SUPPORTING INFORMATION

Aryl fucosides: Synthesis and evaluation of their binding affinity towards DC-SIGN receptor

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Supplementary figures

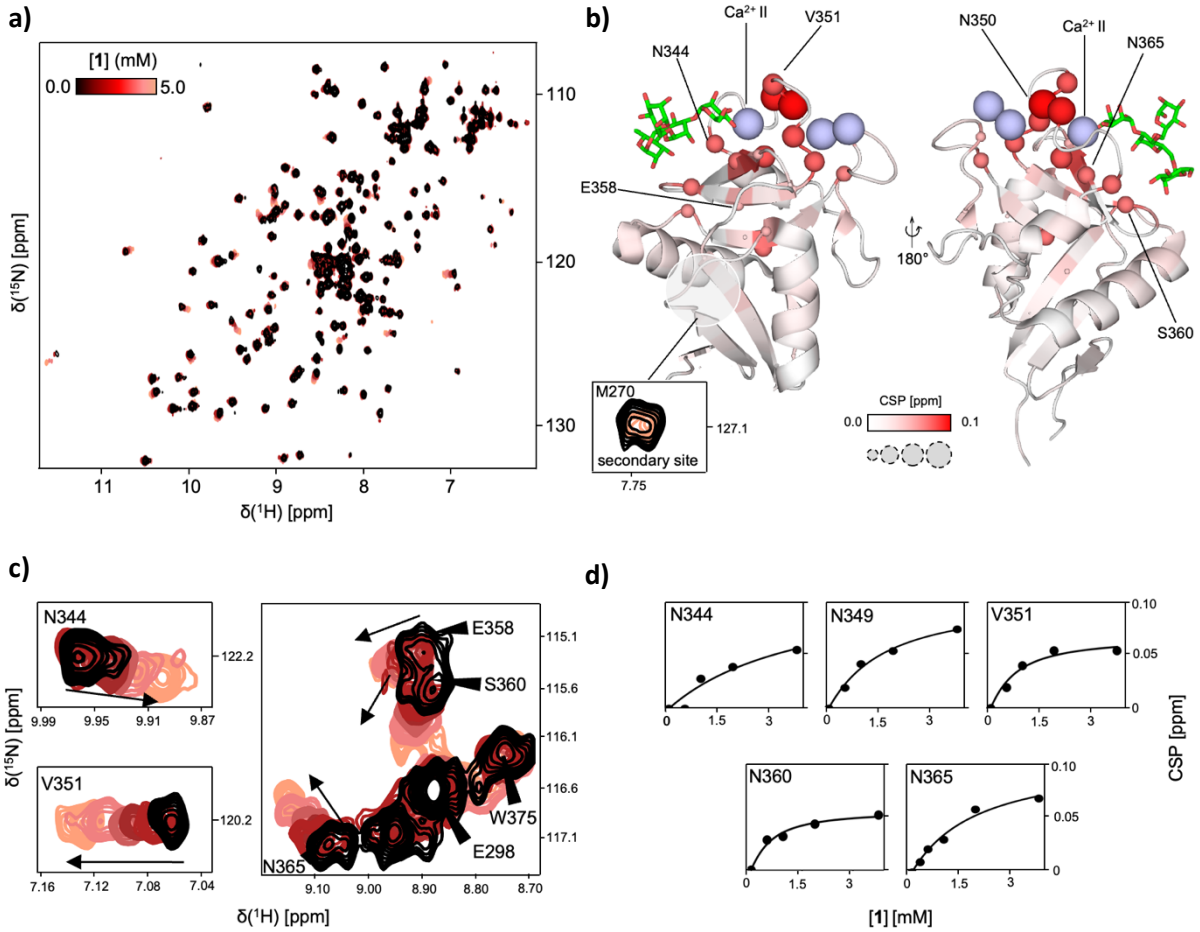


Fig. S1 Interaction of **1** with DC-SIGN CRD. a) Superimposed ^{1}H–^{15}N HSQC NMR titration spectra of ^{15}N-labelled DC-SIGN CRD interacting with increasing concentrations of **1** reveal several concentration-dependent CSPs. b) Mapping of CSPs induced at 5 mM **1** to the structure of DC-SIGN CRD (PDB code: 1SL4) suggests the ligand to interact with the carbohydrate binding site around Ca^{2+} site II in the long loop region and beta strands β3 and β4. No CSP of the secondary site residue M270 is observable. c) Examples of residues in the carbohydrate binding site showing fast exchanging resonances upon titration of increasing ligand concentrations. d) Fitting of CSPs over the increasing concentrations of **1** enabled approximation of the affinity (K_D = 2240 ± 620 µM).

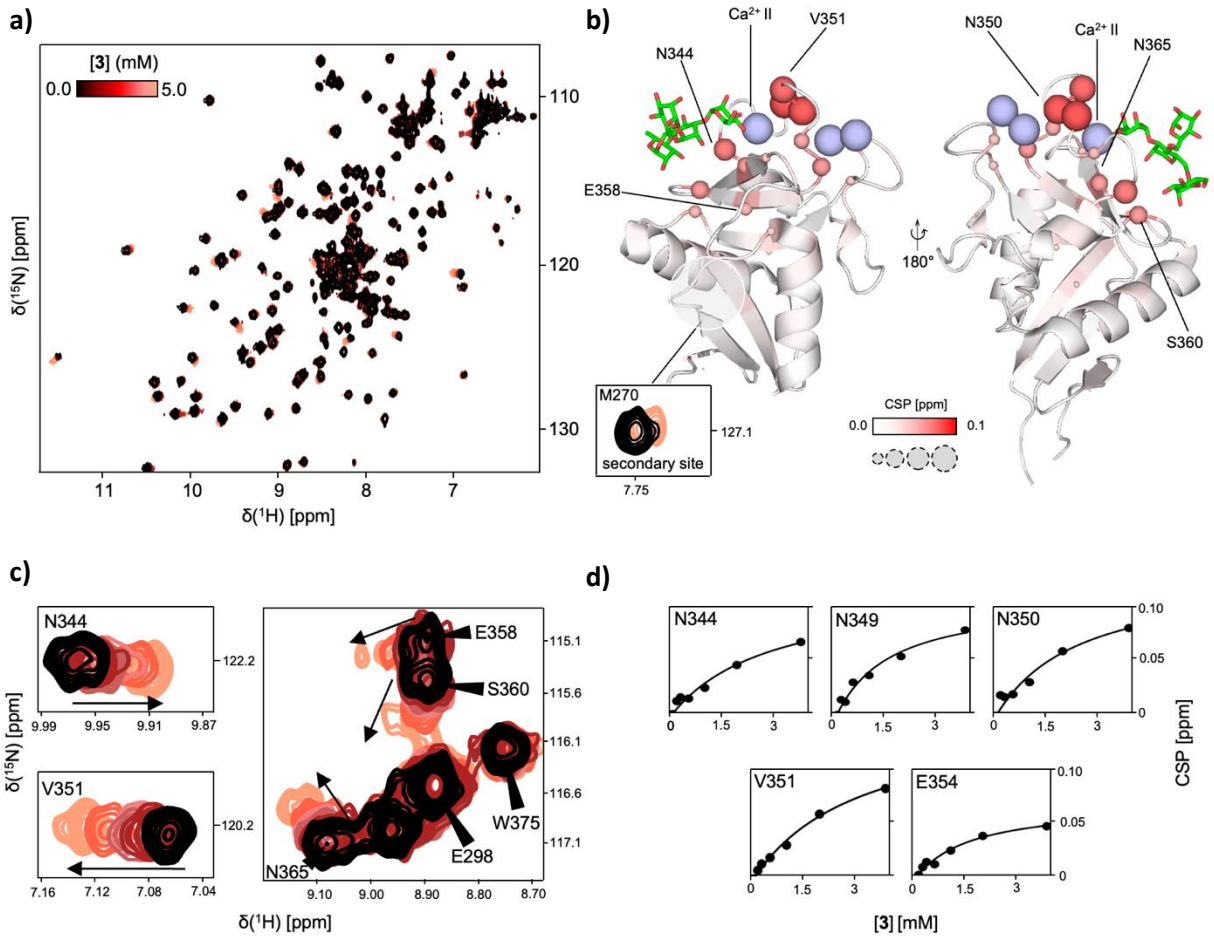


Fig. S2 Interaction of **3** with DC-SIGN CRD. a) Superimposed $^{1}H–^{15}N$ HSQC NMR titration spectra of ^{15}N-labelled DC-SIGN CRD interacting with increasing concentrations of **3** reveal several concentration-dependent CSPs. b) Mapping of CSPs induced at 5 mM **3** to the structure of DC-SIGN CRD (PDB code: 1SL4) suggests the ligand to interact with the carbohydrate binding site around Ca^{2+} site II in the long loop region and beta strands β3 and β4. For the secondary site residue M270, a minor CSP is observable and the peak displays an elongated shape. c) Examples of residues in the carbohydrate binding site showing fast exchanging resonances upon titration of increasing ligand concentrations. d) Fitting of CSPs over the increasing concentrations of **3** enabled approximation of the affinity (K_D = 2600 ± 1240 µM).

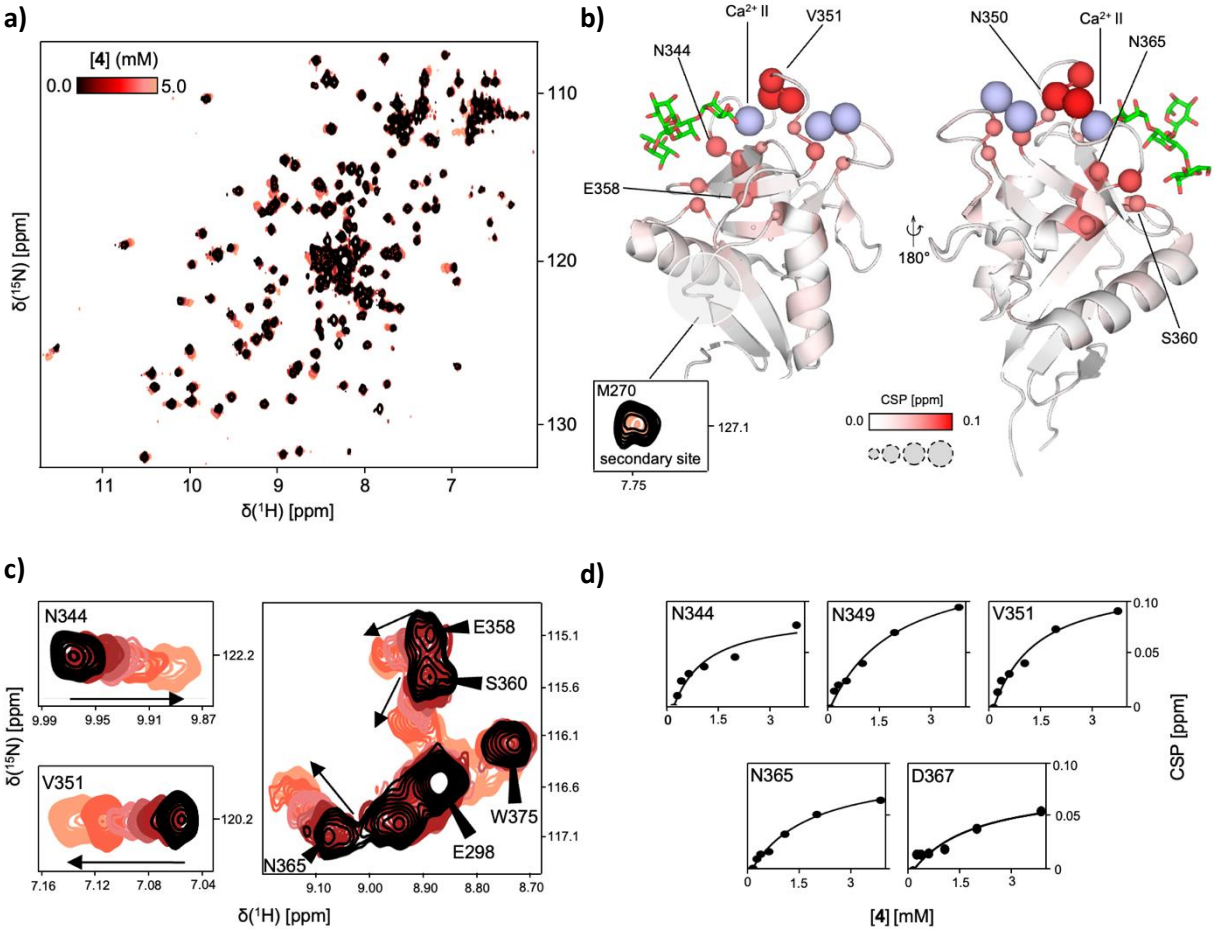


Fig. S3 Interaction of **4** with DC-SIGN CRD. a) Superimposed ^{1}H–^{15}N HSQC NMR titration spectra of ^{15}N-labelled DC-SIGN CRD interacting with increasing concentrations of **4** reveal several concentration-dependent CSPs. b) Mapping of CSPs induced at 5 mM **4** to the structure of DC-SIGN CRD (PDB code: 1SL4) suggests the ligand to interact with the carbohydrate binding site around Ca^{2+} site II in the long loop region and beta strands β3 and β4. No CSP of the secondary site residue M270 is observable. c) Examples of residues in the carbohydrate binding site showing fast exchanging resonances upon titration of increasing ligand concentrations. d) Fitting of CSPs over the increasing concentrations of **4** enabled approximation of the affinity (K_D = 1860 ± 440 µM).

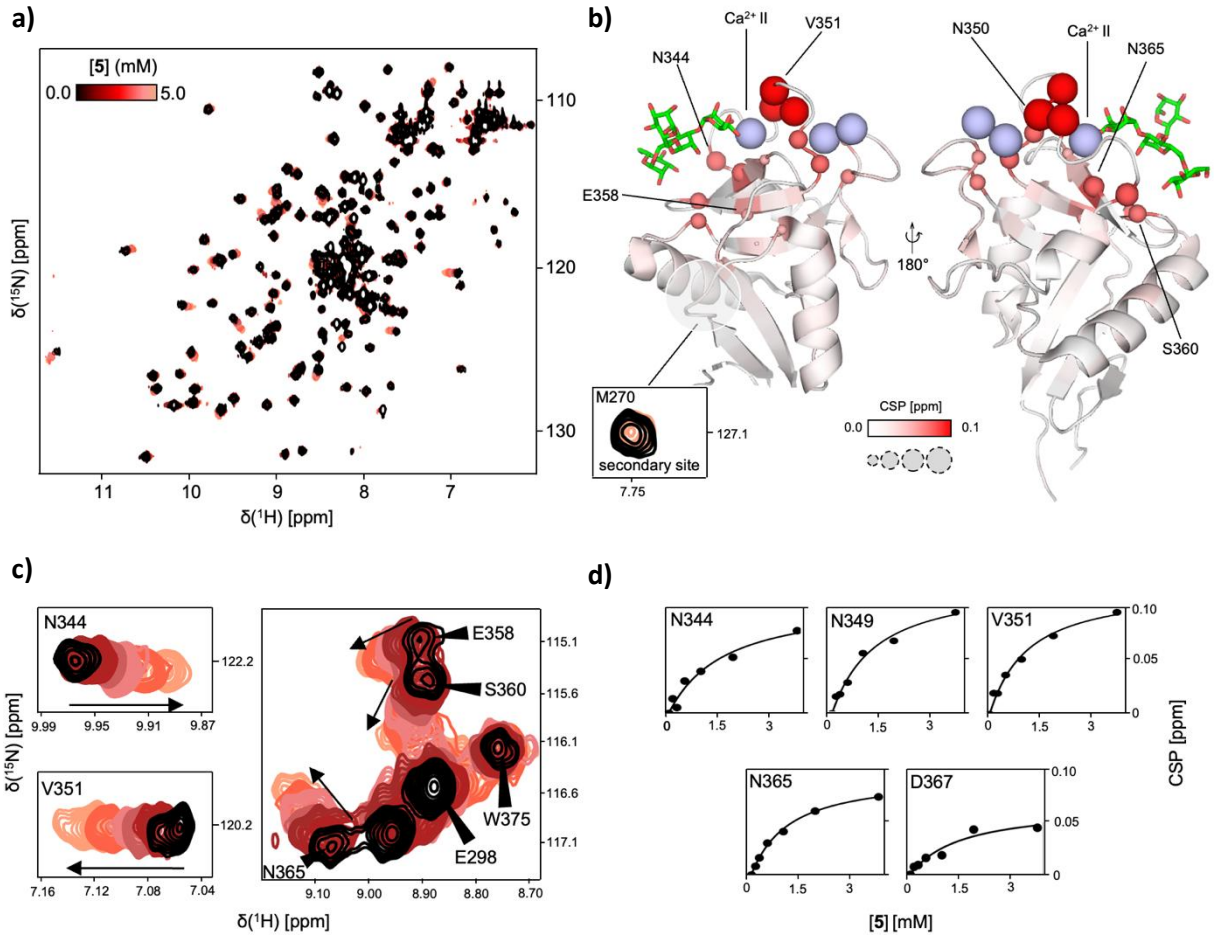


Fig. S4 Interaction of **5** with DC-SIGN CRD. a) Superimposed ^{1}H–^{15}N HSQC NMR titration spectra of ^{15}N-labelled DC-SIGN CRD interacting with increasing concentrations of **5** reveal several concentration-dependent CSPs. b) Mapping of CSPs induced at 5 mM **5** to the structure of DC-SIGN CRD (PDB code: 1SL4) suggests the ligand to interact with the carbohydrate binding site around Ca^{2+} site II in the long loop region and beta strands β3 and β4. No CSP of the secondary site residue M270 is observable. c) Examples of residues in the carbohydrate binding site showing fast exchanging resonances upon titration of increasing ligand concentrations. d) Fitting of CSPs over the increasing concentrations of **5** enabled approximation of the affinity (K_D = 1480 ± 310 µM).

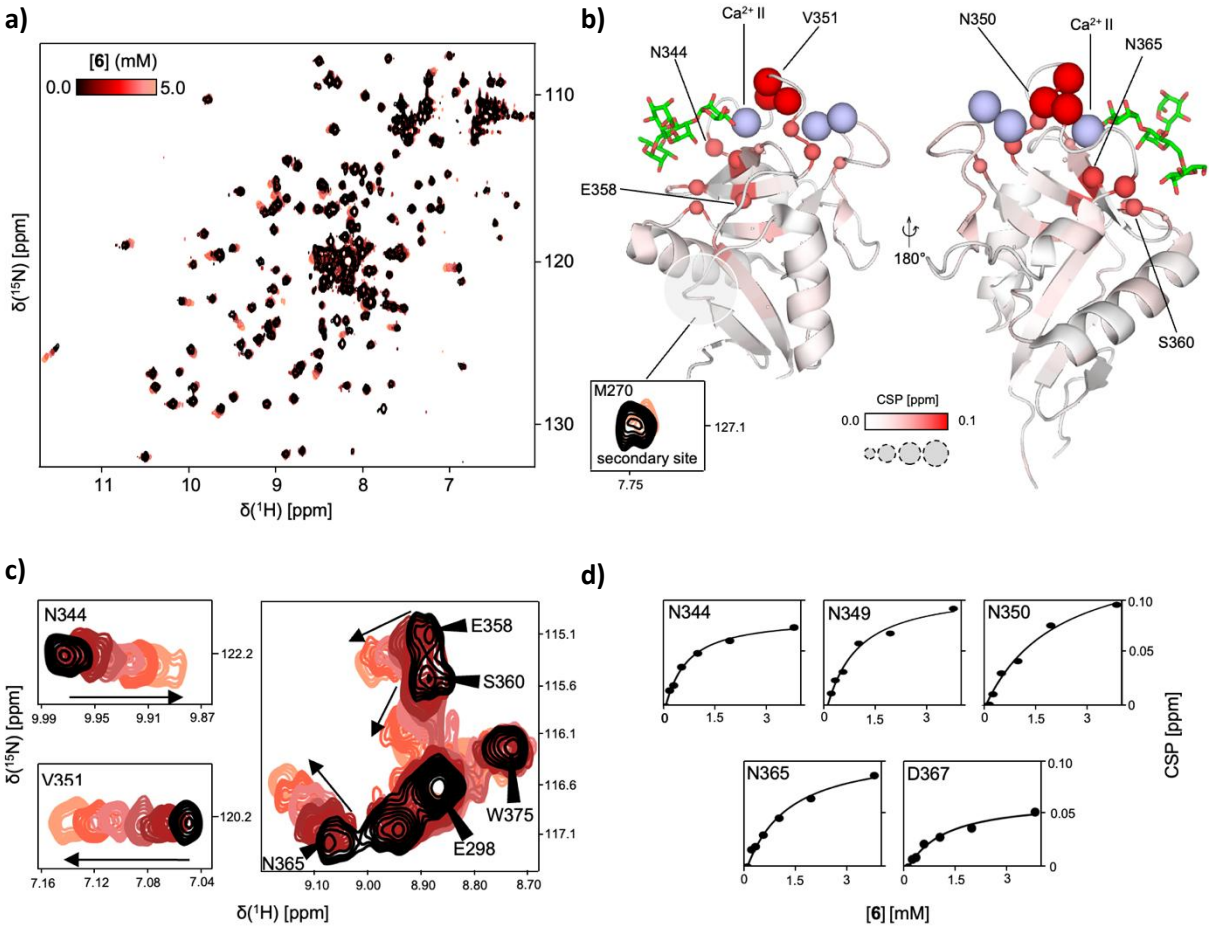


Fig. S5 Interaction of **6** with DC-SIGN CRD. a) Superimposed $^{1}H–^{15}N$ HSQC NMR titration spectra of ^{15}N-labelled DC-SIGN CRD interacting with increasing concentrations of **6** reveal several concentration-dependent CSPs. b) Mapping of CSPs induced at 5 mM **6** to the structure of DC-SIGN CRD (PDB code: 1SL4) suggests the ligand to interact with the carbohydrate binding site around Ca^{2+} site II in the long loop region and beta strands β3 and β4. No CSP of the secondary site residue M270 is observable. c) Examples of residues in the carbohydrate binding site showing fast exchanging resonances upon titration of increasing ligand concentrations. d) Fitting of CSPs over the increasing concentrations of **6** enabled approximation of the affinity (K_D = 1350 ± 510 µM).

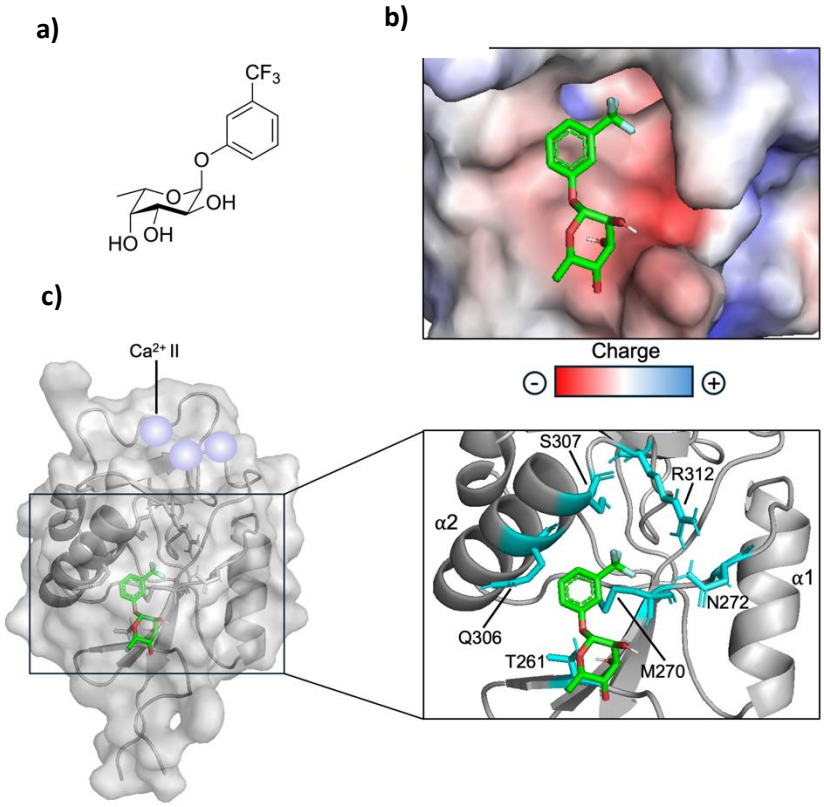


Fig. S6 Molecular docking of **2** to the secondary site of DC-SIGN. a) Compound **2** identified to bind the allosteric secondary site. b) Binding pose of compound **2** in the allosteric secondary site with mapped surface charge. The fucose moiety is oriented towards the negatively charged patch. c) Structure of DC-SIGN CRD with docked compound **2** and a zoom on the allosteric secondary site. The aglycone was identified to present a rather rigid orientation in the secondary site gated by M270 which is in line with the important CSP observed for this residue. The fucose moiety is more flexible and possibly interacts with polar residues adjacent to the secondary site.

Synthetic procedures

General

All reagents and starting materials were purchased from commercial sources and used without further purification. The solvents were purified and dried using standard procedures.[1]

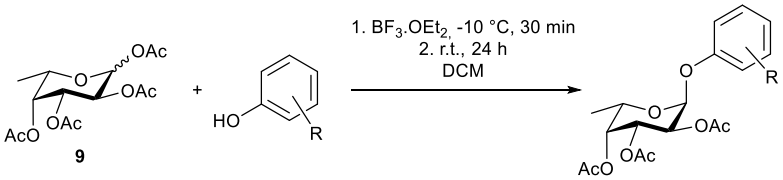
Preparative column chromatography was performed on Kieselgel 60 0.040–0.063 mm (Merck). TLC analyses were carried out on DC Alufolien Kieselgel 60 F254 (Merck). The TLC plates were visualized with a sugar stain solution (5% H_2SO_4, 0.5% 3-methoxyphenol in EtOH). Reversed-phase separations were performed on C18 Sep-Pak 6cc (Waters) 1 g cartridges.

^1H and ^{13}C NMR spectra were measured on Agilent 400-MR DDR2 and JEOL-ECZL400G at 25 °C. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ 7.26 in ^1H and δ 77.16 in ^{13}C; CD_3OD δ 3.31 in ^1H and δ 49.00 in ^{13}C and H_2O δ 4.79 in ^1H). The following abbreviations are used to indicate peak multiplicities: *s* singlet, *br s* broad singlet, *d* doublet, *dd* doublet of doublets, *ddd* doublet of doublets of doublets, *t* triplet, *q* quartet, *p* pentet, *dt* doublet of triplets, *td* triplet of doublets, *m* multiplet. The coupling constants (*J*) are reported in hertz (Hz). NMR spectra were evaluated using MestreNova 14.1.1 (MestreLab Research SSL).

Both low- and high-resolution mass spectra MS were obtained by using electrospray ionization (ESI). The spectra were measured on LTQ Orbitrap XL (Thermo Fisher Scientific).

Synthesis of compounds

General procedure 1: Synthesis of 2,3,4-tri-*O*-acetyl-1-*O*-aryl-α-L-fucopyranosides

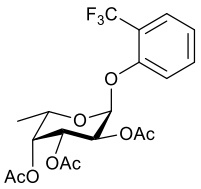


9

10 R = 2-CF_3
11 3-CF_3
12 4-CF_3
13 2-CH_3
14 2-Br
15 2-Cl

Tetra-*O*-acetyl-L-fucopyranose (**9**, 1.0 equiv.) and the respective aglycone (2.0 equiv.) were dried by co-evaporation with anhydrous toluene (3 × 5 mL) and subsequently were placed under high vacuum for 30 min. Dry DCM (5 mL) was added and the flask was purged with argon for 5 min. Activated 4 Å molecular sieves were added and the mixture was stirred at room temperature for 30 min. Next, the reaction mixture was cooled to −10 °C and $BF_3{\cdot}OEt_2$ (5.5 to 6.0 equiv.) was added dropwise. The reaction mixture was stirred at −10 °C for 30 min and then was gradually warmed up to room temperature and was stirred for 24 h. The reaction was quenched with Et_3N (0.2 mL), the mixture was filtered and the filtrate was concentrated *in vacuo* to provide a crude product.

2,3,4-Tri-*O*-acetyl-1-*O*-(2-(trifluoromethyl)phenyl)-α-L-fucopyranoside (10)

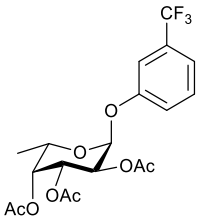


10

Compound **10** was prepared from 100 mg (0.31 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 97 mg (0.60 mmol, 2.0 equiv.) of 2-trifluoromethylphenol, and 210 µL (1.70 mmol, 5.5 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 45 to 50% EtOAc in hexane to provide product **10** as a light brown syrup (137 mg, 31%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.52 – 7.41 (m, 1H), 7.28 – 7.20 (m, 1H), 7.13 – 7.02 (m, 1H), 5.83 (d, *J* = 3.6 Hz, 1H), 5.49 (dd, *J* = 10.8, 3.3 Hz, 1H), 5.39 (dd, *J* = 3.4, 1.3 Hz, 1H), 5.22 (dd, *J* = 10.8, 3.6 Hz, 1H), 4.36 – 4.16 (m, 1H), 2.17 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.13 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.0, 170.6, 170.1, 154.5 (q, *J* = 1.9 Hz), 133.6, 127.2 (q, *J* = 5.1 Hz), 123.5 (q, *J* = 272.3 Hz), 122.1, 119.8 (q, *J* = 31.2 Hz), 115.4, 95.2, 70.8, 68.1, 67.8, 65.9, 20.9, 20.8, 20.7, 16.0. MS (ESI$^+$): calculated for $C_{19}H_{21}O_8F_3Na^+$ 457.1 $[M+Na]^+$, found *m/z* (%) 457.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{19}H_{21}O_8F_3Na^+$ 457.1078, found *m/z* 457.1081 $[M+Na]^+$.

2,3,4-Tri-*O*-acetyl-1-*O*-(3-(trifluoromethyl)phenyl)-α-L-fucopyranoside (11)

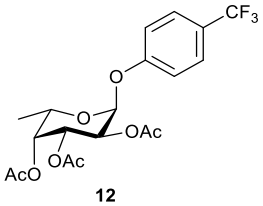


11

Compound **11** was prepared from 85 mg (0.26 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 83 mg (0.51 mmol, 2.0 equiv.) of 3-trifluoromethylphenol, and 180 µL (1.46 mmol, 5.6 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 45 to 50% EtOAc in hexane to provide product **11** as a light brown syrup (39 mg, 35%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.38 (m, 1H), 7.33 – 7.28 (m, 2H), 7.26 – 7.21 (m, 1H), 5.78 (d, *J* = 3.6 Hz, 1H), 5.57 (dd, *J* = 11.0, 3.3 Hz, 1H), 5.37 (d, *J* = 3.4, 1H), 5.30 (dd, *J* = 10.9, 3.6 Hz, 1H), 4.30 – 4.19 (m, 1H), 2.20 (s, 3H), 2.07 (s, 3H), 2.03 (s, 3H), 1.13 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ δ 170.7, 170.6, 170.3, 156.7, 132.22 (q, *J* = 32.5 Hz), 130.4, 123.8 (q, *J* = 272.2 Hz), 119.7 (q, *J* = 1.5 Hz), 119.45 (q, *J* = 3.8 Hz), 113.68 (q, *J* = 3.9 Hz), 95.0, 70.9, 67.9, 67.8, 65.9, 20.9, 20.8, 20.7, 16.0. MS (ESI$^-$): calculated for $C_{19}H_{21}O_8F_3^-$ 433.0 [M−H]$^-$, found *m/z* (%) 433.1 (100) [M−H]$^-$. HRMS (ESI$^-$): calculated for $C_{19}H_{21}O_8F_3^-$ 433.1114, found *m/z* 433.1116 [M−H]$^-$.

2,3,4-Tri-*O*-acetyl-1-*O*-(4-(trifluoromethyl)phenyl)-α-L-fucopyranoside (12)

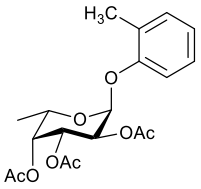


12

Compound **12** was prepared from 60 mg (0.18 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 59 mg (0.36 mmol, 2.0 equiv.) of 4-trifluoromethylphenol, and 135 µL (1.09 mmol, 6.1 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 45 to 50% EtOAc in hexane. Despite repeated attempts to purify the compound, the product was contaminated with some aromatic impurity that originated from the starting compound. Impure product **12** was obtained as a light brown syrup (30 mg, <39%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.54 (m, 2H), 7.17 – 7.11 (m, 2H), 5.80 (d, *J* = 3.7 Hz, 1H), 5.57 (ddd, *J* = 10.9, 3.5, 1.3 Hz, 1H), 5.37 (dt, *J* = 3.1, 1.4 Hz, 1H), 5.29 (ddd, *J* = 10.8, 3.8, 1.3 Hz, 1H), 4.25 – 4.18 (m, 1H), 2.20 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.12 (d, *J* = 6.5 Hz, 3H). MS (ESI$^-$): calculated for $C_{19}H_{21}O_8F_3^-$ 433.0 [M−H]$^-$, found *m/z* (%) 433.1 (100) [M−H]$^-$. HRMS (ESI$^-$): calculated for $C_{19}H_{21}O_8F_3^-$ 433.1113, found 433.1116 [M−H]$^-$.

2,3,4-Tri-*O*-acetyl-1-*O*-(2-tolyl)-α-L-fucopyranoside (13)

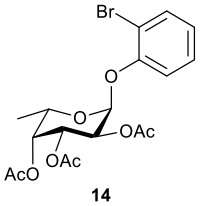


13

Compound **13** was prepared from 50 mg (0.15 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 33 mg (0.30 mmol, 2.0 equiv.) of 2-methylphenol, and 100 µL (0.81 mmol, 5.4 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 12 to 15% EtOAc in hexane to provide product **13** as an off-white solid (40 mg, 70%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 – 7.12 (m, 2H), 7.09 (d, *J* = 7.9 Hz, 1H), 6.96 (t, *J* = 7.4 Hz, 1H), 5.68 (d, *J* = 3.4 Hz, 1H), 5.54 – 5.59 (m, 1H), 5.39 (d, *J* = 3.4, 1H), 5.33 – 5.27 (m, 1H), 4.36 – 4.29 (m, 1H), 2.28 (s, 3H), 2.20 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.16 (d, *J* = 6.6 Hz, 3H).^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.5, 170.3, 155.2, 131.0, 127.8, 127.2, 122.6, 114.6, 95.6, 70.9, 68.3, 68.1, 65.4, 20.9 (2C), 20.8, 16.3, 16.1. MS (ESI$^+$): calculated for $C_{19}H_{24}O_8Na^+$ 403.1 $[M+Na]^+$, found *m*/*z* (%) 403.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{19}H_{24}O_8Na^+$ 403.1361, found *m*/*z* 403.1363 $[M+Na]^+$.

2,3,4-Tri-*O*-acetyl-1-*O*-(2-bromophenyl)-α-L-fucopyranoside (14)

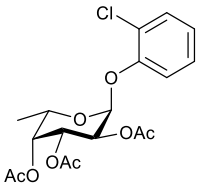


14

Compound **14** was prepared from 100 mg (0.3 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 104 mg (0.60 mmol, 2.0 equiv.) of 2-bromophenol, and 210 µL (1.70 mmol, 5.7 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 20 to 30% EtOAc in hexane. Despite numerous attempts to purify the product, it was obtained in a mixture with $NHEt_3^+$ salt (ca. 1:1.3). Impure product **14** was obtained as a pale yellow syrup (54 mg, <40%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.50 (m, 1H), 7.29 – 7.21 (m, 1H), 7.12 (dt, *J* = 8.2, 1.5 Hz, 1H), 6.97 – 6.89 (m, 1H), 5.78 (d, *J* = 3.7 Hz, 1H), 5.62 (dd, *J* = 10.8, 3.4 Hz, 1H), 5.40 (dd, *J* = 3.3, 1.4 Hz, 1H), 5.32 – 5.19 (m, 1H), 4.41 – 4.32 (m, 1H), 2.18 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.13 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.6, 170.1, 153.3, 133.6, 128.7, 124.0, 116.7, 113.3, 96.0, 70.9, 68.1, 67.9, 65.9, 21.0, 20.8, 20.7, 16.0. MS (ESI$^+$): calculated for $C_{18}H_{21}O_8BrNa^+$ 467.0 $[M+Na]^+$, found *m*/*z* (%) 467.0 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{18}H_{21}O_8BrNa^+$ 467.0314, found *m*/*z* 467.0312 $[M+Na]^+$.

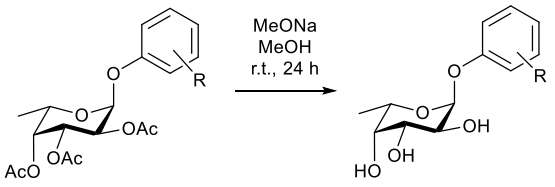
2,3,4-Tri-*O*-acetyl-1-*O*-(2-chlorophenyl)-α-L-fucopyranoside (15)



15

Compound **15** was prepared from 95 mg (0.29 mmol) of tetra-*O*-acetyl-L-fucopyranose (**9**), 74 mg (0.57 mmol, 2.0 equiv.) of 2-chlorophenol, and 200 µL (1.62 mmol, 5.6 equiv.) of $BF_3{\cdot}OEt_2$ according to General procedure 1. The crude product was purified by column chromatography, eluting with 25 to 30% EtOAc in hexane. Despite numerous attempts to purify the product, it was obtained in a mixture with $NHEt_3^+$ salt (ca. 1:0.9). Impure product **15** was obtained as a colourless syrup (23 mg, <20%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.20 (ddd, *J* = 8.1, 7.3, 1.7 Hz, 1H), 7.13 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.00 (ddd, *J* = 7.9, 7.3, 1.6 Hz, 1H), 5.76 (d, *J* = 3.7 Hz, 1H), 5.60 (dd, *J* = 10.9, 3.3 Hz, 1H), 5.41 (dd, *J* = 3.6, 1.3 Hz, 1H), 5.26 (dd, *J* = 10.9, 3.7 Hz, 1H), 4.43 – 4.35 (m, 1H), 2.19 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.15 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.9, 170.7, 170.2, 152.5, 130.6, 128.0, 124.5, 123.8, 117.5, 96.3, 71.0, 68.1, 68.0, 65.9, 21.0, 20.9, 20.8, 16.0. MS (ESI$^+$): calculated for $C_{18}H_{21}O_8ClNa^+$ 423.1 $[M+Na]^+$, found *m*/*z* (%) 423.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{18}H_{21}O_8ClNa^+$ 423.0819, found *m*/*z* 423.0817 $[M+Na]^+$.

General procedure 2: Synthesis of aryl-α-L-fucopyranosides

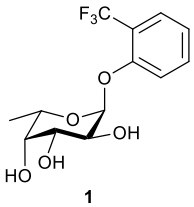


10	R =	2-CF_3	
11		3-CF_3	
12		4-CF_3	
13		2-CH_3	
14		2-Br	
15		2-Cl	

1	R =	2-CF_3
2		3-CF_3
3		4-CF_3
4		2-CH_3
5		2-Br
6		2-Cl

Target compounds **1**–**6** were synthesized by stirring acetylated precursors in a sodium methoxide solution in dry methanol (0.2 M) at room temperature for 24 h. Afterwards, the pH of the reaction mixture was adjusted to 5–6 with Amberlite H^+ resin and the reaction mixture was filtered. The filtrate was concentrated *in vacuo* to provide a crude product.

1-*O*-(2-(Trifluoromethyl)phenyl)-α-L-fucopyranoside (1)

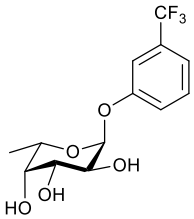


1

Compound **1** was prepared from 40 mg (0.13 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(2--(trifluoromethyl)phenyl)-α-L-fucopyranoside (**10**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 15 to 25% acetonitrile in water to provide product **1** as a pale yellow syrup (18 mg, 63**%**).

^{1}H NMR (400 MHz, CD_3OD) δ 7.60 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.58 – 7.53 (m, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 5.72 (d, *J* = 2.2 Hz, 1H), 4.08 – 3.92 (m, 3H), 3.74 (q, *J* = 1.3 Hz, 1H), 1.16 (d, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, CD_3OD) δ 156.1, 134.7, 127.9 (q, *J* = 5.3 Hz), 125.3 (q, *J* = 271.7 Hz), 122.3, 120.2 (q, *J* = 30.8 Hz), 116.4, 98.6, 73.4, 71.5, 69.5, 69.3, 16.6. MS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.2 $[M+Na]^+$, found *m*/*z* (%) 331.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.0765, found *m*/*z* 331.0764 $[M+Na]^+$.

1-*O*-(3-(Trifluoromethyl)phenyl)-α-L-fucopyranoside (2)

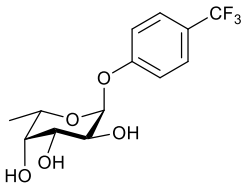


2

Compound **2** was prepared from 39 mg (0.13 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(3--(trifluoromethyl)phenyl)-α-L-fucopyranoside (**11**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 18 to 30% acetonitrile in water to provide product **2** as a pale yellow syrup (14 mg, 50**%**).

^{1}H NMR (400 MHz, CD_3CN) δ 7.54 – 7.47 (m, 1H), 7.39 – 7.32 (m, 3H), 5.55 (d, *J* = 3.6 Hz, 1H), 4.00 – 3.94 (m, 1H), 3.87 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.77 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.70 (dd, *J* = 3.4, 1.3 Hz, 1H), 1.12 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR {^{19}F} (101 MHz, CD_3CN) δ 158.5, 132.1, 131.5, 125.1, 121.5, 119.5, 114.5, 99.1, 72.5, 71.1, 69.5, 68.3, 16.6. MS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.2 $[M+Na]^+$, found *m*/*z* (%) 331.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.0765, found *m*/*z* 331.0764 $[M+Na]^+$.

1-*O*-(4-(Trifluoromethyl)phenyl)-α-L-fucopyranoside (3)

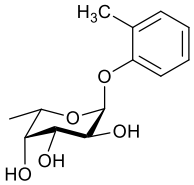


3

Compound **3** was prepared from 30 mg (0.10 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(4--(trifluoromethyl)phenyl)-α-L-fucopyranoside (**12**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 18 to 30% acetonitrile in water to provide product **3** as a pale yellow syrup (8 mg, 38**%**).

^{1}H NMR (400 MHz, D_2O) δ 8.02 – 7.93 (m, 2H), 7.21 – 7.11 (m, 2H), 5.70 (d, *J* = 3.9 Hz, 1H), 4.13 – 4.02 (m, 2H), 3.92 (dd, *J* = 10.3, 3.8 Hz, 1H), 3.81 (d, *J* = 3.4 Hz, 1H), 1.09 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR {^{19}F} (101 MHz, D_2O) δ 169.0, 160.3, 131.6 (2C), 123.5, 116.5 (2C), 96.5, 71.6, 69.5, 67.9, 67.7, 15.2. MS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.2 $[M+Na]^+$, found *m/z* (%) 331.1 (100). $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{13}H_{15}F_3O_5Na^+$ 331.0765, found *m/z* 331.0764 $[M+Na]^+$.

1-*O*-(2-Tolyl)-α-L-fucopyranoside (4)

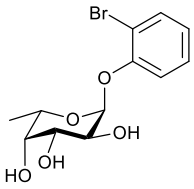


4

Compound **4** was prepared from 24 mg (0.09 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(2-tolyl)-α-L--fucopyranoside (**13**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 18 to 30% of acetonitrile in water to provide product **4** as an off-white solid (9 mg, 52**%**).

^{1}H NMR (400 MHz, CD_3CN) δ 7.19 – 7.11 (m, 2H), 7.07 (dd, *J* = 8.2, 1.3 Hz, 1H), 6.92 (td, *J* = 7.3, 1.4 Hz, 1H), 5.49 (d, *J* = 3.7 Hz, 1H), 4.00 (qd, *J* = 6.6, 1.3 Hz, 1H), 3.92 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.79 (dd, *J* = 10.0, 3.7 Hz, 1H), 3.71 (dd, *J* = 3.4, 1.4 Hz, 1H), 2.24 (s, 3H), 1.12 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 156.1, 131.7, 128.7, 127.8, 122.8, 115.6, 98.5, 72.7, 71.3, 69.8, 68.0, 16.6, 16.4. MS (ESI$^+$): calculated for $C_{13}H_{18}O_5Na^+$ 277.1 $[M+Na]^+$, found *m/z* (%) 277.1 (100) $[M+Na]^+$. HRMS (ESI$^+$): calculated for $C_{13}H_{18}O_5Na^+$ 277.1048, found *m/z* 277.1049 $[M+Na]^+$.

1-*O*-(2-Bromophenyl)-α-L-fucopyranoside (5)

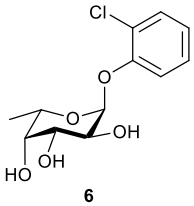


5

Compound **5** was prepared from 50 mg (0.16 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(2-bromophenyl)-α-L--fucopyranoside (**14**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 35 to 40% of acetonitrile in water to provide product **5** as an off-white solid (28 mg, 78**%**).

^{1}H NMR (400 MHz, CD_3CN) δ 7.58 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.31 (d, *J* = 1.6 Hz, 1H), 7.23 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.95 (td, *J* = 7.6, 1.5 Hz, 1H), 5.60 (d, *J* = 3.6 Hz, 1H), 4.04 (qd, *J* = 6.6, 1.4 Hz, 1H), 3.94 (dd, *J* = 10.0, 3.4 Hz, 1H), 3.84 – 3.78 (m, 1H), 3.73 (dd, *J* = 3.4, 1.4 Hz, 1H), 1.12 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 154.2, 134.2, 129.8, 124.4, 117.7, 113.6, 99.4, 72.5, 71.3, 69.5, 68.7, 16.5. MS (ESI^+): calculated for $C_{12}H_{15}O_5BrNa^+$ 341.1 $[M+Na]^+$, found *m/z* (%) 341.1 (100) $[M+Na]^+$. HRMS (ESI^+): calculated for $C_{12}H_{15}O_5BrNa^+$ 341.0004, found *m/z* 341.0005 $[M+Na]^+$.

1-*O*-(2-Chlorophenyl)-α-L-fucopyranoside (6)



6

Compound **6** was prepared from 22 mg (0.08 mmol) of 2,3,4-tri-*O*-acetyl-1-*O*-(2-chlorophenyl)-α-L--fucopyranoside (**15**) and 5 mL of 0.2 M sodium methoxide in methanol according to General procedure 2. The crude product was purified by reverse phase column chromatography (Sep-Pack C_{18}, 1.0 g), eluting with 32 to 38% of acetonitrile in water to provide product **6** as a colourless syrup (7 mg, 49**%**).

^{1}H NMR (400 MHz, CD_3CN) δ 7.44 – 7.38 (m, 1H), 7.31 – 7.23 (m, 2H), 7.05 – 6.99 (m, 1H), 5.58 (d, *J* = 3.7 Hz, 1H), 4.09 – 4.01 (m, 1H), 3.92 (dd, *J* = 10.0, 3.3 Hz, 1H), 3.81 (dd, *J* = 10.1, 3.7 Hz, 1H), 3.72 (dd, *J* = 3.5, 1.4 Hz, 1H), 1.12 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (101 MHz, CD_3CN) δ 153.5, 131.1, 129.1, 124.5, 124.1, 118.2, 99.7, 72.5, 71.2, 69.6, 68.6, 16.6. MS (ESI^+): calculated for $C_{12}H_{15}O_4ClNa^+$ 297.1 $[M+Na]^+$, found *m/z* (%) 297.1 (100) $[M+Na]^+$. HRMS (ESI^+): calculated for $C_{12}H_{15}O_4ClNa^+$ 297.0501, found *m/z* 297.0500 $[M+Na]^+$.

Evaluation of DC-SIGN binding affinity

Protein expression and purification

Unless stated otherwise, all chemicals, growth media and enzymes used for protein expression and purification were purchased from Sigma Aldrich or Carl Roth. Codon-optimized genes cloned into a pET28a vector for the bacterial expression of ^{15}N-labelled DC-SIGN CRD were purchased from GenScript.

^{15}N-labelled DC-SIGN CRD was produced as previously described.[2] In brief, the protein was expressed insolubly in BL21 (DE3) *E. coli* transformed with a pET28a vector encoding amino acids 253–404 of DC-SIGN and a *N*-terminal His-tag. Bacteria grown in M9 minimal medium supplemented with $^{15}NH_4Cl$ were lysed and inclusion bodies were harvested by centrifugation. Following solubilization, the protein was refolded overnight *via* rapid dilution. Next, the protein was dialyzed against 100 mM Tris-HCl, 150 mM NaCl, 10 mM $CaCl_2$ (pH 7.8) and purified *via* Ni^{2+} NTA affinity chromatography. Purified protein was pooled and dialyzed against 20 mM MES, 40 mM NaCl, 10 mM $CaCl_2$ (pH 6.0), concentrated using a centrifugal spin filter, snap frozen and then stored at −80°C.

NMR binding experiments

Unless stated otherwise, all chemicals for buffers used in NMR measurements were purchased from Sigma Aldrich or Carl Roth. NMR tubes were purchased from Bruker. Ligands were dissolved at 100 mM in D_2O. All samples contained 0.2 mM ^{15}N-labelled DC-SIGN CRD in 20 mM MES, 40 mM NaCl, 10 mM $CaCl_2$ (pH 6.0) supplemented with 10% D_2O and varying concentrations of ligand.

^{1}H–^{15}N HSQC NMR ligand titration experiments with compounds **1**–**6** were performed on a Bruker 500 MHz system equipped with a TCI H/F-C-N Prodigy probe. Spectra processing and analysis was done in TopSpin (Bruker) and CCPNMR 3.2.[3] Resonances were assigned by transferring a previously published assignment (BMRB Entry 27854) to the nearest neighbour in a reference spectrum containing no ligand.[4] Overlapping or disappearing peaks were not assigned. Further CSP analysis and curve fitting to estimate affinities was performed using Python scripts. CSPs were calculated according to **Equation 1** as previously described.[5]

$$\mathrm{CSP} = \sqrt{\frac{(\partial(^{1}\mathrm{H}))^2 + (0.15 \cdot \partial(^{15}\mathrm{N}))^2}{2}}$$

Equation 1

Resonances in fast exchange upon addition of ligand at different concentration $[L]_T$ and constant protein concentration $[P]_T$, were used to calculate K_D values in a global two parameter fit using **Equation 2**.

$$\mathrm{CSP} = \mathrm{CSP}_{\max} p_b$$

Equation 2

with

$$p_b = \frac{[P]_T + [L]_T + K_D - \sqrt{([P]_T + [L]_T + K_D)^2 - 4[P]_T[L]_T}}{2[L]_T}$$

Binding-mode analysis was conducted by mapping CSPs induced by 3.125 mM ligand on the X-ray structure of DC-SIGN CRD (PDB: 1SL4) using PyMOL (Schrödinger).

Molecular modelling

Molecular modelling for compound **2** was done in Molecular Operating Environment 2022 (MOE) (Chemical Computing Group ULC, 2019) using the X-ray structure of DC-SIGN CRD (PDB: 1SL4). Default options and parameters were used, and structural corrections and protonation states were implemented by MOE's *Structure Preparation* and *Protonate 3D* applications, respectively. The structures were minimized using the *QuickPrep* application. Compound **2** was prepared for docking with the *Database Wash* application. The AMBER10:EHT forcefield was used for all refinement/minimization steps. No pharmacophoric constraints were applied for docking of **2**, but the initial positioning of the ligand was constrained to residues previously defined as secondary site.[2] Initially generated docking poses were scored with the London ΔG function and highly scored poses were refined in molecular mechanics simulations. Refined poses were scored *via* the GBIV/WSA ΔG function and evaluated by visual inspection.

Plate-based competition assay

Unless stated otherwise, all chemicals for were purchased from Sigma Aldrich or Carl Roth.

The competitive binding assay was designed to evaluate the interaction between immobilized DC-SIGN extracellular domain (ECD) and horseradish peroxidase (HRP), a glycoprotein known to bind DC-SIGN. A 96-well transparent Nunc Maxisorp plate (Thermo Fisher) was coated with DC-SIGN ECD (100 µL/well, 0.1 mg/mL) in immobilization buffer (25 mM HEPES, 150 mM NaCl, 25 mM $CaCl_2$, pH 7.4). The plate was sealed with a membrane and incubated overnight at 4 °C.

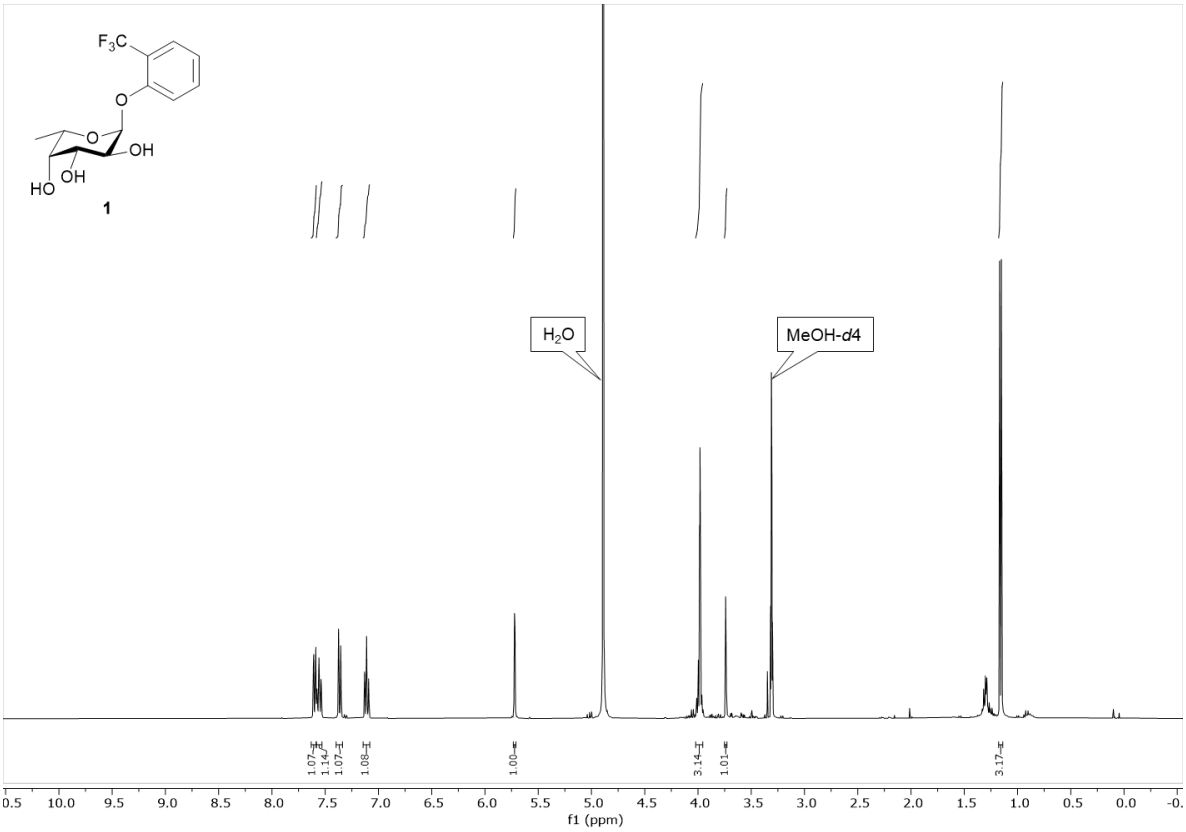
Following incubation, the protein solution was removed and wells were blocked with 100 µL/well of HRP blocking buffer (25 mM HEPES, 150 mM NaCl, 25 mM $CaCl_2$, pH 7.4, supplemented with 2% BSA and 0.01% Tween-20). After sealing, the plate was incubated for 2 h at room temperature.

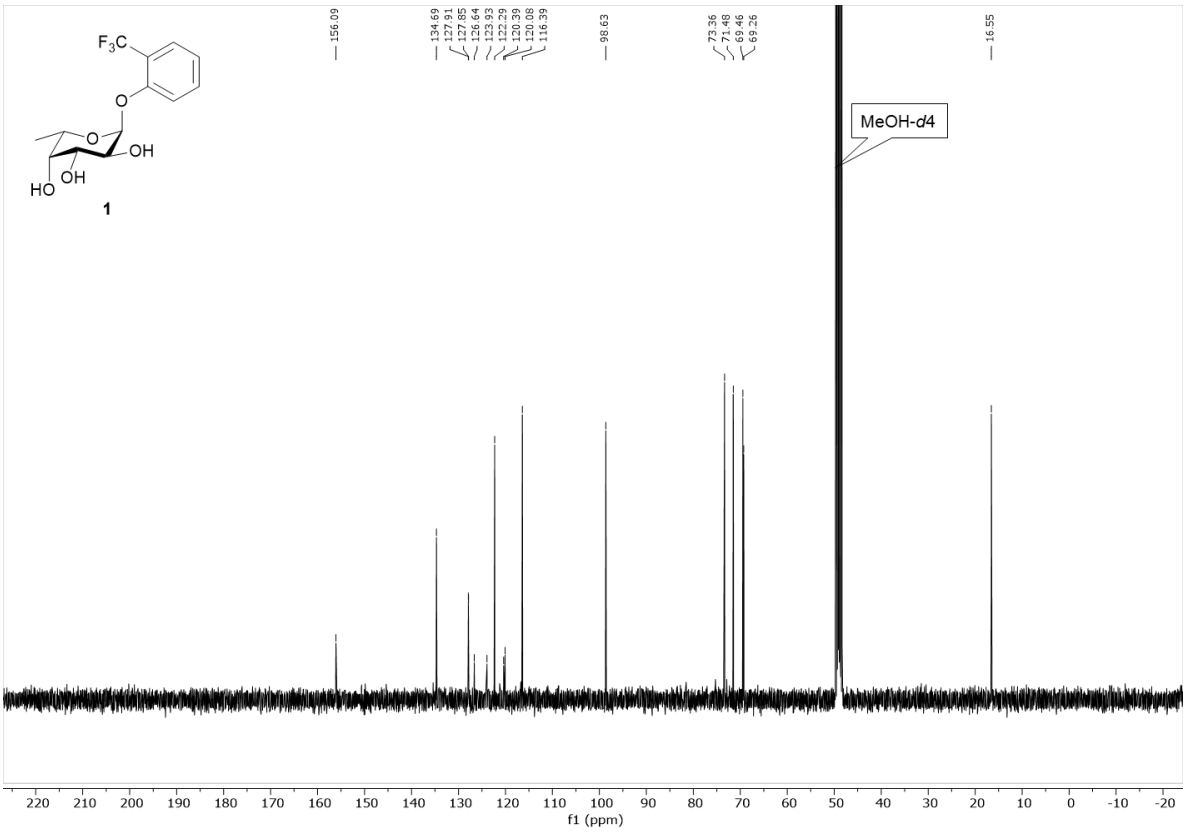
The blocking buffer was removed and wells were washed three times with 50 µL wash buffer (25 mM HEPES, 150 mM NaCl, 25 mM $CaCl_2$, pH 7.4).

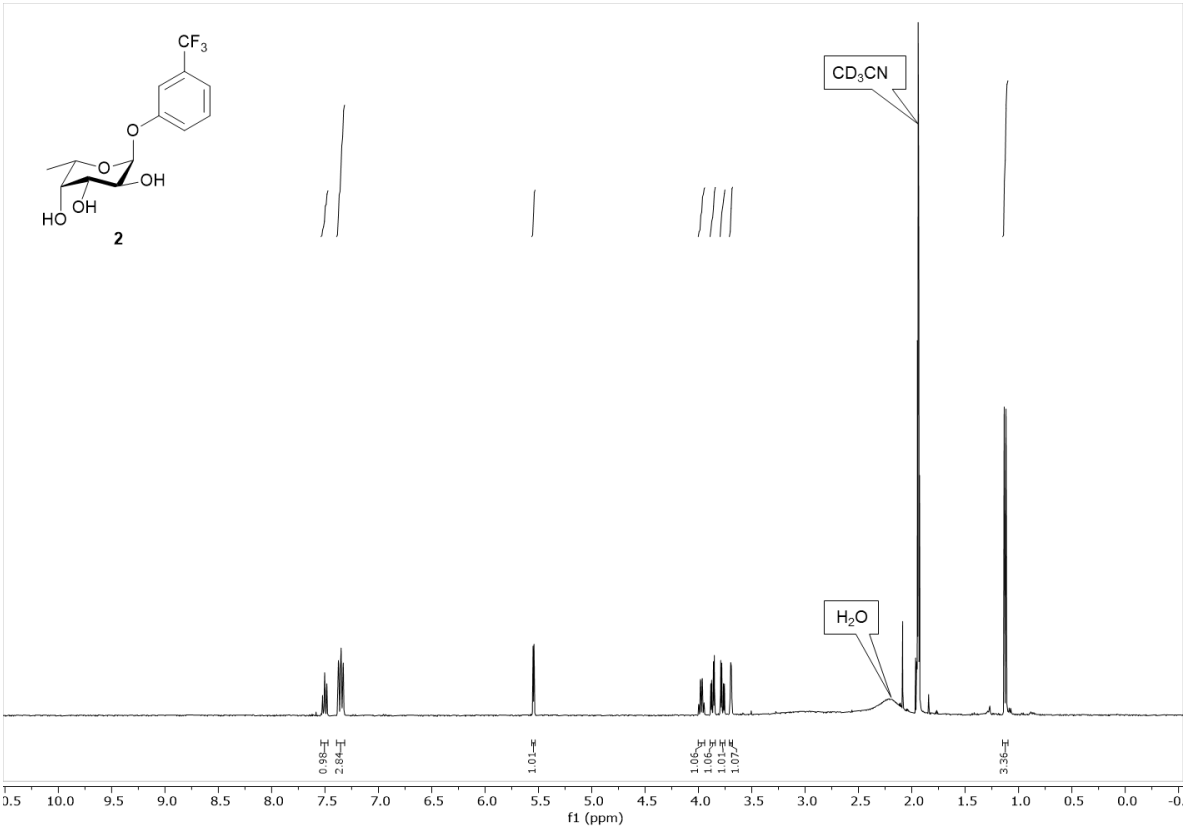
For the competition assay, a mixture containing HRP (1 µg/mL) and the inhibitor at the highest tested concentration was prepared in compound buffer (25 mM HEPES, 150 mM NaCl, 25 mM $CaCl_2$, pH 7.4). This mixture was added to the protein-coated wells at a final volume of 50 µL/well. Each compound was tested in duplicate, with a seven-point serial dilution. The DMSO concentration was maintained constant at 5% per well, and a 5% DMSO control without inhibitor was included. The plate was incubated at room temperature for 2 h in the dark, followed by three washing steps with 100 µL wash buffer.

To detect HRP binding, 50 µL of pre-mixed TMB substrate (TMB Substrate Kit, Thermo Fisher) was added per well. After 1 min incubation time, 100 µL of a 0.18 M phosphoric acid was added to stop the reaction. Absorbance was measured at 450 nm using a 96-well plate reader. Data were fitted to a logistic function using Origin software to determine the half-maximal inhibitory concentration (IC_{50}) of the tested inhibitors.[6]

^{1}H and ^{13}C NMR spectra of new compounds



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HO
OH
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CD3CN
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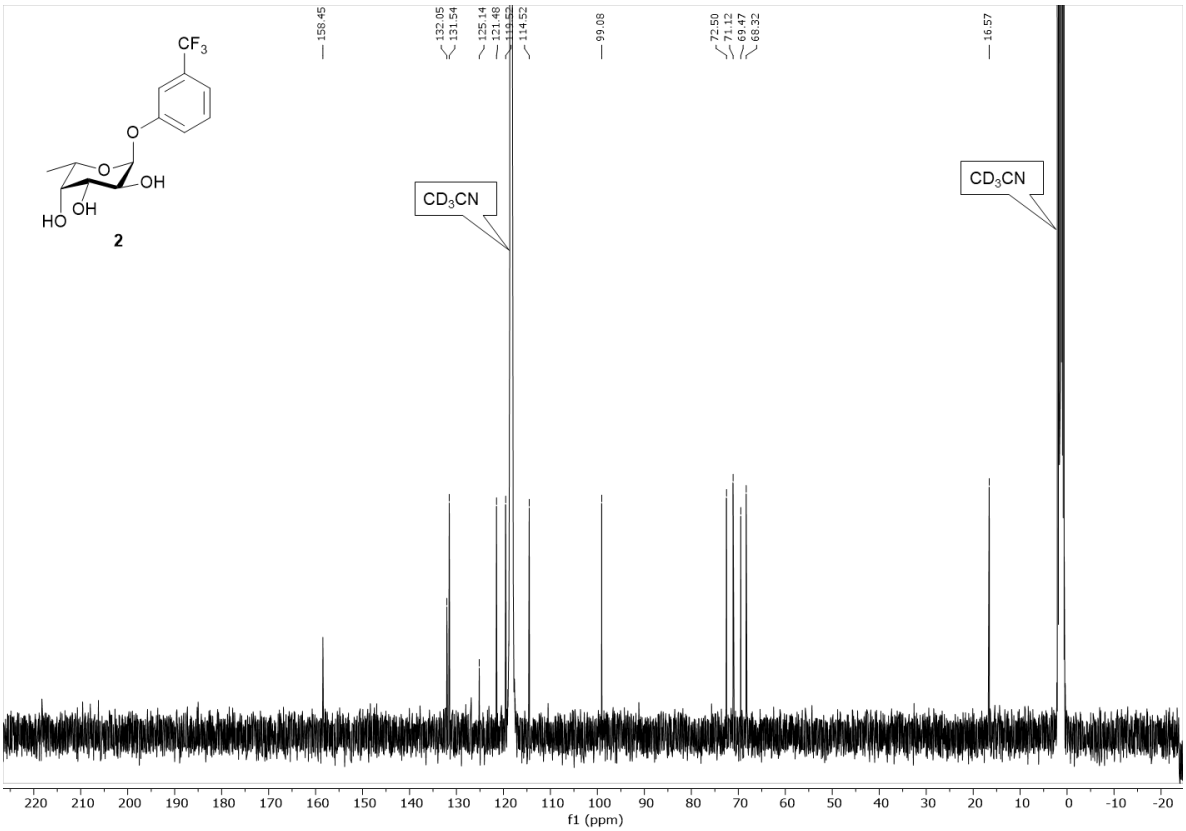
1.06
1.06
1.01
1.07
3.36
f1 (ppm)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HO
OH
2
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114.52
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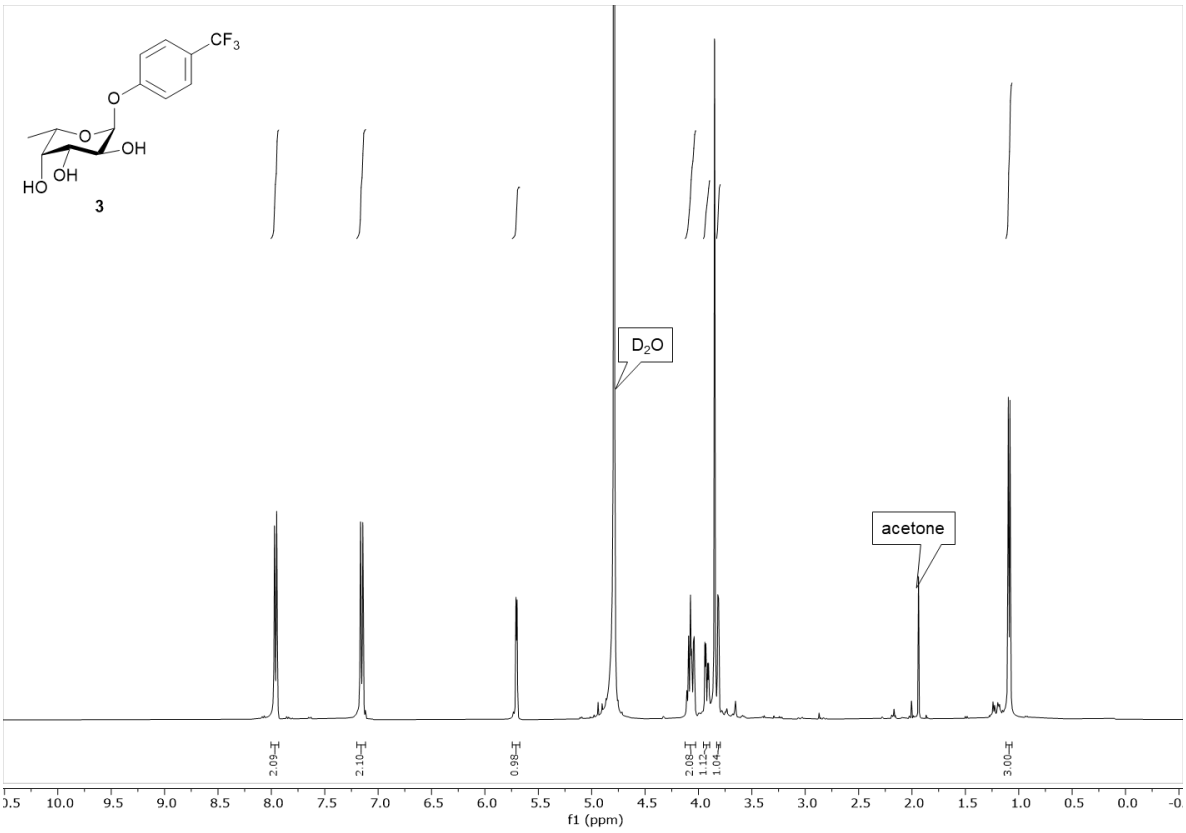
CD3CN
CD3CN
f1 (ppm)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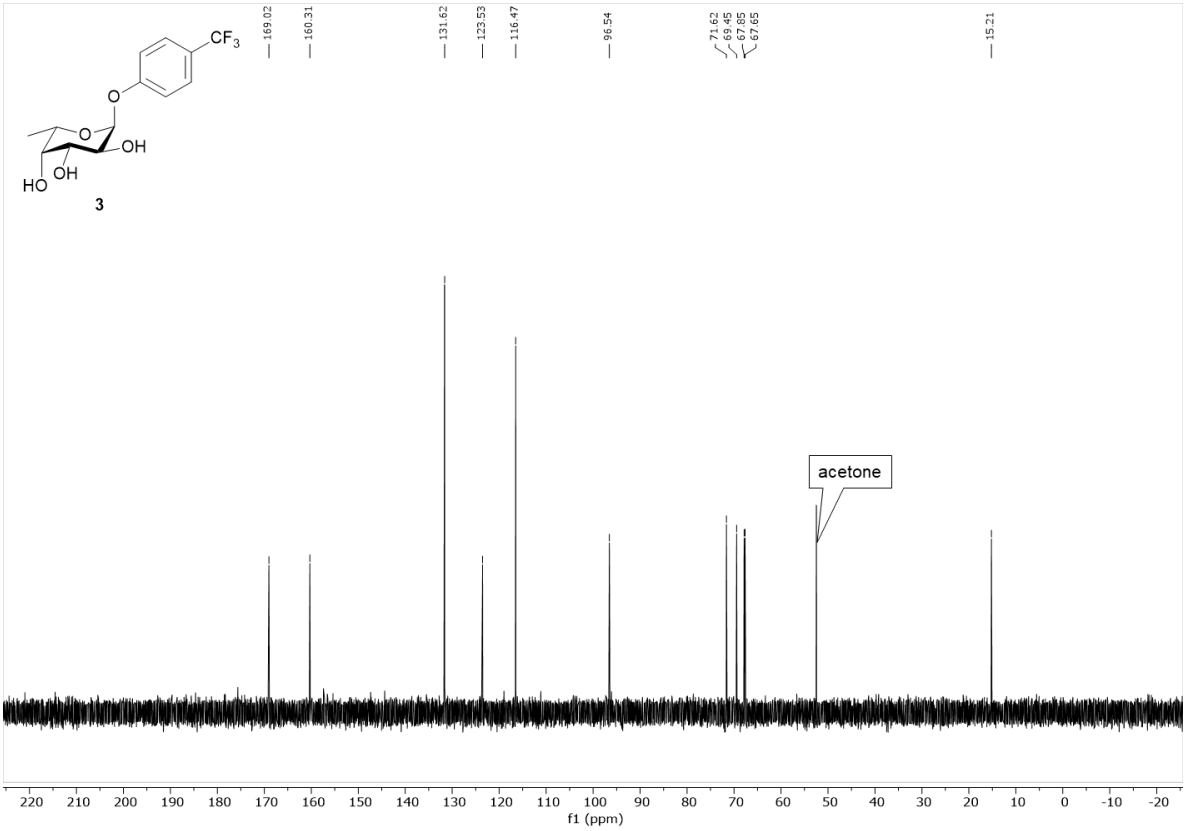
0.98
2.08
1.12
1.04
3.00
f1 (ppm)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HO
OH
3
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71.62
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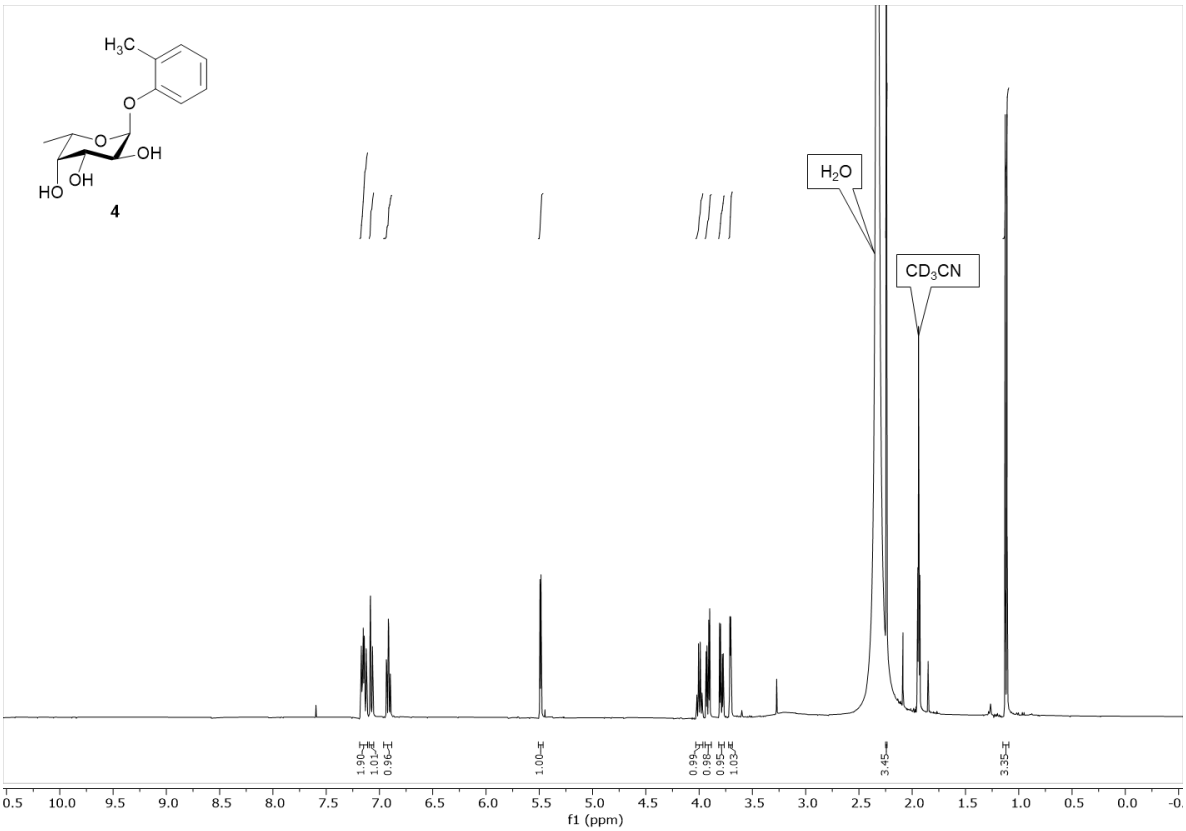
67.85
67.65
15.21
acetone
f1 (ppm)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OH
OH
HO
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H2O
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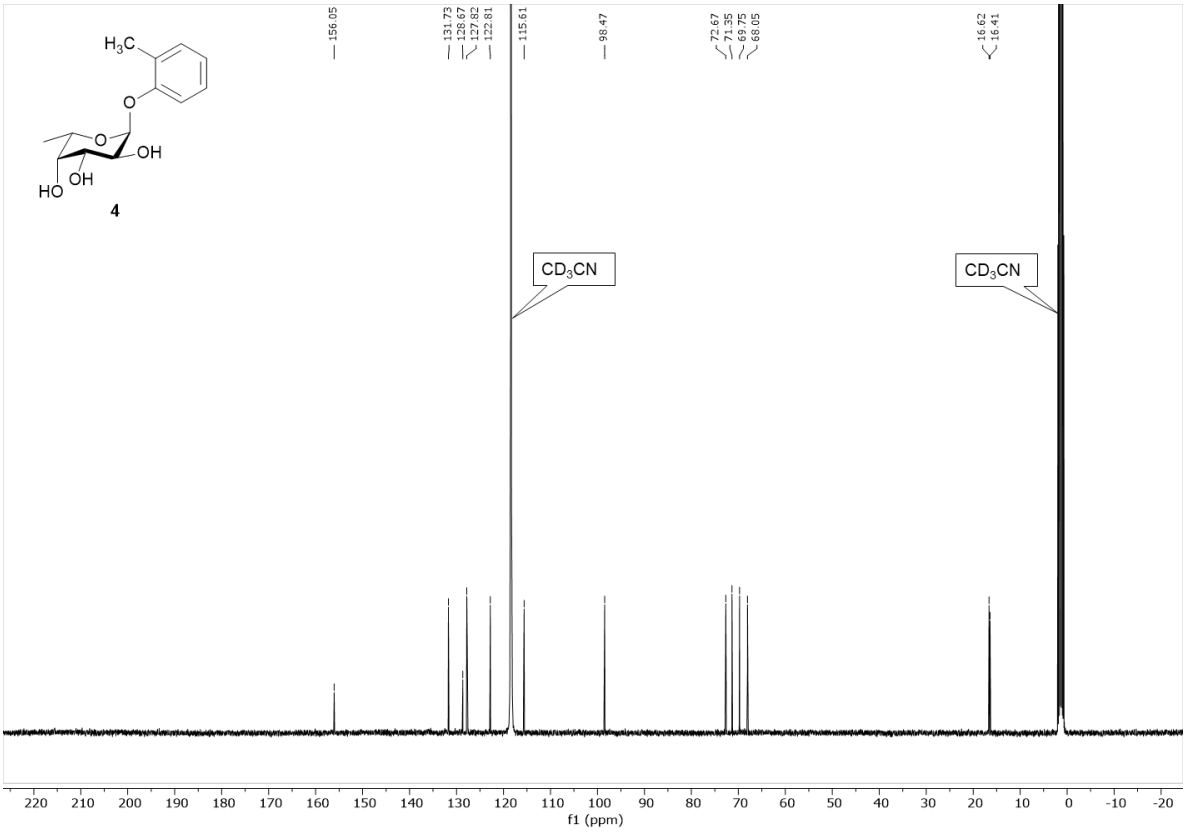
3.45
3.35
f1 (ppm)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OH
OH
HO
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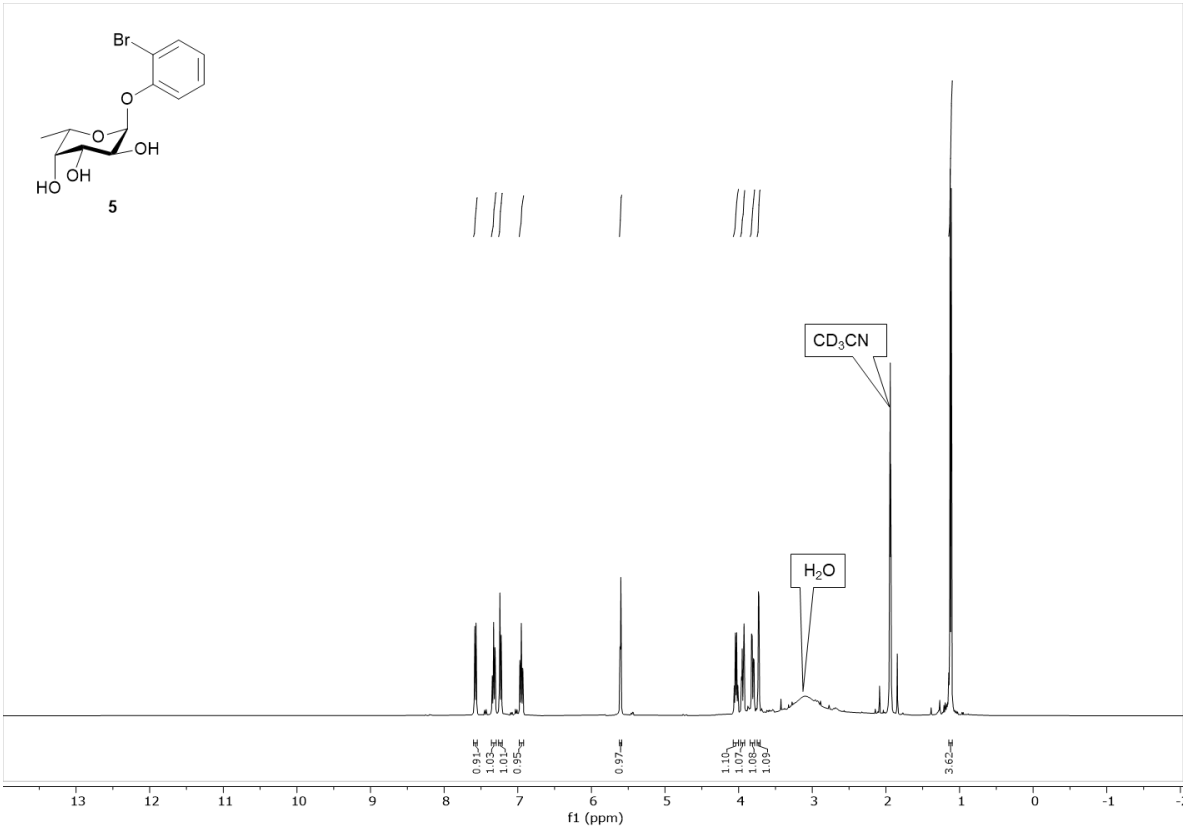
16.62
16.41
CD3CN
CD3CN
f1 (ppm)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OH
HO
5
CD3CN
H2O
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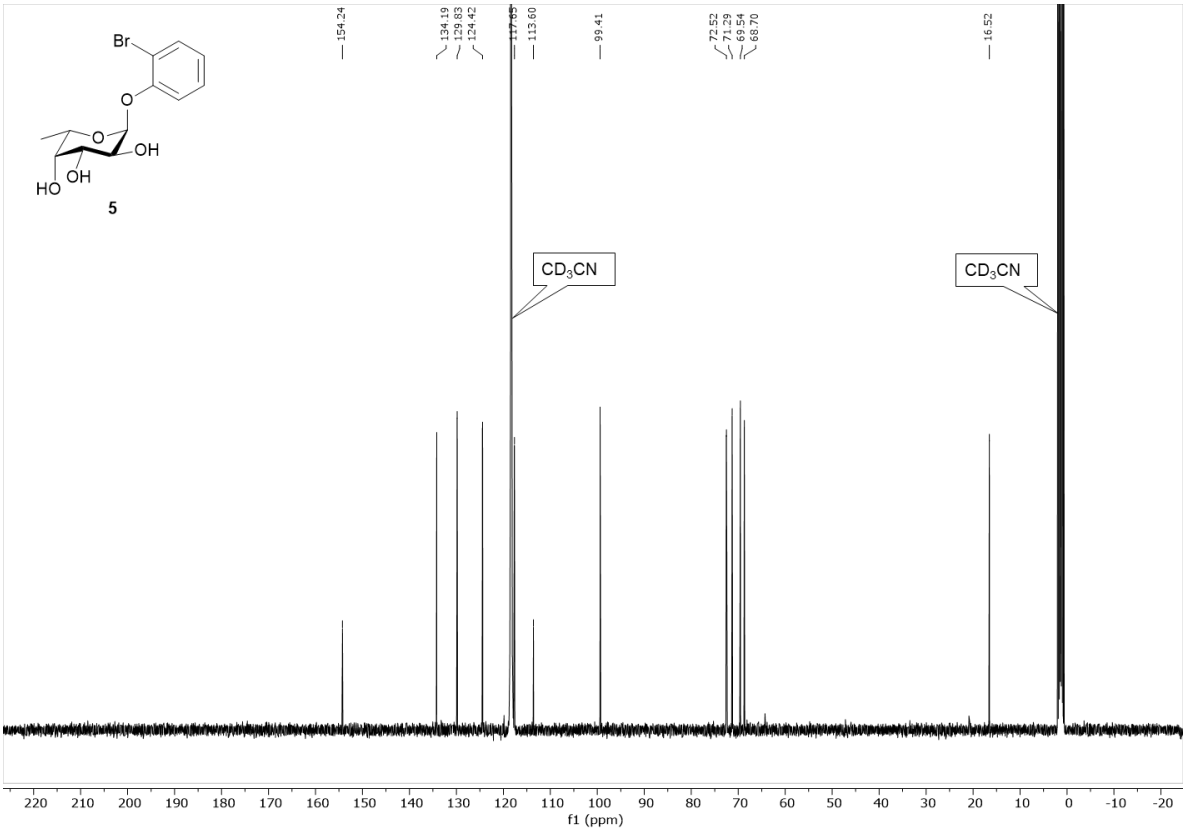
1.01
0.95
0.97
1.10
1.07
1.08
1.09
3.62
f1 (ppm)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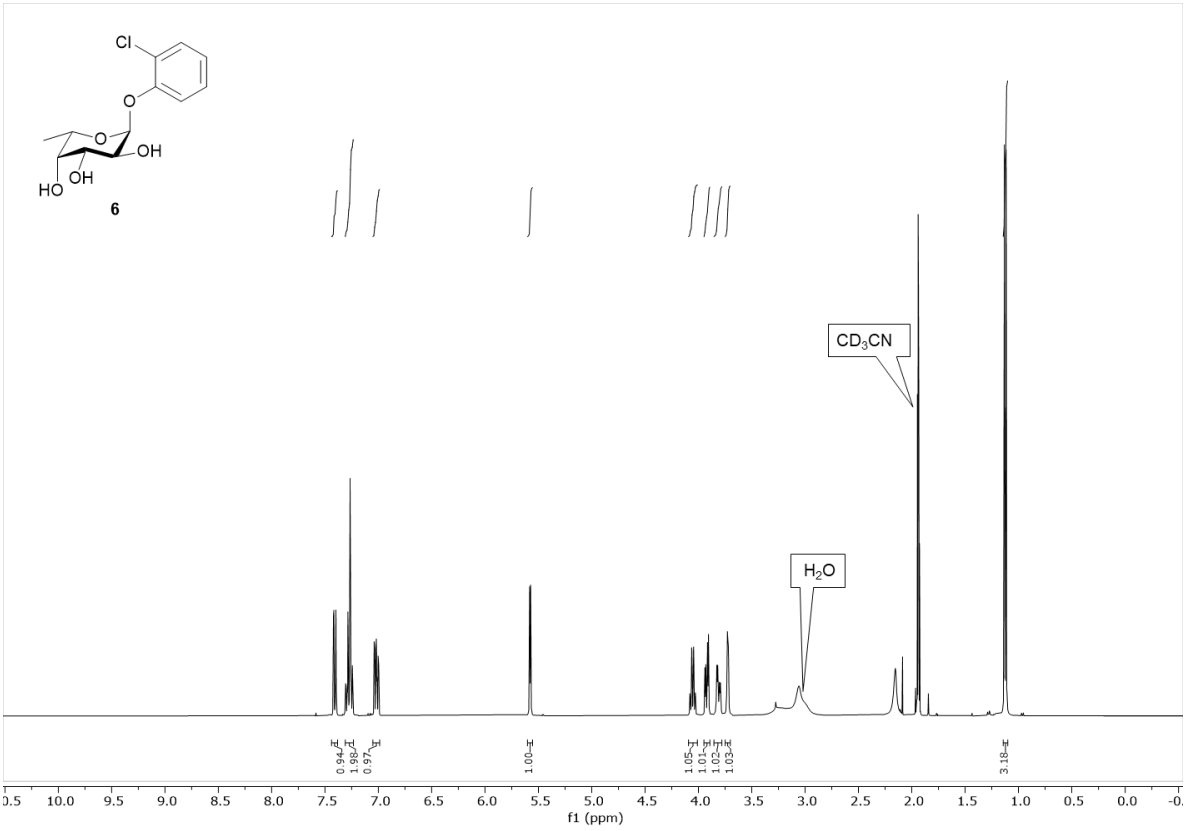
68.70
16.52
CD3CN
CD3CN
f1 (ppm)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HO
OH
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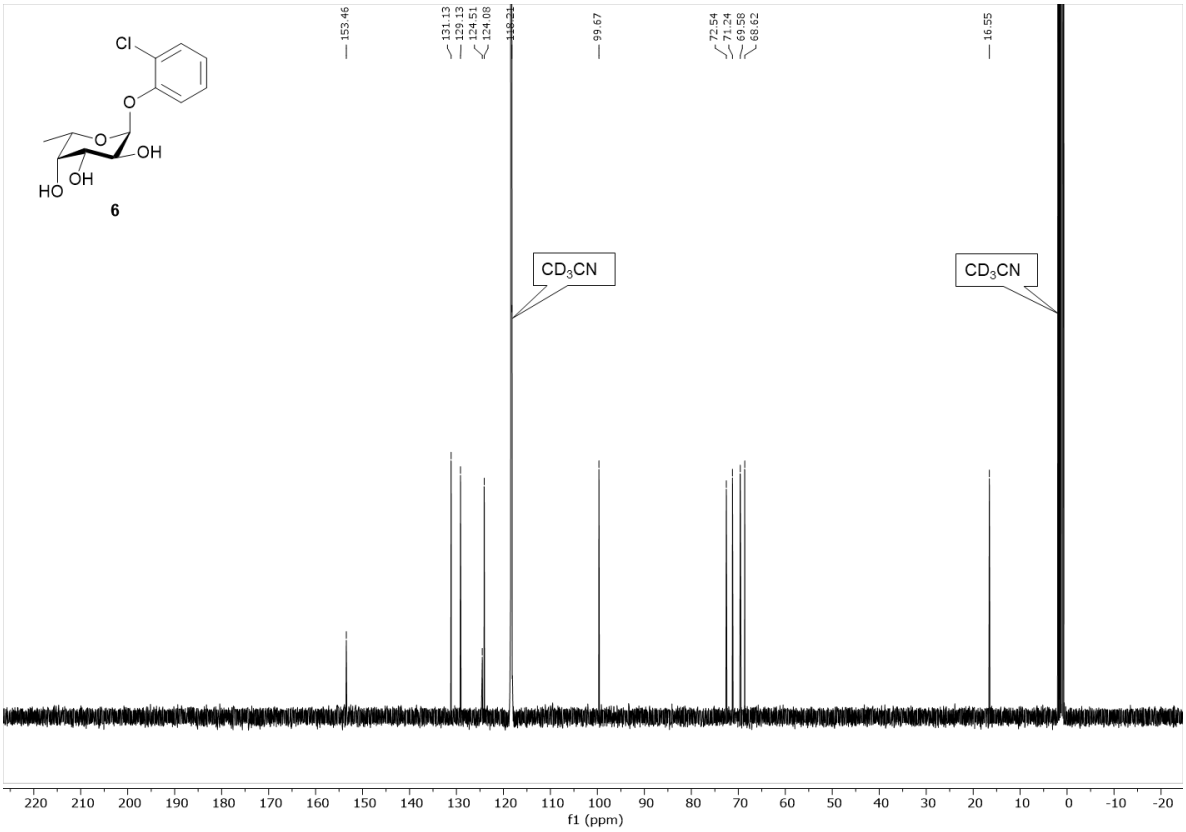
1.00
1.05
1.01
1.02
1.03
3.18
f1 (ppm)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HO
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129.13
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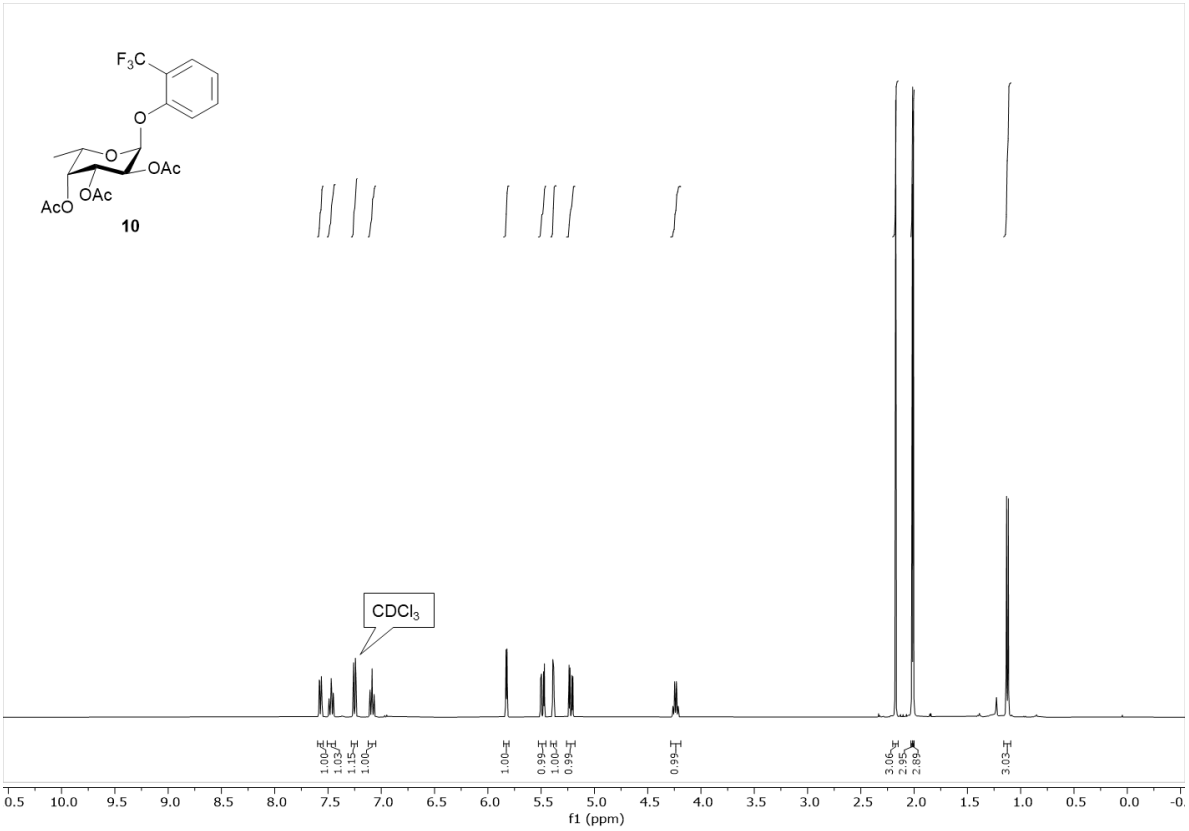
16.55
CD3CN
CD3CN
f1 (ppm)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1.03
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1.00
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0.99
0.99
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2.95
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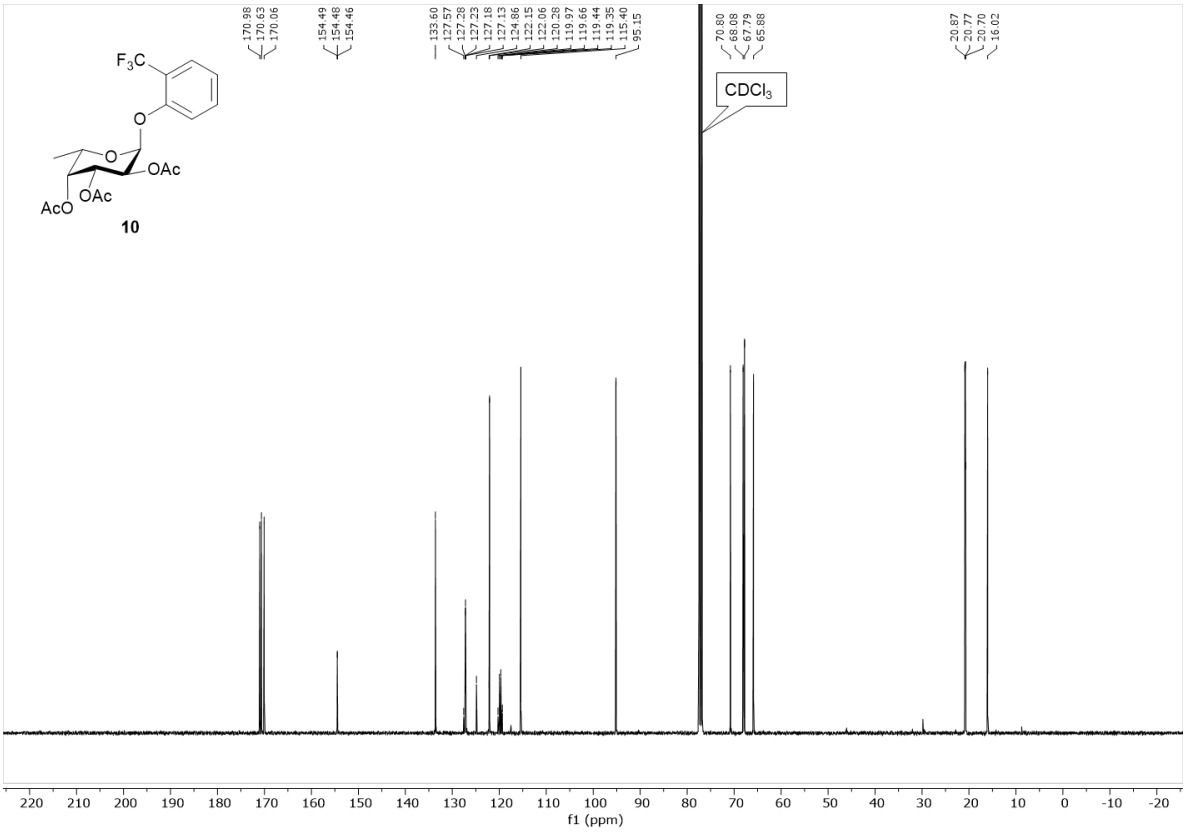
3.03
f1 (ppm)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154.48
154.46
133.60
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127.23
127.18
127.13
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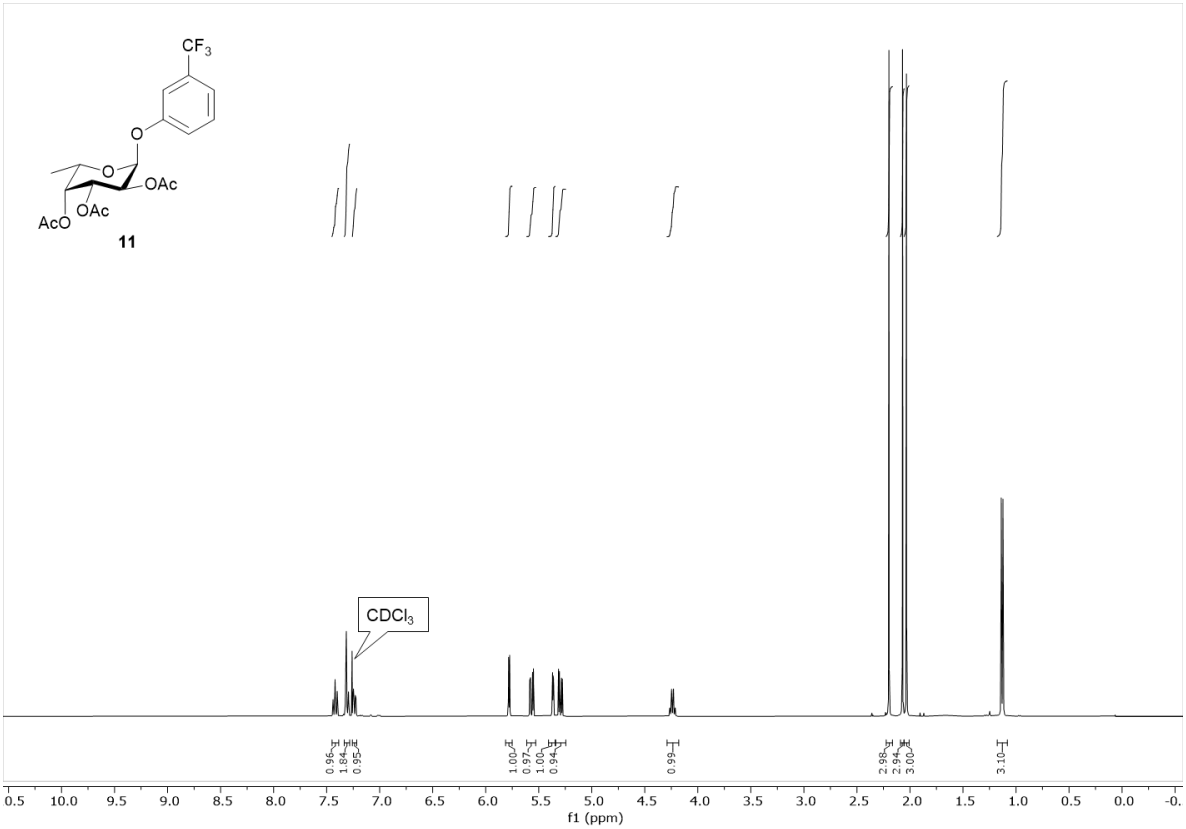
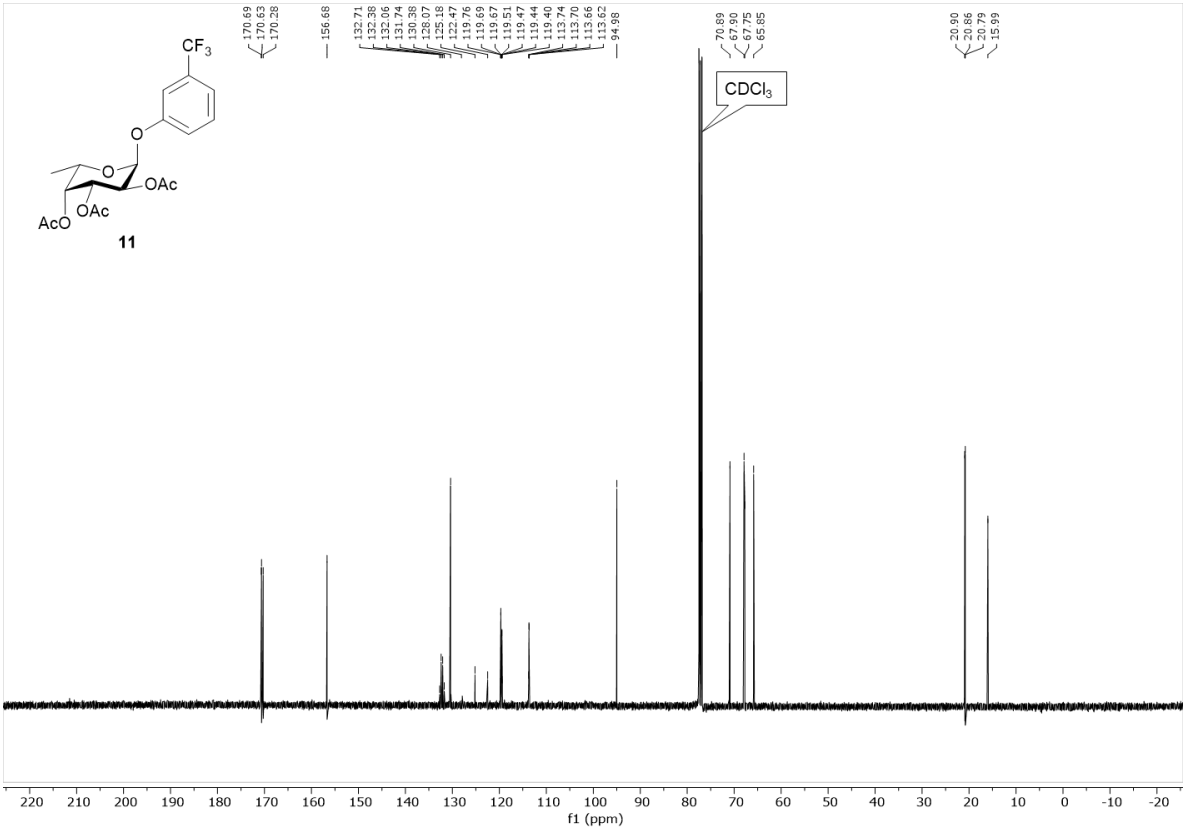
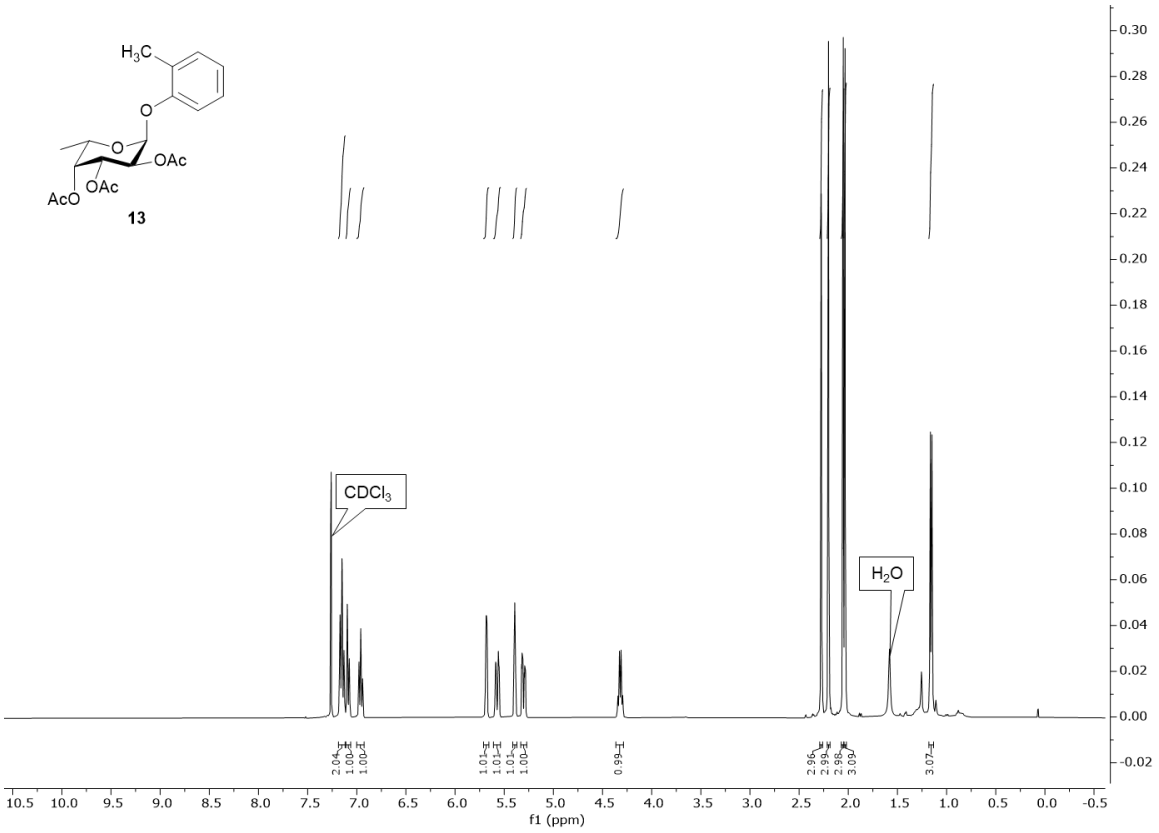
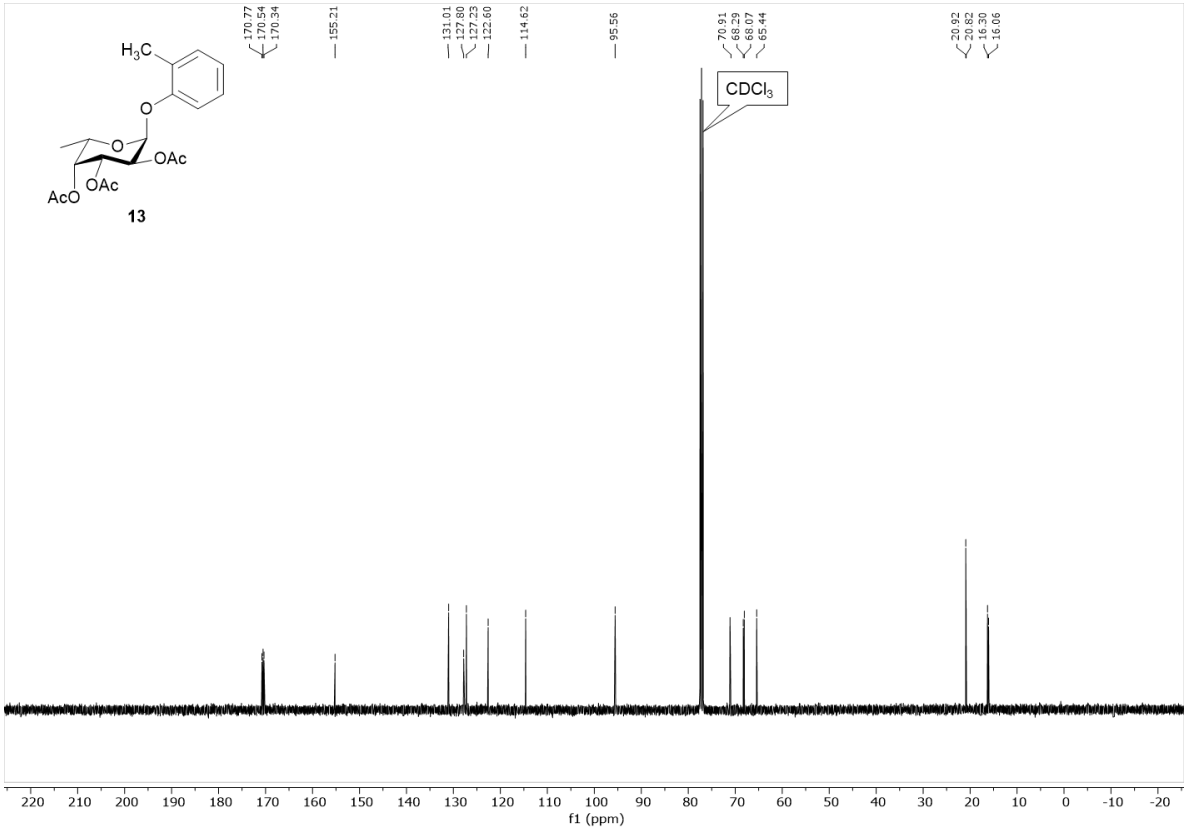
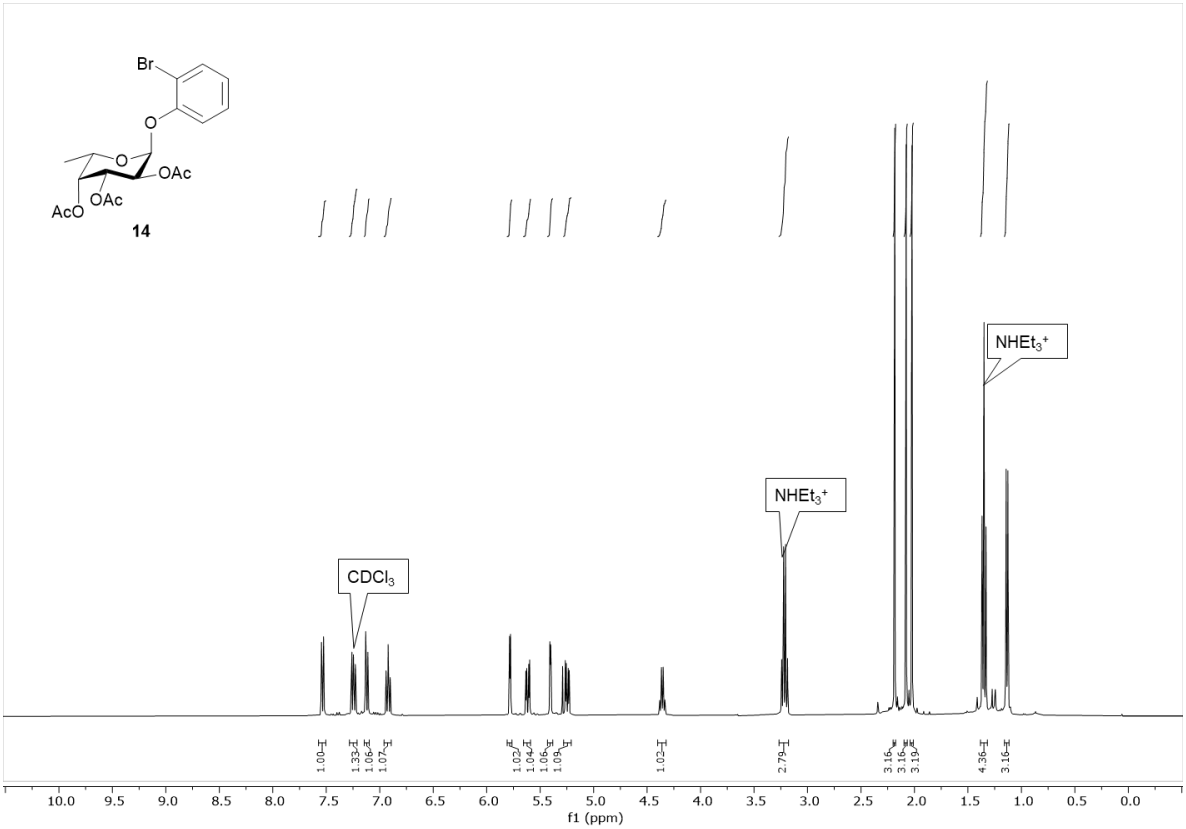
122.15
122.06
120.28
119.97
119.66
119.44
119.35
115.40
95.15
70.80
68.08
67.79
65.88
20.87
20.77
20.70
16.02
F3C
O
O
OAc
OAc
AcO
10
CDCl3
f1 (ppm)

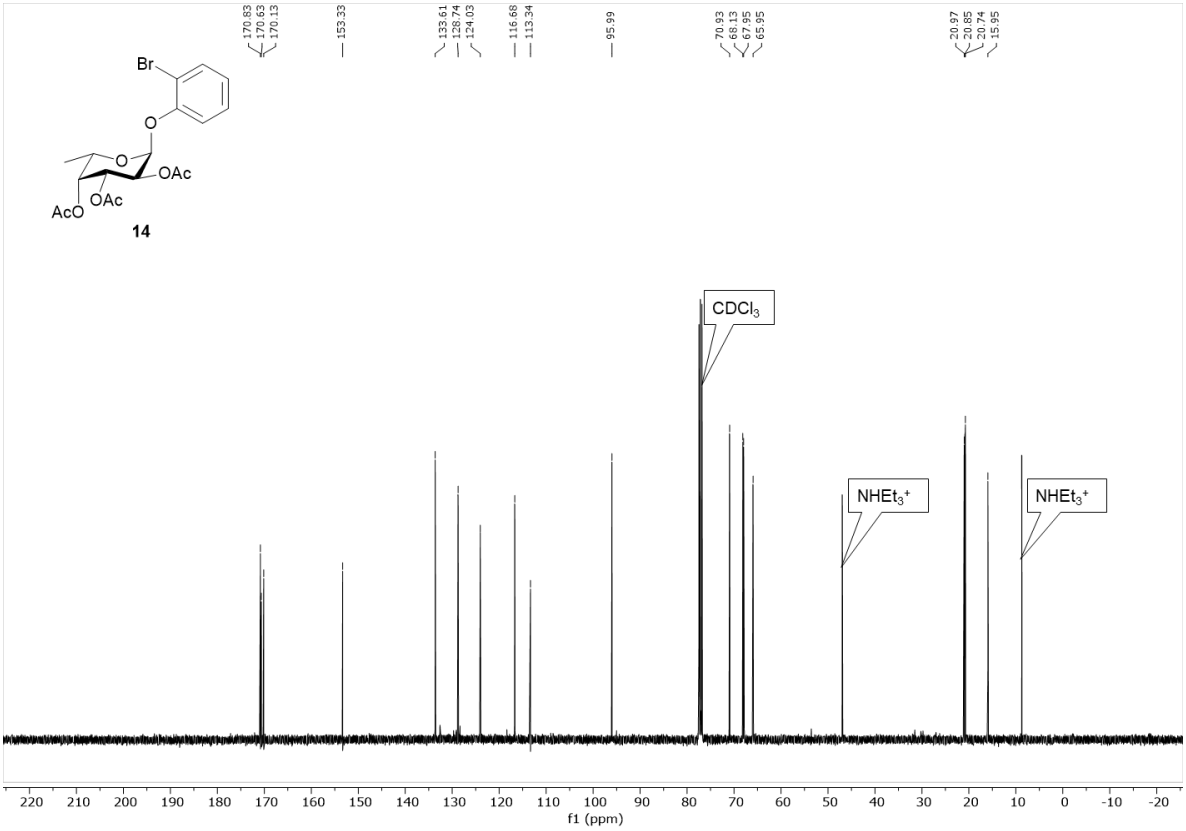

CF3
O
O
OAc
AcO
OAc
11
CDCl3
0.96
1.84
0.95
1.00
0.97
1.00
0.94
0.99
2.98
2.94
3.00
3.10
0.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.
f1 (ppm)

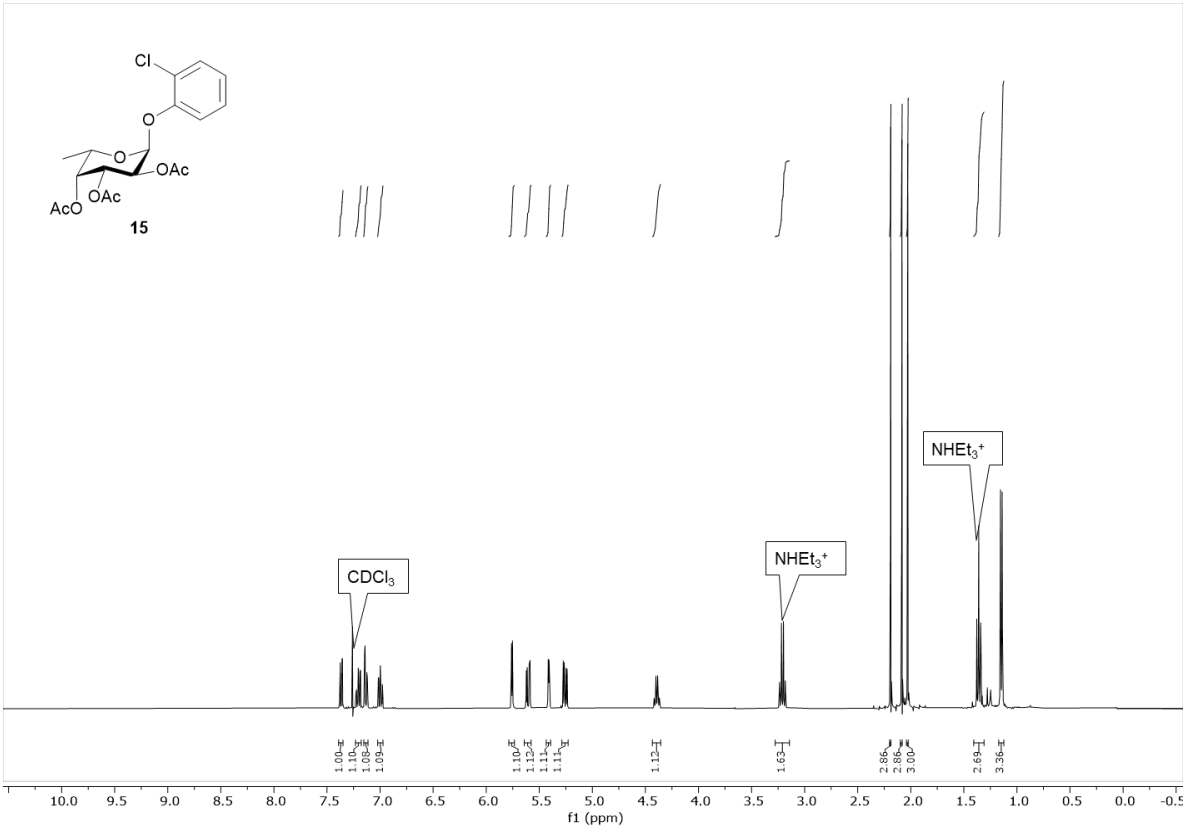

170.69
170.63
170.28
156.68
132.71
132.38
132.06
131.74
130.38
128.07
125.18
122.47
119.76
119.69
119.67
119.51
119.47
119.44
119.40
113.74
113.70
113.66
113.62
94.98
70.89
67.90
67.75
65.85
20.90
20.86
20.79
15.99
CF3
O
O
OAc
AcO
OAc
11
CDCl3
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

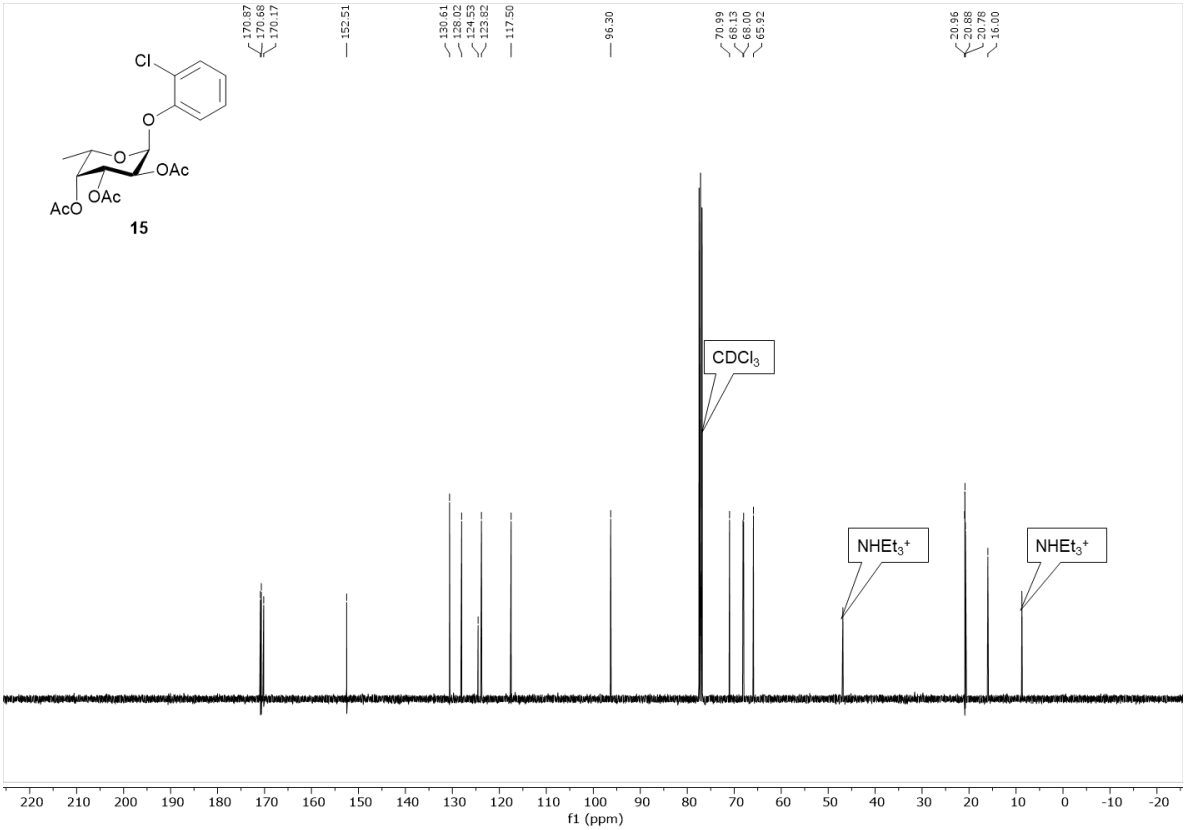

H3C
O
O
OAc
AcO
OAc
13
CDCl3
H2O
f1 (ppm)


H3C
O
O
OAc
AcO
OAc
13
170.77
170.54
170.34
155.21
131.01
127.80
127.23
122.60
114.62
95.56
70.91
68.29
68.07
65.44
20.92
20.82
16.30
16.06
CDCl3
f1 (ppm)


Br
O
O
OAc
AcO
OAc
14
NHEt3+
NHEt3+
CDCl3
1.00
1.33
1.06
1.07
1.02
1.04
1.06
1.09
1.02
2.79
3.16
3.16
3.19
4.36
3.16
f1 (ppm)


170.83
170.63
170.13
153.33
133.61
128.74
124.03
116.68
113.34
95.99
70.93
68.13
67.95
65.95
20.97
20.85
20.74
15.95
Br
O
O
OAc
AcO
OAc
14
CDCl3
NHEt3+
NHEt3+
f1 (ppm)


Cl
O
O
OAc
AcO
OAc
15
CDCl3
NHEt3+
NHEt3+
1.00
1.10
1.08
1.09
1.10
1.12
1.11
1.11
1.12
1.63
2.86
2.86
3.00
2.69
3.36
f1 (ppm)


170.87
170.68
170.17
152.51
130.61
128.02
124.53
123.82
117.50
96.30
70.99
68.13
68.00
65.92
20.96
20.88
20.78
16.00
Cl
O
O
OAc
AcO
OAc
15
CDCl3
NHEt3+
NHEt3+
f1 (ppm)

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