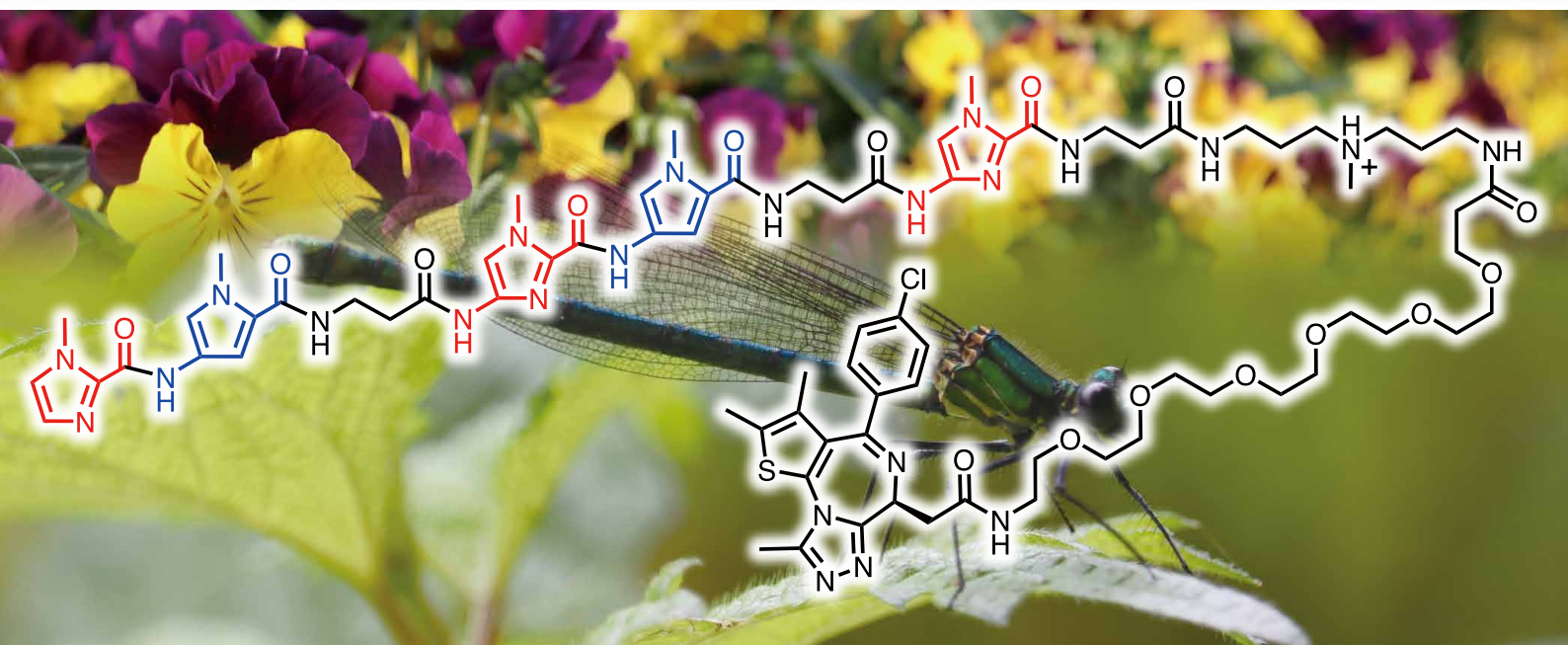# TCI

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Research Article

Pyrrole-Imidazole Polyamide
– A Front Runner in Mid-Molecular Pharmaceuticals

Thangavel Vaijayanthi[1, 2], Ganesh N. Pandian[*2], Hiroshi Sugiyama[*2]

[1]Chief Executive officer, Regugene Co. Ltd., 8F, 134 Chudoji Minamimachi, Shimogyo-ku, Kyoto-City, 600-8813, Japan.
[2]Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Yoshida-Ushinomaecho, Sakyo-Ku, Kyoto 606-8501, Japan.
E-mail: hs@kuchem.kyoto-u.ac.jp; namasivayam.ganeshpandian.5z@kyoto-u.ac.jp

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1. Introduction

Several disruptive innovations in mid-molecular drug discovery are taking place, as exemplified by the success of mRNA vaccines against the COVID-19 pandemic. Future precision medicine approaches that utilize genetic knowledge to develop nucleic acid-based designer drugs have the potential to treat and even cure currently incurable diseases. Several tools have recently been developed to target gene transcription at the molecular level of DNA, including the Nobel Prize-winning CRISPR-Cas9 and transcription activators (TALEs). Programmable small molecules based on nucleic acid sequence information can mimic the structure and function of natural transcription factors (TFs), selectively targeting DNA-protein interactions and potentially modifying the transcriptional mechanisms associated with incurable diseases.[1] This process of modulating transcription factors to control gene expression without editing the DNA sequence itself is called "transcription therapy." Here, we discuss the potential of pyrrole-imidazole polyamides (PIPs) as synthetic transcription factors and activators as transcription therapy agents to regulate genes on demand.

2. History of Development of 2-Pyrrole-Imidazole Polyamide

In 1985, Dickerson *et al*. demonstrated that netropsin, an antibiotic which has two *N*-methylpyrrole (Py) groups, forms a 1:1 complex with A/T in the minor groove of DNA by X-ray crystallography.[2] Subsequently, Dickerson and Dabrowiak showed that conversion of netropsin's Py to imidazole (Im) enabled it to hydrogen bond with the 2-amino group of guanine, enabling specific recognition of the G/C sequence.[3] A detailed examination of netropsin and distamycin A displacement experiments with Im showed that the bimolecules bind to the minor groove of DNA with a 2:1 complex formation and G/C specificity.[4] Based on this result, Dervan *et al*. proposed a new class of major groove-binding pyrrole-imidazole polyamides (PIPs) that can selectively recognize four Watson-Crick base pairs in double-stranded DNA. In linear PIPs that bind in one molecule, Py recognizes A/T base pairs and Im recognizes G/C base pairs. Recognizes A/T or T/A base pairs (**Figure 1**).[5] A pair of hydroxypyrroles Hp and Py was also found to specifically discriminate between T/A and A/T, but has been rarely used since then due to the instability of the compound. Hairpin PIPs are programmable middle molecules with binding affinities and sequence specificities comparable to those of natural transcription factors.[6]
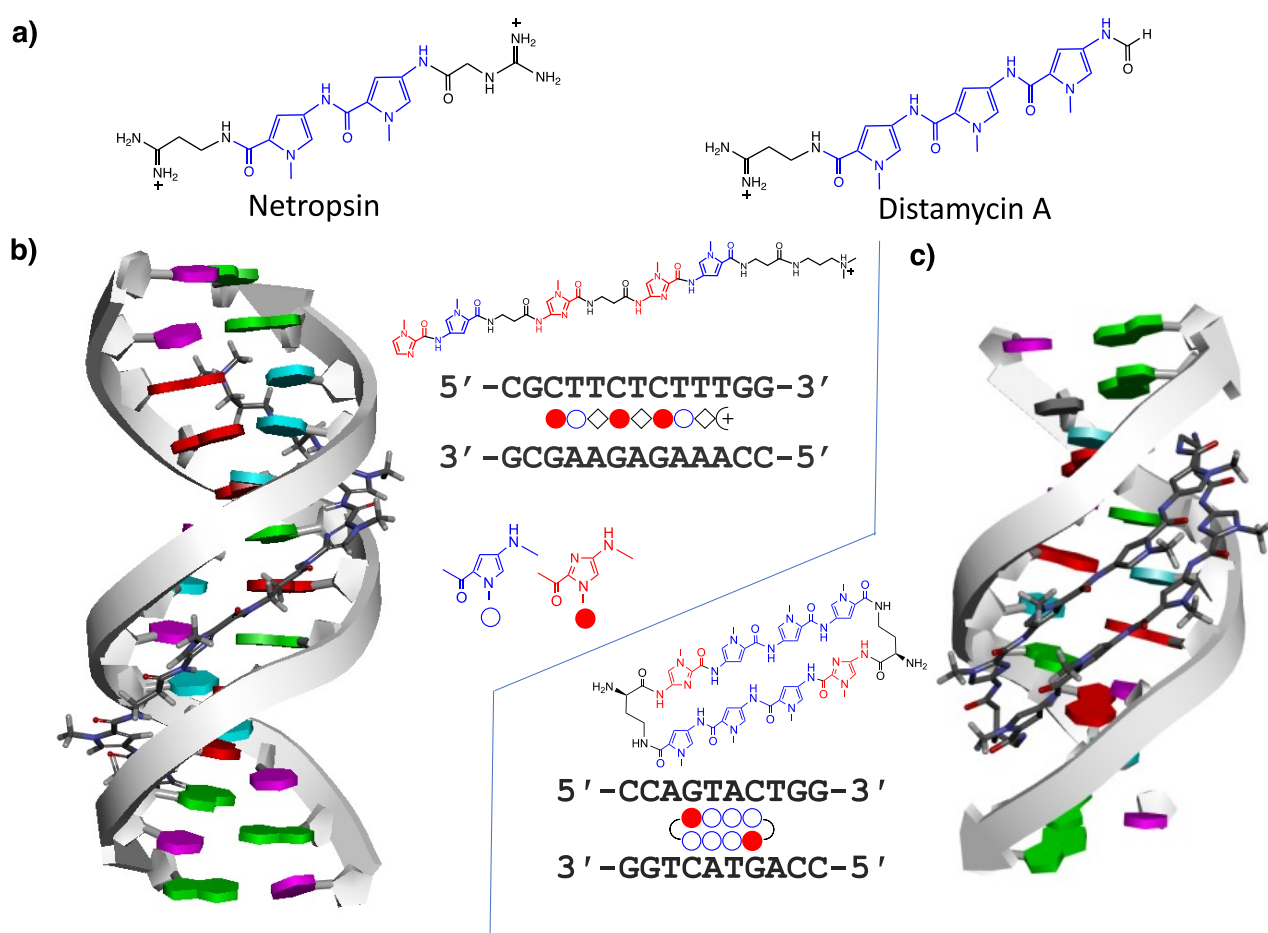

Figure 1. a) Chemical structures of the natural products netropsin and distamycin A. b) Molecular recognition of double-stranded DNA by linear PIPs. ^{1}H NMR structure 1LEJ. c) Molecular recognition of dsDNA by circular PIPs. X-ray crystal structure 3OMJ.

3. Binding Orientation of PIPs: Parallel or Antiparallel?

In the beginning stage of development, it was common for hairpin PIPs and cyclic PIPs to bind to DNA with the N-terminus to C-terminus of the PIPs aligned in the 5′to 3′ direction of DNA (**Figure 2a**).[7] However, subsequent studies revealed that some PIPs bind to DNA in the opposite direction (from the N-terminus to the C-terminus of PIP in the 3' to 5' direction of DNA).[8] Interestingly, it was shown that the parallel and antiparallel orientation can be controlled by the chirality of the amino group of the γ-turn portion of the cyclic PIP. cPIPs with (R)-α-amino groups bound in parallel, whereas cPIPs with (S)-α-amino groups showed antiparallel binding (**Figure 2**). Therefore, the orientation of PIP can be controlled by the chirality of the amino group in the γ-turn portion.[9] Antiparallel binding of chiral cPIP was confirmed by the X-ray crystal structure of the cPIP complex with DNA at 1.5 Å resolution (**Figure 2b**).[10] From the crystal structure, it was found that the positions of the hydrogen bonds between the base and cPIP are very similar in the parallel and antiparallel cases.[11] The formation of hydrogen bonds between amino groups on the γ-turn unit was also revealed.
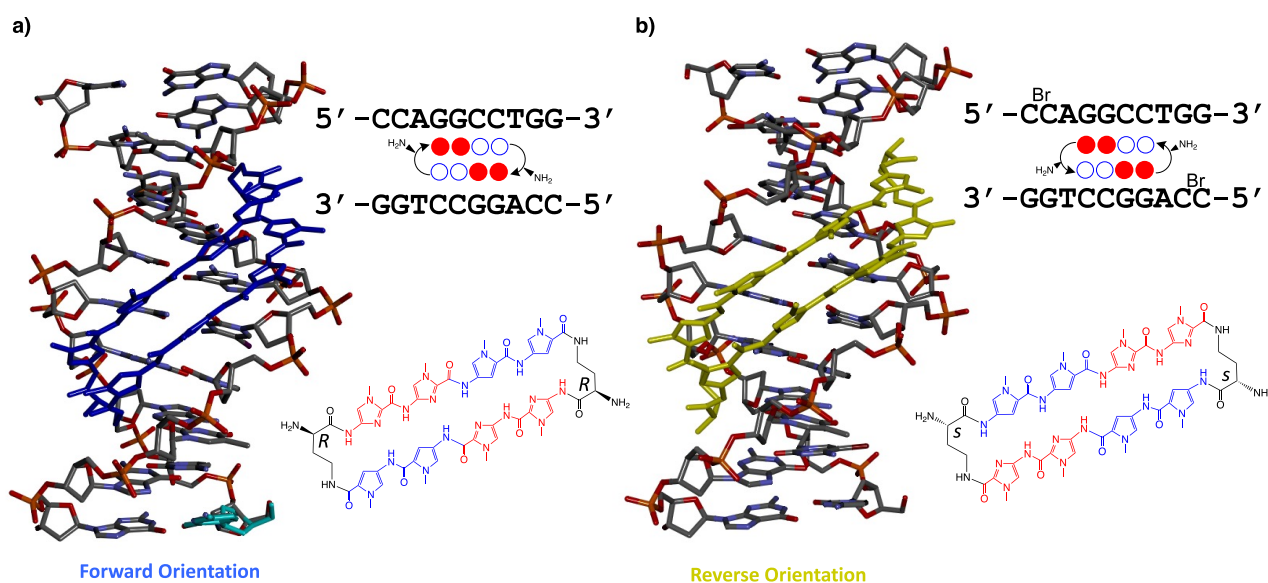

Figure 2. a) Structure of parallel binding of cPIP to DNA. X-ray crystal structure PDB 3I5L.[11] The arrow on the turn indicates the direction from the *N*-terminus to the *C*-terminus of PIP. b) Antiparallel binding structure of cPIP. X-ray crystal structure PDB 6M5B.[10]

4. PIP as DNA-Binding Molecules - Progress and Prospects

PIPs can be designed for any sequence and can compete with transcription factors to cause targeted repression of downstream genes. For example, the hairpin PIP (Soxi), which targets a SOX2 binding sequence (5'-CTTTTGTT), can differentiate induced pluripotent stem (iPS) cells to differentiate into the mesoderm lineages.[12] We also showed that Soxi can be used as an anticancer agent in mouse models by altering downstream genes.[13] Similarly, PIP, which binds to REL / ELK1 in the EV1 gene promoter sequence, effectively suppressed metastasis of breast cancer cells.[14] Thus, even a hairpin PIP of this size can have DNA binding affinity and functional properties similar to natural TFs. Recognition sequences of natural transcription factors are only on the order of 4-10 base pairs. Even with such short recognition sequences, transcription factors can accurately regulate gene expression. To achieve this precision, transcription factors often work as cooperative dimers. In mammalian cells, among about 1000 transcription factors, 55-70% form homo/ heterodimers to cooperatively regulate gene expression by extending recognition sequences and ensuring high binding affinity. In 2018, we developed a "PIP-HoGu (host-guest)" system (**Figure 3a**), to bind cyclodextrin and adamantane molecules and artificially assemble them side-by-side to cooperatively bind to target sequences.[15] In fact, the PIP-HoGu system was shown to bind cooperatively in cells by luciferase reporter assays. Furthermore, a cooperative dimeric ePIP-HoGu system with epigenetic activity was created by

combining PIP-HoGu with improved inclusion capacity cucurbituril (CB7) with a bromodomain binding agent (Bi).[16] The results showed that this ePIP-HoGu system can induce acetylation of targeted histones. We also created a cooperative system "PIP-NaCo" that can be modulated by sequence by replacing the cyclodextrin-adamantane pair with a left-handed γPNA pair (**Figure 3b**).[17] Duplex

formation between complementary PNA chains drove cooperative dimer formation, allowing programmable cooperative binding of PIP pairs. Importantly, since this system does not interact with natural nucleic acids such as DNA or RNA, it can be applied to target sequences more precisely, potentially leading to selective disease treatment.

Figure 3. a) Development of a PIP-HoGu system constructed to mimic the cooperative regulatory capacity of natural transcription factor pairs.[15,16] b) Structure and dimerization region of PIP-NaCo with bioorthogonal left-handed γPNA strand.[17] c) Structure of the PIP-cIKP conjugate that simultaneously recognizes the G4 structure and the flanking double-stranded DNA sequence.[43]

Furthermore, we extended the cooperative dimerization system using PIPs to target recognition of DNA secondary structures. Although the B-form duplex is the main structure of DNA, it has been pointed out that local structures such as left-handed Z-forms and guanine quadruplex (G4) structures are also formed depending on the sequence. We have developed a cyclic compound cIKP containing lysine

and N-methylimidazole that binds to the G4 structure.[18] We synthesized a hybrid molecule, PIP-cIKP, covalently bound to PIPs and demonstrated that it can simultaneously recognize double-stranded DNA and G4 structures (**Figure 3c**).[19] This approach, which utilizes the local structure of DNA for molecular recognition, shows the potential for enhancing the genomic specificity of PIPs.

5. Synthesis and Biological Evaluation of DNA Alkylating PIPs

DNA alkylating agents react mainly with purine bases and covalently bind to DNA, resulting in replication and transcription inhibition. Therefore, DNA alkylating agents have long been used as anticancer drugs, but their side effects have been problematic due to their low selectivity; combining DNA alkylating agents with sequence-specific PIPs to selectively alkylate mutant sequences unique to cancer cells may reduce the side effects of DNA alkylating agents.[20] Since KRAS mutations are found in many cancers, suppression of their expression has attracted attention as a target for anti-cancer drugs. We designed and synthesized KR12, an alkylated PIP that selectively reacts with this mutated sequence, which was confirmed by gel electrophoresis for DNA sequencing. In fact, KRAS expression was efficiently suppressed in experiments using human cancer cell lines. Furthermore, cancer growth was dropped in experiments with carcinoma-bearing mice bearing human colon cancer.[21] Next-generation sequencing analysis has confirmed that KR12 can target GTT mutations with much higher affinity than wild-type GGT sequences.[22] We are investigating the anticancer properties of a commercially available alkylating agent, chlorambucil (Chb), bound to PIP. Runt-related transcription factors (RUNX) 1-3 are known to be involved in the progression of hematologic cancers. We synthesized Chb-M', which recognizes and alkylates the consensus sequence of RUNX1-3 (5'-TGTGGT-3' and 5'-TGCGGT-3'), and showed that Chb-M' effectively inhibits RUNX targets in many cancer cell lines. In experiments using mouse models, Chb-M' was shown to effectively inhibit RUNX targets in many cancer cell lines, with significant effects in acute myeloid leukemia, acute lymphoblastic leukemia, noncellular lung cancer, and gastric cancer (**Figure 4**).[23]
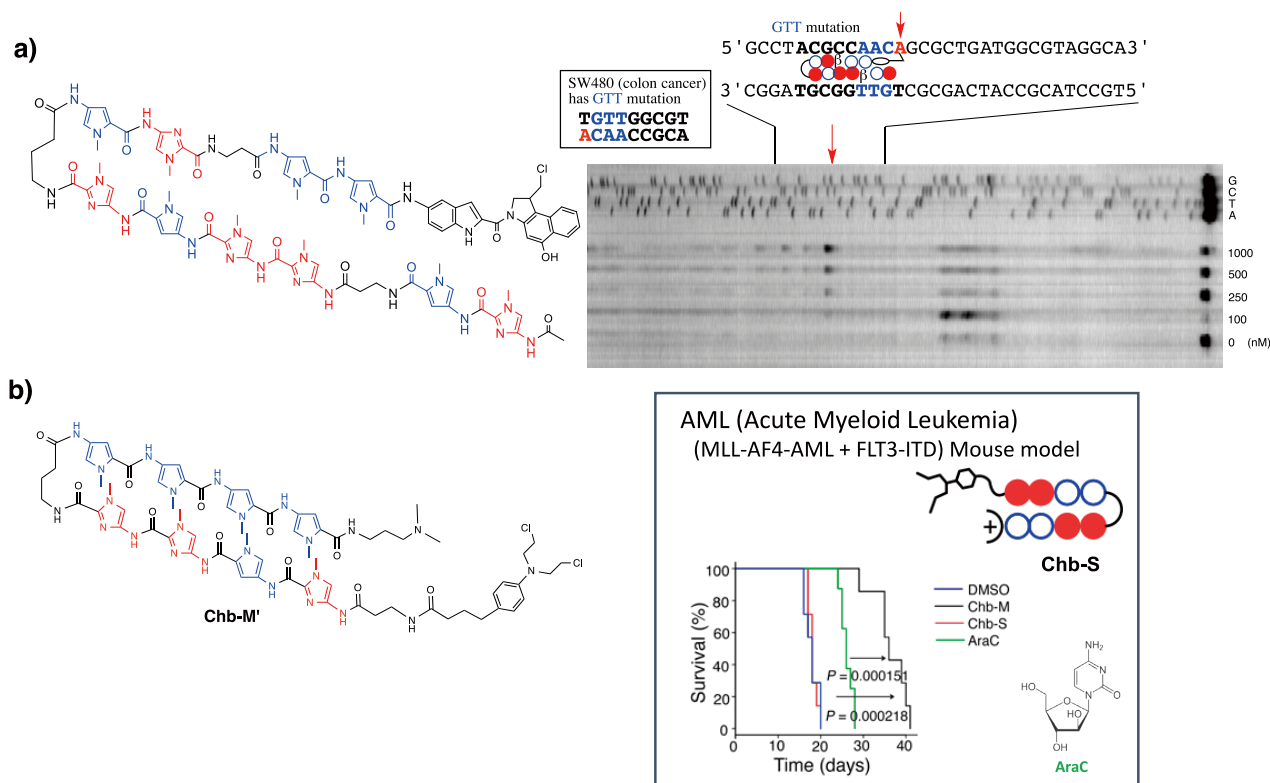

Figure 4. a) Evaluation of the chemical structure of KR12 targeting KRAS mutations and DNA sequence-selective alkylation by gel electrophoresis for sequencing. Targeted alkylation at the adenine N3 position is cleaved by subsequent heat treatment to give a band. b) Chemical structure of Chb-M' that inhibits RUNX1-3 binding. Results in a mouse model of acute myeloid leukemia. All model mice usually die within 20 days, but mice injected with Chb-M' from the tail vein were found to survive for 20 days. This effect is stronger than AraC, which is currently used clinically, and Chb-S, which alkylates with a different nucleotide sequence, has no effect at all.[23]

6. Potential Treatment of Triplet Repeat Disease

Triplet repeat diseases are caused by abnormal elongation of triplet repeat sequences in the genome. Expansion of CAG and CTG is known to cause several neurological disorders, including fragile X syndrome and myotonic dystrophy. The mechanism by which expanded CAG repeats cause disease can be explained via polyglutamine (polyQ) toxicity. Expanded CAG repeats are translated into uninterrupted glutamine residues, forming the expanded polyQ tracts and becoming amyloid cores and causes protein misfolding and aggregation, leading to neurodegeneration. DM1, which is also induced by CTG repeat expansion of the 3'-untranslated region (3'-UTR) of DMPK, is the most common neuromuscular disorder (**Figure 5a**). Thus, CWG repeat disorders are thought to be caused by highly complex intracellular mechanisms, and effective therapies have not been developed yet. Therefore, we have synthesized PIPs that bind to various repeats.[24]
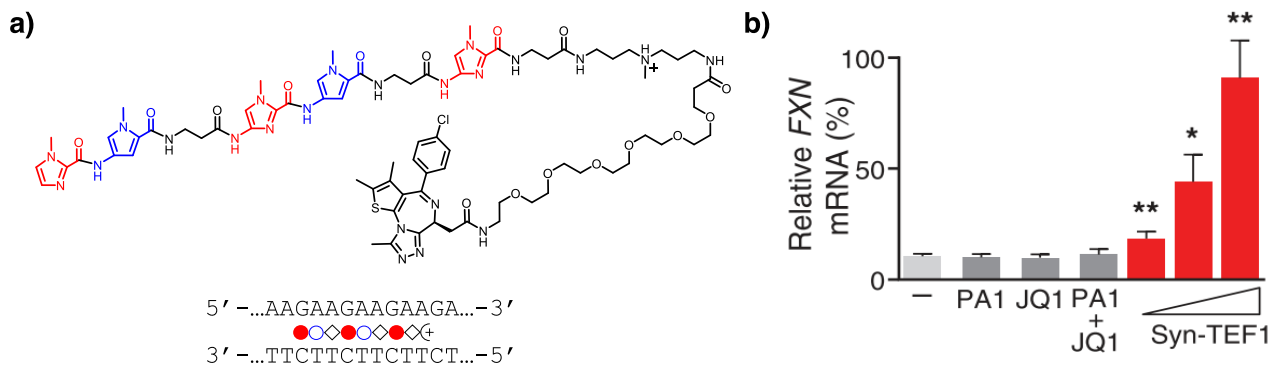

Figure 5. a) Chemical structure and binding mode of synTEF1. b) The amount of FXN, which had been suppressed by administration of synTEF1, returned to normal.[25]

Ansari *et al*. investigated the therapeutic potential of synTEF1, a PIP and binding to GAA repeats, and JQ1 binding to BrD4, a transcription elongation factor, for Friedreich's ataxia. It was shown that synTEF1 restores the expression level of frataxin (FXN), which is heterochromatinized and downregulated, to almost normal values.[25] Designer therapeutics, a startup company, has been conducting clinical trials for the treatment of Friedreich's ataxia since March of last year and has reported good interim results.[26]

7. Telomeres - An Attractive Target for PIP Technology?

Tandem repeats of d(TTAGGG)/d(CCCTAA) sequences at the telomeric ends of chromosomes are essential for chromosomal stability.[27] During cell division, telomere length is shortened because the ends of chromosomes cannot be replicated by DNA polymerase. Therefore, in normal cells, the number of divisions is limited to 30 to 60 times, and apoptosis, which is cell death, occurs. However, telomerase expressed in stem cells and germ cells can restore and maintain telomere length. Cancer cells can proliferate indefinitely because apoptosis does not occur due to telomere shortening. Approximately 85% of cancer cells express telomerase, and the remaining 15% avoid telomere shortening by recombination.[28] Therefore, targeting the telomeric repeat sequences of cancer cells is expected to have an antitumor effect.[29]

Therefore, we developed a DNA alkylating agent using a tandem hairpin PIP motif with 12-base recognition ability, targeted the 5'-d(AACCCT)n-3' sequence, and showed that it could induce apoptosis.[30] Furthermore, in collaboration with Maeshima et al., we synthesized various tandem-type fluorescent PIPs such as TH59, and developed a method for selectively targeting telomeres in live cells by optimizing the fluorescent site and hinge region.[31] Telomere length of HeLaS3, HeLa1.3, and U2OS ALT cells was measured using fluorescently labeled TH59. This indicated that telomeres in tumor cells are relatively shorter than in normal tissues. Strong signals with low background noise were observed in brain and lung tissues by the TH59 system when mouse tissue sections were simultaneously stained with an antibody to the telomere binding protein TRF1. On the other hand, TRF1 gave a weak signal with high background noise, indicating that the staining ability of the TH59 system is superior to conventional immunostaining techniques. In addition, we synthesized TT59 (3 hairpins and 2 hinges), a tandem trimer that can target the 18-nt human telomeric repeat sequence TTAGGG, and showed that it could stain telomeres with even lower background (**Figures 6a** and **b**).[32] Furthermore, we designed and synthesized a tetrameric PIPs, TTet59 (having 4 hairpin units and 3 hinges) that targets 24 bp of human telomeric 5'-(TTAGGG)n-3' repeats.[33] TAMRA TTet59-B was shown to have higher specificity with lower background signal than previously reported trimeric and dimeric probes. Probes with excitation and emission wavelengths in the near-infrared region (NIR) exhibit excellent fluorescence with low phototoxicity, making them suitable for live cell imaging. Near-infrared silicon rhodamine (SiR) is widely used in live cells as a fluorescent group with excellent fluorescence. Therefore, TTet59B was modified with SiR (SiR-TTet59B), and excellent telomere visualization was demonstrated.[34] In studies with U2OS cells, SiR-TTet59B enabled us to observe telomere length and dynamics in mitotic and interphase cells (**Figure 6c**).
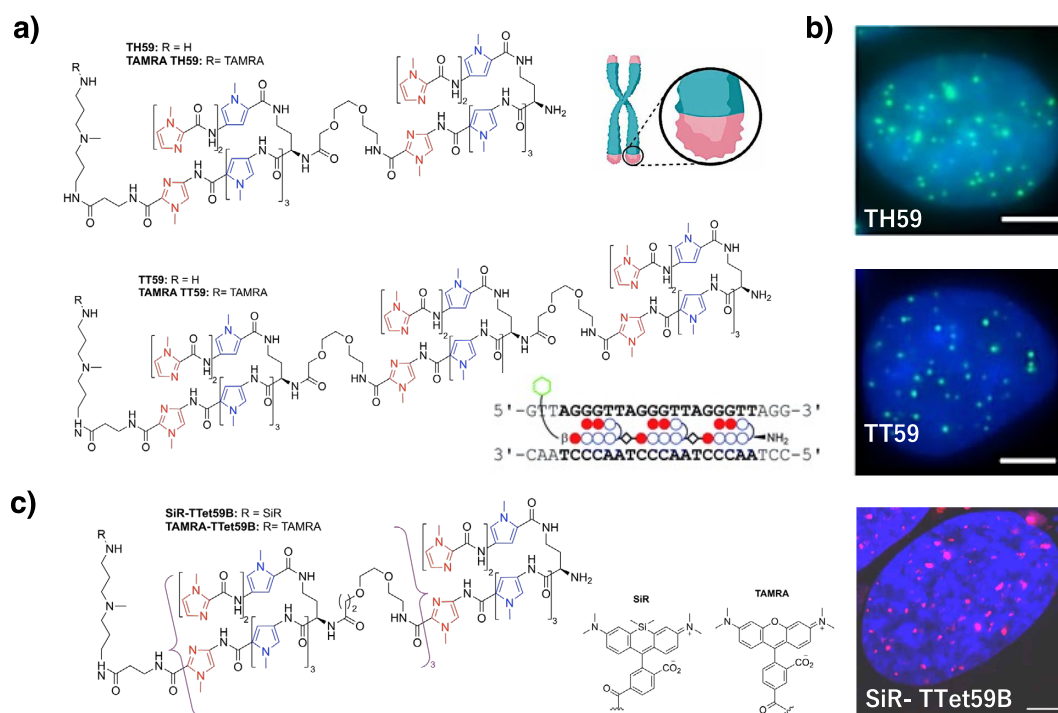

Figure 6. a) Chemical structures of telomere staining PIPs TH59 and TT59. b) Stained image of telomeres in HeLa 1.3 cells. Strong fluorescence is seen at individual chromosome ends, indicating high selectivity. c) Chemical structures of 24 bp sequences targeting TAMRA® TTet59-B and SiR-TTet59B and telomere visualization at the interphase stage of live U2OS cells.[34]
* TAMRA is a trademark of trademark Applied Biosystems, Inc.

In general, as the molecular weight of PIP increases, it becomes difficult to incorporate it into the nucleus.[35] Dervan *et al.* showed that introduction of isophthalic acid at the *C*-terminus or an aryl group in the turn structure can improve the uptake of PIP into the cell and into the nucleus.[36] We have also introduced a triarginine group into a 6-base recognizing PIP, and demonstrated improved cellular uptake and nuclear localization using flow cytometry and confocal microscopy.[37]

8. Delivery of PIP to Mitochondria

Although PIP targets nuclear DNA, it can also target mitochondria with a circular mtDNA of 16.6 kbp. We synthesized a new type of MITO-PIP that localizes to mitochondria. MITO-PIP was preferentially localized in mitochondria and caused selective transcriptional repression of the ND-6 gene on the mitochondrial genome (**Figure 7**).[38] Reactive oxygen species such as superoxide are generated in mitochondria that produce ATP through oxidative phosphorylation, and mtDNA mutation occurs. Mitochondrial diseases caused by such mutations are known. We developed MITO-PIP-Chb by introducing the alkylating agent chlorambucil (Chb) into MITO-PIP, and investigated whether the mutant adenine can be repaired by alkylating it.[39] MITO-PIP-Chb was confirmed to selectively alkylate the adenine of the m.8950G>A mutant sequence. Moreover, using cells with m.8950G>A mutated sequences, MITO-PIP-Chb did reduce the proportion of mutated sequences. Future developments are expected as there is still no good treatment for refractory mitochondrial diseases.
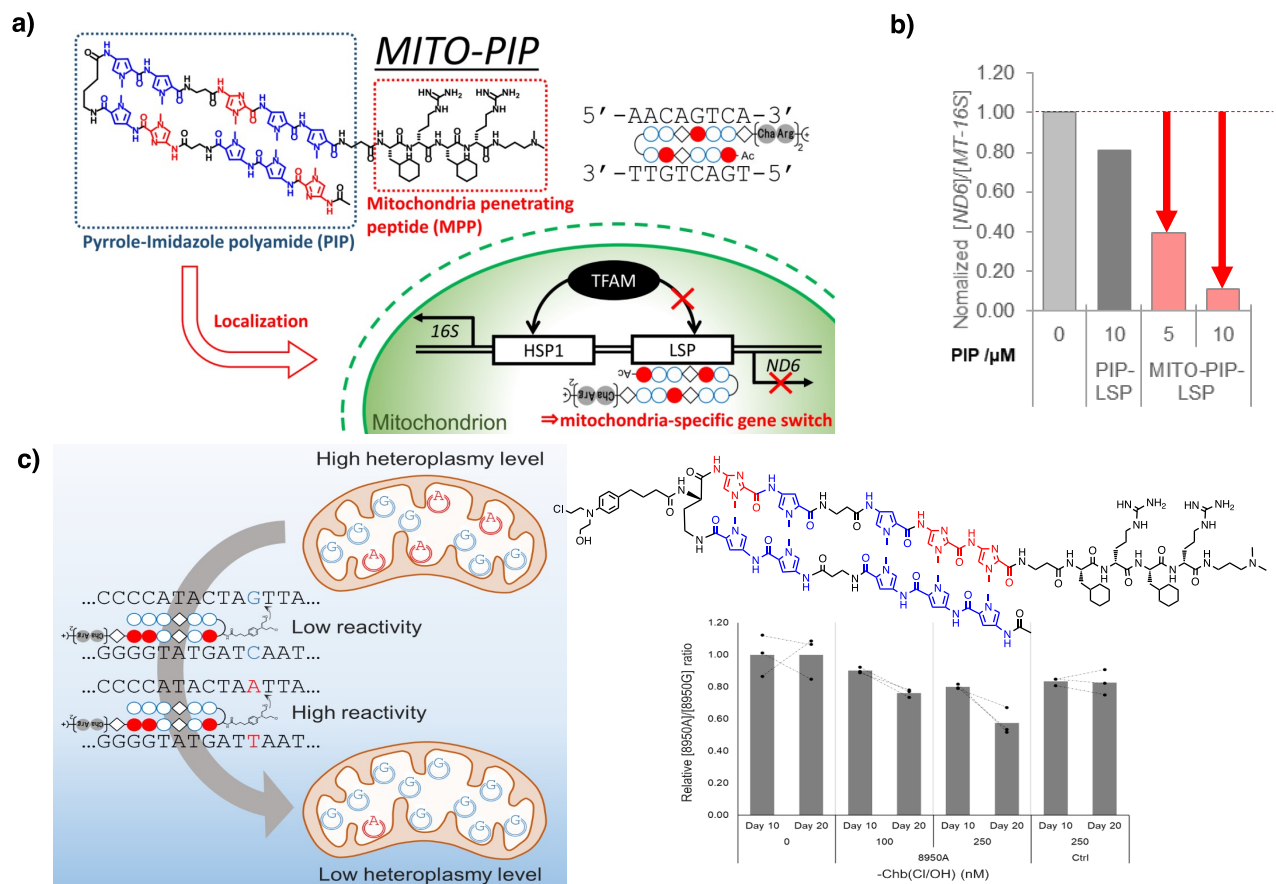

Figure 7. a) Chemical structure and mechanism of action of MITO-PIP-LSP[38]. b) MITO-PIP-LSP dose-dependently suppressed the expression of ND6 by binding to the light chain promoter (LSP). On the other hand, PIP-LSP has almost no effect. c) Structure of MITO-PIP-Chb targeting mutated adenine and schematic diagram of reducing mutations.[39] Treatment with MITO-PIP-Chb reduced mutant 8950A and increased 8950G.

9. PIP Functions as an Epigenetic Modifier

Gene expression is controlled epigenetically in addition to the base sequence of DNA. Epigenetic gene regulation is coordinated by protein complexes with writer, reader, and eraser functions. For example, in histone acetylation, histone deacetylase (HDAC), an eraser that removes acetyl groups from histone proteins, and histone acetyltransferase (HAT) function as writers to write acetyl groups. There is also a reader protein that reads each state. We bound various epigenetic modifiers to PIP and induced histone acetylation and gene expression.[40]
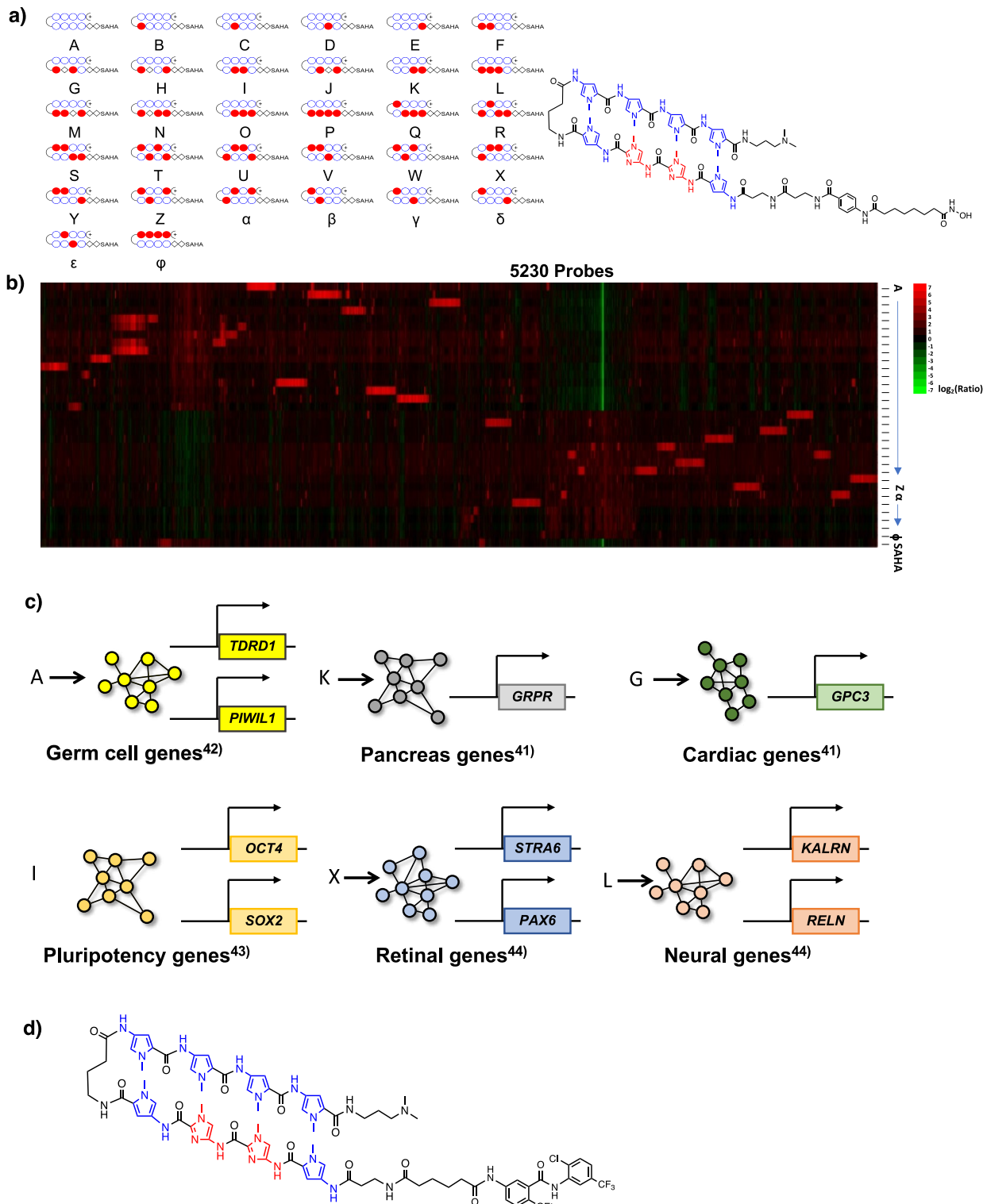

Figure 8. a) SAHA-PIP chemical structure and SAHA-PIP library. b) Results of gene expression analysis by microarray of HDF processed with SAHA-PIP library. c) Activation of typical gene clusters. d) Chemical structures of PIPHAT activators.[42]

We conjugated 32 different PIPs with SAHA, a known HDAC inhibitor, and tested their gene expression on human dermal fibroblasts (HDFs) two days later. As a result, expression of 100 to 200 genes was increased by 10-fold or more. Interestingly, almost all SAHA-PIPs activated different gene clusters.[41] **Figure 8** summarizes the activation of typical gene clusters by SAHA-PIP. In addition, PIP was combined with a HAT activator to examine the enhancement of gene expression, and it was confirmed that it has the same activity as SAHA-PIP.[42]

Furthermore, we attempted to increase gene expression by binding Bi, a molecule that binds to the bromodomain present in HAT, with PIP.[43] The bromodomain present in HAT has the function of recognizing the acetyl group of lysine and further

acetylating neighboring lysines to expand euchromatin. In fact, Bi-PIP induced acetyl group at the PIP-binding sequence and enhanced gene expression. PD-1-based cancer immunotherapy represented by Opdivo developed by Professor Honjo has attracted attention for its efficacy.[44] However, it does not show efficacy in half of the cancers, where exhaustion of T cells is thought to be the cause. Therefore, we tried to activate PGC-1, which regulates mitochondrial biogenesis, to activate T cells. As a result, it was shown that EnPGC-1 with Bi and enhanced nuclear localization ability with triarginine enhanced immunotherapy at the mouse level.[45] EnPGC-1 induced mitochondrial activation, energy metabolism, and proliferation of CD8+ T cells under in vitro conditions.
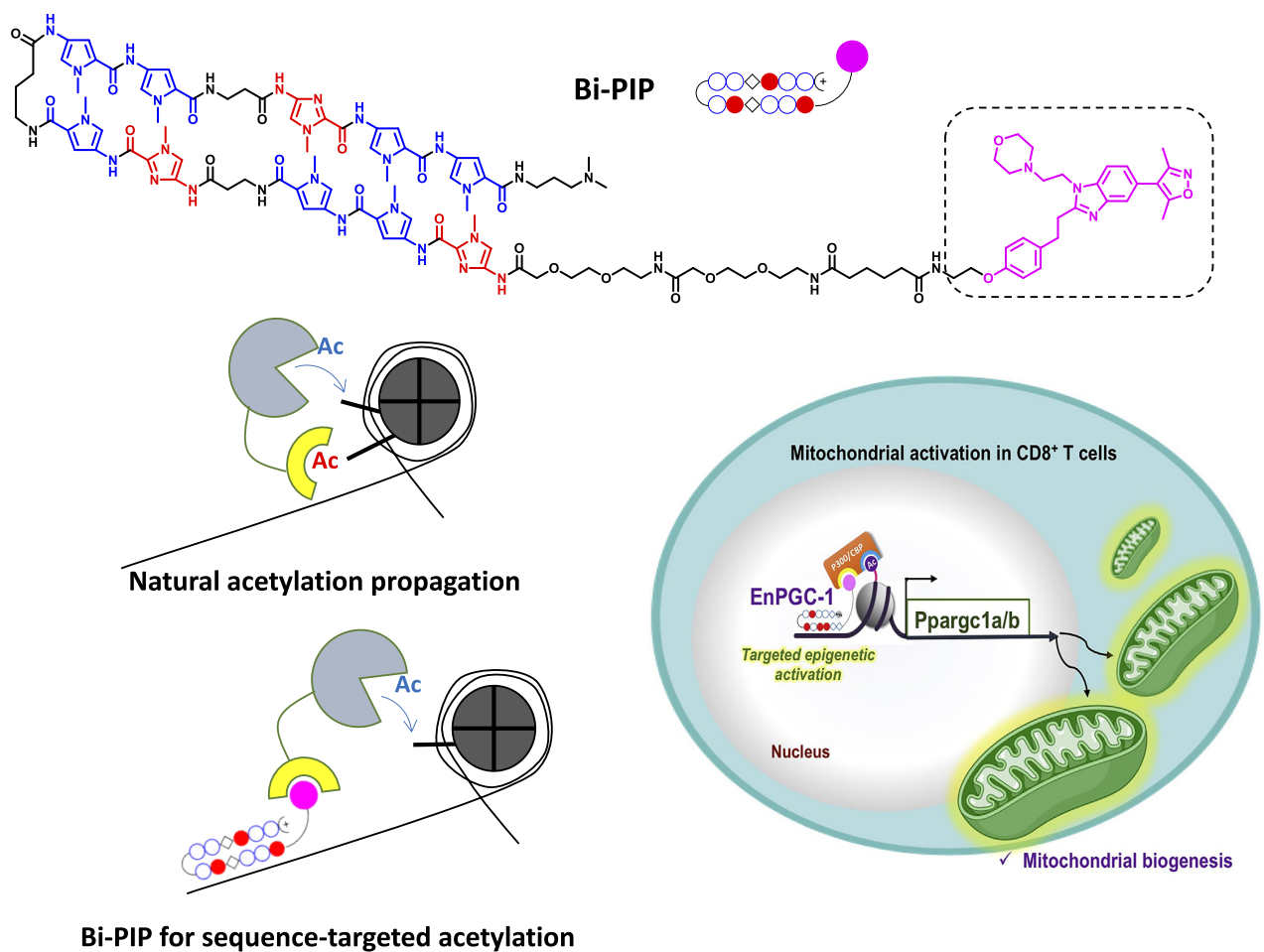

Figure 9. Schematic representation of PIP coupled with the acetyl-lysine mimetic EnPGC1 induced targeted epigenetic induction of PGC1 family genes and mitochondrial biogenesis in T cells, providing a synergistic combination therapy.

10. Summary and Outlook

Since his 2020 Nobel Prize for CRISPR-Cas9, there has been interest in developing nucleic acid therapeutics based on genetic information. In particular, middle-molecule modulators of gene transcription may reset the dysfunctional transcriptional machinery of diseased cells. Among nucleic acid-based small-molecule regulators, his PIP technology, invented by Prof. Peter Dervan, shows potential for Phase 1 clinical trials in the treatment of trinucleotide diseases. Designer PIPs are attracting attention as targeted transcriptional therapeutics because they can regulate the transcription of target genes without altering the nucleotide sequence of the genomic DNA. PIP has shown a remarkable ability to induce promoter-specific transcriptional regulation of oncogenes, cellular reprogramming genes, and mitochondrial genes. Alkylating PIPs have successfully targeted point mutations in cancer-associated genes such as KRAS and RUNX1. In particular, PIP targeting RUNX1 has demonstrated its biological efficacy in treating a wide range of cancer cells at the animal model level. A multifunctional PIP alkylator successfully achieved targeted removal of mutated mitochondrial DNA, further demonstrating the potential of PIP as a promising transcriptional therapeutic that can be personalized on demand. It has also been demonstrated to have a synergistic effect in anti-cancer immunotherapy. This groundbreaking study opens the possibility of using this technology as a stand-alone therapy, as well as combining the therapy with already available conventional therapies. Although the PIP technology is promising, there are some improvements to be made, such as water solubility, balance between recognition and permeation, and limitations to achieve multifunctionality. In addition, there are typical challenges of therapeutic approaches such as low cost of manufacture, sustained bioavailability, minimization of off-target toxicity, and socioeconomic issues. As technology advances, PIPs, unique middle molecules, are poised to provide a paradigm shift in the treatment of diseases that currently have limited treatment options.

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Author Information

Vaijayanthi Thangavel obtained her Ph.D. in Chemistry at the Indian Institute of Technology, Madras, Chennai, India, in 2008 under the supervision of Prof. Anju Chadha. She then conducted postdoctoral research in the group of Prof. Andreas S. Bommarius at the Georgia Institute of Technology, Atlanta, USA. She continued her research as a Special Assistant Professor (Research) in the group of Prof. Hidetaka Hori, Niigata University, Japan. Since 2011 she has researched with Prof Hiroshi Sugiyama at the Graduate School of Science, Kyoto University, and Dr. Ganesh N. Pandian at iCeMS, Kyoto University. From 2021, She has also taken charge as CEO at ReguGene, a Kyoto University-based Startup company.

Contact E-mail address: thangavel.vaijayanthi.8v@kyoto-u.ac.jp

Ganesh N. Pandian obtained his Ph.D. (Applied biosciences) in 2009 from Niigata University under Prof. Hidetaka Hori with a Monbukagakusho (Japanese Government) scholarship. He continued his research as Assistant Professor (Research) and served as a visiting scientific advisor in the Ushiki patent office. In 2010, he joined Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University as Research Associate under Prof. Hiroshi Sugiyama and was promoted to Assistant Professor in 2014. Ganesh is now a Principal Investigator and Junior Associate Professor at Kyoto University iCeMS and is continuing his research on artificial genetic switches for cell fate and disease control.

Contact E-Mail address: namasivayam.ganeshpandian.5z@kyoto-u.ac.jp

Hiroshi Sugiyama obtained his Ph.D. in 1984 with Prof. Teruo Matsuura at Kyoto University. After postdoctoral studies at the University of Virginia with Prof. Sydney M. Hecht, he returned to Kyoto University in 1986 as an Assistant Professor and became Associate Professor in 1993. In 1996, he joined the Institute of Biomaterials and Bioengineering at Tokyo Medical and Dental University. He has been a Professor of Chemical Biology at Kyoto University from 2003-2022. In 2022, he moved to iCeMS, Kyoto University.

Contact E-mail address: sugiyama.hiroshi.3s@kyoto-u.ac.jp

Related Products

Boc-Im-OH	250mg	1g	B6350
Boc-Py-OH	250mg	1g	B6351
Fmoc-Im-OH	1g	5g	F1313
Fmoc-Py-OH	250mg	1g	F1314
Fmoc-PyIm-OH	1g	5g	F1315

Chemistry Chat

My Familiar Compound Family
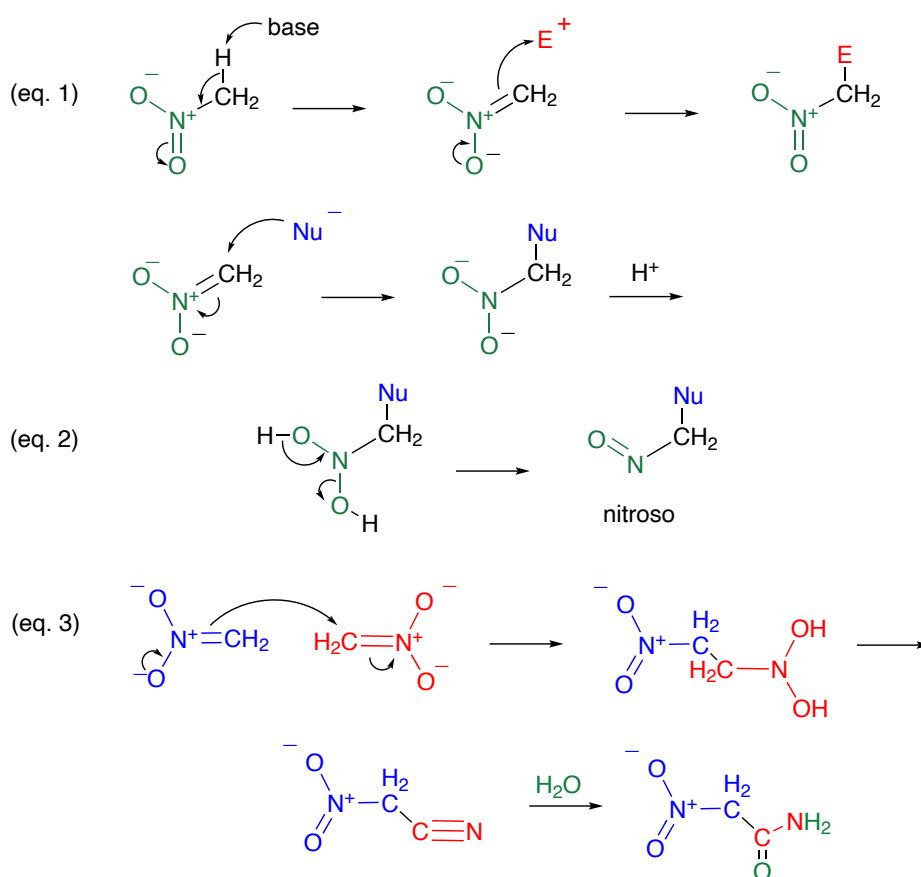### – Nitro Compounds –

Nagatoshi Nishiwaki

School of Science and Engineering, Kochi University of Technology

I encountered nitro compounds at the first university I was employed at after completing graduate school. Nitro compounds (like cyano compounds) are only regarded as compounds related to amines without being devoted to a single chapter in textbooks. They were completely unknown to me, as I myself had hardly ever used them, even when I was a student. It is strange that now, after 30 years, nitro compounds have been the central focus of my research. Furthermore, I have written a review article on nitro compounds.[1]

Diverse Reactivity

Nitro groups exhibit diverse reactivity similar to carbonyl groups.[2] The first thing that comes to mind is that they can make substrates electron-deficient by inductive electron-withdrawing and resonance effects. Nitro groups are also often used as nucleophiles because of their high acidity at the α-position and the stable anions they produce (**Scheme 1**, eq. 1). Furthermore, they have a similar reactivity to carbonyl compounds, such as acting as


Scheme 1. Reactions of nitro compounds with electrophiles or nucleophiles

electrophiles when the other reactants are nucleophiles (eq. 2) and reacting with other nitro groups (eq. 3). However, the major difference between a nitro group and a carbonyl group is that a nitro group can act as a leaving group. The nitro group itself may be substituted directly, or it may leave together with the hydrogen at the adjacent position as a nitrite, forming a double bond. Furthermore, a nitro group is a useful functional group in synthetic chemistry because it can be derivatized into various frameworks by chemical transformation, including reduction.

Electron-withdrawing Effect

As mentioned above, a nitro group acts as a strong electron-withdrawing group. Even when only looking at the inductive effect, the pKa of nitroacetic acid is 1.68, which is comparable to that of dichloroacetic acid (1.29), indicating an affinity equivalent to that of two chloro groups. Furthermore, if the resonance effect is added, the substrate becomes highly electron-deficient.

2-Methyl-4-nitro-3-isoxazolin-2(5H)-one (nitroisoxazolone) is the compound that made me realize the electron-withdrawing effect of the nitro group in particular: the ring nitrogen at the 2-position is electron-withdrawn by the nitro group and carbonyl group through a double bond. In addition, a highly electronegative oxygen is bonded to the adjacent position, and a carbonyl group is bonded further ahead. Indeed, the ring nitrogen is highly electrophilic, and in the reaction of 1,3-dicarbonyl compounds with enolate ions, ring transformation proceeds accompanied by decarboxylation to give polysubstituted pyrroles (**Scheme 2**, eq. 1).[3]

The acidity of the hydrogen at the 3-position of nitroisoxazolone is also quite high, and water acts as a base to deprotonate it. Subsequent ring-opening, ring-reclosing, dehydration, and decarboxylation would produce the nitrile oxide (eq. 2).[4] The 3-position proton of pyridinium salt,[5] the precursor of this isoxazolone, is also highly acidic even though it is anionic. Indeed, it can be deprotonated by organic bases such as pyrrolidine, which undergoes ring-opening to give dianionic cyanoacinitroacetates (eq. 3).[6,7] This compound can be used as a cyano(nitro)methylating agent that can be safely handled instead of nitroacetonitrile, which is explosive.[6]

Lesson learned: "We are the ones not taking full advantage of the compound's specs."

Scheme 2. Chemical transformations of nitroisoxazolone

Leaving Ability

The nitro group works as a leaving group: in the reaction of ethyl α-nitrocinnamate with acetylacetone, the enolate ion intramolecularly displaces the nitro group to give dihydrofuran (**Scheme 3**, eq. 1).[8] We hypothesized that when ethyl α-nitrocinnamate is in the presence of acetylide, a conjugate addition proceeds. We thought that the functionalized enyne could be obtained by elimination of nitrous acid, since the conjugate addition proceeds when the acetylide reacts with ethyl α-nitro cinnamate. However, we had to struggle with the quirky behavior of the elimination reaction not occurring not eliminating when we wanted it to. Thinking that the lack of elimination was due to an insufficient amount of base, we used an excess amount of acetylide, but no change at all was observed (eq. 2). After various trials, we almost gave up, but then we tried using triethylamine, a weak base, at

last. As a result, the deprotonation proceeded efficiently and we succeeded in obtaining enyne in high yield (eq. 3).[9] In the case of acetylide, the equilibrium was biased toward the anion from which the α-hydrogen of the nitro group was withdrawn because it is a strong base. So, the reaction did not proceed. To the contrary, triethylamine is a weak base and there is an equilibrium between deprotonation and protonation. When the base then withdraws the β-hydrogen, the deprotonation proceeds to afford enynes. We learned that when the reaction does not proceed, we often use more reagents with higher reactivity or harsher reaction conditions, but sometimes the reaction proceeds by decreasing the reactivity.

Lesson learned, "If you push and it does not work, try pulling."
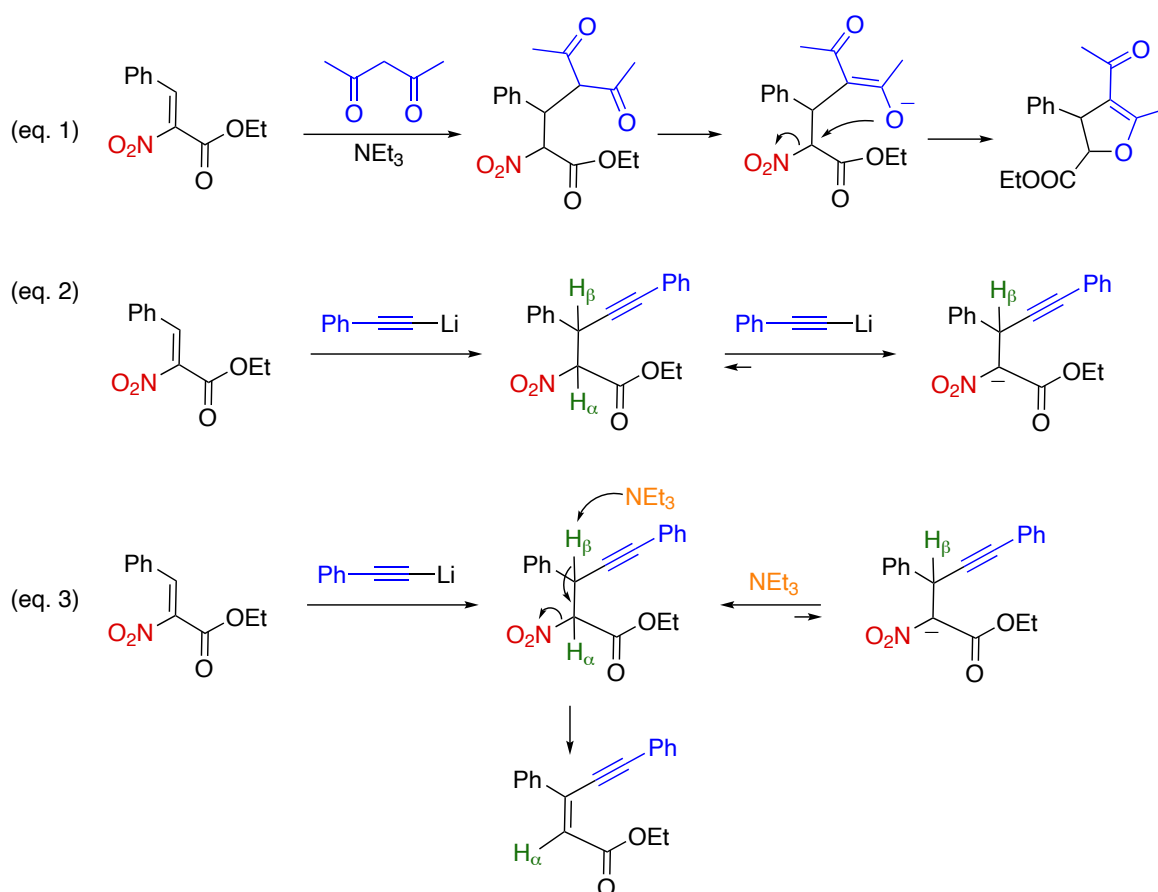
Scheme 3. Reactions using a nitro group as a leaving group

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Author Information

Professor Nagatoshi Nishiwaki received a Ph.D. in 1991 from Osaka University. He worked in Professor Ariga's group in the Department of Chemistry, Osaka Kyoiku University, as an assistant professor (1991-2000) and associate professor (2001-2008). From 2000 to 2001, he was with Karl Anker Jørgensen's group at Århus (Aarhus) University in Denmark. He worked at the Center for Collaborative Research, Anan National College of Technology as an associate professor from 2008 to 2009. Then, he moved to the School of Environmental Science and Engineering, Kochi University of Technology in 2009, where he has been a professor since 2011. His research interests comprise synthetic organic chemistry using nitro compounds, heterocycles (synthesis, ring transformation, 1,3-dipolar cycloaddition, application as tools in organic synthesis), pseudo-intramolecular reactions.

New Products Information

Air-Stable Ni(0) Complex Ni(COD)(DQ), a Useful Precatalyst

Ni(COD)(DQ) (1)　　　　　　　　　　　　　　　　Product Number: **N1198**

1g　5g

　　(1,5-Cyclooctadiene)(duroquinone)nickel(0) (Ni(COD)(DQ), **1**) was reported to be an air-stable precatalyst for a variety of nickel-catalyzed reactions by Engle *et al*.[1] For example, **1** gave comparable results to the Ni(0) complex bis(1,5-cyclooctadiene)nickel (Ni(COD)$_2$), which is sensitive to air and heat, and other nickel(II) complexes in various reactions, including Suzuki-Miyaura cross-coupling and Buchwald-Hartwig amination. In addition, the synthesis of *N,N*-diarylsulfonamides through C-N cross-coupling using **1** as a catalyst[2] and the deoxygenative alkenylation of carboxylic acids to give all-carbon tetrasubstituted alkenes using **1** and a photoredox catalyst[3] have been reported. Therefore, further development and application of reactions using **1** are anticipated in the future.
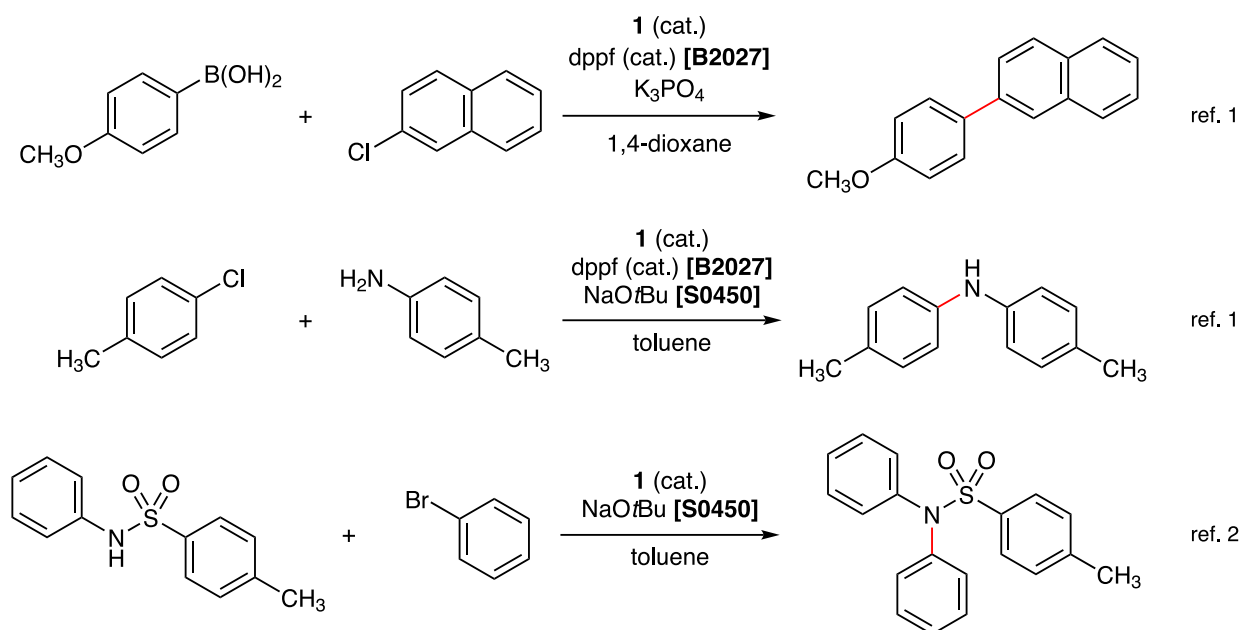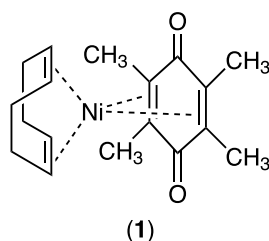
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Related Products

Bis(1,5-cyclooctadiene)nickel(0) (= Ni(COD)$_2$)			5g	B6553
1,1'-Bis(diphenylphosphino)ferrocene (= dppf)	1g	5g	25g	B2027
Sodium *tert*-Butoxide	25g	100g	500g	S0450

Naphthalene Carboxylic Anhydride for the Synthesis of Functional Materials

1,2,5,6-Naphthalenetetracarboxylic Dianhydride (1)

Product Number: **N1247**
1g

TCI offers naphthalene tetracarboxylic anhydrides and its derivatives with various substitution patterns and 1,2,5,6-naphthalene tetracarboxylic dianhydride (1,2,5,6-NTCDA, **1**) has newly been commercialized. Unlike other NTCDA derivatives, **1** has a folded structure and is expected to improve solubility. Polyimides, electrochromic material,[1] and n-type organic semiconductors[2] have been reported using **1**. In addition, it has been reported that **1** can be regioselectively brominated and utilized in the cross-coupling to synthesize donor-acceptor polymers.[3]

Polyimides

Electrochromic material

Donor-Acceptor Polymers

1,2,5,6-NTCDA
(1)
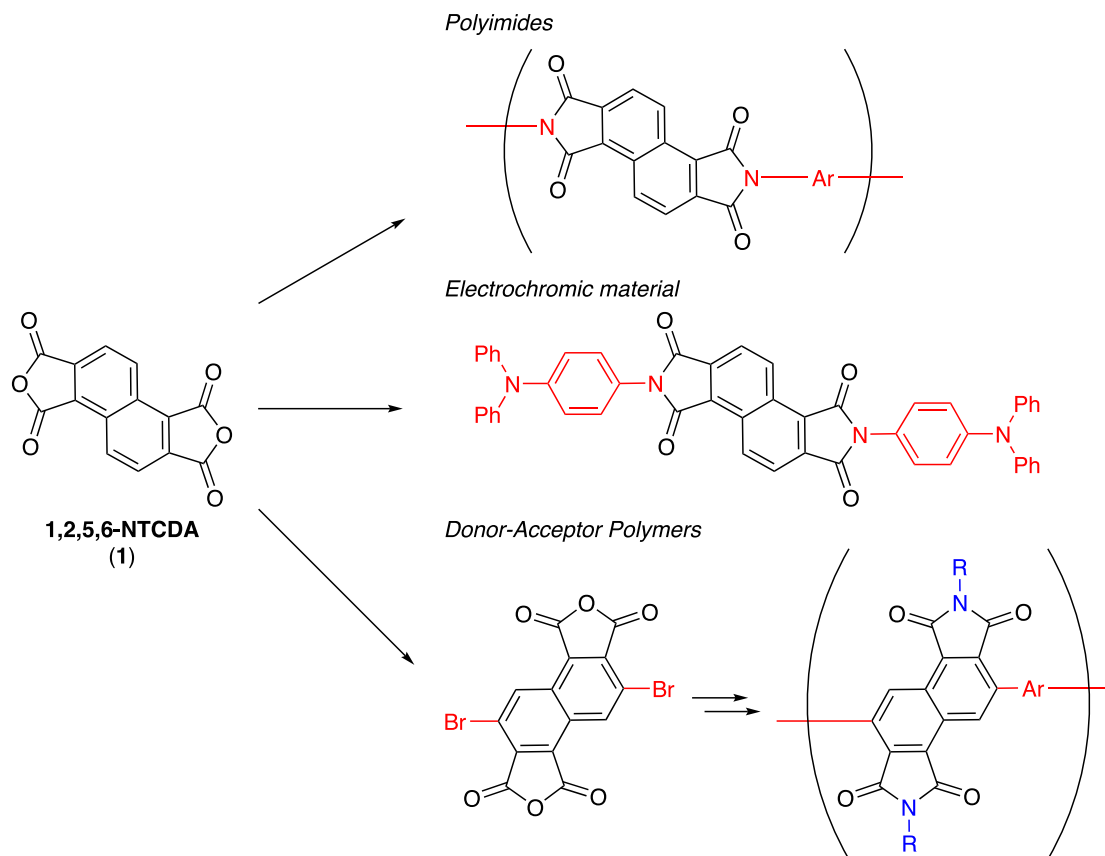
TCI's other NTCDAs
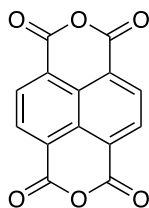
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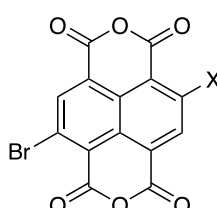
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[D4399] (X = Br)
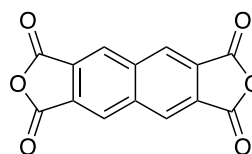
[N1128]

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3) Z. Li, K. Feng, J. Liu, J. Mei, Y. Lia Q. Peng, *J. Mater. Chem. A* **2016**, *4*, 7372.

Related Products

	25g	250g	
Naphthalene-1,4,5,8-tetracarboxylic Dianhydride (= NTCDA)	25g	250g	N0369
NTCDA (purified by sublimation)	1g	5g	N0755
2-Bromonaphthalene-1,4,5,8-tetracarboxylic 1,8:4,5-Dianhydride	1g	5g	B5756
2,6-Dibromonaphthalene-1,4,5,8-tetracarboxylic Dianhydride	1g	5g	D4339
2,3,6,7-Naphthalenetetracarboxylic 2,3:6,7-Dianhydride (= 2,3,6,7-NTCDA)	1g	5g	N1128

Graphitic Carbon Nitride and Heptazine-Based Building Blocks

Graphitic Carbon Nitride (1)

Product Number: **G0539**
200mg

Melem (2)

Product Number: **M3538**
**1g**

Heptazine Chloride (3)

Product Number: **T4145**
1g

Graphic carbon nitride (g-C$_3$N$_4$, **1**) has received a great deal of attention as a metal-free photocatalyst due to promising properties such as water splitting and organic matter removal in recent years.[1] Melem (**2**) and heptazine chloride (**3**), which are partial structures of g-C$_3$N$_4$, are useful for a precise synthesis of g-C$_3$N$_4$ inspired materials and comparison of its physical properties. They are also applied for a monomer unit of MOF and COF.[2] Moreover, in light-emitting material derivatized from **3**, a delayed fluorescence from inverted singlet - triplet excited states (DFIST) has been demonstrated; in other words, it has negative ΔE_{ST}.[3] The development of a stable and efficient OLED based on DFIST mechanism is expected.

(**1**)

(**2**)
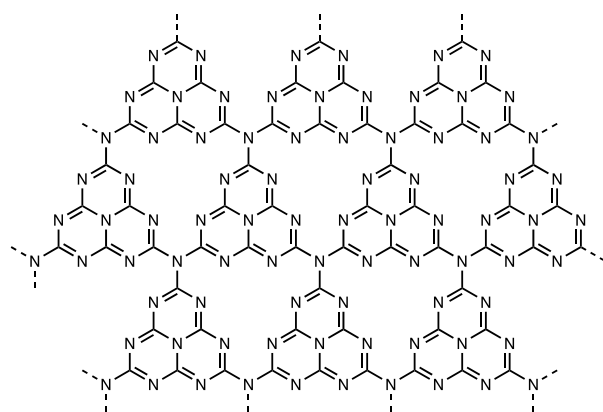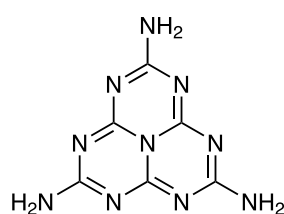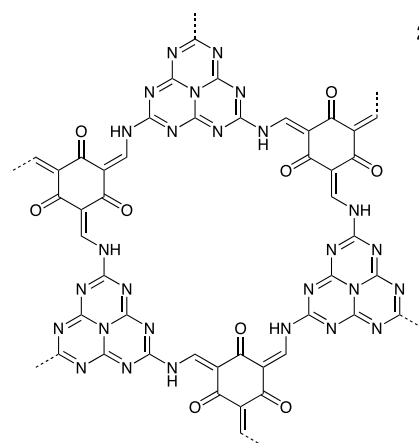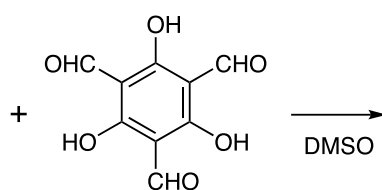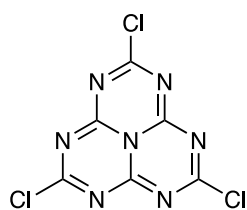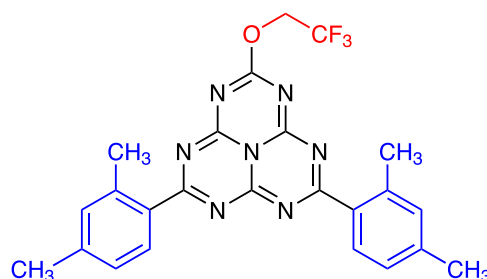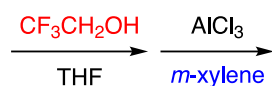
(**3**)

References

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Related Products

Melamine Monomer	25g	500g	T0337
2,4,6-Triformylphloroglucinol	200mg	1g	T3688

Measuring Relative Cell Number with Our ATP-Luciferase Assay Reagent

ATP-Luciferase Cell Viability Assay Solution (1.0mL×10) (1)
Product Number: A3495
1set

ATP-Luciferase Cell Viability Assay Solution (2)
Product Number: A3519
10mL

ATP-luciferase cell viability assay solution (**1**, **2**) takes advantage of the firefly luciferase reaction, in which luciferase is reacted in the presence of ATP, magnesium, and molecular oxygen using the luciferase enzyme to produce light at roughly 560 nm. Given that the strength of the resulting bioluminescent signal scales linearly with ATP concentration, and that cells contain a more-or-less constant amount of ATP throughout the cell cycle, this reaction can be used to measure not only ATP concentration but also relative cell number (linear range = 20 - 10000 cells).[1,2] Due to the chemiluminescent nature of the reaction, no special filters are required. Additionally, direct application to plated cells changes the color of the medium to yellow, aiding in correct application.

+Light (~560 nm)

$$\text{Luciferin} \xrightarrow[\substack{+ATP \\ +O_2}]{\text{Luciferase, } Mg^{2+}}$$
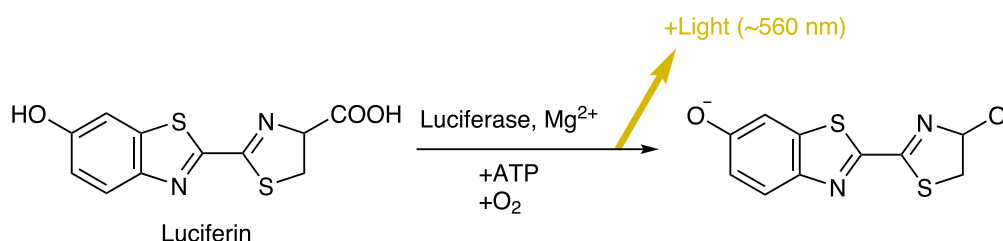
Luciferin

* **1** and **2** include luciferin, luciferase and Mg^{2+}.

References
1) A. Lundin, *Methods Enzymol*. **2000**, *305*, 346.
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Related Products

MTT Solution [for Cell proliferation assay] (1mL×5)		1set	M3353
Resazurin (Ready-to-use solution) [for Cell proliferation assay]		25mL	R0195
D-(-)-Luciferin [Chemiluminescence Reagent]	10mg	50mg	A5030

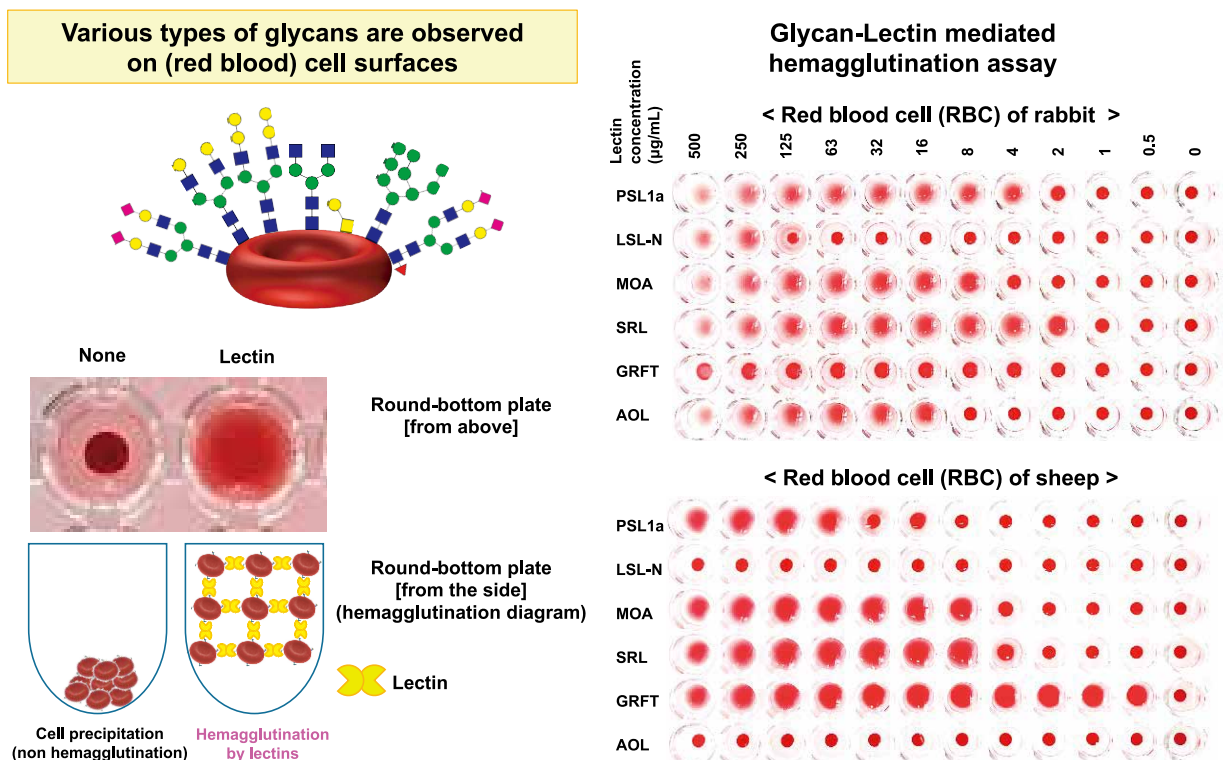# Hemagglutinin: Recombinant Lectins

Recombinant *Polyporus squamosus* lectin (= rPSL1a) expressed in *Escherichia coli* (1)

Product Number: **R0225**
1mL

Recombinant *Laetiporus sulphureus* lectin N-Terminal Domain (= rLSL-N) expressed in *Escherichia coli* (2)

Product Number: **R0226**
1mL

Recombinant *Marasmius oreades* agglutinin (= rMOA) expressed in *Escherichia coli* (3)

Product Number: **R0227**
1mL

Recombinant *Sclerotium rolfsiilectin* (= rSRL) expressed in *Escherichia coli* (4)

Product Number: **R0228**
1mL

Recombinant *Griffithsia* sp. lectin (= rGRFT) expressed in *Escherichia coli* (5)

Product Number: **R0229**
1mL

Lectin, Fucose specific from *Aspergillus oryzae* (= AOL) (5mg/mL, PBS pH6.5) (6)

Product Number: **L0169**
1mL

Lectin, well known as hemagglutinin, is widely distributed in plants, fungi like mushrooms, and animals. It has been used as a versatile tool in the glycoscience and cell research fields for a long time. TCI has a lineup of recombinant lectins whose genes have been cloned because they provide more stable quality rather than extract ones (**1**-**6**).

The glycan structure and expression level on the surface of erythrocytes are diverse depending on the animals (see below **figure**), and the profiling of hemagglutination caused by lectins is different. As other applications of lectin reagents, they have been utilized in lectin-staining of tissues, mitogens, cell profiling, prognosis with glyco-biomarker, signal induction of cells and so on.
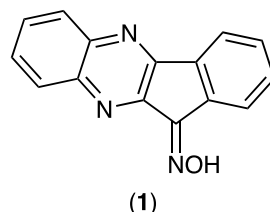
1-**5** and **6** were commercialized under licenses from National Institute of Advanced Industrial Science and Technology (AIST) and Gekkeikan Sake Company, LTD., respectively.

Various types of glycans are observed on (red blood) cell surfaces

None Lectin

Round-bottom plate [from above]

Round-bottom plate [from the side] (hemagglutination diagram)

Lectin

Cell precipitation (non hemagglutination)

Hemagglutination by lectins

Glycan-Lectin mediated hemagglutination assay

< Red blood cell (RBC) of rabbit >

< Red blood cell (RBC) of sheep >

c-Jun N-Terminal Kinase (JNK) Inhibitor

IQ-1 (1)

Product Number: **I1125**
10mg 50mg

(1)

IQ-1 (**1**) is an inhibitor of LPS-induced NF-κB/AP-1 activation with IC$_{50}$ of 2.3±0.41 µM,[1] and binds to the c-Jun N-terminal kinase (JNK) family with the affinities as shown in Table 1. According to molecular docking analysis, the amine moiety in the Asn152 side-chain of JNK3 forms hydrogen bonds with both nitrogen and oxygen atoms in the oxime moiety of **1**. **1** also inhibits pro-inflammatory cytokines* and nitric oxide production by human and murine monocyte/macrophages. In addition, **1** allows for the Wnt/β-catenin-driven long-term expansion of mouse ESCs and prevents spontaneous differentiation.[2]

*In reference 1, the authors show that interleukin (IL)-1α, IL-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon-γ, and granulocyte-macrophage colony-stimulating factor are inhibited by **1**.

Table 1. Binding affinity of **1** to each kinase[1]

Kinases	Kd (µM)
JNK1	0.24
JNK2	0.36
JNK3	0.10
CK1δ	0.38
PI3Kγ	0.47
MKNK2	0.92

References

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Activin Receptor-Like Kinase (ALK) 4/5/7 Inhibitor

A 83-01 (1)

Product Number: **A3324**
25mg

(**1**)
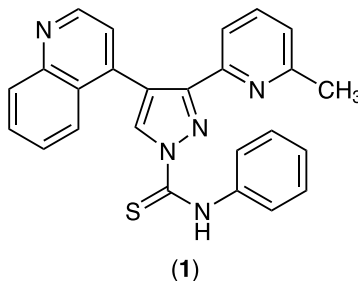
Activin receptor-like kinases (ALKs) are members of the type I activin receptor family, and belong to the serine/threonine kinase receptors of the transforming growth factor-β (TGF-β) superfamily.[1] A 83-01 (**1**) inhibits ALK 4/5/7 and the IC_{50} values are 45, 12, 7.5 nM, respectively. On the other hand, **1** shows weak inhibition for other ALKs.[2] It was reported that **1** could help stabilize rat induced pluripotent stem cells (iPSC) in combination with other chemicals[3] and that **1** converts epiblast stem cells (EpiSCs) to an earlier pluripotency state.[4] It was also reported that the combination of **1** and PD0325901 enables reprogramming of human primary somatic cells to iPSCs with exogenous expression of only OCT4.[5]

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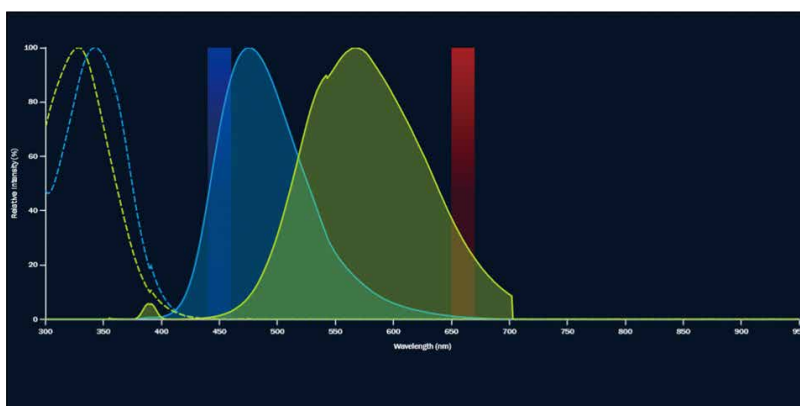
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