Pergamon

Tetrahedron 57 (2001) 1515–1524

TETRAHEDRON

Regioselective synthesis of $^{13}C_1$-labeled 2-deoxyribonolactones

Michael P. Hayes, Paul J. Hatala, Brian A. Sherer, Xiaohong Tong, Nilo Zanatta, Philip N. Borer and James Kallmerten*

Department of Chemistry, Syracuse University, 1-014 Center for Science and Technology, Syracuse, NY 13244-4100, USA

Received 6 July 2000; accepted 6 December 2000

Abstract—Syntheses of the five regioselectively $^{13}C_1$-labeled 5-*O*-benzyl-2-deoxyribonolactones are described. $^{13}C_1$-Labeled deoxyribonolactones were prepared by addition of KCN to epoxides **7** and subsequent lactonization of the resulting nitriles. Integration of the independent schemes leading to the five isotopomers of **9** results in an efficient and cost effective preparation of labeled mixtures of ^{13}C mono-labeled deoxyribonolactones. These mixtures are the pivotal intermediates in the preparation of 'population labeled' ^{13}C-labeled nucleoside phosphoramidites for solid-phase oligonucleotide synthesis. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Multidimensional heteronuclear NMR spectroscopy has emerged as a powerful tool for the investigation of the solution structure and dynamics of biological macromolecules.[1] An intrinsic limitation of heteronuclear NMR analysis of biopolymers is the low natural abundance of ^{13}C and ^{15}N, and recent attention has focused on strategies for isotopic enrichment of proteins and oligonucleotides. NMR analysis of isotopically enriched biopolymers is facilitated by an enhanced sensitivity over natural abundance spectra that simplify assignments and spectral editing by heteronuclear frequencies. The observed NMR sensitivity of enriched systems additionally allows for sensitive measurement of $^{13}C-^{1}H$, $^{13}C-^{31}P$, and $^{13}C-^{13}C$ coupling constants, and provides $^{13}C-^{1}H$ relaxation data for interpreting the dynamic properties of macromolecular systems.

Proteins enriched in ^{13}C- and/or ^{15}N are readily obtained from organisms grown in isotope exclusive media and NMR studies of these uniformly labeled peptides have been employed in the study of protein structure.[2] The analogous labeling of DNA and RNA using biosynthetic techniques is more problematic. Microorganisms typically produce significantly more protein than DNA and there are relatively few naturally occurring nucleic acids with molecular weights in the range amenable to NMR study. Uniformly labeled RNA oligonucleotides, prepared from degradation of relatively abundant ribosomal RNA, have been used to construct short oligonucleotides of defined sequence.[3] A similar degradation-reconstitution approach has been employed to synthesize uniformly labeled DNA oligomers.[4]

The advent of effective techniques for the solid-phase synthesis of oligonucleotides via the coupling of nucleoside phosphoramidites has fostered efforts focused on the regioselective preparation of isotopically labeled nucleosides for use as monomer building blocks in the synthesis of small DNA and RNA molecules.[5] Synthetic protocols incorporating stable isotopes into the purine and pyrimidine bases of nucleosides have emerged,[6] however, the regioselective incorporation of ^{13}C labels into the deoxyribose backbone of nucleosides has received less attention. Serianni and co-workers have reported the preparation of the 1-^{13}C and 2-^{13}C labeled deoxyriboses,[7] and NMR studies of deoxyoligonucleotides generated from regioselectively labeled[8] and synthetically derived, uniformly labeled[9] deoxyribose have appeared. Surprisingly, effective preparations of each of the $^{13}C_1$-labeled deoxyriboses and derived oligonucleotides have not been described.

As part of our program to investigate the conformational dynamics of DNA oligomers[10] we sought a strategy for the ^{13}C enrichment of the carbohydrate backbone which would broadly enhance the sensitivity of multidimensional ^{13}C NMR experiments and at the same time isolate individual ^{13}C nuclei, simplifying interpretation of relaxation properties. An attractive strategy for isotopic enrichment of the carbohydrate backbone of deoxyribonucleotides would be realized by the synthesis of labeled oligonucleotide *pl*-**1**[11] from monomer reagents which were themselves mixtures of the five different $^{13}C_1$-labeled phosphoramidite[12] reagents *pl*-**2**, a labeling scheme we have designated 'population labeling.' As shown in Fig. 1, the preparation of such population labeled oligonucleotides, *pl*-**1** would involve three distinct synthetic phases: (1) regioselective synthesis of the five $^{13}C_1$-labeled deoxyriboses, (2) combination of suitably derivatized variants of these monolabeled sugars and conversion of the resulting mixture to the corresponding nucleoside phosphoramidite *pl*-**2**, and (3)

Keywords: nucleic acids; labeling; NMR; regioselection.

* Corresponding author. Tel.: +315-443-2854; fax: +315-443-4070; e-mail: jkallmer@syr.edu

PII: S0040-4020(00)01148-0

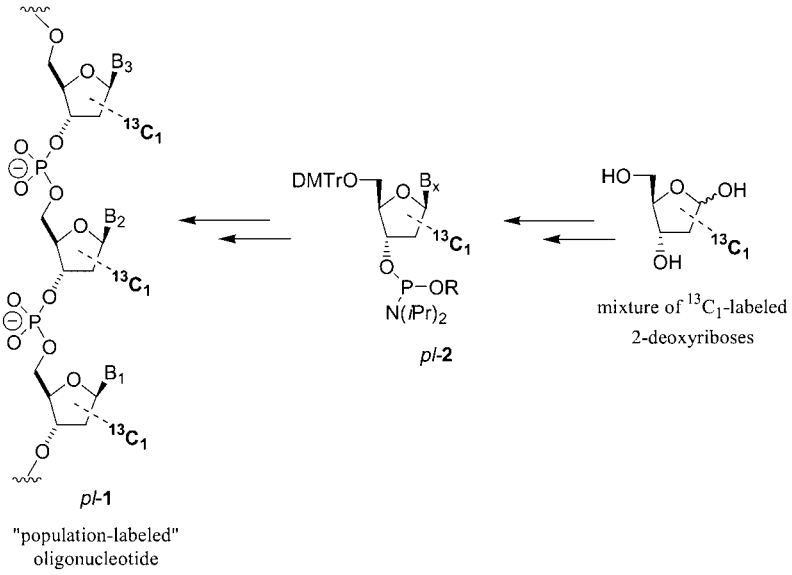


Figure 1.

solid-phase synthesis of a oligonucleotide *pl*-**1** obtained from the above phosphoramidite reagents. Each deoxyribose subunit of the oligonucleotide **1** obtained from such a sequence would contain a single, randomly distributed ^{13}C atom. If equimolar amounts of all five isotopomers were used, this would result in a net ^{13}C enrichment of up to 21% at each of the carbon atoms of the phosphodeoxyribose backbone.[13]

For a population labeled oligomer, only ^{13}C–^{13}C coupling would occur at natural abundance ensuring that the dominant NMR relaxation pathway involves the easily interpreted ^{13}C–^{1}H dipolar mechanism. Other advantages of site-specifically labeled DNA include simple measurement of one- and multiple-bond couplings between the ^{13}C-label and ^{1}H, ^{13}C, or ^{31}P at natural abundance to determine chemical shifts, coupling constants, and estimate dihedral angles. The increased sensitivity of population labeled oligomers would allow segmental labeling of larger DNA molecules, which are currently inaccessible due to spectral overlap. Population labeled oligomers also represent excellent candidates for measurement of residual dipolar couplings in oriented systems to accurately determine long-range geometrical and dynamical information.[14] Additionally, synthetic access to selectively monolabeled deoxyribose subunits presents opportunities for extension of the population labeling strategy to the preparation of DNA oligomers incorporating multiply-labeled deoxyribose units, further enhancing spectroscopic sensitivity and economy of preparation.

Successful implementation of our plan to prepare population labeled deoxyoligonucleotides requires that two key issues be addressed. Oligonucleotide synthesis via the phosphoramidite method is intrinsically inefficient with respect to the phosphoramidite monomers. Typical experimental procedures call for the use of up to a 10-fold excess of the phosphoramidite for each coupling step, a stoichiometry that is incompatible with the use of ^{13}C-labeled monomers prepared by multistep synthesis. Fortunately, for our purposes, a recently described modification of the phosphoramidite protocol allows for efficient recovery and recycling of phosphoramidite reagents.[15] A potentially more difficult problem involves the formidable logistics of preparing population labeled phosphoramidite reagents. Full implementation of a population-labeling scheme would require the formal preparation of 20 different regioisomerically-labeled monomer reagents (four bases each having five potential labeling sites). Our plan to prepare population labeled oligonucleotides hinged on our development of an economically practical and logistically manageable access to the five $^{13}C_1$-deoxyribose isomers.

2. Results and discussion

2.1. Synthesis of $^{13}C_1$-labeled deoxyribonolactones

The ultimate objective of preparing mixtures of optically active, $^{13}C_1$-labeled 2-deoxypentose derivatives suggested asymmetric synthesis as an attractive source for the individual monolabeled sugars.[16] To minimize the total number of transformations required to prepare population labeled phosphoramidite reagents *pl*-**2**, attention was focused on the development of an integrated synthetic plan that

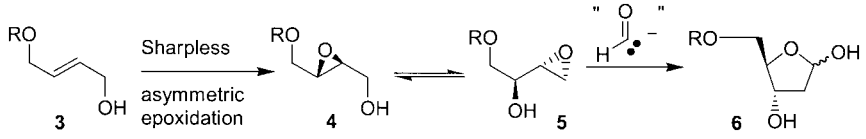
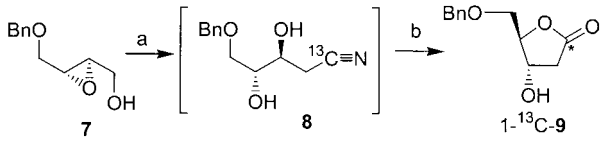


Figure 2.

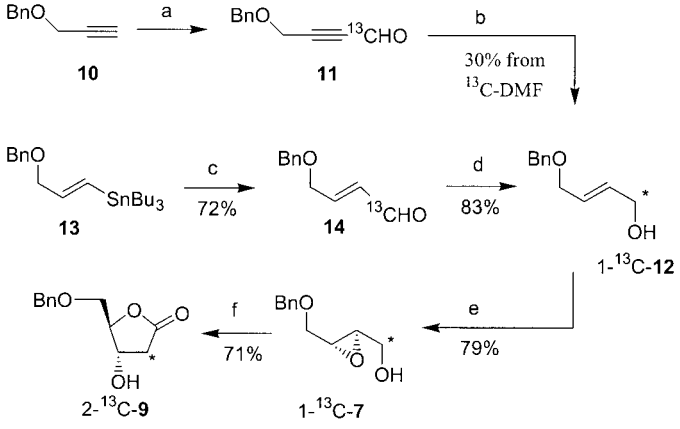


Scheme 1. *Reagents*: (a) K^{13}CN, KI, EtOH–H_2O, Δ, (b) HCl, H_2O.

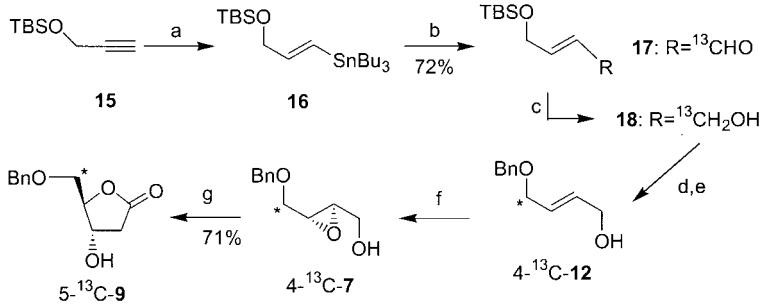
would accommodate the combination of mono-labeled intermediates at early stages of our overall scheme. From the dual perspective of convergence and conceptual simplicity, the well-studied nucleophilic homologation of optically active epoxy alcohols **4**, prepared by Sharpless asymmetric epoxidation,[17] presented an attractive scenario for the asymmetric construction of $^{13}C_1$-deoxyribose derivatives.[18] Capture of $^{13}C_1$-labeled epoxide **4** by a suitable formate anion equivalent would establish the deoxyribose system of **6**, reducing the task of preparing five $^{13}C_1$-labeled deoxyribonolactones to the more manageable challenge of accessing $^{13}C_1$-labeled *E*-1,4-butenediols, **3** (Fig. 2).

The efficacy of this scheme was demonstrated by the synthesis of C1-labeled lactone 1-^{13}C-**9**. Epoxide (2*R*,3*R*)-**7**, prepared in 94% enantiomeric excess by Sharpless epoxidation of *O*-benzyl-1,4-butendiol,[17] was treated with ^{13}C-labeled potassium cyanide using Ganem's general protocol (Scheme 1) to effect Payne rearrangement and subsequent incorporation of the cyano group.[18] Hydrolysis of the resulting hydroxy nitrile **8** under acidic conditions was accompanied by lactonization to yield the C1-labeled deoxyribonolactone 1-^{13}C-**9** in 71% yield from **7**. An enantiomeric excess of 92% was indicated for 1-^{13}C-**9** by analysis with (+)-Eu(hfc)$_3$.[19]

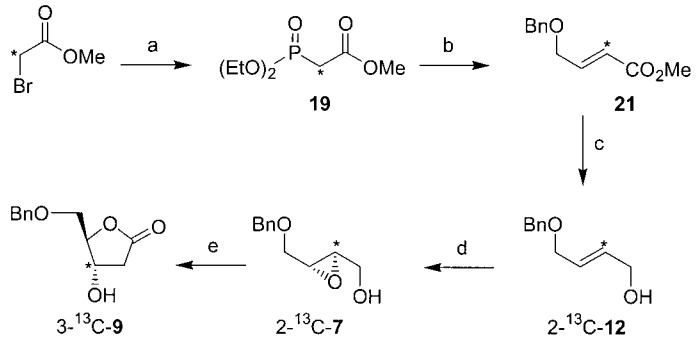
With an effective protocol for the asymmetric construction of the deoxyribonolactone system in hand, we turned our attention to the regioselective synthesis of the $^{13}C_1$-labeled isomers of masked butenediol **3**. As an initial approach to $^{13}C_1$-labeled **3**, we examined the addition of the anion obtained from lithiation of *O*-benzyl propynol **10**[20] to $^{13}C_1$-dimethylformamide (DMF), affording the sensitive C1-labeled aldehyde **11** (Scheme 2). Reduction of **11** and stereoselective transformation of the resulting propargyl alcohol to the desired *E*-alkenol were accomplished in a single step by treatment with $LiAlH_4$, to give allylic alcohol 1-^{13}C-**12** in 30% overall yield, based on consumed $^{13}C_1$-DMF. We attribute the relative inefficiency of this sequence to the marginally effective addition of alkyne **10** to DMF, a transformation typically accompanied by poor yields.[21] Addition of alkenyl anions to DMF constitutes a more reliable synthetic entry to unsaturated aldehydes, and we therefore examined a more efficient route to 1-^{13}C-**12** based on addition of a suitably functionalized propenyl nucleophile to $^{13}C_1$-labeled DMF. Transmetalation of the readily available stannane **13**[22] and addition of the resulting alkenyl lithium reagent to $^{13}C_1$-DMF gave aldehyde **14** which was reduced without further purification to afford the desired alcohol 1-^{13}C-**12** in 60% overall yield. Epoxidation of 1-^{13}C-**12** afforded epoxide 1-^{13}C-**7** (94% enantiomeric excess by Mosher ester analysis[23]) which was subjected to



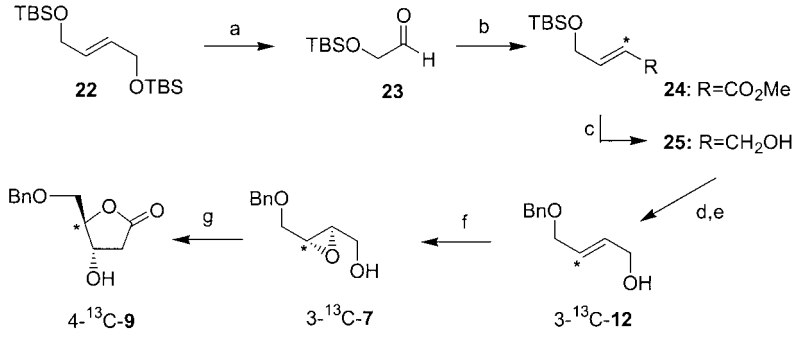
Scheme 2. *Reagents*: (a) *n*BuLi, THF, −78°C, H^{13}C(O)NMe$_2$; (b) $LiAlH_4$, Et_2O, 0° to 25°C; (c) MeLi, Et_2O, −78°C, H^{13}C(O)NMe$_2$; (d) DIBAL, Et_2O, −78°C; (e) TBHP, Ti(O*i*Pr)$_4$, D-(−)-DIPT, DCM, −20°C; (f) KCN, KI, EtOH–H_2O, Δ, then HCl, H_2O.



Scheme 3. *Reagents*: (a) Bu_3SnH, (1.3 equiv.), AIBN, 80°C; (b) *n*BuLi, THF, −78°C, H^{13}C(O)NMe$_2$; (c) DIBAL, Et_2O, −78°C; (d) KH, BnBr, DME; (e) TBAF, H_2O; (f) TBHP, Ti(O*i*Pr)$_4$, D-(−)-DIPT, DCM, −20°C; (g) KCN, KI, EtOH–H_2O, Δ then HCl, H_2O.



Scheme 4. *Reagents*: (a) $P(OEt)_3$, reflux; (b) LDA, THF, $BnOCH_2CHO$ (**20**); (c) DIBAL, Et_2O, −78°C; (d) TBHP, $Ti(OiPr)_4$, D-(−)-DIPT, DCM, −20°C; (e) KCN, KI, EtOH–H_2O, Δ, then HCl, H_2O.



Scheme 5. *Reagents*: (a) O_3, DCM, −78°C; (b) LDA, THF, **19**, −78°C; (c) DIBAL, Et_2O, −78°C; (d) KH, BnBr, DME; (e) TBAF; (f) TBHP, $Ti(OiPr)_4$, D-(−)-DIPT, DCM, −20°C; (g) KCN, KI, EtOH–H_2O, Δ, then HCl, H_2O.

nucleophilic homologation with cyanide ion and subsequent acid-catalyzed lactonization to give the C2-labeled deoxyribonolactone 2-^{13}C-**9**.

The latent symmetry of the 1,4-butenediol system suggested a conceptually similar scheme for the preparation of the C5-labeled lactone 5-^{13}C-**9** (Scheme 3) of **12**. Hydrostannylation of silyl-protected **15** produced a 7:1 mixture of **16** and the regioisomeric 2-tributylstannylpropene. Lithiation of **16** and addition to ^{13}C-DMF yielded **17**, which was reduced without further purification to the silyl-protected alcohol **18**. At this juncture, the butenediol system of **18** was inverted by benzylation and desilylation to form the C4-labeled alcohol 4-^{13}C-**12**. Sharpless epoxidation of 4-^{13}C-**12**, followed by cyanation and lactonizaion of the resulting oxirane 4-^{13}C-**7**, afforded the C5-labeled deoxyribonolactone 5-^{13}C-**9**.

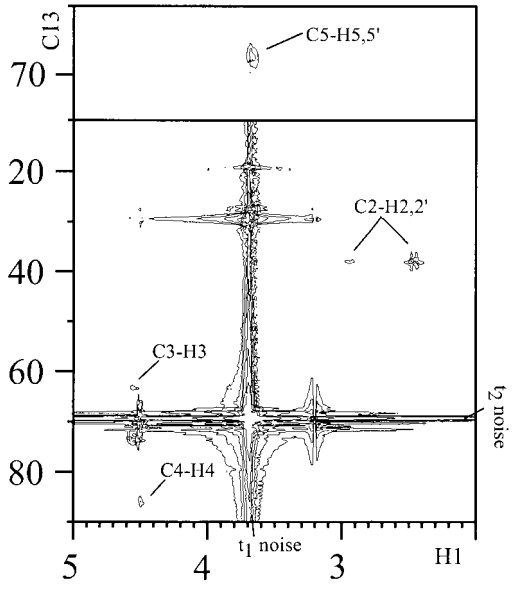


Figure 3. ^{1}H-^{13}C HSQC of 5-^{13}C-**9**.

Our strategy for introduction of ^{13}C-label at C3 and C4 of the deoxyribose system focused on the preparation of the C2 and C3-^{13}C-labeled analogs of **3**. Incorporation of ^{13}C label from phosphonate **19** (prepared in three steps from 2-^{13}C acetic acid[24]) was accomplished by Wadsworth–Emmons–Horner addition of **19** to *O*-benzylglycaldehyde **20**[25] to give the unsaturated ester **21** (Scheme 4). Further chemoselective reduction with DIBAL yielded allylic alcohol 2-^{13}C-**12** in 49% overall yield from **19**. Conversion of 2-^{13}C-**12** to deoxyribonolactone 3-^{13}C-**5** was accomplished by our standard sequence via epoxide 2-^{13}C-**7**.

An analogous scheme was employed for the preparation of lactone 4-^{13}C-**9**. Addition of **19** to silyl-protected aldehyde **23** yielded *E*-unsaturated ester **24**, which upon reduction furnished alcohol 3-^{13}C-**12**. Conversion to 4-^{13}C-**9** was accomplished as described above (Scheme 5).

A representative 2D-spectrum of 5-^{13}C-**9** (Fig. 3) illustrates the ~90-fold increase in sensitivity compared to natural abundance for a regioselectively ^{13}C-labeled ribonolactone. The upper panel shows a region from the HSQC acquired with 4 scans per increment (the minimum number for the pulse program), and shows only ^{1}H–^{13}C-5. The lower

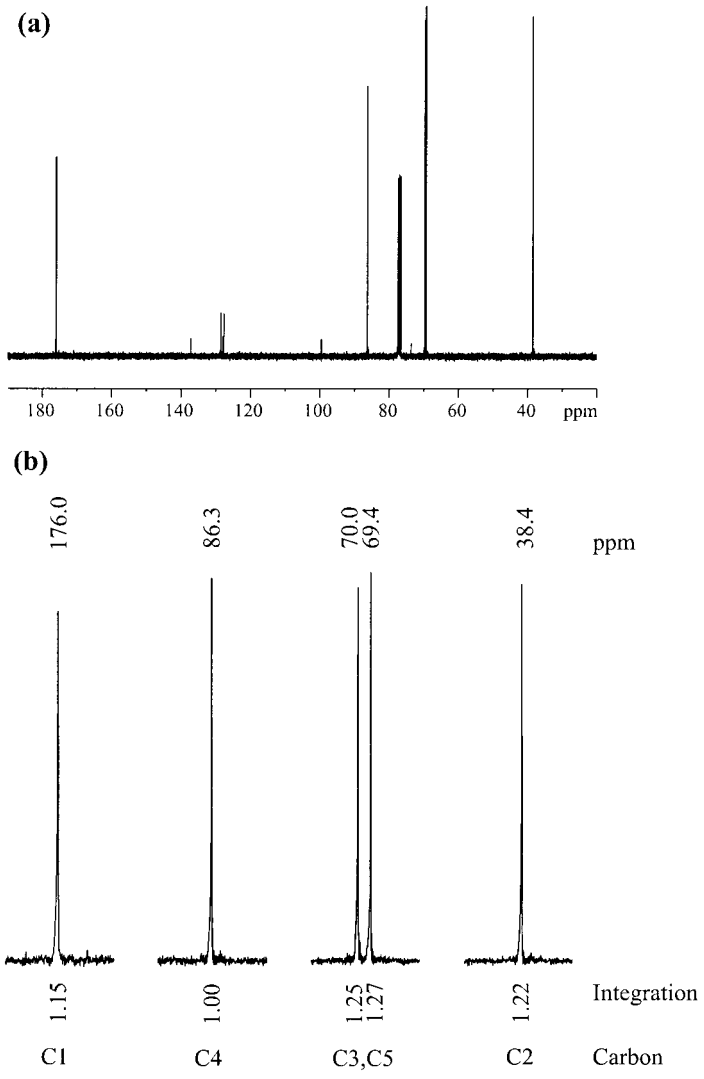
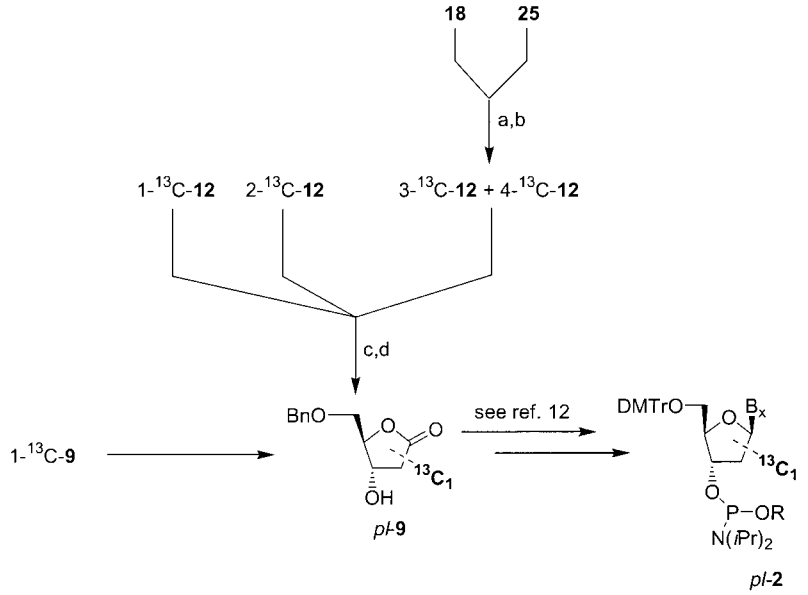


Figure 4. (a) The complete ^{13}C NMR spectra of *pl*-**9**. Non-enriched peaks are as follows: benzyl group-137.2, 128.5, 128.0, 127.6, 73.7; DC offset glitch-100.0; $CDCl_3$-triplet at 77.0. (b) Quantitative ^{13}C of *pl*-**9**. Each panel is a 3 ppm portion of the entire spectrum.



Scheme 6. *Reagents*: (a) KH, BnBr, DME; (b) TBAF; (c) TBHP, $Ti(OiPr)_4$, D-(−)-DIPT, DCM, −20°C; (d) KCN, KI, EtOH–H_2O, Δ, then HCl, H_2O.

HSQC spectrum required 64 scans per increment. The natural abundance signals are shown with only one or two contours; the labeled peak is so intense that its t_2-and t_1-noise ridges dominate the spectrum.

Combination of equimolar quantities of the five $^{13}C_1$-labeled lactones affords the corresponding population-labeled mixture, *pl*-**9**. The ^{13}C NMR spectrum of this mixture, shown in Fig. 4a, demonstrates the 20-fold enhanced ^{13}C sensitivity of at each position of the lactone ring in comparison to the unlabeled 5-*O*-benzyl group. Relative values for the degree of isotopic substitution at each labeled position were obtained by quantitative ^{13}C NMR (Fig. 4b). The chemical shift, integration, and position of each carbon are indicated. An integrated scheme, in which labeled intermediates are strategically combined at key stages of the synthesis, offers an alternative entry to *pl*-**9** with significant gains in efficiency and economy (Scheme 6). Thus, $^{13}C_1$-labeled alcohols **18** and **25** can be combined and the resulting mixture converted to the mixture of alcohols 3-^{13}C-**12** and 4-^{13}C-**12**. Further combination with stoichiometric quantities of alcohols 1-^{13}C-**12** and 2-^{13}C-**12**, followed by our standard sequence, yields a mixture of the 4 mono labeled lactones **9**. Final addition of a stoichiometric amount of 1-^{13}C-**9** yields the population-labeled mixture *pl*-**9**. Overall, this integrated scheme results in the savings of 8 synthetic operations over the independent syntheses and combination of the individual $^{13}C_1$-labeled lactones, and provides an efficient route to population-labeled mixtures of **9**, where each ^{13}C is present at ~20%.

3. Conclusions

In conclusion, we have successfully prepared a series of regioselectively labeled $^{13}C_1$-deoxyribonolactones, compounds that represent key intermediates for the preparation of ^{13}C-labeled nucleotides and carbohydrate-backbone-labeled DNA oligomers. Simple modifications of our general synthetic scheme can be used to prepare all possible ^{13}C-isotopomers of deoxyribose, and will provide access to multiply-labeled deoxyribose derivatives and a wide range of alternative population-labeling scenarios. For example, doubly labeled $^{13}C_1$,$^{13}C_x$-deoxyribose derivatives (x=2,3,4,5) are readily available from the four ^{13}C-**7** isotopomers by using K^{13}CN in the final homologation/ring-closure step of our sequence. Similar modifications to the general synthetic scheme described herein will yield other multiply-labeled schemes of increased efficiency and economy and which represent useful tools for the NMR spectroscopy of nucleic acids. Efforts in these areas are the subject of ongoing investigations and will be the subject of future reports from these laboratories.

4. Experimental

4.1. General

IR spectra were recorded on a Perkin Elmer Paragon 1000 FT spectrometer, with sodium chloride plates, and are reported in wavenumbers (cm^{-1}). ^1H- and ^{13}C NMR were taken on Bruker Avance 300, and General Electric QE-300 MHz spectrometers unless 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. Optical rotations were measured on either Jasco DIP-1000 or a Perkin–Elmer 241 spectrometer. Enantiomeric excess was determined by either Mosher ester derivatization (ME),[23] 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 one of the following: anisaldehyde (method A), phosphomolybdic acid (PMA) (method B). Reactions were performed in flame or oven dried glassware under a nitrogen or argon atmosphere where appropriate. Reagents were used from suppliers without purification unless otherwise indicated.

HSQC spectra were acquired on a Bruker Avance 500 MHz spectrometer, using standard pulse programs with gradients. HSQC spectra were collected with 4 scans per increment, or 64 scans per increment (depending on whether the natural abundance peaks were desired), into 2048×512 points with no sample spinning and no zero-filling. 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.

In contrast to quantitative ^{13}C NMR acquisition parameters typically employed for unlabeled compounds,[26] we have found the following parameters practical for ^{13}C-labeled compounds: an inverse gated pulse program collected 32 k points per scan with a 60 second delay time and a sweep width of 22675 Hz. Data so acquired was zero-filled to 64 k and transformed with no window function.

4.1.1. [1-^{13}C]-(*E*)-4-Benzyloxy-but-2-en-1-ol. Route A (1-^{13}C-12). To a solution of benzyl ether **10** (1.02 g, 7.02 mmol) in 5 mL THF at −78°C was added dropwise *n*BuLi (2.37 mL, 2.5 M in hexanes). The resulting solution was stirred at −78°C for 30 min, at which time ^{13}C-DMF (0.4 g, 5.4 mmol) was added and the mixture was allowed to warm to ambient temperature over 3 h. The mixture was diluted with 10 mL ether and washed successively with 5 mL portions of 10% HCl, sat. aq. $NaHCO_3$, and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated to give the crude aldehyde **11** as a dark oil. The crude reaction product was typically subjected directly to $LiAlH_4$ reduction, but could be further purified by flash chromatography (5:1 hexanes/EtOAc) to give **11** as a pale oil. IR (neat): 1632; ^1H NMR: 4.37 (s, 2H), 4.63 (s, 2H), 7.36 (s, 5H), 9.25 (d, J=194.8 Hz, 1H); ^{13}C NMR: 176.2.

To a 0°C solution of aldehyde **11** in 8 mL ether was slowly added $LiAlH_4$ (0.204 g, 5.4 mmol) and the resulting mixture was allowed to warm to ambient temperature and stirred for 12 h. The reaction mixture was re-cooled to 0°C and quenched by sequential addition of 1 mL of H_2O, 2 mL

20% aq. NaOH, and 1 mL brine. The resulting mixture was diluted with 30 mL ether, filtered, and concentrated to give a pale oil. Flash chromatography (5:1 hexanes/EtOAc) afforded (288 mg, 30% based on consumed ^{13}C DMF) of alcohol 1-^{13}C-12 as a pale oil. R_f=0.29 (1:1 hexanes/EtOAc) method A; IR (neat): 3427; ^{1}H NMR: 1.82 (br s, 1H), 4.04 (d, J=4.8 Hz, 1H), 4.15 (dd, J=142.4, 4.5 Hz, 2H), 5.89 (m, 2H), 7.34 (m, 5H); ^{13}C NMR: 62.9; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_2$, 179.1027; found, 179.0998.

4.1.2. [1-^{13}C]-(*E*)-4-Benzyloxy-but-2-en-1-ol. Route B (1-^{13}C-12). To a solution of stannane **13** (1.765 g, 4.09 mmol) in 5 mL THF at −78°C was added *n*BuLi (1.84 mL, 2.5 M in hexanes). The mixture was stirred for 2 h at −78°C, and was then added to a 0°C solution of ^{13}C-DMF (200 mg, 2.70 mmol) in 1 mL THF. The reaction mixture was stirred for 2.5 h and poured into a separatory funnel containing 5 mL 10% HCl and 10 mL ether. The organic layer was separated and washed with two additional 5 mL portions of HCl. The aqueous layer was extracted with 25 mL of ether, and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give crude aldehyde **14** as a yellow oil. The crude reaction product was typically subjected directly to DIBAL reduction, but could be further purified by flash chromatography (4:1 hexanes/EtOAc) to give (344 mg, 72%) of **14** as a pale oil. IR (neat): 1652; ^{1}H NMR: 4.30 (dd, J=1.45, 5.54 Hz, 2H), 4.60 (s, 2H), 6.45 (m, 2H), 6.89 (m, 2H), 9.59 (dd, J=7.85, 172.5 Hz, 1H); ^{13}C NMR: 193.2.

Crude aldehyde **14** was taken up in 5 mL anhydrous ether, cooled to −78°C and treated with 3.9 mL DIBAL (2 equiv., 1.0 M in hexanes). The reaction mixture was stirred for 2 h at −78°C and quenched by careful addition of 2 mL MeOH and 1 mL H_2O. The resulting mixture was diluted with 20 mL ether and treated with 3 mL aq. Rochelle salt. After transferring to a separatory funnel the aqueous layer was extracted with 10 mL ether and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residual oil was purified by flash chromatography (1:1 hexanes/EtOAc) to give (288 mg, 60% based on consumed ^{13}C-DMF) of 1-^{13}C-12 as a yellow oil. IR (neat): 3427; ^{1}H NMR: 1.82 (br s, 1H), 4.04 (d, J=4.8 Hz, 1H), 4.15 (dd, J=4.5 and 142.4 Hz, 2H), 5.89 (m, 2H), 7.34 (m, 5H); ^{13}C NMR: 62.9; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_2$, 179.1027; found, 179.0998.

4.1.3. *tert*-Butyl-dimethyl-(3-tributylstannanyl-allyloxy)-silane (16). To a round bottom flask containing silyl ether **15** (3.15 g, 18.5 mmol) was added Bu_3SnH (7.01 g, 24.1 mmol) and AIBN (300 mg, 1.85 mmol). The resulting mixture was heated to 80°C for 2 h and then cooled to ambient temperature resulting in a 7:1 mixture of products, as determined by GC. Preparative GC (20% SE-30 on 60/80 Chromosorb W) gave two clear oils. Major product: R_f=0.90 (2:1 hexanes/EtOAc) method B; IR (neat): 2989.5; ^{1}HNMR: 0.07 (s, 6H), 0.88 (m, 6H), 0.91 (s, 9H), 0.96 (s, 9H), 1.29 (m, 6H), 1.54 (m, 6H), 4.21 (d, J=3.7 Hz, 2H), 6.07 (m, 1H), 6.15 (d, J=19.1 Hz, 1H); ^{13}C NMR: 9.4, 13.7, 26.0, 27.3, 29.1, 66.7, 126.9, 147.3. Minor product: IR (neat): 2989.5; ^{1}H NMR: 0.01 (s, 3H), 0.60 (s, 3H), 0.884 (m, 6H), 0.912 (s, 9H), 0.930 (s, 9H), 1.297 (m, 6H), 1.552 (m, 6H), 4.281 (m, 2H), 5.171 (d, J=2.15 Hz, 2H), 5.86 (d, J=2.15 Hz, 2H); ^{13}C NMR: 3.4, 13.7, 26.0, 29.1, 29.7, 61.4, 104.9, 148.5.

4.1.4. [1-^{13}C]-(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-but-2-en-1-ol (18). To a stirred solution of vinyltin reagent (1.806 g, 4.05 mmol) in 6 mL THF was added *n*-BuLi (1.62 mL, 2.5 M in hexanes) at −78°C for 1 h. ^{13}C-DMF, which was prestirred in BaO for 2 h at −78°C, in 2 mL THF was added to the vinyltin solution and the resulting mixture was stirred at −78°C for 2 h. The reaction was quenched by pouring into a separatory funnel containing 10 mL, 10% HCl, and diluted with 50 mL ether. The organic layer was washed with another portion of 10 mL 10% HCl. The organic layer was then separated and dried with $MgSO_4$, filtered, and concentrated to give a yellow oil, which was purified by flash chromatography to give a yellow oil (374 mg, 69% from ^{13}C-DMF). IR (neat): 1659; ^{1}H NMR: 0.08 (s, 6H), 0.90 (s, 9H), 4.44 (m, 2H), 6.42 (m, 1H), 6.89 (m, 1H), 9.60 (dd, J=8.0 and 171.3 Hz, 1H); ^{13}C NMR: 193.2.

The aldehyde was taken up in 9 mL CH_2Cl_2, and treated with DIBAL (2 eq, 3.72 mL, 1.0 M in hexanes) at −78°C. After 35 min, the reaction was quenched by addition of 3 mL MeOH. The quenched reaction mixture was diluted with 40 mL of CH_2Cl_2, 3 mL of 3.0 M Rochelle salt, and stirred overnight. The layers were separated and the aqueous layer was extracted with two 35 mL portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give a yellow oil which was purified by flash chromatography (2:1 hexanes/EtOAc) to provide a yellow oil (339 mg, 90%). Overall yield from two steps: 62%. R_f=0.48 (2:1 hexanes/EtOAc) method A; IR (neat): 3365; ^{1}H NMR: 0.08 (s, 6H), 0.90 (s, 9H), 4.16 (dd, J=4.8 and 142.2 Hz, 2H), 4.39 (d, J=4.8 Hz, 2H), 5.84 (m, 2H); ^{13}C NMR: 63.2; HRMS (m/z): $[M+H]^+$ calcd for $[^{13}C]C_{10}H_{15}O_2$, 204.1600; found, 204.1493.

4.1.5. [4-^{13}C]-(*E*)-4-Benzyloxy-but-2-en-1-ol (4-^{13}C-12). To a stirred solution of alcohol **17** (205 mg, 1.0 mmol) in 5 mL DME was added NaH (27 mg, 1.1 mmol) at 0°C. The reaction was stirred for 10 min, at which time benzyl bromide (207 mg, 1.2 mmol) was added and the mixture allowed to warm to ambient temperature. After 3 h, the mixture was diluted with ether and transferred to a separatory funnel. The organic layer was washed with 10 mL H_2O, and then dried with $MgSO_4$, filtered, and concentrated to give a yellow oil which was purified by flash chromatography (10:1 hexanes/EtOAc) to give of the benzyl ether as a yellow oil. IR (neat): 3395; ^{1}H NMR: 014 (s, 6H), 0.98 (s, 9H), 4.14 (dd, J=142.3 and 4.8 Hz, 2H), 4.33 (d, J=4.8 Hz, 2H), 4.56 (d, J=5.3 Hz, 2H), 5.89 (m, 2H), 7.36 (m, 5H); ^{13}C NMR: 70.2.

To a solution of benzyl ether (400 mg, 1.52 mmol) in 10 mL THF at 0°C was added 3.0 mL TBAF (1.0 M, 3.0 mmol). After stirring for 30 min, the reaction mixture was diluted with ether and transferred to a separatory funnel where the organic layer was washed with H_2O. The aqueous layer was extracted with ether and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated to give a pale oil. Purification by flash chromatography (1:1 hexanes/EtOAc) afforded (280 mg, 63%) of allylic alcohol, 4-^{13}C-

12 as a pale yellow oil. IR (neat): 3380; ^{1}H NMR: 1.69 (br s, 1H), 4.04 (dd, J=5.1, 141.4 Hz, 2H), 4.16 (d, J=4.4 Hz, 2H), 4.54 (s, 2H), 5.89 (m, 2H), 7.34 (m, 5H); ^{13}C NMR: 70.2; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_2$, 179.1027; found, 179.1007.

4.1.6. [2-^{13}C]-(*E*)-4-Benzyloxy-but-2-en-1-ol (2-^{13}C-12). [2-^{13}C]-*(E)*-4-Benzyloxy-but-2-enoic acid methyl ester (100 mg, 0.49 mmol) was dissolved in 5 mL ether, cooled to 0°C and then treated with DIBAL (1.3 mL, 1.0 M in hexanes). The reaction was stirred at 0°C for about 10 min. The reaction was quenched with H_2O, and treated with Rochelle salt (satd. soln., 5 mL) and stirred for another 1 h. The organic phase was then separated, dried, and concentrated. The crude product was purified by flash chromatography (10:1 hexanes/EtOAc) to afford the allylic alcohol (55 mg, 76%). IR (neat): 3536.2; ^{1}H NMR: 1.61 (br s, 1H), 4.04 (m, 2H), 4.16 (m, 2H), 4.53 (s, 2H), 5.85 (m, 1H), 5.92 (dm, J=149.0 Hz, 1H), 7.34 (m, 5H); ^{13}C NMR: 132.2; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_2$, 179.1027; found, 179.1001.

4.1.7. [2-^{13}C]-(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-but-2-enoic acid methyl ester (24). To a suspension of washed NaH (90 mg, 3.75 mmol) in 8 mL DME at ambient temperature was added dropwise a solution of [2-^{13}C]-(diethoxy-phosphoryl)-acetic acid methyl ester (784 mg, 3.7 mmol) in 2 mL DME. The resulting solution was stirred for 1 h, at which time a solution of (*tert*-butyl-dimethyl-silanyloxy)-acetaldehyde (974 mg, 5.6 mmol) in 2 mL DME was added dropwise. The reaction mixture was stirred at ambient temperature for 1 h and then quenched by addition of 1 mL H_2O. The mixture was transferred to a separatory funnel and extracted with two 10 mL portions of ether and then combined organic phases washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated. The residual dark oil was purified by flash chromatography to afford (550 mg, 64%) as an oil. R_f=0.58 (3:1 hexanes/EtOAc) method A; IR (neat): 1723; ^{1}NMR: 0.10 (s, 6H), 0.94 (s, 9H), 3.76 (s, 3H), 4.35 (m, 2H), 6.12 (ddt, J=165.33, 15.5, 2.27 Hz, 1H), 7.03 (ddd, J=15.5, 3.24, 3.12 Hz, 1H); ^{13}C NMR: 119.2; HRMS (m/z): $[M+H]^+$ calcd for $[^{13}C]C_{10}H_{22}O_3Si$, 232.1450; found, 232.1449.

4.1.8. [2-^{13}C]-(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-but-2-en-1-ol (17). The [2-^{13}C]-(*E*)-4-(*tert*-Butyl-dimethyl-silanyloxy)-but-2-enoic acid methyl ester **19** (200 mg, 0.87 mmol) was dissolved in 10 mL ether, cooled to 0°C and then treated with DIBAL (1.74 mL, 1.0 M in hexanes). The reaction was stirred at 0°C for about 30 min. The reaction was then quenched with H_2O, treated with Rochelle salt (sat. soln., 5 mL) and stirred for 2 h. The organic phase was separated, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography (10:1 hexanes/EtOAc) to afford (126 mg, 72%). IR (neat): 3346.7, 2919.8, 2848.6, 1463.7, 1250.2; ^{1}H NMR: 0.09 (s, 6H), 0.92 (s, 9H), 4.20 (m, 4H), 5.80 (m, 1H), 5.62 and 6.15 (dm, J=154.8 Hz, 1H); ^{13}C NMR: 128.9; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{22}O_2Si$, 203.1423; found, 203.1424.

4.1.9. [3-^{13}C]-(*E*)-4-Benzyloxy-but-2-en-1-ol (3-^{13}C-12). The [2-^{13}C]-(*E*)-4-(*tert*-butyl-dimethyl-silanyloxy)-but-2-en-1-ol (1.327 mg, 6.5 mmol) was dissolved in 25 mL DME and cooled to 0°C and then treated with washed NaH (334 mg, 14.3 mmol). The mixture was stirred at 0°C for 5 min and benzyl bromide (1.34 g, 7.85 mmol) was added in 2 mL DME. The reaction was then stirred at ambient temperature for 10 h. The reaction was quenched with H_2O, separated and the organic layer extracted with ether, dried over $MgSO_4$, filtered, and concentrated. The crude product was dissolved in 70 mL THF and then treated with TBAF (20 mL, 1.0 M in THF). The mixture was stirred at ambient temperature for 5 h. The reaction was then transferred to a separatory funnel and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography to afford (920 mg, 79%) of a yellow oil. IR (neat): 3537.0; ^{1}NMR: 1.54 (br s, 1H), 4.04 (m, 2H), 4.18 (m, 2H), 4.53 (s, 2H), 5.86 (dm, 155.5, 1H), 5.92 (m, 1H), 7.34 (m, 5H); ^{13}C NMR: 127.8; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_2$, 179.1027; found, 179.1010.

4.2. Representative procedure for Sharpless epoxidation of 4-benzyloxy-but-2-en-1-ols

4.2.1. [1-^{13}C]-(2*R*,3*R*)-(3-Benzyloxymethyl-oxiranyl)-methanol (1-^{13}C-7). To a round bottom flask was added powdered, activated 4 Å molecular sieves (120 mg), and a stir bar. The round bottom flask was then flame dried and allowed to cool to ambient temperature under nitrogen. Methylene chloride (3.5 mL), D-(−)-diisopropyl tartrate (34 mg, 0.146 mmol), and Ti(O*i*Pr)$_4$ (32 mg, 0.114 mmol) 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 (100 mg, 0.558 mmol) was diluted in 2.5 mL CH_2Cl_2 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 2 h. The quenched mixture was filtered through a frit and concentrated to yield a yellow crude product that was purified by flash chromatography to afford (86.6 mg, 79%) as a colorless oil. R_f=0.25 (1:1 hexanes/EtOAc); ee=94% (ME); $[\alpha]_D^{25}$=+19.6[27] (c 0.24, $CHCl_3$); IR (neat): 3427.9, 2862.7, 1496.9, 1103.4; ^{1}H NMR: 1.75 (br s, 1H), 3.10 (m, 1H), 3.25 (m, 1H), 3.52 (dd, J=5.5 Hz, 11.5, 1H), 3.66 (dm, J=142.7 Hz, 1H), 3.77 (dd, J=2.9, 11.5 Hz, 1H), 3.95 (m, J=143.3 Hz, 1H), 4.57 (ABq, J=12.2 Hz, 2H), 7.32 (m, 5H); ^{13}C NMR: 61.1; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_3$, 195.0976; found, 195.1014.

4.2.2. [2-^{13}C]-(2*R*,3*R*)-(3-Benzyloxymethyl-oxiranyl)-methanol (2-^{13}C-7). Ee=90%(ME); $[\alpha]_D^{25}$=+19.0 (c 0.31, $CHCl_3$); IR (neat): 3427.9, 2862.7, 1496.9, 1103.4; ^{1}H NMR: 1.75 (br t, 1H), 3.10 (dm, J=173.4 Hz, 1H), 3.25 (m, 1H), 3.52 (ddd, J=2.5,5.5, 11.5 Hz, 1H), 3.66 (m, 1H), 3.77 (dt, J=2.7, 11.5 Hz, 1H), 3.95 (m, 1H), 4.57 (ABq, J=12.2 Hz, 2H), 7.32 (m, 5H); ^{13}C NMR: 55.7; HRMS (m/z): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_3$, 195.0976; found, 195.1011.

4.2.3. [3-^{13}C]-(2*R*,3*R*)-(3-Benzyloxymethyl-oxiranyl)-methanol (3-^{13}C-7). Ee=93%(ME); $[\alpha]_D^{25}$=+18.6 (*c* 0.42, $CHCl_3$); IR (neat): 3427.9, 2862.7, 1496.9, 1103.4; ^{1}H NMR: 1.75 (br s, 1H), 3.10 (m, 1H), 3.25 (m, *J*=226.5 Hz, 1H), 3.52 (m, 1H), 3.66 (m, 1H), 3.77 (m, 1H), 3.95 (dm, *J*=143.3 Hz, 1H), 4.57 (ABq, *J*=12.2 Hz, 2H), 7.32 (m, 5H); ^{13}C NMR: 54.7; HRMS (*m/z*): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_3$, 195.0976; found, 195.0994.

4.2.4. [4-^{13}C]-(2*R*,3*R*)-(3-Benzyloxymethyl-oxiranyl)-methanol (4-^{13}C-7). Ee=95%(ME); $[\alpha]_D^{25}$=+19.0 (*c* 0.28, $CHCl_3$); IR (neat): 3427.9, 2862.7, 1496.9, 1103.4; ^{1}H NMR: 1.75 (br t, 1H), 3.10 (m, 1H), 3.25 (m, 1H), 3.52 (ddd, *J*=5.3, 11.5, 141.4 Hz, 1H), 3.66 (m, 1H), 3.77 (ddd, *J*=2.6, 11.2, 126.8 Hz, 1H), 3.95 (m, 1H), 4.57 (dABq, *J*=4.4, 12.2 Hz, 2H), 7.32 (m, 5H); ^{13}C NMR: 69.6; HRMS (*m/z*): $[M]^+$ calcd for $[^{13}C]C_{10}H_{14}O_3$, 195.0976; found, 195.1015.

4.3. Representative procedure for preparation of [^{13}C]-5-*O*-benzyl-2-deoxyribonolactones

4.3.1. [1-^{13}C]-(4*S*,5*R*)-5-Benzyloxymethyl-4-hydroxy-dihydro-furan-2-one (1-^{13}C-9). To a solution of epoxide 7 (2.94 g, 15 mmol) in 120 mL of 2:1 EtOH/H_2O, was added $K^{13}CN$ (1.5 g, 22.5 mmol) and KI (24 mg, 0.15 mmol). The reaction mixture was heated at reflux for 48 h, cooled to ambient temperature and carefully acidified to pH 2 with 10% HCl. The resulting mixture was warmed to 60°C and stirred for 16 h, at which time the reaction mixture was cooled and transferred to a separatory funnel and the aqueous layer was extracted with three 50 mL 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 (2.19 g, 71%) of lactone **1**-^{13}**C-9** as a colorless oil. ee=92% (CSA); $[\alpha]_D^{25}$=+2.3 (*c* 0.23, $CHCl_3$); R_f=0.27 (1:1 hexanes/EtOAc); IR (neat): 3434, 1777; ^{1}NMR: 2.09 (br d, 1H), 2.48 (ddd, *J*=2.6, 5.1, 17.9 Hz, 1H), 2.98 (td, *J*=6.6, 18.0 Hz, 1H), 3.68 (dd, *J*=3.5, 10.5 Hz, 1H), 3.74 (dd, *J*=3.0, 10.5 Hz, 1H), 4.49 (m, 1H), 4.56 (ABq, *J*=11.8 Hz, 2H), 4.59 (m, 1H), 7.34 (m, 5H); ^{13}C NMR: 176.0; HRMS (*m/z*): $[M+H]^+$ calcd for $[^{13}C]C_{11}H_{15}O_4$, 224.1004; found, 224.1005.

4.3.2. [2-^{13}C]-(4*S*,5*R*)-5-Benzyloxymethyl-4-hydroxy-dihydro-furan-2-one (2-^{13}C-9). Ee=94%(CSA); $[\alpha]_D^{25}$=+2.3 (*c* 0.18, $CHCl_3$); IR (neat): 3433.7, 1776.7; ^{1}NMR: 2.11 (br s, 1H), 2.48 (ddd, *J*=2.7, 18.0, 136.1 Hz, 1H), 2.98 (ddd, *J*=6.8, 18.0, 133.7 Hz, 1H), 3.68 (dd, *J*=3.7, 10.5 Hz, 1H), 3.74 (dd, *J*=3.7, 10.5 Hz, 1H), 4.49 (m, 1H), 4.56 (ABq, *J*=11.8 Hz, 2H), 4.59 (m, 1H), 7.34 (m, 5H); ^{13}C NMR: 38.4; HRMS (*m/z*): $[M+H]^+$ calcd for $[^{13}C]C_{11}H_{15}O_4$, 224.1004; found, 224.1004.

4.3.3. [3-^{13}C]-(4*S*,5*R*)-5-Benzyloxymethyl-4-hydroxy-dihydro-furan-2-one (3-^{13}C-9). Ee=90%(CSA); $[\alpha]_D^{25}$=+2.2 (*c* 0.14, $CHCl_3$); IR (neat): 3433.7, 1776.7; ^{1}NMR: 2.09 (br s, 1H), 2.48 (ddd, *J*=2.4, 5.3, 18.0 Hz, 1H), 2.98 (ddd, *J*=0.9, 6.6, 18.0 Hz, 1H), 3.68 (m, 1H), 3.74 (m, 1H), 4.49 (m, 1H), 4.56 (ABq, *J*=11.8 Hz, 2H), 4.59 (dm, *J*=153.4 Hz, 1H), 7.34 (m, 5H); ^{13}C NMR: 69.6; HRMS (*m/z*): $[M+H]^+$ calcd for $[^{13}C]C_{11}H_{15}O_4$, 224.1004; found, 224.1008.

4.3.4. [4-^{13}C]-(4*S*,5*R*)-5-Benzyloxymethyl-4-hydroxy-dihydro-furan-2-one (4-^{13}C-9). Ee=93%(CSA); $[\alpha]_D^{25}$=+2.3 (*c* 0.21, $CHCl_3$); IR (neat): 3433.7, 1776.7; ^{1}NMR: 2.29 (br s, 1H), 2.48 (td, *J*=2.7, 18.0 Hz, 1H), 2.98 (ddd, *J*=2.2, 6.7, 18.0 Hz, 1H), 3.68 (dd, *J*=3.7, 10.5 Hz, 1H), 3.74 (m, 1H), 4.49 (m, 1H), 4.56 (ABq, *J*=11.8 Hz, 2H), 4.59 (dm, *J*=153.4 Hz, 1H), 7.34 (m, 5H); ^{13}C NMR: 86.4; HRMS (*m/z*): $[M+H]^+$ calcd for $[^{13}C]C_{11}H_{15}O_4$, 224.1004; found, 224.1008.

4.3.5. [5-^{13}C]-(4*S*,5*R*)-5-Benzyloxymethyl-4-hydroxy-dihydro-furan-2-one (5-^{13}C-9). Ee=95%(CSA); $[\alpha]_D^{25}$+2.4 (*c* 0.16, $CHCl_3$); IR (neat): 3433.7, 1776.7; ^{1}NMR: 1.07 (br s, 1H), 2.48 (dd, *J*=2.8, 18.0, 1H), 2.98 (dd, *J*=6.8, 18.0, 1H), 3.68 (dd, *J*=10.6, 143.2H), 3.74 (dd, *J*=2.9, 10.5 Hz, 1H), 4.49 (m, 1H), 4.56 (ABq, *J*=11.9, 2H), 4.59 (m, 1H), 7.34 (m, 5H); ^{13}C NMR: 69.4; HRMS (*m/z*): $[M+H]^+$ calcd for $[^{13}C]C_{11}H_{15}O_4$, 224.1004; found, 224.1005.

Acknowledgements

Support for this work in the form of research grants from the National Institutes of Health (GM-35069, GM-32691). A generous gift of 2-^{13}C acetic acid from Cambridge Isotope Laboratories, through their Grants Program is gratefully acknowledged.

References

1. (a) Ikura, M.; Kay, L. E.; Bax, A. *Biochemistry* **1990**, *29*, 4659. (b) Kay, L. E.; Clore, G. M.; Bax, A.; Gronenborn, A. M. *Science* **1990**, *249*, 411. (c) Robertson, A. D.; Rhyu, G. I.; Westler, W. M.; Markley, J. L. *Biopolymers* **1990**, *29*, 461. (d) Cavanagh, J.; Fairbrother, W. J.; Palmer III, A. G.; Skelton, N. J. *Protein NMR Spectroscopy: Principles and Practice*, Academic: San Diego, CA, 1996. (e) Clore, G. M.; Gronenborn, A. M. *NMR of Proteins*, CRC: Boca Raton, FL, 1993.
2. (a) Reilly, D.; Fairbrother, W. J. *J. Biomol. NMR* **1994**, *4*, 459. (b) Hansen, A. P.; Petros, A. M.; Mazer, A. P.; Pederson, T. M.; Rueter, A.; Fesik, S. W. *Biochemistry* **1992**, *31*, 12713.
3. (a) Batey, R. T.; Battiste, J. L.; Williamson, J. R. *Methods Enzymol.* **1995**, *261*, 300. (b) King, G. C.; Harper, J. W.; Xi, Z. *Methods Enzymol.* **1995**, *261*, 436. (c) Nikonowicz, E. P.; Sirr, A.; Legault, P.; Jucker, F. M.; Baer, L. M.; Pardi, A. *Nucleic Acid Res.* **1992**, *20*, 4507.
4. (a) Masse, J. E.; Bortmann, P.; Dieckmann, T.; Feigon, J. *Nucleic Acids Res.* **1998**, *26*, 2618. (b) Louis, J. M.; Martin, R. G.; Clore, G. M.; Gronenborn, A. M. *J. Biol. Chem.* **1998**, *273*, 2374. (c) Smith, D. E.; Su, J. Y.; Jucker, F. M. *J. Biomol. NMR* **1997**, *10*, 245. (d) Zimmer, D. P.; Crothers, D. M. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3091.
5. For a review see: Serianni, A. S.; Barker, R. 11. In *Isotopes in the Physical and Biomedical Sciences*, Buncel, E., Jones, J. R., Eds.; Elsevier: Amsterdam, 1987; pp 211.
6. (a) SantaLucia, Jr., J.; Shen, L. X.; Cai, Z.; Lewis, H.; Tinoco,

Jr., I. *Nucleic Acids Res.* **1995**, *23*, 4913. (b). Williamson, J. R.; Boxer, S. G. *Nucleic Acid Res.* **1988**, *16*, 1529.

7. (a) Synder, J. R.; Serianni, A. S. *Carbohydr. Res.* **1987**, *163*, 169. (b) Hayes, M. L.; Pennings, N. J.; Serianni, A. S.; Barker, R. *J. Am. Chem. Soc.* **1982**, *104*, 6764. (c) Kline, P. C.; Serianni, A. S. *J. Am. Chem. Soc.* **1990**, *112*, 7373. (d) Wu, J.; Serianni, A. S. *Biopolymers* **1994**, *34*, 1175. (e) In addition, Serianni and Bondo have described the syntheses of 26 of the 32 ^{13}C-labeled isotopomers of D-ribose. See: Serianni, A. S.; Bondo, P. B. *J. Biomol. Struct. Dyn.* **1994**, *11*, 1133.
8. (a) Gaudin, R.; Paquet, F.; Chanteloup, L.; Beau, J. -M.; Thuong, N. T.; Lancelot, G. *J Biomol. NMR* **1995**, *5*, 49. (b) Bornet, O.; Lancelot, G.; Chanteloup, L.; Nguyen, T. T.; Beau, J. M. *J. Biomol. NMR* **1994**, *4*, 575.
9. (a) Ono, A.; Tate, S.; Ishide, Y.; Kainosho, M. *J. Biomol. NMR* **1994**, *4*, 581. (b) Tate, S.; Ono, A.; Kainosho, M. *J. Magn. Reson. B* **1995**, *106*, 89. (c) Tolbert, T. J.; Williamson, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 12100.
10. (a) Borer, P. N.; LaPlante, S. R.; Hakkinen, A.; Kumar, A.; Levy, G. C.; Martin, A.; Zanatta, N. *Biochemistry* **1994**, *33*, 2441. (b) LaPlante, S. R.; Hakkinen, A.; Levy, G. C.; van der Marel, J.; Wang, A. H. -J.; Zanatta, N.; Borer, P. N. *Biochemistry* **1994**, *33*, 2430. (c) Ashcroft, J.; LaPlante, S. R.; Borer, P. N.; Cowburn, D. *J. Am. Chem. Soc.* **1989**, *111*, 363. (d) LaPlante, S. R.; Ashcroft, J.; Cowburn, D.; Levy, G. C.; Borer, P. N. *J. Biomol. Struct. Dyn.* **1988**, *5*, 1089. (e) Borer, P. N.; LaPlante, S. R.; Zanatta, N.; Levy, G. C. *Nucleic Acids Res.* **1988**, *16*, 2323. (f) Stone, M. P.; Winkle, S. A.; Borer, P. N. *J. Biomol. Struct. Dyn.* **1986**, *3*, 767. (g) Borer, P. N.; Zanatta, N.; Holak, T. A.; Levy, G. C.; van Boom, J. H.; Wang, A. H. -J. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1373.
11. Labels for compounds are given two designations: (1) either the position of the ^{13}C-label, or *pl* for population labeled (e.g. *pl*-**1**), and (2) a number for the class of compound. Therefore, all epoxides are **7**, but 4-^{13}C-**7** is ^{13}C labeled at the 4 position. In figures the ^{13}C-label is indicated by *.
12. (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859. (b) McBride, L. J.; Caruthers, M. H. *Tetrahedron Lett.* **1983**, *24*, 245. (c) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223. (d) Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987**, *154*, 287. (e) Tanaka, T.; Letsinger, R. L. *Nucleic Acids Res.* **1984**, *12*, 6827. (f) Hubbard, A. J.; Jones, A. S.; Walker, R. T. *Nucleic Acids Res.* **1984**, *12*, 6827.
13. Unlabeled sites still contribute 1.1% natural abundance. Therefore, if 1/5 of the mixture is a 3-$^{13}C_1$-labeled lactone, and the other 4/5 of the mixture is 1.1% then $(1/5)^{*}99\%+(4/5)^{*}1.1\approx 21\%$.
14. (a) Tjandra, N.; Bax, A. *Science* **1997**, *278*, 1111. (b) Hansen, M. R.; Hanson, P.; Pardi, A. *Methods Enzymol.* **2000**, *317*, 220.
15. Scremin, C. L.; Zhou, L.; Srinivasacher, K.; Beaucage, S. L. *J. Org. Chem.* **1994**, *59*, 1963.
16. McGarvey, G. J.; Kimura, M.; Oh, T.; Williams, J. M. *J. Carbohydr. Chem.* **1984**, *3*, 125.
17. (a) Katsuki, T.; Lee, A. W. M.; Ma, P.; Martin, V. S.; Masamune, S.; Sharpless, K. B.; Tuddenham, D.; Walker, F. J. *J. Org. Chem.* **1982**, *47*, 1373. (b) Ma, P.; Martin, V. S.; Masamune, S.; Sharpless, K. B.; Viti, S. M. *J. Org. Chem.* **1982**, *47*, 1378.
18. Wrobel, J. E.; Ganem, B. *J. Org. Chem.* **1983**, *48*, 3761. A conceptually similar approach was employed for the synthesis of a perdeuterated deoxyribose derivative: see Goering, B. K.; Lee, K.; An, B.; Cha, J. K.; *J. Org. Chem.* **1993**, *58*, 1100.
19. Fraser, R. R.; Petit, M. A.; Saunders, J. K. *J. Chem. Soc., Chem. Commun.* **1971**, 1450.
20. Benzyl ether **10** and silyl ether **15** were synthesized from propargyl alcohol and the appropriate protecting group using common conditions.
21. (a) Jones, E. R. H.; Skattebol, L.; Whiting, M. C. *J. Chem. Soc.* **1958**, 1054. (b) Journet, M.; Dongwei, C.; DiMichele, L. M.; Larsen, R. D. *Tetrahedron Lett.* **1998**, *39*, 6427.
22. Jung, M. E.; Light, L. A. *Tetrahedron Lett.* **1982**, 3851.
23. (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512. (b) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143.
24. Samuel, S. P.; Niu, T.-Q.; Erickson, K. L. *J. Am. Chem. Soc.* **1989**, *111*, 1429.
25. Both **20** and **23** were synthesized by the following procedure: (1) di-protection of 2-butene-1,4-diol with either TBSCl or BnBr and (2) ozonolysis of the double bond.
26. Braun, S.; Kalinowski, H.-O.; Berger, S. 150 and More Basic NMR Experiments. Wiley-VCH: New York, 1998; pp 298.
27. The literature value for (2*S*,3*S*)-(3-benzyloxymethyl-oxiranyl)-methanol: ($\alpha_D^{24}=-21$ see Ref. 15b).