Supporting Information for:

Bicyclic isourea derived from 1-deoxynojirimycin are potent inhibitors of β-glucocerebrosidase

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General Procedures

Reagents, solvents and solutions. Unless stated otherwise, chemicals were obtained from commercial sources and used without further purification. All solvents were purchased from Biosolve (Valkenswaard, The Netherlands) and were stored on molecular sieves (4 Å). 2,3,4,6-Tetra-O-benzyl-D-glucopyranose was obtained from Carbosynth Limited (MT06691). 2,3,4,6-Tetra-O-benzyl-1-deoxynojirimycin[1] (**10**), Cbz-NCS[2] and compounds **11a**[3], **12a**[3], **13a**[3] were prepared as previously described. The preparation of compounds **11e**, **12e**, **13e** and **14e** required access to non-commercial amine building block **16** that was prepared according to established literature procedures.

Purification Techniques. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F254). One or more of the following methods were used for visualization: 10% H_2SO_4 in MeOH, molybdenum blue, $KMnO_4$ or ninhydrine followed by warming until spots could be visible detected under UV light. Flash chromatography was performed using Merck type 60, 230–400 mesh silica gel. Removal of solvent was performed under reduced pressure using a rotary evaporator.

Instrumentation for Compound Characterization. For LC–MS analysis, an HPLC system (detection simultaneously at *l* 214, 254 nm and evaporative light detection) equipped with an analytical C_{18} column (100 Å pore size, 4.6 mm (⌀) x 250 mm (l), 10 µm particle size) or C_8 column (100 Å pore size, 4.6 mm (⌀) x 250 mm (l), 10 µm particle size) in combination with buffers A: H_2O, B: MeCN, A and B with 1.0% aqueous trifluoroacetic acid (TFA), in some cases - coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications (detection simultaneously at *l* 213, 254 nm), an automated HPLC system equipped with a preparative C_{18} column (20 mm (⌀) x 250 mm (l), 5 µm particle size) or C_8 column (20 mm (⌀) x 250 mm (l), 5 µm particle size) in combination with buffers A: H_2O, B: MeCN, A and B with 1.0% aqueous trifluoroacetic acid (TFA). High-resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in H_2O/MeCN 50:50 *v/v* and 0.1% formic acid) on a mass spectrometer.

^1H and ^{13}C NMR spectra were recorded on 500–125 MHz or 400–100 MHz spectrometers. Chemical shifts *(d)* are given in ppm relative to tetramethylsilane (TMS) as internal standard. All ^{13}C NMR spectra are proton decoupled. ^1H NMR data are reported in the following order: number of protons, multiplicity (*s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet and *m*, multiplet) and coupling constant (*J*) in Hertz (*Hz*). When appropriate, the multiplicity is preceded by *br*, indicating that the signal was broad. ^{13}C NMR spectra were recorded at 75.5 MHz with chemical shifts reported relative to $CDCl_3$ d 77.0. ^{13}C NMR spectra were recorded using the attached proton test (APT) sequence. All literature compounds had ^1H NMR and mass spectra consistent with the assigned structures.

References

1 Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. Development of Adamantan-1-Yl-Methoxy-Functionalized 1-Deoxynojirimycin Derivatives as Selective Inhibitors of Glucosylceramide Metabolism in Man. *J. Org. Chem.* **2007**, *72*, 1088–1097.

2 Martin, N. I.; Woodward, J. J.; Marletta, M. A. N G-Hydroxyguanidines from Primary Amines. *Org. Lett.* **2006**, *8*, 1–4.

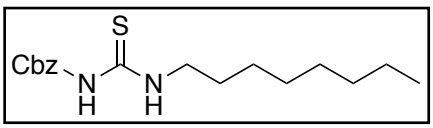
3 Kooij, R.; Branderhorst, H. M.; Bonte, S.; Wieclawska, S.; Martin, N. I.; Pieters, R. J. Glycosidase Inhibition by Novel Guanidinium and Urea Iminosugar Derivatives. *Medchemcomm* **2013**, *4*, 387.

Experimental Details

General procedure for synthesis of Thiourea Intermediates 11(a-e).

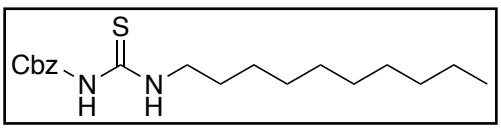
The amine of choice (1.2 eq) was dissolved in CH_2Cl_2 (100 mL) and treated with a 0.5 M solution of CbzNCS in CH_2Cl_2 (1 eq) and NEt_3 (10 eq). After stirring for 12 h at room temperature, TLC analysis indicated complete conversion to thiourea species. The CH_2Cl_2 was then removed under reduced pressure, and the residue dissolved in chloroform, and applied directly to a silica column, eluting with EtOAc/hexanes. Analytical data and characterization data for compounds **11(a-e)** are given below.

N-(Benzyloxycarbonyl)-N'-(octyl) thiourea (11a).



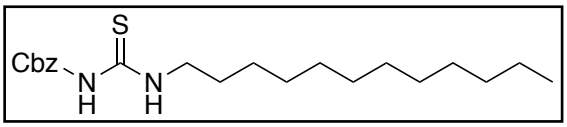
According to the literature procedure.[3] Yield: 1.6 g, 42%. R_f (EtOAc/hexanes = 1:8) = 0.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70 - 0.98 (m, 3H), 1.12 - 1.47 (m, 10H), 1.60 - 1.71 (m, 2H), 3.63 (td, *J* = 5.4, 7.2 Hz, 2H), 5.18 (s, 2H), 7.27 - 7.47 (m, 5H), 8.05 (br s, 1H), 9.61 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 152.5, 134.5, 129.1, 128.9, 128.8, 128.7, 128.6, 128.51, 128.46, 128.3, 77.3, 68.1, 45.8, 31.7, 29.1, 28.2, 26.9, 22.6, 14.1. HRMS (ESI, $[M+Na]^+$), calculated for $C_{17}H_{26}N_2O_2S$, 345.1613; found, 345.1580.

N-(Benzyloxycarbonyl)-N'-(decyl) thiourea (11b).



Yield: 2.7 g, 65%. R_f (EtOAc/hexanes = 1:8) = 0.33. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 3H), 1.20 - 1.44 (m, 14H), 1.57 - 1.71 (m, 2H), 3.63 (td, *J* = 5.4, 7.2 Hz, 2H), 5.17 (s, 2H), 7.31 - 7.47 (m, 5H), 8.07 (br s, 1H), 9.62 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 152.5, 134.5, 129.1, 129.0, 128.9, 128.8, 128.7, 128.3, 68.1, 45.8, 38.4, 31.9, 29.5, 29.2, 28.2, 26.9, 22.7, 14.1. HRMS (ESI, $[M+Na]^+$), calculated for $C_{19}H_{30}N_2O_2S$, 373.1926; found, 373.1890.

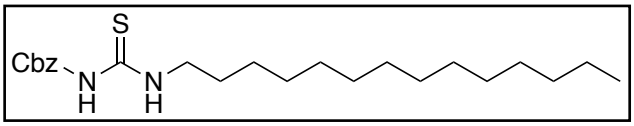
N-(Benzyloxycarbonyl)-N'-(dodecyl) thiourea (11c).



Yield: 3.4 g, 75%. R_f (EtOAc/hexanes = 1:8) = 0.56. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.4 Hz, 3H), 1.16 - 1.47 (m, 18H), 1.56 - 1.77 (m, 2H), 3.56 - 3.72 (m, 2H), 5.17 (s, 2H), 7.20 - 7.50 (m, 5H), 8.12 (s, 1H), 9.62 (br s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 152.5, 134.5, 134.3, 129.1, 129.0, 128.9, 128.8, 128.7, 128.3, 111.9, 77.4, 77.0,

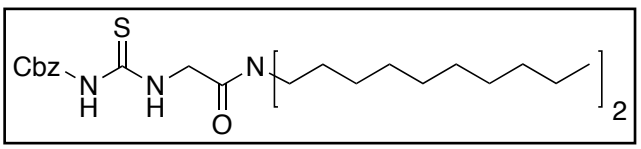
76.7, 68.1, 45.8, 38.4, 31.9, 29.61, 29.60, 29.53, 29.45, 29.3, 29.2, 28.2, 26.9, 22.7, 14.1. HRMS (ESI, $[M+Na]^+$), calculated for $C_{21}H_{34}N_2O_2S$, 401.2239; found, 401.2209.

N-(Benzyloxycarbonyl)-N'-(tetradecyl) thiourea (11d).



Yield: 3.9 g, 80%. R_f(EtOAc/hexanes = 1:8) = 0.6. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.8 Hz, 3H), 1.20 - 1.41 (m, 22H), 1.55 - 1.75 (m, 2H), 3.63 (td, *J* = 5.4, 7.2 Hz, 2H), 5.17 (s, 2H), 7.17 - 7.49 (m, 5H), 8.05 (s, 1H), 9.61 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.7, 152.4, 134.5, 134.3, 129.1, 128.94, 128.89, 128.8, 128.7, 128.3, 68.1, 45.8, 41.2, 38.4, 31.9, 29.7, 29.64, 29.63, 29.61, 29.5, 29.4, 29.3, 29.2, 28.2, 26.9, 22.7, 14.1. HRMS (ESI, $[M+Na]^+$), calculated for $C_{23}H_{38}N_2O_2S$, 429.2552; found, 429.2502.

Benzyl 2-(didecylamino)-(2-oxoethyl)aminothionylcarbamate (11e).

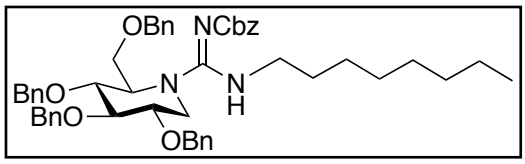


Yield: 0.86 g, 86%. R_f(EtOAc/hexanes = 1:8) = 0.16. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.7 Hz, 6H), 1.20 - 1.41 (m, 28H), 1.49 - 1.64 (m, 4H), 3.19 (t, *J* = 7.6 Hz, 2H), 3.35 (t, *J* = 7.8 Hz, 2H), 4.33 - 4.42 (m, 2H), 5.20 (s, 2H), 7.31 - 7.43 (m, 5H), 8.07 (s, 1H), 10.60 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 165.9, 152.0, 134.6, 129.0, 129.0, 128.8, 128.64, 128.62, 128.57, 128.5, 128.4, 128.2, 127.4, 77.3, 77.0, 76.7, 68.2, 67.9, 47.7, 46.9, 46.2, 34.6, 31.9, 31.8, 29.53, 29.51, 29.47, 29.4, 29.28, 29.26, 29.2, 28.6, 27.5, 27.0, 26.8, 22.7, 22.6, 14.1. HRMS (ESI, $[M+Na]^+$), calculated for $C_{31}H_{53}N_3O_3S$, 548.3886; found, 548.3897.

General Procedure for synthesis of Benzyl protected N-Substituted Guanidine Compounds 12(a-e).

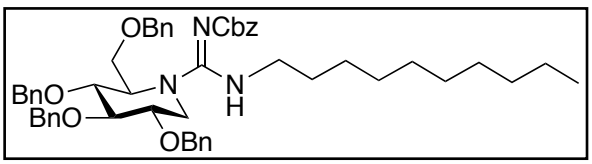
Thioureas **11(a-e)** (1.1 eq), OBn-DNJ (**10**, 1 eq) and EDCI (1.5 eq) were dissolved in CH_2Cl_2 (20 mL), followed by addition of NEt_3 (3 eq). Reaction mixture was stirred for 18h at room temperature, after which TLC analysis confirmed total consumption of starting material. The solvent was then removed under reduced pressure, and the residue, dissolved in $CHCl_3$, was applied directly to a silica column, eluting with hexanes chaser and subsequently with EtOAc/hexanes. Analytical data and characterization data for compounds **12(a-e)** are given below.

Benzyl ((Z)-(octylamino)((2S, 3S, 4R)-3,4,5-tris(benyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (12a).



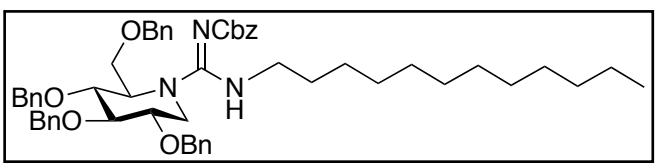
According to the literature procedure.[3] Yield: 340 mg, 75%. R_f (EtOAc/hexanes = 2:3) = 0.30. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.0 Hz, 3H), 1.07 - 1.48 (m, 12H), 2.92 - 3.14 (m, 2H), 3.43 (dd, *J* = 3.5, 13.8 Hz, 1H), 3.53 - 3.86 (m, 6H), 4.01 (dd, *J* = 5.2 Hz, 1H), 4.30 - 4.80 (m, 8H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.16 (d, *J* = 12.6 Hz, 1H), 6.90 - 7.70 (m, 25H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 160.9, 138.0, 137.88, 137.86, 137.6, 128.40, 128.35, 128.2, 127.93, 127.90, 127.86, 127.82, 127.80, 127.74, 127.72, 127.70, 127.68, 127.4, 80.7, 77.8, 77.4, 77.0, 76.7, 75.4, 73.4, 73.1, 72.7, 71.3, 69.8, 66.6, 58.7, 44.5, 44.0, 31.8, 29.6, 29.2, 29.1, 26.8, 22.6, 14.1. HRMS (ESI, $[M+H]^+$), calculated for $C_{51}H_{61}N_3O_6$, 812.4639; found, 812.4623.

Benzyl ((Z)-(decylamino)((2S, 3S, 4R)-3,4,5-tris(benyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (12b).



Yield: 790 mg, 85%. R_f (EtOAc/hexanes = 2:3) = 0.32. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.05 - 1.39 (m, 16H), 2.89 - 3.19 (m, 2H), 3.43 (dd, *J* = 3.5, 13.7 Hz, 1H), 3.56 - 3.85 (m, 6H), 4.00 (ddd, *J* = 5.2 Hz, 1H), 4.30 - 4.78 (m, 8H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.16 (d, *J* = 12.6 Hz, 1H), 7.13 - 7.51 (m, 25H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 160.9, 138.0, 137.9, 137.9, 137.5, 128.4, 128.3, 128.3, 128.1, 127.92, 127.89, 127.84, 127.80, 127.78, 127.72, 127.70, 127.68, 127.66, 127.3, 80.7, 77.8, 77.3, 77.0, 76.7, 75.4, 73.4, 73.1, 72.7, 71.3, 69.8, 66.6, 58.7, 44.5, 44.0, 31.9, 29.7, 29.51, 29.46, 29.3, 29.2, 26.8, 22.7, 14.1. HRMS (ESI, $[M+H]^+$), calculated for $C_{53}H_{65}N_3O_6$, 840.4952; found, 840.4916.

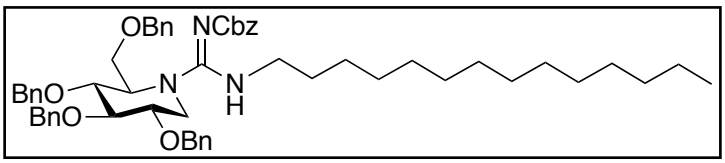
Benzyl ((Z)-(dodecylamino)((2S, 3S, 4R)-3,4,5-tris(benyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (12c).



Yield: 420 mg, 86%. R_f (EtOAc/hexanes = 2:3) = 0.33. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (d, *J* = 7.1 Hz, 3H), 1.02 - 1.54 (m, 20H), 2.89 - 3.20 (m, 2H), 3.43 (dd, *J* = 3.7, 13.1 Hz, 1H), 3.54 - 3.88 (m, 6H), 3.93 - 4.08 (m, 1H), 4.31 - 4.80 (m, 8H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.16 (d, *J* = 12.6 Hz, 1H), 7.08 - 7.71 (m, 25H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.0, 160.9, 138.0, 137.9, 137.9, 137.6, 128.39, 128.35, 128.2, 127.93, 127.90, 127.86, 127.8, 127.74, 127.72, 127.69, 127.67,

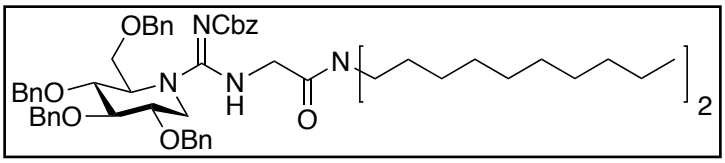
127.4, 80.7, 77.8, 77.3, 77.0, 76.7, 75.4, 73.4, 73.1, 72.7, 71.3, 69.8, 66.6, 58.7, 44.5, 44.0, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 26.8, 22.7, 14.1. HRMS (ESI, $[M+H]^+$), calculated for $C_{55}H_{69}N_3O_6$, 868.5265; found, 868.5234.

Benzyl ((Z)-(tetradecylamino)((2S, 3S, 4R)-3,4,5-tris(benyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (12d).



Yield: 392 mg, 79%. R_f (EtOAc/hexanes = 2:3) = 0.35. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 - 0.98 (m, 3H), 1.05 - 1.52 (m, 24H), 2.87 - 3.19 (m, 2H), 3.43 (dd, *J* = 3.5, 13.7 Hz, 1H), 3.56 - 3.85 (m, 6H), 3.94 - 4.09 (m, 1H), 4.35 - 4.74 (m, 8H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.16 (d, *J* = 12.6 Hz, 1H), 7.16 - 7.51 (m, 25H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.1, 161.0, 138.1, 138.0, 137.9, 137.9, 137.6, 128.40, 128.36, 128.35, 128.2, 127.94, 127.91, 127.86, 127.82, 127.80, 127.74, 127.72, 127.70, 127.68, 127.4, 80.7, 77.9, 77.4, 77.0, 76.7, 75.4, 73.4, 73.1, 72.8, 71.3, 69.8, 66.6, 58.7, 44.5, 44.0, 31.9, 29.71, 29.69, 29.67, 29.6, 29.5, 29.4, 29.2, 26.8, 22.7, 14.1. HRMS (ESI, $[M+H]^+$), calculated for $C_{57}H_{73}N_3O_6$, 896.5578; found, 896.5557.

Benzyl ((Z)-((2-(didecylamino)-2-oxoethyl)amino)((2S,3S,4R)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidin-1-yl)methylene)carbamate (12e).

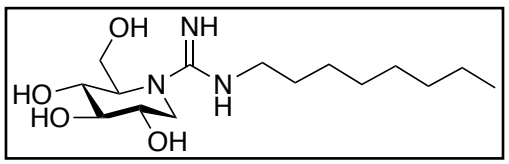


Yield: 622 mg, 54%. R_f (EtOAc/hexanes = 2:3) = 0.52. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.0 Hz, 6H), 1.06 - 1.36 (m, 28H), 1.42 - 1.54 (m, 4H), 2.91 (t, *J* = 7.6 Hz, 2H), 3.15 - 3.37 (m, 2H), 3.47 (dd, *J* = 3.7, 14.1 Hz, 1H), 3.57 - 4.01 (m, 8H), 4.20 (q, *J* = 5.0 Hz, 1H), 4.33 - 4.78 (m, 8H), 5.11 (d, *J* = 12.6 Hz, 1H), 5.17 (d, *J* = 12.6 Hz, 1H), 7.10 - 7.49 (m, 25H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 160.3, 138.5, 138.2, 138.1, 138.0, 128.48, 128.45, 128.4, 128.3, 128.2, 128.13, 128.10, 127.9, 127.84, 127.79, 127.7, 127.5, 81.7, 78.4, 77.5, 77.2, 76.8, 75.1, 73.4, 73.2, 73.0, 71.3, 69.4, 66.7, 58.4, 46.7, 46.1, 44.5, 43.9, 32.02, 32.01, 29.73, 29.70, 29.68, 29.6, 29.5, 29.4, 28.7, 27.7, 27.2, 27.0, 22.8, 14.3. HRMS (ESI, $[M+H]^+$), calculated for $C_{65}H_{88}N_4O_7$, 1037.6731; found, 1037.6685.

General procedure for Pd/C catalyzed hydrogenolysis for synthesis of N-Substituted Guanidine Compounds 13(a-e).

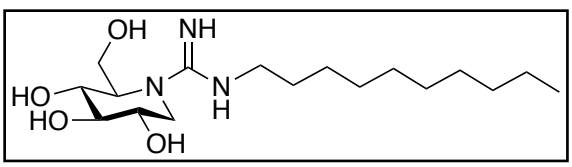
The perbenzylated iminosugar was dissolved in a mixture of glacial AcOH in MeOH (1/1, v/v) and transferred to a Parr high-pressure hydrogenation flask. A catalytic amount of 10% palladium on carbon was added to the flask (ca. 10 mg catalyst/mg of benzylated starting material). The flask was put under reduced pressure and ventilated with hydrogen gas. This procedure was repeated twice after which the pressure was adjusted to 4.5-5.0 bar hydrogen pressure. The reaction was allowed to proceed for 6-12 h while mechanically shaken and the pressure maintained at the value initially set. The mixture was filtered over celite on a glass microfiber filter, followed by rinsing the filter with MeOH. The mixture was concentrated under reduced pressure. The crude products thus obtained were purified using RP-HPLC employing a preparative C8 column and an H_2O/MeCN gradient moving from 5% to 95% MeCN (0.1% TFA) over 60 min (flow rate, 18.0 mL/min). Product **13e** was purified using an H_2O/MeCN gradient moving from 50% to 95% MeCN (0.1% TFA) over 90 min (flow rate, 18.0 mL/min). Fractions containing the desired product were combined and lyophilized to yield the pure compounds as amorphous white powders. Analytical data and in-depth characterization data for compounds **13(a-e)** are given below.

(2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)-N-octylpiperidine-1-carboximidamide (13a).



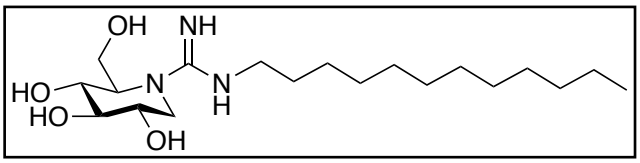
According to previously reported procedure.[3] Yield: 16 mg, 82%. ^{1}H NMR (500 MHz, D_2O) 0.74-0.86 (t, 3H), 1.16-1.34 (m, 12H), 1.50-1.62 (m, 2H), 3.17-3.26 (m, 2H), 3.50-3.55 (m, 2H), 3.60 (d, 2H), 3.76- 3.82 (m, 2H), 3.82-3.90 (m, 2H). ^{13}C NMR (126 MHz, D_2O) 87.5, 85.0, 82.6, 78.3, 73.8, 59.8, 56.8, 45.6, 42.8, 42.5, 40.3, 36.6, 28.0. HRMS (ESI, $[M+H]^+$), calculated for $C_{15}H_{31}N_3O_4$, 318.2393; found, 318.2414.

(2R,3R,4R,5S)-N-decyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide (13b).



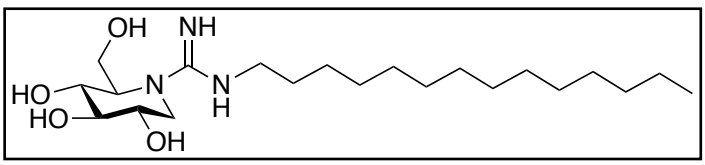
Yield: 8 mg, 75%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.8 Hz, 3H), 1.16 - 1.37 (m, 14H), 1.40 - 1.59 (m, 2H), 3.12 - 3.24 (m, 2H), 3.37 - 3.56 (m, 4H), 3.57 - 3.68 (m, 2H), 3.68 - 3.78 (m, 2H), 5.19 - 5.40 (m, 1H), 5.40 - 5.60 (m, 1H), 7.35 - 7.53 (m, 3H), 7.67 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 87.4, 85.1, 83.9, 78.5, 74.6, 74.0, 60.0, 59.4, 57.1, 46.4, 44.0, 43.6, 41.1, 37.3, 29.1. HRMS (ESI, $[M+H]^+$), calculated for $C_{17}H_{35}N_3O_4$, 346.2706; found, 346.2721.

(2R,3R,4R,5S)-N-dodecyl-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamide (13c).



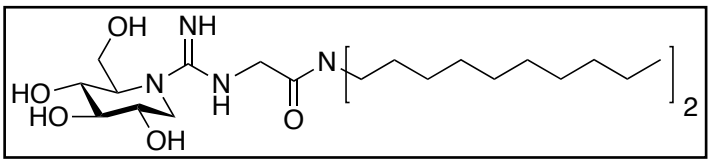
Yield: 12 mg, 82%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.8 Hz, 3H), 1.18 - 1.35 (m, 18H), 1.41 - 1.59 (m, 2H), 3.10 - 3.22 (m, 2H), 3.35 - 3.56 (m, 4H), 3.58 - 3.68 (m, 2H), 3.70 - 3.79 (m, 2H), 5.29 (dd, J = 4.0, 5.6 Hz, 2H), 5.39 - 5.55 (m, 2H), 7.36 - 7.50 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 87.4, 85.1, 83.9, 78.6, 74.66, 74.65, 60.0, 57.1, 46.5, 44.0, 43.6, 41.2, 36.6, 29.1. HRMS (ESI, [M+H]$^+$), calculated for $C_{19}H_{39}N_3O_4$, 374.3019; found, 374.3021.

(2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)-N-tetradecylpiperidine-1-carboximidamide (13d).



Yield: 7 mg, 79%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (t, J = 6.7 Hz, 3H), 1.17 - 1.33 (m, 22H), 1.42 - 1.56 (m, 2H), 3.12 - 3.21 (m, 2H), 3.42 - 3.56 (m, 4H), 3.57 - 3.69 (m, 2H), 3.70 - 3.78 (m, 2H), 5.19 - 5.39 (m, 2H), 5.47 (s, 1H), 7.33 - 7.56 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 86.8, 85.1, 83.3, 78.6, 74.6, 74.1, 59.38, 57.1, 46.5, 43.9, 43.6, 41.2, 37.2, 29.1. HRMS (ESI, [M+H]$^+$), calculated for $C_{21}H_{43}N_3O_4$, 402.3332; found, 402.3328.

N,N-didecyl-2-((2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidine-1-carboximidamido)acetamide (13e).



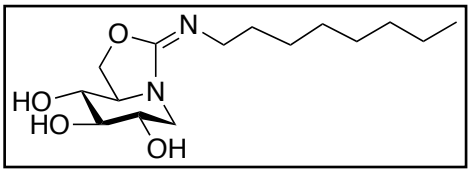
Yield: 15 mg, 89%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.7 Hz, 6H), 1.13 - 1.36 (m, 28H), 1.37 - 1.65 (m, 4H), 3.20 (t, J = 7.8 Hz, 4H), 3.39 - 3.53 (m, 4H), 3.60 - 3.79 (m, 4H), 4.14 (dd, J = 4.8 Hz, 2H), 5.23 - 5.39 (br s, 2H), 5.55 - 5.69 (s, 2H), 7.51 - 7.69 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 87.7, 85.3, 83.8, 79.4, 74.64, 74.63, 61.4, 60.7, 60.3, 58.9, 45.9, 44.0, 43.8, 43.4, 42.3, 41.51, 41.52, 37.2, 29.1. HRMS (ESI, [M+H]$^+$), calculated for $C_{29}H_{58}N_4O_5$, 543.4485; found, 543.4470.

General procedure for Pd/C catalyzed hydrogenolysis for synthesis of N-Substituted Urea Compounds 14(a-e).

Similarly to the guanidine compounds **13(a-e)**, the bicyclic isourea analogs **14(a-e)** were prepared using the same procedure with the exception of employing a longer reaction time in the final

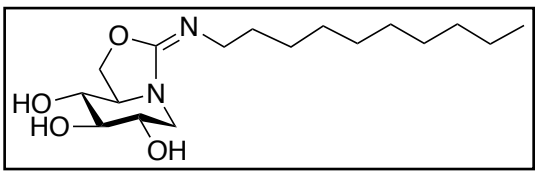
deprotection step to facilitate intramolecular cyclization. Reactions were stirred for 72-144 hours until fully converted to the cyclized compounds **14(a-e)**. Analytical data and in-depth characterization data for isourea compounds **14(a-e)** are given below.

(6S,7R,8R,8aR,E)-3-(octylimino)hexahydro-3H-oxazolo[3,4-a]pyridine-6,7,8-triol (14a).



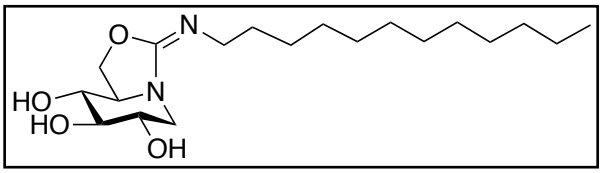
Yield: 21 mg, 82%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.87 (t, J = 7.2 Hz, 3H), 1.23 - 1.31 (m, 12H), 1.43 - 1.60 (m, 2H), 2.92 (dd, J = 10.8, 13.2 Hz, 1H), 3.15 (ddd, J = 4.8, 9.0, 13.6 Hz, 1H), 3.22 (ddt, J = 6.2 Hz, 2H), 3.27 - 3.31 (m, 1H), 3.35 - 3.41 (m, 1H), 3.88 (ddd, J = 6.8, 8.8, 9.3 Hz, 1H), 3.98 (dd, J = 5.7, 13.1 Hz, 1H), 4.56 (dd, J = 6.8, 8.7 Hz, 1H), 4.92 (t, J = 8.6 Hz, 1H), 5.32 (d, J = 4.6 Hz, 1H), 5.46 (d, J = 5.4 Hz, 1H), 5.63 (d, J = 5.3 Hz, 1H), 9.39 (s, NH$^+$, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.6, 77.1, 72.6, 68.4, 60.6, 45.1, 42.9, 31.6, 28.98, 28.95, 26.4, 22.5, 14.4. HRMS (ESI, [M+H]$^+$), calculated for $C_{15}H_{28}N_2O_4$, 301.2127; found, 301.2147.

(6S,7R,8R,8aR,E)-3-(decylimino)hexahydro-3H-oxazolo[3,4-a]pyridine-6,7,8-triol (14b).



Yield: 16 mg, 85%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.2 Hz, 3H), 1.19 - 1.33 (m, 14H), 1.45 - 1.56 (m, 2H), 2.91 (t, J = 11.6, 12.1 Hz, 1H), 3.11 - 3.18 (m, 1H), 3.21 (d, J = 6.7 Hz, 2H), 3.27 - 3.31 (m, 1H), 3.35 - 3.42 (m, 1H), 3.82 - 3.92 (m, 1H), 3.98 (dd, J = 5.7, 13.1 Hz, 1H), 4.56 (t, J = 7.7 Hz, 1H), 4.92 (t, J = 8.7 Hz, 1H), 5.32 (d, J = 4.6 Hz, 1H), 5.46 (d, J = 5.4 Hz, 1H), 5.63 (d, J = 5.3 Hz, 1H), 9.38 (s, NH$^+$, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.2, 76.6, 73.3, 72.1, 67.9, 60.2, 44.6, 42.5, 31.3, 28.93, 28.91, 28.7, 28.6, 25.9, 22.1, 14.0. HRMS (ESI, [M+H]$^+$), calculated for $C_{17}H_{32}N_2O_4$, 329.2440; found, 329.2442.

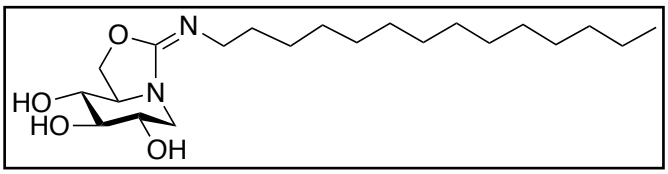
(6S,7R,8R,8aR,E)-3-(dodecylimino)hexahydro-3H-oxazolo[3,4-a]pyridine-6,7,8-triol (14c).



Yield: 11 mg, 80%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, J = 6.7 Hz, 3H), 1.18 - 1.35 (m, 18H), 1.44 - 1.58 (m, 2H), 2.91 (dd, J = 10.8, 13.1 Hz, 1H), 3.12 - 3.18 (m, 1H), 3.18 - 3.25 (m, 2H), 3.27 - 3.31 (m, 1H), 3.35 - 3.42 (m, 1H), 3.83 - 3.91 (m, 1H), 3.97 (dd, J = 5.6, 13.1 Hz, 1H), 4.56 (dd, J = 6.8, 8.8 Hz, 1H), 4.92 (t, J = 8.7 Hz, 1H), 5.31 (d, J = 4.6 Hz, 1H), 5.46 (d, J = 5.4 Hz, 1H), 5.62 (d, J = 5.3 Hz, 1H), 9.36 (s, NH$^+$, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.2, 76.6, 73.3, 72.1,

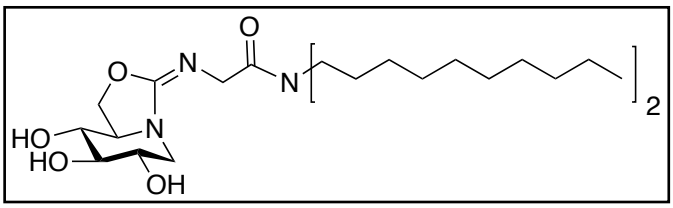
67.9, 60.2, 44.6, 42.5, 31.3, 29.03, 28.99, 28.98, 28.9, 28.7, 28.6, 25.9, 22.1, 14.0. HRMS (ESI, $[M+H]^+$), calculated for $C_{19}H_{36}N_2O_4$, 357.2753; found, 357.2770.

(6S,7R,8 R,8aR,E)-3-(tetradecylimino)hexahydro-3H-oxazolo[3,4-a]pyridine-6,7,8-triol (14d).



Yield: 18 mg, 86%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (t, J = 6.7 Hz, 3H), 1.18 - 1.30 (m, 22H), 1.50 (d, J = 7.6 Hz, 2H), 2.91 (dd, J = 10.9, 13.1 Hz, 1H), 3.12 - 3.18 (m, 1H), 3.18 - 3.26 (m, 2H), 3.27 - 3.30 (m, 1H), 3.34 - 3.41 (m, 1H), 3.81 - 3.93 (m, 1H), 3.97 (dd, J = 5.7, 13.2 Hz, 1H), 4.55 (dd, J = 7.4, 8.2 Hz, 2H), 4.92 (t, J = 8.5 Hz, 2H), 5.31 (d, J = 4.7 Hz, 1H), 5.46 (d, J = 5.4 Hz, 1H), 5.62 (d, J = 5.3 Hz, 1H), 9.36 (s, NH^+, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.2, 76.6, 73.3, 72.1, 67.9, 60.2, 44.6, 42.5, 31.3, 29.04, 29.03, 29.00, 28.98, 28.9, 28.7, 28.6, 26.0, 22.1, 14.0. HRMS (ESI, $[M+H]^+$), calculated for $C_{19}H_{36}N_2O_4$, 385.3066; found, 385.3068.

N,N-didecyl-2-(((6S,7R,8R,8aR,E)-6,7,8-trihydroxyhexahydro-3H-oxazolo[3,4-a]pyridin-3-ylidene)amino)acetamide (14e).

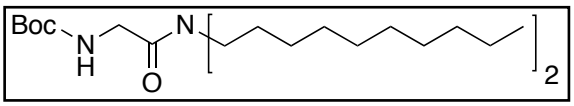


Yield: 15 mg, 91%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (2 x t, J = 6.7 Hz, 6H), 1.16 - 1.32 (m, 28H), 1.38 - 1.54 (m, 4H), 3.03 (t, J = 10.8, 13.2 Hz, 1H), 3.14 - 3.29 (m, 6H), 3.35 - 3.42 (m, 1H), 3.96 (ddd, J = 6.0, 8.9 Hz, 1H), 4.06 (dd, J = 5.6, 13.3 Hz, 1H), 4.16 (s, 2H), 4.56 (dd, J = 5.9, 8.8 Hz, 1H), 4.88 (dd, J = 8.6 Hz, 1H), 5.36 (d, J = 4.6 Hz, 1H), 5.53 (d, J = 5.3 Hz, 1H), 5.69 (d, J = 5.2 Hz, 1H), 9.78 (s, NH^+, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.5, 159.3, 104.18, 104.17, 76.5, 73.3, 72.1, 68.0, 60.4, 46.11, 46.10, 45.5, 44.8, 43.5, 43.5, 31.3, 28.97, 28.95, 28.92, 28.8, 28.71, 28.68, 28.67, 28.2, 27.1, 26.3, 26.1, 22.1, 14.0. HRMS (ESI, $[M+H]^+$), calculated for $C_{29}H_{55}N_3O_5$, 526.4220; found, 526.4229.

Scheme S1. Synthetic route towards tiourea compound **11e**.

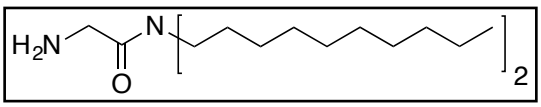
15 16 11e

***Tert*-butyl (2-(didecyclamino)-2-oxoethyl)carbamate (15).**



Boc-Gly-OH (0.88 g, 5 mmol, 1 eq) was dissolved in CH_2Cl_2 (60 mL). Didecylamine (1.64 g, 5.5 mmol, 1.1 eq) and EDCI (1.05 g, 5 mmol, 1 eq) were added and the reaction mixture was stirred for 24h. Consumption of starting material was confirmed with TLC analysis, followed by extraction with HCl (3x200 mL), sat. aq. $NaHCO_3$ (3x200 mL) and sat. aq. NaCl (200 mL). The combined organic phases were dried with Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexanes = 4:1). Yield: 3.64 g, 92%. R_f(EtOAc/hexanes = 4:1) = 0.82. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (2 x t, *J* = 7.1 Hz, 6H), 1.21 - 1.38 (m, 28H), 1.45 (s, 9H), 1.47 - 1.62 (m, 4H), 3.09 - 3.19 (m, 2H), 3.27 - 3.36 (m, 2H), 3.94 (d, *J* = 4.2 Hz, 2H), 5.57 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 155.8, 79.4, 77.3, 77.0, 76.7, 46.9, 46.1, 42.1, 31.83, 31.81, 29.50, 29.48, 29.4, 29.34, 29.28, 29.25, 29.2, 28.7, 28.3, 27.6, 26.94, 26.85, 22.6, 14.1. HRMS (ESI, $[M+H-Boc]^+$), calculated for $C_{27}H_{54}N_2O_3$, 355.3688; found, 355.3639.

2-amino-*N*,N-didecylacetamide (16).



TFA (15 mL) was added to previously isolated **15** (3.27 g, 7.19 mmol, 1 eq), dissolved in CH_2Cl_2 (15 mL) and stirred for 24 h at room temperature. After consumption of starting material was observed with TLC, the residue was purified by flash column chromatography to obtain a yellow oil which was concentrated and co-evaporated with $CHCl_3$. Yield: 2.55 g, quant. yield, R_f(EtOAc/hexanes = 8:1) = 0.12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (2 x t, *J* = 7.0 Hz, 6H), 1.18 - 1.38 (m, 28H), 1.40 - 1.69 (m, 4H), 3.12 (t, *J* = 7.7 Hz, 2H), 3.30 (t, *J* = 7.6 Hz, 2H), 3.87 (s, 2H), 8.21 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.9, 77.3, 77.0, 76.7, 47.2, 46.5, 40.0, 31.9, 31.8, 29.5, 29.44, 29.41, 29.34, 29.28, 29.21, 29.17, 28.3, 27.2, 26.9, 26.7, 22.63, 22.61, 14.0. HRMS (ESI, $[M+H]^+$), calculated for $C_{22}H_{46}N_2O$, 355.3688; found, 355.3633.

Kinetic characterization of cyclization to form isoureas 14(a-e)

Introduction

The kinetic characterization of cyclization to form isoureas **14(a-e)** in aqueous solutions was studied by ultra-high performance liquid chromatography – mass spectrometry (UHPLC-MS). A stability indicating method was developed and used to test the chemical stability of the compounds at pH 5.2 and pH 7.0 during a maximum period of time of 24 days.

Reagents

Acetonitrile (ACN; LC-MS Chromasolv) was purchased from Biosolve BV (Valkenswaard, The Netherlands). Formic acid (FA; LC-MS grade), acetic acid, sodium acetate and ammonium acetate were acquired from Sigma-Aldrich (St. Louis, MA, USA). Ultra-pure water was obtained from a Synergy UV water delivery system from Millipore (Billerica, MA, USA). Buffer solutions of 200 mM ammonium acetate (pH 7.0) and 21 mM acetic acid with 85 mM sodium acetate (pH 5.2) were prepared in ultra-pure water. Standard solutions of 1 mM guanidine compounds **13(a-e)** were prepared in buffer solutions with pH 5.2 and pH 7.0.

Chromatography

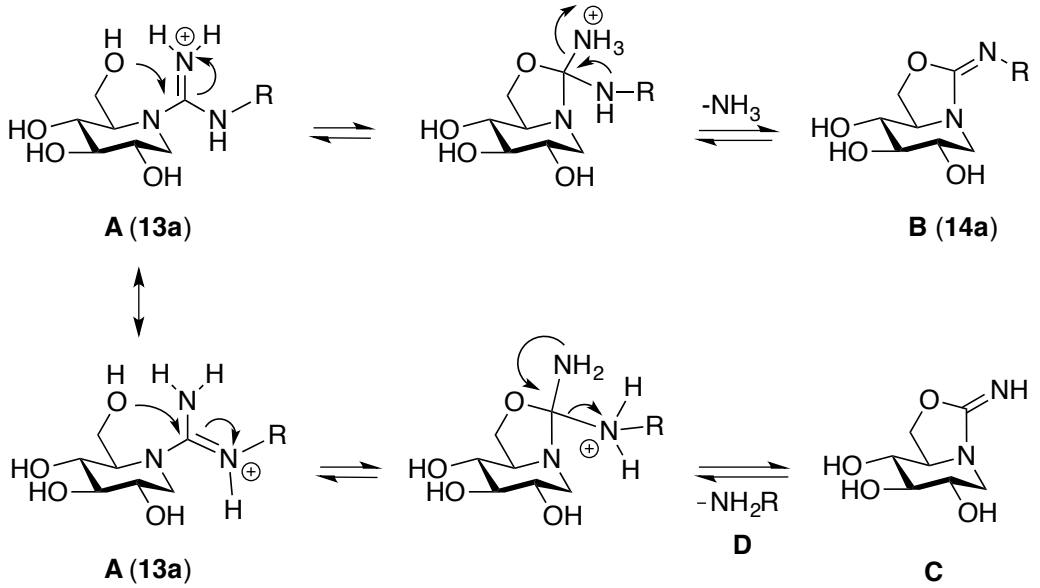
Separation of guanidine compounds and their degradation products was achieved on a Zorbax Eclipse plus C_{18} column (4.6 x 50 mm, 1.8 μm particles). A 2-μL injection volume was used of all samples. UHPLC was performed on a 1290 Infinity UHPLC system (Agilent Technologies, Waldbronn, BW, Germany) consisting of a binary pump and an autosampler. The optimal separation was achieved with a binary gradient with 0.5% FA (% v/v) in water (eluent A) and ACN (eluent B) at a flow rate of 0.5 mL/min. Detection was performed on a quadrupole-time-off-flight mass spectrometer, equipped with an electrospray ionization source (Bruker Daltonics, Bremen, HB, Germany). Masses were acquired from *m/z* 50-700 at a spectra rate of 1.5 Hz, nebulizer pressure was 4 bar, gas flow was 10 L/min, gas temperature was 2000 °C and capillary voltage was 3 kV. Guanidine compounds **13(a-e)** and their degradation products (Scheme S2), were detected as positive ions ($[M+H]^+$).

Procedure

A sample of 1 mM **13a**, degraded in buffer solution (pH 7.0) at 21 °C for 2 days, was used to develop the stability indicating method. The separation of **13a** and the degradation products was optimized by adjusting the amount and type of acidifier and organic modifier and the gradient time and slope. Good selectivity for all high and low polar compounds was achieved in one run with 0.5% FA/ACN gradients (Figure S1). The optimized gradient started with a composition of 70%

eluent A and 30% eluent B (% v/v) for 3 min, increased linearly to 100% B in 3.5 min and remained at 100% B for 2 min. MS setting were optimized to obtain maximum detector response for all compounds. The optimized method was used to study the degradation of the synthesized guanidine compounds. Samples were dissolved in buffer solutions with pH 5.2 or pH 7.0, kept at 21 °C and analysed at regular time intervals.

Scheme S2. Formation of guanidine iminosugars to bicyclic isoureas driven *via* ammonia elimination.



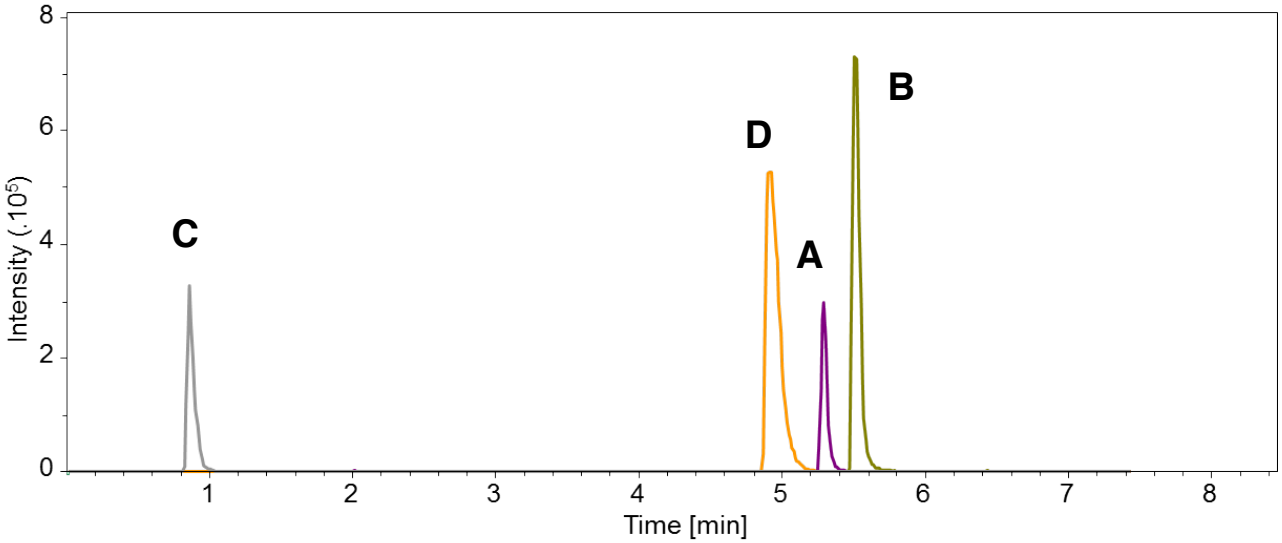


Figure S1. Extracted ion chromatogram for compound **A** (**13a**) obtained with the optimized UHPLC-MS method of a 1mM solution (pH 7.0), degraded at 21 °C for 2 days. The following compounds were identified by their *m/z* value: **A**) *m/z* 318 for compound **13a**; **B**) *m/z* 301 for compound **14a**; **C**) *m/z* 189 for isourea free amine after alkylamine elimination; **D**) *m/z* 130 for alkylamine.

The isourea compounds **14(a-e)** were identified by their *m/z* value as the main degradation products. The sum of peak areas in the chromatograms of the guanidine and the isourea compounds remained constant in time in the samples analysed at different time points which indicates that their detector responses are similar. This allows for the application of relative responses for quantitative analysis of these compounds to determine the degradation process as described in Scheme S2. Figure S2 shows the first-order degradation data of **13a** and the formation of **14a** at pH 7.0, both expressed as % area. The half-life time, where the concentration of **13a** and **14a** where equal, was > 20h. By using the logarithm (to base-e) of the concentration a linear graph was obtained (Figure S3), confirming that the degradation of **13a** was a first-order reaction with equation:

$$[\mathbf{13a}(t)] = [\mathbf{13a}]_0 \cdot e^{-k.t}$$

The slopes of the Ln-linearized curves of all guanidine compounds **13(a-e)** were used to calculate the degradation rate constants (k) and the half-times ($t_{0.5}$=(Ln2/k)) for each compound.

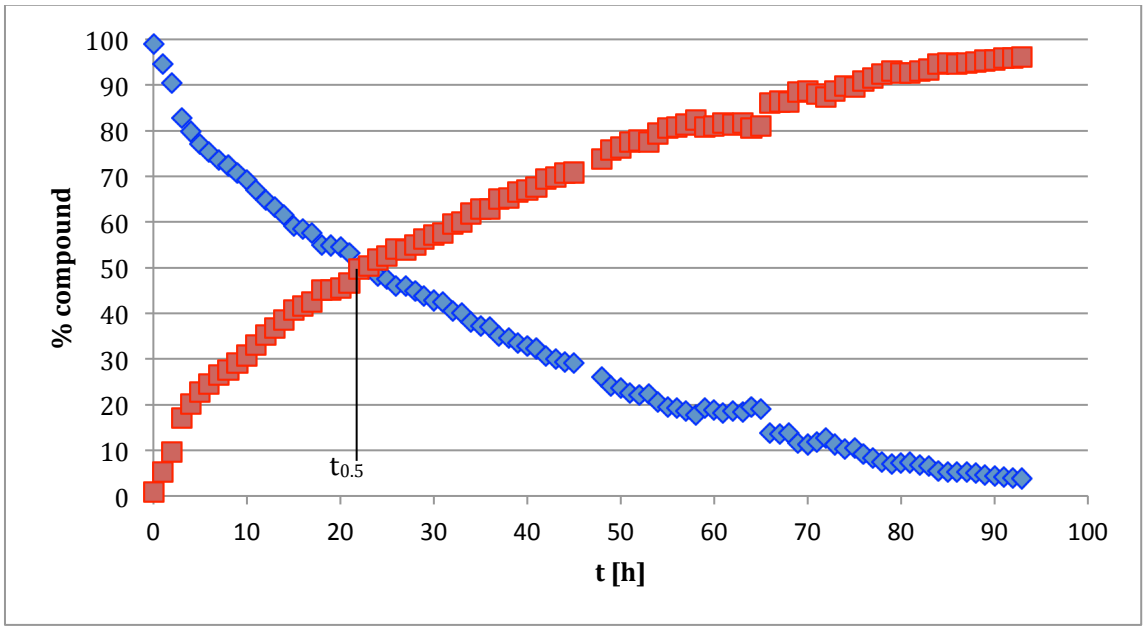


Figure S2. First-order degradation of **13a** and formation of **14a** in pH 7.0 buffer solution at 21 °C.

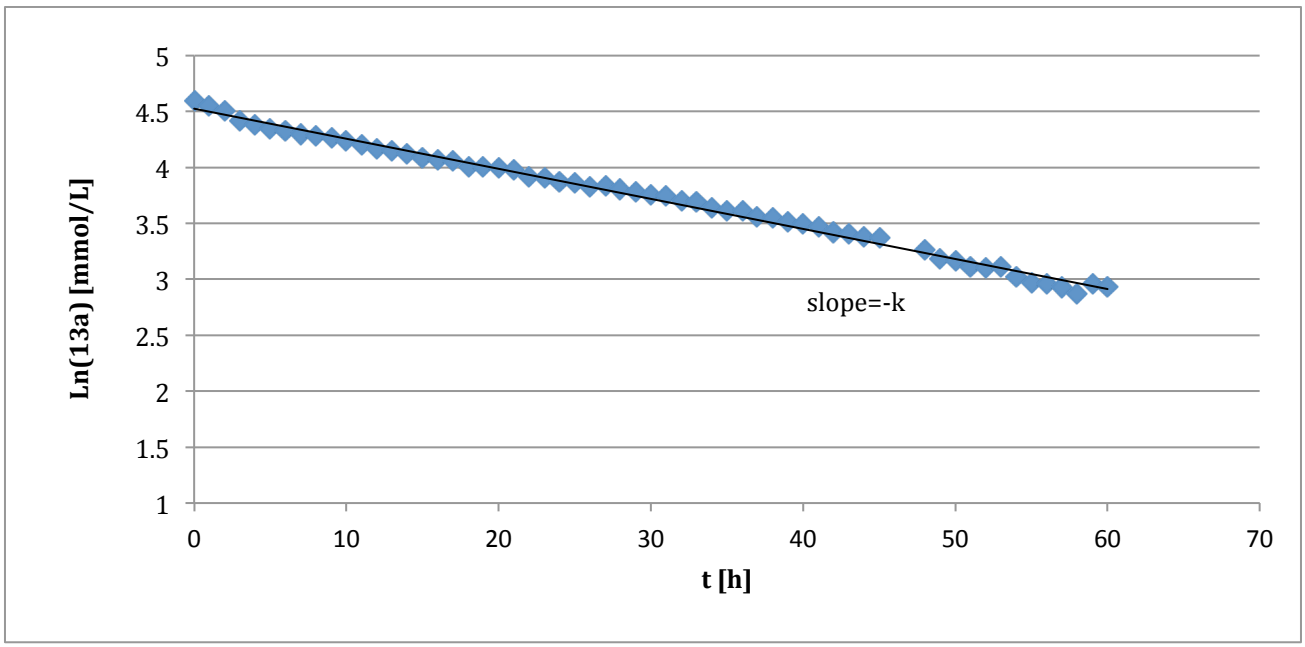


Figure S3. Ln-linearization of the first-order degradation data of **13a** in pH 7.0 buffer solution at 21 °C.

Table S1 shows the $t_{0.5}$ values for compounds **13(a-e)** for the degradation at pH 5.2 and pH 7.0. Degradation was much slower at pH 5.2, compared to pH 7.0 and compounds with shorter lipophilic tails appeared to be more stable than compounds with extended alkyl chains. Compounds **14(a-e)** showed no degradation at both pH even after 14 days.

Table S1: $t_{0.5}$ values for guanidine compounds **13(a-e)** at pH 7.0 and 5.2.

	$t_{0.5}$ in buffer pH 7.0 (h)	$t_{0.5}$ in buffer pH 5.2 (h)
Compound		
13a	21.4	519.4
13b	17.4	465.5
13c	14.1	226.8
13d	2.9	37.2
13e	1.0	9.7

Biochemical Assays

Inhibition Assays against Commercial Glycosidases and Human Recombinant GBA (R&D 7410-GH)

Inhibition assays against commercial glycosidases were performed in either phosphate or acetate buffer at the optimum pH for each enzyme (See below for enzyme specific data). Determination of the IC_{50} values of the iminosugars was carried out by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases on the corresponding p-nitrophenyl glycoside substrates in the presence of a concentration range of iminosugar derivatives. The incubation mixture consisted of 50 μL of inhibitor solution in buffer (0.1 U mL^{-1}) and 50 μL of enzyme solution. The concentrations of the enzyme were adjusted so that the reading for the final absorbance was in the range of 0.5–1.5 units. Inhibitor and enzyme solutions were mixed in a disposable 96-well microtiter plate and then incubated at room temperature for 5 minutes. Next, the reactions were initiated by addition of 50 μL of a solution of the corresponding p-nitrophenyl glycoside substrates solution in the appropriate buffer at the optimum pH for the enzyme. After the reaction mixture was incubated at 37 ^{0}C for 30 min, the reaction was quenched with 0.5 M Na_2CO_3 (150 μL) and the absorbance of 4-nitrophenol released from the substrate was read immediately at 405 nm using a BioTek mQuant Microplate Spectrophotometer.

IC_{50} values were determined graphically with GraphPad Prism (version 6.0) by making a plot of percentage inhibition versus the log of inhibitor concentration, using at least 8 different inhibitor concentrations. IC_{50} values were presented as a concentration of the iminosugars that inhibits 50% of the enzyme activity under the assay conditions. NN-DNJ was used as a reference compound. All materials were purchased from Sigma-Aldrich. All data are reported in Table S2.

The commercial glycosidase solutions were prepared as following:

[b]For **α-glucosidase** (from *baker's yeast,* Sigma G5003, 0.05 U/mL) the activity was determined with p-nitrophenyl-α-D-glucopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2).

[c]For **α-galactosidase** (from *green coffee beans*, Sigma G8507, 0.05 U/mL) activity was determined with p-nitrophenyl-α-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 6.8).

[d]For **β-glucosidase** (from *almond*, Sigma G4511, 0.05 U/mL) the activity was determined with p-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0).

[e]For **β-galactosidase** (from *bovine liver*, Sigma G1875, 0.05 U/mL) activity was determined with p-nitrophenyl-β-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2).

[f]For **Naringinase** (from *penicillium decumbens,* Sigma N1385, 0.06 U/mL) the activity was determined with p-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0).

[g]**Recombinant Human Glucosylceramidase/β-glucocerebrosidase/GBA** (7410-GH), purchased from R&D was also used in the inhibition studies. The used substrate 4-methylumbelliferyl-β-D-glucopyranoside was purchased by Sigma-Aldrich. GBA activity was determined with 4-methylumbelliferyl-β-D-glucopyranoside as reported in (A. Trapero, J. Med. Chem. 2012, 55, 4479-4488)**.**[2] Briefly, enzyme solutions (25 µL from a stock solution containing 0.6 µg/mL) in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2 or pH 7.0) were incubated at 37°C without (control) or with inhibitor at a final volume of 50 µL for 30 min. After addition of 25 µL 4-methylumbelliferyl-β-D-glucopyranoside (7.2 mM, McIlvaine buffer pH 5.2 or pH 7.0), the samples were incubated at 37°C for 10 min. Enzymatic reactions were stopped by the addition of aliquots (100 µL) of Glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methylumbelliferone formed was determined with a FLUOstar microplate reader (BMG Labtech) at 355 nm (excitation) and 460 nm (emission).

[g]**Recombinant Human Galactosylceramidase/GALC** (7310-GH), purchased from R&D was used in the inhibition studies. The used substrate 4-methylumbelliferyl-β-D-galactopyranoside was purchased by Sigma-Aldrich. GALC activity was determined with 4-methylumbelliferyl-β-D-galactopyranoside as reported in assay procedure R&D product 7310-GH. Briefly, enzyme solutions (25 µL from a stock solution containing 60 ng/mL) in the presence of 0.5% (v/v) Triton X-100 in Assay buffer (50 mM sodium citrate and 125 mM NaCL, pH 4.5) were incubated at 37°C without (control) or with inhibitor at a final volume of 50 µL for 10 min. After addition of 25 µL 4-methylumbelliferyl-β-D-galactopyranoside (0.75mM, Assay buffer), the samples were incubated at 37°C for 20 min. Enzymatic reactions were stopped by the addition of aliquots (50 µL) of Glycine/NaOH buffer (100 mM, pH 10.6). The amount of 4-methylumbelliferone formed was determined with a FLUOstar microplate reader (BMG Labtech) at 355 nm (excitation) and 460 nm (emission).

Table S2. Glycosidase inhibition values obtained for **14a-e**.[a]

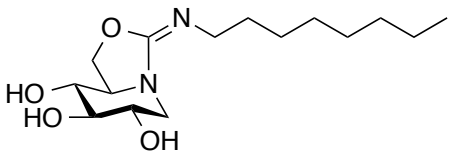
Enzyme	14a	14b	14c	14d	14e	NNDNJ
α-glu[b]	> 100000	> 100000	> 100000	> 100000	> 100000	> 100000
α-gal[c]	> 100000	> 100000	> 100000	> 100000	> 100000	> 100000
β-glu[d]	5650±387	2080±95	1650±70	661±30	> 100000	> 100000
β-gal[e]	420±9	136±4	88±10	138±34	938±91	> 100000
Nar[f]	128±6	117±6	141±7	195±1	518±7	116±5
GBA[g] (pH 7.0)	**20.8±1.3**	**2.6±0.9**	**1.8±0.1**	**1.5±0.2**	**1.7±0.1**	562.5±56.6
GBA[g] (pH 5.2)	**135.5±3.9**	**16.7±0.5**	**12.8±1.2**	**15.4±1.7**	**14.0±1.2**	1293.0±55.3
GALC[g]	> 10000	> 10000	> 10000	> 10000	> 10000	> 10000

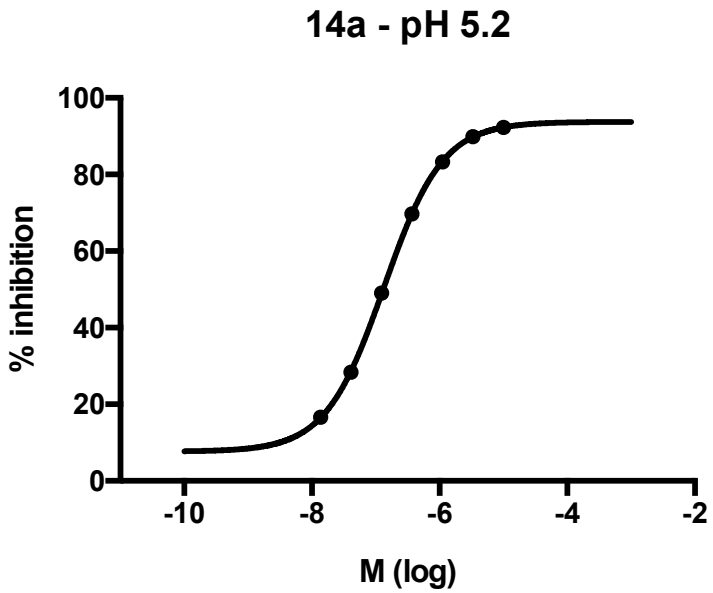
[a] IC_{50} values are reported in nM and are averages obtained from triple independent duplicate analysis of each compound. For ease of comparison, the IC_{50} values obtained for all compounds shown in Table SI 2 are compared to a reference compound NNDNJ.
[b] α-glucosidase (from *baker's yeast,* Sigma G5003): 0.05 U/mL, the activity was determined with p-nitrophenyl-α-D-glucopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2).
[c] α-galactosidase (from *green coffee beans*, Sigma G8507): 0.05 U/mL; α-galactosidase activity was determined with p-nitrophenyl-α-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 6.8).
[d] β-glucosidase (from *almond*, Sigma G4511): 0.05 U/mL; the activity was determined with p-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0).
[e] β-galactosidase (from *bovine liver*, Sigma G1875): 0.05 U/mL; activity was determined with p-nitrophenyl-β-D-galactopyranoside (0.7 mM final conc. in well) in sodium phosphate buffer (100 mM, pH 7.2).
[f] Naringinase (from *penicillium decumbens,* Sigma N1385): 0.06 U/mL. the activity was determined with p-nitrophenyl-β-D-glucopyranoside (0.7 mM final conc. in well) in sodium acetate buffer (100 mM, pH 5.0).
[g] β-glucocerebrosidase (GBA) and β-galactocerebrosidase (GALC) activities were determined using 4-methylumbelliferyl-β-D-glucopyranoside and 4-methylumbelliferyl-β-D-galactopyranoside respectively using assay conditions based on those previously reported.[4]

References

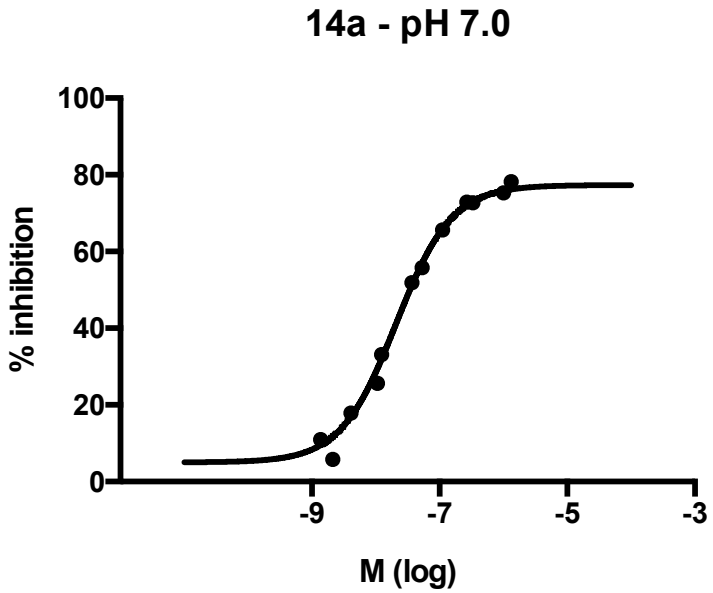
4 Trapero, A.; Gonzalez-Bulnes, P.; Butters, T. D.; Llebaria, A. Potent aminocyclitol glucocerebrosidase inhibitors are subnanomolar pharmacological chaperones for treating Gaucher disease *J. Med. Chem.* **2012**, 55, 4479-88.

Inhibition of GBA Assay

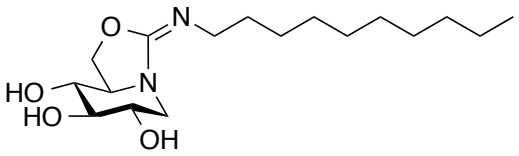




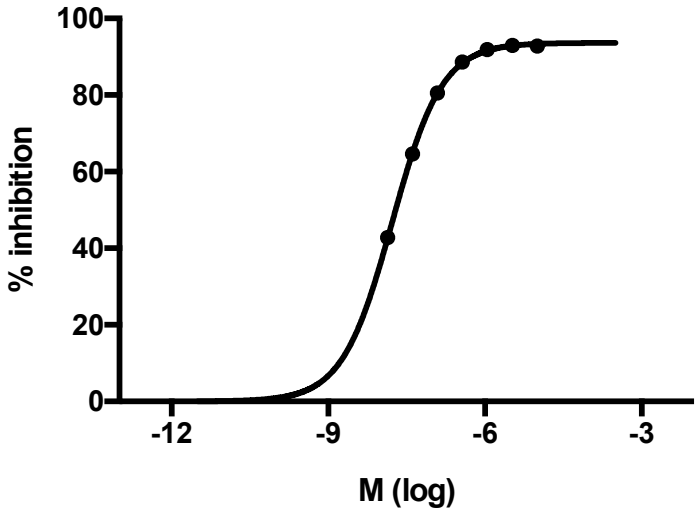
IC_{50} = 1.355e-007 ± 3.892e-009



IC_{50} = 2.084e-008 ± 1.328e-009

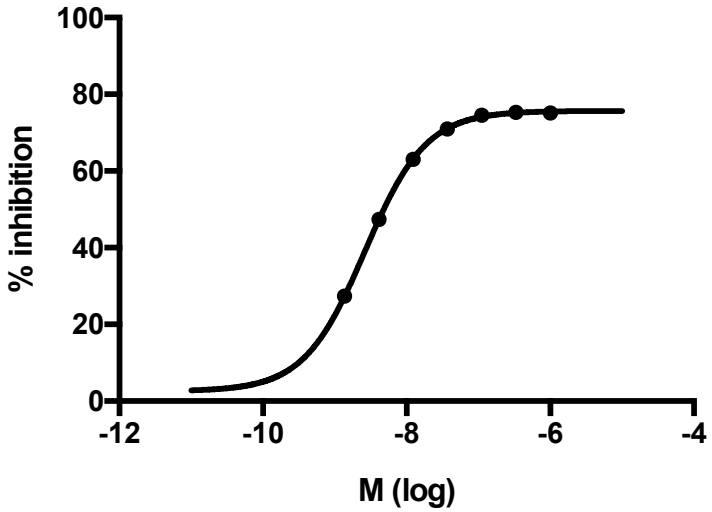


14b - pH 5.2

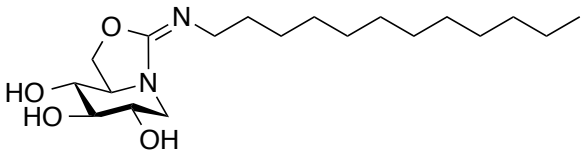


IC_{50} = 1.673e-008 ± 4.915e-010

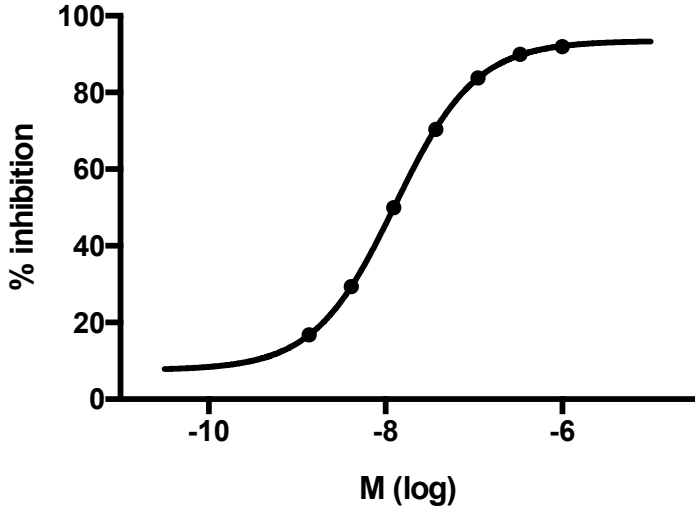
14b - pH 7.0



IC_{50} = 2.633e-009 ± 9.009e-010

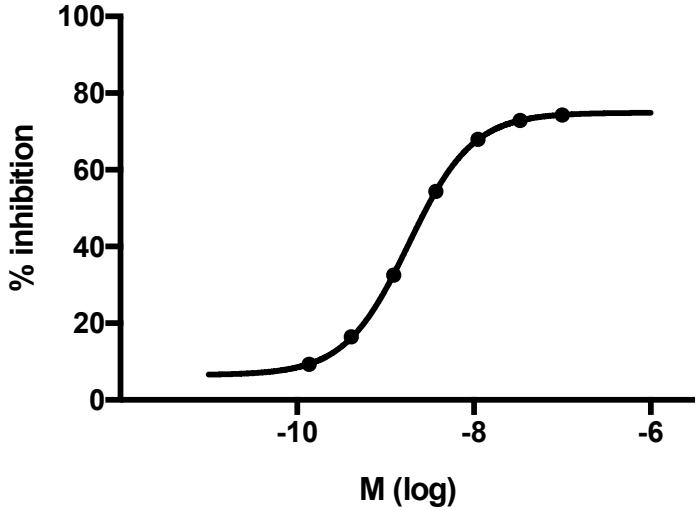


14c - pH 5.2

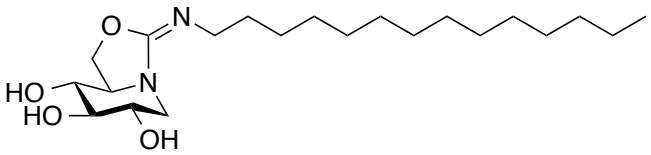


IC_{50} = 1.276e-008 ± 1.226e-009

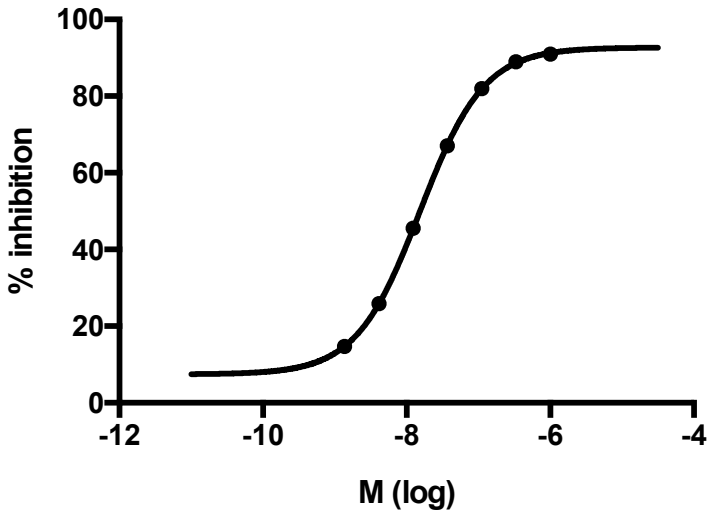
14c - pH 7.0



IC_{50} = 1.832e-009 ± 1.141e-010

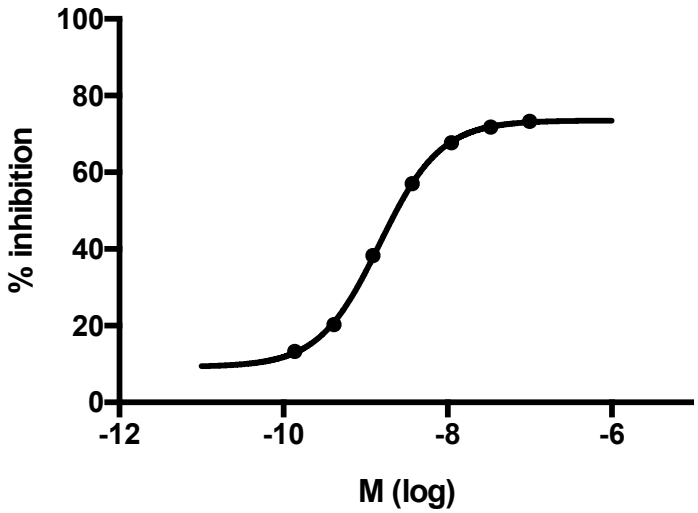


14d - pH 5.2

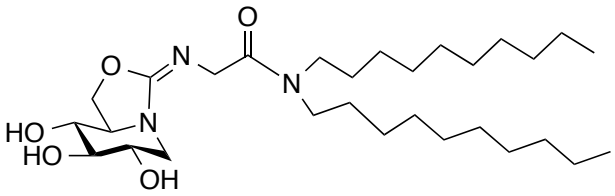


IC_{50} = 1.542e-008 ± 1.720e-009

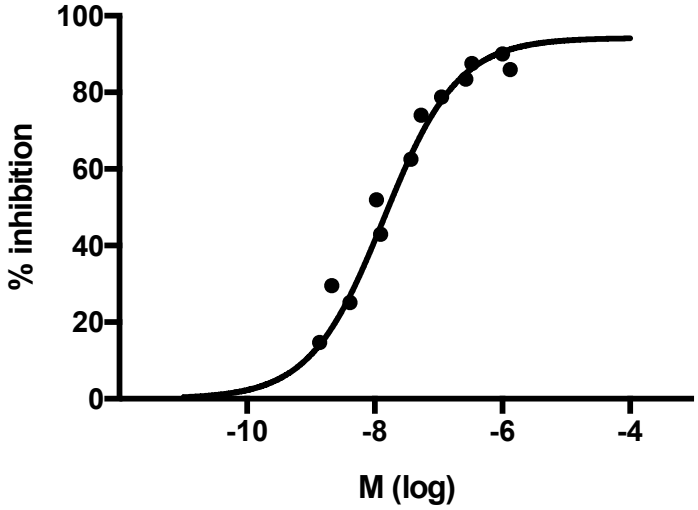
14d - pH 7.0



IC_{50} = 1.489e-009 ± 2.189e-010

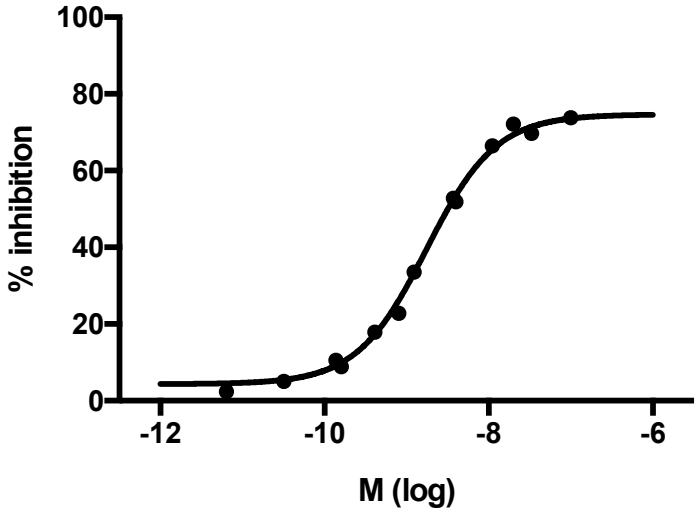


14e - pH 5.2

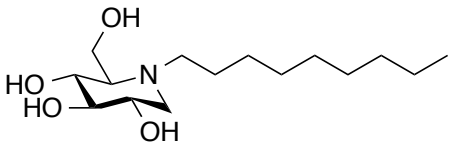


IC_{50} = 1.395e-008 ± 1.193e-009

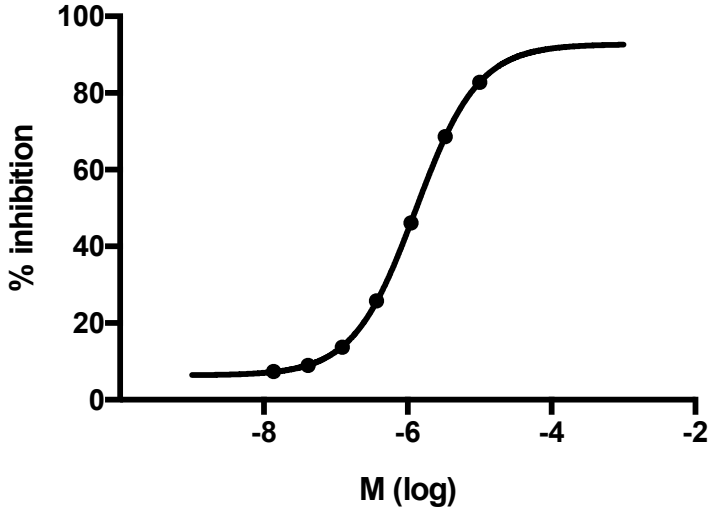
14e - pH 7.0



IC_{50} = 1.744e-009 ± 1.488e-010

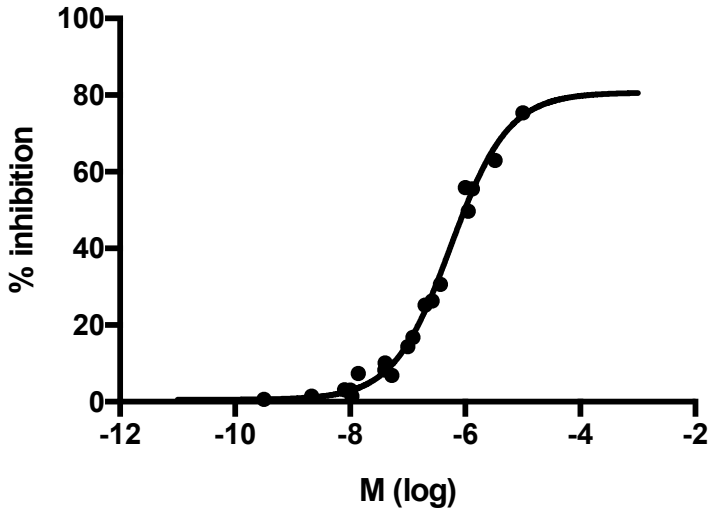


NN-DNJ - pH 5.2



IC_{50} = 1.293e-006 ± 5.526e-008

NN-DNJ - pH 7.0



IC_{50} = 5.625e-007 ± 5.664e-008

Potentiometric Analysis to determine pKa of 14a.

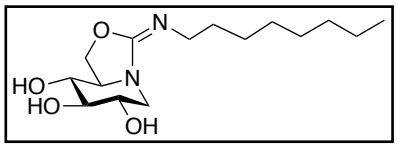
Potentiometric pKa determination of 14a.

The sample pKa was determined *via* the potentiometric (pH-metric) technique[5] using the SiriusT3 instrument from Sirius Analytical. The potentiometric titrations were carried out at 25.0±0.1°C under argon atmosphere. Ionic strength was adjusted to 0.15 M using KCl. A 10mM stock solution of the sample was pre-acidified to pH 1.8 with 0.5 M aqueous HCl and titrated with 0.5 M aqueous KOH to pH 12.2. Sample concentration was approximately 1.2 – 1.6 mM and the sample was titrated in triplicate under aqueous conditions. The pKa values were obtained using SiriusT3 software (v1.1, Sirius) and these values were used to calculate the pKa values. A UV-turbidity probe was used to detect any precipitation, however no precipitation of the sample from solution was observed under the conditions used. The pKa values were determined potentiometrically, as the initial fast UV-metric assay showed extremely low absorbance, indicating that no sufficiently UV-active chromophores were in close proximity to the pKa's determined.

References

5 Reijenga, J.; van Hoof, A.; van Loon, A.; Teunissen, B. Development of Methods for the Determination of pK_a Values in Anal. Chem. Insights. **2013**, *8*, 53–71.

Potentiometric Analysis Results for 14a.



Analytical Service Report

Customer Details	
Name:	Tim Potter
Company/Institution:	Cyprotex Discovery Ltd
Email Address:	T.Potter@cyprotex.com
Telephone Number:	01625 505 100
Purchase Order No.:	C18792
Sample Details	
Sample name:	Sample1
Batch Id/Ref No.:	-
Sirius Ref. No.:	160080
Analysis Details	
Type of Analysis:	pK_a
Analysis Codes:	1801010
Date:	25th February – 1st March 2016
Analyst Name:	Rebeca Ruiz (Senior Chemist, Applications Team Leader)
Laboratory Notebook:	RR001-072
Checked By:	Breeze Outhwaite (Applications Chemist)

Results

pK_a:

pK_a	Type*	T / °C	Ionic Environment	Method
9.31 ± 0.01	Base	25.0	0.15 M KCl	pH-metric

**The type of ionisable groups predicted by the programme ACD/Percepta 2012 Release*

Instrument ID: SiriusT309014
Traceability: 16B-25006, 16C-01011

Comments

pK_a: The sample pK_a was determined using the potentiometric (pH-metric) technique.

UV-metric:

The sample was initially titrated in a fast-UV triple titration between pH 2 – 12 at concentrations of 31 – 19 µM, under aqueous conditions. No precipitation of the sample from solution was observed and no evidence of any sample ionisation within the investigated pH range was inferred from the spectroscopic data obtained. Therefore, the sample was analysed using the pH-metric method.

pH-metric:

The sample was subsequently titrated in a potentiometric triple titration, under aqueous conditions from pH 2.0 – 12.0 at concentrations of 1.6 – 1.2 mM. No precipitation of the sample from solution was observed and one pK_a, with an average value of 9.31 ± 0.01 was determined from the potentiometric data collected.

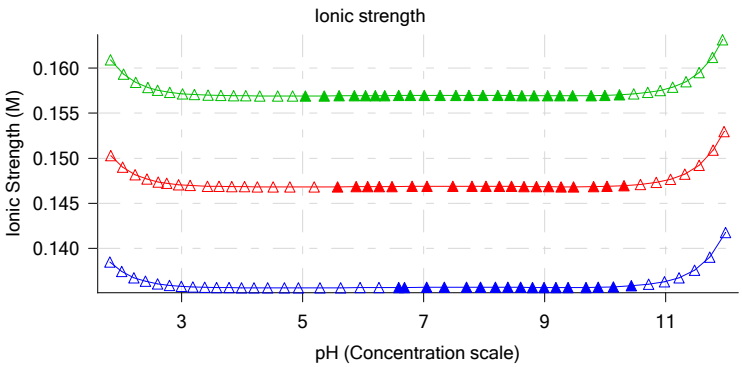
pH-metric Result

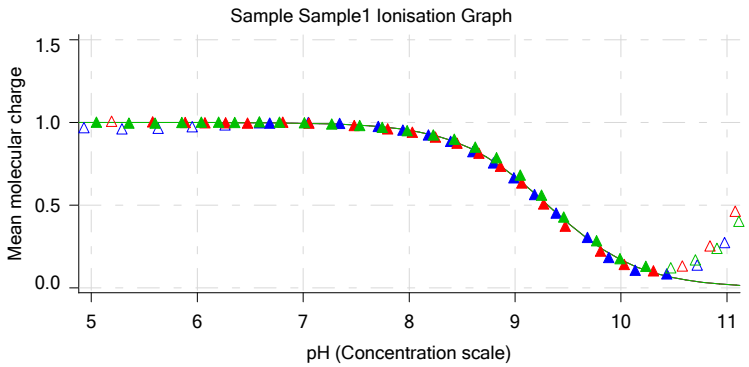
Sample name:	**Sample1**	Experiment start time:	**01/03/2016 13:40:48**
Assay name:	**pH-metric pKa**	Analyst:	**RR**
Assay ID:	**16C-01011**	Instrument ID:	**T309014**
Quality:	**Good**		
Filename:	**I:\Analytical Services Lab\Data 2016\AS_Customer Samples\Cyprotex\Cyprotex UK\February\C18792_work order 45_GS\16C-01011_Sample1_pH-metric pKa.t3r**		

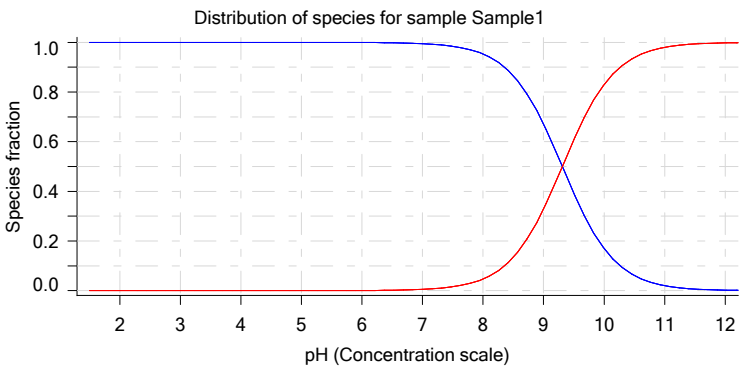
pH-metric Result

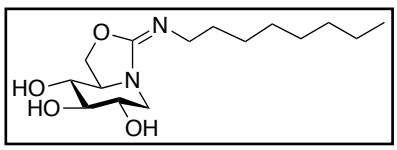
Base pKa 1 9.31 ±0.01 (n=50)

Graphs









Cytotoxicity assay in Wild-Type and GD derived Fibroblasts.

Cells were seeded at a density of 10000 cells per well in 96-well plates. Media were renewed after 24 h and compounds were added to give final concentrations of 10, 1, 0.1 or 0.01 μM (for compounds **13a**, **14a**, **NNDNJ**) and 1, 0.1 or 0.01 μM **(**for compounds **13b-e** and **14b-e**).

All compounds were dissolved in DMSO (final conc <1%) and control experiments were performed with DMSO. Cells were incubated at 37 °C in 5% CO_2 for 24 h. The cytotoxicity was measured by using the CytoTox 96 non-radioactive cytotoxicity assay and the Celltiter-Blue Cell viability assay from Promega.

No toxicity was found for compounds **13a-e** and **14a-e** at 1μM or lower.

Measurements of GBA Activity in Intact Human (Wild-Type) Fibroblasts and N370S Fibroblasts Derived from Gaucher Disease Patients

Cell lines and culture. Wild-type fibroblasts (GM 05659) and from Gaucher patients derived fibroblasts (homozygous for N370S GBA (GM00372) were obtained from Coriell Institute, Camden, USA. Fibroblast cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) and penicillin-streptomycin (100 U/ml resp. 0.1 mg/ml, Sigma-Aldrich) at 37°C in 5% CO_2 and all cells used in this study were between the 5th and 15th passages.

The Fibroblasts assay (*chaperone assay)* was performed according to a modified version as described in a paper published by Trapero, et. al.[4]

GBA Inhibition in Wild-Type Fibroblasts. Cells were plated into 24-well assay plates and incubated at 37°C in 5% CO_2 until a monolayer of at least 50% confluency was reached. The media were then replaced with fresh media with or without a test compound and incubated at 37°C in 5% CO_2 for 4 days. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed twice with phosphate buffered saline (PBS) solution. Then, 80 µl of PBS and 80 µl of 200 mM acetate buffer (pH 4.0) were added to each well. The reaction was started by addition of 100 µl of 7.2 mM 4-methylumbelliferyl-β-D-glucopyranoside (200 mM acetate buffer pH 4.0) to each well, followed by incubation at 37°C for 2h. Enzymatic reactions were stopped by lysing the cells with 0.9 ml glycine/NaOH buffer (100 mM pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission at 460 nm) with the Fluoroskan Ascent FL plate reader (Labsystems) in 96-well format. Data are reported in Table S3 and Figure S4.

References

4 Trapero, A.; González-Bulnes, P.; Butters, T. D.; Llebaria, A. Potent Aminocyclitol Glucocerebrosidase Inhibitors are Subnanomolar Pharmacological Chaperones for Treating Gaucher Disease in *J. Med. Chem.* **2012**, 55, 4479–4488.

Table S3. **14(a-e)** Inhibition Data of GBA in Wild-Type Fibroblasts

Inhibition of GBA in Wild-Type Fibroblasts				
Compound	**Concentration [nM]**			
	100	**10**	**1**	**0,1**
NN-DNJ	9,3 ± 0,4	19,2 ± 2,4	17,8 ± 1,9	14,2 ± 3,8
14a	11,2 ± 0,7	16,1 ± 0,9	17,2 ± 2,4	14,2 ± 2,8
14b	61,4 ± 1,1	35,8 ± 3,6	22,1 ± 8,1	20,4 ± 4,6
14c	84,1 ± 1,5	49,8 ± 6,1	27,0 ± 3,3	13,7 ± 3,6
14d	86,3 ± 0,8	53,9 ± 1,9	24,4 ± 4,0	13,0 ± 1,8
14e	90,0 ± 0,8	67,2 ± 0,9	25,0 ± 2,5	9,8 ± 5,0

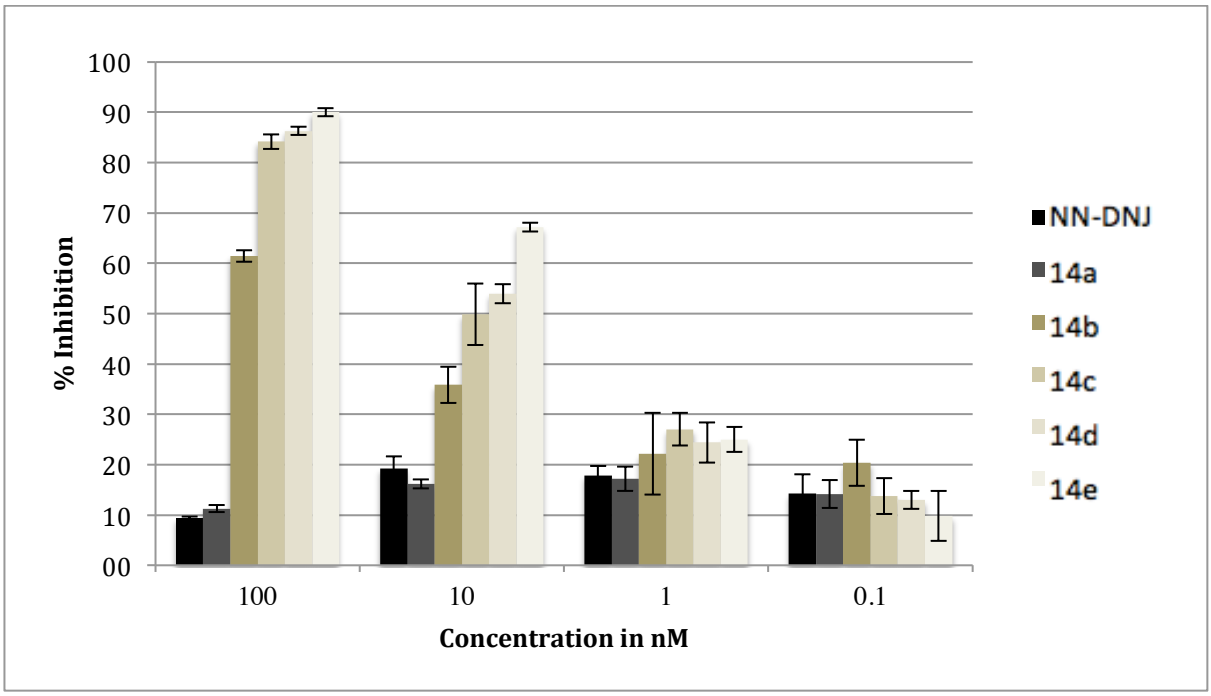


Figure S4. GBA inhibition of **14(a-e)** in wild-type fibroblasts (GM 05659) after 24h incubation time at the indicated inhibitor concentrations.

Measurement of N370S GBA Activity in Fibroblasts Derived from Patients with GD. The Chaperone assay was performed according to a modified version as previously described.[4] Fibroblasts were plated into 24-well assay plates and incubated at 37°C under 5% CO_2 atmosphere until a monolayer of at least 50% confluency was reached. The media were then replaced with fresh media with or without various concentrations of test compounds and incubated at 37°C in 5% CO_2 for 4 days. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed twice with phosphate buffered saline (PBS) solution. Then, 80 µl of PBS and 80 µl of 200 mM acetate buffer (pH 4.0) were added to each well. The reaction was started by addition of 100 µl of 7.2 mM 4-methylumbelliferyl-β-D-glucopyranoside (200 mM acetate buffer pH 4.0) to each well, followed by incubation at 37°C for 2h. Enzymatic reactions were stopped by lysing the cells with 0.9 ml glycine/NaOH buffer (100 mM pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission at 460 nm) with the Fluoroskan Ascent FL plate reader (Labsystems) in 96-well format. Data are reported in Table S4 and Figure S5.

Table S4. Measurement of N370S GBA Activity in Fibroblasts Derived from Patients with GD.

Compound	Concentration nM			
	100,00	10,00	1,00	0,10
NN-DNJ	1,11 ± 0,03	0,99 ± 0,04	0,90 ± 0,09	0,99 ± 0,04
14a	1,17 ± 0,07	1,02 ± 0,08	0,95 ± 0,01	0,97 ± 0,05
14b	0,64 ± 0,08	0,91 ± 0,11	1,00 ± 0,15	1,07 ± 0,05
14c	0,40 ± 0,06	0,77 ± 0,09	0,99 ± 0,08	1,01 ± 0,04
14d	0,34 ± 0,04	0,67 ± 0,05	0,95 ± 0,06	1,02 ± 0,07
14e	0,29 ± 0,03	0,55 ± 0,05	0,97 ± 0,04	1,02 ± 0,05

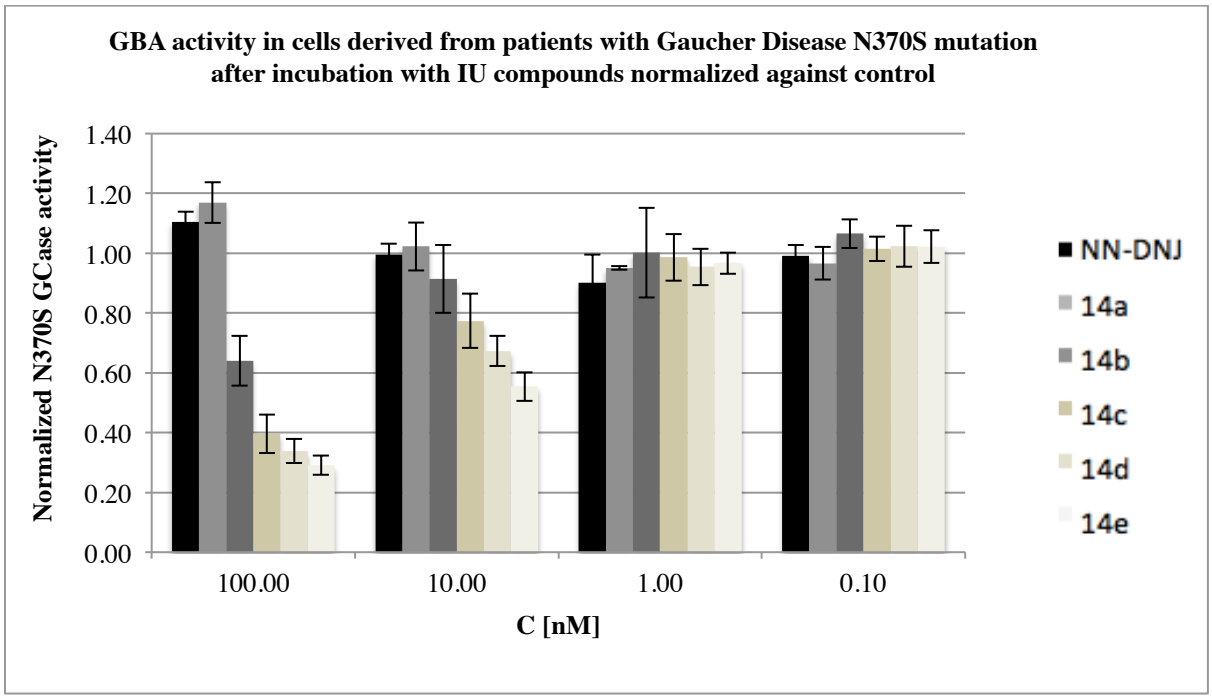
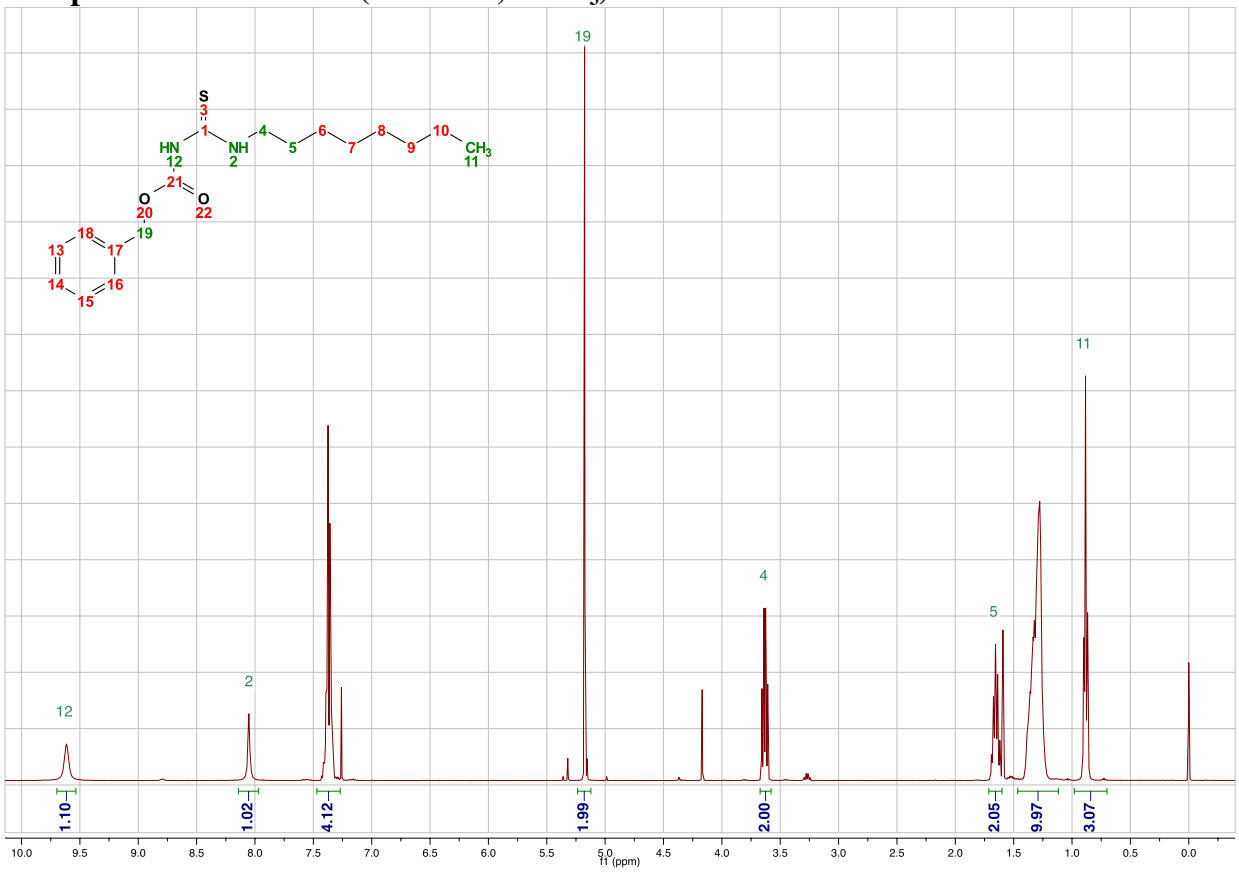


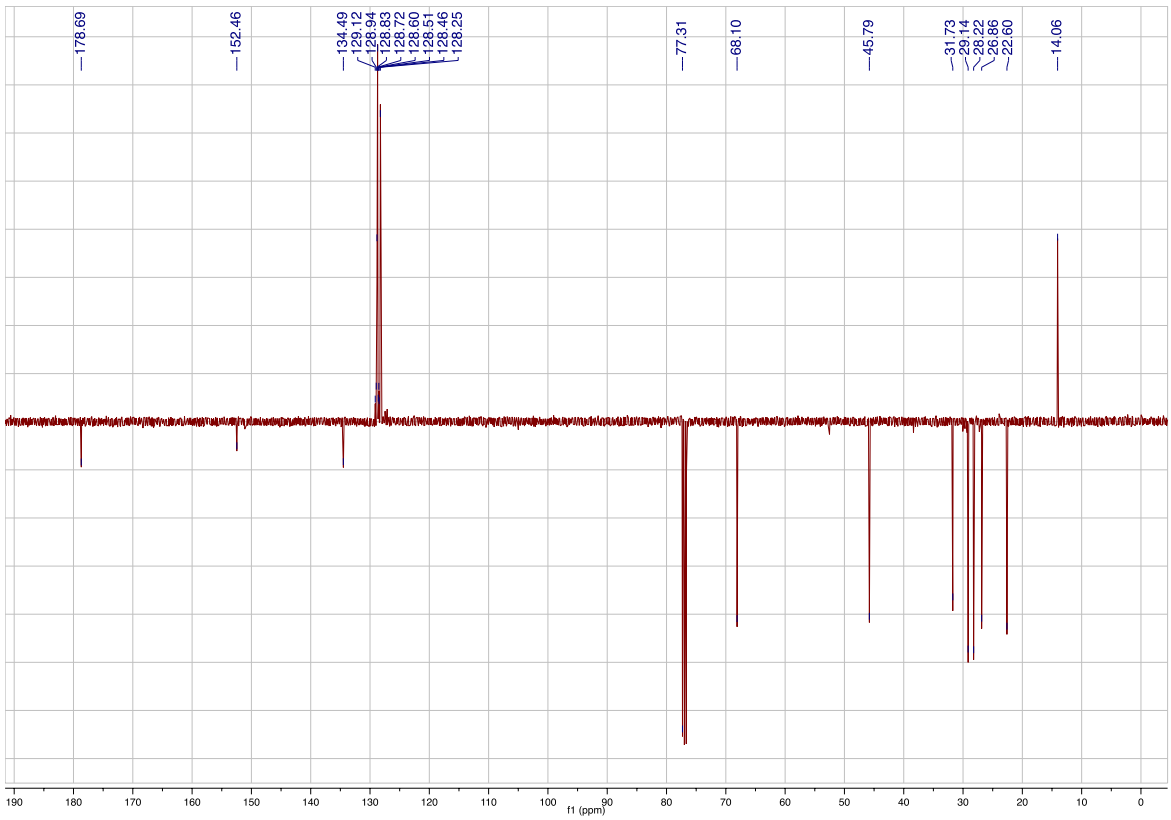
Figure S5. The effect of compounds **14(a-e)** on GBA activity in N370S fibroblasts (GM00372) from Gaucher patients. Cells were cultured for 4 days in the absence or presence of increasing concentrations (nM) of the compounds before GBA activity was measured. Experiments were performed in triplicate, and each bar represents the mean ± SD. Enzyme activity is normalized to untreated cells, assigned a relative activity of 1.

^{1}H and ^{13}C NMR spectra for compounds 11(a-e), 12(a-e), 13(a-e), 14(a-e), 15 and 16.

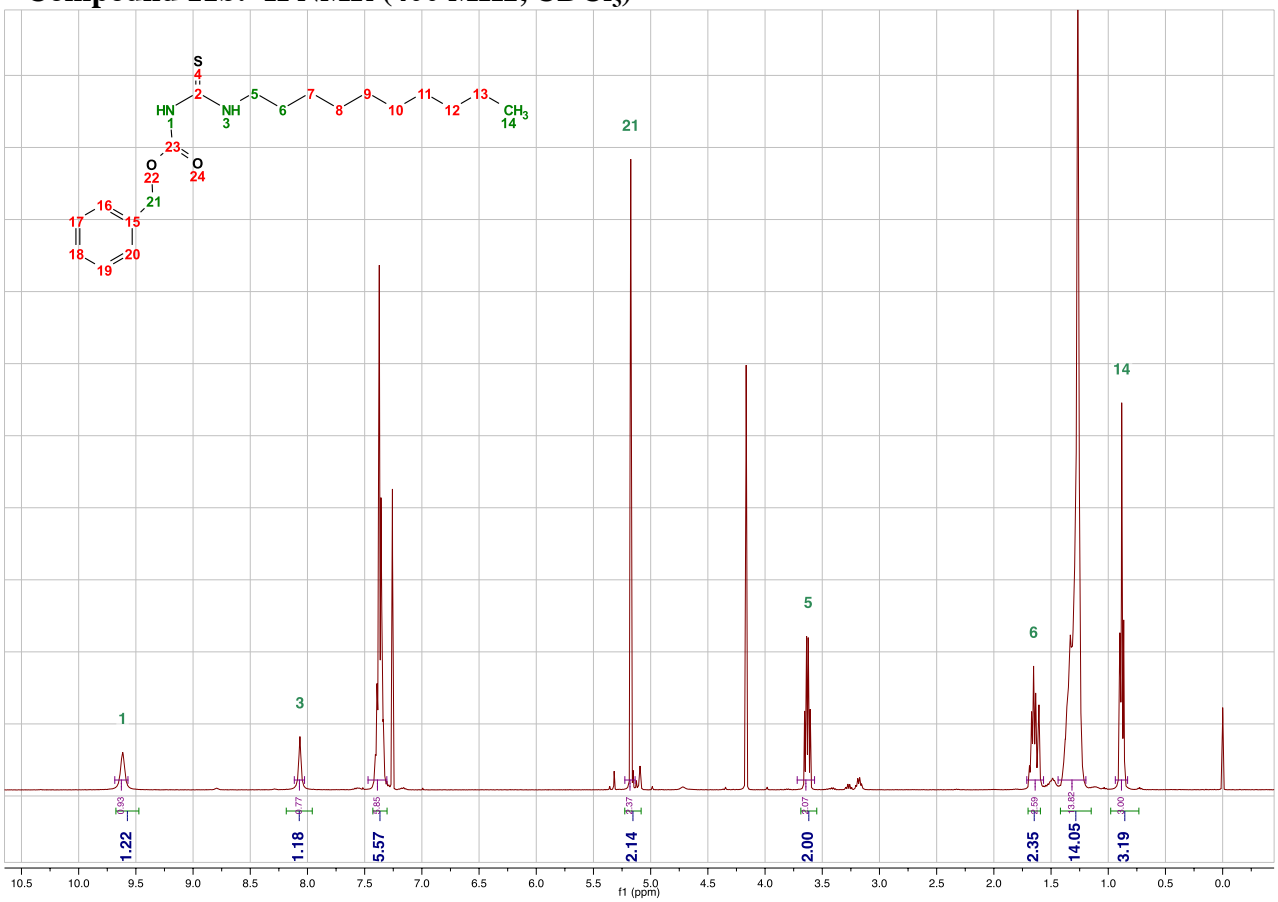
Compound 11a: ^{1}H NMR (400 MHz, $CDCl_3$)



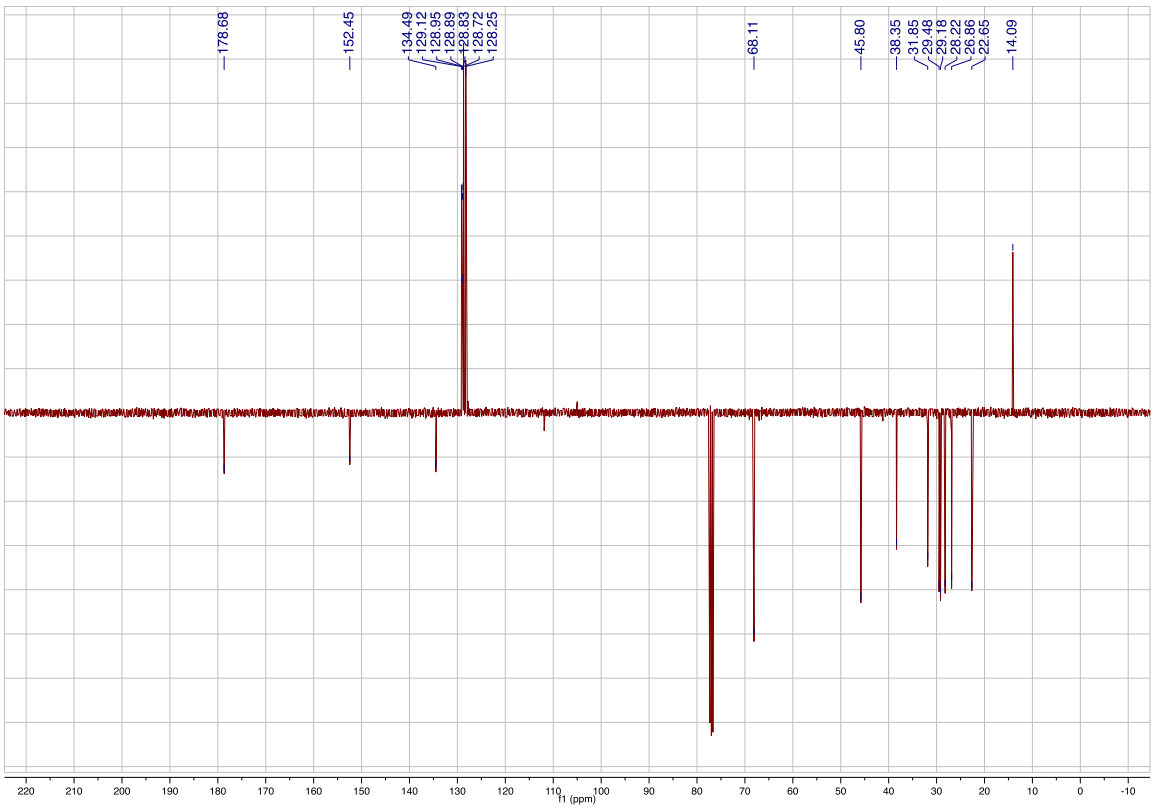
Compound 11a: ^{13}C NMR (101 MHz, $CDCl_3$)



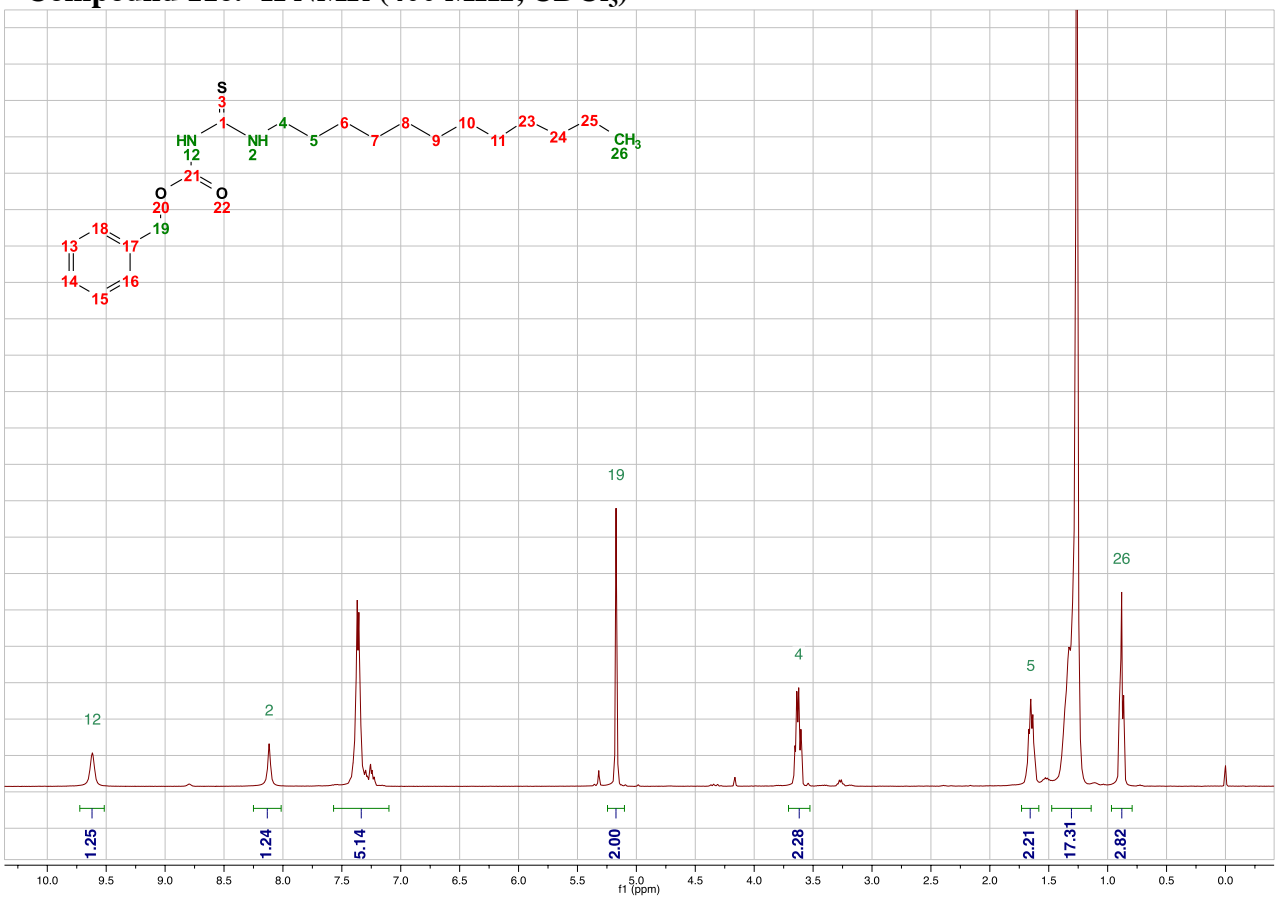
Compound 11b: ^{1}H NMR (400 MHz, $CDCl_3$)



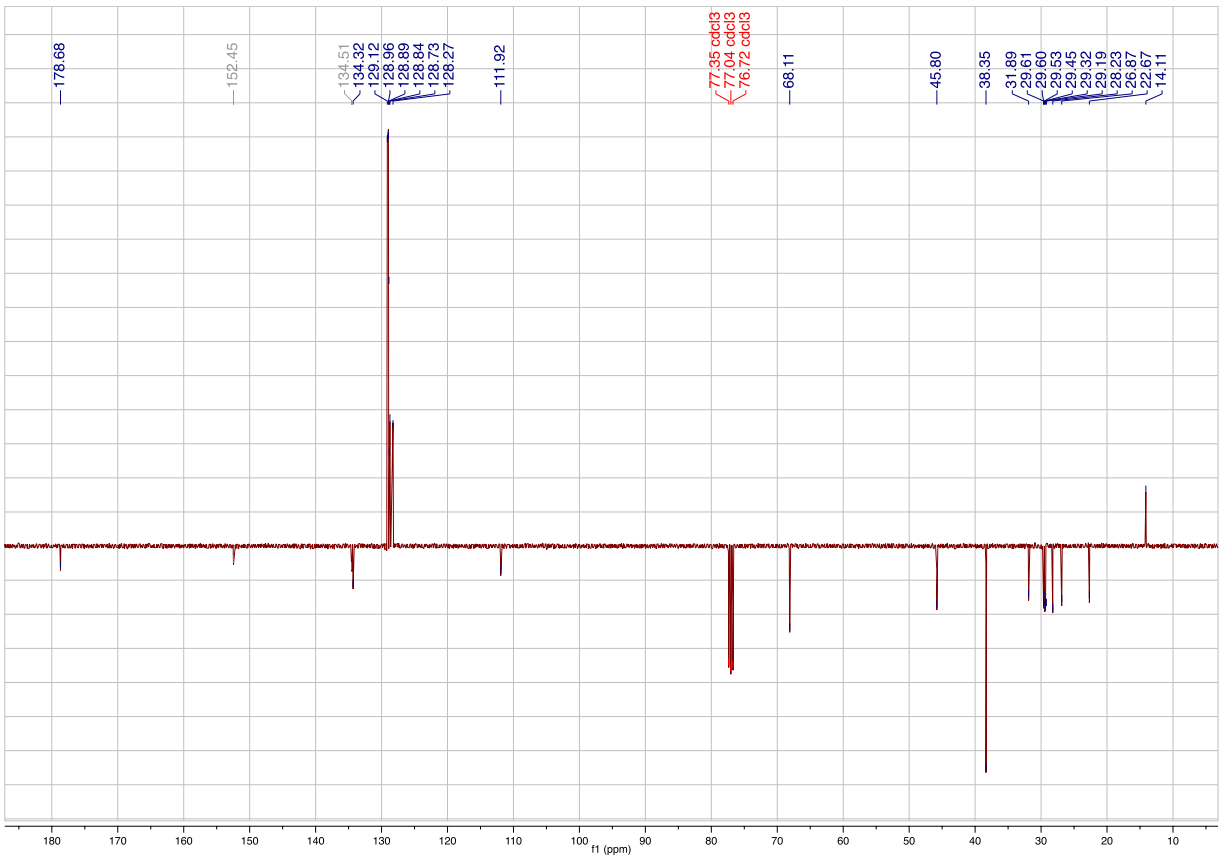
Compound 11b: ^{13}C NMR (101 MHz, $CDCl_3$)



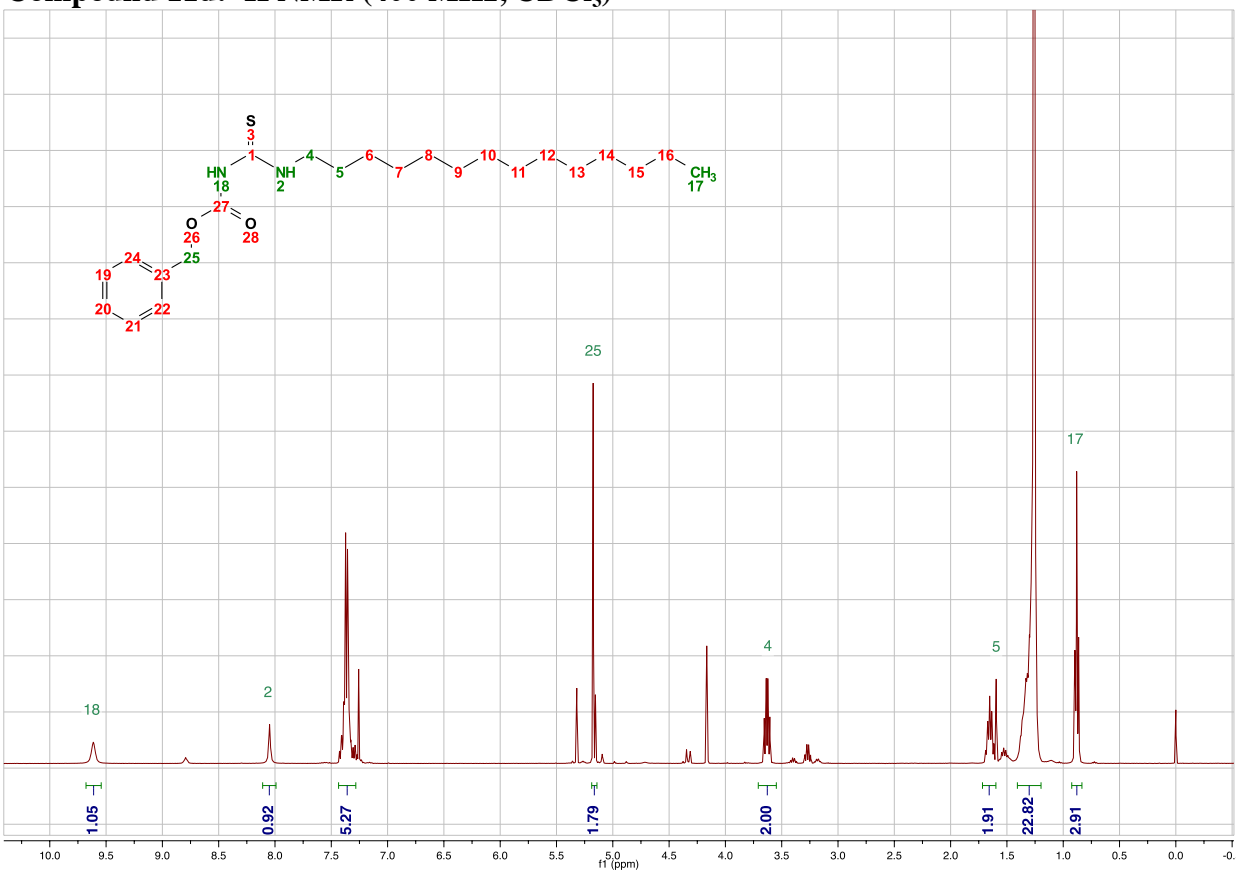
Compound 11c: ^{1}H NMR (400 MHz, $CDCl_3$)



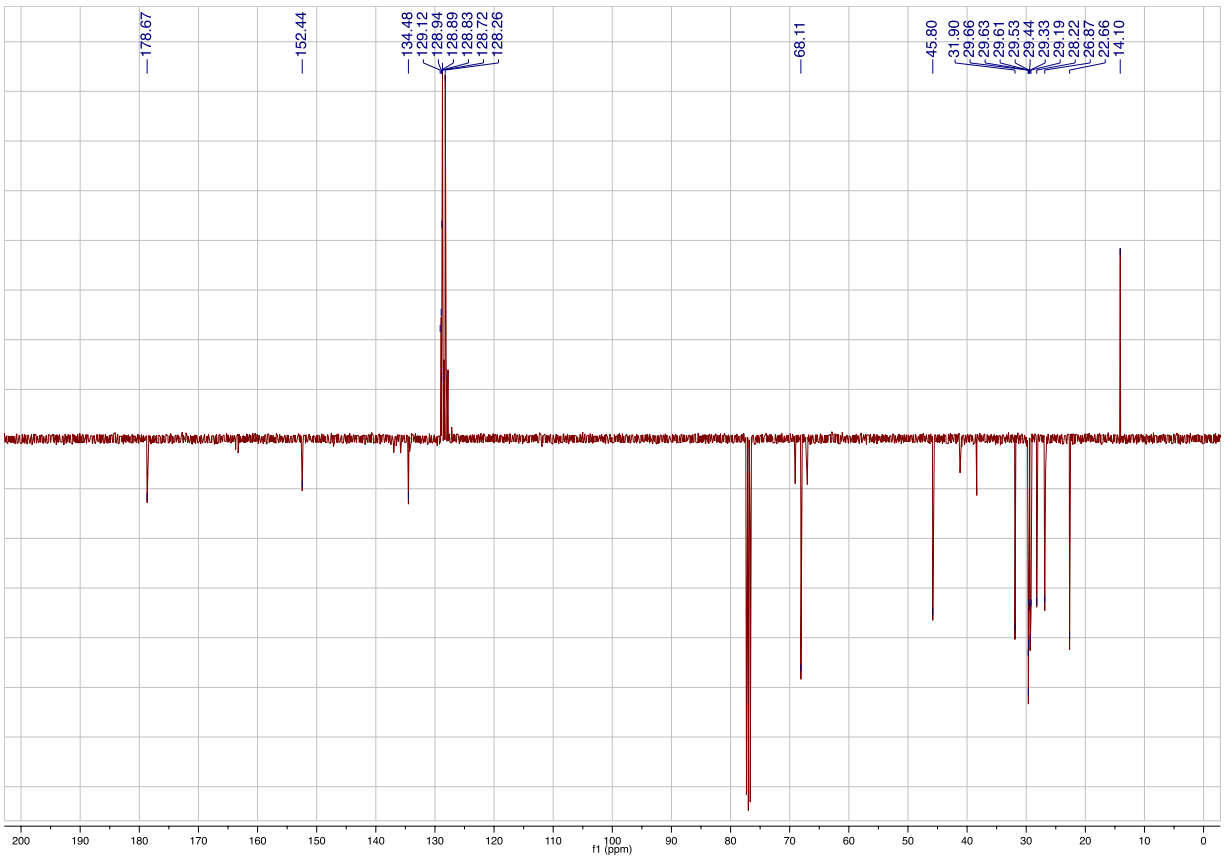
Compound 11c: ^{13}C NMR (101 MHz, $CDCl_3$)



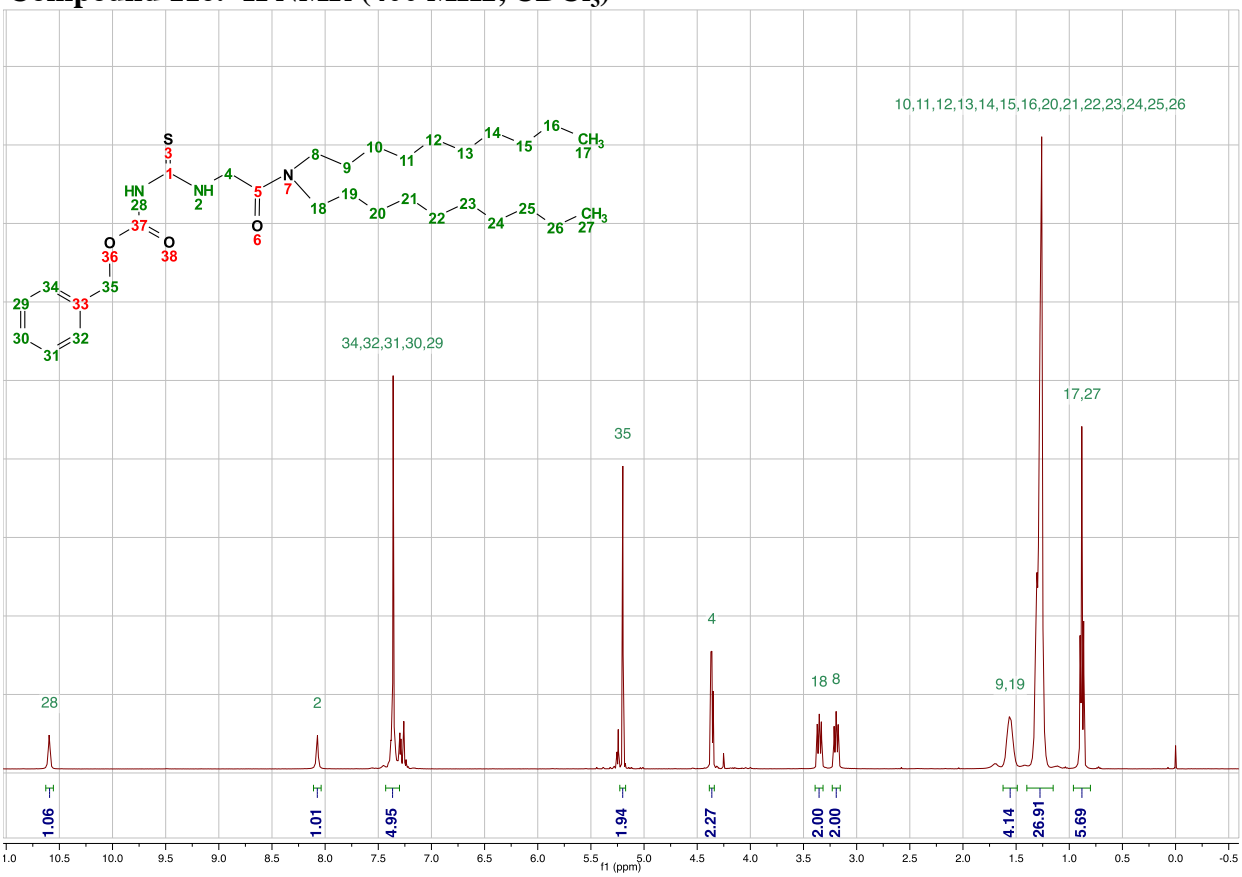
Compound 11d: ^{1}H NMR (400 MHz, $CDCl_3$)



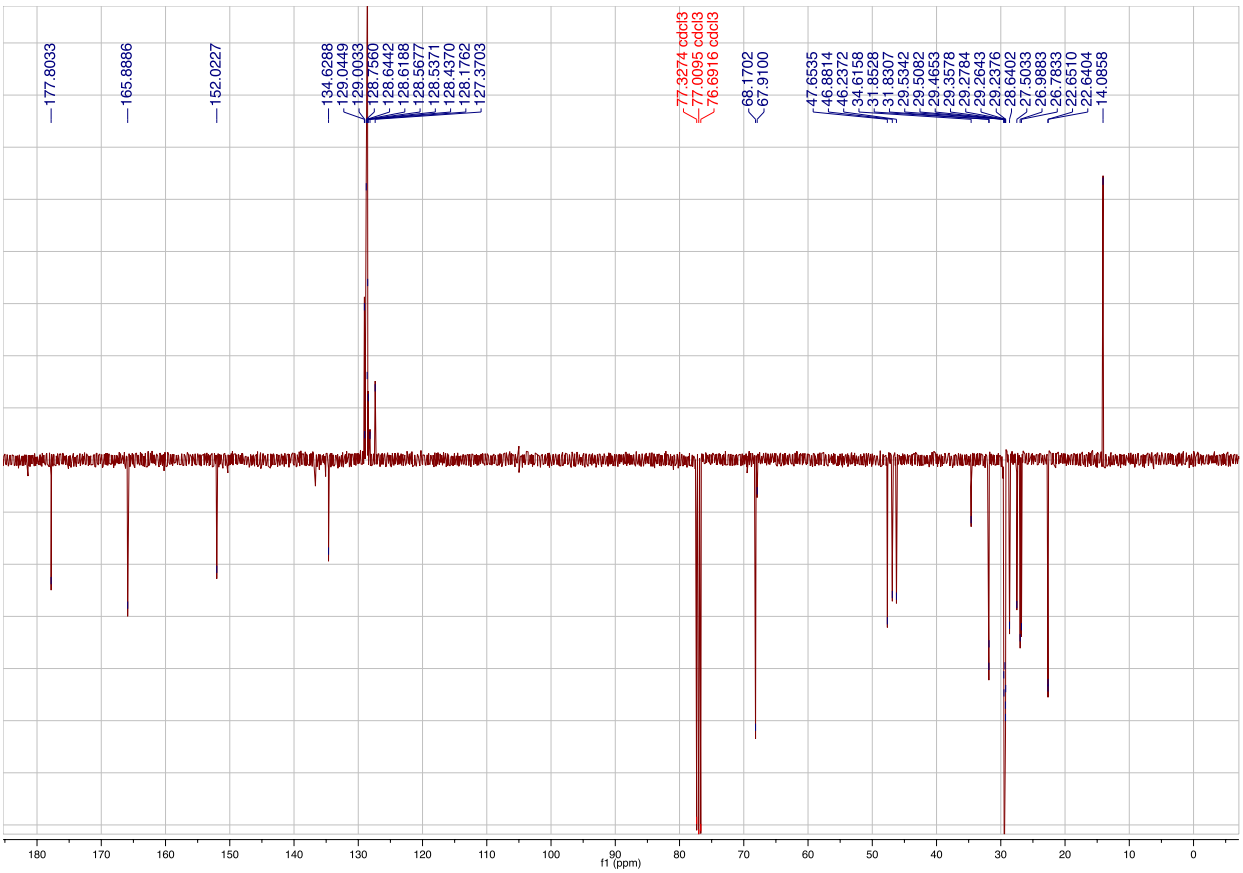
Compound 11d: ^{13}C NMR (101 MHz, $CDCl_3$)



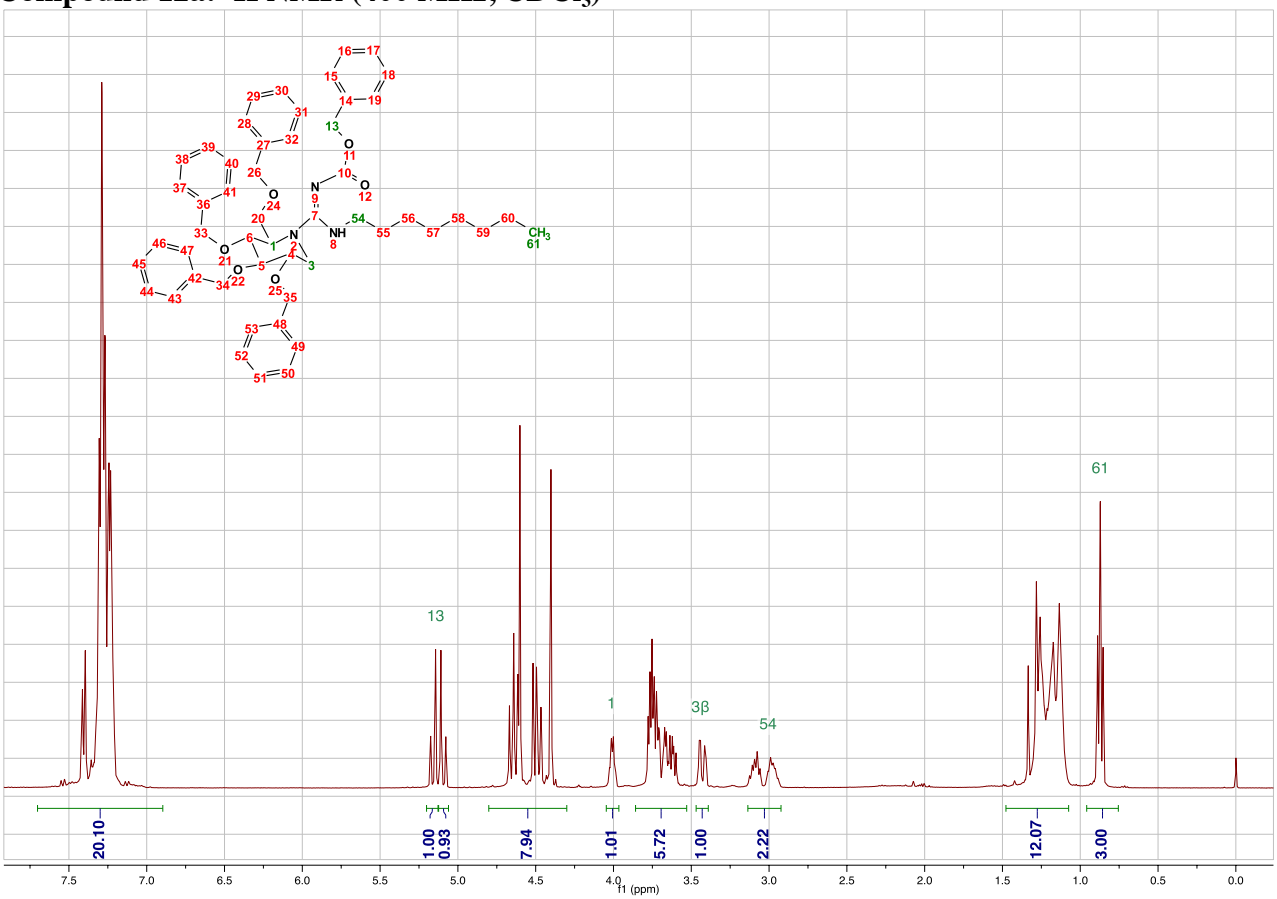
Compound 11e: ^{1}H NMR (400 MHz, $CDCl_3$)



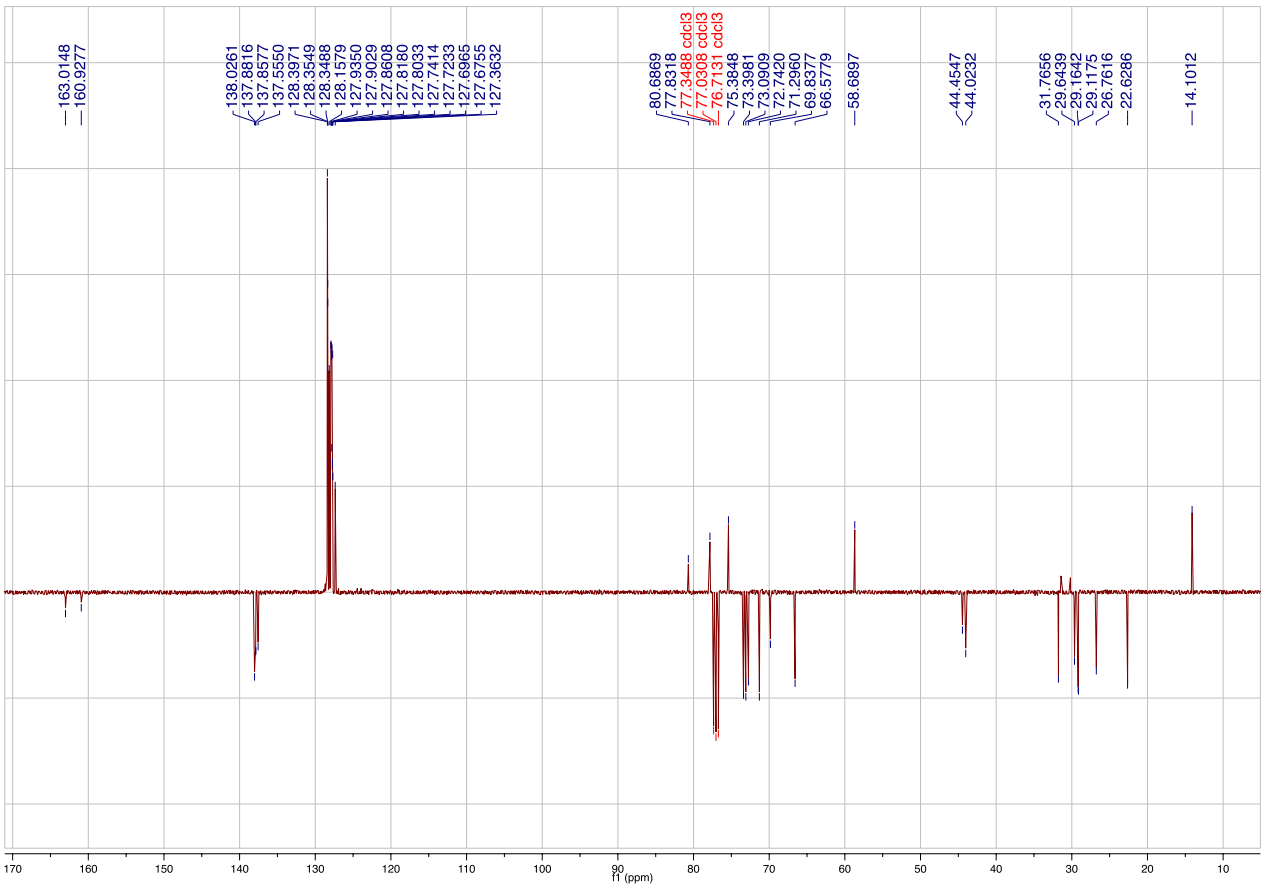
Compound 11e: ^{13}C NMR (101 MHz, $CDCl_3$)



Compound 12a: ^{1}H NMR (400 MHz, $CDCl_3$)



Compound 12a: ^{13}C NMR (101 MHz, $CDCl_3$)



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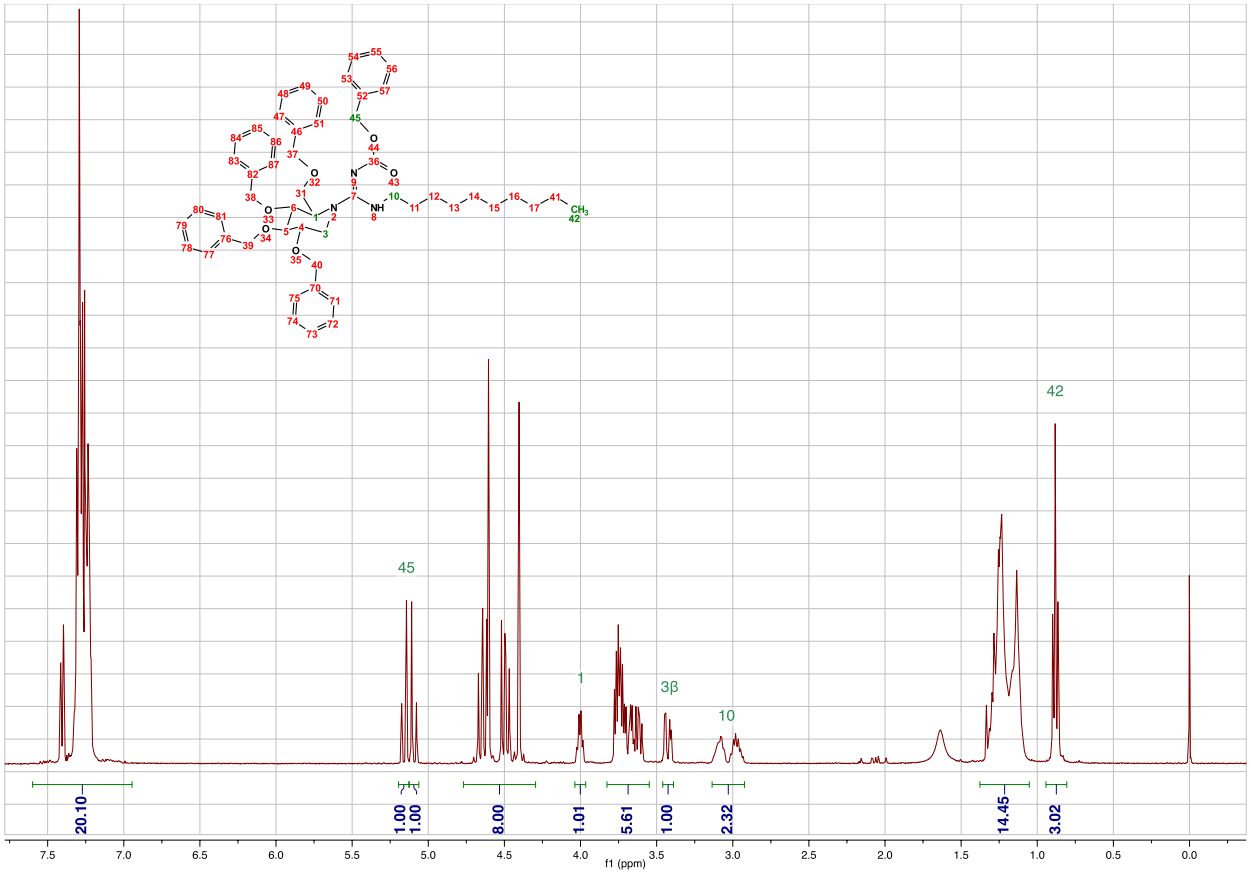
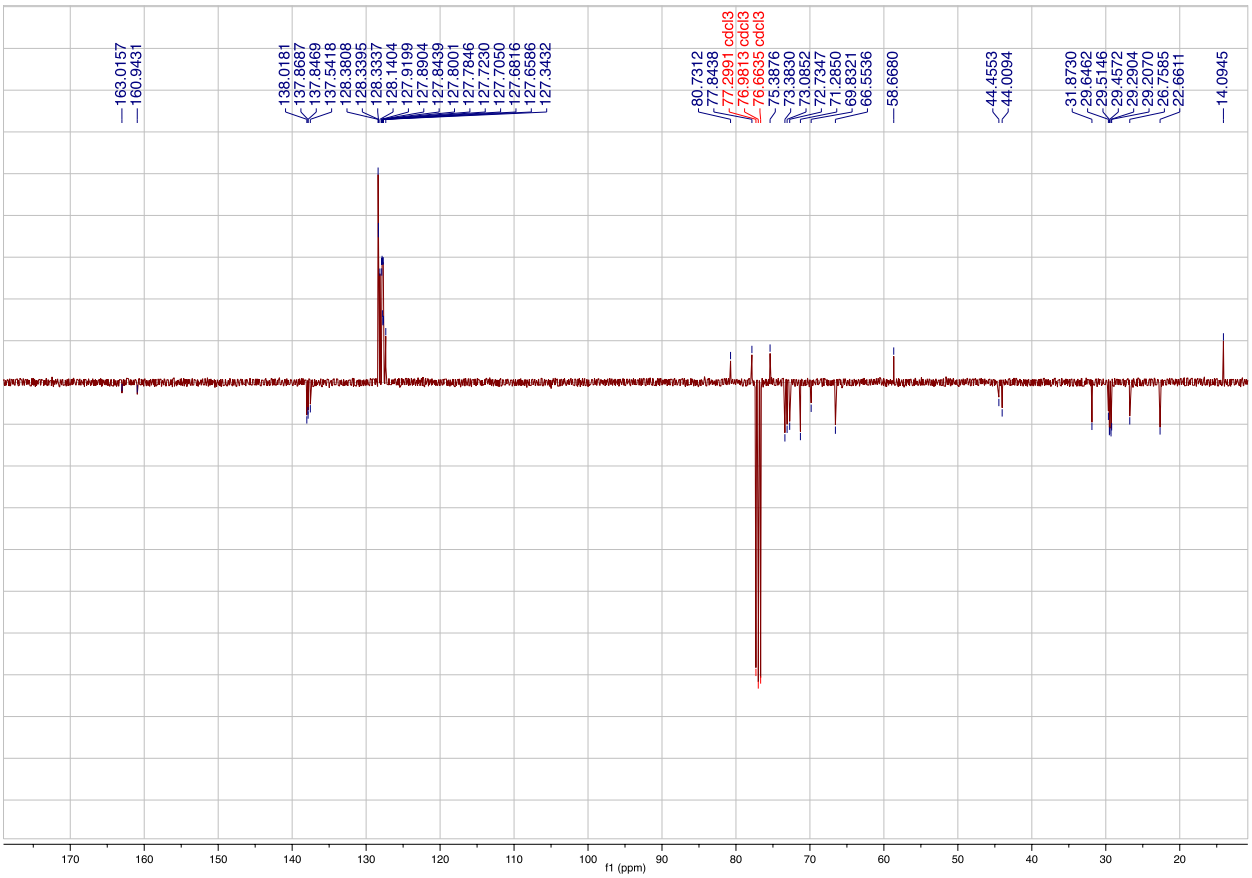


Compound 12b: ^{13}C NMR (101 MHz, $CDCl_3$)



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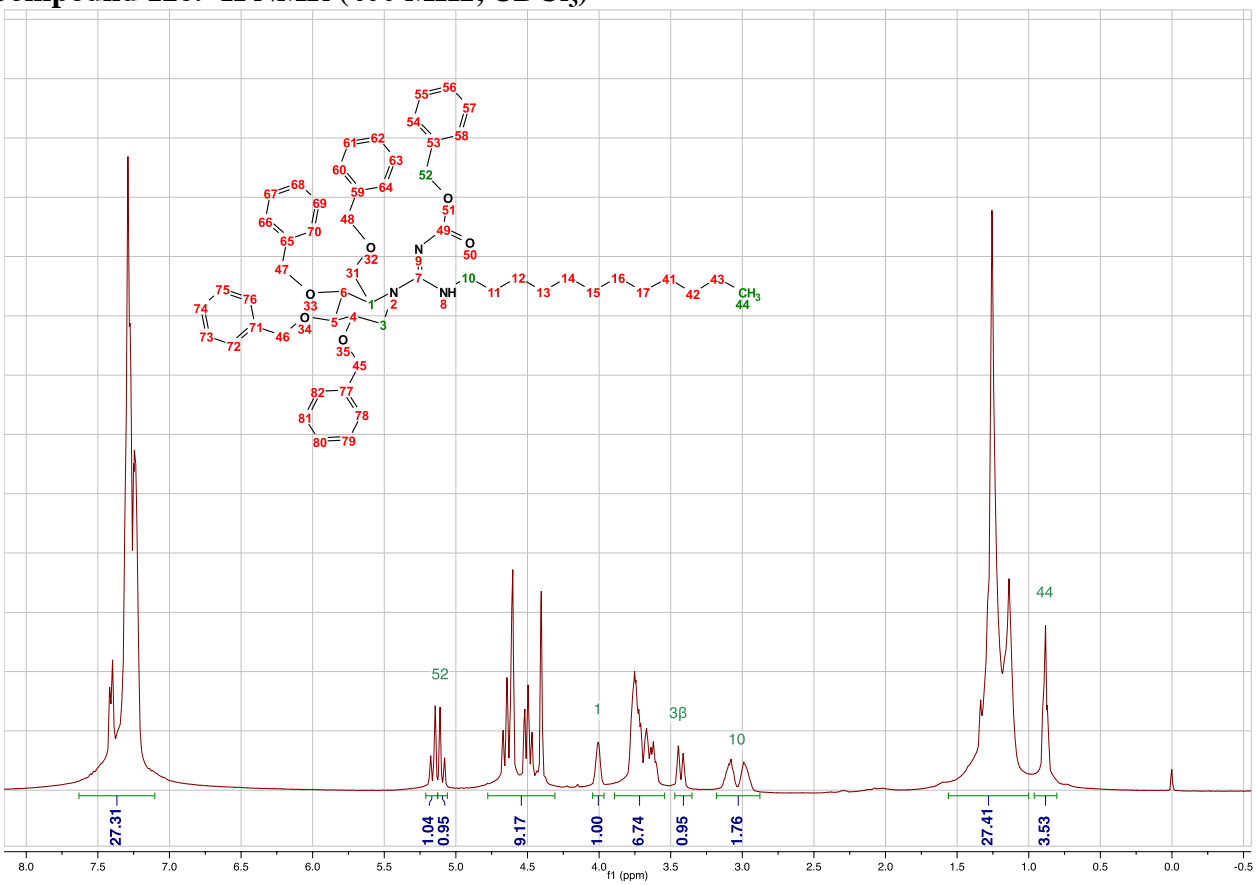

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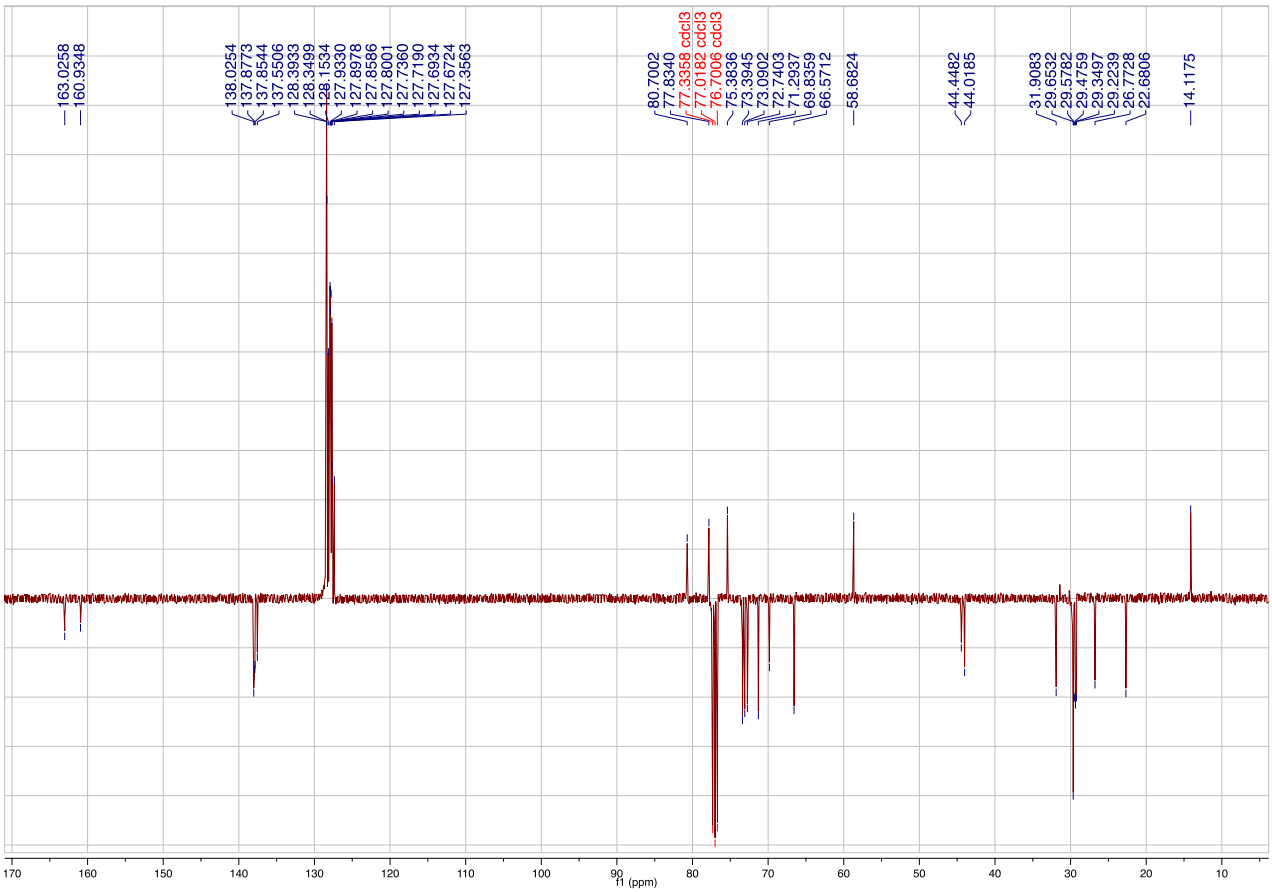

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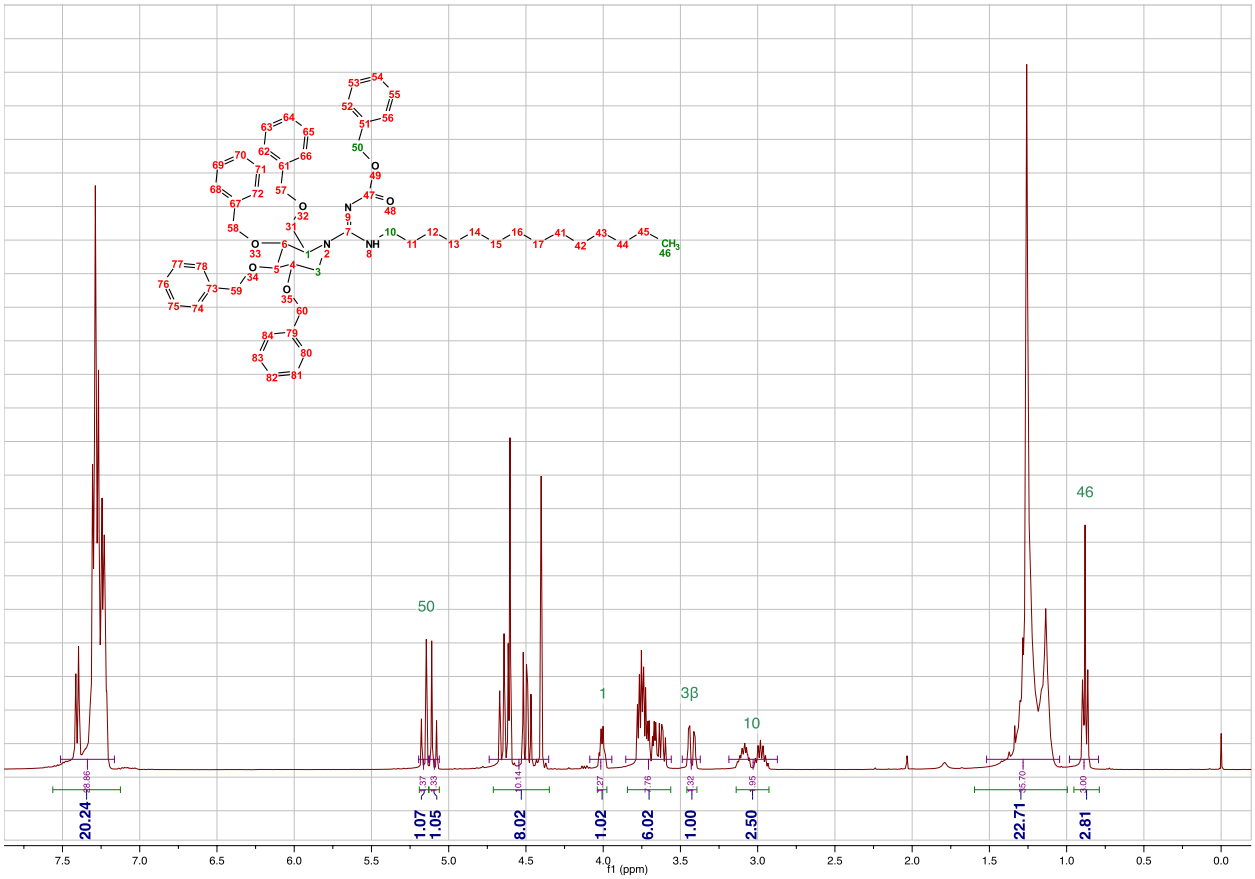

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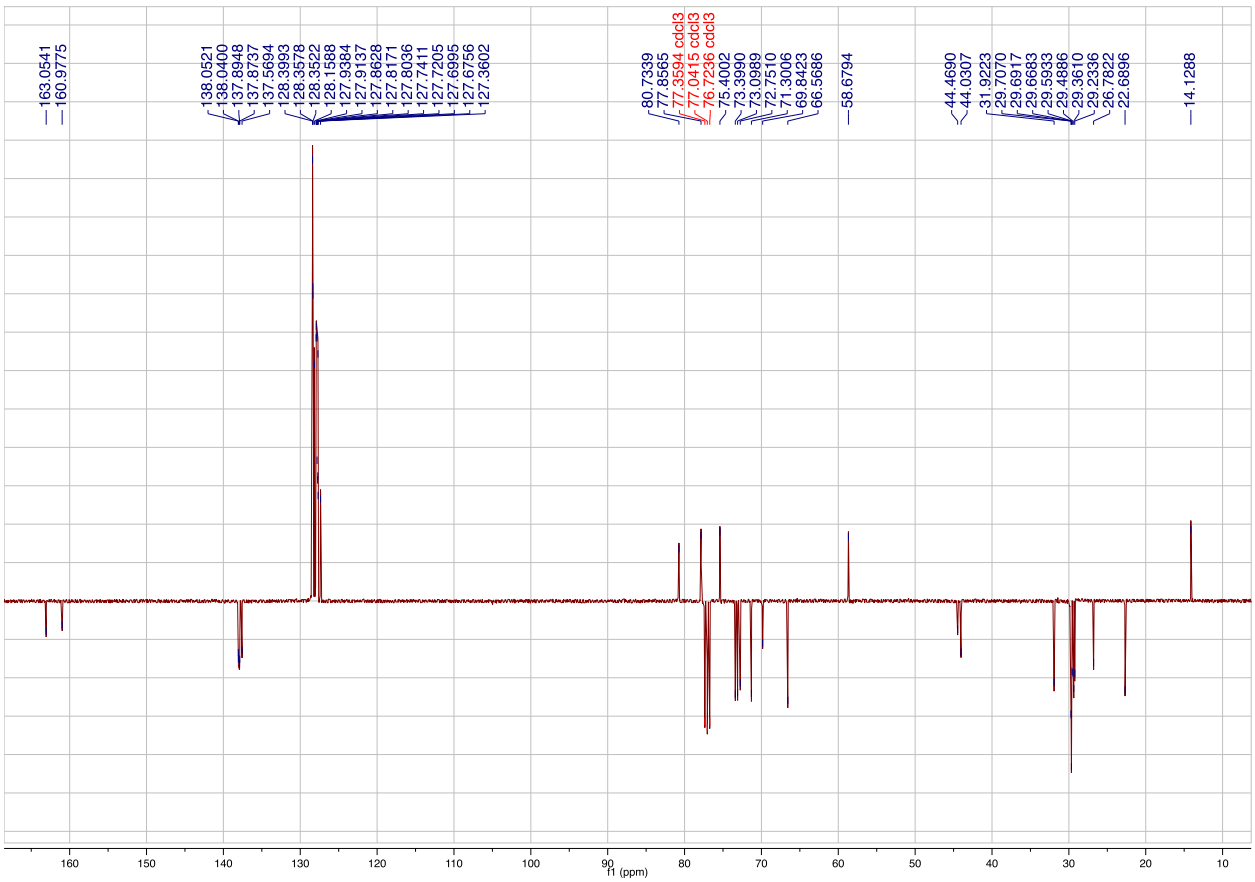
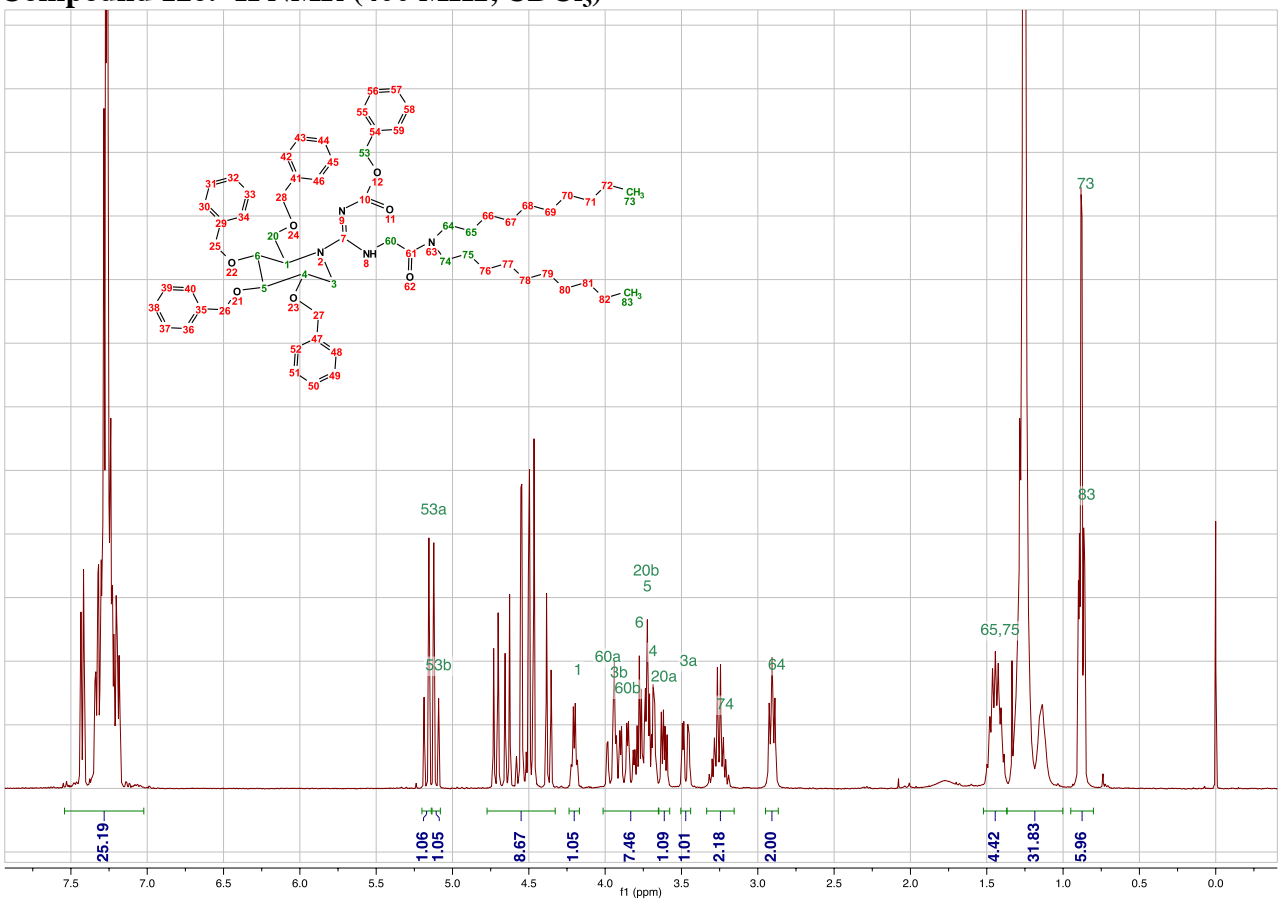


Compound 12e: ^{1}H NMR (400 MHz, $CDCl_3$)



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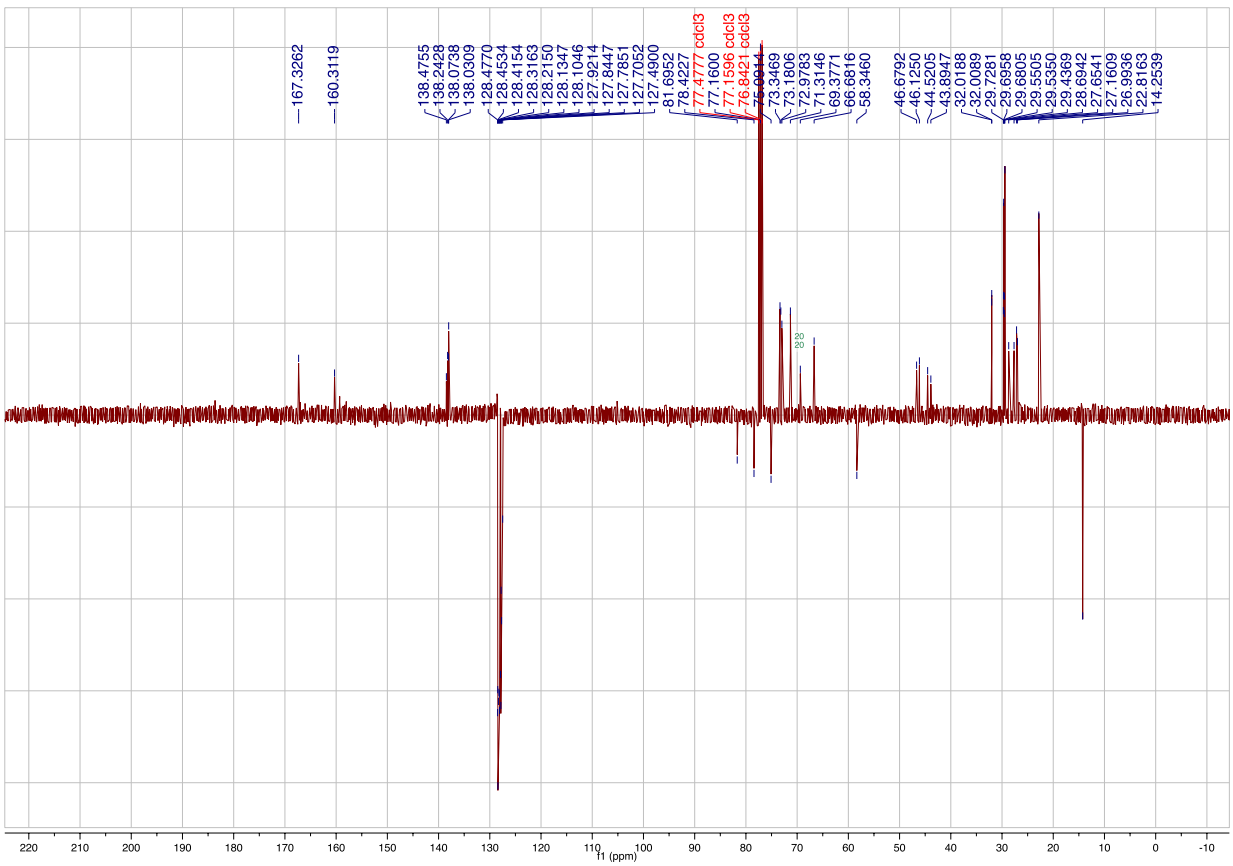
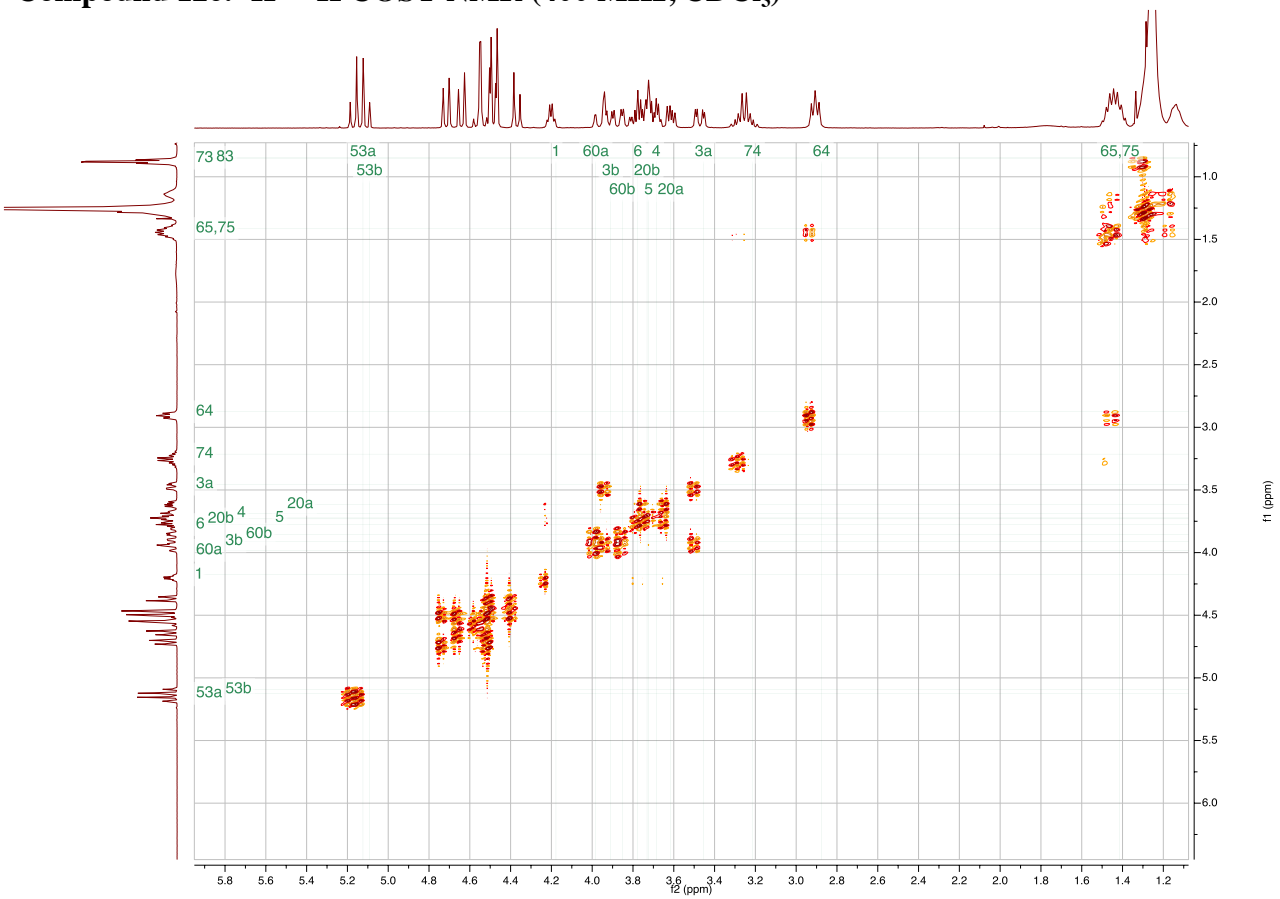
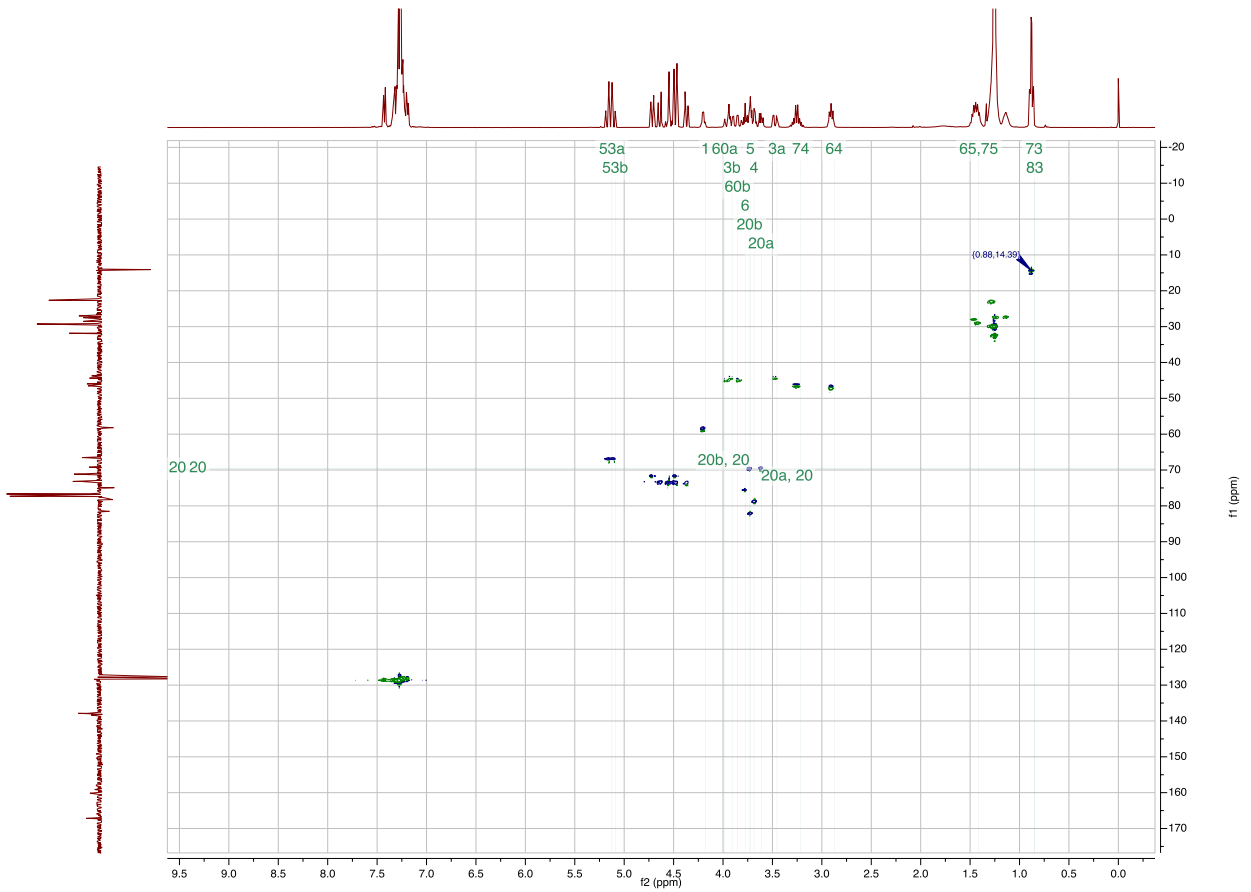


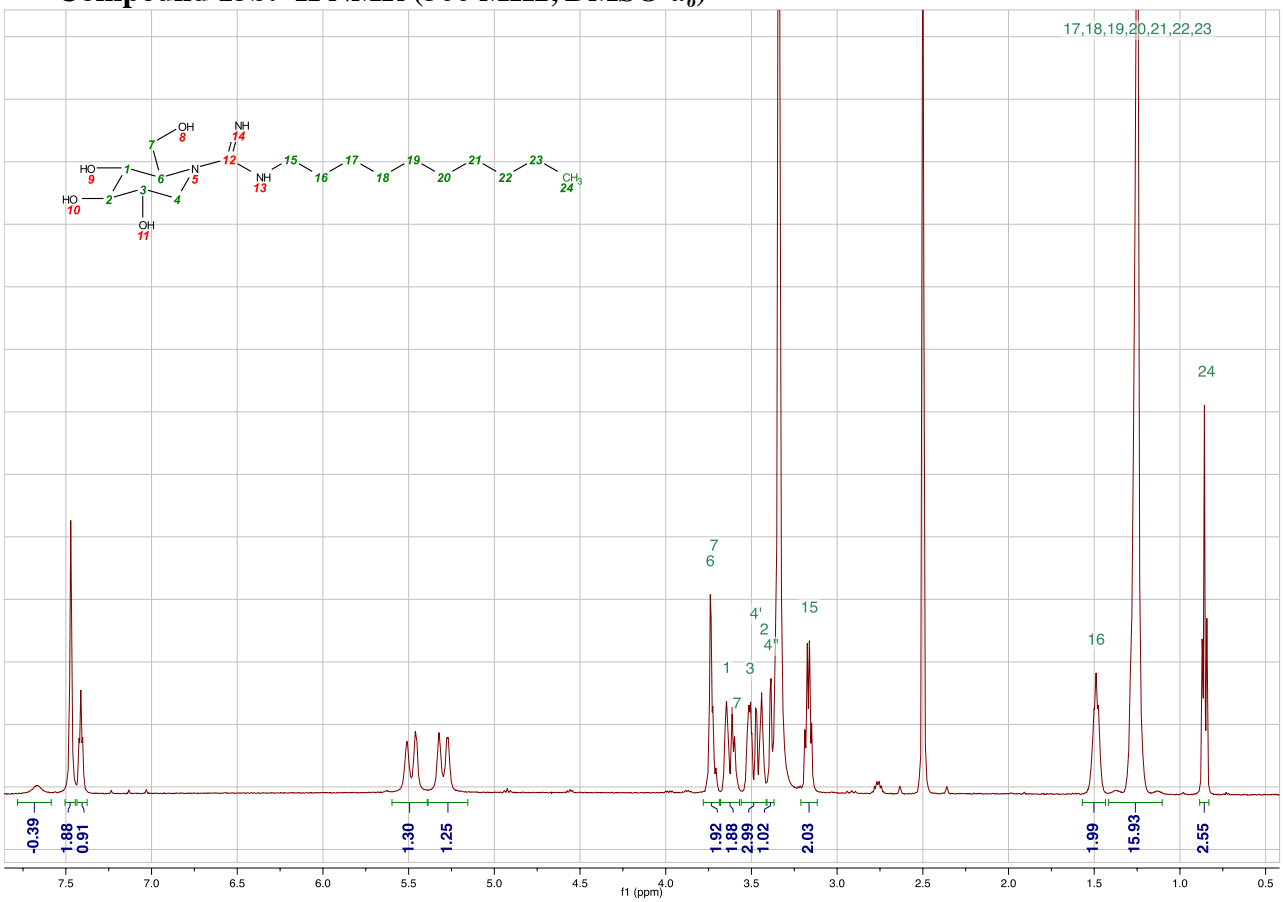
Compound 12e: $^{1}H - ^{1}H$ COSY NMR (400 MHz, $CDCl_3$)



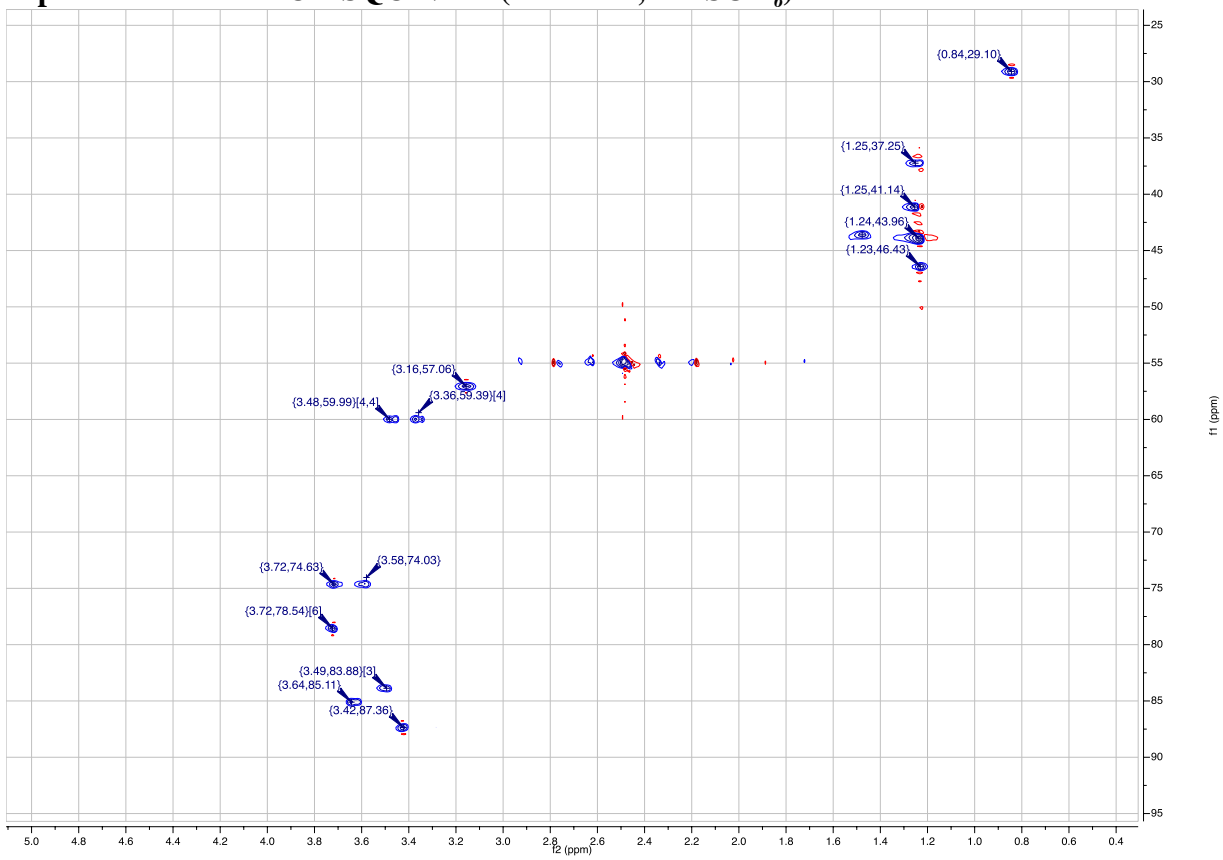
Compound 12e: $^{1}H - ^{13}C$ HSQC NMR (400 MHz, $CDCl_3$)



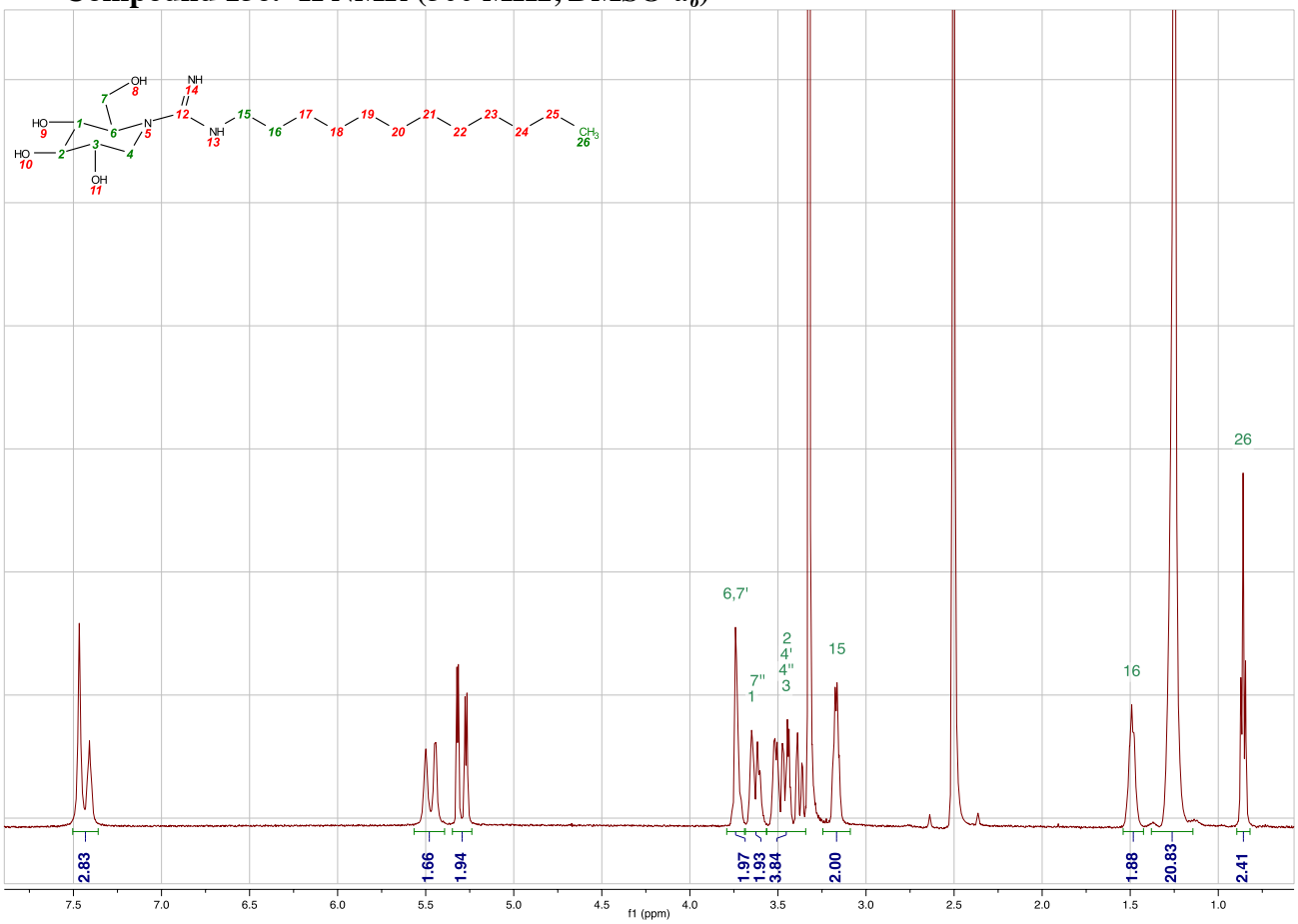
Compound 13b: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound 13b: ^{1}H – ^{13}C HSQC NMR (126 MHz, DMSO-d_6)

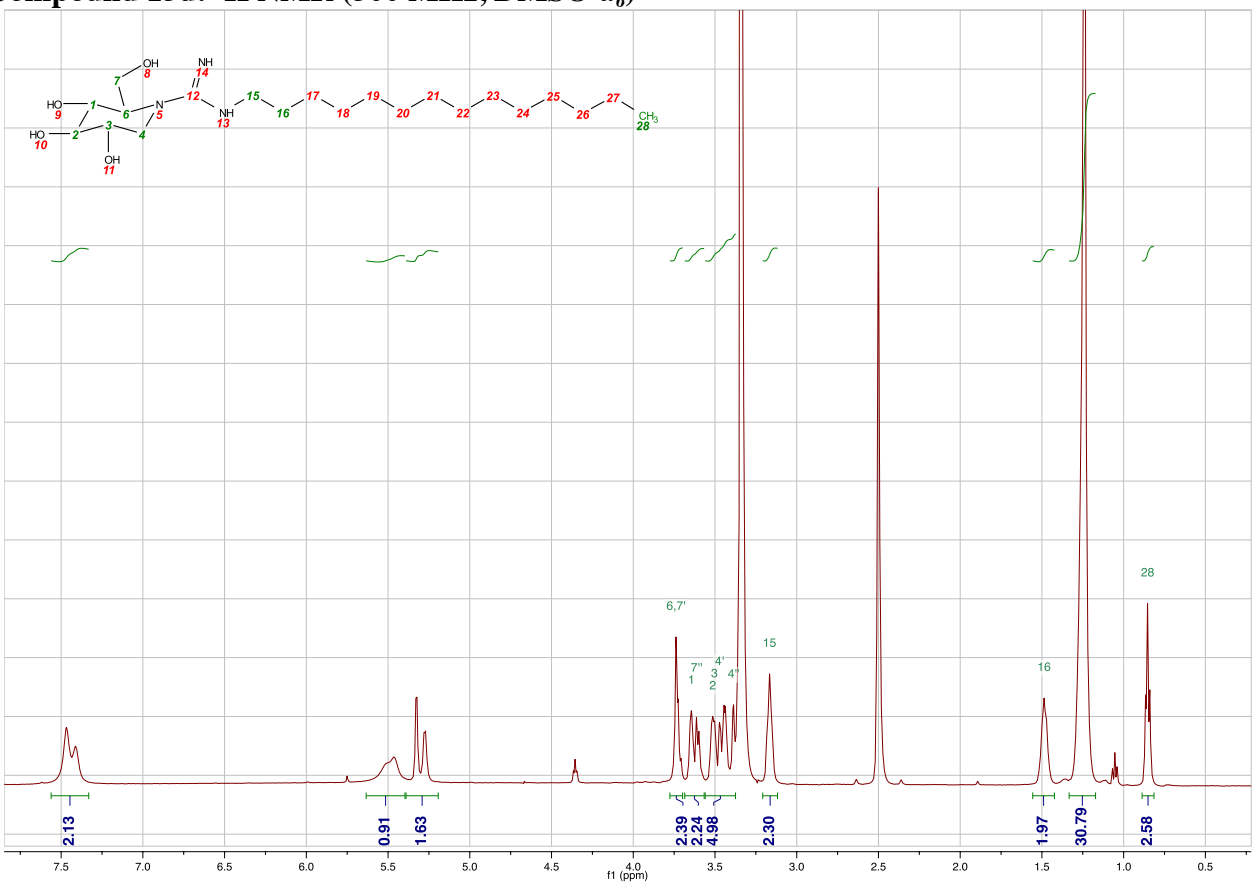


Compound 13c: ^{1}H NMR (500 MHz, DMSO-d_6)



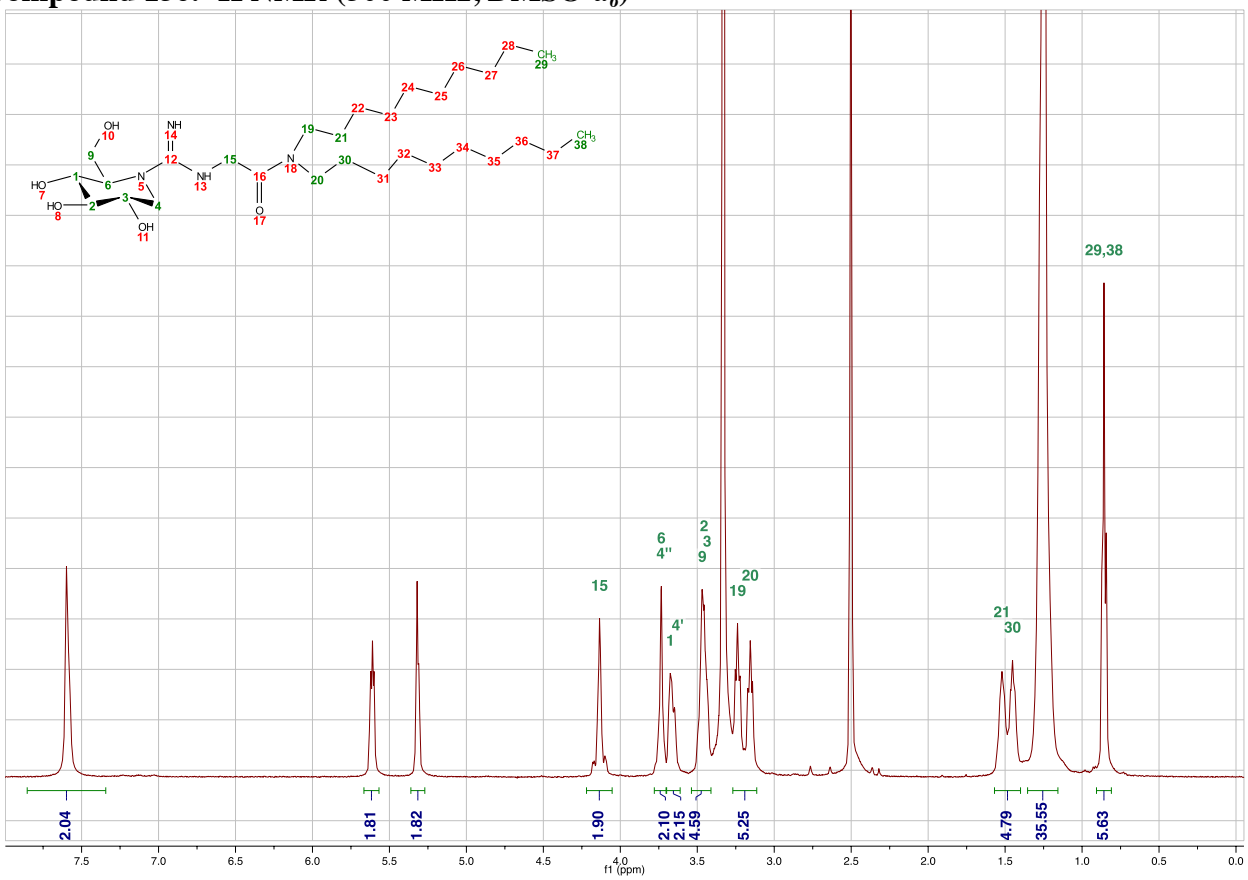
Compound 13c: ^{1}H – ^{13}C HSQC NMR (126 MHz, DMSO-d_6)

Compound 13d: ^{1}H NMR (500 MHz, DMSO-d_6)



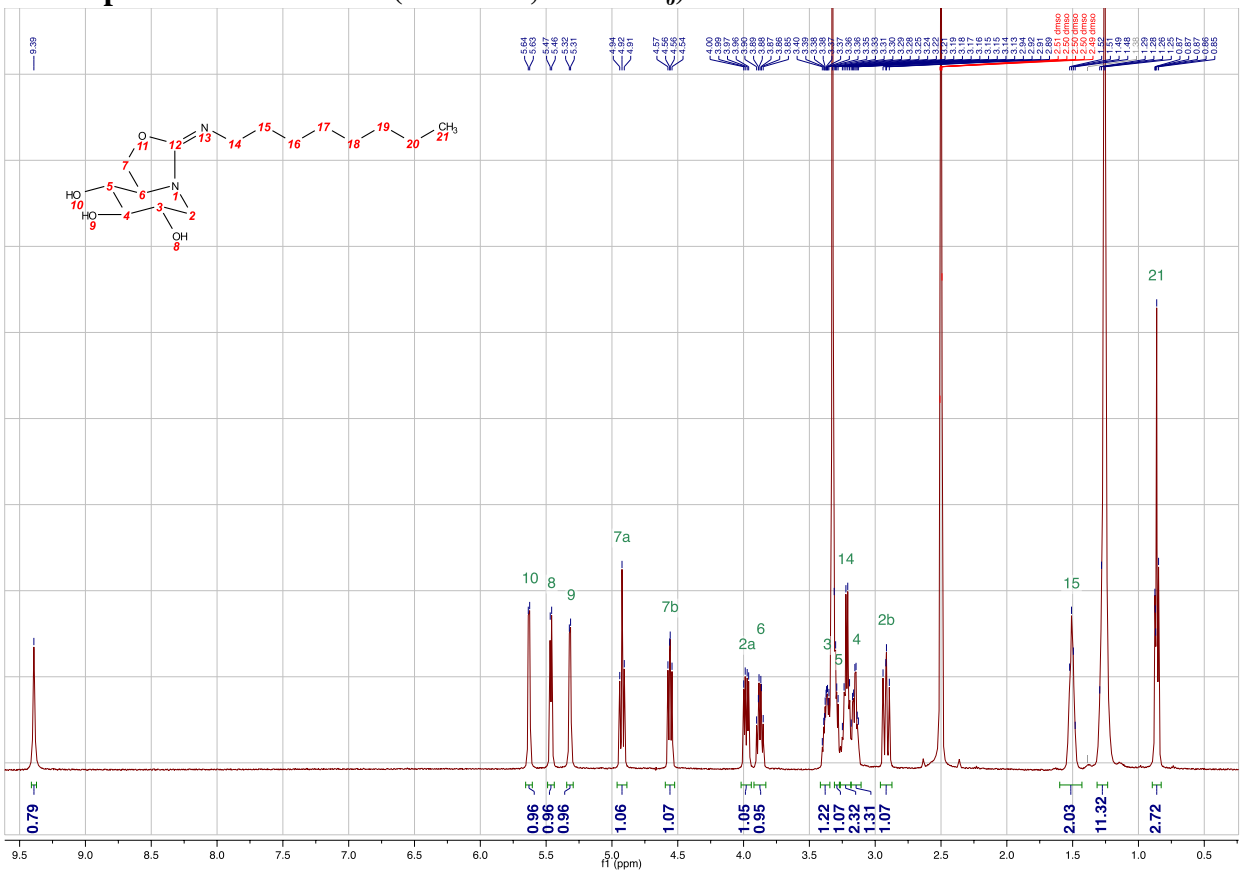
Compound 13d: ^{1}H – ^{13}C HSQC NMR (126 MHz, DMSO-d_6)

Compound 13e: ^{1}H NMR (500 MHz, DMSO-d_6)

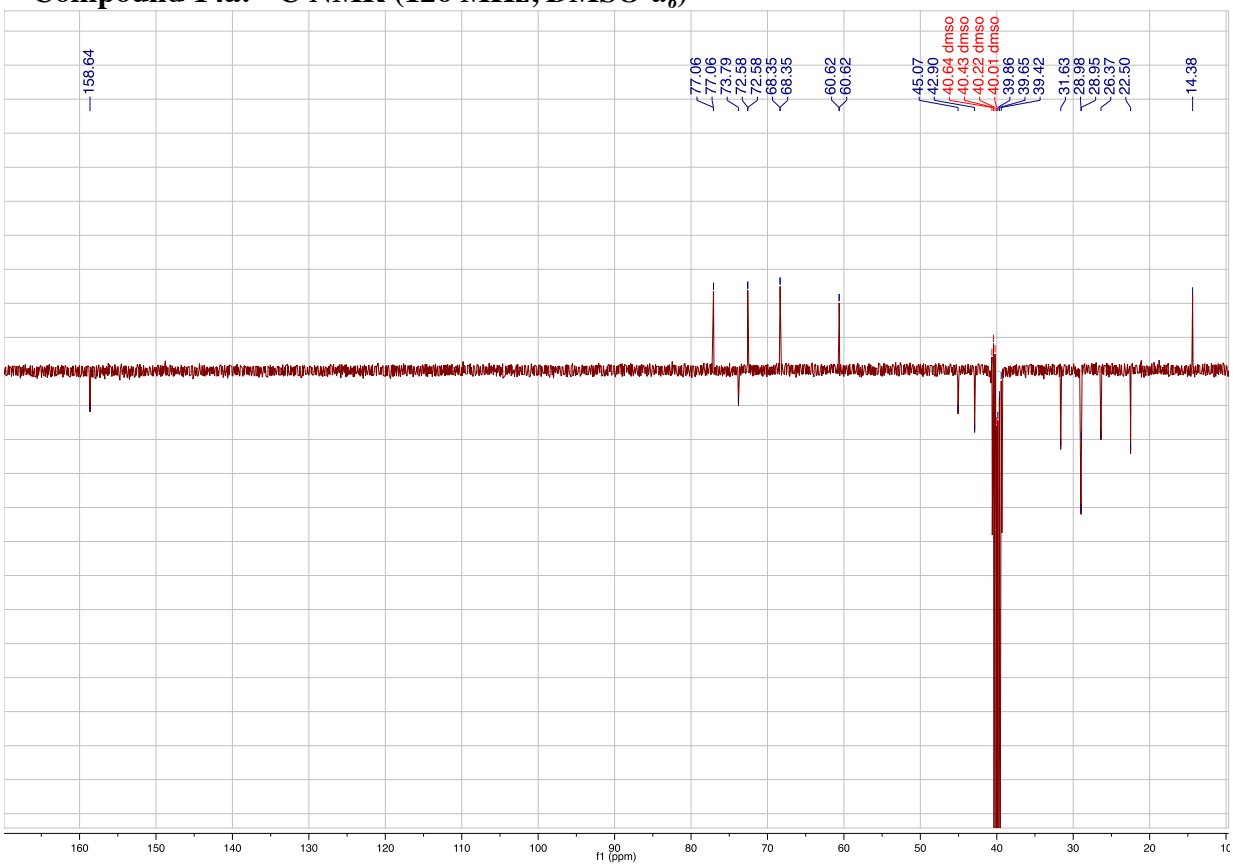


Compound 13e: ^{1}H – ^{13}C HSQC NMR (126 MHz, DMSO-d_6)

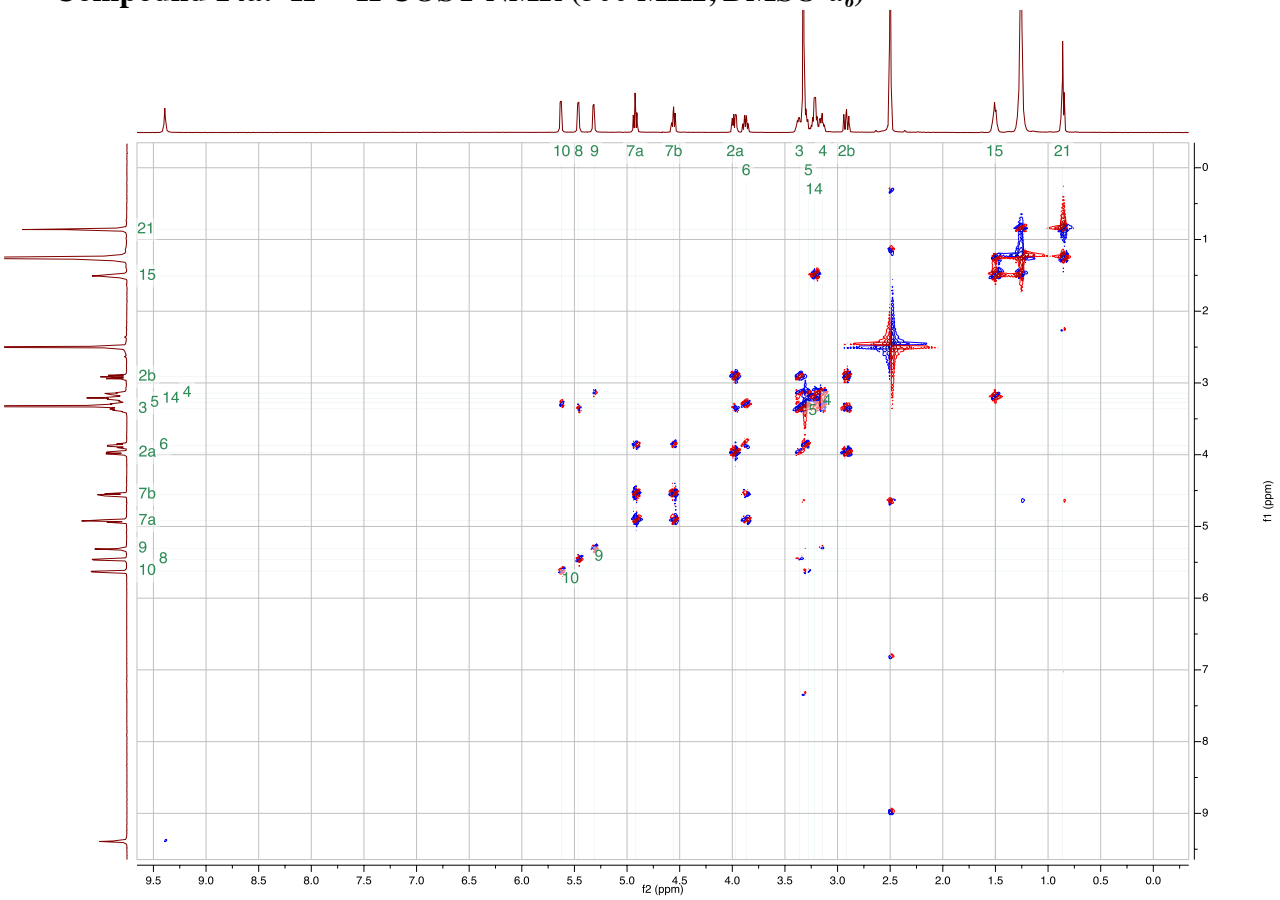
Compound 14a: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound 14a: ^{13}C NMR (126 MHz, DMSO-d_6)



Compound 14a: $^{1}H - ^{1}H$ COSY NMR (500 MHz, DMSO-d_6)



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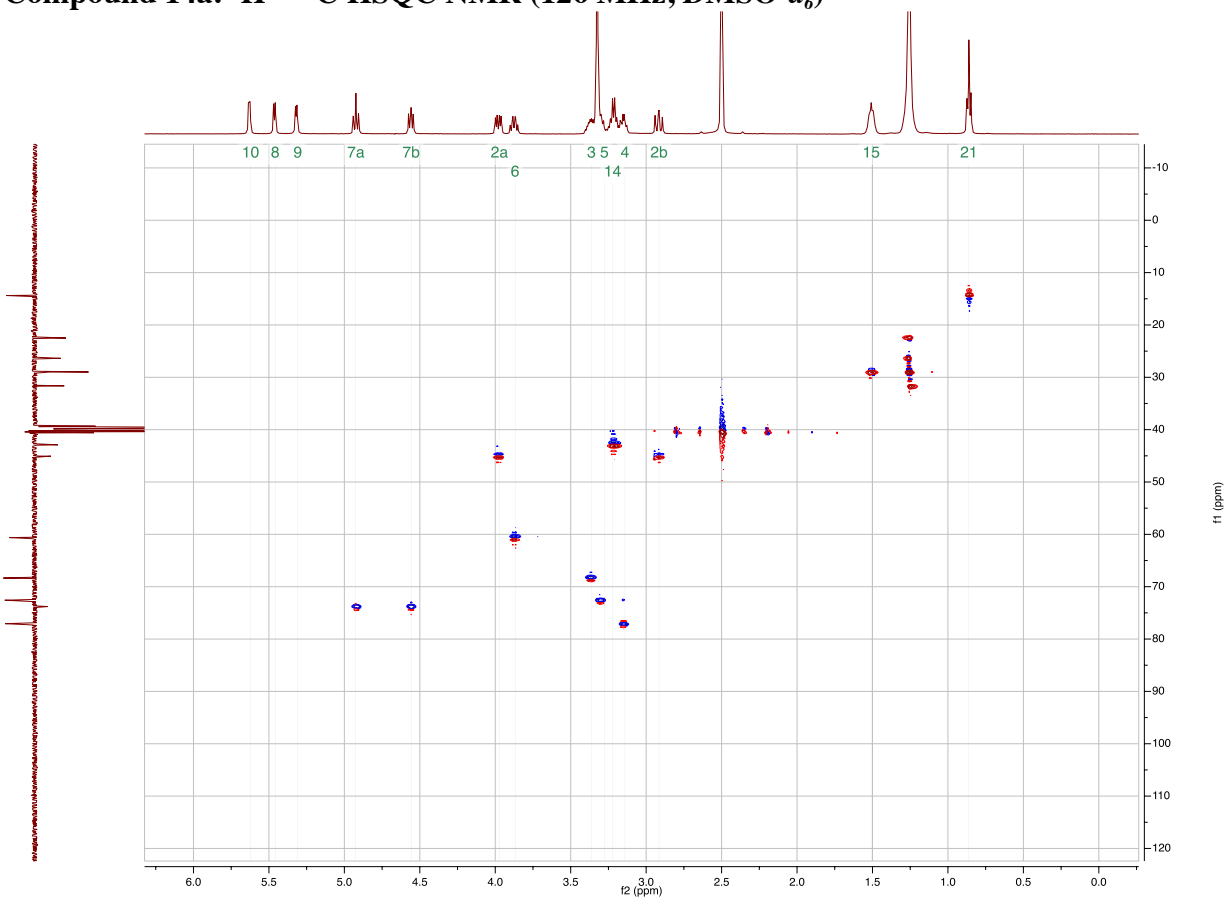
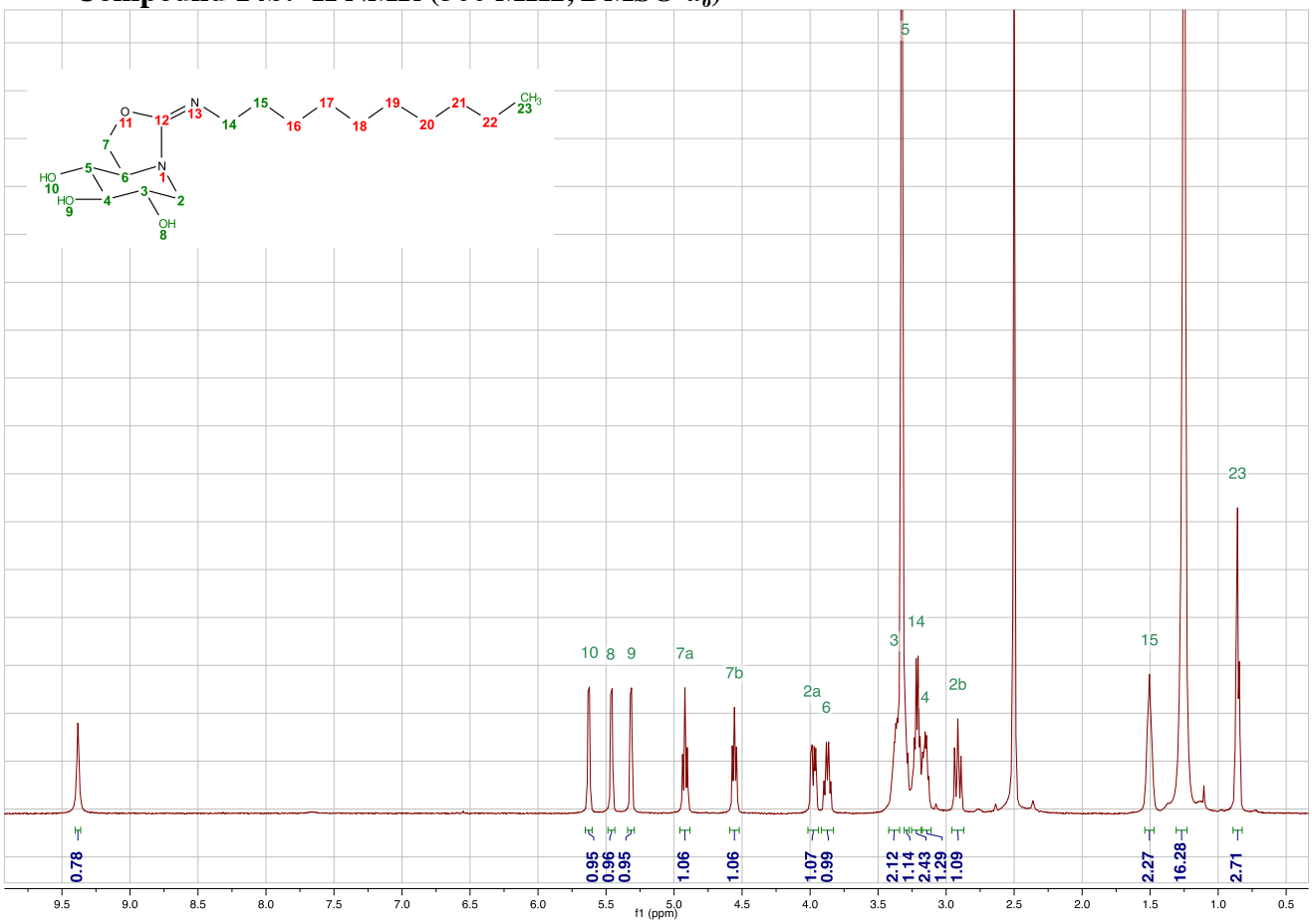
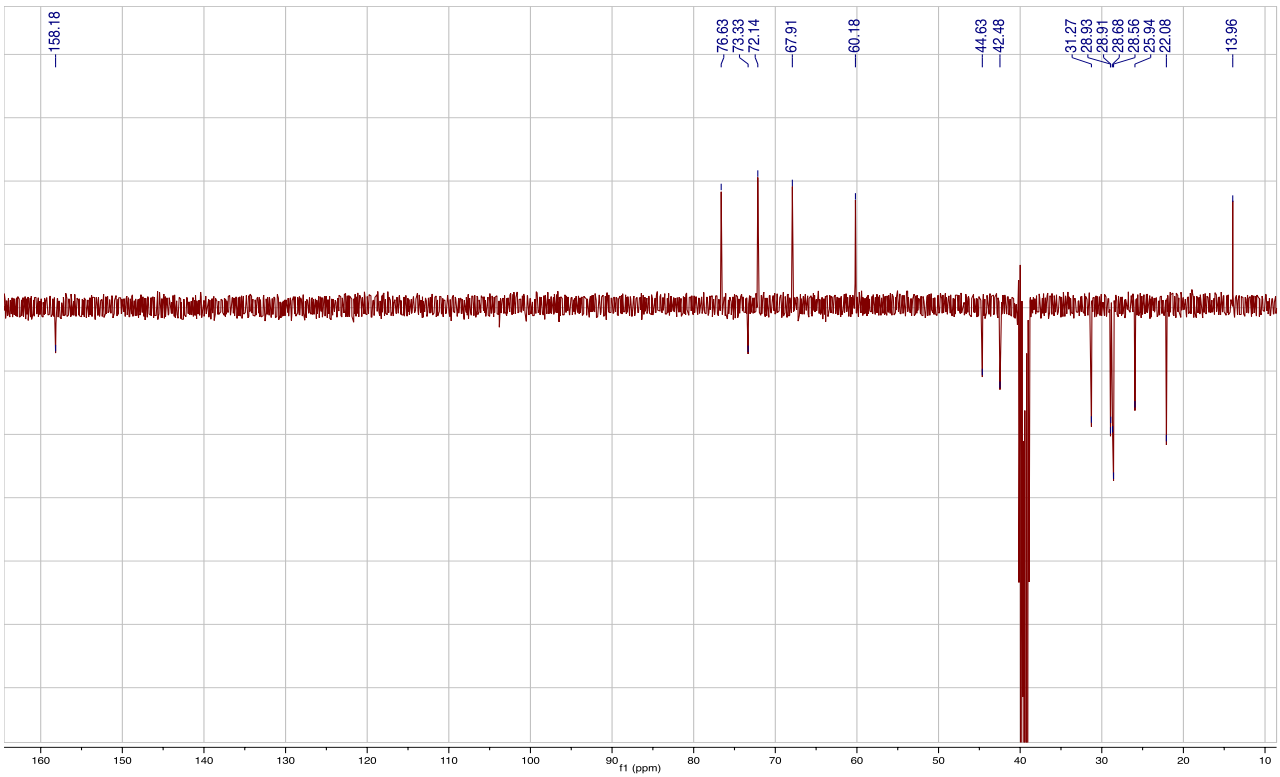


Compound 14b: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound 14b: ^{13}C NMR (126 MHz, DMSO-d_6)



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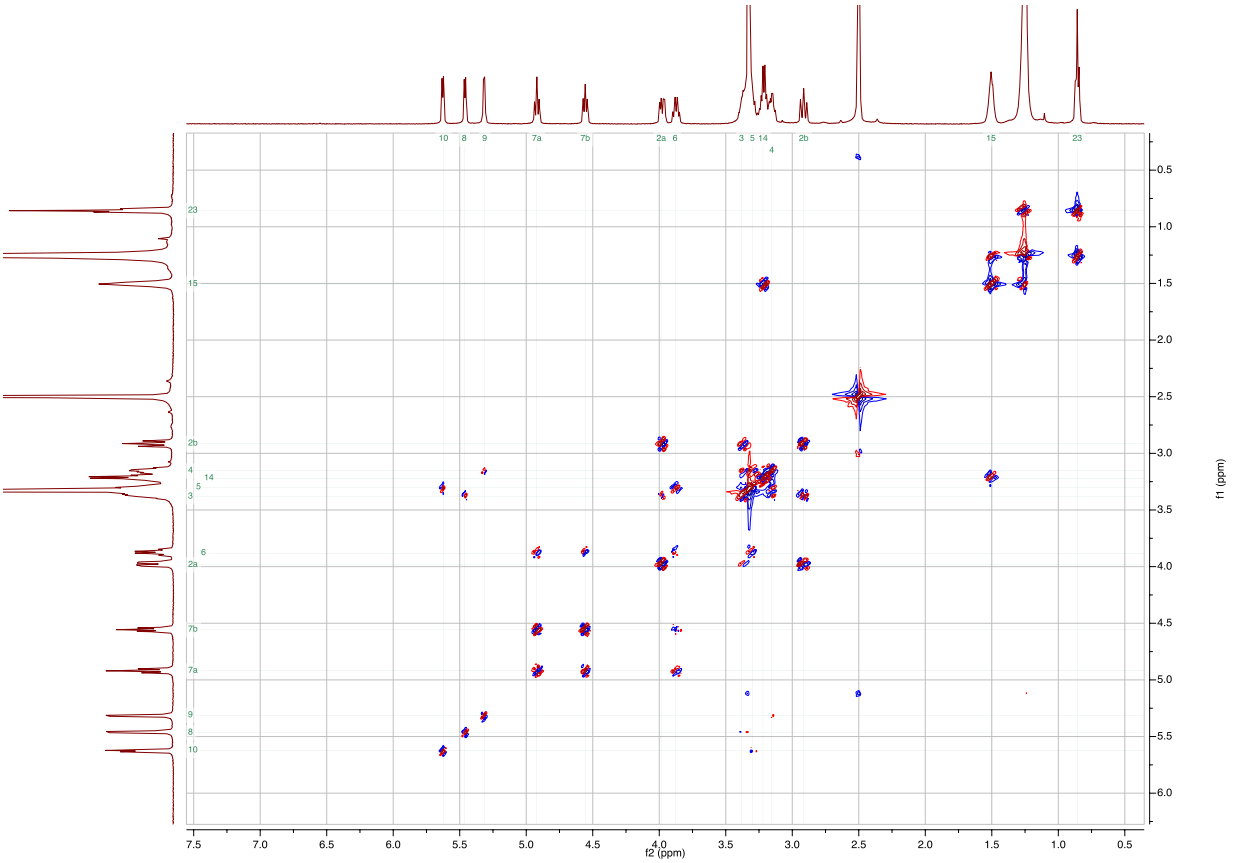
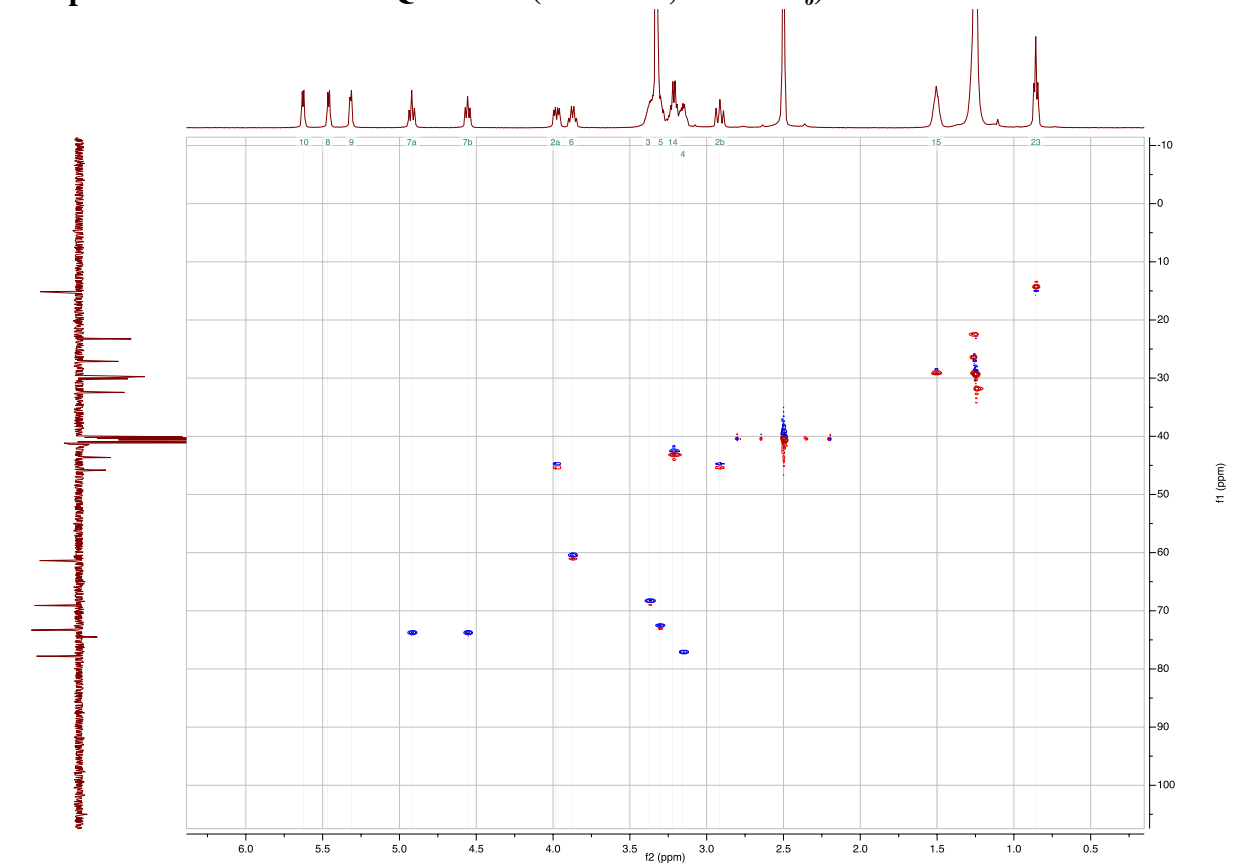
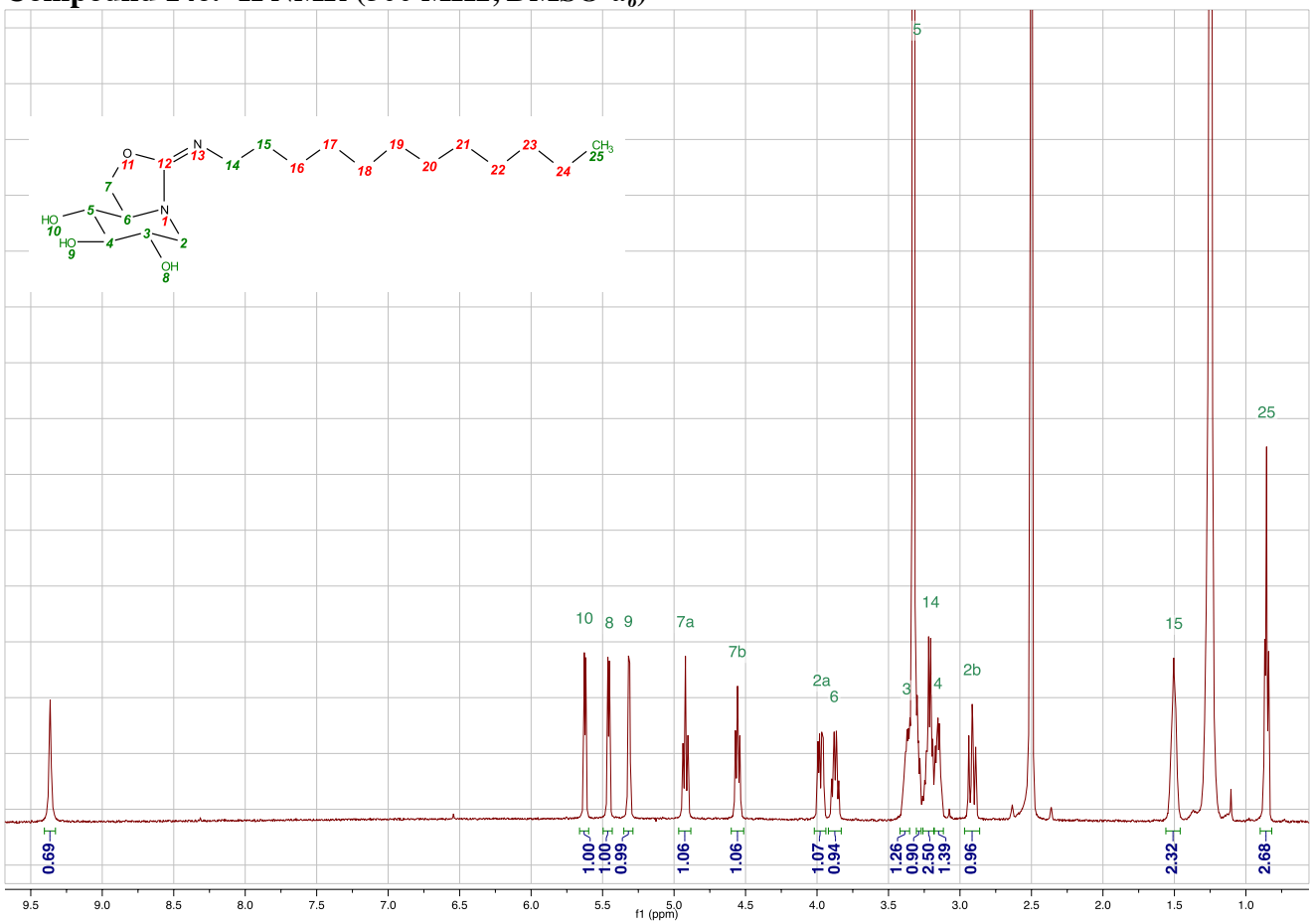


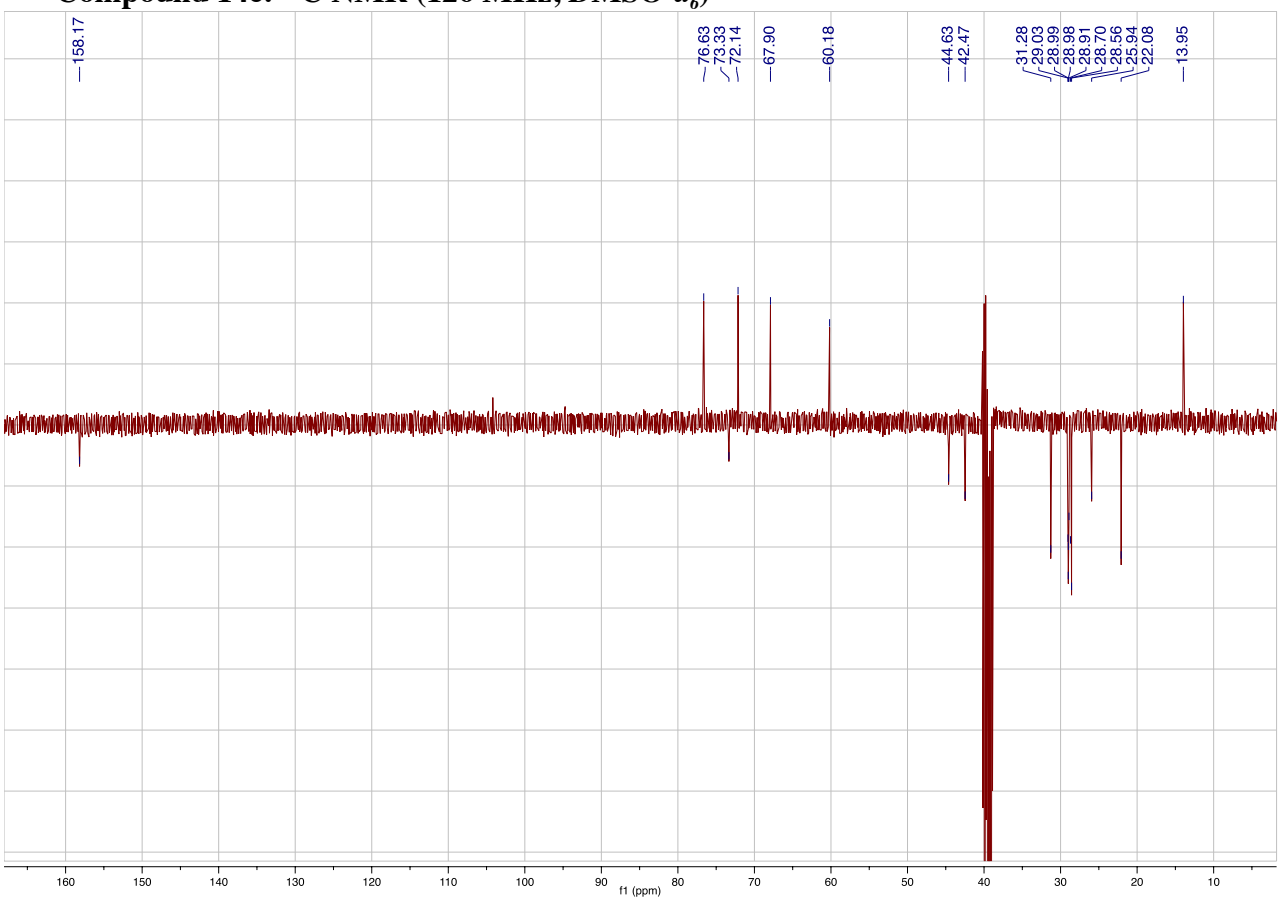
Compound 14b: $^{1}H - ^{13}C$ HSQC NMR (126 MHz, DMSO-d_6)



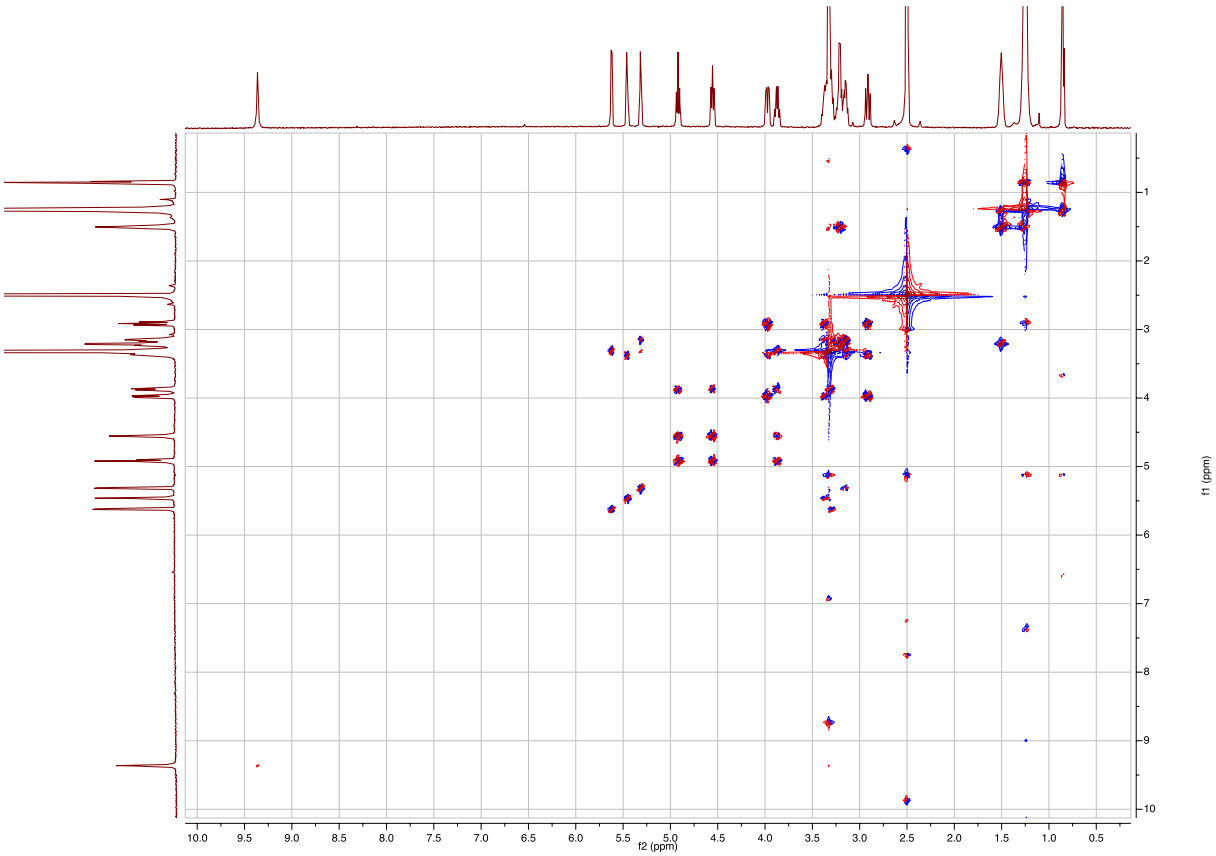
Compound 14c: ^{1}H NMR (500 MHz, DMSO-d_6)



Compound 14c: ^{13}C NMR (126 MHz, DMSO-d_6)



Compound 14c: ^{1}H – ^{1}H COSY NMR (500 MHz, DMSO-d_6)



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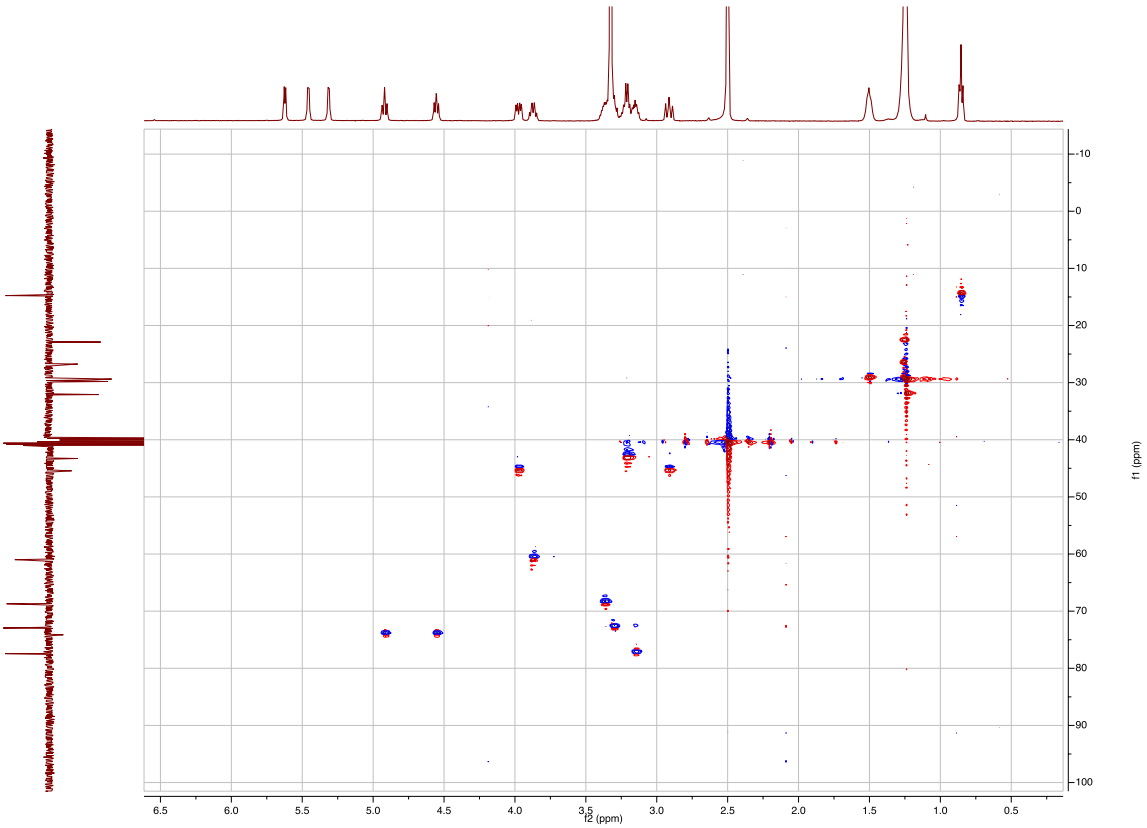
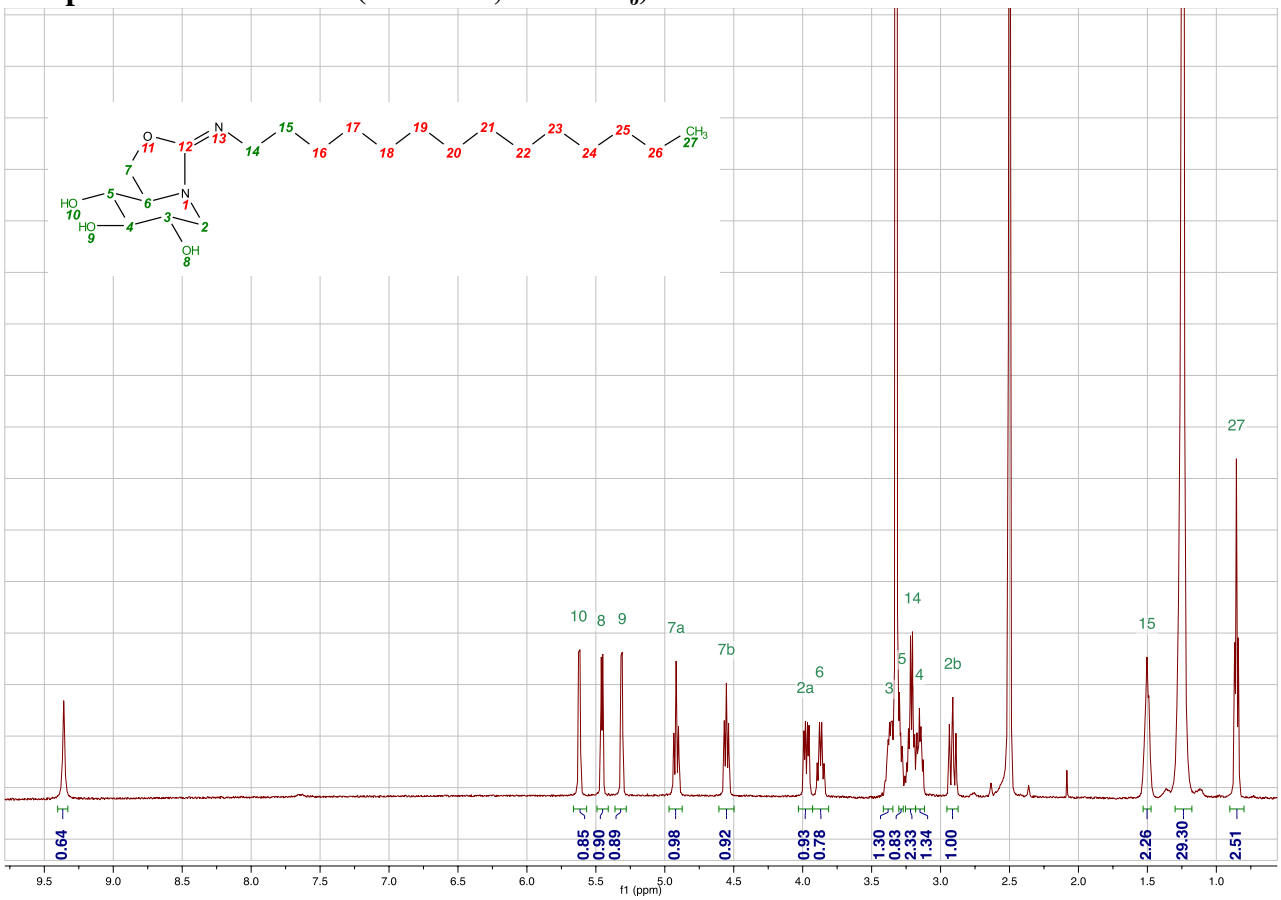
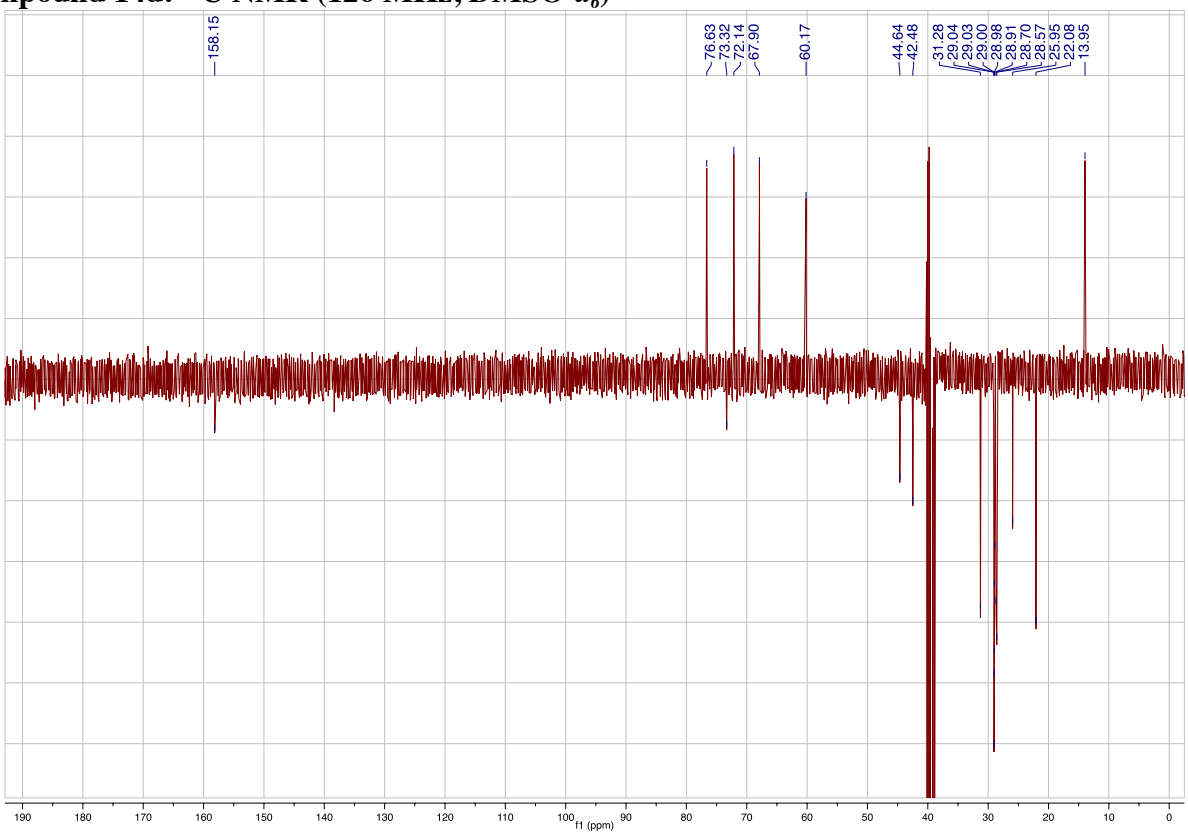


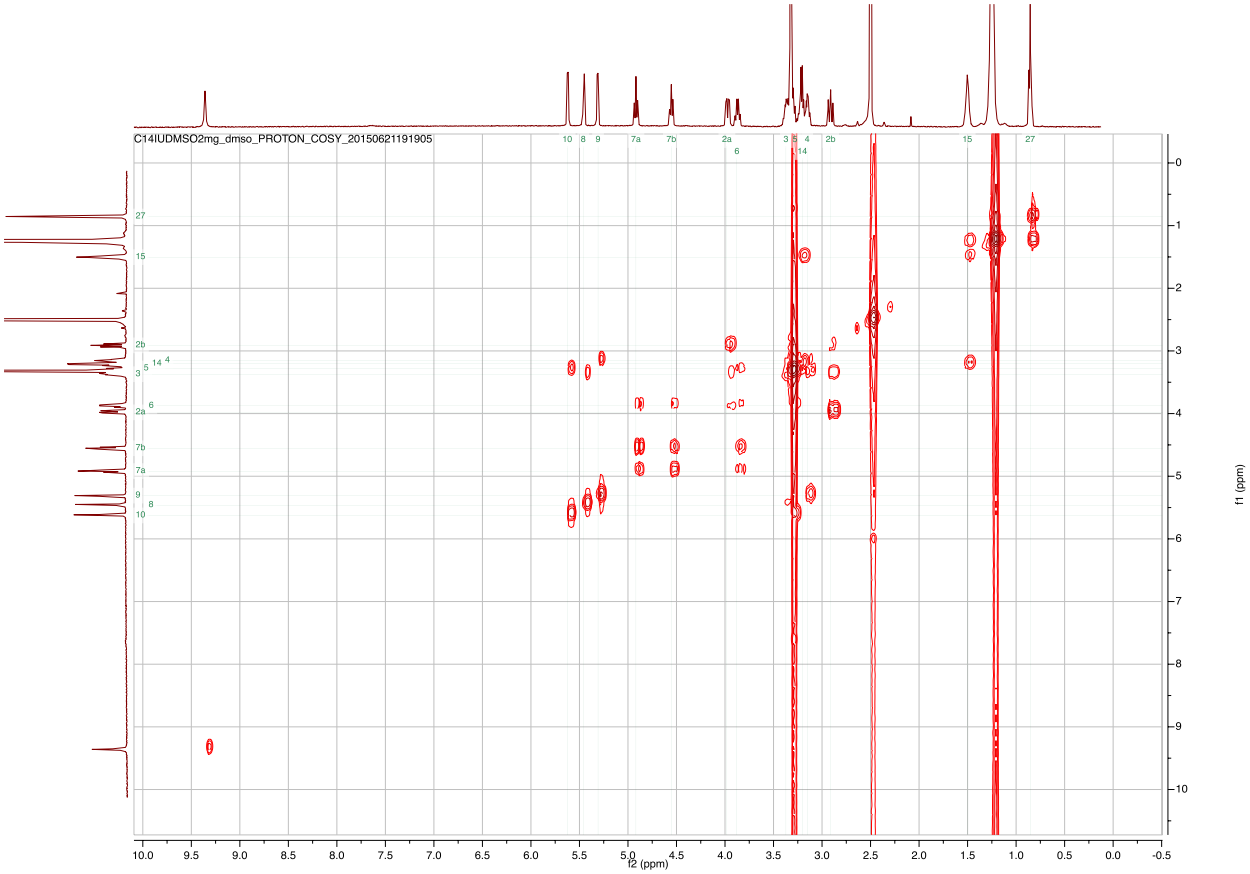
Compound 14d: ^{1}H NMR (500 MHz, DMSO-d_6)



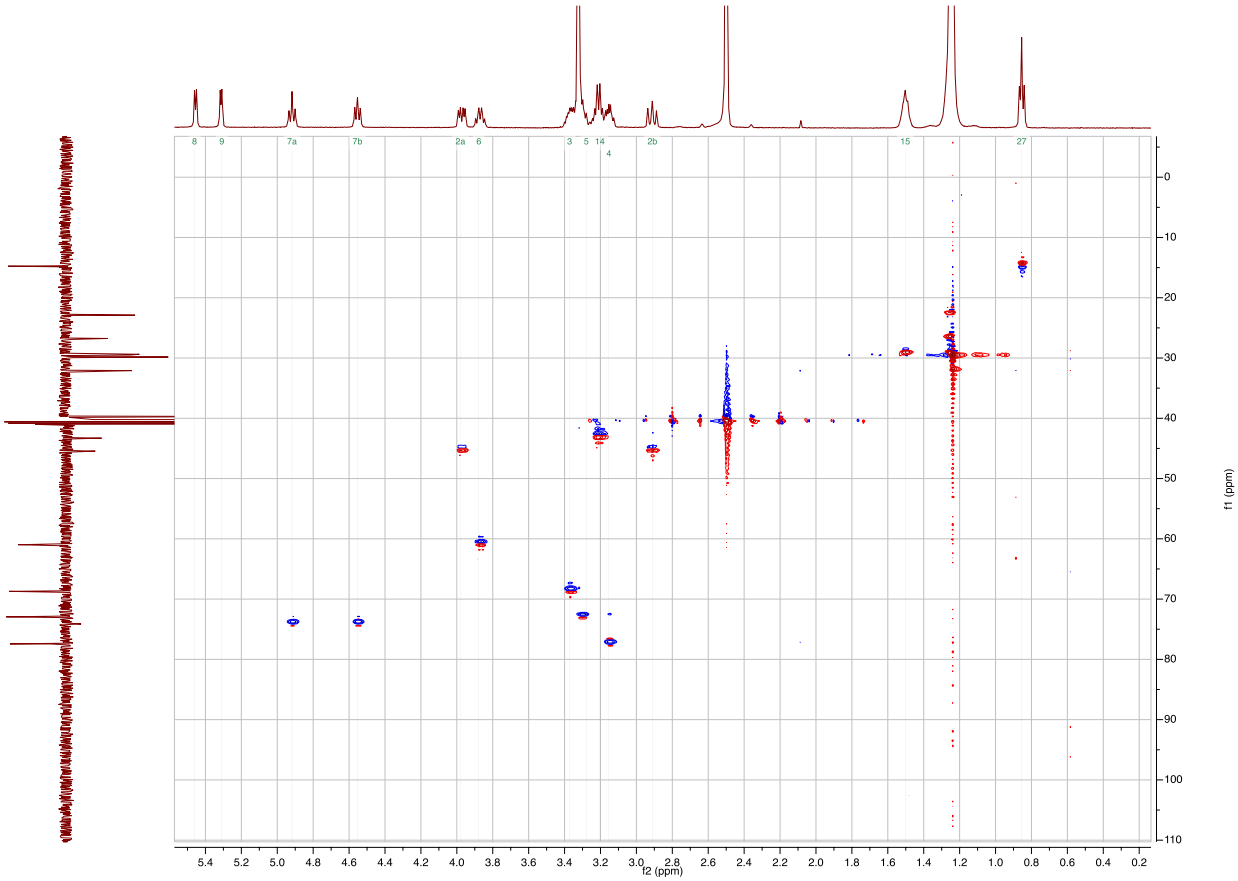
Compound 14d: ^{13}C NMR (126 MHz, DMSO-d_6)



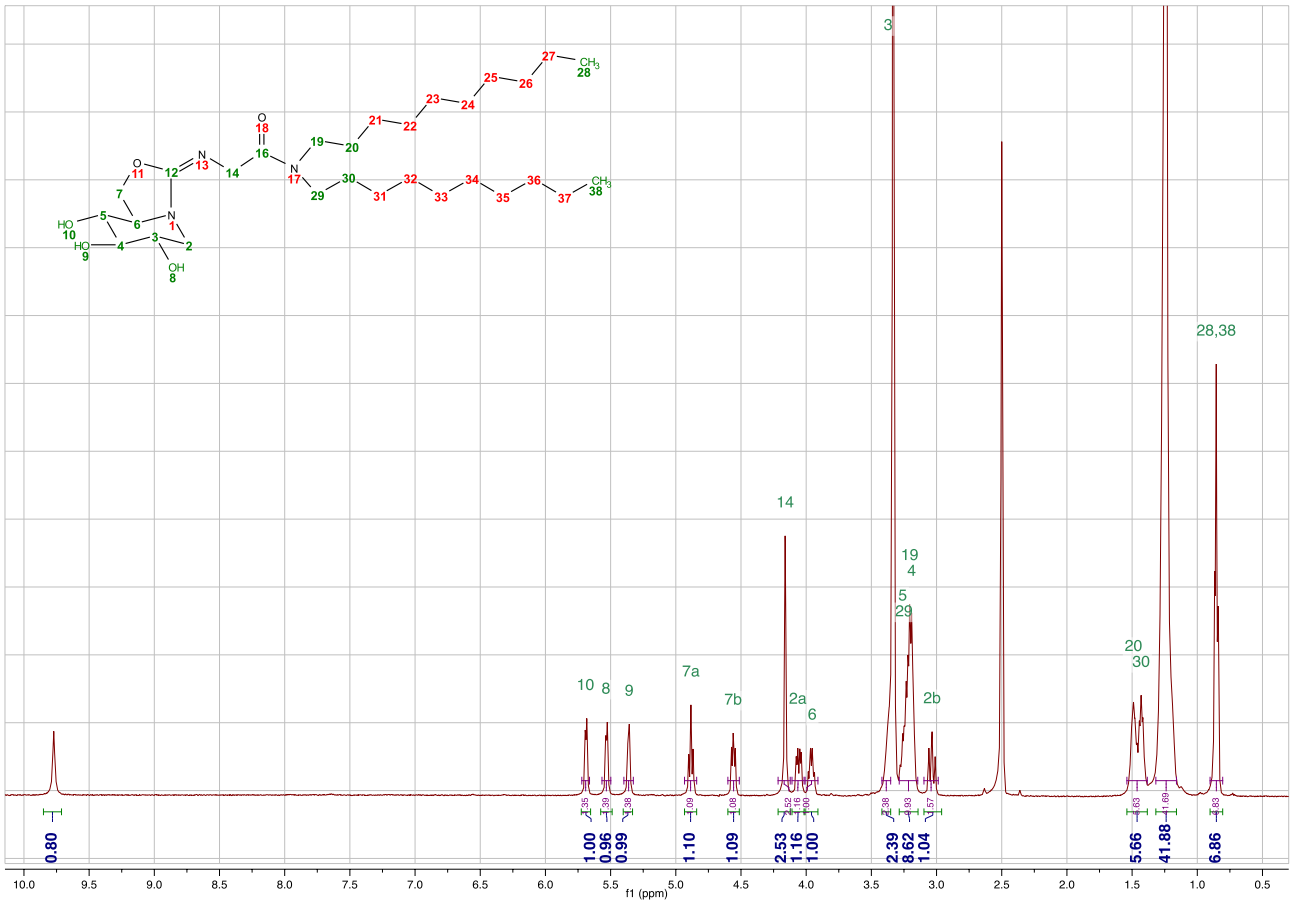
Compound 14d: $^{1}H - {}^{1}H$ COSY NMR (500 MHz, DMSO-d_6)



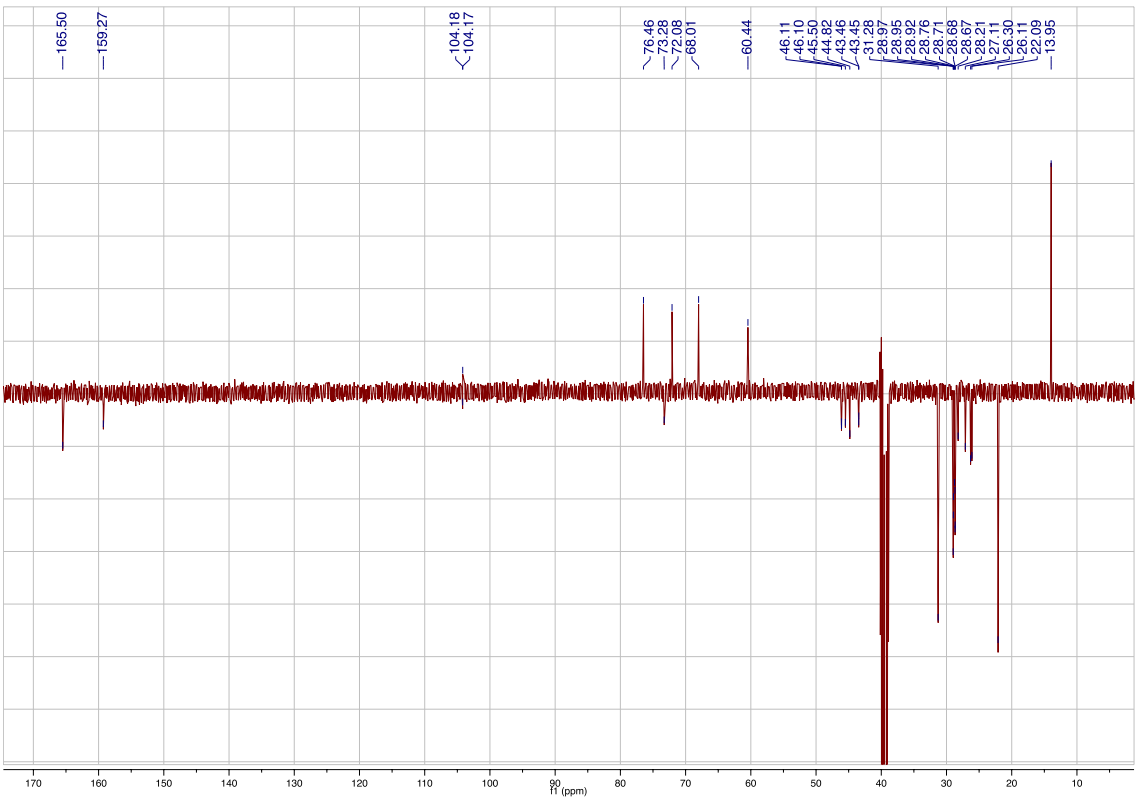
Compound 14d: $^{1}H - {}^{13}C$ HSQC NMR (126 MHz, DMSO-d_6)



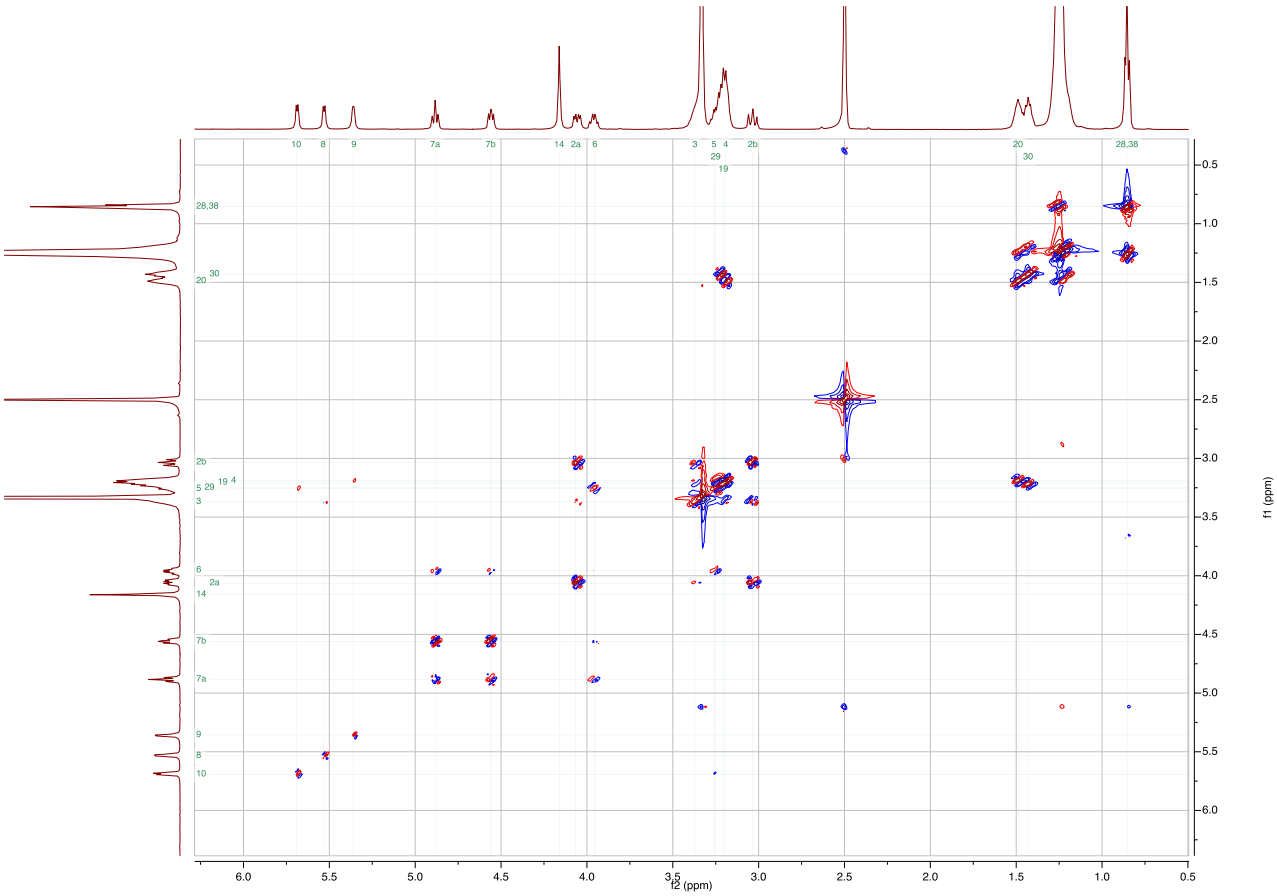
Compound 14e: ^{1}H NMR (500 MHz, DMSO-d_6)



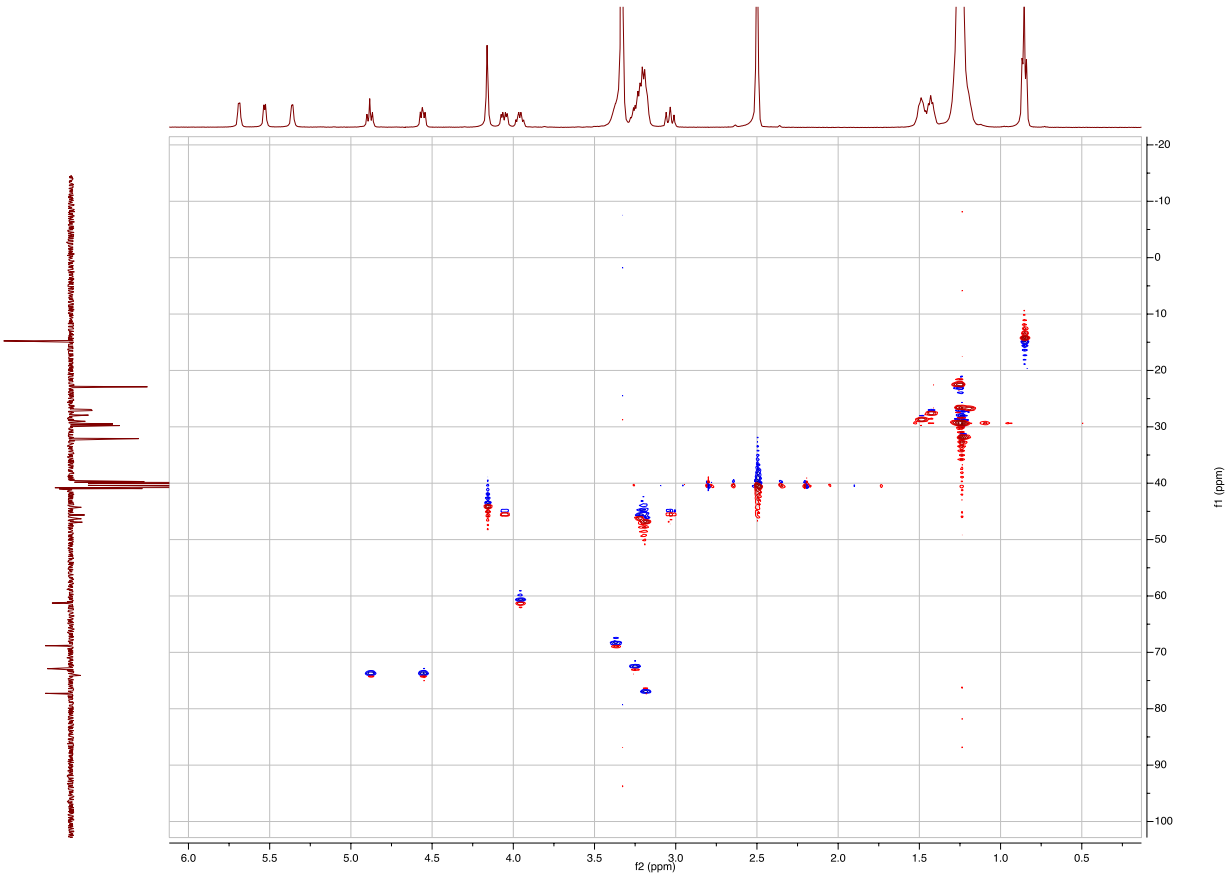
Compound 14e: ^{13}C NMR (126 MHz, DMSO-d_6)



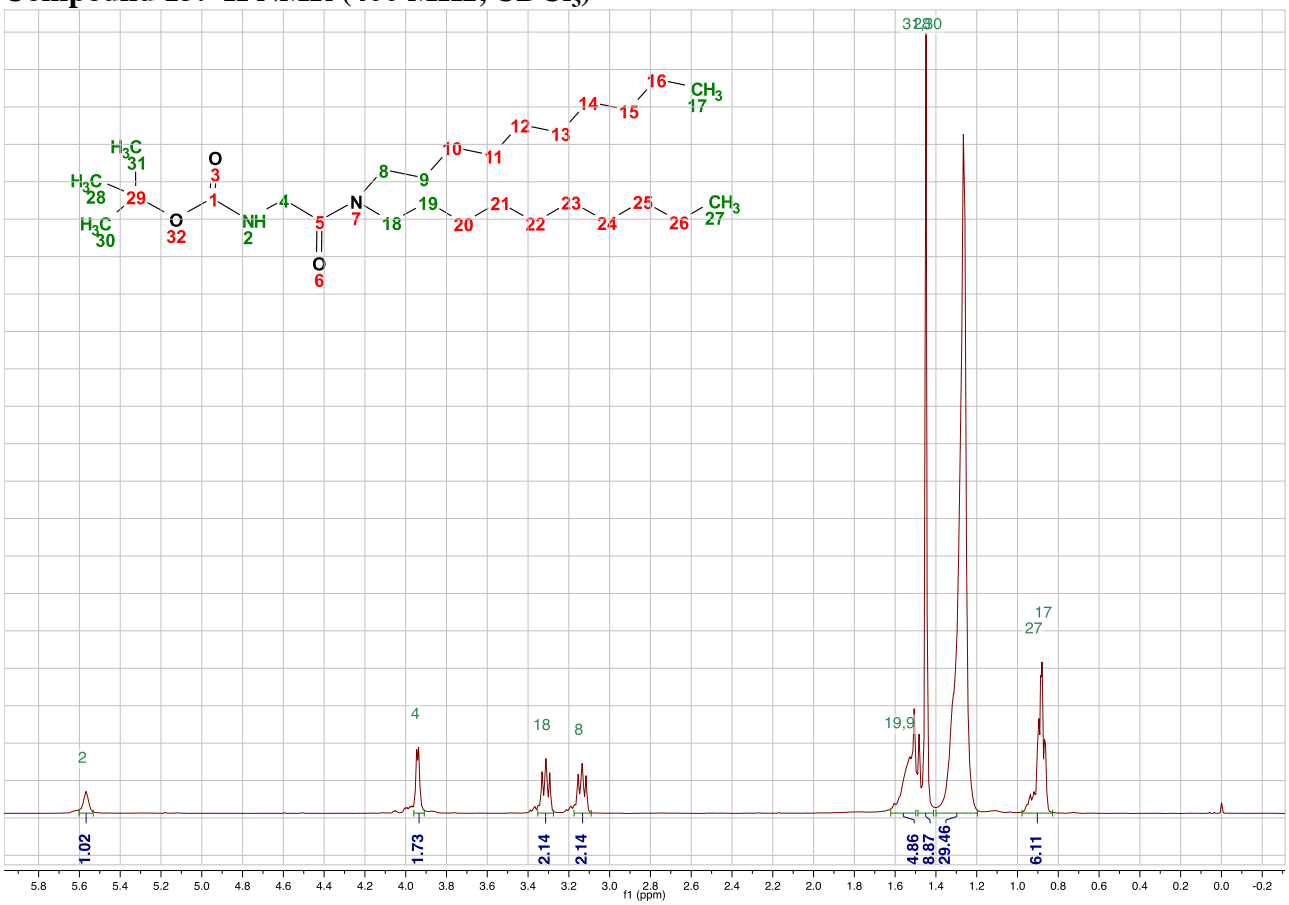
Compound 14e: ^{1}H – ^{1}H COSY NMR (500 MHz, DMSO-d_6)



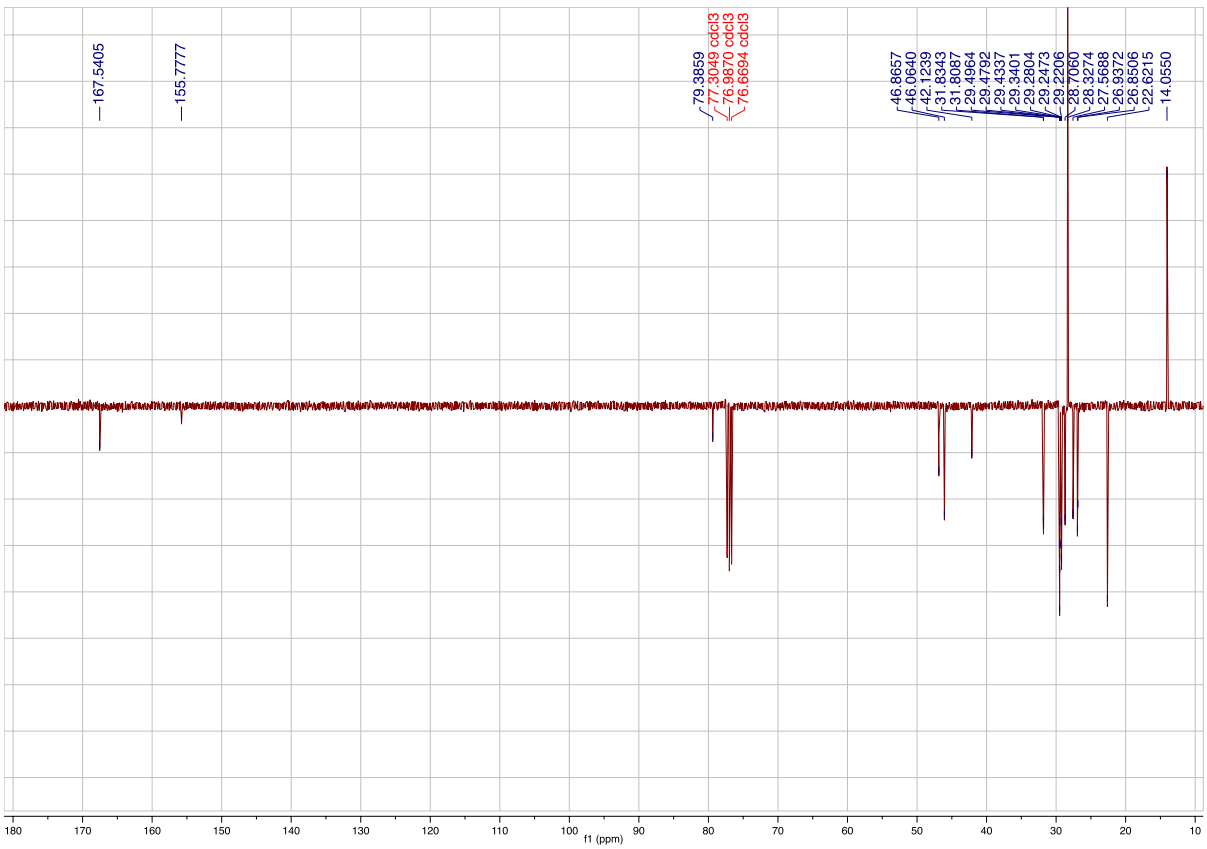
Compound 14e: ^{1}H – ^{13}C HSQC NMR (126 MHz, DMSO-d_6)



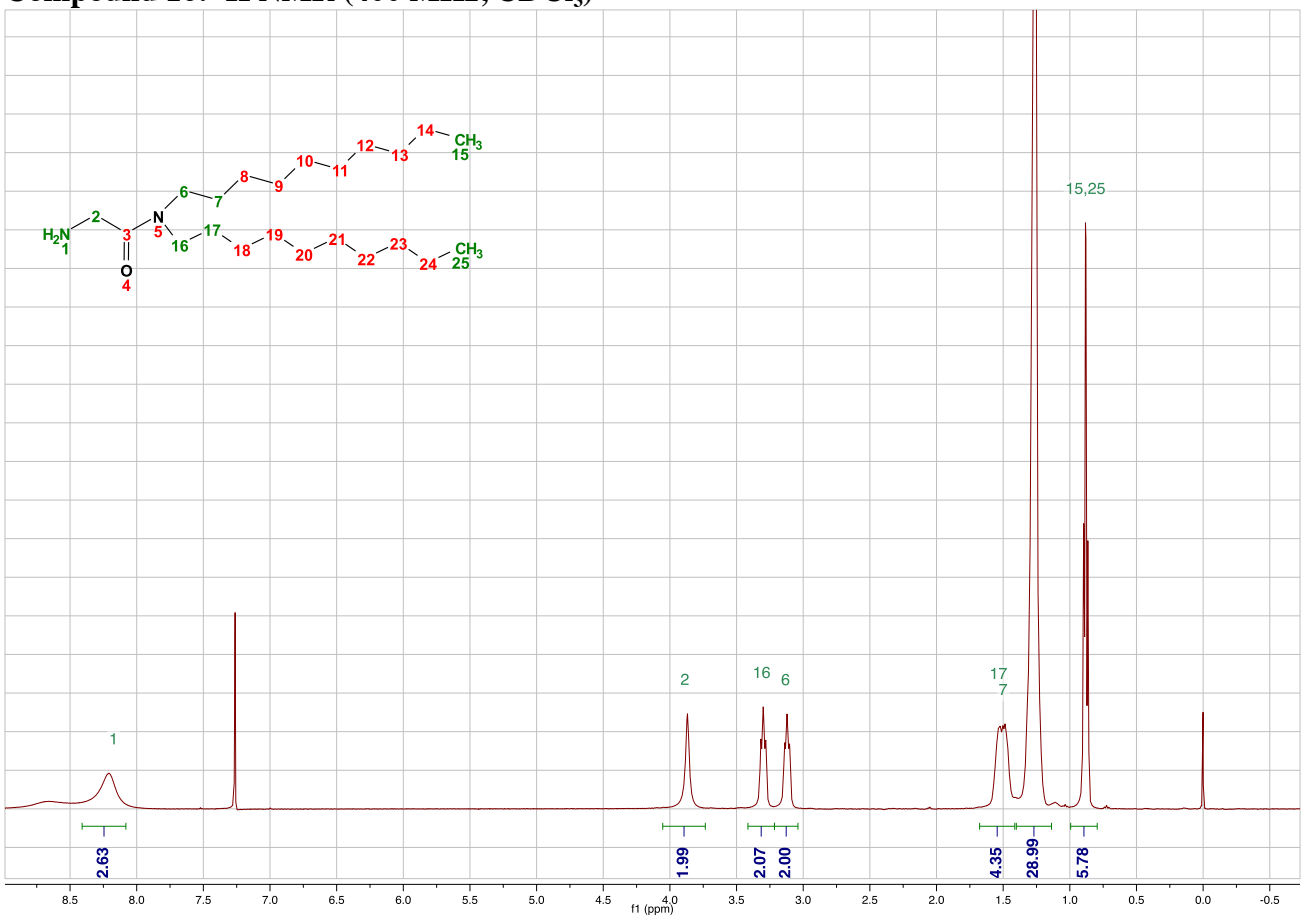
Compound 15: ^{1}H NMR (400 MHz, $CDCl_3$)



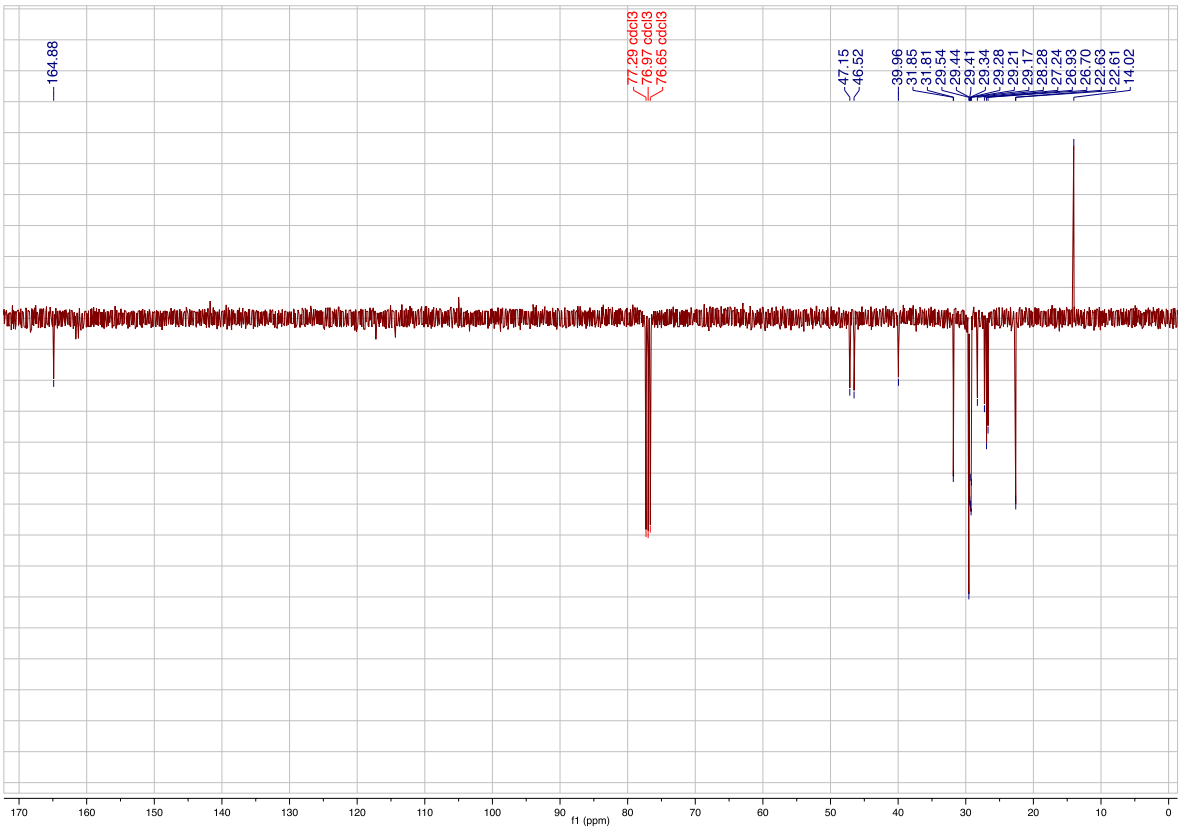
Compound 15: ^{13}C NMR (101 MHz, $CDCl_3$)



Compound 16: ^{1}H NMR (400 MHz, $CDCl_3$)

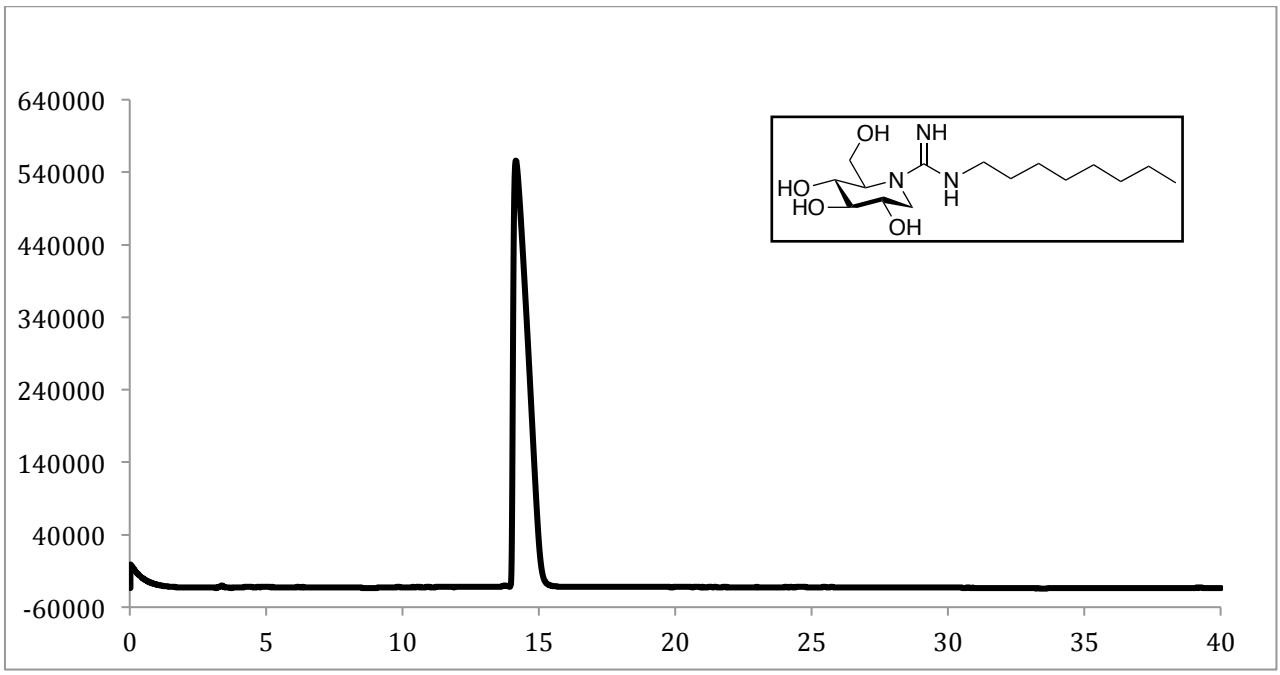


Compound 16: ^{13}C NMR (101 MHz, $CDCl_3$)

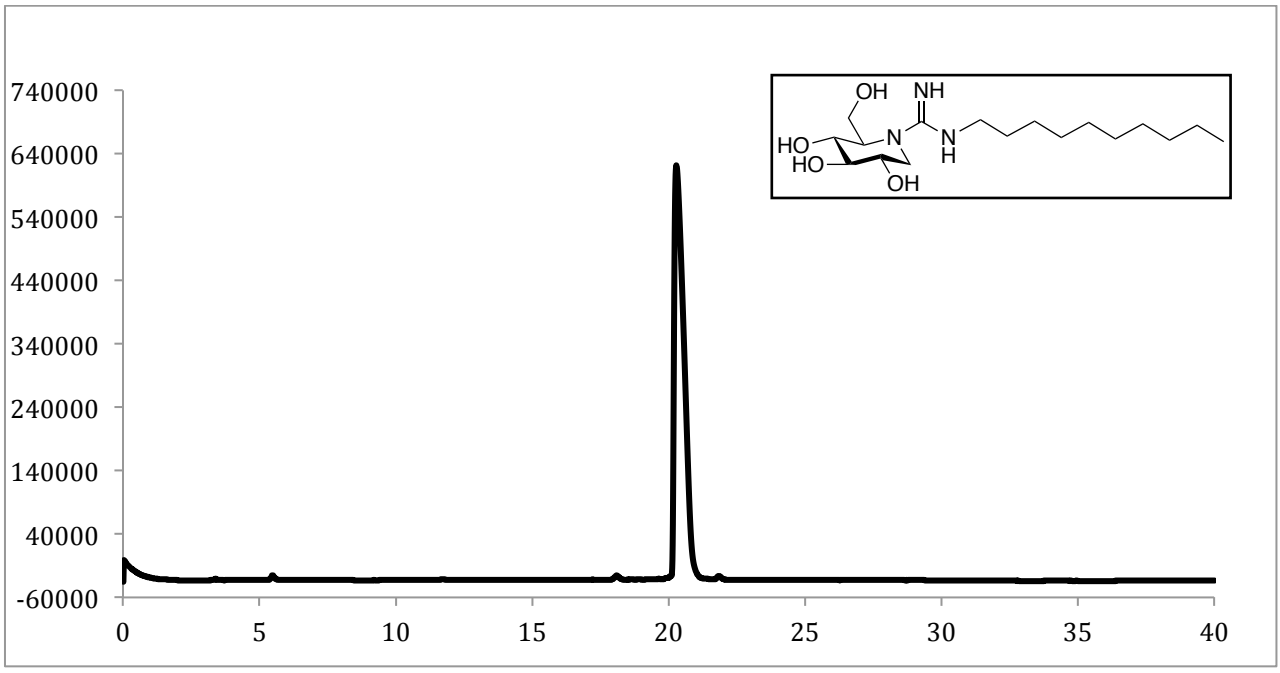


Analytical RP-HPLC traces for compounds 13(a-e) and 14(a-e).

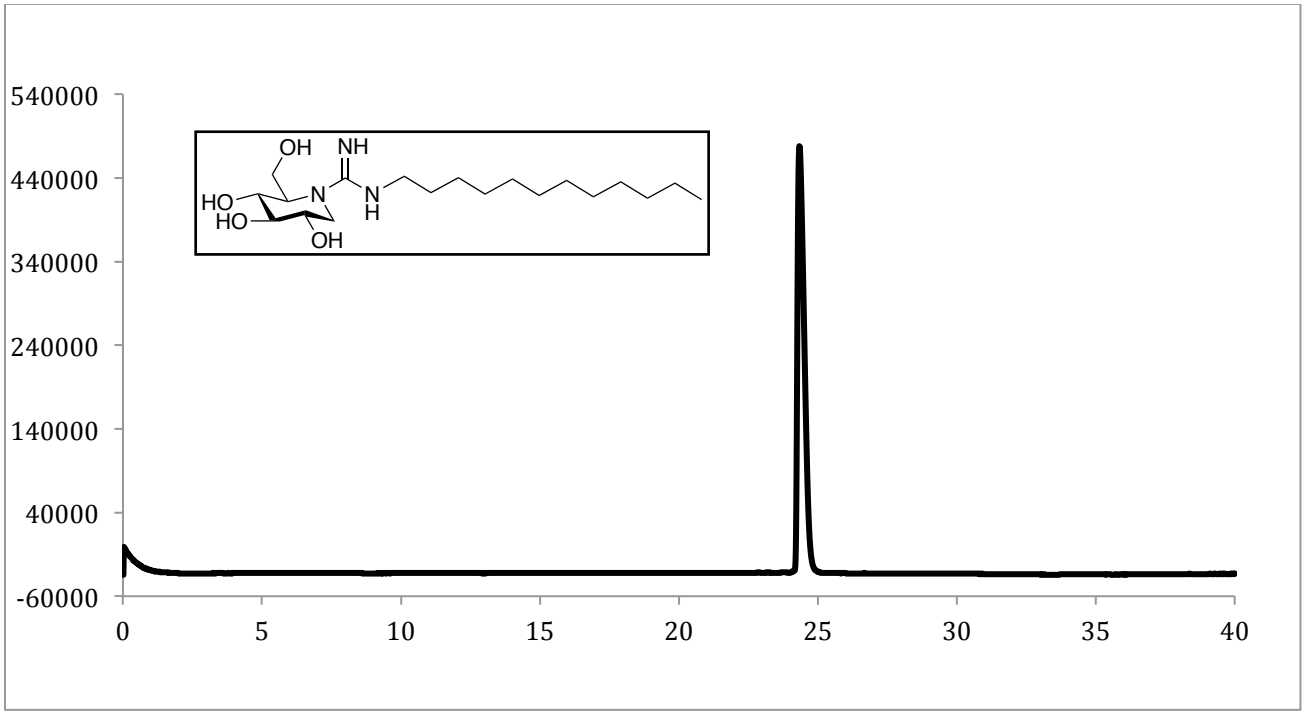
Compound 13a



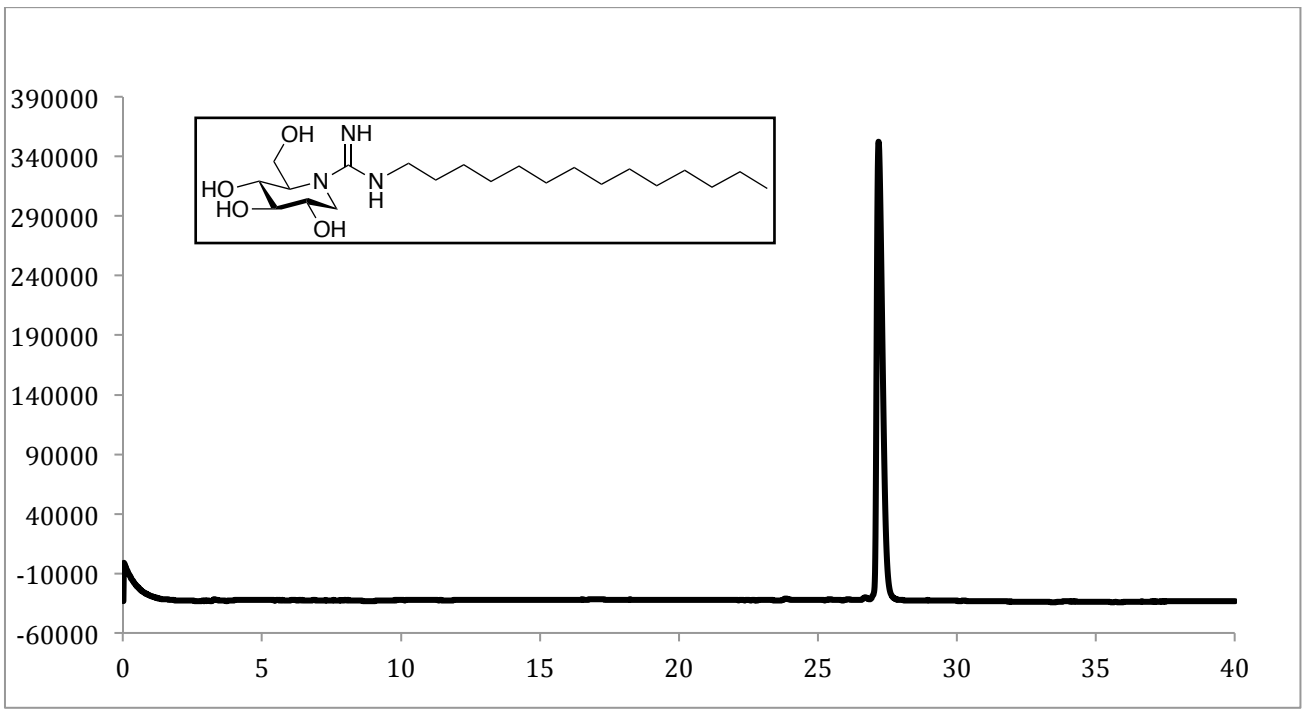
Compound 13b



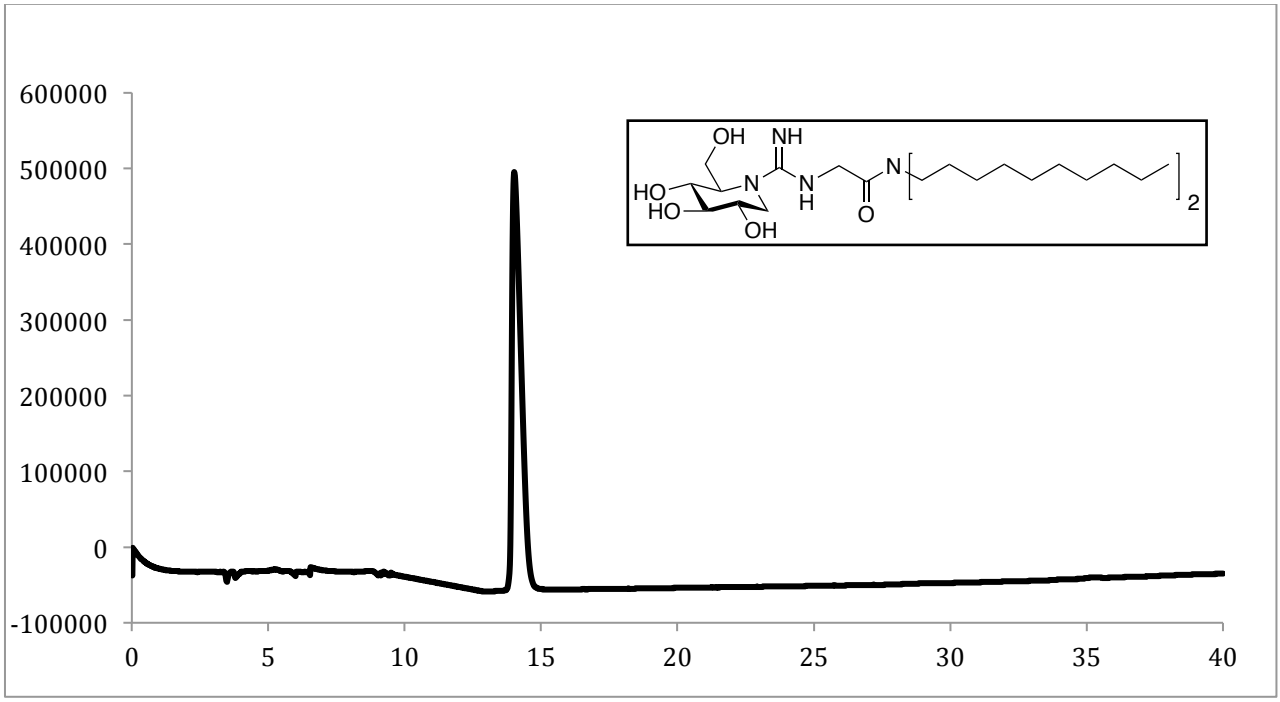
Compound 13c



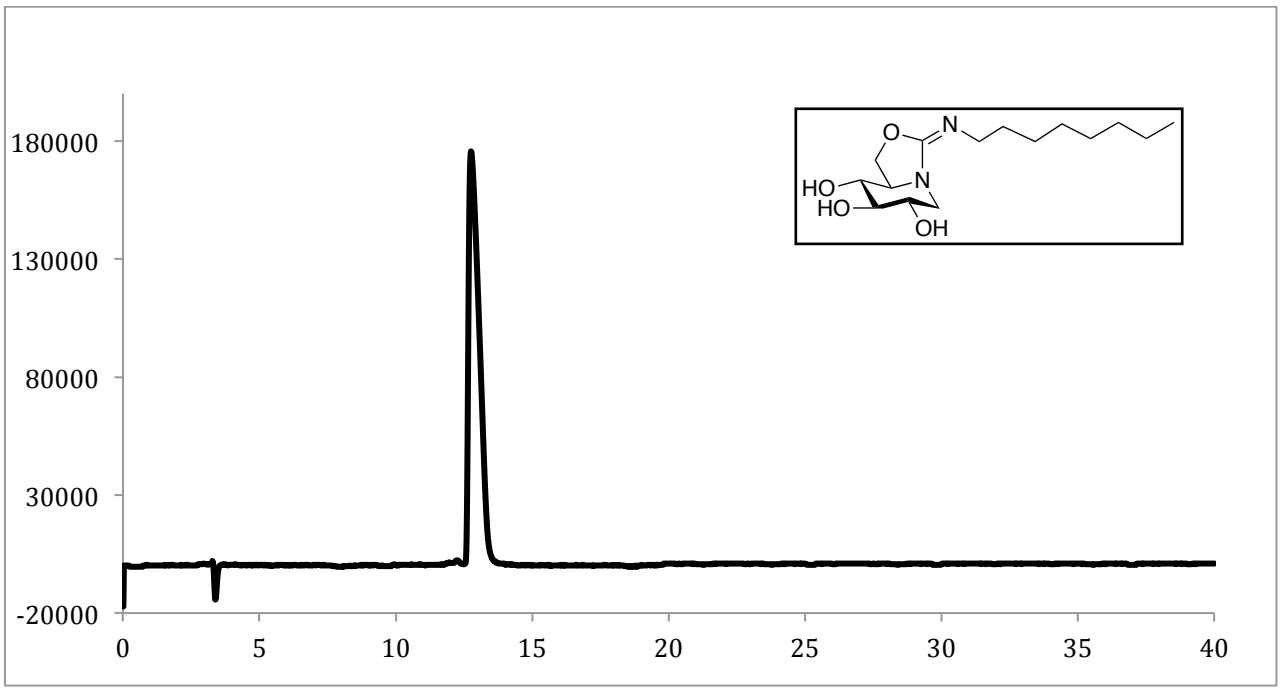
Compound 13d



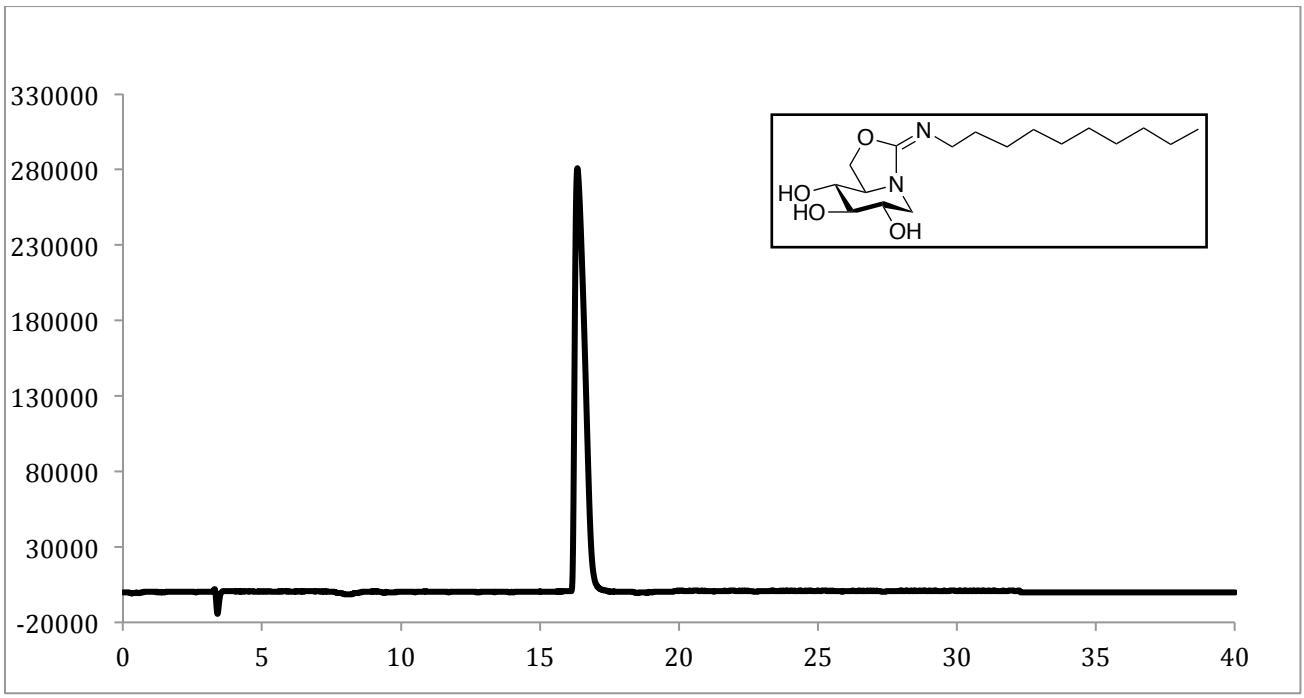
Compound 13e



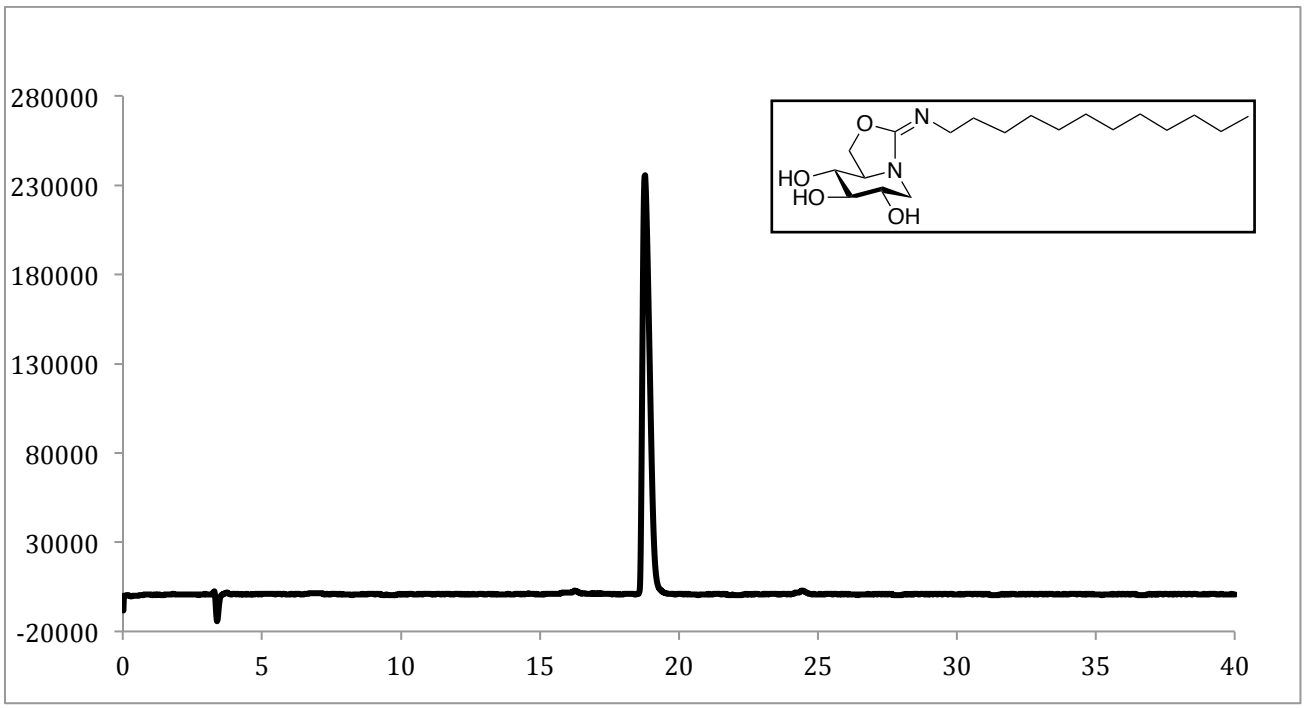
Compound 14a



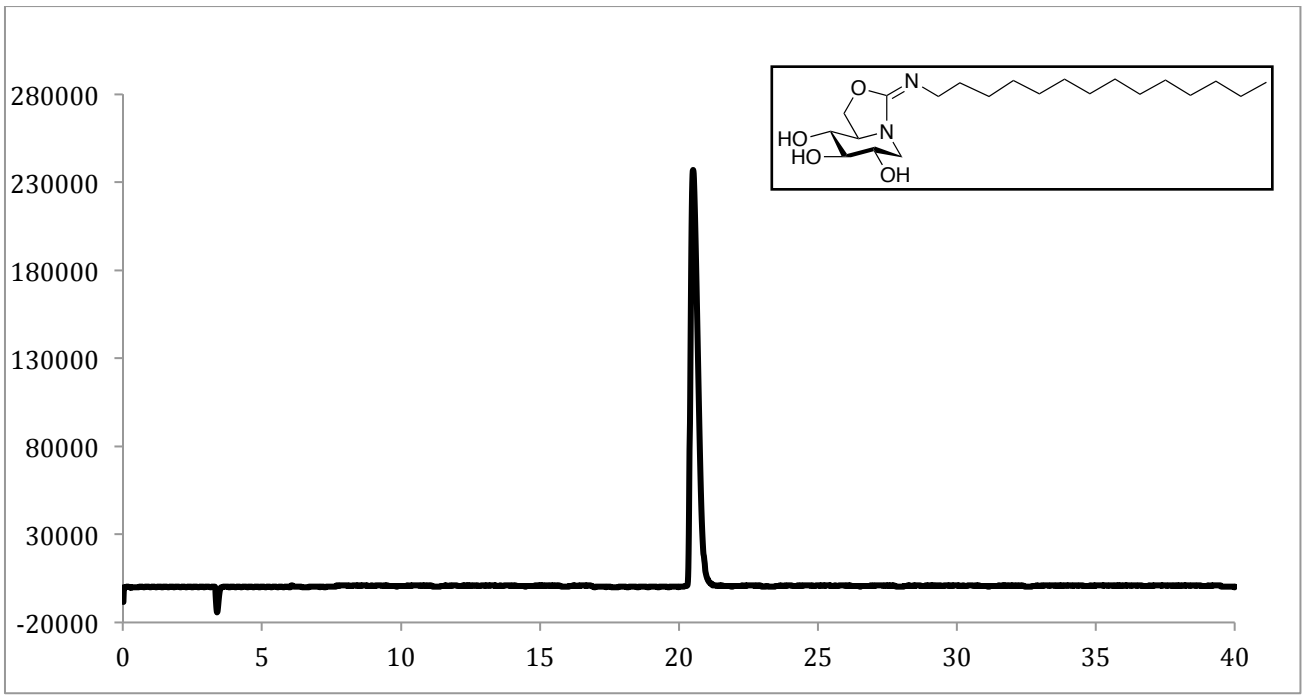
Compound 14b



Compound 14c



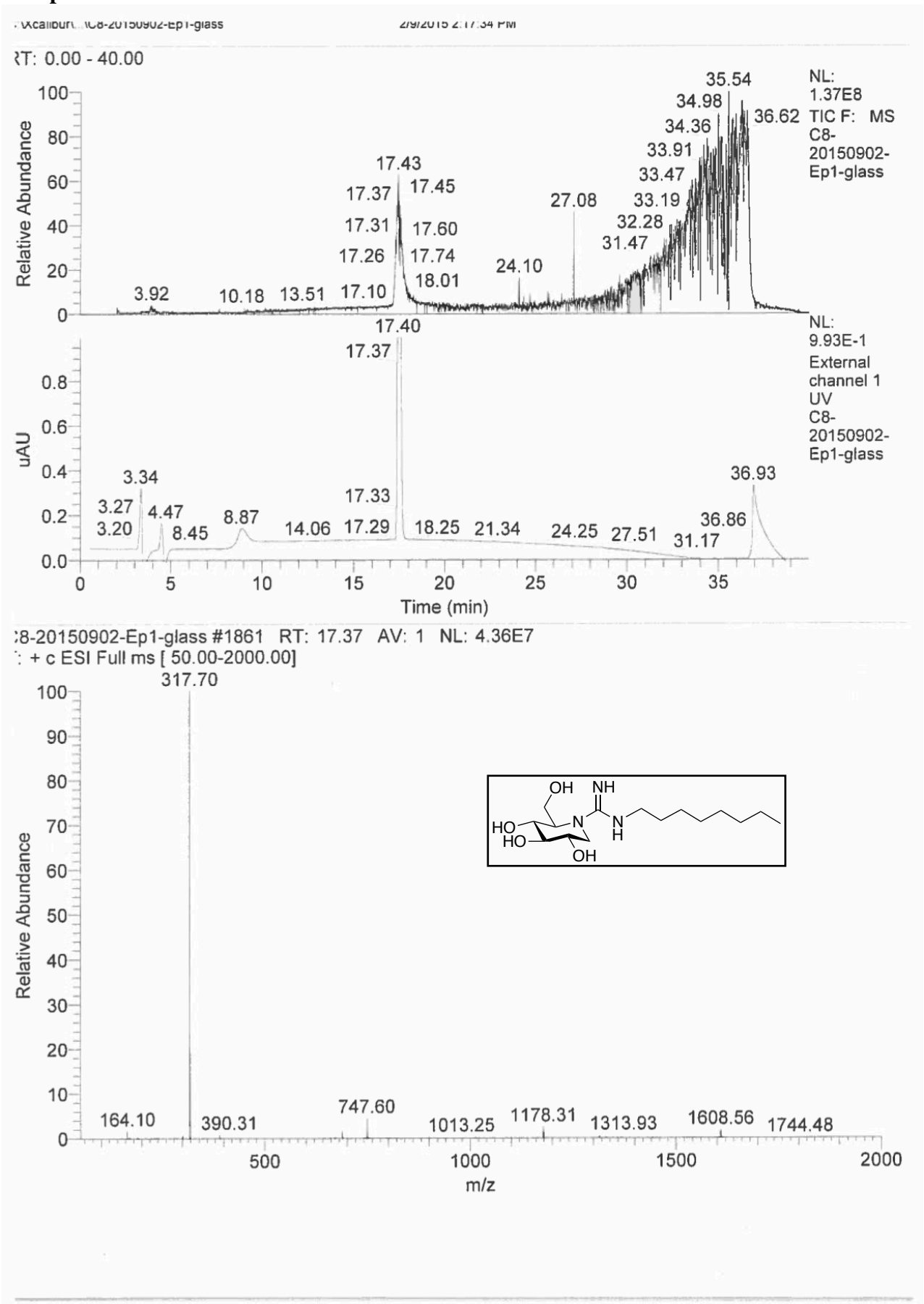
Compound 14d



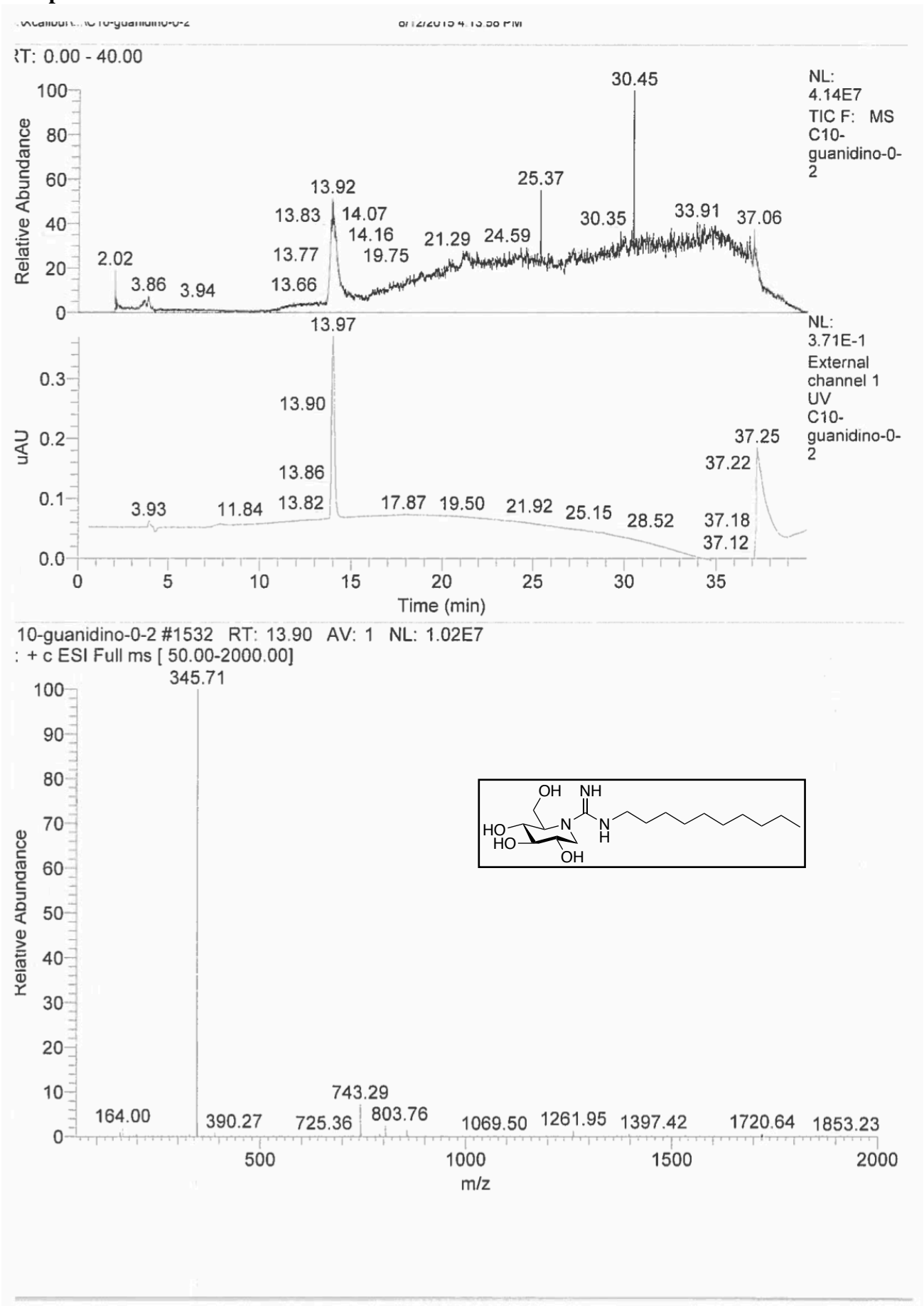
Compound 14e

Analytical LCMS results for compounds 13(a-e) and 14(a-e).

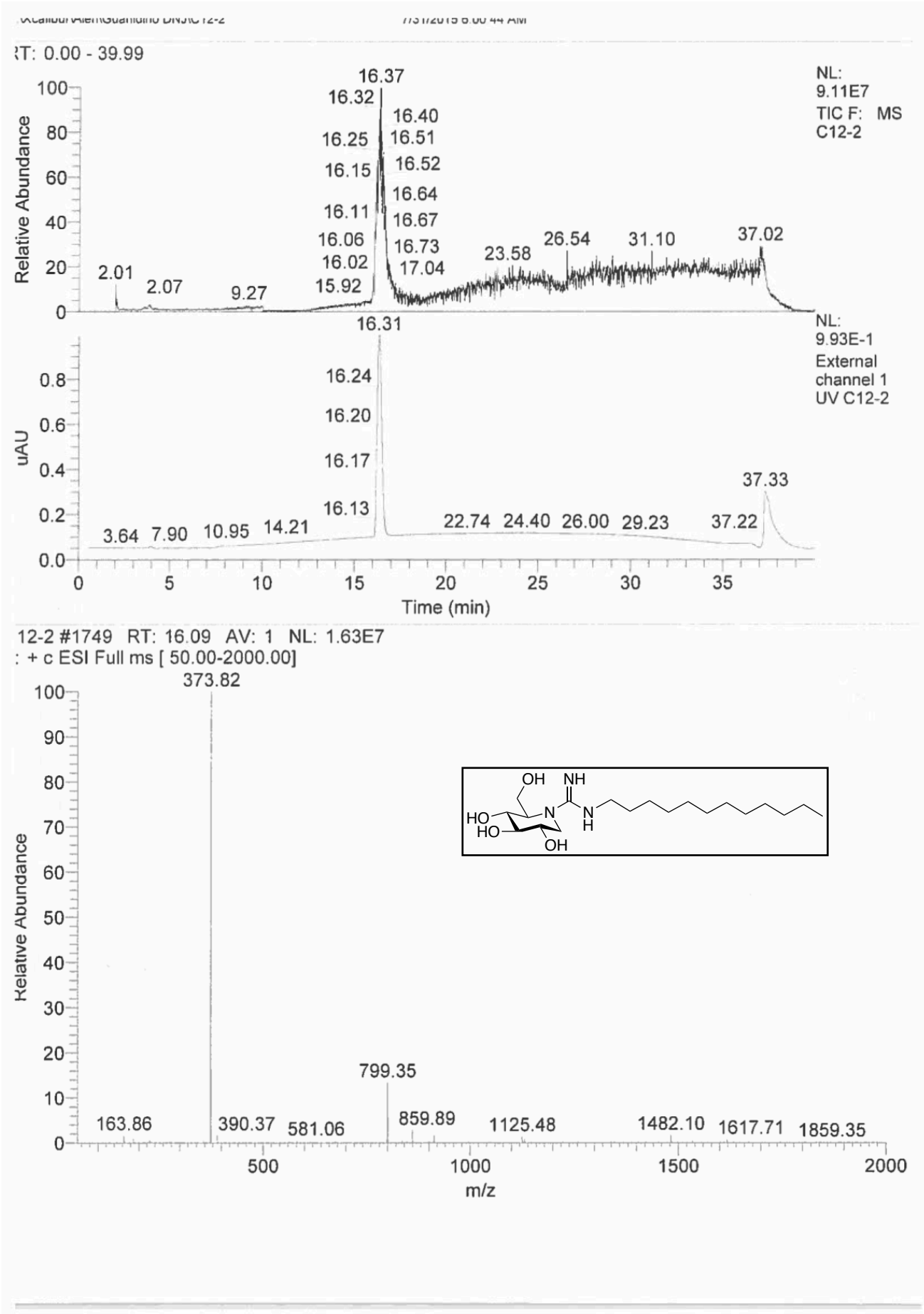
Compound 13a



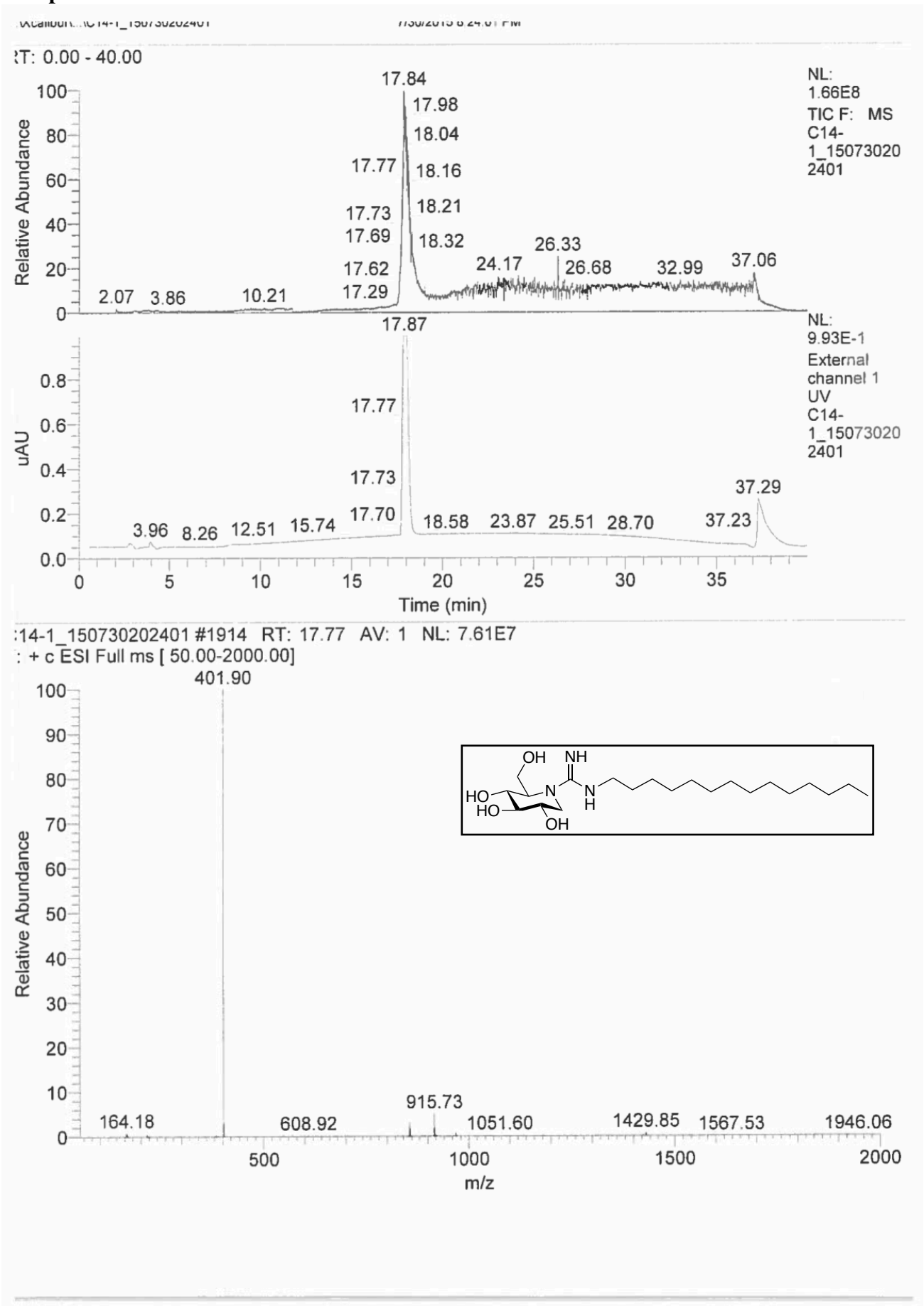
Compound 13b



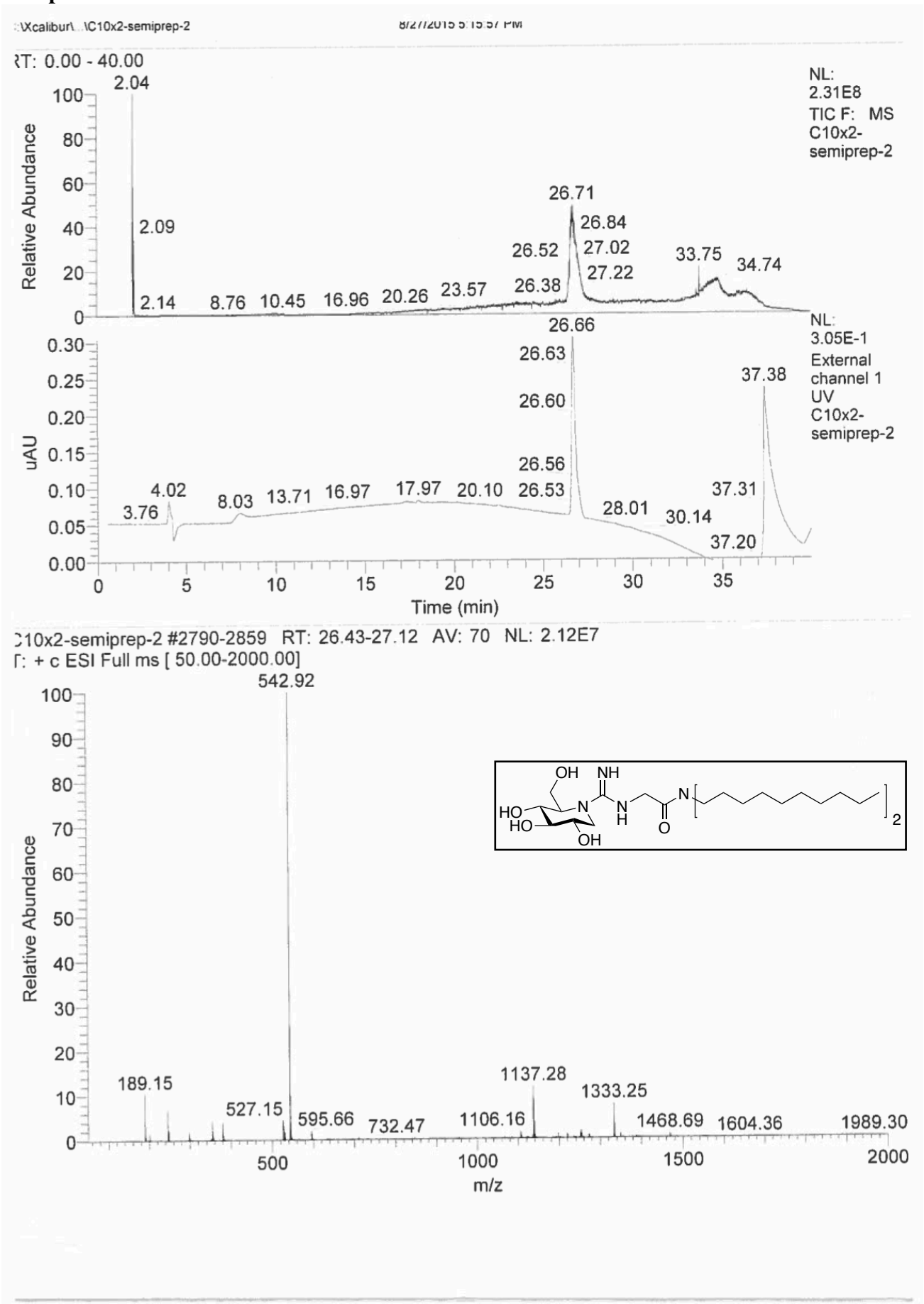
Compound 13c



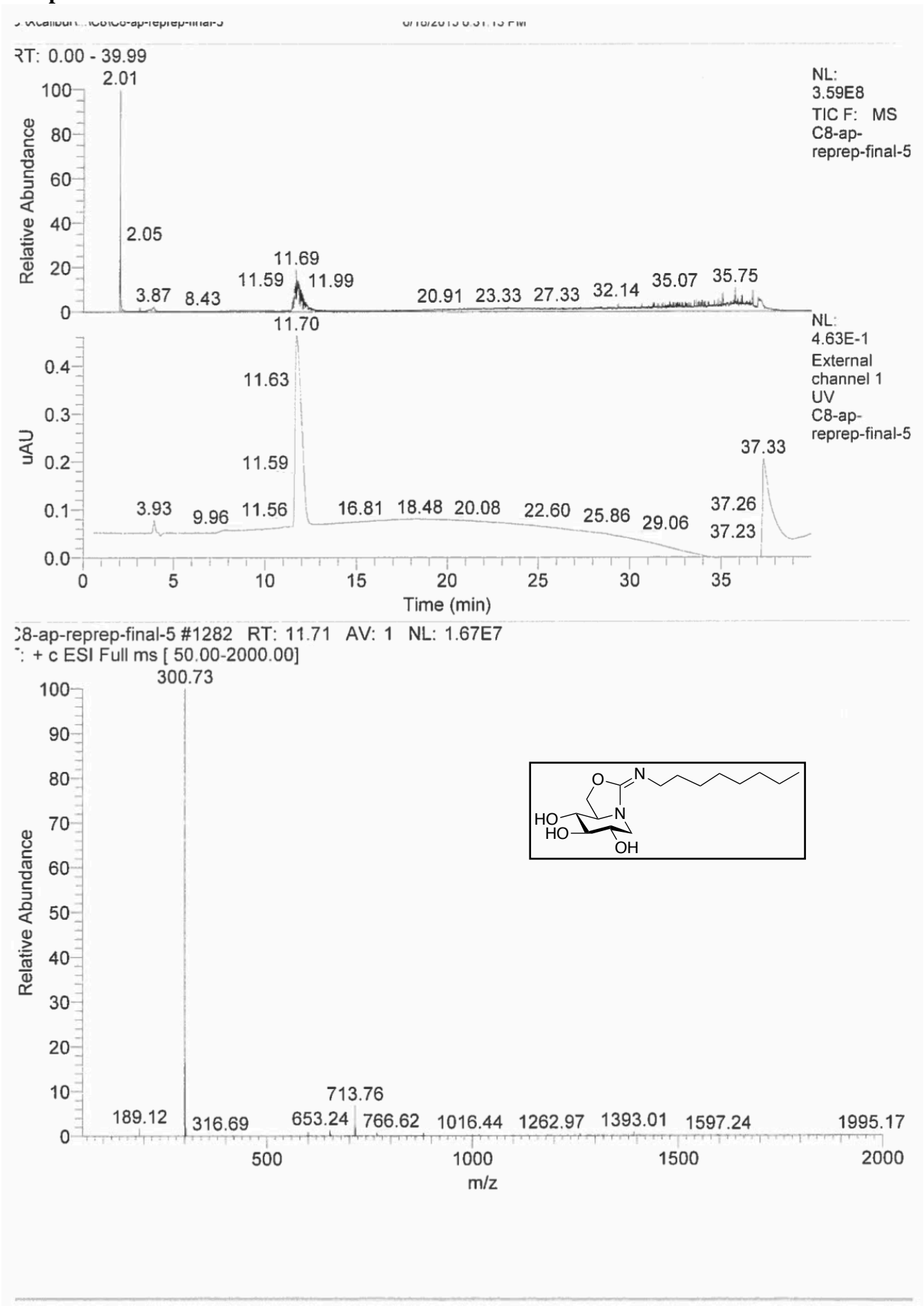
Compound 13d



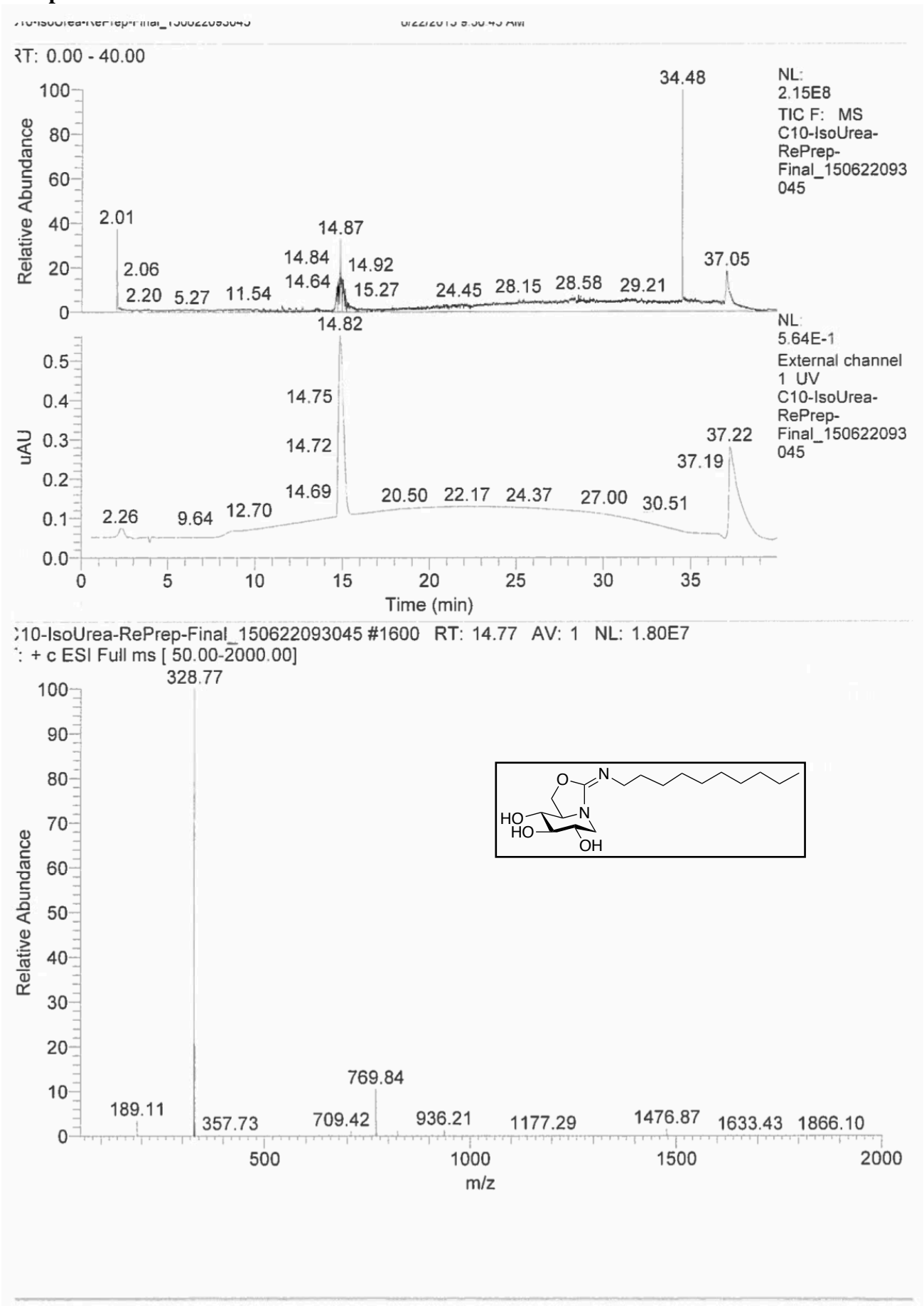
Compound 13e



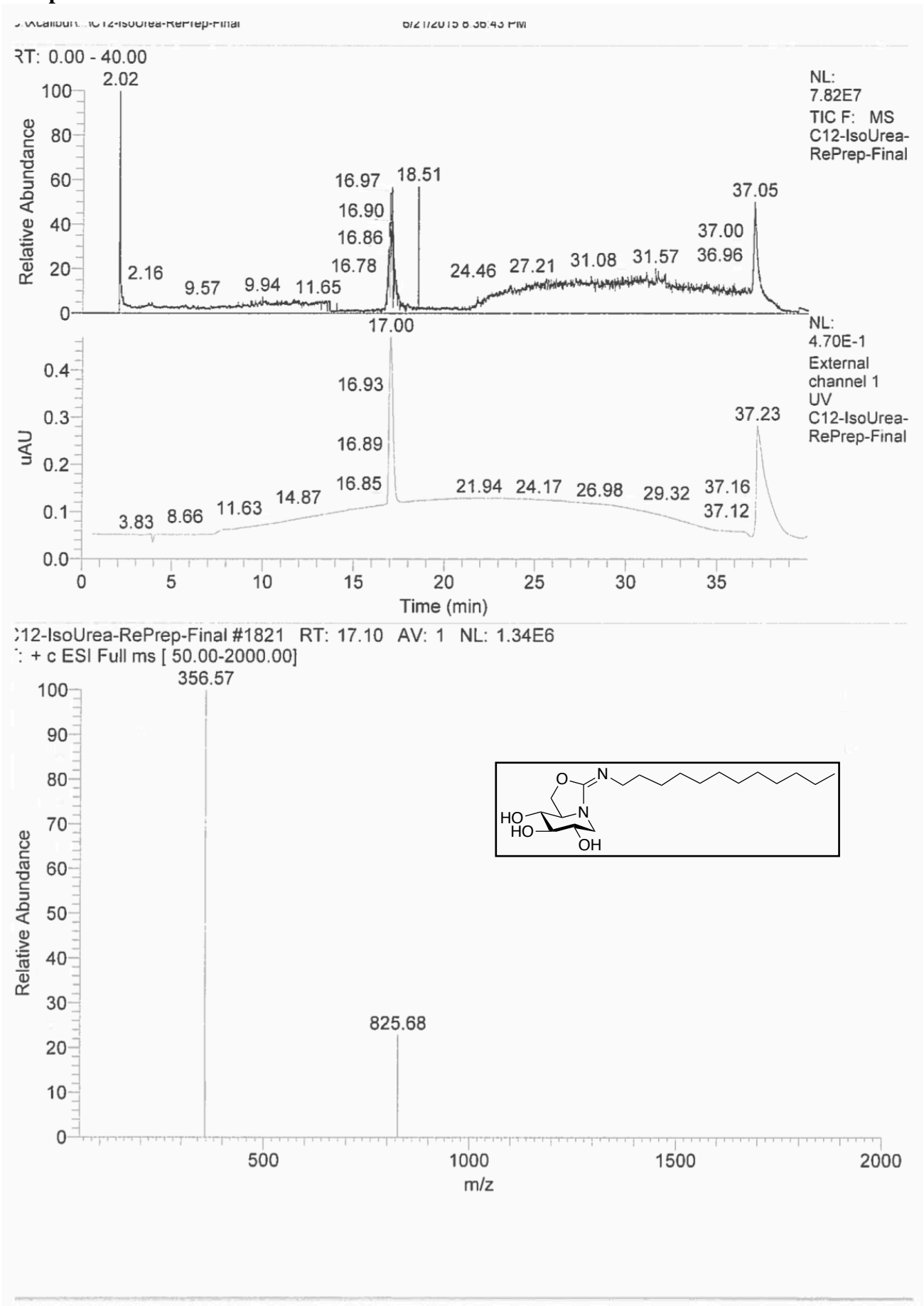
Compound 14a



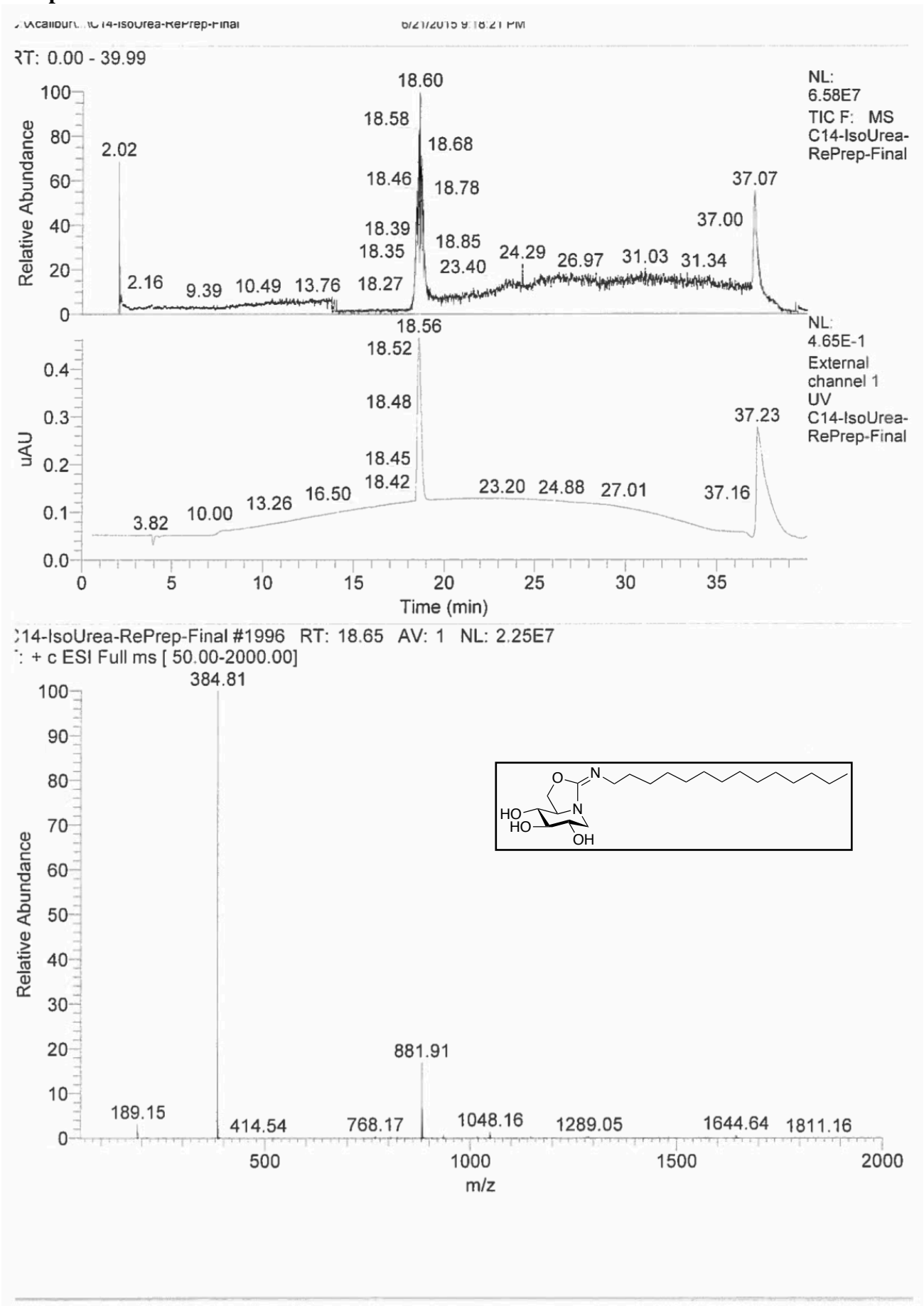
Compound 14b



Compound 14c



Compound 14d



Compound 14e