Biocatalytic Syntheses of Protected D-Mannose-d_5, D-Mannose-d_7, D-Mannitol-2,3,4,5,6-d_5, and D-Mannitol-1,1,2,3,4,5,6,6-d_8

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Specifically labeled carbohydrates constitute a group of compounds that are both difficult to prepare and highly desirable for various biological studies. For example, labeled glucoses were utilized to help obtain dynamic measurements of the cerebral pentose phosphate pathway both *in vivo*[1] and in cultured cells.[2] Deuterated sugars have been applied to study the pathway of hepatic glycogen synthesis in rats[3] and in humans.[4] d_7-Glucose was employed to study the biodistribution of glucose in rats,[5] and a labeled galactitol was used to help diagnose disorders of galactose metabolism of fetuses.[6] Finally, labeled glucose has been used to study glucose metabolism in animals and man.[7]

Deuterated carbohydrates that are available commercially include, but are not limited to, such sugars as D-glucose-6,6-d_2 ($86.60/500 mg), D-glucose-1-*d* ($68.49/250 mg), and D-glucose-*2*-*d* ($169.40/250 mg). Perfluorinated derivatives are not available presumably because of the expected facile elimination of HF from unprotected species. Of the eight simple D-sugars, only glucose had been previously synthesized as its hepta-*C*-deuteride.[8] Analogs of the simple D-sugars will be eminently useful in protein binding studies as well as in instances where deuterium incorporation originating in carbohydrate metabolism is desired. Deuterated sugars will also be helpful in understanding the dynamics of carbohydrates in solution.[9]

For the past several years we have devoted considerable time and effort to the development of concise syntheses of carbohydrates from halocyclohexadiene-*cis*-diols.[10] Among these accomplishments are the brief preparations of cyclitols,[11] aminocyclitols,[12] monosaccharides,[13] aza sugars,[14] a novel alkaloid kifunensine,[15] pseudo-sugars,[16] and, most recently, amino sugars.[17] For this study deuterated derivatives of the four biologically important hexoses, D-glucose, D-mannose, D-galactose, and D-fucose, were selected as targets in which the synthesis of perdeuteriomannose would be evaluated to determine the feasibility of this approach. Herein we report the first application of our general protocol to the synthesis of d_5- and d_7-mannoses and perdeuterated mannitol derivatives via toluene dioxygenase-mediated oxidation of d_5-halobenzenes.

Perdeuteriochlorobenzene (**1a**) was subjected to whole cell oxidation by *Pseudomonas putida* 39/D[18] or *Escherichia coli* JM109(pDTG601)[19] as previously described,[20] yielding the corresponding halocyclohexadiene-*cis*-dihydrodiol **2a** in approximate yields of 1 g/L (Scheme 1). Protection of diols **2a** and **2b**[21] as their acetonides followed by osmylation provided the key intermediates: *cis*-diol **4a** as a colorless oil (85% overall yield) and *cis*-diol **4b** as a white solid (68% overall yield). Diols **4** were subjected to ozonolysis and reductive workup, giving a mixture of compounds which were acetylated under standard conditions providing a mixture of anomers of acetyl 4,6-diacetyl-2,3-*O*-isopropylidene-D-mannoside-*2,3,4,5,6*-d_5 (**5**) as the major identifiable product. This compound was isolated as a 3:2 mixture of C6 epimers, indicated by a heteronuclear multiple quantum correlation experiment (HMQC). Alternatively, ozonolysis of diol **4a** and subsequent reduction with sodium borodeuteride gave the intermediate alcohol which was acetylated to yield acetyl 4,6-diacetyl-2,3-*O*-isopropylidene-D-mannoside-*1,2,3,4,5,6,6*-d_7 (**6**). In order to provide a viable route to perdeuteriomannose, diol **4a** was subjected to a similar ozonylitic/reductive sequence employing sodium borodeuteride to give the intermediate triol which was converted to the glycoside **7** in two steps. Comparison of the ^{1}H NMR of this molecule **7** with that

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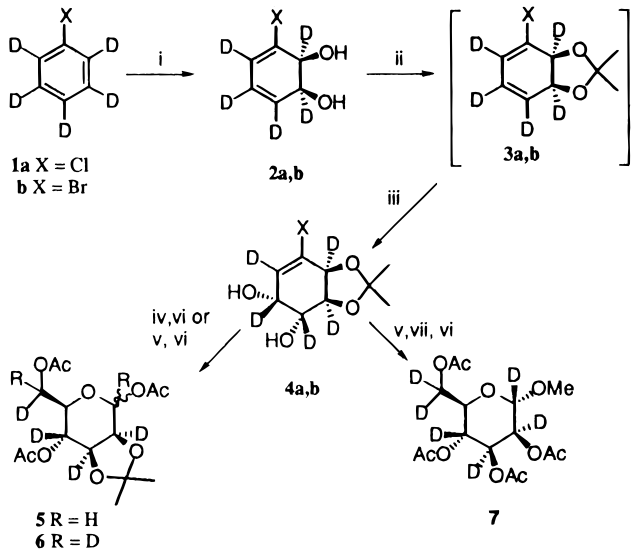
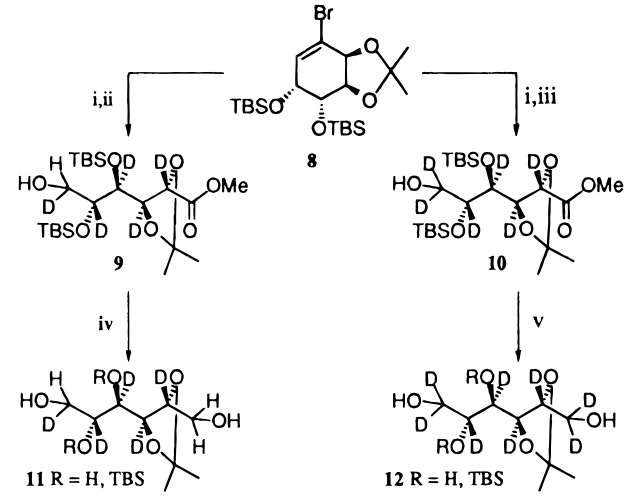
Scheme 1[a]



[a] Reagents: (i) *Pseudomonas putida* 39/D or *Escherichia coli* JM109; (ii) DMP, *p*-TSA, CH_2Cl_2; (iii) OsO_4, *t*-BuOH/H_2O (5:2); (iv) (a) O_3, MeOD, −78 °C, (b) $NaBH_4$, 0 °C−rt; (v) (a) O_3, MeOD, −78 °C, (b) $NaBD_4$, 0 °C−rt; (vi) Ac_2O, pyridine, CH_2Cl_2; (vii) MeOH, HCl (concd), rt.

Scheme 2[a]



[a] Reagents: (i) O_3, MeOH, −78 °C; (ii) $NaBH_4$, 0 °C−rt; (iii) $NaBD_4$, 0 °C−rt; (iv) LAH, THF, rt; (v) LAD, THF, rt.

of the corresponding hydrido derivative[22] establishes the stereochemistry of the methylglycoside to be as depicted.

The syntheses of deuterated mannitols were also undertaken from deuteriodiol **4b** (Scheme 2). Diol **4b** was protected as its disilyl ether **8** and subjected to ozonolysis and reductive workup. When sodium borohydride was employed as the reducing agent, methyl (2*S*,3*S*,4*R*,5*S*)-6-hydroxy-4,5-bis((*tert*-butyldimethylsily)-loxy)-2,3-*O*-isopropylidenehexanoate-*2,3,4,5,6*-d_5 (**9**) was the major product; with sodium borodeuteride, methyl (2*S*,3*S*,4*R*,5*S*)-6-hydroxy-4,5-bis((*tert*-butyldimethylsilyl)-oxy)-2,3-*O*-isopropylidenehexanoate-*1,2,3,4,5,6,6*-d_6 (**10**) was obtained.

Esters **9** and **10** were reduced with lithium aluminum hydride ($LiAlH_4$) and lithium aluminum deuteride ($LiAlD_4$), respectively, to give the target mannitols: ((*tert*-butyldimethylsilyl)oxy)-2,3-*O*-isopropylidene-D-mannitol-*2,3,4,5,6*-d_5 (**11**) (44% overall yield from **8**) and ((*tert*-butyldimethylsilyl)oxy)-2,3-*O*-isopropylidene-D-mannitol-*1,1,2,3,4,5,6,6*-d_8 (**12**) (34% overall yield from **8**).[23]

In conclusion, the synthesis of perdeuterated carbohydrates from d_5-halobenzenes proved to be reasonably facile, certainly when compared to any preparations of the title compounds from natural sugars by oxidation/reduction sequences. Studies leading to other labeled carbohydrates by application of the previously disclosed general method of synthesis coupled with biooxidation of specificaly labeled substrates are in progress.

Experimental Section

General experimental procedures have been published previously.[11c]

(1*S*,2*S*)-3-Chlorocyclohexa-3,5-diene-1,2-diol-*1,2,4,5,6*-d_5 (2a). Perdeuterated diol **2a** was used without purification:mp 91−94 °C; $[\alpha]^{26}_D$ +59.0 (*c* 1.06, $CHCl_3$); IR (KBr) cm^{-1} 3294, 2777, 2191, 1604, 1566; ^1H NMR ($CDCl_3$) δ 4.4 (s, OH), 4.4 (s, OH); ^{13}C NMR ($CDCl_3$) δ 135.9, 131.1, 130.8, 130.4, 123.5, 123.1, 122.8, 122.5, 122.1, 121.8, 71.8, 71.5, 71.2, 70.3, 70.0, 69.7; MS (EI) *m/z* (rel intensity) 151 (M^+) (60), 133 (23), 105 (100), 85 (21), 70 (40); HRMS calcd for $C_6D_5H_2ClO_2$ 151.0448, found 151.0450, error 1.3 ppm.

(1*S*,2*S*)-3-Bromocyclohexa-3,5-diene-1,2-diol-*1,2,4,5,6*-d_5 (2b). Perdeuterated diol **2b** was obtained from Genencor International and used without further purification: mp 92−95 °C; $[\alpha]^{27}_D$ +23.8 (*c* 1.73, $CHCl_3$); IR (KBr) cm^{-1} 3348, 2262, 1561, 1406, 1352; ^1H NMR ($CDCl_3$) δ 2.26 (bs, OH); ^{13}C NMR ($CDCl_3$) δ 131.2, 130.9, 130.7, 128.1, 127.9, 127.6, 126.8, 123.7, 123.4, 123.2, 73.6, 73.4, 73.1, 70.8, 70.6, 70.4; MS (CI) *m/z* (rel intensity) 197 ($M^+ + 1$)(4), 195 (4), 180 (13), 178 (18), 148 (5), 116 (7), 99 (57), 41 (100).

(1*S*,2*S*,3*S*,4*S*)-5-Chloro-3,4-*O*-isopropylidenecyclohex-5-ene-1,2,3,4-tetrol-*1,2,3,4,6*-d_5 (4a). Procedures for acetonide formation and subsequent diol formation have been previously described.[11e,24] Spectral and physical data for diol **4a**: $[\alpha]^{26}_D$ −33.1 (*c* 2.07, $CHCl_3$); IR cm^{-1} 3405, 2988, 2936, 2168, 1632, 1373; ^1H NMR ($CDCl_3$) δ 2.96 (s, 2OH), 1.41 (s, 3H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 132.4, 126.8, 126.5, 126.3, 110.3, 75.7, 75.5, 75.3, 74.7, 74.4, 74.2, 69.2, 68.9, 68.7, 66.2, 66.0, 65.7, 27.5, 25.9; MS (CI) *m/z* (rel intensity) 226 ($M^+ + 1$) (100), 210 (30), 150 (65), 103 (32); HRMS calcd for $C_9D_5H_9ClO_4$ 226.0894, found 226.0901, error 2.7 ppm.. Anal. Calcd for $C_9D_5H_8ClO_4$: C, 47.90; H, 5.80. Found: C, 47.70; H, 5.89.

(1*S*,2*S*,3*S*,4*S*)-5-Bromo-3,4-*O*-isopropylidenecyclohex-5-ene-1,2,3,4-tetrol-*1,2,3,4,6*-d_5 (4). Procedures for acetonide formation and subsequent diol formation have been previously described.[11e, 24] Spectral and physical data for diol **4b**: mp 122−124 °C; $[\alpha]^{27}_D$ −8.5 (*c* 1.00, $CHCl_3$); IR (KBr) cm^{-1} 3507, 3388, 2986, 2933, 2162, 1629, 1370; ^1H NMR ($CDCl_3$) δ 4.12 (s, OH), 4.04 (s, OH), 1.44 (s, 3H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 130.9, 130.6, 130.5, 123.4, 110.2, 75.9, 75.7, 75.5, 69.1, 68.8, 68.6, 67.0, 66.8, 66.5, 27.6, 26.1; MS (CI) *m/z* (rel intensity) 270 ($M^+ + 1$) (100), 254 (27), 194 (5), 103 (57). Anal. Calcd for $C_9D_5H_8BrO_4$: C, 40.01; H, 4.85. Found: C, 39.92; H, 4.86.

(1*S*,2*S*,3*S*,4*S*)-5-Bromo-1,2-bis((*tert*-butyldimethylsilyl)-oxy)-3,4-*O*-isopropylidenecyclohex-5-ene-*1,2,3,4,6*-d_5 (8). Diol **4b** (916.2 mg, 3.392 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and after 5 min, TBSCl (2.159 g, 4.22 equiv) was added followed by imidazole (1.1781 g, 5.1 equiv). Stirring was continued for 9 h whereupon more TBSCl (1.0854 g, 2.12 equiv) and imidazole (522.5 mg, 2.26 equiv) were added. After an additional 23 h 45 min of stirring, the reaction was filtered, concentrated onto silica gel, and flash chromatographed (19:1 hexane:ethyl acetate) to yield the title compound as a white solid in 100% yield: $[\alpha]^{27}_D$ −45.6 (*c* 1.01, $CHCl_3$); IR (KBr) cm^{-1} 2928, 2891, 2856, 2137, 1627, 1472; ^1H NMR ($CDCl_3$) δ 1.42 (s, 3H), 1.39 (s, 3H), 0.91 (s, 9H), 0.87 (s, 9H), 0.097 (s, 6H), 0.089 (s, 3H), 0.084 (s, 3H);

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^{13}C NMR ($CDCl_3$) δ 121.4, 110.0, 27.5, 26.2, 26.0, 25.7, 18.3, 18.1, −4.4, −4.5, −4.8, −4.9; MS (CI) *m*/*z* (rel intensity) 498 ($M^+ + 1$) (7), 442 (15), 440 (14), 426 (20), 424 (20) 384 (63), 382 (60), 360 (100); HRMS calcd for $C_{21}D_5H_{37}BrO_4Si_2$ 498.2119, found 498.2101, error 3.6 ppm. Anal. Calcd for $C_{21}D_5H_{36}BrO_4Si_2$: C, 50.58; H, 8.29. Found: C, 50.67; H, 8.26.

General Procedure for Ozonolysis. The compound was dissolved in methyl alcohol-*d* and cooled to −78 °C. A stream of O_3/O_2 was passed until a dark yellow/green color persisted for 5 min. After the excess O_3 was removed at −78 °C, the temperature was raised to 0 °C and $NaBH_4$ ($NaBD_4$) was added. The reaction was brought to ambient temperature, and more $NaBH_4$ ($NaBD_4$) (equivalents varied) was added until thereduction was complete. The reaction was diluted with water and brought to pH ∼2 with HCl (1 N). The solution was then extracted with ethyl acetate. The combined organic layers were washed with brine (2×), dried ($MgSO_4$), filtered, and concentrated.

Acetyl 4,6-Diacetyl-2,3-*O*-isopropylidene-D-mannoside-*2,3,4,5,6-d*$_5$ (5). The crude ozonolysis product (prepared from 97.2 mg of diols **4**) was dissolved in excess acetic anhydride and pyridine and permitted to stir for approximately 12 h whereupon ethanol was added followed by water. The aqueous layer was extracted with ethyl acetate (5×). The combined organic layers were washed with brine, dried, and concentrated onto silica gel and subjected to flash chromatography (3:2 ether:hexane) to give a 3:2 epimeric ratio of triacetyl-d_5 mannose **5** as a colorless oil (9 mg, 7%): IR cm^{-1} 2991, 1747, 1458, 1438, 1374; ^{1}H NMR ($CDCl_3$) δ 6.16 (s, 1H), 4.59 (s, 0.6H), 4.15 (s, 0.4H), 2.08 (s, 6H), 2.06 (s, 3H), 1.47 (s, 3H), 1.30 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6, 169.6, 169.2, 113.5, 100.5, 25.9, 24.9, 21.0, 20.9, 20.8; MS (CI) *m*/*z* (rel intensity) 336 ($M^+ - 15$) (10), 292 (84), 234 (29), 61 (38), 43 (100). Anal. Calcd for $C_{15}D_5H_{17}O_9$; C, 51.27; H, 6.31. Found: C, 51.36; H, 6.27.

Acetyl 4,6-Diacetyl-2,3-*O*-isopropylidene-D-mannoside-*1,2,3,4,5,6,6-d*$_7$ (6). Compound **6** (331.3 mg, 35%) was prepared following the above procedures from compound **4a** (601.3 mg, 2.7 mmol). **6**: $[\alpha]^{26}_D$ +22.0 (*c* 2.06, $CHCl_3$); IR cm^{-1} 2991, 2940, 2188, 1747, 1434; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 2.02 (s, 3H), 1.42 (s, 3H), 1.26 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6, 169.5, 169.1, 113.4, 25.9, 24.8, 20.94, 20.87, 20.7; MS (EI) *m*/*z* (rel intensity) 338 ($M^+ - 15$) (40), 294 (10), 266 (12), 174 (18), 43 (100); HRMS calcd for $C_{14}H_{12}D_7O_9$ 338.1468, found 338.1468, error 0.0 ppm. Anal. Calcd for $C_{15}H_{15}D_7O_9$: C, 50.97; H, 6.28. Found: C, 50.69; H, 6.35.

Methyl 2,3,4,6-Tetraacetyl-D-mannoside-*1,2,3,4,6,6-d*$_6$ (7). To the crude ozonolysis product (113.4 mg, 0.5 mmol) in methanol (1 mL) was added several drops of concentrated hydrochloric acid. After 24 h the solution was concentrated and the residue dissolved in excess pyridine and acetic acid. After 5 h the solution was concentrated and the residue flash chromatographed (2:1 hexane:ethyl acetate) to yield the title compound (42.4 mg, 23%) which exhibited spectral properties similar to those described previously for nondeuterated analog:[22] ^{1}H NMR ($CDCl_3$) 3.41 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H).

Methyl (2*S*,3*S*,4*R*,5*S*)-6-Hydroxy-4,5-bis((*tert*-butyldimethylsilyl)oxy)-2,3-*O*-isopropylidenehexanoate-*2,3,4,5,6-d*$_5$ (9). Crude ester **9** was prepared from acetonide **8** (459.6 mg, 0.9217 mmol) according to the general ozonolysis procedure and was subjected to flash chromatography (17:3 hexane:ethyl acetate) to yield pure ester **9** as a colorless oil (78.6 mg, 67% yield): $[\alpha]^{23.5}_D$ +12.9 (*c* 1.68, $CHCl_3$); IR cm^{-1} 3504, 2954, 2930, 2881, 2856, 2136, 1735, 1473; ^{1}H NMR ($CDCl_3$) δ 3.75 (s, 3H), 3.69 (d, 1H, J = 6 Hz), 2.38 (d, OH, J = 5.6 Hz), 1.58 (s, 3H), 1.36 (s, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.121 (s, 3H), 0.118 (s, 3H), 0.099 (s, 3H), 0.094 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 110.0, 63.3, 63.1, 62.9, 52.0, 26.4, 26.0, 25.9, 25.6, 18.4, 18.1, −4.3, −4.4, −4.8, −4.9; MS (CI) *m*/*z* (rel intensity) 468 ($M^+ - 15$) (2), 428 (15), 353 (9), 295 (20), 143 (28), 59 (100). Anal. Calcd for $C_{22}D_5H_{41}O_7Si_2$: C, 54.61, H, 9.58. Found: C, 54.78, H, 9.59.

Methyl (2*S*,3*S*,4*R*,5*S*)-6-Hydroxy-4,5-bis((*tert*-butyldimethylsilyl)oxy)-2,3-*O*-isopropylidenehexanoate-*2,3,4,5,6,6-d*$_6$ (10). Ester **10** was prepared from acetonide **8** (211.6 mg, 0.424 mmol) following the general ozonolysis procedure and was subjected to flash chromatography (17:3 hexane:ethyl acetate) to yield pure ester **10** as a colorless oil (81.1 mg, 39.4%): $[\alpha]^{22}_D$ +12.1 (*c* 2.09, $CHCl_3$); IR cm^{-1} 3499, 2953, 2930, 2886, 2857, 2116, 1736, 1472; ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H), 2.31 (bs, OH), 1.56 (s, 3H), 1.34 (s, 3H), 0.89, (s, 9H), 0.87 (s, 9H), 0.099 (s, 3H), 0.096 (s, 3H), 0.077 (s, 3H), 0.072 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8, 110.0, 52.0, 26.5, 26.0, 25.9, 25.6, 18.5, 18.1, −4.3, −4.4, −4.8, −4.9; MS (CI) *m*/*z* (rel intensity) 485 ($M^+ + 1$) (16), 469 (18), 453 (10), 427 (100), 353 (27), 295 (45). Anal. Calcd for $C_{22}D_6H_{40}O_7Si_2$: C, 54.50; H, 9.56. Found: C, 54.61; H, 9.59.

General Procedure for Lithium Aluminum Hydride/Lithium Aluminum Deuteride Reductions. The ester was dissolved in freshly distilled THF and cooled to 0 °C. After ∼3 min, LAH (LAD) was added. The reaction was slowly brought to room temperature and LAH (LAD) was again added (1 equiv). The reaction was cooled to −78 °C, and the reaction was quenched with water (1 μL/mg LAH), NaOH (10% aqueous, 1 μL/mg LAH), and water (3 μL/mg LAH). The reaction was brought to room temperature and stirred until a white paste formed. The paste was filtered, concentrated onto silica gel, and flash chromatographed (3:2 ethyl acetate:hexane) to give pure mannitols.

((*tert*-Butyldimethylsilyl)oxy)-2,3-*O*-isopropylidene-D-mannitol-*2,3,4,5,6-d*$_5$ (11). Mannitol **11** (13.9 mg, 44%) was prepared from ester **9** (44.9 mg, 0.0928 mmol) as a colorless oil following the general procedure for LAH reductions. **11**: mp 44−47 °C; $[\alpha]^{22}_D$ +4.77 (*c* 1.09, $CHCl_3$); IR cm^{-1} 3403, 2953, 2930, 2886, 2856, 2173, 1472; ^{1}H NMR ($CDCl_3$) δ 3.81 (m, 3H), 3.04 (s, OH), 2.73 (s, OH), 2.69 (bs, OH), 1.51 (s, 3H), 1.40 (s, 3H), 0.90 (s, 9H), 0.091 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 108.1, 64.0, 63.8, 63.6, 61.2, 27.0, 25.8, 24.9, 18.2, −5.4, −5.5; MS (CI) *m*/*z* (rel intensity) 344 ($M^+ + 3$) (4), 343 ($M^+ + 2$) (8), 342 ($M^+ + 1$) (10), 326 (5), 308 (4), 284 (34), 134 (54), 73 (95), 59 (100). Anal. Calcd for $C_{15}D_5H_{27}O_6Si$: C, 52.75; H, 9.45. Found: C, 52.65; H, 9.40.

((*tert*-Butyldimethylsilyl)oxy)-2,3-*O*-isopropylidene-D-mannitol-*1,1,2,3,4,5,6,6-d*$_8$ (12). Mannitol **12** (37 mg, 34%) was prepared from ester **10** (159.4 mg, 0.3197 mmol) as a colorless oil following the general procedure for LAD reductions. **12**: $[\alpha]^{25}_D$ +2.70 (*c* 2.04, $CHCl_3$); IR cm^{-1} 3440, 2954, 2930, 2884, 2857, 2101, 1471; ^{1}H NMR ($CDCl_3$) δ 3.06 (s, OH), 2.74 (s, OH), 2.70 (s, OH), 1.51 (s, 3H), 1.40 (s, 3H), 0.91 (s, 9H), 0.095 (s, 3H), 0.092 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 108.1, 27.0, 25.8, 24.9 18.2, −5.4, −5.5; MS (CI) *m*/*z* (rel intensity) 346 ($M^+ + 2$) (2), 345 ($M^+ + 1$) (5), 344 (M^+) (1), 287 (8), 250 (4), 155 (10), 137 (13), 73 (40), 59 (72), 41 (100). Anal. Calcd for $C_{15}D_8H_{24}O_6Si$: C, 52.29, H, 9.36. Found: C, 52.04; H, 9.28.

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