Synthesis of (1, 2-$^{13}C_2$) 2-Phosphoglycolic acid.

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SUMMARY

The synthesis of (1,2-$^{13}C_2$) 2-phosphoglycolic acid, **5**, from commercially-available (1,2-$^{13}C_2$) bromoacetic acid is described along with some of its solution NMR characteristics.

Key Words: (1,2-$^{13}C_2$) Glycolic acid; (1,2-$^{13}C_2$) 2-phosphoglycolic acid; synthesis; triosephosphate isomerase; NMR; spin-coupling.

INTRODUCTION

2-Phosphoglycolic acid is a transition-state analogue inhibitor ($K_i = 7\mu m$) of the enzyme triosephosphate isomerase (TIM)[1]. This glycolytic enzyme catalyzes the reversible conversion of dihydroxyacetone phosphate (DHAP) to D-glycleraldehyde-3-phosphate (GAP). We are probing the stereochemical question of the phosphate orientation of substrates in the active site of TIM with solution and solid-state NMR methods. It has been proposed[2] that orbital interactions favoring the conversion of DHAP to GAP and disfavoring phosphate elimination are present when the dihedral angle between the phosphate and the adjacent carbon-oxygen bond is close to zero. Since three-bond, vicinal, spin couplings, which can be related to dihedral angles via Karplus relationships, are not available in **5**, we are attempting to use one-bond, ^{13}C-^{13}C couplings [3] to assess the bound geometry using solid-state NMR methods.[4] This application required doubly, ^{13}C-labelled **5**, whose synthesis is described in this report.

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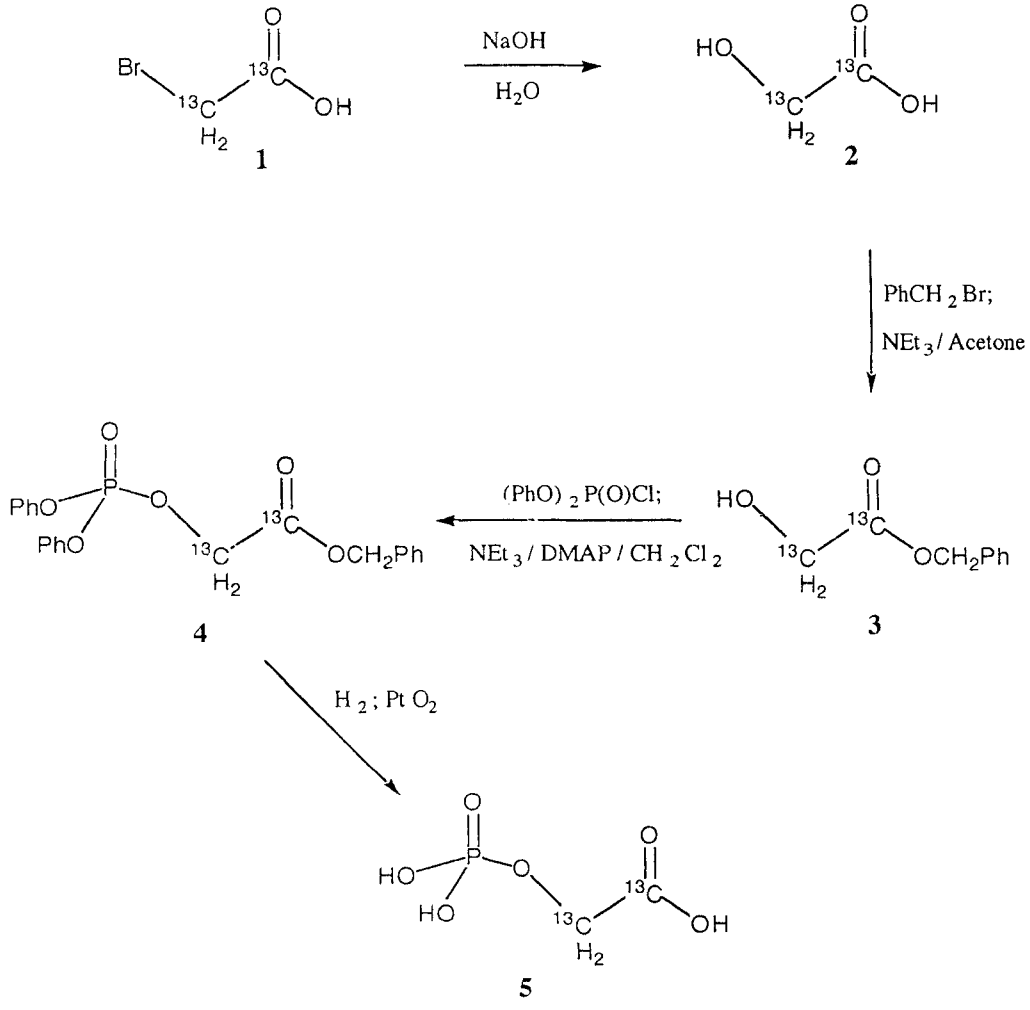
RESULTS AND DISCUSSION

The synthesis of (1,2-$^{13}C_2$) 2-phosphoglycolic acid, **5**. (Scheme 1) began with the hydrolysis of the commercially-available (1,2-$^{13}C_2$) bromoacetic acid, **1**, with excess sodium hydroxide at 50°C to give the (1,2-$^{13}C_2$) glycolic acid, **2**, in quantitative yield after work-up[5]. Compound **2** was esterified[6] with benzyl bromide, under reflux conditions in acetone with triethylamine as the base, affording **3**. Further reaction of compound **3** with 1.1 equivalents of diphenylchlorophosphate in the presence of a catalytic amount of N,N-dimethylaminopyridine,



Scheme 1.

and two equivalents of triethylamine yielded, after aqueous work-up and flash chromatography on silica gel, phosphate triester, **4** (63% yield)[7]. Hydrogenolysis of **4** over platinum oxide afforded the desired compound, **5**, in 78% yield (13% overall yield from **1**) and >99% isotopic purity as determined by NMR and mass spectra.

It should be noted that our attempts to effect the direct displacement of the bromine in **1** with aqueous sodium phosphate were unsuccessful. The lengthier route, described here, provided us, however, with synthetic intermediates which could be easily purified by normal-phase silica-gel chromatography and, ultimately , provided better control of final product purity.

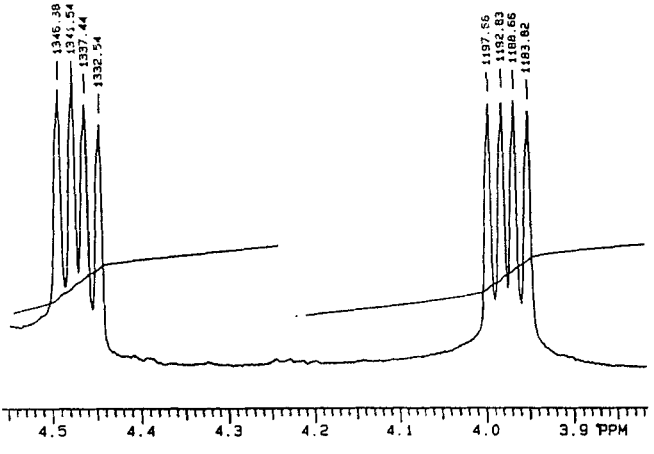


Fig. 1. The ^{1}H NMR spectrum of (1,2-$^{13}C_2$) 2-phosphoglycolic acid, **5** , shows the expected splitting from carbon and phosphorus coupling (in D_2O, 300 MHz): δ 4.22 (ddd, $J_{C\text{-}H}$ =148.7, 4.8 Hz, $J_{H\text{-}P}$ = 8.9 Hz, 2H).

Figure 1 illustrates the ^{1}H NMR spectrum of **5** in D_2O. Since the methylene protons are magnetically equivalent, the observed signal multiplicity may be attributed to the presence of $^3J_{HCOP}$, $^1J_{CH}$, and $^2J_{CCH}$, producing a ddd pattern. The ^{13}C-^{13}C coupling constants for **2** and **5** , in aqueous solution, decrease as the pH is raised from 1 to 6.5. This behavior may be attributed to the deprotonation of the carboxyl group, which could affect the solution conformation of the compound and/or the s-character of the C-C bond [3].

EXPERIMENTAL

Materials and instrumentation. (1,2-$^{13}C_2$) Bromoacetic acid, diphenylphosphochloridate, triethylamine, and 4-dimethylaminopyridine were purchased from Aldrich Chemical Co. and used without further purification. Solvents and additional reagents were used as purchased from the supplier. NMR spectra were obtained on Varian VXR-300 or Bruker AM-300 spectrometers. Mass Spectra were obtained on a Finnigan 4600 (chemical ionization) and on a TSQ 70 (negative ion FAB).

(1,2-$^{13}C_2$) Glycolic acid (2).

(1,2-$^{13}C_2$) Bromoacetic acid (**1**) (0.170g, 1.22 mmol) was mixed with 5ml of 3N NaOH and stirred at 50°C for 3 hours. After acidification to pH 1 with 1N HCl, the solution was extracted with ethyl acetate and the solvent removed from the extract to dryness under vacuum to obtain yellow crystals (0.1g, quantitative yield). ^{1}H NMR (300 MHz, D_2O) δ 4.17 (J = 144.8 , 4.44 Hz). ^{13}C NMR (75 MHz, D_2O): δ 62.2 (d, J_{C-C} = 58.8 Hz at pH 1; 54.9 Hz at pH 6.5), 178.9 (d, J_{C-C} = 58.8 Hz at pH 1; 54.9 Hz at pH 6.5).

(1,2-$^{13}C_2$) Glycolic acid, phenylmethyl ester (3).

To a solution of (**2**) (0.200 g, 2.56 mmol) in acetone (10 mL), were added benzylbromide (0.479 g, 2.80 mmol) and triethylamine (0.408 mL, 2.93 mmol). The reaction mixture was heated at reflux under nitrogen for 24 h then allowed to cool to room temperature and filtered. The solvent was removed from the filtrate by rotary evaporation affording a yellow, oily solid. The crude product was taken up in ethyl acetate (20 mL) and washed with water (5 mL X 2). The layers were separated and the aqueous phase extracted with ethyl acetate (10 mL X 3). The combined, organic phase was dried over anhydrous $MgSO_4$, filtered, and the solvent removed by rotary evaporation to give a light, yellow oil (0.221g). The product was further purified by flash-column chromatography (hexanes:ethyl acetate; 75:25 v/v)to obtain (**3**) (0.154g, 40% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.36 (m, 5H), 5.25 (d, J = 3.2 Hz, 2H), 4.20 (dd, J_{C-H} =146 Hz, J_{H-H} = 4.9 Hz, 2H), 2.46 (br. s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.2 (d, J_{C-C} = 59 Hz), 128.7, 128.6, 128.5, 67.3, 60.7 (d, J_{C-C} = 59 Hz). MS (CI, NH_3): *m/z* 169 ($M{\cdot}H^+$), 186 ($M{\cdot}NH_4^+$).

(1,2-$^{13}C_2$) 2- Phosphoglycolic acid, phenylmethyl ester(4).

A solution of (**3**) (0.154 g, 0.92 mmol), 4-dimethylaminopyridine (0.035 g, 0.290 mmol) and triethylamine (250 μL, 1.89 mmol) in methylene chloride (3 mL), was prepared and cooled to 0°C under nitrogen. Diphenylphosphochloridate (0.22 mL; 1.06 mmol) in methylene chloride (1 mL) was added dropwise and stirring continued under these conditions for one hour. The solution was concentrated by rotary evaporation and the resulting yellow, oily solid was taken up in methyl-t-butyl ether. The ethereal solution was washed with water (3 mL), followed by aqueous HCl (1N; 3 mL) and then saturated, aqueous $NaHCO_3$ (3 mL). The ethereal layer was dried over anhydrous $MgSO_4$ and concentrated to give a yellow oil which was further purified by flash column chromatography on silica gel (hexanes:ethyl acetate; 90:10 to 75:25) to obtain (**4**) (0.23 g, 63% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34-7.16 (m, 15H), 5.21 (d, J = 3.1 Hz, 2H), 4.77 (ddd, J_{C-H} =151 Hz, J_{H-P} = 10.9 Hz, J_{H-H} = 4.93 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 167.0 (dd, J_{C-C} = 66.4 Hz, J_{C-P} = 7.1 Hz), 129.9, 128.8, 128.6, 125.6, 120.3 120.2, 67.5, 64.5 (dd, J_{C-C} = 66.4 Hz, J_{C-P} = 5.3 Hz). ^{31}P NMR ($CDCl_3$, 121 MHz) δ -11.6 (J_{C-P} = 5.7, 5.4 Hz). MS (CI, NH_3): *m/z* 401 ($M{\cdot}H^+$), 418 ($M{\cdot}NH_4^+$).

(1,2-$^{13}C_2$) 2-Phosphoglycolic acid, (5).

To a stirring solution of platinum oxide (0.100 g) in ethyl acetate (10 ml) under a hydrogen atmosphere, was added an ethyl acetate solution of (**4**) (0.130 g in 1 ml). The heterogeneous mixture was stirred at room temperature for 22 hours and then filtered through Whatman 1 filter paper. The recovered metal was washed with additional ethyl acetate and the combined organic phase was concentrated by rotary evaporation affording a waxy, yellow-white solid. The solid was triturated with methylene chloride (3mL) and the remaining solvent removed under oil-pump vacuum to afford phosphoglycolic acid (**5**) (0.040 g, 78%). ^{1}H NMR (D_2O, 300 MHz): δ 4.22 (ddd, J_{C-H} = 148.7, 4.8 Hz, J_{H-P} = 8.9 Hz, 2H). ^{13}C NMR (D_2O, 75 MHz): δ 176 (dd, J_{C-C} = 61.9 Hz at pH1, 57.4 Hz at pH 6.5; 57.8 Hz at pH 8.8, J_{C-P} = 8.1 Hz at pH 1; 9.8 Hz at pH 6.5; 10.3 Hz at pH 8.8), 64.5 (dd, J_{C-C} = 61.9 Hz at pH1, 57.4 Hz at pH 6.5; 57.8 Hz at pH 8.8, J_{C-P} = 4.8 Hz at pH 1; 5.0 Hz at pH 6.5; 4.6 Hz at pH 8.8). ^{31}P NMR (121 MHz, D_2O) δ 2.05 (J couplings were not well resolved). MS (-ve FAB): *m/z* 157 ($M-H^-$), 179 ($M+Na-2H^-$).

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