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^{CNV}K and ^{CNV}D - ultrafast reversible photo-cross-linkers for DNA or RNA

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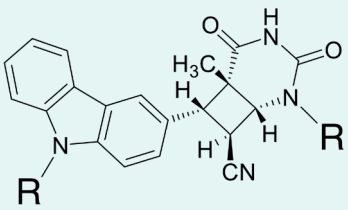
Introduction

3-cyanovinylcarbazole nucleoside (^{CNV}K[1]) and D-threoninol (^{CNV}D[2]) are members of a novel class of photo-cross-linkers for DNA or RNA strands (Figure 1, Page 2). Cross-linking technology is useful for the detection, regulation, and manipulation of DNA or RNA. In particular, photo-irradiation induced cross-linking is useful from the perspective of regulation, and photo-cross-linkers such as psoralen and coumarin have been used in traditional methods. However, many improvements remain necessary in terms of photoreactivity, sequence specificity, and so on. We report that ^{CNV}K and ^{CNV}D have very high photoreactivity and they enable selective photo-cross-linking of target DNA or RNA by photo-irradiation for a few seconds. We expect that they could be successfully applied in the photochemical regulation of nucleic acids in living cells, which has been difficult with traditional methods.

Ultrafast Photo-cross-linking

The photo-cross-linkers ^{CNV}K and ^{CNV}D can be included in DNA or RNA strands according to the usual DMTr-phosphoramidite method. They also have very high photoreactivity and it is possible to photo-cross-link them to a pyrimidine base by photoirradiation for a few seconds at 365nm, as well as to the pyrimidine base in the complementary strand. In addition, ^{CNV}K can photo-cross-link to a pyrimidine base via [2+2] photocycloaddition, similar to psoralen and thymidine dimers. Therefore, it is possible to photo-split the cross-link by a 3 min, 312 nm irradiation (Figure 2, Page 2).



Structure of ^{CNV}K cross-link to Thymidine

The sequence selectivity of photo-cross-linking of ODN containing ^{CNV}K or ^{CNV}D was evaluated (Figure 3, Page 2). ^{CNV}K can photo-cross-link with a position of -1 in the target strand. Therefore, we investigated the effect of varying the ^{CNV}K pairing base and surrounding bases on photo-cross-linking. Photo-irradiation was performed for 10 seconds using 64 sequences obtained by changing the bases of X and Y and Z to A, T,

Continued on Page 2

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G, and C and their complementary strands. As a result, it was found that photo-cross-linking occurs only in the case of thymine or cytosine without being affected by the pairing base or adjacent bases. Compared with ^{CNV}K, ^{CNV}D has faster photo-cross-linking, and for cytosine ^{CNV}D is used, and a significant acceleration of photo-cross-linking reaction can be confirmed.

Photochemical labeling of plasmid

This photo-cross-linking reaction had high sequence selectivity and operability, and it could photo-cross-link a targeted site in a plasmid[3]. Results of the atomic force microscopy (AFM) imaging indicated that a biotin-modified ODN can be photo-cross-linked to a single-stranded or double-stranded plasmid. It was possible to photo-cross-link only one selective site, even on a long plasmid (Figure 4, Page 3).

Acceleration of DNA strand displacement

This ultrafast photo-cross-linking reaction is an effective tool for multiple methods involving nucleic acids. For example, DNA strand displacement is a fundamental reaction for both *in vivo* and *in vitro* biological events such as genomic DNA replication, transcription, and PCR. It is usually a very fast reaction with enzyme assistance *in vivo*, but it takes time to proceed *in vitro*. Therefore, the use of an ultrafast photo-cross-linking reaction would greatly improve stability, and possibly significantly accelerate the DNA strand exchange reaction (Figure 5, Page 3).[4]

Photochemical regulation of antisense effect

This ultrafast photo-cross-linking reaction is also capable of photo-cross-linking to RNA[5]. We evaluated

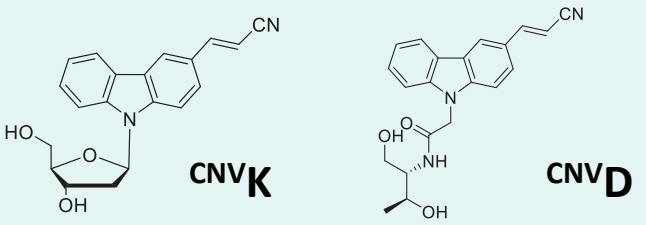

Figure 1. Structure of 3-cyanovinylcarbazole with nucleoside and D-threoninol

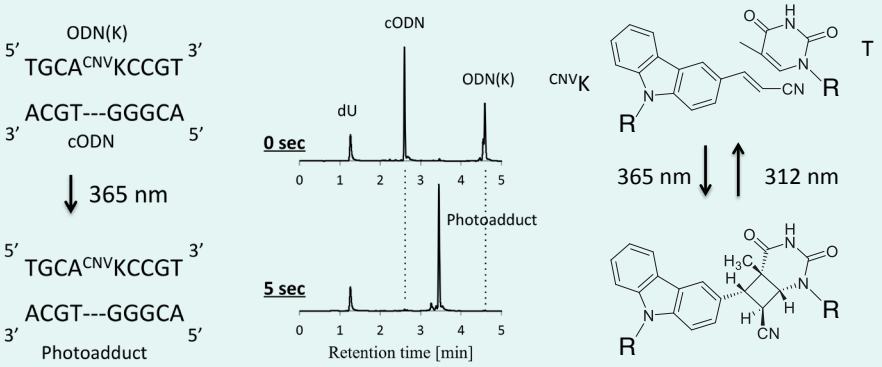

Figure 2. Ultrafast photo-cross-linking

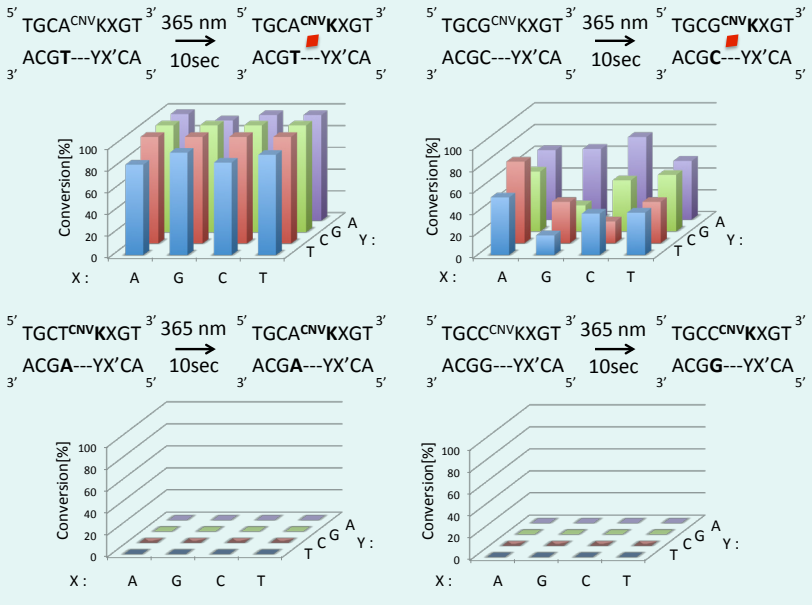

Figure 3. Sequence selective photo-cross-linking

the antisense effect using the photo-cross-linking reaction to mRNA. Ultrafast photo-cross-linking using ^{CNV}K or ^{CNV}D greatly improved the stability of the duplex, and therefore, when it was used as an antisense nucleic acid, it exerted a large antisense effect. A ^{CNV}K probe was converted to phosphorothioate and was introduced into HeLa cells stably expressing GFP. The intensity of GFP fluorescence and the amount of GFP mRNA was decreased by a 10 sec photoirradiation at 365 nm[6], which indicated that GFP gene expression was inhibited by photoirradiation. In addition, it is also possible to regulate its antisense effect by the desired timing of photoirradiation (Figure 6).

Photo-cross-linkable RNA FISH

The stabilization of double-strand formation using ultrafast photo-cross-linking can also be applied to RNA fluorescence in situ hybridization (FISH). In the RNA FISH method, the wash steps are necessary to remove nonspecific signals but can also cause problems such as low detection signal and poor reproducibility. A photo-cross-linkable FISH probe containing ^{CNV}K was introduced into the immobilized E. coli, and RNA FISH was performed using 16S rRNA as a target (Figure 7, Page 4). Fluorescence was confirmed even when the wash steps utilized buffer containing formamide, indicating that stable detection is possible without wash conditions[7]. In addition, strong fluorescence was confirmed even for targets with undetectable sensitivity.

Conclusion

^{CNV}K and ^{CNV}D are photo-cross-linkers capable of photo-cross-linking at an extremely high photo-reactivity compared with conventional optical crosslinkers such as psoralens. This

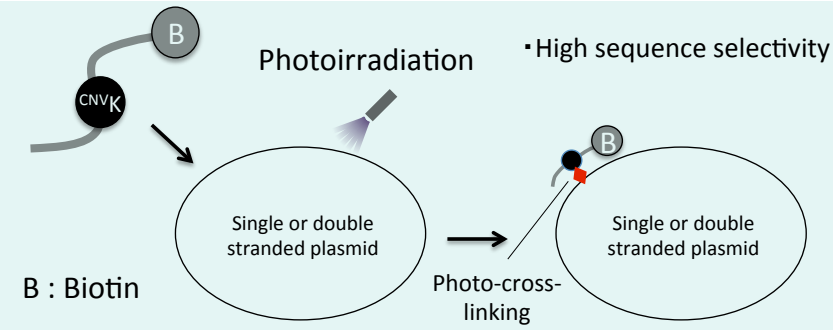

Figure 4. Scheme of photochemical labeling of plasmid using ultrafast photo-cross-linking

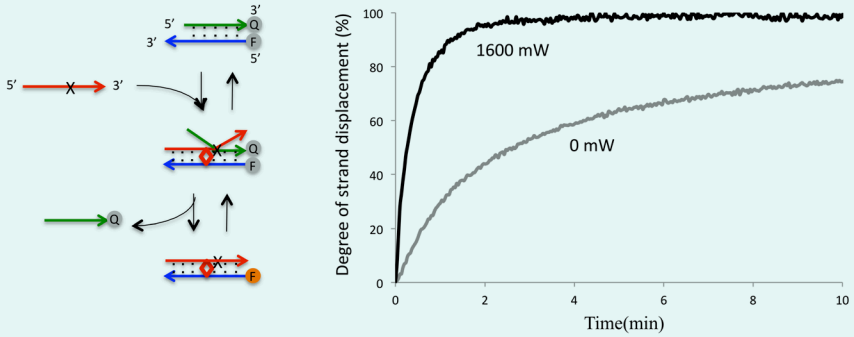

Figure 5. Acceleration of DNA strand displacement using ultrafast photo-cross-linking

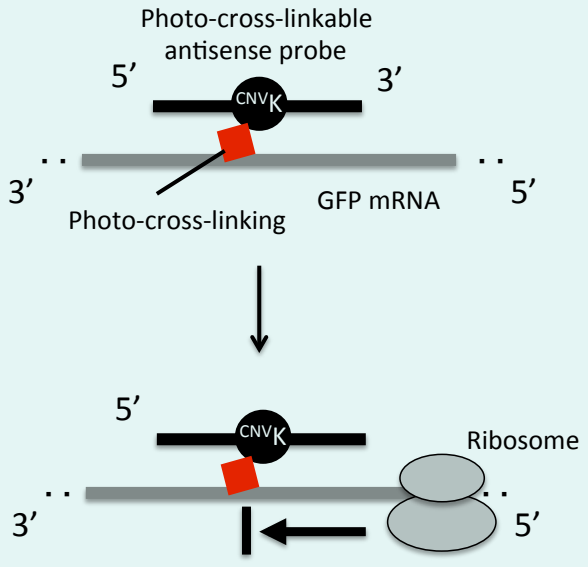

Figure 6. Photochemical regulation of antisense effect

technology could be applied to many fields such as those of antisense nucleic acids and FISH.

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Intellectual property rights

The ultrafast photo-cross-linker has been granted the following patents: US8697357 and US7972792.

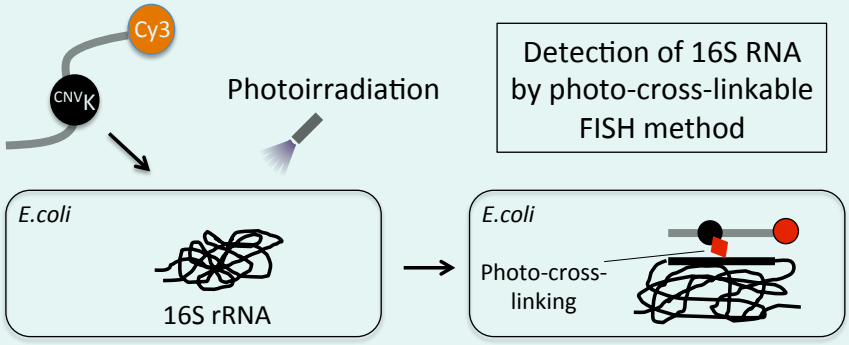

Figure 7. Ultrafast photo-cross-linking as applied to RNA fluorescence in situ hybridization (FISH).

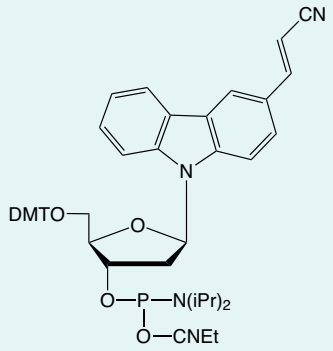

Figure 8. Structure of 3-Cyanovinyl-carbazole Phosphoramidite (^{CNV}K)

Reintroducing ^{CNV}K Phosphoramidite

Glen Research and Maravai LifeSciences have completed an agreement with Nicca Chemical to begin supplying ^{CNV}K Phosphoramidite to the research market worldwide, with the exception of Japan.

Use of ^{CNV}K

For coupling of ^{CNV}K Phosphoramidite (Figure 8), regular coupling times are suggested. However, the use of UltraMILD monomers is preferred. (Catalog Numbers: dA: 10-1601-xx, dC: 10-1015-xx, dG: 10-1621-xx, dT: 10-1030-xx). To avoid any exchange of the iPr-Pac group on the dG with acetyl, use the UltraMild Cap Mix A (40-4210-xx/ 40-4212-xx).

For deprotection: If UltraMILD reagents were used, use 0.05M Potassium Carbonate in Methanol for 4 hours at Room Temperature OR for 2 hours at Room Temperature in 30% Ammonium Hydroxide. If standard bases were used, deprotection in Ammonium Hydroxide at Room Temperature for 24-36 hours will give acceptable yields.

Item	Catalog No.	Pack	Price ($)
3-Cyanovinylcarbazole Phosphoramidite (^{CNV}K)	10-4960-95	50 µmole	200.00
	10-4960-90	100 µmole	390.00
	10-4960-02	0.25g	1125.00

Technical Brief: Non-Aqueous Oxidation using CSO

In previous Glen Report articles, we have demonstrated that the iodine oxidation step during DNA synthesis cycles has the potential to damage some minor bases and modifiers. In this article, we have compiled some of the previous information to show that (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) (1) is an ideal non-aqueous oxidizer for oligonucleotide synthesis.

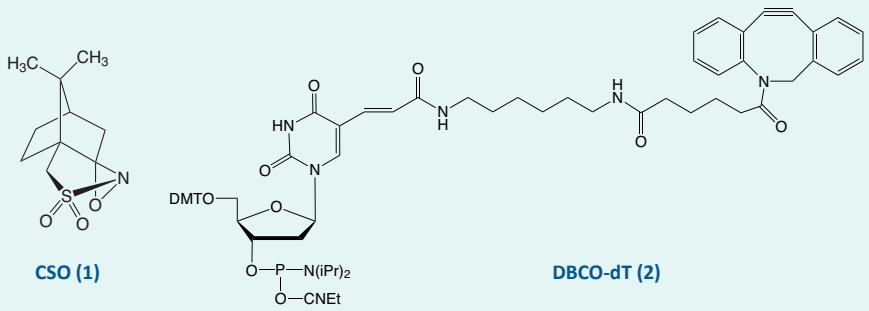

Figure 1: Structures of CSO and DBCO-dT

Non-Aqueous Alternatives

Iodine-based oxidizers have been the standard for DNA and RNA synthesis since the advent of automated synthesizers. They are fast and efficient oxidizers, typically requiring less than 30 seconds for complete oxidation of phosphite triesters to phosphate triesters. However, while iodine-based oxidizers work well for most applications, there are some circumstances where non-aqueous oxidizers may be advantageous, especially where the bases or linkages being produced are sensitive to the presence of water and/or iodine during synthesis.

Non-aqueous oxidizers, typically peroxides, including tert-butyl hydroperoxide, cumene hydroperoxide, hydrogen peroxide, and bis-trimethylsilyl peroxide, among others, have also been employed in DNA synthesis. These peroxides tend to be unstable, requiring that they be freshly formulated just prior to use, and so are difficult to use in routine automated synthesis, hence the need for a stable, effective non-aqueous oxidizer.

Methyl Phosphonate Linkages

In 1996, we investigated the use of (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (CSO) as a non-aqueous oxidizer for the synthesis of oligonucleotides containing methyl phosphonate linkages. It was already known that the use of low water oxidizers improved the synthesis of oligos containing methyl phosphonates. However, we were able to show that a 0.5M solution of CSO in acetonitrile (40-4632-xx) gave exellent results in the synthesis of chimeric oligonucleotides containing methyl phosphonate linkages.

Sensitivity of Purine Bases

Some bases that show instability to multiple successive synthesis cycles exhibit better stability with a low water oxidizer. At the same time as investigating methyl phosphonates, we also found that a 0.5M solution of CSO in acetonitrile worked well as an oxidizer for the synthesis of oligos containing multiple incorporations of 7-deaza-dG, compared with iodine oxidation which caused substantial degradation.

Recently a customer noted difficulty in preparing oligo-dI. Because of the known sensitivity of purine bases to iodine oxidation, we suggested using 0.5M CSO (with a 3 minute oxidation time) instead of the standard Iodine based oxidizer. Using CSO, the customer found that the synthesis was much improved and the oligo was successfully isolated.

Since Inosine is somewhat susceptible to damage by iodine during oxidation, we now recommend the use 0.5M CSO in anhydrous acetonitrile with a 3 minute oxidation time, if there are >6 incorporations of inosine within a sequence.[1]

DBCO

In a recent article,[2] we described the sensitivity of the copper-free click reagent DBCO to iodine oxidation. The occurrence of a side reaction came to our attention[3] during the earlier investigation of a customer problem preparing a relatively long oligo (63-mer) containing DBCO-dT (2). The customer's report seemed to indicate that the DBCO moiety was being cleaved during repetitive synthesis cycles. Conceptually, this was unexpected since amide linkages are resistant to hydrolysis, which implied that DBCO-dT is sensitive to one or more of the synthesis reagents and that the repeated exposure during the synthesis of long oligos led to cleavage of the DBCO.

To test this hypothesis, CPG from a simple 12-mer dT synthesis containing three additions of DBCO-dT was used. The RP HPLC of the test oligo synthesis is shown in Figure 2a. This oligo was then subjected to treatment with standard DNA synthesis oxidizer, 0.02 M Iodine, for 5 minutes at

room temperature. This exposure is equivalent to roughly 20 synthesis cycles. As shown in Figure 2b, the resulting degradation was quite dramatic.

As described above, with other analogues with sensitivity to iodine, we have achieved good results using CSO. So, when a 63-mer was re-synthesized using 0.5 M CSO in acetonitrile and a 3 minute oxidation time, the hoped-for improvement was very significant indeed.

Figure 3 shows the deconvolved electrospray MS data for the same sequence synthesized using standard 0.02 M Iodine versus 0.5 M CSO with the target mass being 20,511 Da. It is clear that the DBCO moiety is being cleaved when exposed to iodine-based oxidizers. What appears to have occurred during oxidation with iodine is the formation of an N-iodo amide, making the amide linkage unstable. During deprotection, the DBCO is eliminated, leaving a hexamido linker present. (The splitting of the -DBCO peaks is 14 Da, indicating the formation of both the amide and N-methylamide linkers which results from the oligo being deprotected in AMA). The lower molecular weight peaks associated with the CSO-oxidized oligo are deletion mutants (-1, -2 and -3 dTs), which suggests the oxidation time of 3 minutes should have been increased slightly for an oligo of this length.

As a result of these data, we now recommend that synthesis of oligos containing DBCO-dT be completed using 0.5 M CSO oxidizer. Acceptable results can be achieved with iodine oxidation if DBCO-dT is subjected to no more than 8-10 further cycles.

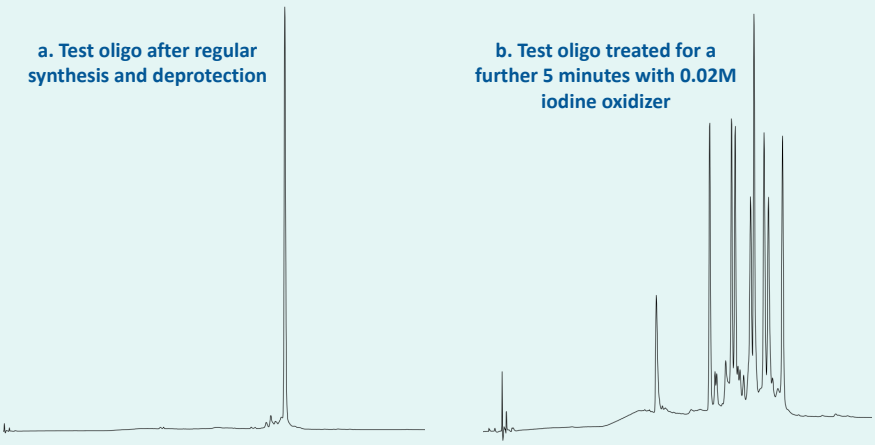

Figure 2: RP HPLC of Test 12-mer containing three DBCO-dT additions

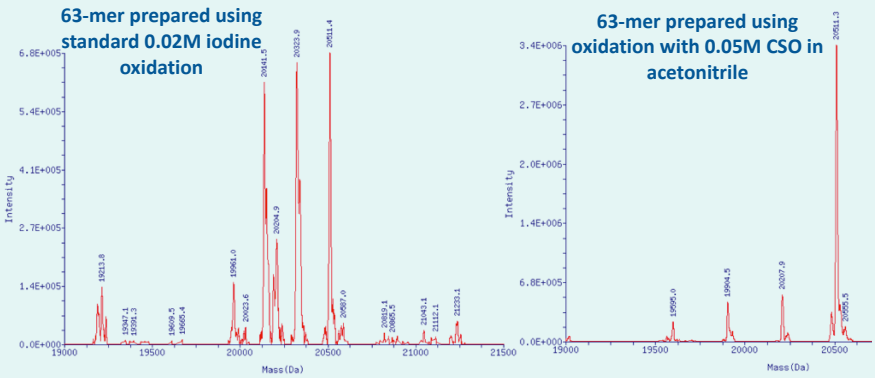

Figure 3: MS analysis of 63-mer containing three DBCO-dT additions

PACE modification

As with methyl phosphonates, the PACE modification is degraded by N-methylimidazole during capping and is susceptible to cleavage during aqueous oxidation using iodine. For this reason, we recommend using Unicap (40-4410-XX), a phosphoramidite-based capping reagent, and 0.5 M CSO (40-4632-XX), a non-aqueous oxidizer, for best results.[4]

Following coupling of the 2'-OMe PACE monomer, the recommended procedure is to cap using Unicap with a regular coupling time and then oxidize using 0.5 M CSO for 3 minutes.

$CDPI_3$ MGB™ CPG

As noted, the iodine oxidation step during DNA synthesis cycles has the potential to damage minor bases and modifiers. So it was no surprise when it was found that the indole residues of $CDPI_3$ MGB CPG (Figure 4, Page 7) are susceptible to iodination when standard 0.02 M Iodine oxidizer is used during synthesis. (This is only observed in the $CDPI_3$ MGB CPG which lacks the ethoxycarbonyl protecting groups on the nitrogens of the indole rings of the 5'-$CDPI_3$ MGB phosphoramidite.)

us Oxidation using CSO (cont.)

Figure 5, Page 7 shows chromatograms 1) and 2) of the sequence 5'-T8-$CDPI_3$ MGB-3' deprotected in 30% ammonium hydroxide for 2 hours at room temperature. The first oligo was synthesized using non-iodine oxidation with 0.5 M CSO and a 3 minute oxidation time while the second used 0.02 M iodine oxidizer. Chromatogram 2) illustrates the multiplicity of iodination on the indole rings. However in this case, as shown in the third chromatogram, the iodination is mostly reversible when the oligo is deprotected for 17 hr at 55 °C in EtOH/NH4OH 1:3 (v/v).[5]

Conclusion

In this review article, we have demonstrated several examples of situations where side reactions in minor bases and modifiers are essentially eliminated by the use of a non-aqueous and non-iodine containing oxidizer. While these side reactions are relatively minor in the case of simple oligonucleotides with a single addition of the minor base or modifier, multiple additions and/or multiple further cycles of oligonucleotide synthesis revealed extensive modification by iodine. Our advice is to consider the use of the non-aqueous oxidizer CSO when unusual and unexpected results manifest themselves in the synthesis of more complex or longer oligonucleotides.

References

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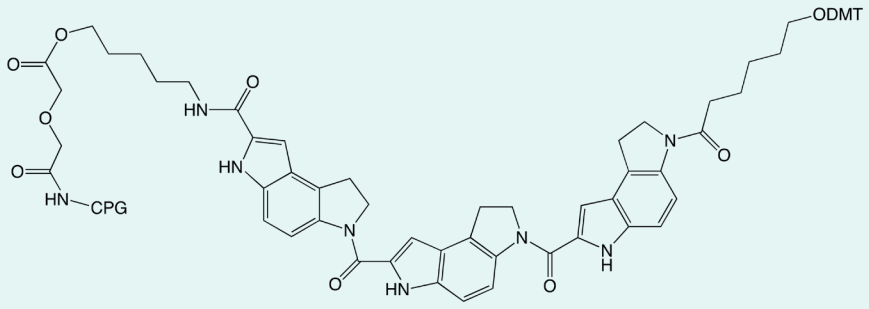
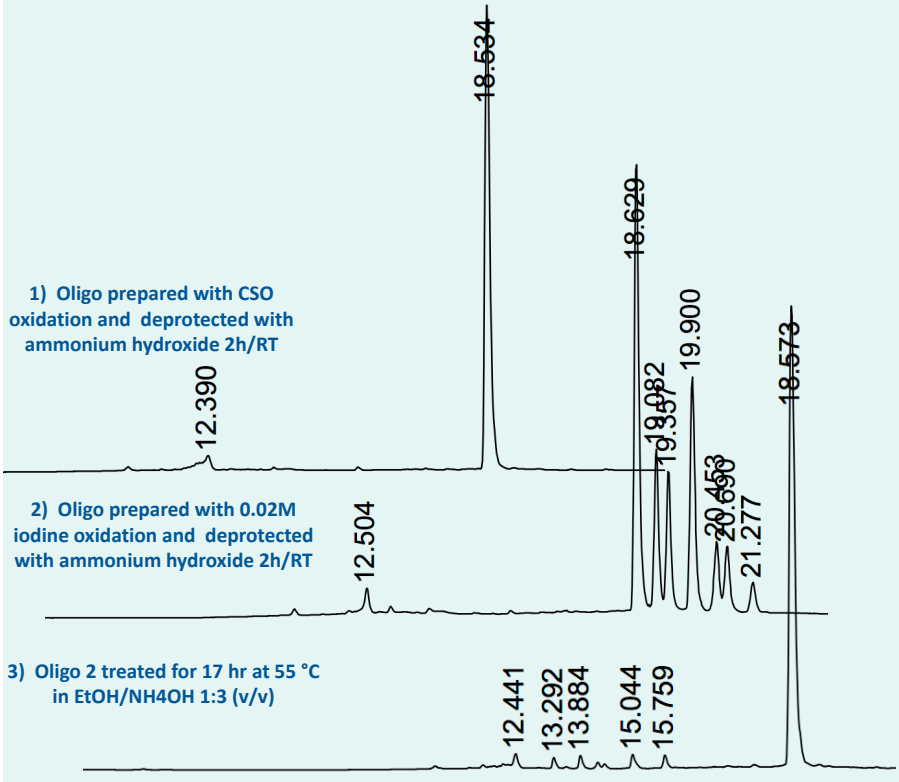


$CDPI_3$ MGB™ CPG

Figure 4: Structure of $CDPI_3$ MGB™ CPG



Chromatogram 1 shows the oligo prepared using CSO oxidation. Chromatogram 2 shows the result of iodine oxidation with the various permutations of 0, 1, 2 or 3 iodines coupled to the indoles of the $CDPI_3$ MGB - as determined by ESI MS - most likely at the 3 position of the indoles as described by Boger and Sakya *J. Org Chem*. 1992, 57, 1277-1284. Chromatogram 3 shows the oligo of Chromatogram 2 after deprotection with ethanolic ammonium hydroxide to reverse the iodination reactions.

Figure 5: Chromatograms of 5'-T8-$CDPI_3$ MGB-3'

New Product - Locked Ana

Introduction

Locked nucleic acid (LNA) is a type of modified RNA that consists of nucleotides with a bicyclic sugar unit where the 2'-oxygen and the 4'-carbon atoms are connected with a methylene unit, as detailed in Figure 1. These structures were originally developed by Wengel and co-workers[1, 2] and since then, LNA has proven to be quite popular, particularly in the field of therapeutics where LNA has been evaluated in several clinical trials.[3-6] The most attractive aspect of LNA is that it exhibits unprecedented thermal stability towards complementary DNA and RNA. The additional ring conformationally restricts the sugar into an orientation that is particularly favorable for hybridization. A single insertion of an LNA nucleotide can enhance the melting temperature by more than 9 °C against RNA. This enhancement is sequence dependent and does decrease with increasing LNA content.[7] It should be pointed out that this stabilization does not come at the expense of specificity. In fact, LNA's exhibit mismatch specificity that is more often than not superior to that of DNA.

In addition to its ability to bind very tightly to DNA and RNA, LNA also has several other desirable traits. Like DNA and RNA, LNA is highly soluble in water and can be therapeutically delivered using standard DNA/RNA strategies. Also, the nucleoside phosphoramidites of LNA are commercially available and can be readily used with well-established solid phase synthesis methods to synthesize all-LNA or LNA/DNA/RNA chimeras. Not only does this make LNA widely available, but it also allows LNA nucleotides to be interspersed among DNA and RNA nucleotides, giving researchers a high degree of design control for their desired applications. Furthermore, LNA is well-tolerated in biological systems. A lot of this is because LNA oligonucleotides do not need to be as long to exhibit tight binding to their targets. Finally, as a backbone modification, LNA is highly nuclease resistant. Fully modified LNA is very stable in the presence of intra- and extracellular endo- and exonucleases while LNA/RNA and LNA/DNA chimeras show stabilities that are sequence dependent. For example, mixmers with alternating LNA and DNA residues will be much more stable than gapmers containing a stretch DNA flanked by LNA on the 5' and 3' termini.

Applications

The outstanding properties of LNA have been exploited in a wide range of applications. One of these areas involves the detection of single nucleotide polymorphisms (SNPs). A SNP is a variation in a single nucleotide that occurs at specific positions between members of the same species. These can occur in coding or regulatory regions and are associated with a wide range of human diseases. The use of LNA for SNP detection applications relies on the enhanced mismatch recognition relative to native DNA and have been carried out in various formats.

In allele-specific PCR, primers containing one single LNA residue across from the point of mutation allow PCR product to be generated only for the desired allele of interest. In one investigation, an allele specific LNA qPCR assay was designed to study mutations in oncogenes and had an analytical sensitivity of 0.1 %.[8]

In other experiments, LNA probes have also been used as capture probes for ELISA-like assays and fluorescence in situ hybridization (FISH) experiments.

Another area where the use of LNA has been investigated thoroughly is in the field of antisense to modulate gene expression by inactivating mRNA. This has been predominantly done in one of two ways. In the first, all-LNA or LNA/DNA mixmers are used as steric blocks to inhibit therapeutically interesting RNA targets, particularly those that have complex structures that make them inaccessible to typical antisense agents. In the second, DNA gapmers are used to facilitate RNase H cleavage of the RNA target. These sequences are usually composed of an internal DNA sequence of at least 7 nt that is sandwiched between two sections of LNA of 2-5 nt. The LNA termini provide affinity while the DNA region triggers cellular RNase H activity. As an example, Koch and coworkers have shown that a 13 nt gapmer containing an 8 nt DNA sequence was able to significantly lower non-high-density lipoprotein in both mice and non-human primates.[9]

Other areas where LNA has been used include the cleavage of RNA by DNAzymes, the detection of microRNA as well as the capture of RNA. LNA continues to be a highly desirable oligonucleotide modification.

In the past, Glen Research was able to provide LNA phosphoramidites for the research and development community, but, unfortunately, they had to be discontinued. Recent developments have allowed us to again provide the tools for this technology in the form of our LA phosphoramidites, as described below. As was the case in the past, the pyrimidine bases are thymine and 5-methylcytosine rather than uracil and cytosine, respectively, as shown in Figure 1.

log Phosphoramidites

Use of LA Phosphoramidites

The synthesis of LNA oligonucleotides is very similar to that of DNA oligonucleotides. The LA phosphoramidites can be dissolved in anhydrous acetonitrile to standard concentrations, except for 5-Me-C, which requires the use of a 1:1 mixture of methylene chloride and acetonitrile.

They are more sterically hindered compared to standard DNA phosphoramidites and therefore require a longer coupling time. Coupling times of 3 and 4 min are recommended for ABI and Expedite synthesizers, respectively, using tetrazole activator.

The oxidation of the phosphite after LA coupling is also slower compared to the similar DNA phosphite, and therefore a longer oxidation time is suggested. Using standard iodine oxidation procedures (0.02 M), 45 seconds has been found to be the optimal oxidation time on both ABI and Expedite instruments.

LA-containing oligonucleotides are deprotected following standard protocols. It is, however, advisable to avoid the use of methylamine when deprotecting oligos containing Me-Bz-C-LA, since this can result in introduction of an N4-methyl modification. LA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogues, modifiers and labels.

LNA oligonucleotides are water soluble, can be separated by gel electrophoresis and precipitated by ethanol.

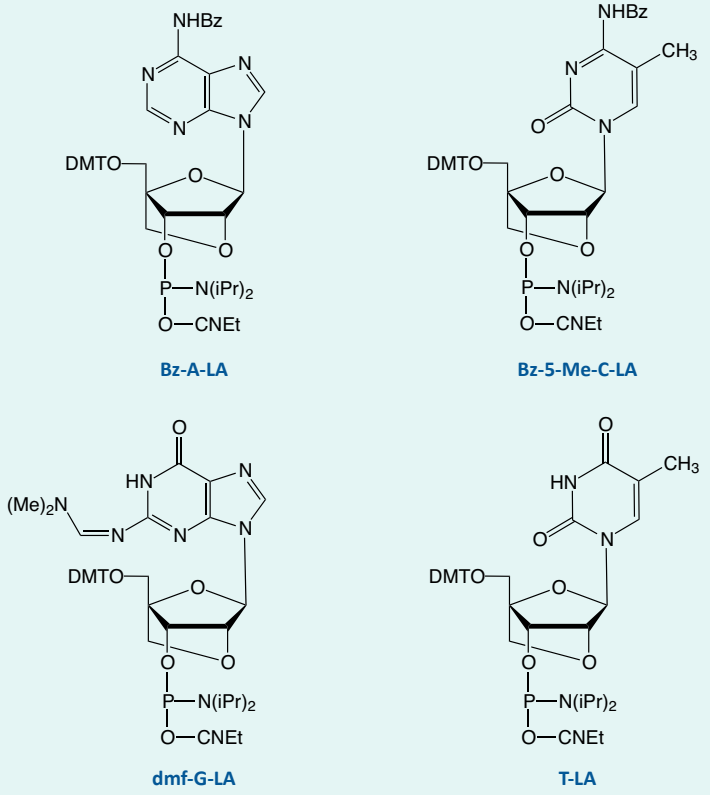


Figure 1: Structures of Locked Analog Phosphoramidites

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Item	Catalog No.	Pack	Price ($)
Bz-A-LA Phosphoramidite	10-2000-05	0.5g	75.00
	10-2000-10	1.0g	150.00
Bz-5-Me-C-LA Phosphoramidite	10-2011-05	0.5g	75.00
	10-2011-10	1.0g	150.00
dmf-G-LA Phosphoramidite	10-2029-05	0.5g	75.00
	10-2029-10	1.0g	150.00
T-LA Phosphoramidite	10-2030-05	0.5g	75.00
	10-2030-10	1.0g	150.00

Introduction

In a previous Glen Report article, Misha Shchepinov reviewed the variety of strategies available for the preparation and use of oligonucleotide dendrimers.[1] In that article, he defined dendrimers as discrete, highly branched, monodispersed polymers that possess patterns reminiscent of the branching of trees. He also postulated that dendrimer oligonucleotides would be representative of a new segment of polymer science.

DNA molecules are well suited for use in nanotechnology because of their unique molecular recognition features. For example, dendrimers with arms terminating in oligonucleotides of the same or of different sequences could be used to build cages, cryptands, tubes, nets, scaffolds and other more complex 3-D structures (Figure 1).[2,3] In the field of oligonucleotide arrays, where the signal is originally limited by the surface density of the device, the multiplicity of labeling afforded by dendritic structures allows detection at much lower concentrations. In the case of fluorescence detection, these structures allow amplified detection with adequate separation of labels available to avoid self-quenching. Similarly, dendritic labeling of probes allows for amplification of signal without affecting the behavior of the core oligonucleotide probe sequence (Figure 2).

Glen Research offers a set of phosphoramidites for the synthesis of oligonucleotide dendrimers. These phosphoramidites, shown in Figure 4, Page 11, allow the dendritic segment to be readily prepared without affecting the structure of the core oligonucleotide.

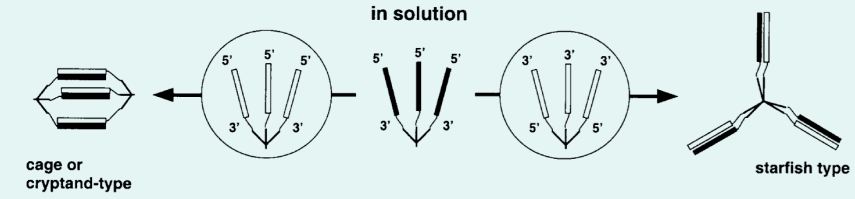

Figure 1. Formation of Complementary Dendritic Complexes

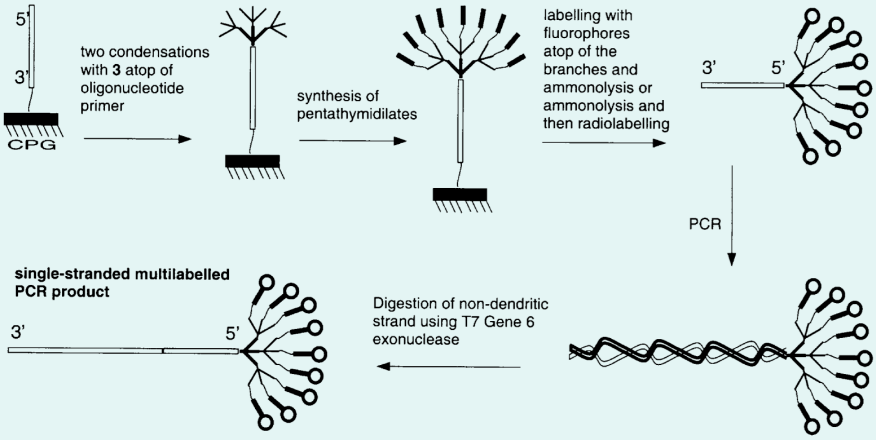

Figure 2. Conversion of Polylabeled PCR Primer into a Single-Stranded Polylabeled PCR Product

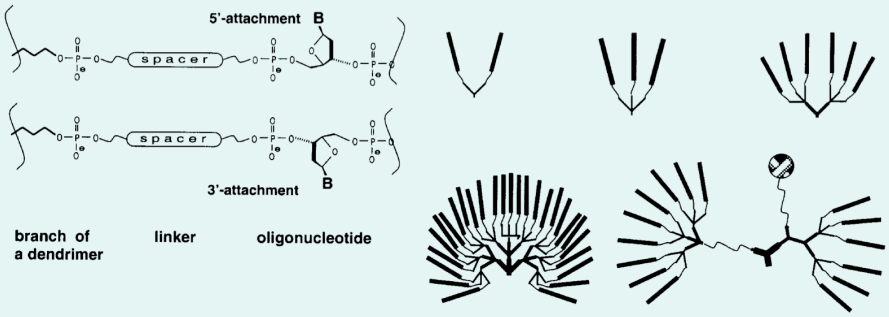

Figure 3. Examples of Plain and Mixed Dendrimers of Different Generations

ers – An Update

Symmetrically Branched Dendrimers

Symmetrically branched structures can be readily prepared using Symmetric Doubler (1), which simply doubles branches in the growing oligonucleotide chain with every addition, as the name implies. More complex branches can be prepared by trebling the 5' termini using Trebler (2) and Long Trebler (3), as illustrated in Figure 3.

As you can imagine, coupling branching species to the end of a growing oligonucleotide will potentially lead to the synthesis grinding to a halt for steric reasons. This was one of the reasons for adding Long Trebler (3) to the set since this molecule incorporates longer spacer arms in an attempt to alleviate steric effects. Regardless, the addition of intermediate spacer molecules, such as Spacer 18, is highly recommended.

As the density of oligonucleotides increases on the solid support, steric hindrance causes reactions to slow so complex dendritic oligonucleotides may need some optimization of the synthesis cycles. Certainly, the synthesis should be carried out on at least 1000Å CPG and preferably 2000Å CPG for more complex dendrimers.

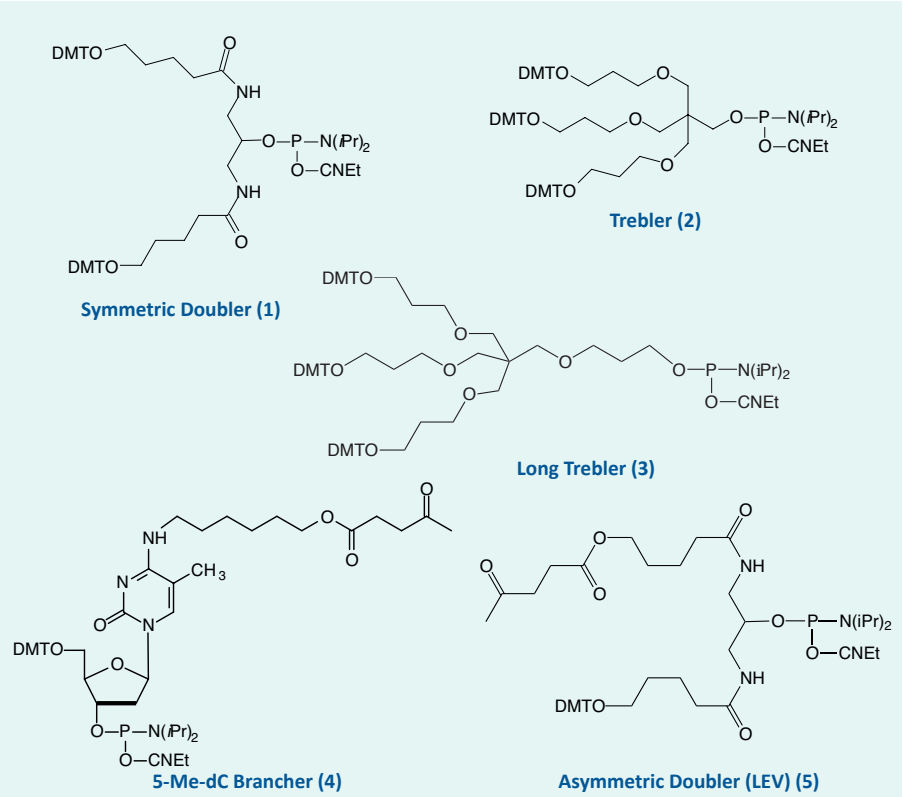

Figure 4. Structures of Symmetric and Asymmetric Branchers

Asymmetrically Branched Dendrimers

By far the most common approach to DNA diagnostics is amplification of the target sequence to produce enough copies for a signal to be observed using conventional detection systems. However, an alternative approach does exist - direct analysis of the target DNA by signal amplification.[4-7] This latter technique requires that the synthetic oligonucleotide should contain the primary sequence attached to many identical copies of the secondary sequence. It is the detection of the many copies of the secondary sequence that serves to amplify the signal. The resulting branched oligonucleotide has been aptly described as comb-like, with the primary sequence in the handle and the secondary sequences being the teeth of the comb. The synthesis of comb-like structures requires the use of an asymmetric branching phosphoramidite.

Glen Research previously offered an Asymmetric Brancher with the site destined to become part of the comb sequence blocked with the ubiquitous Fmoc protecting group.[8] Unfortunately, this structure proved to have limited stability as a phosphoramidite and was discontinued a few years ago. Following the success and inherent stability of the 5-Me-dC Brancher Phosphoramidite (4), we have now introduced Asymmetric Doubler (Lev) (5), also protected with a levulinyl (Lev) protecting group. Once the primary oligonucleotide sequence 'handle' of the comb structure has been synthesized, the Lev protecting group can be specifically removed with a hydrazine reagent and the oligonucleotide 'teeth' can then be synthesized.

Synthesis of these comb structures has been described in detail for dC

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Brancher and is included on Page 2 of its Technical Bulletin - http://www.glenresearch.com/Technical/TB_dC_Brancher.pdf.

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Item	Catalog No.	Pack	Price ($)
Symmetric Doubler Phosphoramidite	10-1920-90	100 µmole	150.00
	10-1920-02	0.25g	240.00
Asymmetric Doubler (LEV) Phosphoramidite	10-1981-90	100 µmole	105.00
	10-1981-02	0.25g	250.00
Trebler Phosphoramidite	10-1922-90	100 µmole	180.00
	10-1922-02	0.25g	300.00
Long Trebler Phosphoramidite	10-1925-90	100 µmole	200.00
	10-1925-02	0.25g	300.00
5-Me-dC Brancher Phosphoramidite	10-1018-90	100 µmole	205.00
	10-1018-02	0.25g	505.00