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(20) GC–MS work was done at the Mass Spectral Facility, Cornell University.
(21) $[CpFe(CO)_2]_2$ was enriched to 75% as determined by infrared spectroscopy by treatment of a toluene solution at 80 °C with two successive portions of 99% ^{13}CO.
(22) Three species exist in THF solution, $(Cp)(CO)_2Fe$–Li (I), $[(Cp)(CO)_2Fe^-](Li^+)$ (II), and Cp(CO)Fe–CO–Li (III), with ~60% III being present.[16]
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(26) The chloride, $CpFe(CO)_2Cl$, is also formed.

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Oxygen Chiral Phosphodiesters. 1. Synthesis and Configurational Analysis of Cyclic [^{18}O]-2′-Deoxyadenosine 3′,5′-Monophosphate

Sir:

It is now generally accepted that stereochemical investigation of the mechanism of a phosphotransferase reaction is the most direct method for determining whether the mechanism involves the formation of a covalent adduct between the enzyme and substrate. The majority of these experiments have been carried out with phosphorothioate mono- and diesters. The use of sulfur analogues of the normal substrates simplifies the synthesis and configurational analysis of the chiral substrate and product, but the presence of sulfur often decreases the velocity of the enzymic reaction and may introduce some uncertainty in interpretation of results when coordination of the substrate by a metal ion is required for catalysis.[1] Phosphate esters which are chiral by virtue of oxygen isotopes cannot pose such problems. Knowles' group recently reported the syntheses of a number of ^{16}O,-^{17}O,^{18}O-labeled chiral phosphate monoesters and described an elegant mass spectral technique for the configurational analysis of such esters.[2] These esters have been used to probe the mechanisms of a number of enzyme-catalyzed reactions, including demonstration of a retention of configuration at phosphorus during the reaction catalyzed by the alkaline phosphatase from *Escherichia coli*[3] and an inversion of configuration in the reaction catalyzed by glycerol kinase.[4] However, many phosphotransferases catalyze reactions involving phosphate diesters, and Knowles' synthetic method is not amenable to their preparation. In this communication we report the stereospecific synthesis of both diastereomers of cyclic [^{18}O]-2′-deoxy-AMP and a simple method for the direct determination of their absolute configurations at phosphorus.

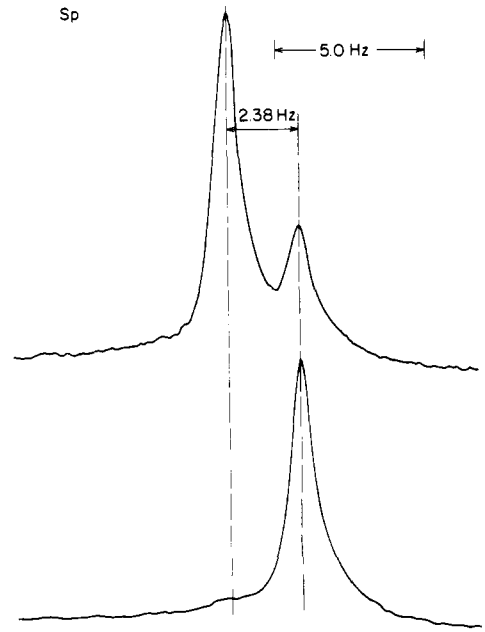

Figure 1. 80.9-MHz ^{31}P NMR spectra of cyclic [^{18}O]-2′-deoxy-AMP prepared from the axial *P*-anilidate diluted (top) and undiluted (bottom) with unlabeled cyclic 2′-deoxy-AMP. The samples were prepared in 0.10 M EDTA, pH 7.0, containing 20% D_2O. The approximate chemical shift of the unlabeled cyclic 2′-deoxy-AMP is 2.6 ppm (upfield shift relative to an external capillary containing 85% H_3PO_4). The total concentration of cyclic 2′-deoxy-AMP is 20 mM in each sample.

The diastereomeric *P*-anilidates of cyclic 2′-deoxy-AMP can be readily prepared by the stereospecific potassium *tert*-butoxide catalyzed cyclization of diastereomerically pure samples of 2′-deoxy-3′-(*o*-chlorophenyl-*N*-phenyl phosphoramidate)adenosine.[5,6] Stec's group has demonstrated that *P*-anilidates react smoothly with carbon disulfide after treatment with sodium hydride to provide the corresponding phosphorothioates.[7] This reaction was found to proceed with retention of configuration at phosphorus, as would be predicted on the basis of apical attack–apical departure from a pentacoordinate intermediate. We have found that the sodium salts of *P*-anilidates also react smoothly with carbon dioxide in pyridine to provide the phosphate diesters in quantitative yield.

When the *P*-anilidates of cyclic 2′-deoxy-AMP were reacted separately with a tenfold excess of 99% enriched $C^{18}O_2$ and the products purified by chromatography on DEAE-Sephadex A-25,[8] phosphodiesters were obtained which were identical with authentic cyclic 2′-deoxy-AMP using the criteria of TLC, ^{1}H NMR at 270 MHz, and ^{31}P NMR at 32 MHz. The presence of ^{18}O in the diesters was confirmed by examination of ^{31}P spectra which were obtained at 80.9 MHz using a 250-Hz sweep width, 16K data points (0.03 Hz/real data point), and 40 transients. In Figures 1 and 2 we present these spectra and those obtained under identical conditions when the two labeled diesters were mixed with a threefold molar excess of unlabeled cyclic 2′-deoxy-AMP. In the spectra recorded on the undiluted samples, one major resonance and one minor resonance are observed. In the spectra recorded on the isotopically diluted samples, the same two resonances are observed, with the upfield resonance being associated with the labeled diester.[9] The minor resonance in the spectra of the undiluted samples is due to a small amount of unlabeled cyclic 2′-deoxy-AMP. The ^{18}O perturbation in the diester prepared from the axial anilidate is 2.38 Hz, and that in the diester prepared from the equatorial anilidate is 2.56 Hz. A spectrum was obtained at 145.7 MHz on a sample containing 12 mM unlabeled cyclic 2′-deoxy-AMP

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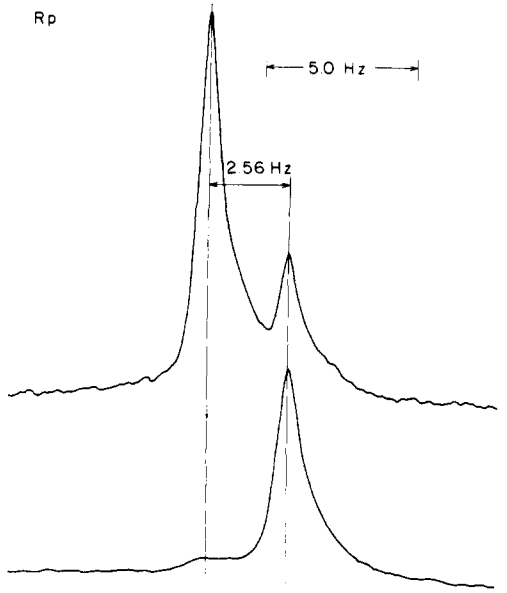

Figure 2. 80.9-MHz ^{31}P NMR spectra of cyclic [^{18}O]-2′-deoxy-AMP prepared from the equatorial *P*-anilidate diluted (top) and undiluted (bottom) with unlabeled cyclic 2′-deoxy-AMP. The total concentration of cyclic 2′-deoxy-AMP is 20 mM in each sample.

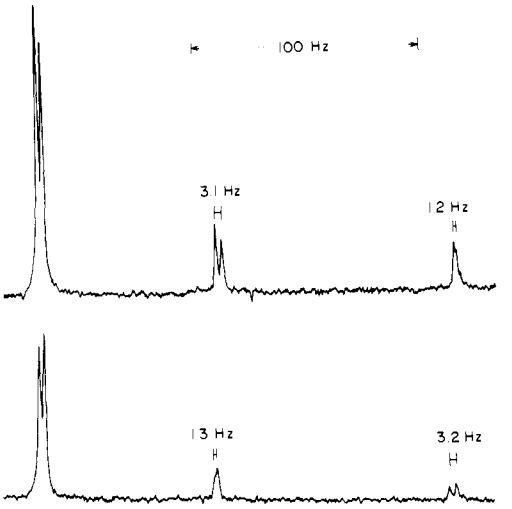

Figure 3. 80.9-MHz ^{31}P NMR spectra of ethyl esters of cyclic 2′-deoxy-AMP prepared from equimolar mixtures of unlabeled cyclic 2′-deoxy-AMP and the cyclic [^{18}O]-2′-deoxy-AMP samples: top, the ^{18}O-labeled ester from the axial *P*-anilidate; bottom, the ^{18}O-labeled ester from the equatorial *P*-anilidate. Sample preparation is described in the text. The spectra were obtained with a 500-Hz sweep width and 8K data points (0.12 Hz/real data point). The approximate chemical shift of the unlabeled diester is 3.0 ppm in this solvent, that of the equatorial ester is 4.5 ppm, and that of the axial ester is 6.5 ppm.

and 2 mM of each ^{18}O-labeled diester at pH 7.0; the line widths of the ^{16}O and ^{18}O resonances were identical within error, indicating that the observed difference in the ^{18}O perturbations of the two ^{18}O-labeled diesters is not significant.

Although the reaction of *P*-anilidates with carbon dioxide would be predicted to occur with retention of configuration at phosphorus, it is important to verify this prediction if these esters are to be used in stereochemical studies of enzymic reactions. One method of performing the configurational analysis would be to hydrolyze the diesters in $H_2{}^{17}O$ using the bovine heart cyclic AMP phosphodiesterase as catalyst and analyze the chirality of the resulting ^{16}O,^{17}O,^{18}O,-labeled chiral samples of 5′-dAMP with Knowles' procedure[2] (the cyclic AMP phosphodiesterase is known to catalyze the hydrolysis of the S_P diastereomer of cyclic adenosine 3′,5′-phosphorothioate with inversion of configuration[10]); however, we sought an alternate method of analysis which would be more direct and require less material.

Samples of the triethylammonium salts of each of the two labeled diesters (50 μmol) were mixed with equimolar amounts of the salt of the unlabeled diester and acidified with 2 equiv of aqueous HCl. After drying, the two samples were suspended in anhydrous ethanol and treated with an excess of diazoethane. The water-soluble triesters and unreacted diesters[11] were dissolved in a mixture of 2 mL of aqueous 0.02 M EDTA, pH 7.0, containing 80% D_2O, and 1.5 mL of anhydrous ethanol; the ^{31}P NMR spectra were then obtained at 80.9 MHz.[12]

The spectra obtained are shown in Figure 3. The top spectrum is that of the mixture prepared from the diester obtained from the axial *P*-anilidate. Three sets of resonances are observed, the most downfield being that of unreacted diester, the middle that of the equatorial ester, and the most upfield that of the axial ester[14]. The ^{18}O perturbation in the equatorial is 3.1 Hz and that in the axial ester is 1.2 Hz. This difference in the magnitudes of the perturbations can be explained on the basis of recent reports that the magnitude of the perturbation increases with the order of the bond between the ^{18}O and phosphorus nucleus[15] and the fact that the incorporation of ^{18}O proceeded stereospecifically. The relative values of the ^{18}O perturbations in this spectrum indicate that the ^{18}O is located in the axial exocyclic position, demonstrating that the reaction with carbon dioxide proceeds with the predicted retention of configuration. Thus, this diastereomer has the S_P configuration at phosphorus.

The bottom spectrum in Figure 3 shows the products of the reaction of the ^{18}O-labeled diester prepared from the equatorial anilidate. The ^{18}O perturbations in this spectrum (1.3 Hz for the equatorial ester and 3.2 Hz for the axial ester) demonstrate that the absolute configuration at phosphorus in this ester is R_P.

In an accompanying communication results are reported from the laboratory of Stec[16] which demonstrate that the diastereomeric *P*-anilidates of 2-oxo-2-hydroxy-4-methyl-1,3,2-dioxaphosphorinane react with [^{18}O]benzaldehyde with retention of configuration at phosphorus, in agreement with the results reported in this communication.

We have also found that the sodium salts of acyclic *P*-anilidates, e.g., the *P*-anilidates of thymidine 3′- and 5′-(4-nitrophenyl phosphate), react smoothly with carbon dioxide to provide quantitative yields of the phosphate diesters.[6] Thus, the demonstration that the reaction of *P*-anilidates with carbonyl compounds proceeds with retention of configuration permits the confident synthesis of a wide variety of oxygen chiral phosphate diesters for use in studying the mechanisms of a large number of enzyme-catalyzed reactions.

The application of ^{18}O perturbations of ^{31}P NMR chemical shifts to deduce absolute configurations at phosphorus as described in this communication should be useful for other ^{18}O-labeled esters, e.g., chiral [α-^{18}O]-ATP.[17]

Acknowledgments. We thank Professor Wojciech J. Stec for providing us with details of his unpublished work. We are grateful to Professor Ian M. Armitage, Dr. James Otvos, and Dr. George McDonald for their generous assistance in obtaining the high-field ^{31}P NMR spectra. This research was supported by a grant (GM-22350) from the National Institutes of Health. The high-field NMR spectrometers used in this research are supported by grants from the Biomedical Technology Program of the National Institutes of Health (Bruker HX-270, Grant RR-798, and Bruker WH-360, Grant RR-542) and the National Science Foundation (Bruker CXP-200, Grant CHE-7916120).

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(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.
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(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.
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(18) NIH Research Career Development Awardee (CA-00499), 1978–1983.

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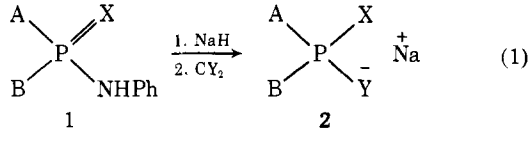
Received February 19, 1980

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).

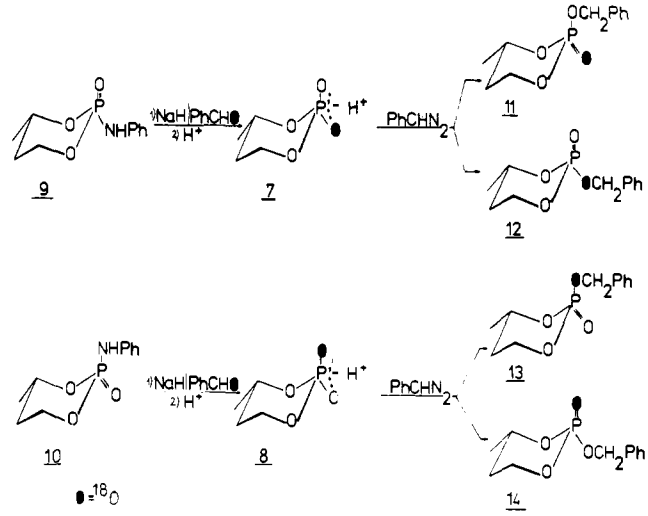


1 2 (1)

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

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