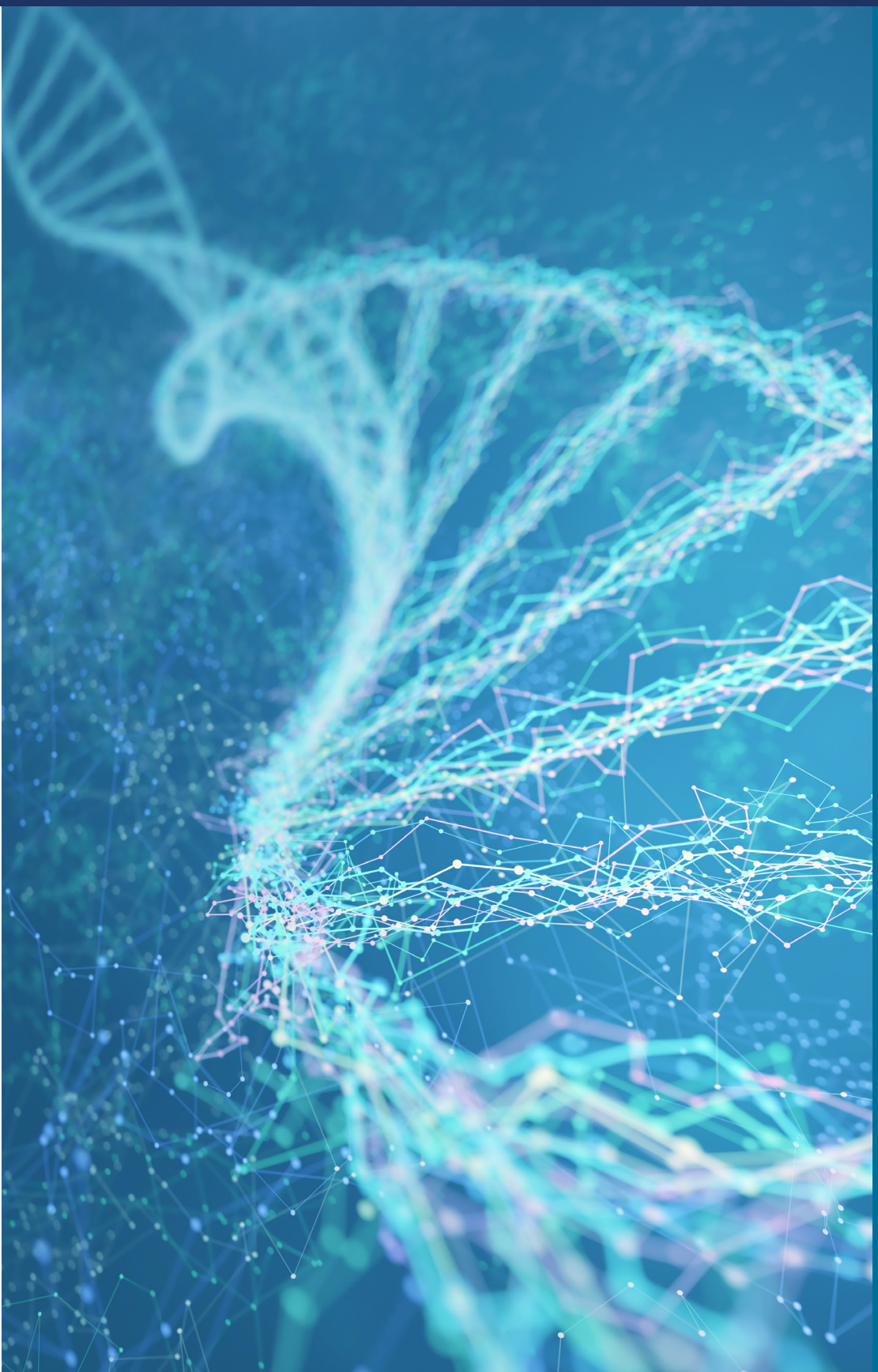
The **Glen Report**

Volume 35.2 | December 2023



See technical snippets on back cover

GLEN RESEARCH
part of Maravai LifeSciences

CleanCap® M6

Author:
Chunping Xu, Ph.D., TriLink BioTechnologies

Introduction:

The m7G (N7-Methyl guanosine) cap structure is present in all eukaryotic mRNAs and plays an essential role in the cap-dependent initiation of protein synthesis. Another role of the mRNA cap is to stabilize mRNA and prevent it from 5' to 3' exonuclease cleavage. Various dinucleotide and trinucleotide cap analogs have been reported in the literature so far for co-transcriptional mRNA capping.[1,2] Compared to dinucleotide cap analogs such as ARCA and related derivatives, TriLink's CleanCap AG (m7GpppAG) can enable more efficient mRNA capping since T7 RNA polymerase initiates mRNA synthesis on CleanCap AG by hybridizing two nucleotides (AG) to the DNA template. In contrast, ARCA initiates mRNA synthesis by binding a single nucleotide (G) to the DNA template, and there is competition from GTP, which will result in lower capping efficiency (Figure 1).

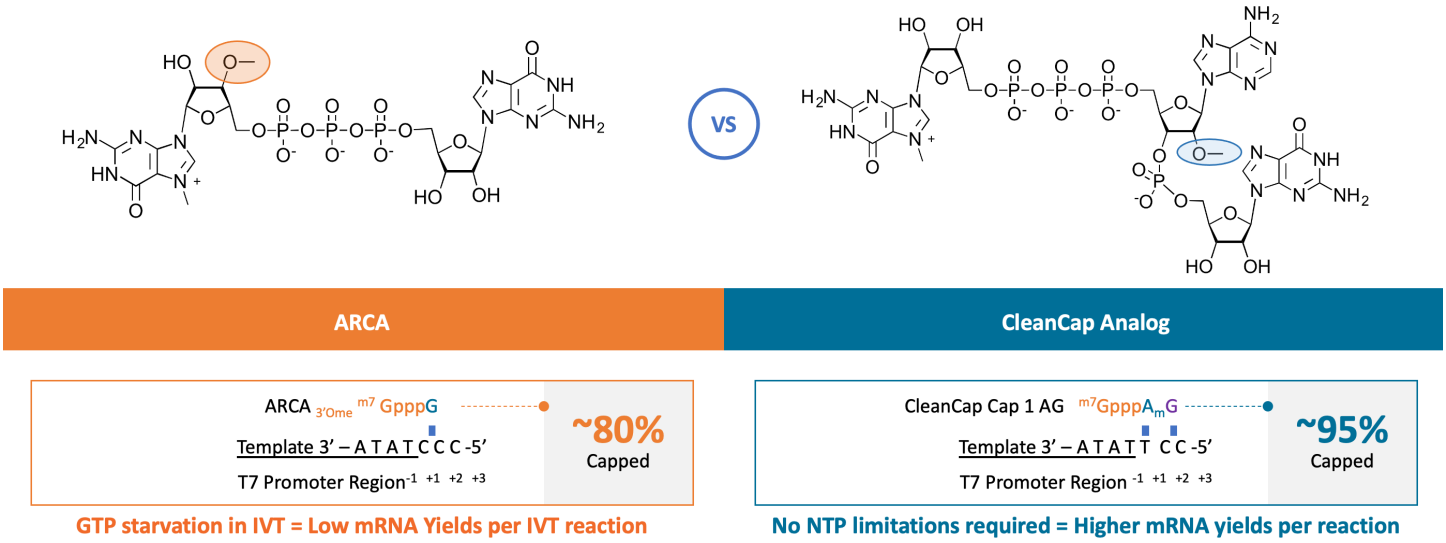
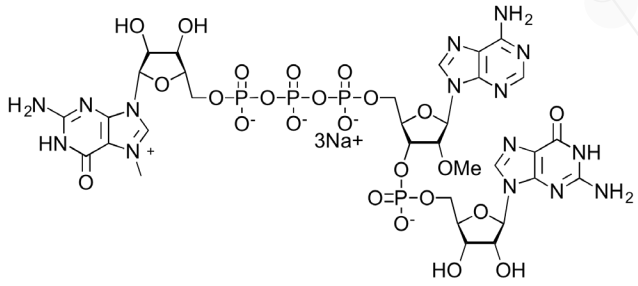


Figure 1. ARCA versus CleanCap reagent

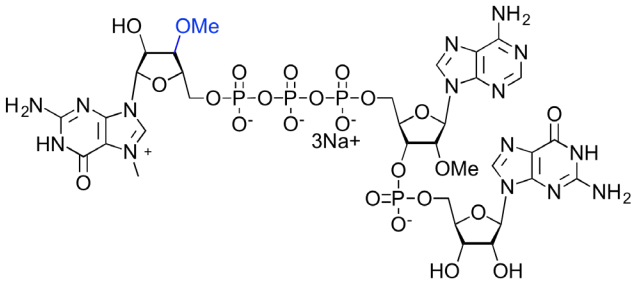
N6-Methyl adenosine (^{m6}A) is the most abundant internal modification in mammalian mRNA and modulates numerous cellular processes such as nuclear export, mRNA splicing, polyadenylation, stability and translation. N6, 2'-O-Dimethyl adenosine ($^{m6}A_m$) is an extension of the 5' cap that can occur on the first transcribed nucleotide if it is an adenosine. The addition of $^{m6}A_m$ to 5'-UTR has been shown to play a role in both mRNA translation and stability. Even though most translation is achieved through recognition of the 5' cap, for select mRNA, the ^{m6}A modification has been shown to facilitate cap-independent translation during stress.[3-6]

CleanCap M6 development:

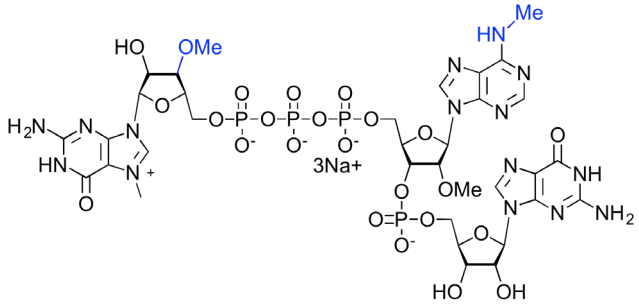
In our efforts to find more efficient cap analogs, we wanted to introduce the $^{m6}A_m$ modification into CleanCap AG and study the effect of the $^{m6}A_m$ on mRNA translation. Using our standard mRNA *in vitro* transcription (IVT) conditions, this cap results in low yield and low capping efficiency. As a result, new IVT conditions had to be developed.[7]



CleanCap AG



CleanCap AG 3'OMe



CleanCap M6

Figure 2. CleanCap AG Analogs

CleanCap AG 3'OMe and CleanCap M6 (Figure 2) have been incorporated into Wasabi mRNA (modified GFP) and tested in a cell-based assay for protein expression in HeLa cells. Significant enhancement in protein expression was observed. Protein expression of mWasabi by *in vitro* translation was increased by more than two-fold when CleanCap M6 was utilized, relative to that of CleanCap AG 3'-OMe (Figure 3). The three versions of CleanCap analogs (CleanCap AG, CleanCap AG 3'OMe and CleanCap M6, Figure 2) were also evaluated *in vivo*. When incorporated into FLuc mRNA and tested in mice for protein expression, we observed a significant increase in protein translation going from CleanCap AG to CleanCap AG 3'OMe and also from CleanCap AG 3'OMe to CleanCap M6 (Figures 4A and 4B). CleanCap M6 incorporated into other mRNA constructs, such as EPO, also show enhancement in protein expression.

CleanCap M6 has been shown to give the best protein expression in all the cap analogs we have tested so far (Figures 4A and 4B). The impact of $^{m6}A_m$ on protein translation has been reported in the literature by other groups.[3-6] Although, the exact mechanism is not clear yet, we believe the $^{m6}A_m$ nucleotide hinders the decapping process and stabilizes mRNA in cells, which will lead to protein translation improvement.

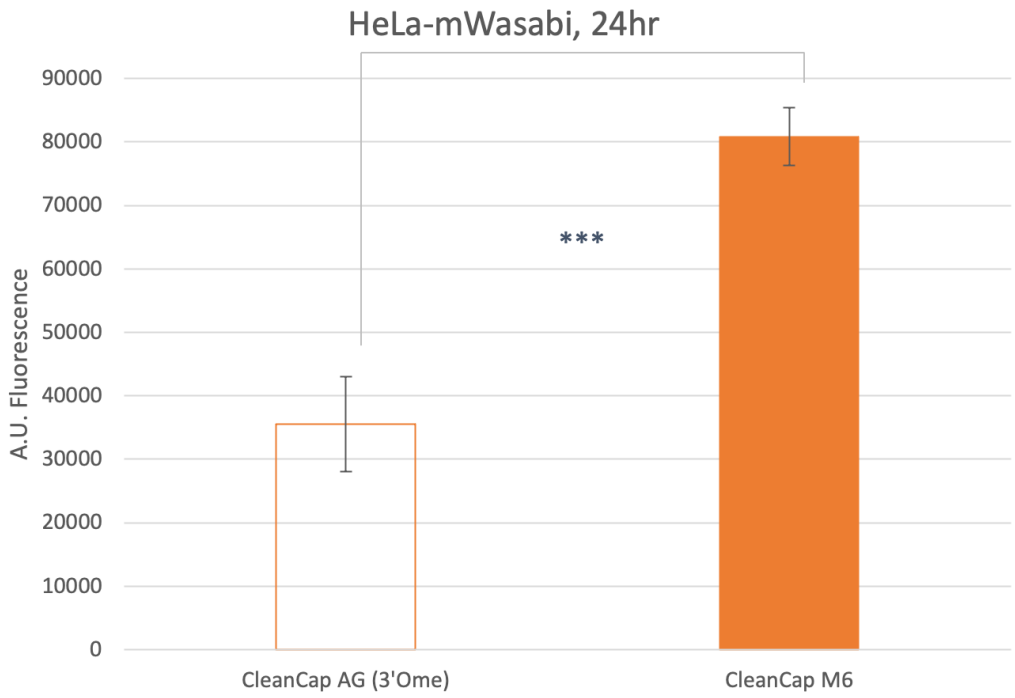


Figure 3: CleanCap M6 modification promotes *in vitro* protein translation (mWasabi). All groups are significantly different, technical replicates *** p < 0.001, t-tailed T-test. Error bars are standard error of mean. n = 5/group. HeLa cells were transfected with mRNA coding mWasabi, capped with either CleanCap AG 3'OMe or CleanCap M6, and fluorescence was measured at 24 hours.

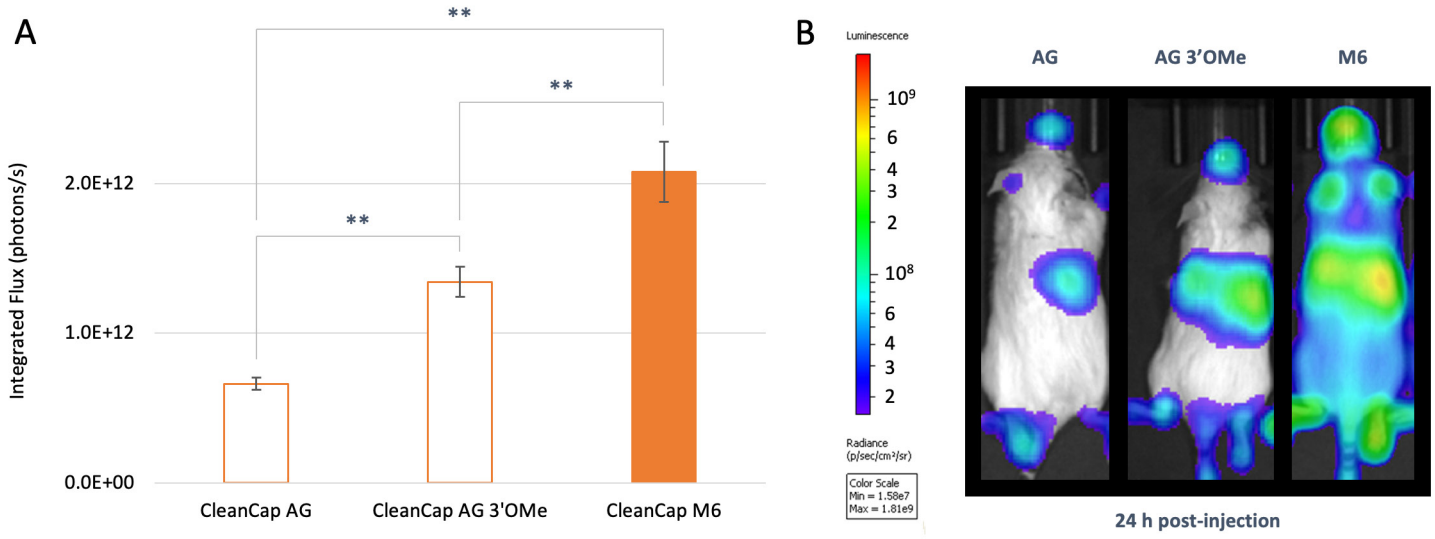
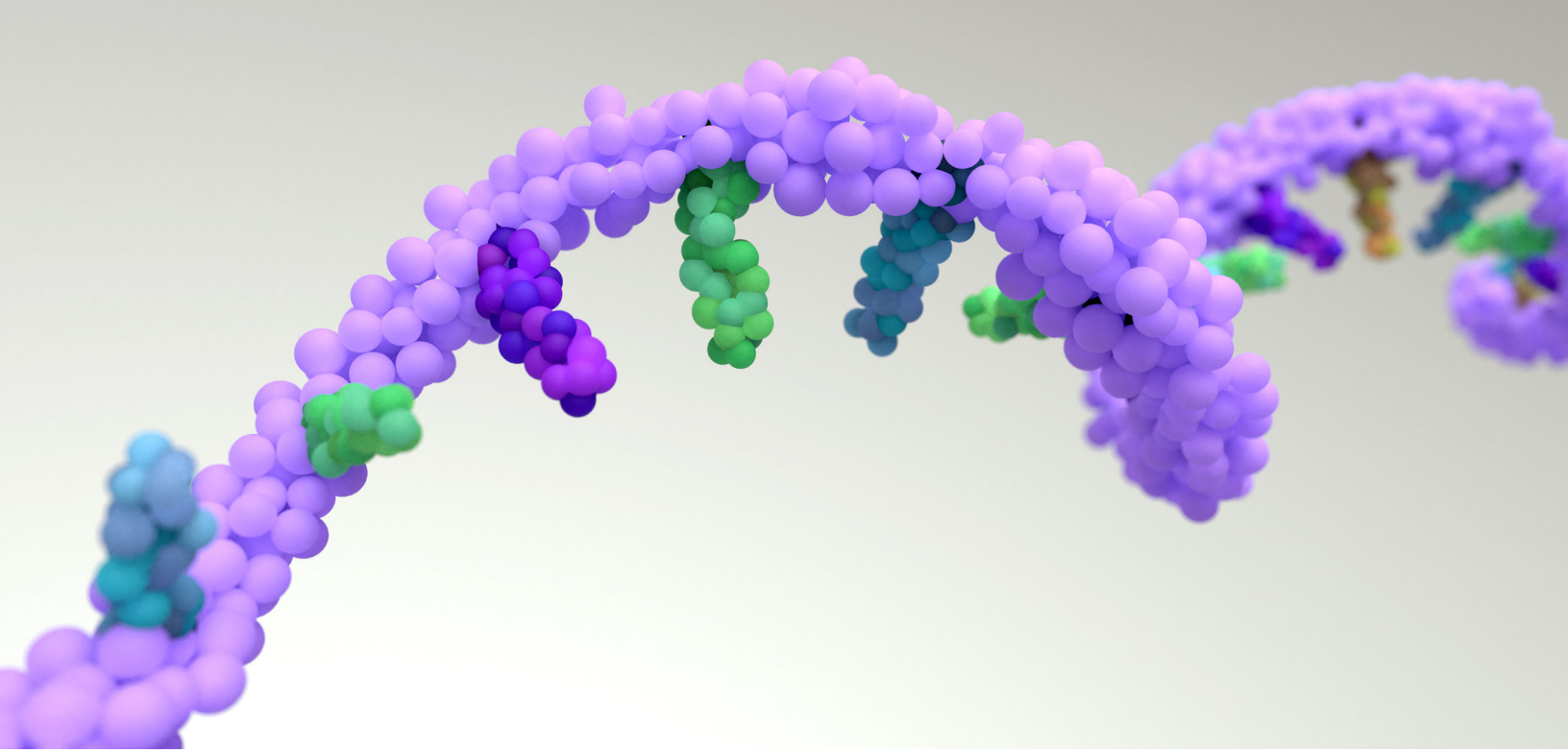


Figure 4: CleanCap M6 mRNA promotes *in vivo* Luciferase translation in mice. Performance of FLuc mRNA in an LNP-formulated, tail vein delivered mouse model, 1 mg/kg dose per group. Luciferase activity, as photons per second, was measured after luciferin injection. The difference between the groups was cap analog structure only. All other variables were controlled. A. All groups are significantly different, ** p < 0.01, one-way ANOVA. Error bars are standard error of mean. n = 5/group. B. Whole body luminescence.



Applications:

With TriLink's streamlined mRNA co-transcriptional capping process, CleanCap M6 can be widely used in mRNA capping to facilitate mRNA vaccine or therapeutic development. After we launched this new product in May, many customers have already tested it. CleanCap M6 can be used to replace the current enzymatic or ARCA capping technology due to the benefits associated with CleanCap M6:

1. Enhanced protein expression
2. High capping efficiency
3. Low levels of double-stranded RNA
4. Faster turn-around time compared to both ARCA and enzymatic capping
5. Lower capping cost at a larger scale compared to enzymatic capping

For therapeutic development, due to the higher potency, CleanCap M6 capped mRNA can be used at a lower dose to minimize an immune response.

References:

1. Muttach, F., et al. (2017). "Synthetic mRNA capping." Beilstein J Org Chem **13**: 2819-2832.
2. Kowalska, J., et al. (2008). "Synthesis and characterization of mRNA cap analogs containing phosphorothioate substitutions that bind tightly to eIF4E and are resistant to the decapping pyrophosphatase DcpS." RNA **14**(6): 1119-1131.
3. Mauer, J., et al. (2017). "Reversible methylation of m(6)A(m) in the 5' cap controls mRNA stability." Nature **541**(7637): 371-375.
4. Liu, A. and X. Wang (2022). "The Pivotal Role of Chemical Modifications in mRNA Therapeutics." Front Cell Dev Biol **10**: 901510.
5. Catacalos, C., et al. (2022). "Epitranscriptomics in parasitic protists: Role of RNA chemical modifications in posttranscriptional gene regulation." PLoS Pathog **18**(12): e1010972.
6. Sikorski, P. J., et al. (2020). "The identity and methylation status of the first transcribed nucleotide in eukaryotic mRNA 5' cap modulates protein expression in living cells." Nucleic Acids Res **48**(4): 1607-1626.
7. CleanCap Reagent M6-(N-7453), https://www.trilinkbiotech.com/cleancap-reagent-m6.html.

NEW PRODUCT

2'-OMe-N6-Me-A-CE ($^{m6}A_m$) Phosphoramidite

The mRNA cap is a highly methylated 5'-modification that enhances the efficiency of mRNA translation. In nature, the mRNA cap initiates translation into proteins, protects mRNA from degradation, recruits protein complexes for processing mechanisms, and marks cellular mRNA to avoid immune system responses.[1] Multiple cap structures exist based on the amount of methylation present (Figure 1). Cap 0 contains an N7-G cap connected by a 5'-5'-triphosphate bridge and initiates translation and protects RNA from degradation. Additional methylation at the 2'-position of the first transcribed nucleotide yields Cap 1. Cap 2 is present when the second transcribed nucleotide is methylated at the 2'- hydroxyl group. We would like to thank Dr. Chunping Xu and the TriLink team for their work and overview on mRNA capping reagents and their applications in mRNA studies.[2]

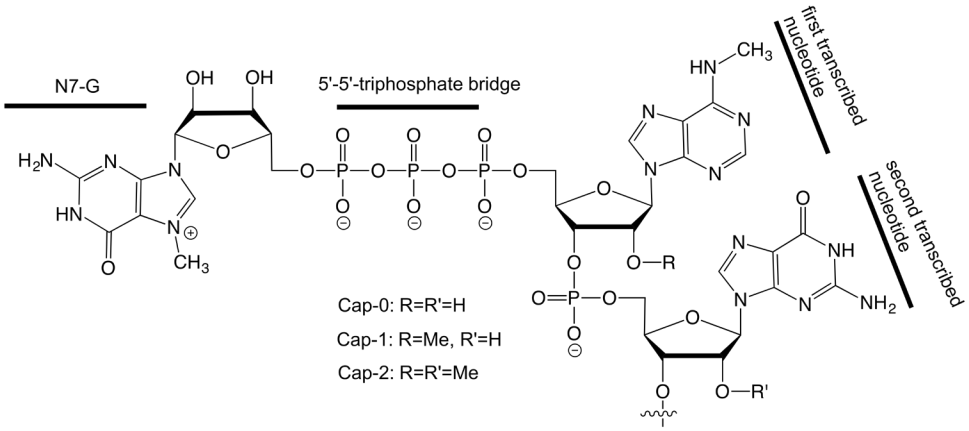
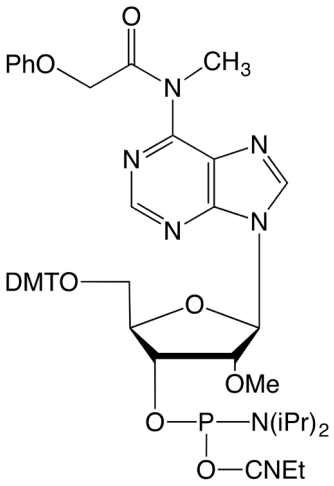


Figure 1. mRNA 5'-cap structures. In this rendering, the first transcribed nucleoside (adenosine) is further methylated to show $^{m6}A_m$.

To further support this research, we are introducing a 2'-OMe-N6-Me-A-CE ($^{m6}A_m$) phosphoramidite. This modification is crucial to the development of novel cap structures bearing the $^{m6}A_m$N dimer (in the case of CleanCap M6, N = G). In addition to its importance in the mRNA cap, $^{m6}A_m$ has also been implicated in gene regulation.[3] Several sequencing methods have been developed to detect and quantify $^{m6}A_m$ levels. Results of $^{m6}A_m$ mapping experiments have led to somewhat controversial findings. For example, the presence of $^{m6}A_m$ enhances or reduces mRNA stability in certain genes. It's likely that the effect of $^{m6}A_m$ on stability depends on outside factors, such as tissue. In addition, internal $^{m6}A_m$ modification was found to negatively impact splicing regulation.

In a recent study, it was shown that downregulation of an $^{m6}A_m$ writer, PCIF1, promoted cancer cell proliferation. In contrast, overexpression of the methyltransferase responsible for $^{m6}A_m$ induces apoptosis.[4]

In our hands, the $^{m6}A_m$ phosphoramidite is easy to use in oligonucleotide synthesis and deprotection. For coupling, a 6-minute coupling time was required using standard tetrazole activator. This monomer is protected with phenoxyacetyl (Pac) to minimize potential branching. If combined with other UltraMild phosphoramidites, use UltraMild Cap Mix A (40-4210-xx/40-4212-xx) to avoid exchange of the iPr-Pac group on G with acetyl. The $^{m6}A_m$ can be deprotected using UltraMild conditions (0.05M Potassium Carbonate in Methanol, 4 h, RT or 30% Ammonium Hydroxide, 2 h, RT). This modification is also compatible with standard deprotection in AMA.



Item	Pack Size	Catalog No.
2'-OMe-N6-Me-A-CE Phosphoramidite	50 µmol	10-3105-95
	100 µmol	10-3105-90
	0.25 g	10-3105-02

References

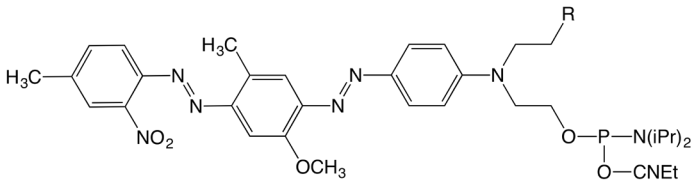
1. A. Galloway, and V.H. Cowling, *Biochim Biophys Acta Gene Regul Mech*, 2019, **1862**, 270-279.
2. *The Glen Report*, 2023, **35.2**, 1-5.
3. B. Cesaro, M. Tarullo, and A. Fatica, *Int J Mol Sci*, 2023, **24**, 2277-2290.
4. S. Gao, et al., *Biochim Biophys Acta Mol Basis Dis*, 2022, **1868**, 166498.

NEW PRODUCTS

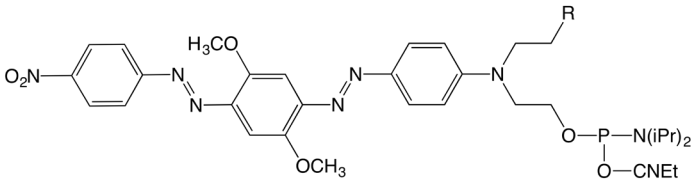
BHQ-1 Phosphoramidite and BHQ-2 Phosphoramidite

Glen Research introduced many versions of the Black Hole Quencher™ (BHQ) dyes in 2002.[1] Among those products are the BHQ-1 and BHQ-2 5'-Phosphoramidites, dT, and the 3' CPG versions. In this article, we are introducing two new non-nucleoside versions: BHQ-1 and BHQ-2 Phosphoramidites. The only difference in the structure between the 5'-BHQ-1 and 2 phosphoramidites and the structure of BHQ-1 and 2 phosphoramidites is the addition of the O-4,4'-dimethoxytrityl "O-DMT" in the new versions (Figure 1). The addition of the DMT group will allow the incorporation of these modifications internally or at the 3'-end of the oligonucleotide by using one of our universal supports.

Like other dark quenchers, such as Dabcyl, BHQ products consist of a polyaromatic-azo backbone (Ph-N=N-Ph). This allows them to absorb a broader range of wavelengths in the visible spectrum, resulting in the absorption of many colors. Alteration of the electron-withdrawing group (e.g., NO_2) and the electron-donating group (e.g., Me) in the aromatic rings of the BHQ's structure provides many BHQs, such as BHQ-1 and BHQ-2 with shifted spectral properties (Table 1 and Figure 2).[1]



BHQ-1



BHQ-2

R=H=5'-BHQ Phosphoramidite
R=ODMT=BHQ Phosphoramidtie

Figure 1. Structures of the BHQ-1 and BHQ-2 Phosphoramidites

Table 1. Physical properties of BHQ-1 and BHQ-2

BHQ Quencher	λ_{max} (nm)	E_{260} (L/mol•cm)	E_{max} (L/mol•cm)
BHQ-1	534	8,000	34,000
BHQ-2	579	8,000	38,000

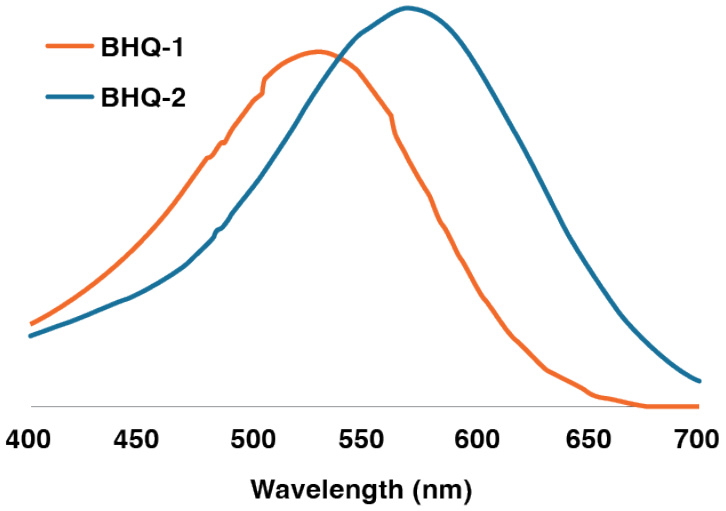


Figure 2. Visible spectra of BHQ-1 and BHQ-2

When first introduced to the market in 2000, researchers believed that BHQ probes operated only via the FRET quenching mechanism in which the excited fluorophore "donor" transfers energy to a quencher "acceptor" when they are both in close proximity (~20-100 Å). However, in 2002, Marras, Kramer, and Tyagi[2] concluded that the quenching in BHQ dual-labeled probes occurs through a combination of FRET and static quenching mechanisms. The affinity of the fluorophore for BHQs in the probe leads to the two binding each other, producing a non-fluorescent intramolecular dimer in the static quenching mechanism.

BHQs Applications

In addition to their broad absorption spectra, BHQs have large extinction coefficients, excellent coupling efficiencies, are compatible with ammonium hydroxide deprotection, and are completely non-fluorescent quenchers. These factors contributed to the popularity of BHQs in building dual probes with several fluorophores. As a result, BHQs were used extensively during the COVID-19 pandemic. Most of the World Health Organization's COVID-19 testing protocols used BHQs, as they became favorable quenchers for quantitative real-time PCR (qPCR).[3] Moreover, most of the manufacturers in the molecular diagnostic field use BHQ in their assays.

BHQs have not only played an essential role during the COVID-19 pandemic in diagnosing patients with COVID-19, but they are now playing another important role in the early detection of disease outbreaks. Non-fluorescent quenchers, such as BHQs, are recommended in the recent Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines for hydrolysis probes used in Digital PCR (dPCR).[4] Currently, the BHQs are used with dPCR to check the concentration of the SARS-CoV-2 in wastewater.

Use of BHQ-1 and BHQ-2 Phosphoramidites

Much like the 5'-BHQ-1 and 2 phosphoramidites, no changes are needed from the standard coupling method recommended by the synthesizer manufacturer. The deprotection also follows the standard method recommended by synthesizer manufacturers, except that the use of methylamine in ultrafast deprotection protocols will result in degradation to the dye and is not recommended in the case of the BHQ-2 phosphoramidite. The optimal deprotection conditions for probes that contain BHQ-1 or BHQ-2 and other dyes in the dual-labeled probes can be found in our recent Glen Report (34.2).[5]

"Black Hole Quencher" is a trademark of Biosearch Technologies, Inc., Novato, CA.

Item	Pack Size	Catalog No.
BHQ-1 Phosphoramidite	50 µmol	10-1961-95
	100 µmol	10-1961-90
	0.25 g	10-1961-02
BHQ-2 Phosphoramidite	50 µmol	10-1962-95
	100 µmol	10-1962-90
	0.25 g	10-1962-02

References

1. *The Glen Report*, 2004, **17.1**, 4-6.
2. S.A. Marras, F.R. Kramer, and S. Tyagi, *Nucleic Acids Res*, 2002, **30**, e122.
3. S.A. Bustin, and T. Nolan, *Int J Mol Sci*, 2020, **21**, 3004-3012.
4. dMIQE Group and J.F. Huggett, *Clin Chem*, 2020, **66**, 1012-1029.
5. *The Glen Report*, 2022, **34.2**, 1-2.

APPLICATION NOTE

Degenerate Oligonucleotides

A degenerate oligonucleotide is a mixture of sequences that differ in one or more positions. If there is a single degenerate position that contains only purines, the mixture contains roughly equal amounts of two sequences (2^1). If there are ten completely random degenerate positions, the mixture contains over a million sequences (4^{10}). To describe these degenerate positions, one of eleven possible single-letter, mixed-base codes is used. The standard nomenclature for these degenerate positions is defined by the International Union of Pure and Applied Chemistry (IUPAC, Table 1). Pu**R**ine-only positions would be denoted as "R" while completely random (a**N**y) positions would be denoted as "N". Degenerate oligonucleotides serve many special applications, and the role these degenerate oligonucleotides play in several applications will be discussed briefly below.

1. Degenerate PCR

For certain PCR amplifications, the target template sequence can either be unknown or vary (e.g., SNPs, codon degeneracy, different alleles, etc.). For the former, conserved sequences from related species can be used for primer design while for the latter, primer design would try to accommodate all possibilities. In either scenario, degenerate primers can be used. The primers are designed to minimize degeneracy, and the degenerate positions are usually placed away from the 3'-end to promote proper annealing. As an example, an 18 nt primer corresponding to Lys-Ile-Asp-Trp-Phe-Trp would have a degeneracy of 24 to cover all possible codons (Table 2). Degenerate PCR can be very effective. In one multiplex PCR assay investigation, primers of up to 64-fold degeneracy were used to detect a panel of bacteria and viruses, including some that had significant sequence diversity.[1]

2. Whole Genome Amplification

Whole genome amplification is a method for indiscriminately amplifying the whole genome rather than small, distinct sections. The method is typically employed in situations where nanograms of material are amplified into micrograms of material for subsequent manipulations. Two popular approaches that use degenerate primers to accomplish this are **P**rimer **E**xtension **P**reamplification (PEP)[2] and **D**egenerate **O**ligonucleotide **P**rimed PCR (DOP-PCR).[3] The primers used in these two methods are significantly more degenerate than those employed in degenerate PCR. PEP uses completely random 15 nt primers for amplification (Table 2). The one billion primer mixture (4^{15}) binds at random locations on the DNA template, and annealing temperatures are initially very low to promote hybridization. For a single human cell, it has been estimated that at least 78% of the genomic material has been replicated at least 30 times over a 50-cycle procedure.[2] For DOP-PCR, a partially degenerate primer is used instead (Table 2). Six completely degenerate positions are flanked by ten defined nucleotides on the 5'-end and six defined nucleotides on the 3'-end. Like PEP, the initial annealing temperature is very low, and the 4,096 primers (4^6) allow species-independent general DNA amplification. As expected, both techniques generate smears of DNA fragments when analyzed by agarose gel electrophoresis.

3. Mutagenesis

Site-directed mutagenesis is a method of introducing specific changes to plasmid DNA, and degenerate oligonucleotides are one way of generating libraries for this process.[4] Input oligonucleotides contain a central random codon region with constant 5'- and 3'-ends of 15 nt each that are complementary to regions adjacent to the target plasmid site. While the use of completely degenerate regions (NNN) is possible, this kind of approach has drawbacks due not only to the degeneracy of the genetic code but also to the presence of three stop codons. Instead, one can use NNS codons (20 amino acids and only one stop codon) or other more biased degenerate codons such as TDK (hydrophobic amino acids only) or VVC (hydrophilic amino acids only). Fifteen or more codons can be randomized in this manner at a time. For more control over codon composition in libraries, Glen Research also offers trimer phosphoramidites.[5]

4. SELEX

SELEX (**S**ystematic **E**volution of **L**igands by **EX**ponential enrichment) is an *in vitro* selection method for aptamers and nucleic acid enzymes that begin with a very large random pool of sequences.[6-8] These sequences typically have a 30-80 nt central random region and primer binding regions to facilitate PCR amplification (Table 2). Libraries can consist of up to 10^{15} different sequences (~1 nmol), and multiple rounds of selective partitioning and amplification whittles the original library down to a considerably smaller number of sequences that bind to a desired target or catalyze a certain reaction.

5. Next Generation Sequencing

The arrival of next generation sequencing has allowed researchers to obtain massive sets of data, currently up to billions of reads at a time. Looking through these sizable data sets, one may want to quantify individual oligonucleotides based on the number of reads. However, since most, if not all, sequencing methods require amplification, and such amplification is not uniform, this does not work well. To address this, **U**nique **M**olecular **I**dentifiers (UMIs) can be used.[9] A 6-12 random nucleotide region is appended to every original strand of template as a molecular barcode to change every original strand into a unique sequence. The UMI, which is sequenced along with the target, becomes part of the data for processing. Instead of counting reads of a sequence, the count measures how many different UMIs exist for that same sequence.

Oligonucleotide Synthesis

Degenerate oligonucleotide mixtures are typically synthesized on a single column. When a position requires two bases, then two phosphoramidites are simultaneously injected into the synthesis column. While the synthesizer can draw from two or more phosphoramidite bottles, as needed, the results vary depending on the synthesizer and are rarely homogeneous. A much more effective solution is to prepare a special premixed bottle of phosphoramidites for degenerate positions, and up to eleven of these would be required (Table 1). Over the years, our customers have asked us to prepare DNA phosphoramidite mixes for them, and we have provided them as custom products. Now, as the need for degenerate oligonucleotides continues to grow, we have decided to offer these as standard products to cover all eleven possible DNA combinations in equimolar mixes using the most popular versions of our dA, dC and dG. If one would like customized ratios to compensate for different phosphoramidite coupling rates or different protecting groups on the bases, please reach out to our Customer Service Team. All equimolar and customized ratio phosphoramidite mixes will require at least a two-week lead time.

Table 1. Standard nomenclature for degenerate positions.

Symbol	Bases	Origin of designation
R	A, G	pu**R**ine
Y	C, T	p**Y**rimidine
M	A, C	a**M**ino
K	G, T	**K**eto
S	C, G	**S**trong interaction (3 hydrogen bonds)
W	A, T	**W**eak interaction (2 hydrogen bonds)
H	A, C, T	not G, **H** follows G in the alphabet
B	C, G, T	not A, **B** follows A
V	A, C, G	not T (not-U), **V** follows U
D	A, G, T	not C, **D** follows C
N	A, C, G, T	a**N**y

Table 2. Examples of degenerate oligonucleotides.

Application	Example Sequences (5' – 3')
Degenerate PCR	AA**R** AT**H** GA**Y** TGG TT**Y** TGG Lys Ile Asp Trp Phe Trp
PEP	**NNN** **NNN** **NNN** **NNN** **NNN**
DOP-PCR	CCG ACT CGA G**NN** **NNN** **N**AT GTG G
Site-directed Mutagenesis	15nt **NNS** **NNS** **NNS** **TDK** **VVC** 15nt
SELEX (N_{30})	Primer (**NNN**)$_{10}$ Primer

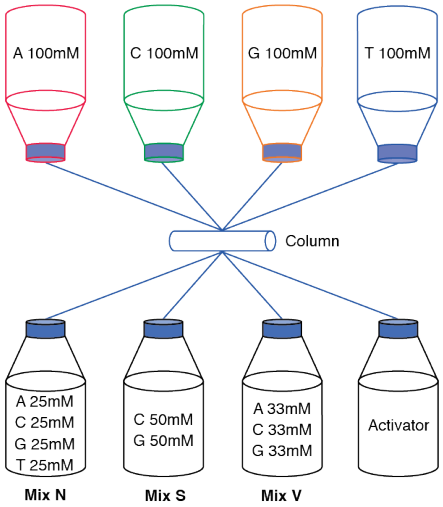


Figure 1. Degenerate oligonucleotide synthesis with mixes

Item	Catalog No.
Mix R: dA+dmf-dG-CE Phosphoramidite Mix	Mix R
Mix Y: Ac-dC+dT-CE Phosphoramidite Mix	Mix Y
Mix M: dA+Ac-dC-CE Phosphoramidite Mix	Mix M
Mix K: dmf-dG+dT-CE Phosphoramidite Mix	Mix K
Mix S: Ac-dC+dmf-dG-CE Phosphoramidite Mix	Mix S
Mix W: dA+dT-CE Phosphoramidite Mix	Mix W
Mix H: dA+Ac-dC+dT-CE Phosphoramidite Mix	Mix H
Mix B: Ac-dC+dmf-dG+dT-CE Phosphoramidite Mix	Mix B
Mix V: dA+Ac-dC+dmf-dG-CE Phosphoramidite Mix	Mix V
Mix D: dA+dmf-dG+dT-CE Phosphoramidite Mix	Mix D
Mix N: dA+Ac-dC+dmf-dG+dT-CE Phosphoramidite Mix	Mix N

References

1. M.A. Poritz, *et al.*, *PLoS One*, 2011, **6**, e26047.
2. L. Zhang, *et al.*, *Proc Natl Acad Sci U S A*, 1992, **89**, 5847-51.
3. H. Telenius, *et al.*, *Genomics*, 1992, **13**, 718-25.
4. S.S. Sidhu, and G.A. Weiss, Constructing phage display libraries by oligonucleotide-directed mutagenesis. In *Phage Display: A practical approach*, T. Clackson; H. B. Lowman, Eds. 2004; pp 27-42.
5. *The Glen Report*, 2020, **32.1**, 8-11.
6. A.D. Ellington, and J.W. Szostak, *Nature*, 1990, **346**, 818-22.
7. C. Tuerk, and L. Gold, *Science*, 1990, **249**, 505-10.
8. D.L. Robertson, and G.F. Joyce, *Nature*, 1990, **344**, 467-8.
9. T. Kivioja, *et al.*, *Nat Methods*, 2011, **9**, 72-4.

PRODUCT REVIEW

DBCO

In recent years, one type of Click Chemistry called Strain-Promoted Azide-Alkyne Cycloaddition (SPAAC)[1] has become particularly popular. The conjugation reaction is relatively fast, selective, and compatible with biological environments. The reaction involves an azide and a strained alkyne to form a stable 1,2,3-triazole linkage (Figure 1), and the strained alkyne is the key chemical group that makes it all possible.

Alkynes are ideally linear, and when they are incorporated into cyclic analogs, particularly smaller ones, the alkyne will have angle strain. While linear alkynes require toxic copper catalysts to facilitate conjugation, strained alkynes will spontaneously react. The formation of the triazole ring converts the higher energy, strained alkyne to an alkene, thereby relieving the ring strain.

We offer dibenzocyclooctyne, DBCO, as a modification for facilitating SPAAC with oligonucleotides. There are three phosphoramidites: 5'-DBCO-TEG Phosphoramidite, DBCO-dT-CE Phosphoramidite, and DBCO-Serinol Phosphoramidite for 5', internal, and general oligonucleotide labeling, respectively (Figure 2). These products work great. The only caveat to using them is that iodine oxidizer and DBCO are not compatible, which necessitates the use of our mild oxidizing agent, CSO, instead. For a 5' addition, only one cycle of CSO oxidation is required whereas for a 3' or internal insertion, CSO oxidation will be required throughout the whole synthesis. For those who would like to avoid the use of CSO, we also offer an NHS ester (Figure 2) that will label any amino modifier group post oligonucleotide deprotection. For example, one can pair the NHS ester with 5'-Amino-Modifier TEG CE-Phosphoramidite or Amino-Modifier C6 dT (Figure 2) to give the same exact structures obtained from two of the three DBCO phosphoramidites.

Our DBCO products have been successfully used in a wide range of investigations. In one project, DBCO facilitated the labeling of catalase enzymes with oligonucleotides for protein-protein and protein-gold nanoparticle superlattice formation.[2] Another group of researchers used DBCO to attach an RNA adaptor sequence to an mRNA 5'-cap to enable nanopore sequencing of full-length mRNA sequences.[3] In a third study, DBCO allowed antisense oligonucleotides to be attached to monoclonal antibodies for leukemia-targeting.[4] The resulting antibody-drug conjugate was effective in both *in vitro* and *in vivo* mouse model experiments. Finally, DBCO facilitated the engraftment of proteins onto modular DNA scaffolds for the analysis of protein-protein interactions at single-molecule resolution.[5]

For those considering conjugations and bioconjugations, SPAAC and DBCO should be one approach worthy of strong consideration.

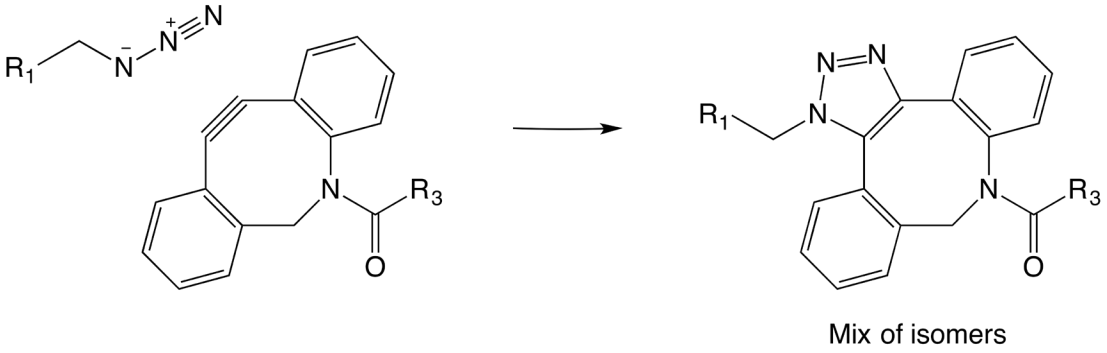
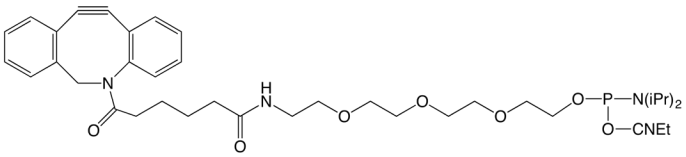
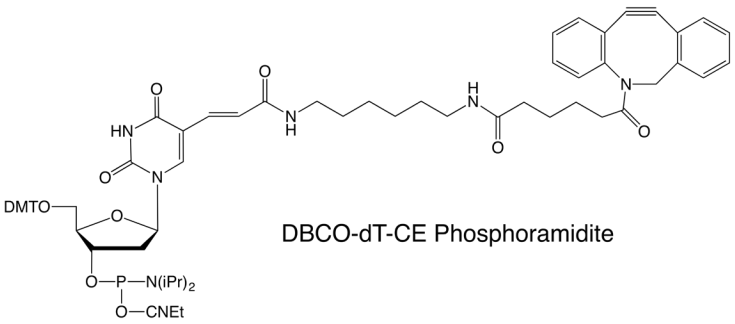


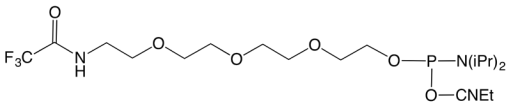
Figure 1. SPAAC with the strained alkyne DBCO



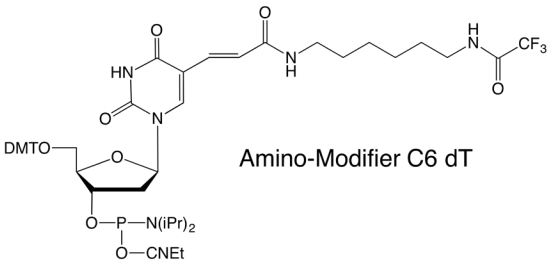
5'-DBCO-TEG Phosphoramidite



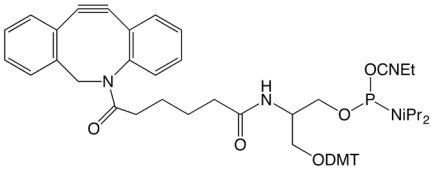
DBCO-dT-CE Phosphoramidite



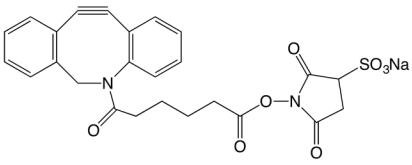
5'-Amino-Modifier TEG CE-Phosphoramidite



Amino-Modifier C6 dT



DBCO-Serinol Phosphoramidite



DBCO-sulfo-NHS Ester

Figure 2. DBCO products and possible amino-modifier precursors

Item	Pack Size	Catalog No.
5'-DBCO-TEG Phosphoramidite	50 µmol	10-1941-95
	100 µmol	10-1941-90
	0.25 g	10-1941-02
DBCO-dT-CE Phosphoramidite	50 µmol	10-1539-95
	100 µmol	10-1539-90
	0.25 g	10-1539-02
DBCO-Serinol Phosphoramidite	50 µmol	10-1998-95
	100 µmol	10-1998-90
	0.25 g	10-1998-02
DBCO-sulfo-NHS Ester	5.2 mg	50-1941-23
	52 mg	50-1941-24
5'-Amino-Modifier TEG CE-Phosphoramidite	100 µmol	10-1917-90
	0.25 g	10-1917-02
Amino-Modifier C6 dT	100 µmol	10-1039-90
	0.25 g	10-1039-02
	0.5 g	10-1039-05
0.5M CSO in Anhydrous Acetonitrile	200 mL	40-4632-52
	450 mL	40-4632-57
	1000 mL	40-4632-71
	2000 mL	40-4632-62

References

1. J. Dommerholt, F. Rutjes, and F.L. van Delft, *Top Curr Chem (Cham)*, 2016, **374**, 16.
2. J.D. Brodin, E. Auyeung, and C.A. Mirkin, *Proc Natl Acad Sci U S A*, 2015, **112**, 4564-9.
3. L. Mulroney, *et al., RNA*, 2022, **28**, 162-176.
4. N. Satake, *et al., Mol Med*, 2016, **22**, 632-642.
5. D. Kostrz, *et al., Nat Nanotechnol*, 2019, **14**, 988-993.

GLEN RESEARCH

part of Maravai LifeSciences

PRESORTED STANDARD
US POSTAGE
PAID
RESTON VA
PERMIT NO 536

Technical Snippets

Why is 5-Me-C the only cytosine version in certain backbones?

We typically receive this question as it pertains to our Locked Nucleic Acid (LA) and MOE monomers. LA and MOE oligonucleotides were developed for therapeutic applications. In the case of LA, both 5-Me-C LNA and C LNA oligonucleotides have been studied in the literature. However, 5-Me-C offers certain benefits, including reduced immune response and stronger base pairing. In some product lines, 5-methylated U is also offered instead of the unmethylated version.

Relevant products:

Bz-5-Me-C-LA-CE Phosphoramidite (10-2011)

Bz-5-Me-C-LA-CPG (20-2511)

5-Me-C-2'-MOE-Phosphoramidite (10-3211)

Is it possible to desalt short oligonucleotides (≤10mer) using Glen Gel-Pak™ columns?

Glen Gel-Pak™ columns are not compatible for desalting short oligonucleotides (≤10mer). The desalting concept for these columns relies on size exclusion chromatography. Molecules above the exclusion limit of the resin elute early from the column whereas molecules below the exclusion limit are retained in the matrix longer, effecting the separation between large and small molecules. Small oligonucleotides (≤10mer) are small enough to enter the matrix, preventing the proper desalting of the short oligonucleotide. Alternatively, our Glen-Pak™ cartridges are efficiently able to purify both short and long oligonucleotides. The principle of the Glen-Pak™ cartridge purification relies on the DMT-ON oligonucleotides. As long as the short oligos are DMT-ON then Glen-Pak™ cartridges should be able to purify them following the DMT-ON purification procedure. There is no need for further desalting after the Glen-Pak™ purifications.

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)

glenresearch.com

Glen Research
22825 Davis Drive, Sterling, VA 20164
703.437.6191
support@glenresearch.com