Supplementary material

for

Synthesis and structural analysis of D-fructofuranosylated compounds for the analysis of GH172 difructose dianhydride I synthase/hydrolase

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General procedures:

All reactions sensitive to air and/or moisture were carried out under argon atmosphere with anhydrous solvents. Substrates of glycosylations were dried by azeotropic removal with toluene. Column chromatography was performed on silica gel 60N, 100–210 mesh (Kanto Kagaku Co., Ltd.). Preparative thin layer chromatography was performed on silica gel 60 F254, 0.5 mm (E. Merck). ^{1}H NMR spectra were recorded at 400 MHz on a JEOL ECX 400 spectrometer and ^{1}H NMR spectra were referenced to $CHCl_3$ at 7.24 and MeOH at 3.31 ppm. ^{13}C NMR at 100 MHz spectra were referenced to the central peak of $CDCl_3$ at 77.0 ppm and CD_3OD at 49.0 ppm. ESI-TOF mass spectra were recorded on a Waters Synapt G2 in positive mode with leucine-enkephalin as the internal standard by Dr. Toshihiko Nogawa (Molecular Structure Characterization Unit, RIKEN CSRS). All other reagents were purchased from the Wako Pure Chemical Industries Ltd., Kanto Chemicals Co. Inc., Tokyo Chemical Industry Co., Ltd. and Aldrich Chemical Company.

Experimental procedure for the synthesis of 3.

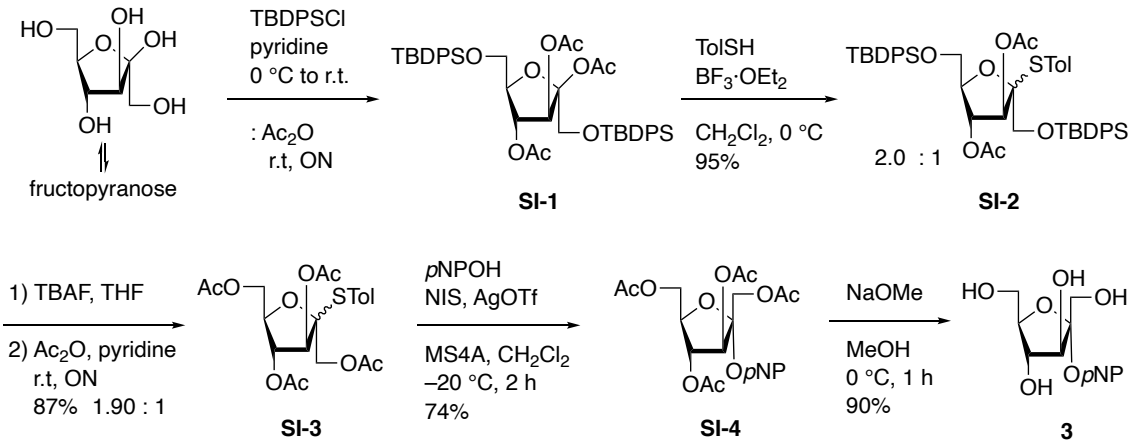


Figure SI-1. Synthesis of **3**.

Tolyl 3,4-di-*O*-acetyl-1,6-di-*O*-*t*-butyldiphenylsilyl-2-thio-D-fructofuranoside (SI-2).

To a solution of D-fructose (10.0 g, 55.5 mmol) in pyridine (50 mL) was added TBDPSCl (31.3 mL, 122.3 mmol) at 0 °C under an Ar atmosphere. The reaction mixture was stirred at the same temperature for overnight and then to the mixture was added acetic anhydride (20 mL) at 0 °C. The reaction mixture was stirred at room temperature for overnight and quenched with ice water, extracted with ethyl acetate, washed with sat. $KHSO_4$ aq, H_2O, sat. $NaHCO_3$ aq, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography using a gradient solvent system (hexane/ethyl acetate = 100/1 to 50/1 to 25/1 to 10/1 to 2/1) to give 2,3,4-tri-*O*-acetyl-1,6-di-*O*-*t*-butyldiphenylsilyl-D-fructofuranose (**SI-1**, 35.2 g, 81%) which was used as the anomeric mixture (α : β = 1 : 2.2): ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01–1.08 (m, 39.6 H, *t*-Bu$^{\beta}$ x2 + 18 H, *t*-Bu$^{\alpha}$ x2), 1.82 (s, 3 H, Ac$^{\alpha}$), 2.026 (s, 6.6 H, Ac$^{\beta}$ + 3 H, Ac$^{\alpha}$), 2.031 (s, 6.6 H, Ac$^{\beta}$), 2.07 (s, Ac$^{\beta}$, 6.6 H), 2.10 (s, Ac$^{\alpha}$, 3 H), 3.70 (d, *J* = 11.2 Hz, C1$^{\beta}$-H, 2.2 H), 3.74 (d, *J* = 11.2 Hz, C1$^{\beta}$-H, 2.2 H), 3.73 (d, *J* = 10.8 Hz, C1$^{\alpha}$-H, 1 H), 3.77 (d, *J* = 10.8 Hz, C1$^{\alpha}$-H, 1 H), 3.77–3.85 (m, C6$^{\beta}$-H, 4.4 H), 3.84 (d, *J* = 11.2, 4.0 Hz, C6$^{\alpha}$-H, 1 H), 3.89 (d, *J* = 11.2, 4.0 Hz, C6$^{\alpha}$-H, 1 H), 4.05–4.12 (m, C5$^{\beta}$-H, 2.2 H), 4.24 (dt, *J* = 5.2, 4.0 Hz, C5$^{\alpha}$-H, 1 H), 5.21 (dd, *J* = 5.2, 1.2 Hz, C4$^{\alpha}$-H, 1 H), 5.30 (d, *J* = 1.2 Hz,[SI-1, SI-2] C3$^{\alpha}$-H, 1 H), 5.57 (t, *J* = 5.2 Hz, C4$^{\beta}$-H, 2.2 H), 5.74 (d, *J* = 5.2 Hz, [SI-1, SI-2] C3$^{\beta}$-H, 2.2 H), 7.28−7.75 (m, Ar$^{\alpha}$, 22 H + Ar$^{\beta}$, 10 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.0 (*t*Bu$^{\alpha}$), 19.12 (*t*Bu$^{\beta}$), 19.15 (*t*Bu$^{\alpha}$), 19.23 (*t*Bu$^{\beta}$), 20.7 (Ac$^{\beta}$), 20.9 (Ac$^{\alpha}$), 26.6 (*t*Bu$^{\alpha}$, *t*Bu$^{\beta}$), 63.0 (C$^{\alpha}$6), 64.7 (C$^{\alpha}$1), 64.9 (C$^{\beta}$6), 65.0 (C$^{\beta}$1), 76.6 (C$^{\beta}$3), 77.2 (C$^{\alpha}$4), 77.8 (C$^{\beta}$4), 81.5 (C$^{\alpha}$3), 81.8 (C$^{\beta}$5), 82.5(C$^{\alpha}$5), 103.2 (C$^{\beta}$2), 104.0 (C$^{\alpha}$2), 127.5, 127.6, 127.8, 127.9, 129.6, 129.6, 129.9, 130.0, 131.9, 132.1, 132.3, 132.5, 132.9, 133.0,

133.2, 135.4, 135.5, 135.6, 135.7 (Ar), 169.1 (C^{α}=O), 169.7 (C^{β}=O), 169.9 (C^{α}=O), 170.4 (C^{β}=O); ESI-TOF MS: calcd for $C_{44}H_{54}Na_1O_9Si_2$ $[M+Na]^+$ 805.32, found 805.32; HRMS (ESI-TOF): calcd for $C_{44}H_{54}Na_1O_9Si_2$ $[M+Na]^+$ 805.3204, found 805.3206. To a solution of 2,3,4-tri-*O*-acetyl-1,6-di-*O*-*t*-butyldiphenylsilyl-D-fructofuranose (10.0 g, 12.8 mmol) in CH_2Cl_2 (25 mL) were added 4-methylbenzenethiol (TolSH) (1.80 g, 14.5 mmol) and $BF_3{\cdot}OEt_2$ (4.94 mL, 39.2 mmol) at 0 °C and the mixture was stirred for 2 h at room temperature and quenched with triethylamine (5 mL), extracted with ethyl acetate, washed with sat. $NaHCO_3$ aq, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography using a gradient solvent system (hexane/ethyl acetate = 100/1 to 50/1 to 25/1 to 10/1 to 2/1) to give the title compound (**SI-2**) (10.3 g, 95%) as the anomeric mixture (α : β = 2.0 : 1).

SI-2:^{1}H NMR (400 MHz, $CDCl_3$): **α-isomer**: δ 1.83 (s, Ac, 3 H), 2.04 (s, Ac, 3 H), 2.29 (s, Me, 3 H), 3.70 (d, *J* = 10.8 Hz, C1-H, 1 H), 3.79 (d, *J* = 11.6, 3.6 Hz, C6-H, 1 H), 3.84 (d, *J* = 10.8 Hz, C1-H, 1 H), 3.86–3.90 (m, C6-H, 1 H), 3.69 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.31 (td, *J* = 6.8, 3.6 Hz, C5-H, 1 H), 5.22 (dd, *J* = 6.8, 4.4 Hz, C4-H, 1 H), 5.42 (d, *J* = 4.4 Hz,[SI-1, SI-2] C3-H, 1 H), 6.94 (d, *J* = 8.0 Hz, Ar, 2 H), 7.26–7.72 (m, Ar, 12 H); **β-isomer**: δ 1.96 (s, Ac, 3 H), 2.13 (s, Ac, 3 H), 2.23 (s, Me, 3 H), 3.58 (d, *J* = 10.8 Hz, C1-H, 1 H), 3.71 (d, *J* = 10.8 Hz, C1-H, 1 H), 3.86–3.90 (m, C6-H, 2 H), 4.16 (q, *J* = 6.0 Hz, C5-H, 1 H), 5.55 (d, *J* = 6.4, 6.0 Hz, C4-H, 1 H), 6.04 (d, *J* = 6.4 Hz,[SI-1, SI-2] C3-H, 1 H), 6.85 (d, *J* = 8.0 Hz, Ar, 2 H), 7.26–7.72 (m, Ar, 12 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.9, 19.10, 19.14, 19.16 (*t*Bu), 20.6, 20.7 (Ac), 21.0 ($C^{\beta}H_3Ar$), 21.1 ($C^{\alpha}H_3Ar$), 26.5, 26.6, 26.7 (*t*Bu), 63.3 ($C^{\alpha}1$), 63.6 ($C^{\alpha}6$), 64.0 ($C^{\beta}6$), 66.0 ($C^{\beta}1$), 76.9 ($C^{\beta}4$), 77.3 ($C^{\beta}3$), 77.8 ($C^{\alpha}4$), 81.17 ($C^{\alpha}3$/$C^{\alpha}5$), 81.19 ($C^{\alpha}3$/$C^{\alpha}5$), 81.8 ($C^{\beta}5$), 95.7 ($C^{\beta}2$), 96.6 ($C^{\alpha}2$), 126.19, 126.82, 127.42, 127.49, 127.54, 127.62, 127.63, 129.00, 129.27, 129.30, 129.42, 129.47, 129.56, 129.64, 132.78, 132.92, 133.00, 133.06, 133.10, 133.13, 133.18, 134.69, 135.32, 135.38, 135.51, 135.64, 135.72, 135.97, 138.18, 138.28 (Ar), 169.4 (C^{α}=O), 169.7 (C^{β}=O), 169.9 (C^{β}=O), 170.0 (C^{α}=O). ESI-TOF MS: calcd for $C_{49}H_{58}Na_1O_7S_1Si_2$ $[M+Na]^+$ 869.33, found 869.34; HRMS (ESI-TOF): calcd for $C_{49}H_{58}Na_1O_7S_1Si_2$ $[M+Na]^+$ 869.3340, found 869.3339.

Tol 1,3,4,6-tetra-*O*-acetyl-2-thio-D-fructofuranoside (SI-3).

To a solution of tolyl 3,4-di-*O*-acetyl-1,6-di-*O*-*t*-butyldiphenylsilyl-D-fructofuranose (**SI-2**) (6.21 g, 7.33 mmol) in THF (100 mL) was added TBAF (1 M in THF, 22 mL, 22 mmol) at room temperature and the mixture was stirred for 2 h at room temperature. After evaporation, to a solution of the resultant residue in pyridine (20 mL) was added acetic anhydride (5.0 mL) at 0 °C and the mixture was stirred overnight at room temperature and quenched with ice water, extracted with ethyl acetate, washed with sat. $KHSO_4$ aq, H_2O, satd $NaHCO_3$ aq, and brine, dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by silica gel column chromatography using a gradient solvent system (hexane/ethyl acetate = 25/1 to 10/1 to 5/1 to 3/1 to 1/1) to give the title compound (**SI-3**) (2.90 g, 87%, α : β = 1.9 : 1):

SI-3: ^{1}H NMR (400 MHz, $CDCl_3$): **α-isomer**: δ 2.02 (s, Ac, 3 H), 2.03 (s, Ac, 3 H), 2.06 (s, Ac, 3 H), 2.08 (s, Ac, 3 H), 2.31 (s, Me, 3 H), 4.00 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.20 (d, *J* = 12.4, 6.4 Hz, C6-H, 1 H), 4.21 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.38 (d, *J* = 12.4, 3.2 Hz, C6-H, 1 H), 4.45–4.50 (m, C5-H, 1 H), 5.10 (dd, *J* = 7.2, 4.0 Hz, C4-H, 1 H), 5.40 (d, *J* = 4.0 Hz, C3-H,[SI-1, SI-2] 1 H), 7.10 (d, *J* = 8.0 Hz, Ar, 2 H), 7.36 (d, *J* = 8.0 Hz, Ar, 2 H); **β-isomer**: δ 2.03 (s, Ac, 3 H), 2.07 (s, Ac, 3 H), 2.10 (s, Ac, 3 H), 2.14 (s, Ac, 3 H), 2.30 (s, Me, 3 H), 4.04 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.09 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.25 (td, *J* = 6.4, 5.2 Hz, C5-H, 1 H), 4.39 (d, *J* = 12.4, 6.4 Hz, C6-H, 1 H), 4.45–4.50 (m, C6-H, 1 H), 5.54 (t, *J* = 6.4 Hz, C4-H, 1 H), 5.70 (d, *J* = 6.4 Hz, C3-H,[SI-1, SI-2] 1 H), 7.10 (d, *J* = 8.0 Hz, Ar, 2 H), 7.36 (d, *J* = 8.0 Hz, Ar, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$): **α-isomer**: δ 20.5 (Ac), 20.7 (Ac x2), 20.8 (Ac), 21.2 (CH_3Ar), 62.6 (C1), 62.8 (C6), 77.0 (C4), 77.4 (C5), 80.5 (C3), 93.6 (C2), 125.3 (Tolyl), 129.7 (Tolyl), 136.4 (Tolyl), 139.8 (Tolyl). 169.1 (C=O), 169.9 (C=O), 170.0 (C=O), 170.4 (C=O); **β-isomer**: δ 20.62 (Ac), 20.66 (Ac), 20.70 (Ac), 20.74 (Ac), 21.1 (CH_3Ar), 63.7 (C6), 65.2 (C1), 76.2 (C4), 76.5 (C5), 79.3 (C3), 93.6 (C2), 124.8 (Tolyl), 129.6 (Tolyl), 136.3 (Tolyl), 139.5 (Tolyl). 169.6 (C=O), 169.9 (C=O), 170.0 (C=O), 170.6 (C=O); ESI-TOF MS: calcd for $C_{21}H_{26}Na_1O_9S_1$ $[M+Na]^+$

477.12, found 477.12; HRMS (ESI-TOF): calcd for $C_{21}H_{26}Na_1O_9S_1$ $[M+Na]^+$ 477.1195, found 477.1192.

4-Nitrophenyl 1,3,4,6-tetra-*O*-acetyl-α-D-fructofuranoside (SI-4).

The title compound was synthesized from **SI-3** according to the synthesis of 4-nitrophenyl 1,3,4,6-tetra-*O*-acetyl-α-D-arabinofuranoside.[SI-1] To a mixture of a tolyl 1,3,4,6-tetra-*O*-acetyl-2-thio-D-fructofuranoside (α : β = 1.9 : 1, 0.60 g, 1.32 mmol) and *p*-nitrophenol (204 mg, 1.45 mmol) and freshly activated molecular sieve (4 Å, 0.25 g) in CH_2Cl_2 (3.0 mL) were added NIS (569 mg, 2.50 mmol) and AgOTf (34.6 mg, 0.132 mmol) at –20 °C under an argon atmosphere. The reaction mixture was stirred at the same temperature for 2 h and quenched with Et_3N. The suspension was diluted with $CHCl_3$ and filtered through a Celite pad, the filtrate was washed successively with 10% $Na_2S_2O_3$ aq, saturated $NaHCO_3$ aq, and brine. The organic layer was dried through Na_2SO_4 and concentrated in *vacuo*. The residue was purified by preparative thin layer chromatography (ethyl acetate/hexane = 1/2) to afford the title compound (458 mg, 74 %).

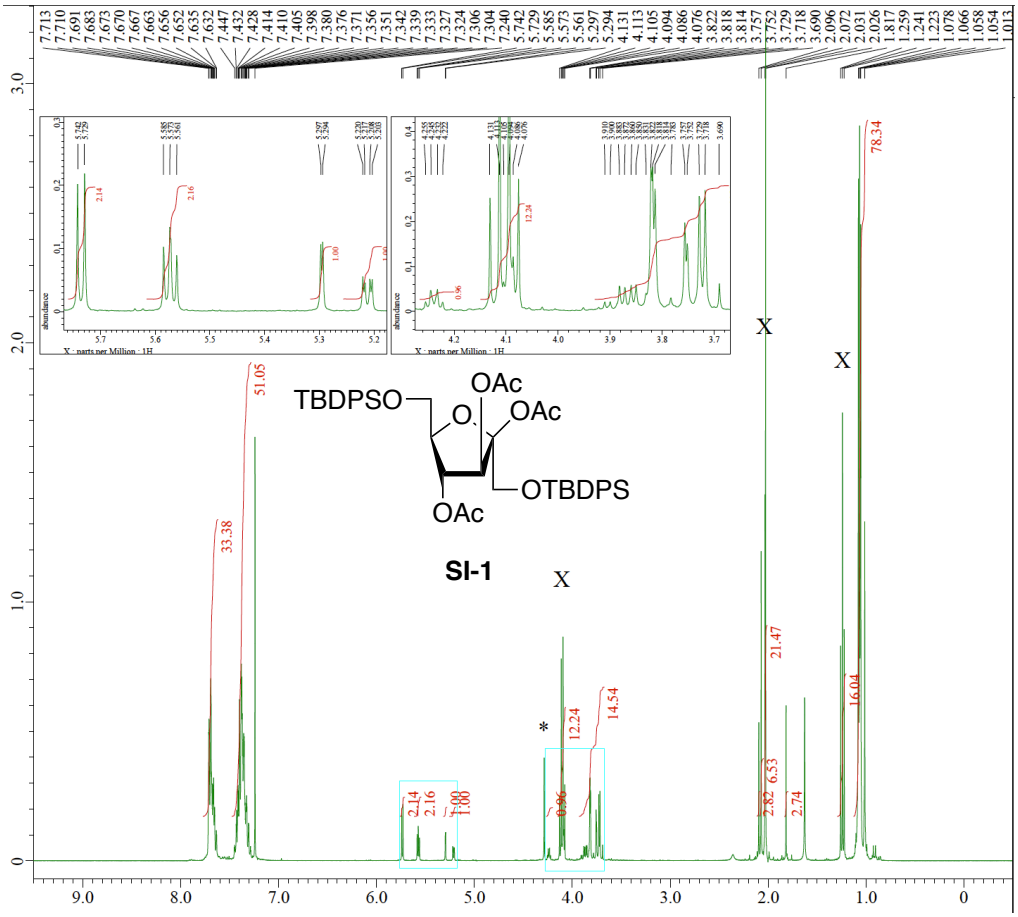
SI-4: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99 (s, Ac, 3 H), 2.09 (s, Ac, 3 H), 2.10 (s, Ac, 3 H), 2.12 (s, Ac, 3 H), 4.24 (dd, *J* = 12.0, 6.0 Hz, C6-H, 1 H), 4.33–4.38 (m, C5-H, 1 H), 4.45 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.41 (d, *J* = 12.4 Hz, C1-H, 1 H), 4.45 (dd, *J* = 12.0, 3.6 Hz, C6-H, 1 H), 5.00 (dd, *J* = 4.8, 1.6 Hz, C4-H, 1 H), 5.62 (d, *J* = 1.6 Hz, C3-H,[SI-1, SI-2] 1 H), 7.24–7.29 (m, *p*NP 2 H), 8.14–8.20 (m, Ar, 2 H); 1D-NOE difference: irradiated 5.62 (C3-H) → enhanced 8.19 (3.4%, *p*NP), 7.27 (4.1%, *p*NP); irradiated 7.27 (*p*NP) → enhanced 5.62 (1.4%, C3-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5 (*Me*CO), 20.6 (*Me*CO), 20.66 (*Me*CO), 20.71 (*Me*CO), 59.2 (C1), 62.8 (C6), 77.4 (C4), 80.0 (C3), 82.1 (C5), 110.0 (C2), 120.4 (*p*NP), 125.5 (*p*NP), 143.5 (*p*NP), 158.7 (*p*NP), 168.6 (Me*C*O), 169.6 (Me*C*O), 170.0 (Me*C*O), 170.4 (Me*C*O); ESI-TOF MS: calcd for $C_{20}H_{23}N_1Na_1O_{12}$ $[M+Na]^+$ 492.11, found 492.11; HRMS (ESI-TOF): calcd for $C_{20}H_{23}N_1Na_1O_{12}$ $[M+Na]^+$ 492.1118, found 492.1115.

4-Nitrophenyl α-D-fructofuranoside 3.

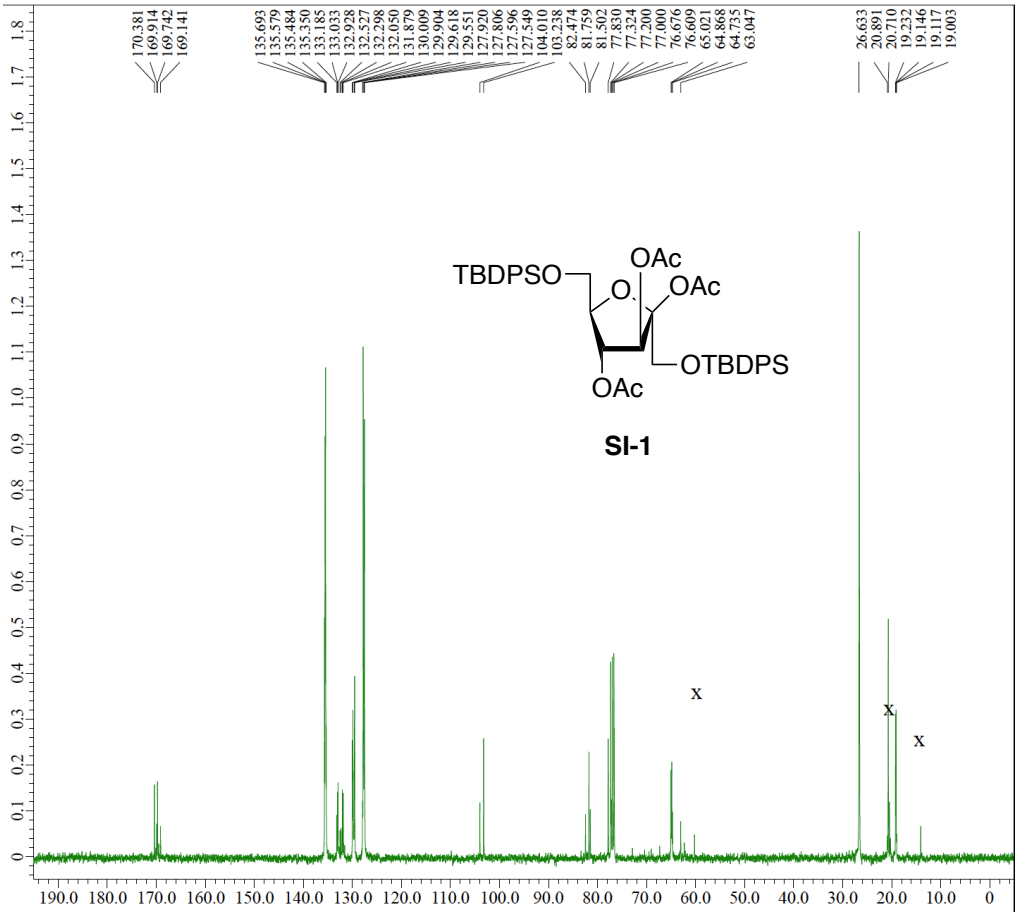
The title compound **3** was synthesized from **SI-4** according to the synthesis of 4-nitrophenyl α-D-arabinofuranoside.[SI-3] To a solution of **SI-4** (30.0 mg, 63.9 μmol) in MeOH (2.0 mL) at 0 °C was added a solution of MeONa (5.0 μL, 28% in MeOH). The reaction mixture was stirred for 30 min at the same temperature, then neutralized with Amberlyst® 15 H^+ form, filtered through Celite® pad and concentrated in *vacuo*. The residue was purified by preparative thin layer chromatography ($CHCl_3$/MeOH = 10/1) to afford the title compound (17.3 mg, 90%).

3: ^{1}H NMR (400 MHz, CD_3OD): δ 3.60 (dd, *J* = 12.4, 4.0 Hz, C6-H, 1 H), 3.70–3.75 (m, C1-H, C6-H, 2 H), 3.79 (d, *J* = 12.4 Hz, C1-H, 1 H), 3.90–3.94 (m, C4-H, C5-H, 2 H), 4.21 (d, *J* = 3.6 Hz,[SI-1, SI-2] C3-H, 1 H), 7.28–7.33 (m, *p*NP, 2 H), 8.06–8.10 (m, *p*NP, 2 H); ^{13}C NMR (100 MHz, CD_3OD): δ 61.3 (C1), 62.7 (C6), 78.0 (C4), 83.9 (C3), 85.5 (C5), 113.2 (C2), 122.3 (*p*NP), 126.0 (*p*NP), 144.2 (*p*NP), 161.8 (*p*NP); ESI-TOF MS: calcd for $C_{12}H_{15}N_1Na_1O_8$ $[M+Na]^+$ 324.07, found 324.08; HRMS (ESI-TOF): calcd for $C_{12}H_{15}N_1Na_1O_8$ $[M+Na]^+$ 324.0695, found 324.0697.

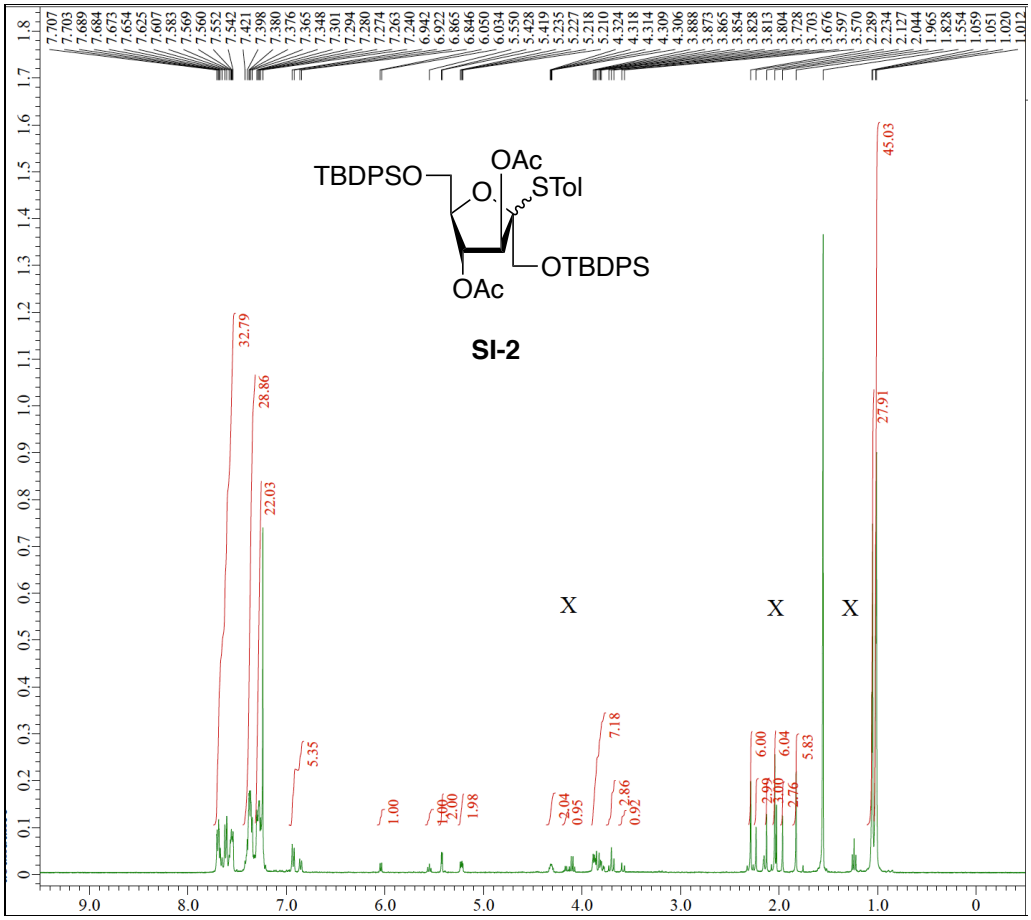
^{1}H NMR spectrum of **SI-1** in $CDCl_3$ (400 MHz). (X : peaks of residual EtOAc; * : peak of impurity <not assigned>).



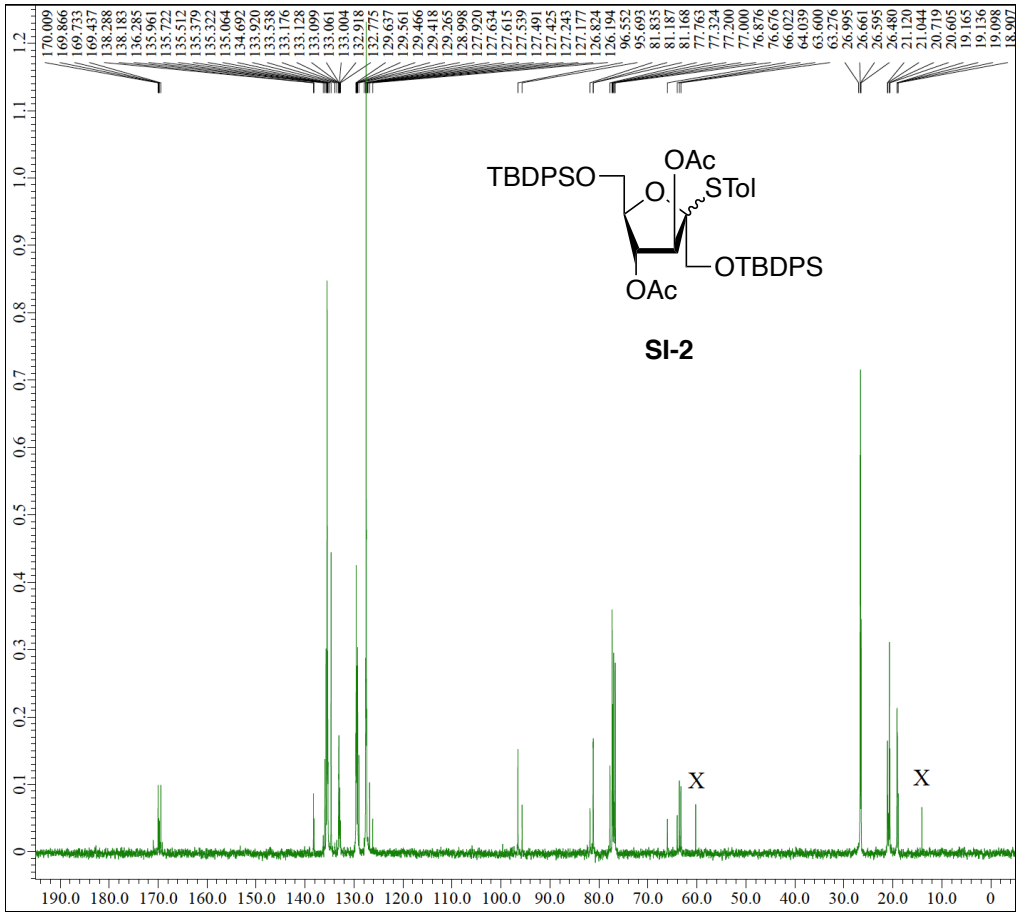
^{13}C NMR spectrum of **SI-1** in $CDCl_3$ (100 MHz). (X : peaks of residual EtOAc.)



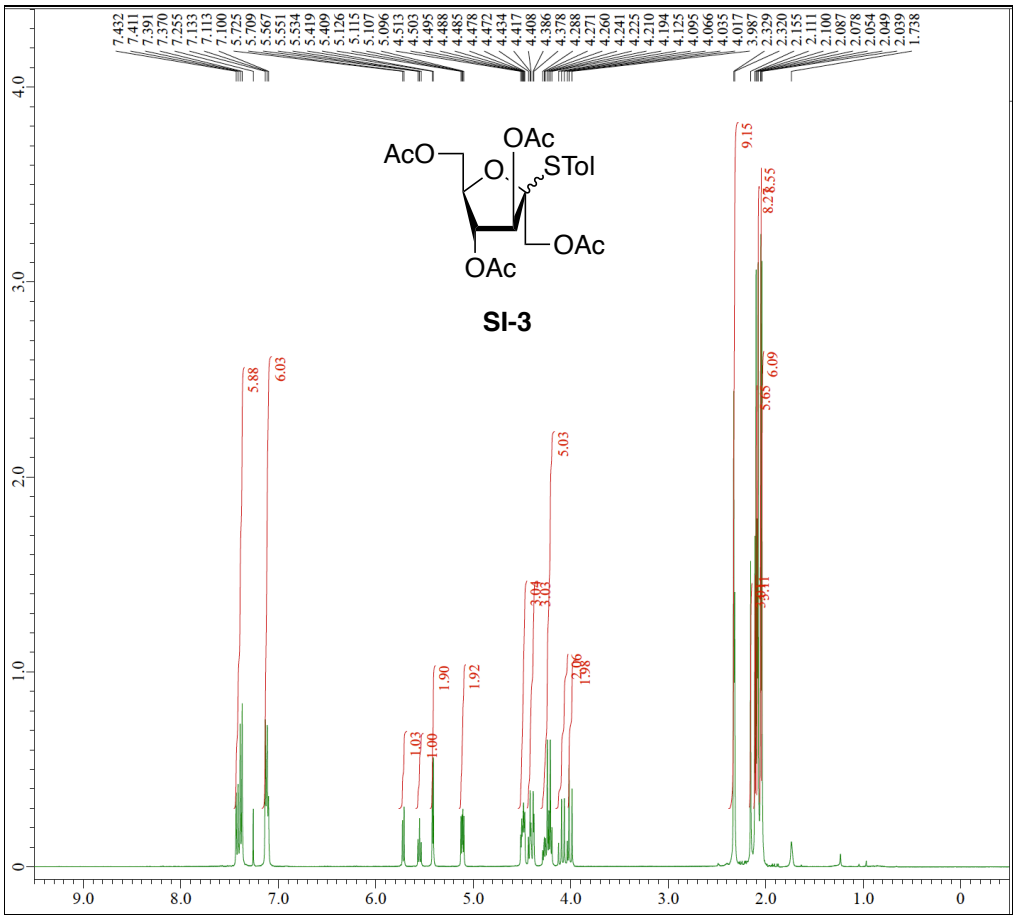
^{1}H NMR spectrum of **SI-2** in $CDCl_3$ (400 MHz). (X : peaks of residual EtOAc)



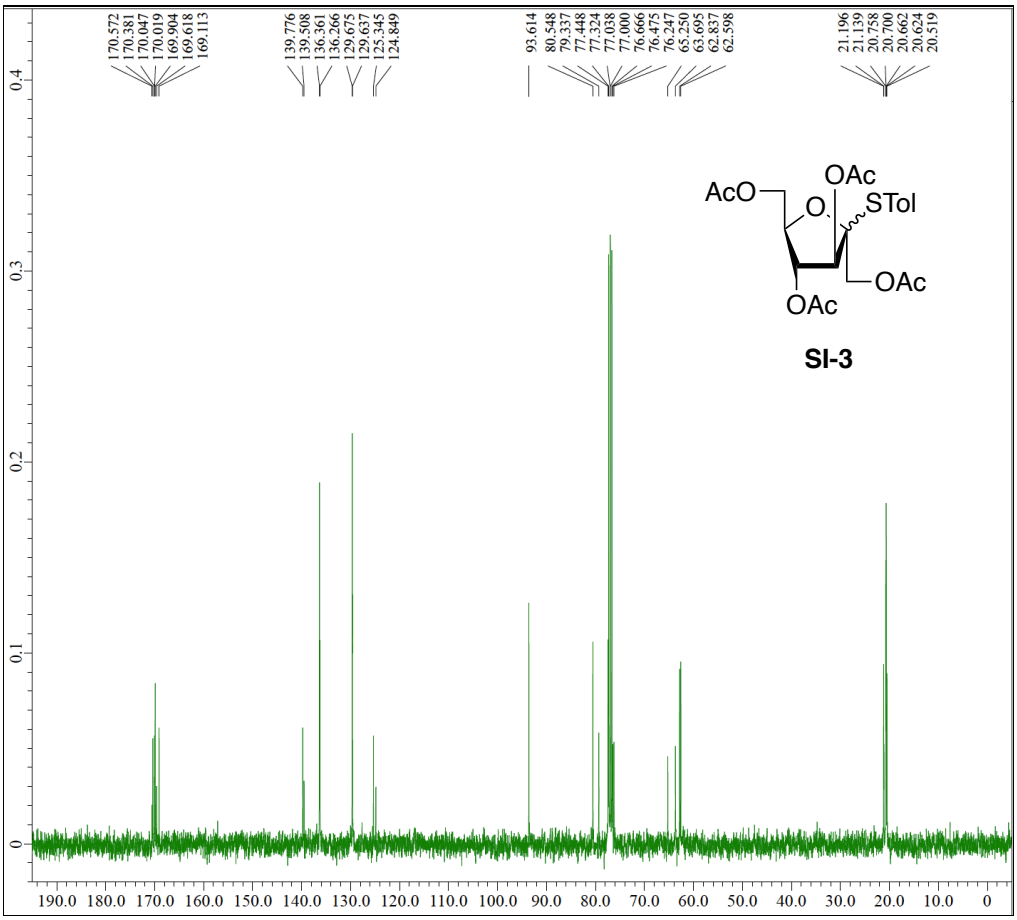
^{13}C NMR spectrum of **SI-2** in $CDCl_3$ (100 MHz). (X : peaks of residual EtOAc)



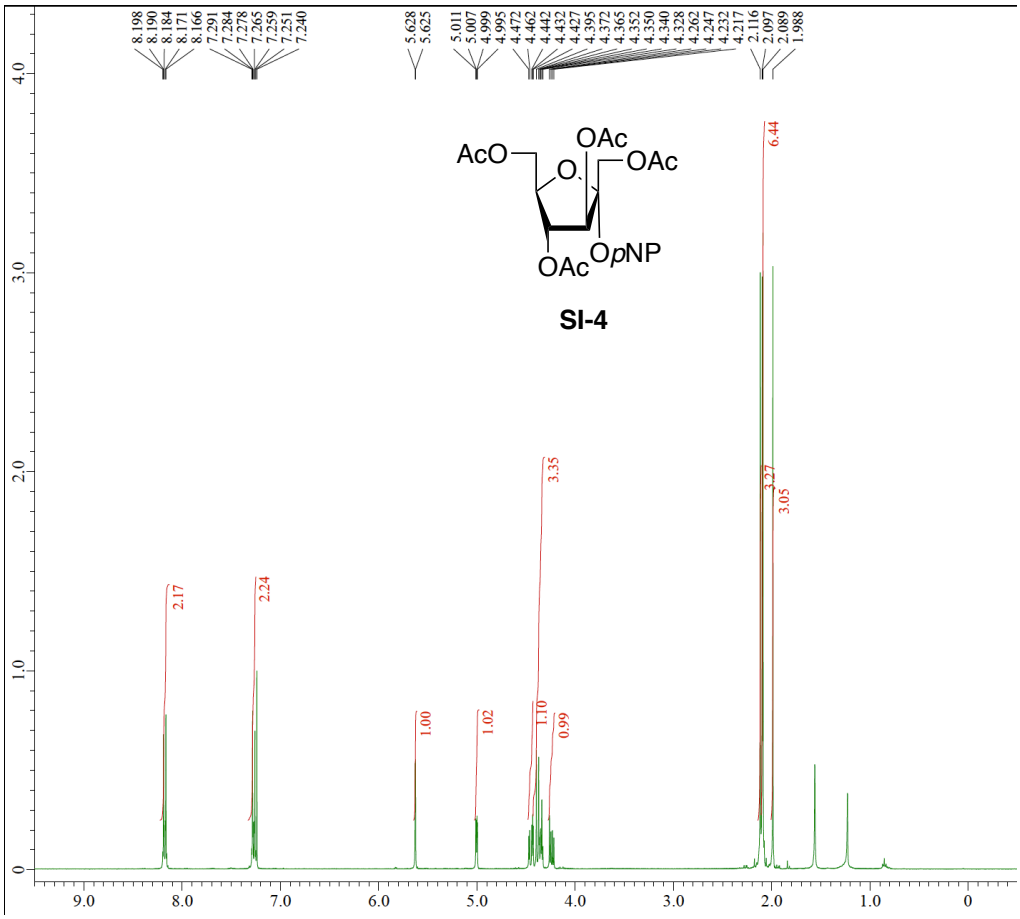
^{1}H NMR spectrum of **SI-3** in $CDCl_3$ (400 MHz).



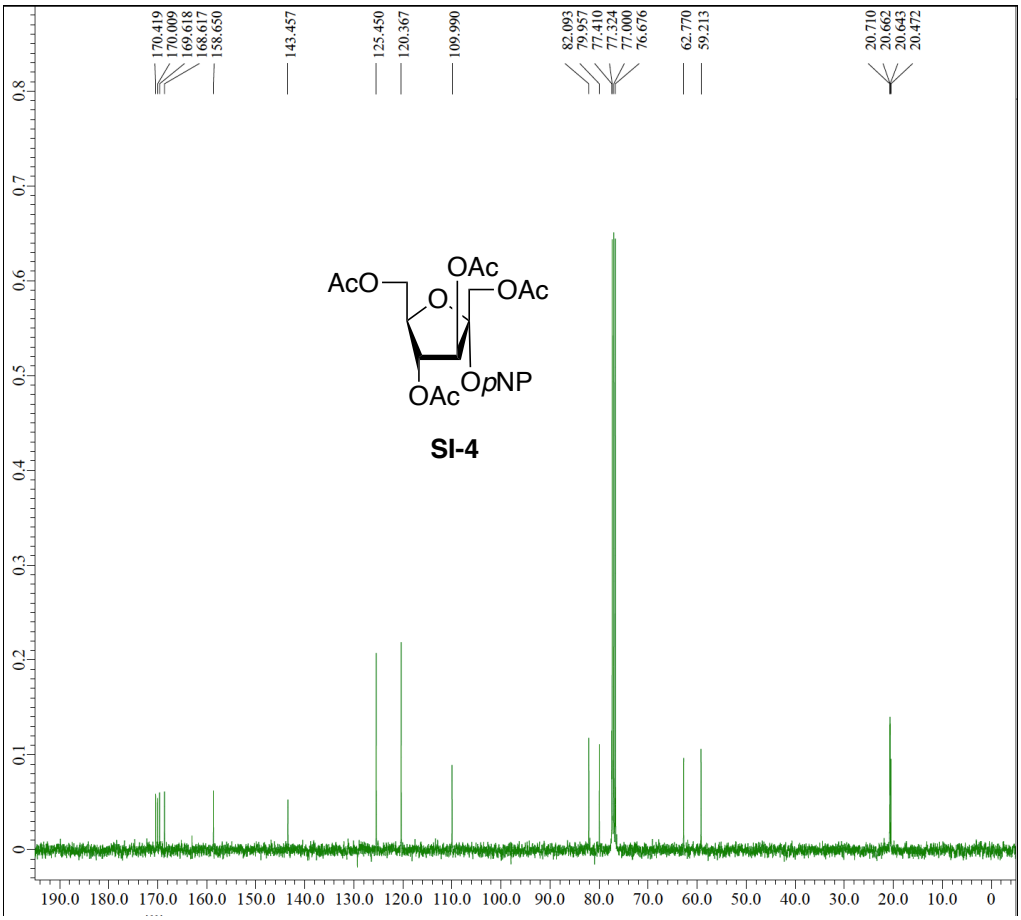
^{13}C NMR spectrum of **SI-3** in $CDCl_3$ (100 MHz).



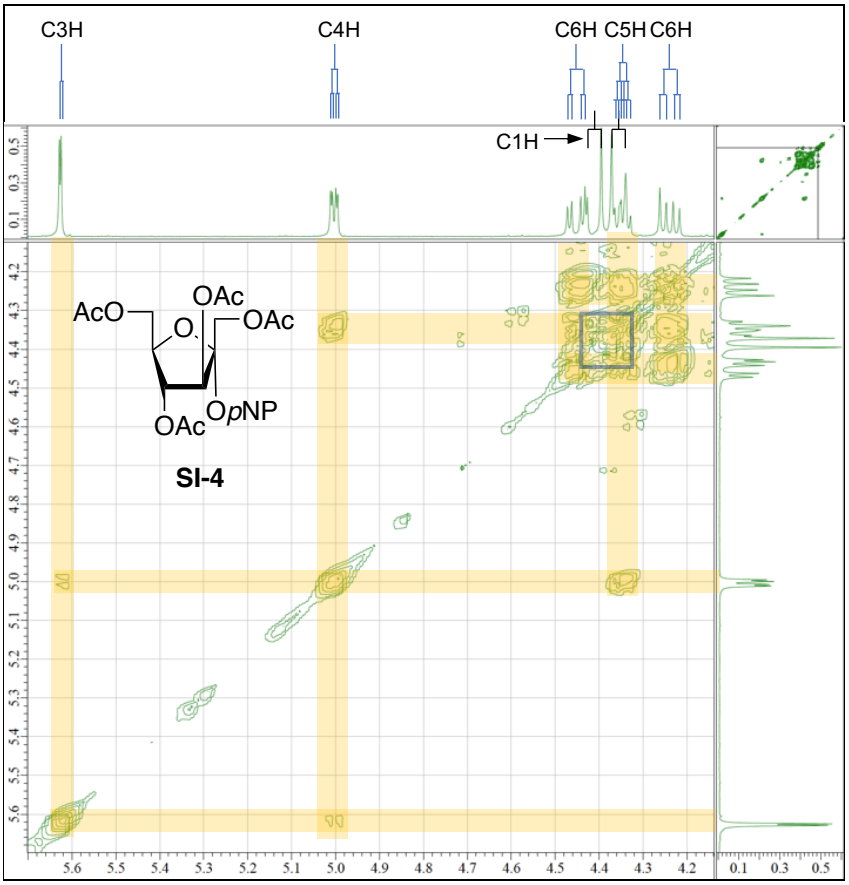
^{1}H NMR spectrum of **SI-4** in $CDCl_3$ (400 MHz).



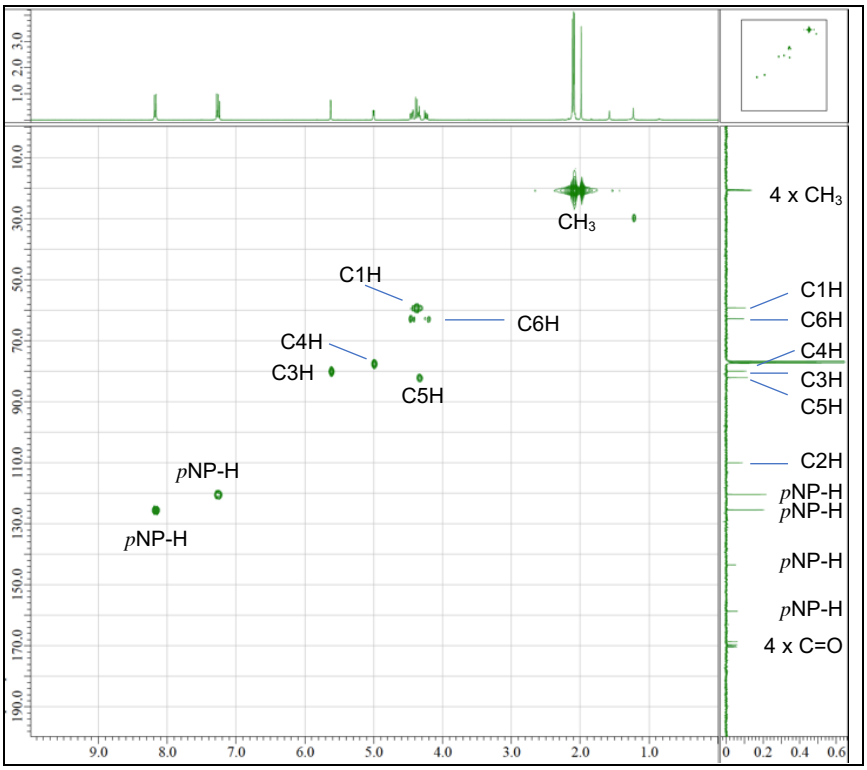
^{13}C NMR spectrum of **SI-4** in $CDCl_3$ (100 MHz).



^{1}H-^{1}H COSY spectrum of **SI-4** in $CDCl_3$ (400 MHz).



HMQC spectrum of spectrum of **SI-4** in $CDCl_3$ (400 MHz).



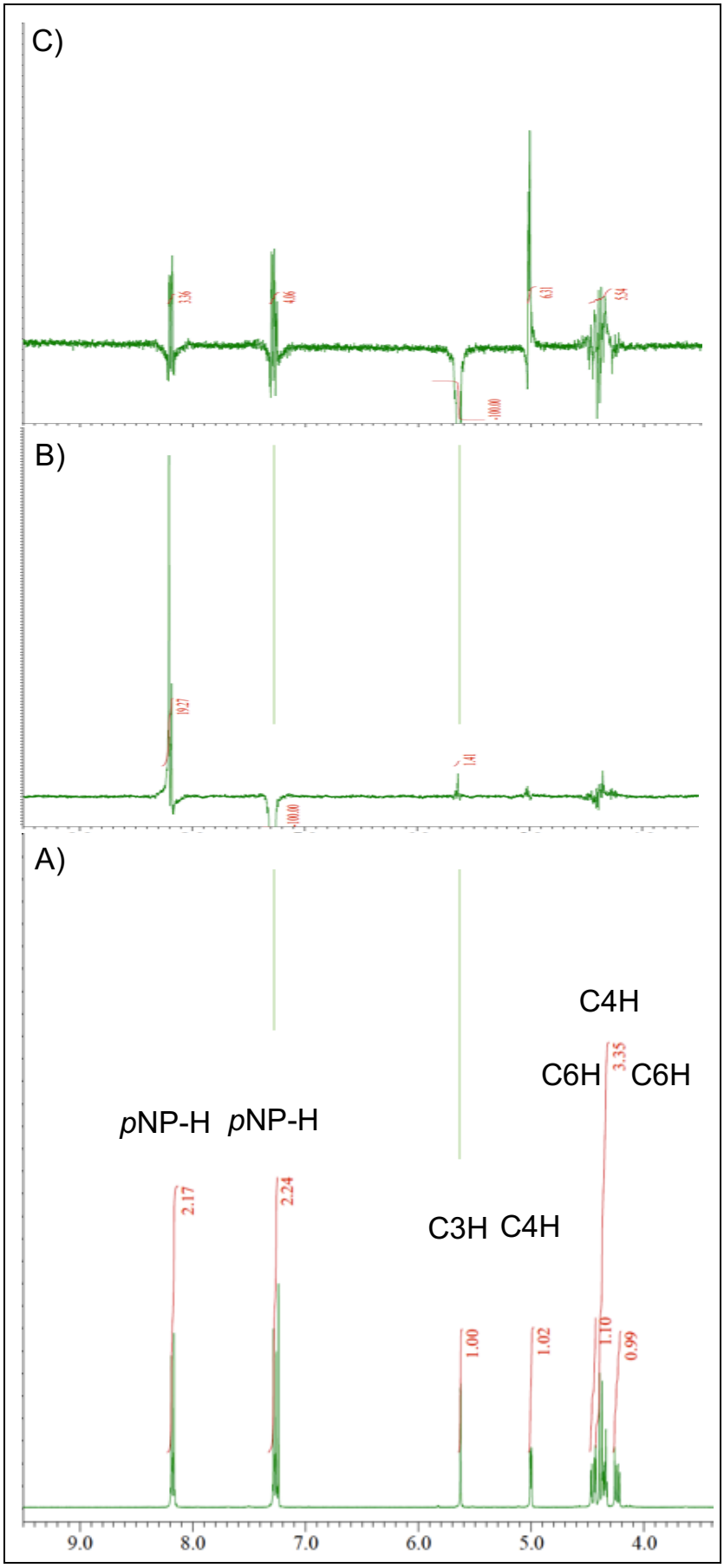
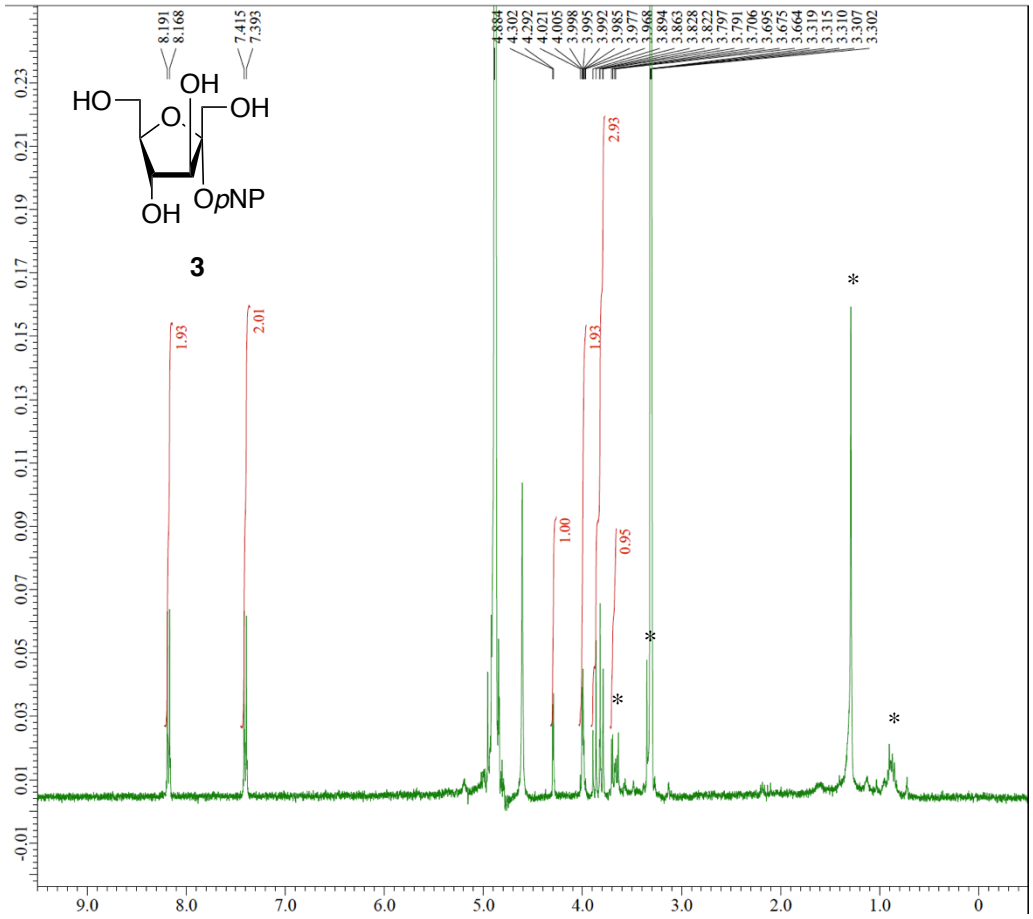


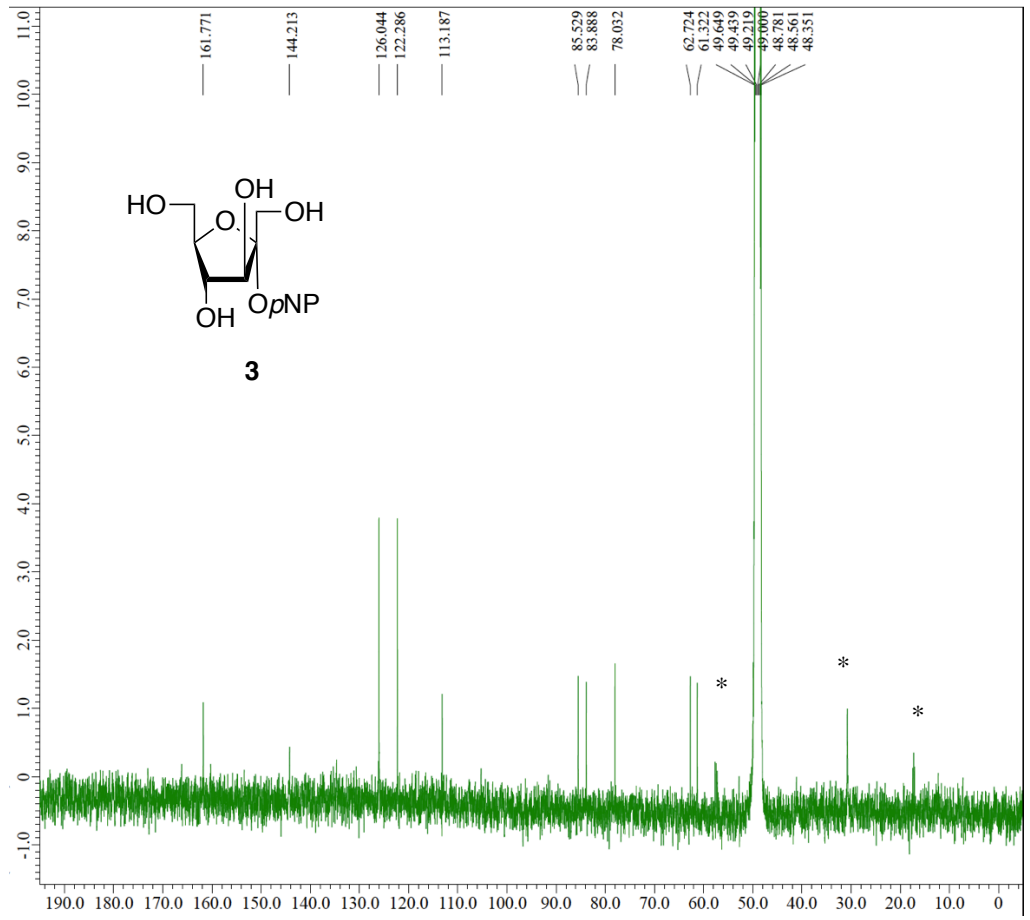
Figure SI-1. 1D NOE difference spectra of **SI-4**.

A) ^{1}H NMR spectra of **SI-4**; B) irradiated at 7.27 ppm (*p*NP-C2'-H) in $CDCl_3$ (100 MHz); C) irradiated at 3.44 ppm (C3H) in $CDCl_3$ (400 MHz). Enhancements (%) were estimated by comparing to an irradiated peak as the standard (100%).

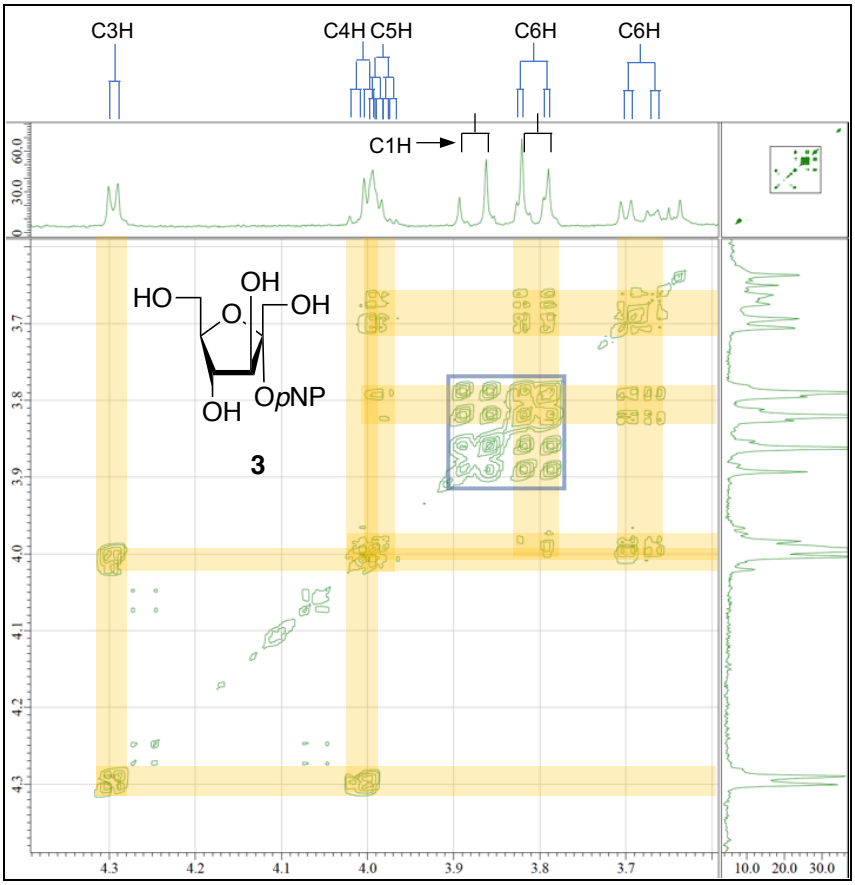
^{1}H NMR spectrum of **3** in CD_3OD (400 MHz). (* : peaks of impurity <not assigned>).



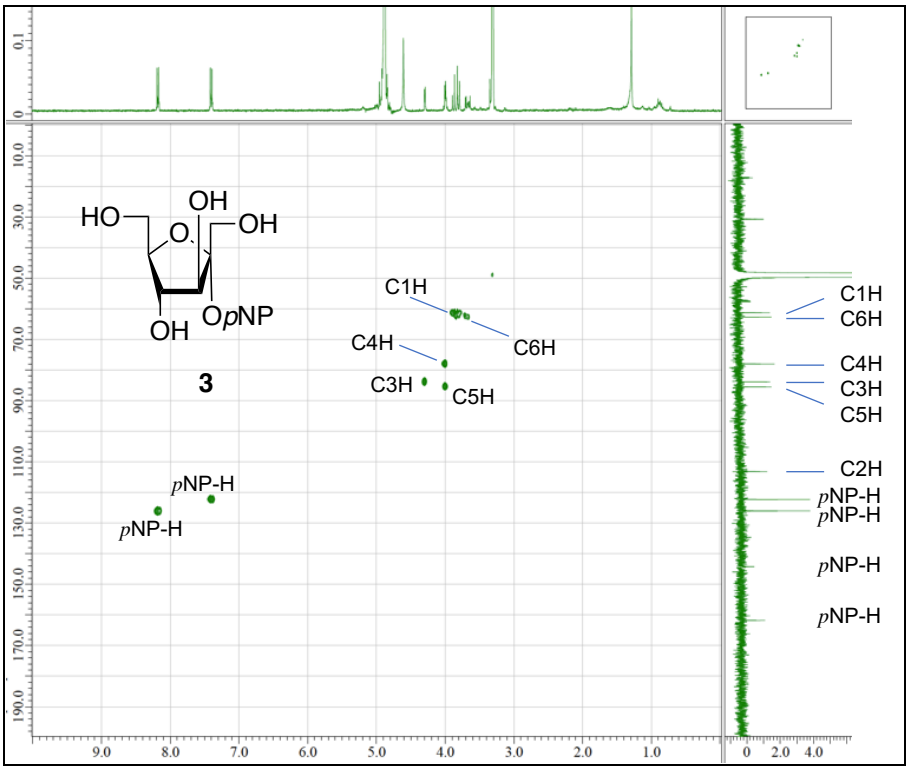
^{13}C NMR spectrum of **3** in CD_3OD (100 MHz). (* : peaks of impurity <not assigned>)



^{1}H-^{1}H COSY spectrum of **3** in CD_3OD (400 MHz).



HMQC spectrum of **3** in CD_3OD (400 MHz).



Reference for SI.

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