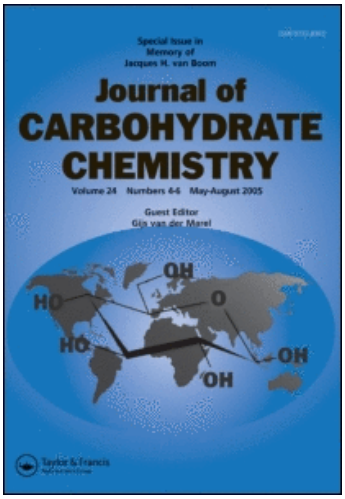

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SYNTHESIS OF TRIDEUTERIOMETHYL 5-DEUTERIUM-β-D-MANNOPYRANOSIDE.

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ABSTRACT

The title compound was prepared by first converting trideuteriomethyl 2,3,4-tri-*O*-benzyl-β-**D**-mannopyranoside to a 6-bromo-6-deoxy derivative which on elimination by using DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) or DBN (1,5-diazabicyclo[4.3.0]non-5-ene) gave a hex-5-enopyranoside derivative. The deuteroboration of the hex-5-enopyranoside followed by oxidation and subsequent deblocking produced trideuteriomethyl 5-deuterium-β-**D**-mannopyranoside.

INTRODUCTION

In our long term project on the three-dimensional studies of oligosaccharides of *N*-linked glycoproteins by ^{1}H NMR, we often require the synthesis of oligosaccharides in which specific isotopes, such as deuterium or ^{13}C, have been incorporated. Deuteration simplifies the coupling pattern of the remaining protons in the molecule since, proton-deuterium couplings are one sixth of the normal proton-proton couplings. Most importantly, for studies of three dimensional oligosaccharide structure, the NOE's (Nuclear Overhauser Enhancements) become larger. This arises because incorporation of deuterium

in a hexose ring isolates the protons on either side of it, and reduces the number of relaxation pathways of the remaining proton spin systems.

As mannose is one of the important monosaccharides in glycoproteins, we have been actively engaged in developing different methods for the synthesis of mannose with deuterium at selective sites.[1] This work describes the incorporation of deuterium at C–5 of trideuteriomethyl β–D–mannopyranoside.

RESULTS AND DISCUSSION

As shown in Fig.1, the synthesis of **6** was carried out from trideuteriomethyl 2,3,4–tri–*O*–benzyl–β–D–mannopyranoside (**1**) which was easily prepared by the acetolysis[2] of methyl 2,3,4,6–tetra–*O*–benzyl–α–D–mannopyranoside followed by glycosidation[3] with methanol–d_4. Compound **1** was reacted with methanesulfonyl chloride in the presence of triethylamine to give the 6–*O*–mesylate derivative (**2**), which on direct treatment with triethylamine and lithium bromide in methyl ethyl ketone,[4] produced the 6–bromo–6–deoxy compound (**3**) in 90% yield. The ^{1}H NMR spectrum of the latter intermediate is easily distinguished from its predecessor in the 4.0 to 3.6 ppm region where all of the individual resonances attributed to H–2, H–4 and H–6 can be seen without overlap. The resonance for H–6', shifts from 3.8 ppm in the hydroxy compound (**1**) to 3.65 ppm in the bromo compound (**3**), where it overlaps with its H–3 resonance.

The elimination reaction of compound **3** was successfully carried out with the use of DBU (1,8–diazabicyclo[5.4.0]undec–7–ene) in toluene and gave compound **4** in 51% yield. We observed this reaction to be more efficient (75% yield) with the use of DBN (1,5–diazabicyclo[4.3.0]non–5,6–ene). It was interesting to note that the reaction occurred with poor yield using pyridine and silver fluoride, which are the commonly known reagents for making hex–5–enopyranoside derivatives.[5]

The ^{1}H–^{13}C correlated NMR spectrum of **4** clearly shows the three benzyl methylene carbon doublets centered at 70.5, 71.4, and 71.9 ppm with J_{CH} ranging from 140 to 143 Hz. The corresponding ^{1}H NMR resonances of the three sets of geminal protons on these benzyl carbons are summarized in the experimental section. The ^{13}C resonances for carbons C–1 through C–4 are typical of those found in pyranoses. The unique chemical shift of C–5 at 137.2 ppm and C–6 at 98.4 ppm support the presence of the exocyclic double bond at C–5. The absence of a resonance for C–5 in the ^{1}H–^{13}C correlated NMR spectrum corroborates this conclusion. Similarly, the proton spectrum (substantiated by the ^{1}H–^{13}C correlation) shows resonances for H6 and H6' at 4.84 and 4.67 ppm as singlets. In general, geminal coupling constants range from –3 to 7 Hz for exocyclic methylene groups such as >C=CH_2. The magnitude of J_{gem} for these groups varies with electronegative substituents such as oxygen and can fall in the range 0–1 Hz as observed here. The chemical shifts of the aromatic carbons were found in the range from 126 to 127.5 ppm while the corresponding hydrogens were found at 7.2 ppm.

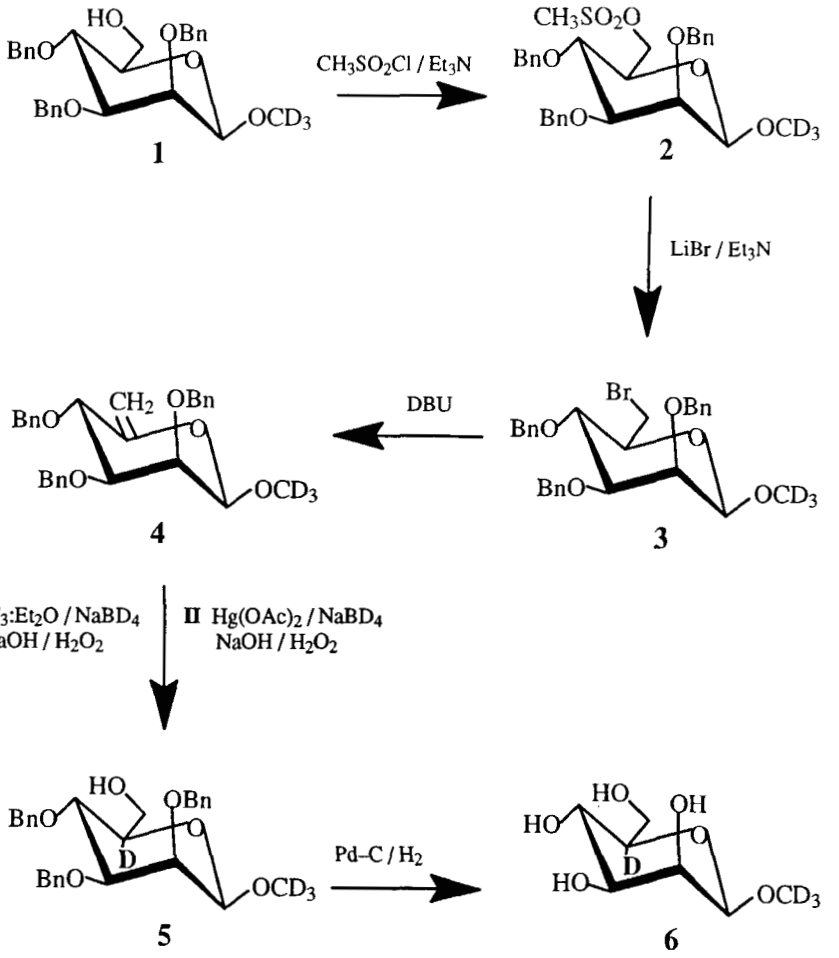

Figure 1 : Synthesis of trideuteriomethyl 5–deuterium–β–D–mannopyranoside **6**.

The next important step in the synthesis was to introduce a deuterium at C–5 by deuteroboration of compound **4** followed by the oxidation of the organoborane formed *in situ*. Since hydroboration reactions are slow and very sensitive to steric factors, many new hydroborating agents with specialized properties have been developed.[6] However, to explore these reagents in order to find the optimum deuteroborating agent for **4** would be difficult and expensive. As a result, we selected two different sets of reaction conditions, **I** and **II**, where reagents are easy to make and the source of deuterium is easily available. In reaction **I**, the reagent BF_3:Et_2O/$NaBD_4$, which has been reported for the stereoselective organoboration of alkenes,[7] was used on the hex–5–enopyranoside **4** in diglyme. This reaction produced mainly compound **5** in 40% yield. The ^1H NMR spectrum of purified **5**

was virtually identical to **1** except that the integration of H–5 gave only 0.2H, representing 80% deuteration at C–5. As a result, H–6' appears as a simple broad doublet at 3.77 ppm with a geminal coupling constant, $J_{6,6'}$ = 11.79 Hz, whereas in the starting compound **1**, it appears as a doublet of doublets at 3.77 ppm with $J_{6,6'}$ = 11.75 and $J_{5,6'}$ = 5.15 Hz. The mass spectral data, in addition to NMR spectral data, confirm the structure of **5** as trideuteriomethyl 2,3,4–tri–*O*–benzyl–5–deuterium–β–**D**–mannopyranoside.

In reaction **II**, the choice of acetoxyborodeuteride ($CH_3COOB^-D_3$) was made primarily for its convenient preparation, mildness, simple reaction procedure and novelty for the deuteroboration of hex–5–enopyranoside. The literature[8] suggested that the remarkable selectivity of acetoxyborodeuteride resulted in the exclusive deuteroboration at the internal, more reactive, carbon–carbon double bond of a diene. However, it was unclear what the effect would be on compound **4** where one carbon of the disubstituted exocyclic 5,6–ene is linked to the ring oxygen. Reaction **II** was very slow and the starting compound **4** was recovered in 69% yield. The desired compound **5** was obtained in only 14% yield. The much lower yield of the latter, relative to that obtained using reaction **I**, suggests that the double bond in **5** has the characteristics of a terminal double bond with respect to its reactivity with acetoxyborodeuteride. However, the integration of H–5 in the NMR spectrum of this product also gave 0.20 H, corresponding to 80% deuterium incorporation, indicating that the efficiency of the two methods is equivalent.

Compound **5** obtained by reaction **I** and **II** was hydrogenated in separate experiments under 45 psi with 10% palladium on carbon as the catalyst to give the title compound **6**. Because the percentage of deuterium incorporation at C–5 in compound **6** was found by NMR to be only 80% in both reactions **I** and **II**, we suspected that the boron–deuterium was undergoing exchange to boron–proton from traces of H_2O in the solvent. On the assumption that traces of moisture in deuterated diglyme (d_{14}) would form D_2O, we repeated reaction **I** with the deuterated solvent. The ^1H NMR spectrum of the title product obtained after deblocking now showed 85% deuterium incorporation at C–5. Subsequent repetition of the same experiment with the addition of 0.05% D_2O, along with diglyme (d_{14}) produced the title compound with 90% deuterium incorporation at C–5 (Fig. 2 B.). The increases in percent deuteration most likely arise from a reduction in the rate of the exchange of boron–deuterium to boron–proton during the formation of diborane (B_2D_6) or organoborane complex. An investigation will be undertaken to explore the mechanistic aspects of the role of D_2O in deuteroboration of hex–5–enopyranosides.

EXPERIMENTAL

General Procedures. Optical rotations were measured with a Perkin–Elmer polarimeter (model 140) at 25 °C. ^1H NMR and ^{13}C NMR spectra were recorded at 27 °C in $CDCl_3$ and D_2O with Bruker AM 500 MHz and AM 300 MHz spectrometers.

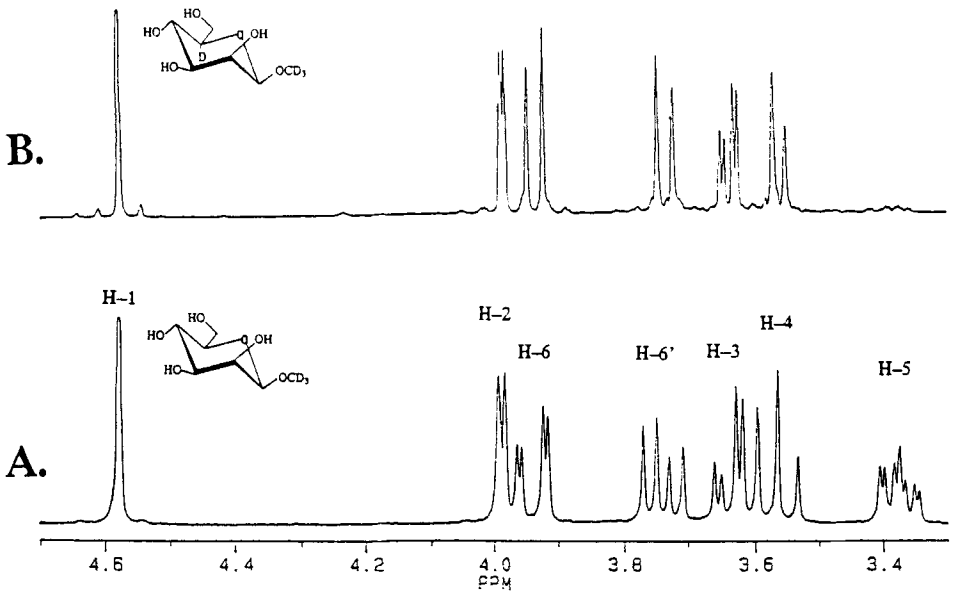

Figure 2 : 500 MHz ^{1}H NMR of **6**, (**A.**) with proton at C–5; (**B.**) with deuterium, 90% atom incorporation at C–5.

Tetramethylsilane (TMS) was used as the internal reference for solutions in $CDCl_3$ while internal acetone was used for D_2O solutions (acetone, 2.225 ppm relative to internal DSS). Ring proton assignments were made by first–order analysis of the spectra and were supported by homonuclear decoupling, two–dimensional COSY or HETCOR, as required. High Resolution Mass Spectroscopy (HRMS) was obtained on a VG–Analytical ZAB–SE spectrometer. The samples were ionized by Fast Atom Bombardment (FAB). All reagents were purchased from Aldrich and solvents from BDH, the latter were dried by conventional methods.

Trideuteriomethyl 2,3,4–Tri–*O*–benzyl–6–bromo–6–deoxy–β–D–mannopyranoside (3). Trideuteriomethyl 2,3,4–tri–*O*–benzyl–β–D–mannopyranoside **1** (1.18 g, 0.228 mmole), prepared by an earlier method,[3] was dissolved in dichloromethane (32.5 mL) and cooled to 0° C under an argon atmosphere. To this solution, triethylamine (0.53 mL, 3.8 mmole) and methanesulfonyl chloride (0.23 mL, 3.0 mmole) were added. The reaction mixture was stirred at the same temperature for 30 min after which it was diluted with dichloromethane. The organic layer was washed successively with an aqueous $NaHCO_3$ solution, water, and brine, and kept over anhydrous $MgSO_4$. After filtration and concentration, the solution yielded a syrupy mesylate (**2**) which was dried by co–evaporation with toluene (3 x 25 mL) and left under vacuum for 45 min. The

mesylate (**2**) was then dissolved in methyl ethyl ketone (16.5 mL) and the solution was refluxed for 90 min in the presence of triethylamine (0.33 mL, 2.4 mmole) and lithium bromide (4.53 g, 52.1 mmole) under anhydrous condition. The reaction mixture was worked up in the manner described above which yielded a thick yellow oil. Purification by flash chromatography gave compound **3** (1.18 g, 90% yield), Rf = 0.69 in hexane/ethyl acetate (2 : 1), $[\alpha]_D$ = – 47.6° (c = 0.46, $CHCl_3$). ^1H NMR δ 7.16 to 7.35 (aromatic, m, 15H); 5.08 and 4.93 ($PhCH_2$, AB quartet, 2H, J_{gem} = 11.7 Hz); 4.76 and 5.08 ($PhCH_2$, AB quartet, 2H, J_{gem} = 10.9 Hz); 4.64 and 4.56 ($PhCH_2$, AB quartet, 2H, J_{gem} = 11.9 Hz); 4.56 (H–1, s, 1H, $J_{1,2}$ < 0.8 Hz); 4.02 (H–2, d, 1H, $J_{2,3}$ = 3.0 Hz); 3.94 (H–4, t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz); 3.84 (H–6, dd, 1H, $J_{5,6}$ = 2.1 Hz, $J_{6,6'}$ = 10.7 Hz); 3.65 (H–6', dd, 1H, $J_{5,6'}$ = 7.5 Hz, $J_{6,6'}$ = 10.7 Hz); 3.63 (H–3, dd, 1H, $J_{3,4}$ = 9.1 Hz, $J_{2,3}$ = 3.0 Hz); 3.56 (H–5, m, 1H, $J_{5,6}$ = 7.5 Hz, $J_{5,6'}$ = 2.1 Hz). HRMS 495.1192 (2.1 mmu, $C_{27}H_{28}O_4Br$, M – OCD_3).

Trideuteriomethyl 6–Deoxy–2,3,4–tri–*O*–benzyl–β–D–lyxo–hex–5–enopyranoside (4). Compound **3** (0.40 g, 0.768 mmol) and DBU (3.7 mL, 2.4 mmol) were dissolved in dry toluene (2mL) and stirred at 105 °C for 3 h. After cooling, the reaction mixture was diluted with dichloromethane (65 mL), washed consecutively with water, 1N HCl, aqueous $NaHCO_3$ and water. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated to a syrup which was purified on silica gel by flash chromatography (hexane/ethyl acetate 3 : 1) to give 4 (0.175 g, 51%), Rf = 0.63 (hexane/ethyl acetate 2 : 1), $[\alpha]_D$ = – 85.4° (c = 0.83, $CHCl_3$). ^1H NMR δ 7.0–7.3 (aromatic, 15H); 4.645 (H–1, s, 1H, $J_{1,2}$ < 1 Hz); 4.84 (H–6, s, 1H, $J_{6,6'}$ < 1 Hz); 4.65 (H–6', s, 1H, $J_{6,6'}$ < 1 Hz); 4.49 and 4.64 ($PhCH_2$, AB quartet, 2H, J_{gem} = 11.8 Hz); 4.69 and 4.66 ($PhCH_2$, AB quartet, 2H, J_{gem} = 12.4 Hz); 4.66 ($PhCH_2$, s, 2H); 4.11 (H–4, 1H, $J_{3,4}$ = 5.8 Hz); 3.95 (H–2, t, 1H, $J_{1,2}$ = $J_{2,3}$ = 3.1 Hz); 3.74 ppm (H–3, ddd, 1H, $J_{2,3}$ = 3.4 Hz, $J_{3,4}$ = 5.8 Hz). ^{13}C NMR δ 137.2 (C–5, s); 101.5 (C–1, d, $J_{C1,H1}$ = 166 Hz); 98.4 (C–6, $J_{C6,H6}$ = 160 Hz); 76.0 (C–3, d, $J_{C3,H3}$ = 143 Hz); 75.2 (C–4, d, $J_{C4,H4}$ = 146 Hz); 73.0 (C–2, d, $J_{C2,H2}$ = 138 Hz) ; 71.9(PhCH, d, $J_{C,H}$ = 141 Hz); 71.4 (PhCH, d, $J_{C,H}$ = 140.2 Hz); 70.5 (PhCH, d, $J_{C,H}$ = 140 Hz). HRMS 523.2179 (2.5 mmu, $C_{30}H_{35}O_6S$, M – OCD_3 + thioglycerol cluster). In thioglycerol matrix, m/z 523 was the dominant ion in the spectrum, whereas when *m*–nitrobenzyl alcohol was used as a matrix, (M – Na^+) (m/z 472) was observed.

Trideuteriomethyl 2,3,4–Tri–*O*–benzyl–5–deuterium–β–D–mannopyranoside (5). *Hydroboration of Compound 4.* [I] BF_3:Et_2O (50 μL) was added dropwise to a well stirred suspension of $NaBD_4$ (0.006 g, 0.14 mmol) and compound **4** (0.1 g, 0.22 mmol) in anhydrous diglyme (1 mL) at 0 °C under argon. The mixture was then stirred at room temperature for 3 h. The organoborane thus formed was oxidized by the consecutive addition of H_2O (0.2 mL), NaOH (3N, 0.2 mL) and H_2O_2 (36 %, 0.2 mL) at 0 °C followed by heating at 60 – 70 °C for 3 h. After cooling, the reaction mixture was extracted with diethyl ether (10 mL) which was washed with water and a brine solution.

The organic layer was separated and dried over anhydrous Na_2SO_4, filtered and concentrated to a syrup. The latter was purified on silica gel by flash chromatography using hexane/ethyl acetate (1 : 1) as the eluant to give compound **5** (0.04 g, 40%), Rf = 0.28 (hexane/ethyl acetate 1 : 1), $[\alpha]_D = -69.5°$ (c = 0.48, $CHCl_3$). ^1H NMR δ 4.942 and 4.646 ($PhCH_2$, AB quartet, 2H, J_{gem} = 10.88 Hz); 4.942 and 4.819 ($PhCH_2$, AB quartet, 2H, J_{gem} = 12.29 Hz); 4.537 and 4.481($PhCH_2$, AB quartet, 2H, J_{gem} = 11.85 Hz); 4.331 (H–1, d, 1H, $J_{1,2} < 0.5$ Hz); 3.9 – 3.85 (H–2, H–4 and H–6, m, 3H); 3.774 (H–6', d, 1H, $J_{6,6'}$ = 11.79 Hz); 3.523 (H–3, dd, 1H, $J_{3,4}$ = 9.44 Hz, $J_{3,2}$ = 3.0 Hz); 3.326 (H–5, ddd, 0.2H, $J_{4,5}$ = 9.35 Hz, $J_{5,6}$ = 5.32 Hz, $J_{5,6'}$ = 3.02 Hz). HRMS 434.2098 (2.0 mmu, $C_{27}H_{28}O_5D$, M – OCD_3); 467.2381 (– 0.6 mmu, $C_{28}H_{27}O_6D_4$, M – H).

[II] In an oven dried 50 mL round bottom flask, anhydrous THF (0.8 mL) and $NaBD_4$ (0.007 g, 0.16 mmol) were placed under argon. After cooling the flask at 0 °C, mercuric acetate (0.024 g, 0.075 mmol) was added with care. The mixture in the flask was stirred at 0 °C for 1 h and then brought to room temperature. To this reaction mixture was added compound **4** (0.1 g, 0.22 mmol) in dry THF (1 mL) dropwise. The contents were further stirred for 16 h at room temperature. The organoborane thus prepared in THF was cooled to 0 °C, and aqueous NaOH (3 N, 0.1 mL) was added, followed by an addition of H_2O_2 (36 %, 0.1 mL). The flask was allowed to attain room temperature and heated for 1 h at 70 °C during which time the mercury was coagulated. The contents were brought to room temperature and the solution was decanted from the mercury. The work up was then repeated in the same way as above to give compound **5** (0.015 g, 14%). ^1H NMR δ 4.493 and 4.645 ($PhCH_2$, AB quartet, 2H, J_{gem} = 10.86 Hz); 4.493 and 4.812 ($PhCH_2$, AB quartet, 2H, J_{gem} = 12.25 Hz); 4.533 and 4.486 ($PhCH_2$, AB quartet, 2H, J_{gem} = 11.80 Hz); 4.334 (H–1, d, 1H, $J_{1,2} < 1$ Hz); 3.95 – 3.85 (H–2, H–4 and H–6, m, 3H); 3.773 (H–6', d, 1H, $J_{6',6}$ = 11.33 Hz); 3.524 (H–3, dd, 1H, $J_{3,4}$ = 9.49 Hz, $J_{3,2}$ = 2.91 Hz); 3.329 (H–5, ddd, 0.08 H, $J_{4,5}$ = 9.00 Hz, $J_{5,6}$ = 5.44 Hz, $J_{5,6'}$ = 3.00 Hz). In addition, the TLC revealed the presence of the starting compound (Rf = 0.8 in hexane/ethyl acetate 1 : 1, 69 %).

Trideuteriomethyl 5–Deuterium–β–D–mannopyranoside (6). A solution of compound **5** (0.03 g, 0.064 mmol) in ethanol (12.5 mL), ethyl acetate (2.5 mL), and water (2.5 mL) with two drops of acetic acid was shaken in the presence of 10% Pd–C (0.05 g) under 3 atmospheres of hydrogen at room temperature. After 24 hours, the suspension was filtered through Celite and the filtrate was concentrated to a syrup which was redissolved in aqueous methanol and treated with mixed bed resin (BioRad AG501–X8, 1 mL) to give the pure compound **6** (0.011 g, 90%), $[\alpha]_D = -34.9°$ (c = 0.76, H_2O). ^1H NMR δ 4.574 (H–1, s, 1H, $J_{1,2} < 1$ Hz); 3.982 (H–2, d, 1H, $J_{2,3}$ = 3.20 Hz); 3.930 (H–6, d, 1H, $J_{6,6'}$ = 12.23 Hz); 3.730 (H–6', d, 1H, $J_{6',6}$ = 12.21 Hz); 3.634 (H–3, dd, 1H, $J_{2,3}$ = 3.22 Hz, $J_{3,4}$ = 9.65 Hz); 3.558 (H–4, d, 1H, $J_{3,4}$ = 9.67 Hz); 3.372 (H–5, ddd, 0.2 H, $J_{4,5}$ = 9.29 Hz, $J_{5,6}$ = 2.27 Hz, $J_{5,6'}$ = 6.82 Hz). HRMS 199.1136 (2.0 mmu, $C_7H_{11}O_6D_4$, MH^+); 221.09675 (3.2mmu, $C_7H_{10}O_6D_4Na$, MNa^+).

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