Syntheses of New 2-Hydroxythiazol-5-yl and 3-Hydroxy-1,2,4-triazol-5-ylphosphonic Acids as Potential Cyclic Spatial Mimics of Glyphosate

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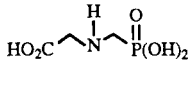
Received January 10, 1995

A low temperature lithiation/phosphorylation procedure has been successfully applied to 2-methoxythiazole and 1-substituted 3-alkoxy or 3-silyloxy-1*H*-1,2,4-triazoles to provide a series of novel hydroxy-substituted heterocyclic phosphonates and phosphonic acids as potental cyclic spatial mimics of glyphosate.

J. Heterocyclic Chem., **32**, 893 (1995).

Introduction.

Glyphosate (*N*-phosphonomethylglycine) [2,3] (**1**), the active ingredient in Roundup® herbicide, is an extremely effective and environmentally compatible, broad spectrum herbicide. The commercial success of glyphosate has stimulated the search for other phosphonate structures with improved biological properties [4]. For example, various saturated cyclic phosphonates, which link the two glyphosate methylene carbons, such as 5-phosphonoproline (**2**), have been prepared and evaluated [5], but none to date exhibits biological activity comparable to glyphosate. Consequently, we sought to identify an alternative strategy which would lead to more effective cyclic spatial mimics of glyphosate. As described in more detail below, a consideration of glyphosate's molecular mode of action in combination with molecular modeling experiments led us to design and synthesize 2-hydroxythiazol-5-ylphosphonic acid (**3**) and 3-hydroxy-1,2,4-triazol-5-ylphosphonic acid (**4**) as potential new spatial forms of glyphosate.



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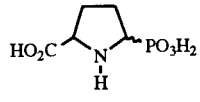

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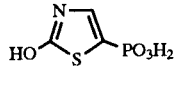

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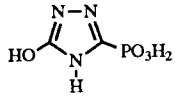

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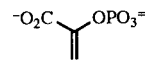
Glyphosate functions as a selective and highly specific inhibitor of the enzyme, EPSP synthase [6,7]. In all plant and most bacterial systems examined to date, glyphosate is a potent, competitive inhibitor versus one of this enzyme's natural substrates, phosphoenolpyruvate (PEP, **5**) [8]. Glyphosate exhibits a highly specific interaction with enzyme, since minor structural variations induce major changes in enzyme affinity [9,10]. Glyphosate does not simply function as a ground-state analog of PEP, since it does not inhibit any other PEP-utilizing enzyme [9]. These observations have been used to support the proposal that glyphosate functions as a transition-state analog inhibitor of the putative PEP oxonium ion (**6**) formed transiently during EPSP synthase catalysis [9,11].



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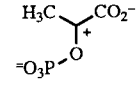


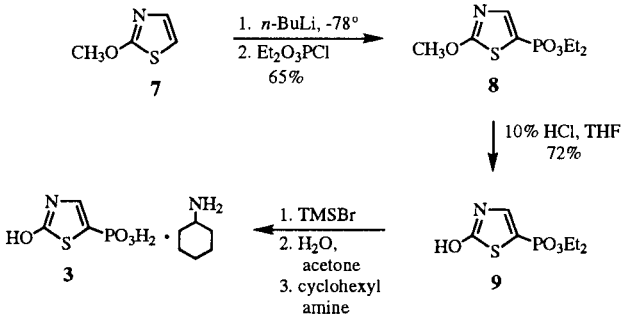
6

To properly function as a transition-state analog inhibitor, the glyphosate binding site should be virtually superimposable with the presumably planar **6**, even though it contains an extra atom between the anionic centers. The planar configuration of **6** requires that the ionic phosphonic and carboxylate functionalities of glyphosate be bound in a "pinched" conformation with each anionic group on the same side of the nitrogen atom. X-ray crystal structures of glyphosate have been reported in both "pinched" [12] and extended [13] conformations. Molecular modeling studies indicate that the "pinched" conformation of glyphosate can be superimposed on **5** without any significant energy penalty [14,15]. The flexibility of glyphosate allows it to adapt a conformation where each anionic center can be superimposed on the corresponding group in PEP, with the methylene groups oriented above and below the plane. The need for an out of plane twist of the glyphosate backbone at the methylene centers may explain why cyclic compounds such as **2** are poor inhibitors of this system. Nevertheless, a more compact, planar form of glyphosate which could closely conform to the three dimensional orientation of the PEP oxonium ion **6** could be an extremely potent inhibitor of this system. Molecular modeling experiments strongly suggested that the 2-hydroxythiazol-5-ylphosphonic acid (**3**) and 3-hydroxy-1,2,4-triazol-5-ylphosphonic acid (**4**) satisfied many of the key spatial criteria imposed by the planar configuration in **6**.

Results and Discussion.

The synthesis of **3** (Scheme I) started by reacting commercially available 2-bromothiazole with hot anhydrous sodium methoxide in methanol to give the known [16] 2-methoxythiazole derivative **7** in 70% yield. The low temperature metalation of 2-methoxythiazole can be achieved selectively at the 5-position [17] with *n*-butyllithium, and subsequent electrophilic trapping with diethyl chlorophosphate produces the desired 2-methoxythiazol-5-ylphosphonic acid diethyl ester (**8**) in 65% yield. Removal of the *O*-methyl protecting group in **8** can be accomplished either stepwise with mild acid to produce the 2-hydroxythiazol-5-ylphosphonate derivative **9**, or directly with trimethylsilyl bromide (TMSBr) to give the desired 2-hydroxythiazol-5-ylphosphonic acid **3**, which was isolated as its cyclohexylammonium salt.

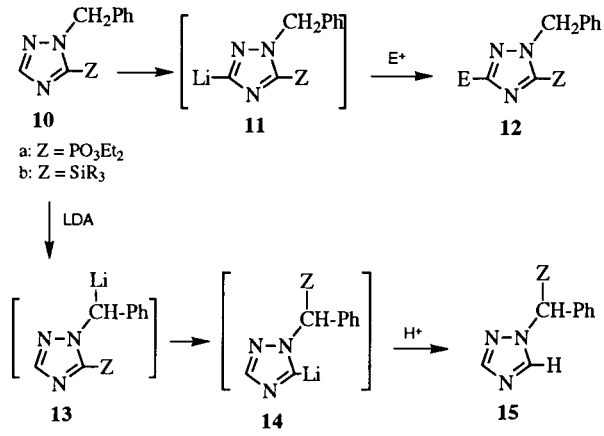
Scheme I



We originally envisioned a sequential metalation and electrophilic trapping approach (Scheme II) for the synthesis of a suitably protected precursor of 3-hydroxy-1,2,4-triazol-5-ylphosphonic acid, such as **12a**, starting from the known 1-benzyl-1,2,4-triazol-5-ylphosphonic acid diethyl ester (**10a**) [18]. However, no conditions could be identified to generate **11a** by lithiation at the triazole 3-position. Instead, the phosphonate functionality in **10a** directs metalation to the benzylic carbon leading to the lithiated intermediate **13a**, which spontaneously rearranges at low temperature to give the lithiated triazole **14a** by an unexpected carbon to carbon migration of the phosphonate group [19]. Subsequent acidification of the resulting solution produces the rearranged 1,2,4-triazol-1-ylbenzylphosphonic acid diethyl ester (**15a**). Similar rearranged products were also obtained starting from 1-benzyl-1-1,2,4-triazol-5-yl silanes **10b** [20]. Consequently, we concluded from these results that any lithiation strategy to introduce the phosphonate group would have to begin with a suitably protected hydroxyl functionality already in place on the triazole ring.

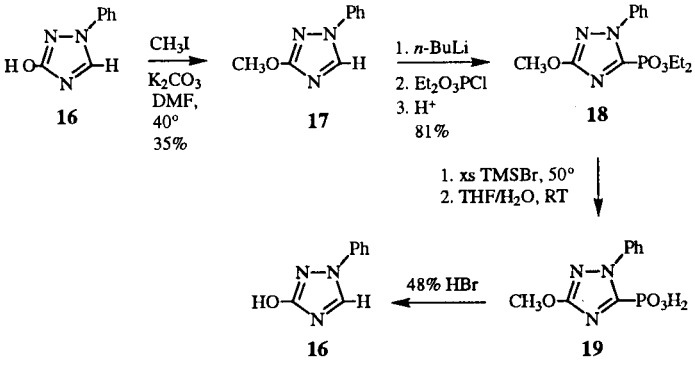
Since no lithiation studies have been reported using 3-alkoxy-substituted 1,2,4-triazoles, we prepared 1-phenyl-3-methoxy-1,2,4-triazole (**17**) to test the viability of this approach (Scheme III). The cyclization of commercially available 1-phenylsemicarbazide with a mixture of formic acid and sulfuric acid gave the known [21] 1-phenyl-3-hydroxy-1,2,4-triazole (**16**) in high yield. Subsequent methylation of **16** with dimethyl sulfate and potassium carbonate in warm dimethylformamide produced **17** in moderate yield. The low temperature lithiation of **17** with *n*-butyllithium followed by phosphorylation with diethyl chlorophosphate gave very good isolated yields of 1-phenyl-3-methoxy-1,2,4-triazol-5-ylphosphonic acid diethyl ester (**18**) after workup. Whereas trimethylsilyl bromide had efficiently cleaved the *O*-methyl ether in **8**, treatment of **18** with this reagent in excess cleanly produced 1-phenyl-3-methoxy-1,2,4-triazol-5-ylphosphonic acid (**19**). More forcing acidic reaction conditions using 48% hydrobromic acid led only to C-P bond cleavage, regenerating **16** in the process. These results indicated that the C-P bond in hydroxylated triazoles is much more susceptible to acid catalyzed cleavage than had been previously observed [18] with the simpler 3-unsubstituted analog **10a**. A hydroxyl protecting goup which could be cleaved under milder conditions was obviously needed.

Scheme II

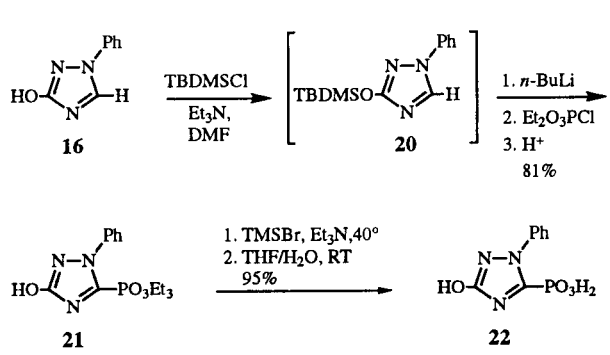


Fortunately, many of these problems could be easily circumvented by employing a *t*-butyldimethylsilyl (TBDMS) ether protecting group (Scheme IV). Reaction of **16** with *t*-butyldimethylsilyl chloride (TBDMSCl) and triethylamine generated the silyl ether **20** (^{1}H-nmr), which was immediately lithiated under nitrogen at low temperature with *n*-butyllithium and subsequently phosphorylated with diethyl chlorophosphate. In this case, quenching with mild acid simultaneously removed the silyl ether protecting group to give good isolated yields of 1-phenyl-3-hydroxy-1,2,4-triazol-5-ylphosphonic acid diethyl ester (**21**). Removal of the ethyl phosphonate esters from **21** with warm excess trimethylsilyl bromide under the usual conditions gave a reasonable yield of the

Scheme III



Scheme IV

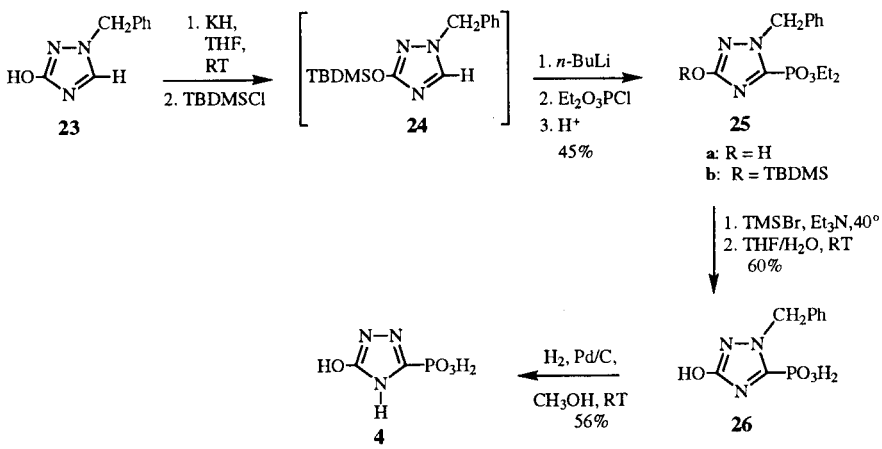


desired 1-phenyl-3-hydroxy-1,2,4-triazol-5-ylphosphonic acid (**22**), which was always accompanied by 20-30% of **16**. However, when triethylamine was added as an acid scavenger to the TMSBr cleavage reaction, nearly quantitative isolated yields of the deprotected product **22** were obtained. As expected, all attempts to remove the *N*-phenyl group from **22** led to significant decomposition.

Based on these results, the synthesis of **4** required that a more easily removable nitrogen protecting group be incorporated into this sequence. Consequently, the preparation of **4** began (Scheme V) from the known [22] 1-benzyl-3-hydroxy-1,2,4-triazole (**23**). First, the potassium salt was generated with potassium hydride, and then prolonged reaction with *t*-butyldimethylsilyl chloride (TBDMSCl) at room temperature gave nearly quantitative (^{1}H-nmr) conversion to the silyl ether **24**. Lithiation of **24** occurred under nitrogen at low temperature with *n*-butyllithium, and subsequent phosphorylation with diethyl chlorophosphate produced a mixture of phosphonates **25a,b** after workup. Desilylation with potassium fluoride and a catalytic amount of tetrabutylammonium fluoride gave the hydroxytriazole intermediate **25a** along with several impurities. Purification of **25a** was extremely difficult and could only be accomplished by a low temperature fractional recrystallization from methylene chloride, which resulted in considerable loss of material (15-20% yield). All attempts to purify **25a** by chromatography or distillation led to considerable decomposition. Consequently, the crude mixture containing both **25a** and **25b** was hydrolyzed with TMSBr and triethylamine to the desired 1-benzyl-3-hydroxy-1,2,4-triazol-5-ylphosphonic acid (**26**). In this case, 10-20% of C-P bond cleavage products were still observed despite the presence of the acid scavenger. Utilization of more hindered amines did not improve this transformation. The crude hydrolysis products were purified by cation exchange chromatography to give **26** in 25% overall yield from **23**.

The debenzylation of **26** was accomplished by catalytic hydrogenation with 10% palladium on carbon at 60 psi. The low solubility of this substrate in either methanol or water required prolonged reaction times of several days at room temperature. Best results were obtained in methanol after three days to give 3-hydroxy-1,2,4-triazol-5-ylphosphonic acid (**4**), in 56% isolated yield. The utilization of acid catalysts (aqueous hydrochloric acid) or elevated temperatures led to a significant amount of C-P bond cleavage by-products.

Scheme V



Compounds **3** and **4** were evaluated as EPSP synthase inhibitors using standard kinetic assays [23,24]. No significant enzyme inhibition was observed with either of these materials when tested at 10 m*M* concentration. Glyphosate exhibits essentially 100% inhibition of enzyme activity at low μ*M* concentrations. Consequently, these compounds are at least 1000-fold weaker than glyphosate as EPSP synthase inhibitors. These results indicate that conformationally constrained, planar analogs of glyphosate are essentially inactive as EPSP synthase inhibitors. Thus, the flexibility of the glyphosate backbone must be an extremely important feature of its enzyme inhibition properties.

EXPERIMENTAL

The ^{1}H-nmr spectra were recorded either at 60 MHz or 360 MHz, and the ^{13}C-nmr spectra were recorded at 75 MHz, using TMS as an internal standard. The ^{31}P-nmr spectra were recorded at 40.5 MHz with chemical shifts reported in ppm relative to phosphoric acid (external coaxial standard). Melting points were determined on a Mel-Temp (Laboratory Devices, Inc.) apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc. or by Galbraith Laboratories, Inc.

Anhydrous tetrahydrofuran was obtained from Aldrich (Sure-Seal® bottles). In all metalation reactions, the results were generally improved if the tetrahydrofuran was further dried in the reaction flask by the addition of *n*-butyllithium using triphenylmethane (5-10 mg) as an indicator. All other solvents were Fisher reagent grade.

2-Methoxythiazol-5-ylphosphonic Acid, Diethyl Ester (**8**).

A solution of 2-methoxythiazole **7** [16] (28.0 g, 243 mmoles) in 500 ml of anhydrous tetrahydrofuran was cooled to -78° under a dry nitrogen atmosphere and treated with *n*-butyllithium (109 ml of a 2.3 *M* solution in hexane, 250 mmoles) dropwise over a 5 minute period. The resulting yellow solution was stirred for 1 hour at -78°, then treated with diethyl chlorophosphate (43.0 g, 250 mmoles) *via* syringe. After an additional hour at -78°, the solution was allowed to slowly warm to room temperature and stir overnight. The solution was concentrated *in vacuo*, and the residue was layered between dichloromethane and water. The organic layer was washed with brine, dried through sodium sulfate and concentrated to yield 47.5 g of a yellow oil. Purification by kugelrohr distillation (109-110°/0.2 mm Hg) yielded 47.5 g (78%) of **8** as a colorless liquid, $n_D^{25} = 1.494$. ^{1}H-nmr (deuteriochloroform): 7.6 (d, J = 5 Hz, 1H), 4.1 (d of q, J = 7 Hz, J = 7 Hz, appears as pentuplet overlapped by singlet, 4H), 4.1 (s, overlapped by pentuplet, 3H), 1.3 (t, J = 7 Hz, 6H); ^{31}P-nmr (deuteriochloroform, decoupled): 9.8 (s).

Anal. Calcd. for $C_8H_{14}NO_4PS$: C, 38.25; H, 5.62; N, 5.58; S, 12.76. Found: C, 38.29; H, 5.62; N, 5.53; S, 12.66.

2-Hydroxythiazol-5-ylphosphonic Acid, Diethyl Ester (**9**).

A solution of **8** (6 g, 23.9 mmoles) in a 1:1 (v/v) mixture of tetrahydrofuran and 10% aqueous hydrochloric acid was stirred for about 72 hours at room temperature. The solution was poured into cold aqueous sodium bicarbonate (saturated), and the aqueous mixture was washed with an equal volume of diethyl ether. The aqueous layer was cooled to 0° and carefully acidified to a *p*H of 2 with concentrated hydrochloric acid. The product was then extracted from the cold aqueous mixture with dichloromethane. The organic layer was dried through sodium sulfate and concentrated *in vacuo* to yield a tan solid. Recrystallization from ether/petroleum ether yielded 4.1 g (72%) of **9** as an off-white solid, mp 80-82°. ^{1}H-nmr (deuteriochloroform): 7.4 (d, J = 9 Hz, 1H), 4.15 (d of q, J = 7 Hz, J = 7 Hz, appears as pentuplet, 4H), 1.35 (sept, J = 7 Hz, 6H); ^{31}P-nmr (deuteriochloroform, decoupled): 9.0 (s).

Anal. Calcd. for $C_7H_{12}NO_4PS$: C, 35.44; H, 5.10; N, 5.90; S, 13.52. Found: C, 35.52; H, 5.15; N, 5.87; S, 13.45.

2-Hydroxythiazol-5-ylphosphonic Acid, Cyclohexylamine Salt (**3**).

A solution of **8** (10 g, 40 mmoles) in trimethylsilyl bromide (Aldrich, 24.4 g, 159 mmoles) was warmed to 40° under nitrogen with stirring. After 3 hours at 40°, the reaction was complete by ^{31}P-nmr. The excess trimethylsilyl bromide was removed *in vacuo*, and the residue was dissolved in 10 ml of acetone and cooled to 0°. The solution was treated with 1 ml of water and stirred for 10 minutes. The mixture was then concentrated overnight at room temperature and 0.5 mm of Hg. The clear, glassy residue was dissolved in 100 ml of methanol and treated with cyclohexylamine which produced a white precipitate. The solid was filtered and recrystallized from a 1:1:1 (v/v) mixture of water:isopropyl alcohol:acetone. The solid must be brought out very slowly to obtain pure **3** as a white solid, mp 161-163° dec; ^{1}H-nmr and combustion analysis indicates that the product is a mixture of monoamine and diamine salts (average = 1.8 equivalents); ^{1}H-nmr (deuterium oxide): 6.89 (d, J = 8 Hz, 1H), 3.08 (unresolved multiplet, 1.1H), 1.96 (unresolved multiplet, 3.9H), 1.77 (unresolved multiplet, 3.9H), 1.62 (unresolved multiplet, 2.2H), 1.32 (unresolved multiplet, 7.6H), 1.12 (unresolved multiplet, 1.1H). ^{31}P-nmr (deuterium oxide, decoupled): -1.1; ^{13}C-nmr (methanol-d_4, decoupled): 129.8 (d, $J_{C\text{-}S\text{-}C\text{-}P}$ = 8 Hz), 124.6 (d, $J_{C\text{-}C\text{-}P}$ = 13 Hz), 120.8 (d, $J_{C\text{-}P}$ = 180 Hz), 51.3, 31.5, 25.4, 24.9.

Anal. Calcd. for $C_3H_4NO_4PS \cdot 1.8\ C_6H_{13}N \cdot 0.5\ H_2O$: C, 44.95; H, 7.78; N, 10.64; P, 8.40. Found: C, 44.74, H, 8.04; N, 10.21; P, 8.52.

3-Methoxy-1-phenyl-1*H*-1,2,4-triazole (**17**).

A solution of 1-phenyl-3-hydroxy-1,2,4-triazole [21] **16** (10.0 g, 62 mmoles) in dimethylformamide (200 ml) was treated with potassium carbonate (12.8 g, 93 mmoles) and dimethyl sulfate (11.7 g, 93 mmoles) and warmed to 80° under nitrogen. After 48 hours the reaction was cooled, and the mixture was concentrated *in vacuo* at 40°. The residue was dissolved in a mixture of methylene chloride and water, and the layers were separated. The organic layer was concentrated *in vacuo*, and the residue was purified by flash chromatography with ethyl acetate: cyclohexane (40:60, v/v) as eluant. This gave 6.2 g (57%) of **17** which was used without further purification. Washing the column with neat ethyl acetate gave 2.6 g of recovered **16**. The ^{1}H-nmr (deuteriochloroform) of **17** showed: 8.0 (s, 1H), 6.9-7.5 (m, 5H), 3.7 (s, 3H).

1-Phenyl-3-methoxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid, Diethyl Ester (**18**).

A solution of 3-methoxy-1-phenyl-1*H*-1,2,4-triazole **17** (6.9 g, 39 mmoles) and *N,N,N'N'*-tetramethylethylene diamine (12

ml) in 200 ml of anhydrous tetrahydrofuran was treated with *n*-butyllithium (20.5 ml of a 2.3 *M* solution in hexane, 47 mmoles) over 10 minutes at -78° under nitrogen. The resulting orange solution was stirred for 2 hours, then was treated with diethyl chlorophosphate (8.1 g, 47 mmoles) and allowed to slowly warm to room temperature and stirred overnight. The reaction was quenched with saturated aqueous ammonium chloride and concentrated *in vacuo*. The residue was taken up in methylene chloride, washed with brine and dried through sodium sulfate. Concentration yielded 12.9 g of a tan oil, which was purified *via* flash chromatography on silica gel with ethyl acetate:cyclohexane (80:20, v/v) as the eluant. This gave 9.9 g (81%) of **18** as a light yellow oil, n_D^{25} = 1.5221; ^{1}H-nmr (deuteriochloroform): 7.4-7.8 (m, 5H), 3.9-4.4 (d of q, J = 7 Hz, J = 7 Hz, appears as pentuplet, overlapped by singlet, 4H), 4.0 (s, overlapped by pentuplet, 3H), 1.2 (t, J = 7 Hz, 6H); ^{31}P-nmr (deuteriochloroform, decoupled): -0.9.

Anal. Calcd. for $C_{13}H_{18}N_3O_4P$: C, 50.16; H, 5.83; P, 9.95. Found: C, 49.95; H, 5.85; P, 9.84.

1-Phenyl-3-methoxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid (**19**).

A solution of **18** (5.6 g, 18 mmoles) in trimethylsilyl bromide (16.5 g, 108 mmoles) was heated to 50° under nitrogen with stirring. After 3 hours the reaction was cooled to room temperature, and the excess trimethylsilyl bromide was removed *in vacuo*. The residue was dissolved in tetrahydrofuran and treated with 0.5 ml of water. After an hour, the solid was filtered, washed with tetrahydrofuran, and dried *in vacuo* (0.1 mm Hg at 60°). This gave 4.3 g (98%) of **19** as a white solid, mp 217-218°; ^{1}H-nmr (dimethyl sulfoxide-d_6): 11.6 (broad s, 2H), 7.4-7.9 (m, 5H), 3.9 (s, 3H); ^{31}P-nmr (dimethyl sulfoxide-d_6, decoupled): -5.4.

Anal. Calcd. for $C_9H_{10}N_3O_4P$: C, 42.36; H, 3.95; N, 16.47; P, 12.14. Found: C, 42.40; H, 3.90; N, 16.44; P, 12.14.

1-Phenyl-3-*t*-butyldimethylsilyloxy-1*H*-1,2,4-triazole (**20**).

A suspension of **16** (40 g, 250 mmoles) in anhydrous dimethylformamide (250 ml) was treated with *t*-butyldimethylsilyl chloride (75 g, 500 mmoles) and triethylamine (50.5 g, 500 mmoles) and warmed to 80° for 48 hours. The solvent was removed *in vacuo* at 40°, and the residue was layered between methylene chloride and water. The organic layer was washed three additional times with an equal volume of water and then dried over sodium sulfate. After filtration the solution was concentrated *in vacuo* to yield an oily solid. Silanol was removed by Kugelrohr distillation (50° at 0.5 mm) leaving a residue which was >95% pure **20** based on ^{1}H-nmr (dimethyl sulfoxide-d_6): 8.6 (s, 1H), 7.1-7.5 (m, 5H), 0.7 (s, 9H), -0.1 (s, 6H). This material was used directly in the next step without further purification.

1-Phenyl-3-hydroxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid, Diethyl Ester (**21**).

A solution of 1-phenyl-3-*t*-butyldimethylsilyloxy-1*H*-1,2,4-triazole **20** (5 g, 18 mmoles) in 200 ml of anhydrous tetrahydrofuran was treated, dropwise, with *n*-butyllithium (8.3 ml of a 2.3 *M* solution in hexane, 19 mmoles) at -78° under nitrogen over 15 minutes. After 2 hours the solution was treated with diethyl chlorophosphate (3.14 g, 18 mmoles) and stirring was continued for an additional hour at -78°. The reaction was warmed to room temperature, quenched with methanol, and concentrated *in vacuo*. The residue was worked up (methylene chloride, water, brine, dried (sodium sulfate), and concentrated) to yield 4.6 g of a brown oil. Recrystallization from ether/tetrahydrofuran (slowly, over several days) gave 2.3 g (43%) of **21**, mp 118-120°. ^{1}H-nmr (dimethyl sulfoxide-d_6): 8.9 (s, 1H), 7.4-8.0 (m, 5H), 4.1 (d of q, J = 7 Hz, J = 7 Hz, appears as pentuplet, 4H), 1.2 (t, J = 7 Hz, 6H); ^{31}P-nmr (dimethyl sulfoxide-d_6, decoupled): -1.57.

Anal. Calcd. for $C_{12}H_{16}N_3O_4P$: C, 48.49; H, 5.43; P, 10.42. Found: C, 48.38; H, 5.45; P, 10.57.

1-Phenyl-3-hydroxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid (**22**).

A solution of **21** (3.7 g, 12.5 mmoles) in triethylamine (3.5 ml) was treated with trimethylsilyl bromide (20 ml, 92 mmoles) and heated to 40° under nitrogen with stirring. After 4 hours the reaction was cooled to 0° and diluted with 50 ml of anhydrous tetrahydrofuran. The precipitated triethylamine hydrobromide was removed by filtration under nitrogen, and the filtrate was concentrated *in vacuo*. The residue was redissolved in tetrahydrofuran (20 ml) and treated with 5 ml of propylene oxide. After 20 minutes the solution was concentrated *in vacuo,* and the residue was treated with tetrahydrofuran:water (50 ml of 95:5 mixture, v/v). A white precipitate formed immediately and was collected by filtration. Recrystallization from hot water and drying *in vacuo* gave 2.85 g (95%) of pure **22**, mp 205-206°; ^{1}H-nmr (dimethyl sulfoxide-d_6): 12.3 (broad s, 3H), 7.3-7.8 (m, 5H); ^{31}P-nmr (dimethyl sulfoxide-d_6, decoupled): -5.5; ^{13}C-nmr (dimethyl sulfoxide-d_6, decoupled): 165.6 (d, $J_{C\text{-}N\text{-}C\text{-}P}$ = 22 Hz), 149.5 (d, $J_{C\text{-}P}$ = 217 Hz), 138.2 (s), 132.0 (s), 128.8 (s), 124.9 (s).

Anal. Calcd. for $C_8H_8N_3O_4P$: C, 39.85; H, 3.34; N, 17.43; P, 12.84. Found: C, 39.97; H, 3.33; N, 17.46; P, 12.60.

1-Benzyl-3-hydroxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid, Diethyl Ester (**25a**).

A suspension of **23** [22] (32.4 g, 0.185 mole) in 2000 ml of anhydrous tetrahydrofuran was treated with a slurry of potassium hydride (22.9 g of 35% oil dispersion, washed free of oil with hexane) in 50 ml of tetrahydrofuran *via* syringe under nitrogen. The mixture was stirred at 25° for 8 hours then treated with a solution of *t*-butyldimethylsilyl chloride (31 g, 0.22 mole) in tetrahydrofuran (135 ml). Stirring was continued for 12 hours. The mixture was cooled to -100° and treated with *n*-butyllithium (95 ml of a 2.3 *M* solution in hexane, 0.22 mole) over a 15 minute period. The temperature remained below -95° throughout the addition. The reaction was stirred for 2 hours at -100° then treated with diethyl chlorophosphate (41.4 g, 0.24 moles) and allowed to slowly warm to -78°. After 2 hours at -78°, the reaction was warmed to room temperature and quenched with ammonium chloride. The mixture was filtered, and the filtrate was treated with potassium fluoride (26 g of dihydrate) and a catalytic amount of tetrabutylammonium fluoride. After 24 hours the mixture was concentrated *in vacuo*, and the residue was layered between ethylacetate and water. The organic layer was washed with brine and dried over sodium sulfate. Concentration yielded 28 g of a brown oil. Recrystallization from methylene chloride at -78° yielded 18 g (31%) of pure **25a**, mp 59-61°; ^{1}H-nmr (deuteriochloroform): 10.2 (broad, 1H), 7.3-7.7 (m, 5H), 5.6 (s, 2H), 4.2 (pentuplet, J = 7 Hz, 4H), 1.3 (t, J = 7 Hz, 6H): ^{31}P-NMR (deuteriochloroform, decoupled): -2.4.

Anal. Calcd. for $C_{13}H_{18}N_3O_4P$: C, 50.16; H, 5.83; N, 13.50; P, 9.95. Found: C, 50.52; H, 5.75; N, 13.61; P, 10.00.

1-Benzyl-3-hydroxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid Hemihydrate (**26**).

A mixture of **25a** (18 g, 58 mmoles) and triethylamine (11.7 g, 116 mmoles) was cooled to 0° and treated with trimethylsilyl bromide (53 g, 348 mmoles). The resulting suspension was heated to 45° and stirred for 24 hours (monitored by ^{31}P-nmr). The excess trimethylsilyl bromide was removed *in vacuo*, and the residue was dissolved in 200 ml of anhydrous tetrahydrofuran. The precipitated triethylamine hydrobromide was removed by filtration under nitrogen. The filtrate was treated with propylene oxide (3 ml) and was concentrated *in vacuo* (0.5 mm of Hg). The residue was dissolved in tetrahydrofuran (100 ml) and treated with 5 ml of water. A gummy precipitate formed and was collected. The gum was dissolved in 200 ml of 5% aqueous sodium hydroxide and was passed through a column of Dowex 50 x 8-200 cation exchange resin in 8 separate runs. This yielded 11.0 g (74%) of **26** as a white solid, mp 170°; ^{1}H-nmr (dimethyl sulfoxide-d_6): 9.2 (s, 3H), 7.3 (s, 5H), 5.5 (s, 2H); ^{31}P-nmr (dimethyl sulfoxide-d_6, decoupled): -10.6; ^{13}C-nmr (dimethyl sulfoxide-d_6, decoupled): 163.5 (d, $J_{C\text{-}N\text{-}C\text{-}P}$ = 18.5 Hz), 149.1 (d, $J_{C\text{-}P}$ = 205 Hz), 135.8, 128.4, 128.3, 127.9, 52.7.

Anal. Calcd. for $C_9H_{10}N_3O_4P{\cdot}0.5\ H_2O$: C, 40.92; H, 4.20; P, 11.72. Found: 40.98; H, 4.26; P, 11.60.

3-Hydroxy-1*H*-1,2,4-triazol-5-ylphosphonic Acid (**4**).

A mixture of **26** (5.0 g, 19.6 mmoles), and 10% palladium on carbon (1 g) in 250 ml of methanol was hydrogenated at 60 psi for 3 days. The catalyst was removed by filtration through celite under nitrogen, and the filtrate was concentrated *in vacuo*. The resulting foam was dissolved in a minimal amount of methanol, and treated with acetone until cloudy. This yielded 1.2 g of pure **4** as a white solid after sitting for several days. The mother liquor was passed through a Dowex 50 x 8-200 column to yield an additional 0.6 g of product. Total yield = 1.8 g (56%), mp 255° w/dec; ^{31}P-nmr (dimethyl sulfoxide-d_6, decoupled): -5.2; ^{13}C-nmr (methanol-d_4, decoupled): 158.6, 144.5 (d, $J_{C\text{-}P}$ = 240 Hz).

Anal. Calcd. for $C_2H_4N_3O_4P{\cdot}0.1H_2O$: C, 14.40; H, 2.54; N, 25.19; P, 18.56. Found: C, 14.60; H, 2.46; N, 24.96; P, 18.37.

Acknowledgment.

The authors are grateful to Mr. Joel Ream of Monsanto Company for completing the enzyme evaluations of these compounds.

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