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Spirocyclopropylene bridged nucleic acid (scpBNA™) Phosphoramidites

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Introduction

2'-O,4'-C-Spirocyclopropylene bridged nucleic acid (scpBNA™)[1,2] is an analogue of 2'-O,4'-C-methylene bridged nucleic acid (2',4'-BNA/LNA), which is characterized by a cyclopropane ring at the 6'-position of its sugar moiety (Figure 1). The scpBNA™-modified oligonucleotides can show excellent duplex-forming ability with complementary single-stranded RNA. They can also exhibit highly increased enzymatic stability of the oligonucleotide as compared to that of the phosphorothioate-, 2'-methoxylethyl-RNA- (2'-MOE), and LNA-containing oligonucleotides.

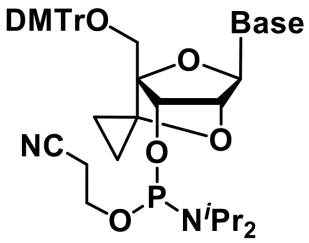

Figure 1. scpBNA™ monomer structure

1. scpBNA™ exhibits high binding affinity towards complementary strands with high RNA selectivity

Compared to the natural DNA-oligonucleotides, the scpBNA™-modified oligonucleotides show excellent binding affinity toward complementary ssRNA with a ΔT_m 4.8 – 5.1 °C per modification, which is comparable to those of LNA-modified oligonucleotides. As for the ssDNA complements, scpBNA™ displayed similar but slightly lower duplex-forming ability (ΔT_m 1 – 2 °C per mismatch) relative to the LNA counterpart, exhibiting its RNA selectivity. This reduced binding affinity of scpBNA™ toward ssDNA is possibly due to the conformational change of the sugar moiety upon incorporation of the highly strained cyclopropane unit. It is also possible that the bulky bridge structure of scpBNA™ produces large steric perturbations in the narrow minor groove of the duplex.

2. scpBNA™ modification is useful to reduce the number of phosphorothioate bonds of therapeutic oligonucleotides

One of the important applications of scpBNA™ is that it reduces the number of phosphorothioate bonds from an oligonucleotide without compromising metabolic stability. The phosphorothioate bonds are generally introduced to enhance the metabolic stability of an oligonucleotide. The phosphorothioate modification, however, is also known to cause toxicity due to their non-specific binding towards proteins. Therefore, reducing the number of phosphorothioates is beneficial to reduce toxicity of an oligonucleotide.[3] Since scpBNA™ can exhibit excellent nuclease resistance, it can be incorporated at the 3'-end or both the 3'-and 5'-ends of an oligonucleotide while removing phosphorothioate bonds and retaining metabolic stability. In a model sequence with scpBNA™ at the 3'-end without phosphorothioate bonds, the exonuclease resistance was found to be equal or slightly better than that of a single phosphorothioate modification. More than 80% of the scpBNA™-modified oligonucleotide remained intact even after 60 min of incubation with snake venom phosphodiesterase (Figure 2). Other modifications such as LNA and MOE at the same position without the phosphorothioate modification did not resist enzymatic digestion, and the oligonucleotides were rapidly degraded within 30 min.

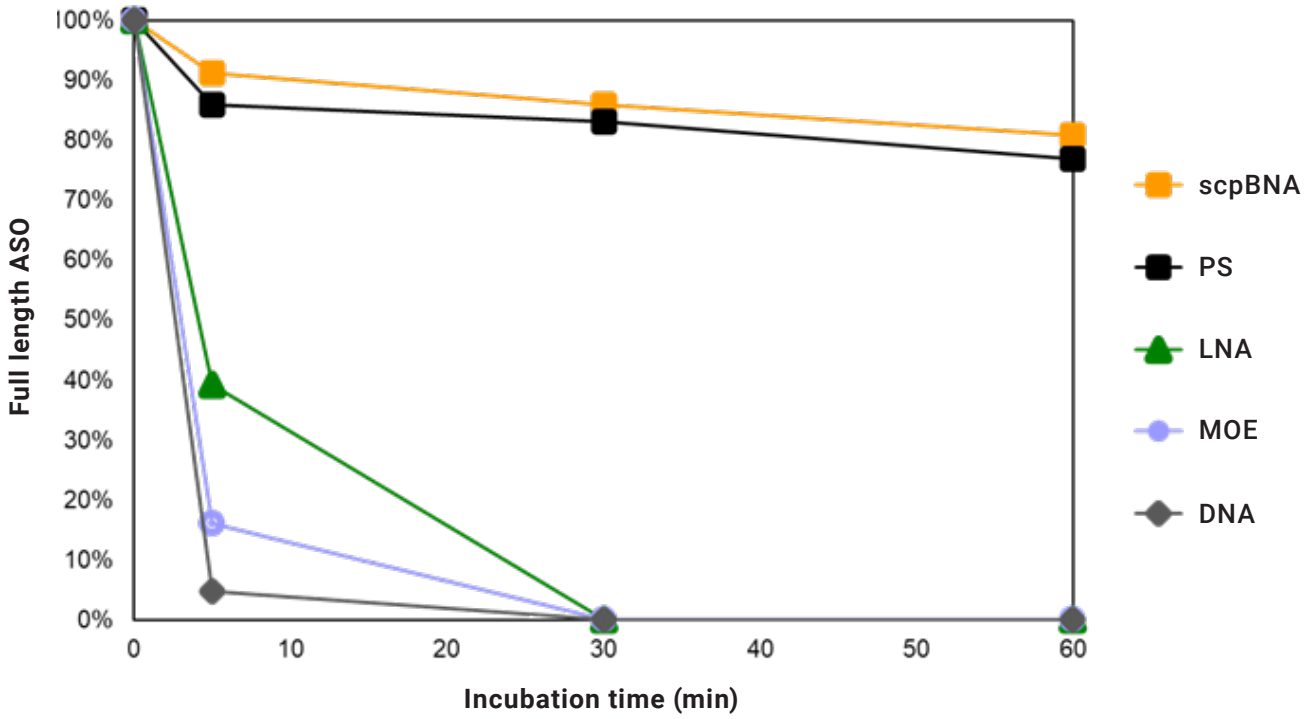

Figure 2. Exonuclease stability of oligonucleotide containing scpBNA™.

GCGTTTTTTGC**T**; where **T** = DNA, DNA-PS, LNA, MOE, scpBNA™

Conditions: Each oligonucleotide (1 nmol) was incubated with 200 µL of 0.004 unit Crotalus adamanteus venom phosphodiesterase (CAVP) in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$ at 37 °C for up to 60 min. The remaining intact oligonucleotide was calculated from the area under the peaks analyzed by HPLC.

3. scpBNA™ can be easily incorporated into an oligonucleotide

The scpBNA™-modified oligonucleotides can be synthesized in an automated DNA synthesizer following the standard phosphoramidite protocol. scpBNA™ is compatible with other modified monomers, modifiers, and labels. The phosphoramidites can be dissolved in anhydrous acetonitrile to standard concentrations, except for the 5-methylcytosine, which requires an acetonitrile-THF (3:1, v/v) or acetonitrile-dichloromethane (1:1, v/v) solution. Similar to LNA-phosphoramidites, scpBNA™-phosphoramidites are sterically hindered and require a prolonged coupling time of 8 – 12 min for their incorporation into an oligonucleotide. The solid-supported scpBNA™-oligonucleotides can be treated with concentrated ammonium hydroxide at 55 °C, 12 h for cleavage and deprotection. Although scpBNA™-modifications introduce hydrophobicity, scpBNA™-oligonucleotides with a few scpBNA™ monomers are soluble in water and can be purified and analyzed using the same methods used for other modified oligonucleotides.

References

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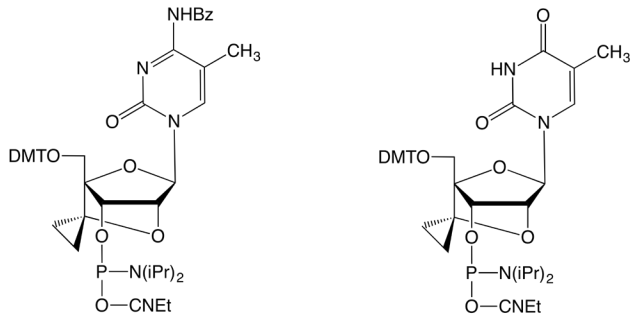
3. US9611479 – Crosslinked nucleoside and nucleotide.

NEW PRODUCTS

Spirocyclopropylene Bridged Nucleic Acids

Locked nucleic acid (LNA) is a popular bicyclic backbone that significantly increases duplex stability. A single substitution can increase melting temperatures by as much as 9 °C without sacrificing specificity,[1] and as such, LNA is usually one of the first modifications that come to mind for increasing target affinity regardless of the application. Since LNA was first described,[2,3] there have been many other similarly locked or bridged nucleic acid backbones that have been published such as S-constrained ethyl (cEt), ethylene-bridged nucleic acid (ENA) and more.[4] Another example of bridged nucleic acid is the tricyclic spirocyclopropylene-bridged nucleic acid (scpBNA™).[5]

As described in the preceding article, scpBNA exhibits high binding affinity and excellent metabolic stability that reduces the number of phosphorothioate bonds required in the oligonucleotide sequence. In collaboration with Summit Pharmaceuticals as well as Luxna Biotech, we are happy to share that we are introducing the scpBNA phosphoramidites of 5-methyl-C and T (Figure 1) to give customers the opportunity to use this unique backbone in their applications. In the future, there are also plans to introduce the purine phosphoramidites. Please stay tuned!



5-Me-C-scpBNA-CE Phosphoramidite

T-scpBNA-CE Phosphoramidite

Figure 1. scpBNA Pyrimidine Phosphoramidites

Item	Pack Size	Catalog No.
5-Me-C-scpBNA™-CE Phosphoramidite	100 µmol	10-2211-90
	0.25 g	10-2211-02
T-scpBNA™-CE Phosphoramidite	100 µmol	10-2230-90
	0.25 g	10-2230-02

IP Statement:

scpBNA™ is a trademark owned by Luxna Biotech Co., Ltd. (Luxna).

scpBNA™ phosphoramidites (Products) are protected by WO2015/125783A1, WO2020/204022A1 and their foreign patents/applications controlled by Luxna.

Glen Research sells the Products under contract with Summit Pharmaceuticals International Corporation, which has the license from Luxna. The resale of the Products is prohibited. The Products can be used only for experimental or research use, and the uses of the Products, including processed products thereof, for any purposes other than experimental or research use is prohibited. The purchase of the Products does not grant any right for the Patents, except for experimental or research use. For any other use, the customer must obtain a license from Luxna (sales@luxnabiotech.co.jp, +81-6-6170-1228).

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APPLICATION NOTE

Polymerase Chain Reaction (PCR) Optimization

Polymerase Chain Reaction (PCR) is a nucleic acid amplification technique that is used in various applications, such as diagnostics, forensics, agriculture, and basic research. PCR uses a polymerase to synthesize many, many copies of a target sequence over repeated cycles of denaturing and renaturing the sample (Figure 1). The PCR polymerase relies on DNA primers and deoxynucleotide triphosphates (dNTPs) to recognize the target nucleic acid sequence and generate the complementary strand to the template, respectively. PCR primer design is where Glen Research comes into play.

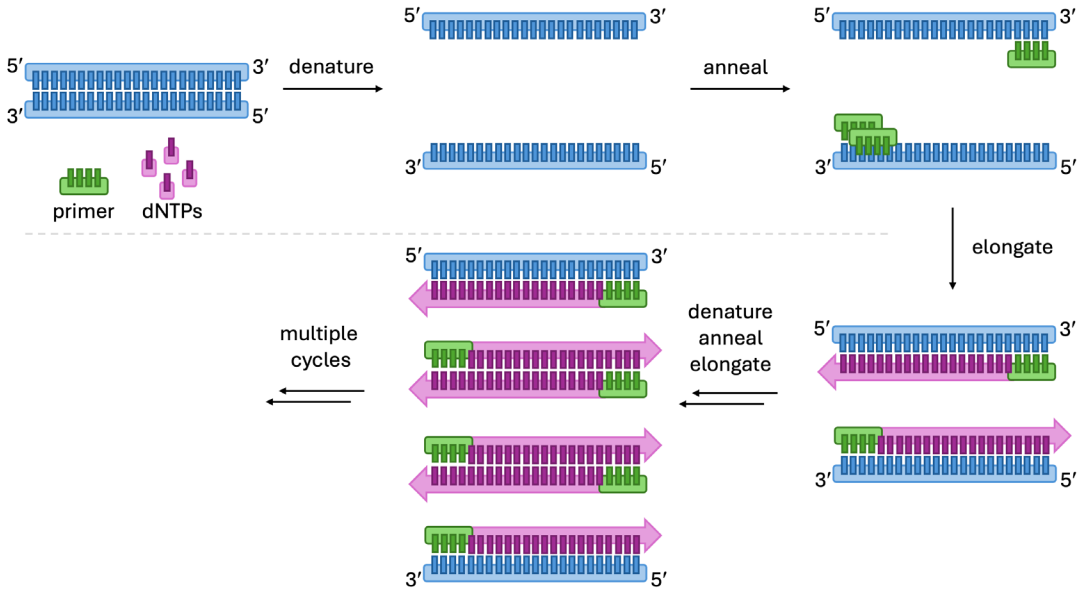

Figure 1. Illustration of PCR

PCR primers must be able to selectively recognize the target sequence to obtain optimal amplification. Obviously, the primer must have the right base composition to hybridize to the 3' region of the target for polymerase extension. In addition to the correct sequence, several base modifications have been evaluated in their ability to improve PCR efficiency.

When it comes to PCR modifications, some obvious backbones come to mind. Anything that can enhance hybridization and melting temperatures (T_m) has been used to enhance PCR efficiency. These include Locked Analog, 2'-OMe, and 2'-F. However, for the scope of this article, we want to focus on some of our less well-known modifications for PCR primers. It's worth pointing out that these modifications can also apply to quantitative PCR (qPCR) probes, which use the same strategy but contain a reporter molecule.

Self-Avoiding Molecular Recognition Systems (SAMRS)

Self-Avoiding Molecular Recognition Systems, or SAMRS, utilize modified bases that form more stable base pairs with the natural complement than with their SAMRS complement.[1] The SAMRS bases analogs include 2-Aminopurine (A*), N4-Et-dC (C*), dI (G*), and 2-Thio-dT (T*) (Figure 2). The A analog, 2-Aminopurine, preferentially binds natural thymidine over 2-Thio-dT due to one less hydrogen bond (Figure 3). Similarly, C* binds stronger to G than G* and G* binds stronger to C than C*, based on melting temperatures.[1]

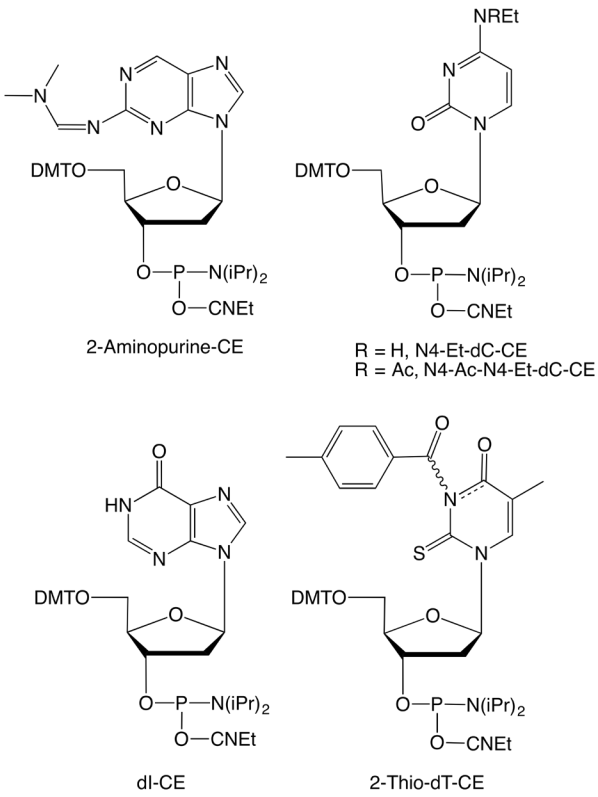

Figure 2. SAMRS base phosphoramidites

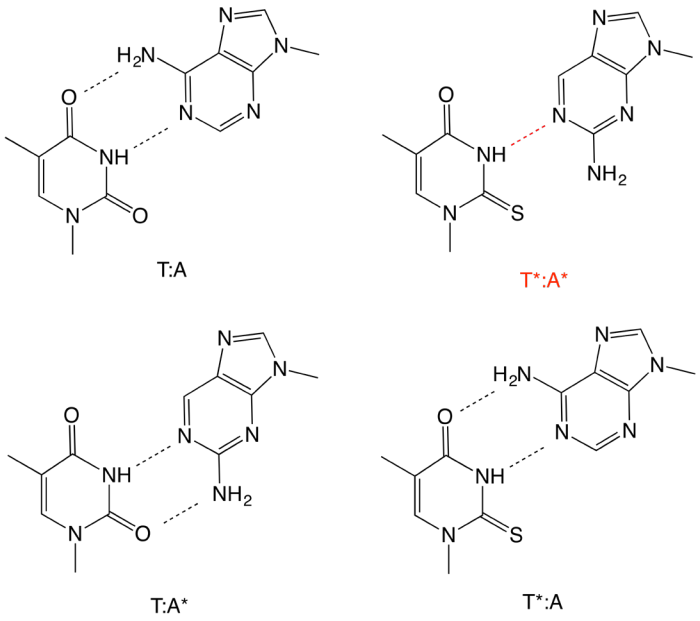

Figure 3. Base pairing motifs

This technology enables PCR primers using SAMRS bases to avoid undesired intra- and intermolecular hybridization between primers. This is particularly beneficial in multiplexed PCR techniques.[1, 2] Primer dimer amplification is often more efficient than the desired target, so primer-primer interactions should be avoided to prevent reagent consumption. Preventing the formation of primer dimers can improve discrimination between single nucleotide polymorphisms (SNPs).[2, 3]

PCR primers are not made fully with SAMRS modifications. The amount of SAMRS in each primer must be optimized to enhance target binding and discourage primer dimer formation. For this system to work best, primers should be at least 20 nucleotides long and contain 1-3 SAMRS modifications.[2] Generally, the least destabilizing monomer is T*, followed by A* and C*, and lastly G*. G-rich primers have lower amplification efficiency than other sequences. Ideally, the 3'-most base should be natural DNA.

Notably, SAMRS technology has also been used in isothermal amplification techniques, including loop-mediated isothermal amplification (LAMP) and recombinase-based isothermal amplification.[4, 5]

Other Base Modifications

In addition to SAMRS, other base modifications have been used to improve PCR efficiency. A PCR method, termed Snake, used forward primers with a 5'-flap bearing base modification that is recognized by the 5'-nuclease activity of the popular Taq DNA polymerase.[6] The 5'-flap sequence enables a stem-loop structure in the Snake PCR amplicon, which improved signal productivity and SNP discrimination. Certain positions of the 5'-flap sequence were substituted with 2-Amino-dA and/or 5-propynyl dU (pdU) to enhance binding (Figure 4). Each 2-Amino-dA increases Tm by 3.0 °C while a pdU addition yields a +1.7 °C per substitution.[7] Similar to SAMRS, the amount of these modifications must be optimized to prevent too stable complexes from forming and some destabilizing modifications (dI and dU) may be added to regulate this.

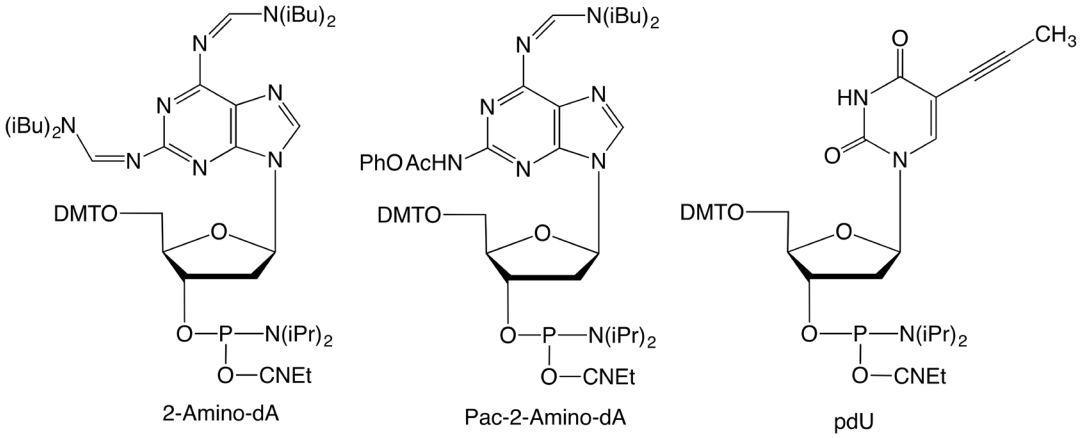

Figure 4. Base modifications for Snake primer 5'-flap

Non-base Modifications

Lastly, the addition of a simple 5'-thiol to the end of the PCR has been shown to enhance PCR sensitivity and yield.[8] The proposed mechanism for this enhancement is due to the interaction between the primer and the PCR enzymes, namely the polymerase.

The major benefit of this approach is that it does not need significant optimization to identify how many and which base modifications are needed to improve PCR outcomes. This technique required contaminant-free reactions as the presence of other proteins in the sample inhibited PCR with thiol-modified primers.

Technique	Product	Catalog No.
SAMRS	2-Aminopurine-CE Phosphoramidite	10-1046
	N4-Et-dC-CE Phosphoramidite	10-1068
	N4-Ac-N4-Et-dC-CE Phosphoramidite	10-1513
	dI-CE Phosphoramidite	10-1040
	2-Thio-dT-CE Phosphoramidite	10-1036
Snake	2-Amino-dA-CE Phosphoramidite	10-1085
	Pac-2-Amino-dA-CE Phosphoramidite	10-1585
	pdU-CE Phosphoramidite	10-1054
Thiol	Thiol-Modifier C6 S-S	10-1936

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NEW PRODUCTS

Catalyst-free Click Reactions

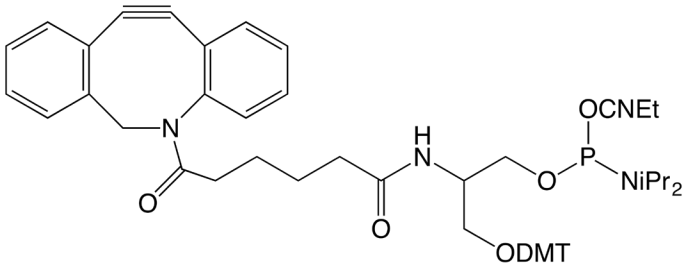
The Copper(I) Catalyzed Azide-Alkyne Cycloaddition (CuAAC) is the most recognizable example of click reactions.[1] However, the presence of copper may not be favorable in oligonucleotide research or biological samples due to its known toxicity. To circumvent this issue, strain-assisted labels have been developed to facilitate click reactions without requiring any catalyst. We are happy to introduce two new products towards this effort: 3'-DBCO-Serinol CPG (20-2998) and 5'-TCO C6 Phosphoramidite (10-1943).

3'-DBCO-Serinol CPG

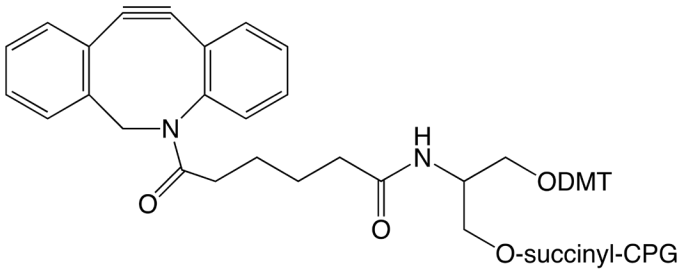
Dibenzocyclooctyne (DBCO) is no stranger to the Glen Research catalog and is a popular choice when looking for copper-free click chemistry between an alkyne and an azide. This is referred to as Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC). Copper-free click chemistry offers several advantages to users, including stability in solution on the synthesizer, stability to standard deprotection conditions, and efficiency in click reaction performance.[3]

With this new addition, we now offer five DBCO products: 5'-DBCO-TEG Phosphoramidite (10-1941), DBCO-dT-CE Phosphoramidite (10-1539), DBCO-Serinol Phosphoramidite (10-1998), DBCO-sulfo-NHS Ester (50-1941), and our new 3'-DBCO-Serinol CPG. Various applications that have used our DBCO products have been described previously.[2]

3'-DBCO-Serinol CPG is a solid support version of our DBCO-Serinol Phosphoramidite (Figure 1). The introduction of this 3'-DBCO-Serinol CPG allows researchers to place the DBCO modifier at the 3'-end of their oligonucleotide without the need for multiple synthesis reagents. Previously, to incorporate DBCO at the 3'-end, one needed to use the phosphoramidite with universal support or the NHS ester with a 3'-amino modifier.



DBCO-Serinol Phosphoramidite



3'-DBCO-Serinol CPG

Figure 1. 3'-DBCO-Serinol Products

Recommended Protocols for 3'-DBCO-Serinol CPG

DBCO is susceptible to damage from standard iodine oxidation and our mild oxidizer, CSO in anhydrous acetonitrile, is required. This is especially crucial since the DBCO is at the 3'-end and must survive several rounds of coupling and oxidation. 3'-DBCO-Serinol CPG is compatible with standard phosphoramidites, including dmf-dG but not iBu-dG. DBCO-oligonucleotides are stable to ammonium hydroxide deprotection for 2 h at 65 °C or overnight at room temperature. If iBu-dG is used, deprotection with AMA for 2 h at room temperature may slightly degrade the cyclooctyne.[3] UltraMild deprotection conditions are also compatible with 3'-DBCO-Serinol CPG.

5'-TCO C6 Phosphoramidite

Bioorthogonal reactions used in imaging, diagnostics, and therapy applications require rapid kinetics and high specificity to optimize the time required and amount of labeling agent needed to achieve high coupling yields.[4] TCO phosphoramidite is a new addition to our line-up and offers our customers another approach to conjugation chemistry (Figure 2). TCO is a strained alkene that reacts

with tetrazines in an inverse electron-demand Diels-Alder cycloaddition (iedDA) reaction. TCO-tetrazine ligation is ultrafast, selective, and works well in mild conditions (e.g. room temperature, neutral pH, and aqueous solutions).[5, 6] DBCO and TCO reactions occur very rapidly in practice. In the literature, it has been reported that DBCO derivatives typically display second-order rate constants in the 1-2 $M^{-1}s^{-1}$ range while TCO-based click reactions exhibit very high bimolecular rate constants (10^3-10^5 $M^{-1}s^{-1}$).[4-6] Rate constants depend on both components that are part of the click reaction and conditions of the reaction. For example, in a separate study, DBCO and TCO exhibited more comparable rate constants, 0.1-1 $M^{-1}s^{-1}$.[7]

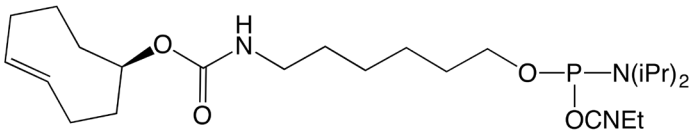


Figure 2. 5'-TCO C6 Phosphoramidite

In the iedDA cycloaddition, an electron-poor diene, commonly 1,2,4,5-Tetrazine, reacts with an electron-rich dienophile, such as TCO, via a normal [4+2] Diels-Alder cycloaddition and then immediately undergoes a subsequent, irreversible retro Diels-Alder step, which releases a molecule of nitrogen (Figure 3).[4-7] The reaction can be observed spectroscopically by the disappearance of the visible absorption band around 510-550 nm, which comes from the tetrazine chromophore, as long as the tetrazine label does not interfere with this absorption range.[4]

Applications

Certain tetrazine fluorophores that are only fluorogenic upon cycloaddition improve signal-to-noise ratio for fluorescence microscopy.[7] The tetrazine-TCO cycloaddition has been used to fluorescently label live cells,[4] radiolabel biomolecules,[5] and in triggered conjugation reactions, activate the tetrazine moiety enzymatically.[7, 8]

Specificity is obviously important when it comes to labeling DNA probes. Selectively coupling different labels site-specifically to a single oligonucleotide is commonly achieved through two different approaches: (1) two different conjugation reactions are carried out sequentially with or without purification steps or (2) protecting groups must be removed between successive conjugation steps to direct reactivity. The tetrazine-TCO cycloaddition is fully orthogonal to copper-catalyzed azide-alkyne reaction and both conjugations can take place in a one-pot reaction without cross reactivity.[9] The iedDA cycloaddition was even shown to be orthogonal to the strain-promoted alkyne-azide cycloaddition (SPAAC) between an azide and DBCO, albeit requiring a more careful choice of reactants based on their reactivity to prevent interference.[10] This can be particularly useful in designing oligonucleotide FRET probes.[7, 9-10]

The tetrazine-TCO conjugation strategy was also employed to ligate modified oligonucleotides into a fully functional sgRNA for CRISPR. The oligonucleotides contained nucleobase modifications, including N1-methyladenosine (m^1A), N6-methyladenosine (m^6A), and 4-thiouridine (s^4U), all modifications that we also offer, to evaluate the impact of RNA modifications of CRSIPR activity.[11]

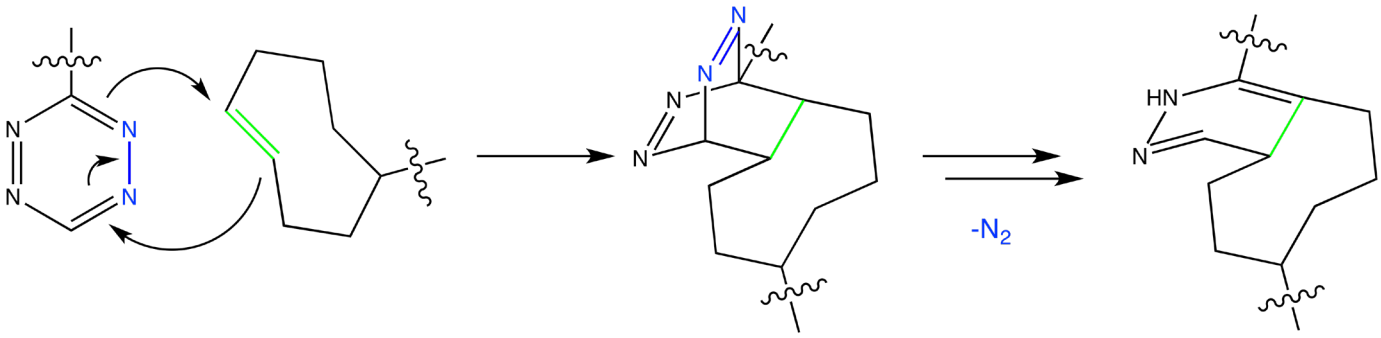


Figure 3. Tetrazine Diels-Alder cycloaddition with trans-cyclooctene

Recommended Protocols for 5'-TCO C6 Phosphoramidite

One major benefit to TCO over DBCO-containing oligonucleotides is its relative compatibility with iodine oxidation. Prolonged exposure to iodine does induce some degradation of TCO. However, this is a 5'-modifier and must only be capable of surviving a single oxidation cycle. In our hands, we found either CSO or iodine oxidation (0.02 M) yielded equivalent coupling efficiency and oligonucleotide purity using 5'-TCO C6 Phosphoramidite. A 10 min coupling time is recommended and the TCO modification is compatible with standard deprotection conditions.

TCO reacts readily with modified tetrazines in organic solvents or aqueous solutions. It is worth mentioning that highly substituted and sterically hindered tetrazines may have difficulty reacting with TCO.[5] In our hands, we coupled FAM-tetrazine to a 5'-TCO oligonucleotide using the following procedure.

For a 0.2 µmole synthesis of a TCO-modified oligonucleotide:

1. Dissolve oligonucleotide in 500 µL of 0.1 M potassium phosphate buffer (pH 6.9-7.2).
2. Dissolve 10 eq of tetrazine label in 250 µL DMSO.
3. Add tetrazine solution to oligonucleotide solution.
4. Agitate the mixture and incubate at room temperature for 60 min.
5. Separate oligo-conjugate from salts and excess label by size exclusion on a Glen Gel-Pak™ desalting column or equivalent.

Summary

These new additions benefit researchers taking advantage of copper-free click reactions for their oligonucleotide conjugations. DBCO and TCO react readily and selectively with their respective substrates. The biocompatibility of the two types of click reactions (SPAAC and iedDA) has the potential to lead to research where multiple click reactions take place on the same oligonucleotide. We're excited to see where our customers can go with this.

Item	Pack Size	Catalog No.
3'-DBCO-Serinol CPG	0.1 g	20-2998-01
	1.0 g	20-2998-10
1 µmol columns	Pack of 4	20-2998-41
0.2 µmol columns	Pack of 4	20-2998-42
10 µmol columns	Pack of 1	20-2998-13
15 µmol columns	Pack of 1	20-2998-14
0.5M CSO in Anhydrous Acetontrile	200 mL	40-4632-52
	450 mL	40-4632-57
	1000 mL	40-4632-71
	2000 mL	40-4632-62
5'-TCO C6 Phosphoramidite	50 µmol	10-1943-95
	100 µmol	10-1943-90
	0.25 g	10-1943-02

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NEW PRODUCT

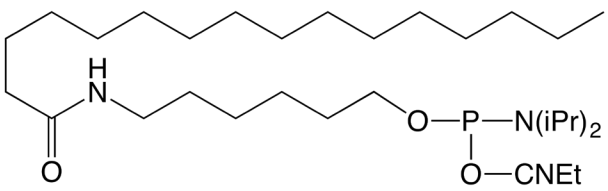
Palmitate Serinol Phosphoramidite

Our serinol product line has been developed over the past few years through a strong collaboration with Glen Research and Nelson Biotechnologies.[1]

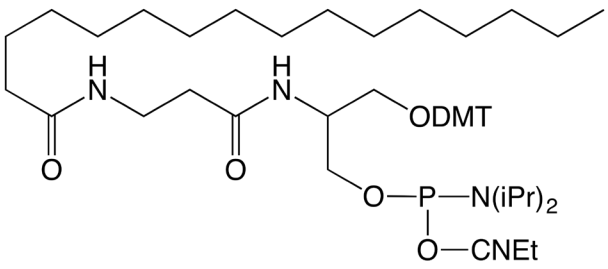
Currently, Glen Research offers a diverse range of serinol phosphoramidites and solid supports, including:

- Five click chemistry products for oligonucleotide conjugation, including our latest addition, 3'-DBCO-Serinol CPG, introduced in this Glen Report
- Three biotin products
- Two dithiol products
- Two amino modifiers
- One fluorescein reagent

In this article, we are pleased to introduce Palmitate Serinol Phosphoramidite (Figure 1) to our product portfolio. This is the first serinol member to join our line of hydrophobic modifications, like fatty acids and cholesterol, which enhance cellular uptake and activity in various tissues. It is a serinol-based version of our Palmitate Phosphoramidite (10-1978).[2]

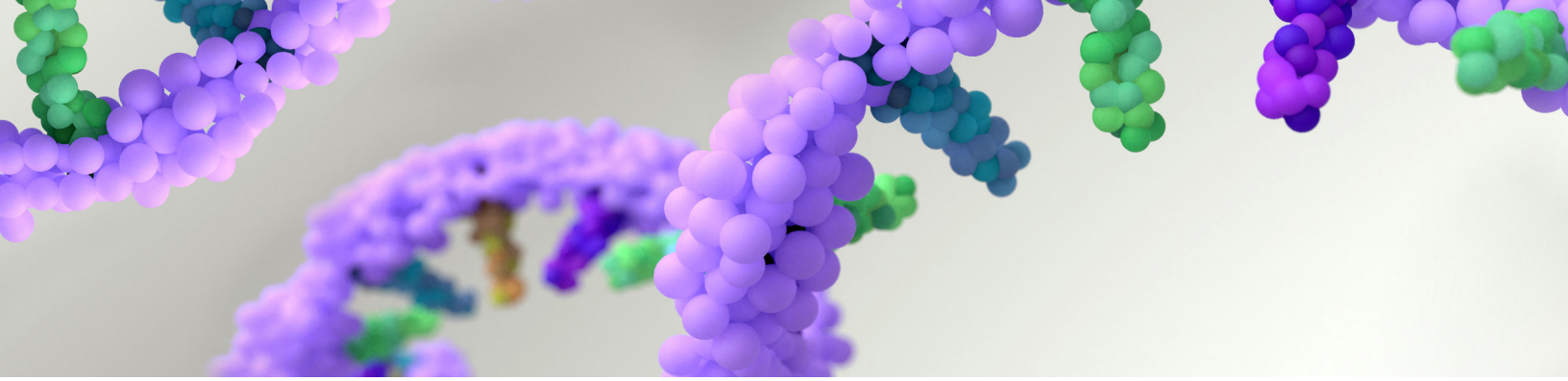


Palmitate Phosphoramidite



Palmitate Serinol Phosphoramidite

Figure 1. Palmitate Structures



The addition of the DMT group in the serinol version allows for the incorporation of the modification internally, or at the 3'-end of the oligonucleotide by using one of our universal supports. The DMT group also facilitates multiple incorporations of the modification.

Palmitate-modification of oligonucleotides continues to be popular. Since the introduction of Palmitate Phosphoramidite in May 2022,[2] ongoing research has focused on improving cellular uptake and delivery of palmitate-oligonucleotide conjugates to specific tissues. This research has resulted in many new scientific publications. We have selected two of these publications to highlight. One approach improved siRNA delivery by attaching palmitate to the 5'-end of the sense strands targeting HPRT1 in HeLa cells. Additionally, the effectiveness of these palmitate-conjugated siRNAs was enhanced by using a synthetic sphingolipid analog, which inhibits endocytic recycling.[3]

In the second approach, a palmitate phosphoramidite monomer was synthesized and incorporated multiple times into antisense oligonucleotides (ASOs) using standard phosphoramidite chemistry. The addition of multiple palmitates increased the lipophilicity of the ASOs, which in turn enhanced their cellular uptake compared to ASOs without palmitate. The position of palmitate insertion within the ASO sequence also affected cellular uptake. Specifically, inserting two palmitates at the 5'-end of the ASO improved cellular uptake while placing one palmitate at the 5'-end and another at the 3'-end resulted in reduced cellular uptake compared to ASOs without fatty acids.[4]

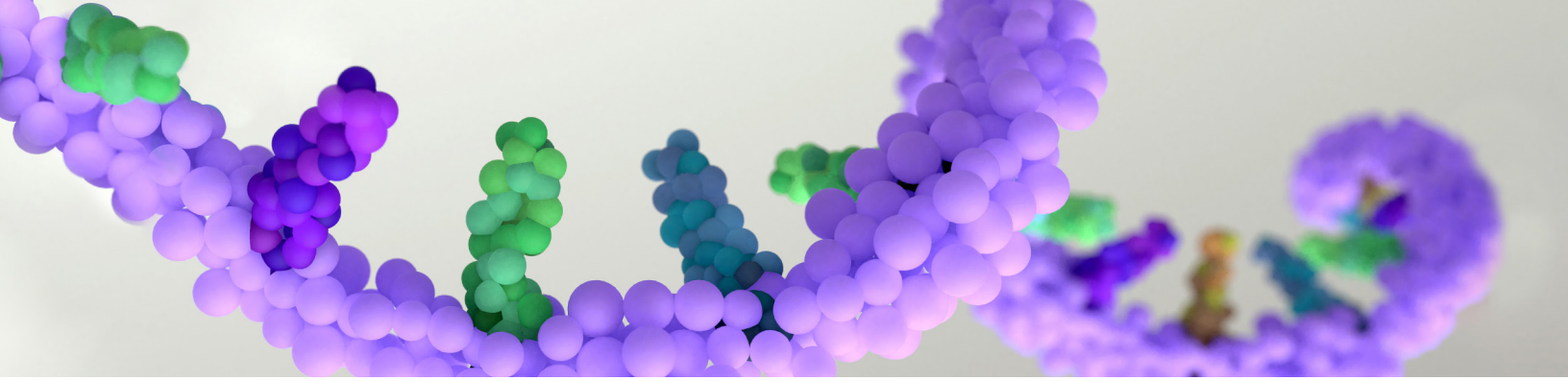
Use of Palmitate Serinol Phosphoramidite

The Palmitate Serinol Phosphoramidite has an advantage over the Palmitate Phosphoramidite in that it dissolves fully in acetonitrile, whereas the latter requires a mixture of acetonitrile and dichloromethane (1:3). Like the Palmitate Phosphoramidite, a 6-minute coupling time is recommended for the serinol version. No additional changes are necessary beyond the standard deprotection and cleavage methods required for the nucleobases.

Item	Pack Size	Catalog No.
Palmitate Phosphoramidite	50 µmol	10-1978-95
	100 µmol	10-1978-90
	0.25 g	10-1978-02
Palmitate Serinol Phosphoramidite	50 µmol	10-1990-95
	100 µmol	10-1990-90
	0.25 g	10-1990-02

References

1. *The Glen Report*, 2013, **25.1**, 4-5.
2. *The Glen Report*, 2022, **34.1**, 9-10.
3. B.T. Finicle, et al., *Nucleic Acids Res*, 2023, **51**, 1583-1599.
4. Y. Tanaka, et al., *Bioorg Med Chem*, 2023, **81**, 117192.



TECHNICAL NOTE

Preventing Detritylation During RNA Deprotection

In our last issue, we reported on coupling efficiency using various monomers (DNA vs. TOM vs. TBDMS).[1] As expected, TOM performed better than TBDMS, which has a more significant impact for longer oligonucleotides. During our experiments, we observed some unavoidable loss of the DMT group while drying the oligonucleotide down between global deprotection/cleavage and 2'-desilylation. This is not ideal, especially for those relying on DMT-ON purification for their RNAs. To follow up on this, we sought a method to minimize detritylation during the workup of RNA.

It turns out, we didn't have to look too far. About 15 years ago, we developed a method to prevent detritylation caused by drying down and tested this on trityl-protected amino-modifiers.[2] In DNA oligonucleotides, a nonvolatile base, such as TRIS, is added prior to drying down to prevent detritylation. However, the presence of TRIS inhibits the subsequent 2'-desilylation reaction with TEA•3HF for RNA.

The obvious next step was to test this on our RNA sequence:

5'-UUG UUC UUA UUG UUC UUA UU-3'

After synthesis on our ABI394 with TBDMS phosphoramidites, we used the following procedure:

- Deprotect oligonucleotide as necessary according to nucleobase protecting groups and support.
- Add 45 mg Tris base/mL.
- Desalt on Glen-Pak™ DNA Cartridge.
  - Condition cartridge with 0.5 mL ACN, followed by 1 mL 2M TEAA.
  - Load oligonucleotide drop-wise onto Glen-Pak DNA Cartridge.
  - Rinse with 2 mL RNase-free water.
  - Rinse twice with 2 mL 0.5M aqueous sodium hydroxide.*
  - Rinse with 2 mL RNAse-free water.
  - Elute with 1 mL 75% ACN/RNase-free water.
- Dry down.
- 2'-Desilylation.
  - Dissolve in DMSO (115 µL) and warm in a 65 °C water bath until fully dissolved.
  - Add TEA (60 µL) and TEA•3HF (75 µL).
  - Heat in a 65 °C water bath for 2.5 h.
  - Quench with RNA quenching buffer (750 µL).
- Desalt on Glen Gel-Pak™ following recommended conditions and elute crude oligonucleotide in 0.1M RNase-free TEAA.

The resulting oligonucleotide was analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC).

* In general, NaOH treatment is rather harsh for RNA oligonucleotides and we don't typically see recommendations like this. Due to the short contact time while on the column, the sodium hydroxide did not negatively impact the RNA integrity, confirmed by the chromatograms. However, it is worth pointing out that this step should be done quickly. While we did not test other possibilities, alternative sodium-based solutions may work just as well to convert the oligonucleotide to the sodium salt on the Glen-Pak cartridge (e.g. 0.1M NaOH, 0.5M $NaCO_3$, or 0.5M $NaHCO_3$).

Following this method, we observed much better results by HPLC (Figure 1). In our standard method (green), the RNA was dried down prior to 2'-desilylation and desalting, and we saw failure sequences and a DMT-off full-length peak that eluted with the failures, according to MS analysis. However, when the RNA was converted to the nonvolatile sodium salt (gray) prior to drying down, we still saw the failure sequences but the DMT-off full length was significantly reduced. The effective average coupling efficiency was improved by 0.5%.

An alternative comparison method is to look at the area under the main peak. Converting the oligonucleotide to the sodium salt increased the percent area of the main peak by almost 10%. Explicitly, the main peak in the standard method totaled 77% and the main peak in the method evaluated in this article was 85%.

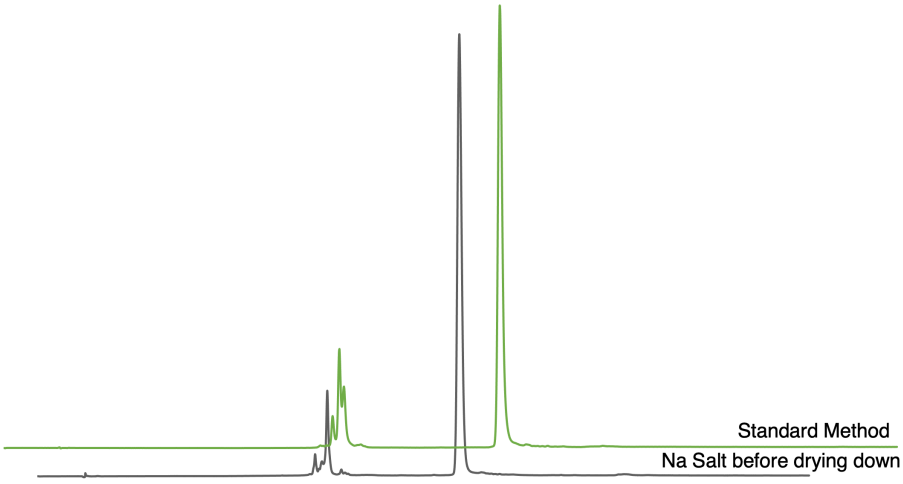


Figure 1. HPLC of 20 mer RNA oligonucleotides from TBDMS monomers with standard method (green) or converting oligonucleotide to the sodium salt before drying down (gray).

While we did not test this on TOM oligonucleotides, we previously observed the same loss of DMT using TOM phosphoramidites and expect this method to equally improve the overall coupling efficiency and yield of the DMT-ON full-length species.

References

1. *The Glen Report*, 2024, **36.1**, 9-13.
2. *The Glen Report*, 2009, **21.1**, 16.

Notes

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Technical Snippets

What is the oligonucleotide synthesis scale for the Glen Gel-Pak™ desalting columns?

The oligonucleotide synthesis scale for the Glen Gel-Pak™ desalting columns is based on the oligonucleotide loading volume (mL), rather than the oligonucleotide synthesis scale (µmol). However, these two metrics are equivalent. Therefore:

- 0.2 Column = 0.2 µmol
- 1.0 Column = 1.0 µmol
- 2.5 Column = 2.5 µmol

Relevant products:

Glen Gel-Pak™ 1.0 Desalting Column (61-5010)

Glen Gel-Pak™ 2.5 Desalting Column (61-5025)

Glen Gel-Pak™ 0.2 Desalting Column (61-5002)

How does the HAA buffer compare to the TEAA buffer in my HPLC?

A solution of HAA (0.1M) behaves differently than TEAA at the same concentration. HAA is comprised of hexyl ammonium acetate and TEAA is made up of triethylammonium acetate. Hexylamine has a 6-carbon chain and the longer alkyl group interacts more strongly with reverse phase resins. As a result, HAA provides higher resolving power and is often preferred for DMT-OFF purifications.

HAA has a different and higher absorbance profile at 254 nm than TEAA. This is normal and should not interfere with your analysis. We have observed anywhere from 0-0.035 AU at 254 nm.

Relevant products:

2.0M Hexylammonium Acetate, HPLC grade, pH=7 (60-4210)

2.0M Triethylamine Acetate, HPLC grade, pH=7 (60-4110)

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