Supplementary Information

For

Synthesis of a Monophosphoryl Lipid A Derivative and Its Conjugation to a Modified Form of Tumor-Associated Carbohydrate Antigen GM3

Qianli Wang, Jie Xue and Zhongwu Guo*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA

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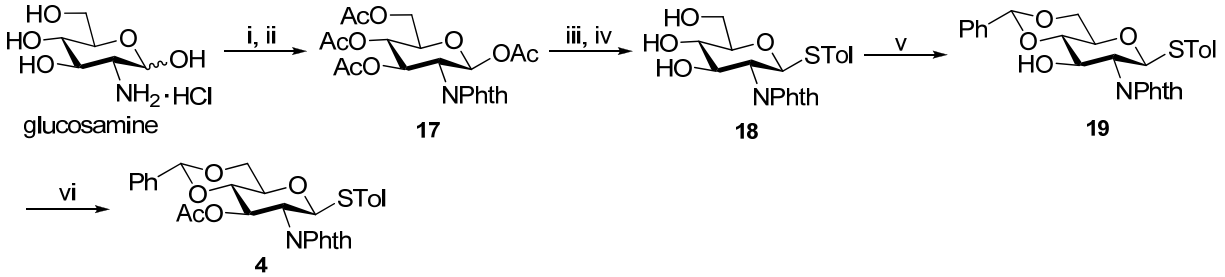
1. Experimental

General Methods. NMR spectra were recorded on a 400 or 500 MHz instrument with chemical shifts reported in ppm (δ) in reference to Me_4Si if not specified otherwise. Coupling constants (*J*) are reported in hertz (Hz). Optical rotations were determined using an Autopol III polarimeter. High resolution mass spectra (HRMS) were obtained with a Waters Micromass-LCTPremier-XE instrument, and MALDI-TOF MS were performed with a Bruker Ultraflex mass spectrometer. Thin layer chromatography (TLC) was performed on silica gel GF254 plates with detection by phosphomolybdic acid in EtOH or 1% H_2SO_4 in EtOH. Molecular sieves were dried under high vacuum at 170-180 ^oC for 6-10 h immediately before use. Commercial anhydrous solvents and other reagents were used without further purification.

Scheme S-1: Synthesis of Compound **4**.



Reagents and Conditions: i) Phthalic anhydride, NaOH, H_2O-MeOH; ii) Ac_2O, AcONa; iii) TolSH, $BF_3 \cdot Et_2O$, CH_2Cl_2; iv) MeONa, MeOH-CH_2Cl_2; v) $PhCH(OMe)_2$, TsOH, DMF; vi) Ac_2O, TEA, CH_2Cl_2

Compound 17. After the mixture of D-glucosamine hydrochloride (80.0 g, 0.37 mol), NaOH (15.6 g, 0.39 mol), H_2O (350 mL), and MeOH (150 mL) was stirred at rt for 1 h, phthalic anhydride (63.2 g, 0.43 mol) was added, and the reaction was stirred at rt overnight. The solid materials were filtered, washed with a small amount of H_2O, and then dried to produce a solid

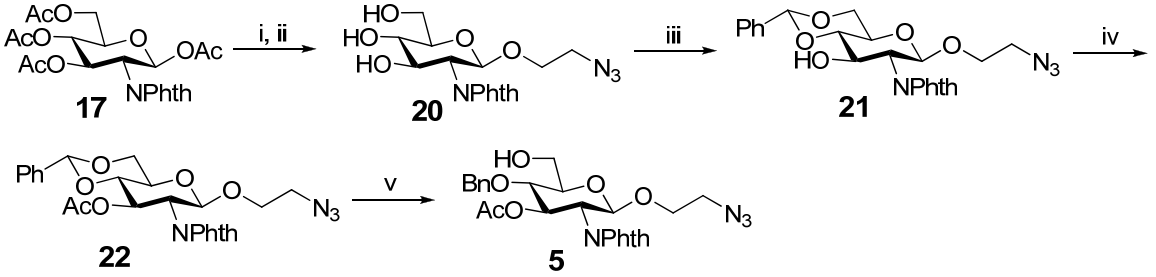
intermediate (77.0 g). The mixture of this intermediate (20.0 g, 0.061 mol) and AcONa (13.5 g, 0.164 mol) in Ac_2O (270 mL) was refluxed for 10 h. After removing most of Ac_2O in vacuum, the residue was poured into ice-water, and the mixture was extracted with DCM (300 mL). The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The residue was recrystallized from EtOAc and hexane to give **17** as a light yellow solid (14.9 g, 52%).

Compound 18. To the stirred solution of **17** (26.7 g, 0.056 mol) and *p*-toluenethiol (9.0 g, 0.073 mol) in anhydrous DCM (90 mL) at 0 °C, $BF_3{\cdot}Et_2O$ (10.6 mL, 0.084 mol) was added dropwise. When TLC showed the reaction was completed, the reaction mixture was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was then dissolved in DCM (50 mL) and treated with CH_3ONa/CH_3OH solution (20 mL, 0.4 M) at rt for 1.5 h. After most of the solvent was removed, the mixture was put in the refrigerator for 2 h, and the mixture was filtered to give **18** as a white solid (10.8 g, 66.7% for two steps). Ref. S. G. Hansen, T. Skrydstrup. *Eur. J. Org. Chem.*, 2007, 3392.

Compound 19. The solution of **18** (10.8 g, 26.0 mmol), benzaldehyde dimethyl acetal (5.9 mL, 39.0 mmol) and $TsOH{\cdot}H_2O$ (0.29 g, 1.3 mmol) in anhydrous DMF (50 mL) was stirred at 50 °C with occasional vacuum application until TLC showed the reaction was complete. The reaction was quenched with triethylamine, and the mixture was diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The residue was purified by flash column chromatography to give **19** as a white solid (11.4 g, 87.0%). Ref. Y. Niu, N. Wang, X. Cao, X. Ye. *Synlett*, 2007, 2116.

Compound 4. The mixture of **19** (13.0 g, 26.0 mmol), Ac_2O (3.7 mL, 39.0 mmol), triethylamine (7.9 mL, 78.0 mmol) and DMAP (12 mg, catalytic amount) in DCM (30 mL) was stirred at rt for 5 h. The reaction mixture was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was recrystallized from EtOAc and hexane to give **4** as a white solid (11.0 g, 78.0%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.86 (m, 2 H, aromatic H of Phth), 7.75 (m, 2 H, aromatic H of Phth), 7.43 (m, 2 H, aromatic H), 7.35 (m, 3 H, aromatic H), 7.27 (d, *J* = 8.0 Hz, 2 H, aromatic H), 7.07 (d, *J* = 8.0 Hz, 2 H, aromatic H), 5.88 (t, *J* = 9.6 Hz, 1 H, H-3), 5.76 (d, *J* = 10.8 Hz, 1 H, H-1), 5.52 (s, 1 H, PhCH), 4.42 (dd, *J* = 10.4 and 4.8 Hz, 1 H, H-6), 4.33 (t, *J* = 10.8 Hz, 1 H, H-2), 3.85-3.71 (m, 3 H, H-4, H-5, H-6'), 2.32 (s, 3 H, CH_3), 1.87 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.9, 134.4, 133.7, 129.7, 128.2, 127.2, 123.7, 101.7, 84.0, 79.0, 70.7, 68.8, 54.4, 21.2, 20.5.

Scheme S-2: Synthesis of Compound **5**.



Reagents and Conditions: i) 2-azidoethanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2; ii) MeONa, MeOH; iii) $PhCH(OMe)_2$, TsOH, DMF; iv) Ac_2O, TEA, DMAP, CH_2Cl_2; v) BH_3 / THF, TMSOTf

Compound 20. To the stirred mixture of **17** (13.5 g, 0.028 mol), 2-azidoethanol (10.0 g, 0.113 mol) and molecular sieve (4 Å, 3.5 g) in anhydrous DCM (40 mL) under argon, $BF_3 \cdot Et_2O$ (5.4 mL, 0.042 mol) was added dropwise. After the mixture was stirred at rt for 2 days, the reaction was quenched with saturated $NaHCO_3$ solution. The mixture was diluted with DCM and filtered through a Celite pad. The filtrate and washings were combined and washed with brine, dried over Na_2SO_4, and concentrated. The residue was then dissolved in MeOH, to which was added CH_3ONa/CH_3OH solution (0.4 M) until pH = 9. After at rt for 20 min, the reaction mixture was neutralized with Amberlite IR-120 (H^+) resin, concentrated in vacuum, and finally purified by flash column chromatography to give **20** as syrup (9.0 g, 83.9% for two steps). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.85 (m, 2 H, aromatic H), 7.80 (m, 2 H, aromatic H), 5.25 (d, *J* = 8.5 Hz, 1 H, H-1), 4.23 (dd, *J* = 11.0 and 8.5 Hz, 1 H, H-2), 4.04-3.98 (m, 2 H, H-3, H-6), 3.94 (dd, *J* = 10.0 and 4.0 Hz, 1 H, H-6'), 3.74 (m, 1 H, O$\underline{CH_2}CH_2N_3$), 3.65-3.60 (m, 1 H, H-5), 3.47-3.38 (m, 2 H, H-4, O$\underline{CH_2}CH_2N_3$), 3.21-3.17 (m, 1 H, $\underline{CH_2}N_3$). ^{13}C NMR (CD_3OD, 125 MHz): δ 134.2, 132.1, 98.6, 77.3, 71.5, 71.4, 68.4, 61.5, 57.3, 50.5, 8.5.

Compound 21. It was prepared from **20** (85.5%) following the same procedure described for **19**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2 H, aromatic H of Phth), 7.73 (m, 2 H, aromatic H of Phth), 7.46 (m, 2 H, aromatic H), 7.37 (m, 3 H, aromatic H), 5.55 (s, 1 H, Ph$\underline{CH}$), 5.32 (d, *J* = 8.5 Hz, 1 H, H-1), 4.61-4.56 (m, 1 H, H-3), 4.36 (dd, *J* = 10.4 and 4.0 Hz, 1 H, H-6), 4.24 (dd, *J* = 10.4 and 8.8 Hz, 1 H, H-6'), 3.98-3.93 (m, 1 H, O$\underline{CH_2}CH_2N_3$), 3.83-3.79 (m, 1 H, H-2), 3.65-3.57 (m, 3 H, H-4, H-5, O$\underline{CH_2}CH_2N_3$), 3.37-3.30 (m, 1 H, $\underline{CH_2}N_3$), 3.23 (d, 1 H, OH), 3.19-3.13 (m, 1 H, $\underline{CH_2}N_3$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.9, 137.3, 134.3, 132.0, 129.5, 128.6, 128.4, 126.6, 123.6, 102.1, 99.2, 82.3, 68.8, 68.7, 66.5, 56.8, 50.7, 36.7, 31.6. Ref. J. Xue, J. Zhu, R. E. Marchant, Z. Guo. *Org. Lett*., 2005, ***7***, 3753.

Compound 22. It was prepared from **21** (77.4%) following the same procedure described for **4**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85 (m, 2 H, aromatic H of Phth), 7.73 (m, 2 H, aromatic H of Phth), 7.46 (m, 2 H, aromatic H), 7.37 (m, 3 H, aromatic H), 5.85 (t, *J* = 9.2 Hz, 1 H, H-3), 5.55 (s, 1 H, Ph$\underline{CH}$), 5.53 (d, *J* = 8.4 Hz, 1 H, H-1), 4.41 (dd, *J* = 10.4 and 4.8 Hz, 1 H, H-6), 4.32 (dd, *J* = 10.4 and 8.0 Hz, 1 H, H-6'), 4.02-3.98 (m, 1 H, O$\underline{CH_2}CH_2N_3$), 3.88-3.74 (m, 3 H, H-2, H-4, O$\underline{CH_2}CH_2N_3$), 3.68-3.63 (m, 1 H, H-5), 3.41-3.34 (m, 1 H, $\underline{CH_2}N_3$), 3.19-3.13 (m, 1 H, $\underline{CH_2}N_3$), 1.89 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.5, 129.4, 128.5, 126.5, 123.8, 101.9, 99.0, 79.5, 69.9, 69.1, 68.9, 66.6, 55.5, 50.6, 20.8.

Compound 5. To a stirred solution of **22** (3.0 g, 6.0 mmol) in $BH_3 \cdot THF$ at 0 ^{o}C, TMSOTf (1.5 mL) was added dropwise. After the mixture was stirred at 0 ^{o}C for another hour, the reaction was quenched by trietylamine and MeOH. The solution was then concentrated and purified by flash column chromatography to give **5** as a white solid (2.0 g, 66.0%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.82 (m, 2 H, aromatic H of Phth), 7.70 (m, 2 H, aromatic H of Phth), 7.32 (m, 2 H, aromatic H), 7.26 (m, 3H, aromatic H), 5.77 (dd, *J* = 10.5 and 9.0 Hz, 1 H, H-3), 5.50 (d, *J* = 9.6 Hz, 1 H, H-1), 4.66 (d, *J* = 11.0 Hz, 2 H, Ph$\underline{CH_2}$), 4.20 (dd, *J* = 10.5 and 8.5 Hz, 1 H, H-2), 3.99-3.89 (m, 2 H, H-6, O$\underline{CH_2}CH_2N_3$), 3.82 (m, 2 H, H-5, H-6'), 3.67-3.62 (m, 2 H, H-4, O$\underline{CH_2}CH_2N_3$), 3.35-3.30 (m, 1 H, $\underline{CH_2}N_3$), 3.18-3.14 (m, 1 H, $\underline{CH_2}N_3$), 1.76 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.5, 134.4, 134.3, 128.8, 128.7, 128.2, 128.0, 123.7, 98.4, 79.2, 76.4, 75.7, 75.6, 75.0, 73.3, 72.0, 69.0, 61.9, 56.9, 56.9, 55.3, 50.6, 20.8.

Compound 6. After a mixture of **4** (1.6 g, 2.94 mmol), **5** (1.0 g, 1.96 mmol) and 4Å molecular sieves (4 g) was stirred at rt in anhydrous dichloromethane (DCM) for 2 h under an Argon atmosphere, it was cooled to -50 ^{o}C, and then NIS (1.34 g, 5.88 mmol) and AgOTf (0.05 g, 0.2 mmol) were added. The mixture was stirred at rt for 2 days and then quenched by the addition of triethylamine. The molecular sieves were filtered off with a Celite pad and washed with DCM. The filtrate and washings were combined and washed with saturated $Na_2S_2O_3$ solution and brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by flash column chromatography (toluene and AcOEt 10:1) to give **6** as a white solid (0.98 g, 54%). R_f = 0.40 (toluene and AcOEt 4:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.82-7.78 (m, 4 H), 7.70-7.66 (m, 4 H), 7.46 (m, 2 H), 7.37-7.35 (m, 3 H), 7.26-7.23 (m, 3 H), 7.06-7.03 (m, 2 H), 5.88 (t, *J* = 8.8 Hz, 1 H, H-3'), 5.65 (dd, *J* = 10.4, 8.8 Hz, 1 H, H-3), 5.58 (d, *J* = 8.8 Hz, 1 H, H-1'), 5.56 (s, 1 H, PhCH), 5.35 (d, *J* = 8.8 Hz, 1 H, H-1), 4.46-4.36 (m, 4 H, H-6', H-2', PhCH$_2$), 4.14-4.08 (m, 2 H, H-6, H-2), 3.88 (d, *J* = 10.4 Hz, 1 H, H-4'), 3.84-3.73 (m, 4 H, H-6, H-6', H-5', OCH$_2$CH$_2$N$_3$), 3.67-3.62 (m, 1 H, H-5), 3.58-3.51 (m, 2 H, H-4, OCH$_2$CH$_2$N$_3$), 3.31-3.24 (m, 1 H, CH$_2$N$_3$), 3.08-3.03 (m, 1 H, CH$_2$N$_3$), 1.90 (s, 3 H, OAc), 1.71 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.5, 170.2, 137.6, 137.1, 134.5, 134.3, 129.4, 128.6, 128.5, 128.1, 127.7, 126.5, 123.8, 123.6, 101.9, 98.7, 98.1, 79.4, 77.6, 74.8, 73.2, 70.0, 68.9, 68.8, 68.2, 66.6, 55.5, 55.1, 50.1, 20.8, 20.7. HR ESI MS (*m*/*z*) calcd. for $C_{48}H_{45}N_5O_{15}Na$ $(M + Na)^+$ 954.2810, found 954.2813.

Compound 7. After a mixture of **6** (0.58 g, 0.63 mmol) and hydrazine monohydrate (5.5 mL) in ethanol (30 mL) was refluxed for 2 h, it was concentrated in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2 and MeOH 15:1 to 10:1) to afford **7** as a white solid (0.27 g, 73%). R_f = 0.65 (CH_2Cl_2 and MeOH 7:1). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.50-7.47 (m, 2 H), 7.40-7.28 (m, 8 H), 5.57 (s, 1 H, PhCH), 4.95 (d, *J* = 11.2 Hz, 1 H, PhCH$_2$), 4.64 (d, *J* = 10.4 Hz, 1 H, PhCH$_2$), 4.37 (d, *J* = 8.4 Hz, 1 H, H-1'), 4.27 (d, *J* = 8.0 Hz, 1 H, H-1), 4.24 (dd, *J* = 10.4, 4.8 Hz, 1 H, H-6'), 4.09 (dd, *J* = 11.2 and 2.4 Hz, 1 H, H-6), 4.06-4.01 (m, 1 H, OCH$_2$CH$_2$N$_3$), 3.79-3.68 (m, 3 H, H-6, H-6', OCH$_2$CH$_2$N$_3$), 3.55-3.34 (m, 8 H, H-3, H-3', H-4, H-4', H-5, H-5', CH$_2$N$_3$), 2.73 (t, *J* = 8.8 Hz, 1 H, H-2'), 2.65 (dd, *J* = 10.4, 8.0 Hz, 1 H, H-2). ^{13}C NMR (CD_3OD, 100 MHz): δ 138.0, 128.7, 128.2, 127.9, 127.8, 127.6, 126.3, 104.4, 103.4, 101.9, 81.6, 78.7, 76.6, 74.8, 74.6, 72.9, 68.9, 68.7, 68.5, 66.9, 57.9, 57.4, 50.8. HR ESI MS (*m*/*z*): calcd for $C_{28}H_{38}N_5O_9$ $(M + H)^+$, 588.2670; found, 588.2647.

Compound 9. After the solution of EDC·HCl (391 mg, 2.0 mmol) and **8** (260 mg, 0.6 mmol) in anhydrous DCM (8 mL) and DMF (0.5 mL) was stirred at rt for 0.5 h, it was cooled to 0 ^{o}C, and then a solution of **7** (120 mg, 0.2 mmol) in DMF (1.5 mL) was added. The mixture was stirred at rt overnight and then diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2 and MeOH 40:1) to give **9** as a white solid (230 mg, 80%). R_f = 0.40 (CH_2Cl_2 and MeOH 20:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.50-7.47 (m, 2 H), 7.37-7.29 (m, 8 H), 6.19 (d, *J* = 4.8 Hz, 1 H, NH'), 5.99 (d, *J* = 6.8 Hz, 1 H, NH), 5.53 (s, 1 H, PhCH), 5.19-5.13 (m, 1 H, lipid), 5.11-5.05 (m, 1H, lipid), 4.98 (d, *J* = 11.2 Hz, 1 H, PhCH$_2$), 4.81 (d, *J* = 8.8 Hz, 1 H, H-1), 4.64 (d, *J* = 11.2 Hz, 1 H, PhCH$_2$), 4.62 (d, *J* = 3.2 Hz, 1 H, 3-OH), 4.58 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.30 (dd, *J* = 10.8 and 4.0 Hz, 1 H, H-6), 4.17 (dt, = *J* 9.2 and 3.2 Hz, 1 H, H-3'), 4.09 (dd, *J* = 11.2 and 2.4 Hz, 1 H, H-6'), 4.06-4.01 (m, 1 H, OCH$_2$CH$_2$N$_3$), 3.99 (d, *J* = 3.2 Hz, 1 H, 3'-OH), 3.93 (dt, *J* = 9.6 and 3.2 Hz, 1 H, H-3), 3.77-3.70 (m, 3 H, H-6, H-6', OCH$_2$CH$_2$N$_3$), 3.56-3.31 (m, 8 H, H-2, H-2', H-4, H-4', H-5, H-5', OCH$_2$CH$_2$N$_3$), 2.48 (d, *J* = 6.4 Hz, 2 H, lipid), 2.37-2.26 (m, 6 H, lipid),

1.66-1.50 (m, 8 H, lipid), 1.25 (br, 68 H, 34 x CH_2, lipid), 0.87 (t, *J* 6.4 Hz, 12 H, 4 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.6, 174.5, 172.1, 171.5, 138.5, 137.3, 129.3, 128.7, 128.5, 128.3, 128.1, 126.6, 102.1, 100.8, 100.3, 81.7, 78.5, 76.2, 74.9, 74.7, 71.7, 71.5, 71.1, 68.8, 68.2, 66.5, 59.1, 58.3, 50.9, 42.6, 36.7, 34.8, 32.1, 31.7, 29.9, 29.7, 29.6, 29.4, 25.5, 25.2, 22.9, 14.3. HR ESI MS (*m*/*z*): calcd for $C_{80}H_{133}N_5NaO_{15}$ $(M + Na)^+$, 1426.9696; found, 1426.9696.

Compound 10. After the solution of EDC·HCl (205 mg, 1.07 mmol) and lauric acid (142 mg, 0.712 mmol) in anhydrous DCM (5 mL) was stirred at rt for 20 min, a solution of **9** (100 mg, 0.07 mmol) and N,N-dimethylaminopyridine (DMAP, 8.7 mg, 0.07 mmol) in DCM (5 mL) was added. The mixture was stirred at 45 °C overnight, and it was then diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2 and acetone 30:1 to 20:1) to give **10** as a white solid (110 mg, 87.3%). R_f = 0.40 ($CH_2Cl_2$and acetone 20:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.43-7.40 (m, 2 H), 7.35-7.21 (m, 8 H), 5.97 (d, *J* = 7.2 Hz, 2 H, NH, NH'), 5.48 (s, 1 H, Ph<u>CH</u>), 5.24 (t, *J* = 10.0 Hz, 1 H, H-3'), 5.17-5.00 (m, 3 H, H-3, 2 H lipid), 4.70 (d, *J* = 8.4 Hz, 1 H, H-1), 4.61-4.52 (m, 3 H, H-1', Ph<u>CH_2</u>), 4.32 (dd, *J* = 10.4, 4.8 Hz, 1 H, H-6'), 4.06-3.90 (m, 4 H, H-2, H-2', H-6, O<u>CH_2</u>CH_2N_3), 3.79-3.64 (m, 4 H, H-4', H-6, H-6', O<u>CH_2</u>CH_2N_3), 3.61-3.42 (m, 4 H, H-4, H-5, H-5', <u>CH_2N_3</u>), 3.37-3.31 (m, 1 H, <u>CH_2N_3</u>), 2.47-2.40 (m, 2 H, lipid), 2.34-2.15 (m, 6 H, lipid), 1.64-1.48 (m, 12 H, lipid), 1.24 (br, 104 H, 52 x CH_2, lipid), 0.87 (t, *J* = 6.4 Hz, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.0, 173.9, 173.8, 169.9, 169.7, 137.8, 137.1, 129.3, 128.7, 128.4, 128.2, 127.9, 126.3, 102.2, 101.5, 100.7, 79.0, 76.4, 75.3, 74.6, 71.5, 71.3, 71.2, 68.8, 68.1, 67.1, 66.7, 54.7, 53.6, 51.0, 42.1, 41.8, 34.8, 34.7, 34.5, 34.3, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 25.5, 25.3, 25.0, 22.9, 14.3. HR ESI MS (*m*/*z*): calcd for $C_{104}H_{177}N_5NaO_{17}$ $(M + Na)^+$, 1791.3037; found, 1791.3024.

Compound 11. After the mixture of **10** (85 mg, 48 μmol), $NaBH_3CN$ (45 mg, 0.72 mmol) and 4Å molecular sieves (1 g) in dry THF (10 mL) was stirred at rt for 2 h, it was cooled to 0 °C, and then HCl (1 M in dry ether) was added dropwise until the pH = 2. After the reaction mixture was stirred at 0 °C for 1 h and at rt for 3 h, triethylamine (0.5 mL) was added to terminate the reaction. The molecular sieves were filtered off through a Celite pat and washed with DCM. The filtrate and washings were combined and washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and finally evaporated in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2 and acetone 15:1) to give **11** as a white solid (60 mg, 70.6%). R_f = 0.25 (CH_2Cl_2 and acetone 15:1). $[\alpha]^{24}_D$ -15.0 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.36-7.19 (m, 10 H), 5.96-5.90 (m, 2 H, NH', NH), 5.15-4.98 (m, 4 H, H-3', H-3, and 2 H of lipid), 4.64 (d, *J* = 8.8 Hz, 1 H, H-1), 4.60-4.50 (m, 5 H, H-1', 2 x Ph<u>CH_2</u>), 4.04-3.85 (m, 4 H, H-2', H-2, H-6', O<u>CH_2</u>CH_2N_3), 3.79-3.63 (m, 5 H, H-4, 2 x H-6, H-6', O<u>CH_2</u>CH_2N_3), 3.60-3.48 (m, 3 H, H-4', H-5', H-5), 3.46-3.38 (m, 1 H, <u>CH_2N_3</u>), 3.33-3.26 (m, 1 H, <u>CH_2N_3</u>), 2.46-2.12 (m, 8 H, lipid), 1.66-1.46 (m, 12 H, lipid), 1.25 (br, 104 H, 52 x CH_2, lipid), 0.87 (t, *J* = 6.4 Hz, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.8, 174.0, 173.9, 169.9, 169.8, 137.8, 128.7, 128.6, 128.1, 128.0, 127.9, 101.4, 100.8, 76.5, 75.2, 74.7, 74.2, 74.0, 71.3, 71.2, 70.7, 67.9, 67.3, 54.0, 53.7, 50.9, 42.0, 41.8, 34.7, 34.4, 34.3, 32.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.5, 25.3, 25.2, 25.0, 22.9, 14.3. MALDI-TOF MS (*m*/*z*): calcd for $C_{104}H_{179}N_5O_{17}$, 1770.3; found, 1793.3 $(M + Na)^+$.

Compound 3. To the stirred solution of **11** (38 mg, 21 μmol) in dry DCM (3 mL), dibenzyl diisopropylphosphoramidite **12** (21 μL, 64 μmol) and 1*H*-tetrazole (~0.45 M in CH_3CN, 0.24 mL,

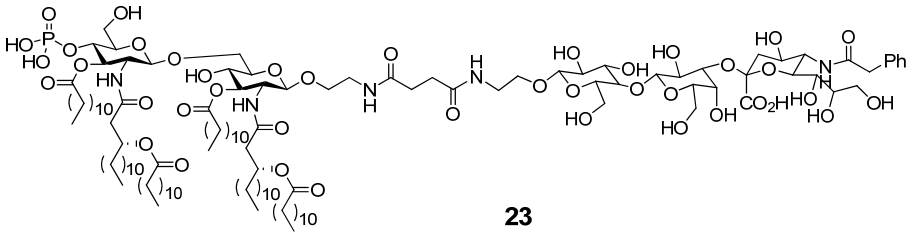
0.107 mmol) were added. After the mixture was stirred at rt for 2 h and then cooled to -20 °C, t-BuOOH (~5.5 M in CH_3CN, 39 μL, 0.214 mmol) was added, and the mixture was stirred at rt for another 2 h. The solvent was removed in vacuo, and the residue was purified by flash column chromatography (CH_2Cl_2 and MeOH 60:1) to give **3** as syrup (36.5 mg, 84%). R_f = 0.45 (CH_2Cl_2 and MeOH 40:1). $[\alpha]^{24}_D$ -9.0 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33-7.19 (m, 20 H), 5.87 (d, *J* = 8.8 Hz, 1 H, NH'), 5.80 (d, *J* = 8.8 Hz, 1 H, NH), 5.31 (dd, *J* = 10.4 and 8.8 Hz, 1 H, H-3'), 5.13-5.04 (m, 2 H, H-3 and 1 H of lipid), 5.02-4.95 (m, 1 H, lipid), 4.91-4.85 (m, 4 H, $(PhCH_2O)_2P$), 4.77 (d, *J* = 8.4 Hz, 1 H, H-1'), 4.54 (s, 2 H, $PhCH_2$), 4.51 (d, *J* = 8.0 Hz, 1 H, H-1), 4.48-4.41 (m, 3 H, H-4', $PhCH_2$), 4.05-3.88 (m, 3 H, H-2, H-6, $OCH_2CH_2N_3$), 3.84-3.68 (m, 3 H, H-2', H-6', H-6), 3.67-3.47 (m, 5 H, $OCH_2CH_2N_3$, H-6', H-5', H-5, H-4), 3.46-3.39 (m, 1 H, CH_2N_3), 3.32-3.25 (m, 1 H, CH_2N_3), 2.45-2.35 (m, 2 H, lipid), 2.32-2.12 (m, 8 H, lipid), 1.64-1.36 (m, 10 H, lipid), 1.34-1.08 (br, 104 H, 52 x CH_2, lipid), 0.87 (t, *J* = 6.4 Hz, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 128.8, 128.7, 128.6, 128.1, 128.0, 127.8, 101.0, 100.8, 76.5, 75.2, 74.7, 74.6, 74.5, 74.3, 73.6, 72.6, 71.3, 71.1, 70.7, 69.8, 69.7, 68.9, 68.3, 67.3, 54.8, 53.7, 50.9, 42.0, 41.9, 34.7, 34.4, 34.1, 32.1, 29.9, 29.8, 29.7, 29.6, 29.4, 25.6, 25.2, 25.0, 24.8, 22.9, 14.4. ^{31}P NMR ($CDCl_3$, 161 MHz): δ -1.11. HR ESI MS (*m*/*z*): calcd for $C_{118}H_{192}N_5NaO_{20}P$ $(M + Na)^+$, 2053.3796; found, 2053.3835.

Compound 13. A suspension of **3** (25 mg, 12 μmol), active zinc dust (25.0 mg, 0.38 mmol), and acetic acid (7 μL, 0.12 mmol) in DCM (2 mL) was stirred at rt for 24 h, and then solid materials were removed by filtration and washed with DCM. The combined filtrates were neutralized with DIPEA, washed with brine, dried over anhydrous Na_2SO_4, and then concentrated in vacuo. The product [HR ESI MS (*m*/*z*): calcd for $C_{118}H_{195}N_3O_{20}P$ $(M + H)^+$, 2005.4072, found, 2005.4142] was used for the next step of reaction without further purification. The solution of the obtained crude amine, succinic anhydride (5 mg, 49 μmol), DIPEA (20 μL, 0.12 mmol) and a catalytic amount of DMAP in DCM (2 mL) and DMF (0.5 mL) was stirred at rt overnight. The mixture was concentrated in vacuo and co-evaporated with toluene a couple of times, and the residue was purified by flash column chromatography (CH_2Cl_2 and MeOH 30:1) to give **13** as a white solid (18 mg, 70%). R_f = 0.3 (CH_2Cl_2 and MeOH 20:1). $[\alpha]^{24}_D$ -12.0 (*c* 0.25, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34-7.16 (m, 20 H), 6.61 (m, 1 H, OCH_2CH_2NH), 6.34 (d, *J* = 8.8 Hz, 1 H, NH), 5.94 (d, *J* = 8.8 Hz, 1 H, NH), 5.43 (t, *J* = 9.6 Hz, 1 H, H-3'), 5.10-4.97 (m, 3 H, H-3 and 2 H of lipid), 4.93-4.83 (m, 4 H, $(PhCH_2O)_2P$), 4.78 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.57-4.43 (m, 5 H, H-4', 2 x $PhCH_2$), 4.34 (d, *J* = 8.0 Hz, 1 H, H-1), 4.06-3.95 (m, 2 H, H-2, H-6), 3.84-3.76 (m, 2 H, H-2', H-6'), 3.74-3.61 (m, 5 H, OCH_2CH_2NH, H-5', H-6, H-6'), 3.60-3.45 (m, 3 H, OCH_2CH_2NH, H-5, H-4), 3.28-3.20 (m, 1 H, OCH_2CH_2NH), 2.82-2.72 (m, 1 H, CH_2CH_2COOH), 2.64-2.46 (m, 3 H, CH_2CH_2COOH), 2.46-2.38 (m, 2 H, lipid), 2.34-2.10 (m, 8 H, lipid), 1.64-1.35 (m, 10 H, lipid), 1.34-1.02 (br, 104 H, 52 x CH_2, lipid), 0.87 (t, *J* = 5.4 Hz, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.3, 174.3, 174.2, 173.9, 170.3, 170.3, 138.3, 137.8, 135.7, 128.8, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 127.8, 101.4, 100.9, 75.0, 74.8, 74.7, 74.3, 73.6, 72.7, 71.5, 69.9, 69.8, 68.9, 68.3, 54.8, 54.0, 42.0, 41.6, 40.2, 34.8, 34.5, 34.3, 34.2, 32.2, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.6, 25.5, 25.3, 25.0, 24.8, 22.9, 14.4. ^{31}P NMR ($CDCl_3$, 161 MHz): δ -1.36. HR ESI MS (*m*/*z*): calcd for $C_{122}H_{198}N_3NaO_{23}P$ $(M + Na)^+$, 2127.4051;, found, 2127.4089.

Compound 14. To a stirred solution of **13** (18 mg, 8 μmol) and *p*-nitrophenol (5.9 mg, 42 μmol) in DCM (5 mL) was added EDC·HCl (8.2 mg, 42 μmol) in an ice bath. After the mixture was stirred at rt for 5 h, it was diluted with DCM, washed with brine, dried over anhydrous Na_2SO_4,

and condensed in vacuo. The residue was purified on a TLC plate (CH_2Cl_2 and MeOH 20:1) to give **14** as a white solid (16 mg, 83.5%). R_f = 0.55 (CH_2Cl_2 and MeOH 20:1). $[\alpha]^{24}_D$ -10.0 (*c* 0.65, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.24-8.19 (m, 2 H), 7.34-7.16 (m, 22 H), 6.73 (m, 1 H, $OCH_2CH_2\underline{NH}$), 6.09 (d, *J* 8.8 Hz, 1 H, NH), 5.78 (d, *J* = 9.2 Hz, 1 H, NH), 5.32 (t, *J* = 8.8 Hz, 1 H, H-3'), 5.08-5.03 (m, 1 H, lipid), 5.00-4.94 (m, 2 H, H-3 and 1 H of lipid), 4.92-4.83 (m, 4 H, $(Ph\underline{CH_2}O)_2P$), 4.71 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.57-4.49 (m, 2 H, $Ph\underline{CH_2}$), 4.48-4.40 (m, 3 H, H-4', $Ph\underline{CH_2}$), 4.31 (d, *J* = 8.0 Hz, 1 H, H-1), 4.06-3.88 (m, 2 H, H-6, H-2), 3.83-3.71 (m, 3 H, H-2', H-6', $O\underline{CH_2}CH_2NH$), 3.69-3.58 (m, 4 H, $O\underline{CH_2}CH_2NH$, H-5', H-6, H-6'), 3.55-3.34 (m, 4 H, H-5, H-4, $OCH_2\underline{CH_2}NH$), 2.92 (t, *J* = 7.6 Hz, 2 H, $\underline{CH_2}COOPhNO_2$), 2.62 (t, *J* = 7.6 Hz, 2 H, $\underline{CH_2}CH_2COOPhNO_2$), 2.44-2.35 (m, 2 H, lipid), 2.30-2.12 (m, 8 H, lipid), 1.70-1.36 (m, 10 H, lipid), 1.34-1.02 (br, 104 H, 52 x CH_2, lipid), 0.87 (t, *J* = 6.4 Hz, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.2, 174.1, 173.9, 173.8, 171.7, 171.4, 170.4, 170.2, 155.7, 145.5, 138.2, 137.7, 135.6, 135.5, 128.9, 128.8, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 125.4, 122.7, 101.5, 101.0, 76.4, 74.8, 74.5, 74.4, 73.6, 72.5, 71.3, 71.2, 70.0, 69.9, 69.8, 68.9, 68.5, 68.0, 54.8, 54.3, 42.2, 41.9, 40.2, 34.8, 34.5, 34.4, 34.2, 32.2, 30.5, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 25.6, 25.3, 25.0, 24.8, 22.9, 14.4. ^{31}P NMR ($CDCl_3$, 161 MHz): δ -1.04. HR ESI MS (*m*/*z*): calcd for $C_{128}H_{201}N_4NaO_{25}P$ $(M + Na)^+$, 2248.4215; found, 2248.4226.

Compound 16. To a stirred solution of **14** (12 mg, 5 μmol) and **15** (6 mg, 8 μmol) in DMF (1.5 mL), *N*-methylmorpholine (NMM, 6 μL, 54 μmol) was added at 0 °C. The reaction mixture was stirred at rt overnight, and then DMF was removed in vacuo. The residue was purified on a TLC plate (CH_2Cl_2 and MeOH 3:1) to give **16** as a white solid (10 mg, 65%). R_f = 0.2 (CH_2Cl_2 and MeOH 3:1). $[\alpha]^{24}_D$ -4.5 (*c* 0.38, $CHCl_3$ and MeOH 4:1). ^{1}H NMR ($CDCl_3$ and CD_3OD 6:1, 500 MHz): δ 7.24-7.09 (m, 25 H), 5.30 (t, *J* = 10.0 Hz, 1 H, H-3'), 5.02-4.91 (m, 3 H, H-3, and 2 H of lipid), 4.81-4.73 (m, 4 H, $(Ph\underline{CH_2}O)_2P$), 4.68 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.47-4.40 (m, 2 H, $Ph\underline{CH_2}$), 4.39-4.33 (m, 3 H, H-4', $Ph\underline{CH_2}$), 4.32 (d, *J* = 8.0 Hz, 1 H, H-1''), 4.28 (d, *J* = 9.0 Hz, 1 H, H-1), 4.17 (d, *J* = 7.5 Hz, 1 H, H-1'''), 3.99-3.94 (m, 2 H, H-6), 3.94-3.40 (m, 31 H), 3.38-3.16 (m, 5 H), 2.40-2.32 (m, 4H), 2.29 (dd, *J* = 15.0 and 6.0 Hz, 1 H, H-3''e of GM_3), 2.25-2.00 (m, 11 H, lipid and H-3''a of GM_3), 1.67-1.34 (m, 10 H, lipid), 1.30-0.98 (br, 104 H, 52 x CH_2, lipid), 0.84-0.70 (m, 18 H, 6 x CH_3, lipid). ^{13}C NMR ($CDCl_3$ and CD_3OD 6:1, 125 MHz): δ 129.0, 128.8, 128.6, 128.5, 128.4, 128.0, 127.8, 127.7, 127.6, 127.1, 103.9, 102.9, 101.2, 100.5, 80.0, 77.5, 76.5, 76.1, 75.4, 74.9, 74.6, 74.2, 73.9, 73.7, 73.4, 73.3, 72.4, 72.3, 71.5, 71.2, 70.9, 69.9, 69.8, 69.2, 68.8, 68.5, 68.1, 67.5, 63.4, 61.7, 60.9, 54.4, 53.7, 52.7, 49.4, 49.2, 49.1, 48.9, 42.8, 41.1, 40.9, 39.6, 39.4, 34.5, 34.2, 34.0, 31.9, 31.0, 30.9, 29.7, 29.5, 29.4, 29.3, 25.4, 25.1, 24.8, 24.6, 22.7, 14.0. ^{31}P NMR ($CDCl_3$ and CD_3OD 6:1, 161 MHz): δ -1.52. HR ESI MS (*m*/*z*): calcd for $C_{153}H_{244}N_5Na_2O_{41}P$ $(M + 2Na)^{2+}$, 1442.3342; found, 1442.3287.

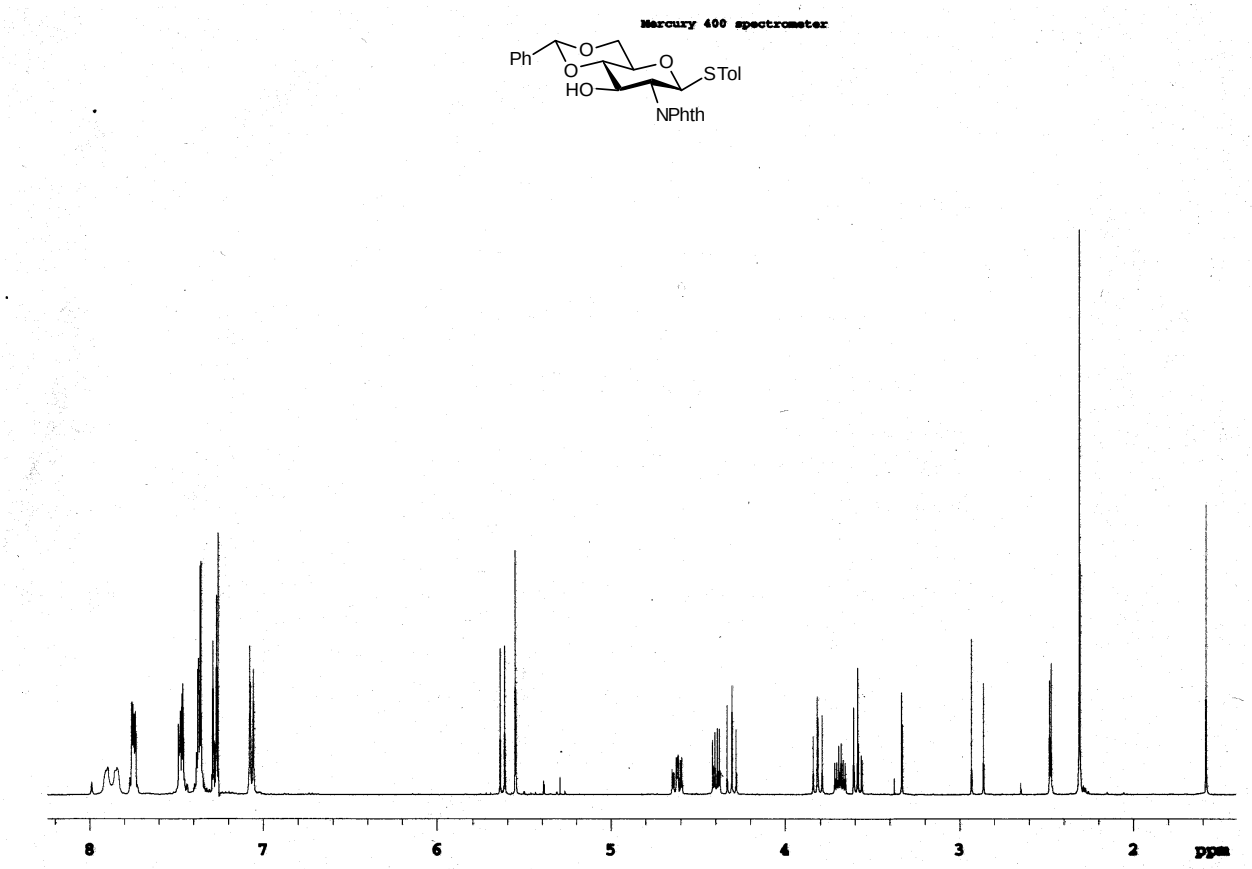


23

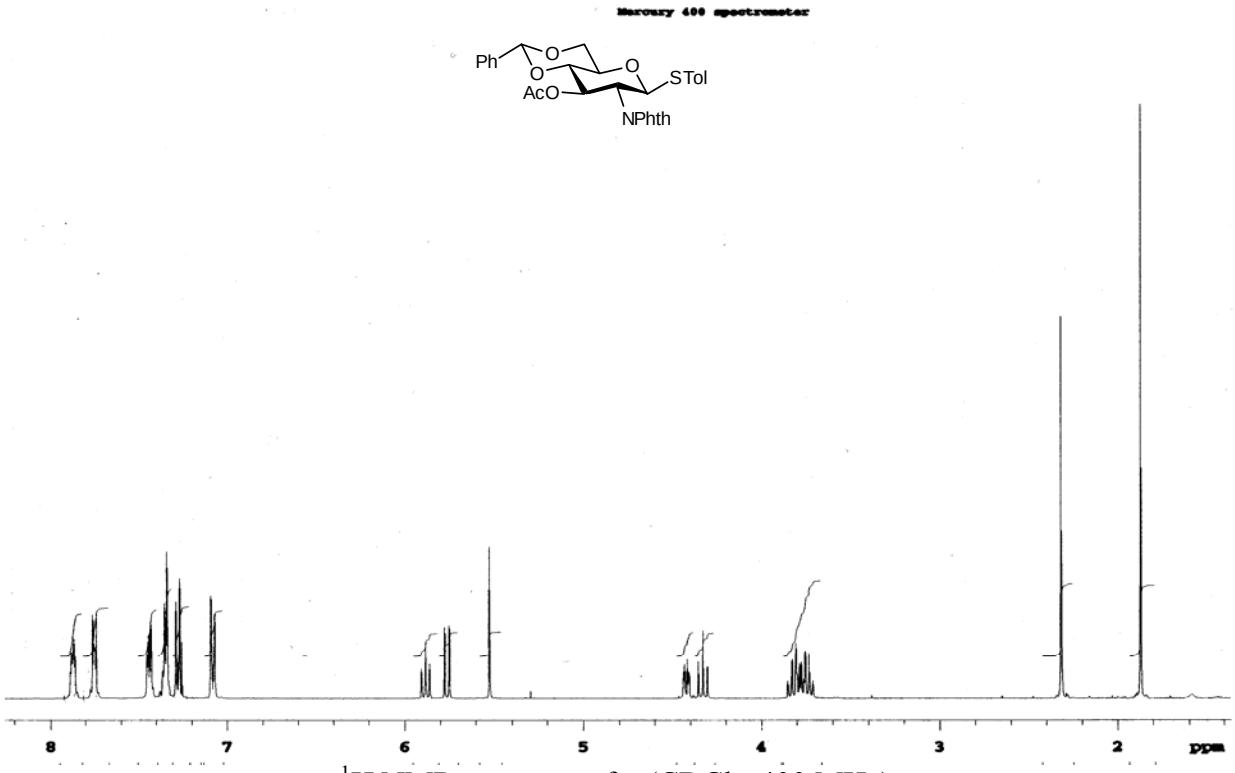
Compound 23. A mixture of **16** (7.5 mg, 2.64 μmol) and 10% Pd-C (5.0 mg) in DCM-MeOH (1:1, 4 mL) was stirred under an atmosphere of H_2 at rt for 1 day. Thereafter, the catalyst was

removed by filtration through a Celite pad, and the Celite pad was subsequently washed with DCM-MeOH (1:1) and MeOH. The combined filtrates were concentrated in vacuum, and the residue was purified by a short silica gel column (eluent: DCM/MeOH 1:3) to give **23** as a white solid (4.0 mg, 61.5%). R_f = 0.25 (CH_2Cl_2/MeOH 1:3). ^{1}H NMR ($CDCl_3$-CD_3OD, 500 MHz): δ ^{31}P-NMR($CDCl_3$-CD_3OD, 161 MHz): δ 2.875.

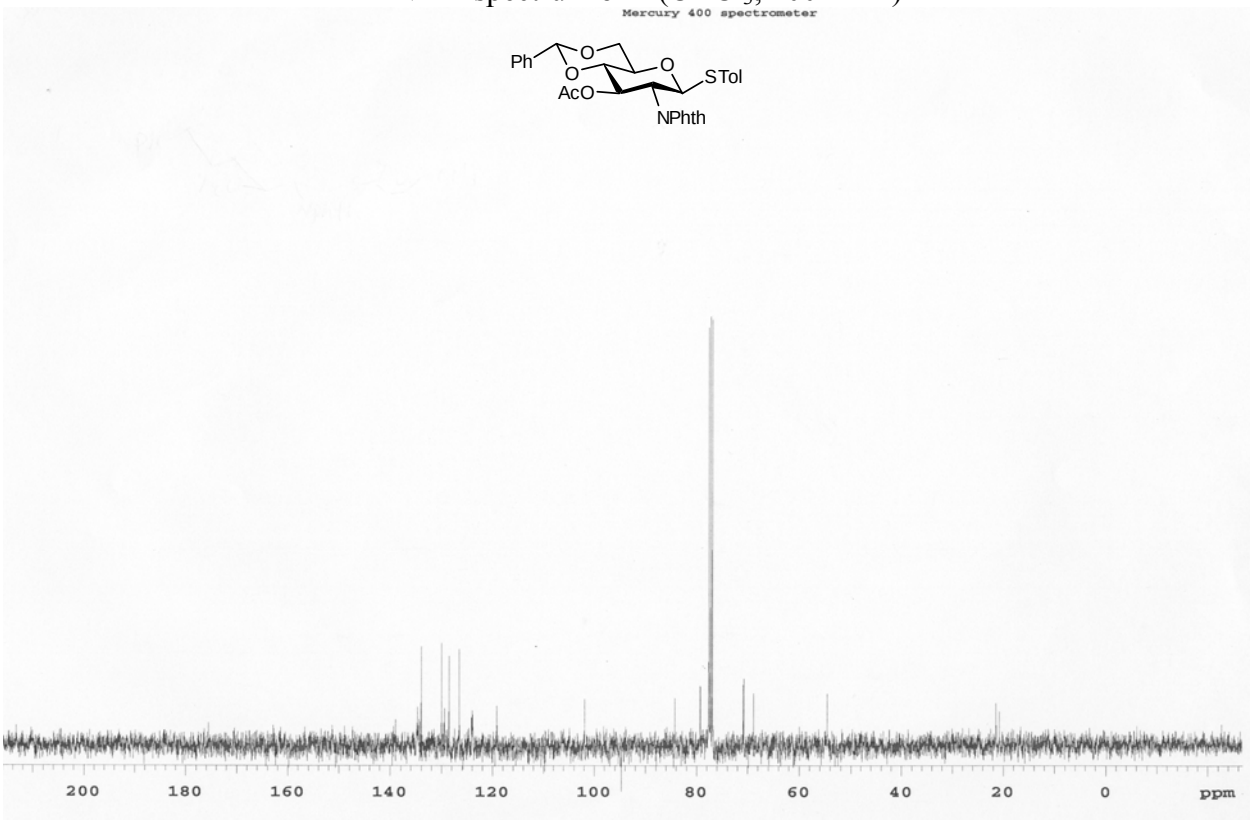
2. NMR and MS Spectra



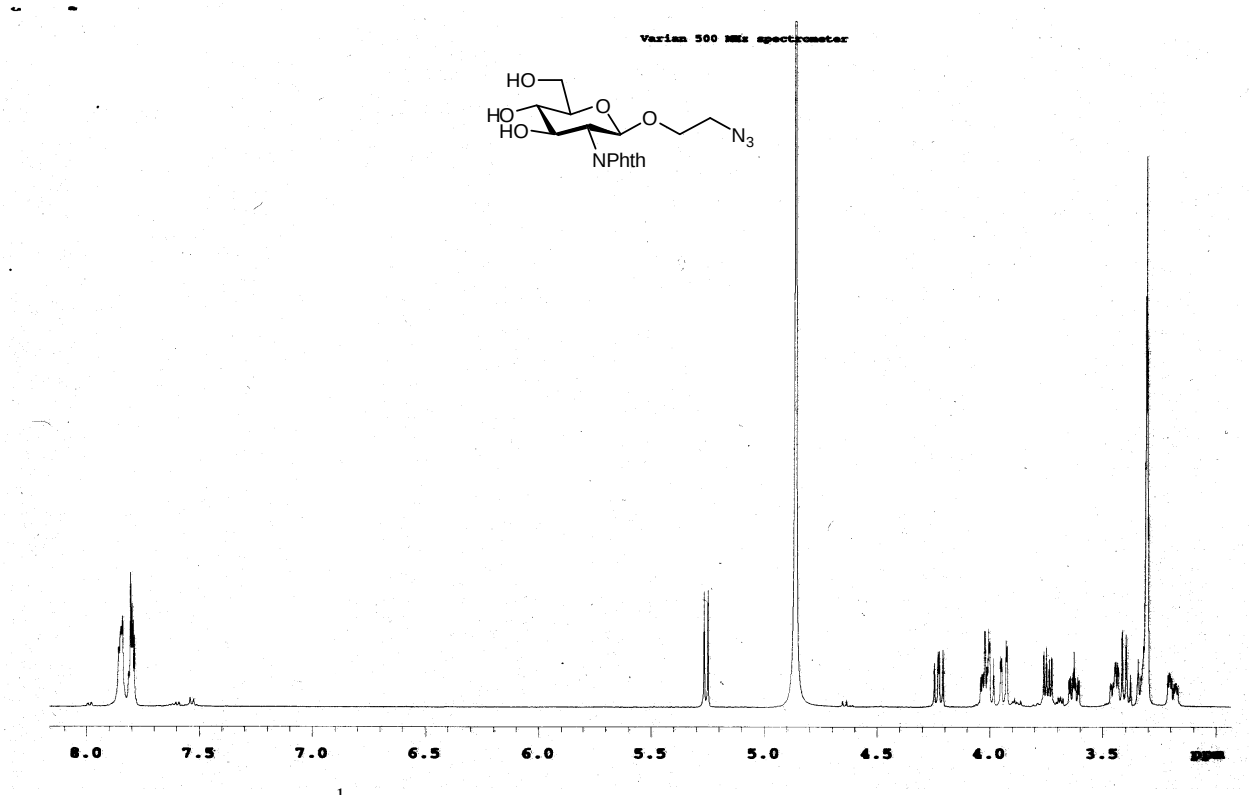
^{1}H NMR spectrum of **19** ($CDCl_3$, 400 MHz)



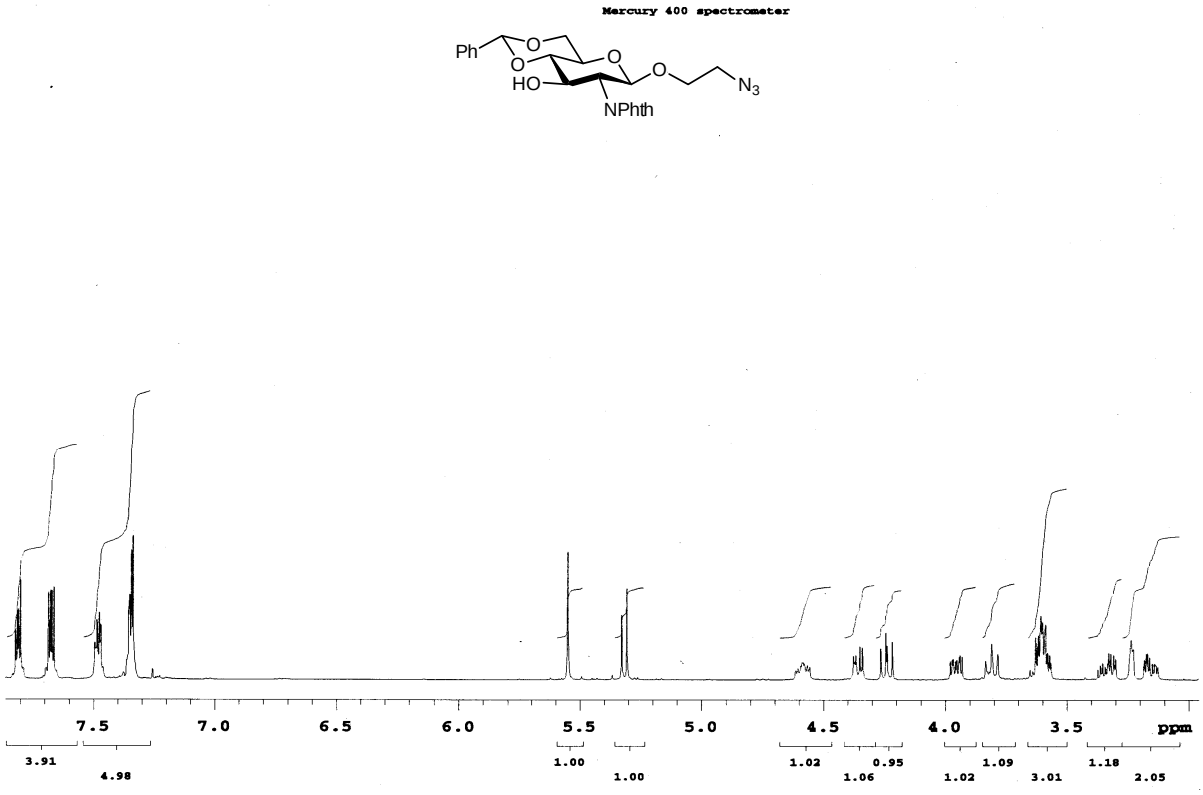
^{1}H NMR spectrum of **4** ($CDCl_3$, 400 MHz)



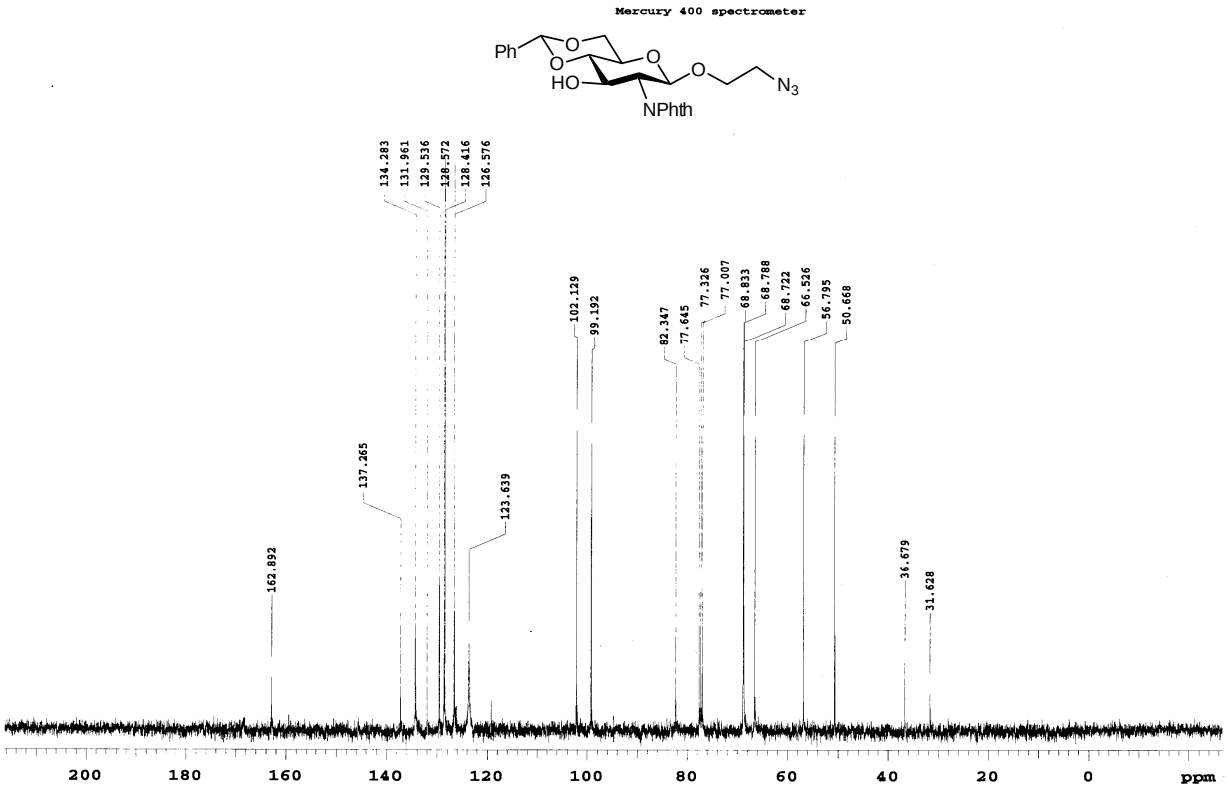
^{13}C NMR spectrum of **4** ($CDCl_3$, 100 MHz)



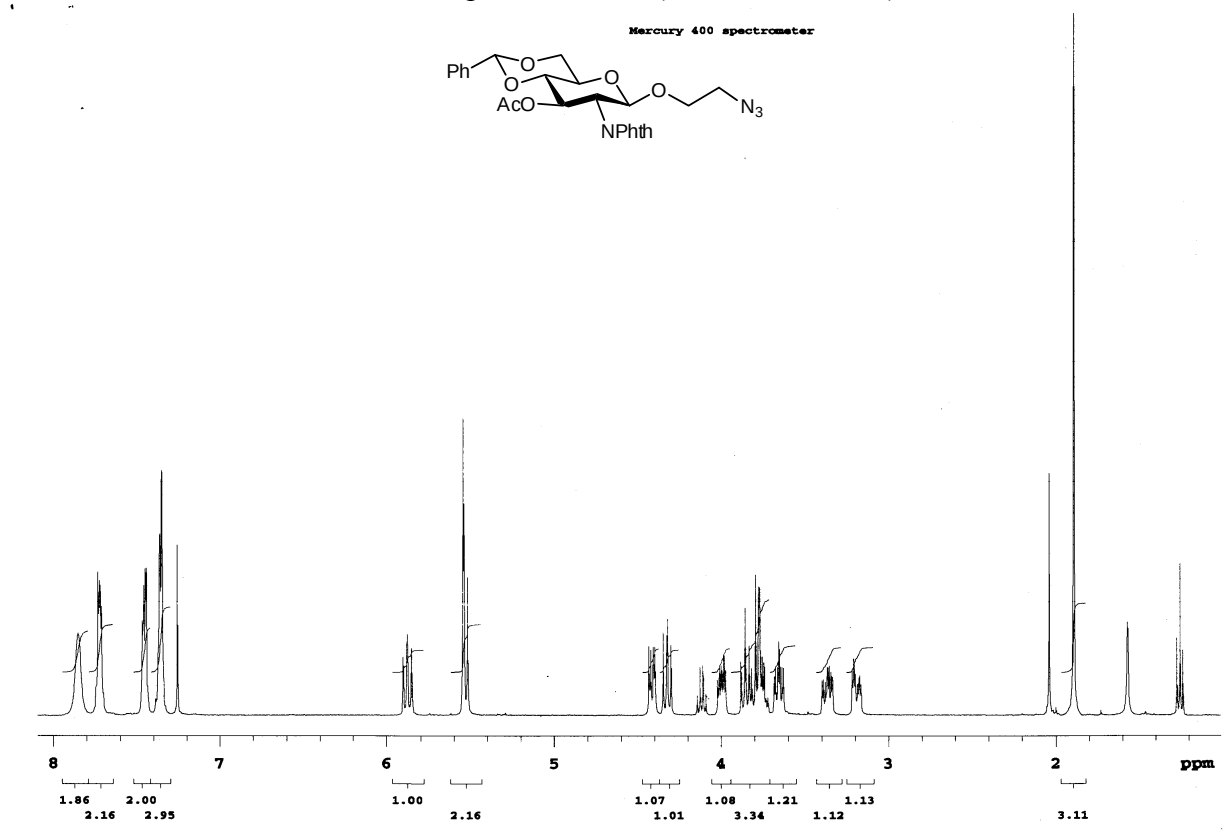
^{1}H NMR spectrum of **20** (CD_3OD, 500 MHz)



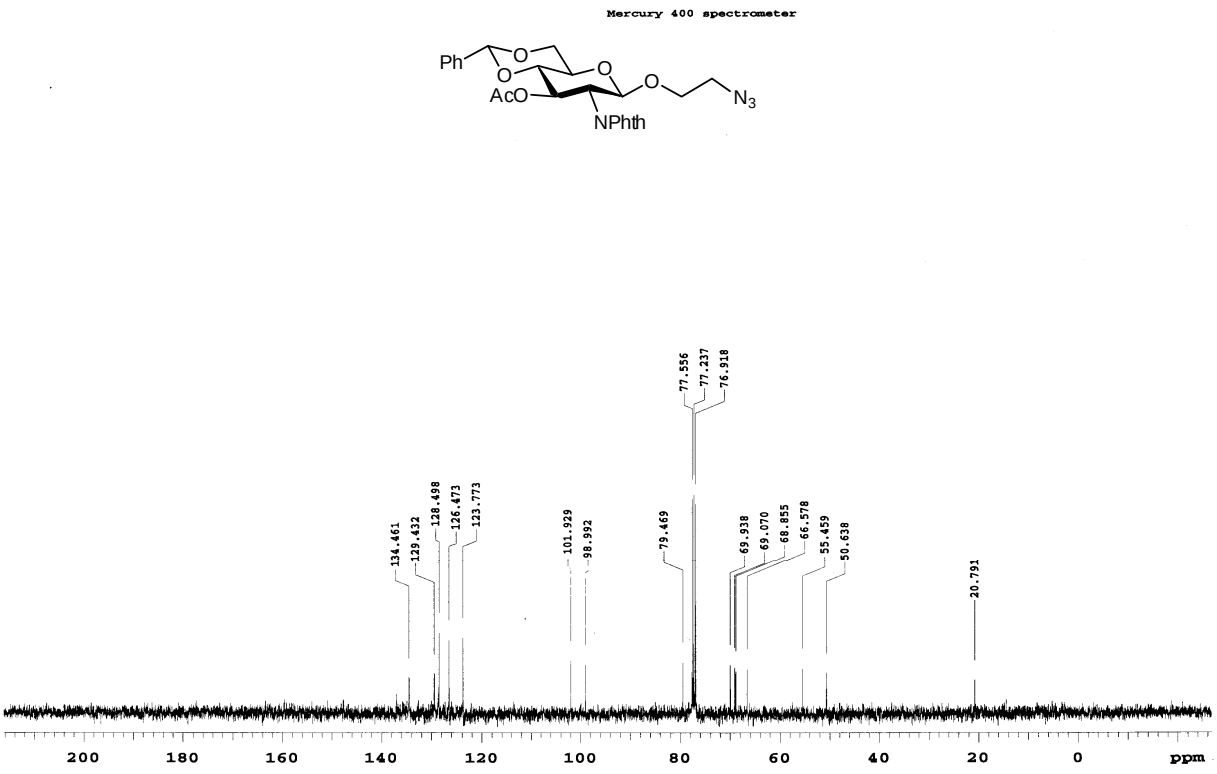
^{1}H NMR spectrum of **21** ($CDCl_3$, 400 MHz)



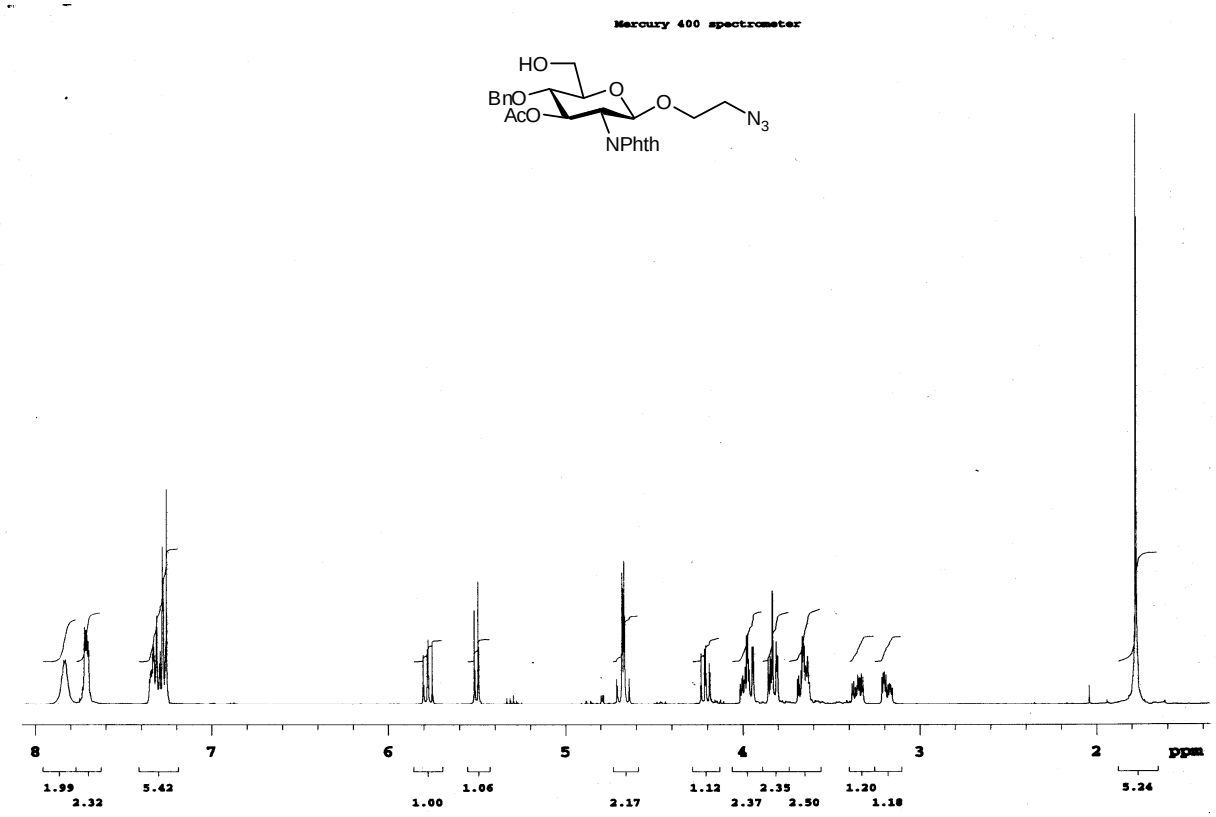
^{13}C NMR spectrum of **21** ($CDCl_3$, 100 MHz)



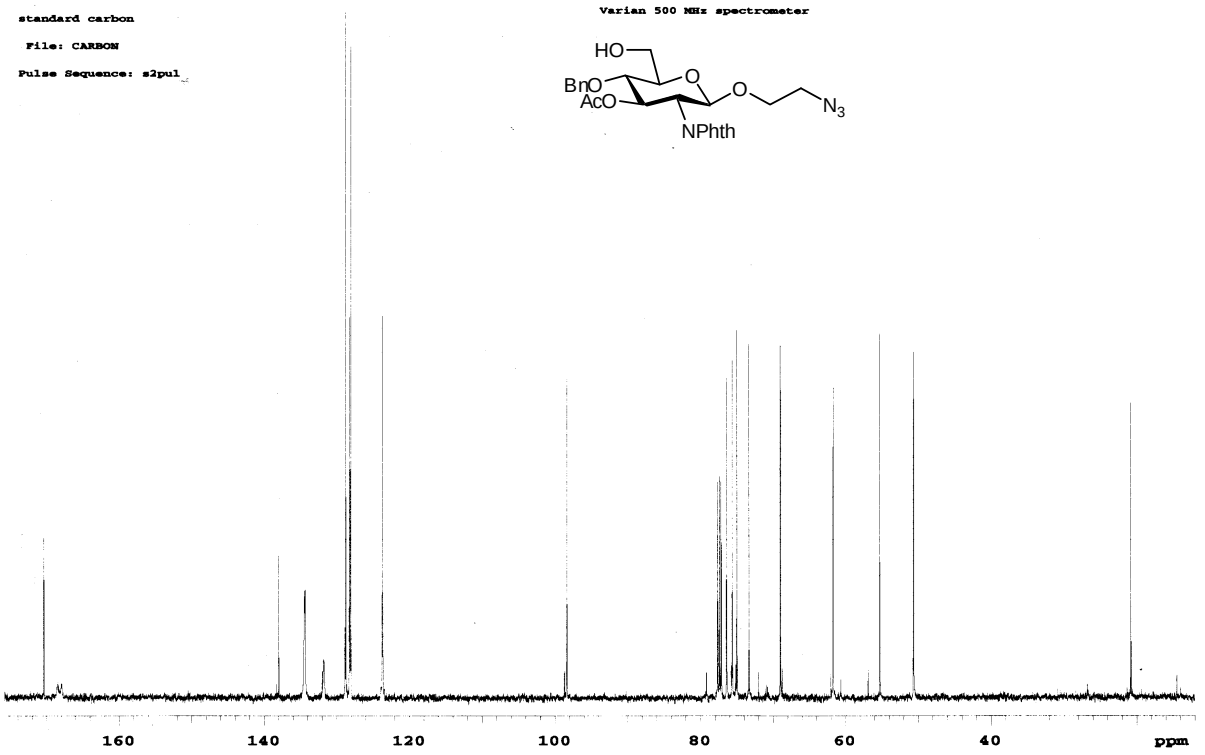
^{1}H NMR spectrum of **22** ($CDCl_3$, 400 MHz)



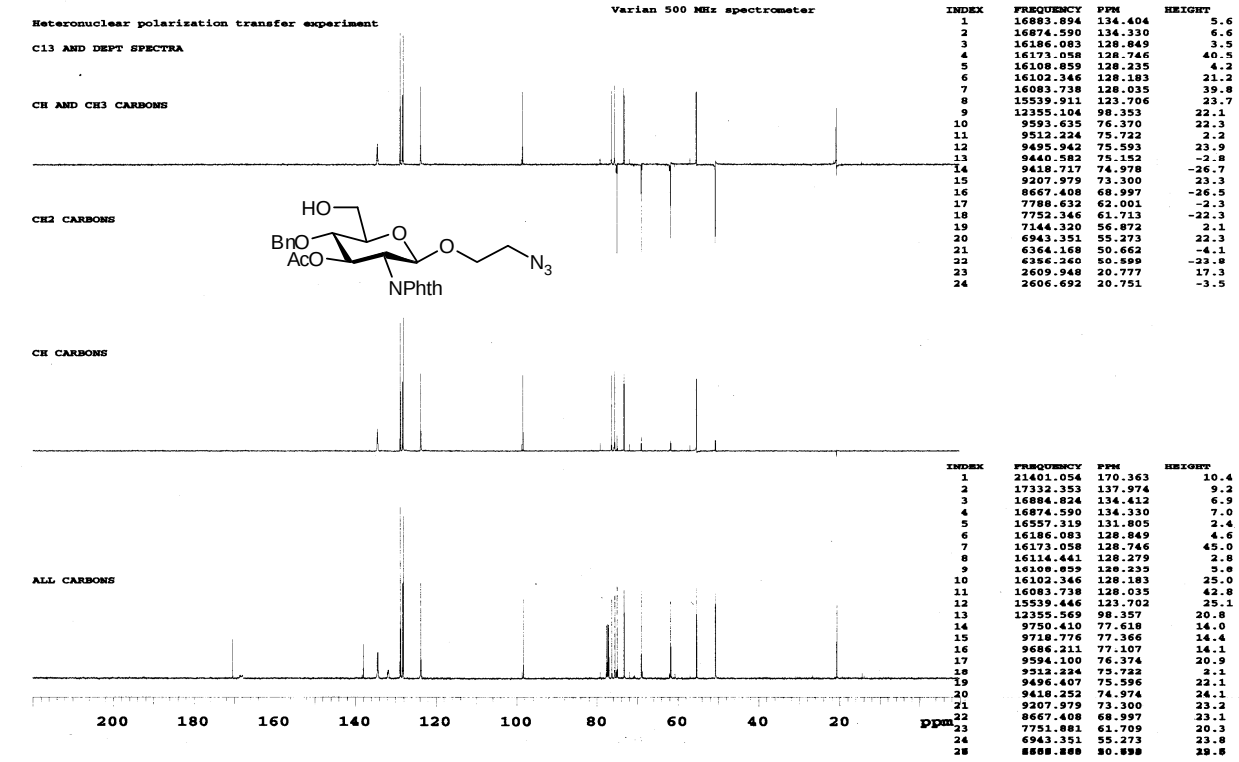
^{13}C NMR spectrum of **22** ($CDCl_3$, 100 MHz)



^{1}H NMR spectrum of **5** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **5** ($CDCl_3$, 125 MHz)



INDEX	FREQUENCY	PPM	HEIGHT
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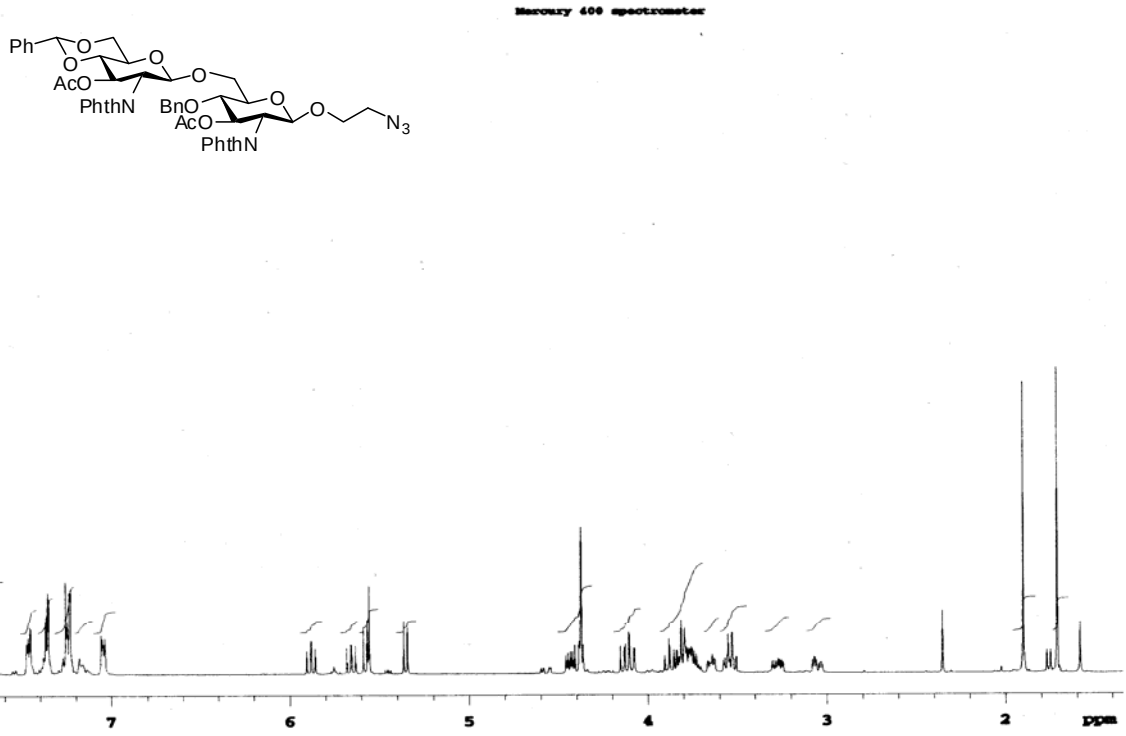
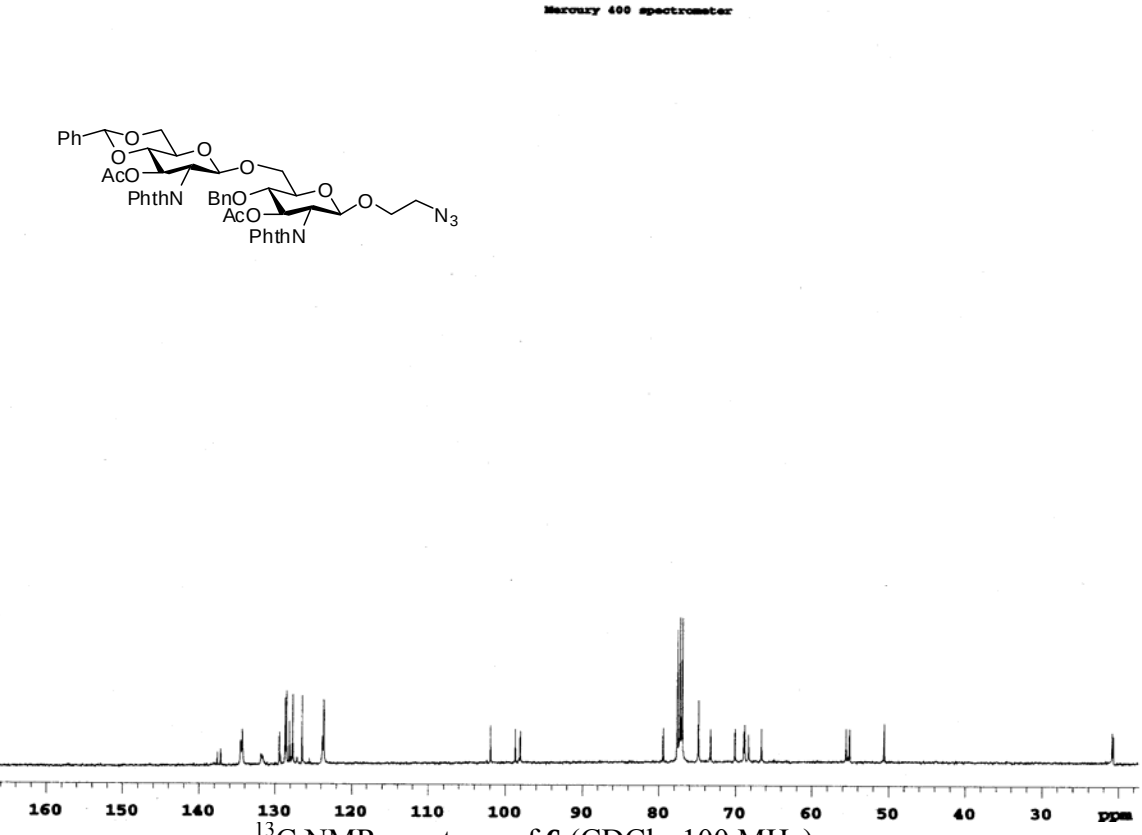
2	16874.590	134.330	6.6
3	16186.083	128.849	3.5
4	16173.058	128.746	40.5
5	16108.859	128.235	4.2
6	16102.346	128.183	21.2
7	16083.738	128.035	39.8
8	15539.911	123.706	23.7
9	12355.104	98.353	22.1
10	9593.635	76.370	22.3
11	9512.224	75.722	2.2
12	9495.942	75.593	23.9
13	9440.582	75.152	-2.8
14	9418.717	74.978	-26.7
15	9207.979	73.300	23.3
16	8667.408	68.997	-26.5
17	7788.632	62.001	-2.3
18	7752.346	61.713	-22.3
19	7144.320	56.872	2.1
20	6943.351	55.273	22.3
21	6364.168	50.662	-4.1
22	6356.260	50.599	-23.8
23	2609.948	20.777	17.3
24	2606.692	20.751	-3.5

INDEX	FREQUENCY	PPM	HEIGHT
1	21401.054	170.363	10.4
2	17332.353	137.974	9.2
3	16884.824	134.412	6.9
4	16874.590	134.330	7.0
5	16557.319	131.805	2.4
6	16186.083	128.849	4.6
7	16173.058	128.746	45.0
8	16114.441	128.279	2.8
9	16108.859	128.235	5.8
10	16102.346	128.183	25.0
11	16083.738	128.035	42.8
12	15539.446	123.702	25.1
13	12355.569	98.357	20.8
14	9750.410	77.618	14.0
15	9718.776	77.366	14.4
16	9686.211	77.107	14.1
17	9594.100	76.374	20.9
18	9512.224	75.722	2.1
19	9496.407	75.596	22.1
20	9418.252	74.974	24.1
21	9207.979	73.300	23.2
22	8667.408	68.997	23.1
23	7751.881	61.709	20.3
24	6943.351	55.273	23.8
25	6668.868	50.898	29.5

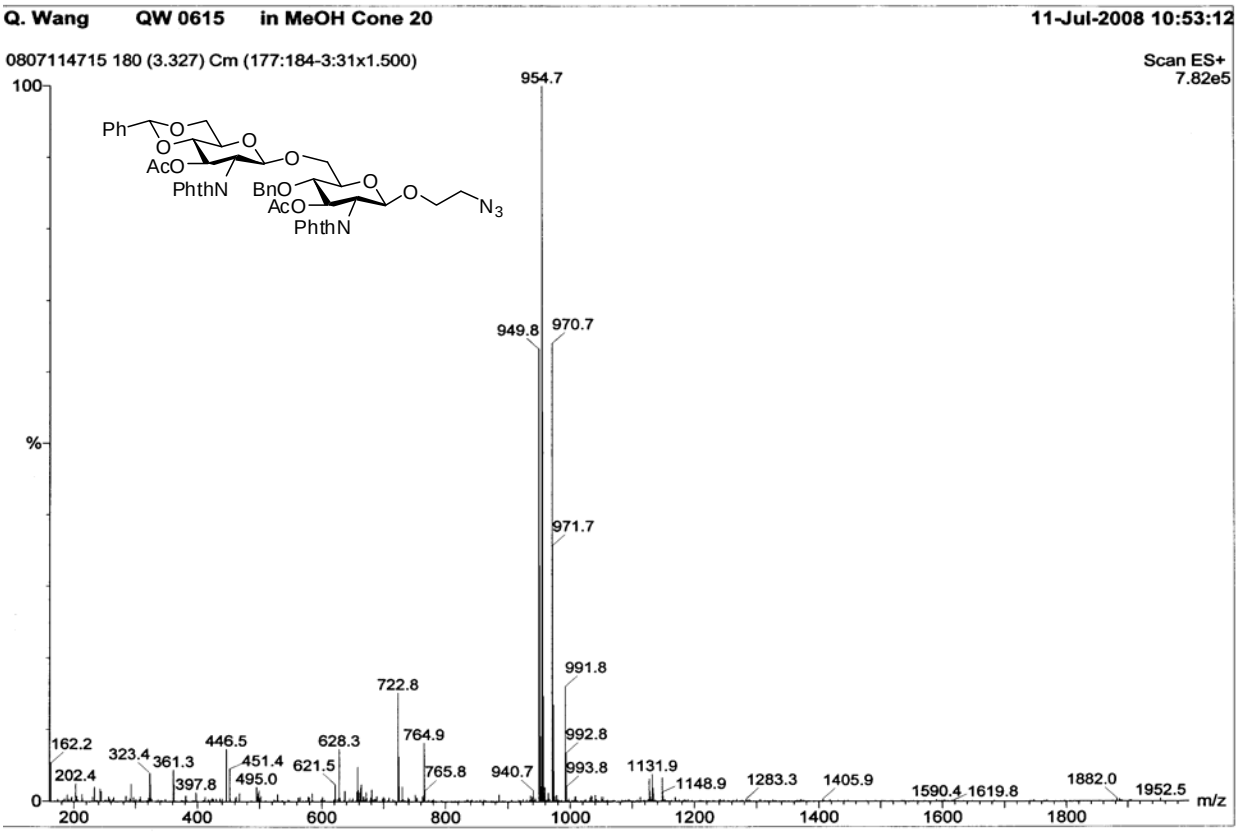
^{13}C DEPT spectrum of **5** ($CDCl_3$, 100 MHz)



^{1}H NMR spectrum of **6** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **6** ($CDCl_3$, 100 MHz)



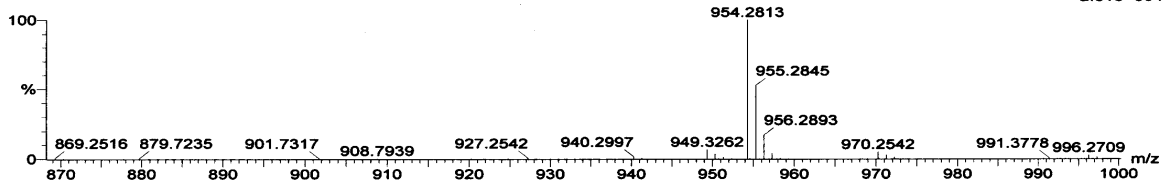
ESI MS spectrum of **6**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 500.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

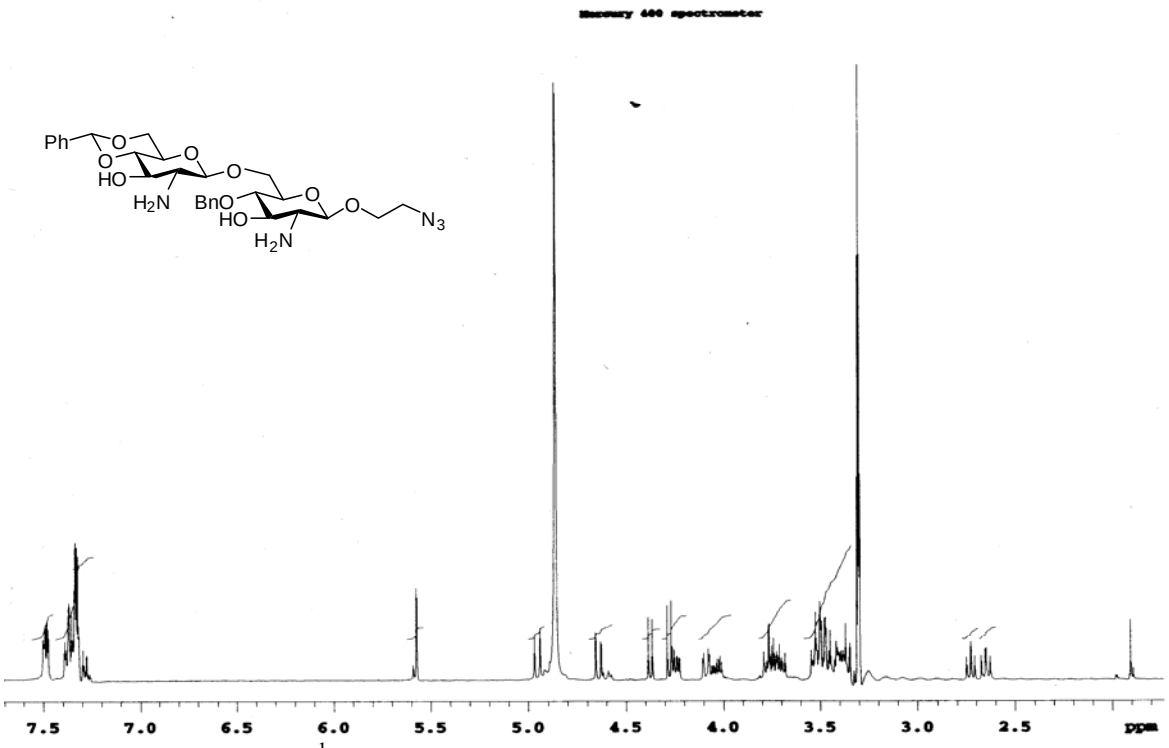
Monoisotopic Mass, Even Electron Ions
637 formula(e) evaluated with 7 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-50 H: 0-50 N: 0-6 O: 0-20 Na: 0-2
Q. Wang QW 0615
Shay 2008-07b.pro
2009_0108_0267 12 (0.246) Cm (10:12-1:5x2.000)

LCT Premier 08-Jan-2009 11:48:17
1: TOF MS ES+
2.81e+004

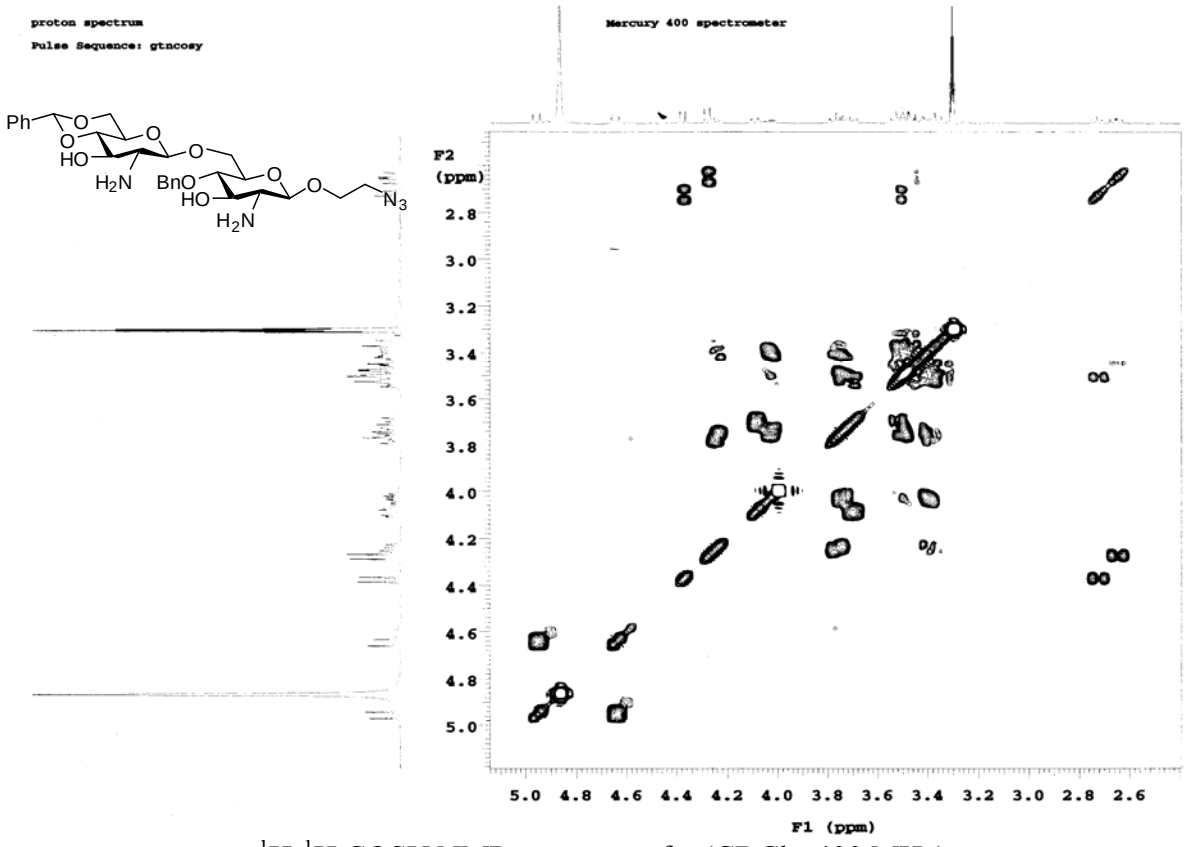


Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		5.0	5.0	500.0			
954.2813	954.2810	0.3	0.3	28.5	47.1	4.6	C48 H45 N5 O15 Na
	954.2821	-0.8	-0.8	26.5	47.5	4.9	C49 H48 N O19
	954.2796	1.7	1.8	23.5	45.3	2.7	C47 H49 N O19 Na
	954.2834	-2.1	-2.2	31.5	49.1	6.6	C50 H44 N5 O15
	954.2786	2.7	2.8	25.5	45.0	2.5	C46 H46 N5 O15 Na2
	954.2845	-3.2	-3.4	16.5	49.6	7.1	C39 H50 N5 O20 Na2
	954.2772	4.1	4.3	20.5	42.7	0.2	C45 H50 N O19 Na2

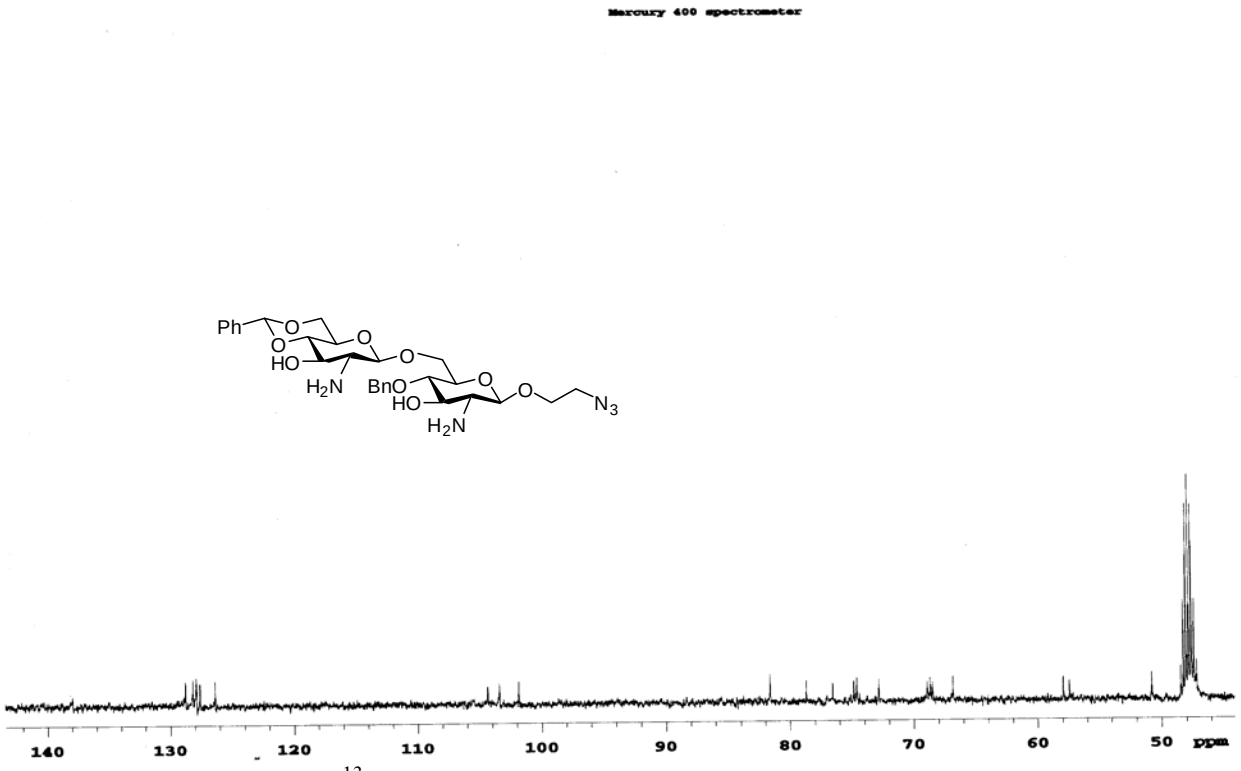
HR ESI MS spectrum of **6**



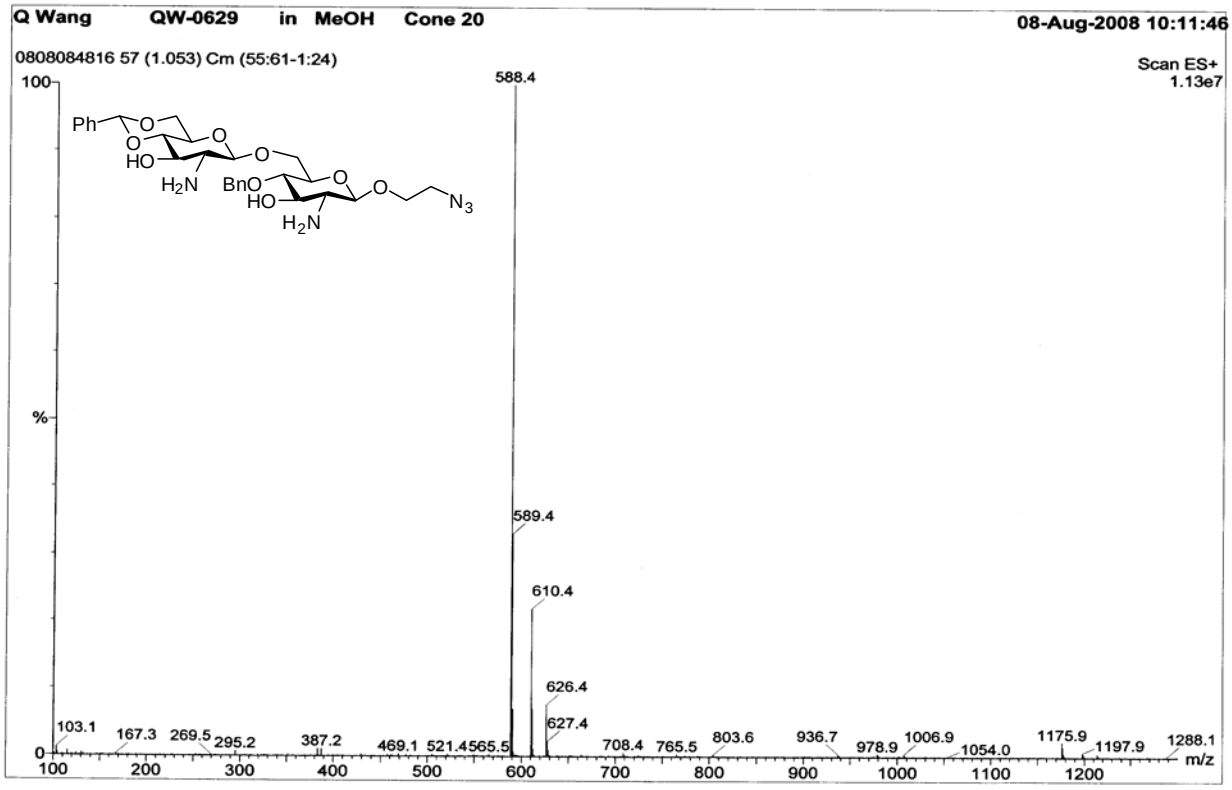
^{1}H NMR spectrum of **7** ($CDCl_3$, 400 MHz)



^{1}H-^{1}H COSY NMR spectrum of **7** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **7** ($CDCl_3$, 100 MHz)



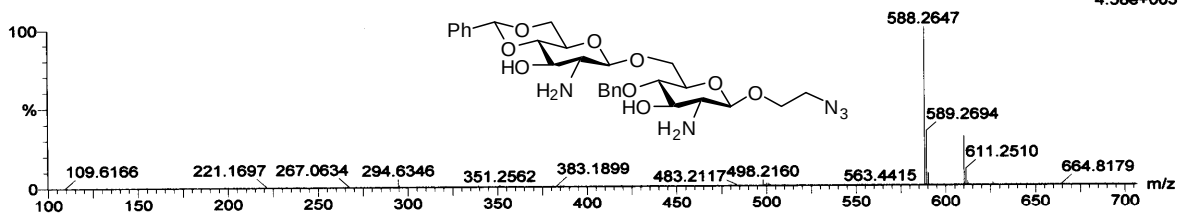
ESI MS spectrum of **7**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 60.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

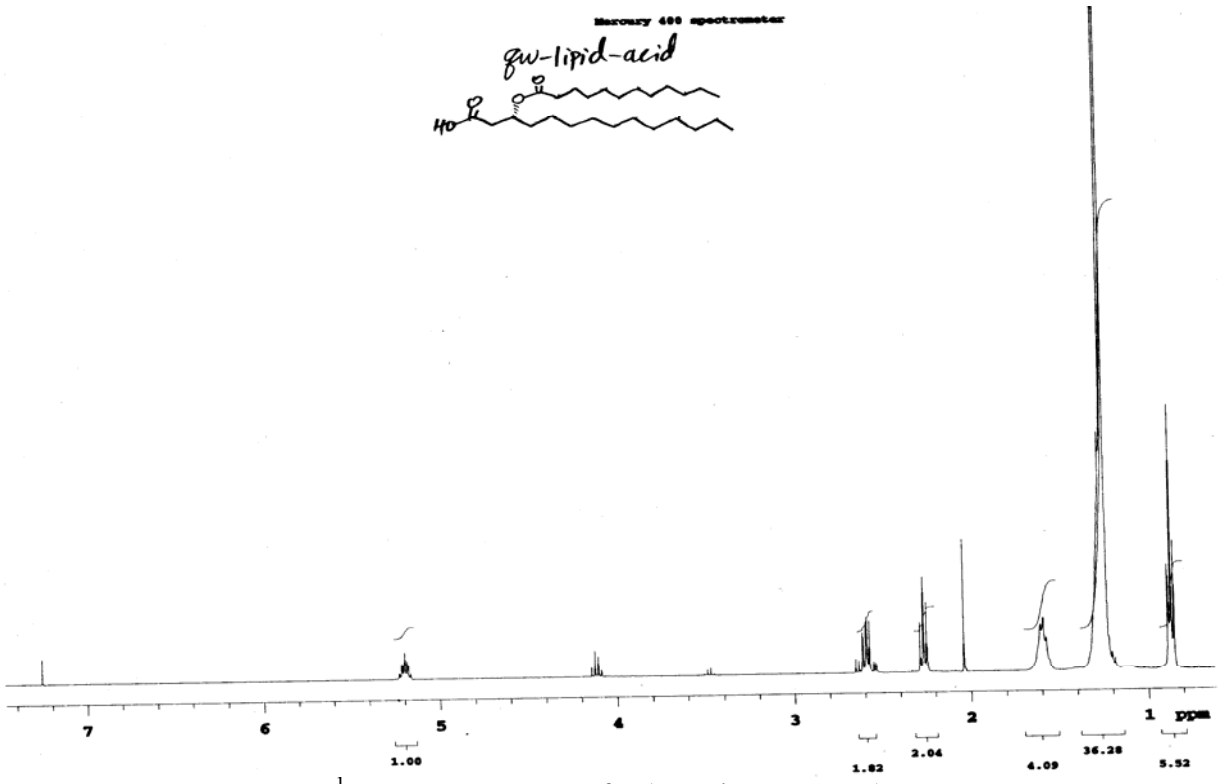
Monoisotopic Mass, Even Electron Ions
1070 formula(e) evaluated with 7 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-113 H: 0-116 N: 0-5 O: 0-13 23Na: 0-1
guo- Qwang QW0629 mw587 LCT0109 10pg/ul meoh 1ul 1ul stk
Shay 2008-07b.pro
2008_0822_0109_26 12 (0.246) Cm (9:12-33:45x2.000)

LCT Premier 22-Aug-2008 16:33:32
1: TOF MS ES+
4.58e+003

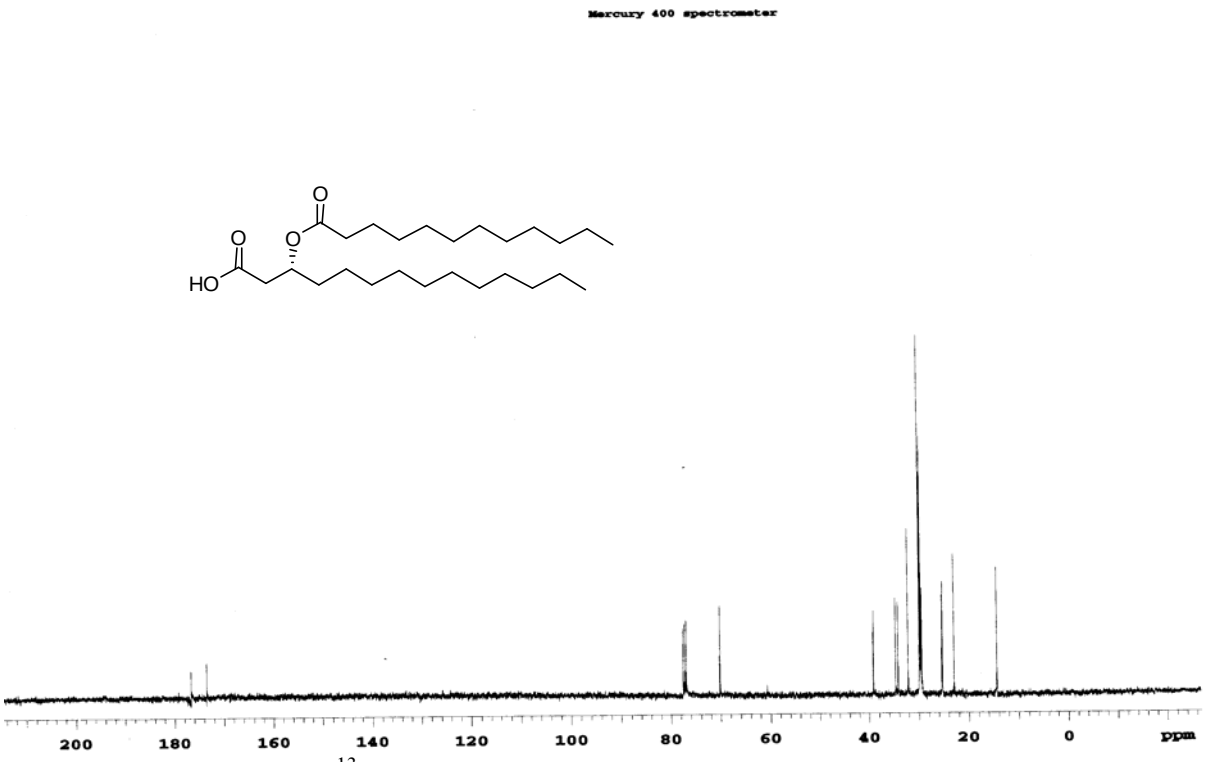


Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
Minimum:				-1.5			
Maximum:		8.0	5.0	60.0			
588.2647	588.2670	-2.3	-3.9	12.5	52.4	0.8	C28 H38 N5 O9
	588.2656	-0.9	-1.5	7.5	52.8	1.2	C27 H42 N O13
	588.2645	0.2	0.3	9.5	53.1	1.5	C26 H39 N5 O9 23Na
	588.2632	1.5	2.5	4.5	56.1	4.5	C25 H43 N O13 23Na
	588.2651	-0.4	-0.7	25.5	59.7	8.1	C40 H34 N3 O2
	588.2627	2.0	3.4	22.5	60.2	8.6	C38 H35 N3 O2 23Na
	588.2667	-2.0	-3.4	26.5	61.3	9.7	C43 H35 N 23Na

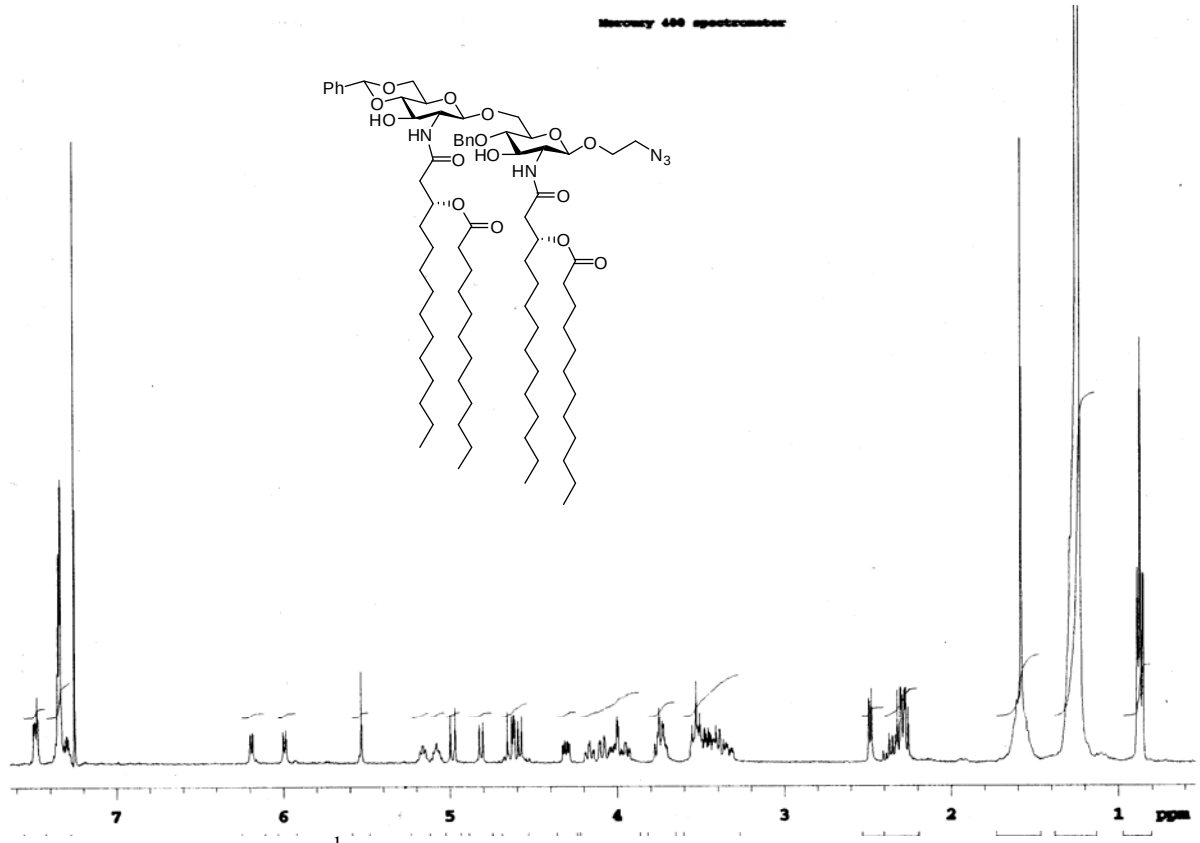
HR ESI MS spectrum of **7**



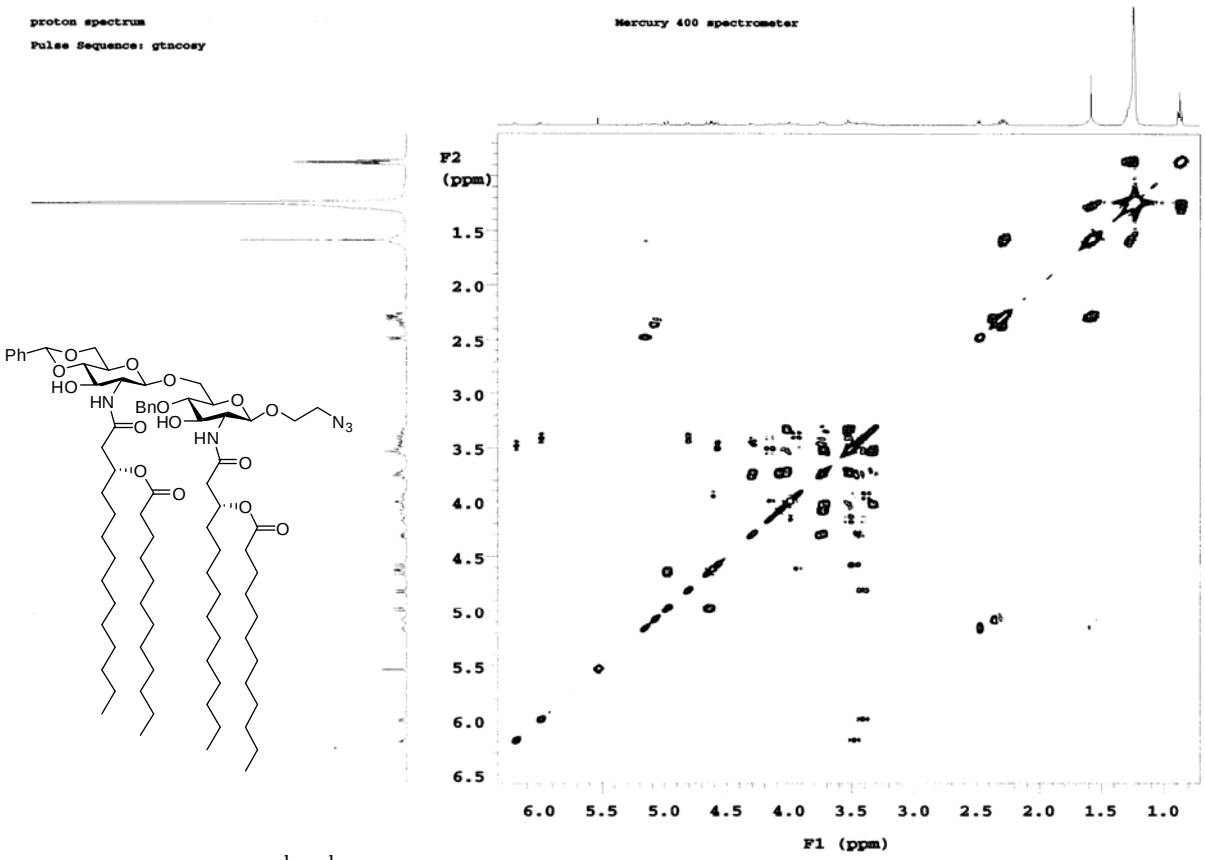
^{1}H NMR spectrum of **8** ($CDCl_3$, 400 MHz)



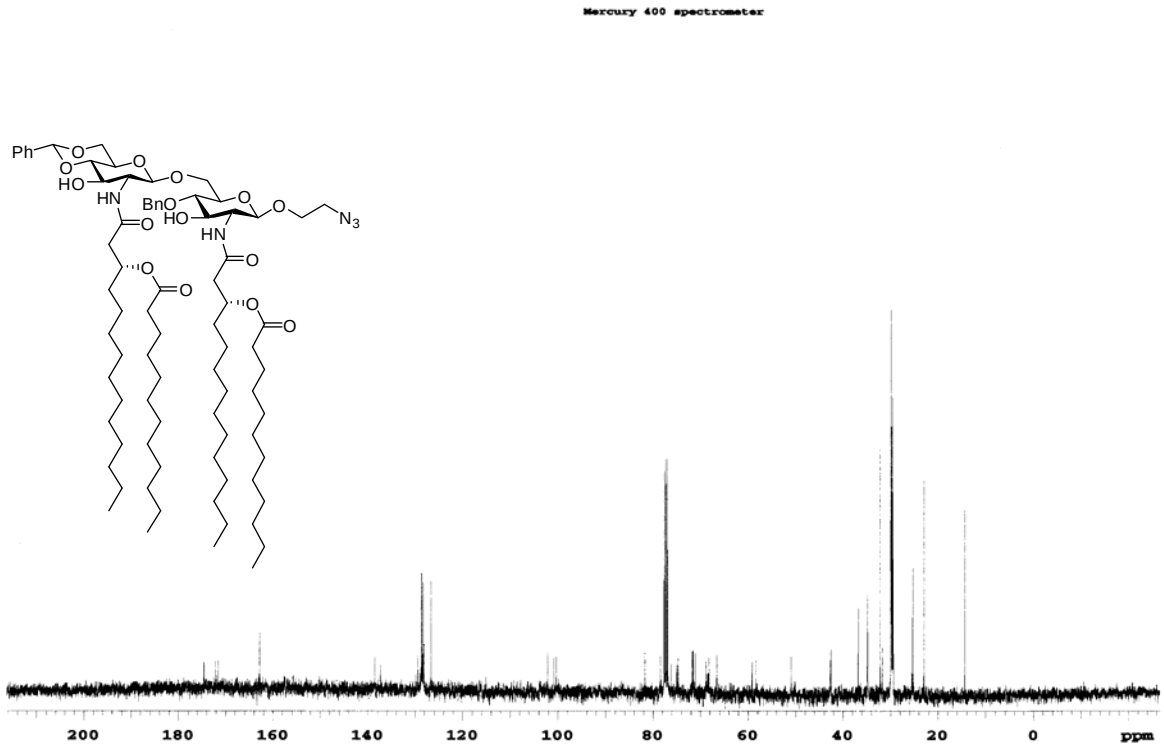
^{13}C NMR spectrum of **8** ($CDCl_3$, 100 MHz)



^{1}H NMR spectrum of **9** ($CDCl_3$, 400 MHz)

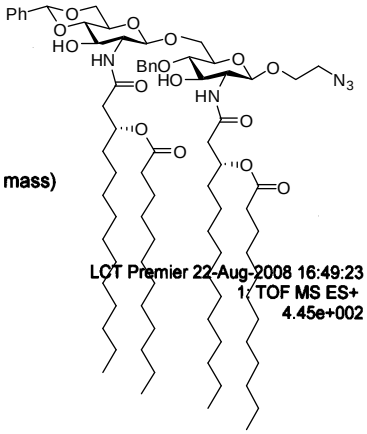


^{1}H-^{1}H COSY NMR spectrum of **9** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **9** ($CDCl_3$, 100 MHz)

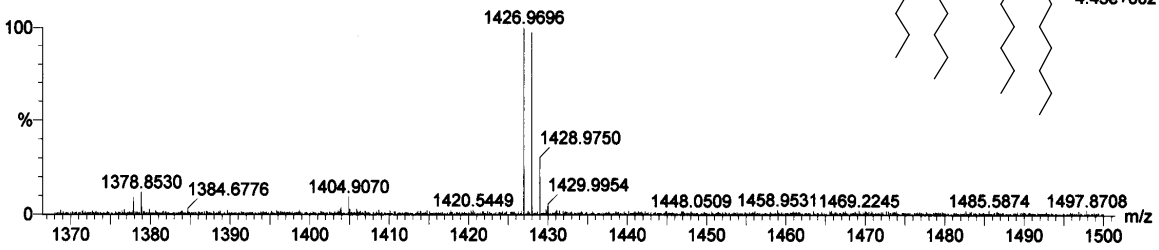
Elemental Composition Report **Page 1**



Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 60.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

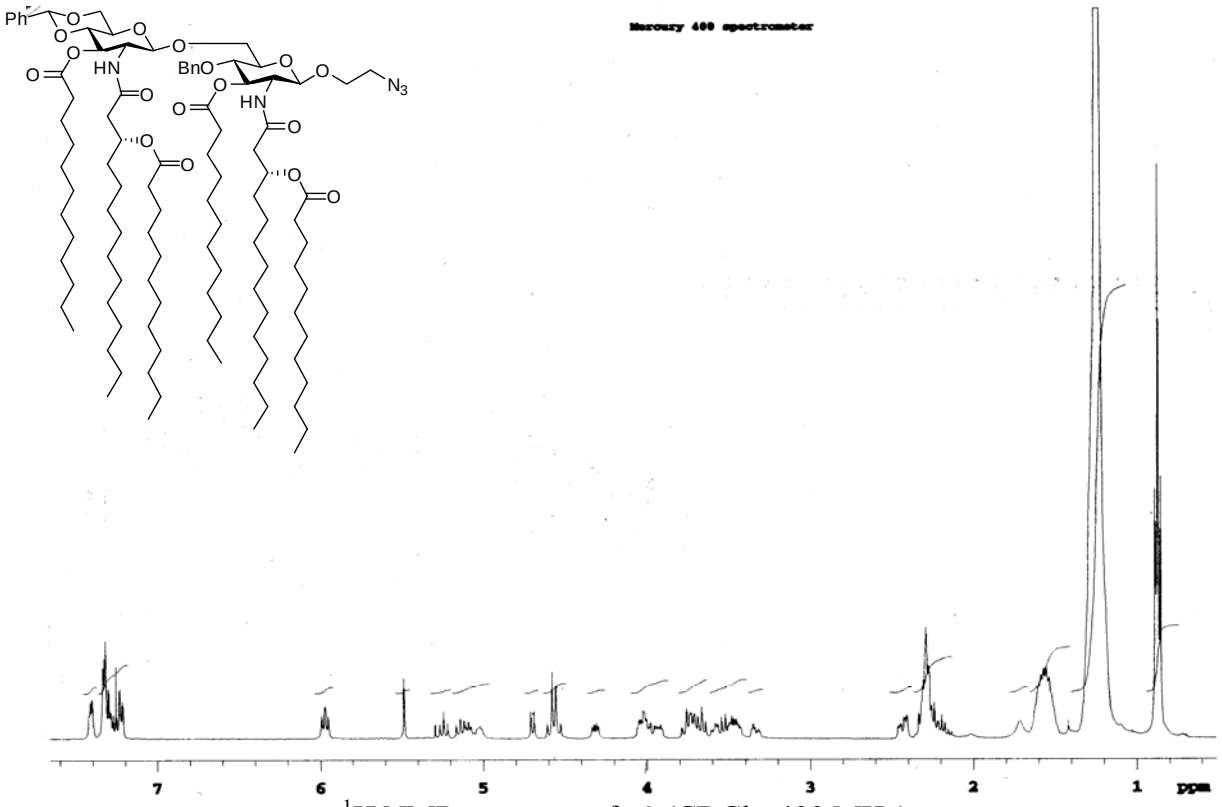
Monoisotopic Mass, Even Electron Ions
199 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-80 H: 0-200 N: 0-5 O: 0-15 23Na: 0-1
guo- Qwang QW0632 mw1403.9 LCT0110 10pg/ul meoh 1ul 1ul stk
Shay 2008-07b.pro
2008_0822_0110_28 14 (0.300) Cm (10:14-1:6x2.000)

LCT Premier 22-Aug-2008 16:49:23
1: TOF MS ES+
4.45e+002

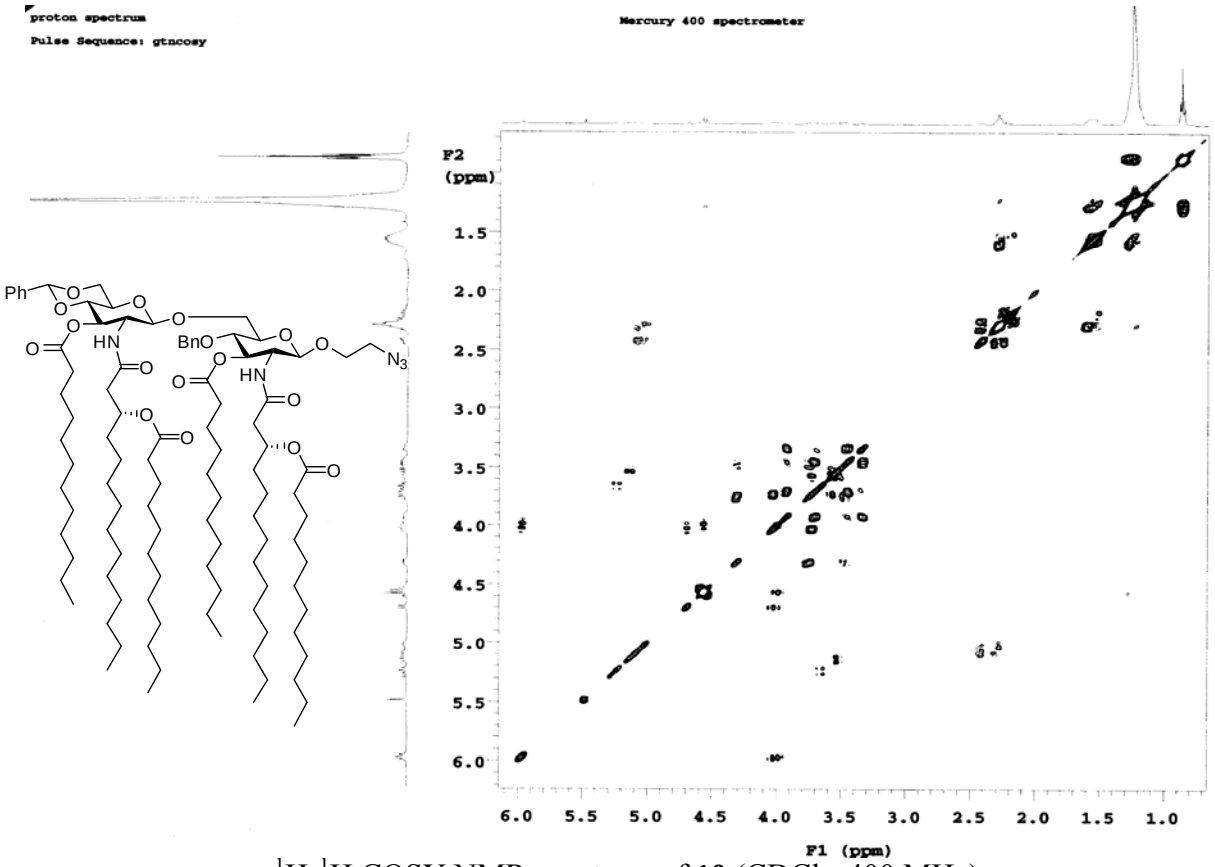


Minimum:				-1.5			
Maximum:		8.0	5.0	60.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1426.9696	1426.9696	0.0	0.0	16.5	94.1	0.0	C80 H133 N5 O15 23Na

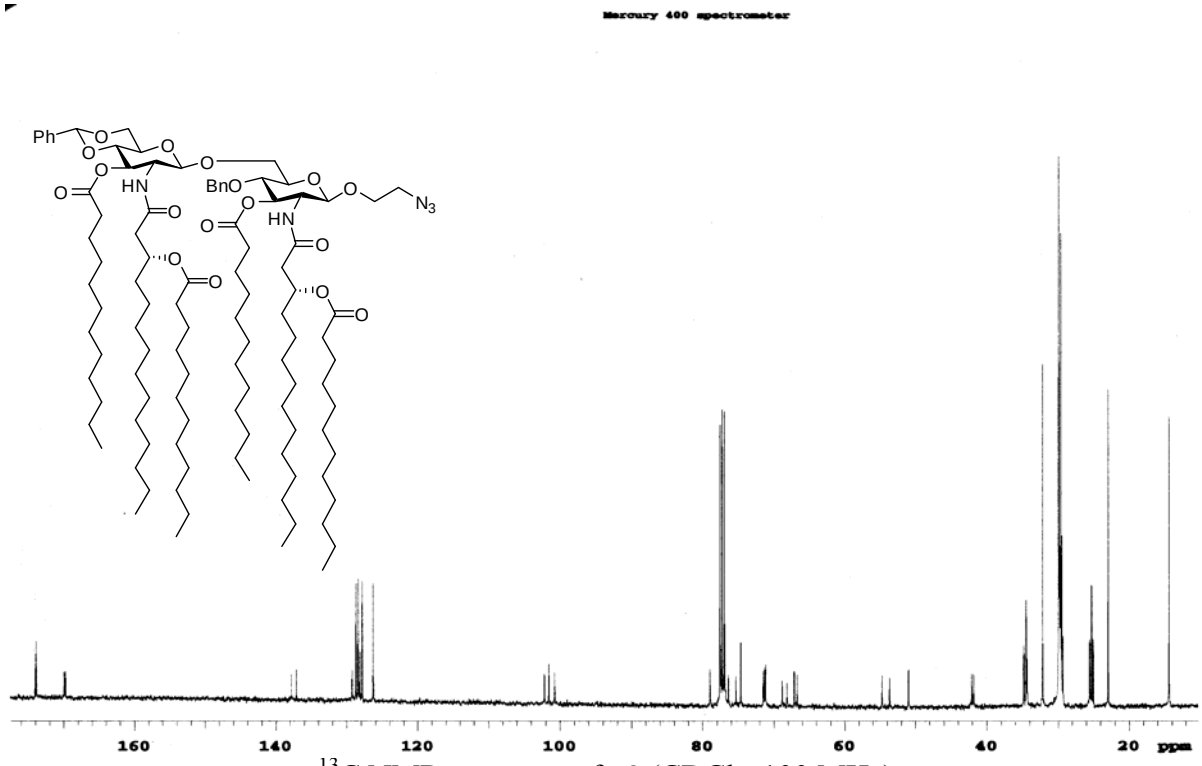
HR ESI MS spectrum of **9**



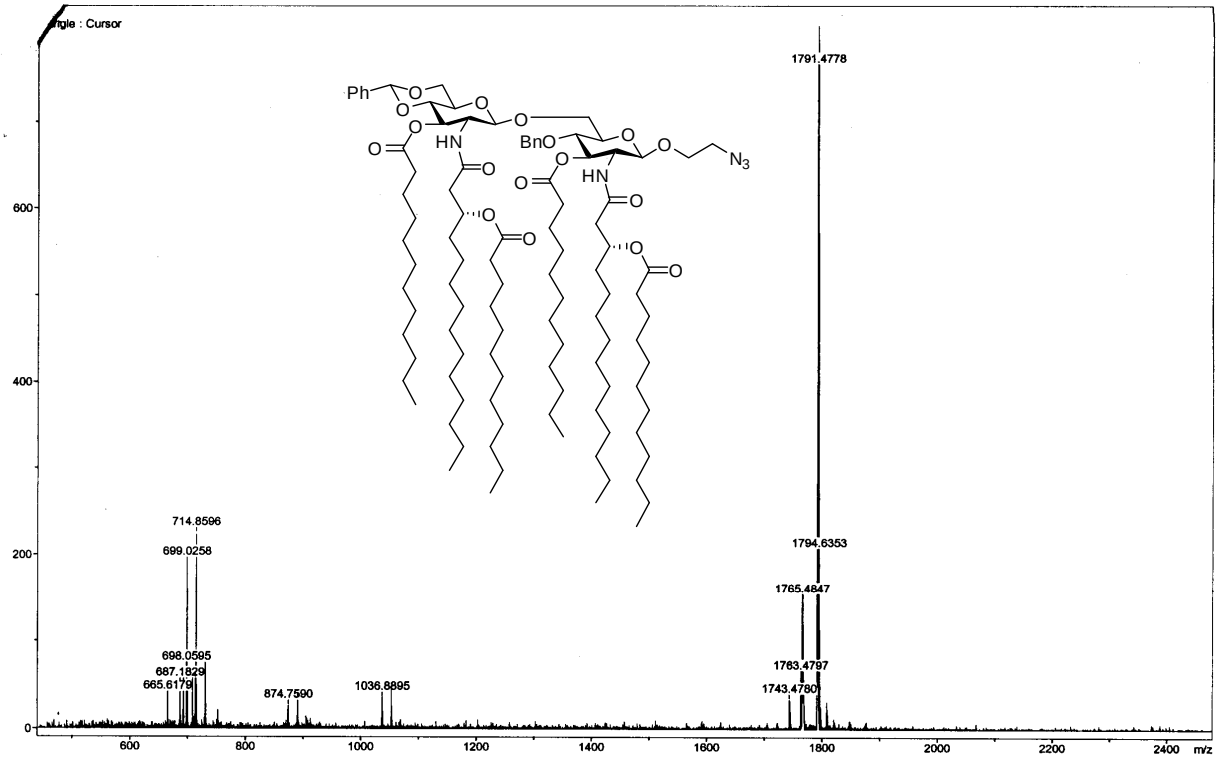
^{1}H NMR spectrum of **10** ($CDCl_3$, 400 MHz)



^{1}H-^{1}H COSY NMR spectrum of **10** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **10** ($CDCl_3$, 100 MHz)

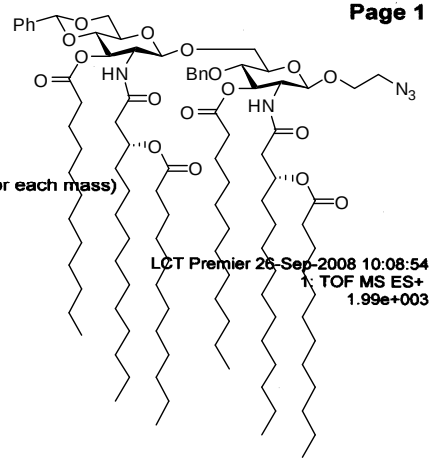


MALTI-TOF MS spectrum of **10**

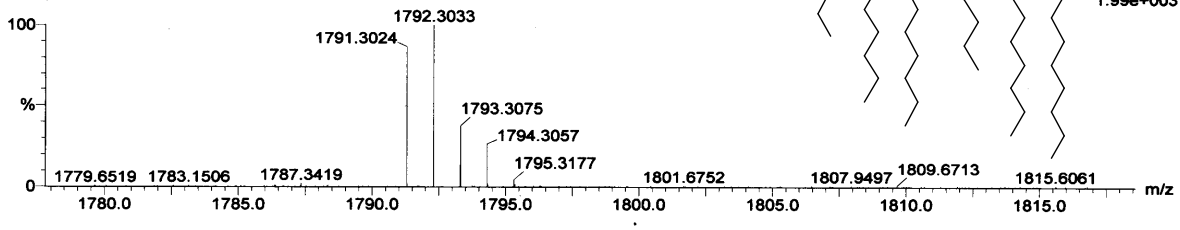
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
426 formula(e) evaluated with 4 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-110 H: 0-500 N: 0-5 O: 0-17 23Na: 0-1
26-Sep-200810:08:54Guo- quanli wang qwo640 mw1768.3 LCT0144 5uL meoh 4x stk
Shay 2008-07b.pro
2008_0926_0144_02 16 (0.317) Cm (13:21-(3:8+38:43)x3.000)

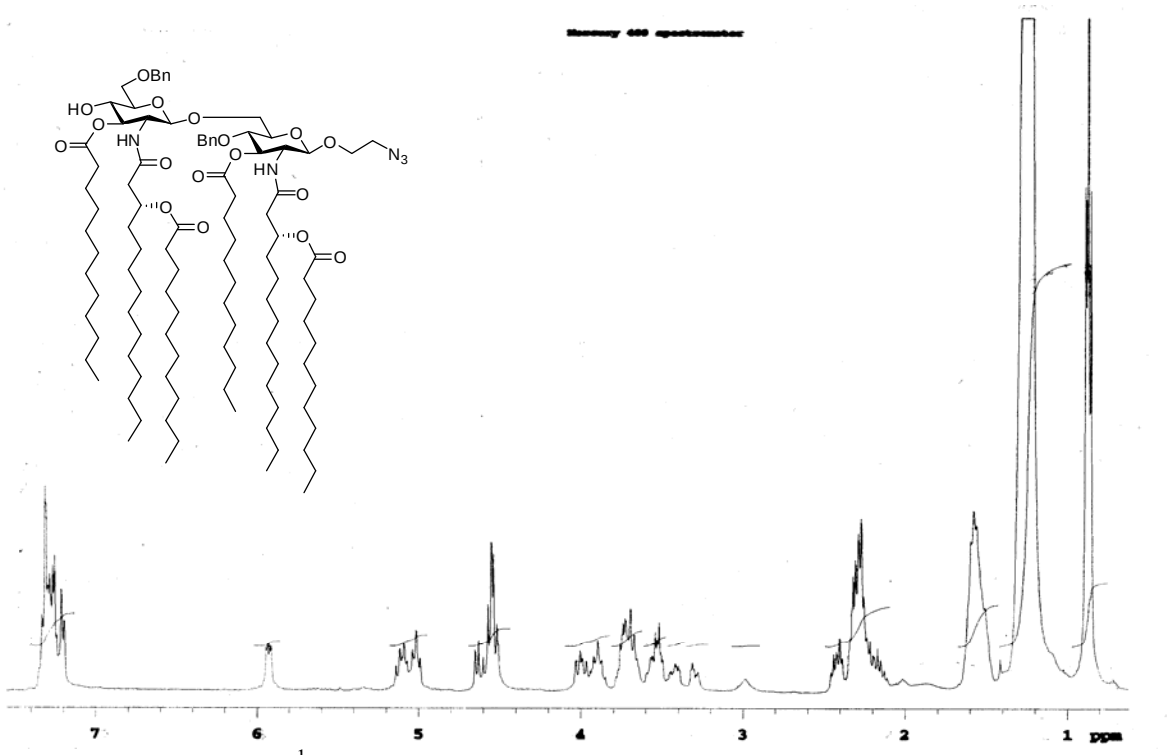


LCT Premier 26-Sep-2008 10:08:54
1: TOF MS ES+
1.99e+003

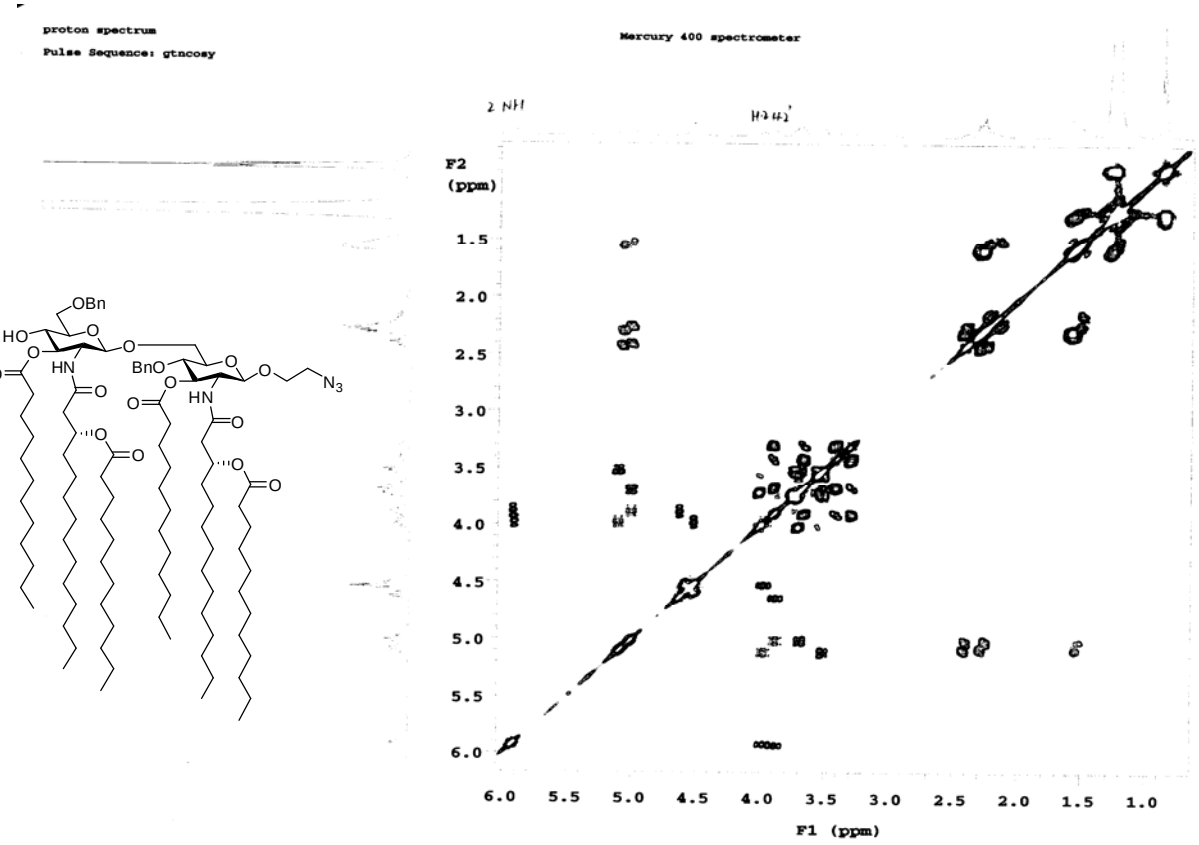


Minimum:				-1.5			
Maximum:		5.0	5.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
1791.3024	1791.3037	-1.3	-0.7	18.5	63.2	0.6	C104 H177 N5 O17 23Na
	1791.3061	-3.7	-2.1	21.5	64.2	1.6	C106 H176 N5 O17
	1791.2965	5.9	3.3	22.5	64.3	1.7	C110 H177 N O16 23Na
	1791.3077	-5.3	-3.0	22.5	65.2	2.6	C109 H177 N3 O15 23Na

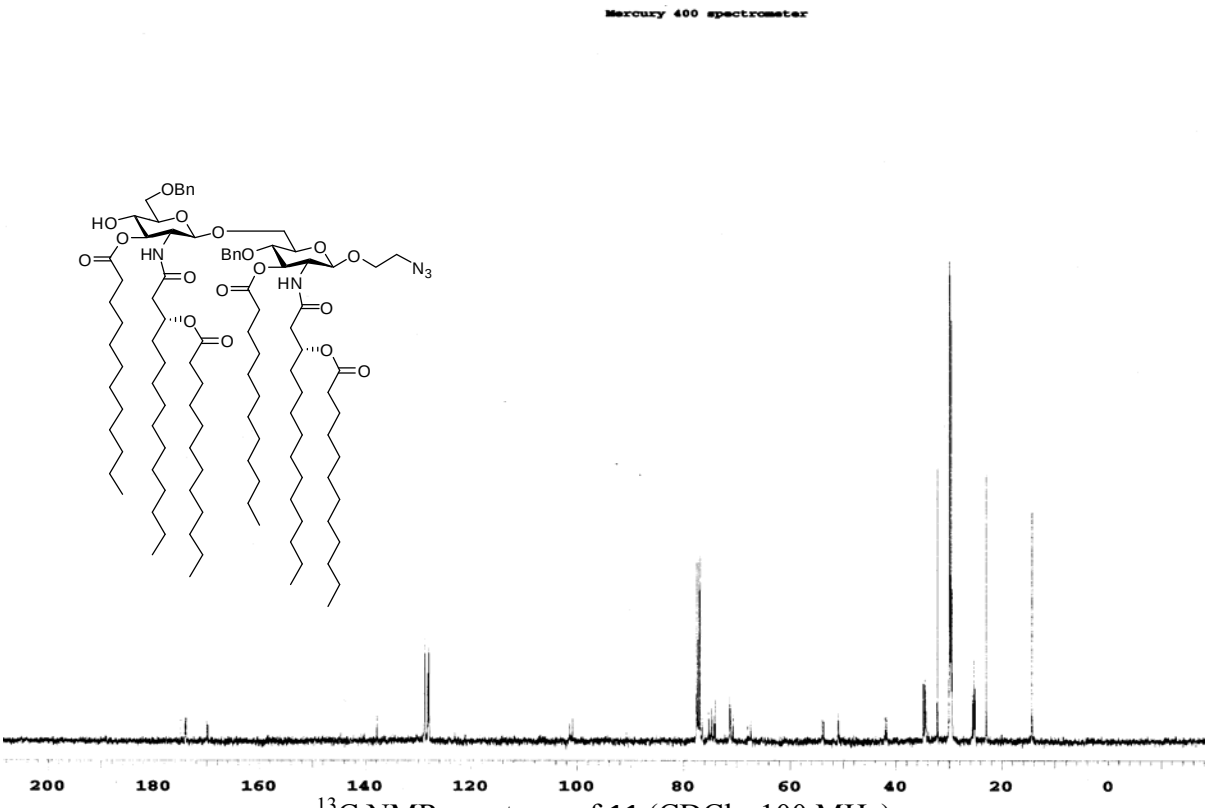
HR ESI MS spectrum of **10**



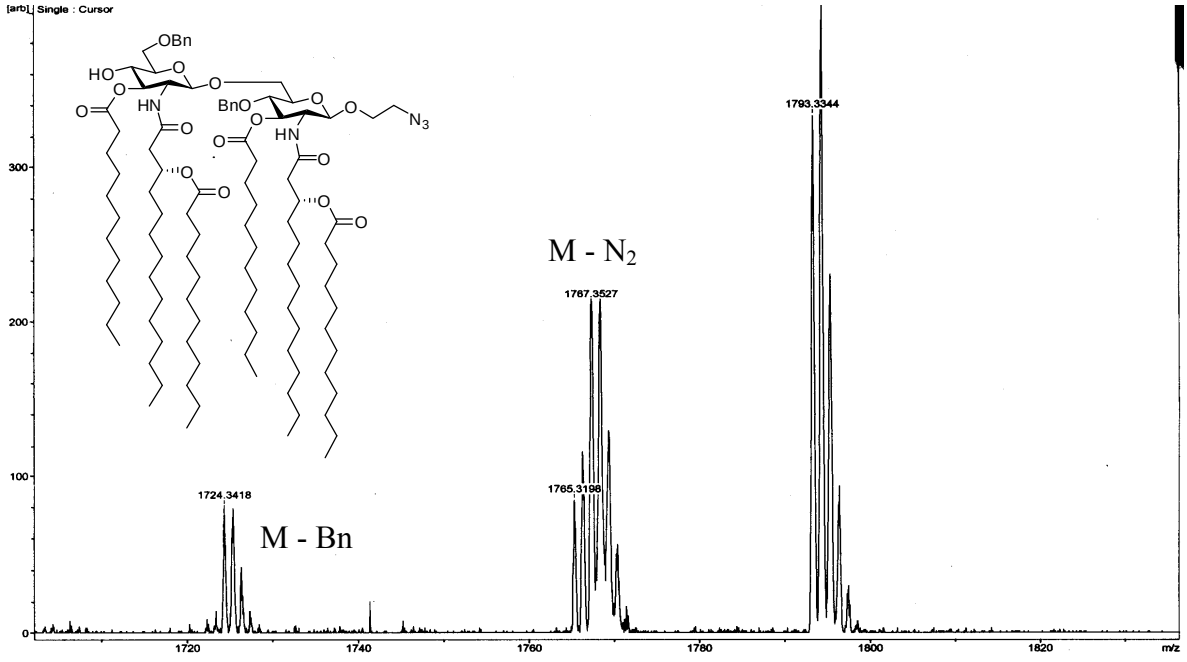
^{1}H NMR spectrum of **11** ($CDCl_3$, 400 MHz)



^{1}H-^{1}H COSY NMR spectrum of **11** ($CDCl_3$, 400 MHz)



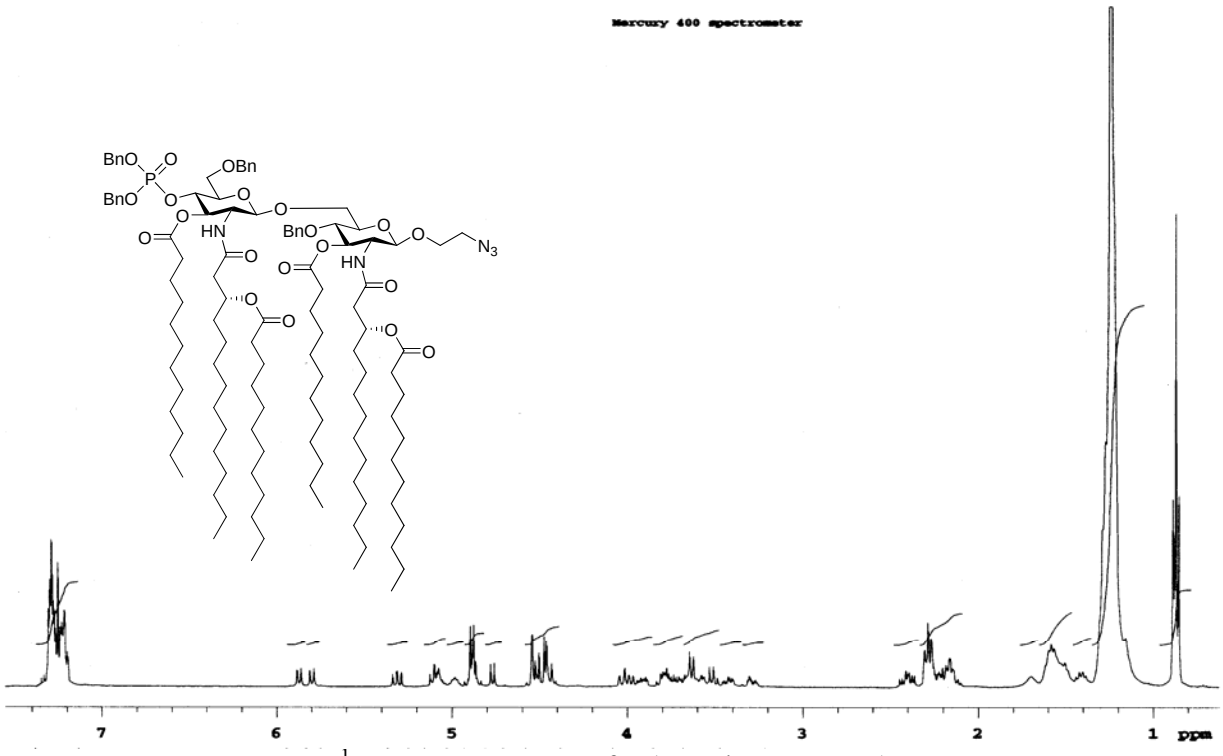
^{13}C NMR spectrum of **11** ($CDCl_3$, 100 MHz)



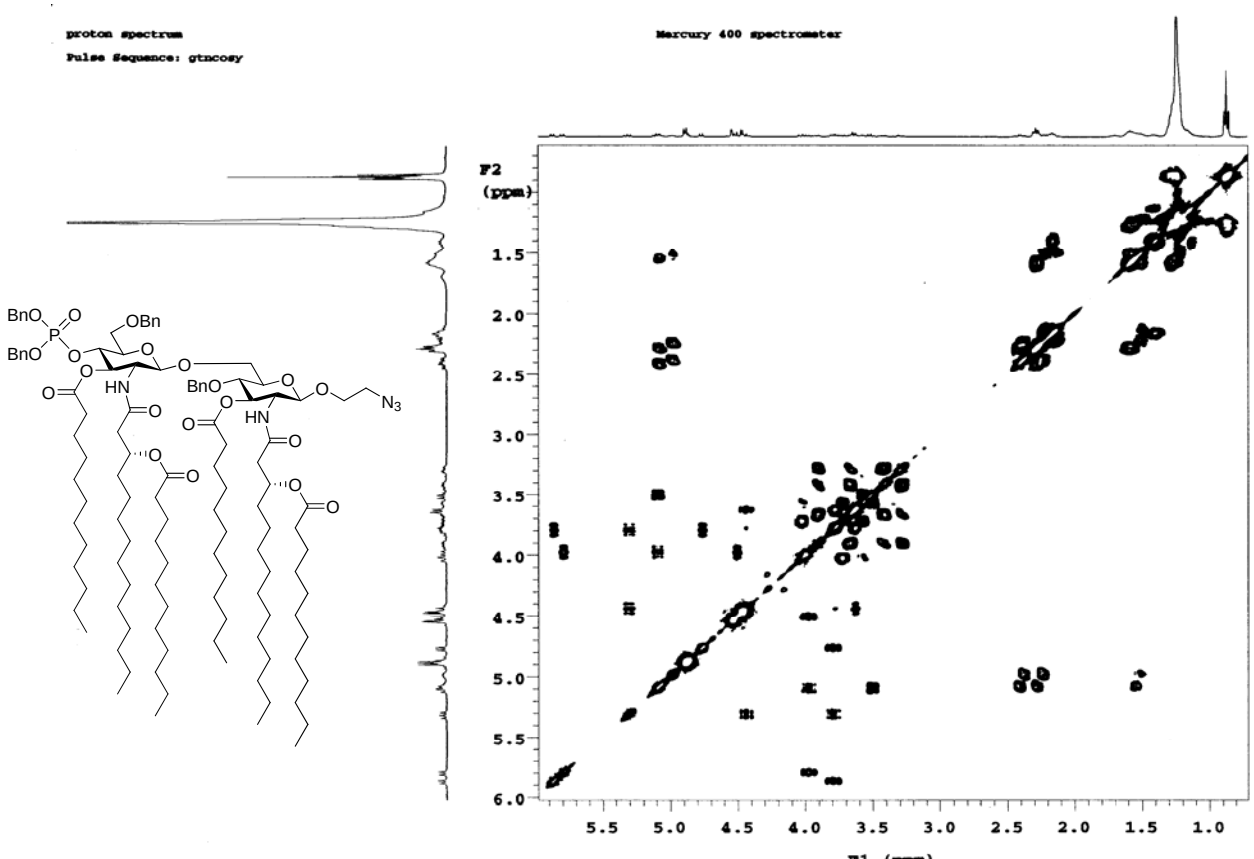
Bruker Daltonics flexControl

Display Screenshot - Generated On 2008-09-25 11h49m56s

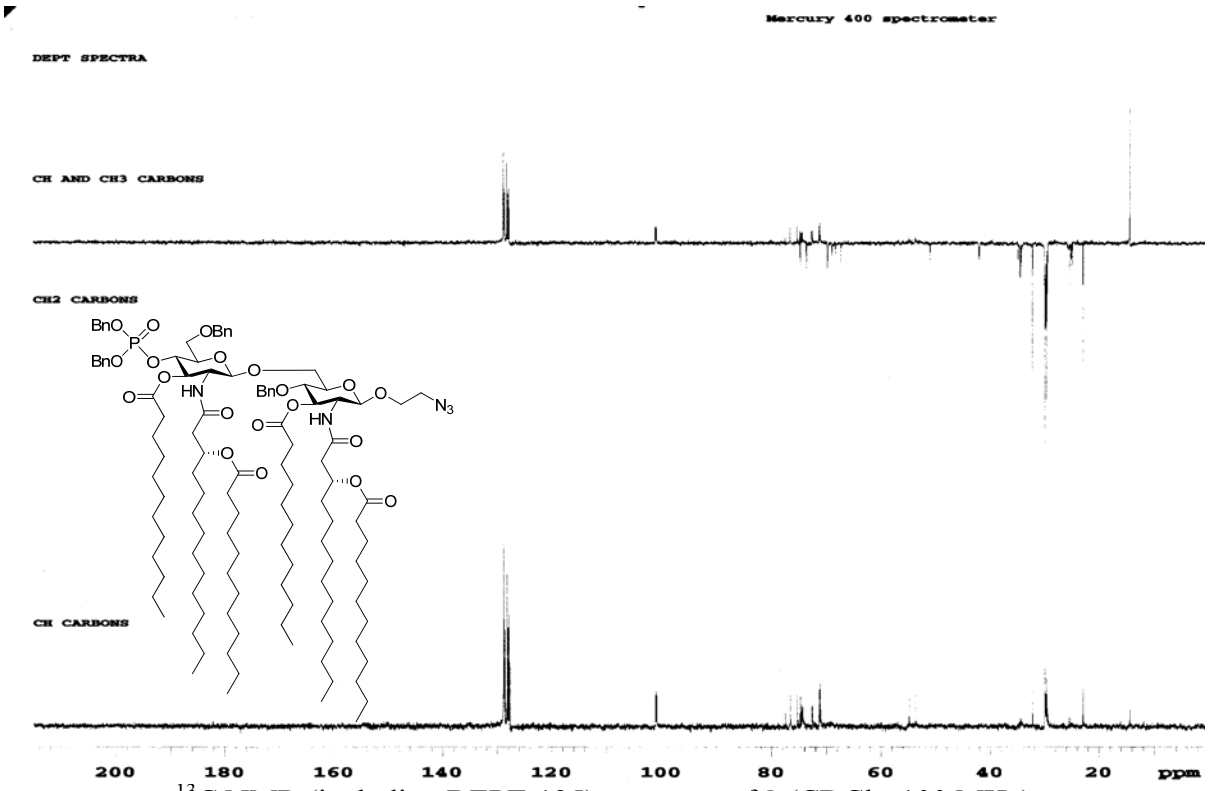
MALTI-TOF MS spectrum of **11**



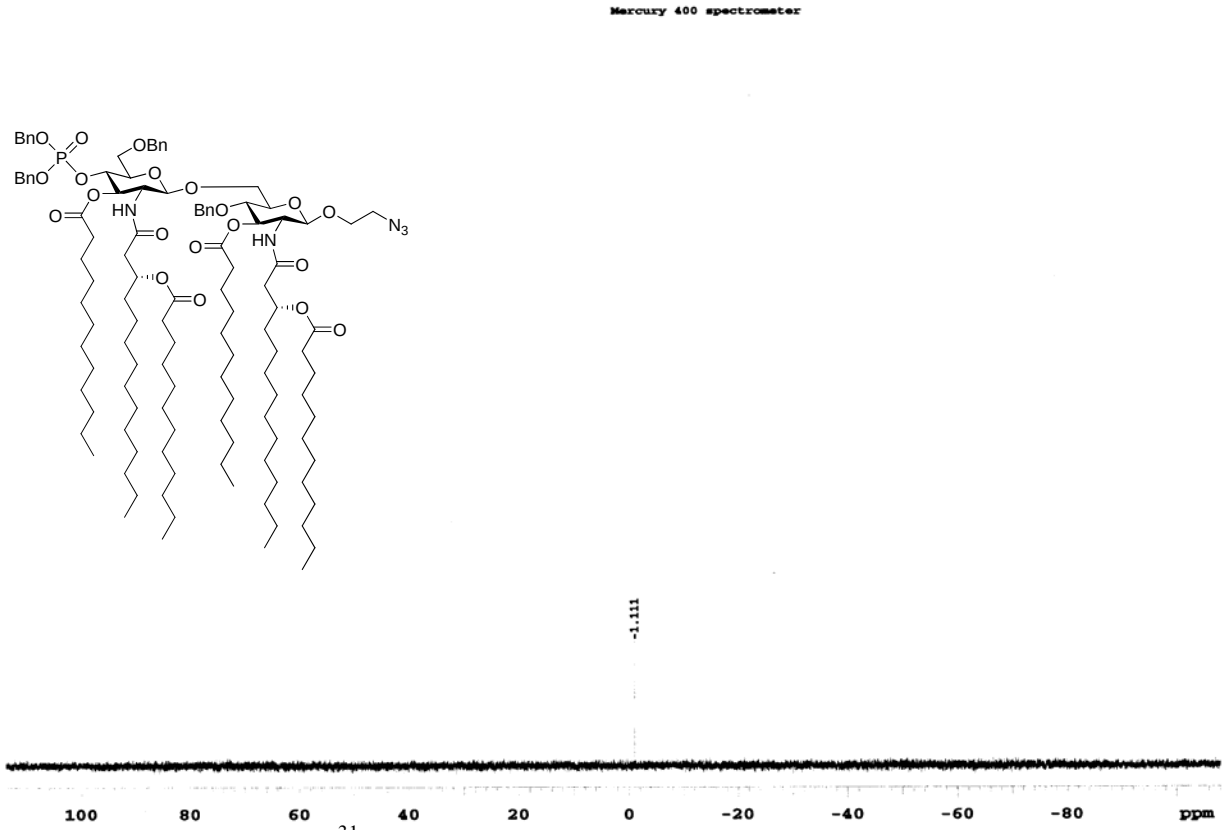
^{1}H NMR spectrum of **3** ($CDCl_3$, 400 MHz)



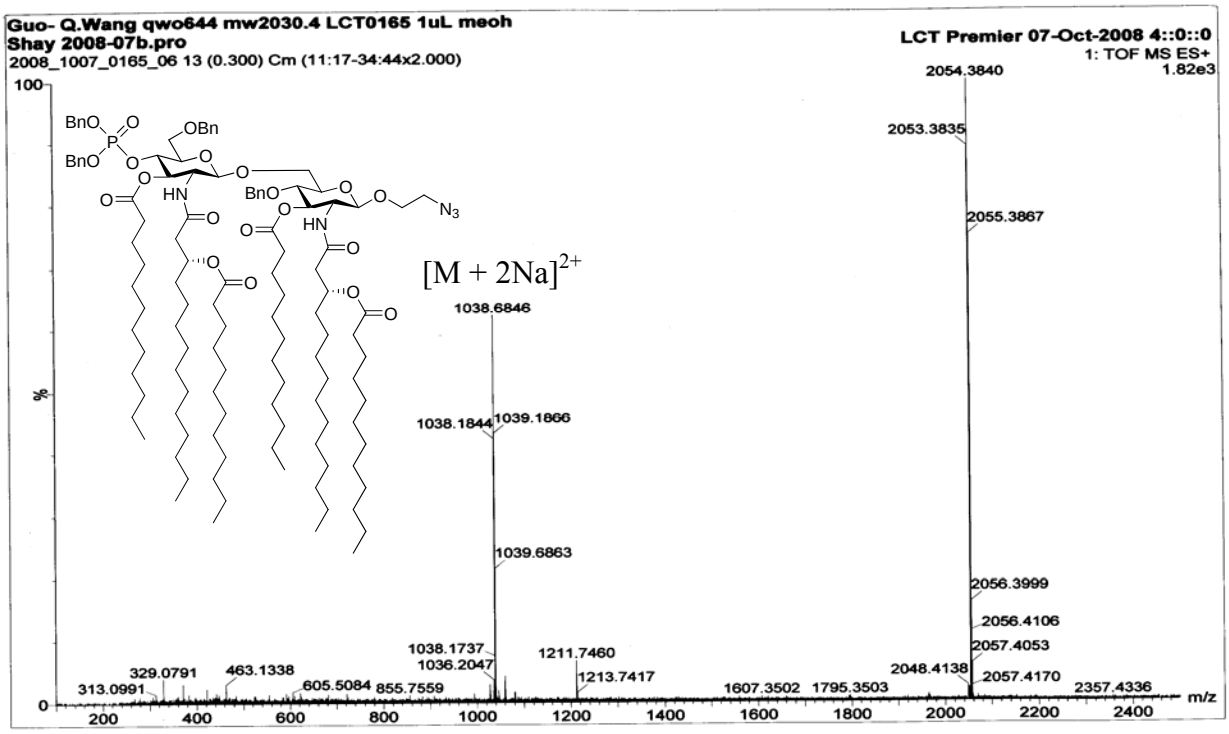
^{1}H-^{1}H COSY NMR spectrum of **3** ($CDCl_3$, 400 MHz)



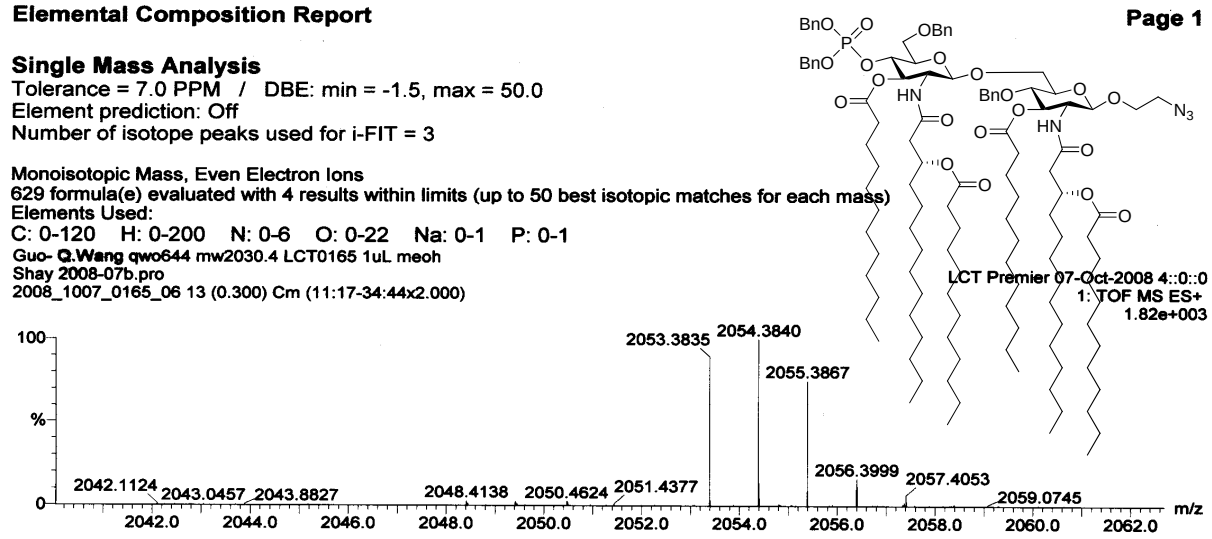
^{13}C NMR (including DEPT-135) spectrum of **3** ($CDCl_3$, 100 MHz)



^{31}P NMR spectrum of **3** ($CDCl_3$, 161 MHz)

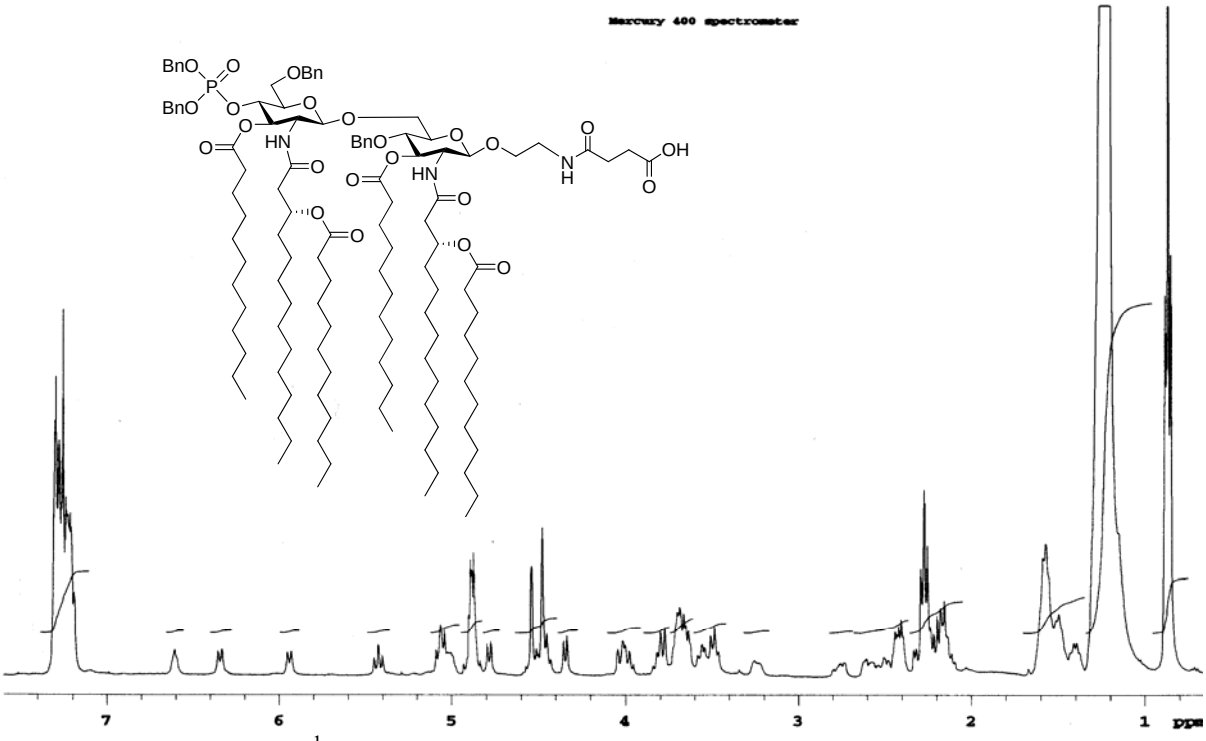


HR ESI MS spectrum of **3**

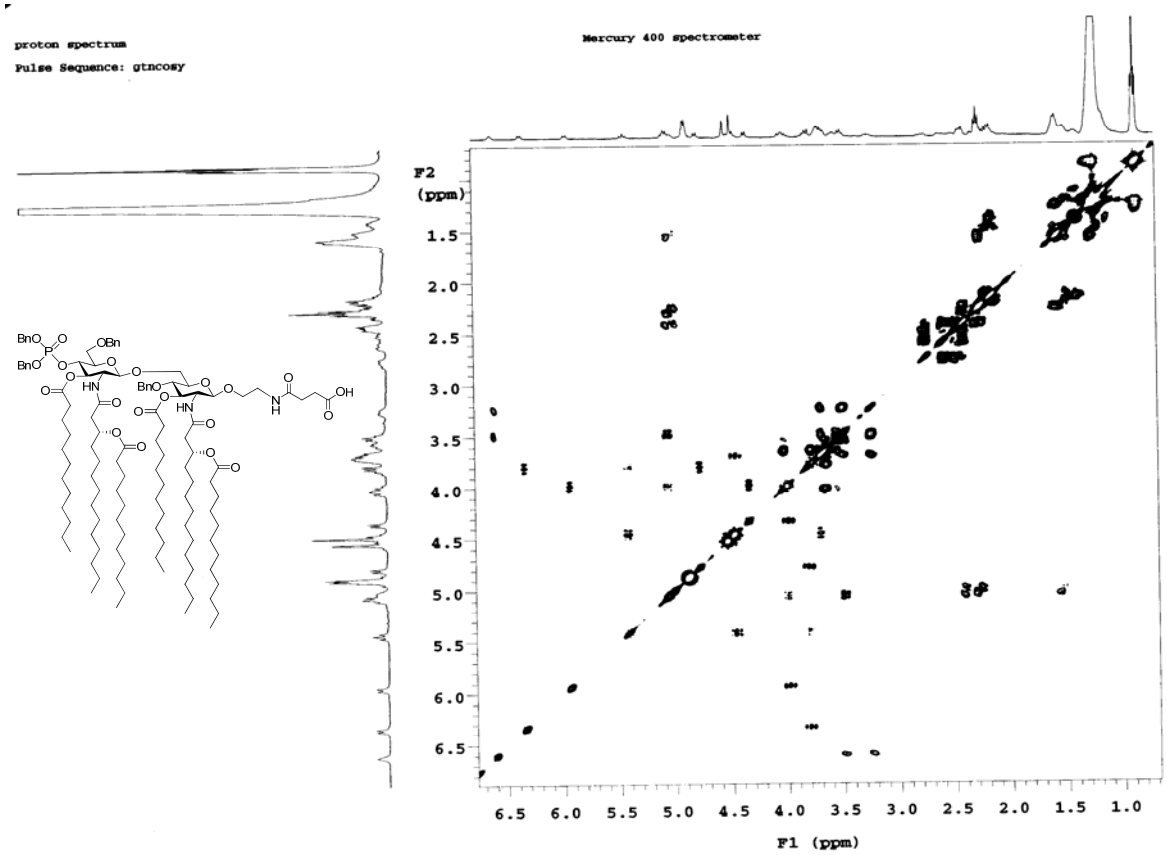


Minimum:				-1.5			
Maximum:		5.0	7.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2053.3835	2053.3796	3.9	1.9	25.5	82.9	0.9	C118 H192 N5 O20 Na P
	2053.3820	1.5	0.7	28.5	83.2	1.2	C120 H191 N5 O20 P
	2053.3878	-4.3	-2.1	25.5	83.6	1.6	C118 H191 N5 O22 Na
	2053.3902	-6.7	-3.3	28.5	84.6	2.6	C120 H190 N5 O22

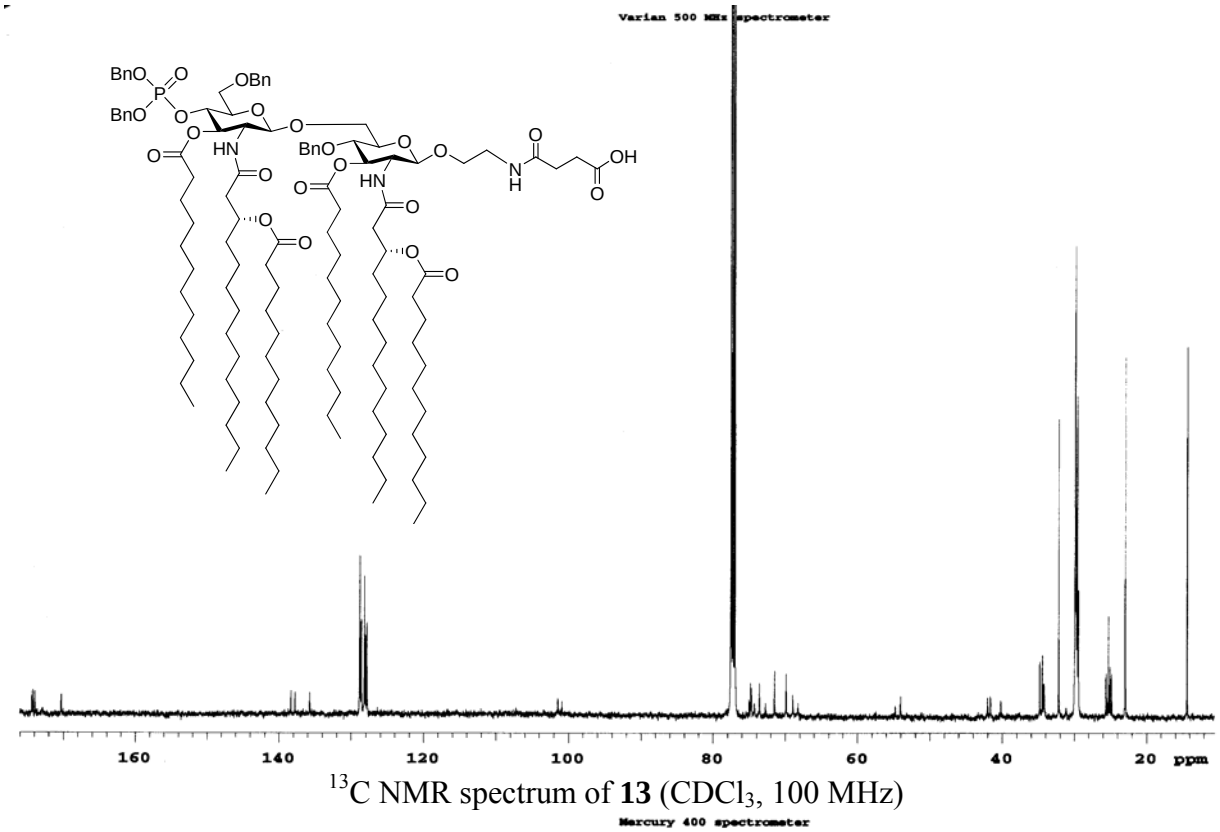
HR ESI MS spectrum of **3** (expansion)



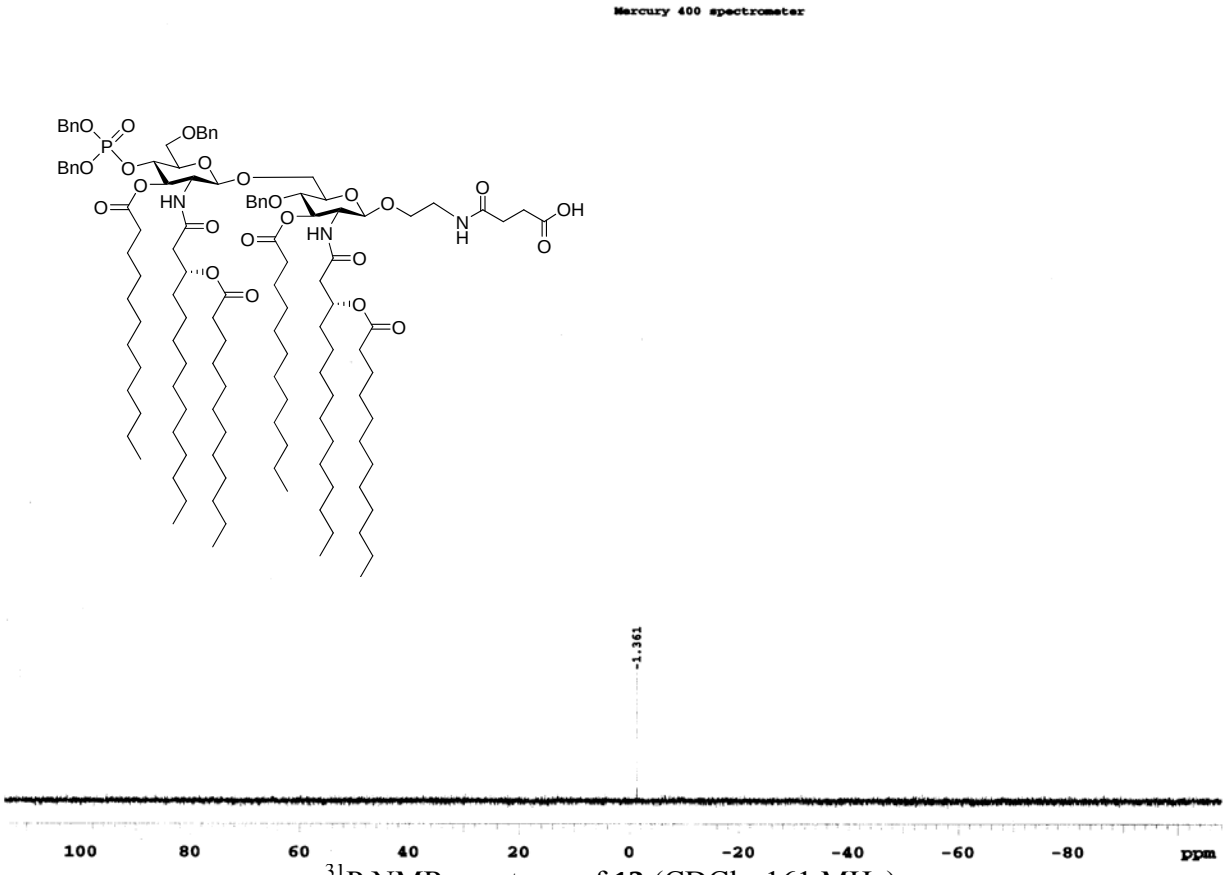
^{1}H NMR spectrum of **13** ($CDCl_3$, 400 MHz)



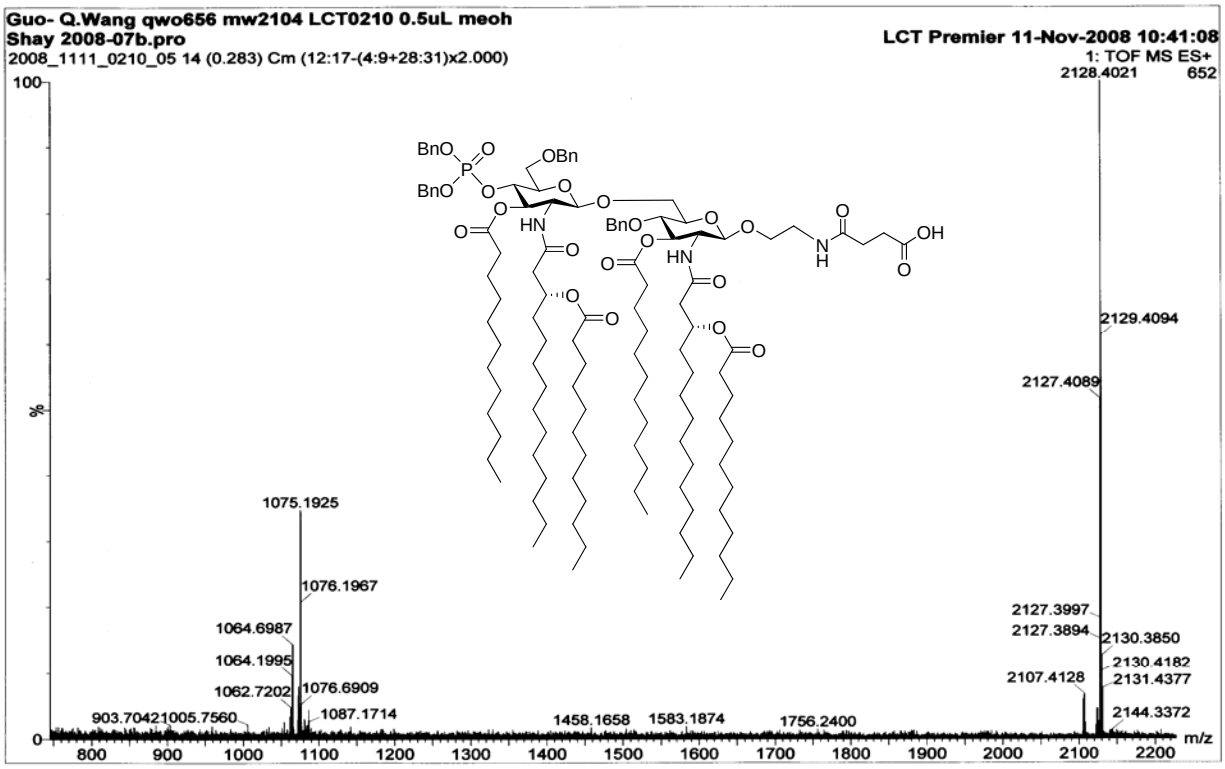
^{1}H-^{1}H COSY NMR spectrum of **13** ($CDCl_3$, 400 MHz)



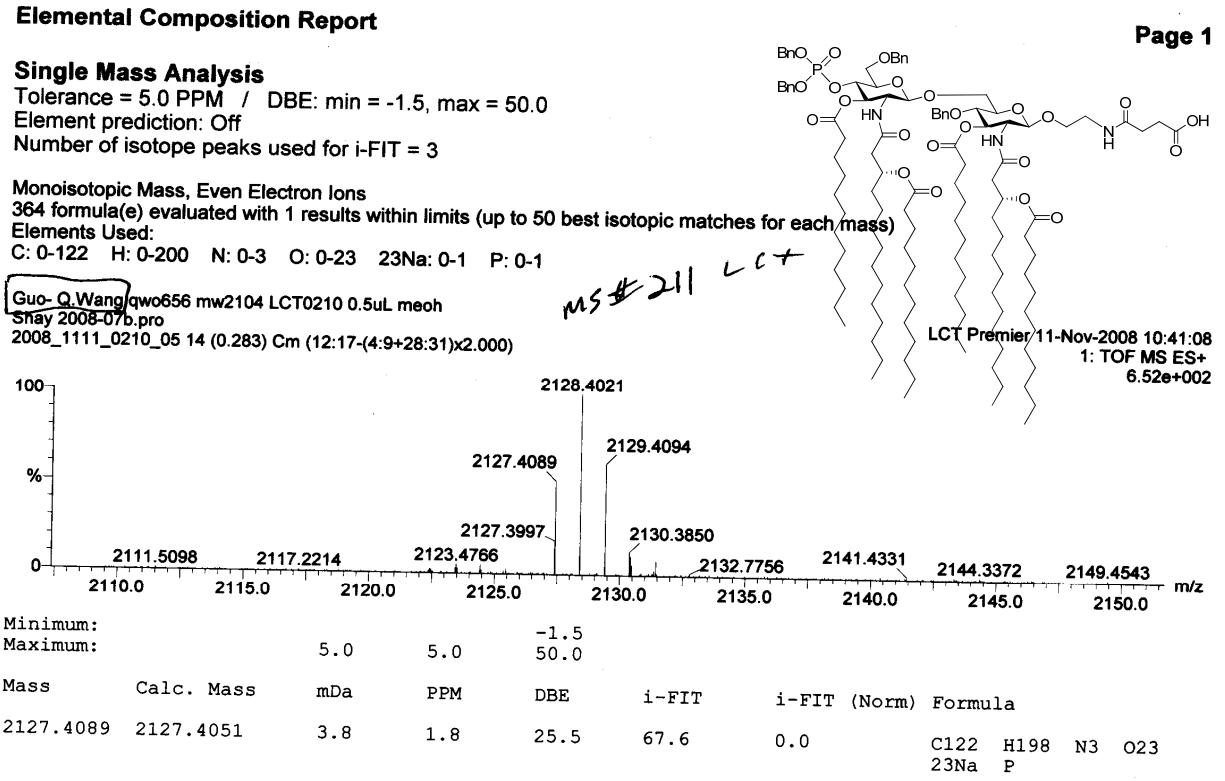
^{13}C NMR spectrum of **13** ($CDCl_3$, 100 MHz)



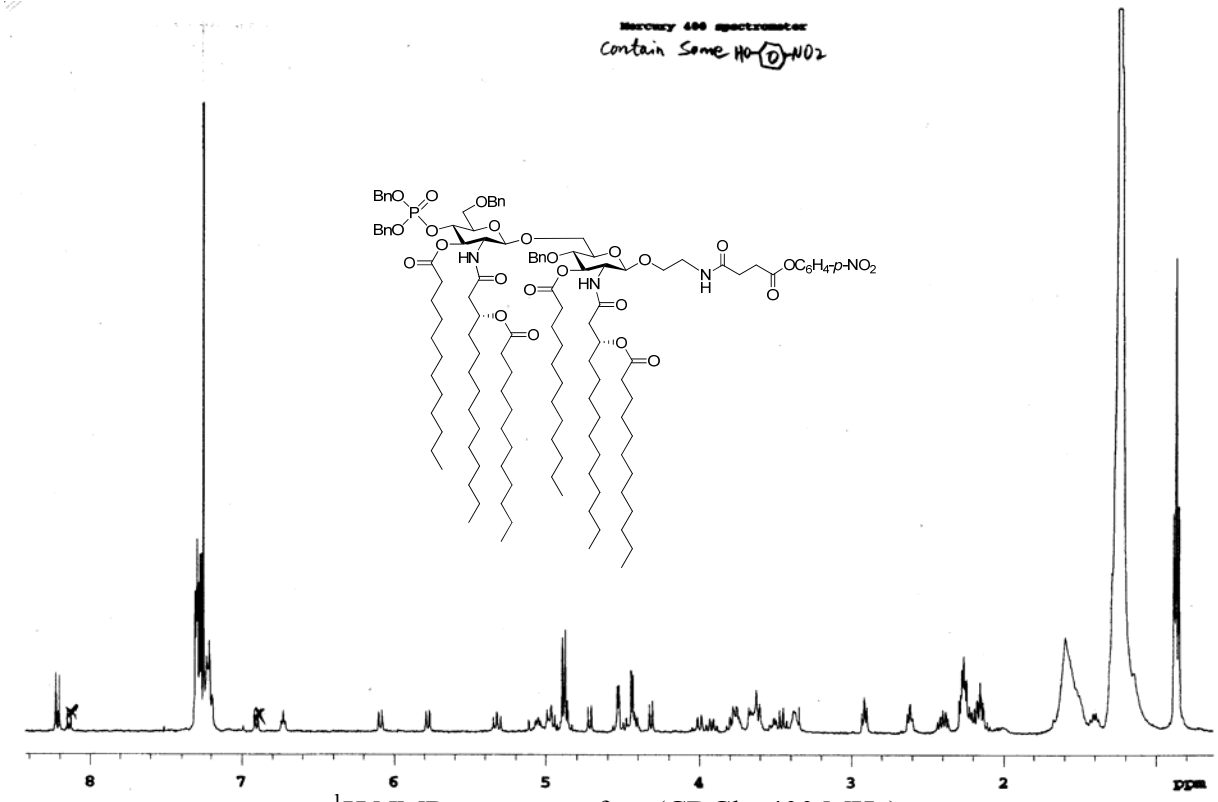
^{31}P NMR spectrum of **13** ($CDCl_3$, 161 MHz)



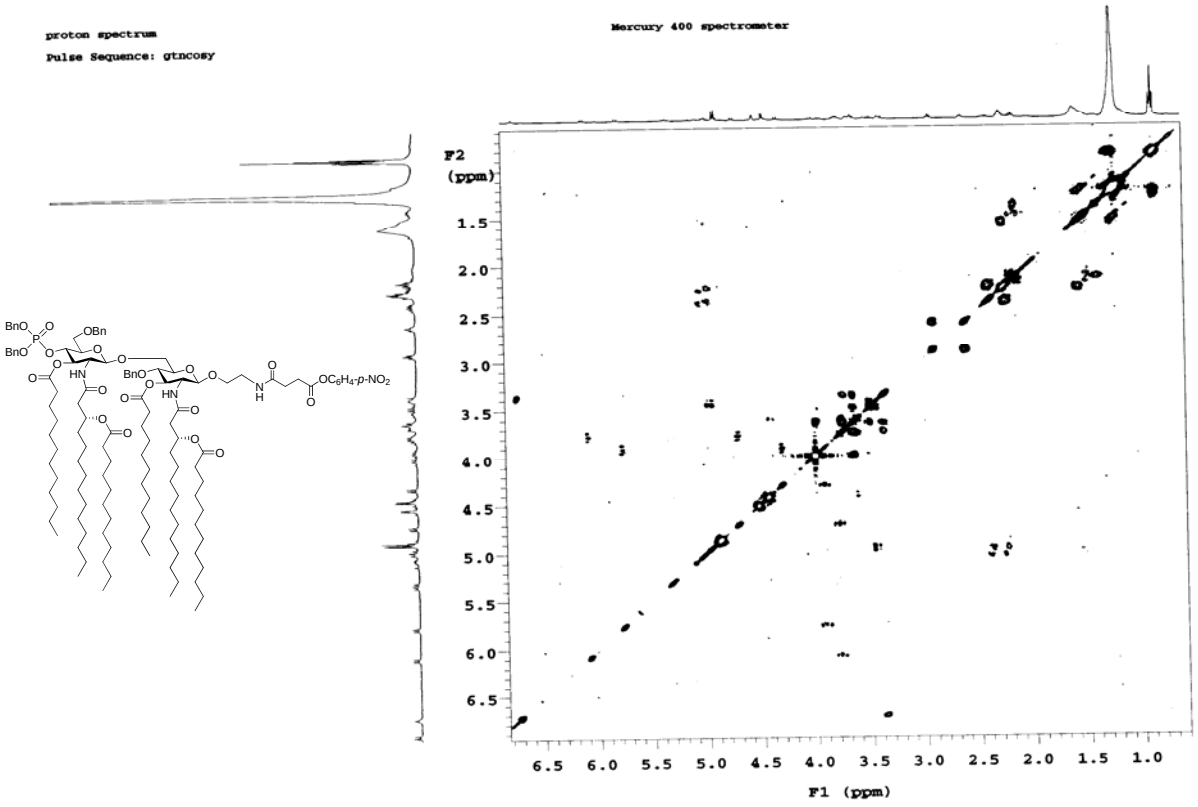
HR ESI MS spectrum of **13**



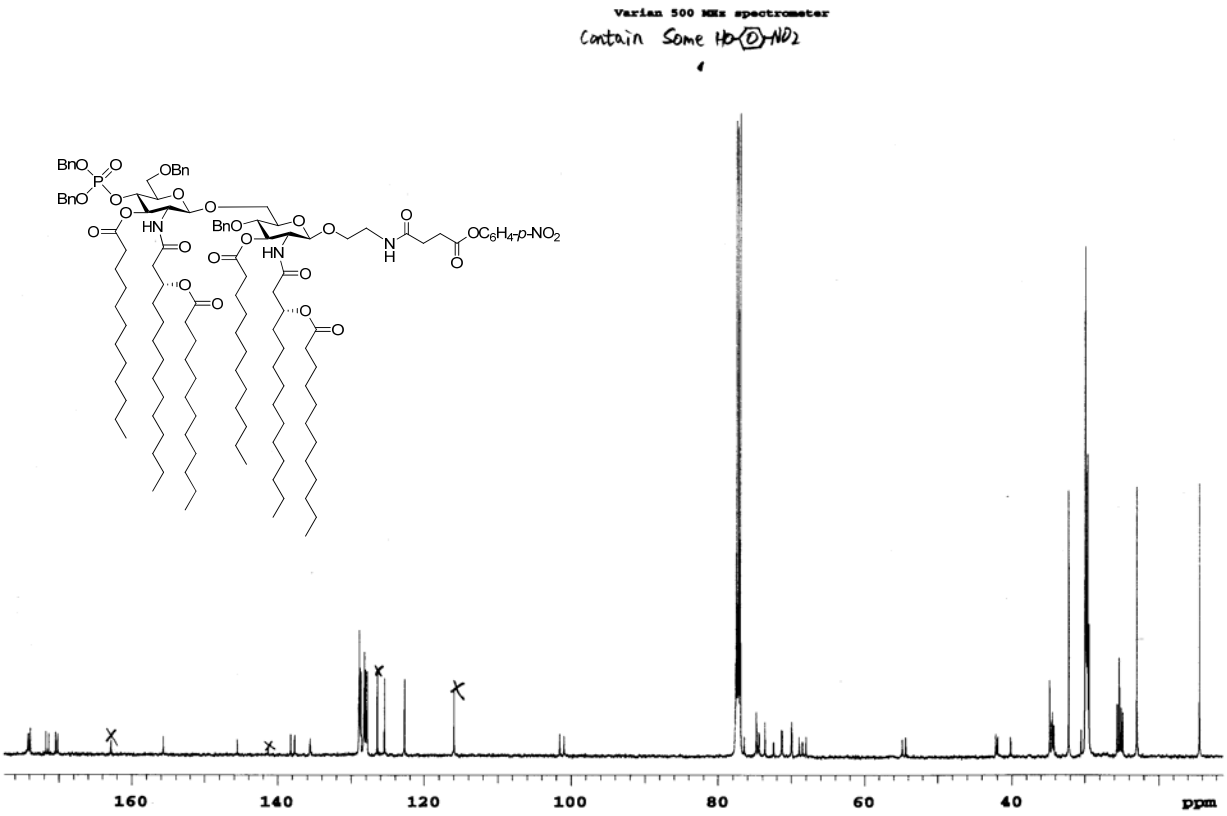
HR ESI MS spectrum of **13** (expansion)



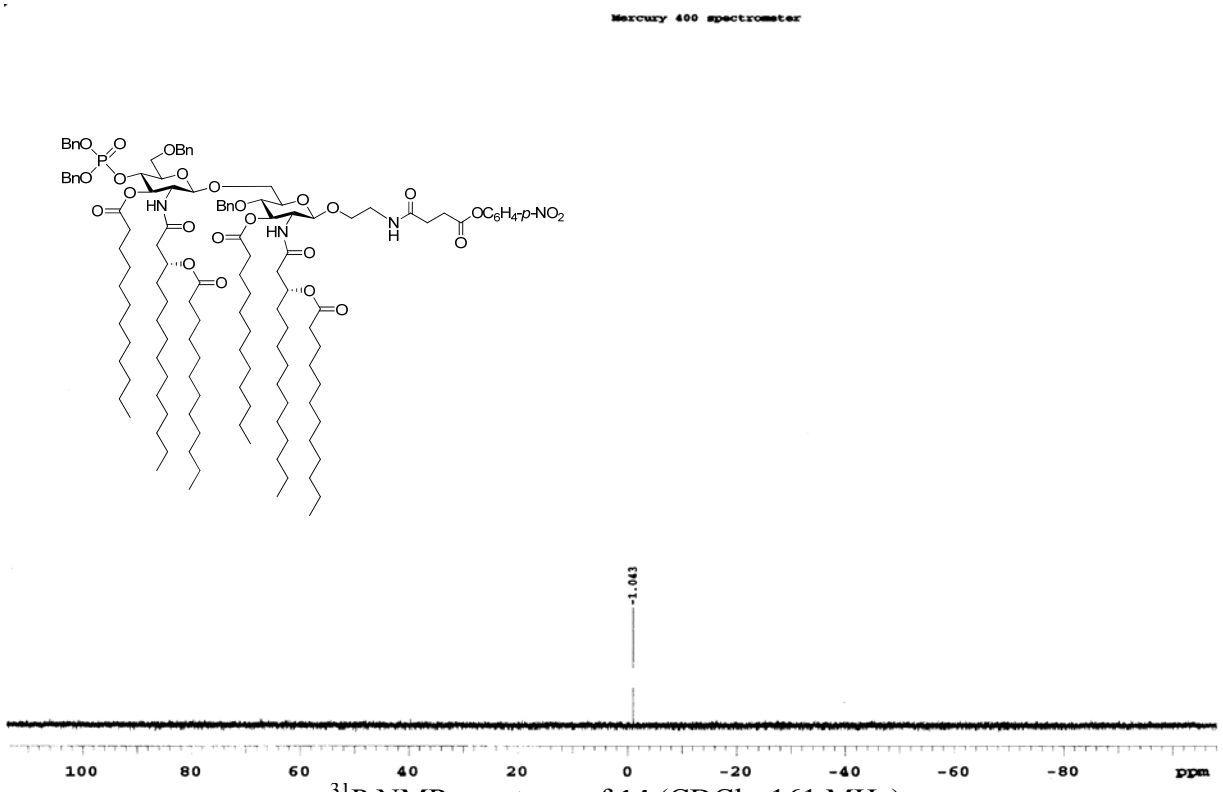
^{1}H NMR spectrum of **14** ($CDCl_3$, 400 MHz)



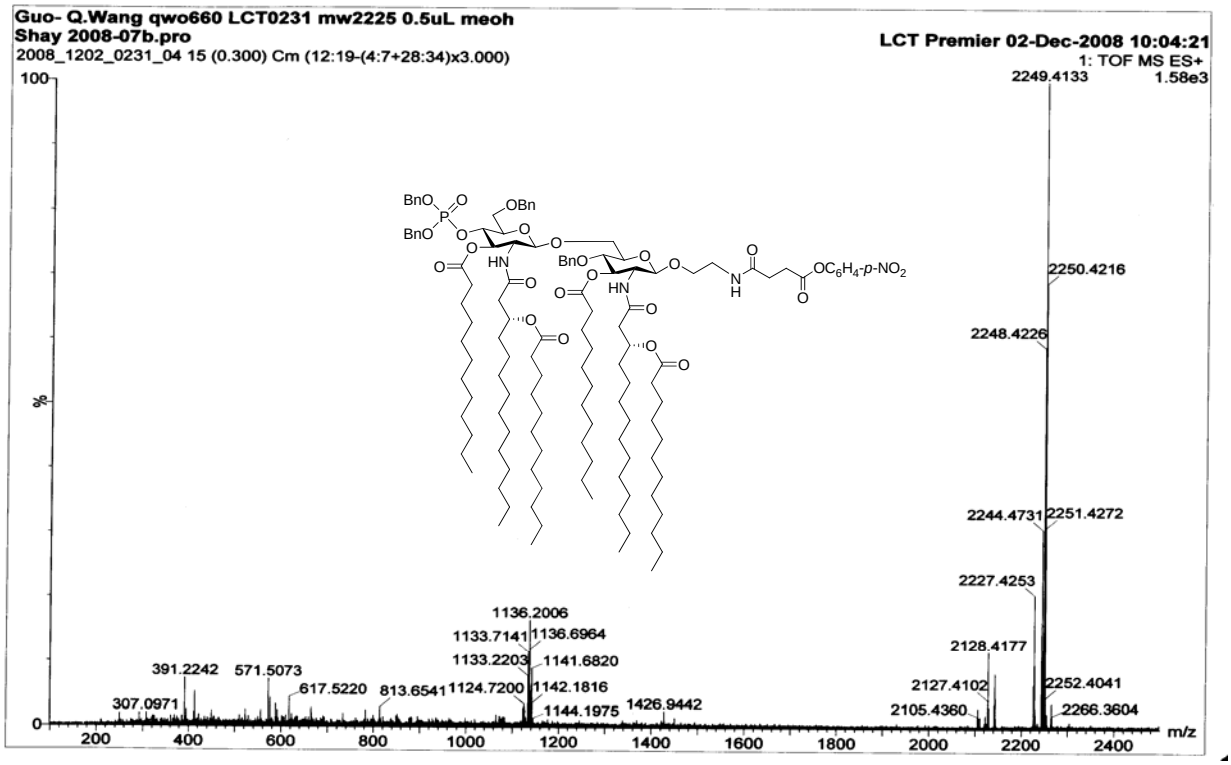
^{1}H-^{1}H COSY NMR spectrum of **14** ($CDCl_3$, 400 MHz)



^{13}C NMR spectrum of **14** ($CDCl_3$, 125 MHz)



^{31}P NMR spectrum of **14** ($CDCl_3$, 161 MHz)

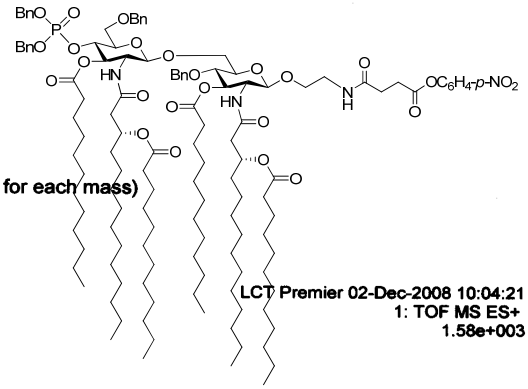


HR ESI MS spectrum of **14**

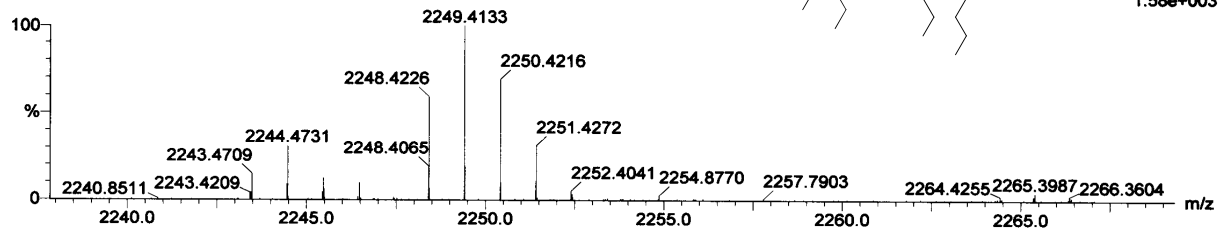
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
468 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-130 H: 0-210 N: 0-4 O: 0-25 23Na: 0-1 P: 0-1

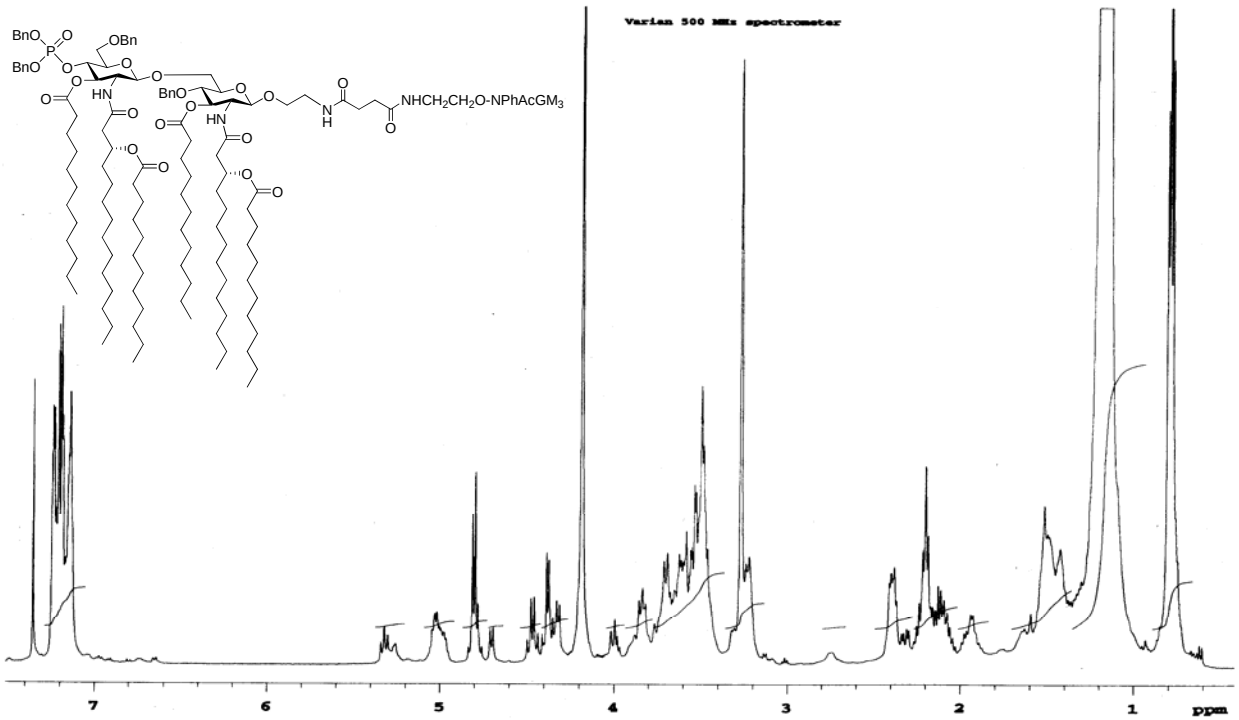


Guo- Q.Wang qwo660 LCT0231 mw2225 0.5uL meoh
Shay 2008-07b.pro
2008_1202_0231_04 15 (0.300) Cm (12:19-(4:7+28:34)x3.000)

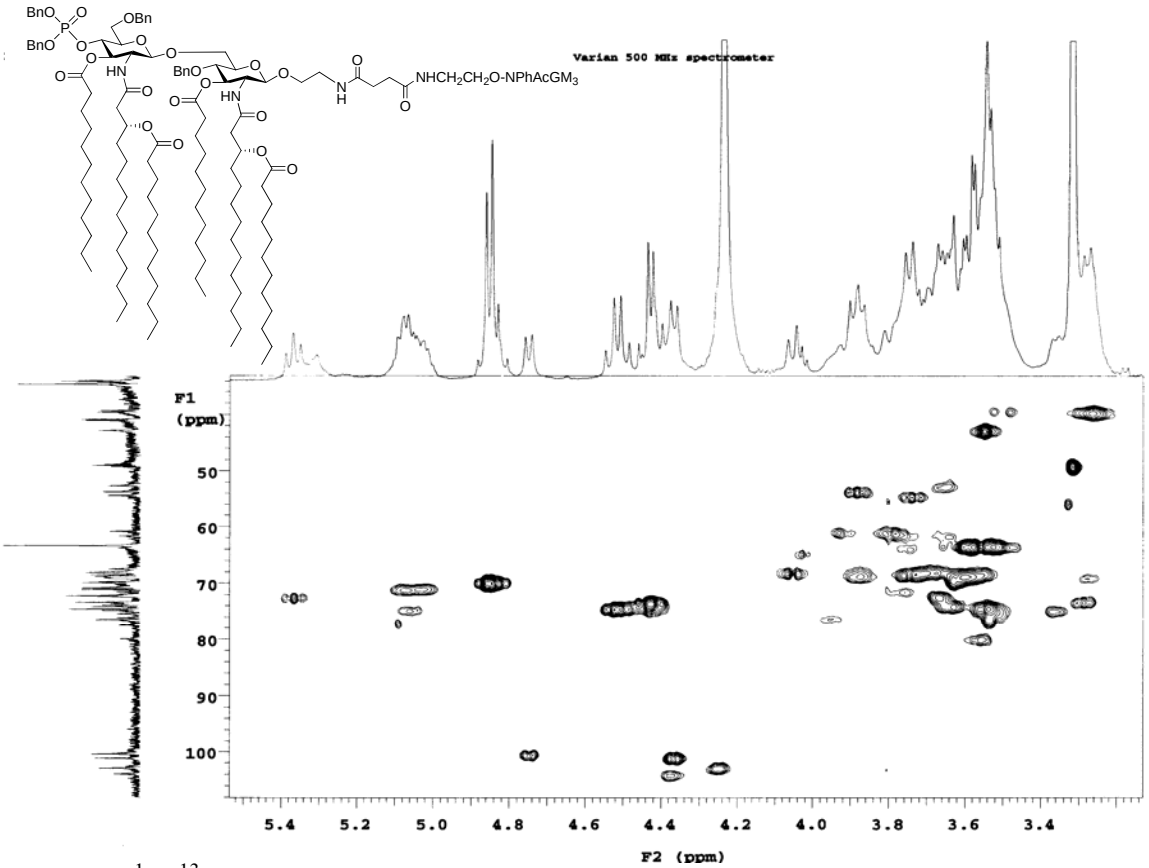


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2248.4226	2248.4215	1.1	0.5	30.5	70.2	0.5	✓C128 H201 N4 O25 23Na P
	2248.4239	-1.3	-0.6	33.5	70.5	0.9	C130 H200 N4 O25 P

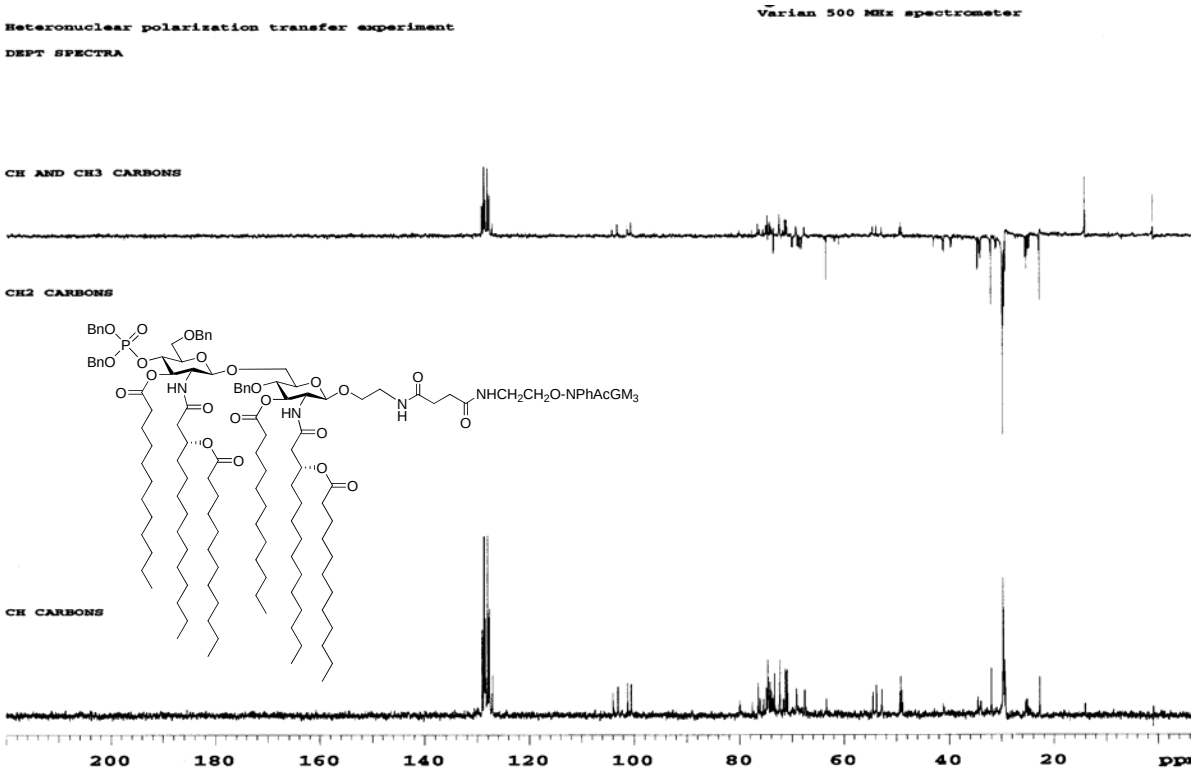
HR ESI MS spectrum of **14** (expansion)



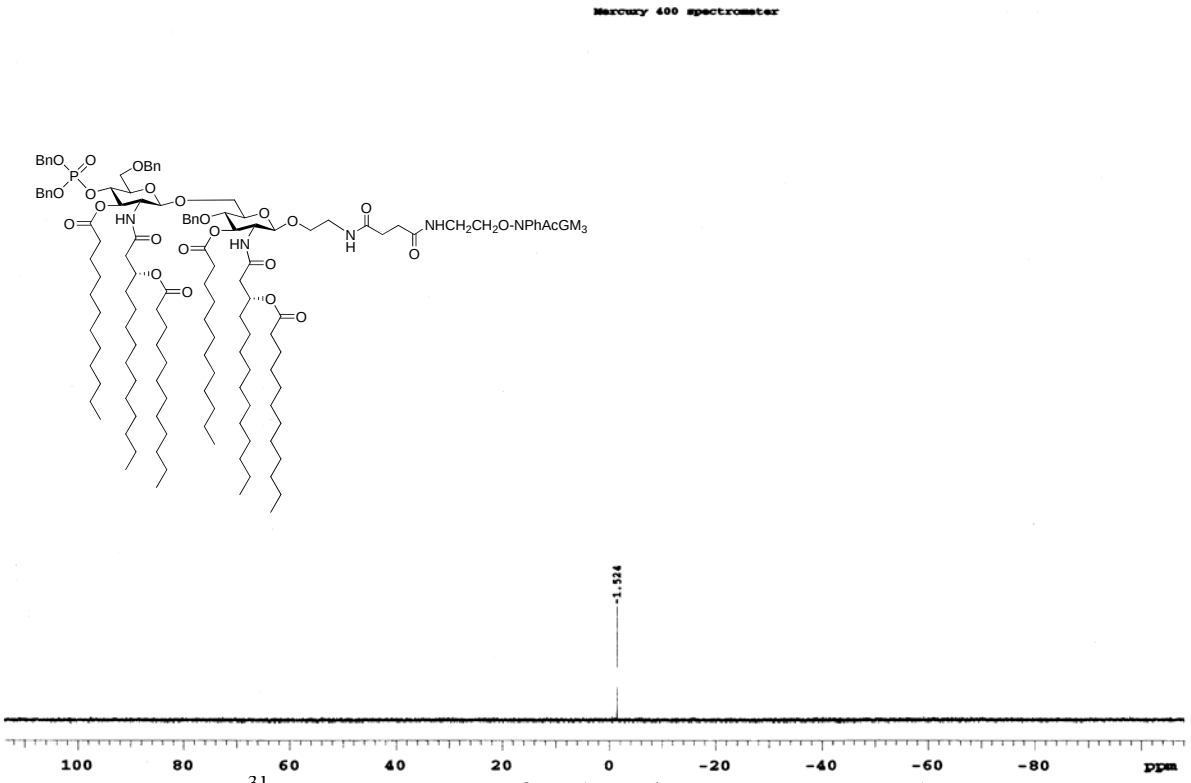
^{1}H NMR spectrum of **16** ($CDCl_3$-CD_3OD, 500 MHz)



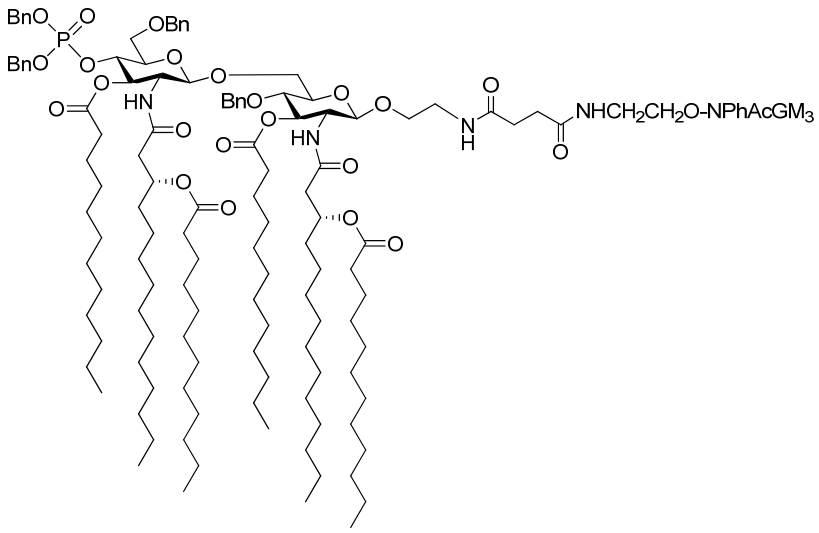
^{1}H-^{13}C HMQC NMR spectrum of **16** ($CDCl_3$-CD_3OD, 500/125 MHz)



^{13}C NMR spectrum of **16** (including DEPT-135, $CDCl_3$-CD_3OD, 125 MHz)



^{31}P NMR spectrum of **16** ($CDCl_3$-CD_3OD, 161 MHz)

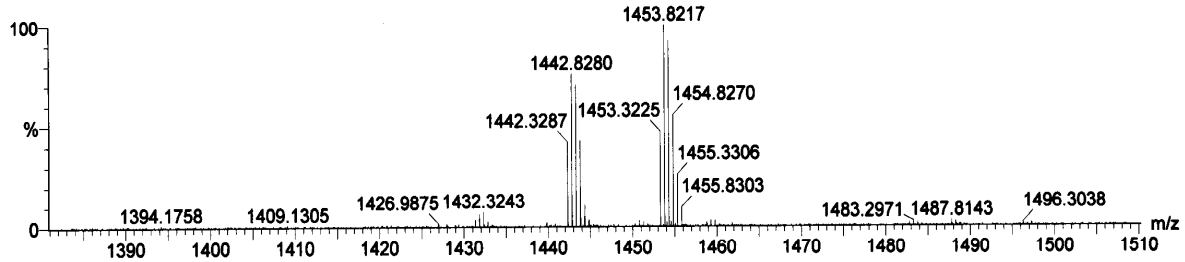


Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

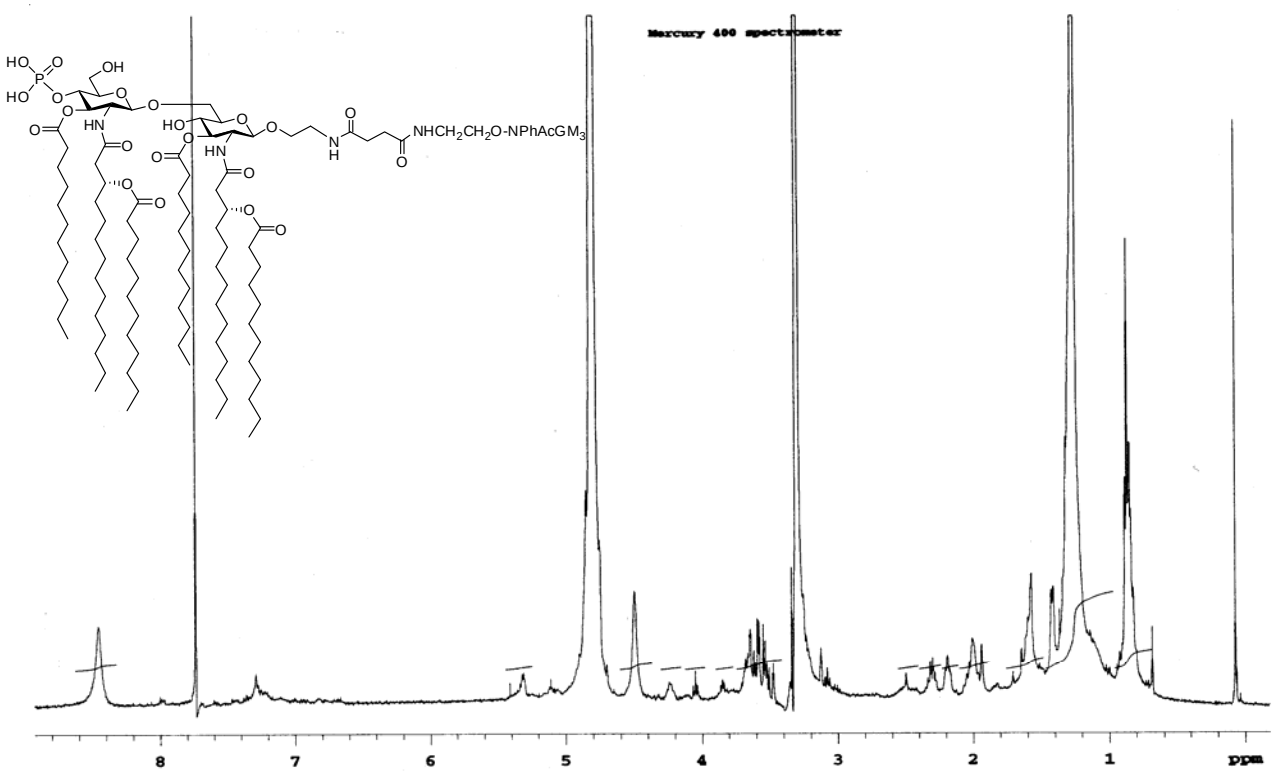
Monoisotopic Mass, Odd Electron Ions
1512 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 153-153 H: 0-1000 N: 0-5 O: 0-41 23Na: 0-2 P: 0-1
Guo- Q.Wang qwo663 LCT0232 mw2838 5uL meoh + Na
Shay 2008-07b.pro
2008_1202_0232_08 16 (0.317) Cm (10:22-(1:7+31:41)x3.000)

LCT Premier 02-Dec-2008 10:25:52
1: TOF MS ES+
3.10e+003

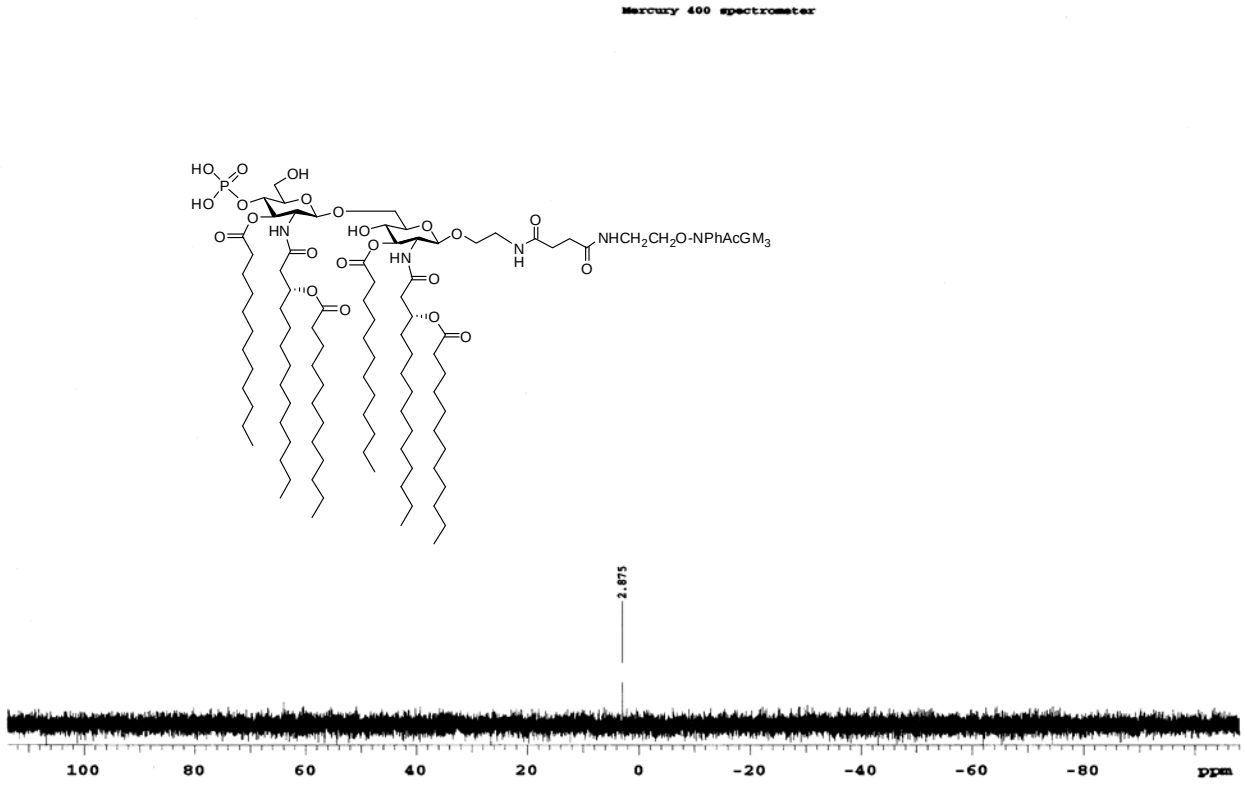


Minimum:				-1.5			
Maximum:		5.0	20.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
2884.6574	2884.6695	-12.1	-4.2	34.0	-1.$	n/a	C153 H244 N5 O41 23Na2 P

HR ESI MS spectrum of **16** (expansion)



^{1}H NMR spectrum of **23** ($CDCl_3$-CD_3OD, 400 MHz)



^{31}P NMR spectrum of **23** ($CDCl_3$-CD_3OD, 161 MHz)