Bis[2,2,2-trifluoroethyl] Phosphite, a New Reagent for Synthesizing Mono- and Diesters of Phosphorous Acid

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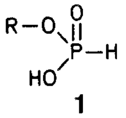
Monoesters of phosphorous acid (**1**) are of interest as analogues of biologically important phosphates and as starting materials for the synthesis of phosphoric monoesters. Diesters of phosphorous acid are highly reactive precursors of phosphoramidates and phosphoric triesters. Mono- and diesters of phosphorous acid have not been used widely in synthesis because a general, efficient method for their preparation has not been available.

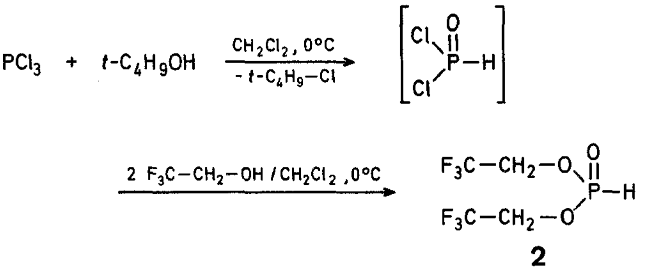


1

Monophosphites (**1**) have been prepared by condensing alcohols with phosphorous acid in the presence of carbodiimides[2] or arylsulfonylimidazoles[3]. Transesterification of triphenyl phosphite with alcohols followed by an alkaline work-up yields monophosphites[4]. Reaction of equimolar amounts of some alcohols with phosphorus trichloride and aqueous work-up gives phosphite monoesters[5,6]. Treatment of phosphorus trichloride successively with water and two alcohols gives unsymmetrical phosphite diesters[7]. Such esters have also resulted from reaction of a tetraalkylammonium salt of a monophosphite with an alkyl iodide[8] and by the carbodiimide condensation of an alcohol with a monophosphite[9]. Ribonucleoside 5′-benzyl phosphites have been made by reacting the nucleoside with *O*-benzylphosphorous-*O*,*O*-diphenylphosphoric anhydride[10].

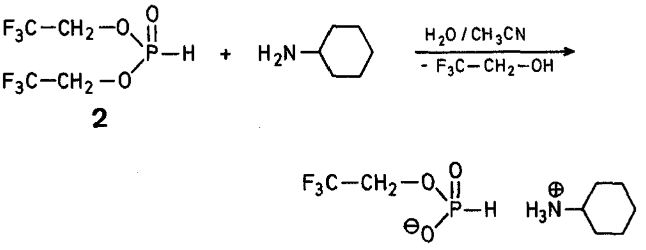
In a search for a more general synthesis applicable to sensitive biological compounds and involving a simpler purification procedure we have studied the transesterification of phosphite diesters with alcohols and phenols. Transesterification of diethyl phosphite[11] and dimethyl phosphite[12] has been reported. In some cases, catalysis by a sodium alkoxide is required[13].

We have found that transesterification of a new reagent, bis[2,2,2-trifluoroethyl] phosphite (**2**) is a simple way of preparing many phosphite esters. The reagent is obtained in good yield by reaction of one equivalent of 2-methyl-2-propanol with phosphorus trichloride followed by two equivalents of 2,2,2-trifluoroethanol.



2

Compound **2** can be stored unchanged in screw-cap vials in a desiccator for at least one year. Treament of **2** with cyclohexylamine in acetonitrile in the presence of water yields the cyclohexylammonium salt of 2,2,2-trifluorethyl phosphite (**3**).



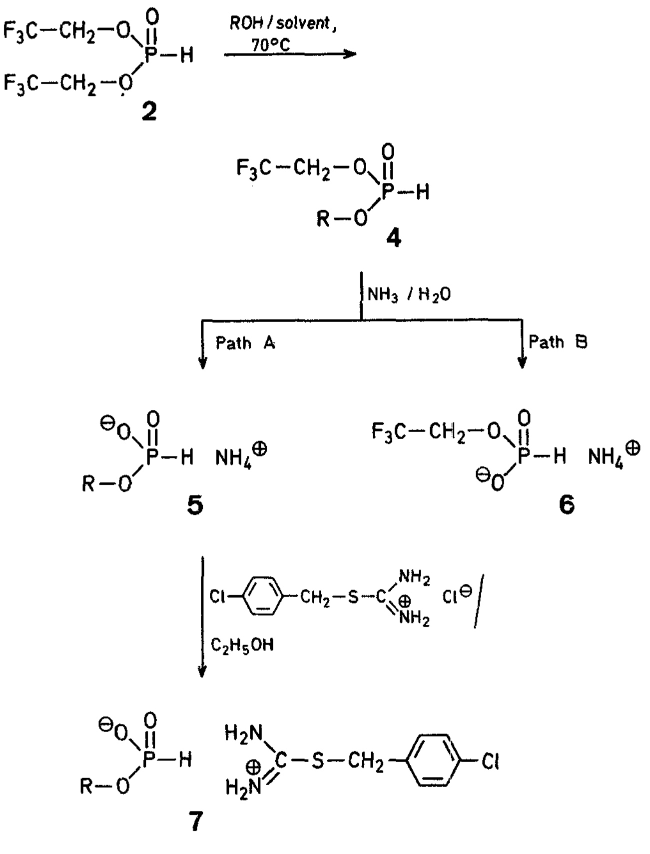
2, **3**

Transesterification procedes easily when a 1 : 2 to 1 : 3 molar ratio of hydroxy compound and **2** are heated at 60–70 °C in an anhydrous solvent for 2–3 h. Yields of the unsymmetrical diester **4** are practically quantitative. Reaction time is reduced to 30–60 min by a catalytic amount of triethylamine. Solvents found to be suitable include acetonitrile, 1,4-dioxan, dimethylformamide, and pyridine. The usual work-up procedure involves simply applying a vacuum (1–5 torr) to the reaction mixture. Most of the unreacted **2** and trifluoroethanol are thus removed. The product is then suitable for many reactions. Phosphites of type **4** decompose quickly in the atmosphere and should be used without delay. The transesterification method has been applied successfully to the synthesis of phosphites of carbohydrates, steroids, and nucleosides. Common hydroxy protecting groups which are compatible with **2** found so far are the acetate, benzoate, triphenylmethyl ether, dimethoxytriphenylmethyl ether, *t*-butyldimethylsilyl ether, isopropylidene, and ethoxymethylidene groups. The only unsuitable protecting groups discovered thus far are the 2,2,2-tribromoethoxycarbonyl and 2,4-dinitrophenylsulfenyl groups, and the isopropylidene group involving the anomeric carbon of a carbohydrate.

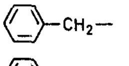
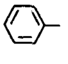
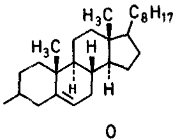
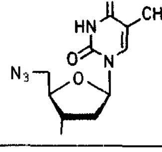
Saponification of the phosphite diesters is of preparative value. The diesters **4** react with aqueous ammonia in two ways. Path A, the major reaction, is a useful synthesis of ammonium salts of monoesters of phosphorous acid (**5**). Path B leads to the original hydroxy compound plus the ammonium salt of 2,2,2-trifluoroethyl phosphite (**6**).

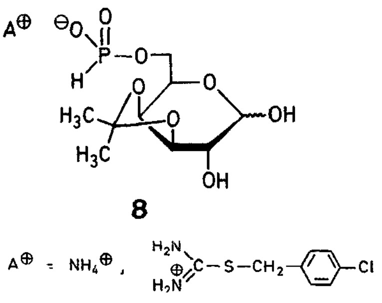
The phosphite monoesters **5** are most conveniently isolated for analytical purposes as their *S*-(*p*-chlorobenzyl)-thiuronium salts **7** following saponification of **4** with aqueous ammonia. The method is illustrated by the synthesis of the ammonium salts **5a–d** and the corresponding thiuronium derivatives **7a–d**.

When our method was applied to 1,2,3,4-di-*O*-isopropylidene-D-galactopyranose, the 1,2-*O*-isopropylidene group was lost, presumably through formation of a labile 1-phosphite which was hydrolyzed during work-up. The final product, based on quantitative U.V. analysis of the thiuronium salt and reducing sugar reaction with Benedict's reagent, appears to be the 6-phosphite **8**.



2, **4**, **5**, **6**, **7**

4,5,7	
a	
b	
c	
d	



8

Analytical yields in excess of 95 % have been obtained starting from 5′-monomethoxytriphenylmethyl derivatives of deoxynucleosides with protected bases (i.e., A^{Bz}, C^{Bz}, and $G^{i\text{-}C_4H_9}$). The synthesis of oligonucleotides using nucleoside trifluoroethyl phosphites is in progress.

Melting points were measured on a Fisher-Johns hotplate and are corrected. Infrared spectra were measured with a Perkin-Elmer 237 B grating spectrophotometer and ultraviolet spectra, with a Unicam SP800 instrument. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Molecular weights were determined by U.V. spectral analysis of thiuronium salts. All products were homogeneous in several chromatographic systems. Compounds on chromatograms were visualized either by U.V. absorbances or by a modified Hanes-Isherwood spray reagent[14].

Bis[2,2,2-trifluoroethyl] Phosphite (2):

A solution of anhydrous 2-methyl-2-propanol (37.0 g, 0.5 mol) in dry dichloromethane (100 ml) is added dropwise to a stirred solution of phosphorus trichloride (43.5 ml, 0.5 mol) in dichloromethane (100 ml) over a period of 45 min. The mixture is maintained at 0–5 °C under a nitrogen atmosphere. Stirring is continued for an additional 30 min at 0 °C. A solution of anhydrous 2,2,2-trifluoroethanol (100.0 g, 1 mol) in dichloromethane (100 ml) is added to the mixture at 0–5 °C over a period of 30 min. Stirring is continued under a stream of nitrogen at 25 °C for 16 h to remove hydrogen chloride. Dichloromethane is removed by distillation at atmospheric pressure. The product is distilled through a 10-cm Vigreux column; yield: 100.8 g (82 %); b.p. 43–44 °C/2 torr; d_4^{25}: 1.6686.

$C_4H_5FO_3P$	calc.	C 19.52	H 2.05	F 46.33	P 12.59
(246.1)	found	19.46	1.97	46.53	12.47

I.R. (film): ν_{max} = 2485 (PH), 1250 (P = O), 1150 (CF₃), 1080 cm⁻¹ (P-O-C).

2,2,2-Trifluoroethyl Phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt:

Bis[2,2,2-trifluoroethyl] phosphite (2; 1.40 ml, 9.1 mmol) in acetonitrile (30 ml) is treated with concentrated aqueous ammonia (4 ml). Removal of the solvent leaves the hygroscopic ammonium salt of trifluoroethyl phosphite; m.p. 103–104 °C. This product is mixed with *S*-(*p*-chlorobenzyl) thiuronium chloride (2.15 g, 9.1 mmol) in ethanol (30 ml). The ethanol is removed and the residue is extracted with acetone (3 × 15 ml). Ammonium chloride is removed by centrifuging; evaporation of acetone from the supernatant gives the thiuronium phosphite; yield: 3.00 g (81 %). An analytical sample is recrystallized from acetonitrile; m.p. 149–150 °C.

$C_{10}H_{13}ClF_3N_2O_3PS$	calc.	C 32.93	H 3.59	N 7.68
(364.7)	found	33.12	3.59	7.60

I.R. (KBr): ν_{max} = 2400 (PH), 1670 (C = S), 1220 (P = O), 1170 (CF₃), 1080 cm⁻¹ (P-O-C).

2,2,2-Trifluoroethyl Phosphite, Cyclohexylammonium Salt (3):

A solution of bis[2,2,2-trifluoroethyl] phosphite (2; 0.12 g, 0.5 mmol), in acetonitrile (10 ml) is treated with of water (9 µl) and cyclohexylamine (0.057 ml). The cyclohexylammonium salt is deposited immediately as large needles; yield: 0.125 g (95 %). The analytical sample is recrystallized from ethanol/acetone; mp. 160–160.5 °C.

$C_8H_{17}NO_3P$	calc.	C 36.50	H 6.51	N 5.32	P 11.77
(263.2)	found	36.58	6.67	5.19	11.57

I.R. (Nujol): ν_{max} = 2420 (PH), 1210 (P = O), 1170 (CF₃), 1080 cm⁻¹ (P-O-C).

Benzyl Phosphite, Ammonium Salt (5a):

A mixture of anhydrous benzyl alcohol (0.31 ml, 3 mmol) and bis[2,2,2-trifluoroethyl] phosphite (2; 1.60 ml, 10 mmol) in anhydrous acetonitrile (15 ml) is heated in a heavy wall 50-ml centrifuge tube with screw cap at 70 °C (oil bath) for 4 h. Volatile material is removed on a rotary evaporator (bath, 50 °C) and two 20-ml portions of acetonitrile are evaporated from the residue. The residue is taken up in acetonitrile (20 ml), mixed with conc. aqueous ammonia (2 ml) and then taken to dryness. Two portions (20 ml) of ethanol are evaporated from the residue which is then washed with ether (50 ml), and dried in vacuo with phosphorus pentoxide at 25 °C for 18 h; yield of the ammonium salt of benzyl phosphite: 0.54 g (95 %); m.p. 142–145 °C (with previous softening). The product is free of trifluoroethyl phosphite and inorganic phosphite as shown by paper chromatography (developer: 1-butanol/acetic acid/water, 4 : 1 : 1).

Benzyl Phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt (7a):

For analytical purposes the ammonium salt (**5a**) is converted to the thiouronium salt as follows. The ammonium salt (0.262 g, 1.30 mmol) is mixed with *p*-chlorobenzylthiuronium chloride (0.329 g, 1.39 mmol) and ethanol (10 ml) at 60 °C. The solvent is evaporated and the residue washed successively with acetone and water and finally recrystallized from ethanol; yield: 0.311 g (60 %): m.p. 195–196 °C.

$C_{15}H_{18}ClN_2O_3PS$	calc.	C 48.32	H 4.87	N 7.51
(372.8)	found	48.31	5.01	7.39

U.V. (CH₃OH): λ_{max} = 223 nm (ε = 14300); 205 nm (ε = 8500).

Phenyl Phosphite, Ammonium Salt (5b):

With phenol (3 mmol) in place of benzyl alcohol, the procedure just described gives phenyl phosphite, ammonium salt; yield: 0.420 g (80 %); m.p. 172–173 °C (from ethanol/ether).

U.V. (H₂O) λ_{max} = 204 nm (ε = 7150), 260 nm (ε = 290).

Phenyl Phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt (7b):

The thiuronium salt is prepared as described for benzyl phosphite and recrystallized from ethanol; m.p. 187.5–188.5 °C.

$C_{14}H_{16}ClN_2O_3PS$	calc.	C 46.86	H 4.50	N 7.81	P 8.63
(358.8)	found	46.99	4.49	7.72	8.67

U.V. (CH₃OH): λ_{max} = 223 nm (ε = 14200).

Cholesteryl Phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt (7c):

A solution of anhydrous cholesterol (0.780 g, 2 mmol) and bis[2,2,2-trifluoroethyl] phosphite (2; 1.28 ml, 8 mmol) in dimethylformamide (10 ml) is heated in a stoppered flask at 70 °C for 4 h. The volatile components are removed by coevaporation with toluene (30 ml). Acetonitrile (20 ml) and concentrated aqueous ammonia (2 ml) are added to the residue, and the resulting suspension is stirred for 16 h. The ammonium salt of cholesteryl phosphite thus formed is too hygroscopic to handle so the entire amount is converted to the thiuronium salt.

The ammonia reaction mixture is evaporated to dryness, the residue is dissolved in ethanol (25 ml) and mixed with a solution of *S*-(*p*-chlorobenzyl) thiuronium chloride (0.474 g, 2 mmol) in ethanol (10 ml). Precipitation of the thiuronium phosphite begins immediately. After 20 min, the solvent is evaporated and the residue extracted successively with ether (3 × 25 ml) and water (4 × 20 ml) and dried; yield: 0.820 g (63 %): m.p. 200–201 °C. An analytical sample is obtained by recrystallization from 95 % ethanol; m.p. 203–204 °C.

$C_{35}H_{56}ClN_2O_3PS$	calc.	C 64.54	H 8.67	N 4.30	P 4.76
(651.3)	found	64.81	8.89	4.17	4.69

U.V. (H₂O): λ_{max} = 223 nm (ε = 14100).

5′-Azido-5′-deoxythymidine-3′-phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt (7d):

A suspension of 5′-azido-5′-deooxythymidine[15] (1.335 g, 5 mmol) and bis[2,2,2-trifluoroethyl] phosphite (2; 2.40 ml, 15 mmol) in anhydrous acetonitrile (25 ml) is heated at 70 °C for 3 h. All of the solid dissolves within the first 10 min. Volatile material is removed in vacuo and finally by coevaporation with additional portions of acetonitrile. The residue is dissolved in acetonitrile (25 ml) and treated with concentrated aqueous ammonia (5 ml). Solvent is evaporated and the resulting gum is extracted with acetone (2 × 20 ml) leaving the ammonium salt of 5′-azido-5′-deoxythymidine-3′-phosphite as an extremely hygroscopic white powder; yield: 1.70 g (98 %).

The ammonium salt is converted to the thiuronium salt for analysis by reaction with *S*-(*p*-chlorobenzyl) thiuronium chloride (1.185 g, 5 mmol) in absolute ethanol (20 ml). After centrifuging to remove ammonium chloride, the supernatant is concentrated and purified by short-column chromatography on silica gel, using a gradient of chloroform and methanol for elution. The product is homogeneous by paper chromatography (2-propanol/ammonia/water, 7 : 1 : 2) and electrophoresis (r_m = 0.60 relative to thymidine 5'-phosphate, pH 7.1); yield: 1.87 g (60% based on dihydrate).

$C_{18}H_{27}ClN_7O_8PS$	calc.	C 38.05	H 4.79	P 5.45
(568.1)	found	38.24	4.57	5.83

Mol. Weight: 561

I. R. (Nujol): ν_{max} = 2360 (PH), 2100 (N_3), and 1650 cm^{-1} (C=S).

U. V. (CH_3OH): λ_{max} = 223 and 263 nm. For ε_{223} = 20000, which is the sum of contributions from the thiuronium ion (ε_{223} = 14200) and the nucleoside (ε_{223} = 5800).

3,4-*O*-Isopropylidene-D-galactopyranose-6-phosphite, *S*-(*p*-Chlorobenzyl) Thiuronium Salt (8):

1,2,3,4-Di-*O*-isopropylidene-D-galactopyranose (1.062 g, 4 mmol) is dissolved in a mixture of bis[2,2,2-trifluoroethyl] phosphite (**2**; 1.60 ml, 10 mmol) and anhydrous acetonitrile (15 ml). The solution is heated at 70 °C for 4 h. Volatile material is removed on a rotary evaporator (1 torr, bath 50 °C, 30 min). The residue is taken up in acetonitrile (20 ml) and treated with concentrated aqueous ammonia (5 ml). After the volatile material has been removed in vacuo, a mixture of *S*-(*p*-chlorobenzyl) thiuronium chloride (0.948 g, 4 mmol) and acetone (25 ml) is added to the residue. The precipitated ammonium chloride is removed by centrifuging. The supernatant is taken to dryness and the residue stirred with ethyl acetate (20 ml). Further precipitate is removed by centrifuging. To the supernatant is added ether (50 ml). The resulting precipitate is washed with ether and dried in vacuo over phosphorus pentoxide at 25 °C for one day; yield: 1.327 g (69 %); m.p. 100–101 °C.

Mol. Weight: 480.

I. R. (KBr): ν_{max} = 3400 (OH), 3250 (NH_2), 2360 (PH), 1670 (C=S), 1370 cm^{-1} [$C(CH_3)_2$].

U. V. (CH_3OH): λ_{max} = 223 nm (ε = 14200).

The product gave a positive reaction with Benedict's reagent (80 °C 0.5 min). Neither the starting materials nor monoalkyl esters of phosphorous acid gave any reaction.

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