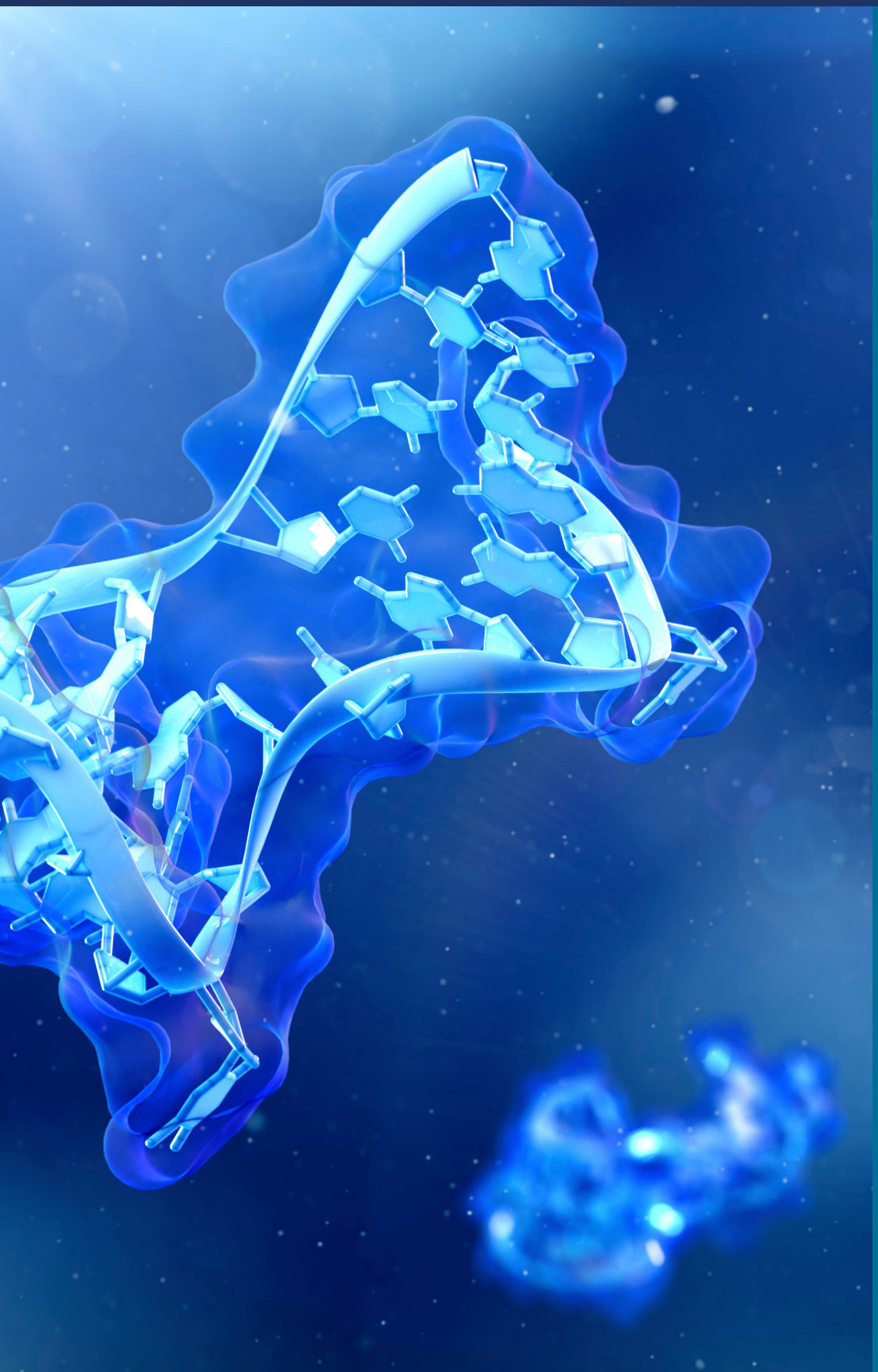
The Glen Report

Volume 37.1 | May 2025



See technical snippets on back cover

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NEW PRODUCT

N3-Methyl-C-CE Phosphoramidite

N3-Methyl-C (m^3C) is a naturally occurring ribonucleoside that is mostly found in tRNA but has recently been described in mRNA as well.[1] In tRNA, m^3C is often located in the anticodon loop (position 32) where its absence can affect translation. In humans, four methyltransferases have thus far been identified for m^3C. Methyltransferase-like 2A (METTL2A), METTL2B and METTL6 methylate tRNA while METTL8 methylates mRNA. In addition, human alkylation B (AlkB) proteins are possible demethylases for m^3C, suggesting that m^3C may be a dynamic modification. Under physiological conditions, m^3C is positively charged (Figure 1). The methylation is on the base pairing face, and as such, base pairing is significantly disrupted. For a single substitution of m^3C, melting temperatures can be reduced by as much as 20 °C and mismatch specificity can be lowered by 10-16 °C.[2]

While m^3C was identified over 60 years ago, many questions remain regarding its function, and research on this modification continues to grow.[1] To facilitate these efforts, Glen Research is introducing the phosphoramidite of m^3C (Figure 1, Table 1). This phosphoramidite employs acetyl protection on the nucleobase and TBDMS protection on the 2'-OH and can be coupled using standard RNA coupling times. Of note, the recommended diluent is anhydrous acetonitrile/dichloromethane 1:1 by volume. For deprotection, UltraMild and standard deprotection conditions can be used. As reported earlier, even with acetyl protection, AMA deprotection is not compatible.[3] Under such conditions, we also observed predominantly N4-methylated product ($m^{3,4}C$). As such, all deprotection options containing methylamine should be avoided.

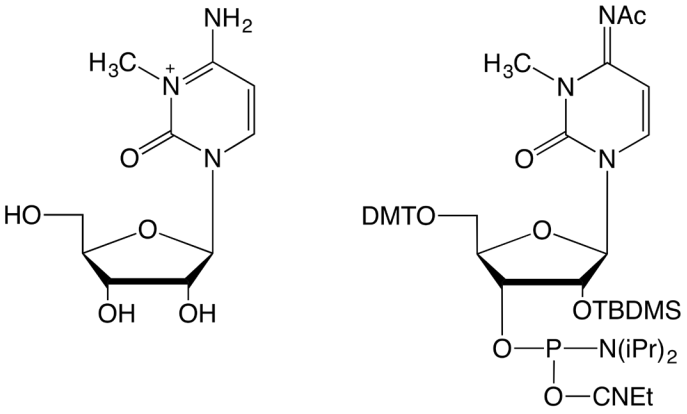


Figure 1. m^3C. Left, nucleoside; right, phosphoramidite.

Item	Pack Size	Catalog No.
N3-Methyl-C-CE Phosphoramidite	50 µmol	10-3013-95
	100 µmol	10-3013-90
	0.25 g	10-3013-02

Table 1. N3-Methyl-C-CE Phosphoramidite ordering information

References

1. K.E. Bohnsack, N. Kleiber, N. Lemus-Diaz, and M.T. Bohnsack, *Trends Biochem Sci*, 2022, **47**, 596-608.
2. S. Mao, *et al.*, *ACS Chem Biol*, 2021, **16**, 76-85.
3. S. Moreno, L. Flemmich, and R. Micura, *Monatsh Chem*, 2022, **153**, 285-291.

PRODUCT REVIEW

Post-transcriptional RNA Modifications

After transcription, RNA will go through several processing steps, one of which is chemical modification. Transfer RNA (tRNA), on average, have more than 10% of their nucleotides modified while messenger RNA (mRNA) will typically have less than a tenth of that. Regardless of which type of RNA, the chemical modifications that are added can be very diverse, and each of the modifications play a role in the function and/or regulation of the resulting RNA. To date, more than 170 modifications have been characterized ranging from a single methyl group to multiple heteroatomic/heterocyclic chains.[1-3]

A couple of the most abundant modifications are N6-methyladenosine (m^6A) and pseudouridine (ψ, Figure 1). The m^6A modification is the most prevalent modification in mRNA but can also be found in tRNA, ribosomal RNA (rRNA) and small nuclear RNA (snRNA). In mRNA, m^6A is involved in mRNA splicing, stability, structure, translation and more. The modification is reversible, and there are numerous proteins and enzymes involved in the methylation (writing), recognition (reading) as well as demethylation (erasing) in regards to m^6A. m^6A has been linked to many diseases including obesity, cardiovascular diseases and multiple cancers.[4]

Pseudouridine is the most abundant RNA modification in non-coding RNAs. Pseudouridine is an isomer of uridine where the N1 and C5 have essentially swapped locations with each other (Figure 1). This change allows for enhanced base stacking interactions that makes ψ particularly important for stability and folding in tRNA and rRNA structures. Unlike m^6A, ψ is a permanent modification. Pseudouridine has also been linked to numerous diseases including cancer and genetic disorders.[5]

It should be noted that m^6A and ψ are among a list of natural RNA modifications that play important roles in immune function, as was demonstrated by the Nobel Prize-winning research of Kariko and Weissman.[6] In their work, prokaryotic and *in vitro*-transcribed RNA stimulated an immune response while RNA bearing such modifications did not. For these reasons, both mRNA COVID vaccines from Moderna and Pfizer/BioNTech use N1-methylpseudouridine ($m^1ψ$), a methylated version of ψ that elicits an even lower innate immune response, as a complete replacement for uridine (U). Likewise, oligonucleotide drugs often use 5-methyl-substituted pyrimidines for similar reasons.

As a lot of the biology of these RNA modifications have yet to be understood, it would be very beneficial to be able to synthesize oligonucleotides with these modifications to further study them. Currently, Glen Research offers 17 of the more common RNA modifications as phosphoramidites (Figure 2, Table 1). That includes the newly introduced m^3C as well as m^6A, ψ and $m^1ψ$. Six of them are found in mRNA, tRNA, snRNA and tRNA while the remaining 11 are found in a subset of these four types of RNA. There are six modifications of A, four modifications of C, one modification of G and six modifications of U. These 17 are of course only a small subset of the possible modifications, and we continue to look for opportunities to offer more of these post-transcriptional modifications to support the growing field of RNA research.

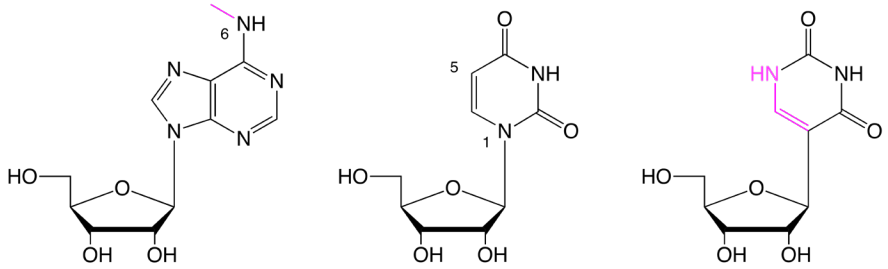


Figure 1. Structures of m^6A and ψ. Modifications are highlighted in purple.

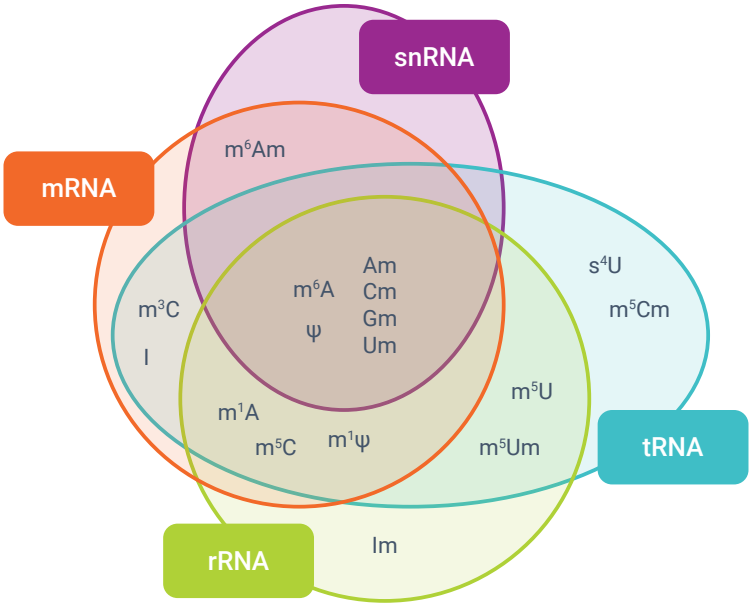


Figure 2. Post-transcriptional RNA modifications available at Glen Research, as grouped by RNA type.

Base	Modification	Item	Catalog No.
A	Am	2'-OMe-A-CE Phosphoramidite	10-3100
	m^1A	1-Me-A-CE Phosphoramidite	10-3501
	m^6A	N6-Methyl-A-CE Phosphoramidite	10-3005
	m^6Am	2'-OMe-N6-Me-A-CE Phosphoramidite	10-3105
	I	I-CE Phosphoramidite	10-3040
	Im	2'-OMe-I-CE Phosphoramidite	10-3140
C	Cm	2'-OMe-Ac-C-CE Phosphoramidite	10-3115
	m^5C	5-Me-C-TOM-CE Phosphoramidite	10-3064
	m^5Cm	2'-OMe-5-Me-C-CE Phosphoramidite	10-3160
	m^3C	N3-Methyl-C-CE Phosphoramidite	10-3013
G	Gm	2'-OMe-G-CE Phosphoramidite	10-3121
U	Um	2'-OMe-U-CE Phosphoramidite	10-3130
	m^5U	5-Me-U-CE Phosphoramidite	10-3050
	m^5Um	2'-OMe-5-Me-U-CE Phosphoramidite	10-3131
	s^4U	4-Thio-U-TOM-CE Phosphoramidite	10-3052
	ψ	PseudoUridine-CE Phosphoramidite	10-3055
	$m^1ψ$	1-Methyl-PseudoUridine Phosphoramidite	10-3056

Table 1. Post-transcriptional RNA modification products

References

1. A. Cappannini, *et al., Nucleic Acids Res*, 2024, **52**, D239-D244.
2. P.A. Limbach, P.F. Crain, and J.A. McCloskey, *Nucleic Acids Res*, 1994, **22**, 2183-2196.
3. P.J. McCown, *et al., Wiley Interdiscip Rev RNA*, 2020, **11**, e1595.
4. X. Jiang, *et al., Signal Transduct Target Ther*, 2021, **6**, 74.
5. J. Cerneckis, Q. Cui, C. He, C. Yi, and Y. Shi, *Trends Pharmacol Sci*, 2022, **43**, 522-535.
6. K. Kariko, M. Buckstein, H. Ni, and D. Weissman, *Immunity*, 2005, **23**, 165-175.

NEW PRODUCT

Ac-5-Me-C-LA-CE Phosphoramidite

Glen Research is excited to introduce a new addition to its Locked Nucleic Acid (LNA) line, Ac-5-Me-C-LA-CE Phosphoramidite (Figure 1). This new version of LNA-C features an acetyl protecting group (Ac), compared to the benzoyl (Bz) group found in our existing product, Bz-5-Me- C-LA-CE Phosphoramidite (Table 1). The Bz-version is incompatible with the methylamine deprotection method, as it forms 5-10% of an undesired transamination product, 5,N4-dimethyl-C, when the methylamine displaces benzamide.[1] The new Ac-version addresses this issue, allowing customers to utilize UltraFast deprotection method with AMA.

Similar to the current Bz-version, this new version of LNA incorporates 5-methylcytosine instead of standard cytosine. The methylation is crucial in therapeutic applications. A study by Kariko et al. demonstrated that unmodified RNA activates toll-like receptor (TLR) proteins such as TLR7 and TLR8, stimulating the innate immune system, while RNA containing 5-methylcytosine is not stimulatory.[2]

In addition to AMA deprotection compatibility, this new Ac-version requires only anhydrous acetonitrile as a diluent. In contrast, the Bz-version performs best with an anhydrous Acetonitrile/Dichloromethane 1:1 (v/v) mixture. Aside from this change in diluent, the Ac-version can be used in the same manner as the Bz-version for coupling. A three-minute coupling time is recommended, and it is advisable to triple the oxidation time for optimal performance. For deprotection, no changes are needed from the standard method recommended by the synthesizer manufacturer.

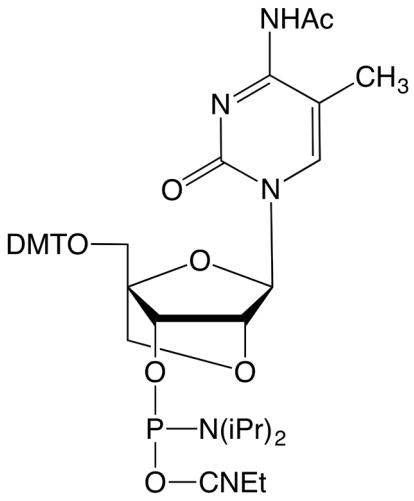


Figure 1. Ac-5-Me-C-LA-CE Phosphoramidite

Item	Pack Size	Catalog No.
Ac-5-Me-C-LA-CE Phosphoramidite	0.25 g	10-2015-02
	0.5 g	10-2015-05
Bz-5-Me-C-LA-CE Phosphoramidite	0.5 g	10-2011-05
	1.0 g	10-2011-10

Table 1. Ac and Bz 5-Me-C-LA-CE Phosphoramidites

References

1. The Glen Report, 2014, **26.1**, 4-6.
2. K. Kariko, M. Buckstein, H. Ni, and D. Weissman, Immunity, 2005, **23**, 165-175.

APPLICATION NOTE

Enzymatic Recognition of Nucleic Acid Modifications

DNA is an exceptionally stable molecule, a property that is essential for its role in storing and transmitting all genetic information across generations. Its stability allows it to withstand various environmental conditions and ensures the integrity of genetic material over time. Despite this stability, DNA is not invulnerable; specific enzymes, known as nucleases, can recognize and cleave DNA strands. This process occurs both naturally, as part of cellular processes such as DNA repair and replication, and artificially, in laboratory settings for research and genetic engineering purposes.

Many enzymes recognize DNA by detecting specific modifications or unique sequences within the molecule. Modifications such as methylation, which involves the addition of a methyl group to certain bases like cytosine or adenine, serve as signals for these enzymes. For instance, restriction enzymes, commonly used in molecular biology, bind to specific palindromic sequences of DNA and cut at or near these sites. Similarly, DNA repair enzymes can detect abnormalities like mismatches, oxidative damage, or breaks in the DNA backbone, often relying on the chemical alterations present in damaged bases. This ability to detect modifications ensures that cellular processes such as DNA repair, replication, and transcription proceed with high fidelity. In gene editing technologies like CRISPR-Cas9, the system uses guide RNA to direct the enzyme to a precise DNA sequence, demonstrating how enzymatic recognition is harnessed for precise genome modification.

Natural modifications

Damage to the nucleobase results in a lesion that is recognized as part of the DNA damage repair pathway. Some examples include 8-oxo-deoxyGuanosine (8-oxo-dG), Thymidine Glycol (Tg), and deoxyuridine (dU) lesions (Table 1). Oxidative damage produces 8-oxo-dG and Tg, while uracil in DNA forms when cytosine undergoes spontaneous deamination. Nucleobase excision, the first step of base excision repair (BER), is performed by glycosylases. In mammals, 8-oxo-dG is removed by 8-oxoguanine DNA glycosylase 1 (OGG1), Tg is excised by thymine DNA glycosylase (TDG), and dU is recognized and removed by Uracil DNA Glycosylase (UDG). Once the glycosidic bond is cleaved, an abasic or apurinic/apyrimidinic (AP) site is formed (Figure 1).

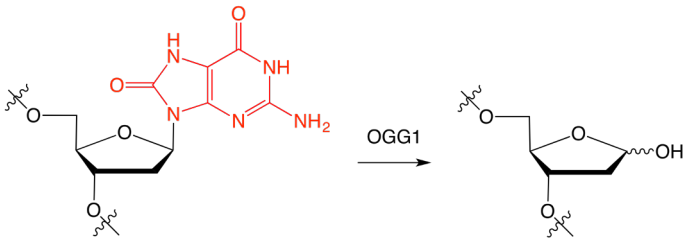


Figure 1. Abasic site formation

AP sites are locations lacking a nucleobase (A, C, G, T) and are perhaps the most common DNA lesions. This can occur spontaneously due to cleavage of the glycosidic bond or it can arise as an intermediate in the BER pathway. It is estimated that 10,000 to 30,000 AP sites are formed per cell per day.[1] AP sites are located internally and are recognized and cleaved by AP endonuclease (APE1). AP sites are unstable and sensitive to basic cleavage. The easiest way to introduce an AP site into an oligonucleotide is using our Abasic II Phosphoramidite (Table 1).

Certain nucleases exhibit a preference for either DNA or RNA as their substrate, reflecting their specialized roles in cellular processes. These enzymes are broadly classified into two main types: endonucleases and exonucleases. Endonucleases cleave nucleic acid strands at internal sites, breaking phosphodiester bonds within the molecule. A prominent example of this type are restriction enzymes, which induce precise strand breaks at or near their target sites.

In contrast, exonucleases degrade nucleic acids by sequentially removing individual nucleotides from the ends of a strand. These enzymes are further categorized based on their directionality: some exonucleases act on the 5'-end of the strand, while others target the 3'-end. This directional specificity is crucial for processes like DNA proofreading and repair, where precise removal of nucleotides is required to maintain genetic integrity.

Some nucleases exhibit remarkable substrate specificity, targeting distinct chemical groups on nucleic acids

such as a 5'-phosphate. For instance, Terminator 5'-Phosphate-Dependent Exonuclease exclusively digests RNA molecules that possess a 5'-monophosphate group, while leaving RNA with a 5'-triphosphate, 5'-cap, or 5'-hydroxyl group untouched. This specificity is critical for certain experimental applications, such as selectively degrading specific RNA species. Similarly, nucleases like Mung Bean Nuclease and BAL 31 Nuclease require both a 5'-phosphate and a 3'-hydroxyl group for activity. These enzymes are commonly used in molecular biology for tasks like removing single-stranded overhangs from DNA, highlighting their importance in precise nucleic acid manipulation. Creating a 5'-phosphorylated oligonucleotide is simple when using one of our chemical phosphorylating reagents, such as our Solid Chemical Phosphorylation Reagent II (Table 1).

Unnatural Modifications

Extensive research has focused on identifying nuclease-resistant modifications. Common strategies include incorporating phosphorothioate (PS) linkages or introducing structural modifications to the sugar-phosphate backbone, such as Locked Nucleic Acids (LNAs), 2'-O-methyl (2'-OMe), and 2'-methoxyethyl (2'-MOE) modifications. These alterations aim to improve biostability, protecting therapeutic oligonucleotides from degradation by nucleases in biological environments.

However, it is important to note that many unnatural modifications do not inherently confer nuclease resistance. These modifications often serve other purposes without significantly impacting or are designed to be susceptible to nuclease activity.

Oligonucleotides containing the synthetic bases isodC and isodG are subject to digestion by exonucleases.[2] This non-natural base pair was specifically engineered to expand the genetic code, introducing new possibilities for synthetic biology and molecular engineering.[3] The sensitivity of this artificial base pair to nucleases is particularly relevant in the context of sequencing technologies, as it highlights both the challenges and potential opportunities for the development of new methods for reading and utilizing expanded genetic systems.

dR-Amino modification is an internal amino-modifier that resembles an abasic site (Figure 2, Table 1). The amino modifier enables conjugation of a specific label at a terminal or internal position of the oligonucleotide via NHS chemistry. This can be useful in various applications, such as RNA interference or diagnostic assay development. The amino linker is recognized by nucleases.

The beta anomer of dR-Amino is susceptible to cleavage by 8-oxoguanine DNA glycosylase (fpg) and Endonuclease IV (Nfo).[4,5] This feature is particularly useful and can create nuclease-responsive probes. This allows for reversible NHS ester labeling because nuclease activity on dR will cleave the amino-label linker, resulting in an AP site (Figure 2). Fluorogenic conjugates of the dR-amine have been used to evaluate fluorescent probes for real-time polymerization chain reaction (RT-PCR).[6,7] Derivatives of dR to incorporate biotin, digoxigenin, and GalNAc are used for diagnostics, sequencing, in situ hybridization, and delivery applications.[8] Any of our NHS esters can be used to install various modifications, including fluorophores, biotin, maleimide, and click chemistry handles.

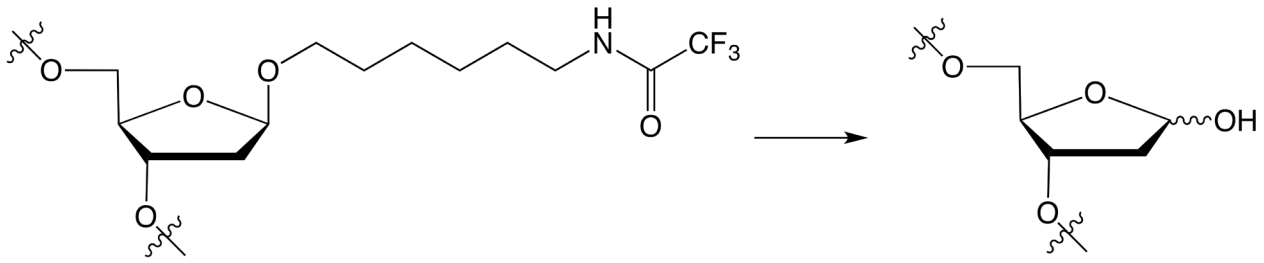


Figure 2. dR-Amino cleavage by nuclease to form abasic site

Modification	Product	Catalog No.
Tg	Thymidine Glycol CE Phosphoramidite	10-1096
8-oxo-dG	8-oxo-dG-CE Phosphoramidite	10-1028
dU	dU-CE Phosphoramidite	10-1050
AP	Abasic II Phosphoramidite	10-1927
Phosphate	Chemical Phosphorylation Reagent	10-1900
	Chemical Phosphorylation Reagent II	10-1901
	Solid Chemical Phosphorylation Reagent II	10-1902
Unnatural Base Pair	dmf-5-Me-isodC-CE Phosphoramidite	10-1065
	dmf-isodG-CE Phosphoramidite	10-1078
dR	Amino-Modifier C6 dR	10-1911

Table 1. Relevant products

References

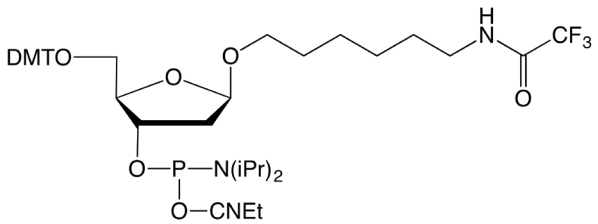
1. J. Nakamura, *et al., Cancer Res*, 1998, **58**, 222-225.
2. G. Lohman. New England Biolabs, Inc. The effect of nucleic acid modifications on digestion by DNA exonucleases. https://www.neb.com/en-us/tools-and-resources/feature-articles/the-effect-of-nucleic-acid-modifications-on-digestion-by-dna-exonucleases.
3. J.A. Piccirilli, T. Krauch, S.E. Moroney, and S.A. Benner, *Nature*, 1990, **343**, 33-37.
4. O. Piepenburg, N. Armes. US 2011/0053153.
5. O. Piepenburg, N. Armes. US 2017/0175173.
6. C.M. McKeen, L.J. Brown, J.T. Nicol, J.M. Mellor, and T. Brown, *Org Biomol Chem*, 2003, **1**, 2267-2275.
7. J.P. May, *et al., Org Biomol Chem*, 2005, **3**, 2534-2542.
8. A. Debacker, J. Voutila, L. Mitchell, C. McKeen. US 2022/0281911.

NEW PRODUCT

Amino-Modifier C6 dR

Amino-Modifier C6 dR is now available in our catalog for purchase (Table 1). This new amino modifier offers an additional conjugation strategy to our customers, one that can be useful in probe design by taking advantage of its nuclease sensitivity. Amino-Modifier C6 dR can be incorporated multiple times in an oligonucleotide.

In our hands, no changes are needed for coupling apart from the standard method recommended by the synthesizer manufacturer. Standard deprotection is also compatible with this modification. If using ammonia deprotection, a minor side reaction can lead to irreversible capping of the amine. If multiple additions are made, this may be significant. To prevent the reaction, synthesize using acetyl-protected dC and deprotect in 30% ammonia/40% methylamine 1:1 (AMA) at 65 °C for 15 minutes.



Item	Pack Size	Catalog No.
Amino-Modifier C6 dR	50 µmol	10-1911-95
	100 µmol	10-1911-90
	0.25 g	10-1911-02

Table 1. Amino-Modifier C6 dR ordering information

LITERATURE HIGHLIGHT

Highly-modified mRNA Capture Sequences

At Glen Research, we have a large catalog of products for oligonucleotide synthesis. Depending on their needs, customers will determine which products to use as well as where to place them in a sequence. This can result in some very complex oligonucleotides, as was the case in an ACS Chemical Biology article from a few years ago.[1]

The authors were working to optimize an *in vivo*, single cell transcriptome analysis assay that the same group described in earlier publications.[2,3] The final mRNA capture sequence (Figure 1) had a total of 12 different products incorporated, six of which were non-nucleosidic modifications. There was a thiol modifier that was conjugated to a cell penetrating peptide (CPP) to facilitate cellular uptake. There was of course a poly-U sequence that is used to capture the poly-A tails of mRNA. That region was blocked with a photo-cleavable cage of two segments of A's held together with a spacer and two photocleavable linkages. There was also a Cyanine 5 / Cyanine 3 FRET pair that can be used to monitor both cellular uptake as well as photo-decaging. Finally, there was a biotin to facilitate capture of the desired mRNA sequences onto streptavidin. A full list of the products used can be found on the next page (Table 1).

As one can imagine, synthesizing such an oligonucleotide requires a bit of planning. First and foremost, the synthesizer may not have 11 phosphoramidite ports, as was the case for the authors who were using an ABI394. That meant that the synthesis had to be performed over at least two runs. One must also consider how the deprotection needs to be done. Of the modifications present, the Cyanine 5 is the most deprotection-sensitive. We usually recommend an UltraMild synthesis, but since UltraMild versions of the 2'-F phosphoramidites are not available, deprotection with ammonium hydroxide at room temperature was used instead. Purification was performed with RP-HPLC, which allows for simultaneous monitoring of UV as well as the absorbances of the Cyanine 5 and Cyanine 3.

This is just one example of what our customers can do with our products, and we look forward to seeing many more examples in the future.

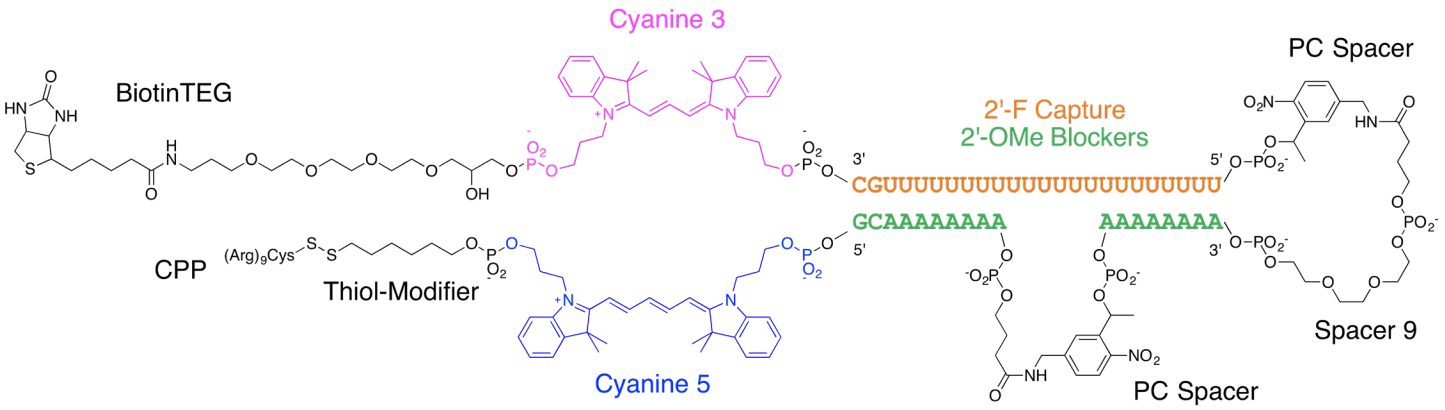


Figure 1. mRNA capture sequence

Product	Catalog No.
3'-BiotinTEG CPG	20-2955
Cyanine 3 Phosphoramidite	10-5913
2'-F-Ac-C-CE Phosphoramidite	10-3415
2'-F-G-CE Phosphoramidite	10-3420
2'-F-U-CE Phosphoramidite	10-3430
PC Spacer Phosphoramidite	10-4913
Spacer Phosphoramidite 9	10-1909
2'-OMe-A-CE Phosphoramidite	10-3100
2'-OMe-Ac-C-CE Phosphoramidite	10-3115
2'-OMe-G-CE Phosphoramidite	10-3121
Cyanine 5 Phosphoramidite	10-5915
Thiol-Modifier C6 S-S	10-1936

Table 1. Products used for mRNA capture sequence

References

1. S.B. Yeldell, L. Yang, J. Lee, J.H. Eberwine, and I.J. Dmochowski, ACS Chem Biol, 2020, **15**, 2714-2721.
2. D. Lovatt, et al., Nat Methods, 2014, **11**, 190-196.
3. S.B. Yeldell, B.K. Ruble, and I.J. Dmochowski, Org Biomol Chem, 2017, **15**, 10001-10009.

APPLICATION NOTE

Aptamers in Diagnostics

When it comes to oligonucleotides in the field of diagnostics, polymerase chain reaction, or PCR for short, is certainly quick to come to mind. What about point-of-care diagnostics? Traditionally, antibodies detect protein levels in a sample on a lateral flow strip. This is the case for common at-home tests for pregnancy or coronavirus.

In recent years, more studies are coming out that use aptamer-based sensing to detect specific targets linked to a disease or condition. Aptamers are preferable over antibodies because they are cheaper, easier to prepare, more stable, and do not produce an immune response (Table 1).[1-3]

	Aptamers	Monoclonal Antibodies
Stability	Can withstand several rounds of denaturation	Easily denatured
	Long shelf life	Short shelf life
	Stable at room temperature	Requires refrigeration
	Degradable by nucleases	Degradable by proteases
Preparation	Chemical production (*in vitro* SELEX) requires 2-8 weeks	Biological production (*in vivo*) requires 6+ months
	Inexpensive	High cost of synthesis
	Low batch-to-batch variation	High batch-to-batch variation
Other Properties	Targets range from ions to small molecules to whole cells	Targets must induce small immune response for antibody production
	Can be easily modified	Modifications can reduce activity
	Fast cellular internalization	Slow cellular internalization
	Specificity can distinguish single-point mutations	Different antibodies may bind same target

Table 1. Properties of aptamers vs. antibodies[2-3]

Aptamers are molecular recognition elements that, ideally, have high binding affinity and specificity for their targets. In the presence of their target, the single-stranded nucleic acid folds into a 3-dimensional structure that recognizes the intended analyte. Target binding relies on tertiary structure rather than primary sequence. This is often coupled with a signal to report detection. Aptamers are compatible with numerous diagnostic formats, such as colorimetric assays, fluorescence-based assays, lateral flow platforms, and nanoparticles.[1]

Detecting Small Molecules

Dopamine is a neurotransmitter that regulates important biological processes, including movements, cognition, motivation, and mood. Dopamine is also a biomarker for stress, and decreased levels have been linked to neurodegenerative diseases. Detection of dopamine in biological samples can be useful for early diagnostics, something that is especially crucial for diseases like Alzheimer's, Parkinson's, and Huntington's. Recently, a dopamine aptamer was used in a lateral flow assay to detect dopamine levels in urine samples (Table 2).[4] In this system, the aptamer is hybridized to a complementary strand bound to a gold nanoparticle (AuNP) (Figure 1A). In the presence of its antigen, the aptamer strand dissociates from the duplex to bind its target, freeing up the AuNP-bound strand to bind a complementary probe linked to the test strip and induce a positive signal. The limit of detection in urine was roughly ~10 ng/mL dopamine, which is clinically relevant as the typical concentration range for the biomarker under healthy physiological conditions is 52-480 ng/mL.

In a similar AuNP-aptameric-based assay, a fluorescently tagged aptamer was used to monitor ampicillin levels in urine to determine ideal antibiotic dosage and patient compliance during treatment (Table 2).[5] This is particularly valuable as it pertains to drug-resistant infections caused by the misuse of antibiotics. In the absence of ampicillin, the non-specifically bound AuNP quenched fluorescence. At increasing concentrations of ampicillin, the aptamer dissociates from the AuNP

surface leading to an increase in fluorescence (Figure 1B). This aptamer was specific to ampicillin when compared to structurally similar β-lactams (i.e. cephalexin, amoxicillin, or hydrolyzed ampicillin) (Figure 2). Clinical samples generally have ampicillin concentrations that range from 1 µM - 1 mM, and this assay detected as low as 21 nM ampicillin in biological samples.[5]

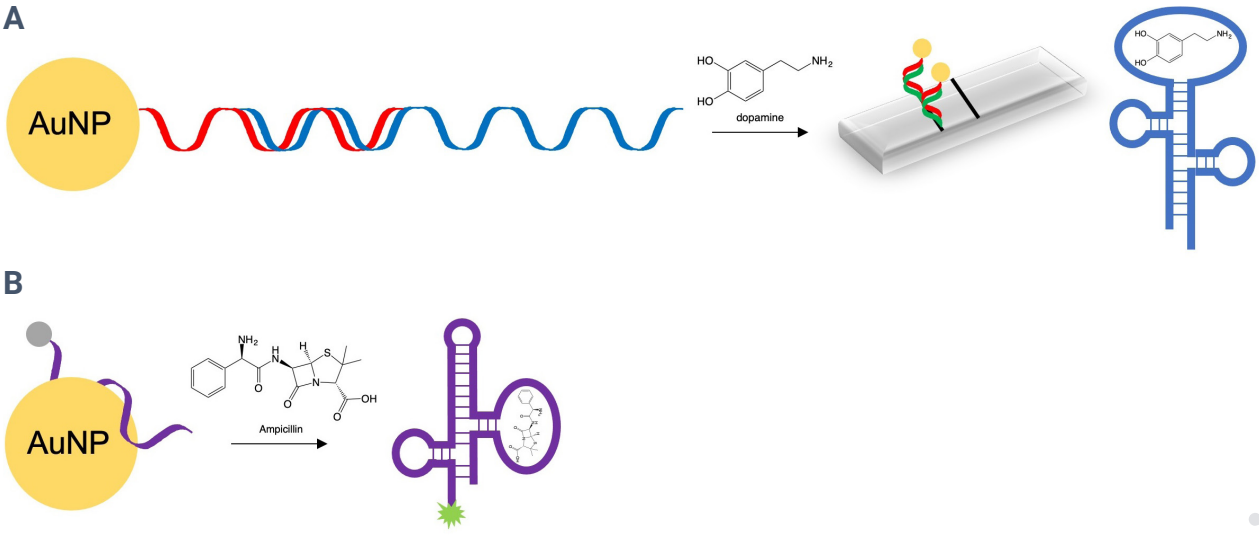


Figure 1. AuNP-based aptamer assay schemes

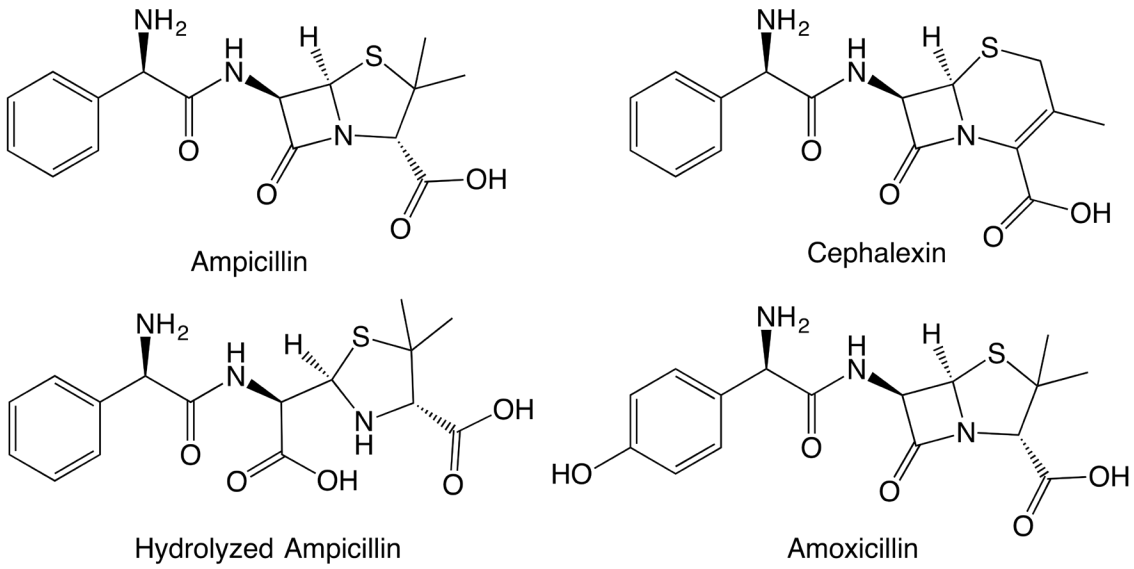


Figure 2. β-lactam structures

Detecting Proteins

Similar fluorescent-based aptamer strategies have been used to target clinically relevant proteins. This is the case for detecting the peanut protein, Ara 1, in food samples. In this application, a portable, consumer-friendly device was engineered for sample preparation and protein detection in just three minutes.[6] The fluorescently labeled aptamer is anchored to a solid support until target binding leads to dissociation and washing away of the fluorophore. The loss of fluorescence indicates the presence of the peanut allergen. This detection method was capable of detecting protein concentrations as low as 12.5 ppm in a food sample and was specific to peanut over other nuts.

After SELEX, the aptamer was modified to be G/C-rich at the 5' and 3' ends to enhance binding to the anchors, made of complementary DNA bearing a 5'-amine modification used for chip printing.

SomaLogic has leveraged nucleobase modifications to enhance aptamer binding affinity to their protein target. Replacing dT bases with various 5-modified dU provided "slow off-rate aptamers" called SOMAmers. When applied to a DNA microarray, their SOMAscan was capable of detecting thousands of proteins for disease-related proteomics and clinical applications. This strategy has been reviewed previously.[7]

Target	Application	Sequence (5'-3')
Dopamine	Neurodegenerative diseases	CTC TCG GGA CGA CGC GAG TTT GAA GGT TCG TTC GCA GGT GTG GAG TGA CGT CGT CCC
Ampicillin	Antibiotic resistance	FAM-GGC GAT ATG TTG GCG GGC G
Ara 1	Peanut Allergen	GGG TCG AGC TGA GTG GAA GCG TTT CTC CGT

Table 2. Summary of aptamers mentioned

Chemical Modifications

Where does Glen Research come into play for the aptamer field? Whether the aptamer application is in diagnostics or therapeutics, the initial discovery typically relies on modified and unmodified triphosphates for enzymatic synthesis during selection. After identifying a hit, the aptamer sequence is chemically synthesized using phosphoramidite chemistry. This is also the time for optimization of the aptamer, such as installing attachment chemistry for immobilization or improving stability as well as detection modifications, such as fluorophores or conjugation modifications. To immobilize aptamers onto a solid surface for diagnostic applications, popular candidates include thiol and biotin labels.

While aptamers offer several benefits compared to antibodies, a major disadvantage is poor stability in biological samples. Chemical modifications can mitigate this drawback.[8] One strategy to prevent exonuclease activity is to cap the 3'-end with a 3'-3'- linkage or a 3'-biotin. For the former, this can be achieved by using inverted dT, inverted dSpacer, or a Spacer C3.[9]

Backbone modifications that were identified during selection by using modified triphosphates can also be incorporated easily using phosphoramidites (i.e. 2'-Fluoro, 2'-amino, 2'-O-methyl). Some of these modifications have also been shown to optimize aptamer-antigen affinity. Internucleotide triazole modifications have been shown to prevent nuclease hydrolysis.[8] Lastly, L-DNA monomers have been used in the development of Spiegelmers, which have been optimized to recognize novel targets.[8,10] These L-aptamer constructs are not recognized by bodily enzymes.

We offer many modifications that support aptamer development (Table 3).

Modification	Product	Catalog No.
Fluorescein	5'-Fluorescein Phosphoramidite	10-5901
Cyanine	Cyanine 5 Phosphoramidite	10-5915
5'-Amine	5'-Amino-Modifier C6-TFA	10-1916
Thiol	Thiol-Modifier C6 S-S Phosphoramidite	10-1936
	Dithiol Serinol Phosphoramidite	10-1991
	3'-Thiol-Modifier 6 S-S CPG	20-2938
	3'-Dithiol Serinol CPG	20-2991
Biotin	5'-Biotin Phosphoramidite	10-5950
	5'-Biotin II Phosphoramidite	10-1954
	PC Biotin Phosphoramidite	10-4950
	3'-BiotinTEG CPG	20-2955
	3'-Protected Biotin Serinol CPG	20-2993
3'-Cap	dT-5'-CPG	20-0302
	dSpacer-5'-CE Phosphoramidite	10-4191
	3'-Spacer C3 CPG	20-2913
2'-F RNA	2'-F-A-CE Phosphoramidite	10-3400
	2'-F-Ac-C-CE Phosphoramidite	10-3415
	2'-F-G-CE Phosphoramidite	10-3420
	2'-F-U-CE Phosphoramidite	10-3430
2'-OMe	2'-OMe-A-CE Phosphoramidite	10-3100
	2'-OMe-Ac-C-CE Phosphoramidite	10-3115
	2'-OMe-G-CE Phosphoramidite	10-3121
	2'-OMe-U-CE Phosphoramidite	10-3130
L-DNA	Beta-L-Pac-dA-CE Phosphoramidite	10-2101
	Beta-L-Ac-dC-CE Phosphoramidite	10-2115
	Beta-L-iPr-Pac-dG-CE Phosphoramidite	10-2121
	Beta-L-dT-CE Phosphoramidite	10-2130

Table 3. Products used for aptamer development and optimization

References

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Notes

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Technical Snippets

How can I make a dual-labeled oligo using two different NHS esters?

To incorporate two labels on the same oligonucleotide when the labels are only available as NHS esters, orthogonal amino modifiers must be used. For example, an fmoc-protected amino modifier at the 3'-end can be selectively cleaved using piperidine for the first NHS ester conjugation while the oligonucleotide is still attached to the solid support. Subsequently, an amino modifier protected with TFA or MMT at the 5'-end can be removed during normal oligonucleotide deprotection prior to the second NHS ester conjugation, which occurs off the CPG. In this scenario, the 3'-label must be compatible with deprotection conditions.

Alternatively, a 5'-MMT amino modifier can be paired with a 3'-PT amino modifier. The MMT can be cleaved selectively on synthesizer using a standard deblock cycle and is ready for the first on-column NHS ester conjugation. Subsequent oligonucleotide cleavage and deprotection will reveal the 3'-amine for the second NHS ester reaction. In this case, the 5'-label must be compatible with deprotection conditions.

The above schemes could also be adapted for an oligo containing an internal label and a terminal label, where the internal label uses a TFA-protected amino modifier, which we have available in all major bases (A, C, G, and T/U).

Relevant products:

All Amino-Modifiers

What is the recommended amount of deprotection solution to use for my oligonucleotide?

The recommended volume of deprotection solution varies based on the oligonucleotide synthesis scale, as outlined below:

40-200 nmole → 0.5 mL

1.0 µmol → 1.0 mL

10-20 µmol → 5 mL per 10 µmole synthesis scale

Relevant products:

0.05M Potassium Carbonate in Methanol (60-4600)

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