New product development

GLEN RESEARCH

part of Maravai LifeSciences

Innovation for
nucleic acid chemistry

Three years of product innovation at Glen Research

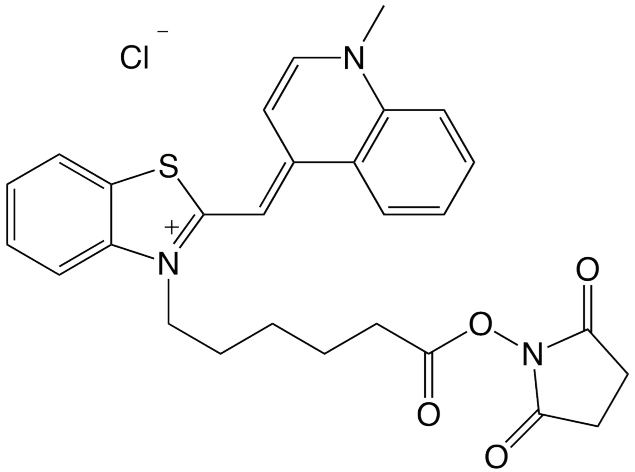
We are unlocking the power of genomics with academic and commercial research professionals worldwide.

As the leader in DNA/RNA nucleotide synthesis supplies and support for more than three decades, Glen Research offers the industry's broadest range of oligonucleotide materials and custom products, as well as hands-on, scientific consultation.

Thiazole Orange NHS Ester

Up to 8.6x hybridization fluorescent enhancement

Improvements in fluorogenic hybridization probe methodologies have great potential in the field of nucleic acid-based diagnostics, and in this context, thiazole orange has been the focus of intense study. Thiazole orange (TO) is a fluorescent asymmetric cyanine dye with an excitation peak at 514 nm and an emission peak at 533 nm and produces far greater fluorescence upon hybridization. Glen Research has added Thiazole Orange NHS Ester to our offerings in order for researchers to better explore the use of TO in the fluorescence imaging of DNA and RNA.



- Hybridization of TO is accompanied by a fluorescent enhancement of up to 8.6-fold, with an average of 3.9-fold
- Strongly stabilizes DNA duplexes which allows for the use of shorter probes in sequence specific recognition of target nucleic acids; the stabilizing effects of TO are additive when tethered to triplex forming oligonucleotides
- TO oligo conjugates have been used in combination with other fluorophores for highly sensitive, multi-color detection of DNA and RNA targets

References:

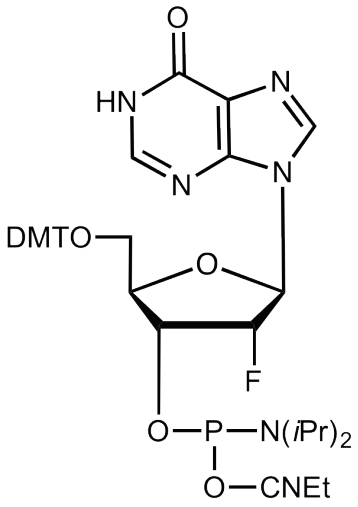
Qiu, J. Q., Wilson, A., El-Sagheer, A. H. & Brown, T. (2016). Combination probes with intercalating anchors and proximal fluorophores for DNA and RNA detection. Nucleic Acids Res. 44, e138.

Klimkowski, P., De Ornellas, S., Singleton, D., El-Sagheer, A. H. & Brown, T. (2019). Design of thiazole orange oligonucleotide probes for detection of DNA and RNA by fluorescence and duplex melting. Org. Biomol. Chem. 17, 5943-5950.GR 32.12

2’-Fluoro-Inosine-CE Phosphoramidite

Degenerate base replacement with greater stability over traditional inosine

Inosine and 2’-deoxyinosine are non-standard, naturally occurring nucleotides that contain the nucleobase hypoxanthine. Inosine is considered a universal base, as it can interact with each of the standard bases via two hydrogen bonds. The interactions in order of decreasing stability are I-C > I-A > I-T ≈ I-G. To complement our existing DNA, RNA, and 2’-OMe versions of inosine, we have added the 2’-F version.



- Due to its base pairing properties, inosine is used extensively in applications involving synthetic primers or probes (e.g. PCR)
- When the target position is ambiguous, an inosine can be used to maximize hybridization, as an alternative to degenerate oligonucleotides
- This 2’-F version of inosine can be used in the same way as standard 2’-F phosphoramidites and has greater stability over inosine in oligonucleotides

References:

F.H. Martin, M.M. Castro, F. Aboul-ela, and I. Tinoco, Jr., Nucleic Acids Res, 1985, 13, 8927-38.

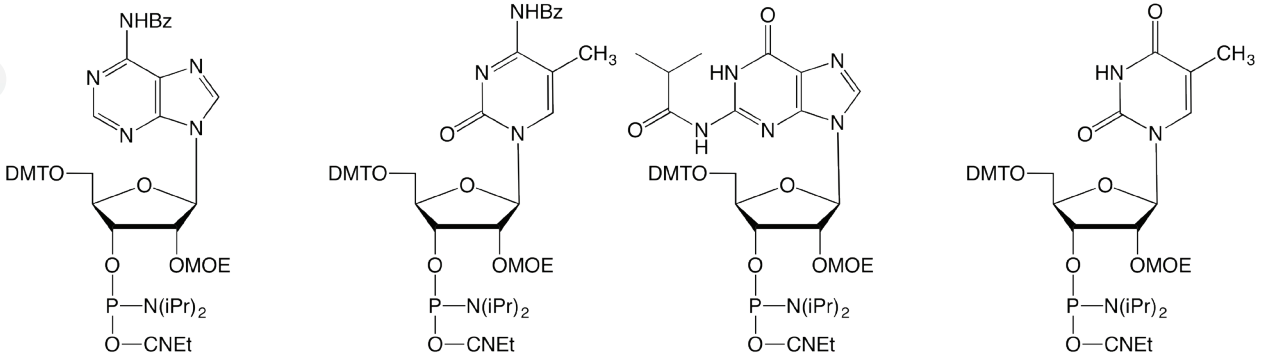
E. Ohtsuka, S. Matsuki, M. Ikehara, Y. Takahashi, and K. Matsubara, J Biol Chem, 1985, 260, 2605-8.

R.V. Patil, and E.E. Dekker, Nucleic Acids Res, 1990, 18, 3080.

2’-MOE RNA Phosphoramidites

Affinity, specificity, and nuclease stability

2’-O-methoxyethyl-RNA (2’-MOE) favors the formation of A-form, RNA-like double helices, which results in enhanced duplex stability when paired with RNA targets. Increased nuclease resistance and low toxicity make 2’-MOE an attractive backbone modification for many oligonucleotide drug candidates, three of which have been approved by the FDA. As research with 2’- MOE continues to be conducted, Glen Research made the backbone more accessible by adding the 2’-MOE phosphoramidites of A, 5-Me-C, G and 5-Me-U.



- Enhanced duplex stability
- Significantly increased nuclease resistance
- Relatively non-toxic

References:

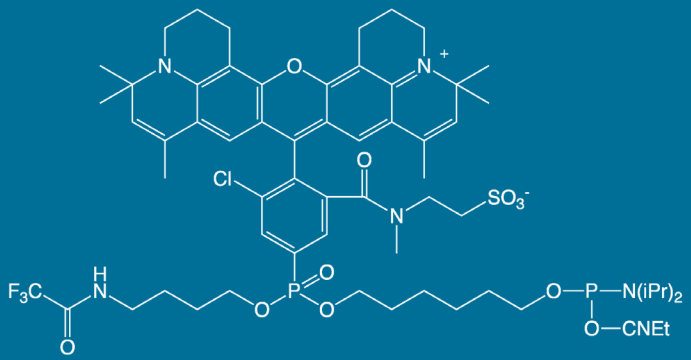
C.A. Stein, and D. Castanotto, Mol Ther, 2017, 25, 1069-1075.

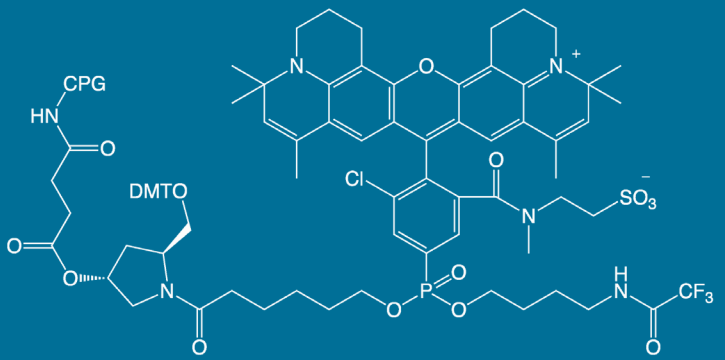
V. Mathew, and A.K. Wang, Drug Des Devel Ther, 2019, 13, 1515-1525.

AquaPhluor® 639 5’-Phosphoramidite and CPG

The logical alternative to Cyanine 5 for fluorescence-based applications

AquaPhluor® 639 (AP 639) is a red-shifted rhodamine dye and a logical alternative to Cyanine 5. Like Cyanine 5, AP639 is relatively bright and exhibits sequence- and pH-independent fluorescence. Unlike Cyanine 5, AP639 also shows excellent chemical and photochemical stability as well as temperature-independent fluorescence. In collaboration with ELITechGroup, Glen Research is pleased to offer the phosphoramidite and CPG versions of this dye for 5’ or 3’ labeling, respectively.





- AP639 is much more stable to deprotection conditions than Cyanine 5, which allows for improved synthesis yields and fewer restrictions during oligonucleotide manufacturing
- Highly resistant to photobleaching for high intensity illumination applications
- Temperature-independent fluorescence increases effectiveness significantly in higher temperature applications such as PCR

ÄKTA Capping Reagents

More effective formulations for ÄKTA capping reagents

The ÄKTA OligoPilot Plus (10/100) oligonucleotide synthesizers are popular for small to medium scale operations. The instruments are supported by UNICORN control software, and its HPLC-driven system makes it flexible and reagent-efficient. Many of our customers use ÄKTA OligoPilot systems, and we have a set of standard ÄKTA-compatible reagents. For capping, Glen Research has historically offered the following:

- Cap A 20 % Methylimidazole in Acetonitrile
- Cap B 20 % Acetic Anhydride and 30 % 2,6-Lutidine in Acetonitrile

The Cap B mixture is actually an equal mix of two solutions: 40 % acetic anhydride/acetonitrile and 60 % 2,6-lutidine/acetonitrile. Ideally, these two would be mixed by the end user prior to loading onto the instrument, but regulatory restrictions make such an option challenging. Instead, the mixing is performed at Glen Research just prior to shipping. The resulting mixture has a relatively short half-life and we recommend that the solution is used within 8 weeks of the mixing date.

To address this short shelf life, Glen Research is introducing a new set of capping reagents for the ÄKTA instrument line:

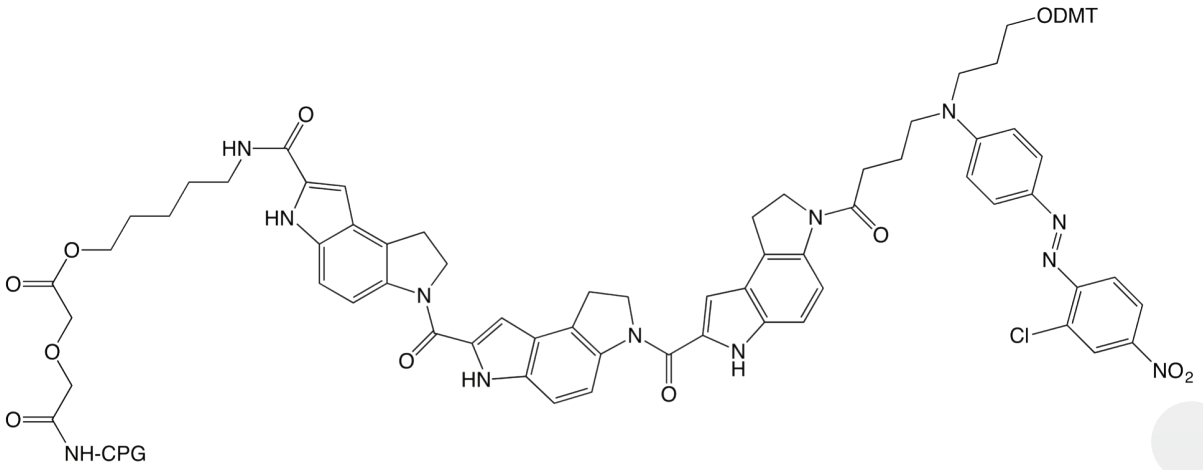
- Cap A 30 % 2,6-Luditine/20 % Methylimidazole/Acetonitrile
- Cap B 20 % Acetic Anhydride in Acetonitrile
- Cap B 5 % Phenoxyacetic Anhydride in Acetonitrile

These Cap B formulations are much more stable than our traditional offerings, with a reanalysis date of one year following release. In addition to standard capping, we are also making an UltraMild Cap B available for syntheses that require UltraMild conditions. We hope that users of ÄKTA instruments find these new formulations useful in the capping step of the oligonucleotide synthesis cycle.

MGB Eclipse® CPG

Design shorter probes with higher specificity

MGB Eclipse pairs a minor groove binding (MGB) tripeptide with a dark quencher. The MGB significantly enhances hybridization, while the Eclipse® has a broad absorption range that quenches many of the most common fluorophores, including FAM, HEX, TET and Yakima Yellow®. Together, MGB Eclipse is an attractive group for the synthesis of hydrolysis probes, the most common probe detection method for qPCR.

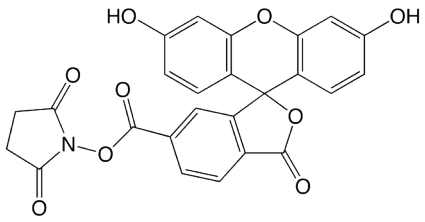


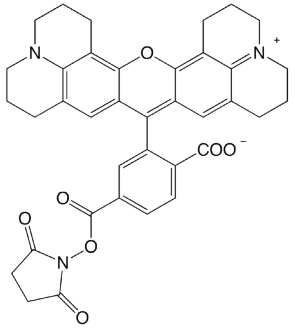
- MGB Eclipse hydrolysis probes have been used in a wide range of applications, including pathogen detection, SNP detection, viral load quantification and mutation detection
- As a combination of two modifications, MGB Eclipse exhibits spectral properties that retain the absorbances of both of its two components
- The already assembled MGB Eclipse support matches the standard MGB Eclipse structure used in qPCR probes

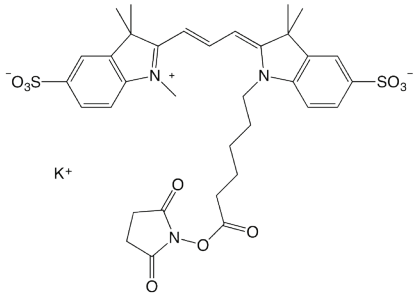
Dye NHS Esters

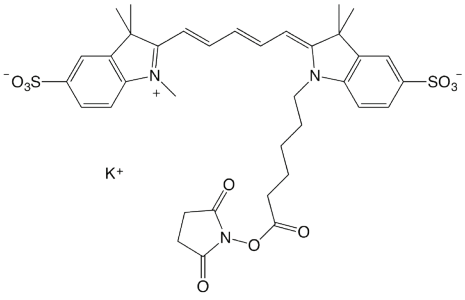
Fluorophores only available as post-synthetic modifications

Our product line of NHS ester post-synthetic modifications for fluorophore labeling has been extended with the following additions: Fluorescein, ROX, sulfoCyanine 3 and sulfoCyanine 5. Each of these dyes fluoresce in different regions of the visible spectrum and have been used extensively for the labeling of oligonucleotides, peptides, proteins and other amine-containing molecules/biomolecules. While fluorescein is widely available in phosphoramidite form, the other three dyes can only be attached to oligonucleotides by post-synthetic modification.







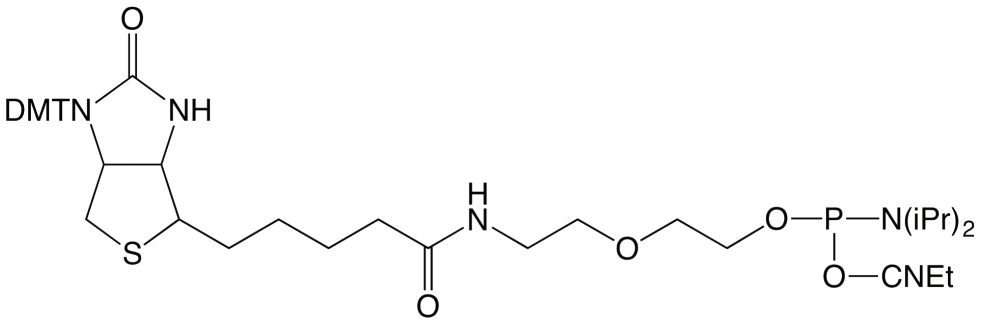


- Fluorescein NHS Ester
  - Using a 6-FAM isomer, the NHS ester approach bypasses the issue of non-fluorescent impurities forming, due to methylamine reacting with fluorescein during oligonucleotide deprotection
- ROX NHS Ester
  - Despite its significant role in dideoxy Sanger sequencing and use in qPCR applications, ROX is not available as a phosphoramidite, and as such, this NHS ester is the standard method of incorporation into oligonucleotides
- sulfoCyanine 5/3 NHS Esters
  - These NHS esters give users of indocyanine (Cyanine 5 and 3) fluorophores an alternative to cyanine 5, which is one of the more deprotection sensitive dyes we offer

5’-Biotin II Phosphoramidite

Another versatile option for biotinylation of oligonucleotides

Biotin is a popular oligonucleotide modification and is used in a wide range of applications, including enrichment, detection, and immobilization assays. Glen Research’s first biotin product was released in 1991, and our collection has grown substantially since then. 5’-Biotin II consists of an ethylene glycol linker, an alternative structure to the all-carbon linker in our traditional 5’-Biotin Phosphoramidite. The addition of 5’-Biotin II phosphoramidite allows researchers accustomed to using the amino-modifier 5 linker to continue doing so in their experiments.

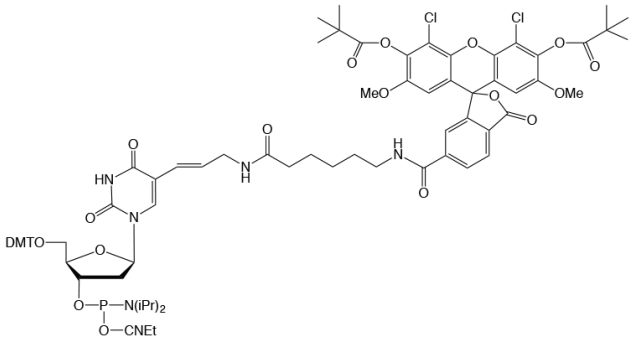


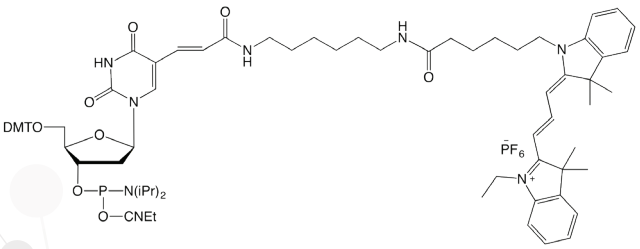
- Convenient, on-column automated incorporation – as opposed to post-synthetic conjugations – such as click chemistry or NHS ester couplings
- Contains a DMT-group that enables reverse phase cartridge (such as Glen-Pak) and HPLC purification techniques
- Matches the standard 5’-biotin structure used by major oligonucleotide synthesis providers

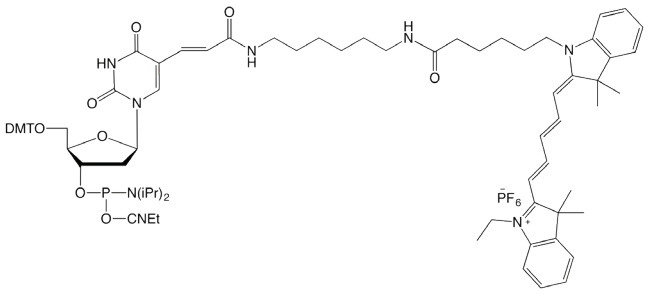
Dye-dT Phosphoramidites

Insert fluorophores into any oligonucleotide sequence position

Fluorescent oligonucleotide probes have a long-standing history in detecting and visualizing nucleic acids. Fluorophore labels are integral to multiplex reactions, fluorescent microscopy, PCR, and many other applications. Glen Research is excited to expand our current line of internal fluorophore-dT phosphoramidites products with the addition of JOE-dT, Quasar® 570-dT, and Quasar® 670-dT. Quasar 570 and 670 fluorophores are derivatives of cyanine 3 and 5, respectively. Each fluorophore possesses unique spectroscopic characteristics and offers multiple benefits.







- Internal labels yield the same oligonucleotide as an amino modifier dT and dye NHS ester, without requiring additional post-synthetic reactions
- Replace dT residues without affecting the ability of labeled thymidine to form base pairs
- Compatible with DMT-ON purification

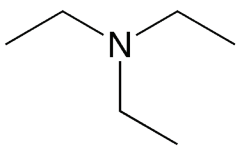
References:

Xu, W., Chan, K., Kool, T. Nat. Chem, 2017, 9, 1043-1055.

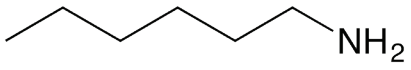
2.0M Hexylammonium Acetate, HPLC Grade, pH=7

Improve chromatographic analysis and purification of DMT-OFF oligonucleotides

To give our customers more options in terms of analyzing and purifying oligonucleotides, we have added a 2.0M Hexylammonium Acetate (HAA) buffer to our offerings. Hexylamine and triethylamine share the same chemical formula, but the longer alkyl chain of hexylamine interacts more strongly with typical reversed-phase resins, making the ion pairing process in reversed-phase high performance liquid chromatography (RP-HPLC) with HAA more effective than triethylammonium acetate (TEAA).



Triethylamine
$C_6H_{15}N$



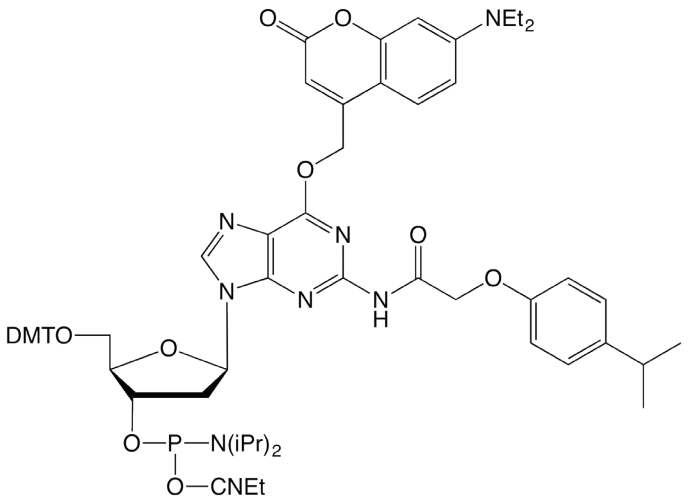
Hexylamine
$C_6H_{15}N$

- Much higher resolving power for both polythymidine sequences and sequences of mixed base composition
- Increased ion-pairing ability compared with traditional buffers such as TEAA
- The boiling point of hexylamine HAA is notably higher than that of triethylamine, making it a less volatile buffer as well

DEACM Caged-dG Phosphoramidite

Versatile tool for light-activatable (“caged”) oligonucleotides

Photocaging of oligonucleotides is a powerful method for structural and kinetic investigations, or gene regulation. For this reason, we introduced NPOM Caged-dT to give our customers the ability to perform such experiments. The NPOM is an o-nitrobenzyl (ONB) group that is attached to the base pairing face of thymine. In their caged form, the oligos are unable to participate in base pairing – it is only after exposure to non-invasive light that the native oligo is released. We now provide a second option for synthesizing photocaged oligonucleotides, DEACM Caged-dG, which gives our customers access to another nucleobase as well as a very different photoremovable protecting group.



- Major drawbacks of ONB cages such as 1-(2-nitrophenyl)ethyl (NPE) are poor photorelease quantum yields and absorption maxima in the UV region
- The uncaging efficiency of DEACM Caged-dG was shown to be 17x higher than in a nitrophenethyl photocage control
- Faster uncaging offers better resolution than common ONB cages for kinetic studies
- Absorption and fluorescence profile allows for easy monitoring during oligonucleotide synthesis and purification, or tracking in cells

References:

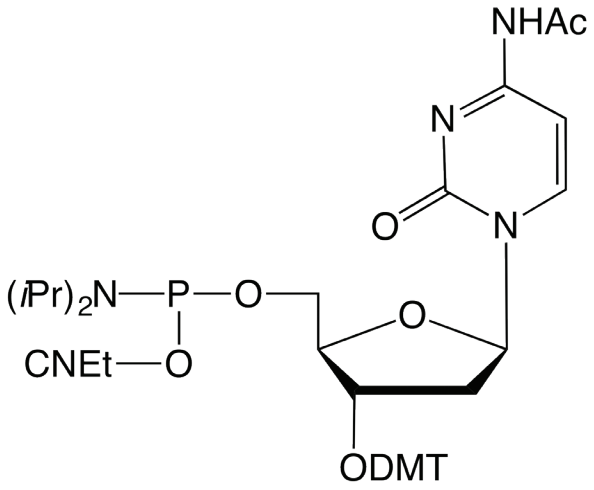
The Glen Report, 2011, 23.2, 7.

The Glen Report, 2022, 34.11.

Ac-dC-5’-CE Phosphoramidite

Reverse DNA synthesis phosphoramidite

For many years now, Glen Research has been providing reverse DNA synthesis reagents to the research community, and in recent years, the need for 5’-3’ direction synthesis reagents has increased significantly. These reagents have protecting groups and attachment points between the 5’ and 3’ positions that have been reversed – everything else is identical – the protection groups are similar and the synthesizer protocols are the same. One option that users of our DNA reverse synthesis reagents have not had up until recently is methylamine (AMA) deprotection compatibility.



- Glen Research 5’-CE phosphoramidites are designed for the production of 5’-5’ or 3’-3’ linkages, useful in antisense studies, or to synthesize oligonucleotide segments in the opposite sense from normal synthesis, for special applications
- Benzoyl-dC protected phosphoramidites, like our traditional offering of dC, are incompatible with any deprotection method that involves methylamine
- Usage is the same as our reverse Bz-dC phosphoramidite and is compatible with all standard deprotection methods, including AMA

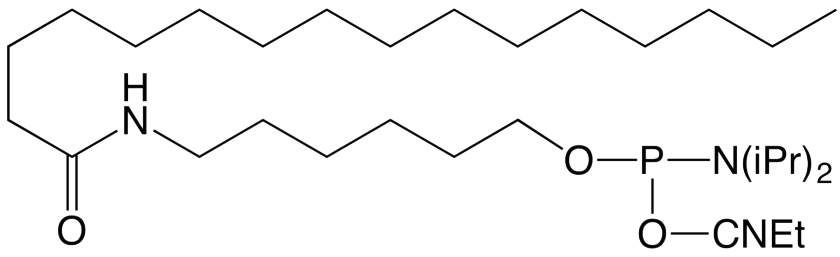
References:

The Glen Report, 2022, 34.14.

Palmitate Phosphoramidite

Increase cellular uptake of oligonucleotides

Palmitic acid is a saturated long-chain fatty acid with a 16-carbon backbone. It is naturally found in palm oil and is the most common fatty acid found in the human body. The palmitate phosphoramidite consists of the C16 chain connected to a C6 linker via a traditional amide bond. This lipophilic modification can improve cellular uptake and activity in multiple tissues.



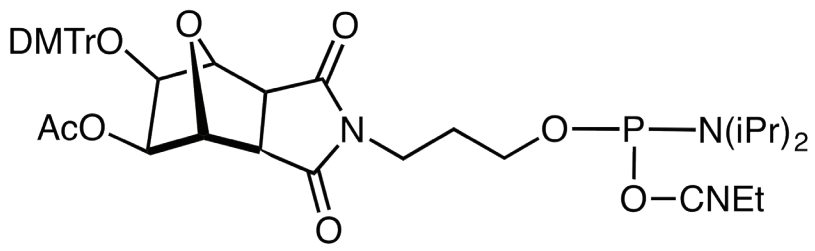
- Palmitate conjugates offer cellular uptake and delivery to extrahepatic tissues
- Recent studies using palmitoyl-oligonucleotides suggest promising outcomes as the use of therapeutic oligonucleotides continues to rise
- Non-toxic

References:
The Glen Report, 2022, 34.16.

Universal-CE Phosphoramidite

Dual-purpose linker

We offer a range of linkers compatible with multiple cleavage methods, including enzymatic, hydrolysis, photolabile, and disulfide. Universal-CE Phosphoramidite is a new cleavable linker that yields two different end moieties, one bearing a 3’-OH and one bearing a 5’-amino modifier with a C3 linker, upon cleavage. No phosphoramidite products in our current offerings directly yield a 3’-OH upon cleavage, which may be crucial for certain downstream applications, such as primer extension or ligation assays.



- Based on the cleavage products, Universal-CE can also be used as a 5’-modification to yield an oligonucleotide with a 5’-amino modifier C3
- Universal-CE could also be used to modify a hydroxyl- or amine-containing surface, creating a universal-like support, capable of oligonucleotide synthesis and subsequent cleavage
- Universal-CE is based on the same chemistry as Glen UnySupport, which is compatible with multiple standard deprotection and cleavage conditions

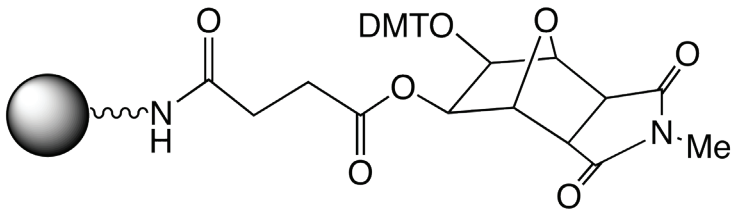
References:

The Glen Report, 2022, 34.17.

UnySupport™ CPGs

Synthesize long oligos without compromising loading

Due to their versatility, it's no surprise that universal supports remain popular over requiring a support for each 3'-nucleoside or modification. Particularly when a special modification is only available as a phosphoramidite, a reliable universal support is essential. Our UnySupport, which is based on UnyLinker chemistry, was first introduced in 2008. Since then, we have included multiple versions: 500Å CPG, 1000Å CPG, high load CPG, and polystyrene. We are pleased to introduce two new versions of UnySupport: 1400Å CPG and 2000Å CPG.



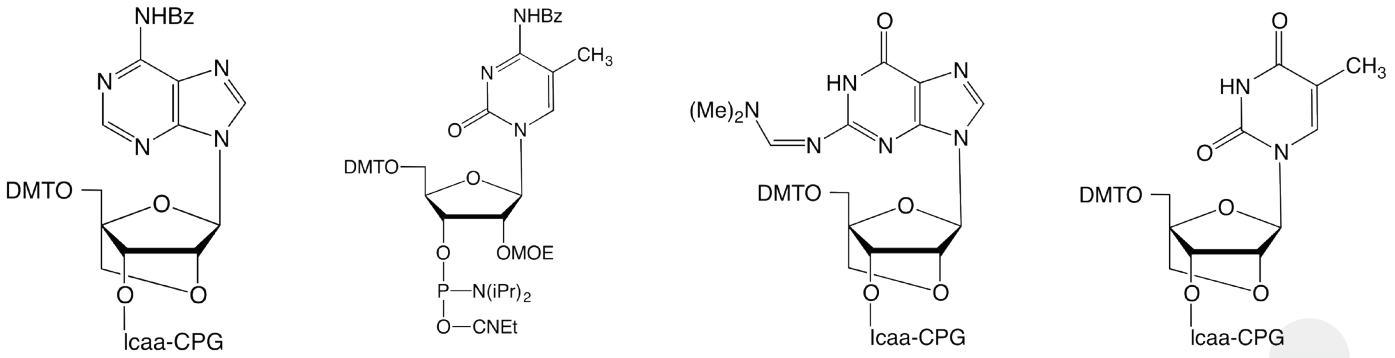
- Our 2000Å CPG supports are no longer limited to main DNA bases (A, C, G, and T) – the introduction of UnySupport 2000Å CPG allows researchers to use all of our modifiers in a 2000Å environment
- The 2000Å supports are best for very long (>150 mer) oligonucleotides
- UnySupport 1400Å CPG can synthesize long oligonucleotides without compromising loading

References:
The Glen Report, 2022, 34.22.
The Glen Report, 2008, 20.2, 10-11.
The Glen Report, 2009, 21.1, 14.

Locked Analog (LA) CPGs

Synthesize 3’-LNA modified oligonucleotides without a universal support

Locked Nucleic Acid (LNA) is a popular modified ribonucleic acid containing a methylene bridge connecting the 2’-oxygen and 4’-carbon atoms. The bicyclic structure introduces conformationally restrained units, locking the modified ribose into a C3’-endo conformation. LNA-modified oligos are increasingly prominent for hybridization assays and probes due to enhanced thermal stability towards complementary oligos, without compromising base pairing specificity.



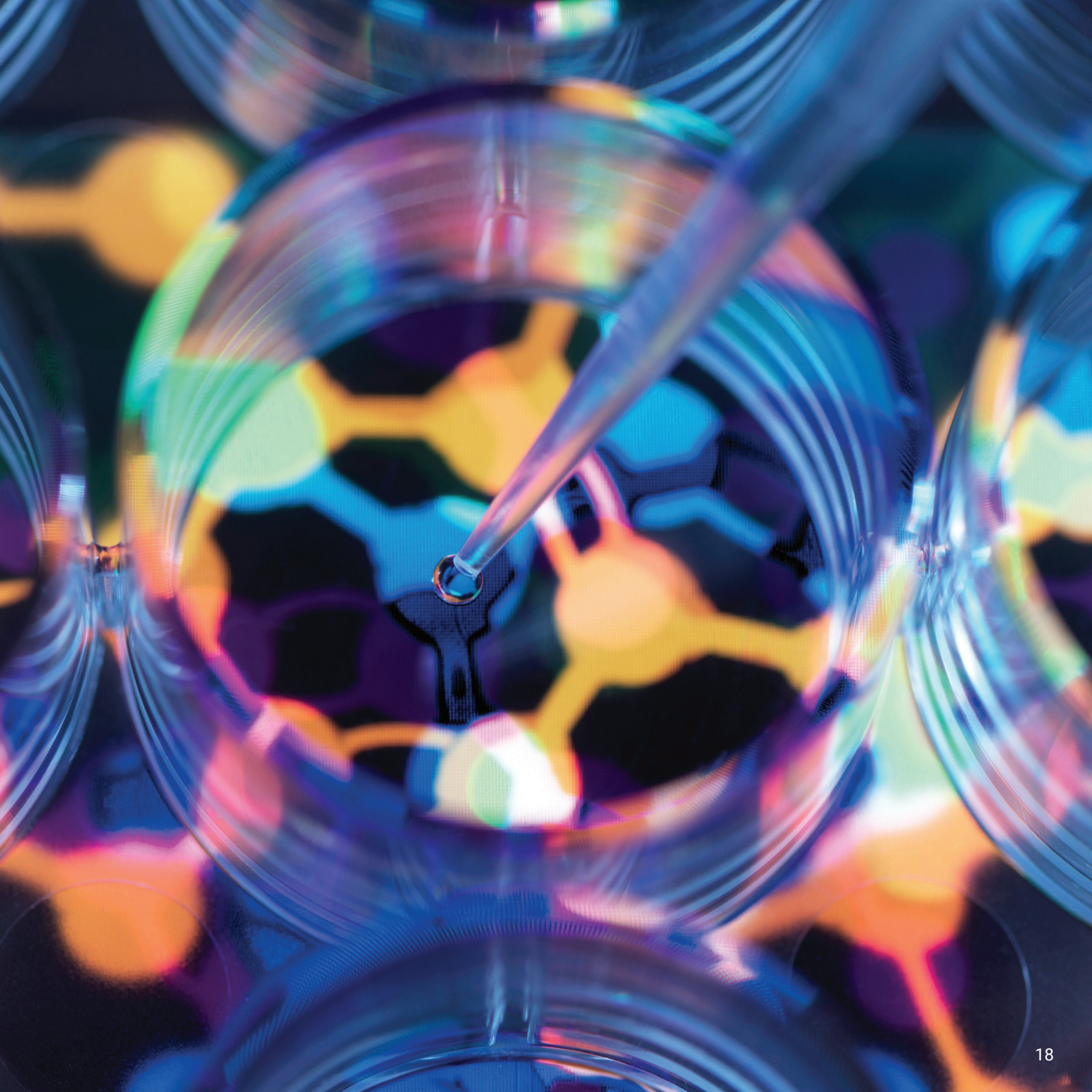
- LNA is recommended for use in any hybridization assay that requires high specificity and/or reproducibility, e.g., dual-labeled probes, in situ hybridization probes, molecular beacons and PCR primers
- LA CPGs negate the need for universal supports, which typically require special cleavage conditions, to synthesize 3’-LNA modified oligonucleotides
- LNA can be mixed with DNA and RNA, as well as other nucleic acid analogs, modifiers, and labels

References:

The Glen Report, 2022, 34.23.

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

M. Petersen, et al., J Mol Recognit, 2000, 13, 44-53.



Contact us at **703.437.6191** or **support@glenresearch.com** to speak with an expert.

Visit **glenresearch.com** to learn more.

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