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THE RAPID CHEMICAL SYNTHESIS OF ARABINONUCLEOTIDES

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Abstract: A fast and convenient procedure for the chemical synthesis of arabinonucleotides which eliminates the multistep protection of the arabinonucleoside building blocks is described. Both solution and solid phase phosphite triester procedures are described[1].

Because of the biological activity of arabinonucleosides[2], the chemical synthesis of small units of arabinonucleic acids (ANA) has attracted considerable interest[3].

The synthesis of arabinonucleotides, like that of ribonucleotides, is complicated by the presence of the 2'-hydroxyl group. The selective protection of the 2'-hydroxyl group in arabinonucleosides is generally difficult due to steric hindrance[4]. In the original work by Wechter[5], the 2'- and 3'-hydroxyls were left unprotected during nucleotide coupling steps and as a result, mixtures of 2'-5' and 3'-5' linked nucleotides were obtained. There has been one report of a stepwise synthesis of 3'-5' linked arabinonucleotides of defined sequence but the protection of the nucleoside monomers required numerous steps[6]. We wish to describe a method which minimizes the protection of the arabinonucleoside units and affords 3'-5'-arabinonucleotide oligomers in good yields.

The key reagents used were the nucleoside 3'-phosphoramidite derivatives **2a,b** which could be easily prepared in good yields in a manner analogous to the preparation of 2'-deoxyribo-[7] or ribonucleoside 3'-phosphoramidites[8]. Thus N,N-diisopropylcyanoethylphosphamidic chloride selectively reacted with **1a** and **1b** to afford the 3'-phosphoramidites **2a** and **2b** in 66 and 90% yields, respectively[9] (SCHEME 1). The 2',3'-bisphosphoramidites were also isolated (5-10% yield) and were easily separated from the desired 3'-phosphoramidites by silica gel chromatography. The 2'-phosphoramidite of uridine was formed in 3% yield (^{31}P-NMR) while that of adenosine could not be detected indicating a marked difference in the steric environments of the 2'- and 3'-hydroxyl groups.

In order to evaluate **2a,b** as building blocks for nucleotide synthesis they were activated (0.50 mmol.) with tetrazole (2.0 mmol.) in THF (3.0 mL.) in the presence of the 2',3'-diacetylated nucleosides **4a,b** (0.67 mmol.) respectively, (SCHEME 1). After 30 min., the resulting 3'-5' dinucleoside phosphite triester intermediate **5a,b** was reacted *in situ* with Ac_2O/DMAP/collidine/THF

SCHEME 1

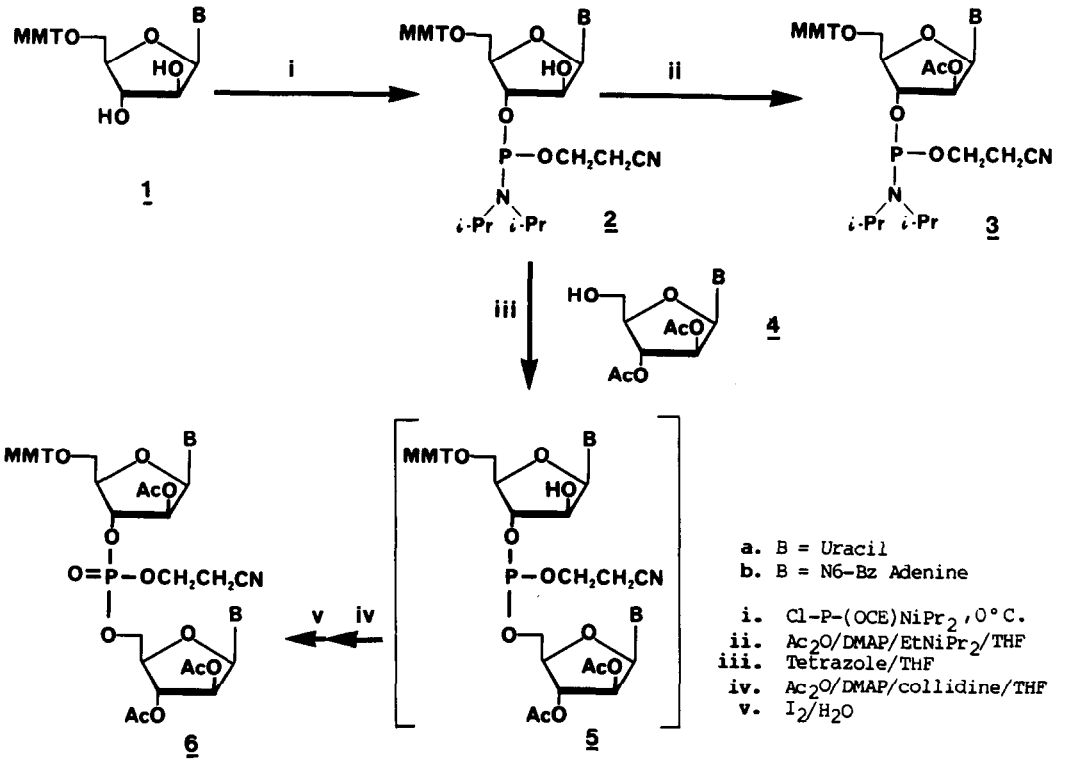

TABLE 1. AUTOMATED ANA SYNTHESIS CYCLE.

Step	Reagent*	Time (sec.)
1	Acetonitrile wash	30
2	5% Trichloroacetic acid/Dicholoroethane	90
3	Acetonitrile wash	60
4	0.05M phosphoramidite/0.17M Tetrazole/ Acetonitrile	15
5	Recycle (see text)	15-285
6	0.25M Ac_2O/DMAP/collidine/THF	60
7	0.1M I_2 H_2O/pyridine/THF 1:2:7	30
8	Acetonitrile wash	50
9	0.25M Ac_2O/DMAP/collidine/THF	30

*Flow Rate = 5 mL. $min.^{-1}$ Total cycle time = 5.3 - 10.8 min.

(2.7 mmol./0.27 mmol./0.5 mL./2.7 mL., 30min.) to block the 2'-hydroxyl position. Treatment with an excess iodine/water/THF solution (5 min.) gave the protected phosphotriesters **6a,b** in 60-80% yields. Thus activation of the 3'-amidites with tetrazole, acetylation of the 2'-hydroxyl and iodine oxidation are all carried out sequentially without intermediate product isolation to give a fully protected 3'-5' linked nucleotide. The dimers **6a,b** were detritylated by treatment with 80% AcOH (90° , 15 min.) in 70-80% yields.

This methodology was also applied to the solid phase synthesis of oligomers. The use of solid phase synthesis provided many advantages inherent to the technique such as low consumption of reagents, high coupling yields, automation using a DNA/RNA synthesizer[10] and the lack of purification required between each coupling. The solid support used was Vydac TP silica gel[11] which was derivatized to a nucleoside loading of 20-25 micromol g^{-1}.

The synthesis cycle shown in TABLE 1 was used to prepare the arabinonucleotides ara-**ApA**, ara-**ApApA**, ara-**UpU**, ara-**UpUpU**. At the end of each coupling cycle any unreacted 5'- and all of the 2'-hydroxyls of the newly added nucleoside were acetylated using 0.25M Ac_2O/DMAP/collidine/THF solution.

During the coupling step one may envisage the possibility of the formation of branches at the unprotected 2'-hydroxyl of the newly added nucleoside. This would result in coupling yields greater than 100% and the formation of branched sequences. To investigate this possibility the trinucleotide ara-**UpUpU** was synthesized using coupling cycles of 0.5, 1 and 5 min. Chain branching was indeed observed (gel electrophoresis and HPLC analysis) and became more important with increasing coupling times. The average coupling yields of these syntheses (determined by spectrophotometric quantitation of the released MMT cation) were 75%, 94% and 115%, respectively. HPLC analysis of these syntheses after deprotection (vide infra), showed ara-**UpUpU**/ara-**UpU**/ branching sequences in a ratio of 16:6:1, 4:1:1 and 2:0.5:1, respectively.

The branching can be entirely eliminated by an alternate synthetic strategy. In this approach the 3'-phosphoramidites **2a,b** (0.50 mmol.) were reacted with Ac_2O/DMAP/$EtNiPr_2$/THF (2.0 mmol./0.2 mmol./4.0 mmol./4.0 mL., 45 min.) to give the corresponding 2'-acetylated 3'-phosphoramidites **3a,b** as white foams in quantitative yields. The purity of the crude products judged by TLC and ^{31}P-NMR was greater than 95% and therefore they were used without further purification. The synthesis of ara-**UpUpU** (5 min. coupling cycle) with the 2'-acetylated phosphoramidite **3a** gave a 96% average coupling yield and no branching. Phosphoramidites **3a,b** were used to prepare the hexanucleotide 5'-ara-**UpApUpApUpA**-3' using the synthesis cycle shown in Table 1 (5 min. coupling cycle) with an average coupling yield of 98%.

All arabinonucleotides were deprotected in a single step by treatment with NH_4OH/EtOH (3/1; 50° , 16 hr., concomitant decyanoethylation, debenzoylation and cleavage from the polymer support). As found by Wechter[5], all of the nucleotides were stable to alkaline treatment (0.1 N KOH, 37°, 16 hr. which degrades RNA) and were degraded by spleen phosphodiesterase (2'-5'-linked aranucleotides are not degraded by this enzyme) and snake venom diasterase to the corresponding monomer components (HPLC analysis). In addition, the deprotected ara-**UpU** and ara-**ApA** were characterized by 2D-NMR methods. The ^1H-NMR spectra of the latter was identical to that reported by Doornbos et al[12].

The procedure described in this report permits the rapid and efficient synthesis of 3'-5' linked arabinonucleotides with a minimum effort involved in the protection of monomer units.

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