Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Integrated Approach to the Discovery of Potent Agelastatin A Analogues for Brain Tumors: Chemical Synthesis and Biological, Physicochemical and CNS Pharmacokinetic Analyses

Zhimin Li,[c,‡] Daisuke Shigeoka,[a‡] Thomas R. Caulfield,[d] Takashi Kawachi,[a] Yushi Qiu,[c] Takuma Kamon,[a] Masayoshi Arai,[a] Han W. Tun*[,b] and Takehiko Yoshimitsu*[,a]

[a] *Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan*

[b] *Department of Hematology/Oncology, Mayo Clinic, Jacksonville, Florida, 32224, USA*

[c] *Department of Cancer Biology, Mayo Clinic, Jacksonville, Florida, 32224,USA*

[d] *Mayo Clinic College of Medicine, Dept. of Neuroscience, Jacksonville, Florida, 32224, USA*

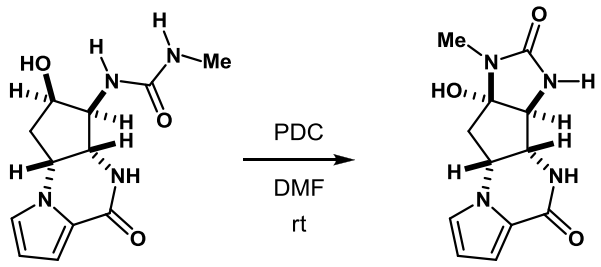
yoshimit@phs.osaka-u.ac.jp, tun.han@mayo.edu

Supplementary Information

Chemical synthesis of AA analogues:

General. Melting points are uncorrected. All reagents were used as received from commercial suppliers unless otherwise noted. ^{1}H NMR spectra (500 or 400 MHz) and ^{13}C NMR spectra (125 or 100 MHz) were measured in the specified solvents. Chemical shifts are reported in ppm relative to the internal solvent signal [chloroform-*d*: 7.26 ppm (^{1}H NMR), 77.0 ppm (^{13}C NMR); methanol-*d*: 3.30 ppm (^{1}H NMR), 49.0 ppm (^{13}C NMR)]. The proton signal of TMS (0.00 ppm) or DMSO (2.50 ppm) was also used in some cases as the internal standard for ^{1}H NMR spectra. FT-IR spectra were recorded for samples loaded on KBr powder using the diffuse reflectance method, dispersed in KBr pellet, or loaded as neat film on NaCl plate. Mass spectra were obtained according to the specified technique. Analytical thin layer chromatography (TLC) was performed using Kieselgel 60 F_{254}. Compounds were visualized with UV light and stained with anisaldehyde solution, phosphomolybdic acid in EtOH, iodine, or $KMnO_4$ solution. The preparation of compounds **1**, **2**, **3**, **5** and **11** has previously been reported.[1] A modified protocol for oxidation of compound **3** using PDC in DMF was described below.

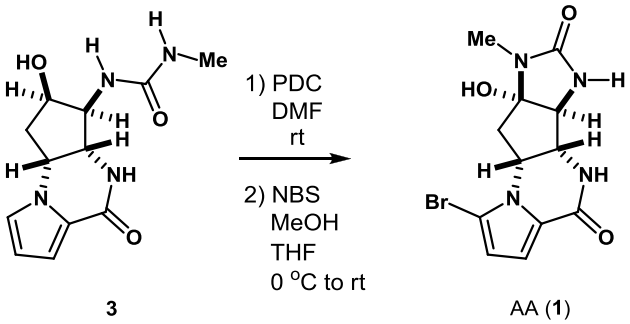
Chemical synthesis of AA analogues:



3 **5**

(5a*S*,5b*S*,8a*S*,9a*R*)-8a-Hydroxy-8-methyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5] cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Debromoagelastatin A (DeBAA)] (5): A single-necked, 100 mL round-bottomed flask equipped with a magnetic stir bar was charged with the known urea **3** (140 mg, 0.53 mmol)[1a,b] and DMF (27 mL) at room temperature. To the mixture was added pyridinium dichromate (PDC) (598 mg, 1.59 mmol), and the mixture was stirred at room temperature. After 21 h, *i*-PrOH (0.12 mL) was added, and the mixture was stirred for an additional 10

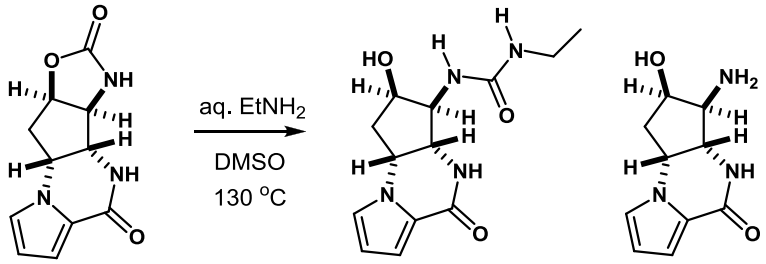
min and concentrated under reduced pressure. The residue was charged onto a column of flash silica gel/florisil (slurry packed) and eluted with MeOH/EtOAc (1:10→1:3v/v) to give debromoagelastatin A (DeBAA) (**5**) (86 mg, 62%) as a colorless solid. **Debromoagelastatin A (DeBAA) (5)**: $[\alpha]^{21}_{D}$ –68.1 (*c* 0.775, MeOH); IR (KBr) ν 3279, 2924, 1690, 1645 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.02 (dd, 1H, *J* = 2.7, 1.4 Hz), 6.88 (dd, 1H, *J* = 4.1, 1.4 Hz), 6.23 (dd, 1H, *J* = 4.1, 2.7 Hz), 4.65 (ddd, 1H, *J* = 10.1, 6.4, 6.0 Hz), 3.99 (dd, 1H, *J* = 6.0, 1.4 Hz), 3.81 (d, 1H, *J* = 1.2 Hz), 2.79 (s, 3H), 2.61 (dd, 1H, *J* = 13.7, 6.4 Hz), 2.27 (dd, 1H, *J* = 13.7, 10.1 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 162.1, 161.3, 125.7, 122.8, 115.4, 111.1, 95.8, 68.0, 62.8, 55.6, 41.6, 24.2. MS *m/z*: 263 $[M+1]^+$, 59 (100%); HRMS (FAB) calcd for $C_{12}H_{15}N_4O_3$ $[M+H]^+$: 263.1144, found: 263.1146.[1a]



3 AA (**1**)

Synthesis of AA (1) by a modified protocol (2 step conversion of urea 3 to AA): (5a*S*,5b*S*,8a*S*,9a*R*)-1-Bromo-8a-hydroxy-8-methyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo [4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Agelastatin A (AA)] (1): A single-necked, 200 mL round-bottomed flask equipped with a magnetic stir bar was charged with alcohol **3** (310 mg, 1.17 mmol) and DMF (60 mL) at room temperature. To the mixture was added pyridinium dichromate (PDC) (1.33 g, 3.52 mmol), and the mixture was stirred at room temperature. After 18 h, *i*-PrOH (0.3 mL) was added, and the mixture was stirred for an additional 10 min and concentrated under reduced pressure. The residue was charged onto a column of flash silica gel/florisil (slurry packed) and eluted with MeOH/CH_2Cl_2 (1:5 v/v) to give debromoagelastatin A (DeBAA) (**5**) (328 mg; containing traces of inseparable unreacted urea **3** and unidentified impurities) as solids. The material **5** was dissolved in a mixed solvent of MeOH (35 mL) and THF (70 mL). Then NBS (63 mg, 0.354 mmol) was added to the mixture at 0°C, and

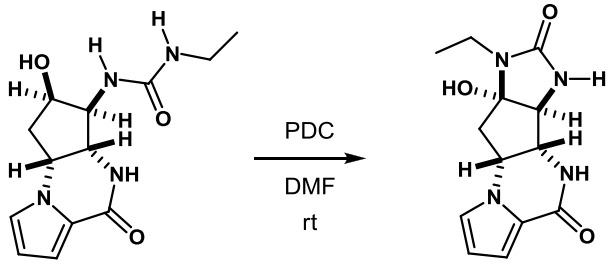
the whole mixture was allowed to warm to room temperature. After 40 min of stirring, the mixture was again cooled to 0°C, and NBS (21 mg, 0.118 mmol) was added. Stirring was continued for 30 min at room temperature, and the mixture was again cooled to 0°C. To this was added NBS (21 mg, 0.118 mmol), and the mixture was allowed to warm to room temperature. After being stirred at the same temperature for 20 min, the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($MeOH/CH_2Cl_2$ 1:10)* to give agelastatin A (**1**) (AA) (164 mg, 41% in 2 steps from urea **3**) as a white solid. *$MeOH/H_2O$/EtOAc (1:1:33 v/v) was also suitable for purification of the synthetic agelastatin by flash silica gel column chromatography. ***Recrystallization:*** A crystalline sample of agelastatin A (210 mg) was obtained by recrystallization of 258 mg of agelastatin A from MeOH. **Agelastatin A (AA) (1):** Mp 203-205 ºC; $[\alpha]_D^{27}$ -82.2 (*c* 0.14, MeOH); IR (KBr) ν 3258, 2953, 1643 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.91 (d, 1H, *J* = 4.3 Hz), 6.32 (d, 1H, *J* = 4.3 Hz), 4.59 (ddd, 1H, *J* = 12.2, 6.1, 5.5 Hz), 4.07 (dd, 1H, *J* = 5.5 Hz), 3.87 (s, 1H), 2.80 (s, 3H), 2.64 (dd, 1H, *J* = 12.8, 6.1 Hz), 2.09 (dd, 1H, *J* = 12.8, 12.2 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 161.1, 124.1, 116.0, 113.8, 107.3, 95.7, 67.4, 62.2, 54.3, 40.0, 24.2. MS *m/z*: 341 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{12}H_{14}N_4O_3{}^{79}Br$ $[M+H]^+$: 341.0249, found 341.0271.[1a]



2 4 11

1-Ethyl-3-((2*R*,3*S*,3a*S*,9a*R*)-2-Hydroxy-5-oxo-2,3,3a,4,5,9a-hexahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-*a*]pyrazin-3-yl)urea [Urea (4)]: To a solution of compound **2** (36.4 mg, 0.165 mmol)[1] in DMSO (2.28 mL) in a stainless steel tube was added 70% aq. $EtNH_2$ (2.28 mL, 28 mmol) at room temperature, and the mixture was heated at 130 ºC for 10 h. Additional aq. $EtNH_2$ (0.5 mL, 6.14 mmol; 70% v/v) was added and heating was continued at 130 ºC for further 6 h. After concentration of the mixture under reduced

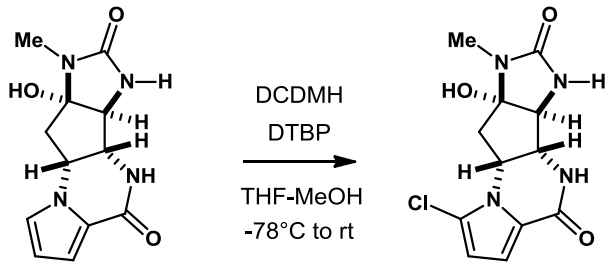
pressure, the residue was rinsed with MeOH to leave unreacted compound **2** (11.8 mg, 32% recovered) as a colorless solid. Concentration of the MeOH extracts under reduced pressure followed by flash column chromatography ($MeOH/CH_2Cl_2$ 1:6→1:5 v/v) of the resultant residue afforded urea **4** (18.7 mg, 43%) as a colorless solid. Further elution using MeOH as eluent afforded β-aminoalcohol **11** (5.7 mg, 18%) as a colorless solid. **Urea 4**: $[\alpha]^{25}_D$ -177.0 (*c* 0.565, MeOH); IR (KBr) ν 3279, 1634 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.00 (dd, 1H, *J* = 2.7, 1.8 Hz), 6.85 (dd, 1H, *J* = 3.7, 1.8 Hz), 6.27 (dd, 1H, *J* = 3.7, 2.7 Hz), 4.70 (dt, 1H, *J* = 6.9, 4.6 Hz), 4.17 (m, 1H), 3.97 (m, 2H), 3.13 (q, 2H, *J* = 7.3 Hz), 2.52 (m, 1H), 2.38 (ddd, 1H, *J* = 15.1, 7.3, 2.7 Hz), 1.09 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 161.36, 160.83, 124.45, 123.55, 114.83, 111.51, 69.35, 60.59, 59.43, 53.12, 41.00, 35.80, 15.66; MS *m/z*: 279 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{13}H_{19}N_4O_3$ $[M+H]^+$: 279.1457, found: 279.1483. The spectral and analytical data of β-aminoalcohol **11** were identical with those previously reported.[7j]



4 6

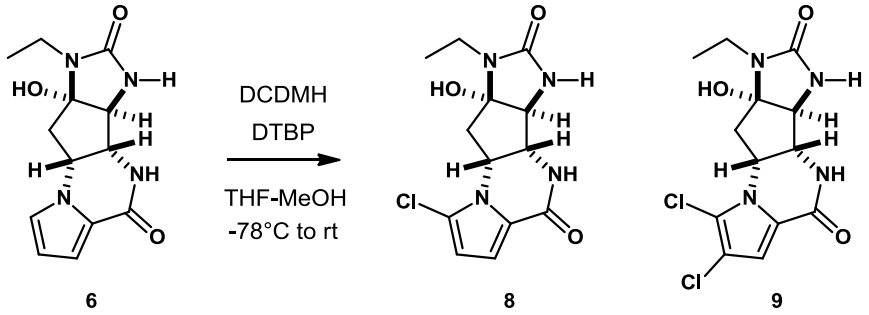
(5a*S*,5b*S*,8a*S*,9a*R*)-8-Ethyl-8a-hydroxy-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Debromoethylagelastatin A (DeBEAA)](6): To a stirred solution of urea **4** (32.3 mg, 0.116 mmol) in DMF (3.3 mL) was added pyridinium dichromate (PDC) (131 mg, 0.35 mmol). After 73 h, *i*-PrOH (27 µL) was added, and the mixture was stirred for additional 10 min and concentrated under reduced pressure. The residue was charged onto a column of flash silica gel/florisil (slurry packed) and eluted with $MeOH/CH_2Cl_2$ (1:12 v/v) to give debromoethylagelastatin A (DeBEAA) (**6**) (18 mg, 57%) as a colorless solid. **Debromoethylagelastatin A (DeBEAA) (6)**: $[\alpha]^{25}_D$ -54.4 (*c* 0.20, MeOH); IR (KBr) ν 3236, 1653 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.02 (dd, 1H, *J* = 2.8, 1.2 Hz), 6.88 (dd,

1H, *J* = 3.6, 1.2 Hz), 6.23 (dd, 1H, *J* = 3.6, 2.8 Hz), 4.65 (ddd, 1H, *J* = 10.0, 6.0, 6.0 Hz), 3.99 (dd, 1H, *J* = 4.8, 1.2 Hz), 3.77 (d, 1H, *J* = 1.2 Hz), 3.36 (m, 1H), 3.20 (m, 1H), 2.59 (dd, 1H, *J* = 13.2, 6.4 Hz), 2.37 (dd, 1H, *J* = 13.2, 10.0 Hz), 1.25 (t, 3H, *J* = 7.2 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 162.00, 161.28, 125.61, 122.94, 115.44, 111.13, 96.37, 68.31 62.96, 55.66, 42.86, 35.20, 15.87; MS *m/z*: 277 $[M+1]^+$, 154 (100%); HRMS (FAB) calcd for $C_{13}H_{17}N_4O_3$ $[M+H]^+$: 277.1301, found: 277.1310.



5 **7**

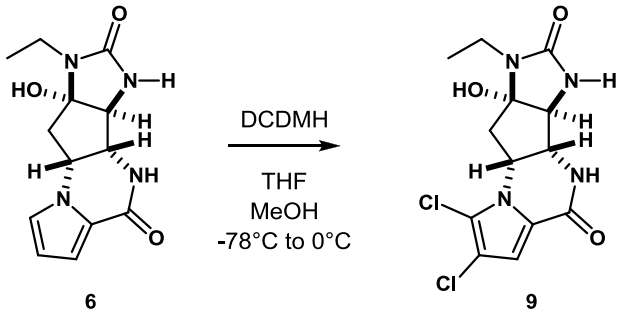
(5a*S*,5b*S*,8a*S*,9a*R*)-1-Chloro-8a-hydroxy-8-methyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Chloroagelasttain A (CAA)] (7): To a stirred solution of debromoagelastatin A (DeBAA) (**5**)[1] (40 mg, 0.153 mmol) in THF-MeOH (4.5 mL; 2:1 v/v) in a dry ice/acetone cooling bath (-78 ºC) were added 2,6-di-*tert*-butylpyridine (DTBP) (51 µL; 0.230 mmol) and dichlorodimethylhydantoin (DCDMH) (30.1 mg in THF-MeOH 500 µL; 2:1 v/v, 0.153 mmol). Following removal of the dry ice/acetone bath, stirring was continued for 45 min at room temperature. The mixture was quenched with Et_3N (213 µL; 1.53 mmol) and 2-methyl-2-butene (162 µL; 1.53 mmol, and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (MeOH/EtOAc/H_2O 1:50:1 v/v/v) to give chloroagelastatin A (CAA) (**7**) (29.2 mg, 67%) as a colorless solid. **Chloroagelasttain A (CAA) (7):** $[\alpha]^{25}_D$ -29.1 (*c* 0.225, MeOH); IR (KBr) ν 3333, 1636 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.85 (d, 1H, *J* = 4.1 Hz), 6.19 (d, 1H, *J* = 4.1 Hz), 4.58 (ddd, 1H, *J* = 12.4, 6.4, 5.5 Hz), 4.04 (d, 1H, *J* = 5.5 Hz), 3.83 (s, 1H), 2.75 (s, 3H), 2.59 (dd, 1H, *J* = 13.3, 6.4 Hz), 2.06 (dd, 1H, *J* = 12.8, 12.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 161.1, 122.4, 120.7, 115.3, 110.0, 95.8, 67.4, 62.3, 53.1, 40.0, 24.2; MS *m/z*: 297 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{12}H_{14}ClN_4O_3$ $[M+H]^+$: 297.0754, found: 297.0762.



6 8 9

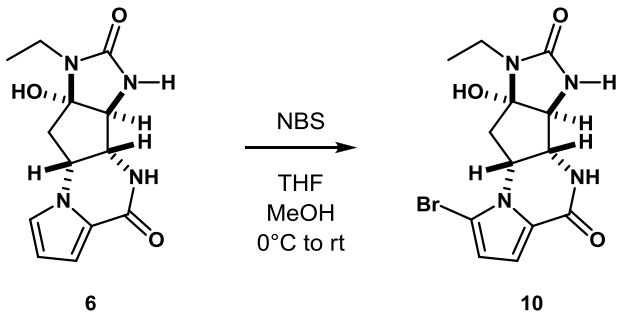
(5a*S*,5b*S*,8a*S*,9a*R*)-1-Chloro-8-ethyl-8a-hydroxy-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Chloroethylagelastatin A (CEAA)] (8) and (5a*S*,5b*S*,8a*S*,9a*R*)-1,2-Dichloro-8-ethyl-8a-hydroxy-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Dichloroethylagelastatin A (DCEAA)] (**9**)**:** To a stirred solution of debromoethylagelastatin A (DeBEAA) (**6**) (36 mg, 0.130 mmol) in THF-MeOH (24.6 mL; 2:1 v/v) in a dry ice/acetone cooling bath (-78 ºC) were added 2,6-di-tert-butylpyridine (DTBP) (44 µL; 0.195 mmol) and dichlorodimethylhydantoin (DCDMH) (25.6 mg in THF-MeOH 500 µL; 2:1 v/v, 0.130 mmol). Following removal of the dry ice/acetone bath, stirring was continued for 45 min at room temperature. The mixture was quenched with Et_3N (181 µL; 1.30 mmol) and 2-methyl-2-butene (138 µL; 1.30 mmol), and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($MeOH/CH_2Cl_2$ 1:15 v/v) to give chloroethylagelastatin A (CEAA) (**8**) (18.3 mg, 45%) as a colorless solid and dichloroethylagelastatin A (DCEAA) (**9**) (6.7 mg, 15%) as a colorless solid. **Chloroethylagelastatin A (CEAA) (8)**: $[\alpha]^{25}_D$ -59.6 (*c* 0.355, MeOH); IR (KBr) ν 3265, 1664 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.89 (d, 1H, *J* = 4.3 Hz), 6.23 (d, 1H, *J* = 4.3 Hz), 4.65 (ddd, 1H, *J* = 12.2, 6.1, 4.9 Hz), 4.07 (d, 1H, *J* = 4.9 Hz), 3.84 (s, 1H), 3.37-3.23 (m, 2H), 2.62 (dd, 1H, *J* = 12.8, 6.1 Hz), 2.18 (dd, 1H, *J* = 12.8, 12.2 Hz), 1.27 (t, 3H, *J* = 7.3 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 161.4, 161.1, 122.5, 120.6, 115.3, 110.0, 96.1, 67.5, 62.4, 53.0, 41.0, 34.9, 15.9; MS *m/z*: 311 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{13}H_{16}ClN_4O_3$ $[M+H]^+$: 311.0911, found: 311.0905. **Dichloroethylagelastatin A (DCEAA)** (**9**): $[\alpha]^{25}_D$ -47.2 (*c* 0.445, MeOH); IR (KBr) ν 3352, 1653 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 6.87 (s, 1H), 4.64 (ddd, 1H, *J* = 12.4, 6.0, 5.6 Hz), 4.10 (d, 1H, *J* =

5.6 Hz), 3.83 (s, 1H), 3.36-3.23 (m, 2H), 2.64 (dd, 1H, *J* = 12.8, 6.0Hz), 2.19 (dd, 1H, *J* = 12.8, 12.4 Hz), 1.27 (t, 3H, *J* = 6.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.4, 160.0, 121.7, 117.7, 113.8, 112.8, 96.0, 67.5, 62.2, 53.6, 40.9, 34.9, 15.9; MS *m/z*: 345 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{13}H_{15}Cl_2N_4O_3$ $[M+H]^+$: 345.0521, found: 345.0527.



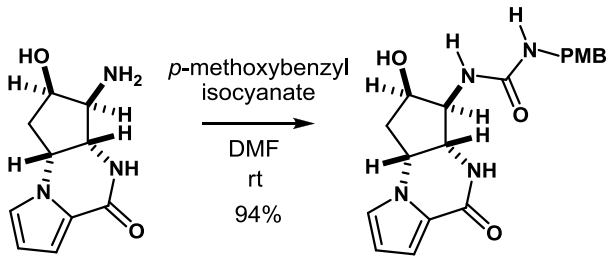
6 9

(5a*S*,5b*S*,8a*S*,9a*R*)-1,2-Dichloro-8-ethyl-8a-hydroxy-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Dichloroethylagelastatin A (DCEAA)] (9): To a stirred solution of debromoethylagelastatin A (DeBEAA) (**6**) (15 mg, 0.0543 mmol) in THF-MeOH (10.5 mL; 2:1 v/v) was added dichlorodimethylhydantoin (DCDMH) (10.7 mg in THF-MeOH 100 µL; 2:1 v/v, 0.0543 mmol) at -78 ºC, and the whole mixture was warmed to 0 ºC. After 40 min of stirring, the mixture was again cooled to -78 ºC, and DCDMH (8.6 mg in THF-MeOH 100 µL; 2:1 v/v, 0.0437 mmol) was added. Stirring was continued for additional 30 min at 0 ºC. Following quenching with Et_3N (76 µL; 0.543 mmol) and 2-methyl-2-butene (58 µL; 0.0.543 mmol), the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography ($MeOH/CH_2Cl_2$ 1:12 v/v) to give dichloroethylagelastatin A (DCEAA) (**9**) (10.5 mg, 56%) as a colorless solid.



6 10

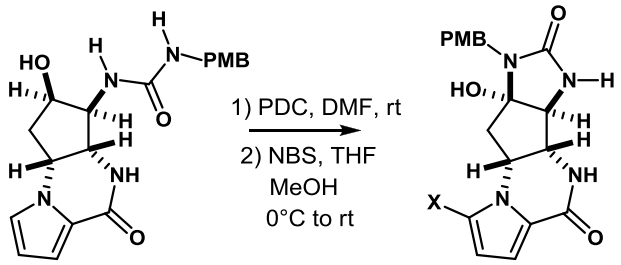
(5a*S*,5b*S*,8a*S*,9a*R*)-1-Bromo-8-ethyl-8a-hydroxy-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Ethylagelastatin A (EAA)] (10): To a solution of debromoethylagelastatin A (DeBEAA) (**6**) (2.5 mg, 0.009 mmol) in THF-MeOH (1.5 mL; 2:1 v/v) was added NBS (1 mg in THF-MeOH 100 µL; 2:1 v/v, 0.005 mmol) at 0 ºC, and the mixture was allowed to warm to room temperature. After being stirred for 50 min, the mixture was again cooled to 0 ^{o}C, and NBS (0.32 mg in THF-MeOH 100 µL; 2:1 v/v, 0.0018 mmol) was added. After stirring for additional 1 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (MeOH/CH_2Cl_2 1:10) to give ethylagelastatin A (EAA) (**10**) (2.1 mg, 65%) as a colorless solid. **Ethylagelastatin A (EAA) (10):** $[\alpha]^{25}_{D}$ -38.2 (*c* 0.055, MeOH); IR (KBr) ν 3381, 3069, 1655 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.90 (d, 1H, *J* = 4.1 Hz), 6.33 (dd, 1H, *J* = 4.1 Hz), 4.63 (ddd, 1H, *J* = 11.9, 6.0, 6.0 Hz), 4.08 (d, 1H, *J* = 5.5 Hz), 3.84 (s, 1H), 3.37-3.24 (m, 2H), 2.63 (dd, 1H, *J* = 12.8, 6.4 Hz), 2.16 (dd, 1H, *J* = 12.8, 10.0 Hz), 1.30 (t, 3H, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.5, 161.0, 124.2, 116.0, 113.8, 107.2, 96.0, 67.5, 62.3, 54.3, 41.1, 34.9, 16.0; MS *m/z*: 355 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{13}H_{16}BrN_4O_3$ $[M+H]^+$: 355.0406, found: 355.0423.



11 12

1-((2*R*,3*S*,3a*S*,9a*R*)-2-Hydroxy-5-oxo-2,3,3a,4,5,9a-hexahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-*a*]pyrazin-3-yl)-3-(4-methoxybenzyl)urea [Urea (12)]: To a solution of β-aminoalcohol **11** (9 mg, 0.0403 mmol) in DMF (1 mL) was added *p*-methoxybenzyl isocyanate (6.3 µL, 0.0443 mmol). After stirring for 80 min at room temperature, the mixture was concentrated under reduced pressure to give a sufficiently pure urea **12** (14.1 mg, 94%) as a colorless solid. **Urea 12:** IR (KBr) ν 3285, 1636 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-*d*6) δ 7.72 (d, 1H, *J* = 3.4 Hz), 7.16 (d, 2H, *J* = 8.6 Hz), 7.04

(dd, 1H, *J* = 2.3, 1.7 Hz), 6.86 (d, 2H, *J* = 8.6 Hz), 6.69 (dd, 1H, *J* = 6.3, 5.7 Hz), 6.61 (dd, 1H, *J* = 4.0, 1.7 Hz), 6.18 (dd, 1H, *J* = 3.4, 2.2 Hz), 5.93 (d, 1H, *J* = 8.0 Hz), 5.32 (d, 1H, *J* = 4.6 Hz), 4.57 (m, 1H), 4.16-4.09 (m, 2H), 3.97 (m, 1H), 3.83-3.73 (m, 2H), 3.71 (s, 3H), 2.42 (ddd, 1H, *J* = 14.9, 6.3, 4.0 Hz), 2.22 (ddd, 1H, *J* = 14.3, 7.4, 2.3 Hz); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 158.04, 157.69, 132.49, 128.47, 123.39, 122.44, 113.63, 111.84, 109.50, 67.37, 58.60, 57.42, 55.05, 51.39, 42.41; MS *m/z*: 371 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{19}H_{23}N_4O_4$ $[M+H]^+$: 371.1719, found: 371.1728.

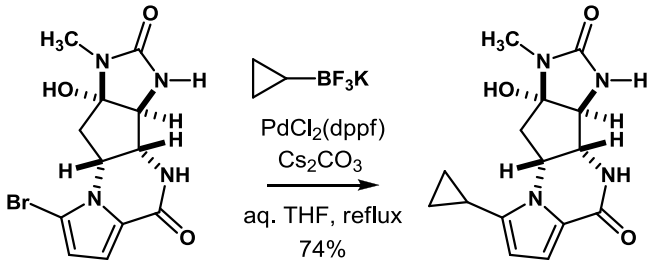


12

13 X = H
14 X = Br

(5a*S*,5b*S*,8a*S*,9a*R*)-8a-Hydroxy-8-(4-methoxybenzyl)-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Hemiaminal (13)]: To a stirred solution of urea **12** (10 mg, 0.027 mmol) in DMF (1 mL) at room temperature was added pyridinium dichromate (PDC) (30.5 mg, 0.081 mmol), and the mixture was stirred at room temperature. After 28 h, *i*-PrOH (6.2 µL) was added, and the mixture was stirred for additional 10 min and concentrated under reduced pressure. The residue was charged onto a column of flash silica gel/florisil (slurry packed) and eluted with MeOH/CH_2Cl_2 (1:15 v/v) to give hemiaminal **13** (3 mg, 30%) as a pale yellow solid. **Hemiaminal 13:** pale yellow solid; $[\alpha]^{25}_D$ -7.0 (*c* 0.165, MeOH); IR (KBr) ν 3457, 1633 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (d, 2H, *J* = 8.7 Hz), 6.93 (d, 2H, *J* = 8.7 Hz), 6.81 (dd, 1H, *J* = 3.8, 1.4 Hz), 6.50 (dd, 1H *J* = 2.3, 1.8 Hz), 6.15 (dd, 1H, *J* = 3.8, 2.3 Hz), 4.64 (d, 1H, *J* = 15.6 Hz), 4.40 (m, 1H), 4.20 (d, 1H, *J* = 15.6 Hz), 3.96 (dd, 1H, *J* = 5.0, 1.4 Hz), 3.81-3.76 (m, 4H), 2.18-2.10 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ161.87, 161.57, 160.67, 132.67, 130.29, 124.94, 122.84, 115.29, 115.01, 111.11, 95.83, 68.08, 62.83, 55.77, 55.38, 42.43, 42.26; MS *m/z*: 369 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{19}H_{21}N_4O_4$ $[M+H]^+$: 369.1563, found: 369.1549.

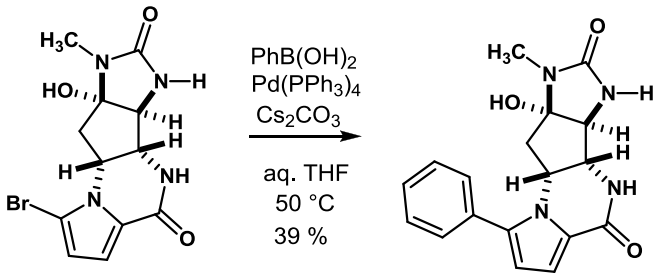
(5a*S*,5b*S*,8a*S*,9a*R*)-1-Bromo-8a-hydroxy-8-(4-methoxybenzyl)-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [*N*-*p*-methoxybenxylagelasttain A (*N*-PMBAA)] (14): To a solution of hemiaminal **13** (1.6 mg, 0.004 mmol) in THF-MeOH (0.75 mL; 2:1 v/v) was added NBS (0.386 mg in THF-MeOH 100 µL; 2:1 v/v, 0.0022 mmol) at 0 ºC, and the mixture was allowed to warm to room temperature. After being stirred for 15 min, the mixture was again cooled to 0 ºC, and NBS (0.386 mg in THF-MeOH 100 µL; 2:1 v/v, 0.0022 mmol) was added. After stirring for additional 45 min at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (MeOH/EtOAC/H_2O 1:80:1 v/v/v) to give *N*-*p*-methoxybenzyl agelastatin A (*N*-PMBAA) (**14**) (1.7 mg, 88%) as a colorless solid. ***N*-*p*-methoxybenxylagelasttain A (*N*-PMBAA) (14):** colorless solid; $[\alpha]^{25}_{D}$ -5.8 (*c* 0. 05, MeOH); IR (KBr) 3340, 1651 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.36 (d, 2H, *J* = 8.0 Hz), 6.90 (d, 2H, *J* = 8.0 Hz), 6.82 (dd, 1H, *J* = 4.0, Hz), 6.18 (d, 1H *J* = 4.0 Hz), 4.66 (d, 1H, *J* = 15.6 Hz), 4.40 (ddd, 1H, *J* = 11.6, 6.0, 5.6 Hz), 4.15 (d, 1H, *J* = 15.6 Hz), 4.05 (d, 1H, *J* = 5.6 Hz), 3.87 (s, 1H), 3.77 (s, 3H), 2.35 (dd, 1H, *J* = 12.8, 5.6 Hz), 1.93 (dd, 1H, *J* = 12.8, 12.4 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ161.84, 160.98, 160.61, 133.00, 130.40, 124.01, 115.88, 115.44, 113.52, 107.08, 95.91, 67.77, 62.08, 55.78, 54.12, 42.14, 40.87; MS *m/z*: 447 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{19}H_{20}BrN_4O_4$ $[M+H]^+$: 447.0668, found: 447.0667.



1 15

(5a*S*,5b*S*,8a*S*,9a*R*)-1-Cyclopropyl-8a-hydroxy-8-methyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Cyclopropylagelastatin (CPAA)] (15): To a stirred solution of agelastatin A (**1**) (14.8 mg, 0.0434 mmol) in THF-H_2O (4 mL; 3:1 v/v) were added potassium cyclopropyl trifluoroborate (7.4 mg, 0.0521 mmol), Cs_2CO_3 (46.7 mg, 0.143 mmol), and $PdCl_2$(dppf)

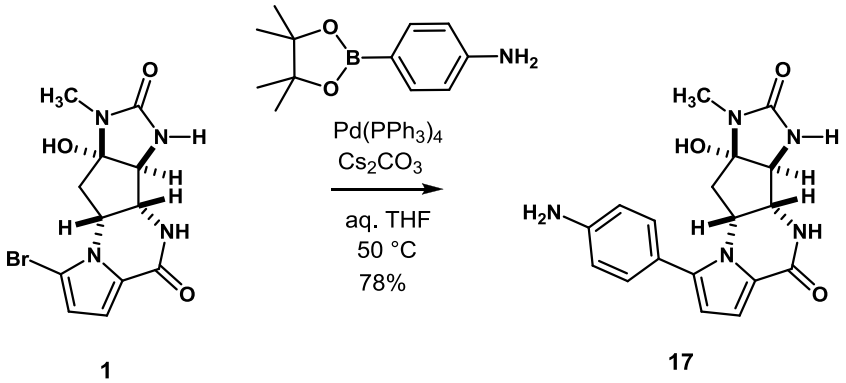
(17.7 mg, 0.0217 mmol). After stirring at 100 °C for 24 h, the mixture was cooled to room temperature and additional amounts of potassium cyclopropyl trifluoroborate (7.4 mg, 0.0521 mmol) and $PdCl_2$(dppf) (17.7 mg, 0.0217 mmol) were added. After stirring for additional 12 h at 100 °C, the whole mixture was cooled to room temperature and transferred to a separatory funnel where it was partitioned between CH_2Cl_2 and H_2O. The aqueous layer was separated and concentrated. The residue was purified by flash silica gel column chromatography (MeOH/CH_2Cl_2 1:7) to give cyclopropylagelastatin A (CPAA) (**15**) (9.7 mg, 74%) as a colorless solid. **Cyclopropylagelastatin (CPAA) (15):** colorless solid; $[\alpha]^{25}_D$ -43.3 (*c* 0.065, MeOH); IR (KBr) ν 3383, 1633 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 6.79 (d, 1H, *J* = 4.0 Hz), 5.86 (d, 1H, *J* = 4.0 Hz), 4.74 (ddd, 1H, *J* = 11.6, 6.4, 6.0 Hz), 4.04 (d, 1H, *J* = 5.2 Hz), 3.86 (s, 1H), 2.80 (s, 3H), 2.69 (dd, 1H, *J* = 13.2, 6.4 Hz), 2.12 (dd, 1H, *J* = 12.8, 12.4 Hz), 1.81 (m, 1H), 0.96-0.92 (m, 2H), 0.70 (m, 1H), 0.60 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.10, 161.51, 141.04, 121.88, 115.23, 107.50, 95.90, 67.53, 62.48, 52.99, 40.34, 24.20, 7.39, 7.19, 6.31; MS *m/z*: 303 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{15}H_{19}N_4O_3$ $[M+H]^+$: 303.1457, found: 303.1482.



1 **16**

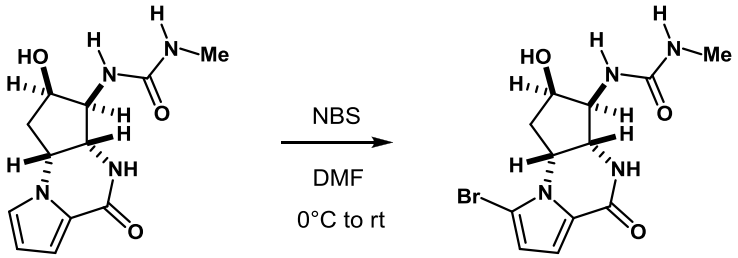
(5a*S*,5b*S*,8a*S*,9a*R*)-8a-Hydroxy-8-methyl-1-phenyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Phenylagelastatin A (PAA)] (16): To a stirred solution of agelastatin A (**1**) (19.2 mg, 0.0563 mmol) in THF-H_2O (2 mL; 1:1 v/v) were added phenylboronic acid (20.6 mg, 0.169 mmol), Cs_2CO_3 (91.7 mg, 0.282 mmol), and $Pd(PPh_3)_4$ (19.5 mg, 0.0169 mmol). After stirring at 50 °C for 40 min, the mixture was cooled to room temperature and transferred to a separatory funnel where it was partitioned between CH_2Cl_2 and H_2O. The

aqueous layer was separated and concentrated. The residue was purified by flash silica gel column chromatography (MeOH/CH_2Cl_2 1:8) to give phenylagelastatin A (PAA) (**16**) (7.4 mg, 39%) as a colorless solid. **Phenylagelastatin A (PAA) (16):** $[\alpha]^{24}_D$ -82.5 (*c* 0.225, MeOH); IR (KBr) ν 3198, 1680 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 7.51-7.38 (m, 5H), 6.99 (d, 1H, *J* = 4.1 Hz), 6.32 (d, 1H, *J* = 4.1 Hz), 4.52 (ddd, 1H, *J* = 11.5, 6.0, 5.5 Hz), 4.05 (d, 1H, *J* = 5.0 Hz), 3.83 (s, 1H), 2.58 (s, 3H), 2.38 (dd, 1H, *J* = 13.2, 6.4 Hz), 2.19 (dd, 1H, *J* = 12.8, 12.4 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 162.13, 161.38, 139.28, 132.76, 129.96, 129.86, 129.68, 123.56, 115.98, 111.76, 95.41, 67.21, 62.43, 53.85, 40.82, 23.97; MS *m/z*: 339 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{18}H_{19}N_4O_3$ $[M+H]^+$: 339.1457, found: 339.1447.



(5a*S*,5b*S*,8a*S*,9a*R*)-1-(4-Aminophenyl)-8a-hydroxy-8-methyl-5,5a,5b,6,8,8a,9,9a-octahydroimidazo[4',5':4,5]cyclopenta[1,2-*e*]pyrrolo[1,2-*a*]pyrazine-4,7-dione [Aminophenylagelastatin A (APAA)] (17): To a stirred solution of agelastatin A (**1**) (8.7 mg, 0.0255 mmol) in THF-H_2O (2 mL; 1:1 v/v) were added *p*-aminophenylboronic acid (6.1 mg, 0.0281 mmol), Cs_2CO_3 (41.5 mg, 0.128 mmol), and $Pd(PPh_3)_4$ (8.8 mg, 0.0077 mmol). After stirring at 50 °C for 1 h, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography (MeOH/CH_2Cl_2 1:20) to give *p*-aminophenylagelastatin A (APAA) (**17**) (7 mg, 78%) as a pale yellow solid. **Aminophenylagelastatin A (APAA) (17):** $[\alpha]^{25}_D$ -45.6 (*c* 0.05, MeOH); IR (KBr) ν 3288, 1647 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.15 (d, 2H, *J* = 8.5 Hz), 6.94 (d, 1H, *J* = 4.5 Hz), 6.76 (d, 2H, *J* = 8.5 Hz), 6.18 (d, 1H, *J* = 4.5 Hz), 4.50 (ddd, 1H, *J* = 11.5, 6.0, 5.5 Hz), 4.01 (d, 1H, *J* = 4.5 Hz), 3.82 (s, 1H), 2.62

(s, 3H), 2.39 (dd, 1H, *J* = 12.5, 6.0 Hz), 2.17 (dd, 1H, *J* = 13.0, 12.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 162.31, 161.42, 149.89, 140.34, 130.74, 122.50, 121.34, 116.08, 116.05, 110.91, 95.45, 67.16, 62.45, 53.67, 40.79, 24.04; MS *m/z*: 354 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{18}H_{20}N_5O_3$ $[M+H]^+$: 354.1566, found: 354.1570.



3 18

1-((2*R*,3*S*,3a*S*,9a*R*)-8-Bromo-2-hydroxy-5-oxo-2,3,3a,4,5,9a-hexahydro-1*H*-cyclopenta[*e*]pyrrolo[1,2-*a*]pyrazin-3-yl)-3-methylurea [Urea agelastatin A (UAA)] (18): To a solution of urea **3** (20 mg, 0.0757 mmol) in DMF (1 mL) at 0 ^{o}C was added NBS (13.5 mg, 0.0757 mmol). After stirring for 40 min at room temperature, the mixture was concentrated under reduced pressure. The residue was rinsed with EtOAc to leave sufficiently pure urea agelastaitin A (UAA) (**18**) (25.4 mg, 97%) as a colorless solid. **Urea agelastatin A (UAA) (18):** $[\alpha]^{25}_{D}$ -26.3 (*c* 0.245, MeOH); colorless solid; IR (KBr) ν 3310, 1618 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 6.82 (d, 1H, *J* = 4.3 Hz), 6.27 (d, 1H, *J* = 4.3 Hz), 4.93 (dt, 1H, *J* = 10.4, 7.3 Hz), 4.34 (ddd, 1H, *J* = 5.5, 4.9, 1.8 Hz), 4.08-4.02 (m, 2H), 2.67 (s, 3H), 2.44 (ddd, 1H, *J* = 13.4, 7.3, 1.8 Hz), 1.91 (ddd, 1H, *J* = 13.4, 10.4, 5.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 161.79, 160.15, 123.82, 115.38, 113.88, 106.93, 69.80, 62.66, 61.11, 53.78, 41.12, 26.88; MS *m/z*: 343 $[M+1]^+$, 93 (100%); HRMS (FAB) calcd for $C_{12}H_{16}BrN_4O_3$ $[M+H]^+$: 343.0406, found: 343.0412.

Cell proliferation assay of AA and AA analogues:

1) *Raji cells and MDA-MB-132 cells* were purchased from American Tissue Culture (Manassas, VA). They were maintained in RPMI-1640 media with L-glutamine and supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin. Raji cells or MDA-MB-132 were seeded in triplicate at 50,000 cells per mL per well of a 24-well tissue culture plate in 0.5 mL of media. The next day, the cells were treated with vehicle

control or a drug (AA or one of its 18 analogues) at 18.75, 37.5, 75, 150, 300, 600, 1200, 2400, 5000, or 10,000 nM. Cells were harvested and counted on the third day in a Coulter Particle Counter (Beckman-Coulter Corp.:Brea, CA). IC50 values were calculated using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA).

2) *Human DU145 prostate cancer cells* were cultured in RPMI 1640 supplemented with heat-inactivated 10% FBS and kanamycin (50 µg/mL) in a humidified atmosphere of 5% CO_2 at 37 ºC. The cell suspension in the culture medium was plated into each well of 96-well plates (10,000 cells/well/100 µL). After 24 h, testing compounds were added, and then the plates were incubated for an additional 24 h in a humidified atmosphere of 5 % CO_2 at 37 ºC. The cell proliferation was detected according to an established MTT method, as previously described.[2] The IC_{50} value was determined by linear interpolation from the growth inhibition curve.

Chemoinformatic analysis:

Methods and materials: All AA analogues were analyzed for chemoinformatic properties and physical descriptors. Chemoinformatic properties for AA analogues were then compared with a pool of commercial drugs (>1712 different drug molecules) and ranked based on chemoinformatic statistics that fall within the range formed from greater than 95% of commercial drugs. The analyses examined a broad range of chemoinformatic descriptors with an emphasis on log BB, but other contributing factors were weighted as well. All initial structures were derived in ChemDraw and optimized using ChemBio 3D and GAMESS for optimization of the structure at both RHF/3-21G level of theory and PM6, R-closed shell, PCM solvent for semi-empirical optimization.[3-5] The root mean square deviation (r.m.s.d.) difference between the all optimized structures was <0.05 Angstroms. The optimized structures were imported into Schrodinger's Maestro (MAE format) module for chemical profiling with QikProp.[6] Additionally, we conducted a similarity comparison against the most common pharmaceutical drugs, screening for top drugs with the most similar physical descriptors to each AA analogue. Such a comparison does not preclude a common activity, but does indicate the likelihood of drug-like ability for these AA analogues. Additional considerations for optimal chemoinformatic

behaviour of CNS penetration were investigated, in part with novel algorithmic approaches, per the literature.[3-5]

Table S1. Chemoinformatic analyses of CAA (**7**) with Schrödinger's QikProp. Resulting statistical data were generated on a QM optimized version of CAA (**7**).

Principal Descriptors:		**(Range 95% of Drugs)**
Solute	molecular weight	296.713 (130.0/725.0)
Solute	dipole moment (D)	3.125 (1.0/12.5)
Solute	total SASA	460.284 (300.0/1000.0)
Solute	hydrophobic SASA	136.270 (0.0/750.0)
Solute	hydrophilic SASA	159.482 (7.0/330.0)
Solute	carbon Pi SASA	95.619 (0.0/ 450.0)
Solute	weakly polar SASA	68.913 (0.0/175.0)
Solute	molecular volume (Å^3)	791.990 (500.0/2000.0)
Solute	vdW polar SA (PSA)	99.131 (7.0/200.0)
Solute	no. of rotatable bonds	1.000 (0.0/15.0)
Solute as donor -hydrogen bonds		3.000 (0.0/6.0)
Solute as acceptor - hydrogen bonds		5.250 (2.0/20.0)
Solute globularity (sphere = 1)		0.899 (0.75/0.95)
Solute ionization potential (eV)		8.906 (7.9/10.5)
Solute electron affinity (eV)		-0.157 (-0.9/1.7)
Predictions for properties:		**(Range 95% of drugs)**
QP polarizability (Å^3)		25.813*M* (13.0/70.0)
QP log P for hexadecane-gas		8.701*M* (4.0/18.0)
QP log P for octanol-gas		16.070*M* (8.0/35.0)
QP log P for water-gas		11.931*M* (4.0/45.0)
QP log P for octanol-water		1.153 (-2.0/6.5)
QP log S for aqueous solubility		-3.098 (-6.5/0.5)
QP log S- conformation-independent		-3.648 (-6.5/0.5)
QP log K hsa serum protein binding		-0.227 (-1.5/1.5)
QP log BB for brain-blood		-0.642 (-3.0/1.2)
No. of primary metabolites		1 (1.0/8.0)
HERG K+ channel blockage: log IC50		-3.616 (<-5 poor)
Apparent Caco-2 permeability (nm/sec)		304 (<25 poor, >500 excellent)
Apparent MDCK permeability (nm/sec)		326 (<25 poor, >500 excellent)
QP log Kp for skin permeability		-4.026 (Kp in cm/hr)
Jm, max transdermal transport rate		0.022 (μg/cm^2 hr)
Lipinski's rule of 5 violations		0 (maximum is 4)
Jorgensen's rule of 3 violations		0 (maximum is 3)

QP Breakdown (< for descriptor over training max)

log Po/w:		**-log S:**	
H-bond donor	-0.900	H-bond donor	-1.205
H-bond acceptor	-2.557	H-bond acceptor	-2.749
Volume	5.167	SASA	8.722
Ac x Dn$^{0.5}$/SASA	0.876	Ac x Dn$^{0.5}$/SASA	1.975
FISA	-1.104	Rotor bonds	-0.163
Non-con amines	0.000	N protonation	0.000
Non-con amides	0.000	Non-con amides	0.000
WPSA and PISA	0.375	WPSA	0.300
Constant	-0.705	Constant	-3.783

Total	1.153	Total	3.098

log BB:

Hydrophilic SASA	-1.314
WPSA	0.169
Rotor bonds	-0.060
N protonation	0.000
FOSA	0.000
Constant	0.564
Total	-0.642

Table S2. Chemoinformatic analyses of CEAA (**8**) with Schrödinger's QikProp. Resulting statistical data were generated on a QM optimized version of CEAA (**8**).

Principal Descriptors:		**(Range 95% of Drugs)**
Solute	molecular weight	310.739 (130.0/725.0)
Solute	dipole moment (D)	3.015 (1.0/12.5)
Solute	total SASA	483.566 (300.0/1000.0)
Solute	hydrophobic SASA	173.830 (0.0/750.0)
Solute	hydrophilic SASA	145.205 (7.0/330.0)
Solute	carbon Pi SASA	95.619 (0.0/ 450.0)
Solute	weakly polar SASA	68.912 (0.0/175.0)
Solute	molecular volume ($Å^3$)	844.677 (500.0/2000.0)
Solute	vdW polar SA (PSA)	95.795 (7.0/200.0)
Solute	no. of rotatable bonds	2.000 (0.0/15.0)
Solute as donor -hydrogen bonds		3.000 (0.0/6.0)
Solute as acceptor - hydrogen bonds		5.250 (2.0/20.0)
Solute globularity (sphere = 1)		0.894 (0.75/0.95)
Solute ionization potential (eV)		8.902 (7.9/10.5)
Solute electron affinity (eV)		-0.163 (-0.9/1.7)
Predictions for properties:		**(Range 95% of drugs)**
QP polarizability ($Å^3$)		27.279*M* (13.0/70.0)
QP log P for hexadecane-gas		9.077*M* (4.0/18.0)
QP log P for octanol-gas		16.455*M* (8.0/35.0)
QP log P for water-gas		11.669*M* (4.0/45.0)
QP log P for octanol-water		1.553 (-2.0/6.5)
QP log S for aqueous solubility		-3.282 (-6.5/0.5)
QP log S- conformation-independent		-3.912 (-6.5/0.5)
QP log K hsa serum protein binding		-0.158 (-1.5/1.5)
QP log BB for brain-blood		-0.600 (-3.0/1.2)
No. of primary metabolites		1 (1.0/8.0)
HERG K+ channel blockage: log IC50		-3.709 (<-5 poor)
Apparent Caco-2 permeability (nm/sec)		415 (<25 poor, >500 excellent)
Apparent MDCK permeability (nm/sec)		457 (<25 poor, >500 excellent)
QP log Kp for skin permeability		-3.667 (Kp in cm/hr)
Jm, max transdermal transport rate		0.035 (μg/cm^2 hr)
Lipinski's rule of 5 violations		0 (maximum is 4)
Jorgensen's rule of 3 violations		0 (maximum is 3)

QP Breakdown (< for descriptor over training max)

log Po/w:		**-log S:**	
H-bond donor	-0.900	H-bond donor	-1.205
H-bond acceptor	-2.557	H-bond acceptor	-2.749
Volume	5.511	SASA	9.164
Ac x $Dn^{0.5}$/SASA	0.834	Ac x $Dn^{0.5}$/SASA	1.880

FISA	-1.005	Rotor bonds	-0.326
Non-con amines	0.000	N protonation	0.000
Non-con amides	0.000	Non-con amides	0.000
WPSA and PISA	0.375	WPSA	0.300
Constant	-0.705	Constant	-3.783
Total	1.553	Total	3.282

log BB:

Hydrophilic SASA	-1.212
WPSA	0.169
Rotor bonds	-0.121
N protonation	0.000
FOSA	0.000
Constant	0.564
Total	-0.600

Table S3. Chemoinformatic analyses of DCEAA (**9**) with Schrödinger's QikProp Resulting statistics were generated on a QM optimized version of DCEAA (**9**).

Principal Descriptors:		**(Range 95% of Drugs)**
Solute	molecular weight	345.185 (130.0/725.0)
Solute	dipole moment (D)	3.056 (1.0/12.5)
Solute	total SASA	505.874 (300.0/1000.0)
Solute	hydrophobic SASA	165.316 (0.0/750.0)
Solute	hydrophilic SASA	151.919 (7.0/330.0)
Solute	carbon Pi SASA	52.495 (0.0/ 450.0)
Solute	weakly polar SASA	136.144 (0.0/175.0)
Solute	molecular volume ($Å^3$)	888.714 (500.0/2000.0)
Solute	vdW polar SA (PSA)	96.648 (7.0/200.0)
Solute	no. of rotatable bonds	2.000 (0.0/15.0)
Solute as donor -hydrogen bonds		3.000 (0.0/6.0)
Solute as acceptor - hydrogen bonds		5.250 (2.0/20.0)
Solute globularity (sphere = 1)		0.884 (0.75/0.95)
Solute ionization potential (eV)		8.860 (7.9/10.5)
Solute electron affinity (eV)		-0.003 (-0.9/1.7)
Predictions for properties:		**(Range 95% of drugs)**
QP polarizability ($Å^3$)		28.629*M* (13.0/70.0)
QP log P for hexadecane-gas		9.660 *M* (4.0/18.0)
QP log P for octanol-gas		17.206 *M* (8.0/35.0)
QP log P for water-gas		11.499 *M* (4.0/45.0)
QP log P for octanol-water		1.963 (-2.0/6.5)
QP log S for aqueous solubility		-3.914 (-6.5/0.5)
QP log S- conformation-independent		-4.572 (-6.5/0.5)
QP log K hsa serum protein binding		-0.066 (-1.5/1.5)
QP log BB for brain-blood		-0.520 (-3.0/1.2)
No. of primary metabolites		1 (1.0/8.0)
HERG K+ channel blockage: log IC50		-3.664 (<-5 poor)
Apparent Caco-2 permeability (nm/sec)		359 (<25 poor, >500 excellent)
Apparent MDCK permeability (nm/sec)		910 (<25 poor, >500 excellent)
QP log Kp for skin permeability		-3.942 (Kp in cm/hr)
Jm, max transdermal transport rate		0.00 (μg/cm^2 hr)
Lipinski's rule of 5 violations		0 (maximum is 4)
Jorgensen's rule of 3 violations		0 (maximum is 3)

QP Breakdown (< for descriptor over training max)

log Po/w:		**-log S:**	
H-bond donor	-0.900	H-bond donor	-1.205
H-bond acceptor	-2.557	H-bond acceptor	-2.749
Volume	5.798	SASA	9.586
Ac x $Dn^{0.5}$/SASA	0.797	Ac x $Dn^{0.5}$/SASA	1.797
FISA	-1.051	Rotor bonds	-0.362
Non-con amines	0.000	N protonation	0.000
Non-con amides	0.000	Non-con amides	0.000
WPSA and PISA	0.581	WPSA	0.593
Constant	-0.705	Constant	-3.783
Total	1.963	Total	3.914

log BB:	
Hydrophilic SASA	-1.297
WPSA	0.334
Rotor bonds	-0.121
N protonation	0.000
FOSA	0.000
Constant	0.564
Total	-0.520

Methods on CNS pharmacokinetic (PK) analysis of Agelastatin A and its analogues (CAA, CEAA, and DCEAA):

Animals and housing: Male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) were used in this CNS pharmacokinetic study on Agelastatin A and its three analogues (CAA, CEAA, and DCEAA). Six rats were used for each drug. Animal use was approved by Mayo Foundation Institutional Animal Use and Care Committee (IACUC) and was consistent with the NIH guidelines for the care and use of laboratory animals. The rats were approximately 2 months old and weighed 300 ± 25 g at the beginning of the study. All rats were housed in a temperature-controlled room (23±2 °C) with a 12:12 light dark cycle (lights off at 6:00pm). Purina 5001 Rodent Chow and tap water were available ad libitum at all times.

Microdialysis surgery and procedure:

Rats were anesthetized with gasiform isoflurane (1% isoflurane in a mixture of 20% oxygen and 80% nitrogen gas) and immobilized in a stereotaxic frame (KOPF Instruments, Tujunga, CA). Anesthesia was maintained during the entire procedure. The guide cannula (CMA Microdialysis Inc., Acton, MA) was stereotactically implanted into the lateral ventricle (AP -0.9, L 1.6, V 3.4, relative to bregma and skull), and then secured

to the skull by screws and dental cement. Following surgery, each rat was housed individually with food and water ad libitum for 3 days for recovery from cannulation surgery. Microdialysis experiments were carried out on conscious, freely moving rats. On the day of the experiment, the stylet in the guide cannula was replaced with the microdialysis probe (CMA/11 with 4 mm membrane, CMA Microdialysis Inc., Acton, MA) and a vascular microdialysis probe (CMA/20 with 4 mm membrane, CMA Microdialysis Inc, Acton, MA)) was implanted into a jugular vein. The probes had inlet tubes connected to syringes to deliver artificial cerebrospinal fluid (146 mM NaCl, 1.2 mM $CaCl_2$, 3 mM KCl, 1 mM $MgCl_2$, 1.9 mM Na_2HPO_4, 0.1 mM NaH_2PO_4, pH 7.4) into the ventricle and Dulbecco's phosphate-buffered saline (D-PBS) into the blood at 0.5 µl/min flow rate. The outlet tubes were connected to a microfraction collector and the dialysates were collected at 4°C. Rats were allowed to recover for at least 24 hours prior to the administration of drugs. Single 2.5 mg/kg dose administered intravenously was used for all the drugs. Three baseline fractions were collected before the drug injection and then 22 samples were collected over 18 hours after the injection. All samples were applied to the capillary electrophoresis with UV detection (CE-UV) for the determination of concentration of the drug in CSF and blood. The rats were sacrificed using CO_2 inhalation after the experiment. The position of the probe was verified by visual inspection at the end of each experiment.

Determination of Agelastatin A and its three analogues with the use of CE-UV:

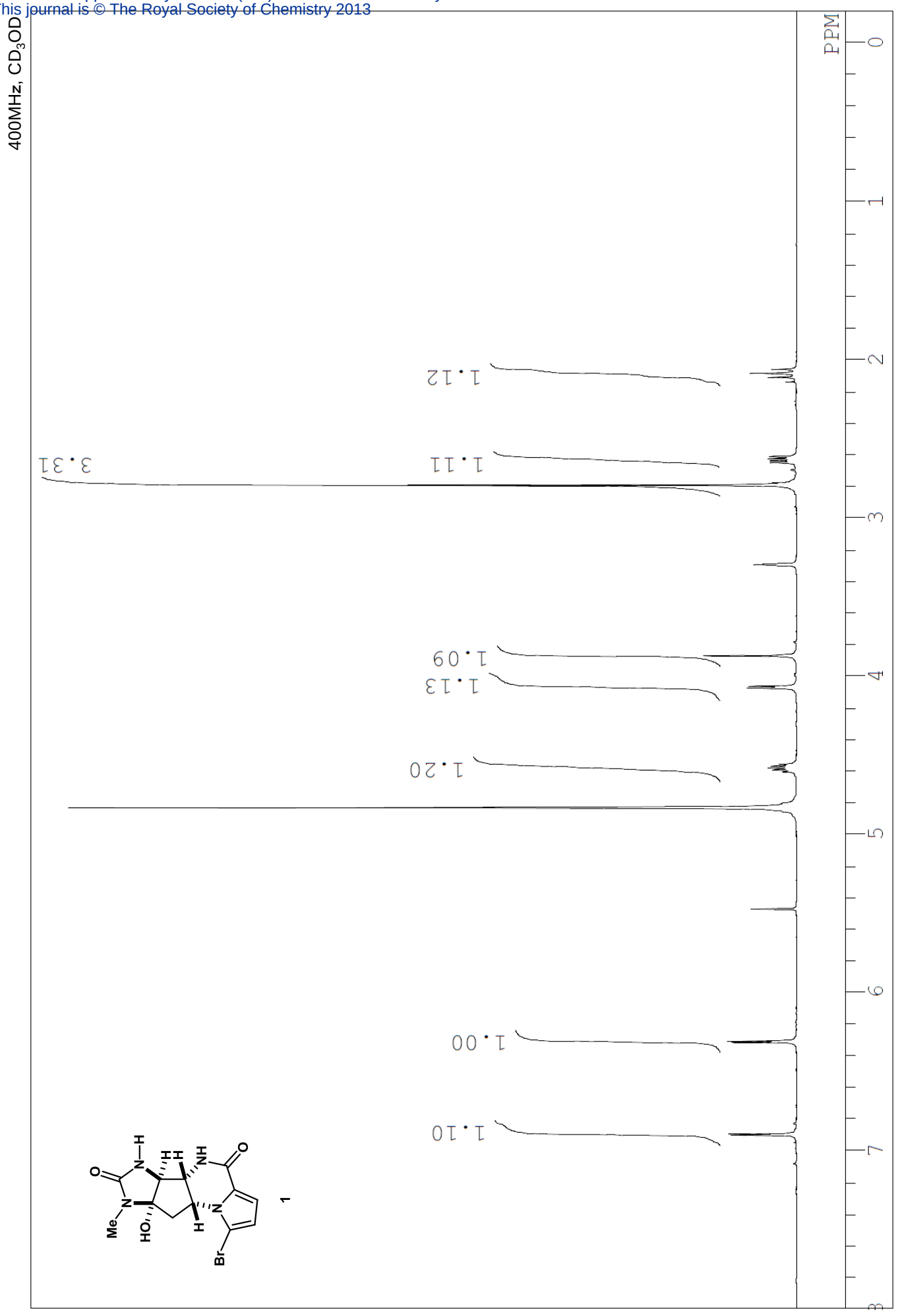
The drug concentration in the microdialysate was measured by CE-UV (Agilent 3D CE). Briefly, the capillaries were preconditioned with 1 M sodium hydroxide for 2 min, water for 2 min and running buffer [100 mmol/L solution of ammonium acetate (adjusted to pH 3.1 with acetic acid)-acetonitrile (50:50, v/v)] for 3 min. The samples were injected at a pressure of 0.7 psi for 5 s and the injection volume was approximately 5 nl. After injection, the drug was separated in a fused silica capillary of 50 µm I.D. and 50/65 cm length (effective length/total length) under 15 kv and 25°C. The absorbance from the drug was detected with UV at 280 nm. The emission was collected on a photomultiplier tube (PMT). The detection limit of AA, CAA, CEAA and DCEAA are 3.8 nM, 1.9 nM, 1.2 nM and 2.9 nM, respectively.

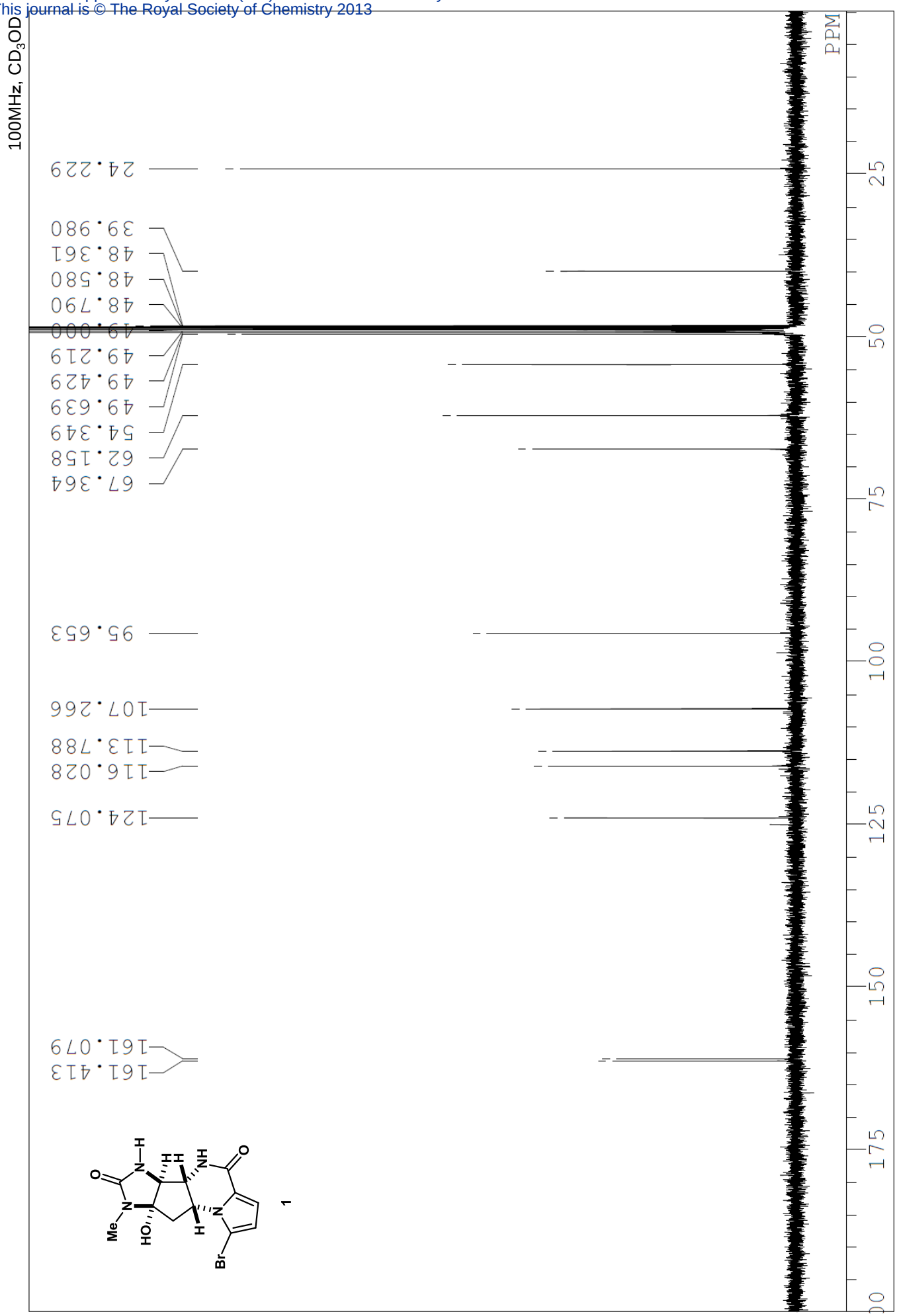
Statistical analysis:

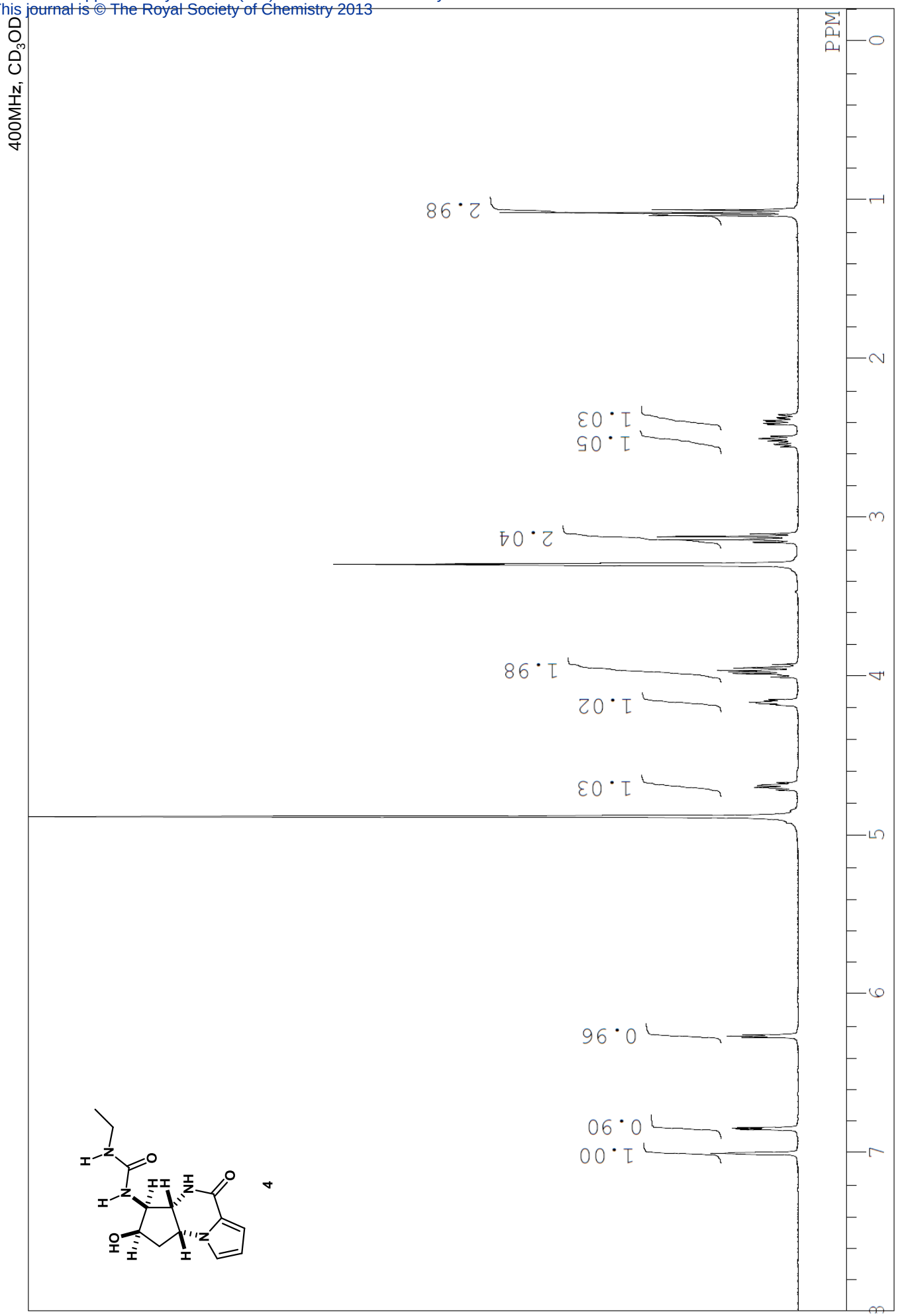
Two-way repeated measures ANOVA followed by Tukey's test was used. $P<0.05$ was considered significant. CNS penetration is determined as the ratio of CSF and blood area under the curve (AUC).

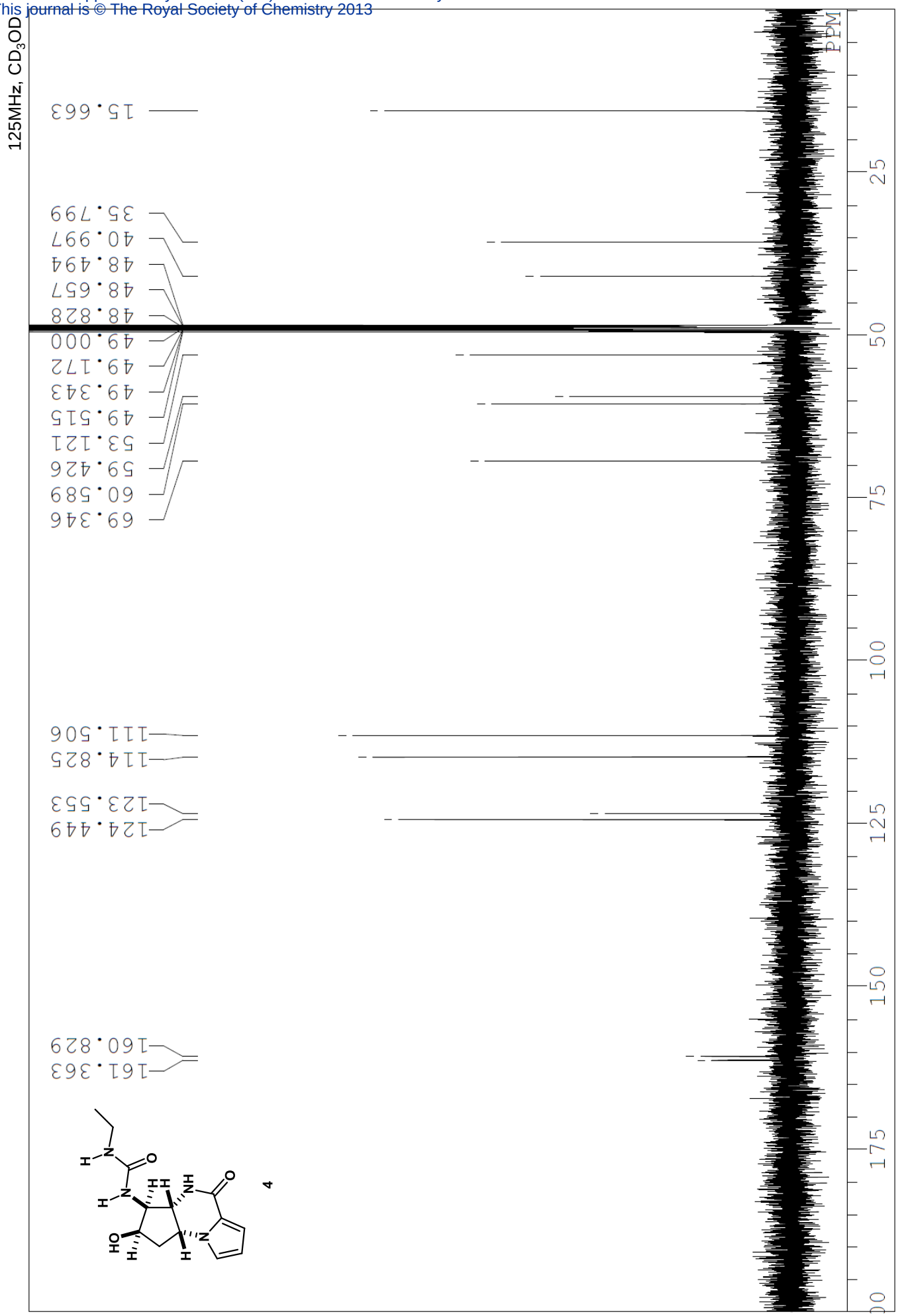
References

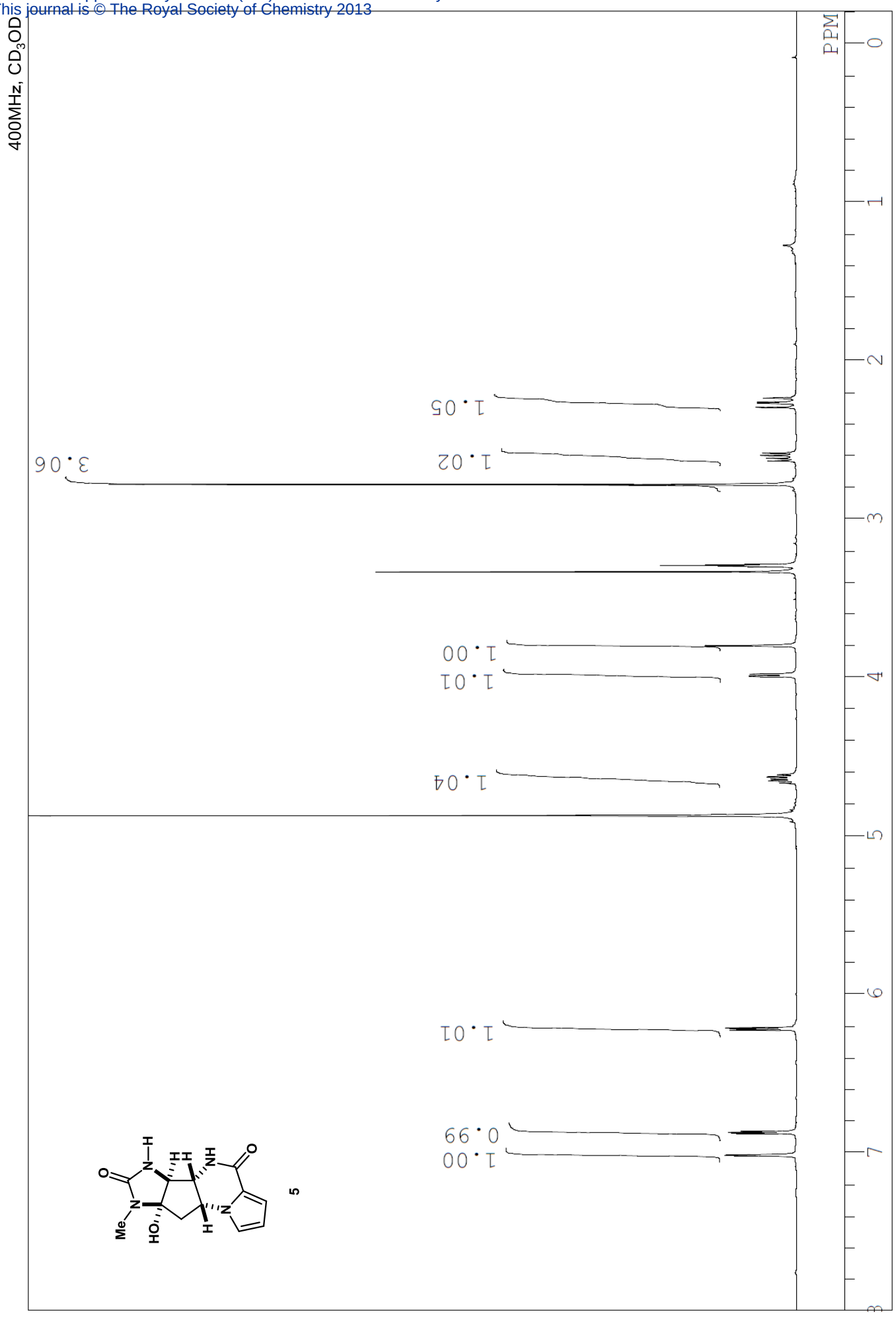
1. (a) T. Yoshimitsu, T. Ino, T. Tanaka, *Org. Lett.* 2008, **10**, 5457. (b) T. Yoshimitsu, T, Ino, N. Futamura, T. Kamon, T. Tanaka, *Org. Lett.* 2009, **11**, 3402.
2. T. Mosmann, *J. Immunol. Methods* 1983, **65**, 55.
3. T. Caulfield, B. Devkota, *Proteins*, 2012, 2489.
4. Z. Li, T. Kamon, D. A. Personett, T. Caulfield, J. A. Copland, T. Yoshimitsu, H. W. Tun, *Med. Chem. Commun.* 2012, **3**, 233.
5. Z. Li, T. Caulfield, Y. Qiu, J. A. Copland, H. W. Tun, *Med. Chem. Commun.* 2012, **3**, 1526.
6. Suite 2012: Qikprop, version 3.5, 2011, Schrödinger, LLC, New York, NY, 2012.

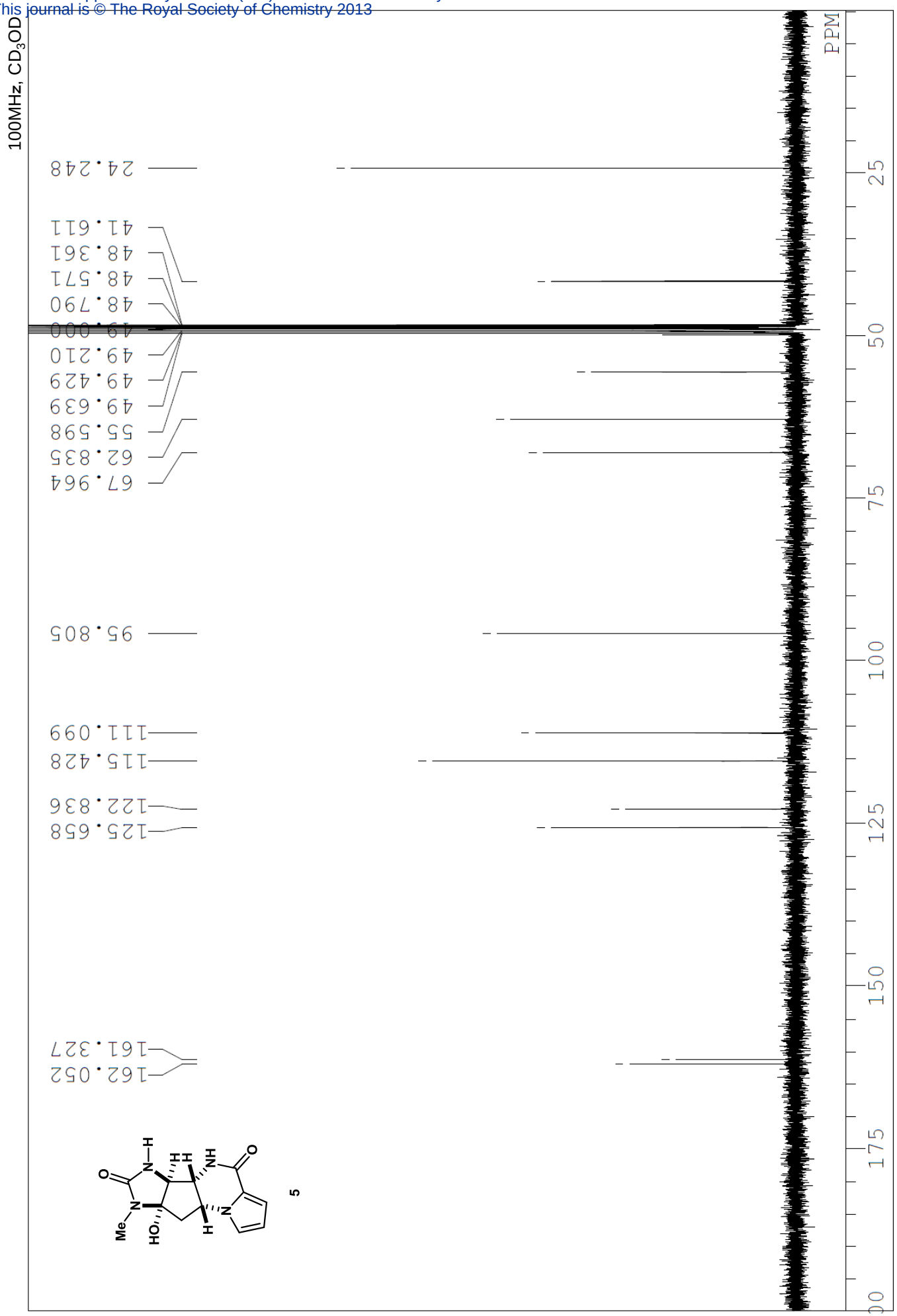


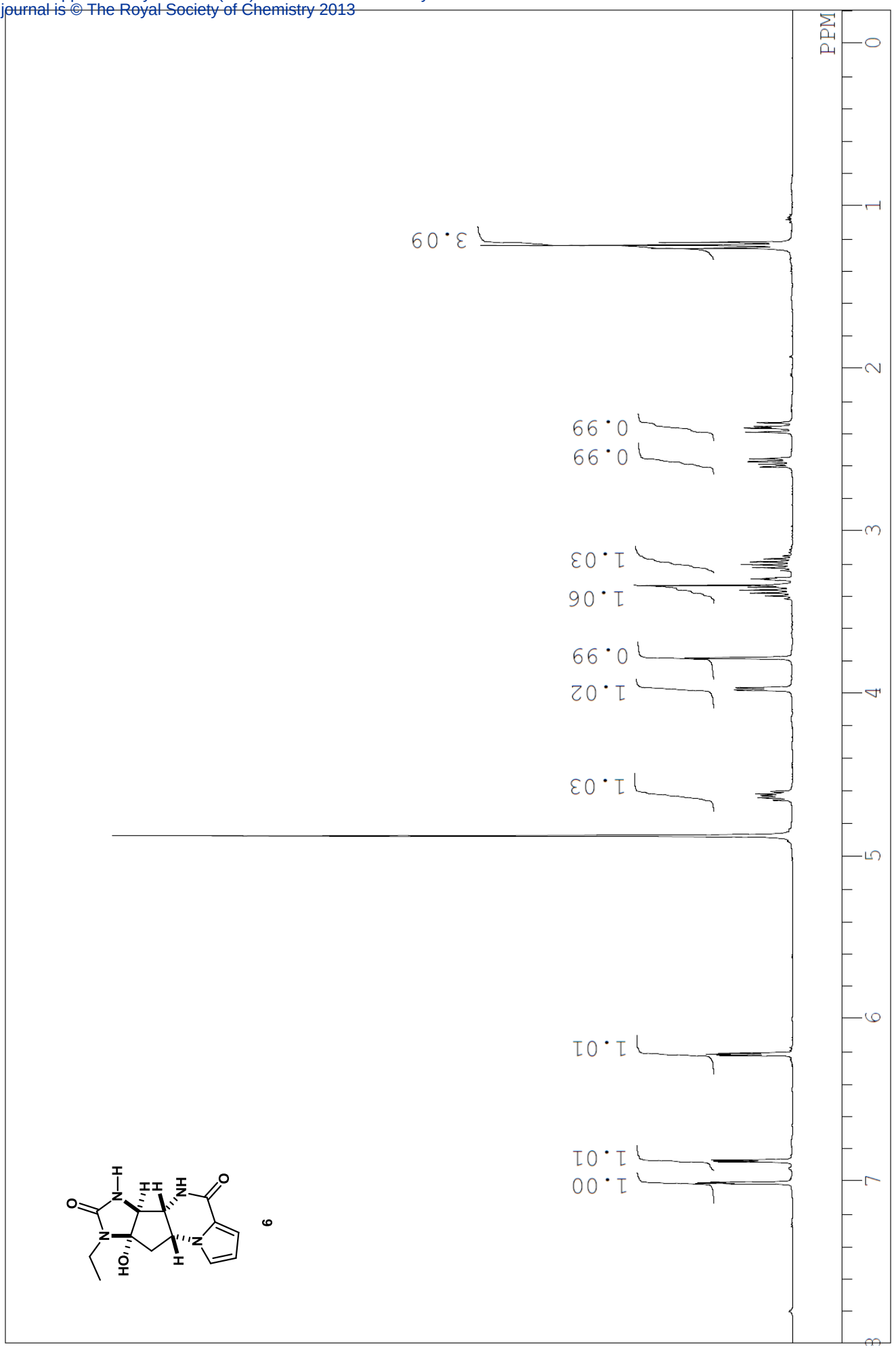










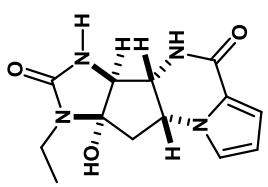


9

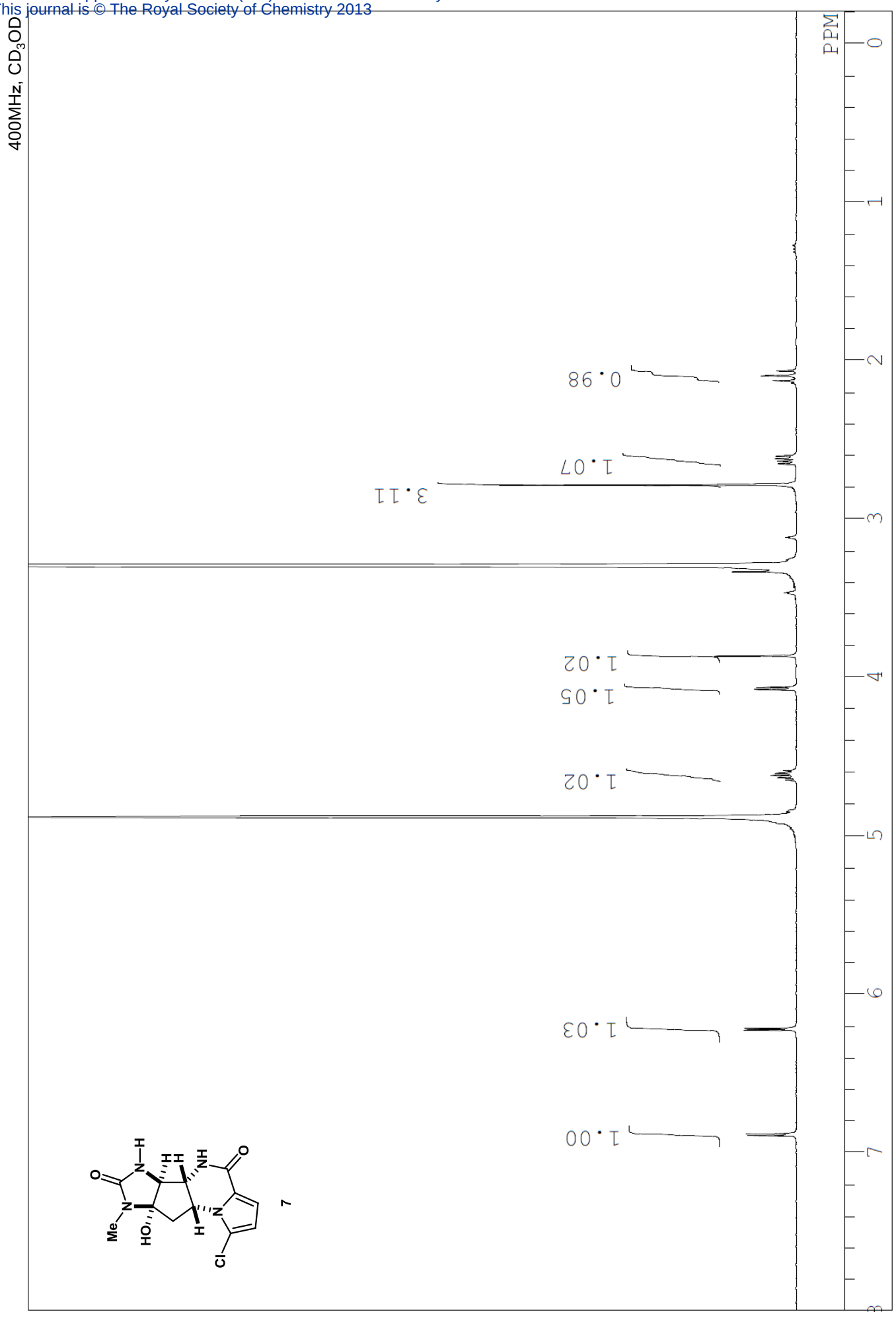
125MHz, CD_3OD

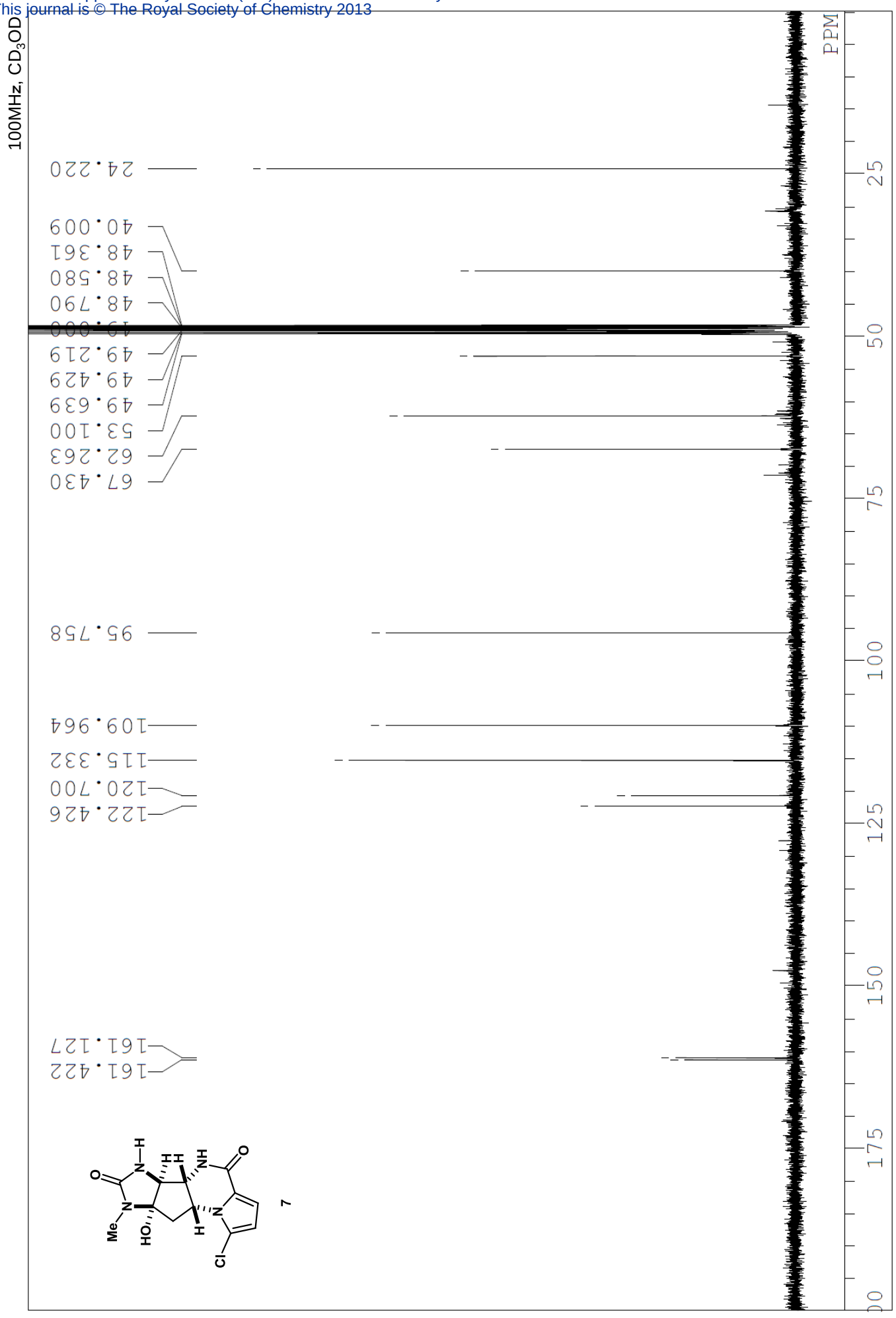
15.867
35.197
42.855
48.490
48.655
48.827
49.000
49.165
49.337
49.510
55.663
62.959
68.314
96.371
111.128
115.438
122.940
125.605
162.003
161.279

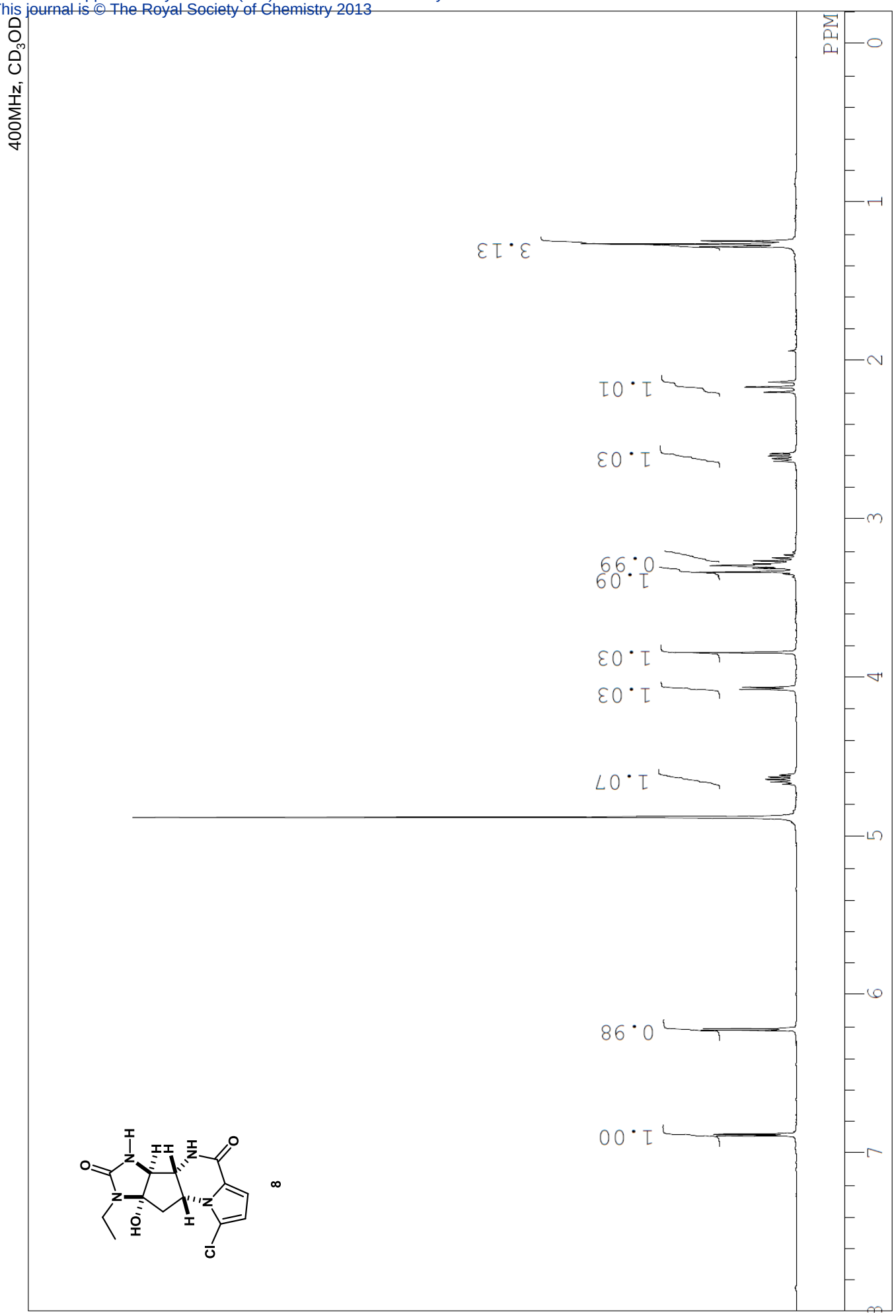
PPM 0 25 50 75 100 125 150 175 200



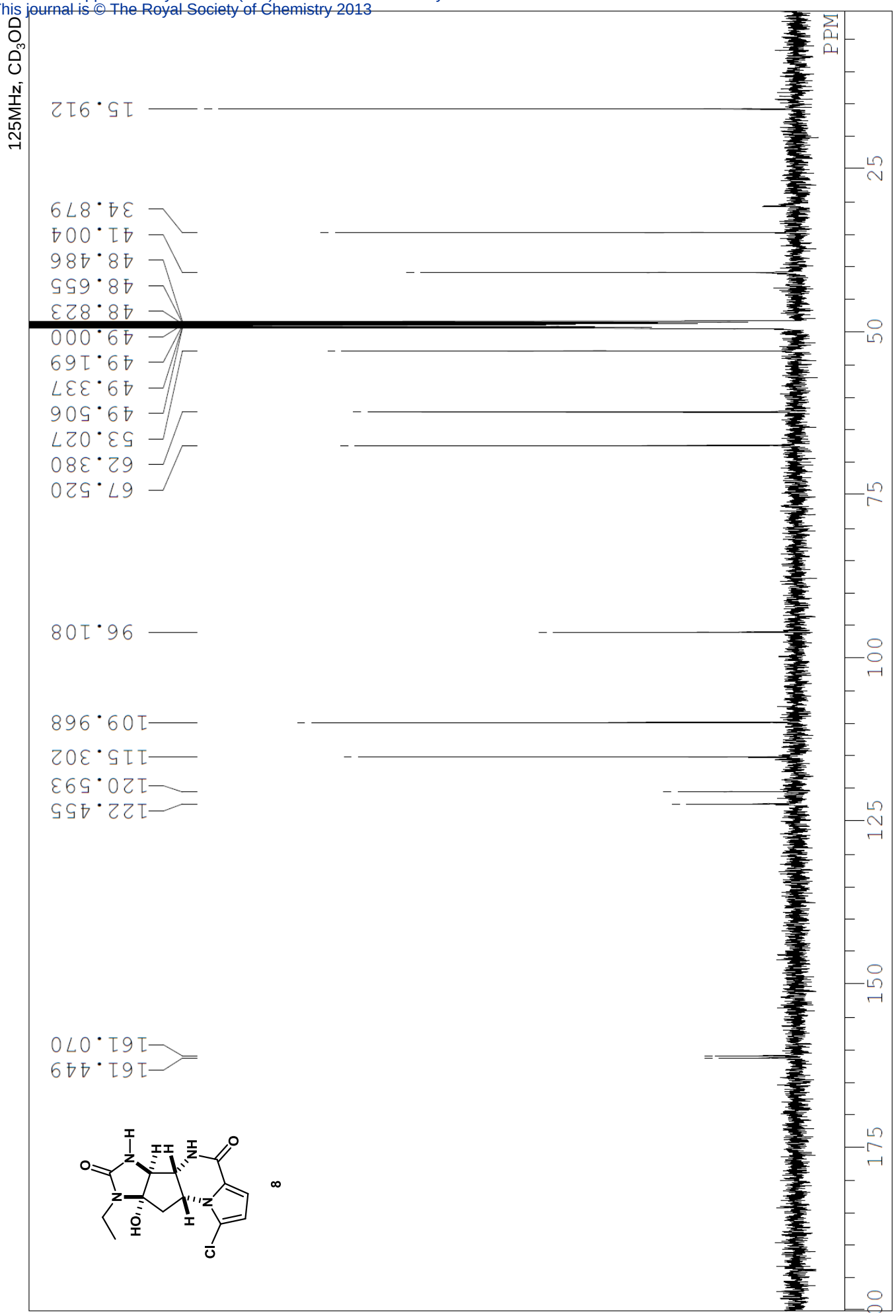
6

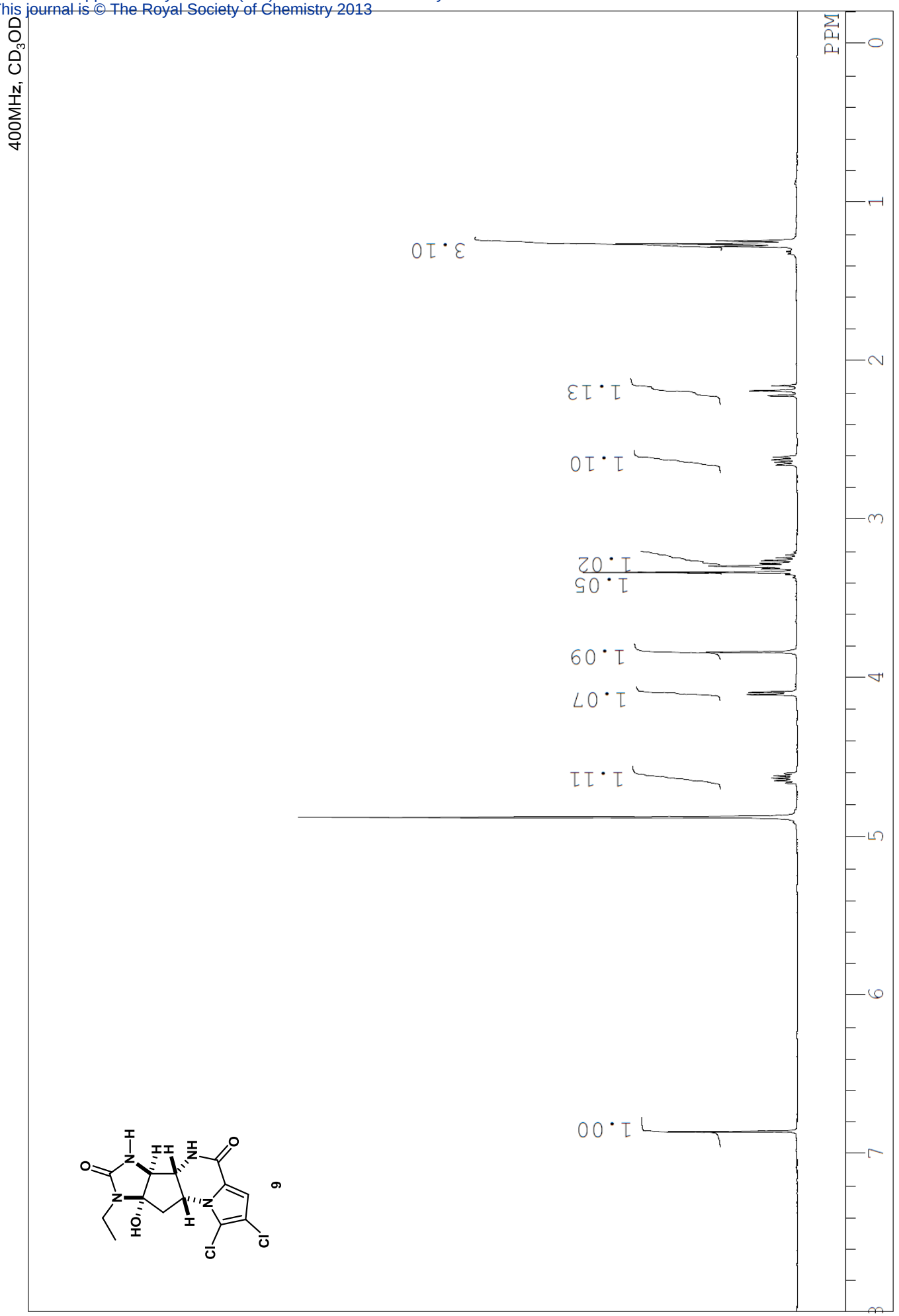


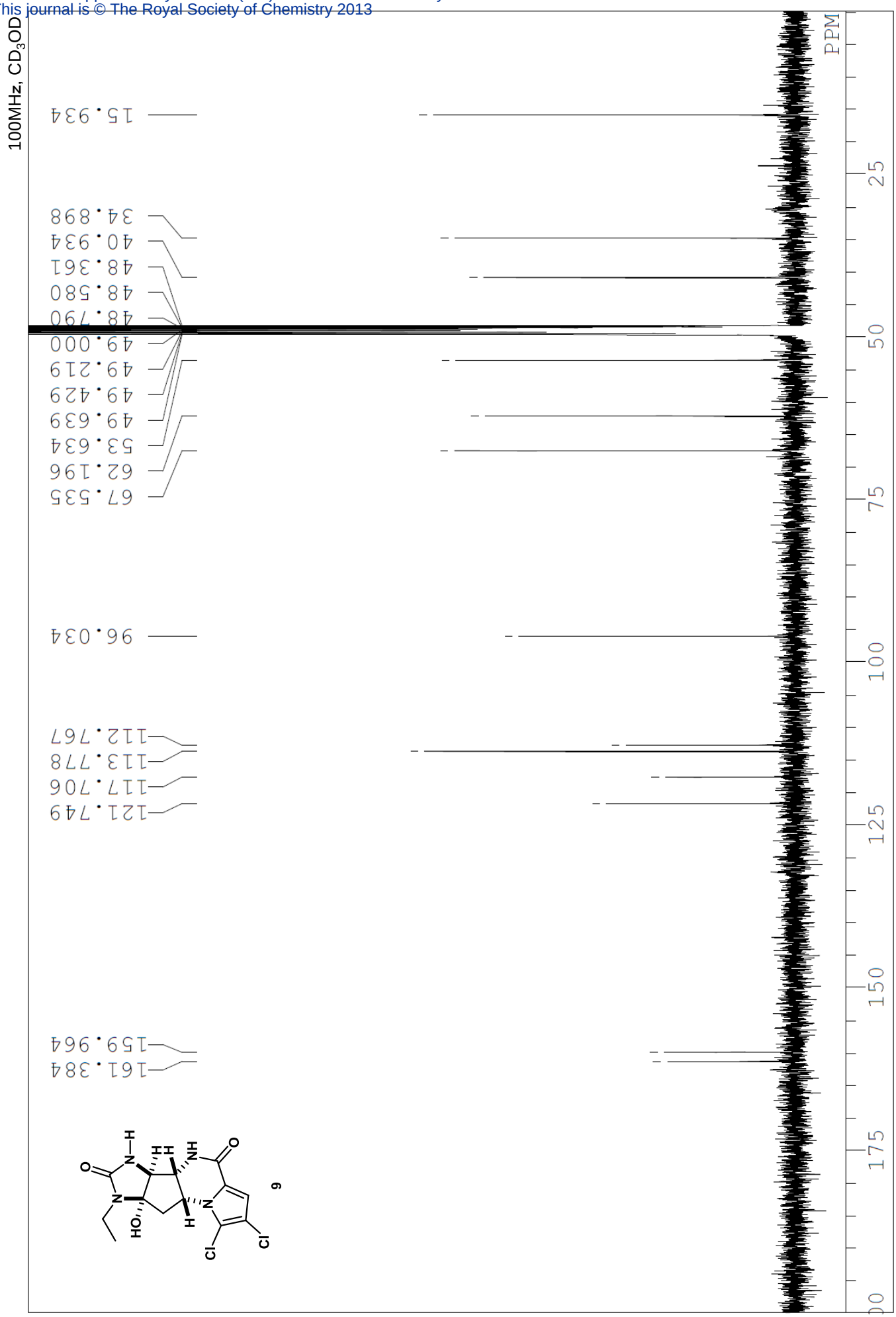


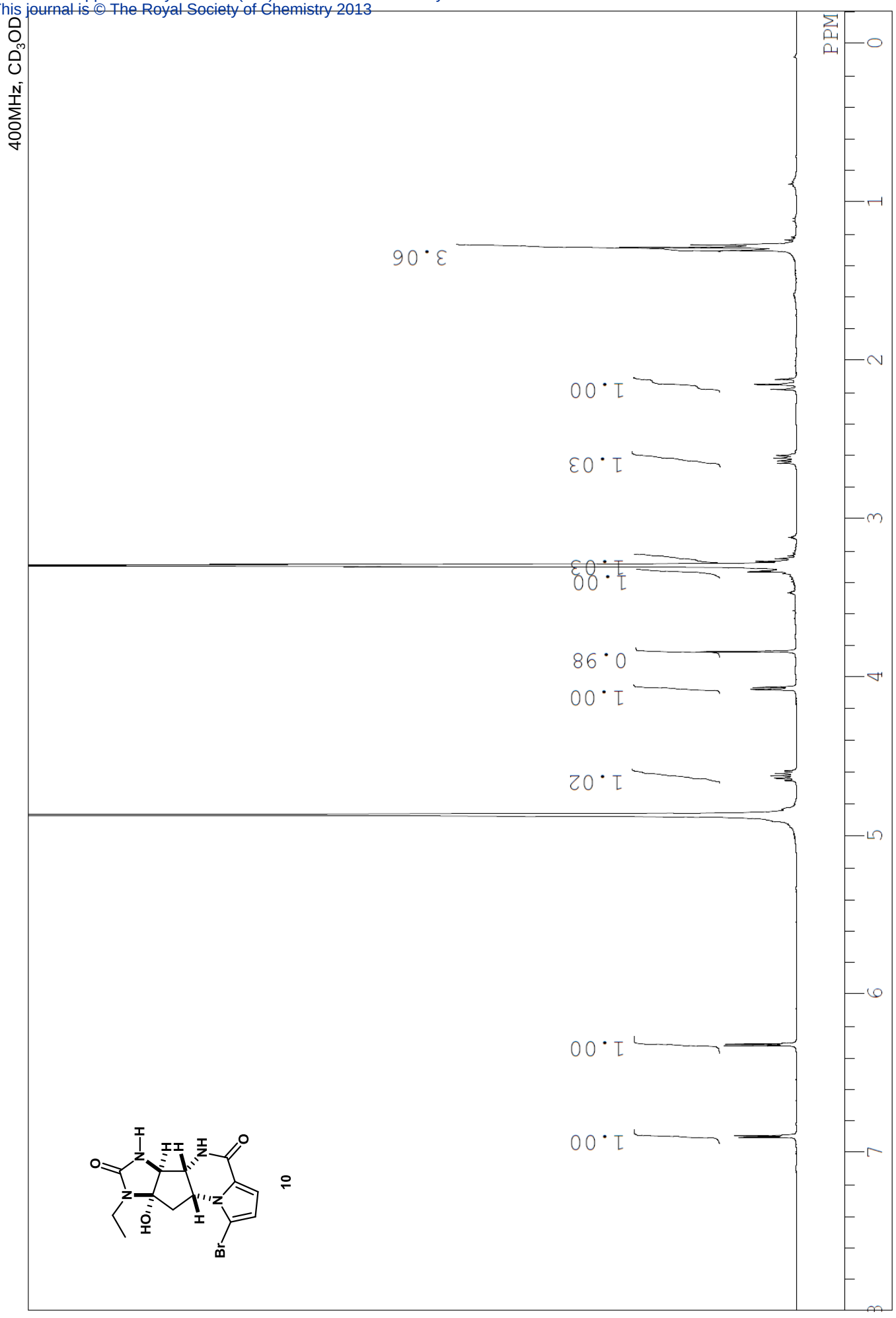


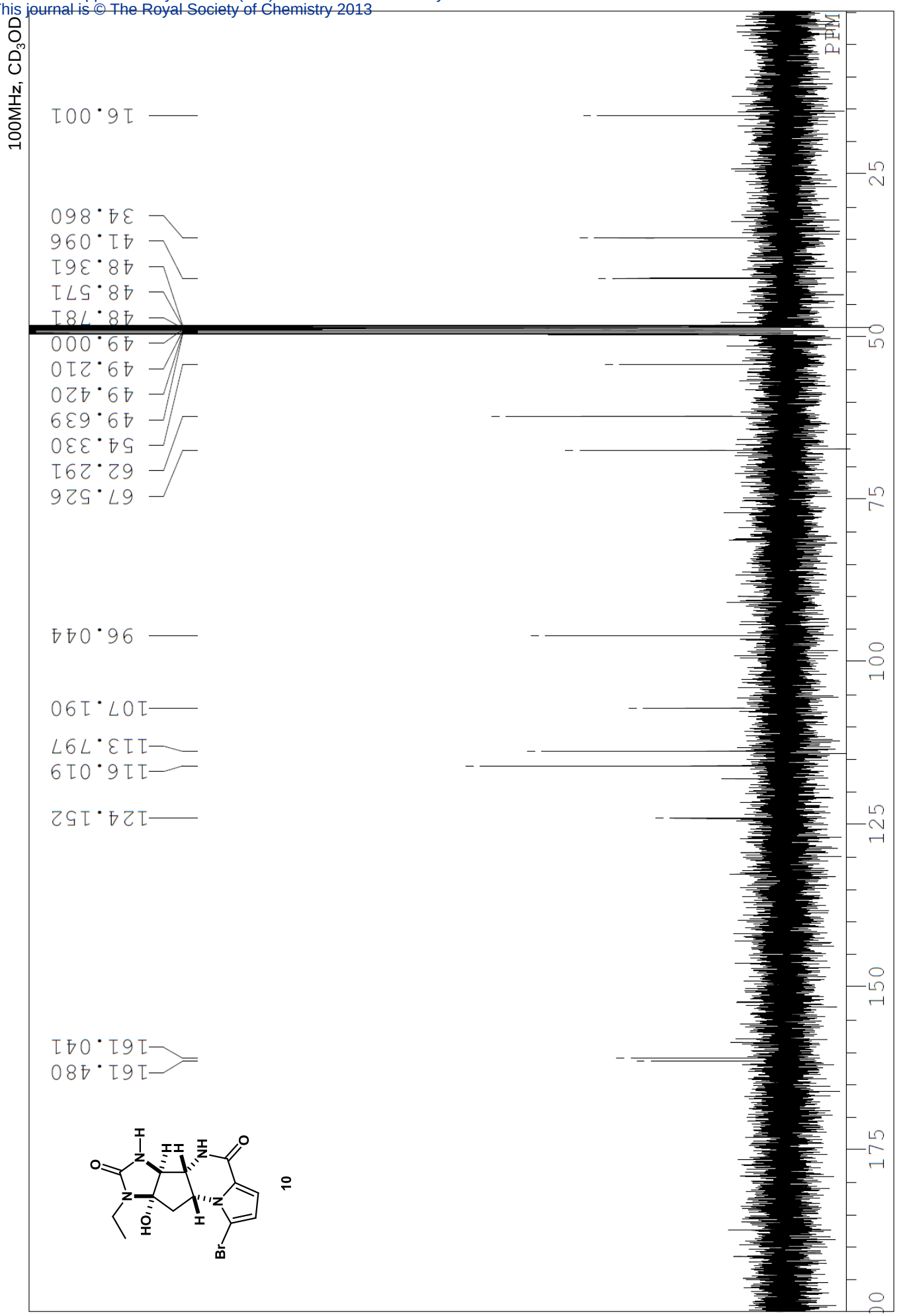
8

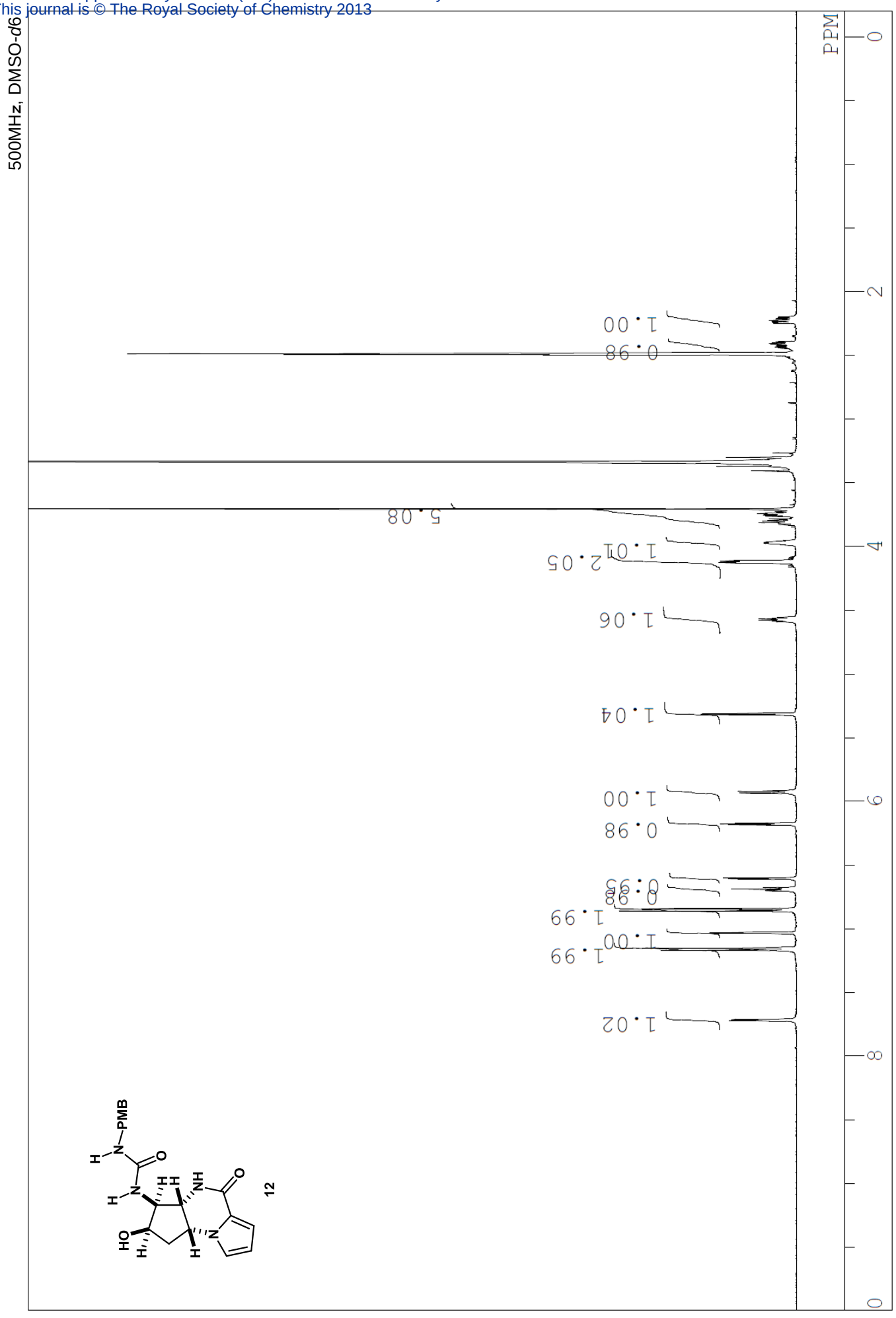


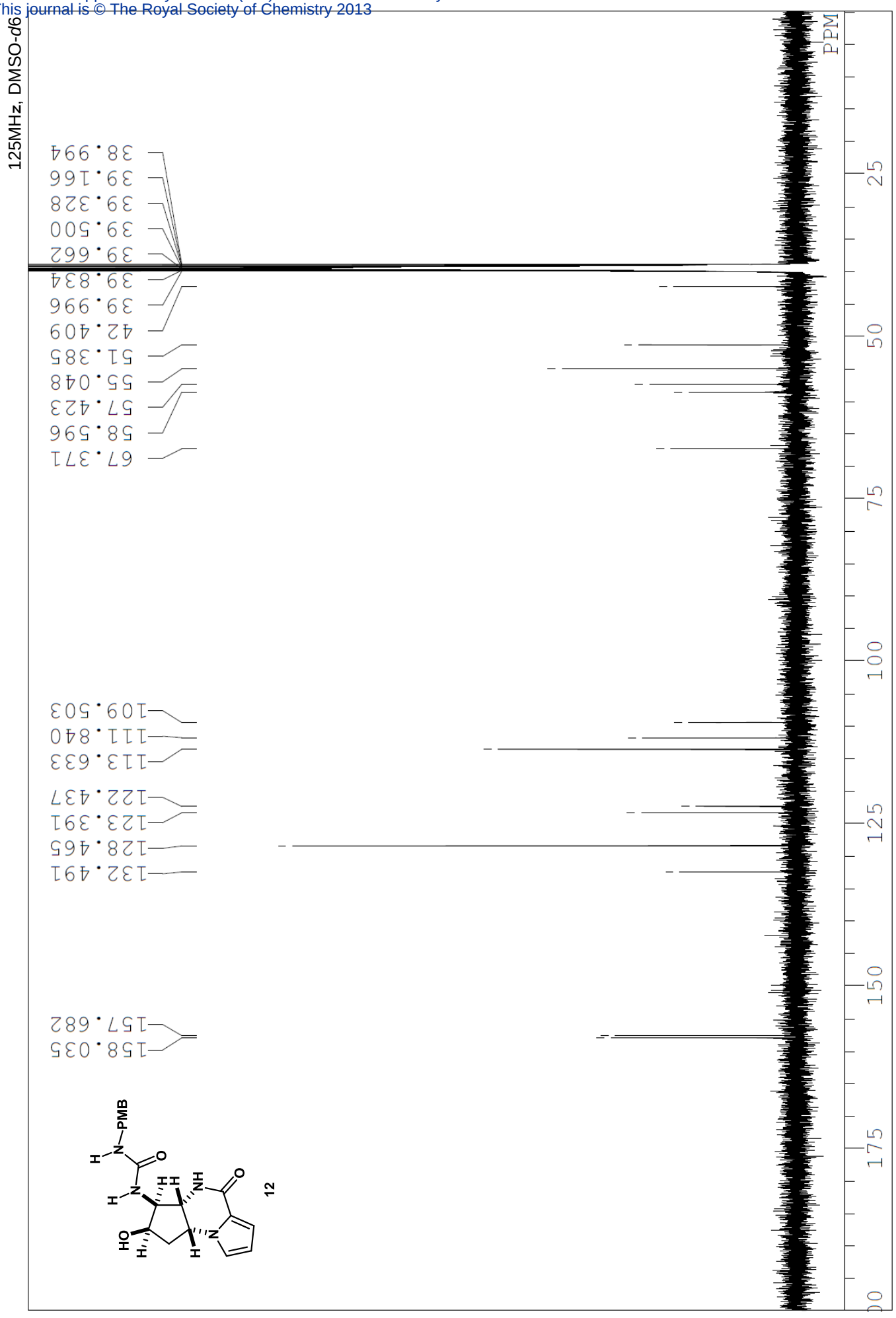


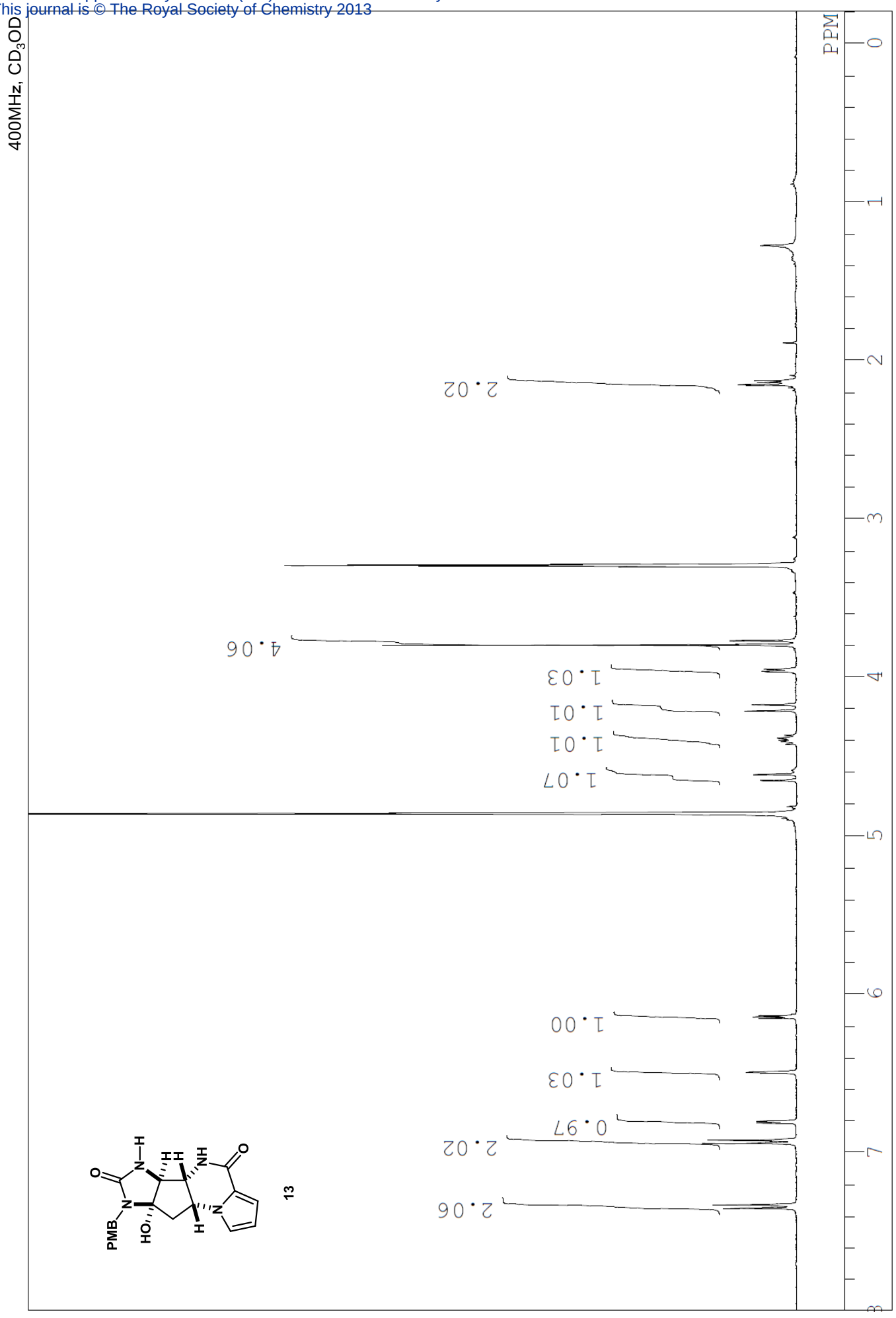


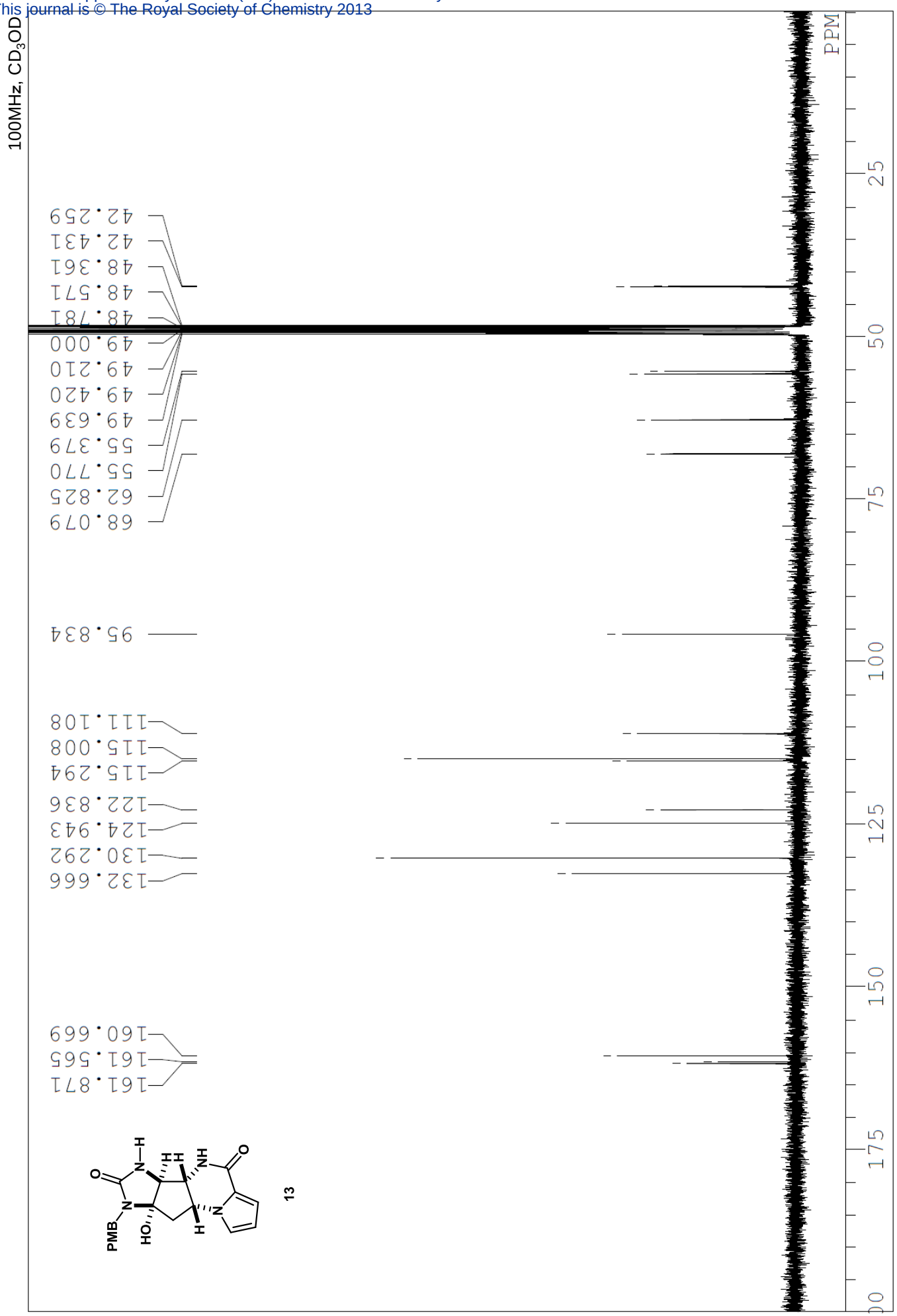


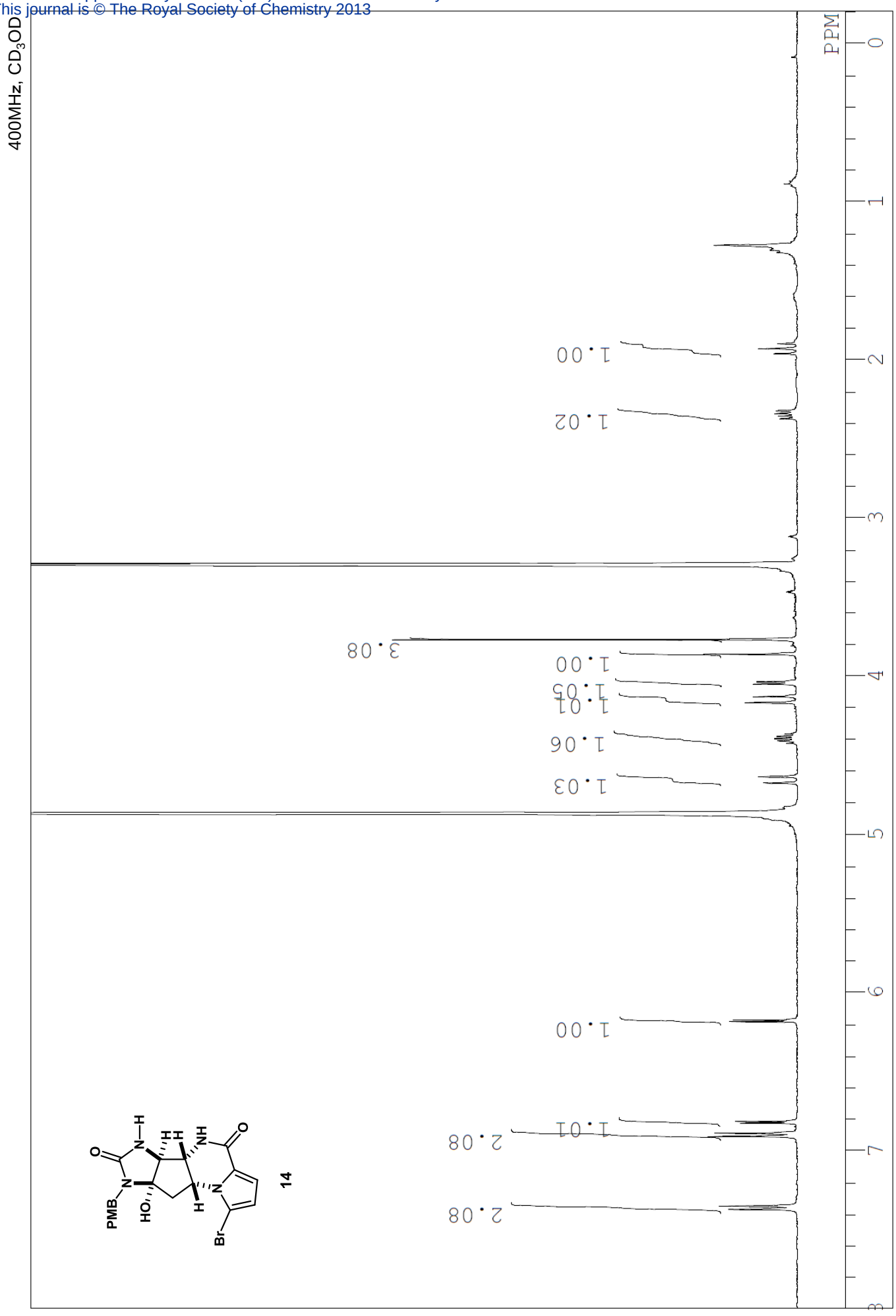


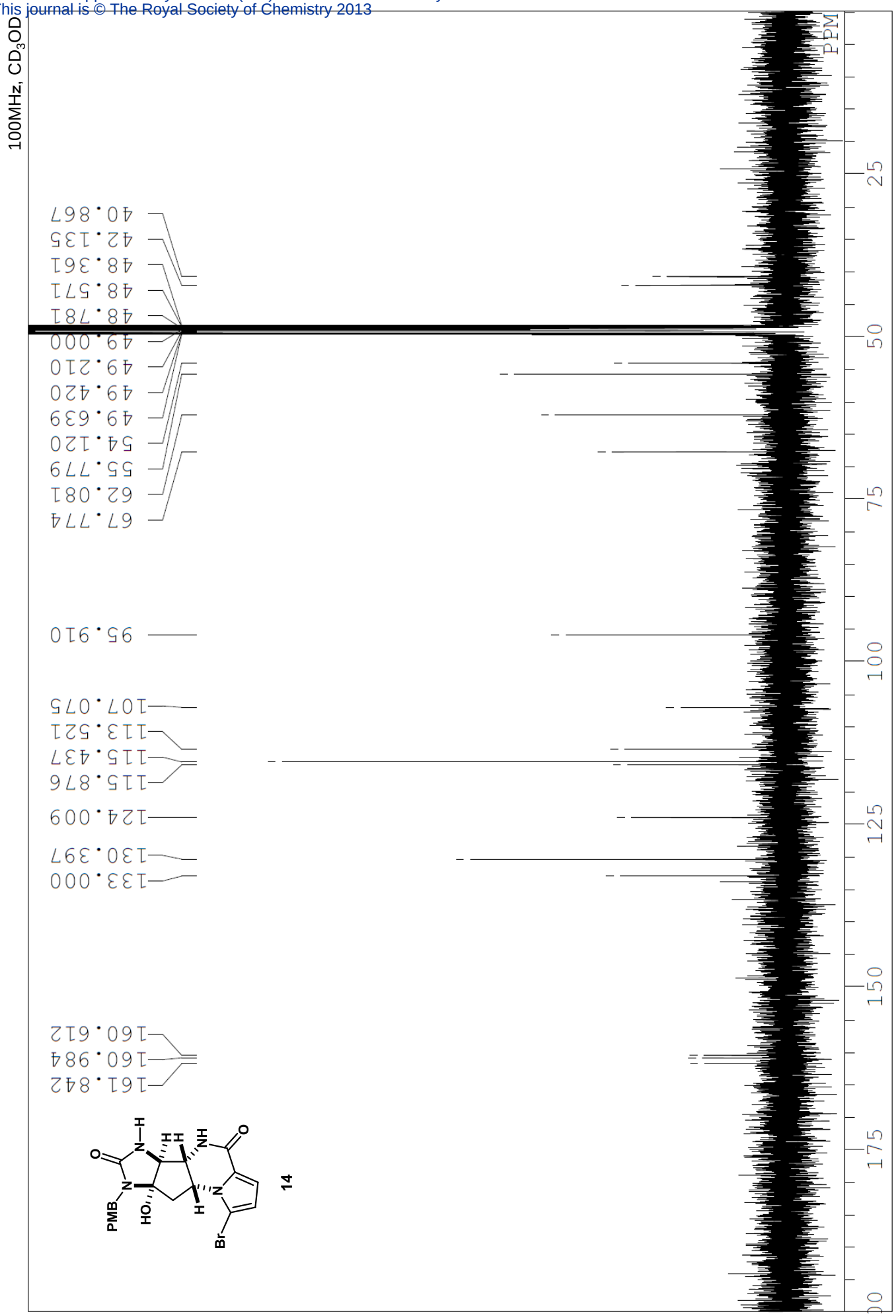


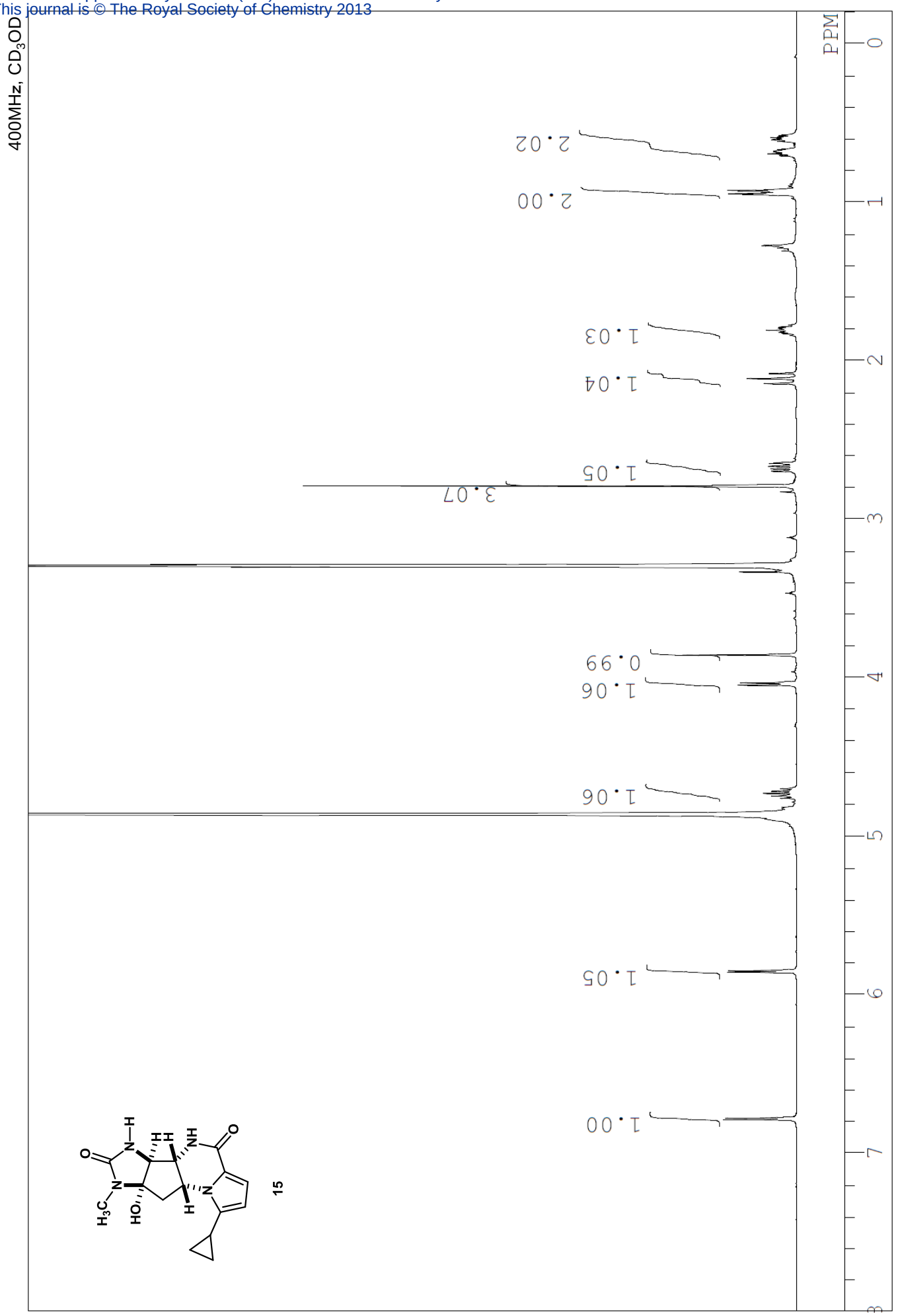




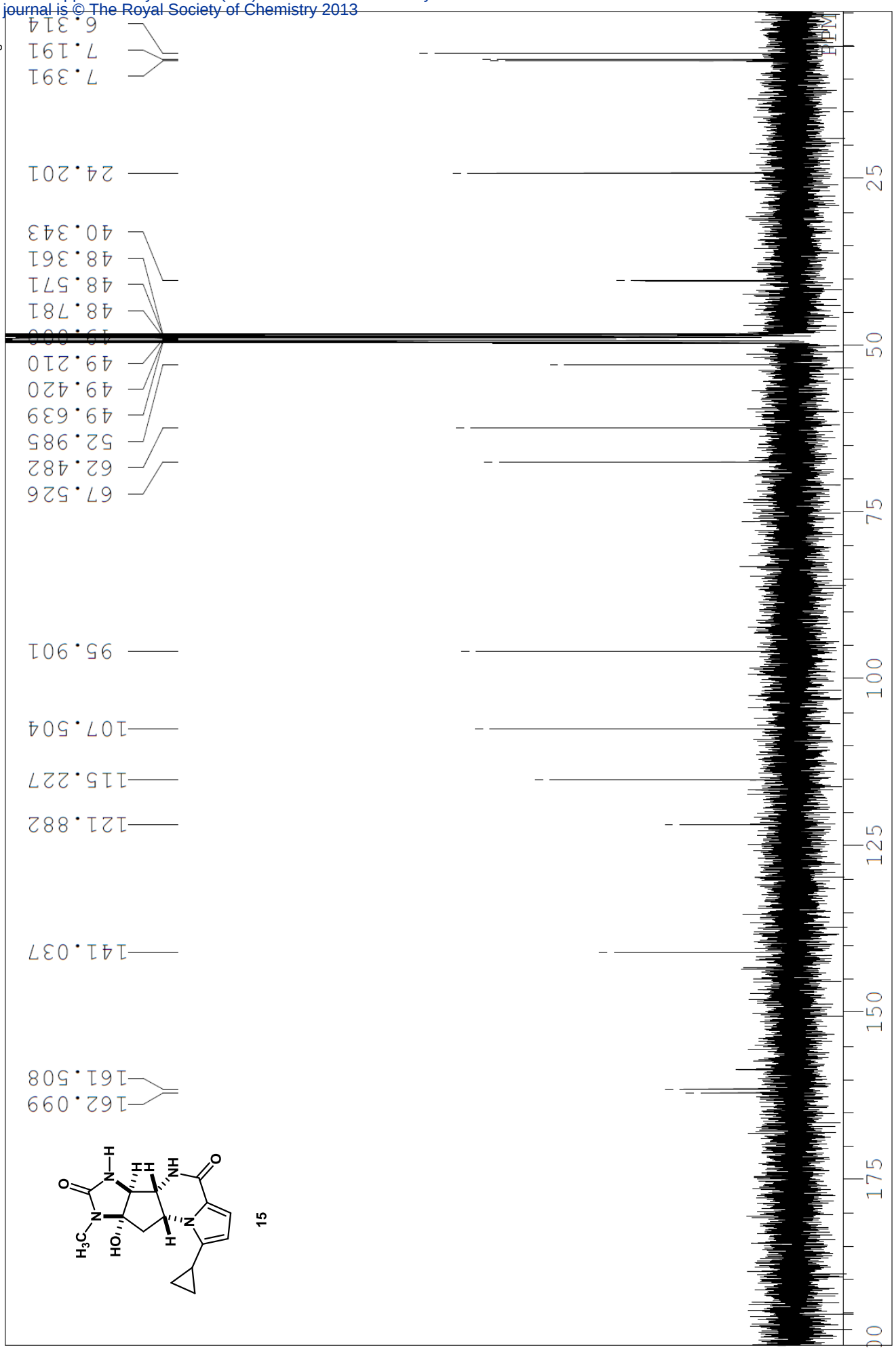




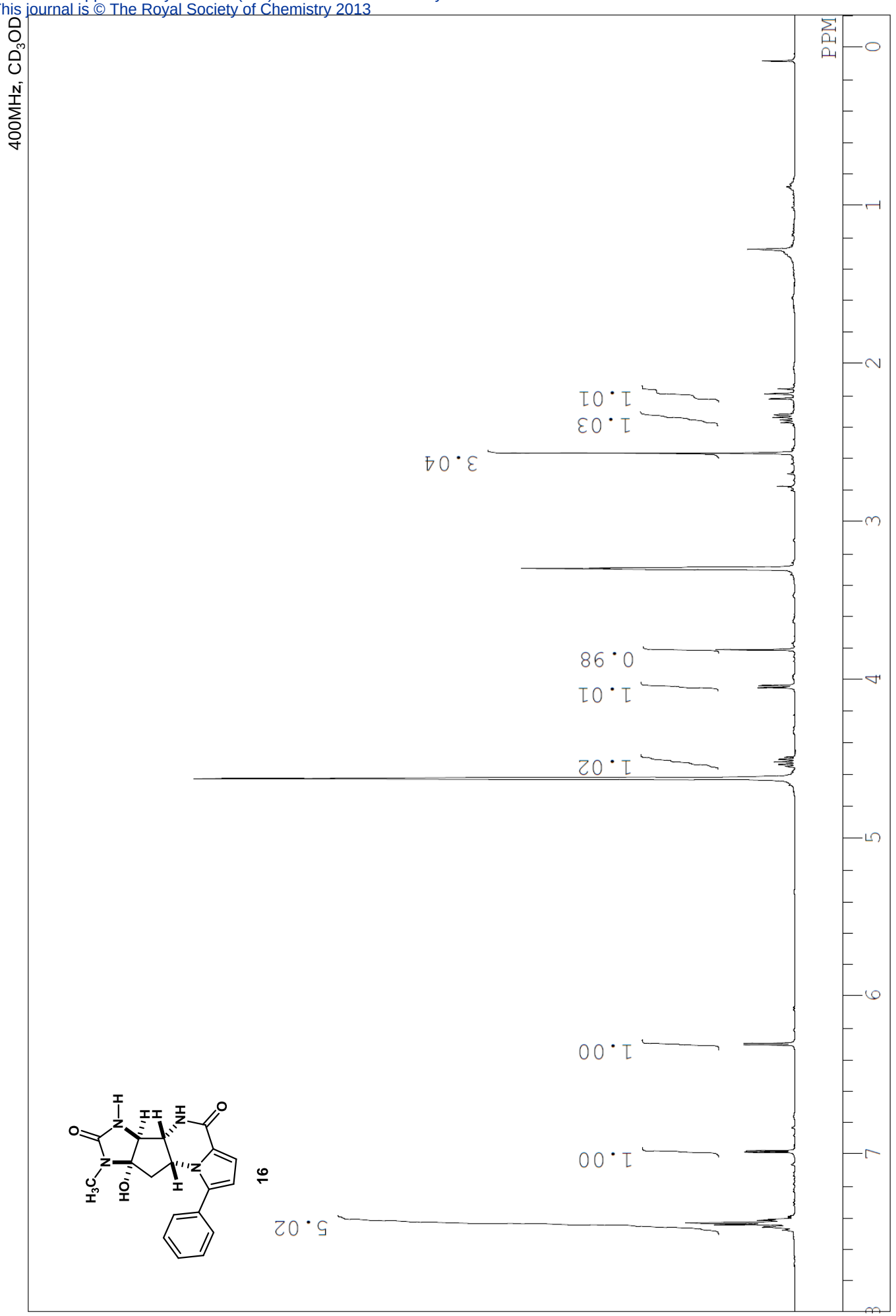


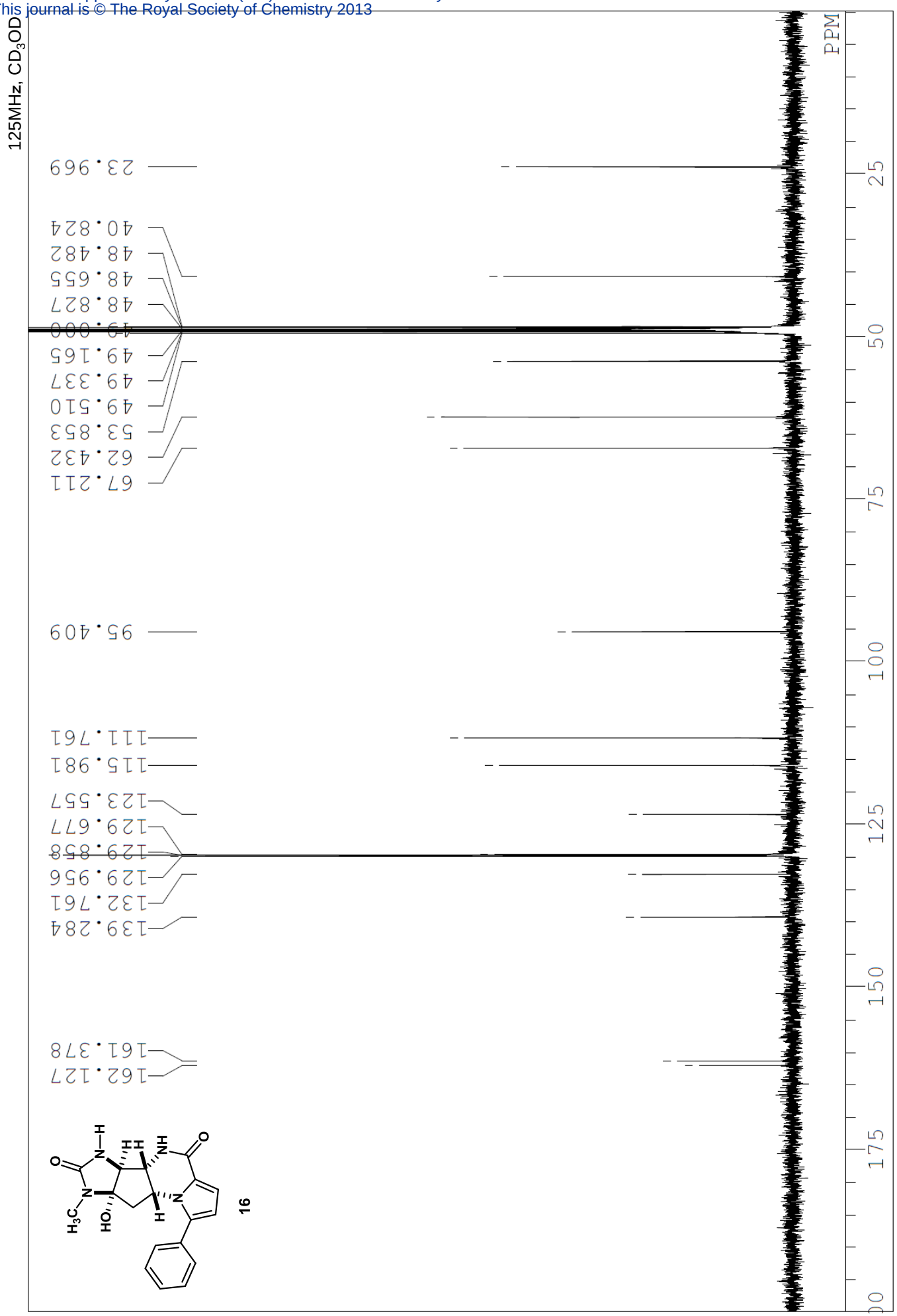


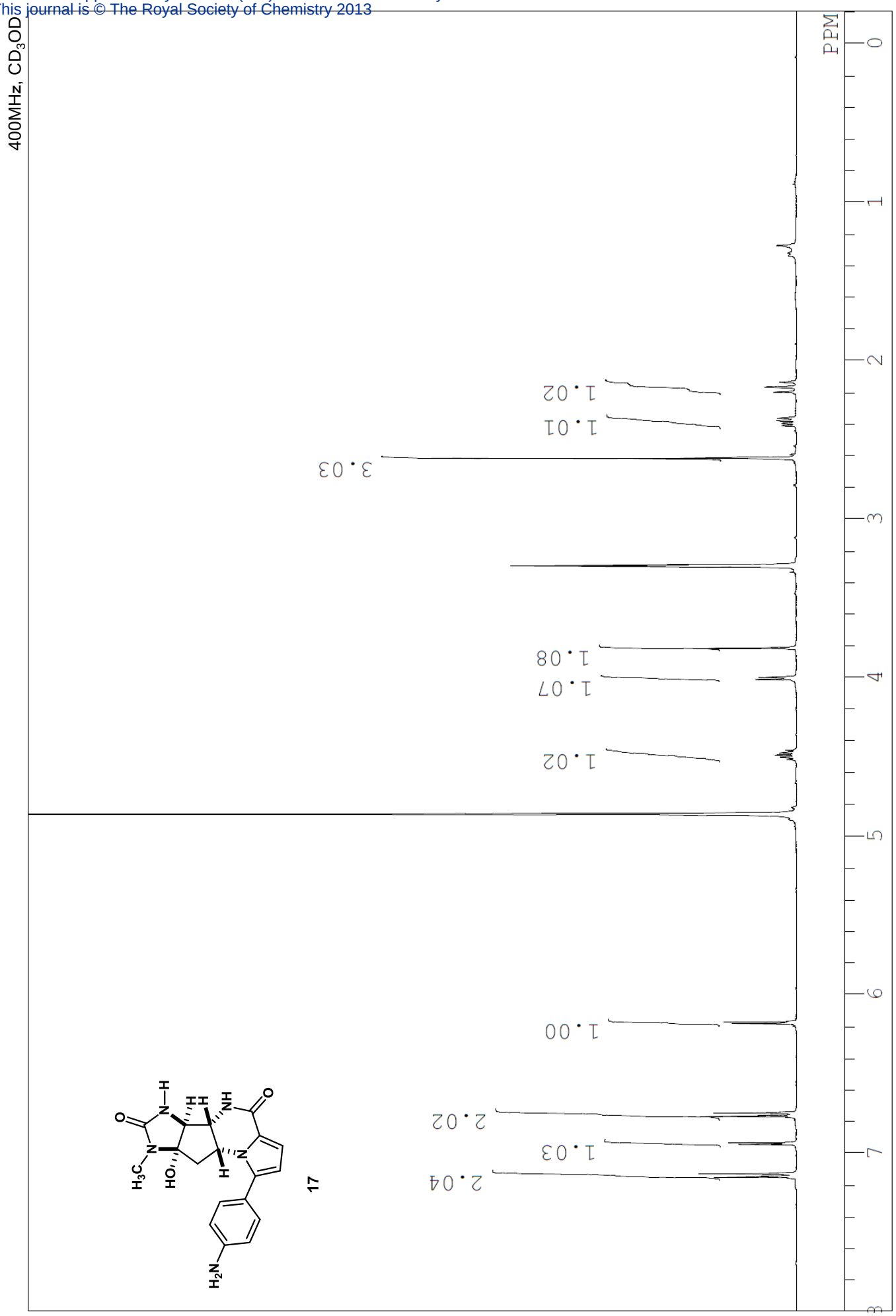
100MHz, CD_3OD



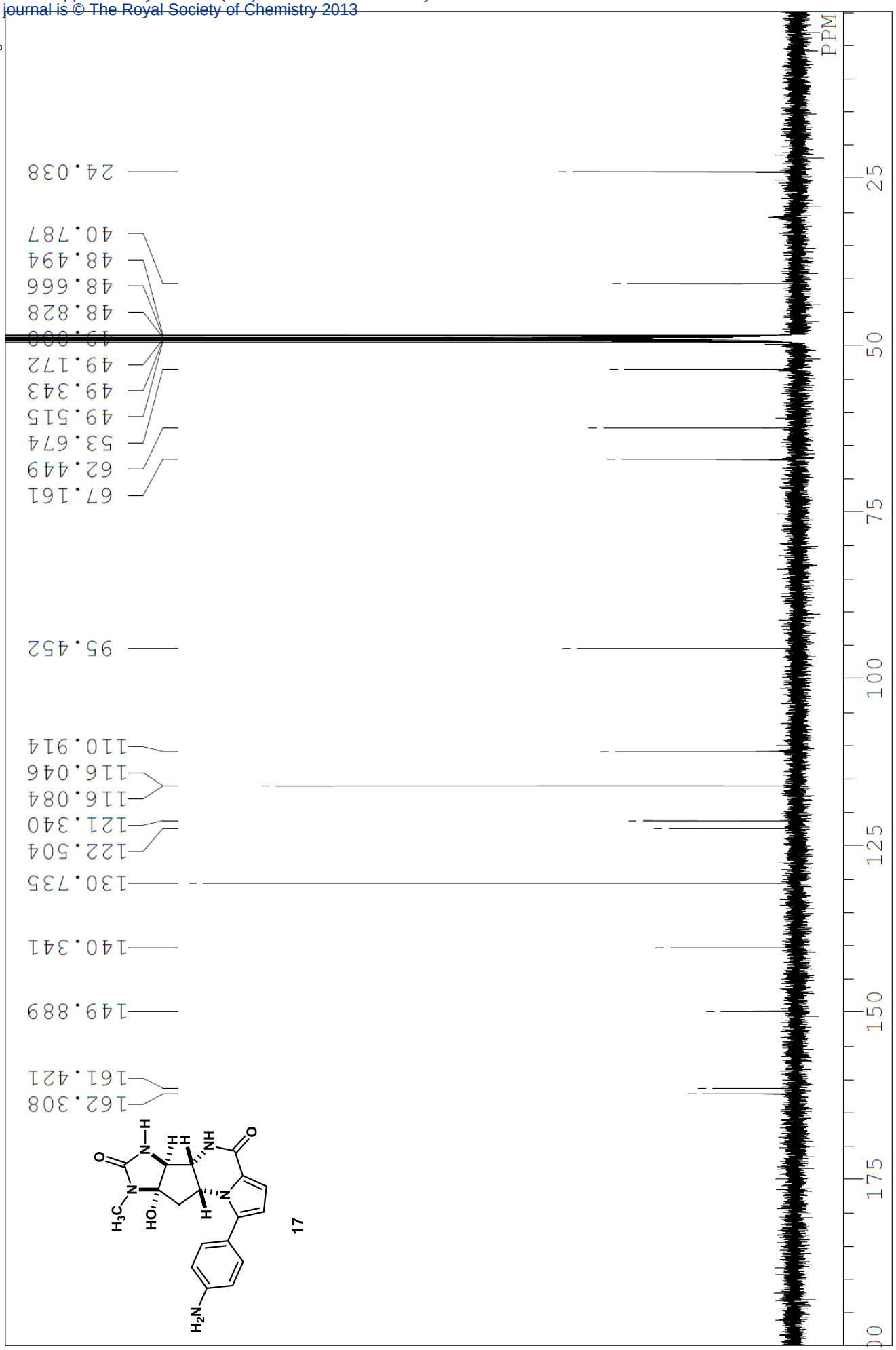
15

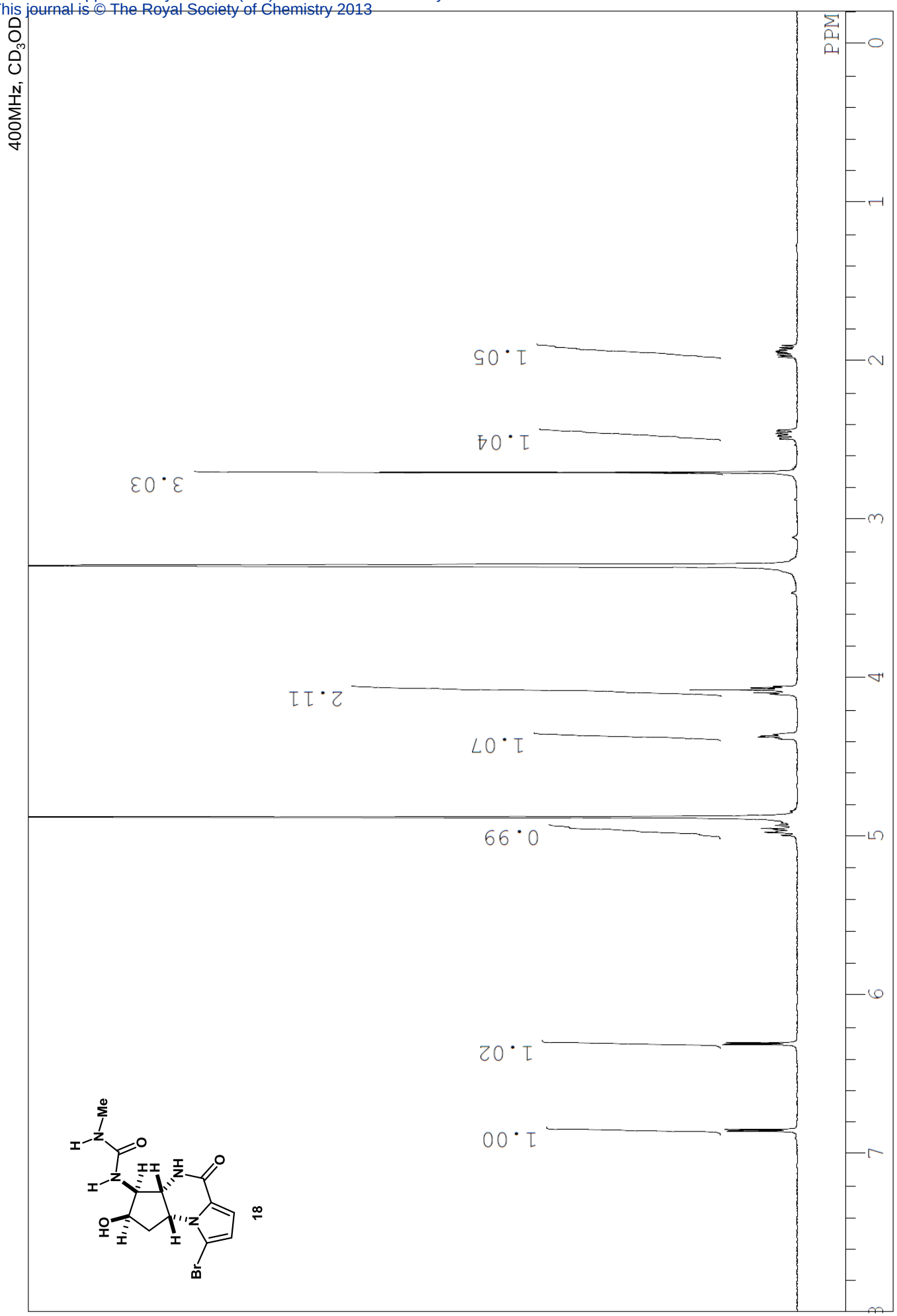




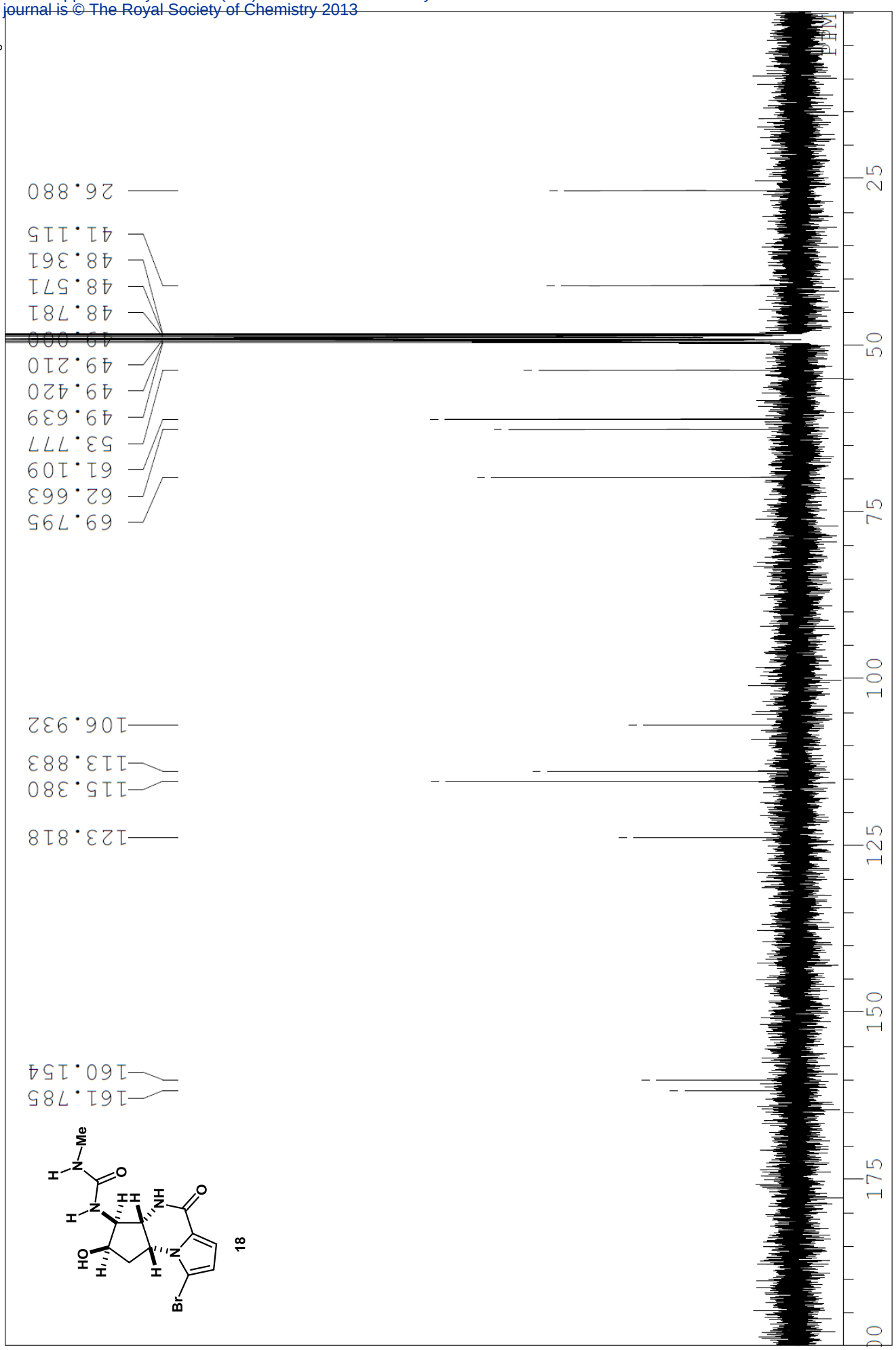


125MHz, CD_3OD





100MHz, CD_3OD



18