# Novel Synthesis of [$^{33}$P]-(2-Chloroethyl)phosphonic Acid

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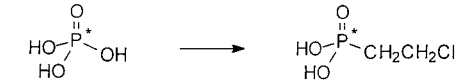
Phosphonic acids and their derivatives play an important role in the regulation of biological processes.[1] They are widely used as pharmaceuticals and agrochemicals, e.g., glyphosate [*N*-(phosphonomethyl)glycine] as a herbicide and ethephon [(2-chloroethyl)phosphonic acid] as a plant growth regulator.[2] Their safe and effective use requires a thorough understanding of their metabolic fate and toxicology. For example, ethephon inhibits plasma butyrylcholinesterase in mammals including humans, presumably by phosphorylation of a serine residue at the active site.[3] Further study of the mechanism and consequences of this enzyme inhibition and possible phosphorylation of other proteins required the radiosynthesis of [$^{33}$P]-ethephon.

The challenge for radiosynthesis of ethephon lies in formation of the phosphorus−carbon bond starting from the most readily available precursor, [$^{33}$P]-phosphoric acid ($H_3PO_4$). In addition, the acid nature of ethephon as well as its instability under neutral and basic conditions[4] require a clean and efficient procedure to minimize purification. Earlier multistep syntheses of $^{32}$P- and $^{33}$P-labeled phosphonic acid derivatives did not meet these specifications.[5]




phosphoric acid 1

We report here a novel and convenient route, starting from [$^{33}$P]-$H_3PO_4$, to synthesize [$^{33}$P]-ethephon with high yield and purity, which can also be applied to a variety of phosphonic acids and their derivatives.

## Results and Discussion

There are two major approaches to form the phosphorus−carbon bond: (1) the attack of a phosphorus or phosphoric acid derivative (a halide or an ester) by an organometallic reagent and (2) the nucleophilic attack by a trivalent phosphorus center on electrophilic carbon.[1,6] Direct synthesis of ethephon by the first approach is not achievable due to the nonavailability of a $\beta$-chloro organometallic reagent. Indirect synthesis, e.g., through the sequence aldehyde, alcohol, and chloride, requires several reaction and purification steps with only moderate yield,[7] which is not suitable for radiolabeling.

Ethephon is normally synthesized by the second approach through an Arbuzov reaction,[8] either via coupling of a trialkyl phosphite with 1-bromo-2-chloroethane or by intramolecular rearrangement of tris(2-chloroethyl) phosphite. In both cases, the final step is acid hydrolysis or reaction with trimethylsilyl bromide[9] for dealkylation of the resulting phosphonate to the phosphonic acid. We examined the Arbuzov reaction of several trialkyl phosphites [trimethyl, triethyl, triisopropyl, and tris(2-chloroethyl)] and found that, besides the long time (>16 h) and high temperature (~160 °C), the procedure gives a series of byproducts presumably from attack at the chloro-substituted carbon or intermolecular Arbuzov reaction.[10] Impurities in trialkyl phosphites from the reaction of phosphorus trichloride ($PCl_3$) with alcohol significantly increase the amounts of side products. Difficulties are also reported in completely hydrolyzing to the desired phosphonic acids.[10b,11]

We therefore chose to use a more bulky and more reactive phosphite, tris(trimethylsilyl) phosphite [$P(OTMS)_3$], expecting that it would provide higher selectivity between the carbons with bromide and chloride and also a milder reaction condition. This proved to be the case, and the Arbuzov reaction of $P(OTMS)_3$[12] with 1-bromo-2-chloroethane in 1,2-dichloroethane was almost complete on refluxing for 2 h, giving the desired compound exclusively, but the reaction with 1,2-dichloro-

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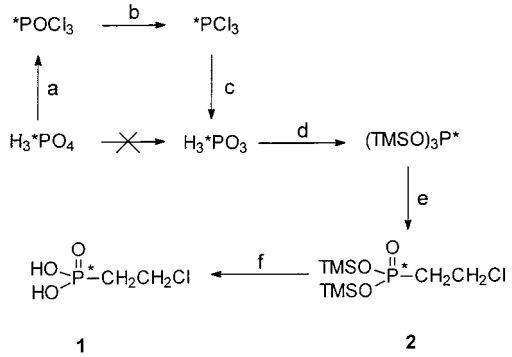
**Scheme 1**[a]




[a] Reagents and conditions: (a) $PCl_5$, −78 °C, then rt and 90 °C; (b) $PPh_3$, toluene, reflux, 4.5 h; (c) $H_2O$, −78 °C, 15 min; (d) BSTFA, $BrCH_2CH_2Cl$; (e) $BrCH_2CH_2Cl$, reflux, 2 h; (f) $H_2O$/MeOH, rt, overnight.

ethane alone gave no Arbuzov product under the same condition. In addition, the phosphonate product can be hydrolyzed completely with methanol/water in 1 h at room temperature.

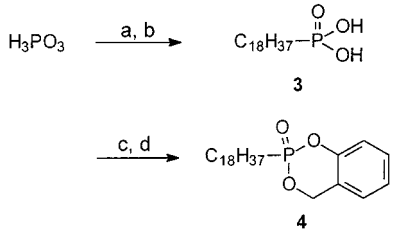
The conversion of quinquevalent $H_3PO_4$ to trivalent $P(OTMS)_3$ requires reduction, but no procedure is reported to transform phosphoric acid and its derivatives to the corresponding phosphorous acid and its derivatives. We discovered that phosphorus oxychloride ($POCl_3$) can be reduced to $PCl_3$ by triphenylphosphine ($PPh_3$) under mild conditions. However, direct reduction of $H_3PO_4$ or trialkyl phosphate with $PPh_3$ is not successful.

Straightforward transformation of $PCl_3$ to $P(OTMS)_3$ by direct reaction with either sodium trimethylsilanolate or hexamethyldisiloxane with the catalysis of a variety of Lewis acids was not satisfactory.[13] The conversion was finally achieved by an indirect approach via first hydrolyzing $PCl_3$ to phosphorous acid ($H_3PO_3$) and then converting to $P(OTMS)_3$ by *N*,*O*-bis(trimethylsilyl)acetamide or *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) under mild conditions. BSTFA provides the advantage of giving volatile byproducts easily removed under vacuum.

Thus, [$^{33}$P]-ethephon was synthesized from [$^{33}$P]-$H_3PO_4$ as shown in Scheme 1. The aqueous solution of [$^{33}$P]-$H_3PO_4$ was treated with phosphorus pentachloride ($PCl_5$), resulting in conversion not only of $H_2O$ (the solvent) to $POCl_3$ but also of [$^{33}$P]-$H_3PO_4$ to [$^{33}$P]-$POCl_3$.[14] Reaction with $PPh_3$ in toluene under reflux condition reduced [$^{33}$P]-$POCl_3$ to [$^{33}$P]-$PCl_3$ which was then hydrolyzed to [$^{33}$P]-$H_3PO_3$. Subsequent reaction of [$^{33}$P]-$H_3PO_3$ with BSTFA afforded [$^{33}$P]-$P(OTMS)_3$; using 1-bromo-2-chloroethane as the solvent, the reaction proceeded directly in situ to give phosphonate **2**. Mild hydrolysis gave essentially pure [$^{33}$P]-ethephon **1** in 70% overall chemical and radiochemical yields. The incorporation efficiency of radioactivity was nearly quantitative. Repeated reactions gave yields of 60−80%.

The conversion of [$^{33}$P]-$H_3PO_4$ to [$^{33}$P]-$P(OTMS)_3$ plays a key role in the radiosynthesis. $P(OTMS)_3$ is a very versatile reagent to form the phosphorus−carbon bond[15]

**Scheme 2**[a]




[a] Reagents and conditions: (a) BSTFA/$C_{18}H_{37}Br$, 120 °C, overnight; (b) MeOH/$H_2O$, rt, 2 h; (c) $PCl_5$/$POCl_3$, 120 °C, 2 h; (d) 2-hydroxybenzyl alcohol, Py, DMAP, THF, rt, 2 days.

and accordingly the procedure is not only applicable to the synthesis of ethephon but also to a variety of other phosphonic acids and their derivatives. This is illustrated by synthesis of 2-stearyl-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (**4**) (Scheme 2), a very potent inhibitor of fatty acid amide hydrolase.[16]

Synthesis of **4** started from $H_3PO_3$, which for radiosynthesis could be made from [$^{33}$P]-$H_3PO_4$ by the method described above. Treatment of $H_3PO_3$ with 1 equiv of stearyl bromide and excess BSTFA followed by hydrolysis with methanol and water gave stearylphosphonic acid (**3**) in nearly quantitative yield. Phosphonic acid **3** was activated with $PCl_5$ and then reacted with 2-hydroxybenzyl alcohol in the presence of pyridine and DMAP to give **4**.

In summary, a novel and efficient route has been established for the synthesis of $^{33}$P-labeled phosphonic acids and their derivatives. It involves the conversion of [$^{33}$P]-$H_3PO_4$ to [$^{33}$P]-$PCl_3$ through an unprecedented reduction of quinquevalent $POCl_3$ with $PPh_3$ and uses a facile conversion to $P(OTMS)_3$, a key intermediate to form the phosphorus−carbon bond. In the example of [$^{33}$P]-ethephon, the two-stage conversion provides high yield, high purity, and high efficiency of radioactive incorporation with little purification.

## Experimental Section

**General.** All reactions were conducted with magnetic stirring in oven-dried glassware under nitrogen or argon. Anhydrous toluene was from Aldrich Chemical Co. in a Sure/Seal bottle. THF was distilled over sodium with benzophenone as the indicator. $^1$H, $^{13}$C, and $^{31}$P NMR spectra were recorded on a Bruker AMX-300 NMR spectrometer. Chemical shifts for $^1$H NMR are relative to $CHCl_3$ ($\delta$ = 7.27 ppm), for $^{13}$C NMR are relative to $CDCl_3$ ($\delta$ = 77.0 ppm), and for $^{31}$P NMR are relative to 85% $H_3PO_4$ ($\delta$ = 0.0 ppm). All intermediates, i.e., $POCl_3$, $PCl_3$, $H_3PO_3$, $P(OTMS)_3$, and $(TMSO)_2P(O)CH_2CH_2Cl$, have been confirmed by NMR in preliminary experiments without radioactivity. [$^{33}$P]-$H_3PO_4$ was from NEN Life Science Products Inc.

**[$^{33}$P]-(2-Chloroethyl)phosphonic Acid (1).** An aqueous solution of [$^{33}$P]-$H_3PO_4$ (2.0 mCi, about 10 ng in 1.0 mL) in a 50-mL pear-shaped flask was mixed with $K_2CO_3$ (1.0 mg) and taken to dryness by rotary evaporator at 30 °C. Water (80 $\mu$L, 4.4 mmol) was added into the flask. $PCl_5$ (1.0 g granules, 4.8 mmol) was placed in a dropping funnel connected to the flask through a condenser. The flask was immersed in a dry ice−acetone bath, freezing the water. The reaction was initiated by addition of a small portion of $PCl_5$, removing the cooling bath and allowing the ice to thaw and react with $PCl_5$. This cycle was repeated until the reaction with $PCl_5$ was no longer violent before the addition of all the remaining $PCl_5$. The flask was then heated with an oil bath at 90 °C for 15 min, after which toluene (10 mL) was added and this temperature was maintained for

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another 15 min. The reaction was then cooled to room temperature, and $PPh_3$ (1.5 g, 5.7 mmol) was introduced. The solution was gently refluxed for 4.5 h and then distilled into a round-bottom flask (100 mL) cooled by a dry ice−acetone bath. Into the distillate was added a solution of water (0.25 g, 14 mmol) in THF (3.0 mL) at −78 °C. The solution was stirred at this temperature for 15 min, and then the solvents were removed by vacuum, leaving [$^{33}$P]-$H_3PO_3$ as a residue.

1-Bromo-2-chloroethane (12 mL) was added into the flask. The air was completely removed with vacuum, and argon was introduced. BSTFA (4.4 mL, 16 mmol) was then dropped in through a syringe. The resulting solution was refluxed for 2 h and then cooled. Residual reactants and some byproducts were removed by vacuum at room temperature. The remaining liquid (∼3 mL) was treated with a mixture of methanol (10 mL) and water (2 mL) and kept at room-temperature overnight. Methanol and water were removed by rotary evaporator. The residue (∼0.5 mL) was further azeotropically dried with absolute ethanol. The byproduct trifluoromethylacetamide was completely removed at 60 °C by vacuum (∼0.1 mmHg). The final product was essentially pure ethephon (**1**) (445 mg, 3.1 mmol, purity >97%) labeled with phosphorus-33. $^{31}$P ($CD_3OD$): $\delta$ +23.9 ppm. $^1$H ($CD_3OD$): $\delta$ 3.74 (q, 2H, $J$ = 8.7 Hz), 2.23 (dt, 2H, $J$ = 8.1, 18.4 Hz). The specific activity was 0.42 mCi/mmol, and the overall chemical and radiochemical yields were 70%.[17]

**2-Stearyl-4*H*-1,3,2-benzodioxaphosphorin 2-Oxide (4).** Into a mixture of $H_3PO_3$ (82 mg, 1.0 mmol) and stearyl bromide (332 mg, 1.0 mmol) under argon was added BSTFA (1.2 mL, 4.4 mmol). The reaction mixture was kept at 120 °C overnight before the introduction of a mixture of methanol (4.0 mL) and water (1.0 mL) and additional stirring at room temperature for 2 h. Removal of solvents by rotary evaporator followed by removal of byproduct trifluoromethylacetamide at 60 °C by a high-vacuum pump gave stearylphosphonic acid (**3**) (>99%). $^{31}$P ($CD_3OD$): $\delta$ +31.4 ppm. $PCl_5$ (417 mg, 2.0 mmol) was then added in $POCl_3$ (2.0 mL) as a carrier. The solution was heated at 120 °C for 2 h followed by the removal of $POCl_3$ with a vacuum pump. The residue was dissolved in anhydrous THF (10 mL) and treated with a solution of 2-hydroxybenzyl alcohol (124 mg, 1.0 mmol), pyridine (0.20 g, 2.5 mmol), and DMAP (10 mg, 0.08 mmol) in THF (5.0 mL). The reaction mixture was kept stirring at room temperature for 2 days. Salts that had formed were precipitated by addition of ether (20 mL) and filtered off. Removal of solvents followed by column chromatography with ether/hexane (10:1) gave pure **4** (94 mg, 23%). $^{31}$P ($CDCl_3$): $\delta$ +28.9 ppm. $^1$H ($CDCl_3$): $\delta$ 7.40−7.04 (m, 4H), 5.45 (dd, 1H, $J$ = 8.2, 13.8 Hz), 5.07 (dd, 1H, $J$ = 13.8, 18.5 Hz), 1.98 (dt, 2H, $J$ = 9.2, 17.4 Hz), 1.87−1.25 (m, 32H), 0.88 (t, 3H, $J$ = 6.4 Hz). $^{13}$C ($CDCl_3$): $\delta$ 150.0 (d, $J$ = 6.7 Hz), 129.9, 125.6, 123.8, 122.6 (d, $J$ = 9.0 Hz), 118.5 (d, $J$ = 6.7 Hz), 65.6 (d, $J$ = 6.7 Hz), 31.9, 30.4, 30.2, 29.7, 29.3 and 29.2 (region of several carbons), 29.0, 26.3, 24.5, 22.7, 22.1, 14.1. FAB-HRMS calcd for $C_{25}H_{44}O_3P$ ($MH^+$): 423.3030, found: 423.3026.

(17) The product was ethephon with a tracer level of phosphorus-33. The chemical yield was determined by $^1$H NMR using an internal standard and the radiochemical yield by liquid scintillation counting. The specific activity for a mixture of labeled ethephon and unlabeled ethephon did not change on recrystallization from chloroform. The high radiochemical purity was also confirmed by TLC on silica gel, $R_f$ 0.7 in *n*-butanol−acetic acid−water (2:1:1).

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