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Note

Synthesis of site-specific, deuterium-substituted α-L-Rha*p*-(1→2)-α-L-Rha*p*-OMe

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Abstract

The disaccharide α-L-Rha*p*-(1→2)-α-L-(2-^{2}H)Rha*p*-OMe has been synthesized. The site-specific deuteration was performed at C-2 by reduction of a suitably protected β-linked 2-ulose derivative. In addition, α-L-Rha*p*-(1→2)-α-L-(2-^{2}H)Rha*p*-O($^2H_3$)Me was also synthesized. © 1998 Elsevier Science Ltd. All rights reserved

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The predominant technique used for conformational studies of carbohydrates in solution is NMR spectroscopy. It is versatile and can give information on proton–proton internuclear distances by the employment of NOESY experiments. Information on dihedral angles can be obtained from measurement of coupling constants, which are used both in homo- and heteronuclear cases. Studies of nuclear spin relaxation rates by investigation of spin–lattice and spin–spin relaxation times can give information on dynamics of the molecule. For carbohydrates, intramolecular hydrogen bonding can occur for certain geometries. These conformers may be stabilized by a hydrogen bond and the conformational equilibrium shifted as a result. Hydrogen bonding can for hydroxyl or amide protons be studied by NMR spectroscopy through the investigation of ^{1}H/^{2}H exchange rates, chemical shift displacement as a function of temperature or by ^{1}H/^{2}H chemical shift isotope effects.

In some cases, the problem under investigation cannot be fully resolved due to the inability of the technique to differentiate between various cases. One such problem has been observed in the conformational analysis of α-L-Rha*p*-(1→2)-α-L-Rha*p*-OMe [1], where two possible conformational states could not be differentiated due to a 'three spin effect' between protons, which rendered the NOEs for the two conformational states essentially identical under the experimental conditions employed. By simulation of the NOE build-up curves from both conformers, it became apparent that, if the H-2 proton at the glycosidic linkage could be eliminated, then differentiation between conformers should be possible. This could be performed by a MINSY NMR experiment or by substitution of H-2 by a deuterium atom. Here, we report the synthesis of site-specific, deuterated analogues of α-L-Rha*p*-(1→2)-α-L-Rha*p*-OMe.

Site-specific deuteration of the title compound **1** [2] was targeted to H-2 (**1-*d***) and to H-2 and the

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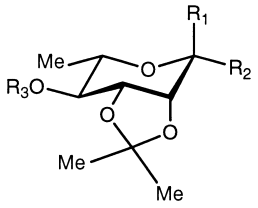
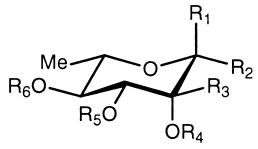
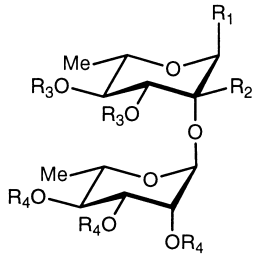
O-methyl group (**1-*d*$_4$**). In order to obtain a good yield of product with the *manno*-configuration by stereoselective reduction of a 2-ulose derivative with sodium borodeuteride, it is necessary that the anomeric center should have the β-configuration. A key intermediate in the synthesis was therefore methyl 3,4-di-*O*-benzyl-6-deoxy-β-L-*arabino*-hexopyranosid-2-ulose.



2 R_1 = OAll, R_2 = H, R_3 = Bn
3 R_1 = OH, R_2 = H, R_3 = Bn
4-*d*$_3$ R_1 = H, R_2 = O(^{2}H$_3$)Me, R_3 = Bn



5-*d*$_3$ R_1 = H, R_2 = O(^{2}H$_3$)Me, R_3 = R_4 = R_5 = H, R_6 = Bn
6-*d*$_3$ R_1 = H, R_2 = O(^{2}H$_3$)Me, R_3 = R_4 = H, R_5 = R_6 = Bn
7-*d*$_4$ R_1 = H, R_2 = O(^{2}H$_3$)Me, R_3 = ^{2}H, R_4 = H, R_5 = R_6 = Bn
8-*d* R_1 = OMe, R_2 = H, R_3 = ^{2}H, R_4 = H, R_5 = R_6 = Bn
8-*d*$_4$ R_1 = O(^{2}H$_3$)Me, R_2 = H, R_3 = ^{2}H, R_4 = H, R_5 = R_6 = Bn



9-*d* R_1 = OMe, R_2 = ^{2}H, R_3 = Bn, R_4 = Bz
9-*d*$_4$ R_1 = O(^{2}H$_3$)Me, R_2 = ^{2}H, R_3 = Bn, R_4 = Bz
1 R_1 = OMe, R_2 = R_3 = R_4 = H
1-*d* R_1 = OMe, R_2 = ^{2}H, R_3 = R_4 = H
1-*d*$_4$ R_1 = O(^{2}H$_3$)Me, R_2 = ^{2}H, R_3 = R_4 = H

This intermediate was synthesized from the starting material, allyl 4-*O*-benzyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**2**), obtained in a three-step synthesis from L-rhamnose according to the procedure of Gigg et al. [3]. Isomerization of the allyl group using the Wilkinson catalyst [tris(triphenylphosphine)rhodium(I) chloride] and ethyldiisopropylamine in ethanol [4,5], followed by hydrolysis of the prop-1-enyl glycoside with $HgCl_2$–HgO in aqueous acetone [6,7], gave crystalline 4-*O*-benzyl-2,3-*O*-isopropylidene-α-L-rhamnopyranose (**3**) in 70% yield. Preparation of (^{2}H$_3$)methyl 4-*O*-benzyl-2,3-*O*-isopropylidene-β-L-rhamnopyranoside (**4-*d*$_3$**) was accomplished under conditions described by Haines et al. [8], in which the alcohol was methylated using sodium hydride and (^{2}H$_3$)methyl iodide in tetrahydrofuran to give **4-*d*$_3$** in 91% yield.

Acidic hydrolysis of the isopropylidene group gave (^{2}H$_3$)methyl 4-*O*-benzyl-β-L-rhamnopyranoside (**5-*d*$_3$**) (92%). (^{2}H$_3$)Methyl 3,4-di-*O*-benzyl-β-L-rhamnopyranoside (**6-*d*$_3$**) was first prepared by the original method of Handa et al. [9] which gave, after activation by dibutyltin oxide and subsequent regioselective benzylation of the diol in *N*,*N*-dimethylformamide and benzyl bromide, only a low yield (<40%) of the desired regioisomer. However, when the activated stannylene intermediate was benzylated using neat benzyl bromide, the yield was raised to 92%.

Swern oxidation (dimethyl sulfoxide/oxalyl chloride/triethylamine) [10] of **6-*d*$_3$** afforded the 2-ulo intermediate, which was not isolated. Subsequent reduction with sodium borodeuteride in methanol-d_4 gave the isotopically enriched (^{2}H$_3$)methyl 3,4-di-*O*-benzyl-β-L-(2-^{2}H)rhamnopyranoside (**7-*d*$_4$**) in 77% yield. The extent of deuteration was >98% as calculated by integration of the remaining H-2 signal in the ^{1}H NMR spectrum. Anomerization of **7-*d*$_4$** with non deuterated or deuterated methanolic hydrogen chloride gave the glycosyl acceptors methyl 3,4-di-*O*-benzyl-α-L-(2-^{2}H)rhamnopyranoside (**8-*d***) and (^{2}H$_3$)methyl 3,4-di-*O*-benzyl-α-L-(2-^{2}H)rhamnopyranoside (**8-*d*$_4$**) in 80 and 82%, respectively.

Glycosylation of compounds **8-*d*** and **8-*d*$_4$** with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide [11] using silver triflate as a promoter [12,13] afforded the disaccharides **9-*d*** and **9-*d*$_4$** in 81 and 82%, respectively. Hydrogenolysis of **9-*d*** and **9-*d*$_4$** over Pd–C and subsequent removal of the benzoate protecting groups with methanolic sodium methoxide gave after gel filtration the target molecules **1-*d*** and **1-*d*$_4$** in 87 and 89%, respectively. The overall yield from **2** was ~26% for the two target compounds. The ^{1}H NMR spectra of **1**, **1-*d*** and **1-*d*$_4$** are given in Fig. 1. We are presently investigating the three-dimensional structure of the title compound making use of these site-specific deuterated analogues.

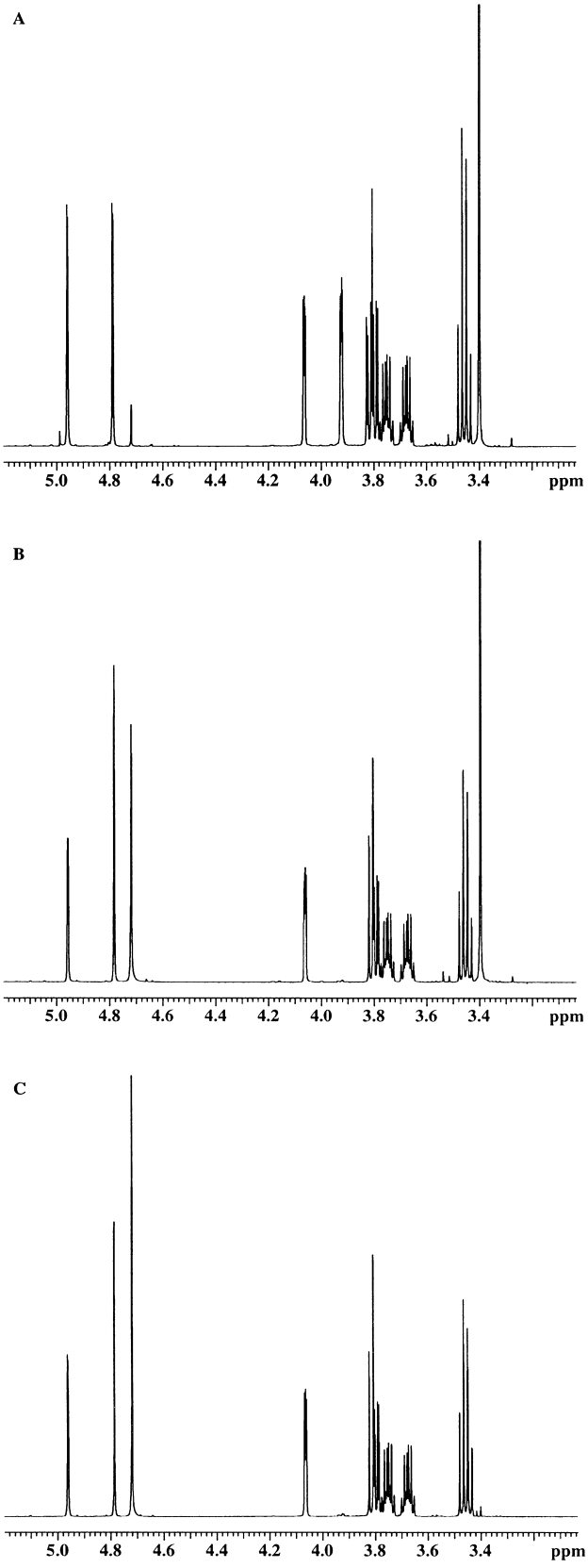


Fig. 1. ^{1}H NMR spectra of α-L-Rhap-(1→2)-α-L-Rha*p*-OMe (A), α-L-Rha*p*-(1→2)-α-L-(2-^{2}H)Rha*p*-OMe (B) and α-L-Rha*p*-(1→2)-α-L-(2-^{2}H)Rha*p*-O(^{2}H$_3$)Me (C).

1. Experimental

General methods.—Concentrations were performed under reduced pressure at temperatures <40 °C (bath). Optical rotations were measured at 22 °C for solutions in $CHCl_3$ or water with a Perkin–Elmer 241 polarimeter. NMR spectra were recorded at 30 °C for solutions in $CDCl_3$, Me_2SO-d_6 or D_2O using JEOL GSX-270 or Varian Inova 600 spectrometers. Chemical shifts are reported in ppm relative to internal $SiMe_4$ (δ_H 0.00), internal sodium 4,4-dimethyl-4-sila-2,2,3,3-($^2H_4$)pentanoate (δ_H 0.00), residual Me_2SO-d_5 (δ_H 2.57) or $CDCl_3$ (δ_C 77.17). Electrospray-Ionization mass spectrometry (ESIMS) was performed in the negative-ion mode on a VG Quattro triple quadrupole mass spectrometer (Micromass) with 1:1 MeOH–water as the mobile phase. High-resolution fast atom bombardment mass spectrometry (HR-FABMS) was performed in the positive-ion mode at a resolution of 10 000 on a JEOL SX-102 using glycerol or *m*-nitrobenzylalcohol as a matrix. Selected intermediates **4**, **5**, **6** and **8** were also prepared non-deuterated (data not shown).

4-O-*Benzyl-2,3*-O-*isopropylidene*-α-L-*rhamnopyranose* (**3**).—A mixture of **2** [3] (4 g, 12 mmol), ethyldiisopropylamine (1.55 g, 12 mmol) and tris-(triphenylphosphine)rhodium(I) chloride (0.11 g, 0.12 mmol) in EtOH (50 mL) was boiled under reflux for 3 h. The mixture was concentrated, and the residue was dissolved in 10:1 acetone–water (25 mL) containing HgO (3.37 g, 16 mmol). A solution of $HgCl_2$ (3.57 g, 13 mmol) in 10:1 acetone–water (25 mL) was added dropwise to the suspension. When TLC (5:1 toluene–EtOAc) showed the reaction to be complete, the mixture was concentrated, and the residue was dissolved in Et_2O, washed with a saturated KI solution, dried, and concentrated. Silica gel column chromatography (7:1 toluene–EtOAc) gave **3** (2.46 g, 70%); $[\alpha]_D$ +21° (*c* 1.0, $CHCl_3$); ^{1}H NMR (Me_2SO-d_6): δ 1.22 (H-6), 1.35 and 1.47 (CMe_2), 3.18 (H-4), 3.81 (H-5), 4.09 (H-2), 4.21 (H-3), 4.64 and 4.84 (CH_2Ph), 5.17 (H-1); ^{13}C NMR ($CDCl_3$): δ 18.1 (C-6), 26.4 and 28.0 (CCH_3), 65.1, 73.1, 76.3, 78.2, 80.8, 92.1 (C-1), 109.4, 127.8, 128.2, 128.4, 138.2. ESIMS: $[M-H]^-$ *m*/*z* 293.3; HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{16}H_{22}O_5Na$ 317.1365; found 317.1351.

($^2H_3$)Methyl 4-O-*benzyl*-β-L-*rhamnopyranoside* (**5-d$_3$**).—MeI-d_3 (1.39 mL, 21.8 mmol) was added to a mixture of **3** (2.26 g, 7.7 mmol) and NaH (0.52 g, 21.8 mmol) in THF (50 mL), and the

mixture was stirred at room temperature. When TLC (7:1 toluene–EtOAc) showed the reaction to be complete, MeOH (2 mL) was added and the mixture was concentrated. The residue was dissolved in CH_2Cl_2 and washed with water, dried, concentrated and applied to a silica gel column (10:1 toluene–EtOAc) to give ($^2H_3$)methyl 4-*O*-benzyl-2,3-*O*-isopropylidene-β-L-rhamnopyranoside (**4-*d*$_3$**) (2.18 g, 91%); ^{13}C NMR ($CDCl_3$): δ 18.3 (C-6), 26.3 and 27.8 (C*C*H$_3$), 56.5 (OMe-*d*$_3$, t), 70.5, 72.7, 74.5, 80.0, 81.0, 99.5 (C-1), 110.4, 127.6, 127.8, 128.2, 138.1.

Compound **4-*d*$_3$** (2.0 g, 6.4 mmol) was dissolved in $CHCl_3$ (10 mL) and aq 90% CF_3COOH (10 mL) was added. The mixture was stirred for 2 h at room temperature, then washed with saturated $NaHCO_3$, dried, concentrated, and purified by silica gel column chromatography (1:1 toluene–EtOAc) to give **5-*d*$_3$** as a white solid (1.61 g, 92%); $[\alpha]_D$ +48° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 17.9 (C-6), 56.0 (OMe-*d*$_3$, t), 71.1, 71.2, 74.2, 75.0, 81.4, 100.6 (C-1), 127.8, 128.0, 128.4, 138.4. HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{14}H_{17}D_3O_5Na$ 294.1397; found 294.1426.

($^2H_3$*)Methyl 3,4-di*-O-*benzyl*-β-L-*rhamnopyranoside* (**6-d$_3$**).—A suspension of compound **5-*d*$_3$** (1.53 g, 5.6 mmol) and dibutyltin oxide (1.54 g, 6.2 mmol) in anhydrous MeOH (50 mL) was boiled under reflux for 3 h. The solvent was evaporated, and the resulting syrup (crude) was dried in a vacuum line and thereafter dissolved in benzyl bromide (10 mL, 8.4 mmol). The mixture was heated at 90 °C for 30 min, then diluted with toluene and put onto a silica gel column and eluted with toluene followed by 2:1 toluene–EtOAc to give after concentration **6-*d*$_3$** as a solid (1.87 g, 92%); $[\alpha]_D$ +36° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.37 (H-6), 3.33 (H-5), 3.53 (H-3,4), 4.09 (H-2), 4.29 (H-1), 4.65, 4.68, 4.76, 4.94 (C*H*$_2$Ph); ^{13}C NMR ($CDCl_3$): δ 18.0 (C-6), 68.5, 71.6, 76.7, 79.8, 81.5, 100.6 (C-1), 127.9–128.6, 138.0, 138.5. HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{21}H_{23}D_3O_5Na$ 384.1866; found 384.1875.

($^2H_3$*)Methyl 3,4-di*-O-*benzyl*-α-L-*(2-*^2H*)rhamnopyranoside* (**8-d$_4$**).—Dimethyl sulfoxide (0.63 mL, 8.8 mmol) in CH_2Cl_2 (3 mL) was added to a cooled solution (−60 °C) of oxalyl chloride (0.38 mL, 4.4 mmol) in CH_2Cl_2 (15 mL), and the mixture was stirred for 5 min. Thereafter compound **6-*d*$_3$** (1.45 g, 4.0 mmol) in CH_2Cl_2 (10 mL) was added dropwise during 15 min. After stirring for 30 min at −60 °C, Et_3N (2.8 mL, 20 mmol) was added and the solution was allowed to reach 0 °C. Water was added and the two phases were separated, the organic phase dried (Na_2SO_4) and concentrated. The residue was immediately dissolved in MeOH-*d*$_4$ (40 mL) and $NaBD_4$ (0.185 g, 4.4 mmol) was added. After 15 min the mixture was concentrated. Silica gel column chromatography (4:1 toluene–EtOAc) gave ($^2H_3$)methyl 3,4-di-*O*-benzyl-β-L-(2-^{2}H)rhamnopyranoside (**7-*d*$_4$**) (1.12 g, 77%); R_f (3:1 toluene–EtOAc): 0.19; $[\alpha]_D$ +40° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.37 (H-6), 3.33 (H-5) 3.53 (H-3,4), 4.29 (H-1, s), 4.65, 4.68, 4.76, 4.94 (C*H*$_2$Ph).

Compound **7-*d*$_4$** (0.500 g, 1.38 mmol) was dissolved in MeOH-*d*$_4$ and acidified (0.5 mL 2 M acetyl chloride in MeOH-*d*$_4$). The mixture was boiled under reflux for 1 h, then it was concentrated. The residue was purified on a silica gel column (4:1 toluene–EtOAc) to give **8-*d*$_4$** (0.410 g, 82%); R_f (3:1 toluene–EtOAc): 0.37; ^{13}C NMR ($CDCl_3$): δ 18.0 (C-6), 67.3, 68.2 (C-2, t), 72.1 and 75.4 (C*H*$_2$Ph), 80.1, 100.1 (C-1), 127.8-128.6, 138.1, 138.5. ESIMS: $[M-H]^-$ *m*/*z* 361.1. HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{21}H_{22}D_4O_5Na$ 385.1929; found 385.1922.

Methyl 3,4-di-O-*benzyl*-α-L-*(2-*^2H*)rhamnopyranoside* (**8-d**).—This compound was prepared as described for **8-*d*$_4$**, but using MeOH, starting from **7-*d*$_4$** (0.500 g, 1.38 mmol) to give **8-*d*** (0.396 g, 80%); $[\alpha]_D$ −40° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 54.9 (OMe). ESIMS: $[M-H]^-$ *m*/*z* 358.2. HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{21}H_{25}DO_5Na$ 382.1741; found 382.1781.

($^2H_3$*)Methyl (2,3,4-tri*-O-*benzoyl*-α-L-*rhamnopyranosyl)-(1→2)-3,4-di*-O-*benzyl*-α-L-*(2-*^2H*)rhamnopyranoside* (**9-d$_4$**).—A mixture of **8-*d*$_4$** (0.350 g, 0.97 mmol), 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (0.679 g, 1.26 mmol), collidine (59 μL, 0.49 mmol) and 4Å molecular sieves (0.5 g) in CH_2Cl_2 (20 mL) was stirred for 15 min. Then, the temperature was lowered to −40 °C and AgOTf (0.325 g, 1.26 mmol) was added. When TLC (10:1 toluene–EtOAc) showed a complete reaction, pyridine (50 μL) was added, and the mixture was filtered through Celite and concentrated. Silica gel chromatography of the residual syrup (toluene, 20:1 toluene–EtOAc) gave **9-*d*$_4$** (0.650 g, 82%); $[\alpha]_D$ +96° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 17.9 and 18.1 (C-6,6′), 67.3, 68.2, 70.1, 70.8, 72.2, 72.7, 75.7, 79.9, 80.3, 99.6 and 100.0 (C-1,1′), 127.6–133.2, 138.5, 138.8, 165.4, 165.6, 166.0. HRFABMS: $[M+Na]^+$ *m*/*z* calcd for $C_{48}H_{44}D_4O_{12}Na$ 843.3295, found 843.3306.

Methyl (2,3,4-tri-O-*benzoyl*-α-L-*rhamnopyranosyl)-(1→2)-3,4-di*-O-*benzyl*-α-L-*(2-^{2}H)rhamnopyranoside* (**9-d**).—This compound was prepared as described for **9-*d*$_4$** starting from **8-*d*** (0.320 g, 0.9 mmol) to give **9-*d*** (0.600 g, 81%). ^{1}H NMR ($CDCl_3$): δ 1.34 and 1.39 (H-6,6′), 3.36 (OMe). HRFABMS: $[M+Na]^+$ *m/z* calcd for $C_{48}H_{47}DO_{12}Na$ 840.3106; found 840.3134.

($^2H_3$)Methyl α-L-*rhamnopyranosyl-(1→2)*-α-L-*(2-^{2}H)rhamnopyranoside* (**1-d$_4$**).—Compound **9-*d*$_4$** (0.300 g, 0.366 mmol) was dissolved in a mixture of 1:1 EtOAc–EtOH (20 mL) and hydrogenolysed over 10% Pd–C (catalytic amount) for 16 h. Then, the mixture was filtered through Celite and concentrated. The residue was dissolved in 0.1 M NaOMe/MeOH (25 mL). When TLC (12:3:3:1 EtOAc–HOAc–MeOH–H_2O) showed that the debenzoylation was complete, the mixture was passed through a column of Dowex-50 (H^+) and concentrated to dryness. Gel filtration through a Bio-Gel P-2 column eluted with water and freeze-drying gave 107 mg of **1-*d*$_4$** (89%). NMR data and optical rotation are in agreement with those previously published [2]. HRFABMS: $[M+Na]^+$ *m/z* calcd for $C_{13}H_{20}D_4O_9Na$ 351.1569; found 351.1554.

Methyl α-L-*rhamnopyranosyl-(1→2)*-α-L-*(2-^{2}H) rhamnopyranoside* (**1-d**).—This compound was prepared as described for **1-*d*$_4$** starting from **9-*d*** (0.300 g, 0.367 mmol), to give **1-*d*** (104 mg, 87%). HRFABMS: $[M+Na]^+$ *m/z* calcd for $C_{13}H_{23}DO_9Na$ 348.1381, found 348.1374.

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