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Supporting Information

Synthesis of a β-CCT-lanthanide conjugate for binding the dopamine transporter

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I. General Considerations

Reagents

All substrates, with the exception of maleimido-mono-amide-DOTA-tris(*t*-butyl)ester (Macrocyclics) and cocaine•HCl (Sigma-Aldrich), were purchased from Fisher Scientific. Flash chromatography was performed on Silicycle silica gel (60Å, 40- 63 μm). All reactions were carried out in oven dried glassware under N_2 atmosphere.

Instrumentation

NMR spectra were acquired using a Bruker 300 MHz Avance III spectrometer and a Varian Inova 500 MHz spectrometer. ^{1}H and ^{13}C NMR were referenced to TMS or the residual solvent signal. GC/MS analysis was performed using an Agilent Technologies 6890 GC System with a 5973 Mass Selective Detector. All high resolution mass spectra were acquired using an Applied Biosystems QSTAR Elite Hybrid LC/MS/MS system.

Cell Cultures

Human embryonic kidney-293 (HEK293) cells were maintained using HyClone DMEM/High Glucose Modified media (ThermoFisher) supplemented with 10% fetal bovine serum, 2 mM L-glutamate, 100 units/ml of penicillin and 0.1 mg/ml streptomycin. The rat dopamine transporter (DAT) cDNA was cloned into the pcDNA3.1/Zeo plasmid vector (Invitrogen) and used to transfect HEK293 cells using Lipofectamine transfection reagent (Invitrogen) according to the manufacturer's instructions. A colony of cells that stably incorporated the DAT cDNA-containing plasmid vector was selected for using the antibiotic Zeocin (500 μg/ml, Invitrogen) and expanded. The amount of DAT in a sample of 250,000 cells (0.5514 nmol) was obtained by a standard binding experiment using ^{3}H-β-CIT (Perkin-Elmer).

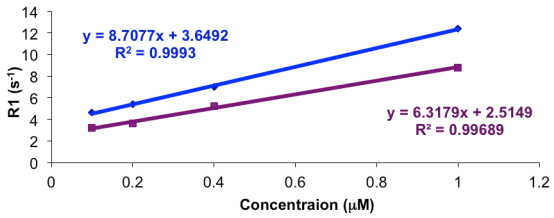
II. Relaxometry Experiments

T1 Experiments

All T_1 experiments were performed using a standard T_1 spin-flip echo experiment in a 300 MHz Bruker Avance NMR.[17] The PBS buffer for these experiments contained a 95:5 ratio of D_2O/H_2O. This allowed for the ability to lock on the ^2H signal during the course of the experiment as well as suppression of the signal for easier detection. The T1 relaxation times were measured across a range of concentrations for **1** and **2** at room temperature. A PBS buffer solution containing 250,000 of the aforementioned DAT-transfected cells (50 µL, 0.55 nmol DAT) were then added to the NMR tubes containing the DCA and the T_1 relaxation times were immediately measured. The consequent data are shown below. The control experiment corresponds to the addition of non-transfected cells resulting in no change in the observed R_1.

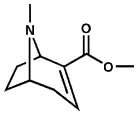
[probe]	1 uM, (2)	1 uM, (1)	1 uM, (1)	400 nM (1)	200 nM (1)	100 nM (1)	100 nM, (1)	10 nM, (1)	1 nM, (1)	100 pM, (1)	10 pM, (1)	control
[probe] (mM)	1.0E-03	1.0E-03	1.0E-03	4.0E-04	2.0E-04	1.0E-04	1.0E-04	1.0E-05	1.0E-06	1.0E-07	1.0E-08	1.0E-05
-log[probe]	3.0	3.0	3.0	3.4	3.7	4.0	4.0	5.0	6.0	7.0	8.0	-5.0
T1 (obs)	2.12	2.27					6.32	7.72	7.57	7.41	7.47	2.27
T1 (obs) w/ DAT	1.77	1.60					4.23	4.82	5.12	6.54	6.63	2.27
R1(obs)	0.47	0.44	0.44	0.26	0.18	0.16	0.16	0.13	0.13	0.13	0.13	0.44
R1(obs) w/ DAT	0.56	0.63	0.62	0.35	0.27	0.23	0.24	0.21	0.20	0.15	0.15	0.44
R1(obs)*	9.43	**8.81**	**8.80**	**5.20**	**3.60**	**3.20**	**3.16**	**2.59**	**2.64**	**2.70**	**2.68**	8.81
R1(obs)* w/ DAT	11.30	**12.50**	**12.40**	**7.00**	**5.40**	**4.60**	**4.73**	**4.15**	**3.91**	**3.06**	**3.02**	8.81
Δ R1*	1.87	**3.69**	**3.60**	**1.80**	**1.80**	**1.40**	**1.56**	**1.56**	**1.26**	**0.36**	**0.34**	0.00

* Relaxivity corrected for 95:5 D2O:H2O



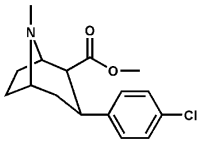
III. Synthesis of Cocaine-Derived DCA, 1

Compound 3: R-(−)-Anhydroecgonine Methyl Ester



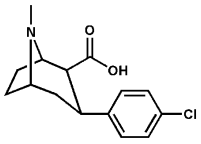
Cocaine•HCl (7.35 mmol, 2.5 g) was dissolved in 6 N HCl (15 mL) and heated to reflux for hours. Upon cooling to room temperature the reaction mixture was washed with diethyl ether (2 x 15 mL) and concentrated to dryness under reduced pressure. Phosphorous oxychloride (15 mL) was then added to the residue, and the mixture was refluxed for four hours. The solvent was then removed under reduced pressure and the resulting dark brown oil was cooled to -40ºC. Anhydrous methanol (25 mL) was then added to the residue and was subsequently removed under reduced pressure. The residue was the dissolved in water (30 mL) and the pH was raised to 9 by ammonium hydroxide. The aqueous solution was the extracted with $CHCl_3$ (3 x 30 mL) and dried over K_2CO_3. The organic phase was then concentrated under reduced pressure to yield a dark brown oil. The crude product was purified by distillation under reduced pressure using a Kugelrhor apparatus to give ecgonidine methyl ester **3** (1.13 g, 84%), a clear oil. All spectral information matched literature values.[1]

Compound 5: 2β-Carbomethoxy-3β-(4-chlorophenyl)tropane



To a magnetically stirred solution of 4-chlorophenylmagnesium bromide (9.36 mmol, 9.36 mL) was added **3** (6.24 mmol, 1.13 g) in TBME (60 mL) dropwise at -40ºC. The reaction mixture was stirred vigorously for three hours and was then quenched with TFA (5 mL) at -78ºC. The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 x 30 mL). The combined organic layers were dried with K_2CO_3 and concentrated under reduced pressure to yield a crude yellow oil. The product was purified by flash chromatography (5% Et_3N, 30% diethyl ether, 65% hexane) to give pure **5** (878 mg, 48%), a white solid. All spectral information matched literature values.[3]

Compound S1: 2β-Carboxylic Acid-3β-(4-chlorophenyl)tropane



Compound **5** (1.88 mmol, 550 mg) was refluxed in dioxane/water (1:1, 15 mL) for 24 hours. The reaction mixture was then cooled to room temperature and washed with diethyl ether (2 x 25 mL). The aqueous layer was then concentrated under reduced pressure, and the crude residue was recrystallized in methanol and diethyl ether to give pure **S1** (462 mg, 88%), a white solid. All spectral information matched literature values.[3]

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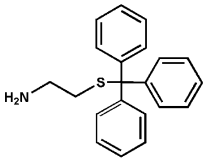
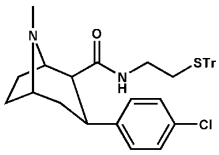
[1] Zou, M.-F.; Kopajtic, T.; Katz, J. L.; Wirtz, S.; Justice, J.B., Jr.; Newma, A. H. *J. Med. Chem.* **2001**, *44*, 4453–4461.

Compound S2: 2-(Tritylthio)ethanamine



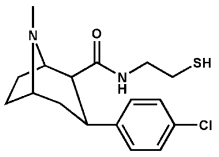
To a magnetically stirred solution of TFA (36 mL) and 2-aminoethanethiol HCl (50.7 mmol, 5.76 g) was added trityl chloride (50.7 mmol, 14.1 g). The reaction mixture was stirred for three hours at room temperature at which time it was concentrated under reduced pressure. The residue was rediluted in EtOAc (50 mL), and the organic phase was washed with 3N NaOH (4 x 15 mL), water (15 mL), saturated $NaHCO_3$ (2 x 15 mL) and brine (3 x 15 mL). The organic phase was then dried over $MgSO_4$, filtered over celite and concentrated under reduced pressure. The resulting oil was then recrystallized with CH_2Cl_2 and hexane and filtered to give pure **S2**, a light orange solid (15.31 g, 95%). All spectral information matched literature values.[2]

Compound S3: 2-β-*N*-(2-(tritylthiol)ethylamine)carboxamide-3β-(4-chlorophenyl)tropane



To a magnetically stirred solution of **S1** (0.93 mmol, 259 mg) in DMF (10 mL) was added sequentially **S1** (0.93 mmol, 300 mg), HOBt (1.7 mmol, 260 mg), and EDC HCl (3.16 mmol, 606 mg). The reaction mixture was stirred at room temperature under N_2 for 48 hours, at which time it was diluted with ethyl acetate (20 mL) and washed with saturated sodium bicarbonate (2 x 10 mL), water (10 mL) and brine (10 mL). The organic layer was then dried over magnesium sulfate and concentrated under reduced pressure to yield a yellow solid. The product was purified by flash chromatography (60:30:10 hexanes : ethyl acetate : triethylamine) to give pure **S3** (76%), a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.78 (s, 1H), 7.45 (d, 6H, J=4Hz), 7.34-7.25 (m, 9H), 7.17 (d, 2H, J=8Hz), 7.08 (d, 2H, J= 8Hz), 3.35-3.31 (m, 2H), 3.22-2.93 (m, 3H), 2.48 (d, 1H, J=4Hz), 2.47-2.39 (m, 3H), 2.97 (s, 3H), 2.22-2.07 (m, 2H), 1.75-1.62(m, 4H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.32, 144.79, 139.72, 132.21, 129.51, 129.02, 128.25, 127.91, 126.70, 66.55, 63.74, 61.17, 54.13, 41.26, 37.80, 35.08, 34.72, 32.25, 26.05, 24.80. HRMS ESI (m/z): $[M+H]^+$ calculated for $C_{36}H_{37}ClN_2OS$ 581.23 m/z, observed 581.2306 m/z.

Compound 6: 2-β-*N*-(2-(thiol)ethylamine)carboxamide-3β-(4-chlorophenyl)tropane

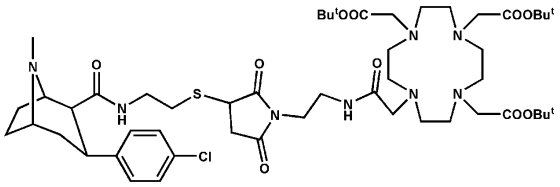


To a magnetically stirred solution of **S3** (0.18 mmol, 107 mg) dissolved in CH_2Cl_2 (3 mL) and TFA (3 mL) was added triethylsilane (0.36 mmol, 42 mg). The reaction mixture was allowed to stir at room temperature under N_2 for three hours at which time it was concentrated under reduced pressure. The resulting yellow oil was dissolved in water and the resulting precipitate was removed by filtration. The filtrate was then concentrated under reduced pressure, diluted in saturated $NaHCO_3$, extracted with CH_2Cl_2, dried over $MgSO_4$

[2] Ruggles, E.L.; Deker, P. B.; Hondal, R. J. *Tetrahedron* **2009**, 65, 1257-1267.

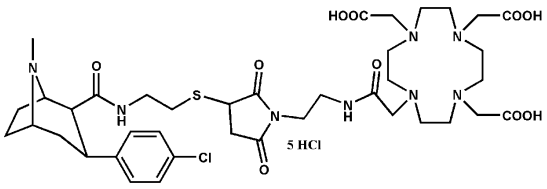
and evaporated to dryness to yield **6** (98%), a white solid. The product was immediately carried on to the next reaction. HRMS ESI (m/z): $[M+H]^+$ calculated for $C_{17}H_{23}ClN_2OS$ 339.12 m/z, observed 339.1237 m/z.

Compound S4: β-CCT DCA precursor



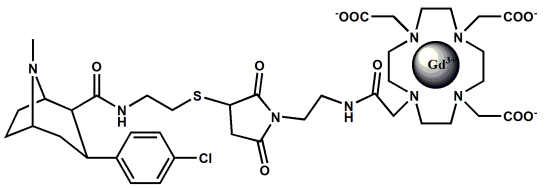
To a magnetically stirred solution of **6** (0.125 mmol, 57 mg) in DMF (5 mL) was added maleimido-mono-amide-DOTA-tris(*t*-butylester) (0.10 mmol, 70 mg). The reaction mixture was allowed to stir at room temperature overnight at which time it was diluted in water, extracted with CH_2Cl_2 (3 x 10 mL), and the combined organic layers were washed with saturated sodium bicarbonate (1 x 10 mL) and water (1 x 10 mL), dried over magnesium sulfate, and concentrated under reduced pressure to yield a yellow solid. The product was purified by flash chromatography (90:10:1 CH_2Cl_2 : MeOH : NH_4OH) to give pure **S4** (34 mg, 33%), an off-white solid. Both ^1H and ^{13}C NMR spectra depicted the characteristic peak broadening and overlap associated with tetra-substituted DOTA ligands. ^1H-NMR (500 MHz, $CDCl_3$) δ 7.64 (bs, 2H), 7.27 (d, 2H, J=10Hz), 7.21 (d, 2H, J=8Hz), 4.07-1.60 (m, 48 H), 1.48 (s, 9H), 1.46 (s, 18H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.81, 177.76, 175.59, 173.01, 172.45, 132. 65, 130.90, 129.06, 128.56, 81.85, 81.69, 58.18, 55.99, 55.75, 52.63-52.09 (br), 50.69, 48.50-47.87(br), 38.60, 37.32, 36.65, 36.34, 34.14, 32.14, 31.91, 29.90, 29.39, 28.85, 28.04, 27.87, 24.28. HRMS ESI (m/z): $[M+2H]^{2+}$ calculated for $C_{51}H_{81}ClN_8O_{10}S$ 517.2750 m/z, observed 517.2756 m/z.

Compound S5: De-protected β-CCT DCA precursor



S4 (0.00823 mmol, 8.5 mg) was refluxed in 6 N HCl (2 mL) for six hours (reaction progress monitored via MS), at which time the reaction mixture was cooled to room temperature, washed with diethyl ether (2 x 10 mL), filtererd through a 0.45 μm syringe filter, and evaporated to dryness under reduced pressure using a rotary evaporator to yield **S5** (8.6 mg, 99%), a white solid. HRMS ESI (m/z): $[M-H]^-$ calculated for $C_{51}H_{81}ClN_8O_{10}S$ 863.36 m/z, observed 863.3610 m/z.

Compound 1: β-CCT DCA

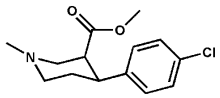


To a magnetically stirred solution of **S5** (0.0082 mmol, 8.6 mg) in water (2 mL) was added $GdCl_3$ (0.0082 mmol, 1.9 mg). The reaction mixture's pH was adjusted and maintained between 5 and 6.5 using 0.1M HCl.

After 24 hours, the pH was raised to 8 by adding 0.1M NaOH, and the reaction mixture was filtered through a 0.45μm syringe filter and evaporated to dryness using a rotary evaporator to give pure **1** in quantitative yield, a white solid. LRMS ESI (m/z): $[M-H]^-$ calculated for $C_{39}H_{54}ClGdN_8O_{10}S$ 1019.66, observed 1019.61 m/z.

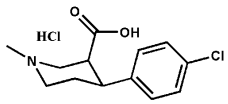
IV. Synthesis of Arecoline-Derived DCA 2

Compound 9: (+)-4β-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylate



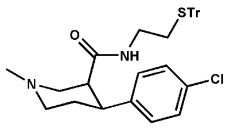
To a magnetically stirred solution of 4-chlorophenylmagnesium bromide (18 mmol, 18 mL) was added free-based arecoline (12.0 mmol, 2.58 g) in TBME (18 mL) dropwise at -40ºC. The reaction mixture was stirred vigorously for three hours and was then quenched with saturated ammonium chloride (40 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 x 30 mL). The combined organic layers were then concentrated under reduced pressure to yield a yellow oil. The product was purified by flash chromatography (5% Et_3N, 30% diethyl ether, 65% hexane) to give pure **9** (1.15 g, 36%), a white solid. All spectral information matched literature values.[3]

Compound S6: (+)-4β-(4-chlorophenyl)-1-methylpiperidine-3α-carboxylic acid hydrochloride



Compound **9** (4.2 mmol, 1.12 g) was refluxed in 6 N HCl (25 mL) for six hours. The reaction mixture was then cooled to room temperature and washed with diethyl ether (2 x 25 mL). The aqueous layer was then concentrated under reduced pressure, and the residue was recrystallized in methanol and diethyl ether to give pure **S6** (1.18 g, 98%), a white solid. All spectral information matched literature values.[2]

Compound S7: (+)-4β-(4-chlorophenyl)-1-methyl-N-(2-(tritylthio)ethyl) piperidine-3α-carboxamide

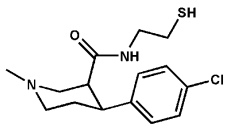


To a magnetically stirred solution of **S6** (0.69 mmol, 200mg) in CH_2Cl_2 (10 mL) was added sequentially DIPEA (0.83 mmol, 107 mg), EDC (0.69 mmol, 132 mg), HOBt (0.69 mmol, 106 mg), and **S1** (0.69 mmol, 220 mg). The reaction mixture was allowed to stir at room temperature under N_2 for 24 hours, at which time it was washed with saturated sodium bicarbonate (2 x 10 mL), dried over magnesium sulfate and concentrated under reduced pressure to yield an orange oil. The product was purified by flash chromatography (65:30:5

[3] Mobele, B. I.; Kinahan, T.; Ulysse, L. G.; Gagnier, S. V.; Ironside, M. D.; Knox, G. S.; Mohammadi, F. *Org. Process Res. Dev.* **2006**, *10*, 914-920

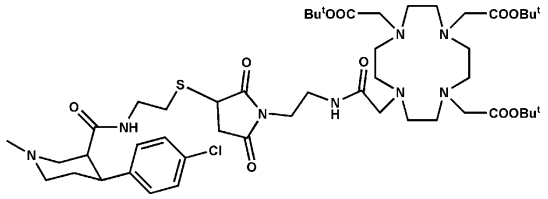
hexane:ethyl acetate:triethylamine) to give pure **S7** (292 mg, 76%), a white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.01 (bs, 1H), 7.51 (d, 6H, J=8Hz), 7.36-7.27 (m, 9H), 7.24 (d, 2H, J=8Hz), 7.11 (d, 2H, J=8Hz), 3.31-3.26 (m, 1H), 3.12-3.05 (m, 3H), 2.83-2.80 (m, 1H), 2.70 (bs, 1H), 2.43 (t, 2H, J=4Hz), 2.34 (s, 3H), 2.31-2.28 (m, 2H), 2.13 (t, 1H, J=8Hz), 1.77 (d, 1H, J=12 Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 172.28, 144.84, 140.90, 132.29, 129.58, 128.82, 128.37, 128.00, 126.80, 66.55, 57.77, 55.66, 48.25, 46.08, 42.74, 37.79, 32.47, 26.85. HRMS ESI (m/z): $[M+H]^+$ calculated for $C_{34}H_{35}ClN_2OS$ 555.22 m/z, observed 555.2210 m/z.

Compound 8: (4R)-4-(4-chlorophenyl)-N-(2-mercaptoethyl)-1-methylpiperidine-3-carboxamide



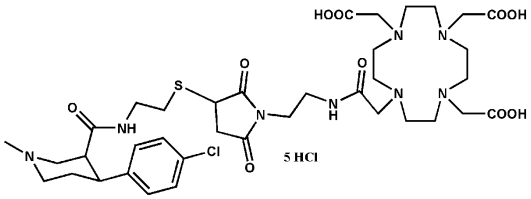
To a magnetically stirred solution of **S7** (0.45 mmol, 252 mg) dissolved in CH_2Cl_2 (5 mL) and TFA (5 mL) was added triethylsilane (0.90 mmol, 105 mg). The reaction mixture was allowed to stir at room temperature under N_2 for three hours, at which time it was concentrated under reduced pressure. The resulting yellow oil was dissolved in water and the resulting precipitate was removed by filtration. The filtrate was then concentrated under reduced pressure to dryness, diluted in saturated $NaHCO_3$, extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated under reduced pressure to yield **8** (190 mg, 98%), an off-white solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.07 (bs, 1H), 7.24 (d, 2H, J=8Hz), 7.10(d, 2H, J=8Hz), 3.42-3.32 (m, 2H), 3.07 (d, 2H, J=12Hz), 2.81-2.62 (m, 4H), 2.33 (s, 3H), 2.34-2.24 (m, 2H), 2.13 (t, 1H, J=12Hz), 1.76 (d, 1H, J=12Hz), 1.35 (t, 1H, J=8Hz). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 173.10, 163.19-161.76 (q, TFA) 138.57, 132.66, 128.73, 128.71, 121.87-110.30 (q, TFA), 55.52, 54.47, 43.73, 43.63, 41.79, 39.04, 23.20, 22.88. LRMS EI (m/z): $[M]^+$ calculated for $C_{15}H_{21}ClN_2OS$ 312.11 m/z, observed 312.10 m/z.

Compound S8: Arecoline-derived DCA precursor



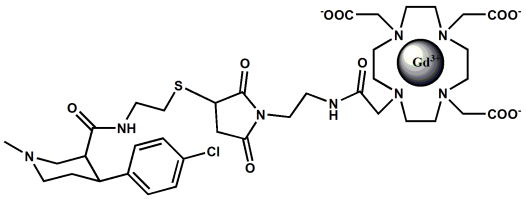
To a magnetically stirred solution of **8** (0.090 mmol, 38.4 mg) in DMF (4.8 mL) was added maleimido-mono-amide-DOTA-tris(*t*-butylester) (0.072 mmol, 50 mg). The reaction mixture was allowed to stir at room temperature overnight at which time it was diluted in water, extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers were washed with saturated sodium bicarbonate (1 x 10mL) and water (1 x 10 mL), dried over magnesium sulfate, and concentrated under reduced pressure to yield a yellow solid. The product was purified by flash chromatography (90:10:1 CH_2Cl_2:MeOH:NH_4OH) to give pure **S8** (24 mg, 33%), an off-white solid. Both ^{1}H and ^{13}C NMR spectra depicted the characteristic peak broadening and overlap associated with tetra-substituted DOTA ligands. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.24 (d, 2H, J=5Hz), 7.13 (d, 2H, J=5Hz), 7.02 (bs, 1H), 4.11-4.04 (m, 1H), 3.60-2.60 (m, 27H), 2.42-2.35 (m, 10H), 2.22-1.75 (m, 9H), 1.48 (s, 9H), 1.46 (s, 18H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.69, 177.53, 175.54, 172.75, 172.75, 172.49, 140.76, 132.19, 128.80, 128.33, 81.82, 81.65, 57.60, 55.77, 55.34, 53.14-51.70 (br), 49.26-47.34 (br), 45.72, 45.65, 42.18, 39.10, 38.24, 38.18, 37.43, 36.30, 36.21, 32.17, 31.64, 28.02, 27.91, 26.33. HRMS ESI (m/z): $[M+2H]^{2+}$ calculated for $C_{49}H_{79}ClN_8O_{10}S$ 504.2690 m/z, observed 504.2700 m/z.

Compound 9: Deprotected arecoline-derived DCA precursor



S8 (0.024 mmol, 24 mg) was refluxed in 6 N HCl (2 mL) for six hours (reaction progress was monitored via MS) at which time the reaction mixture was cooled to room temperature, washed with diethyl ether (2 x 10 mL), filtererd through a 0.45μm syringe filter, and evaporated to dryness under reduced pressure using a rotary evaporator to give pure **9** (24 mg, 99%), a white solid. The product was then immediately used in the next reaction. HRMS ESI (m/z): $[M-H]^-$ calculated for $C_{37}H_{55}ClN_8O_{10}S$ 837.35 m/z, observed 837.3506 m/z.

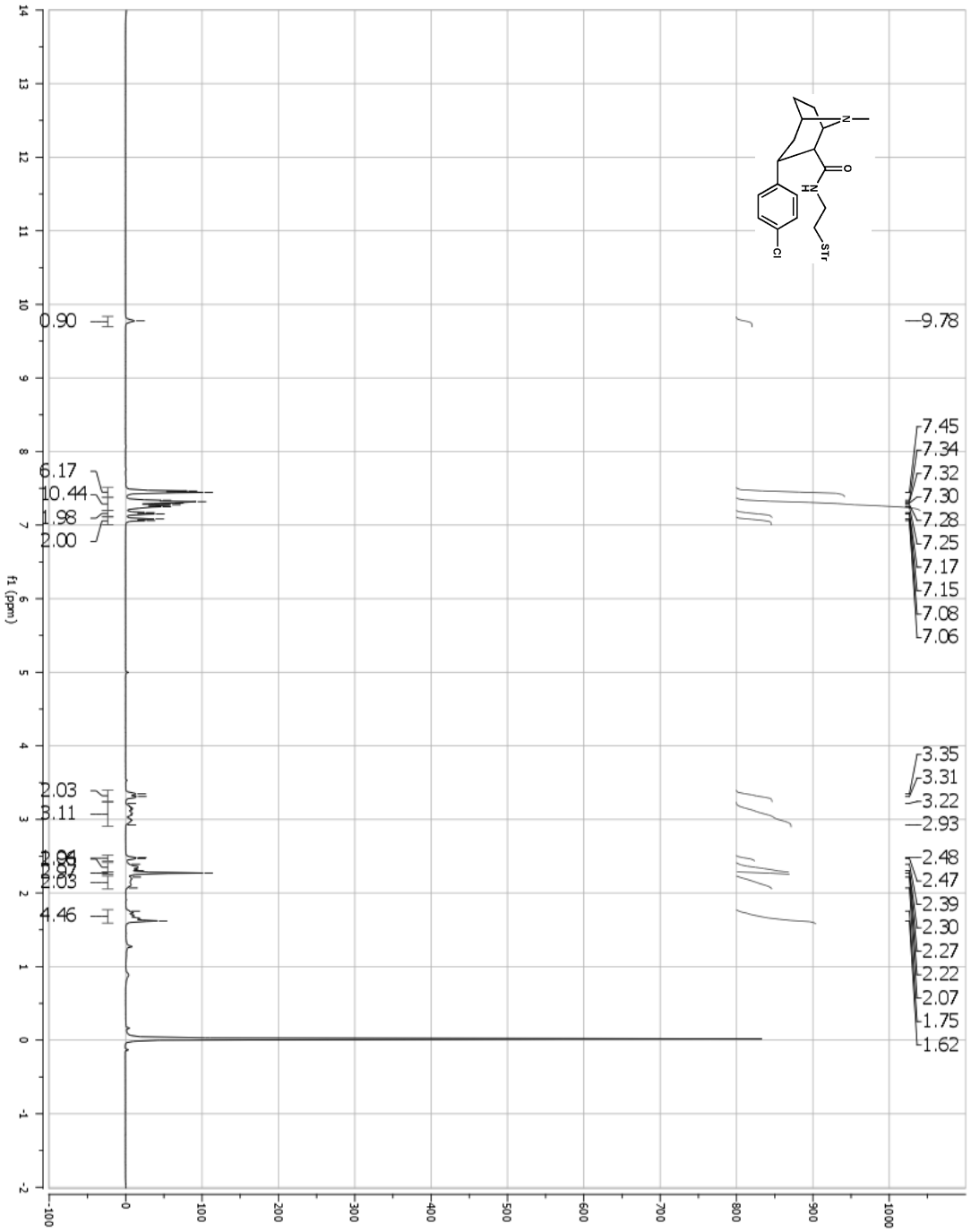
Compound 2: Arecoline-derived DCA



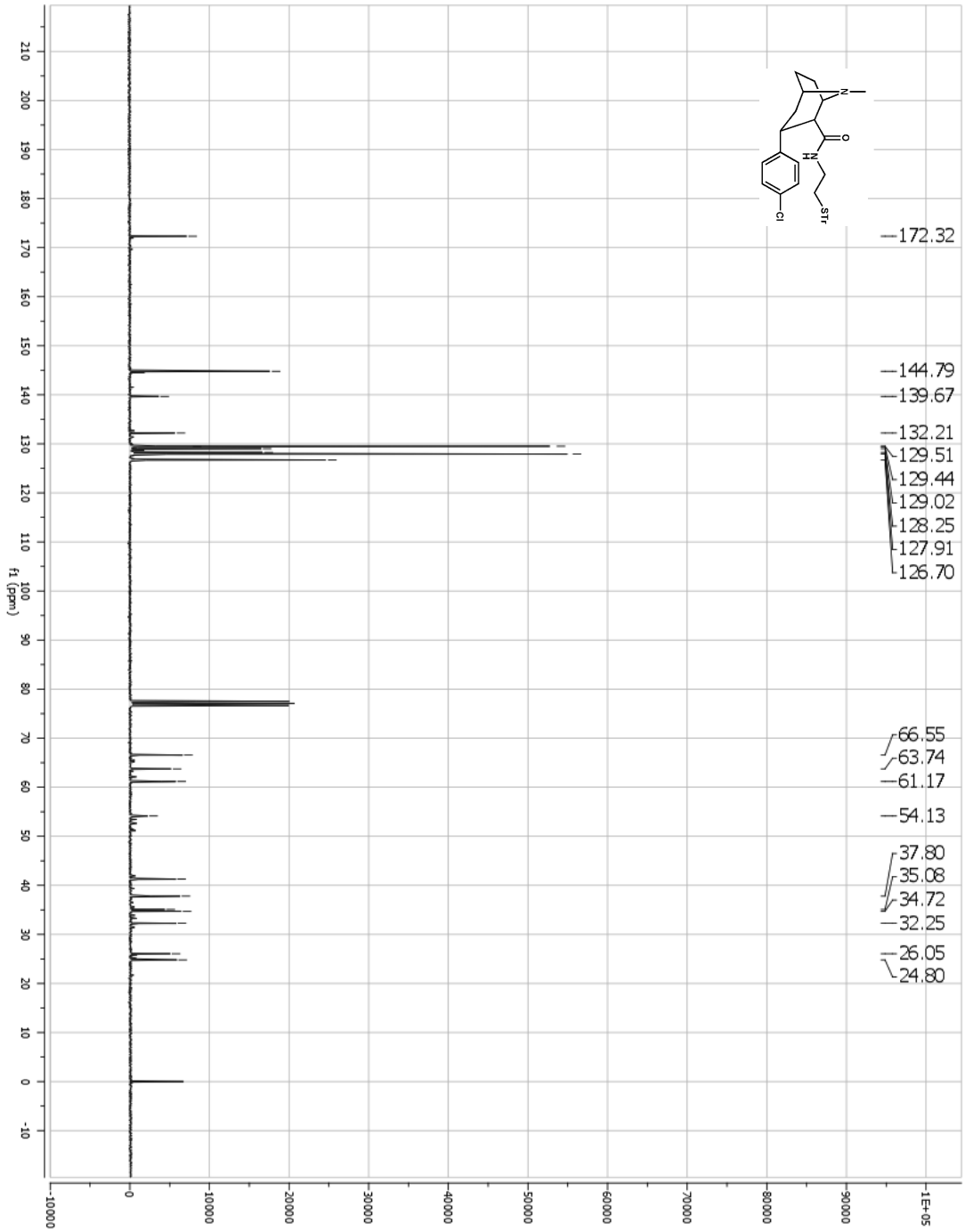
To a magnetically stirred solution of **S9** (0.012 mmol, 12.5 mg) in water (1.2 mL) was added $GdCl_3$ (0.012 mmol, 2.8 mg). The reaction's pH was adjusted and maintained between 5 and 6.5 using 0.1M HCl. After 24 hours, the pH was raised to 8 by addition of 0.1M NaOH, and the reaction mixture was filtered through a 0.45μm syringe filter and evaporated to dryness using a rotary evaporator to give pure **2**, a white solid. LRMS ESI (m/z): $[M-H]^-$ calculated for $C_{37}H_{52}ClGdN_8O_{10}S$ 992.25, observed 992.24 m/z.

V. ^{1}H and ^{13}C NMR Spectra of Previously Unreported Compounds

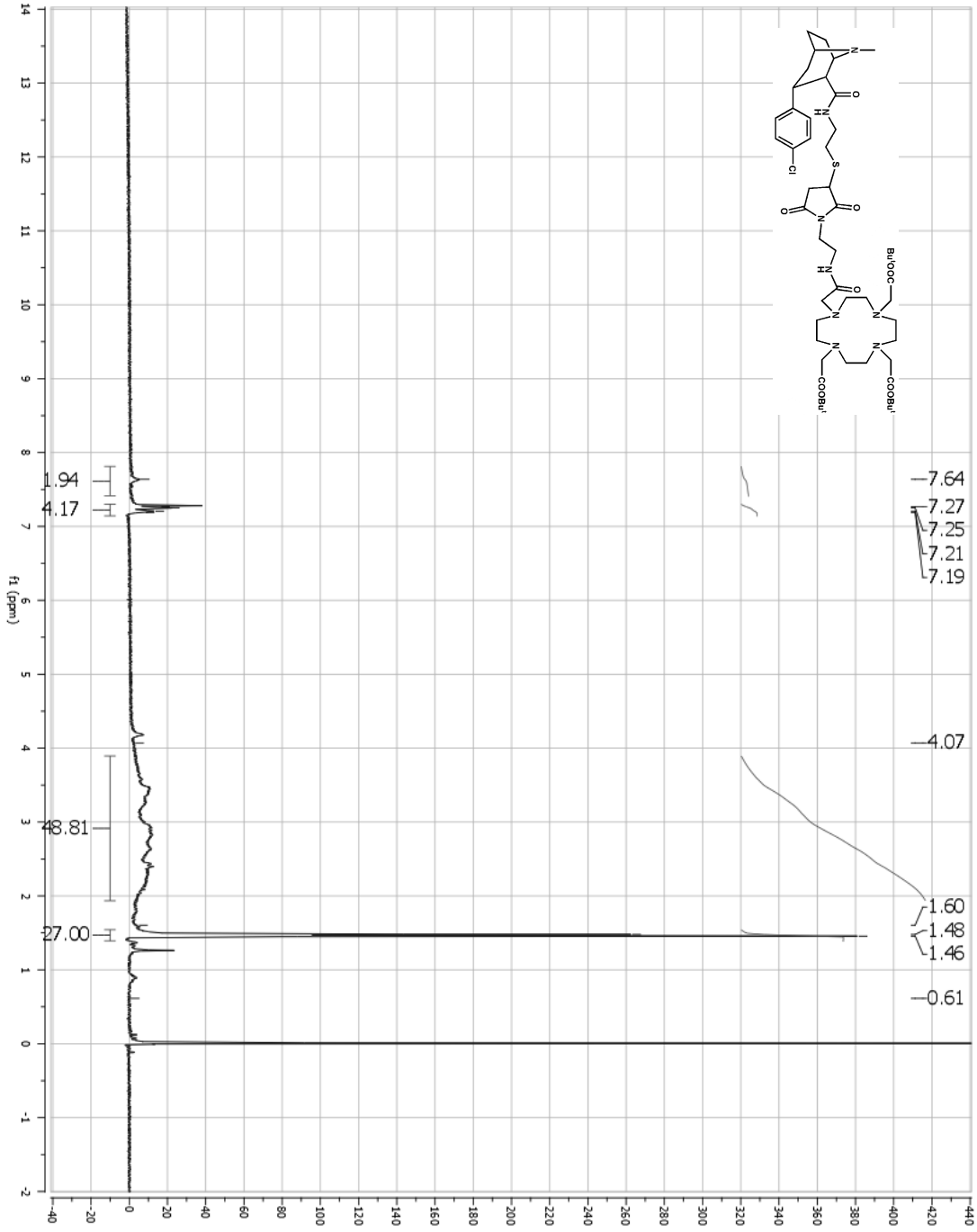
Compound S3 ^{1}H NMR



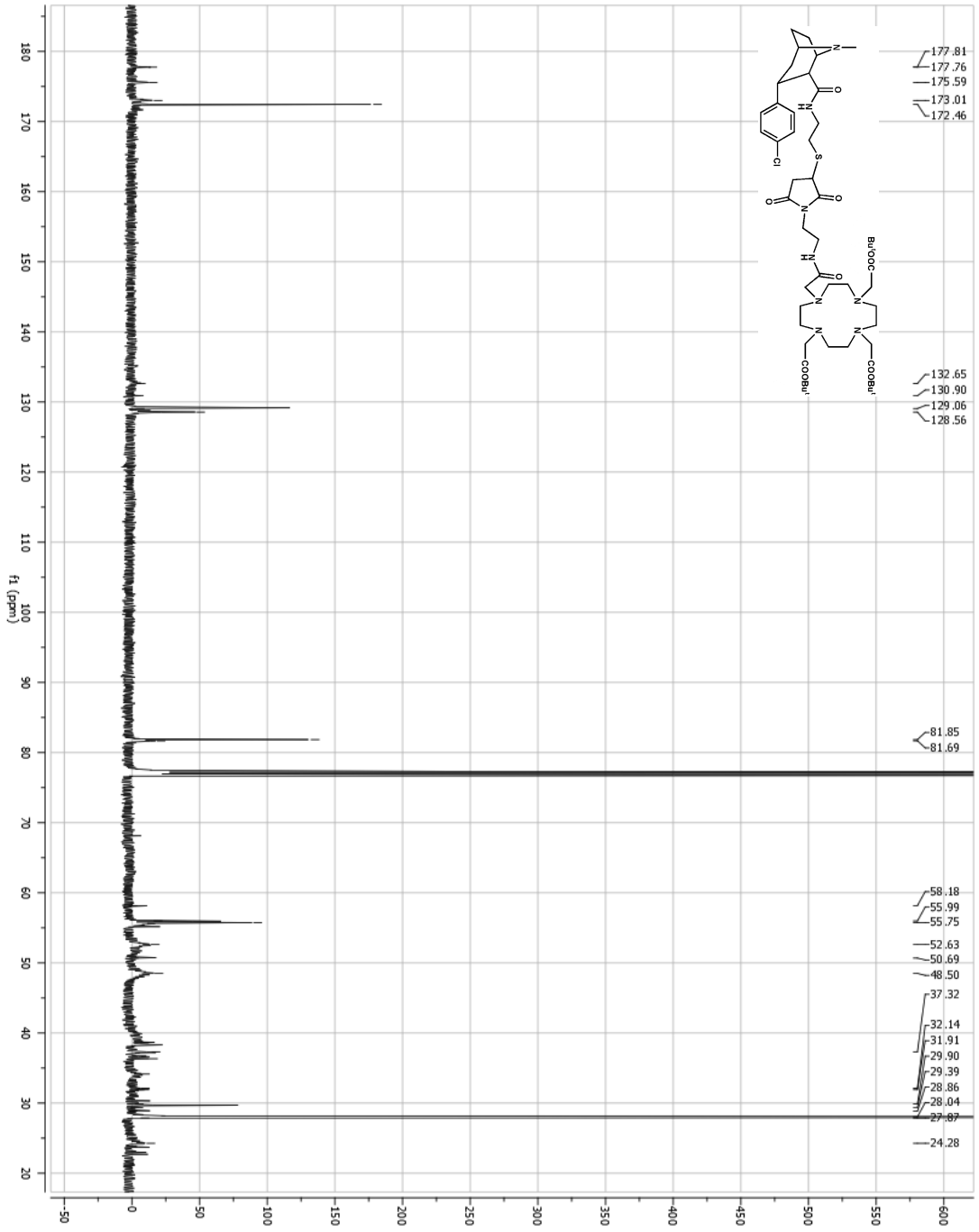
Compound S3 ^{13}C NMR



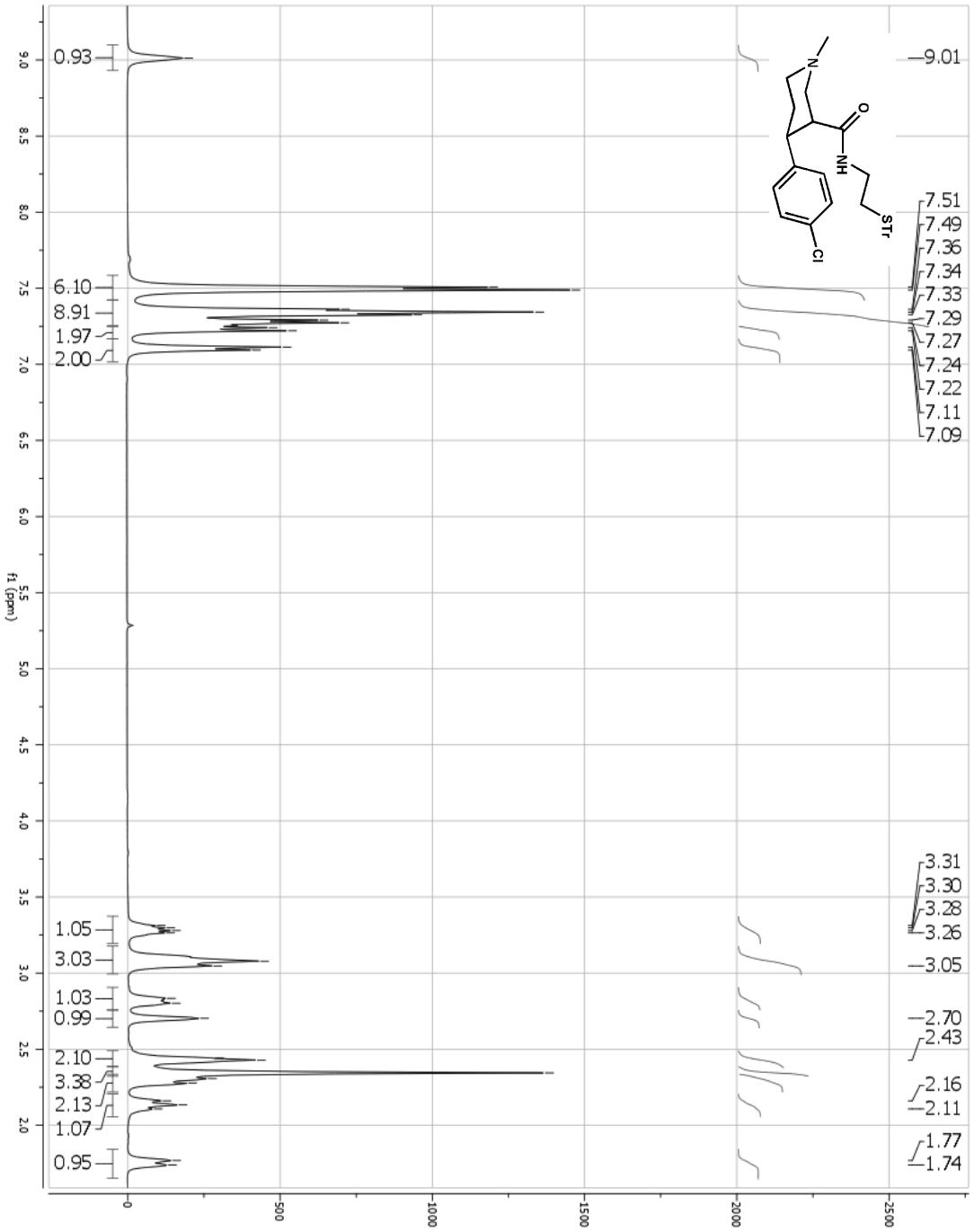
Compound S4 ^{1}H NMR



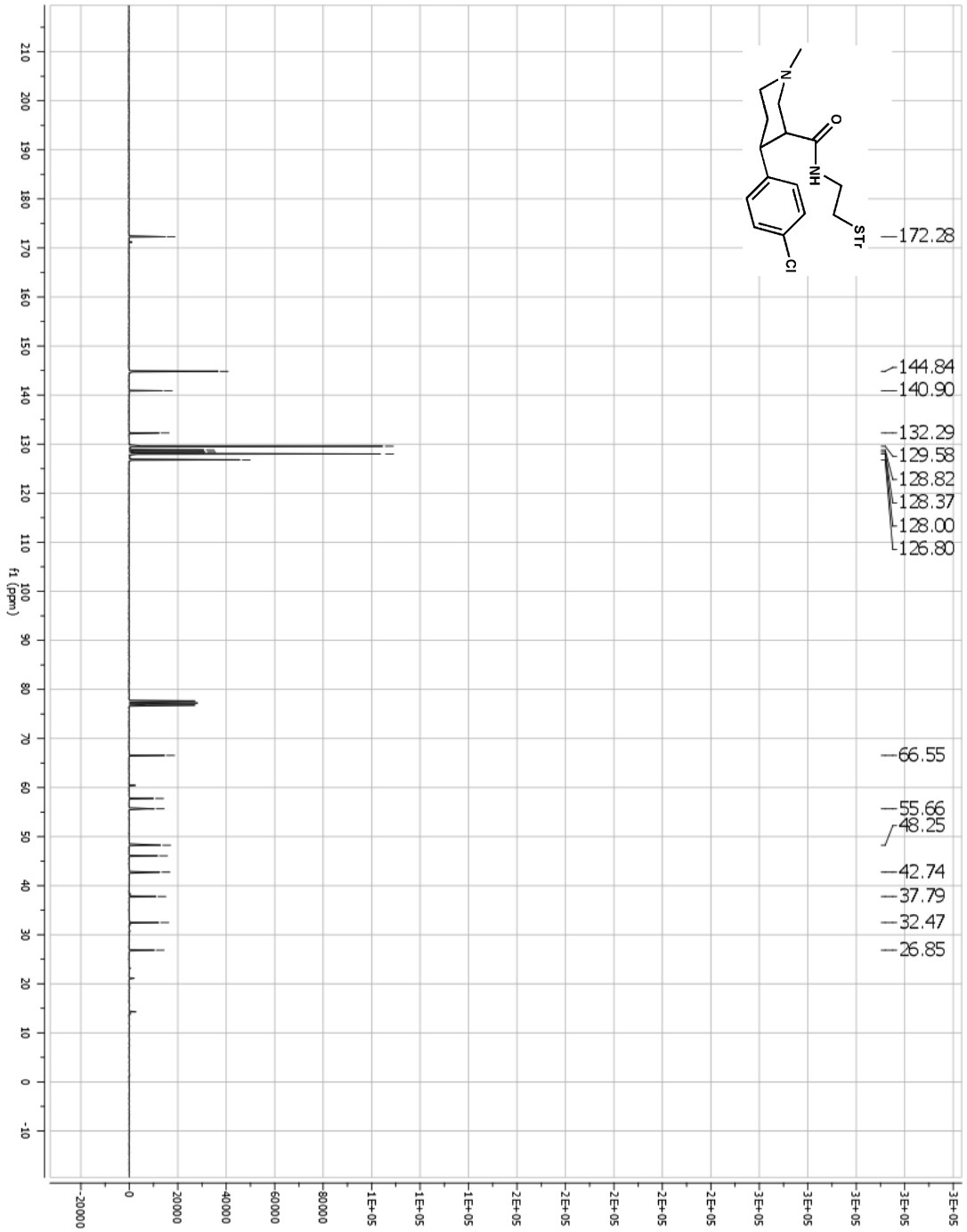
Compound S4 ^{13}C NMR



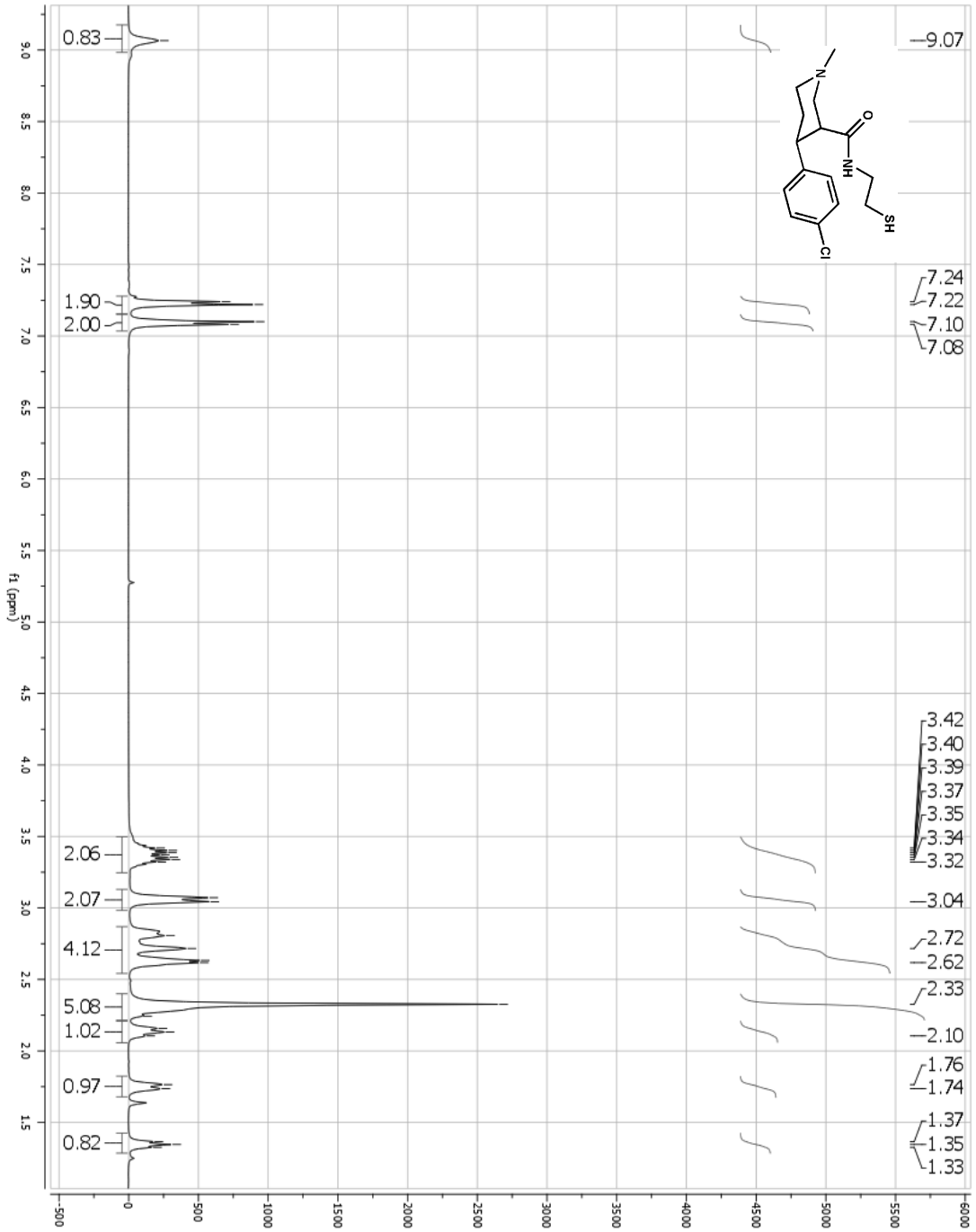
Compound S7 ^{1}H NMR



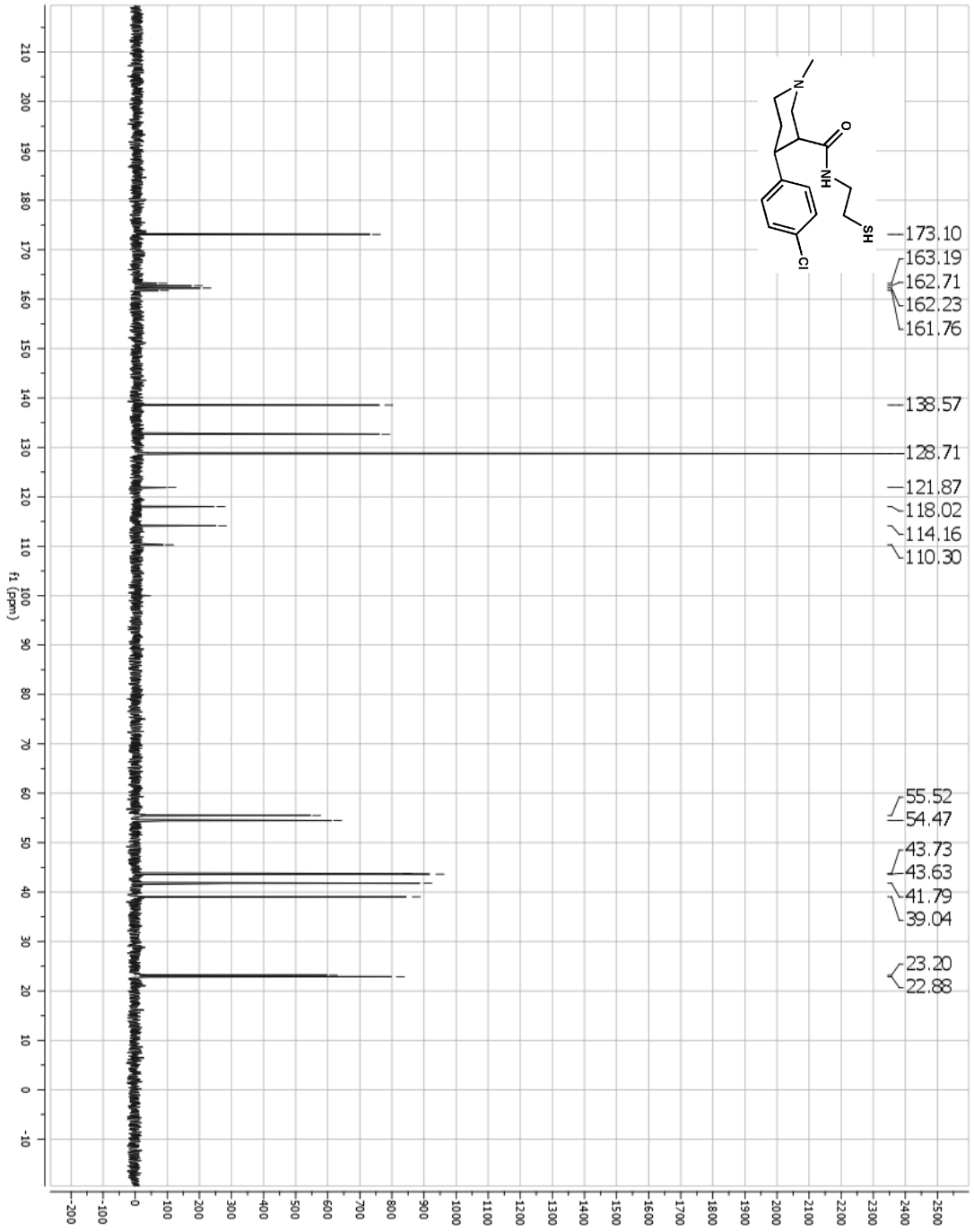
Compound S7 ^{13}C NMR



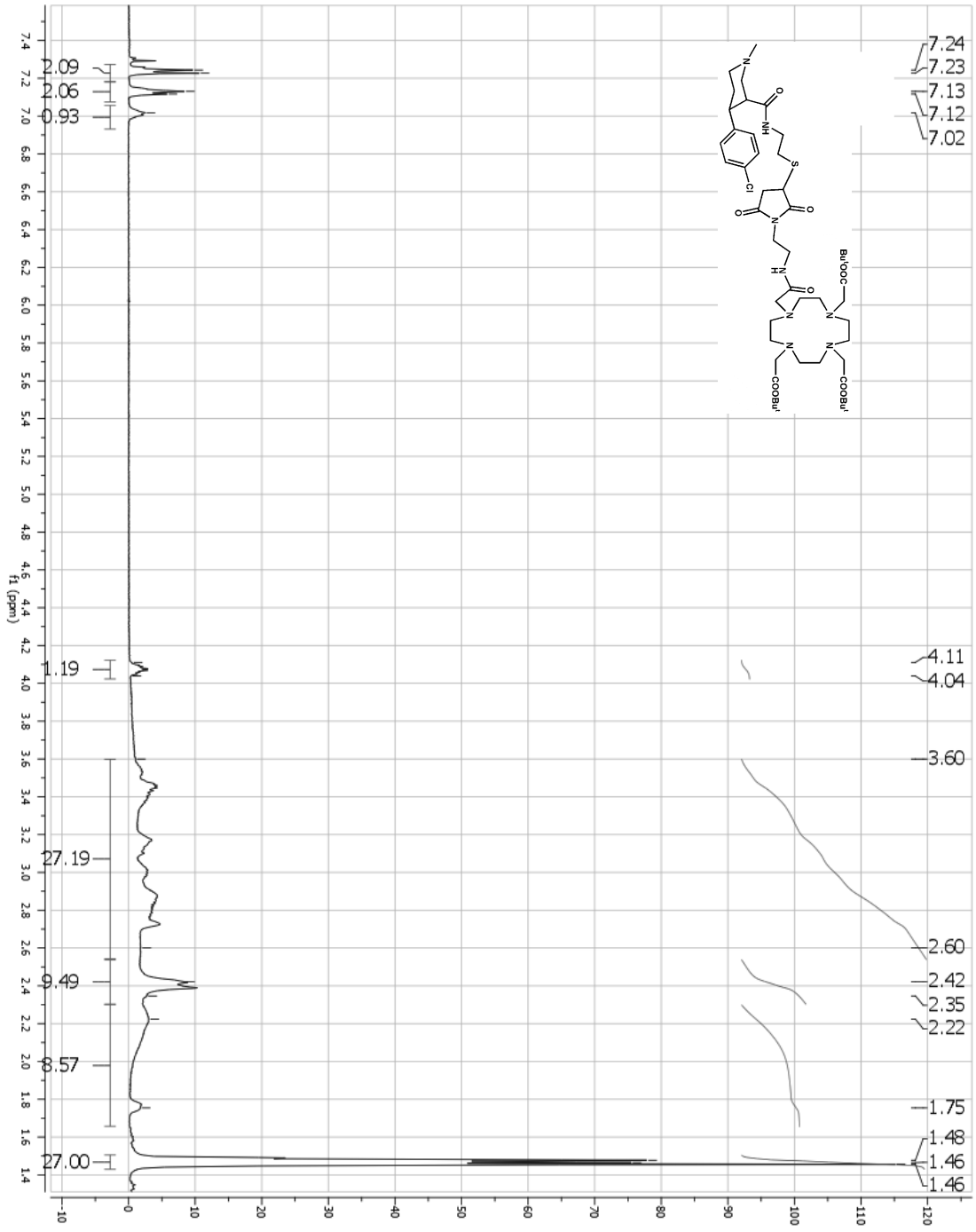
Compound 8 ^{1}H NMR



Compound 8 ^{13}C NMR



Compound S8 ^{1}H NMR



Compound S8 ^{13}C NMR