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SYNTHESIS OF DINUCLEOSIDE MONOPHOSPHOROTHIOATES VIA ADDITION OF SULPHUR TO PHOSPHITE TRIESTERS.

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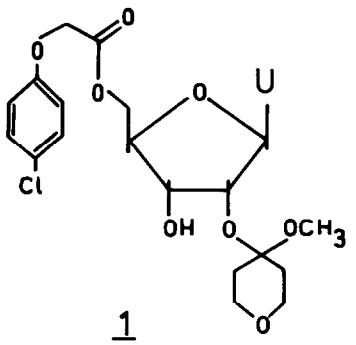
Thiophosphate analogs of dinucleoside monophosphates possess a chiral phosphorus atom and, as such, are important analogs for the elucidation of the stereochemistry of action of those enzymes in which reaction at this phosphorus atom occurs. They have been prepared by condensing a protected nucleoside having a free 3'-hydroxy function with an appropriately protected nucleoside 5'-O-phosphorothioate using 2,4,6-triisopropylbenzenesulphonyl chloride[1,2]. This dehydrative coupling, however, led predominantly to desulphurized products, and another synthetic route had to be developed.

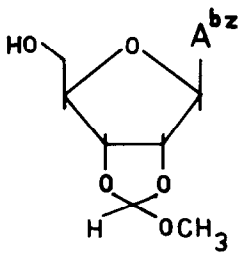
At first we attempted to synthesize the desired diribonucleoside monophosphorothioates, in an analogous way to the phosphotriester method[3], by reaction of a nucleoside having a free 3'-hydroxy (e.g. 1) with O-phenyl phosphorodichloridothioate ($C_6H_5OP(S)Cl_2$), followed by condensation with a 5'-hydroxy nucleoside (e.g. 2). This approach failed because no reaction occurred under the conditions that were successful for the oxygen analog O-phenyl phosphorodichloridate[3].

The problem was solved when we found that elementary sulphur adds to phosphite triesters in pyridine solution under very mild conditions to give phosphorothioate triesters in quantitative yields. For instance, when an 0.1 M solution of triethyl phosphite in pyridine was stirred at room temperature with a tenfold excess of sulphur the addition was shown, by ^{31}P NMR, to be complete within 5 min. Isomerization of the thiono- to the thiolo-

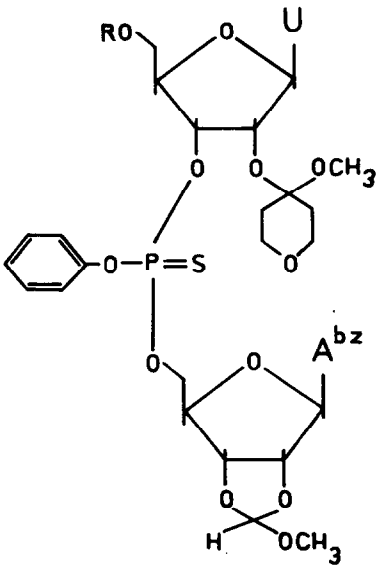


1

a) $PhOPCl_2$, -78°
b) 2
c) S_8



2



3 a R= $ClC_6H_4OCH_2\overset{O}{\overset{\|}{C}}-$
b R= H
c R= 

compound, reported for sulphur addition in the neat liquid[4], did not occur. Moreover, sulphur addition to triaryl phosphites, which was reported to take place only at elevated temperatures (100-150°)[5], was complete in 2 h at room temperature when carried out in pyridine solution.

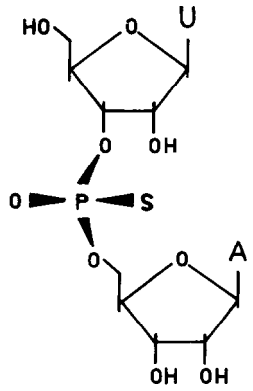
Application of the above reaction to the method published recently by Letsinger et al. for the synthesis of oligonucleotides via phosphite triester intermediates[6] should thus provide a convenient way to prepare thiophosphate analogues of dinucleoside monophosphates.

In the synthesis of oligonucleotides via phosphotriester intermediates aryl groups have been shown to be excellent protective groups for the phosphotriester function[7]. Phenyl was thus chosen in the present work to serve as the protective group for the third acidic phosphorothioate function. Thus, a solution of 5'-O-(p-chlorophenoxyacetyl)-2'-O-(methoxytetrahydropyranyl)uri-

dine[8] (1, 0.2 mmole) in 1 ml pyridine and 1 ml tetrahydrofuran was added during 2 min to a stirred solution of O-phenyl phosphorodichloridite ($C_6H_5OPCl_2$, 0.3 mmole) in 0.6 ml tetrahydrofuran and 0.12 ml pyridine at -78°. After another 2 min at -78°, N^6-benzoyl-2',3'-O-methoxymethylideneadenosine[9] (2, 0.45 mmole) was added. Both steps were complete in a total reaction time of 12 min at -78°. Pyridine (2 ml) and 150 mg sulphur (10 eq.) were then added and the mixture was stirred further at room temperature. ^{31}P NMR after 1.5 h showed ca. 60 % conversion of products with chemical shifts at ca. 132 ppm (relative to 85 % H_3PO_4) to products with chemical shifts at ca. 62 ppm. After 16 h at 20°, when ^{31}P NMR showed the addition of sulphur to be complete, the reaction mixture was worked up and chromatographed over a silica-gel column to give 3a, contaminated with the 5'-5' symmetrical O-phenyl phosphorothioate of 2.

The protective p-chlorophenoxyacetyl group which was included in the synthetic scheme to serve as a "purification handle", was removed by a short base-treatment with 2.5 % ammonia in dioxan-water (1:1 v/v) for 16 min at 20°. A second chromatographic step allowed us to isolate pure 3b in 61 % yield (based on 1). The ^{31}P and ^{1}H NMR spectra showed the presence of the two phosphorus diastereomers[10] which, however, could not be separated by chromatographic means.

Before alkaline deblocking the partially-protected dinucleotide 3b was tetrahydropyranylated (yield 91 %) to 3c to prevent isomerization of the dinucleotide during the base treatment[11]. Complete deprotection of 3c was accomplished as follows: (i) 0.2N NaOH in dioxan-water (1:4 v/v) at 40° for 20 h removed the phosphorothioate protecting phenyl group; (ii) 25 % aqueous ammonia at 50° for 4 h removed the N-benzoyl group from adenine; (iii) acid treatment (0.01 N HCl, pH 2.0) at 20° for 16 h removed the remaining acid-labile protective groups. After neutralization (pH=8) of the reaction with dilute ammonia the desired dinucleoside monophosphorothioate uridylyl (3'-5') adenylyl O,O-phosphorothioate (Up(S)A) was obtained as a mixture of R_p and S_p diastereomers with a chemical purity of ca. 90 %. This material was further purified by DEAE-Sephadex chromatography to give the pure mixture of diastereomers of Up(S)A in the ratio 61:39, as determined by ^{31}P NMR and high performance liquid



Up(S)A (R_p)

chromatography.

The ^{31}P chemical shift for Up(S)A (56.1 and 55.5 ppm at pH 7.5) are in agreement with those for O,O-dialkyl phosphorothioates (55-60 ppm at neutral pH) but not with those for O,S-dialkyl phosphorothioates (ca. 25 ppm)[12] and thus confirm the correct identity of the phosphorothioate group.

The product was completely digested by limited treatment with Pancreatic RNase A to give uridine 2',3'-cyclic O,O-phosphorothioate (mixture of diastereomers)[13] and adenosine in the ratio 0.95: 1.00.

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