GLYCOMIMETIC LIGANDS BLOCK THE INTERACTION OF SARS-COV-2 SPIKE PROTEIN WITH C-TYPE LECTIN CO-RECEPTORS

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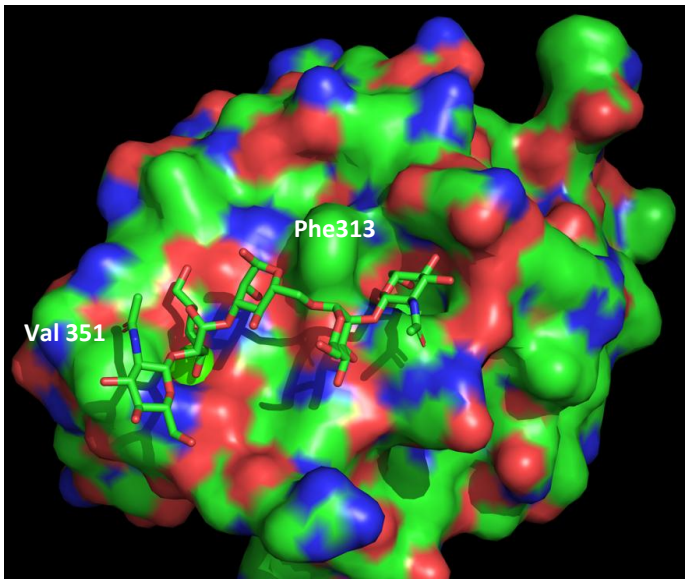
Figure SI-1. X-ray structures of A) DC-SIGN (pdb 1K9I) and B) L-SIGN (pdb 1K9J) in complex with $GlcNAc_2Man_3$. The solvent accessible surface area of the proteins is shown. The Ca^{2+} ions are shown in green. The carbohydrate-binding region is large, shallow and remarkably similar in shape in the two proteins. C) X-ray structure of DC-SIGN in complex with **7** (pdb 6GHV). The Ca^{2+} ion is shown in magenta

A



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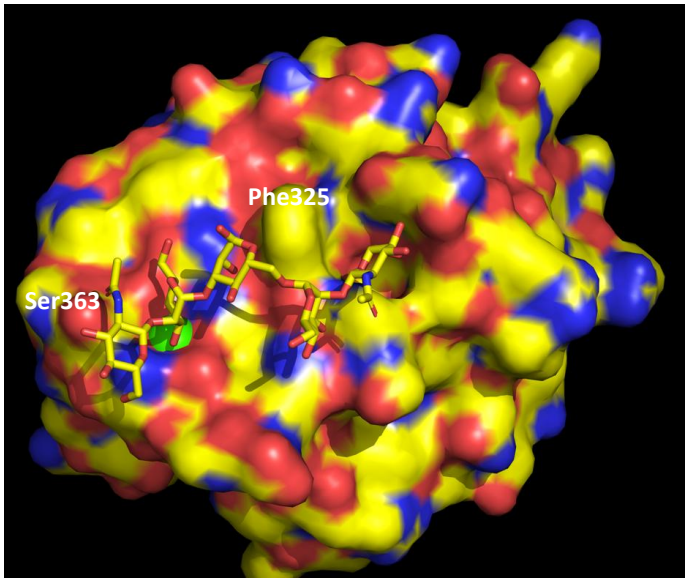

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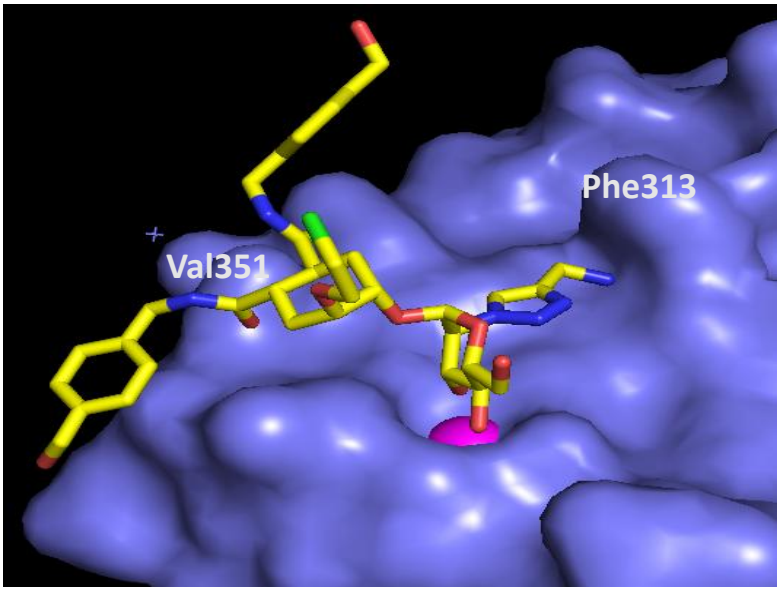
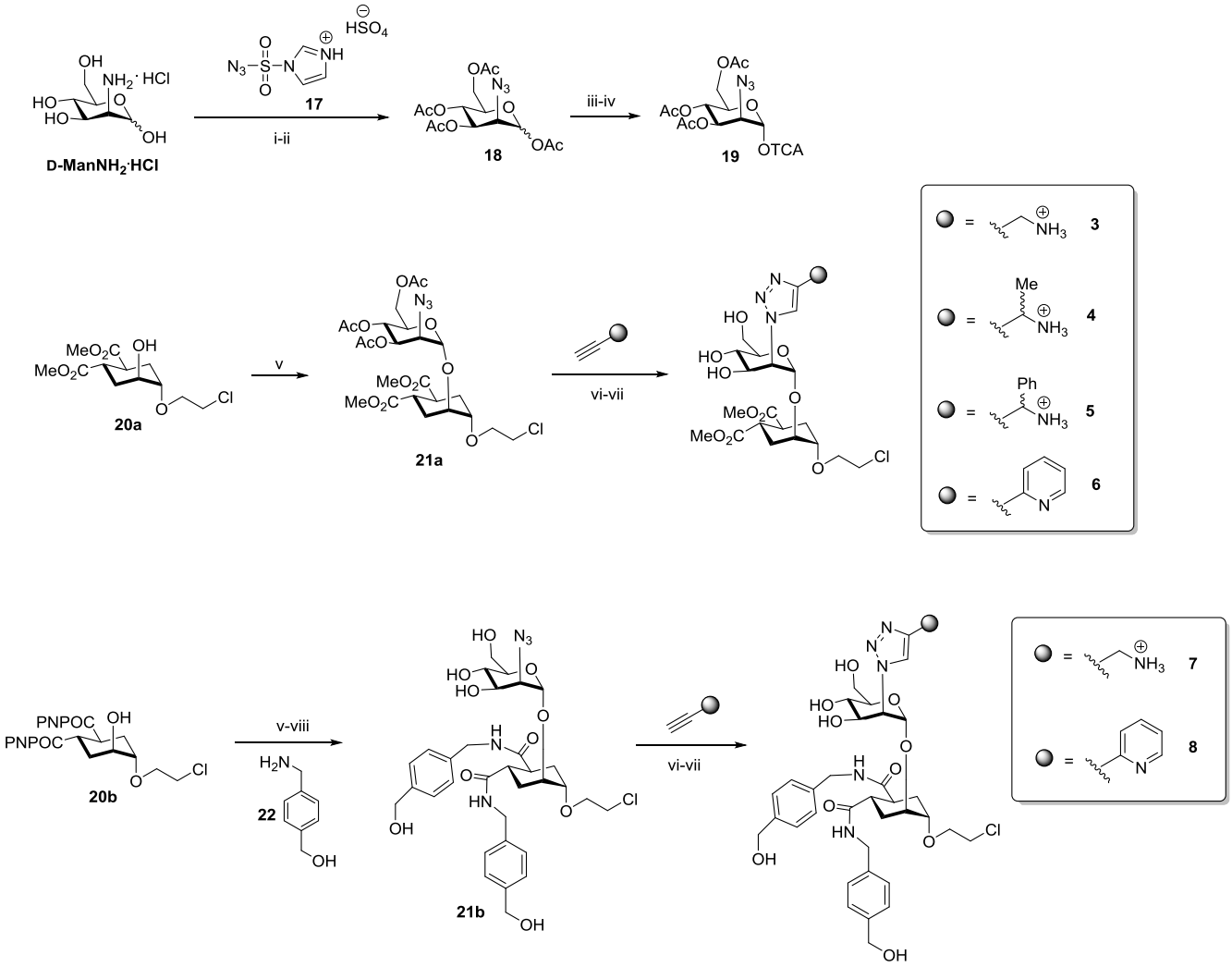


Synthesis :general procedures and materials

All commercial reagents (Abcr, Carbosynth and Merck) were used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed under a nitrogen atmosphere. Anhydrous solvents were purchased from Merck with water content ≤ 0.0005%. Et_3N, CH_2Cl_2, CH_3CN and MeOH were dried over calcium hydride, THF was dried over sodium/benzophenone and freshly distilled. All solvents were of reagent grade or HPLC grade. Reactions were monitored by analytical thin-layer chromatography (TLC) performed on Silica Gel 60 F254 plates (Merck) or Silica Gel 60 RP-18F254s plates (Merck) with UV detection (254 nm and 366 nm) and/or staining with ceric ammonium molybdate reagent, potassium permanganate or ninhydrin. Flash chromatography was performed according to Still's procedure[1] using Silica gel Macherey-Naegel 60 (40-63 μm, 230-400 mesh, Merck). Automated chromatography was performed on a Biotage Isolera Prime system with double UV detection; Biotage SNAP KP-Sil and SFAR cartridges were employed. NMR experiments were recorded on a Bruker Avance 400 MHz instruments at 298 K (unless otherwise stated). Chemical shifts (δ) are expressed in ppm and are referred to internal standards (TMS). The δ (ppm) axis has been calibrated on the solvent residual signal for which every spectrum was recorded. The signal shapes (^{1}H-NMR) are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), qui (quintet), sex (sextet), m (multiplet), dd (doublet of doublets), dt (doublet of triplets). COSY and HSQC experiments were used to assist the ^{1}H and ^{13}C resonance assignments. Mass spectra were recorded on a ThermoFisherLCQ apparatus (ESI ionization); high-resolution mass spectra (HRMS) were acquired on a Waters SYNAPT G2 Si ESI QTof instrument. Specific optical rotation values were measured using a Perkin-Elmer 241 polarimeter, at 589 nm in a 1 dm cell.

Synthesis of ps-diMan based compounds **3-8** - Scheme SI-1



Scheme SI-1 *Synthesis of the ps-diMan based ligands* ***3-8****. i)K_2CO_3, $CuSO_4.5H_2O$, MeOH, -20 °C to RT, over night; ii) Ac_2O, pyridine, 0 °C to RT, overnight,; iii) $MeNH_2$, THF, 0 °C to RT, 5h; iv) Cl_3CCN, DBU, CH_2Cl_2, 0°C to RT, 30 min; v)* ***19****, TMSOTf, CH_2Cl_2, -30 °C, 1h; vi) alkyne from Figure SI-2, $CuSO_4.5H_2O$, Na-ascorbate, TBTA, THF/H_2O; vii) MeONa, MeOH, RT; viii)* ***22****, THF/DMF, 2d.*

Imidazole sulfonyl azide (**17**) was synthesized according to Potter *et al.*[2] . Compounds **3-7** and **18**, **19**, **20a**, **21b** were synthesized according to Medve *et al.*[3]. The glycosyl acceptor **20b** was synthesized according to Varga *et al.*[4] The ps-diMan ligand **1** perform as a structural and functional mimic of the corresponding di-mannoside Manα1–2Man and bind in the carbohydrate recognition domain of DC-SIGN, as shown by X-ray crystallography.[5a] They include a 1,2-dicarbomethoxycyclohexane scaffold, conformationally locked to mimic a mannose residue, which maintains the shape of the structure, while increasing the lipophilicity and reducing the susceptibility to enzymatic hydrolysis.[5b]

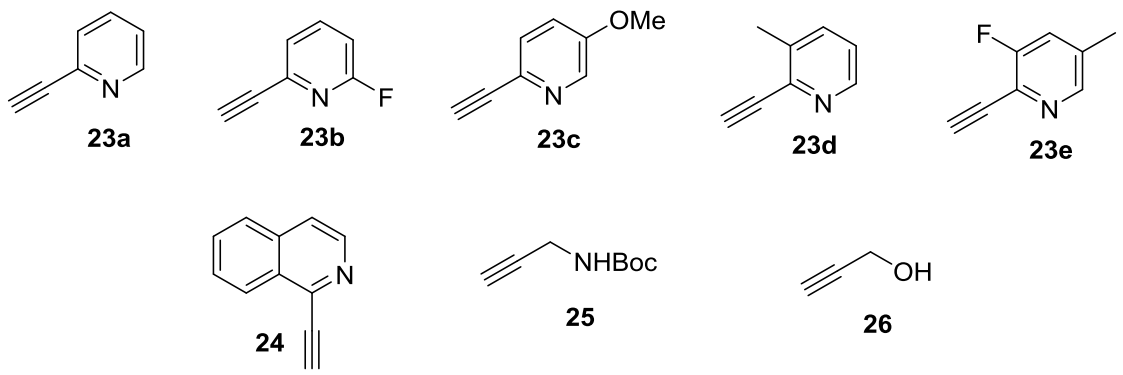
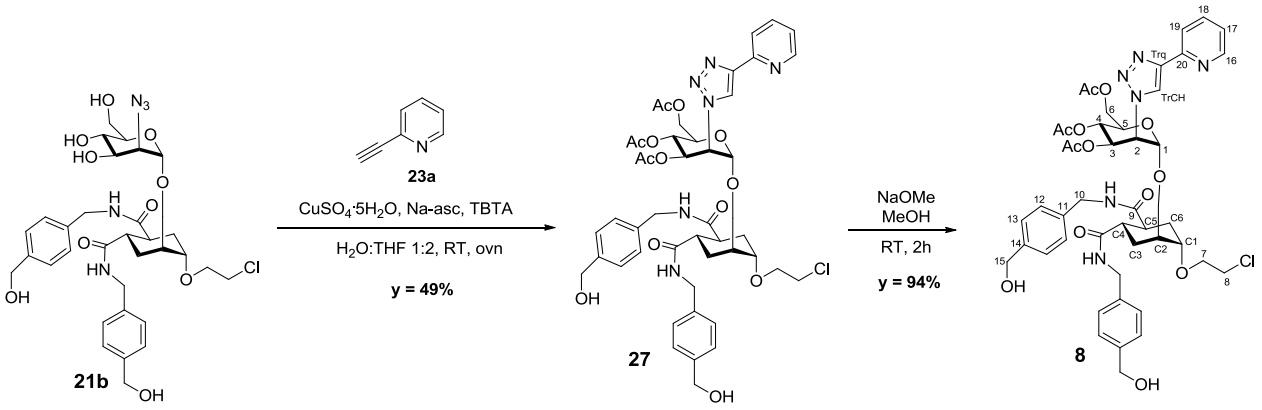


Figure SI-2 *Alkynes used in CuAAC reaction. Alkynes* ***23a, 23c, 23d, 23e, 25*** *and* ***26*** *were purchased from Merck. Alkynes* ***23b*** *and* ***24*** *were purchased from Abcr*

Synthesis of 8



1) *CuAAC reaction*:

A 0.04 M $CuSO_4 \cdot 5H_2O$ solution and a 0.16 M Na-ascorbate solution were prepared in deoxygenated water. A 1 M solution of 2-ethynylpyridine **23a**, a 0.038 M solution of Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (**TBTA**) and a 0.31 M solution of **21b** were prepared in deoxygenated THF.

To the alkyne solution (39 μL, 0.039 mmol, 1 mol eq) were added 0.2 mol eq of TBTA solution (205 μL, 0.0078 mmol), 0.1 mol eq of $CuSO_4 \cdot 5H_2O$ solution (97.5 μL, 0.0039 mmol) and 0.4 mol eq of Na-ascorbate solution (97.5 μL, 0.0156 mmol). The mixture was stirred at room temperature for 10 min, then 1 mol eq of the azide **21b** solution was added (125.8 μL, 0.039 mmol). The reaction was stirred at room temperature, under N_2 and protected from light overnight. Upon completion (TLC $CHCl_3$:MeOH 9:1, R_f(**27**): 0.36), the solvents were evaporated, and the crude product purified via flash chromatography ($CHCl_3$ with a MeOH gradient from 0% to 30%) to obtain the pure product as a white foam (17.5 mg, 49%).

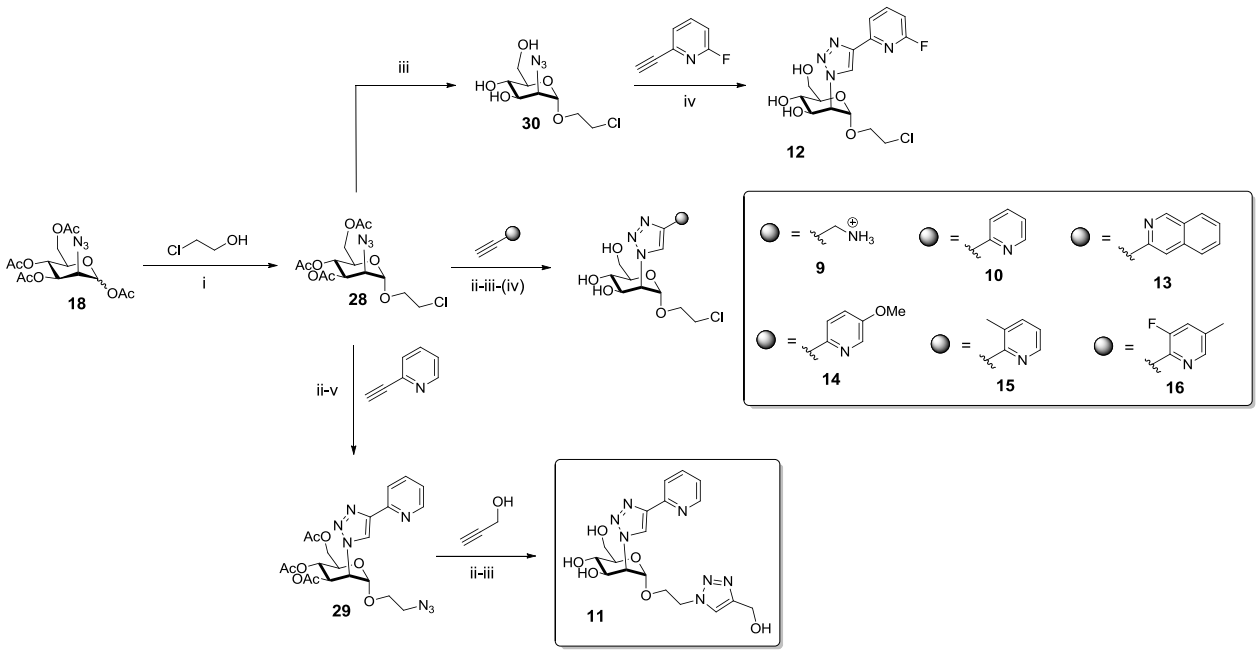
^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.79 (s, 1H, H_{TrCH}), 8.57 (d, 1H, $J_{16\text{-}17}$ = 4.2 Hz, H_{16}), 8.10 (d, 1H, $J_{19\text{-}18}$ = 7.9 Hz, H_{19}), 7.91 (dt, 1H, $J_{18\text{-}19}$ = 7.9 Hz, $J_{18\text{-}17}$ =1.5 Hz, H_{18}), 7.39-7.34 (m, 1H, H_{17}), 7.33-7.16 (m, 8H, H_{12} + H_{13}), 5.64-5.58 (m, 2H, H_3 + H_2), 5.55 (s, 1H, H_1), 5.33 (t, 1H, $J_{4\text{-}3}$ = 9.4 Hz, H_4), 4.55 (s, 2H, H_{15a}), 4.53 (s, 2H, H_{15b}) 4.41 (dd, 1H, $J_{6a\text{-}6b}$ = 11.4 Hz, $J_{6a\text{-}5}$ = 4.4 Hz, H_{6a}), 4.34-4.21 (m, 6H, H_9 + H_5 + H_{6b}), 4.15 (d, 1H, $J_{C2\text{-}C1}$ = 2.9 Hz, H_{C2}), 3.90 (m, 1H, H_{7a}), 3.84-3.74 (m, 2H, H_{7b} + H_{C1}), 3.72-3.66 (m, 2H, H_8), 3.06-2.88 (m, 2H, H_{C4} + H_{C5}), 2.18 (s, 3H, OAc), 2.11-1.97 (m, 7H, OAc + H_{C3} + H_{C6}), 1.93 (s, 3H, OAc)
MS (ESI): m/z calculated for $[C_{45}H_{53}ClN_6NaO_{13}]^+$: 943.33 $[M + Na]^+$, found: 943.40

2) <u>*Zemplèn deacetylation*</u>:

To a solution of **27** (17.5 mg, 0.019 mmol) in dry MeOH (920 μL) a freshly prepared 1M NaOMe solution in dry MeOH was added (1.5 mol eq NaOMe, 29 μL) to a 0.02M final concentration of the substrate (0.03M final concentration of NaOMe). The reaction was stirred at room temperature, under N_2 for 2h. Upon completion (TLC $CHCl_3$:MeOH 8:2, R_f(**8**): 0.53), the reaction was neutralized with Amberlite® IR120 hydrogen form ion-exchange resin, filtered and concentrated to obtain the pure product as a white foam (14.2 mg, 94%).

^{1}H-NMR (400 MHz, CD_3OD): 8.70 (s, 1H, H_{TrCH}), 8.56 (br s, 1H, H_{16}), 8.06 (d, 1H, $J_{19\text{-}18}$ = 7.6 Hz, H_{19}), 7.91 (t, 1H, $J_{18\text{-}19}$ = 7.6 Hz, H_{18}), 7.42-7.33 (m, 1H, H_{17}), 7.32-7.16 (m, 8H, H_{12} + H_{13}), 5.43 (s, 1H, H_1), 5.28 (d, 1H, $J_{2\text{-}3}$ = 4.9 Hz, H_2), 4.58 (s, 2H, H_{15a}), 4.52 (s, 2H, H_{15b}), 4.33 (s, 2H, H_{10a}), 4.30-4.23 (m, 3H, H_{10b} + H_3), 4.20-4.14 (m, 2H, H_{C2}), 4.01-3.84 (m, 3H, H_6 + H_{7a}), 3.83-3.76 (m, 3H, H_{7b} + H_5 + H_{C1}), 3.75-3.64 (m, 3H, H_8 + H_4), 3.06-2.92 (m, 2H, H_{C4} + H_{C5}), 2.11-1.92 (m, 4H, H_{C3} + H_{C6}). ^{13}C-NMR (400 MHz, CD_3OD): 175.7 (C_{9a}), 175.4 (C_{9b}), 148.9 (C_{16}), 140.1 (C_{Trq} + C_{20}), 137.7 (C_{11a}), 137.5 (C_{18}), 137.4 (C_{11b}), 128.6 (C_{17}), 127.6 (C_{19}), 127.0 (C_{12a}), 126.9 (C_{12b}), 126.8 (C_{13a}), 126.7 (C_{13b}), 123.2 (C_{TrCH}), 96.0 (C_1), 74.9 (C_{C1}), 74.5 (C_5), 70.6 (C_{C2}), 69.3 (C_7), 68.9 (C_3), 66.7 (C_4), 64.3 (C_2), 63.6 (C_{15a}), 63.5 (C_{15b}), 61.1 (C_6), 42.9 (C_{10}), 42.3 (C_8), 40.2 (C_{C4} + C_{C5}), 28.6 (C_{C6}), 27.0 (C_{C3}).
MS (HRMS): m/z calculated for $[C_{39}H_{47}ClN_6NaO_{10}]^+$: 817.2940 $[M + Na]^+$, found: 817.2940
$[\alpha]_D^{27} = +11^\circ$ $(c = 0.5, \mathrm{MeOH})$

Synthesis of monomannosides **9-16** – Scheme SI-2



Scheme SI-2 *Synthesis of the monomannoside ligands* ***9-16****. i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to RT, overnight, ii) $CuSO_4{\cdot}5H_2O$, Na-ascorbate, THF/H_2O; iii) MeONa, MeOH, RT; iv) TFA, CH_2Cl_2, RT; v) NaN_3, DMF, 55 °C, 3d*

General procedure for CuAAC reaction

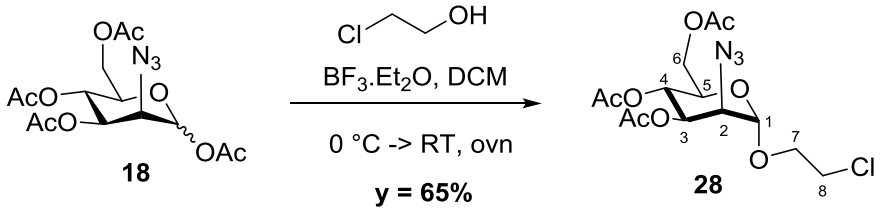
A 0.04 M $CuSO_4{\cdot}5H_2O$ and a 0.16 M Na-ascorbate solutions were prepared in deoxygenated water. A 0.4 M alkyne and a 0.4 M azide solutions were prepared in deoxygenated THF. To the alkyne solution (1 mol eq) were added: 0.1 mol eq of $CuSO_4{\cdot}5H_2O$ solution, 0.4 mol eq of Na-ascorbate solution and 1 mol eq of the azide solution. The reaction was stirred at room temperature, under N_2 and protected from light. Upon completion (TLC analysis), the solvents were evaporated and the crude product purified via flash chromatography (Hex:AcOEt or CH_2Cl_2:MeOH or $CHCl_3$:MeOH).

General procedure for Deacetylation

A) Zemplèn conditions: 1 mol eq of acetylated compound was dissolved in dry MeOH to 0.1 M concentration and a freshly prepared 0.1 M NaOMe solution in dry MeOH was added to 0.05 M final concentration of the substrate. The reaction was stirred at room temperature, under N_2. Upon completion (TLC analysis), the reaction was neutralized with Amberlite® IR120 ion-exchange resin (hydrogen form), filtered and concentrated *in vacuo* to yield the pure product (unless otherwise stated).

B) 1 mol eq of acetylated compound was dissolved in 10 mol eq of a 1 M $MeNH_2$ solution in EtOH. The reaction was stirred at room temperature, under N_2. Upon completion (TLC analysis), the solvent was removed *in vacuo* and the residue was co-evaporated with MeOH several times. Freeze-drying cycles were employed to completely remove the AcNHMe by-product.

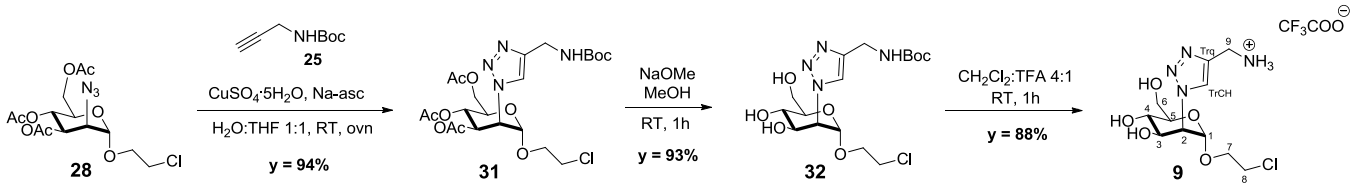
Synthesis of 28



Compound **18** (5.6 g, 15.0 mmol, 1 eq.) was dissolved in dry CH_2Cl_2 (17 mL, [**18**] = 0.57M) and 2-chloroethanol (2 mL, 30.0 mmol, 2 eq.) was added. The mixture was cooled at 0 °C, then $BF_3{\cdot}Et_2O$ (7.4 mL, 60 mmol, 4 eq.) was slowly added. The reaction was stirred at room temperature, under N_2 overnight. Upon completion (TLC monitoring, toluene:AcOEt 7:3, R_f(**28**)=0.51, staining by ceric ammonium molybdate reagent) the mixture was extracted with cold H_2O (x2), $NaHCO_3$ sat. sol. (x1) and cold H_2O (x1). The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Crude product was purified via automated flash chromatography (toluene with AcOEt gradient from 0% to 30%) to obtain the pure product as a white foam (3.82 g, 65%).

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 5.39 (dd, 1H, $J_{3\text{-}4}$= 9.7 Hz, $J_{2\text{-}3}$= 3.8 Hz, H_3), 5.32 (t, 1H, $J_{4\text{-}3}$= $J_{4\text{-}5}$= 9.7 Hz, H_4), 4.89 (d, 1H, $J_{1\text{-}2}$= 1.3 Hz, H_1), 4.23 (dd, 1H, $J_{6a\text{-}6b}$ = 12.3 Hz, $J_{6a\text{-}5}$ = 4.9 Hz, H_{6a}), 4.12-4.04 (m, 2H, H_5 + H_{6b}), 3.96-3.89 (m, 1H, H_{7a}), 3.84-3.76 (m, 1H, H_{7b}), 3.67 (t, 2H, $J_{8\text{-}7}$ = 5.5 Hz, H_8), 2.10 (s, 6H, 2x OAc), 2.05 (s, 3H, OAc). ^{13}C-NMR (400MHz, $CDCl_3$): δ(ppm)= 170.8 ($CH_3\underline{CO}$), 170.0 ($CH_3\underline{CO}$), 169.7 ($CH_3\underline{CO}$), 98.7 (C_1), 71.0 (C_3), 68.9 (C_5), 65.6 (C_7), 65.9 (C_4), 62.2 (C_6), 61.5 (C_2), 42.6 (C_8), 20.8 ($\underline{CH_3}CO$), 20.7 ($\underline{CH_3}CO$), 20.6 ($\underline{CH_3}CO$)
MS (ESI): m/z calculated for $[C_{14}H_{20}ClN_3NaO_8]^+$: 416.08 $[M + Na]^+$, found: 416.26
$[\alpha]_D^{15} = +68°$ $(c = 2.0, CHCl_3)$

Synthesis of 9



1) *CuAAC reaction*:

Synthesized from **28** and N-Boc-propargylamine **25** according to general procedure for CuAAC reaction. Purified via automated flash chromatography (Hexane with AcoEt gradient from 50% to 100%) to obtain pure product as a white foam (Yield: 94%).

R_f(**31**): 0.21 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.10 (s, 1H, H_{TrCH}), 5.51-5.45 (m, 1H, H_3), 5.43-5.39 (m, 1H, H_2), 5.35-5.25 (m, 2H, H_4 + H_1), 4.40-4.30 (m, 4H, H_5 +H_{6a} + H_9), 4.22 (d, 1H, J_{6b-6a} = 10.6 Hz, H_{6b}), 4.06- 3.98 (m, 1H, H_{7a}), 3.94-3.87 (m, 1H, H_{7b}), 3.80 (t, 2H, J_{8-7} = 5.7 Hz, H_8), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.89 (s, 3H, OAc), 1.44 (s, 9H, 3xCH_3).
MS (ESI): m/z calculated for $[C_{22}H_{33}ClN_4NaO_{10}]^+$: 571.18 $[M + Na]^+$, found: 571.08

2) *Deacetylation:*

Performed on intermediate **31** according to general procedure A for deacetylation. (Yield: 93%)

R_f(**32**): 0.35 in $CHCl_3$:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.10 (s, 1H, H_{TrCH}), 5.13 (s, 1H, H_1), 5.08 (d, 1H, J_{2-3} = 5.3 Hz, H_2), 4.31 (s, 2H, H_9), 4.22-4.16 (m, 1H, H_3), 4.05-3.97 (s, 1H, H_{7a}), 3.92-3.78 (m, 4H, H_{7b} + H_6 + H_5), 3.77-3.68 (m, 3H, H_8 + H_4), 1.44 (s, 9H, 3xCH_3).
MS (ESI): m/z calculated for $[C_{16}H_{27}ClN_4NaO_7]^+$: 445.15 $[M + Na]^+$, found: 445.07

3) *Boc removal:*

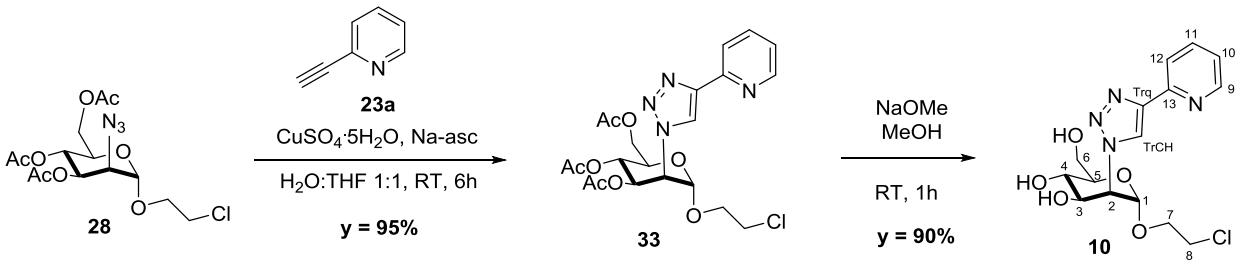
Compound **32** (53 mg, 0.13 mmol, 1 eq.) was dissolved in a 4:1 mixture of dry CH_2Cl_2 and TFA (2.5 mL, [**32**]= 0.05M). The reaction was stirred at room temperature, under N_2 for 1h. Upon completion (TLC monitoring, CH_2Cl_2:MeOH 8:2, R_f(**9**)=0.26, staining ceric ammonium molybdateic reagent and/or Ninhydrin) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times to obtain the pure product as a white foam (48.3 mg, 88%).

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.31 (s, 1H, H_{TrCH}), 5.17-5.11 (m, 2H, H_2 + H_1), 4.29- 4.19 (m, 3H, H_9 + H_3), 4.06-3.98 (m, 1H, H_{7a}), 3.90 (d, 2H, $J_{6\text{-}5}$ = 3.1 Hz, H_6), 3.86-3.79 (m, 2H, H_{7b} + H_4), 3.78-3.69 (m, 3H, H_8 + H_5). ^{13}C-NMR (400MHz, DMSO-d^6): δ(ppm)= 158.0 ($CF_3\underline{C}OO^-$)139.8 (C_{trq}), 124.3 (C_{TrCH}), 97.45 (C_1), 74.2 (C_5), 68.2 (C_3), 67.2 (C_7), 66.2 (C_4), 63.7 (C_2), 60.5 (C_6), 43.5 (C_8), 33.9 (C_9)

MS (HRMS): m/z calculated for $[C_{11}H_{19}ClN_4NaO_5]^+$: 345.0942 $[M+ Na]^+$, found: 345.0934

$[\alpha]_D^{15} = +13°$ $(c = 0.92, \text{MeOH})$

Synthesis of 10



1) *CuAAC reaction*:

Synthesized from **28** and 2-ethynylpyridine **23a** according to general procedure for CuAAC reaction. Purified via flash chromatography (Hex with AcoEt gradient from 50% to 100%) to obtain pure product as a white foam (Yield: 95%).

R_f(**33**): 0.31 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 8.76 (s, 1H, H_{TrCH}), 8.53 (d, 1H, $J_{9\text{-}10}$ = 4.7 Hz, H_9), 8.18 (d, 1H, $J_{12\text{-}11}$ = 8.1 Hz, H_{12}), 7.79 (dt, 1H, $J_{11\text{-}12}$ = 8.1 Hz, $J_{11\text{-}10}$ = 1.5 Hz, H_{11}), 7.25-7.22 (m, 1H, H_{10}), 5.61-5.52 (m, 2H, H_3 + H_2), 5.41 (t, 1H, $J_{4\text{-}3}$ = 10.0 Hz, H_4), 5.19 (s, 1H, H_1), 4.32-4.25 (m, 3H, H_6 + H_5), 4.06-3.99 (m, 1H, H_{7a}), 3.94-3.86 (m, 1H, H_{7b}), 3.77 (t, 2H, $J_{8\text{-}7}$ = 5.6 Hz , H_8), 2.31 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.97 (s, 3H, OAc).

MS (ESI): m/z calculated for $[C_{21}H_{25}ClN_4NaO_8]^+$: 519.13 $[M + Na]^+$, found: 519.38

2) *Deacetylation:*

Performed on intermediate **33** according to general procedure A for Deacetylation. (Yield: 90%)

R_f(**10**): 0.41 in $CHCl_3$:MeOH 9:1

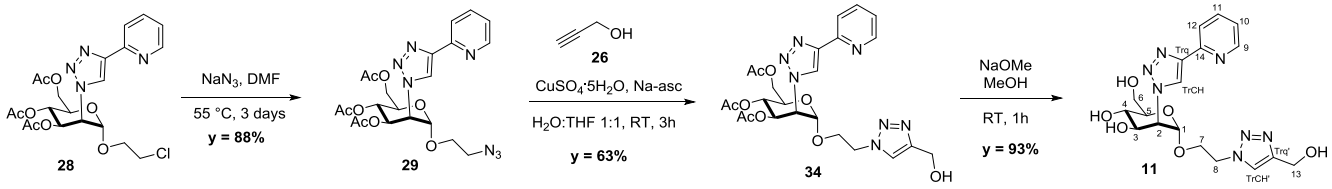
^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.73 (s, 1H, H_{TrCH}), 8.57 (brs, 1H, H_9), 8.10 (brs, 1H, H_{12}), 7.92 (m, 1H, H_{11}), 7.41-7.35 (brs, 1H, H_{10}), 5.22 (s, 1H, H_1), 5.18 (d, 1H, $J_{2\text{-}3}$ = 5.3 Hz, H_2), 4.28-4.22 (m, 1H, H_3), 4.08-4.02 (m, 1H, H_4), 3.97-3.90 (m, 2H, H_8), 3.90-3.75 (m, 5H, H_5 + H_6 + H_7). ^{1}H-NMR (400MHz, DMSO-d^6, 370K): δ(ppm)= 8.61 (d, 1H, $J_{9\text{-}10}$ = 4.2 Hz, H_9), 8.57 (s, 1H, H_{TrCH}), 8.03 (d, 1H, $J_{12\text{-}11}$ = 7.9 Hz, H_{12}), 7.87 (dt, 1H, $J_{11\text{-}12}$

= 7.9 Hz, $J_{11\text{-}10}$ = 1.5 Hz, H_{11}), 7.35-7.29 (m, 1H, H_{10}), 5.22 (d, 1H, $J_{1\text{-}2}$ = 1.7 Hz, H_1), 5.08 (d, 1H, $J_{OH\text{-}3}$ = 4.7 Hz, OH-3), 5.04 (dd, 1H, $J_{2\text{-}3}$ = 5.1 Hz, $J_{2\text{-}1}$ = 1.7 Hz, H_2), 4.83 (d, 1H, $J_{OH\text{-}4}$ = 3.1 Hz, OH-4), 4.42 (t, 1H, $J_{OH\text{-}6}$ = 5.3 Hz, OH-6), 4.14-4.06 (m, 1H, H_3), 4.01-3.91 (m, 1H, H_{7a}), 3.88-3.78 (m, 3H, H_8 + H_{7b}), 3.78-3.68 (m, 4H, H_5 + H_6 + H_4). ^{13}C-NMR (400MHz, DMSO-d^6): δ(ppm)= 150.1 (C_{13}), 149.7 (C_9), 146.9 (C_{Trq}), 137.2 (C_{11}), 123.0 (C_{10}), 122.9 (C_{TrCH}), 119.5 (C_{12}), 97.4 (C_1), 73.9 (C_5), 68.4 (C_3), 67.3 (C_7), 65.9 (C_4), 63.8 (C_2), 60.0 (C_6), 43.6 (C_8)

MS (HRMS): m/z calculated for $[C_{15}H_{19}ClN_4NaO_5]^+$: 393.0942 $[M + Na]^+$, found: 393.0942

$[\alpha]_D^{27} = +1$ $(c = 1.1, \text{MeOH})$

Synthesis of 11



1) *Chlorine-azide exchange*:

Compound **28** (580 mg, 1.17 mmol, 1 eq.) was dissolved in dry DMF ([**28**]= 0.2M), then NaN_3 (380 mg, 5.84 mmol. 5 eq) was added. The reaction was stirred at 55 °C, under N_2 for 3 days. Upon completion (NMR analysis) the mixture was concentrated *in vacuo.* Crude product was purified via automated flash chromatography (Hex:AcOEt 1:1) to obtain the pure product as a white foam (520 mg, 88%).

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.81 (s, 1H, H_{TrCH}), 8.57 (d, 1H, $J_{9\text{-}10}$ = 4.7 Hz, H_9), 8.12 (d, 1H, $J_{12\text{-}11}$ = 8.0 Hz, H_{12}), 7.93 (dt, 1H, $J_{11\text{-}12}$ = 8.1 Hz, $J_{11\text{-}10}$ = 1.7 Hz, H_{11}), 7.40-7.35 (m, 1H, H_{10}), 5.59-5.54 (m, 1H, H_3), 5.51 (dd, 1H, $J_{2\text{-}3}$ = 5.5 Hz, $J_{2\text{-}1}$ = 1.3 Hz, H_2), 5.44-5.36 (m, 2H, H_4 + H_1), 4.43-4.36 (m, 1H, H_{6a}), 4.35-4.28 (m, 2H, H_5 + H_{6b}), 4.05-3.96 (m, 1H, H_{7a}), 3.86-3.78 (m, 1H, H_{7b}), 3.65-3.51 (m, 2H, H_8), 2.21 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.93 (s, 3H, OAc).

MS (ESI): m/z calculated for $[C_{21}H_{25}N_7NaO_8]^+$: 526.17 $[M + Na]^+$, found: 526.01

2) *CuAAC reaction*:

Synthesized from **29** and propargyl alcohol **26** according to general procedure for CuAAC reaction. Purified via flash chromatography ($CHCl_3$ with MeOH gradient from 0% to 10%) to obtain pure product as a white foam (Yield: 63%).

R_f(**34**): 0.54 in $CHCl_3$:MeOH 9:1

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 8.64 (s, 1H, H_{TrCH}), 8.49 (d, 1H, $J_{9\text{-}10}$ = 4.4 Hz, H_9), 8.13 (d, 1H, $J_{12\text{-}11}$ = 7.9 Hz, H_{12}), 7.77 (dt, 1H, $J_{11\text{-}12}$ = 7.9 Hz, $J_{11\text{-}10}$ = 1.5 Hz, H_{11}), 7.72 (s, 1H, $H_{TrCH'}$), 7.24-7.18 (m, 1H, H_{10}), 5.41-5.33 (m, 2H, H_3 + H_2), 5.29 (t, 1H, $J_{4\text{-}3}$ = 10.0 Hz , H_4), 5.07 (s, 1H, H_1), 4.79 (s, 2H, H_9), 4.64 (t, 2H, $J_{8\text{-}7}$ = 4.9 Hz, H_8), 4.25-4.14 (m, 3H, H_6 + H_{7a}), 3.99 (m, 1H, H_{7b}), 3.62-3.56 (m, 1H, H_5), 2.25 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc).

MS (ESI): m/z calculated for $[C_{24}H_{29}N_7NaO_9]^+$: 582.19 $[M + Na]^+$, found: 582.08

3) *Deacetylation*

Performed on intermediate **34** according to general procedure A for deacetylation. (Yield: 93%)

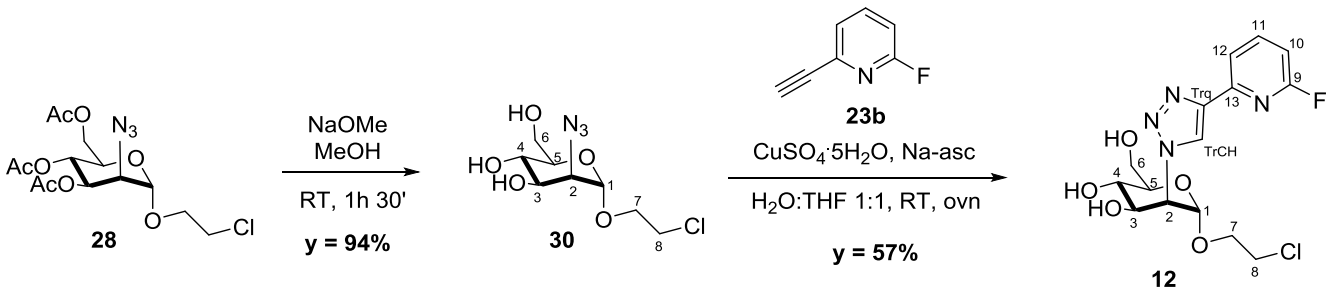
R_f(**11**): 0.11 in $CHCl_3$:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.67 (s, 1H, H_{TrCH}), 8.55 (d, 1H, $J_{9\text{-}10}$ = 4.8 Hz, H_9), 8.06 (d, 1H, $J_{12\text{-}11}$ = 8.1 Hz, H_{12}), 8.00 (s, 1H, $H_{TrCH'}$), 7.90 (dt, 1H, $J_{11\text{-}12}$ = 8.1 Hz, $J_{11\text{-}10}$ = 1.2 Hz, H_{11}), 7.39-7.33 (m, 1H, H_{10}), 5.15 (s, 1H, H_1), 5.11 (d, 1H, $J_{2\text{-}3}$ = 5.3 Hz, H_2), 4.72-4.66 (m, 4H, H_8 + H_{13}), 4.23-4.09 (m, 2H, H_{7a} + H_3), 4.01-3.94 (m, 1H, H_{7b}), 3.88-3.81 (m, 2H, H_6), 3.74 (t, 1H, $J_{4\text{-}3}$ = 9.7 Hz, H_4), 3.35-3.32 (m, 1H, H_5). ^{13}C-NMR (400MHz, DMSO-d^6): δ(ppm)= 150.0 (C_{14}), 149.6 (C_9), 147.9 (C_{Trq}), 146.9 ($C_{Trq'}$), 137.1 (C_{11}), 123.1 (C_{10}), 123.0 ($C_{TrCH'}$), 122. 9 (C_{TrCH}), 119.5 (C_{12}), 97.2 (C_1), 73.8 (C_5), 65.7 (C_3), 65.4 (C_7), 66.2 (C_4), 63.6 (C_2), 59.9 (C_6), 55.0 (C_{13}), 49.0 (C_8)

MS (HRMS): m/z calculated for $[C_{18}H_{23}ClN_7NaO_6]^+$: 456.1607 $[M + Na]^+$, found: 456.1609

$[\alpha]_D^{25} = +1$ $(c = 0.9, \text{MeOH})$

Synthesis of 12



1) *Deacetylation*:

Performed on intermediate **28** according to general procedure A for deacetylation. (Yield: 94%).

R_f(**30**): 0.29 in Toluene:AcOEt 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 4.86 (d, 1H, $J_{1\text{-}2}$ = 1.3 Hz, H_1), 3.99-3.91 (m, 2H, H_3 + H_{7a}), 3.87 (dd, 1H, $J_{2\text{-}3}$ = 4.05 Hz, $J_{2\text{-}1}$ = 1.3 Hz, H_2), 3.82 (d, 1H, $J_{6a\text{-}6b}$ = 11.8 Hz, H_{6a}), 3.78-3.63 (m, 4H, H_8 + H_{7b} + H_{6b}), 3.62-3.53 (m, 2H, H_4 + H_5). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 99.75 (C_1), 75.03 (C_5), 72.4 (C_3), 69.1 (C_7), 68.6 (C_4), 65.6 (C_2), 62.7 (C_6), 43.8 (C_8).

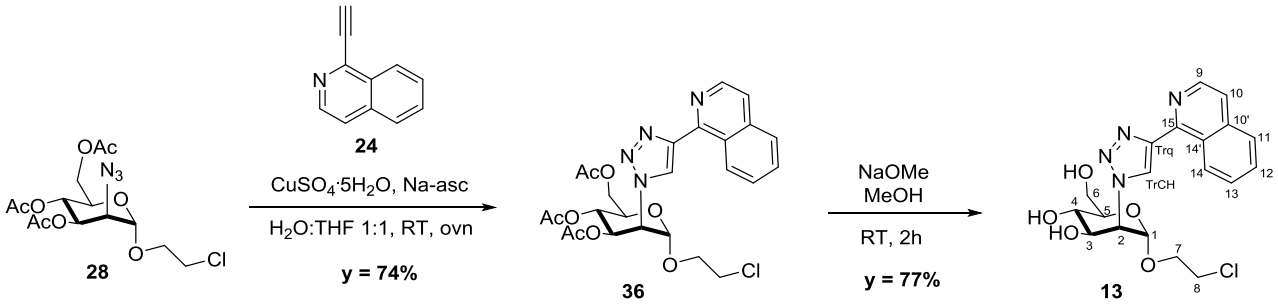
2) *CuAAC reaction*:

Synthesized from **30** and 2-ethynyl-6-fluoropyridine **23b** according to general procedure for CuAAC reaction. Purified via flash chromatography ($CHCl_3$ with MeOH gradient from 0% to 20%) to obtain pure product as a white foam (Yield: 57%).

R_f(**12**): 0.37 in $CHCl_3$:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.70 (s, 1H, H_{TrCH}), 8.01 (ddd, 1H, $J_{11\text{-}10}$ = 8.1 Hz, $J_{11\text{-}12}$ = 7.6 Hz, $J_{11\text{-}F}$ = 7.5 Hz, H_{11}), 7.95 (dd, 1H, $J_{12\text{-}11}$ = 7.6 Hz, $J_{12\text{-}10}$ = 1.8 Hz, H_{12}), 7.01 (dd, 1H, $J_{10\text{-}11}$ = 8.1 Hz, $J_{10\text{-}F}$ = 1.9 Hz, H_{10}), 5.21 (s, 1H, H_1), 5.18 (d, 1H, $J_{2\text{-}3}$ = 5.1 Hz, H_2), 4.24 (dd, 1H, $J_{3\text{-}4}$ = 8.6 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 4.07-3.99 (m, 1H, H_{7a}), 3.91 (d, 2H, $J_{6\text{-}5}$ = 3.1 Hz, H_6), 3.88-3.75 (m, 5H, H_4 + H_5 + H_{7b} + H_8). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 164.7 (d, $J_{9\text{-}F}$ = 240 Hz, C_9). 149.8 (d, $J_{13\text{-}F}$ = 14.2 Hz, C_{13}), 147.5 (C_{Trq}), 143.9 (d, $J_{11\text{-}F}$ = 8.1 Hz, C_{11}), 125.1 (C_{TrCH}), 118.3 (d, $J_{12\text{-}F}$ = 3.8 Hz, C_{12}), 109.5 (d, $J_{10\text{-}F}$ = 37.6 Hz, C_{10}), 99.4 (C_1), 75.2 (C_5), 70.2 (C_3), 69.3 (C_7), 67.7 (C_4), 65.6 (C_2), 61.9 (C_6), 43.9 (C_8). ^{19}F-NMR (300 MHz, CD_3OD): δ(ppm)= -70.01
MS (HRMS): m/z calculated for $[C_{15}H_{18}ClFN_4NaO_5]^+$: 411.0847 $[M + Na]^+$, found: 411.0844
$[\alpha]_D^{26} = +4 \ (c = 0.41, \text{MeOH})$

Synthesis of 13



1) *CuAAC reaction*:

Synthesized from **28** and 1-ethynylisoquinoline **24** according to general procedure for CuAAC reaction. Purified via flash chromatography (Hex: AcoEt gradient 1:1) to obtain pure product as a white foam (Yield: 74%).

R_f(**36**): 0.38 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 9.17 (d, 1H, $J_{14\text{-}13}$ = 8.6 Hz, H_{14}), 8.89 (s, 1H, H_{TrCH}), 8.51 (d, 1H, $J_{9\text{-}10}$ = 5.6 Hz, H_9), 8.02 (d, 1H, $J_{11\text{-}12}$ = 8.1 Hz, H_{11}), 7.86-7.78 (m, 2H, H_{10} + H_{12}), 7.77-7.71 (m, 1H, H_{13}), 5.64-5.58 (m, 2H, H_2 + H_3), 5.49 (m, 2H, H_4 + H_1), 4.43-4.36 (m, 2H, H_5 + H_{6a}), 4.33-4.26 (m, 1H, H_{6b}), 4.13-4.05 (m, 1H, H_{7a}), 4.01-3.94 (m, 1H, H_{7b}), 3.84 (t, 2H, $J_{8\text{-}7}$ = 5.3 Hz, H_8), 2.11 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.97 (s, 3H, OAc).

2) *Deacetylation:*

Performed on intermediate **36** according to general procedure A for deacetylation. (Yield: 77%)

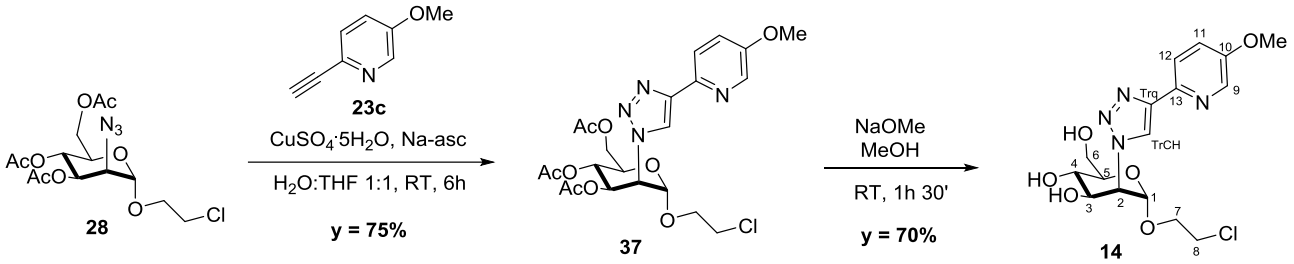
R_f(**13**): 0.59 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.96 (d, 1H, $J_{14\text{-}13}$ = 8.5 Hz, H_{14}), 8.79 (s, 1H, H_{TrCH}), 8.49 (brs, 1H, H_9), 7.94 (d, 1H, $J_{11\text{-}12}$ = 8.0 Hz, H_{11}), 7.82-7.73 (m, 2H, H_{10} + H_{12}), 7.70-7.65 (m, 1H, H_{13}), 5.29-5.26 (m, 2H, H_1 + H_2), 4.34-4.28 (m, 1H, H_3), 4.08-4.01 (m, 1H, H_{7a}), 3.95-3.82 (m, 5H, H_6 + H_4 + H_5 + H_{7b}), 3.77 (t, 2H, $J_{8\text{-}7}$ = 5.3 Hz, H_8). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 149.7 ($C_{14'}$), 146.9 ($C_{10'}$), 140.9 (C_9), 137.2 (C_{Trq}), 130.6 (C_{12}), 127.9 (C_{13}), 127.3 (C_{14}), 126.8 (C_{11}), 125.9 (C_{10}), 98.2 (C_1), 73.9 (C_5), 68.9 (C_3), 67.9 (C_7), 66.3 (C_4), 64.3 (C_2), 60.6 (C_6), 42.4 (C_8)

MS (HRMS): m/z calculated for $[C_{19}H_{22}ClN_4O_5]^+$: 421.1279 $[M + H]^+$, found: 421.1283; m/z calculated for $[C_{19}H_{21}ClN_4NaO_5]^+$: 443.1098 $[M + Na]^+$, found: 443.1103

$[\alpha]_D^{31} = +8$ $(c = 0.5, \text{MeOH})$

Synthesis of 14



1) *CuAAC reaction*:

Synthesized from **28** and 2-ethynyl-5-methoxypyridine **23c** according to general procedure for CuAAC reaction. Purified via flash chromatography (Hex with AcoEt gradient from 50% to 60%) to obtain pure product as a white foam (Yield: 75%).

R_f(**37**): 0.24 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 8.61 (s, 1H, H_{TrCH}), 8.18 (d, 1H, $J_{9\text{-}11}$ = 2.9 Hz, H_9), 8.07 (d, 1H, $J_{12\text{-}11}$ = 8.7 Hz, H_{12}), 7.27 (dd, 1H, $J_{11\text{-}12}$ = 8.7 Hz, $J_{11\text{-}9}$ = 2.9 Hz, H_{11}), 5.53 (dd, 1H, $J_{3\text{-}4}$ = 9.9 Hz, $J_{3\text{-}2}$ = 4.9 Hz, H_3), 5.47 (d, 1H, $J_{2\text{-}3}$ = 4.9 Hz, H_2), 5.37 (t, 1H, $J_{4\text{-}3}$ = 9.9 Hz, H_4), 5.16 (s, 1H, H_1), 4.29-4.21 (m, 3H, H_6 + H_5), 4.03-3.94 (m, 1H, H_{7a}), 3.90-3.82 (m, 4H, H_{7b} + O<u>Me</u>), 3.71 (t, 2H, $J_{8\text{-}7}$ = 5.4 Hz, H_8), 2.26 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.93 (s, 3H, OAc)

2) *Deacetylation*:

Performed on intermediate **37** according to general procedure A for deacetylation. (Yield: 70%)

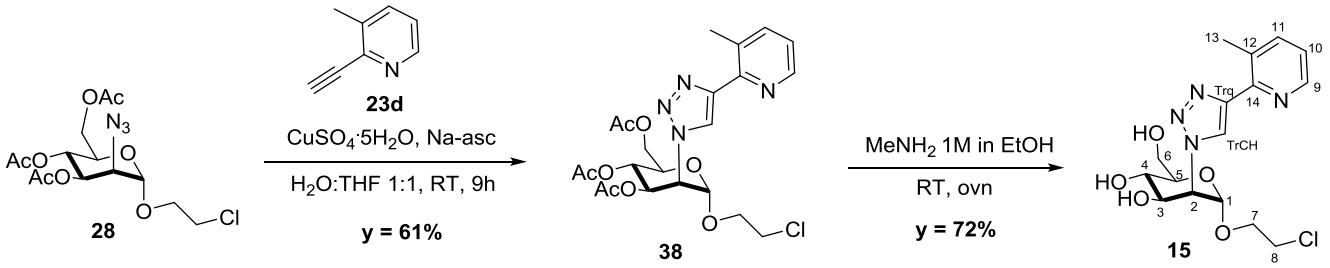
R_f(**14**): 0.48 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.61 (s, 1H, TrCH), 8.25 (brs, 1H, H_9), 7.99 (d, 1H, $J_{12\text{-}11}$ = 8.6 Hz, H_{12}), 7.49 (dd, 1H, $J_{11\text{-}12}$ = 8.9 Hz, $J_{11\text{-}9}$ = 2.8 Hz, H_{11}), 5.21 (s, 1H, H_1), 5.17 (d, 1H, $J_{2\text{-}3}$ = 5.1 Hz, H_2), 4.24 (dd, 1H, $J_{3\text{-}4}$ = 9.1 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 4.07-3.99 (m, 1H, H_{7a}), 3.95-3.89 (m, 5H, H_6 + O<u>Me</u>), 3.88-3.81 (m, 3H, H_{7b} + H_4 + H_5), 3.80-3.74 (m, 2H, H_8). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 138.1 (C_9), 123.6 (C_{11}), 122.9 (C_{12}), 99.7 (C_1), 75.4 (C_5), 70.3 (C_3), 69.4 (C_7), 67.9 (C_4), 65.6 (C_2), 62.2 (C_6), 56.4 (OMe), 43.8 (C_8).

MS (HRMS): m/z calculated for $[C_{16}H_{22}ClN_4O_6]^+$: 401.1228 $[M + H]^+$, found: 401.1230; m/z calculated for $[C_{16}H_{21}ClN_4NaO_6]^+$: 423.1047 $[M + Na]^+$, found: 423.1049

$[\alpha]_D^{31} = +3$ $(c = 0.34, \text{MeOH})$

Synthesis of 15



1) *CuAAC reaction*:

Synthesized from **28** and 2-ethynyl-3-methylpyridine **23d** according to general procedure for CuAAC reaction. Purified via flash chromatography (Hex:AcoEt 1:1) to obtain pure product as a yellowish foam (Yield: 61%).

R_f(**37**): 0.29 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.69 (s, 1H, H_{TrCH}), 8.42 (d, 1H, $J_{9\text{-}10}$ = 3.1 Hz, H_9), 7.75 (d, 1H, $J_{11\text{-}10}$ = 7.7 Hz, H_{11}), 7.34-7.26 (m, 1H, H_{10}), 5.60-5.50 (m, 2H, H_3 + H_2), 5.48-5.35 (m, 2H, H_4 + H_1), 4.41-4.33 (m, 2H, H_5 + H_{6a}), 4.31-4.24 (m, 1H, H_{6b}), 4.11-4.02 (m, 1H, H_{7a}), 3.99-3.91 (m, 1H, H_{7b}), 3.83 (t, 2H, $J_{8\text{-}7}$ = 5.2 Hz, H_8), 2.65 (s, 3H, H_{13}), 2.13 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.94 (s, 3H, OAc)

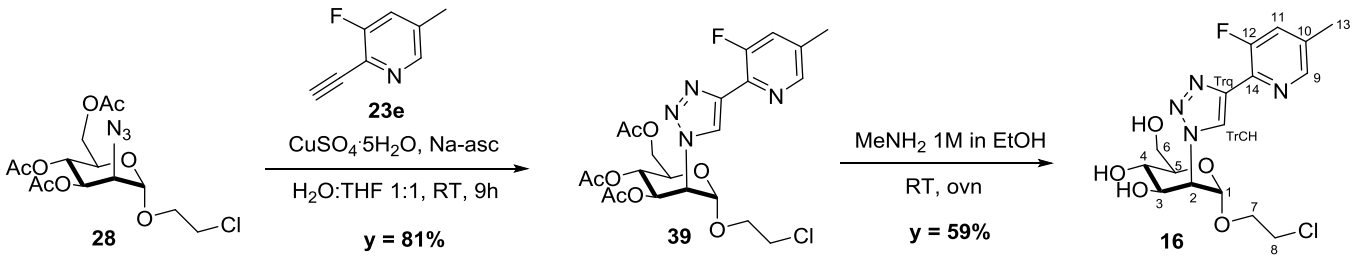
2) *Deacetylation*:

Performed on intermediate **38** according to general procedure B for deacetylation. (Yield: 72%)

R_f(**15**): 0.40 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.61 (s, 1H, H_{TrCH}), 8.44 (brs, 1H, H_9), 7.76 (d, 1H, $J_{11\text{-}10}$ = 7.7 Hz, H_{11}), 7.35-7.28 (m, 1H, H_{10}), 5.24-5.19 (m, 2H, H_1 + H_2), 4.30-4.23 (m, 1H, H_3), 4.07-3.99 (m, 1H, H_{7a}), 3.92-3.90 (m, 2H, H_6), 3.89-3.81 (m, 3H, H_{7b} + H_4 + H_5), 3.77 (t, 2H, $J_{8\text{-}7}$ = 4.3 Hz, H_8), 2.59 (s, 3H, H_{13}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 148.1 (C_{14}), 146.8 (C_{Trq}), 146.2 (C_9), 139.6 (C_{11}), 132.4 (C_{12}), 124.8 (C_{TrCH}), 123.1 (C_{10}), 98.2 (C_1), 73.8 (C_5), 68.9 (C_3), 68.1 (C_7), 66.3 (C_4), 64.2 (C_2), 60.6 (C_6), 42.4 (C_8), 19.1 (C_{13})
MS (HRMS): m/z calculated for $[C_{16}H_{22}ClN_4O_5]^+$: 385.1279 $[M + H]^+$, found: 385.1272; m/z calculated for $[C_{16}H_{21}ClN_4NaO_5]^+$: 407.11 $[M + Na]^+$, found: 407.1091
$[\alpha]_D^{27} = +20 \ (c = 0.2, \text{MeOH})$

Synthesis of 16



1) *CuAAC reaction*:

Synthesized from **28** and 2-Ethynyl-3-fluoro-5-methylpyridine **23e** according to general procedure for CuAAC reaction. Purified via flash chromatography (Hex with AcoEt gradient from 50% to 60%) to obtain pure product as a white foam (Yield: 81%).

R_f(**39**): 0.20 in Hex:AcoEt 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.68 (d, 1H, J_{TrCH-F} = 1.7 Hz, H_{TrCH}), 8.33 (s, 1H, H_9), 7.56 (d, 1H, J_{11-F} = 11.6 Hz, H_{11}), 5.58-5.51 (m, 2H, H_3 + H_2), 5.44-5.36 (m, 2H, H_4 + H_1), 4.43-4.34 (m, 2H, H_5 + H_{6a}), 4.27-4.21 (m, 1H, H_{6b}), 4.11-4.02 (m, 1H, H_{7a}), 3.99-3.91 (m, 1H, H_{7b}), 3.83 (t, 2H, J_{8-7} = 5.3 Hz, H_8), 2.41 (s, 3H, H_{13}), 2.15 (s, 3H, OAc), 2.04 (s, 3H, OAc), 1.92 (s, 3H, OAc)

2) *Deacetylation*

Performed on intermediate **38** according to general procedure B for deacetylation. Purified via reverse-phase chromatography (H_2O with MeOH gradient from 0% to 100%) to obtain pure product as a white foam (Yield: 59%).

R_f(**16**): 0.16 in H_2O:MeOH 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.68 (d, 1H, J_{TrCH-F} = 2.3 Hz, H_{TrCH}), 8.32 (s, 1H, H_9), 7.55 (d, 1H, J_{11-F} = 11.4 Hz, H_{11}), 5.24-5.19 (m, 2H, H_1 + H_2), 4.30-4.22 (m, 1H, H_3), 4.06-3.99 (m, 1H, H_{7a}), 3.93-3.88 (m, 2H, H_6), 3.87-3.81 (m, 3H, H_{7b} + H_5 + H_4), 3.77 (t, 2H, J_{8-7} = 5.4 Hz, H_8), 2.41 (s, 3H, H_{13}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm)= 157.6 (d, J_{12-F} = 256.9 Hz, C_{12}), 147.0 (d, J_{9-F} = 4.0 Hz, C_9), 143.2 (d, J_{10-F} = 6.8 Hz, C_{10}), 137.1 (d, J_{Trq-F} = 3.4 Hz, C_{Trq}), 136.3 (d, J_{14-F} = 13.0 Hz, C_{14}), 126.2 (d, J_{TrCH-F} = 10.1 Hz, C_{TrCH}), 125.8 (d, J_{11-F} = 18.7 Hz, C_{11}), 99.5 (C_1), 75.2 (C_5), 70.2 (C_3), 69.4 (C_7), 67.7 (C_4), 65.6 (C_2), 61.9 (C_6), 43.8 (C_8), 17.8 (C_{13})

^{19}F-NMR (300 MHz, CD_3OD): δ(ppm)= -124.52

MS (HRMS): m/z calculated for $[C_{16}H_{20}ClFN_4NaO_5]^+$: 425.10 [M + Na]$^+$, found: 425.1001; m/z calculated for $[C_{16}H_{19}ClFN_4Na_2O_5]^+$: 447.08 [M + 2Na - H]$^+$, found: 447.0822

$[\alpha]_D^{27} = +2 \ (c = 0.6, \text{MeOH})$

SPR experiments

Lectin production and purification

DC-SIGN ECD has been expressed, refolded, purified as previously described.[6] Using an expression vector previously described,[7] the Extracellular Domain (ECD) of L-SIGN (residue 78-399) was expressed in *E. coli* BL21(DE3) as inclusion bodies. Typically, bacteria were cultured in 4 L of LB medium and 50 µg.mL^{-1} of kanamycin at 37°C. Expression is induced with 1 mM IPTG at OD_{600}=1.2. After 3 hours, cells were recovered at 3000 g for 20 min at 4°C. Bacterial pellets were resuspended in 30 mL of Buffer A (25 mM Tris pH 8, 150 mM NaCl, 4 mM $CaCl_2$), supplemented with one tablet of cOmplete EDTA-free protease inhibitor cocktail each (Roche).

Cells were lysed by sonication and centrifuged at 100.000 g for 30 minutes, 4°C. Pellet was resuspended using a Potter in Urea 2M, 25 mM Tris pH 8, 150 mM NaCl, 4 mM$CaCl_2$, Triton 1%. After another centrifugation (100 000 g, 30 min, 4°C), the pellet was resuspended in Buffer A. After a third centrifugation, the inclusion bodies were solubilized in Guanidine 6M, 25 mM Tris, 150 mM NaCl, 0,01% β-mercaptoethanol. Solubilized proteins were recovered in the supernatant of a fourth centrifugation. Refolding was performed by a drop by drop dilution to reach a final 5-time dilution in 1.25 M NaCl, 25 mM Tris pH 8, 25 mM $CaCl_2$. The mixture is then dialyzed against 25 mM Tris pH 8 overnight, and then two more 3-hour dialysis to equilibrium against buffer A. Insoluble precipitate was removed by centrifugation at 100 000 g, 30 min, 4°C.

Supernatant was first loaded on a Mannan-Agarose column equilibrated with Buffer A. L-SIGN ECD was eluted in the same buffer without $CaCl_2$ and supplemented with 1 mM EDTA. As a second step, the eluted pool of L-SIGN ECD was loaded onto a Superose 12 equilibrated in Buffer A. Fractions of 1 mL were obtained and analyzed by SDS-PAGE (12%). Those containing purified L-SIGN ECD were pooled and concentrated by ultrafiltration using a polyethersulfone membrane MWCO 30,000. Storage of L-SIGN ECD was done at -80°C.

Spike HexaPro production and purification

Spike protein used for this work is Spike Hexapro, described in the paper of Hsiesh *et al*.[8]. Its production and purification were done as described for Spike 2P in Thépaut *et al*.[9]

Competition experiments followed by Surface Plasmon Resonance

Surface Plasmon Resonance (SPR) experiments were performed on Biacore T200 instrument at 25°C. Two types of competition experiments were conducted using either Man-BSA or Spike functionalized surfaces. Interaction tests on α1-3, α1-6 Mannotriose BSA (14 atoms spacer, from Dextra.here after called Man-BSA) surfaces were carried out on a CM3 sensorchip. Flow cell 1 (Fc1) to 3 were activated with 60 µL of a mixture of EDC-NHS (according to manufacturer). Fc1 was functionalized with 16.7 µL of BSA at 20 µg/mL in 10 mM NaOAc pH 4.

Fc1 will be used as a reference surface. Man-BSA was immobilized at 5 µL/min on Fc2 and 3, using around 15 µL at 60 µg/mL. Remaining activated groups were neutralized with 60 µL of 1 M Ethanolamine pH 8.5. After functionalization, all surfaces were washed with 100 µL of 10 mM HCl followed by 100 µL of 50 mM NaOH/1M NaCl at 100 µL/min. Finally, Fc1, 2 and 3 were functionalized respectively at 2751, 2689, and 2692 RU.

Spike functionalized surface were done using a CM4 sensorchip. Fc 1 to 3 were activated as described above. BSA was immobilized on Fc1 following same protocol as previously. Fc2 and 3 were functionalized with Spike HexaPro at 20 µg/mL in 100 mM NaOAc pH 5, reaching 1766 and 1913 RU respectively.

Inhibitory potency of compounds towards DC-SIGN and L-SIGN ECD was evaluated by competition tests on both type of surfaces, BSA-man or Spike.

Competition experiments were performed with decreasing concentrations of compounds from 5 mM to 9.7656 µM, using a 1:2 dilution. Compounds were co-injected with 20 µM of DC-SIGN whatever the surface and 70 µM of L-SIGN over Man-BSA surface or 20 µM of L-SIGN over Spike surface. Flow was set at 5 µL/min in Buffer A supplemented with 0,05% Tween, with an association and dissociation time set to 100 seconds each. For first series of compounds on Man-BSA surface, an association and dissociation time of 150 seconds was used. Surface regeneration was done for 10 seconds at 100 µL with a 100-second stabilization period, using 50 mM EDTA for DC-SIGN tests and 50 mM glycine NaOH pH 12 0.15% Triton 25 mM EDTA for L-SIGN.

Surface properties – Figures SI-3 and SI-4

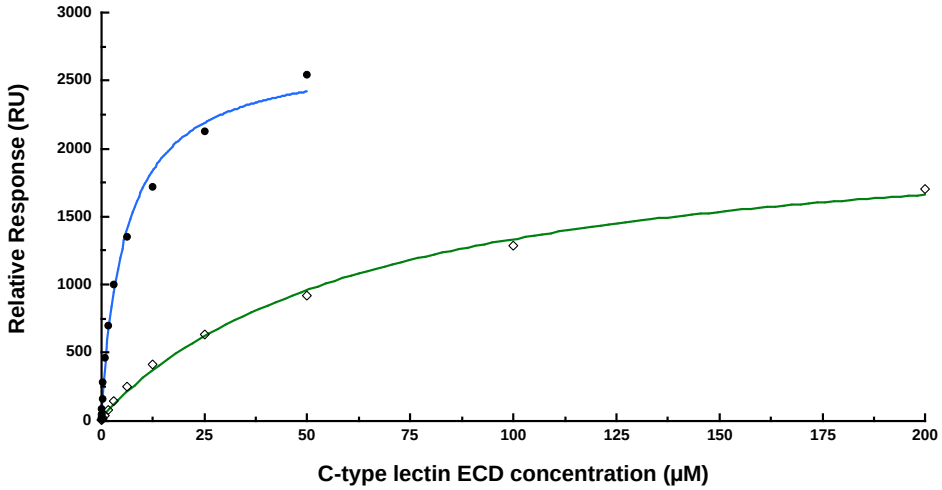


Figure SI-3 *: DC-SIGN and L-SIGN ECD titrations over Man-BSA surface. DC-SIGN ECD titration is represented by the blue curve and L-SIGN by the green curve.*

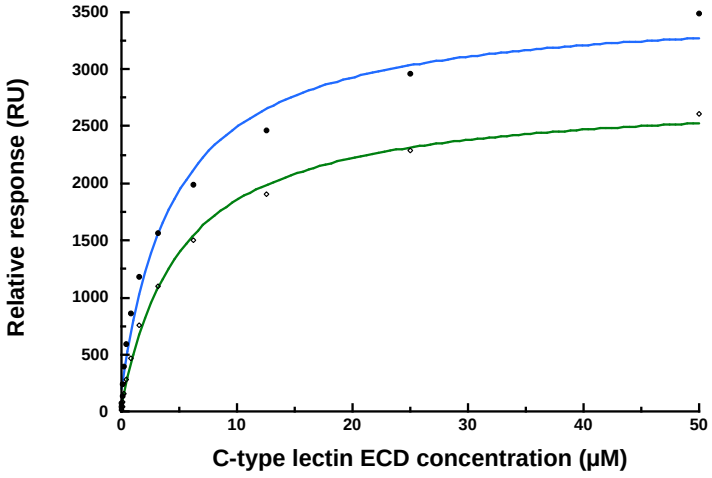
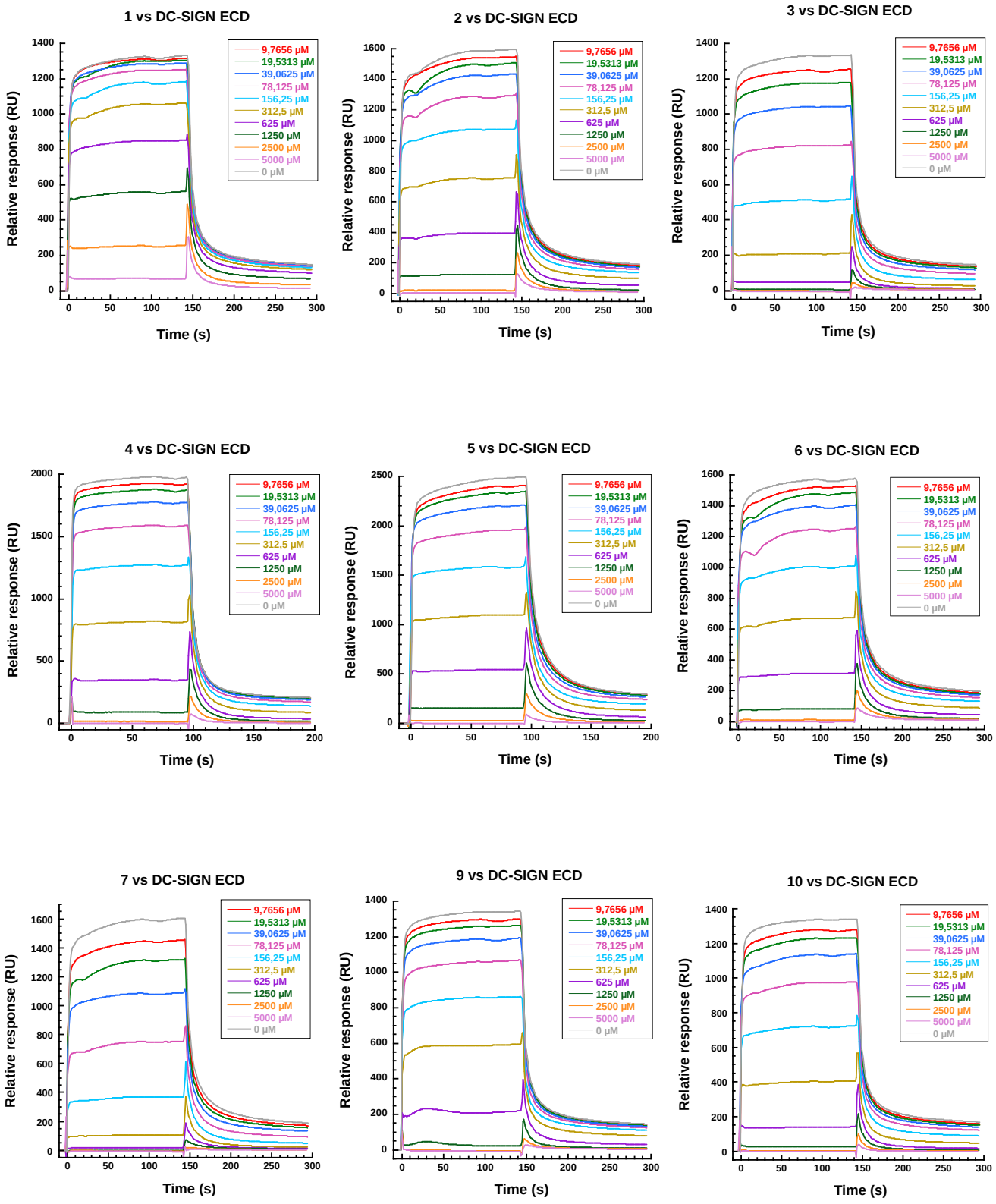
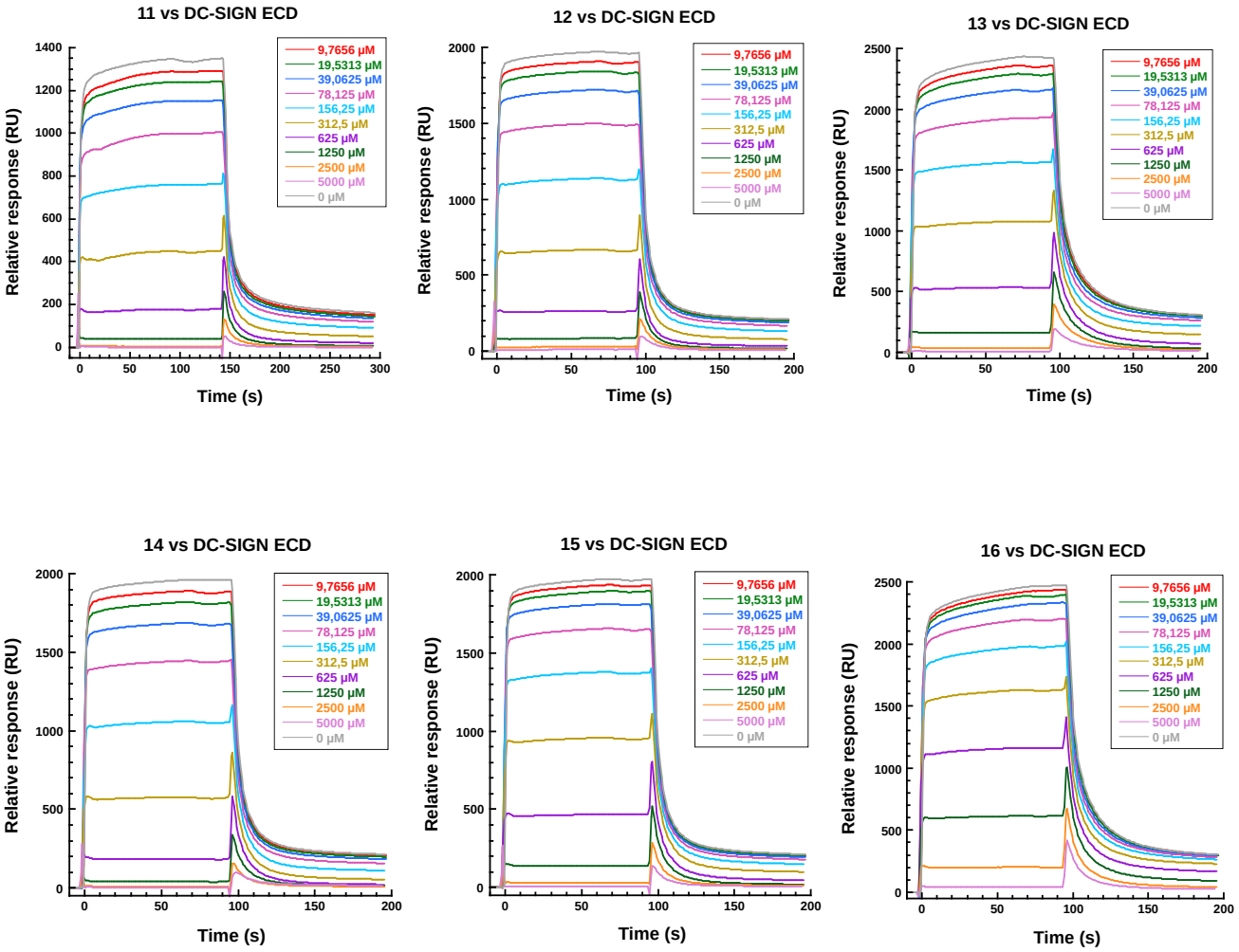


Figure SI-4 *: DC-SIGN and L-SIGN ECD titrations over Spike surface. DC-SIGN ECD titration is represented by the blue curve and L-SIGN by the green curve.*

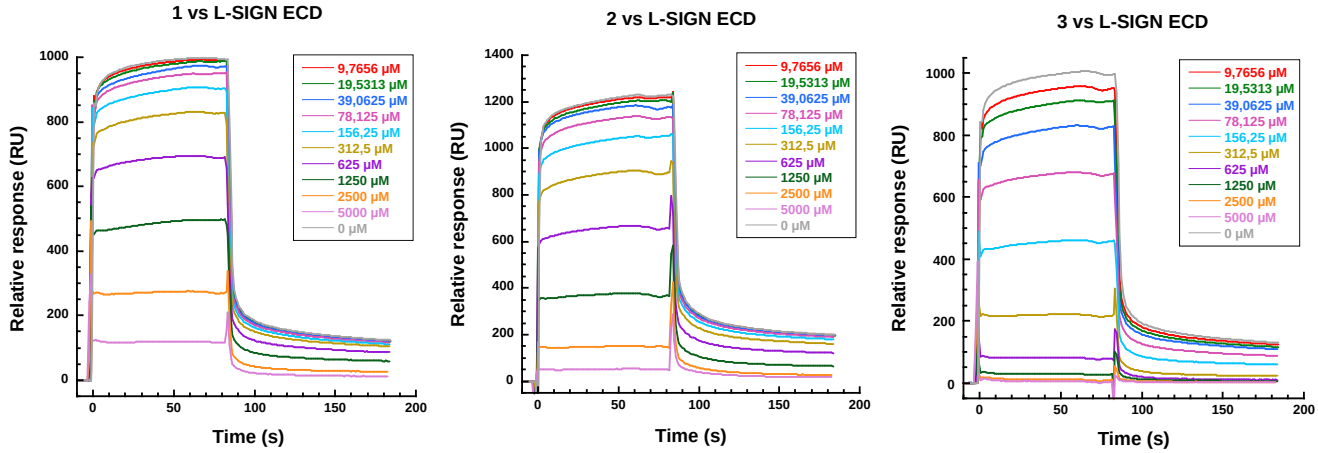
Sensorgrams of competition experiments

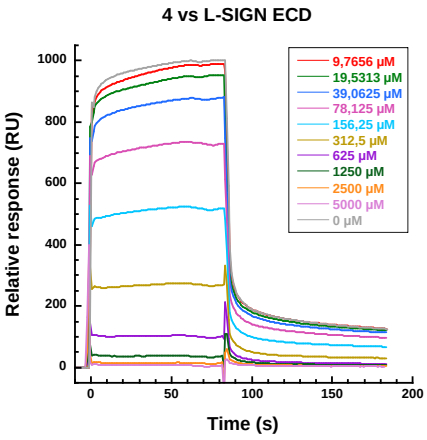
1) *DC-SIGN tests on Man-BSA surface*:

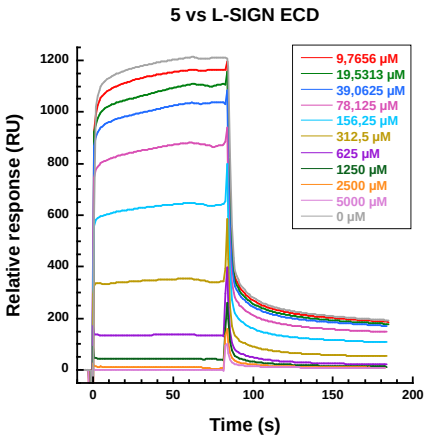


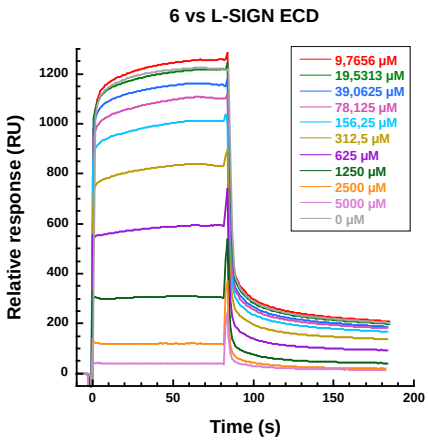


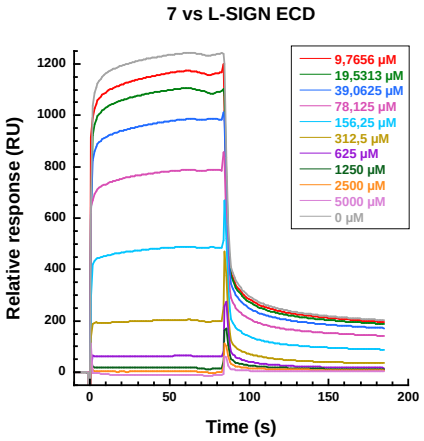
2) *L-SIGN tests on Man-BSA surface*:

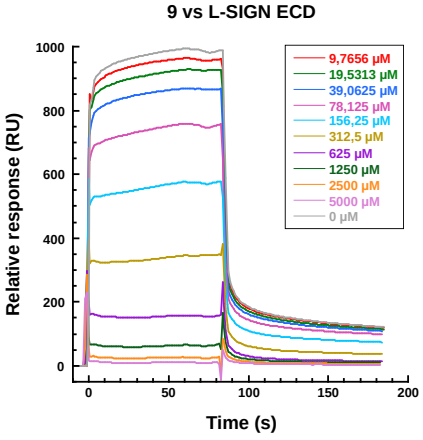


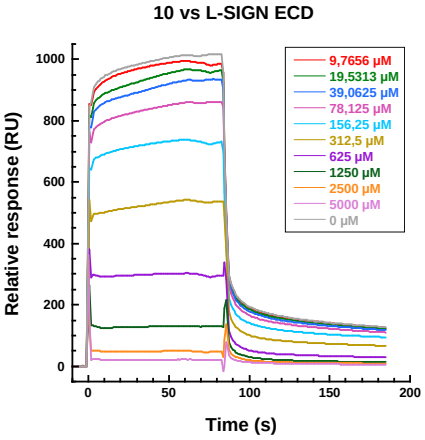

4 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

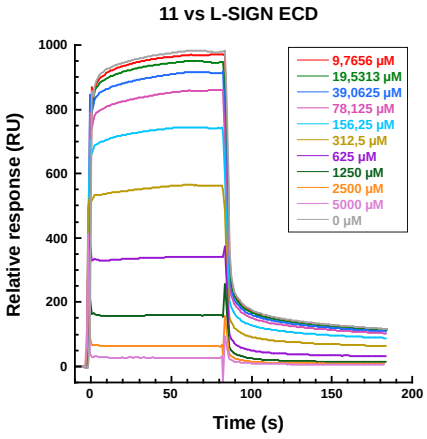

5 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

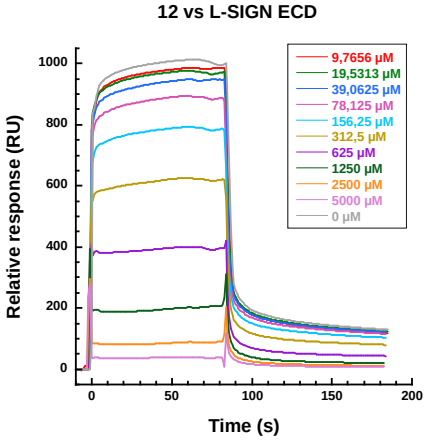

6 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

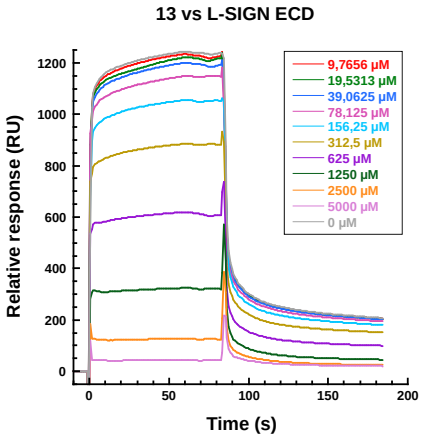

7 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

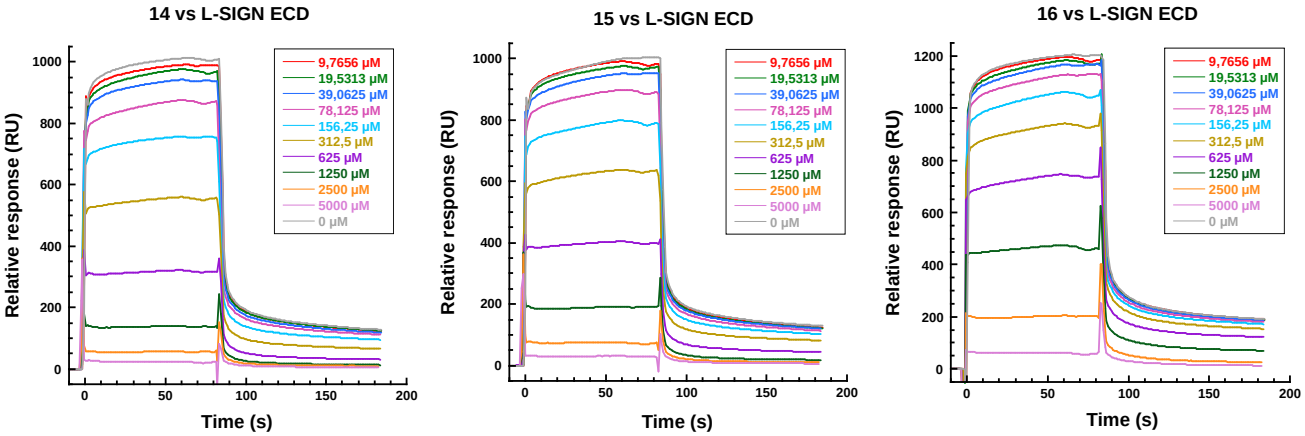

9 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM


10 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

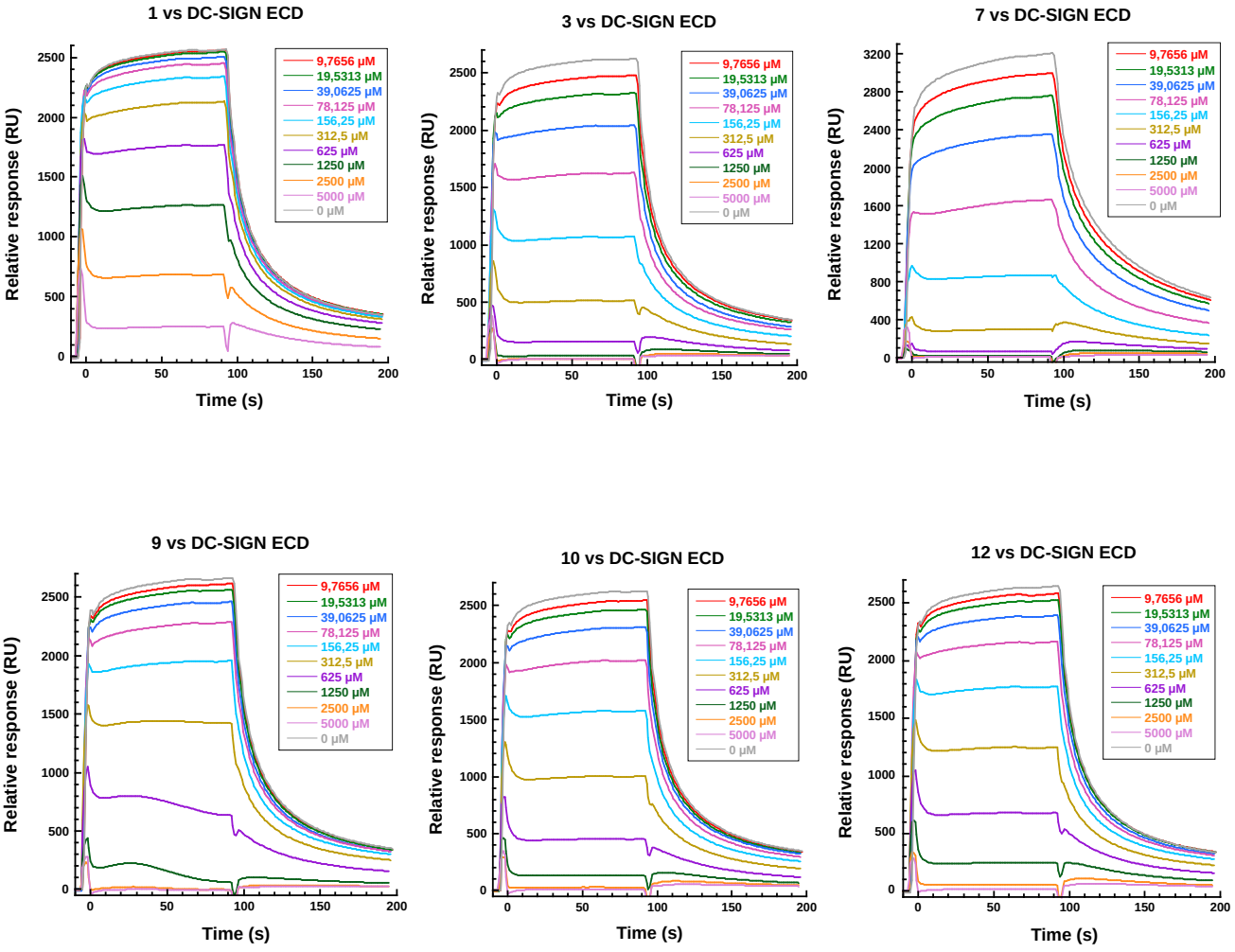

11 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

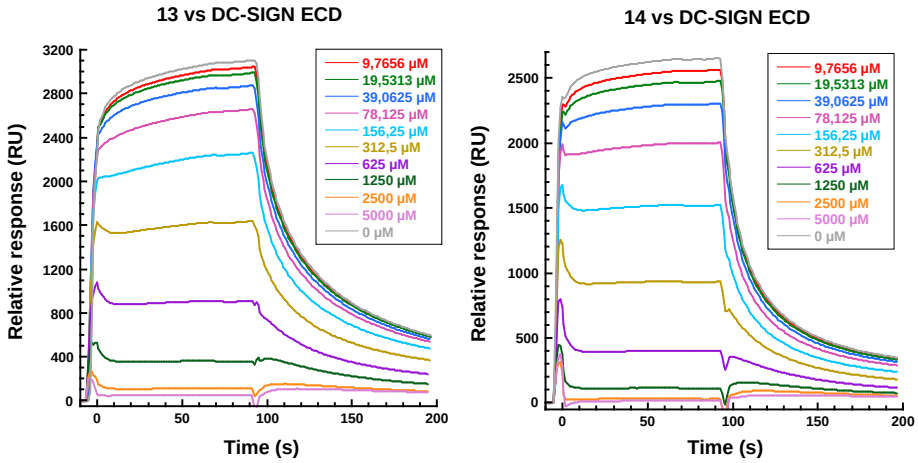

12 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM


13 vs L-SIGN ECD
Relative response (RU)
Time (s)
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM

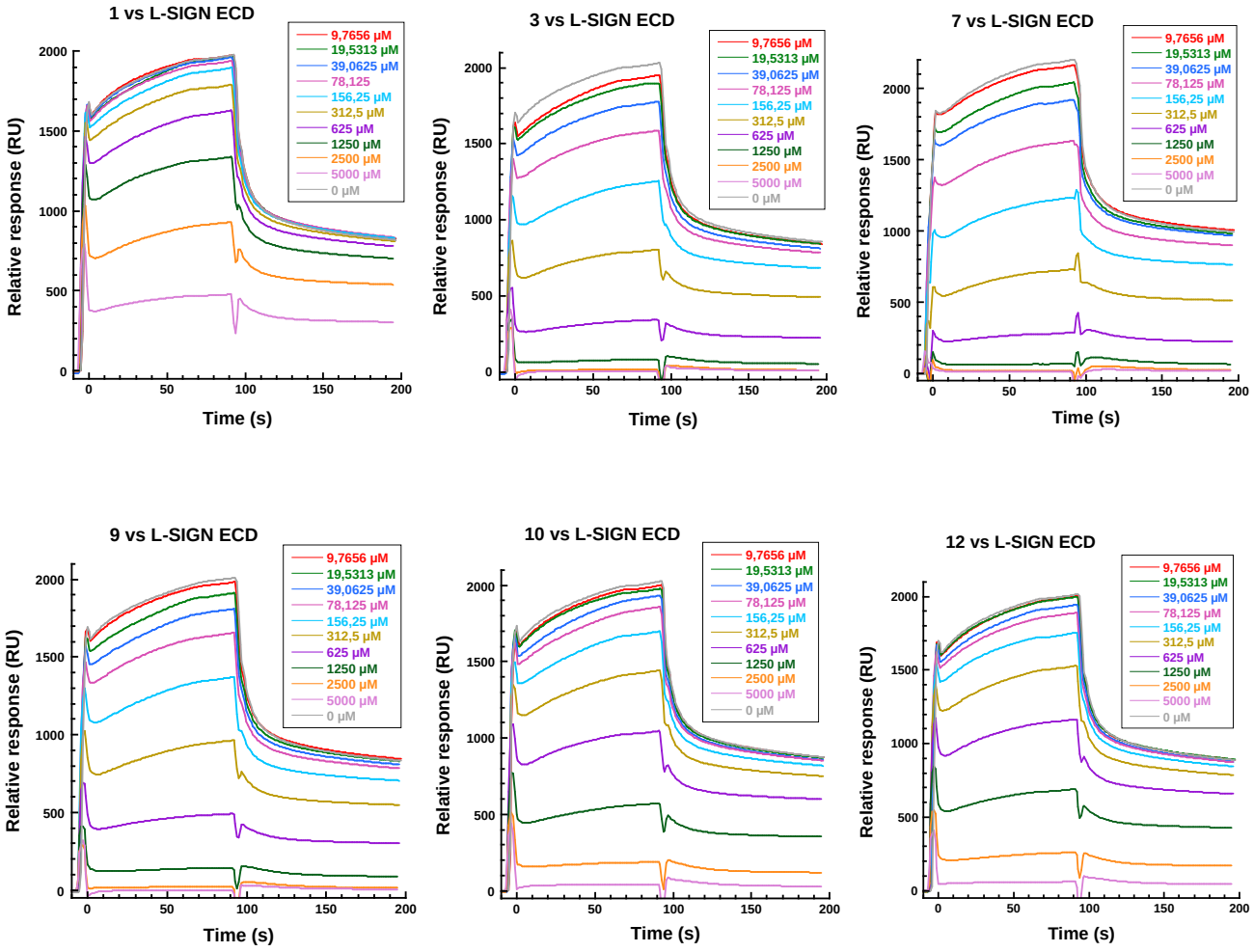


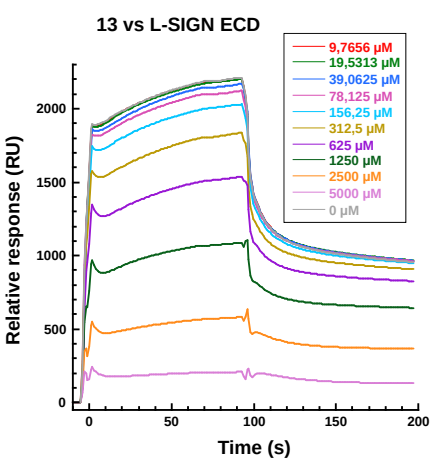
3) *DC-SIGN tests on Spike surface*:

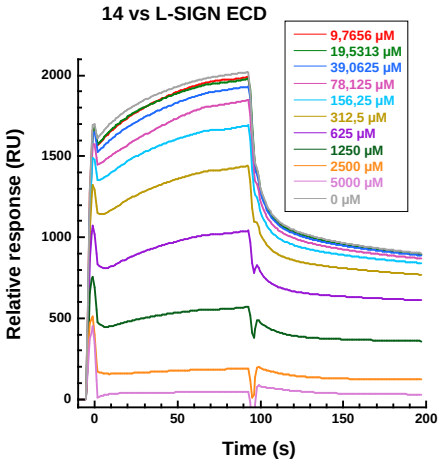




4) *L-SIGN tests on Spike surface*:




13 vs L-SIGN ECD
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM
Relative response (RU)
Time (s)


14 vs L-SIGN ECD
9,7656 µM
19,5313 µM
39,0625 µM
78,125 µM
156,25 µM
312,5 µM
625 µM
1250 µM
2500 µM
5000 µM
0 µM
Relative response (RU)
Time (s)

Inhibition curves

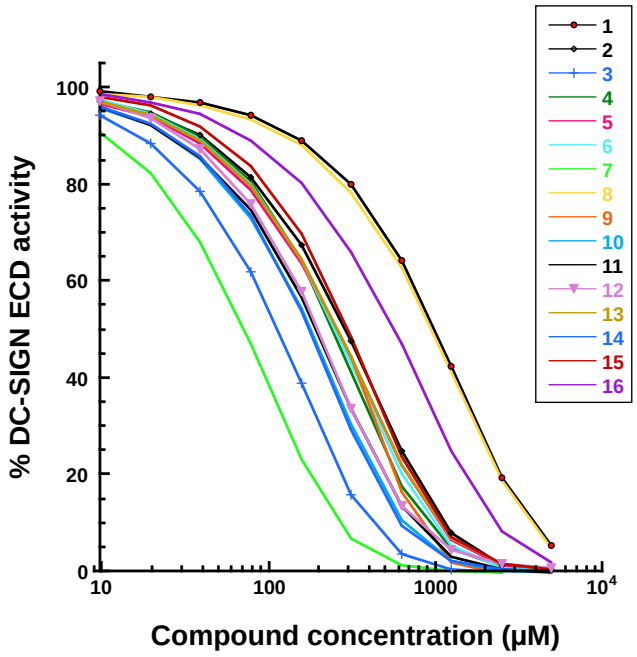


Figure SI-5 *: Inhibition of DC-SIGN interaction with Man-BSA surface.*

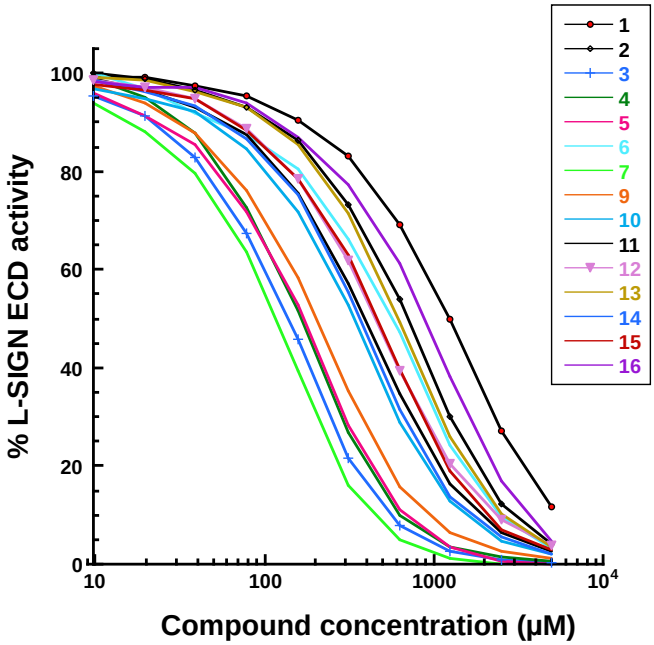


Figure SI-6 *: Inhibition of L-SIGN interaction with Man-BSA surface.*

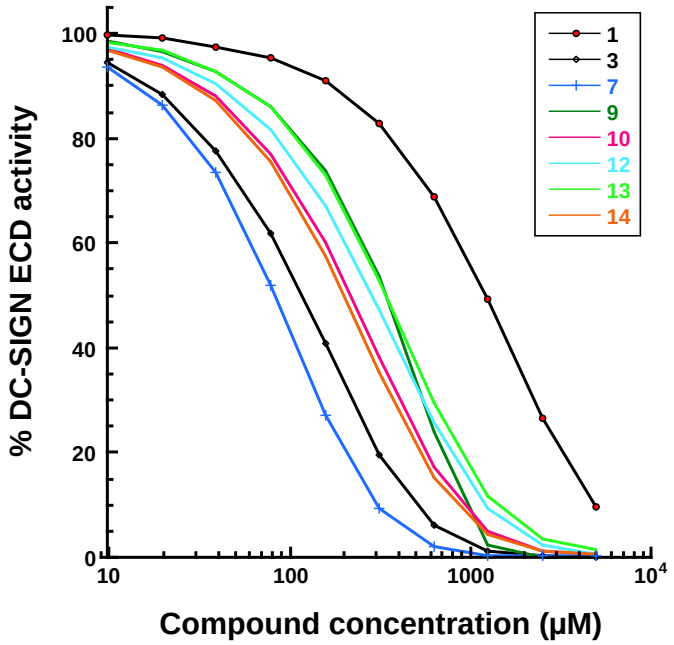


Figure SI-7 *: Inhibition of DC-SIGN interaction with Spike surface.*

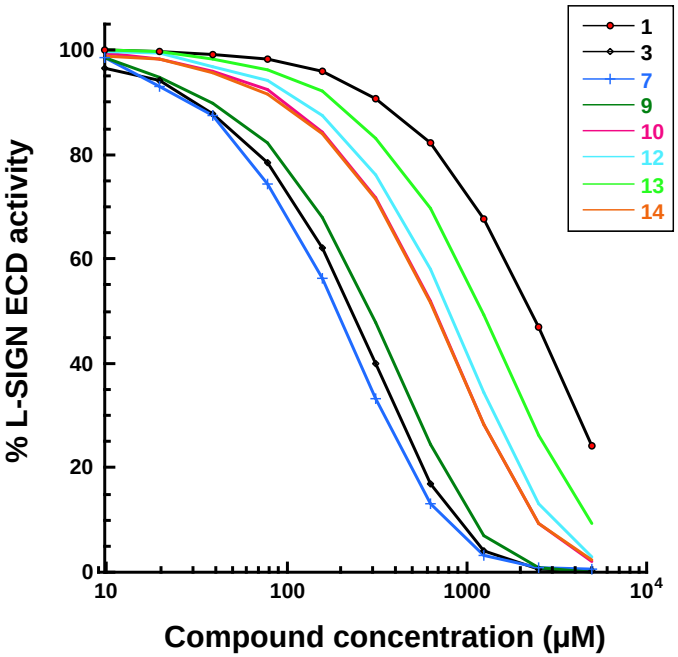
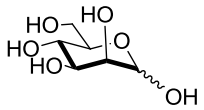
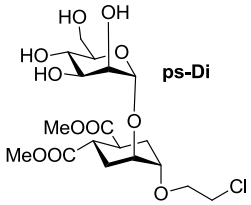
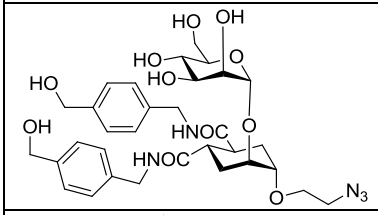
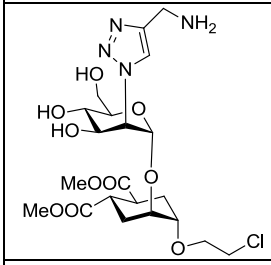
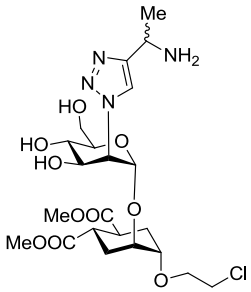
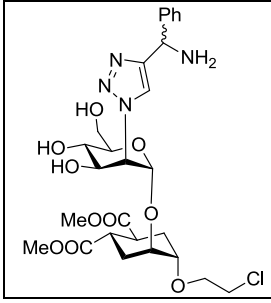
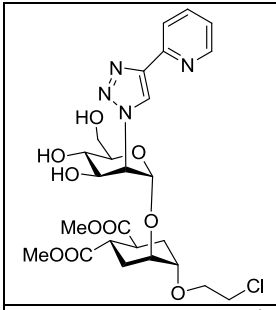
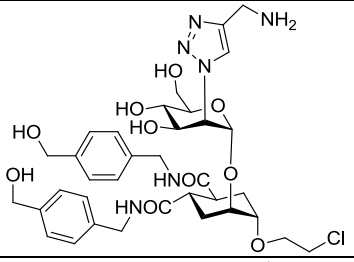
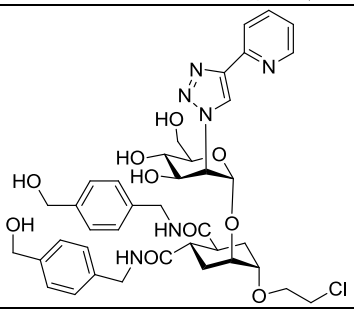
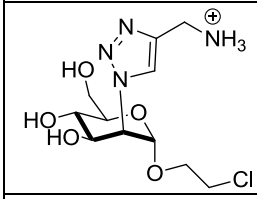
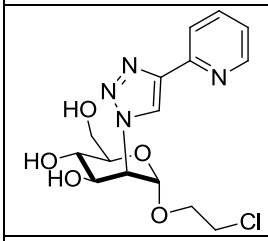
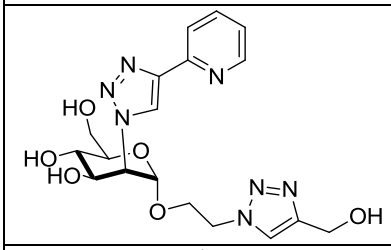
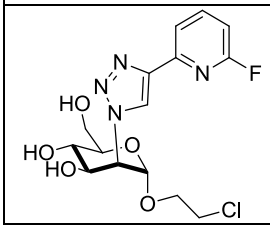


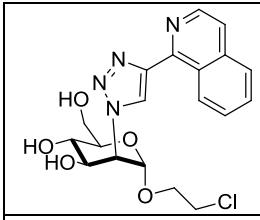
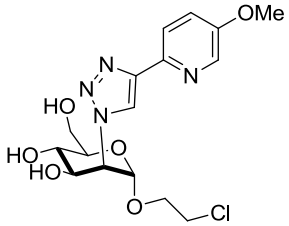
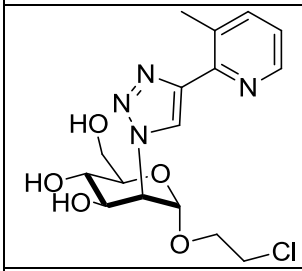
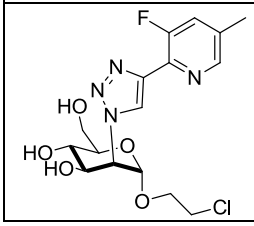
Figure SI-8 *: Inhibition of L-SIGN interaction with Spike surface.*

Inhibition data - Table SI-1

Table SI-2 Inhibitory potency ($IC_{50,}$ μM) of the ligands towards L-SIGN and DC-SIGN. SPR competition assays were performed with 70 μM L-SIGN and 20 μM DC-SIGN on the Man-BSA chip. Experiments on the Spike chip were performed with both L-SIGN and DC-SIGN at 20 μM

Compound	Number	IC_{50} (μM) L-SIGN	IC_{50} (μM) DC-SIGN
	**D-Man**	Man-BSA: n.d. Spike: 1939 ± 19 μM	Man-BSA: 3005[5a] Spike: 3026 ± 63 μM
	**1**	Man-BSA: 1202 ± 22.5 Spike: 2200 ± 92	Man-BSA: 965 ± 27 Spike: 1199 ± 49
	**2**	Man-BSA: 680 ± 0.05 Spike /	Man-BSA: 279 ± 4.8 Spike /
	**3**	Man-BSA: 132 ± 0.9 Spike: 218 ± 2.8	Man-BSA: 105 ± 4.3 Spike: 113 ± 0.02
	**4**	Man-BSA: 157 ± 0.4 Spike /	Man-BSA: 235 ± 0.1 Spike /
	**5**	Man-BSA: 163 ± 0.5 Spike /	Man-BSA: 238 ± 1.7 Spike /

	**6**	Man-BSA: 542 ± 3.3 Spike /	Man-BSA: 240 ± 1.4 Spike /
	**7**	Man-BSA: 113 ± 0 Spike: 180 ± 1.7	Man-BSA: 69 ± 0.5 Spike: 82 ± 1.3
	**8**	Man-BSA: 1285 Spike /	Man-BSA: 956 ± 7.6 Spike /
	**9**	Man-BSA: 193 ± 2.6 Spike: 278 ± 6.9	Man-BSA: 232 ± 7.7 Spike: 318 ± 1.2
	**10**	Man-BSA: 326 ± 1 Spike: 628 ± 12.05	Man-BSA: 163 ± 7 Spike: 210 ± 0.3
	**11**	Man-BSA: 378 ± 4.6 Spike 685.6 ± 2.9 µM	Man-BSA: 178 ± 6.6 Spike 240.2 ± 1.7 µM
	**12**	Man-BSA: 450 ± 8.2 Spike: 760 ± 14.2	Man-BSA: 188 ± 0.3 Spike: 281 ± 0.9

	**13**	Man-BSA: 601 ± 0.6 Spike: 1235 ± 13	Man-BSA: 240 ± 1.4 Spike: 334 ± 3.3
	**14**	Man-BSA: 354 ± 2.7 Spike: 627 ± 8.2	Man-BSA: 172 ± 5.1 Spike: 192 ± 0.9
	**15**	Man-BSA: 459 ± 11.7 Spike /	Man-BSA: 286 ± 1 Spike /
	**16**	Man-BSA: 875 ± 2.05 Spike /	Man-BSA: 545 ± 2.5 Spike /

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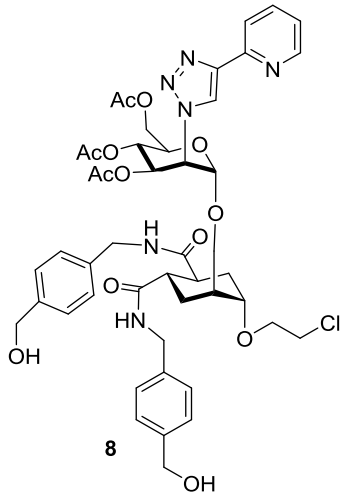
NMR Spectra of ligands



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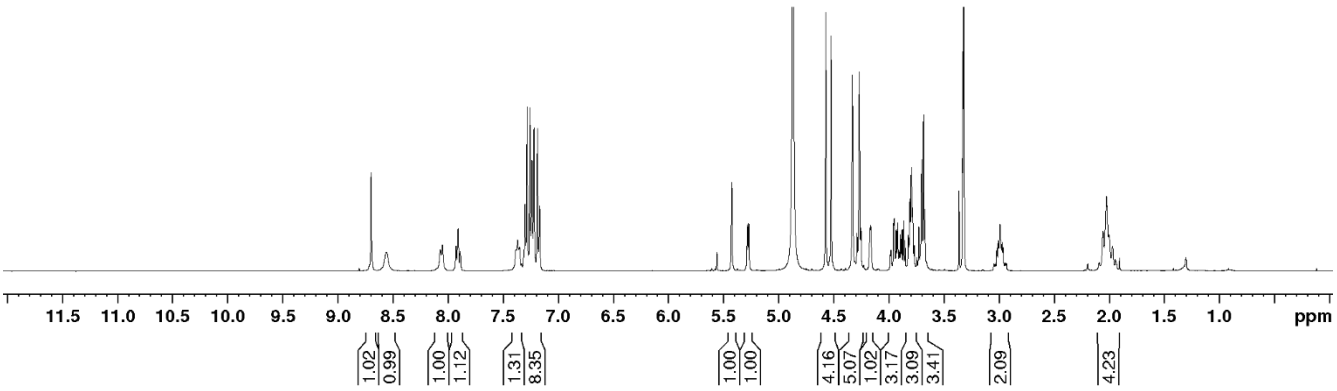
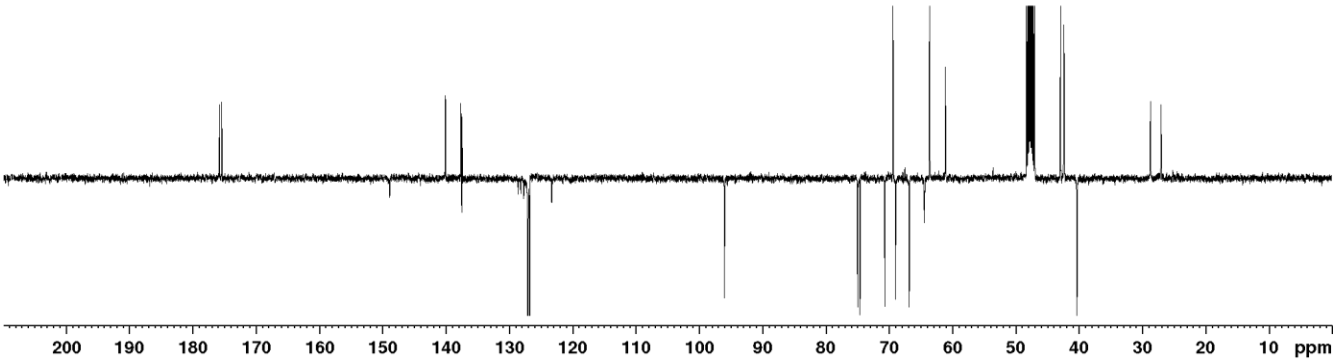
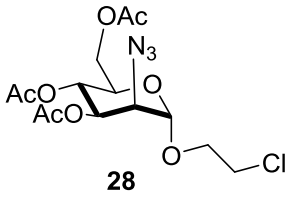
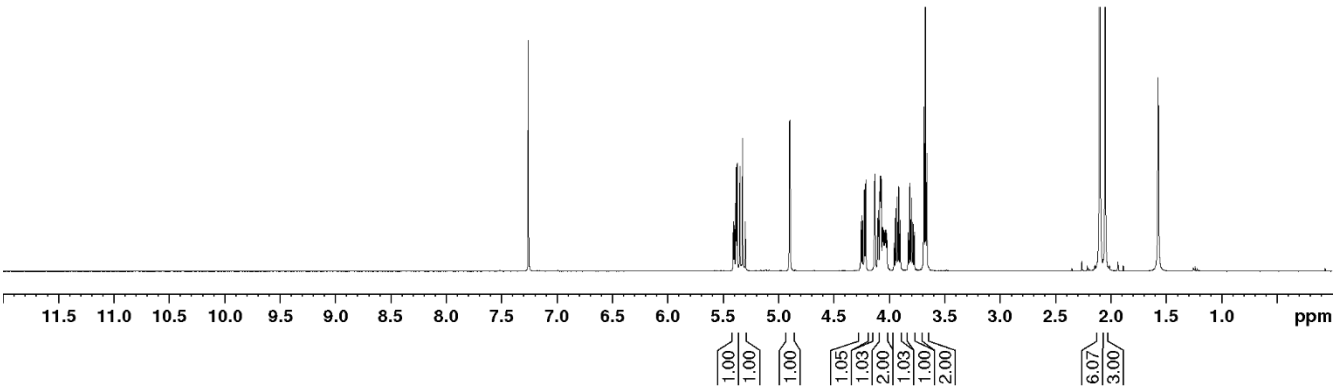
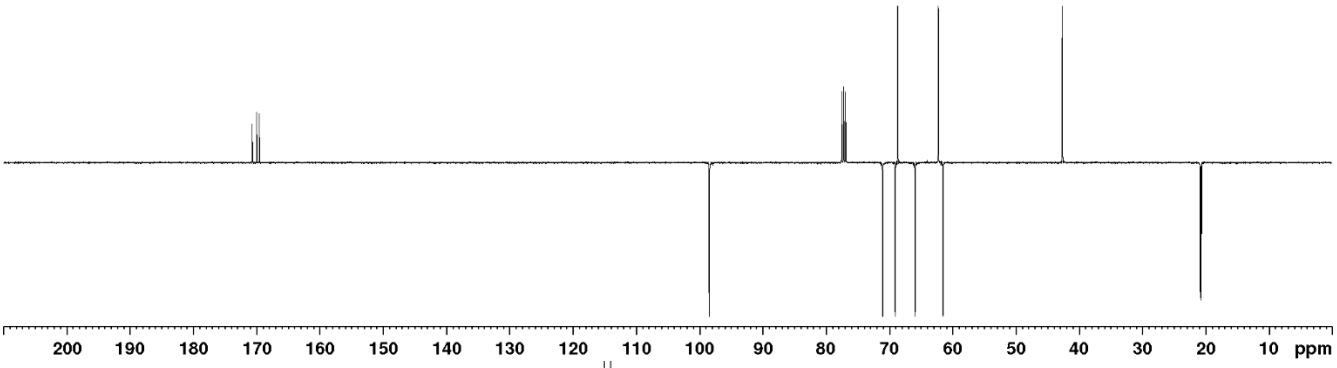





OAc
N3
AcO
AcO
O
O
Cl

28


11.5 11.0 10.5 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 ppm
1.00
1.00
1.00
1.05
1.03
2.00
1.03
1.00
2.00
6.07
3.00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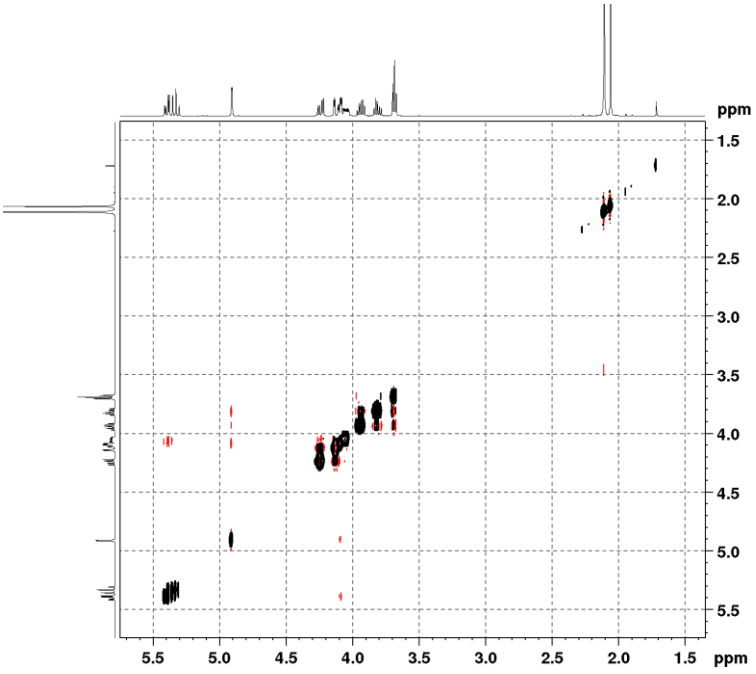
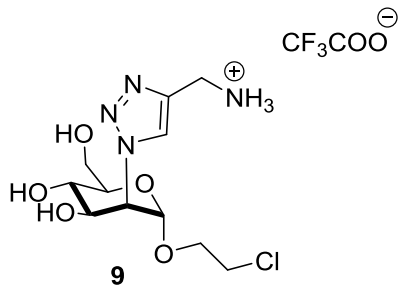
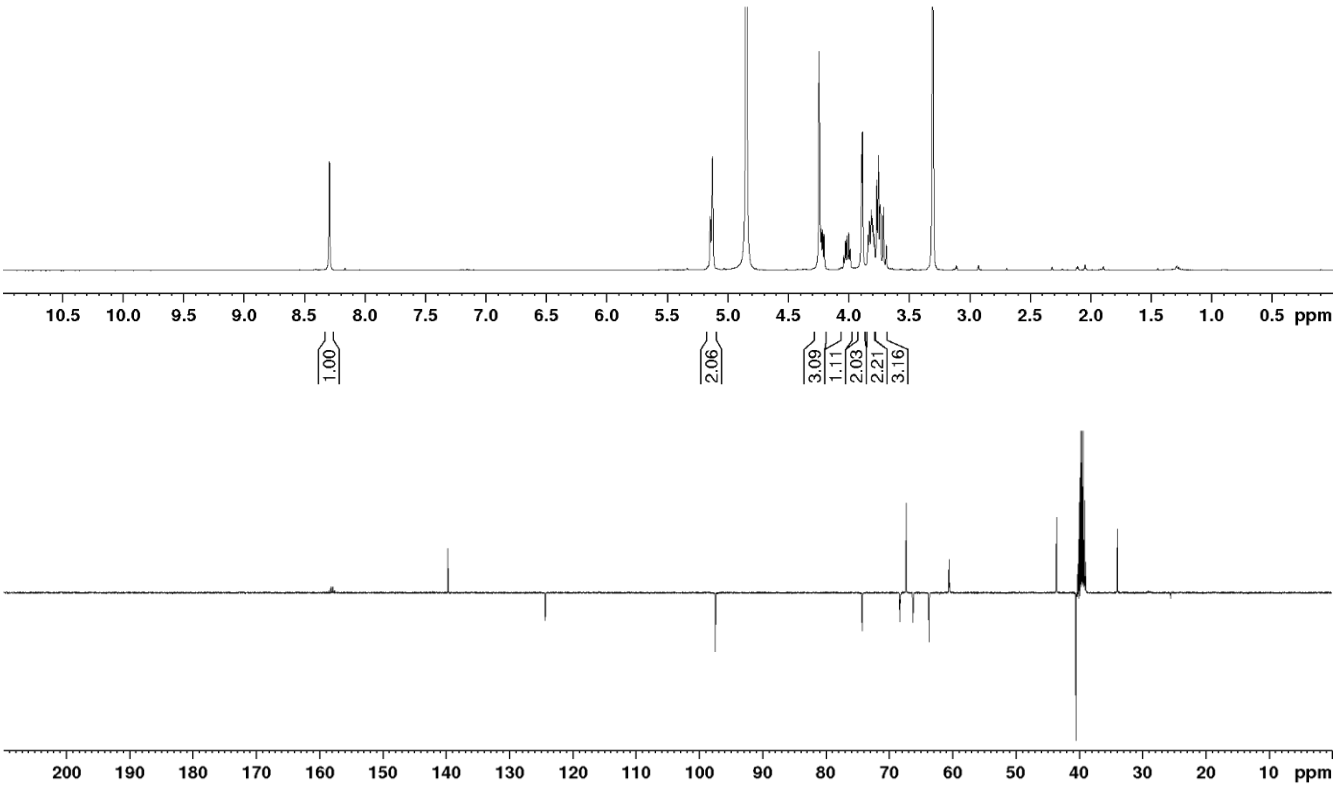
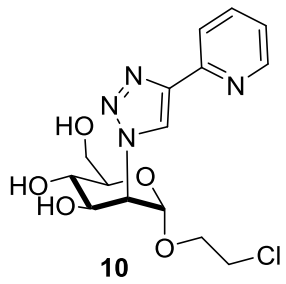

ppm
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 ppm


CF3COO
NH3
N
N
N
HO
HO
HO
O
O
Cl

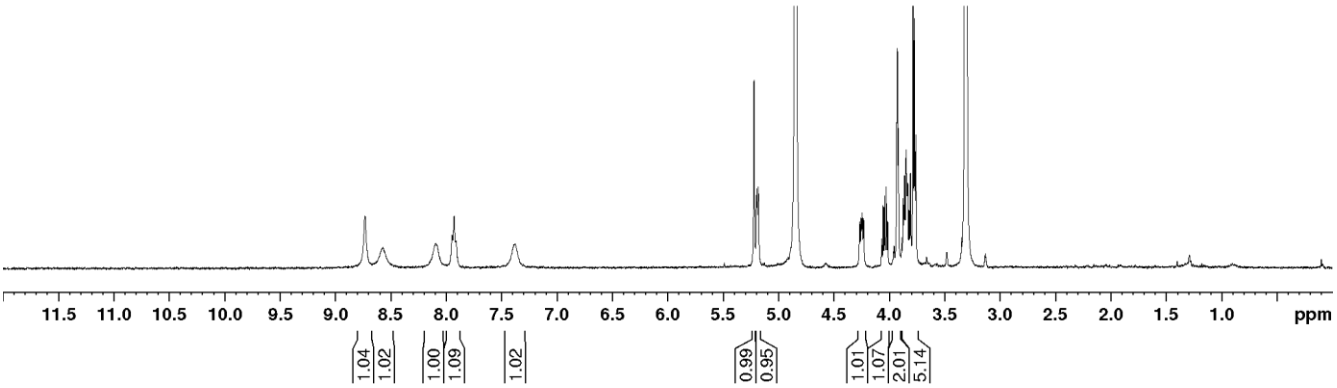
9

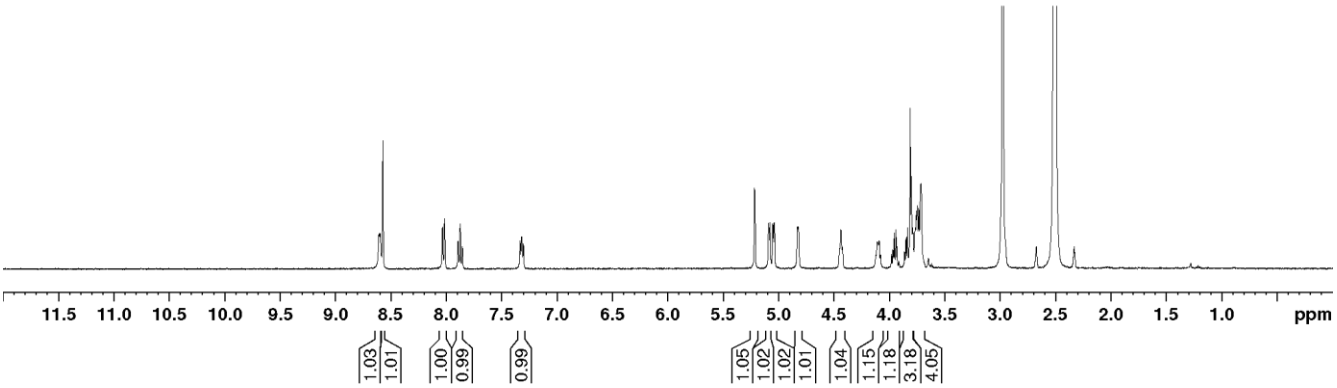

10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
2.06
3.09
1.11
2.03
2.21
3.16

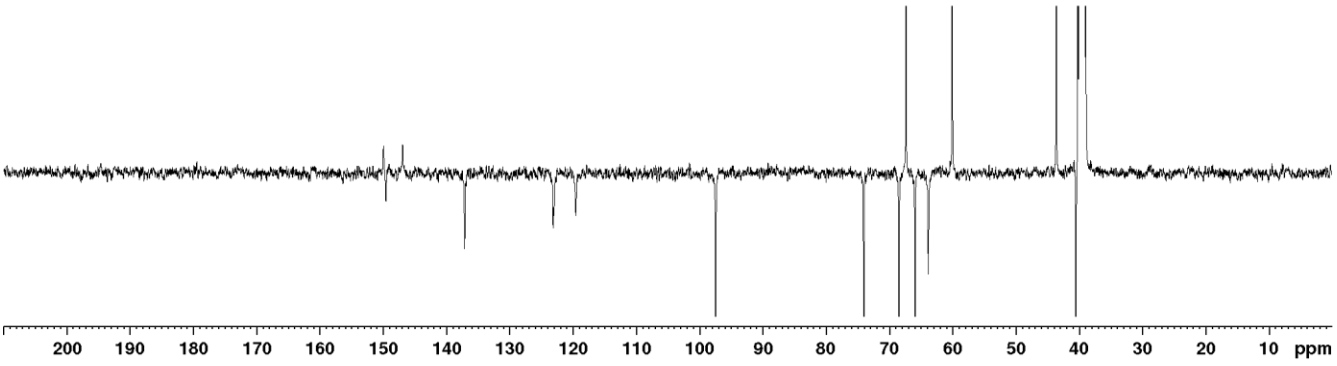
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


HO
N
N
N
N
HO
HO
O
O
Cl

10


11.5 11.0 10.5 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 ppm
1.04 1.02 1.00 1.09 1.02 0.99 0.95 1.01 1.07 2.01 5.14


11.5 11.0 10.5 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 ppm
1.03 1.01 1.00 0.99 0.99 1.05 1.02 1.02 1.01 1.04 1.15 1.18 3.18 4.05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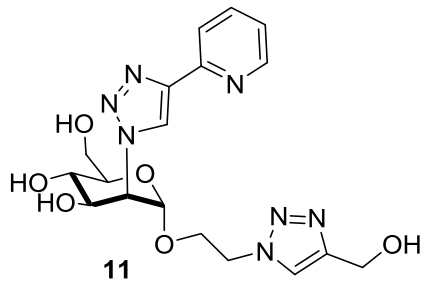
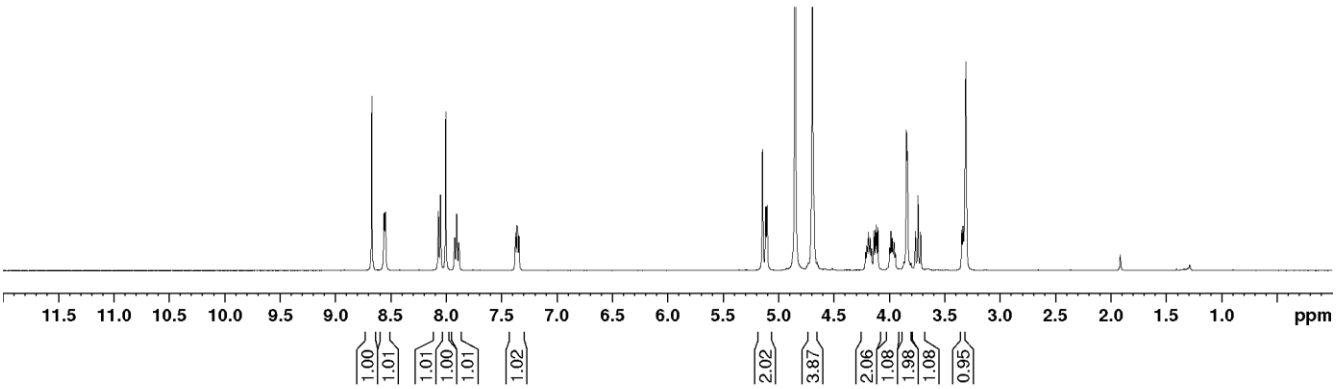
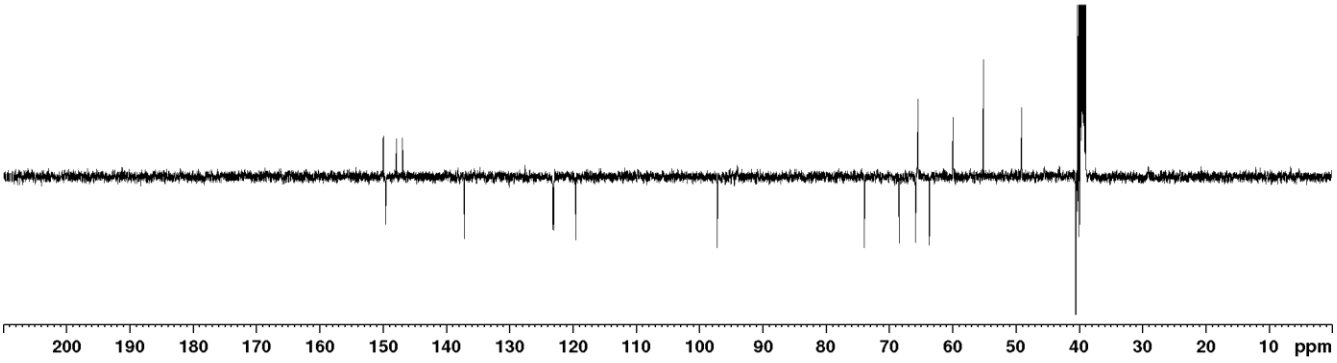

HO
HO
HO
O
N
N
N
O
N
N=N
N
OH

11


11.5 11.0 10.5 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 ppm
1.00 1.01 1.01 1.00 1.01 1.02 2.02 3.87 2.06 1.08 1.98 1.08 0.95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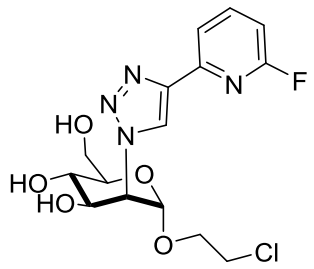
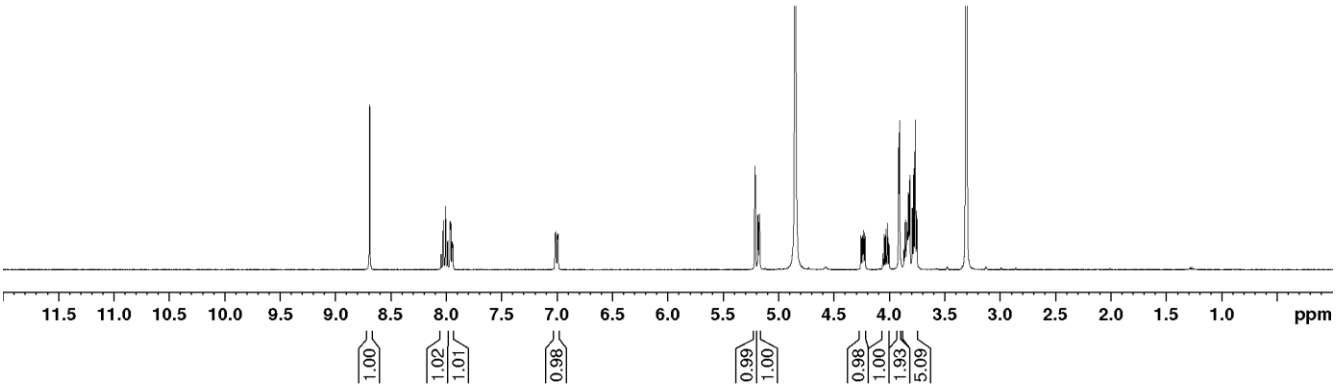
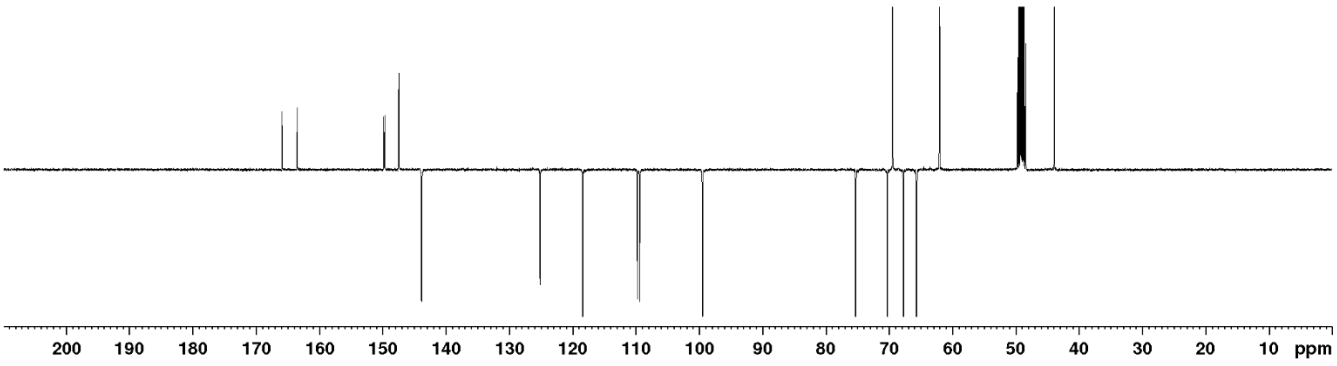

N
N
N
F
HO
N
HO
O
HO
O
Cl

12


11.5 11.0 10.5 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 ppm
1.00
1.02
1.01
0.98
0.99
1.00
0.98
1.00
1.93
5.09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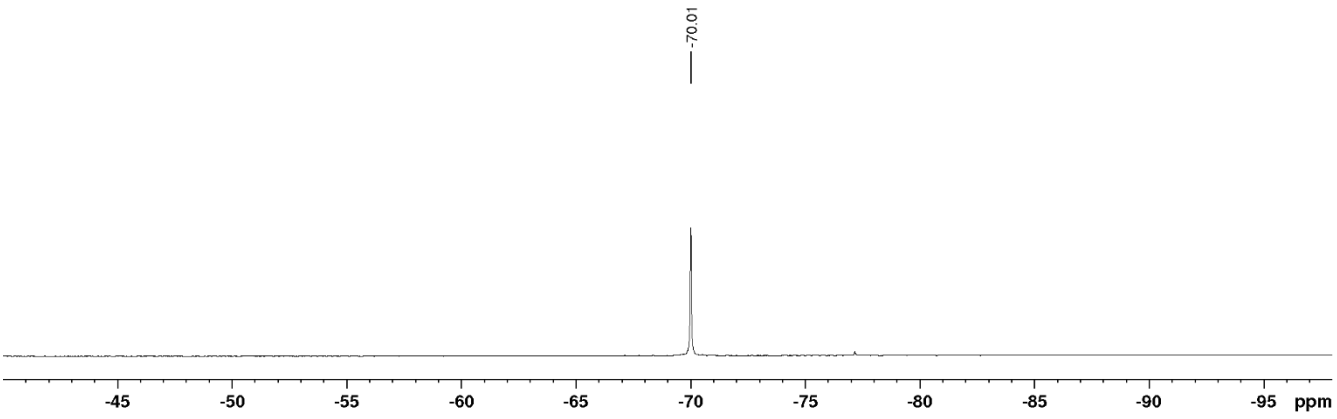
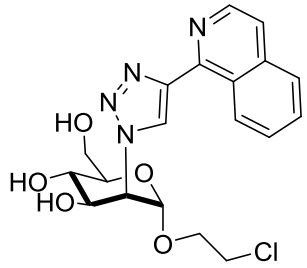
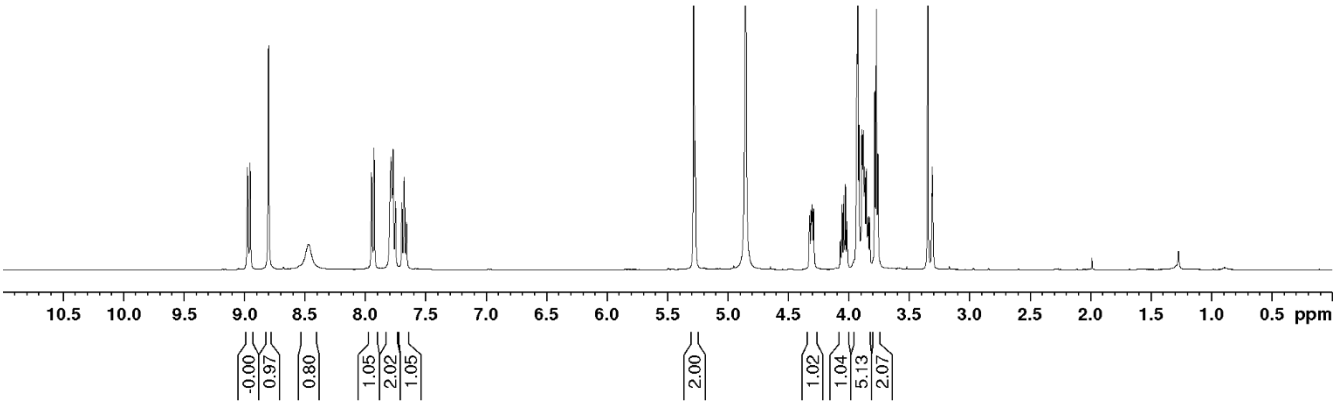
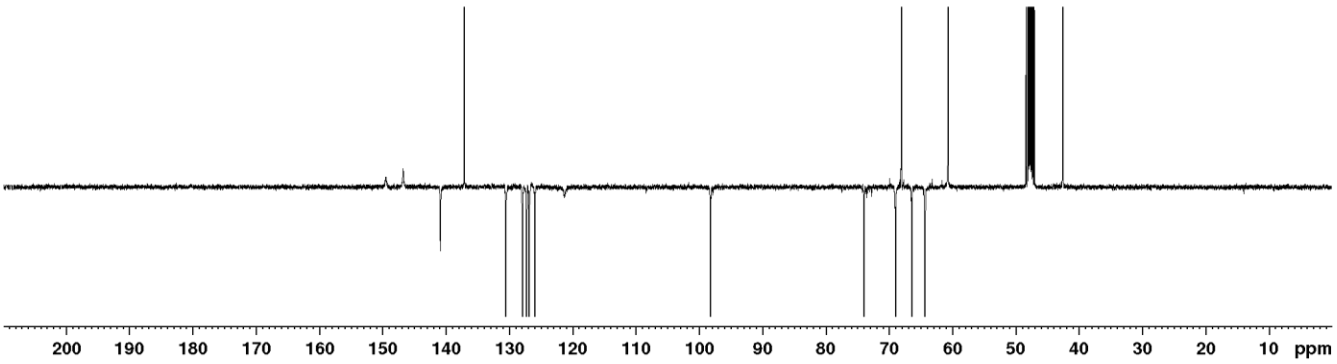

-70.01
-45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 ppm


N
N
N
HO
HO
HO
O
O
Cl

13


10.5 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 ppm
-0.00
0.97
0.80
1.05
2.02
1.05
2.00
1.02
1.04
5.13
2.07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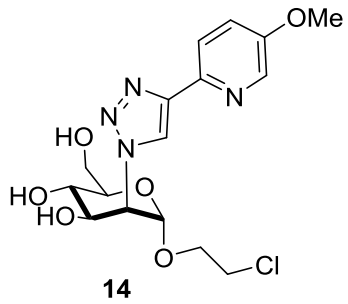
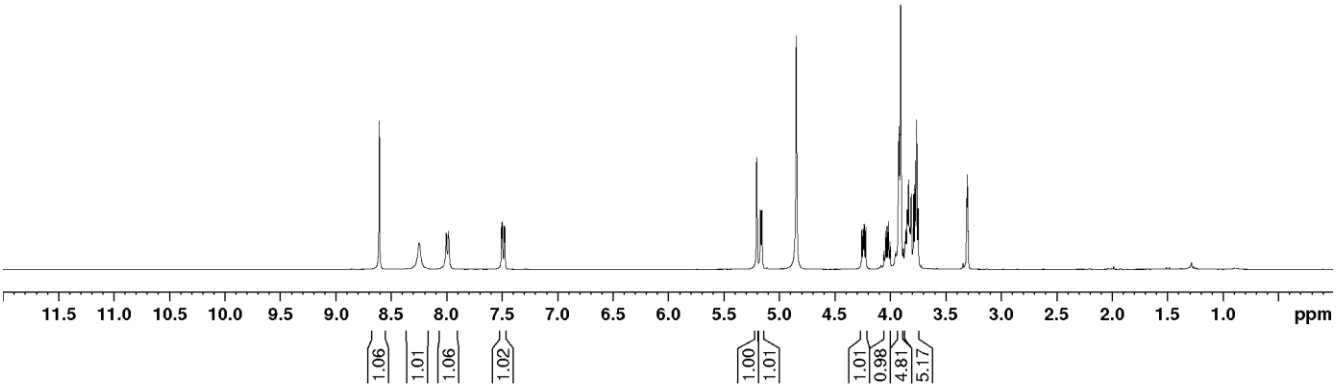
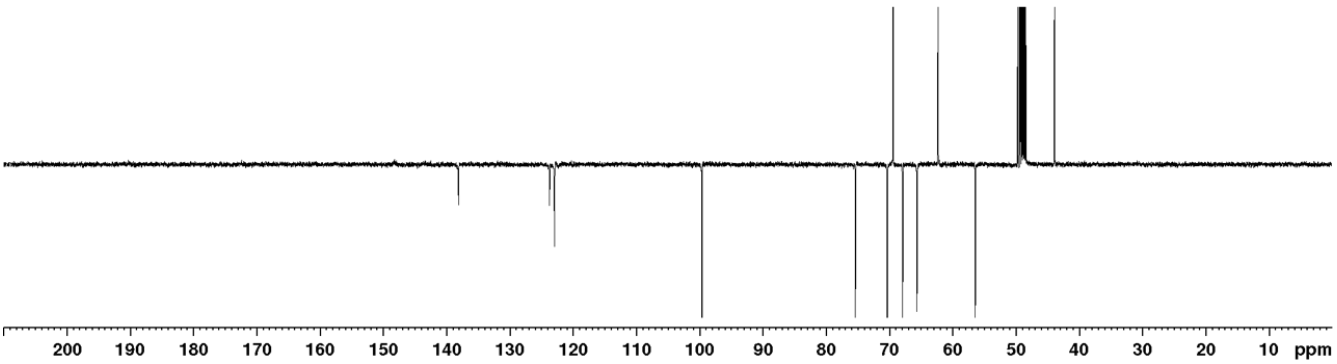

OMe
N
N
N
HO
N
HO
HO
O
O
Cl

14


11.5 11.0 10.5 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 ppm
1.06
1.01
1.06
1.02
1.00
1.01
1.01
0.98
4.81
5.17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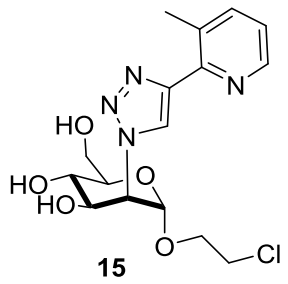
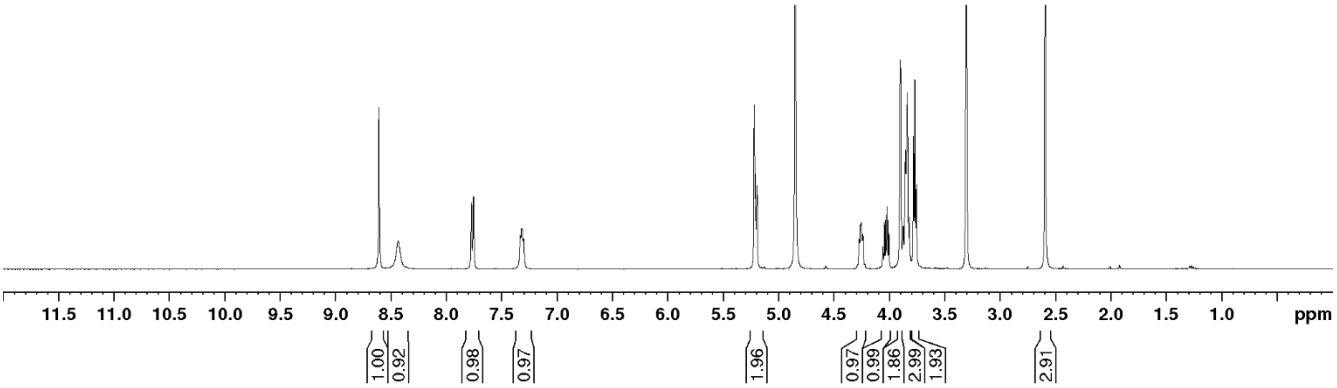
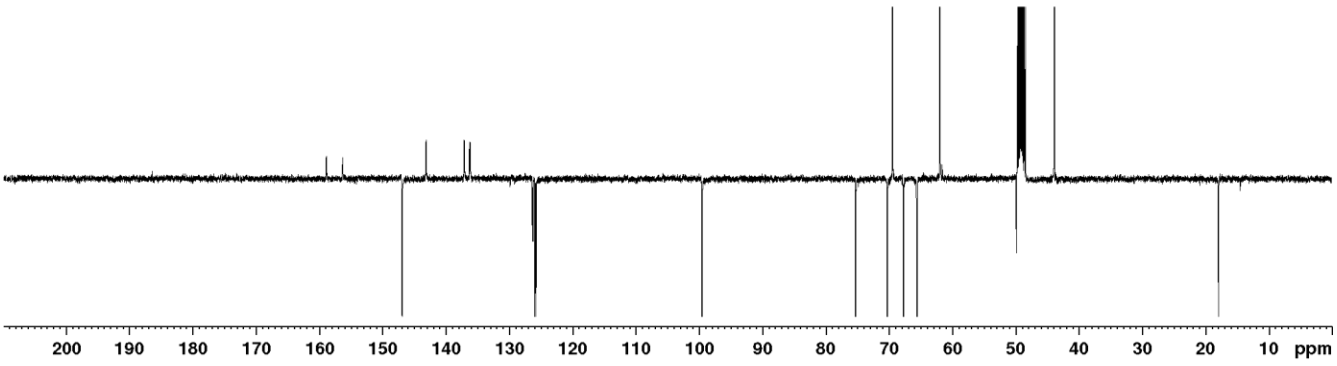

HO
N
N
N
N
HO
HO
O
O
Cl

15


11.5 11.0 10.5 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 ppm
1.00
0.92
0.98
0.97
1.96
0.97
0.99
1.86
2.99
1.93
2.91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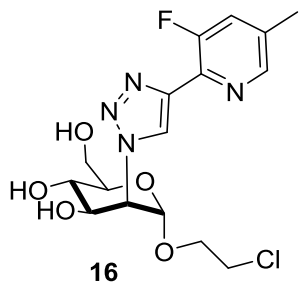
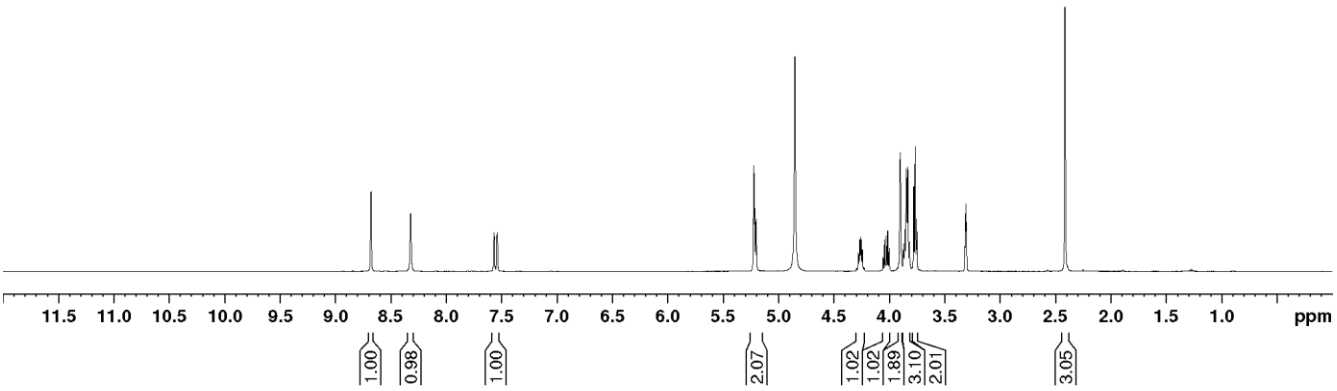
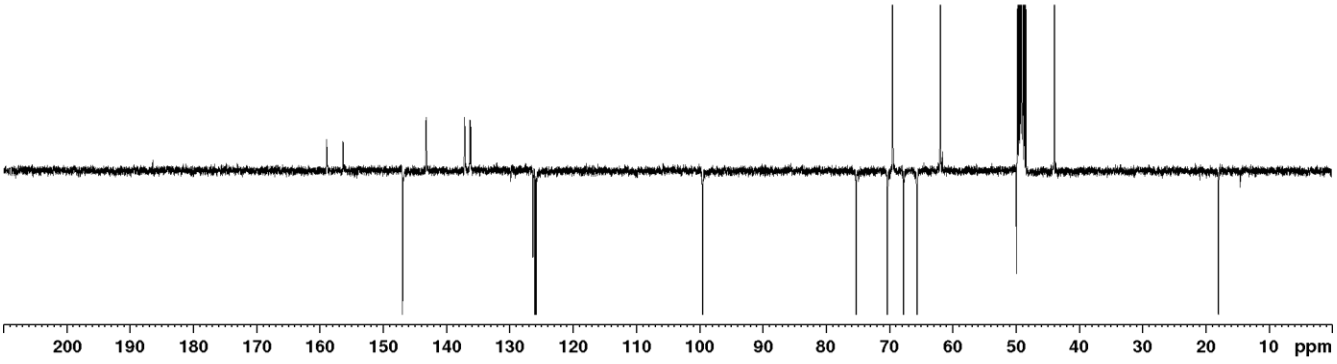
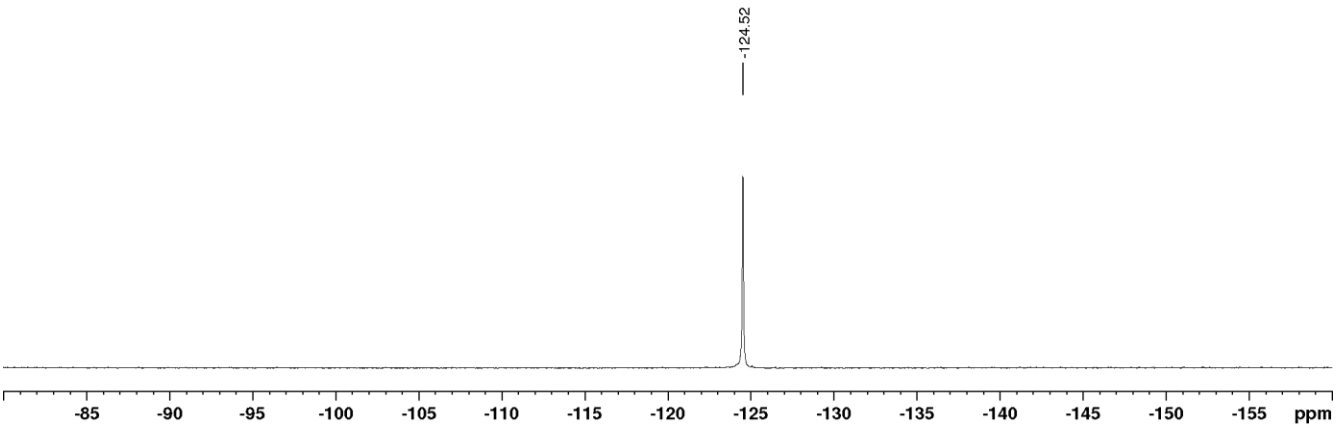

F
N
N
N
HO
N
HO
HO
O
O
Cl

16


11.5 11.0 10.5 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 ppm
1.00
0.98
1.00
2.07
1.02
1.02
1.89
3.10
2.01
3.05


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


-124.52
-85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -135 -140 -145 -150 -155 ppm