Supporting Information

A 'catch-and-release' receptor for the cholera toxin

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Characterisation of polymer **P1**

The molecular weight and monomer composition of **P1** was determined by end-group analysis, comparing the integration of the terminal C*H*$_3$ protons with the C*H*(CH$_3$)$_2$ protons of NIPAm and the C*H*CH$_2$ and CHC*H*$_2$ protons of NIPAm and **M1**. The monomer composition of **P1** was determined to be 4:1 NIPAm:**M1**, in contrast to the feed ratio of 8:1 NIPAm:**M1**, most likely as a consequence of a difference in reactivity of the two monomers.

polymer	chain transfer agent	monomers	initiator	solvent	time / h	temp / °C	M_n[a] / g mol^{-1}	M_n[b] / g mol^{-1}	M_w[b] / g mol^{-1}	PDI[b] (M_w/M_n)
P1	DDMAT (1 eq)	NIPAm (160 eq) **M1** (20 eq)	AIBN (0.2 eq)	DMSO	18	70	13,400	20,300	22,700	1.12

Table 1 Characterisation of copolymer **P1**. [a] As determined by ^{1}H NMR spectroscopy. [b] As determined by gel permeation chromatography in DMF (0.6 mL/ min) calibrated against near monodisperse methyl methacrylate standards. AIBN: azobis(isobutyronitrile), DMSO: dimethylsulfoxide, DMA: *N*,*N*-dimethylacrylamide, DDMAT: *S*-1-dodecyl-*S*''-(α,α-dimethyl-α''-acetic acid)trithiocarbonate.

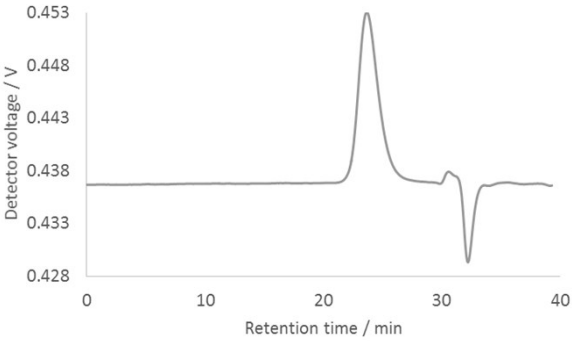


Fig. S1 Differential refractive index gel permeation chromatography (GPC) trace of **P1** in DMF (0.6 mL/min, containing 1.0 g/L LiBr).

Characterisation of GM1os functionalised polymer **P2-GM1os**

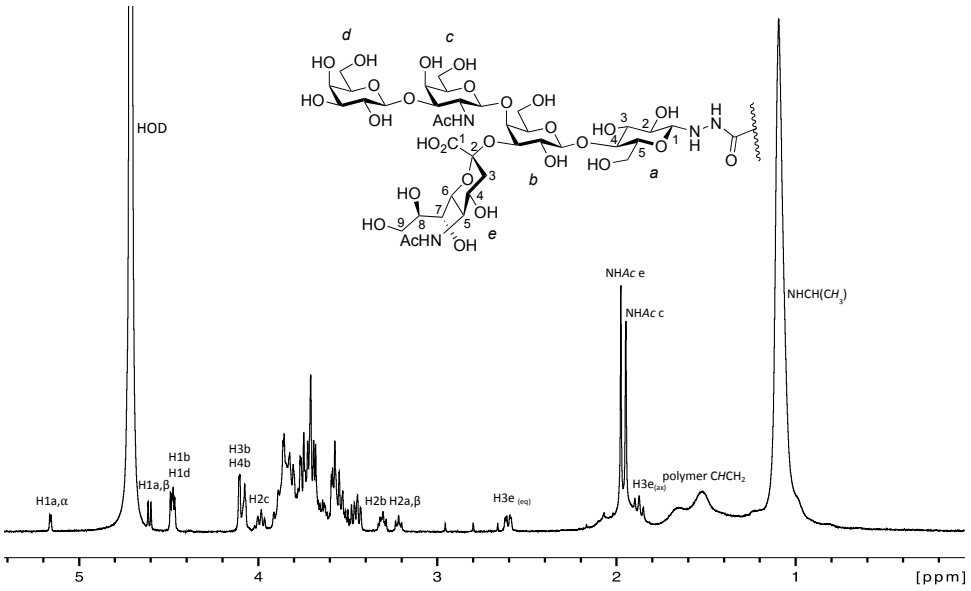


Fig S2 ^{1}H NMR spectrum of **P2-GM1os** (500 MHz, D_2O)

The concentration of **P2-GM1os** solutions used for ITC and ELLA experiments was determined by quantification of the trithiocarbonate end groups. Solutions of known concentration of **P2** between 104 µM and 3.26 µM were prepared in triplicate and their absorbance at 309 nm was determined (Fig. S2) allowing the molar extinction coefficient ε_{309} to be determined to be 9920 M^{-1} cm^{-1}.

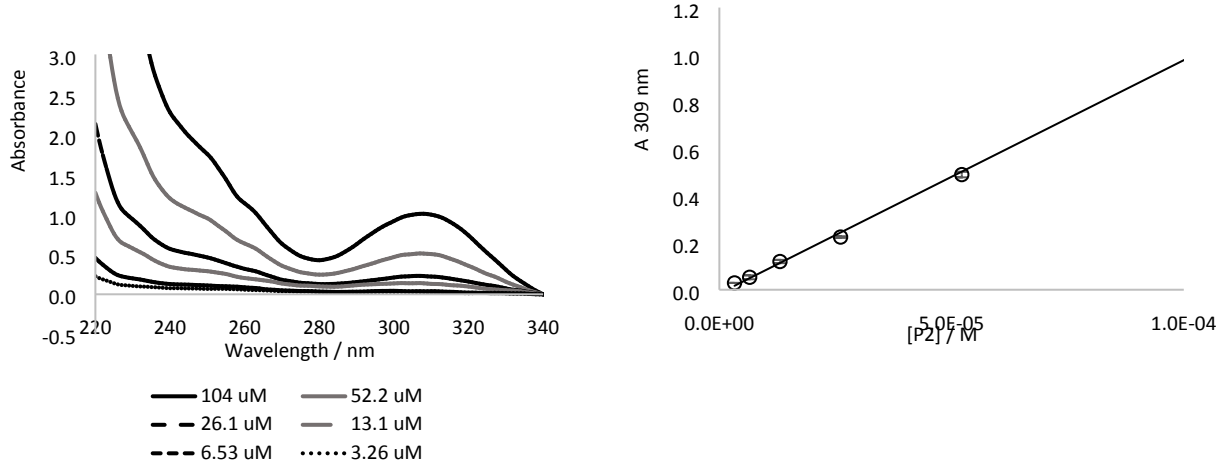


Fig. S3 (a) UV-Vis absorbance spectra for solutions of **P2**. (b) Beer-Lambert plot for **P2**.

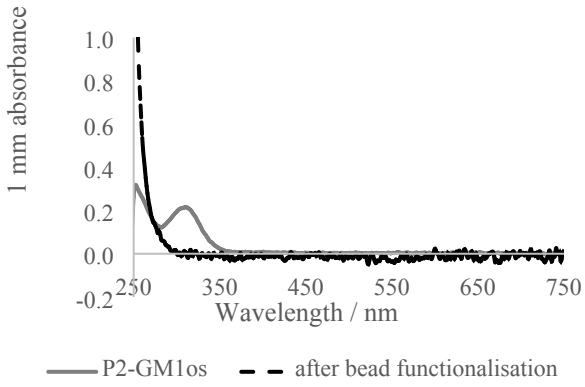


Fig. S4 UV-Vis absorbance spectra for a solution of **P2-GM1os**, and the same solution after aminolysis and exposure to maleimide-functionalise agarose beads.

Development of organoid swelling assay

To develop the organoid assay, intestinal organoids were stimulated dose dependently with cholera toxin to select a nonsaturating concentration for inhibitor testing while retaining maximal assay sensitivity (Fig. S5). 3 µg/mL of cholera toxin was required to induce sufficient swelling of organoids which is 30 times higher than used in the earlier experiments.[1] We next assessed dose-dependent inhibition of cholera toxin- mediated swelling of **P2-GM1os** for binding cholera toxin B subunit. GM1os and free galactose was measured as a reference inhibitors. Organoids were stimulated with cholera toxin, with or without inhibitors.

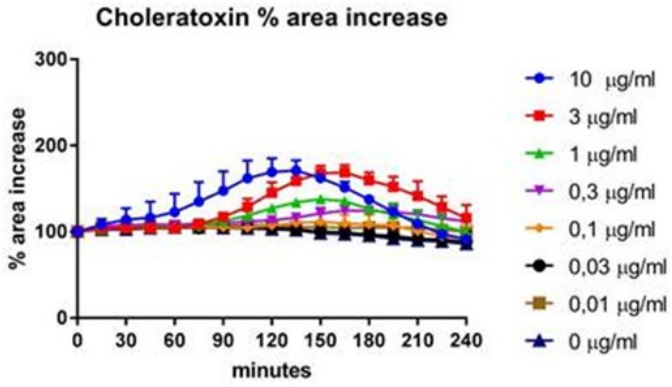
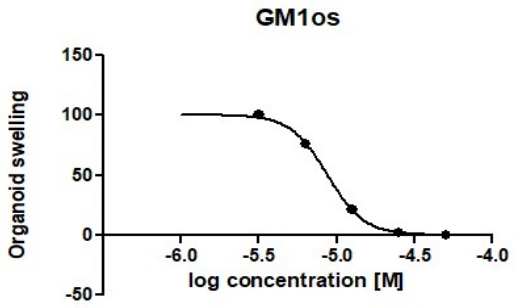


Fig. S5 Organoids were stimulated with cholera toxin as indicated (µg/mL), and organoid swelling was measured by relative area increase in time (t = 0 min: 100%), n = 1 triplicate ± SD.



log(inhibitor) vs. response -- Variable slope (met IC50 errors)	
Best-fit values	
BOTTOM	= 0.0
TOP	= 100.0
LOGIC50	-5.059
HILLSLOPE	-3.656
IC50	8.739e-006
Span	= 100.0
Std. Error	
LOGIC50	0.1066
HILLSLOPE	2.453
IC50	2.144e-006
95% Confidence Intervals	
LOGIC50	-5.274 to -4.843
HILLSLOPE	-8.606 to 1.295
IC50	4.412e-006 to 1.307e-005
Goodness of Fit	
Degrees of Freedom	43
R²	0.2850
Absolute Sum of Squares	187088
Sy.x	65.96
Constraints	
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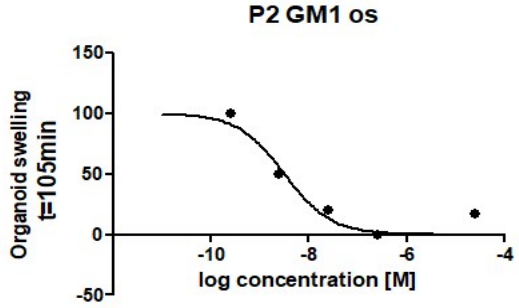
TOP	TOP = 100.0
Number of points	
Analyzed	45



log(inhibitor) vs. response -- Variable slope (met IC50 errors)	
Best-fit values	
BOTTOM	= 0.0
TOP	= 100.0
LOGIC50	-8.492
HILLSLOPE	-0.9095
IC50	3.222e-009
Span	= 100.0
Std. Error	
LOGIC50	0.1970
HILLSLOPE	0.3547
IC50	1.462e-009
95% Confidence Intervals	
LOGIC50	-8.917 to -8.066
HILLSLOPE	-1.676 to -0.1433
IC50	6.507e-011 to 6.380e-009
Goodness of Fit	
Degrees of Freedom	13
R²	0.7613
Absolute Sum of Squares	5391
Sy.x	20.36
Constraints	
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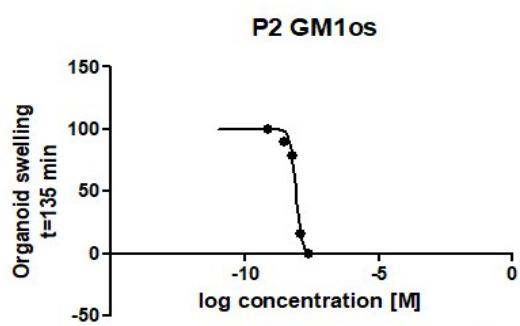
Number of points	
Analyzed	15



	P2 GM1 os
log(inhibitor) vs. response -- Variable slope (met IC50 errors)	
Best-fit values	
BOTTOM	= 0.0
TOP	= 100.0
LOGIC50	-8.089
HILLSLOPE	-4.098
IC50	8.149e-009
Span	= 100.0
Std. Error	
LOGIC50	0.1267
HILLSLOPE	3.409
IC50	2.378e-009
95% Confidence Intervals	
LOGIC50	-8.363 to -7.815
HILLSLOPE	-11.46 to 3.267
IC50	3.013e-009 to 1.328e-008
Goodness of Fit	
Degrees of Freedom	13
R²	0.4725
Absolute Sum of Squares	27695
Sy.x	46.16
Constraints	
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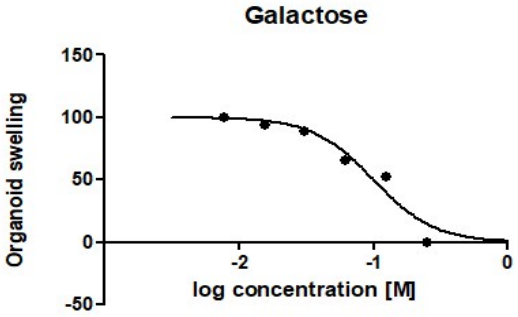
TOP	TOP = 100.0
Number of points	
Analyzed	15



log(inhibitor) vs. response -- Variable slope (met IC50 errors)	
Best-fit values	
BOTTOM	= 0.0
TOP	= 100.0
LOGIC50	-0.9968
HILLSLOPE	-1.920
IC50	0.1007
Span	= 100.0
Std. Error	
LOGIC50	0.07190
HILLSLOPE	0.5772
IC50	0.01668
95% Confidence Intervals	
LOGIC50	-1.141 to -0.8523
HILLSLOPE	-3.080 to -0.7592
IC50	0.06720 to 0.1343
Goodness of Fit	
Degrees of Freedom	50
R²	0.4959
Absolute Sum of Squares	54894
Sy.x	33.13
Constraints	
BOTTOM	BOTTOM = 0.0
TOP	TOP = 100.0
Number of points	
Analyzed	52

Organoid swelling assays

Fig. S6 Organoid swelling assays indicate the inhibitory potency of **P2-GM1os** compared to GM1os and galactose.

NMR spectra

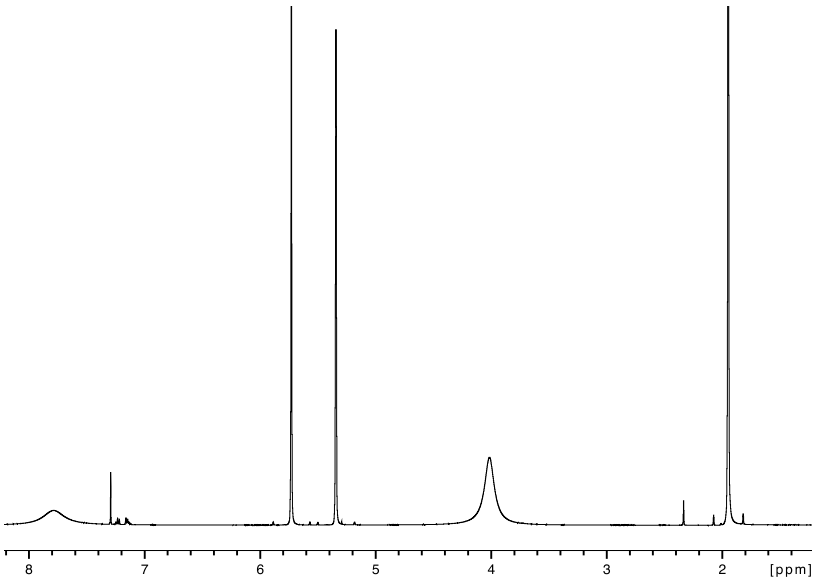


Fig S7 ^{1}H NMR spectrum of **1** (500 MHz, $CDCl_3$)

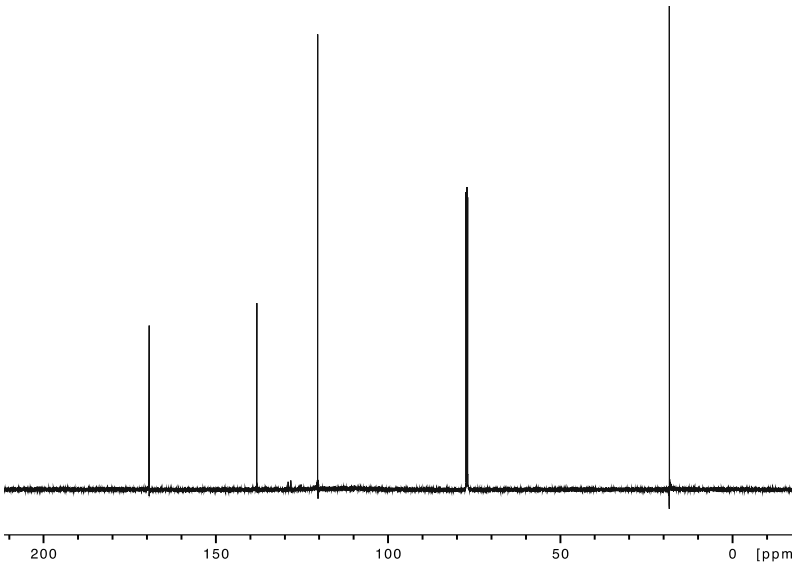


Fig S8 ^{13}C NMR spectrum of **1** (125 MHz, $CDCl_3$)

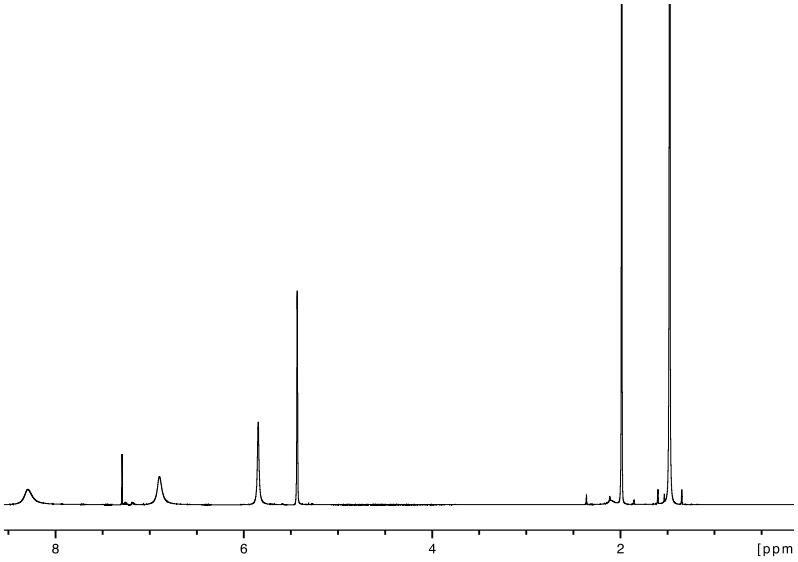


Fig S9 ^{1}H NMR spectrum of **M1** (500 MHz, $CDCl_3$)

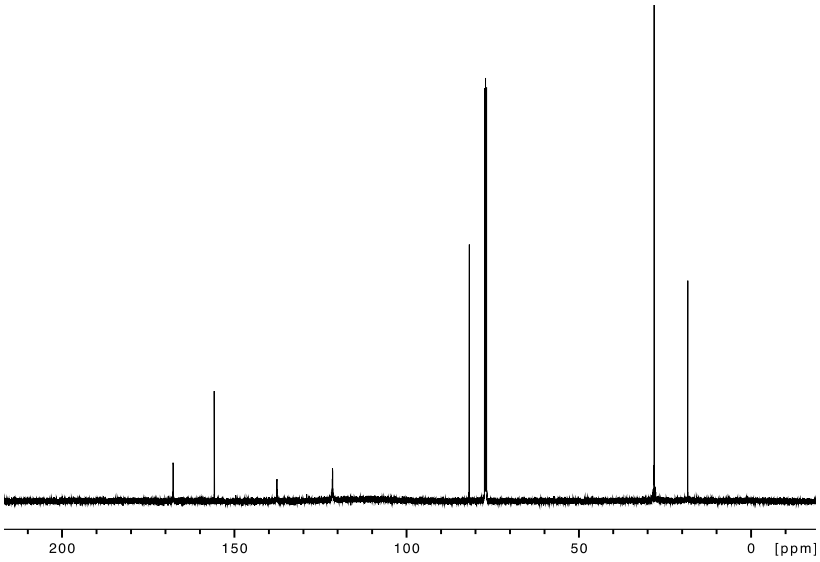


Fig S10 ^{13}C NMR spectrum of **M1** (125 MHz, $CDCl_3$)

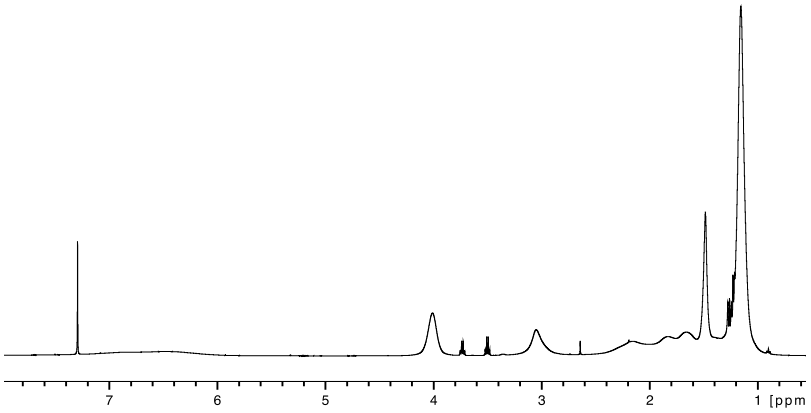


Fig S11 ^{1}H NMR spectrum of **P1** (500 MHz, $CDCl_3$)

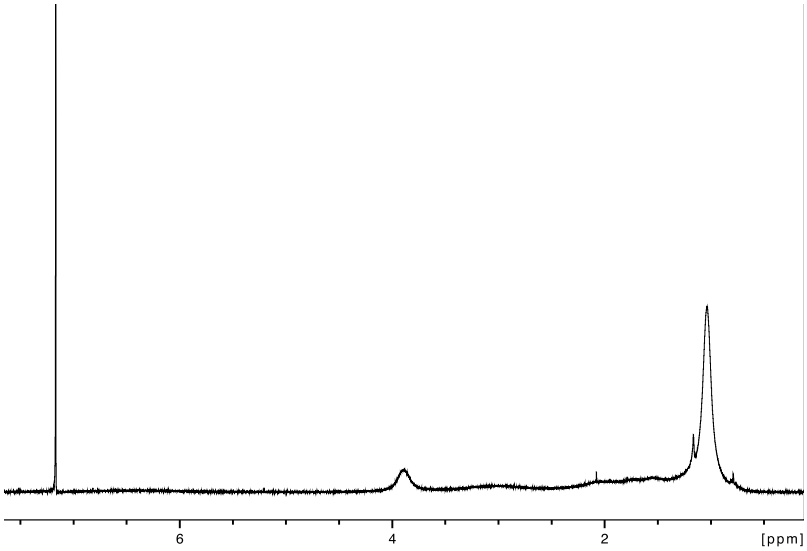


Fig S12 ^{1}H NMR spectrum of **P2** (500 MHz, $CDCl_3$)

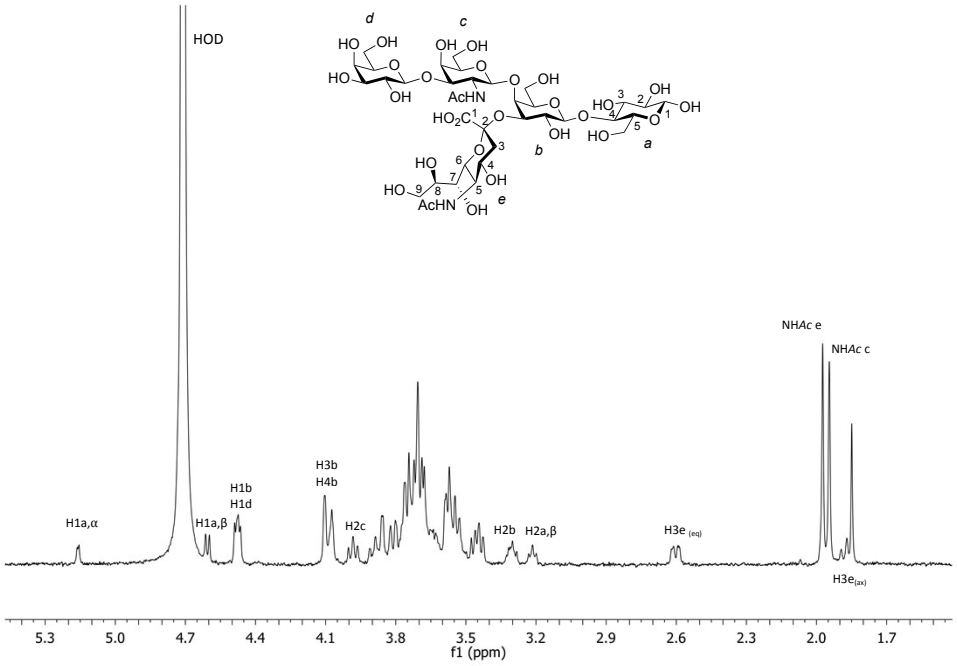


Fig S13 ^{1}H NMR spectrum of GM1os (500 MHz, D_2O)

References

1. D. D. Zomer-van Ommen, A. V. Pukin, O. Fu, L. H. C. Quarles van Ufford, H. M. Janssens, J. M. Beekman and R. J. Pieters, *J. Med. Chem.*, 2016, **59**, 6968-6972.