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PREPARATION OF GLYCERONUCLEOSIDE PHOSPHORAMIDITE SYNTHONS AND THEIR USE IN THE SOLID PHASE SYNTHESIS OF ACYCLIC OLIGONUCLEOTIDES

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Abstract The syntheses of N,N-diisopropylmethylphosphoramidites (**3a,b**) of 9-{[2-dimethoxytrityl-hydroxy-1-(hydroxymethyl)ethoxy]-methyl}N^6-benzoyladenine, **2a**, and 1-{[2-dimethoxytrityl-hydroxy-1-(hydroxymethyl)ethoxy]-methyl}thymine, **2b**, are described. Oligoacyclonucleotides, 2 - 8 units long, were synthesized using these synthons on either controlled pore glass or silica gel supports with average coupling yields up to 98%. The preparation of 1-{[2-dimethoxytrityl-hydroxy-1-(pentachlorophenylsuccinyl-hydroxymethyl)ethoxy]-methyl}thymine, **5**, for the derivatization of long chain alkylamine controlled pore glass is also described.

Glyceronucleosides, acyclic analogues of naturally occuring deoxynucleosides have been synthesized in our research group[1,2,3,4] and are potent antiviral agents. In these molecules, **1**, the usual sugars, 2-deoxy-D-ribose and D-ribose of deoxynucleosides and ribonucleosides respectively, are replaced by an acyclic glycerol side chain. These compounds, especially those which have purines as the base component have been found to be active *in vitro* and *in vivo* against a range of viral pathogens[5].

The synthesis of oligonucleotides in which these acyclic residues are substituted for the normal DNA and RNA sugars would be of interest since the product glyceronucleotides might also possess antiviral activity. Furthermore the preparation of acyclic nucleoside phosphoramidites would add a fourth possibility to the repetoire of nucleic acid synthons presently available (*vide infra*) for nucleic acid synthesis. The synthesis of glyceronucleotides in solution is difficult due to the fact that the protected glyceronucleotides contain an unresolved chiral center at the C-3'. Therefore each time a unit is added to a growing nucleotide chain the number of diastereomers increases twofold. Another difficulty is the availability of the starting materials since the acyclic glycerol and base portions must be prepared separately, then condensed and purified. The use of a solid phase approach would eliminate the need for intermediate purifications of the protected nucleotides and the scale of the synthesis would drastically reduce the amount of starting material needed.

The reagents of choice in the synthesis of oligonucleotides, in the ribo[6,7], deoxyribo[8,9,10], and arabino[11] series, are the 3'-O-(N,N-diisopropylalkyl)phosphoramidite derivatives. We have adopted a similar strategy for the synthesis of acyclic oligonucleotides.

The phosphoramidite derivatives of the adenine and thymine acyclic nucleosides **2a,b** were prepared in a manner similar to that described for the preparation of ribonucleoside phosphoramidites[7] and is illustrated in **Scheme 1**. The suitably protected glyceronucleoside (1 eq.) was dissolved in THF and the resulting solution

added, dropwise, to a stirred THF solution of diisopropylethylamine (4 eq.) and N,N-diisopropylaminomethyl-phosphonamidic chloride (1.3 eq.) at room temperature. The reactions were checked by TLC and were complete in 1.5 h. The workup consisted of a simple extraction between ethyl acetate and 5% $NaHCO_3$. The crude products were purified by silica gel chromatography using $EtOAc/CH_2Cl_2/NEt_3$ mixtures as eluent. Selected spectroscopic and physical properties of **3a,b** are shown in **Table 1**[12].

The next step in this approach was to derivatize a suitable solid support with the acyclic nucleosides. Two supports were evaluated for this purpose, long chain alkylamine controlled pore glass beads and Vydac TP silica gel. The derivatization involving the LCAA-CPG beads required the preparation of the pentachlorophenylsuccinates of the acyclic nucleosides. The thymine compound **5** was prepared first to test the methodology. Its preparation paralleled the procedure used for ribonucleosides[7] (**Scheme 1**). Protected **2b** was treated with succinic anhydride and a catalytic amount of DMAP in pyridine to yield **4**. Following workup crude **4** was then treated with pentachlorophenol and DCC to yield **5**. Compound **5** was precipitated in hexane and used without further purification. LCAA-CPG was derivatized as described previously[7]. The loading obtained was 16 $\mu mol\ g^{-1}$.

Carboxyl functionalized Vydac TP silica gel support[13,14,15,16] was then evaluated as a solid support. Esterification of the polymer-bound carboxyl group with the hydroxyl group of **2a** or **b** was accomplished using DCC[17]. The loadings obtained, 63 $\mu mol\ g^{-1}$ for both the adenine and thymine derivatives, were much improved over the results of the LCAA-CPG experiment.

Both types of derivatized support were tested in the synthesis of a thymine acyclic nucleotide trimer using the cycle shown in **Table 2.** The automated solid phase syntheses were performed as described in reference 7. The average coupling yield, monitored by spectrophotometric quantitation of released dimethoxytrityl cation at 504 nm, was 98% in the case of the LCAA-CPG support and 93% in the Vydac TP silica case. These yields were equivalent to the coupling yields achieved in both the RNA, DNA, and ANA (arabino nucleic acid) syntheses in general, using the phosphoramidite procedure. The silica gel support did offer the advantage of a much higher loading and therefore a larger amount of final product, thus further syntheses were performed on the silica support. Subsequently a series of oligomers, the dimers, trimers, and tetramers of both the adenine and thymine acyclic nucleotides was prepared. In addition a self complementary mixed hexamer, acyclo(**TATATA**) and an octamer of the thymine acyclic nucleoside were synthesized. Average coupling yields of 93-96% were obtained.

The acyclic oligomers were deprotected in only two steps. Thiophenoxide ion was used to remove the methyl phosphate protecting group, followed by $NH_4OH/EtOH$ treatment @ 55 °C for 18h to simaltaneously cleave the oligomer from the support and remove the benzoyl protecting group from the adenine residues. The residue resulting from the evaporation of the ammoniacal solution was extracted with H_2O and the resulting extracts lyophilized and reconstituted as 1 ODU 10 μl^{-1} aqueous solutions. Typical yields of 17-20 ODU per base residue were obtained.

All sequences were checked by electrophoresis on a 25% polyacrylamide/8M urea gel[18] and were compared with a series dimers, trimers, and tetramers prepared in solution[19]. All of the solid phase prepared sequences were identical to the sequence prepared in solution. These results were confirmed by HPLC analysis of the oligomers.

The sequences were also assayed for nuclease sensitivity and were found to be resistant to snake venom and spleen phosphodiesterases, as expected from earlier work[3]. The acyclic oligonucleotides were, however,

Scheme 1

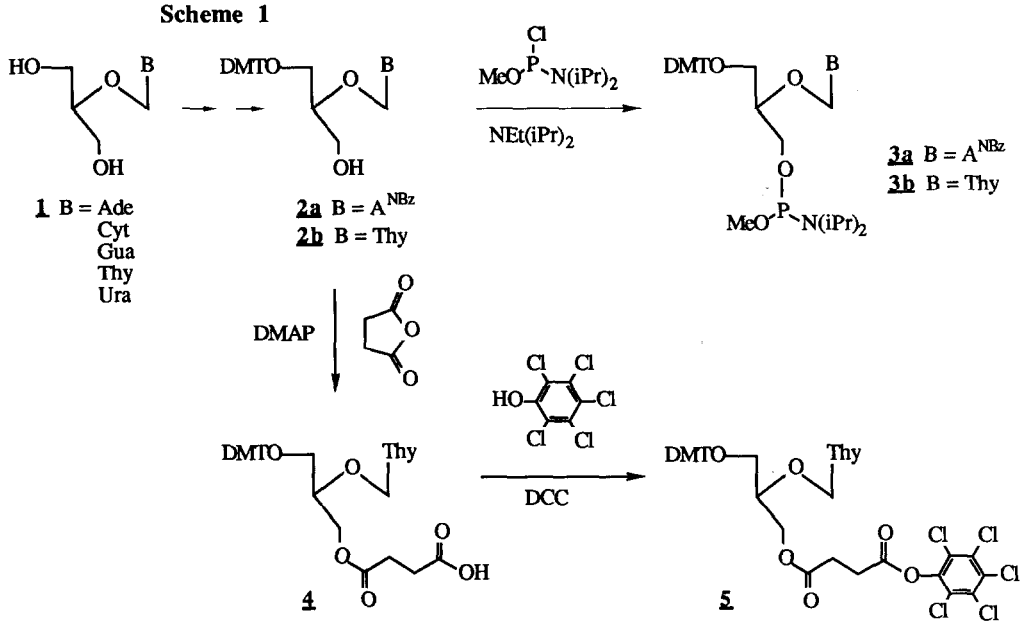

Table 1: Selected Physical, Chromatographic, and UV Properties of Glyceronucleoside Phosphoramidites

Compound	Isolated Yield (%)	R_f[a]	m.p. °C	UV (max; min)[b]	^{31}P NMR (ppm)[c]
3a	93	0.79	57-59	282; 259	149.29, 149.21, 149.16, 149.07
3b	92	0.61	56-60	268; 236	149.97, 149.92, 149.75, 149.56
4	87	0.26	na	254; 248	na
5	99	0.83	na	322, 254; 305, 248	na

na = not applicable a = ethylacetate b = 95%EtOH c = Downfield from 85% H_3PO_4, in $CDCl_3$ @ 80.98 MHz

Table 2: Synthesis Cycle for the Synthesis of Acyclic Oligonucleotides

Step	Reagent*	Time (sec.)
1	3% TCA/DCE	90
2	Acetonitrile	40
3	0.075M Amidite + 0.5M Tetrazole : 2/1	15
4	Recycle	105
5	0.25M Ac_2O/DMAP/Collidine THF	60
6	0.1M I_2 THF/Pyridine/H_2O : 7/2/1	30
7	Acetonitrile	20
8	0.25M Ac_2O/DMAP/Collidine THF	30
9	Acetonitrile	30
	Total	420

* Flow Rate = 5 ml min^{-1}

substrates for polynucleotide kinase and were labelled with [γ-^{32}P] ATP under standard conditions (assayed by gel electrophoresis[18,19]).

In this note we have described methods for the preparation of acyclic nucleoside phosphoramidite reagents and attachment of acyclic nucleosides to both long chain alkylamine controlled pore glass beads and Vydac TP silica gel. The conditions for the solid phase synthesis and deprotection of acyclic nucleotide oligomers have been determined. This method allows for the preparation of analogue sequences not easily accessible using solution phase techniques and for the incorporation of non-standard nucleic acid fragments in normal DNA, RNA, or ANA chains.

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References and Notes

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