Disodium Salt of 2-[(Dihydroxyphosphinyl)difluoromethyl]-propenoic Acid: An Isopolar and Isosteric Analogue of Phosphoenolpyruvate

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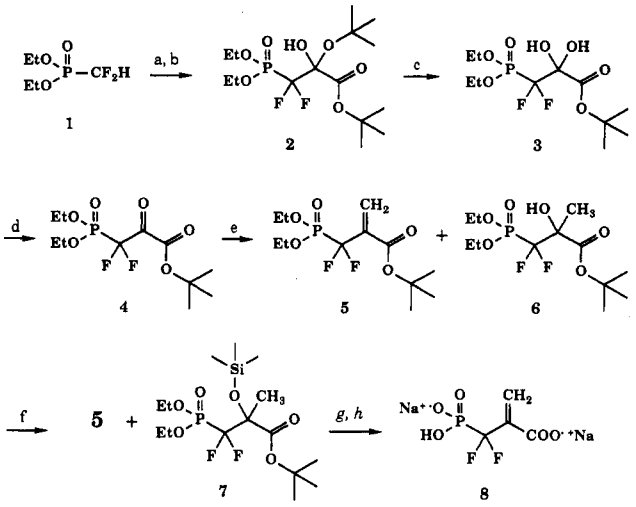
Phosphonate analogues of biological phosphate intermediates have been widely studied[1] in an effort to design useful biologically active molecules. The corresponding in vivo activity of these analogues could potentially be enhanced if the steric and electronic properties of the phosphonate more closely mimicked those of the phosphate being modeled. A number of years ago, Blackburn demonstrated that the electronic properties of pyrophosphate were more effectively mimicked by its difluoromethylene bis-phosphonic acid analogue than by methylene bis-phosphonic acid.[2] His results raised the possibility that the (difluoromethyl)phosphonate moiety could be an effective steric and electronic mimic of phosphate, possibly with applicability for designing biologically active molecules. Recently, the second pK_a values of two biochemical phosphate intermediates were found to compare favorably with the second pK_a values of their analogous (difluoromethylene)phosphonate analogues.[3] The X-ray structures of a phosphate and its (difluoromethylene)phosphonate analogue have also been found to compare favorably.[4]

This paper describes the synthesis of the difluoromethylene analogue of phosphoenolpyruvate (PEP), a ubiquitous molecule in nature. In addition to being isopolar and isosteric with PEP, this analogue was also envisioned to be a potential Michael acceptor which could bind irreversibly to an enzyme active site for which PEP is a substrate. Some initial biochemical results are also described.

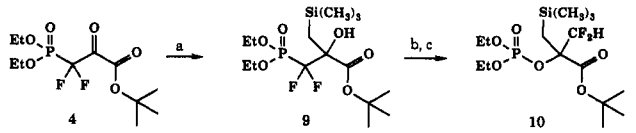
The synthesis of the disodium salt of 2-[(dihydroxyphosphinyl)difluoromethyl]propenoic acid, **8**, is shown in Scheme I. Diethyl (difluoromethyl)phosphonate,[5] **1**, was metalated and added to di-*tert*-butyl oxalate to afford exclusively the unusually stable *tert*-butyl hemiketal **2**. When a solution of this material was heated at reflux in benzene or chromatographed on silica gel, only a few percent was converted to hydrate **3**. The complete hydrolysis to **3** was effected with $NaHCO_3$ in aqueous acetonitrile, and subsequent azeotropic dehydration with benzene afforded the ketone **4**. Both of these latter reactions were easily monitored by ^{19}F-NMR. Reaction of ketone **4** with freshly prepared Tebbe reagent (μ-chloro-μ-methylenebis(cyclopentadienyl)titaniumdimethylaluminum) gave an inseparable 3:1 mixture of **5** and **6**. On reaction with *N,O*-bis(trimethysilyl)acetamide, the olefin **5** was entirely converted to its Michael adduct with acetamide. However, the chemical conversion of this mixture to **5** and **7** was successfully achieved in *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), where no reaction

Scheme I. Synthesis of the Disodium Salt of 2-[(Dihydroxyphosphinyl)difluoromethyl]propenoic Acid[a]



[a] Key: (a) LDA, ≤−70 °C; (b) di-*tert*-butyl oxalate, ≤−70 °C, then acidic workup; (c) saturated aqueous $NaHCO_3/CH_3CN$; (d) benzene azeotrope; (e) Tebbe reagent; (f) *N,O*-Bis(trimethylsilyl)-trifluoroacetamide, rt for 6 d; (g) TFA, 8 h at reflux; (h) $NaHCO_3$.

Scheme II. Attempted Peterson Olefination[a]



[a] Key: (a) $(CH_3)_3SiCH_2MgCl$, ≤−60 °C; (b) LDA, −78 °C–rt; (c) H_2O.

of the BSTFA with **5** occurred over the 6 days required to completely convert **6** to **7**. Purification of the mixture afforded **5** which was cleanly and completely deprotected to 2-(phosphonodifluoromethyl)acrylic acid by heating to reflux in trifluoroacetic acid.[6] Reaction of this material with 2 equiv of $NaHCO_3$ gave analytically pure disodium salt **8**[7] in an overall yield of 12%.

The conversion of **4** to **5** was also attempted via a traditional Wittig reaction and via a Peterson olefination sequence. Neither of these methods afforded any **5**, demonstrating the highly effective nature of the Tebbe reagent for achieving this transformation. Ketone **4** gave an uncharacterized product mixture in an attempted Wittig reaction with methylene triphenylphosphorane. As shown in Scheme II, an attempted Peterson olefination reaction with **9** afforded the rearranged phosphate **10** in excellent yield. This type of rearrangement has previously been reported only as a minor byproduct.[5]

The enzyme EPSP synthase, which catalyzes the reaction of shikimate 3-phosphate (S3P) with phosphoenolpyruvate (PEP) to produce 5-enolpyruvoylshikimate 3-phosphate as an intermediate in the biosynthesis of essential aromatic amino acids, demonstrated time-dependent inhibition by **8**.[8–10]

(1) Engel R. *Chem. Rev.* **1977**, *77*, 349.
(2) Blackburn, M. G.; England, D. A.; Kolkman, F. *J. Chem. Soc., Chem. Commun.* **1981**, 1169.
(3) Chambers, R. D.; Jaouhari, R.; O'Hagan, D. *Tetrahedron* **1989**, *45*, 5101.
(4) Chambers, R. D.; O'Hagan, D.; Lamont, R. B.; Jain, S. C. *J. Chem. Soc., Chem. Commun.* **1990**, 1053.
(5) Obayashi, M.; Ito, E.; Matsui, K.; Kondo, K. *Tetrahedron Lett.* **1982**, *23*, 2323.
(6) A neighboring carboxyl group is known to assist the hydrolysis of phosphonate esters. See: Blackburn, M. G.; Brown, M. J. *J. Am. Chem. Soc.* **1969**, *91*, 525.
(7) For biochemical studies, DEAE sephadex A-25 ion-exchange chromatography and subsequent treatment with AG 50W-X8 (Na^+) resin was also used to purify 2-phosphonodifluoromethylacrylic acid.
(8) Inhibition studies with 2-(phosphonomethyl)acrylate had no effect on the initial rate of EPSP synthase or under incubation conditions with or without S3P after 120 h at room temperature. In contrast, compound **8** in the presence of S3P irreversibly inhibited EPSP synthase 50% in 6 h with inactivation stopping at 20% activity remaining after 120 h. The activity could not be recovered after extensive dialysis.

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Experimental Section

Melting points are uncorrected. ^{19}F NMR shifts are expressed in ppm relative to CCl_3F, with upfield shifts taken as negative. ^{31}P NMR shifts are expressed in ppm relative to 85% H_3PO_4, with upfield shifts taken as negative. Elemental analyses were performed by Atlantic Microlab, Inc. Anhydrous THF was distilled from the benzophenone/sodium ketyl. All other solvents were used without drying. Organic solvents from aqueous extraction were routinely dried with $MgSO_4$. As appropriate, reactions were run under a positive pressure of nitrogen.

***tert*-Butyl 2-Hydroxy-2-(*tert*-butoxy)-3,3-difluoro-3-(diethoxyphosphinyl)propionate (2) and *tert*-Butyl 2,2-Dihydroxy-3,3-difluoro-3-(diethoxyphosphinyl)propionate (3).** A solution of *n*-BuLi in hexanes (1.55 M, 103 mL, 160 mmol) was added dropwise to an ice–water cooled solution of diisopropylamine (16.96 g, 168 mmol) in THF (20 mL). The resulting yellow solution was cooled with dry ice/acetone, and a solution of diethyl (difluoromethyl)phosphonate (30.0 g, 160 mmol) in THF (30 mL) was added dropwise, maintaining an internal reaction temperatures ≤−70 °C. This solution was cannulated into a −78 °C precooled solution of di-*tert*-butyl oxalate (35.46 g, 160 mmol) in THF (150 mL). Little exotherm occurred so the resulting solution was stirred at −78 °C for 30 min and then was quenched by the dropwise addition of AcOH (9.58 g, 160 mmol), diluted with Et_2O, thoroughly extracted with saturated aqueous $NaHCO_3$, dried, and concentrated to afford the *tert*-butyl hemiketal **2** as an oil. The conversion to the hydrate was best effected by dissolving hemiketal **2** in CH_3CN followed by reaction with saturated aqueous $NaHCO_3$. The reaction was conveniently monitored to completion by ^{19}F-NMR, usually within about 1 h. Concentration to remove most of the CH_3CN was followed by partitioning between CH_2Cl_2 and H_2O. The aqueous solution was extracted with additional CH_2Cl_2, and the combined organic extracts were dried, concentrated, and crystallized from EtOAc/hexanes to afford compound **3** (36.7 g, 60%). Data for compound **2**: ^{19}F-NMR δ 117.72 (q of d, J_{F-F} = 300.4 Hz, J_{F-P} = 98.5 Hz). Data for compound **3**: ^1H-NMR δ 5.14 (br s, 2.7 H), 4.31 (m, 4 H), 1.51 (s, 9 H), 1.36 (t, J = 10.8 Hz, 6 H); ^{13}C-NMR δ 206.11, 166.41, 116.80 (d of t, J_{C-F} = 273.6 Hz, J_{C-P} = 200.98 Hz), 93.09 (d of t, J_{C-F} = 25.89 Hz, J_{C-P} = 14.03 Hz), 83.57, 65.09, 27.49, 16.19; ^{19}F-NMR δ 124.67 (d, J_{F-P} = 96.0 Hz); ^{31}P-NMR δ 6.03 (t, J_{P-F} = 96.4 Hz).

Anal. Calcd for $C_{11}H_{19}F_2O_6P_1 + 0.7H_2O$: C, 40.18; H, 6.25. Found: C, 40.21; H, 6.39.

***tert*-Butyl 2-Oxo-3,3-difluoro-3-(diethoxyphosphinyl)propionate (4).** With periodic monitoring by ^{31}P-NMR, a solution of **3** (27.8 g, 83 mmol) in benzene was azeotroped to afford a solution of ketone **4**: ^{31}P-NMR δ 1.85 (t, J_{P-F} = 95.2 Hz).

***tert*-Butyl 2-[(Diethoxyphosphinyl)difluoromethyl]propenoate (5) and *tert*-Butyl 2-[(Trimethylsilyl)oxy]-2-methyl-3,3-difluoro-3-(diethoxyphosphinyl)propionate (7).** Most of the benzene from the solution of **4** above was removed and replaced with toluene. A solution of the Tebbe reagent (about 100 mmol)[11] in toluene (50 mL) was added dropwise, maintaining the internal reaction temperature <−90 °C with an Et_2O/N_2 cooling bath. The resulting solution was warmed to 0 °C and poured into a vigorously stirred two-phase mixture of CH_2Cl_2 and saturated aqueous $NaHCO_3$ to afford an emulsion. After filtering through Celite to remove most of the titanium and aluminum salts, the phases were separated and the aqueous phase was extracted twice with additional CH_2Cl_2. The combined organic extracts were dried, concentrated, and dissolved in a small volume of EtOAc, and more salts were precipitated by the addition of hexanes. This mixture was filtered, concentrated, and purified by preparative LC with 1:3 EtOAc/hexanes to afford 11.2 g of a yellow oil. A solution of this oil in *N,O*-bis(trimethylsilyl)trifluoromethylacetamide (46.75 mL, 176 mmol) was stirred at room temperature for 6 days and then was concentrated under vacuum and purified by preparative LC with 1:3 EtOAc/hexanes to afford **7** (4.3 g, 13%) as a colorless oil plus **5** contaminated with CF_3CONH_2. The CF_3CONH_2 was completely removed by concentrating the mixture from xylenes and afforded pure **5** (5.2 g, 20%) as a yellow oil. Data for compound **5**: ^1H-NMR δ 6.53 (br s, 1 H), 6.37 (m, 1 H), 4.29 (m, 4 H), 1.51 (s, 9 H), 1.38 (t, J = 10.2 Hz, 6 H); ^{31}P-NMR δ 5.81 (t, J_{P-F} = 109.86 Hz); ^{13}C-NMR δ 161.44, 135.58 (d of t, J_{C-F} = 21.09 Hz, J_{C-P} = 13.69 Hz), 131.13, 116.70 (d of t, J_{C-F} = 264.69 Hz, J_{C-P} = 216.36 Hz), 82.21, 64.69, 27.82, 16.21; ^{19}F-NMR δ 156.18 (d, J_{F-P} = 107.4 Hz); MS, m/e 315 ($M^+ + 1$).

Anal. Calcd for $C_{12}H_{21}F_2O_5P_1$: C, 45.86; H, 6.74. Found: C, 45.67; H, 6.69.

Data for compound **7**: ^1H-NMR δ 4.23 (m, 4 H), 1.66 (br s, 3 H), 1.61 (br s, H_2O), 1.49 (s, 9 H), 1.35 (t, J = 7.2 Hz, 6 H), 0.19 (s, 9 H); MS m/e 405 ($M^+ + 1$).

Anal. Calcd for $C_{15}H_{31}F_2O_6P_1Si_1 + 0.4\ H_2O$: C, 43.76; H, 7.79. Found: C, 43.48; H, 7.39.

2-[(Dihydroxyphosphinyl)difluoromethyl]propenoate, Disodium Salt (8). A solution of **5** (2.3007 g, 7.327 mmol) in trifluoroacetic acid (100 g) was refluxed for 8 h and then stirred at room temperature overnight. Most of the TFA was removed under vacuum, and the resulting mixture was partitioned between CH_2Cl_2 and H_2O. The aqueous phase was extracted with additional CH_2Cl_2 (2x) and then was filtered through sintered glass and concentrated to a yellow oil. The residual TFA was completely removed by lyophilization as evidenced by ^{19}F-NMR and then was dissolved in a small volume of H_2O and treated with $NaHCO_3$ (1.231 g, 14.65 mmol). Lyophilization of this solution afforded a quantitative yield of **8** as a hygroscopic, yellow solid: ^1H-NMR δ 6.15 (br s, 1 H), 6.04 (br s, 1 H); ^{31}P-NMR δ 4.72 (t, J_{P-F} = 85.4 Hz); ^{13}C-NMR δ 176.28, 144.04 (d of t, J_{C-F} = 22.6 Hz, J_{C-P} = 12.3 Hz), 128.05 (t, J = 9.0 Hz), 123.47 (d of t, J_{C-F} = 256.2 Hz, J_{C-P} = 181.8 Hz); negative FAB MS m/e 223 ($M^+ - Na^+$).

Anal. Calcd for $C_4H_3F_2Na_2O_5P_1 + 1.5H_2O$: C, 17.60; H, 2.22. Found: C, 17.89; H, 2.59.

***tert*-Butyl 2-[(Diethoxyphosphinyl)difluoromethyl]-2-hydroxy-3-(trimethylsilyl)propionate (9).** A solution of $(CH_3)_3SiCH_2MgCl$ (31.7 mmol) in THF (15 mL) was added dropwise to a dry ice/acetone cooled solution of the ketone **4** (10.0 g, 31.6 mmol) in THF (40 mL), maintaining the internal reaction temperature <−60 °C. The resulting mixture was quenched by the dropwise addition of AcOH (2.0 mL, 34.9 mmol) in THF (5 mL) at −78 °C and then was diluted with Et_2O and thoroughly extracted with saturated aqueous $NaHCO_3$, concentrated, and purified by preparative LC with 3:17 EtOAc/hexanes to afford 8.25 g (65%) of **9** as a white solid: mp 52–54 °C; ^1H-NMR δ 4.28 (m, 4 H), 3.91 (br s, 1 H), 1.51 (s, 9 H), 1.37 (m, 8 H), −0.04 (s, 9 H); ^{31}P-NMR δ 5.88 (t, J_{P-F} = 101.3 Hz); CI MS m/e 405 ($M^+ + 1$).

Anal. Calcd for $C_{15}H_{31}F_2O_6P_1Si_1$: C, 44.54; H, 7.73. Found: C, 44.54; H, 7.69.

Diethyl 1-(*tert*-Butoxycarbonyl)-1-(difluoromethyl)-2-(trimethylsilyl)ethyl Phosphate (10). A solution of *n*-BuLi in hexanes (1.55 M, 6.7 mL, 10.4 mmol) was added dropwise to an ice–water cooled solution of diisopropylamine (1.5 mL, 10.7 mmol) in THF (5 mL). The resulting yellow solution was cooled to −78 °C while a solution of **9** (4.0 g, 9.9 mmol) in THF (5 mL) was added dropwise. After the solution was stirred at room temperature overnight, the ^{31}P-NMR showed no phosphorus rearrangement, possibly due to a strong intramolecular lithium coordination complex. A few milliliters of H_2O was added to break up this complex, and within 10 min complete rearrangement occurred. The reaction mixture was diluted with Et_2O, thoroughly extracted with 10% aqueous HCl (2x) followed by extraction with saturated aqueous $NaHCO_3$ (2x), dried, and concentrated to afford 3.67 g of an oil and then purified by preparative LC with 3:17 EtOAc/hexanes to afford 3.48 g (87%) of **10** as a colorless oil: ^1H-NMR δ 6.25 (AB quartet, J = 57.5 Hz, J = 57.5 Hz), 4.13 (m, 4 H), 1.51 (s, 9 H), 1.33 (m, 6 H), 0.11 (s, 9 H); ^{31}P-NMR: δ 5.45 (s); CI MS m/e 377 ($M^+ + 1$).

Anal. Calcd for $C_{15}H_{31}F_2O_6P_1Si_1$: C, 44.54; H, 7.73. Found: C, 44.70; H, 7.71.

(9) For a study of the competitive binding of other PEP analogues with EPSP synthase, see: Walker, M. C.; Ream, J. E.; Sammons, R. D.; Logusch, E. W.; O'Leary, M. H.; Somerville, R. L.; Sikorski, J. A. *Bioorg. Med. Chem. Lett.* **1992**, *1*, 683.

(10) We gratefully acknowledge Dr. Robert D. Sammons for biochemical measurements.

(11) This material was prepared by carefully adding a 2 M solution of trimethylaluminum in toluene (100 mL, 200 mmol) to an ice–water cooled slurry of titanocene dichloride (25 g, 100 mmol) in toluene (30 mL). The resulting red solution was reacted at room temperature for 4 days and then was concentrated under vacuum to a dark red solid which was used immediately.