Found: C, 67.77; H, 7.91; N, 2.11; S, 4.86.

The assignments for the preceding ^1H NMR data were confirmed by decoupling experiments, of particular significance being the observation that irradiation at δ 2.43 caused collapse of the signal at δ 3.49/3.51.

9α-Methoxy-9β,6-nitrilo- and 9β-Methoxy-9α,6-nitrilo-7-thia-PGF$_1$ Methyl Ester (47) and Their 15β-Epimers (48). Compound **46** was deprotected with *p*-toluenesulfonic acid as above to give **47** and **48** (73%), which were separated by column chromatography (silica gel, 2% methanol/ether).

47: R_f 0.25 (silica gel; ether); IR ($CHCl_3$) 3680 (m, OH), 3490 (m, OH), 3450 (m, OH), 1730 (s, C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3 H, s, CH_3-20), 1.24–1.78 (12 H, CH_2-3,4,16,17,18,19), 1.90 (dd, J = 14.0, 10.5 Hz, CH_2-10α of cis isomer), 2.10 (dd, J = 13.0, 9.0 Hz, CH_2-10α of trans isomer) (δ 1.90/δ 2.10 ratio of 2:1, total 1 H), 2.22 (m, CH-12 of cis isomer), 2.52 (m, CH-12 of trans isomer) (δ 2.22/δ 2.52 ratio of ca. 2:1, total 1 H), 2.36 (2 H, t, CH_2-2), 2.58 (2 H, m, CH_2-5), 2.78 (1 H, m, CH_2-10β), 3.14 (s, CH_3O-9 of cis isomer), 3.26 (s, CH_3O-9 of trans isomer) (δ 3.14/δ 3.26 ratio of 2:1, total 3 H), 3.51 (d, J = 10.0 Hz, CH-8 of cis isomer), 4.20 (d, J = 7.2 Hz, CH-8 of trans isomer) (δ 3.51/δ 4.20 ratio of ca 2:1, total 1 H), 3.94 (1 H, m, CHOH-11), 4.09 (1 H, m, CHOH-15), 5.55–5.70 (2 H, m, CH=CH); mass spectrum, m/e 413 (M$^+$), 395, 382, 364, 351, 310, 239, 221; exact mass, m/e 413.2226 (calcd for $C_{21}H_{35}NO_5S$ 413.2236).

48: R_f 0.30 (silica gel, ether); spectral data for this isomer were almost identical with those of **47**.

9α-Methoxy-9β,6-nitrilo- and 9β-Methoxy-9α,6-nitrilo-7-thia-PGF$_1$ (49). Saponification of **47** by the above procedure gave **49**: ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3 H, t, CH_3-20), 1.22–1.80 (12 H, m, CH_2-3,4,16,17,18,19), 1.95 (dd, CH_2-10α of cis isomer), 2.08 (dd, CH_2-10α of trans isomer), 2.22 (m, CH-12 of cis isomer), 2.38 (2 H, t, CH_2-2), 2.53 (m, CH-12 of trans isomer), 2.62 (2 H, m, CH_2-5), 2.78 (1 H, dd, CH_2-10β), 3.12 (s, CH_3O-9 of cis isomer), 3.26 (s, CH_3O-9 of trans isomer), 3.49 (d, CH-8 of cis isomer), 3.90 (1 H, m, CHOH-11), 4.06 (1 H, m, CHOH-15), 4.18 (d, CH-8 of trans isomer), 4.40–4.90 (3 H, br, OH's, CO_2H), 5.50–5.68 (2 H, m, CH=CH); mass spectrum, m/e 339 (M$^+$), 381, 367, 349, 296, 278, 250, 221.

9α-Methoxy-9β,6-nitrilo- and 9β-Methoxy-9α,6-nitrilo-7-thia-15β-PGF$_1$ (50). Saponification of **48** as above gave **50** with spectral data almost identical with those of **49**.

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Supplementary Material Available: Experimental data for 1-(phenylthio)-2(*E*)-octene, **25**, **26**, **28**, **29**, **31**, and **32** (2 pages). Ordering information is given on any current masthead page.

Registry No. **2**, 82945-91-3; **3**, 82945-92-4; **6**, 7129-41-1; **7**, 85250-37-9; 3β-**8**, 85281-34-1; 3α-**8**, 82945-80-0; 1β-**9**, 82978-40-3; 1α-**9**, 82945-81-1; 1β-**10**, 85250-38-0; 1α-**10**, 85281-35-2; 1β-**11**, 85281-36-3; 1α-**11**, 85281-37-4; 3β-**12**, 85281-38-5; 3α-**12**, 85281-39-6; 3β-**13**, 82978-09-4; 3α-**13**, 82978-04-9; **14**, 53398-57-5; **15**, 82948-77-4; **16**, 85250-39-1; **17**, 85250-40-4; **18**, 74365-04-1; **19**, 56831-18-6; **20**, 85250-41-5; 1β-**21**, 85250-42-6; 1α-**21**, 85281-40-9; 1β-**22**, 85250-43-7; 1α-**22**, 85281-41-0; 3β-**23**, 85281-42-1; 3α-**23**, 85281-43-2; 3β-**24**, 82978-10-7; 3α-**24**, 82945-83-3; 3β-**25**, 85250-44-8; 3α-**25**, 85281-44-3; 3β-**26**, 85250-45-9; 3α-**26**, 85281-45-4; 3β-**27**, 82978-11-8; 3α-**27**, 82945-84-4; 3β-**28**, 85250-46-0; 3α-**28**, 85281-46-5; 3β-**29**, 85250-47-1; 3α-**29**, 85281-47-6; 3β-**30**, 82978-12-9; 3α-**30**, 82945-85-5; 3β-**31**, 85250-48-2; 3α-**31**, 85281-48-7; 3β-**32**, 85250-49-3; 3α-**32**, 85281-49-8; **33** (isomer 1), 82978-13-0; **33** (isomer 2), 82945-86-6; **33** (isomer 3), 82978-15-2; **33** (isomer 4), 82978-14-1; 15β-**35**, 85250-50-6; 15α-**35**, 85281-50-1; 15β-**36**, 85250-51-7; 15α-**36**, 85281-51-2; 3β-**37**, 85250-52-8; 3α-**37**, 85281-54-5; **38**, 82945-87-7; **39**, 82945-96-8; 15β-**40**, 82978-17-4; 15α-**40**, 82945-89-9; 15β-**41**, 82978-18-5; 15α-**41**, 82945-90-2; 15β-**42**, 85250-53-9; 15α-**42**, 85281-52-3; 15β-**43**, 85250-54-0; 15α-**43**, 85281-53-4; **44**, 82978-05-0; **45**, 82978-06-1; *cis*-15β-**46**, 82978-19-6; *cis*-15α-**46**, 82945-93-5; *trans*-15β-**46**, 82978-21-0; *trans*-15α-**46**, 82978-20-9; *cis*-**47**, 82945-94-6; *trans*-**47**, 82978-22-1; *cis*-**48**, 82978-07-2; *trans*-**48**, 82978-23-2; *cis*-**49**, 82945-95-7; *trans*-**49**, 82978-24-3; *cis*-**50**, 82978-08-3; *trans*-**50**, 82978-25-4; 3-(1-ethoxyethoxy)-1-octyne, 60741-07-3; 1-octyn-3-ol, 818-72-4; 1-octen-3-ol, 3391-86-4; 1-chloro-2(*E*)-octene, 68883-76-1; 2-mercaptopyridine–sodium, 13327-62-3; 1-(2-pyridylthio)-2-(*E*)-octene, 85250-55-1; 1-(phenylthio)-2(*E*)-octene, 85250-56-2; cyclopentene oxide, 285-67-6; 3-[(*tert*-butyldimethylsilyl)oxy]-1-iodo-1(*E*)-octene, 39178-66-0; methyl hydrogen adipate, 627-91-8; monomethyl adipate acid chloride, 35444-44-1; methyl adipamate, 40760-22-3; glutaric acid monomethyl ester, 1501-27-5.

Stereoselective Deuterium Exchange of Methylene Protons in Methyl Tetrofuranosides: Hydroxymethyl Group Conformations in Methyl Pentofuranosides[1a]

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Methyl D-tetro- and pentofuranosides were treated with Raney nickel in hot ^2H_2O, and the resulting C-deuterated products were characterized by ^{13}C, ^1H, and ^2H NMR. In two tetrofuranosides, methyl β-D-erythrofuranoside (**1**) and methyl α-D-threofuranoside (**3**), ^1H–^2H exchange at C4 was stereoselective; the C4 protons of **1** and **3** were unequivocally assigned from this reaction with the aid of ^{13}C enrichment. Exchange at C4 occurs also in methyl β-D-ribofuranoside (**11**) and methyl β-D-xylofuranoside (**13**) but in none of the other pentofuranosides. Exchange occurs in all furanosides having H4 cis to O2 and trans to O1. A convenient synthesis of perdeuterated D-ribose is described on the basis of the Raney Ni exchange results with **11**. By use of the selectively C4-deuterated tetrofuranosides derived from **1** and **3**, eight pentofuranosides were prepared and the C5 protons stereochemically assigned. ^1H–^1H coupling constants and ^{13}C spin–lattice relaxation times (T_1) were measured and are interpreted in terms of the mobility and conformational preferences of the C4–C5 bond in these compounds, and factors affecting these preferences are discussed.

Carbohydrates enriched with deuterium have been widely used to elucidate reaction pathways,[2,3] interpret complex ^1H NMR spectra,[4,5] and assign ^{13}C chemical shifts.[6,7] In addition, deuterium spin–lattice relaxation

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time measurements are facilitated with [^{2}H]-enriched compounds, and these data can be used to evaluate molecular motion.[8] Perdeuterated derivatives may be particularly useful for NMR studies of oligomers such as oligosaccharides and oligonucleotides where selective incorporation of perdeuterated residues can aid in the interpretation of otherwise complex spectra.

Two complementary chemical methods offer an attractive route for the synthesis of perdeuterated carbohydrates. Koch et al.[9,10] have described the preparation of multiply deuterated glycosides through ^{1}H–^{2}H exchange, catalyzed by deuterated Raney Ni in hot ^2H_2O. Only those hydrogens bonded to carbons having free hydroxyl groups are reported to exchange with deuterium, leaving C1 and the ring carbon undeuterated. Deuteration at the ring carbon can be achieved by sequential exchange reactions on both furanoside and pyranoside forms,[11] leaving only C1 undeuterated. Aldoses selectively deuterated at C1 can be prepared by reduction of aldonitriles in ^2H_2O with Pd/$BaSO_4$ and $^2H_2$.[5] It is clear that serial application of Raney nickel exchange reactions to pyranosides and furanosides of [1-^{2}H]aldoses will permit the synthesis of perdeuterated carbohydrates.

We were interested in the large-scale preparation of perdeuterated D-ribose for incorporation into nucleotides and oligonucleotides. Although perdeuterated nucleotides have been prepared biosynthetically for incorporation into oligonucleotides,[12,13] large-scale synthesis using this approach appears impractical. We initiated the synthesis of perdeuterated D-ribose by treating methyl β-D-erythrofuranoside (**1**) with deuterated Raney Ni. The expected [2,3-$^2H_2$] product was to be converted to the pentose with incorporation of ^{2}H at C1 by using the chain-extension reaction,[5] followed by Raney Ni ^{1}H–^{2}H exchange on the [1,3,4-$^2H_2$]furanoside to yield, after glycoside hydrolysis, perdeuterated D-ribose. Unexpectedly, the exchange reaction on **1** yielded methyl β-D-[2,3,4S-$^2H_3$]erythrofuranoside (**2**) with apparently complete stereoselective deuterium substitution at C4.

Methyl α-D-threofuranoside (**3**) yielded methyl α-D-[2,3,4R-$^2H_3$]threofuranoside (**4**). In order to define the general characteristics of the exchange reaction, we examined the following compounds: methyl α-D-erythrofuranoside (**5**), methyl β-D-threofuranoside (**6**), methyl α-D-arabinofuranoside (**7**), methyl β-D-arabinofuranoside (**8**), methyl α-D-lyxofuranoside (**9**), methyl α-D-ribofuranoside (**10**), methyl β-D-ribofuranoside (**11**), methyl α-D-xylofuranoside (**12**), and methyl β-D-xylofuranoside (**13**). The observation of H4 exchange in **11** led to a more efficient approach to the preparation of perdeuterated D-ribose.

Compounds **2** and **4** were used to prepare methyl D-pentofuranosides that are deuterated stereoselectively at C5. The C5 protons were assigned and the conformational preferences of the exocyclic C4-C5 bond assessed from ^{1}H–^{1}H coupling data. ^{13}C spin–lattice relaxation times of methyl D-pentofuranosides were measured to evaluate the degree of mobility of the C4–C5 fragment. Factors affecting C4–C5 bond conformation in pentofuranosides are identified and discussed.

Experimental Section

Materials. Nickel–aluminum alloy (50/50) was obtained from Alfa Products. Deuterium oxide (^2H_2O, 99.8 atom %) was purchased from Aldrich Chemical Co. D-Pentoses were obtained from Sigma Chemical Co. Potassium [^{13}C]cyanide (K^{13}CN, 99.7 atom %) was supplied by the Los Alamos Scientific Laboratory, University of California, Los Alamos, NM.

D-Erythrose and D-threose were prepared from 4,6-*O*-ethylidene-D-glucose[14] and 4,6-*O*-ethylidene-D-galactose,[15] respectively.

Methyl D-erythrofuranosides were prepared from D-erythrose by treatment with methanol–sulfuric acid. D-Erythrose (3.0 g, 25 mmol) was dissolved in anhydrous methanol (175 mL), 2.0 mL of 18 M H_2SO_4 was added dropwise with stirring, and the reaction mixture was left at room temperature for 30 h. The solution was passed through a column containing excess Amberlite IRA-68 (OH^-) resin (Sigma), and the column was washed with 300 mL of H_2O. Effluents were combined, concentrated at 30 °C in vacuo to a thick syrup, and loaded on a Dowex 1-X2 (200–400 mesh) column (2.5 × 31 cm) in the hydroxide form.[16] The column was eluted with decarbonated distilled water at ~1 mL/min, 10 mL fractions were collected and assayed for glycoside with phenol–sulfuric acid.[17] Three compounds eluted in the following order: D-erythrose dimethyl acetal, methyl α-D-erythrofuranoside (**5**), methyl β-D-erythrofuranoside (**1**). Approximate yields as syrups were as follows: acetal (8%), α (11%), β (65%).

Methyl D-threofuranosides were prepared from D-threose in a similar fashion. Compounds eluted from the Dowex 1-X2 column in the following order: D-threose dimethyl acetal, methyl β-D-threofuranoside (**6**), methyl α-D-threofuranoside (**3**). Approximate yields as syrups were as follows: acetal (8%), α (56%), β (20%).

Tetrofuranoside anomers were identified by their characteristic ^{13}C chemical shifts.[18]

Preparation of Deuterated Raney Ni. The preparation is adapted from that described by Augustine[19] for the preparation of W2 Raney Ni. Sodium hydroxide (31.7 g, 0.8 mol) was dissolved in 125 mL of H_2O in a beaker and the solution cooled to 10 °C (ice bath). With mechanical stirring, nickel–aluminum alloy (50/50, 25 g) was added batchwise at a rate to maintain the temperature ≤20 °C (addition takes ~2 h). The suspension was left at room temperature until hydrogen evolution slowed and was then heated to 60 °C for 9 h. Volume was maintained with frequent additions of water. After 9 h of digestion the mixture was cooled and transferred to a stoppered graduated cylinder, the water was decanted, and the catalyst was washed with 42 mL of 2.4 M NaOH. After decantation of the alkaline wash, the catalyst was suspended repeatedly in H_2O (100 mL) until the washes were neutral (20–25 times). The Raney Ni was transferred to a glass column with a fritted-glass plug, the water was drained to the bed, and the catalyst was washed four or five times with 10–15 mL of ^2H_2O, draining to the column bed between washes. The catalyst was stored at room temperature in the securely stoppered column. For use in exchange reactions, Raney Ni was measured by volume with a pipet.

Reactivity was affected by the method of preparation and age. Therefore, exchange reactions should be monitored with time by

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^1H or ^{13}C NMR to determine the completeness of reaction.

Reaction of Deuterated Raney Ni with Methyl β-D-Erythrofuranoside (1). To deuterated Raney Ni (9 mL) in a 100-mL round-bottomed flask was added methyl β-D-erythrofuranoside (1; 0.58 g, 4.3 mmol) in 30 mL of ^2H_2O, and the reaction flask was fitted with a condenser and drying tube. The suspension was refluxed for 14 h with stirring, and samples were removed every 3–4 h for assay by ^1H NMR. After 14 h the suspension was cooled, the liquid was removed by pipet, and the catalyst was washed three times with 3–5 mL of ^2H_2O, heating the suspension to reflux each time. The solutions were combined, centrifuged for 20 min at 5900*g* to remove a fine white precipitate, and the precipitates were washed once with 3–5 mL of ^2H_2O and recentrifuged. The supernatants were combined and concentrated in vacuo at 30 °C to a syrup which was dissolved in 3–4 mL of H_2O and loaded on a Dowex 1X2 (200–400 mesh) column (2.4 × 55 cm) in the hydroxide form. The column was eluted with decarbonated distilled water at ~0.5 mL/min, and 5-mL fractions were collected and assayed for glycosides as described above. Three peaks eluted: fractions 28–34, minor byproducts; 80–90, methyl α-L-[2,3,4S-$^2H_3$]threofuranoside (**14**); 95–120, methyl β-D-[2,3,4S-$^2H_3$]erythrofuranoside (**2**). The fractions were pooled and concentrated to syrups at 30 °C in vacuo. Compound **2**: yield 2.1 mmol (49%); [α] −129.4°; standard methyl β-D-erythrofuranoside [α] −133.5° (lit.[20] [α] −148.0°). Compound **14**: yield 0.5 mmol (12%); [α] −90.3°, standard methyl α-D-threofuranoside [α] +96.5° (lit.[20] D isomer [α] +97°).

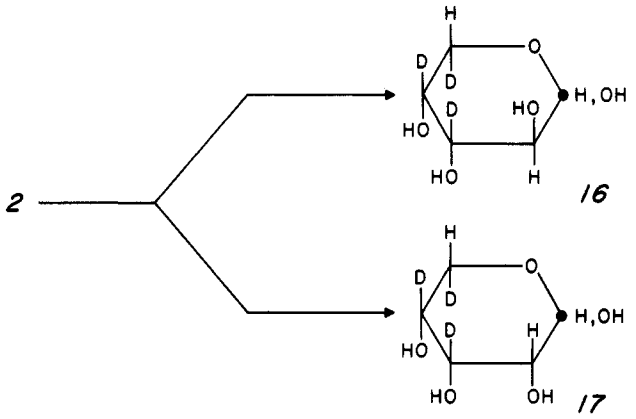
Hydrolysis of 2 to D-[2,3,4S-$^2H_3$]Erythrose (15) and Conversion to D-[1-^{13}C; 3,4,5S-$^2H_3$]Arabinose and Ribose (16, 17). Compound **2** (0.27 g, 2.0 mmol) was dissolved in 50 mL of aqueous 0.1 N H_2SO_4, and the solution was refluxed for 30 min. Hydrolysis was complete as determined by ferricyanide assay.[21] The solution was cooled, and $BaCO_3$ was added slowly with stirring until the pH rose to ~6. The white suspension was centrifuged, and the precipitate was washed once with water and recentrifuged. The slightly turbid supernatants were combined and filtered through a glass-fiber filter, and the clear filtrate was treated separately and batchwise with excess Dowex 1-X8 (200–400 mesh, OAc^-) and Dowex 50-X8 (20–50 mesh, H^+). The clear solution was concentrated to ~3 mL at 30 °C in vacuo and loaded on a Dowex 50-X8 (200–400 mesh) column (2.4 × 46 cm) in the barium form.[22] Fractions (14 mL) were collected at 15-min intervals and assayed with silver nitrate.[23] Fractions 12–16 contained **15** as determined by ^1H and ^{13}C NMR.[5,24]

D-[2,3,4S-$^2H_3$]Erythrose (**15**) was treated with $K^{13}CN$ (0.14 g, 2.2 mmol) in 10 mL of H_2O at pH 7.8 for 15–20 min, and the 2-epimeric [^{13}C, ^2H]pentononitriles were converted to 2-epimeric [^{13}C, ^2H]pentoses and purified as described previously.[5] Yields from **2** after chromatography on Dowex 50-X8 (200–400 mesh, Ba^{2+}): peak 1, D-[1-^{13}C; 3,4,5S-$^2H_3$]arabinose (**16**), 0.09 g (0.6 mmol, 30%); peak 2, D-[1-^{13}C; 3,4,5S-$^2H_3$]ribose (**17**), 0.13 g (0.8 mmol, 40%).

Reaction of Deuterated Raney Ni with Methyl α-D-Threofuranoside (3). To deuterated Raney Ni (8 mL) in a 100-mL flask was added methyl α-D-threofuranoside (**3**; 3.7 mmol, 0.49 g) in 40 mL of ^2H_2O, and the reaction flask was fitted with a condenser and drying tube. The mixture was refluxed for 60 h with stirring, and samples were removed after 18, 40, and 60 h for assay by ^1H NMR. After 60 h, the suspension was cooled, and the products were purified as described for **1**. One major peak eluted from the Dowex 1-X2 (200–400 mesh, OH^-) column. Fractions containing methyl α-D-[2,3,4R-$^2H_3$]threofuranoside (**4**) were pooled and concentrated to a syrup at 30 °C in vacuo: yield 0.33 g (2.4 mmol, 65%); [α] +92.0°; standard methyl α-D-threofuranoside [α] +96.5° (lit.[20] [α] +97.0°).

Hydrolysis of 4 to D-[2,3,4R-$^2H_3$]Threose (18) and Conversion to D-[1-^{13}C; 3,4,5R-$^2H_3$]Lyxose and Xylose (19, 20).

Scheme I



Compound **4** (0.33 g, 2.4 mmol) was dissolved in 50 mL of 0.1 N H_2SO_4, and the solution was refluxed for 45 min. Hydrolysis was complete as determined by ferricyanide assay.[21] The solution was cooled, and the products were purified as described for the hydrolysis of **2**. Chromatography on Dowex 50-X8 (200–400 mesh, Ba^{2+}) yielded **18** as determined by ^1H and ^{13}C NMR.[5,24,25]

D-[2,3,4R-$^2H_3$]Threose (**18**) was treated with $K^{13}CN$ as described above for the preparation of **16** and **17**. Yields from **4** after chromatography on Dowex 50-X8 (200–400 mesh, Ba^{2+}): peak 1, D-[1-^{13}C; 3,4,5R-$^2H_3$]xylose (**20**), 0.17 g (1.1 mmol, 46%); peak 2, D-[1-^{13}C; 3,4,5R-$^2H_3$]lyxose (**19**), 0.14 g (0.9 mmol, 38%).

Glycosidation of D-Pentoses and Deuterated D-Pentoses. D-Pentoses and deuterated D-pentoses (**16, 17, 19, 20**) were converted to methyl D-pentofuranosides by methods described previously: D-arabino,[26] D-lyxo,[27] D-ribo,[28] D-xylo.[26] Anomers were purified by chromatography on Dowex 1-X2 (200–400 mesh) in the hydroxide form[16] and were identified by their characteristic ^{13}C chemical shifts.[18]

Instrumentation. Proton-decoupled ^{13}C (75 MHz), ^1H (300 MHz), and ^2H {^1H} (46 MHz) NMR spectra were obtained with a Bruker WM-300 Fourier transform spectrometer equipped with quadrature detection. ^{13}C and ^1H NMR spectra were obtained with the spectrometer locked to the resonance of ^2H_2O in the sample. ^2H {^1H} NMR spectra[29] were obtained with samples dissolved in deuterium-depleted water (Sigma) and with the spectrometer unlocked.

^1H {^2H} NMR spectra were obtained in an unlocked mode with the output of a Hewlett-Packard frequency synthesizer, tuned at the appropriate decoupling frequency, fed into the ^2H-lock channel of the probe. These spectra were required to determine $^3J_{C1,H5}$ values in **16, 17, 19,** and **20**. Deuterium decoupling removed the severe line broadening of the H5 resonance caused mainly by two-bond ^1H–^2H coupling and permitted the evaluation of the residual ^{13}C–^1H coupling.

^{13}C NMR chemical shifts (±0.1 ppm) are referenced to the anomeric carbon of β-D-[1-^{13}C]glucopyranose (97.4 ppm). ^1H NMR chemical shifts (±0.01 ppm) are referenced to internal sodium 3-(trimethylsilyl)-1-propanesulfonate. ^1H–^1H and ^{13}C–^1H coupling constants are accurate to ±0.1 Hz.

Results and Discussion

The Raney Ni catalyzed exchange of protons in glycosides in ^2H_2O offers a convenient route to the large-scale synthesis of specifically or perdeuterated aldoses. Koch et al.[9-11] have reported that exchange is limited to protons on carbons bearing a free hydroxyl group. This seems to

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Table I. ^1H Chemical Shifts for Methyl D-Tetrofuranosides and Methyl D-Pentofuranosides in ^2H_2O

compd	chemical shift,[a] ppm							
	H1	H2	H3	H4R	H4S	H5S	H5R	OCH_3
α-erythro	4.90	4.12	4.24	4.13	3.87			3.40
β-erythro	4.90	4.04	4.35	4.11	3.81			3.39
α-threo	4.91	4.06	4.20	4.27	3.84			3.37
β-threo	5.00	4.09	4.28	4.15	3.67			3.42
α-arabino[b]	4.91	4.04	3.93	4.02		3.80	3.69	3.40
β-arabino[b]	4.89	4.13	4.00	3.88		3.76	3.61	3.41
α-lyxo	4.95	4.11	4.32	4.24		3.81	3.73	3.44
β-lyxo	4.91	4.19	4.24	4.15		3.83	3.72	3.40
α-ribo[b]	4.99	4.11	4.03	4.09		3.73	3.66	3.43
β-ribo[b]	4.88	4.02	4.14	4.00		3.78	3.59	3.38
α-xylo	4.99	4.14	4.29	4.23		3.76	3.69	3.44
β-xylo	4.89	4.12	4.21	4.35		3.83	3.73	3.39

[a] Relative to internal sodium 3-(trimethylsilyl)-1-propanesulfonate and accurate to ±0.01 ppm. [b] Values taken from ref 5.

Table II. Exchange Results for Methyl D-Tetrofuransoides and Methyl D-Pentofuranosides

compd	exchange sites[a]	byproducts[c]
α-D-erythro (5)	H2, H3	β-threo (48%, 25 h), α-threo (17%, 25 h)
β-D-erythro (1)	H3 > H4S > H2	α-threo (~45%, 12 h)
α-D-threo (3)	H3 > H2 ≅ H4R	β-erythro (34%, 27 h)
β-D-threo (6)	H2 > H3 > H1	α-erythro (15%, 24 h) β-erythro (9%, 24 h) α-threo (8%, 24 h)
α-D-arabino (7)	H2 ≅ H3 > H5R, H5S	α-D-lyxo (11%, 26 h)
β-D-arabino (8)	H2 > H3, H5R, H5S > H1	
α-D-lyxo (9)	H2, H3, H5R, H5S	α-D-arabino (75%, 25 h)
α-D-ribo (10)	H2, H3, H5R, H5S	α-D-arabino (40%, 20 h), α-D-xylo (~20%, 20 h)
β-D-ribo (11)	H5R, H5S > H2 ≅ H3 > H4	β-D-xylo or β-D-arabino[b] (12%, 27 h)
α-D-xylo (12)	H2, H3, H5R, H5S	α-D-ribo (~50%, 7 h)
β-D-xylo (13)	H2, H3, H4, H5R, H5S	β-D-ribo (>80%, 8 h)

[a] Relative rate of exchange as indicated for β-D-erythro, α-D-threo, β-D-threo, α-D-arabino, β-D-arabino, and β-D-ribo. Exchange at other sites was <5%. [b] Configuration of byproduct could not be identified. [c] Yield and reaction time are given in parentheses.

Table III. NMR Parameters for D-[1-^{13}C; 3,4,5-$^2H_3$]Pentopyranoses 16, 17, 19, and 20

compd		^1H chemical shifts,[a] ppm			coupling constants,[b] Hz			
		H1	H2	H5 (residual)	H1-H2	C1-H1	C1-H2	C1-H5
arabinose	α	4.49	3.47	3.62	7.8	160.9	5.8	2.8
	β	5.22	3.78	3.97	3.4	169.2	br	2.2
lyxose	α	5.05	3.84	3.71	4.4	167.3	3.3	4.6
	β	4.90	3.96	3.28	1.5	162.1	1.7	3.1
ribose	α	4.84	3.79	3.57	2.2	164.6		4.2
	β	4.91	3.49	3.79	6.5	165.6	4.9	7.0
xylose	α	5.17	3.50	3.64	3.6	169.7	0	1.7
	β	4.55	3.21	3.28	7.9	161.5	6.0	2.9

[a] Relative to internal sodium 3-(trimethylsilyl)-1-propanesulfonate and accurate to ±0.01 ppm. [b] Accurate to ±0.1 Hz; br = broadened.

be the case for pyranosides, but it is clear that in certain furanosides H4 and/or H1 can exchange.

Primary ^2H-Exchange Products from Tetrofuranosides. ^1H Chemical shifts in ^2H_2O for methyl D-tetro- and -pentofuranosides are listed in Table I and were used to determine sites of exchange.

(A) Methyl β-D-Erythrofuranoside (1). The ^1H–^2H exchange reaction yields methyl β-D-erythrofuranoside deuterated at H2, H3, and H4S (Figure 1, Table II); H3 exchanges slightly faster than H4S and H4S more readily than H2, while H1, H4R, and the methyl protons do not exchange. The more shielded of the H4 resonances was assigned to H4S (the proton cis to O3) on the basis of substituent effects and coupling to C1.[30] This assignment was confirmed by converting **2** to **16** and **17** (Scheme I) and measuring the three-bond coupling between C1 and the remaining H5. For example, α-D-arabinopyranose exists in the $^1C_4$ conformation.[31] If H4S exchanges, the dihedral angle between C1 and the axial H5R is 60°, and $^3J_{C1,H5}$ = 2.0–2.5 Hz.[32] On the other hand, if H4R exchanges, the dihedral angle is 180°, and $^3J_{C1,H5}$ = 6.5–7.0 Hz. The observed $^3J_{C1,H5}$ value of 2.8 Hz (Table III) confirms H4S exchange.

β-D-Ribopyranose exists in the $^4C_1$ conformation[31] in which the remaining C5 proton (H5R) is antiperiplanar (180°) with respect to C1. $^3J_{C1,H5}$ is equal to 7.0 Hz (Table III) as expected for H4S exchange in **1**.

$^3J_{C1,H5}$ values for α-D-ribopyranose and α-D-arabinopyranose (Table III) probably reflect an average of both

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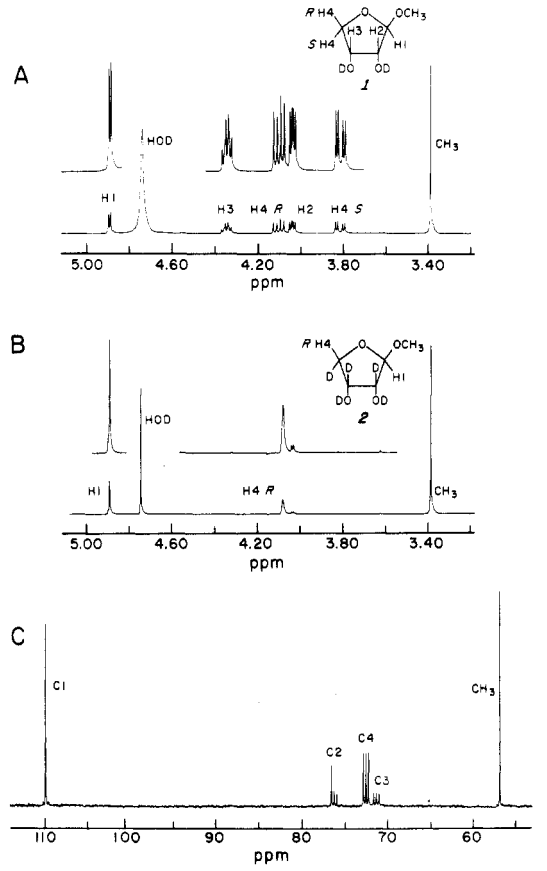
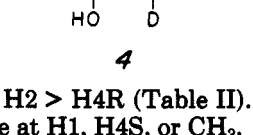


Figure 1. NMR spectra of 1 and 2 in ^2H_2O. (A) 300-MHz ^1H NMR spectrum of 1 showing all resonance assignments. (B) 300-MHz ^1H NMR spectrum of 2 showing the loss of resonances of H2, H3, and H4S. The resonance of H4R is broadened by ^1H–^2H coupling. The small doublet at 4.04 ppm is due to residual H2 protons coupled to H1 ($^3J_{H1,H2}$ = 2.9 Hz). (C) Proton-decoupled ^{13}C NMR spectrum (75 MHz) of 2, showing singlets for C1 and CH_3. Resonances of C2, C3, and C4 are split by the directly bonded deuterium ($^1J_{CD}$ = 23 Hz). The C4 resonance would be split into five lines had both H4S and H4R fully exchanged. The isotope shift for C2 upon deuteration (~0.36 ppm) is apparent since residual protonated C2 appears as the singlet slightly downfield from the deuterated C2 triplet.

$^4C_1$ and $^1C_4$ conformations[31] and cannot be used for assignment.

(B) From Methyl α-D-Threofuranoside (3). Treatment of 3 with deuterated Raney Ni yields methyl α-D-[2,3,4R-$^2H_3$]threofuranoside (4). Relative rates of exchange are H3 > H2 > H4R (Table II). There is no evidence of exchange at H1, H4S, or CH_3. With short incubation times methyl α-D-[3-^2H]threofuranoside (22) can be isolated in good yield (>85%).



4

The site of deuteration at C4 of 3 was confirmed by converting 4 to 19 and 20. For α- and β-xylopyranose, the $^4C_1$ conformation is preferred,[31] and the dihedral angle between C1 and the remaining H5 (H5S) is 60° if H4R exchanges. Observed $^3J_{C1,H5}$ values of 1.7 and 2.9 Hz (Table III), respectively, confirm this assignment and the site of exchange.

L-[4S-^2H]Threose has been prepared by Maradufu et al.[33] via stereoselective reduction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with $LiAl^2H_4$. The H4S proton (anti to O3) was replaced and found to be the less shielded C4 proton. Our results with the D isomer agree with their assignment.

(C) From Methyl α-D-Erythrofuranoside (5) and Methyl β-D-Threofuranoside (6). Exchange with 5 and 6 yielded methyl α-D-[2,3-$^2H_2$]erythrofuranoside (23) and methyl β-D-[2,3-$^2H_2$]threofuranoside (24), respectively. In 6, H2 exchanges more rapidly than H3, and with extended incubation times, H1 exchanges (38% in 24 h).

Primary ^2H-Exchange Products from Pentofuranosides. Results from exchange reactions with methyl D-pentofuranosides are summarized in Table II. In these compounds protons at C2, C3, and C5 exchange. Exchange also occurs at C4 in 11 and 13, which are the pentofuranoside analogues of 1 and 3, respectively. In 8, the pentofuranoside analogue of 6, H1 exchanges slowly. Exchange of H4 in 11, which was not observed by Koch et al.,[9] permits the synthesis of methyl β-D-[1,2,3,4,5R,5S-$^2H_6$]ribofuranoside (21) by a more efficient

D-erythrose ⟶

$$\text{methyl } \beta\text{-D-[1-}^2\text{H]ribofuranoside} \xrightarrow[^2H_2O]{\text{Raney Ni}} \mathbf{21}$$

path and in a twofold greater yield from methyl β-D-[1-^2H]ribofuranoside than is possible from 1 via 2.

Characterization of Secondary ^2H-Exchange Products. Prolonged incubation of methyl D-tetro- and -pentofuranosides with deuterated Raney Ni in ^2H_2O at 80 °C gives rise to various deuterated byproducts (Table II). From a synthetic standpoint, exchange times generally should be kept short to minimize their accumulation.

In tetrofuranosides, C3 epimers form most readily (Table II). For example, 1 epimerizes to methyl α-L-[2,3,4S-$^2H_3$]threofuranoside (14) as established by ^1H, ^2H, and ^{13}C NMR and by optical rotation. The α-L-threo isomer can be isolated early in the reaction with H3 exchange >95% complete, H4S partly deuterated, and H2 <5% deuterated. With time, H2 and H4S exchange completely while no exchange of H4R occurs as determined by ^2H NMR. Exchange reactions with 3, 5, and 6 produce the 3-epimers methyl β-erythrofuranoside, methyl β-threofuranoside, and methyl α-erythrofuranoside, respectively. Although optical rotation measurements were not made, these byproducts probably have the L configuration.

From relative stabilities of furanosyl rings predicted solely from the number of *cis*-1,2 hydroxyl interactions, 2-epimers of α-erythro- and β-threofuranoside should be at least as stable as 3-epimers. The latter are the principal epimerization products after ~25 h, however, indicating that the reactions are not driven by thermodynamic stabilities and have not reached thermodynamic equilibrium.

Byproducts formed by 3-epimerization are also found in reactions with pentofuranosides (Table II). When arabino compounds react, 3-epimers are present in small amounts after 26 h. In the same period, lyxo compounds epimerize to a large extent to arabino compounds, as expected on the basis of thermodynamic stabilities. In the xylo–ribo interconversion the ribo configuration is favored; after 26 h little 3-epimer (β-xylo) is formed from the β-ribo compound, whereas considerable β-ribo byproduct is formed from the β-xylo compound after 8 h. In this case, the mixture expected from thermodynamic considerations is not obtained.

In 8, 10, and 12, 2-epimers should be more stable than

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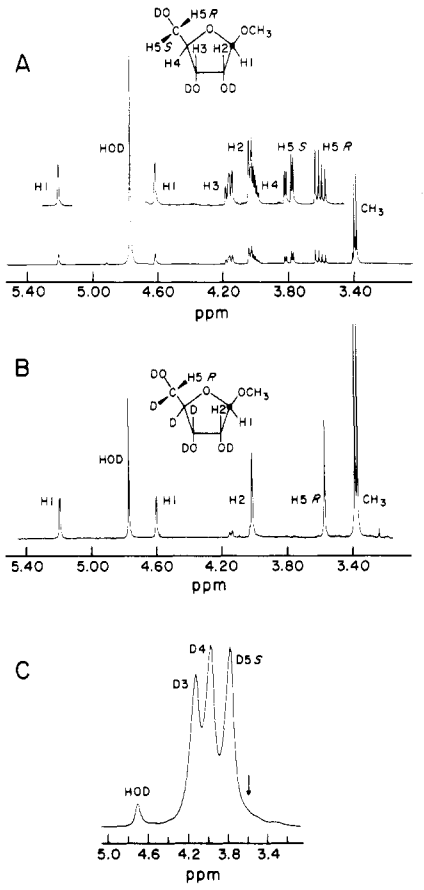


Figure 2. NMR spectra of methyl β-D-[1-^{13}C]ribofuranoside and methyl β-D-[1-^{13}C; 3,4,5S-$^{2}H_3$]ribofuranoside prepared from **2**. (A) 300-MHz ^{1}H NMR spectrum of methyl β-D-[1-^{13}C]ribofuranoside showing assignments for all protons. ^{13}C–^{1}H couplings were obtained from this spectrum: $^1J_{C1,H1}$ = 174.2 Hz, $^2J_{C1,H2}$ = 0 Hz, $^2J_{C1,CH3}$ = 4.3 Hz, $^3J_{C1,H3}$ = 0.9 Hz, $^3J_{C1,H4}$ = 3.0 Hz. (B) 300-MHz ^{1}H {^{2}H} NMR spectrum of methyl β-D-[1-^{13}C; 3,4,5S-$^2H_3$]ribofuranoside, showing resonances of H1, H2, and H5R. (C) 46-MHz ^{2}H {^{1}H} NMR spectrum of methyl β-D-[1-^{13}C; 3,4,5S-$^2H_3$]ribofuranoside showing resonances of D3, D4, and D5S. The resonance of D5R would appear upfield from D5S (arrow). Its absence confirms the exchange stereoselectivity at C4 in **1**.

3-epimers. Only in **10**, however, does substantial 2-epimerization occur.

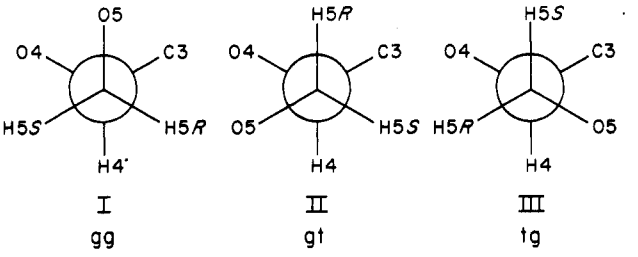
Structural Requirements for Stereoselective Exchange at C4 of Furanosides. Only four methyl D-tetro- and -pentofuranosides react with Raney Ni to form products deuterated at C4: **1**, **3**, **11**, and **13**. Several aspects of the exchange reaction should be noted. First, H4 exchanges only when it is cis to O2 and trans to O1. Second, in **3**, exchange at H3 is rapid and occurs with little C3 epimerization or H4 exchange. Third, in **1**, exchange at H3 is rapid but is accompanied by either C3 epimerization or by H4S exchange. Fourth, inversions occur mainly at C3, and even after 24 h the reaction has not reached thermodynamic equilibrium between the erythro and threo isomers. Fifth, selective binding of the starting material to the catalyst must occur to effect stereoselective deuteration at C4 of **1** and **3**.

Deuterium exchange at positions geminal to a hydroxyl has been proposed to involve keto intermediates. These keto forms may undergo enolization and rehydrogenation, thereby mediating vicinal exchange. 3-Keto compounds formed from **1** to **3** are enantiomeric, and their reduction will give both erythro and threo configurations. Although formation of the latter from a 3-keto intermediate is thermodynamically and perhaps kinetically favored, so that C3 epimerization is faster in **1** than in **3**, the involvement of a 3-keto compound cannot explain the relative rates of C3 and C4 exchange in **1** and **3**. If 3-keto (or 3,4-enol) forms were key intermediates, both **1** and **3** should give the same mixture of [3,4-$^2H_2$] product throughout the reaction. They do not, which argues against mechanisms of H4 exchange involving common intermediates. It is simpler to assume that exchange at C4 involves specific binding directed by the structure at C1 and C2 of the starting material and a direct (hydride) exchange.

Assignment of Hydroxymethyl Protons of Methyl D-Pentofuranosides and Conformational Implications. Carbohydrates stereospecifically deuterated at the hydroxymethyl group can be prepared from **2** and **4** (Figure 2). Previous methods used to accomplish this substitution have been based on the exchange specificity of alcohol dehydrogenase[34,35] or on the stereospecific attack of reducing agents on dialdo compounds.[36,37]

Stereospecifically labeled hydroxymethyl groups are valuable in the study of carbohydrate conformation. Pentofuranosyl and hexopyranosyl rings contain these moieties as exocyclic fragments in which conformational preferences can be determined if the ^{1}H resonances of the two protons can be assigned. Assignments are made either through selective substitution with deuterium,[34-37] through substituent effects on ^{1}H chemical shifts,[38] or from ^{13}C–^{1}H coupling between these protons and a vicinal carbon.[39,40]

Hydroxymethyl conformations in β-ribofuranosides are particularly important in the study of the conformations of nucleosides and their derivatives. Three minimal energy (staggered) rotamers (I–III) describe C4–C5 bond con-



formations. Rotamer I (gg) contains two gauche interactions between H4–H5R and H4–H5S, whereas rotamers II (gt) and III (tg) contain one gauche and one anti interaction. Gerlt and Youngblood[34] have calculated rotamer populations for **11** on the basis of eq 1–3.[41] Rotamers I–III

$$1.3P_{gg} + 2.7P_{gt} + 11.7P_{tg} = {}^3J_{H4,H5S} \quad (1)$$

$$1.3P_{gg} + 11.5P_{gt} + 5.8P_{tg} = {}^3J_{H4,H5R} \quad (2)$$

$$P_{gg} + P_{gt} + P_{tg} = 1 \quad (3)$$

were calculated to be in the ratio 38:44:18. Using their values of $^3J_{H4,H5R}$ (6.6 Hz) and $^3J_{H4,H5S}$ (3.3 Hz), we determine this rato to be 41:46:13.

In uridine the β-ribofuranosyl hydroxymethyl group populates rotamers I–III in the approximate ratio 63:25:12.[34,42] It has been suggested that rotamer I is

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Table IV. ^1H-^1H Vicinal Coupling Constants between H4 and the H5 Protons in Methyl D-Pentofuranosides in ^2H_2O

compd	coupling constant,[a] Hz	
	$^3J_{H4,H5S}$	$^3J_{H4,H5R}$
α-arabino[b]	3.1	6.1
3-deoxy-α-threopento	3.5	5.9
β-arabino[b]	3.2	7.4
α-lyxo	4.4	6.7
β-lyxo	4.5	7.6
α-ribo[b]	3.1	4.8
β-ribo[b]	3.1	6.6
α-xylo	3.8	6.0
β-xylo	4.4	7.6

[a] Accurate to ±0.2 Hz. [b] Values taken from ref 5.

Table V. Rotamer Distribution about the C4–C5 Bond in Methyl D-Pentofuranosides Determined from ^1H-^1H Couplings in Table IV

compd	P_{gg}[a] (I)	P_{gt}[a] (II)	P_{tg}[a] (III)
α-arabino	0.35 (0.46)	0.48 (0.42)	0.17 (0.12)
β-arabino	0.20 (0.34)	0.62 (0.55)	0.18 (0.11)
α-lyxo	0.14 (0.34)	0.55 (0.42)	0.31 (0.24)
β-lyxo	0.04 (0.25)	0.64 (0.51)	0.32 (0.24)
α-ribo	0.48 (0.59)	0.35 (0.28)	0.17 (0.13)
β-ribo	0.29 (0.42)	0.54 (0.47)	0.17 (0.11)
α-xylo	0.29 (0.43)	0.47 (0.38)	0.24 (0.19)
β-xylo	0.05 (0.25)	0.64 (0.52)	0.31 (0.23)

[a] Calculated from eq 3–5. The values in parentheses were calculated from eq 1–3.

preferred by virtue of electrostatic and/or hydrogen bonding interactions between the substituent on C5′ and the base.[34] Ritchie and Perlin[43] have prepared [5′-^2H]-adenosine from D-[5-^2H]xylose and determined that rotamer I is predominant (approximately 73%).

We were interested in examining how C4–C5 bond conformation in pentofuranosyl rings would be affected by substituent geometry. The eight methyl D-pentofuranosides derived from **2** and **4** were prepared to unequivocally assign the C5 protons. In all cases, the more shielded C5 proton is the H5R proton. This result confirms the assignments by Gerlt and Youngblood[34] which were based on ^1H chemical shift comparisons with (*S*)-tetrahydrofuran-[H5R-^2H]methanol.

Rotamer distributions were calculated by using two sets of equations for comparison. Equations 1–3 and 3–5 were

$$^3J(60°)(P_{gg} + P_{gt}) + {}^3J(180°)(P_{tg}) = {}^3J_{H4,H5S} \quad (4)$$

$$^3J(60°)(P_{gg} + P_{tg}) + {}^3J(180°)(P_{gt}) = {}^3J_{H4,H5R} \quad (5)$$

used with $^3J_{H4,H5S}$ and $^3J_{H4,H5R}$ values listed in Table IV. Equations 1–3 account for differences in the magnitude of gauche couplings caused by the different arrangements of substituents,[41] whereas a simpler treatment is used in eq 3–5. Values for $^3J(60°)$ and $^3J(180°)$ of 1.5 and 11.0 Hz, respectively, were used in eq 4 and 5.[44] Results are given in Table V.

Both treatments of the coupling data predict the same general distribution of rotamers but yield different absolute percentages. Rotamers I and II are stabilized by "gauche effects", which may account for their higher proportion in the rotamer population. Rotamer distribution is sensitive also to the configuration of hydroxyl groups on the ring. Extremes of substituent orientation occur in β-lyxo and α-ribo configurations where all substituents are cis and trans to C5, respectively. The proportion of rotamer I, P_{gg}, is larger in α-ribo than in β-lyxo, while both P_{gt} and P_{tg} are smaller. Rotamer I has O5 oriented "over" the ring, and its proportion would be expected to increase as the number of hydroxyls cis to C5 decreases. This trend is observed in the series β-lyxo → α-lyxo → α-xylo → α-ribo where P_{gg} increases in the order 0.04 (0.25) → 0.14 (0.34) → 0.29 (0.43) → 0.48 (0.59) (Table V).

Table VI. ^{13}C Spin-Lattice Relaxation Times[a] for Methyl D-Pentofuranosides

compd	T_1, s				
	C1	C2	C3	C4	C5
α-arabino	2.5	2.4	2.4	2.4	1.4
β-arabino	2.3	2.4	2.4	2.2	1.4
α-lyxo	2.6	2.6	2.5	2.6	1.5
α-ribo	2.5	2.5	2.2	2.5	1.3
β-ribo	2.3	2.2	2.3	2.2	1.2
α-xylo	2.5	2.4	2.4	2.5	1.4
β-xylo	2.3	2.2	2.4	2.4	1.4

[a] Measured by inversion-recovery at 75 MHz (36 ± 1 °C) in 50 mM NaOAc/HOAc (pH 5.0), 3 mM EDTA, and 50:50 $H_2O/^2H_2O$. [Glycoside] = 0.2 M. Samples were degassed with Ar for 5 min prior to measurement. Values are accurate to ±10%.

The effects of O1 and O2 orientation can be determined by comparing the following pairs: for O1, α-xylo-β-xylo and α-ribo-β-ribo; for O_2, α-xylo-α-lyxo and α-ribo-α-arabino. As either O1 or O2 changes from trans to C5 to cis to C5, P_{gg} decreases and P_{gt} increases, with P_{tg} remaining relatively constant. It is unclear why P_{gt} is affected by this change in substituent orientation, but furanosyl ring conformation must play a substantial role in limiting hydroxymethyl group conformation. Although we cannot assess this contribution, overall trends can be discerned and interpreted in terms of configuration alone.

The effect of orientation of O3 on the distribution of rotamers can be assessed by comparing four pairs of isomers: β-arabino-β-lyxo, α-ribo-α-xylo, β-ribo-β-xylo, and α-arabino-α-lyxo. These comparisons show that P_{gt} remains relatively constant while P_{gg} decreases and P_{tg} increases when O3 is cis to C5. Two effects probably destabilize rotamer III when O3 is trans to C5: an unfavorable 1,3-interaction between O3 and O5[45] and a negative "gauche effect" between O3 and the ring oxygen.[46] When O3 is cis to C5, a positive "gauche effect" results while the 1,3-interaction is removed, thereby stabilizing rotamer III and destabilizing rotamer I (steric hindrance of O3).

The effect of interactions between O3 and O5 on C4–C5 bond conformation can, in principle, be examined by interpreting the ^1H NMR spectrum of methyl 3-deoxy-α-D-*threo*-pentofuranoside (**25**). If the most shielded C5 proton in **25** is H5R, then, from eq 1–3, P_{gg}:P_{gt}:P_{tg} = 46:38:16. This distribution is similar to that for **7**, where O3 is trans to C5 and P_{gg}:P_{gt}:P_{tg} = 44:41:16. On the other hand, if H5R is the less-shielded proton, P_{gg}:P_{gt}:P_{tg} = 54:2:44, a very different and less probable outcome.

Finally, we note that the C4–C5 bond in methyl D-pentofuranosides does not rotate freely in solution. If rotation was completely free and rapid, $^3J_{H4,H5}$ should equal $^3J_{H4,H5'}$ and be the average $^3J_{H,H}$ over the entire Karplus curve. Use of the three-state rapid-transit model for C4–C5 rotamer analysis, implicit in the preceding discussion, is further supported by ^{13}C spin–lattice relaxation time (T_1)

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measurements (Table VI). Motion of monosaccharides in solution is generally isotropic;[47,48] that is, it is described by a single correlation time, τ_c. Furthermore, ^{13}C relaxation of protonated carbons is predominantly dipolar.[47,48] In the absence of free rotation about the C4–C5 bond, C5 will have the same correlation time as the ring carbons, and its T_1 will be half that of the ring carbons. Relaxation data in Table VI suggests that C5 has the same correlation time as the ring carbons and that motion about the C4–C5 bond is restricted, possibly through intramolecular or intermolecular (solvent) hydrogen bonding of the C5 hydroxyl group. Similar observations have been made for hydroxymethyl groups in pyranosyl rings.[40]

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Transformation of Methionine into *S*-*tert*-Butylhomocysteine. Application to a Methionine-Containing Peptide: Substance P

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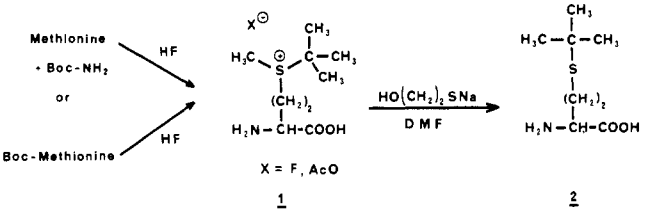
We have developed a general methodology to transform methionine into *S*-*tert*-butylhomocysteine via the *tert*-butyl sulfonium salt of methionine. This sequence of reactions can also be applied to methionine-containing peptides, as illustrated by the case of substance P (SP). Cleavage of the *S*-*tert*-butyl protecting group yields [Hcy[11]]-SP. The thiol group may undergo various reactions leading to S-modified analogues, for instance, fluorescent SP.

A large number of biologically active peptides have a methionine residue in their active core, and this amino acid is sometimes essential for the bioactivity, as for example in substance P (SP): Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2,[1] a member of the tachikinins family.[2] We have been interested in facile modification of this methionine residue either for structure–activity relationship studies or for binding studies after labeling this position with a fluorescent or a radioactive group.

A few methionine-modified analogues of eledoisin, another member of the tachikinins group, have already been described by Bernardi et al.,[3] but all these *S*-alkylhomocysteine analogues have been obtained by total synthesis. The *S*-alkylhomocysteines were synthesized via *S*-benzylhomocysteine, which was first debenzylated by sodium in liquid ammonia, yielding the thiol, which was then realkylated in situ.[4] Since we were interested in obtaining a large number of methionine-modified analogues of SP starting from the same substrate, we first considered the synthesis of [Hcy[11]]-SP using *S*-benzylhomocysteine. But deprotection by sodium in liquid ammonia of the *S*-benzyl group had to be excluded in the case of SP which contains two proline residues. Furthermore, we observed that removal of the *S*-benzyl protecting group by HF, which is used to cleave the peptide from a methylbenzhydrylamine resin (MBHA)[5] with concomitant removal of all the protecting groups, was too slow and required too long an exposure in HF, leading to many byproducts. Hence we transformed the methionine residue into *S*-*tert*-butylhomocysteine which can be used, after N-α-protection, in peptide synthesis. The *S*-*tert*-butyl group can then be easily removed.

Scheme I



Furthermore, we have established that this sequence of reactions can also be applied to peptides and thus provides a general methodology to prepare, from any methionine-containing peptide, a large variety of S-modified analogues.

Results and Discussion

S-*tert*-Butylhomocysteine (**2**) was synthesized from methionine, according to Scheme I. The use of an intermediate *S*-*tert*-butyl sulfonium salt was expected to offer two main advantages: good regioselectivity in the

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