Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 4

SYNTHESIS OF NON-BRIDGING $^{18}O_3$ LABELED PHOSPHATE MONOESTERS

Kim C. Calvo
Department of Chemistry, University of Akron, Akron, OH 44325

SUMMARY

A new method is reported for the synthesis of tri-^{18}O substituted phosphate monoesters where the phosphoryl oxygen ester bond is ^{16}O. The method relies on the reactivity of trivalent phosphorus toward hydrolysis and oxidation to produce dimethyl [$^{18}O_3$]-phosphorochloridate with a minimal excess of labeled water. This chlorophosphate diester can be used to prepare a wide variety of phosphate monoesters where the three peripherial oxygens are labeled.

Key words: synthesis, oxygen isotopes, phosphate esters

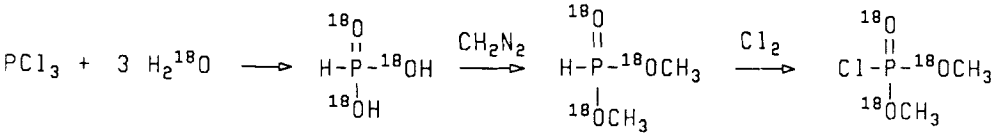
INTRODUCTION

Oxygen isotope labeled esters of phosphoric acid have found wide use in the study of enzyme mechanisms, microbial metabolism, and kinetic isotope effect studies (1,2). The methods used to produce ^{18}O labeled phosphate esters generally employ the hydrolysis of PCl_5 with labeled water and the subsequent phosphorylation of the alcohol of interest using either a dehydrating agent or an enzymatic reaction. The difficulities associated with these methods are 1) low chemical yield, 2) loss of isotopic label and 3) the use of a large excess of labeled water to minimize pyrophosphate formation (1). Because of our interest in the kinetics of the solvolysis of phosphate esters, we required an efficient, general chemical method for the synthesis of ^{18}O labeled phosphate monoesters. Our approach to this problem involves the synthesis of a reactive, protected phosphorochloridate.

As outlined in Scheme 1, the isotopes of oxygen are introduced to the phosphorus atom by hydrolyzing the extremely reactive phosphorus

0362–4803/89/040395–05$05.00

Received March 31, 1988
Revised August 22, 1988

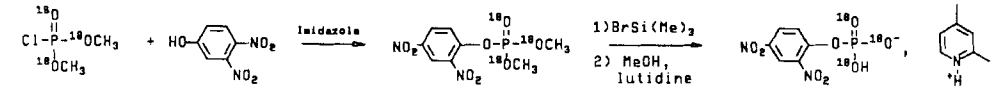


trichloride. Because of the reactivity pyrophosphorus acid (3), only a slight excess (5 mole percent) of the labeled water is required for a stoichiometric formation of [$^{18}O_3$] phosphorus acid. This was demonstrated by observing the ^{31}P NMR of a reaction mixture formed using unlabeled water. Only a single phosphorus peak was observed at δ 4.65 (J_{HP}=700 Hz).

Without isolating the labeled phosphorus acid, the dimethyl ester is formed by reaction with diazomethane. It is important that the diazomethane used at this step is produced by distillation (e.g. by the method outlined in technical information bulletin no. AL-134 distributed by the Aldrich Chemical Co.).

Again without isolation of the intermediate, the phosphorochloridate is formed by chlorine oxidation of the dimethyl phosphorus acid. The end point of the reaction is easily observed by the yellowish color produced by an excess of the chlorine. A small excess of chlorine at this point is not deliterious as the P(V) product is resistant to further oxidation. The dimethyl-[$^{18}O_3$] phosphorochloridate is then purified by bulb to bulb distillation at 0.1 mm. By using a high vacuum and a well cooled receiver (dry ice), the product can be distilled at at relatively low temperature. This minimizes side reactions (pyrophosphate formation) which can occur when the phosphorochloridate is heated. ^{31}P NMR of the dimethylphosphorochloridate demonstrates a major peak at δ 8.95 (>95%) and a minor peak at δ -9.17 (<5%) which is apparently due to tetramethylpyrophosphate. The desired product is obtained in 94% yield and is greater than 95% pure. The isotopic incorporation is very high as 98% ^{18}O water produces 93% dimethyl-[$^{18}O_3$] phosphorochloridate and 7% of the $^{18}O_2$,^{16}O species (theoretically we expect 94% of the tri-^{18}O compound).

The utility of the labeled phosphorochloridate is demonstrated by its use in the synthesis of monohydrogen, monolutidinium 2,4-dinitrophenyl-[$^{18}O_3$] phosphate. Using the basic conditions of Ramierez (4), the synthesis is outlined in Scheme 2. Reaction of the labeled phosphorochloridate with



2,4-dinitrophenol is carried out in methylene chloride. Using imidazole as the base easily generates the dimethyl 2,4-dinitrophenyl [$^{18}O_3$] phosphate. Treatment of this triester with bromotrimethylsilane followed by ethanol containing 1 equivalent of 2,4-lutidine produces the required salt. This method is quite general as the ester cleavage reaction of bromotrimethylsilane demonstrates a high degree of selectivity for methyl esters (5) (methyl esters react about 4 times faster than ethyl esters) and should be applicable to the synthesis of a variety of oxygen labeled phosphate esters.

EXPERIMENTAL

General All reagents were of the highest purity commercially available and were used without purification except as indicated. Ether was distilled from benzophenone ketyl, 2,4-lutidine was distilled from CaH_2, dichloromethane was distilled from P_2O_5 and stored over potassium carbonate, ethanol was distilled from magnesium ethoxide, phosphorus trichloride was distilled from PCl_5 and stored in a dessicator at -20°C and benzene was distilled from sodium metal, all under nitrogen. Imidazole and 2,4-dinitrophenol were recrystallized. Chlorine gas was passed through a $CaCl_2$ drying column. Melting points were determined using a Mel-Temp melting point apparatus and are uncorrected. Mass spectra were obtained on a Finnigin MAT 700 ion trap detector attached to a Hewlett-Packard 5890 GC. An RSL-300 capillary column was used for the GC separation and the ionization voltage of the ion trap was set at 70 ev. ^1H and ^{31}P NMR were obtained on a Varian FT-80A, proton spectra are referenced to TMS and phosphorus spectra are referenced to external trimethylphosphite at 140 ppm.

Dimethyl [$^{18}O_3$] phosphorochloridate. To 20 mL of dry ether in a 100 mL round bottom flask is added 0.250 mL (12.75 mmol) $H_2{}^{18}O$ (98 atom percent, Mound Research Labs). While the two phase system is magnetically stirred in an ice bath, 0.371 mL (4.25 mmol) of phosphorus trichloride is slowly added under nitrogen. A vigorous reaction ensues. After the addition of PCl_3 is complete, the reaction mixture is slowly allowed to warm to room temperature over 20 min. An etheral solution of diazomethane is added till a yellow color persists. The ether is removed under a stream of dry nitrogen. Benzene, 20 mL, is added and chlorine gas passed into the solution until a faint yellow color remains. The mixture is

evaporated in vacuo and the residual oil distilled bulb to bulb (bath temperature 60°C, 0.1 mm Hg) yielding 0.606 gm of product (94%). ^{1}H NMR ($CDCl_3$) δ 3.80 (d, J=13.7 Hz). ^{31}P NMR ($CDCl_3$) δ 8.95 (s, >95%), -9.17 (s,<5%). m/e (rel. int.) 148 (9.84), 150 (100), 152 (30.71).

monohydrogen monolutidinium 2,4-dinitrophenyl-[$^{18}O_3$] phosphate. To a solution containing 0.552 gm (3.0 mmol) 2,4-dinitrophenol and .204 gm (3.0 mmol) imidazole in 45 mL dichloromethane is added a solution of 0.451 gm (3.0 mmol) dimethyl-[$^{18}O_3$]-phosphorochloridate in 4 mL of dichloromethane. The orangish solution is stirred under nitrogen for 8 hours then filtered through 1 gm of silica gel. The silica gel is washed with 10 mL of ethylacetate and the combined filtrates are evaporated. The yellowish oil, dimethyl 2,4-dinitrophenyl-[$^{18}O_3$] phosphate shows a single ^{31}P NMR ($CDCl_3$) resonance at δ -6.68. ^{1}H NMR ($CDCl_3$) δ 8.72-7.50 (m, 3H), 3.88 (d, J=10 Hz, 6H). Yield 0.671 gm (75%). The methyl esters were cleaved by reaction with bromotrimethylsilane. A solution of 0.298 gm (1.0 mmol) of the triester in 15 mL of dichloromethane was treated with 0.264 mL (2.0 mmol) of bromotrimethylsilane. The reaction was stirred under nitrogen for 6 hours, rotoevaporated and treated with a solution of 0.110 gm (1.0 mmol) of 2,4-lutidine in 10 mL of dry ethanol. The solution was cooled on ice, filtered and the resulting pale yellow crystals were recrystallized from ethanol. mp 140-141 °C (lit. (6) 142°C). ^{31}P NMR (CD_3OD/CH_3OH 9:1 v/v) δ -6.87 (s). The salt is very hydroscopic. Prolonged storage in a dessicator results in considerable decomposition. The ^{18}O incorporation was measured after solvolysis of the salt in ethanol. The salt, 10 mg, was dissolved in dry ethanol and allowed to sit at 45°C for 10 days in a dessicator, at which time the formation of 2,4-dinitrophenol had ceased. The solution was concentrated in a stream of dry nitrogen, acidified with dry HCl gas and treated with an excess of diazoethane (generated from 1-ethyl-3-nitro-1-nitrosoguanidine) in ether. GC/MS analysis demonstrated a peak corresponding to [$^{18}O_3$]-triethylphosphate. m/e (rel. int.) 104 (100%), 102 (7.98%).

REFERENCES

1) Hackney, D., Stempel, K. and Boyer, P.- Methods in Enzymology, vol. 64, (Purich, D. ed), Academic Press, NY, pp 60-83 (1980)

2) Weiss, P., Knight, W. and Cleland, W.-J. Am. Chem. Soc. 108: 2761-2 (1986)

3) Kosalopoff, G.- J. Am. Chem. Soc. 73: 4101-2 (1951)

4) Ramierez, F. and Maracek, J.-Synthesis: 601 (1978)

5) McKenna, C. and Schmidhauser, J.-J. Chem. Soc. Chem. Commun.: 739 (1979)

6) Kirby, A. and Varvoglis, A.-J. Am. Chem. Soc. 89: 415-23 (1967)