

# Efficient Syntheses of Multiply $^2\text{H}$ - and $^{13}\text{C}$ -Labeled Acrylic Acid, Glyceric Acid, Glycidic Acid and Glycerol

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## SUMMARY

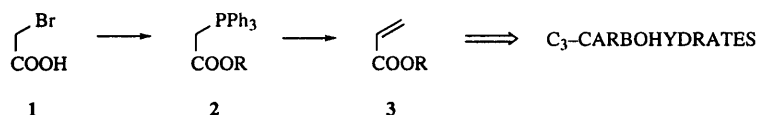
Syntheses of multiply  $^2\text{H}$ - and  $^{13}\text{C}$ -labeled C-3 carbohydrates have been carried out from commercially available  $[1,2-^{13}\text{C}_2]$ bromoacetic acid. The syntheses are illustrated for  $[1,2-^{13}\text{C}_2]$ acrylate,  $[2,3,3-^2\text{H}_3, 1,2-^{13}\text{C}_2]$ acrylate,  $[1,2-^{13}\text{C}_2]$ glycerate,  $[2,3,3-^2\text{H}_3, 1,2-^{13}\text{C}_2]$ glycerate,  $[1,2-^{13}\text{C}_2]$ glycidate and  $[1,1-^2\text{H}_2, 1,2-^{13}\text{C}_2]$ glycerol..

**Key words:** paired isotope ( $^2\text{H}, ^{13}\text{C}$ ) synthesis, acrylic acid, glyceric acid, glycidic acid, glycerol

## INTRODUCTION

The use of paired, stable isotopes to monitor bond-breaking, rearrangement and bond-forming processes by NMR spectroscopy has become a fundamental tool of natural product biosynthetic studies. Particularly useful have been fundamental precursors as, for example,  $[1,2-^{13}\text{C}_2]$ acetate,  $[1-^{13}\text{C}, ^{18}\text{O}_2]$ acetate,  $[2,2,2-^2\text{H}_3, 1-^{13}\text{C}]$ acetate,  $[\text{U}-^{13}\text{C}]$ glycerol or  $[\text{U}-^{13}\text{C}]$ glucose (1). The appearance of paired isotopes in a metabolic intermediate or end product can be detected by the observation of spin-spin coupling or  $\alpha$ - and  $\beta$ -isotopic shifts typically of  $^{13}\text{C}$ -resonances. The sensitivity of these measurements can be an order of magnitude or greater than enhancements of signal intensity (through biochemical incorporation of a singly-labeled precursor) at natural abundance. Moreover, relatively NMR-insensitive nuclei as  $^2\text{H}$  or  $^{15}\text{N}$  can be detected by their interaction with a more NMR-sensitive nucleus as, for example,  $^{13}\text{C}$ . Thus, in a paired isotope experiment the experimentally more readily observed nucleus can be used to monitor the nearby presence or absence of a less readily detected nucleus of biochemical interest.

Scheme 1

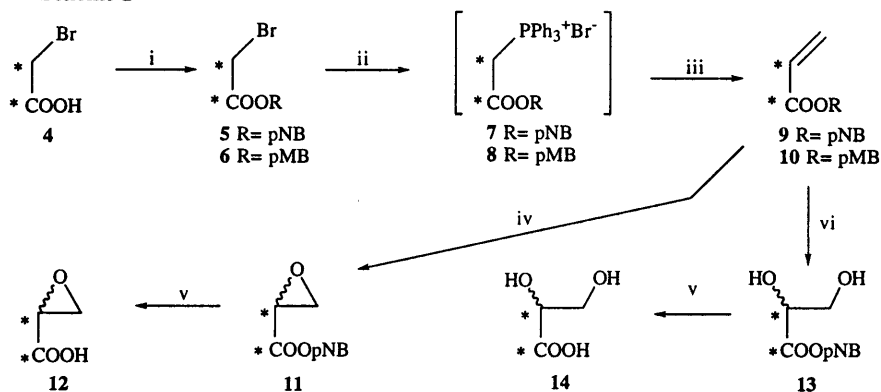


In this paper we describe an efficient, general synthesis of [1,2- $^{13}\text{C}_2$ ]acylate, [1,2- $^{13}\text{C}_2$ ]glycerate, [1,2- $^{13}\text{C}_2$ ]glycidate and [1,2- $^{13}\text{C}_2$ ]glycerol, as well as routes to these fundamental  $\text{C}_3$ -carbohydrates specifically deuterium labeled at high extents of incorporation. The syntheses begin with commercially available [1,2- $^{13}\text{C}_2$ ]bromoacetic acid (**1**) and take advantage of both the stability, and the facile hydrogen exchange properties of the corresponding triphenylphosphonium bromide (**2**) to allow rapid access to acrylate esters **3** under aqueous conditions (Scheme 1). The synthetic usefulness of this reactive ester is readily apparent, but is illustrated here in the preparation of multiply  $^2\text{H}$ ,  $^{13}\text{C}$ -labeled glycerates, glycidates and glycerol, biochemical probes and synthetic intermediates of broad, general use.

## RESULTS AND DISCUSSION

[1,2- $^{13}\text{C}_2$ ]Bromoacetic acid (**4**) was protected as its crystalline p-nitrobenzyl (**5**) ( $J_{\text{C-C}} = 65.6 \text{ Hz}$ ) and oily p-methoxybenzyl esters (**6**) in high yield using either DCC/DMAP or EDC/DMAP (Scheme 2). Arbuzov reaction with triphenylphosphine provided ylides **7** and **8**. Use of sodium iodide as a co-reagent was found to be useful in reducing the reaction time. However, the intermediate iodide formed was unstable and prone to unwanted side reactions. The phosphonium salts **7** and **8** were not isolated

Scheme 2



**Reagents and conditions:** i) pNB-OH or pMB-OH, EDC, DMAP,  $\text{CH}_2\text{Cl}_2$ , r.t., 3 h; ii)  $\text{PPh}_3$ ,  $\text{CH}_2\text{Cl}_2$ , r.t., 24 h; iii) aq. 37%  $\text{CH}_2\text{O}$ ,  $\text{CH}_2\text{Cl}_2$ , 10% aq.  $\text{NaHCO}_3$ , 24 h, ~ 90% (for 3 steps); iv) aq.  $\text{NaOCl}$ , DMF, r.t., 48 h, 48%; v) 1 atm.  $\text{H}_2$ , 10% Pd-C, THF,  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , then RP HPLC, ~ 100% for **12**, 70 % for **14**; vi)  $t\text{BuOOH}$ , cat.  $\text{OsO}_4$ ,  $\text{Et}_4\text{NOAc}$ , acetone, 0 °C to r.t., 6 h, 66%.

but were used immediately for Wittig reaction with 37% aqueous formaldehyde. Acrylates **9** ( $J_{C-C} = 73.5$  Hz) and **10** were obtained in >90 % overall yield. The most convenient way to obtain the acrylate (**9**) was a one-pot approach where all the reactants of the Arbuzov and Wittig reactions were stirred at room temperature for *ca.* 36 h. Prolonged reaction times and the use of high concentrations of reagents led to side reactions, including formation of the corresponding acetates rather than acrylates.

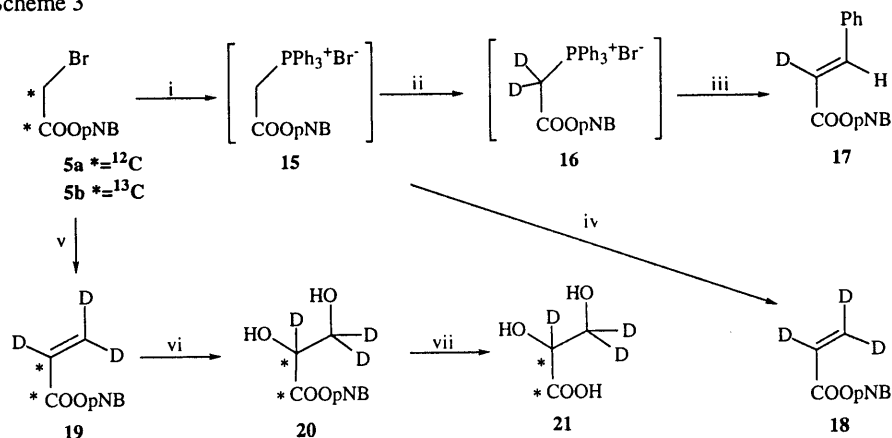
Epoxidation of the acrylate ester proved to be difficult. Several oxidizing agents reportedly useful for epoxidation of electron-deficient alkenes were examined [*e.g.* mCPBA/reflux (**2**), UHP/Ac<sub>2</sub>O (**3**), UHP/maleic anhydride (**4**), H<sub>2</sub>O<sub>2</sub>/KOH (**5**), H<sub>2</sub>O<sub>2</sub>/NaWO<sub>4</sub> (**6**) dimethyldioxirane (**7**), *t*-BuOOH/*n*-BuLi (**8**), NaOCl/THF (**9**)] with only limited success. However, sodium hypochlorite in DMF (**10**) provided the desired glycidate ester (**11**) ( $J_{C-C} = 78.6$  Hz) in moderate yield. The relatively low yield was due principally to benzylic oxidation and subsequent cleavage of the ester moiety. The *p*-nitrobenzyl ester was found to be more stable under these conditions compared to the *p*-methoxybenzyl. The ester of **11** was cleaved by hydrogenolysis and [1,2-<sup>13</sup>C<sub>2</sub>]-*D,L*-glycidic acid (**12**) was obtained in essentially quantitative yield.

To obtain the corresponding labeled glyceric acid, acrylate **9** was subjected to Sharpless dihydroxylation (**11**) to provide **13** ( $J_{C-C} = 56.9$  Hz) in good yield. Hydrogenolysis of the ester functionality proceeded smoothly and RP HPLC purification gave [1,2-<sup>13</sup>C<sub>2</sub>]-*D,L*-glyceric acid (**14**) ( $J_{C-C} = 55.5$  Hz) in 70% yield.

In a model experiment *p*-nitrobenzyl bromoacetate (**5a**, Scheme 3) was dissolved in toluene at room temperature. Triphenylphosphine was added and the reaction mixture was stirred for a day. A fine white solid **15** precipitated in virtually quantitative yield. The phosphonium salt was dissolved in a mixture of D<sub>2</sub>O (99.9% D) and dry THF (5:1) at 50 °C under an argon atmosphere (**12**, **13**). The solution was filtered and treated over a second day under an argon atmosphere with benzaldehyde in deuteriochloroform in the presence of anhydrous potassium carbonate. Work-up and column chromatography provided *p*-nitrobenzyl [2-<sup>2</sup>H]cinnamate (**17**) in excellent yield. The level of deuterium incorporation was determined by proton NMR and mass spectrometry to be >92%.

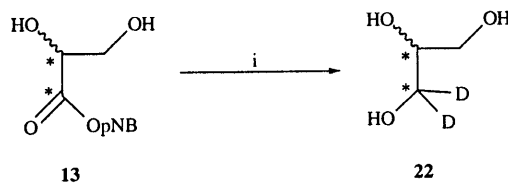
In order to avoid loss of deuterium at C-2 in the envisioned synthesis of labeled phosphonium salt **16** in aqueous solution, commercially available D<sub>2</sub>CO (98% D<sub>2</sub>) in D<sub>2</sub>O was used for the Wittig reaction. In a trial experiment **15** in THF was treated under an argon atmosphere with D<sub>2</sub>CO in D<sub>2</sub>O in the presence of anhydrous potassium carbonate. After stirring overnight, the reaction mixture was worked up and the product (**18**) was subjected to NMR and MS analysis. We were pleased to

Scheme 3



observe that the  $^1\text{H}$ -NMR spectrum of the product lacked all the olefinic resonances indicating a very high deuterium content at all three vinyl positions. These findings were corroborated by mass spectrometry (98%  $\text{D}_3$ , 2%  $\text{D}_2$ ). These results meant that *deuterium exchange at C-2 must have been significantly faster than Wittig reaction*. Encouraged by the fast relative rate of deuterium exchange and the stability of the ylide to aqueous conditions, it was decided to use a one-pot procedure to eliminate isolation/purification steps and increase the overall efficiency of the synthesis. Thus, p-nitrobenzyl [1,2- $^{13}\text{C}_2$ ]bromoacetate (**5b**) was dissolved in dry tetrahydrofuran and methylene chloride and treated with triphenylphosphine under an argon atmosphere. After stirring overnight,  $\text{D}_2\text{O}$  was added and stirring was continued for an additional 24 h. Then  $\text{D}_2\text{CO}$  in  $\text{D}_2\text{O}$  and anhydrous  $\text{K}_2\text{CO}_3$  were added and stirring was continued for yet another 24 h. Aqueous work-up and column chromatography yielded **19** ( $J_{\text{C-C}} = 72.5$  Hz,  $J_{\text{C2-D}} = 25.8$  Hz) in 80 % yield. The levels of  $^2\text{H}$  at C-2 and C-3 were found to be >98%/site.

Scheme 4



Reagents and conditions: i) 2 eq.  $\text{NaBD}_4$ , THF, 0.1 M phosphate buffer, pH 7.0, r.t., 1.5 h, 76%

Sharpless dihydroxylation of **19** provided glyceric ester **20** ( $J_{C-C} = 58.7$  Hz,  $J_{C2-D} = 21.8$  Hz) in very good yield. Catalytic hydrogenation and subsequent RP HPLC purification provided free acid **21** ( $J_{C-C} = 54.2$  Hz,  $J_{C2-D} = 22.1$  Hz) in nearly 60% overall yield from [1,2- $^{13}C_2$ ]bromoacetate.

Glyceric ester **13** was conveniently reduced to the corresponding multiply labeled glycerol (**22**) ( $J_{C-C} = 41.2$  Hz) (14, 15) in 76% yield using excess sodium borodeuteride (98% D) in THF-phosphate buffer. Use of ethanol, generally the solvent of choice for such reductions, led to the intermediate formation of the ethyl ester, longer reaction times, and reduced the overall yield for **22**. Careful integration of the  $\beta$ -shifted resonances (1) for C-2 revealed that **22** contained >92% dideuterated and <8% monodeuterated species. No undeuterated material was detectable.

## EXPERIMENTAL

The  $^1H$ - and  $^{13}C$ -NMR spectra were recorded on a Varian Unity<sup>plus</sup> 400 MHz spectrometer. Deuteriochloroform containing 0.03% (for  $^1H$ -NMR) and 1% TMS (for  $^{13}C$ -NMR), deuterium oxide ( $D_2O$ ) containing 0.05% (for  $^1H$ -NMR) and 0.75% 3-(trimethylsilyl)-propionic-2,2,3,3- $d_4$  acid sodium salt (for  $^{13}C$ -NMR), and methylsulfoxide- $d_6$  containing 1% TMS were used as NMR solvents. The spectra were referenced to TMS and TMSPA (0 ppm). IR spectra were obtained on a Perkin Elmer 1600 series FT-spectrophotometer as thin films between NaCl discs. Mass spectral analyses were obtained on a VG Instruments 70-S GC/MS spectrometer using EI, EI/CI, CI, and FAB techniques. For TLC Analtech Uniplate Silica gel GHLF 250  $\mu m$  precoated glass plates were used (UV light, ammonium molybdate/heat visualization). For flash chromatography E. Merck silica gel 60 adsorbent (particle size 0.040-0.063 mm, 230-400 mesh) was used. In general 100 x (v/w) of silical gel was used based on the crude weight of reaction product to be separated. For reverse phase (RP) HPLC a Waters 600 multisolvent delivery system was used equipped with a Waters 490 programmable multi-wavelength detector (210 nm) and a Whatman C-18 Partisil-10 ODS-1 or ODS-3 column. Spectral characterizations described below are for unlabeled materials prepared in a manner identical to the entitled labeled compounds. Key spectral observations for labeled synthetic intermediates and final products are given in the text.

### *p*-Nitrobenzyl [1,2- $^{13}C_2$ ]bromoacetate (**5**)

[1,2- $^{13}C_2$ , 99%]Bromoacetic acid (**4**; 2.78 g, 20 mmol), *p*-nitrobenzyl alcohol (3.06 g, 20 mmol) and DMAP (245 mg, 2 mmol) were dissolved in methylene chloride (100 ml). The reaction flask was kept in a water bath to maintain room temperature. Dicyclohexylcarbodiimide (4.54 g, 22 mmol) in methylene chloride (50 ml) was added dropwise. The reaction mixture was stirred for 1 h. The white precipitate was filtered and washed with methylene chloride. The combined organic filtrates were washed with 10% sodium bicarbonate (2 x 150 ml), water (150 ml), 10% HCl (150 ml) and brine (150 ml) and dried over anhydrous sodium sulfate. The solvent was removed *in vacuo* to

give an oil, which solidified upon standing [4.18 g, 76%; m.p. 50-51 °C;  $R_f$  = 0.41 (hexane:ethyl acetate = 4:1)]. *IR* (NaCl, film): 2940, 2860, 1747, 1607, 1519, 1346, 1278, 1159, 1110, 860, 847, 805, 738  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  4.01 (s, 2H,  $\text{BrCH}_2$ ), 5.33 (s, 2H,  $\text{CH}_2\text{O}$ ), 7.57 (d, 2H,  $J$  = 8.4 Hz, aryl CH), 8.17 (d, 2H,  $J$  = 8.4 Hz, aryl CH),  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  25.86 (C-2), 66.16 ( $\text{CH}_2\text{O}$ ), 123.69, 128.37, 142.48, 147.57, 166.84 (CO); *MS* (EI, 70/150 eV):  $m/z$  273 ( $\text{M}^+$ , 1%), 214 (M-59, 2%), 194 (M-81, 55%), 152 (M-121, 100%).

Exact mass calc'd for  $\text{C}_9\text{H}_8\text{NO}_4\text{Br}$  272.9637, found 272.9641.

*p*-Methoxybenzyl [1,2- $^{13}\text{C}_2$ ]bromoacetate (**6**)

[1,2- $^{13}\text{C}_2$ , 99%]Bromoacetic acid (**4**; 5.0 g, 36 mmol), *p*-methoxybenzyl alcohol (6.22 g, 5.6 ml, 45 mmol) and DMAP (10 mol%, 440 mg, 3.6 mmol) were dissolved in methylene chloride (150 ml). EDC (7.67 g, 40 mmol) was added in one portion and the reaction mixture was stirred at room temperature overnight. The mixture was diluted with methylene chloride and washed with 10% sodium hydrogencarbonate (2 x 200 ml), water (200 ml), dilute HCl (200 ml) and brine (200 ml). The solution was dried over anhydrous sodium sulfate. The product was obtained by silica gel chromatography on a short, thick column using 30% ethyl acetate in hexanes as eluent to give the product as a pale yellow oil [6.5 g, 70%;  $R_f$  = 0.66 (hexane:ethyl acetate = 4:1)]. *IR* (NaCl, film): 3000, 2950, 2900, 2840, 1738, 1607, 1586, 1514, 1463, 1455, 1408, 1374, 1302, 1277, 1243, 1163, 1111, 1027, 957, 820  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.78 (s, 3H, OMe), 3.93 (s, 2H,  $\text{CH}_2\text{Br}$ ), 5.12 (s, 2H,  $\text{OCH}_2$ ), 6.87 (d, 2H,  $J$  = 8.5 Hz, aryl CH), 7.29 (d, 2H,  $J$  = 8.5 Hz, aryl CH);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  26.0 (C-2), 55.21 (OMe), 67.74 ( $\text{OCH}_2$ ), 113.93, 127.02, 130.26, 159.83, 166.9 (CO); *MS* (EI, DEEP.CI):  $m/z$  258 ( $\text{M}^+$ , 6%), 214 (M-44, 15%), 179 (M-79, 24%), 137 (M-121, 34%), 121 (M-137, 100%).

Exact mass calc'd for  $\text{C}_{10}\text{H}_{11}\text{BrO}_3$  257.9892, found 257.9894.

*p*-Nitrobenzyl [1,2- $^{13}\text{C}_2$ ]acrylate (**9**)

*p*-Nitrobenzyl [1,2- $^{13}\text{C}_2$ ]bromoacetate (**5**; 18 g, 78.4 mmol) was dissolved in methylene chloride (150 ml). Triphenylphosphine (22.64 g, 86.3 mmol) was added and the solution was stirred for 24 h. Then 37% aqueous formaldehyde (10 ml) and 10% sodium bicarbonate (200 ml) were added and the reaction mixture was stirred for a further 24 h. The phases were separated and the organic layer was washed with brine (2 x 100 ml) and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to give a yellow solid, which was chromatographed on a short, thick column of silica gel using 20% ethyl acetate in hexanes as eluent. The solvent was removed to give an oil which slowly crystallized [11.9 g, 73%; m.p. 51-2 °C;  $R_f$  = 0.66 (hexane:ethyl acetate = 4:1)]. *IR* (NaCl, film): 3120, 3035, 3050, 2950, 2940, 1730, 1633, 1603, 1517, 1400, 1341, 1325, 1291, 1187, 991, 843, 809, 738  $\text{cm}^{-1}$ ;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.31 (s, 2H,  $\text{CH}_2\text{O}$ ), 5.92 (dd, 1H,  $J$  = 10.4, 1.2 Hz, H-3 *cis*), 6.21 (dd, 1H,  $J$  = 17.1, 10.4 Hz, H-2), 6.49 (dd, 1H,  $J$  = 17.1, 1.2 Hz, H-3 *trans*), 7.55 (d, 2H,  $J$  = 8.6 Hz, aryl CH), 8.22 (d, 2H,  $J$  = 8.6 Hz, aryl CH);

$^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  64.77 ( $\text{CH}_2\text{O}$ ), 123.75, 127.70 (C-2), 131.90 (C-3), 134.18, 147.68, 165.55 (CO); MS (EI/CI,  $\text{NH}_3$ , 70/150 eV):  $m/z$  225 ( $\text{MNH}_4^+$ , 31%), 207 ( $\text{M}^+$ , 1%), 155 (M-52, 11%), 135 (M-72, 12%), 122 (M-85, 47%), 121 (M-86, 14%), 109 (M-98, 13%), 108 (M-99, 100%).

Exact mass calcd for  $\text{C}_{10}\text{H}_9\text{NO}_4$  207.0532, found 207.0536.

*p*-Methoxybenzyl [1,2- $^{13}\text{C}_2$ ]acrylate (**10**)

*p*-Methoxybenzyl [1,2- $^{13}\text{C}_2$ ]acrylate (**6**; 6.51 g, 25.2 mmol) and triphenylphosphine (7.27 g, 27.7 mmol) were dissolved in methylene chloride (100 ml), and the reaction mixture was stirred at room temperature for 24 h. Then 37% aqueous formaldehyde (20 ml) and 10% sodium bicarbonate (100 ml) were added and the two-phase system was stirred vigorously overnight. The phases were separated, and the organic layer was washed with water (100 ml), brine (100 ml) and dried over anhydrous sodium sulfate. The product was obtained by silica gel chromatography on a short, thick column [4.42 g, 91%;  $R_f$  = 0.68 (hexane:ethyl acetate = 4:1)]. IR (NaCl, film) 3035, 3000, 2950, 2940, 2840, 1731, 1634, 1614, 1516, 1463, 1404, 1371, 1296, 1247, 1173, 1035, 985, 810  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.76 (s, 3H, OMe), 5.11 (s, 2H,  $\text{OCH}_2$ ), 5.78 (dd, 1H,  $J$  = 10.4, 1.8 Hz, H-3 *cis*), 6.12 (dd, 1H,  $J$  = 10.4, 17.1 Hz, H-2), 6.40 (dd, 1H,  $J$  = 17.1, 1.8 Hz, H-3 *trans*), 6.87 (d, 2H,  $J$  = 9.6 Hz, aryl CH), 7.30 (d, 2H,  $J$  = 9.6 Hz, aryl CH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.16 (OMe), 66.10 ( $\text{OCH}_2$ ), 113.89, 127.94, 128.40 (C-2), 130.08, 130.80 (C-3), 159.61, 165.96 (CO); MS (EI, 70 eV):  $m/z$  192 ( $\text{M}^+$ , 47%), 174 (M-18, 2%), 147 (M-45, 19%), 137 (M-55, 7%), 121 (M-71, 100%).

Exact mass calcd for  $\text{C}_{11}\text{H}_{12}\text{O}_3$  192.0786, found 192.0787.

*p*-Nitrobenzyl *D,L*-[1,2- $^{13}\text{C}_2$ ]glycidate (**11**)

*p*-Nitrobenzyl [1,2- $^{13}\text{C}_2$ ]acrylate (**9**; 2.82 g, 13.5 mmol) was dissolved in DMF (100 ml). 5% Sodium hypochlorite solution (10 ml) was added in one portion and the reaction mixture was allowed to stand at room temperature overnight. The mixture was poured into brine (300 ml) and extracted with ethyl acetate (2 x 200 ml). The combined organic layers were washed with brine (2 x 10 ml) and dried over anhydrous sodium sulfate. The product was obtained by silica gel chromatography using 10% ethyl acetate in hexanes as eluent (1.46 g, 48%). The product was recrystallized from ethyl acetate-hexanes to give white needles, m.p. 52.5–53.5 °C. IR (NaCl, film): 3115, 3100, 2950, 1749, 1605, 1518, 1405, 1250, 1187, 1027, 856  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.02 (m, 2H, H-3), 3.58 (m, 1H, H-2), 5.31 (s, 2H,  $\text{OCH}_2$ ), 7.54 (d, 2H,  $J$  = 8.7 Hz, aryl CH), 8.24 (d, 2H,  $J$  = 8.7 Hz, aryl CH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  46.52 (C-3), 47.24 (C-2), 65.62 ( $\text{CH}_2\text{O}$ ), 123.79, 128.58, 142.32, 147.76, 168.91 (CO); MS (EI/CI,  $\text{NH}_3$ , 70/150 eV):  $m/z$  241 ( $\text{MNH}_4^+$ , 30%), 223 ( $\text{M}^+$ , 1%), 196 (M-27, 2%), 155 (M-68, 10%), 122 (M-101, 86%), 108 (M-115, 100%).

Exact mass calcd for  $\text{C}_{10}\text{H}_9\text{NO}_5$  ( $\text{MNH}_4^+$ ) 241.0824, found 241.0827.

*Sodium D,L-[1,2-<sup>13</sup>C<sub>2</sub>]glycidate (12).*

p-Nitrobenzyl D,L-[1,2-<sup>13</sup>C<sub>2</sub>]glycidate (**11**; 676 mg, 3 mmol) was dissolved in THF (50 ml). 10% Pd-C (50 mg) and NaHCO<sub>3</sub> (252 mg, 3 mmol) in water (10 ml) were added, and the resulting heterogeneous mixture was stirred under a hydrogen atmosphere overnight. The mixture was filtered through Celite, which was thoroughly washed with 10% aqueous ammonium carbonate and acetone. The volatile organic components of the filtrate were removed under reduced pressure and the aqueous solution was thoroughly washed with ethyl acetate and freeze-dried. The slightly yellow-colored solid was redissolved in water (30 ml) and filtered through a 0.22 µm syringe filter. Lyophilization gave a colorless solid (254 mg, 94%). The physical data obtained for **12** were identical to those of an authentic sample (**16**).

*p-Nitrobenzyl D,L-[1,2-<sup>13</sup>C<sub>2</sub>]glycerate (13).*

p-Nitrobenzyl [1,2-<sup>13</sup>C<sub>2</sub>]acrylate (**9**; 1.216 g, 5.81 mmol) and Et<sub>4</sub>NOAc•4H<sub>2</sub>O (380 mg, 1.45 mmol) were dissolved in acetone (50 ml) and the solution was cooled to 0 °C. Then 90% *t*-BuOOH (1.7 eq., 890 mg, 9.9 mmol, 990 µl) was added in one portion followed by the dropwise addition of 5% OsO<sub>4</sub> (w/v) in THF (100 µl). The reaction temperature was slowly allowed to rise to room temperature. After *ca.* 5 h (carefully monitored by TLC) the reaction was complete. The reaction mixture was preadsorbed onto silica gel and purified by flash chromatography on silica gel using 30% hexanes in ethyl acetate. The product solidified upon standing at 4 °C in the refrigerator [936 mg, 66%; m.p. 91–2 °C, *R*<sub>f</sub> = 0.55 (hexane:ethyl acetate:methanol = 6:3:1). IR (NaCl, film): 3460 (br), 2915, 1731, 1607, 1520, 1447, 1341, 1240, 1222, 1116, 1061, 1011, 988, 841 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 3.63 (t, 2H, *J* = 4.8 Hz, H-3); 4.18 (dt, 1H, *J* = 6.2, 4.8 Hz, H-2), 4.96 (t, 1H, *J* = 5.9 Hz, OH), 5.30 (s, 2H, OCH<sub>2</sub>), 5.55 (d, 1H, *J* = 6.2 Hz, OH), 7.65 (d, 2H, *J* = 8.9 Hz, aryl CH), 8.24 (d, 2H, *J* = 8.9 Hz, aryl CH); <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 63.71 (CH<sub>2</sub>O), 64.32 (C-3), 72.08 (C-2), 123.45, 128.22, 144.0, 146.98, 172.7 (CO); MS (EI/CI, DCI, NH<sub>3</sub>): *m/z* 259 (MNH<sub>4</sub><sup>+</sup>, 100%), 225 (M-16, 5%), 171 (M-70, 11%), 153 (M-88, 34%), 136 (M-105, 63%), 107 (M-134, 58%).

Exact mass calcd for C<sub>10</sub>H<sub>11</sub>NO<sub>6</sub> 241.0586, found 241.0582.

*Sodium D,L-[1,2-<sup>13</sup>C<sub>2</sub>]glycerate (14).*

p-Nitrobenzyl D,L-[1,2-<sup>13</sup>C<sub>2</sub>]glycerate (**13**; 1.672 g, 6.87 mmol) was dissolved in THF (50 ml). 10% Pd-C (50 mg) and NaHCO<sub>3</sub> (577 mg, 6.87 mmol) in water (15 ml) were added, and the resulting heterogeneous mixture was hydrogenated at atmospheric pressure overnight. The mixture was filtered through Celite, which was thoroughly washed with water and acetone. The organic components of the solvent system were removed under reduced pressure and the aqueous solution was thoroughly washed with ethyl acetate and freeze-dried. The sodium salt was purified by RP HPLC [Whatman Partisil 10 ODS-3 (25 x 250 mm), dH<sub>2</sub>O, 15 ml/min, ret. time: 4.5–5 min] (629 mg, 70%). The physical and spectroscopic data were identical with those of an authentic sample.



*p*-Nitrobenzyl [2-<sup>2</sup>H]cinnamate (**17**).

*p*-Nitrobenzyl bromoacetate (**5a**; 6.82 g, 24.9 mmol) was dissolved in toluene (50 ml). Triphenylphosphine (6.85 g, 26 mmol) was added in one portion, and the reaction mixture was stirred at room temperature for two days. The white solid obtained was removed by filtration and thoroughly washed with hexanes. Drying in air provided the title compound in essentially quantitative yield, which was used for Wittig reaction without further purification.

Phosphonium salt **15** (1.5 g, 2.79 mmol) was dissolved in D<sub>2</sub>O (50 ml, 99.9% D) under an argon atmosphere at 50 °C. Freshly distilled THF (10 ml) was added to ensure complete dissolution. After 1 h, the slightly cloudy solution was filtered through a fritted funnel (fine), and the filtrate was allowed to stand overnight at room temperature under an argon atmosphere. Then deuteriochloroform (100 µl, 99.9% D) and benzaldehyde (341 µl, 356 mg, 3.35 mmol) was added followed by anhydrous potassium carbonate (386 mg, 2.79 mmol). After stirring overnight, the two phases were separated and the aqueous layer was washed with methylene chloride (2 x 100 ml). The combined organic layers were washed with water (3 x 100 ml) and brine (100 ml) and dried over anhydrous sodium sulphate. The solvent was removed and the product was obtained by flash chromatography on silica gel using 10% ethyl acetate in hexanes as solvent. Based on mass spectrometric analysis 91-92% deuterium enrichment was achieved, *R*<sub>f</sub> = 0.49 (hexane/ethyl acetate = 4/1). *IR* (NaCl, film): 2950, 2915, 1705, 1606, 1513, 1447, 1345, 1317, 1250, 1188, 1159, 1102, 1020, 800, 690 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.34 (s, 2H, OCH<sub>2</sub>), 7.38 (m, 3H, aryl CH), 7.53 (m, 4H, aryl CH), 7.75 (s, 1H, H-3; H-2 at δ 6.52 was absent), 8.23 (d, 2H, *J* = 8.7 Hz, aryl CH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 64.73 (OCH<sub>2</sub>), 123.73, 128.16, 128.28 (C-2), 128.91, 130.60, 134.01, 143.40, 145.83, 147.60, 166.30 (CO); *MS* (SHL.EI, 9.9 eV): *m/z* 284 (M<sup>+</sup>, 23%), 267 (M-17, 46%), 239 (M-45, 27%), 238 (M-46, 23%), 237 (M-47, 14%), 193 (M-91, 19%), 148 (M-136, 11%), 132 (M-152, 100%).

Exact mass calcd for C<sub>16</sub>H<sub>12</sub>DO<sub>4</sub> (M<sup>+</sup>) 284.0907, found 284.0909.

*p*-Nitrobenzyl [2,3,3-<sup>2</sup>H<sub>3</sub>, 1,2-<sup>13</sup>C<sub>2</sub>]acrylate (**19**).

For the <sup>12</sup>C-isotope containing analogue (**18**): **15** (1.29 g, 2.4 mmol) was dissolved in freshly distilled THF (30 ml) under an argon atmosphere. Anhydrous potassium carbonate (332 g, 2.4 mmol) and D<sub>2</sub>CO (98% D<sub>2</sub>) in D<sub>2</sub>O (1 ml, 99.9% D) were added and the reaction mixture was stirred at room temperature overnight. The THF was removed under reduced pressure and the residue was partitioned between ethyl acetate (100 ml) and water (100 ml). The aqueous layer was washed with ethyl acetate (100 ml) and the combined organic layers were washed with water (100 ml) and brine (100 ml) and dried over anhydrous sodium sulphate. The solvent was removed and the oily residue was filtered through a short column of silica gel to separate the phosphonium salt. <sup>1</sup>H NMR spectral analysis showed that all of the vinyl hydrogens were replaced with deuterium. Mass spectrometric analysis revealed D<sub>3</sub>-species constituted 98% of the product and D<sub>2</sub>-species the remaining 2%.

For the  $^{13}\text{C}$ -labeled analogue (**19**): Compound **5b** (4.14 g, 15 mmol) was dissolved in dry THF (100 ml) and dry methylene chloride (100 ml) under an argon atmosphere. Triphenylphosphine (4.33 g, 16.5 mmol) was added and the solution was stirred at room temperature overnight. Then  $\text{D}_2\text{O}$  (50 ml, 99.9%) was added in one portion and the reaction mixture was stirred for another 24 h. The solution was treated with  $\text{D}_2\text{CO}$  (98%  $\text{D}_2$ ) in  $\text{D}_2\text{O}$  (10 ml) followed by anhydrous potassium carbonate (2.07 g, 15 mmol) and the reaction mixture was stirred overnight. The volatile organics were removed under reduced pressure and the aqueous solution was extracted with ethyl acetate (3 x 100 ml). The combined organic layers were washed with water (100 ml) and brine (100 ml) and dried over anhydrous sodium sulphate. The product was obtained by flash chromatography on silica gel using 10% ethyl acetate in hexanes as eluent (2.55 g, 80%). *IR* (NaCl, film): 3120, 3035, 3050, 2950, 2940, 1724, 1603, 1563, 1516, 1342, 1237, 1109, 1075, 994, 844, 764, 738  $\text{cm}^{-1}$ ;  $^1\text{H}$  *NMR* (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  5.31 (s, 2H,  $\text{OCH}_2$ ), 7.55 (d, 2H,  $J = 8.8$  Hz, aryl CH), 8.22 (d, 2H,  $J = 8.8$  Hz, aryl CH);  $^{13}\text{C}$  *NMR* (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  64.75 ( $\text{OCH}_2$ ), 123.79 (aryl CH), 127.4 (dt,  $J = 72.5$ , 25.8 Hz, C-2); 128.28, 143.10, 147.68, 165.7 (d,  $J = 72.5$  Hz, CO); *MS* (SHL.EI, 20 eV):  $m/z$  210 ( $\text{M}^+$ , 13%), 193 (M-17, 19%), 165 (M-45, 8%), 152 (M-58, 8%), 136 (M-74, 19%), 119 (M-91, 16%), 58 (M-152, 100%).

Exact mass calcd for  $\text{C}_{10}\text{H}_6\text{D}_3\text{NO}_4$  ( $\text{M}^+$ ) 210.0720, found 210.0721.

*p*-Nitrobenzyl *D,L*-[2,3,3- $^2\text{H}_3$ , 1,2- $^{13}\text{C}_2$ ]glycerate (**20**).

Labeled acrylate **19** (1.16 g, 5.49 mmol) and  $\text{Et}_4\text{NOAc}$  (359 mg, 1.37 mmol) were dissolved in acetone (50 ml) and the solution was cooled to 0 °C. Then 90% *t*-BuOOH (1.7 eq., 934  $\mu\text{l}$ , 841 mg, 9.33 mmol) was added in one portion followed by the dropwise addition of 5%  $\text{OsO}_4$  (w/v) in THF (100  $\mu\text{l}$ ). The reaction mixture was allowed to rise to room temperature and stirring was continued until all of the starting material was consumed. After preadsorption onto silica gel, the product was obtained by silica gel chromatography using 30% hexanes in ethyl acetate as eluent (1.07 g, 79%). *IR* (NaCl, film): 3400 (br), 2950, 2915, 1733, 1607, 1525, 1497, 1446, 1347, 1290, 1163, 1139, 988, 849, 738  $\text{cm}^{-1}$ ;  $^1\text{H}$  *NMR* (400 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  4.90 (s, 1H, OH), 5.30 (s, 2H,  $\text{OCH}_2$ ), 5.51 (s, 1H, OH), 7.65 (d, 2H,  $J = 8.6$  Hz, aryl CH); 8.23 (d, 2H,  $J = 8.6$  Hz, aryl CH);  $^{13}\text{C}$  *NMR* (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  66.02 ( $\text{OCH}_2$ ), 71.2 (dt,  $J = 58.7$ , 21.8 Hz, C-2), 123.89 (aryl CH), 128.37 (aryl CH), 142.09 (aryl), 172.7 (d,  $J = 58.7$  Hz, CO).

Exact mass calcd for  $\text{C}_{10}\text{H}_8\text{D}_3\text{NO}_6$  244.0775, found 244.0779.

Sodium *D,L*-[2,3,3- $^2\text{H}_3$ , 1,2- $^{13}\text{C}_2$ ]glycerate (**21**).

Labeled *p*-nitrobenzyl glycerate **20** (1.07 g, 4.37 mmol) was dissolved in THF (30 ml). Sodium hydrogencarbonate (367 mg, 4.37 mmol) in water (10 ml) was added followed by 10% Pd-C (50 mg). The heterogeneous mixture was hydrogenated at atmospheric pressure overnight. The solution was filtered through Celite, which was subsequently washed with acetone (50 ml) and water (50 ml). The organic components of the solvent system were removed under reduced pressure and

the aqueous layer was washed with ethyl acetate (2 x 100 ml) and lyophilized to provide labeled sodium glycerate in effectively quantitative yield;  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  75.4 (dt,  $J = 54.2$ , 21.8 Hz, C-2), 181.6 (d,  $J = 54.2$  Hz, C-1).

[1,1- $^2\text{H}_2$ , 1,2- $^{13}\text{C}_2$ ]glycerol (**22**).

Labeled p-nitrobenzyl glyceric acid **13** (1.49 g, 6.18 mmol) was dissolved in the mixture of THF (20 ml) and 0.1 M phosphate buffer (1 ml, pH 7.0). Sodium borodeuteride (2 eq., 468 mg, 12.4 mmol; 98 % D) was added in portions while the reaction mixture was vigorously stirred. After ca. 1.5 h, the reaction was complete, and a few drops of dilute HCl were added and the reaction mixture was stirred for 5 min. The crude reaction mixture was evaporated onto silica gel and the product was obtained by column chromatography on silica gel eluting with 20% hexanes in ethyl acetate and 50% methanol in methylene chloride. The solvents were removed under reduced pressure and the residue was dissolved in water. The solution was filtered through a syringe filter and lyophilized (434 mg, 76%). The residue showed  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR resonances in accord with an authentic sample (14, 15).  $\beta$ -Deuterium shifts on the  $^{13}\text{C}$ -resonance of C-2 were used to determine the extent of deuterium enrichment at C-1.

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