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GLEN RESEARCH
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NEW PRODUCT

Expanding the Toolbox: Introducing AGC Trimer Phosphoramidite

Golden Gate Assembly has revolutionized synthetic biology by enabling fast, modular, and scarless DNA assembly.[1] This technique, which leverages the precision of Type IIS restriction enzymes like BsaI and BsmBI, has become a preferred method for applications such as protein engineering, phage display, and mRNA display. As the adoption of Golden Gate Assembly grows in combinatorial library design and high-throughput cloning workflows, so too does the importance of codon compatibility with this method.

One particular area of growing concern is codon coverage in trimer phosphoramidite libraries. Specifically, the need for better support of serine codons that are compatible with Golden Gate cloning strategies. Our current trimer library includes 29 of the 64 possible codons, which is practical for reducing redundancy and optimizing cost, but leaves gaps in codon flexibility.

The Golden Gate Bottleneck: Restriction Site Interference

Golden Gate Assembly relies on the use of Type IIS restriction enzymes, which cut outside of their recognition sequences and enable seamless, scarless ligation of DNA fragments. Among these, BsaI (GGTCTC) and BsmBI (CGTCTC) are the most widely used enzymes for Golden Gate protocols. However, the power of this method comes with strict sequence constraints—internal BsaI and BsmBI sites must be avoided within inserts, including within coding regions, to ensure efficient and accurate assembly.[1]

This poses a challenge for our existing offering of trimer phosphoramidites. Across all three reading frames in the sense direction, several codons can unintentionally contribute to the formation of BsaI or BsmBI restriction sites when combined with common flanking sequences. Examples include GTC (Val), CTC (Leu), and TCT (Ser). The presence of these codons in inserts can lead to off-target cleavage, loss of clones, or failed assemblies.

We do not use GTC and CTC as our standard Val and Leu codon selections. However, TCT, our only forward-sense codon for serine, remains a vulnerability. Serine can be encoded by five alternative codons (TCC, TCA, TCG, AGC, and AGT), none of these are currently available in our trimer pool.

On the antisense strand, we face a similar issue. AGA, our standard reverse codon for serine, is also incompatible with Golden Gate due to its potential to create restriction sites. However, we've identified a workaround: GCT, which normally encodes for alanine, can function as a reverse codon for serine without generating BsaI or BsmBI sites if paired correctly.

For researchers using Golden Gate Assembly, this means the only viable trimer-based solution works in antisense mode. Those building sense-oriented libraries, or libraries requiring both sense and antisense coverage, are left without a compatible option. The missing piece? A forward-facing serine codon that matches GCT in the antisense direction and avoids problematic restriction sites.

AGC: A Golden Codon for Golden Gate Assembly

This is where AGC comes in. AGC is a sense codon for serine that solves multiple problems at once. First, it does not contain any sequences that could contribute to BsaI or BsmBI sites, making it compatible with Golden Gate Assembly. Second, AGC is well tolerated and efficiently translated in *E. coli*. According to the Codon Adaptation Index (CAI)[2], a measure of how frequently a codon is used in highly expressed genes—AGC ranks as the third-best codon for serine, and in many codon usage tables, it ranks even higher.

Importantly, AGC on the sense strand pairs with GCT on the antisense strand, which is already part of our existing library. This pairing creates a dual benefit: improved sequence compatibility in both directions for double-stranded oligonucleotide applications, and increased flexibility in codon usage. AGC is a backup codon for serine and a backup codon for alanine in the antisense lineup. This redundancy helps reduce sequence constraints during library design. Including AGC allows researchers to maintain amino acid diversity in their constructs while fully leveraging the benefits of Golden Gate Assembly.

Introducing AGC Trimer Phosphoramidite

The increasing use of Golden Gate Assembly in next-generation library construction demands more thoughtful design of trimer phosphoramidite sets. The absence of AGC limits options for serine incorporation, and therefore, the robustness of randomized libraries, particularly when used in evolving proteins where every position matters. It was a simple decision to add AGC to our trimer line-up to significantly improve the versatility of oligonucleotide libraries for today's cutting-edge applications (Table 1).

AGC Trimer Phosphoramidite can be used in the same manner as our other trimer phosphoramidites. A 15 min coupling time is recommended and deprotection is carried out using 30% NH4OH for 17 hours at room temperature followed by an additional 4 hours at 55 °C. We determined the reaction factor of AGC to be 0.41, based on coupling data relative to our other trimers.[3]

To support modern, restriction-site-sensitive cloning methods like Golden Gate Assembly, and to provide redundancy and flexibility in synthetic oligonucleotide design, we are thrilled to make AGC our newest trimer phosphoramidite. It fills a critical gap left by the incompatibility of TCT and AGA, ensures compatibility with antisense GCT, and aligns with *E.coli* expression needs.

For a custom trimer mix of a particular subset of codons or a trimer mix that represents a set of trimers that is biased toward a particular codon or codons, please contact support@glenresearch.com for a quotation and projected delivery date.

Table 1. New Product Information

Item	Pack Size	Catalog No.
AGC Trimer Phosphoramidite	50 µmol	13-1021-95
	100 µmol	13-1021-90
Custom Trimer Mix	Custom	13-9999-SP

References

1. New England Biolabs, Inc. NEBridge® Golden Gate Assembly. https://www.neb.com/en-us/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/golden-gate-assembly.
2. P.M. Sharp, and W.H. Li, *Nucleic Acids Res*, 1987, **15**, 1281-95.
3. *The Glen Report*, 2013, **25.1**, 2.

NEW PRODUCT

N4-Methyl-C-CE Phosphoramidite

To support the expanding field of RNA research, we are pleased to introduce the natural monomethylated cytidine, N4-Methyl-C-CE Phosphoramidite (m^4C) (Figure 1, Table 1). This addition complements our existing portfolio, which already includes three other isomers with methylation at N3 (m^3C), C5 (m^5C), and 2'-O (Cm).

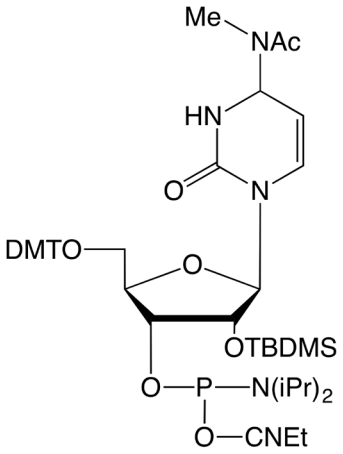


Figure 1. N4-Methyl-C-CE Phosphoramidite (m^4C)

The methyl group in this new modification is attached to the exocyclic amine and can adopt two conformations (*anti* and *syn*) with respect to the nitrogen at position 3 (Figure 2).[1] Studies have shown that the nucleoside favors the syn conformation by a ratio of 20:1 due to steric hindrance.[2] Since the syn conformation places the methyl group on the base pairing face, m^4C destabilizes base pairing relative to that of C.

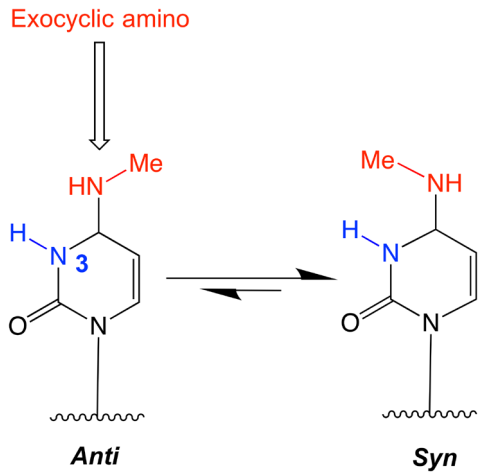


Figure 2. N4-Methyl-Cytosine Conformations

Methylation is one of the most common and important modifications found in RNA.[3] Examples include m⁶A, Cm, m³C, and m⁵C. The m⁴C modification has been identified in the bacterial cytoplasmic rRNA as well as in eukaryotic mitochondrial rRNA. The enzyme RsmH methyltransferase is responsible for N⁴-methylation at position C1402 in bacterial 16S ribosomal RNA (rRNA).[4] Its human ortholog, methyltransferase-like 15 (METTL15), catalyzes the methylation at C839 in 12S mitochondrial rRNA (mt-rRNA).[5] This methylation process is essential for mitochondrial protein synthesis, and disruption of it impairs mitochondrial function. Such impairment has been linked to diseases, including obesity.[5]

The m⁴C phosphoramidite features acetyl protection on the nucleobase and TBDMS protection at the 2'-OH. A 15-minute coupling time is recommended for this modification, and the preferred diluent is a 1:1 (v/v) mixture of anhydrous acetonitrile and dichloromethane. m⁴C phosphoramidite is compatible with AMA deprotection conditions.

Table 1. N4-Methyl-C-CE Phosphoramidite ordering information

Item	Pack Size	Catalog No.
N4-Methyl-C-CE Phosphoramidite	50 µmol	10-3016-95
	100 µmol	10-3016-90
	0.25 g	10-3016-02

References

1. A. Liaqat, M.V. Sednev, C. Stiller, and C. Hobartner, *Angew Chem Int Ed Engl*, 2021, **60**, 19058-19062.
2. D. Engel, and P.H. von Hippel, *Biochemistry*, 1974, **13**, 4143-58.
3. P. Boccaletto, *et al., Nucleic Acids Res*, 2018, **46**, D303-D307.
4. S. Kimura, and T. Suzuki, *Nucleic Acids Res*, 2010, **38**, 1341-52.
5. H. Chen, *et al., J Biol Chem*, 2020, **295**, 8505-8513.

APPLICATION NOTE

Study and Use of Natural RNA Modifications

RNA can be modified post-transcriptionally in more than 170 different ways at the nucleotide level. Of these, Glen offers a small subset as phosphoramidites, and they have recently been highlighted.[1] With the addition of N4-methyl-C (m⁴C) to our portfolio,[2] Glen Research now has a total of 18 of these natural RNA modifications available to support the growing field of RNA research (Table 1), and they continue to enable important and impactful studies.

As most of our customers know, messenger RNA (mRNA) is typically produced by *in vitro* transcription, an enzymatic process that is not very flexible when it comes to introducing modifications. Any modified nucleotide would either have to be spiked in (random sites) or completely replace the standard one. To address this, a chemo-enzymatic approach was used to synthesize mRNA instead.[3]

The researchers chemically synthesized short, capped strands of RNA (10-23 nt) and enzymatically-ligated them to *in vitro* transcribed mRNA. In this way, a medicinal chemistry-like approach was possible for exploring modification preferences at or near the 5'-end. Modifications on the cap, the first base identity (+1 position), the phosphodiester linkages, and the sugar backbone were all explored. The long list of modifications that were used included 2'-OMe A (Am), N6-methyl A (m⁶A), N6, 2'-O-dimethyl A (m⁶Am),

inosine (I), Cm, Gm, Um, and N1-methyl pseudoU ($m^1\psi$). Ultimately, the optimized construct consisted of an LNA m7G cap, an A in the +1 position and either a single LNA substitution at the +1 position or six 2'-OMe substitutions at positions +1 to +6. These sequences gave translation enhancement of 8.6- and 7.5-fold, respectively.

As part of the same work, a branched oligonucleotide strategy was used to assemble dual-capped mRNA as well as capped circular RNA (Figure 1). For these RNAs, translation was elevated 17- and 3.7-fold, respectively, relative to controls. It should be noted that standard circular RNA is not very compatible with $m^1\psi$ whereas the capped circular RNA does not share this issue, making capped circular RNAs particularly attractive.

In another recent publication, *in silico* λ-dynamics was investigated for predicting binding between antibodies and modified residues of RNA.[4] Antibodies are versatile affinity agents that can be used to enrich for, capture, or directly identify different RNA modifications in RNA. While antibodies can be developed for this application, the techniques to evaluate their binding and specificity are limited. *In silico* λ-dynamics is a possible solution to this. To investigate, the crystal structures of antibody fragments bound to the free nucleosides of I and m^6A were determined, and these data were used as a foundation for the method. The *in silico* model was used to analyze the binding of the two antibodies to 44 nucleobase-modified and 4 unmodified RNA nucleosides relative to that of m^6A and I in terms of binding free energies. Notable differences in specificity of the two antibodies were observed, and some of these specificities were verified with modified RNA oligonucleotides in Enzyme-Linked Immunosorbent Assay (ELISA) and MicroScale Thermophoresis (MST) experiments.

Continuing on the theme of RNA modification analysis, a third study developed an improved procedure for 5-methyl-C (m^5C) sequencing.[5] Bisulfite sequencing is a standard and popular method for the identification of m^5C residues. In the presence of cytosine and m^5C, bisulfite selectively reduces cytosine to uracil (Figure 2). By sequencing both RNA before and after bisulfite treatment, the locations of m^5C residues can be readily deduced. While this method is highly effective, there are limitations. These include long reaction times, damage to the RNA, and incomplete C-to-U conversion. To address these, a modified bisulfite method was developed termed ultrafast bisulfite sequencing (UBS-seq). Using a more highly soluble ammonium bisulfite/sulfite mixture in place of sodium bisulfite, all three limitations were notably improved. Compared to commercially available m^5C sequencing kits, UBS-seq gave much lower false positive m^5C sites while yielding more detected fractions for known m^5C sites. The new method was applied to the mRNA of HeLa cells, and analysis of the data revealed that NSUN2 is the principal methyl transferase responsible for installing m^5C sites in HeLa mRNA. The authors were also able to apply a similar method on DNA for the identification of 5-methylcytosine.

In all these recent investigations, modified RNA phosphoramidites played key roles in facilitating the research conducted. As more of these modifications become available, one should expect many more exciting developments in this area.

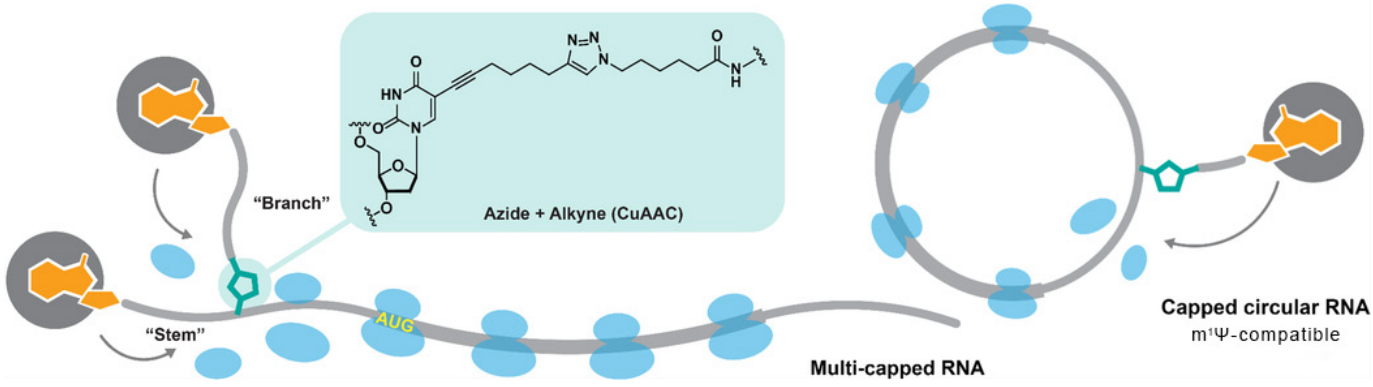


Figure 1. Expanded mRNA topologies. [Credit: Wang Lab]

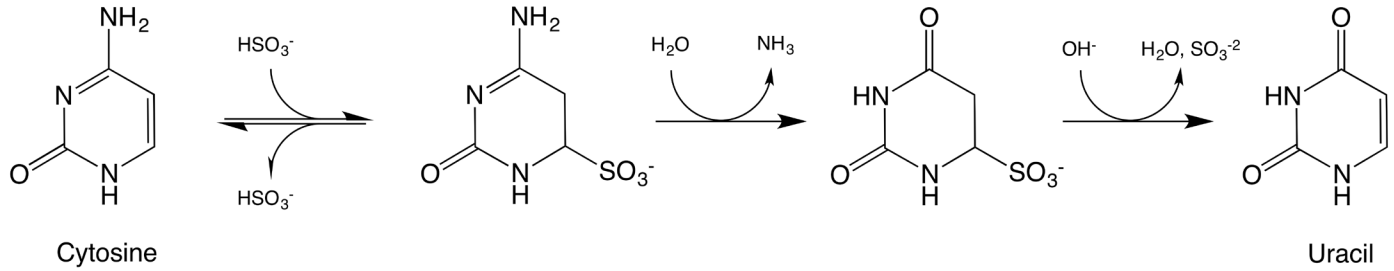


Figure 2. Bisulfite conversion of cytosine to uracil.

Table 1. Natural RNA Modifications

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
	m^4C	N4-Methyl-C-CE Phosphoramidite	10-3016
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

References

1. *The Glen Report*, 2025, **37.1**, 3-5.
2. *The Glen Report*, 2025, **37.2**, 3-4.
3. H. Chen, *et al.*, *Nat Biotechnol*, 2025, **43**, 1128-1143.
4. M. Angelo, W. Zhang, J.Z. Vilseck, and S.T. Aoki, *Nucleic Acids Res*, 2025, **53**.
5. Q. Dai, *et al.*, *Nat Biotechnol*, 2024, **42**, 1559-1570.

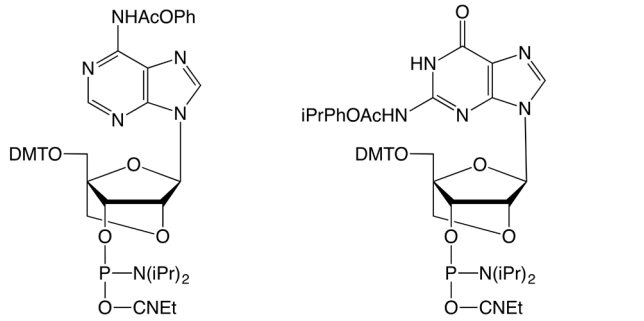
NEW PRODUCTS

UltraMild Locked Analog Phosphoramidites

The standard basic deprotection conditions of AMA or ammonium hydroxide at elevated temperatures can be detrimental to certain sensitive modifications such as Cyanine 5, TAMRA, methylene blue, and others. To address this, Glen offers a line of UltraMild phosphoramidite products that allow for much milder deprotection conditions. Adenine is protected with a phenoxyacetyl group (Pac), cytosine is protected with an acetyl group (Ac), and guanine is protected with an isopropylphenoxyacetyl group (iPr-Pac). Together with thymine/uracil, which do not require protection, oligonucleotides can be deprotected in either concentrated ammonium hydroxide for two hours at room temperature or 50 mM potassium carbonate in methanol for four hours at room temperature. Glen's UltraMild offerings have included DNA, RNA, 2'-OMe as well as L-DNA, and they have all been popular.

Locked nucleic acid (LNA) or simply locked analog (LA) is a widely used nucleic acid backbone modification. It uses a bicyclic sugar that significantly enhances duplex stabilities and has been discussed in recent Glen articles in depth.[1-3] To allow customers to pair more delicate modifications with LNA, two new products are being introduced: Pac-A-LA-CE and iPr-Pac-G-LA-CE Phosphoramidites (Figure 1, Table 1). These can be used with the existing Ac-C-LA-CE and T-LA-CE Phosphoramdites as a full UltraMild synthesis option.

UltraMild syntheses of LNA should follow our general recommendations. Coupling times should be extended to at least three minutes, oxidation times need to be tripled (45 seconds with 0.02 M iodine on an ABI394), and phenoxyacetic anhydride should be used in place of acetic anhydride for capping.



Pac-A-LA-CE Phosphoramidite

iPr-Pac-G-LA-CE Phosphoramidite

Figure 1. UltraMild LA Phosphoramidites

Table 1. UltraMild LA Phosphoramidite ordering information

Item	Pack Size	Catalog No.
Pac-A-LA-CE Phosphoramidite	0.25g	10-2601-02
	0.5g	10-2601-05
iPr-Pac-G-LA-CE Phosphoramidite	0.25 g	10-2621-02
	0.5g	10-2621-05

References

1. *The Glen Report*, 2018, **30.2**, 8-9.
2. *The Glen Report*, 2022, **34.2**, 3-5.
3. *The Glen Report*, 2025, **37.1**, 5.

PRODUCT REVIEW

UltraMild Capping

Capping is an essential step in the oligonucleotide synthesis cycle. Monomer additions are never quantitative, and capping ensures that failure sequences are blocked from further extensions and can be readily removed during downstream purification. Capping is typically performed with acetic anhydride, which converts the 5'-OH of failed coupling sites to acetyl esters.

For UltraMild syntheses, in addition to using phosphoramidites with labile protecting groups, phenoxyacetic (Pac) anhydride must be used in place

of acetic anhydride as well. During synthesis, the isopropylphenoxyacetyl (iPr-Pac) group on the guanine can exchange with the capping agent.[1] The resulting acetyl-protected guanine would be much more difficult to deprotect and defeat the purpose of an UltraMild synthesis. Using Pac anhydride fixes this. While exchange is still possible, it is inconsequential.

Glen carries three Pac anhydride capping mixes that can be substituted for their acetic anhydride capping equivalents (Tables 1 and 2), which can be Cap A or Cap B depending on the formulation. If there is ever any confusion as to which capping mixture to choose, one needs to make sure that between the Cap A and Cap B mixes, there are all three key reagents present: capping agent (Pac anhydride), capping activator (N-methylimidazole or DMAP) and a weak base (pyridine or 2,6-lutidine). Alternatively, compatible capping reagent pairs can be found on our instrument-specific product lists.

Table 1. UltraMild capping pairs. Color denotes capping agent, capping activator, and weak base.

Cap A	Cap B
5% Phenoxyacetic anhydride in THF/Pyridine (40-4210)	16% 1-Methylimidazole in THF (40-4220)
5% Phenoxyacetic anhydride in THF (40-4212)	10% 1-Methylimidazole in THF/Pyridine (40-4122)
20% 1-Methylimidazole in Acetonitrile/ 2,6-Lutidine (40-4115)	5% Phenoxyacetic Anhydride in Acetonitrile (40-4128)

Table 2. Phenoxyacetic anhydride cap mix ordering information.

Item	Pack Size	Catalog No.
5% Phenoxyacetic anhydride in THF/Pyridine	200 mL	40-4210-52
	450 mL	40-4210-57
5% Phenoxyacetic anhydride in THF	200 mL	40-4212-52
	450 mL	40-4212-57
5% Phenoxyacetic Anhydride in Acetonitrile	1 L	40-4128-71

References

1. C. Chaix, D. Molko, and R. Téoule, *Tetrahedron Letters*, 1989, **30**, 71-74.

APPLICATION NOTE

Greener Roads Ahead: Sustainable Advances in Solid-Phase Oligonucleotide Synthesis

Synthetic oligonucleotides lie at the heart of modern biotechnology and therapeutics—from qPCR probes to antisense drugs and mRNA vaccines. Over the decades, solid-phase phosphoramidite chemistry has remained the gold standard for their assembly. However, the environmental and operational burdens of this approach (waste solvents, specialized reagents, and chromatographic purifications) galvanized efforts to "green" the process. Recent efforts alongside process-focused initiatives are redefining synthesis through safer solvents, innovative purification, and waste reduction.

To truly evaluate and improve solid-phase oligonucleotide synthesis (SPOS) sustainability, many researchers are turning to green chemistry metrics such as the E-factor (mass of waste per mass of product) and PMI (process mass intensity). Another way to monitor the greenness of a technique is atom economy, which measures how much of the reactants' mass ends up in the end product. Lifecycle assessments, solvent replacement scorecards, and reagent hazard ratings are increasingly used to guide improvements. These metrics also help translate academic advances into practical, scalable improvements for industrial production—ensuring that sustainability efforts are not only theoretical but measurable.

SPOS consists of well-defined stages: repetitive coupling cycles on a solid support, followed by cleavage, deprotection, and purification. Each stage consumes large amounts of solvents such as acetonitrile, dichloromethane (DCM), and toluene. Depending on the instrument and usage, each synthesis cycle can produce up to 3-5 mL of waste. Even at a small scale (1.0 µmol), the process is highly reagent-intensive, expending ~7-8 mg of excess phosphoramidite. These reagents not only contribute significantly to process mass intensity, but also pose toxicity and disposal concerns. Optimizing any of these steps requires balancing the need for high-purity oligonucleotides with the environmental and operational costs of synthesis.[1] The industry's challenge is to maintain efficiency and reliability while reducing its ecological footprint.

One of the most effective ways to green SPOS is to reduce or eliminate hazardous solvents. DCM, historically used in DMT detritylation and wash steps, is being replaced with greener alternatives or eliminated entirely in some workflows. Chlorinated solvents have particularly come under scrutiny due to their environmental persistence and health hazards. Swapping to toluene-based deblocking reagents, a less hazardous organic solvent, has been widely adopted in large scale oligonucleotide manufacturing.[1,2] The caveat of this is that toluene is incompatible with conductivity-based trityl monitoring systems employed by many commercial synthesizers. Removing acidic deblocking reagents altogether and exploring thermal detritylation has been proposed.[3] Another area of innovation is greener activators. Newer activators like 4,5-dicyanoimidazole (DCI) offer faster coupling, lower toxicity, and improved safety profiles.[2,4] Moreover, minimizing reagent equivalents through more efficient chemistry has become a key goal. One opportunity is to use CSO oxidizer to eliminate the introduction of water, iodine, and pyridine into the synthesis cycle, potentially reducing the solvent washes needed to restore anhydrous conditions in the system. Together, these improvements aim to simplify reagent handling and reduce the chemical footprint of each synthesis cycle without compromising oligonucleotide quality.

Researchers are now experimenting with strategies that reduce overall solvent consumption. For example, solvent-recycling systems are increasingly integrated

into commercial synthesizers, especially for acetonitrile, arguably the most heavily used solvent in SPOS.[1,2] Even seemingly minor changes can make an impact. Minimizing or eliminating wash solvent volumes after noncritical steps (coupling, sulfurization, end-cycle) in the synthesis cycle is another route to reduce waste generation.[5] These shifts reflect a broader commitment to minimizing the environmental impact of synthesis at both lab and industrial scales.

Purification, typically achieved through reverse phase high-performance liquid chromatography (RP-HPLC) or anion exchange (AEX) chromatography, is one of the most resource-intensive steps of oligonucleotide production. To mitigate this, researchers are developing purification alternatives that require less solvent, fewer steps, or both. For instance, solid-phase extraction (SPE), such as our Glen-Pak™ cartridges and membrane-based filtration approaches are emerging as scalable options that reduce the need for high-volume chromatography. Additionally, solvent-efficient chromatographic systems minimize waste by continuously recycling mobile phases and reprocessing impure fractions, enabling the recovery of highly pure full-length product without the need for multiple chromatographic runs.[6] By streamlining purification, these strategies reduce overall solvent use and allow for cleaner, more compact workflows.

Atom economy of oligonucleotide synthesis is typically very low. Despite a high yield per coupling step, the use of protecting groups that are lost at each cycle (e.g. 5'-DMT, exocyclic base protecting groups, 2'-OH protecting groups, and phosphate protecting groups) dramatically reduces the atom efficiency of the overall process. Ligation-based strategies, both enzymatic and chemical, offer significant improvements in atom economy for oligonucleotide synthesis by enabling the efficient assembly of fully deprotected fragments without the extensive use of protecting groups, excess reagents, or wasteful byproducts. Enzymatic ligation, using enzymes like DNA ligase, joins DNA strands with high specificity under mild conditions, incorporating nearly all atoms of the starting materials and generating only minimal byproducts such as adenosine monophosphate or pyrophosphate. Similarly, chemical ligation methods, including click chemistry (e.g., CuAAC or SPAAC), allow for the modular, high-yield coupling of oligonucleotide blocks via bioorthogonal reactions that proceed cleanly and quantitatively. Both approaches circumvent the atom inefficiencies of traditional phosphoramidite synthesis—where multiple protecting groups and stoichiometric reagents are discarded at each step, and enable more sustainable, scalable assembly of complex oligonucleotides in line with green chemistry principles.

Another strategy for improving sustainability in oligonucleotide therapeutics lies in optimized compound design. Advances in tissue-targeting conjugates, such as GalNAc, and in chemical modifications to the sugar and backbone, such as 2'-MOE, 2'-OMe, LNA, and phosphorothioate (PS) linkages, have significantly enhanced potency, stability, and bioavailability. These improvements reduce the required dosing and can ultimately lower the total production volume of oligonucleotides needed for therapeutic effect, thereby contributing to a more sustainable manufacturing paradigm.

Solid-phase phosphoramidite chemistry remains the backbone of oligonucleotide synthesis—but it's not static. A growing ecosystem of greener solvents, safer activators, and efficient purification methods is reshaping SPOS into a more sustainable craft (Table 1). While challenges remain like matching chromatographic purity at scale and scaling solvent recovery, the direction is clear. Through deliberate, iterative improvements informed by green chemistry and cross-discipline insights, SPOS is evolving to meet the environmental demands of tomorrow's biotech landscape.

Table 1. Relevant Product Information

Product Type	Item	Catalog No.
Deblock	3% DCA in Toluene	40-4240
Activator	4,5-Dicyanoimidazole (DCI), Crystalline	30-3050
	0.25M DCI in Anhydrous Acetonitrile	30-3150
Oxidizer	0.5M CSO in Anhydrous Acetonitrile	40-4632
Chemical Ligation	3'-Propargyl-5-Me-dC CPG	20-2982
	5'-Hexynyl Phosphoramidite	10-1908
	5'-I-dT-CE	10-1931
	3'-Azido-Modifier Serinol CPG	20-2999
Chemical Modifications	5'-GalNAc C3 Phosphoramidite	10-1974
	A-2'-MOE	10-3200
	5-Me-C-2'-MOE	10-3211
	G-2'-MOE	10-3220
	5-Me-U-2'-MOE	10-3231
	Bz-A-LA-CE	10-2000
	Ac-5-Me-C-LA-CE	10-2015
	Bz-5-Me-C-LA-CE	10-2011
	dmf-G-LA-CE	10-2029
	T-LA-CE	10-2030
	2'-OMe-A-CE	10-3100
	2'-OMe-Ac-C-CE	10-3115
	2'-OMe-G-CE	10-3121
	2'-OMe-ibu-G-CE	10-3120
	2'-OMe-U-CE	10-3130
	Sulfurizing Reagent II	40-4037
	0.05M Sulfurizing Reagent II in Pyridine/Acetonitrile	40-4137
Purification	Glen-Pak™ 50 mg Purification Cartridge	60-5000
	Glen-Pak™ 150 mg Purification Cartridge	60-5100
		60-5200
	Glen-Pak™ 3 g Purification Cartridge	60-5300
	Glen-Pak™ 30 mg 96-Well Plate	60-5400
	Glen-Pak™ 3 mg 384-Well Plate	60-5500

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1. B.I. Andrews, *et al., J Org Chem*, 2021, **86**, 49-61.
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3. *The Glen Report*, 2024, **36.1**, 13-15.
4. *The Glen Report*, 1997, **10.1**, 1-2.
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Technical Snippets

What are the common additional mass peaks observed in mass spectrometry due to incomplete deprotection of synthesized oligonucleotides?

Solid-phase synthesis of oligonucleotide uses phosphoramidte chemistry and requires the use of a selection of protecting groups that need to be removed after synthesis. Incomplete deprotection of synthesized oligonucleotides can result in a portion of the oligonucleotides with higher expected mass than the full-length product (M+). Common protecting groups and their corresponding mass are listed in Table 1 below (note: this is not a comprehensive list).

Table 1. Common protecting groups

Protecting Group Name	Abbreviation	Mass (Da)
4,4'-dimethoxytrityl	DMT	302.39
Acetyl	Ac	42.05
Benzoyl	Bz	104.12
beta (β)-cyanoethyl	CE	53.08
Isobutyryl	iBu	70.11
Phenoxyacetyl	Pac	134.15
tert-butyldimethylsilyl	TBDMS	114.28

Incomplete deprotection of an oligonucleotide can result in several mass peaks, depending on how many protecting groups remain on the sequence. For example, if a sequence contains multiple guanines protected with iBu groups, one may observe peaks at +70.11, +140.22, and +210.33.

What is the best way to make a 3'-methylene blue oligonucleotide?

We have two methylene blue products: a phosphoramidite and an NHS ester. For 3'-labeling, we prefer an amino modifier and the NHS ester because branching is known to occur when methylene blue is incorporated internally or at the 3'-end. In addition, methylene blue requires ultramild deprotection so the use of the NHS ester in a post-synthetic reaction precludes the need for special conditions.

Relevant products:

Methylene Blue II Phosphoramidite (10-5961)

Methylene Blue NHS Ester (50-1960)

glenresearch.com

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