CHEMISTRY
A European Journal

Supporting Information

Fluoroacetamide Moieties as NMR Spectroscopy Probes for the Molecular Recognition of GlcNAc-Containing Sugars: Modulation of the CH–π Stacking Interactions by Different Fluorination Patterns

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SUPPORTING INFORMATION

1. **Homo- and heteronuclear ^{19}F-^{19}F and ^{19}F-^{1}H correlation experiments and STD experiments**
2. **Theoretical electron density surfaces and charge distribution of (fluoro)acetamide moieties**
3. **Glycan array experiments**
4. **NMR spectra of new compounds**
5. **^{1}H STD-NMR**

1. Homo- and heteronuclear ^{19}F-^{19}F and ^{19}F-^{1}H correlation experiments and STD experiments

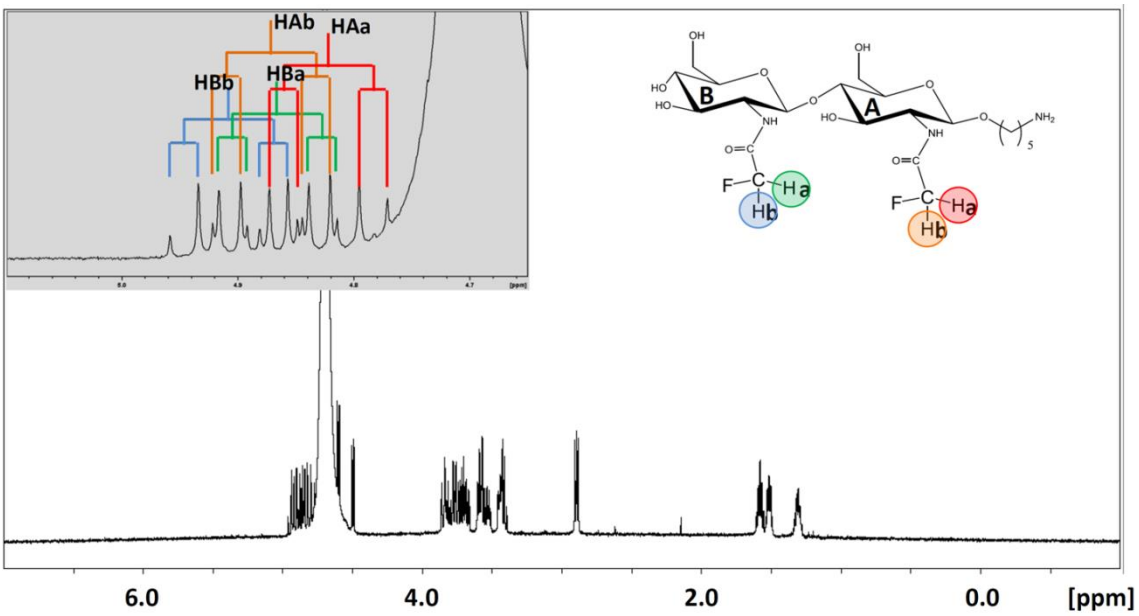


Figure S1. 600 MHz ^{1}H NMR spectrum of **1**. The spectral region for the acetamide protons is shown in the inset. The homo- and hetero-nuclear (J_{HH}, J_{HF}) coupling constants for every residue are specified using a color code.

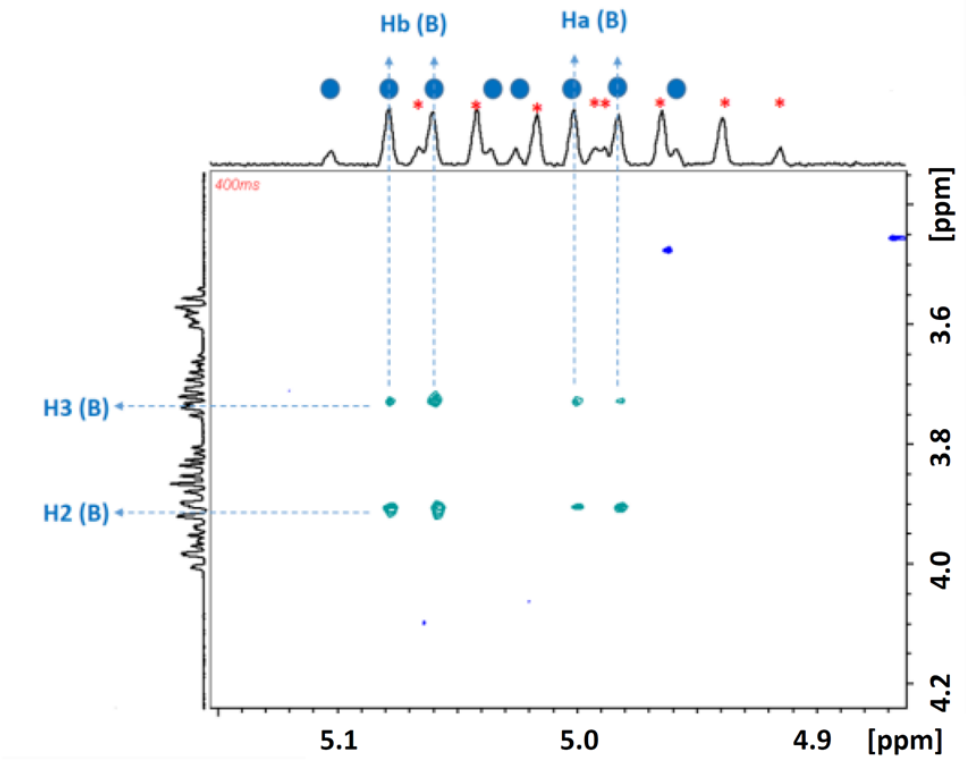


Figure S2. ^{1}H-^{1}H NOESY spectrum (400ms mixing time) of compound **1**. Correlation between both protons of the –CH_2F group (Hb and Ha) and H2 and H3 of the non-reducing end unit (B). Only crosspeaks with the strongest components of the multiplets are observed. The blue spots indicate the components of the multiplets for the non-reducing end (B), while red stars refer to the multiplets of the reducing end (A).

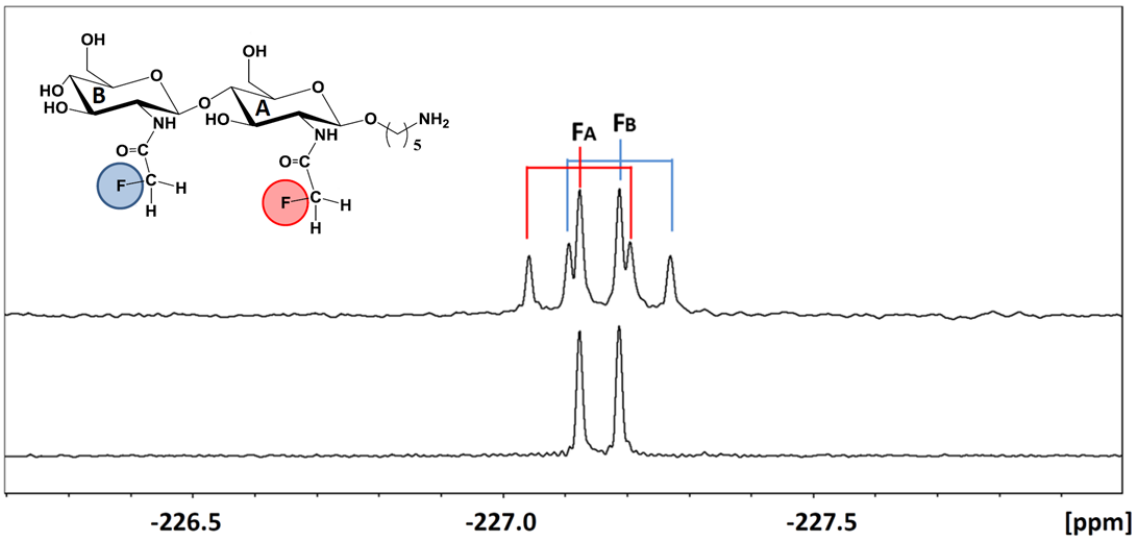


Figure S3. 564 MHz ^{19}F NMR spectrum of **1**. Bottom panel, ^{19}F-{^{1}H} decoupled NMR spectrum. Top panel ^{19}F-{^{1}H} coupled NMR spectrum, with the heteronuclear J_{FH} couplings for every residue specified in color code.

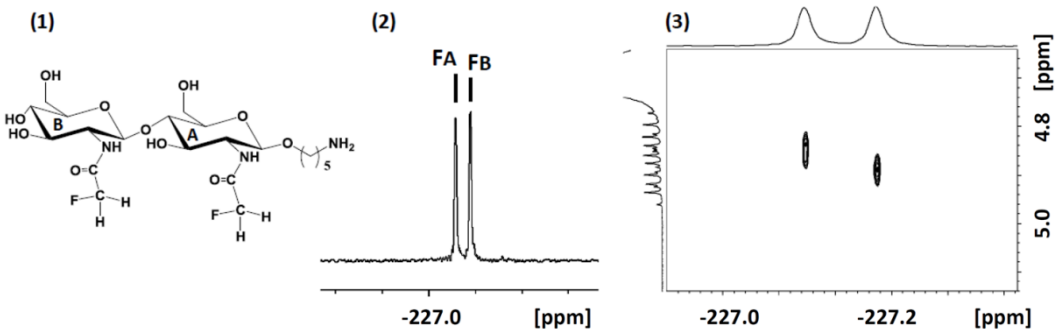


Figure S4. (1) Residue label for molecule **1**. (2) 546 MHz ^{19}F-{^{1}H} decoupled NMR spectra. (3) ^{19}F-^{1}H heteronuclear correlation spectrum of **1**. The correlation between the fluoroacetamide protons and their respective fluorine signals are evidenced.

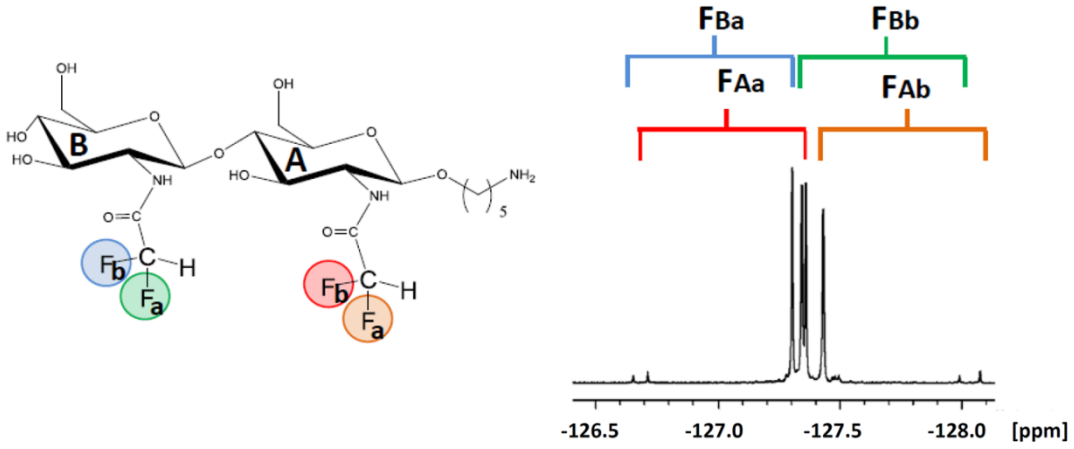


Figure S5. 564 MHz ^{19}F-{^{1}H}-decoupled NMR spectrum of **2**. The analysis of the multiplicity is shown.

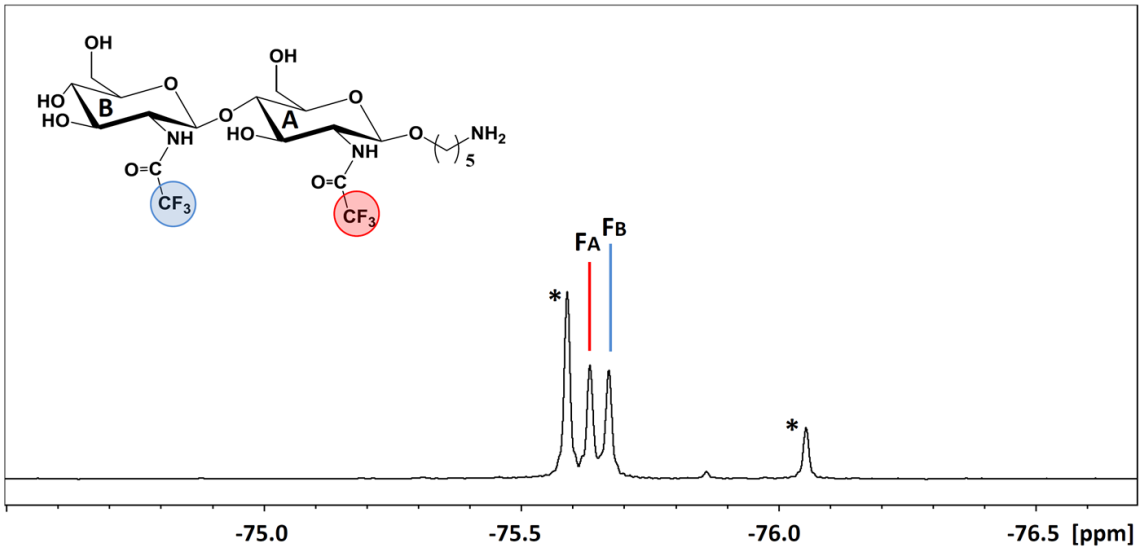


Figure S6. 564 MHz ^{19}F-{^{1}H} decoupled of **3**. The signals for every residue specified in a color code. Two impurities are labeled with (*) symbol.

Table S1. ^{1}H STD competition experiments for K_D determination of compounds 1 and 2.

^{1}H-STD relative Intensity (%)		
CH_3 (4) : CHxFy	CH_2F (1)	CHF_2 (2)
0	100	100
1	60	85
3	33	55
6	25	33

The K_D dissociation constants for the complexes of **1** and **2** with WGA were estimated by competitive STD experiments with the natural ligand, **4** (K_D = 0.19 mM).[19] The absolute concentration of compounds **1** and **2** is 0.5 mM, while that of WGA protein is 10 µM.

The target K_D values can be determined using the usual equation for competitive STD experiments:[28, 29] $K_D = (iK_i \cdot C_L)/(C_i\text{-}iC_i\text{-}iK_i)$

where K_i refers to the dissociation constant of **4,** C_i and C_L are the concentrations of **4** and the fluorinated analogue (**1** or **2**), respectively, and i is the fractional inhibition, defined as $i = (L_0\text{-}L_i)/L_0$, where L_0 and L_i are the ^{1}H-STD signal intensities of the corresponding CH protons at the fluoroacetamide moiety in the absence and presence of **4**, respectively.

Table S2. ^{1}H STD competition experiments for K_D determination of compound 3.

^{1}H-STD relative Intensity (%)	
[-CF_3 (3) : -CH_3 (4)]	CH_3 (4)
0	100
0,5	90
0,75	85
1	80
2	70
5	50

The K_D dissociation constant for compound **3** has been estimated by competitive STD experiments on the WGA-**4** complex. The absolute concentration of compounds **4** is 1.5 mM, while that of WGA protein is 30 µM.

The target K_i value can be determined using the equation for competitive STD experiments

$$K_i = C_i\,(K_D - i\cdot K_D)/\,i \cdot (C_L + K_D)$$

where K_D refers to the dissociation constant of **4**, C_i and C_L are the concentration of **3** and **4**, respectively, and i is the fractional inhibition, defined as $i = (L_0\text{-}L_i)/L_0$, where L_0 and L_i are the ^{1}H-STD signal intensities of the corresponding CH_3 protons at the acetamide moiety of compound **4** in the absence and presence of **3**, respectively.

2. Theoretical electron density surfaces and charge distribution of (fluoro)acetamide moieties

Table S3. Charge distribution showing Mulliken-type atomic charge for model compounds N-methyl (fluoro)acetamides at M06-2X/6-31G(d,p) level of theory (including solvation).[30]

Molecule	π-interaction type	$\delta^{+}_{Mulliken}$ activated H	$\delta^{+}_{Mulliken}$ (-CX_3)
***N*-Me-acetamide**	CH-π	0.152	-0.219
***N*-Me-fluoroacetamide**	CH-π	0.160	0.031
***N*-Me-difluoroacetamide**	CH-π	0.162	0.427
***N*-Me-trifluoroacetamide**	CF-π	-[a]	0.782

[a]Calculated Mulliken charge for F atoms is -0.264

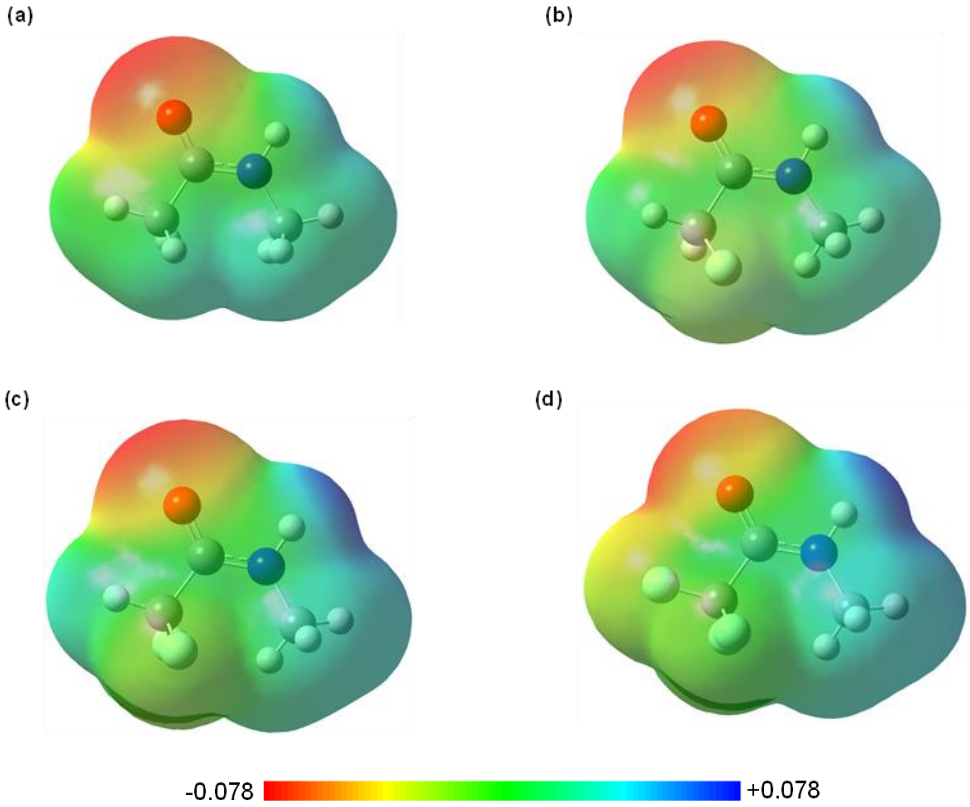
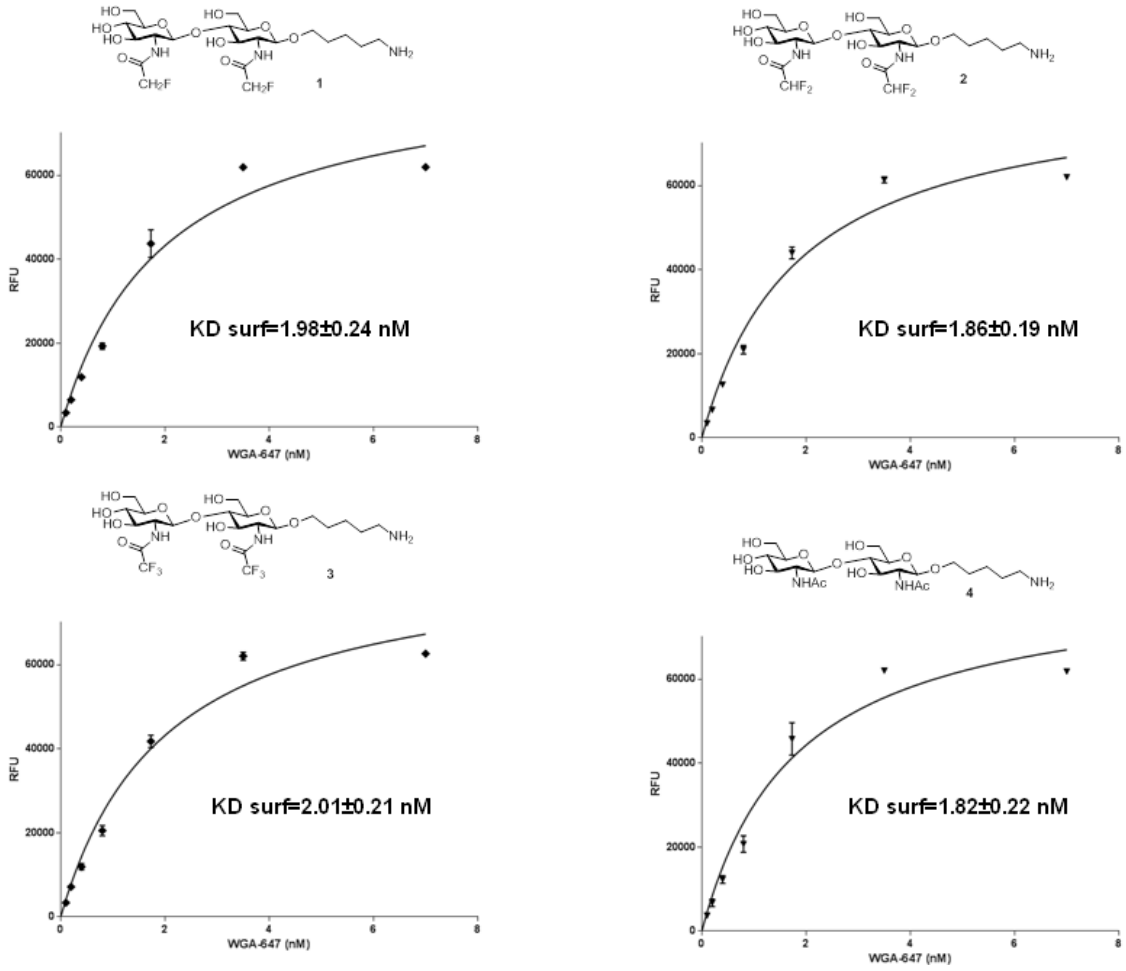


Figure S7. Electron density surface from total SFC density mapped with electrostatic potential at M06-2X/6-31G(d,p) level of theory (including solvation) for: (a) *N*-methyl acetamide; (b) *N*-methyl fluoroacetamide; (c) *N*-methyl difluoroacetamide and (d) *N*-methyl trifluoroacetamide.

3. Glycan array experiments

Figure S8. Binding curves for compounds **1**, **2**, **3** and **4** obtained after incubation with different concentrations of WGA-647 on the glycan microarray. The K_D surf values were obtained by fitting the curves to Langmuir isotherms.



4. NMR spectra of new compounds

Figure S9. ^{1}H NMR spectrum of compound **1** (D_2O, 500 MHz)

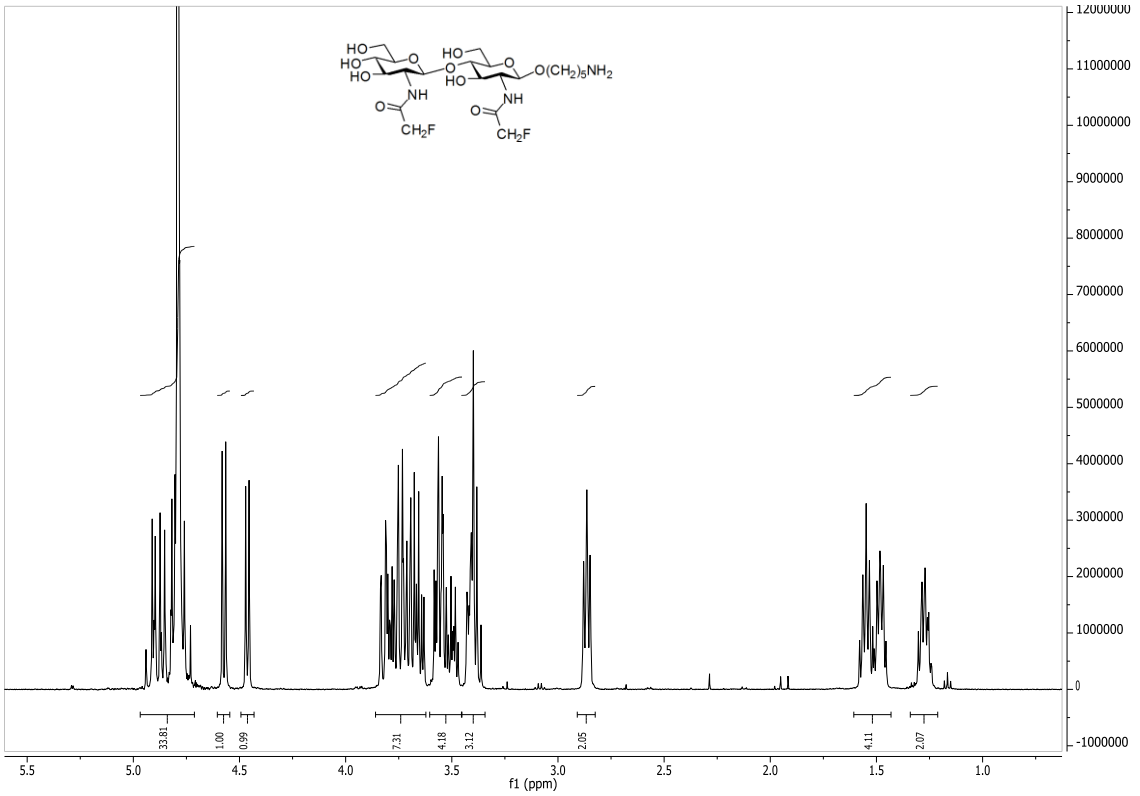


Figure S10. ^{13}C NMR spectrum of compound **1** (D_2O, 126 MHz)

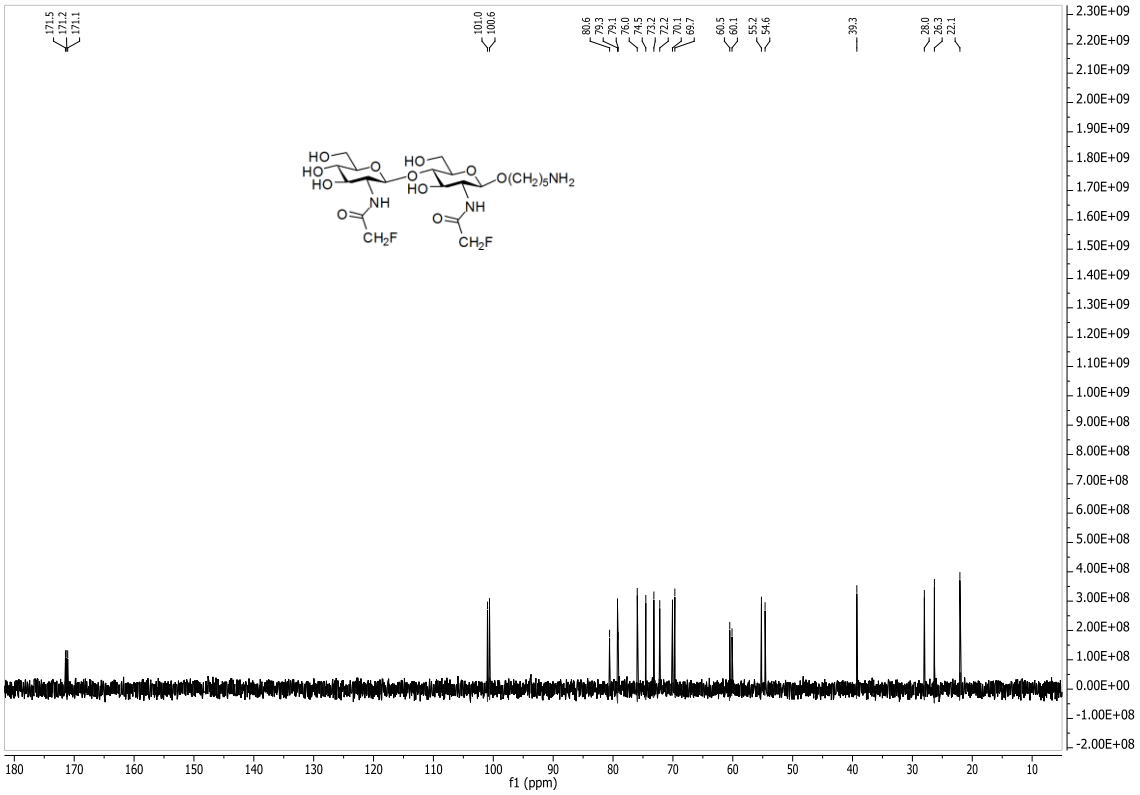


Figure S11. ^{1}H NMR spectrum of compound **3** (D_2O, 500 MHz)

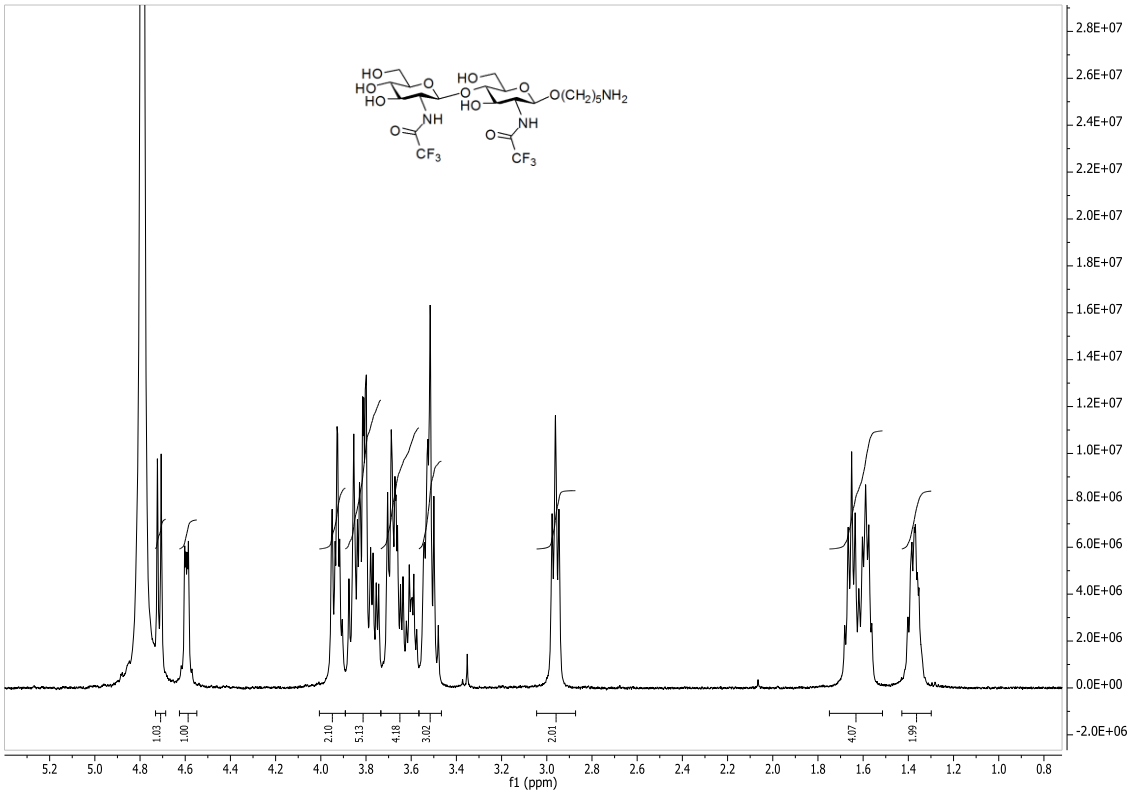
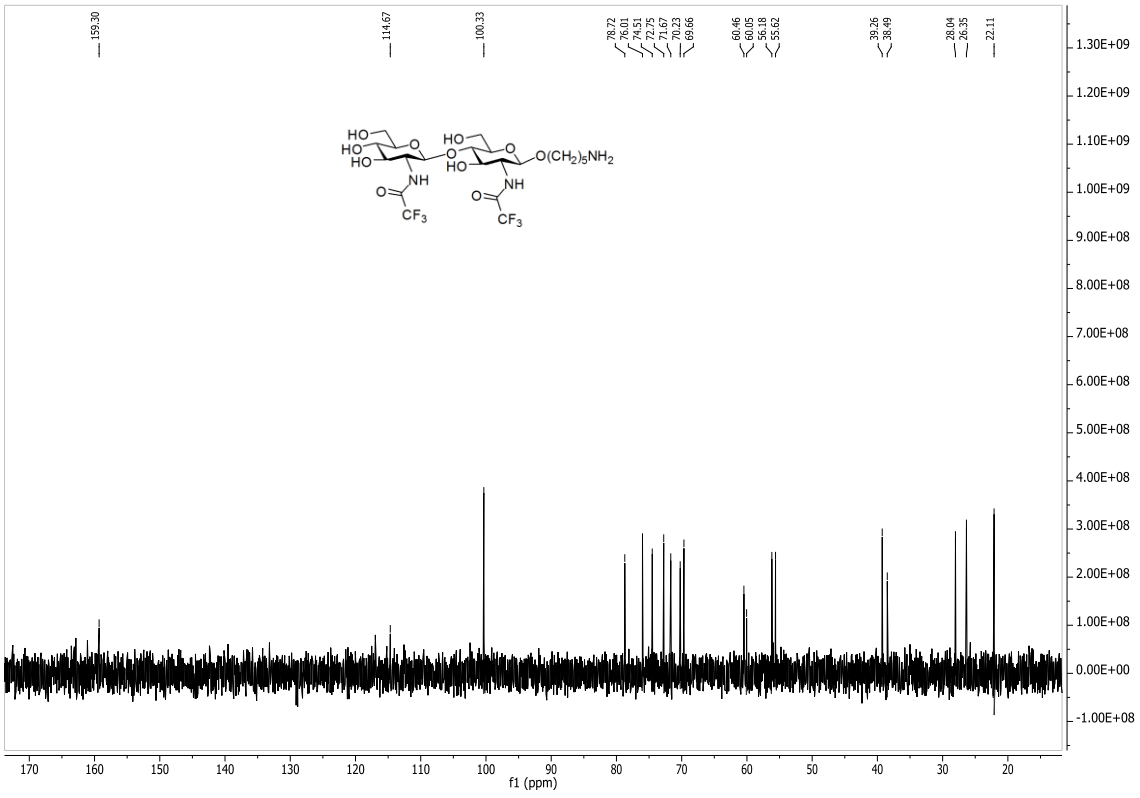


Figure S12. ^{13}C NMR spectrum of compound **3** (D_2O, 126 MHz)



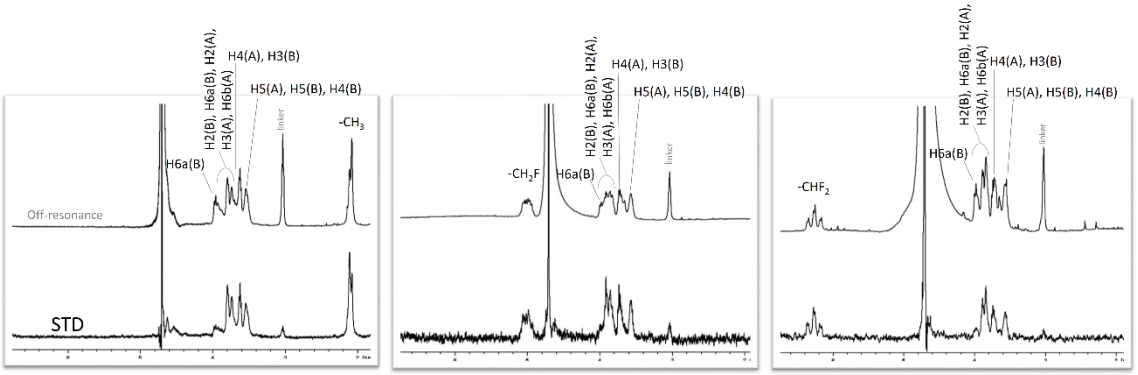


Figure S13. ^{1}H-STD-NMR (top) and off-resonance (bottom) spectra for (A) □-chitobioside (**4**), (B) fluorinated (-CH_2F) ligand **2**, and (C) fluorinated (-CHF_2) ligand **3.**

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