LINK

Guidebook for the Synthesis of Oligonucleotides

Product Guide 2015/16

www.linktech.co.uk | +44 (0) 1698 849911 | sales@linktech.co.uk

Exceptional as standard

LINK

Don't let paperwork slow down the real work.

Order reagents in seconds for next-day delivery.

Synthesis of oligonucleotides is complicated enough without the chore of ordering reagents getting in the way. Buying oligo reagents from LINK is simple and straightforward. In the time it takes to download a couple of music tracks online, you could be sorted for next-day delivery, anywhere in mainland Europe.

Our extensive catalogue of products for the synthesis of oligonucleotides (including DNA, RNA, PNA and UNA products) has all the reagents you need. What's more, our quality control ensures impeccable consistency of product.

Opening an online account with LINK is easy – and you can place your first order in minutes. Popular products are always held in stock, ensuring a fast and reliable turnaround from order to delivery. And, for many common products, highly attractive multi-pack discounts are available immediately online – there's no need to request a quote.

All of which leaves you to concentrate on making your oligos. While we concentrate on making your life easier.

Find out more: **www.linktech.co.uk** | **+44 (0) 1698 849911** | **sales@linktech.co.uk**

Exceptional as standard

Guidebook for the Synthesis of Oligonucleotides

Product Guide 2015/16

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LINK

WE TAKE QUALITY TO A HIGHER LEVEL

At LINK, we work to one standard.

And that's exceptional.

We manufacture reagents for oligonucleotide synthesis to an unrivalled level of quality. We control and check the whole manufacturing process, repeatedly, at every stage. We put 25 years' experience in chemistry - in particular with respect to oligo synthesis reagents - at your disposal, and whether you want 1g or 1kg, we're with you all the way.

We combine a comprehensive catalogue of off-the-shelf materials together with a full bespoke service – including our specification-tailored LINK+ reagents – so you can order precisely what you need, every time.

As one of the biggest specialist companies in our field, we ensure great value for money.

Our team is made up of passionate, committed professionals who deliver the most efficient, helpful service you could imagine. And don't just take our word for it: when we asked our customers to rate our service, the average score was a rather impressive 9.3 out of 10 (we're working on the other 0.7).

If it says LINK on the label, you know you can trust it.

A little bit of history...

LINK began operations in 1989 in laboratories within Glasgow University with an initial emphasis on the provision of reagents for DNA synthesis. In 1992 the Company expanded to occupy custom-equipped laboratories in Cumbernauld near Glasgow. In 2000 we moved to newly-built premises at the Strathclyde Business Park in central Scotland, where we are still located.

Initially the Company operated primarily as a contract manufacturer with a number of key clients, however in 2001 we launched our first catalogue of reagents offering our manufactured products direct to customers. Since then the catalogue has grown to hundreds of products, many of them unique to us, whilst our custom and contract business goes from strength to strength.

Throughout this expansion, the Company goal has remained the same: to provide the unique link between scientists working in the life sciences and the chemical tools and technologies that they require. Such a bridge forms the basis of the multi-disciplinary approach vital to the growing biotechnology industry.

LINK now boasts a strong portfolio of joint-venture development projects and research collaborations with a number of companies and research institutions worldwide.

Our quality guarantee

At LINK, we are very customer focused and strive to not only meet but exceed the requirements and expectations of our customers. As a measure of this, our Quality Management System (QMS) is certified to ISO9001 standard.

While continuing to provide robust and reliable service and products, we are committed to continual improvement and as such once an improvement project has been determined, we put together Continual Improvement Groups consisting of key people to work on the project. Many of these project ideas come from the customer. In conjunction with our QMS, our Customer Relationship Management (CRM) System allows all customer contact (sales, enquiries, technical support) to be monitored as a means of identifying trends which allows us to act faster in terms of any changes that may be required as a result of a trend, *e.g.* scale up of a particular product. This then becomes a continual improvement project.

Clearly LINK is committed to providing high quality products therefore our QC processes allow for testing of all key raw materials and all intermediates in addition to our final products. After QC all batches undergo QA review prior to release.

Facilities

LINK operates from a single 7500 sq. ft. custom-built facility in central Scotland. In 2008 the upgrading of these facilities to include two large-scale laboratories was completed, giving the company the ability to manufacture from small, development scale right up to the kilo scale to meet our customers' growing needs.

Our QC operation, and associated QA quarantine areas, are housed in defined areas of the facility separate from process development and routine manufacturing.

Not only do we have a wide variety of in-house analytical techniques such as HPLC, LCMS-TOF and UV/vis, we also work with external partners to provide *e.g.* NMR and fluorescence measurement, enabling us to fully characterise and analyse all our products, intermediates and raw materials. All our oligonucleotide synthesiser reagents products are functionally tested on one of our in-house oligo synthesisers and the resulting oligo subsequently analysed.

Society & the environment

At LINK we are very much aware of our responsibility to the environment and consider the impact of our operations on society very seriously. We strive to minimise our waste production and maximise recycling at all times - whether in our laboratories, canteen, or paper for our marketing materials.

Of course, chemical production will always produce waste but we always ensure that the hazardous material element of what waste we do produce is properly contained and dealt with responsibly.

We also have an annual charities budget that supports charitable organisations chosen by our staff, including Cancer Research UK (www.cancerresearchuk.org), and, locally, the Scottish Centre for Children with Motor Impairments (www.craighalbert.org.uk).

LINK+

YOUR WISH IS OUR COMMAND

We just love a demanding customer.

That's precisely why LINK+ evolved.

At LINK, our catalogue range of oligo reagents is exceptional. But it may not be enough for our more specialist clients. You know who you are – CMOs, GMP manufacturers, diagnostic oligo manufacturers and others.

You may work to ISO 13485, but you're certainly a customer with highly specialised, possibly unique, requirements. You need a partner you can trust to deliver the highest quality products, to your exacting standard, *via* a managed supply chain.

Our LINK+ service has been designed just for you. It brings you enhanced specification, scaled manufacturing and the option for custom and bulk packaging, as well as scheduled deliveries.

LINK+ reagents take high-end specification to a whole new level. A LINK+ product has a base specification set at the highest available on the market, but we'll adapt it to exactly what you need – using your test methods if that's what you want. We'll supply a comprehensive analytical report, including impurity profiling. And as you'd expect, at LINK we're certified to ISO 9001.

So be as demanding as you like. We'll rise to the challenge.

LINK+ reagents

Through working with several key clients, we have begun the creation of the LINK+ range of products. These have a high-grade minimum specification, however this can be further customised to your requirements if necessary. Through continued consultation with customers we will add to this range of products over time. Full specifications are available on request, however the headline values are shown on our website at www.linktech.co.uk/linkplus.

Ordering LINK+ reagents

As these are specialised products, we normally manufacture to order therefore there will be a short lead time. Stocks are not held in the same way as regular catalogue products, although we do hold residual stock where appropriate. Pack sizing and pricing for LINK+ reagents is dependent on your specific requirements. Please therefore contact us, quoting the item numbers you are interested in.

Enhanced specification

Although at LINK our QMS is certified to ISO9001:2008 standard, we understand that many of our customers are certified to other quality standards (*e.g.* ISO13485) and therefore have different requirements. For these customers, we have implemented processes which will allow us to meet these specific needs.

For instance, all our products undergo QC testing and QA review to ensure they meet a defined specification and a Certificate of Analysis (CoA) is issued and shipped to the customer along with the product. For many customers this is sufficient. However, for others there is a benefit for LINK to align our QC analysis for a product (or set of products) to that of the client's own testing and copies of the analytical data along with the CoA are sent along with the product. This is in place for many of our customers.

For other clients we go one step further and carry out impurity profiling based on LCMS, NMR and HPLC data. Where applicable we can provide the analytical data and interpretation prior to shipping the material for pre-approval by the customer. This is something we have done when *e.g.* the purchase is for a new product or the customer is evaluating us as a new supplier.

Scaled manufacturing

LINK's facilities give us the capability to manufacture from the small research gram scale right up to kilo scale batches for commercial manufacture. Other suppliers can offer either end of that spectrum but very few can do both. Through tested process optimisation methods we can scale up our production, taking your product from initial testing right up to early phase clinical trials of the oligonucleotide, thus providing the essential continuity of your supply chain through these stages.

Custom and bulk packaging

We take care to provide our off-the-shelf products in popular pack sizes and packing formats. As your needs grow so do the options available to you. We can supply any manufactured product in bulk quantities, with a significant reduction in pricing/gram from our standard catalogue packs.

We can either ship in single containers or aliquot material; the unit size and type of packaging is entirely at the discretion of the customer. For any bulk order, the packaging will be agreed at the outset of manufacturing. This is particularly important for oils as the final dispensing of the material generally has to be into synthesiser-ready bottles for dilution and use without further handling.

Planned deliveries

No one likes to try to predict the future. However, regardless of product unit size (therefore this could equally apply to small vials and bulk containers) the efficiencies gained from forward ordering and planned deliveries are significant for both us and you as the customer. For us it allows us to plan production effectively and to maximise scale thereby reducing costs. Consequently for you it provides lower price and confidence to your supply chain, through preferred discounting and lowered administration costs, and the knowledge that our production schedule is being tailored to your needs.

For further information, including a list of LINK+ products, see **www.linktech.co.uk/linkplus**

Contents

Legal Statements

The small print.

Black Hole Quenchers®, CAL Fluor® Dyes and Quasar® Dyes.

Black Hole Quencher, BHQ, CAL Fluor and Quasar, are registered trademarks of Biosearch Technologies, Inc. (BTI).

All BTI products offered for sale by LINK are sold under the condition that they be used for research purposes only and are prohibited from use in diagnostic or other applications unless explicitly authorized by separate written agreement with BTI.

Black Hole Quencher® (BHQ®) CAL Fluor®, and Quasar® dyes (referred to collectively as "BTI Dyes") are sold to the purchaser for internal R&D use only and are not to be used for clinical or clinical diagnostic purposes. Neither the BTI Dyes nor the compounds synthesized with them are to be re-packaged or re-sold. Separate licences for other than the aforementioned internal R&D applications of the BTI Dyes may be available. Please inquire *via* info@biosearchtech.com.

The Black Hole Quencher® dye technology is protected in the United States and other countries by U.S. patents and continuations numbered 7,019,129, 7,019,129B1, 7,109,312B2, 7,582,432, 8,410,255B2 and 8,440,399B2 issued to Biosearch Technologies, Inc. The CAL Fluor® technology is covered by U.S. patent number 7,344,701B2. The Quasar® technology is covered by U.S. Patent numbers 7,705,150B2, 7,868, 157B2 and 8,436,153B2.

dPEG® Reagents

dPEG® products are sold under licence from Quanta Biodesign, Ltd, (Plain City, OH), are sold for laboratory use only and are not intended to be used for any other purposes, including but not limited to, *in vitro* diagnostic purposes, in foods, drugs, medical devices, cosmetics or commercial use. A separate licencing agreement/supply agreement with Quanta BioDesign, Ltd. is required to use the products in applications beyond laboratory use. Customers are responsible for informing Quanta BioDesign, Ltd when products are being used beyond lab use. dPEG® technology is protected by US Patents #7,888,536 and 8,637,711 and US Patent Pending #2013/0052130. dPEG® is a registered trademark of Quanta BioDesign, Ltd.

EDITH

The use of EDITH in the US is protected under US Patent No. 5,852,168 and licence for such use must be sought from The University of Minneapolis.

2'-Fluoro

A licence may be required from Isis Pharmaceuticals, Inc. to incorporate 2'-fluoro modified nucleosides into oligonucleotides as claimed in US Patent Numbers 5,670,633, 6,005,087, 6,531,584 and foreign equivalents.

Photocleavable (PC) Modifiers

The Photocleavable (PC) Modifiers were developed by Ambergen Inc., Massachusetts, US, Link Technologies Ltd, Bellshill, Scotland and Glen Research Corp., Virginia, US and are made available under licence from Ambergen Inc. These products or the use of these products may be covered by one or more patents including: US Patent Nos. 6,218,530; 7,195,874; 8,906,700; 8,932,879 and International Patent Nos. EP1086251; JP,4058704,B; JP,4147230,B.

PNA

PNA monomers are manufactured and sold pursuant to licence under one or more of US Patents Nos. 5,773,571, 6,133,444, 6,172,226, 6,395,474, 6,414,112, 6,613,873, 6,710,163 and 6,713,602, or corresponding patent claims outside the US. PNA Monomers are sold to be used for internal research use only, and are not to be resold unless by separate licence.

SolidCPR™

SolidCPR™ is sold by agreement with Glen Research, Corp., Virginia, US under EP Patent No. 0816368.

SynBase™

SynBase™ is a trademark of Link Technologies Ltd, Bellshill, Scotland.

UNA

UNA oligonucleotide reagents are protected by patent applications owned by Arcturus Therapeutics, Inc. (www.arcturusrx.com) and which, within the reagents field, have been exclusively licenced to RiboTask ApS (www.ribotask.com) with the opportunity of further sublicencing. A licence from RiboTask ApS enables Link Technologies Ltd to provide to customers UNA phosphoramidite monomer reagents for use in oligonucleotides for research uses only. The products in their original or any modified form may be used only for the customer's internal research purposes and not for commercial, diagnostic, clinical, therapeutic, or other use, including use in humans. The purchase of products does not include or carry an implied right or licence for the buyer to use such products in their original or any modified form for commercial use. Customers may not distribute or provide the products in their original or any modified form to any third party.

Vitamin Products

Use of 5'-Niacin-CE Phosphoramidite, 5'-Niacin-C6-CE Phosphoramidite and bisDMT-Pyridoxine-C6-CE Phosphoramidite (Patent Pending PCT/GB2015/050611) is only permitted for incorporation into oligonucleotides for private purposes, including Research Use Only (RUO) oligonucleotides, *i.e.* not for commercial applications. Purchase of these products from LINK allows the purchaser the right to sell an oligonucleotide modified with the products, but only for RUO by the end-user. This right only applies to product purchased from Link Technologies or a licenced vendor. For all other uses of these products, in particular commercial applications, licencing programs are available from Link Technologies Ltd. For the avoidance of doubt, a separate licence must be sought for any synthesis and commercial sale of any oligonucleotide modified with the product for any non-RUO purpose, such as, but not limited to, therapeutic and diagnostic applications. Please contact us for further details.

Ordering Information

All you need to know about getting your oligonucleotide reagents from us.

Product Packaging

There are a number of different automated DNA/RNA synthesisers in current use. Our standard product packaging is designed to be compatible with the original configuration of as many of the popular small to medium scale models as possible, the most common being the ABI391/392/394 series, the Expedite 8909 and the MerMade range. The different bottles used in our packaging are listed in Table 1 below, whilst Table 3 on page 20 shows the standard configuration for the majority of synthesisers available. Please refer to these tables, particularly when ordering ancillary reagents.

Unmodified DNA phosphoramidites (page 26) are packaged in ABI, Expedite and MerMade vials. Customers should therefore note the relevant catalogue numbers in the ordering information for these products. All other phosphoramidites (up to 250mg) are packed in an 8ml ABI compatible vial, bottle A in Table 1 below. An empty Expedite vial, for transfer of the amidites by the customer, can be supplied free of charge if requested. Please indicate this requirement by explicitly ordering the vial pack (2100-Z001; contains bottle C). Similarly, empty MerMade bottle packs can be supplied free of charge (order 2173-Z001, bottle D).

Our column-packed CPG supports are routinely packaged in ALL-FIT columns suitable for use with most automated synthesisers which use the luer format, including ABI and Expedite instruments. Pipette-tip columns (for use with MerMade, ABI 3900 *etc*), packed with base functionalised CPGs and popular 3'-modifier CPGs, are also available and are listed where appropriate. Where not listed these can normally be packed by request.

PNA products (**5001** - **5004**, page 57) are packed in bottle C, as is EDITH (**2171**, page 62).

Ancillary reagents are packaged in a number of bottle sizes and type. These are summarised on page 27.

To order empty stock bottles and columns see details on page 115.

Given the variety of instruments available, we do not list every possible bottle size. In many instances, however, where we do not list a type of packaging or bottle type we may still be able

Table 1. The standard bottle types used for packaging synthesiser-ready reagents are denoted in the Guide by code letters as listed here. All modifier phosphoramidites up to 250mg are packed in bottle A.

Code	Item No.	Size	Type	Neck	Closure
A	0189	8ml	Amber glass	20mm	Septum
B	0190	15ml	Amber glass	20mm	Septum
C	0230	30ml (1oz)	Amber glass	18mm	Screw
D	0411	30ml (1oz)	Amber glass	28-405 (28-400)	Screw
E	0229	60ml	Amber glass	18mm	Screw
F	0105	60ml	Amber glass	20mm	Septum
G	0107	100ml	Amber glass	20mm	Septum
H	0109	200ml (8oz)	Amber glass	24-405 (24-400)	Screw
J	0110	200ml (8oz)	Amber glass	28-405 (28-400)	Screw
K	0111	450ml (16oz)	Amber glass	28-405 (28-400)	Screw
L	0747	500ml	Clear glass (Schott)	DIN45 (GL45)	Screw
M	0448	1L	Amber glass	DIN45 (GL45)	Screw
P	0911	500ml	Amber glass (Schott)	DIN45 (GL45)	Screw

to assist you with what you require. Therefore please ask and we'll do our best to help.

Where we supply bulk quantities of a product this will be supplied either in HDPE Nalgene® bottles (powders) or Schott/Duran bottles (oils) unless otherwise specified.

For most products, custom packaging is generally available on request.

Pricing & Payment

Current prices (in £, € and $) are available on our web site. A number of core products have advertised quantity discounts (up to multiples of 50). These are clearly shown on the web site. Further discounts will be considered on regular and bulk purchases for any product. An automatic 10% discount on our list prices will be applied to customers from academic institutions, except where alternative or additional discounts have been agreed, or where quantity discounting is already in place. We reserve the right to alter or remove discounts on certain products at our discretion. Customers with agreed discounts are able to view these prices when logged in to the web site.

Invoices are sent by e-mail. Customers can be invoiced in GBP, Euro or USD according to their preference. By default, invoices should be settled by bank transfer. Bank payment details are provided with invoices.

Customers can now pay invoices by credit card *via* invoice from our PayPal account. If you wish to use this method, please indicate at the time of ordering and clearly state the e-mail address to which the invoice should be sent. Invoices will be sent when the goods are ready to ship. Payment by this method is due immediately on receipt of invoice, and goods will be shipped once confirmation of payment has been received, unless otherwise agreed. Paypal invoices are raised in GBP. Customers may request an invoice in either Euro or USD, however there is unfortunately a 2.5% surcharge for this option due to charges incurred.

Ordering

Online

Orders can be placed online at **www.linktech.co.uk**. Printed orders for faxing to us can also be generated easily on our web site. Customers registered with the site can log in to view their agreed discounts and can order products instantly at these prices. If you wish assistance with registering on the web site then please contact webmaster@linktech.co.uk.

Payment for orders made *via* the site is collected by invoice. At the time of writing, there is no immediate payment facility on the web site, however invoices can be requested *via* PayPal for credit card payment (see above).

By Fax, Telephone or E-mail

When using any of these methods, please ensure that you provide us with the following information:

- Customer number (if known)
- Contact name and telephone number
- E-mail address for order confirmation, shipping notification and invoice
- Purchase order number
- Catalogue numbers and product descriptions
- Order quantity and unit size of products

For current customers delivery and invoice addresses will not be required unless different from previous orders.

Orders can be placed by:

Telephone: +44 (0) 1698 849911
Fax: +44 (0) 1698 849922
E-mail: sales@linktech.co.uk

Confirmation of Orders

Orders will be confirmed by e-mail upon receipt, giving an estimated shipping date if possible. Receipt of orders placed on the web site will also be confirmed automatically by e-mail. Please advise immediately if there are any discrepancies. Where an estimated shipping date is not provided with the order confirmation, one will be provided ASAP. If you have not received an e-mail confirmation within 24 business hours then please contact us in case we have not received your order.

Shipping

Shipping costs are summarised in Table 2 below. In each of the UK, EU and US territories there is a standard shipping rate for non-hazardous goods. Regardless of storage conditions, most of our products are perfectly stable for ambient temperature shipment. However, a small number of products require shipment on ice. For these an additional shipment charge is necessary. We do not routinely ship on dry ice, however this can be arranged at the customer's expense.

Due to carrier restrictions and legal requirements a higher shipment cost is incurred on shipments of hazardous products. This is variable and will be confirmed on receipt of order. Please note the vast majority of our products are classed as non-hazardous, however all solvents and liquid reagents are classed as hazardous. An updated list of countries we are able to ship hazardous goods to is now also included below. Please do note that the regulations for shipping hazardous goods change frequently, so it is always worth checking the web site for updates.

Hazardous products, and those requiring shipment on ice, are noted as such on the web site and identified in the Product Guide. Where multiple shipments are required on a single order, we only charge the highest shipment component to a customer. For example, an order containing both hazardous and non-hazardous goods will be shipped in two shipments: the hazardous shipment will be charged accordingly, however the non-hazardous shipment will incur no further charge.

To guarantee same-day shipment for non-hazardous stock items, orders must be placed by 14:00 (UK time). For hazardous items this cut-off is 11:00 (UK time). Stock items are normally shipped within 24 hours of receipt of order (except where perishable products or hazardous products require alternative shipping arrangements). Where products are not in stock, customers will be advised within 24 hours of expected shipping dates. Notification of shipping will be made by e-mail, providing full Internet package tracking information where available.

Terms and Conditions of Sale

A full description of our terms and conditions is available at **www.linktech.co.uk/terms**.

Table 2. Summary of Shipping Costs.

	UK	Europe	US	ROW
Orders containing no hazardous goods, shipped at ambient temperature.	£10	€25	$30	Variable
Orders containing products requiring shipment on ice.	£15	€50	$45	Variable
Orders containing hazardous goods.*	£30	Variable - see included countries below	Not available	Not available

* The cost of hazardous shipments outside of UK (Scotland, England, Wales, Northern Ireland) is variable and sent by a non-guaranteed delivery service. Delivery is typically up to 1-2 weeks. Hazardous goods can only be shipped to the following countries: UK, Andorra, Austria, Belgium, Bulgaria, Croatia, The Czech Republic, Denmark, Estonia, Finland, France, Germany, Gibraltar, Greece, Guernsey, Hungary, The Republic Of Ireland, Italy, Jersey, Latvia, Lithuania, Luxembourg, The Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and Switzerland.

Delivery methods and cost of shipments outside of the UK, Europe and the US will be confirmed upon receipt of order if available. In all cases where costs are variable the actual cost will be confirmed upon receipt of order.

These costs are subject to change therefore please do check the web site for our latest charges.

Technical Support

In the first instance, technical support is available *via* our online Help Centre at **http://helpcentre.linktech.co.uk**. If the information you require is not already detailed on the website, then a facility is provided to submit a technical support ticket.

Whilst we think this is an excellent online resource, please feel free to contact our support team by telephone on +44 (0) 1698 849911 with any query you might have.

What We Don't Do...

At LINK we can do most things when it comes to reagents for oligo synthesis. One thing we don't do, however, is provide a custom oligo synthesis service. After all, this would just put us in competition with our own customers!

If you don't have a synthesiser and are looking to get your oligos made for you then a quick search on the Internet should give you the name of a custom oligo company in your area. If they don't provide the modification you're after then please let us know and we'll try and sort that out for you. For more information see **www.linktech.co.uk/custom_oligos**.

Distributors

Although we are happy to ship worldwide, you may find the convenience of a local distributor beneficial. For this reason we are appointing partners outside of the UK and Europe to offer this service. Current partners are listed below, however please check **www.linktech.co.uk/catalogue/distributors** for an updated list.

Please note that some catalogue products may not be available in some territories due to licencing restrictions.

North America and Canada

American International Chemical, Inc.
Advanced Technology Division
135 Newbury St.
Framingham
MA 01701
USA

Toll Free: 800-238-0001 EXT: 2287

E-mail: orders@aicma.com

Fax: 508 872 5737

Web: **www.aicma/atd**

Japan

GeneDesign Co. Ltd.
7-7-29 Saitoasagi
Ibraki
Osaka 567-0085
Japan

Tel: +81-72-640-5180

Fax: +81-72-640-5181

E-mail: info-e@genedesign.co.jp

Web: **www.genedesign.co.jp**

Table 3. Summary of automated DNA/RNA synthesiser types (A - Z by manufacturer). This table is for reference purposes only and should not be used for instrument buying decisions. The data is correct to the best of our knowledge at the time of writing, however please check with the relevant manufacturer for detailed and current specification information as this may change.

Manufacturer	Model	Scale	Format	Trityl monitor	Amidite ports (A, G, C, T/U)	Amidite Ports (modifiers)	Ancillary Reagent Ports
ABI	ABI 391	40nmol - 15µmol	Column	No	4	1 or 4	5 + 2 external for MeCN and DCM
ABI	ABI 392	40nmol - 15µmol	Column	Yes	4	1 or 4	5 + 1 for deprotection solution + 2 external for MeCN and DCM
ABI	ABI 394	40nmol - 15µmol	Column	Yes	4	1 or 4	5 + 1 for deprotection solution + 2 external for MeCN and DCM
ABI	ABI 3400	40nmol - 1µmol	Column	Yes	4	1 or 4	5 + 1 for deprotection solution + 2 external for MeCN and DCM
ABI	ABI 3900	40nmol - 1µmol	Column	No	8	6	2 banks of 4 + 3 external (1 x Deblock, 2 x MeCN)
Azco	OligoArray	~0.1 pMoles	Chip	No	4	2	7
Azco	Oligo 800	200-400nmol or 1-15µmol	Column	Yes	4	4	2 Activator + 8 others
Bioautomation	MerMade 4	50nmol - 50µmol	Column	Yes	10		7
Bioautomation	MerMade 6	50nmol - 200µmol	Column	Yes	10 to 20		7
Bioautomation	MerMade 12	50nmol - 200µmol	Column	Yes	10 to 20		7
Bioautomation	MerMade 48	50nmol - 1µmol	Column	No	2 x 4	6	7
Bioautomation	MerMade 192	5nmol - 1µmol	Plate or Column	No	10 to 20		7
Bioautomation	MerMade 192E	50nmol - 1µmol	Column	No	4	4	7
Bioautomation	MerMade 384	5nmol - 1µmol	Plate	No	10		7
Biolytic	Dr Oligo 96/192	10nmol - 2µmol	Plates or column	No	up to 23	6 to 16	4 plus 10 external
Biolytic	Dr Oligo XC	10nmol - 2µmol	Plates or column	No	up to 23	6 to 16	6 to 8
Biosset	ASM-800	40nmol – 0.4µmol or 1-15µmol	Column	No	4	4	10

Standard Column Type	Standard Bottle Type (Amidites)	Standard Bottle Type (Ancillary Reagents)	Synthesis positions	Notes & Availability
Luer	20mm slider	24-405 or 28-405 screw	1	Discontinued. Used instrument may be available on second-hand market.
Luer	20mm slider	24-405 or 28-405 screw	2	Discontinued. Used instrument may be available on second-hand market.
Luer	20mm slider	24-405 or 28-405 screw	4	Discontinued. Used instrument may be available on second-hand market.
Luer	20mm slider	24-405 or 28-405 screw	4	Discontinued. Used instrument may be available on second-hand market.
Pipette-tip	20mm slider	28-405 screw	48	Discontinued. Used instrument may be available on second-hand market. Original MerMade 48 was based on this.
N/A	Information not available	Information not available	1 to 12 chips; each containing 2,000; 12,000; or 90,000 oligos	Deblocking requires special reagent for electrochemistry, all other reagents are "off-the-shelf" synthesis reagents. For availability see www.azcobiotech.com.
Pipette-tip	Information not available	Information not available	8	Column fittings can be converted to ALL-FIT. For availability see www.azcobiotech.com.
Pipette-tip	45mm (GL45) or 28-405 screw	28-405 screw	4	Dedicated sulphurisation port. For availability see www.bioautomation.com.
Pipette-tip	45mm (GL45) or 28-405 screw	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	6 expandable to 12	Dedicated sulphurisation port. For availability see www.bioautomation.com.
Pipette-tip	45mm (GL45) or 28-405 screw	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	12	Dedicated sulphurisation port. For availability see www.bioautomation.com.
Pipette-tip	20mm slider	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	48	3900-based version now discontinued; being redesigned at time of writing. For availability see www.bioautomation.com.
Pipette-tip (if Column)	45mm (GL45) or 28-405 screw	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	2 x 96	Dedicated sulphurisation port. For availability see www.bioautomation.com.
Pipette-tip	45mm (GL45) or 28-405 screw	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	192	For availability see www.bioautomation.com.
N/A	45mm (GL45) or 28-405 screw	Activator/Caps/Oxidiser: 28-405 screw; Wash/ Deblock: 45mm (GL45) screw	4 x 96	For availability see www.bioautomation.com.
Pipette-tip	28mm screw	Activator/Caps/Oxidiser: 45mm (GL45) screw; Wash/Deblock: 38mm screw	2 x 96	Can add an extra oxidiser, premixed Capping reagents. Only high-throughput system that includes trityl monitoring. Also available as 384 and 768 versions. For availability see www.biolytic.com.
Pipette-tip	28mm screw	Activator/Caps/Oxidiser: 45mm (GL45) screw; Wash/Deblock: 38mm screw	2 x 96	Features of Dr Oligo 96/192 plus synthesis, cleave and deprotection all on the same instrument. For availability see www.biolytic.com.
Luer	Mix of 45mm (GL45) and 38mm 2.5L/4L jug	Mix of 45mm (GL45) and 38mm 2.5L/4L jug	8	For availability see www.biosset.com.

Manufacturer	Model	Scale	Format	Trityl monitor	Amidite ports (A, G, C, T/U)	Amidite Ports (modifiers)	Ancillary Reagent Ports
Biosset	ASM-2000	10nmol – 1µmol	Plates or column	No	4	8	9
GE	ÄKTA oligopilot plus 10	1 - 50µmol	Column	Yes	4	4 to 8	6
GE	ÄKTA oligopilot plus 100	50µmol - 9mmol	Column	Yes	4	4 to 8	6
GE	OligoPilot 400	4 - 30mmol	Column	Yes	4	7	9 + 3 MeCN
GE	OligoProcess	Custom	Column	Custom	Custom	Custom	Custom
K&A Laborgeraete	S-4-LC	40nmol - 10µmol	Column	No	4	6	6
K&A Laborgeraete	S-8-LC	40nmol - 10µmol	Column	No	4	6	6
K&A Laborgeraete	H-6	40nmol - 10µmol	Column	Yes	4	8	6
K&A Laborgeraete	H-8 Standard	40nmol - 10µmol	Column	Yes	4	8	6
K&A Laborgeraete	H-8 SE	40nmol - 10µmol	Column	Yes	4	16	10
K&A Laborgeraete	H-16	40nmol - 10µmol	Column	Yes	4	12	10
K&A Laborgeraete	H-32	40nmol - 10µmol	Column	Yes	4	1	6
K&A Laborgeraete	H-96/192	Variable	Plate	No	Custom	6	Custom
Perceptive Biosystems	Expedite 8905	50nmol - 15µmol	Column	Yes	4	5	8
Perceptive Biosystems	Expedite 8909	50nmol - 15µmol	Column	Yes	4	5	8
Polygen	10-Column	10nmol - 1µmol or 1-5µmol	Slider Block	Yes	4	2	6
Polygen	12-Column	10nmol - 1µmol or 1-5µmol	Slider Block	Yes	4	4	6
Polygen	96-Column	10nmol - 1µmol or 1-5µmol	Slider Block	Yes	4	4	6
Polygen	384-Column	10nmol - 1µmol or 1-5µmol	Slider Block	Yes	4	4	6

Standard Column Type	Standard Bottle Type (Amidites)	Standard Bottle Type (Ancillary Reagents)	Synthesis positions	Notes & Availability
N/A	Mix of 45mm (GL45) and 38mm 2.5L/4L jug	Mix of 45mm (GL45) and 38mm 2.5L/4L jug	96	For availability see www.biosset.com.
Various	20mm slider	45mm (GL45) screw	7	For availability see www.gelifesciences.com.
Various	20mm slider	45mm (GL45) screw	7	For availability see www.gelifesciences.com.
Various	45mm (GL45) screw	45mm (GL45) screw	1	Can be customised to synthesise on a 60mmol scale. 21CFR part 11 compliant software. For availability see www.gelifesciences.com.
Various	45mm (GL45) screw	45mm (GL45) screw	1	Industrial, late phase clinical trial use. For availability see www.gelifesciences.com.
Luer	18mm screw	28-405 or 45mm (GL45) screw	4	For availability see www.dna-synthesizer.de.
Luer	18mm screw	28-405 or 45mm (GL45) screw	8	For availability see www.dna-synthesizer.de.
Luer	18mm screw	28-405 or 45mm (GL45) screw	6	For availability see www.dna-synthesizer.de.
Luer	18mm screw	28-405 or 45mm (GL45) screw	8	For availability see www.dna-synthesizer.de.
Luer	18mm screw	28-405 or 45mm (GL45) screw	8	For availability see www.dna-synthesizer.de.
Luer	18mm screw	45mm (GL45) screw	16	For availability see www.dna-synthesizer.de.
Luer	18mm or 28-405 screw	45mm (GL45) screw	32	For availability see www.dna-synthesizer.de.
N/A	Custom	Custom	2 x 96	Discontinued. For availability see www.dna-synthesizer.de.
Luer	18mm screw	28-405 screw	2	Aux position used for sulphurisation. Discontinued. Used instrument may be available on second-hand market.
Luer	18mm screw	28-405 screw	2	Aux position used for sulphurisation. Discontinued. Used instrument may be available on second-hand market.
N/A	18mm screw	28-405 screw	10	Proprietary slider block; does not use columns. For availability see www.polygen.de.
N/A	18mm screw	28-405 screw	12	Proprietary slider block; does not use columns. For availability see www.polygen.de.
N/A	18mm screw	28-405 or 45mm (GL45) screw	96	Proprietary slider block; although columns can be custom fitted. For availability see www.polygen.de.
N/A	18mm screw	28-405 or 45mm (GL45) screw	384	Proprietary slider block; does not use columns. For availability see www.polygen.de.

Chemistry of Oligonucleotide Synthesis

Over 30 years since its introduction, the use of phosphoramidite chemistry remains the method of choice for the automated synthesis of oligonucleotides.

Oligonucleotide Synthesis Cycle

Most techniques used in molecular biology today rely on synthetic oligonucleotides, including PCR, DNA sequencing, and Single-Nucleotide Polymorphism (SNP) assays. The vast majority of oligonucleotides are synthesised on automated synthesisers using phosphoramidite methodology.

Oligonucleotide phosphoramidite chemistry was first introduced 30 years ago.[1] The method is based on the use of DNA phosphoramidite nucleosides which are modified with a 4,4'-dimethoxytrityl (DMTr) protecting group on the 5'-OH, a ß-cyanoethyl-protected 3'-phosphite, and appropriate conventional protecting groups on the reactive primary amines in the heterocyclic nucleobase. The four classic protected DNA nucleoside phosphoramidites are benzoyl-dA, benzoyl-dC, iso-butyryl-dG and dT (which requires no base protection), products **2003**, **2004**, **2002** and **2001** respectively. As discussed in the following pages, both acetyl-dC (**2034**) and dimethylformamidine-dG (**2030**) are now also routinely used.

The phosphoramidite approach is today carried out almost exclusively on automated synthesisers using controlled-pore glass (CPG) or polystyrene solid supports.[2] These supports are held in small synthesis 'columns' that act as the reaction vessel. These columns are attached to the synthesiser and phosphoramidite and ancillary reagents are passed through the column in cycles thus extending the oligonucleotide chain.

The synthesis cycle consists of four steps: deblocking (detritylation); activation/coupling; capping; and oxidation. These steps are shown in Figure 1. Synthesis occurs in the 3' to 5' direction; this is in fact opposite to enzymatic synthesis by DNA polymerases.

Conventionally, the 3' base in the sequence is incorporated by use of a base-functionalised CPG or polystyrene support (1), although 'universal' supports are available (see below). Synthesis initiates with removal ('deblocking' or 'detritylation') of the 5'-dimethoxytrityl group by treatment with acid (classically 3% trichloroacetic acid in DCM (**4140**)[3]) to afford the reactive 5'-OH group (2). The phosphoramidite corresponding to the second base in the sequence (3) is activated[4] (using a tetrazole-like product such as ETT (**0237** or **3140**/**3142**/**3145**/**3146**) or BTT (**0234** or **3160**/**3162**), then coupled to the first nucleoside *via* the 5'-OH to form a phosphite linkage (4).

1 **An investigation of several deoxynucleoside phosphoramidites useful for synthesising deoxyoligonucleotides**, L.J. McBride and M.H. Caruthers, *Tetrahedron Lett.*, **24**, 245-248, 1983.

2 For a recent review see: **A brief review of DNA and RNA chemical synthesis**, M.H. Caruthers, *Biochem. Soc. Trans.*, **39**, 575-580, 2011.

3 In larger production environments 2-5% dichloroacetic acid in toluene is commonly used; we can provide this by request - for 5% quote item **4500**. 3% DCA in DCM (**4040**) is also available.

4 A description of the mechanism of activation *via* the phosphorotetrazolide intermediate can be found in **Studies on the role of tetrazole in the activation of phosphoramidites**, S. Berner, K. Mühlegger and H. Seliger, *Nucleic Acids Research*, **17**, 853-864, 1989.

Solid phase phosphoramidite coupling usually proceeds to around 99% efficiency. If the 1% of molecules remaining with reactive 5'-OH groups are left untreated, unwanted side-products will result. To prevent this, a 'capping' step is introduced prior to the oxidation to acetylate the unreacted 5'-OH (5). This is done using a solution containing acetic anhydride (Cap Mix A -**4010**/**4110**/**4012**) and the catalyst N-methylimidazole (Cap Mix B – **4120**/**4122**). Unless blocked these truncated oligos can continue to react in subsequent cycles giving near full-length oligos with internal deletions (species referred to as (N-1)mers).

The unstable trivalent phosphite triester linkage is oxidised, *via* an iodine-phosphorous adduct, to the stable pentavalent phosphotriester (6) using iodine in a THF/(pyridine or lutidine)/water solution (**4230**/**4330**/**4132**). After oxidation the cycle is repeated, starting with detritylation of the second molecule and so on.

The synthesis cycle is repeated until the desired length of oligonucleotide is achieved. At this point the synthesis is complete.

At this point there are two choices: either the final 5'-DMTr group can be left in place as a purification 'handle' (DMT ON option on the synthesiser; see below) or it can be removed by a final acid treatment (DMT OFF) The oligonucleotide can then be cleaved from the solid support using a suitable deprotection solution, *e.g.* ammonium hydroxide solution at room temperature.

If desired, cleavage and deprotection can be carried out simultaneously. In addition to cleaving the support, the cyanoethyl groups are removed from the sugar-phosphate

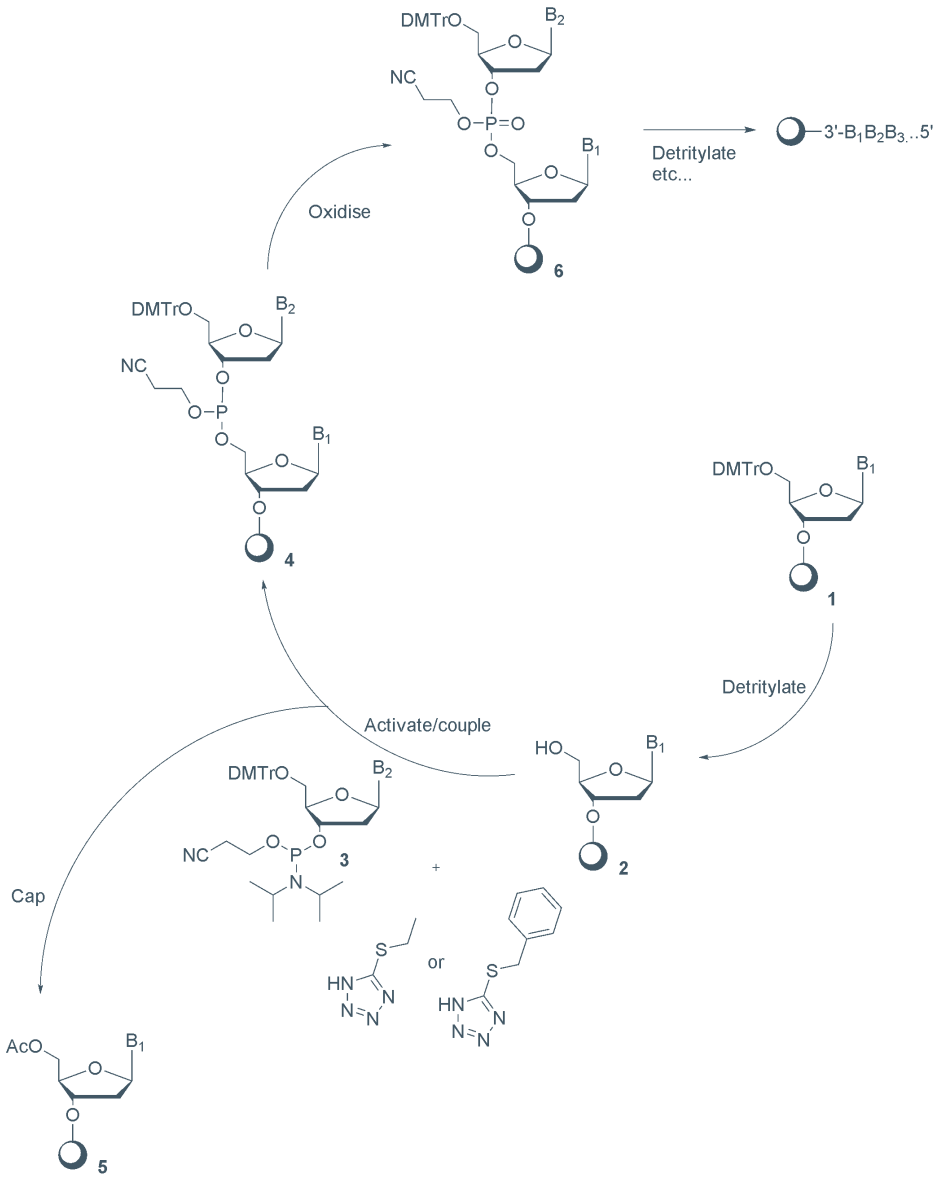


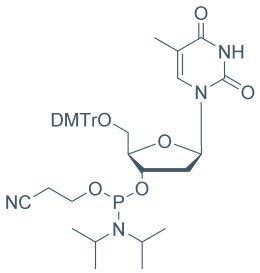
Figure 1. The oligonucleotide synthesis cycle using phosphoramidite chemistry.

backbone. Nucleobase protection is also removed at this time. The specific cleavage and deprotection conditions will vary from oligo to oligo depending on the nucleobase protection employed and any modifiers present. This is typically done by heating the resin in the deprotection solution or in gaseous ammonia.

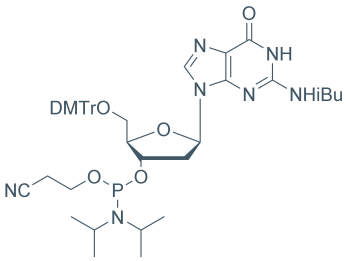
After deprotection, oligos are typically de-salted (also removes small-molecule side products) or purified by methods such as Polyacrylamide Gel Electrophoresis (PAGE), reverse-phase (RP) HPLC, cartridge methods or ion-exchange (IE) HPLC.

Leaving the DMTr protecting group in place aids the purification of full-length sequences, since these contain a hydrophobic group that is retained by reverse phase chromatographic media. In contrast, the failure sequences do not possess a hydrophobic group and so are much less retained on chromatography. This is the basis on which oligonucleotides are purified by reverse-phase (RP) HPLC. Note that if the capping or detritylation steps are inefficient, N-1, N-2, ... species can occur but the 5'-end of the failures is still protected with DMTr.

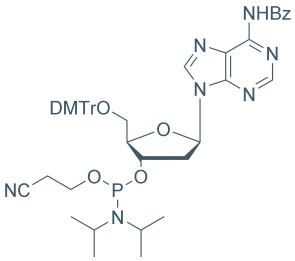
Classically, after preparative chromatography the 5'-DMTr group would be removed by acetic acid treatment to give the biologically active oligonucleotide. Whilst this is still a valid method, it is not now commonly carried out in this manner. There are many available preparative columns (*e.g.* Hamilton PRP-3, ABI POROS, Waters X-Bridge) that will allow DMT ON purification



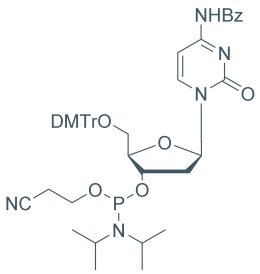
2001



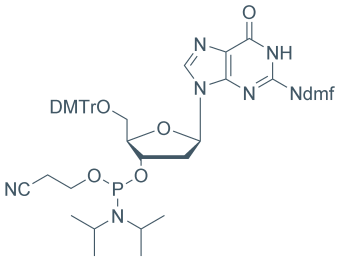
2002



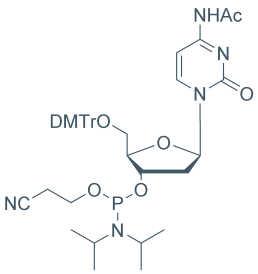
2003



2004



2030



2034

Ordering Unmodified DNA Phosphoramidites

Product	Pack Size	Cat. No. 28-405 screw (*e.g.* MerMade)	Cat. No. 20mm slider (*e.g.* ABI)	Cat. No. 18mm screw (*e.g.* Expedite)
Bz-dA-CE Phosphoramidite	1g	2003-C001M[D]	2003-C001[B]	2003-C001E[C]
	2g	2003-C002M[J]	2003-C002[F]	2003-C002E[E]
Bz-dC-CE Phosphoramidite	1g	2004-C001M[D]	2004-C001[B]	2004-C001E[C]
	2g	2004-C002M[J]	2004-C002[F]	2004-C002E[E]
Ac-dC-CE Phosphoramidite	1g	2034-C001M[D]	2034-C001[B]	2034-C001E[C]
	2g	2034-C002M[J]	2034-C002[F]	2034-C002E[E]
iBu-dG-CE Phosphoramidite	1g	2002-C001M[D]	2002-C001[B]	2002-C001E[C]
	2g	2002-C002M[J]	2002-C002[F]	2002-C002E[E]
dmf-dG-CE Phosphoramidite	1g	2030-C001M[D]	2030-C001[B]	2030-C001E[C]
	2g	2030-C002M[J]	2030-C002[F]	2030-C002E[E]
dT-CE Phosphoramidite	1g	2001-C001M[D]	2001-C001[B]	2001-C001E[C]
	2g	2001-C002M[J]	2001-C002[F]	2001-C002E[E]

Note that the superscript letters denote the bottle type as per Table 1 on page 16.

and the detritylation to be carried out on the column, thus allowing the product to be collected already detritylated. This is fast and less likely to lead to depurination than solution-phase acetic acid treatment.

Many cartridge purification systems based on this principle are available. In this case the crude oligonucleotide is adsorbed on to the cartridge and failure sequences are washed out by elution with water, leaving the pure full-length product on the solid medium. The cartridge is then treated with acid to remove the DMTr group and the pure oligonucleotide is eluted with acetonitrile/water.

The synthesis of RNA (where the chemistry is complicated by the presence of an additional 2'-OH functional group) is discussed on page 32.

Ordering Ancillary Reagents

Product	Pack Size	Bottle Code	Neck†	Cat. No.
Diluent: Acetonitrile, anhydrous*	100ml	G	20mm	4050-D100
ETT Activator (Crystalline)	1g	Nalgene®	N/A	0237-C001
	10g	Nalgene®	N/A	0237-C010
	25g	Nalgene®	N/A	0237-C025
BTT Activator (Crystalline)	1g	Nalgene®	N/A	0234-C001
	10g	Nalgene®	N/A	0234-C010
	25g	Nalgene®	N/A	0234-C025
ETT Activator (0.25M)*	200ml	H	24-405	3140-D200
	200ml	J	28-405	3142-D200
	450ml	K	28-405	3140-D450
ETT Activator (0.5M)*	200ml	H	24-405	3145-D200
	200ml	J	28-405	3146-D200
	450ml	K	28-405	3145-D450
BTT Activator (0.3M)*	200ml	H	24-405	3160-D200
	200ml	J	28-405	3162-D200
	450ml	K	28-405	3160-D450
Oxidiser: 0.1M Iodine in THF/pyridine/water (78:20:2)*	200ml	H	24-405	4230-D200
	450ml	K	28-405	4230-D450
Oxidiser: 0.02M Iodine in THF/pyridine/water (7:2:1)*	200ml	H	24-405	4330-D200
	450ml	K	28-405	4330-D450
Oxidiser: 0.02M Iodine in THF/pyridine/water (89.6:0.4:10)*	200ml	J	28-405	4132-D200
Deblock Mix: 3% TCA in DCM*	450ml	K	28-405	4140-D450
	900ml	M	45mm (GL45)	4140-D900
Cap Mix A: THF/pyridine/acetic anhydride (8:1:1)*	200ml	H	24-405	4110-D200
	450ml	K	28-405	4110-D450
Cap Mix A: THF/lutidine/acetic anhydride (8:1:1)*	200ml	H	24-405	4010-D200
	450ml	K	28-405	4010-D450
Cap Mix A: THF/acetic anhydride (9:1)*	200ml	J	28-405	4012-D200
Cap Mix A: THF/pyridine/Pac-anhydride (85:10:5)*	200ml	H	24-405	4210-D200
	450ml	K	28-405	4210-D450
Cap Mix B: 10% Methylimidazole in THF*	200ml	H	24-405	4120-D200
	450ml	K	28-405	4120-D450
Cap Mix B: 10% Methylimidazole in THF/pyridine (8:1)*	200ml	J	28-405	4122-D200
Anhydrous Wash: Acetonitrile, anhydrous*	300ml	K	28-405	4050-D300
	500ml	P	45mm (GL45)	4050-D500
	1L	M	45mm (GL45)	4050-L001
DEA Wash: 20% Diethylamine in anhydrous acetonitrile*	1L			4028-L001

Note that the Bottle Code letters denote the bottle type as per Table 1 on page 16.
*Classified as hazardous for shipping. †All necks have screw closures except bottle G which has a septum/slider closure.

Choosing a Solid Support

LINK's SynBase™ CPG solid supports are available in a variety of pore sizes and functionalised nucleoside loadings. Three pore sizes are offered: 500Å, 1000Å, and 3000Å. The 500Å support comes in standard (35-50µmol/g) and high (60-100µmol/g) loading varieties (product names are appended with either an 'S' or 'H' respectively where there is a choice). The range has been designed to offer optimum performance depending on the application, and the required nucleoside loading and length of sequence. The range is summarised below.

Shorter primer molecules (ca. 20 bases) can be synthesised on the 500Å support. Large-scale synthesis (typically of oligos 40 bases or less in length) is most economical using the higher loading. Medium-length DNA (20-80 bases) for the widest range of applications, is best synthesised using the 1000Å support. Most companies in truth use 1000Å for all small scale synthesis (40nmol - 10µmol) regardless of oligo length. Indeed, this support is the one we routinely use when preparing all 3'-modifier supports.

For very long sequences (>80 bases) we recommend the 3000Å support. This CPG has a formidable ability to allow synthesis of oligos up to 250 bases long. It is now also available functionalised as a Phosphate (**2398**, page 77) and Spacer C3 (**2395**, page 38) CPG. Other

Table 4. Summary of SynBase™ CPG Supports.

	Features			Optimum Use			
Product	Average Pore Size (Å)	Nominal Particle Size (µm)	Nucleoside Loading (µmol/g)	<20 Bases	20-80 Bases	>80 Bases	Larger-scale Synthesis
SynBase™ CPG 500/110 S	500	110	35-50	•			
SynBase™ CPG 500/110 H	500	110	60-100	•			•
SynBase™ CPG 1000/110	1000	110	25-40	•	•		
SynBase™ CPG 3000/110	3000	110	10-25			•	

Ordering SynBase™ CPG Functionalised Supports

		Cat. No.					
Product	**Pack Size**	**Bz-dA**	**Bz-dC**	**Ac-dC**	**iBu-dG**	**dmf-dG**	**dT**
SynBase™ CPG 500/110 S	1g	2263-C001	2264-C001	2357-C001	2262-C001	2277-C001	2261-C001
	5g	2263-C005	2264-C005	2357-C005	2262-C005	2277-C005	2261-C005
	25g	2263-C025	2264-C025	2357-C025	2262-C025	2277-C025	2261-C025
ALL-FIT Columns	10 x 40nmol	2263-P007	2264-P007	2357-P007	2262-P007	2277-P007	2261-P007
	10 x 0.2µmol	2263-P002	2264-P002	2357-P002	2262-P002	2277-P002	2261-P002
	10 x 1µmol	2263-P008	2264-P008	2357-P008	2262-P008	2277-P008	2261-P008
SynBase™ CPG 500/110 H	1g	2267-C001	2268-C001	2375-C001	2266-C001	2278-C001	2265-C001
	5g	2267-C005	2268-C005	2375-C005	2266-C005	2278-C005	2265-C005
	25g	2267-C025	2268-C025	2375-C025	2266-C025	2278-C025	2265-C025
SynBase™ CPG 1000/110	1g	2273-C001	2274-C001	2275-C001	2272-C001	2317-C001	2271-C001
	5g	2273-C005	2274-C005	2275-C005	2272-C005	2317-C005	2271-C005
	25g	2273-C025	2274-C025	2275-C025	2272-C025	2317-C025	2271-C025
ALL-FIT Columns	10 x 40nmol	2273-P007	2274-P007	2275-P007	2272-P007	2317-P007	2271-P007
	10 x 0.2µmol	2273-P002	2274-P002	2275-P002	2272-P002	2317-P002	2271-P002
	10 x 1µmol	2273-P008	2274-P008	2275-P008	2272-P008	2317-P008	2271-P008
MerMade Columns	10 x 50nmol	2273-P018	2274-P018	2275-P018	2272-P018	2317-P018	2271-P018
	10 x 0.2µmol	2273-P016	2274-P016	2275-P016	2272-P016	2317-P016	2271-P016
	10 x 1µmol	2273-P022	2274-P022	2275-P022	2272-P022	2317-P022	2271-P022
SynBase™ CPG 3000/110	1g	2381-C001	2382-C001	2383-C001	2384-C001	-	2386-C001
	5g	2381-C005	2382-C005	2383-C005	2384-C005	-	2386-C005
	25g	2381-C025	2382-C025	2383-C025	2384-C025	-	2386-C025
ALL-FIT Columns	10 x 40nmol	2381-P007	2382-P007	2383-P007	2384-P007	-	2386-P007
	10 x 0.2µmol	2381-P002	2382-P002	2383-P002	2384-P002	-	2386-P002

Note that in addition to the column types listed above, we can also supply other configurations such as the ABI3900 pipette-tip columns. For ordering information please check with a sales representative or online for up to date details.

3’-modifier CPGs prepared with 3000Å support may be available as custom products so please contact us with your requirements.

Employing Universal Supports

As outlined above, in oligo synthesis the 3’-end is generally determined by the base or modifier functionalisation of the solid support. Whilst the synthesis of any oligo is efficient, given the choice of functionalised supports available, there are advantages in using a ‘universal’ support where there is no nucleobase or modification already present. This can be used for the synthesis of any oligo sequence as the first base at the 3’-end is determined by the first addition in the synthesis cycle.

When preparing wells in plate synthesisers this eliminates the possibility of the incorrect resin being placed in a well. It also allows automated preparation of the plates. There is an added benefit in large scale syntheses, where inventory costs are lowered as only the one support is required.

A universal support can also be applied in situations where a 3’-modification support is not available, using a phosphoramidite modifier as the first addition in the cycle (this will only work with phosphoramidites capable of extending the oligo chain, *i.e.* not 5’-modifiers).

For these purposes we offer Universal SynBase™ CPG 1000/110 (**2304**)[5] and Universal-Q SynBase ™ CPG (**2300**/**2410**/**2411**).

Where the support is functionalised with a nucleoside or modifier, this is usually attached *via* a succinate linkage (as in our unmodified base products).[6] Cleavage with ammonium hydroxide at room temperature can take about an hour. The alternative oxalate group has been shown to be very labile during cleavage, but its stability to the synthesis cycle is unsatisfactory.

The Q-supports were developed[7] with fast, mild cleavage in mind, however we have observed mixed results. The linker is stable to capping mixtures, but is slightly labile in oxidiser solution (8% cleavage overnight which is the equivalent of approximately 2000 synthesis cycles on an average program).

For both these universal supports a high temperature or extended time is required to completely remove the universal linker. For **2300** one of the following needs to be used: ammonium hydroxide solution, 17h at 80°C; AMA, 5h at 80°C; or AMA, overnight at 55°C.

Contrary to previously published mild methods, the best conditions we have found to completely remove the Q linker during deprotection are AMA at 70°C for 2.5h or AMA at 80°C for 2h. For this reason it is inadvisable to use this support with modifications that require mild, ultramild or room temperature deprotection. Compatibilty with RNA is therefore mixed. We would not recommend the use of the support with TBDMS chemistry.

In summary, despite some innovations, there remains the need for a truly universal support that has wide application. Most recently a candidate has emerged based on a molecule which is “conformationally preorganized” to accelerate the dephosphorylation reaction.[8] The rigid bicyclic molecule is designed to facilitate the formation of the cyclic phosphate transition state, thereby stimulating the rate of dephosphorylation. The use of this support has been

5 **A universal support for oligonucleotide synthesis**, S. Scott, P. Hardy, R.C. Sheppard, and M.J. McLean, in *Innovation and Perspectives in Solid-Phase Synthesis. Peptides, Proteins, and Nucleic Acids, Biological and Biomedical Applications* (R. Epton, ed.), 115-124, 1994. Mayflower Worldwide, Ltd., Birmingham, UK.

6 **Linkers and cleavage strategies in solid-phase organic synthesis and combinatorial chemistry**, F. Guillier, D. Orain and M. Bradley, *Chemical Reviews*, **100**, 2091-2158, 2000.

7 **Hydroquinone-O,O’-diacetic acid as a more labile replacement for succinate acid linkers in solid-phase oligonucleotide synthesis**, R.T. Pon and S.Y. Yu, *Tetrahedron Lett.*, **38**, 3327-3330, 1997.

8 **A conformationally preorganized universal solid support for efficient oligonucleotide synthesis**, A.P. Guzaev and M. Manoharan, *J. Am. Chem. Soc.*, **125**, 2380-2381, 2003.

demonstrated in antisense phosphorothioate synthesis[9] and in drug development.[10]

The methyl version of the support (preferable over the phenyl version since methylamine rather than aniline is formed on deprotection) is available as UnySupport from Glen Research under licence from Isis Pharmaceuticals.

Protection Group Strategies

UltraMILD Deprotection

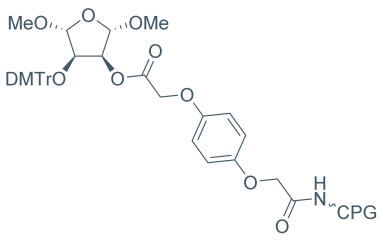
In oligonucleotide synthesis the classic heterocyclic base protection groups (Bz-dA, Bz-dC and iBu-dG) are routinely removed using ammonium hydroxide solution with heating. Unfortunately many modifiers and labels used in oligonucleotide synthesis will not withstand prolonged exposure to such strongly alkaline conditions. The UltraMILD monomers - phenoxyacetyl (Pac)-dA (**2059**), acetyl (Ac)-dC (**2034**), and iso-propyl-phenoxyacetyl (iPr-Pac)-dG (**2060**) - were developed to alleviate this. This alternative protection allows milder deprotection conditions to be used where sensitive labels and tags have been incorporated into the oligonucleotide. This strategy allows the use of very mild deprotection conditions such as 0.05M potassium carbonate in methanol at room temperature. The UltraMILD monomers can also be deprotected using ammonium hydroxide solution, and, in fact, acetyl- is currently the protecting-group of choice for dC since this is compatible with all deprotection conditions.

The corresponding Pac-dA, Ac-dC, and iPr-Pac-dG functionalised SynBase™ supports are also available for UltraMILD compatibility of the first 3' base, as are UltraMILD capping reagents.

It should be noted that using the alternative capping solution containing Pac-anhydride (**4210**) avoids the possibility of formation of acetyl-dG by exchange in regular capping solutions. N2-acetyl-dG would not be deprotected under UltraMILD conditions.

9 **Efficient synthesis of antisense phosphorothioate oligonucleotides using a universal solid support**, R. Krishna Kumar, A.P. Guzaev, C. Rentel and V.T. Ravikumar, *Tetrahedron*, **62**, 4528–4534, 2006.

10 **UnyLinker: An efficient and scaleable synthesis of oligonucleotides utilizing a universal linker molecule: A novel approach to enhance the purity of drugs**, V.T. Ravikumar, R. Krishna Kumar, P. Olsen, M.N. Moore, R.L. Carty, M. Andrade, D. Gorman, X. Zhu, I. Cedillo, Z. Wang, L. Mendez, A.N. Scozzari, G. Aguirre, R. Somanathan and S. Berneès, *Org. Process Res. Dev.*, **12**, 399-410, 2008.



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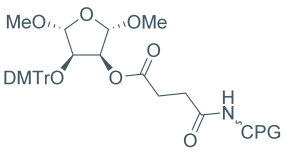


2304

Ordering Universal Supports

Product	Pack Size	Cat. No.
Universal SynBase™ CPG 1000/110	500mg	2304-B500
	1g	2304-C001
	5g	2304-C005
	25g	2304-C025
Universal Q SynBase™ CPG 500/110	250mg	2300-B250
	1g	2300-C001
ALL-FIT Columns	10 x 1µmol	2300-P008
MerMade Columns	10 x 1µmol	2300-P022

Product	Pack Size	Cat. No.
Universal Q SynBase™ CPG 1000/110 S	250mg	2410-B250
	1g	2410-C001
Universal Q SynBase™ CPG 1000/110 H	250mg	2411-B250
	1g	2411-C001

FAST Deprotection

The use of dimethylformamidine-(dmf)-dG (**2030**) has in recent years gained favour over iBu-dG (**2003**), originally due to its ability to deprotect with ammonium hydroxide in 1h at 65°C (or 2h at 55°C). This, together with the availability of the Ac-dC phosphoramidite developed for UltraMILD protocols, led to the creation of a new monomer set allowing rapid deprotection by the FAST method. By using Ac-dC, Bz-dA and dmf-dG monomers, FAST cleavage and deprotection can be effected by a 1:1 mixture of aqueous ammonium hydroxide and aqueous methylamine (known as AMA) in 10 minutes. Cleavage takes place over 5 minutes at room temperature, then deprotection follows by heating to 65°C for a further 5 minutes.[11] Deprotection also takes place at room temperature if left for 120 minutes. AMA deprotection is not recommended for use in the presence of sensitive labels such as cyanine or rhodamine (TAMRA) dyes, or where there are Bz-protected C nucleosides as this will result in transamidation with methylamine.

dmf-dG works particularly well with ^{t}butylamine/methanol/water (1:1:2) as used for rhodamine containing modifiers (*e.g.* TAMRA).

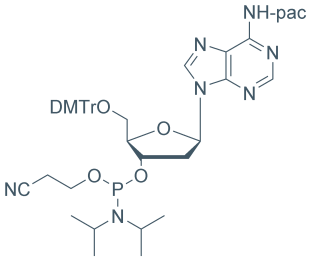
The Ac-dC and dmf-dG protected SynBase™ supports are also available.

Other Methods

While all common deprotection methods require purification to remove the residual protective groups (*e.g.* benzamide) and insoluble silicates, an ammonia-free reagent mixture that allows avoidance of additional purification has been reported.[12] The method, which uses a mixture of lithium hydroxide and triethylamine, can be applied to deprotect oligos synthesised

11 Using AMA, the order of hydrolysis of the base protecting groups is the acetyl group on dC, followed by the benzoyl group on dA, and then the dmf groups from dG. The hydrolysis of Ac-dC is almost instantaneous, thereby precluding the unwanted transamidation reaction to the side-product N-Me-dC possible with alkylamine deprotection.

12 **Advanced method for oligonucleotide deprotection**, S.A. Surzhikov, E.N. Timofeev, B.K. Chernov, J.B. Golova and A.D. Mirzabekov, *Nucleic Acids Research*, **28**, e29, 2000.



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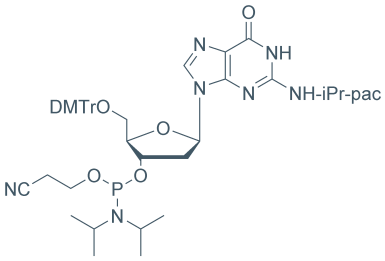

2060

Ordering Unmodified UltraMILD Phosphoramidites and Supports

Product	Pack Size	Cat. No.
Pac-dA-CE Phosphoramidite	250mg	2059-B250
	500mg	2059-B500
	1g	2059-C001
iPr-Pac-dG-CE Phosphoramidite	250mg	2060-B250
	500mg	2060-B500
	1g	2060-C001

Product	Pack Size	Cat. No.
Pac-dA SynBase™ CPG 1000/110	1g	2290-C001
	5g	2290-C005
ALL-FIT Columns	10 x 0.2µmol	2290-P002
	10 x 1µmol	2290-P008
MerMade Columns	10 x 0.2µmol	2290-P016
	10 x 1µmol	2290-P022
iPr-Pac-dG SynBase™ CPG 1000/110	1g	2292-C001
	5g	2292-C005
ALL-FIT Columns	10 x 0.2µmol	2292-P002
	10 x 1µmol	2292-P008
MerMade Columns	10 x 0.2µmol	2292-P016
	10 x 1µmol	2292-P022

For details of the UltraMILD Cap A solution see page 27.

using the classical or Pac-protected phosphoramidites.

A modified "ultra-mild" protocol has also been reported recently that is compatible with the known UltraMILD monomers.[13] This method, utilising 10% diisopropylamine in methanol with 0.25M of ß-mercaptoethanol, was developed for studies incorporating the base-sensitive dG-AAF into DNA; it could well be of more general use for the incorporation of base-labile functionalities into DNA.

Oligoribonucleotide Synthesis

There are many uses of RNA oligonucleotides such as understanding the role of ribozymes (catalytic RNA) and cellular RNA as a target for antisense therapeutics. However the need for chemically synthesised RNA oligonucleotides has become increasingly important since the advent of synthetic siRNA for use in antisense technologies such as gene silencing, and the therapeutic application of RNAi.[14]

Chemistry and Protection Strategies

DNA and RNA have very similar structures and differ only in the presence of the 2'-OH moiety in the latter, and that in RNA thymidine (T) is replaced with uridine (U). As such, the chemistries in terms of synthesis and deprotection of the oligonucleotides differ.[15]

There are many 2'-OH protection chemistries available: ^{t}butyldimethylsilyl (TBDMS)[16], Xeragon's 2'-O-triisopropylsilyoxymethyl, (TOM™)[17], Dharmacon's 2'-O-bis(2-acetoxyethoxy) methyl (ACE™)[18] and very recently Agilent's 2'-O-thiomorpholine-4-carbothioate (TC).[19] However, to date TBDMS chemistry remains the most widely accepted and utilised in RNA synthesis, particularly where the RNA is used in therapeutic applications.

The coupling reaction for RNA synthesis is much longer in comparison with DNA synthesis. Using TBDMS chemistry coupling times of up to 12 minutes are required depending on the choice of activator, although with 0.25M BTT, 3-5 minute couplings are possible.

Until recently the synthesis of longer RNA oligos (~80mers) in reasonable yields was met with some difficulty due to the premature partial deprotection of the TBDMS group during the step to remove the nucleobase protection with ammonium hydroxide solution with heating. Reaction between the now free 2'-OH group and the 3'-phosphate resulted in either cleavage of the oligo at this point or rearrangement to 2'-phosphate and 3'-OH. The desilylation can be suppressed by the use of anhydrous ethanolic ammonia or ethanolic ammonium

13 **Site-specific incorporation of N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-AAF) into oligonucleotides using modified 'ultra-mild' DNA synthesis**, L.C.J. Gillet, J. Alzeer and O.D. Schärer, *Nucleic Acids Research*, **33**, 1961-1969, 2005.

14 **RNA interference in the clinic: challenges and future directions**, C.V. Pecot, G.A. Calin, R.L. Coleman, G. Lopez-Berestein and A.K. Sood, *Nat. Rev. Cancer*, **11**, 59-67, 2011.

15 For a recent overview of this area see: **Current Strategies for the Synthesis of RNA**, S. Muller, J. Wolf and S.A. Ivanov, *Current Organic Synthesis*, **1**, 293-307, 2004.

16 **Chemical synthesis of biologically active oligoribonucleotides using beta-cyanoethyl protected ribonucleoside phosphoramidites**, S.A. Scaringe, C. Francklyn and N. Usman, *Nucleic Acids Research*, **18**, 5433-5441, 1990.

17 **Synthesis and pairing properties of oligoribonucleotide analogues containing a metal-binding site attached to beta-D-allofuranosyl cytosine**, X. Wu and S. Pitsch, *Nucleic Acids Research*, **26**, 4315-4323, 1998.

18 **Novel RNA synthesis method using 5'-O-silyl-2'-O-orthoester protecting groups**, S.A. Scaringe, F.E. Wincott and M.H. Caruthers, *J. Amer. Chem. Soc.*, **120**, 11820-11821, 1998. The ACE™ method also requires that the 5'-OH is protected with a silyl ether rather than the common DMTr group. Moreover, at present the phosphoramidites are not commercially available.

19 **The Development of a Cost-Effective Large Scale Synthesis Process for RNA Therapeutics**, D. Dellinger, Presentation at TIDEs®, May 18, 2009.

hydroxide.[20] However, the most significant improvement is with the use of AMA[21] (aqueous ammonium hydroxide/methylamine 1:1) or ethanolic AMA[22] in conjunction with the use of fast deprotection amidites which allows the nucleobases to be deprotected in 10 minutes at 65˚C. The use of DMSO/ethanolic methylamine (1:1) has also been reported.[23] It must be noted that the use of Bz-dC or Bz-C with AMA leads to transamidation with methylamine and these monomers are therefore not suited to this deprotection method.

The most critical step in obtaining high quality RNA is removing the 2'-TBDMS group. Before starting on this part of the deprotection process it is important that the sample is completely dry and all the nucleobase deprotection solutions have been removed. Removal of the TBDMS groups is most commonly achieved using N-methylpyrrolidone/triethylamine/ triethylamine trihydrofluoride (NMP/Et_3N/Et_3N.3HF) or with either DMSO or DMF to replace the NMP.

While to date this has been the most widely used RNA chemistry the two step deprotection is time consuming and detrimental to more sensitive modifications (*e.g.* cyanine dyes). A one-pot deprotection method for TBDMS chemistry which uses anhydrous methylamine and neat Et_3N/Et_3N.3HF has been described.[24] The deprotection time is still 2-3 hours and is still incompatible with sensitive modifications.

Recently TC-RNA chemistry has been introduced. This has a one-step deprotection method that removes both the nucleobase and 2'-OH protection simultaneously; this technology however is not widely available for commercial use.

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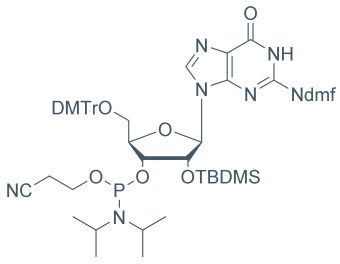
20 **Prevention of chain cleavage in the chemical synthesis of 2'-silylated oligoribonucleotides**, T. Wu, K.K. Ogilvie and R.T. Pon, *Nucleic Acids Research*, **17**, 3501-3517, 1989.

21 **Methylamine deprotection provides increased yield of oligoribonucleotides**, M.P. Reddy, F. Farooqui and N.B. Hanna, *Tetrahedron Lett., 36*, 8929-8932, 1995. For an account of the use of this protection approach in DNA synthesis see: **Fast cleavage and deprotection of oligonucleotides**, M.P. Reddy, N.B. Hanna and F. Farooqui, *Tetrahedron Lett.*, **35**, 4311-4314, 1994.

22 **Synthesis, deprotection, analysis and purification of RNA and ribozymes**, F. Wincott, A. DiRenzo, S. Scaringe and N. Usman, *Nucleic Acids Research*, **23**, 2677-2684, 1995.

23 **Comparison of coupling and deprotection protocols for RNA synthesis**, R.T. Pon and S. Yu, Poster Presentation at ABRF 2004, Integrating Technologies in Proteomics & Genomics, Portland, Oregon, Feb. 28 – Mar. 2, 2004.

24 **"One-pot" oligoribonucleotide deprotection with anhydrous methylamine and neat triethylamine trihydrofluoride**, L. Bellon, in *Current Protocols in Nucleic Acid Chemistry*, Unit 3.6, Eds. S.L. Beaucage, D.E. Bergstrom, G.D. Glick and R.A. Jones, John Wiley & Sons, 2000.



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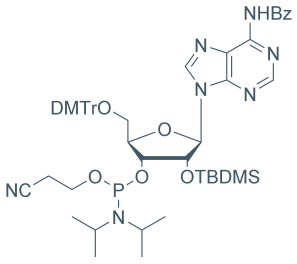

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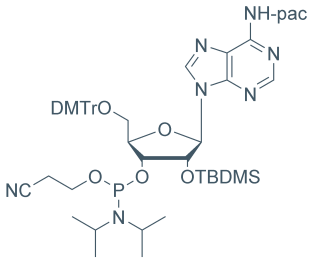

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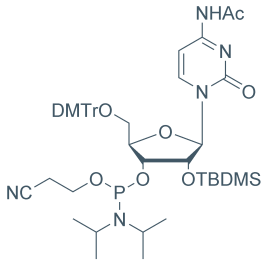

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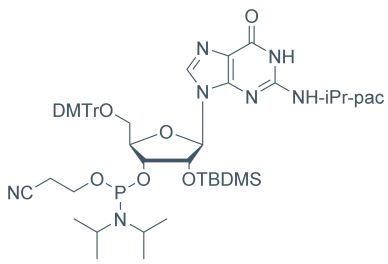

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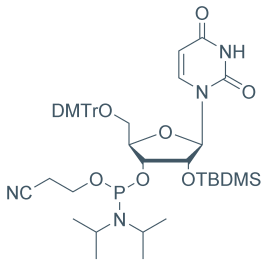

2040

Ordering 2'-OTBDMS RNA Phosphoramidites

Product	Pack Size	Cat. No.
Bz-A-CE Phosphoramidite	250mg	2036-B250
	500mg	2036-B500
	1g	2036-C001
Pac-A-CE Phosphoramidite	250mg	2037-B250
	500mg	2037-B500
	1g	2037-C001
Ac-C-CE Phosphoramidite	250mg	2038-B250
	500mg	2038-B500
	1g	2038-C001
dmf-G-CE Phosphoramidite	250mg	2033-B250
	500mg	2033-B500
	1g	2033-C001
iPr-Pac-G-CE Phosphoramidite	250mg	2039-B250
	500mg	2039-B500
	1g	2039-C001
U-CE Phosphoramidite	250mg	2040-B250
	500mg	2040-B500
	1g	2040-C001

Ordering Unmodified RNA SynBase™ CPG Supports

		Cat. No.					
Product	Pack Size	Bz-A	Pac-A	Ac-C	dmf-G	iPr-Pac-G	U
RNA SynBase™ CPG 1000/110	100mg	2321-B100	2319-B100	2309-B100	2318-B100	2320-B100	2295-B100
	250mg	2321-B250	2319-B250	2309-B250	2318-B250	2320-B250	2295-B250
	1g	2321-C001	2319-C001	2309-C001	2318-C001	2320-C001	2295-C001
ALL-FIT Columns	10 x 0.2µmol	2321-P002	2319-P002	2309-P002	2318-P002	2320-P002	2295-P002
	10 x 1µmol	2321-P008	-	2309-P008	2318-P008	-	2295-P008
MerMade Columns	10 x 0.2µmol	2321-P016	2319-P016	2309-P016	2318-P016	2320-P016	2295-P016
	10 x 1µmol	2321-P022	-	2309-P022	2318-P022	-	2295-P022

Alternative Activators

Activators containing tetrazole (traditionally as a 0.45M solution in anhydrous acetonitrile) have classically been the reagents of choice in routine automated DNA & RNA synthesis. There are, however, two main disadvantages to using this product. Firstly, at lower laboratory temperatures (typically 18°C), solid tetrazole can crystallise from the near-saturated solution causing blockage of delivery lines.

Secondly, the product has become more difficult to obtain because of shipping restrictions due to its classification as an explosive (current UK law permits shipping of tetrazole only as a solution; even this cannot be shipped by air). As a consequence, we now no longer offer this product.

An alternative activator, 5-Ethylthio-1H-tetrazole (ETT, **3140**/**3142**/**3145**/**3146**), can offer more effective activation than tetrazole without crystallisation problems. In particular it has been shown to decrease the coupling times in both RNA synthesis[25] and DNA synthesis. However, it is believed that shipping restrictions will also be imposed on this product in the future. It has also been demonstrated that the acidity of tetrazole is sufficient to deprotect the trityl group in monomer solution - to a small extent - leading to some dimer (n+1) formation.[26]

The less acidic 4,5-dicyanoimidazole (DCI) has been used as an alternative activator to avoid this side-reaction.[27] The increased nucleophilicity of this molecule also increases the rate of

25 (a) **Synthesis, deprotection, analysis and purification of RNA and ribozymes**, F. Wincott, A. DiRenzo, C. Shaffer, S. Grimm, D. Tracz, C. Workman, D. Sweedler, C. Gonzalez, S. Scaringe and N. Usman, *Nucleic Acids Research*, **23**, 2677-2684, 1995; (b) **An efficient method for the isolation and purification of oligoribonucleotides**, B. Sproat, F. Colonna, B. Mullah, D. Tsou, A. Andrus, A. Hampel and R. Vinayak, *Nucleosides & Nucleotides*, **14**, 255-273, 1995; (c) **Large-scale synthesis of oligoribonucleotides on high-loaded polystyrene (HLP) support**, D. Tsou, A. Hampel, A. Andrus and R. Vinayak, *Nucleosides & Nucleotides*, **14**, 1481-1492, 1995.

26 **On the formation of longmers in phosphorothioate oligodeoxyribonucleotide synthesis**, A.H. Krotz, P.G. Klopchin, K.L. Walker, G.S. Srivatsa, D.L. Cole and V.T. Ravikumar, *Tetrahedron Lett.*, **38**, 3875-3878, 1997.

27 **Efficient activation of nucleoside phosphoramidites with 4,5-dicyanoimidazole during oligonucleotide synthesis**, C. Vargeese, J. Carter, J. Yegge, S. Krivjansky, A. Settle, E. Kropp, K. Petersen and W. Pieken, *Nucleic Acids Research*, **26**, 1046-1050, 1998.

activation. We offer 0.25M dicyanoimidazole in anhydrous acetonitrile (**3150**). This is available in a 450ml (16oz), 28-405 screw neck, amber bottle, suitable for Expedite 8909, upgraded ABI 392/394, ABI 3400 and 3900, and all MerMade synthesisers.

Despite these advances, a need remains for additional activators, particularly in RNA synthesis in which the longer coupling times, due to the steric effects of protecting the 2'-OH, ideally could still be reduced. We provide 5-Benzylthio-1H-tetrazole (BTT) activator (**3160**/**3162**) to specifically meet this need.

BTT has been classed as a non-explosive material and therefore availability of the product is not restricted. Furthermore, this product is available at a cost equivalent to other tetrazole activators. In DNA synthesis, coupling efficiency is routinely at least as good as with tetrazole, and often better. In RNA synthesis, the coupling of TBDMS or TOM monomers with 1H-tetrazole activation conditions can require 12-15min. Using BTT 3min coupling times are recommended, although 90s has been used effectively using 6.5eq of 0.25M BTT and 6eq of 0.1M phosphoramidite.[28] BTT is, in fact, very slightly more acidic than ETT, however it has been shown that N+1 peaks are no more significant using BTT with shorter coupling times than ETT with a 6min coupling time or 1H-tetrazole for 12min.

Note that we now also provide crystalline BTT (**0234**) and ETT (**0237**) - these can ship as non-hazardous products thereby reducing costs.

Although we still find BTT to be the most widely effective activator, some customers still prefer to use DCI or ETT in certain situations, therefore we are happy to support this.

Bespoke 3'-incorporation: Employing Unfunctionalised Amino-SynBase™

The use of our Amino-SynBase™ products (**1034** and **1049**) allows the user to directly incorporate a bespoke nucleobase or modifying unit of their own choosing at the 3'-end.

Synthetically this is done by reacting the Amino-SynBase™ with the succinate (or other suitable derivative) of the nucleoside (or modifier) in the presence of *e.g.* a carbodiimide and base in a suitable solvent. The modifying unit of course requires a protected alcohol available for further chain extension in the synthesis cycle (*i.e.* in nucleosides the 5'-OH is protected by a DMTr group). This 3'-modified CPG can then be used to synthesise an oligonucleotide.

Our Amino-SynBase™ products are fully activated and are used without further treatment.

28 **The synthesis of 2'-O-[(triisopropylsilyl)oxy]methyl (TOM) phosphoramidites of methylated ribonucleosides for the use in automated RNA solid-phase synthesis**, C. Höbartner, C. Kreutz, E. Flecker, E. Ottenschläger, W. Pils, K. Grubmayr and R. Micura, *Monatchefte für Chemie*, **134**, 851-873, 2003.

Ordering SynBase™ CPG Unfunctionalised Supports

Product	Pack Size	Cat. No.
Amino-SynBase™ CPG 500/110	1g	1034-C001
	5g	1034-C005
	25g	1034-C025
Amino-SynBase™ CPG 1000/110	1g	1049-C001
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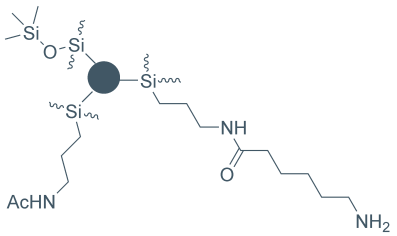
	25g	1049-C025



1034/1049

Modifiers and Their Use in Oligonucleotide Synthesis

Modifying oligonucleotide structure is becoming an increasingly important tool in a vast range of applications, as the breadth of modifications continues to grow.

Introduction

Although only ~10% of oligonucleotides manufactured are modified, this is an extremely important sector of the oligonucleotide market. Modifying an oligonucleotide enables the development of diagnostic tests, therapeutics, detection methods and genetic analysis tools. As these develop, the need for new improved modifiers grows. For instance, the first examples of phosphorylated oligonucleotides introduced 5'-phosphate enzymatically. Today there are many examples of phosphorylating reagents (*e.g.* **2101**, **2127**) available for direct incorporation during oligonucleotide synthesis. When used in combination with a sulphurisation reagent, the resulting thiophosphate was once a common means of conjugating biomolecules (*e.g.* HRP) to oligonucleotides. This was often inefficient due to the proximity of the biomolecule to the oligonucleotide. Since then, a range of amino and thiol linkers have been developed to allow more efficient coupling and, when used in conjunction with a spacer, can not only improve the conjugation efficiency further, depending on the nature and length of the spacer, can improve the efficiency of the oligonucleotide in its intended application.

While post-synthetic labelling is still important, many phosphoramidites have been developed to eliminate the need for this. 5'-FAM (**2134**) phosphoramidite for instance now allows the incorporation of fluorescein to the 5'-end of an oligonucleotide without the need for a linker and an active form of the dye and the additional downstream processing associated with post labelling.

The need to improve hybridisation properties to obtain higher specificity in terms of detection or a more stable duplex for therapeutic use has driven the development of modified bases and modified backbones. PNA for instance forms a very strong duplex as a result of the lack of charge on the backbone. It is now possible to fine tune the T_m of a duplex with the use of modifiers. For instance 2'-OMe nucleosides will increase the T_m of a duplex by 1-4ºC per addition whereas the incorporation of UNA nucleosides will decrease the T_m of a duplex by 5-10ºC. Therefore it is possible that a precise T_m can be dictated by the use of such modifications. Where the sugar is modified, this provides a means of protecting the oligonucleotide against nucleases.

In short, although the majority of oligonucleotides manufactured are unmodified there is an important and continually evolving need for modifiers.

Spacers

In general terms a spacer is introduced into an oligonucleotide to add distance between the oligonucleotide and a modifier. This reduces the possibility of any adverse interaction between the modifier and the sequence. For instance, G-rich sequences are known to quench fluorescein therefore the use of a suitable spacer will remove the dye label from the proximity

of the oligonucleotide minimising the quenching effect. In a similar fashion, spacers are often used to distance between multiple additions of self-quenching dyes *e.g.* fluorescein.[29]

The application of the modified oligonucleotide will dictate whether a hydrophilic (Spacer 18; **2129**, Spacer 9; **2128**) or hydrophobic spacer (Spacer C3; **2113**, Spacer C12; **2147**) is required. Multiple incorporations of varying lengths of these spacers allow the precise length of the spacer arm to be controlled. This can be important in hairpin loop[30] and duplex studies[31] of DNA.

Several spacers have specific uses. Spacer C3 phosphoramidite (**2113**), when incorporated into an oligonucleotide, mimics the three carbon spacing between the 3' and 5' hydroxyls of sugar unit[32]. Although useful where the base at a specific site is unknown, the flexibility of the alkyl chain distorts the sugar-phosphate backbone. This can be alleviated with the use of dSpacer (**2146**) since incorporation of this modifier sits directly into the natural sugar-phosphate backbone with no adverse effect. This modifier mimics abasic sites[33] and is useful in the study of mutations resulting from depurination.

In some cases it is advantageous to remove the modifier from the oligonucleotide *e.g.* if using biotin as a means of capture, the target is bound to the biotin labelled probe. This is then captured using a streptavidin affinity column and the target-probe duplex can be eluted if a cleavable spacer is used (*e.g.* our photocleavable spacer **2131**; see page 66 for more information).

Although less common than terminal spacing, but equally important, spacers have been incorporated within an oligonucleotide. This adds distance between sections of the sequence. For instance Cytocell's SMART detection assay[34] uses spacer 18 or HEG (**2129**) in the template probe where one section acts as an anchor in binding to the target leaving the other section free for hybridisation to the extension probe to allow amplification during PCR. In this case the spacer gives flexibility to the template probe to enable hybridisation to both the target and the extension probe.

In similar way, HEG is used in Scorpion Primers® to separate the probe and primer section. However in this case, this not only provides the flexibility to allow the probe to flip back to hybridise to the amplicon but also acts as a PCR blocker to prevent read through to the probe.[35]

3'-Spacers such as **2245** and **2395** are often used as an alternative to 3'-phosphate as blockers since, when incorporated at the 3'-end, the resulting oligonucleotide shows nuclease and polymerase resistance. In fact spacer C3 is often incorporated at the 3'-end of an oligonucleotide for use with restriction enzymes rather than phosphate since the latter is thought to partially cleave during the assay.

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29 See for example: **Design of multidye systems for FRET-based applications**, M.S. Shchepinov and V.A. Korshun, *Nucleosides, Nucleotides & Nucleic Acids*, **20**, 369-374, 2001.

30 **Circular dichroism studies of an oligodeoxyribonucleotide containing a hairpin loop made of a hexaethylene glycol chain: conformation and stability**, M. Durand, K. Chevrie, M. Chassignol, N.T. Thuong and J.C. Maurizot, *Nucleic Acids Research*, **18**, 6353-6359, 1990.

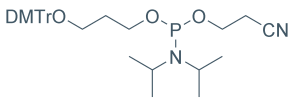
31 **A nicked duplex decamer DNA with a PEG6 tether**, L. Kozerski, A.P. Mazurek, R. Kawecki, W. Bocian, P. Krajewski, E. Bednarek, J. Sitkowski, M. P. Williamson, A.J.G. Moir and P.E. Hansen, *Nucleic Acids Research*, **29**, 1132-1143, 2001.

32 **Enhancing sequence-specific cleavage of RNA within a duplex region: Incorporation of 1,3-propanediol linkers into oligonucleotide conjugates of serinol-terpyridine**, B.N. Trawick, T.A. Osiek and J.K. Bashkin, *Bioconjugate Chem.*, **12**, 900-905, 2001.

33 (a) **Oligodeoxynucleotides containing synthetic abasic sites model substrates for DNA-polymerases and apurinic apyrimidinic endonucleases**, M. Takeshita, C.N. Chang, F. Johnson, S. Will and A.P. Grollman, *J. Biol. Chem.*, **262**, 10171-10179, 1987; (b) **NMR-studies of abasic sites in DNA duplexes deoxyadenosine stacks into the helix opposite the cyclic analog of 2-deoxyribose**, M.W. Kalnik, C.N. Chang, A.P. Grollman and D.J. Patel, *Biochemistry*, **27**, 924-931, 1988.

34 **Detection of virus mRNA within infected host cells using an isothermal nucleic acid amplification assay: marine cyanophage gene expression within *Synechococcus sp***, S.D. Wharam, M.J. Hall and W.H. Wilson, *Virology Journal*, **4**, 52-59, 2007.

35 **Duplex Scorpion primers in SNP analysis and FRET applications**, A. Solinas, L.J. Brown, C. McKeen, J.M. Mellor, J.T.G. Nicol, N. Thelwell and T. Brown, *Nucleic Acids Research*, **29** (20), e96, 2001.



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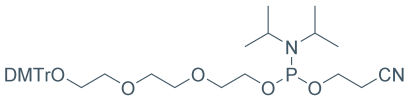

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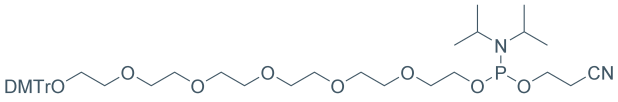

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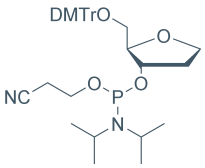

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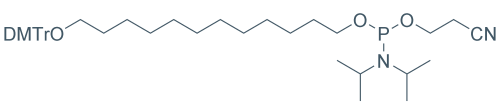

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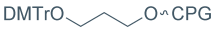


2245/2395

Ordering Spacer Modifiers

Product	Pack Size	Cat. No.
dSpacer-CE Phosphoramidite	100µmol	2146-F100
	250mg	2146-B250
Spacer-CE Phosphoramidite C3	100µmol	2113-F100
	250mg	2113-B250
Spacer-CE Phosphoramidite 9	100µmol	2128-F100
	250mg	2128-B250
Spacer-CE Phosphoramidite C12	100µmol	2147-F100
	250mg	2147-B250
Spacer-CE Phosphoramidite 18	100µmol	2129-F100
	250mg	2129-B250

Product	Pack Size	Cat. No.
3'-Spacer-C3 SynBase™ CPG 1000/110	100mg	2245-B100
	1g	2245-C001
ALL-FIT Columns	4 x 0.2µmol	2245-P001
	10 x 0.2µmol	2245-P002
	4 x 1µmol	2245-P010
	10 x 1µmol	2245-P008
MerMade Columns	4 x 0.2µmol	2245-P015
	10 x 0.2µmol	2245-P016
	4 x 1µmol	2245-P026
	10 x 1µmol	2245-P022
3'-Spacer-C3 SynBase™ CPG 3000/110	100mg	2395-B100
	1g	2395-C001
ALL-FIT Columns	4 x 0.2µmol	2395-P001
	10 x 0.2µmol	2395-P002
MerMade Columns	4 x 0.2µmol	2395-P015
	10 x 0.2µmol	2395-P016

Conjugation Reagents

Incorporation of reactive functional groups, particularly primary amine, thiol, or carboxylate, at specific sites within an oligonucleotide allows for subsequent post-synthesis conjugation of the oligo with a number of different affinity, reporter or protein labels, depending on the application. Such labels need to be reactive towards the incorporated functional group, for example, NHS esters or isothiocyanates will react with primary amines, and iodoacetamides or maleimides will conjugate with primary thiols. This approach is often necessary where the desired label or tag is either not available as a phosphoramidite, or is sensitive or unstable to the conditions of oligonucleotide synthesis or deprotection. A common example is the attachement of a rhodamine dye using the TAMRA NHS ester. Functionally-derivitized oligos can also be covalently attached to surfaces such as glass slides or gold microspheres for use in various microarray or nanoelectronic applications.

Amino Modification

5'-Amino Linkers

One of the most common modifications is the incorporation of a primary amine at the 5'-terminus of the oligonucleotide using an 'amino-linker' phosphoramidite, protected with either the base labile trifluoroacetate[36] (TFA) (*e.g.* 5'-TFA-Amino-Modifier C6-CE Phosphoramidite, **2124**) or the acid-labile monomethoxytrityl[37] (MMT) (*e.g.* 5'-MMT-Amino-Modifier C6-CE Phosphoramidite, **2123**, or 5'-MMT-Amino-Modifier C12-CE Phosphoramidite, **2133**) groups. Both classes of monomer are used in automated synthesis.

The choice between the MMT and TFA-protected C6 amino modifiers is dependent on the purification strategy used on the oligo, or whether on-column or solution-phase conjugation is required. If this is purified, the MMT protection is preferable since the trityl group, stable to the basic cleavage and deprotection conditions, can be used as a 'handle' in *e.g.* cartridge purification where the MMT group is removed during the purification process. Otherwise, the TFA protection is perfectly suitable. A variety of molecules can be attached to the liberated 5'-amine such as fluorescent dyes or haptens such as biotin.

The MMT group can also be removed by extended deblocking on the synthesiser, allowing a solid-phase conjugation of a label containing *e.g.* an activated carboxylic acid. However, in this case it is important to remember that the conjugate must be stable to the subsequent cleavage and deprotection conditions.

The shorter C5 or C6 carbon chain linkers may be used to attach compounds where proximity to the oligonucleotide causes no problem. The longer C12 analogue has specific applications in *e.g.* affinity chromatography, where the oligo must be sufficiently distanced from the surface, and in some cases labelling with fluorescent tags, where close interaction may lead to partially quenched fluorescence.

Our hydrophilic Amino-Modifier 11 product is particularly useful for solution-phase couplings of labels to oligos. It is often found that when using hydrophobic amino-linkers, *e.g.* **2123**, an additional hydrophilic spacer is required. This extends the distance of the label from the oligo. Our product has this hydrophilicity "built-in" and can therefore be used where a hydrophilic linker is required. Once incorporated into an oligo this linker is equivalent to ~2 base units. It is available in both TFA (**2182**) and MMT (**2193**) protected forms, the latter allowing oligo purification based on exploiting the trityl group, or on-column conjugations as described above.

Internal Amino Linkers

Internal amino-functions, ready for further post-synthetic modification, can be introduced to oligonucleotides by a number of products. Amino-Modifier C6-dC-CE Phosphoramidite (**2141**) and both the Amino-Modifier C2-dT (**2149**) and C6-dT (**2135**) products can be added in place

36 This is a standard amino-protecting group in organic synthesis. See for example: *Greene's Protective Groups in Organic Synthesis*, 5th Edition, P.G.M. Wuts (Ed.), Wiley-Blackwell, 2013.

37 **The synthesis of oligonucleotides containing a primary amino group at the 5'-terminus**, B.A. Connolly, *Nucleic Acids Research*, **15**, 3131-3139, 1987.

of a 2'-deoxycytidine and a thymidine residue, respectively, during oligonucleotide synthesis. In the case of the C6 analogues, after deprotection, the primary amine is distanced from the oligonucleotide by a total of 10 atoms and can be labelled or attached to a biomolecule such as an enzyme. The C2 analogue is more appropriate for applications where the attached label is designed to interact with the oligonucleotide. It has been shown that duplexes containing a modified T base have no adverse affect on melting behaviour.[38]

However, there are times when it is advantageous to have an interaction between the duplex and the label. For instance, incorporating dansyl directly onto the 5-position of dU allows the study of the interaction of antibiotics with the minor groove by measuring the change in fluorescence signal.[39]

The related Amino-Modifier C6-dA-CE Phosphoramidite (**2071**) is useful for introducing an amino function at a dA site, although the linker on the 8-position does cause some destabilisation of the duplex pairing to T (approximately 2°C per insertion).

Applications of the internal modification technique are varied. For example, an internally amino-modified oligo is ideal for incorporating dyes not normally available as phosphoramidites, such as ROX, *via* an NHS ester. For example, when synthesising wavelength shifting FRET probes using FAM/ROX, combine the ROX-modified amino-dT with a FAM modification at the 5'-end.

3'-Amino-Linkers

The most commonly used product for introducing a 3'-amino functionality is Fmoc-protected 3'-Amino-Modifier C7 SynBase™ CPG 1000 (**2350**). Use of an Fmoc-protected amine has both advantages and disadvantages. This is quite stable to oligo synthesis conditions however, if not handled correctly, some loss of Fmoc may occur. This leads to capping of the free amine with acetic anhydride and hence loss of functionality. The main advantage of Fmoc is that it can be removed selectively without cleavage from the support allowing solid-phase conjugation of the desired label. This can be done prior to or subsequent to oligonucleotide synthesis. It should be noted that, due to the 1,3-diol configuration, **2350** contains a chiral centre and will generate a pair of diastereomers in oligo synthesis, although this is rarely observed in HPLC.

Alternatives to Fmoc protection have been investigated. Phthalimide (PT) chemistry has been used in the development of 3'-PT-Amino-Modifier C6 CPG[40] (**2365**), where the nitrogen which will ultimately provide the 3'-amino function is part of the PT group attached to the support through an imide group attached to the aromatic ring. This linkage is stable to all conditions of oligo synthesis and the resulting amino functionality does not add any additional chiral centres/diastereomers to the oligo. Cleavage and deprotection is achieved using an extended ammonium hydroxide treatment. A completely analogous C3 product (**2371**) is also available.

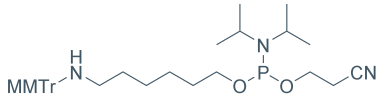
Two additional products are available for introducing 3'-amino functionality without blocking the terminus from any desired enzymatic activity. These are 3'-Amino-Modifier C6-dC CPG (**2369**) and the equivalent dT analogue (**2367**).

38 **Synthesis and characterization of DNA oligomers and duplexes containing covalently attached molecular labels: comparison of biotin, fluorescein, and pyrene labels by thermodynamic and optical spectroscopic measurements**, J. Telser, K.A. Cruickshank, L.E. Morrison and T.L. Netzel, *J. Amer. Chem. Soc.*, **111**, 6966-6976, 1989.

39 **Fluorescent d(CGCGAATTCGCG): characterization of major groove polarity and study of minor groove interactions through a major groove semantophore conjugate**, D.A. Barawkar and K.N. Ganesh, *Nucleic Acids Research*, **23**, 159-164, 1995.

40 **An improved CPG support for the synthesis of 3'-amine-tailed oligonucleotides**, C.R. Petrie, M.W. Reed, A.D. Adams and R.B. Meyer, Jr., *Bioconjugate Chem.*, **3**, 85-87, 1992.

5'-Amino-Linkers



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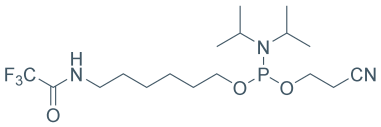

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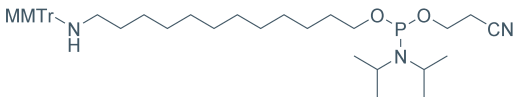

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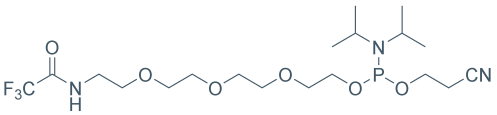

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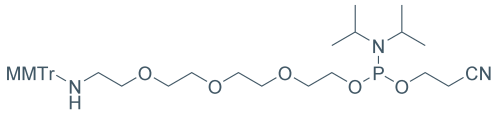

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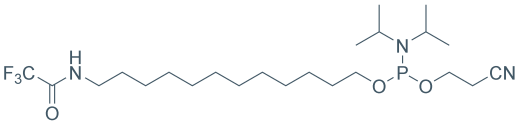

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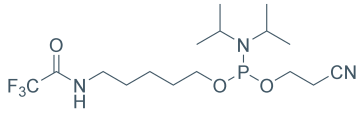

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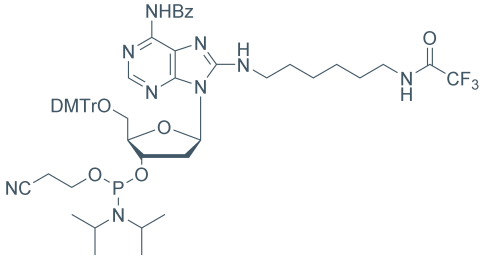
Internal Amino-Linkers



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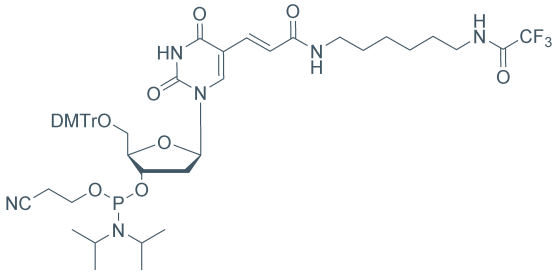

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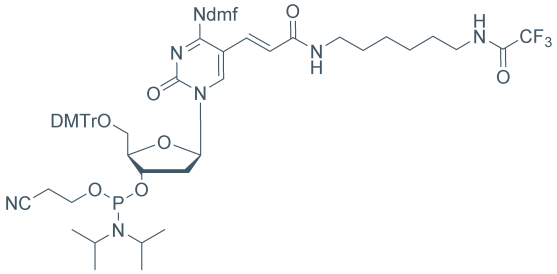

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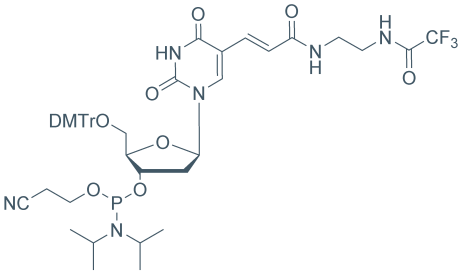

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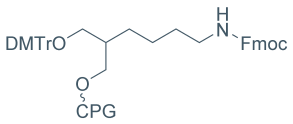
3'-Amino-Linkers



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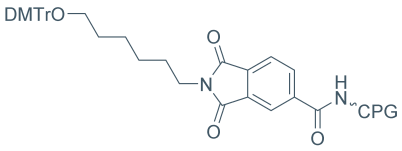

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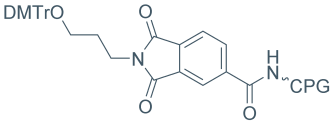

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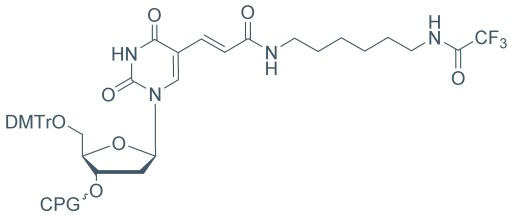

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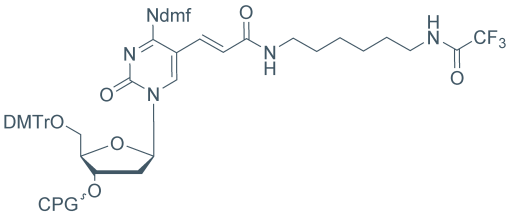

2369

Ordering Amino-Linkers

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5'-TFA-Amino-Modifier-C6-CE Phosphoramidite*	100µmol	2124-F100
	250mg	2124-B250
5'-TFA-Amino-Modifier-11-CE Phosphoramidite	100µmol	2182-F100
	250mg	2182-B250
5'-MMT-Amino-Modifier-11-CE Phosphoramidite	100µmol	2193-F100
	250mg	2193-B250
5'-MMT-Amino-Modifier-C12-CE Phosphoramidite	100µmol	2133-F100
	250mg	2133-B250
5'-TFA-Amino-Modifier-C12-CE Phosphoramidite	100µmol	2532-F100
	250mg	2532-B250
5'-TFA-Amino-Modifier-C5-CE Phosphoramidite	100µmol	2534-F100
	250mg	2534-B250
Amino-Modifier-C2-dT-CE Phosphoramidite	100µmol	2149-F100
	250mg	2149-B250
	500mg	2149-B500
Amino-Modifier-C6-dA-CE Phosphoramidite	100µmol	2071-F100
	250mg	2071-B250
	500mg	2071-B500
Amino-Modifier-C6-dC-CE Phosphoramidite	100µmol	2141-F100
	250mg	2141-B250
	500mg	2141-B500
Amino-Modifier-C6-dT-CE Phosphoramidite	100µmol	2135-F100
	250mg	2135-B250
	500mg	2135-B500

Product	Pack Size	Cat. No.
3'-Amino-Modifier-C7 CPG 1000	100mg	2350-B100
	1g	2350-C001
ALL-FIT Columns	4 x 0.2µmol	2350-P001
	10 x 0.2µmol	2350-P002
	4 x 1µmol	2350-P010
	10 x 1µmol	2350-P008
MerMade Columns	4 x 0.2µmol	2350-P015
	10 x 0.2µmol	2350-P016
	4 x 1µmol	2350-P026
	10 x 1µmol	2350-P022
3'-PT-Amino-Modifier-C3 CPG	100mg	2371-B100
	1g	2371-C001
ALL-FIT Columns	4 x 0.2µmol	2371-P001
	10 x 0.2µmol	2371-P002
	4 x 1µmol	2371-P010
	10 x 1µmol	2371-P008
3'-PT-Amino-Modifier-C6 CPG	100mg	2365-B100
	1g	2365-C001
ALL-FIT Columns	4 x 0.2µmol	2365-P001
	10 x 0.2µmol	2365-P002
	4 x 1µmol	2365-P010
	10 x 1µmol	2365-P008
3'-Amino-Modifier-C6-dC CPG	100mg	2369-B100
	1g	2369-C001
ALL-FIT Columns	4 x 0.2µmol	2369-P001
	10 x 0.2µmol	2369-P002
	4 x 1µmol	2369-P010
	10 x 1µmol	2369-P008
3'-Amino-Modifier-C6-dT CPG	100mg	2367-B100
	1g	2367-C001
ALL-FIT Columns	4 x 0.2µmol	2367-P001
	10 x 0.2µmol	2367-P002
	4 x 1µmol	2367-P010
	10 x 1µmol	2367-P008

* Requires to be shipped on ice.

Thiol Modification

5'-Thiol-Linkers

5'-Thiol-Modifiers, phosphoramidites used to introduce a 5'-thio functionality[41] to an oligo, have very similar applications to amino-modifiers. The thiol group is used to attach labels such as fluorescent tags[42] and biotin.[43] Conjugation to fluorescent markers is possible, for example, *via* reactions of the thiol with iodoacetate and maleimide derivatives to form thioether linkages.

Since conjugation to a thiol is orthogonal to that of an amino functionality, it is not uncommon to have both an amino (*e.g.* 3') and a thiol (*e.g.* 5') in the same oligo.

In general, thiol modification at the 5'-end of the oligonucleotide is achieved with 5'-Thiol-Modifier C6-CE Phosphoramidite (**2125**) or, more commonly, the Thiol-Modifier C6 S-S CE Phosphoramidite (**2126**). As with the MMT protected amino-modifiers, the trityl group on **2125** is usually retained after cleavage of the oligonucleotide to assist purification. However, because the S-trityl group is not acid labile, it must be removed by treatment with silver nitrate. Although this procedure is commonly used it must be very carefully carried out. Use of **2126** offers an alternative and more robust protocol, whereby the thiol is liberated by use of tris(2-carboxyethyl)phosphine (TCEP). This disulphide product can also be used to modify the 3'-position by using the phosphoramidite as the first adduct in the oligo sequence. Incorporation of **2126** at the 5'-end allows the possibility of DMT-ON purification prior to reduction of the disulphide bridge.

Following on from the hydrophilic amino modifiers described above, we have prepared a hydrophilic thiol product, S-Bz TEG-CE Phosphoramidite (**2187**), which offers all the same advantages of the analogous amino products, but for use in applications where conjugation to a thiol is preferred or necessary.

We have shown that the post-labelling efficiency of the hydrophilic products is comparable with the current most commonly used amino and thiol linkers, used on their own or in combination with a HEG spacer.[44] **2187** is not compatible with thiotetrazoles as activators therefore DCI is recommended for this modification.

An alternative route to thiol modification can be used with our thioctic acid product (**2166**, see below).

Modification of Oligonucleotides with Thioctic Acid for Gold and Silver Bioconjugation

Immobilisation of DNA and other biopolymers on solid surfaces has wide application in microarrays, biosensors and related technologies. Recently, the detection of specific DNA sequences—a central theme of molecular diagnostics—has been achieved using oligonucleotide probes conjugated to metallic nanoparticle substrates.[45]

Attachment of molecules to gold surfaces (planar or nanoparticle) can be achieved *via* thiol-

41 **Chemical synthesis of oligonucleotides containing a free sulphydryl group and subsequent attachment of thiol specific probes**, B.A. Connolly and P. Rider, *Nucleic Acids Research*, **13**, 4485-4502, 1985.

42 **Efficient methods for attachment of thiol specific probes to the 3'- ends of synthetic oligodeoxyribonucleotides**, R. Zuckermann, D. Corey and P. Schultz, *Nucleic Acids Research*, **15**, 5305-5321, 1987.

43 **The synthesis of protected 5'-mercapto-2',5'-dideoxyribonucleoside-3'-O-phosphoramidites; uses of 5'-mercapto-oligodeoxyribonucleotides**, B.S. Sproat, B. Beijer, P. Rider and P. Neuner, *Nucleic Acids Research*, **15**, 4837-4848, 1987.

44 **A comparison of hydrophilic and hydrophobic amino and thiol linkers for use in post-synthetic labelling of oligonucleotides**, S. Aitken, U. Ixkes, C. McKeen and D. Picken, poster presented at IS3NA XIX IRT, Lyon, 2010. Available online: https://linktechsupport.zendesk.com/hc/en-us/articles/200143418-A-Comparison-of-Hydrophilic-and-Hydrophobic-Amino-and-Thiol-Linkers-for-use-in-Post-Synthetic-Labelling-of-Oligonucleotides.

45 For reviews of this area see: (a) **Nanostructures in biodiagnostics**, N.L. Rosi and C.A. Mirkin, *Chem. Rev.*, **105**, 1547-1562, 2005; (b) **Surface recognition of bio-macromolecules using nanoparticle receptors**, A. Verma and V. Rotello, *Chem. Comm.*, 303-312, 2005; and (c) **Nanoparticles, proteins and nucleic acids: Biotechnology meets materials science**, C.M. Niemeyer, *Angew. Chem. , Int. Ed.*, **40**, 4128-4156, 2001.

based linkers that have a natural affinity for the metal.[46] Recognised 5'-thiol modifiers such as the aforementioned 5'-Thiol Modifier C6 CE Phosphoramidite (**2125**) have been used in such applications.[47] Alternatively, Yoo *et al*[48] have demonstrated the modification of a planar gold surface using the dithiol-containing thioctic acid, which was then activated as an NHS ester to allow attachment of an anti-DNA antibody. Taira and Yokoyama have also reported DNA-conjugated polyallylamines employing thioctic acid-based amides as side-chains.[49]

Researchers at the University of Strathclyde have further investigated the use of Thioctic Acid NHS Ester (**2166**) in oligonucleotide immobilisation[50] and, in particular, demonstrate the superior conjugate stability afforded by the dithiol modification when compared to mono-thiols. Furthermore, they extend the use of this modification to silver conjugation, until now difficult to achieve successfully.[51] Sharma *et al* have also recently used this molecule in gold nanoparticle patterning on self-assembled DNA.[52]

2166 can be attached after cleavage to the 3'-end of an oligonucleotide using a 3'-amino-modified solid support, or to the 5'-end post-synthetically to an amino-modified oligo in the same way as, *e.g.*, TAMRA NHS ester. Availability of this product therefore allows the simple synthesis of dithiol-modified oligos for attachment to gold and silver surfaces.

Internal Thiol-Linkers

Although thiol-modified oligonucleotides are routinely used to introduce labels such as dyes, haptens and enzymes *via* reaction with maleimides or haloacetamides, this method has been traditionally limited to the 5' or 3' end of the oligonculeotide.

The use of thiol reactive labels for internal modification until now required the conversion of an amino functionality, *e.g.* amino-dT (**2135** or **2149**) with thioctic acid (**2166**). To overcome this we have developed a thiol-dT modification (**2191**) that can be incorporated within an oligonucleotide and reacts directly with maleimides and haloacetamides.

3'-Thiol-Linkers

Using different strategies, both the Thiol-Modifier C6 S-S CE-Phosphoramidite (**2126**, see above) and the 3'-Thiol-Modifier C3 S-S CPG (**2361**) can be used to introduce a 3'-thio functionality. The latter is simply used as any other support, with subsequent cleavage of the disulphide linkage affording the free thiol. This functionality can be exploited in much the same way as 5'-thiol modification. In addition, 3'-conjugation of phosphorothioates

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46 **Some recent advances in nanostructure preparation from gold and silver particles: a short topical review**, M. Brust and C.J. Kiely, *Colloids Surf., A: Physicochemical and Engineering Aspects*, **202**, 175-186, 2002. For a recent RNA application see: **Enzyme-free interrogation of RNA sites *via* primers and oligonucleotides 3'-linked to gold surfaces**, U. Plutowski, S.R. Vogel, M. Bauer, C. Deck, M.J. Pankratz and C. Richert, *Org. Lett.*, **9**, 2187-2190, 2007 and references therein.

47 See for example: (a) **A multi-step chemical modification procedure to create DNA arrays on gold surfaces for the study of protein-DNA interactions with surface plasmon resonance imaging**, J.M. Brockman, A.G. Frutos and R.M. Corn, *J. Amer. Chem. Soc.*, **121**, 8044-8051, 1999; (b) **Formation, spectroscopic characterization and application of sulfhydryl-terminated alkanethiol monolayers for the chemical attachment of DNA onto gold surfaces**, E.A. Smith, M.J. Wanat, Y. Cheng, S.V.P. Barreira, A.G. Frutos and R.M. Corn, *Langmuir*, **17**, 2502-2507, 2001; and (c) **The effect of surface probe density on DNA hybridisation**, A.W. Peterson, R.J. Heaton and R.M. Georgiadis, *Nucleic Acids Research*, **29**, 5163-5168, 2001.

48 **A radioimmunoassay method for detection of DNA based on chemical immobilization of anti-DNA antibody**, S.-K. Yoo, M. Yoon, U.J. Park, H.S. Han, J.H. Kim, and H.J. Hwang, *Exp. Mol. Medicine*, **31**, 122-125, 1999.

49 **DNA-conjugated polymers for self-assembled DNA chip fabrication**, S. Taira and K. Yokoyama, *Analytical Sciences*, **20**, 267-271, 2004.

50 (a) **Enhanced oligonucleotide-nanoparticle conjugate stability using thioctic acid modified oligonucleotides**, J.A. Dougan, C. Karlsson, W.E. Smith and D. Graham, *Nucleic Acids Research*, **35**, 3668-3675, 2007; (b) **Highly sensitive detection of dye-labelled DNA using nanostructured gold surfaces**, R.J. Stokes, A. Macaskill, J.A. Dougan, P.G. Hargreaves, H.M. Stanford, W.E. Smith, K. Faulds and D. Graham, *Chem. Commun.*, 2811-2813, 2007.

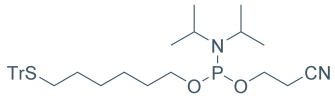
51 **Ultrasensitive DNA detection using oligonucleotide-silver nanoparticle conjugates**, D.G. Thompson, A. Enright, K. Faulds, W.E. Smith and D. Graham, *Anal. Chem.*, **80**, 2805-2810, 2008.

52 **Toward reliable gold nanoparticle patterning on self-assembled DNA nanoscaffold**, J. Sharma, R. Chhabra, C.S. Andersen, K.V. Gothelf, H. Yan and Y. Liu, *J. Amer. Chem. Soc.*, **130**, 7820-7821, 2008.

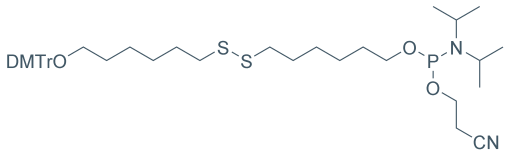
to peptides *via* a disulphide linkage has been reported.[53] The 3'-phosphorothioate (thiophosphate) is generated by the addition of the first base to the 3'-phosphate resin (*e.g.* **2279**), but one linkage is sulphurised with *e.g.* EDITH (**2171**). Cleavage and deprotection releases the 3'-thiophosphate modified oligo. In a similar way, thiol-modified oligos are conjugated to maleimide-modified enzymes or peptides.

53 (a) **Synthesis of peptide-oligonucleotide phosphorothioate conjugates by convergent or step-wise solid-phase strategies**, M. Antopolsky and A. Azhayev, *Nucleosides, Nucleotides & Nucleic Acids*, **20**, 539-550, 2001; (b) **Efficient synthesis of oligonucleotide-peptide conjugates on large scale**, S.O. Doronina, A.P. Guzaev and M. Manoharan, *Nucleosides, Nucleotides & Nucleic Acids*, **20**, 1007-1010, 2001.

5'-Thiol-Linkers



2125



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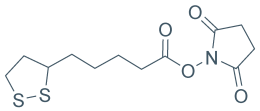
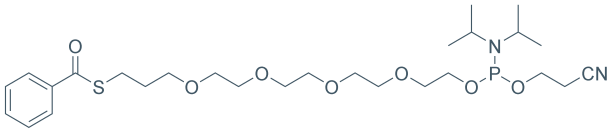
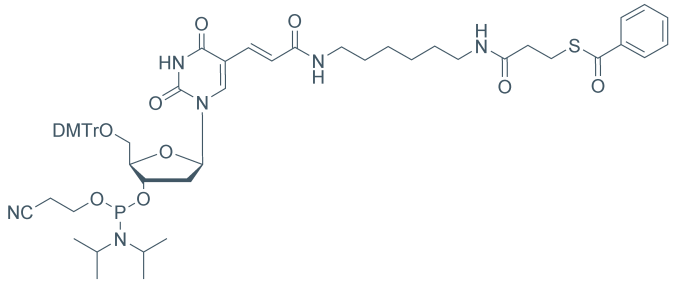


2166



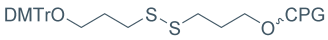
2187

Internal Thiol-Linker



2191

3'-Thiol-Linker



2361

Ordering Thiol-Linkers

Product	Pack Size	Cat. No.
5'-Thiol-Modifier-C6-CE Phosphoramidite	100µmol	2125-F100
	250mg	2125-B250
Thiol-Modifier-C6 S-S CE Phosphoramidite	100µmol	2126-F100
	250mg	2126-B250
S-Bz-TEG-CE Phosphoramidite	100µmol	2187-F100
	250mg	2187-B250
Thioctic Acid NHS Ester	100mg	2166-B100
	250mg	2166-B250
Bz-S-C6-dT-CE Phosphoramidite	100µmol	2191-F100
	250mg	2191-B250

Product	Pack Size	Cat. No.
3'-Thiol-Modifier-C3 S-S CPG	100mg	2361-B100
	1g	2361-C001
ALL-FIT Columns	4 x 0.2µmol	2361-P001
	10 x 0.2µmol	2361-P002
	4 x 1µmol	2361-P010
	10 x 1µmol	2361-P008
MerMade Columns	4 x 0.2µmol	2361-P015
	10 x 0.2µmol	2361-P016
	4 x 1µmol	2361-P026
	10 x 1µmol	2361-P022

Carboxylate Modification

The use of the 5'-carboxylate modifier (**2057**), first described by Kachalova *et al*,[54] allows the introduction of a carboxylic acid function at the 5'-end of an oligonucleotide that is available for conjugation to amines whilst still on the solid support. This strategy avoids the problems of low yields, long reaction times and the need for excess reactants often encountered by other post-cleavage solution methods.

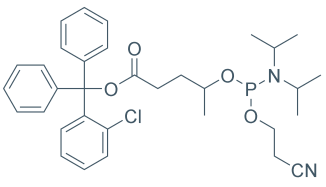
This non-nucleosidic building block is incorporated into the final step of automated DNA synthesis using phosphoramidite chemistry. The 2'-chlorotrityl protecting group is stable during coupling, capping and oxidation or sulphurisation but is easily removed during the deblock step; typically 3% TCA in DCM. At this stage the 5'-carboxylate oligonucleotide can be cleaved and deprotected or further modified by on-column conjugation. Alternatively the 2-chlorotrityl protection can be retained until after cleavage and deprotection.

If the conjugation step has already been completed, provided the label is stable, most deprotection conditions are applicable. Otherwise it is best to use 0.4M NaOH in methanol / water (4:1) overnight at room temperature. This will avoid the formation of an amide as would be the case using ammonium hydroxide or AMA deprotection conditions.

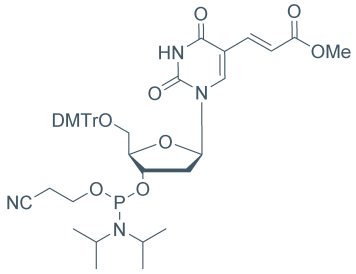
Subsequent conjugation of the 5'-carboxylic acid function to a range of primary and secondary aliphatic amines can be achieved through amide bond formation on the solid support. We have demonstrated the flexibility of this modifier through reactions with the diene furfurylamine, an aminocaproic ester spacer and ß-casomorphin-5-amide, the latter providing a simple and expedient synthesis of an oligonucleotide-peptide conjugate. Coupling yields up to 93% have been attained after cleavage from the solid support.

Internal carboxylate functions can be achieved using Carboxy-dT-CE Phosphoramidite (**2142**). The methyl ester is hydrolysed during deprotection and can be coupled directly to a molecule containing a primary amino group by *via* a peptide coupling reaction.

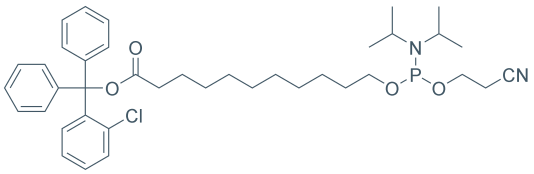
54 **A new and efficient method for the synthesis of 5'-conjugates of oligonucleotides through amide-bond formation on solid phase**, A.V. Kachalova, D.A. Stetsenko, E.A. Romanova, V.N. Tashlitsky, M.J. Gait and T.S. Oretskaya, *Helvetica Chimica Acta*, **85**, 2409-2416, 2002.



2057



2142



2531

Ordering Carboxylate Modifiers

Product	Pack Size	Cat. No.
5'-Carboxylate-Modifier-CE Phosphoramidite	150µmol	2057-F150
	250mg	2057-B250
Carboxy-dT-CE Phosphoramidite	100µmol	2142-F100
	250mg	2142-B250

Product	Pack Size	Cat. No.
5'-Carboxy-C10-CE Phosphoramidite	150µmol	2531-F150
	250mg	2531-B250

Aldehyde Modification

The aldehyde function is often used to conjugate biopolymers to other molecules by processes such as reductive amination or adduct formation with hydroxylamines, hydrazines and semicarbazides. Aldehydes have also been used as a means of immobilising oligonucleotides onto solid surfaces.[55]

The use of this functionality has been hampered by the complexity of existing routes such as post synthetic periodate oxidation of a diol to produce the aldehyde, and the lack of conveniently available ready-made phosphoramidites or supports to incorporate an aldehyde functionality into an oligonucleotide.

Researchers at Kyoto University in Japan described the first facile incorporation of an aldehyde function into DNA without any protection/deprotection of the aldehyde by using 3-formylindole 2'-deoxynucleoside (**2056**).[56] This formylindole modifier can be placed either in the centre of or at the 5'-end of an oligonucleotide, but an extended coupling time of 15min for this modifier is recommended to provide a coupling efficiency of >95%.

Since the sugar unit of the pseudo nucleoside is unmodified, multiple incorporations of dR-formylindole are possible. This not only provides multiple conjugation sites but formylindole is known to act as a universal base resulting in destabilisation of the duplex by 7-10ºC per addition when compared with the natural duplex. This modification is stable to most cleavage and deprotection conditions.

Post-synthetic modification of oligonucleotides bearing this moiety, and still bound to the solid support, has also been achieved. In essence, the options for post-synthetic modification of the aldehyde functionalised oligonucleotide are limited only by the reactive nature of aldehydes and the conditions to which the conjugate is stable.

We have used the aldehyde function to conveniently attach molecules such as O-benzylhydroxylamine and diphenylhydrazine. The use of DMT ON oligonucleotides produced extremely hydrophobic material with these substituents, however, DMT OFF oligonucleotides reacted in a mixture of acetate buffer (pH 4.7) and DMSO (1:1) at 37°C overnight to give conjugation yields in excess of 70% when modified in the centre and in excess of 80% when modified at the 5'-end of the oligonucleotide. Alternative examples of aldehyde conjugations are available in the literature.[57] Aldehyde functionalised oligos have also successfully been coupled with 6-hydrazine nicotinamide (HyNic) modified labels.[58]

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55 For a review of this area see: **Use of carbonyl group addition-elimination reactions for synthesis of nucleic acid conjugates**, T.S. Zatsepin, D.A. Stetsenko, M.J. Gait and T.S. Oretskaya, *Bioconjugate Chemistry*, **16**, 471-489, 2005.

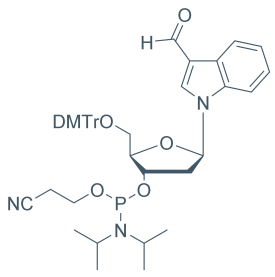
56 **A facile incorporation of the aldehyde function into DNA: 3-formylindole nucleoside as an aldehyde containing universal nucleoside**, A. Okamoto, K.Tainaka and I. Saito, *Tetrahedron Lett.*, **43**, 4581-4583, 2002.

57 (a) **Synthesis of peptide-oligonucleotide conjugates with single and multiple peptides attached to 2'-aldehydes through thiazolidine, oxime, and hydrazine linkages**, T.S. Zatsepin, D.A. Stetsenko, A.A. Arzumanov, E.A. Romanova, M.J. Gait and T.S. Oretskaya, *Bioconjugate Chemistry*, **13**, 822-830, 2002; (b) **Hydrazine oligonucleotides: new chemical modification for chip array attachment and conjugation**, S. Raddatz, J. Mueller-Ibeler, J. Kluge, L. Wäß, G. Burdinski, J.R. Havens, T.J. Onofrey, D. Wang and M. Schweitzer, *Nucleic Acids Research*, **30**, 4793-4802, 2002.

58 **Biomolecule/polymer conjugates**, D.A. Schwartz, US Patent No. 6,911,535 B2, 2005.

Ordering Aldehyde Modifier

Product	Pack Size	Cat. No.
Formylindole-CE Phosphoramidite	100µmol	2056-F100
	250mg	2056-B250



2056

On-Column Oligonucleotide Conjugations

Modified oligonucleotides where labels (*e.g.* reporter, carrier, biomolecule) have been conjugated to the oligonucleotide have a vast array of applications such as diagnostics, capture and therapeutics.[59]

While the preferred method of conjugation is *via* solid phase synthesis using phosphoramidite chemistry, there remain many examples where this method is not feasible, either because the label does not exist as a phosphoramidite or solid support, or that the label is not compatible with oligonucleotide synthesis and/or deprotection. In these cases there are two choices:

1. The label is conjugated on solid phase after oligonucleotide synthesis but prior to cleavage and deprotection.

In this case the active functional group in the oligonucleotide must be easily deprotected without cleaving the oligonucleotide from the resin and the label must be compatible with the required deprotection conditions.

or

2. The label is conjugated in solution phase after cleavage and deprotection.

In this case the label must have some degree of solubility and must be stable in aqueous solution even if mixed with a co-solvent such as DMSO or DMF.

In either scenario, the oligonucleotide is most commonly functionalised with one reactive group and the label functionalised with a complementary reactive group. The most commonly used pairings are amines/NHS esters and thiols/maleimides, although many others are available *e.g.* alkynes/azides (click chemistry) and furan/maleimides (Diels-Alder chemistry).

Of these scenarios, solution phase conjugation is the most common but on-column conjugations are particularly useful where the label and the conjugation product are difficult to separate or where the label is not soluble in aqueous phase - hence solution phase coupling is not feasible. On completion of the coupling reaction, excess label is washed from the column prior to cleavage and deprotection leaving only separation of the conjugate, unlabelled oligonucleotide and failure sequences from the reaction mixture. Typical labels include dyes such as ROX and TMR, lipophilic compounds that are not available as synthesis reagents, and amino acids or small peptides.

A number of LINK's phosphoramidites and solid supports allow on-column conjugations with many of the amino, thiol, carboxy and aldehyde modified oligonucleotides discussed previously in this section.

Amino Modified Oligonucleotides

The Fmoc group of 3'-Amino-C7 CPG (**2350**) is easily removed with 20% piperidine in MeCN with no cleavage of the oligonucleotide from the support.[60] The MMT group of 5'-MMT-Amino-Modifier C6-CE Phosphoramidite (**2123**), 5'-MMT-Amino-Modifier C12-CE Phosphoramidite (**2133**) and 5'-MMT-Amino-Modifier-11-CE Phosphoramidite (**2193**) is removed using an elongated detritylation step. In this case it is recommended that the resin is washed with 20% diethylamine in acetonitrile to ensure the free amine is not in the protonated form.

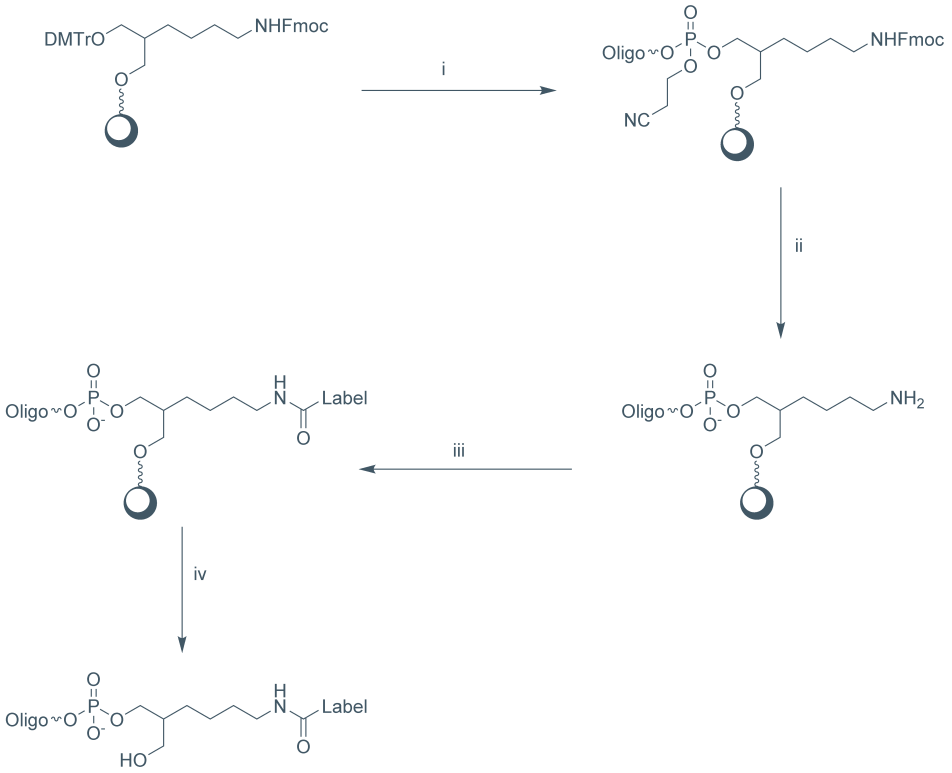
A label, typically an active ester such as an NHS ester, can then be conjugated to the free amine. For small molecules such as fluorescent dyes, this is often carried out in DMF with up to six equivalents of the NHS ester. Short peptides and amino acid residues are generally added *via* a typical peptide coupling using a coupling agent (*e.g.* HATU or DCC) or a crosslinker (such as DSS) where the C-terminus of the peptide is coupled to the amino functionality of the oligonucleotide. Figure 2 depicts on-column labelling of an oligonucleotide modified with **2350**.

Thiol Modified Oligonucleotides

Thioctic Acid NHS Ester (**2166**) must be used in conjunction with one of the amino-modifiers

59 **Bioconjugate Techniques**, 3rd Edition, G.T. Hermanson, 2013.

60 US Patent no. 5736626, 1998; Solid Support Reagents for the Direct Synthesis of 3'-Labelled Polynucleotides.



i. Oligo Synthesis, ii. 20% Piperidine/MeCN, iii. Label-NHS Ester, iv. Oligo Deprotection

Figure 2. On-column conjugation to an amino-modified oligonucleotide.

mentioned previously, but thereafter has the ability to be used as a thiol reactive site. However, this is generally used as a means of conjugating oligonucleotides to silver or gold nanoparticles.[61]

Thiol-Modifier C6 S-S CE Phosphoramidite (**2126**), once incorporated into an oligonucleotide, introduces the possibility of reducing the disulphide bridge with *e.g.* TCEP in water or mercaptoethanol followed by conjugation to a maleimide or acetamide active label. See Figure 3.

Carboxylate Modified Oligonucleotides

In the case of 5'-Carboxylate Modifier-CE Phosphoramidite (**2057**), the oligonucleotide is synthesised 'DMT OFF' to remove the chlorotrityl group and conjugation of the label carried out prior to cleavage and deprotection. Typically the coupling reaction is carried out using a peptide coupling reagent such as HATU to an amino functionalised label to form a stable amide linkage. This is indicated in Figure 4. Labels in this case are generally amino

61 (a) **Enhanced oligonucleotide-nanoparticle conjugate stability using thioctic acid modified oligonucleotides**, J.A. Dougan, C. Karlsson, W.E. Smith and D. Graham, *Nucleic Acids Research*, **35**, 3668-3675, 2007; (b) **Highly sensitive detection of dye-labelled DNA using nanostructured gold surfaces**, R.J. Stokes, A. Macaskill, J.A. Dougan, P.G. Hargreaves, H.M. Stanford, W.E. Smith, K. Faulds and D. Graham, *Chem. Commun.*, 2811-2813, 2007.

i. Disulphide Reduction, ii. Label Coupling, iii. Cleavage and Deprotection

Figure 3. On-column conjugation to a thiol-modified oligonucleotide.

functionalised dyes such as the near infrared dye Cyanine 7 amine, or amino acids and small peptides where coupling occurs between the N-terminus of the peptide and the 5'-end of the oligonucleotide.

Aldehyde Modified Oligonucleotides

Among other functional groups, aldehydes, such as that in Formylindole Modifier-CE Phosphoramidite (**2056**), will react with amines to form an imine (Schiff's base) which is generally followed by a borohydride reduction due to the instability of the imine bond.[62] These will also react with a hydrazine to form a hydrazone.[63] Solulink HyNic™ conjugation technology[64] is derived from this type of coupling. Although semi-carbizide couplings are commonly used to attach oligonucleotides to glass slides, it is feasible the reaction of an aldehyde with a semi-carbizide to form a semi-carbizone[65] can be applied to on-column coupling. These couplings are shown in Figure 5.

By combining both options, *i.e.* on-column and solution phase conjugations, it is possible to incorporate the same functional group with orthogonal protection into an oligonucleotide where each position can be labelled in turn. This is illustrated in Figure 6; an oligonucleotide modified at the 3'-end with **2350**, the 5'-end with **2193**, and internally with Amino-Modifier C6-dT-CE Phosphoramidite (**2135**), allows stepwise conjugation in three positions. The oligonucleotide is synthesised 'DMT-ON' and the Fmoc group at the 3'-end is removed and labelled at this position followed by a capping step as per solid phase oligonucleotide synthesis. Detritylation to remove the MMT group from the 5'-end is then carried out which is in turn labelled followed by a capping step. The resin is then treated with 20% DEA in MeCN then cleaved, deprotected and - if necessary - purified. The final conjugation step can now be carried out resulting in an oligonucleotide modified in three positions.

Alternatively, more than one functional group can be incorporated into the oligonucleotide, *e.g.* **2135** can be replaced with Bz-S-C6-dT-CE Phosphoramidite (**2191**) or **2193** replaced with **2126**. Here one of the conjugation reactions would become thiol/maleimide or thiol/acetamide.

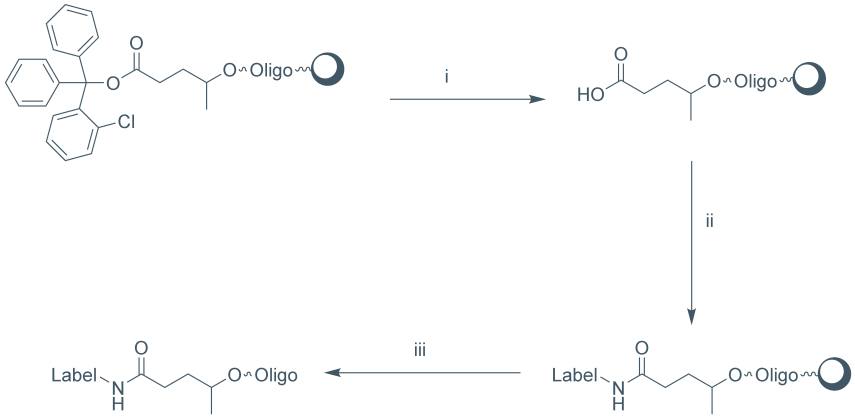
While on-column post synthetic conjugations are not the most widely used method of labelling an oligonucleotide, this method opens up the ability to improve on solution phase couplings where the label is either unstable or has poor solubility in aqueous buffers. This also opens up the possibility of carrying out multiple post synthetic coupling reactions on the same oligonucleotide.

62 **Use of carbonyl group addition-elimination reactions for the synthesis of nucleic acid conjugates**, T.S. Zatsepin, D.A. Stetsenko, M.J. Gait and T.S. Oretskaya, *Bioconjugate Chemistry*, **16**, 471-489, 2005.

63 **Rapid oxime and hydrazone ligations with aromatic aldehydes for biomolecular labelling**, A. Dirksen and P.E. Dawson, *Bioconjugate Chemistry*, **19**, 2543-2548, 2008.

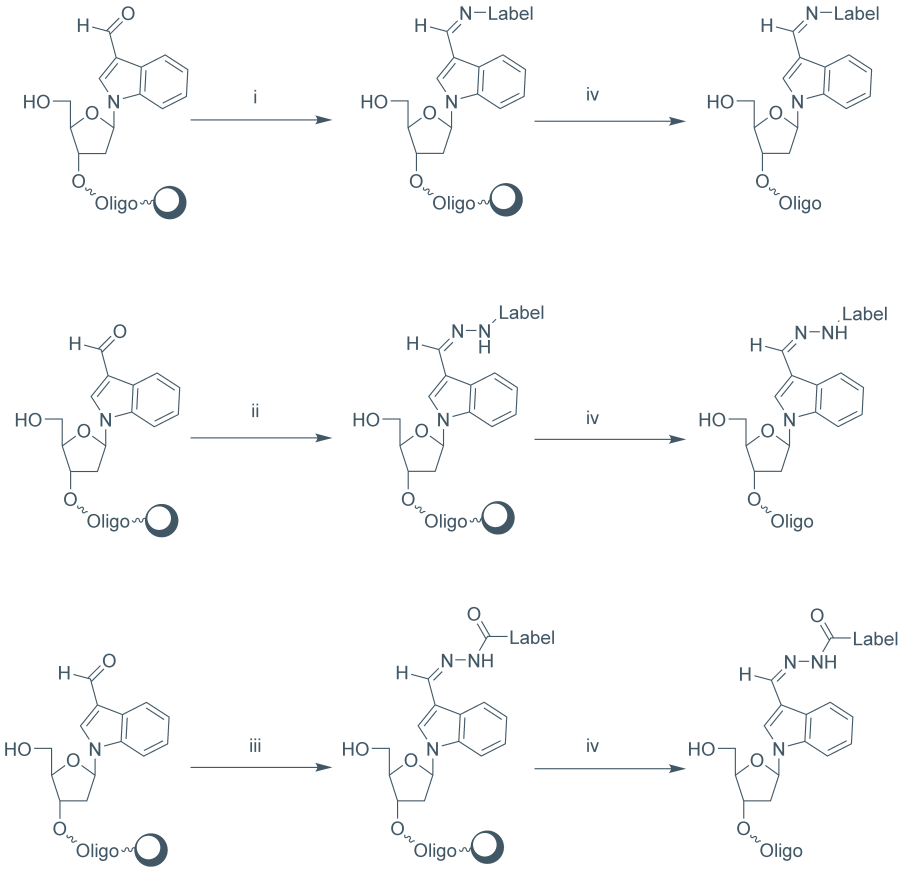
64 **Technetium 99m human polyclonal IgG radiolabeled *via* the hydrazino nicotinamide**, M.J. Abrams, M. Juweid, C.I. TenKate, D.A. Schwartz, M.M. Hauser, F.E. Gaul, J. Fuccello, R.H. Rubin, H.W. Strauss and A.J. Fischman, *J. Nuclear Med.*, **31**, 2022-2028, 1990.

65 **Attachment of benzaldehyde-modified oligonucleotide probes to semi-carbazide coated glass**, M.A. Podyminogin, E.A. Lukhtanov and M.W. Reed, *Nucleic Acids Research*, **29**, 5090-5098, 2001.



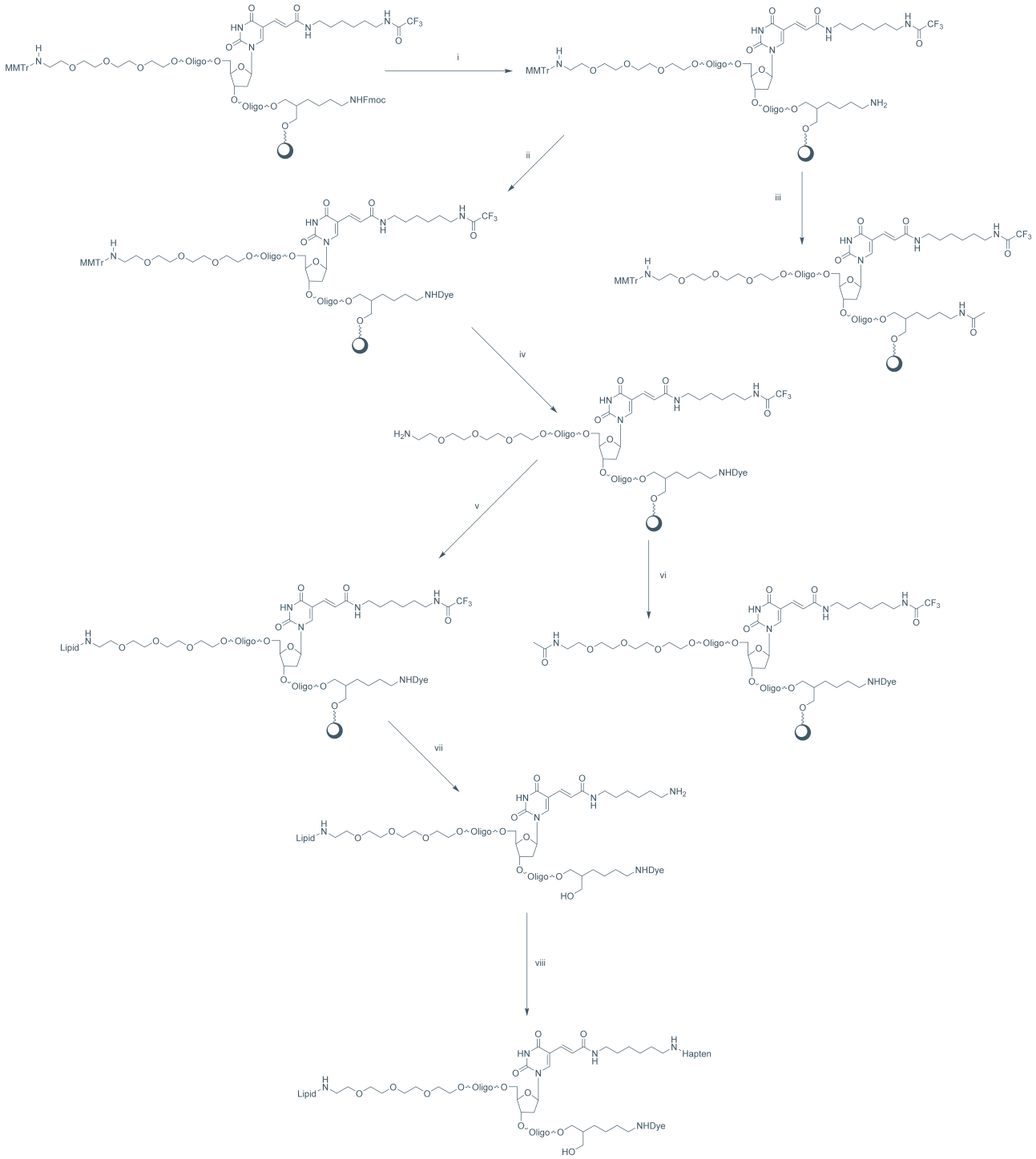
i. Detritylation, ii. Label Coupling, iii. Cleavage and Deprotection

Figure 4. On-column conjugation to a carboxylate-modified oligonucleotide.



i. Label-NH_2, ii. Label-$NHNH_2$, iii. Label-$NHCONHNH_2$, iv. Cleavage and Deprotection

Figure 5. On-column conjugation to an aldehyde-modified oligonucleotide.



i. 20% Piperidine/MeCN, ii. Dye Coupling, iii. Capping, iv. 3% TCA/DCM, v. Lipid Coupling, vi. Capping, vii. Cleavage and Deprotection, viii. Solution-Phase Coupling of Hapten

Figure 6. Conjugation at three amino-functionalised positions within an oligonucleotide.

Backbone Modification

PNA

Introduction

Peptide Nucleic Acid (PNA) was originally conceived as a ligand for the recognition of double-stranded DNA.[66] The concept was to mimic an oligonucleotide binding to double stranded DNA *via* Hoogsteen base pairing. However it is the favourable properties of PNA when mimicing and/or binding to single strands of DNA that have seen PNA gather interest in many areas of modern chemical biology.

Structure and Properties of PNA

The structure of PNA is quite simple (see Figure 7), consisting of repeating N-(2-aminoethyl)-glycine units linked by amide bonds. The purine (A, G) and pyrimidine (C, T) bases are attached to the backbone by methylene carbonyl linkages. Unlike DNA or its analogues, PNAs do not contain any sugar moieties or phosphate groups. Again, unlike DNA, the backbone is acyclic, achiral and neutral.

It is tempting to regard PNA as a DNA analogue, however its chemical structure shows that it is in fact more similar to a protein or peptide molecule. Nevertheless, for applications using PNA the basis of analysis is using sequence information just like with DNA *etc.* By convention, PNAs are represented like peptides, with the N-terminus (or pseudo 5') at the left hand side position and the C-terminus (pseudo 3') at the right.

PNA oligomers are less soluble in water than DNA, and in some aqueous buffers (especially phosphate) poor solubility can be an issue. This is particularly true with increasing length (>12 units) and purine content (especially G above 60%). Often the inclusion of one or two lysine residues can alleviate this problem, as can use of the AEEA spacer (**5005**).

The neutrality of the PNA backbone is a significant feature that has several consequences. One of the most important is the stronger binding between complementary PNA/DNA strands than between DNA/DNA strands at low to medium ionic strength. This can be attributed to the lack of charge repulsion between PNA and DNA. This is also thought to be the reason that

66 (a) **Sequence selective recognition of DNA by strand displacement with a thymine-substituted polyamide**, P.E. Nielsen, M. Egholm, R.H. Berg and O. Buchardt, *Science*, **254**, 1497-1500, 1991; (b) **Peptide nucleic acids (PNA). Oligonucleotide analogues with an achiral peptide backbone**, M. Egholm, O. Buchardt, P.E. Nielsen and R.H. Berg, *J. Amer. Chem. Soc.*, **114**, 1895-1897, 1992; (c) **Peptide nucleic acids (PNA). DNA analogues with a polyamide backbone**, P.E. Nielsen, M. Egholm, R.H. Berg and O. Buchardt, In "Antisense Research and Application", S. Crook and B. Lebleu (eds.), CRC Press, Boca Raton, pp. 363-373.

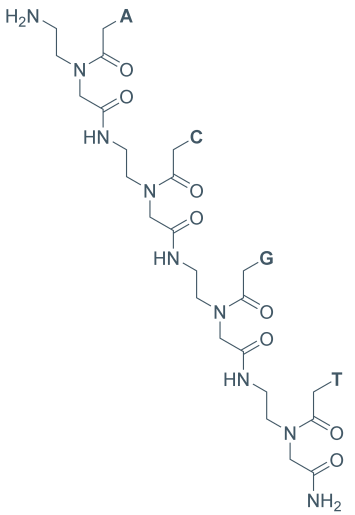


Figure 7. General structure of PNA, where A, C, G and T are the standard purine and pyrimidine nucleobases.

the sequence specificity of PNA to DNA is also higher than in native DNA/DNA strands.[67]

In general, homopyrimidine PNAs form extremely stable triplexes that have sufficient stability to invade intact double stranded DNA. Studies have also shown that 2PNA/DNA triplex formation follows the rules of homopyrimidine DNA triplex formation, *i.e.* with an antiparallel Watson-Crick duplex and a parallel bound Hoogsteen strand. Even more stable triplexes can be formed when the Watson-Crick PNA strand is connected by continuous synthesis *via* ethylene glycol type linkers (*e.g.* AEAA, **5005**) to the Hoogsteen strand. Such constructs are called bis-PNAs.[68]

Applications of PNA

PNA may be used in many of the same applications as synthetic DNA, but with the additional benefits gained from tighter binding and greater specificity. It has therefore become a versatile tool in genetic diagnostics and a variety of molecular biology techniques; particularly *in situ* hybridisation and PCR clamping, but also nucleic acid capture, plasmid vector tagging, duplex DNA targeting, and solution-phase hybridisation detection.[69] bis-PNAs, particularly, provide a tool for selectively targeting any short homopurine sequence in intact double stranded DNA with very high specificity and efficacy.

The ability to bind to both DNA and RNA is a key feature of PNA, as compared to other analogues that favour RNA. In a typical *in situ* hybridisation probing application of mRNA, PNA probes offer faster hybridisation, higher signal, and better specificity. In this application, a set of longer DNA probes with multiple labels can be substituted by a single PNA 15mer with one label.[70] Using one shorter probe improves sequence discrimination, and the PNA has added advantages of increasing overall specificity of the assay, lower background signal and long term stability of the probes.

The lack of a sugar-phosphate backbone makes PNA resistant to nucleases and polymerases. As a result, unmodified PNAs cannot be used as primers in PCR (or other amplification techniques). However, the improved hybridisation properties of PNA are utilised in PCR clamping assays. This technique involves PNA blocking extension of a DNA primer by competing for binding at, or around, the primer site. It has been shown that the superior specificity of the competing PNA results in an assay that allows for discrimination of single base pair differences.[71] This technique has recently been extended to using PNA as both a PCR clamp and probe.[72]

In a similar way PNA-DNA chimeras have been used to enhance DNA amplification.[73] The PNA

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67 **PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen bonding rules**, M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S.M. Freier, D.A. Driver, R.H. Berg, S.K. Kim, B. NordJn and P.E. Nielsen, *Nature*, **365**, 556-568, 1993.

68 **Single and bis peptide nucleic acids as triplexing agents: binding and stoichiometry**, M.C. Griffith, L.M. Risen, M.J. Greig, E.A. Lesnik, K.G. Sprangle, R.H. Griffey, J.S. Kiely and S.M. Freier, *J. Amer. Chem. Soc.*, **117**, 831-832, 1995.

69 For a review see: **Peptide nucleic acid: a versatile tool in genetic diagnostics and molecular biology**, P.E. Nielsen, *Curr. Opin. Biotechnol.*, **12**, 16-20, 2001 and references therein.

70 **Cellular uptake and intracellular fate of antisense oligonucleotides**, A.R. Thierry, E. Vives, J.P. Richard, P. Prevot, C. Martinand-Mari, I. Robbins and B. Lebleu, *Curr. Opin. Mol. Ther.*, **1**, 226-243, 1999.

71 (a) **Single base pair mutation analysis by PNA directed PCR clamping**, H. Ørum, P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt and C. Stanley, *Nucleic Acid Research*, **21**, 5332-5336, 1993; (b) **Simple and sensitive detection of mutations in ras proto-oncogenes using PNA-mediated PCR clamping**, C. Thiede, E. Bayerdörffer, R. Blasczyk, B. Wittig and A. Neubauer, *Nucleic Acids Research*, **24**, 983-984, 1996; (c) **Facilitated detection of oncogene mutations from exfoliated tissue material by a PNA-mediated 'enriched PCR' protocol**, M. Behn, C. Thiede, A. Neubauer, W. Pankow and M. Schuermann, *J. Pathol.*, **190**, 69-75, 2000; (d) **Peptide nucleic acid-mediated PCR clamping as a useful supplement in the determination of microbial diversity**, F. Von Wintzingerode, O. Landt, A. Ehrlich and U.B. Göbel, *Appl. Environ. Microbiol.*, **66**, 549-557, 2000; (e) **The age-related accumulation of a mitochondrial DNA control region mutation in muscle, but not brain, detected by a sensitive PNA-directed PCR clamping based method**, D.G. Murdock, N.C. Christacos and D.C. Wallace, *Nucleic Acids Research*, **28**, 4350-4355, 2000.

72 **Single-tube reaction using peptide nucleic acid as both PCR clamp and sensor probe for the detection of rare mutations**, C.-C. Chiou, J.-D. Luo and T.-L. Chen, *Nature Protocols*, **1**, 2604-2612, 2006.

73 **PNA-DNA oligomers and methods of use thereof**, L.T. Bortolin, C.M. Rudzinski and A.L. Stephens, US Patent No. 2008/0131880 A1.

part binds to the target with greater specificity and the DNA part is amplified.

More recently attention has also turned to the chemical modification of PNA to improve cellular uptake and binding to double-stranded DNA and RNA.[74]

PNA vs LNA

Locked Nucleic Acid (LNA)[75], like PNA, is a DNA analogue of much interest. Structurally these analogues are very different, however their application is in many respects very similar. Each technology has its own advantages and choice between them principally depends upon the experimental conditions and specifics of the application.[76]

In diagnostics, LNA has found particular use in single nucleotide polymorphism (SNP) assay analysis, owing to its excellent thermal stability and mismatch discrimination.[77] LNA-DNA chimera also exhibit RNase H activity (PNA does not) and this can be exploited in therapeutic applications.[78] A significant advantage of PNA is its neutral backbone. This greatly assists in cell delivery when combined with cell-penetrating peptides in antisense therapeutics.[79]

The synthesis of LNA homo-oligomers is less common than with PNAs. DNA (or RNA) is usually "modified" with LNA by incorporation of LNA units into a DNA oligomer to form a chimera. This is easily done as LNAs are synthesised using conventional phosphoramidite chemistry. PNA synthesis, on the other hand, more closely resembles peptide chemistry but it is possible to synthesise PNA-DNA chimera using modified PNA monomers.[59, 80]

PNA Synthesis by Fmoc-Chemistry

Although PNA was first synthesised using ^{t}Boc/Z chemistry[81], the milder chemistry of the Fmoc/Bhoc protection allows the synthesis of PNA with *e.g.* sensitive reporter groups. The simplified final cleavage and deprotection can also be achieved in minutes, provided a suitable resin is used.

After extensive screening, the benzhydryloxycarbonyl (Bhoc) group was selected as the best choice for protecting the exocyclic amino groups of the nucleobases. This group provides sufficient protection during synthesis, is readily removed under the cleavage conditions, and renders solubility to the monomers. For PNA synthesis, therefore, we provide the four Fmoc/Bhoc monomers (items **5001**—**5004**) and a hydrophilic spacer molecule, AEEA (**5005**).

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74 **Recent advances in chemical modification of peptide nucleic acids**, E. Rozners, *J. Nucleic Acids*, **2012**, Article ID 518162, 8pp, 2012.

75 LNA is locked by means of a methylene bridge that connects the 2'-oxygen atom to the 4' carbon atom. This bridge 'locks' the structure conferring a RNA-like C3'-endo conformation to the sugar part of the molecule. LNA products are available exclusively from Exiqon A/S. Also see: **LNA (Locked Nucleic Acids): Synthesis of the adenine, cytosine, guanine, 5-methylcytosine, thymine and uracil bicyclonucleoside monomers, oligomerisation, and unprecedented nucleic acid recognition**, A.A. Koshkin, S.K. Singh, P. Nielsen, V.K. Rajwanshi, R. Kumar, M. Meldgaard, C.E. Olsen, and J. Wengel, *Tetrahedron*, **54**, 3607-3630, 1998.

76 For a comparative review of the two technologies see: **Promising nucleic acid analogs and mimics: characteristic features and applications of PNA, LNA, and morpholino**, S. Karkare and D. Bhatnagar, *Appl. Microbiol. Biotechnol.*, **71**, 575-586, 2006.

77 **Detection of the Factor V Leiden mutation by direct allele-specific hybridization of PCR amplicons to photo immobilized locked nucleic acids**, H. Ørum, M.H. Jakobsev, T. Koch, J. Vuust and M.B. Borre, *Clin. Chem.*, **45**, 1898-1905, 1999.

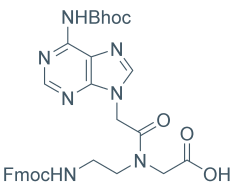
78 **Design of antisense oligonucleotides stabilized by locked nucleic acids**, J. Kurreck, E. Wyszko, C. Gillenand and V.A. Erdmann, *Nucleic Acids Research*, **30**, 1911-1918, 2002.

79 **Effects in live cells of a c-myc anti-gene PNA linked to a nuclear localization signal**, G. Cutrona, E.M. Carpaneto, M. Ulivi, S. Roncella, O. Landt, M. Ferrarini and L.C. Boffa, *Nature Biotechnol.*, **18**, 300-303, 2000.

80 (a) **Novel synthetic routes to PNA monomers and PNA-DNA linker molecules**, G. Breipohl, D.W. Will, A. Peyman and E. Uhlmann, *Tetrahedron*, **53**, 14671-14686, 1997; (b) **New synthesis of PNA–3' DNA linker monomers, useful building blocks to obtain PNA/DNA chimeras**, D. Musumeci, G.N. Roviello, M. Valente, R. Sapio, C. Pedone and E.M. Bucci, *Peptide Science*, **76**, 535–542, 2004.

81 **Synthesis of peptide nucleic acid monomers containing the four natural nucleobases: thymine, cytosine, adenine and guanine, and their oligomerization**, K.L. Dueholm, M. Egholm, C. Behrens, L. Christensen, H.F. Hansen, T. Vulpius, K. Petersen, R.H. Berg, P.E. Nielsen and O. Buchardt, *J. Org. Chem.*, **59**, 5767-5773, 1994.

The latter is used in bis PNA and can be added to PNA to aid solubility. It is also useful to add to the N-terminus (pseudo 5') when labelling PNA with *e.g.* biotin, ROX, TAMRA *etc.*



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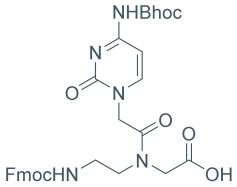

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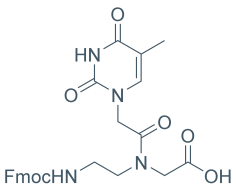

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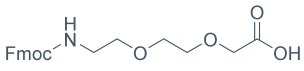


5005

dPEG® Spacers

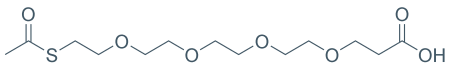
"dPEG®" (discrete poly-(ethylene glycol) or discrete PEG) products, invented by QuantaBiodesign, like traditional PEGs, contain a hydrophilic, water-soluble backbone consisting of repeating ethylene oxide units; however, unlike traditional PEGs, each of the products represents a single compound with a unique, specific, single molecular weight. These products have been used for the modification of therapeutic macromolecules, as linkers in antibody-drug conjugates (ADCs), for bioconjugation of biologics, for surface modification, and in diagnostics, amongst other things.

dPEG® products convey the beneficial properties of traditional PEGs, such as increased water solubility, reduced aggregation, increased hydrodynamic volume, and reduced immunogenicity, but they do so without the complications of polydispersity and allow for the rational design of dPEG® conjugates and analysis of their structure-activity relationships. Two of the products have specific utility in PNA oligomer design.

While the AEEA linker (**5005**) is the most commonly used linker in PNA synthesis, it is possible to use the thiol modified dPEG® linkers, dPEG®$_4$-SATA-Acid (S-acetyl-dPEG®$_4$-acid) (**5010**) and SPDP-dPEG®$_4$-Acid (**5011**). These are particularly useful where a label is only reactive with a thiol and the use of Cys residues is not optimal, *e.g.* where this would interfere with the functionality of a PNA-peptide. They also have potential as a means of attaching PNA to gold surfaces *via* the thiol linker, and the in-built PEG spacer adds distance between the surface and the oligo in a similar way to that described by Anstaett *et al.*[82] These can be incorporated either at the N-terminus or on a branching point incorporated as described above. The thiol linkers are coupled in the same way as the AEEA linker **5005**.

Both linkers are suitable for on-column conjugation where **5010** is deprotected using hydroxylamine or **5011** using TCEP.

82 **Synthesis of stable peptide nucleic acid-modified gold nanoparticles and their assembly onto gold surfaces**, P. Anstaett, Y. Zheng, T. Thai, A.M. Funston, U. Bach and G. Gasser, *Angew. Chem. Int. Ed.*, **52**, 4217 –422, 2013.



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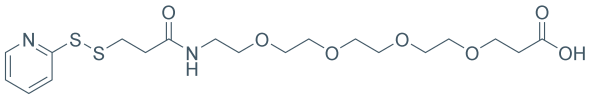


5011

Ordering Peptide Nucleic Acids (PNA) and PNA Linkers

Product	Pack Size	Cat. No.
Fmoc-A(Bhoc)-PNA-OH	700µmol	5001-F700
	500mg	5001-B500
	1g	5001-C001
Fmoc-C(Bhoc)-PNA-OH	700µmol	5002-F700
	500mg	5002-B500
	1g	5002-C001
Fmoc-G(Bhoc)-PNA-OH	700µmol	5003-F700
	500mg	5003-B500
	1g	5003-C001
Fmoc-T-PNA-OH	700µmol	5004-F700
	500mg	5004-B500
	1g	5004-C001
Fmoc-AEEA-OH Spacer	500µmol	5005-F500
dPEG®$_4$-SATA-Acid	100mg	5010-B100
	1g	5010-C001
SPDP-dPEG®$_4$-Acid	100mg	5011-B100
	1g	5011-C001

Unlocked Nucleic Acids

As far back as 1995 the research group of Professor Jesper Wengel introduced the thymine UNA monomer as a modification in DNA oligonucleotides.[83] Along with many other acyclic nucleotide modifications, UNA was shown to induce decreased binding affinity towards a complementary strand. UNA has subsequently been studied as a constituent in gapmer antisense oligonucleotides, and compatibility with RNase H recognition and RNA cleavage has been reported.[84]

UNA (Unlocked Nucleic Acid) is an acyclic analogue of RNA in which the bond between the C2' and C3' atoms is not present (see Figure 8).

Like LNA, UNA is an RNA analogue.[85] However, whereas the additional methylene group linking the O2' and C4' atoms of LNA locks its furanose ring into a C3'-endo conformation, the lack of the C2'-C3' bond in UNA makes this molecule very flexible. It can be helpful to think of LNA as 'locked RNA' and UNA as 'unlocked RNA'.

UNA enables fine tuning of duplex thermodynamic stabilities. Their antipodal structural characteristics make UNA and LNA complementary with respect to effect on binding affinity

83 **Synthesis and evaluation of oligodeoxynucleotides containing acyclic nucleosides: Introduction of three novel analogues and a summary**, P. Nielsen, L.H. Dreiøe and J.Wengel, *Bioorg. Med. Chem.*, **3**, 19-28, 1995. For a recent review of the field see: **Unlocked nucleic acid - an RNA modification with broad potential**, A. Pasternak and J. Wengel, *Org. Biomol. Chem.*, **9**, 3591-7, 2011.

84 **Efficient RNase H-Directed Cleavage of RNA Promoted by Antisense DNA or 2'F-ANA Constructs Containing Acyclic Nucleotide Inserts**, M.M. Mangos, K.-L. Min, E. Viazovkina, A. Galarneau, M.I. Elzagheid, M.A. Parniak, and M.J. Damha, *J. Amer. Chem. Soc.*, **125**, 654-661, 2003.

85 For a comparison review see: **Locked and unlocked nucleosides in functional nucleic acids**, H. Doessing and B. Vester, *Molecules*, **16**, 4511-4526, 2011.

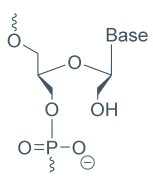


Figure 8. General structure of UNA.

towards a DNA or RNA target: T_m is decreased by 5-10˚C per UNA monomer; T_m is increased by 3-10˚C per LNA monomer.

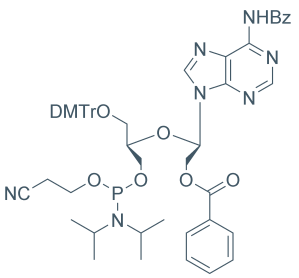
Since the effect of UNA incorporation is additive, by varying the number of incorporations into DNA or RNA oligonucleotides, the stability of the resulting duplexes can be reduced. Since UNA bases have been shown to form Watson-Crick base pairs with complementary bases, the destabilisation effect clearly arises from the flexibility of the acyclic backbone. This feature allows the introduction of local structural flexibility into a single or double stranded nucleic acid.

UNA monomers can be positioned strategically to induce either lack of discrimination of mismatches, *i.e.* universal base behavior, or increased discrimination of mismatches, *i.e.* improved hybridisation specificity. UNA-modified RNA duplexes have been shown by CD spectroscopy to structurally mimic unmodified RNA duplexes.[86]

UNA monomers have been found to be a useful modification in siRNA-based gene-silencing technology as they can protect the siRNA from serum degradation and offer reduced off-target effects while retaining potency.[87] UNA modified siRNAs exhibit a number of key

86 **UNA (unlocked nucleic acid): A flexible RNA mimic that allows engineering of nucleic acid duplex stability**, N. Langkjær, A. Pasternak and J. Wengel, *Bioorg. Med. Chem.*, **17**, 5420-5425, 2009.

87 **A screen of chemical modifications identifies position-specific modification by UNA to most potently reduce siRNA off-target effects**, J.B. Bramsen, M.M. Pakula, T.B. Hansen, C. Bus, N. Langkjær, D. Odadzic, R. Smicius, S.L. Wengel, J. Chattopadhyaya, J.W. Engels, P. Herdewijn, J. Wengel and J. Kjems, *Nucleic Acids Research*, **38**, 5761-5773, 2010.



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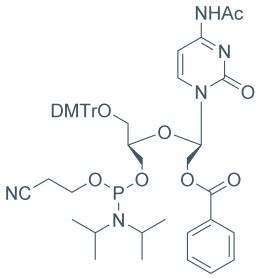

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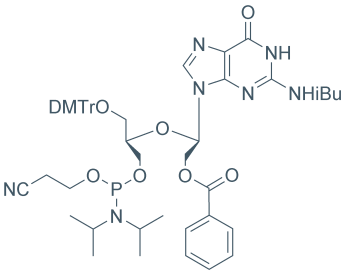

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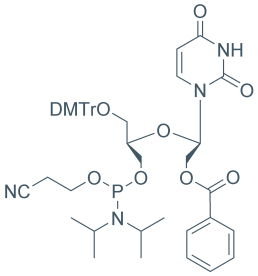


2186

Ordering Unlocked Nucleic Acids (UNA)

Product	Pack Size	Cat. No.
Bz-A-UNA-CE Phosphoramidite	250mg	2183-B250
	500mg	2183-B500
	1g	2183-C001
Ac-C-UNA-CE Phosphoramidite	250mg	2184-B250
	500mg	2184-B500
	1g	2184-C001

Product	Pack Size	Cat. No.
iBu-G-UNA-CE Phosphoramidite	250mg	2185-B250
	500mg	2185-B500
	1g	2185-C001
U-UNA-CE Phosphoramidite	250mg	2186-B250
	500mg	2186-B500
	1g	2186-C001

characteristics:[88]

- One or two UNA monomers in the 3'-overhangs stabilise against degradation by nucleases;
- One UNA monomer in position 6 or 7 in the antisense strand alleviates miRNA-type off-target effects and leads to very potent gene silencing;
- UNA monomers in the antisense strand can be combined with other modifications.

Enzymatic recognition studies have also been reported.[89]

We offer the four UNA phosphoramidites (**2183**-**2186**) employing Ac-C, Bz-A and iBu-G base protection.

H-Phosphonates

H-phosphonate monomers (**2005**-**7**) and (**2035**) are useful for the preparation of internucleotide linkages that are not attainable by phosphoramidite chemistry.[90] The advantage of this chemistry over phosphoramidite chemistry is that in one reaction the backbone of the entire oligonucleotide is converted to the required form. This is typically oxidation to give a sugar-phosphate backbone or sulphurised to give a thiophosphate-sugar backbone, or conversion to the silyl phosphite triester which provides a useful means of

88 **A large-scale chemical modification screen identifies design rules to generate siRNAs with high activity, high stability and low toxicity**, J.B. Bramsen, M.B. Laursen, A.F. Nielsen, T.B. Hansen, C. Bus, N. Langkjær, B.R. Babu, T. Højland, M. Abramov, A. Van Aerschot, D. Odadzic, R. Smicius, J. Haas, C. Andree, J. Barman, M. Wenska, P. Srivastava, C. Zhou, D. Honcharenko, S. Hess, E. Müller, G.V. Bobkov, S.N. Mikhailov, E. Fava, T.F. Meyer, J. Chattopadhyaya, M. Zerial, J.W. Engels, P. Herdewijn, J. Wengel and J. Kjems, *Nucleic Acids Research*, **37**, 2867-2881, 2009.

89 **Stepping towards highly flexible aptamers: enzymatic recognition studies of unlocked nucleic acid nucleotides**, C. Dubois, M.A. Campbell, S.L. Edwards, J. Wengel and R.N. Veedu, *Chem. Commun.*, **48**, 5503-5505, 2012.

90 **Nucleoside H-phosphonates. Chemical synthesis of oligodeoxyribonucleotides by the hydrogenphosphonate approach**, P.J. Garegg, I. Lidh, T. Regberg, J. Stawinski and R. Strömberg, *Tetrahedron Lett.*, **27**, 4051-4054, 1986.

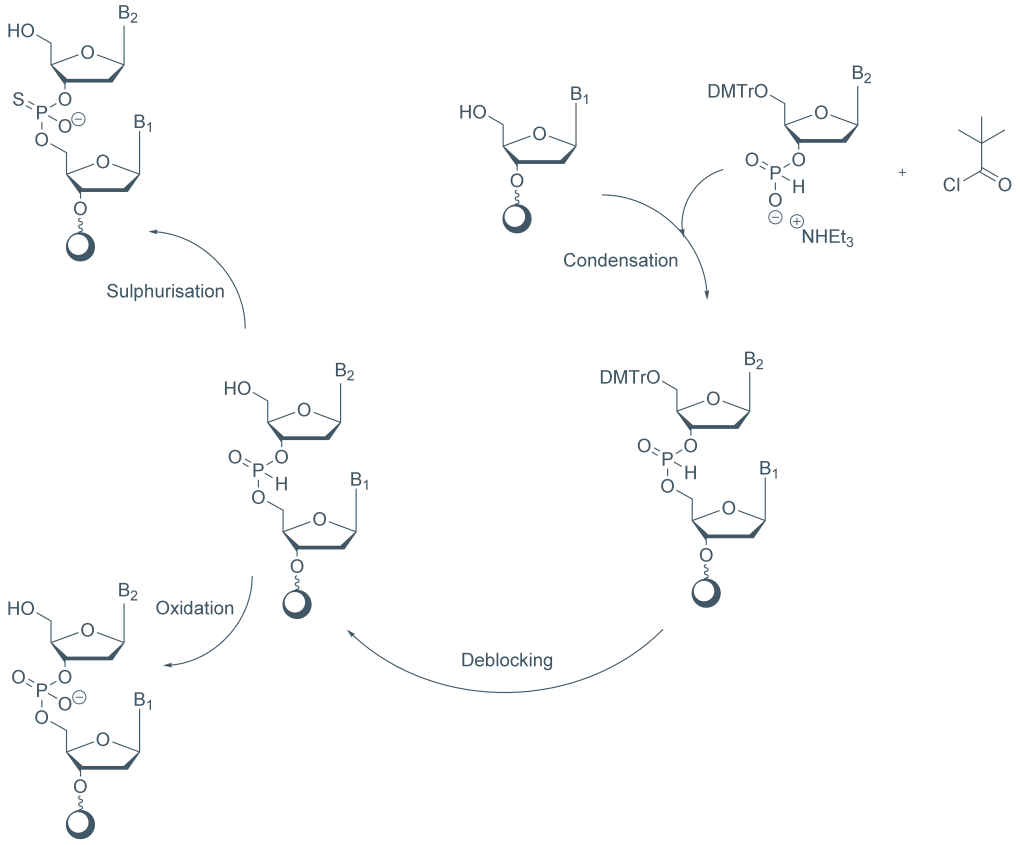


Figure 9. H-phosphonate synthesis cycle.

generating a variety of phosphorus analogues.[91]

The H-phosphonate moiety renders phosphate protection unnecessary and the nucleobases are deprotected using ammonium hydroxide conditions applicable to any unmodified or phosphorothioate oligonucleotide. A popular application of H-phosphonate method is the synthesis of radiolabelled phosphorothioates.[92]

Phosphorothioates

Introduction

Phosphorothioate-containing oligonucleotides (PS-Oligos), containing one sulphur atom in place of an oxygen atom (see Figure 10), have found widespread use in molecular biology. The increased resistance to nuclease digestion that is exhibited by sulphur-containing backbone analogues has prompted consideration of these molecules for medical applications. Phosphorothioate-containing antisense oligos have been used *in vitro* and *in vivo* as inhibitors of gene expression.[93]

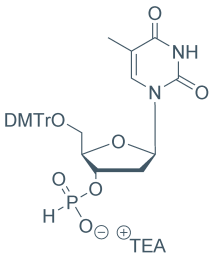
Site-specific attachment of reporter groups onto the DNA or RNA backbone is facilitated by the introduction of single phosphorothioate sites.[94] Phosphorothioates have also been

91 **Synthesis of DNA/RNA and their analogs *via* phosphoramidite and H-phosphonate chemistries**, S. Roy and M. Caruthers, *Molecules*, **18**, 14268-14284, 2013.

92 **Preparation of 35S-labelled polyphosphorothioate oligodeoxyribonucleotides by the use of H-phosphonate chemistry**, C.A.Stein, C.A. Iversen, C. Subashinge, J.S. Cohen, W.J. Stec and G. Zon, *Analytical Biochem.*, **188**, 11-16, 1990.

93 See for example: **Improved biological activity of antisense oligonucleotides conjugated to a fusogenic peptide**, J.-P. Bongartz, A.-M. Aubertin, P.G. Milhaud and B. Lebleu, *Nucleic Acids Research*, **22**, 4681-4688, 1994.

94 **Acceptor helix interactions in a Class II tRNA synthetase: Photoaffinity crosslinking of an RNA miniduplex substrate**, K. Musier-Forsyth and P. Schimmel, *Biochemistry*, **33**, 773-779, 1994.



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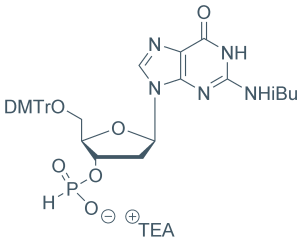
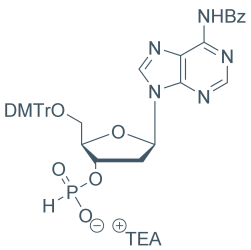
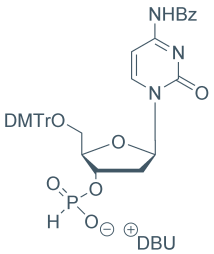


2006



2007



2035

Ordering H-Phosphonates

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Bz-dA-H Phosphonate, TEA Salt	250mg	2007-B250
	500mg	2007-B500
Bz-dC-H Phosphonate, DBU Salt	250mg	2035-B250
	500mg	2035-B500

Product	Pack Size	Cat. No.
iBu-dG-H Phosphonate, TEA Salt	250mg	2006-B250
	500mg	2006-B500
dT-H Phosphonate, TEA Salt	250mg	2005-B250
	500mg	2005-B500

incorporated into oligos for mechanistic studies on DNA-protein[95] and RNA-protein[96] interactions. Backbone modifications, including phosphorothioate substitutions, are also being explored as an approach for increasing the nuclease resistance, and therefore enhancing the therapeutic potential, of ribozymes.[97]

Using solid-phase oligonucleotide assembly, phosphorothioates can be prepared in two ways: by use of H-phosphonates (see above) or by using a sulphurising reagent in conjunction with phosphoramidite chemistry, discussed below.

Sulphurisation

During synthesis using the phosphoramidite approach, the backbone of either DNA or RNA can be modified by sulphurisation (or sulphur-transfer) reagents to replace one non-bridging oxygen atom in the phosphodiester, thus creating a phosphorothioate (PS) linkage.

This makes this method more suitable than H-phosphonate chemistry for controlling the state of each linkage [P=O versus P=S] in a site-specific manner. Compatibility with automated protocols is what gives this technique widest appeal.

Classically, elemental sulphur has been used as a sulphurising reagent,[98] however it is not an efficient process due to poor solubility and slow kinetics. It is imperative that an efficient sulphurisation reagent is used in phosphorothioate synthesis, particularly as synthesis scale and cost increase during commercial oligo production.

A number of sulphurising reagents have been described. These include phenylacetyl disulphide (PADS),[99] tetraethylthiuram disulphide (TETD),[100] 3H-1,2-benzodithiol-3-one

95 **Application of phosphate-backbone-modified oligonucleotides in the studies on EcoRI endonuclease mechanism of action**, M. Koziolkiewicz and W.J. Stec, *Biochemistry*, **31**, 9460-9466, 1992.

96 **Determination of RNA-protein contacts using thiophosphate substitutions**, J.F. Milligan and O.C. Uhlenbeck, *Biochemistry*, **28**, 2849-2855, 1989.

97 **Ribozymes as human therapeutic agents**, R.E. Christoffersen and J.J. Marr, *J. Med. Chem.*, **38**, 2023-2037, 1995.

98 **Synthesis of dinucleoside monophosphorothioates *via* addition of sulphur to phosphite triesters**, P.M. Burgers and F. Eckstein, *Tetrahedron Lett.*, **40**, 3835-3838, 1978.

99 (a) **An efficient approach toward the synthesis of phosphorothioate diesters *via* the Schönberg reaction**, P.C.J. Kamer, H.C.P.F. Roelen, H. van den Elst, G.A. van der Marel and J.H. van Boom, *Tetrahedron Lett.*, **30**, 6757-6760, 1989; (b) **A study on the use of phenylacetyl disulfide in the solid-phase synthesis of oligodeoxynucleoside phosphorothioates**, H.C.P.F. Roelen, P.C.J. Kamer, H. van den Elst, G.A. van der Marel and J.H. van Boom, *Recl. Trav. Chim. Pays-Bas.*, **110**, 325-331, 1991.

100 **Internucleotide phosphite sulfurization with tetraethylthiuram disulfide. Phosphorothioate oligonucleotide synthesis *via* phosphoramidite chemistry**, H. Vu and B.L. Hirschbein, *Terahedron Lett.*, **32**, 3005-3008, 1991.

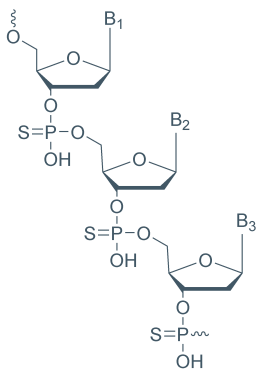


Figure 10. General structure of phosphorothioates.

1,1-dioxide (Beaucage Reagent),[101] 3-ethoxy-1,2,4-dithiazolidine-5-one (EDITH),[102a-d] 1,2,4-dithiazolidine-3,5-dione (DtsNH),[89a] 3-methyl-1,2,4-dithiazolin-5-one (MEDITH),[103] dibenzoyl tetrasulphide,[104] bis(O,O-diisopropoxyphosphinothioyl) disulphide (S-Tetra),[105] benzyltriethylammonium tetrathiomolybdate (BTTM),[106] bis(p-toluenesulphonyl) disulphide[107] and 3-amino-1,2,4-dithiazole-5-thione (ADTT).[108]

Of these, Beaucage Reagent (**0023**) and TETD were the first commercially available, although the former has been most widely used until now, principally due to its better performance and better stability under comparable conditions.

Beaucage Reagent is a relatively efficient sulphurising agent, however it is inherently unstable and has a tendency to precipitate from solution and therefore clog the delivery lines of an automated DNA synthesiser. Furthermore, the by-product formed in the sulphurisation reaction (3H-2,1-benzoxanthiolan-3-one-1-oxide) is a potent oxidising agent, leading to

101 (a) **3H-1,2-benzodithiole-3-one 1,1-dioxide as an improved sulfurizing reagent in the solid-phase synthesis of oligodeoxyribonucleoside phosphorothioates**, R.P. Iyer, W. Egan, J.B. Regan and S.L. Beaucage, *J. Amer. Chem. Soc.*, **112**, 1253-1254, 1990; (b) **The automated synthesis of sulfur-containing oligodeoxyribonucleotides using 3H-1,2-benzodithiole-3-one 1,1-dioxide**, R.P. Iyer, L.R. Phillips, W. Egan, J.B. Regan and S.L. Beaucage, *J. Org. Chem.*, **55**, 4693-4699, 1990.

102 (a) **Use of 1,2,4-dithiazoline-3,5-dione (DtsNH) and 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH) for synthesis of phosphorothioate-containing oligodeoxyribonucleotides**, Q. Xu, K. Musier-Forsyth, R.P. Hammer and G. Barany, *Nucleic Acids Research*, **24**, 1602-1607, 1996; (b) **Efficient introduction of phosphorothioates into RNA oligonucleotides by 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH)**, Q. Xu, G. Barany, R.P. Hammer and K. Musier-Forsyth, *Nucleic Acids Research*, **24**, 3643-3644, 1996; (c) **Synthetic, mechanistic, and structural studies related to 1,2,4-dithiazolidine-3,5-dione**, L. Chen, T.R. Thompson, R.P. Hammer and G. Barany, *J. Org. Chem.*, **61**, 6639-6645, 1996; (d) **Evaluation of 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH) as a new sulfurizing reagent in combination with labile exocyclic amino protecting groups for solid-phase oligonucleotide synthesis**, M.Y.-X. Ma, J.C. Dignam, G.W. Fong, L. Li, S.H. Gray, B. Jacob-Samuel and S.T. George, *Nucleic Acids Research*, **25**, 3590-3593, 1997.

103 **Solid phase synthesis of oligonucleotide phosphorothioate analogues using 3-methyl-1,2,4-dithiazolin-5-one (MEDITH) as a new sulfur-transfer reagent**, Z. Zhang, A. Nichols, J.X. Tang, Y. Han and J.J. Tang, *Tetrahedron Lett.*, **40**, 2095-2098, 1999.

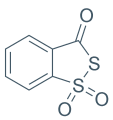
104 **Dibenzoyl tetrasulphide—A rapid sulphur transfer agent in the synthesis of phosphorothioate analogues of oligonucleotides**, M.V. Rao, C.B. Reese and Z. Zhengyun, *Tetrahedron Lett.*, **33**, 4839-4842, 1992.

105 **Bis(O,O-diisopropoxy phosphinothioyl) disulfide—a highly efficient sulfurizing reagent for cost-effective synthesis of oligo(nucleoside phosphorothioate)s**, W.J. Stec, B. Uznanski and A. Wilk, *Tetrahedron Lett.*, **34**, 5317-5320, 1993.

106 **Solid phase synthesis of phosphorothioate oligonucleotides using benzyltriethylammonium tetrathiomolybdate as a rapid sulfur transfer reagent**, M.V. Rao and K. Macfarlane, *Tetrahedron Lett.*, **36**, 6741-6744, 1994.

107 **New efficient sulfurizing reagents for the preparation of oligodeoxyribonucleotide phosphorothioate analogues**, V.A. Efimov, A.L. Kalinkina, O.G. Chakhmakhcheva, T.S. Hill and K. Jayaraman, *Nucleic Acids Research*, **23**, 4029-4033, 1995.

108 **Large-scale synthesis of oligonucleotide phosphorothioates using 3-amino-1,2,4-dithiazole-5-thione as an efficient sulfur-transfer reagent**, J.-Y. Tang, Y. Han, J.X. Tang and Z. Zhang, *Org. Proc. Res. Dev.*, **4**, 194-198, 2000.



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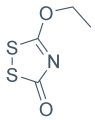

2171

Ordering Sulphurising Reagents

Product	Pack Size	Cat. No.
Beaucage Reagent	1g	0023-C001
	2g	0023-C002
Silanised Bottle - Expedite/MerMade (Bottle Type J)	240ml	0228-D240
Silanised Bottle - ABI (Bottle Type H)	240ml	0227-D240

Product	Pack Size	Cat. No.
EDITH	5mmol	2171-G005
	10mmol	2171-G010
	22.5mmol	2171-G023

side-products, *e.g.* phosphodiesters, which are difficult to separate from the desired product. TETD's sulphurisation rate is slow and therefore a significant molar excess of this reagent is required. Even with this excess, the sulphurisation yields are low.

The shortcomings of these reagents, particularly evident in large-scale synthesis, has seen increased interest in the alternatives to Beaucage and TETD. PADS, for example, has found favour in some quarters for the synthesis of antisense oligonucleotides[109] and siRNA,[110] although there is a requirement to "age" the solution prior to synthesis to achieve optimum results.[111] More recently, effective sulphurisation using 3-((N,N-dimethyl-aminomethylidene) amino)-3H-1,2,4-dithiazole-5-thione (DDTT) has been described commercially, however the use of this reagent is dependant on dilution with a mixture of anhydrous pyridine in acetonitrile or THF.

It is EDITH (**2171**), however, that is now emerging as the reagent of choice amongst users due to its all-round capability and ease of use. This product is soluble in acetonitrile (other Beaucage alternatives require either pyridine or picoline), reportedly stable in solution for several months (a silanised bottle is not required), and exhibits high sulphurisation efficiency with both DNA and RNA. Its high efficiency in RNA synthesis, often unobtainable with other reagents, is of particular benefit, giving a reported >99% sulphurisation efficiency. [89b]

EDITH's efficiency in sulphurising DNA in comparison to TETD has been demonstrated by Xu *et al*, [89a] who reported a 0.5M solution of TETD and contact time of 15min giving 96% PS, whilst EDITH at 0.05M and contact time 30s gave >98% PS.

The compatibility of EDITH with labile (fast) deprotection chemistry has also been noted. [89d] Some deleterious G modification has been observed, however this can be eliminated by using a modified coupling-cap-thio-cap cycle. This allows the preparation of certain phosphorothioates that may be sensitive to prolonged ammonium hydroxide solution treatment. However, it should be noted that capping prior to sulphurisation can lead to some oxidation of the P^{III} species.

Creating Methylated and Ethylated Backbones

Methyl Phosphonamidites

Since methyl (Me) phosphonate linkages are uncharged and nuclease resistant, oligonucleotides containing these have many applications, particularly in developing novel strategies for targeted cellular delivery of antisense therapeutic agents.[112] These were among the first modified oligonucleotides shown to inhibit protein synthesis *via* an antisense mechanism.

Synthesis using these monomers requires a low water content oxidiser and changes

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109 **Synthesis of antisense oligonucleotides: Replacement of 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage Reagent) with phenylacetyl disulfide (PADS) as efficient sulfurization reagent: From bench to bulk manufacture of active pharmaceutical ingredient**, Z.S. Cheruvallath, R.L. Carty, M.N. Moore, D.C. Capaldi, A.H. Krotz, P.D. Wheeler, B.J. Turney, S.R. Craig, H.J. Gaus, A.N. Scozzari, D.L. Cole and V.T. Ravikumar, *Org. Proc. Res. Dev.*, **4**, 199-204, 2000.

110 **Development of siRNA for therapeutics: Efficient synthesis of phosphorothioate RNA utilizing phenylacetyl disulfide (PADS)**, V.T. Ravikumar, M. Andrade, R.L. Carty, A. Dan and S. Barone, *Bioorganic & Medicinal Chem. Lett.*, **16**, 2513-2517, 2006.

111 (a) **Phosphorothioate oligonucleotides with low phosphate diester content: Greater than 99.9% sulfurization efficiency with "aged" solutions of phenylacetyl disulfide (PADS)**, A.H. Krotz, D. Gorman, P. Mataruse, C. Foster, J.D. Godbout, C.C. Coffin and A.N. Scozzari, *Org. Proc. Res. Dev.*, **8**, 852-858, 2004; (b) **An alternative advantageous protocol for efficient synthesis of phosphorothioate oligonucleotides utilizing phenylacetyl disulfide (PADS)**, R.K. Kumar, P. Olsen and V.T. Ravikumar, *Nucleosides, Nucleotides and Nucleic Acids*, **26**, 181-188, 2007.

112 See for example: (a) **Comparative hybrid arrest by tandem antisense oligodeoxyribonucleotides or oligodeoxyribonucleoside methylphosphonates in a cell-free system**, L.J. Maher, III and B.J. Dolnick, *Nucleic Acids Research*, **16**, 3341-3358, 1988; (b) **Solid-phase synthesis of oligo-2-pyrimidinone-2'-deoxyribonucleotides and oligo-2-pyrimidinone-2'-deoxyribose methylphosphonates**, Y. Zhou and P.O.P. Ts'o, *Nucleic Acids Research*, **24**, 2652-2659, 1996; and (c) **Nuclear antisense effects of neutral, anionic and cationic oligonucleotide analogs**, P. Sazani, S.-H. Kang, M.A. Maier, C. Wei, J. Dillman, J. Summerton, M. Manoharan and R. Kole, *Nucleic Acids Research*, **19**, 3965-3974, 2001.

are necessary from commonly used deprotection procedures because the linkages are more base-labile. EDA in 95% EtOH (1:1) is typically used, but other methods have been reported.[113]To help in purification and isolation of these oligos, it is best to incorporate as many phosphodiester linkages (prepared from ß-cyanoethyl phosphoramidites) into each oligo as possible.

Methyl Phosphoramidites

The UltraMILD set of methyl phosphoramidites, in conjunction with UltraMILD deprotection conditions, can be used to prepare the interesting, nuclease resistant methyl phosphotriester linkage. Again, these have potential therapeutic applications.

Ethyl Phosphoramidites

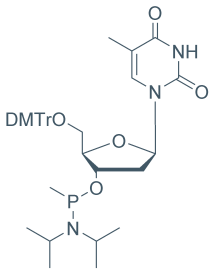
As a result of several customer requests we have introduced a range of ethyl phosphoramidites with classical nucleobase protection (iBu-dG, Bz-dA, Bz-dC) (**2516** - **2519**).

Nuclease resistant P-ethoxy oligonucleotides (a hydrophobic analogue of phosphodiesters) have been shown, through incorporation into liposomes, to be effective in the inhibition of protein expression and cell growth in therapeutic applications.[114,115] The neutral charge and slight lipophilic character appears to improve the delivery of the oligonucleotide into a cell.

113 **Deprotection of methyiphosphonate oligonucleotides using a novel one-pot procedure**, R.I. Hogrefe, M.M. Vaghefi, M.A. Reynolds, K.M. Young and L. Arnold Jr, *Nucleic Acids Research*, **21**, 2031-2038, 1993.

114 **Cellular pharmacology of P-ethoxy antisense oligonucleotides targeted to Bcl-2 in a follicular lymphoma cell line**, Y. Gutiérrez-Puente, A.M. Tari, R.J. Ford, R. Tamez-Guerra, R. Mercado-Hernandez, M. Santoyo-Stephano, and G. Lopez-Berestein, *Leuk. Lymphoma.*, **44**, 1979-85, 2003.

115 **Safety, pharmacokinetics, and tissue distribution of liposomal P-ethoxy antisense oligonucleotides targeted to Bcl-2**, Y. Gutiérrez-Puente, A.M. Tari, C. Stephens, M. Rosenblum, R.T. Guerra, G. Lopez-Berestein, *J. Pharmacol. Exp. Ther.*, **291**, 865-9, 1999.



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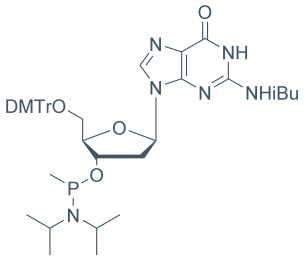

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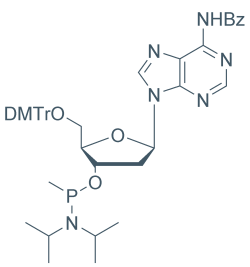

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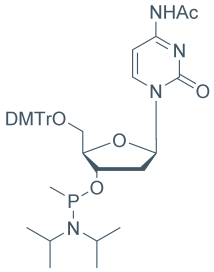

2077

Ordering Methyl Phosphonamidites

Product	Pack Size	Cat. No.
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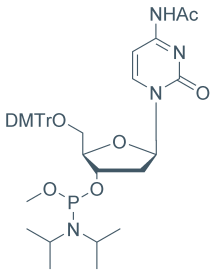
	500mg	2075-B500
Ac-dC-Me Phosphonamidite	250mg	2077-B250
	500mg	2077-B500

Product	Pack Size	Cat. No.
iBu-dG-Me Phosphonamidite	250mg	2074-B250
	500mg	2074-B500
dT-Me Phosphonamidite	250mg	2073-B250
	500mg	2073-B500



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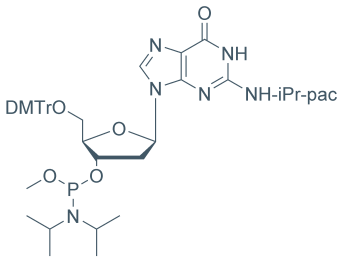

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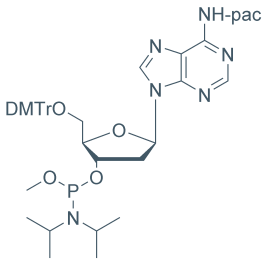

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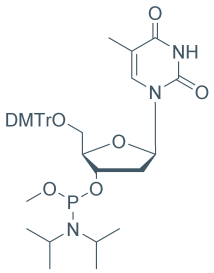

2078

Ordering Methyl Phosphoramidites

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	500mg	2052-B500
	1g	2052-C001
Ac-dC-Me Phosphoramidite	250mg	2050-B250
	500mg	2050-B500
	1g	2050-C001

Product	Pack Size	Cat. No.
iPr-Pac-dG-Me Phosphoramidite	250mg	2051-B250
	500mg	2051-B500
	1g	2051-C001
dT-Me Phosphoramidite	250mg	2078-B250
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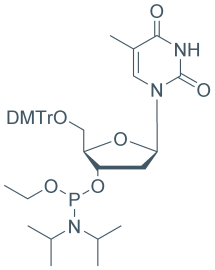

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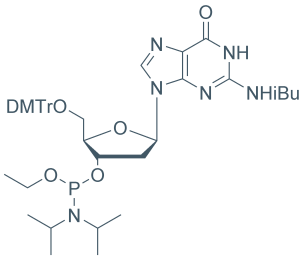

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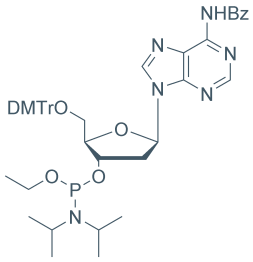

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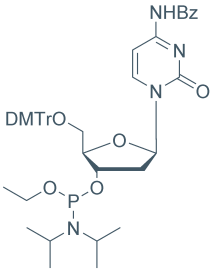

2519

Ordering Ethyl Phosphoramidites

Product	Pack Size	Cat. No.
dT-Et Phosphoramidite	500mg	2516-B500
	1g	2516-C001
iBu-dG-Et Phosphoramidite	500mg	2517-B500
	1g	2517-C001

Product	Pack Size	Cat. No.
Bz-dA-Et Phosphoramidite	500mg	2518-B500
	1g	2518-C001
Bz-dC-Et Phosphoramidite	500mg	2519-B500
	1g	2519-C001

Using Photocleavable (PC) Modifiers

Introduction

The versatility of common modifiers and labels has been extended by the introduction of several photocleavable analogues of the Amino- and Spacer-Modifiers and the Biotin label. This range is complemented by a PC linker molecule, suited to a wide variety of applications.

Design of Photocleavable Modifiers

The general design of the PC monomers is based on an α-substituted 2-nitrobenzyl group.[116] The photo-reactive group is derivatised as a cyanoethyl phosphoramidite for use in automated DNA synthesis.[117]

The PC 5'-Biotin-CE Phosphoramidite (**2122**) contains a biotinyl moiety that bears a trityl group on the N-1 nitrogen atom. This is primarily for N-protection (cf. **2109**) rather than to facilitate coupling efficiency monitoring by trityl cation assay. However, as with **2109**, the N-DMTr group enables cartridge purification of the oligo.

5' Addition of PC Amino-Modifier-CE Phosphoramidite (**2130**) to an oligonucleotide, followed by cleavage from the support and deprotection, results in an aminolinker separated from the oligo by a photocleavable linker. The amino group is then used in post-synthetic modification with amine reactive reagents or to attach synthetic oligonucleotides to activated solid supports. This is particularly useful for capturing DNA or RNA where the oligonucleotide/DNA duplex is cleaved from the surface by photolysis of the PC linker.

While the biotin and amino modifiers are both 5'-terminus modifiers, both the PC Spacer (**2131**) and PC Linker (**2066**) Phosphoramidites can be used as mid-sequence modifiers (for example for use with mass markers).

Upon irradiating a PC-modified oligo with near-UV light, the phosphodiester bond between the linker and the phosphate is cleaved, resulting in the formation of a 5'-monophosphate on the released oligonucleotide. **2066** has the added advantage in that photocleavage results in monophosphate fragments at both the 3'- and 5'-termini (see Figure 11).

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116 (a) **Photocleavage of a 2-nitrobenzyl linker bridging a fluorophore to the 5' end of DNA**, X. Bai, Z. Li, S. Jockusch, N. J. Turro, and J. Ju, *PNAS*, **100**, 409–413, 2003; (b) **Model studies for new o-nitrobenzyl photolabile linkers: substituent effects on the rates of photochemical cleavage**, C.P. Holmes, *J. Org. Chem.*, **62**, 2370-2380, 1997.

117 For examples of applications of related, non-phosphoramidite, molecules see: (a) **Photochemical control of the infectivity of adenoviral vectors using a novel photocleavable biotinylation reagent**, M.W. Pandori, D.A. Hobson, J. Olejnik, S. Sonar, E. Krzymańska-Olejnik, K.J. Rothschild, A.A. Palmer, T.J. Phillips and T. Sano, *Chemistry & Biology*, **9**, 567-573, 2002; and (b) **Design and synthesis of a photocleavable biotinylated nucleotide for DNA analysis by mass spectrometry**, X. Bai, S. Kim, Z. Li, N. J. Turro, and J. Ju, *Nucleic Acids Research*, **32**, 535-541, 2004.

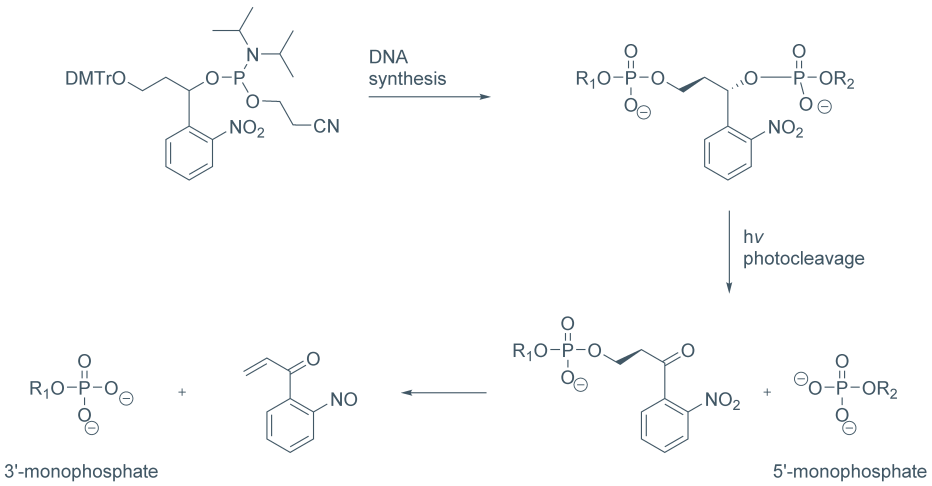


Figure 11. Photocleavage using PC Linker Phosphoramidite.

Application of Photocleavable Modifiers

Affinity Conjugation & Purification

Photocleavable amino-tag phosphoramidites represent a more general approach compared to PC biotin.[118] 5'-PC amino oligonucleotides can be reacted with a wide variety of activated molecules and surfaces, facilitating the formation of a variety of photocleavable conjugates.[119] Upon exposure to near UV light the unmodified oligonucleotide and/or marker molecule can be released and recovered.

The 5'-PC amino group can be used as an affinity tag for photo-cleavage-mediated affinity purification and phosphorylation of synthetic oligonucleotides in conjunction with activated supports. 5'-PC amino labelled oligos suggest applications including multiple non-radioactive probing of DNA/RNA blots, affinity isolation and purification of nucleic acids binding proteins, diagnostic assays requiring release of probe-target complex or specific marker, cassette mutagenesis and PCR.

Oligonucleotide Isolation & Purification

PC Biotin-labelled DNA can be captured with streptavidin beads in a similar fashion to oligonucleotides modified with conventional 5'-biotin phosphoramidite (see Figure 12)[120] and therefore biotin PC linkers are particularly useful in capture probes. The PC biotin is rapidly and quantitatively cleaved from the 5'-terminus, releasing the DNA into solution, by simply illuminating with a hand-held UV light source at 300-350nm. After photo-cleavage the DNA is suitable for further biological manipulations like gene construction and cloning after ligation. However, more commonly this technique is used to isolate DNA by first hybridisation to the biotin labelled probe, then release of the probe/DNA duplex after photolysis.

Avidin-biotin technology has found applications as diverse as detection of proteins by non-radioactive immunoassays, cytochemical staining, cell separation, isolation of nucleic acids, detection of specific DNA/RNA sequences by hybridisation, and probing conformational changes in ion channels.

The use of PC biotin facilitates these applications with the additional benefit of providing an easily removable label. More advanced applications now envisaged include the selective release of biomolecules from 2-dimensional arrays and the assembly of biomolecular constructs at the nanometer scale. PC 5'-Biotin labelled oligonucleotides are useful in a variety of applications in molecular biology including cassette mutagenesis and PCR, where the biotin is used as a means of capture. PC 5'-Biotin Phosphoramidite has been used for the synthesis, purification and phosphorylation of 50mer and 60mer oligonucleotides.

See page 91 for other biotin products.

Photo-triggered Strand Cleavage

Photo-triggered DNA cleavage is a major tool used for studying conformational changes and strand breaks, as well as for studying activation of nucleic-acid-targeted drugs, such as antisense oligonucleotides.[121] The PC Linker Phosphoramidite (**2066**), first described for

118 **Photocleavable aminotag phosphoramidites for 5'-termini DNA/RNA labelling**, J. Olejnik, E. Krzymańska-Olejnik, and K.J. Rothschild, *Nucleic Acids Research*, **26**, 3572-3576, 1998.

119 **Photocleavable peptide-DNA conjugates: synthesis and applications to DNA analysis using MALDI-MS**, J. Olejnik, H.-C. Lüdemann, E. Krzymańska-Olejnik, S. Berkenkamp, F. Hillenkamp and K.J. Rothschild, *Nucleic Acids Research*, **27**, 4626-4631, 1999.

120 (a) **Photocleavable affinity tags for isolation and detection of biomolecules**, J. Olejnik, E. Krzymańska-Olejnik, and K.J. Rothschild, *Methods in Enzymology*, **291**, 135-154, 1998; (b) **Photocleavable biotin phosphoramidite for 5'-end-labelling, affinity purification and phosphorylation of synthetic oligonucleotides**, J. Olejnik, E. Krzymańska-Olejnik, and K.J. Rothschild, *Nucleic Acids Research*, **24**, 361-366, 1996; (c) **Photocleavable biotin derivatives: A versatile approach for the isolation of biomolecules**, J. Olejnik, S. Sonar, E. Krzymańska-Olejnik, and K.J. Rothschild, *Proc. Natl. Acad. Sci. USA*, **92**, 7590-7594, 1995.

121 **Using photolabile ligands in drug discovery and development**, G. Dormán and G.D. Prestwich, *Trends in Biotechnology*, **18**, 64-77, 2000.

use in phototriggered hybridisation,[122] has also been used in the design of multifunctional DNA and RNA conjugates for the *in vivo* selection of new molecules catalysing biomolecular reactions.[123]

The genoSNIP method[124] for single-nucleoside polymorphism (SNP) genotyping by MALDI-TOF mass spectrometry utilises this modification; the method uses size reduction of primer extension products by incorporation of the PC Linker for photo-triggering strand breaks near the 3'-end of the extension primer.

In addition, the PC Spacer Phosphoramidite, can be used as an intermediary to attach any modified phosphoramidite to the terminus of the oligonucleotide. This allows the modification to be removed by photolysis if required.

122 **Design and synthesis of a versatile photocleavable DNA building block. Application to phototriggered hybridization**, P. Ordoukhanian and J-S. Taylor, *J. Amer. Chem. Soc.*, **117**, 9570-9571, 1995.

123 (a) **Libraries of multifunctional RNA conjugates for the selection of new RNA catalysts**, F. Hausch and A. Jäschke, *Bioconjugate Chem.*, **8**, 885-890, 1997; (b) **A novel carboxy-functionalized photocleavable dinucleotide analog for the selection of RNA catalysts**, F. Hausch and A. Jäschke, *Tetrahedron Lett.*, **39**, 6157-6158, 1998; (c) **Multifunctional DNA conjugates for the *in vitro* selection of new catalysts**, F. Hausch and A. Jäschke, *Nucleic Acids Research*, **28**, e35, 2000; (d) **Multifunctional dinucleotide analogs for the generation of complex RNA conjugates**, F. Hausch and A. Jäschke, *Tetrahedron*, **57**, 1261-1268, 2001.

124 **genoSNIP: SNP genotyping by MALDI-TOF MS using photocleavable oligonucleotides**, T. Wenzel, T. Elssner, K. Fahr, J. Bimmler, S. Richter, I. Thomas, and M. Kostrzewa, *Nucleosides, Nucleotides and Nucleic Acids*, **22**, 1579-1581, 2003.

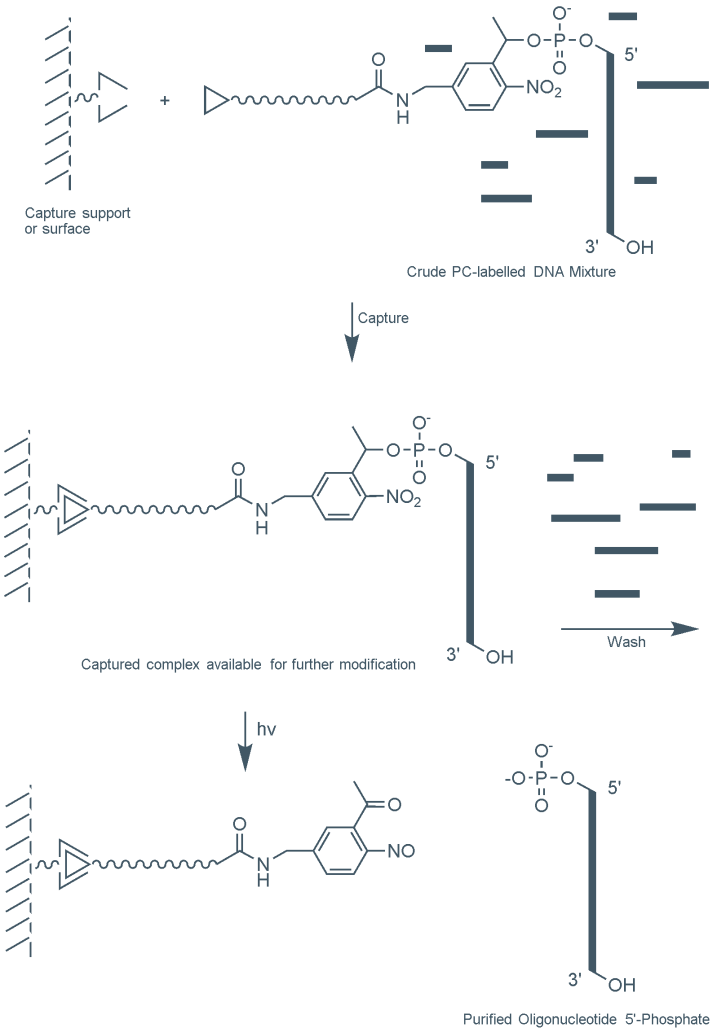
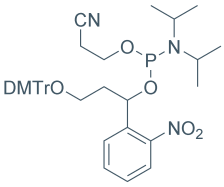
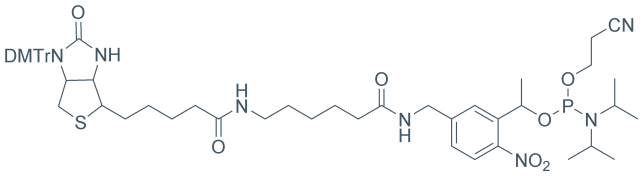


Figure 12. DNA purification using PC-5'-Biotin Phosphoramidite.



2066



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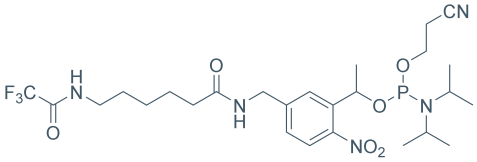
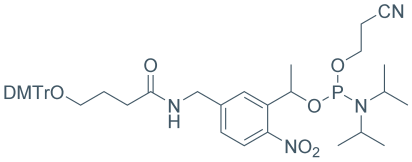


2130



2131

Ordering Photocleavable Modifiers

Product	Pack Size	Cat. No.
PC 5'-Biotin-CE Phosphoramidite	50µmol	2122-F050
	100µmol	2122-F100
	250mg	2122-B250
PC 5'-Amino-Modifier-CE Phosphoramidite	100µmol	2130-F100
	250mg	2130-B250

Product	Pack Size	Cat. No.
PC Spacer-CE Phosphoramidite	100µmol	2131-F100
	250mg	2131-B250
PC Linker-CE Phosphoramidite*	100µmol	2066-F100
	250mg	2066-B250

* Requires to be shipped on ice.

Modifications for Nuclease Resistance

Synthetic oligonucleotides, just like their natural counterparts, are prone to degradation once introduced into a cell. This degradation is due to the presence of exo- and endonuclease enzymes, as well as inherent chemical instability (particularly for RNA). Under cellular conditions, this leads to fast *in vivo* degradation of oligos and a short half-life.[125] To reduce or eliminate this susceptibility, nuclease-resistant modifications can be introduced into oligonucleotides. For antisense or RNAi applications, incorporation of modifications conferring nuclease resistance is essential and such modifications are used routinely. There are a number of ways to introduce nuclease resistance into a synthetic oligonucleotide.

When considering such an oligo, one must also try to minimise potential deleterious side-effects (such as reduced duplex stability, increased toxicity, or induction of off-target biological effects). One method of achieving this is by creating a 'gapmer', in which the linkages of the three terminal 5'- and 3'-bases are phosphorothiolated (see phosphorothioates on page 60), with the remaining bases in the middle having phosphorodiester linkages. Such oligos are highly resistant to both 5'- and 3'-exonuclease degradation. In addition, because phosphorothiolation lowers the binding affinity of the oligo for its target (T_m of the oligo-target duplex is lowered between 0.5ºC and 1.5ºC per linkage), use of as few as six such linkages can give an acceptable balance between nuclease resistance and binding affinity.[126] If increased binding affinity is required, other modifications can also be incorporated into the oligo, such as 2'-fluoro pyrimidines or 2'-OMe bases. The downside of using phosphorothiolation is that sulphur-containing linkages can be toxic, limiting the applicability of this approach.

Alternatively, methylphosphonates (see page 63) can be used for the 5'- and 3'-end positions of the 'gapmer'. Methylphosphonates lower an oligo's binding affinity more than phosphorothiolation, therefore the use of additional modifications, such as 2'-fluoro nucleosides, is used to counteract this effect. Most commonly, the substitution of 2'-OMe bases at some or all positions of an oligo is used as the preferred route to inducing nuclease resistance.[127] Since the nuclease resistance conferred by 2'-OMe lies between that of unmodified nucleosides (no resistance) and phosphorothiolation (highly resistant), extensive/complete 2'-O-methylation is frequently chosen when a high level of nuclease resistance is required. 2'-O-methylation also confers the desirable property of higher binding affinity (that is, higher duplex T_m) to the oligo for its target. For these reasons, 2'-OMe nucleosides are extensively used in siRNA and aptamer applications.

2'-O-Methyl Modifications

2'-O-Methyloligoribonucleotides[128] are extremely useful reagents for a variety of molecular biology applications. The 2'-OMe RNA-RNA duplex is more thermally stable than the corresponding DNA-RNA one.[129] In addition, 2'-OMe-RNA is chemically more stable than either DNA or RNA and is resistant to degradation by RNA- or DNA-specific nucleases.[130] It is worth noting though that duplexes formed between oligos having 2'-OMe bases at all positions and

125 **Rate of degradation of [alpha]- and [beta]-oligodeoxynucleotides in Xenopus oocytes. Implications for anti-messenger strategies**, C. Cazenave, M. Chevrier, T.T. Nguyen and C. Helene, *Nucleic Acids Research*, **15**, 10507-10521, 1987.

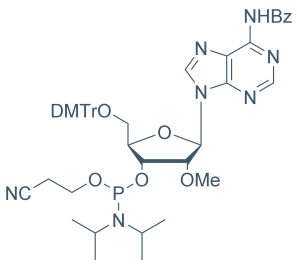
126 **Evaluation of different types of end-capping modifications on the stability of oligonucleotides toward 3'- and 5'-exonucleases**, D. Pandolfi, F. Rauzi and M.L. Capobianco, *Nucleosides & Nucleotides*, **18**, 2051-2069, 1999.

127 (a) **Evaluation of 2'-Modified Oligonucleotides Containing 2'-Deoxy Gaps as Antisense Inhibitors of Gene Expression**, B.P. Monia, E.A. Lesnik, C. Gonzalez, W.F. Lima, D. McGee, C.J. Guinosso, A.M. Kawasaki, P.D. Cook and S.M. Frier, *J. Biol. Chem.*, **268**, 14514-14522, 1993; (b) **Nuclease Resistance and Antisense Activity of Modified Oligonucleotides Targeted to Ha-ras**, B.P. Monia, J.F. Johnston, H. Sasmor and L.L. Cummins, *J. Biol. Chem.*, **271**, 14533-14540, 1996.

128 Note this is not a 2'-OH protecting group strategy; the 2'-OMe group cannot be cleaved under RNA synthesis and deprotection conditions.

129 **Synthesis and hybridization studies on two complementary nona(2'-O- methyl)ribonucleotides**, H. Inoue, Y. Hayase, A. Imura, S. Iwai, K. Miura, and E. Ohtsuka, *Nucleic Acids Research*, **15**, 6131-6148, 1987.

130 **Highly efficient chemical synthesis of 2'-O-methyloligoribonucleotides and tetrabiotinylated derivatives; novel probes that are resistant to degradation by RNA or DNA specific nucleases**, B.S. Sproat, A.I. Lamond, B. Beijer, P. Neuner and U. Ryder, *Nucleic Acids Research*, **17**, 3373-3386, 1989.



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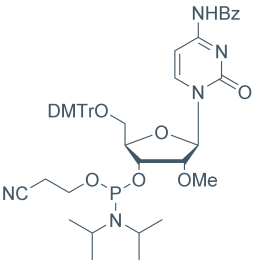

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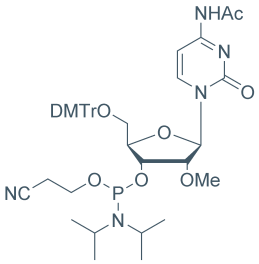

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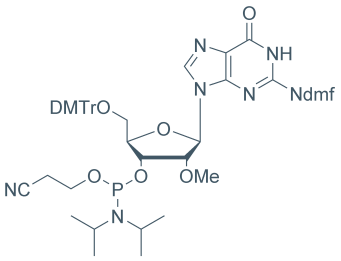

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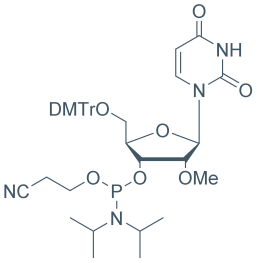

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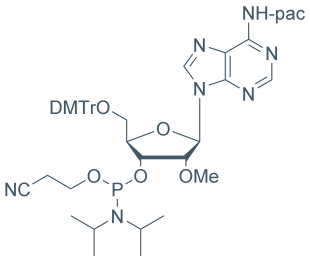

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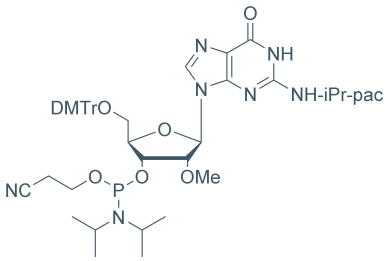

2084

Ordering Unmodified 2'-OMe RNA Phosphoramidites

Product	Pack Size	Cat. No.
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	500mg	2041-B500
	1g	2041-C001
2'-OMe-Pac-A-CE Phosphoramidite	250mg	2083-B250
	500mg	2083-B500
	1g	2083-C001
2'-OMe-Bz-C-CE Phosphoramidite	250mg	2042-B250
	500mg	2042-B500
	1g	2042-C001
2'-OMe-Ac-C-CE Phosphoramidite	250mg	2043-B250
	500mg	2043-B500
	1g	2043-C001

Product	Pack Size	Cat. No.
2'-OMe-dmf-G-CE Phosphoramidite	250mg	2044-B250
	500mg	2044-B500
	1g	2044-C001
2'-OMe-iPr-Pac-G-CE Phosphoramidite	250mg	2084-B250
	500mg	2084-B500
	1g	2084-C001
2'-OMe-U-CE Phosphoramidite	250mg	2045-B250
	500mg	2045-B500
	1g	2045-C001

Ordering Unmodified 2'-OMe RNA SynBase™ CPG Supports

		Cat. No.				
Product	Pack Size	Bz-A	Bz-C	Ac-C	dmf-G	U
2'-OMe RNA SynBase™ CPG 1000/110	100mg	2312-B100	2313-B100	2314-B100	2311-B100	2310-B100
	250mg	2312-B250	2313-B250	2314-B250	2311-B250	2310-B250
	1g	2312-C001	2313-C001	2314-C001	2311-C001	2310-C001
ALL-FIT Columns	10 x 0.2µmol	2312-P002	2313-P002	2314-P002	2311-P002	2310-P002
	10 x 1µmol	2312-P008	2313-P008	2314-P008	2311-P008	2310-P008

RNA are incapable of RNase H activity, thus making them ineffective in RNaseH dependent antisense applications[131], although they can suppress gene expression by blocking the mRNA translation process *via* steric hindrance.[132] The enhanced RNase and DNase resistance, and the increased thermal stability of their duplexes and triplexes, have been examined in a number of ways.[133,134] Applications range from simple antigene type experiments to the correction of aberrant splicing. Researchers have also made use of biotinylated 2'-OMe RNA for the affinity selection or affinity depletion of ribonucleoprotein complexes, most notably in the field of RNA processing.[135]

We provide a range of 2'-OMe phosphoramidites (**2041** - **2045**, **2083**/**2084**) and CPG supports (**2310** - **2313**) with a variety of protecting groups. Whilst we have for some time offered these, increasingly researchers are looking for nucleobase modifications to RNA-type molecules to extend the experimentation available. In fact, many of these modifiers are increasingly being used in larger scale oligonucleotide manufacture. To this end we have introduced a number of other 2'-OMe RNA products.

Oligonucleotides containing 2'-OMe-5-Me-U (2'-OMe-T) (**2099**), 2'-OMe-N-Ac-5-Me-C (**2192**) or 2'-OMe-I (**2098**) are particularly applicable to triplex and antisense studies using 2'-OMe-RNA. For example, the immune stimulatory activity of CpG containing oligonucleotides in which C or G was substituted with 2'-OMe ribonucleotides, 5-Me-dC, or 2'-OMe-5-Me-C has been studied alone and in combination with TLR agonists.[136]

When 2'-OMe residues are incorporated into triplex forming oligonucleotides[137] (TFOs), similar trends in nuclease resistance and triplex stability are seen as with duplexes. Hence

131 **Sequence-dependent hydrolysis of RNA using modified oligonucleotide splints and RNase H**, H. Inoue, Y. Hayase, S. Iwai and E. Ohtsuka, *FEBS Lett.*, **215**, 327-330, 1987.

132 **Antisense technologies. Improvement through novel chemical modifications**, J. Kurreck, *Eur. J. Biochem.*, **270**, 1628-1644, 2003.

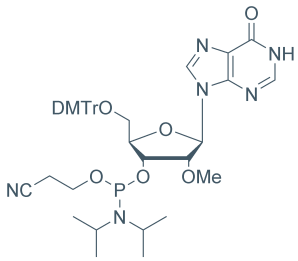
133 **Effective incorporation of 2'-O-methyl-oligoribonucleotides into liposomes and enhanced cell association through modification with thiocholesterol**, B. Oberhauser and E. Wagner, *Nucleic Acids Research*, **20**, 533-538, 1992.

134 **Enhancement of ribozyme catalytic activity by a contiguous oligodeoxynucleotide (facilitator) and by 2'-O-methylation**, J. Goodchild, *Nucleic Acids Research*, **20**, 4607-4612, 1992.

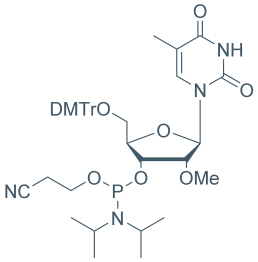
135 See for example: **Mapping U2 snRNP – premRNA interactions using biotinylated oligonucleotides made of 2'-OMe RNA**, S.M.L. Barabino, B.S. Sproat, U. Ryder, B.J. Blencowe, A.I. Lamond, *The EMBO Journal*, **8**, 4171-4178, 1989.

136 **Modifications incorporated in CpG motifs of oligodeoxynucleotides lead to antagonist activity of toll-like receptors 7 and 9**,
D. Yu, D. Wang, F.-G. Zhu, L. Bhagat, M. Dai, E. R. Kandimalla and S. Agrawal, *J. Med. Chem.*, **52**, 5108-5114, 2009.

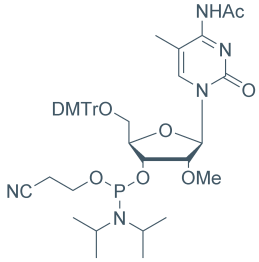
137 **Pyrimidine motif triplexes containing polypurine RNA or DNA with oligo 2'-O-Methyl or DNA triplex forming oligonucleotides**, M. Behan and P.S. Miller, *Biochim. Biophys. Acta.*, **1492**, 155-162, 2000.



2098



2099



2192

Ordering Modified 2'-OMe RNA Phosphoramidites

Product	Pack Size	Cat. No.
2'-OMe-I-CE Phosphoramidite	100µmol	2098-F100
	250mg	2098-B250
2'-OMe-5-Me-U Phosphoramidite	100µmol	2099-F100
	250mg	2099-B250

Product	Pack Size	Cat. No.
2'-OMe-N-Ac-5-Me-C Phosphoramidite	100µmol	2192-F100
	250mg	2192-B250

they have been used to develop TFOs for use as gene targeting reagents.[138] Triplex stability can be increased further with the incorporation of 2'-OMe-5-Me-U residues into the oligonucleotide[139] using **2099**. Interestingly, 2'-OMe-5-Me C (using **2192**) can have the opposite effect and destabilise the triplex, yet is still more stable than a DNA and/or RNA triplex. Incorporation of both 2'-OMe-5-Me U and 2'-OMe-5-Me C enables fine-tuning of the T_m of the resulting triplex.

Conveniently, the deprotection of 2'-OMe oligoribonucleotides are exactly the same as for unmodified oligodeoxynucleotides. However, due to the higher degree of hydrophobicity, some alterations may be required in terms of purification. Because the 2'-OMe oligos are nuclease resistant, unless the oligo contains RNA residues, the need to decontaminate equipment and glassware with *e.g.* RNase away is not an absolute requirement.

2'-Fluoro Phosphoramidites

2'-F-RNA oligonucleotides (synthesised using **2079** and **2080**) adopt an A-form helix on hybridisation to a target. Whereas a hydroxyl group of RNA is a hydrogen bond donor, fluorine appears to be a weak acceptor. These features of 2'-F-RNA oligonucleotides lead to certain interesting properties. For example, it was demonstrated that oligonucleotides hybridise to a RNA oligonucleotide in the following order of increasing stability: DNA < RNA < 2'-OMe-RNA < 2'-F-RNA.[140]

Aptamers composed of 2'-F–RNA bind targets with higher affinities and are more resistant to nucleases, compared to RNA aptamers.[141] In addition, 2'-F-RNA can be effectively used in siRNA applications. It has been shown that siRNA synthesised with 2'-F pyrimidine nucleosides are more inhibitory, and show considerably increased stability in human plasma, compared to siRNA.[142] 2'-F-RNA is now finding a number of applications, especially in RNA interference for the specific silencing of genes in cells and *in vivo*.[143]

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138 **The development of bioactive triple helix-forming oligonucleotides**, M.M. Seidman, N. Puri, A. Majumdar, B. Cuenoud, P.S. Miller and R. Alam, in volume 1058, *Therapeutic Oligonucleotides: Transcriptional and Translational Strategies for Silencing Gene Expression*, 119–127, November 2005, Wiley-Blackwell.

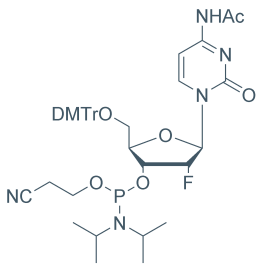
139 **Effects of 5-methyl substitution in 2'-O-methyl oligo(pyrimidine)nucleotides on triple-helix formation**, M. Shimizu, T. Koizumi., H. Inoue and E. Ohstuka, *Bioorg. Med. Chem. Letts.*, **4**, 1029-1032, 1994.

140 **Uniformly modified 2'-deoxy-2'-fluoro phosphorothioate oligonucleotides as nuclease-resistant antisense compounds with high affinity and specificity for RNA targets**, A.M. Kawasaki, M.D. Casper, S.M. Freier, E.A. Lesnik, M.C. Zounes, L.L. Cummins, C. Gonzalez and P.D. Cook, *J. Med. Chem.*, **36**, 831-841, 1993.

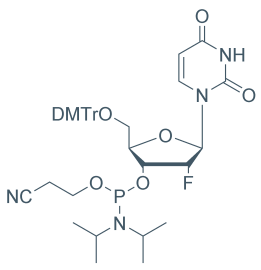
141 **Neutralization of infectivity of diverse R5 clinical isolates of human immunodeficiency virus type 1 by gp120-binding 2'-F-RNA aptamers**, M. Khati, M. Schüman, J. Ibrahim, Q. Sattentau, S. Gordon and W. James, *J. Virology*, **77**, 12692-12698, 2003.

142 ***In vivo* activity of nuclease-resistant siRNAs**, J.M. Layzer, A.P. McCaffrey, A.K. Tanner, Z. Huang, M.A. Kay and B.A. Sullenger, *RNA*, **10**, 766-771, 2004.

143 **Molecular requirements for degradation of a modified sense RNA strand by Escherichia coli ribonuclease H1**, D.R. Yazbeck, K.-L. Min and M.J. Damha, *Nucleic Acids Research*, **30**, 3015-3025, 2002.



2079



2080

Ordering 2'-F Phosphoramidites

Product	Pack Size	Cat. No.
2'-F-Ac-dC-CE Phosphoramidite	100µmol	2079-F100
	250mg	2079-B250

Product	Pack Size	Cat. No.
2'-F-dU-CE Phosphoramidite	100µmol	2080-F100
	250mg	2080-B250

Reverse (5' to 3') Oligonucleotides

The chemical synthesis of DNA using the phosphoramidite method proceeds in a 3' to 5' direction principally as a consequence of the use of building blocks activated as 3'-O-phosphoramidites. The primary 5'-OH group is significantly more reactive than the secondary 3'-OH (or 2'-OH) group, making it straightforward to protect with the DMT group leaving the 3'-OH available to form the phosphoramidite. In contrast, 'reverse' oligonucleotide synthesis (*i.e.* in a 5' to 3' direction) has not been utilised to nearly the same extent. Nevertheless, there are several applications of this chemistry, most notably in nuclease resistance.

An interesting addition to the protection of antisense oligonucleotides is to modify the terminal linkages from the natural 3'-5' to 3'-3' and/or 5'-5' linkages. In this way, the oligonucleotides are protected against exonuclease activity, especially 3'-exonuclease activity which is by far the most significant enzymatic degradation route, resulting in nucleosides with no toxicity concerns. This strategy has been applied by Beaucage and co-workers who have used 5'-O-phosphoramidites in the formation of oligonucleotides having alternating 3'-3' and 5'-5' linkages to maintain effective hybridisation.[144] A simpler approach is in fact to modify only the linkage at the 3' terminus.[145] This is conveniently carried out and results in effective resistance with minimal disruption to hybridisation.

In addition to the established applications in nuclease resistance and hairpin loops, other technologies exploit the flexibility of reverse oligo synthesis. For example, with the increasing use of DNA chip technology, interest has focused upon the synthesis of support-bound, fully deprotected oligonucleotides.[146] Such molecules are accessible through the use of 2-(4-nitrophenyl)-ethyl/[2-(4-nitrophenyl)ethoxy]carbonyl (npe/npeoc) protecting groups[147] on the nucleobase.

The use of 5'-O-phosphoramidites has not generally been used for the elaboration of oligonucleotides, even though this approach offers a facile route to 3'-modified oligodeoxynucleotides. The potential for this approach has recently been demonstrated by Hecht and co-workers using a phosphoramidite derived from tyrosine.[148] The derived oligonucleotide was shown to have chromatographic and electrophoretic properties identical with the modified oligo resulting from the proteinase K digestion of a topoisomerase-DNA complex.

We offer reverse phosphoramidites (**2020** - **2023**, **2093**) and solid supports (**2294**, **2298**, **2355**, **2356**), with classical heterocyclic base protection groups.

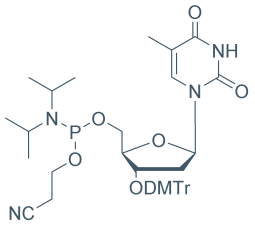
144 (a) **Alternating α,β-oligothymidylates with alternating (3'-3')- and (5'-5')-internucleotidic phosphodiester linkages as models for antisense oligodeoxyribonucleotides**, M. Koga, M.F. Moore and S.L. Beaucage, *J. Org. Chem.*, **56**, 3757-3759, 1991; (b) **Synthesis and physicochemical properties of alternating α,β-oligodeoxyribonucleotides with alternating (3'-3')- and (5'-5')-internucleotidic phosphodiester linkages**, M. Koga, A. Wilk, M.F. Moore, C.L. Scremin, L. Zhou and S.L. Beaucage, *J. Org. Chem.*, **60**, 1520-1530, 1995.

145 (a) **Antisense effect of oligodeoxynucleotides with inverted terminal internucleotidic linkages: a minimal modification protecting against nucleolytic degradation**, J.F.R. Ortigao, H. Rosch, H. Selter, A. Frohlich, A. Lorenz, M. Montenarh and H. Seliger, *Antisense Res. & Dev.*, **2**, 129-146, 1992; (b) **Oligonucleotide analogs with terminal 3'-3'- internucleotidic and 5'-5'-internucleotidic linkages as antisense inhibitors of viral gene-expression**, H. Seliger, A. Frohlich, M. Montenarh, J.F.R. Ortigao and H. Rosch, *Nucleosides & Nucleotides*, **10**, 469-477, 1991.

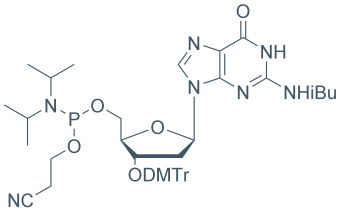
146 **Synthesis of 2'-deoxyribonucleoside 5'-phosphoramidites: New building blocks for the inverse (5'-3')-oligonucleotide approach**, T. Wagner and W. Pfleiderer, *Helv. Chim. Acta.*, **83**, 2023-2035, 2000.

147 **Improved synthesis of oligodeoxyribonucleotides**, K.P. Stengele and W. Pfleiderer, *Tetrahedron Lett.*, **31**, 2549-2552, 1990.

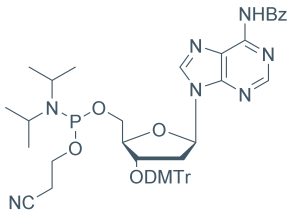
148 **3'-Modified oligonucleotides by reverse DNA synthesis**, C.D. Claeboe, R. Gao and S. M. Hecht, *Nucleic Acids Research*, **31**, 5685-5691, 2003.



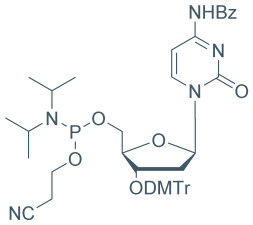
2020



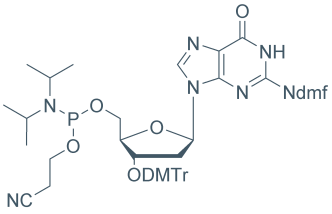
2021



2022



2023



2093

Ordering 5' to 3' Synthesis Reagents

Product	Pack Size	Cat. No.
Bz-dA-5'-CE Phosphoramidite	250mg	2022-B250
	500mg	2022-B500
	1g	2022-C001
Bz-dC-5'-CE Phosphoramidite	250mg	2023-B250
	500mg	2023-B500
	1g	2023-C001
iBu-dG-5'-CE Phosphoramidite	250mg	2021-B250
	500mg	2021-B500
	1g	2021-C001
dmf-dG-5'-CE Phosphoramidite	250mg	2093-B250
	500mg	2093-B500
	1g	2093-C001
dT-5'-CE Phosphoramidite	250mg	2020-B250
	500mg	2020-B500
	1g	2020-C001
Bz-dA-5'-SynBase™ CPG 1000/110	100mg	2355-B100
	1g	2355-C001
ALL-FIT Columns	10 x 0.2µmol	2355-P002
	10 x 1µmol	2355-P008
MerMade Columns	10 x 0.2µmol	2355-P016
	10 x 1µmol	2355-P022

Product	Pack Size	Cat. No.
Bz-dC-5'-SynBase™ CPG 1000/110	100mg	2356-B100
	1g	2356-C001
ALL-FIT Columns	10 x 0.2µmol	2356-P002
	10 x 1µmol	2356-P008
MerMade Columns	10 x 0.2µmol	2356-P016
	10 x 1µmol	2356-P022
iBu-dG-5'-SynBase™ CPG 1000/110	100mg	2298-B100
	1g	2298-C001
ALL-FIT Columns	10 x 0.2µmol	2298-P002
	10 x 1µmol	2298-P008
MerMade Columns	10 x 0.2µmol	2298-P016
	10 x 1µmol	2298-P022
dT-5'-SynBase™ CPG 1000/110	100mg	2294-B100
	1g	2294-C001
ALL-FIT Columns	10 x 0.2µmol	2294-P002
	10 x 1µmol	2294-P008
MerMade Columns	10 x 0.2µmol	2294-P016
	10 x 1µmol	2294-P022

Chemical Phosphorylation

Chemical phosphorylation is a cost-effective alternative to enzymatic methods (using T4 polynucleotide kinase and ATP), allowing efficient introduction of terminal phosphate groups. Oligonucleotides containing a 5'-phosphate group have various applications, being most widely used as a means of ligating one oligo to another, *e.g.* as linkers and adapters, in cloning, gene construction, and ligation in general. This is still the most common method of gene synthesis. 3'-Phosphorylations, however, are used to block enzyme activity. For example, this is an efficient and commonly used PCR blocking technique.

Phosphorylation of the 5'-terminus on oligonucleotides is routinely achieved, with higher yields than using kinase, using Phosphate-ON (**2101**) (also known as Chemical Phosphorylation Reagent (CPR))[149]. Aside from its inherent convenience, CPR also has the advantage over enzymatic methods in allowing determination of the phosphorylation efficiency due to the presence of the DMTr protecting group. However, the trityl group cannot be used as a purification handle. It is eliminated along with the sulphonyl ethyl group to produce the 5'-phosphate during the ammonium hydroxide deprotection.

2101 can also be used at the 3'-end to incorporate a 3'-phosphate by addition to any support (*e.g.* a dT column). This is particularly useful for labelling long oligos where higher pore sized resins for modification are not available. It is for this reason we introduced the 3000Å phosphate support (see below).

This technique is not only limited to phosphate modification, since any modifying phosphoramidite can be added to the phosphate-ON-T. In this case the oligo will be terminated at the 3'-end with "modifier-phosphate-3'".

Another phosphorylation reagent, known as *CPR II* (Figure 13), has been described by researchers at the University of Turku in Finland.[150]

With this product, conventional ammonium hydroxide cleavage gives rise to an oligonucleotide protected at the 5'-phosphate with a DMTr-ether. At this stage, the oligo may be easily separated from truncated impurities by *e.g.* RP-HPLC or cartridge-purification. The DMTr-group is then removed by aqueous acid and brief ammonium hydroxide treatment yields the 5'-phosphate. Alternatively, the yield of the last coupling may be quantified by detritylation of the oligo whilst still on the support. Deprotection then leads to the 5'-phosphorylated oligo.

This *CPR II* reagent has been further refined (by substituting the ethyl esters for methyl amides) to provide a product, *solidCPR*™ (**2127**, also known as *CPRIIa*),[151] that offers all the benefits of *CPR II* whilst also being a stable solid that permits easy weighing, handling and dissolution (**2101** and *CPR II* are both viscous glasses). This product also allows the option of DMT ON purification (see Figure 14).

Although **2101** can be used in 3'-phosphorylations, 3'-Phosphate SynBase™ CPG 1000/110 and 3000/110 (**2279** and **2398**), allow direct preparation of oligonucleotides with a 3'-phosphate group. **2127** cannot be used for 3'-phosphorylation since the DMTr-protected OH is required to release the phosphate group.

149 **A chemical 5'-phosphorylation of oligodeoxyribonucleotides that can be monitored by trityl cation release**, T. Horn and M. Urdea, *Tetrahedron Lett.*, **27**, 4705-4708, 1986.

150 **A new approach for chemical phosphorylation of oligonucleotides at the 5'-terminus**, A. Guzaev, H. Salo, A. Azhayev and H. Lonnberg, *Tetrahedron*, **51**, 9375-9384, 1995.

151 **Chemical phosphorylation of oligonucleotides and reactants therefor**, A. Guzaev, A. Azhayev and H. Lonnberg, US Patent No. 5959090, 1999.

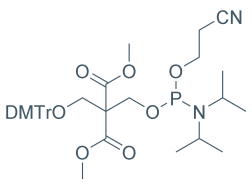


Figure 13. *CPR II.*

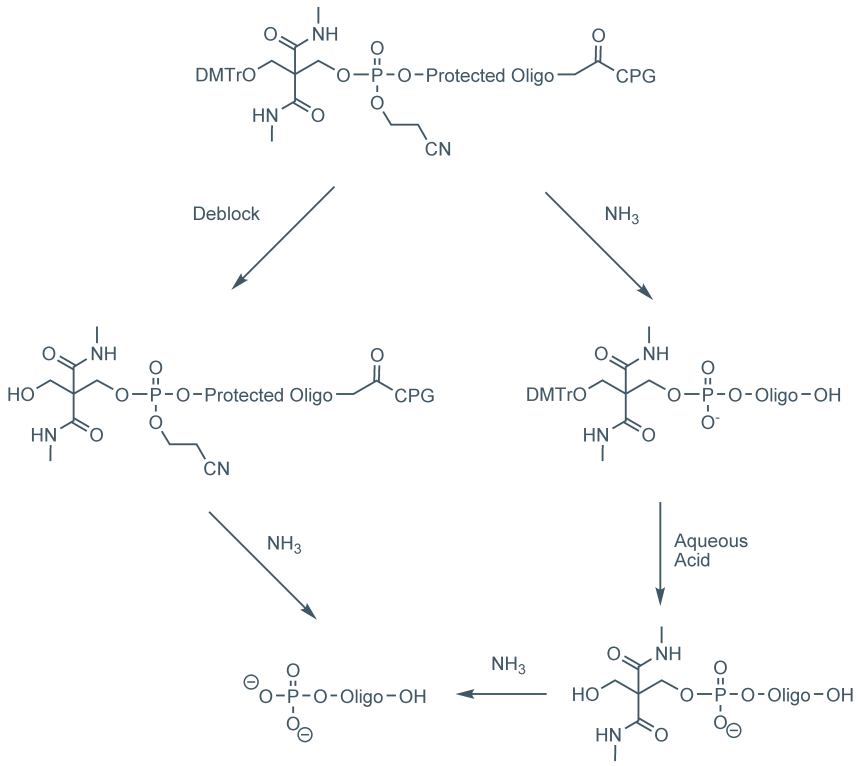
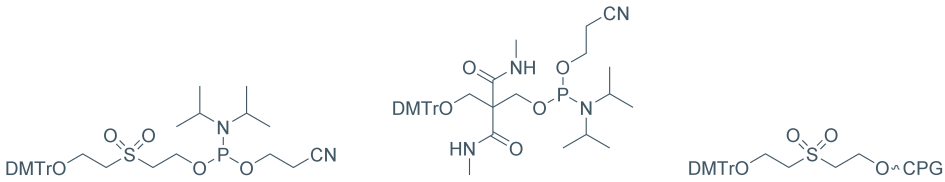


Figure 14. 5'-Phosphorylation using *solidCPR*™.

The presence of the methylamides in **2127** protects the modification from ß-elimination reactions until the base hydrolysis during deprotection. This can therefore be used in conjunction with Fmoc or levulinyl protected branching monomers (*e.g.* **2150**, see page 94) without forming the phosphate moiety until the deprotection step. **2101** is not protected against ß-elimination and would form the phosphate moiety during deprotection of the branching point. Hence if this is used at the 3' end it would cleave the oligo from the support (as would **2279**/**2389**).



2101 **2127** **2279/2398**

Ordering Chemical Phosphorylation Reagents

Product	Pack Size	Cat. No.
CPR (Phosphate-ON)	100µmol	2101-F100
	250mg	2101-B250
solidCPR™	100µmol	2127-F100
	250mg	2127-B250
3'-Phosphate SynBase™ CPG 1000/110	100mg	2279-B100
	1g	2279-C001
ALL-FIT Columns	4 x 0.2µmol	2279-P001
	10 x 0.2µmol	2279-P002
	4 x 1µmol	2279-P010
	10 x 1µmol	2279-P008

Product	Pack Size	Cat. No.
MerMade Columns	4 x 0.2µmol	2279-P015
	10 x 0.2µmol	2279-P016
	4 x 1µmol	2279-P026
	10 x 1µmol	2279-P022
3'-Phosphate SynBase™ CPG 3000/110	100mg	2398-B100
	1g	2398-C001
ALL-FIT Columns	4 x 0.2µmol	2398-P001
	10 x 0.2µmol	2398-P002
MerMade Columns	4 x 0.2µmol	2398-P015
	10 x 0.2µmol	2398-P016

Applications of Modified Oligonucleotides

Modified oligonucleotides are now being used in many applications, most notably diagnostics and therapeutics.

Introduction

As we have seen in the preceding section, modifying an oligonucleotide enables the development of diagnostic tests, therapeutics, detection methods and genetic analysis tools. Whilst there is a multitude of applications such as gene synthesis, genetic profiling, biosensors, cosmetics and agriculture, the two main sectors utilising modified oligonucleotides are diagnostics and therapeutics.

Diagnostics

Introduction

The diagnostics sector is the fastest growing sector in the oligonucleotide market. This is primarily driven by the advances in terms of detection methods, particularly with respect to qPCR and sequencing techniques. These improvements in turn demand the need for new and improved detection labels such as fluorophores or electrochemical labels to fine tune these techniques to give higher sensitivity and selectivity.

There is a vast array of oligonucleotide related diagnostic tests but, with the exception of one or two techniques, they fall into one of three categories: fluorescence (*e.g.* probe based qPCR); electrochemical (*e.g.* CombiMatrix microarrays); or colourimetric detection (*e.g.* ELISA assay).

Fluorescence Detection

Introduction

Dye-labelled oligonucleotides have many important biochemical and analytical uses. For certain applications, such as DNA Sanger sequencing and *in situ* hybridisation (*e.g.* FISH), oligos are required to be singly labelled. Subsequent detection and analysis rely on the fluorescent properties of the dye, most of which emit light in the visible spectrum.

Other types of oligonucleotide - *e.g.* probes for Real-time quantification of DNA and RNA[152] (Fluorophore-Quencher (FQ) probes) and allele discrimination[153] (Molecular Beacons™) - are doubly labelled, one dye acting as a fluorophore, the other as a quencher.

Where a fluorophore/quencher pair is used in such applications dynamic quenching occurs *via* either FRET (Fluorescence Resonance Energy Transfer) or by collisional quenching. For

152 **Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems**, S.A. Bustin, J. Molecular *Endocrinology*, **29**, 23-39, 2002.

153 **Multiplex detection of single-nucleotide variations using molecular beacons**, S.A.E. Marras, F.R. Kramer and S. Tyagi, *Genetic Analysis*, **14**, 151-156, 1999.

the most part, the mechanism is dependent on the quencher. For example, BHQ®s are FRET quenchers, whereas dabcyl works *via* collision. The latter is thought to work independently of the extinction coefficient, whereas in FRET a high extinction coefficient is important. This is thought to be the reason for the high quenching efficiency of BHQ®s.

Consideration must also be given to the overlap of the donor emission and acceptor absorption spectra. It is crucial that this is effective to generate efficient quenching.

Regardless of the quenching mechanism, the result is essentially the same. A dual-labelled probe (Taqman Probes™, Molecular Beacons™, Scorpion Primers™ *etc*) hybridise to the amplicon formed during PCR and the fluorophore becomes separated from the quencher. This mechanism is dependent on the probe type but in general either the probe opens, increasing the distance between the fluorophore and quencher such that quenching no longer occurs (Molecular Beacons™), or the fluorophore (or quencher) is cleaved from the probe (Taqman Probes™) (most common).

The fluorophore/quencher pair of choice is dependent on the emission wavelength of the fluorophore, *i.e.* the detection signal required. However, there are cases where there is a requirement to modify the emission wavelength of the fluorophore. Typically a combination of FAM/ROX/quencher (*e.g.* DDQ-I) is used. In this case there are two donor-acceptor interactions. FAM/ROX shifts the wavelength of the signal and the quencher acts as the acceptor for the FAM/ROX emission.

While the use of a quencher in this type of application is the most widely used, FRET studies are known where two fluorophores are used.[154] This is often applied in structural studies, *e.g.* RNA/proteins[155], although it has been shown that FRET signal is dependent on the position of the dyes in the oligo.[156]

This section gives an overview of the dye labelled products available from LINK that can be used in such applications.

Fluorophores

TAMRA Labelling

This is the most commonly used rhodamine dye in oligonucleotide based applications. The fluorescent properties of TAMRA *are* sometimes used in oligonucleotide labelling, however TAMRA is more often used as a quencher (see page 85).

Fluorescein Labelling

There are several ways of labelling an oligonucleotide with fluorescein-type dyes. The choice of label is diverse, depending on the degree of chlorination of the aromatic rings. This determines the fluorescence emission of the dye. 5'-Fluorescein-CE Phosphoramidite (6-FAM) (**2134**), derived from the single isomer 6-carboxyfluorescein, 5'-Hexachloro-fluorescein-CE Phosphoramidite (HEX) (**2136**) and 5'-Tetrachlorofluorescein-CE Phosphoramidite (TET) (**2137**), can all be used to efficiently label an oligonucleotide at the 5'-end.

There are two other phosphoramidites available from LINK that can be used for labelling an oligonucleotide with fluorescein. While both 6-Fluorescein-CE Phosphoramidite (**2139**) and Fluorescein-CE Phosphoramidite (**2148**) incorporate the same fluorescent dye as **2134**, the linking backbone differs. **2139** has a 1,3-diol structure, where the additional OH is protected with DMTr. This not only allows coupling efficiency monitoring by DMTr release, it allows the possibility of multiple additions within the oligo for use in, *e.g.* chromosome painting. However, this often requires a linker (*e.g.* spacer-18 **2129**) to be incorporated between each addition to prevent self-quenching of fluorescein. In the same way spacer-C3 (**2113**) is used to

154 **Spectroscopic investigation of a FRET molecular beacon containing two fluorophores for probing DNA/RNA sequences**, S. Jockusch, A.A. Marti, N.J. Turro, Z. Li, X. Li, J. Ju, N. Stevens and D.L. Akins, *Photochemical & Photobiological Sciences*, **5**, 493-498, 2006.

155 **Single-Molecule Observation of the Induction of k-Turn RNA Structure on Binding L7Ae Protein**, J. Wang, T. Fessl, K.T. Schroeder, J. Ouellet, Y. Liu, A.D.J. Freeman and D.M.J. Lilley, *Biophysical Journal*, **103**, 2541–2548, 2012.

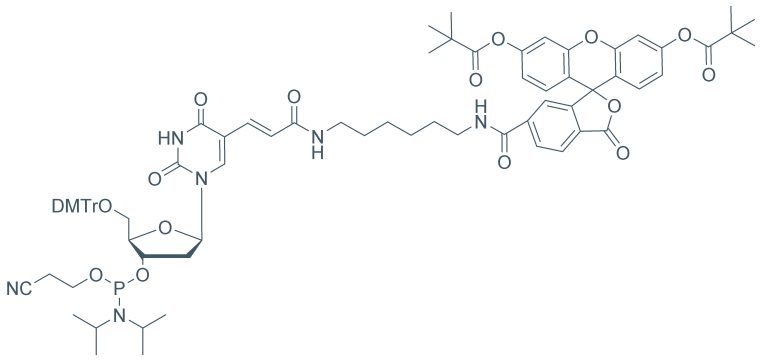
156 **Orientation dependence in fluorescent energy transfer between Cy3 and Cy5 terminally attached to double-stranded nucleic acids**, A. Iqbal, S. Arslan, B. Okumus, T.J. Wilson, G. Giraud, D.G. Norman, T. Ha and D.M. J. Lilley, *PNAS*, **105**, 11176-11181, 2008.

mimic the distance between the 3' and 5'-O of dR, the 1,3-diol arrangement of **2139** provides the same scenario. It must be noted that, as with spacer-C3, a distortion of the backbone occurs, particularly with multiple incorporations. As with all 5'-DMTr protected (or pseudo 5' species), the DMTr group can be used to aid purification.

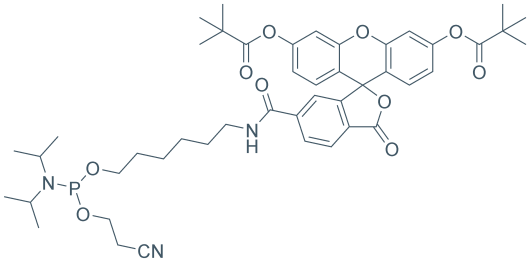
Fluorescein-CE Phosphoramidite (**2148**) offers the same possibilities as **2139** but in this case the linker is attached to the fluorescein *via* a thiourea linkage. This mimics the original method of incorporating fluorescein to an amino-modified oligo. It must be noted however that the linkage is attached *via* the 5 position of the ring system in this case.

Internal sequence additions of Fluorescein are achieved using Fluorescein-dT-CE Phosphoramidite (**2068**), by substituting any suitable dT residue. Again, multiple additions can be carried out but the spacing between each fluorescein-dT is crucial to prevent self-quenching.

Labelling the 3'-end of an oligo with fluorescein can be achieved using one of four available supports. 3'-Fluorescein CPG (**2359**), based on the 5-isomer of the substituted fluorescein, and 3'-(6-Fluorescein) CPG (**2368**), prepared from 6-FAM, are commonly chosen for this purpose. In addition, we offer 3'-(6-FAM) CPG (**2366**) and Fluorescein-dT CPG (**2370**), which are also derived from 6-carboxy fluorescein. **2366** also allows the effective blockage of the 3'-terminus from polymerase extension, as well as exonuclease activity. **2370** allows both of these activities to proceed. Cleavage and deprotection, typically with ammonium hydroxide, liberates the fluorescein-labelled oligo when using any of these supports.



2068



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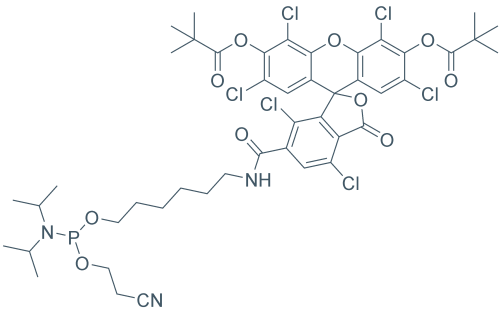

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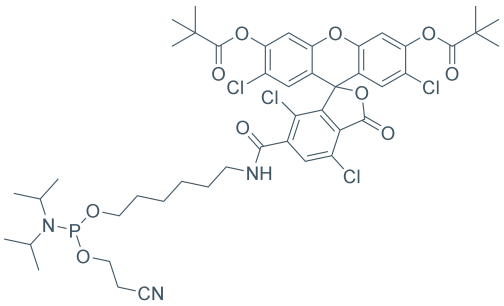

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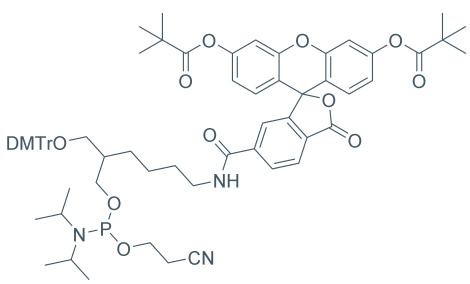

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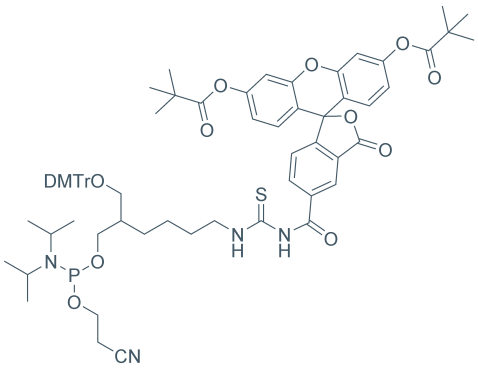

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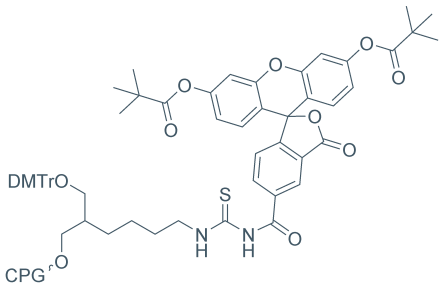

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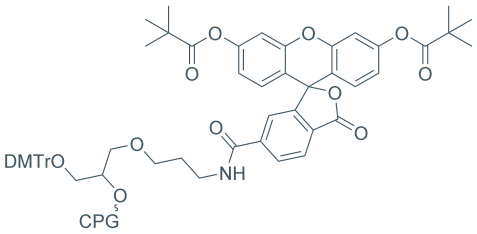

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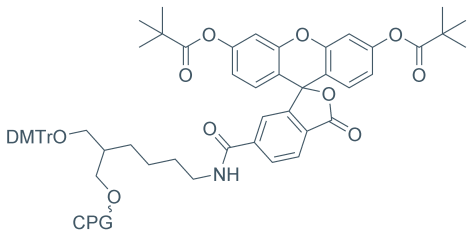

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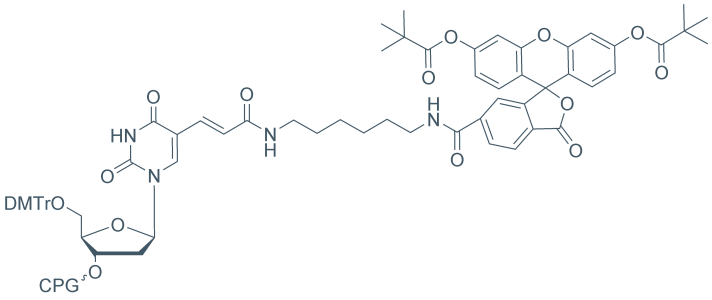


2370

Ordering Fluorescein Labelling Reagents

Product	Pack Size	Cat. No.
5'-Fluorescein-CE Phosphoramidite (6-FAM)	50µmol	2134-F050
	100µmol	2134-F100
	250mg	2134-B250
3'-(6-FAM) CPG	100mg	2366-B100
	1g	2366-C001
ALL-FIT Columns	4 x 0.2µmol	2366-P001
	10 x 0.2µmol	2366-P002
	4 x 1µmol	2366-P010
	10 x 1µmol	2366-P008
MerMade Columns	4 x 0.2µmol	2366-P015
	10 x 0.2µmol	2366-P016
	4 x 1µmol	2366-P026
	10 x 1µmol	2366-P022
5'-Hexachloro-Fluorescein-CE Phosphoramidite (HEX)	50µmol	2136-F050
	100µmol	2136-F100
	250mg	2136-B250
5'-Tetrachloro-Fluorescein-CE Phosphoramidite (TET)	50µmol	2137-F050
	100µmol	2137-F100
	250mg	2137-B250
Fluorescein-CE Phosphoramidite	50µmol	2148-F050
	100µmol	2148-F100
	250mg	2148-B250

Product	Pack Size	Cat. No.
3'-Fluorescein CPG	100mg	2359-B100
	1g	2359-C001
ALL-FIT Columns	4 x 0.2µmol	2359-P001
	10 x 0.2µmol	2359-P002
	4 x 1µmol	2359-P010
	10 x 1µmol	2359-P008
6-Fluorescein-CE Phosphoramidite	50µmol	2139-F050
	100µmol	2139-F100
	250mg	2139-B250
3'-(6-Fluorescein) CPG	100mg	2368-B100
	1g	2368-C001
ALL-FIT Columns	4 x 0.2µmol	2368-P001
	10 x 0.2µmol	2368-P002
	4 x 1µmol	2368-P010
	10 x 1µmol	2368-P008
Fluorescein-dT-CE Phosphoramidite	50µmol	2068-F050
	100µmol	2068-F100
	250mg	2068-B250
3'-Fluorescein-dT CPG	100mg	2370-B100
	1g	2370-C001
ALL-FIT Columns	4 x 0.2µmol	2370-P001
	10 x 0.2µmol	2370-P002
	4 x 1µmol	2370-P010
	10 x 1µmol	2370-P008

Cyanine Dyes (including Quasar® Dyes)

Cyanine-based dyes have been used for many years in areas such as textiles. Their application in nucleic acid labelling[157] increased firstly with their commercial availability as succinimidyl esters, then latterly as phosphoramidites. Today they are a central part of many diagnostic platforms and assays based on fluorophore labelling and detection.

Cyanine dyes are used as fluorescent markers in oligonucleotide synthesis,[158] primarily for molecular diagnostics such as the preparation of probes used in monitoring real-time PCR, fluorescence *in situ* hybridisation (FISH) and in Surface-Enhanced Resonance Raman Spectroscopy (SERRS) based DNA detection assays. Their emission spectra can be tuned by altering the length of the polymethine chain and solubility in organic or aqueous solvents can be altered *via* the substituents on the aromatic ring.

The most commonly used phosphoramidite dyes are Cyanine-3 (**2520**) and Cyanine-5 (**2521**). These are generally attached to the 5'-end of an oligonucleotide during synthesis, however are easily incorporated within the sequence. The MMT-protected hydroxyl group is removed in the same way as DMTr protection. Internal incorporation is not common due to the lack of heterocyclic base in their structure and as such they do not have the ability to participate in base pairing. This destabilises any duplexes formed.

For 3'-attachment, we have introduced the equivalent 3'-modified 1000Å CPG supports, 3'-Cyanine-3 (**2412**) and 3'-Cyanine-5 (**2413**). Previously this was done by adding the dye post-synthetically onto an amino-modified oligonucleotide or by adding the amidite to a support functionalised with a modification that will not interfere with the use of the oligonucleotide (*e.g.* phosphate, spacer). 3'-Labelling is particularly useful in FRET where the FRET partner is incorporated either at the 5'-end or within the oligonucleotide sequence.[159]

Quasar® dyes 570 and 670 (available as phosphoramidites **2158** and **2159** respectively) are fluorescent indocarbocyanines, which fluoresce in the yellow-orange (**2158**) and red (**2159**) regions of the visible spectrum. Both dyes are directly analogous in application to the common cyanine (Cy™) dyes, Quasar® 570 for cyanine-3/Cy™3 and Quasar® 670 for cyanine-5/Cy™5 in the labelling of fluorescent probes. The dye phosphoramidites are used directly in automated oligo synthesis. Note, unlike **2520** and **2521**, Quasar® dyes do not have the ability to be added internally within an oligo sequence. Both Quasar® 570 and 670 are quenched by BHQ®-2 (see page 87).

CAL Fluor® Dyes

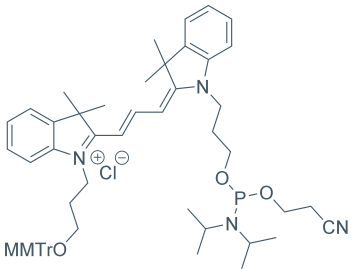
CAL Fluor® Dyes are a set of fluorescent dyes specifically designed for qPCR instruments. These novel xanthene fluorophores can replace previous dyes as a lower-cost alternative. The dyes can be efficiently manufactured, and remain stable to the conditions of oligo synthesis and work up. The attachment chemistry linking the CAL Fluor® Dyes to biomolecules eliminates the problem of multiple isomers. This results in dye labels that are easier to manufacture, have a single RP-HPLC peak and have well-defined emission spectra.

CAL Fluor® Dyes are available as CPGs and phosphoramidites, allowing facile incorporation during oligo synthesis, which, as we have noted with other products, results in more efficient label incorporation and fewer purification steps than post-synthesis labelling. The CAL Fluor® Dyes have emission maxima from 520nm to 635nm and can be paired with BHQ®-1 or BHQ®-2 for efficient quenching in a variety of probe formats.

157 **Molecular Probes Based on Cyanine Dyes for Nucleic Acid Research**, T.G. Deligeorgiev, p125, in *Near Infrared Dyes for High Technology Applications*, Ed. S. Daehne, U. Resch-Genger and O. Wolfbeis, NATO ASI Series Publ., Kluwer Academic, 1998.

158 **Fluorescence based strategies for genetic analysis**, R.T. Ranasinghe and T. Brown, *Chem. Commun.*, 5487-5502, 2005.

159 **Fluorescence resonance energy transfer in near-infrared fluorescent oligonucleotide probes for detecting protein-DNA interactions**, S. Zhang, V. Metelev, D. Tabatadze, P.C. Zamecnik, A. Bogdanov Jr, *Proc. Natl. Acad. Sci. USA*, **105**, 4156-4161, 2008.



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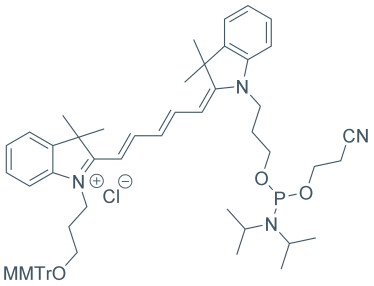

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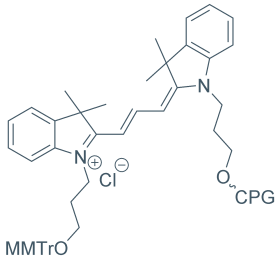

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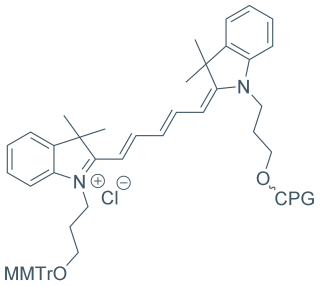

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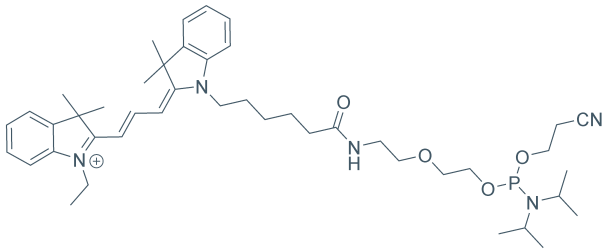

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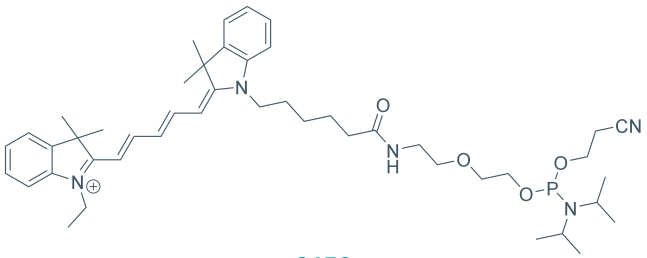


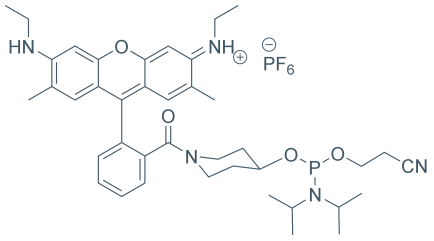
2159

Ordering Cyanine Dyes (including Quasar® Dyes)

Product	Pack Size	Cat. No.
Cyanine-3-CE Phosphoramidite	50µmol	2520-F050
	100µmol	2520-F100
Cyanine-5-CE Phosphoramidite	50µmol	2521-F050
	100µmol	2521-F100
3'-Cyanine-3 SynBase CPG 1000	100mg	2412-B100
	1g	2412-C001
3'-Cyanine-5 SynBase CPG 1000	100mg	2413-B100
	1g	2413-C001

Product	Pack Size	Cat. No.
Quasar® 570-CE Phosphoramidite	100µmol	2158-F100
	250mg	2158-B250
Quasar® 670-CE Phosphoramidite	100µmol	2159-F100
	250mg	2159-B250

All cyanine products require to be shipped on ice.



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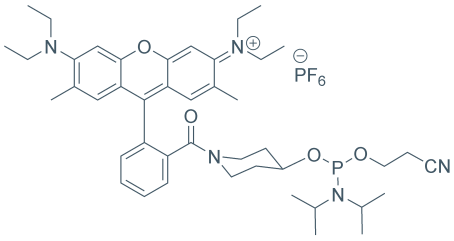

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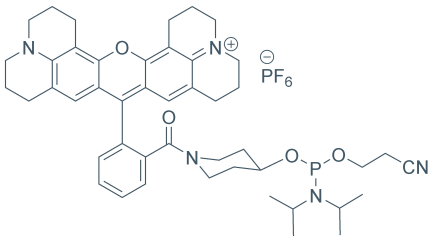

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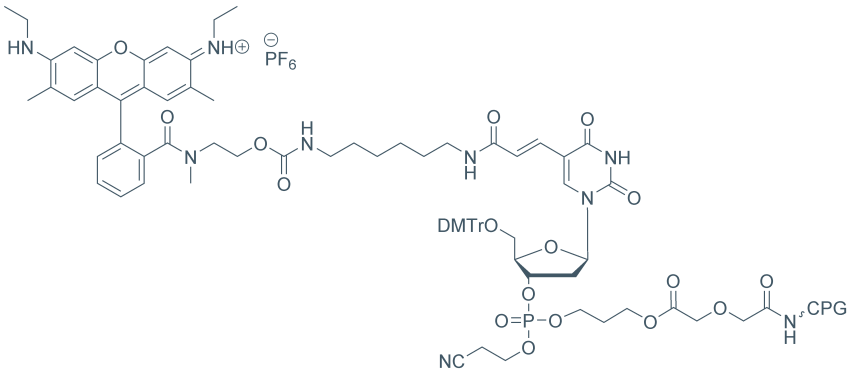

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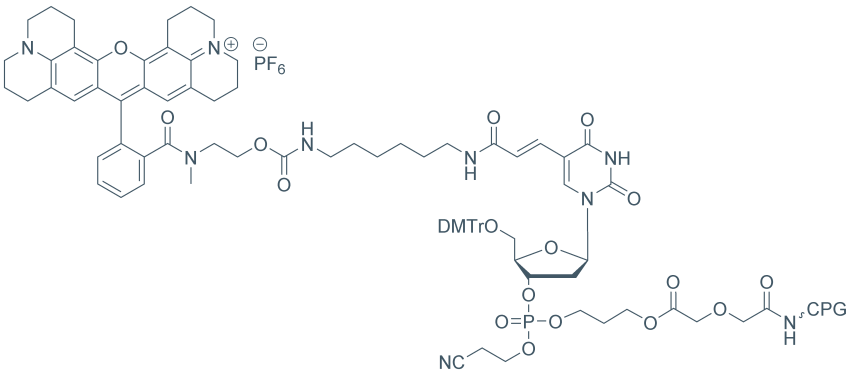


2424

Ordering CAL Fluor® Dyes

Product	Pack Size	Cat. No.
CAL Fluor® Orange 560-CE Phosphoramidite	50µmol	2538-F050
	100µmol	2538-F100
CAL Fluor® Red 590-CE Phosphoramidite	50µmol	2539-F050
	100µmol	2539-F100
CAL Fluor® Red 610-CE Phosphoramidite	50µmol	2540-F050
	100µmol	2540-F100

Product	Pack Size	Cat. No.
CAL Fluor® Orange 560 CPG 500	100mg	2423-B100
	1g	2423-C001
CAL Fluor® Red 610 CPG 500	100mg	2424-B100
	1g	2424-C001

All CAL Fluor® products require to be shipped on ice.

Table 5. Dye Selection Chart. Note the dyes are listed in order of absorbance maxima; the colour scale is used only as a pictorial representation. Dyes in bold are available from LINK.

Fluorophore	Abs. Max. (nm)	Em. Max. (nm)
Cy5.5™	675	694
Alexa 647	650	668
Quasar® 670 / Cyanine-5 (Cy5™)	647	667
BODIPY® 650/665-X	646	660
BODIPY® 630/650-X	625	640
CAL Fluor® Red 635	618	637
BODIPY® TR-X/Alexa 594	590	617
CAL Fluor® Red 610	590	610
Cy3.5™	581	596
BODIPY® 581/591	581	591
Redmond Red™	580	594
Texas Red X/Alexa 568	578	603
ROX	575	602
CAL Fluor® Red 590	569	591
BODIPY® 564/570	563	569
Alexa 546	556	573
TAMRA/Rhodamine Red-X	555	580
Alexa 555	555	565
Quasar® 570	548	566
BODIPY® TMR/ **Cyanine-3** (Cy™3)	544	570
CAL Fluor® Orange 560	538	559

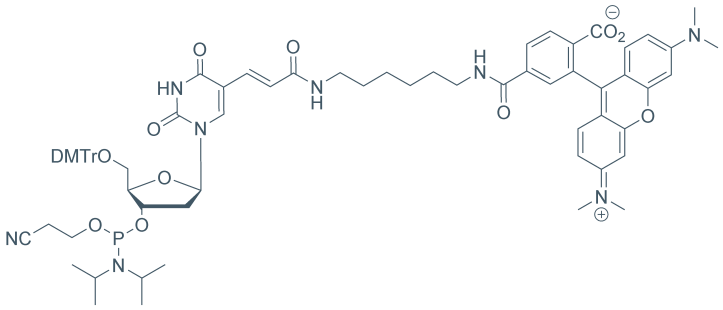
Fluorophore	Abs. Max. (nm)	Em. Max. (nm)
HEX	535	556
Alexa 532/VIC/ BODIPY® 530/550	532	554
Yakima Yellow™	531	549
Rhodamine 6G	528	550
CAL Fluor® Gold 540	522	544
TET	521	536
JOE	520	548
Oregon Green® 514	506	526
Rhodamine Green-X	503	528
BODIPY® FL	502	513
Alexa 488	495	519
6-FAM	494	525
BODIPY® 493/503	493	503
Cy2™	489	506
Pulsar® 650	460	650
Alexa 430	433	539
Coumarin	432	472
Pacific Blue	416	451
Acridine	362	462
Marina Blue	362	459
Alexa 350	346	442
Edans	336	468
Fluorescein/ DANSYL	335	518

Quenchers

TAMRA Labelling

The light-absorbing properties of TAMRA, and spectral overlap with several commonly used fluorophores - including FAM, HEX, TET and JOE, make it useful as a quencher for the design of dual-labelled probes. The usefulness of TAMRA is, however, limited because of its broad emission spectrum, which reduces its capabilities in multiplexing. Its intrinsic fluorescence contributes to the background signal, potentially reducing the sensitivity of assays based on TAMRA. Despite these limitations, TAMRA has been used extensively in the design of probe-based assays, perhaps most notably in Taqman™ probes for Real-Time PCR.

Oligonucleotides can be labelled with TAMRA using two distinct methodologies. TAMRA is not sufficiently stable to strong bases; the molecule degrades in the presence of ammonium hydroxide. If this deprotection is required, the oligonucleotide is synthesised with an amino group at either the 3'- (most common), or 5'-end and labelled with TAMRA post-synthetically using TAMRA-NHS Ester (**0251**). Oligonucleotides synthesised using mild deprotection monomers can be labelled directly with TAMRA, either internally by substituting any suitable dT residue with TAMRA-dT-CE Phosphoramidite (**2143**), or at the 3'-end using 3'-TAMRA CPG support (**2372**). Subsequent deprotection of the oligo is achieved with ^{t}butylamine/methanol/water (1:1:2) for 2.5h at 70˚C. Although there is still a small amount of TAMRA degradation, this is easily removed during purification. As previously mentioned, TAMRA is also a fluorophore and, although one of the most widely used quenchers, applications often require the use of a dark quencher.



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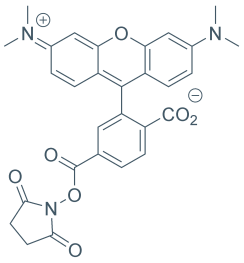
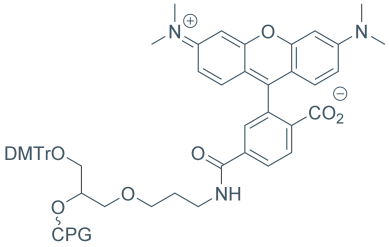


0251



2372

Ordering TAMRA Labelling Reagents

Product	Pack Size	Cat. No.
TAMRA-dT-CE Phosphoramidite	50µmol	2143-F050
	100µmol	2143-F100
TAMRA NHS Ester (0.17M in DMSO)	60µl	0251-M060

Product	Pack Size	Cat. No.
3'-TAMRA CPG	100mg	2372-B100
	1g	2372-C001
ALL-FIT Columns	4 x 0.2µmol	2372-P001
	10 x 0.2µmol	2372-P002
	4 x 1µmol	2372-P010
	10 x 1µmol	2372-P008

Non-Fluorescent (Dark) Quenchers

Dabcyl Labelling

Dabcyl, because of its light absorbance properties and lack of residual fluorescence, has been widely used as a quencher in diagnostic probes such as Molecular Beacons™.[160] A Molecular Beacon™ is a hybridisation probe consisting of a fluorophore, a quencher and a defined section of the oligonucleotide sequence complementary to that of the target nucleic acid. In the inactive state, when the probe is not hybridised to its target sequence, the fluorescence energy of the fluorophore is transferred to the quencher by a process of collisional quenching. For light energy transfer to take place efficiently, both fluorophore and quencher have to be in close proximity. This requirement is accounted for in the design of Molecular Beacons™ in that the two parts of the stem hybridise to hold the F/Q pair in close proximity. Hybridisation of the Molecular Beacon™ probe to its target sequence results in the separation of the stem and hence the F/Q pair, resulting in fluorescence.

Since dabcyl is stable to oligo synthesis it can be incorporated at any point in the sequence *via* one of Link's modifiers: at the 5' end using 5'-Dabcyl-CE Phosphoramidite (**2085**) - *e.g.* for use in TwistAmp™ fpg probes; at the 3'-end using 3'-Dabcyl CPG (**2374**) - *e.g.* for Taqman™ probes, duplex Scorpions™ and Molecular Beacons™); or internally using Dabcyl-dT-CE Phosphoramidite (**2144**) - *e.g.* in Scorpion Primers™. Deprotection of the oligo is dependent on the F/Q pair but in general is as per unmodified oligos.

Dabcyl's absorption properties limit the range of dyes it can quench to those emitting at 400-550nm (absorption maximum, 471nm). However, when used in Molecular Beacons™, the

160 **Molecular beacons: probes that fluoresce upon hybridisation**, S. Tyagi and F.R. Kramer, *Nature Biotechnology*, **14**, 303-308, 1996.

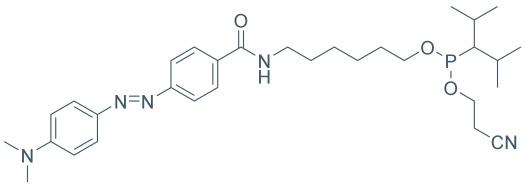
fluorophore and dabcyl are brought close enough to allow a slightly broader spectrum of dyes to be quenched, thereby increasing the versatility of the dabcyl molecule.

Although dabcyl was originally the quencher of choice, for the most part this has been superceded by Black Hole Quenchers® (BHQ®s).

Black Hole Quenchers®

The demands of modern genomic and diagnostic applications, which are typically centred around an ever increasing need for greater assay sensitivity, has led to the development of a series of new non-fluorescent quenchers. Some of the best known of these are the Black Hole Quenchers® (BHQ®s) that have been specifically optimised for FRET-based quenching.

Due to the high extinction co-efficiency and the broad spectral overlap covered by each BHQ®, the efficiency of quenching is increased, when compared to molecules like dabcyl. This in turn means that BHQ®s provide access to a much larger range of wavelengths for detection purposes, covering visible into near IR regions of the spectrum (480-730nm). Coupling this with the fact that these molecules have no residual background fluorescence (they are true dark quenchers) makes BHQ®s a favourable choice for RT-PCR applications. Of the four



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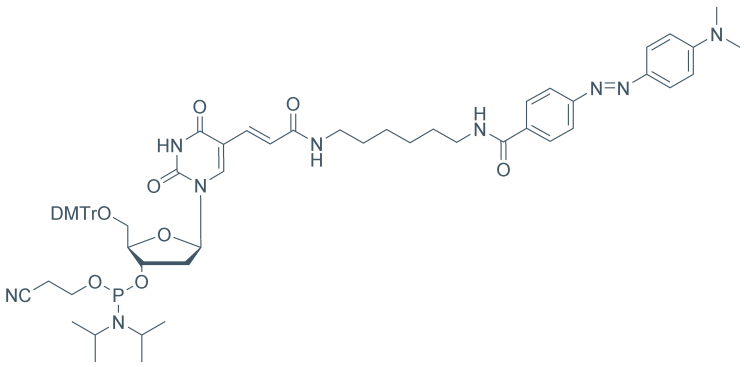

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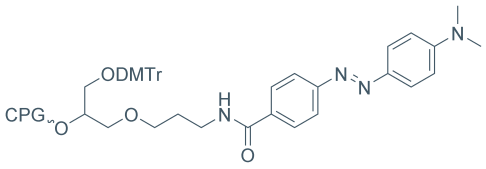


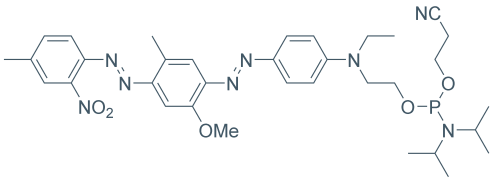
2374

Ordering Dabcyl Labelling Reagents

Product	Pack Size	Cat. No.
5'-Dabcyl-CE Phosphoramidite	50µmol	2085-F050
	100µmol	2085-F100
	250mg	2085-B250
Dabcyl-dT-CE Phosphoramidite	50µmol	2144-F050
	100µmol	2144-F100
	250mg	2144-B250

Product	Pack Size	Cat. No.
3'-Dabcyl CPG	100mg	2374-B100
	1g	2374-C001
ALL-FIT Columns	4 x 0.2µmol	2374-P001
	10 x 0.2µmol	2374-P002
	4 x 1µmol	2374-P010
	10 x 1µmol	2374-P008
MerMade Columns	4 x 0.2µmol	2374-P015
	10 x 0.2µmol	2374-P016
	4 x 1µmol	2374-P026
	10 x 1µmol	2374-P022

original BHQ®s available, BHQ®-1 and BHQ®-2 are currently available from LINK, either as the 5'-Phosphoramidites (items **2154** and **2155** respectively), the dT-Phosphoramidites (**2156** and **2157**) or the 3'-CPGs (**2379** and **2380**). Only considering the excitation and emission values suggests Cy5™, Cyanine-5 and Quasar® 670 require BHQ®-3 for efficient quenching, however BHQ®-2 is recommended because it is less susceptible to degradation. BHQ®-1 is typically used to quench in the range 480-580nm and can be used in conjunction with the commonly used fluorophores; *e.g.* FAM, TET, JOE and HEX. BHQ®-2 is used to quench in the range 550-650nm and is most effective in quenching fluorophores such as TAMRA, ROX, Cyanine-3, Cy3™, Cy3.5™ and Red 640. Each of the available BHQ® phosphoramidites and CPGs are used directly in automated synthesis.



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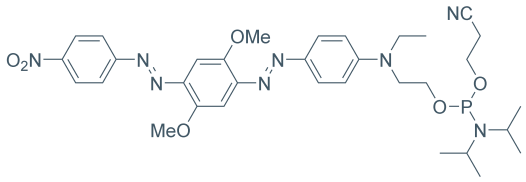

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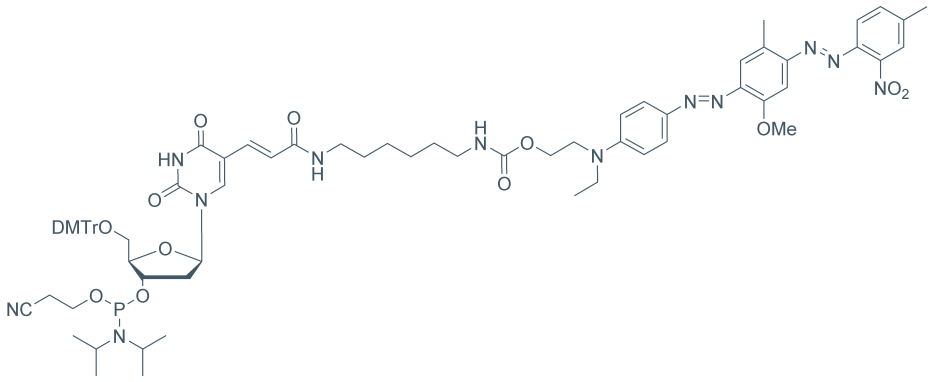

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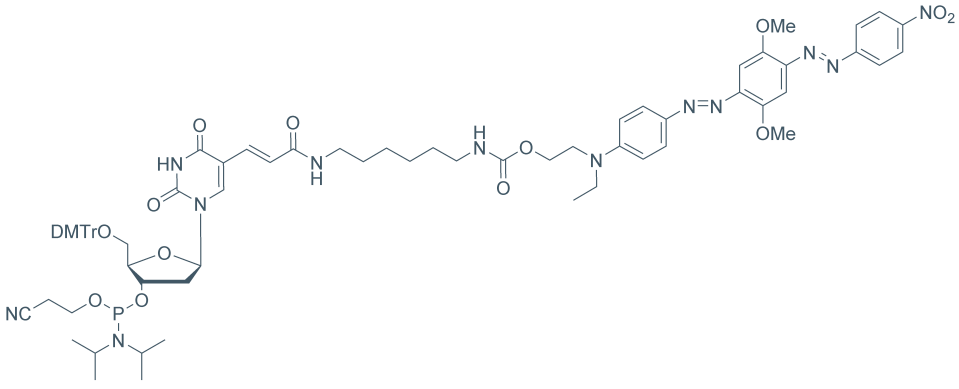

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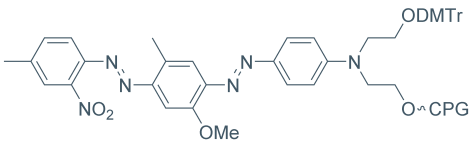

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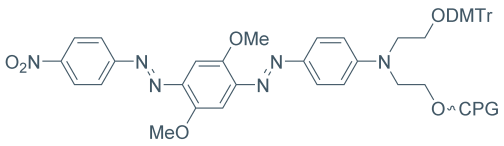


2380

Ordering Black Hole Quenchers®

Product	Pack Size	Cat. No.
5'-BHQ®-1-CE Phosphoramidite	50µmol	2154-F050
	100µmol	2154-F100
	250mg	2154-B250
5'-BHQ®-2-CE Phosphoramidite	50µmol	2155-F050
	100µmol	2155-F100
	250mg	2155-B250
BHQ®-1-dT-CE Phosphoramidite	50µmol	2156-F050
	100µmol	2156-F100
	250mg	2156-B250
BHQ®-2-dT-CE Phosphoramidite	50µmol	2157-F050
	100µmol	2157-F100
	250mg	2157-B250

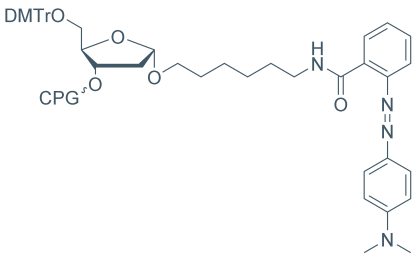
Product	Pack Size	Cat. No.
3'-BHQ®-1 CPG	100mg	2379-B100
	1g	2379-C001
ALL-FIT Columns	4 x 0.2µmol	2379-P001
	10 x 0.2µmol	2379-P002
	4 x 1µmol	2379-P010
	10 x 1µmol	2379-P008
3'-BHQ®-2 CPG	100mg	2380-B100
	1g	2380-C001
ALL-FIT Columns	4 x 0.2µmol	2380-P001
	10 x 0.2µmol	2380-P002
	4 x 1µmol	2380-P010
	10 x 1µmol	2380-P008

All BHQ® products require to be shipped on ice.

Deep Dark Quencher 1

Deep Dark Quencher 1 is a non-fluorescent molecule quenching the shorter wavelength dyes such as FAM. As such, its quenching properties are very similar to that of dabcyl (see Figure 15 below). This modification is available as a 3'-modifier CPG 1000Å support (**2349**).

In this case the quencher is attached to the anomeric position of dRibose. This removes the possibility of losing the label during deprotection, a problem often associated with dabcyl due to the 1,2-diol configuration. Additionally, incorporation of DDQ-1 results in preservation of the natural sugar-phosphate backbone meaning there is no adverse effect on the structure of the oligo.



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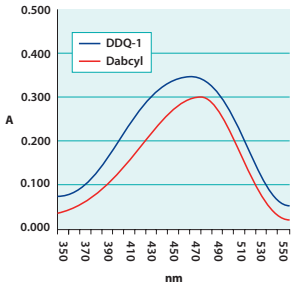

Figure 15. Comparative UV absorption spectra of 3'-Dabcyl– and 3'-DDQ-1- labelled oligonucleotides (Dabcyl λ_{max} 471nm, DDQ λ_{max} 473nm).

Ordering Deep Dark Quencher 1

Product	Pack Size	Cat. No.
3'-DDQ-1 SynBase™ CPG 1000/110	100mg	2349-B100
	1g	2349-C001
ALL-FIT Columns	4 x 0.2µmol	2349-P001
	10 x 0.2µmol	2349-P002
	4 x 1µmol	2349-P010
	10 x 1µmol	2349-P008

Table 6. Dye/Quencher Selection Chart. Note the dyes are listed in order of emission maxima; the colour scale is used only as a pictorial representation. Dyes and quenchers in bold are available from LINK.

Fluorophore	Abs. Max. (nm)	Em. Max. (nm)	Quencher
Cy5.5™	675	694	
Alexa 647	650	668	
Quasar® 670/Cyanine-5 (Cy5™)	647	667	
BODIPY® 650/665-X	646	660	
Pulsar® 650	460	650	
BODIPY® 630/650-X	625	640	
CAL Fluor® Red 635	618	637	
BODIPY® TR-X/Alexa 594	590	617	
CAL Fluor® Red 610	590	610	
Texas Red X/Alexa 568	578	603	
ROX	575	602	**BHQ®-2** 559-670 Abs. Max. 579nm
Cy3.5™	581	596	
Redmond Red™	580	594	
BODIPY® 581/591	581	591	
CAL Fluor® Red 590	569	591	
TAMRA/Rhodamine Red-X	555	580	
Alexa 546	556	573	
BODIPY® TMR / **Cyanine-5** (Cy3™)	544	570	
BODIPY® 564/570	563	569	
Quasar® 570	548	566	
Alexa 555	555	565	
CAL Fluor® Orange 560	538	559	
HEX	535	556	
Alexa 532/VIC/BODIPY® 530/550	532	554	
Rhodamine 6G	528	550	**BHQ®-1** 480-580 Abs. Max. 534nm
Yakima Yellow™	531	549	
JOE	520	548	
CAL Fluor® Gold 540	522	544	
Alexa 430	433	539	
TET	521	536	
Rhodamine Green-X	503	528	
Oregon Green® 514	506	526	
6-FAM	494	525	
Alexa 488	495	519	
Fluorescein/DANSYL	335	518	
BODIPY® FL	502	513	
Cy2™	489	506	
BODIPY® 493/503	493	503	
Coumarin	432	472	**Dabcyl/ DDQ-1** 400-550 Abs. Max. 479/410nm
Edans	336	468	
Acridine	362	462	
Marina Blue	362	459	
Pacific Blue	416	451	
Alexa 350	346	442	

Colourimetric Detection & Capture

Colourimetric detection is one of the oldest diagnostic techniques. This is based on the interaction of an enzyme *e.g.* HRP interacting with a substrate. This also requires some form of capture of the target with a hapten labelled probe and the duplex captured using an affinity column or matrix loaded with a suitable protein or antibody. Examples of haptens are biotin, DNP and DIG, the most commonly used being biotin in conjunction with streptavidin or avidin. The enzyme labelled oligo then hybridises to another part of the immobilised target and treatment with the substrate produces a distinctive colour.

Biotin Labelling

The uses of avidin-biotin technology are diverse.[161] Applications include the detection of proteins by non-radioactive immunoassays, cytochemical staining, cell separation, isolation of nucleic acids, detection of specific DNA/RNA sequences by hybridisation, and probing of conformational changes in ion channels.

Many of these applications require the use of oligos containing biotin at one or more positions. The availability of functional biotin, in turn, provides the opportunity for immobilisation on pre-coated solid surfaces.[162] An extension of this technology using the photocleavable biotin product (**2122**) is described on page 66.

Several different reagents are available for labelling nucleic acids with biotin. Choosing the right one will depend largely on the position within the oligonucleotide requiring to be labelled. Biotin-CE Phosphoramidite (**2140**) is based on a 1,3-diol structure where one hydroxyl is protected with DMTr and the other is the phosphoramidite, hence it can be used for adding multiple biotins to either the 3', or 5' end of an oligonucleotide. It has been suggested that this property could be exploited in the development of diagnostic probes, in applications such as ELISA, in which signal amplification is often beneficial. This has been shown using *in situ* hybridisation studies where three biotins at either end of the oligo gives the optimal signal.[163]

Biotin-TEG-CE Phosphoramidite (**2132**) can be used in a similar way to **2140** for adding biotin to the 3'- and 5'- ends of an oligo. This phosphoramidite also has an extended 15 atom mixed polarity spacer arm based on a triethylene glycol linker. The benefits of an extended spacer arm separating the biotin function from the rest of the oligo may be seen in applications where possible steric hindrance effects could be reduced as a result, *e.g.* when dual-labelling with bulky reporter molecules, such as haptens, dyes, or enzymes. Note the 1,2-diol arrangement makes cleavage during deprotection possible therefore it is advisable to keep the 5'-DMTr group on until after deprotection.

5'-Biotin-CE Phosphoramidite (**2109**) can also be used for adding biotin to an oligo, but only to the 5'-end.[164] The DMTr protection on the N1 of biotin prevents branching during coupling. The DMTr group can, however, be used to assist in reverse-phase cartridge and HPLC purification although biotin is hydrophobic enough to obtain good separation of biotin labelled oligos (DMT OFF) and unlabelled oligos.

The addition of biotin internally within an oligonucleotide sequence is achieved using Biotin-dT-CE Phosphoramidite (**2067**), where any suitable dT position within the sequence can be replaced with biotin-dT. The tert-butylbenzoyl group, used to increase solubility and to protect

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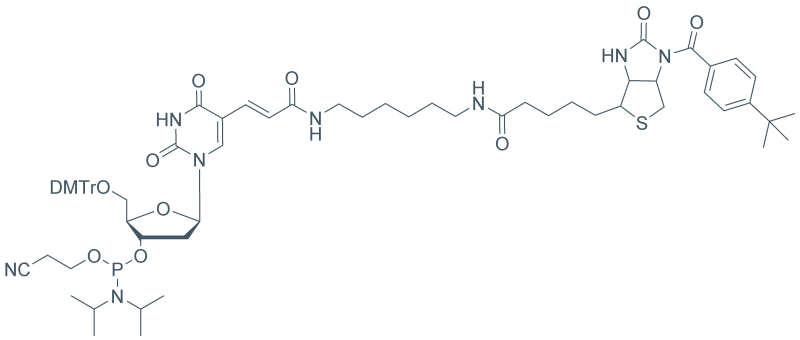
161 See for example: (a) **Avidin-Biotin Technology**, M. Wilchek and E.A. Bayer (Eds.), in *Methods in Enzymology*, J.N. Abelson and M.I. Simon (Series Eds.), Volume 184, 671pp, Academic Press, 1990; (b) **The biotin-(strept)avidin system: Principles and applications in biotechnology**, E.P. Diamandis and T.K. Christopoulos, *Clinical Chem.*, **37**, 625-636, 1991.

162 See for example: **Electrochemical detection of non-labelled oligonucleotide DNA using biotin-modified DNA(ss) on a streptavidin-modified gold electrode**, J.W. Park, H.-Y. Lee, J.M. Kim, R. Yamasaki, T. Kanno, H. Tanaka, H. Tanaka and T. Kawai, *J. Bioscience and Bioengineering*, **97**, 29-32, 2004.

163 **A comparative study of digoxigenin, 2,4-dinitrophenyl, and alkaline phosphatase as deoxyoligonucleotide labels in non-radioisotopic *in situ* hybridisation**, S.J. Harper, E. Bailey, C.M. McKeen, A.S. Stewart, J.H. Pringle, J. Feeholly and T. Brown, *J. Clinical Pathology*, **50**, 686-690, 1997.

164 For a recent diagnostic application see: **Detection and differentiation of Plasmodium species by polymerase chain reaction and colorimetric detection in blood sample of patients with suspected malaria**, D.M. Whitely, G.M. LeCornec, A. Baddeley, J. Savill, M.W. Syrmis, I.M. Mackay, D.J. Siebert, D. Burns, M. Nissen and T.P. Sloots, *Parasitology*, **49**, 25-29, 2004.



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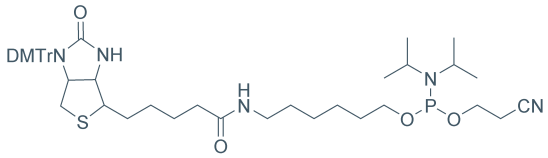
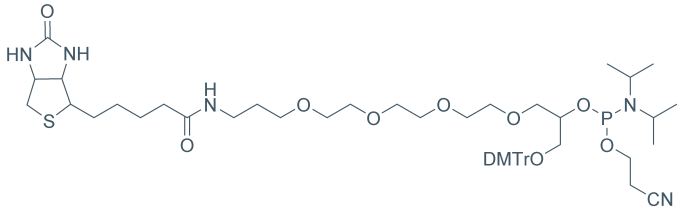


2109



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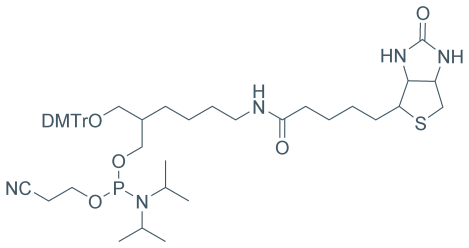
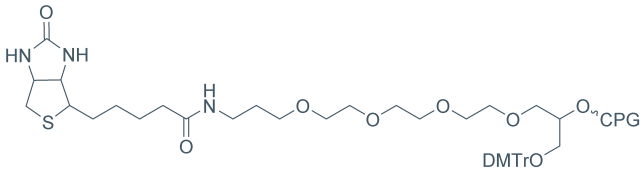


2140



2353

Ordering Biotin Labelling Reagents

Product	Pack Size	Cat. No.
Biotin-CE Phosphoramidite	50µmol	2140-F050
	100µmol	2140-F100
	250mg	2140-B250
Biotin-TEG-CE Phosphoramidite	50µmol	2132-F050
	100µmol	2132-F100
	250mg	2132-B250
3'-Biotin-TEG CPG	100mg	2353-B100
	1g	2353-C001
ALL-FIT Columns	4 x 0.2µmol	2353-P001
	10 x 0.2µmol	2353-P002
	4 x 1µmol	2353-P010
	10 x 1µmol	2353-P008

Product	Pack Size	Cat. No.
Biotin-dT-CE Phosphoramidite	50µmol	2067-F050
	100µmol	2067-F100
	250mg	2067-B250
5'-Biotin-CE Phosphoramidite	50µmol	2109-F050
	100µmol	2109-F100
	250mg	2109-B250

the biotin, is removed in the ammonium hydroxide deprotection step.

Finally, the direct labelling of the 3'-end of an oligonucleotide sequence with biotin is also possible and is routinely achieved using 3'-Biotin-TEG CPG (**2353**), which incorporates biotin at the first step in the synthesis process.

Electrochemical Detection

Ferrocene Labelling

Ferrocene (Fc) and its derivatives are attractive electrochemical probes for nucleic acid analysis because of their stability and convenient synthetic chemistry. Early examples of Fc labelling have utilised the conjugation of carboxy-Fc to 5'-amino-modified oligos.[165] Internal post-synthetic labelling of DNA probes has been obtained by reaction with ferrocenecarboxaldehyde or aminoferrocene.[166] For direct incorporation into oligonucleotides, Fc phosphoramidites[167] and monomers with a ferrocenyl moiety linked to position 5 of 2'-dU[168(a)] and dC[147(a,b)] or the 2' sugar position of dA and dC[169] have been described, as has on column derivatisation of I-dU with ferrocenyl propargylamide.[170] Methods using redox tagging have also been employed.[171]

Recently Brisset and co-workers[172] have described the synthesis and use of abasic Fc-modified phosphoramidites, including the preparation of Fc-modified phosphorothioates[173] however, to our knowledge, these are not commercially available. In any case, reported coupling efficiencies and oligo synthesis yields are relatively low.

To provide a robust phosphoramidite for direct incorporation into oligos we chose a structure (item **2167**) analogous to our current dT products (amino, dabcyl, biotin, fluorescein *etc*). This both simplifies its synthesis and imparts the benefits of having a nucleobasic structure consistent with natural DNA-sugar-phosphate backbone. Further, as the Fc-modification is on the 5-position of the pyrimidine, natural base-pairing to dA will still occur.

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165 (a) **Electrochemically active DNA probes: Detection of target DNA sequences at femtomole level by high-performance liquid chromatography with electrochemical detection**, S. Takenaka, Y. Uto, H. Kondo, T. Ihara and M. Takagi, *Anal. Biochem.*, **218**, 436-443, 1994; (b) **Ferrocene-oligonucleotide conjugates for electrochemical probing of DNA**, T. Ihara, Y. Maruo, S. Takenaka and M. Takagi, *Nucleic Acids Research*, **24**, 4273-4280, 1996; (c) **Electrochemical analysis of DNA amplified by the polymerase chain reaction with a ferrocenylated oligonucleotide**, Y. Uto, H. Kondo, M. Abe, T. Suzuki and S. Takenaka, *Anal. Biochem.*, **250**, 122-124, 1997.

166 **Electrochemical detection of sequence-specific DNA using a DNA probe labelled with aminoferrocene and chitosan modified electrode immobilized with ssDNA**, C. Xu, H. Cai, P. He and Y. Fang, *Analyst*, **126**, 62-65, 2001.

167 (a) M. Wiessler and D. Schutte, European Patent WO9709337 (1997); (b) T.Chunlin, US Patent Application US2009/0155795 A1 (2009).

168 (a) **Uridine-conjugated ferrocene DNA oligonucleotides: Unexpected cyclization reaction of the uridine base**, C.J. Yu, H. Yowanto, Y. Wan, T.J. Meade, Y. Chong, M. Strong, L.H. Donilon, J.F. Kayyem, M. Gozin and G.F. Blackburn, *J. Amer, Chem. Soc.*, **122**, 6767-6768, 2000; (b) **Ferrocene-modified pyrimidine nucleosides: synthesis, structure and electrochemistry**, H. Song, X. Li, Y. Long, G. Schatte and H.-B. Kraatz, *Dalton Trans.*, 4696-4701, 2006.

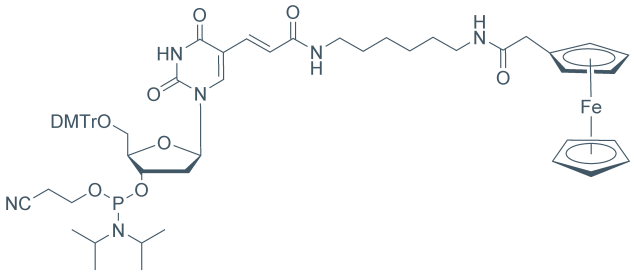
169 **2'-Ribose-ferrocene oligonucleotides for electronic detection of nucleic acids**, C.J. Yu, H. Wang, Y. Wan, H. Yowanto, J.C. Kim, L.H. Donilon, C. Tao, M. Strong and Y. Chong, *J. Org. Chem.*, **66**, 2937-2942, 2001.

170 **On-column derivatization of oligodeoxynucleotides with ferrocene**, A.E. Beilstein and M.W. Grinstaff, *Chem. Commun.*, 509-510, 2000.

171 (a) **Synthesis of the first ferrocene-labelled dideoxynucleotide and its use for the 3'-redox end-labelling of 5'-modified single-stranded oligonucleotides**, A. Anne, B. Blanc and J. Moiroux, *Bioconjug. Chem.*, **12**, 396-405, 2001; (b) **Ferrocene conjugates of dUTP for enzymatic redox labelling of DNA**, W.A Wlassoff and G.C. King, *Nucleic Acids Research*, **30**, e58, 2002.

172 (a) **Automated synthesis of new ferrocenyl-modified oligonucleotides: study of their properties in solution**, A.E. Navarro, N. Spinelli, C. Moustrou, C. Chaix, B. Mandrand and H. Brisset, *Nucleic Acids Research*, **32**, 5310-5319, 2004; (b) **Supported synthesis of ferrocene modified oligonucleotides as new electroactive DNA probes**, A.-E. Navarro, N. Spinelli, C. Chaix, C. Moustrou, B. Mandrand and H. Brisset, *Bioorg. Med. Chem. Lett.*, **14**, 2439-2441, 2004; (c) C. Chaix-Bauvais *et al*, US Patent Application US2005/0038234 A1 (2005).

173 **The first automated synthesis of ferrocene-labelled phosphorothioate DNA probe: A new potential tool for the fabrication of microarrays**, H. Brisset, A.-E. Navarro, N. Spinelli, C. Chaix and B. Mandrand, *Biotechnol. J.*, **1**, 95-98, 2006.



2167

Ordering Electrochemical Labelling Reagent

Product	Pack Size	Cat. No.
Ferrocene-dT-CE Phosphoramidite	100µmol	2167-F100
	250mg	2167-B250

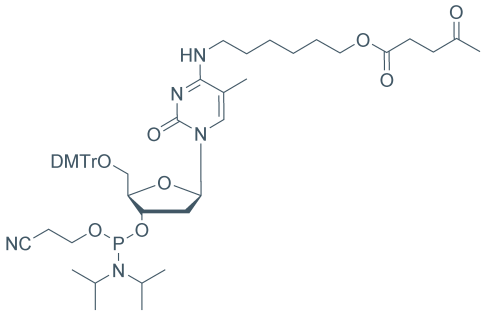
Branching Modification

Branched DNA (bDNA) has become a significant tool in diagnostics research and, in particular, gene expression analysis.[174] For example, branching possibilities can be exploited to achieve multiplicity of labelled probe hybridisation to target sequences leading to enhanced signals. 5-Me-dC-Brancher CE Phosphoramidite (**2150**)[175] has been designed to provide a facile route to incorporate branching capability into an oligonucleotide.

The Me-dC Brancher is a 5'-trityl-protected, 3'-phosphoramidite dT analogue, that can be incorporated into an oligonucleotide during synthesis. The levulinyl group on the branching chain is removed with buffered hydrazine at neutral pH—conditions that do not affect any other groups (*e.g.* it does not cleave from the support)—yet it does not degrade during

174 (a) **Nucleic Acid Detection Technologies—Labels, Strategies, and Formats**, L.J. Kricka, *Clinical Chemistry*, **45**, 453-458, 1999; (b) **Signal amplification through nucleotide extension and excision on a dendritic DNA platform**, S. Capaldi, R.C. Getts, and S.D. Jayasena, *Nucleic Acids Research*, **28**, e21, 2000.

175 (a) **Forks and combs and DNA: The synthesis of branched oligodeoxyribonucleotides**, T. Horn and M.S. Urdea, *Nucleic Acids Research*, **17**, 6959-6967, 1989; (b) **An improved divergent synthesis of comb-type branched oligodeoxyribonucleotides (bDNA) containing multiple secondary sequences**, T. Horn, C-A. Chang and M.S. Urdea, *Nucleic Acids Research*, **25**, 4835-4841, 1997; (c) **Chemical synthesis and characterization of branched oligodeoxynucleotides (bDNA) for use as signal amplifiers in nucleic acid quantification assays**, T. Horn, C-A. Chang and M.S. Urdea, *Nucleic Acids Research*, **25**, 4842-4849, 1997.



2150

Ordering Branching Modifier

Product	Pack Size	Cat. No.
5-Me-dC-Brancher-CE Phosphoramidite	100µmol	2150-F100
	250mg	2150-B250

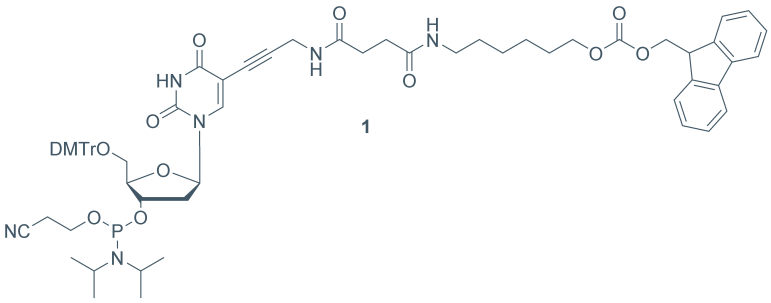
storage and synthesis, unlike the Fmoc protection used on other commercially available branching phosphoramidites. This product has the advantage of being nucleosidic, thereby preserving internucleotide distance, therefore perturbs DNA structure less than, for example, a non-nucleosidic doubler or trebler molecule.

It must be noted that, although visually this structure resembles Me-dC, the linker on the N-4 position results in hybridisation akin to dT. Therefore if hybridisation is required at the branching point, this modifier must replace a T base within the natural DNA sequence.

Use of Branching Me-dC CE Phosphoramidites to Incorporate Site Specific Modifiers

It is widely known that branching monomers are used in the preparation of oligonucleotide dendrimers[176] and **2150** has been used to generate comb and fork like oligonucleotide structures for use in nucleic acid hybridisation assay as a means of signal amplification.[175(a)]

However, it is also possible to incorporate reporter groups into specific sites within an oligonucleotide sequence using this monomer in an analogous manner to the method reported by Brown *et al.*[177] Here they used a branching dT phosphoramidite (1) to incorporate dyes such as Cyanine-5 (**2521**) within the sequence for use in real-time probes such as HyBeacon or Angler probes.



1

In this case 1 was incorporated within an oligonucleotide where the 5'-end is blocked either by retaining the DMT group, capping with acetyl protection or by the incorporation of a terminal modifier *e.g.* 6-FAM CE Phosphoramidite (**2134**). While keeping the oligonucleotide on the column, the Fmoc group is removed with 20% piperidine in MeCN or DMF and the cyanine dye phosphoramidite is added to the branching point under the same conditions as incorporation at the 5'-end. This is outlined in Figure 16 overleaf.

Although it is possible to incorporate **2521** within an oligonucleotide sequence, this results in a destabilised duplex whereas the use of the modified dT has no adverse effect. This is also true when **2150** is used in the same way. Although a Me-dC anaolgue, the presence of the branching chain on the N4 position of the pyrimidine results in this modifier having hybridisation properties akin to dT rather than dC hence is incorporated as a 'dT' position of the oligonucleotide sequence. In this case (see Figure 17 overleaf), the levulinyl protection is removed using 0.5M hydrazine hydrate in pyridine/acetic acid 1:1.

The use of such branching monomers opens up the possibility of incorporating modifiers only available as 5'-addition amidites internally within the sequence. For instance this gives a means of generating HEX-dT (2) using **2150** and **2136** or cholesteryl dT (3) using **2150** and **2170** as shown in Figure 18 on page 98. Neither of these dT modifiers are commercially available as amidites.

This can be particularly useful in evaluating which marker works best in a given application

176 (a) **Oligonucleotide dendrimers: Synthesis and use as polylabelled DNA probes**, M. S. Shchepinov, I. A. Udalova, A. J. Bridgman and E. M. Southern, *Nucleic Acids Research*, **25**, 4447-4454, 1997; (b) **Branched oligonucleotides induce *in vivo* gene conversion of a mutated EGFP reporter**, P. A. Olsen, C. McKeen and S. Krauss, *Gene Therapy*, **10**, 1830-1840, 2003.

177 **Synthesis of a modified thymidine monomer for site-specific incorporation of reporter groups into oligonucleotides**, L. J. Brown, J. P. May, T. Brown, *Tetrahedron Letters*, **42**, 2587-2591, 2001.

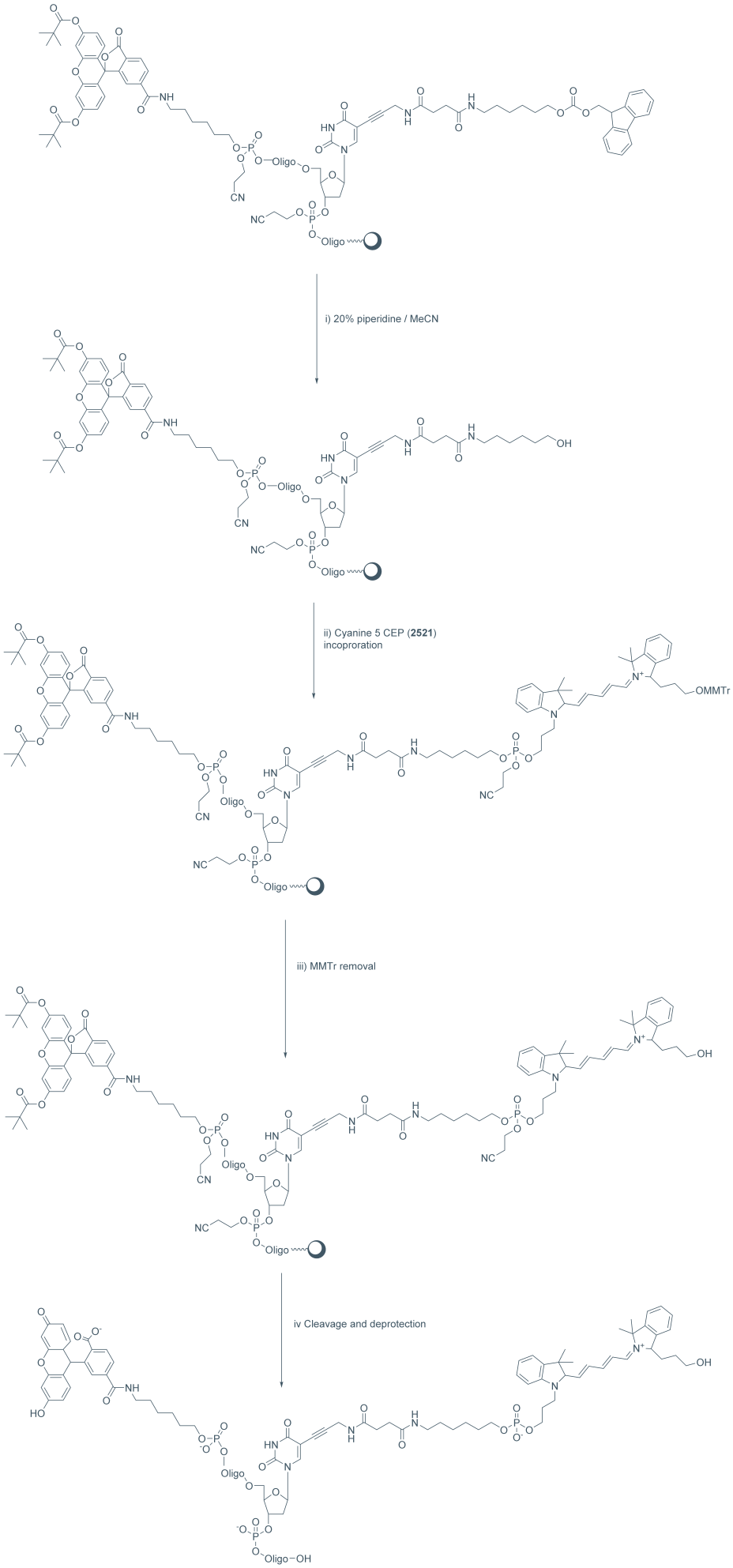


Figure 16. Use of Fmoc protected branching dT amidite for incorporation of cyanine-5.

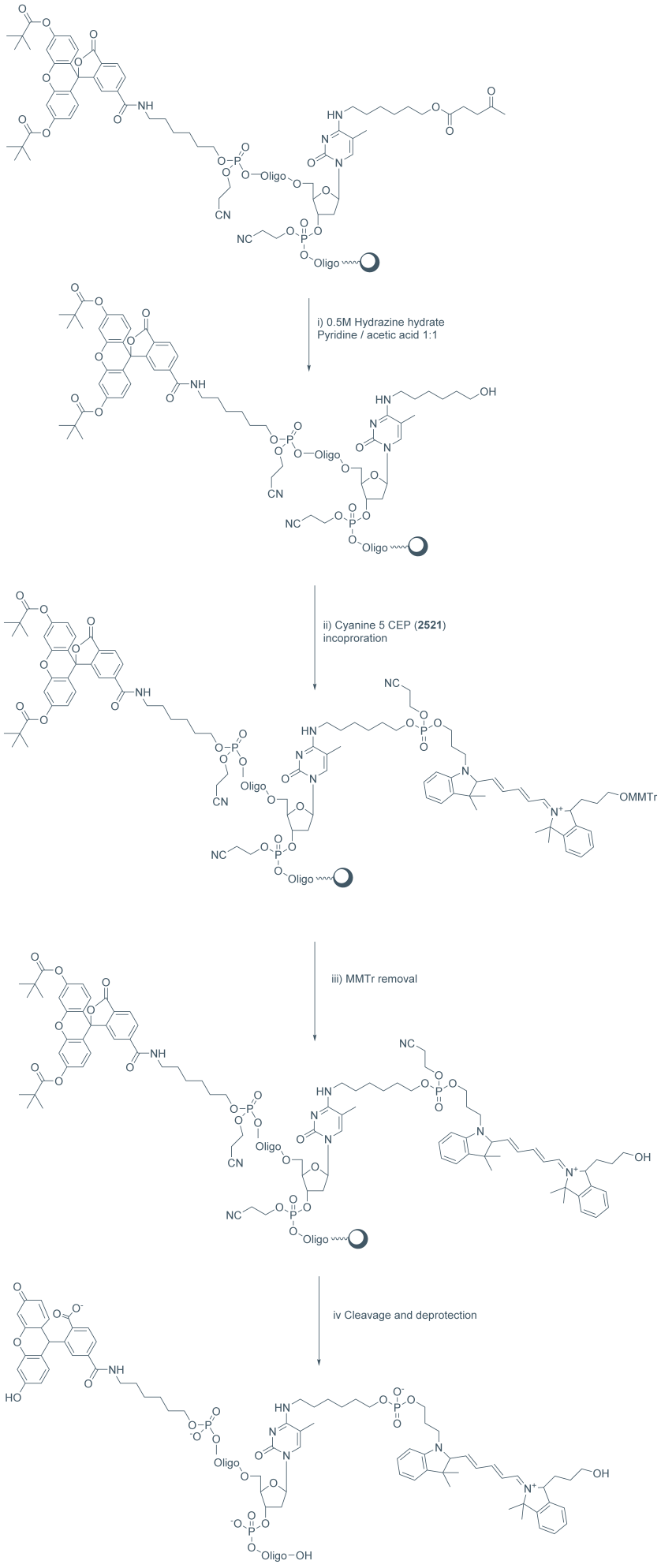


Figure 17. Use of Levulinyl protected branching Me-dC amidite for incorporation of cyanine-5.

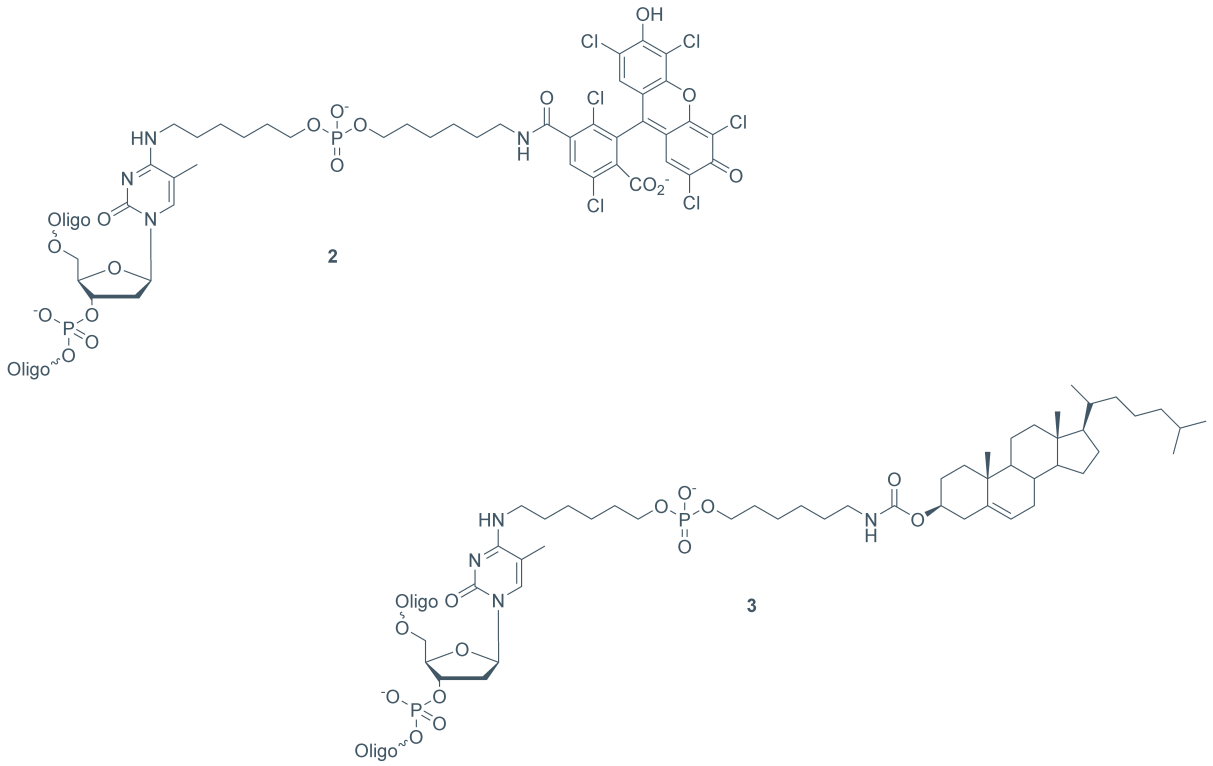


Figure 18. Generation of HEX dT (2) and cholesteryl dT (3) within an oligonucleotide sequence using 2150.

without the expense of synthesising a range of modified dT amidites to get the same result. Using this information, the preferred modified dT amidite can be synthesised with the peace of mind of knowing that the resulting oligonucleotide will give the desired result when used in an assay.

Although originally **2150** was designed for the preparation of highly branched oligonucleotides at LINK we see the potential of this product to allow the incorporation of modified dT bases not commercially available within the sequence.

Therapeutics

Introduction

Oligonucleotide therapeutics[178] is a broad term which actually covers a number of modes of action of similarly structured molecules. Therefore, whilst the design and construction of the oligos is often similar, there are several ways in which therapeutic effects can be induced. These include antisense, anti-miRs, aptamers,[179] DNAzymes & ribozymes, exon skipping, siRNA, transcription factor decoys (TFD) and immunostimulatory effects. Currently, the main focus in terms of oligonucleotide therapeutics is on antisense and siRNA technologies.

Antisense Therapy

The concept underlying antisense technology is relatively straightforward: the use of a sequence, complementary by virtue of base pair hybridization, to a specific mRNA can inhibit its expression and then induce a blockade in the transfer of genetic information from DNA to protein.[180] The development of antisense oligonucleotide technologies as therapeutics agents in recent years led to the first FDA approval for the commercialization of an antisense oligonucleotide, Vitravene (for cytomegalovirus retinitis[181]), and to numerous clinical trials of therapeutic oligonucleotides.[182]

Phosphorothioate oligonucleotides (see page 60) were the first modified oligos to be used in antisense applications (*e.g.* Vitravene). Their endonuclease resistance and their RNase H activity make them suitable candidates for this purpose, for both RNase H directed and steric block antisense applications.

However, where the oligonucleotide has a high degree of phosphorothiolation, non-specific binding is known to occur.[183] There is also the issue where binding efficiency to RNA is much lower than that of DNA. In spite of this, full phosphorothioate and part-phosphorothioate oligos are still used in the development of antisense oligonucleotide therapeutics.

In order to resolve these issues base, sugar and other phosphate modifications have been developed. These "second-generation" oligonucleotides are resistant to degradation by cellular nucleases (see page 70) and hybridize specifically to their target mRNA with higher affinity than the isosequential phosphodiester or phosphorothioate. However, such antisense effects result from RNase H-independent mechanisms.

In this respect the most common oligonucleotide modification involves use of 2-O-methyl groups (see page 70). These oligonucleotides form high melting heteroduplexes with targeted

178 **Therapeutic Oligonucleotides**, *RSC Biomolecular Sciences*, Ed. J. Kurreck, 2008, ISBN 978-0-85404-116-9.

179 **Aptamers as therapeutics**, A.D. Keefe, S. Pai and A. Ellington, *Nature Reviews Drug Discovery*, **9**, 537-550, 2010.

180 **Antisense Oligonucleotides: Basic Concepts and Mechanisms**, N. Dias and C. A. Stein, *Mol. Cancer Ther.*, **1**, 347, 2002.

181 (a) **Technology evaluation: fomivirsen. Isis Pharmaceuticals Inc/CIBA vision**, R.M. Orr, *Curr. Opin. Mol. Ther.*, **3**, 288 –294, 2001; (b) **Fomivirsen approved for CMV retinitis**, B. Roehr, *J. Int. Assoc. Physicians AIDS Care*, **4**, 14 –16, 1998.

182 **Antisense therapeutics: is it as simple as complementary base recognition?**, S. Agrawal and E.R. Kandimalla, *Mol. Med. Today*, **6**, 72 –81, 2000.

183 (a) **Mac-1 (CD11b/CD18) is an oligodeoxynucleotide-binding protein**, L. Benimetskaya, J.D. Loike, Z. Khaled, G. Loike, S.C. Silverstein, L. Cao, J. el Khoury, T.Q. Cai and C.A. Stein, *Nat. Med.*, **3**, 414 –420,1997; (b) **Controversies in the cellular pharmacology of oligodeoxynucleotides**, C.A. Stein, *Ciba Found. Symp.*, **209**, 79 –89, 1997; (c) **Phosphorothioate oligodeoxynucleotides bind to basic fibroblast growth factor, inhibit its binding to cell surface receptors, and remove it from low affinity binding sites on extracellular matrix**, M.A. Guvakova, L.A. Yakubov, I. Vlodavsky, J.L. Tonkinson and C.A. Stein, *J. Biol. Chem.*, **270**, 2620 –2627, 1995; (d) **Inhibition of high affinity basic fibroblast growth factor binding by oligonucleotides**, S.M. Fennewald and R.F. Rando. *J. Biol. Chem.*, **270**, 21718 –21721, 1995.

mRNA[184] and induce an antisense effect by a non-RNase H-dependent mechanism[185], *i.e. via* a steric blocking mechanism.

Stable oligos have also been produced that do not possess the natural phosphate-ribose backbone. PNAs (see page 53) have an uncharged, flexible, polyamide backbone comprised of repeating N-(2-aminoethyl)glycine units to which the bases are attached. These oligomers can form very stable duplexes or triplexes with nucleic acids: single or double-strand DNA or RNA.[186] The property of high-affinity nucleic acid binding can be explained by the lack of electrostatic repulsion because of the absence of negative charges on the PNA oligomers. Because PNAs are not substrates for the RNase H or other RNases, the antisense mechanism of PNAs depends on steric hindrance. PNAs can also bind to DNA and inhibit RNA polymerase initiation and elongation,[187] as well as the binding and action of transcription factors.[188] PNAs can also bind mRNA and inhibit splicing[189] or translation initiation and elongation.[190]

Although such modifications in their own right have proved efficient in terms of antisense applications, it is the combination of these modifiers, including the use of phosphorothioate linkages, which have given the most dramatic improvements. Specificity, as well as efficacy, can be increased by using a chimeric oligonucleotide, in which the RNase H-competent segment, usually a phosphorothioate moiety, is flanked on one or both termini by a higher-affinity region of modified RNA,[191] frequently 2'-O-alkyloligoribonucleotides. This substitution not only increases the affinity of the oligonucleotide for its target but reduces the cleavage of

184 **Evaluation of 2'-modified oligonucleotides containing 2'-deoxy gaps as antisense inhibitors of gene expression**, B.P. Monia, E.A. Lesnik, C. Gonzalez, W.F. Lima, D. McGee, C.J. Guinosso, A.M. Kawasaki, P.D. Cook and S.M. Freier, *J. Biol. Chem.*, **268**, 14514 –14522, 1993.

185 **2'-O-(2-Methoxy)ethyl-modified anti-intercellular adhesion molecule 1 (ICAM-1) oligonucleotides selectively increase the ICAM-1 mRNA level and inhibit formation of the ICAM-1 translation initiation complex in human umbilical vein endothelial cells**, B.F. Baker, S.S. Lot, T.P. Condon, S. Cheng-Flournoy, E.A. Lesnik, H.M. Sasmor and C.F. Bennett, *J. Biol. Chem.*, **272**, 11994 –12000, 1997.

186 (a) **Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide**, P.E. Nielsen, M. Egholm, R.H. Berg and O. Buchardt, *Science*, **254**, 1497 –1500, 1991; (b) **Kinetics for hybridization of peptide nucleic acids (PNA) with DNA and RNA studied with the BIAcore technique**, K.K. Jensen, H. Orum, P.E. Nielsen and B. Norden. *Biochemistry*, **36**, 5072 –5077, 1997.

187 **Effects in live cells of a c-myc anti-gene PNA linked to a nuclear localization signal**, G. Cutrona, E.M. Carpaneto, M. Ulivi, S. Roncella, O. Landt, M. Ferrarini and L.C. Boffa, *Nat. Biotechnol.*, **18**, 300 –303, 2000; (b) **Invasion of the CAG triplet repeats by a complementary peptide nucleic acid inhibits transcription of the androgen receptor and TATA-binding protein genes and correlates with refolding of an active nucleosome containing a unique AR gene sequence**, L.C. Boffa, P.L. Morris, E.M. Carpaneto, M. Louissaint and V.G. Allfrey, *J. Biol. Chem.*, **271**, 13228 –13233, 1996; (c) **Antisense and antigene properties of peptide nucleic acids**, J.C. Hanvey, N.J. Peffer, J.E. Bisi, S.A. Thomson, R. Cadilla, J.A. Josey, D.J. Ricca, C.F. Hassman, M.A. Bonham, K.G. Au *et al*, *Science*, **258**, 1481 –1485, 1992.

188 **Inhibition of NF-$_{\kappa}$B specific transcriptional activation by PNA strand invasion**, T.A. Vickers, M.C. Griffith, K. Ramasamy, L.M. Risen and S.M. Freier, *Nucleic Acids Research*, **23**, 3003 –3008, 1995.

189 **Peptide nucleic acids are potent modulators of endogenous pre-mRNA splicing of the murine interleukin-5 receptor-α chain**, J.G. Karras, M.A. Maier, T. Lu, A. Watt and M. Manoharan, *Biochemistry*, **40**, 7853 –7859, 2001.

190 (a) **Inhibition of promyelocytic leukemia (PML)/retinoic acid receptor-α and PML expression in acute promyelocytic leukemia cells by anti-PML peptide nucleic acid**, L. Mologni, E. Marchesi, P.E. Nielsen and C. Gambacorti-Passerini, *Cancer Res.*, **61**, 5468 –5473, 2001; (b) ***In vitro* transcriptional and translational block of the bcl-2 gene operated by peptide nucleic acid**, L. Mologni, P.E. Nielsen and C. Gambacorti-Passerini, *Biochem. Biophys. Res. Commun.*, **264**, 537 –543,1999; (c) **Antisense PNA tridecamers targeted to the coding region of Ha-ras mRNA arrest polypeptide chain elongation**, N. Dias, S. Dheur, P.E. Nielsen, S. Gryaznov, A. Van Aerschot, P. Herdewijn, C. Helene and T.E. Saison-Behmoaras, *J. Mol. Biol.*, **294**, 403 –416, 1999; (d) **Antisense inhibition of gene expression in bacteria by PNA targeted to mRNA**, L. Good and P.E. Nielsen, *Nat. Biotechnol.*, **16**, 355 –358, 1998; (e) ***In vitro* transcription and translation inhibition by anti-promyelocytic leukemia (PML)/retinoic acid receptor α and anti-PML peptide nucleic acid**, C. Gambacorti-Passerini, L. Mologni, C. Bertazzoli, P. le Coutre, E. Marchesi, F. Grignani and P.E. Nielsen, *Blood*, **88**, 1411 –1417, 1996.

191 (a) **Characterization of a potent and specific class of antisense oligonucleotide inhibitor of human protein kinase C-α expression**, R.A McKay, L.J. Miraglia, L.L. Cummins, S.R. Owens, H. Sasmor and N.M. Dean, *J. Biol. Chem.*, **274**, 1715 –1722, 1999; (b) **Selecting optimal oligonucleotide composition for maximal antisense effect following streptolysin O-mediated delivery into human leukaemia cells**, R.V. Giles, D.G. Spiller, J. Grzybowski, R.E. Clark, P. Nicklin and D.M. Tidd, *Nucleic Acids Research*, **26**, 1567 –1575, 1998.

nontargeted mRNAs by RNase H.[192]

Other examples of "second-generation" antisense oligonucleotides include phosphorodiamidate morpholino oligomers,[193] and N3'-P5' PN, which result from the replacement of the oxygen at the 3' position on ribose by an amine group.[194]

siRNA

Unlike antisense oligonucleotides, siRNA is a duplex made up of a sense (passenger) and antisense (guide) strands. In this case the mechanism of gene silencing is more complex. First the duplex loads onto the RNA-Induced Silencing Complex (RISC) where the strands separate. The antisense strand then guides sequence specific cleavage of the target mRNA with the protein Agronaute, the latter being the catalytic component of RISC.

Just like antisense oligonucleotides, it is important to build in nuclease resistance to the therapeutic. Similarly, high binding efficiency between the antisense strand and the target mRNA is highly desirable. As a consequence, the aforementioned modifiers developed to improve antisense technology are equally applicable to siRNA. Typical modifications are combinations of 2'- or sugar modified nucleosides such as 2'-OMe, 2'-F, LNA and phosphorothioate.

Cell Delivery and Uptake

Despite advances in oligonucleotide therapeutics, the main issues remain cell delivery and cellular uptake. A number of strategies have been developed to combat this, the most widely used being the conjugation of a 'delivery' reagent to the oligonucleotide. In general the reagent is hydrophobic in nature, *e.g.* cholesterol, and is often attached *via* a cleavable linker.

This is typically incorporated at the 5'-end of the oligo, and for siRNA is incorporated on the sense (passenger) strand.

Lipophilic Modification

The introduction of hydrophobic (lipophilic) residues into oligonucleotides with a view to improving their penetration into cells has recently met with some success.[195] Cholesteryl-conjugated oligonucleotides have in particular been the subject of substantial interest in antisense and other studies due to the lipophilicity and good availability of cholesterol. One such study[196] has shown the use of cholesteryl-modified siRNA in therapeutic gene silencing.

Historically this has been attached by post-synthetic conjugation of an amino-modified oligo

192 (a) **Mixed-backbone oligonucleotides as second generation antisense oligonucleotides: *in vitro* and *in vivo* studies**, S. Agrawal, Z. Jiang, Q. Zhao, D. Shaw, Q. Cai, A. Roskey, L. Channavajjala, C. Saxinger and R. Zhang, *Proc. Natl. Acad. Sci. USA*, **94**, 2620 –2625, 1997; (b) **Impact of mixed-backbone oligonucleotides on target binding affinity and target cleaving specificity and selectivity by Escherichia coli RNase H**, L.X. Shen, E.R. Kandimalla and S. Agrawal, *Bioorg. Med. Chem.*, **6**, 1695 –1705, 1998.

193 **Morpholino antisense oligomers: design, preparation, and properties**, J. Summerton and D. Weller, *Antisense Nucleic Acid Drug Dev.*, **7**, 187 –195, 1997.

194 (a) **Oligonucleotide N3'–>P5' phosphoramidates**, S.M. Gryaznov, D.H. Lloyd, J.K. Chen, R.G. Schultz, L.A. DeDionisio, L. Ratmeyer and W.D. Wilson, *Proc. Natl. Acad. Sci. USA*, **92**, 5798 –5802, 1995; (b) **Synthesis of oligodeoxyribonucleotide N3'–>P5' phosphoramidates**, J.K. Chen, R.G. Schultz, D.H. Lloyd and S.M. Gryaznov, *Nucleic Acids Research*, **23**, 2661 –2668, 1995; (c) **Oligonucleotide N3'–>P5 phosphoramidates as antisense agents**, S. Gryaznov, T. Skorski, C. Cucco, M. Nieborowska-Skorska, C.Y. Chiu, D. Lloyd, J.K. Chen, M. Koziolkiewicz and B. Calabretta, *Nucleic Acids Research*, **24**, 1508 –1514, 1996; (d) **Antileukemia effect of c-myc N3'–>P5' phosphoramidate antisense oligonucleotides *in vivo***, T. Skorski, D. Perrotti, M. Nieborowska-Skorska, S. Gryaznov and B. Calabretta, *Proc. Natl. Acad. Sci. USA*, **94**, 3966 –3971, 1997.

195 See for example: **Cholesterol conjugated oligonucleotide and LNA: A comparison of cellular and nuclear uptake by Hep2 cells enhanced by Streptolysin-O**, Š. Holasová, M. Mojžíšek, M. Bunček, D. Vokurková, H. Radilová, M. Šafářová, M. Červinka and R. Haluza, *Molecular and Cellular Biochem.*, **276**, 61-69, 2005.

196 **Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs**, J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R.K. Pandey, T. Racie, K.G. Rajeev, I. Röhl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan and H.-P. Vornlocher, *Nature*, **432**, 173-178, 2004.

to cholesterol chloroformate,[197] however direct attachment during synthesis is much more convenient. 5'-Attachment is possible *via* a modified phosphoramidite.[198,199,200] By comparison to other cholesterol amidites available we have found 5'-Cholesterol-CE Phosphoramidite (**2170**) to offer specific advantages in oligo synthesis.[201]

Since the cholesterol is attached directly to aminohexanol, it is not susceptible to 1,2-diol elimination as observed in some other products. Lack of a trityl group simplifies purification; some cholesterol products must be used in trityl-on mode (to prevent 1,2-diol elimination during deprotection), then detritylated, and can subsequently be very difficult to purify.

Significantly, its coupling efficiency (final modification step) is routinely >90% giving a high yield of modified product. This compares favourably to final (5') modification efficiencies we have observed with other commercial products. This product can readily be used in automated synthesis. Although, like most cholesterol products, it is not soluble in acetonitrile, it is easily dissolved using dichloromethane as the diluent. Unlike competing products, there is no requirement for solvent mixtures that include THF (this solvent can cause problems in some large-scale automated instruments).

Aside from other oligonucleotide design criteria, 3'-modification can offer the added benefit of, at least partially, protecting the oligo from exonucleases in the cell. For this purpose we offer 3'-Cholesterol SynBase™ CPG 1000/110 (**2394**).

This product has a couple of notable advantages over competing products. Like the phosphoramidite (**2170**), it is not susceptible to the 1,2-diol elimination observed in some other supports. Furthermore, since the modification is based on the natural sugar-phosphate backbone, there are no adverse structural effects on the oligo. The product can also be used without IP restriction.

At the request of several customers, we have extended our cholesterol-modification range to include a TEG-based product, 5'-Cholesterol-TEG-CE Phosphoramidite (**2189**). This, too, is a simple 5'-modifier without the complications of a 1,2-diol and trityl protection. This product has the added benefit of solubility in acetonitrile.[201]

The strict guidelines imposed by regulatory authorities now make it essential to use non-animal based products in pharmaceutical drug development for humans. With increasing frequency, therefore, our customers are requesting that we supply products with BSE/TSE statements. We have now developed an alternative route to these products that uses entirely plant-derived cholesterol, making them even better choices for modification of oligos.

Of similar application, but comparatively less studied to date, is the incorporation of the palmitoyl moiety into oligonucleotides. One such use employs an oligonucleotide conjugate

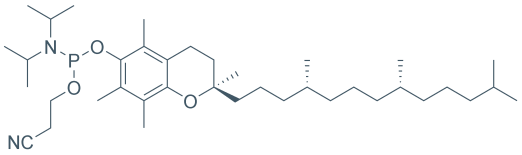
197 See for example: (a) **Cholesteryl-conjugated oligonucleotides: synthesis, properties, and activity as inhibitors of replication of human immunodeficiency virus in cell culture**, R.L. Letsinger, G. Zhang, D.K. Sun, T. Ikeuchi and P.S. Sarin, *Proc. Natl. Acad. Sci.*, **86**, 6553-6556, 1989; and (b) **A simplified synthesis of acridine and/or lipid containing oligodeoxynucleotides**, C.J. Marasco, Jnr., N.J. Angelino, B. Paul and B.J. Dolnick, *Tetrahedron Lett.*, **35**, 3029-3032, 1994. Other methods, such as conjugation *via* a disulphide bond to terminal phosphate groups, have also been used. See for example: **Antisense effects of cholesterol-oligodeoxynucleotide conjugates associated with poly(alkylcyanoacrylate) nanoparticles**, G. Godard, A.S. Boutorine, E. Saison-Behmoaras and C. Hélène, *Eur. J. Biochem.*, **232**, 404-410, 1995.

198 **Assembling liposomes by means of an oligonucleotide tagged with a lipophilic unit**, N. Maru, K. Shohda and T. Sugawara, *Nucleic Acids Symposium Series No. 48*, 95-96, 2004.

199 **Synthesis and physical properties of anti-HIV antisense oligonucleotides bearing terminal lipophilic groups**, C. MacKellar, D. Graham, D.W. Will, S. Burgess and T. Brown, *Nucleic Acids Research*, **20**, 3411-3417, 1992.

200 **Mode of action of 5'-linked cholesteryl phosphorothioate oligodeoxynucleotides in inhibiting syncytia formation and infection by HIV-1 and HIV-2 *in vitro***, C. A. Stein, Ranajit Pal, A. L. DeVico, G. Hoke, S. Mumbauer, O. Kinstler, M. G. Sarngadharan and R. L. Letsinger, *Biochemistry*, **30**, 2439 - 2444, 1991.

201 For a detailed assessment of our cholesterol modifications see: **Plant derived cholesterol modifications: Comparative use in oligonucleotide synthesis**, S. Aitken, D. Hannah, U. Ixkes, C. McKeen and D. Picken, available online: https://linktechsupport.zendesk.com/hc/en-us/articles/200143398-Plant-Derived-Cholesterol-Modifications-A-Comparison-of-Commercially-Available-Cholesterol-Phosphoramidites-and-Solid-Supports-for-use-in-Oligonucleotide-Synthesis-of-DNA-and-RNA-TC-and-TBDMS-chemistries-.



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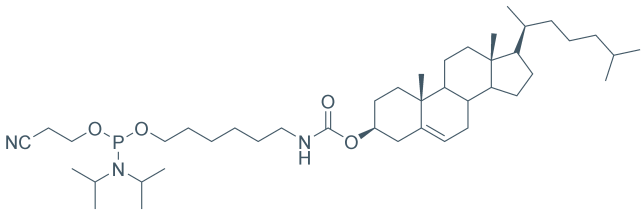

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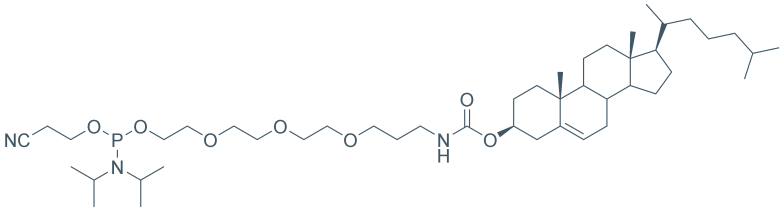
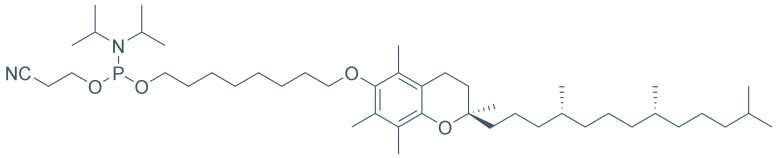


2189



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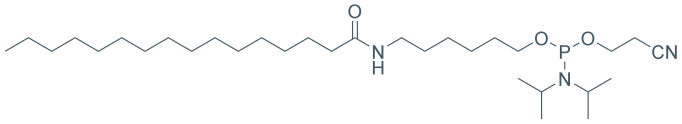

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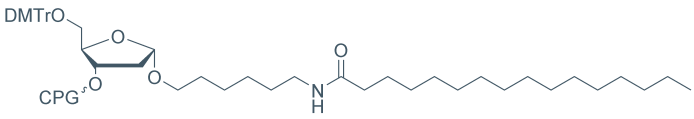
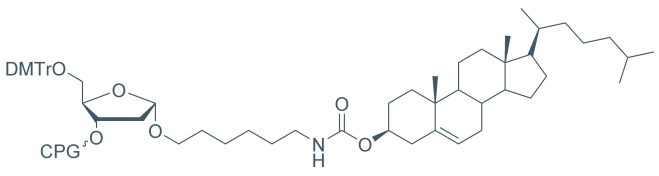


2393



2394

Ordering Lipophilic Modifiers

Product	Pack Size	Cat. No.
5'-Cholesterol-CE Phosphoramidite	100µmol	2170-F100
	250mg	2170-B250
5'-Colesterol-TEG-CE Phosphoramidite	100µmol	2189-F100
	250mg	2189-B250
5'-Tocopherol-CE Phosphoramidite	100µmol	2163-F100
	250mg	2163-B250
5'-Octyl-tocopherol-CE Phosphoramidite	100µmol	2194-F100
	250mg	2194-B250
5'-Palmitate-CE Phosphoramidite	100µmol	2199-F100
	250mg	2199-B250

Product	Pack Size	Cat. No.
3'-Cholesterol SynBase™ CPG 1000/110	100mg	2394-B100
	1g	2394-C001
ALL-FIT Columns	4 x 0.2µmol	2394-P001
	10 x 0.2µmol	2394-P002
	4 x 1µmol	2394-P010
	10 x 1µmol	2394-P008
MerMade Columns	4 x 0.2µmol	2394-P015
	10 x 0.2µmol	2394-P016
	4 x 1µmol	2394-P026
	10 x 1µmol	2394-P022
3'-Palmitate SynBase™ CPG 1000/110	100mg	2393-B100
	1g	2393-C001
ALL-FIT Columns	4 x 0.2µmol	2393-P001
	10 x 0.2µmol	2393-P002
	4 x 1µmol	2393-P010
	10 x 1µmol	2393-P008

with a 5'-palmitoyl group attached through an amide bond.[202] This has been used to modify GRN163, a thio-phosphoramidate oligonucleotide, to enhance the potency of telomerase inhibition. We offer both 5'-Palmitate-C6-CE Phosphoramidite (**2199**) and 3'-Palmitate SynBase™ CPG 1000/110 (**2393**) for direct incorporation of a palmitoyl group during oligo synthesis, at the 5' and 3' end respectively.

As with cholesterol modifications, other lipophiles such as tocopherol (vitamin E) have been shown to have potential use in the delivery of oligonucleotides into cells. Vitamins such as tocopherol are not produced by the target cells, but are used by the latter and therefore vitamins are recognised. They are thought to be internalised by cells only after interaction with a binding protein and therefore have the potential for specific targeting of a cell type.[203,204,205,206]

We have extended our line of lipophilic modifiers to include two products, namely 5'-Tocopherol-CE Phosphoramidite (**2163**) and the analogous 5'-Octyltocopherol-CE Phosphoramidite (**2194**). These can be used to introduce tocopherol at the 5' end, either directly on the 5'-OH of the final base or in conjunction with a linker such as C6 S-S thiol (**2126**). This latter approach enables the tocopherol to be cleaved *via* the disulphide bridge, for example once the oligo has been delivered to the cell. As a spacer arm is often required for label distancing, **2194** was developed with a "built in" C8 spacer.[207]

As an aside, the hydrophobic nature of tocopherol has also been utilised as a means of

202 **Lipid modification of GNR163, an N3' P5' thio-phosphoramidate oligonucleotide, enhances the potency of telomerase inhibition**, B.-S. Herbert, G.C. Gellert, A. Hochreiter, K. Pongracz, W.E. Wright, D. Zielinska, A.C. Chin, C.B. Harley, J.W. Shay and S.M. Gryaznov, *Oncogene*, **24**, 5262-5268, 2005.

203 **Delivery of oligonucleotides and analogues: The oligonucleotide conjugate-based approach**, F. Marlin, P. Simon, T. Saison-Behmoaras and C. Giovannangeli, *ChemBioChem.*, **11**, 1493-1500, 2010.

204 **Efficient *in vivo* delivery of siRNA to the liver by conjugation to alpha-tocopherol**, K. Nishina, T. Unno, Y. Uno, T. Kubodera, T. Kanouchi, H. Mizusawa and T. Yokota, *Mol. Ther.*, **16**, 734-740, 2008.

205 **Resolution of liver cirrhosis using vitamin-A coupled liposomes to deliver siRNA against a collagen-specific chaperone**, Y. Sato, K. Murase, J. Kato, M. Kobune, T. Sato, Y. Kawano, R. Takimoto, K. Takada, K. Miyanishi, T. Matsunaga, T. Takayama and Y. Niitsu, *Nat. Biotechnol.*, **26**, 431-442, 2008.

206 **Attachment of vitamin E derivatives to oligonucleotides during solid-phase synthesis**, D. Will and T. Brown, *Tet. Letts .*, **33**, 2729-2732, 1992.

207 **Tocopherol (Vitamin E) modified oligonucleotides**, S. Aitken, R. Archer, G. McGeoch, C. McKeen and D. Picken, poster presented at TIDES 2011. Available online: https://linktechsupport.zendesk.com/hc/en-us/articles/200143388-Tocopherol-Vitamin-E-Modified-Oligonucleotides.

improving the purification of ribozymes.[208] We have also demonstrated the use of tocopherol products as a means of allowing an initial purification of thiol-modified oligos with a view to improving the efficiency of a second, *e.g.* ion-exchange, purification.[209,210]

Vitamin Modification

Inspired by the applicability of tocopherol, to extend our vitamin-based reagents we have developed two pyridine based synthesis reagents derived from niacin, 5'-Niacin-CE Phosphoramidite (**2530**) and 5'-Niacin-C6-CE Phosphoramidite (**2537**). Niacin is important in the biosynthesis of NAD and NADH, hence is recognised by cells. Being much less hydrophobic and bulky than more commonly used delivery reagents it is anticipated the need to remove modifiers derived from niacin will no longer be necessary. Also since these are important in many biological pathways, once in the cell, it is possible that these will be removed by hydrolysis of the phosphate linkage.

Niacin is an essential vitamin and is one of only five where deficiency results in a pandemic deficiency disease, in this case pellagra. It is known for its therapeutic effects as a lipid lowering drug.[211] This works by inhibiting the production of cyclic AMP and as a result the liver is unable to produce LDL. Recently niacin has been shown to improve the ability of immune cells to kill staphylococcus bacteria (responsible for MRSA).[212]

Since the niacin molecule is very small compared to an oligonucleotide, it can be difficult to get sufficient resolution between a niacin labelled oligonucleotide and the analogous unlabelled sequence. We have found that in these cases, the introduction of a C6 linker between the pyridine ring and the phosphoramidite resolves this issue. We have therefore also introduced 5'-Niacin-C6-CE Phosphoramidite (**2537**).[213]

To add to the range of vitamin based delivery reagents, we have also used a C6 linker attached to a pyridoxine phosphoramidite (bisDMT-Pyridoxine-C6-CE Phosphoramidite, **2536**). The DMT protection allows DMT-ON purification. Pyridoxine (vitamin B6) has several biological functions: red blood cell production; homocysteine reduction; inflammation reduction and neurotransmitter production. It is known that almost all eukaryotic cells have receptor mediated transport system for this vitamin where pyridoxine acts as a carrier and, once the biomolecule is in the cell or cell compartment, the carrier is lost as pyridoxial phosphate. There is evidence that cellular uptake of peptide-oligonucleotide conjugates has been improved where pyridoxine has been conjugated to the peptide. Although the vitamin is attached *via* an

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208 **Fast and simple purification of chemically modified hammerhead ribozymes using a lipophilic capture tag**, B.S. Sproat, T. Rupp, N. Menhardt, D. Keane, and B. Beijer, *Nucleic Acids Research*, **27**, 1950-1955, 1999.

209 **Tocopherol (Vitamin E) modified oligonucleotides II: Utilising hydrophobicity to aid purification**, S. Aitken, R. Archer, G. McGeoch, C. McKeen and D. Picken, poster presented at the 6th Cambridge Symposium on Nucleic Acids Chemistry and Biology, 2011. Available online: https://linktechsupport.zendesk.com/hc/en-us/articles/200143428-Tocopherol-Vitamin-E-Modified-Oligonucleotides-II-Utilising-Hydrophobicity-to-Aid-Purification.

210 **Oligonucleotide delivery and purification: Tocopherol modification improves product purification and aids delivery into cells**, C. McKeen, *Gen. Eng. News*, **32**(3), 22-23, February 1, 2012. Available online: www.genengnews.com/gen-articles/oligonucleotide-delivery-and-purification/3991/.

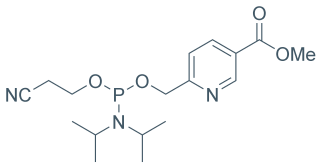
211 (a) **Influence of nicotinic acid on serum cholesterol in man**, R. Altschul, A. Hoffer and J.D. Stephen, *Arch. Biochem. Biophys.*, **54**, 558–559, 1955; (b) **Extended-release niacin vs gemfibrozil for the treatment of low levels of high-density lipoprotein cholesterol**, J.R. Guyton, M.A. Blazing, J. Hagar, M.L. Kashyap, R.H. Knopp, J.M. McKenney, D.T. Nash and S.D. Nash, *Arch. Intern. Med.*, **160**, 1177–1184, 2000; (c) **Effectiveness of once-nightly dosing of extended-release niacin alone and in combination for hypercholesterolemia**, J.R. Guyton, A.C. Goldberg, R.A. Kreisberg, D.L. Sprecher, H.R. Superko and C.M. O'Connor, *Am. J. Cardiol.*, **82**, 737–743, 1998; (d) **Effect of low-dose niacin on high-density lipoprotein cholesterol and total cholesterol/high-density lipoprotein cholesterol ratio,** M.H. Luria, Arch. *Intern. Med.*, **148**, 2493–2495, 1988; (e) **Lipoprotein responses to treatment with lovastatin, gemfibrozil, and nicotinic acid in normolipidemic patients with hypoalphalipoproteinemia**, G.L. Vega and S.M. Grundy, *Arch. Intern. Med.*, **154**, 73–82, 1994.

212 **C/EBPε mediates nicotinamide-enhanced clearance of Staphylococcus aureus in mice**, P. Kyme, N.H. Thoennissen, C.W. Tseng, G.B. Thoennissen, A.J. Wolf, K. Shimada, U.O. Krug, K. Lee, C. Müller-Tidow, W.E. Berdel, W.D. Hardy, A.F. Gombart, H.P. Koeffler, G.Y. Liu, *Journal of Clinical Investigation*, **122**, 3316-3329, 2012.

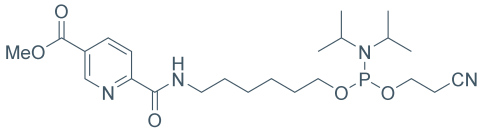
213 **Vitamin Based Phosphoramidites for use as Potential Oligonucleotide Delivery Reagents**, C.M. McKeen, A. Osnowski, R. Archer, K. Mullaney, S. Aitken and J. Wilson, poster presented at OTS Annual Meeting, San Diego, 2014. Available online: https://linktechsupport.zendesk.com/hc/en-us/articles/202190449-Vitamin-Based-Phosphoramidites-for-use-as-Potential-Oligonucleotide-Delivery-Reagents.

amide, an enzymatic conversion takes place and the carrier is still released as the phosphate.

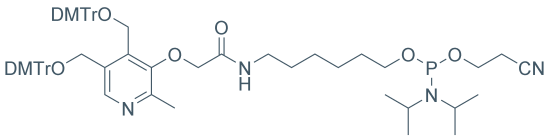
This modification allows the direct incorporation at the 5'-end of an oligo and once in the cell it is thought that the carrier will be hydrolysed from the oligo.



2530



2537



2536

Ordering Vitamin Modifiers

Product	Pack Size	Cat. No.
5'-Niacin-CE Phosphoramidite	100µmol	2530-F100
	250mg	2530-B250
	500mg	2530-B500
5'-Niacin-C6-CE Phosphoramidite	100µmol	2537-F100
	250mg	2537-B250

Product	Pack Size	Cat. No.
bisDMT-Pyridoxine-C6-CE Phosphoramidite	100µmol	2536-F100
	250mg	2536-B250

Structural Studies

Duplex Stability/Instability

The hybridisation properties of synthetic oligonucleotides are crucial for almost all applications. Optimisation of base pairing, and subsequent duplex stabilisation, is therefore desirable.

C-5 Methyl pyrimidine nucleosides are known to stabilise duplexes relative to the non-methylated bases. Therefore the use of 5-Me-dC-CE Phosphoramidite (available either as N-Bz (**2017**) or N-Ac (**2529**))[214] rather than dC results in enhanced binding (a similar comparison can be made between thymidine and 2'-deoxyuridine). This increase in duplex stabilisation is attributed to the hydrophobic nature of the methyl groups that helps eliminate water molecules from the duplex. We also offer a 5-Me-dC CPG (**2323**) for modification at the 3'-end. The stabilisation properties of Me-dC make this a suitable modification for stabilisation of triplex strands, where its presence raises the melting temperature of the third strand.

During duplex hybridisation of unmodified oligos, A-T base pairs have two hydrogen bonds, whereas G-C base pairs have three. One of the simplest methods of improving duplex stabilisation is the use of 2-Amino-dA-CE Phosphoramidite (**2145**) (2,6-diaminopurine) in place of dA.[215] This forms an additional hydrogen bond with thymidine (see Figure 19). However, **2145** also destabilises A-G wobble mismatches, thus increasing specificity.

It is also worth noting that 2'-OMe modifications, primarily used to confer nuclease resistance, have the complementary property of duplex stabiliation (see page 70 for more details).

Other Duplex Effects

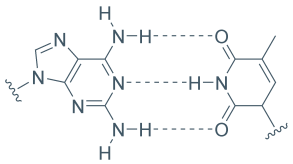
In sequencing applications, the design of primers can be complicated by the degeneracy of the genetic code (there are 64 possible 3-base codon configurations and only 21 amino acids, and therefore the third base in a sequence codon is often unknown). The problem of degeneracy can also be tackled by the use of universal bases.[216] Deoxyinosine is often used

214 **Effect of 5-methylcytosine on the stability of triple-stranded DNA-a thermodynamic study**, L.E. Xodo, G. Manzini, F. Quadrifoglio, G.A. van der Marel and J.H. van Boom, *Nucleic Acids Research*, **19**, 5625-5631, 1991.

215 **Oligonucleotides containing 2-aminoadenine and 5-methylcytosine are more effective as primers for PCR amplification than their non-modified counterparts**, Y. Lebedev, N. Akopyants, T. Azhikina, Y. Shevchenko, V. Potapov, D. Stecenko, D. Berg and E. Sverdlov, *Genetic Analysis – Biomolecular Engineering*, **13**, 15-21, 1996.

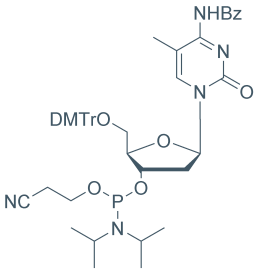
216 **The applications of universal DNA base analogues**, D. Loakes, *Nucleic Acids Research*, **29**, 2437-2447, 2001.

G C A T

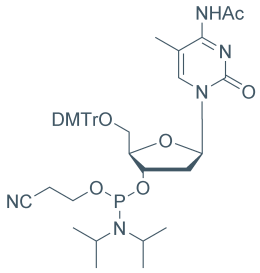


2-amino-dA T

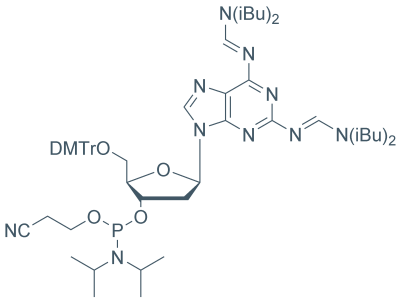
Figure 19. Hydrogen bonding patterns.



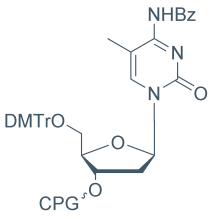
2017



2529



2145



2323

as a degenerate base in an oligonucleotide to alleviate this problem.[217] This is possible since its structure allows it to base pair with all four bases in various 'wobble' structures. However, the base-pairing is not equivalent with each of the 4 naturally occurring bases. The overall preferential order of base-pairing is: dI-dC > dI-dA > dI-dG = dI-dT. We provide both dI-CE Phosphoramidite (**2016**) and dI SynBase™ CPG 1000/110 (**2293**). 2'-Deoxynebularine[218] is another example. Incorporation of a deoxyuridine base within a DNA sequence can be used to induce mutagenic effects. The enzyme uracil-N-glycosylase (UNG) can specifically remove uracil to create abasic sites at the deoxyuridine positions. This property is used to generate site-specific strand breaks in the oligonucleotide. We provide both dU-CE Phosphoramidite (**2013**) and dU SynBase™ CPG 1000/110 (**2287**).

Deoxyxanthosine

Xanthosine (Figure 20) is a naturally occurring nucleoside containing a purine heterocycle that presents an H-bonding pattern to a complementary strand distinct from that presented by unmodified purines found in encoded oligonucleotides.

Xanthosine has been proposed as a 'universal base', *i.e.* a heterocycle that can pair equally well

217 (a) **Base pairing involving deoxyinosine: implications for probe design**, F.H. Martin, M.M. Castro, F. Aboul-ela and I. Tinoco, Jr, *Nucleic Acids Research*, **13**, 8927-8938, 1985; (b) **Studies on the base pairing properties of deoxyinosine by solid phase hybridisation to oligonucleotides**, S.C. Case-Green, E.M. Southern, *Nucleic Acids Research*, **22**, 131-136, 1994.

218 (a) **Synthesis and properties of oligonucleotides containing 2'-deoxynebularine and 2'-deoxyxanthosine**, R. Eritja, D.M. Horowitz, P.A. Walker, J.P. Ziehler-Martin, M.S. Boosalis, M.F. Goodman, K. Itakura and B.E. Kaplan, *Nucleic Acids Research*, **14**, 8135-8153, 1986; (b) As a custom item we have prepared the phosphoramidite (**2024**), see: **A convenient synthesis of deoxynebularine phosphoramidite**, D. Picken and V. Gault, *Nucleosides, Nucleotides and Nucleic Acids*, **16**, 937-939, 1997. Please enquire regarding availability.

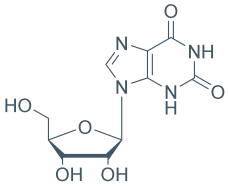
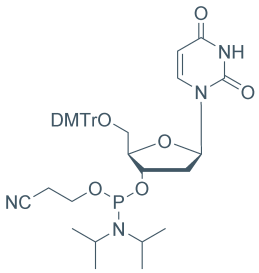
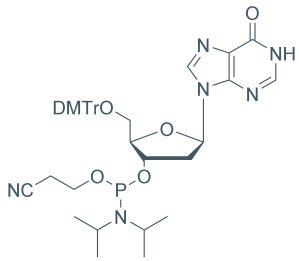


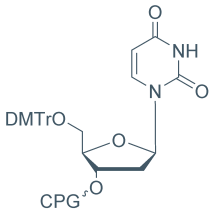
Figure 20. Xanthosine.



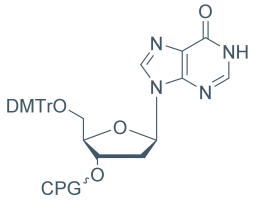
2013



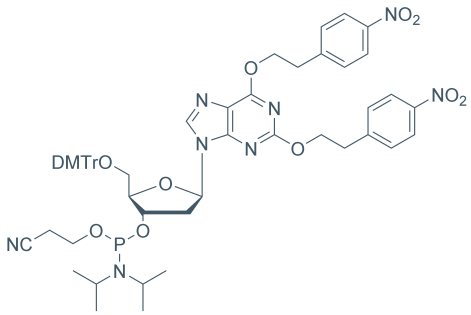
2016



2287



2293



2164

with all four natural nucleosides.[219] As such, several studies have been carried out (as far back as the mid-1980s), incorporating deoxyxanthosine (dX) into oligonucleotides. However the expected base-pairing properties were not observed.

Other notable properties have been reported however. Benner and co-workers have described the extension of the 'genetic alphabet' by purine partnering dX with 5-(ß-D-ribofuranosyl)pyrimidine-2,4-diamine, a pyrimidine analogue presenting an H-bonding pattern complementary to dX.[220] Recently dX has been used in the study of the physiologically important nitrosative deamination of DNA which is one of the main causes of genomic mutations.[221, 222(b)]

Although a number of monomers for the incorporation of dX have been reported (using phosphotriester or phosphoramidite chemistry), the most effective of these is the 2-(4-nitrophenyl)ethyl (NPE) O^2/O^6 doubly-protected monomer, our product

219 (a) **Double protection of the heterocyclic base of xanthosine and 2'-deoxyxanthosine**, A. van Aerschot, M. Mag, P. Herdewijn and H. Vanderhaeghe, *Nucleosides & Nucleotides*, **8**, 159-178, 1989; (b) **Synthesis and properties of oligonucleotides containing 2'-deoxynebularine and 2'-deoxyxanthosine**, R. Eritja, D.M. Horowitz, P.A. Walker, J.P. Ziehler-Martin, M.S. Boosalis, M.F. Goodman, K. Itakura and B.E. Kaplan, *Nucleic Acids Research*, **14**, 8135-8153, 1986.

220 **Differential discrimination of DNA polymerases for variants of the non-standard nucleobase pair between xanthosine and 2,4-diaminopyridine, two components of an expanded genetic alphabet**, M.J. Lutz, H.A. Held, M. Hottiger, U. Hübscher and S.A. Benner, *Nucleic Acids Research*, **24**, 1308-1313, 1996.

221 (a) **Stability of 2'-deoxyxanthosine in DNA**, V. Vongchampa, M. Dong, L. Gingipalli and P. Dedon, *Nucleic Acids Research*, **31**, 1045-1051, 2003 ; (b) **A bifunctional DNA repair protein from *Ferroplasma acidarmanus* exhibits O^6-alkylguanine-DNA alkyltransferase and endonuclease V activities**, S. Kanugula, G.T. Pauly, R.C. Moschel and A.E. Pegg, *PNAS*, **102**, 3617-3622, 2005.

2'-Deoxyxanthosine-CE Phosphoramidite (**2164**).[222] **2164** is used as per standard protocols, with an extra deprotection reagent to remove the NPE groups (see page 152 for details).

Photocrosslinking

Halogenated nucleosides are versatile reagents in oligo applications. We provide a wide range of halogenated nucleoside phosphoramidites and CPG supports.

Photocross-linking is a useful technique for the partial definition of the nucleic acid-protein interface of nucleoprotein complexes.[223] Photoactive bases may also be used to probe the crystal structure of the protein-DNA complexes.[224] Photoactive analogues of dC (5-Iodo- and 5-Bromo-dC (**2009** and **2011**)) and dT analogues (5-Iodo- and 5-Bromo-dU (**2014** and **2012**)) are available as phosphoramidites. The Br-dU support (**2325**) is also available.

8-Br-dA (**2054**)[225] and 8-Br-dG (**2055**) phosphoramidites have been proposed to complete the set of the four photoactive bases required to examine base to amino acid contact pairs, although work in this regard has been limited. 8-Br-dG is also useful in promoting the formation of Z-form DNA structures and for locating subtle differences in DNA polymerases and repair enzymes.[226]

Sulphur modified bases are of particular use for cross-linking. 4-Thio-dT-CE Phosphoramidite (**2070**) provides a convenient modification for photo cross-linking and photo-affinity labelling applications.

Other Structural Studies

The three-dimensional structure of DNA can be probed by x-ray crystallography using several halogenated nucleoside phosphoramidites.[227] In addition, antibodies exist which are specific for Br-dU so that oligonucleotides containing Br-dU can be used as probes.

5-Fluoro-deoxyuridine (**2010**) is a base analogue that has the potential to bind to A and G. It does not destabilise duplex formation, and is an alternative to using mixed bases A/G for degeneracy.[228]

In addition to halogenated nucleosides, LINK offers several other phosphoramidites that have uses in various structural studies.

2-Aminopurine-CE Phosphoramidite (**2069**) is useful for investigating structural changes, as the base is deficient in hydrogen bonding sites. It is also mildly fluorescent.

8-oxo-dG-CE Phosphoramidite (**2072**) allows investigation of the structure and activity of oligonucleotides containing an 8-oxo mutation. This is formed naturally when DNA is subjected to oxidative conditions or ionising radiation. The resulting 8-oxo modification is significant in mutagenesis and ultimately carcinogenesis.

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222 (a) **Synthesis and characterisation of oligonucleotides containing 2'-deoxyxanthosine using phosphoramidite chemistry**, S.C. Jurczyk, J. Horlacher, K.G. Devined, S.A. Benner and T.R. Battersby, *Helv. Chim. Acta.*, **83**, 1517-1524, 2000; (b) **Stability, miscoding potential and repair of 2'-deoxyxanthosine in DNA: Implications for nitric oxide-induced mutagenesis**, G.E. Weunschell, T.R. O'Connor and J. Termini, *Biochemistry*, **42**, 3608-3616, 2003

223 **Photocross-linking of nucleic acids to associated proteins**, K.M. Meisenheimer and T.H. Koch, *Critical Reviews in Biochemistry and Molecular Biology*, **32**, 101-140, 1997.

224 **Crystal structure of chromomycin-DNA complex**, C.M. Ogata, W.A. Hendrickson, X. Gao and D. Patel, *J. Abstr. Amer. Cryst. Assoc. Mtg. Ser.*, **2**, 1753, 1989.

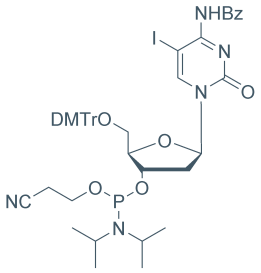
225 **Synthesis of photoactive DNA: Incorporation of 8-bromo-2'-deoxyadenosine into synthetic oligonucleotides**, J. Liu and G.L. Verdine, *Tetrahedron Lett.*, **33**, 4265-4268, 1992.

226 **Synthesis and properties of oligonucleotides containing 8-bromo-2'-deoxyguanosine**, C. Fàbrega, M.J. Macías and R. Eritja, *Nucleosides, Nucleotides & Nucleic Acids*, **20**, 251-260, 2001.

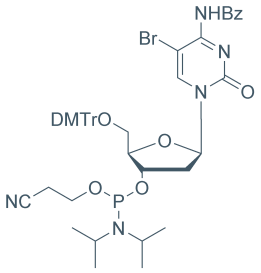
227 **Effects of cationic charge on three-dimensional structures of intercalative complexes: structure of a bis-intercalated DNA complex solved by MAD phasing**, X. Shui, M.E. Peek, L.A. Lipscomb, Q. Gao, C. Ogata, B.P. Roques, C. Garbay-Jaureguiberry, A.P. Wilkinson and L.D. Williams, *Curr. Med. Chem.*, **7**, 5971, 2000.

228 The less studied halogenated minor base phosphoramidites 5-I-U (**2031**) and 5-Br-U (**2032**) (structures not drawn) can be useful in cross-linking and x-ray studies. Please contact us regarding their availability.

Photocrosslinking Modifiers



2009



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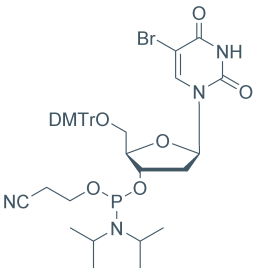

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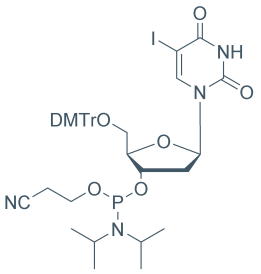
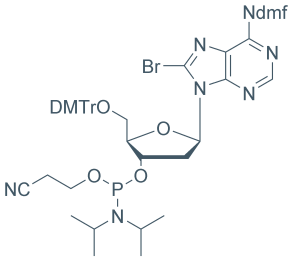
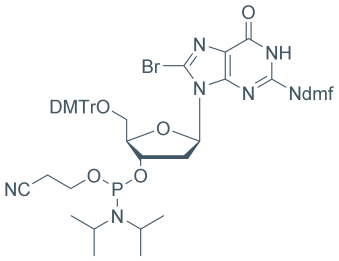


2014



2054



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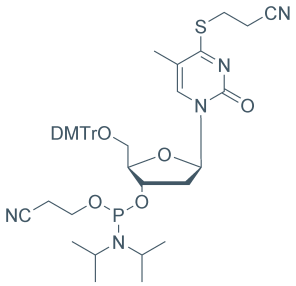

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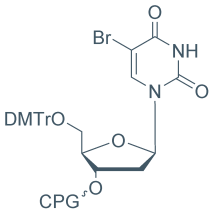
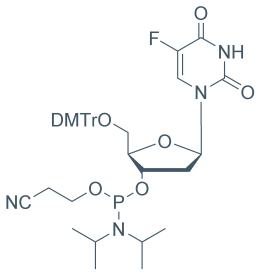
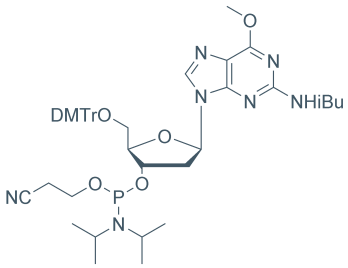


2325

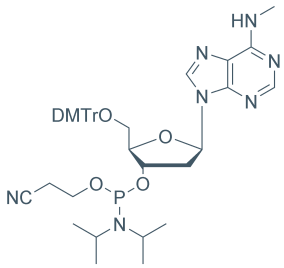
Other Structural Study Modifiers



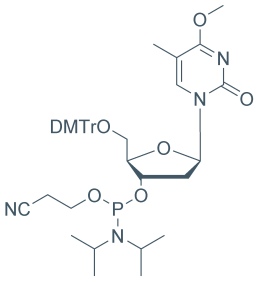
2010



2018



2019



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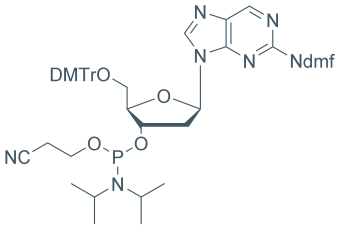

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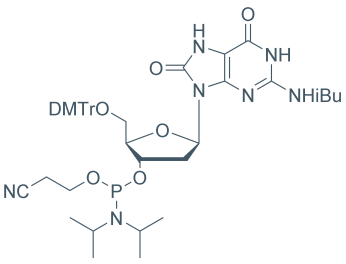


2072

Methylating agents are common carcinogens which function by methylation of nucleobases in DNA. To examine the resulting mutagenic effects, the methylated products O6-Me-dG-CE Phosphoramidite (**2018**), N6-Me-dA-CE Phosphoramidite (**2019**), and O4-Me-dT-CE Phosphoramidite (**2025**) can be incorporated in oligonucleotides.

Oligonucleotides containing a hairpin loop are used routinely for structural studies of duplex formation. The hairpin loop allows the oligonucleotide to bend back on itself thereby forming a duplex in an anti-parallel formation. The hairpin may be nucleosidic or it may consist of a polyethylene glycol spacer.[229] By using "reverse" 5'-O-phosphoramidites (see page 74) for part of the synthesis, oligos with hairpin loops can be formed in which the strands are parallel.[230] These parallel stranded oligos can be readily prepared with 5'-5' or 3'-3' sense. Parallel stranded oligos are now also used in triplex formation studies.

229 **Triple-helix formation by an oligonucleotide containing one (dA)12 and two (dT)12 sequences bridged by two hexaethylene glycol chains**, M. Durand, S. Peloille, N.T. Thuong and J.C. Maurizot, *Biochemistry*, **31**, 9197-9204, 1992.

230 **Parallel Stranded DNA**, J.H. van der Sande, N.B. Ramsing, M.W. Germann, W. Elhorst, B.W. Kalisch, E. van Kitzing, R.T. Pon, R.C. Clegg and T.M. Jovin, *Science*, **241**, 551-557, 1988.

Ordering Structural Study Modifiers

Product	Pack Size	Cat. No.
5-Me-Bz-dC-CE Phosphoramidite	250mg	2017-B250
	1g	2017-C001
5-Me-Ac-dC-CE Phosphoramidite	250mg	2529-B250
	1g	2529-C001
5-Me-dC SynBase™ CPG 1000/110	100mg	2323-B100
	10 x 0.2µmol	2323-P002
	10 x 1µmol	2323-P008
2-Amino-dA-CE Phosphoramidite	100µmol	2145-F100
	250mg	2145-B250
dI-CE Phosphoramidite	250mg	2016-B250
	500mg	2016-B500
	1g	2016-C001
dI SynBase™ CPG 1000/110	100mg	2293-B100
	1g	2293-C001
	10 x 0.2µmol	2293-P002
	10 x 1µmol	2293-P008
dU-CE Phosphoramidite	250mg	2013-B250
	500mg	2013-B500
	1g	2013-C001
dU SynBase™ CPG 1000/110	100mg	2287-B100
	1g	2287-C001
	10 x 0.2µmol	2287-P002
	10 x 1µmol	2287-P008
Deoxyxanthosine-CE Phosphoramidite	100µmol	2164-F100
	250mg	2164-B250
5-I-dC-CE Phosphoramidite	100µmol	2009-F100
	250mg	2009-B250

Product	Pack Size	Cat. No.
5-F-dU-CE Phosphoramidite	100µmol	2010-F100
	250mg	2010-B250
5-Br-dC-CE Phosphoramidite	100µmol	2011-F100
	250mg	2011-B250
5-Br-dU-CE Phosphoramidite	100µmol	2012-F100
	250mg	2012-B250
5-Br-dU SynBase™ CPG 1000/110	100mg	2325-B100
	1g	2325-C001
5-I-dU-CE Phosphoramidite	100µmol	2014-F100
	250mg	2014-B250
8-Br-dA-CE Phosphoramidite	100µmol	2054-F100
	250mg	2054-B250
8-Br-dG-CE Phosphoramidite	100µmol	2055-F100
	250mg	2055-B250
2-Amino-purine-CE Phosphoramidite	100µmol	2069-F100
	250mg	2069-B250
4-Thio-dT-CE Phosphoramidite	100µmol	2070-F100
	250mg	2070-B250
8-oxo-dG-CE Phosphoramidite	50µmol	2072-F050
	100µmol	2072-F100
	250mg	2072-B250
O6-Me-dG-CE Phosphoramidite	100µmol	2018-F100
	250mg	2018-B250
N6-Me-dA-CE Phosphoramidite	100µmol	2019-F100
	250mg	2019-B250
O4-Me-dT-CE Phosphoramidite	100µmol	2025-F100
	250mg	2025-B250

Miscellaneous Products

We also provide purification cartridges, nucleoside synthesis reagents, plus empty synthesiser bottles and columns.

Oligonucleotide Purification

For a simple, cost-effective solution for DNA and RNA DMT-ON oligonucleotide purification we provide a range of TOP cartridges. Features include:

- Superior yield and purity come from the proprietary polymeric resins and optimized buffers
- Typical yield is more than 85% and typical purity is over 90%, eliminating the need for multiple sample loading steps
- TOP cartridges use up to two thirds less reagent than products from other vendors

Modified Nucleosides

A number of halogenated nucleosides (5-I-dU (**0020**), 5-Br-dC (**0065**), 5-Br-dU (**0079**) and 5-I-dC (**0120**)) are available giving a convenient starting point for further synthetic work. Various other intermediates, such as 5'-DMTr-T (**0001**), 5'-DMTr-iBu-dG (**0002**), 5'-DMTr-Bz-dA (**0003**) and 5'-DMTr-Bz-dC (**0004**), although not listed opposite, are available in bulk on request.

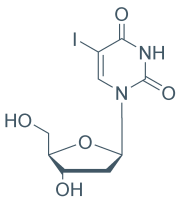
Nucleoside Synthesis Reagents

Nucleosides are prepared for oligonucleotide synthesis using 4,4'-dimethoxytrityl chloride (**0021**) for DMTr-protection of the 5'-OH. The 3'-phosphoramidite functionality is achieved using a phosphitylating reagent (**1002**) in the presence of an activator, DIHT (**1001**). For ribonucleosides, or other cases where the 2' position is hindered - or where rapid reaction is required, a more reactive chlorophosphitylating reagent (**1028**) is used instead of **1002**.

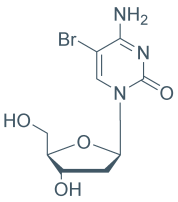
Empty Synthesis Bottles & Columns

Our unmodified DNA phosphoramidites are packaged ready for use on either ABI, MerMade, or Expedite synthesisers. All other products are packaged by default in ABI compatible bottles, however other bottles can be provided on request (see detailed information on product packaging on page 16).

Similarly, synthesis supports are routinely packed in ALL-FIT luer columns, compatible with most ABI & Expedite instruments (not ABI 3900), and many are available as pipette-tip (*e.g.* MerMade or ABI3900) columns. Customers buying bulk supports may wish to source empty columns.



0020



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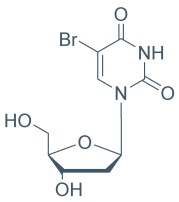
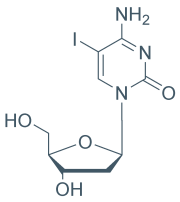
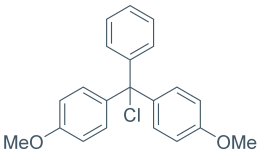


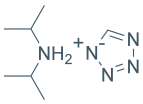
0079



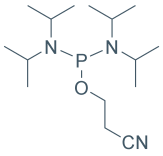
0120



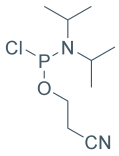
0021



1001



1002



1028

Ordering Miscellaneous Products

Product	Pack Size	Cat. No.
TOP DNA Tubes, 150mg	Pack of 96	9003-P038
TOP RNA Tubes, 100mg	Pack of 96	9004-P038
TOP DNA Jr, Bond-Elut®, 150mg	Pack of 50	9005-P039
TOP Tubes, 50mg (Gravity Flow)	Pack of 96	9006-P038
5-I-dU	10g	0020-C010
	25g	0020-C025
5-Br-dC	5g	0065-C005
	10g	0065-C010
5-Br-dU	5g	0079-C005
	10g	0079-C010
5-I-dC	5g	0120-C005
	10g	0120-C010

Product	Pack Size	Cat. No.
4,4'-Dimethoxy-trityl chloride	100g	0021-C100
	250g	0021-C250
DIHT**	10g	1001-C010
Phosphitylating Reagent	10g	1002-C010
Chloro-phosphitylating Reagent*	10g	1028-C010
Expedite Vial Pack	30ml	2100-Z001
MerMade Vial Pack	15ml	2174-Z001
MerMade Vial Pack	30ml	2173-Z001
Empty ABI3900 Columns (w/ frits)	10 x 0.2/1µmol	0849-Z002
Empty ALL-FIT Luer Columns (ABI394 & Expedite 8909) (w/ frits)	10 x 0.2/1µmol	0256-Z002
Empty MerMade Columns (w/ frits)	10x50nmol	0287-Z002
	10 x 0.2/1µmol	0271-Z002

**Only available in mainland UK. *Classified as hazardous for shipping.

Oligonucleotide Synthesis & Bioconjugation Protocols

Completely revised and updated for this edition of the Guide, full details for the use of all our products.

Introduction

Unless otherwise stated, all phosphoramidites are dissolved in anhydrous acetonitrile, according to the dissolution data starting on page 170, for use on an automated oligo synthesiser. Protocols for the coupling, cleavage and deprotection for all products are listed below. Unless otherwise stated, standard deblock, capping and oxidation conditions are as per your synthesiser instructions.

The page reference given for each product refers to the associated product ordering information in the main section of the Guide.

Please note that the information provided here is accurate to the best of our knowledge. Protocols may require optimisation depending on the synthesis platform used and customers are encouraged to consult the documentation provided by the instrument manufacturer.

Please refer to our Terms & Conditions of Sale on our web site for further details.

Dilution of non-crystalline phosphoramidites

Some users have found poor synthetic results when using an "autodilute" function on their synthesiser for non-crystalline phosphoramidites. In our experience phosphoramidites that take the form of oils, gums or glasses must be dissolved in the appropriate diluent, left for 10min, then vortexed prior to attachment of the bottle to the oligo synthesiser. Do not use autodilute.

Common products in this category include CPR, spacer phosphoramidites (including dSpacer), 5'-thiol- & amino-modifiers, 5'-carboxylate modifier, and the 5'-cholesterol modifier.

See the relevant product protocol for further information.

Protocols for Solid-Phase Oligonucleotide Synthesis using Unmodified Phosphoramidites

2003	Bz-dA-CE Phosphoramidite	26
2004	Bz-dC-CE Phosphoramidite	26
2034	Ac-dC-CE Phosphoramidite	26
2002	iBu-dG-CE Phosphoramidite	26
2030	dmf-dG-CE Phosphoramidite	26
2001	dT-CE Phosphoramidite	26
3140/2	ETT Activator (0.25M)	27
3145/6	ETT Activator (0.5M)	27
3160/2	BTT Activator (0.3M)	27
4010	Cap Mix A: THF/lutidine/acetic anhydride (8:1:1)	27
4012	Cap Mix A: THF/acetic anhydride (9:1)	27
4050	Diluent/Anhydrous Wash	27
4110	Cap Mix A: THF/pyridine/acetic anhydride (8:1:1)	27

4120	Cap Mix B: 10% Methylimidazole in THF	27
4122	Cap Mix B: 10% Methylimidazole in THF/pyridine (8:1)	27
4132	Oxidiser: 0.02M Iodine in THF/ pyridine/water (89.6:0.4:10)	27
4140	Deblock Mix: 3% TCA in DCM	27
4230	Oxidiser: 0.1M Iodine in THF/ pyridine/water (78:20:2)	27
4330	Oxidiser: 0.02M Iodine in THF/ pyridine/water (7:2:1)	27
Various	SynBase™ CPG Solid Supports	27

The Synthesis Cycle

Conventional automated solid-phase oligonucleotide synthesis is performed in a small synthesis column into which the solid support (typically CPG or polystyrene) has been packed. A solid support is selected functionalised with the first base (or modification) required at the 3'-end of the oligonucleotide. The synthesis cycle is then carried out on the instrument as per the manufacturer's instructions. All steps are carried out under positive argon pressure, principally to prevent exposure of the reactive P^{III} species to air. Timings of the steps will vary with instrument type, but will in general consist of the following:

Deblocking

The 5'-DMTr group of the 5'-terminal base is removed by brief exposure to a 'deblocking' acid, typically 3% trichloroacetic acid (TCA) in dichloromethane (DCM) (**4140**), but also 3% dichloroacetic acid (DCA) in DCM (**4040**) or 5% DCA in toluene (**4500**).[231] The spectrophotometric assay of the resultant trityl cation can be measured to monitor the efficiency of the synthesis reaction.

Activation & Coupling

To add the next base to the deblocked 5'-OH, the appropriate phosphoramidite is first activated. This is typically achieved using either a tetrazole-type (0.3M BTT (**3160**/**3162**), 0.25M ETT (**3140**/**3142**) or 0.5M ETT (**3145**/**3146**)) or an imidazole-type (0.25M DCI). The activated species is then reacted with the 5'-OH to give a trivalent phosphite triester. Typically the coupling reaction uses a 20-fold molar excess of activator and a 5-fold molar excess of phosphoramidite with respect to the starting scale of the synthesis column. Coupling times vary depending on the phosphoramidite being used. Unmodified base coupling is typically 30s but modifiers tend to need much longer (typically 5-10min). It must also be noted that some modifiers require specific activators for efficient coupling.

Capping

While the coupling reaction is very efficient (generally 98-99%), a very small percentage of 5'-OH remains unreacted. These reactive groups are capped, typically by acetylation, to prevent reaction during subsequent couplings leading to deletion sequences. The acetylation is achieved using a mixture of acetic anhydride (Cap Mix A (**4110**/**4012**)) and N-methylimidazole (Cap Mix B (**4120**/**4122**)) in the presence of base (typically pyridine or lutidine). There is no noticeable difference in the choice of base. Note that for fast deprotection amidites, and some modifiers, Pac-anhydride (**4210**) is required to avoid transamination.

Oxidation

The unstable trivalent phosphite triester is oxidised to the stable pentavalent phosphotriester, by use of either 0.1M or 0.02M iodine in THF/pyridine/water (**4230**/**4330**/**4132**). The oxidation step completes a single base cycle of the oligonucleotide synthesis (although for longer oligos a second capping step is often carried out after the oxidation). Care must be taken with some sensitive modifiers in choosing an oxidiser, avoiding the use of 0.1M iodine.

For the synthesis of phosphorothioate oligos (see page 138), the oxidation step is replaced by a sulphurisation. However, the capping step must be carried out after the sulphurisation, not before.

Cleavage & Deprotection

After synthesis, the oligonucleotide is cleaved from the support and deprotected. Traditionally, this is a two-step process; cleave then deprotect. However, today it is more usual for this to be carried out in one step. This is particularly true where gas phase deprotections are employed using gaseous anhydrous ammonia. In this case, the fully deprotected oligonucleotide is eluted from the support either with water, ready for quantification, or buffer, ready for purification. This method is particularly useful for high throughput synthesis. In general the deprotection conditions are determined by the modification type incorporated into the oligonucleotide. They can also be determined by the nucleobase protection used to synthesise the oligo backbone. See information

231 Please note that items **4040** and **4500** are not listed as standard catalogue items, but are available in bottle sizes and types by request.

on pages 156-169 for an overview of commonly used solution phase cleavage/deprotection conditions and the modifications they are compatible with.

Standard DNA bases protected with traditional groups (Bz-dA (**2003**), Bz-dC (**2004**) and iBu-dG (**2002**)) are generally deprotected using ammonium hydroxide solution. This deprotection is generally slow and not compatible with all modifications. The introduction of Ac-dC (**2034**) and dmf-dG (**2030**), and the corresponding RNA bases, allow much faster deprotection using AMA (1:1 mixture of 28% (or higher) ammonium hydroxide and 40% aqueous methylamine). Additionally these allowed deprotection of oligonucleotides containing sensitive modifcations such as TAMRA where deprotection with ^{t}butylamine/MeOH/water (1:1:2) is required. However, there was still the need for even milder deprotection. This became possible with Pac-protected amidites where it is possible to deprotect the nucleobases with potassium carbonate solution.

The use of the monomer set Bz-dA, Ac-dC and dmf-dG allows deprotection in AMA (10min at 65°C) provided any modifications present are compatible with this.

At this point, oligos are generally purified and 'desalted' (see below).

DMT ON Synthesis

For various reasons (*e.g.* to aid purification), an oligonucleotide is synthesised "DMT ON" and the final dimethoxytrityl group only removed during or after purification. To achieve this there is no treatment with deblock after the final phosphoramidite addition. The oligo is cleaved and deprotected as required. If the DMTr group is retained during purification this can be removed by treatment with acid (80% acetic acid in water).

Storage & Stability

Unmodified phosphoramidites are refrigerated at 2 to 8°C. Stability in anhydrous acetonitrile solution is 2-3 days. Modified phosphoramidites are generally less stable and, in some cases, must be used on the day of dilution. Refer to the information in the subsequent pages for guidance on each modifier.

Use of Activators

Traditionally, tetrazole was used as the activator during oligonucleotide synthesis. Although this was chemically efficient for DNA synthesis, there were several drawbacks. Tetrazole has a tendency to precipitate from acetonitrile solution due to its low solubility. This is especially true in winter months, where it was not uncommon for tetrazole to precipitate on the synthesiser overnight resulting in blocked lines. Coupling efficiency during RNA synthesis, or while using other sterically hindered phosphoramidites, was greatly reduced in comparison to DNA synthesis. Finally, tetrazole is classed as an explosive which makes transportation of the product, even in solution, costly or impossible.

There are many alternative activators available today, the most common used being 0.25M ETT (**3140**/**3142**), 0.3M BTT (**3160**/**3162**) and DCI (**3150**). All of these are much more soluble than tetrazole in acetonitrile thus resolving the crystallisation problem. In terms of coupling efficiency in DNA synthesis, they are all comparable with tetrazole. However, they are much more efficient when used with sterically hindered phosphoramidites such as 2'-OTBDMS RNA monomers. In fact, using 0.3M BTT has been reported to reduce the coupling time of 2'-OTBDMS protected RNA amidites to 3min rather than the 12-15min required when using tetrazole. In RNA synthesis, using BTT as an activator has brought coupling efficiencies in line with DNA synthesis, i.e >99%. The same applies to the use of 0.5M ETT (**3145**/**3146**).

One drawback is using ETT or BTT rather than tetrazole is that they are more acidic (pK_a: ETT 4.3; BTT 4.1; tetrazole 4.89). This can be problematic when synthesising on a larger scale (>10-15µmol) or for very long oligos. In this case DCI (pK_a 5.2) is recommended to avoid depurination or loss of trityl groups during coupling.

Preparation of BTT & ETT Solutions

Rather than use pre-mixed solutions, some customers prefer to prepare their own activator solutions. This gives the flexibility of making only the quantity required for synthesis. There is also the added advantage that shipping the solids does not incur a hazardous charge.

Crystalline BTT (**0234**) and ETT (**0237**) are provided in three pack sizes (1g, 10g and 25g) for dilution to customers' required concentrations in whatever synthesiser bottle is appropriate (larger bulk amounts can be provided on request). Typical dilution information is given below (these examples equate to routine bottle sizes and concentrations, however, obviously a range of pack sizes is provided to give customers flexibility in this).

Product	M.Wt.	200ml	450ml
BTT (0.3M)	192.24	11.53g	25.95g
ETT (0.25M)	130.17	6.51g	14.64g
ETT (0.5M)	130.17	13.02g	29.29g

To prepare the solution simply weigh out the desired amount of product into your instrument bottle and dilute by adding filtered, anhydrous acetonitrile (Diluent, **4050**) as indicated. Ensure that the solid is entirely dissolved prior to use. This is essential to avoid blockages on the synthesiser. Use end line filters on the activator bottle position, again to prevent any blockages. Filtering under argon after dissolution is preferable, but must be completed under anhydrous and inert conditions.

Manual Phosphoramidite Addition

Sometimes, perhaps to minimise the use of an expensive modifier, you may wish to perform a manual addition of a phosphoramidite in oligonucleotide synthesis. A protocol for this using a ABI 394 DNA/RNA synthesiser is detailed below (but can be adapted for use with other synthesisers).

1. Synthesise the oligo up to the point of the addition of the modification (DMT OFF).
2. Take up 250µl of activator in a syringe and pass this through the column.
3. Place the column back on the line that attaches to the top of the synthesis column on one of the column positions on the synthesiser (do not attach the bottom of the column to the synthesiser).
4. Carry out a reverse flush (on an ABI 394: Manual, Function 2 and choose the column position you need to use).
5. Remove the column from the synthesiser.
6. Take up 150µl of the amidite in a syringe and load this onto the column containing the part-synthesised oligo.
7. Take up 150µl of the activator and load this onto the other end of the column such that the two reagents (amidite and activator) mix on the column.
8. Pass the reagent backwards and forwards through the column with the syringes for around 1min.
9. Leave the coupling reaction to stand for the desired coupling time occasionally passing the reagents between the syringes.
10. Remove the reagent from the column and place on one of the column positions on the synthesiser.
11. Wash the excess reagents from the column with acetonitrile (on an ABI 394: Manual, Function 42 and choose the column position you need to use).
12. Carry out a reverse flush as per step 4.

You can now either carry out the oxidation and capping steps between syringes with washing and drying steps to remove the excess reagents in between or you can run a "synthesis" (*e.g.* 5T) where there is a connector (union) in the column position and acetonitrile in the "amidite" position (on an ABI 394: bottle position 5) and swap the connector for the column just after the coupling step so that all other steps are carried out on the synthesiser.

If the modification is at an internal position within the oligo then the remainder of the sequence must now be completed.

Purification Methods

Solvents are removed from oligonucleotides by evaporation under vacuum or freeze-drying. Before an oligonucleotide is purified the sequence must be considered carefully for secondary structure. This includes runs of three or more G units, hairpin loops, or other self-complementary regions. Secondary structure in an oligonucleotide will determine which chromatographic method is chosen for purification.

Modifications must also be considered since some require very specific purification protocols, especially where combinations of modifiers are used.

Ethanol Precipitation

This is primarily used when no purification is required on the oligonucleotide. In this case the oligo is dissolved in a high salt concentration (typically 0.3M sodium acetate) and precipitated from the solution by the addition of ethanol. This removes residual salts and by-products from the deprotection step. For this reason ethanol precipitations are often carried out prior to chromatographic purifications (*e.g.* HPLC, PAGE).

Gel Filtration (or Gel Permeation Chromatography)

This is one of the simplest forms of chromatography. It is based on separation of molecules by size. It is particularly useful in removing buffer salts such as sodium chloride and ammonium acetate, a process know as 'desalting'. As such this is generally used after purification to remove residual salts.

Gel filtration is also useful in removing very small (<10 bases) failure sequences from oligo mixtures. This is often carried out prior to purification or if an oligo is going to be used in its crude form.

Commonly used are Nap 10 or Nap 25 sephadex columns. The column type is dependent on the quantity of oligo and the quantity of salt being removed. FPLC and IE purified oligos require two gel filtration steps due to the large amount of salt used in the buffers.

Although dependent on the oligo, it is sometimes advantageous to precipitate the oligo prior to gel filtration, *e.g.* where high salt concentrations have been used.

Reverse-Phase HPLC

Reverse-phase high-performance liquid chromatography (RP-HPLC) is effective in purifying oligonucleotides by separation through hydrophobic differences in the molecules. Crude mixtures of oligos will contain the main product and failure sequences, all of which will be of varying sequence and length and therefore hydrophobicity. These differences can be very subtle, however the technique is generally sensitive enough providing that the oligo is under 40 bases in length.

Again, the sequence and modifications used must also be considered in choosing the RP-HPLC conditions.

The limitations of RP-HPLC are that long oligos, due to steric hindrance, cannot enter the pores of the chromatography column and resolution is decreased. In addition, any secondary structure in the oligo can mask the hydrophobicity of chemically similar oligos to the extent that separation of these is virtually impossible. If the secondary structure is severe the product can elute a series of peaks that represents a population of oligos of identical length and sequence that have different degrees of secondary structure.

Ion-exchange (IE) HPLC

This technique separates oligonucleotides on the basis of charge differences. Each oligo in the crude mixture has a different net charge based on the number of phosphate groups in the molecule (base length) and on the respective charges on the heterocyclic bases (base composition).

Separation of the crude mixture is accomplished by slowly increasing the ionic strength of the mobile phase (*i.e.* increasing the percentage of salt). By doing so, the longer, more charged oligos will elute later than the shorter ones. This method minimises the effect of secondary structure due to the high salt concentration, which is further reduced by running the purification at elevated temperatures.

However, there are limits in terms of the length of the oligo that can be successfully purified using this technique. In general, oligos >80 bases do not separate well from N-1 failures and other techniques have to be used.

FPLC

FPLC (Fast-protein Liquid Chromatography) is the purification method of choice for oligonucleotides that are particularly long (>40 bases) and/or have extensive secondary structure. This is because FPLC allows the use of high pH buffers, thus ensuring that the oligo is completely denatured during purification (*i.e.* there is no secondary structure as the pH is too high for duplex formation).

This has been successfully used to purify oligos >100 bases. However, delicate modifications such as cyanine dyes or TAMRA do not withstand the high pH conditions. This method is not suitable for RNA.

Polyacrylamide Gel Electrophoresis (PAGE)

The separation of oligos using PAGE utilises the effect of the charge and the molecular weight of the oligo under the influence of an electric field.

The acrylamide content of the gel is adjusted to suit the length of the oligo being purified to ensure that the slowest running product on the gel is the desired one. This is particularly good for purifying oligos >80 bases and, in general, N-1 failures are easily removed.

There are very few modifications that are not compatible with PAGE purification. In general this is the result of a combination of modifications, *e.g.* 2'-OMe with full PS linkages.

Cartridge Purification

There are many purification cartridges commercially available such as PolyPak, MOP, TOPs and Clarity. These all work on the same principle.

The oligo is synthesised DMT ON and the hydrophobicity of the DMTr group holds the full length oligo on the column and the non-DMTr containing failures are eluted from the the column. 2% TFA or DCA solution is passed through the column, removing the DMTr group from the oligo which is then eluted in buffer.

There are many advantages to using cartridge purification: fast; high-throughput; one column per oligo therefore low risk of cross-contamination. The disadvantages are that it is not suitable for all modifications and, if the oligo is purine-rich, there is a risk of depurination when removing the DMTr group.

Oligonucleotide purification using TOP-DNA, TOP-RNA and TOP-DNA Jnr cartridges

Solution Preparation details

100mg/mL NaCl

1. Weigh 10g of sodium chloride into a 100mL volumetric flask.
2. Add water to give a total volume of 100mL.
3. Mix thoroughly.

2% TFA solution

NOTE this solution needs to be made fresh on the day of use.

1. Measure 2mL of TFA and add to a 100mL bottle.
2. Add 98mL of water.

MeCN/water

1. Measure 500mL of MeCN and add to a 1L bottle.
2. Measure 500mL of water and add to the MeCN.
3. Mix thoroughly.

2M Tris HCl(aq)

1. Weigh out 31.53g into a 100mL volumetric flask.
2. Add water to give a final volume of 100mL.
3. Mix thoroughly.

1M ammonium bicarbonate / 30% MeCN

1. Weigh out 7.91g into a 100mL volumetric flask.
2. Add water to give a final volume of 100mL.
3. Mix thoroughly.

TOP-DNA Purification

Do not remove the deprotection solution or desalt the oligo. The deprotection solution needs to be present.

1. Add 1mL of aqueous sodium chloride solution (100mg/mL) to each oligo.
2. Place one column for each oligo onto the vacuum manifold.
3. Turn the vacuum pump on.
4. Adjust the pressure to 7.0in (178mm) Hg using the vacuum control valve.
5. Add 0.5mL of acetonitrile to the cartridge.
6. Immediately then add 1mL of 2MTEAA to condition the medium.
7. Add the oligo solution to the cartridge in 1mL aliquots.
8. Add 1mL of NaCl(aq) solution (100mg/mL) to the column.
9. Add 1mL of NaCl(aq) solution (100mg/mL) to the column.
10. Add 1mL of 2% TFA/water solution to the column.
11. Add 1mL of 2% TFA/water solution to the column.
12. Add 1mL of water to the column.
13. Add 1mL of water to the column.
14. Release the vacuum.
15. Place an appropriately labelled tube beneath each of the columns.
16. Add 1mL of MeCN/water 1:1 to elute the DNA oligo under vacuum.

TOP-DNA Jnr (Bond Elut®) Purification

Do not remove the deprotection solution or desalt the oligo. The deprotection solution needs to be present.

1. Add 1mL of aqueous sodium chloride solution (100mg/mL) to each oligo.
2. Appropriately label one cartridge for each oligo to be purified.
3. Pass 0.5mL of acetonitrile slowly through the cartridge.
4. Immediately then pass 1mL of 2MTEAA to condition the medium.
5. Add the oligo solution to the cartridge in 1mL aliquots by slowly passing through with a syringe.
6. Slowly pass 1mL of NaCl(aq) solution (100mg/mL) through the cartridge.
7. Slowly pass 1mL of NaCl(aq) solution (100mg/mL) through the cartridge.
8. Slowly pass 1mL of 2% TFA/water solution through the cartridge.
9. Slowly pass 1mL of 2% TFA/water solution through the cartridge.
10. Slowly pass 1mL of water through the cartridge.
11. Slowly pass 1mL of water through the cartridge.
12. Place an appropriately labelled tube beneath each of the cartridges.
13. Slowly pass 1mL of MeCN/water 1:1 to elute the DNA oligo collecting into the labelled tube.

TOP-RNA Purification (with TBDMS RNA Chemistry)

Do not remove the deprotection solution or desalt the oligo. The deprotection solution needs to be present. Use RNase free solutions for this procedure.

1. Add 1.75mL of aqueous 2M TrisHCl solution to each oligo.
2. Place one column for each oligo onto the vacuum manifold.
3. Turn the vacuum pump on.
4. Adjust the pressure to 7.0in (178mm) Hg using the vacuum control valve.
5. Add 0.5mL of acetonitrile to the cartridge.
6. Immediately then add 1mL of 2M TEAA to condition the medium.
7. Add the oligo solution to the cartridge in 1mL aliquots.

8. Add 1mL of MeCN/2MTEAA, pH7.0 solution (1:9, v:v) to the column.
9. Add 1mL of water to the column.
10. Add 1mL of 2% TFA/water solution to the column.
11. Add 1mL of 2% TFA/water solution to the column.
12. Add 1mL of water to the column.
13. Add 1mL of water to the column.
14. Release the vacuum.
15. Place an appropriately labelled tube beneath each of the columns.
16. Add 1mL of 1M ammonium bicarbonate(aq)/30% MeCN solution to elute the RNA oligo under vacuum.

Use of Universal Supports

2304	Universal SynBase™ CPG 1000/110	30
2300	Universal Q SynBase™ CPG 500/110	30
2410	Universal Q SynBase™ CPG 1000/110 S	30
2411	Universal Q SynBase™ CPG 1000/110 H	30

Rather than having predetermined bases or modifications on the solid support, the use of universal supports allows the addition of 3'- base or modification to the support. This is useful for many reasons. It allows preparation of plates (96, 384 *etc*) in an easy and efficient manner without the risk of the wrong solid support being added to the well. It also allows the incorporation of base modifications at the 3'-end of an oligo where no modified solid support is commercially available, *e.g.* 2'-F-dU.

Deblocking

An initial additional detritylation is recommended as this step is much slower on universal supports than on nucleoside supports. This prevents N-1 oligos from the 3'-end.

Coupling

No changes are required from the standard synthesis method recommended by the synthesiser manufacturer. This is therefore determined by the oligo type and any modification present.

Oxidation

2304 - No changes are required from the standard synthesis method recommended by the synthesiser manufacturer.

2300/2410/2411 - The linker is stable to capping mixtures, but is slightly labile in oxidiser solution (8% cleavage overnight which is the equivalent of approximately 2000 synthesis cycles on an average program).

Cleavage & Deprotection

2304 - In order to completely remove the universal linker one of the following need to be used: (1) ammonium hydroxide solution, 17h at 80°C; (2) AMA, 5h at 80°C; or (3) AMA, overnight at 55°C.

2300/2410/2411 - Contrary to previously published mild methods, the best conditions we have found to completely remove the Q linker during deprotection are AMA at 70°C for 2.5h or AMA at 80°C for 2h. For this reason it is inadvisable to use this support with modifications that require mild, ultramild or room temperature deprotection. Compatibilty with RNA is therefore mixed. We would not recommend the use of the support with TBDMS chemistry.

Storage & Stability

Refrigerate at 2-8°C. Material is stable for several years.

Use of UltraMILD DNA Reagents

2059	Pac-dA-CE Phosphoramidite	31
2060	iPr-Pac-dG-CE Phosphoramidite	31
2290	Pac-dA-SynBase™ CPG 1000/110	31
2292	iPr-Pac-dG-SynBase™ CPG 1000/110	31
4210	Cap Mix A: THF/pyridine/Pac-anhydride (85:10:5)	27

Although Ac-dC-CE Phosphoramidite (**2034**) can be deprotected under UltraMILD conditions this monomer is routinely used under standard and UltraFAST conditions. Customers should refer to page 116 for use of this product and the analogous CPG supports.

Coupling

No changes are required from the standard synthesiser procedure. If many dG residues are included in the oligonucleotide, we recommend the use of phenoxyacetic anhydride in Cap Mix A (**4210**). This removes the possibility of exchange of the iPr-Pac protecting group on the dG moiety with acetate from the commonly used acetic anhydride capping mix.

Deprotection & Purification

We no longer stock UltraMILD Deprotection solution (0.05M Potassium Carbonate in Methanol). This is because in our experience this reagent works best when freshly prepared just prior to use. To prepare 100ml of such a solution:

1. To a suitable volumetric calibrated vessel, add 0.69g potassium carbonate (K_2CO_3).
2. Add dry methanol to a volume of 100ml.
3. Stir, protected from moisture, until dissolved leaving overnight if necessary.

Cleavage and deprotection can be carried out in 4-17h at room temperature with this solution (alternatively use AMA at room temperature for 2h; ultimately the overall protocol will be dependent on the requirements of the base-sensitive modifications being used). Cartridge purification can be performed directly after deprotection.

Typical Protocol

1. Carry out the synthesis of the modified oligonucleotide.
2. Open the synthesis column, or push the resin out and transfer the support to a suitable reaction vial.
3. Treat the support with 0.5ml of 0.05M potassium carbonate in anhydrous methanol for a minimum of 4h at room temperature. For oligos with a high dG content reaction overnight is recommended.
4. Desalt with a G25 column.

Storage & Stability

Phosphoramidites and supports are stored refrigerated at 2 to 8°C. Phosphoramidite solutions are best used within 24h.

RNA Synthesis

RNA Synthesis using 2'-O-TBDMS Protection

2033	dmf-G-CE Phosphoramidite	34
2036	Bz-A-CE Phosphoramidite	34
2037	Pac-A-CE Phosphoramidite	34
2038	Ac-C-CE Phosphoramidite	34
2039	iPr-Pac-G-CE Phosphoramidite	34
2040	U-CE Phosphoramidite	34
2295	U SynBase™ CPG 1000/110	34
2309	Ac-C SynBase™ CPG 1000/110	34
2318	dmf-G SynBase™ CPG 1000/110	34
2319	Pac-A SynBase™ CPG 1000/110	34
2320	iPr-Pac-G SynBase™ CPG 1000/110	34
2321	Bz-A SynBase™ CPG 1000/110	34

Coupling

The steric hindrance introduced by the additional 2'-protecting group reduces the coupling efficiency of the phosphoramidite, and is limited to >97% using tetrazole activator even with extended reaction times. When using 0.45M tetrazole, 0.25M ETT (**3140**/**3142**) or 0.25M DCI (**3150**), to optimise efficiency a 12min coupling time is recommended. It has been shown that this coupling time can be reduced to 3min when using 0.3M BTT as the activator (**0234**/**3160**/**3162**).

Cleavage & Deprotection

Removal of the base protecting groups, cleavage of the linkage to the support and removal of the ß-cyanoethyl groups are all achieved routinely with aqueous 20% NH_3/methylamine (1:1). The cleavage is fast (10-30min) at

65°C.[232]

The deprotection strategies used are dependent on the base-protection utilised. Bz-A (**2036**), dmf-G (**2033**) and Ac-C (**2038**) can also be deprotected using AMA (1:1 ammonium hydroxide solution in aqueous methylamine) at 65°C for 10min, however it should be noted that the use of ethanol in the deprotection solution aids solubility for full RNA sequences. When synthesising RNA chimera, *e.g.* RNA/DNA or RNA/2'-OMe RNA, the C amidite cannot be Bz protected otherwise deprotection with AMA will result in transamidation.

Pac-A (**2037**), iPr-Pac-G (**2039**) and Ac-C (**2038**) can be deprotected using UltraMILD conditions such as 0.05M potassium carbonate in methanol at room temperature for 4h.

Desilylation

The desilylation procedure is the same for DMT ON and DMT OFF oligos. Suspend the residue in dry N-methyl pyrrolidone/Et_3N/Et_3N.3HF (6:3:4 v/v/v) and deprotect silyl groups for 2.5h at 65°C in a sealed sterile tube. Alternatively, DMSO (or DMF)/Et_3N.3HF (3:1 v/v) can be used.

Detritylation & Purification

Retaining the DMTr group after oligonucleotide synthesis is advantageous to the purification of RNA. This allows failures with no 5'-DMTr protection to be easily removed from the crude mixture. Traditionally this was carried out by RP-HPLC where the full-length DMTr-protected oligonucleotide was collected then detritylated using 10% acetic acid at pH 3.5 (30-45min) then quenched with ammonium bicarbonate followed by desalting.

Today it is possible to carry out the purification by RP-HPLC which includes the detritylation step and the full-length detritylated product is collected. Also, cartridge purification - *e.g.* TOPS, PolyPak or GlenPak - allow fast processing of oligonucleotides where DMTr-oligos are loaded onto the column and the full-length detritylated product eluted. This is particularly useful in preventing cross contamination since a new column is used for each oligo. DMT-ON purification, although useful, does not remove the N-1 sequences where the DMTr protection is retained. In this case cartridge purification can be useful to remove all other failures prior to purification by IE-HPLC. The latter is also the recommended method for purification of RNA where the DMTr group has been removed, or where the sequence is longer (≥40 bases). The most commonly used IE columns for purifying RNA are Dionex DNA-PAC (100 or 200) columns although there are alternatives from Phenomenex and other suppliers.

Typical buffers are:

Anion-exchange HPLC—A: 20mM aqueous Tris-HCl pH 7.6 + 1mM EDTA + 10mM sodium perchlorate; B: As A with 600mM sodium perchlorate. This system may require alteration for longer oligos.

RP-HPLC—A: 95% 0.1M triethylammonium acetate (TEAA) + 5% acetonitrile; B: 95% acetonitrile + 5% water (or 5% 0.1M TEAA). Gradient of 0-50% B during 20min.

Much longer oligos are best synthesised DMT-OFF and purified by PAGE.

Storage & Stability

All solid phosphoramidites and RNA supports are stored dry in a freezer at –10 to –30°C. The phosphoramidites are stable in anhydrous acetonitrile solution for 24h.

Unlike DNA, RNA is highly unstable in basic media and must not be exposed to high pH. Traces of heavy metal ions present in various salts used also lead to degradation. Buffers containing 1mM EDTA are used to prevent this.

RNA is very sensitive to degradation by nucleases. All glass (and plastic) used must be rinsed in water containing diethylpyrocarbonate (DEPC) (2%) and autoclaved before re-use. All reagents, water and consumables used in subsequent handling must be sterile and certified RNAse free. Anion-exchange and desalting columns must be stored in sterile water/acetonitrile (1:1), except Q-res type which are stored in water/ethanol (4:1).

232 Heating can be continued up to 1h, however prolonged treatment will cause gradual loss of TBDMS groups resulting in degradation.

Spacer Modification

2113	Spacer-CE Phosphoramidite C3	38
2128	Spacer-CE Phosphoramidite 9	38
2129	Spacer-CE Phosphoramidite 18	38
2146	dSpacer-CE Phosphoramidite	38
2147	Spacer-CE Phosphoramidite C12	38
2245	3'-Spacer-C3 SynBase™ CPG 1000/110	38
2395	3'-Spacer-C3 SynBase™ CPG 3000/110	38

Dissolution

Prepare the amidite solutions 5-10min before use. It is recommended these are vortexed to ensure complete dissolution before placing on the synthesiser. See notes on page 116.

Coupling, Cleavage & Deprotection

For all spacer phosphoramidites, no changes are required from standard synthesiser protocols. Likewise, these are all compatible with most deprotection conditions. See information on pages 156-169 for more details.

As with all non-nucleosidic modifications, using **2245** or **2395** requires an additional detritylation prior to use in synthesis. It is therefore important not to use a cycle with an initial capping step. This minimises the formation of N-1 product at the 3' end. Cleavage and deprotection is achieved using the protocol required by the nucleobases. The cleavage and deprotection can also be done in one step by placing the resin in the deprotection solution then using the conditions required for deprotecting the nucleobases. See information on pages 156-169.

Storage & Stability

The products are stored dry in a freezer at –10 to –30°C. Phosphoramidite stability in solution is 2-3 days.

Modification for Conjugation

5'-Amino Modification

2123	5'-MMT-Amino-Modifier-C6-CE Phosphoramidite	42
2124	5'-TFA-Amino-Modifier-C6-CE Phosphoramidite	42
2133	5'-MMT-Amino-Modifier-C12-CE Phosphoramidite	42
2182	5'-TFA-Amino-Modifier 11 CE Phosphoramidite	42
2193	5'-MMT-Amino-Modifier 11 CE Phosphoramidite	42
2532	5'-TFA-Amino-Modifier-C12-CE Phosphoramidite	42
2534	5'-TFA-Amino-Modifier-C5-CE Phosphoramidite	42

Dissolution

For all, prepare the amidite solution 5-10min before placing on the synthesiser to ensure complete dissolution. See notes on page 116.

Coupling

A 5min coupling time is recommended for all except **2193**.

2193 - A 2min (120s) coupling time is recommended.

Deblocking

For TFA-protected 5'-amino modifiers (**2124** and **2182**) this final step is not required. For MMT 5'-modifiers (**2123**, **2133** and **2193**) it is better to retain the MMT protection and remove this during or after purification.

Cleavage, Deprotection & Purification

For all amino modifiers it is recommended that the synthesis column is washed with 10-20% DEA/acetonitrile for 3min prior to cleavage from the support. This will remove the cyanoethyl groups that can react with the free amino or thio groups during deprotection, blocking these sites with acrylonitrile. The resin must then be

thoroughly washed (acetonitrile) and dried (Ar, He, N_2, air) prior to cleavage and base deprotection.

If fast deprotection on the nucleobases (G, C) is used, *e.g.* Ac-dC and/or dmf-dG, cleavage and deprotection can be carried out in either AMA (35min at 65˚C or 2h at RT) or ammonium hydroxide solution (2h at 55˚C). If Bz-dC and iBu-dG are used then cleavage and deprotection is achieved using ammonium hydroxide solution (2.5h at 70˚C, 4h at 55˚C, or overnight at 55˚C).

For TFA-protected modifiers the TFA group is lost during deprotection. If the intended use is post-synthesis labelling the oligo with an active ester (*e.g.* TAMRA, **0251**), it is recommended that the oligo is desalted prior to the labelling reaction.

MMT protection is stable to base treatment and this can be used to aid purification in *e.g.* "DMT ON" HPLC or cartridge purification. If, after purification or desalting, the MMT group still needs to be removed this is typically carried out using 80% acetic acid/water for 30min and the oligo is immediately desalted.

Determination of coupling efficiency for MMT Modifiers

This procedure is used solely for the purpose of determining coupling efficiency; it should not be used for routine deprotection. The calculation is based on a comparison of the absorbance of the MMT cation at 472nm versus the absorbance of the DMTr cation at 497nm. The ratio factor (RF) of the absorbance of a standard solution is:

$$RF = A\text{-}DMT(497)/A\text{-}MMT(472) = 1.33$$

1. Carry out the synthesis (1µmol) in the DMT ON mode.
2. Collect the last DMTr solution in a 100ml volumetric flask and make up to the mark with 0.1M toluenesulphonic acid in anhydrous acetonitrile (TSA). Zero the instrument with TSA at 497nm and then measure the absorbance (A1) at 497nm.
3. After the synthesis is complete, remove the synthesis column and manually deblock the MMT group with aliquots of the normal deblocking mix until all the yellow colour has eluted. This process takes up to 15min. Collect the solution in a 100ml volumetric flask and make to 100ml with TSA. Measure the absorbance (A2) at 472nm.
4. Calculate the coupling efficiency using the formula:

$$\text{Coupling (\%)} = A2/A1 \times 1.33 \times 100$$

Note that, due to incomplete deblocking of the MMT group, this procedure yields a coupling efficiency determination about 5% below the actual coupling efficiency.

The modified oligonucleotide may be purified using a cartridge, HPLC, or gel electrophoresis. Cartridge purification is accomplished using the DMT ON procedure. MMT removal on the cartridge is not reliable with a standard 2% aqueous trifluoroacetic acid (TFA) wash, therefore 2 additional TFA washes are required.

Alternatively, treatment of the purified DMT ON oligo (see below) with acetic acid:water (80:20) in solution at room temperature for 1h will remove the MMT group. Precipitate the oligo with an ethanol/2.5M sodium acetate precipitation. Again, complete removal is not achieved therefore additional HPLC is required.

RP-HPLC may be performed either before or after attachment of the label. If purification is desired prior to a label attachment, the MMT group should not be removed from the oligo as the lipophilic nature of the group aids in HPLC purification. RP-HPLC is best carried out using a C18 or equivalent column. The MMT group can be removed in cartridge purification or by treatment with acetic acid in solution as above.

Storage & Stability

The oils are stored dry in a freezer at –10 to –30°C. Acetonitrile solutions must be used within 24h.

Internal Amino Modification

2071	Amino-Modifier-C6-dA-CE Phosphoramidite	42
2135	Amino-Modifier-C6-dT-CE Phosphoramidite	42
2141	Amino-Modifier-C6-dC-CE Phosphoramidite	42
2149	Amino-Modifier-C2-dT-CE Phosphoramidite	42

Coupling

No changes are required from the standard method recommended by the synthesiser manufacturer. Coupling is as per unmodified nucleoside amidites.

Capping

Some users have experienced small amounts of transamidation using acetic anhydride capping reagents. To avoid this use Pac-anhydride Cap A (**4210**).

Cleavage & Deoprotection

The trifluoroacetyl (TFA) protecting group on the primary amine is removed during ammonium hydroxide solution

deprotection. However, a minor side reaction during deprotection can lead to irreversibly capping 2-5% of the amine. This could be significant if multiple additions of the modifier are made. To prevent the reaction, synthesise using acetyl-protected C and, prior to cleavage and deprotection, wash the column with 10-20% DEA/acetonitrile. Deprotect in AMA at 65°C for 15min.

Storage & Stability

Store in a freezer below -10°C. Diluted samples must be freshly prepared for use and used within 24h.

3'-Amino Modification

2350	3'-Amino-Modifier-C7 CPG 1000	42
2365	3'-PT-Amino-Modifier-C6 CPG	42
2367	3'-Amino-Modifier-C6-dT CPG	42
2369	3'-Amino-Modifier-C6-dC CPG	42
2371	3'-PT-Amino-Modifier-C3 CPG	42

Coupling

All supports are used as per unmodified nucleoside supports. However, non-nucleosidic modifications are slow to detritylate and require an additional detritylation prior to use in synthesis. In this case it is important not to use a cycle with an initial capping step. AMA deprotection will require dmf-G and Ac-C to be used.

Capping

2367 & **2369** - Some users have experienced small amounts of transamidation using acetic anhydride capping reagents. To avoid this use Pac-anhydride Cap A (**4210**).

Cleavage & Deprotection

2350 —Cleavage of the oligonucleotide requires 2h at room temperature with ammonium hydroxide or AMA. It is important to wash the column with 10-20% diisopropylamine in acetonitrile or 10-20% DEA/acetonitrile to prevent blocking with acrylonitrile during deprotection. Alternatively, 20% piperidine in DMF can be used, rather than DEA. In fact this will simultaneously remove Fmoc and cyanoethyl protection, plus remove the vinylfluorene by-product from the Fmoc deprotection reaction.

For the PT-Amino CPGs (**2371** & **2365**)—Cleavage of the oligonucleotide requires an overnight reaction at 55°C with ammonium hydroxide. This treatment will complete the deprotection of the nucleobases for all protection types. It is important to wash the column with 10-20% diisopropylamine in acetonitrile or 10-20% DEA/acetonitrile to prevent blocking with acrylonitrile during deprotection.

2369 & **2367**—Use deprotection conditions as required by the nucleobases. It is important to wash the column with 10-20% diisopropylamine in acetonitrile or 10-20% DEA/acetonitrile to prevent blocking with acrylonitrile during deprotection.

Storage & Stability

The supports are stored in a freezer at –10 to –30°C.

3'- and 5'-Thiol-Modification

2125	5'-Thiol-Modifier-C6-CE Phosphoramidite	45
2126	Thiol-Modifier-C6 S-S CE Phosphoramidite	45
2166	Thioctic Acid NHS Ester	45
2187	(Hydrophilic) S-Bz TEG-CE Phosphoramidite	45
2361	3'-Thiol-Modifier-C3 S-S CPG	45

Strategies

Unlike terminal amino linkers, which are generally designed for specific 3'- or 5'-modification, the various available terminal thiol linkers can often be used for both, depending on the sequence.

2187 is used for 5'-modification. The benzoyl protecting group was selected such that the free thiol is formed during oligonucleotide deprotection, but adding TCEP to the deprotection solution is recommended to prevent dimerisation of the oligo *via* a disulphide bridge. Therefore there is no need for the use of silver salts as is required to remove the trityl group when using **2125**. The lack of the disulphide bridge, as is present in **2126**, means better stability hence there is no drop in this amidite's performance even after being on the synthesiser for 48h.

5'-Modification can also be achieved using either **2125** or **2126** using the standard synthesis cycle. The latter offers the advantage of avoiding the use of silver nitrate in the final deprotection. Both **2126** and **2361** can be used

to achieve 3'-modification. To do this either use the **2361** support as standard or add **2126** as the first monomer to any support and continue the sequence from there. 3'-Thiol-modified oligos are particularly useful in cases where a different post-synthetically added label is desired for the 5'-terminus, in which case an amino linker is used to achieve the latter.

Whereas an amino modifier (see above) is used to add a label *via* an active ester, thiol modification allows labelling *via* a haloacetamide or maleimide derivatised label.

Terminal modification (3' or 5') with a thiol for gold or silver conjugation is easily achieved with the aforementioned modifications or **2166** can be added post-synthetically to an amino-functionalised oligo.

Use of Thiol Modifier Phosphoramidites

Dissolution

For all, prepare the amidite solution 5-10min before placing on the synthesiser to ensure complete dissolution. See notes on page 116.

Coupling

A minimum coupling time of 5min (300s) is recommended for thiol modifiers. For **2187** use 0.3M BTT or 0.25M DCI in acetonitrile. **Do not use ETT** as this reacts with the Bz-S group. A 15min (900s) coupling time is recommended.

Oxidation

2125 – It is recommended the final cycle oxidation is carried out with 0.02M (**4132/4330**) iodine to minimise oxidative cleavage of the trityl-S linkage.

2126 – For 5'-modification 0.02M iodine is used in the final cycle. For 3'-modification it is used in all oxidation steps.

Deprotection & Purification

2125 – The trityl group used to protect the thiol is not acid labile and therefore cannot be removed on a DNA synthesiser during the detritylation step. Cleavage of the oligonucleotide from the support and removal of the base protecting groups are carried out with ammonium hydroxide solution or AMA if fast deprotecting amidites are used. If purification is desired, it is best done before removing the trityl group. The presence of the trityl group allows DMT-ON RP-HPLC purification techniques to be used. Note this is not suitable for cartridge purification since, as previously mentioned, the trityl group is not acid labile.

Final deblocking of the oligonucleotide involves cleavage of the trityl-sulphur bond. This is achieved with silver nitrate, the excess silver being precipitated with TCEP. Excess TCEP is removed by desalting or by ethanol precipitation.

Typical Protocol:

1. Deprotect with ammonium hydroxide solution or AMA.
2. Purify the trityl-containing oligo by HPLC.
3. Evaporate the product solution to dryness.
4. Suspend the product in 0.1M triethylammonium acetate (TEAA), pH 6.5 at a concentration of approximately 100 ODs/ml.
5. Add 0.15 volumes of 1M aqueous silver nitrate solution, mix thoroughly, and leave to react at room temperature for 30min.
6. Add 0.20 volumes of 1M aqueous TCEP solution, mix thoroughly, and leave at room temperature for 5min.
7. Centrifuge the suspension to remove the silver-TCEP complex. Remove the supernatant. Wash the precipitate with 1 volume of 0.1M TEAA. Centrifuge and combine the supernatant with the first wash. (Alternatively, vortex the suspension and apply to a desalting column equilibrated with conjugation buffer.)
8. Remove the excess TCEP from the supernatant by desalting using a NAP-25 column and proceed directly to the conjugation reaction.

2126 – 5'-Modification:

(a) Typical Protocol – DMT OFF synthesis:

1. Add the Thiol-Modifier C6 S-S at the 5'-terminus of the oligo in the automated DMT OFF synthesis mode. The DMTr release from the last cycle can be used to determine coupling efficiency.
2. Deprotect the oligo using AMA solution or ammonium hydroxide. AMA for 2h at RT works best, however other modifications in the oligo must also be compatible.
3. Isolate, desalt, and, if necessary, purify by HPLC.
4. Evaporate to dryness.
5. Add 200µl of 87mM TCEP in water (29mg/ml) and allow to stand for 1h. Mixing the solution with the pipette aids the reaction, and frothing is generally observed.
6. Desalt. The thiol-modified oligo is ready for use.

(b) Typical Protocol – DMT ON synthesis

1. Add the Thiol-Modifier C6 S-S at the 5'-terminus of the oligo in the automated DMT ON synthesis mode.
2. Carry out deprotection in AMA for 2h at RT.
3. Purify the trityl-containing oligo on a cartridge and evaporate the solution to dryness.
4. Cleave the disulphide linkage using 87mM TCEP in water for 1h at room temperature (see notes in Step 5 above).
5. Desalt the oligo.

2126 – 3'-Modification: Similar procedures as used for **2361** are used, noting strategies above.

2187 - Treat the column with 20% DEA/MeCN prior to deprotection. This is extremely important since the free thiol is readily capped by acrylonitrile if the cyanoethyl protection is not removed prior to cleavage and deprotection of the nucleobases.

For deprotection in AMA, the temperature and time will depend on the other modifications and protecting group chemistry of the other amidites. Typically this would be AMA, 10min at 65˚C.

The addition of 100mM TCEP to the deprotection solution has been shown to prevent the formation of the dimerised oligonucleotide *via* the disulphide bridge.

Immediate conjugation is recommended otherwise treatment with TCEP to cleave the disulphide bridge is required.

Storage & Stability

The oils are stored dry in a freezer at –10 to –30°C. Stability in solution is 2-3 days.

Thiol-modified oligos are best kept dry under an inert atmosphere in the Tr-S or S-S state until ready to use. Treat with TCEP immediately before use. Note even if the oligo has previously been treated TCEP (or DTT) this will readily form a disulphide bridge to form the dimerised oligo, therefore TCEP treatment is required before use.

Oligonucleotide Modification with Thioctic Acid NHS Ester (2166) and Conjugation to Gold & Silver

3'-Modification

A published protocol for preparing an oligo modified with thioctic acid at the 3'-end is freely available.[233] This simple procedure utilises our 3'-Amino-Modifier C7 CPG product (**2350**), however either of the methods described below are applicable.

5'-Modification

These procedures assume synthesis of the oligonucleotide on a 1µmol scale. If synthesis on an alternative scale is required adjust the amounts of reagents accordingly. Expected yield from either conjugation method is approx. 50%.

(a) In-solution conjugation with 5'-amino-modified oligo:

1. Prepare 0.1M Sodium Bicarbonate solution, pH ~8.0: Weigh sodium bicarbonate solution (0.84g, 0.01mol) into a 250ml beaker. Add water (90ml) and mix thoroughly. Transfer to a 100ml volumetric flask and make up to a final volume of 100ml.
2. Prepare 0.1M Sodium Carbonate solution pH ~12: Weigh sodium carbonate monohydrate solution (1.24g, 0.01mol) into a 250ml beaker. Add water (90ml) and mix thoroughly. Transfer to a 100ml volumetric flask and make up to a final volume of 100ml.
3. Prepare 0.1M Sodium carbonate/bicarbonate buffer: Place 0.1M sodium bicarbonate solution (10ml) into a screwtop bottle. Add 0.1M sodium carbonate solution until the pH reaches 9.75.
4. Prepare 0.1M TEAA: Place 2M TEAA (50ml) into a 2L beaker. Add water (950ml) and mix thoroughly. Adjust the pH to 7.0 - 7.2 (add acetic acid to lower and triethylamine to raise the pH). Filter directly into a 1L Duran bottle.
5. Prepare 80mM thioctic acid/DMSO: Add DMSO (120µl) to a 2.5 - 3mg sample of thioctic acid NHS ester (**2166**).
6. Dry the oligonucleotide(s) on a freeze drier or evaporator.
7. To each of the oligonucleotides, add 0.1M sodium carbonate/bicarbonate buffer pH 9.75 (75µl).
8. Add thioctic acid/DMSO solution (30µl).
9. Allow to stand overnight.
10. Prepare a G25 column pre-treated with 0.1M TEAA for each of the oligos.
11. Label a 2ml sample tube for each of the oligonucleotide.
12. Place the tubes from Step 11 into the larger centrifuge tubes used for G25 columns.
13. Place a G25 column (prepared in step 10) on top of the sample tubes
14. Add the oligonucleotide/thioctic acid mixture to the top of the G25 column and centrifuge at 9000rpm.
15. The oligonucleotide is now ready for analysis or purification.

233 **Enhanced oligonucleotide-nanoparticle conjugate stability using thioctic acid modified oligonucleotides**, J.A. Dougan, C. Karlsson, W.E. Smith and D. Graham, *Nucleic Acids Research*, **35**, 3668-3675, 2007.

(b) On-column conjugation with 5'-amino-modified oligo

On-column conjugation can be achieved using a trityl-protected amino-modifier. Note, however, that removal of the MMT-protecting group on the synthesiser is slow, inefficient and difficult to measure. TFA-protected amino-modifiers are incompatible with on-column conjugations as the TFA group is not removed until cleavage and deprotection of the oligo.

1. Preparation of Modified Oligo: Synthesise the 5'-amino-modified oligo (DMT OFF).
2. Wash the synthesis column with approx. 2ml of Cap Mix B (10% methylimidazole/pyridine/THF) (**4122**) for 2-3 min.
3. Wash the column with 10ml of HPLC-grade acetonitrile.
4. Preparation of Thioctic Acid NHS Ester: Dissolve 20mg (66µmol) of **2166** in 1ml of acetonitrile.
5. Conjugation: Pass the thioctic acid NHS ester solution prepared in Step 4 over the immobilised modified oligo by passing the solution over the resin between two syringes.
6. Incubate at room temperature overnight.
7. Wash the column with 10ml of HPLC-grade acetonitrile.
8. Blow the column dry with argon.
9. Cleave and deprotect the oligo using the conditions recommended for the modifier and bases used.

A similar protocol can be applied to on-column labelling at the 3'-end using **2350**. In this case, after oligo synthesis, the Fmoc protection is removed by treating the column with 20% piperidine/MeCN. Leave for 5min then wash with MeCN. The above conjugation protocol can then be followed from Step 2 onwards.

Purification

Modified oligonucleotides are purified, if required, by known chromatographic methods such as RP-HPLC.

Storage & Stability

This product is stored dry in a freezer at –20°C. Solutions in acetonitrile must be prepared fresh for immediate use and not stored.

Gold & Silver Bioconjugation Protocols

Customers wishing to use oligonucleotides modified with **2166** to conjugate to gold or silver nanoparticles should refer to reference 233 for applicable methods.

Use of Thiol-Modifier CPG (2361)

Coupling

Use as per unmodified nucleoside supports. However, non-nucleosidic modifications are slow to detritylate and require an additional detritylation prior to use in synthesis. In this case it is important not to use a cycle with an initial capping step. AMA deprotection will require dmf-G and Ac-C to be used.

Oxidation

0.02M iodine (**4132** & **4330**) is used in all oxidation steps.

Cleavage & Deprotection

Deprotect the oligo using conditions for unmodified bases, *e.g.* AMA at room temperature for 2h or ammonium hydroxide solution at 55˚C for 4h. This is dependent on the nucleobase protection and other modifications in the oligo.

Desalt the deprotected oligo and evaporate to dryness. Purify the oligo if required (RP-HPLC or cartridge), desalt and dry.

Add 200µl of a solution of 87mM TCEP in water (29mg/ml) and leave (preferably with agitation) for 1h.

Pass the oligo down a G25 or Nap 10 column equilibrated with water or the buffer being used in the subsequent conjugation reaction. It is recommended this is carried out immediately.

Storage & Stability

Store in a freezer at –10 to –30°C.

Thiol-modified oligos are best kept dry under an inert atmosphere in the Tr-S or S-S state until ready to use. Treat with TCEP immediately before use. Note even if the oligo has previously been treated TCEP (or DTT) this will readily form a disulphide bridge to form the dimerised oligo, therefore TCEP treatment is required before use.

Internal Thiol Modification

2191	Bz-S-dT-CE Phosphoramidite	45

Coupling

BTT or DCI can be used as activators. Do not use ETT. A coupling time of 10min (600s) is recommended. To

facilitate AMA deprotection, dmf-G and Ac-C must be used.

Cleavage, Deprotection & Purification

Before carrying out the cleavage and deprotection, wash with a 20% DEA/MeCN solution, then:

1. Cleave and deprotect using 100mM TCEP in AMA at room temperature for 2h.
2. Desalt using a G25 column.
3. Purify if required.
4. Dry the oligo.
5. Add 100µl of 87mM TCEP in water.
6. Leave for 1h at room temperature.
7. Pass through a G25 column pre-equilibrated with the conjugation buffer.

Conjugation

To conjugate, add the appropriate maleimide, haloacetamide or equivalent to the above solution and react as appropriate. Once the reaction is complete, pass through a G25 column pre-equilibrated with 0.1M TEAA or, if conjugation is on a solid surface, thoroughly with water.

Storage & Stability

Store in a freezer below -10°C. Diluted samples must be freshly prepared for use and used within 24h.

Thiol-modified oligos are best kept dry under an inert atmosphere in the Tr-S or S-S state until ready to use. Treat with TCEP immediately before use. Note even if the oligo has previously been treated TCEP (or DTT) this will readily form a disulphide bridge to form the dimerised oligo, therefore TCEP treatment is required before use.

Carboxylate Modification

2057	5'-Carboxylate-Modifier-CE Phosphoramidite	46
2142	Carboxy-dT-CE Phosphoramidite	46
2531	5'-Carboxy-C10-CE Phosphoramidite	46

Dissolution

Phosphoramidites are best dissolved in anhydrous acetonitrile, although for **2057** and **2531** increased concentrations of 0.15M and 0.1M solutions are recommended for ABI/MerMade and Expedite synthesisers respectively (hence the provision of a 150µmol pack).

Coupling

2057 & **2531** - An extended coupling time of 15min is recommended. For **2142** use 25-60s.

Cleavage & Deprotection

For **2057** & **2531**, the 2'-chlorotrityl protecting group is stable to oligonucleotide coupling conditions, but is easily removed by acidic detritylating conditions (3% w/v trichloroacetic acid in DCM). Deprotection with 0.4M NaOH in methanol (4:1) overnight is recommended to ensure the free carboxylate is released. Ammonium hydroxide or AMA deprotection can result in amide formation.

2142 - Cleavage and deprotection is carried out using a mild deprotection: 0.4M methanolic sodium hydroxide (methanol:water 4:1) for 17h at room temperature. Remove the support and neutralise with 2M TEAA. Note: The methyl-ester is hydrolysed during this deprotection and can therefore be coupled directly to a molecule containing a primary amino group by standard peptide coupling or *via* the intermediate N-hydroxy-succinimide (NHS) ester. Use of ammonium hydroxide or AMA must be avoided, otherwise the amide or methylamide derivative will be formed in preference to the free acid.

Storage & Stability

Store in a freezer below -10°C. Diluted samples must be freshly prepared for use and used within 24h.

Example conjugation using 2057 to conjugate an amine

1. Synthesise the 5'-carboxylate-modified oligo as per notes above. Retain on the support.
2. Activate the carboxylic acid by treating the support-bound oligo with HATU (100 eq.), HOBt (100 eq.), and dry DMF (100µl).
3. Warm the reaction to 35°C and shake for 35min.
4. After activation of the acid is complete, add triethylamine (100 eq.) and the amino-functionalised label (100 eq.).
5. Warm the conjugation mixture to 35°C and shake for 1h.
6. The unbound amine can easily be removed from the solid support by washing successively with DMF (2 x 100µl), ethanol (2 x 200µl), and distilled water (2x 200µl).

7. The conjugate can then be deprotected and removed from the solid support using deprotection conditions suitable for the modified oligo

8. The conjugate is now ready for purification.

Further examples of the use of **2057**[234] are available in the literature.

Aldehyde Modification

2056	Formylindole-Modifier-CE Phosphoramidite	47

Coupling

An extended coupling time of 15min is recommended.

Cleavage & Deprotection

Deprotection is dictated by other modifiers and nucleobase protection. This is stable to all commonly used deprotection strategies.

Storage & Stability

Stable in solution for up to 4 days; store at –20°C.

Example conjugation using 2056 with a functionalised hydrazine [235]

1. Synthesise the 5'- or internally modified aldehyde-modified oligo as per notes above. Retain on the support.

2. Prepare a solution of the reagents used for the conjugation (10mM of the hydrazine hydrochloride, *e.g.* N,N-diphenylhydrazine hydrochloride, and 10mM sodium acetate in 1.5ml ethanol).

3. To the solution add the support-bound oligo (ca. 0.5µmol) then shake at 60°C for 24h. Alternatively the solution from Step 2 can be passed through the column using two syringes.

4. Evaporate the solution under vacuum, or (if using syringes), remove the solution from the column and wash the resin in the column with MeCN. Dry the resin by passing argon through the column.

5. The conjugate can then be deprotected and cleaved from the solid support using deprotection conditions suitable for the modified oligo

6. Pass through a G25 column to remove the deprotection solution.

7. The modified oligo is now ready for purification.

Further examples of the use of an alternative aldehyde modifier[236] (whose protocols could be extended to **2056**) are available in the literature.

Backbone Modification

Peptide Nucleic Acid (PNA) Synthesis

5001	Fmoc-A(Bhoc)-PNA-OH	57
5002	Fmoc-C(Bhoc)-PNA-OH	57
5003	Fmoc-G(Bhoc)-PNA-OH	57
5004	Fmoc-T-PNA-OH	57
5005	Fmoc-AEAA-OH Spacer	57
5010	dPEG®$_4$-SATA-Acid	57
5011	SPDP-dPEG®$_4$-Acid	57

Peptide nucleic acids mimic oligonucleotides in that the ability to base pair remains while the sugar phosphate backbone has been replaced by a charge neutral polyamide. The neutral charge facilitates a more stable duplex when hybridised to DNA or RNA. As a result, PNA lends itself well to antisense or diagnostic technologies.

Guidelines for synthesis of PNA using an automated DNA synthesiser are provided here, however these could

234 **A new and efficient method for synthesis of 5'-conjugates of oligonucleotides through amide-bond formation on solid phase**, A.V. Kachalov, D.A. Stetsenko, E.A. Romanova, V.N. Tashlitsky, M.J. Gait, and T.S. Oretskaya, *Helvetica Chimica Acta*, **85**, 2409-16, 2002.

235 **A facile incorporation of the aldehyde function into DNA: 3-formylindole nucleoside as an aldehyde containing universal nucleoside**, A. Okamoto, K.Tainaka and I. Saito, *Tetrahedron Lett.*, **43**, 4581-4583, 2002.

236 **Synthesis of peptide-oligonucleotide conjugates with single and multiple peptides attached to 2'-aldehydes through thiazolidine, oxime, and hydrazine linkages**, T.S. Zatsepin, D.A. Stetsenko, A.A. Arzumanov, E.A. Romanova, M.J. Gait and T.S. Oretskaya, *Bioconjugate Chemistry*, **13**, 822-830, 2002.

be easily applied to other automated instruments capable of synthetic chemistry, in fact many users have successfully transferred this synthesis to microwave assisted peptide synthesisers. Information on obtaining protocols for manual PNA synthesis or synthesis by alternative automated means is provided below.

General Guidelines for PNA Design

PNA has a higher binding affinity to a DNA strand in a duplex than DNA itself. Therefore, for most applications, a short oligomer length of 12-15 units is sufficient, and in many cases even shorter probes will work well. Longer oligomers tend to aggregate and are difficult to purify and characterise.

Purine-rich PNA tends to aggregate, particularly with G-rich oligomers. As a rule, limit the number of purines to 6 in any 10 units and do not include more than 3 G units in a row. The shorter the oligomer, the less susceptible to aggregation it will be. Avoid, if possible, self-complementary sequences as PNA/PNA interactions are even stronger than those of PNA/DNA.

Incorporating lysine at one or both ends of the PNA oligomer can greatly assist solubility without any adverse effect on the hybridsation properties, as is the incorporation of multiple AEAA (**5005**) linkers. In reality, these guidelines cannot always be adhered to, but there are ways to improve the synthesis and downstream handling of more complex PNA oligos.

Labelling of PNA

PNA can be labelled with *e.g.* fluorescein and biotin, however this can decrease the solubility of the oligomer, especially with purine-rich sequences. When labelling PNA, adhere to the following:

- Incorporate two or more spacer monomers (**5005**) between the label and the PNA oligomer;
- If you are labelling with biotin, fluorescein, rhodamine, or other acid-resistant reporter groups, PNA oligomer labelling is best carried out on the resin before cleavage & deprotection;
- If you are labelling with acid-sensitive compounds, labels must be attached in solution after cleavage & deprotection.

C-terminal labels may be similarly prepared by the incorporation of *e.g.* Lysine.

Direction of Synthesis

In PNA synthesis the amino-terminal is analogous with the 5'-end of a DNA sequence. Using methods conventional to DNA synthesis, therefore, the PNA will be synthesised starting with the C-terminus towards the N-terminus (pseudo 3'-5').

General PNA Oligomer Synthesis Protocols[237]

Fmoc-based PNA synthesis consists of a repetitive cycle of deblocking, activation/coupling, and capping. Typically this is done on a 2µmol scale.

Choice of Solid Support

In general, an appropriate solid support must be cleavable in acid, leaving a C-terminal amide, and be compatible with Fmoc chemistry. Both PAL-PEG-PS and XAL-PEG-PS have been found to be suitable in this regard.

Despite the advantages of XAL-handled supports outlined later, it should be remembered that either the PAL or XAL handles may be placed on a wide range of resin types. Best results, however, have been reported with polyethylene glycol derivatised polystyrene (PEG-PS). Note also that PNA synthesis is optimally performed on resins loaded below 0.2mmol/g. Higher loadings can lead to aggregation and poor synthesis quality.

Finally, unlike the most common solid supports used in DNA synthesis, the PNA supports are universal – the first monomer is not contained within the support - and therefore only a single support is needed rather than a range of four. Note that, due to the universal support, the C terminal is an amide rather than a free acid.

Dissolution

PNA monomers are prepared for coupling as 0.2M solutions in peptide-grade N-methylpyrrolidone (NMP). Our PNA monomers are provided in 500mg and 1g bulk sizes, and in a convenient 700µmol size ready-packaged in a 30ml vial for use on an Expedite DNA synthesiser (enough for around 30 additions). The AEEA spacer (**5005**) is provided in Expedite-ready 500µmol vials (also 30ml).

Fmoc-Removal (Deblocking)

Removal of the Fmoc from the primary aliphatic amine, required for subsequent monomer coupling, is achieved by treatment with 20% piperidine in DMF for 30s (flow rate 0.4ml/min). Transacylation is possible at this step (A>G>T, but not with C), however this is not a significant issue if brief basic treatments are employed. A deblock step is required to remove the final Fmoc group prior to cleavage, unless Fmoc-ON purification is desired.

237 Protocols are based on those provided in **Synthesis of PNA Oligomers by Fmoc Chemistry**, R. Casale, I.S. Jensen and M. Egholm, in *Peptide Nucleic Acids: Protocols and Applications*, P.E. Nielsen (Ed.), Second Edition, Garland Science, 2003.

Activation/Coupling

The coupling conditions were initially based on methods employed for Boc synthesis.[238] Activation of the monomer carboxylic acid function is achieved with HATU (or PyBOP®) and a base mixture of 0.2M DIPEA and 0.3M lutidine for 2.5min.[239] This mixture requires to "pre-activate" for this time before introduction to the support for coupling. A slight excess of monomer is used in the coupling (prevents the undesired tetramethylguanidine capping arising from direct reaction of HATU with the primary amine on the growing PNA chain). A minimum activation-coupling time of 7.5min (or 8.25min with washing steps) is suggested.

Capping (Acetylation)

Capping of unreacted product is carried out with a solution of 5% acetic anhydride and 6% lutidine in DMF. This mixture has been found to have a longer shelf life (months) than acetic anhydride and bases such as DIPEA or pyridine. Note also that the piperidine wash step, found to be useful in Boc protocols for elimination of products stemming from acylation of the exocyclic and cyclic amino groups of the nucleobases, is effectively accomplished during Fmoc synthesis during each subsequent deprotection.

Labelling

PNA oligomers can be labelled at the N-terminus by attachment *via* the free amine generated on removal of the final Fmoc group. This is best accomplished whilst the PNA is still attached to the solid support (as outlined above, two or more spacer (**5005**) molecules incorporated into the sequence, prior to addition of the label increases the conjugation efficiency). Labelling is then carried out using *e.g.* an activated NHS ester (5 to 10-fold excess) of biotin, fluorescein, *etc* using the protocols applicable to those materials. If the PNA is lysine terminated, then the lysine residue can be considered a substitute for one spacer residue. After labelling, oligos are cleaved and deprotected as below. Where multiple Lys incorporations are required, it is important to use orthogonal protection to obtain selective labelling.

Acid-sensitive labels cannot be incorporated on-column as they will not survive the cleavage conditions. Such labels are attached post-synthetically in solution. Again, follow the protocol appropriate to the label being used.

Labelling can also be accomplished in solution *via* the ε-amino group of an incorporated lysine. This is particularly useful for introducing a C-terminal label or second label to the oligomer. Also, since there are several orthogonal protection strategies applicable to Lys, it is possible to use this as a means of adding multiple labels. In a similar way, it is also possible to use suitably protected Cys residues.

LINK has introduced dPEG® thiol linkers (**5010** and **5011**). Both of these are suitable for on-column labelling where **5010** is deprotected using hydroxylamine and **5011** using TCEP to give the free thiol. These are specifically for use at the N-terminus.

Cleavage and Bhoc Deprotection

PNA is both deprotected and cleaved from the solid support by treatment with TFA containing 5% m-cresol, the latter a scavenger for the resultant benzhydryl cations. However, depending on the amino acids used in PNA-petide synthesis, other scavengers such as 10% phenol and/or TIS (triisopropylsilane) may be required. Deprotection is complete within 1min, however the cleavage takes longer and is dependent on the support used. Approximately 400µl of cleavage solution is required for a 2µmol synthesis.

Generally, PAL-PEG-PS is used for Fmoc-based PNA assembly; this requires treatment with 5% m-cresol in TFA (or similar) for 90min to complete cleavage. The XAL synthesis handle can be employed for more rapid cleavage, complete in 5min. The XAL[240] synthesis handle, like PAL, produces PNA with a carboxy terminal amide. The PNA product is isolated from the cleavage mixture by precipitation with diethyl ether.

Typical Cleavage & Deprotection Protocol (0.2µmol Scale):

1. After drying the resin, transfer to a microfilter tube.
2. Add 200µl of the 5% m-cresol in TFA cleavage mixture (or similar) to the top of the microfilter tube. Allow to sit at room temperature for:

   PAL resin — 90min.
   XAL resin — 5min.

3. Centrifuge the resin for 5min.
4. Repeat steps 2 and 3.

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238 (a) **Improved synthesis, purification and characterisation of PNA oligomers,** L. Christensen, R. Fitzpatrick, B. Gildea, B. Warren and J. Coull, *In Solid Phase Synthesis. Peptides, Proteins and Nucleic Acid*, R.Epton, Ed., pp149-156, Mayflower Worldwide Ltd, Birmingham, 1994; (b) **Solid-phase synthesis of peptide nucleic acids**, L. Christensen, R. Fitzpatrick, B. Gildea, K.H. Petersen, H.F. Hansen, T. Koch, M. Egholm, O.Buchardt, P.E. Neilsen, J. Coull and R.H. Berg, *J. Peptide Sci.*, **3**, 175-183, 1995.

239 **Effect of Tertiary Bases on O-Benzotriazolyluronium Salt-Induced Peptide Segment Coupling**, L.A.Carpino and A. El-Faham, *J. Org. Chem.*, **59**, 695-698, 1994.

240 **Preparation and Applications of Xanthenylamide (XAL) Handles for Solid-Phase Synthesis of C-Terminal Peptide Amides under Particularly Mild Conditions**, Y. Han, S.L. Bontems, P. Hegyes, M.C. Munson, C.A. Minor, S.A. Kates, F. Albericio and G. Barany, *J. Org. Chem.*, **61**, 6326-6339, 1996.

5. Remove the filter insert from the microcentrifuge tube.
6. Add 1 ml of diethyl ether.
7. Shake or vortex for about 1min.
8. Centrifuge for 5min. The crude PNA forms a pellet at the bottom of the microcentrifuge tube.
9. Decant the supernatant. Retain this until cleavage and deprotection is complete, and you have successfully recovered the PNA.
10. Repeat steps 6-9 twice more.
11. Dry the PNA by removing the cap from the tube and leaving open for about an hour to allow excess diethyl ether to evaporate.

Analysis and Purification

PNA, like DNA, can be analysed for purity by RP-HPLC and MALDI-TOF mass spectroscopy. Reconstitution of the PNA for analysis is best done in a solution of 0.1% TFA in water. The quantity of PNA produced is measured as a total optical density (OD) at 260nm. This ranges between 80 and 180OD for a 2µmol synthesis depending on the length of sequence.

Suggested HPLC conditions: C8 or C18, A = 0.1% TFA in water, B = 0.1% TFA in acetonitrile. Gradient of 19:1 to 13:7 over 35min at 1ml/min. HPLC analysis is performed at 55°C to avoid aggregation of the PNA.

Purification is best carried out by RP-HPLC using conditions similar to the analytical method. Fmoc-ON purification may be performed. If the PNA has an N-terminal O-spacer unit or an α-amino acid, Fmoc-ON purification is very useful to separate the modified PNA from the unmodified PNA.

Handling, Storage & Stability

The monomers are stored dry in a freezer at –20°C. The dry monomers are prone to static. Typically, 0.2M PNA monomer solutions are stable for 1-2 weeks in peptide-grade NMP.

PNA oligomers have a high affinity for glass surfaces and polystyrene. Where possible use polypropylene or polyethylene materials when working with low (sub-micromolar) concentrations.

Crude PNA oligomers can be aliquoted from 0.1% TFA/water solutions, however if they are not to be used immediately then drying the aliquots prevents degradation. PNA purified by RP-HPLC with TFA present will be protonated at all the basic amino groups (A, C and the amino terminus) and hence carry trifluoroacetate counter-ions when dissolved in water. This aids solubility at high concentrations, however users should be aware of possible toxic effects of TFA in cells. Buffered solutions can be used to regulate pH.

In cases where the oligomer is difficult to dissolve, addition of 10-20% acetonitrile to the aqueous solution and heating to 50°C for 10min aids solubility.

Further Information

Alternative Synthesis Platforms

If you are familiar with DNA synthesis then synthesis of PNA on an Expedite instrument may be your simplest "entry-point" for this chemistry. However, other platforms are available.

A recent example Fmoc-based protocol of PNA synthesis on a peptide synthesiser (Advanced ChemTech Omega 396) has been reported.[241] The Liberty range of microwave-assisted peptide synthesisers is also capable of PNA synthesis (see www.cem.com).

Goodwin *et al* have described a method applicable to general PNA synthesis.[242] Hüsken *et al* have also described a manual method utilised when labelling PNA with ferrocene.[243]

Acknowledgement

LINK would like to thank Donna Williams of the MRC in Cambridge, UK, and Professor James Schneider of Carnegie Mellon University in Pittsburgh, US, for their helpful discussions on the use of these products.

241 **Effect of terminal amino acids on the stability and specificity of PNA-DNA hybridisation**, N.C. Silvester, G.R. Bushell, D.J. Searles and C.L. Brown, *Org. & Biomol. Chem.*, **5**, 917-923, 2007.

242 **A simple procedure for the solid-phase synthesis of peptide nucleic acids with N-terminal cysteine**, T.E. Goodwin, R.D. Holland, J.O. Lay and K.D. Raney, *Bioorganic and Medicinal Chem. Lett.*, **8**, 2231-2234, 1998.

243 **"Four-Potential" ferrocene labeling of PNA oligomers *via* click chemistry**, N. Hüsken, G. Gasser, S.D. Köster and N. Metzler-Nolte, *Bioconjuagte Chem.*, **20**, 1578-1586, 2009.

Unlocked Nucleic Acid (UNA) Synthesis

2183	Bz-A UNA CE Phosphoramidite	58
2184	Ac-C UNA CE Phosphoramidite	58
2185	iBu-G UNA CE Phosphoramidite	58
2186	U UNA CE Phosphoramidite	58

Dissolution

UNA phosphoramidites are dissolved in dry acetonitrile to a concentration of 0.1M.

Coupling

A coupling time of 3-5min is recommended for up to 1µmol synthesis. Use 0.25M ETT as activator.

Cleavage & Deprotection

UNA is amenable to cleavage and deprotection methods as used for unmodified DNA chemistry, *e.g.* AMA at RT for 2h, or at 65˚C for 10min - 4h depending on the nature of the other modifications in the oligo. Typically this would be AMA, at 65˚C for 35min. With reference to the synthesis of UNA-RNA chimeras, this is also compatible with TBDMS-RNA chemistry deprotection.

Purification

No change is required to the purification method as for unmodified DNA or RNA.

Storage & Stability

All phosphoramidites are stored at -10 to -30°C. They are stable in solution for 2-3 days.

Methyl Phosphonamidites

2073	dT-Me Phosphonamidite	64
2074	iBu-dG-Me Phosphonamidite	64
2075	Bz-dA-Me Phosphonamidite	64
2077	Ac-dC-Me Phosphonamidite	64

Dissolution

dG-Me Phosphonamidite (**2074**) is dissolved in anhydrous THF prior to use in synthesis. Other monomers are dissolved in anhydrous acetonitrile.

Coupling

A coupling time of 6min is recommended for syntheses of up to 1µmol. The use of the Ac-dC (**2077**) monomer is preferred to avoid base modification of dC residues during deprotection with ethylenediamine.[244] Trityl monitors may understate the coupling efficiency, presumably due to a difference in the rate of release of the trityl group.

Cleavage & Deprotection

A one-pot procedure for cleavage and deprotection has been described.[245] This is preferred to the procedure[246] used in the past since it leads to less cleavage of the methyl phosphonate backbone during the ammonium hydroxide solution cleavage step and evaporation of the ethylenediamine.

Typical Protocol

1. Air-dry the support in the synthesis column, open the column and transfer the support to a deprotection vial.
2. Add 0.5ml of an ammonium hydroxide solution consisting of acetonitrile/ethanol/ammonium hydroxide (45:45:10) to the support. Seal the vial and leave it at room temperature for 0.5h.
3. Add 0.5ml of ethylenediamine to the vial and reseal it. Leave it at room temperature for a further 6h.

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244 (a) **Elimination of transamination side product by the use of dC(Ac) methylphosphonamidite in the synthesis of oligonucleotide methylphosphonates**, M.P. Reddy, F. Farooqui and N.B. Hanna, *Tetrahedron Lett.*, **37**, 8691-8694, 1996; (b) **Correction to above**, M.P. Reddy, F. Farooqui and N.B. Hanna, *Tetrahedron Lett.*, **38**, 1101-1101, 1997.

245 (a) **Deprotection of methylphosphonate oligonucleotides using a novel one- pot procedure**, R.I. Hogrefe, M.M. Vaghefi, M.A. Reynolds, K.M. Young and L.J. Arnold, *Nucleic Acids Research*, **21**, 2031-2038, 1993; (b) R.I. Hogrefe, M.A. Reynolds, M.M. Vaghefi, K.M. Young, T.A. Riley, R.E. Klem and L.J. Arnold, **Protocols for Oligonucleotides and Analogs**, Vol. 20 in the series *Methods in Molecular Biology*, 143-164, 1993, S. Agrawal, editor, Humana Press Inc., Totowa, NJ.

246 **Oligodeoxynucleoside methylphosphonates–synthesis and enzymatic degradation**, S. Agrawal and J. Goodchild, *Tetrahedron Lett.*, **28**, 3539-3542, 1987.

4. Filter in a 0.2µm microfilter tube then desalt on a G25 sephadex column and wash the support twice with 0.5ml acetonitrile/water (1:1).
5. Dilute the combined supernatant and washes to 15ml with water.
6. Adjust the pH to 7 with 6M hydrochloric acid (1 part) in a 1:1 mix of acetonitrile/water (9 parts) (ca. 2ml).
7. Desalt.

Purification

Most oligo purification procedures can be used if the oligo contains several phosphodiester linkages. If the oligo contains a very high percentage of Me phosphonate linkages, RP-HPLC purification is preferred. However, these oligos have poor solubility characteristics and have been known to precipitate in the sample loop, HPLC column, or other surfaces. More detailed descriptions of HPLC purification have been published. [203]

Note: These procedures have been shown to work well for small scale synthesis (1µmol or below). For larger synthesis scales, changes may be required in the capping and oxidation steps. See reference 246 for more details.

Storage & Stability

All phosphonamidites are stored refrigerated at a maximum of 2-8°C. They are stable in solution for 24h.

Methyl Phosphoramidites

2050	Ac-dC-Me Phosphoramidite	65
2051	iPr-Pac-dG-Me Phosphoramidite	65
2052	Pac-dA-Me Phosphoramidite	65
2078	dT-Me Phosphoramidite	65

Coupling

Coupling is as per unmodified nucleoside amidites. If the oligo contains many dG residues, use phenoxyacetic anhydride in the Cap Mix A (**4210**) to avoid the exchange of the iPr-Pac group on the dG with acetate from the acetic anhydride capping mix.

Deprotection

Use UltraMILD deprotection with 0.05M potassium carbonate in methanol to leave the methyl triester intact.[247] Alternatively, room temperature deprotection with ammonium hydroxide, followed by removing the deprotection solution by G25, can be used. In this case it is important not to remove the ammonium hydroxide solution with heat to avoid hydrolysis.

Storage & Stability

Refrigerate the solids at a maximum of 2-8°C. Stability in solution is 2-3 days.

Ethyl Phosphoramidites

2516	dT-Ethyl Phosphoramidite	65
2517	iBu-dG-Ethyl Phosphoramidite	65
2518	Bz-dA-Ethyl Phosphoramidite	65
2519	Bz-dC-Ethyl Phosphoramidite	65

Coupling

A coupling time of 6min (360s) is recommended.

Cleavage & Deprotection

Assuming your strategy is to retain the ethyl groups, carry out deprotections at room temperature. Heating the oligos during deprotection will lead to loss of OEt from the phosphate backbone.

2516 & **2518** - Use ammonium hydroxide solution overnight at room temperature.

2517 - Use ammonium hydroxide solution at room temperature for 48h. Overnight deprotection does not completely remove the iBu nucleobase protection (~13% remains).

2519 - Use ammonium hydroxide solution overnight at room temperature. Note that deprotection with AMA will lead to transamination.

After deprotection remove the deprotection solution by G25. Do not heat the sample to remove deprotection reagents.

247 See also: T. Atkinson and M. Smith in *Oligonucleotide synthesis: a Practical Approach*, M.J. Gait (editor), IRL Press Limited, Oxford, 1984, pages 68-70.

As with methyl phosphoramidites, deprotection with 0.05M potassium carbonate is also possible.

Storage & Stability

All phosphoramidites are stored refrigerated at a maximum of 2-8°C. Stability in solution is 2-3 days.

H-Phosphonates

2005	dT-H Phosphonate, TEA Salt	60
2006	dG-H Phosphonate, TEA Salt	60
2007	dA-H Phosphonate, TEA Salt	60
2035	dC-H Phosphonate, DBU Salt	60

Please note that the pre-formulated ancillary reagents used in H-phosphonate synthesis are unfortunately not available from LINK. Please contact us for further advice if required.

Dissolution

All monomers must be dissolved in acetonitrile/pyridine (1:1).

Coupling & Capping [248]

Deblock with 3% TCA/DCM (**4140**) is used, however activation is carried out with 1-adamantane carbonyl chloride dissolved in acetonitrile/pyridine (95:5). Capping is carried out with the TEA salt of isopropyl phosphite.

Oxidation

There are two oxidation steps and they need only be carried out at the end of the synthesis. The first is accomplished using 0.1M iodine in pyridine/N-methylimidazole/water/THF (5:1:5:89), and the second with 0.1M iodine in triethylamine/water/THF (5:5:90).

Deprotection

Most deprotection conditions used for phosphodiester oligonucleotides can be applied. See information on pages 156-169 for more details.

Storage & Stability

Refrigerate monomers. Stability in solution is approximately 1 week.

Sulphurisation

0023	Sulphurising Reagent	62
2171	EDITH	62

Dissolution

EDITH (**2171**) is available in millimolar pack sizes ready for dilution with convenient amounts of anhydrous acetonitrile to a 0.05M concentration, according to the following:

Cat. No.	Pack Size	Dilute with:
2171-G005	5mmol	100ml
2171-G010	10mmol	200ml
2171-G023	22.5mmol	450ml

Beaucage Reagent (**0023**) is supplied in solid form in 1g and 2g packs, together with a silanised bottle suitable for direct attachment to an automated DNA synthesiser (different bottle types are available). Simply pour the reagent into the silanised bottle and add anhydrous acetonitrile (100ml/g) to obtain a 0.05M solution. For smaller scale syntheses, smaller quantities can be prepared (*e.g.* 0.5g/50ml). After use, the silanised bottle may be rinsed with acetonitrile, dried and stored for future use.

Sulphurisation

Routinely sulphurisation reagents are used on an automated DNA synthesiser, utilising the dedicated thio port where available, following the instrument manufacturer's recommendations for sulphurisation cycles. Depending on the type of synthesiser, there may be a sulphurising cycle available. If a dedicated port and line is not available, the oxidiser port and line may be used. In this case, the line must first be flushed extensively with acetonitrile followed by liberal flushing with the sulphurising reagent. After use, remove the reagent immediately from the instrument and flush the line with acetonitrile, prior to replacing the oxidiser.

When using EDITH (**2171**) a sulphurisation time of 30s is recommended, although longer times will not have any

248 **Novel activating and capping reagents for improved hydrogenphosphonate DNA synthesis**, A. Andrus, J.W. Efcavitch, L.T. McBride and B. Giusti, *Tetrahedron Lett.*, **29**, 861-864, 1988.

adverse affect on the oligo if reducing the sulphurisation time in the cycle is not possible.

For optimum results, it is recommended that the sulphurisation is carried out prior to the capping step in order to minimise competitive oxidation.

Some deleterious G modification has been observed when using EDITH with certain labile (fast) deprotection chemistry.[249] This can be eliminated by using a modified coupling-cap-thio-cap cycle, thus allowing preparation of phosphorothioate oligos without the need for prolonged ammonium hydroxide deprotection. The cap-thio-cap cycle is possible using EDITH due to the short sulphurisation time required.

Cleavage & Deprotection

Phosphorothioate oligos can be cleaved from the support and deprotected using ammonium hydroxide, therefore follow any necessary guidelines applicable to the nucleobase protection used. Full PS oligonucleotides may require the addition of 10-20% EtOH to the deprotection solution to aid solubility. This is particularly true of PS-RNA oligonucleotides.

Purification

Phosphorothioate purification is routinely carried out by RP-HPLC or cartridge methods. SAX[250] and WAX[251] HPLC has also been described, however the former requires high salt concentrations. FPLC at high pH is not recommended.

Note that high purity or 2'-OMe phosphorothioates are best achieved by PAGE purification.

Analysis

The best means of identifying the presence of oxidised linkages in the oligo is by MS. Either ESI or MALDI are effective for this purpose.

Storage & Stability

2171 - Store the solid in a glass container in the freezer at –10 to –30°C. Although EDITH is reportedly stable in solution for several months, we recommend, as with all other oligonucleotide synthesis reagents, that solutions are freshly prepared as close to use as possible. It can be used on the synthesiser for several days, however, without any drop in performance provided no precipitate is observed.

0023 - Store the solid reagent in a freezer at –10 to –30˚C, and dry. In solution in acetonitrile, it must be stored in plastic or silanised glass containers. It will remain stable under these conditions for several months (precipitation will accompany decomposition). Where the solution has been used on an oxidiser port, the reagent will decompose over 1-2 days. Where a spare port is routinely used for this reagent, the solution will be stable for up to a month.

Photocleavable (PC) Modification

2066	PC Linker-CE Phosphoramidite	69
2122	PC 5'-Biotin-CE Phosphoramidite	69
2130	PC 5'-Amino-Modifier-CE Phosphoramidite	69
2131	PC Spacer-CE Phosphoramidite	69

Coupling

PC 5'-Biotin (**2122**), PC Amino (**2130**) & PC Spacer (**2131**) products require a 2min coupling time. For coupling efficiencies >95% with PC Linker (**2066**), an extended coupling time of 15min is recommended.

Detritylation

A second deblock step is recommended for the biotin product (**2122**) if the final DMTr group is to be removed on the synthesiser (the DMTr group is slow to detritylate from the N1 position of biotin). This is not required for the other PC products.

249 **Evaluation of 3-ethoxy-1,2,4-dithiazoline-5-one (EDITH) as a new sulfurizing reagent in combination with labile exocyclic amino protecting groups for solid-phase oligonucleotide synthesis**, M.Y.-X. Ma, J.C. Dignam, G.W. Fong, L. Li, S.H. Gray, B. Jacob-Samuel and S.T. George, *Nucleic Acids Research*, **25**, 3590-3593, 1997.

250 **Separation of synthetic phosphorothioate oligonucleotides from phosphodiester-defect species by strong-anion exchange HPLC**, B.J. Bergot and G. Zon, *Annals of the New York Academy of Sciences*, **660**, 310-312, 1992.

251 **Ion-exchange high-performance liquid chromatography analysis of oligodeoxyribonucleotide phosphorothioates**, V. Metelev and S. Agrawal, *Anal. Biochem.*, **200**, 342-346, 1992.

Deprotection

For **2130** a column wash with 10-20% diispropylamine in acetonitrile or 10-20% DEA/acetonitrile is required prior to cleavage and deprotection of the oligo.

The modifiers deprotect under most deprotection conditions. The trifluoroacetyl (TFA) group in the amino product (**2130**) is base labile and is therefore removed during the ammonium hydroxide or AMA deprotection leaving the 5'-amine.

Photocleavage

Photocleavage is carried out simply by exposure of the oligo in 0.1M TEAA solution to a hand-held UV light source (~365nm) at room temperature. Quantitative cleavage occurs with a 1mW/cm^2 lamp after irradiation for 10min when using products **2122**, **2130** and **2131**. Up to 30min with a 25mW/cm^2 lamp may be necessary for **2066**. The time taken for photocleavage will depend on the intensity of the lamp used. More powerful UV light sources can be used, although to avoid damaging the oligo (thymidine dimer formation) a 300nm cut-out filter is required.

When using **2066** the release of the 3'-phosphate oligo has been shown to be pH dependent. Conversion rates are higher at pH 9.4 than at 7.4. Release of the 5'-phosphate occurs directly upon photocleavage

Opaque magnetic particles are not recommended in PC-biotin-avidin capture applications. Glass particles, *e.g.* CPG, are best used otherwise photocleavage will be restricted to only 5-10%.

Storage & Stability

All modifiers are stored dry and protected from exposure to light in a freezer at –10 to –30°C. Stability in solution is 2-3 days. PC-modified oligos are best protected from over-exposure to light where possible.

Nuclease Resistance

2'-OMe Incorporations

2041	2'-OMe-Bz-A-CE Phosphoramidite	71
2042	2'-OMe-Bz-C-CE Phosphoramidite	71
2043	2'-OMe-Ac-C-CE Phosphoramidite	71
2044	2'-OMe-dmf-G-CE Phosphoramidite	71
2045	2'-OMe-U-CE Phosphoramidite	71
2083	2'-OMe-Pac-A-CE Phosphoramidite	71
2084	2'-OMe-iPr-Pac-G-CE Phosphoramidite	71
2310	2'-OMe-U SynBase™ CPG 1000/110	71
2311	2'-OMe-dmf-G SynBase™ CPG 1000/110	71
2312	2'-OMe-Bz-A SynBase™ CPG 1000/110	71
2313	2'-OMe-Bz-C SynBase™ CPG 1000/110	71
2314	2'-OMe-Ac-C SynBase™ CPG 1000/110	71

Coupling

To optimise efficiency a 6min coupling time is recommended. Use of the supports does not require any change to the synthesis cycle.

Cleavage & Deprotection

Most oligonucleotide deprotection conditions can be applied. See information on pages 156-169 for more details.

Ac-C (**2043**) must be used when employing AMA deprotection (10min at 65°C). UltraMILD deprotection can be used with Pac-A (**2083**), iPr-Pac-G (**2084**) and **2043**. Bz-C (**2042** or **2313**) cannot be used with AMA deprotection.

Storage & Stability

All products are stored refrigerated at 2 to 8˚C. Stability in solution is 3-5 days.

Modified 2'-OMe Incorporations

2098	2'-OMe-I-CE Phosphoramidite	72
2099	2'-OMe-5-Me-U-CE Phosphoramidite (2'-OMe-T)	72
2192	2'-OMe-N-Ac-5-Me-C-CE Phosphoramidite	72

Coupling

ETT, BTT or DCI can be used as activators. A coupling time of 6min (360s) is recommended.

Cleavage & Deprotection

Most oligonucleotide deprotection conditions can be applied. See information on pages 156-169 for more details.
In general, AMA at 65°C for 10min is used, employing dmf-G and Ac-C in the sequence.

Storage & Stability

Store in a freezer below -10°C. Diluted samples are freshly prepared and used within 24h.

2'-Fluoro Modifications

2079	2'-F-Ac-dC-CE Phosphoramidite	73
2080	2'-F-dU-CE Phosphoramidite	73

Coupling

A 3min coupling time is recommended.

Cleavage & Deprotection

Use AMA for 2h at room temperature. Do not heat the oligos. It is necessary to use Fast (or UltraFast deprotection amidites for all other nucleoside incorporations.

Purification

Cartridge or RP-HPLC is generally used as a means of purification.

Storage & Stability

The solids are stored in the freezer at –10 to –30°C. The dissolved amidites must be used within 24h.

Reverse (5' to 3') Synthesis

2020	dT-5'-CE Phosphoramidite	75
2021	iBu-dG-5'-CE Phosphoramidite	75
2022	Bz-dA-5'-CE Phosphoramidite	75
2023	Bz-dC-5'-CE Phosphoramidite	75
2093	dmf-dG-5'-CE Phosphoramidite	75
2294	dT-5'-SynBase™ CPG 1000/110	75
2298	iBu-dG-5'-SynBase™ CPG 1000/110	75
2355	Bz-dA-5'-SynBase™ CPG 1000/110	75
2356	Bz-dC-5'-SynBase™ CPG 1000/110	75

Coupling

Coupling is as per unmodified nucleoside amidites and supports.

Cleavage & Deprotection

Most oligonucleotide deprotection conditions can be applied. See information on pages 156-169 for more details.

Storage & Stability

All phosphoramidites are stored refrigerated at a maximum of 2-8°C. Their stability in solution is the same as unmodified dA, dC, dG and dT phosphoramidites. CPGs are stored in the fridge or freezer.

Chemical Phosphorylation

2101	Chemical Phosphorylation Reagent (CPR)	77
2127	Solid Chemical Phosphorylation Reagent (solidCPR™)	77
2279	3'-Phosphate SynBase™ CPG 1000/110	77
2398	3'-Phosphate SynBase™ CPG 3000/110	77

Coupling & Deprotection using CPR or the 3'-Phosphate CPGs

Note that **2101** must be dissolved in acetonitrile at least 10min prior to placing on the synthesiser. See notes on page 116.

5'-Phosphorylation

CPR (**2101**) is coupled and deprotected using the standard instrument cycle. Note that the DMTr-group is lost during ammonium hydroxide deprotection and thus is not available for cartridge purification. It is important to heat the deprotection reaction to ensure complete removal of the sulphonyl diethanol groups. For 5'-phosphorylation **2101** is coupled in the final step of the synthesis as with other 5'-modifications. The DMTr group is used to give an indication of the coupling efficiency.

3'-Phosphorylation

In addition, **2101** can also be used to phosphorylate the 3'-terminus. To do this it is introduced as the first addition to the solid support, followed by synthesis of the oligo (any convenient support can be used since the support-bound nucleotide is not incorporated into the sequence). After deprotection, the linkage decomposes and is ß-eliminated leaving a phosphate group at the 3'-end. The final DMTr group may be removed on the synthesiser or it may be retained to aid purification. If retained, it can be removed on a purification cartridge, during HPLC purification, or, following purification, by treatment with acetic acid:water (80:20) at room temperature for 1h.

Alternatively, direct preparation of oligos with a 3'-phosphate can be achieved using 3'-Phosphate CPG (**2279** and **2398**). These supports are used in a manner identical to unmodified nucleoside supports. Cleavage and deprotection of the oligos synthesised with these modifications is defined by the nucleobase protection used. However, to complete the ß-elimination of the sulphonyl diethanol group to form the phosphate heat is required. The minimum deprotection conditions are AMA for 35min at 55°C.

Compatibility with RNA

The phosphorylation reagents are all compatible with TBDMS chemistry, but the DEA wash needs to be eliminated from the cleavage and deprotection step to avoid cleavage from the support where 3'-phosphorylation is required. The addition of the terminal phosphate adds protection against nucleases to the RNA.

Coupling & Deprotection using solidCPR™

The use of solidCPR™ (**2127**) has advantages over CPR (**2101**) and as such has its own distinct protocols. CPR protocols cannot be used as this will lead to poor results. **2127** is coupled in the final step in the synthesis using a 6min coupling time. If wishing to remove the DMTr group, a second deblock step is recommended.

Deprotection & Cleavage DMT OFF in Solution

The support, after detritylation, when treated using most deprotection and cleavage conditions, including fast deprotection, will release the terminal free phosphate. The two most suitable options are either ammonium hydroxide solution for 4h at 55˚C or AMA for 2h at RT. After this cleavage and deprotection step the free 5' phosphate is obtained.

DMT ON Cartridge Purification and Deprotection

The sequence with a 5'-DMTr group is purified, after synthesis and base deprotection, using a modified protocol for cartridge purification. After detritylation on the cartridge by treatment with 2% aqueous trifluoroacetic acid (TFA) the final phosphorylated sequence is obtained *via* the following steps:

1. Prepare a stock solution of either 50mM potassium carbonate, pH 12/1M NaCl or 50mM sodium hydroxide/1M NaCl. Approximately 2ml will be required for a standard size cartridge.
2. Neutralise the residual TFA on the column by passing approx. 0.5ml of the stock solution through the cartridge fairly rapidly (over ca. 10s).
3. Then gradually pass the remainder of the stock solution through the column in aliquots over a period of 20min.
4. Wash the cartridge with water (2ml).
5. Elute the purified phosphorylated oligo with 20% aqueous acetonitrile (1-3ml depending on the size of the cartridge).

Storage & Stability

Store both CPRs and the solid support in a freezer dry at –10 to -30°C. Dissolved **2101** must be used within 24h.

2127 is stable in solution for 2-3 days.

Diagnostics Applications

Fluorescence Detection

Fluorescein Labelling

2068	Fluorescein-dT-CE Phosphoramidite	81
2134	5'-Fluorescein-CE Phosphoramidite (6-FAM)	81
2136	5'-Hexachloro-Fluorescein-CE Phosphoramidite (HEX)	81
2137	5'-Tetrachloro-Fluorescein-CE Phosphoramidite (TET)	81
2139	6-Fluorescein-CE Phosphoramidite	81
2148	Fluorescein-CE Phosphoramidite	81
2359	3'-Fluorescein CPG	81
2366	3'-(6-FAM) CPG	81
2368	3'-(6-Fluorescein) CPG	81
2370	3'-Fluorescein-dT CPG	81

Coupling

6-FAM (**2134**), HEX (**2136**) & TET (**2137**) – A 3min coupling time is recommended.

2148 – A 12-15min coupling time is recommended.

2139 – A 15min coupling time is recommended.

2068 – A 10min coupling time is recommended.

All CPG supports are used in a manner identical to nucleoside supports, with the exception of the deblock step.

Non-nucleosidic CPG supports do not detritylate as rapidly as nucleosidic ones, therefore an additional detritylation step is recommended. It is therefore necessary to use a cycle that does not contain an initial capping step.

Deprotection

2134, **2137**, **2148** & **2139** can be deprotected with ammonium hydroxide solution, although **2137** must not be subjected to prolonged heating at 55°C.

2136 is also deprotected with ammonium hydroxide solution, but at RT for 24h. The ammonia must be removed immediately after deprotection. Do not use ^{t}butylamine/methanol/water (1:1:2) as this will completely degrade the HEX.

Oligos containing **2068** can be deprotected with most oligonucleotide deprotection conditions, removing the fluorescein protecting groups at the same time.

2366, **2359** & **2368** – Cleavage of the oligonucleotide from these supports requires 45min at room temperature with ammonium hydroxide. Complete the deprotection using the protocol required by the nucleobases.

2370 – Deprotect using protocols dictated by the nucleobase protection strategy or other modifiers in the oligo.

Purification

Fluorescently labelled oligonucleotides can be purified and analysed using the same methods employed for unmodified DNA. Note, however, DMT ON purifications are not possible with **2134**, **2136** or **2137** as these are 5'-modifications with no DMTr group. Nevertheless, the hydrophobic nature of these modifications make purification straightforward.

It is also possible to carry out analysis on dilute solutions using fluorescence detection with RP-HPLC (see below for data).

Spectral Data

	Absorbance Max./nm	Emission Max./nm	Colour
6-FAM	494	525	Green
HEX	535	556	Pink
TET	521	536	Orange

Storage & Stability

All phosphoramidites and CPGs are stored dry, frozen at –10°C to –30°C. **2134** and **2137** are stable in solution for 1-2 days and after 4 days show <90% coupling efficiency. **2139** is stable in solution for 2-3 days, **2068** & **2136** only for 24h, and **2148** is unstable and must be used immediately after preparation.

Fluorescently labelled oligonucleotides must be stored in the dark, either dry or in neutral aqueous media at –20°C. Do not store crude fluorescently labelled oligonucleotides in basic solutions.

Fluorescein Labelling of RNA

It is possible to label an RNA oligo at the 3'- or 5'-end with fluorescein and deprotect/desilylate leaving the label intact, provided the desilylation is carried out using $Et_3N.3HF$ in DMSO (1:1) in place of TBAF.

Cyanine Labelling (inc. Quasar® Dyes)

2158	Quasar® 570-CE Phosphoramidite	83
2159	Quasar® 670-CE Phosphoramidite	83
2520	Cyanine-3-CE Phosphoramidite	83
2521	Cyanine-5-CE Phosphoramidite	83
2412	3'-Cyanine-3 SynBase™ CPG 1000	83
2413	3'-Cyanine-5 SynBase™ CPG 1000	83

Dissolution

Although all the phosphoramidites are soluble in acetonitrile, we have found better results for the cyanine 3 and 5 dyes when using anhydrous, alcohol-free dichloromethane. Use of molecular sieves is also recommended with either diluent.

Coupling

A 10min coupling time is recommended for all phosphoramidites. Due to the milder conditions required when deprotecting Quasar®-labelled oligonucleotides, the use of fast deprotecting phosphoramidites is recommended.

Oxidation

The use of 0.1M oxidiser must be avoided to minimise oxidation of cyanine dyes. Hence 0.02M oxidiser is recommended (**4132** or **4330**).

Deprotection

As these reagents require no deprotection themselves, the oligonucleotide deprotection strategy employed is guided by the protective groups on the rest of the oligo. However, the mildest possible conditions are recommended, especially for Quasar® 670 (**2159**) and Cyanine-5 (**2521**) which are susceptible to degradation at elevated temperatures and basic conditions.

Ammonium hydroxide for 8h at room temperature, followed by immediate removal of the deprotection solution by passing through G25 is recommended. For all cyanine dyes it is advisable to remove the deprotection solution without heating, *e.g.* G25 or precipitation.

Spectral Data

	Absorbance Max./nm	Emission Max./nm	Fluorescent Region
Quasar® 570	547	570	Yellow-Orange
Quasar® 670	644	670	Red
Cyanine-3	546	563	Yellow-Red
Cyanine-5	646	662	Violet-Far Red

Storage & Stability

These products are light-sensitive and are susceptible to oxidation when left exposed to air and/or moisture. Store dry in a freezer, in a sealed dark or amber container at –20°C. Similarly, modified oligonucleotides must also be protected from exposure to light. The phosphoramidites are stable in solution under argon for 24h.

CAL Fluor® Dyes

2423	3'-CAL Fluor® Orange 560 CPG 500	84
2424	3'-CAL Fluor® Red 610 CPG 500	84
2538	CAL Fluor® Orange 560-CE Phosphoramidite	84
2539	CAL Fluor® Red 590-CE Phosphoramidite	84
2540	CAL Fluor® Red 610-CE Phosphoramidite	84

Dissolution

2538 - Dilute to 0.1M in dry, alcohol-free DCM.

2539 & **2540** - Dilute to 0.1M in dry acetonitrile.

In all cases the use of 4Å molecular sieves is highly recommended.

Coupling

A 15min coupling is recommended. Due to the mild deprotection conditions we recommend using fast deprotecting amidites. Prior to dilution of amidites ensure that all product is at the bottom of the vial. Dilute to the recommended concentration and mix thoroughly in sealed vial to ensure that all contents are dissolved. Once dissolved, add the sieves. We recommend using ETT (**3140**/**2**/**5**) as the activator for these products.

Cleavage & Deprotection

Cleave and deprotect the CAL dye labelled oligo in 2-methoxyethylamine[252]/MeOH (1:3) for 3h at 60°C (all products) or 6h at 25°C (CPGs). Filter the sample and evaporate the supernatant. Re-dissolve the CAL dye labelled oligo in water. The use of strong bases, such as concentrated ammonia, causes degradation and must be avoided.

Storage & Stability

The products are light sensitive. Prolonged exposure to light may cause photo-bleaching. The products are also susceptible to oxidation when left out in the open or undesiccated. Store at -20°C, desiccated. Amidite solutions should be stored under argon, but used within 24h.

Spectral Data

	Absorbance Max./nm	Emission Max./nm	Fluorescent Region
CAL Fluor® Orange 560	537	558	yellow-orange
CAL Fluor® Red 590	569	591	yellow-orange
CAL Fluor® Red 610	590	610	orange-red

TAMRA Labelling

0251	TAMRA NHS Ester (in DMSO)	86
2143	TAMRA-dT-CE Phosphoramidite	86
2372	3'-TAMRA CPG	86

Synthesis Planning

TAMRA-labelled oligonucleotides require milder deprotection conditions: use ^{t}butylamine/methanol/water (1:1:2).[253] Therefore, use Ac-dC and dmf-dG when synthesising the oligo. Ammonium hydroxide or AMA deprotection significantly degrades TAMRA. Post-synthetic labelling using TAMRA NHS Ester (**0251**) is discussed below.

Dissolution

TAMRA-dT-CE Phosphoramidite (**2143**) is dissolved in 10% THF in anhydrous acetonitrile (**4059**) typically to 0.1M concentration. Allow 15min for the product to completely dissolve.

Coupling

2143 – A 6min coupling time is recommended. Note, as with all dyes, remove the modifier from the synthesiser port and clean thoroughly immediately after synthesis is complete.

252 Available from Sigma-Aldrich, catalogue # 241067-50.

253 **Automated synthesis of double dye-labelled oligonucleotides using tetramethylrhodamine (TAMRA) solid supports**, B. Mullah and A. Andrus, *Tetrahedron Lett.*, **38**, 5751-5754, 1997.

Cleavage & Deprotection

2143 – Deprotection is carried out using ^{t}butylamine/methanol/water (1:1:2) for 2.5h at 70˚C. 0.05M Potassium carbonate deprotection is also possible.

2372 – Typical protocol is as follows:

1. After the oligo has been synthesised, remove the support from the column and add 600µl of ^{t}butylamine/methanol/water (1:1:2) solution.
2. Heat at 70˚C for 2.5h..
3. Pass the sample down a G25 column equilibrated with water or buffer for purification This removes the deprotection solution.
4. Sample is now ready for purification.

TOP-DNA Cartridge Purification

Note this will not remove oligos containing TAMRA degradation. Do not remove the deprotection solution or desalt the oligo. The deprotection solution needs to be present.

1. Add 1mL of aqueous sodium chloride solution (100mg/mL) to each oligo.
2. Place one column for each oligo onto the vacuum manifold.
3. Turn the vacuum pump on.
4. Adjust the pressure to 7.0in (178mm) Hg using the vacuum control valve.
5. Add 0.5mL of acetonitrile to the cartridge.
6. Immediately then add 1mL of 2MTEAA to condition the medium.
7. Add the oligo solution to the cartridge in 1mL aliquots.
8. Add 1mL of NaCl(aq) solution (100mg/mL) to the column.
9. Add 1mL of NaCl(aq) solution (100mg/mL) to the column.
10. Add 1mL of 2% TFA/water solution to the column.
11. Add 1mL of 2% TFA/water solution to the column.
12. Add 1mL of water to the column.
13. Add 1mL of water to the column.
14. Release the vacuum.
15. Place an appropriately labelled tube beneath each of the column.
16. Add 1mL of MeCN/water 1:1 to elute the DNA oligo under vacuum.
17. If all the TMR-oligo is not eluted, repeat Step 16.

Post-Synthesis Labelling Protocol

As noted above, TAMRA-labelled oligos will not survive ammonium hydroxide or AMA deprotection conditions. Therefore, if such conditions are necessary, **0251** can be used to label the oligonucleotide post-synthetically to an amino functionalised oligonucleotide. This is provided as a 0.17M solution in DMSO. A synthesised amino-modified oligonucleotide is then conjugated to TAMRA using the NHS ester in sodium carbonate/bicarbonate buffer (0.1M, pH=9). A typical protocol for the conjugation of an amino-modified oligo (synthesised at 0.2µmol scale) is as follows:

1. Dissolve the amino-modified oligo in 125µl of conjugation buffer.
2. Add 6µl of TAMRA/DMSO solution (ca. 5-fold excess).
3. Vortex the mixture and incubate at 37°C in the dark overnight.
4. Separate the oligo-TAMRA conjugate from salts and free TAMRA by size-exclusion chromatography on a NAP-10 column or equivalent.
5. To do this, equilibrate the NAP column with 0.1M TEAA.
6. Load the reaction mixture onto the column and let this flow into the column.
7. Add 0.5ml TEAA buffer and also let this flow into the column.
8. Elute the conjugate with <1.5ml TEAA buffer.
9. Collect the conjugate and reduce to a suitable volume for HPLC purification.

Spectral Data

	Absorbance Max./nm	Emission Max./nm	Colour
TAMRA	565	580	Pink

Storage & Stability

All products are stored dry in a freezer at –10 to –30°C, protected from light. Labelled oligos are stored in the dark. **2143** is stable in solution for 24h, but it is recommended this is removed from the synthesis port after use and the

port thoroughly cleaned to avoid combination during the next synthesis.

Non-Fluorescent (Dark) Quenchers

General

Today, the most commonly used dark quenchers are all azo based. It must be noted that as a result it is not uncommon to observe fragmentation during mass spectrometry analysis by MALDI-TOF. Dabcyl and DDQ-1 will show a fragment of M-134, BHQ®-1 shows M-298 and BHQ®-2 shows M-300.

Dabcyl Labelling

2085	5'-Dabcyl-CE Phosphoramidite	87
2144	Dabcyl-dT-CE Phosphoramidite	87
2374	3'-Dabcyl CPG	87

Dissolution

Dabcyl-dT-CE Phosphoramidite (**2144**) is dissolved in 10% THF in acetonitrile (**4059**, provided free upon request), 5'-Dabcyl-CE Phosphoramidite (**2085**) in anhydrous acetonitrile.

Coupling

A 6min coupling time is recommended for the phosphoramidites. 3'-Dabcyl CPG (**2374**) is used as per an unmodified nucleoside support. However, since non-nucleosidic modifications detritylate at a slower rate it is recommended to carry out an initial additional detritylation. In this case it is important not to carry out any additional capping.

Deprotection

Most oligonucleotide deprotection conditions can be applied. See information on pages 156-169 for more details.

Storage & Stability

All compounds are stored in a freezer at -10 to -30°C. Stability of the phosphoramidites in solution is 2-3 days.

Black-Hole Quenchers® (BHQ®s)

2154	5'-BHQ®-1-CE Phosphoramidite	89
2155	5'-BHQ®-2-CE Phosphoramidite	89
2156	BHQ®-1-dT-CE Phosphoramidite	89
2157	BHQ®-2-dT-CE Phosphoramidite	89
2379	3'-BHQ®-1 CPG	89
2380	3'-BHQ®-2 CPG	89

Dissolution

The dT amidites (**2156** & **2157**) are dissolved in anhydrous (alcohol-free) dichloromethane, and the 5'-amidites (**2154** & **2155**) are dissolved in anhydrous acetonitrile, all to 0.1M concentration. Prepare these amidite solutions at least 10min before placing on the synthesiser.

Coupling

2154, **2155** & **2156** – A 10min coupling time is recommended.

2157 – A 15min coupling time is recommended.

The CPGs are used per unmodified solid supports following the synthesiser instructions. Non-nucleosidic CPG supports do not detritylate as rapidly as nucleosidic ones, therefore an additional detritylation step is recommended. It is therefore necessary to use a cycle that does not contain an initial capping step.

Cleavage & Deprotection

When selecting a deprotection method for oligos containing the BHQ® phosphoramidites, use conditions suitable for any other modifications on the oligonucleotide. The BHQ® component itself is stable to most deprotection conditions. When using a base-sensitive fluorophore such as TAMRA, UltraMILD deprotection (0.05M potassium carbonate in methanol for 4h at room temperature) or deprotection with ^{t}butylamine/water (1:3) for 2.5h at 70°C is recommended.

The CPGs have a glycolate linkage. This allows for rapid cleavage of the oligonucleotides and is labile enough for base-sensitive oligos. For cleavage use ammonium hydroxide for 20min at 25°C or AMA for 5min at 25°C; or, when using TAMRA, ^{t}butylamine/methanol/water (1:1:2) for 45min at 25°C.

Complete the deprotection with conditions suitable for the other nucleobases or modifications as above.

Spectral Data

	Absorbance Max./nm
BHQ®-1	534
BHQ®-2	579

Analysis

Note that if analysing oligos after purification by MS, an additional peak at M-298 (BHQ®-1) or M-300 (BHQ®-2) is observed. This is attributed to fragmentation within the MS through the diazo bond.

Storage & Stability

Products are stored in light-protected containers in the freezer at –10 to –30°C. All phosphoramidites are susceptible to oxidation when left exposed to air and/or moisture. All phosphoramidites are stable in solution under argon for 2 days.

Deep Dark Quencher 1

2349	3'-DDQ-1 SynBase™ CPG 1000/110	89

Detritylation

Non-nucleosidic CPG supports do not detritylate as rapidly as nucleosidic ones, therefore an additional detritylation step is recommended. It is therefore necessary to use a cycle that does not contain an initial capping step.

Cleavage & Deprotection

UltraFAST conditions can be used and provide the best results: a 1:1 mixture of aqueous ammonium hydroxide and aqueous methylamine (AMA) for 10min at 65°C.

Purification & Analysis

Purification by RP-HPLC or cartridge purification is recommended. IE-HPLC or PAGE for high purity oligos also works very well, however FPLC tends not to give a good separation. This is thought to be due to the zwitterionic nature of this modification.

Note that if analysing oligos after purification by MS, an additional peak at M-134 is observed. This is attributed to fragmentation within the MS through the diazo bond.

Storage & Stability

The support is stored in the fridge or freezer and protected from exposure to light. Similarly, modified oligos are also protected from light.

Biotin Labelling

2067	Biotin-dT-CE Phosphoramidite	92
2109	5'-Biotin-CE Phosphoramidite	92
2132	Biotin-TEG-CE Phosphoramidite	92
2140	Biotin-CE Phosphoramidite	92
2353	3'-Biotin-TEG CPG	92

Oxidation

The use of 0.1M oxidiser must be avoided to prevent oxidation of biotin. However, should this occur, there is no adverse affect on biotin-avidin binding.

Coupling, Deprotection & Purification

Biotin-CE Phosphoramidite (**2140**) – A 15min coupling time is recommended. This is stable to most deprotection conditions.

Biotin-TEG-CE Phosphoramidite (**2132**) – A 12-15min coupling time is recommended. Most deprotection conditions are applicable. For optimal yield, oligos are prepared DMT ON, with removal of the DMTr group after cleavage and deprotection. Otherwise, the 1,2-diol configuration allows cleavage of the biotin TEG during deprotection.

3'-Biotin-TEG CPG (**2353**) – Since non-nucleosidic solid supports have a slower detritylation rate than nucleosidic supports, it is recommended than an additional detritylation be carried out. Note in this case an initial capping step must be avoided. Most cleavage and deprotection conditions are applicable.

Biotin-dT-CE Phosphoramidite (**2067**) – An extended coupling time of 15min is recommended. Most cleavage and deprotection conditions are applicable. The ^{t}butylbenzoyl group, used to aid the solubility and to protect the biotin, is removed during deprotection.

5'-Biotin-CE Phosphoramidite (**2109**) – A 5min coupling time is recommended. The trityl group is slow to detritylate, therefore removal on the synthesiser requires an additional deblocking step. If the biotin DMTr group is retained to aid cartridge purification, we recommend that the oligo is left in contact with the TFA solution for 10min.

Storage & Stability

All compounds are stored in a freezer at –10 to –30°C. **2140** & **2132** are stable in solution for 2-3 days, **2109** for 1-2 days, and **2067** for 3-5 days.

Electrochemical Detection

2167	Ferrocene-dT-CE Phosphoramidite	94

Coupling

A 5min coupling time is recommended. Ac-dC and dmf-dG must be used in the oligo synthesis to allow AMA deprotection.

Oxidation

The use of 0.1M oxidiser must be avoided to minimise oxidation of the ferrocene label.

Cleavage & Deprotection

Cleave and deprotect using AMA for 10min at 65˚C, followed by immediate desalting with G25 to remove the deprotection solution.

Purification

Best results are obtained using ion-exchange HPLC. Note to avoid oxidation of the ferrocene ring system it is recommended all solvents and buffers are deoxygenated with argon.

Storage & Stability

Store in a freezer at -10 to -30˚C. Acetonitrile solutions must be used within 24h.

Branching Modification

2150	5-Me-dC-Brancher-CE Phosphoramidite	94

Due to the possible complexity of these syntheses, we would advise customers to use these recommendations only as a guide and to optimise the conditions for their own use depending on the sequences and other modifiers employed.

Note that 3'-phosphate modification is not stable to the levulinyl deprotection step and the oligo is cleaved from the support.

Synthesis of simple branched structures

The product is simple to use in the synthesis of branched oligos with a small number of Branching Modifier (BM) inclusions (*e.g.* the "fork" structure created by using just one BM). The primary sequence is synthesised, incorporating the BM as follows:

Coupling

Coupling is as per unmodified nucleoside amidites.

Levulinyl Deprotection

If the secondary sequence is not required at the 5' end, this must be capped prior to removing the levulinyl group. Prior to secondary sequence synthesis, the column is removed from the synthesiser and the levulinyl group is selectively removed without cleavage of the oligonucleotide by treatment with 0.5ml freshly-prepared 0.5M hydrazine hydrate in 1:1 pyridine/acetic acid (when using a 40nmol to 1µmol scale; use 10-15ml for 10µmol synthesis columns). To do this, fit the column with syringes and wash the solution back and forth. Allow to react for 15min (note sequences with many BM molecules require treatment for up to 90min to ensure complete levulinyl removal). Rinse the solid support with 10ml acetic acid/pyridine (1:1), followed by extensive rinsing with acetonitrile before drying under a stream of argon.

Secondary Sequence

At this point the column is returned to the synthesiser to proceed with the secondary sequence synthesis. For primary sequences with only one or two BM molecules, the secondary sequence can be carried out using unmodified cycle conditions although optimisation may be required.

It is important that no initial capping step is carried out in the synthesis cycle when synthesising the secondary sequence.

Note that for the secondary sequence, the equivalents of phosphoramidites must be increased to account for the number of growing chains. For example, if there are two branching points, double the molar equivalents of amidite will theoretically be required.

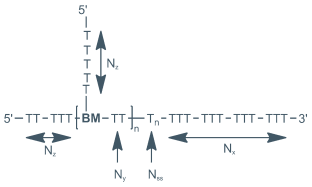
Cleavage & Nucleobase Deprotection

After secondary synthesis is complete, the oligonucleotide is cleaved from the support and base-deprotected. Most deprotection conditions are applicable (although this will be determined by other modifications within the oligo).

However, due to the complex secondary structures that can now form, ammonium hydroxide overnight at 55˚C gives the best results.

Synthesis of complex 'comb'-like structures

Construction of more complex comb-like structures with many BMs in the primary sequence requires greater control both in the initial design of the primary sequence, and in the protocol used for synthesis of the secondary sequences. Horn *et al*[254] have suggested a scheme for doing this, the overall design of which is shown below.



The main recommendations for carrying out these syntheses are as follows: [216, 255]

Primary Sequence

For complex structures, the principal consideration when synthesising the primary target oligonucleotide is to combat the possible adverse effects on oligo yield due to the steric bulk of the oligo. This is done in two main ways: by using a large-pore CPG; and by introducing a spacer sequence to distance the branches from the CPG.

Increasing the pore size of the CPG to 2000Å or even 3000Å has been shown to greatly improve the quality of the synthesis. The steric constraints can be further reduced by using a lower nucleoside loading. A spacer sequence, typically T20, can be added between the primary sequence and inclusion of the branching molecules, and a further T2 spacer between the BMs themselves. The use of HEG spacers (*e.g.* **2129**) is also known to be effective.

The coupling of the phosphoramidites, and subsequent levulinyl deprotection (90min for high BM content) and washing of the support is carried out as described above for simple primary sequences.

Secondary Sequence

Synthesis of the secondary sequences is best carried using a large excess of phosphoramidite reagents (10-fold excess with respect to each hydroxyl site) and a longer coupling time (*e.g.* 60s on an ABI 394 synthesiser). As in simpler structures (see above) the molar equivalents of phosphoramidites employed needs to be increased in accordance with the number of branched chains being extended.

The branched DNA can be detritylated, cleaved from the support and deprotected using ammonium hydroxide although conditions may need to be optimised. Note that for multiple branches the final detritylation may need to be increased.

254 **An improved divergent synthesis of comb-type branched oligodeoxyribonucleotides (bDNA) containing multiple secondary sequences**, T. Horn, C-A. Chang and M.S. Urdea, *Nucleic Acids Research*, **25**, 4835-4841, 1997.

255 **Chemical synthesis and characterization of branched oligodeoxynucleotides (bDNA) for use as signal amplifiers in nucleic acid quantification assays**, T. Horn, C-A. Chang and M.S. Urdea, *Nucleic Acids Research*, **25**, 4842-4849, 1997.

Therapeutic Application Modification

Lipophilic Group Modification

2163	5'-Tocopherol-CE Phosphoramidite	104
2170	5'-Cholesterol-CE Phosphoramidite	104
2189	5'-Cholesterol-TEG-CE Phosphoramidite	104
2194	5'-Octyltocopherol-CE Phosphoramidite	104
2199	5'-Palmitate-C6-CE Phosphoramidite	104
2393	3'-Palmitate SynBase™ CPG 1000/110	104
2394	3'-Cholesterol SynBase™ CPG 1000/110	104

Dissolution

2163, **2170**, **2194** and **2199** - Dilute in anhydrous, alcohol-free DCM to a concentration of 0.1M.

2189 - Use anhydrous acetonitrile to a concentration of 0.1M.

Prepare the amidite solution 5-10min before placing on the synthesiser to ensure complete dissolution. See notes on page 116.

Coupling

2163, **2170** and **2194** - An increased coupling time of 15min is recommended for the phosphoramidites. Contrary to MacKellar *et al*[256], we have found that when using **2170**, column washes with DCM before and after coupling are unnecessary. In our hands, omitting the DCM washes gave the highest final coupling results and there was no evidence of reagent precipitation in the lines.

2199 - 3-5min (3min up to 1µmol).

2393 and **2394** - Both CPG supports are used as per any unmodified nucleoside support as per instrument instructions. However, non-nucleosidic modifications are slow to detritylate and require an additional detritylation prior to use in synthesis. In this case it is important not to use a cycle with an initial capping step.

It is recommended that the oligonucleotide is synthesised DMT OFF when using the 3' modifications (CPGs), otherwise the presence of the DMTr and hydrophobic group can result in difficult purification and solubility issues.

Cleavage & Deprotection

For the amidites no changes are required from your typical method, however the optimum conditions are AMA for 2h at RT. The modifiers - **except for 2189** - are stable to most common deprotection methods *e.g.* AMA, 10min, 65°C (cholesterol-TEG has a tendency to cleave through the carbamate at elevated temperatures, therefore room temperature deprotection is required in this case).

The CPG supports use the succinyl linker which will cleave under most ammonium hydroxide solution and AMA deprotection conditions (typically 1-2h at room temperature with ammonium hydroxide solution, and a few minutes at 65°C with AMA). The linker will also cleave with potassium carbonate at room temperature (>90% after 4h). Therefore cleavage and deprotection of the oligo is carried out according to the deprotection protocols required by the nucleobases and other modifiers (if present). When synthesising short oligos (<15 bases) it is, however, advantageous to add 20% EtOH to the cleavage and deprotection solution to ensure complete dissolution of the oligo.

Purification

Purification by RP-HPLC is recommended for oligonucleotides modified with hydrophobic labels. Where 3'-modifiers are used DMT ON purification is possible but makes the oligonucleotide extremely hydrophobic. As a result short oligos often have solubility issues. Also, removing the DMTr group in aqueous acetic acid can cleave the cholesterol label from the oligo through the carbamate bond.

Where modification is incorporated at the 5' end, there is a significant difference in the retention time between the labelled and unlabelled oligo, making purification simple. In general the HPLC gradient must reach at least 95% MeCN to elute the product.

Whilst this removes the unlabelled failures from the labelled oligo very efficiently, if there is a requirement to remove any labelled deletion sequences IE-HPLC or PAGE is the preferred choice. Similarly, where the modifier is incorporated at the 3' end, the latter is the preferred choice of purification. RP-HPLC gives limited separation in

256 **Synthesis and physical properties of anti-HIV antisense oligonucleotides bearing terminal lipophilic groups**, C. MacKellar, D. Graham, D.W. Will, S. Burgess and T. Brown, *Nucleic Acids Research*, **20**, 3411-3417, 1992.

this case since the full-length and failure sequences are all labelled with the hydrophobic group.

Storage & Stability

All products are stored dry in a freezer at –10 to –30°C and are stable under these conditions for over 12 months. Diluted samples must be used within 24h.

Vitamin Modification

2530	5'-Niacin-CE Phosphoramidite	106
2536	BisDMT-Pyridoxine-C6-CE Phosphoramidite	106
2537	5'-Niacin-C6-CE Phosphoramidite	106

Coupling

A 15min coupling time is recommended for all products.

Cleavage & Deprotection

2530 and **2537** - Use the deprotection conditions consistent with which form of niacin / nicotinamide you wish to use in your application, and the compatibility with other bases and modifications in your oligo:

For -CO_2H use 0.4M NaOH in MeOH/water (4:1), RT, 5h.

For -$CONH_2$ use ammonium hydroxide, 55˚C, overnight.

For -$CONHCH_3$ use ammonium hydroxide/methylamine (AMA), RT for 2h or 65˚C for 10min.

Note that for RNA, to obtain the free acid, sodium hydroxide deprotection is not compatible therefore it is best to use 0.1M DBU in anhydrous MeCN, 18h, RT for the cleavage and nucleobase deprotection.

2536 - Use AMA, 2h, RT. Note that DMT-ON purification can be employed. A double detritylation is required.

Storage & Stability

Store in a freezer at -10 to -30˚C. Note that these products are still in early stage development therefore stability data is still being gathered.

Structural Study Modification

Duplex Effects

2013	dU-CE Phosphoramidite	112
2016	dI-CE Phosphoramidite	112
2017	5-Me-Bz-dC-CE Phosphoramidite	112
2145	2-Amino-dA-CE Phosphoramidite	112
2164	2'-Deoxyxanthosine-CE Phosphoramidite	112
2287	dU SynBase™ CPG 1000/110	112
2293	dI SynBase™ CPG 1000/110	112
2323	5-Me-dC SynBase™ CPG 1000/110	112
2529	5-Me-Ac-dC-CE Phosphoramidite	112

Coupling

Couple the phosphoramidites **2013**, **2016** and **2017** using the standard method as recommended by synthesiser manufacturer with coupling times as per unmodified bases. 90s is recommended for **2529**. A coupling time of 15min is recommended for **2145**. A coupling time of 3min is recommended for **2164**. The solid supports are used as per unmodified nucleoside supports.

Deprotection

The supports are cleaved and deprotection carried out using the protocols required by the nucleobases.

For **2013**, **2016** and **2529** most oligonucleotide deprotection conditions can be applied, however **2013**, **2016** and **2529** are also compatible with AMA deprotection; typically 10min at 55ºC. See information on pages 156-169 for more details.

2017 - This is not stable to AMA deprotection, therefore ammonium hydroxide, 55ºC, overnight is recommended.

2145 - Use AMA deprotection in conjunction with Ac-dC, rather than Bz-dC, or transamidation will occur.

2164 - Cleavage from support and primary deprotection is accomplished by treatment with ammonium hydroxide

solution at room temperature for 24h.

After removing the solvent[257], the NPE groups are removed by treatment with 0.3M tetramethylguanidinium 2-nitrobenzaldoximate solution in water/dioxan (1:1) at 70°C for 48h. We find that this extended treatment is necessary to ensure complete removal of both of the NPE protecting groups.

It should be noted that this treatment generates a variety of low molecular weight by-products which are observed in the HPLC. Satisfactory results are obtained by de-salting the deprotection mixture into water using a NAP or G25 column before HPLC, from which the final oligonucleotide product can then be isolated.

Storage & Stability

Refrigerate dry compounds. Stability of phosphoramidites in solution is similar to dA, dC, dG and dT monomers.

Photocrosslinking

2009	5-I-dC-CE Phosphoramidite	112
2011	5-Br-dC-CE Phosphoramidite	112
2012	5-Br-dU-CE Phosphoramidite	112
2014	5-I-dU-CE Phosphoramidite	112
2054	8-Br-dA-CE Phosphoramidite	112
2055	8-Br-dG-CE Phosphoramidite	112
2325	5-Br-dU SynBase™ CPG 1000/110	112
2070	4-Thio-dT-CE Phosphoramidite	112

Coupling

Coupling is as per unmodified nucleoside amidites.

Oxidation

0.02M Oxidiser is recommended for **2070**.

Deprotection

To avoid ammonolysis at the halogenated site, a mild deprotection step is recommended using ammonium hydroxide for 24h at room temperature, or AMA for 2h at room temperature provided dmf-dG and Ac-dC are used. Bz-protected C amidites are not compatible with AMA deprotection.

For **2070** it is desirable to remove the cyanoethyl protecting group using 1M DBU in acetonitrile for 3h at room temperature, prior to cleavage and deprotection. The latter is achieved with 50mM NaSH in ammonium hydroxide solution for 24h at room temperature.

Storage & Stability

Store the solid phosphoramidites refrigerated, dry, at a maximum of 2-8°C. Store the monomers in solution for no longer than 24h, although 8-Br-dG (**2055**) and 5-Br-dU (**2012**) are stable for 2-3 days. Halogenated nucleosides tend to be photosensitive therefore retain the amidites in amber vials.

Other Structural Studies

2010	5-F-dU-CE Phosphoramidite	112
2018	O6-Me-dG-CE Phosphoramidite	112
2019	N6-Me-dA-CE Phosphoramidite	112
2025	O4-Me-dT-CE Phosphoramidite	112
2069	2-Aminopurine-CE Phosphoramidite	112
2072	8-oxo-dG-CE Phosphoramidite	112

Coupling

Coupling is as per unmodified nucleoside amidites.

Deprotection

2010 - To avoid ammonolysis at the halogenated site, a mild deprotection step is recommended using ammonium hydroxide for 24h at room temperature, or AMA for 2h at room temperature provided dmf-dG and Ac-dC are used. Bz-protected C amidites are not compatible with AMA deprotection.

257 This is best achieved by removing the deprotection solution by G25 then freeze drying to remove the water. Heat cannot be used or the NPE groups would cleave.

2018 and **2025** -

1. Empty the synthesis resin from the column into a sample tube.
2. Add 900µl anhydrous MeOH and 100µl DBU (1,8-Diazabicyclo [5.4.0]undec-7-ene) to the resin in the sample tube and cap tightly.
3. Place the tube in a dry dark place and leave it for 5 days.
4. Reduce to small volume.
5. Add 1ml of 10mM aqueous sodium hydroxide solution and desalt or purify the oligonucleotide using standard procedures.

2019 - Use AMA at room temperature for 2h. As with **2010**, employ dmf-dG and Ac-dC within the sequence.

Most deprotection conditions are applicable to **2069**.

2072 - Cleave and deprotect with ammonium hydroxide containing 0.25M 2-mercaptoethanol for 17h at 55°C to avoid oxidative degradation of the 8-oxo-dG site.

Storage & Stability

Refrigerate dry compounds. Stability in solution is 2-3 days, although **2072** is best used within 24h. Halogenated nucleosides tend to be photosensitive therefore retain **2010** in amber vials.

Summary Protocols

Summarised synthesis and deprotection conditions for all products for your convenience. Products are grouped into phosphoramidites (and similar), pages 156 to 165, and solid supports, pages 166 to 169, ordered by item number.

General Notes

Diluent

It is recommended to dissolve phosphoramidites 5-10min prior to placing on the synthesiser.

Coupling

Coupling times are stated as ranges. The lower end refers to ≤ 1µmol synthesis scales, the higher to scales in the 5 - 10µmol region. Adjust accordingly.

Deprotection

Deprotection conditions, other than those listed, are possible, especially when using combinations of modifications. Where AMA or EDA are used, the standard DNA or RNA bases must have fast deprotection (*i.e.* Ac-dC and dmf-dG, or Ac-C and dmf-G).

Phosphoramidites *etc.*

Item No.	Modification	Is the Modification Suitable for "DMT ON" Purification?	Diluent	Recommended Concentration	Stability In Solution	Coupling Time / s	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2001	dT	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C		26	116
2002	iBu-dG	Yes	MeCN	0.065-0.1M	5-7d	25-60	Ammonium hydroxide, 4h, 55°C		26	116
2003	Bz-dA	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C		26	116
2004	Bz-dC	Yes	MeCN	0.065-0.1M	5-7d	25-60	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur.	26	116
2005	dT H-phosphonate TEA salt	Yes	Pyr/ MeCN 1:1	0.1M	5-7d	300-600	Ammonium hydroxide, 4h, 55°C	Note modified coupling and oxidation conditions are required.	60	138
2006	iBu-dG H-phosphonate TEA salt	Yes	Pyr/ MeCN 1:1	0.1M	5-7d	300-600	Ammonium hydroxide, 4h, 55°C	Note modified coupling and oxidation conditions are required.	60	138
2007	Bz-dA H-phosphonate TEA salt	Yes	Pyr/ MeCN 1:1	0.1M	5-7d	300-600	Ammonium hydroxide, 4h, 55°C	Note modified coupling and oxidation conditions are required.	60	138
2009	5-I-dC	Yes	MeCN	0.065-0.1M	24h	25-60	Ammonium hydroxide, 24h, RT		112	153

Phosphoramidites *etc.*										
Item No.	**Modification**	**Is the Modification Suitable for "DMT ON" Purification?**	**Diluent**	**Recommended Concentration**	**Stability In Solution**	**Coupling Time / s**	**Recommended Deprotection Conditions**	**Comments**	**Ordering Page**	**Protocol Page**
2010	5-F-dU	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	112	153
2011	5-Br-dC	Yes	MeCN	0.065-0.1M	24h	25-60	Ammonium hydroxide, 24h, RT	Ac-dC and dmf-dG or Ac-C and dmf-G are required for standard DNA or RNA bases for deprotection.	112	153
2012	5-Br-dU	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	112	153
2013	dU	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 10min, 65°C	See general deprotection notes above.	112	152
2014	5-I-dU	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	112	153
2016	dI	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	112	152
2017	5-Me-dC	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	112	152
2018	O6-Me-dG	Yes	MeCN	0.065-0.1M	2-3d	25-60	1. 10% DBU/anhydrous MeOH, 5d, RT 2. 10mM NaOH (aq), desalt	As 2025 below.	112	153
2019	N6-Me-dA	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 2h, RT	See general deprotection notes above.	112	153
2020	dT-5'-CEPA	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	75	141
2021	iBu-dG-5'-CEPA	Yes	MeCN	0.065-0.1M	5-7d	25-60	Ammonium hydroxide, 4h, 55°C		75	141
2022	Bz-dA-5'-CEPA	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	75	141
2023	Bz-dC-5'-CEPA	Yes	MeCN	0.065-0.1M	5-7d	25-60	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur.	75	141
2025	O4-Me-dT	Yes		0.065-0.1M	2-3d	25-60	1. 10% DBU/anhydrous MeOH, 5d, RT 2. 10mM NaOH (aq), desalt	Empty the resin from the column into a sample tube and add the deprotection solution (1ml). Leave in the dark for 5d at room temperature and reduce to a small volume. Add 10mM NaOH (1ml) then filter and desalt or purify.	112	153
2030	dmf-dG	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	26	116
2033	dmf-G	Yes	MeCN	0.065-0.1M	4-5d	300-900	1. AMA, 10min, 65°C 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2034	Ac-dC	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	26	116
2035	Bz-dC H-Phosphonate, DBU salt	Yes	Pyr/ MeCN 1:1	0.1M	5-7d	300	Ammonium hydroxide, 4h, 55°C	Note modified coupling and oxidation conditions are required.	60	138

Phosphoramidites *etc.*

Item No.	Modification	Is the Modification Suitable for "DMT ON" Purification?	Diluent	Recommended Concentration	Stability In Solution	Coupling Time / s	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2036	Bz-A	Yes	MeCN	0.065-0.1M	4-5d	300-900	1. AMA, 10min, 65°C 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2037	Pac-A	Yes	MeCN	0.065-0.1M	1-2d	300-900	1. Ethanolic ammonium hydroxide, 16h, RT 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2038	Ac-C	Yes	MeCN	0.065-0.1M	4-5d	300-900	1. AMA, 10min, 65°C 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2039	iPr-Pac-G	Yes	MeCN	0.065-0.1M	1-2d	300-900	1. Ethanolic ammonium hydroxide, 16h, RT 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2040	U	Yes	MeCN	0.065-0.1M	4-5d	300-900	1. AMA, 10min, 65°C 2. Et_3N/Et_3N:3HF/NMP 1.5:2:3, 2.5h, 65°C	The coupling time will depend on the activator being used, BTT can reduce the coupling time to 3min but for ETT it is better to use 10-15min coupling. Also longer oligos will require longer coupling times.	34	123
2041	2'-OMe-Bz-A	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 10min, 65°C	See general deprotection notes above.	71	140
2042	2'-OMe-Bz-C	Yes	MeCN	0.065-0.1M	3-5d	300-500	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur.	71	140
2043	2'-OMe-Ac-C	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 10min, 65°C	See general deprotection notes above.	71	140
2044	2'-OMe-dmf-G	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 10min, 65°C	See general deprotection notes above.	71	140
2045	2'-OMe-U	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 10min, 65°C	See general deprotection notes above.	71	140
2050	Ac-dC-Me-PA	Yes	MeCN	0.065-0.1M	2-3d	25-60	0.05M K_2CO_3 in MeOH, 4h, RT		65	137
2051	iPr-Pac-dG-Me-PA	Yes	MeCN	0.065-0.1M	2-3d	25-60	0.05M K_2CO_3 in MeOH, 4h, RT		65	137

Phosphoramidites *etc.*										
Item No.	**Modification**	**Is the Modification Suitable for "DMT ON" Purification?**	**Diluent**	**Recommended Concentration**	**Stability In Solution**	**Coupling Time / s**	**Recommended Deprotection Conditions**	**Comments**	**Ordering Page**	**Protocol Page**
2052	Pac-dA-Me-PA	Yes	MeCN	0.065-0.1M	2-3d	25-60	0.05M K_2CO_3 in MeOH, 4h, RT		65	137
2054	8-Br-dA	Yes	MeCN	0.065-0.1M	24h	25-60	Ammonium hydroxide, 24h, RT		112	153
2055	8-Br-dG	Yes	MeCN	0.065-0.1M	24h	25-60	Ammonium hydroxide, 24h, RT		112	153
2056	Formylindole	Yes	MeCN	0.065-0.1M	3-4d	900	AMA, 10min, 65°C	See general deprotection notes above.	47	132
2057	Carboxylate	No	MeCN	0.1M-0.15M	2-3d	900	0.4M NaOH in MeOH/water (4:1), overnight, RT		46	131
2059	Pac-dA	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	31	122
2060	iPr-Pac-dG	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	31	122
2066	PC Linker	Yes	MeCN	0.065-0.1M	24h	900	AMA, 10min, 65°C	See general deprotection notes above.	69	139
2067	Biotin-dT	Yes	MeCN	0.1M	2-3d	900	AMA, 10min, 65°C	See general deprotection notes above.	92	148
2068	Fluorescein-dT	Yes	MeCN	0.065-0.1M	24h	300-600	AMA, 10min, 65°C	See general deprotection notes above.	81	143
2069	2-Aminopurine	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	112	153
2070	4-Thio-dT	Yes	MeCN	0.065-0.1M	2-3d	25-60	50mM NaSH / Ammonium hydroxide, 24h, RT		112	153
2071	Amino-C6-dA	No	MeCN	0.065-0.1M	2-3d	25-60	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	126
2072	8-Oxo-dG	Yes	MeCN	0.065-0.1M	24h	25-60	0.25M Mercaptoethanol / Ammonium hydroxide, 17h, 55°C		112	153
2073	dT Me Phosphonamidite	Yes	MeCN	0.065-0.1M	24h	300-400	MeCN/EtOH/Ammonium hydroxide, 0.5h then EDA 6h, RT		64	136
2074	iBu-dG Me Phosphonamidite	Yes	THF	0.065-0.1M	24h	300-400	MeCN/EtOH/Ammonium hydroxide, 0.5h then EDA 6h, RT		64	136
2075	Bz-dA Me Phosphonamidite	Yes	MeCN	0.065-0.1M	24h	300-400	MeCN/EtOH/Ammonium hydroxide, 0.5h then EDA 6h, RT		64	136
2077	Ac-dC Me Phosphonamidite	Yes	MeCN	0.065-0.1M	24h	300-400	MeCN/EtOH/Ammonium hydroxide, 0.5h then EDA 6h, RT		64	136
2078	dT-Me-PA	Yes	MeCN	0.065-0.1M	2-3d	25-60	0.05M K_2CO_3 in MeOH, 4h, RT		65	137
2079	2'-F-Ac-C	Yes	MeCN	0.065-0.1M	24h	300-400	AMA, 2h, RT	Do not heat the oligo. See general deprotection notes above.	73	141
2080	2'-F-U	Yes	MeCN	0.065-0.1M	24h	300-400	AMA, 2h, RT	Do not heat the oligo. See general deprotection notes above.	73	141

Phosphoramidites *etc.*

Item No.	Modification	Is the Modification Suitable for "DMT ON" Purification?	Diluent	Recommended Concentration	Stability In Solution	Coupling Time / s	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2083	2'-OMe-Pac-A	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 2h, RT	See general deprotection notes above.	71	140
2084	2'-OMe-iPr-Pac-G	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 2h, RT	See general deprotection notes above.	71	140
2085	Dabcyl	No	10%THF / MeCN	0.065-0.1M	2-3d	300-500	AMA, 10min, 65°C	N-134 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	87	147
2093	dmf-dG 5'-CEPA	Yes	MeCN	0.065-0.1M	5-7d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	75	141
2098	2'-OMe-I	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 2h, RT	See general deprotection notes above.	72	141
2099	2'-OMe-5-Me-U	Yes	MeCN	0.065-0.1M	3-5d	300-500	AMA, 10min, 65°C	See general deprotection notes above.	72	141
2101	Phosphate	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 35min, 55°C	See general deprotection notes above.	77	142
2109	Biotin	Yes	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	An additional detritylation step is recommended to efficiently remove the N-DMT protection. See general deprotection notes above.	92	148
2113	Spacer-C3	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 10min, 65°C	See general deprotection notes above.	38	125
2122	PC-Biotin	Yes	MeCN	0.065-0.1M	2-3d	120-240	AMA, 2h, RT	An additional detritylation step is recommended to efficiently remove the N-DMT protection. See general deprotection notes above.	69	139
2123	MMT-Amino-C6	Yes	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2124	TFA-Amino-C6	No	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2125	Thiol-C6	No	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Oxidiser containing 0.02M Iodine is recommended with this modification. See general deprotection notes above.	45	127
2126	Thiol-C6 S-S	Yes	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Oxidiser containing 0.02M Iodine is recommended with this modification. See general deprotection notes above.	45	127

Phosphoramidites *etc.*										
Item No.	**Modification**	**Is the Modification Suitable for "DMT ON" Purification?**	**Diluent**	**Recommended Concentration**	**Stability In Solution**	**Coupling Time / s**	**Recommended Deprotection Conditions**	**Comments**	**Ordering Page**	**Protocol Page**
2127	Phosphate	Yes	MeCN	0.065-0.1M	2-3d	300-500	AMA, 2h, RT	If the DMT group is to be removed prior to cleavage and deprotection an additional detritylation step is required. See general deprotection notes above.	77	142
2128	Spacer-9	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 10min, 65°C	See general deprotection notes above.	38	125
2129	Spacer-18	Yes	MeCN	0.065-0.1M	24h	25-60	AMA, 10min, 65°C	See general deprotection notes above.	38	125
2130	PC Amino	No	MeCN	0.065-0.1M	24h	120-240	AMA, 2h, RT	See general deprotection notes above.	69	139
2131	PC Spacer	Yes	MeCN	0.065-0.1M	24h	120-240	AMA, 2h, RT	See general deprotection notes above.	69	139
2132	Biotin-TEG	Yes	MeCN	0.1M	2-3d	720-900	AMA, 2h, RT	The oligo must be deprotected DMT-ON to prevent loss of the label due to the 1,2-diol configuration. See general deprotection notes above.	92	148
2133	Amino-C12	Yes	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2134	6-FAM	No	MeCN	0.065-0.1M	2-3d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	81	143
2135	Amino-C6-dT	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 15min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	126
2136	HEX	No	MeCN	0.1M	24h	180-300	Ammonium hydroxide, 24h, RT	The deprotection solution must be removed immediately after deprotection. This is unstable to prolonged heating in ammonium hydroxide and to ^{t}butylamine/ water/ methanol at 65°C.	81	143
2137	TET	No	MeCN	0.065-0.1M	1-2d	180-300	Ammonium hydroxide, 55°C, 4h		81	143
2139	6-Fluorescein	No	MeCN	0.065-0.1M	1-2d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	81	143
2140	Biotin	Yes	MeCN	0.1M	2-3d	900	AMA, 2h, RT	The oligo must be deprotected DMT-ON to prevent loss of the label due to the 1,2-diol configuration. See general deprotection notes above.	92	148

Phosphoramidites *etc.*

Item No.	Modification	Is the Modification Suitable for "DMT ON" Purification?	Diluent	Recommended Concentration	Stability In Solution	Coupling Time / s	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2141	Amino-C6-dC	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 15min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	126
2142	Carboxy-dT	Yes	MeCN	0.065-0.1M	2-3d	25-60	0.4M Methanolic NaOH (MeOH/Water 4:1), 17h, RT		46	131
2143	TAMRA-dT	Yes	10%THF / MeCN	0.1M	24h	300-500	^{t}Butylamine / water / MeOH (1:2:1), 2.5h, 70°C	This is unstable to ammonium hydroxide and is not compatible with the deprotection of HEX.	86	145
2144	Dabcyl-dT	Yes	MeCN	0.065-0.1M	2-3d	300-500	AMA, 10min, 65°C	N-134 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	87	147
2145	2-Amino-dA	Yes	MeCN	0.1M	4-5d	900	AMA, 10min, 65°C	See general deprotection notes above.	112	152
2146	dSpacer	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	38	125
2147	Spacer-C12	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 10min, 65°C	See general deprotection notes above.	38	125
2148	Fluorescein	Yes	MeCN	0.065-0.1M	2-3h	720-900	AMA, 10min, 65°C	See general deprotection notes above.	81	143
2149	Amino-C2-dT	Yes	MeCN	0.065-0.1M	2-3d	25-60	AMA, 15min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	126
2150	5-Me-dC-Brancher	Yes	MeCN	0.065-0.1M	24h	60-180	Ammonium hydroxide, 16h, 55°C		94	149
2154	BHQ®-1	No	MeCN	0.1M	1-2d	600-720	AMA, 10min, 65°C	N-298 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	89	147
2155	BHQ®-2	No	MeCN	0.1M	1-2d	600-720	AMA, 2h, RT	N-300 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	89	147
2156	BHQ®-1-dT	Yes	DCM, AF	0.1M	1-2d	600-900	AMA, 10min, 65°C	N-298 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	89	147

Phosphoramidites *etc.*										
Item No.	**Modification**	**Is the Modification Suitable for "DMT ON" Purification?**	**Diluent**	**Recommended Concentration**	**Stability In Solution**	**Coupling Time / s**	**Recommended Deprotection Conditions**	**Comments**	**Ordering Page**	**Protocol Page**
2157	BHQ®-2-dT	Yes	DCM, AF	0.1M	1-2d	900	AMA, 2h, RT	N-300 may be observed in MALDI MS analysis. This is due to the azo bond cleaving in the MS. See general deprotection notes above.	89	147
2158	Quasar® 570	No	MeCN	0.1M	24h	600-900	Ammonium hydroxide, 2h, RT	Add 4Å or 3Å molecular sieves to amidite and activator. Removal of the deprotection solution by passing the oligo through a sephadex column immediately after deprotection is essential. See general deprotection notes above.	83	144
2159	Quasar® 670	No	MeCN	0.1M	24h	600-900	Ammonium hydroxide, 2h, RT	Add 4Å or 3Å molecular sieves to amidite and activator. Removal of the deprotection solution by passing the oligo through a sephadex column immediately after deprotection is essential. See general deprotection notes above.	83	144
2163	Tocopherol	No	DCM, AF	0.065-0.1M	1-2d	900	AMA, 2h, RT	May require 10-20% EtOH in the deprotection solution for short oligos. See general deprotection notes above.	104	151
2164	dXanthosine	Yes	MeCN	0.065-0.1M	4-5d	180-300	1. Ammonium hydroxide, 24h, RT 2. 0.3M Tetramethyl-guanidinium-2-nitro-benzaldoximate solution in water/dioxane (1:1), 70°C, 48h	Desalting with a sephadex prior to purification is highly recommended.	112	152
2167	Ferrocene-dT	Yes	MeCN	0.1M	24h	300-500	AMA, 10min, 65°C	Removal of the deprotection solution by passing the oligo through a sephadex column immediately after deprotection is essential. Deoxygenating of the buffer solution with argon is highly recommended to avoid oxidation of the Fc. See general deprotection notes above.	94	149
2170	Cholesterol	No	DCM, AF	0.065-0.1M	2-3d	300-900	AMA, 2h, RT	See general deprotection notes above.	104	151
2182	TFA-Amino-11	Yes	MeCN	0.065-0.1M	1-2d	600-900	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2183	Bz-A-UNA	Yes	MeCN	0.065-0.1M	2-3d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	58	136
2184	Ac-C-UNA	Yes	MeCN	0.065-0.1M	2-3d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	58	136
2185	iBu-G-UNA	Yes	MeCN	0.065-0.1M	2-3d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	58	136

Phosphoramidites *etc.*

Item No.	Modification	Is the Modification Suitable for "DMT ON" Purification?	Diluent	Recommended Concentration	Stability In Solution	Coupling Time / s	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2186	U-UNA	Yes	MeCN	0.065-0.1M	2-3d	180-300	AMA, 10min, 65°C	See general deprotection notes above.	58	136
2187	S-Bz TEG	No	MeCN	0.065-0.1M	1-2d	600-900	AMA, 10min, 65°C + 50mM TCEP	**Do not use ETT** as activator with this modification. Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the thiol functionality with acrylonitrile. See general deprotection notes above.	45	127
2189	Cholesterol-TEG	No	MeCN	0.065-0.1M	2-3d	600-900	AMA, 2h, RT	See general deprotection notes above	104	151
2191	BzS-dT	Yes	MeCN	0.065-0.1M	3-5d	600-900	AMA, 2h, RT + 50mM TCEP	**Do not use ETT** as activator with this modification. Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the thiol functionality with acrylonitrile. See general deprotection notes above.	45	130
2192	2'-OMe-5-Me-C	Yes	MeCN	0.065-0.1M	1-2d	360-900	AMA, 10min, 65°C	See general deprotection notes above.	72	141
2193	MMT-Amino-11	Yes	MeCN	0.065-0.1M	1-2d	600-900	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the thiol functionality with acrylonitrile. See general deprotection notes above.	42	125
2194	Octyl-tocopherol	No	DCM, AF	0.065-0.1M	1-2d	900	AMA, 2h, RT	May require 10-20% EtOH in the deprotection solution for short oligos. See general deprotection notes above.	104	151
2199	5'-Palmitate	No	DCM, AF	0.065-0.1M	24h	180-300	AMA, 2h, RT		104	151
2516	dT EtPA	Yes	MeCN	0.065-0.1M	2-3d	25-60	Ammonium hydroxide, overnight, RT	Do not heat to deprotect as this will remove the P-Ethyl group.	65	137
2517	iBu-dG EtPA	Yes	MeCN	0.065-0.1M	2-3d	25-60	Ammonium hydroxide, 48h, RT	Do not heat to deprotect as this will remove the P-Ethyl group.	65	137
2518	Bz-dA EtPA	Yes	MeCN	0.065-0.1M	2-3d	25-60	Ammonium hydroxide, overnight, RT	Do not heat to deprotect as this will remove the P-Ethyl group.	65	137
2519	Bz-dC EtPA	Yes	MeCN	0.065-0.1M	2-3d	25-60	Ammonium hydroxide, overnight, RT	Do not heat to deprotect as this will remove the P-Ethyl group.	65	137
2520	Cyanine-3	Yes	DCM, AF	0.065-0.1M	24h	600-900	Ammonium hydroxide, 2h, RT	Add 4Å or 3Å molecular sieves to amidite and activator. Removal of the deprotection solution by passing the oligo through a G25 column immediately after deprotection is essential. See general deprotection notes above.	83	144

Phosphoramidites *etc.*										
Item No.	**Modification**	**Is the Modification Suitable for "DMT ON" Purification?**	**Diluent**	**Recommended Concentration**	**Stability In Solution**	**Coupling Time / s**	**Recommended Deprotection Conditions**	**Comments**	**Ordering Page**	**Protocol Page**
2521	Cyanine-5	Yes	DCM, AF	0.065-0.1M	24h	600-900	Ammonium hydroxide, 2h, RT	Add 4Å or 3Å molecular sieves to amidite and activator. Removal of the deprotection solution by passing the oligo through a G25 column immediately after deprotection is essential. See general deprotection notes above.	83	144
2529	5-Me-Ac-dC	Yes	MeCN	0.065-0.1M	24h	90	AMA, 10min, 65°C		112	152
2530	5'-Niacin	No	MeCN	0.065-0.1M	24h	900	Various	See page 152.	106	152
2531	5'-Carboxy-C10	No	MeCN	0.065-0.1M	24h	900	0.4M NaOH in MeOH/water (4:1), overnight, RT		46	131
2532	5'-TFA-Amino-C12	No	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2534	5'-TFA-Amino-C5	No	MeCN	0.065-0.1M	24h	300-500	AMA, 10min, 65°C	Requires a 10-20% DEA/MeCN wash prior to cleavage and deprotection to avoid capping the amino functionality with acrylonitrile. See general deprotection notes above.	42	125
2536	bisDMT-Pyridoxine-C6	Yes	MeCN	0.065-0.1M	24h	900	AMA, 2h, RT	Requires double detritylation.	106	152
2537	5'-Niacin-C6	No	MeCN	0.065-0.1M	24h	900	Various	See page 152.	106	152
2538	CAL Fluor® Orange 560	No	DCM, AF	0.1M	24h	900	2-Methoxyethylamine/MeOH (1:3), 3h, 60°C	Add 4Å molecular sieves to amidite and activator (use ETT). Use fast deprotecting amidites.	84	145
2539	CAL Fluor® Red 590	No	MeCN	0.1M	24h	900	2-Methoxyethylamine/MeOH (1:3), 3h, 60°C	Add 4Å molecular sieves to amidite and activator (use ETT). Use fast deprotecting amidites.	84	145
2540	CAL Fluor® Red 610	No	MeCN	0.1M	24h	900	2-Methoxyethylamine/MeOH (1:3), 3h, 60°C	Add 4Å molecular sieves to amidite and activator (use ETT). Use fast deprotecting amidites.	84	145

Solid supports start overleaf/

Solid Supports

Item No.	Modification	Average Pore Size (Å)	Nominal Particle Size (µm)	Typical Loading (µmol/g)	Support Format/Type	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2245	Spacer C3	1000	110	25-50	CPG	AMA, 35min, 55°C	An additional initial detritylation is recommended for non-nucleosidic solid supports to ensure complete detritylation prior to the initial coupling. See general deprotection notes above.	38	125
2261	dT	500	110	35-50	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2262	iBu-dG	500	110	35-50	CPG	Ammonium hydroxide, 4h, 55°C	Not suitable for oligos >40 bases.	28	117
2263	Bz-dA	500	110	35-50	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2264	Bz-dC	500	110	35-50	CPG	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur. Not suitable for oligos >40 bases.	28	117
2265	dT	500	110	60-100	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2266	iBu-dG	500	110	60-100	CPG	Ammonium hydroxide, 4h, 55°C	Not suitable for oligos >40 bases.	28	117
2267	Bz-dA	500	110	60-100	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2268	Bz-dC	500	110	60-100	CPG	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur. Not suitable for oligos >40 bases.	28	117
2271	dT	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2272	iBu-dG	1000	110	25-40	CPG	Ammonium hydroxide, 4h, 55°C		28	117
2273	Bz-dA	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2274	Bz-dC	1000	110	25-40	CPG	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur.	28	117
2275	Ac-dC	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2277	dmf-dG	500	110	35-50	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2278	dmf-dG	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2279	3'-Phosphate	1000	110	30-50	CPG	AMA, 35min, 55°C	Not recommended for use with branching monomers such as **2150** since the oligo can cleave during the removal of the branching point protecting group. See general deprotection notes above.	77	142
2287	dU	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	112	152
2290	Pac-dA	1000	110	25-40	CPG	0.05M K_2CO_3 in MeOH, 16h, RT	The use of UltraMILD capping reagent is recommended (**4210**).	31	122
2292	iPr-Pac-dG	1000	110	25-40	CPG	0.05M K_2CO_3 in MeOH, 16h, RT	The use of UltraMILD capping reagent is recommended (**4210**).	31	122
2293	dI	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	112	152

Solid Supports									
Item No.	Modification	Average Pore Size (Å)	Nominal Particle Size (µm)	Typical Loading (µmol/g)	Support Format/Type	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2294	dT-5'	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	75	141
2295	U-RNA	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2298	iBu-dG-5'	1000	110	25-40	CPG	Ammonium hydroxide, 4h, 55°C		75	141
2300	Universal Q	500	110	35-50	CPG	AMA, 2.5h, 70°C	Suitable for all DNA and RNA deprotection methods but was designed for use with UltraMILD deprotection chemistry (*e.g.* Pac). Not suitable for oligos >40 bases. See general deprotection notes above.	30	122
2304	Universal	1000	110	25-40	CPG	AMA, overnight, 55°C	See general deprotection notes above.	30	122
2309	Ac-C-RNA	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2310	2'-OMe-U RNA	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	71	140
2311	2'-OMe-dmf-G RNA	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	71	140
2312	2'-OMe-Bz-A RNA	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	71	140
2313	2'-OMe-Bz-C RNA	1000	110	25-40	CPG	Ammonium hydroxide, 4h, 55°C	Never use with AMA or EDA otherwise transamination will occur.	71	140
2314	2'-OMe-Ac-C RNA	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	71	140
2317	dmf-dG	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2318	dmf-G	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2319	Pac-A	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2320	iPr-Pac-G	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2321	Bz-A	1000	110	25-40	CPG	Any RNA deprotection method	See general deprotection notes above.	34	123
2323	5-Me-dC	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	112	152
2325	5-Br-dU	1000	110	25-40	CPG	AMA, 2h, RT	See general deprotection notes above.	112	153
2349	3'-DDQ-1	1000	110	25-50	CPG	AMA, 10min, 65°C	See general deprotection notes above.	89	148
2350	3'-Amino Modifier C7	1000	110	≥25	CPG	AMA, 2h, RT	The resin must be treated with 20% DEA/MeCN prior to cleavage and deprotection to prevent side reactions with acrylonitrile. See general deprotection notes above.	42	127
2353	3'-Biotin-TEG	1000	110	≥25	CPG	AMA, 35min, 55°C	An additional initial detritylation is recommended for non-nucleosidic solid supports to ensure complete detritylation prior to the initial coupling. See general deprotection notes above.	92	148
2355	Bz-dA-5'	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	75	141
2356	Bz-dC-5'	1000	110	25-40	CPG	AMA, 35min, 55°C	See general deprotection notes above.	75	141
2357	Ac-dC	500	110	35-50	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2359	3'-Fluorescein	1000	110	≥25	CPG	AMA, 35min, 55°C	See general deprotection notes above.	81	143

Solid Supports

Item No.	Modification	Average Pore Size (Å)	Nominal Particle Size (µm)	Typical Loading (µmol/g)	Support Format/Type	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2361	3'-Thiol Modifier-C3 S-S	1000	110	≥25	CPG	AMA, 2h, RT	See general deprotection notes above.	45	127
2365	3'-PT-Amino-Modifier-C6	1000	110	≥25	CPG	Ammonium hydroxide, overnight, 55°C	The resin must be treated with 20% DEA/MeCN prior to cleavage and deprotection to prevent side reactions with acrylonitrile.	42	127
2366	3'-(6-FAM)	1000	110	≥25	CPG	AMA, 35min, 55°C	See general deprotection notes above	81	143
2367	3'-Amino-Modifier-C6-dT	1000	110	≥25	CPG	AMA, 35min, 55°C	The resin must be treated with 20% DEA/MeCN prior to cleavage and deprotection to prevent side reactions with acrylonitrile. See general deprotection notes above.	42	127
2368	3'-(6-Fluorescein)	1000	110	≥25	CPG	AMA, 35min, 55°C	See general deprotection notes above	81	143
2369	3'-Amino-Modifier-C6-dC	1000	110	≥25	CPG	AMA, 35min, 55°C	The resin must be treated with 20% DEA/MeCN prior to cleavage and deprotection to prevent side reactions with acrylonitrile. See general deprotection notes above.	42	127
2370	3'-Fluorescein-dT	1000	110	≥25	CPG	AMA, 35min, 55°C	See general deprotection notes above.	81	143
2371	3'-PT-Amino-Modifier-C3	1000	110	≥25	CPG	Ammonium hydroxide, overnight, 55°C	The resin must be treated with 20% DEA/MeCN prior to cleavage and deprotection to prevent side reactions with acrylonitrile.	42	127
2372	3'-TAMRA	1000	110	≥25	CPG	^{t}Butylamine/methanol/water (1:1:2), 2.5h, 70°C	Ac-dC (**2034**) and dmf-dG (**2030**) are recommended with this modification.	86	145
2374	3'-Dabcyl	1000	110	≥25	CPG	AMA, 35min, 55°C	MS analysis (in particularly MALDI) will result in N-134 in the spectra due to fragmentation in the MS through the N=N bond. See general deprotection notes above.	87	147
2375	Ac-dC	500	110	35-50	CPG	AMA, 35min, 55°C	Not suitable for oligos >40 bases. See general deprotection notes above.	28	117
2379	BHQ®-1	1000	110	≥25	CPG	AMA, 35min, 55°C	MS analysis (in particularly MALDI) will result in N-298 in the spectra due to fragmentation in the MS through the N=N bond. See general deprotection notes above.	89	147
2380	BHQ®-2	1000	110	≥25	CPG	AMA, 35min, 55°C	MS analysis (in particularly MALDI) will result in N-300 in the spectra due to fragmentation in the MS through the N=N bond. See general deprotection notes above.	89	147
2381	Bz-dA	3000	110	10-25	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2382	Bz-dC	3000	110	10-25	CPG	Ammonium hydroxide, 4h, 55°C		28	117
2383	Ac-dC	3000	110	10-25	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117
2384	iBu-dG	3000	110	10-25	CPG	Ammonium hydroxide, 4h, 55°C		28	117
2386	dT	3000	110	10-25	CPG	AMA, 35min, 55°C	See general deprotection notes above.	28	117

Solid Supports									
Item No.	Modification	Average Pore Size (Å)	Nominal Particle Size (µm)	Typical Loading (µmol/g)	Support Format/Type	Recommended Deprotection Conditions	Comments	Ordering Page	Protocol Page
2393	3'-Palmitate	1000	110	25-40	CPG	AMA containing 20% EtOH, 2h, RT	See general deprotection notes above.	104	151
2394	3'-Cholesterol	1000	110	25-40	CPG	AMA containing 20% EtOH, 2h, RT	See general deprotection notes above.	104	151
2395	3'-Spacer-C3	3000	110	10-25	CPG	AMA, 35min, 55°C	An additional initial detritylation is recommended for non-nucleosidic solid supports to ensure complete detritylation prior to the initial coupling.	38	125
2398	3'-Phosphate	3000	110	10-25	CPG	AMA, 35min, 55°C	Not recommended for use with branching monomers such as **2150** since the oligo can cleave during the removal of the branching point protecting group. See general deprotection notes above.	77	142
2410	Universal Q	1000	110	25-40	CPG	AMA, 2.5h, 70°C	Suitable for all DNA and RNA deprotection methods but was designed for use with UltraMILD deprotection chemistry (*e.g.* Pac). Not suitable for oligos >40 bases. See general deprotection notes above.	30	122
2411	Universal Q	1000	110	60-100	CPG	AMA, 2.5h, 70°C	Suitable for all DNA and RNA deprotection methods but was designed for use with UltraMILD deprotection chemistry (*e.g.* Pac). Not suitable for oligos >40 bases. See general deprotection notes above.	30	122
2412	Cyanine-3	1000	110	25-40	CPG	Ammonium hydroxide, 2h, RT	Removal of the deprotection solution by passing the oligo through a sephadex column immediately after deprotection is essential. See general deprotection notes above.	83	144
2413	Cyanine-5	1000	110	25-40	CPG	Ammonium hydroxide, 2h, RT	Removal of the deprotection solution by passing the oligo through a sephadex column immediately after deprotection is essential. See general deprotection notes above.	83	144
2423	CAL Fluor® Orange 560	500	70-140	>=25	CPG	2-Methoxyethylamine/MeOH (1:3), 3h, 60°C	Add 4Å molecular sieves to amidite and activator (use ETT). Use fast deprotecting amidites.	84	145
2424	CAL Fluor® Red 610	500	70-140	>=25	CPG	2-Methoxyethylamine/MeOH (1:3), 3h, 60°C	Add 4Å molecular sieves to amidite and activator (use ETT). Use fast deprotecting amidites.	84	145

Physical Data

Summarised molecular weight, oligonucleotide unit weight and dilution data for your convenience. Products are listed by item number.

Introduction

For our phosphoramidites, the molecular weight (Mol. Wt.) is the formula weight of the fully protected monomer as sold. This is used in the table below to show the diluent volume required for 0.1M (ABI and MerMade instruments) and 0.067M (Expedite Instruments) solutions for representative pack sizes (250mg, 500mg and 1g). The dilutions for other pack sizes are easily calculated by extrapolation. Modifier phosphoramidites are commonly available as 100µmol vials. Dilution of these is always 1ml (0.1M) and 1.5ml (0.067M) and is therefore not repeated in the table.

All phosphoramidite reagents should be dissolved in anhydrous acetonitrile (Diluent, **4050**), **unless otherwise indicated in the relevant preceding protocol page**.

The unit weight is the formula weight of each monomer or 3'-CPG fragment once inserted into an oligonucleotide with all protecting groups removed. To obtain the molecular weight of a specific oligo, use the formula:

Oligo Mol. Wt. = Sum of Unit Wts. - 61.96

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
0020	354.10	-	-	-	-	-	-	-
0021	338.83	-	-	-	-	-	-	-
0023*	200.23	-	-	-	-	-	-	-
0065	306.12	-	-	-	-	-	-	-
0079	307.10	-	-	-	-	-	-	-
0120	353.12	-	-	-	-	-	-	-
0251	527.53	412.44	-	-	-	-	-	-
1001	171.25	-	-	-	-	-	-	-
1002	301.41	-	-	-	-	-	-	-
1028	236.68	-	-	-	-	-	-	-
2001	744.83	304.20	3.36	6.71	13.43	5.01	10.02	20.04
2002	839.93	329.21	2.98	5.95	11.91	4.44	8.88	17.77
2003	857.95	313.21	2.91	5.83	11.66	4.35	8.70	17.40
2004	833.92	289.18	3.00	6.00	11.99	4.47	8.95	17.90
2005	709.79	304.20	3.52	7.04	14.09	5.26	10.51	21.03
2006	804.89	329.21	3.11	6.21	12.42	4.64	9.27	18.54
2007	822.90	313.21	3.04	6.08	12.15	4.53	9.07	18.14
2009	959.83	415.08	2.60	5.21	10.42	3.89	7.78	15.55
2010	748.79	308.16	3.34	6.68	13.35	4.98	9.97	19.93
2011	912.82	368.08	2.74	5.48	10.96	4.09	8.18	16.35
2012	809.69	369.07	3.09	6.18	12.35	4.61	9.22	18.43
2013	730.80	290.17	3.42	6.84	13.68	5.11	10.21	20.42
2014	856.69	416.07	2.92	5.84	11.67	4.36	8.71	17.42
2016	754.83	314.19	3.31	6.62	13.25	4.94	9.89	19.77
2017	847.90	303.21	2.95	5.90	11.79	4.40	8.80	17.60
2018	853.97	343.24	2.93	5.86	11.71	4.37	8.74	17.48
2019	767.87	327.24	3.26	6.51	13.02	4.86	9.72	19.44
2020	744.83	304.20	3.36	6.71	13.43	5.01	10.02	20.04

* Note that this product is used at a concentration of 0.05M. For dilution data see page138.

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
2021	839.94	329.21	2.98	5.95	11.91	4.44	8.88	17.77
2022	857.95	313.21	2.91	5.83	11.66	4.35	8.70	17.40
2023	833.93	289.18	3.00	6.00	11.99	4.47	8.95	17.90
2025	758.85	318.22	3.29	6.59	13.18	4.92	9.83	19.67
2030	824.92	329.21	3.03	6.06	12.12	4.52	9.05	18.09
2033	955.18	345.21	2.62	5.23	10.47	3.91	7.81	15.63
2034	771.85	289.18	3.24	6.48	12.96	4.83	9.67	19.34
2035	849.93	289.18	2.94	5.88	11.77	4.39	8.78	17.56
2036	988.21	329.21	2.53	5.06	10.12	3.78	7.55	15.10
2037	1018.23	329.21	2.46	4.91	9.82	3.66	7.33	14.66
2038	902.11	305.18	2.77	5.54	11.09	4.14	8.27	16.54
2039	1076.31	345.21	2.32	4.65	9.29	3.47	6.93	13.87
2040	861.06	306.17	2.90	5.81	11.61	4.33	8.67	17.33
2041	887.97	343.24	2.82	5.63	11.26	4.20	8.40	16.81
2042	863.95	319.21	2.89	5.79	11.57	4.32	8.64	17.28
2043	801.88	319.21	3.12	6.24	12.47	4.65	9.31	18.61
2044	855.95	359.24	2.92	5.84	11.68	4.36	8.72	17.44
2045	760.82	320.20	3.29	6.57	13.14	4.90	9.81	19.62
2050	732.81	303.21	3.41	6.82	13.65	5.09	10.18	20.37
2051	907.01	343.23	2.76	5.51	11.03	4.11	8.23	16.46
2052	848.93	327.23	2.94	5.89	11.78	4.40	8.79	17.58
2054	887.81	392.11	2.82	5.63	11.26	4.20	8.41	16.81
2055	903.90	408.10	2.77	5.53	11.06	4.13	8.26	16.51
2056	763.87	323.24	3.27	6.55	13.09	4.88	9.77	19.54
2057*	595.12	180.10	2.80	5.60	11.20	4.20	8.40	16.80
2059	887.97	313.21	2.82	5.63	11.26	4.20	8.40	16.81
2060	946.05	329.21	2.64	5.29	10.57	3.94	7.89	15.78
2066	699.78	259.15	3.57	7.15	14.29	5.33	10.66	21.33
2067	1285.55	684.70	1.94	3.89	7.78	2.90	5.81	11.61
2068	1425.57	815.71	1.75	3.51	7.01	2.62	5.23	10.47
2069	808.93	313.21	3.09	6.18	12.36	4.61	9.23	18.45
2070	813.95	320.26	3.07	6.14	12.29	4.58	9.17	18.34
2071	1068.14	427.40	2.34	4.68	9.36	3.49	6.99	13.97
2072	855.93	345.21	2.92	5.84	11.68	4.36	8.72	17.44
2073	689.79	302.23	3.62	7.25	14.50	5.41	10.82	21.64
2074	784.89	327.24	3.19	6.37	12.74	4.75	9.51	19.02
2075	802.91	311.24	3.11	6.23	12.45	4.65	9.29	18.59
2077	716.81	287.21	3.49	6.98	13.95	5.21	10.41	20.82
2078	705.79	318.22	3.54	7.08	14.17	5.29	10.57	21.15
2079	789.84	307.18	3.17	6.33	12.66	4.72	9.45	18.90
2080	748.79	308.16	3.34	6.68	13.35	4.98	9.97	19.93
2083	917.99	343.24	2.72	5.45	10.89	4.06	8.13	16.26
2084	976.07	359.24	2.56	5.12	10.25	3.82	7.65	15.29
2085	568.69	430.18	4.40	8.79	17.58	6.56	13.12	26.25
2093	824.92	329.21	3.03	6.06	12.12	4.52	9.05	18.09
2098	784.85	344.22	3.19	6.37	12.74	4.75	9.51	19.02
2099	774.85	334.23	3.23	6.45	12.91	4.82	9.63	19.26
2101	656.77	79.98	3.81	7.61	15.23	5.68	11.36	22.73
2109	846.08	405.45	2.95	5.91	11.82	4.41	8.82	17.64
2113	578.69	138.06	4.32	8.64	17.28	6.45	12.90	25.79
2122	1038.25	597.62	2.41	4.82	9.63	3.59	7.19	14.38
2123	589.76	179.16	4.24	8.48	16.96	6.33	12.65	25.31

* Note that this product is used at a higher concentration (0.15M and 0.1M) on ABI/MerMade and Expedite synthesisers respectively. 150µl packs are therefore provided for dilution in 1ml/1.5ml. Dilution data given for other weights refers to these 0.15M and 0.1M concentrations.

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
2124	413.42	179.16	6.05	12.09	24.19	9.03	18.05	36.10
2125	576.78	196.20	4.33	8.67	17.34	6.47	12.94	25.88
2126	769.05	196.20	3.25	6.50	13.00	4.85	9.70	19.41
2127	692.79	79.98	3.61	7.22	14.43	5.39	10.77	21.54
2128	652.77	212.14	3.83	7.66	15.32	5.72	11.43	22.86
2129	784.93	344.30	3.18	6.37	12.74	4.75	9.51	19.01
2130	605.59	371.32	4.13	8.26	16.51	6.16	12.32	24.65
2131	784.88	344.26	3.19	6.37	12.74	4.75	9.51	19.02
2132	1010.24	569.61	2.47	4.95	9.90	3.69	7.39	14.77
2133	673.92	263.32	3.71	7.42	14.84	5.54	11.07	22.15
2134	843.95	537.46	2.96	5.92	11.85	4.42	8.84	17.69
2135	995.05	458.41	2.51	5.02	10.05	3.75	7.50	15.00
2136	1050.62	744.13	2.38	4.76	9.52	3.55	7.10	14.21
2137	981.73	675.24	2.55	5.09	10.19	3.80	7.60	15.20
2139	1176.35	566.48	2.13	4.25	8.50	3.17	6.34	12.69
2140	876.10	435.48	2.85	5.71	11.41	4.26	8.52	17.04
2141	1049.14	457.42	2.38	4.77	9.53	3.56	7.11	14.23
2142	814.88	360.22	3.07	6.14	12.27	4.58	9.16	18.32
2143	1311.48	870.85	1.91	3.81	7.62	2.85	5.69	11.38
2144	1150.32	709.70	2.17	4.35	8.69	3.24	6.49	12.97
2145	1047.33	328.22	2.39	4.77	9.55	3.56	7.13	14.25
2146	620.73	180.10	4.03	8.06	16.11	6.01	12.02	24.04
2147	704.93	264.30	3.55	7.09	14.19	5.29	10.59	21.17
2148	1207.50	598.56	2.07	4.14	8.28	3.09	6.18	12.36
2149	938.94	402.30	2.66	5.33	10.65	3.97	7.95	15.90
2150	942.10	403.37	2.65	5.31	10.61	3.96	7.92	15.84
2154	676.76	538.49	3.69	7.39	14.78	5.51	11.03	22.05
2155	678.73	540.47	3.68	7.37	14.73	5.50	11.00	21.99
2156	1401.56	960.93	1.78	3.57	7.13	2.66	5.32	10.65
2157	1403.53	962.91	1.78	3.56	7.12	2.66	5.32	10.63
2158	759.00	622.74	3.29	6.59	13.18	4.92	9.83	19.66
2159	785.04	648.78	3.18	6.37	12.74	4.75	9.51	19.01
2163	630.94	492.68	3.96	7.92	15.85	5.91	11.83	23.66
2164	1069.12	330.20	2.34	4.68	9.35	3.49	6.98	13.96
2166	303.40	188.30	8.24	16.48	32.96	12.30	24.60	49.19
2167	1125.09	684.46	2.22	4.44	8.89	3.32	6.63	13.27
2170	730.07	591.81	3.42	6.85	13.70	5.11	10.22	20.44
2171*	163.21	-	-	-	-	-	-	-
2182	489.47	255.21	5.11	10.22	20.43	7.62	15.25	30.49
2183	980.07	331.23	2.55	5.10	10.20	3.81	7.61	15.23
2184	893.97	307.20	2.80	5.59	11.19	4.17	8.35	16.70
2185	962.05	347.23	2.60	5.20	10.39	3.88	7.76	15.51
2186	852.92	308.18	2.93	5.86	11.72	4.37	8.75	17.50
2187	572.70	331.34	4.37	8.73	17.46	6.52	13.03	26.06
2189	820.15	682.90	3.05	6.10	12.19	4.55	9.10	18.20
2191	1091.27	546.53	2.29	4.58	9.16	3.42	6.84	13.68
2192	815.90	333.24	3.06	6.13	12.26	4.57	9.15	18.29
2193	665.81	255.21	3.75	7.51	15.02	5.60	11.21	22.42
2194	759.15	620.89	3.29	6.59	13.17	4.92	9.83	19.66
2199	555.83	417.57	4.50	9.00	17.99	6.71	13.43	26.85
2245	-	138.06	-	-	-	-	-	-
2261	-	304.20	-	-	-	-	-	-
2262	-	329.21	-	-	-	-	-	-

* Note that this product is used at a reduced concentration of 0.05M and supplied in millimolar pack sizes. For dilution data see page 138.

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
2263	-	313.21	-	-	-	-	-	-
2264	-	289.18	-	-	-	-	-	-
2265	-	304.20	-	-	-	-	-	-
2266	-	329.21	-	-	-	-	-	-
2267	-	313.21	-	-	-	-	-	-
2268	-	289.18	-	-	-	-	-	-
2271	-	304.20	-	-	-	-	-	-
2272	-	329.21	-	-	-	-	-	-
2273	-	313.21	-	-	-	-	-	-
2274	-	289.18	-	-	-	-	-	-
2275	-	289.18	-	-	-	-	-	-
2277	-	329.21	-	-	-	-	-	-
2278	-	329.21	-	-	-	-	-	-
2279	-	79.98	-	-	-	-	-	-
2287	-	290.17	-	-	-	-	-	-
2290	-	313.21	-	-	-	-	-	-
2292	-	329.21	-	-	-	-	-	-
2293	-	314.19	-	-	-	-	-	-
2294	-	304.20	-	-	-	-	-	-
2295	-	306.17	-	-	-	-	-	-
2298	-	329.21	-	-	-	-	-	-
2300	-	0.00	-	-	-	-	-	-
2304	-	0.00	-	-	-	-	-	-
2309	-	305.18	-	-	-	-	-	-
2310	-	320.20	-	-	-	-	-	-
2311	-	359.24	-	-	-	-	-	-
2312	-	343.24	-	-	-	-	-	-
2313	-	319.21	-	-	-	-	-	-
2314	-	319.21	-	-	-	-	-	-
2317	-	329.21	-	-	-	-	-	-
2318	-	345.21	-	-	-	-	-	-
2319	-	329.21	-	-	-	-	-	-
2320	-	345.21	-	-	-	-	-	-
2321	-	329.21	-	-	-	-	-	-
2323	-	303.21	-	-	-	-	-	-
2325	-	369.07	-	-	-	-	-	-
2349	-	546.57	-	-	-	-	-	-
2350	-	209.18	-	-	-	-	-	-
2353	-	569.61	-	-	-	-	-	-
2355	-	313.21	-	-	-	-	-	-
2356	-	289.18	-	-	-	-	-	-
2357	-	289.18	-	-	-	-	-	-
2359	-	598.56	-	-	-	-	-	-
2361	-	154.12	-	-	-	-	-	-
2365	-	179.15	-	-	-	-	-	-
2366	-	569.46	-	-	-	-	-	-
2367	-	359.24	-	-	-	-	-	-
2368	-	566.48	-	-	-	-	-	-
2369	-	457.42	-	-	-	-	-	-
2370	-	815.71	-	-	-	-	-	-
2371	-	137.07	-	-	-	-	-	-
2372	-	623.60	-	-	-	-	-	-
2374	-	462.44	-	-	-	-	-	-
2375	-	289.18	-	-	-	-	-	-

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
2379	-	554.49	-	-	-	-	-	-
2380	-	556.47	-	-	-	-	-	-
2381	-	313.21	-	-	-	-	-	-
2382	-	289.18	-	-	-	-	-	-
2383	-	289.18	-	-	-	-	-	-
2384	-	329.21	-	-	-	-	-	-
2386	-	304.20	-	-	-	-	-	-
2393	-	533.69	-	-	-	-	-	-
2394	-	707.93	-	-	-	-	-	-
2395	-	138.06	-	-	-	-	-	-
2398	-	79.98	-	-	-	-	-	-
2410	-	0.00	-	-	-	-	-	-
2411	-	0.00	-	-	-	-	-	-
2412	-	507.59	-	-	-	-	-	-
2413	-	533.63	-	-	-	-	-	-
2423	-	1095.41	-	-	-	-	-	-
2424	-	1171.44	-	-	-	-	-	-
2516	719.82	304.20	3.47	6.95	13.89	5.18	10.37	20.73
2517	814.92	329.21	3.07	6.14	12.27	4.58	9.16	18.32
2518	832.94	313.21	3.00	6.00	12.01	4.48	8.96	17.92
2519	808.91	289.18	3.09	6.18	12.36	4.61	9.23	18.45
2520	953.64	507.59	2.62	5.24	10.49	3.91	7.83	15.65
2521	979.68	533.63	2.55	5.10	10.21	3.81	7.62	15.23
2529	785.88	303.21	3.18	6.36	12.72	4.75	9.50	18.99
2530	367.38	216.01 (CO_2H) 215.02 ($CONH_2$) 229.04 ($CONHCH_3$)	6.80	13.61	27.22	10.16	20.31	40.63
2531*	679.28	265.12 (CO_2H) 264.14 ($CONH_2$) 278.15 ($CONHCH_3$)	2.45	4.91	9.81	3.68	7.36	14.72
2532	497.58	263.32	5.02	10.05	20.10	7.50	15.00	30.00
2534	399.39	165.06	6.26	12.52	25.04	9.34	18.69	37.37
2535	872.05	209.08 (Internal) 210.09 (Terminal)	2.87	5.73	11.47	4.28	8.56	17.12
2536	1067.36	389.15	2.34	4.68	9.37	3.50	6.99	13.98
2537	480.54	329.09 (CO_2H) 328.11 ($CONH_2$) 342.12 ($CONHCH_3$)	5.20	10.40	20.81	7.76	15.53	31.06
2538	843.83	560.60	2.96	5.93	11.85	4.42	8.84	17.69
2539	871.88	588.65	2.87	5.73	11.47	4.28	8.56	17.12
2540	919.93	636.69	2.72	5.44	10.87	4.06	8.11	16.22

* Note that this product is used at a higher concentration (0.15M and 0.1M) on ABI/MerMade and Expedite synthesisers respectively. 150µl packs are therefore provided for dilution in 1ml/1.5ml. Dilution data given for other weights refers to these 0.15M and 0.1M concentrations.

			Dilution (0.1M)/ml			Dilution (0.067M)/ml		
Item No.	Mol. Wt.	Unit Wt.	250mg	500mg	1g	250mg	500mg	1g
5001*	725.76	275.27	-	-	-	1.72	3.44	6.89
5002*	701.74	251.24	-	-	-	1.78	3.56	7.13
5003*	741.76	291.27	-	-	-	1.69	3.37	6.74
5004*	506.52	266.26	-	-	-	2.47	4.94	9.87
5005**	385.42	145.16	-	-	-	3.24	6.49	12.97
5010	324.39	265.11	7.71	15.41	30.83	11.50	23.01	46.01
5011	462.58	336.15	5.40	10.81	21.62	8.07	16.13	32.27

* Note that these products are used at a higher concentration (0.2M), and typically on an Expedite synthesiser. 700µl packs are provided for dilution in 3.5ml. Dilution data given for other weights refers to this 0.2M concentration.

** Note that this product is used at a higher concentration (0.2M), and typically on an Expedite synthesiser. 500µl packs are provided for dilution in 2.5ml. Dilution data given for other weights refers to this 0.2M concentration.

Glossary

Anti-Parallel

Hybridised strands of oligonucleotides that are directionally opposed.

Antisense

Single stranded oligonucleotide complementary to a specific DNA or RNA sequence which upon binding prevents any further action by the sequence (*e.g.* prevention of protein translations of mRNA).

Amplicon

The product of an amplification reaction of a nucleic acid either naturally or by PCR.

Aptamer

Oligonucleotides selected from a random pool as a result of their binding properties to a specific target.

Carcinogenesis

The process by which healthy cells are transformed into cancerous cells.

Cassette Mutagenesis

Insertion of an oligonucleotide carrying a gene mutation into a plasmid where the insertion site is cleaved by a restriction enzyme followed by ligation of the oligonucleotide into the plasmid.

Cell penetrating peptide

A short peptide generally chemically linked to a nucleic acid (*e.g.* DNA fragment or oligonucleotide) which aids cellular uptake *via* endocytosis.

Collisional Quenching

Collisional quenching occurs when a fluorophore and quencher are in close enough proximity to enable molecular interactions (*e.g.* p-π orbital overlapping) allowing non-radiative transitions to the ground state resulting in quenching. In this case quenching is not highly dependent on the wavelength overlap of the fluorophore/quencher pair.

CpG Motif

Unmethylated C-phosphate-G dinucleotides within a nucleic acid sequence.

Degeneracy/Wobble

A defined position or positions within a mixture of oligonucleotides where two or more different bases are possible. For instance the defined position may contain either A or G.

Duplex

The result of the hybridisation of two single complementary single strands of nucleic acids.

ELISA

Enzyme-Linked ImmunoSorbent Assay; detection assay to determine the presence and quantify of a substance (*e.g.* protein). This involves the generation of an antigen-antibody complex where the antibody is linked to an enzyme. Detection is achieved by measuring the product of the enzyme acting on a specific substrate.

FISH

Fluorescence *In-Situ* Hybridisation; employs the hybridisation of a fluorescent probe complementary to a specific sequence on a chromosome which is then visualised by fluorescent microscopy.

FRET

Fluorescence Resonance Energy Transfer; The transfer of energy from a high energy donor (fluorophore) to an acceptor. The latter can be a quencher (non-radiative transfer) or a second fluorophore (radiative transfer). Efficient FRET is achieved when there is good overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor. Where the acceptor acts as a quencher, a high extinction co-efficient is thought to be an important factor is terms of quenching efficiency.

Gapmer

Antisense oligonucleotides where the central region is recognised by RNase H but the flanking 5' and 3' sections are chemically modified to be RNase H resistant.

Gene Silencing

The prevention of gene expression ('switching off' the gene) by interruption or suppression of transcription or translation.

In-situ Hybridisation

The hybridisation of a single stranded probe to denatured cellular DNA or RNA in order to detect a specific sequence. Visualisation is achieved with microspcopy.

mRNA

Messenger RNA; is transcribed from genes then involved in the transcription of proteins.

miRNA

MicroRNAs; these are highly conserved small RNA molecules which regulate gene expression by binding to 3'-untranslated regions of specific mRNA molecules.

Mass Marker

A modifier attached to an oligonucleotide whereby after hybridisation to a specific target the marker can be released (usually by photolysis) and detected by mass spectroscopy.

Molecular Beacons™

This is a hybridisation probe whereby in the absence of target the fluorophore is quenched but as the target is amplified during PCR the probe hybridises to the amplicon separating the fluorophore from the quencher generating a fluorescent signal. In this case the fluorophore and quencher are incorporated at the 5' and 3' ends of the probe which are held in close proximity by the stem (short complementary sequences at the 5' and 3' ends which are hybridised together in the absence of a target). The probe (complementary to the target) is found in the centre of the oligonucleotide.

Mutagenesis

The process by which a stable genetic mutation is generated from a healthy gene.

Parallel

Hybridised strands of oligonucleotides that are directionally identical.

PCR

The Polymerase Chain Reaction (PCR) is a technique widely used to amplify a section of target DNA that is flanked by two known genetic sequences. Two short primers are prepared and are designed such that each is complementary to sections of the known sequences. The latter are typically 18-30 bases in length, with similar (%G+C) content to ensure similar annealing temperatures. The amplification is achieved by thermal cycling using nucleotide triphosphates and a thermally stable enzyme *e.g.* Taq Polymerase.

PCR Blocker

A modification incorporated into an oligonucleotide generally at the 3'-end but in some specific cases internally (*e.g.* Scorpion™ primers) resulting in a DNA polymerase resistant oligonucleotide at the site of the blocker.

PCR clamping

Allows selective amplification of target DNA where sequences differ by a single base pair. This generally involves the use of PNA oligos to block one or more sequence due to the highly stable PNA-DNA duplex formed on hybridisation which is resistant to DNA polymerase. This leaves only the desired target available for amplification.

Photolysis

Chemical reaction induced by light (or photons) resulting in either a rearrangement or division of the molecule.

pKa

Acid dissociation constant, used to define the strength of an acid. A strong acid will have a low pKa value whereas a weak acid will have a high value.

Quadruplex

G-rich nucleic acid sequence with the ability to form a highly stable four sided square planar structure (guanine tetrad) *via* Hoogsteen hydrogen bonding. Where two or more guanine tetrads stack, a G-quadruplex is formed.

RNAi

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression, typically by causing the destruction of specific mRNA molecules.

RT-PCR

Reverse Transcription Polymerase Chain Reaction is a means of quantitatively detecting the level of RNA expression by generating complementary DNA transcripts from RNA using reverse transcriptase followed by PCR.

RT-PCR/qPCR

Real-Time Polymerase Chain Reaction / Quantitative Polymerase Chain Reaction is a means of measuring the amplification of specific DNA sequences using PCR where the formation of the amplicon(s) is measured by the generation of a fluorescent signal by use of a fluorescent probe with a sequence complementary to the target DNA sequence.

Scorpion™ Primer

This is a hybridisation probe whereby in the absence of target the fluorophore is quenched but as the target is amplified during PCR the probe hybridises to the amplicon separating the fluorophore from the quencher generating a fluorescent signal. This is similar to a molecular beacon in that the fluorophore and quencher are held in close proximity *via* a stem. However, it differs from a molecular beacon in that the quencher is placed internally within the sequence connecting the probe to a primer *via* a PCR blocker. In this case when the probe hybridises to the target this is an intramolecular process since after hybridisation the probe is chemically linked to the amplicon.

Sense

Single stranded oligonucleotide complementary to a corresponding antisense strand (*i.e.* has the same sequence as the target *e.g.* mRNA).

SERRS

Surface Enhanced Resonance Raman Scattering; a detection method by which a Raman signal is generated from a Raman-active molecule absorbed onto a metal surface. The Raman-active molecule can be a labelled (*e.g.* TAMRA) oligonucleotide attached to a metal surface (*e.g.* gold) *via* a suitable linkage (*e.g.* thiol-gold).

siRNA

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 base pairs in length. siRNA plays many roles, but its most notable is in the RNA interference (RNAi) pathway, where it interferes with the expression of specific genes with complementary nucleotide sequence.

SNP

Single Nucleotide Polymorphism; variation between two or more nucleic acid sequences (generally genes) by one nucleotide base. This can also be an insertion or deletion.

Splicing

The process by which nucleic acid fragments are combined to form larger fragments. For example the formation of recombinant DNA or chimeric genes.

Taqman™ Probe

This is a hybridisation probe whereby in the absence of target the fluorophore is quenched. During PCR in addition to amplification, Taq polymerase cleaves the probe from the target by way of its 5'-3' exonuclease activity releasing the fluorophore into solution generating a fluorescent signal.

Triplex

A structure in which three oligonucleotides are hybridised together to form a triple helix where the third strand is bound to the duplex by Hoogsteen hydrogen bonding.

Universal Base

A base with the ability to base pair with any of the four natural bases with minimal detriment to the stability or functionality of the resulting duplex.

Wobble/Degeneracy

A defined position or positions within a mixture of oligonucleotides where two or more different bases are possible. For instance the defined position may contain either A or G.

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0227	Silanised Bottle - ABI (Bottle Type H)	62	-
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0271	Columns (Empty w/frits): MerMade 0.2/1.0µmol	115	-
0287	Columns (Empty w/frits): MerMade 50nmol	115	-
0849	Columns (Empty w/frits): ABI3900 0.2/1.0µmol	115	-
1001	DIHT	115	-
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2034	Ac-dC-CE Phosphoramidite	26	116
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2037	Pac-A-CE Phosphoramidite	34	123
2038	Ac-C-CE Phosphoramidite	34	123
2039	iPr-Pac-G-CE Phosphoramidite	34	123
2040	U-CE Phosphoramidite	34	123
2041	2'-OMe-Bz-A-CE Phosphoramidite	71	140
2042	2'-OMe-Bz-C-CE Phosphoramidite	71	140
2043	2'-OMe-Ac-C-CE Phosphoramidite	71	140
2044	2'-OMe-dmf-G-CE Phosphoramidite	71	140
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2077	Ac-dC-Me Phosphonamidite	64	136
2078	dT-Me Phosphoramidite	65	137
2079	2'-F-Ac-dC-CE Phosphoramidite	73	141
2080	2'-F-dU-CE Phosphoramidite	73	141
2083	2'-OMe-Pac-A-CE Phosphoramidite	71	140
2084	2'-OMe-iPr-Pac-G-CE Phosphoramidite	71	140
2085	5'-Dabcyl-CE Phosphoramidite	87	147
2093	dmf-dG-5'-CE Phosphoramidite	75	141
2098	2'-OMe-I-CE Phosphoramidite	72	141
2099	2'-OMe-5-Me-U-CE Phosphoramidite	72	141
2100	Expedite Vial Pack - 30ml	115	-
2101	Phosphate-ON (Chemical Phosphorylation Reagent, CPR)	77	142
2109	5'-Biotin-CE Phosphoramidite	92	148
2113	Spacer-CE Phosphoramidite C3	38	125
2122	PC 5'-Biotin-CE Phosphoramidite	69	139
2123	5'-MMT-Amino-Modifier-C6-CE Phosphoramidite	42	125
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2129	Spacer-CE Phosphoramidite 18 (HEG)	38	125
2130	PC Amino-Modifier-CE Phosphoramidite	69	139
2131	PC Spacer-CE Phosphoramidite	69	139
2132	Biotin-TEG-CE Phosphoramidite	92	148
2133	5'-MMT-Amino-Modifier-C12-CE Phosphoramidite	42	125
2134	5'-Fluorescein-CE Phosphoramidite (6-FAM)	81	143
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2136	5'-Hexachloro-Fluorescein-CE Phosphoramidite (HEX)	81	143
2137	5'-Tetrachloro-Fluorescein-CE Phosphoramidite (TET)	81	143
2139	6-Fluorescein-CE Phosphoramidite	81	143
2140	Biotin-CE-Phosphoramidite	92	148
2141	Amino-Modifier-C6-dC-CE Phosphoramidite	42	126
2142	Carboxy-dT-CE Phosphoramidite	46	131
2143	TAMRA-dT-CE Phosphoramidite	86	145
2144	Dabcyl-dT-CE Phosphoramidite	87	147
2145	2-Amino-dA-CE Phosphoramidite	112	152
2146	dSpacer-CE Phosphoramidite	38	125
2147	Spacer-CE Phosphoramidite C12	38	125
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2156	BHQ®-1-dT-CE-Phosphoramidite	89	147
2157	BHQ®-2-dT-CE-Phosphoramidite	89	147
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2159	Quasar® 670-CE Phosphoramidite	83	144
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2164	Deoxyxanthosine-CE Phosphoramidite	112	152
2166	Thioactic Acid NHS Ester	45	127
2167	Ferrocene-dT-CE Phsophoramidite	94	149
2170	5'-Cholesterol-CE Phosphoramidite	104	151
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2173	MerMade Vial Pack - 30ml	115	-
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2183	Bz-A-UNA-CE Phosphoramidite	58	136
2184	Ac-C-UNA-CE Phosphoramidite	58	136
2185	iBu-G-UNA-CE Phosphoramidite	58	136
2186	U-UNA-CE Phosphoramidite	58	136
2187	Bz-S-TEG-CE Phosphoramidite	45	127
2189	5'-Cholesterol-TEG-CE Phosphoramidite	104	151
2191	Bz-S-C6-dT-CE Phosphoramidite	45	130
2192	2'-OMe-N-Ac-5-Me-C-CE Phosphoramidite	72	141
2193	5'-MMT-Amino-Modifier-11-CE Phosphoramidite	42	125
2194	5'-Octyltocopherol-CE Phosphoramidite	104	151
2199	5'-Palmitate-CE Phosphoramidite	104	151
2245	3'-Spacer-C3 SynBase™ CPG 1000/110	38	125
2261	dT SynBase™ CPG 500/110 S	28	117
2262	iBu-dG SynBase™ CPG 500/110 S	28	117
2263	Bz-dA SynBase™ CPG 500/110 S	28	117
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2266	iBu-dG SynBase™ CPG 500/110 H	28	117
2267	Bz-dA SynBase™ CPG 500/110 H	28	117
2268	Bz-dC SynBase™ CPG 500/110 H	28	117
2271	dT SynBase™ CPG 1000/110	28	117
2272	iBu-dG SynBase™ CPG 1000/110	28	117
2273	Bz-dA SynBase™ CPG 1000/110	28	117
2274	Bz-dC SynBase™ CPG 1000/110	28	117
2275	Ac-dC SynBase™ CPG 1000/110	28	117
2277	dmf-dG SynBase™ CPG 500/110 S	28	117
2278	dmf-dG SynBase™ CPG 500/110 H	28	117
2279	3'-Phosphate SynBase™ CPG 1000/110	77	142
2287	dU SynBase™ CPG 1000/110	112	152
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2292	iPr-Pac-dG SynBase™ CPG 1000/110	31	122
2293	dI SynBase™ CPG 1000/110 S	112	152
2294	dT-5'-SynBase™ CPG 1000/110	75	141
2295	U RNA SynBase™ CPG 1000/110	34	123
2298	iBu-dG-5'-SynBase™ CPG 1000/110	75	141
2300	Universal Q SynBase™ 500/110	30	122
2304	Universal SynBase™ CPG 1000/110	30	122
2309	Ac-C RNA SynBase™ CPG 1000/110	34	123
2310	2'-OMe-U RNA SynBase™ CPG 1000/110	71	140
2311	2'-OMe-dmf-G RNA SynBase™ CPG 1000/110	71	140
2312	2'-OMe-Bz-A RNA SynBase™ CPG 1000/110	71	140
2313	2'-OMe-Bz-C RNA SynBase™ CPG 1000/110	71	140
2314	2'-OMe-Ac-C RNA SynBase™ CPG 1000/110	71	140
2317	dmf-dG SynBase™ CPG 1000/110	28	117
2318	dmf-G RNA SynBase™ CPG 1000/110	34	123
2319	Pac-A RNA SynBase™ CPG 1000/110	34	123
2320	iPr-Pac-G RNA SynBase™ CPG 1000/110	34	123
2321	Bz-A RNA SynBase™ CPG 1000/110	34	123
2323	5-Me-dC SynBase™ CPG 1000	112	152
2325	5-Br-dU SynBase™ CPG 1000/110	112	153
2349	3'-DDQ-I SynBase™ CPG 1000/110	89	148
2350	3'-Amino-Modifier-C7 CPG 1000	42	127
2353	3'-Biotin-TEG CPG	92	148
2355	Bz-dA-5'-SynBase™ CPG 1000/110	75	141
2356	Bz-dC-5'-SynBase™ CPG 1000/110	75	141
2357	Ac-dC SynBase™ CPG 500/110 S	28	117
2359	3'-Fluorescein CPG	81	143
2361	3'-Thiol-Modifier-C3 S-S CPG	45	127
2365	3'-PT-Amino-Modifier-C6 CPG	42	127
2366	3'-(6-FAM) CPG	81	143
2367	3'-Amino-Modifier-C6-dT CPG	42	127
2368	3'-(6-Fluorescein) CPG	81	143
2369	3'-Amino-Modifier-C6-dC CPG	42	127
2370	3'-Fluorescein-dT CPG	81	143
2371	3'-PT-Amino-Modifier-C3 CPG	42	127
2372	3'-TAMRA CPG	86	145
2374	3'-Dabcyl CPG	87	147
2375	Ac-dC SynBase™ CPG 500/110 H	28	117
2379	BHQ®-1 CPG 1000	89	147
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2384	iBu-dG SynBase™ CPG 3000/110	28	117
2386	dT SynBase™ CPG 3000/110	28	117
2393	3'-Palmitate SynBase™ CPG 1000/110	104	151
2394	3'-Cholesterol SynBase™ CPG 1000/110	104	151
2395	3'-Spacer-C3 SynBase™ CPG 3000/110	38	125
2398	3'-Phosphate SynBase™ CPG 3000/110	77	142
2410	Universal Q SynBase™ 1000/110 S	30	122
2411	Universal Q SynBase™ 1000/110 H	30	122
2412	3'-Cyanine-3 SynBase™ 1000	83	144
2413	3'-Cyanine-5 SynBase™ 1000	83	144
2423	3'-CAL Fluor® Orange 560 CPG 500	84	145
2424	3'-CAL Fluor® Red 610 CPG 500	84	145
2516	dT-Et Phosphoramidite	65	137
2517	iBu-dG-Et Phosphoramidite	65	137
2518	Bz-dA-Et Phosphoramidite	65	137
2519	Bz-dC-Et Phosphoramidite	65	137
2520	5'-Cyanine-3-CE Phosphoramidite	83	144
2521	5'-Cyanine-5-CE Phosphoramidite	83	144
2529	5-Me-Ac-dC-CE Phosphoramidite	112	152
2530	5'-Niacin-CE Phosphoramidite	106	152
2531	5'-Carboxy-C10-CE Phosphoramidite	46	131
2532	5'-TFA-Amino-Modifier-C12-CE Phosphoramidite	42	125
2534	5'-TFA-Amino-Modifier-C5-CE Phosphoramidite	42	125
2536	bisDMT-Pyridoxine-C6-CE Phosphoramidite	106	152
2537	5'-Niacin-C6-CE Phosphoramidite	106	152
2538	CAL Fluor® Orange 560-CE Phosphoramidite	84	145
2539	CAL Fluor® Red 590-CE Phosphoramidite	84	145
2540	CAL Fluor® Red 610-CE Phosphoramidite	84	145
3140	ETT Activator (0.25M)	27	116
3142	ETT Activator (0.25M)	27	116
3145	ETT Activator (0.5M)	27	116
3146	ETT Activator (0.5M)	27	116
3160	BTT Activator	27	116
3162	BTT Activator	27	116
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4012	Cap Mix A: THF/acetic anhydride (9:1)	27	116
4028	DEA Wash: 20% Diethylamine in DCM	27	-
4050	Anhydrous Wash: Acetonitrile, anhydrous	27	116
4050	Diluent: Acetonitrile, anhydrous	27	116
4110	Cap Mix A: THF/pyridine/acetic anhydride (8:1:1)	27	116
4120	Cap Mix B: 10% Methylimidazole in THF	27	117
4122	Cap Mix B: 10% Methylimidazole in THF/pyridine (8:1)	27	117
4132	Oxidiser: 0.02M Iodine in THF/pyridine/water (89.6:0.4:10)	27	117
4140	Deblock Mix: 3% TCA in DCM	27	117
4210	Cap Mix A: THF/pyridine/Pac-anhydride (85:10:5)	27	122
4230	Oxidiser: 0.1M Iodine in THF/pyridine/water (78:20:2)	27	117
4330	Oxidiser: 0.02M Iodine in THF/pyridine/water (7:2:1)	27	117
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9005	TOP DNA Jr, Bond- Elut®, 150mg	115	120
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1049	Amino-SynBase™ CPG 1000/110	35	-
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2379	BHQ®-1 CPG 1000	89	147
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2156	BHQ®-1-dT-CE-Phosphoramidite	89	147
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2157	BHQ®-2-dT-CE-Phosphoramidite	89	147
2109	5'-Biotin-CE Phosphoramidite	92	148
2140	Biotin-CE-Phosphoramidite	92	148
2067	Biotin-dT-CE Phosphoramidite	92	148
2353	3'-Biotin-TEG CPG	92	148
2132	Biotin-TEG-CE Phosphoramidite	92	148
2054	8-Br-dA-CE Phosphoramidite	112	153
2011	5-Br-dC-CE Phosphoramidite	112	153
2055	8-Br-dG-CE Phosphoramidite	112	153
2325	5-Br-dU SynBase™ CPG 1000/110	112	153
2012	5-Br-dU-CE Phosphoramidite	112	153
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3160	BTT Activator	27	116
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0234	BTT Activator (Crystalline)	27	118
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2314	2'-OMe-Ac-C RNA SynBase™ CPG 1000/110	71	140
2313	2'-OMe-Bz-C RNA SynBase™ CPG 1000/110	71	140
2309	Ac-C RNA SynBase™ CPG 1000/110	34	123
2323	5-Me-dC SynBase™ CPG 1000	112	152
2275	Ac-dC SynBase™ CPG 1000/110	28	117
2274	Bz-dC SynBase™ CPG 1000/110	28	117
2383	Ac-dC SynBase™ CPG 3000/110	28	117
2382	Bz-dC SynBase™ CPG 3000/110	28	117
2375	Ac-dC SynBase™ CPG 500/110 H	28	117
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2357	Ac-dC SynBase™ CPG 500/110 S	28	117
2264	Bz-dC SynBase™ CPG 500/110 S	28	117
2023	Bz-dC-5'-CE Phosphoramidite	75	141
2356	Bz-dC-5'-SynBase™ CPG 1000/110	75	141
2423	3'-CAL Fluor® Orange 560 CPG 500	84	145
2538	CAL Fluor® Orange 560-CE Phosphoramidite	84	145
2539	CAL Fluor® Red 590-CE Phosphoramidite	84	145
2424	3'-CAL Fluor® Red 610 CPG 500	84	145
2540	CAL Fluor® Red 610-CE Phosphoramidite	84	145
4012	Cap Mix A: THF/acetic anhydride (9:1)	27	116
4010	Cap Mix A: THF/lutidine/acetic anhydride (8:1:1)	27	116

Item No.	Product	Ordering Page	Protocol Page
4110	Cap Mix A: THF/pyridine/acetic anhydride (8:1:1)	27	116
4210	Cap Mix A: THF/pyridine/Pac-anhydride (85:10:5)	27	122
4120	Cap Mix B: 10% Methylimidazole in THF	27	117
4122	Cap Mix B: 10% Methylimidazole in THF/pyridine (8:1)	27	117
2531	5'-Carboxy-C10-CE Phosphoramidite	46	131
2142	Carboxy-dT-CE Phosphoramidite	46	131
2057	5'-Carboxylate-Modifier-CE Phosphoramidite	46	131
2150	5-Me-dC-Brancher-CE Phosphoramidite	94	149
2043	2'-OMe-Ac-C-CE Phosphoramidite	71	140
2042	2'-OMe-Bz-C-CE Phosphoramidite	71	140
2192	2'-OMe-N-Ac-5-Me-C-CE Phosphoramidite	72	141
2529	5-Me-Ac-dC-CE Phosphoramidite	112	152
2017	5-Me-Bz-dC-CE Phosphoramidite	112	152
2038	Ac-C-CE Phosphoramidite	34	123
2034	Ac-dC-CE Phosphoramidite	26	116
2004	Bz-dC-CE Phosphoramidite	26	116
2519	Bz-dC-Et Phosphoramidite	65	137
1028	Chlorophosphitylating Reagent	115	-
2394	3'-Cholesterol SynBase™ CPG 1000/110	104	151
2170	5'-Cholesterol-CE Phosphoramidite	104	151
2189	5'-Cholesterol-TEG-CE Phosphoramidite	104	151
2035	Bz-dC-H-Phosphonate, DBU Salt	60	138
2077	Ac-dC-Me Phosphonamidite	64	136
2050	Ac-dC-Me Phosphoramidite	65	137
0849	Columns (Empty w/frits): ABI3900 0.2/1.0µmol	115	-
0256	Columns (Empty w/frits): ALL-FIT Luer (ABI394 & Expedite 8909) 0.2/1.0µmol	115	-
0271	Columns (Empty w/frits): MerMade 0.2/1.0µmol	115	-
0287	Columns (Empty w/frits): MerMade 50nmol	115	-
2184	Ac-C-UNA-CE Phosphoramidite	58	136
2412	3'-Cyanine-3 SynBase™ 1000	83	144
2520	5'-Cyanine-3-CE Phosphoramidite	83	144
2413	3'-Cyanine-5 SynBase™ 1000	83	144
2521	5'-Cyanine-5-CE Phosphoramidite	83	144
	D		
2374	3'-Dabcyl CPG	87	147
2085	5'-Dabcyl-CE Phosphoramidite	87	147
2144	Dabcyl-dT-CE Phosphoramidite	87	147
2349	3'-DDQ-I SynBase™ CPG 1000/110	89	148
4028	DEA Wash: 20% Diethylamine in DCM	27	-
4140	Deblock Mix: 3% TCA in DCM	27	117
2164	Deoxyxanthosine-CE Phosphoramidite	112	152
1001	DIHT	115	-
4050	Diluent: Acetonitrile, anhydrous	27	116
0021	4,4'-Dimethoxytrityl Chloride	115	-
5010	dPEG®$_4$-SATA-Acid	57	132
	E		
2171	EDITH	62	138
3140	ETT Activator (0.25M)	27	116
3142	ETT Activator (0.25M)	27	116
3145	ETT Activator (0.5M)	27	116

Item No.	Product	Ordering Page	Protocol Page
3146	ETT Activator (0.5M)	27	116
0237	ETT Activator (Crystalline)	27	118
2100	Expedite Vial Pack - 30ml	115	-
	F		
2079	2'-F-Ac-dC-CE Phosphoramidite	73	141
2366	3'-(6-FAM) CPG	81	143
2080	2'-F-dU-CE Phosphoramidite	73	141
2010	5-F-dU-CE Phosphoramidite	112	153
2167	Ferrocene-dT-CE Phsophoramidite	94	149
2359	3'-Fluorescein CPG	81	143
2368	3'-(6-Fluorescein) CPG	81	143
2139	6-Fluorescein-CE Phosphoramidite	81	143
2148	Fluorescein-CE Phosphoramidite	81	143
2134	5'-Fluorescein-CE Phosphoramidite (6-FAM)	81	143
2370	3'-Fluorescein-dT CPG	81	143
2068	Fluorescein-dT-CE Phosphoramidite	81	143
2056	Formylindole-Modifier-CE Phosphoramidite	47	132
	G		
2311	2'-OMe-dmf-G RNA SynBase™ CPG 1000/110	71	140
2318	dmf-G RNA SynBase™ CPG 1000/110	34	123
2320	iPr-Pac-G RNA SynBase™ CPG 1000/110	34	123
2317	dmf-dG SynBase™ CPG 1000/110	28	117
2272	iBu-dG SynBase™ CPG 1000/110	28	117
2292	iPr-Pac-dG SynBase™ CPG 1000/110	31	122
2384	iBu-dG SynBase™ CPG 3000/110	28	117
2278	dmf-dG SynBase™ CPG 500/110 H	28	117
2266	iBu-dG SynBase™ CPG 500/110 H	28	117
2277	dmf-dG SynBase™ CPG 500/110 S	28	117
2262	iBu-dG SynBase™ CPG 500/110 S	28	117
2093	dmf-dG-5'-CE Phosphoramidite	75	141
2021	iBu-dG-5'-CE Phosphoramidite	75	141
2298	iBu-dG-5'-SynBase™ CPG 1000/110	75	141
2044	2'-OMe-dmf-G-CE Phosphoramidite	71	140
2084	2'-OMe-iPr-Pac-G-CE Phosphoramidite	71	140
2072	8-oxo-dG-CE Phosphoramidite	112	153
2033	dmf-G-CE Phosphoramidite	34	123
2030	dmf-dG-CE Phosphoramidite	26	116
2002	iBu-dG-CE Phosphoramidite	26	116
2039	iPr-Pac-G-CE Phosphoramidite	34	123
2060	iPr-Pac-dG-CE Phosphoramidite	31	122
2018	O6-Me-dG-CE Phosphoramidite	112	153
2517	iBu-dG-Et Phosphoramidite	65	137
2006	iBu-dG-H-Phosphonate, TEA Salt	60	138
2074	iBu-dG-Me Phosphonamidite	64	136
2051	iPr-Pac-dG-Me Phosphoramidite	65	137
2185	iBu-G-UNA-CE Phosphoramidite	58	136
	H		
2136	5'-Hexachloro-Fluorescein-CE Phosphoramidite (HEX)	81	143

Item No.	Product	Ordering Page	Protocol Page
	I		
2293	dI SynBase™ CPG 1000/110 S	112	152
2098	2'-OMe-I-CE Phosphoramidite	72	141
2016	dI-CE Phosphoramidite	112	152
2009	5-I-dC-CE Phosphoramidite	112	153
2014	5-I-dU-CE Phosphoramidite	112	153
0120	5-Iodo-2'-deoxycytidine	115	-
0020	5-Iodo-2'-deoxyuridine	115	-
	M		
2174	MerMade Vial Pack - 15ml	115	-
2173	MerMade Vial Pack - 30ml	115	-
	N		
2537	5'-Niacin-C6-CE Phosphoramidite	106	152
2530	5'-Niacin-CE Phosphoramidite	106	152
	O		
2194	5'-Octyltocopherol-CE Phosphoramidite	104	151
4330	Oxidiser: 0.02M Iodine in THF/pyridine/water (7:2:1)	27	117
4132	Oxidiser: 0.02M Iodine in THF/pyridine/water (89.6:0.4:10)	27	117
4230	Oxidiser: 0.1M Iodine in THF/pyridine/water (78:20:2)	27	117
	P		
2393	3'-Palmitate SynBase™ CPG 1000/110	104	151
2199	5'-Palmitate-CE Phosphoramidite	104	151
2122	PC 5'-Biotin-CE Phosphoramidite	69	139
2130	PC Amino-Modifier-CE Phosphoramidite	69	139
2066	PC Linker-CE Phosphoramidite	69	139
2131	PC Spacer-CE Phosphoramidite	69	139
2279	3'-Phosphate SynBase™ CPG 1000/110	77	142
2398	3'-Phosphate SynBase™ CPG 3000/110	77	142
2101	Phosphate-ON (Chemical Phosphorylation Reagent, CPR)	77	142
2127	Phosphate-ON (Solid) (solidCPR)	77	142
1002	Phosphitylating Reagent	115	-
5001	Fmoc-PNA-A(Bhoc)-OH	57	132
5002	Fmoc-PNA-C(Bhoc)-OH	57	132
5003	Fmoc-PNA-G(Bhoc)-OH	57	132
5004	Fmoc-PNA-T-OH	57	132
2536	bisDMT-Pyridoxine-C6-CE Phosphoramidite	106	152
	Q		
2158	Quasar® 570-CE Phosphoramidite	83	144
2159	Quasar® 670-CE Phosphoramidite	83	144

Item No.	Product	Ordering Page	Protocol Page
	S		
2191	Bz-S-C6-dT-CE Phosphoramidite	45	130
0227	Silanised Bottle - ABI (Bottle Type H)	62	-
0228	Silanised Bottle - Expedite/MerMade (Bottle Type J)	62	-
2245	3'-Spacer-C3 SynBase™ CPG 1000/110	38	125
2395	3'-Spacer-C3 SynBase™ CPG 3000/110	38	125
2146	dSpacer-CE Phosphoramidite	38	125
2129	Spacer-CE Phosphoramidite 18 (HEG)	38	125
2128	Spacer-CE Phosphoramidite 9	38	125
2147	Spacer-CE Phosphoramidite C12	38	125
2113	Spacer-CE Phosphoramidite C3	38	125
5011	SPDP-dPEG®$_4$-Acid	57	132
2187	Bz-S-TEG-CE Phosphoramidite	45	127
0023	Sulphurising Reagent	62	138
	T		
2271	dT SynBase™ CPG 1000/110	28	117
2386	dT SynBase™ CPG 3000/110	28	117
2265	dT SynBase™ CPG 500/110 H	28	117
2261	dT SynBase™ CPG 500/110 S	28	117
2020	dT-5'-CE Phosphoramidite	75	141
2294	dT-5'-SynBase™ CPG 1000/110	75	141
2372	3'-TAMRA CPG	86	145
0251	TAMRA NHS Ester (0.17M in DMSO)	86	145
2143	TAMRA-dT-CE Phosphoramidite	86	145
2001	dT-CE Phosphoramidite	26	116
2025	O4-Me-dT-CE Phosphoramidite	112	153
2516	dT-Et Phosphoramidite	65	137
2137	5'-Tetrachloro-Fluorescein-CE Phosphoramidite (TET)	81	143
2166	Thioactic Acid NHS Ester	45	127
2070	4-Thio-dT-CE Phosphoramidite	112	153
2361	3'-Thiol-Modifier-C3 S-S CPG	45	127
2126	Thiol-Modifier-C6 S-S CE Phosphoramidite	45	127
2125	5'-Thiol-Modifier-C6-CE Phosphoramidite	45	127
2005	dT-H-Phosphonate, TEA Salt	60	138
2073	dT-Me Phosphonamidite	64	136
2078	dT-Me Phosphoramidite	65	137
2163	5'-Tocopherol-CE Phosphoramidite	104	151
9005	TOP DNA Jr, Bond- Elut®, 150mg	115	120
9003	TOP DNA Tubes, 150mg	115	120
9004	TOP RNA Tubes, 100mg	115	120
9006	Top Tubes, 50mg (Gravity Flow)	115	120
	U		
2310	2'-OMe-U RNA SynBase™ CPG 1000/110	71	140
2295	U RNA SynBase™ CPG 1000/110	34	123
2287	dU SynBase™ CPG 1000/110	112	152
2045	2'-OMe-U-CE Phosphoramidite	71	140
2099	2'-OMe-5-Me-U-CE Phosphoramidite	72	141
2013	dU-CE Phosphoramidite	112	152
2040	U-CE Phosphoramidite	34	123
2411	Universal Q SynBase™ 1000/110 H	30	122

Item No.	Product	Ordering Page	Protocol Page
2410	Universal Q SynBase™ 1000/110 S	30	122
2300	Universal Q SynBase™ 500/110	30	122
2304	Universal SynBase™ CPG 1000/110	30	122
2186	U-UNA-CE Phosphoramidite	58	136

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