Supporting Information:

Non-Carbohydrate Glycomimetics and Glycoprotein Surrogates as DC-SIGN Antagonists and Agonists

*Lynne R. Prost[†], Joseph C. Grim[‡], Marco Tonelli[†] and Laura L. Kiessling[†,‡,]**
Departments of [†]Biochemistry and [‡]Chemistry, University of Wisconsin - Madison, Madison, Wisconsin 53706.

*Corresponding author, kiessling@chem.wisc.edu

Content

S1. Supplemental Methods

Protein NMR Spectroscopy

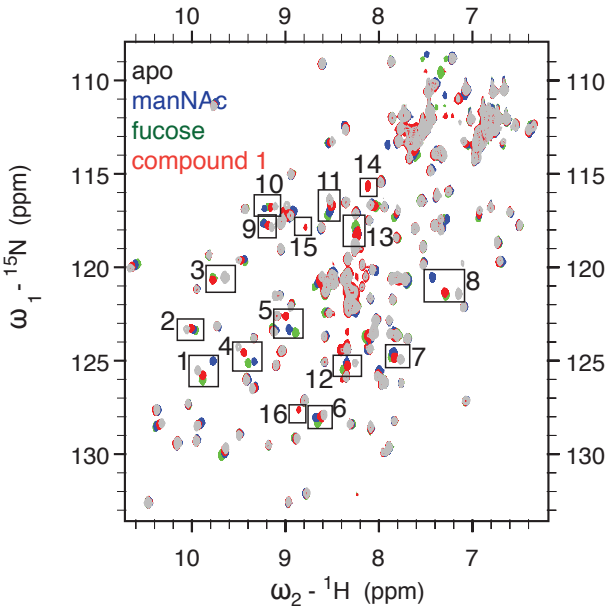
Bacteria were grown at 37 °C in MOPS-minimal medium supplemented with 1 g/L ^{15}N-ammonium chloride to mid-log phase, when IPTG was added to 100 mg/L. Cultures were grown an additional 4 hr, harvested by centrifugation, and lysed by sonication. Inclusion bodies were isolated by centrifugation at 7000 × g and solubilized by resuspension in 50 mL 10 mM Tris pH 7.8, 4 M guanidine HCl, 0.01% BME. Samples were then ultracentrifuged at 40,000 rpm for 40 min. The supernatant was diluted two-fold into cold high salt loading buffer (HSLB, 25 mM Tris pH 7.8, 5 mM $CaCl_2$, 1 M NaCl) and dialyzed against 2.5 L HSLB with three buffer exchanges. After dialysis, precipitate was removed with another ultracentrifugation at 40,000 rpm for 1 hour. Soluble protein was concentrated to about 30 mL and then purified by running 10 mL at a time over a 10 mL mannose-substituted Sepharose column pre-equilibrated with HSLB. The column was washed in 2.5 column volumes HSLB and protein was eluted using 25 mM Tris pH 7.8, 1 M NaCl, 5 mM EDTA. Fractions containing protein were pooled, concentrated, diluted 10-fold in cold low salt loading buffer (LSLB, 25 mM Tris pH 7.8, 5 mM $CaCl_2$, 125 mM NaCl) and concentrated again to 0.2 mM. For data collection, 10% DMSO was added to each sample along with 20 mM ligand, where indicated. All NMR spectra were collected on a Varian

VNMRS spectrometer operating at 600MHz and equipped with a cryogenic probe. The spectra were then processed with NMRPipe (Delaglio, NIH) and analyzed with Sparky (Goddard, UCSF).

Confocal microscopy. Cells were washed and resuspended in PBS containing $CaCl_2$ (Gibco) and 1% BSA at 1 million cells per mL. AF488-bearing probes were added to 200,000 cells to final concentrations of 25 g/mL (mannose-BSA), 5 g/mL (fucose-BSA) or 17.2 mg/mL (compound **3**). Because glycan conjugation is not well described for the purchased conjugates, concentrations are somewhat arbitrary and were chosen experimentally based on optimized cell uptake. Probes were incubated with cells for 40 minutes at 37 °C. Cells were then washed and transferred to eight-chambered cover glass slides (Nunc). Cells were visualized on a Nikon Eclipse Ti-E confocal microscope with a 60x oil-immersion objective and the NIS Elements software package.

Western blotting. Raji and Raji/DC-SIGN cells were washed with PBS containing $CaCl_2$ (Gibco) and resuspended at 2.5 million cells per mL. Man-BSA was added to a final concentration of 50 μg/mL and glycomimetic **3** to 34.4 μg/mL. Samples were collected at indicated time points and lysed in 1% Triton X-100 and treated with PhosSTOP phosphatase and cOmplete Mini protease inhibitors (Roche). DNA was sheared using a 27 gauge needle. Samples were subjected to SDS-PAGE and transferred to a PVDF membrane. Membranes were blocked in TBS-T with 5% milk, incubated with a primary antibody to phospho-JNK (T183/Y185, Cell Signaling), then with a horseradish-peroxidase-conjugated goat anti-rabbit secondary antibody (Jackson Immunoresearch). After detection, blots were stripped at pH 2.2 and re-probed using a JNK primary antibody (Cell Signaling).

S2. HSQC Peak Shifts



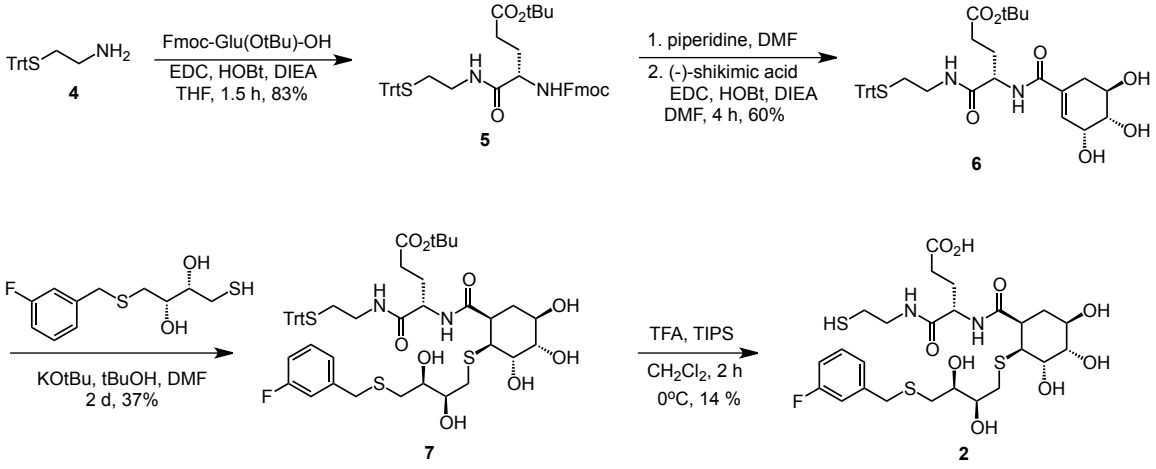
	Apo		Mannose		Fucose		JCG3026	
Peak #	15N	1H	15N	1H	15N	1H	15N	1H
1	125.518	9.938	125.018	9.75	126.047	9.887	125.779	9.882
2	123.329	9.652	123.326	9.98	123.383	9.958	123.274	10.008
3	120.542	9.652	120.527	9.66	120.569	9.773	120.782	9.758
4	124.267	9.503	125.262	9.326	125.125	9.401	124.545	9.448
5	121.53	9.108	123.331	8.96	123.508	8.892	122.819	8.992
6	127.896	8.59	128.046	8.67	128.399	8.64	128.195	8.601
7	124.938	7.77	124.577	7.848	124.795	7.868	124.84	7.836
8	121.424	7.149	120.551	7.429	121.464	7.288	121.363	7.299
9	117.931	9.131	117.631	9.234	117.621	9.234	117.931	9.172
10	116.75	9.113	116.869	9.222	116.771	9.184	116.786	9.157
11	116.367	8.529	117.026	8.53	117.223	8.548	116.66	8.514
12	125.123	8.254	125.149	8.349	125.503	8.376	125.813	8.351
13	118.849	8.259	118.193	8.233	117.842	8.252	118.313	8.241
14							115.664	8.118
15							117.869	8.786
16							123.435	8.339

Figure S1. HSQC peak shifts. HSCQ spectra from Figure 2. Key peaks are highlighted with boxes and numbered. Table shows chemical shift values for each key peak.

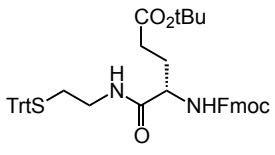
S3. Synthetic Procedures

General Procedures and Materials. All moisture- and oxygen-sensitive reactions were carried out in flame-dried glassware under a nitrogen atmosphere. Unless otherwise noted, all reagents and solvents were the highest commercially available grades and used without further purification. All chemicals were purchased from Sigma Aldrich with the exception of 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (Chem Impex) and Alexa Fluor® 488 5-SPD ester (Life Technologies). Protein bioconjugation was performed with the Imject® Maleimide Activation BSA Spin Kit (Thermo Scientific). Dichloromethane (CH_2Cl_2) and diisopropylethylamine (DIEA) were distilled from calcium hydride, tetrahydrofuran (THF) was distilled from sodium/benzophenone, and water (H_2O) was purified with a MilliQ purification system (Millipore). Analytical thin layer chromatography (TLC) was used to monitor reactions and was performed on 0.25 mm pre-coated Silica Gel 60 F254 (Merck). Compounds were visualized with ultraviolet light (254 nm) and/or charring with *p*-anisaldehyde (15 g *p*-anisaldehyde, 5 mL H_2SO_4, 1 mL AcOH, 250 mL ethanol). Flash chromatography was performed on 230 – 400 mesh SiliaFlash® P60 silica gel (Silicycle).

^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-300 or Varian MercuryPlus 300 spectrometers, and chemical shifts were reported relative to trimethylsilane or residual solvent peaks in parts per million ($CHCl_3$: ^1H δ 7.26, ^{13}C δ 77.0; CH_3OH: ^1H δ 3.31, ^{13}C δ 49.0). Peak multiplicity is reported as singlet (s), doublet (d), triplet (t), multiplet (m), doublet of doublet (dd), etc. High resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT (electrospray ionization, time-of-flight analyzer).



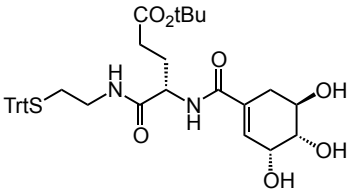
Fmoc-Glu(OtBu)-*N*-(2-*S*-tritylmercaptoethylamide) (5)



To a flask containing 2-*S*-tritylmercaptoethylamine **4**[1] (0.94 g, 2.96 mmol), Fmoc-Glu(OtBu)-OH (1.88 g, 4.44 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.41 g, 7.40 mmol) and hydroxybenzotriazole (HOBt) (1.12 g, 8.88 mmol) was added THF (59 mL) and diisopropylethylamine (DIEA) (3.07 mL, 17.76 mmol). The solution was stirred for 1.5 h at which point analysis by thin-layer chromatography indicated the starting material was

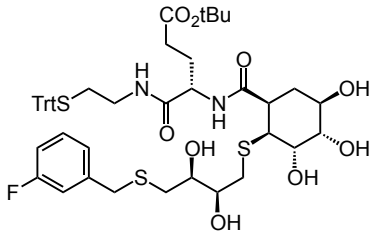
consumed. Aqueous 25% ammonium chloride (w/w) was added to quench the reaction, and diethyl ether (Et_2O) (120 mL) was added. The organic layer was washed with water and brine, and the separated organic layer was dried over magnesium sulfate. The solution was filtered and concentrated under reduced pressure. The residue was purified by column chromatography (1:9 to 3:7 EtOAc:hexanes) to afford compound **5** as a white solid (1.79 g, 2.47 mmol, 83%).
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4, 2H), 7.55 (t, J = 5.3, 2H), 7.42–7.34 (m, 8H), 7.29-7.14 (m, 11H), 6.59 (bs, 1H), 5.81 (d, J = 7.5, 1H), 4.35 (bd, J = 7, 2H), 4.15 (t, J = 7, 1H), 4.10 (q, J = 6.4, 2H), 3.06 (q, J = 6.2, 2H), 2.47-2.20 (m, 4H), 2.03 (s, 3H), 1.89 (p, J = 7.0, 1H), 1.44 (s, 9H), 1.25 (t, J = 7.0, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7, 171.0, 156.2, 144.6, 143.8, 143.7, 141.3, 129.5, 127.9, 127.7, 127.1, 126.7, 125.1, 120.0, 80.9, 67.0, 66.8, 60.4, 54.2, 47.1, 38.3, 31.7, 31.6, 28.0, 21.0, 14.2.
HRMS (EMM): calc'd for $C_{45}H_{46}N_2O_5S$ $[M+Na]^+$: 749.3020, found 749.3199.

Shi-Glu(OtBu)-*N*-(2-*S*-tritylmercaptoethylamide) (6)



To a solution of compound **5** (0.97 g, 1.33 mmol) in anhydrous dimethylformamide (DMF) (5 mL) was added piperidine (1.25 mL). The solution was stirred for 20 minutes at which point analysis by thin-layer chromatography indicated the starting material was consumed. The solution was concentrated under reduced pressure to afford a white solid.
To a flask containing the crude product was added (-)-shikimic acid (0.35 g, 1.99 mmol), EDC (0.64 g, 3.33 mmol) and HOBt (0.54 g, 3.49 mmol). The mixture was dissolved in anhydrous DMF (27 mL) and DIEA (1.4 mL, 7.98 mmol) was added. After 4 h, analysis by thin-layer chromatography indicated the starting material was consumed. The solution was concentrated under reduced pressure to afford a yellow oil. The crude product was purified by column chromatography (100% CH_2Cl_2 to 1:9 MeOH:CH_2Cl_2) to afford **6** as a white solid (0.53 g, 0.80 mmol, 60%).
^{1}H NMR (300 MHz, CD_3OD) δ 7.41 (m, 6H), 7.38-7.19 (m, 9H), 6.50 (t, J = 1.7, 1H), 4.39 (m, 2H), 4.05 (q, J = 6.3, 1H), 3.63 (dd, J = 7.7, 4.1, 1H), 3.39 (m, 1H), 3.10 (qt, J = 13.6, 6.8, 2H), 2.91 (dd, J = 17.8, 4.9, 1H), 2.42-2.32 (m, 4H), 2.21 (dd, J = 17.8, 6.1, 1H), 2.10 (dt, J = 14.2, 7.9, 1H), 1.95 (dt, J = 15.4, 8.3, 1H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, CD_3OD) δ 172.8, 172.4, 169.0, 145.0, 132.6, 132.3, 129.5, 127.8, 126.7, 80.7, 71.9, 67.1, 86.5, 66.2, 53.1, 38.2, 31.5, 30.9, 27.2.
HRMS (EMM): calc'd for $C_{37}H_{44}N_2O_7S$ $[M+Na]^+$: 683.2762, found 683.2751.

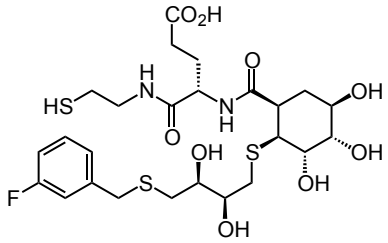
3FB-DTT-shikimate-Glu(OtBu)-*N*-(2-*S*-tritylmercaptoethylamide) (7)



To a flask containing compound **6** (0.33 g, 0.66 mmol) and *m*-fluoro-α-dithiothreitol toluene[2] (0.516 g, 1.97 mmol) was added DMF (6.6 mL). A 1M solution of potassium tert-butoxide (KOtBu) in tetrahydrofuran (THF) (0.66 mL, 0.66 mmol) was added to the mixture, and the resulting solution was stirred for 2 days. The reaction mixture was concentrated under reduced pressure to afford a clear residue. The residue was purified by column chromatography (100% CH_2Cl_2 to 1:9 MeOH:CH_2Cl_2) to afford **7** a colorless syrup (0.22 g, 0.24 mmol, 37%).

^{1}H NMR (300 MHz, CD_3OD) δ 7.39 (m, 6H), 7.30-7.06 (m, 12H), 6.40 (dt, *J* = 8.9, 2.1, 1H), 4.35 (dd, *J* = 8.1, 5.4, 1H), 4.12 (ddd, *J* = 9.0, 3.3, 2.2, 1H), 3.85-3.70 (m, 5H), 3.35 (m, 2H), 3.24-2.90 (m, 1H), 2.82-2.50 (m, 4H), 2.42-2.28 (m, 4H), 2.15-1.80 (m, 3H), 1.70 (m, 1H), 1.45 (s, 9H). ^{13}C NMR (75 Hz, CD_3OD) δ 174.0, 172.9, 172.3, 145.0, 142.0, 130.0, 129.6, 127.6, 127.7, 124.8, 115.7, 115.4, 113.6, 113.4, 80.7, 73.0, 72.1, 71.6, 71.3, 69.2, 66.6, 53.0, 38.2, 36.5, 36.4, 35.7, 34.4, 34.3, 31.6, 31.7, 30.3, 27.3.
HRMS (EMM): calc'd for $C_{48}H_{59}FN_2O_9S_3$ $[M+Na]^+$: 945.3259, found 945.3296.

3FB-DTT-shikimate-Glu-*N*-(2-mercaptoethylamide) (2)



To a flask containing **7** (0.22 g, 0.24 mmol) was added CH_2Cl_2 (0.5 mL) and triisopropylsilane (0.2 mL). The solution was cooled to 0 °C. Trifluoroacetic acid (TFA) (1.5 mL) was added dropwise to the solution. After 2 h, analysis by thin-layer chromatography indicated the starting material was consumed. The reaction mixture was then concentrated under reduced pressure. The pale yellow oil was purified by column chromatography (1:12:87 AcOH:MeOH:CH_2Cl_2) to afford **2** as a colorless residue (0.020 g, 0.032 mmol, 14%).
^{1}H NMR (300 MHz, CD_3OD) δ 7.30 (m, 1H), 7.17 (d, *J* = 7.7, 1H), 7.12 (dd, *J* = 10.2, 2.5, 1H), 6.96 (td, *J* = 9.2, 2.7, 1H), 4.39 (dd, *J* = 8.6, 4.8, 1H), 4.11 (m, 1H), 3.88-3.60 (m, 5H), 3.47-3.30 (m, 2H), 3.19 (dt, *J* = 7.4, 4.4, 1H), 2.80-2.50 (m, 6H), 2.45 (t, *J* = 7.3, 2H), 2.21-1.92 (m, 3H), 1.62 (m, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.6, 174.2, 172.7 129.9, 124.8, 115.7, 115.4, 113.6, 113.3, 72.9, 72.8, 72.5, 72.0, 71.5, 71.2, 69.2, 53.4, 42.7, 36.4, 35.7, 34.4, 30.2, 27.0, 23.3.
HRMS (EMM): calc'd for $C_{25}H_{37}FN_2O_9S_3$ $[M+Na]^+$: 647.1538, found 647.1529.

S4. Bioconjugation procedures

Bovine serum albumin (BSA) conjugate **3** was prepared using the Pierce Imject® Maleimide Activation BSA Spin Kit (cat # 776677). The protocol provided with the kit was followed for the bioconjugation. A 10-fold excess of glycomimetic **2** (with respect to maleimide) was used for conjugation.

The product was analyzed by gel electrophoresis on Novex® 4-20% Tris-glycine gels (Life Technologies) by comparing the bands of BSA prior conjugation and after conjugation (**Figure S2**). These results indicate the bioconjugation occurred efficiently.

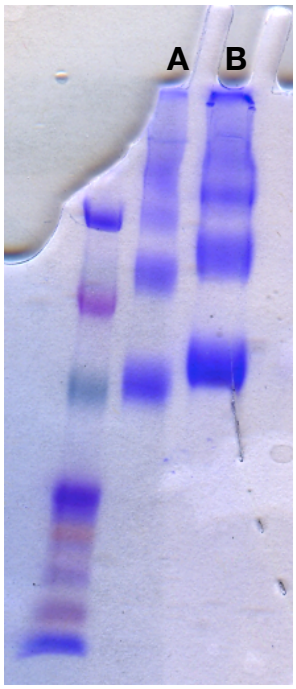


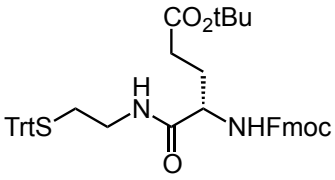
Figure S2. Gel electrophoresis on Novex® 4-20% Tris-glycine gel and and Precision Protein Plus™ Kaleidescope ladder (BioRad) stained with coomassie blue. (A) Maleimide-BSA prior to conjugation and (B) Maleimide-BSA post-conjugation.

The resulting solution of the BSA conjugate was diluted in a 0.1M aqueous $NaHCO_3$ pH 8.3 buffer, and the mixture was concentrated to 1 mL using Amicon Ultra 30K MWCO (Millipore) at 3200 rpm. To the resulting BSA conjugate solution was added an aqueous solution of Alexa Fluor® 488 5-SPD ester (10 mg/mL, 25 μL), and the solution was spun gently on rotisserie for 5 h with protection from light. BSA conjugate **3** was then exposed to three rounds of dialysis into phosphate buffer saline (PBS) pH 7.4 to remove unconjugated fluorophore. The resulting BSA conjugate **3** solution was visibly fluorescent.

S5. Suporting information references.

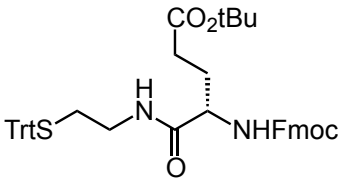
1. Mandel, A. L., LaClair, J. J., and Burkhart, M. D. (2004) The modular synthesis of pantetheine and phosphopantetheine, *Org Lett 6,* 4801-4803.
2. Garber, K. C., Wangkanont, K., Carlson, E. E., and Kiessling, L. L. (2010) A general glycomimetic strategy yields non-carbohydrate inhibitors of DC-SIGN, *Chem Commun 46*, 6747-6749.

S6. NMR spectra



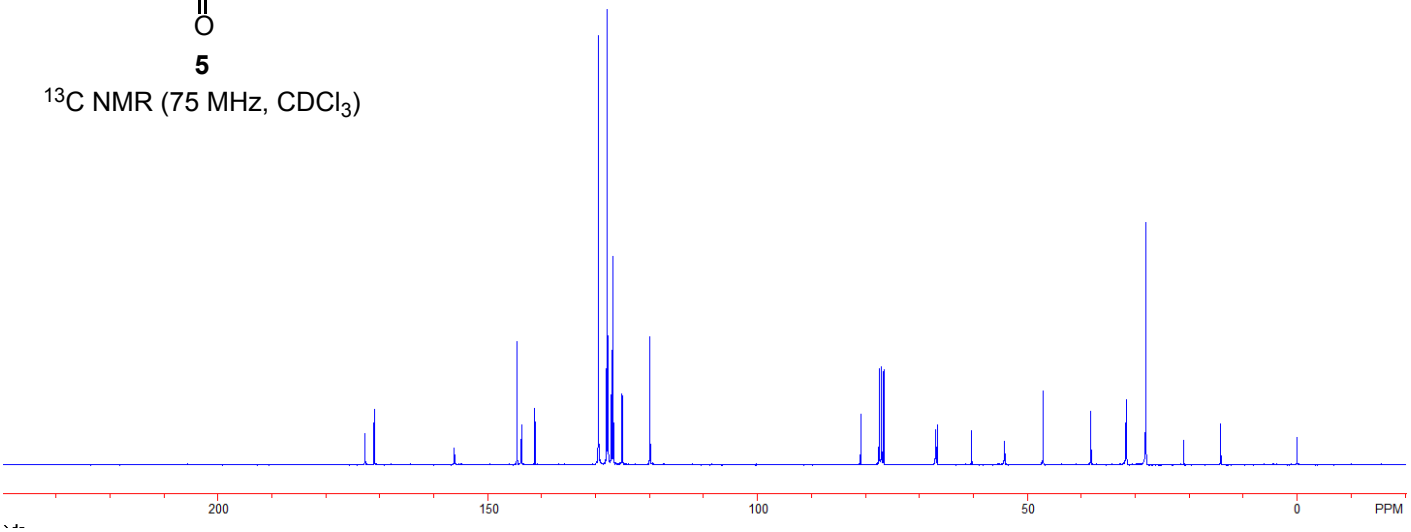
5

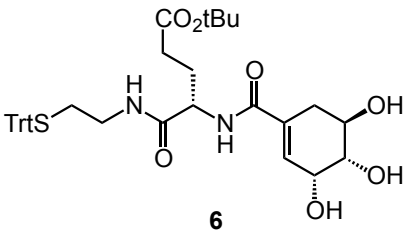
^{1}H NMR (300 MHz, $CDCl_3$)



5

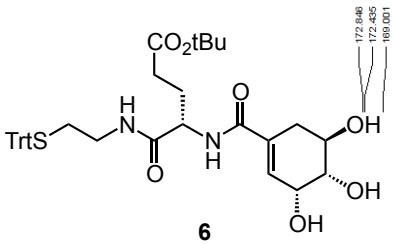
^{13}C NMR (75 MHz, $CDCl_3$)





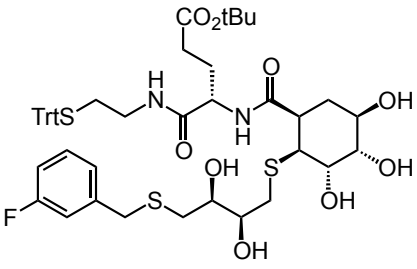
6

^{1}H NMR (300 MHz, CD_3OD)



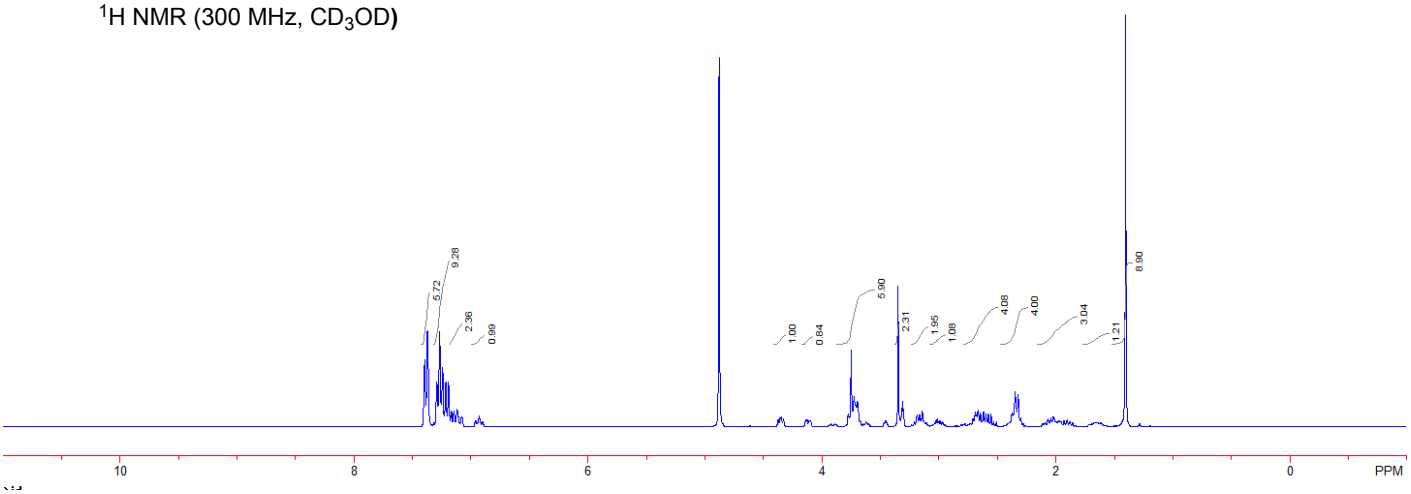
6

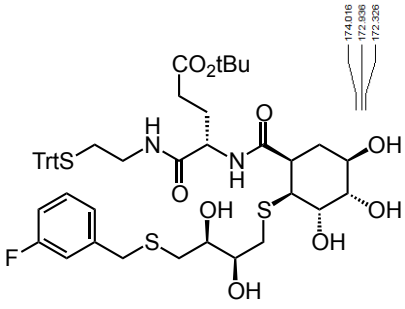
^{13}C NMR (75 MHz, CD_3OD)



7

^{1}H NMR (300 MHz, CD_3OD)





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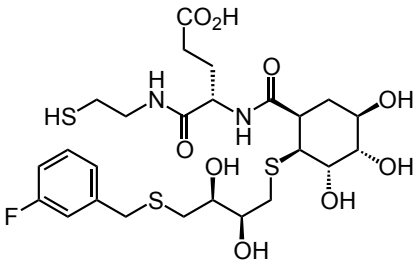
^{13}C NMR (75 MHz, CD_3OD)



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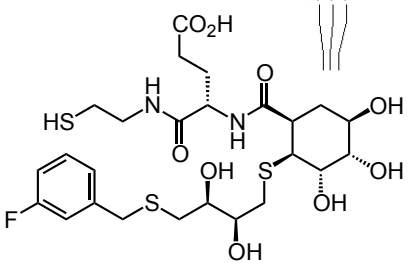
^{1}H NMR (300 MHz, CD_3OD)



2

^{13}C NMR (75 MHz, CD_3OD)