

# The GLEN REPORT Newsletter

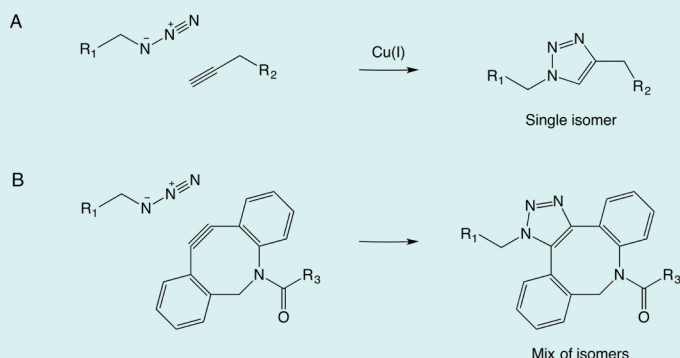
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## New Product — Azido-Modifier Serinol CPG

Since it was first coined by Sharpless over 20 years ago,<sup>1</sup> click chemistry has quickly become an invaluable approach to synthesizing and manipulating organic molecules. The reactions described by click chemistry have been used extensively in materials science, bioconjugation, drug discovery and much more. Click chemistry continues to become more popular and was awarded the 2022 Nobel Prize in Chemistry.

Of the reactions that fall under the click chemistry umbrella, the cycloaddition between an azide and an alkyne, to form a stable 1,2,3-triazole linkage, has been by far the most prevalent. Any entity with an azide can be linked to another entity that has an alkyne. The reaction is specific, high yielding and rapid. In addition, there are also no byproducts generated. Standard alkynes will require a copper (I) catalyst while strained alkynes will react with an azide without any additional inputs beyond solvent.

Azide alkyne cycloadditions are excellent methods for oligonucleotide conjugations,<sup>2</sup> and Glen Research has a long list of reagents to facilitate such reactions. There are numerous alkynes for copper (I)-catalyzed conjugation (Figure 1A). There are also several strained alkynes (DBCO) for copper-free conjugation (Figure 1B). What might be harder to find are azide-containing reagents. This is mostly because azides can react with phosphoramidite groups (Staudinger reaction), and as a result, azide-containing phosphoramidites are not very stable. There are a couple of workarounds (Figure 2). Oligonucleotides containing amino modifiers can be post-synthetically labeled with Azidobutyrate NHS Ester (50-1904). Alternatively, the bromide from 5'-Bromohexyl Phosphoramidite (10-1946) can be displaced by azide prior to oligonucleotide deprotection. Both approaches work well, but is there a way to avoid additional post-synthesis manipulations?



**Figure 1. Azide alkyne cycloaddition. A, copper (I) catalyzed; B, strained alkyne (DBCO).**

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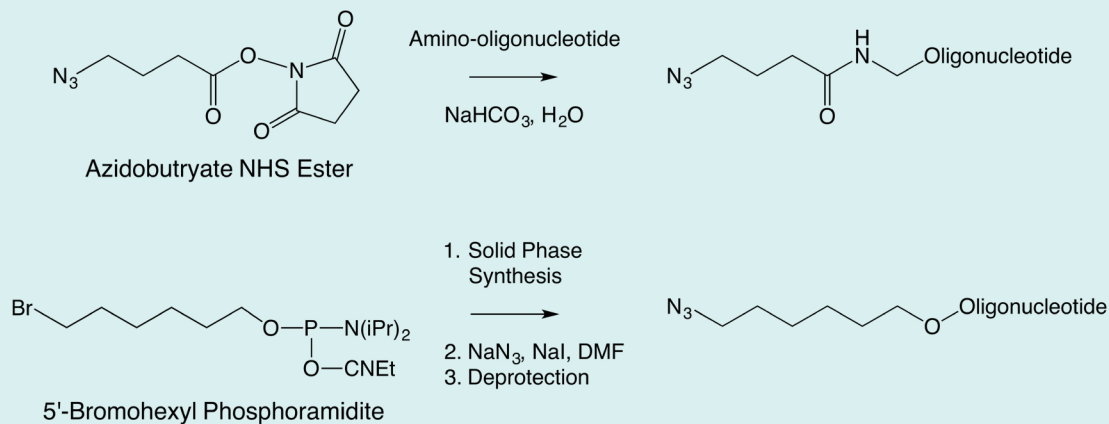
*See technical snippets  
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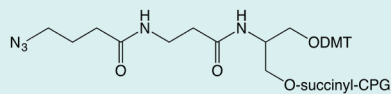


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**Figure 2. Strategies for introducing azides to oligonucleotides**

Years ago, researchers at the University of Montpellier demonstrated that a synthesis support functionalized with an azide modifier will survive oligonucleotide synthesis and deprotection.<sup>3</sup> Subsequently, another team from the University of Innsbruck extended this concept to an azidonucleoside-loaded support.<sup>4</sup> With these developments in mind, we showed that an azide support could be prepared using our 3'-Amino-Modifier C7 CPG (20-2958) and Azidobutyrate NHS Ester (50-1904),<sup>5</sup> and customers have since had a third approach for labeling oligonucleotides with azides. To make azido oligonucleotides even more readily available, we are now introducing 3'-Azido-Modifier Serinol CPG (Figure 3), essentially an amalgam of Azidobutyrate NHS Ester and Amino-Modifier Serinol CPG (20-2997).



**Figure 3. Azido-Modifier Serinol CPG**

The support works very well. It can be used in the same way as any of our other Serinol supports. For coupling, the support should be used in a manner identical to a normally protected nucleoside support since it contains the DMT group. Deprotection

should be carried out as required by the nucleobases. In the past, we have observed a small amount of displacement of the azide by ammonia during deprotection using the bromohexyl approach described earlier. Fortunately, this was not observed for this support, even with an ammonium hydroxide deprotection at 55 °C for 17 hours.

3'-Azido-Modifier Serinol CPG is convenient to use and produces excellent synthesis results. Due to its advantages over other approaches, it is possible that this new support will become the most popular method for preparing azide-labeled oligonucleotides.

## References

1. H.C. Kolb, M.G. Finn, and K.B. Sharpless, *Angew Chem Int Ed Engl*, 2001, **40**, 2004-2021.
2. N.Z. Fantoni, A.H. El-Sagheer, and T. Brown, *Chem Rev*, 2021, **121**, 7122-7154.
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4. T. Santner, M. Hartl, K. Bister, and R. Micura, *Bioconjug Chem*, 2014, **25**, 188-95.
5. *The Glen Report*, 2014, **26.2**, 11.

Item	Pack Size	Catalog No.
Azidobutyrate NHS Ester	2.3 mg	50-1904-23
	23 mg	50-1904-24
5'-Bromohexyl Phosphoramidite	100 µmol	10-1946-90
	0.25 g	10-1946-02
3'-Azido-Modifier Serinol CPG	0.1 g	20-2999-01
	1.0 g	20-2999-10
1 µmol columns	Pack of 4	20-2999-41
0.2 µmol columns	Pack of 4	20-2999-42
10 µmol columns (ABI)	Pack of 1	20-2999-13
15 µmol columns (Expedite)	Pack of 1	20-2999-14

## New Products — NHS Esters

Glen Research offers several N-Hydroxysuccinimide (NHS) Esters for post-synthetic oligonucleotide labeling. Most of our options are fluorophores, due to their increasing popularity. Of our non-dye NHS esters, only click reaction handles are available: alkyne (50-1905), azide (50-1904), and DBCO (50-1941). As we continue to support our customers and provide diverse options for their oligonucleotide synthesis and labeling needs, we are introducing two different NHS Esters: PC Biotin NHS Ester (50-4950) and Maleimide NHS Ester (SMCC) (50-1938).

### PC Biotin

PC Biotin is a photocleavable biotin tag that is already available as a phosphoramidite (10-4950). The structures of PC Biotin Phosphoramidite and PC Biotin NHS ester differ quite a bit (Figure 1). The first difference is the presence of an ethylene glycol linker in the NHS ester. In addition, the nitrophenyl photocleavable moiety of each product differs in substituents and regiochemistry. Lastly, the phosphoramidite contains a DMT group on the biotin, which requires cleavage and is not present in the NHS Ester. Regardless of these structural differences, the functionality of the biotin's affinity for streptavidin remains unchanged.

Relative to other biotin products, a major advantage of PC Biotin is the ability to release the oligonucleotide into solution after biotin capture with streptavidin beads by cleaving the linker between the biotin tag and the oligonucleotide using light.<sup>1</sup> Once cleaved, the oligonucleotide can be used for activity or further analysis. Our PC Biotin Phosphoramidite can only be used as a 5'-modifier and yields a 5'-phosphate after cleavage (~350 nm). PC Biotin NHS Ester reacts anywhere an amino modifier is placed in the sequence to form a carbamate linkage. Upon photocleavage and subsequent decarboxylation, the primary amine target is released (Figure 2).

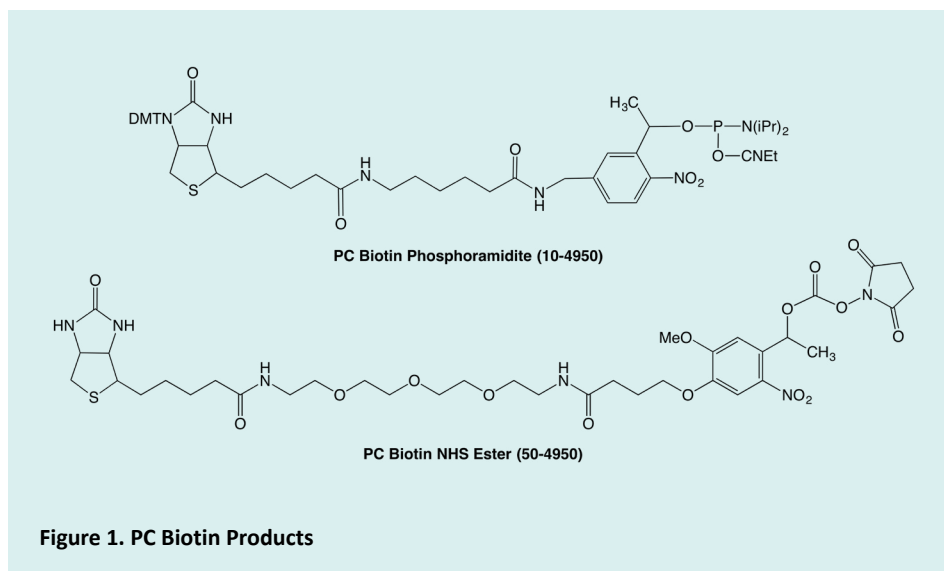


Figure 1. PC Biotin Products

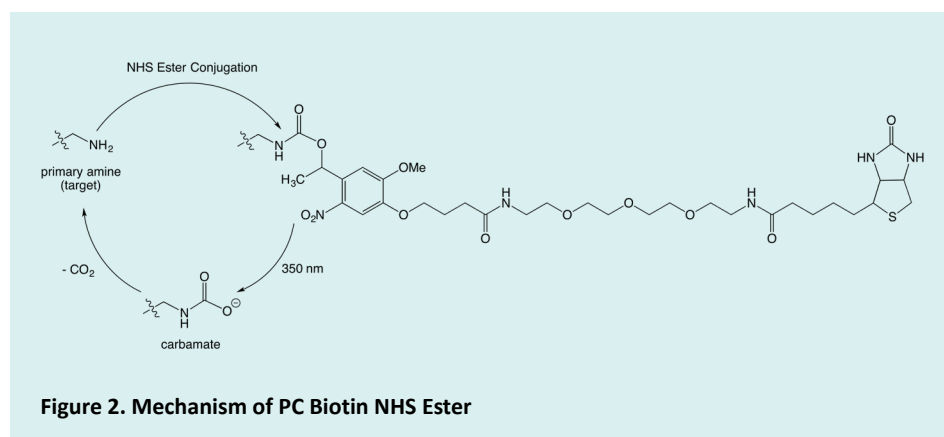


Figure 2. Mechanism of PC Biotin NHS Ester

PC Biotin NHS ester can be used to label a protein or oligonucleotide. Upon cleavage, the target is released unmodified. For proteins, that is ideal. In the case of an oligonucleotide, the starting amino-oligonucleotide post-conjugation could potentially be detected as a result of non-quantitative conjugation or photocleavage. This has the potential to complicate the reaction yield. With that said, we found that PC Biotin NHS Ester was stable to ambient lighting.

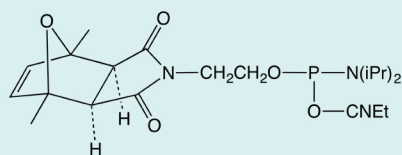
PC Biotin NHS Ester is compatible with our general protocol, previously described.<sup>2</sup> For a 0.2  $\mu$ mole synthesis of an amine-modified oligonucleotide:

1. Dissolve oligonucleotide in 500  $\mu$ L of 0.1 M sodium bicarbonate (pH 9).
2. Dissolve 5–10 eq of NHS ester in 25  $\mu$ L DMF or DMSO.
3. Add NHS ester solution to oligonucleotide solution.
4. Agitate the mixture and incubate at room temperature for 1–2 hrs.
5. Separate oligo-conjugate from salts and excess label by size exclusion on a Glen Gel-Pak™ desalting column or equivalent.

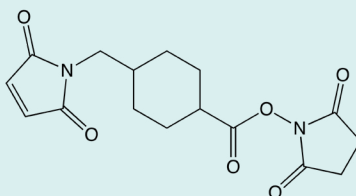
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**5'-Maleimide Modifier Phosphoramidite (10-1938)**



**Maleimide NHS Ester (50-1938)**

**Figure 3. Maleimide Products**

### Maleimide NHS Ester (SMCC)

Maleimide NHS Ester (SMCC) is similar to our other non-dye NHS esters, in that it is a dual reactive molecule used in a subsequent conjugation reaction. An amine-containing target reacts with the NHS ester group before the maleimide reaction occurs, which selectively targets free thiols at neutral pH.

We already have a protected maleimide modifier available as a phosphoramidite (10-1938).<sup>3</sup> This modifier amidite requires a two-step deprotection. First the oligonucleotide deprotection takes place in ammonia and then a retro Diels-Alder reaction must occur before the maleimide can be conjugated to a thiol. This retro Diels-Alder deprotection takes place in toluene at high heat (90 °C) or microwave irradiation. For those who wish to avoid this second deprotection scheme, the Maleimide NHS Ester is an excellent alternative as the maleimide is not introduced until after oligonucleotide deprotection. The Maleimide NHS Ester contains a cyclohexane linker, which decreases the rate of maleimide hydrolysis compared to similar compounds lacking this ring (Figure 3).

Maleimides are very susceptible to hydrolysis at high pH. This conflicts with our previous protocol for NHS ester reaction at basic pH. Therefore, we have an updated protocol for the use of Maleimide NHS Ester at neutral pH to prevent hydrolysis of the maleimide during the NHS ester conjugation reaction.

For a 0.2 μmole synthesis of an amine-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 Maleimide NHS ester (SMCC) in 250 μL DMSO.
3. Add NHS ester solution to oligonucleotide solution.
4. Agitate the mixture and incubate at room temperature for 30-60 min.
5. Separate oligo-conjugate from salts

and excess label by size exclusion on a Glen Gel-Pak™ desalting column or ethanol precipitation.

Due to maleimide instability to hydrolysis, we recommend maleimide conjugation soon after the NHS ester reaction. For the conjugation of the maleimide with small molecule thiols, our general protocol can be used. For a 0.2 μmole synthesis of a maleimide-containing oligonucleotide:

1. Dissolve oligonucleotide in 450 μL of aqueous buffer\* (pH 6.5-7.8).
2. Dissolve 5-10 eq. of thiol compound in 50 μL of compatible solvent.
3. Add thiol solution to oligonucleotide solution.
4. Agitate the mixture and incubate at room temperature for 30-60 min.
5. Separate oligo-conjugate from salts and excess label by size exclusion on a Glen Gel-Pak™ desalting column or equivalent.

\*We have successfully used sodium phosphate buffer (pH 6.5) and triethylammonium acetate buffer (pH 7.0) for maleimide functionalization.

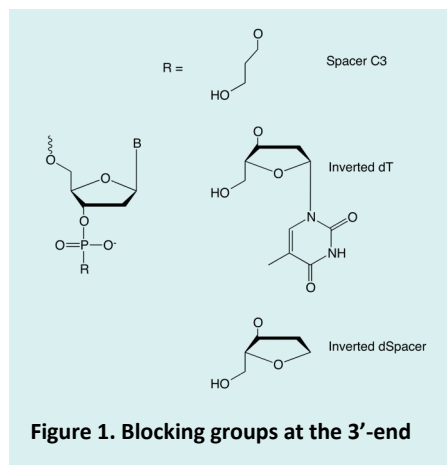
### References

1. *The Glen Report*, 2001, **14.1**, 8-9.
2. *The Glen Report*, 2020, **32.2**, 9-10.
3. *The Glen Report*, 2011, **23.2**, 6.

Item	Pack Size	Catalog No.
PC Biotin NHS Ester	4.2 mg	50-4950-22
Maleimide NHS Ester (SMCC)	3.3 mg	50-1938-23

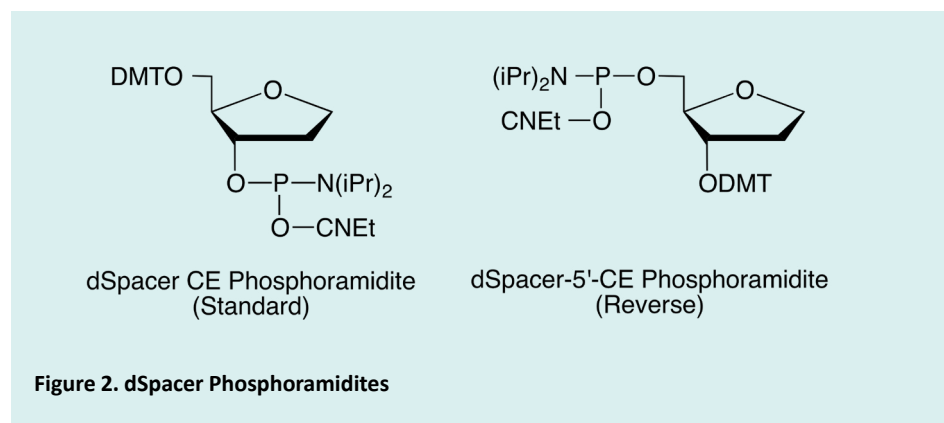
## New Product — dSpacer-5'-CE Phosphoramidite (Reverse dSpacer)

When researchers are synthesizing oligonucleotides for their therapeutic studies, they will often have to place blocking groups on the 3'-end and sometimes at the 5'-end as well. An inverted dT is commonly used to protect oligonucleotides from exonucleases, and a Spacer C3 is often used to prevent polymerase extension. A third option that one might consider is the use of an inverted dSpacer. It shares the same three carbon skeleton of Spacer C3 while omitting the thymine base from the inverted dT (Figure 1).



**Figure 1. Blocking groups at the 3'-end**

There are many examples of researchers using inverted dSpacer modifications in the literature. This has been particularly true for siRNA.<sup>1-3</sup> Both ends of the passenger strand and the 3'-end of the guide strand can all be capped by the addition of an inverted dSpacer to enhance nuclease stability. Incorporating an inverted dSpacer at the 5'-end of the passenger strand also serves to block loading into Ago2, which minimizes competition between the guide and



**Figure 2. dSpacer Phosphoramidites**

passenger strands. All three incorporations do not reduce the gene silencing effect. Similarly, the passenger strand of small activating RNAs (saRNAs) can also be modified with 5'/3' inverted dSpacers.<sup>4</sup> Finally, the hammerhead ribozyme has been modified with a 3' inverted dSpacer.<sup>5, 6</sup> Studies using inverted dT have demonstrated greater than 30-fold increase in serum half-times with no reduction in catalysis,<sup>7</sup> and the inverted dSpacer should yield similar stability enhancement.

There are two approaches to introducing an inverted dSpacer into oligonucleotides. The first approach would be to use our dSpacer CE Phosphoramidite (Figure 2) along with reverse phosphoramidites. The standard dSpacer is typically used to give stable abasic sites for the study of structure as well as DNA damage. When paired with a reverse 5'-3' synthesis direction, inverted dSpacer linkages are obtained. The second, and probably more logical approach would be to use a reverse dSpacer phosphoramidite in the standard 3'-5' synthesis direction. This way, only one reverse phosphoramidite is required.

To give our customers more access to the inverted dSpacer modification as well as blocking groups in general, we are introducing dSpacer-5'-CE Phosphoramidite (Figure 2). Like our standard version, our reverse dSpacer does not require any extended coupling times and is compatible with all standard deprotection conditions.

### References

1. H.J. Haringsma, *et al.*, *Nucleic Acids Res*, 2012, **40**, 4125-36.
2. Y. Pei, *et al.*, *RNA*, 2010, **16**, 2553-63.
3. F. Czauderna, *et al.*, *Nucleic Acids Res*, 2003, **31**, 2705-16.
4. S. Yoon, and J.J. Rossi, *Curr Pharm Biotechnol*, 2018, **19**, 604-610.
5. T.C. Jarvis, *et al.*, *J Biol Chem*, 1996, **271**, 29107-12.
6. D.G. Macejak, *et al.*, *Hepatology*, 2000, **31**, 769-76.
7. L. Beigelman, *et al.*, *J Biol Chem*, 1995, **270**, 25702-8.

Item	Pack Size	Catalog No.
dSpacer CE Phosphoramidite	100 µmol	10-1914-90
	0.25 g	10-1914-02
dSpacer-5'-CE Phosphoramidite	100 µmol	10-4191-90
	0.25 g	10-4191-02



## Technical Note — Purification of 5'-Labeled Oligonucleotides Using Glen-Pak DNA Purification Cartridges

Reverse-phase chromatography is a powerful technique that involves the separation of molecules based on their degree of hydrophobicity. It can be used to separate a wide range of molecules, including proteins and nucleic acids. Many purification tools utilize this method such as reverse-phase high-performance liquid chromatography (RP-HPLC) and solid phase extraction (SPE) cartridges. For the former, a hydrophobic reverse-phase column “stationary phase” is used to retain hydrophobic molecules while allowing the hydrophilic molecules to elute quickly through the column by a hydrophilic “mobile phase”. Molecules containing different hydrophobicity levels elute from the column by the gradual increase of organic solvent in the mobile phase. For the latter, the same principles apply, except there is no gradient. Typically, the initial mobile phase allows the molecule of interest to remain bound to the column while unwanted, more polar entities are washed away. The column is then treated with a much more hydrophobic solvent to recover the desired material. In some ways, SPE cartridges are a crude method of performing RP-HPLC for purification of species where there is a very large difference in polarity.

In the oligonucleotide purification space, an SPE cartridge such as our Glen Pak™ cartridge is often all that is required. The 5'-dimethoxytrityl (DMT) group retained on the oligonucleotide during solid phase synthesis can be a significant source of the oligonucleotide's hydrophobicity, allowing differentiation between desired full length DMT-ON sequences versus undesired shorter DMT-OFF sequences. Upon loading onto the cartridge, the DMT-ON sequence binds to the support while the failure

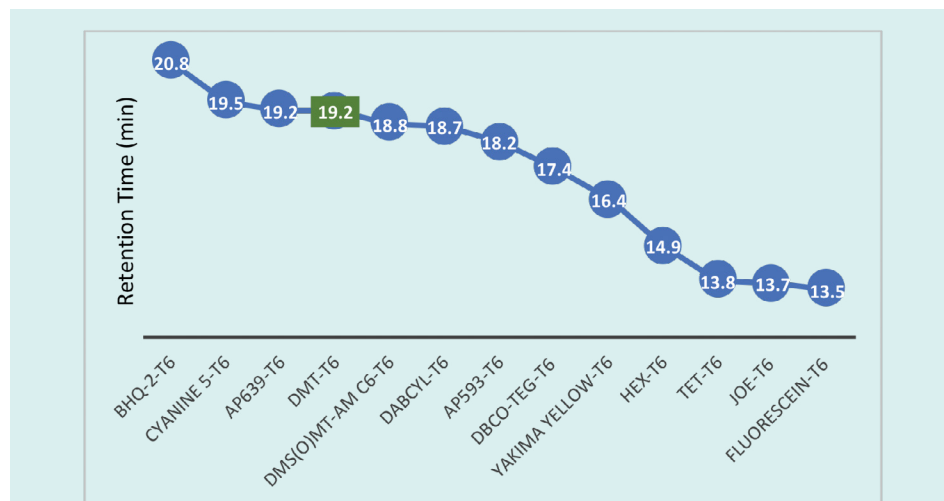


Figure 1. 5'-Labeled T<sub>6</sub> Oligonucleotide Retention Times (3-40 % ACN gradient)

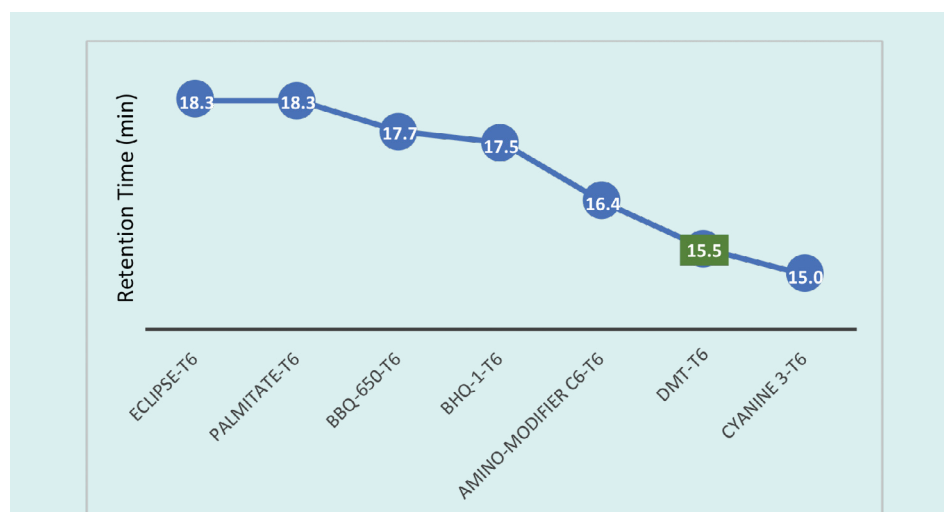


Figure 2. 5'-Labeled T<sub>6</sub> Oligonucleotide Retention Times (3-60 % ACN gradient)

sequences are washed away. As a bonus, the removal of the DMT group can also be performed on the cartridge, allowing full length, DMT-OFF product to be isolated without any additional downstream steps. While the DMT group is an excellent hydrophobic handle for purification on cartridges, in theory, any modification of equivalent or greater hydrophobicity should be able to serve the same purpose.

In this article, we aim to assess what other 5'-hydrophobic modifications may be compatible with the Glen-Pak purification process. The

retention times (RT) observed during RP-HPLC analysis offer good estimates of hydrophobicity for SPE cartridge purification purposes. Figures 1 and 2 show the retention times of many 5'-labeled T<sub>6</sub> sequences, relative to a 5'-DMT-T<sub>6</sub> standard. This represents a starting point to determine which modifications are hydrophobic enough to consider purifying with cartridges. RP-HPLC was performed with a gradient of acetonitrile (ACN) in 0.1 M triethylammonium acetate (TEAA). Figure 1 data was generated with a gradient of 3-40 % ACN over 20 minutes while Figure 2 data was generated with a gradient of 3-60 % ACN also over 20 minutes.





Looking at the data, the observed retention times support what we already know. Our User Guide to Glen-Pak™ Purification provides detailed purification methods that cover, among others, the 5'-modifications of DMS(O)MT-Amino-Modifier C6, 5'-Amino-Modifier C6, Cyanine 5, and Dabcyl.<sup>1</sup> The RT's for these 5'-modifications are either later than or comparable to that of the DMT-T<sub>6</sub>. For instance, Cyanine 5 (Figure 3) has an RT that is slightly later than that of the DMT-T<sub>6</sub>, and the 5'-Cyanine oligonucleotide is indeed compatible with the Glen-Pak purification method. On the other end of the spectrum, the 5'-Fluorescein, TET, and HEX labels, which we know do not work with this purification process,<sup>1</sup> have much earlier RTs compared to that of the DMT-T<sub>6</sub>. These fluorescein type dyes are simply not very hydrophobic, and our recommendation has always been to use Poly-Pak™ II Cartridges instead, where a somewhat different reverse phase purification is carried out.<sup>2</sup>

We can now apply this knowledge to other modifications. Based on the RT data and the relevant hydrophobicity to the DMT-T<sub>6</sub>, we anticipate that the rhodamine dyes AquaPhluor® 593 and 639 (AP593/AP639) should work fine with Glen-Pak cartridges (Figure 1). Quenchers (BHQ-1, BHQ-2, Eclipse, and BBQ-650) also appear to be compatible (Figure 2). With a relatively late RT, Palmitate should work as well. Furthermore, DBCO- and Yakima Yellow-labeled oligonucleotides are less hydrophobic and are probably more challenging for Glen-Pak purification. RP-HPLC purification may be a safer/better alternative for these two modifications. Finally, the JOE modification (Figure 3) yielded a RT consistent with the rest of the fluorescein family and will not be compatible with Glen-Pak cartridges.

Although this is not a comprehensive list of all the labels we offer, we believe the major groups are covered, and the compatibility of other groups can be deduced accordingly. For example, if the Palmitate (Figure 3) is compatible with Glen-Pak purification, other fatty groups like Stearyl and Cholesterol should also work. Likewise, if AP593 (Figure

3) is compatible, then fellow rhodamine TAMRA is likely to work as well.

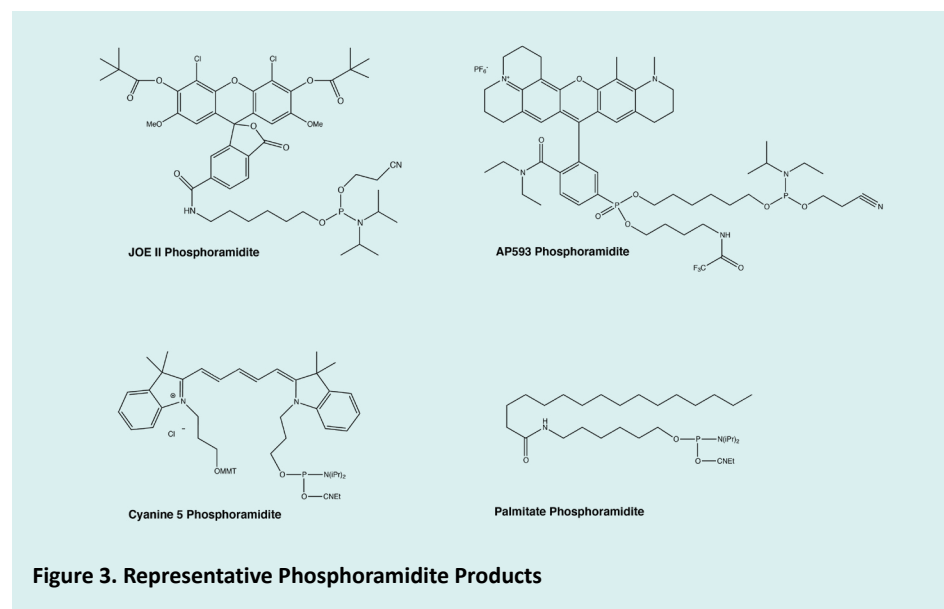
As noted earlier, cartridge purification is a crude but convenient purification method. Even if the purification handles are sufficiently hydrophobic, performance can still vary based on the sequence length as well as sequence content. Longer oligonucleotides will be more hydrophilic, reducing the impact of hydrophobic labels on overall polarity. Also, the presence of other modifications can interfere as in the case of dual-labeled fluorescent probes, such as those used in Fluorescence Resonance Energy Transfer (FRET).<sup>3</sup> If the hydrophobicity of the 3'-label is similar to or greater than that of DMT (Figures 1-2), the DMT-OFF failure oligonucleotide sequences will bind to the support and co-purify with the desired product. The

same effect applies to internally placed hydrophobic modifications.

In summary, the relative RP-HPLC RT's of 5'-labeled oligonucleotides appear to be reliable predictors of Glen-Pak compatibility, and this should be considered as a planning guide as one decides what purification methods to employ. As always, one may also consider using Glen-Pak purification in tandem with another purification method in order to achieve their ultimate desired purity.

## References

1. *The Glen Report*, 2010, **22.1**, 15-16.
2. *The Glen Report*, 1999, **12.1**, 6-8.
3. *The Glen Report*, 2004, **17.1**, 4-6.



Item	Pack Size	Catalog No.
5'-Amino-Modifier C6	100 µmol	10-1906-90
	0.25 g	10-1906-02
5'-AquaPhluor® 593 CE Phosphoramidite	50 µmol	10-5923-95
	100 µmol	10-5923-90
	0.25 g	10-5923-02
5'-AquaPhluor® 639 CE Phosphoramidite	50 µmol	10-5926-02
	100 µmol	10-5926-95
	0.25 g	10-5926-90

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Item	Pack Size	Catalog No.
5'-BBQ-650®-CE Phosphoramidite	50 µmol	10-5934-95
	100 µmol	10-5934-90
	0.25 g	10-5934-02
5'-BHQ-1 Phosphoramidite	50 µmol	10-5931-95
	100 µmol	10-5931-90
	0.25 g	10-5931-02
5'-BHQ-2 Phosphoramidite	50 µmol	10-5932-95
	100 µmol	10-5932-90
	0.25 g	10-5932-02
5'-DabcyI Phosphoramidite	50 µmol	10-5912-95
	100 µmol	10-5912-90
	0.25 g	10-5912-02
5'-DBCO-TEG Phosphoramidite	50 µmol	10-1941-95
	100 µmol	10-1941-90
	0.25 g	10-1941-02
5'-Dichloro-dimethoxy-Fluorescein Phosphoramidite II (JOE II)	50 µmol	10-5906-95
	100 µmol	10-5906-90
	0.25 g	10-5906-02
5'-DMS(O)MT-Amino-Modifier C6	100 µmol	10-1907-90
	0.25 g	10-1907-02
5'-Fluorescein Phosphoramidite	50 µmol	10-5901-95
	100 µmol	10-5901-90
	0.25 g	10-5901-02
5'-Hexachloro-Fluorescein Phosphoramidite	50 µmol	10-5902-95
	100 µmol	10-5902-90
	0.25 g	10-5902-02
5'-Tetrachloro-Fluorescein Phosphoramidite	50 µmol	10-5903-95
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	0.25 g	10-5903-02
Cyanine 3 Phosphoramidite	50 µmol	10-5913-95
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Cyanine 5 Phosphoramidite	50 µmol	10-5915-95
	100 µmol	10-5915-90
	0.25 g	10-5915-02
Eclipse® Quencher Phosphoramidite	50 µmol	10-5925-95
	100 µmol	10-5925-90
	0.25 g	10-5925-02
Palmitate Phosphoramidite	50 µmol	10-1978-95
	100 µmol	10-1978-90
	0.25 g	10-1978-02
Yakima Yellow® Phosphoramidite	50 µmol	10-5921-95
	100 µmol	10-5921-90
	0.25 g	10-5921-02

## Product Review — Rhodamine Dyes

In our previous volume, 34.2, fluorescein dyes were discussed.<sup>1</sup> Another popular family of fluorophores is the rhodamine dyes. They are bright dyes that are used in many of the same applications where fluorescein dyes are used including water tracing, paper/textile coloring, food additives (illegally) and biotechnology. The last of these is, of course, the most interesting for our customers.

TAMRA is the most common of the rhodamine dyes, at least for oligonucleotide labeling. It is short for TetrA Methyl RhodAmine and consists of rhodamine and four methyl substitutions on the two exocyclic amines (Figure 1). Like most rhodamine dyes, the structure exists as predominantly an equilibrium between the open, zwitterionic fluorescent form (right) and the closed non-fluorescent lactone (left). The small molecule dye has excitation and emission wavelengths of 556 and 580 nm, respectively, and exhibits a pink color. It should be noted that the TAMRA excitation overlaps well with the emission of fluorescein. Although we typically refer to TAMRA as a fluorophore, it is very commonly used as a quencher for fluorescein and its substituted derivatives such as TET and Yakima Yellow® in dual-labeled probes.

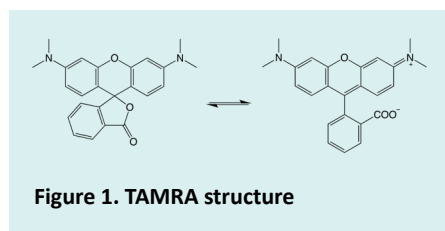
Glen Research offers three options for TAMRA labeling. There are 3'-TAMRA CPG/PS supports to facilitate 3'-labeling. There is also a TAMRA-dT that can be placed anywhere in the oligonucleotide. Finally, there is an NHS ester that can be attached to any one of our many amino-modifiers via post-synthetic modification. This last approach serves two important purposes. 1. Since there is no 5'-TAMRA phosphoramidite available, the NHS ester is the default choice for 5'-labeling. 2. Due to the incompatibility of TAMRA with standard ammonia-based deprotection methods, the NHS ester approach gives researchers additional deprotection options for more challenging to synthesize sequences.





Glen Research also carries other derivatives of rhodamine (Figure 2). Just like our fluorescein family, these derivatives have substitutions on the aromatic rings that red-shift the spectral properties of the fluorophores (Table 1). ROX has additional alkyl rings derived from julolidine and is available as an NHS ester to facilitate post-synthetic conjugation. The core structure of ROX is also available in the form of a 5'-phosphoramidite and CPG as AquaPhluor® 593 (AP593). Further substitution and modification of AP593 gives rise to AquaPhluor® 639 (AP639).

AP593 and AP639 are particularly robust products in the fluorophore offerings. Although they share the same core structure as TAMRA, the AquaPhluors do not share the same deprotection condition susceptibilities. In general, both AMA and ammonium hydroxide are compatible. AP593 essentially allows ROX to be incorporated with no post-synthetic manipulations while AP639 is a rhodamine alternative to Cyanine 5, which is another popular fluorophore that is not very stable under oligonucleotide deprotection conditions.



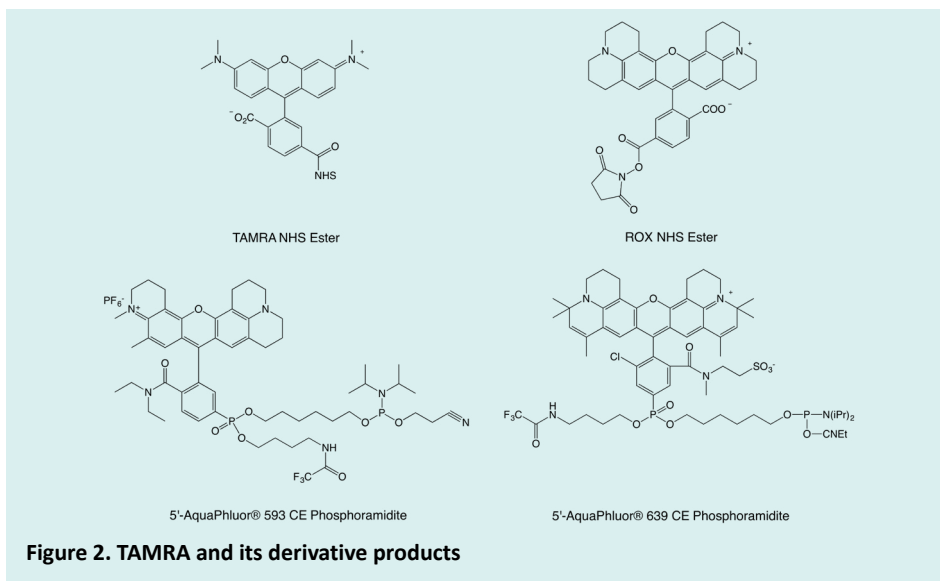
Rhodamine dyes continue to serve as important members of our fluorophore offerings and complement the other dye families very well.

## References

1. *The Glen Report*, 2022, **34.2**, 8-9.

**Table 1. Spectral properties of TAMRA and its derivatives**

	Absorbance Maximum (nm)	Emission Maximum (nm)	Color
TAMRA	556	580	Pink
ROX	588	608	Magenta
AquaPhluor® 593	593	613	Purple
AquaPhluor® 639	639	655	Blue



Item	Pack Size	Catalog No.
3'-TAMRA CPG	0.1 g	20-5910-01
	1.0 g	20-5910-10
1 µmol columns	Pack of 4	20-5910-41
0.2 µmol columns	Pack of 4	20-5910-42
TAMRA-dT	50 µmol	10-1057-95
	100 µmol	10-1057-90
	0.25 g	10-1057-02
TAMRA NHS Ester	60 µL	50-5910-66
ROX NHS Ester	3.2 mg	50-5911-22
5'-AquaPhluor® 593 CE Phosphoramidite	50 µmol	10-5923-95
	100 µmol	10-5923-90
	0.25 g	10-5923-02
5'-AquaPhluor® 639 CE Phosphoramidite	50 µmol	10-5926-95
	100 µmol	10-5926-90
	0.25 g	10-5926-02



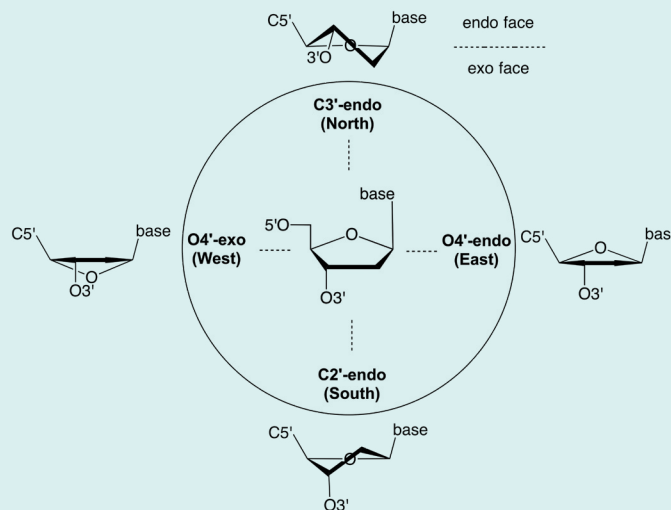
## Technical Note — Sugar Conformations and Modifications

In Glen Report 33.1 we discussed the standard numbering system for nucleobases in DNA and RNA nucleotide building blocks.<sup>1</sup> In this note, we would like to take a closer look at another necessary component in the nucleotide: the sugar ring. One of the few structural differences between DNA and RNA is the lack of a 2'-hydroxyl group in the ribose ring of DNA. This seemingly minor change in the structure has significant biological consequences.

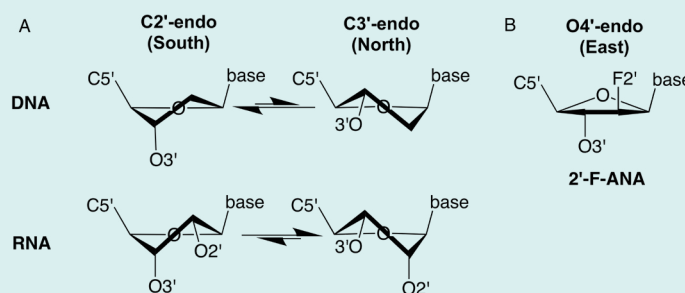
Conformation and dynamics of the (deoxy)ribose impact the overall structure of the nucleic acid, which contributes to recognition and function of the genomic material. The presence of a 2'- functional group influences the more favorable sugar pucker conformation.<sup>2</sup> In turn, the sugar pucker defines the predominant structure adopted upon base pairing interactions (Figure 1). Four conformations are possible: C3'-endo, C2'-endo, O4'-exo and O4'-endo.

DNA adopts a C2'-endo sugar pucker (also referred to as the South conformer), which corresponds to the favored B-form of DNA, where base pairs are almost centered over the helical axis (Figure 2A). In contrast, RNA consists of the C3'-endo sugar pucker (North conformer) due to the size of the C2'-substitution (Figure 2A). As a result, the nucleic acid adopts an A-form, where base pairs are displaced away from the central axis and closer to the major groove. A-form RNA resembles a ribbon-like helix with an open center whereas B-form DNA is the canonical double helix.

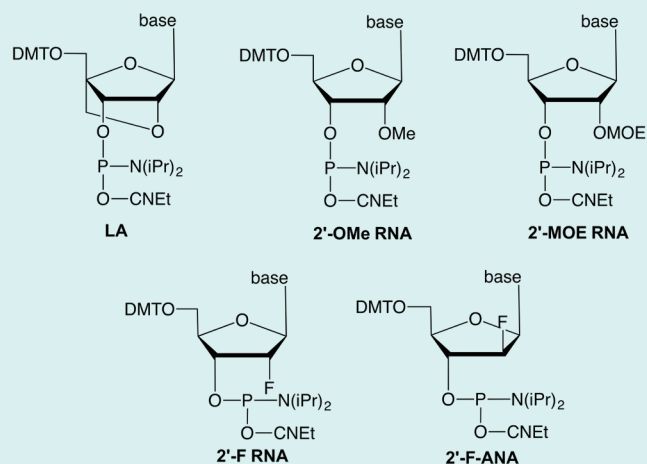
Recently, more sugar modifications have been described and studied, especially due to their relevancy in oligonucleotide therapeutics. For this article, we will focus on the sugar modifications that we offer: Locked Analog (LA), 2'-OMe, 2'-MOE, 2'-F RNA, and 2'-F-ANA (Figure 3).



**Figure 1. Sugar Pucker Conformations**



**Figure 2. Sugar Conformers**



**Figure 3. Sugar modifications available at Glen Research**



We have previously reported on the benefits of our locked analog series.<sup>3,4</sup> Locked nucleic acids offer enhanced binding and allow controlled manipulation of melting temperatures. Our LA modifications are often used in hybridization probes but are also becoming more popular in antisense oligonucleotide therapy. LNA modifications reinforce the C3'-endo conformation. This backbone is so popular that we recently introduced the LA solid supports to accompany our LA phosphoramidites.<sup>4</sup>

Our 2'-alkoxy modifications include 2'-OMe and 2'-MOE. Nucleic acids bearing a 2'-OMe occur naturally in tRNA and other post-translationally modified small RNAs. The same cannot be said for 2'-MOE. The substitution of 2'-hydroxyl to 2'-O-alkyl results in increased chemical and duplex stability and nuclease resistance. The low toxicity of these modifications makes them attractive backbones for therapeutic candidates. Duplexes with 2'-OMe or 2'-MOE may give rise to additional stabilizing forces from base stacking and hydrophobic interactions in the minor groove.<sup>2</sup> However, the 2'-O-alkyl substituents have not been shown to enhance base pairing.

Depending on the orientation of the substituent, the C2'-F group will determine the sugar conformation due to its polarity, rather than its size. The high electronegativity of the fluoride draws the C2'-bonds towards it.<sup>2</sup> Due to these interactions, 2'-F-RNA modifications adopt the standard C3'-endo pucker. Conversely, 2'-F-ANA adopt a B-form helix, although it is through an O4'-endo pucker (Figure 2B). The 2'-F substitutions provide enhanced thermal stability and binding effects. Out of the two, only the 2'-F arabinose substituents are resistant to nucleases. Modifications with fluorine substituents can be monitored by NMR.

Overall, modifications on the furanose ring can yield subtle to major effects on the structure and function of the nucleic acid. Properties such as resistance to

nucleases, enhanced chemical and thermal stability, and the strength of base pairing interactions, can easily be fine-tuned to best fit one's application needs (Table 1).

## References

1. *The Glen Report*, 2021, **33.1**, 8.

2. Evich, M., Spring-Connell, A., Germann, M. *Heterocyclic Communications*, 2017, **23**, 155-165.

3. *The Glen Report*, 2018, **30.2**, 8-9.

4. *The Glen Report*, 2022, **34.3**, 3-5.

**Table 1. Overview of standard and modified sugar backbones available at Glen Research**

Backbone	Sugar Pucker	Form (A or B)	Nuclease Resistance	Thermal Stability	Provides Stronger Base Pairing
DNA	C2'-endo	B			
RNA	C3'-endo	A			
LA	C3'-endo	A	✓	✓	✓
2'-OMe	C3'-endo	A	✓	✓	
2'-MOE	C3'-endo	A	✓	✓	
2'-F	C3'-endo	A		✓	✓
2'-F-ANA	O4'-endo	B	✓	✓	✓

Modification	Product (Phosphoramidites only)	Catalog No.
Locked Analog (LA)	Bz-A-LA-CE	10-2000
	Bz-5-Me-C-LA-CE	10-2011
	Dmf-G-LA-CE	10-2029
	T-LA-CE	10-2030
2'-OMe	2'-OMe-A-CE	10-3100
	2'-OMe-Ac-C-CE	10-3115
	2'-OMe-iBu-G-CE	10-3120
	2'-OMe-G-CE	10-3121
	2'-OMe-U-CE	10-3130
	2'-OMe-Pac-A-CE	10-3601
	2'-OMe-iPr-Pac-G-CE	10-3621
2'-MOE	A-2'-MOE	10-3200
	5-Me-C-2'-MOE	10-3211
	G-2'-MOE	10-3220
	5-Me-U-2'-MOE	10-3231
2'-F-RNA	2'-F-A-CE	10-3400
	2'-F-Ac-C-CE	10-3415
	2'-F-G-CE	10-3420
	2'-F-U-CE	10-3430
2'-F-ANA	2'-F-A-ANA-CE	10-3800
	2'-F-Ac-C-ANA-CE	10-3815
	2'-F-G-ANA-CE	10-3820
	2'-F-U-ANA-CE	10-3830
	2'-F-5-Me-U-ANA-CE	10-3850

## Technical Snippets

### What is the effect of particulates on oligonucleotide synthesis?

The existence of particulates in oligonucleotide reagents such as diluents, deblocking solutions, activators, capping reagents, and oxidizers can cause the clogging of the oligonucleotide synthesizer lines. The clogging of any individual line would of course be highly detrimental to the synthesis. For instance, if the deblock line is fully clogged, the deblock will not reach the column, and the synthesis will not proceed any further.

To prevent particulate contamination, we filter all our reagents through 0.2  $\mu\text{M}$  filters. We also perform a visual examination to confirm that all our reagents are clear and free from particulate contamination.

#### Products:

Ancillary Reagents

### How do I determine reagent consumption?

You have likely seen Coupling Data tables on our product pages and quality documents that describe roughly how many additions one can achieve using a certain pack size on various synthesis scales for ABI and Expedite instruments. These tables account for material required for sufficient priming. If you are looking to estimate the amount of material you need for a synthesis for parameters outside of those tables, here are some tips:

- At 0.1M amidite, one coupling that uses about 100  $\mu\text{L}$  consumes roughly 10 mg (10  $\mu\text{mol}$ ) amidite. This approximation is based on a 1  $\mu\text{mol}$  scale synthesis and assumes the amidite has a molecular weight of  $\sim 1000$  g/mol. In this case, 10 mg comes out to 10 equivalents.
- Amidite consumption will also depend on the scale you are working on. For any given instrument, amidite usage is less efficient at smaller scales (Table 1). These are rough numbers and are meant to represent a good place to start when trying to estimate how much material you need for your synthesis.
- Liquid reagent consumption is largely dictated by the synthesizer. See below for some estimates on reagent consumption for two different instruments that operate on different scales (Table 2).
- Please keep in mind that reagent consumption is ultimately determined by the synthesizer. When in doubt, consider reaching out to your synthesizer manufacturer for a clear estimate of how much reagent is needed for your synthesis.

**Table 1. Amidite consumption by scale**

Scale ( $\mu\text{mol}$ )	Amidite Consumption (mg)
1.0	10
0.2	7.5
0.05	5

**Table 2. Reagent consumption estimations**

Reagent	ABI (1.0 $\mu\text{mol}$ )	ABI (0.2 $\mu\text{mol}$ )	Dr. Oligo (0.2 $\mu\text{mol}$ )
Amidite	150 $\mu\text{L}$	110 $\mu\text{L}$	100 $\mu\text{L}$
Deblock	1.86 mL	1.32 mL	400 $\mu\text{L}$
Activator	370 $\mu\text{L}$	400 $\mu\text{L}$	200 $\mu\text{L}$
Cap A	250 $\mu\text{L}$	290 $\mu\text{L}$	100 $\mu\text{L}$
Cap B	230 $\mu\text{L}$	250 $\mu\text{L}$	100 $\mu\text{L}$
Oxidizer	420 $\mu\text{L}$	410 $\mu\text{L}$	150 $\mu\text{L}$