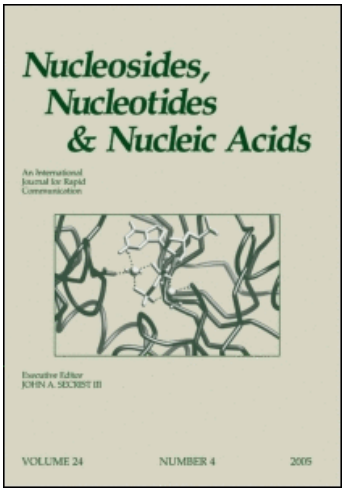

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CYCLIC AMP DIPHENYL PHOSPHORIC MIXED ANHYDRIDE: SYNTHESIS, ^{31}P NMR CHARACTERIZATION AND REACTION WITH DIMETHYLAMINE

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Abstract. Phosphorus diastereoisomers, R_P and S_P of P^1-adenosine cyclic 3',5' P^2-diphenylpyrophosphate (cyclic AMP diphenylphosphoric mixed anhydride) (1) were prepared from adenosine cyclic 3',5'-monophosphate (cyclic AMP) and diphenyl phosphorochloridate and characterized by ^{31}P NMR. The synthesis preferentially gave R_P-1. Reaction of 1 with dimethylamine resulted in the formation of a ($\sim$3:1) mixture of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate and diphenyl-N,N-dimethylphosphoramidate and occurred with inversion of configuration at cyclic AMP phosphorus.

Nucleotide diphenyl phosphoric mixed anhydrides are useful synthetic intermediates. They can be readily prepared from nucleotides with diphenyl phosphorochloridate and, under suitable conditions, quantitatively converted by nucleophiles to P-substituted nucleotide derivatives, since the attack of the nucleophile occurs at the less electrophilic, nucleotide phosphorus atom of the mixed anhydride.[1] The latter reaction is the basis of one of the simplest routes to nucleoside phosphoramidates, when ammonia, a primary or secondary amine is used as nucleophile.[1] While numerous nucleoside 5'-phosphoramidates have been obtained according to this route,[2] to our best knowledge, only two attempts have been made to employ the method for the preparation of nucleoside cyclic 3',5'-phosphoramidates. Preobrazhenskaya et al. successfully synthesized uridylyl

cyclic-(3',5'⟶N)-phenylalanine methyl ester.[3] In the hands of Meyer et al., however, the method failed to apply to the preparation of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate.[4] Since we cannot see any reason why the method would be inapplicable to the preparation of this compound, we have reinvestigated the reaction of adenosine cyclic 3',5'-mono phosphate (cyclic AMP) with diphenyl phosphorochloridate and the aminolysis of the reaction product with dimethylamine.[5]

Cyclic AMP was reacted with 2.2 or 0.5 molar equivalents of diphenyl phosphorochloridate in anhydrous trimethyl phosphate at $0^{o}C$ for 10 min, and the reaction was monitored by ^{31}P NMR. As shown in FIG. 1. the cyclic AMP signal completely disappeared when 2.2 molar equivalents of diphenyl phosphorochloridate was used. At the same time, four doublets about -20 ppm as well as the signal of tetraphenyl pyrophosphate appeared in the reaction mixture.

The four doublets may be assigned to the two phosphorus atoms of P^1-adenosine cyclic 3',5' P^2-diphenyl pyrophosphate (cyclic AMP diphenyl phosphoric mixed anhydride) diastereoisomers ($\underline{R}_P$-**1** and $\underline{S}_P$-**1**), the anticipated products of the reaction between cyclic AMP and diphenyl phosphorochloridate. The two upfield doublets may be attributed to the P^2 phosphorus atom of $\underline{R}_P$-**1** and $\underline{S}_P$-**1** on the basis of their unchanged multiplicity under proton coupling conditions. As a consequence, the two downfield doublets may be ascribed to the chiral P^1 phosphorus atoms. According to the ^{31}P chemical shift criterion established for 2-substituted 2-oxo-1,3,2-dioxaphosphorinanes,[6-12] the P^1 phosphorus atom of $\underline{R}_P$-**1** should absorb at higher field, because this phosphorus atom has the diphenyl phosphate residue in the axial position. In this way, the two, more intensive doublets may be ascribed to $\underline{R}_P$-**1**, while the less intensive doublets may be assigned to $\underline{S}_P$-1.

Doublets corresponding to the P^1 atoms show splittings under proton coupling conditions. The doublet assigned to

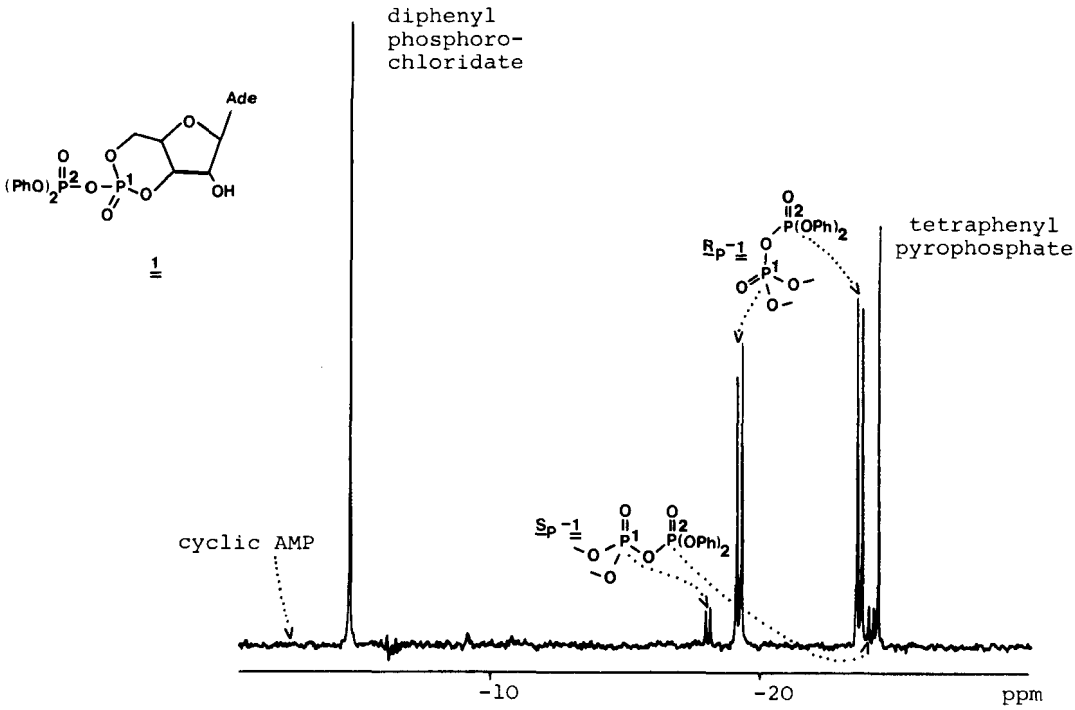

FIG. 1.
The proton-decoupled ^{31}P NMR spectrum of the reaction mixture of cyclic AMP with 2.2 molar equivalents of diphenyl phosphorochloridate in trimethyl phosphate after standing 10 min at 0°C. The broad solvent resonance about +3.40 ppm is not shown.

R_P-1 is split into doublets of a doublet, while the doublet ascribed to S_P-1 appears as a multiplet. Proton-phosphorus couplings may indicate highly populated chair conformation for the dioxaphosphorinane ring of R_P-1 ($^3J_{PH}$ = 22.7 Hz) and mixed chair and twist-boat conformations for the dioxaphosphorinane ring of S_P-1. ($^3J_{PH}$ = 9.1 and 10.9 Hz). This supposition is based on Gorenstein's work on the analogous R_P and S_P-2-aryloxy-2-oxo-*trans*-5,6-tetramethylene-1,3,2-dioxaphosphorinanes[11] as well as on Bentrude's studies on R_P and S_P methyl thymidine cyclic 3',5'-monophosphates.[12]

The two diastereoisomers were formed in a ratio of R_P-1/S_P-1 = (92:8). The preferential formation of R_P-1 is expected on the basis of the greater basicity of the axial P-O oxygen atom in 1,3,2-dioxaphosphorinanes.[13] The appearance of tetraphenyl pyrophosphate is very probably due to the presence of traces of water in the reaction mixture.

When cyclic AMP was reacted with 0.5 molar equivalent of diphenyl phosphorochloridate, diphenyl phosphorochloridate was quantitatively consumed, and a mixture of **1**, cyclic AMP symmetrical anhydride[14] and diphenyl phosphate ∿ (7:1:1) was formed. In this case, **1** that had been produced in the first step, was, in part, transformed by the action of excess cyclic AMP into cyclic AMP symmetrical anhydride with the liberation of diphenyl phosphate.

The reaction mixture prepared with 0.5 molar equivalent of diphenyl phosphorochloridate, was treated with dimethylamine at -20°C. Adenosine cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers and diphenyl-N,N-dimethylphosphoramidate were the phosphoramidate products of the reaction. Diphenyl-N,N-dimethylphosphoramidate can only be derived from the nucleophilic attack of dimethylamine at the P^2 atom of **1**, since diphenyl phosphorochloridate was quantitatively consumed, and tetraphenyl pyrophosphate was not formed during the synthesis of **1** with 0.5 molar equivalent of diphenyl phosphorochloridate. At the same time, the formation of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate may be the result of the nucleophilic attack of dimethylamine either at the P^1 atom of **1** or at one of the phosphorus atoms of cyclic AMP symmetrical anhydride. Taking into account these considerations and the composition of the anhydride mixture, quantitative analysis of the product mixture showed, that nucleophilic attack of dimethylamine occurred at the two phosphorus atoms of **1** in a ratio of P^1/P^2 (∿3:1). Irrespective of whether aqueous or anhydrous dimethylamine was used, this ratio remained unaltered within the limits of experimental errors.

Nucleophilic attack of dimethylamine at P^1 atom of **1** occurs with inversion of configuration: from an $\underline{R}_P/\underline{S}_P$ = (92:8) mixture of **1** prepared with 2.2 molar equivalents of diphenyl phosphorochloridate, an $\underline{R}_P/\underline{S}_P$ = (9:91) mixture of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate was formed.

To our best knowledge, the reaction of 1 with dimethylamine is the first case when a nucleophile attacks both phosphorus atoms of a tetrasubstituted pyrophosphate. For all tetrasubstituted pyrophosphates studied so far, nucleophilic attack at only one, the less electrophilic phosphorus atom with displacement of the better leaving group, was observed. This is due to the fact that, in general, bond breaking between substrate and the leaving group plays the decisive role in displacements at phosphorus.[18-20] The reaction of 1 with dimethylamine may pro forma be regarded as a case where bond forming between substrate and the entering group is also of comparable significance. However, we would prefer the supposition that bond breaking controls this reaction too, and the similar electrophilicity of the two phosphorus atoms is responsible for the two-directional splitting of the pyrophosphate bond.

Relating to the preparative applicability, our results show that the activation of cyclic AMP with diphenyl phosphorochloridate followed by aminolysis of the reactive intermediate 1 with dimethylamine, is a suitable method for the synthesis of adenosine cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers. The theoretical yield of the reaction is 75%. A similar method for the preparation of ribonucleoside cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers that uses 2,4,6-triisopropylbenzenesulfonyl chloride instead of diphenyl phosphorochloridate, has been described recently.[21] In this case, however, the theoretical yield is only 50%, since cyclic AMP symmetrical anhydride is the reactive intermediate of the synthesis.[14]

EXPERIMENTAL

Cyclic AMP tri-n-butylammonium salt was prepared according to Ref. 21. Diphenyl phosphorochloridate (EGA-Chemie) was freshly distilled under reduced pressure, b.p. 140-142°C/52 Pa. Trimethyl phosphate was distilled through an 80x2 cm insulated Vigreux column at reduced pressure prior to use and stored over 0.4 nm molecular sieves.

^{31}P NMR spectra were recorded on a Bruker WM-250 FT spectrometer operating at 101.2 MHz. Positive chemical shifts are downfield from external 85% H_3PO_4.

Reaction of adenosine cyclic 3',5'-monophosphate with diphenyl phosphorochloridate

To a solution of cyclic AMP tri-n-butylammonium salt (0.5 mmol) in trimethyl phosphate (2.5 mL), diphenyl phosphorochloridate A: 227.9 μL (1.1 mmol) or B: 51.8 μL (0.25 mmol) was added. The solution was stirred with the exclusion of atmospheric moisture at 0°C for 10 min then analyzed by ^{31}P NMR. The proton-decoupled ^{31}P NMR spectrum of mixture A is shown in FIG. 1. Common signals in mixtures A and B were: R_P-1, δ -19.28 (dd, $^2J_{PP}$ = 19.8 Hz, $^3J_{PH}$ = 22.7 Hz, P^1), -23.59 (d, $^2J_{PP}$ = 19.0 Hz, P^2); S_P-1, δ -18.09 (m, $^2J_{PP}$ = 17.9 Hz, $^3J_{PH}$s = 9.1 and 10.9 Hz, P^1), -24.07 (d, $^2J_{PP}$ = 17.2 Hz, P^2). Additional signals in the proton-decoupled spectra besides the broad resonance of trimethyl phosphate about +3.40 ppm, were in mixture A: δ -4.89 (s, diphenyl phosphorochoridate) and -24.36 (s, tetraphenyl pyrophosphate); in mixture B: δ -2.56 (s, cyclic AMP), -11.02 (s, diphenyl phosphate), -17.57 (d), -17.91 (s, partly resolved from the P^1 doublet of S_P-1), -18.26 (s) and -19.21 (d, partly buried into the P^1 doublet of R_P-1) (diastereoisomers of cyclic AMP symmetrical anhydride[14]). R_P-1/S_P-1 = 92:8, 1/diphenyl phosphorochloridate/tetraphenyl pyrophosphate = 1.0:0.8:0.2 (in mixture A); 1/cyclic AMP symmetrical anhydride/diphenyl phosphate ∿ 7:1:1 (in mixture B). Cyclic AMP, diphenyl phosphorochloridate, diphenyl phosphate and tetraphenyl pyrophosphate were identified by observing the increase of the respective signal intensities on consecutive addition of authentic samples to the mixtures.

Reaction of P^1-adenosine cyclic 3',5' P^2-diphenyl pyrophosphate with dimethylamine

Mixture B obtained in the reaction of cyclic AMP with diphenyl phosphorochloridate was dropped into 40% (w/v) aqueous dimethylamine solution (25 mL) cooled to -20°C (Ba)

or into a solution of dimethylamine in anhydrous N,N-dimethylformamide of the same molar concentration and temperature (Bb) under vigorous stirring. Mixture A was reacted only with aqueous dimethylamine (Ab). After evaporation aliquots of the residues (one fifth of mixture Ab) were separated by reversed phase MPLC on a LiChroprep RP-18 (25-40 μm, Merck) column (1.5 x 87.0 cm) by using methanol/deionized water (30:70, v/v, 840 mL), then a gradient of the same mixture (320 mL) and methanol (380 mL), as eluent and 1.2 MPa overpressure (elution rate:21.0 mL/2.7 min/fraction). The quantity of products was determined by UV at 260 nm {ε = 15,400 (for adenosine cyclic 3',5'-N,N-dimethylphosphoramidate) and 593 (for diphenyl-N,N-dimethylphosphoramidate)}. Elution order of compounds was: cyclic AMP (in fraction 6-7), unidentified non-nucleotidic material (in fractions 8-9), R_P-adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (in fractions 15-20, 4.73 μmol in mixture Ba, 5.97 μmol in mixture Bb and 6.50 μmol in mixture Ab), S_P-adenosine cyclic 3',5'-N,N-dimethylphosphoramidate (in fractions 24-32, 59.13 μmol in mixture Ba, 47.40 μmol in mixture Bb and 68.30 μmol in mixture Ab) and diphenyl-N,N-dimethylphosphoramidate (in fractions 66-67, 19.39 μmol in mixture Ba and 17.88 μmol in mixture Bb). Overall yield of R_P and S_P-adenosine cyclic 3',5'-N,N-dimethylphosphoramidates in mixture Ab was 74.8 %.

Diphenyl-N,N-dimethylphosphoramidate, UV (MeOH) λ_{max} 268.8, 262.1 and 256.7 nm, λ_{min} 265.5, 258.3 and 229.0 nm; ^{1}H NMR (at 250 MHz in $CDBr_3$), δ 2.78 (s, 3H), 2.83 (s, 3H), 7.13-7.37 (m, 10H); ^{31}P NMR (in trimethyl phosphate) δ +2.63; TLC on silica gel in chloroform/ethylacetate and in chloroform/methanol (9:1 v/v), R_f 0.65 and 0.80, respectively. The compound was in each respect identical with the sample prepared from diphenyl phosphorochloridate and dimethylamine in anhydrous ethyl ether by the analogy of the synthesis of diphenyl-N,N-diethylphosphoramidate[22].

Adenosine cyclic 3',5'-N,N-dimethylphosphoramidate diastereoisomers were identified by TLC and ^{31}P NMR comparisons with authentic samples.[21]

REFERENCES AND NOTES

1. Michelson, A.M. Chem. and Ind. 1960, 1267; "The Chemistry of Nucleosides and Nucleotides", Academic Press, New York, 1963, pp. 230-231; Biochim. Biophys. Acta 1964, 91, 1.
2. Selected examples are: Shabarova, Z.A. Progr.Nucl.Acids Res.Mol.Biol. 1970, 10, 145; Juodka, B.A. Nucleosides and Nucleotides 1984, 3, 445 and references in both papers.
3. Preobrazhenskaya, N.N.; Shabarova, Z.A.; Prokofjev, M.A. Dokl.Akad.Nauk SSSR 1967, 174, 100.
4. Meyer, Jr., R.B.; Shuman, D.A.; Robins, R.K. Tetrahedron Lett. 1973, 269.
5. A part of this work has already been published in a preliminary form: Tomasz, J.; Bottka, S.; Ludwig, J.; Pelczer, I. Phosphorus and Sulfur 1987, 30, 597.
6. Majoral, J.P.; Navech, J. Bull. Soc. Chim. Fr. 1971, 95, 2609.
7. Stec, W.J.; Mikolajczyk, M. Tetrahedron 1973, 29, 539.
8. Bentrude, W.G.; Tan, H.-W. J.Am.Chem.Soc. 1973, 95, 4666.
9. Mosbo, J.A.; Verkade, J.G. J.Am.Chem.Soc. 1973, 95, 4659; J.Org.Chem. 1977, 42, 1549.
10. Cooper, D.B.; Inch, T.D.; Lewis, G.J. J.Chem.Soc.Perkin Trans. I. 1974, 1043.
11. Gorenstein, D.G.; Rowell, R. J.Am.Chem.Soc. 1979, 101, 4925; Gorenstein, D.G.; Rowell, R.; Findlay, J. J.Am. Chem. Soc. 1980, 102, 5077.
12. Sopchik, A.E.; Bajwa, G.S.; Nelson, K.A.; Bentrude, W.G. Phosphorus Chemistry, ACS Symposium Series, No. 171, Quin, L.D. and Verkade, J.G., Eds., American Chemical Society, Washington D.C., 1981, p. 217.
13. Hong, A.-P.; Lee, J.-B.; Verkade, J.G. J.Am.Chem.Soc. 1976, 98, 6547.
14. For ^{31}P NMR characterization of cyclic AMP symmetrical anhydride, see: Tomasz, J.; Bottka, S.; Pelczer, I. Nucleosides and Nucleotides 1987, 6, 3rd issue.

15. Mason, H.S.; Todd, A.R. J.Am.Chem.Soc. 1951, 2267.
16. Corby, N.S.; Kenner, G.W.; Todd, A.R. J.Am.Chem.Soc. 1952, 1234.
17. Michelson, A.M. J.Chem.Soc. 1959, 1371.
18. Todd, A. Proc.Natl.Acad.Sci. U.S.A. 1959, 45, 1389.
19. Kirby, A.J.; Warren, S.G. "The Organic Chemistry of Phosphorus", Elsevier, Amsterdam, 1967, p. 294.
20. Emsley, J.; Hall, D. "The Chemistry of Phosphorus" Harpen & Row, London, 1976, p. 329.
21. Bentrude, W.G.; Tomasz, J. Synthesis 1984, 27.
22. Stock, J.A.; Hopwood, W.J.; Regan, P.D. J.Chem.Soc.C. 1966, 637.

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