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Supporting Information for

Mechanism of 4,6-*O*-Benzylidene Directed β-Mannosylation as Determined by α-Deuterium Kinetic Isotope Effects

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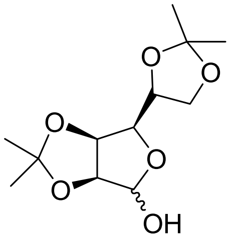
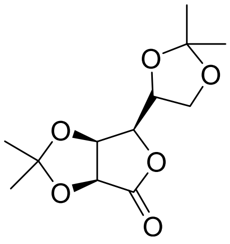
General Procedures. All solvents were dried and distilled by standards. ^{1}H NMR spectra were recorded at 500 MHz and ^{13}C spectra were recorded at 125MHz in $CDCl_3$ solution unless otherwise stated.



2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose[1]

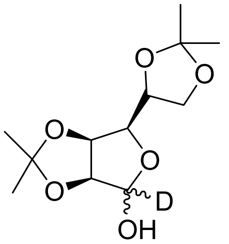
A mixture containing D-mannose (90.0 g, 500 mmol), 2,2-dimethoxypropane (125.0 g, 1.2 mol), PTSA (19.0 g, 100 mmol) in acetone (1L) was stirred at rt for 2 h, concentrated, diluted with dichloromethane and washed with saturated aqueous $NaHCO_3$, and dried (Na_2SO_4). Recrystallization from dichloromethane and hexane provided 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose (121.8 g, 93%) as a white solid. M.p. 119 °C, lit.[2] m.p. 122 °C.

2,3:5,6-Di-*O*-isopropylidene-D-mannono-γ-lactone (1)[1]



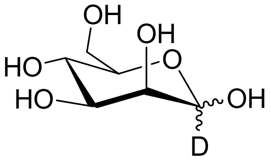
To a solution of 2,3:5,6-Di-*O*-isopropylidene-D-mannofuranose (91.1 g, 350 mmol) in dichloromethane (500 mL) at 0 °C was added bleach (1304 mL, 1.05 mol) NaBr (18.01 g, 175 mmol) and TEMPO (1.09 g, 7.0 mmol) and the reaction mixture was stirred vigorously until the starting material disappeared. The reaction mixture was diluted with EtOAc followed by washing with water, brine and drying over anhydrous Na_2SO_4. Recrystallization from dichloromethane and hexane provided 2,3:5,6-di-*O*-isopropylidene-D-mannono-*γ*-lactone **1** (78.64 g, 87%) as a white solid. M.p. 116 °C, lit.[1] m.p. 116 °C.

1-Deuterio-2,3:5,6-di-*O*-isopropylidene-D-mannofuranose (2)



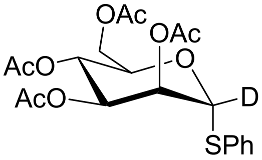
To a solution of **1** (3.0 g, 11.6 mmol) in methanol:water (9:1, 30 mL) was added $NaBD_4$ (0.049 g, 11.6 mmol). The reaction mixture was stirred for 4 h and then was concentrated to dryness. The residue was dissolved in EtOAc and neutralized using 0.2 N HCl and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure to afford 1-deuterio-2,3:5,6-di-*O*-isopropylidene-D-mannofuranose **2** (2.5 g, 83%) as a white solid. $[\alpha]^{25}_D + 17.1°$ (c = 2.5 , absolute ethanol). Lit.[2] $[\alpha]^{21}_D + 16.6°$ (c = 2.5 , absolute ethanol). m.p. 118 °C, lit.[2] m.p. 122 °C . ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 3H), 1.37 (s, 3H), 1.45 (s, 3H), 1.45 (s, 3H), 4.04-4.08 (m, 2H), 4.15 (dd, J = 7.5, 3.6 Hz, 4.38 (m, 1H), 4.61 (d, J = 6.0 Hz) , 4.77 (dd, J = 6.0, 4.0 Hz); ^{13}C NMR ($CDCl_3$): 25.2, 25.6, 26.2, 26.3, 27.2, 27.4, 66.9, 72.2, 73.6, 80.0, 80.7, 85.8, 109.5, 113.1.

1-Deuterio-D-mannose



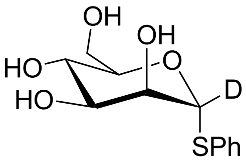
A mixture containing **2** (4.00 g, 15.3 mmol), 2,2-dimethyl-propane-1,3-diol (3.50 g, 33.7 mmol) and PTSA (0.087 g, 4.59 mmol) in dichloromethane (80 mL) was stirred at rt for 1 h. The resulting precipitate was separated to give 1-deuterio-D-mannose (2.33 g, 84%) as a white solid. m.p. 132 °C. Spectral data was identical to that of D-mannose, except that the anomeric proton signal was missing from the ^{1}H NMR spectrum.

S-Phenyl Tetra-*O*-acetyl-1-deuterio-1-thio-α-D-mannopyranoside



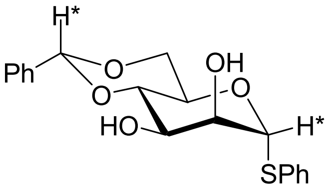
1-Deuterio-D-mannose (3.4 g, 18.9 mmol) was added portion-wise to a 100 mL RB flask containing a stirred solution of acetic anhydride (50 mL) and perchloric acid (0.05 mL), such that the internal temperature of the flask did not exceed 20 °C. Upon completion of the addition, the ice-bath was removed and the mixture was allowed to warm to rt. Once a homogeneous solution was formed, the mixture was poured into an Erlenmeyer flask containing an ice/water mixture and diluted with dichloromethane (25 mL). Saturated aqueous $NaHCO_3$ solution was added to the mixture until the acetic acid was completely neutralized. The organic layer was separated, dried (Na_2SO_4) and concentrated under reduced pressure to give crude pentaacetate (6.74 g) as a syrup. A solution of crude pentaacetate (6.50 g, 17.3 mmol) dissolved in dichloromethane (35 mL) was charged with thiophenol (2.3 mL, 22.4 mmol) under an argon atmosphere. Boron trifluoride etherate (3.1 mL, 24.2 mmol) was added through a pressure-equalizing addition funnel under argon gas line over a period of 10 min. The reaction mixture was stirred over a period of 14 h at rt and then poured into an Erlenmeyer flask containing an ice/water mixture, then neutralized with saturated aqueous $NaHCO_3$ solution. The organic layer was separated and the aqueous phase was further extracted twice with dichloromethane (10 mL). The combined organic layers were washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. The crystalline residue was triturated with diethyl ether:hexanes (1:4) to give the title compound (6.5 g, 86%) as a white powder. $[\alpha\ ]^{20}_{D}$ + 116.7° (c = 0.58 , $CHCl_3$), lit.[3] $[\alpha]^{20}_{D}$ + 107.2° (c = 1.0 , $CHCl_3$). m.p. 85-86 °C, lit.[3] m.p. 87 °C, ^{1}H NMR ($CDCl_3$): δ 2.03 (s, 3H), 2.06 (s, 3H), 2.09 (s, 3H), 2.16 (s, 3H), 4.11 (dd, J = 2.5, 12 Hz, 1H), 4.32 (dd, J = 6.0,12.0 Hz, 1H), 4.54-4.58 (m, 1H), 5.31-5.37 (m, 2H), 5.49 (d, J = 3.0 Hz, 1H), 7.28-7.33 (m, 3H), 7.49-7.51 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 21.0, 21.2, 21.2, 62.8, 66.7, 69.76, 69.8, 71.2, 86.0, 128.5, 129.6, 132.4, 132.9, 170.1, 176.7, 170.3, 170.9.

S-Phenyl 1-Deuterio-1-thio-*α*-D-mannopyranoside (4)



Sodium methoxide (0.13 g, 24.0 mmol), was added to a solution of *S*-phenyl tetra-*O*-acetyl-1-deuterio-1-thio-α-D-mannopyranoside (5.3 g, 12.0 mmol) in methanol (25 mL). After stirring for 4 h, the mixture was acidified with Amberlyst 15 ion exchange resin, filtered and concentrated under reduced pressure. The crude material was triturated with diethyl ether and then dried in the vacuum oven at 45 °C to give **4** (2.80 g, 86%) as a white powder. $[\alpha]^{20}_{D}$ + 203.8° (c = 0.36 in EtOH), lit.[3] $[\alpha]^{20}_{D}$ + 253.2° (c = 3.0 in EtOH). m.p. 126-127 °C, lit.[3] m.p. 128-129 °C. ^{1}H NMR (DMSO): δ 3.43- 3.54 (m, 3H), 3.65 (dd, *J* = 5.0, 2.0 Hz, 1H), 3.76- 3.78 (m, 1H), 3.88-3.90 (m, 1H), 4.53-4.56 (t, *J* = 6.0 Hz, 1H), 4.83 (d, *J* = 5.5 Hz, 1H), 4.92 (d, *J* = 5.5 Hz, 1H), 5.16 (d, *J* = 4.5 Hz, 1H), 7.28-7.35 (m, 3H), 7.50-7.51 (m, 2H); ^{13}C NMR (DMSO): δ 61.7, 67.8, 72.3, 72.7, 76.7, 89.7, 127.9, 129.0, 131.9, 135.7.

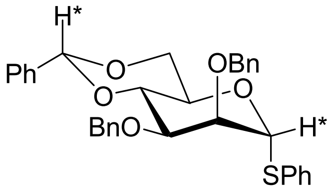
Partially Deuteriated *S*-Phenyl 4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside



A RB flask containing a mixture of deuteriated tetraol **4** (0.50 g, 0.18 mmol), and non-deuterated tetraol (0.50 g, 0.18 mmol), benzaldehyde dimethyl acetal (0.39 mL, 2.57 mmol), 1-deuteriobenzaldehyde dimethyl acetal* (0.39 mL, 2.57 mmol) and PTSA (0.087 g, 0.459 mmol) in N,N-dimethylformamide (20 mL) was heated at 60 °C for 5 h under reduced pressure. The residue was dissolved in EtOAc, washed with water, saturated aqueous $NaHCO_3$ solution and dried (Na_2SO_4). The residue was dissolved in EtOAc and triturated with hexanes to afford partially deuteriated *S*-phenyl 4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside (0.72 g, 55%) as a white solid. $[\alpha]^{20}_{D}$ +215.6° (c = 0.76 , DMSO), lit.[4] $[\alpha]^{20}_{D}$ +289° (c = 0.5 , $CHCl_3$: MeOH, 1:1); m.p. 205- 206 °C, lit.[4] m.p. 213-214 °C. ^{1}H NMR (DMSO): δ 3.73-3.77 (m, 2H), 3.91-4.10 (m, 4H), 5.23 (d, *J* = 6.0 Hz, 1H), 5.33 (s, 1H), 5.58 (d, *J* = 4.0 Hz, 1H), 5.61 (s, 1H), 7.25-7.47 (m, 10H); ^{13}C NMR (DMSO): δ 66.1, 68.4, 68.9, 73.2, 79.3, 90.1, 102.0, 127.26, 128.3, 128.9, 129.7, 130.1, 132.1, 134.4, 138.6.

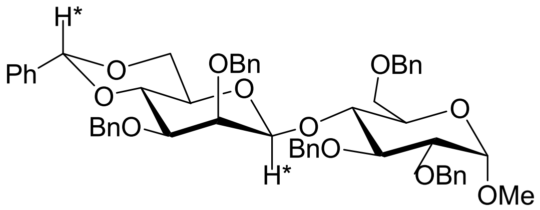
* 1-Deuteriobenzaldehyde dimethyl acetal[5] was synthesized from 1-deuteriobenzaldehyde[6]

Partially Deuteriated *S*-Phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (6).

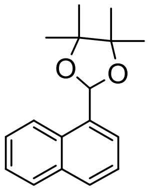


A THF (5mL) solution of partially deuteriated *S*-phenyl-4,6-*O*-benzylidene-1-thio-α-D-mannopyranoside (0.42 g, 1.17 mmol) was added to a suspension of 60% NaH (0.167 g, 4.19 mmol) in THF (5 mL) at 0 °C, and the mixture was stirred for 40 min. To the mixture was added benzyl bromide (0.50 mL, 4.19 mmol) and the reaction mixture was heated at reflux overnight. The reaction mixture was concentrated and MeOH was added followed by water. The aqueous phase was extracted with EtOAc and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc:Hexanes, 1:7) to provide partially deuteriated *S*-phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-*α*-D-mannopyranoside **6** (0.57 g, 91%) as a white solid. $[\alpha]^{20}{}_{D}$ +104.6° (c = 0.59 , $CHCl_3$); lit.[4] $[\alpha]^{20}{}_{D}$ +107° (c = 1.13, $CHCl_3$); m.p. 83-84 °C, lit.[4] m.p. 84-85 °C ^{1}H NMR ($CHCl_3$): δ 3.89 (t, *J* = 10.0 Hz, 1H), 3.97 (dd, *J* = 3.0, 9.0 Hz, 1H), 4.04 (t, *J* = 1.0 Hz, 1H), 4.22-4.33 (m, 3H), 4.66 (d, *J* = 12.0 Hz, 1H), 4.73 (s, 2H), 4.83 (d, *J* = 12.0 Hz, 1H), 5.51 (s, 1H), 5.66 (s, 1H), 7.26-7.51 (m, 20H); ^{13}C NMR ($CHCl_3$): δ 65.8, 68.9, 73.4, 76.6, 78.4, 79.5, 87.5, 101.9, 126.5, 128.0, 128.2, 128.5, 128.6, 128.7, 128.8, 128.2, 129.5, 132.0, 137.9, 138.7.

The KIE Experiment: Partially Deuteriated Methyl 2,3,6-*tri*-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranosyl)-*α*-D-glucopyranoside (11).



A mixture of thioglycoside **6** (0.070 g, 0.13 mmol) and 4,4,5,5-tetramethyl-2-(1-naphthyl)-1,3-dioxolane (0.018 g, 0.065 mmol) was dissolved in $CDCl_3$ and the ^{1}H NMR spectrum recorded. The mixture was then concentrated and activated 3 Å powdered sieves were added before drying under vacuum overnight. To the mixture was added 1-benzenesulfinyl pyrrolidine (0.035 g, 0.18 mmol) and tri-*tert*-butylpyrimidine (0.064 g, 0.26 mmol), followed by drying under vacuum for 45 mins. Dichloromethane (2.5 mL) was added into the mixture under an argon atm followed by cooling to -78 °C and addition of Tf_2O (32.6 *μ*L, 0.19 mmol) at -78 °C. After 35 min, a solution of the glucosyl acceptor **10** (0.030 g, 0.065 mmol) in dichloromethane (0.6 mL) was added and the mixture was further stirred for 1.5 h at -78 °C followed by addition of MeOH (5.2 μL, 0.13 mmol). The resulting reaction mixture was stirred for an additional 1 h at -78 °C before molecular sieves were filtered off, and the organic layer was washed with saturated aqueous $NaHCO_3$ solution and dried (Na_2SO_4). The organic layer was concentrated under reduced pressure and the ^{1}H NMR spectrum of the crude reaction mixture was recorded. The crude mixture was then purified by preparative reversed phase HPLC (C_{18} bonded phase, gradient elution with acetonitrile–water, 1 mL/min. flow, detection at 254nm) to give the title product **11** as colorless oil. $[\alpha]^{27}_D$ -17.1 (c = 0.75 , $CHCl_3$); ^{1}H NMR ($CHCl_3$): δ 3.06 (dt, J = 5.0, 10.0 Hz, 1H), 3.33 (dd, J = 3.0, 10.0 Hz, 1H), 3.46 (dd, J = 3.0, 11.0 Hz, 1H), 3.42 (s, 3H), 3.52-3.57 (m, 3H), 3.60-3.65 (m, 2H), 3.85-3.92 (m, 2H), 4.04-4.11 (m, 2H), 4.29 (d, J = 12.0 Hz, 1H), 4.37 (s, 1H), 4.58-4.66 (m, 5H), 4.74-4.85 (m, 5H), 5.05 (d, J = 4.0 Hz, 1H), 5.52 (s, 1H), 7.26-7.38 (m, 25H); ^{13}C NMR ($CHCl_3$): δ 55.7, 67.6, 68.7, 69.0, 70.0, 72.8, 74.0, 74.0, 75.4, 75.7, 78.1, 78.7, 79.1, 79.4, 80.6, 98.8, 101.7, 101.9, 126.5, 127.6, 127.7, 127.9, 127.9, 128.1, 128.7, 128.5, 128.6, 128.7, 128.8, 128.9, 129.2, 137.8, 138.4, 138.7, 138.0.



4,4,5,5-Tetramethyl-2-(1-naphthyl)-1,3-dioxolane (**7**).

A mixture of 1-naphthaldehyde (1.28 mL, 9.42 mmol), pinacol (2.23 g, 18.84 mmol) and PTSA (0.36 g, 1.89 mmol) in benzene was heated under reflux in a Dean stark apparatus for 6h. The reaction mixture was washed with $NaHCO_3$ and the organic layer was separated and dried (Na_2SO_4). The residue was purified by silica gel column chromatography (30% EtOAc in hexanes) to give 4,4,5,5-tetramethyl-2-(1-naphthyl)-1,3-dioxolane (1.99 g, 75%). M.p. 68 °C, ^{1}H NMR ($CHCl_3$): δ 1.31 (s, 6H), 1.42 (s, 6H), 7.26-7.56 (m, 3H), 7.56-7.89 (m, 3H), 8.2 (d, J = 8.3 Hz); ^{13}C NMR ($CHCl_3$): δ 22.7, 24.6, 83.1, 122.9, 124.5, 125.6, 126.0, 126.6, 128.9, 129.3, 131.5, 134.1, 135.8.

Calculation of the Kinetic Isotope Effect. The ^{1}H NMR spectra of the substrate (**6**)/internal standard mixture, the crude reaction mixture, and the β-mannoside product (**11**) were recorded in $CDCl_3$ at 500 MHz. For each substance, the anomeric, benzylidene acetal, and internal standard resonances were carefully integrated with an integral width of 7 times the peak width at half-height. This integral width was the maximum that could be employed before other signals encroached on the integration. Each spectrum was integrated 10 times and the mean values recorded in the Table 1. The KIE of the β-anomeric product was determined from these vales using the following equation: KIE = $\ln(1 - F)/\ln[1 - (FR_{\mathbf{11}}/R_{\mathbf{6}})]$, wherein F is the fractional conversion of the triflate **9** (yield of **11**) and $R_{\mathbf{11}}$ and $R_{\mathbf{6}}$ the ratios of the benzylidene to anomeric resonances in the product **11** and the thioglycoside **6**, respectively. Integration of the spectra of non labeled **6** and **11**, by the same protocol as employed here, ruled out the possibility of systematic errors in the integration affecting the result.

References

[1] B. S. Moore, H. Cho, R. Casati, E. Kennedy, K. A. Reynolds, U. Mocek, J. M. Beale, H. G. Floss, *J. Am. Chem. Soc.* **1993**, 115, 5254.

[2] L. M. Lerner, P. Kohn, *J. Org. Chem.* **1966**, 31, 339.

[3] S. K. Maity, S. K. Dutta, A. K. Banerjee, B. Achari, M. Singh, *Tetrahedron*. **1994**, 50, 6965.

[4] T. Oshitari, M. Shibasaki, T. Yoshizawa, M. Tomita, K.-i. Takao, S. Kobayashi, *Tetrahedron*, **1997**, 53, 10993.

[5] A. Clerici, N. Pastori, O. Porta, *Tetrahedron* **1998**, 54, 15679.

[6] A. W. Burgstahler, D. E. Walker, J. P. Kuebrich, R. L. Schowen. *J. Org. Chem.* **1972**, 37, 1272.

Table 1

S	S_0	F = S/S_0	1-F	log(1-F)	R (= R_{11})	R_0 (= R_6)	R/R_0	$1-(FR/R_0)$	$\log[1-(FR/R_0)]$	KIE (-78 °C)	KIE (25 °C)
0.53	1.23	0.43	0.57	-0.24	0.71	0.82	0.87	0.63	-0.20	1.20	1.13
0.45	1.15	0.41	0.59	-0.23	0.72	0.83	0.87	0.64	-0.19	1.21	1.13
0.37	0.92	0.40	0.60	-0.22	0.73	0.83	0.88	0.65	-0.19	1.16	1.10

a) S, S_0, R, R_0 are the mean of ten integrations

b) S = Ratio of Benzylidene peak of product/ Internal standard peak measured on the crude reaction mixture

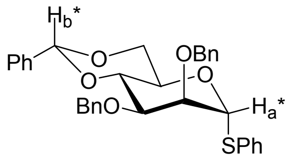
c) S_0 = Ratio of Benzylidene peak of the starting material / Internal standard peak

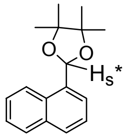
d) R = Ratio of Benzylidene peak / Anomeric peak in the product (β Isomer)

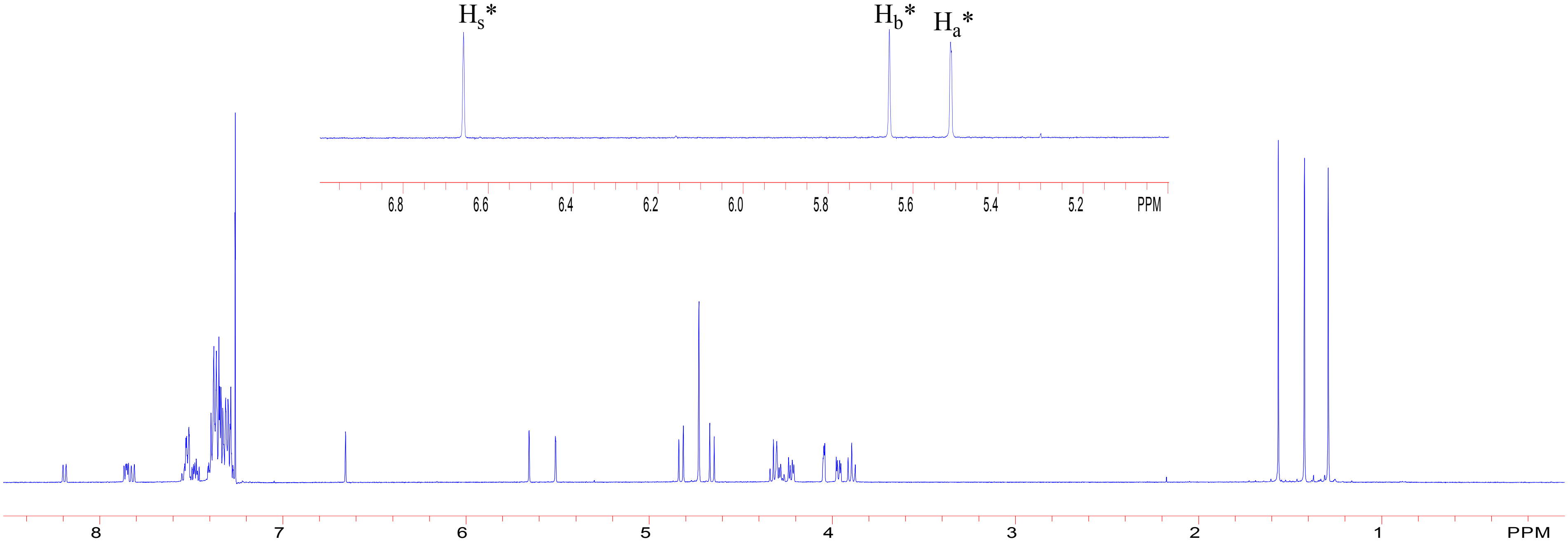
e) R_0 = Ratio of Benzylidene peak / Anomeric peak in the starting material

The KIE at 25 °C was obtained by assuming $T\ln(k_H/k_D)$ = constant.

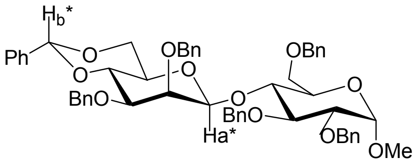
^{1}H NMR Spectrum of a Typical Starting Mixture [Mixture of (6) and (7)]

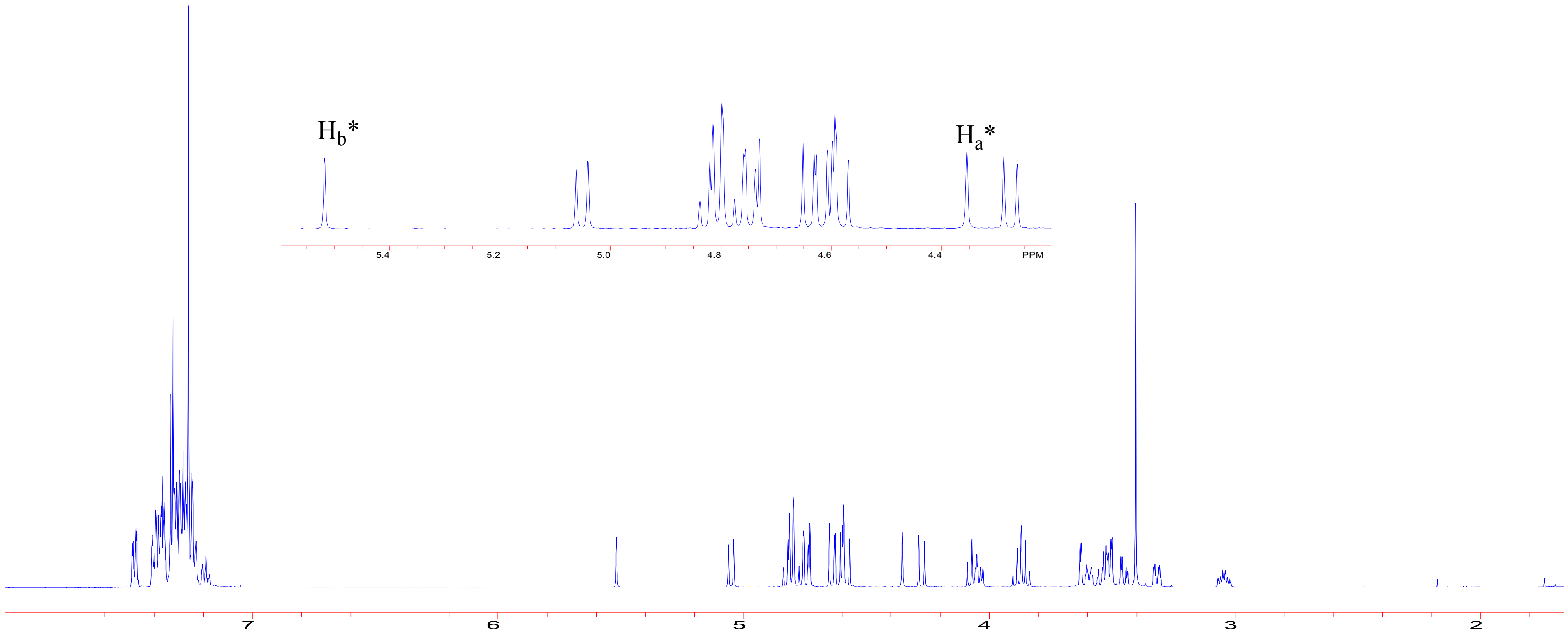




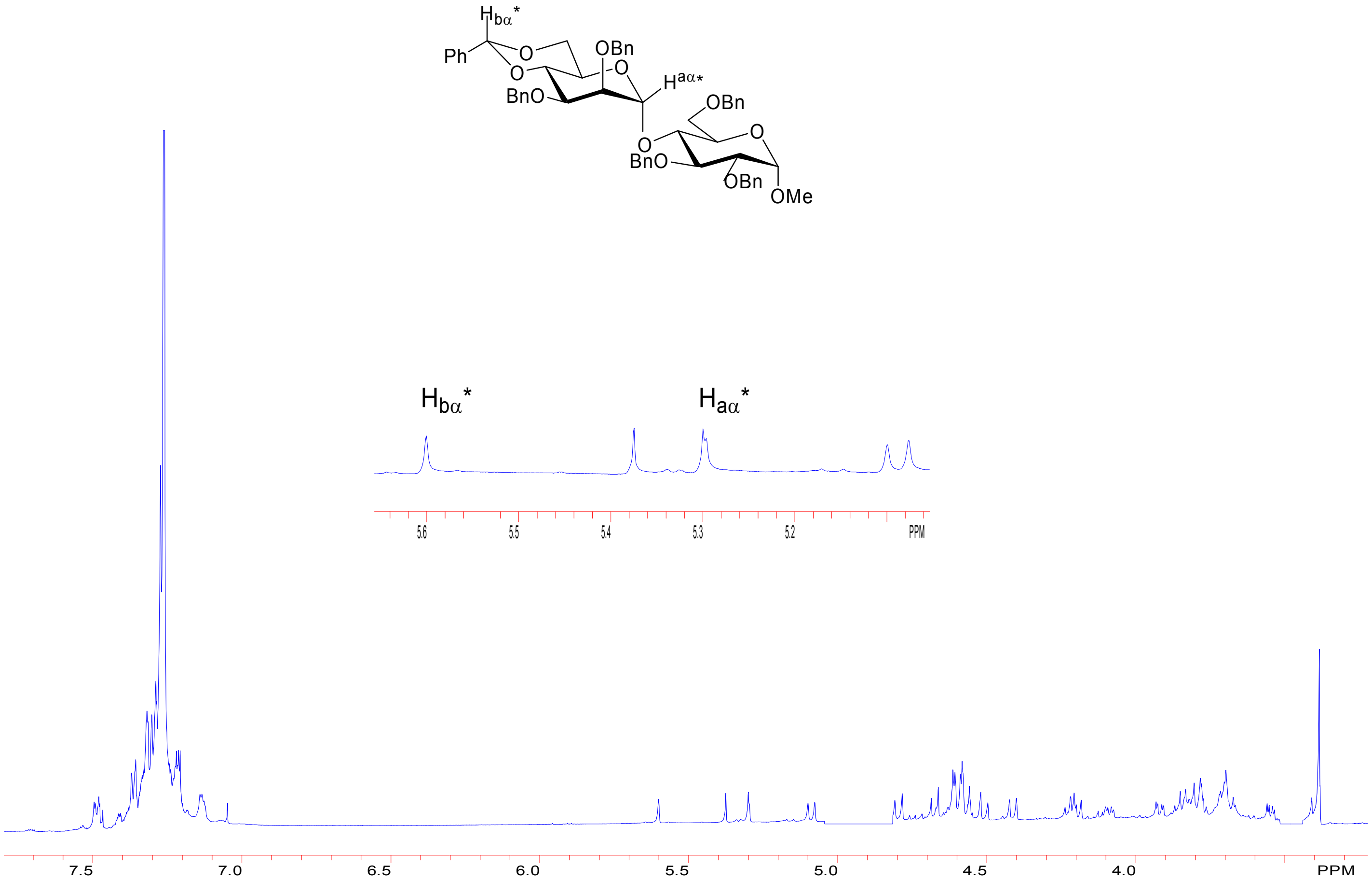


Typical ^{1}H NMR Spectrum of a Typical Purified β-Anomeric Product (11)





^{1}H NMR Spectrum of the Purified α-Anomeric Product (12)



^{1}H NMR Spectrum of a Typical Crude KIE Reaction Mixture (showing minimal α-product 12)