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PREPARATION OF RIBONUCLEOSIDE 3'-O-PHOSPHORAMIDITES AND THEIR APPLICATION TO THE AUTOMATED SOLID PHASE SYNTHESIS OF OLIGONUCLEOTIDES[1]

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Oligoribonucleotides, six to fifteen units long, were synthesized on a controlled pore glass support using ribonucleoside 3'-O-phosphoramidite reagents. Average coupling yields of up to 98% were obtained with diisopropylaminophosphoramidite derivatives.

In light of the increasing interest in the role of RNA in molecular biology (for example its involvement in the self-splicing of pre-rRNA transcripts[2], the emergence of recombinant RNA technology[3], as well as the known role of transfer RNA's in the translation of the genetic code[4]) a facile and rapid procedure for the synthesis of oligoribonucleotides has become an important synthetic goal.

The introduction of the t-butyldimethylsilyl ether as a 2'-hydroxyl protecting group[5] has greatly facilitated the synthesis of oligoribonucleotides. Both solution[6] and solid phase syntheses[7,8], when used in conjunction with the chlorophosphite coupling procedure, have been found to be particularly effective. The nucleoside 3'-O-chlorophosphite reagents are, however, moisture sensitive and difficult to handle.

In the synthesis of oligodeoxyribonucleotides, the preferred reagents are the significantly more stable nucleoside 3'-O-phosphoramidites[9,10]. These phosphoramidite reagents may be prepared free of 3'-3' dimers, are stable to moisture and the conditions of chromatography, may be stored for months, and are easily activated using tetrazole. In this manuscript we would like to describe the preparation of 2'-O-silylated ribonucleoside 3'-O-phosphoramidites and their application to automated solid phase syntheses.

The protected ribonucleoside derivatives **1a-d** of adenosine, cytidine, guanosine, and uridine were used to prepare both morpholino- and diisopropylaminophosphoramidites, **2a-d** and **3a-d**. The morpholine compounds were prepared according to a procedure similar to the method established for deoxyribonucleoside phosphoramidite preparation[11]. The diisopropylamine derivatives could not, however, be prepared in high yields by this procedure. Instead, these compounds were prepared by the addition of a solution of the protected ribonucleoside **1a-d** (1.0 eq.) to a stirred solution of chlorodiisopropylaminomethoxyphosphite (1.3

eq.), anhydrous diisopropylethylamine (4 eq.), and N,N-dimethylaminopyridine (0.2 eq.) in anhydrous THF and the solution stirred for 3 hours. In the case of **3c**, the DMAP was omitted and 2.0 eq. of phosphite were used. Both types of phosphoramidite derivatives were worked up by extraction between ethyl acetate and sat. NaCl solution, and purified by silica gel chromatography using a ternary solvent mixture of CH_2Cl_2/Hexanes/NEt_3. The isolated yields, chromatographic solvent composition, and the ^{31}P NMR shifts are shown in TABLE 1[12].

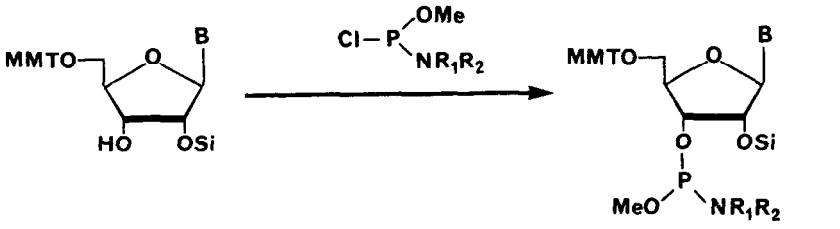
The ribonucleoside phosphoramidite reagents were then evaluated using an automated DNA/RNA synthesizer[8]. The solid support[13] used was either long chain alkylamine controlled pore glass (LCAA-CPG) or Vydac TP silica gel which were derivatized[14] to nucleoside loadings of 20-50 micromoles g^{-1}.

The synthesis cycle shown in TABLE 2 was used with the diisopropylamino phosphoramidites, using tetrazole as the activating reagent, to prepare the three homogeneous pentadecanucleotides: **$(Ap)_{14}A$; $(Cp)_{14}C$; $(Up)_{14}U$;** and **5'-CGG CCCCCGAACCCA** -3' on the LCAA-CPG support. The coupling yield of each cycle was determined by spectrophotometric quantitation of the released MMT cation at 476 nm. The average coupling yields were 95.7, 97.3, 98.1, and 98.0% respectively.

The ribonucleoside morpholinophosphoramidites **2a-d** were found to be less suitable than their diisopropylamine counterparts. The former derivatives were found to be less reactive and more difficult to activate (unpublished ^{31}P NMR studies). Despite the use of a longer coupling step (30 min) than the one shown in TABLE 2, the synthesis of the sequences; **$(Up)_5C$, $(Up)_7U$, $(Up)_5U$,** and **AAGAUC,** resulted in coupling yields of only 75-91%.

All of the oligoribonucleotides were deprotected by successive treatment with: thiophenoxide (30 min., removal of methyl phosphate protection), NH_4OH/EtOH:3/1 (50°, 8 hr., concomitant debenzoylation and cleavage from the support), and 0.1M TBAF/THF solution (2 hr., cleavage of O-silyl ethers). Following the TBAF treatment the completely deprotected oligoribonucleotides were desalted by direct application to a Sephadex G-25F size exclusion column, and subsequently purified by preparative gel electrophoresis (20% polyacrylamide/7M urea). The sequence composition and completeness of the deprotection reactions were confirmed by enzymatic degradation, using snake venom phosphodiesterase/alkaline phosphatase for the mixed sequence and spleen phosphodiesterase for the homopolymers, followed by HPLC analysis[15] in all cases.

These syntheses clearly demonstrate that the automated synthesis of oligoribonucleotides is now greatly improved. The ribonucleoside phosphoramidites have the advantages of being easy to prepare, purify, handle, and are quite stable. Most importantly, the coupling yields achieved using these reagents now approach the nearly quantitative yields which are possible in the synthesis of DNA, and which are necessary for the synthesis of longer RNA sequences such as transfer RNA's.



1.a B = N^6-Benzoyladenine, Si = TBDMS*
b B = N^4-Benzoylcytosine, Si = TBDMS
c B = N^2-Benzoylguanine, Si = TIPS**
d B = Uracil, Si = TBDMS

2.a-d R_1R_2 = 

3.a-d R_1=R_2 = *i*-Pr

* t-butyldimethylsilyl
** triisopropylsilyl

TABLE 1. PREPARATION OF PHOSPHORAMIDITES.

Starting Compound	Product	Isolated Yield (%)	Chromatography Solvent (CH_2Cl_2/Hex/NEt_3)	^{31}P NMR Chemical Shifts*
1. a	2. a	75	40/58/2	-146.26, -145.92
b	b	77	40/58/2	-147.43, -143.77
c	c	65	40/58/2	-147.99, -146.75
d	d	85	50/44/6	-146.84, -144.05
1. a	3. a	96	40/58/2	-151.30, -149.48
b	b	87	40/58/2	-149.97, -148.70
c	c	83	50/40/10	-153.71, -148.96
d	d	99	50/46/4	-149.71, -149.56

*Downfield from 85% H_3PO_4, in $CDCl_3$ @ 80.98 MHz.

TABLE 2. AUTOMATED SYNTHESIS CYCLE

Step	Reagent*	Time (min)
1	5% Trichloroacetic acid/Dichloroethane	3.50
2	Acetonitrile	0.75
3	0.1M Nucleoside Phosphoramidite + 0.5M Tetrazole/Acetonitrile	0.25
4	Recycle	14.75
5	0.05M I_2 THF/Pyridine/H_2O 7/2/1	0.50
6	0.25M Ac_2O/DMAP/Collidine/THF	1.50
7	Acetonitrile	1.25

* Flow Rate = 5 ml min^{-1} Total Cycle Time = 22.50 min

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