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Stereoselective synthesis of chirally deuterated (*S*)-D-(6-$^2H_1$)glucose

Lin Xu and Neil P. J. Price*

Department of Pharmacology and Physiology, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, USA

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Abstract—Chirally deuterated (*S*)-D-(6-$^2H_1$)glucose has been prepared in good overall yield from D-(6,6′-$^2H_2$)glucose by a short, five-step synthesis from D-(6,6-$^2H_2$)glucose utilizing (*R*)-(+)-Alpine-Borane® [(*R*)-9-[(6,6-dimethylbicyclo[3.1.1]hept-2-yl)methyl]-9-borabicyclo[3.3.1]nonane]. Suitably protected methyl 2,3,4-tri-*O*-benzyl-D-(6,6-$^2H_2$)glucopyranoside was prepared and the deuterated O-6 primary alcohol was oxidized to an aldehyde by Swern oxidation. Stereoselective reduction with nondeuterated (*R*)-(+)-Alpine-Borane® gave methyl 2,3,4-tri-*O*-benzyl-(6*S*)-D-(6-$^2H_1$)glucopyranoside, which was deprotected under standard conditions to afford the title compound. The key stereoselective reduction step was achieved in 90% yield. The preparation uses economical, commercially available starting materials and will be useful for elucidating biosynthetic mechanisms.

Keywords: Chirally deuterated glucose; Alpine-Borane®; 9-[(6,6-Dimethylbicyclo[3.1.1]hept-2-yl)methyl]-9-borabicyclo[3.3.1]nonane; NMR spectroscopy; Mass spectrometry

1. Introduction

Chirally deuterated sugars have been widely used to elucidate mechanisms of biosynthesis and of chemical reactions.[1–3] Selective deuteration has also been used to confirm assignments of complex NMR or mass spectra,[4] and have been particularly useful in biosynthetic studies of aminocyclitol antibiotics.[2,5] To date, however, there are very few reported syntheses for regio and stereospecifically deuterated pyranoses. The first reported fully chemical synthesis of (6*R*)- and (6*S*)-(6-$^2H_1$)glucose by Kakinuma required the preparation of an acetylene intermediate, 3-*O*-benzyl-5,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-(6-$^2H_1$)hex-5-ynofuranose, achieved by treatment of the corresponding 6,6′-dibromoolefin with *n*-butyllithium, followed by quenching with deuterated water.[6] The yield for this step was low to moderate, and poorly impacted the overall yield. The key steps here were analogous to the classical synthesis of chiral acetic acid by Cornforth et al.[7]

An alternative procedure was introduced by Ohrui and co-workers.[8] Perbenzoylated 1,6-anhydro-β-D-glucopyranose was photobrominated with bromine in carbon tetrachloride. This reaction proceeded regio and stereospecifically at H-6_{exo} to give (6*S*)-1,6-anhydro-2,3,4-tri-*O*-benzoyl-6-bromo-β-D-glucopyranose in 88% yield. Reduction with tri-*n*-butyltindeuteride also proceeded stereospecifically to generate 1,6-anhydro-2,3,4-tri-*O*-benzoyl-(6*S*)-β-D-(6-$^2H_1$)glucopyranose. This same procedure was later used to synthesize (6*S*)-D-(6-$^2H_1$)galactopyranose[9] and (5*S*)-D-(5-$^2H_1$)ribofuranose.[10] Reported yields were satisfactory, but the photoactivation step required rather specialized apparatus, and the cost of the deuterated reagent is high.

A more recent preparation of *N*-acetyl-(6*R*)-(6-$^2H_1$)glucosamine[11] made use of the stereoselective reducing agent, Alpine-Borane® [(*R*)-9-[(6,6-dimethylbicyclo[3.1.1]hept-2-yl)methyl]-9-borabicyclo-[3.3.1]-nonane† introduced by Brown and Ramachandran.[12] The key step in this synthesis was a stereoselective

* Corresponding author at present address: USDA-ARS-NCAUR, 1815, N. University St., Peoria, IL 61604, USA. Tel.: +1-309-681-6246; fax: +1-309-681-6427; e-mail: pricen@ncaur.usda.gov

† *B*-Isopinocampheyl-9-borabicyclo[3.3.1]nonane is the nomenclature used by Brown and co-workers, as well as by Aldrich Chemical Company.

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reduction of a suitably protected *C*-6-aldehydo-GlcNAc derivative with (*R*)-(+)-Alpine-Borane-*d*® [(*R*-9-[(6,6-dimethylbicyclo[3.1.1]hept-2-yl)methyl]-9-borabicyclo-[3.3.1]nonane-*d*)]. Chirally deuterated *N*-acetyl-(6*R*)-(6-$^2H_1$)glucosamine was obtained in good yield, but required the pre-synthesis of (*R*)-(+)-Alpine-Borane-*d*® from (+)-pinene.[13] A similar approach has been applied to the synthesis of 1,2:3,4-di-*O*-isopropylidene-(6*R*)-α-D-(6-$^2H_1$)galactopyranose.[14]

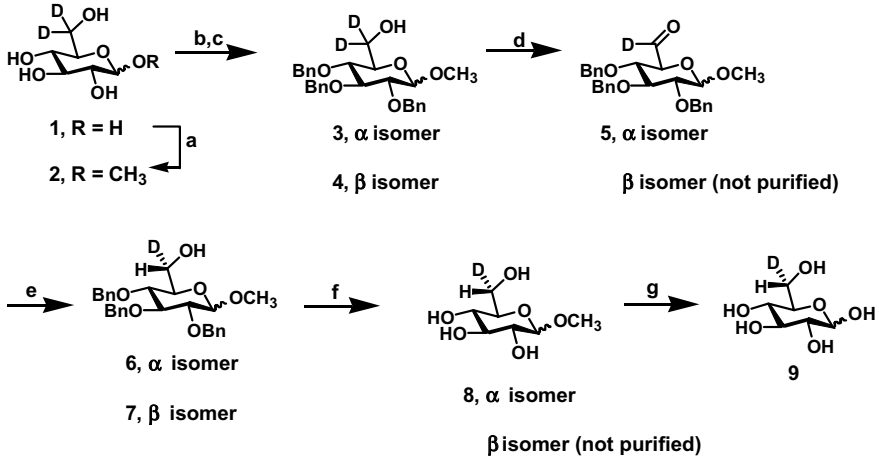
Here we describe a straightforward synthesis of (6*S*)-D-(6-$^2H_1$)glucose from D-(6,6-$^2H_2$)glucose. D-(6,6-$^2H_2$)Glucose was partially protected as methyl 2,3,4-tri-*O*-benzyl-D-(6,6-$^2H_2$)glucopyranoside using the *tert*-butyldimethylsilyl group for transient protection of O-6. The primary alcohol O-6 was subsequently oxidized to the aldehyde by Swern oxidation and stereoselectively reduced with nondeuterated (*R*)-(+)-Alpine-Borane® to yield methyl 2,3,4-tri-*O*-benzyl-(6*S*)-D-(6-$^2H_1$)glucopyranosides **6** and **7**. After deprotection of the methyl and benzyl groups, (6*S*)-D-(6-$^2H_1$)glucose was formed. Two-dimensional NMR techniques such as COSY, HSQC, and HMBC provided fully assigned NMR data for each intermediate and product.

2. Results and discussion

The starting material D-(6,6-$^2H_2$)glucose is commercially available. It can also be simply prepared by reduction of 1,2-*O*-isopropylidene-α-D-glucurono-6,3-lactone with lithium aluminum deuteride and further hydrolysis.[15] D-(6,6-$^2H_2$)Glucose was first converted to the methyl D-(6,6-$^2H_2$)glucopyranosides by refluxing with methanolic 4% HCl. Methyl D-(6,6-$^2H_2$)glucopyranoside was also synthesized by reduction of methyl α-D-glucopyranosiduronic acid methyl ester with sodium borodeuteride.[16] Appropriate protecting groups were chosen to minimize the loss of deuterium. Initially, we used a trityl group to protect O-6 of the glucopyranoside **2**, followed by benzylation at O-2, O-3, and O-4. Subsequent de-tritylation of O-6 with HBr gave mixtures of **3** and **4** in 30% yield. To improve the yield, we chose another approach introduced by Ohrui et al.[1] Primary alcohol O-6 of the methyl D-(6,6-$^2H_2$)glucopyranoside (**2**) was protected by a *tert*-butyldimethylsilyl group. Sodium hydride and benzyl bromide were then added to the reaction mixture to introduce the 2,3,4-tri-*O*-benzyl protecting groups. The *tert*-butyldimethylsilyl group was removed by refluxing in tetrabutylammonium fluoride solution to give mixtures of **3** and **4** in 60% yield (Scheme 1). Methyl 2,3,4-tri-*O*-benzyl-D-(6,6-$^2H_2$)-glucopyranoside can also be prepared with similar yield by stereoselective ring cleavage of methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-glucopyranoside with $LiAlH_4$–$AlCl_3$.[17]

Two isomers **3** and **4** were separated by reversed-phase chromatography to simplify NMR assignment of the products. HPLC analysis showed the isomer ratio of **3** and **4** was 77:23. The anomeric proton of **3** has a chemical shift δ 4.55 and a coupling constant 3.5 Hz. The low vicinal coupling constant for the downfield anomeric proton resonance suggested that **3** is the α-isomer. Accordingly, the anomeric proton of **4** has a chemical shift δ 4.34 and a vicinal coupling constant 7.8 Hz confirming that **4** is the β-isomer. Compound **3** was oxidized to methyl 6-aldehydo-2,3,4-tri-*O*-benzyl-α-D-(6-$^2H_1$)glucopyranoside **5** in 95% yield under the standard Swern conditions.[18] The ^2H NMR spectrum of **5** showed a signal at δ 9.48 ppm indicative of an aldehydic deuterium.

The key step in our synthesis of (6*S*)-D-(6-$^2H_1$)glucose is a stereoselective reduction of the protected *C*-6-aldehydo glycoside **5**. Compound **5** was not especially stable and was easily hydrated to the *gem*-diol. Therefore, **5**



Scheme 1. Reagents and conditions: (a) CH_3OH–4% HCl, reflux; (b) (1) Et_3N, DMAP, *t*-BDMSiCl–DMF, rt, 3 h; (2) Et_3N, NaH, BnBr, rt, 6 h; (c) $(Butyl)_4N^+F^-$, THF–EtOH, reflux, 3 h; (d) (1) DMSO, $(COCl)_2$, −60 °C, 20 min; (2) Et_3N–CH_2Cl_2, −60 °C, 15 min; (e) 1) (*R*)-(+)-Alpine-Borane–CH_2Cl_2, rt, 24 h; (2) NaOH–30% H_2O_2, rt, 1 h; (f) H_2, Pd/C, EtOH, rt, 24 h; (g) 1 N HCl/H_2O, 80 °C, 12 h.

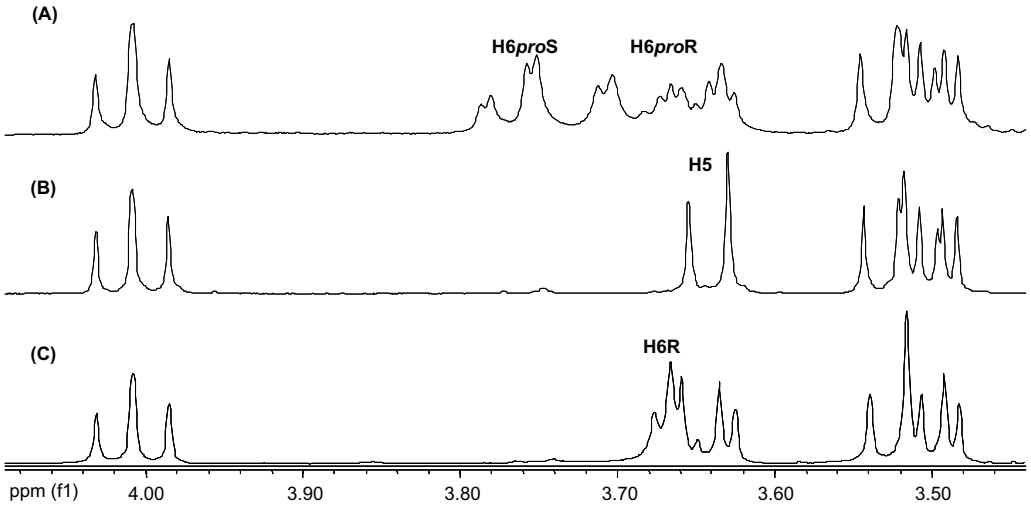


Figure 1. Detail of the 400 MHz ^{1}H NMR spectra of nondeuterated methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (A), methyl 2,3,4-tri-*O*-benzyl-α-D-(6,6-$^2H_2$)glucopyranoside (B), and methyl 2,3,4-tri-*O*-benzyl-(6*S*)-α-D-(6-$^2H_1$)glucopyranoside (C) in $CDCl_3$ is shown. Selected resonances are labeled as H-6*proR* (3.68 ppm), H-6*proS* (3.74 ppm), H-5 (3.63 ppm), and H-6*R* (3.67 ppm).

was reduced immediately with (*R*)-(+)-Alpine-Borane® to form **6** in 90% yield. The ^{1}H and ^{2}H NMR spectra of **6** showed that the reduction was highly stereoselective. In Figure 1, nondeuterated methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside has H-6*proR* and H-6*proS* with chemical shifts δ 3.68 and 3.76 ppm, respectively. Assignment of the chemical shifts of H-6*proR* and H-6*proS* is based on the known methyl 2,3,4-tri-*O*-benzyl-(6*R*)-α-D-(6-$^2H_1$)glucopyranoside,[4] in which H-6(*S*) showed a chemical shift of δ 3.74 ppm. Because both protons on C-6 of **3** are replaced by deuterium, no ^{1}H NMR signal was observed in the range of 3.68–3.74 ppm. H-5 (3.63 ppm) of **3** showed a doublet signal coupled only to H-4 ($J_{4,5}$ 10 Hz), also indicating the absence of H-6 protons. In compound **6**, a proton with chemical shift δ 3.67 ppm was observed coupled to H-5 (3.64 ppm) by ^{1}H–^{1}H COSY. The double-doublet split of H-5 confirmed that H-5 was coupled with H-4 and with a single H-6 proton. The proton was assigned to H-6*R* due to its chemical shift. This assignment was further confirmed by a 2D ^{1}H–^{13}C HSQC experiment, in which H-6*R* was correlated to C-6 (61.5 ppm). The C-6 carbon was observed as a triplet with a normal ^{13}C–^{2}H ^1J coupling constant 21.6 Hz. Furthermore, no proton signal showed at 3.74 ppm (H-6*S*) in the ^{1}H NMR spectrum, and a deuterium signal at 3.77 ppm in the ^{2}H NMR spectrum for **6** suggested that H-6*S* was replaced by deuterium and that the stereoselectivity of reduction by (*R*)-(+)-Alpine-Borane® was almost 100%.

Accordingly, the β-isomer **4** was also oxidized to the aldehyde under Swern conditions, and reduction with (*R*)-(+)-Alpine-Borane® gave **7**. Compared with the ^{1}H NMR spectrum of the known methyl 2,3,4-tri-*O*-benzyl-(6*R*)-β-D-(6-$^2H_1$)glucopyranoside,[4] **7** has all the same proton signals except for H-6. The H-6*S* proton for the known methyl 2,3,4-tri-*O*-benzyl-(6*R*)-β-D-(6-$^2H_1$)-glucopyranoside has a chemical shift at δ 3.87 ppm. However, **7** did not have a proton resonance at δ 3.87 ppm, and instead **7** had H-6 with chemical shift at δ 3.71 ppm. Moreover, the ^{2}H NMR spectrum of **7** showed a deuterium atom with chemical shift at δ 3.87 ppm. The ^{13}C NMR spectrum of **7** was the same as that of the known methyl 2,3,4-tri-*O*-benzyl-(6*R*)-β-D-(6-$^2H_1$)glucopyranoside.[4] These evidences confirm that the absolute configuration of H-6 in **7** is *R*, and therefore compound **7** is assigned as methyl 2,3,4-tri-*O*-benzyl-(6*S*)-β-D-(6-$^2H_1$)glucopyranoside.

The stereoselectivity of Alpine-Borane® is a consequence of coordination between the boron atom and the substrate carbonyl, which holds the substrate in a favored conformation for hydride addition to occur in a highly stereoselective manner. For (*R*)-(+)-Alpine-Borane® this leads to hydride addition to the *si*-face, ultimately resulting in (6*S*)-D-(6-$^2H_1$)glucopyranose. The antipodal reagent, (*S*)-(−)-Alpine-Borane® promotes hydride addition to the carbonyl *re*-face, and assuming no steric restriction to the co-ordination complex, might therefore provide a useful route to the *R*-isomer, (6*R*)-D-(6-$^2H_1$)glucopyranose.

Removal of the 2,3,4-*O*-benzyl groups from **6** was accomplished by reaction with palladium-on-charcoal under hydrogen atmosphere to give methyl (6*S*)-α-D-(6-$^2H_1$)glucopyranoside **(8)** in 92% yield. Compound **8** showed a deuterium resonance at δ 3.60 ppm in the ^{2}H NMR spectrum and a triplet C-6 resonance at δ 62.3 ppm. Acid-catalyzed hydrolysis of **8** produced (6*S*)-D-(6-$^2H_1$)glucose in 85% yield. The normal ratio of α and β-isomers (36:64) in deuterated water was observed in the ^{1}H NMR spectrum. The assignment of NMR data to (6*S*)-α-D-(6-$^2H_1$)glucose

and (6*S*)-β-D-(6-$^2H_1$)glucose was based on ^1H, ^{13}C, and 2D COSY, HSQC, and HMBC spectra. The synthetic (6*S*)-D-(6-$^2H_1$)glucose was successfully incorporated into tunicamycin by fermentation of *S. chartreuses* (work in progress).

3. Experimental

3.1. General methods

All reactions were carried out under dry N_2 except where noted. All solvents were distilled from drying agents. Reagents were purchased from Aldrich Chemical Co. and VWR Scientific. Alpine-Borane® is a trade name of Aldrich Chemical Co. Normal-phase column chromatography was performed on Silica Gel 60 (230–400 mesh, EM Science). Reversed-phase column chromatography was performed on Bakerbond™ C_{18} (40 μm, J. T. Baker). The specific optical rotation values were measured on a Perkin–Elmer 241 polarimeter at 25 °C. ^1H, ^{13}C, COSY, HMBC, and HSQC NMR data were recorded on a Bruker Avance 400 with Me_4Si as the internal standard except where noted. MS analyses were performed with an Agilent LC/MSD ion-trap mass spectrometer (Agilent Technologies) with an electrospray-ionization interface operated in the positive-ion mode.

3.2. Methyl 2,3,4-tri-*O*-benzyl-α-D-(6,6-$^2H_2$)glucopyranoside (3)

D-(6,6-$^2H_2$)Glucose (2 g, 11.1 mmol) was suspended into 4% HCl in abs MeOH solution, and the solution was refluxed until no D-(6,6-$^2H_2$)glucose was detected. After cooling, the solution was evaporated in vacuo to a syrup (2 g) and was used directly in the next reaction without further purification. The synthesis of **3** and **4** from this syrup was accomplished as described by Ohrui et al.[1] Isomers **3** and **4** were separated on a preparative reversed-phase C_{18} column with 68:32 MeOH–water as eluent to yield **3** (2.36 g, 5.06 mmol) and **4** (0.70 g, 1.50 mmol); $[\alpha]_D^{25}$ +19° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 3.35 (s, 3H, OCH_3), 3.48 (dd, 1H, $J_{1,2}$ 3.5, $J_{2,3}$ 9.3 Hz, H-2), 3.51 (dd, 1H, $J_{3,4}$ 9.3, $J_{4,5}$ 10.0 Hz, H-4), 3.63 (d, 1H, $J_{4,5}$ 10.0 Hz, H-5), 3.99 (t, 1H, $J_{2,3} = J_{3,4}$ 9.3 Hz, H-3), 4.55 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.62 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4a-Bn), 4.65 (d, 1H, $J_{3a,3b}$ 12.0 Hz, H-3a-Bn), 4.79 (d, 1H, $J_{3a,3b}$ 12.0 Hz, H-3b-Bn), 4.86 (d, 1H, $J_{2a,2b}$ 10.7 Hz, H-2a-Bn), 4.87 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4b-Bn), 4.98 (d, 1H, $J_{2a,2b}$ 10.7 Hz, H-2b-Bn), 7.27–7.36 (C_6H_5); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.2 (OCH_3), 61.5 (br, C-6), 70.4 (C-5), 73.4 (C-2ab), 75.0 (C-4ab), 75.7 (C-3ab), 77.0 (C-4), 79.9 (C-2), 81.9 (C-3), 98.1 (C-1), 127.6 (Ph), 127.8 (Ph), 127.9 (Ph), 128.0 (Ph), 128.1 (Ph), 128.4 (Ph),128.5 (Ph), 138.1 (Ph), 138.7 (Ph); ^2H NMR ($CDCl_3$, 61.4 MHz): δ 3.75 (br, ^2H-6, *pro-S*), 3.68 (br, ^2H-6, *pro-R*); electrospray-ion trap-MS: calcd for $C_{28}H_{30}{}^2H_2O_6$: *m*/*z* 466. Found: *m*/*z* 467 $[M+H]^+$, 489 $[M+Na]^+$.

3.3. Methyl 2,3,4-tri-*O*-benzyl-β-D-(6,6-$^2H_2$)glucopyranoside (4)

Compound **4** was synthesized as described above for **3**; $[\alpha]_D^{25}$ +7.9° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 3.35 (d, 1H, $J_{4,5}$ 9.6 Hz, H-5), 3.39 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.0 Hz, H-2), 3.56 (s, 3H, OCH_3), 3.58 (dd, 1H, $J_{3,4}$ 9.0, $J_{4,5}$ 9.6 Hz, H-4), 3.66 (t, 1H, $J_{2,3} = J_{3,4}$ 9.0 Hz, H-3), 4.34 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.63 (d, 1H, *J* 10.9 Hz, H-4a-Bn), 4.70 (d, 1H, *J* 10.9 Hz, H-2a-Bn), 4.80 (d, 1H, *J* 10.9 Hz, H-3a-Bn), 4.85 (d, 1H, *J* 10.9 Hz, H-4b-Bn), 4.90 (d, 1H, *J* 10.9 Hz, H-2b-Bn), 4.92 (d, 1H, *J* 10.9 Hz, H-3b-Bn), 7.26–7.34 (Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 57.3 (OCH_3), 61.7 (br, C-6), 74.8 (C-4ab), 74.9 (C-5), 75.1 (C-2ab), 75.7 (C-3ab), 77.5 (C-4), 82.3 (C-2), 84.4 (C-3), 104.8 (C-1), 127.6 (Ph), 127.7 (Ph), 127.8 (Ph), 127.9 (Ph), 128.0 (Ph), 128.4 (Ph), 128.5 (Ph), 137.9 (Ph), 138.4 (Ph), 138.5 (Ph); ^2H NMR ($CDCl_3$, 61.4 MHz): δ 3.86 (br, ^2H-6, *pro-S*), 3.71 (br, ^2H-6, *pro-R*); electrospray-ion trap-MS: calcd for $C_{28}H_{30}{}^2H_2O_6$: *m*/*z* 466. Found: *m*/*z* 467 $[M+H]^+$, 489 $[M+Na]^+$.

3.4. Methyl 2,3,4-tri-*O*-benzyl-α-D-gluco-(6-$^2H_1$)hexodialdo-1,5-pyranoside (5)

Compound **3** (500 mg, 1.07 mmol) was oxidized, according to the procedure described by Singh et al.,[18] to give **5** (470 mg, 1.02 mmol); $[\alpha]_D^{25}$ +14.1° (*c* 1.0, $CHCl_3$); ^1H NMR (C_6D_6, 400 MHz): δ 3.07 (s, 3H, OCH_3), 3.45 (dd, 1H, $J_{1,2}$ 3.38, $J_{2,3}$ 9.56 Hz, H-2), 3.60 (dd, 1H, $J_{3,4}$ 8.75, $J_{4,5}$ 10.2 Hz, H-4), 4.11 (d, 1H, $J_{4,5}$ 10.2 Hz, H-5), 4.24 (dd, 1H, $J_{2,3}$ 9.56, $J_{3,4}$ 8.75 Hz, H-3), 4.47 (d, 1H, $J_{2a,2b}$ 12.0 Hz, H-2a), 4.53 (d, 1H, $J_{2a,2b}$ 12.0 Hz, H-2b), 4.63 (d, 1H, $J_{1,2}$ 3.38 Hz, H-1), 4.68 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4a), 4.82 (d, 1H, $J_{3a,3b}$ 11.3 Hz, H-3a), 4.86 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4b), 5.01 (d, 1H, $J_{3a,3b}$ 11.3 Hz, H-3b), 7.13–7.23 (C_6H_5), 7.32–7.36 (Ph); ^{13}C NMR (C_6D_6, 100 MHz): δ 55.3 (OCH_3), 72.9 (C-2ab), 74.4 (C-5), 75.0 (C-4ab), 75.6 (C-3ab), 77.9 (C-4), 80.3 (C-2), 81.8 (C-3), 98.5 (C-1), 126.2–128.6 (Ph), 138.6 (Ph), 138.9 (Ph), 139.4 (Ph), 196.5 (C-6); ^2H NMR (C_6D_6, 61.4 MHz): δ 9.48 (s, ^2H-6); electrospray-ion trap-MS: calcd for $C_{28}H_{29}{}^2HO_6$: *m*/*z* 463. Found: *m*/*z* 464 $[M+H]^+$, 486 $[M+Na]^+$.

3.5. Methyl 2,3,4-tri-*O*-benzyl-(6*S*)-α-D-(6-$^2H_1$)glucopyranoside (6)

Compound **6** was synthesized as described by Falcone-Hindley and Davis[11] with some modifications. To a solution of aldehyde **5** (470 mg, 1.02 mmol) in CH_2Cl_2 at room temperature was added a 0.5 M solution of *R*-(+)-

Alpine-Borane® in THF (6 mL, 3 mmol). The reaction mixture was stirred at room temperature until **5** was not detected by mass spectrometry (usually 24 h). Acetaldehyde was added to quench the excess Alpine-Borane®. After the solution was stirred for 1 h, the solvent was evaporated in vacuo, and the residue was redissolved in THF (20 mL). To the solution were added 3 M NaOH (20 mL) and 30% H_2O_2 (20 mL). The solution was stirred for 1 h, and THF was removed in vacuo. The aqueous solution was extracted with CH_2Cl_2. Following removal of CH_2Cl_2 the residue was chromatographed on a silica gel column using 60:40 hexane–EtOAc and followed by a preparative reversed-phase C_{18} column with 68:32 MeOH–water as eluent to give **6** (425 mg, 0.91 mmol) in 90% yield; $[\alpha]_D^{25}$ +18.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.36 (s, 3H, OCH_3), 3.50 (dd, 1H, $J_{1,2}$ 3.54, $J_{2,3}$ 9.26 Hz, H-2), 3.52 (t, 1H, $J_{3,4} = J_{4,5}$ 9.46 Hz, H-4), 3.64 (dd, 1H, $J_{4,5}$ 9.46, $J_{5,6}$ 4.05 Hz, H-5), 3.67 (d, 1H, $J_{5,6}$ 4.05 Hz, H-6*R*), 4.01 (dd, 1H, $J_{2,3}$ 9.26, $J_{3,4}$ 9.46 Hz, H-3), 4.56 (d, 1H, $J_{1,2}$ 3.54 Hz, H-1), 4.64 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4a-Bn), 4.67 (d, 1H, $J_{3a,3b}$ 12.0 Hz, H-3a-Bn), 4.81 (d, 1H, $J_{3a,3b}$ 12.0 Hz, H-3b-Bn), 4.84 (d, 1H, $J_{2a,2b}$ 11.0 Hz, H-2a-Bn), 4.89 (d, 1H, $J_{4a,4b}$ 11.0 Hz, H-4b-Bn), 4.99 (d, 1H, $J_{2a,2b}$ 11.0 Hz, H-2b-Bn), 7.27–7.38 (Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 55.1 (OCH_3), 61.5 (t, J_{C-D} 21.6 Hz, C-6), 70.5 (C-5), 73.4 (C-2ab), 75.0 (C-4ab), 75.7 (C-3ab), 77.0 (C-4), 79.9 (C-2), 81.9 (C-3), 98.1 (C-1), 127.6 (Ph), 127.8 (Ph), 127.9 (Ph), 128.0 (Ph), 128.1 (Ph), 128.4 (Ph),128.5 (Ph), 138.1 (Ph), 138.7 (Ph); ^{2}H NMR ($CDCl_3$, 61.4 MHz): δ 3.77 (s, ^{2}H-6, *S*); electrospray-ion trap-MS: calcd for $C_{28}H_{31}{}^2HO_6$: *m*/*z* 465. Found: *m*/*z* 466 $[M+H]^+$, 488 $[M+Na]^+$.

3.6. Methyl 2,3,4-tri-*O*-benzyl-β-D-(6*S*)-(6-$^2H_1$)glucopyranoside (7)

Compound **7** was synthesized as described above for **6**; $[\alpha]_D^{25}$ +8.2° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.37 (dd, 1H, $J_{4,5}$ 9.6, $J_{5,6}$ 4.6 Hz, H-5), 3.40 (dd, 1H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.1 Hz, H-2), 3.57 (s, 3H, OCH_3), 3.58 (dd, 1H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.6 Hz, H-4), 3.67 (t, 1H, $J_{2,3} = J_{3,4}$ 9.1 Hz, H-3), 3.71 (d, 1H, $J_{5,6}$ 4.6 Hz, H-6*R*), 4.36 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.64 (d, 1H, *J* 10.9 Hz, H-4a-Bn), 4.71 (d, 1H, *J* 10.9 Hz, H-2a-Bn), 4.81 (d, 1H, *J* 10.9 Hz, H-3a-Bn), 4.87 (d, 1H, *J* 10.9 Hz, H-4b-Bn), 4.91 (d, 1H, *J* 10.9 Hz, H-2b-Bn), 4.94 (d, 1H, *J* 10.9 Hz, H-3b-Bn), 7.26–7.34 (Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 57.3 (OCH_3), 61.7 (t, J_{C-D} 22 Hz, C-6), 74.8 (C-4ab), 74.9 (C-5), 75.1 (C-2ab), 75.7 (C-3ab), 77.5 (C-4), 82.3 (C-2), 84.4 (C-3), 104.8 (C-1), 127.6 (Ph), 127.7 (Ph), 127.8 (Ph), 127.9 (Ph), 128.1 (Ph), 128.4 (Ph), 128.5 (Ph), 137.9 (Ph), 138.4 (Ph), 138.5 (Ph); ^{2}H NMR ($CDCl_3$, 61.4 MHz): δ 3.87 (s, ^{2}H-6, *S*); electrospray-ion trap-MS: calcd for $C_{28}H_{31}{}^2HO_6$: *m*/*z* 465. Found: *m*/*z* 466 $[M+H]^+$, 488 $[M+Na]^+$.

3.7. Methyl (6*S*)-α-D-(6-$^2H_1$)glucopyranoside (8)

To a solution of **6** (360 mg, 0.77 mmol) in EtOH (40 mL) was added 200 mg of 10% (w/w) palladium-on-charcoal catalyst. The solution was stirred under hydrogen atmosphere (1 L H_2 balloon) for 24 h. The reaction mixture was filtered, and filtrate was concentrated in vacuo. The residue was passed through a Sephadex LH-20 column with MeOH as eluent to yield **8** (138 mg, 0.71 mmol); $[\alpha]_D^{25}$ +158° (*c* 1.1, H_2O); ^{1}H NMR (CD_3OD, 400 MHz): δ 3.28 (dd, 1H, $J_{3,4}$ 8.85, $J_{4,5}$ 9.82 Hz, H-4), 3.37 (s, 3H, OCH_3), 3.40 (dd, 1H, $J_{1,2}$ 3.74, $J_{2,3}$ 9.64 Hz, H-2), 3.50 (dd, 1H, $J_{4,5}$ 9.82, $J_{5,6}$ 5.57, H-5), 3.59 (t, $J_{2,3}$ 9.64, $J_{3,4}$ 8.85 Hz, H-3), 3.64 (d, $J_{5,6}$ 5.57, H-6*R*), 4.65 (d, 1H, $J_{1,2}$ 3.74 Hz, H-1); ^{13}C NMR (CD_3OD, 100 MHz): δ 55.5 (OCH_3), 62.3 (t, J_{C-D} 21.1 Hz, C-6), 71.6 (C-4), 73.3 (C-5), 73.4 (C-2), 75.0 (C-3), 101.1 (C-1); ^{2}H NMR (CD_3OD, 61.4 MHz): δ 3.60 (s, ^{2}H6, *S*); electrospray-ion trap-MS: calcd for $C_7H_{13}{}^2HO_6$: *m*/*z* 195. Found: *m*/*z* 196 $[M+H]^+$, 218 $[M+Na]^+$.

3.8. (6*S*)-D-(6-$^2H_1$)Glucose (9)

Compound **8** (396 mg, 2.02 mmol) was dissolved in 1 M HCl (70 mL) and heated at 80 °C for 12 h. The reaction mixture was cooled to room temperature and neutralized with Amberlite IRA-67 resin. The solution was filtered, and the filtrate was concentrated in vacuo. The residue was chromatographed on Sephadex LH-20 with MeOH as eluent to yield **9** (312 mg, 1.72 mmol) as a white solid; $[\alpha]_D^{25}$ +55° (*c* 1.2, H_2O); ^{1}H NMR (D_2O–acetone-d_6 400 MHz): δ 3.15 (t, 1H, $J_{1,2} = J_{2,3}$ 7.94 Hz, H-2β), 3.28 (m, 1H, H-4α), 3.29 (m, 1H, H-4β), 3.35 (m, 1H, H-3β), 3.36 (m, 1H, H-5β), 3.43 (dd, 1H, $J_{1,2}$ 3.38, $J_{2,3}$ 10.0 Hz, H-2α), 3.61 (d, 1H, H-6β), 3.62 (m, 1H, H-3α), 3.65 (d, 1H, $J_{5,6}$ 3.70 Hz, H-6α), 3.74 (dd, 1H, $J_{4,5}$ 10.0, $J_{5,6}$ 3.70 Hz, H-5α), 4.55 (d, 1H, $J_{1,2}$ 7.94 Hz, H-1β), 5.14 (d, 1H, $J_{1,2}$ 3.38 Hz, H-1α); ^{13}C NMR (D_2O–acetone-d_6 100 MHz): δ 60.5 (t, J_{C-D} 22 Hz, C-6α), 60.8 (t, J_{C-D} 22 Hz, C-6β), 69.9 (C-4α), 69.9 (C-4β), 71.6 (C-5α), 71.7 (C-2α), 73.0 (C-3α), 74.4 (C-2β), 76.0 (C-3β), 76.1 (C-5β), 92.3 (C-1α), 96.1 (C-1β); electrospray-ion trap-MS: calcd for $C_6H_{11}{}^2HO_6$: *m*/*z* 181. Found: *m*/*z* 182 $[M+H]^+$, 204 $[M+Na]^+$.

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