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A HIGHLY EFFICIENT CHEMICAL SYNTHESIS OF Rp AND Sp ADENYL(3'-5')ADENYL-O,O-PHOSPHOROTHIOATE

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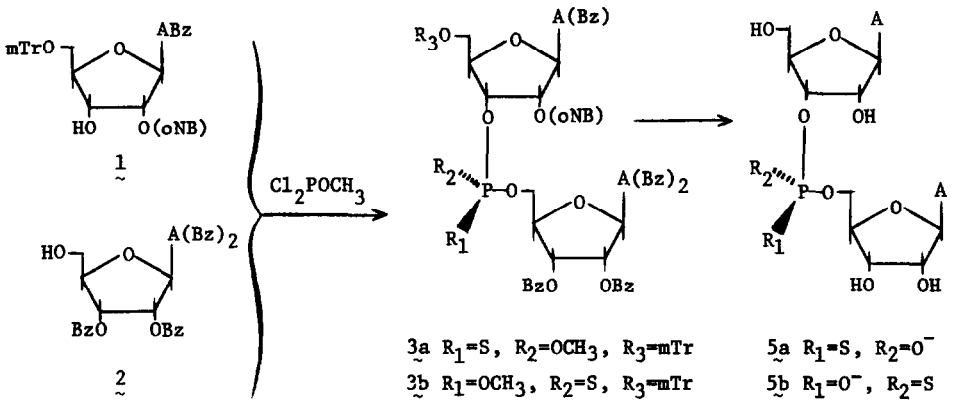
Abstract: A method is described for the chemical synthesis of the title compounds (Ap(S)A) via addition of elemental sulfur to a phosphite triester intermediate. Separation of the diastereomers of phosphorus could be accomplished on silica gel after removal of the terminal 5'-hydroxyl protecting group or on DEAE cellulose after complete deblocking. The triester to diester conversion is readily accomplished by thiophenoxide ion without reprotection of the terminal 5'-hydroxyl. The overall yield of the synthesis (one coupling and four deblocking steps) is 30%.

Dinucleotide monophosphorothioates possess a chiral center at phosphorus. Because of this property, these compounds have played a key role in the elucidation of the stereochemical course of several enzyme catalyzed reactions.[1,2] To facilitate further work in this area it was necessary to develop highly efficient syntheses of these dinucleotides that afforded separation of the diastereomers at phosphorus.

To date only two syntheses of a dinucleotide monophosphorothioate, Up(S)A, have been published. The first of these utilized a novel addition of elemental sulfur to a phosphite triester intermediate, but unfortunately the diastereomers could not be separated at any stage during synthesis.[3] The second synthetic route utilized a more conventional coupling procedure. The phosphorothioate sulfur was protected with a cyanoethyl group during dehydrative coupling.[4] Although the diastereomers could be separated at the triester level on silica gel after deblocking the 5'-terminal hydroxyl, alkaline deblocking of the cyanoethyl group led to a considerable amount of desulfurization via nucleophilic attack at phosphorus. Furthermore, the 5'-hydroxyl had to be reprotected prior to this alkaline deblocking step, since such conditions can lead to formation of a 5'-5' internucleotide bond.[5]

In the synthesis reported here we utilized some recent advances in ribonucleic acid synthesis to overcome the problems encountered above. The requisite nucleoside blocks, 1 and 2, were prepared from literature procedures,[6,7] as was the coupling agent, methoxydichlorophosphite.[8] A mixture of 92.5 µl of methoxydichlorophosphite (0.972 mmoles), 2.5 ml of tetrahydrofuran, and 1 ml of pyridine was stirred under a dry nitrogen atmosphere and cooled to -78°C. To the above, a solution of 500 mg (0.642 mmoles) of the 3'-hydroxy nucleoside, 1, in 5 ml of tetrahydrofuran

was added over a 4-5 minute period and the resulting mixture was allowed to stir for an additional 10 minutes. A solution of 985 mg (1.442 mmoles) of the 5'-hydroxy nucleoside, 2, in 2.5 ml of tetrahydrofuran was then added over a 4 minute period. The resulting mixture was stirred for 15 minutes at -78°C, then warmed to room temperature, and finally, 500 mg of elemental sulfur (64 mmoles), suspended in 10 ml of pyridine, was added. The resulting suspension was stirred for one hour after which the nucleotide products were precipitated by addition of hexane. The overall process is depicted in Scheme I.



mTr = monomethoxytrityl
Bz = benzoyl
oNB = o-nitrobenzyl

3a R_1=S, R_2=OCH_3, R_3=mTr
3b R_1=OCH_3, R_2=S, R_3=mTr
4a R_1=S, R_2=OCH_3, R_3=OH
4b R_1=OCH_3, R_2=S, R_3=OH

5a R_1=S, R_2=O^-
5b R_1=O^-, R_2=S

The products of the coupling reaction include not only the dinucleotides with the correct 3'-5' linkage, 3a and 3b, but also a considerable amount of the symmetrical dinucleotide with a 5'-5' linkage. Since an excess of CH_3OPCl_2 to 1 was used, very little of the symmetrical 3'-3' product could be detected.

After detritylation with 80% acetic acid, all of the dinucleotides, including diastereomers 4a (Rf=0.36) and 4b (Rf=0.28), could be distinguished on TLC (EtOAc:$CHCl_3$, 1:1).[9] These desired diastereomers were separated by silica gel column chromatography (silica gel 60; 30 x 4.0 cm; EtOAc:$CHCl_3$, 1:1). The high Rf diastereomer, 4a, had a P^{31} nmr chemical shift of 71.2 ppm, whereas that for the low Rf diastereomer was 69.1 ppm. The coupling reaction, apparently due to steric considerations, exhibited stereochemical selectivity, since the ratio of high Rf to low Rf diastereomers was 2:1.

Triesters 4a and 4b were separately dissolved in a mixture of dioxane and Et_3N (2.5 ml each). Thiophenol, 1.25 ml, was added and the solution stirred at room temperature for 1 hour after which TLC (EtOAc) indicated complete conversion to a product which remained at the origin of the chromatograph.[10] The product was isolated by precipitation with hexane and P^{31} nmr showed only two resonances: one at 56.6 ppm (from 4a) and one at 55.7 ppm (from 4b). These chemical shifts are consistent with the formation of phosphorothioate diesters[4,5] and no desulfurization was detected. The triester to diester conversion was so mild that, even

though the 5'-hydroxyl was not reprotected prior to treatment with thiophenol, no isomerization of the 3'-5' internucleotide bond linkage was observed by P^{31} nmr.

The benzoyl groups were removed by treatment with saturated NH_3/CH_3OH and the o-nitrobenzyl group by photolysis at 366 nm, essentially as described by Ikehara.[7] The fully deblocked dinucleotide, Ap(S)A, was purified by DEAE cellulose chromatography. The overall yield was 192 μmoles, 30% based on 1. During final purification of 30 μmoles of a 1:1 mixture of the diastereomers of Ap(S)A, 5a and 5b, on DEAE cellulose we found that the diastereomers likewise could be separated at this stage.

Structural proof was accomplished by the following four experiments: i) P^{31} nmr chemical shifts for the diastereomers of Ap(S)A were nearly identical to those reported for the diastereomers of Up(S)A (Table I)[3,4]; ii) The UV spectra of Ap(S)A is identical to that of ApA, as expected; iii) Enzymatic hydrolysis with snake venom phosphodiesterase gave adenosine and AMP as products. In separate control experiments under identical conditions to those above, authentic AMPS, the expected product, was rapidly desulfurized to AMP by the diesterase;[11] iv) Diol determination by periodate[12] gave 1.05 diols per dinucleotide, further ensuring the proper 3'-5' linkage. Separate experiments with deoxynucleoside monophosphorothioates determined the sulfur was not oxidized by periodate treatment.

Table I: Spectral Data for Ap(S)A, ApA and Up(S)A

Compound	Configuration	UV data[a] λ_{max}	UV data[a] λ_{min}	P^{31} chemical Shifts
5a	Rp	256	227	56.8[b]
5b	Sp	256	227	55.7[b]
ApA		256	227	
Up(S)A	Rp			56.1[c]
Up(S)A	Sp			55.5[c]

[a] in H_2O, 25°C.

[b] in 0.1 M Tris·HCl, pH 8.0, 37°C.

[c] Ref. 14.

The P^{31} chemical shift for the Rp diastereomer of Up(S)A occurs at lower field than that for the Sp diastereomer. By analogy, one might expect the resonance at 56.8 ppm for Ap(S)A to be due to the diastereomer with the Rp configuration. To firmly establish the absolute stereochemistry for Ap(S)A, the hydrolysis of a 50:50 mixture of the diastereomers by bovine intestinal phosphodiesterase[13] was followed by P^{31} nmr. After 3 hours the resonance at 56.8 ppm had decreased by approximately one half and that at 55.7 ppm showed no change. Since it is known that the bovine diesterase is stereoselective for dinucleotides with the Rp configuration at phosphorus[2], the resonance at 56.8 ppm is that from the compound with the Rp configuration, 5a, consistent with the above tentative assignment based on the P^{31} data for Up(S)A.[14]

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References

1. Eckstein, F. (1979) Accts. Chem. Res. 12, 204.
2. Bryant, F.R., Marlier, J.F., and Benkovic, S.J. (1979) IUPAC Meeting on Phosphorus in Biology, in press.
3. Burgers, P.M.J. and Eckstein, F. (1978) Tet. Letters 40, 3835.
4. Burgers, P.M.J. and Eckstein, F. (1978) Nucleic Acid Res. Spec. Publ. 4, s43.
5. van Boom, J.H., Burgers, P.M.J., van Deursen, P.H., de Rooy, J.F.M., and Reese, C.B. (1976) Chem. Commun. 167.
6. Lohrmann, R. and Khorana, H.G. (1964) J. Amer. Chem. Soc. 86, 4188.
7. Ohtsuka, E., Tanaka, S. and Ikehara, M. (1977) Chem. Pharm. Bull. 25, 949.
8. Martin, D.R. and Pizzolato, P.J. (1950) J. Amer. Chem. Soc. 72, 4584.
9. The establishment of the absolute stereochemistry of high Rf diastereomer as 4a and the low Rf diastereomer as 4b was accomplished by enzymatic digestion of the final products of the synthesis — 5a and 5b. This is described later in the paper, but for the sake of clarity the absolute stereochemistry of the intermediate products has been assigned throughout the text.
10. Daub, G.W. and van Tamelen, E.E. (1977) J. Amer. Chem. Soc. 99, 3526.
11. Burgers, P.M.J. and Eckstein, F. (1978) Proc. Natl. Acad. Sci. USA 75, 4798.
12. Dixon, J.S. and Lipkin, D. (1954) Anal. Chem. 26, 1092.
13. Landt, M. and Butler, L.G. (1978) Biochem. 17, 4130.
14. Burgers, P.M.J. and Eckstein, F. (1979) Biochem. 18, 592.

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