Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2010

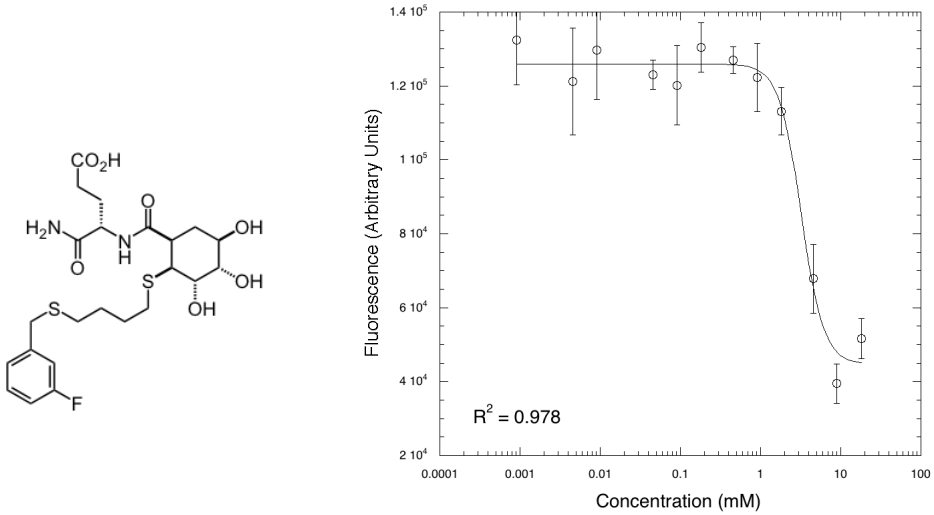
A general glycomimetic strategy yields non-carbohydrate inhibitors of DC-SIGN

Kathleen C. A. Garber,[†] Kittikhun Wangkanont,[†] Erin E. Carlson,[†] and Laura L. Kiessling[†,‡,*]

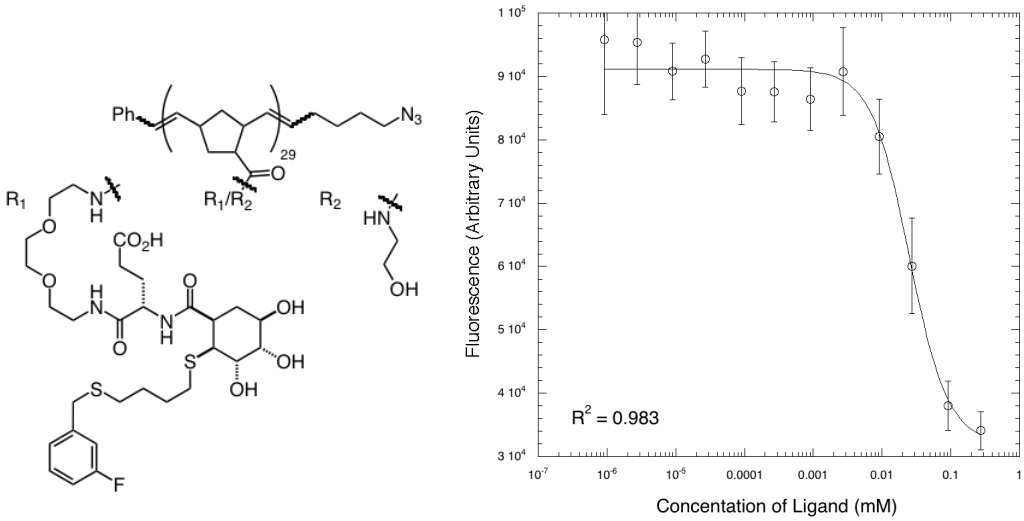
Departments of Chemistry[†] and Biochemistry,[‡] University of Wisconsin—Madison, Madison, Wisconsin 53706

Page	Contents
S2	I. Additional Data & Figures
S3-S9	II. Synthetic Procedures
S10-11	III. Biochemical Procedures
S12-S14	IV. Spectra

I. Additional Data & Figures



SI Figure 1. Binding curve for monomer (**2**).



SI Figure 2. Binding curve for polymer (**3**); concentration shown is that of the glycomimetic residues (not of the polymer).

II. Synthetic Procedures

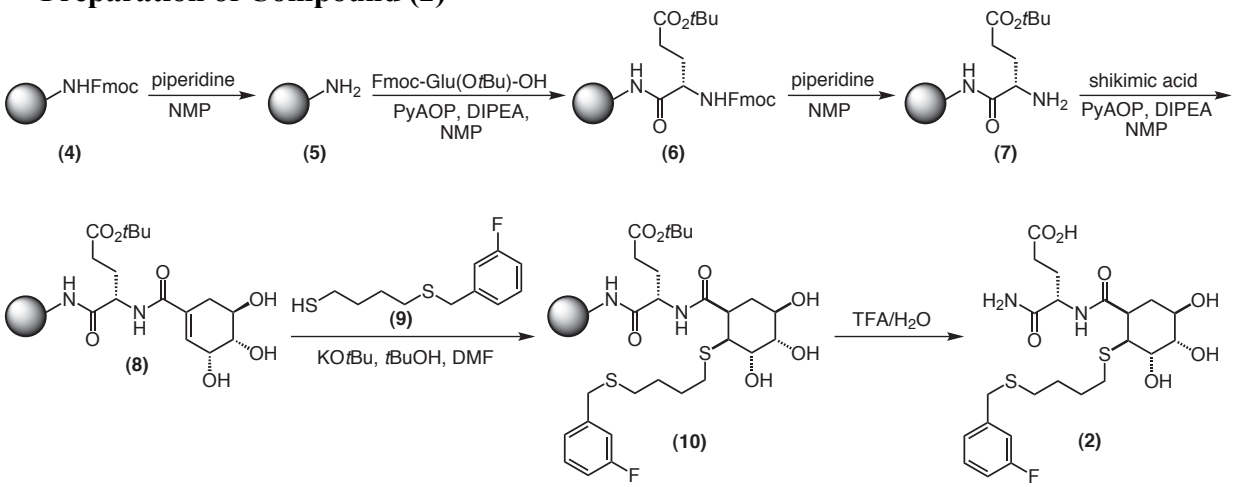
General Procedures

Resin and amino acids were purchased from Novabiochem. Shikimic acid was generously provided by Prof. John Frost (Department of Chemistry, Michigan State University).[1] All other compounds and reagents were purchased from Sigma-Aldrich and used as received.

Flash chromatography was performed using silica gel 60, 230-450 mesh (Sorbent Technologies). Analytical thin-layer chromatography (TLC) was carried out on EM Science TLC plates precoated with silica gel 60 F254 (250-µm layer thickness). Visualization of TLC plates was accomplished using a UV lamp and charring with a *p*-anisaldehyde stain (18 mL *p*-anisaldehyde, 7.5 mL glacial acetic acid (AcOH), 25 mL concentrated H_2SO_4, 675 mL absolute ethanol).

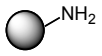
NMR spectra were obtained using a Varian MercuryPlus 300 (^{1}H 300 MHz, ^{13}C 75 MHz), an INOVA 600 (^{1}H 600 MHz, ^{13}C 150 MHz), or UNITY 500 (^{1}H 500 MHz, ^{13}C 125 MHz). Chemical shifts are reported relative to residual solvent signals (CD_2Cl_2): ^{1}H: δ 5.32, ^{13}C: δ 53.5; ($CDCl_3$): ^{1}H: δ 7.27, ^{13}C: δ 77.2; (CD_3OD): ^{1}H: δ 3.31, ^{13}C: δ 49.2; (DMSO-d_6): ^{1}H: δ 2.49, ^{13}C: δ 39.7. ^{1}H NMR data are assumed to be first order with apparent singlets, doublets, and triplets reported as s, d, and t, respectively. Multiplets are reported as m and resonances that appear broad are designated as br. Electron impact mass spectra (EI-MS) were obtained on a Waters (Micromass) AutoSpec, high performance liquid chromatography electrospray ionization mass spectra (LC/MS) were obtained on a Shimadzu LCMS-2010A, and high-resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT.

Preparation of Compound (2)



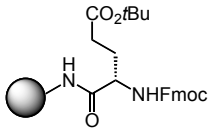
Solid phase reactions were carried out in a ChemGlass 15-mL fritted glass vessel. Resin was washed and swelled in the reaction solvent, dimethylformamide (DMF) or *N*-methylpyrrolidinone (NMP), prior to each reaction. Resin was washed by the addition of solvent to the resin in the fritted vessel followed by vacuum filtration; this process was repeated for a total of three times using each of the following solvents: NMP, DMF, tetrahydrofuran (THF), dichloromethane (DCM), acetonitrile (ACN), and methanol

(MeOH). The volume of solvent used for each wash was roughly 10 mL. The entire washing process was then repeated for a total of three times.



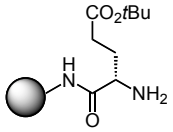
(5)

Rink Amide MBHA (4-methylbenzhydrylamine) resin **4** (1 g, 0.64 mmol/g) was weighed into the fritted glass vessel. NMP (4 mL) and piperidine (4 mL) were added to the resin, and the mixture was sparged for 10 minutes with nitrogen gas. The solution was filtered from the resin, the resin was washed, and the reaction was analyzed for completion using the Kaiser ninhydrin test for free amines.



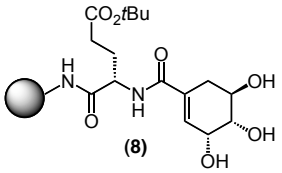
(6)

To PyAOP ((7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, 3 eq.) and the Fmoc-Glu(OtBu)-OH (3 eq.) was added diisopropylethylamine (DIPEA) (6 eq.) and NMP (8 mL), and the solution was stirred for 20 minutes. The solution was then added to the resin (**5**) and the mixture was sparged for 1 hour with nitrogen gas. The solution was filtered from the resin, the resin was washed, and the reaction was analyzed for completion using the Kaiser test.



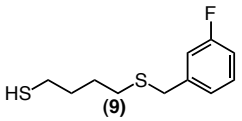
(7)

NMP (4 mL) and piperidine (4 mL) were added to the resin (**6**), and the mixture was sparged for 10 minutes with nitrogen gas. The solution was filtered from the resin, the resin was washed, and the reaction was analyzed for completion using the Kaiser test.



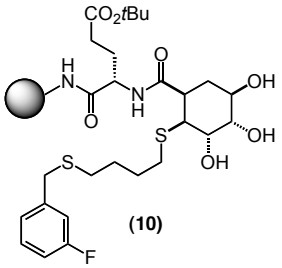
(8)

To PyAOP (3 eq.) and shikimic acid (3 eq.) was added NMP (8 mL) and DIPEA (6 eq.), and the solution was stirred for 20 minutes. The solution was then added to the resin (**7**) and the mixture was sparged for 4 hours with nitrogen gas. The solution was filtered from the resin, the resin was washed, and the reaction was analyzed for completion using the Kaiser test.



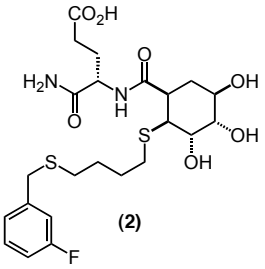
(9)

To a solution of 1,4-butanedithiol (3.00 mL, 25.8 mmol) in ACN (150 mL) was added DIPEA (5.40 mL, 31.0 mmol), and the solution was stirred for 1 hour. A solution of 3-fluorobenzyl bromide (2.85 mL, 23.2 mmol) was prepared in ACN (40 mL), and added dropwise using a cannula over several hours. The reaction was stirred at room temperature overnight. The solvent was removed under reduced pressure, and purification using flash chromatography (20:1 hexanes/ethyl acetate) resulted in a mixture of the desired product **9** and the dibenzylated compound as a yellow oil. As this compound would not affect the next step of the synthesis, the mixture was carried on in the next step. The yield of **9** as determined by ^{1}H NMR was 40 %. ^{1}H NMR (300 MHz, CD_2Cl_2): δ = 7.29 (td, 1H), 7.11 (brd, 1H), 7.06 (dt, 1H), 6.95 (td, 1H), 3.70 (s, 2H), 2.50 (m, 2H), 2.43 (m, 2H), 1.69, (m, 4H), 1.37 (t, 1H). EI-MS calcd for $C_{11}H_{15}FS_2$ $[M]^+$: 230. found 230.



(10)

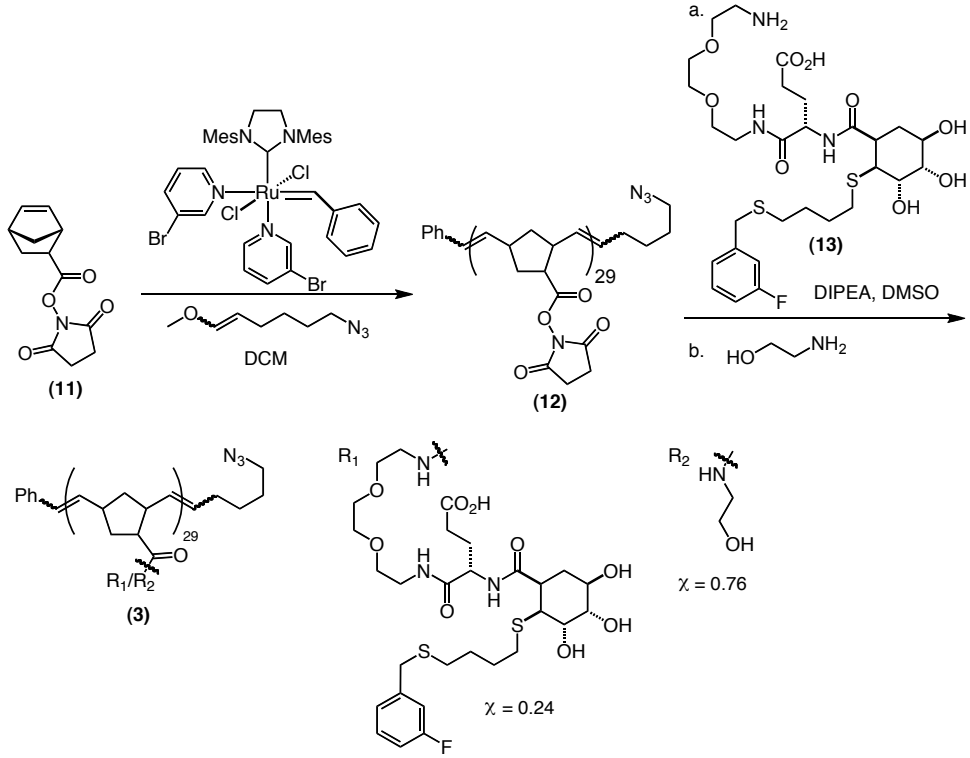
To KO*t*Bu (12 eq.) was added *t*-butanol (2 mL), DMF (7 mL), and **9** (12 eq.). This mixture was added to the resin (**8**), and the mixture was sparged for 48 hours with nitrogen gas. The solution was filtered from the resin and the resin was washed.

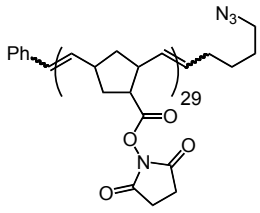


(2)

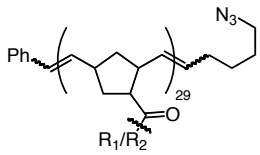
Residual solvent was removed from the resin (**10**) under high vacuum before the product was released from the resin. Cleavage of the product from the resin was carried out by the addition of 6 mL of a trifluoroacetic acid (TFA) cleavage cocktail containing TFA/H_2O (95:5), which was stirred for 1 hour. The resin was filtered, washed with TFA, and the wash solutions were collected and concentrated; the product was purified by column chromatography (90:5:5 DCM/MeOH/AcOH → 85:10:5 DCM/MeOH/AcOH) to yield **2** (44.3 mg, 38.5 % yield) as a clear oil. ^{1}H NMR (600 MHz, CD_3OD) δ 7.30 (td, 1H), 7.12 (br d, 1H), 7.07 (dt, 1H), 6.95 (td, 1H), 4.46 (dd, 1H), 4.07 (t, 1H), 3.69 (m, 4H), 3.35 (s, 2H), 3.26 (dt, 1H), 3.17 (dt, 1H), 2.55 (m, 2H), 2.42 (m, 4H), 2.18 (m, 1H), 1.98 (m, 2H), 1.62 (m, 4H) ^{13}C NMR (150 MHz, CD_3OD) δ 180.89, 179.69, 169.70, 167.76, 147.65, 135.56, 130.31, 121.00, 119.06, 79.55, 78.57, 74.73, 58.45, 56.04, 46.71, 40.81, 38.63, 36.18, 34.37, 33.65, 32.96; ESI-MS calcd for $C_{23}H_{33}FN_2O_7S_2$ $[M + H]^+$: 533.1786 found 533.1782.

Preparation of Compound (3)





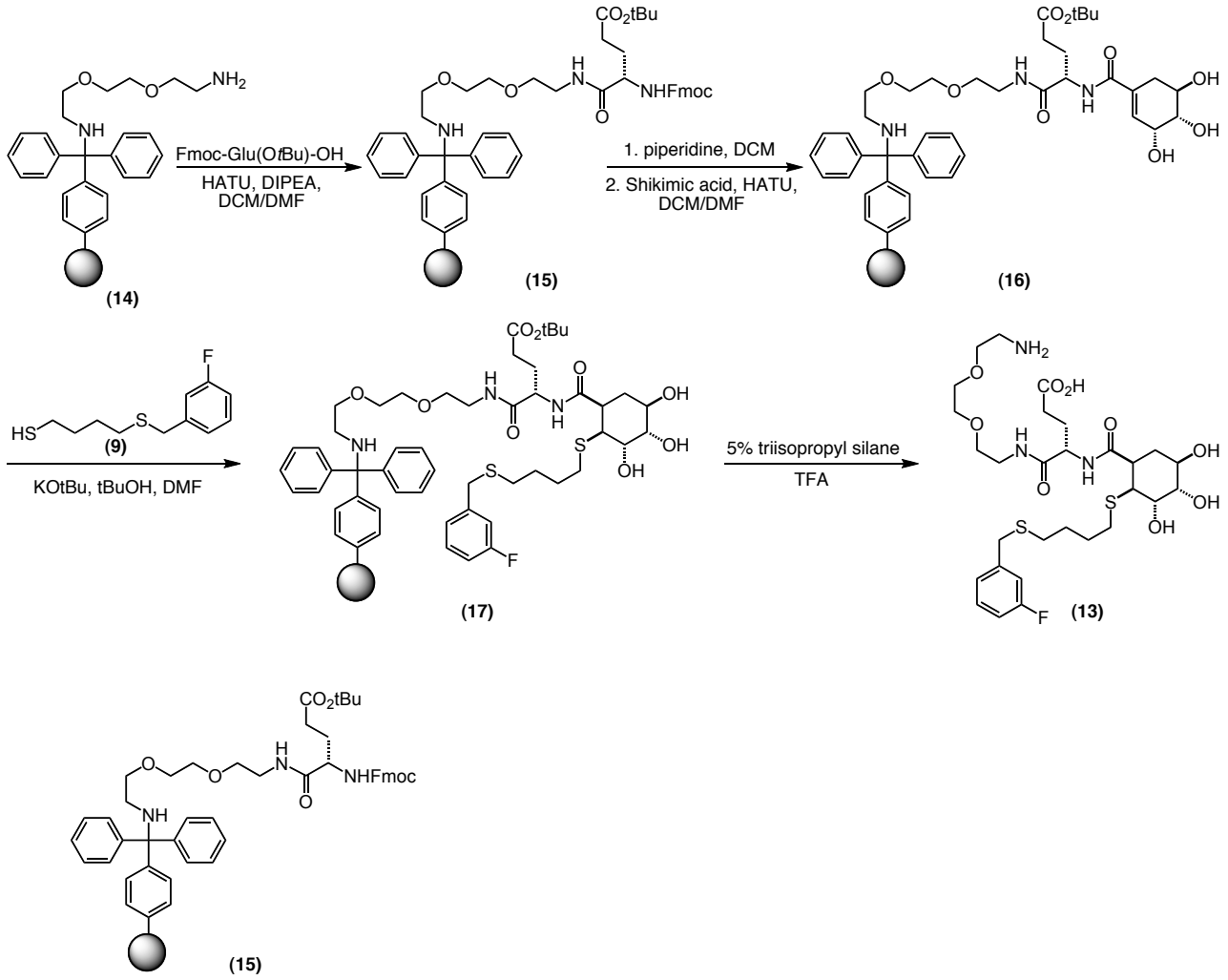
The polymer (**12**) was synthesized following the previously reported general procedure.[2] The product polymer was obtained as brown solid (43.4 mg, 84%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.31 (m, phenyl, 5H), 5.40 (m, alkene, 60.28H), 3.2 (m), 2.95 (br s), 2.79 (br s), 2.61 (br s), 2.06 (br s), 1.95 (br s), 1.81 (m), 1.23 (m). DP = 29, PDI = 1.13, Calculated MW = 7106.74, M_w = 7042, M_n= 6217.



The azide-terminated succinimidyl ester-substituted polymer (**12**, 2.00 mg, 0.281 μmol) was dissolved in anhydrous deoxygenated DMSO (100 μL) under argon. Amine-bearing DC-SIGN ligand **13** (1.81 mg, 2.73 μmol) and DIPEA (2.9 μL, 4.95 μmol) were then added to the polymer solution. The resulting mixture was stirred for 24 hours. To quench any unreacted succinimidyl ester groups, ethanolamine (5 μL) was then added. Mixing was continued for 3 hours. The resulting multivalent ligand was purified with a PD-10

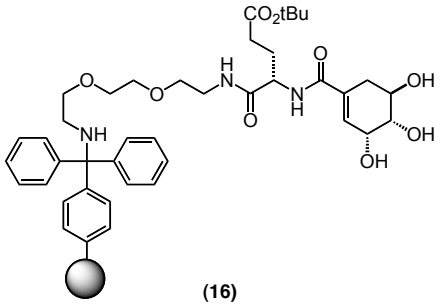
(Sephadex G-25 resin) column using water as the eluent. After lyophilization, **3** was obtained as a white solid (2.73 mg, 98%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.78 (m), 7.34 (br q, fluorophenyl, 7.49H), 7.27 (m, phenyl), 7.15 (br s, fluorophenyl, 14.54H), 7.04 (br t, fluorophenyl, 7.51H), 5.25 (m, alkene, 60.28H), 4.67 (m), 3.84 (s), 3.72 (s), 3.46 (s), 3.12 (br s), 2.90 (br s), 2.45 (s), 2.36 (br s), 2.08 (m), 1.84 (br s), 1.51 (br s), 1.03 (m). Ligand mole fraction = 0.24 Calculated MW = 9883.

Preparation of the Ligand (13)



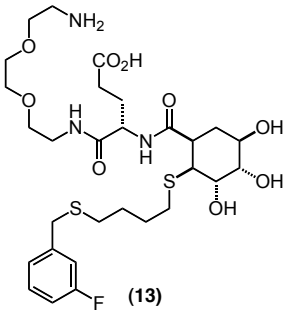
Ortho-bis-(aminoethyl)ethylene glycol trityl resin **14** (Novabiochem, 0.93 mmol/g, 300 mg, 0.279 mmol) was washed and pre-swelled in DCM for 1 hour prior to use. The resin was then suspended in 2 mL DCM. To this mixture, a solution of Fmoc-Glu(O*t*Bu)-OH (356.1 mg, 0.837 mmol), HATU (O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, 318.2 mg, 0.837 mmol), and DIPEA (291 μL, 1.67 mmol) in 2 mL of DCM with a few drops of DMF was added. The mixture was incubated at ambient temperature for 24 hours. The resin was then washed with DCM and DMF. A second batch of the activated amino acid was then added, incubated, and washed as described above. The Kaiser test indicated the reaction was complete. The product was cleaved from the resin in order to verify its identity, and these conditions

also revealed the glutamic acid moiety. Cleavage of the product from the resin was carried out by the addition of 6 mL of a trifluoroacetic acid (TFA) cleavage cocktail containing TFA/H_2O (95:5), which was stirred for 1 hour. The resin was filtered, washed with TFA, and the wash solutions were collected and concentrated. ^{1}H NMR (600 MHz, CD_3OD) δ 7.80 (d, J = 7.6 Hz, 2H), 7.67 (d, J = 7.6 Hz, 2H), 7.39 (t, J= 7.6 Hz, 2H), 7.32 (t, J = 7.6 Hz, 2H), 4.42 (dd, J = 11.1, 7.0 Hz, 1H), 4.34 (dd, J = 11.1, 7.0 Hz, 1H), 4.23 (t, J = 7.0 Hz, 1H), 4.11 (dd, J= 9.2, 5.4 Hz, 1H), 3.65 (t, J = 5.1 Hz, 2H), 3.61 (s, 4H), 3.55 (t, J = 5.4 Hz, 2H), 3.41 (dt, J = 14.1, 5.4 Hz, 1H), 3.36 (dt, J = 14.1, 5.4 Hz, 1H), 3.08 (t, J = 4.9 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.06 (m, 1H), 1.89 (m, 1H); LC/MS calcd for $C_{26}H_{33}N_3O_7$ $[M + H]^+$: 500.2 found 500.1.



(16)

Resin **15**, which bears the immobilized glutamic acid derivative, was swelled in DCM for 5 minutes. Protecting group removal was achieved by treating the resin with a mixture of DCM (2 mL) and piperidine (2 mL) for 10 minutes. After washing, the resin was resuspended in DCM (3 mL). A solution of shikimic acid (145.8 mg, 0.837 mmol), HATU (318.2 mg, 0.837 mmol), and DIPEA (291 μL, 1.67 mmol) in DCM (2 mL) and a few drops of DMF were then added to the resin mixture. The mixture was incubated at ambient temperature for 24 hours. Kaiser ninhydrin confirmed the completion of the reaction. Product was cleaved from resin as above in order to verify identity; glutamic acid was also deprotected in this reaction. ^{1}H NMR (600 MHz, CD_3OD) δ 6.49 (s, 1H), 4.4 (dd, J = 8.9, 5.3 Hz, 1H), 4.38 (s, 1H), 4.01 (dd, J = 11.9, 5.8 Hz, 1H), 3.65 (m, 4H), 3.56 (t, J = 5.4 Hz, 2H), 3.42 (dt, J = 14.2, 5.4 Hz, 1H), 3.38 (dt, J = 14.2, 5.4 Hz, 1H), 2.77 (dd, J = 18.1, 4.6 Hz, 1H), 2.41 (t, J = 7.3, 2H), 2.18 (dd, J = 18.1, 5.4 Hz, 1H), 2.13 (m, 1H), 2.00 (m, 1H).



(13)

Resin **16** was swelled in DMF for 5 minutes before the solvent was removed. To the resin, a solution of **9** (771.3 mg, 3.35 mmol) and KO*t*Bu (409.2 mg, 3.35 mmol) in HO*t*Bu (1.5 mL) and DMF (5 mL) was added. The reaction was left to proceed at ambient temperature for 48 hours. The product was cleaved from the resin as above. The solvent was evaporated under a stream of nitrogen gas and then under vacuum. The

product was purified by extraction from DCM into distilled water. After lyophilization, **13** was obtained as an oil (175.2 mg, 95%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (td, J= 8.4, 6.2 Hz, 1H), 7.13 (br d, J = 7.5 Hz, 1H), 7.07 (br d, J = 9.9 Hz, 1H), 6.94 (td, J = 8.4, 2.1 Hz, 1H), 4.28 (q, J= 6.8 Hz, 1H), 4.06 (br s, 1H), 3.70 (m, 6H), 3.65 (m, 4H), 3.56 (m, 2H), 3.38 (m, 2H), 3.24 (q, J = 3.8 Hz, 2H), 3.17 (dt, J = 12.9, 3.8 Hz, 1H), 3.12 (t, J = 5.4 Hz, 2H), 2.56 (m, 2H), 2.42 (m, 2H), 2.29 (m, 2H), 2.14 (m, 1H), 1.98 (m, 2H), 1.68 (m, 1H), 1.62 (m, 4H) ^{13}C NMR (125 MHz, CD_3OD) δ 178.02, 175.91, 174.32, 165.23, 163.34, 143.21, 130.99, 125.87, 116.45, 114.50, 74.14, 71.40, 70.60, 68.01, 55.55, 51.81, 42.29, 40.72, 40.25, 36.31, 34.39, 34.09, 33.74, 33.59, 31.72, 30.68, 30.54, 29.94, 29.18; HRMS calcd for $C_{29}H_{46}FN_3O_9S_2$ $[M + H]^+$: 664.2733 found 664.2739.

III. Biochemical Procedures

Production of Soluble DC-SIGN/ECD

A plasmid encoding the DC-SIGN extracellular domain (ECD) was provided by Dr. Kurt Drickamer (University of Oxford).[3] The plasmid was transformed into *Escherichia coli* strain BL21/DE3, and the resulting cells were stored in 10% glycerol at -80 °C. This glycerol stock was used to inoculate a flask containing 50 mL Luria-Bertani (LB) media supplemented with ampicillin (100 μg/mL), and the culture was grown overnight at 37 °C. This culture was then used to inoculate 4 liters of LB (100 μg/ml ampicillin), and cells were allowed to grow at 37 °C until an OD_{600} of 0.7 was reached. Gene expression was then induced with 100 mg/L isopropyl-β-D-thiogalactoside, and the culture was incubated with shaking for 3 additional hours at 37 °C. Bacteria were harvested by centrifugation at 8,000*g* for 10 min. Cell pellets were washed with 10 mM Tris-HCl, pH 7.8 and again centrifuged at 8,000*g* for 10 min at 4 °C. Cells were resuspended in 10 mM Tris-HCl, pH 7.8 and sonicated for approximately 2 min. Inclusion bodies were isolated by centrifugation at 10,000*g* for 10 min at 4 °C. This pellet was solubilized in 4 M guanidine-HCl containing 10 mM Tris-HCl (40 mL), pH 7.8, and 0.01% β-mercaptoethanol. This mixture was sonicated briefly and then centrifuged at 137,000*g* for 30 min at 4 °C. The supernatant was diluted with 1 volume of high salt loading buffer (HSLB; 25 mM Tris-HCl, 1M NaCl, 5 mM $CaCl_2$, pH 7.8) and dialyzed overnight against HSLB to initiate protein refolding. After dialysis, the insoluble precipitate was removed by centrifugation at 142,000*g* for 1 hour at 4 °C. This soluble fraction was then further purified over a mannose-substituted Sepharose column. Protein was loaded onto the column and washed with 4-5 column volumes of HSLB. The ECD of DC-SIGN was eluted with 1 M NaCl, 25 mM Tris-HCl, 5 mM ethylenediaminetetraacetic acid (EDTA) at pH 7.8. Purified protein was then dialyzed into low salt loading buffer (LSLB; 25 mM Tris-HCl, 125 mM NaCl, 5 mM $CaCl_2$, pH 7.8) and concentrated with a Centriplus centrifugal filter device (Amicon).

Production of Mannose Binding Protein A (MBP-A)

The pME-366 plasmid encoding MBP-A was transformed into *Escherichia coli* strain JA221, and the resulting cells were stored in 10% glycerol at -80 °C. This glycerol stock was used to inoculate a flask containing 50 mL LB supplemented with ampicillin (100 μg/mL), and the culture was grown overnight at 37 °C. This culture was then used to inoculate 2 L of LB media (100 μg/ml ampicillin), and cells were allowed to grow at 25 °C until an OD_{600} of 0.8 was reached. Gene expression was induced by the addition of isopropyl-β-D-thiogalactoside (final concentration 40 μM) and $CaCl_2$ (final concentration 100 mM), and the culture was incubated with shaking overnight at 25 °C. Bacteria were harvested by centrifugation at 8,000*g* for 10 min at 4 °C. Cells were resuspended in LSLB and frozen at -80 °C. The cell suspension was thawed and sonicated 6 x 2 min and then subjected to centrifugation at 10,000*g* for 10 min. The supernatant was passed through glass wool and then purified over a mannose-substituted Sepharose column. To isolate the protein, the sample was loaded onto the column, which was washed with 4-5 column volumes of HSLB. MBP-A was eluted with 0.5 M α-methyl-D-glucopyranose in LSLB. Purified protein was then dialyzed into LSLB.

Library Screen Assay

Mannan from *Saccharomyces cerevisiae* (Sigma) was immobilized in black Polysorb 384-well plates (Nunc) by overnight incubation at 4 °C. Plates were washed with LSLB (45 μL) and then blocked with 2% BSA in LSLB (45 μL/well) for 2 hours at room temperature. Plates were washed once more with LSLB (45 μL/well) and then incubated with a mixture of Alexa fluor® 594 (Molecular Probes)-labeled DC-SIGN/ECD (1 mg/mL) in 2% BSA solution and compound. After 1 hour, plates were washed 2 times with LSLB (45 μL/well) and the fluorescence emission was determined using a Wallac Envision plate reader (Perkin Elmer) at an excitation wavelength of 600 nm and an emission wavelength of 620 nm. This assay was performed in duplicate.

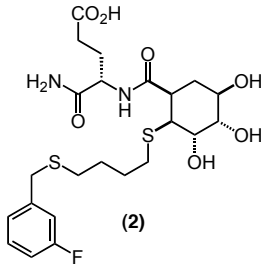
IC_{50} Determination Assay

The protein (DC-SIGN/ECD or MBP-A) was immobilized in black Polysorb 384-well plates (Nunc) by overnight incubation at 4 °C (25 μl/well, 200μg/ml). Plates were washed with LSLB (45 uL) and then blocked with 2% BSA in LSLB (45 μl/well) for 2 hours at room temperature. Plates were washed once more with LSLB (45 μl/well) and then incubated with a mixture of 20 μl Man-Fl-BSA (bovine serum albumin modified with α-D-mannopyranosyl phenyl isothiocyanate and fluorescein isothiocyanate, Sigma; 20 μg/ml) probe in 2% BSA-LSLB and compound (2 μL of DMSO solution) at room temperature. After 1 hour, plates were washed 2 times with LSLB (45 ul/well) and the fluorescence emission was determined using a Wallac Envision plate reader (Perkin Elmer) at an excitation wavelength of 480 nm and an emission wavelength of 520 nm. IC_{50} determination was performed with 5-6 replicates per compound tested.

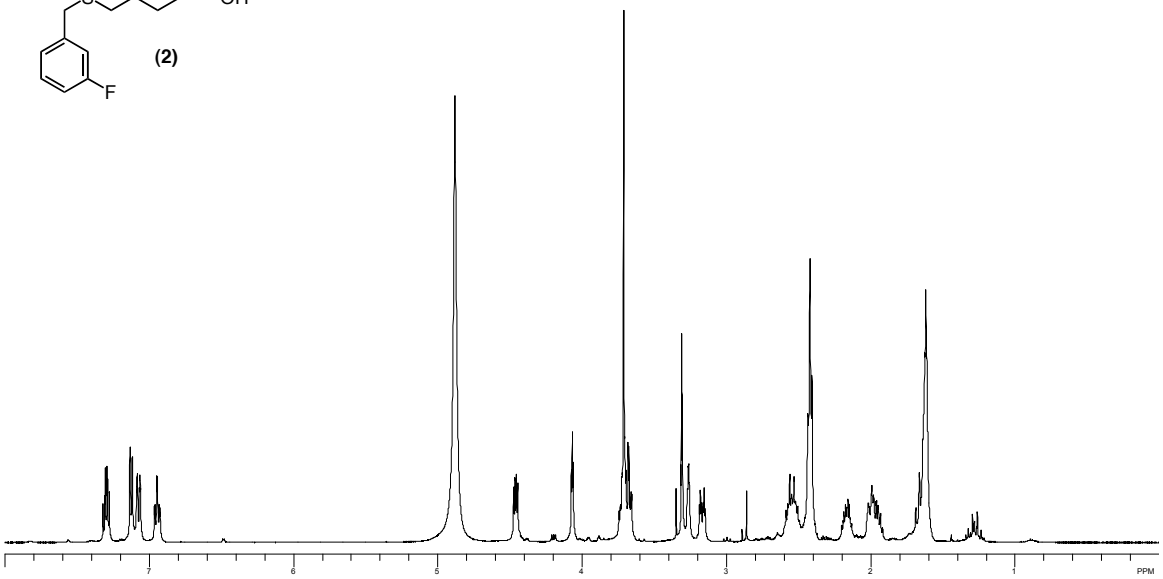
References

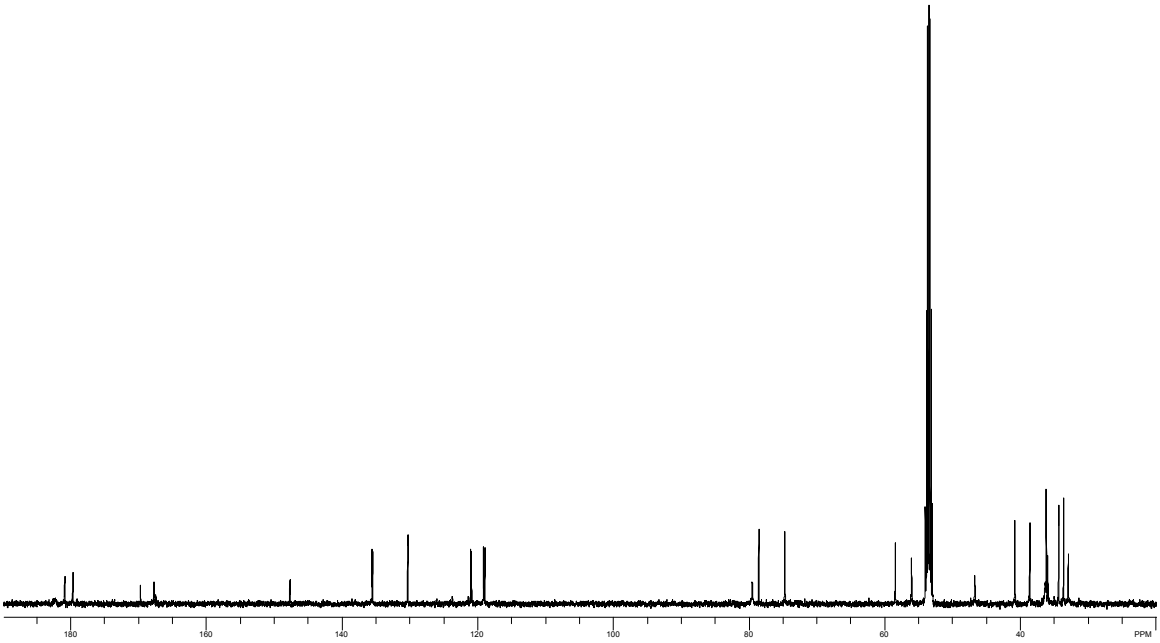
(1) Knop, D. R.; Draths, K. M.; Chandran, S. S.; Barker, J. L.; von Daeniken, R.; Weber, W.; Frost, J. W. *J. Am. Chem. Soc.* **2001**, *123*, 10173-10182.
(2) Mangold, S. L.; Carpenter, R. T.; Kiessling, L. L. *Org. Lett.* **2008**, *10*, 2997-3000.
(3) Mitchell, D. A.; Fadden, A. J.; Drickamer, K. *J. Biol. Chem.* **2001**, *276*, 28939-28945.

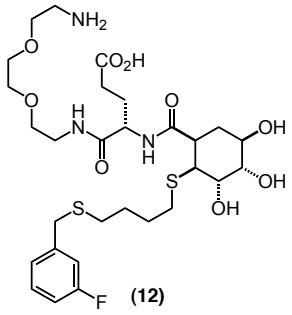
IV. Spectra


CO2H
H2N
O
N
H
O
OH
OH
OH
S
S
F

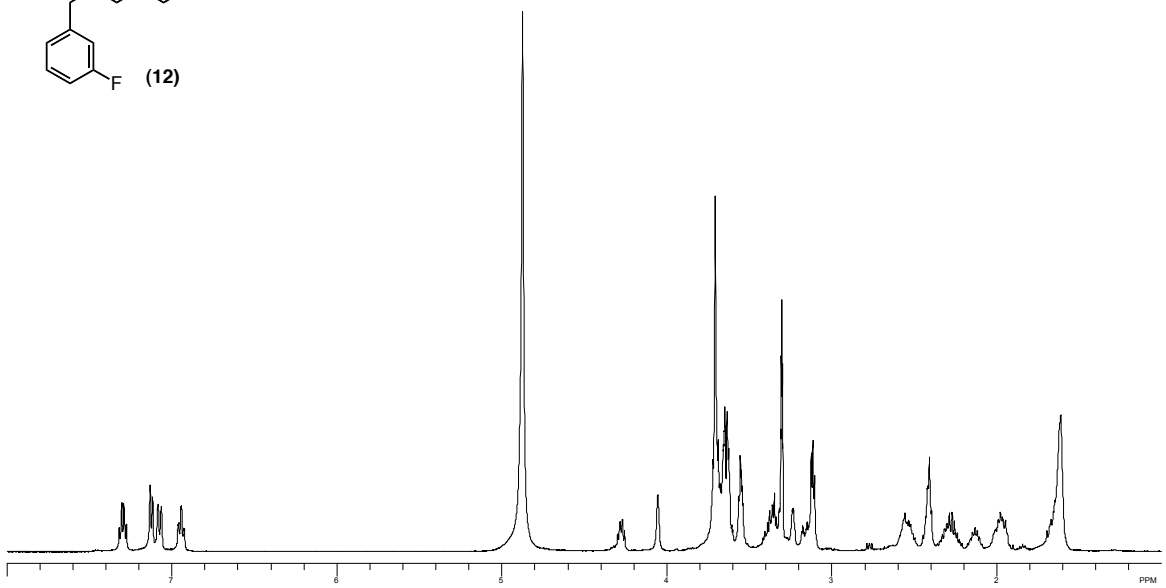
(2)

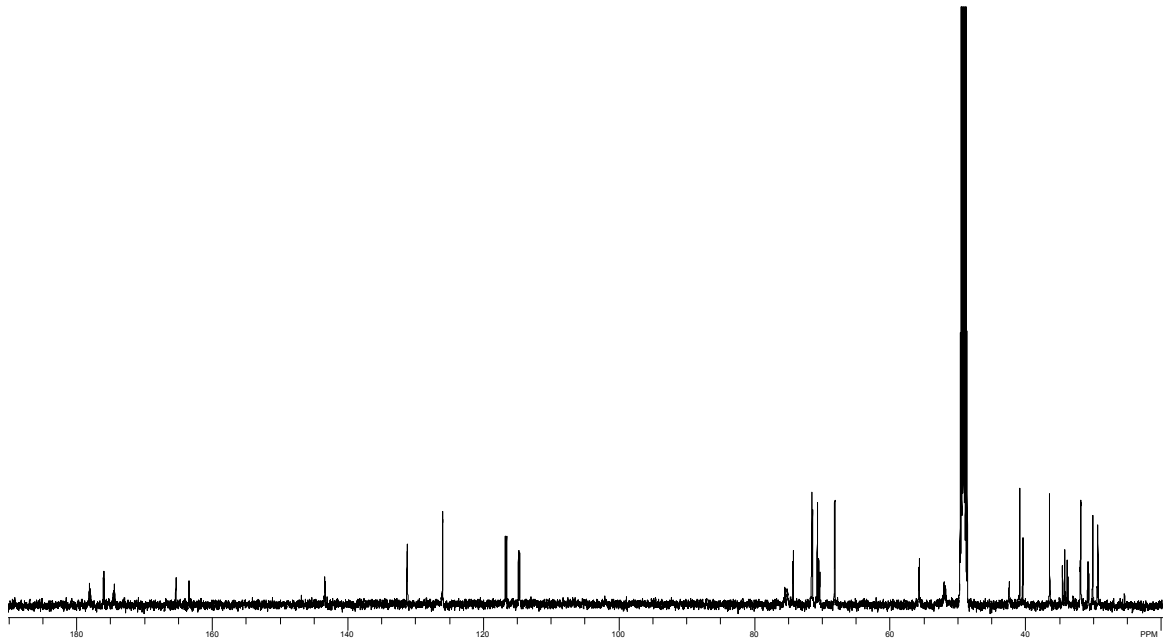

7
6
5
4
3
2
1
PPM

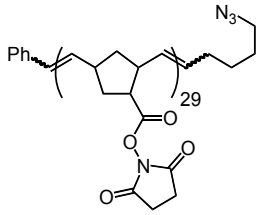

180
160
140
120
100
80
60
40
PPM


NH2
O
CO2H
O
H
N
O
N
H
O
OH
OH
S
OH
S
F

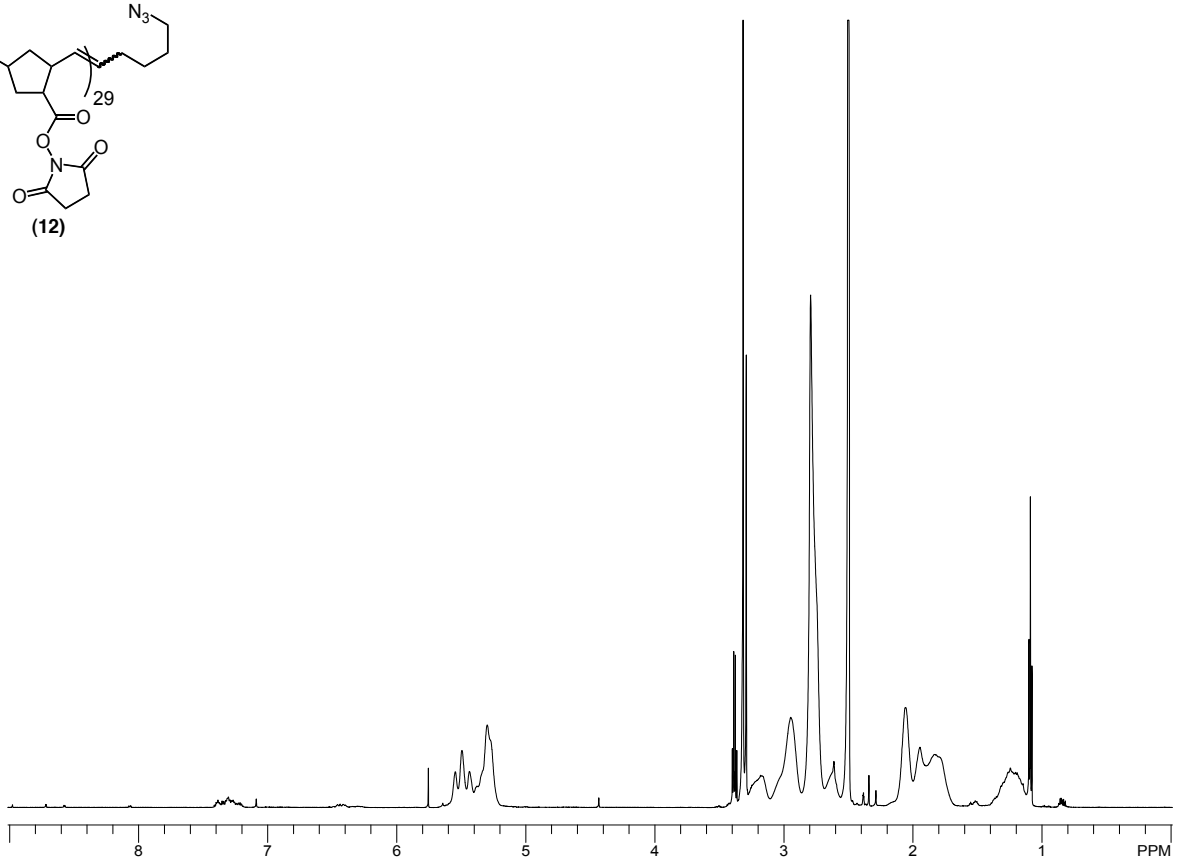
(12)

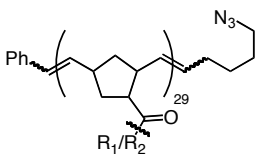

7
6
5
4
3
2
PPM


180
160
140
120
100
80
60
40
PPM


Ph
N3
29
O
O
N
O
O

(12)

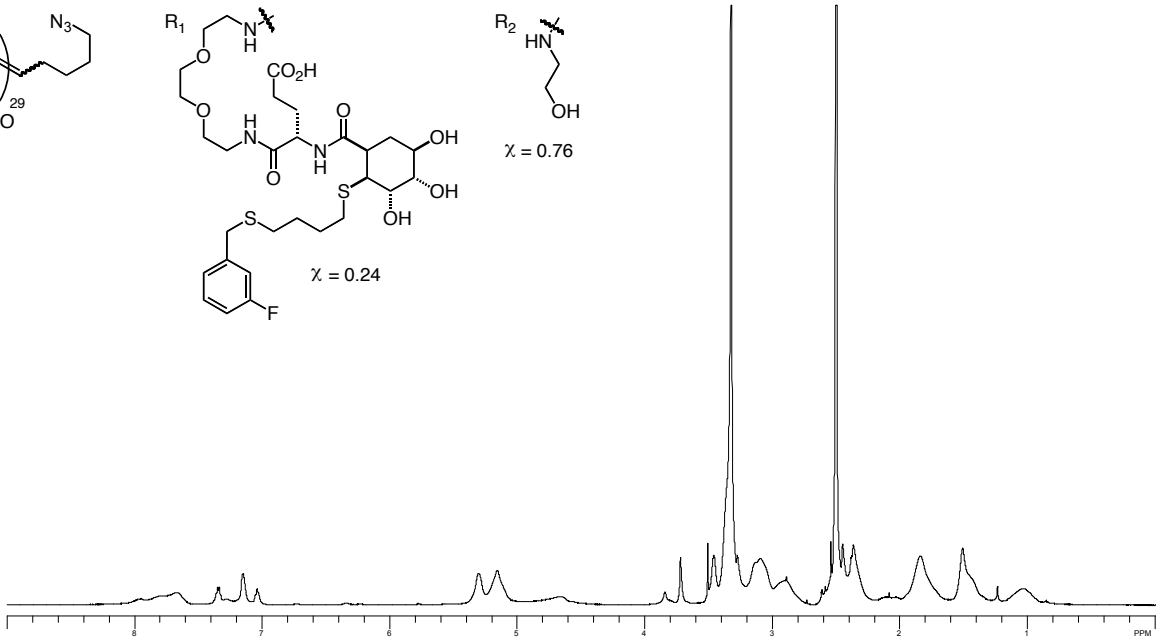

8
7
6
5
4
3
2
1
PPM


Ph
N3
29
O
R1/R2

(3)

R1
N
H
O
CO2H
O
H
N
O
N
H
O
OH
OH
OH
S
S
F
χ = 0.24

R2
HN
OH
χ = 0.76


8
7
6
5
4
3
2
1
PPM