

## Supporting information for

# Sortase A-Mediated Chemoenzymatic Synthesis of Complex Glycosylphosphatidylinositol-Anchored Protein

Zhimeng Wu,<sup>a</sup> Xueqing Guo,<sup>a</sup> Jian Gao,<sup>b</sup> and Zhongwu Guo<sup>a,b,\*</sup>

<sup>a</sup> Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA

<sup>b</sup> National Glycoengineering Research Center, Shandong University, Jinan 250100, China

E-mail: [zwguo@chem.wayne.edu](mailto:zwguo@chem.wayne.edu), [zwguo@sdu.edu.cn](mailto:zwguo@sdu.edu.cn)

### 1. General Experimental Methods

NMR spectra were recorded with a 400 or 500 MHz machine. Proton chemical shifts are reported in ppm ( $\delta$ ) downfield from tetramethylsilane (TMS) or in reference to the proton signal of the solvent DHO ( $\delta$  4.79). Carbon-13 chemical shifts are reported in ppm ( $\delta$ ) in reference to CDCl<sub>3</sub> ( $\delta$  77.16). Coupling constants ( $J$ ) are reported in hertz (Hz). ESI MS spectra were obtained on Waters' ZQ2000 single quadrupole mass spectrometer, and MALDI TOF-MS was performed on Bruker Ultraflex mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel GF254 plate detected by charring with phosphomolibdic acid in EtOH or 1% H<sub>2</sub>SO<sub>4</sub> in EtOH solutions. Molecular sieves were dried under high vacuum at 170-180 °C for 6 to 10 h before use. Commercial anhydrous solvents and other reagents were used without further purification unless otherwise mentioned.

### 2. Synthesis of GPI anchor 1

**1-*O*-(*para*-Methoxybenzyl)-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol**      **2-azido-2-deoxy-3,6-di-*O*-benzyl- $\alpha$ -D-glucopyranoside (5)**. After compounds **3** (1.08 g, 1.64 mmol) and **2** (1.40 g, 2.45 mmol) were co-evaporated with toluene 3 time and then dried under high vacuum for 2 h, a pre-dried mixture solvents of toluene and 1,4-dioxane (20 mL, 2:1) was added. The solution was stirring at r.t. with molecular sieves 4Å for another 2 h under Ar. Then, catalytic amount of TMSOTf (56  $\mu$ L, 0.25 mmol) was added at -15 °C, and the mixture was stirred until the reaction was completed as shown by TLC. The reaction was quenched by adding triethylamine (0.5 mL), and molecular sieves were subsequently removed by filtration through a Celite pad. The organic solution was condensed, and the residue was briefly purified by passing through a silica gel column to give crude product of **4** as an inseparable  $\alpha,\beta$

mixture. This mixture was dissolved in 30 mL of MeOH and dichloromethane (DCM) (2:1), and then a catalytic amount of freshly prepared NaOMe solution was added (pH ~9) to remove the acetyl group. The reaction was stirring at rt for 4 days, and then neutralized with acidic amberlyst 15 resin. After the resin was removed, the solvent was evaporated and the crude product was purified by flash silica gel column chromatograph to give **5** (775 mg, 46%), as well as its  $\beta$  isomer (211 mg, 19%). The spectroscopic data of **5** agreed well with that in literature (K. Pekari, D. Tailler, R. Weingart, and R.R. Schmidt, *J. Org. Chem.* **2001**, *66*, 7432.):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.46-7.44 (m, 32 H), 6.88 (d, 2 H,  $J$  8.0 Hz), 5.76 (d, 1 H,  $J$  3.2 Hz), 5.04-4.98 (dd, 2 H,  $J$  10.4 Hz, 11.2 Hz), 4.91 (s, 2 H), 4.85-4.65 (m, 6 H), 4.52 (s, 2 H), 4.44-4.28 (m, 2 H), 4.26 (m, 1 H), 4.18-4.13 (m, 1 H), 4.12 (s, 1 H), 4.01-3.98 (m, 1 H), 3.83 (s, 3 H), 3.80-3.78 (m, 1 H), 3.76-3.70 (m, 1 H), 3.52-3.48 (m, 2 H), 3.42-3.40 (m, 1 H), 3.29 (dd, 1 H,  $J$  6.8 Hz, 10.8 Hz), 3.24-3.19 (m, 2 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  159.5, 139.1, 138.8, 138.6, 138.5, 138.4, 138.2, 130.0, 129.8-127.1, 114.1, 97.6, 82.2, 82.1, 81.8, 81.1, 79.6, 77.6, 77.3, 77.0, 76.0, 75.9, 75.1, 75.0, 74.5, 73.7, 73.6, 73.0, 72.4, 72.1, 69.6, 69.2, 62.9, 55.5

**1-O-para-Methoxybenzyl-2,3,4,5-tetra-O-benzyl-myo-inositol [2,3,4-tri-O-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 2)-[3,4,6-tri-O-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 6)-[2,3,4-tri-O-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 4)-2-azido-2-deoxy-3,6-di-O-benzyl- $\alpha$ -D-glucopyranoside (**8**).** After **5** (775 mg, 0.76 mmol) and **6** (1.69 g, 1.13 mmol) were co-evaporated with toluene 3 time, they were dried under high vacuum for 2 h and then dissolved in 50 mL of dry diethyl ether. The solution was stirred at rt with molecular sieves 4Å for another 2 h under Ar, and then a catalytic amount of TMSOTf (17  $\mu\text{L}$ , 0.076 mmol) was added at -15 °C. The mixture was stirred until the reaction was completed (TLC). The reaction was quenched by adding triethylamine (0.5 mL), and molecular sieves were removed by filtration through a Celite pad. The organic solution was condensed, and residue was briefly purified by passing through a silica gel column to give crude product of **7**. This mixture was dissolved in 30 mL of MeOH and DCM (2:1), and a catalytic amount of freshly prepared NaOMe solution was added to remove the acetyl group. After the reaction was completed, acidic amberlyst 15 resin was added to neutralize the reaction mixture. The resin was removed by filtration, and the residue was purified by silica gel column chromatograph to give **8** exclusively (1.34g, 76%) along with the recovery of some **5** (182 mg). **8**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.49-7.14 (m, 87 H), 6.94 (d, 2 H,  $J$  8.8 Hz), 5.88 (d, 1 H,  $J$  4 Hz, GlcN<sub>3</sub> H-1), 5.35 (s, 1 H), 5.14 (s, 1 H), 5.08 (d, 1 H,  $J$  11.6 Hz), 5.02 (d, 1 H,  $J$  2.4 Hz), 5.00-4.98 (m, 3 H), 4.96-4.94 (m, 1 H), 4.91-4.90 (m, 2 H), 4.87 (s, 1 H), 4.84-4.79 (m, 2 H),

4.73-4.70 (m, 2 H), 4.67-4.64 (m, 2 H), 4.63-4.58 (m, 2 H), 4.57-4.54 (m, 6 H), 4.52 (d, 1 H,  $J$  4Hz), 4.50-4.49 (m, 4 H), 4.45-4.37 (m, 4 H), 4.23-4.21 (m, 2 H), 4.20-4.19 (m, 2 H), 4.18 (s, 1 H), 4.15-4.08 (m, 3 H), 4.09-4.02 (m, 1 H), 3.99-3.89 (m, 6 H), 3.86 (s, 5 H), 3.84-3.81 (m, 4 H), 3.77-3.70 (m, 2 H), 3.68 (dd, 1 H,  $J$  1.6 Hz, 12.4 Hz), 3.62 (m, 1 H), 3.56-3.54 (m, 3 H), 3.51-3.50 (m, 1 H), 3.48 (s, 1 H), 3.45 (s, 1 H), 3.35 (dd, 1 H,  $J$  3.2 Hz, 11.2 Hz), 3.30 (dd, 1 H,  $J$  4 Hz, 10 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  159.5, 139.1, 138.8, 138.6, 138.5, 138.4, 138.2, 130.0, 129.8-127.1, 114.1, 97.6, 82.2, 82.1, 81.8, 81.1, 79.6, 77.6, 77.3, 77.0, 76.0, 75.9, 75.1, 75.0, 74.5, 73.7, 73.6, 73.0, 72.4, 72.1, 69.6, 69.2, 62.9, 55.5; ESI MS: calcd for  $\text{C}_{143}\text{H}_{153}\text{N}_4\text{O}_{26}$  ( $\text{M} + \text{NH}_4^+$ ), 2342.1, found 2342.1; HR MS: calcd for  $\text{C}_{143}\text{H}_{153}\text{N}_4\text{O}_{26}$  ( $\text{M} + \text{NH}_4^+$ ), 2342.0773, found 2342.0854

**1-*O*-para-Methoxybenzyl-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol [6-*O*-(2-glycylglycylaminoethyl-phosphoryl)-2,3,4-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 2)-[3,4,6-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 6)-[2,3,4-tri-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 4)-[2-azido-2-deoxy-3,6-di-*O*-benzyl- $\alpha$ -D-glucopyranoside (10).** After **8** (98.7 mg, 42  $\mu\text{mol}$ ) and *H*-phosphonate **9** (89.1 mg, 170  $\mu\text{mol}$ ) were co-evaporated with dry pyridine 3 time and dried in high vacuum for 2 h, they were dissolved in dry pyridine (2 mL). Then, a solution of pivaloyl chloride (42.3  $\mu\text{L}$ , 340  $\mu\text{mol}$ ) in pyridine (2 mL) was added at rt under an  $\text{N}_2$  atmosphere. The reaction progress was monitored by TLC. When the reaction was finished, the reaction mixture was cooled to 0  $^\circ\text{C}$ , and a solution of  $\text{I}_2$  (42.5 mg, 170  $\mu\text{mol}$ ) in 1.1 mL of pyridine and water (10:1, v/v) was added. The reaction was quenched 3 h later by addition of saturated aq.  $\text{Na}_2\text{S}_2\text{O}_3$  solution. The mixture was extracted with DCM, and the organic phase was combined, dried and concentrated. The residue was purified by a silica gel column to give **10** (92.8 mg, 74%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  8.45 (s, 1 H, NH), 7.51 (br, 1 H, NH), 7.38-7.04 (m, 82 H), 6.99 (br, 1 H, NH), 6.85 (d, 2 H,  $J$  8.8 Hz), 5.78 (d, 1 H,  $J$  3.6 Hz, GlcN<sub>3</sub> H-1), 5.29 (s, 1 H), 5.10 (s, 1 H), 5.09-5.02 (m, 2 H), 5.00-4.96 (m, 4 H), 4.85 (d, 1 H,  $J$  4.4 Hz), 4.82 (d, 2 H,  $J$  5.2 Hz), 4.77-4.67 (m, 6 H), 4.63 (d, 2 H,  $J$  4.4 Hz), 4.60 (s, 1 H), 4.54 (s, 1 H), 4.50-4.46 (m, 6 H), 4.44-4.39 (m, 6 H), 4.38-4.24 (m, 7 H), 4.13-4.01 (m, 4 H), 4.04-3.98 (m, 3 H), 3.98-3.95 (m, 3 H), 3.92-3.86 (m, 5 H), 3.83 (s, 1 H), 3.81-3.71 (m, 10 H), 3.66 (m, 1 H), 3.57-3.55 (m, 2 H), 3.51-3.48 (m, 1 H), 3.45 (s, 1 H), 3.44-3.35 (m, 4 H), 3.32-3.24 (m, 3 H), 3.20 (dd, 1 H,  $J$  3.6 Hz, 6.4 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  170.2, 169.5, 159.3, 156.9, 138-136.8, 129.6-126.9, 113.9, 100.2, 99.45, 98.9, 97.3, 81.9, 81.5, 80.9, 80.2, 80.0, 79.8, 76.3, 75.9, 75.8, 75.5, 75.2, 74.9, 74.8, 74.6, 74.4, 74.2, 74.1, 73.8, 73.2, 73.1, 72.9, 72.7, 72.3, 72.2, 72.1, 71.8, 71.7, 71.6, 69.5, 68.9, 68.6, 66.6, 66.2, 64.2, 64.0, 63.8, 63.1, 55.3, 52.7, 45.9 [( $\text{CH}_3\text{CH}_2$ )<sub>3</sub>N], 44.9, 42.7, 41.5, 10.3 [( $\text{CH}_3\text{CH}_2$ )<sub>3</sub>N];  $^{31}\text{P}$  NMR (161 Hz,  $\text{CDCl}_3$ ):  $\delta$ : 2.5; ESI MS

(negative mode): calcd for  $C_{157}H_{166}N_6O_{33}P$ , 2694.1, found 2694.1; HR MS: calcd for  $C_{157}H_{166}N_6O_{33}P$ , 2694.1233, found 2694.1362.

**2,3,4,5-tetra-*O*-Benzyl-*myo*-inositol [6-*O*-(2-glycylglycylaminoethylphosphoryl)-2,3,4-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 2)-[3,4,6-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 6)-[2,3,4-tri-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 4)-[2-azido-2-deoxy-3,6-di-*O*-benzyl- $\alpha$ -D-glucopyranoside (11).** After the solution of **10** (92.8 mg, 0.034  $\mu$ mmol) in 4 mL of 5% TFA in DCM was stirred at 0 °C for 0.5 h, the reaction was quenched by addition two drops of  $Et_3N$ . The mixture was extracted with DCM, and the organic phase was combined, dried and concentrated. The residue was purified by a silica gel column to give **11** (58 mg, 69%).  $^1H$  NMR ( $CDCl_3$ , 500 MHz):  $\delta$  8.42 (br, 1 H, NH), 7.53 (br, 1 H, NH), 7.42-7.13 (m, 80 H), 5.57 (d, 1 H,  $J$  2.5 Hz, GlcN<sub>3</sub> H-1), 5.37 (s, 1 H), 5.13 (s, 1 H), 5.10-5.02 (m, 4 H), 5.00-4.96 (m, 3 H), 4.87 (d, 1 H,  $J$  10.5 Hz), 4.82-4.73 (m, 9 H), 4.68 (d, 1 H,  $J$  11 Hz), 4.58-4.53 (m, 4 H), 4.55-4.48 (m, 3 H), 4.45-4.43 (m, 3 H), 4.35-4.30 (m, 6 H), 4.23 (d, 1 H,  $J$  11.5 Hz), 4.13-4.10 (m, 2 H), 4.09-4.03 (m, 4 H), 4.04-3.89 (m, 7 H), 3.88-3.78 (m, 7 H), 3.74 (s, 1 H), 3.72-3.66 (m, 3 H), 3.65-3.63 (m, 1 H), 3.55 (dd, 1 H,  $J$  2.0 Hz, 10 Hz), 3.48-3.43 (m, 3 H), 3.41-3.38 (m, 3 H), 3.34-3.32 (m, 3 H), 3.21-3.19 (m, 1 H);  $^{13}C$  NMR ( $CDCl_3$ , 125 MHz):  $\delta$  170.5, 169.8, 157.2, 139.1-137.6, 137.4, 137.1, 131.2-127.2, 100.1, 99.8, 99.2, 97.8, 82.1, 81.6, 81.2, 80.9, 80.4, 80.2, 80.1, 79.8, 76.5, 76.4, 76.2, 76.1, 75.7, 75.2, 75.1, 74.9, 74.7, 74.5, 74.4, 74.2, 73.6, 73.5, 73.4, 73.3, 73.2, 72.7, 72.6, 72.5, 72.4, 72.1, 72.0, 71.9, 70.5, 69.1, 69.0, 66.9, 66.5, 64.5, 64.3, 64.2, 46.0 [( $CH_3CH_2$ )<sub>3</sub>N], 45.1, 43.1, 41.7, 9.7 [( $CH_3CH_2$ )<sub>3</sub>N];  $^{31}P$  NMR (161 Hz,  $CDCl_3$ ):  $\delta$ : 2.3; ESI MS (negative mode): calcd for  $C_{149}H_{158}N_6O_{32}P$ , 2575.1, found 2574.1; HR MS: calcd for  $C_{149}H_{158}N_6O_{32}P$ , 2574.0658, found 2574.0725.

**1-Octadecylphosphoryl-2,3,4,5-tetra-*O*-benzyl-*myo*-inositol [6-*O*-(2-glycylglycylaminoethylphosphoryl)-2,3,4-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 2)-[3,4,6-tri-*O*-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 6)-[2,3,4-tri-benzyl- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 4)-[2-azido-2-deoxy-3,6-di-*O*-benzyl- $\alpha$ -D-glucopyranoside (13).** After **11** (116 mg, 39.3  $\mu$ mol) and *H*-phosphonate **12** (96.9 mg, 196.3  $\mu$ mol) were co-evaporated with dry pyridine 3 time and dried in high vacuum for 2 h, they were dissolved in dry pyridine (2 mL). Then, to the mixture was added a solution of pivaloyl chloride (25  $\mu$ L, 200  $\mu$ mol) in pyridine (1 mL) at rt under an  $N_2$  atmosphere. The reaction was monitored by TLC. When the reaction was finished, the reaction mixture was cooled to 0 °C, and a solution of  $I_2$  (30 mg, 118  $\mu$ mol) in 1.1 mL of pyridine and water (10:1, v/v) was added. The reaction was quenched 3 h later by addition of saturated aq.  $Na_2S_2O_3$  solution. The mixture was extracted with DCM, and the organic

phase was combined, dried and concentrated. The residue was purified by a silica gel column to give **13** (80 mg, 70%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz): δ 8.31 (br, 1 H, NH), 7.53 (s, 1 H, NH), 7.41 (s, 1 H, NH), 7.35-7.10 (m, 80 H), 5.95 (d, 1 H, *J* 3.5 Hz, GlcN<sub>3</sub> H-1), 5.26 (s, 1 H), 5.09 (s, 1 H), 5.07-5.04 (m, 2 H), 5.02 (s, 2 H), 4.99-4.97 (m, 5 H), 4.90-4.89 (m, 2 H), 4.4.86-4.85 (m, 1 H), 4.82-4.79 (m, 2 H), 4.77-4.73 (m, 2 H), 4.70 (s, 1 H), 4.67 (m, 2 H), 4.59-4.54 (m, 2 H), 4.50 (d, 1 H, *J* 5.5 Hz), 4.49-4.48 (m, 2 H), 4.46-4.44 (m, 4 H), 4.42-4.41 (m, 4 H), 4.38-4.35 (m, 2 H), 4.32-4.24 (m, 5 H), 4.15-4.07 (m, 3 H), 4.05-3.90 (m, 10 H), 3.91-3.86 (m, 2 H), 3.85-3.82 (m, 2 H), 3.81-3.75 (m, 4 H), 3.65-3.60 (m, 3 H), 3.58-3.53 (m, 3 H), 3.50-3.42 (m, 2 H), 3.39-3.35 (m, 1 H), 3.30-3.25 (m, 3 H), 3.19 (dd, 1 H, *J* 3.5 Hz, 10 Hz), 1.65-1.58 (m, 2 H), 1.35-1.20 (m, 32 H), 0.90 (t, 3 H, *J* 7.5 Hz); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz): δ 170.5, 170.4, 170.0, 156.3, 142.2-137.1, 128.8-127.1, 100.9, 99.8, 99.3, 96.7, 82.1, 82.0, 81.2, 80.3, 80.2, 80.1, 76.8, 76.6, 76.3, 76.2, 75.9, 75.1, 74.8, 74.7, 74.5, 74.4, 74.3, 74.1, 73.8, 73.5, 73.3, 73.1, 72.9, 72.6, 72.5, 72.4, 72.3, 72.1, 71.9, 71.8, 70.2, 69.2, 69.1, 66.8, 66.5, 66.1, 66.0, 64.4, 63.4, 45.1 [(CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>N], 43.1, 41.6, 39.0, 32.1, 31.3, 31.2, 30.0, 29.9, 29.8, 28.0, 26.1, 22.9, 14.4, 8.8 [(CH<sub>3</sub>CH<sub>2</sub>)<sub>3</sub>N]; <sup>31</sup>P NMR (161 Hz, CDCl<sub>3</sub>): δ: 2.10, -0.78; ESI MS (negative mode): calcd for C<sub>167</sub>H<sub>195</sub>N<sub>6</sub>O<sub>35</sub>P<sub>2</sub>, 2907.3 found 2906.3; HR MS: calcd for C<sub>167</sub>H<sub>195</sub>N<sub>6</sub>O<sub>35</sub>P<sub>2</sub>, 2906.3139, found 2906.3206.

**1-Octadecylphosphoryl-*myo*-inositol [6-*O*-(2-Glycyl-glycylaminoethylphosphoryl)- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 2)- $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 6)-[ $\alpha$ -D-mannopyranosyl]-(1 $\rightarrow$ 4)-[2-amino-2-deoxy- $\alpha$ -D-glucopyranoside (**1**).** A mixture of **13** (50 mg, 16.3  $\mu$ mol) and 10% Pd(OH)<sub>2</sub>/C (10 mg) in THF, MeOH and H<sub>2</sub>O (2:1:1, 3 mL) and 4% formic acid in H<sub>2</sub>O (120  $\mu$ L) was shaken under an atmosphere of 50 PSI H<sub>2</sub> for 6 h. The reaction mixture was filtered off through a Celite pad, while the pad was washed with the mixture of THF, MeOH, and H<sub>2</sub>O (2:1:1). The filtrates were combined and concentrated in vacuum to afford **1** (17.2 mg, 76%). The NMR signals of **1** were broad because of its aggregation in solution, but its lipid and sugar components were obvious and the number of protons corresponded well with that of the desired product. MALDI-TOF MS (positive mode): calcd for C<sub>54</sub>H<sub>102</sub>N<sub>4</sub>O<sub>33</sub>P<sub>2</sub>, 1396.6, found, 1397.3 (M + H<sup>+</sup>).

### 3. SrtA-mediated ligation of GPI **1** and GFP **14**

The ligation reaction was carried out in 0.3 M Tris-HCl buffer (pH 7.5) containing 0.15 M NaCl, 5 mM CaCl<sub>2</sub> and 0.5 mM mercaptoethanol, and the concentrations of GFP **14**, GPI **1**, and SrtA were 100  $\mu$ M, 2.5 mM, and 50  $\mu$ M, respectively, in 100  $\mu$ L scale. After the reaction mixture was incubated at

37 °C for overnight, while the reaction was monitored and analyzed with HPLC, it was treated with Ni-NTA resin (50 uL, Qiagen) at room temperature for 2 h. The solution was then separated from the resin by centrifugation, and this procedure was repeated once more. The resultant solution was mixed with 1 mL of acetone, and centrifuged for 30 min. After the pellet was collected and dissolved in water, the solution was again treated with acetone and centrifuged. The resultant pellet was dissolved in 50 uL of water containing 0.1% formic acid and finally subjected to HPLC and SDS PAGE analyses.

#### 4. Spectroscopic data of GPI 1 and intermediates involved in its synthesis

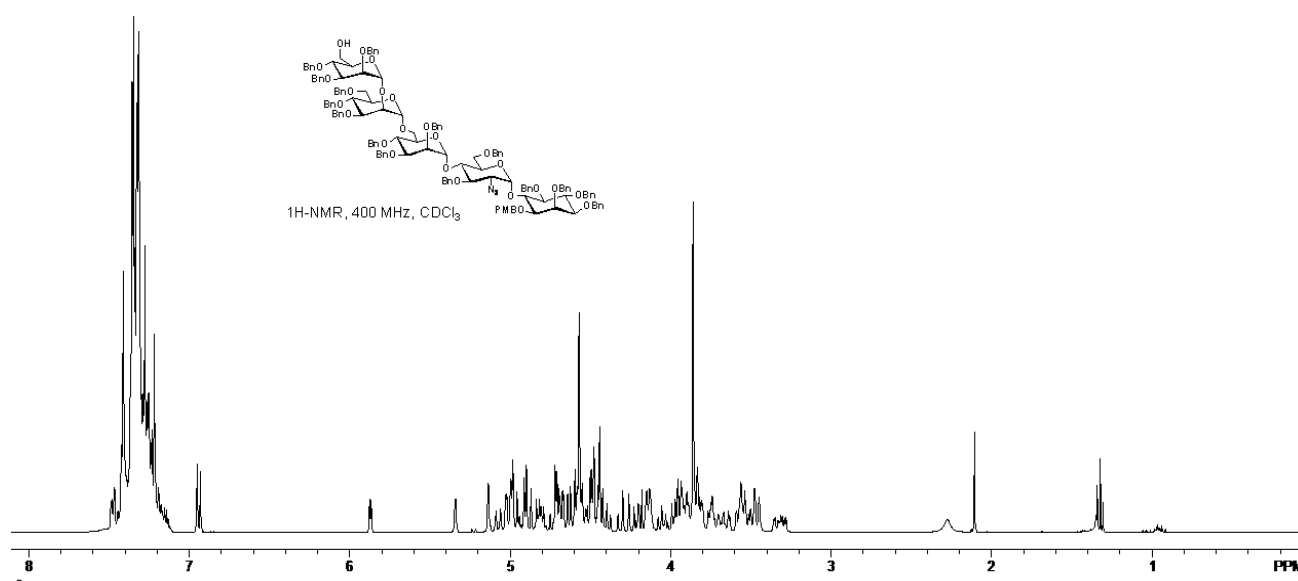


Figure SI-1. <sup>1</sup>H-NMR of **8** at 400 MHz in CDCl<sub>3</sub>.

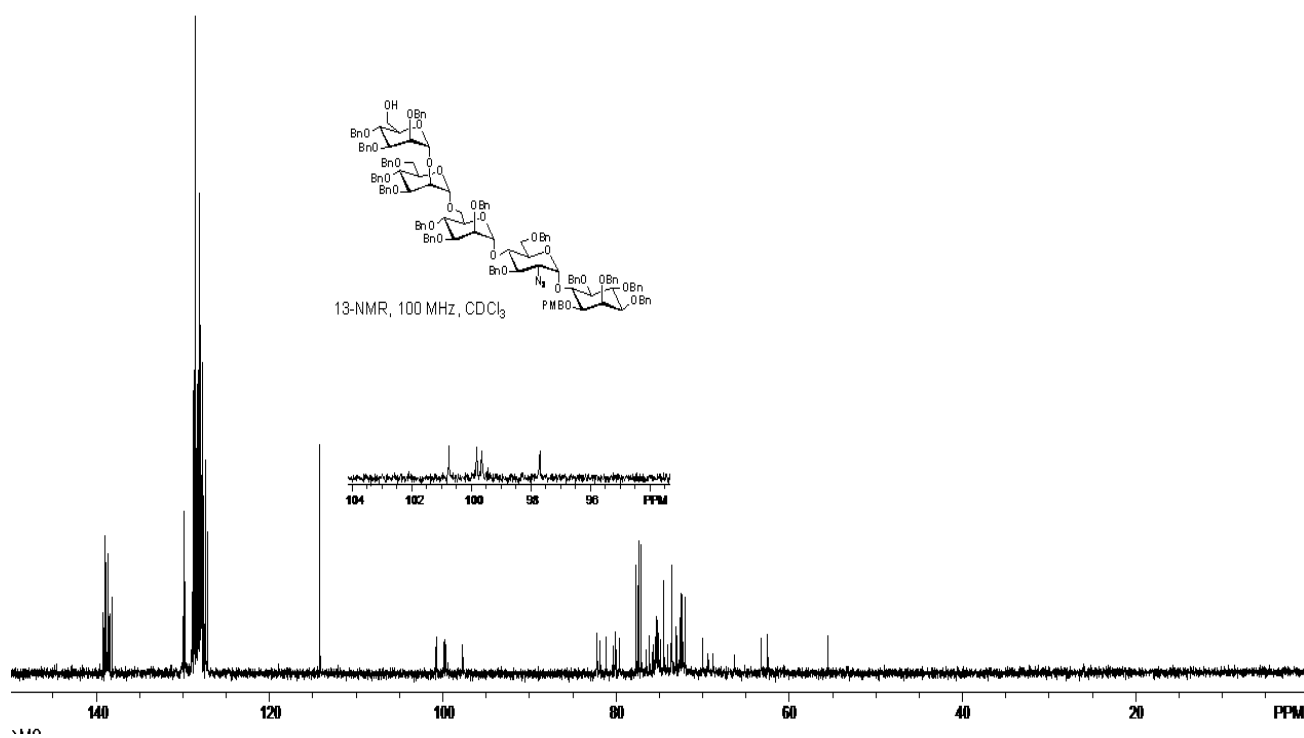


Figure SI-2. <sup>13</sup>C-NMR of **8** at 100 MHz in CDCl<sub>3</sub>.

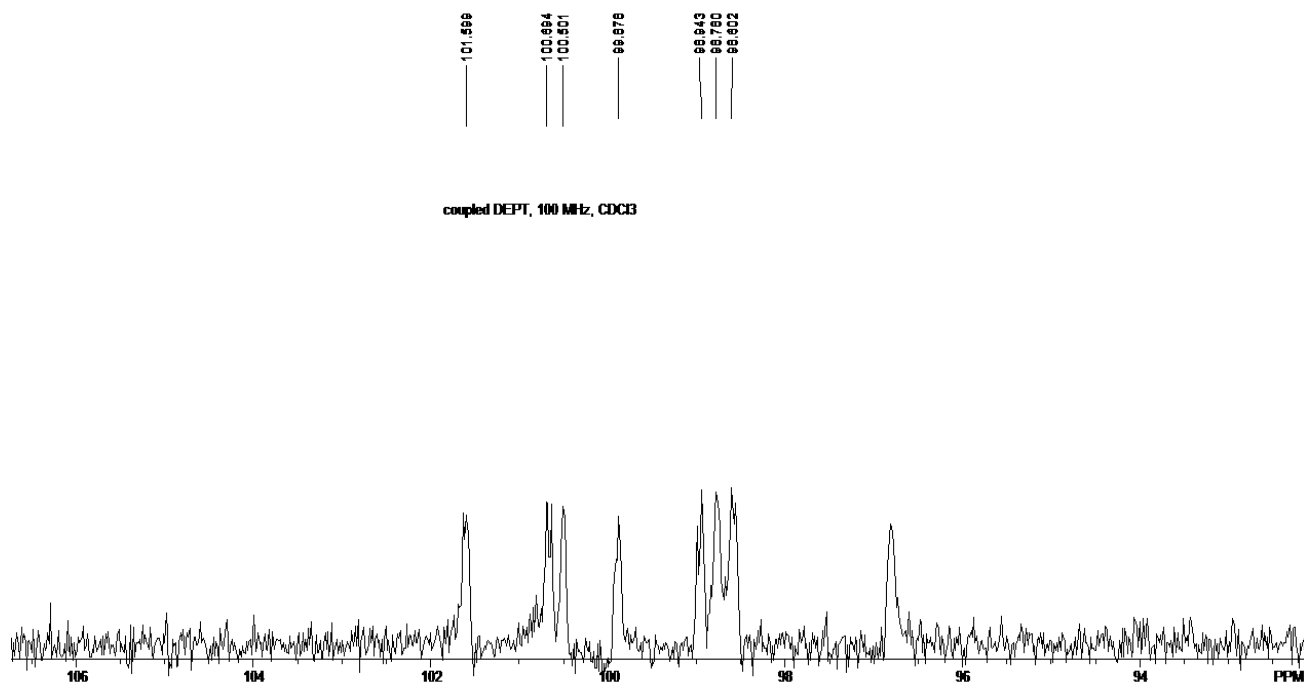


Figure SI-3. Coupled DEPT spectrum of **8** (expansion) at 100 MHz in CDCl<sub>3</sub>.

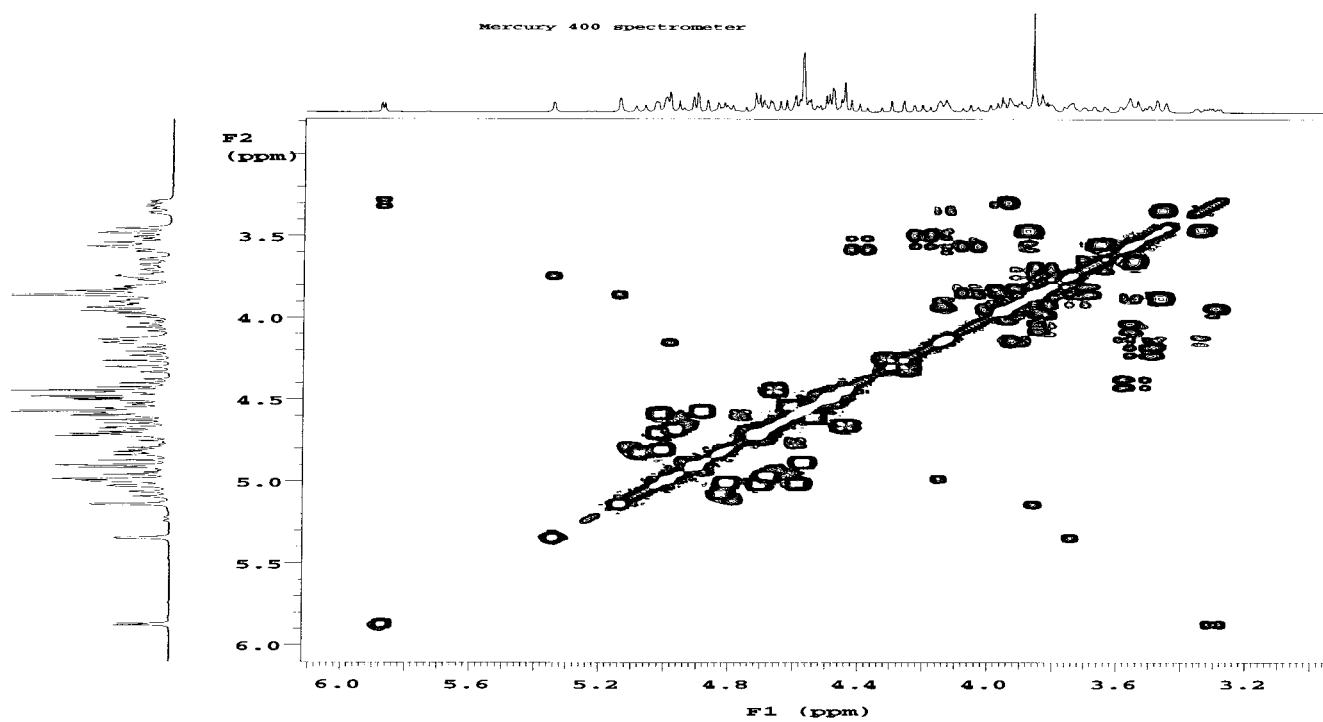


Figure SI-4. H,H-COSY of **8** at 400 MHz in CDCl<sub>3</sub>.

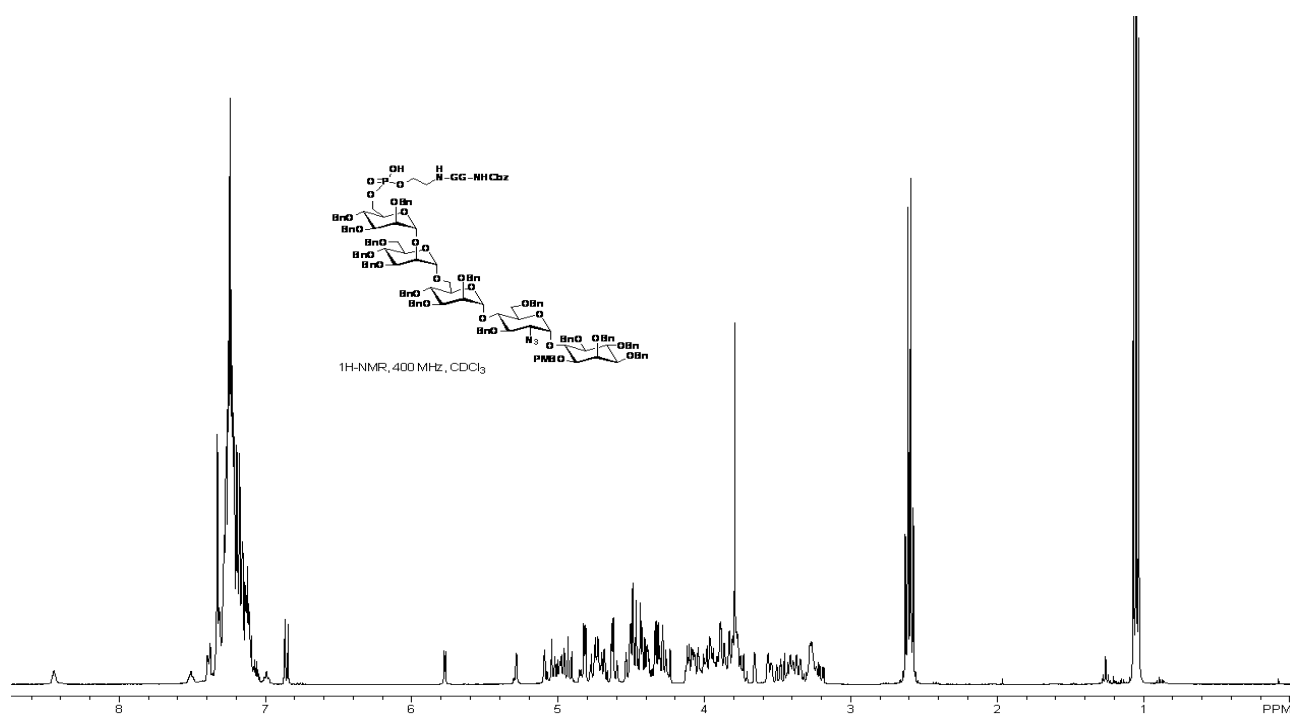


Figure SI-5. <sup>1</sup>H-NMR of **10** at 400 MHz in CDCl<sub>3</sub>.



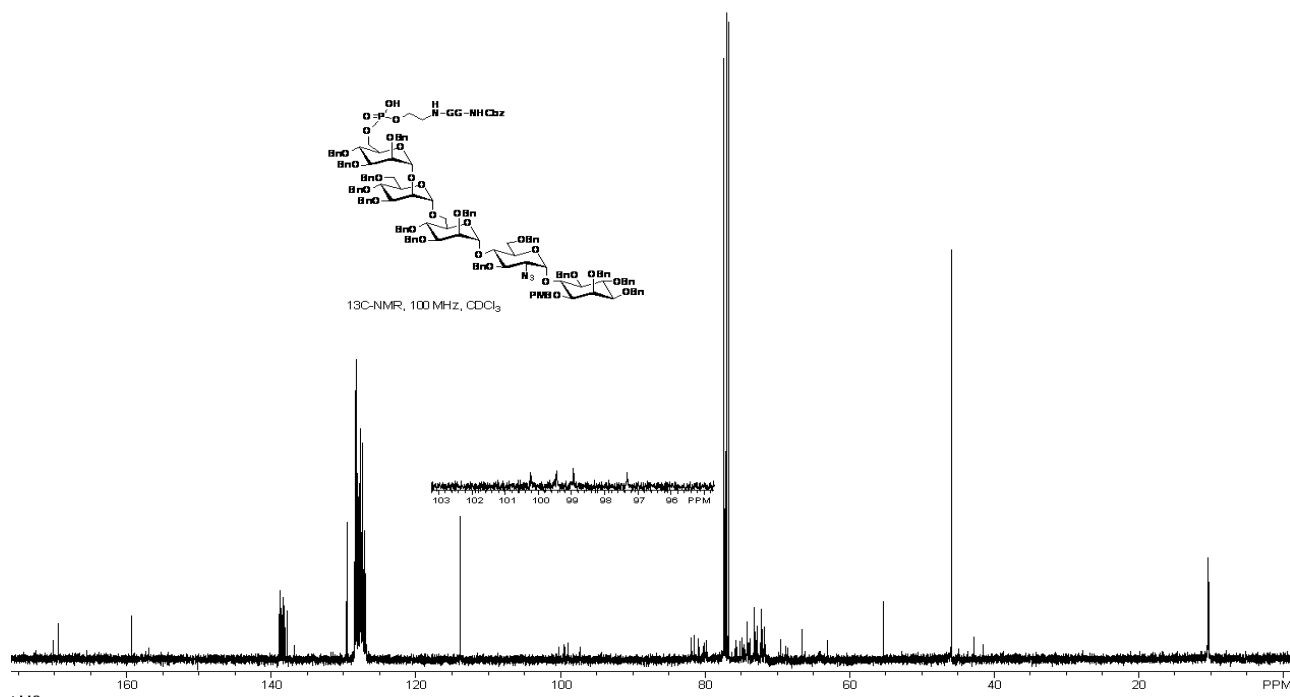


Figure SI-6.  $^{13}\text{C}$ -NMR of **10** at 100 MHz in  $\text{CDCl}_3$ .

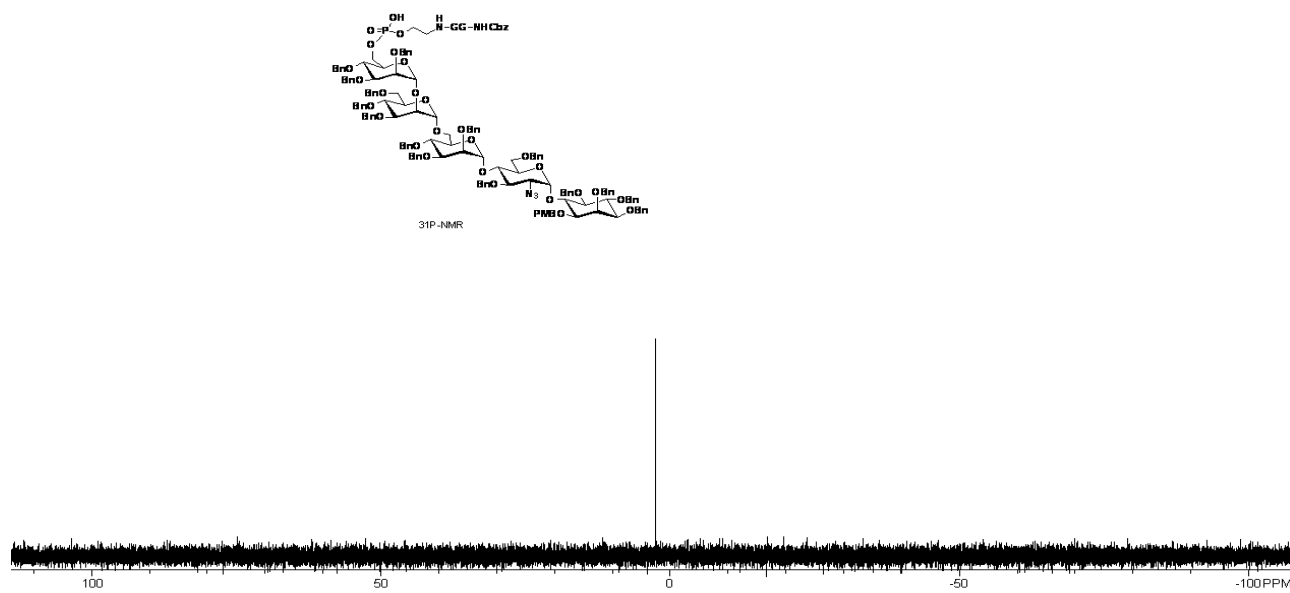


Figure SI-7.  $^{31}\text{P}$ -NMR of **10** at 161 MHz in  $\text{CDCl}_3$ .

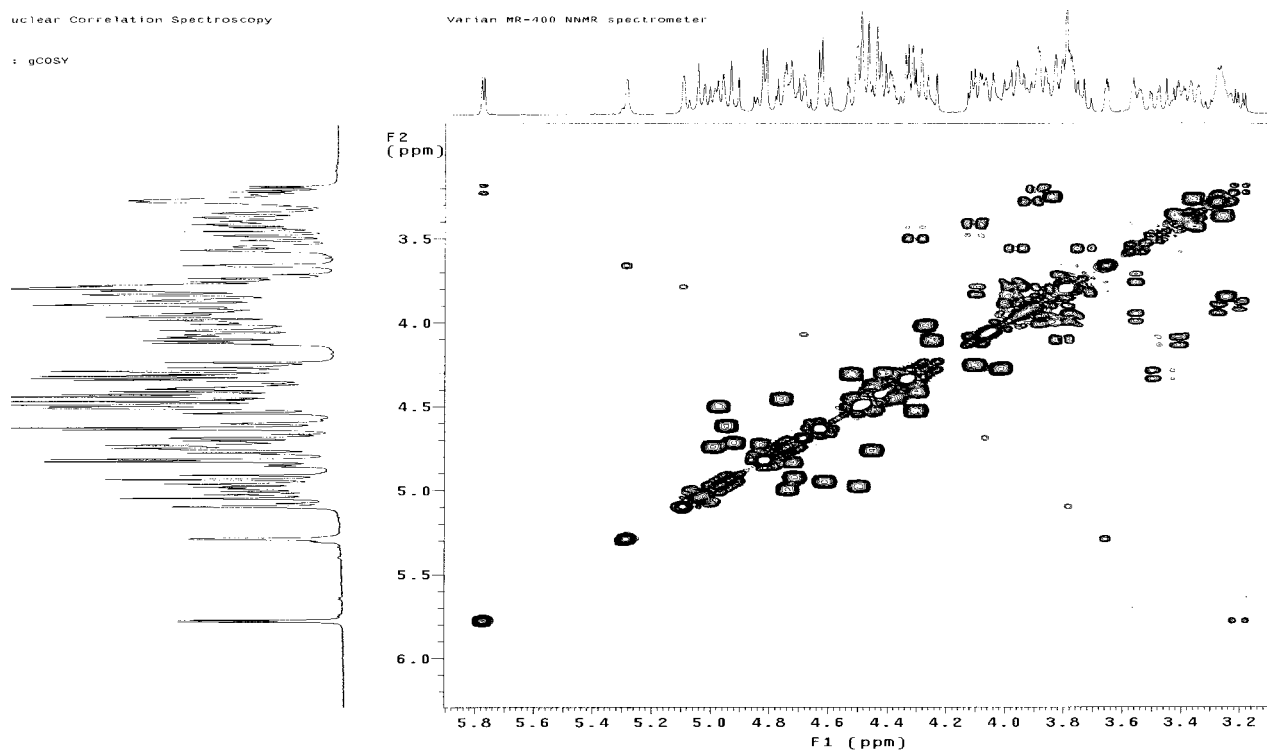


Figure SI-8. H,H-COSY of **10** at 400 MHz in CDCl<sub>3</sub>.

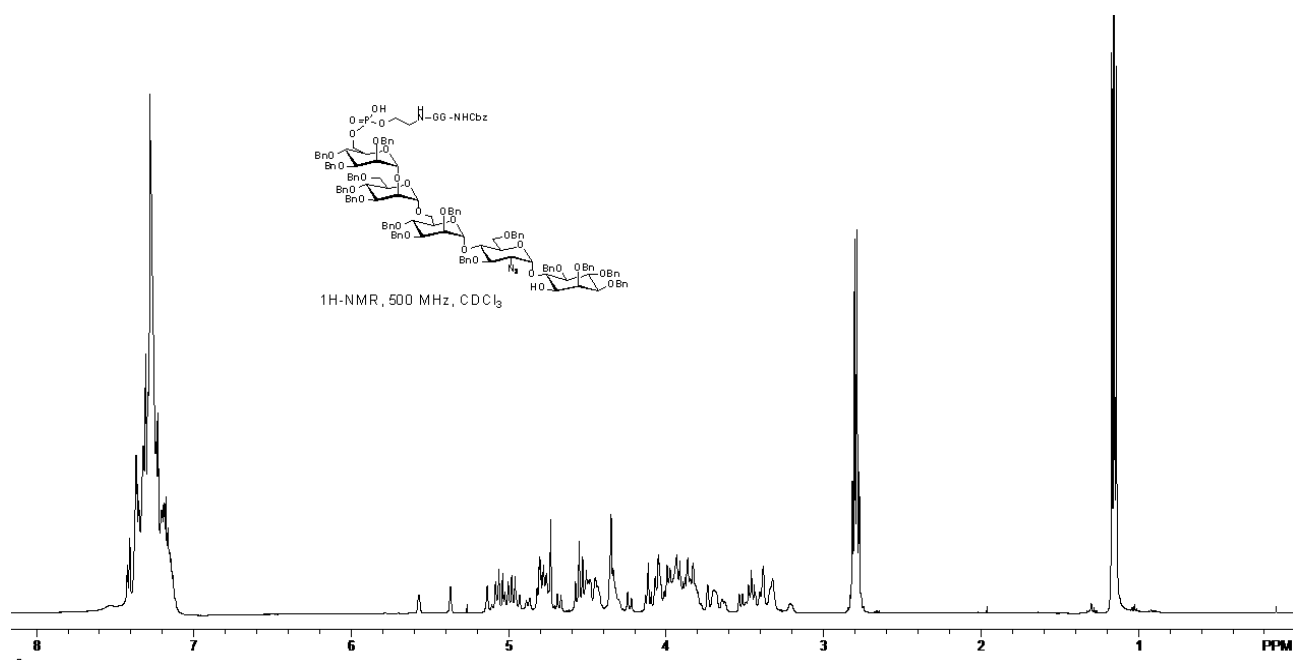


Figure SI-9. <sup>1</sup>H-NMR of **11** at 500 MHz in CDCl<sub>3</sub>.

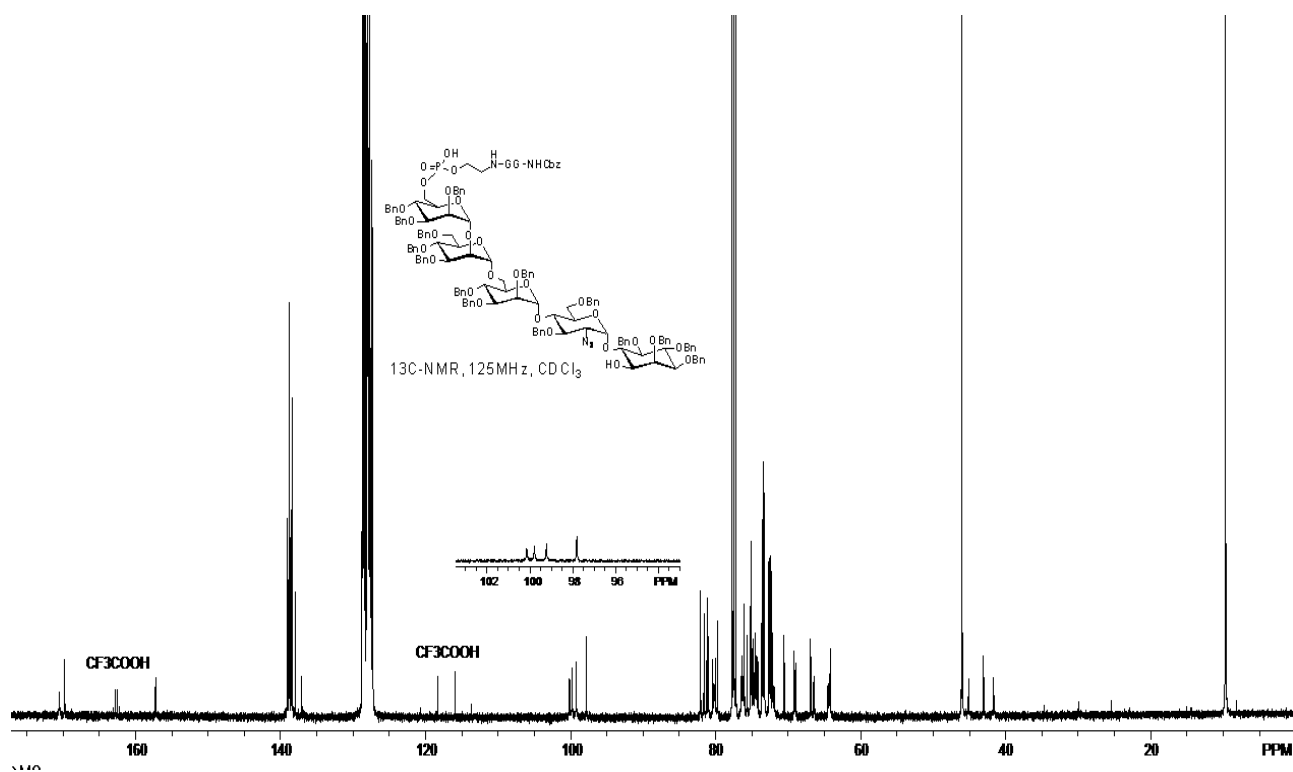


Figure SI-10. <sup>13</sup>C-NMR of **11** at 125 MHz in CDCl<sub>3</sub>.

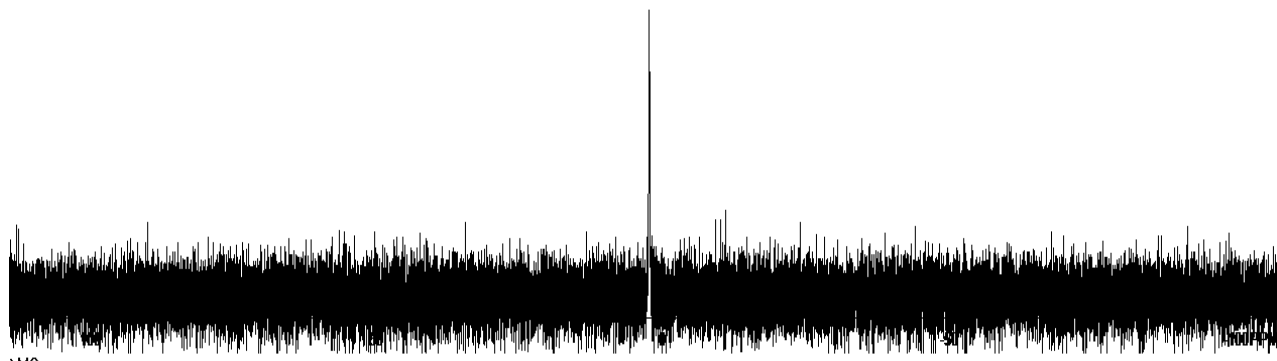
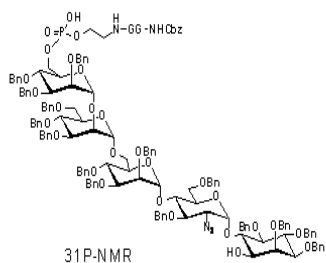


Figure SI-11. <sup>31</sup>P-NMR of **11** at 161 MHz in CDCl<sub>3</sub>.

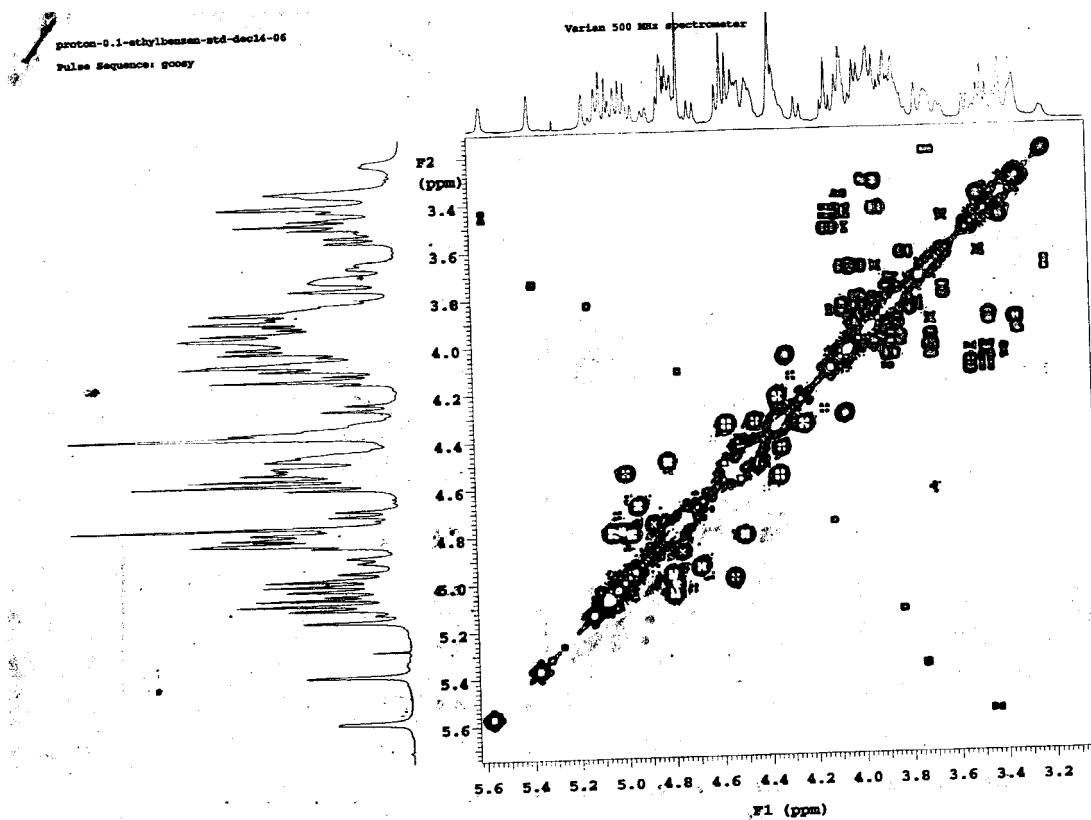


Figure SI-12. H,H-COSY of **11** at 500 MHz in CDCl<sub>3</sub>.

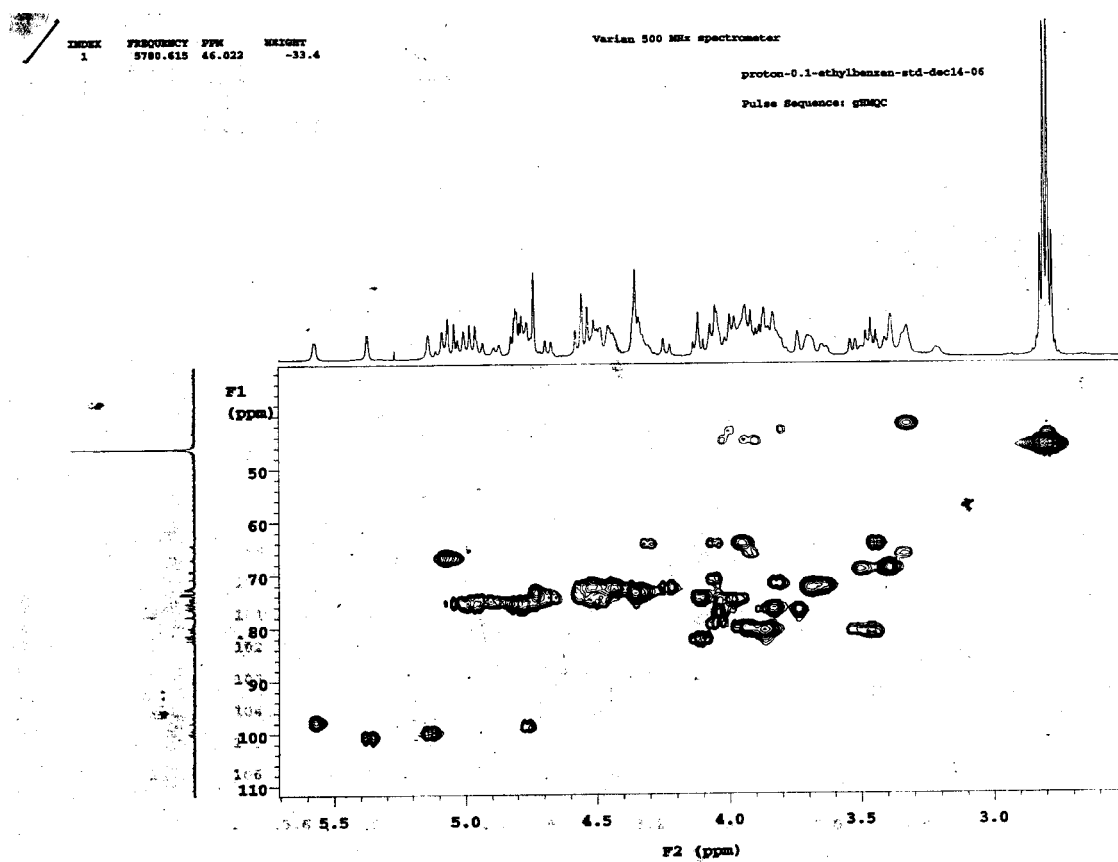


Figure SI-13. HMBC of **11** at 500 MHz in CDCl<sub>3</sub>.

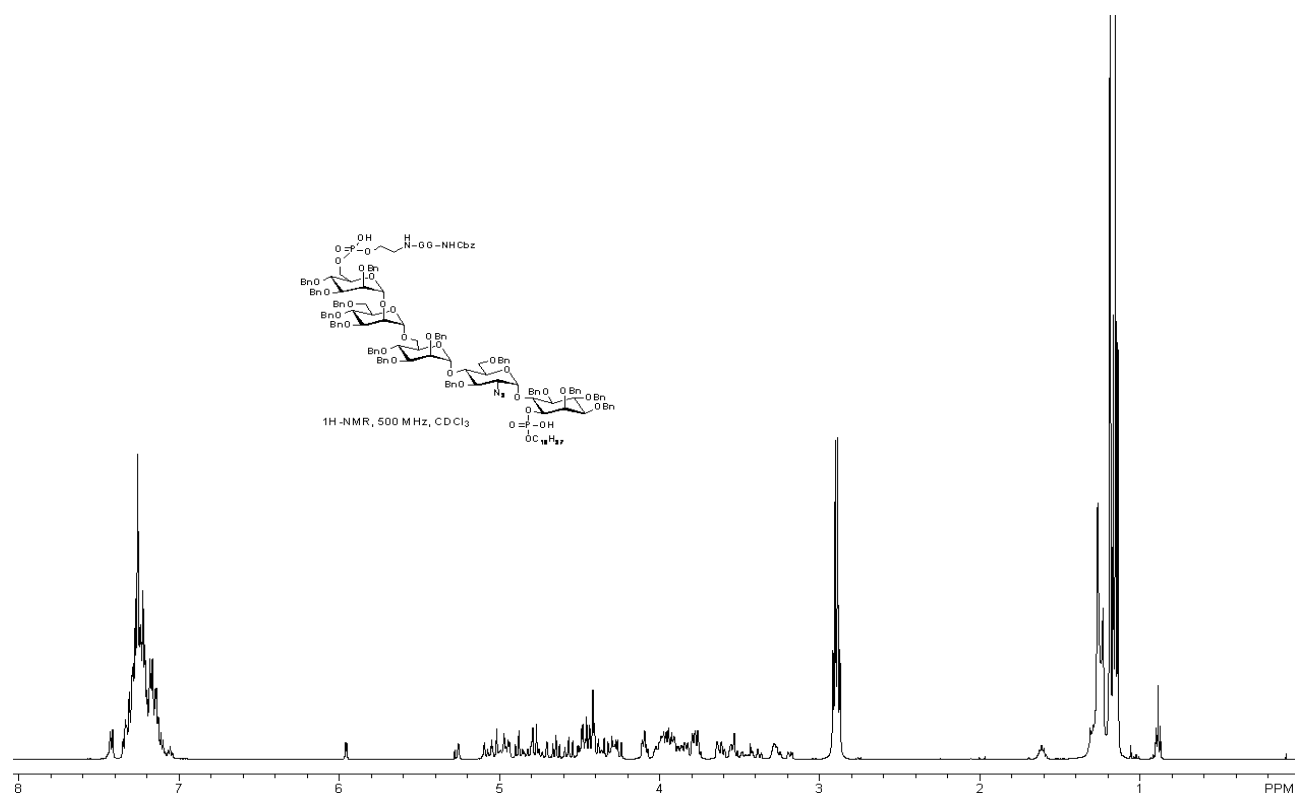


Figure SI-14. <sup>1</sup>H-NMR of **13** at 500 MHz in CDCl<sub>3</sub>.

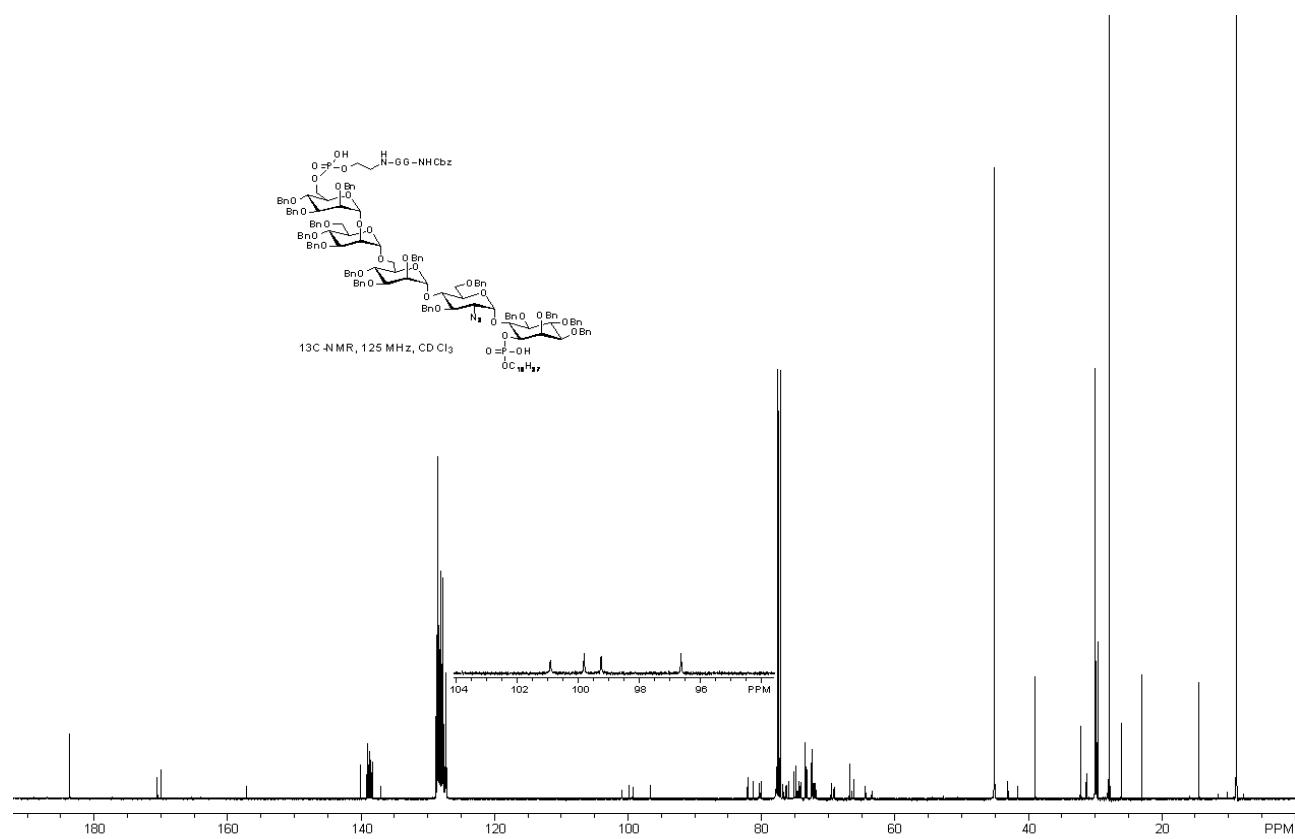


Figure SI-15. <sup>13</sup>C-NMR of **13** at 125 MHz in CDCl<sub>3</sub>.

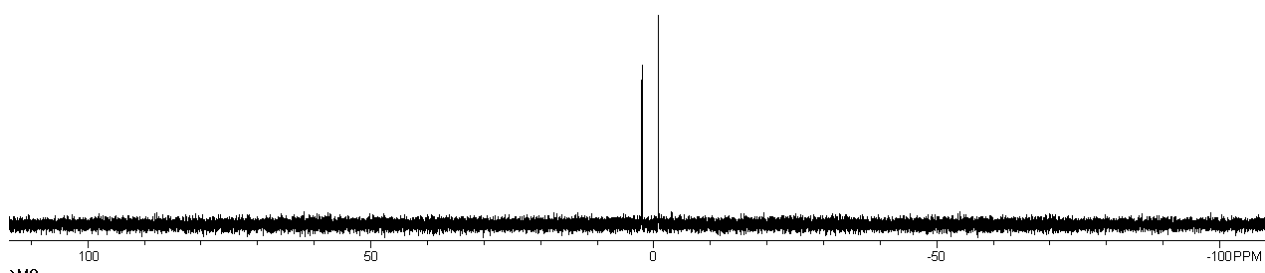
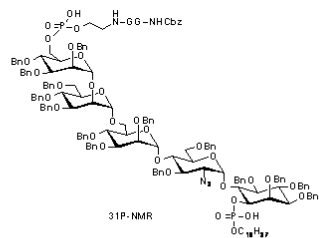


Figure SI-16.  $^{31}\text{P}$ -NMR of **13** at 161 MHz in  $\text{CDCl}_3$ .

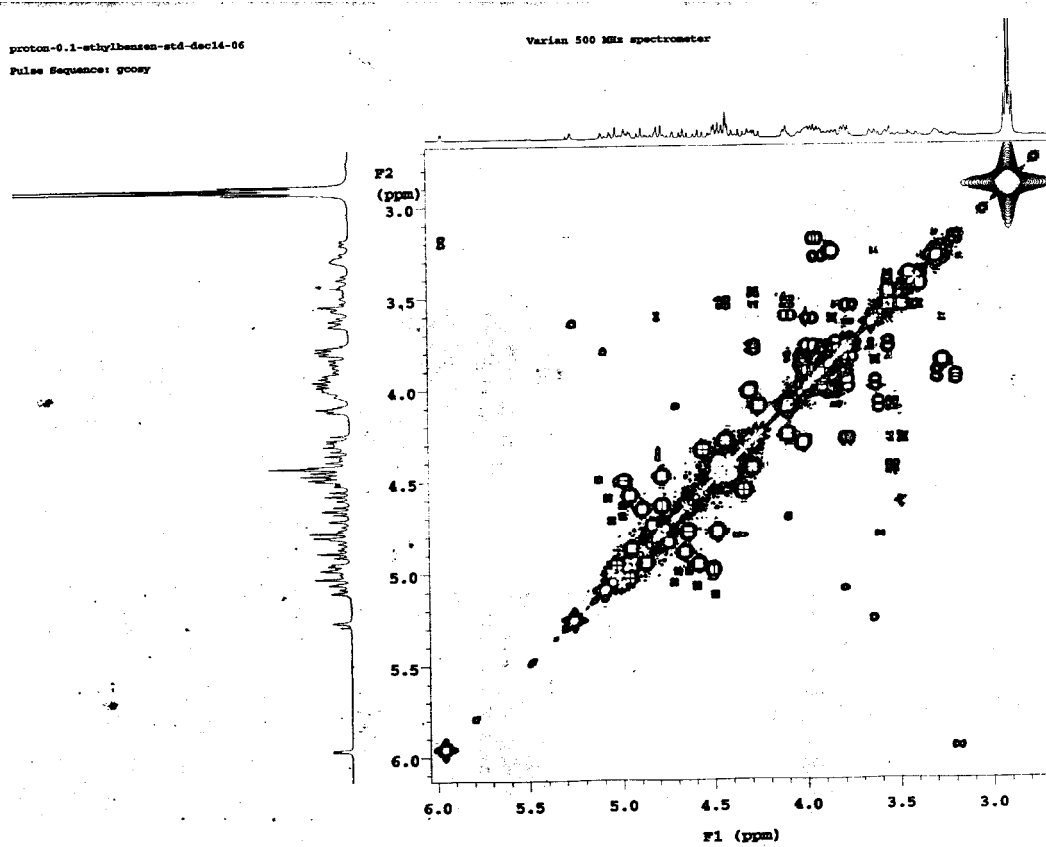


Figure SI-17. H,H-COSY of **13** at 500 MHz in  $\text{CDCl}_3$ .

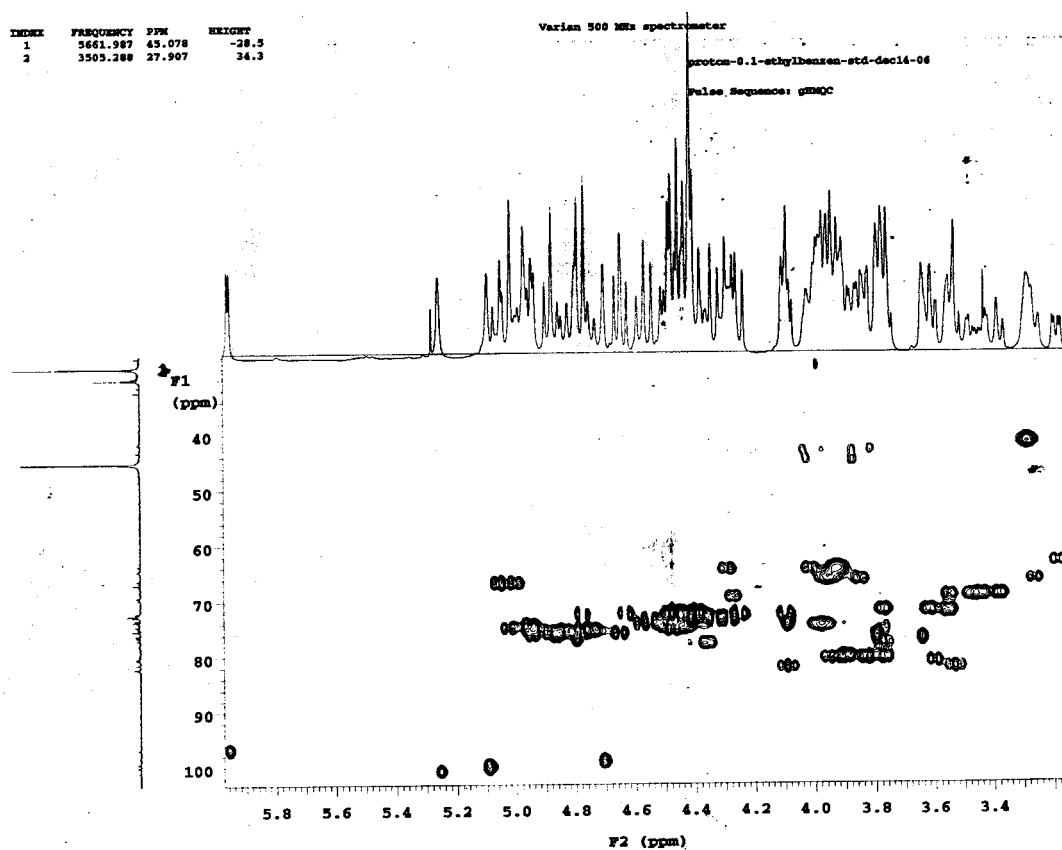


Figure SI-18. HMQC of **13** at 500 MHz in CDCl<sub>3</sub>.

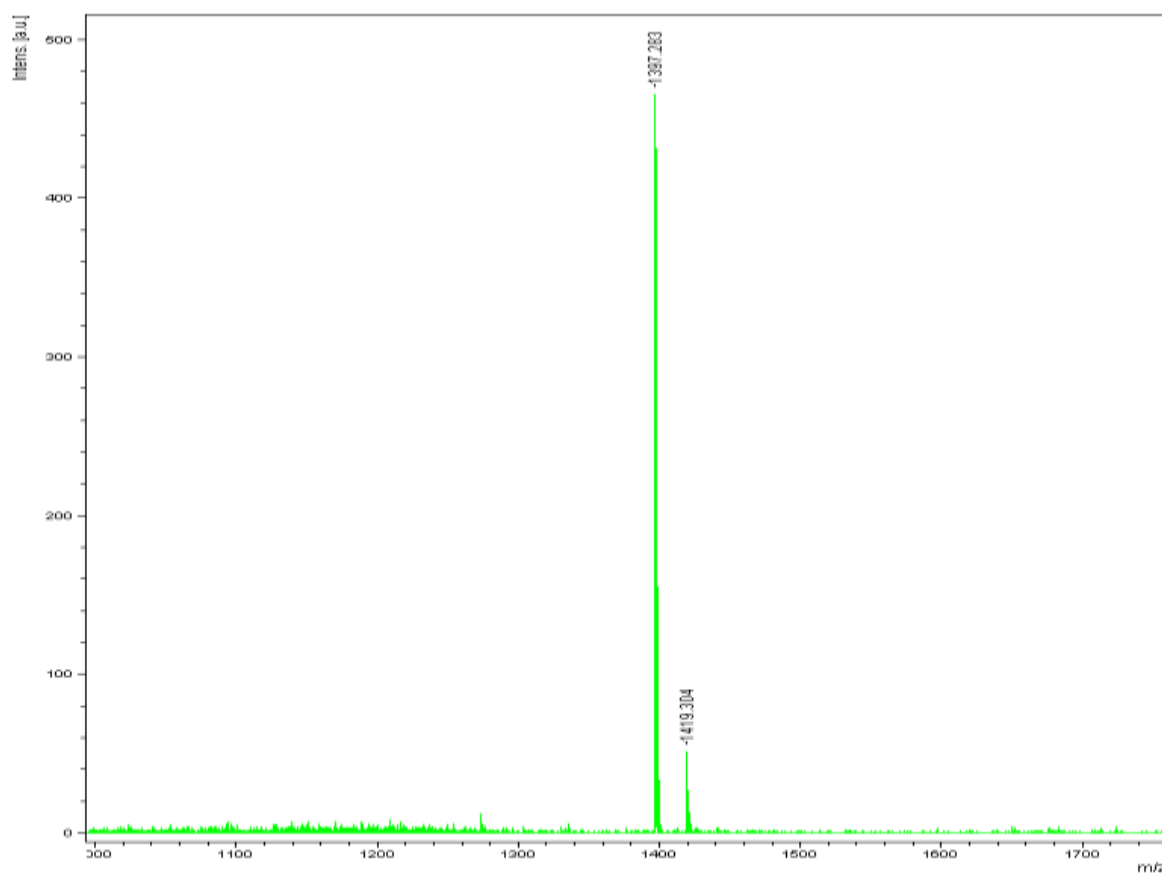


Figure SI-19. MALDI-TOF MS (positive mode) of GPI **1**, calc. for C<sub>54</sub>H<sub>102</sub>N<sub>4</sub>O<sub>33</sub>P<sub>2</sub>, 1396.6; found, 1397.2 [M + H]<sup>+</sup>