Molecular insights into DC-SIGN binding to self-antigens: the interaction with the blood group A/B antigens

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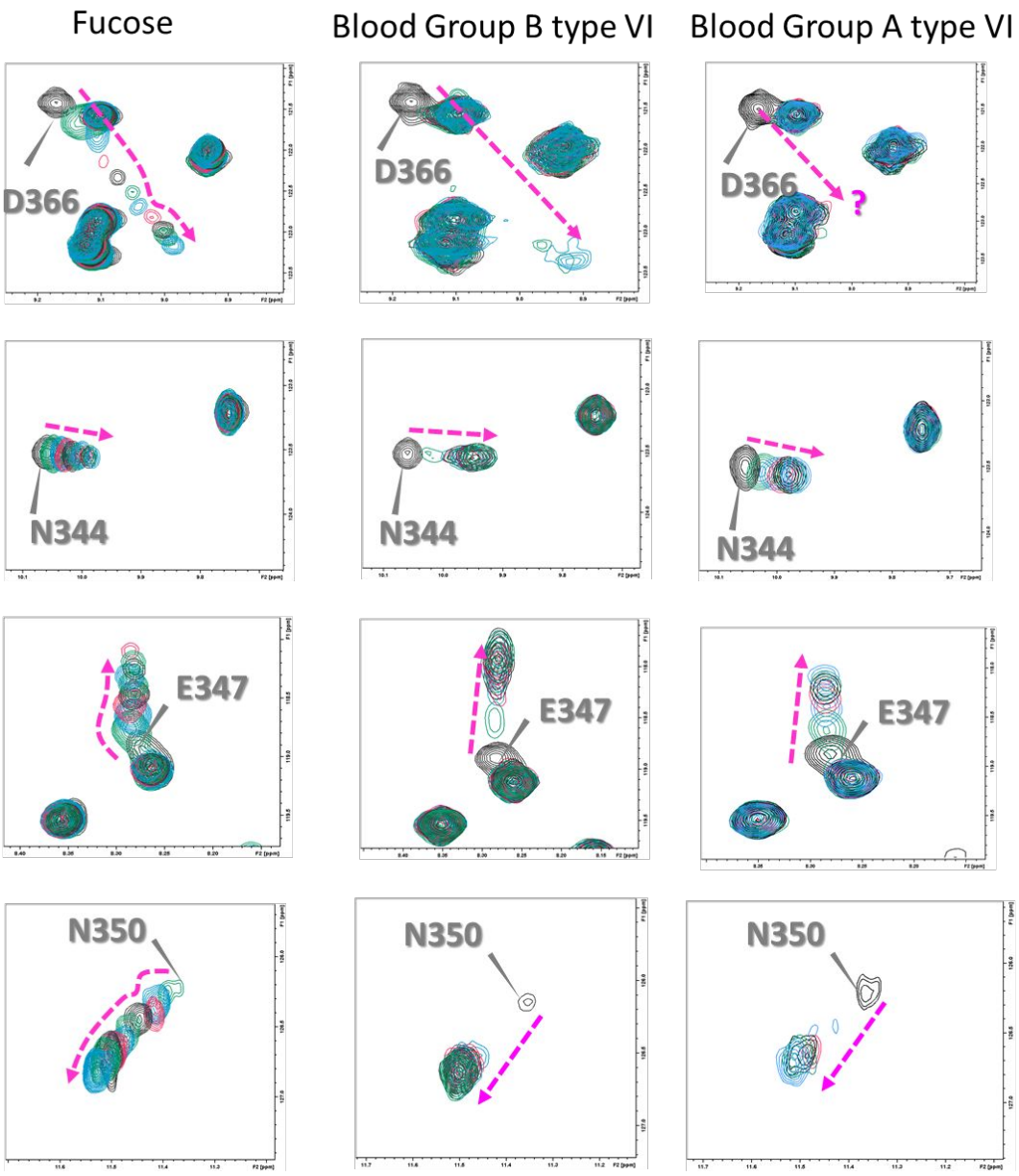

Figure S1. CRD DC-SIGN ^{1}H-^{15}N HQSC: specific aminoacid crosspeak chemical shift perturbation upon increasing additions of ligands: left fucose (**7**), middle blood group B type VI (**2**), right blood group A type VI (**1**).

	Blood Group A type VI	Blood Group B type VI	
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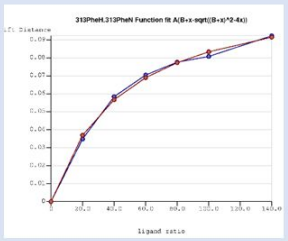
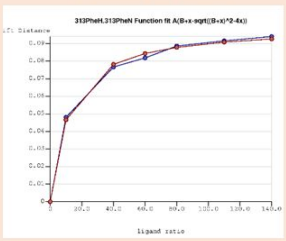
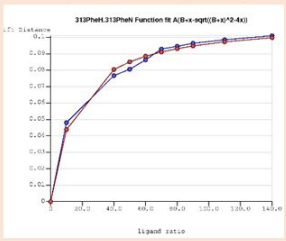
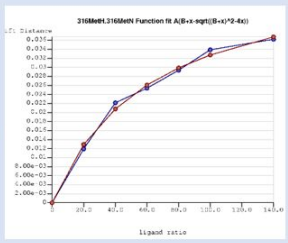
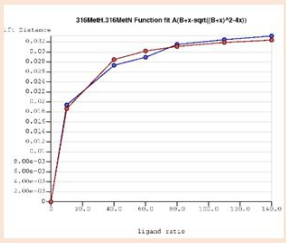
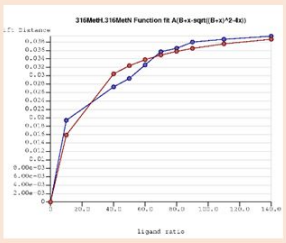
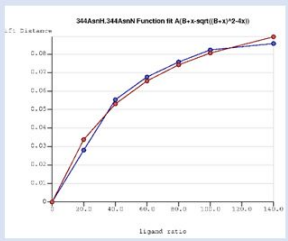
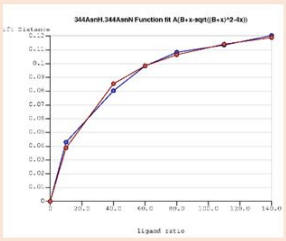
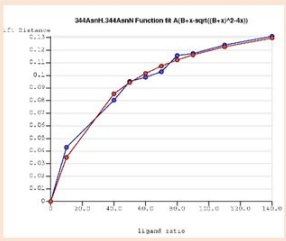
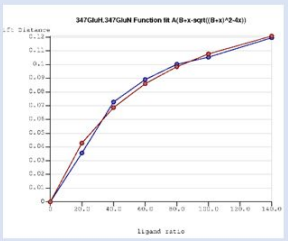
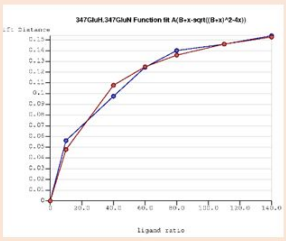
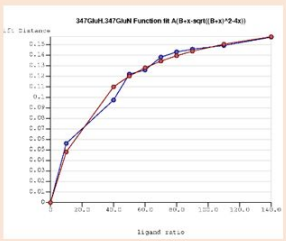
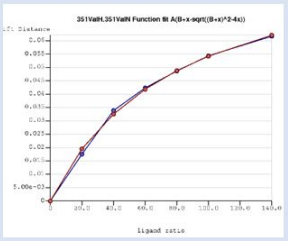
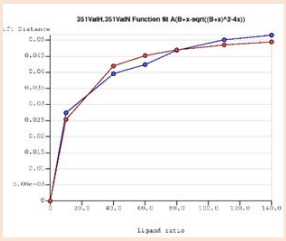
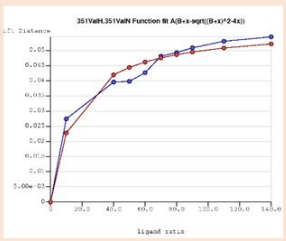
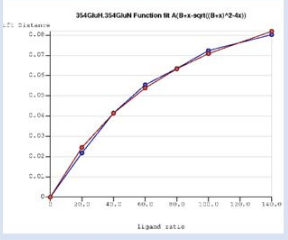
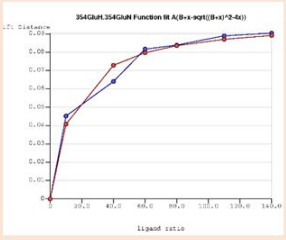
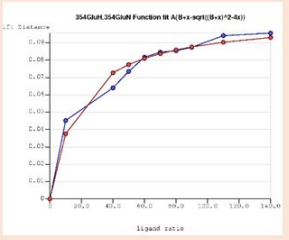
F313			
M316			
N344			
E347			
V351			
E354			

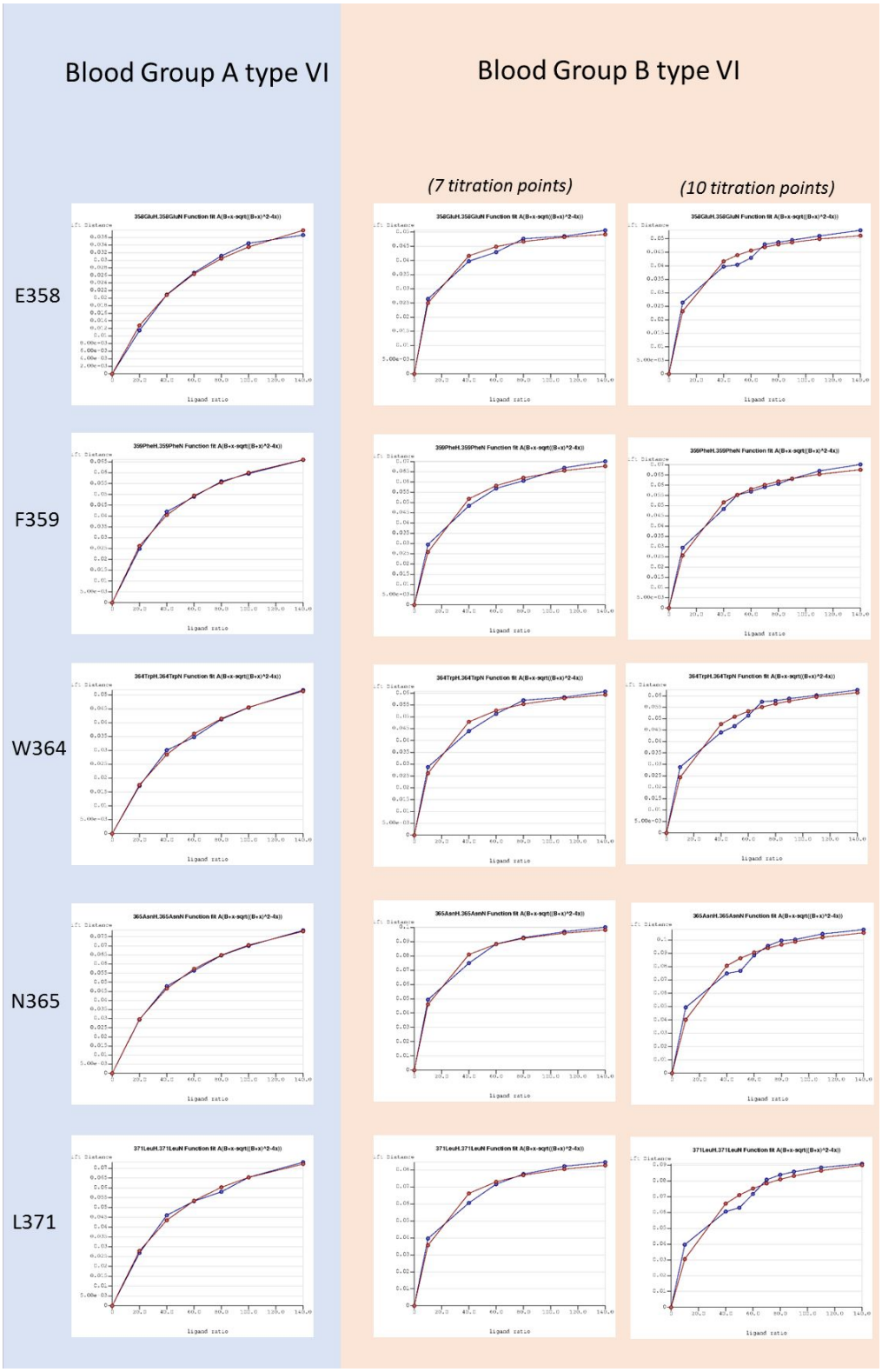


Figure S2. Binding isotherms from CRD DC-SIGN ^{1}H-^{15}N HQSC chemical shift perturbations for specific aminoacid backbone crosspeaks for the interaction with blood group B type VI (**2**) and blood group A type VI (**1**) tetrasaccharides.

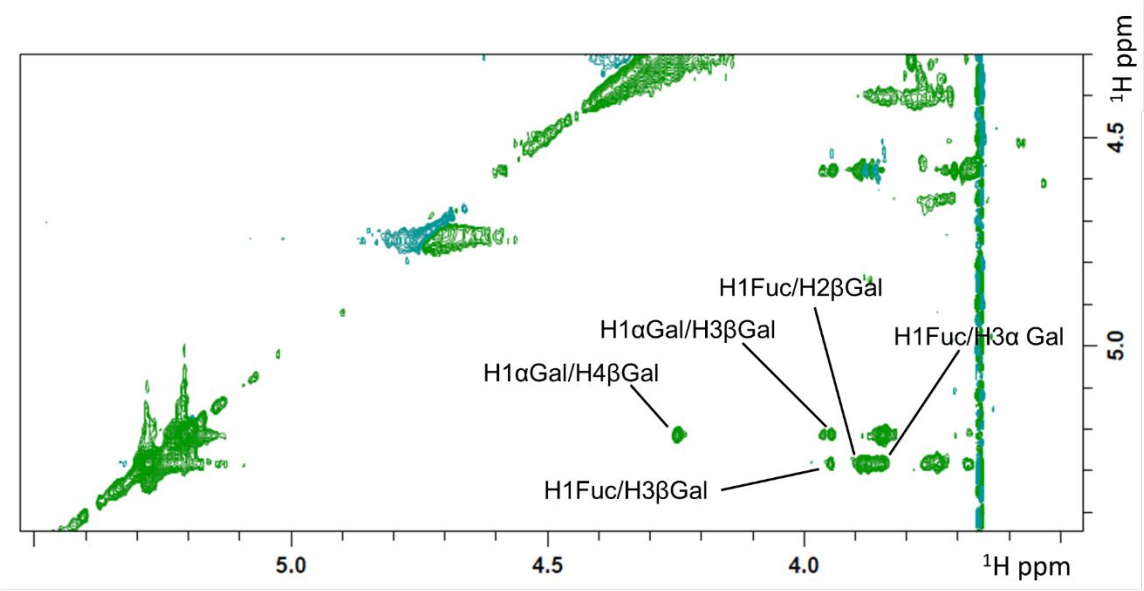


Figure S3. trNOESY spectrum of CRD DC-SIGN with the blood group B type VI tetrasaccharide (compound **2**) with protein/ligand molar ratio 1/5. The key interresidual NOEs are annotated. The spectrum was acquired at 298K with 100 ms of mixing time, in an 800 MHz spectrometer.

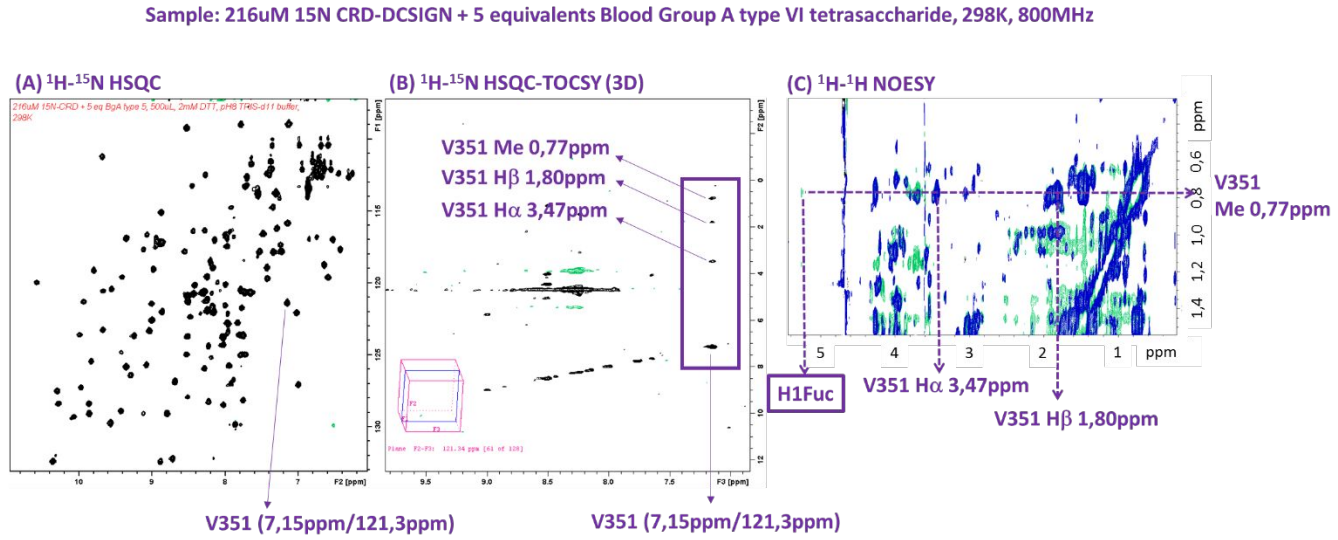


Figure S4. Assignment of the intermolecular NOES H1Fuc/HγV351. (A) ^{1}H-^{15}N HSQC and (B) ^{1}H-^{15}N HSQC-TOCSY of a sample of 216uM ^{15}N CRD-DCSIGN + 5 equivalents Blood Group A type VI tetrasaccharide at 298K at 800MHz, showing the TOCSY correlations of the ^{1}H-^{15}N backbone crosspeak at δ(^{1}H/^{15}N) 7.15/121.3: Hα at 3.47ppm, Hβ at 1.80ppm and Me (Hγ) at 0.77ppm. (C) trNOESY spectrum (green) of 202 CRD-DCSIGN + 5 equivalents Blood Group A type VI tetrasaccharide at 298K at 800MHz and NOESY spectrum (blue) of 202 CRD-DCSIGN, showing the NOE correlations from δ(^{1}H) 0.77ppm: Hα at 3.47ppm, Hβ at 1.80ppm of V351 and the intermolecular H1Fuc.

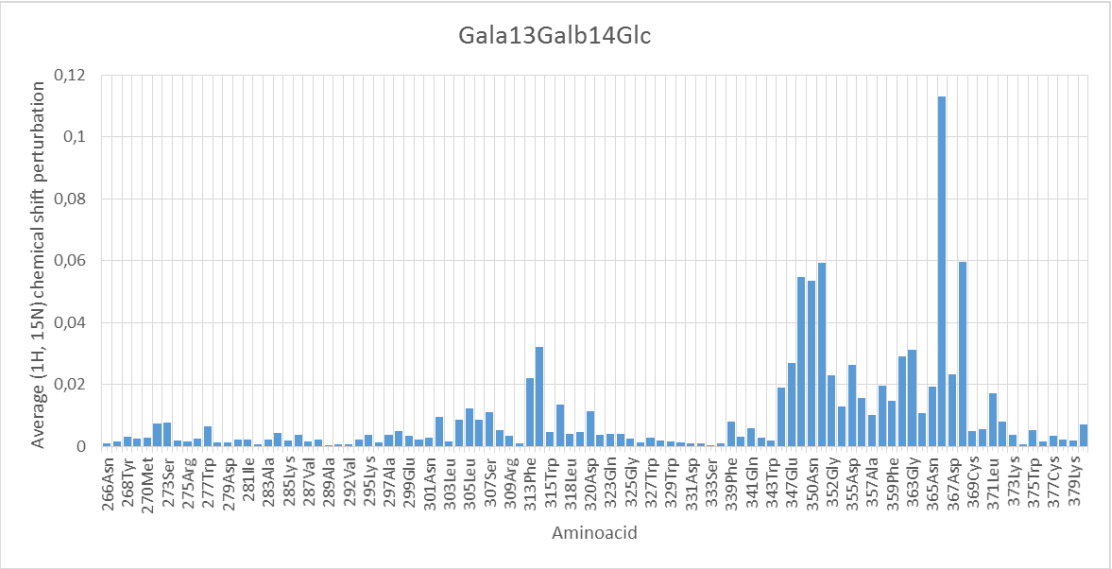


Figure S5. CRD DC-SIGN ^{1}H-^{15}N average chemical shift perturbation for the interaction with the Galα1-3Galβ1-4Glc trisaccharide (compound **3**).

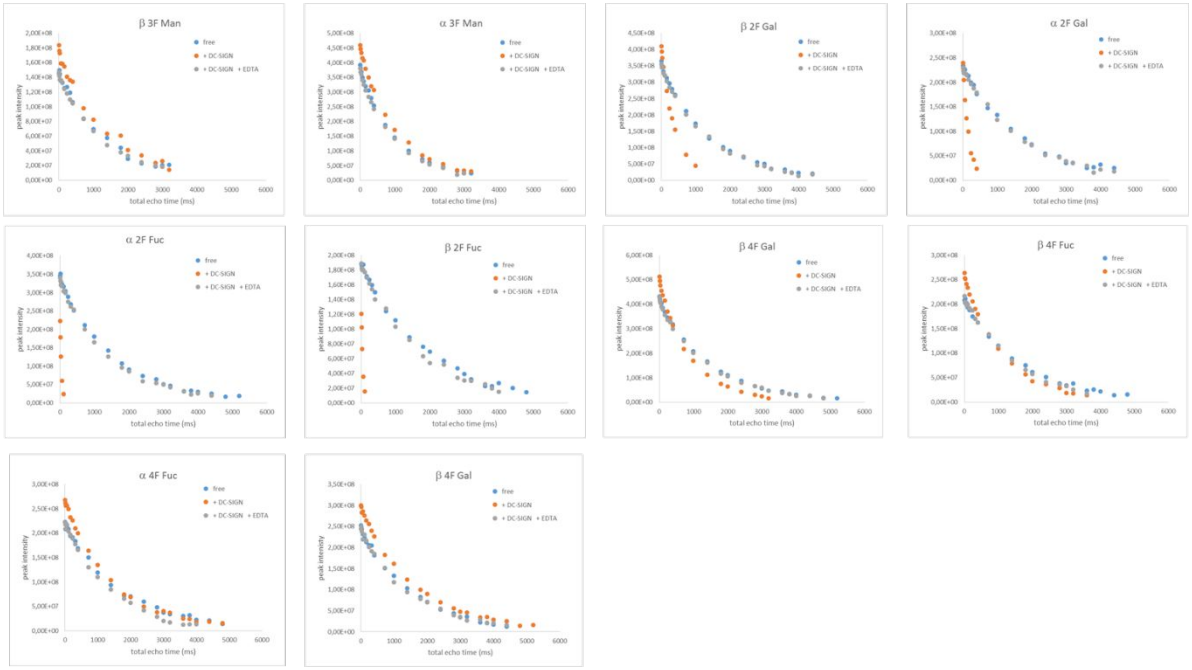


Figure S6. ^{19}F T_2 decay curves from ^{19}F CPMG experiments. In blue, for a sample containing 0.8mM of every monosaccharide (the concentration of each anomer will

depend on the anomerization equilibrium, except for 4F-OMe-Fuc). In orange, for a sample of 80uM CRD DC-SIGN and 0.8mM of every monosaccharide. In grey, after the addition of 20mM EDTA-d12.

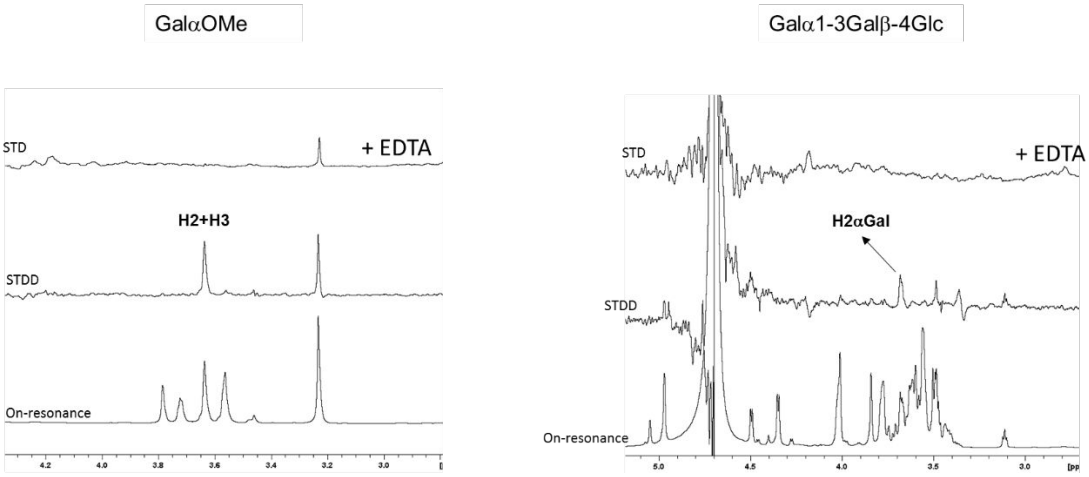


Figure S7. ^{1}H-STD experiments for αGalOMe (left) and Galα1-3Galβ-4Glc (right) in the presence of EDTA. Below: off-resonance spectra for samples containing 80uM of CRD DC-SIGN and 60 equivalents of the corresponding ligand. Middle: STDD spectra of the corresponding ligand (STD of the protein free has been subtracted to the STD of the ligand, acquired under the same conditions). Top: STD of the ligand after the addition of 20mM EDTA-d12. The remaining signal in the STD spectrum of GalaOMe, corresponding to the Me group is an artifact of the pulse sequence used (stddiff.3 from Bruker).

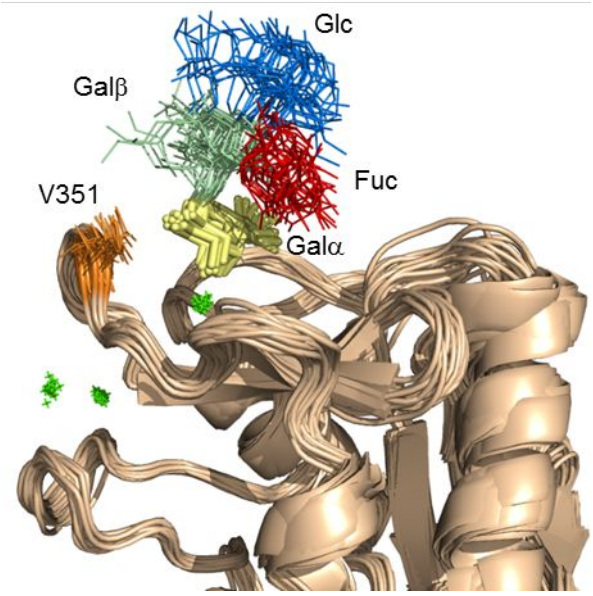
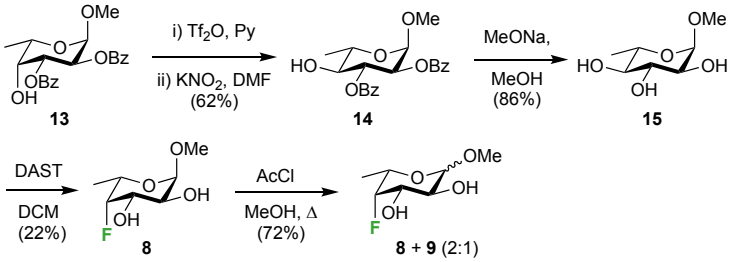


Figure S8. Superimposition of 25 frames along 200ns MD simulation of the complex between CRD DC-SIGN and the blood group B type VI (compound **2**) binding through the terminal αGal residue, coordinating Ca^{+2} with 3-OH/4-OH.

10. Synthetic procedures and characterization data



Scheme S1: The synthesis of methyl 4-deoxy-4-fluoro-α-L-fucopyranoside

Methyl 2,3-di-*O*-benzoyl-α-L-quinovoside 14

To a solution of ester α-**13**[1] (5.80 g, 15.0 mmol) in CH_2Cl_2 (43 mL) and pyridine (11 mL) at 0 °C was added triflic anhydride (4.90 mL, 30.0 mmol) dropwise. After 45 min at 0 °C the reaction was quenched with water (50 mL), the aqueous phase separated and then extracted with CH_2Cl_2 (2 x 50 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The orange crude residue was re-dissolved in DMF (70 mL) then KNO_2 (3.80 g, 45.0 mmol) was added. After 18 h at RT the reaction mixture was diluted with a 1:1 mixture of CH_2Cl_2 and H_2O (120 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 50 mL) and the combined organic phases were washed with sat. $NaHCO_3$ (100 mL), brine (100 mL), dried over $MgSO_4$, filtered, concentrated *in vacuo* then purified by column chromatography (silica, 10-15% EtOAc in petroleum ether) to give the title compound **14** as a colourless foam (3.60 g, 9.3 mmol, 62%). **IR** (neat) 3476 (m br), 2935 (m br), 1721 (s), 1277 (s), 1264 (s), 1053 (s), 708 (s) cm^{-1}; **$[\alpha]_D$** -92 (c 1, pyridine, 25 °C), lit[2] (+107 (c 1, pyridine, D-enantiomer); **^{1}H NMR (400 MHz, $CDCl_3$)** δ 8.03–7.91 (4H, m, CH_{Ar}), 7.52 (2H, tq, *J* 7.6, 1.3 Hz, CH_{Ar}), 7.37 (4H, t, *J* 7.6 , CH_{Ar}), 5.66 (1H, t, *J* 9.9 Hz, H-3), 5.28 (1H, dd, *J* 10.1, 3.7 Hz, H-2), 5.06 (1H, d, *J* 3.7 Hz, H-1), 3.90 (1H, dq, *J* 9.5, 6.2 Hz, H-5), 3.56 (1H, br t, *J* 9.4, H-4), 3.44 (3H, s, OCH_3), 2.88 (1H, br s, OH), 1.41 (3H, d, *J* 6.2 Hz, H-6) ppm; **^{13}C NMR (101 MHz, $CDCl_3$)** δ 167.7 (C=O), 166.0 (C=O), 133.4 (CH_{Ar}), 133.3 (CH_{Ar}), 129.9 (CH_{Ar}), 129.23 (C_{Ar}), 129.19 (C_{Ar}), 128.40 (CH_{Ar}), 128.37 (CH_{Ar}), 96.9 (C-1), 75.2 (C-4), 74.6 (C-3), 71.6 (C-2), 67.6 (C-5), 55.3 ($O\underline{C}H_3$), 17.5 (C-6) ppm; **MS (ESI+)** (m/z) 409 $[M+Na]^+$; **HRMS (ESI+)** for $C_{21}H_{22}NaO_7$ $(M+Na)^+$ calcd. 409.1263, found. 409.1260. ^{1}H-NMR and ^{13}C-NMR data match literature data.[2]

Methyl-α-L-quinovoside (15)

To a solution of ester **14** (3.55 g, 9.2 mmol, 1 eq) in MeOH (74 mL) was added NaOMe (25% wt solution in MeOH, 0.63 mL, 2.8 mmol). After 45 min Amberlite® IR 120 was added until pH = 7 was reached, the RM was filtered, concentrated *in vacuo* and then purified by column chromatography (silica, 0 to 10% MeOH in CH_2Cl_2) to give the title compound **15** as an off-white solid (1.40 g, 7.9 mmol, 86%). **IR** (neat) 3383 (m br), 2907 (m br), 1047 (s), 1277 (s), 1012 (m) cm^{-1}; **$[\alpha]_D$** -155 (c 0.275, chloroform, 25 °C), lit[3] (-154 (c 1, chloroform, 25 °C); **^{1}H NMR (100 MHz, CD_3OD)** δ 4.59 (1H, d, *J* 3.8 Hz, H-1), 3.60 (1H, dq, *J* 9.5, 6.1 Hz, H-5), 3.55 (1H, t, *J* 9.5, 9.3 Hz, H-3), 3.38 (3H, s, O$C\underline{H}_3$), 3.38 (1H, dd, *J* 9.5, 3.8 Hz, H-2), 2.97 (1H, t, *J* 9.5 Hz, H-4), 1.23 (3H, d, *J* 6.1 Hz, H-6) ppm; **^{13}C NMR (101 MHz, CD_3OD)** δ 101.4 (C-1), 77.6 (C-4), 75.0 (C-3), 73.9 (C-2), 68.8 (C-5), 55.6 (O$\underline{C}H_3$), 18.2 (C-6) ppm; **MS (ESI+)** (m/z) 201 $[M+Na]^+$; **HRMS (ESI+)** for $C_7H_{14}NaO_5$ $(M+Na)^+$ calcd. 203.0739, found. 201.0735. NMR data match literature data.[4]

Methyl 4-fluoro-4-deoxy-α-L-fucopyranoside 8

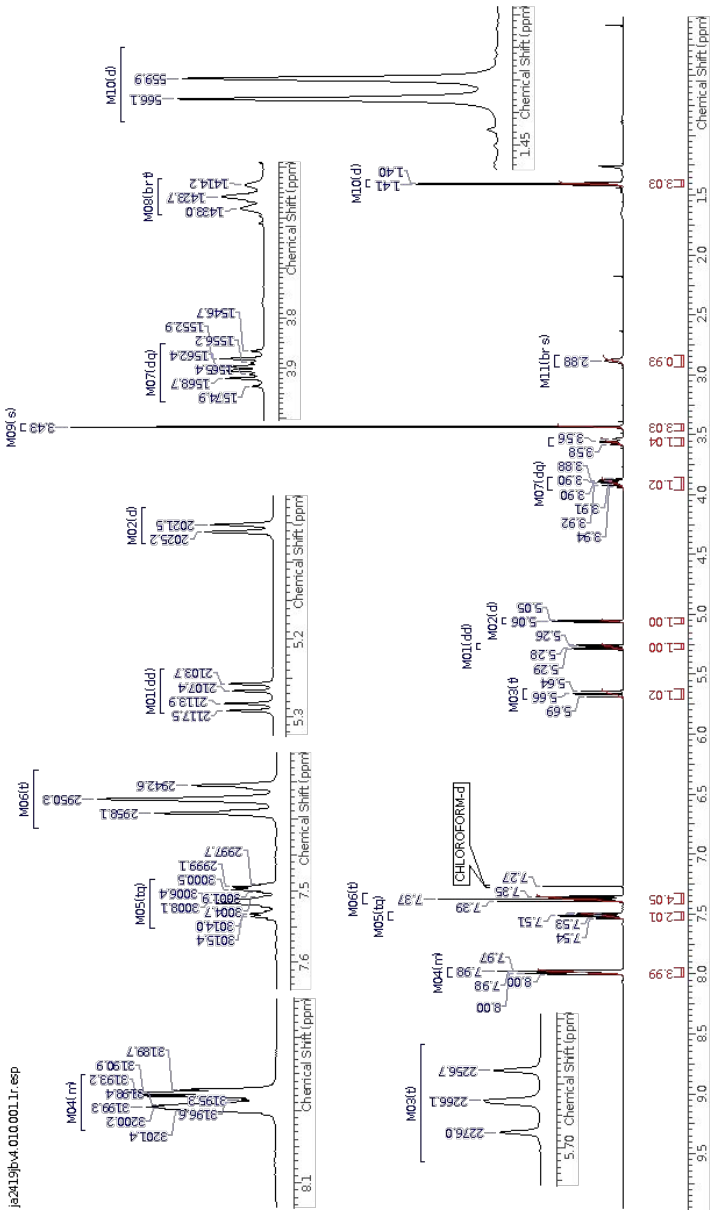
To a solution of **15** (1.4 g, 7.9 mmol) in CH_2Cl_2 (8.5 mL) at 0 °C was added DAST (1.7 mL, 14.2 mmol). After 2 h the reaction mixture was cooled to 0 °C and DAST (1.7 mL, 14.2 mmol) was added. After an additional 20 h the reaction was quenched at 0 °C with MeOH (10 mL), concentrated *in vacuo* and then purified by column chromatography (silica, 30-60% acetone/petroleum ether) to give first the title compound **8** as an off-white solid (310 mg, 1.7 mmol, 22%) and then the starting material (530 mg 2.94 mmol, 37%). **IR** (neat) 3365 (m br), 2943 (m br), 1366 (m), 1084 (s), 1051 (s), 989 (s), 759 (s) cm^{-1}; **$[\alpha]_D$** -191.2 (c 1, MeOH, 25 °C); **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.80 (1H, d, *J* 3.2 Hz, H-1), 4.59 (1 H, dd, *J* 50.5, 2.5 Hz, H-4), 3.93 (1 H, dq, *J* 29.8, 6.7 Hz, H-5), 3.86–3.75 (2H, m, H-2 + H-3), 3.44 (3H, s, O$C\underline{H}_3$), 2.69 (1H, br s, OH-3), 2.36 (1H, br d, *J* 7.8 Hz, OH-2), 1.33 (3H, dd, *J* 6.9, 0.7 Hz, H-6) ppm; **^{13}C NMR (101 MHz, $CDCl_3$)** δ 99.4 (C-1), 91.9 (d, *J* 181.9 Hz, C-4), 70.2 (d, *J* 19.1 Hz, C-3), 69.5 (d, *J* 2.9 Hz, C-2), 65.2 (d, *J* 18.3 Hz, C-5), 55.6 (O$\underline{C}H_3$), 15.8 (d, *J* 5.9 Hz, C-6) ppm; **^{19}F NMR (376 MHz, $CDCl_3$)** δ -221.8 (dt, *J* 50.3, 29.5, 29.5 Hz, F-4) ppm; **^{19}F{^{1}H} NMR (376 MHz, $CDCl_3$)** δ -221.8 (s, F-4) ppm; **MS (ESI+)** (m/z) 203 $[M+Na]^+$; **HRMS (ESI+)** for $C_7H_{13}FNaO_4$ $(M+Na)^+$ calcd. 203.0695, found. 203.0693.

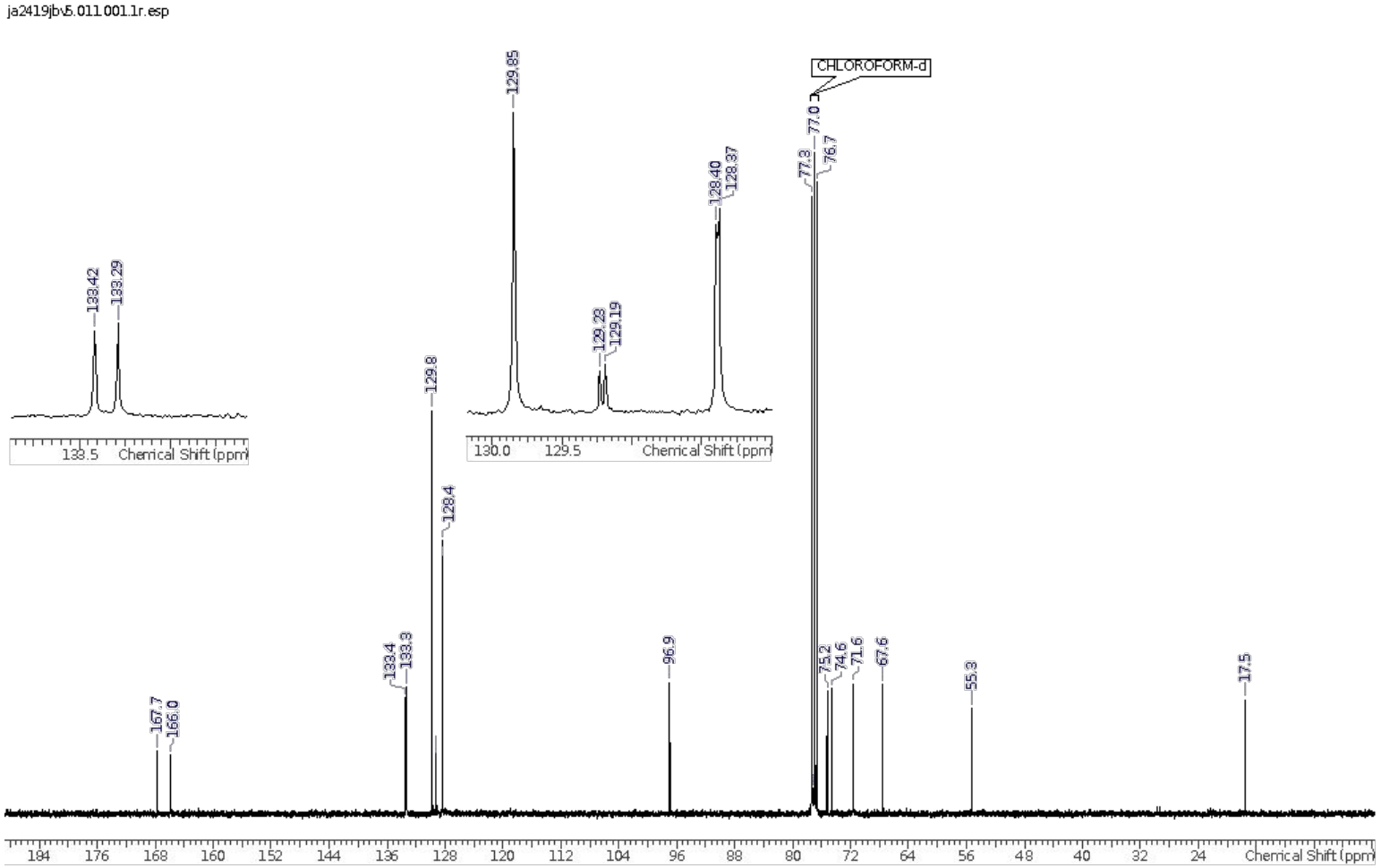
Anomerization of methyl 4-fluoro-4-deoxy-α-L-fucopyranoside 8

To a solution of **8** (31 mg, 0.17 mmol) in MeOH (0.45 mL) was added acetyl chloride (61 mg, 55 μL, 0.77 mmol) dropwise (CAUTION: very exothermic). The reaction mixture was then heated at 65 °C for 16 h. $NaHCO_3$ (72.3 mg, 0.86 mmol) was added followed by concentration *in vacuo* and purification by column chromatography (5 to 10% MeOH in CH_2Cl_2) to give a mixture of α- and β-anomers **8** and **9** as an off white solid. (0.12 mmol, 72%, ratio α:β 2:1). Selected data for β-anomer: **^{1}H NMR (400 MHz, $CDCl_3$)** δ 4.54 (1H, ddd, *J* 49.5, *J* 2.7, *J* 0.6 Hz), 4.17 (1H, dd, *J* 7.3, 1.0 Hz, H-1), 3.68 (H, dq, *J* 27.0, 6.7 Hz, H-5), 3.71-3.61 (2H, m, H-2 + H-3), 3.57 (3H, s, $OC\underline{H}_3$), 2.78 (1H, br s, OH), 2.69 (1H, br s, OH), 1.40 (3H, dd, *J* 6.7, 0.7 Hz) ppm; **^{19}F NMR (376 MHz, $CDCl_3$)** δ -218.3 (dt, *J* 49.4, 29.0, 29.0 Hz, F-4) ppm; **^{19}F{^{1}H} NMR (376 MHz, $CDCl_3$)** δ -218.3 (s, F-4) ppm.

11. Copies of NMR spectra of the synthetic intermediates and final compounds

Methyl 2,3-di-*O*-benzoyl-α-L-quinovoside 14

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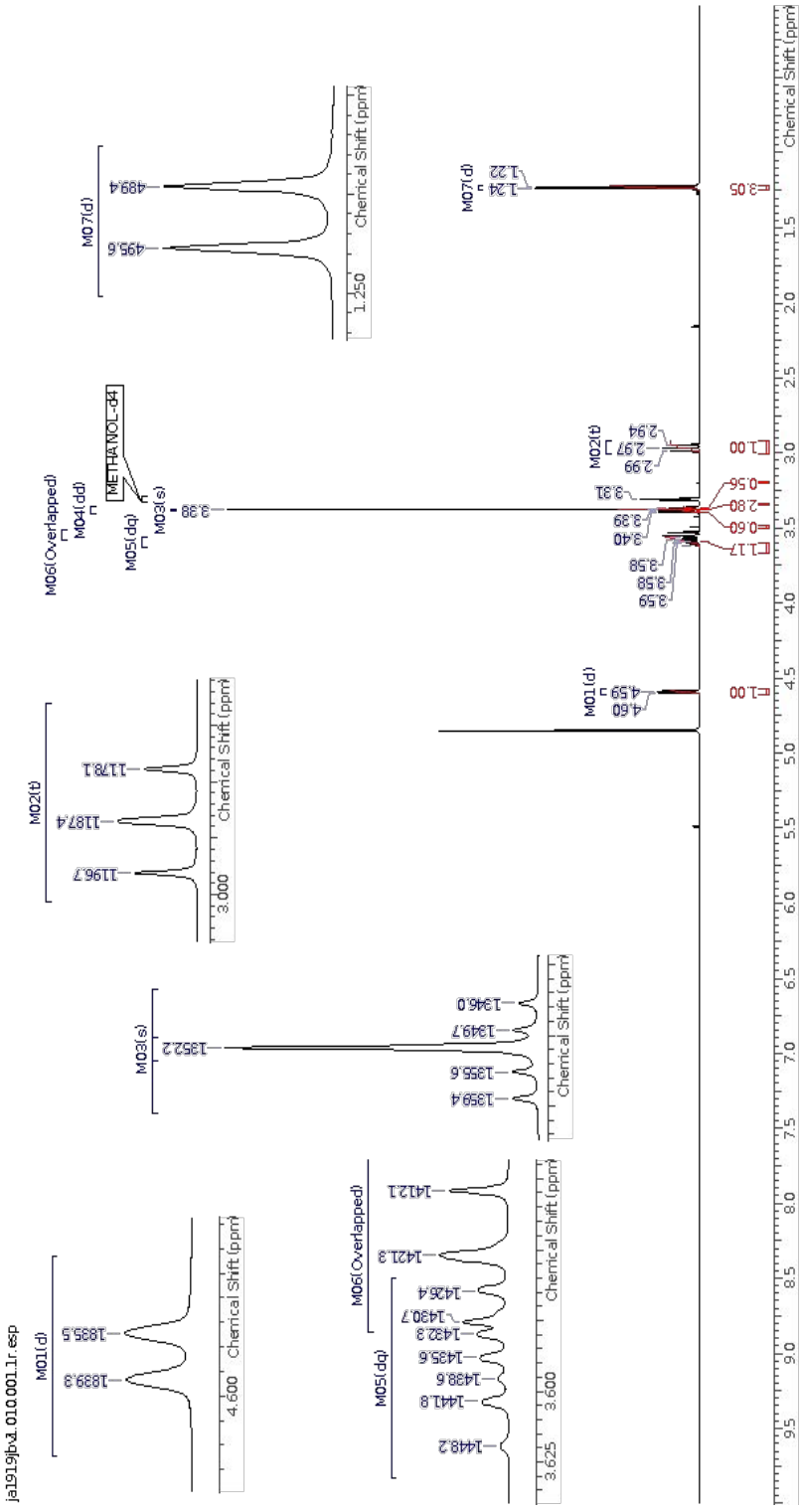
Chemical Shift (ppm)

Methyl-α-L-quinovoside 15



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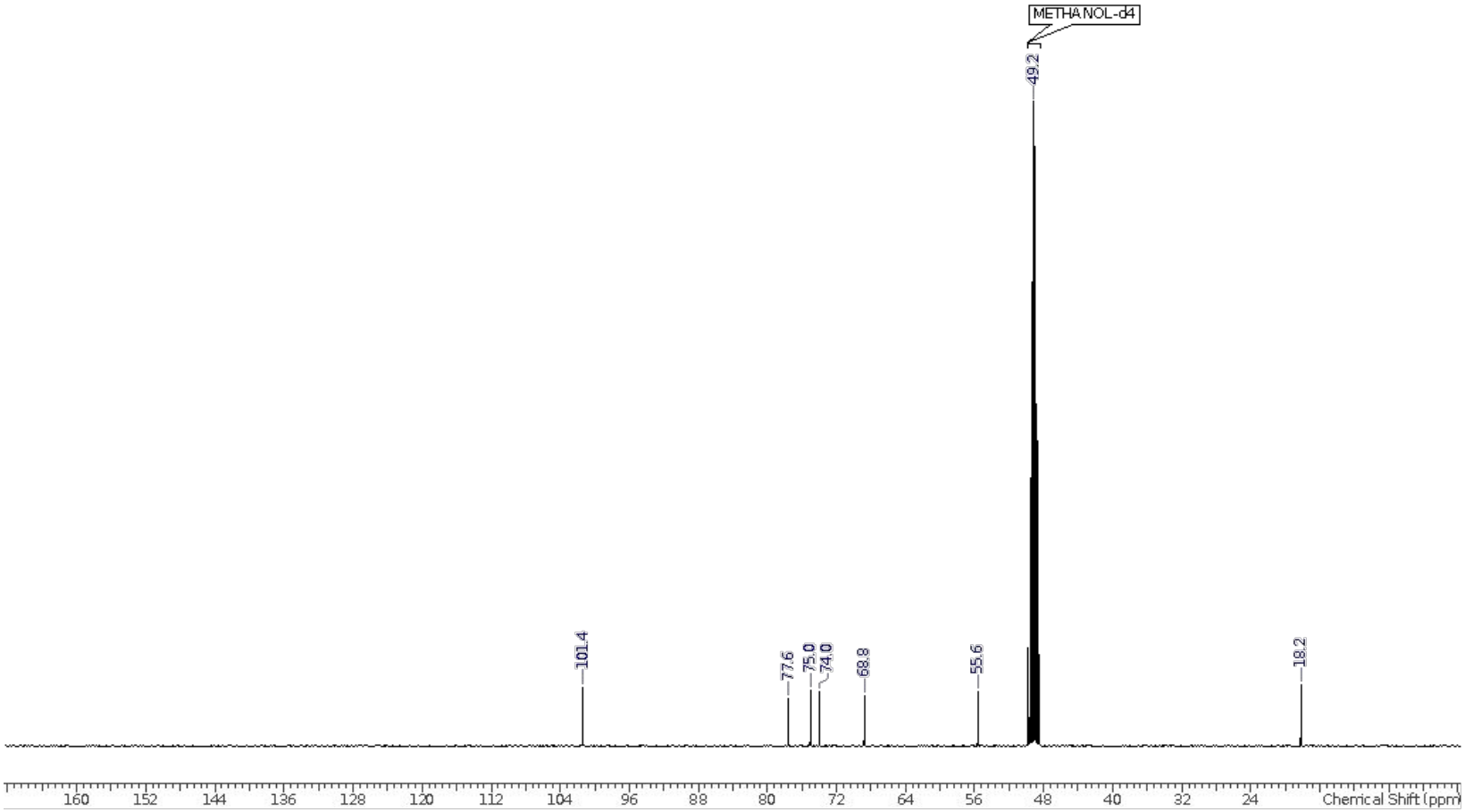
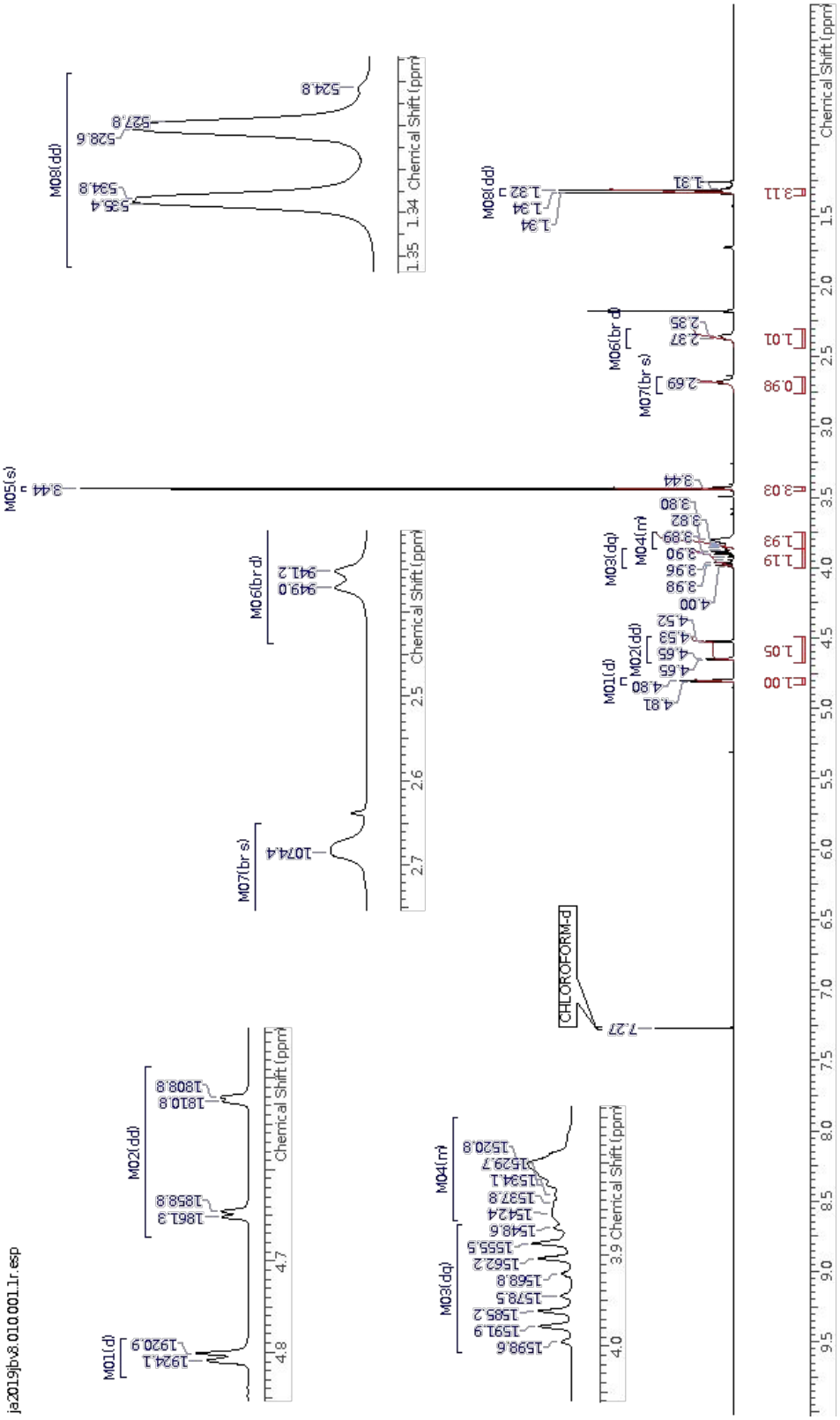
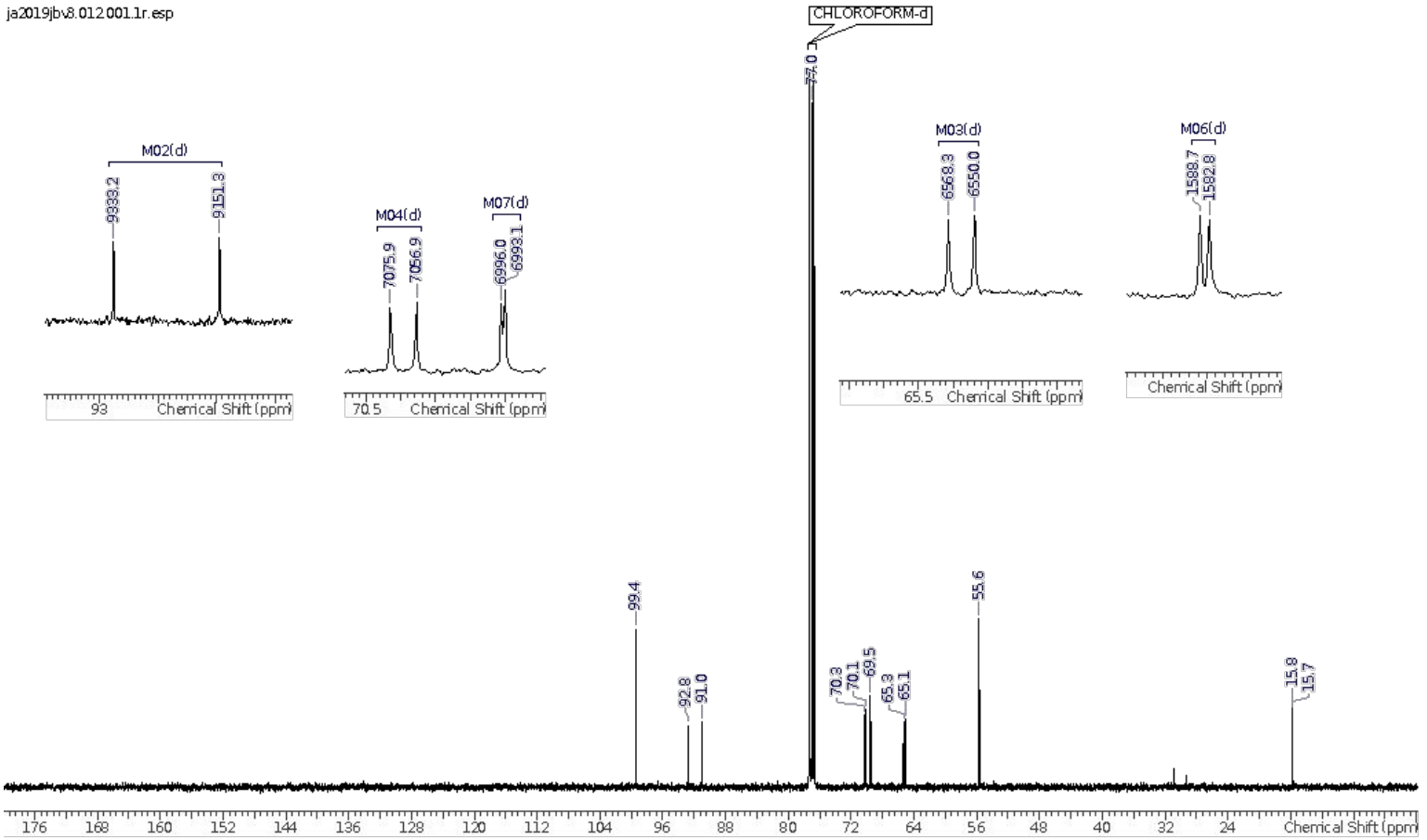
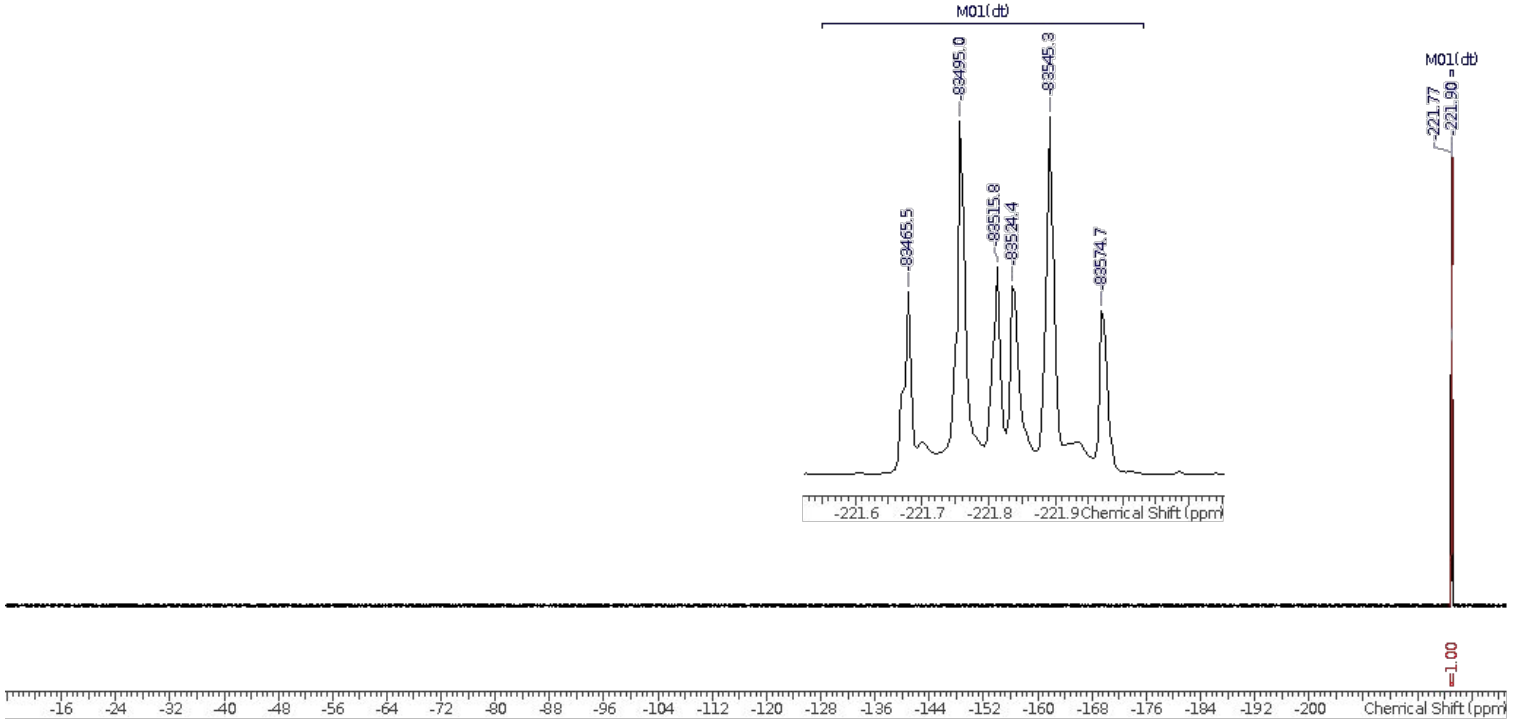


Methyl 4-fluoro-4,6-dideoxy-α-L-fucopyranoside 8

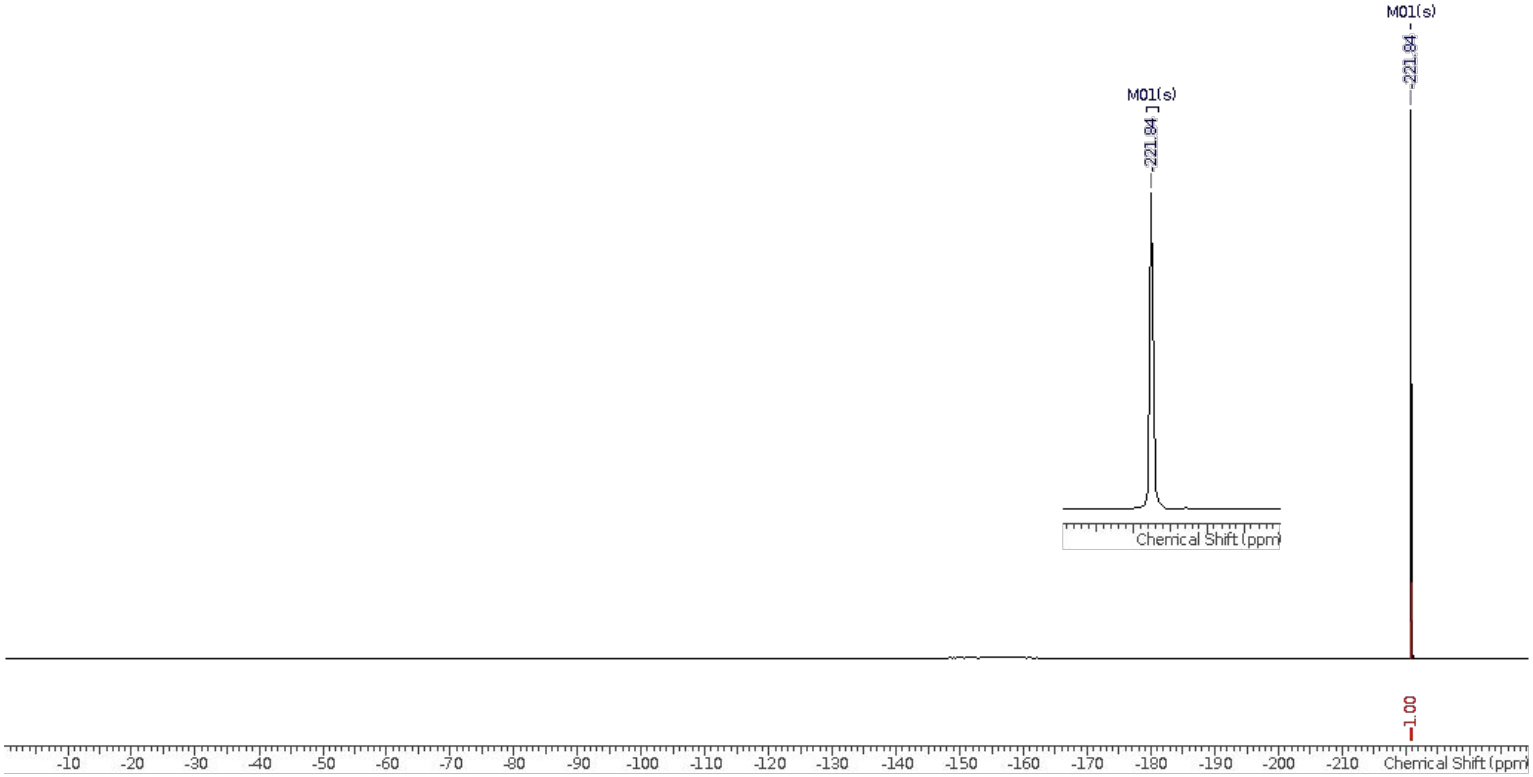



ja2019jbv8.012.001.1r.esp
CHLOROFORM-d
77.0
M02(d)
9338.2
9151.3
M04(d)
7075.9
7056.9
M07(d)
6996.0
6993.1
M03(d)
6568.3
6550.0
M06(d)
1588.7
1582.8
93 Chemical Shift (ppm)
70.5 Chemical Shift (ppm)
65.5 Chemical Shift (ppm)
Chemical Shift (ppm)
99.4
92.8
91.0
70.3
70.1
69.5
65.3
65.1
55.6
15.8
15.7
176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 Chemical Shift (ppm)

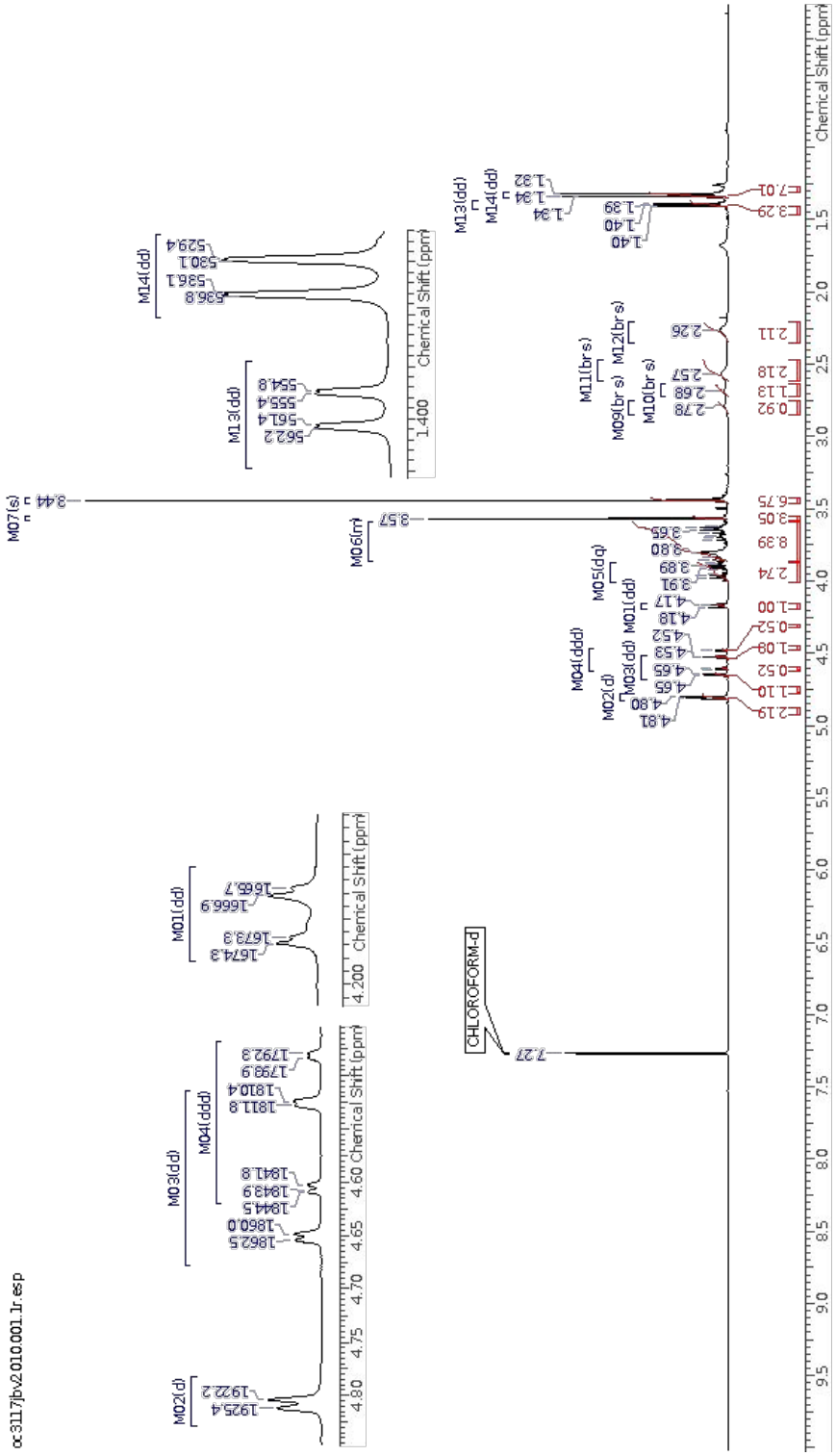
ja2019jbv8.016.001.1r.esp

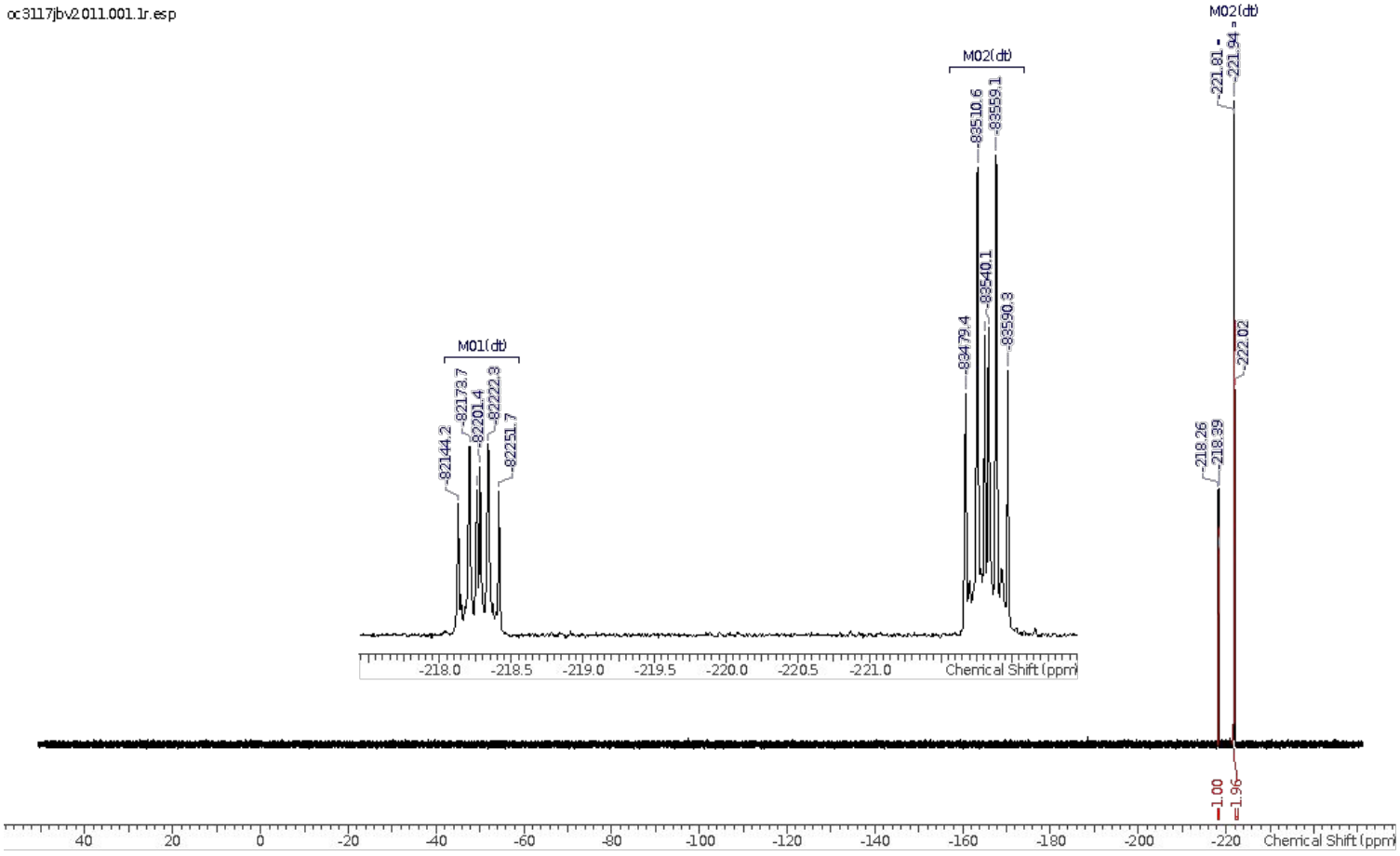


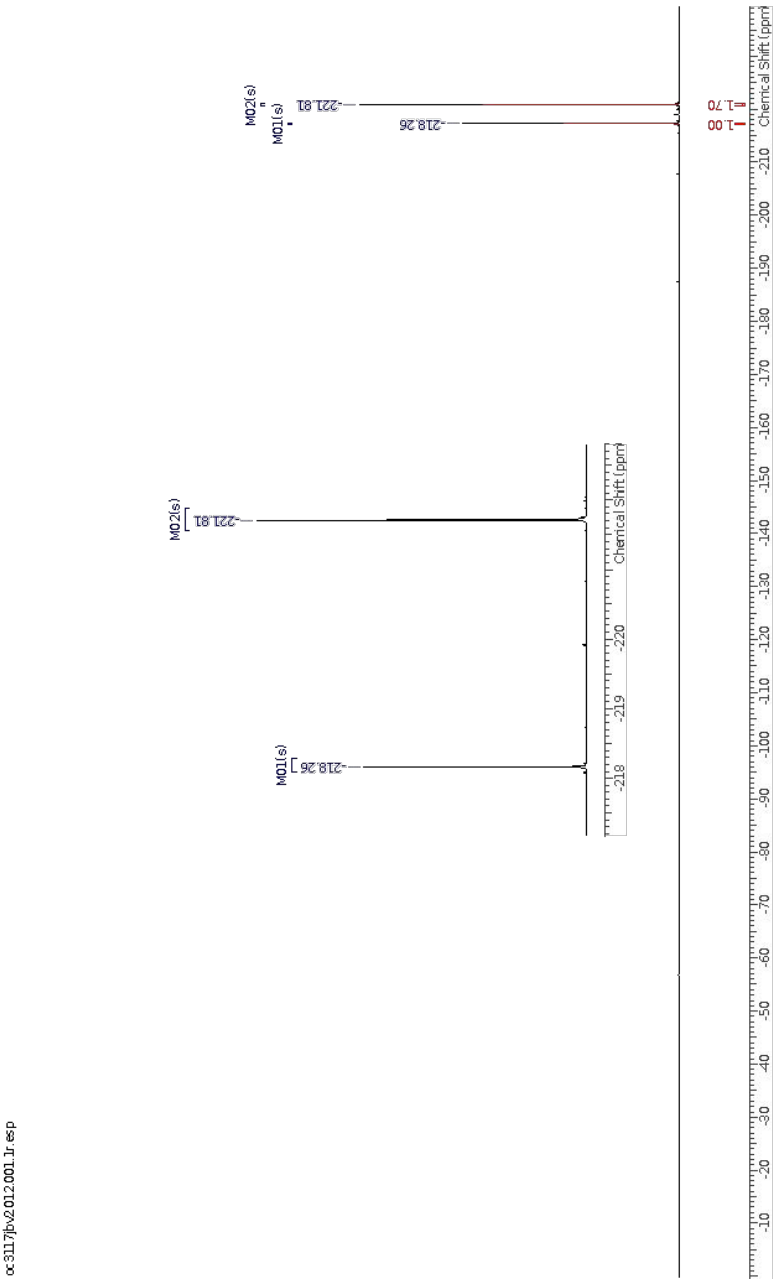
ja2019jbv8.017.001.1r.esp



Methyl 4-fluoro-4,6-dideoxy-L-fucopyranoside, mixture of anomers 8 and 9




oc3117jbv2011.001.1r.esp
M01(dt)
-82144.2
-82178.7
-82201.4
-82222.3
-82251.7
M02(dt)
-83479.4
-83510.6
-83540.1
-83559.1
-83590.3
M02(dt)
-221.81
-221.94
-222.02
-218.26
-218.39
-1.00
-1.96
-218.0 -218.5 -219.0 -219.5 -220.0 -220.5 -221.0 Chemical Shift (ppm)
40 20 0 -20 -40 -60 -80 -100 -120 -140 -160 -180 -200 -220 Chemical Shift (ppm)



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