Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supplementary Information for:

Extending the in vivo persistence of synthetic glycoconjugates using a serum-protein binder

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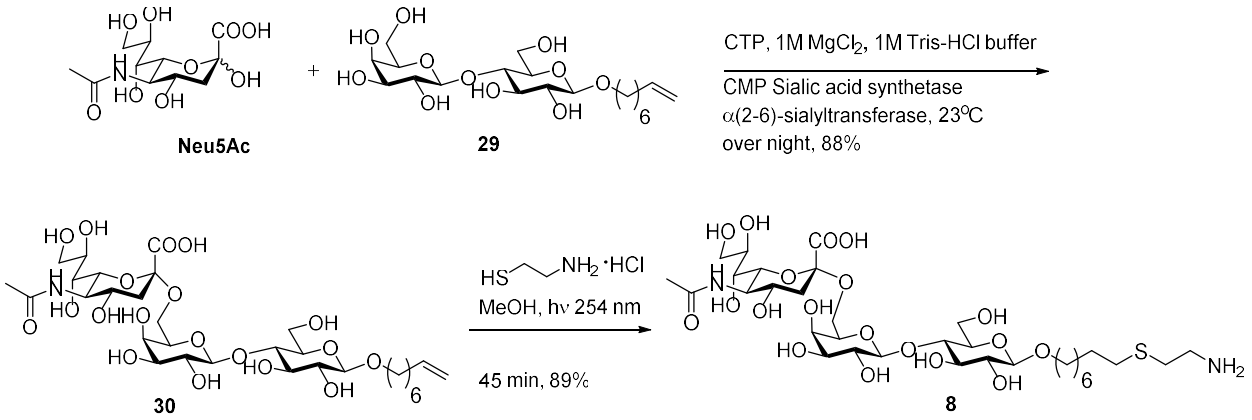
Scheme S1: Synthesis and characterization of **8**



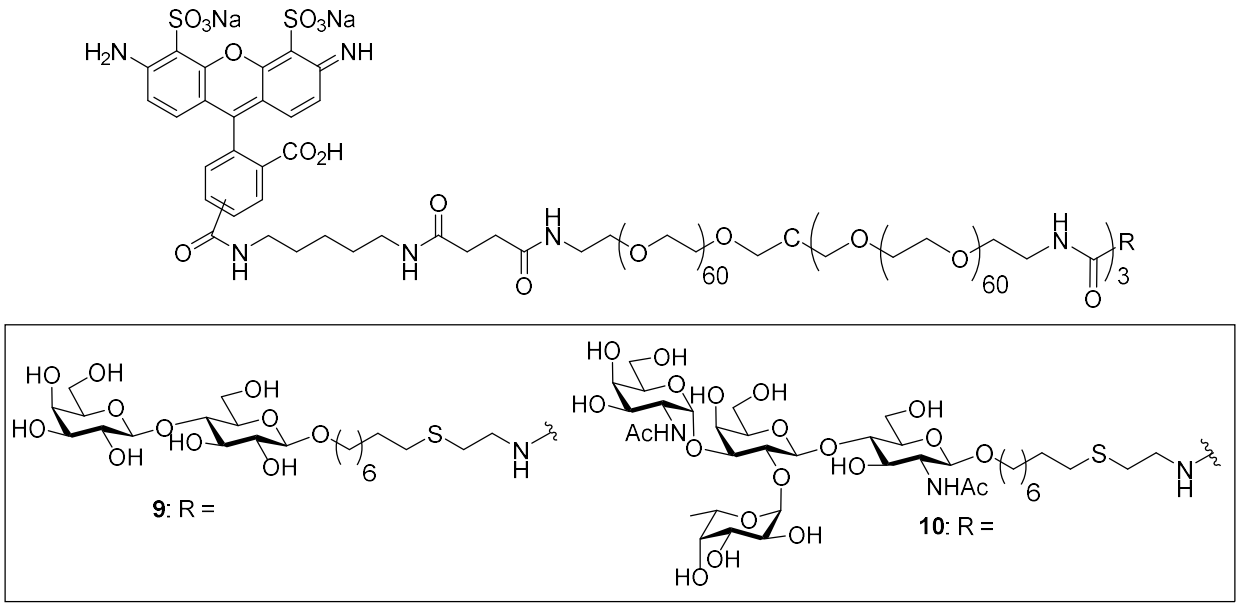
Compound **30** was prepared chemoenzymatically using *N*-Acetyl neuraminic acid (Neu5Ac) (8.7 mg, 28.2 µmol), cytidine triphosphate disodium salt (14.85 mg, 28.2 µmol), 1M $MgCl_2$ (240 µL), distilled H_2O (1.8 mL) and 1M TrisHCl buffer (1.2 mL, pH 8.8) were dissolved in a round bottom flask. *Neisseria meningitides* CMP-Neu5Ac synthetase (NmCSS) (600 µL),[2] octenyl-β-lactoside **29**, (8.4 mg, 18.6 µmol), *Pasteurella multocida* α(2→6)-sialyltransferase (600 µL)[3] and distilled H_2O (1.8 mL) were charged to the reaction mixture. The reaction mixture was stirred overnight. After the reaction was completed, ethanol was added, and the solution was centrifuged, and the supernatant was then lyophilized. The crude product was purified by a sep-pack C-18 reverse phase cartridge eluted with H_2O to MeOH/ H_2O (1:1, v/v) to afford compound **30** (18.50 mg; 88%) as a white solid power after concentration of the fractions containing desired product. HRMS (ESI) calculated for C31H53NO19 [M-H]+ 743.3212, found: 743.3139.

Compound **8** was obtained through irradiation of compound **30**[1] (12 mg, 0.016 mmol) and cysteamine hydrochloride (12.4 mg, 0.16 mmol) in anhydrous MeOH (1 mL) and then solution was bubbled with N_2, and the tube was filled with N_2. The reaction mixture was irradiated under UV light for 45 min, after completion the solution was concentrated, and the crude mixture was purified by C_{18} Sep-pak chromatography using gradient elution (0.5% aq. AcOH to 2:8 v/v

MeOH/0.5% aq. AcOH), followed by treatment with HO^- resin afforded the amine **8** (11.8 mg, 89 %) as a white powder. ^{1}H NMR (600 MHz, D_2O): δ 4.43 (d, *J* = 12.6 Hz, 1H, H-1'), 4.38 (d, *J* = 12.0 Hz, 1H, H-1), 3.92-3,68 (m, 6H), 3.76-3.68 (m, 2H), 3.68-3.64 (m, 1H), 3.66-3.46 (m, 11H), 3.28 (t, *J* = 12.6 Hz, 1H, C*H*$_2$-), 3.17 (t, *J* = 9.6 Hz, 2H, C*H*$_2$-), 3.80 (t, *J* = 10.2 Hz, 2H, C*H*$_2$-), 2.66 (dd, *J* = 6.6, 18.6 Hz, 1H, Heq-3), 3.56 (t, *J* = 10.8 Hz, 2H, C*H*$_2$-),1.98 (s, 3H, NHAc), 1.69 (t, *J* = 18.6 Hz, 1H, Hax-3), 1.59-1.52 (m, 4H), 1.34-1.29 (band, 8H); ^{13}C NMR (126 MHz, D_2O): δ 175.77, 174.34, 104.06, 102.74, 101.16, 80.55, 75.57, 75.48, 74.55, 73.61, 73.38, 73.22, 73.64, 71.64, 71.49, 69.36, 69.24, 69.21, 64.42, 63.50, 61.17, 52.63, 40.95, 39.23, 31.57, 29.51, 29.37, 29.07, 28.97, 28.57, 25.77, 22.91; HRMS (ESI): m/z [M-H]+ calcd for C33H59N2O19S: 819.3431, found: 819.3438.

Scheme S2: Structure of (a) conjugate **9** and A-type II conjugate **10,** (b) conjugate **28**[1]

(a)



(b)

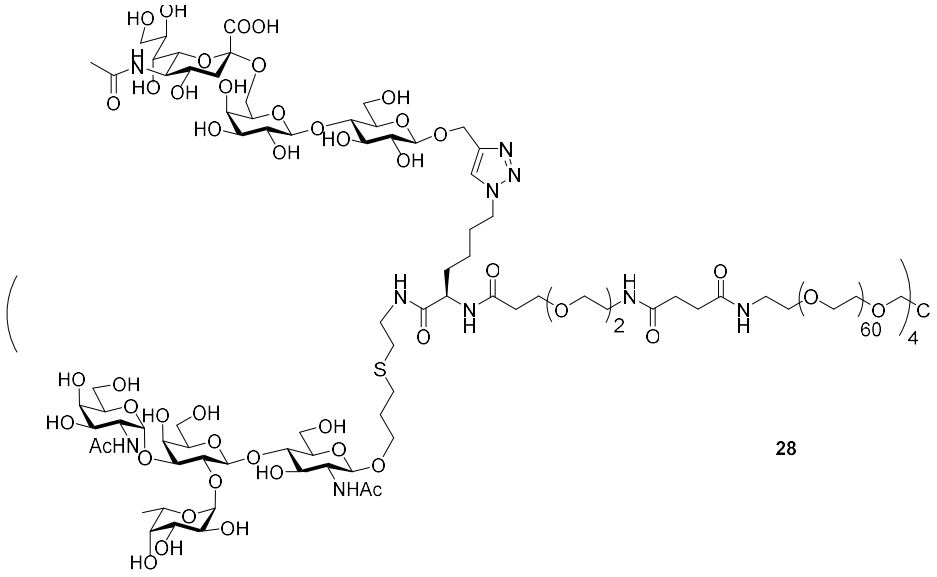
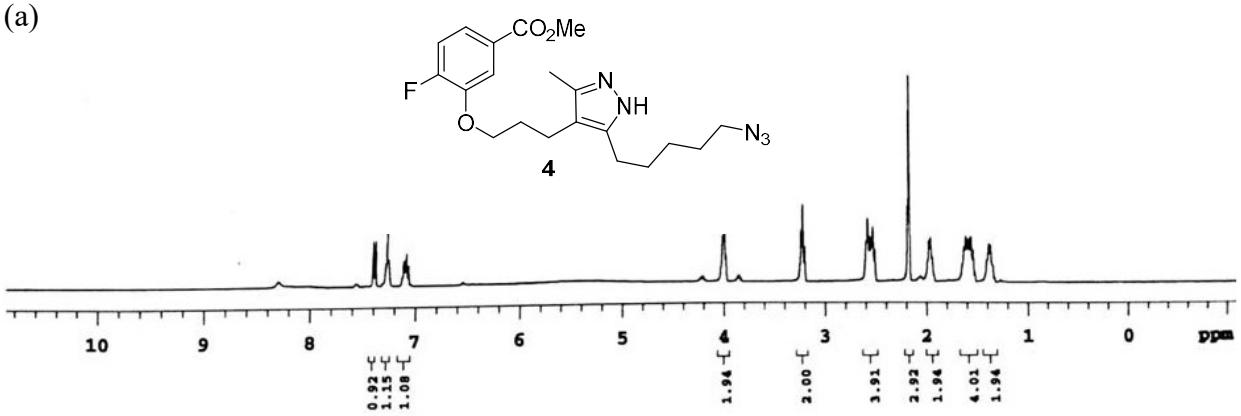


Figure S1: (a) ^{1}H and (b) ^{13}C NMR spectra of compound **4**.

(a)



(b)

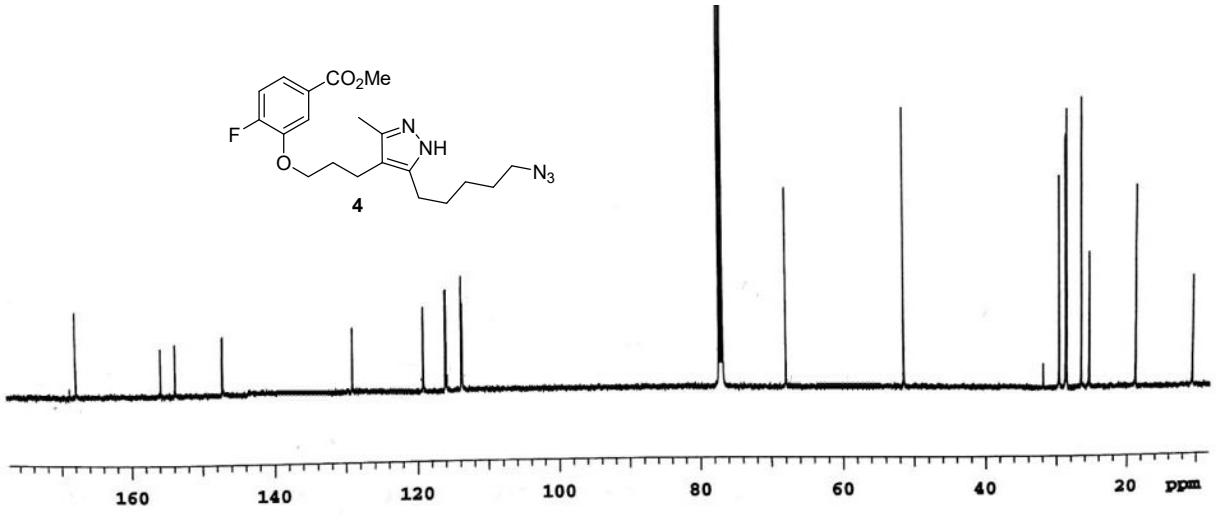
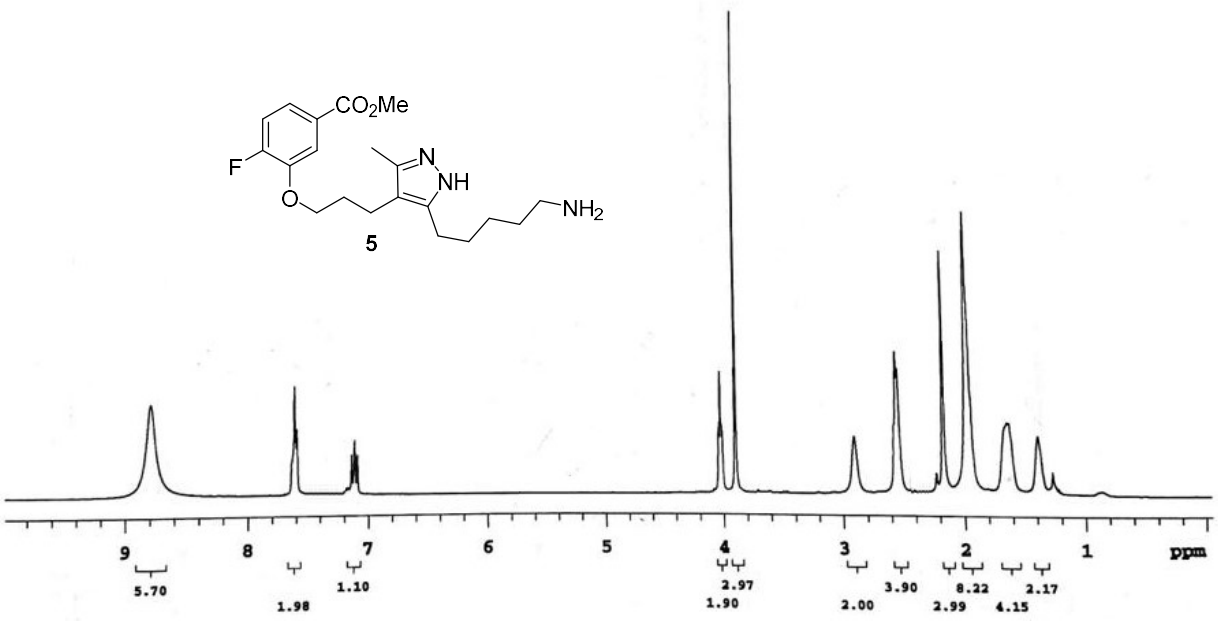


Figure S2: (a) ^{1}H and (b) ^{13}C NMR spectra of compound **5**.

(a)



(b)

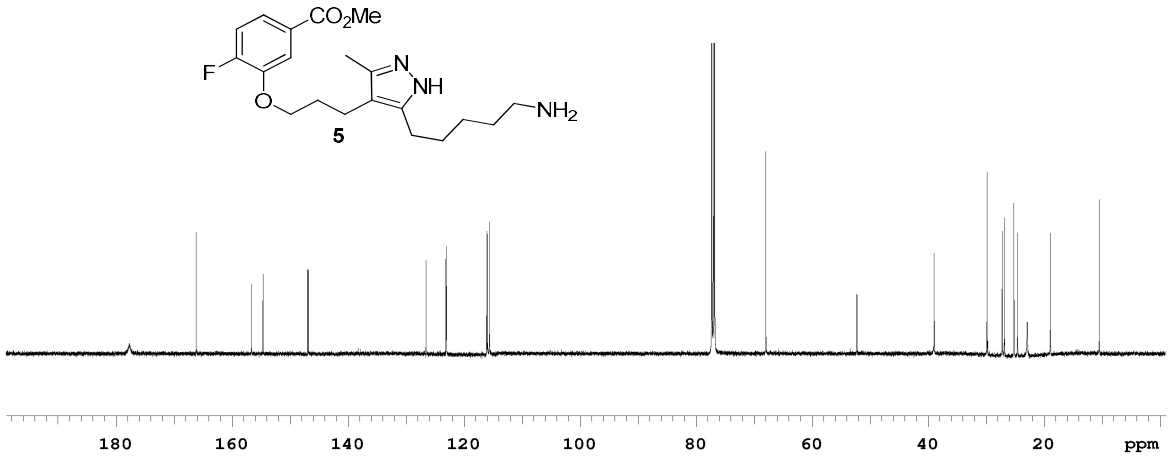
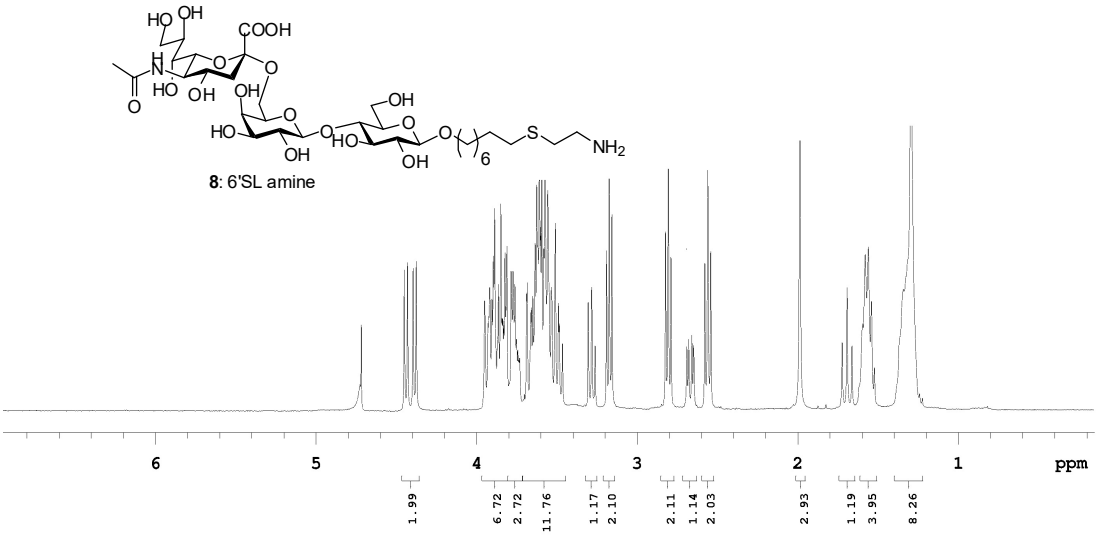


Figure S3: (a) ^{1}H and (b) ^{13}C NMR spectra of compound **8**.

(a)



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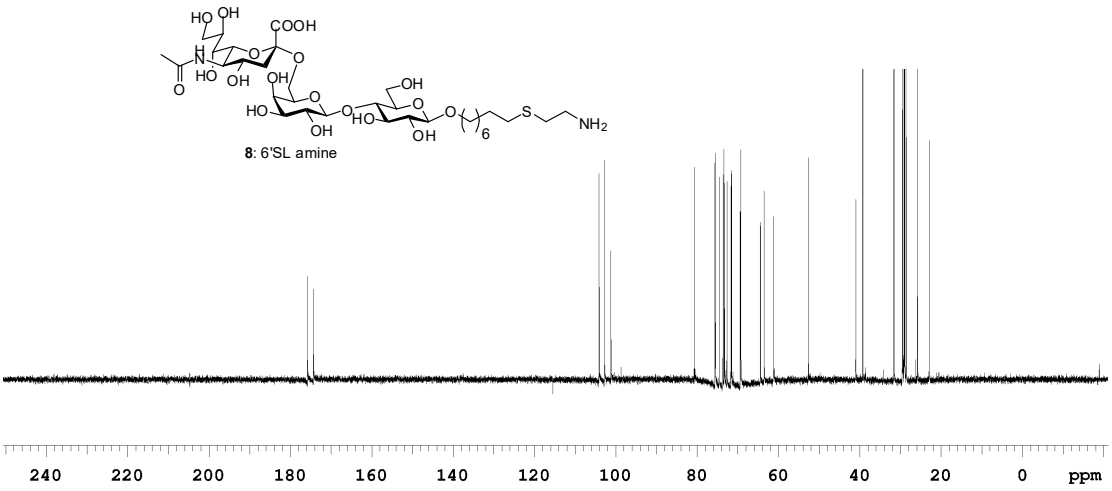
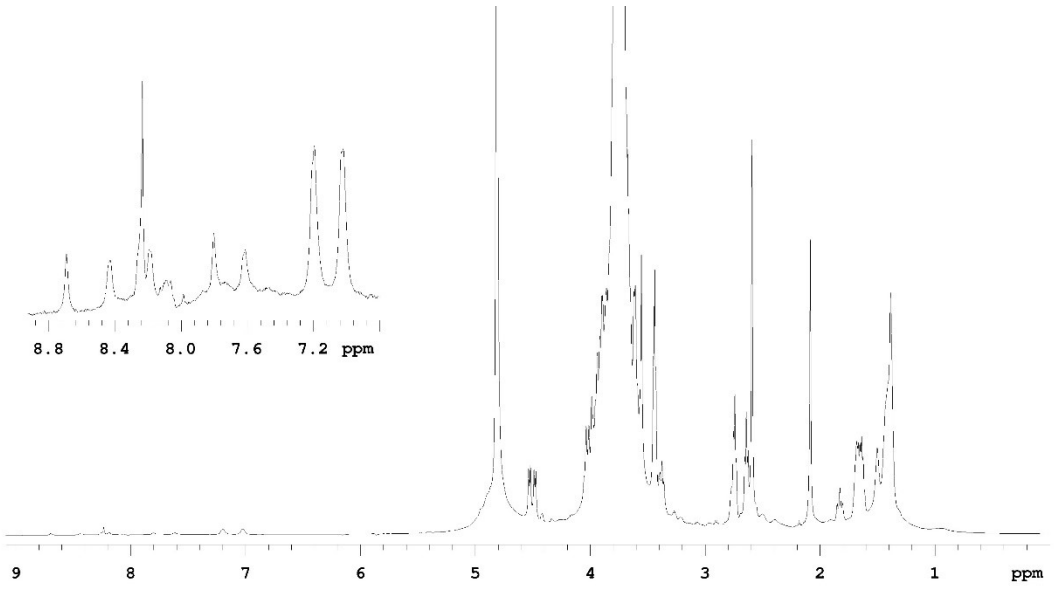


Figure S4: (a) ^{1}H and (b) ^{13}C NMR spectra of compound **11**.

(a)



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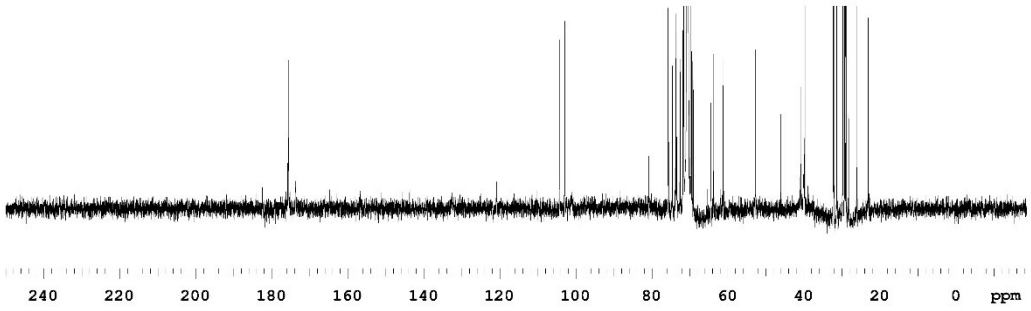
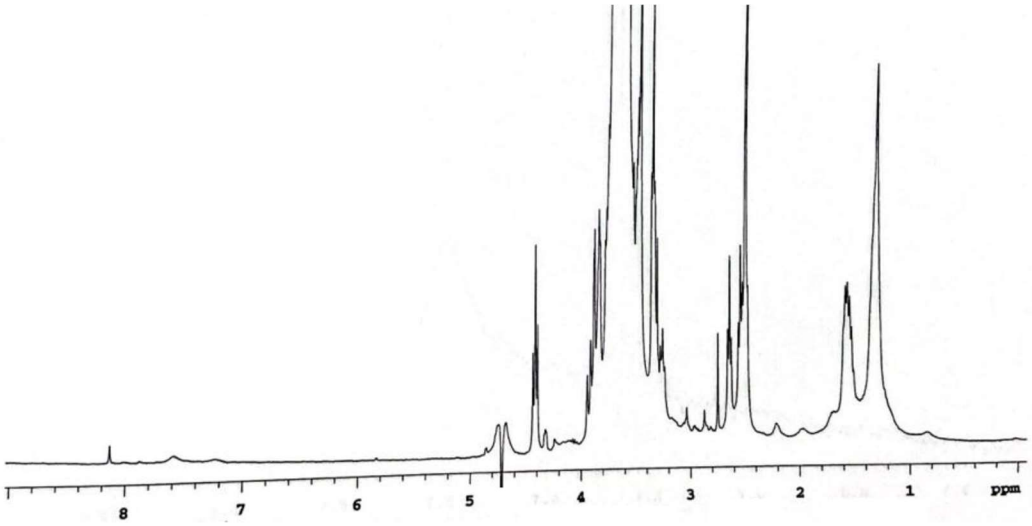


Figure S5: (a) ^{1}H and (b) ^{13}C NMR spectra of compound **16**.

(a)



(b)

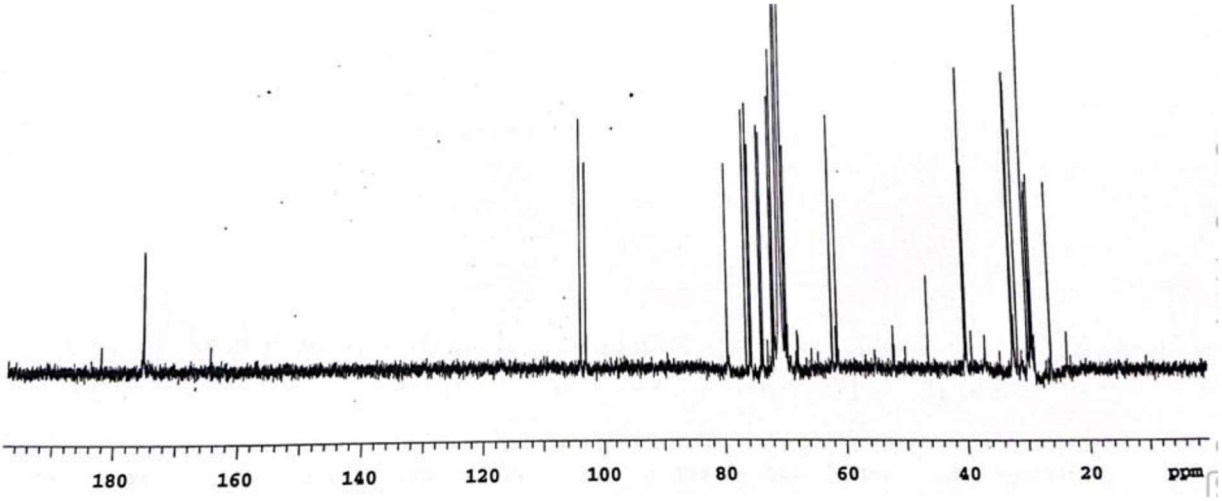
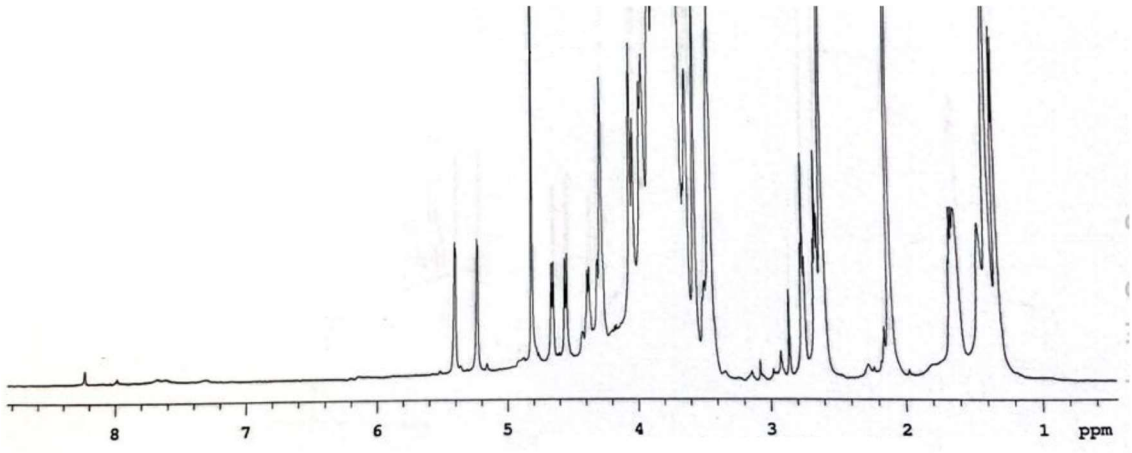


Figure S6: (a) ^{1}H, (b) ^{19}F and (b) ^{13}C NMR spectra of compound **17**.

(a)



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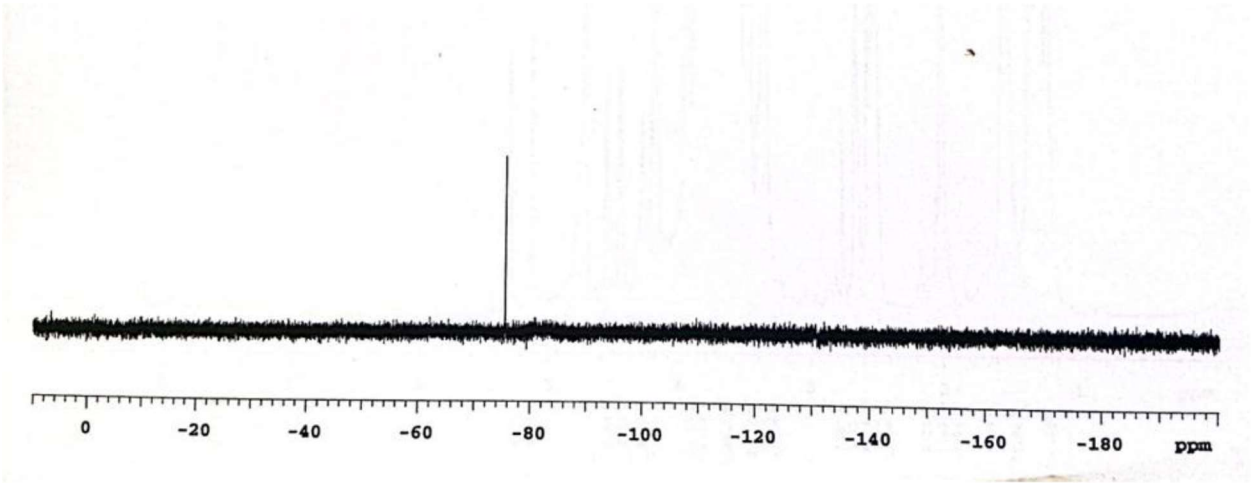


(c)

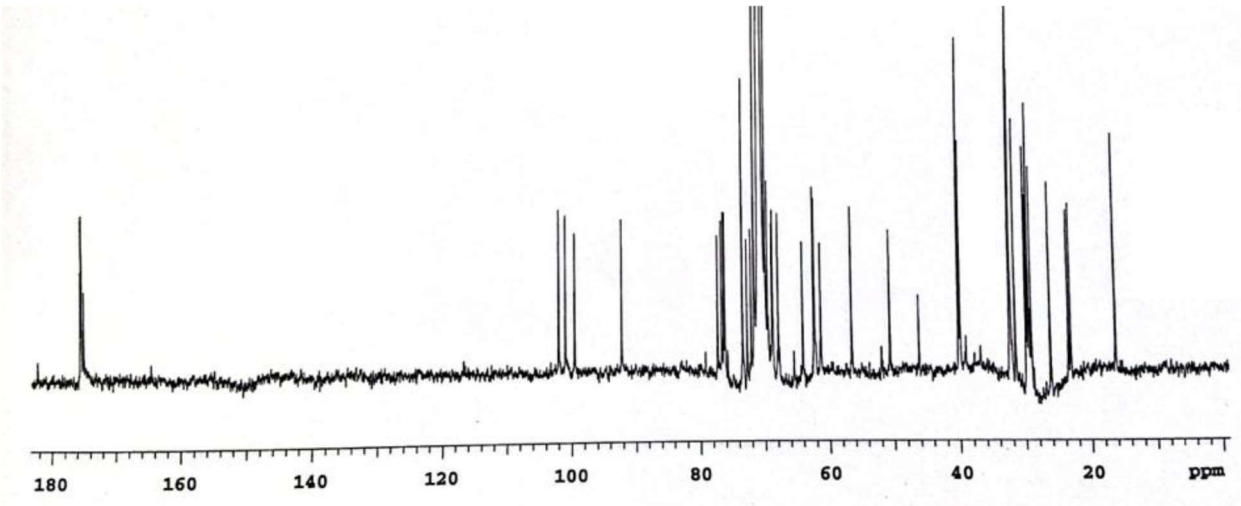
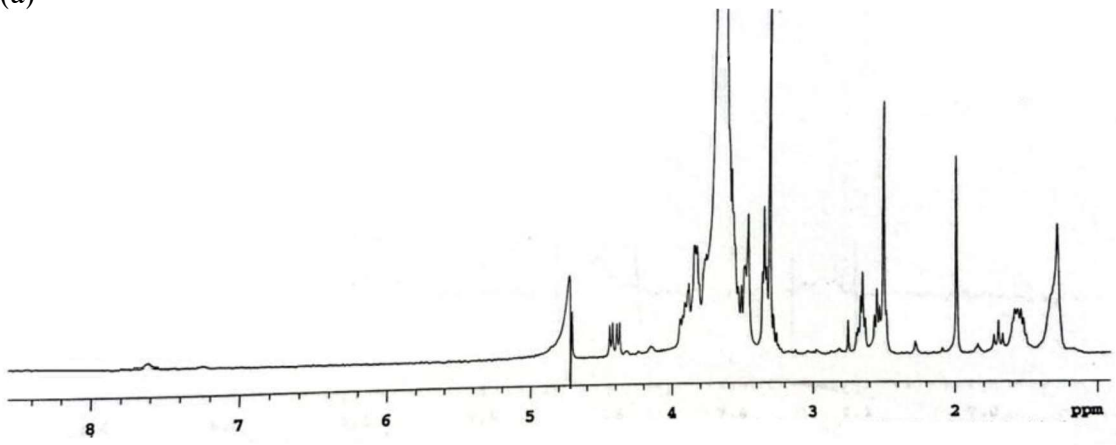


Figure S7: (a) ^{1}H, and (b) ^{13}C NMR spectra of compound **18**.

(a)



(b)

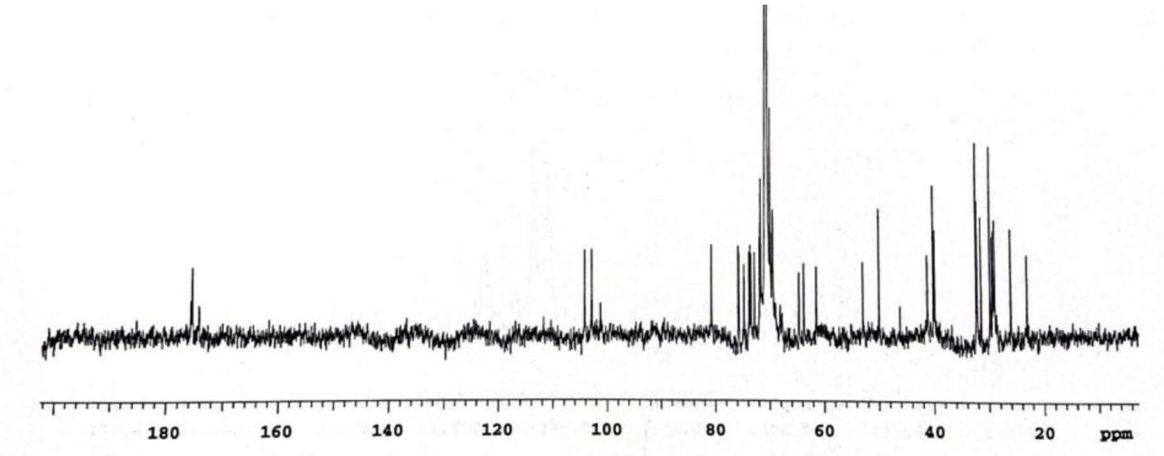
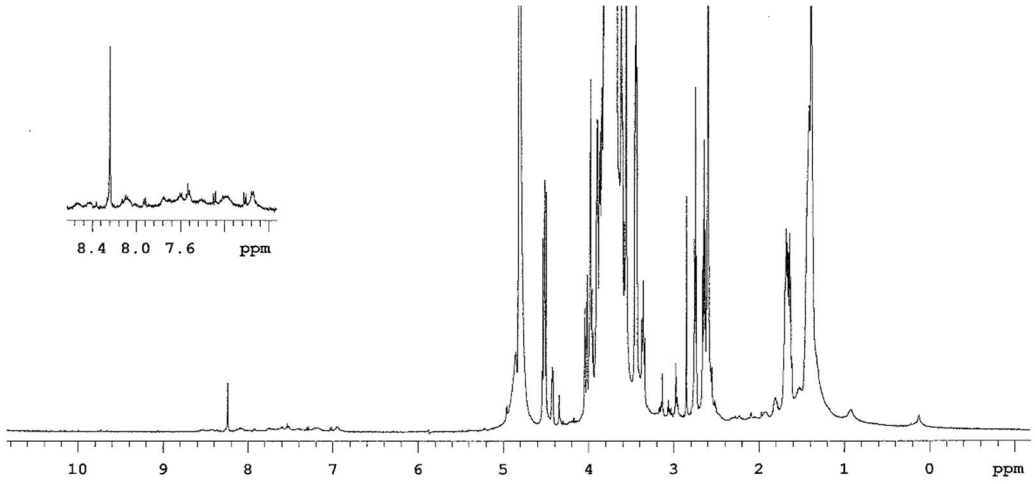
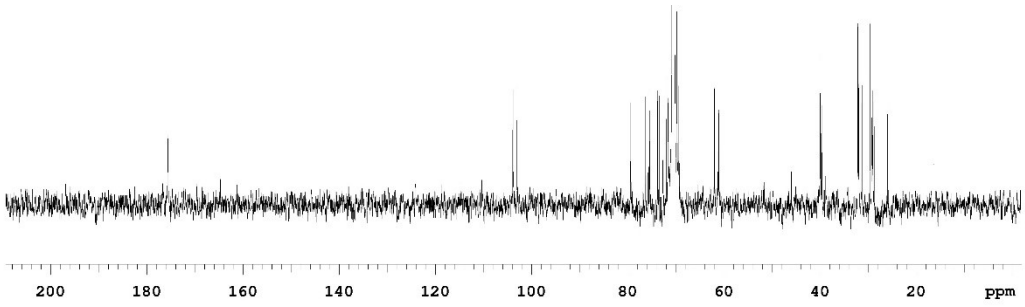


Figure S8 (a) ^{1}H, (b) ^{13}C,(c) ^{19}F NMR spectra of compound **20**.

(a)



(b)



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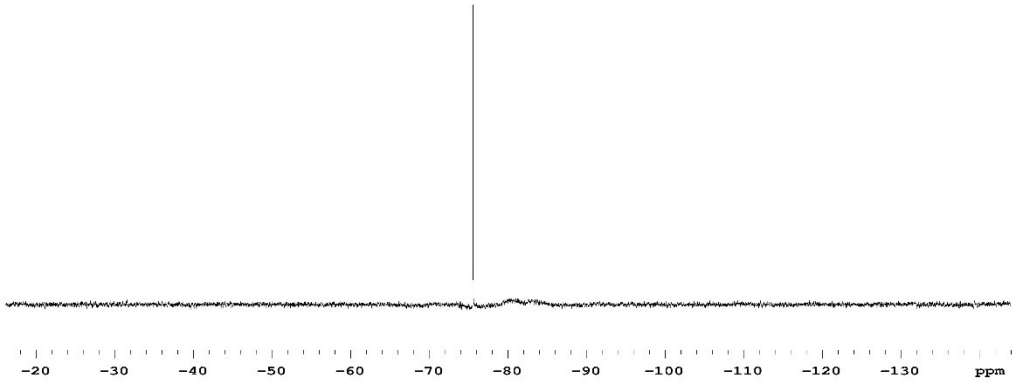
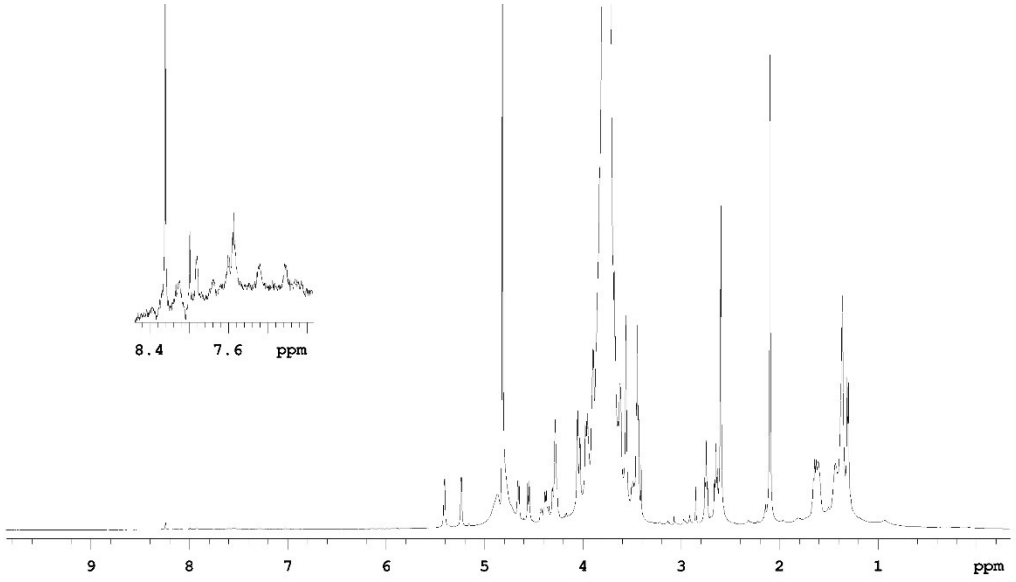
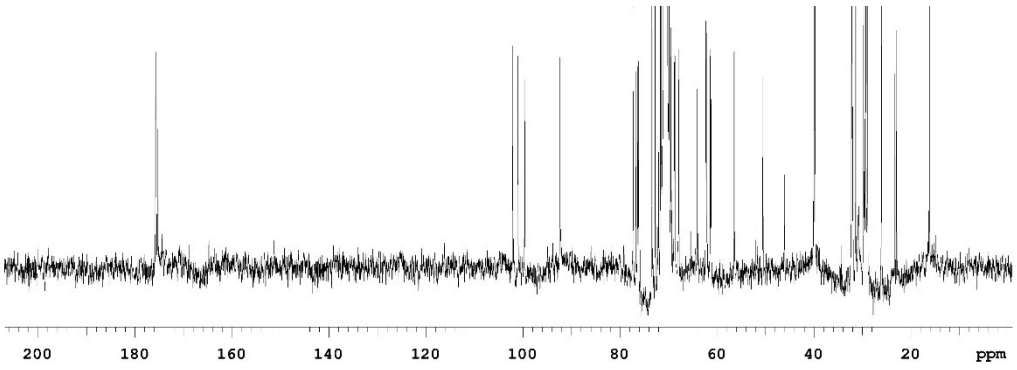


Figure S9: (a) ^{1}H, (b) ^{13}C, (c) ^{19}F NMR spectra of compound **21**.

(a)



(b)



(c)

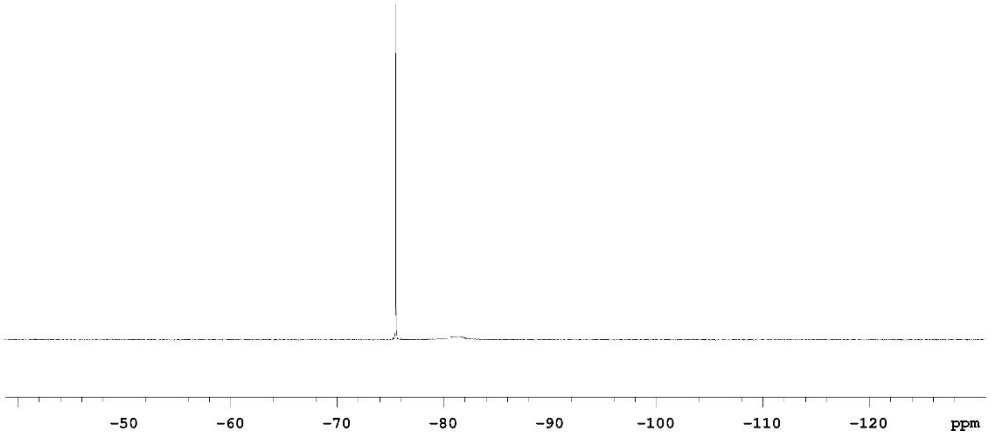
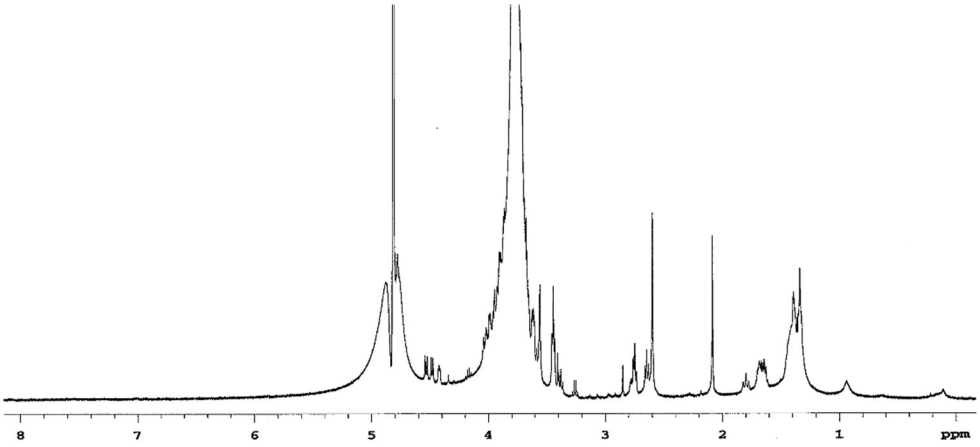
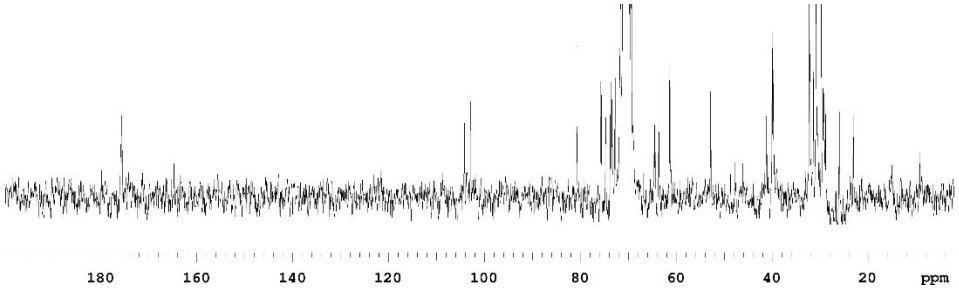


Figure S10: (a) ^{1}H, (b) ^{13}C, (c) ^{19}F NMR spectra of compound **22**.

(a)



(b)



(c)

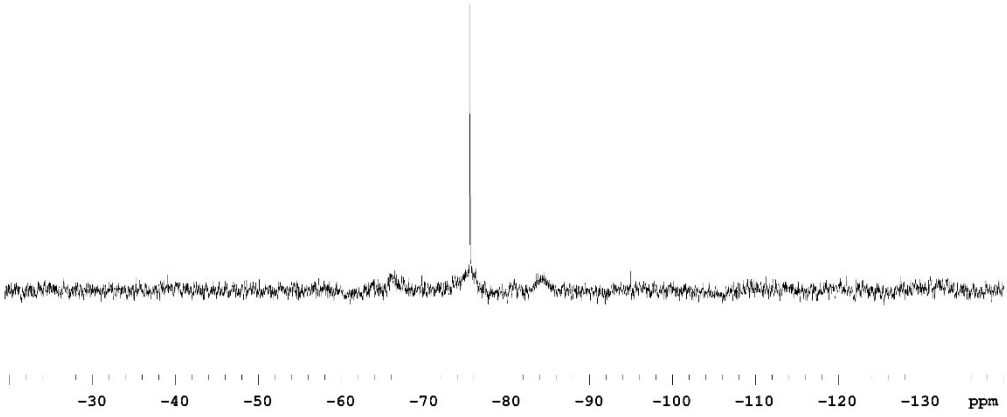


Figure S11: MALDI data of (a) NHS-PEG (**13**) and (b) conjugate **20**.

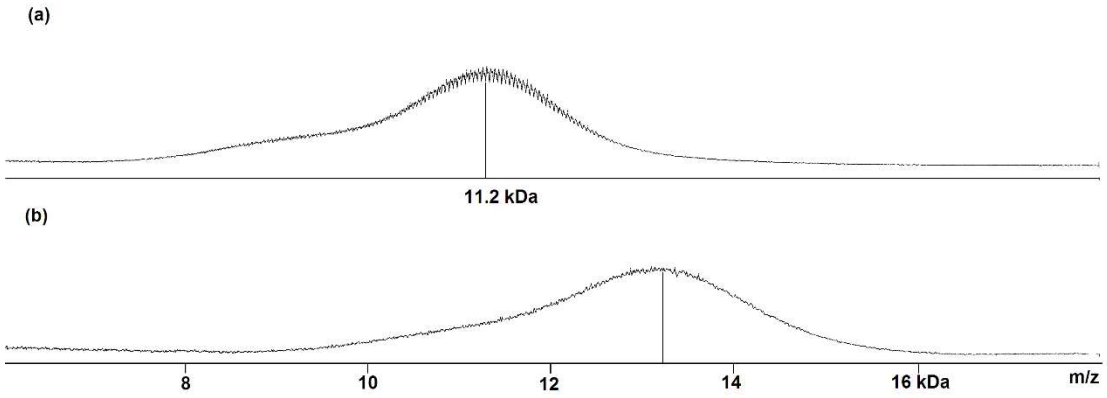


Figure S12: MALDI data of (a) NHS-PEG (**13**) and (b) conjugate **21**.

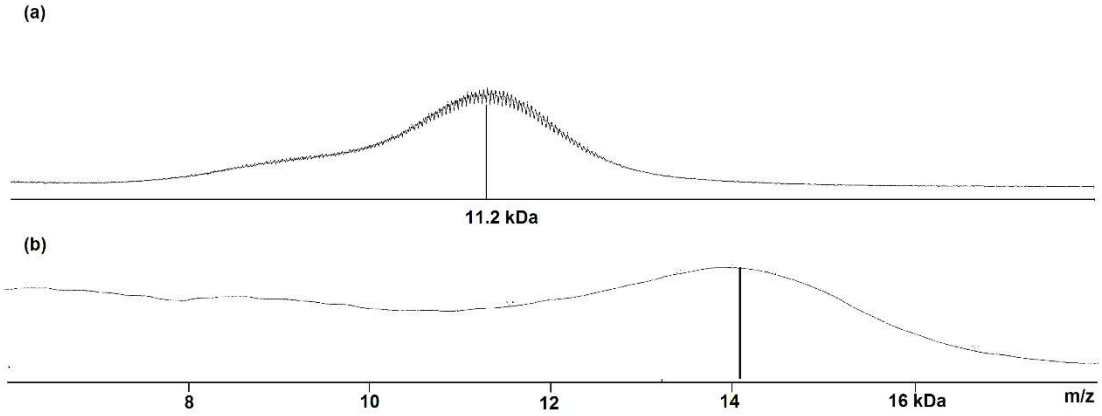


Figure S13: MALDI data of (a) NHS-PEG (**13**) and (b) conjugate **22**.

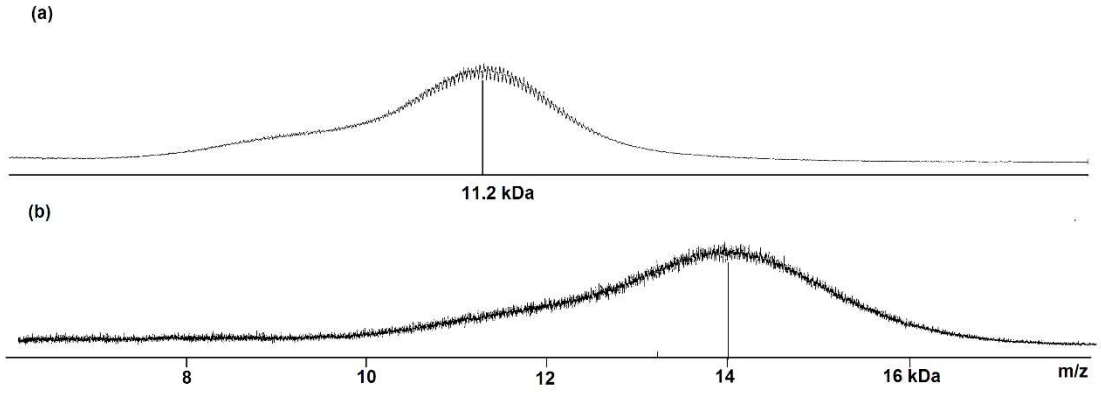
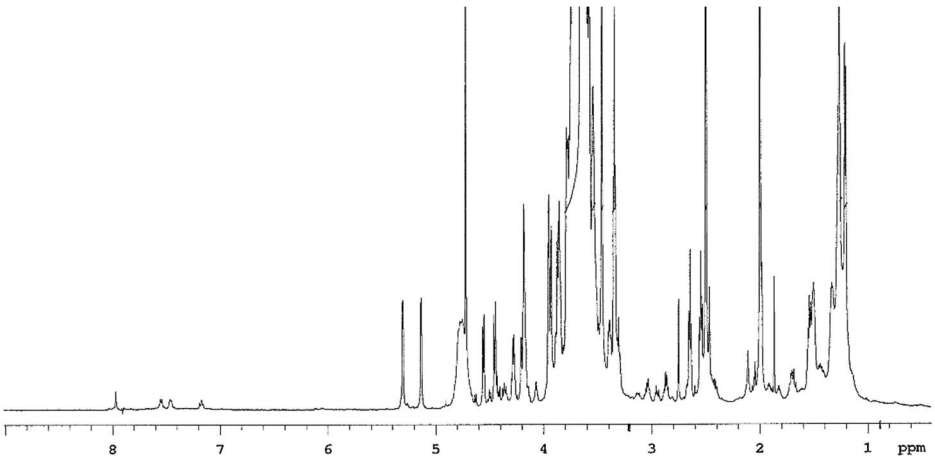
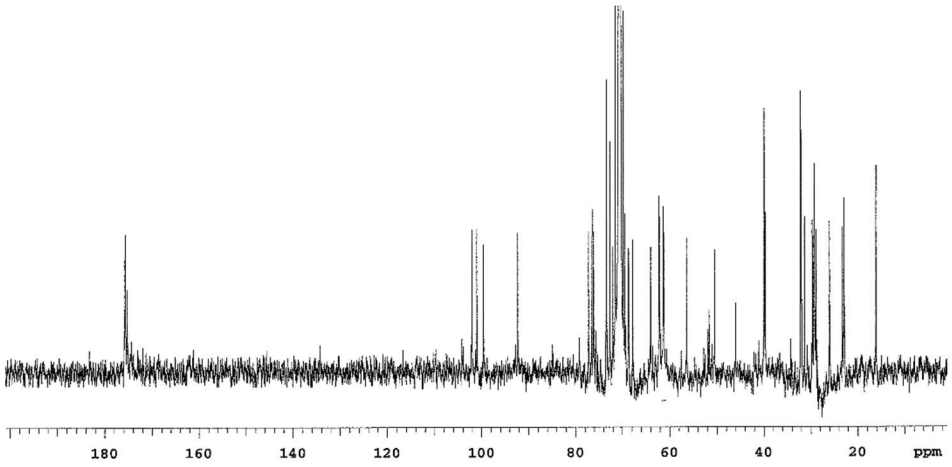


Figure S14: (a) ^{1}H, (b) ^{13}C, (c) ^{19}F NMR spectra of compound **27**.

(a)



(b)



(c)

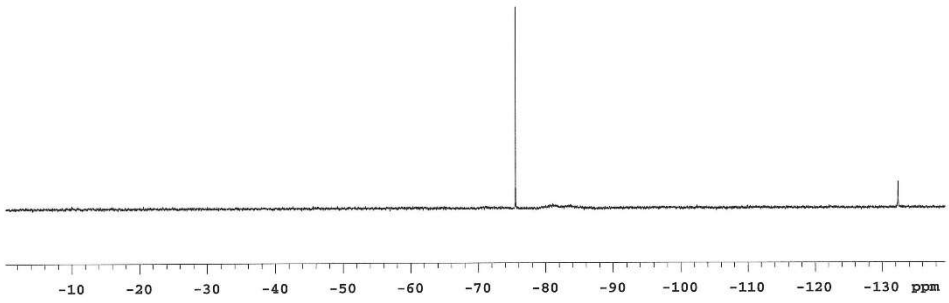
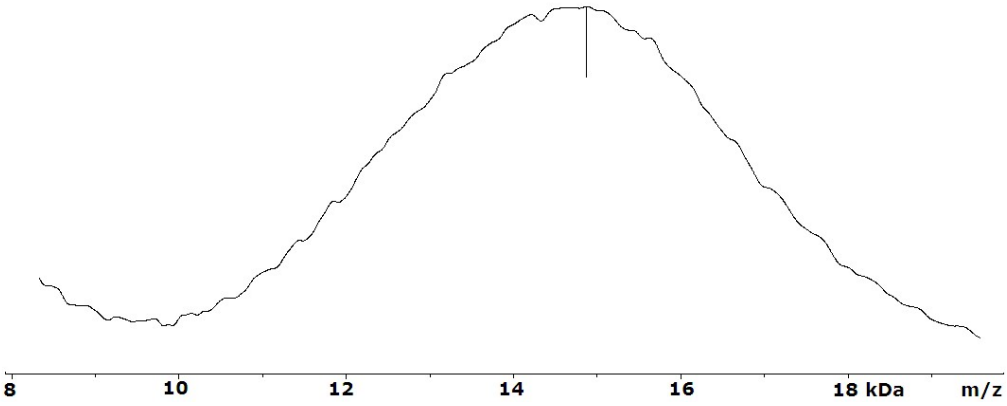
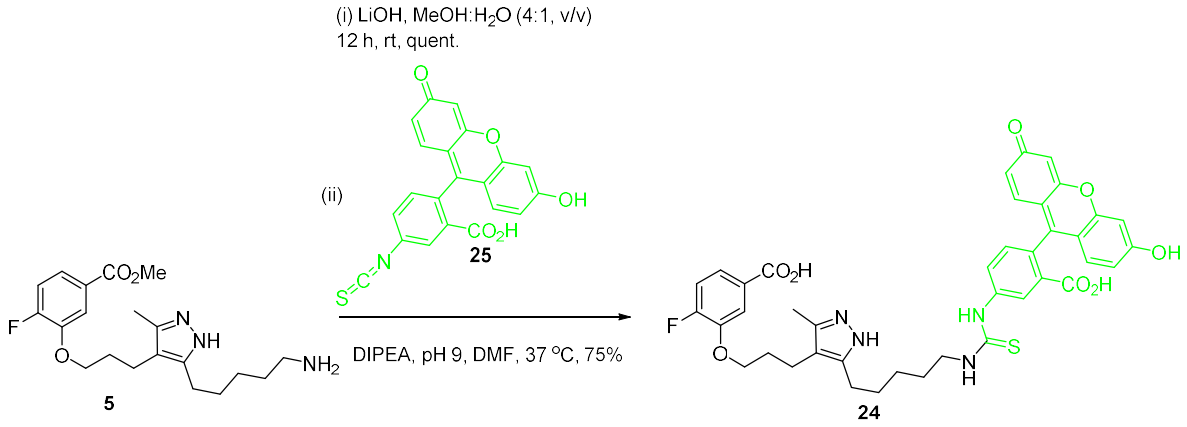


Figure S15: MALDI data of conjugate **27**.



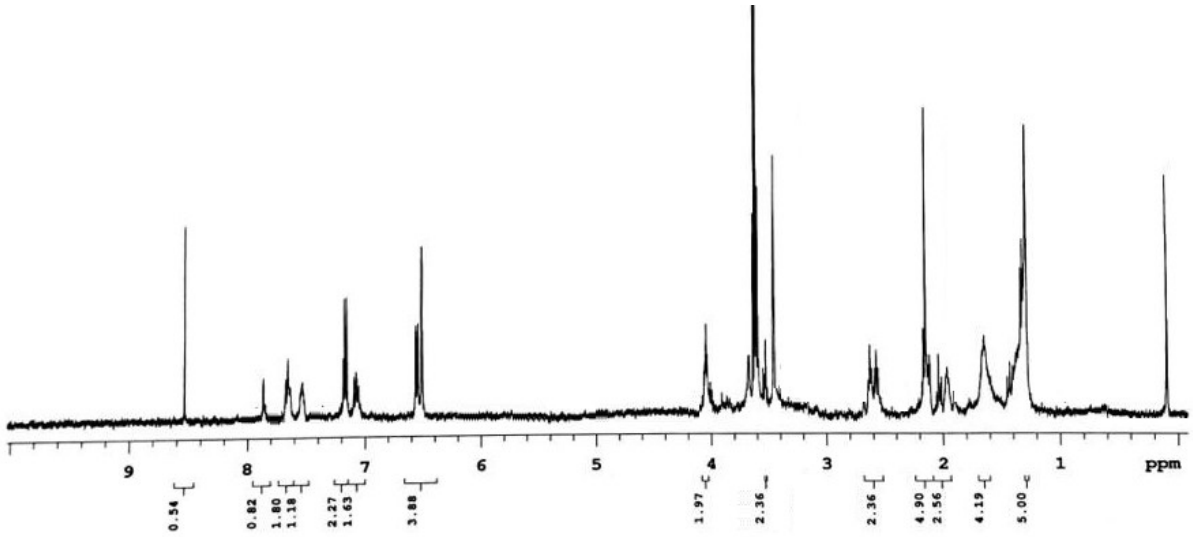
Scheme S3: Synthesis of compound **24**.



Compound **5** (5 mg, 0.01 mmol) was dissolved in a mixture of methanol and water (2 mL, 1:1, v/v). To this suspension was added LiOH (1.5 mg, 0.06 mmol) and the solution was left steering for overnight at room temperature. After completion, the reaction mixture was cooled to 0 ºC in an ice-bath and the solution was neutralized with aq. HCl (1N). The crude product was extracted

with EtOAc (3x 20 mL), washed with water, brine solution, combined organic layers was dried over anhydrous sodium sulfate and concentrated under vacuum to afford NH_2-terminated 4-fluoro-benzoic acid derivative (4 mg) of compound **5**. The compound was used directly for coupling with fluorescein isothiocyanate (FITC, **25**) without further purification. NH_2-terminated 4-fluoro-benzoic acid derivative (2 mg, 0.005 mmol) of compound **5** and FITC **25** (2.4 mg, 0.06 mmol) were dissolved in anhydrous DMF (100 μL). DIPEA (1.7 μL, 0.016, 3 equiv.) was added to the solution and pH of the solution was adjusted to 8.5-9.0. The solution was left stirred for overnight under dark at room temperature. After completion, solvent was removed under reduced pressure and the crude product was purified by C_{18} Sep-pak chromatography using gradient elution (0.1% aq. AcOH to 1:8 v/v MeOH/0.1% aq AcOH) under dark to afford derivative **24** (3.14 mg, 75 %). R*f* = 0.4 (10% MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, CD_3OD): δ 8.53 (s, 1H), 7.86 (s, 1H), 7.67-7.64 (m, 2H), 7.54-7.52 (m, 1H), 7.18-7.15 (m, 2H), 7.09-7.05 (m, 1H), 6.56 (d, *J* = 6.5 Hz, 1H), 6.54 (d, *J* = 6.5 Hz, 1H), 6.51 (d, *J* = 2.0 Hz, 1H), 4.06-4.03 (m, 2H), 3.67-3.64 (m, 2H), 2.62 (t, *J* = 7.0 Hz, 1H), 2.57 (t, *J* = 8.0 Hz, 1H), 2.15 (s, 3H), 2.18-2.11 (m, 2H), 1,98-1.96 (m, 2H), 1.65-1.63 (m, 2H), 1.32-1.29 (m, 4H). LC-MS calcd for C40H39N4FSO8: 753.3, found: 753.1.

Figure S16: ^{1}H NMR spectrum of compound **24**.



References

(1) Daskhan, G. C., Tran, H.-T. T., Meloncelli, P. J., Lowary, T. L., West, L. J., and Cairo, C. W. (2018) Construction of Multivalent Homo- and Heterofunctional ABO Blood Group Glycoconjugates Using a Trifunctional Linker Strategy. *Bioconjugate Chem. 29*, 343-362.

(2) Yu, H., Karpel, R., and Chen, X. (2004) Chemoenzymatic synthesis of CMP-sialic acid derivatives by a one-pot two-enzyme system: comparison of substrate flexibility of three microbial CMP-sialic acid synthetases. *Bioorg. Med. Chem. 12*, 6427-6435.

(3) Yu, H., Huang, S. S., Chokhawala, H., Sun, M. C., Zheng, H. J., and Chen, X. (2006) Highly efficient chemoenzymatic synthesis of naturally occurring and non-natural alpha-2,6-linked sialosides: A P. damsela alpha-2,6-sialyltransferase with extremely flexible donor-substrate specificity. *Angew. Chem., Int. Ed. 45*, 3938-3944.