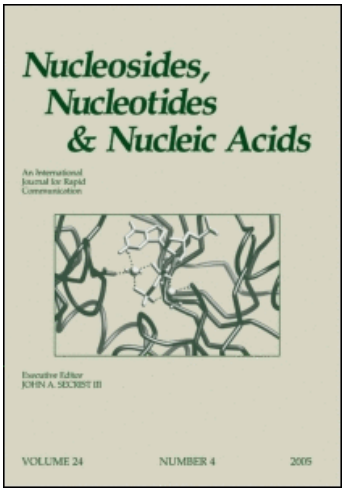

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REGIOSELECTIVE SYNTHESIS OF 1,3,5-$^{13}C_3$ AND 2,4-$^{13}C_2$-LABELED 2-DEOXYRIBONOLACTONES

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REGIOSELECTIVE SYNTHESIS OF 1,3,5-$^{13}C_3$ AND 2,4-$^{13}C_2$-LABELED 2-DEOXYRIBONOLACTONES

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ABSTRACT

The synthesis of 1,3,5-$^{13}C_3$- and 2,4-$^{13}C_2$-labeled 5-O-bromobenzyl-2-deoxyribonolactones **2**, precursors to ^{13}C-enriched nucleoside phosphoramidites for solid-phase synthesis of DNA oligonucletides, is described. An equimolar combination of these two multiply labeled lactones affords a ''population-labeled'' mixture of isotopomers which exhibits an approximately 50-fold increase in the sensitivity of ^{13}C-NMR compared to natural abundance measurements. The ^{13}C-^{13}C 2-bond and 4-bond coupling constants are reported for the lactones; all are < 2 Hz, confirming that this labeling scheme should be especially useful for NMR-relaxation measurements.

INTRODUCTION

As part of our ongoing effort to investigate the structure and dynamics of DNA oligomers[1], we recently reported the preparation of the series of regioselectively $^{13}C_1$-deoxyribonolactones[2]. These labeled deoxyribose derivatives are pivotal intermediates in a novel strategy for ^{13}C labeling of synthetic oligonucleotides, in which a ^{13}C-enriched oligonucleotide is prepared from phophoramidite monomers which are themselves synthesized from equimolar mixtures of the $^{13}C_1$-labeled isotopomers of deoxyribose. Each

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deoxyribose subunit of the oligonucleotide derived from such "population labeled" precursors will contain a single, randomly distributed ^{13}C atom, resulting in a net ^{13}C enrichment of approximately 21% at each of the carbon atoms of the phosphodeoxyribose backbone.

The success of our synthetic efforts directed at monolabeled deoxyribose derivatives has prompted consideration of a second generation strategy for the population labeling of an oligonucleotide, in which incorporation of multiple ^{13}C labels into deoxyribose precursors would enhance both efficiency of our synthetic scheme and the degree to which ^{13}C label is incorporated into synthetic oligonucleotides. Toward this end, we have examined the preparation of 1,3,5-$^{13}C_3$- and 2,4-$^{13}C_2$-labeled deoxyribose systems, reasoning that incorporation of an equimolar mixture of these intermediates into synthetic oligonucleotides would effectively label each of the carbohydrate backbone carbons of the resulting DNA polymer at an overall enrichment of $\sim$50-fold over natural abundance. Besides being an appealing alternative for nucleotide structural determination[3], the 1,3,5-$^{13}C_3$- and 2,4-$^{13}C_2$-labeled deoxyribose systems maximize the contribution of one-bond ^{13}C-^{1}H dipolar effects to NMR relaxation properties. These isotopomers remove one- and three-bond ^{13}C-^{13}C coupling effects that complicate the interpretation of relaxation properties; only natural abundance ^{13}C-^{13}C adjacencies remain. The labeling scheme also provides obvious advantages in sensitivity and reduction of spectral crowding, as well as presenting interesting possibilities for spectral editing and efficient measurement of residual dipolar couplings in oriented systems[4]. Furthermore, recent advances in solid phase oligonucleotide synthesis, indicated that incorporation of precious labeled phosphoramidites into oligonucleotides could be achieved much more efficiently using only 1.75 equivalents[5] of labeled reagent, as opposed to the 10-fold excess normally required[6]. In contrast to the work of Serianni and Bondo[7], and Agrofoglio et al.[8], whose methodology can also be used to create ^{13}C-enriched 2-deoxy-D-ribose, our method does not require the synthesis of D-ribose followed by deoxygenation at C2 to create 2-deoxy-D-ribose.

Our previously reported[2] route to the isotopomeric series of $^{13}C_1$-labeled deoxyribose derivatives, while designed to maximize economy of synthetic effort, is intrinsically limited by the necessity of preparing five unique isotopomers of deoxyribose. An additional liability of our initial approach was the undesired introduction of the minor enantiomer of each isotopomer as the consequence of an early asymmetric transformation that proceeds with high, but not exclusive, stereoselectivity. To address these concerns, we have considered alternative ^{13}C-labeled precursors to population-labeled oligonucleotides that can be accessed with increased efficiency and an improved control of absolute stereochemistry. Attractive in both regards is the labeling scheme depicted in Fig. 1, in which an equimolar mixture of 1,3,5-$^{13}C_3$ and 2,4-$^{13}C_2$-deoxyribonolactones 1,3,5-$^{13}C_3$-**2** and 2,4-$^{13}C_2$-**2** would yield the

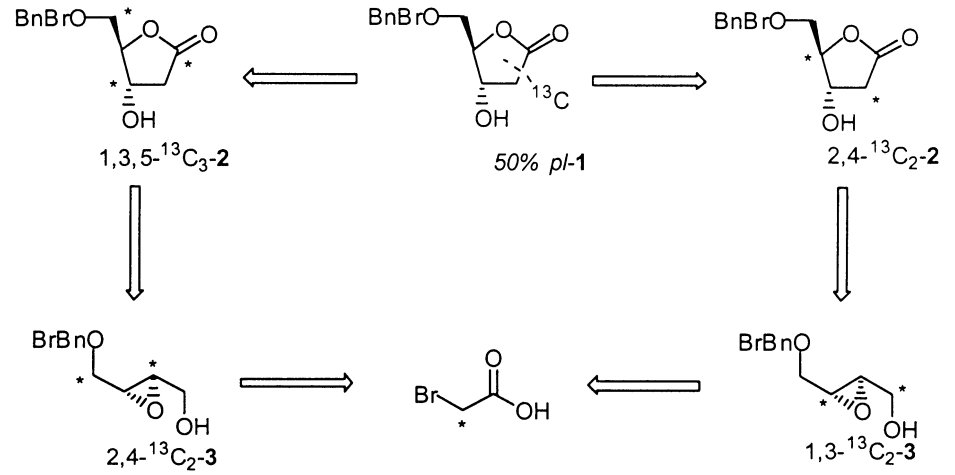


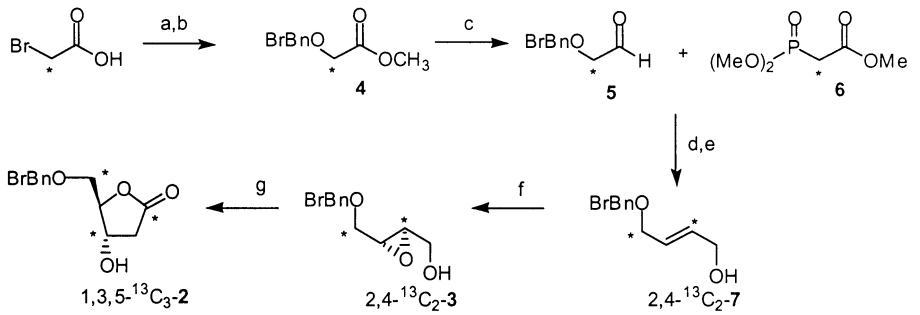
Figure 1. Retrosynthesis of 50% mixture of 1,3,5-$^{13}C_3$- and 2,4-$^{13}C_2$-deoxyribonolactones (top center). The labels originate from 2-^{13}C-bromoacetic acid (bottom center) and K^{13}CN.

population-labeled deoxyribonolactones *pl*-**1**. Oligonucleotides derived from *pl*-**1** would be expected to exhibit an impressive ^{13}C sensitivity enhancement over unlabeled oligomers and over the previously reported monolabeled systems[2]. Herein we describe the short and stereocontrolled routes to deoxyribonolactones 1,3,5-$^{13}C_3$-**2** and 2,4-$^{13}C_2$-**2**, as well as preliminary spectroscopic observations on these compounds and the population-labeled mixtures obtained there from.

RESULTS AND DISCUSSION

Our integrated synthetic plan for preparing multiple ^{13}C-labeled deoxyribose derivatives, based on our previously reported conversion of differentiated 2,3-epoxy butanediols **3** to the corresponding lactones via a modified Payne homologation methodology, is shown in Fig. 1. The substrates for Payne homologation, epoxy alcohols 1,3-$^{13}C_2$-**3** and 2,4-$^{13}C_2$-**3**, were envisioned deriving from a common starting material, the readily available 2-^{13}C-bromoacetic acid. For our preparation of 1,3,5-$^{13}C_3$-**2**, we anticipated introducing the final ^{13}C label at C1 from ^{13}C-labeled cyanide ion. Incorporation of the bromobenzyl ether system for protection of the C4 alcohol was expected to facilitate removal of minor enantiomeric impurities by crystallization, providing optically pure final products[9].

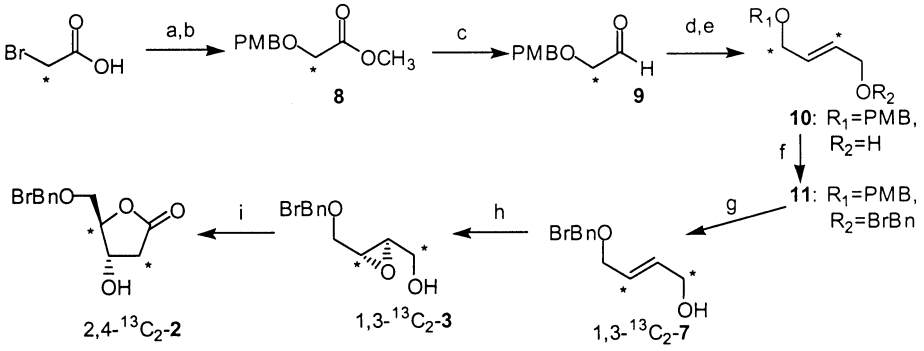
Our synthesis of 1,3,5-$^{13}C_3$-**2** is shown in Scheme 1. O-Alkylation of 2-^{13}C-bromoacetic acid with 4-bromobenzyl alcohol followed by treatment of the crude product with diazomethane yielded methyl ester **4**. Reduction to aldehyde **5**, was achieved with DIBAL at $-78\,^{\circ}$C, trace amounts ($< 10\%$) of the over-reduced alcohol were separated by from **5** by chromatography.



Scheme 1. Reagents: (a) KH, BrBnOH, THF, Δ; (b) CH_2N_2, Et_2O, 0°C; (c) DIBAL, THF, −78°C; (d) NaH, DME, 0°C; (e) DIBAL, THF, 0°C; (f) TBHP, Ti(O*i*Pr)$_4$, D-(-)-DIPT, DCM, −20°C; (g) K^{13}CN, KI, EtOH-H_2O, Δ, then HCl, H_2O.

Homologation of **5** by Wadsworth-Emmons-Horner addition with labeled phosphonate **6**[10] furnished the unsaturated ester (not shown), which was directly reduced with DIBAL to allylic alcohol 2,4-$^{13}C_2$-**7**. Sharpless asymmetric epoxidation[11] of 2,4-$^{13}C_2$-**7** gave epoxide 2,4-$^{13}C_2$-**3** as a single enantiomer[12] after one recrystallization. Payne rearrangement of epoxide 2,4-$^{13}C_2$-**3** and homologation[13] with K^{13}CN, produced a hydroxy nitrile which after hydrolysis and lactonization with aqueous HCl, afforded 1,3,5-$^{13}C_3$-**2**.

For our synthesis of 2,4-$^{13}C_2$-**2**, we proposed to exploit the latent symmetry of the differentiated butendiol **7**, as depicted in Scheme 2. Protection of 2-^{13}C-bromoacetic acid as the 4-methoxybenzyl ether and esterification with diazomethane afforded methyl ester **8**. Reduction to the corresponding aldehyde **9**, addition of phosphonate **6**, and reduction with DIBAL yielded the 4-methoxybenzyl protected butenediol derivative **10**. At this juncture, the diol system of **10** was inverted by protection of the free



Scheme 2. Reagents: (a) KH, PMBOH, THF, Δ; (b) CH_2N_2, Et_2O, 0°C; (c) DIBAL, THF, −78°C; (d) NaH, DME, **6**, 0°C; (e) DIBAL, THF, 0°C; (f) NaH, THF, BrBnBr, 0°C; (g) DDQ, 9:1 DCM:H_2O, 0°C; (h) TBHP, Ti(O*i*Pr)$_4$, D-(-)-DIPT, DCM, −20°C; (i) KCN, KI, EtOH-H_2O, Δ, then HCl, H_2O.

alcohol as the 4-bromobenzyl ether, and selective cleavage of the 4-methoxybenzyl residue[14] gave allylic alcohol 1,3-$^{13}C_2$-**7**. Conversion to 2,4-$^{13}C_2$-**2** was then accomplished as described for Scheme 1[15].

To complete our labeling strategy, equimolar amounts of 1,3,5-$^{13}C_3$-**2** and 2,4-$^{13}C_2$-**2** were combined to produces the population labeled mixture 50% *pl*-**1**. The HSQC spectrum of *pl*-**1** was acquired and compared with the HSQC spectrum for 2,4-$^{13}C_2$-**2** (Fig. 2). All ^{13}C-^{1}H correlations appear in the spectrum of *pl*-**1** (panel a), but only the correlations for C2-H2,H2′ and C4-H4 appear in panel b.

Since we are primarily concerned with the interpretation of relaxation properties, we determined the ^{2}J coupling constants. Multiple-bond pathways through the ring oxygen may also contribute to these small coupling constant values. The values are shown in Table 1. We also measure a mutiple-bond ^{13}C-^{13}C coupling between C1 and C5. Selected spectra are shown (Fig. 3). The larger two-bond and smaller multiple-bond couplings are clearly present for C1 (Fig. 3a). For C3, both $^{2}J_{C3\text{-}C5}$ and $^{2}J_{C3\text{-}C1}$ couplings are visible (Fig. 3b, left), but for C5, the $^{2}J_{C5\text{-}C3}$ and the $^{3}J/^{4}J_{C5\text{-}C1}$ are closer in magnitude so only a triplet appears (Fig. 3b, right). Of course C2 is split by $^{2}J_{C2\text{-}C4}$ as shown (Fig. 2c); the identical coupling constant value was observed at C4 (not shown).

CONCLUSION

We have successfully prepared a new class of population-labeled deoxyribonolactones with $\sim$50-fold increase in ^{13}C sensitivity over natural abundance. The regioselectively labeled lactones are prepared enantiospecifically, and display ^{13}C-^{13}C couplings of no greater than 1.6 Hz. The individual deoxyribonolactones are highly stable intermediates and can be stored for extended periods at $-20^{\circ}C$, prior to combination and conversion to nucleoside phosphoramidites for use in DNA synthesis. The overall synthetic scheme is sufficiently flexible to allow preparation of all ^{13}C-isotopomers of DNA sugars in high yield. In our previous paper, the syntheses of five x-$^{13}C_1$ and four 1,x-$^{13}C_2$-deoxyribonolactone[17] isotopomers is demonstrated. This paper describes the 1,3,5-$^{13}C_3$ and 2,4-$^{13}C_2$-lactones; simple substitution of unlabeled cyanide affords the 3,5-$^{13}C_2$-lactone. This accounts for 12 of the 32 isotopomers of 2-deoxy-D-ribose. Further work in progress demonstrates procedures for the other isotopomers as well as attractive population-labeled mixtures.

EXPERIMENTAL SECTION

General: IR spectra were recorded on a Perkin Elmer Paragon 1000 FT spectrometer, with sodium chloride plates, and are reported in wavenumbers (cm^{-1}). ^{1}H- and ^{13}C NMR were taken on a Bruker Avance 300 unless

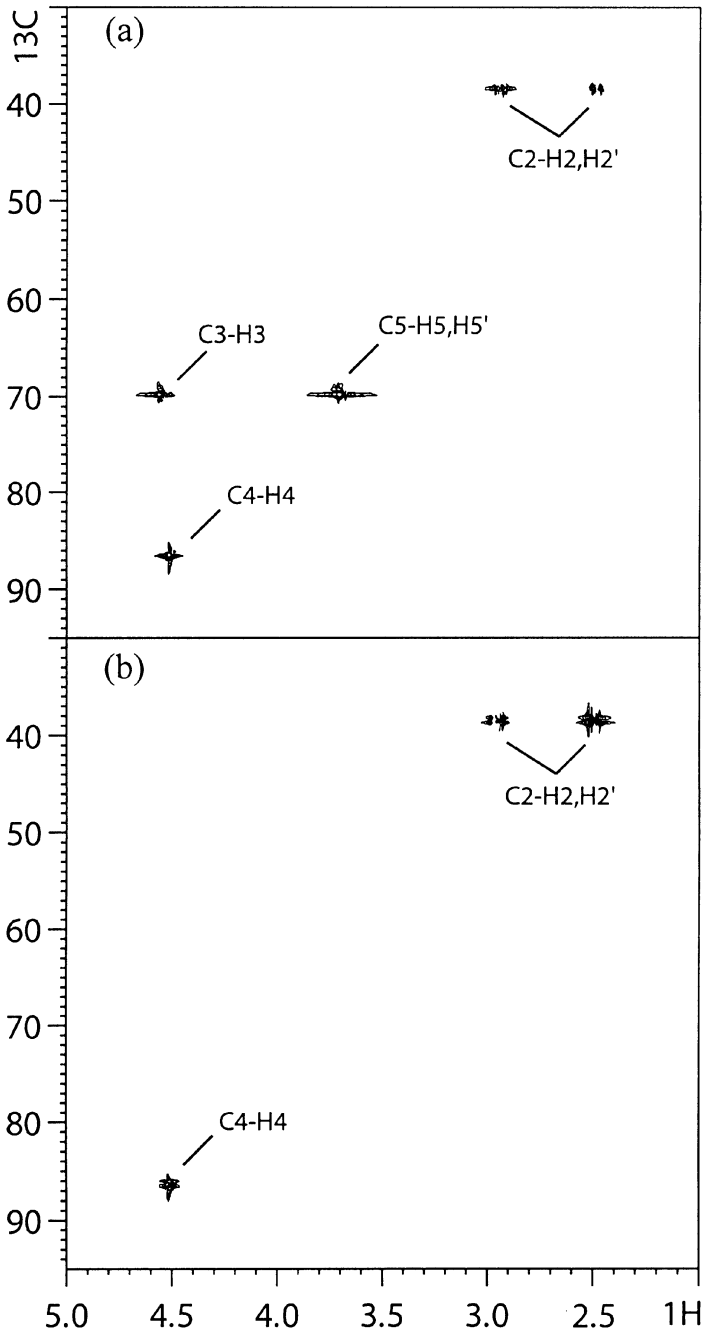


Figure 2. HSQC spectra of 50% *pl*-**1** (panel a), and 2,4-$^{13}C_2$-**2** (panel b).

Table 1. ^{13}C-^{13}C Coupling Constants (in Hz)[16]

	^2J	$^3J/^4J$
C1-C3	1.6	–
C3-C5	0.9	–
C2-C4	0.8	–
C1-C5	–	0.5

otherwise noted. Proton chemical shifts are reported in δ, using the residual $CHCl_3$, as internal reference (7.26 ppm), unless otherwise noted. J values are given in Hz. Carbon chemical shifts are reported in δ, using $CDCl_3$, as an internal reference (77.0 ppm), unless otherwise noted. ^1H and ^{13}C NMR spectra were typically acquired in the same number of scans. Optical rotations were measured on either a Jasco DIP-1000 or a Perkin Elmer 241 spectrometer. Enantiomeric excess was determined by either Mosher ester derivatization (ME)[18], or by chiral shift analysis (CSA) with (+)-Eu(hfc)$_3$[19]. THF and DME were distilled from sodium benzophenone ketyl under a nitrogen atmosphere. Dichloromethane, benzene and pyridine were distilled from calcium hydride before use. Hexanes, ethyl acetate, ether, and anhydrous ether were used from the supplier without further purification. Silica gel (230–400 mesh) was used for flash column chromatography. TLC analysis was performed on Whatman K6F silica gel 60 plates and were stained with the following: anisaldehyde (method A), phosphomolybdic acid (PMA) (method B), $KMnO_4$ (method C), 2,4-DNP (method D), and UV light. Reactions were performed in flame or oven dried glassware under a nitrogen or argon atmosphere where appropriate. Other reagents were used from suppliers without purification unless otherwise indicated.

HSQC spectra were acquired on a Bruker Avance 500 spectrometer, using the pulse program of Palmer et al.[20] HSQC spectra were collected with

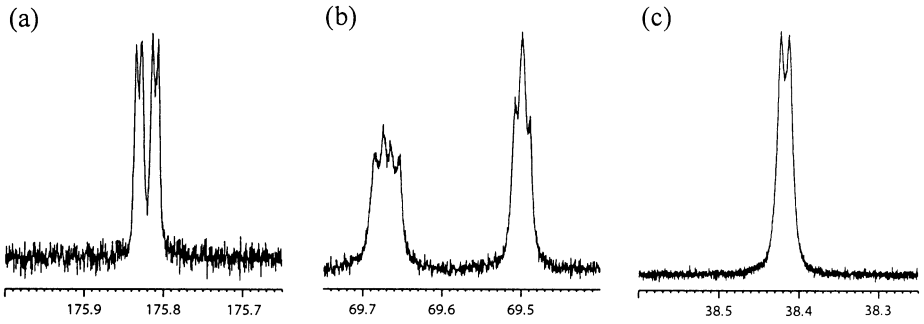


Figure 3. ^{13}C-^{13}C Coupling for (a) C1, (b) C3 (left), and C5 (right), and (c) C2. Each panel is 0.35 ppm.

1 scan per increment, into 2048×512 points with no sample spinning and no zero-filling. These gradient selected acquisitions typically required >15 min. The sweep width was 3255 Hz in F_2 and 12500 Hz in F_1. A squared sine window function was applied in F_2 and F_1.

Data for coupling constants was acquired on a Bruker Avance 300 with a direct detection probe. The sweep width was 450 Hz, and no window function was applied. The peaks were fitted using the NMR1 software package (Tripos Assoc.) and coupling constants calculated assuming first-order coupling.

[2-^{13}C]-(4-Bromo-benzyloxy)-acetic acid methyl ester (4). To a suspension of KH (722 mg, 18 mmol), washed with pentane, in 100 mL of THF at 0°C was added 2-^{13}C-bromoacetic acid (1 g, 0.71 mmol), and then 4-bromobenzyl alcohol (1.46 g, 0.86 mmol) both in 15 mL of THF. The reaction mixture was allowed to warm to room temperature and stirred until the effervescence ceased. The solution was then heated to reflux overnight. After cooling to ambient temperature the reaction mixture was quenched by pouring into a stirring mixture of ice/NH_4Cl (sat.)/ether 30 mL each. The quenched reaction mixture was then transferred to a separatory funnel and the organic layer was washed with $NaHCO_3$ (sat.). The organic layer was then discarded and the aqueous layer acidified with concd. HCl until acidic. The aqueous layer was then extracted with three 50 mL portions of ether, which were dried and concentrated to a white solid. The solid was redissolved in ether, and diazomethane was added to the ether solution at 0°C until a yellow color persisted. Excess diazomethane was quenched with $MgSO_4$, and then filtered and concentrated to yield a slightly yellow oil (1.73 g, 95% over 2 steps). $R_f = 0.64$, 1:1 hexanes:EtOAc, method D; IR (neat): 3000.3, 1759.6; ^{1}H NMR: 3.69 (s, 3H), 4.05 (*d*, $J = 143.8$, 2H), 4,51 (*d*, $J = 4.4$, 2H), 7.18 (m, 2H), 7.41 (m, 2H); ^{13}C NMR: 67.10.

[2-^{13}C]-(*E*)-(4-Methoxy-benzyloxy)-acetic acid methyl ester (8). The procedure for **4** was followed except 4-methoxybenzyl alcohol was substituted for 4-bromobenzyl alcohol. The procedure yielded a slightly yellow solid (1.33 g, 90% over 2 steps). $R_f = 0.60$, 1:1 hexanes:EtOAc, method D; IR (neat): 3005.4, 1760.3; ^{1}H NMR: 3.69 (s, 3H), 4.05 (*d*, $J = 143.8$, 2H), 4.51 (*d*, $J = 4.4$, 2H), 7.18 (m, 2H), 7.41 (m, 2H); ^{13}C NMR: 67.10.

[2,4-$^{13}C_2$]-(*E*)-4-(4-Bromo-benzyloxy)-but-2-enoic acid methyl ester. Methyl ester **4** (1.73 g, 0.66 mmol) was dissolved in 25 mL of THF and added to a round bottomed flask. The resulting homogenous solution was cooled to -78°C and DIBAL (1.2 eq, 8 mL, 1 M in toluene) was added dropwise to the solution The reaction was monitored by TLC, and

when the starting material had been consumed the resulting mixture was quenched with MeOH. The quenched solution was allowed to warm to ambient temperature, and was further diluted with ether and Rochelle salt. After stirring for 2 h the diluted solution was transferred to a separatory funnel and extracted with two 100 mL portions of ether, which were dried over $MgSO_4$, and conc. to yield a yellow oil. The aldehyde (**5**) was flashed in 1:1 hexane:EtOAc. The aldehyde should be used immediately in the next step. $R_f = 0.27$, 1:1 hexane:EtOAc, method D; ^{1}H NMR: 4.12 (*d*, $J = 145.4$, 2H), 4.58 (*d*, $J = 6.9$, 2H), 7.23 (*d*, $J = 8.3$, 2H), 7.50 (*d*, $J = 8.5$, 2H), 9.72 (*d*, $J = 29.7$, 1H); ^{13}C NMR: 75.4.

To a suspension of NaH (155 mg, 0.65 mmol) (washed with pentane) in 25 mL DME was added [2-^{13}C]-(diethoxy-phosphoryl)-acetic acid methyl ester (**6**) (1.37 g, 0.65 mmol). The resulting reaction mixture was equilibrated for 30 min. After equilibration, the solution was cooled to $-78\,^\circ$C and the aldehyde (1.26 g, 0.54 mmol) was added dropwise in 5 mL DME. The reaction mixture was stirred for 30 min and then quenched by pouring into a stirring solution of ice/NH_4Cl (sat)/ether 10 mL each. Separation of the layers and extraction of the organic layer, followed by drying over $MgSO_4$ and conc yielded a clear oil (1.12 g, 59% over 2 steps). $R_f = 0.57$, 1:1 hexanes: EtOAc, method D; IR (neat): 1719.4; ^{1}H NMR: 3.71 (s, 3H), 4.13 (dm, $J = 141.61$, 2H), 4.46 (*d*, $J = 4.12$, 2H), 6.09 (dm, $J = 165.21$, 1H), 6.94 (m, 1H), 7.18 (*d*, $J = 8.23$, 2H), 7.44 (*d*, $J = 8.23$, 2H); ^{13}C NMR: 68.49, 120.79.

[2,4-$^{13}C_2$]-(*E*)-4-(4-Methoxy-benzyloxy)-but-2-enoic acid methyl ester. The above procedure, applied to **8**, yielded a yellow oil. Aldehyde: $R_f = 0.24$, 1:1 hexane:EtOAc, method D; IR (neat): 1723; ^{1}H NMR: 3.81 (s, 3H), 4.06 (dd, $J = 0.5$, 141.3, 2H), 4.56 (*d*, $J = 4.39$, 2H), 6.89 (*d*, $J = 8.8$, 2H), 7.29 (*d*, $J = 8.8$, 2H), 9.69 (*d*, $J = 25.8$, 1H); ^{13}C NMR: 75.0. The above procedure, applied to **9**, yielded a yellow oil (975 mg, 65% over 2 steps). Ester: $R_f = 0.61$, 1:1 hexanes:EtOAc, method D; IR (neat): 1720.2; ^{1}H NMR: 3.74 (s, 3H), 3.81 (s, 3H), 4.14 (dm, $J = 142.43$, 2H), 4.49 (*d*, $J = 4.39$, 2H), 6.11 (dm, $J = 163.57$, 1H), 6.97 (m, 1H), 6.88 (m, 2H), 7.27 (m, 2H); ^{13}C NMR: 68.25, 120.86.

[2,4-$^{13}C_2$]-(*E*)-4-(4-Bromo-benzyloxy)-but-2-en-1-ol (2,4-$^{13}C_2$-7). The unsaturated ester (1.12 g, 0.39 mmol) was dissolved in THF and cooled to $0\,^\circ$C in ice. DIBAL (2.5 eq, 13.5 mL, 1 M in toluene) was added to the stirring solution and the resulting mixture was allowed to warm to ambient temperature. After stirring for 2 h the mixture was again cooled to $0\,^\circ$C and was quenched with MeOH and then diluted with ether and Rochelle salt and stirred overnight. Extraction of the quenched reaction mixture followed by drying over $MgSO_4$, and conc to yield a clear oil (900 mg, 89%). $R_f = 0.35$, 1:1 hexanes:EtOAc, method A; IR (neat): 3388.4, 2853.2; ^{1}H NMR: 1.77 (s, 1H), 4.02 (dt, $J = 5.49$, 141.34, 2H), 4.16 (m, 2H), 4.46

(*d*, $J = 3.57$, 2H), 5.83 (m, 1H), 5.90 (dm, $J = 151.49$, 1H), 7.21 (*d*, $J = 8.51$, 2H), 7.47 (*d*, $J = 8.23$, 2H); ^{13}C NMR: 70.14, 132.38.

[2,4-$^{13}C_2$]-(*E*)-4-(4-Methoxy-benzyloxy)-but-2-en-1-ol (10). The above procedure, applied to the unsaturated ester from **9**, yielded a clear oil (800 mg, 93%). $R_f = 0.29$, 1:1 hexanes:EtOAc, method A; IR (neat): 3388.8, 2838.2; ^{1}H NMR: 1.63 (bs, 1H), 3.80 (s, 3H), 4.00 (dm, $J = 139.96$, 2H), 4.15 (m, 2H), 4.45 (*d*, $J = 4.12$, 2H), 5.83 (m, 1H), 5.89 (dm, $J = 152.31$, 1H), 6.88 (m, 2H), 7.26 (m, 2H); ^{13}C NMR: 69.8, 132.1.

[1,3-$^{13}C_2$]-(*E*)-1-(4-Bromo-benzyloxy)-4-(4-Methoxy-benzyloxy)-but-2-ene (11). To a round bottom flask was added NaH (137 mg, 0.57 mmol) and 5 mL of THF. The resulting solution was cooled to 0°C in ice. Compound **10** was then added followed by bromobenzyl bromide (1.40 g, 0.57 mmol) in 2 portions. The reaction was then allowed to warm to room temperature and monitored by TLC. After the starting material was consumed the reaction was quenched with sat. NH_4Cl. Typical workup provided an oil (1.25 g, 87%). $R_f = 0.84$, 1:1 hexane:EtOAc; IR (neat): 2854.0; ^{1}H NMR: 3.80 (s, 3H), 4.01 (dt, $J = 4.7$, 141.3, 2H), 4.03 (*t*, $J = 4.9$, 2H), 4.45 (m, 2H), 5.85 (dm, $J = 152.3$, 1H), 5.86 (m, 1H), 6.88 (*d*, $J = 8.5$, 2H), 7.24 (m, 4H), 7.46 (*d*, $J = 8.5$, 2H); ^{13}C NMR: 69.8, 129.1.

[1,3-$^{13}C_2$]-(*E*)-4-(4-Bromo-benzyloxy)-but-2-en-1-ol (1,3-$^{13}C_2$-7). **11** was dissolved in 9:1 DCM:H_2O, and cooled to 0°C in ice. DDQ was then added all at once and the reaction was stirred for 1 h. Extraction, drying, and conc yielded an oil (820 mg, 96%). IR (neat): 3388.4, 2853.2; ^{1}H NMR: 1.48 (br s, 1H), 4.03 (*t*, $J = 4.9$, 2H), 4.16 (dt, $J = 5.5$, 142.2, 2H), 4.47 (s, 2H), 5.84 (dm, $J = 153.4$, 1H), 5.91 (m, 1H), 7.21 (*d*, $J = 8.0$, 2H), 7.47 (*d*, $J = 8.2$, 2H); ^{13}C NMR: 63.0, 128.3.

[2,4-$^{13}C_2$]-(2R,3R)-[3-(4-Bromo-benzyloxymethyl)-oxiranyl]-methanol (2,4-$^{13}C_2$-3). To a round bottom flask was added powdered, activated 4 Å molecular sieves, and a stir bar. The round bottom flask was then flame dried and allowed to cool to ambient temperature under nitrogen. Methylene chloride, D-(-)-diisopropyl tartrate, and Ti(O*i*Pr)$_4$ were added sequentially, and the resulting heterogeneous solution was cooled to −25°C (CO_2 (s), CCl_4) and stirred for 30 min. TBHP was then added at −25°C, and the solution was aged for 30 min. Meanwhile, the allylic alcohol 2,4-$^{13}C_2$-**7** was diluted in DCM and stirred over 4 Å molecular sieves while the catalyst was aging. After the aging period the allylic alcohol was added to the catalyst and the reaction mixture was stirred at −25°C for 1 h before being placed in a −20°C freezer overnight. The reaction mixture was quenched by addition of dimethyl sulfide at −20°C and allowing the solution to warm to ambient temperature over

2h. The quenched mixture was filtered through a frit and concentrated to yield a yellow crude product that was purified by flash chromatography to afford as a white solid. Recrystallization was conducted as follows: the solid obtained after flash chromatography was suspended in pentane (1–2mL), and then ether was added until the solution became homogeneous at room temperature. The solution was then placed in a −25°C freezer overnight to allow crystal formation. White needles (826mg, 86%). ee = 100% (ME); $R_f = 0.39$, 2:1 hexane:EtOAc, method A; IR (thin film): 3356.5; ^{1}H NMR: 1.77 (br s, 1H), 3.09 (*d*, $J = 173.7$, 1H), 3.24 (m, 1H), 3.71 (m, 2H), 3.78 (dm, $J = 141.9$, 2H), 3.94 (*d*, $J = 12.6$, 1H), 4.52 (ABq, $J = 13.7$, 2H), 7.21 (*d*, $J = 8.2$, 2H), 7.47 (*d*, $J = 8.5$, 2H); ^{13}C NMR: 55.5, 69.7.

[1,3-$^{13}C_2$]-(2R,3R)-[3-(4-Bromo-benzyloxymethyl)-oxiranyl]-methanol (1,3-$^{13}C_2$-3). The above procedure applied to 1,3-$^{13}C_2$-**7** yielded white needles (704mg, 80%). ee = 100% (ME); IR (thin film): 4305; ^{1}H NMR: 2.12 (br s, 1H), 3.08 (m, 1H), 3.20 (*d*, $J = 158.1$, 1H), 3.63 (dd, $J = 13.2$, 142.7, 2H), 3.77 (*d*, $J = 10.7$, 1H), 4.16 (*d*, $J = 12.9$, 1H), 4.51 (ABq, $J = 11.5$, 2H), 7.20 (*d*, $J = 7.4$, 2H), 7.46 (*d*, $J = 7.4$, 2H); ^{13}C NMR: 54.1, 61.0.

[1,3,5-$^{13}C_3$]-(4S,5R)-5-(4-Bromo-benzyloxymethyl)-4-hydroxy-dihydro-furan-2-one (1,3,5-$^{13}C_3$-2). To a solution of epoxide 2,4-$^{13}C_2$-**3** in 120mL of 2:1 EtOH:H_2O, was added K^{13}CN and KI. The reaction mixture was heated at reflux for 48hr, cooled to ambient temperature and carefully acidified to pH 2 with 10% HCl. The resulting mixture was warmed to 60°C and stirred for 16h, at which time the reaction mixture was cooled and transferred to a separatory funnel and the aqueous layer was extracted with three 50mL portions of Et_2O. The combined ether extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (2:1 hexanes:EtOAc) to give lactone 1,3,5-$^{13}C_3$-**2** as a slightly yellow solid (593mg, 65%). $R_f = 0.31$, 2:1 hexanes:EtOAc, method A; IR (thin film): 3387, 1780; ^{1}H NMR: 2.70 (br s, 1H), 2.45 (dm, $J = 2.7$, 18.0, 1H), 2.91 (dt, $J = 5.8$, 18.3, 1H), 3.66 (dm, $J = 144.0$, 2H), 4.46 (m, 3H), 4.51 (dm, $J = 153.5$, 1H), 7.14 (*d*, $J = 8.2$, 2H), 7.47 (*d*, $J = 8.5$, 2H); ^{13}C NMR: 69.5, 69.6, 175.9.

[2,4-$^{13}C_2$]-(4S,5R)-5-(4-Bromo-benzyloxymethyl)-4-hydroxy-dihydro-furan-2-one (2,4-$^{13}C_2$-2). The above procedure applied to 1,3-$^{13}C_2$-**7** yielded a yellow solid (479mg, 60%). IR (thin film): 3365, 1772; ^{1}H NMR: 2.71 (br s, 1H), 2.45 (ddd, $J = 1.8$, 18.3, 136.8, 1H), 2.92 (ddd, $J = 6.7$, 18.3, 133.7, 1H), 3.67 (m, 2H), 4.46 (ABq, $J = 11.0$, 2H), 4.48 (*d*, $J = 150.5$, 1H), 4.53 (m, 1H), 7.14 (*d*, $J = 8.2$, 2H), 7.47 (*d*, $J = 8.2$, 2H); ^{13}C NMR: 38.4, 86.2.

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