Glen Research REPORT

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Introduction

We are delighted to introduce the first edition of the Glen Research Report but first, some words about our company may be appropriate. Glen Research Corporation was founded in January of this year to provide products and services to researchers in the field of biotechnology. With a background and experience in DNA synthesis, it was natural for us to present an initial product line of reagents for DNA synthesis. As the company grows, we are committed to introduce novel products for DNA manipulation, as well as new products for other areas of biotechnology research.

Already, Glen Research offers one of the most complete lines of reagents for DNA synthesis, both for routine use and for research applications. Our commitment to research applications will, of course, continue and some novel products will be discussed in this and future Research Reports.

In this first edition of the Glen Research Report, we will highlight some recent developments in chemical modification of DNA to produce oligonucleotides which may prove to have utility in AIDS and other anti-viral research.

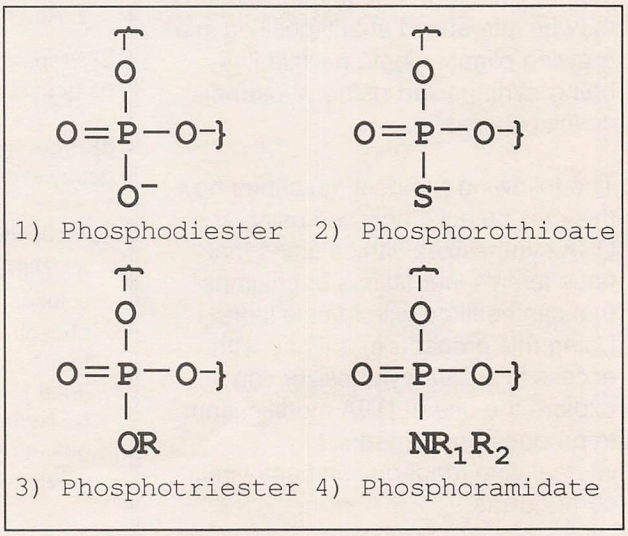
Modified Anti-Sense Oligonucleotides

The potential of anti-sense oligonucleotides to modify viral activity has recently become apparent in a variety of areas of research. Now researchers are beginning to create oligonucleotides with internucleotide phosphate modifications designed to impart specific properties to synthetic DNA: phosphorothioate linkages (2) have similar properties to natural phosphodiesters (1) but offer the advantage of being resistant to cleavage by nucleases; alkyl phosphotriesters (3) and phosphoramidates (4) increase lipophilicity of synthetic DNA, thereby allowing sequences to pass through cell membranes more readily, while also being nuclease resistant.

Future research may focus on the attachment of marker molecules to the phosphate backbone with applications in diagnostic probes or DNA sequencing. These techniques would similarly allow the attachment of amino acids, peptides, polysaccharides, or aromatic intercalating molecules.

It should be noted that the replacement of a pro-chiral oxygen from a natural phosphodiester linkage produces a chiral center at phosphorus. With every phosphate modification a pair of diastereoisomers is therefore formed. If a sequence is modified n times, there will be produced 2^n diastereomers, all of which, in principle, may exhibit differing biological activity.

Modified anti-sense oligonucleotides show potential for the development of novel diagnostic tests and therapeutic agents against viral diseases. A method for point modification of DNA is presented overleaf.



A Practical Application of Nucleoside H-Phosphonates for Point

To date, protocols to use nucleoside H-phosphonates on DNA synthesizers are directed toward the synthesis of unmodified oligonucleotides. These protocols differ from the more common phosphoramidite protocols mainly in that the oxidation step is removed from each cycle and is carried out instead at the end of the synthesis. Despite the clear advantages of this simple, fast synthesis cycle, H-phosphonate chemistry does not appear capable of performing at the level of phosphoramidite chemistry without significant modification. The development of a more hindered activator may increase the coupling efficiency while reducing the number of side reactions, but such an activator may be insufficiently reactive to effect the necessary end-capping. Since nucleoside H-phosphonates are stable in solution, this chemistry may find its place on machines which are used only occasionally for the synthesis of oligonucleotides of less than 50 bases. Contact us for a copy of our review of H-phosphonate chemistry.

The greatest advantage of using nucleoside H-phosphonates may be that they are useful in effecting a variety of backbone modifications of oligonucleotides. These include the preparation of phosphorothioate, phosphoramidate, and phosphotriester linkages. These modifications may be introduced at any position in a growing oligonucleotide while it is being synthesized using phosphoramidite chemistry.

The following protocol describes how this may be accomplished using any DNA synthesizer with columns that have female luer fittings or columns that can be fitted with female luers. Using this procedure, anyone with access to a DNA synthesizer can explore the use of DNA modification to produce novel markers, intercalating reagents, and anti-viral compounds.

Materials

COUPLING REACTION

1) Nucleoside H-phosphonates (Glen Research items 10-1200-05, 10-1210-05, 10-1220-05, and 10-1230-05 or equivalent).
2) Pivaloyl chloride (Aldrich item T7,260-5 or equivalent).
3) Anhydrous acetonitrile (Glen Research item 40-4050-50 or equivalent).
4) Anhydrous pyridine (Aldrich Gold Label item 27,097-0 or equivalent).
5) Several 10mL glass serum vials with rubber septa.
6) Two 2.5mL and one 10mL gas-tight luer syringes (Hamilton or equivalent).
7) Two 2" syringe needles.
8) Luer adaptors (if necessary).

MODIFICATION REACTION

1) Alcohol or amine (these compounds must be pure and extremely dry) or Sulfur (Aldrich Gold Label item 21,392-2 or equivalent).
2) Anhydrous CCl_4 (Aldrich Gold Label item 28,911-6 or equivalent) or Triethylamine (TEA) (Aldrich Gold Label item 23,962-3) and carbon disulfide (Aldrich item 27,066-0).

Procedure

A. Addition of the nucleoside H-phosphonate

1) Prepare a 0.1M solution of the desired nucleoside H-phosphonate in pyridine/acetonitrile 30:70. Transfer the solvents using a dry syringe.
If many syntheses are to be performed within a week, the solution may be prepared in the vial in which the nucleoside H-phosphonate was supplied. (Note: after preparation the solution should be stored cold in a desiccator.) If only a few syntheses are to be performed, it is more economical to weigh an appropriate amount of the nucleoside H-phosphonate as follows:

a. Dry a 10mL serum vial in a 130°C oven for several hours.
b. Cap the vial with a rubber septum, place it in a desiccator containing Drierite® and allow it to cool.
c. Weigh the appropriate amount of nucleoside H-phosphonate. (Note: the vial of nucleoside H-phosphonate must be warmed to room temperature before opening to avoid condensation. Weigh the monomer quickly and under a steady stream of argon or nitrogen to ensure anhydrous conditions.)
d. Open the vial while directing a stream of argon or nitrogen into its mouth and quickly add the nucleoside H-phosphonate; allow the vial to fill with the dry gas and reseal.
e. Add the appropriate amount of solvent mixture using a dry syringe.

2) Prepare a 0.5M solution of pivaloyl chloride in acetonitrile. Handle this preparation as described above to ensure anhydrous conditions.

3) Program the DNA synthesizer to stop just prior to the coupling step (i.e., after detritylation and appropriate washes) at the position where the modification is desired.

4) Coupling Reaction:

a. With a dry syringe draw in an aliquot of the nucleoside H-phosphonate solution. (For a 1 µmole scale synthesis, 0.1mL of the nucleoside H-phosphonate solution should be sufficient; however, because this step is critical to the success of the experiment, it is recommended that 0.2mL to 0.3mL be used.)
b. Remove any reagent from the syringe needle by drawing in a small amount of gas from the sealed vial (this will prevent contamination of the pivaloyl chloride solution).

Modification of Oligonucleotides

c. Draw in a volume of pivaloyl chloride equal to the volume of nucleoside H-phosphonate solution in the syringe. This produces a five-fold molar excess of pivaloyl chloride to the nucleoside H-phosphonate.
d. Invert the syringe quickly two or three times to mix the two solutions.
e. Remove the column from the instrument and attach the syringe to the column using a luer fitting.
f. Attach the second syringe to the other end of the column.
g. Slowly pass the solution back and forth across the support-bound oligonucleotide for two minutes.
h. Remove one of the syringes and draw 2.5mL of air into it. Reconnect this syringe and force the air through the column into the other syringe to remove the coupling solution from the column.
i. Rinse the column and the syringes with anhydrous acetonitrile.

B1. Addition of the alcohol or amine group

1) Prepare a 0.1M solution of the desired alcohol or amine in CCl_4. (Handle this preparation as described in step ***A*-1**)

2) Reaction:

a. Using the same procedure outlined in step ***A*-4**, pass ~0.5mL of the solution back and forth across the support-bound oligonucleotide for ~30 minutes. (Note: the time necessary for quantitative reaction depends on the steric hindrance of the alcohol or amine being used; because this step is crucial to the success of the experiment, a reaction time of 30 minutes is recommended.)
b. Wash the support-bound oligonucleotide by filling one of the syringes with anhydrous acetonitrile and forcing it through the column into the other syringe. Repeat this step several times to thoroughly wash the oligonucleotide.
c. Remove the syringes and reattach the column to the instrument.

B2. Addition of Sulfur

1) Prepare a solution of 0.1M sulfur in TEA/carbon disulfide (1:9).

2) Using the procedure outlined in step ***A*-4**, pass ~0.5mL of the solution back and forth across the support-bound oligonucleotide for ~5 minutes.

3) Follow steps ***B1*-2b** and **2c**.

C. Resuming synthesis

Restart synthesis by skipping the reaction step and continue through the normal cycle.

D. Deprotection

Deprotection may proceed as normal.

References:

Deoxynucleoside H-Phosphonate Diester Intermediates in the Synthesis of Internucleotide Phosphate Analogues. B.C. Froehler, *Tetrahedron Lett.*, 1986, **27**, 5575-5578, and references cited therein.

Literature Review

Solid-Supported Synthesis, Deprotection and Enzymatic Purification of Oligodeoxyribonucleotides. M.S. Urdea and T. Horn, *Tetrahedron Lett.*, 1986, **27**, 2933.

With today's automated synthesizers, oligonucleotides are rapidly and conveniently synthesized with a high degree of efficiency. Using cyanoethyl phosphoramidite chemistry, the deprotection of the synthetic sequences is also rapid and convenient. Thus, purification, if desired, remains the last labor-intensive step for which automation is currently unavailable.

In a recent publication, Urdea and Horn of Chiron Research, describe a procedure for enriching the target oligonucleotide by enzymatic degradation of the failure sequences. The authors suggest that because this procedure involves only reagent addition and wash steps, it should be amenable to automation.

The authors adjust the protection scheme (use N', N'-dibutylformamidine instead of isobutyryl to block the N^6-position of dG) and the end-capping procedure (substitute levulinic anhydride for acetic anhydride) and replace the final 5'-DMT group with benzoate. These adjustments make all protecting groups except the 5'-benzoate of the target sequence and the 3'-succinate linkages to CPG labile to hydrazine.

After deprotection, failure sequences are hydrolyzed on the support with spleen phosphodiesterase, the target sequence being protected by the 5'-benzoate. Standard ammonia treatment releases the fully deprotected product in the presence of failure sequences reduced to 10-mer or less, allowing simple and rapid reverse-phase cartridge purification.

This technique may require further refinement but it does exhibit considerable promise for the eventual total automation of synthesis, deprotection, and purification of DNA.

New Products For DNA Synthesis

Disposable Columns

Glen Research now has disposable columns suitable for use in a variety of DNA synthesizers, including ABI and Vega instruments. Columns are available in 500Å (0.2, 1.0, and 10 µmole synthesis scale) and 1000Å (0.2 µmole synthesis scale) CPG for the synthesis of intermediate (<80-mer) and long (>80-mer) sequences, respectively.

Activator

We also now have available an alternative activator for use in DNA synthesis: 4-nitrophenyltetrazole. This product appears to be a more efficient activator than tetrazole.

Cat. No.	Item	Pack of	Price ($)
20-2100-41	dA-lcaa-CPG 500 1µmole	4	60.00
20-2100-42	dA-lcaa-CPG 500 0.2µmole	4	60.00
20-2100-13	dA-lcaa-CPG 500 10µmole	1	100.00
20-2101-42	dA-lcaa-CPG 1000 0.2µmole	4	60.00
20-2110-41	dC-lcaa-CPG 500 1µmole	4	60.00
20-2110-42	dC-lcaa-CPG 500 0.2µmole	4	60.00
20-2110-13	dC-lcaa-CPG 500 10µmole	1	100.00
20-2111-42	dC-lcaa-CPG 1000 0.2µmole	4	60.00
20-2120-41	dG-lcaa-CPG 500 1µmole	4	60.00
20-2120-42	dG-lcaa-CPG 500 0.2µmole	4	60.00
20-2120-13	dG-lcaa-CPG 500 10µmole	1	100.00
20-2121-42	dG-lcaa-CPG 1000 0.2µmole	4	60.00
20-2130-41	dT-lcaa-CPG 500 1µmole	4	60.00
20-2130-42	dT-lcaa-CPG 500 0.2µmole	4	60.00
20-2130-13	dT-lcaa-CPG 500 10µmole	1	100.00
20-2131-42	dT-lcaa-CPG 1000 0.2µmole	4	60.00

Cat. No.	Item	Pack	Price ($)
30-3020-09	4-Nitrophenyltetrazole, crystalline	0.9g	45.00
30-3120-45	0.1M 4-Nitrophenyltetrazole in Acetonitrile	45mL	55.00

To Our Customers

The response to our initial product launch has been heartening and we would like to thank the many researchers who allowed us the opportunity to be of service. It is never an easy choice to change suppliers, but it is even more difficult to turn over the supply of consumables for your valuable instrumentation to an entirely new company. Fortunately for us, many researchers have found themselves able to place their trust in us and we have endeavored to respond by providing fast, efficient service with deliveries of high purity products at a fair price.

Glen Research

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