References and Notes

(1) Knowles, J. R. *Annu. Rev. Biochem.*, in press.
(2) Abbott, S. J.; Jones, S. R.; Weinman, S. A.; Bockhoff, F. M.; McLafferty, F. W.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 4323.
(3) Jones, S. R.; Kindman, L. A.; Knowles, J. R. *Nature (London)* **1978**, *275*, 564.
(4) Blättler, W. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 510.
(5) Lesnikowski, Z. J.; Stec, W. J.; Zielinski, W. S. *Nucleic Acids Res., Spec. Publ.* **1978**, *4*, S49.
(6) Gerlt, J. A.; Coderre, J. A., unpublished observations.
(7) Baraniak, J.; Kinas, R. W.; Lesiak, K.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1979**, 940.
(8) *P*-Anilidate (1 mmol) is placed in a heavy-walled round-bottom flask fitted with a high vacuum Teflon stopcock and side arm (Ace Glass 7412-07) and dissolved in 15 mL of pyridine freshly distilled from CaH_2. Sodium hydride (3 equiv) is added, and the suspension is stirred for 20 min. Using standard vacuum line techniques, 10 mmol of ^{18}O-labeled carbon dioxide (Prochem) is condensed into the reaction flask. The flask is sealed and allowed to warm to room temperature, and the reaction is stirred for 5 h. Unreacted carbon dioxide (~8 mmol) is removed on the vacuum line, and the reaction is quenched with water. The aqueous solution is washed with CH_2Cl_2 and evaporated to dryness. The residue is dissolved in H_2O and applied to a column of DEAE-Sephadex A-25 (HCO_3^-) (2.5 × 40 cm) and the product is eluted with a 2-L linear gradient of 0–0.25 M triethylammonium bicarbonate. Appropriate fractions are combined and concentrated to afford the solid triethylammonium salt of cyclic [^{18}O]-2′-deoxy-AMP.
(9) Cohn, M.; Hu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 200. Lowe, G.; Sproat, B. S. *J. Chem. Soc., Chem. Commun.* **1978**, 565.
(10) Burgers, P. M. J.; Eckstein, F.; Hunneman, D. H.; Baraniak, J.; Kinas, R. W.; Stec, W. J. *J. Biol. Chem.* **1979**, *254*, 9959.
(11) An aqueous solution of the triethylammonium salts is acidified by addition of 2 equiv of aqueous HCl. The solvent is removed by rotary evaporation, and the residue is dried by repeated evaporation of anhydrous ethanol. The residue is suspended in a 5-mL aliquot of anhydrous ethanol, and an excess of diazoethane prepared in ether is added. After several hours, the reaction mixture becomes homogeneous, and the solvent is removed by rotary evaporation. The residue is suspended in water and centrifuged to remove an insoluble oil; the water is removed by rotary evaporation. Acid (2 equiv) must be added to the triethylammonium salts to produce the strongly acid phosphoric acid which reacts rapidly with diazoethane. The diazoethane was prepared by adding *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine to a mixture of aqueous sodium hydroxide and ether; the ether solution was decanted and dried over potassium hydroxide to avoid distillation and potential explosions. All manipulations were carried out in a fume hood to prevent exposure to the potentially carcinogenic precursor and diazoethane.
(12) In either 0.02 M EDTA solution, pH 7.0, or anhydrous ethanol, narrow line widths could not be obtained. This is presumably explained by micelle formation[13] and paramagnetic impurities, respectively.
(13) Gohil, R. N.; Gillen, R. G.; Nagyvary, J. *Nucleic Acids Res.* **1974**, *1*, 1691.
(14) Engels, J.; Schlaeger, E.-J. *J. Med. Chem.* **1977**, *20*, 907.
(15) Lowe, G.; Potter, B. V. L.; Sproat, B. S.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1979**, 733. Cohn, M.; Hu, A. *J. Am. Chem. Soc.* **1980**, *102*, 913.
(16) Baraniak, J.; Lesiak, K.; Sochacki, M.; Stec, W. J., following paper in this issue.
(17) Gerlt, J. A.; Coderre, J. A.; Wolin, M. S. *J. Biol. Chem.* **1980**, *255*, 331.
(18) NIH Research Career Development Awardee (CA-00499), 1978–1983.

John A. Gerlt,*[18] Jeffrey A. Coderre

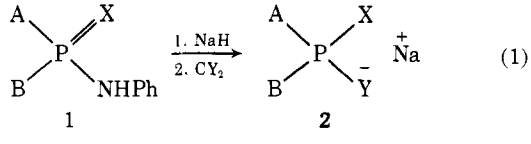
Department of Chemistry, Yale University
New Haven, Connecticut 06511

Received February 19, 1980

Stereospecific Synthesis of Cyclic Adenosine 3′,5′-(S_P)-[^{18}O]Phosphate†

Sir:

We have recently demonstrated that dialkyl phosphoroanilidates **1** can be easily converted into the corresponding phosphorothioates (X = O, Y = S),[1] phosphoroselenoates (X = O, Y = Se),[2] phosphorodithioates (X = Y = S),[1] phosphoroselenothioates (X = S, Y = Se),[2] phosphorodiselenoates (X = Y = Se),[2] and phosphates (X = Y = O)[3] of general formula **2** (eq 1).

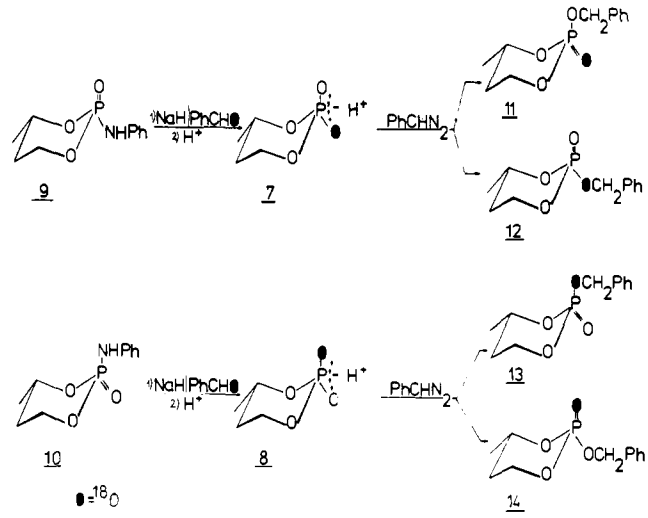


1 2 (1)

A,B = alkyl, alkoxyl, aryloxyl, alkylamino, arylamino
X = O, S, Se; Y = O, S, Se

† Dedicated to Professor Jan Michalski on the occasion of his 60th birthday.

Scheme I



It has been proven that the P-N → P-Y conversion (eq 1, X ≠ Y) is fully stereospecific and proceeds with retention of configuration at the phosphorus atom.[1,4] By means of this synthetic method, the first stereospecific synthesis of both diastereomers of cAMPS has been realized.[5,6] In view of the increasing interest in stereospecific methods for the preparation of biologically relevant phosphate esters which are chiral at phosphorus by virtue of the stable isotopes of oxygen (^{16}O, ^{17}O, ^{18}O),[7,8] we have applied our method of P-N → P-Y conversion to the synthesis of cyclic adenosine 3′,5′-(S_p)-[^{18}O]phosphate (**3**). The recently described cyclic N^6,N^6,$O^{2'}$-tribenzoyladenosine 3′,5′-(R_p)-phosphoranilidate (**4**)[6] has been used as the precursor to **3**. Instead of [^{18}O]carbon dioxide[3] as the ^{18}O source, [^{18}O]benzaldehyde (**5**)[9] has been used. Treatment of a tetrahydrofuran solution (10 mL) of **4** (0.120 g, 0.17 mmol) with sodium hydride followed by **5** (0.184 g, 1.7 mmol) gave cyclic sodium N^6,N^6,$O^{2'}$-tribenzoyladenosine 3′,5′-[^{18}O]-phosphate (**6**). After removal of the benzoyl protecting groups,[10] we obtained [^{18}O]-cAMP (**3**) in 28% yield. The ^{18}O enrichment in **3** was determined after silylation using *tert*-butyldimethylchlorosilane-imidazole-DMF; the mass spectrum of $(TBDMS)_2$-[^{18}O]-cAMP ($M^+ - C_4H_9$, *m/e* 500, 55%) demonstrated 81.7% ^{18}O enrichment. The configuration at phosphorus in **3** is predicted to be *S*.

The assumption of retention of configuration in the conversion of **4** → **3** is based on our previous experiments in which the stereospecificity of the conversion of **1** → **2** (X = ^{16}O, Y = ^{18}O) was supported by the IR spectra of the methyl esters of *cis*- and *trans*-[^{18}O]-2-hydroxy-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes (**7** and **8**)[11] resulting from *trans*- and *cis*-2-phenylamino-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes (**9** and **10**),[12] respectively, after treatment with NaH-**5**. Because the IR method is not adequate for a quantitative determination of the stereospecificities accompanying the conversions of **9** → **7** and **10** → **8**, in this communication we present further experimental evidence that the reaction of phosphoroanilidates **9** and **10** with NaH-**5** proceeds with retention of configuration at the phosphorus atom.

Compound **7** prepared from diastereomerically pure **9** and **5** was treated with an excess of phenyldiazomethane (ethereal solution).[13] The product of this reaction consisted of a mixture of the two diastereomeric triesters **11** and **12** in a ratio of 67:100, respectively (Scheme I). The product mixture was separated into pure triesters **11** and **12** by means of TLC (**11**, $\delta_{^{31}P(CHCl_3)}$ − 7.45 ppm; **12**, $\delta_{^{31}P(CHCl_3)}$ − 5.44 ppm).[14] Using

0002-7863/80/1502-4533$01.00/0

Table I. Positional Incorporation of ^{18}O into Diastereomeric 2-Benzyloxy-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes

compd	^{18}O enrichment measd on molecular ion, %	distribution of ^{18}O in positions: equatorial, %	axial, %
11	76.0	94.8[a]	5.2
12	75.8	97.0	3.0[a]
13	78.7	3.9	96.1[a]
14	78.8	3.6[a]	96.4

[a] Measured on the $(M - C_7H_6O)^+$ ion.

a similar procedure, diastereomerically pure **10** was converted into a mixture of **13** and **14**, which was then separated into pure triesters (**13**, $\delta_{^{31}P(CHCl_3)}$ − 7.45 ppm; **14**, $\delta_{^{31}P(CHCl_3)}$ − 5.44 ppm). Electron impact mass spectra showed that the ^{18}O enrichment of compounds **11–14** was lower than that of **5** (see Table I).

The analysis of the mass spectra of *trans*- and *cis*-2-benzyloxy-2-oxo-4-methyl-1,3,2-dioxaphosphorinanes ([^{16}O]-**11** and [^{16}O]-**12**) clearly shows that, in addition to the molecular ions (*m/e* 242), the fragments corresponding to the loss of benzaldehyde $(M - C_7H_6O)^+$ are fairly abundant (*m/e* 136, 31.2% for [^{16}O]-**11**; *m/e* 136, 25.5% for [^{16}O]-**12**).

Since the spatial orientations of the benzyloxy groups in [^{16}O]-**11** and [^{16}O]-**12** have been assigned[14] and since the benzyloxy group is lost after one hydrogen transfer during the mass spectrum fragmentation process, we have been able to assign the distribution of ^{18}O in the axial and equatorial positions of triesters **11–14** on the basis of their mass spectra. The results are collected in Table I. An inspection of Table I clearly shows that the conversion of P-N → P-^{18}O proceeded with nearly complete retention of configuration at the phosphorus atom.[18] Thus, the S_p absolute configuration can be assigned to **3**.

The lack of complete stereospecificity in the P-N → P-^{18}O conversion is intriguing and must be answered. However, our results allow us to claim that the diastereomeric purity of **3** is at least 94.8% and demonstrate the further applicability of amidodiesters to the preparation of chiral phosphorus compounds. Conversion of deoxyadenosine cyclic 3′,5′-(R_p)- and -(S_p)-phosphoranilidates[19] into [^{18}O]-cdAMP with retention of configuration by means of NaH-C$^{18}O_2$ is described in the accompanying communication.[20]

Acknowledgment. This project was financially assisted by the Polish Academy of Sciences, Grant No. MR-I-12. The authors are indebted to Professor David Gorenstein for his early suggestion of the preparation of [^{18}O]-cAMP.

References and Notes

(1) Stec, W. J.; Okruszek, A.; Lesiak, K.; Uznanski, B.; Michalski, J. *J. Org. Chem.* **1976**, *41*, 227.
(2) Lesiak, K.; Lesnikowski, Z. J.; Stec, W. J.; Zielinska, B. *Pol. J. Chem.* **1979**, *53*, 2041.
(3) Zielinski, W. S.; Lesnikowski, Z. J.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1976**, 772. See also ref 4 therein.
(4) Lesiak, K.; Stec, W. J. *Z. Naturforsch., B* **1978**, *33*, 782.
(5) Baraniak, J.; Lesiak, K.; Stec, W. J. *Pol. J. Chem.* **1979**, *53*, 1387.
(6) Baraniak, J.; Kinas, R. W.; Lesiak, K.; Stec, W. J. *J. Chem. Soc., Chem. Commun.* **1979**, 940.
(7) Cullis, P. M.; Lowe, G. *J. Chem. Soc., Chem. Commun.* **1978**, 512.
(8) Abbot, S. J.; Jones, S. R.; Weinman, S. A.; Knowles, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2558.
(9) Compound **5** (86.5% enriched in ^{18}O) has been obtained by hydrolysis of benzylideneaniline in 98% $H_2{}^{16}O$.
(10) Schaller, H.; Weinmann, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *83*, 3821.
(11) Lesiak K.; Stec, W. J., poster presented at the IUPAC International Symposium on Phosphorus Chemistry Directed Towards Biology, Sept. 25–28, 1979; Burzenin, Poland, Abstract Book, p 66.
(12) Stec, W. J.; Lopusinski, A. *Tetrahedron* **1973**, *29*, 547.
(13) Brown, D. M.; Magrath, D. I.; Todd, A. R. *J. Chem. Soc.* **1955**, 4396
(14) Negative chemical shift values are assigned to compounds absorbing at higher field than H_3PO_4. The assignment of the spatial orientations of the benzyloxy groups in **11** and **12** was achieved by means of a chemical shift criterion[15] and independently by means of the stereospecific (retention) oxidation[16] of *cis*-2-benzyloxy-2-selenono-4-methyl-1,3,2-dioxaphosphorinane [$\delta_{^{31}P(C_6H_6)}$ + 64.9 ppm ($^1J_{^{31}P-^{77}Se}$ = 1006 Hz)] to [^{16}O]-**11** and of the *trans*-2-selenono isomer [$\delta_{^{31}P(C_6H_6)}$ + 68.4 ppm ($^1J_{^{31}P-^{77}Se}$ = 965 Hz)] to [^{16}O]-**12**.[17]
(15) Engels, J.; Schlaeger, E.-J. *J. Med. Chem.* **1977**, *20*, 907.
(16) Stec, W. J.; Okruszek, A.; Michalski, J. *J. Org. Chem.* **1976**, *41*, 233.
(17) Stec, W. J. Z. Naturforsch., *B* **1974**, *29*, 109.
(18) The same stereospecificity was observed in a separate set of experiments performed with benzaldehyde containing 46.9% [^{18}O].
(19) Lesnikowski, Z. J.; Stec, W. J.; Zielinski, W. S. *Nucleic Acids Res., Spec. Publ.* **1978**, *4*, S49.
(20) Gerlt, J. A.; Coderre, J. A. *J. Am. Chem. Soc.*, preceding paper in this issue.

Janina Baraniak, Krystyna Lesiak
Marek Sochacki, Wojciech J. Stec*
Polish Academy of Sciences
Centre of Molecular and Macromolecular Studies
90-362 Lódź, Boczna 5, Poland

Received March 14, 1980

A Method for the Preparation of Unesterified Acyl Phosphates via Stannyl Phosphate Intermediates

Sir:

Acyl phosphates are of importance since the compounds such as acetyl phosphate,[1] adenosine 5′-aminoacyl phosphates,[2] and luciferyladenosine 5′-phosphate[3] are known to be biologically significant. Acyl phosphates belong to a class of mixed anhydrides consisting of carboxylic and phosphoric acids. However, little[4] is known concerning a general method for the synthesis of analytically pure acyl phosphates because of their instability.

We now report a general and practically useful method for the synthesis of unesterified acyl phosphates employing stannyl phosphates via the silyl phosphate intermediates.

Stannyl phosphates were found to react smoothly and stoichiometrically with acyl chlorides.

When diethyl tri-*n*-butylstannyl phosphate (**1**)[5] was treated with 1 equiv of acyl chlorides in dry carbon tetrachloride, the corresponding acyl diethyl phosphates (**2**) were obtained in almost quantitative yields. The reaction proceeded smoothly

$$\underset{}{R\text{-}\overset{O}{\overset{\|}{C}}\text{-}Cl} + \underset{(1)}{Bu_3Sn\text{-}O\text{-}\overset{O}{\overset{\|}{P}}(OEt)_2} \xrightarrow[\text{in } CCl_4]{-Bu_3SnCl} \underset{(2)}{R\text{-}\overset{O}{\overset{\|}{C}}\text{-}O\text{-}\overset{O}{\overset{\|}{P}}(OEt)_2}$$

at room temperature and the complete conversion was monitored by its ^1H NMR spectra; in the case of acetyl diethyl phosphate [NMR (CCl_4, 60 MHz) δ 1.90 (d, 3 H, J_{HP} = 2 Hz, $CH_3C(O)$)] and also in the case of diethyl pivaloyl phosphate [NMR (CCl_4, 60 MHz) δ 1.25 (s, 9 H, $(CH_3)_3CC(O)$)]. Both compounds are not stable during the distillation and partially decomposed. They were isolated in 45 and 66% yields, respectively.

Similarly, aroyl diethyl phosphates such as benzoyl (92%), 4-chlorobenzoyl (86%), toluoyl (89%), 2-methylbenzoyl (83%), and anisoyl (88%) derivatives could be obtained and isolated by means of silica gel column chromatography using a mixture of *n*-hexane and ether (4:1–1:1 v/v). The mixed anhydride structure was confirmed by their IR spectrum ($\nu_{C=O}$ 1750 cm^{-1}, benzoyl).

Several reports have recently appeared employing tri-*n*-butylstannyl group as a leaving group in organic synthesis.[6] In the field of the organophosphorus chemistry, the stannyl groups can be also employed for the synthesis of phosphate esters.

It was found that diethyl tri-*n*-butylstannyl phosphate (**1**) can be converted with trimethylsilyl chloride into diethyl trimethylsilyl phosphate (**3**) almost quantitatively. The conversion of **1** into **3** was ascertained by its ^1H NMR spectra (CCl_4),

0002-7863/80/1502-4534$01.00/0