SPR Methods

Materials and Reagents. Streptavidin-coated SAD 50M sensorchips for a Biacore 2000 (GE) were purchased from XanTec Bioanalytics Gmbh. For running buffer, 20x HBS-EP (0.2 M Hepes, 3 M sodium chloride, 60 mM EDTA, 1.0% polysorbate 20, pH 7.6) (Teknova) was diluted to working concentration, and bovine serum albumin (BSA) (US Biological) was added to 10 mg/mL to approximate the refractive index of 10% serum samples. The running buffer was filtered and deoxygenated. Serum samples were diluted to 10% in running buffer without BSA and filtered. Biotinylated antigens or c(RGDfK)-$(EG)_4$-biotin (Peptides International) were diluted to 5 μM in buffer for immobilization. The cell adhesive cyclic peptide, c(RGDfK)-$(EG)_4$-biotin was used to generate a control surface. Goat anti-human IgG Fcγ-specific and goat anti-human IgM Fc5μ-specific (Jackson Immunoresearch) were diluted to 20 μg/mL in BSA-containing running buffer. Serum was collected from healthy volunteer donors under IRB-approved Human Subjects minimal risk protocol M-2005-1282 at UW-Madison.

Data Collection and Analysis. Ligands were immobilized by using the kinject feature on the Biacore 2000 (GE) (an injection protocol that minimizes artifacts that obscure the first few seconds of the association phase). Kinject was used to apply a volume of 50 μL of biotinylated antigen across the proper flow channel at 10 μL/min in the following order for the first chip: FC4- cRGD, FC3- rhamnose, FC2- αGal, FC1- DNP. The serum sample (125 μL) was then kinjected (300 s on, 300 or 3600 s off) at 25 μL/min. A solution of secondary antibody (75 μL of 20 μg/mL antibody) was injected immediately following the end of serum kinject, still at 25 μL/min. To limit bulk refractive index changes and keep signal within the instrument's detection range, solutions of 10% serum were used. The surface was regenerated by a pulse of 10 mM HCl (50 μL) at 100 μL/min flow across each channel individually to minimize contamination of downstream channels. Over the course of multiple injections, ligands were periodically added to maintain immobilization levels. An example workflow is provided in Figure S1.

FLOW 10 FLOWPATH 4 INJECT cRGD 50 FLOWPATH 3 INJECT Rhamnose 50 FLOWPATH 2 INJECT αGal 50 FLOWPATH 1 INJECT DNP 50 FLOWPATH 1,2,3,4	FLOW 25 KINJECT Serum 125 3600 INJECT anti-IgG 75	KINJECT Serum 125 300 INJECT anti-IgM 75
FLOW 25 KINJECT Serum 125 3600 INJECT anti-IgM 75	FLOW 100 FLOWPATH 4 INJECT 10 mM HCl 50 FLOWPATH 3 INJECT 10 mM HCl 50 FLOWPATH 2 INJECT 10 mM HCl 50 FLOWPATH 1 INJECT 10 mM HCl 50	FLOW 100 FLOWPATH 4 INJECT 10 mM HCl 50 FLOWPATH 3 INJECT 10 mM HCl 50 FLOWPATH 2 INJECT 10 mM HCl 50 FLOWPATH 1 INJECT 10 mM HCl 50 FLOWPATH 1,2,3,4
FLOW 100 FLOWPATH 4 INJECT 10 mM HCl 50 FLOWPATH 3 INJECT 10 mM HCl 50 FLOWPATH 2 INJECT 10 mM HCl 50 FLOWPATH 1 INJECT 10 mM HCl 50 FLOWPATH 1,2,3,4	FLOW 10 FLOWPATH 4 INJECT cRGD 50 FLOWPATH 3 INJECT Rhamnose 50 FLOWPATH 2 INJECT αGal 50 FLOWPATH 1 INJECT DNP 50 FLOWPATH 1,2,3,4	FLOW 25 KINJECT Serum 125 300 INJECT anti-IgG 75
	FLOW 25	FLOW 100 FLOWPATH 4 INJECT 10 mM HCl 50 FLOWPATH 3 INJECT 10 mM HCl 50 FLOWPATH 2 INJECT 10 mM HCl 50 FLOWPATH 1 INJECT 10 mM HCl 50

Fig. S1 Example Biacore 2000 workflow. Squares highlighted in yellow indicate ligand immobilization and refreshing of ligand surface to maintain density and ligand composition. Boxes shaded purple are serum and secondary antibody injections. Boxes shaded blue are regenerations. Flow cells were regenerated individually to minimize ligand scrambling. At the end of each regeneration block, RUs were determined to ensure effective regeneration. For some sera, an extra round of washing was employed to attain surface regeneration.

Data were analyzed using BIAevaluation (Biacore) to generate, align, and reference subtract curves (Fig. S2, S3). Specific values for analysis were retrieved from BIAevaluation manually and plotted in either Excel or Prism 5 (Graph Pad).

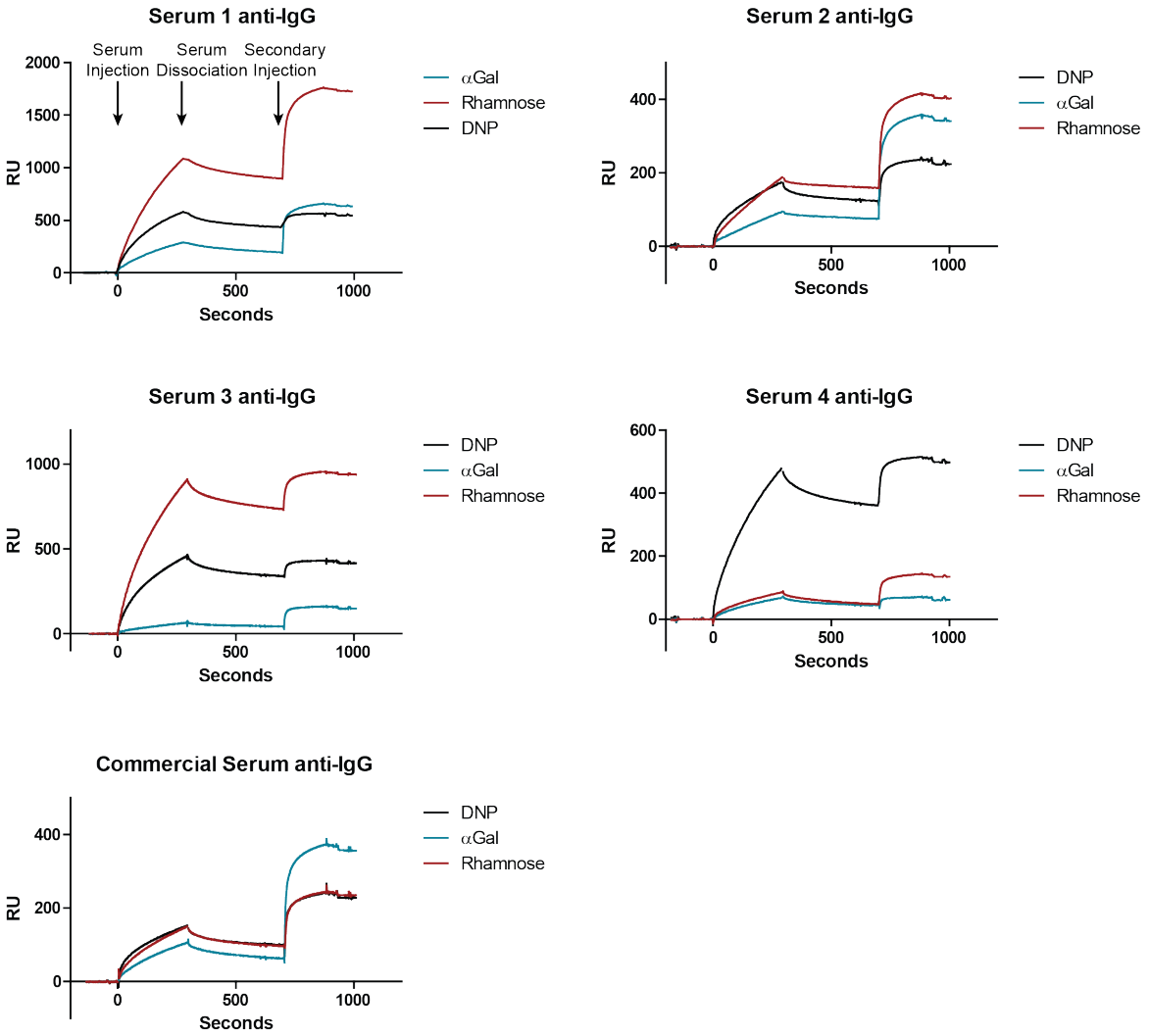


Fig. S2 Representative SPR curves obtained after each surface was exposed to serum and then anti-IgG.

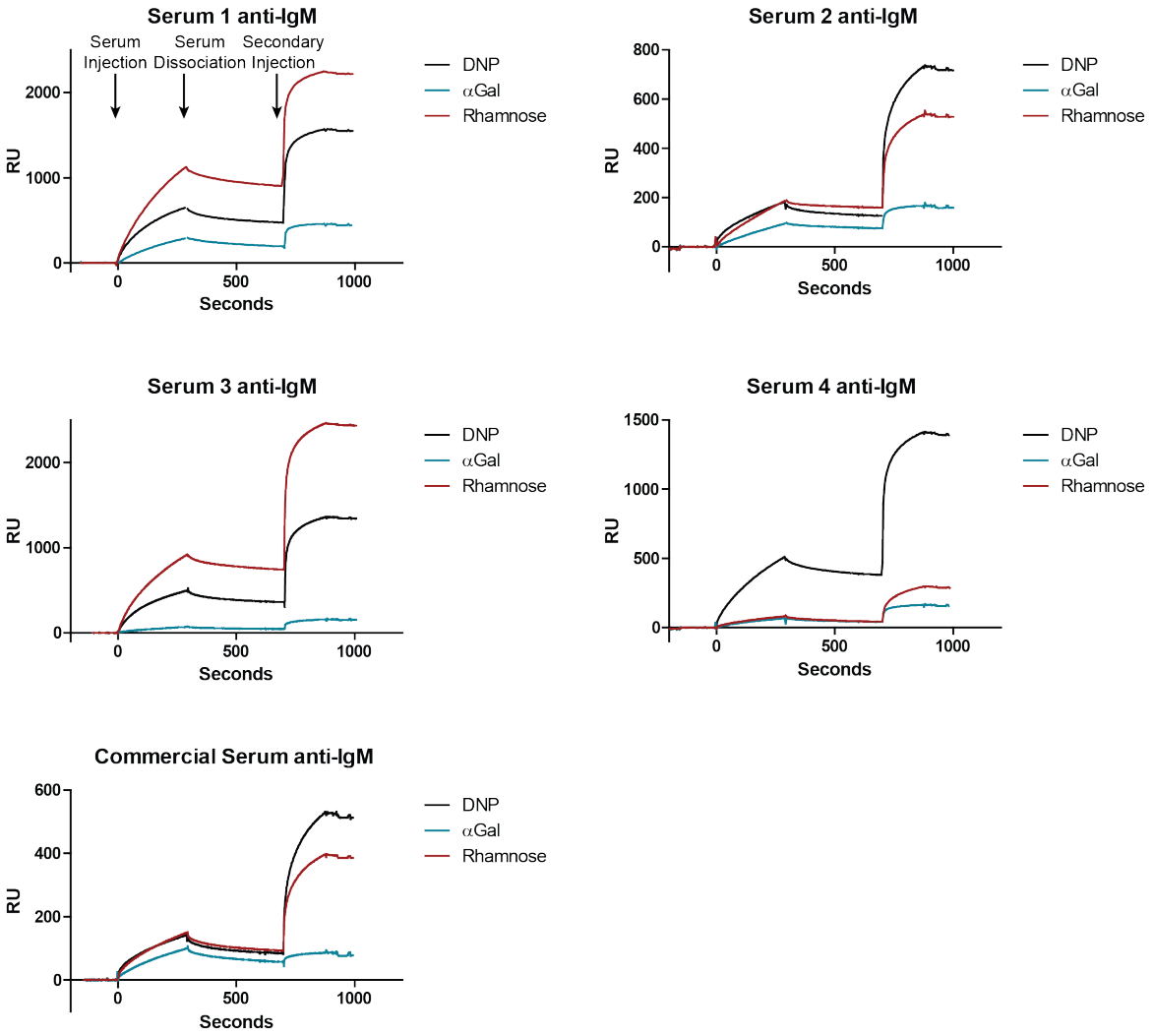
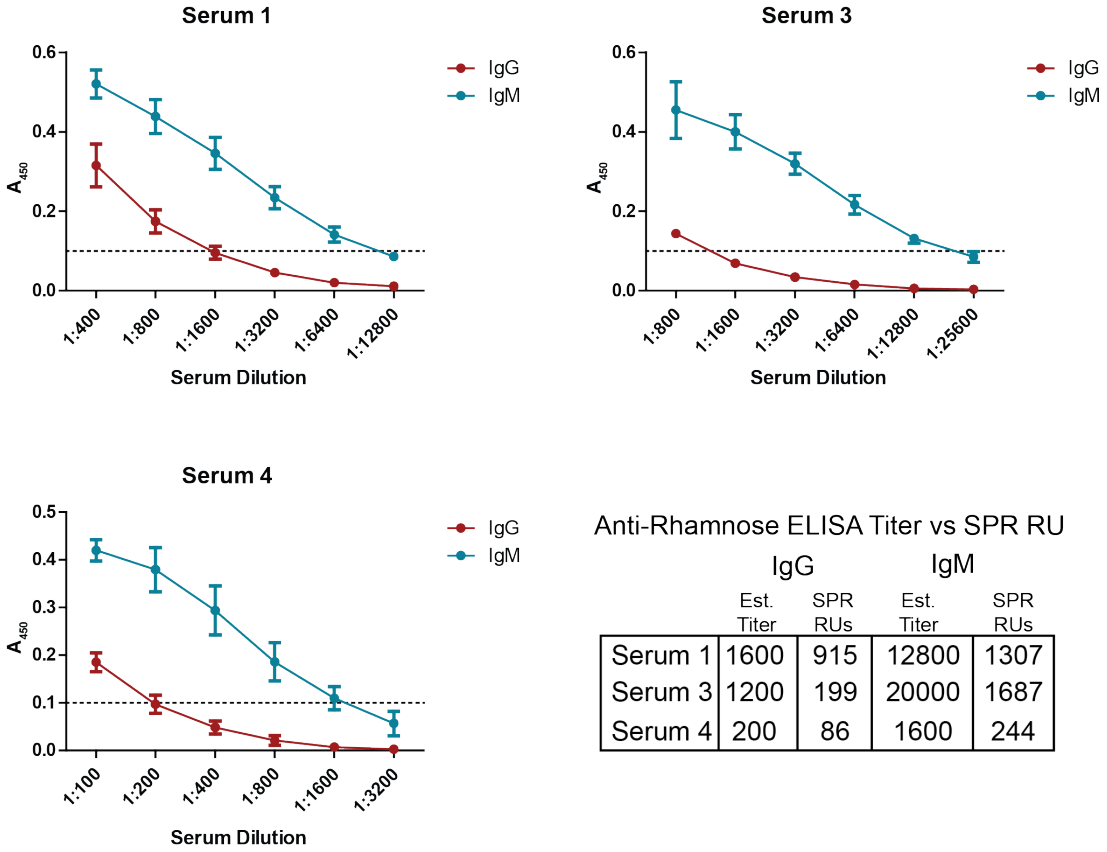


Fig. S3 Representative SPR curves obtained after each surface was exposed to serum and then anti-IgM.

ELISA Methods

To complement the SPR data, sera were analyzed for anti-rhamnose antibodies by an enzyme-linked immunosorbent assay (ELISA). Immunosorp plates 96-well (Nunc) were coated with 100 µL of either 100 µg/mL BSA-rhamnose (see synthetic methods figure **S9**) or BSA alone overnight at 4 °C. Plates were rinsed with phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) and blocked for 2 h at rt with 5% non-fat dry milk in PBS-T (PBS with 0.2% Tween-20). After a rinse with PBS, 100 µL of serum in triplicate two-fold dilutions was added and samples were maintained at rt for 2 h. Plates were washed 4x with PBS-T before the addition of 100 µL 1:2000 goat anti-human IgG-HRP conjugate (horseradish peroxidase) or goat anti-human IgM-HRP conjugate (Caltag). Plates were washed 3x with PBS-T before addition of 100 µL 1-step Turbo TMB (3,3',5,5'-tetramethylbenzidine, a chromogenic substrate for horseradish peroxidase) (Pierce), and the plates were allowed to develop for 3 min before samples were quenched with 1 M sulfuric acid. The absorbance was read at 450 nm on an ELx800 plate reader (BioTek). Results from wells (triplicate) were averaged, and the absorbance values from BSA-only wells were subtracted from those obtained from the wells with rhamnosylated BSA Error bars are ±1 standard deviation from 3 or 4 replicate plates.



Anti-Rhamnose ELISA Titer vs SPR RU

	IgG		IgM	
	Est. Titer	SPR RUs	Est. Titer	SPR RUs
Serum 1	1600	915	12800	1307
Serum 3	1200	199	20000	1687
Serum 4	200	86	1600	244

Fig. S4 ELISA validation of anti-rhamnose biosensor results. Sera showing higher levels of antibody by SPR also had higher titers by traditional ELISA. Titer was estimated as the serum dilution that would give an A_{450} of 0.1

Complement-Dependent Cytotoxicity Assay

M21 melanoma cells (ATCC) were grown overnight in a 12-well plate. Dipalmitoleoylphosphoethanolamine (DPoPE, Avanti Polar Lipids) or Rha_2-DPoPE (see synthetic methods, compound **S.13**) to make 1 mL of 0.1 mM lipid suspension were transferred to clean glass vials and the chloroform was evaporated under argon. Lipids were rehydrated with 1 mL of Hank's balanced salt solution (HBSS, Gibco) 1% BSA pH 7.4 for 30 min then sonicated in a water bath to form the suspension. Growth medium was removed from the M21 cells and 0.5 mL of either lipid suspension or control buffer was added to each well. Cells were treated for 1 hour at 37 °C to allow lipid insertion. The treatment solution was aspirated and cells were removed from the plate with 1 mM EDTA, washed, and resuspended in HBSS 1% BSA at $5x10^5$ cells mL^{-1}. A 50% solution of normal human serum was made by combining equal volumes of normal human serum and HBSS 1% BSA and a 50% heat inactivated serum stock was made similarly. Heat inactivation was performed by heating 100% serum at 56 °C for 30 minutes to inactivate complement components while leaving antibodies intact. An epitube was placed in a rack for each treatment condition. For conditions not receiving blocking antibodies, 200 μL HBSS 1% BSA buffer was added. For conditions receiving blocking antibodies, 156 μL buffer, 4 μL anti-CD59 (clone MEM-43, Novus Biologicals), and 40 μL anti-CD55 (clone 143-30, Southern Biotech) were added to the epitube. The final antibody concentrations in the assay well are 10 μg mL^{-1} of each antibody. 50 μL of each treatment condition was plated into 6 replicate wells of an opaque white 96-well plate. To three of the replicates, 50 μL of 50% normal human serum was added, the other three received 50 μL of 50% heat inactivated serum which lacks functional complement. The plate was held at 37 °C for 2 h to allow for complement-mediated cytotoxicity. The mixture was cooled to rt and 100 μL Cell Titer Glo (Promega) was added. After a short incubation, luminescence was read on an Infinite M1000 (Tecan) using the OD1 filter, 300 ms integration time, and 5 ms settle time after an initial 2 sec shaking step. Data were analyzed using Microsoft Excel. Briefly, results from triplicate experiments were averaged, and the percentage of viable cells was calculated as (100*luminescence $_{\text{Normal Serum}}$/luminescence $_{\text{Heat Inactive Serum}}$). The percentage of cell death was determined by substracting the percentage of cells remaining from the level obtained from viable cells. Biological replicates were performed as indicated and significance was determined by T test.

Complement deposition by flow cytometry

M21 cells were grown in a 12-well plate overnight and treated with suspensions of DPoPE and rha_2-DPoPE as described above for the cytotoxicity assay. Buffer and lipid treated cells were removed from the plate with 1 mM EDTA and resuspended in PBS 1% BSA at $7.5x10^5$ cells mL^{-1}. Into flow tubes containing 300 μL of 50% normal human serum, 300 μL of cell suspension was added to make the final concentration 25% serum. One tube of buffer-treated control cells received 1 μL of MAb 14.18, a positive control for complement activation. Cells were exposed for 1 h to serum at 37 °C, washed with cold PBS 1% BSA, and stained with 3 μL mouse anti-human C4d (Quidel) for 1 h on ice. After cells were washed again, they were treated 3 μL PE-rat anti-mouse (Becton Dickinson) for 1 h on ice and then evalated on a FACSCalibur flow cytometer (Becton Dickinson). Buffer-treated cells were exposed to serum as a measure of background complement activity. Data from all samples were analyzed in FlowJo (TreeStar Inc.).

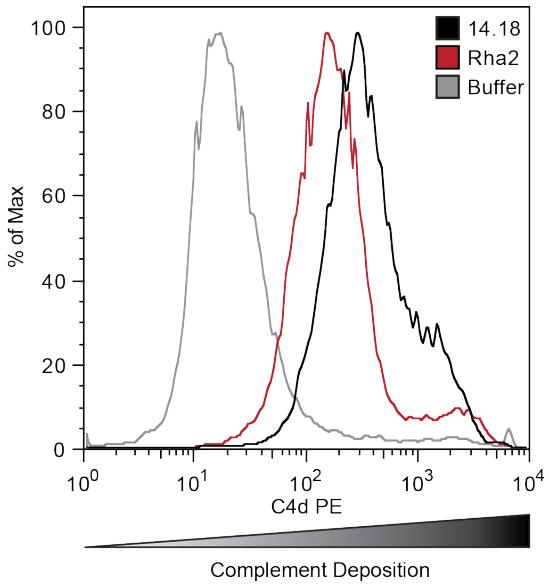


Fig. S5 Assessment of complement activation for M21 cells exposed to Rha_2-DPoPE and human serum (red). Antibody 14.18 (black) was used as a control for postive complement activation.

Synthetic Methods

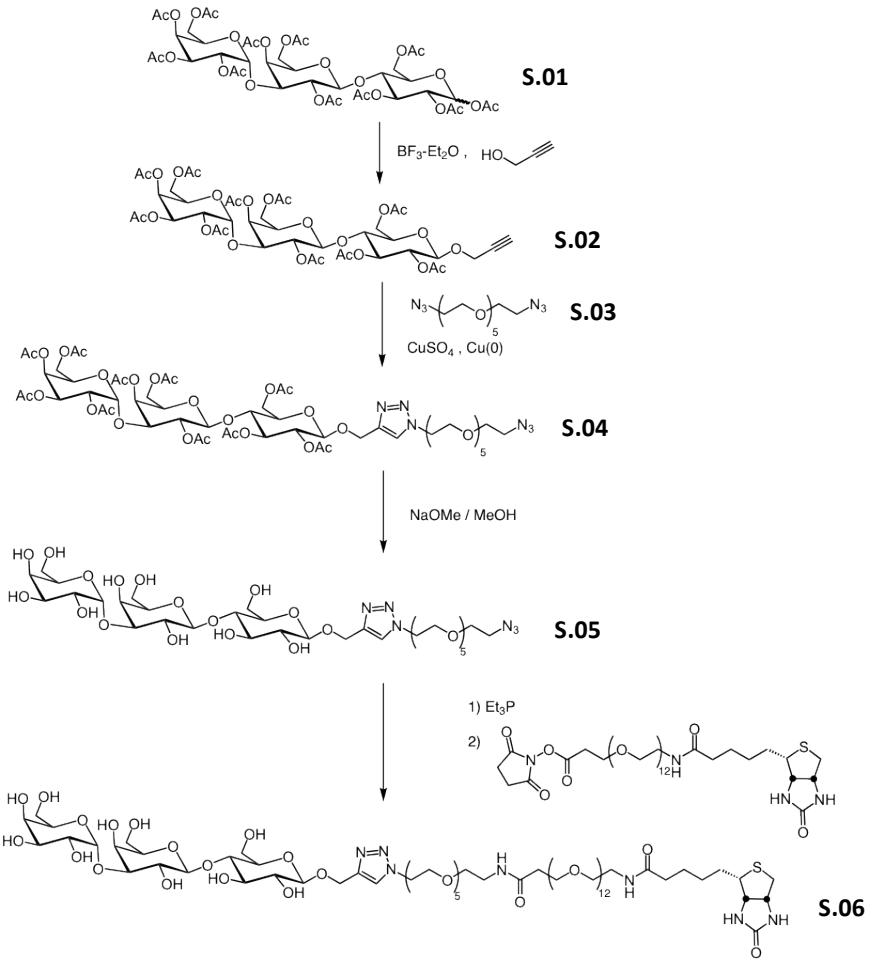
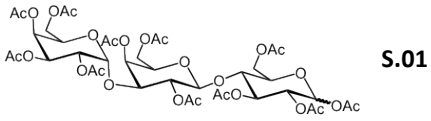
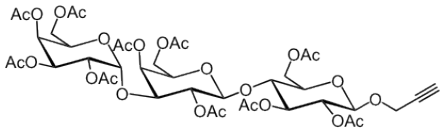


Figure S6 Synthesis of compound α-Gal biotin



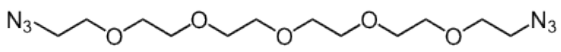
Compound **S.01** was synthesized according to previously published procedures[1] as a 4:6 mixture of alpha/beta anomers.



Compound **S.01** (517.0 mg, 0.54 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and combined with propargyl alcohol (150 uL, 2.6 mmol) and powdered 4 Å molecular sieves (752.6 mg) under a nitrogen atmosphere. The resulting suspension was stirred at rt for 30 min, then cooled to 0 °C and stirred for 1 h. Boron trifluoride diethyl etherate (400 uL, 3.2 mmol) was slowly added over 5 minutes to the cooled suspension, which turned light pink. The reaction mixture was left to warm to rt and stirred overnight. The mixture was diluted with ethyl acetate (50 mL) and quenched with a saturated solution of sodium bicarbonate (50 mL). The solids were filtered and the

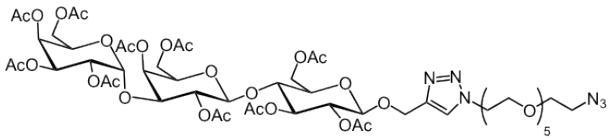
layers separated. The organic layer was washed with brine (5 mL), dried with sodium sulfate, filtered and concentrated in vacuo to a yellow oil. The resulting crude mixture was composed mainly of the desired product and the unreacted alpha anomer of the starting material, components that are difficult to separate by flash chromatography. To facilitate the purification of the product, the residue was solubilized in dry DMF (2.5 mL) and the mixture was exposed to hydrazine acetate (72.7 mg, 0.79 mmol) at room temperature for 2.5 hours, at which point the alpha anomer was completely consumed. The mixture was dissolved in ethyl acetate (25 mL), washed twice with saturated ammonium chloride solution (2 x 10 mL), then brine (5 mL), dried with sodium sulfate, filtered and evaporated in vacuo. The residue was purified by chromatography on silica gel using a gradient of 30→65% ethyl acetate in hexanes, to yield compound **S.02** as a viscous oil (152 mg, 0.16 mmol, 29% yield overall, 49% yield based on the beta anomer in compound **S.01**).

^{1}H NMR (500 MHz, Chloroform-d) δ 5.40 (d, *J* = 3.0 Hz, 1H), 5.29 (d, *J* = 3.1 Hz, 1H), 5.25 – 5.15 (m, 2H), 5.11 (dd, *J* = 10.2, 7.8 Hz, 1H), 5.05 (dd, *J* = 10.1, 3.2 Hz, 1H), 4.87 (dd, *J* = 9.5, 7.9 Hz, 1H), 4.71 (d, *J* = 7.9 Hz, 1H), 4.43 (dd, *J* = 11.9, 2.1 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 1H), 4.30 (d, *J* = 2.5 Hz, 2H), 4.21 – 3.92 (m, 7H), 3.83 – 3.71 (m, 3H), 3.62 (ddd, *J* = 10.2, 5.3, 2.0 Hz, 1H), 2.45 (t, *J* = 2.3 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 6H), 2.07 (s, 3H), 2.03 (s, 3H), 2.02 (s, 6H), 2.01 (s, 3H), 2.00 (s, 3H), 1.91 (s, 3H). MS (ESI) found: 980.3271 ($M+NH_4^+$), calculated: 980.3242



S.03

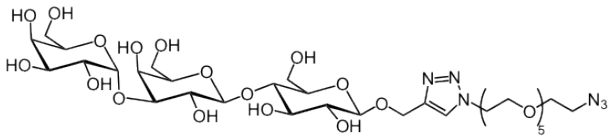
Compound **S.03** was synthesized according to published procedures.[2]



S.04

Alkyne **S.02** (26.2 mg, 0.027 mmol) was dissolved in dry acetonitrile (1 mL) under nitrogen, and azide **S.03** (95.5 mg, 0.29 mmol) in 0.2 mL of dry THF was added. To the resulting solution were added freshly cut copper wire, followed by a 0.3 M solution of copper sulfate (5 uL) in water. The reaction mixture was stirred at 50 °C overnight. The resulting suspension was filtered; the filtrate concentrated in vacuo; and the residue purified by column chromatography on silica gel, using a gradient of 0→8% methanol in dichloromethane to yield 29.6 mg of the desired product **S.04** as a viscous liquid in (0.023 mmol, 85% yield).

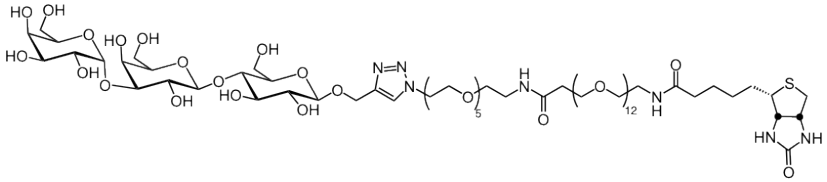
^{1}H NMR (300 MHz, Chloroform-d) δ 7.70 (s, 1H), 5.47 – 5.43 (m, 1H), 5.36 – 5.30 (m, 1H), 5.30 – 5.04 (m, 5H), 4.96 – 4.86 (m, 2H), 4.78 (d, *J* = 12.6 Hz, 1H), 4.64 (d, *J* = 7.9 Hz, 1H), 4.58 – 4.51 (m, 2H), 4.47 (dd, *J* = 12.0, 2.0 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.25 – 3.99 (m, 6H), 3.88 (t, *J* = 5.2 Hz, 2H), 3.85 – 3.74 (m, 3H), 3.71 – 3.56 (m, 19H), 3.43 – 3.34 (m, 2H), 2.15 (s, 3H), 2.13 (s, 6H), 2.12 (s, 3H), 2.06 (s, 9H), 2.03 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H). MS (ESI) found : 1317.4667 ($M+Na^+$), calculated: 1317.4604.



S.05

Acetylated azide **S.04** (28.5 mg, 0.022 mmol) was dissolved in dry methanol (1 mL) under nitrogen, then 100 μL of 1.0 M sodium methoxide in methanol was added. The reaction mixture was stirred at rt overnight, and then quenched using freshly washed Dowex 50WX-8 acidic resin (approximately 100 mg of resin). The reaction was filtered and the resin washed with water (3x5 mL). The combined filtrates were evaporated in vacuo to afford viscous oil (17.5 mg, 0.020 mmol, 91% yield). The crude product **S.05** was used without further purification.

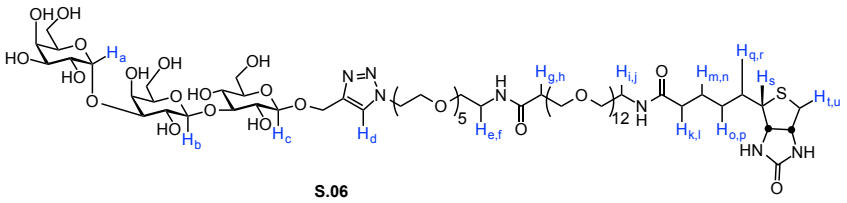
^{1}H NMR (300 MHz, Deuterium Oxide) δ 8.00 (s, 1H), 5.02 (d, *J* = 3.8 Hz, 1H), 4.89 (d, *J* = 12.6 Hz, 1H), 4.76 (d, *J* = 12.6 Hz, 1H), 4.57 – 4.49 (m, 2H), 4.46 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 7.7 Hz, 1H), 4.11 – 4.02 (m, 2H), 3.94 – 3.42 (m, 34H), 3.40 – 3.33 (m, 2H), 3.22 (t, *J* = 8.5 Hz, 1H). MS (ESI) found : 897.3548 ($M+Na^+$), calculated: 897.3547.



S.06

Azide **S.05** (4.4mg, 5 μmol) was dissolved in deionized water (500 μL) and acetonitrile (250 μL) under nitrogen. To this solution was added triethylphosphine (10 uL, 68 μmol), and the resulting mixture was stirred at rt for 5 h. The reaction mixture was concentrated in vacuo to eliminate any remaining triethylphosphine. The resulting mixture was re-solubilized in deionized water (200 μL) to which was added a solution of NHS-PEG_{12}-Biotin (80 μL of solution in DMSO, 2 μmol, Pierce Thermo Scientific, CAS number: 1262633-93-1, full name: 4,7,10,13,16,19,22,25,28,31,34,37-dodecaoxa-40-azapentatetracontanoic acid, 45-[(3a*S*,4*S*,6a*R*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]-41-oxo-,2,5-dioxo-1-pyrrolidinyl ester). The mixture was stirred at rt overnight. Amberlyst A-26(OH) basic resin (approximately 10 mg) was introduced into the reaction mixture, and the resulting suspension was stirred for 10 min, filtered, and concentrated in vacuo. The crude product was purified by reverse-phase HPLC (Vydac C18 column, 2→50% acetonitrile in water, containing 0.01 % formic acid). The product **S.06** was obtained after lyophilization 1.9 mg (1.1 umol, 57% yield) as a colorless film.

^{1}H NMR (500 MHz, deuterium oxide) δ 8.00 (s, 1H, H_d), 5.02 (d, *J* = 3.9 Hz, 1H, H_a), 4.88 (d, *J* = 12.5 Hz, 1H, H_c), 4.76 (d, *J* = 12.6 Hz, 1H, H_d), 4.71 – 4.70 (m, 1H), 4.64 – 4.62 (m, 2H), 4.53 (t, *J* = 5.0 Hz, 2H), 4.51 – 4.48 (m, 1H), 4.46 (d, *J* = 8.1 Hz, 1H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.30 (dd, *J* = 8.0, 4.5 Hz, 1H), 4.10 – 4.03 (m, 2H), 3.92 – 3.79 (m, 6H), 3.74 (dd, *J* = 10.4, 3.8 Hz, 1H), 3.72 – 3.45 (m, ~75H), 3.27 (td, *J* = 5.2, 3.6 Hz, 4H, $H_{e,f}$, $H_{i,j}$), 3.25 – 3.17 (m, 2H, 1H = H_s), 2.87 (dd, *J* = 13.1, 5.1 Hz, 1H, H_t), 2.66 (d, *J* = 13.0 Hz, 1H, H_u), 2.41 (t, *J* = 6.1 Hz, 2H, $H_{g,h}$), 2.15 (t, *J* = 7.3 Hz, 2H, $H_{k,l}$), 1.68 – 1.38 (m, 4H, $H_{m,n,q,r}$), 1.29 (p, *J* = 7.3 Hz, 2H, $H_{o,p}$). MS (ESI) found: 859.8904 [$(M+2Na)^{2+}$], calculated: 859.8914.



S.06

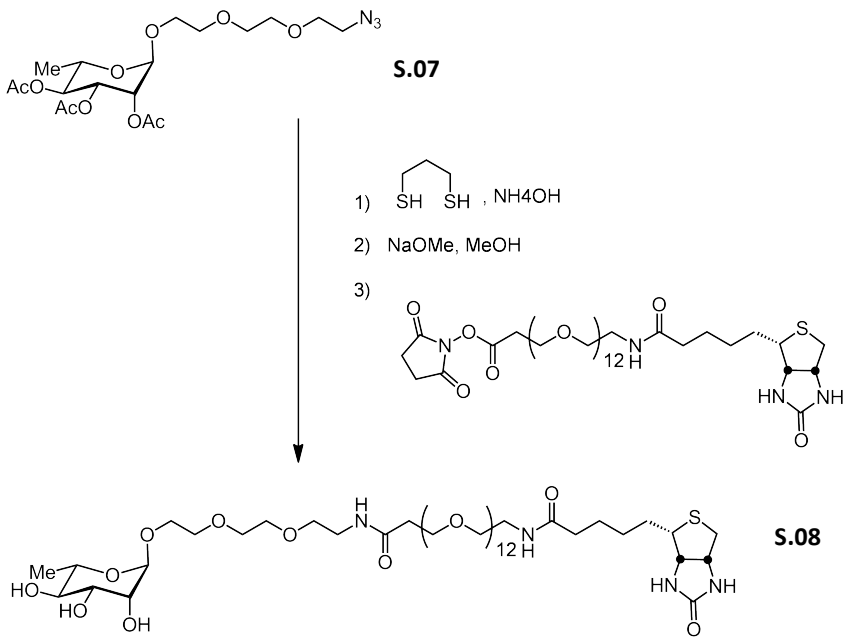
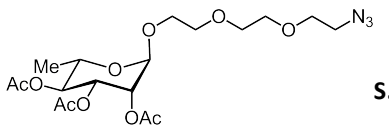
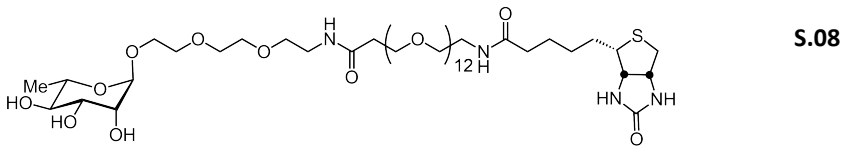


Figure S7 Synthesis of rhamnose-biotin derivative S.08



Compound **S.07** was synthesized according to published procedures.[3]



Azide **S.07** (13.5 mg, 30 μmol) was mixed with a 5% an aqueous solution of ammonium hydroxide (500 μL), acetonitrile (400 μL), and 1,3-propanedithiol (150 μL, 1.5 mmol) and stirred at rt under nitrogen overnight. The next day, LC-MS analysis of the compound revealed the complete reduction of the azide to the corresponding amine, partial acetylation of this newly formed amine, and incomplete removal of the acetate protecting groups. The mixture was evaporated in vacuo and re-dissolved in 500 μL of 1.0 M sodium methoxide in methanol. After two hours, the mixture was filtered through a column of freshly washed DOWEX 50WX8 acidic resin. The loaded resin was first eluted with deionized water and the fractions discarded. The resin was then eluted with a 5% ammonium hydroxide aqueous solution; these fractions were collected and evaporated in vacuo to yield 2.1 mg (~ 7.1 μmol) of a crude product, which was carried forward. This intermediate was dissolved in pyridine (200 μL) and added to 80 uL of a 25 mM DMSO solution of NHS-PEG_{12}-Biotin (2 μmol, purchased from Pierce Thermo Scientific, CAS number: 1262633-93-1, full name: 4,7,10,13,16,19,22,25,28,31,34,37-dodecaoxa-40-azapentatetracontanoic acid, 45-[(3a*S*,4*S*,6a*R*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]-41-oxo-,2,5-dioxo-1-pyrrolidinyl ester). The mixture was stirred at rt for 5 h, the solvent was removed under reduced pressure, and the product was purified by chromatography using a gradient of 0→20 % methanol in ethyl acetate to yield 1.5 mg (1.3 umol, 67%) of compound **S.08** as a tan-colored hygroscopic solid.

^{1}H NMR (500 MHz, Deuterium Oxide) δ 4.69 (s, 1H), 4.49 (t, *J* = 6.8 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.73 (d, *J* = 11.4 Hz, 1H), 3.58 (d, *J* = 4.9 Hz, ~61H), 3.29 (dt, *J* = 11.9, 8.0 Hz, 5H), 2.87 (dd, *J* = 13.3, 4.9 Hz, 1H), 2.66

(d, *J* = 13.1 Hz, 1H), 2.42 (t, *J* = 6.2 Hz, 2H), 2.16 (t, *J* = 7.3 Hz, 2H), 1.68 – 1.40 (m, 4H), 1.37 – 1.23 (m, 2H), 1.17 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d6) δ 172.60, 170.63, 163.18, 100.52, 72.45, 71.18, 70.85, 70.22, 70.17, 70.12, 70.03, 69.95, 69.61, 69.58, 68.79, 67.26, 66.31, 61.50, 60.24, 59.67, 58.44, 55.89, 54.37, 52.98, 52.43, 48.91, 38.96, 38.87, 36.46, 35.55, 28.67, 28.52, 25.73, 21.63, 18.38. MS (ESI) found : 583.2846 [$(M+2Na)^{2+}$], calculated: 583.2854.

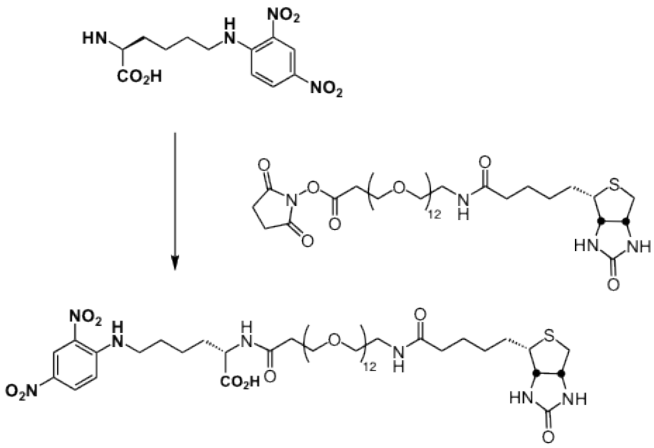
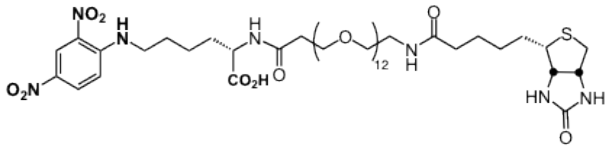


Figure S8 Synthesis of compound DNP biotin



S.09

Nε-(2,4-Dinitrophenyl)-L-lysine hydrochloride (16.8 mg, 48 μmol) was added to a solution of NHS-PEG_{12}-biotin (9.2 μmol, Pierce Thermo Scientific, CAS number: 1262633-93-1, full name: 4,7,10,13,16,19,22,25,28,31,34,37-dodecaoxa-40-azapentatetracontanoic acid, 45-[(3a*S*,4*S*,6a*R*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazol-4-yl]-41-oxo-,2,5-dioxo-1-pyrrolidinyl ester) in pyridine (500 μL) and triethylamine (20 μL). The resulting yellow suspension was stirred at 50 °C under nitrogen for 1 h, cooled to rt, and concentrated in vacuo. The residue was dissolved in methanol (5 mL) and mixed with DOWEX 50-WX8 acidic resin for 5 min. The resin was removed by filtration and the filtrate concentrated in vacuo. The crude product was purified by silica gel chromatography using a gradient of 0 → 30% methanol in dichloromethane, to yield DNP derivative **S.09** as a yellow film (4.1 mg, 3.6 μmol, 39% yield).
^{1}H NMR (400 MHz, Methanol-d4) δ 8.94 (d, *J* = 2.7 Hz, 1H), 8.20 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.14 – 7.02 (m, 1H), 4.38 (td, *J* = 7.7, 4.5 Hz, 1H), 4.33 (dd, *J* = 8.8, 5.0 Hz, 1H), 4.21 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.68 – 3.58 (m, 2H), 3.58 – 3.47 (m, ~42H), 3.43 (dt, *J* = 13.3, 6.3 Hz, 4H), 3.26 (t, *J* = 4.8 Hz, 2H), 3.11 (dt, *J* = 9.7, 4.8 Hz, 1H), 2.83 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.61 (d, *J* = 12.7 Hz, 1H), 2.40 (dtd, *J* = 8.8, 6.0, 3.1 Hz, 2H), 2.12 (t, *J* = 7.4 Hz, 2H), 2.05 – 1.80 (m, 2H), 1.74 – 1.04 (m, ~12H). Structure of **S.09** was verified by ^{1}H-^{13}C HSQC and HMBC analysis. MS (ESI) found : 578.2876 [$(M+2NH_4)^{2+}$], calculated: 578.2888.

Preparation of rhamnosylated BSA:

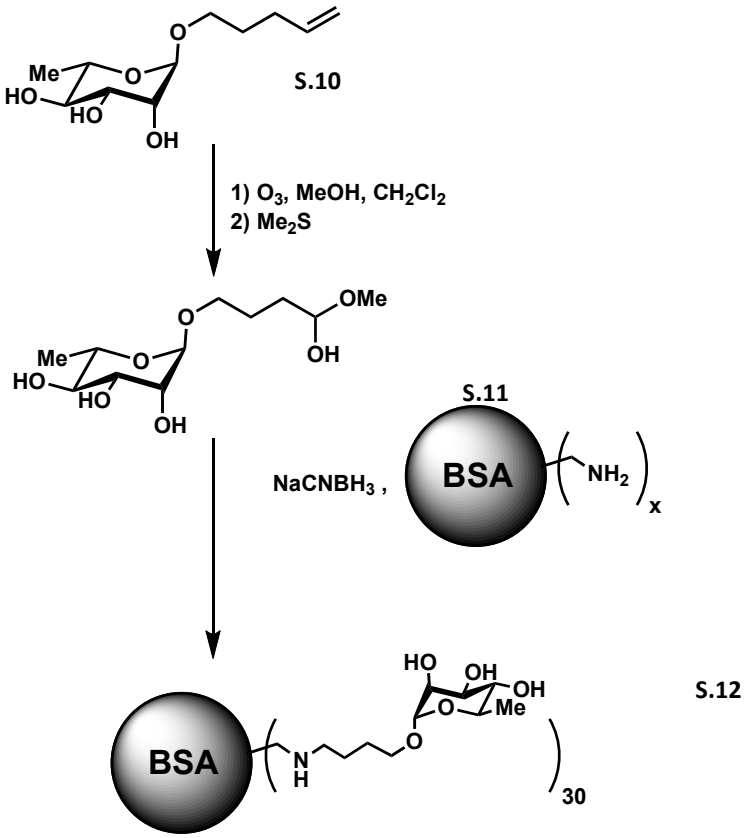
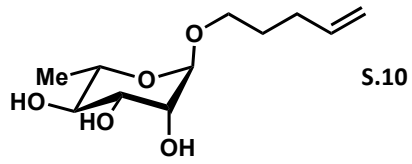
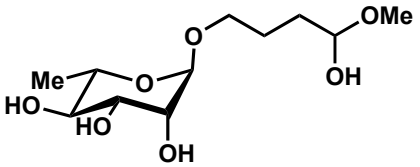


Figure S9 Synthesis of compound S.12



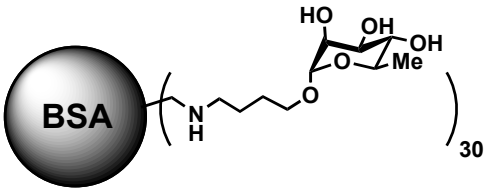
Compound **S.10** was synthesized according to published procedures by Sarkar et al.[4]



S.11

Compound **S.11** was synthesized with a modification on the Sarkar protocol.[4] Briefly, to a stirred solution of compound **S.10** (98.6 mg, 0.42 mmol) in a mixture of dichloromethane (40 mL) and acetonitrile (10 mL) were added 287.0 mg of sodium bicarbonate. The suspension was cooled to -78°C and the solution was sparged with ozone for 3 min, at which point a blue color persisted. The solution was sparged ozone for an additional 2 min, then the solution was sparged with oxygen for 5 min, until the blue color was discharged. To this mixture was added a solution of dimethyl sulfide (5 mL, 68 mmol) in methanol (10 mL), and the mixture was warmed to rt and stirred for 36 h, at which point TLC and ^{13}C NMR confirmed the complete reduction of the intermediate ozonide. The reaction was filtered, the filtrate was concentrated under reduced pressure to yield compound **S.11** as an oil, which was used immediately for the next reaction.

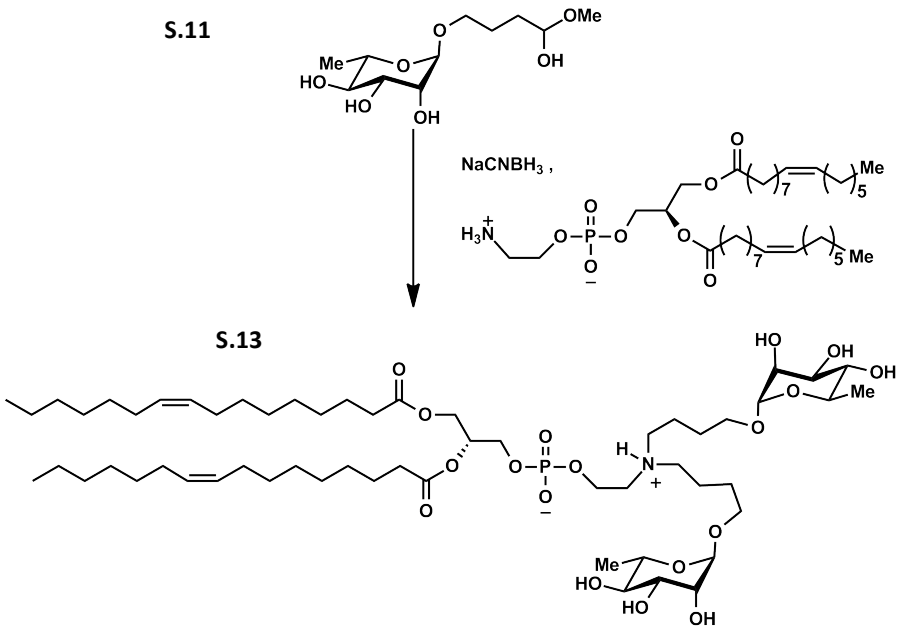
^{1}H NMR (300 MHz, Methanol-d4 + Chloroform-d) δ 4.69 (d, J = 1.8 Hz, 1H), 4.57 (s, 1H), 3.83 (dd, J = 3.5, 1.7 Hz, 1H), 3.67 (dd, J = 9.6, 3.4 Hz, 2H), 3.62 – 3.54 (m, 1H), 3.34 (s, 5H), 1.98 – 1.79 (m, 1H), 1.64 (dd, J = 4.5, 2.5 Hz, 3H), 1.27 (d, J = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, MeOD + $CDCl_3$) δ 100.24, 98.34, 72.61, 71.07, 70.95, 68.39, 66.92, 53.30, 33.30, 24.57, 16.62. MS (ESI) (observed as the free aldehyde) found : 378.1772 ($M+NH_4^+$), calculated: 378.1759



S.12

To 10x phosphate-buffered saline (PBS) adjusted to pH 7.18 (7 mL) was added bovine serum albumin (BSA, 100.0 mg). The protein solution was mixed with freshly prepared compound **S.11** (~0.42 mmol) until the solution was homogeneous. Sodium cyanoborohydride was added (99.9 mg, 1.59 mmol) and the mixture was shaken at 37 ^{o}C for 14 h. An additional portion of sodium cyanoborohydride (98.0 mg, 1.56 mmol) was added, and the mixture was again shaken at 37 ^{o}C for 20 h. The mixture was cooled to room temperature and passed through a 0.2 micron filter. The filtrate was dialyzed at 4 ^{o}C in 2 L of 10 mM NH_4HCO_3 buffer. The solution was changed three times in the course of 72 h. The resulting mixture was lyophilized over 5 days to afford a white amorphous powder (103.8 mg of compound **S.12**), corresponding in mass to a BSA conjugate with ~30 rhamnose moieties (mass shift by MALDI-MS from 66590 daltons for unconjugated BSA, to 73096 daltons for the rhamnose-BSA conjugate).

Synthesis of lipid-rhamnose conjugates



To a stirred solution of compound **S.11** (686 mg, 2.58 mmol) in chloroform (3 mL) and methanol (3 mL) was added 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphoethanolamine (462 mg, 0.67 mmol). The hydride source $NaCNBH_3$ (173 mg, 2.75 mmol) was added, and the reaction mixture was stirred at 45 °C overnight. The reaction mixture was then concentrated under reduced pressure, and the residue was purified by silica gel chromatography using a gradient of 10→50 % methanol in chloroform. The product Rha_2-DPoPE **S.13** was obtained as a colorless foam (456 mg, 0.41 mmol, 61 % yield).
^{1}H NMR (400 MHz, Methanol-d4 + Chloroform-d) δ 5.41 – 5.28 (m, 4H), 5.26 (dd, *J* = 6.6, 3.3 Hz, 1H), 4.72 – 4.68 (m, 3H), 4.44 (dd, *J* = 12.0, 3.2 Hz, 1H), 4.24 – 4.11 (m, 3H), 4.02 (t, *J* = 6.1 Hz, 2H), 3.89 – 3.81 (m, 2H), 3.80 – 3.71 (m, 2H), 3.67 (dd, *J* = 9.5, 3.4 Hz, 2H), 3.57 (dt, *J* = 12.3, 6.2 Hz, 2H), 3.48 (dt, *J* = 10.3, 5.7 Hz, 2H), 3.38 (t, *J* = 9.4 Hz, 4H), 3.20 (t, *J* = 7.5 Hz, 3H), 2.34 (dt, *J* = 9.7, 7.4 Hz, 4H), 2.03 (q, *J* = 5.4, 4.9 Hz, 8H), 1.86 (q, *J* = 6.7 Hz, 3H), 1.71 (dt, *J* = 15.1, 7.2 Hz, 3H), 1.62 (q, *J* = 7.1 Hz, 4H), 1.42 – 1.19 (m, ~40H), 0.90 (t, *J* = 6.6 Hz, 6H). ^{13}C NMR (101 MHz, MeOD + $CDCl_3$) δ 173.77, 173.41, 129.75, 129.49, 100.11, 77.98, 77.66, 77.34, 72.65, 71.06, 70.73, 70.33, 70.25, 68.52, 66.21, 63.82, 63.77, 62.41, 59.49, 55.14, 53.56, 53.49, 48.82, 48.61, 48.40, 48.18, 47.97, 47.76, 47.54, 34.01, 33.85, 31.66, 29.57, 29.11, 29.07, 28.99, 28.96, 28.94, 28.81, 27.01, 26.96, 26.26, 26.17, 24.78, 24.73, 22.48, 21.01, 17.11, 13.57. MS (ESI) found : 1146.7059 ($M+Na^+$), calculated: 1146.7040

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3. Chen, W.; Gu, L.; Zhang, W.; Motari, E.; Cai, L.; Styslinger, T. J.; Wang, P. G., L-rhamnose antigen: a promising alternative to alpha-gal for cancer immunotherapies. *ACS Chem Biol* **2011,** *6*, 185-91.
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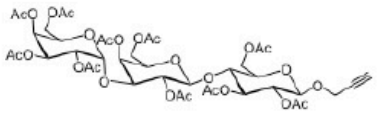

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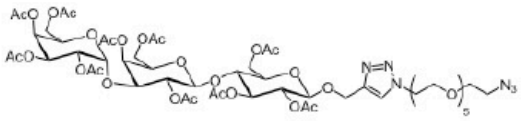

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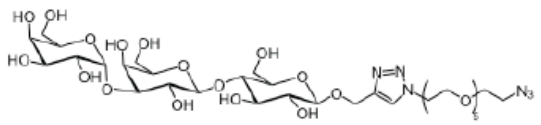

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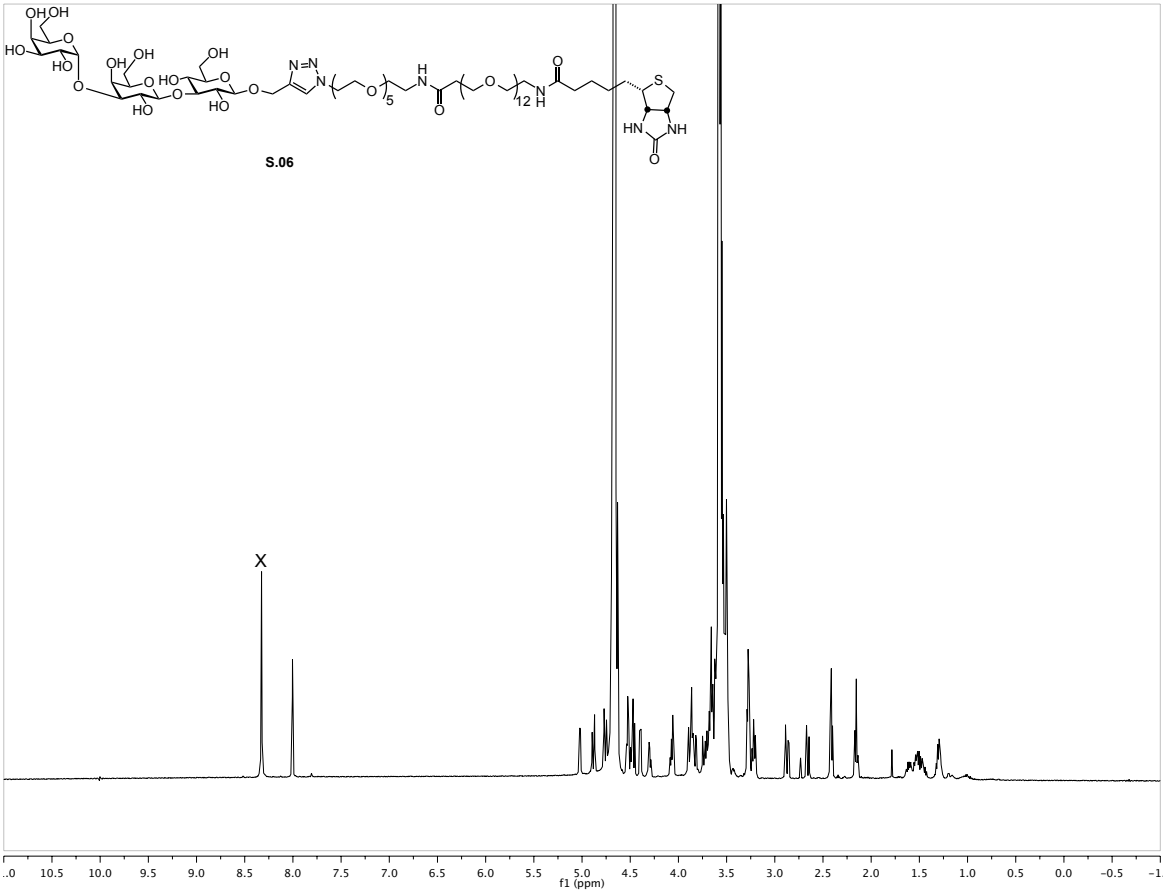

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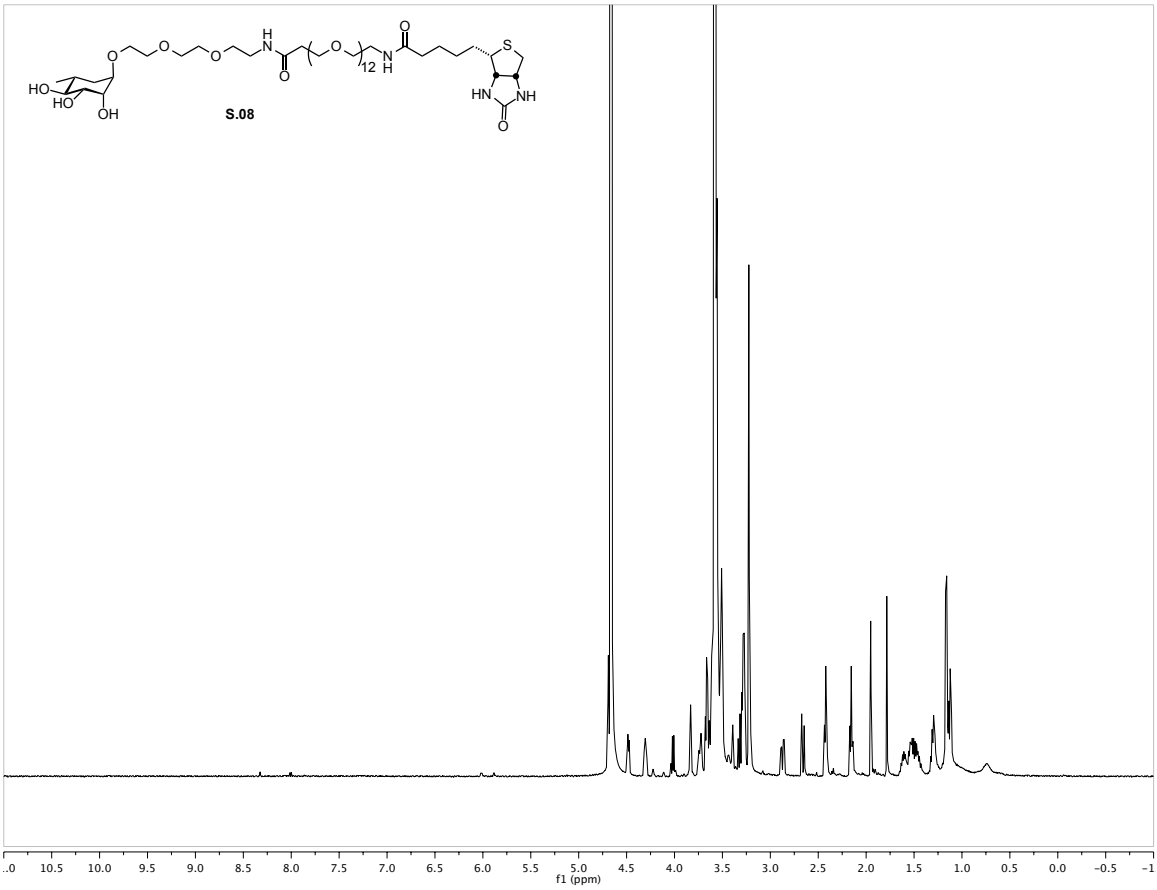
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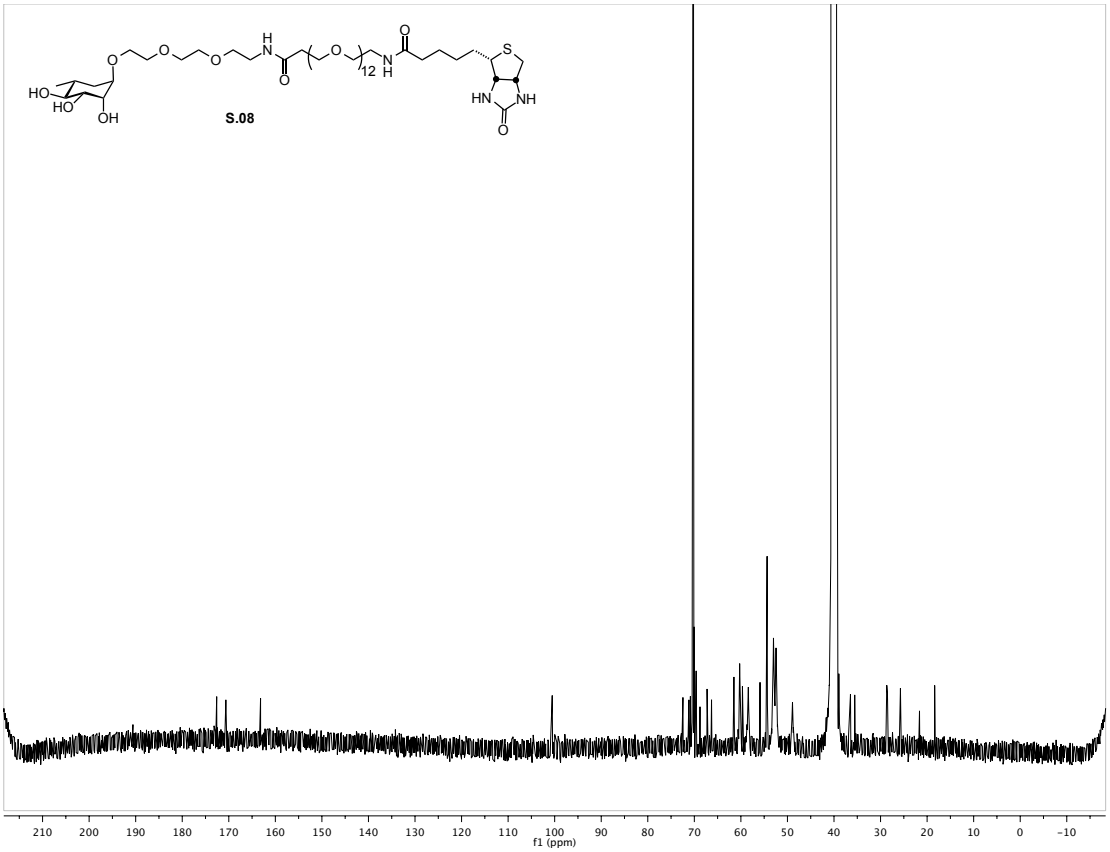
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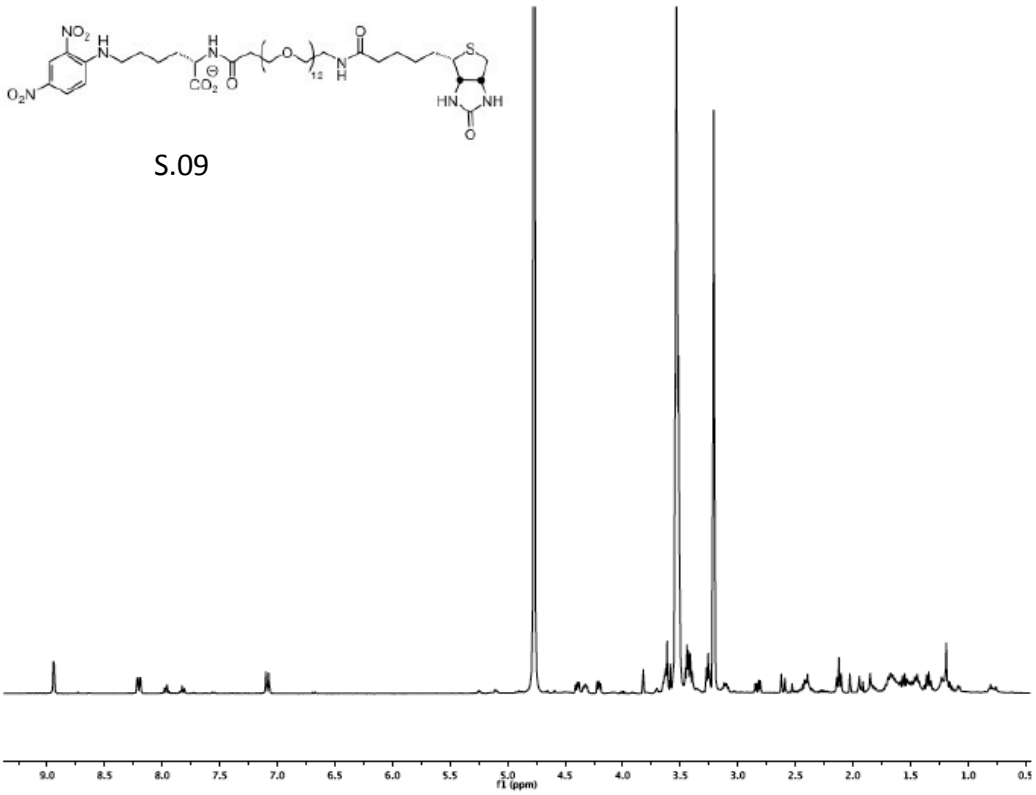

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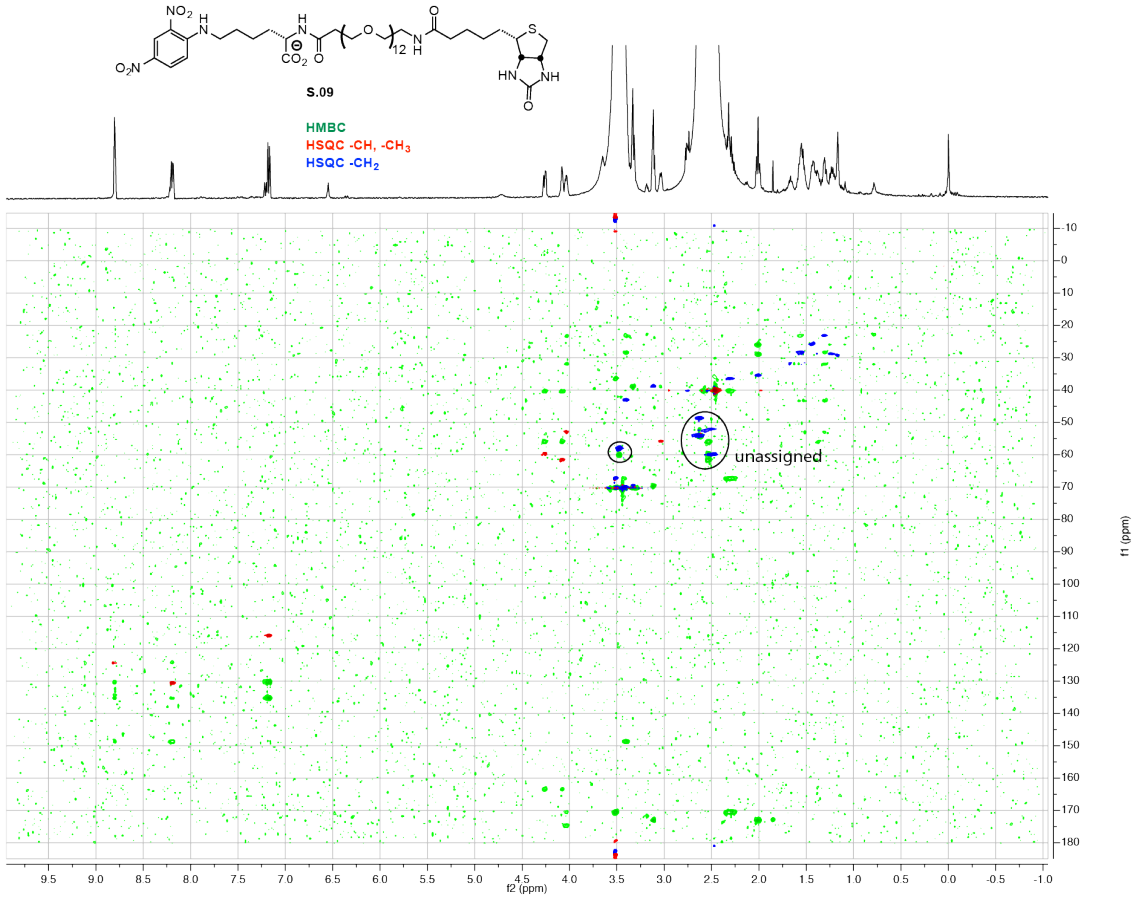

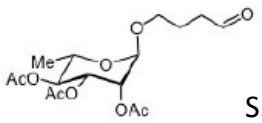
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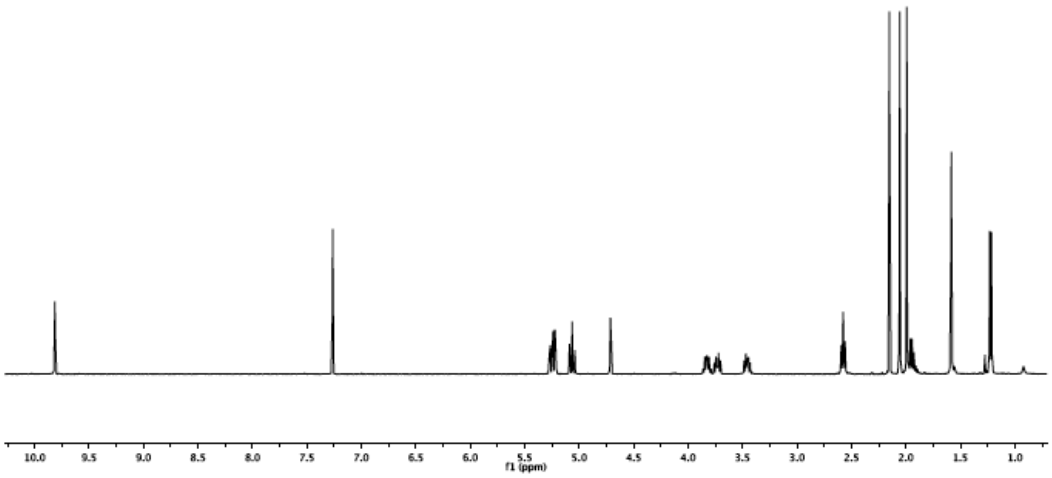
S.10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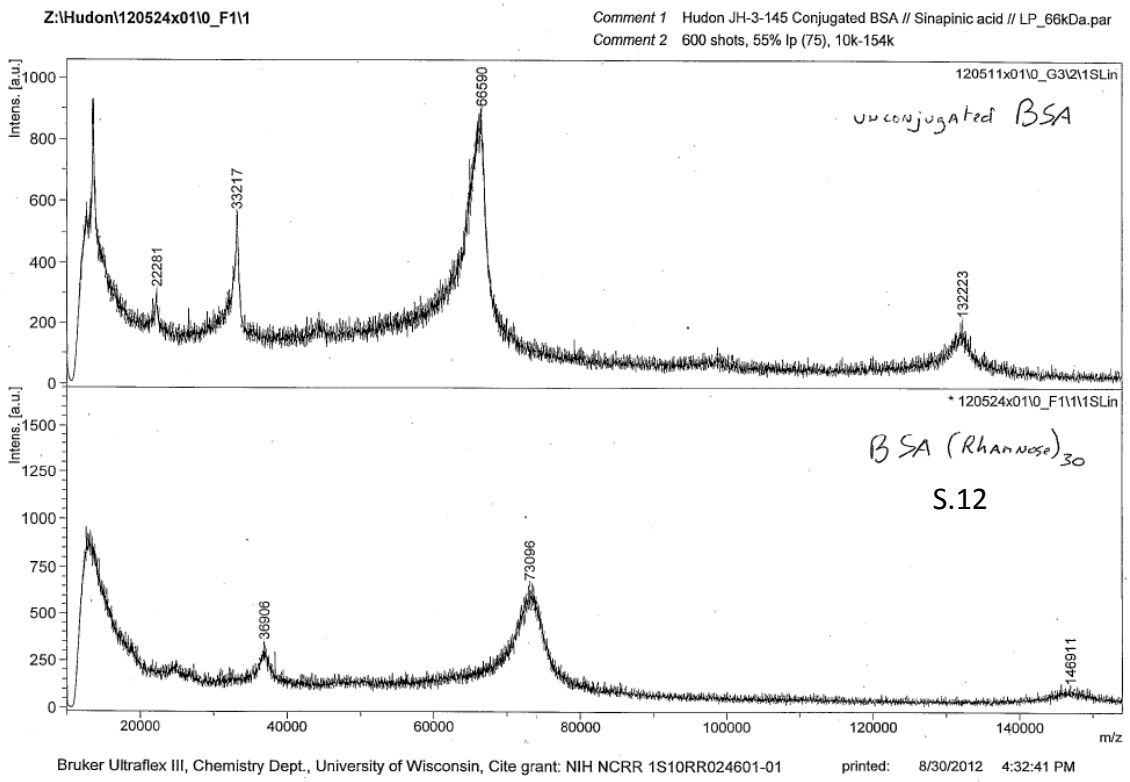
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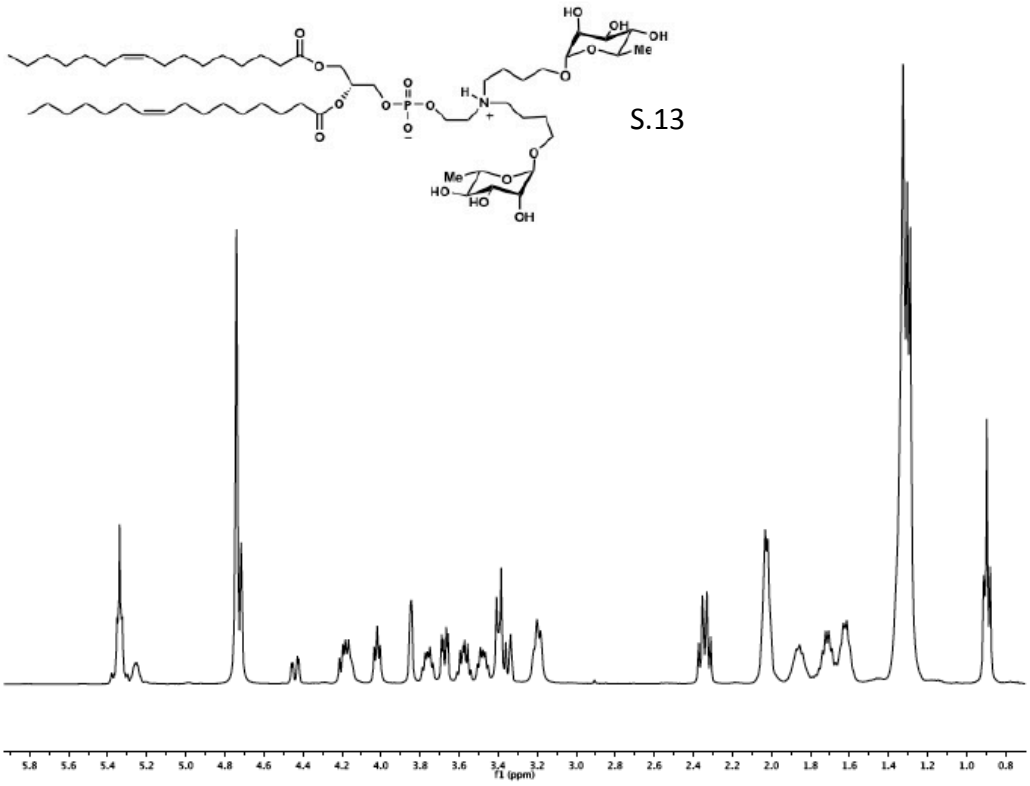
S.12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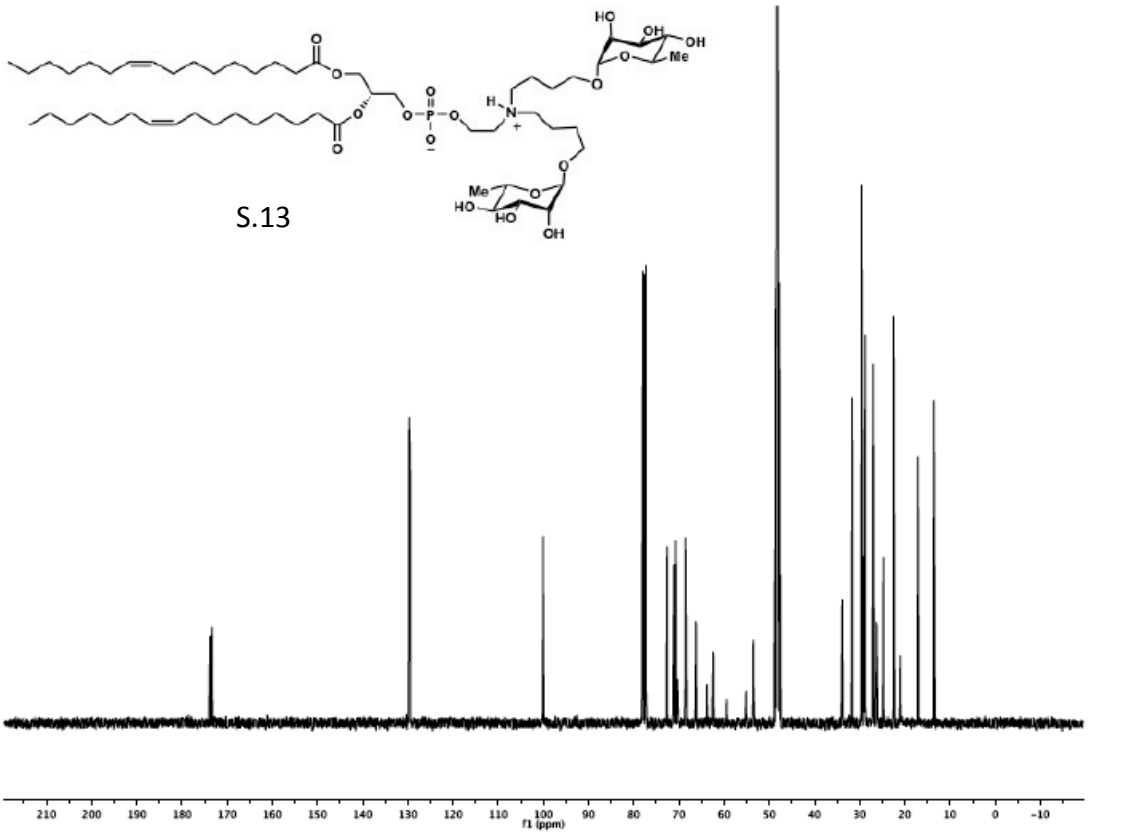
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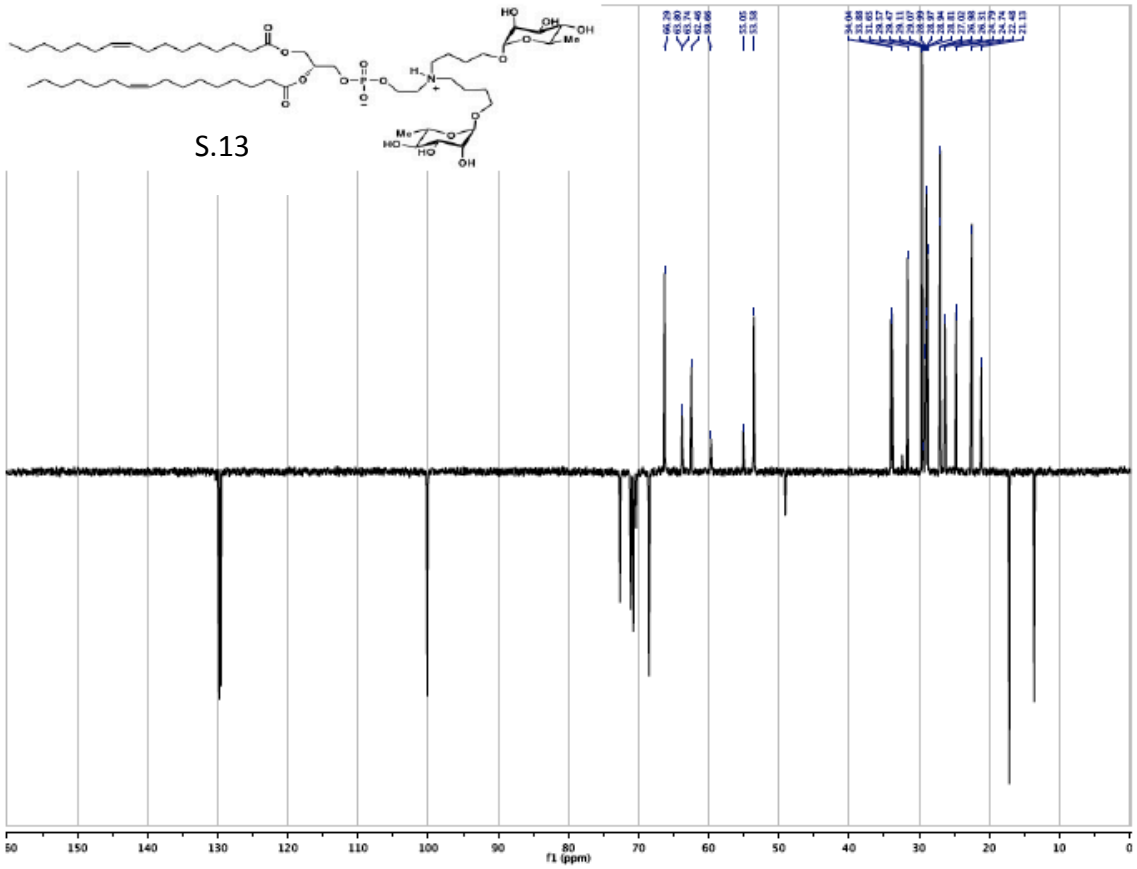
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