Structure-Activity Relationships of Methy-Lysine Reader Antagonists

J. Martin Herold[a], Lindsey Ingerman James[a], Victoria K. Korboukh[a], Cen Gao[a], Kaitlyn E. Coil[b], Dennis J. Bua[b], Jacqueline L. Norris[a], Dmitri B. Kireev[a], Peter J. Brown[c], Jian Jin[a], William P. Janzen[a], Or Gozani[b], Stephen V. Frye[a*]

[a]*Center for Integrated Chemical Biology and Drug Discovery, UNC Eshelman School of Pharmacy, Division of Medicinal Chemistry and Natural Products, University of North Carolina at Chapel Hill Chapel Hill, North Carolina 27599, United States*

[b]*Department of Biology, Stanford University, Stanford, California 94305, United States*

[c]*Structural Genomics Consortium, University of Toronto, Toronto, Ontario, M5G 1L7, Ontario, Canada*

Experimental Section

Protein Purification

L3MBTL1 was expressed and purified as described previously.[1,2] L3MBTL3 was expressed and purified in an analogous fashion. Briefly, cell pellets from a 2 L culture expressing His-tagged L3MBTL3 were lysed with BugBuster protein extraction reagent (EMD Chemicals, Gibbstown, NJ) containing 20 mM imidazole. The cell lysate was clarified by centrifugation and loaded onto a 5 mL HisTrap HP column (GE Healthcare, Piscataway, NJ) equilibrated with binding and wash buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 20 mM imidazole) using an ÄKTA FPLC (GE Healthcare, Piscataway, NJ) at 1 mL/min. His-tagged L3MBTL3 was eluted using a linear gradient of elution buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 500 mM imidazole) over 20 column volumes. Fractions containing L3MBTL3 were confirmed by SDS-PAGE, pooled and loaded at 2 ml/min onto a HiLoad 26/60 Superdex 200 preparative grade size exclusion column (GE Healthcare, Piscataway, NJ) using an ÄKTA FPLC. A constant flow of 2 ml/min size exclusion buffer (10 mM Tris·HCl pH 8.0, 300 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.02% Tween 20) was used to elute L3MBTL3. Fractions containing L3MBTL3 were identified by SDS-PAGE, pooled and subjected to simultaneous concentration and buffer exchange using an Amicon Ultra-15 centrifugal filter unit (Millipore, Billerica, MA) and storage buffer (20 mM Tris·HCl pH 8.0, 250 mM NaCl and 2 mM DTT). Protein concentration was determined by Bradford assay and protein purity was determined to by >95% by Coomassie.

The other proteins included in the Alphascreen assay (L3MBTL4, MBTD1, SFMBT, and CBX7), as well as the constructs for L3MBTL1 and L3MBTL3, were obtained from the SGC in Toronto.

Alphascreen Assay

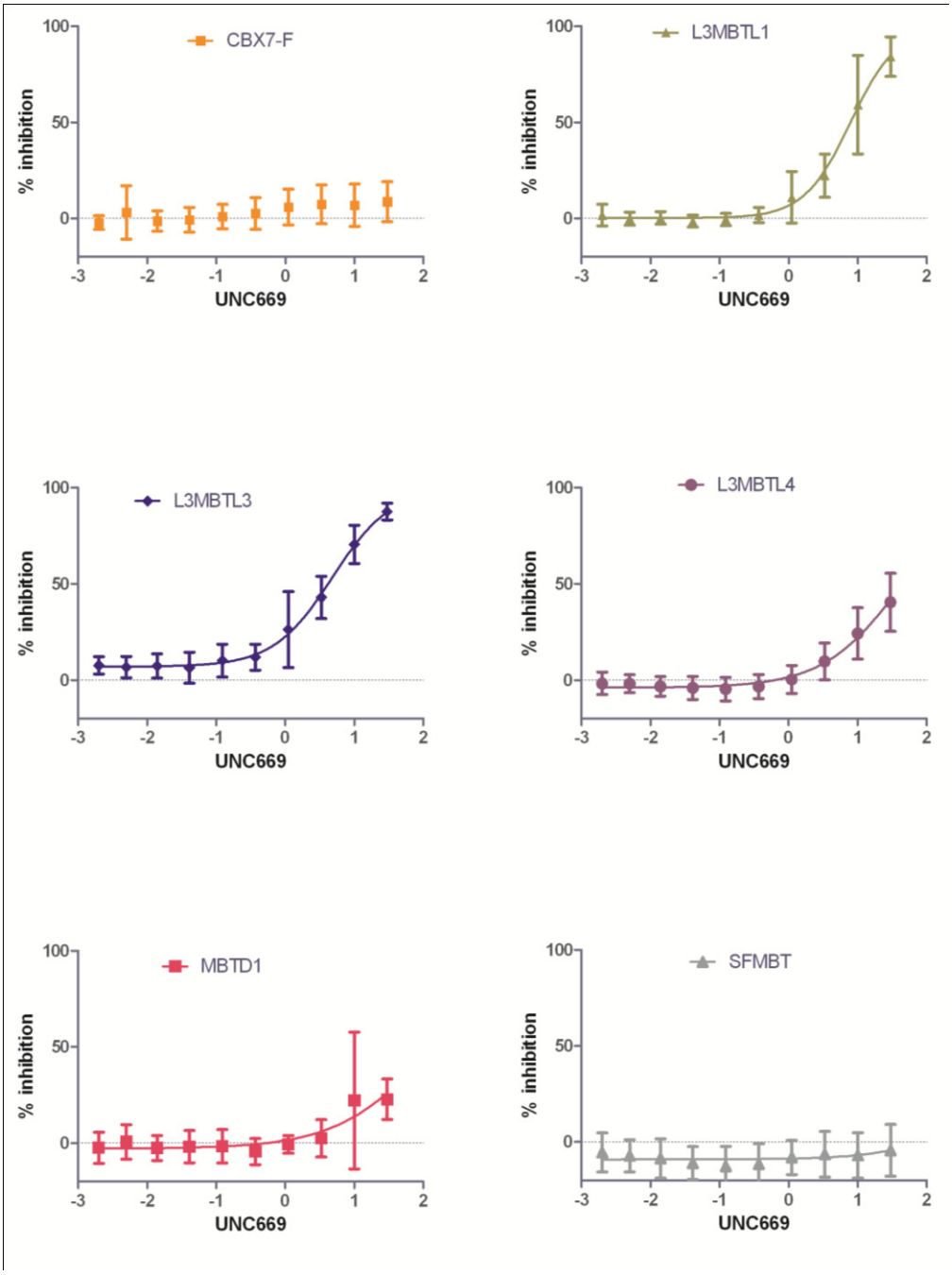
The Alphascreen assay was performed as described elsewhere.[1, 2] In brief, compound plates (1 µl at 30 mM highest concentration) were diluted in 1X assay buffer (20 mM TRIS pH 8.0, 25 mM NaCl, 2 mM DTT and 0.05% Tween-20) over 2 steps using a Multimek robotic pipettor (Nanoscreen) and 1 µl was spotted into the wells of 384-well

assay Proxiplates (PerkinElmer). To these plates 9 µl of protein- peptide mix in 1X assay buffer was added by Multidrop (Thermo) and incubated for 30 min at room temperature. At this point 2 uL of streptavidine- conjugate donor and nickel-chelate acceptor beads (45 µg/mL in 1X assay buffer) were added, the plates were allowed to incubate for an additional 30 min in the dark at room temperature. After incubation the plates were read on EnVision mulilabel reader equipped with HTS alpha screen laser (Perkin Elmer). The screens reported are performed up to 30 µM, and therefore it should be noted that those compounds referred to as inactive are indeed inactive only within the concentration range tested.

IC_{50} data analysis. The data for the IC_{50} values was calculated from replicate runs in the following way: the datapoints for each compound concentration were averaged (at least 5 values from at least 2 independent trials) and plotted using 4-parameters curve fitting (GraphPad Prism 5).

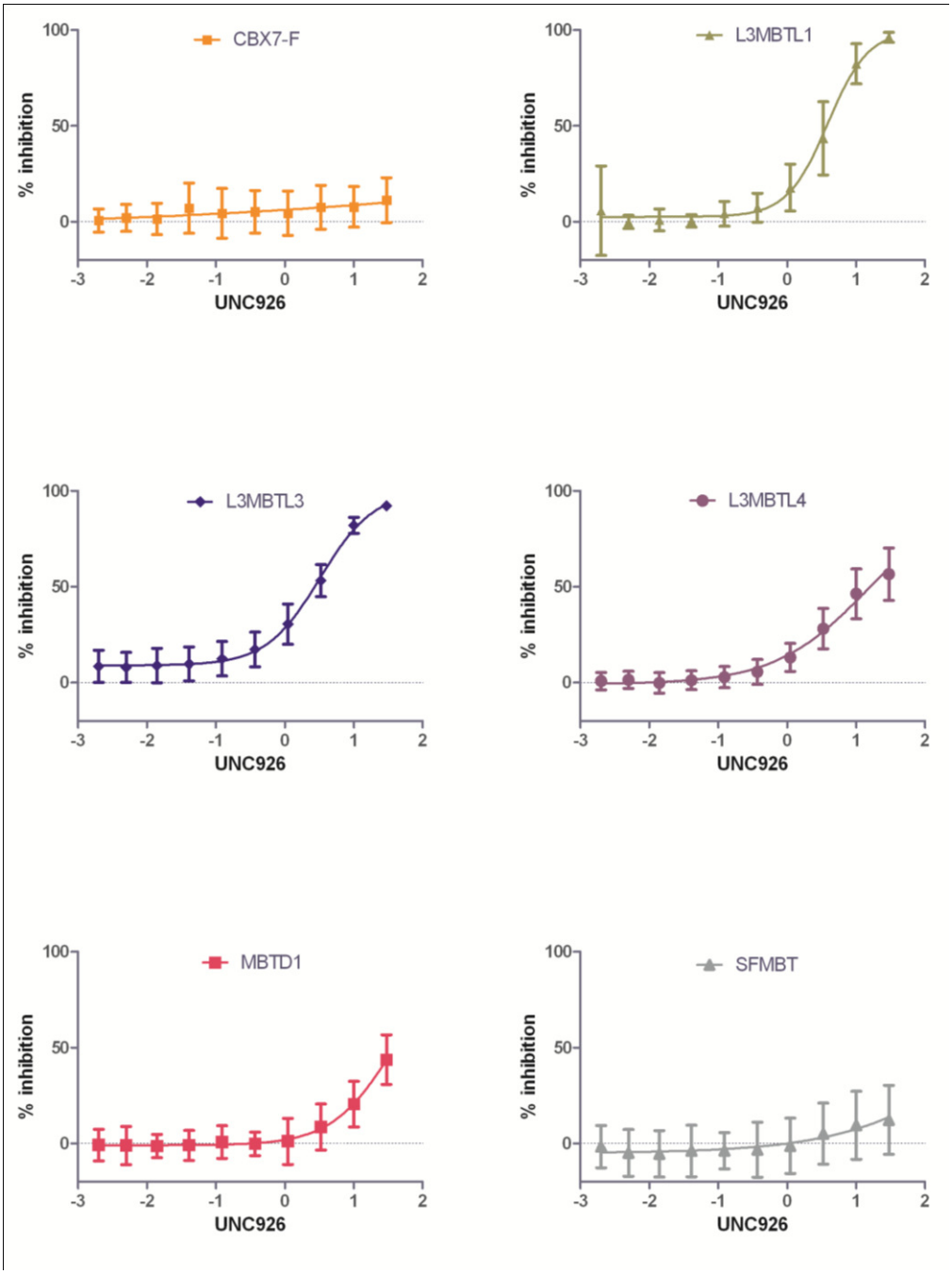
Protein	Peptide	Peptide sequence	Protein final conc. in 10 µl	Peptide final conc. in 10 µl
L3MBTL1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	50 nM	150 nM
L3MBTL3	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH	200 nM	150 nM
L3MBTL4	H2AK36Me1	Biotin-AHA-GRVHRLLRK(Me1)GNYSER-COOH	100 nM	150 nM
MBTD1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	100 nM	150 nM
SFMBT1	H3K9Me1	Biotin-AHA-ARTKQTARK(Me1)STGGKA-COOH	100 nM	150 nM
CBX7-Flag	H3K9Me3	ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH2	100 nM	150 nM

Representative binding curves for 1 (UNC669) against the reader panel:



Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

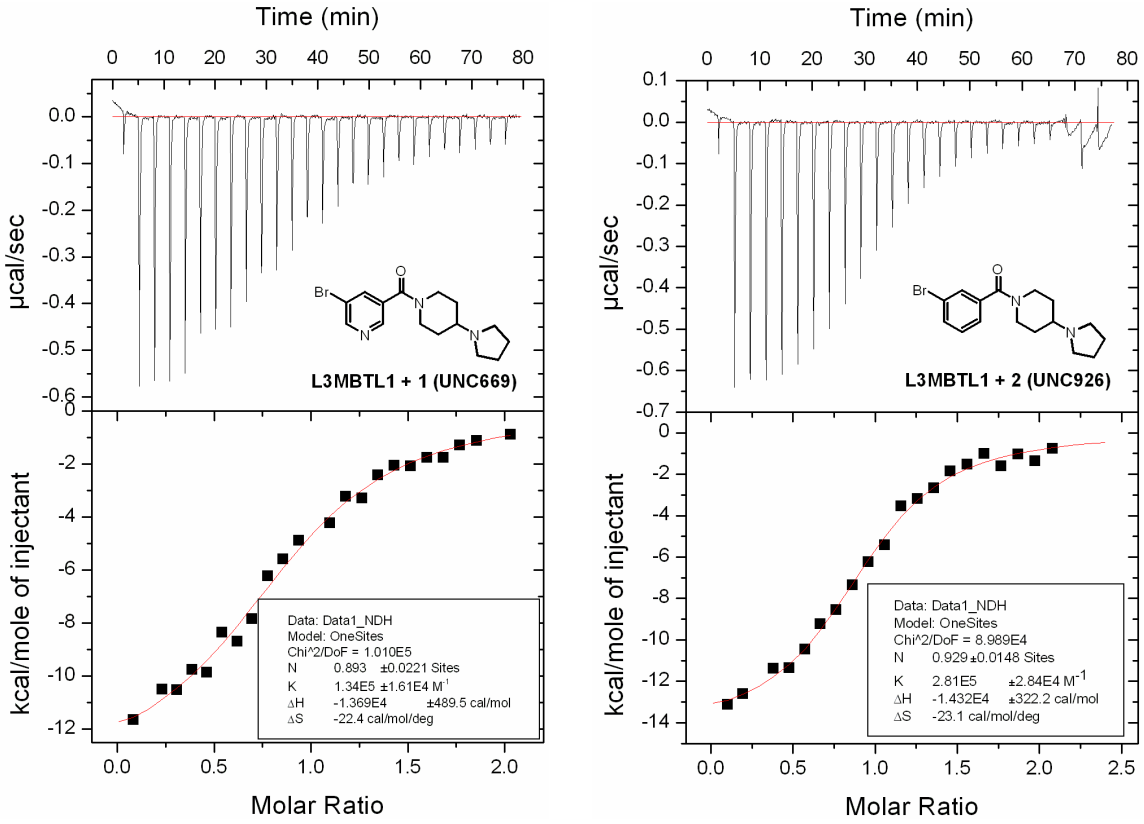
Representative binding curves for 2 (UNC926) against the reader panel:



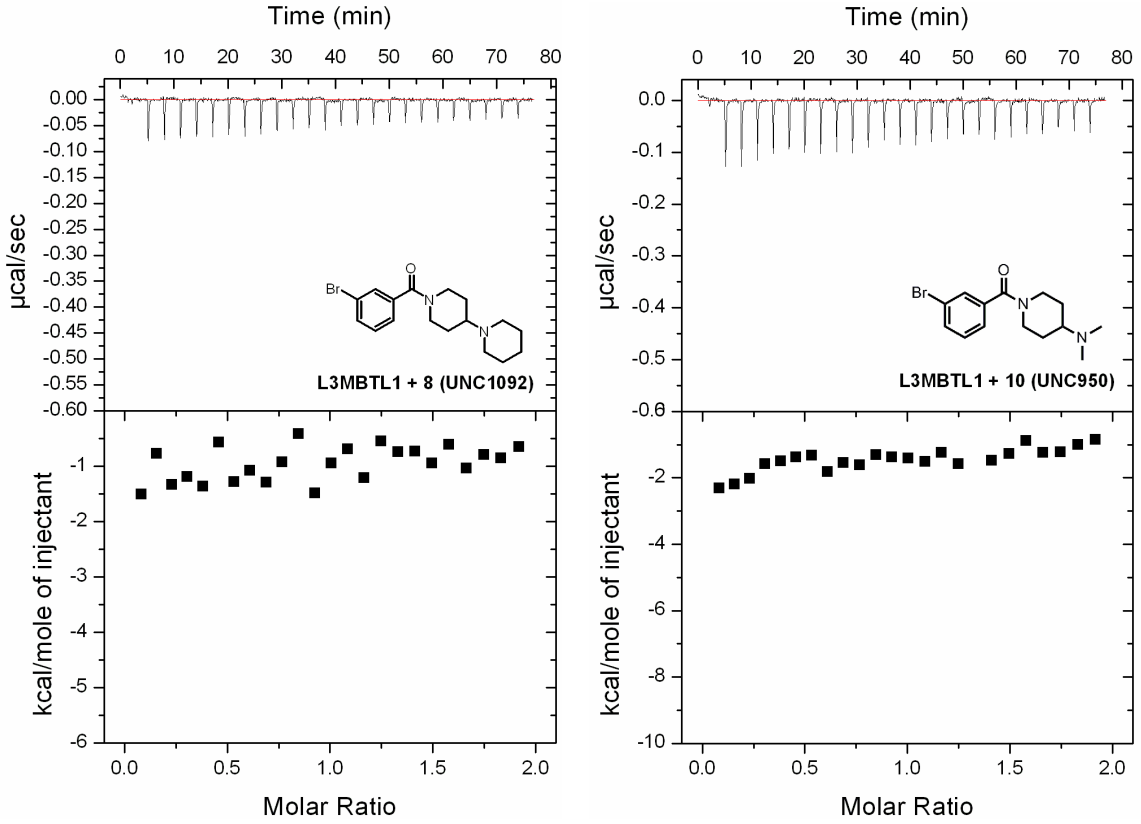
ITC Experiments

All ITC measurements were recorded at 25 °C with an AutoITC200 microcalorimeter (MicroCal Inc., MA). All protein and compound stock samples were in the target buffer (25 mM Tris-HCl, pH 8, 75 mM NaCl, and 2 mM β-mercaptoethanol), and then diluted in the same buffer to achieve the desired concentrations: 40-50 μM L3MBTL1 and 0.5 mM compound. The concentration of the L3MBTL1 stock solution was established using the Edelhoch method, whereas 10 mM compound stock solutions were prepared based on molecular weight.[3] A typical experiment included a single 0.2 μl compound injection into a 200 μl cell filled with L3MBTL1, followed by 26 subsequent 1.5 μl injections of compound. Injections were performed with a spacing of 180 seconds and a reference power of 8 μcal/sec. Control experiments were performed for each compound under identical conditions to determine the heat signals that arise from injecting compound into buffer, and these heats of dilution generated were then subtracted from the protein-compound binding curves. The titration data was analyzed using Origin Software (MicroCal Inc., USA) by non-linear least squares, fitting the heats of binding as a function of the compound:protein ratio to a one site binding model. We should also note that the small difference in potency of **1** (UNC669) for L3MBTL1 in comparison to previously published results is due to a change in the experimental conditions in order to improve protein stability. The NaCl concentration in the buffer was increased from 25 mM previously to 75 mM in the experiments reported herein.

Representative titrations of active compounds 1 (UNC669) and 2 (UNC926):



Representative titrations of inactive compounds 8 (UNC1092) and 10 (UNC950):



Peptide Pull Down Assays

Peptide pull down assays were performed as previously described (Shi et al., 2006).[4] Briefly, 1µg of GST-fusion domain was incubated at 4°C overnight with 1µg of biotinylated peptide in binding buffer (150mM NaCl, 50mM Tris-HCl pH 7.5, 0.05% NP-40). The following day, peptides were precipitated with streptavidin sepharose (GE Healthcare), and then washed three times with binding buffer. The pellets were analyzed by SDS-PAGE. GST-fusions were detected by Western blot using anti-GST antibody (Abcam). Where indicated, UNC compounds, resuspended in sterile PBS, were added at the specified final concentration.

Constructs and protein purification for pull down assays: L3MBTL1 constructs were generated and characterized previously.[5] The 53BP1 construct was generated and

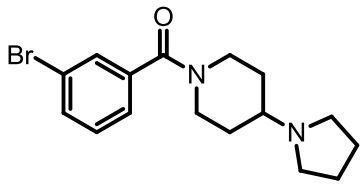
characterized previously.[6] Constructs were expressed in *Escherichia coli* BL21 (DE3) pLysS (Stratagene) grown in LB media. Protein expression was induced using IPTG (1 mM) and cells were lysed by sonication. The GST fusion protein was purified using a glutathione-sepharose resin (GE Healthcare).

General Procedure for Chemical Synthesis

Analytical HPLC data for all compounds were acquired using an Agilent 6110 Series system with the UV detector set to 220 nm. Samples were injected (<10 μL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 μM, C18 column at rt. A mobile phase of A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid) was used. A linear gradient from 10% to 100% B in 5.0 min was followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}H NMR) and 100 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ) relative to the solvent peaks.[7] Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm. Samples were injected onto a Phenomenex Luna 250 × 30 mm, 5 μM, C18 column at room temperature (rt). Mobile phases of A (H_2O + 0.1% TFA) and B (MeOH) were used with a flow rate of 40 mL/min. A linear gradient of 10% to 100% B was used over 17.0 min, followed by a 100% B flush for another 3 minutes. Analytical LCMS (at 220 nm) and NMR were used to establish the purity of targeted compounds. All compounds that were evaluated in biochemical and biophysical assays had >95% purity.

Experimental Procedures for all new compounds

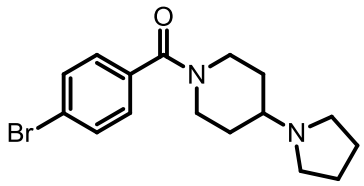
General amide coupling procedure: To a mixture of acid (1 eq.) and TBTU (1.3-1.5 eq.) in DMF, the desired amine (1.2 eq.) was added followed by triethylamine (2-3 eq.). The mixture was stirred at rt for 15 hours. The reaction was quenched by addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with either EtOAc or CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by either column chromatography on silica gel or reverse phase HPLC.



3-Bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide (**UNC926**, Compound **2**): The general procedure was followed with 3-bromobenzoic acid (120 mg, 0.6 mmol) and TBTU (191 mg, 0.6 mmol) in 3 mL of DMF, to which 4-(1-pyrrolidinyl)piperidine was added. The crude mixture was purified by HPLC to afford 252 mg (94%) of the TFA salt of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.62 – 7.55 (m, 1H), 7.54 – 7.50 (m, 1H), 7.36 – 7.29 (m, 2H), 4.74 – 4.56 (m, 1H), 3.85 – 3.65 (m, 1H), 3.65 – 3.46 (m, 2H), 3.35 (tt, J = 11.7, 4.0 Hz, 1H), 3.19 – 2.98 (m, 3H), 2.97 – 2.71 (m, 1H), 2.30 – 1.82 (m, 6H), 1.68 – 1.43 (m, 2H).

MS (ESI): 337 + 339 $[M+H]^+$; HPLC: 100%, t_R: 2.69 min. HRMS calcd. for $C_{16}H_{21}BrN_2O$ + H: 337.0916; found: 337.0925 $[M+H]^+$.

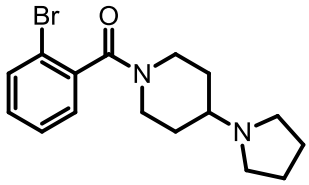


4-Bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide (**3**): The general procedure was followed with 2.15 g (10.7 mmol) of 4-bromobenzoic acid, 1.98 g (12.7 mmol) of 4-(1-pyrrolidinyl)piperidine, 4.12 g (12.7 mmol) of TBTU in 20 mL of DMF and 2.2 mL (16.0 mmol) of NEt_3. After purification by column chromatography on silica gel, 3.07 g (85%) of the title compound were obtained as a off-white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.66 – 7.61 (m, 2H), 7.36 – 7.32 (m, 2H), 4.68 – 4.52 (m, 1H), 3.78 – 3.63 (m, 1H), 3.22 – 3.05 (m, 1H), 2.99 – 2.82 (m, 1H), 2.72 – 2.57 (m, 4H), 2.36 (tt, J = 10.9, 3.9 Hz, 1H), 2.16 – 2.01 (m, 1H), 2.01 – 1.88 (m, 1H), 1.88 – 1.74 (m, 4H), 1.58 – 1.32 (m, 2H).

^{13}C NMR (101 MHz, CD_3OD) δ 171.2, 136.1, 132.9, 129.8, 125.1, 63.1, 52.4, 47.7, 42.2, 32.7, 31.9, 24.0.

MS (ESI): 337 + 339 $[M+H]^+$; HPLC: 100%, t_R: 2.94 min.

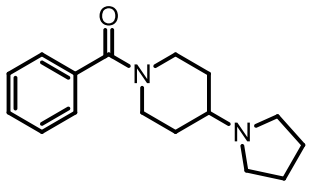


2-Bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide (**4**): The general procedure was followed with 1 g (4.97 mmol) of 2-bromobenzoic acid, 921 mg (5.97 mmol) of 4-(1-pyrrolidinyl)piperidine, 1.92 g (5.97 mmol) of TBTU in 15 mL of DMF and 1.05 mL (7.45 mmol) of NEt_3. After purification by column chromatography on silica gel, 1.24 g (74%) of the title compound were obtained as a white solid. By NMR, it was determined that the compound was isolated as atropisomers.

^{1}H NMR (400 MHz, CD_3OD) *Rotamer A*: δ 7.69 – 7.63 (m, 1H), 7.49 – 7.41 (m, 1H), 7.39 – 7.24 (m, 2H), 4.73 – 4.58 (m, 1H), 3.41 (tt, J = 17.0, 4.8 Hz, 1H), 3.21 – 3.12 (m, 1H), 2.92 (td, J = 12.9, 2.9 Hz, 1H), 2.70 – 2.57 (m, 4H), 2.41 – 2.30 (m, 1H), 2.16 – 2.05

(m, 1H), 1.96 – 1.86 (m, 1H), 1.86 – 1.76 (m, 4H), 1.62 – 1.44 (m, 1H), 1.42 – 1.29 (m, 1H); *Rotamer B*: δ 7.69 – 7.63 (m, 1H), 7.49 – 7.41 (m, 1H), 7.39 – 7.24 (m, 2H), 4.73 – 4.58 (m, 1H), 3.41 (tt, *J* = 17.0, 4.8 Hz, 1H), 3.12 – 3.03 (m, 1H), 2.92 (td, *J* = 12.9, 2.9 Hz, 1H), 2.70 – 2.57 (m, 4H), 2.41 – 2.30 (m, 1H), 2.16 – 2.05 (m, 1H), 1.96 – 1.86 (m, 1H), 1.86 – 1.76 (m, 4H), 1.75 – 1.62 (m, 1H), 1.62 – 1.44 (m, 1H).

MS (ESI): 337 + 339 $[M+H]^+$; HPLC: 100%, t_R: 2.85 min.

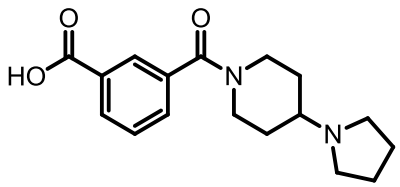


***N*-((4-(Pyrrolidinyl)piperidinyl)benzamide** (**5**): The general procedure was followed with 50 mg (0.4 mmol) of benzoic acid, 82 mg (0.5 mmol) of 4-(1-pyrrolidinyl)piperidine, 158 mg (0.5 mmol) of TBTU in 2 mL of DMF and 0.1 mL (0.8 mmol) of NEt_3. After purification by column chromatography on silica gel, 65 mg (61%) of the title compound were obtained as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.51 – 7.37 (m, 5H), 4.73 – 4.54 (m, 1H), 3.84 – 3.64 (m, 1H), 3.22 – 3.02 (m, 1H), 3.00 – 2.79 (m, 1H), 2.73 – 2.61 (m, 4H), 2.39 (tt, *J* = 11.0, 4.0 Hz, 1H), 2.18 – 2.02 (m, 1H), 2.00 – 1.87 (m, 1H), 1.88 – 1.78 (m, 4H), 1.58 – 1.34 (m, 2H).

^{13}C NMR (101 MHz, cd_3od) δ 172.3, 137.1, 131.0, 129.7, 127.8, 63.2, 52.5, 47.7, 42.1, 32.7, 31.9, 24.0.

MS (ESI): 259 $[M+H]^+$; HPLC: 100%, t_R: 1.87 min.

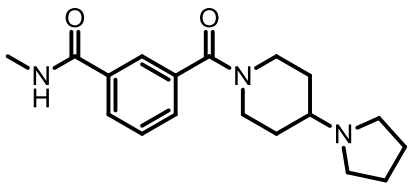


3-Carboxy-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide (**6**): The general procedure was followed with 500 mg (2.3 mmol) of 3-*t*-butylcarbonylbenzoic acid, 382 mg (2.5 mmol)

of 4-(1-pyrrolidinyl)piperidine, 867 mg (2.7 mmol) of TBTU in 15 mL of DMF and 0.4 mL (2.7 mmol) of NEt_3. The crude t-butyl ester was then purified by column chromatography on silica and the ester was subsequentially cleaved by dissolving it in 15 mL of CH_2Cl_2 and adding 2 mL of TFA. After evaporation of the solvent, 1.55 g (quantitative) of acid were obtained of which 60 mg were further purified by HPLC to afford 52 mg of the TFA salt of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (dt, J = 7.6, 1.5 Hz, 1H), 8.07 (t, J = 1.4 Hz, 1H), 7.67 (dt, J = 7.6, 1.5 Hz, 1H), 7.61 (t, J = 7.6 Hz, 1H), 4.83 – 4.70 (m, 1H), 3.94 – 3.76 (m, 1H), 3.76 – 3.57 (m, 2H), 3.45 (tt, J = 11.7, 4.0 Hz, 1H), 3.28 – 3.08 (m, 3H), 3.07 – 2.83 (m, 1H), 2.40 – 1.93 (m, 6H), 1.81 – 1.55 (m, 2H).

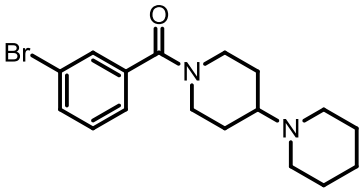
MS (ESI): 303 $[M+H]^+$; HPLC: 100%, t_R: 1.96 min.



***N*-Methyl-3-(4-(pyrrolidin-1-yl)piperidine-1-carbonyl)benzamide** (**7**): The general procedure was followed with 200 mg (0.5 mmol) of acid **6** in 3 mL of DMF, 1 mL (2 mmol) of methylamine (2 M in THF) and 308 mg (1 mmol) of TBTU. After workup, the crude reaction mixture was taken up in 1 mL of methanol, filtered, and purified by HPLC to afford 23 mg (11%) of the TFA salt of the title compound as white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.95 – 7.89 (m, 1H), 7.88 – 7.84 (m, 1H), 7.63 – 7.53 (m, 2H), 4.84 – 4.68 (m, 1H), 3.96 – 3.74 (m, 1H), 3.74 – 3.56 (m, 2H), 3.34 (tt, J = 11.7, 4.0 Hz, 1H), 3.28 – 3.08 (m, 3H), 3.04 – 2.83 (m, 1H), 2.93 (s, 3H), 2.42 – 1.91 (m, 6H), 1.66 (s, 2H).

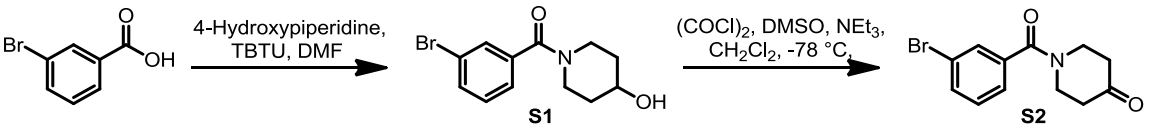
MS (ESI): 316 $[M+H]^+$; HPLC: 100%, t_R: 1.78 min.



3-Bromo-*N*-((4-(piperidinyl)piperidinyl)benzamide (UNC1092, Compound **8**): The general procedure was followed with 100 mg (0.5 mmol) of 3-bromobenzoic acid, 87 mg (0.5 mmol) of 4-piperidinopiperidine, 166 mg (0.5 mmol) of TBTU in 2 mL of DMF and 0.1 mL (0.8 mmol) of NEt_3. After purification by HPLC, 249 mg (quantitative) of the TFA salt of the title compound were obtained as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.64 (m, 1H), 7.64 – 7.61 (m, 1H), 7.44 – 7.37 (m, 2H), 4.83 – 4.69 (m, 1H), 3.95 – 3.73 (m, 1H), 3.58 – 3.43 (m, 3H), 3.29 – 3.10 (m, 1H), 3.10 – 2.97 (m, 2H), 2.97 – 2.79 (m, 1H), 2.29 – 1.92 (m, 4H), 1.91 – 1.64 (m, 5H), 1.60 – 1.44 (m, 1H).

MS (ESI): 351 + 353 $[M+H]^+$; HPLC: 100%, t_R: 3.27 min. HRMS calcd. for $C_{17}H_{23}BrN_2O$ + H: 351.1072; found: 351.1075 $[M+H]^+$.



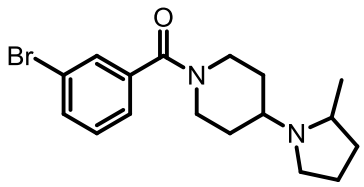
1-(3-Bromobenzoyl)piperidin-4-ol (S1): The general procedure was followed with 3.0 g (14.9 mmol) of 3-bromobenzoic acid, 3.0 g (29.8 mmol) of 4-hydroxypiperidine, 5.7 g (17.9 mmol) of TBTU in 30 mL of DMF and 4.2 mL (29.8 mmol) of NEt_3. The crude reaction was purified by column chromatography on silica gel. The alcohol obtained contained small amounts of TBTU by-products and the mixture (colorless oil) was taken on without further purification.

1-(3-Bromobenzoyl)piperidin-4-one (S2): To a dry flask purged with N_2, 16 mL of dry DCM was added and cooled to -78°C. Oxalyl chloride (352 µl, 4.1 mmol) was slowly added to the flask, followed by dropwise addition of 369 µl of DMSO (5.2 mmol). After stirring for 10 minutes, a solution of **S1** (778 mg, 2.7 mmol) in 9 mL of dry DCM was

added dropwise to the cold solution. After stirring for an additional 30 minutes, 1.9 mL (13.7 mmol) of dry Et_3N was added dropwise. The reaction was stirred for 1 hr at -78°C and then slowly allowed to warm up to room temperature. The reaction was quenched with sat. aq. NH_4Cl and extracted with DCM 3 times. The combined organic extracts were washed with sat. aq. NaCl (2x) and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by column chromatography on silica gel to give 708 mg (91%) of **S2** as a light yellow oil.

^{1}H NMR (400 MHz, CD_3OD) δ 7.67 – 7.61 (m, 1H), 7.60 – 7.57 (m, 1H), 7.41 – 7.35 (m, 2H), 3.73 (s, 2H), 3.50 – 3.34 (m, 2H), 1.95 – 1.63 (m, 4H).

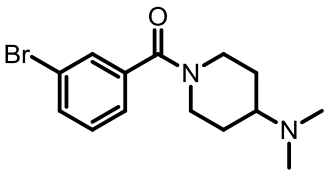
MS (ESI): 282 + 284 $[M+H]^+$; HPLC: 100%, t_R: 1.78 min.



3-Bromo-*N*-((4-(2-methylpyrrolidinyl)piperidinyl)benzamide (**9**): To a solution of 52 mg (0.2 mmol) of ketone **S2** in 3 mL of CH_2Cl_2 was added 0.05 mL (0.6 mmol) of 2-methylpyrrolidine followed by 0.14 mL (0.5 mmol) of $Ti(OiPr)_4$ and 0.06 mL (0.5 mmol) of NEt_3 at rt. The mixture was stirred for 15 h and 117 mg (0.6 mmol) of $NaBH(OAc)_3$ were added in one portion. After 2 h, the mixture was carefully quenched by addition of sat. aq. $NaHCO_3$ and the emulsions were reduced by complexation of the titanium salts with citric acid. The product was extracted with CH_2Cl_2 (6 × 15 mL) and the combined organic extracts were washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered and the crude product was purified by HPLC to afford 7 mg (8%) of the title compound.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.58 (m, 2H), 7.45 – 7.37 (m, 2H), 4.84 – 4.72 (m, 1H), 3.94 – 3.72 (m, 2H), 3.62 (tt, J = 12.0, 3.7 Hz, 1H), 3.57 – 3.48 (m, 1H), 3.29 – 3.14 (m, 1H), 3.04 – 2.80 (m, 1H), 2.36 – 1.93 (m, 6H), 1.84 – 1.62 (m, 3H), 1.44 (d, J = 6.6 Hz, 3H).

MS (ESI): 351 + 353 $[M+H]^+$; HPLC: 100%, t_R: 3.31 min.



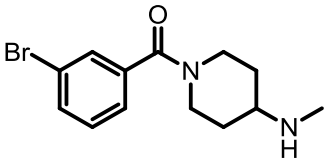
3-Bromo-*N,N*-((4-(dimethylpiperidinyl)benzamide (**10**):

To a solution of 130 mg (0.6 mmol) of ketone **S2** in 2 mL of MeOH were added ca. 1 mL (ca. 15 mmol) of dimethylamine, followed by 0.2 mL (2.9 mmol) of acetic acid. The mixture was stirred for 4 h and 109 mg (1.6 mmol) of $NaBH_3CN$ were added, followed by another 12 h of stirring. Upon completion of the reaction, the solvent was removed, the mixture was taken up in 1 mL of methanol, the solution was filtered, and the crude product was purified by HPLC to afford 121 mg (49%) of the TFA salt of the title compound as white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.65 (m, 1H), 7.65 – 7.62 (m, 1H), 7.44 – 7.37 (m, 2H), 4.84 – 4.71 (m, 1H), 3.95 – 3.75 (m, 1H), 3.50 (tt, J = 12.1, 3.9 Hz, 1H), 3.28 – 3.10 (m, 1H), 2.89 (s, 6H), 3.02 – 2.78 (m, 1H), 2.27 – 1.96 (m, 2H), 1.82 – 1.60 (m, 2H).

^{13}C NMR (101 MHz, CD_3OD) δ 170.63, 138.65, 134.32, 131.68, 130.94, 126.67, 123.66, 64.55, 47.08, 41.62, 40.44, 27.70, 27.09.

MS (ESI): 311 + 313 $[M+H]^+$; HPLC: 100%, t_R: 2.68 min.

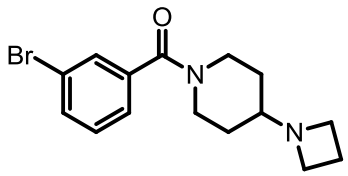


3-Bromo-*N*-(4-methyl)piperidinyl)benzamide (**11**): To a solution of ketone **S2** (93 mg, 0.33 mmol) in 1 mL of DCE, 1.32 mL of methylamine (2 M in THF, 2.64 mmol) was added followed by 0.5 mL (8.73 mmol) of AcOH. The mixture was stirred at rt for 12 h and then 200 mg (0.94 mmol) of $NaBH(OAc)_3$ were added in one portion. After 1 h, the reaction was quenched by addition of sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$, dried over

Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude product was purified by HPLC to afford 19 mg (14%) of the title compound as a light yellow solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.64 (m, 1H), 7.62 – 7.59 (m, 1H), 7.44 – 7.37 (m, 2H), 4.81 – 4.59 (m, 1H), 3.93 – 3.69 (m, 1H), 3.40 – 3.32 (m, 1H), 3.29 – 3.12 (m, 1H), 3.05 – 2.83 (m, 1H), 2.73 (s, 3H), 2.30 – 1.98 (m, 2H), 1.55 (s, 2H).

MS (ESI): 297 + 299 $[M+H]^+$; HPLC: 100%, t_R: 2.51 min.

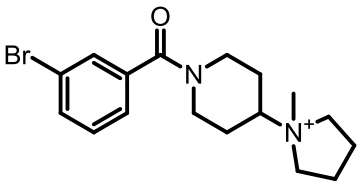


3-Bromo-*N*-((4-(azetidinylpiperidinyl)benzamide (**12**):

To a solution of 108 mg of crude ketone **S2** in 1 mL of EtOH were added 71 mg (0.76 mmol) of azetidine hydrochloride, followed by 0.10 mL (0.76 mmol) of Et_3N and 0.22 mL (0.76 mmol) of $Ti(OiPr)_4$. The mixture was stirred for 15 h, after which 48 mg (0.76 mmol) of $NaBH_3CN$ were added, followed by another 12 h of stirring. Upon completion, the reaction was carefully quenched by addition of sat. aq. $NaHCO_3$ and the emulsions were reduced by complexation of the titanium salts with citric acid. The product was extracted with CH_2Cl_2 (6 × 15 mL) and the combined organic extracts were washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The product was taken up in 1 mL of methanol, the solution was filtered, and the crude product was purified by HPLC to afford 99 mg of the TFA salt of the title compound as colorless solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.63 (m, 1H), 7.61 – 7.57 (m, 1H), 7.43 – 7.36 (m, 2H), 4.82 – 4.55 (m, 1H), 4.31 – 4.07 (m, 4H), 3.95 – 3.62 (m, 1H), 3.47 (tt, J = 11.6, 4.0 Hz, 1H), 3.27 – 2.80 (m, 2H), 2.72 – 2.55 (m, 1H), 2.45 – 2.29 (m, 1H), 2.26 – 1.86 (m, 2H), 1.54 – 1.20 (m, 2H).

MS (ESI): 323 + 325 $[M+H]^+$; HPLC: 100%, t_R: 2.68 min.

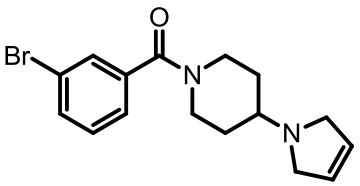


3-Bromo-*N*-((4-(1-methylpyrrolidinyl)piperidinyl)benzamide (**13**):

To a solution of 60 mg (0.2 mmol) of compound **2** in 2 mL of MeOH was added 123 mg (0.89 mmol) of K_2CO_3 followed by 0.06 mL (0.89 mmol) of MeI. The mixture was stirred for 12 h and another 1.5 eq. of the reagents were added until the reaction was completed as judged by LCMS analysis. Upon completion, the mixture was filtered and the crude product was purified by HPLC to afford 80 mg (96%) of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.71 – 7.63 (m, 2H), 7.47 – 7.37 (m, 2H), 4.84 – 4.73 (m, 1H), 3.96 – 3.79 (m, 1H), 3.73 – 3.56 (m, 3H), 3.56 – 3.45 (m, 2H), 3.28 – 3.15 (m, 1H), 2.96 (s, 3H), 2.94 – 2.82 (m, 1H), 2.32 – 2.02 (m, 6H), 2.02 – 1.84 (m, 2H).

MS (ESI): 351 + 353 $[M]^+$; HPLC: 100%, t_R: 2.14 min.

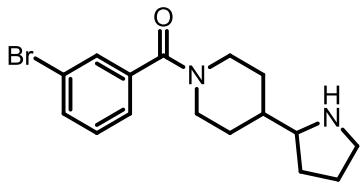


3-Bromo-*N,N*-((4-(3-pyrrolinylpiperidinyl)benzamide (**14**): To a solution of 114 mg of crude ketone **S2** in 1 mL of EtOH were added 61 µl (0.81 mmol) of 3-pyrroline, followed by 0.11 mL (0.81 mmol) of Et_3N and 0.24 mL (.81 mmol) of $Ti(OiPr)_4$. The mixture was stirred for 15 h, after which 51 mg (0.81 mmol) of $NaBH_3CN$ were added, followed by another 12 h of stirring. Upon completion, the reaction was carefully quenched by addition of sat. aq. $NaHCO_3$ and the emulsions were reduced by complexation of the titanium salts with citric acid. The product was extracted with CH_2Cl_2 (6 × 15 mL) and the combined organic extracts were washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The product was taken up

in 1 mL of methanol, the solution was filtered, and the crude product was purified by HPLC to afford 114 mg (85%) of the TFA salt of the title compound as colorless solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.70 – 7.64 (m, 1H), 7.64 – 7.60 (m, 1H), 7.44 – 7.37 (m, 2H), 6.04 – 5.98 (m, 2H), 4.84 – 4.70 (m, 1H), 4.42 – 4.00 (m, 4H), 3.98 – 3.69 (m, 1H), 3.63 (tt, J = 11.8, 4.0 Hz, 1H), 3.29 – 2.85 (m, 2H), 2.43 – 1.93 (m, 2H), 1.84 – 1.44 (m, 2H).

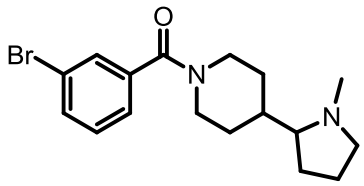
MS (ESI): 335 + 337 $[M+H]^+$; HPLC: 100%, t_R: 3.36 min.



3-Bromo-*N*-((4-(pyrrolidin-2-yl)piperidinyl)benzamide (**15**): The general procedure was followed with 79 mg (0.4 mmol) of 3-bromobenzoic acid, 100 mg (0.4 mmol) of 1-Boc-(4-pyrrolidine-2-yl)-piperidine, 126 mg (0.5 mmol) of TBTU in 2 mL of DMF and 0.01 mL (0.8 mmol) of NEt_3. After purification by column chromatography, 164 mg (95%) of the title compound were obtained as a light yellow oil. The boc-protected amine was taken up in 15 mL of CH_2Cl_2 and 2 mL of TFA were added to cleave the protecting group at rt overnight. Upon completion of the reaction, 0.15 mmol (ca. 6 mL) were purified by HPLC to afford 68 mg (quantitative) of the title compound as a light solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.68 – 7.62 (m, 1H), 7.61 – 7.57 (m, 1H), 7.43 – 7.36 (m, 2H), 4.76 – 4.58 (m, 1H), 3.83 – 3.65 (m, 1H), 3.34 – 3.23 (m, 3H), 3.22 – 3.06 (m, 1H), 2.98 – 2.78 (m, 1H), 2.32 – 2.17 (m, 1H), 2.18 – 1.97 (m, 2H), 1.97 – 1.83 (m, 2H), 1.83 – 1.63 (m, 2H), 1.51 – 1.23 (m, 2H).

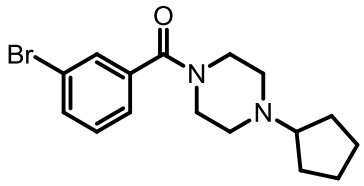
MS (ESI): 337 + 339 $[M+H]^+$; HPLC: 100%, t_R: 3.78 min.



3-Bromo-*N*-((4-(1-methylpyrrolidin-2-yl)piperidinyl)benzamide (**16**): To a solution of 56 mg (0.13 mmol) of 3-bromo-*N*-((4-(pyrrolidin-2-yl)piperidinyl)benzamide (**15**) in 2 mL of MeOH were added ca. 41 µL (0.5 mmol) of formaldehyde (37%), followed by 20 µL (0.38 mmol) of acetic acid. The mixture was stirred for 15 h and 132 mg (0.63 mmol) of $NaBH(OAc)_3$ were added, followed by another 3 h of stirring. Upon completion of the reaction, the solvent was removed, the mixture was taken up in 1 mL of methanol, the solution was filtered, and the crude product was purified by HPLC to afford 41 mg (71%) of the TFA salt of the title compound as white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.68 – 7.62 (m, 1H), 7.61 – 7.57 (m, 1H), 7.43 – 7.36 (m, 2H), 4.80 – 4.59 (m, 1H), 3.89 – 3.68 (m, 1H), 3.68 – 3.58 (m, 1H), 3.35 – 3.32 (m, 1H), 3.26 – 3.08 (m, 2H), 2.96 (s, 3H), 2.92 – 2.73 (m, 1H), 2.32 – 2.19 (m, 1H), 2.19 – 2.06 (m, 2H), 2.06 – 1.97 (m, 1H), 1.97 – 1.58 (m, 3H), 1.55 – 1.25 (m, 2H).

MS (ESI): 351 + 353 $[M+H]^+$; HPLC: 100%, t_R: 3.70 min.

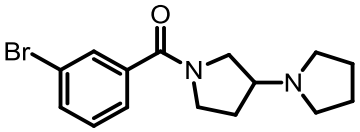


3-Bromo-*N*´-(cyclopentyl)piperazinylbenzamide (**17**): The general procedure was followed with 100 mg (0.5 mmol) of 3-bromobenzoic acid, 80 mg (0.5 mmol) of 1-cyclopentylpiperidine, 166 mg (0.5 mmol) of TBTU in 2 mL of DMF and 0.1 mL (0.8 mmol) of NEt_3. After purification by HPLC, 264 mg (quantitative) of the TFA salt of the title compound were obtained as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.72 – 7.68 (m, 2H), 7.49 – 7.39 (m, 2H), 4.81 – 4.32 (m, 1H), 4.31 – 3.36 (m, 6H), 3.28 – 2.93 (m, 2H), 2.26 – 2.10 (m, 2H), 1.95 – 1.60 (m, 6H).

^{13}C NMR (101 MHz, CD_3OD) δ 170.57, 137.60, 134.76, 131.77, 131.30, 127.00, 123.75, 69.26, 51.91, 29.19, 24.65.

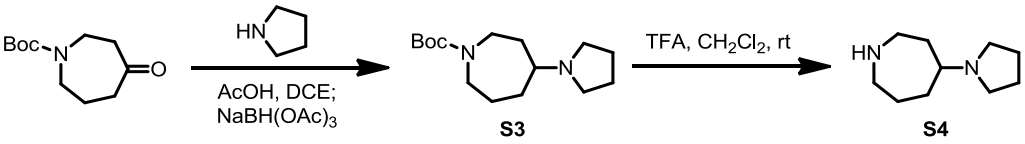
MS (ESI): 337 + 339 $[M+H]^+$; HPLC: 100%, t_R: 3.40 min.



***Rac*-3-Bromo-*N*-(3-pyrrolidinyl)pyrrolidinylbenzamide (18)**: The general procedure was followed with 60 mg (0.3 mmol) of 3-bromobenzoic acid, 42 mg (0.3 mmol) of 1,3′-bipyrrolidine, 96 mg (0.3 mmol) of TBTU in 1.5 mL of DMF and 0.1 mL (0.5 mmol) of NEt_3. After purification by HPLC, 123 mg (94%) of the TFA salt of the title compound were obtained as a white solid.

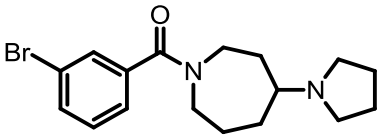
^{1}H NMR (400 MHz, CD_3OD) δ 7.72 (t, J = 1.7 Hz, 1H), 7.68 (dd, J = 8.0, 1.7 Hz, 1H), 7.55 – 7.50 (m, 1H), 7.41 (t, J = 7.8 Hz, 1H), 4.10 – 3.57 (m, 7H), 3.29 – 2.95 (m, 2H), 2.58 – 2.38 (m, 1H), 2.34 – 1.93 (m, 5H).

MS (ESI): 323 + 325 $[M+H]^+$; HPLC: 100%, t_R: 3.28 min.



***Tert*-Butyloxycarbonyl-4-(1-pyrrolidinyl)azepane (S3)**: To a solution of N-Boc-hexahydro-1H-azepin-4-one (950 µl, 4.7 mmol) in 20 mL of DCE, 1.32 mL of pyrrolidine (770 µl, 2.64 mmol) was added followed by 0.75 mL (11.7 mmol) of AcOH. The mixture was stirred at rt for 12 h and then 2.5 g (11.7 mmol) of $NaBH(OAc)_3$ were added in one portion. After 2 h, the reaction was quenched by addition of sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$, dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. Crude **S3** was carried forward without further purification.

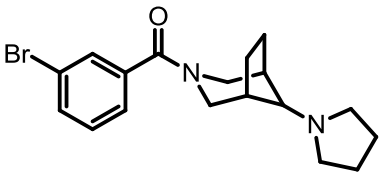
4-(1-Pyrrolidinyl)azepane (S4): To a solution of crude **S3** (1.4 g) in 30 mL of CH_2Cl_2, 3.5 mL of TFA was added. The reaction was stirred for 15 hours and then the solvent was evaporated. Crude **S4** was carried forward without further purification.



3-Bromo-*N*-((4-(piperidinyl)azepane)benzamide (**19**): The general procedure was followed with 50 mg (0.25 mmol) of 3-bromobenzoic acid, 260 mg of crude 4-(1-pyrrolidinyl)azepane **S4**, 279 mg (0.87 mmol) of TBTU in 2 mL of DMF and 0.27 mL (2.0 mmol) of NEt_3. After purification by HPLC, 169 mg of the TFA salt of the title compound were obtained as a light yellow solid. For characterization purposes, 50 mg of **19** was converted to the free base by dissolving in CH_2Cl_2 and then washing with aq. KOH (3 × 10 mL). The solvent was removed by rotary evaporation to give pure **19** as a free base.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.42 (m, 2H), 7.27 – 7.16 (m, 2H), 3.78 – 3.47 (m, 2H), 3.47 – 3.15 (m, 2H), 2.59 – 2.47 (m, 2H), 2.48 – 2.36 (m, 2H), 2.32 – 2.19 (m, 1H), 2.10 – 1.39 (m, 10H).

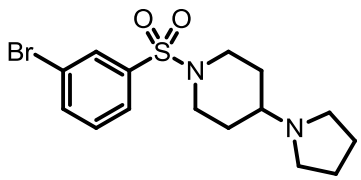
MS (ESI): 351 + 353 $[M+H]^+$; HPLC: 100%, t_R: 2.88 min.



3-Bromo-*N*-(3-(1-pyrrolidinyl-8-azabicyclo[3.2.1]octane))benzamide (**20**):

The general procedure was followed with 40 mg (0.20 mmol) of 3-bromobenzoic acid, 61 mg (0.24 mmol) of 3-(1-pyrrolidinyl-8-azabicyclo[3.2.1]octane, 96 mg (0.30 mmol) of TBTU in 2 mL of DMF and 0.06 mL (0.40 mmol) of NEt_3. After purification by HPLC, 136 mg (quantitative) of the title compound were obtained as an off-white solid.

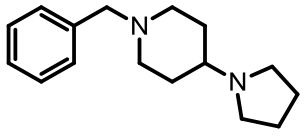
^{1}H NMR (400 MHz, CD_3OD) δ 7.73 – 7.65 (m, 2H), 7.51 – 7.39 (m, 2H), 4.85 – 4.76 (m, 1H), 4.34 – 4.13 (m, 1H), 3.79 – 3.68 (m, 1H), 3.69 – 3.53 (m, 2H), 3.24 – 2.99 (m, 2H), 2.40 – 2.07 (m, 6H), 2.07 – 1.91 (m, 3H), 1.91 – 1.78 (m, 2H), 1.78 – 1.57 (m, 1H).



3-Bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzenesulfonamide (21): To a stirring solution of 108 mg (0.70 mmol) of 4-(1-pyrrolidinyl)piperidine and 0.11 mL of NEt_3 in 2 mL of CH_2Cl_2 was added 100 mg (0.39 mmol) of 3-bromobenzenesulfonylchloride at 0°C. The mixture was allowed to warm to rt and stirred for 14 hours. Upon completion of the reaction, it was quenched by addition of sat. aq $NaHCO_3$ and the layers were separated. The aq. phase was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The product was taken up in 1 mL of methanol, the solution was filtered, and the crude product was purified by HPLC to afford 114 mg (82%) of the TFA salt of the title compound as colorless solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (t, J = 1.8 Hz, 1H), 7.89 – 7.85 (m, 1H), 7.80 – 7.76 (m, 1H), 7.56 (t, J = 7.9 Hz, 1H), 3.97 – 3.89 (m, 2H), 3.70 – 3.56 (m, 2H), 3.17 – 3.03 (m, 3H), 2.43 (td, J = 12.4, 2.3 Hz, 2H), 2.27 – 2.19 (m, 2H), 2.19 – 2.07 (m, 2H), 2.05 – 1.92 (m, 2H), 1.73 (ddd, J = 24.6, 12.4, 4.1 Hz, 2H).

MS (ESI): 373 + 375 $[M+H]^+$; HPLC: 100%, t_R: 3.45 min.

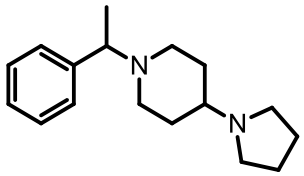


1-Benzyl-4-(pyrrolidin-1-yl)piperidine (22): To a solution of 100 mg (0.53 mmol) of 1-benzyl-4-piperidinone in 2 mL of MeOH was added 56 mg (0.8 mmol) of pyrrolidine and 0.3 mL (5.24 mmol) of AcOH at rt. The mixture was stirred for 15 h and 100 mg

(1.6 mmol) of $NaBH_3CN$ was added in one portion. Upon completion of the reaction, the solvent was removed under reduced pressure, the crude product taken up in 1 mL of MeOH, filter and the purified by HPLC to afford 35 mg (14%) of the bis-TFA salt of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.54 – 7.46 (m, 5H), 4.35 (s, 2H), 3.82 – 3.53 (m, 4H), 3.44 (tt, J = 11.7, 3.9 Hz, 1H), 3.28 – 2.99 (m, 4H), 2.48 – 2.36 (m, 2H), 2.27 – 1.89 (m, 6H).

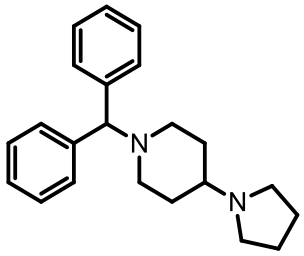
MS (ESI): 245 $[M+H]^+$; HPLC: 90%, t_R: 1.10 min.



1-(1-Phenylethyl)-4-(pyrrolidin-1-yl)piperidine (**23**): To a solution of 60 mg (0.5 mmol) of acetophenone in 5 mL of CH_2Cl_2 was added 154 mg (1 mmol) of 4-(1-pyrrolidinyl)-piperidine and 0.22 mL (0.8 mmol) of Ti(iOPr)$_4$ at rt. The mixture was stirred for 15 h and 528 mg (2.5 mmol) of $NaBH(OAc)_3$ were added. Upon completion, the reaction was quenched by the addition of 10 mL of sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by HPLC to afford 261 mg (quantitative) of the bis-TFA salt of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ δ 7.55 – 7.48 (m, 5H), 4.51 (q, J = 6.9 Hz, 1H), 3.96 – 3.82 (m, 1H), 3.76 – 3.57 (m, 2H), 3.54 – 3.46 (m, 1H), 3.39 – 3.32 (m, 1H), 3.17 – 3.05 (m, 2H), 3.04 – 2.95 (m, 1H), 2.89 (s, 1H), 2.49 – 2.33 (m, 2H), 2.21 – 1.92 (m, 6H), 1.78 (d, J = 7.0 Hz, 3H).

MS (ESI): 259 $[M+H]^+$; HPLC: 100%, t_R: 0.94 min.



1-Benzhydryl-4-(pyrrolidin-1-yl)piperidine (**24**): To a solution of 80 mg (0.4 mmol) of benzophenone in 5 mL of CH_2Cl_2 was added 136 mg (0.9 mmol) of 4-(1-pyrrolidinyl)-piperidine and 0.2 mL (0.7 mmol) of Ti(*i*OPr)$_4$ at rt. The mixture was stirred for 15 h and 465 mg (2.2 mmol) of $NaBH(OAc)_3$ were added. Upon completion, the reaction was quenched by the addition of 10 mL of sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by HPLC to afford 22 mg (9%) of the bis-TFA salt of the title compound as a white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 7.68 – 7.61 (m, 4H), 7.52 – 7.38 (m, 6H), 5.34 (s, 1H), 3.79 – 3.55 (m, 2H), 3.52 – 3.38 (m, 3H), 3.19 – 3.10 (m, 2H), 3.05 (t, J = 13.1 Hz, 2H), 2.39 – 2.29 (m, 2H), 2.25 – 1.95 (m, 6H).

MS (ESI): 321 $[M+H]^+$; HPLC: 100%, t_R: 2.88 min.

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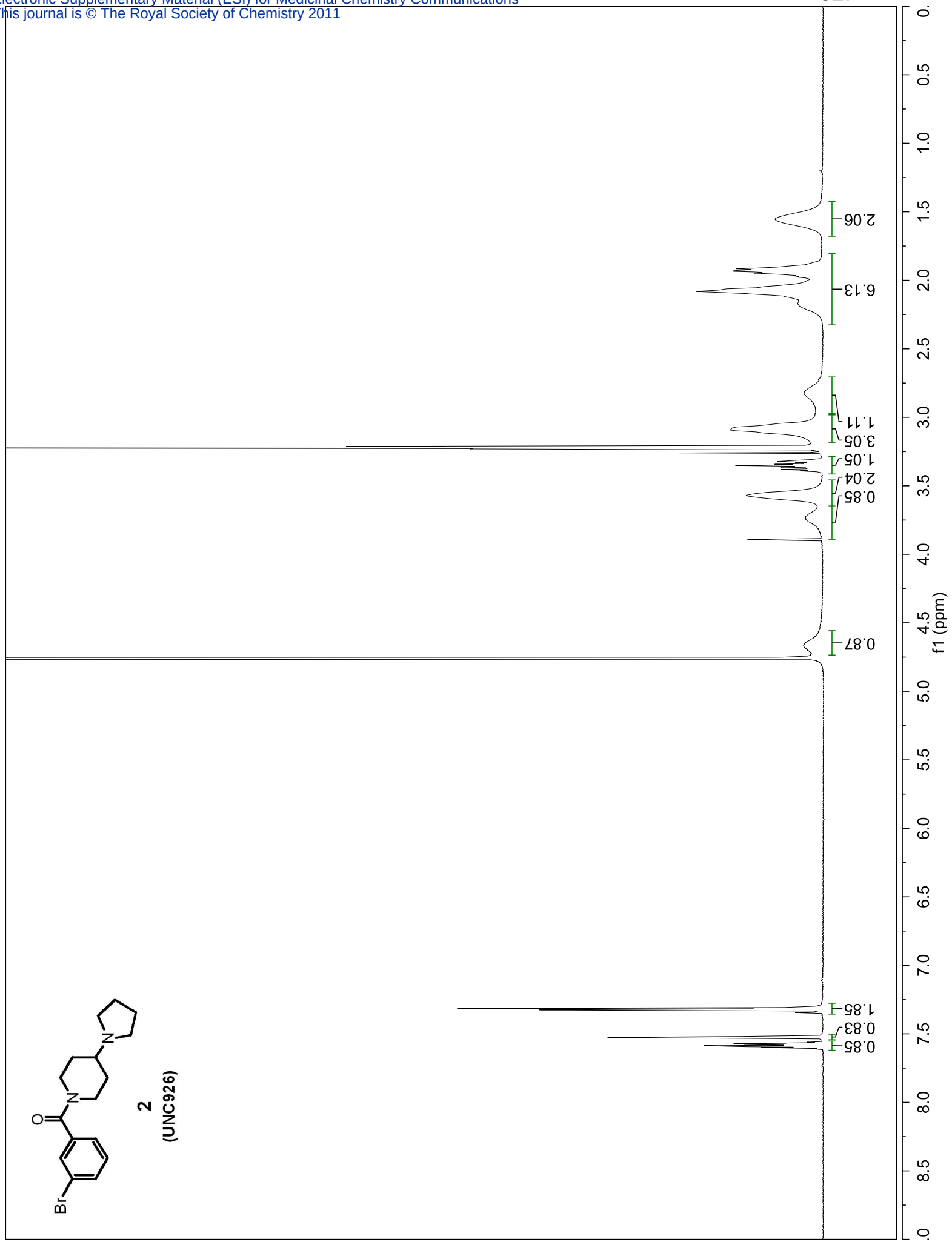

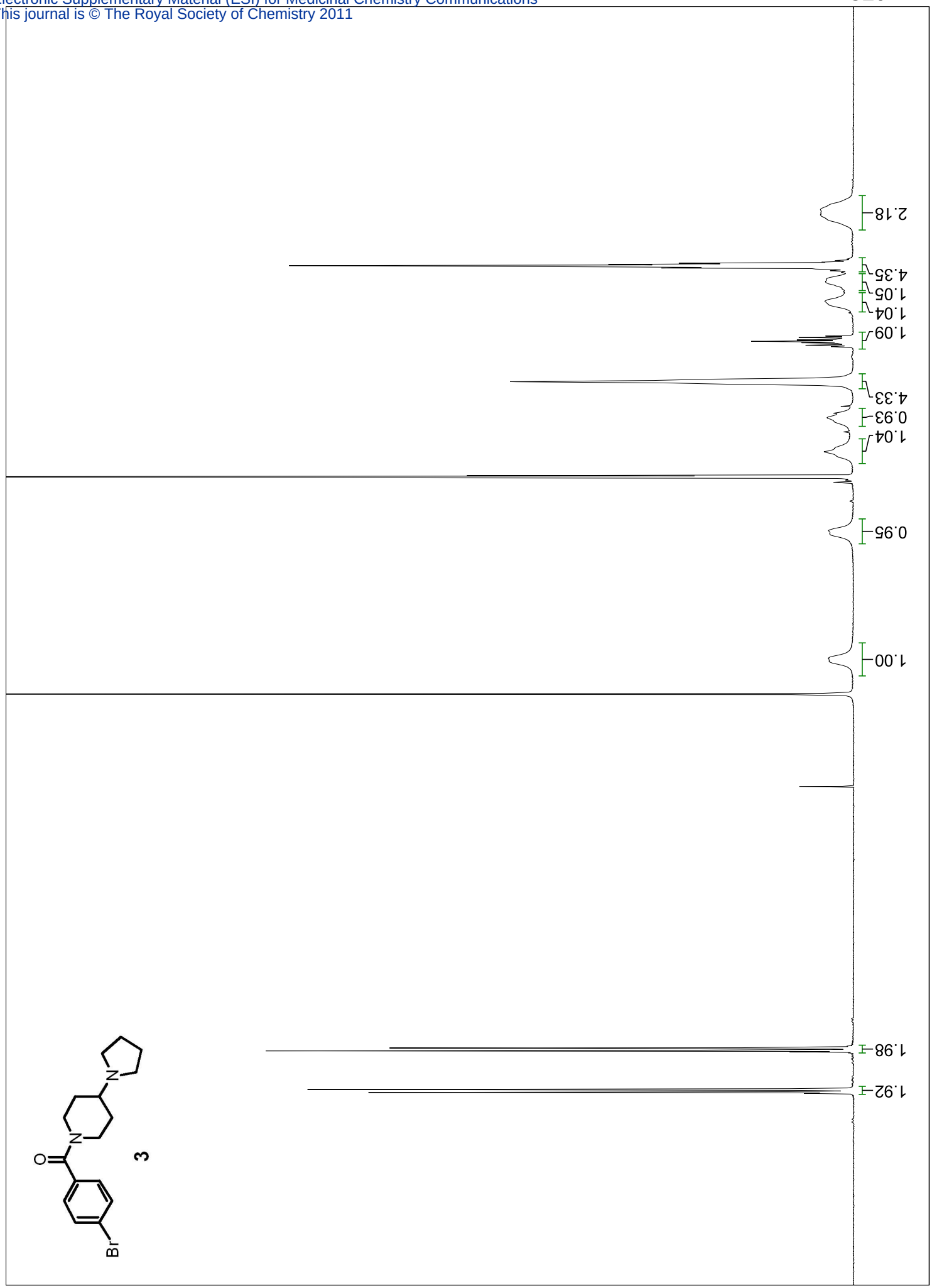

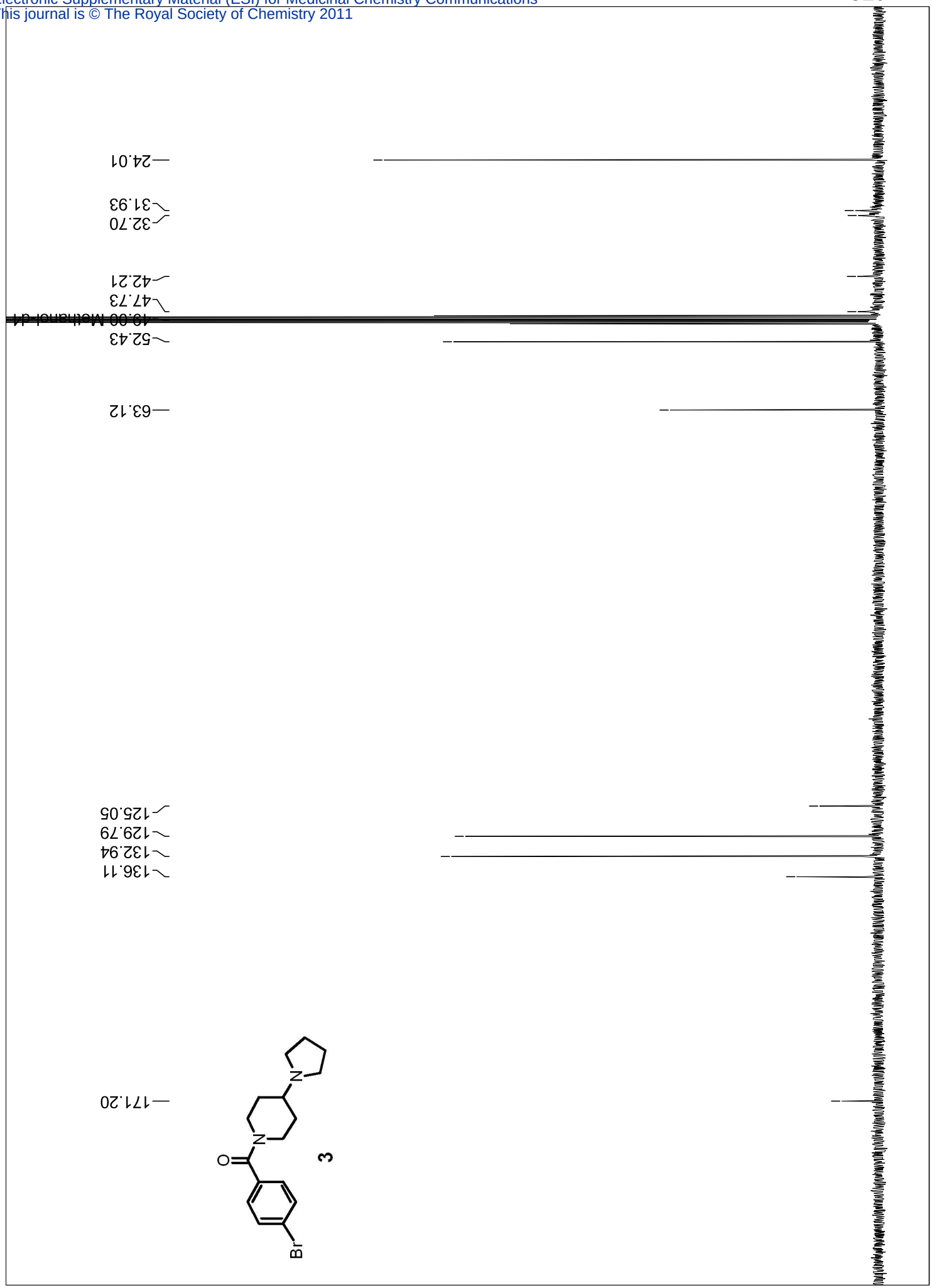

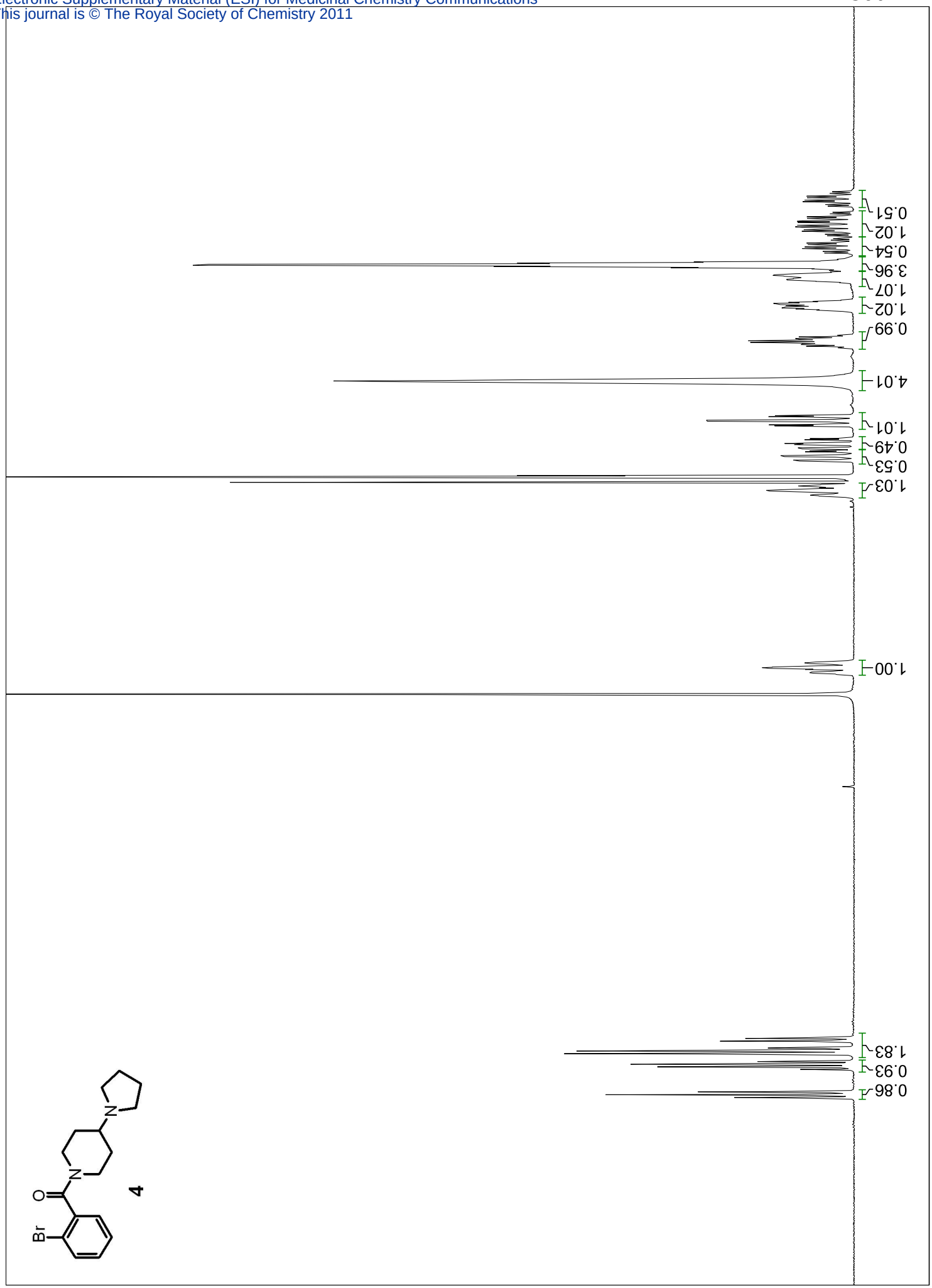

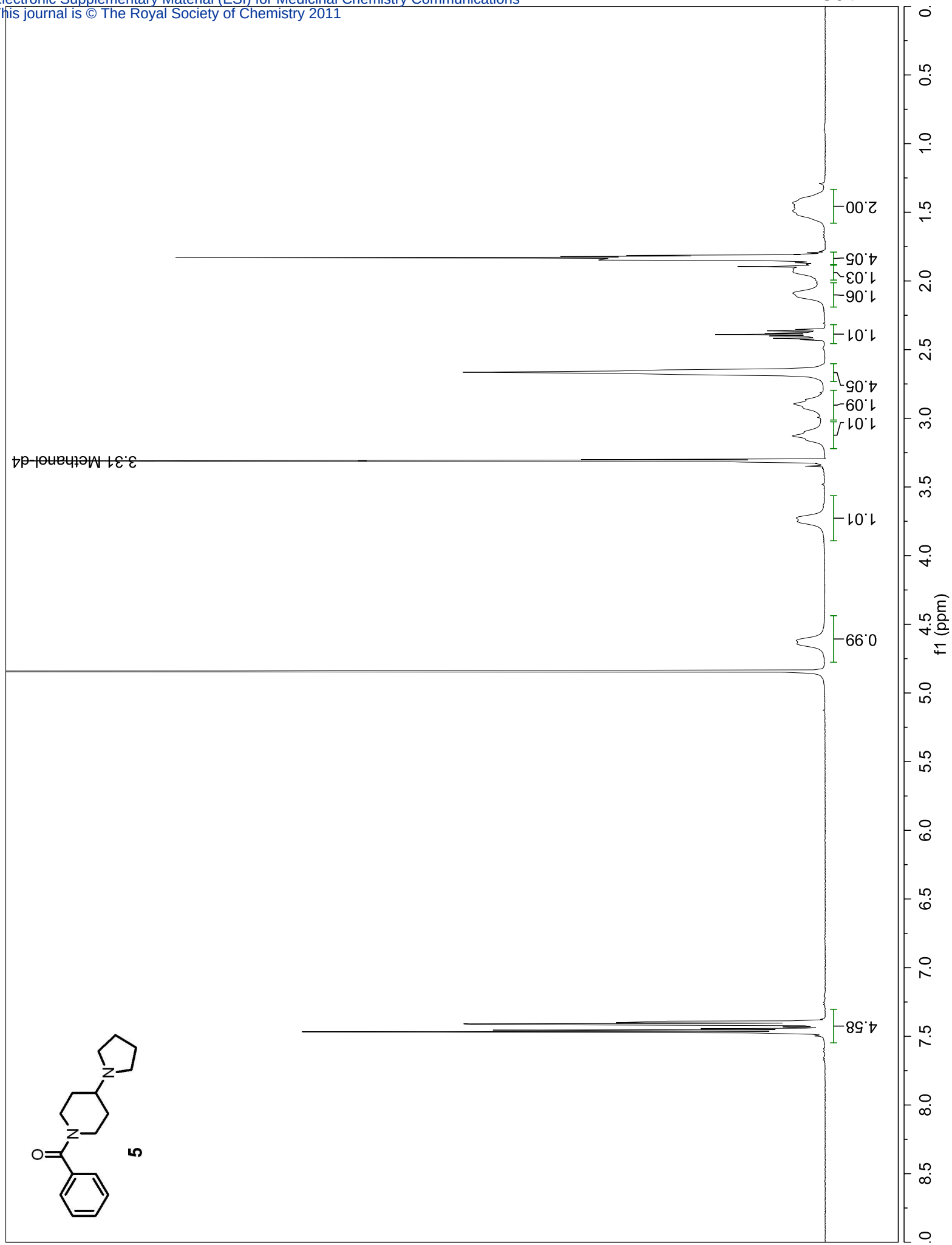

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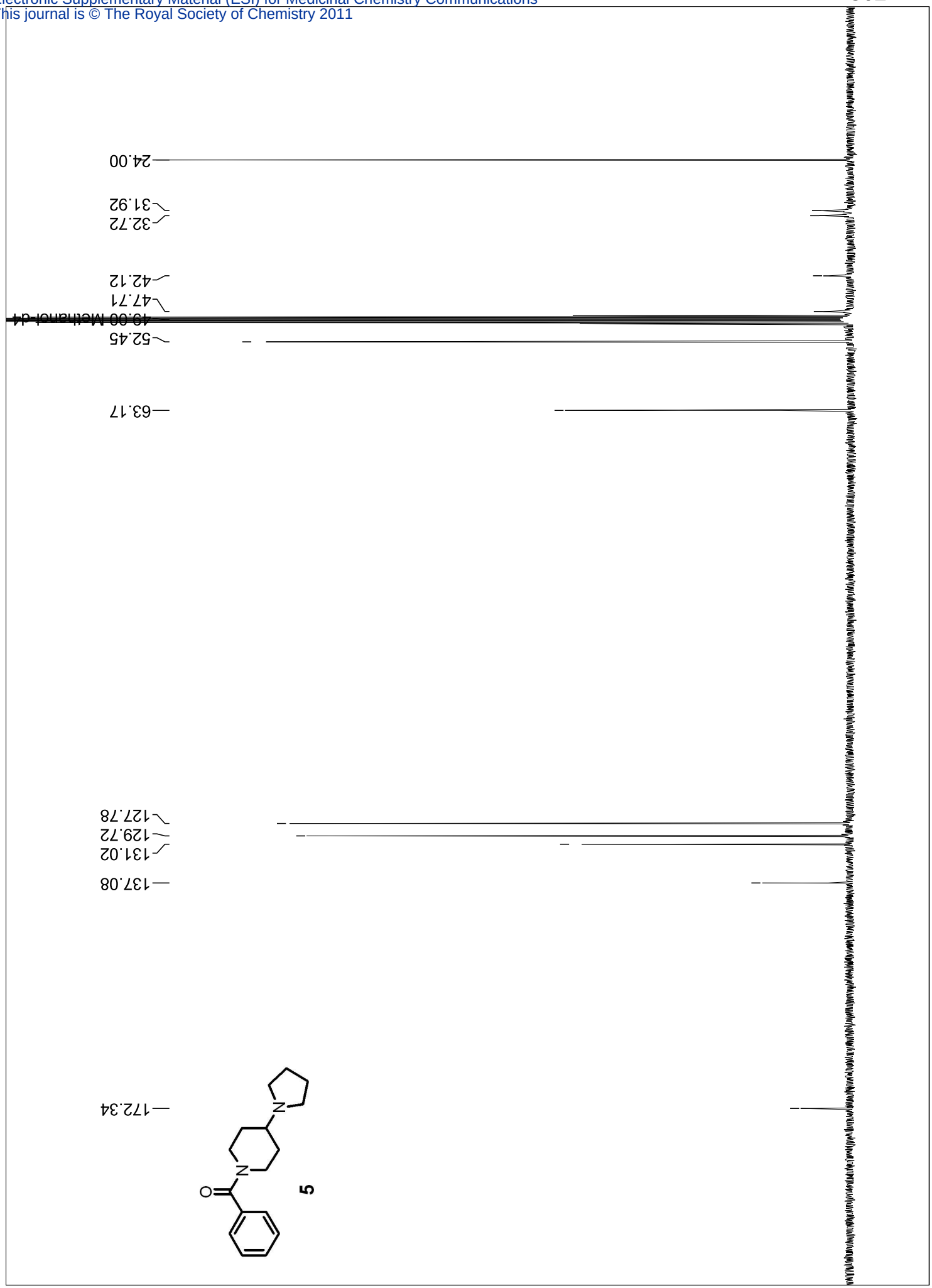

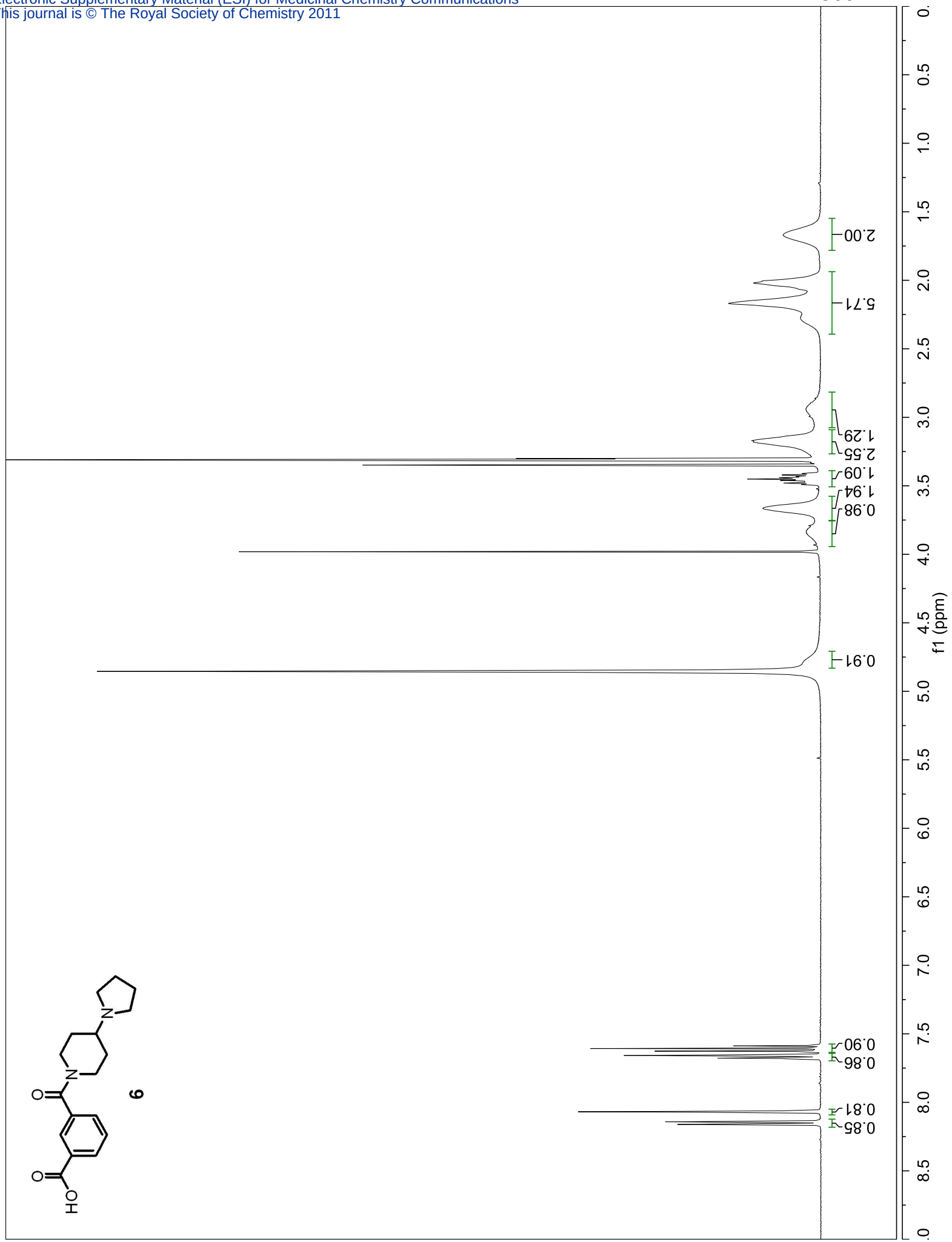

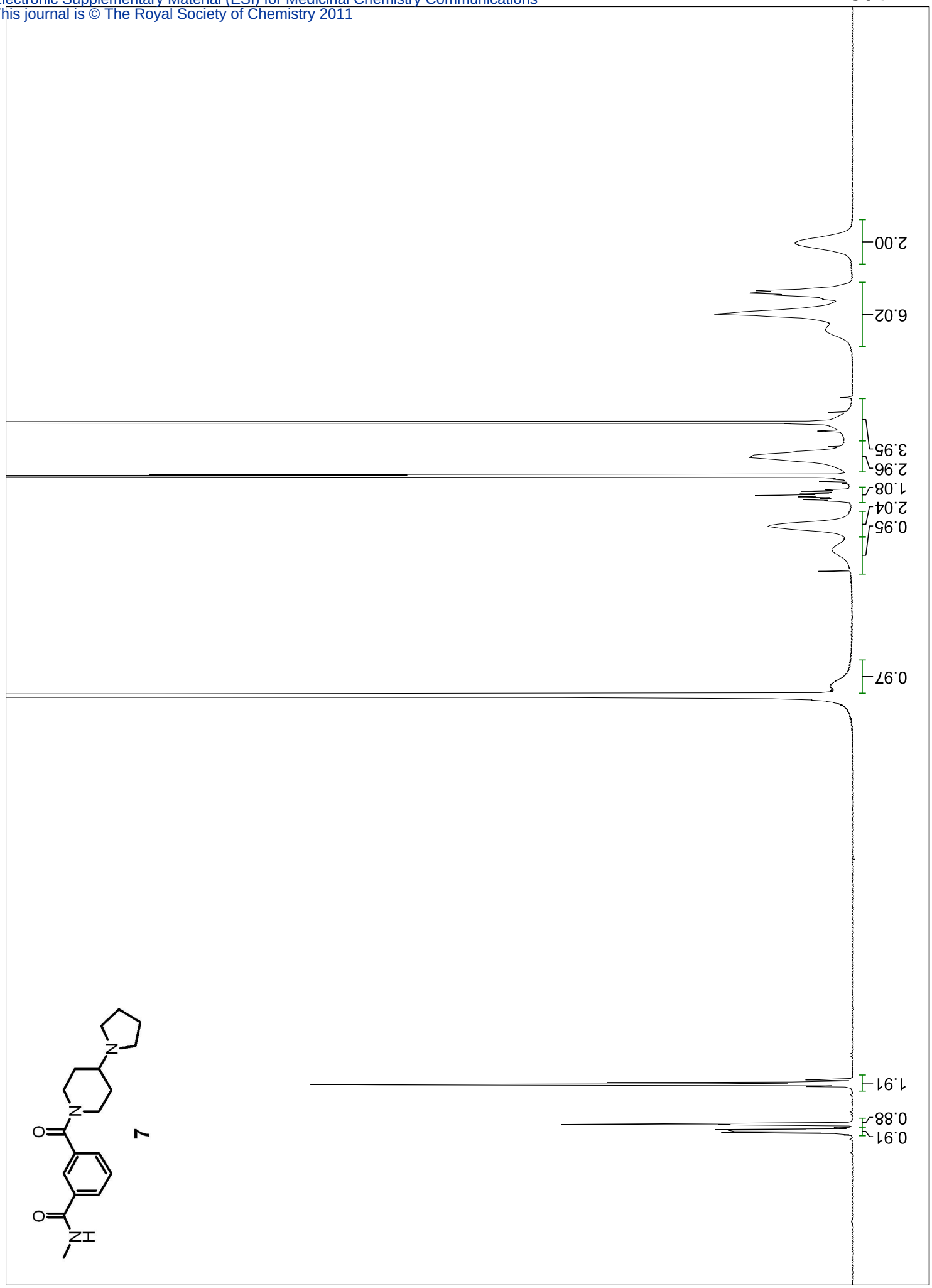

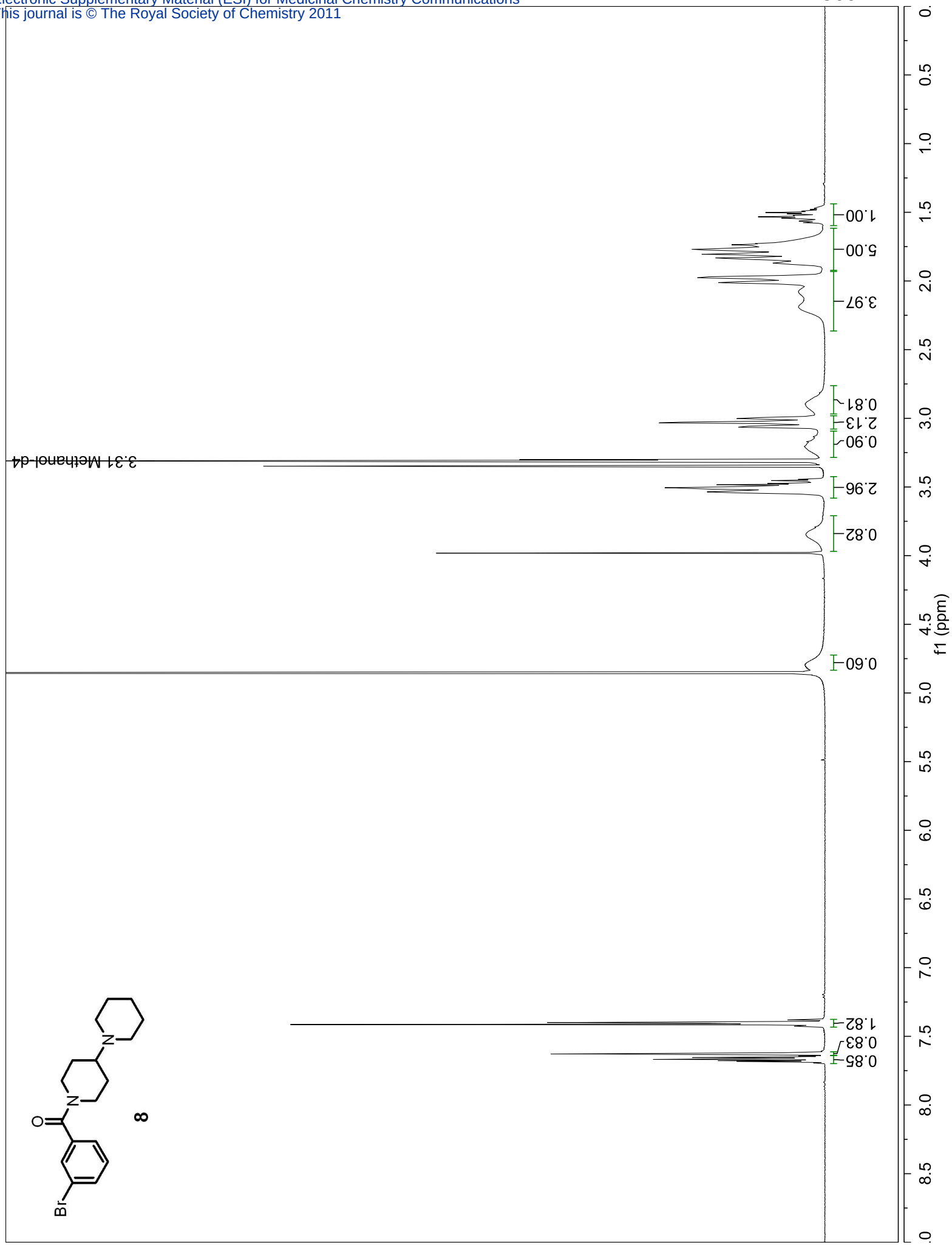

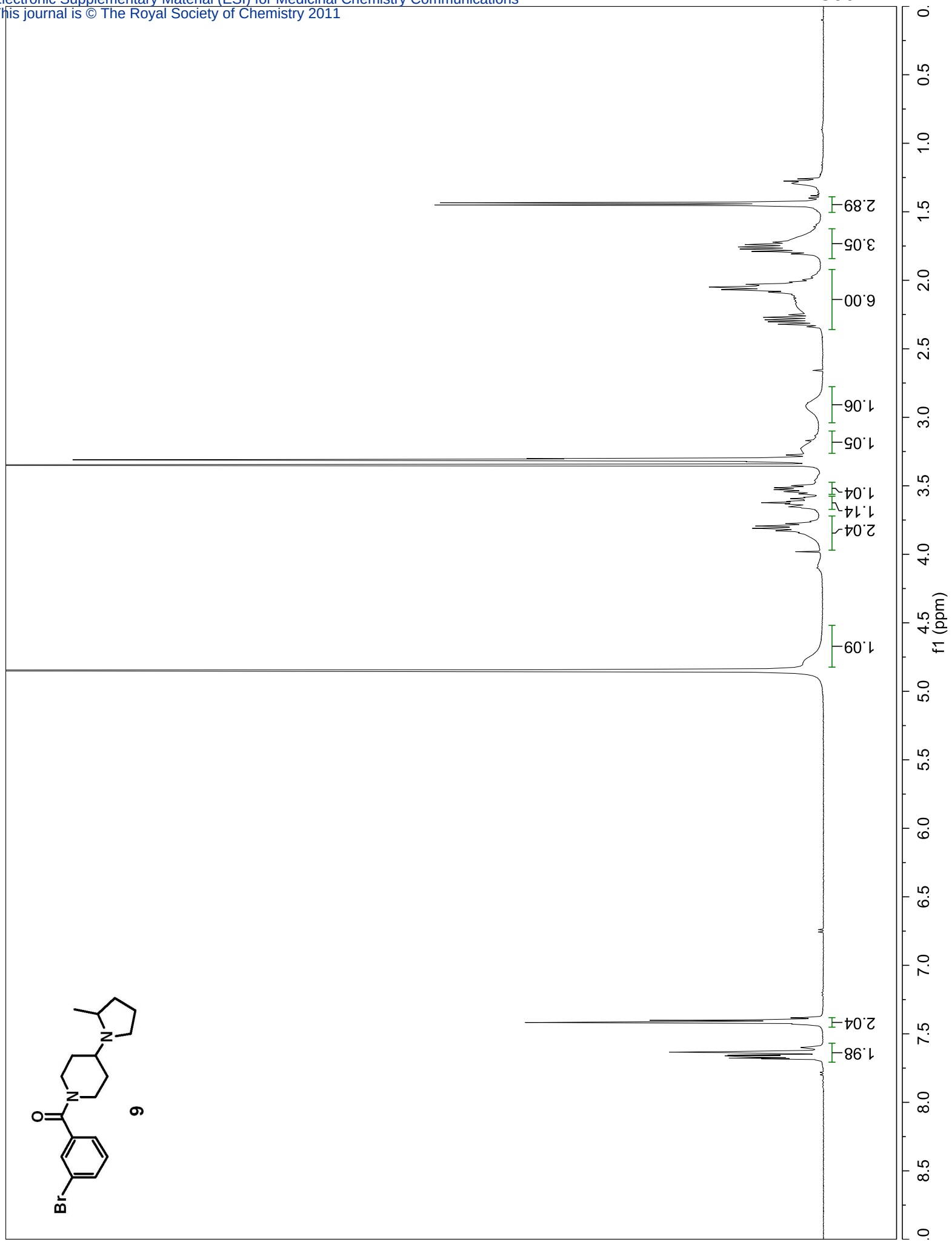

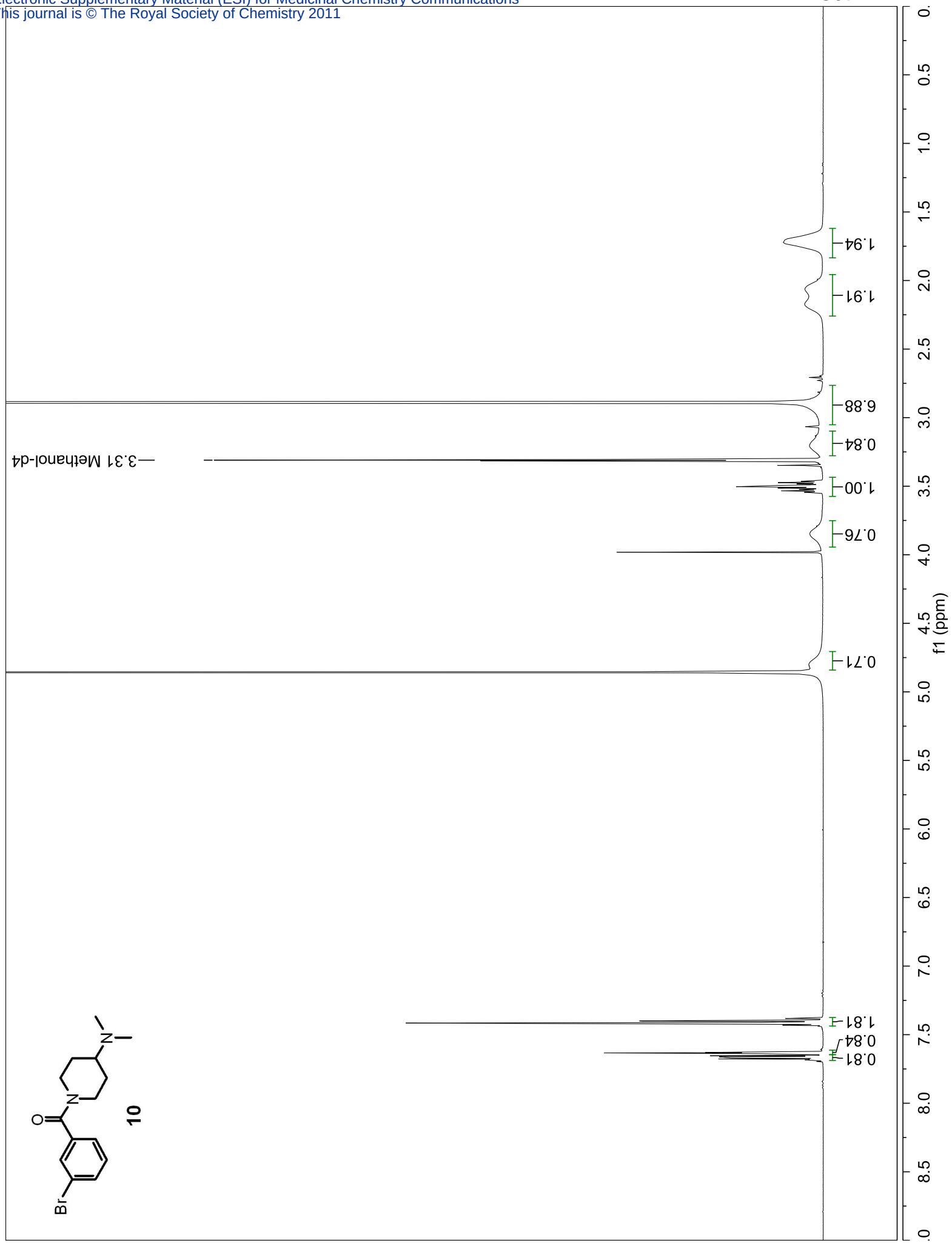

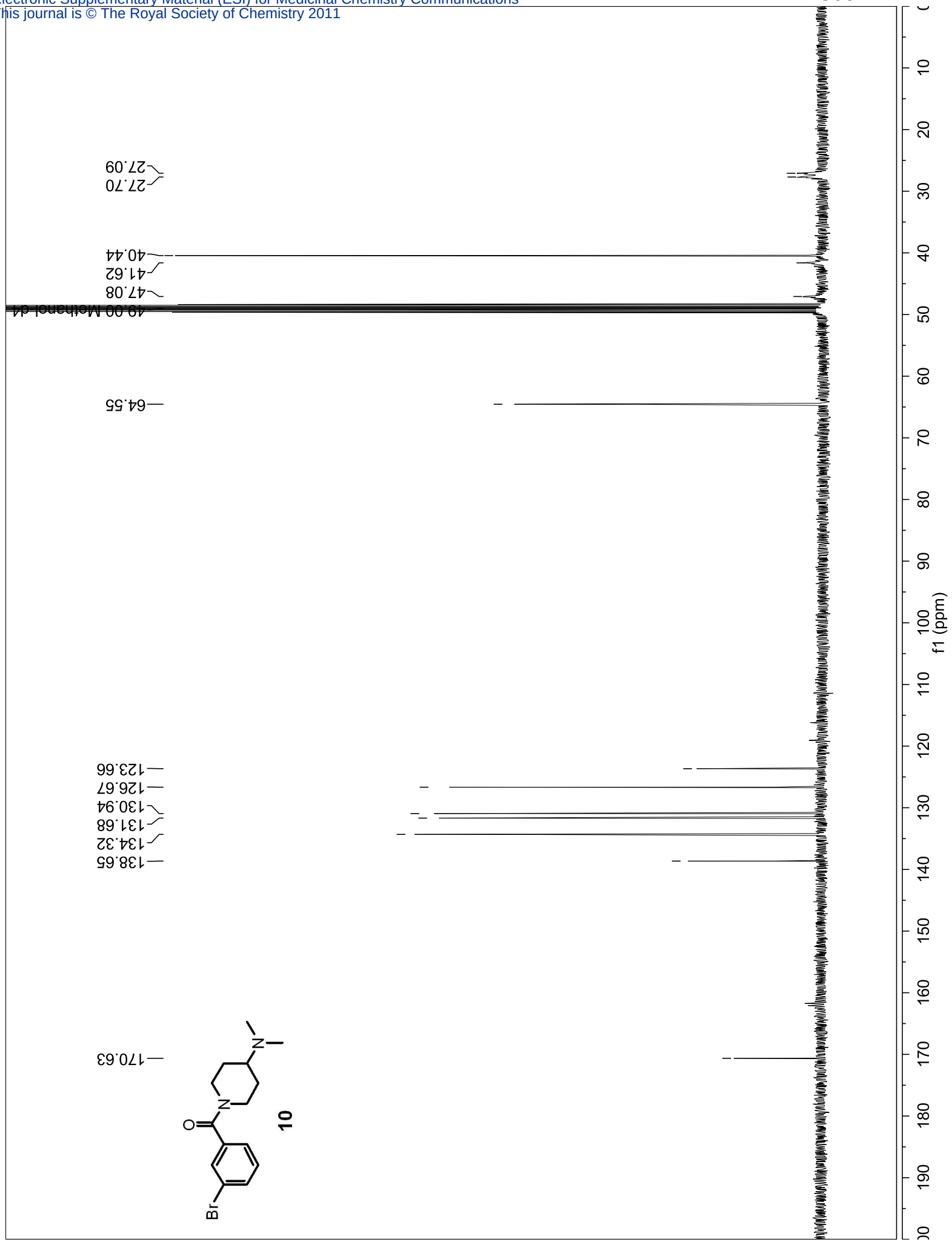

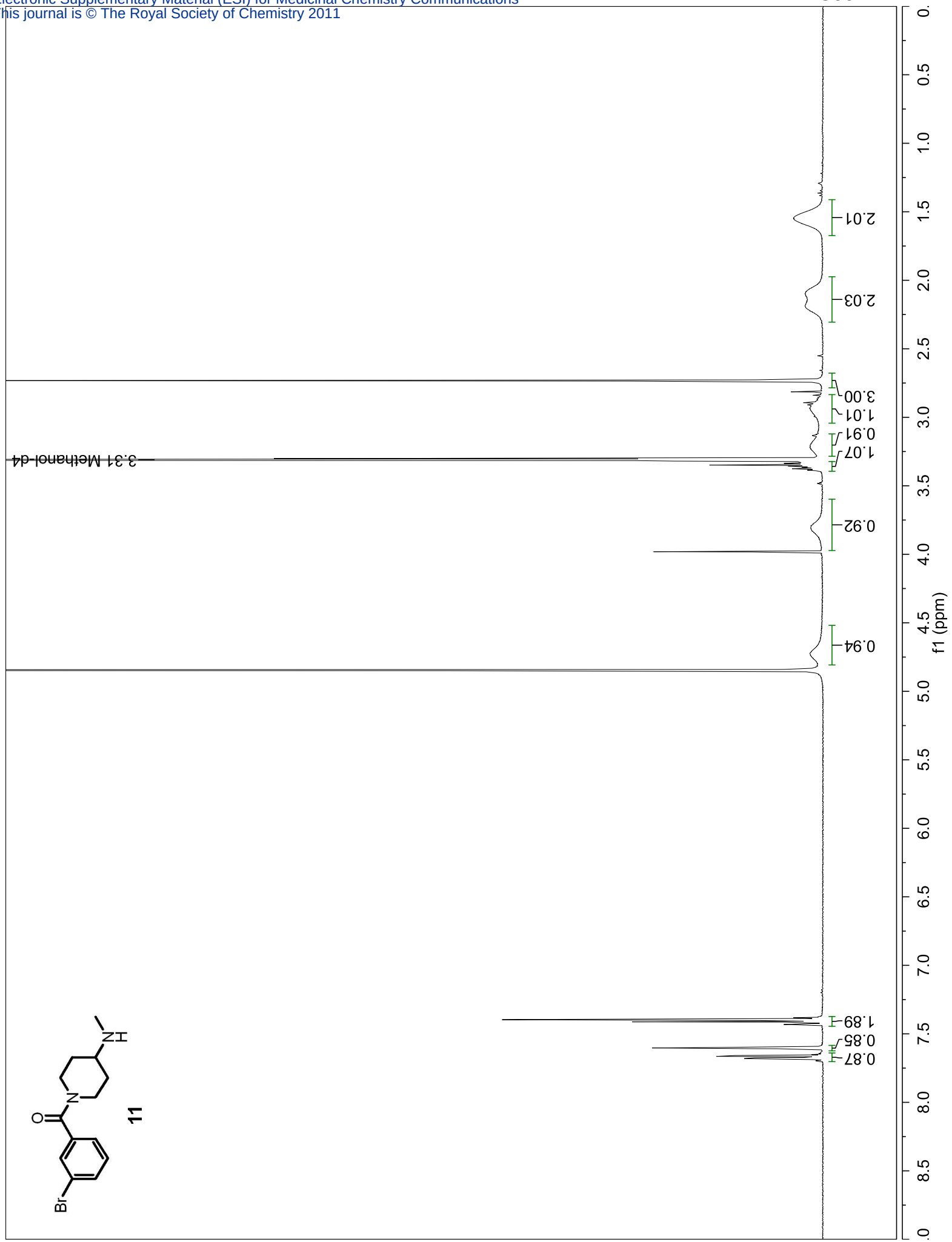

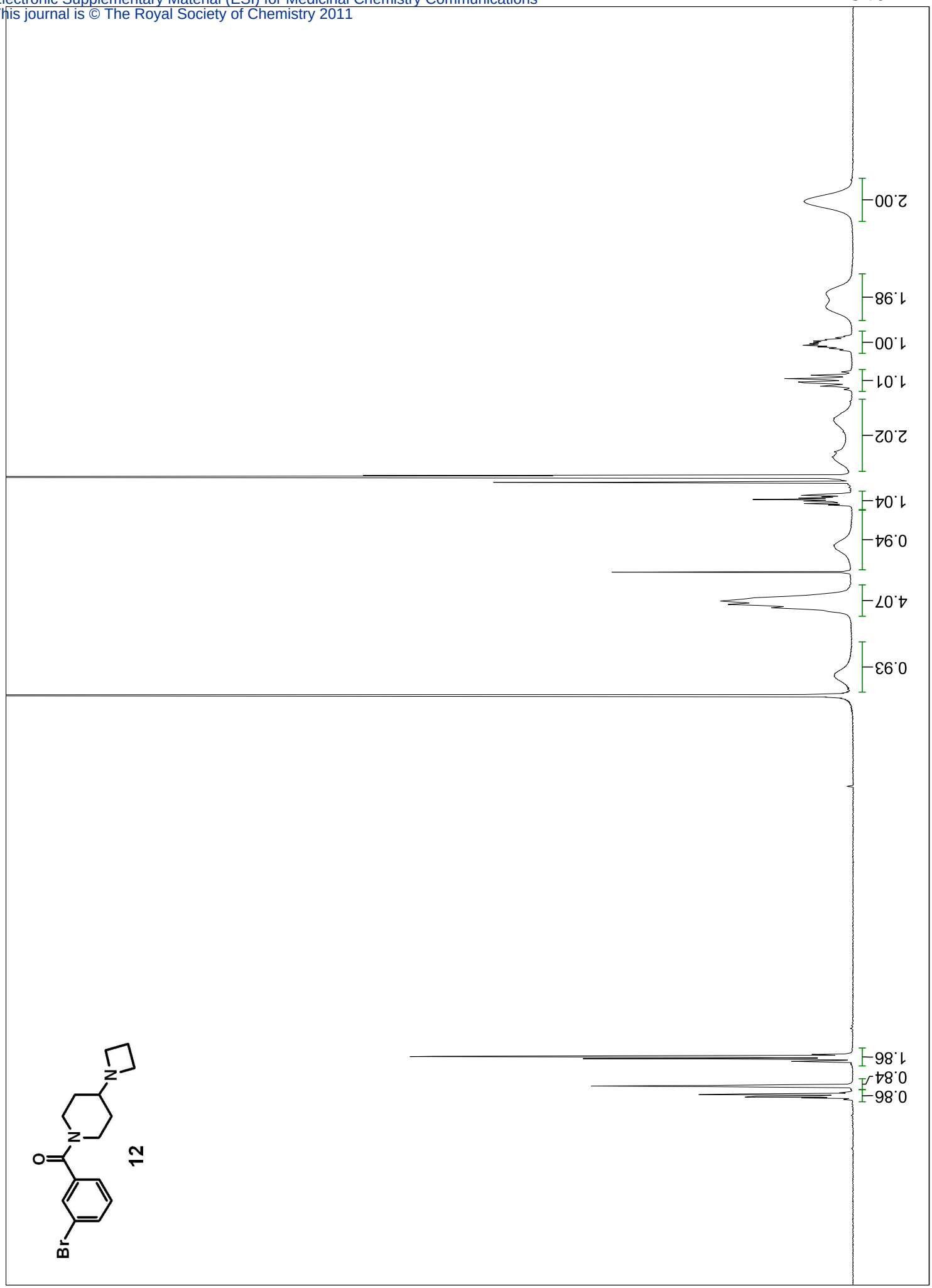

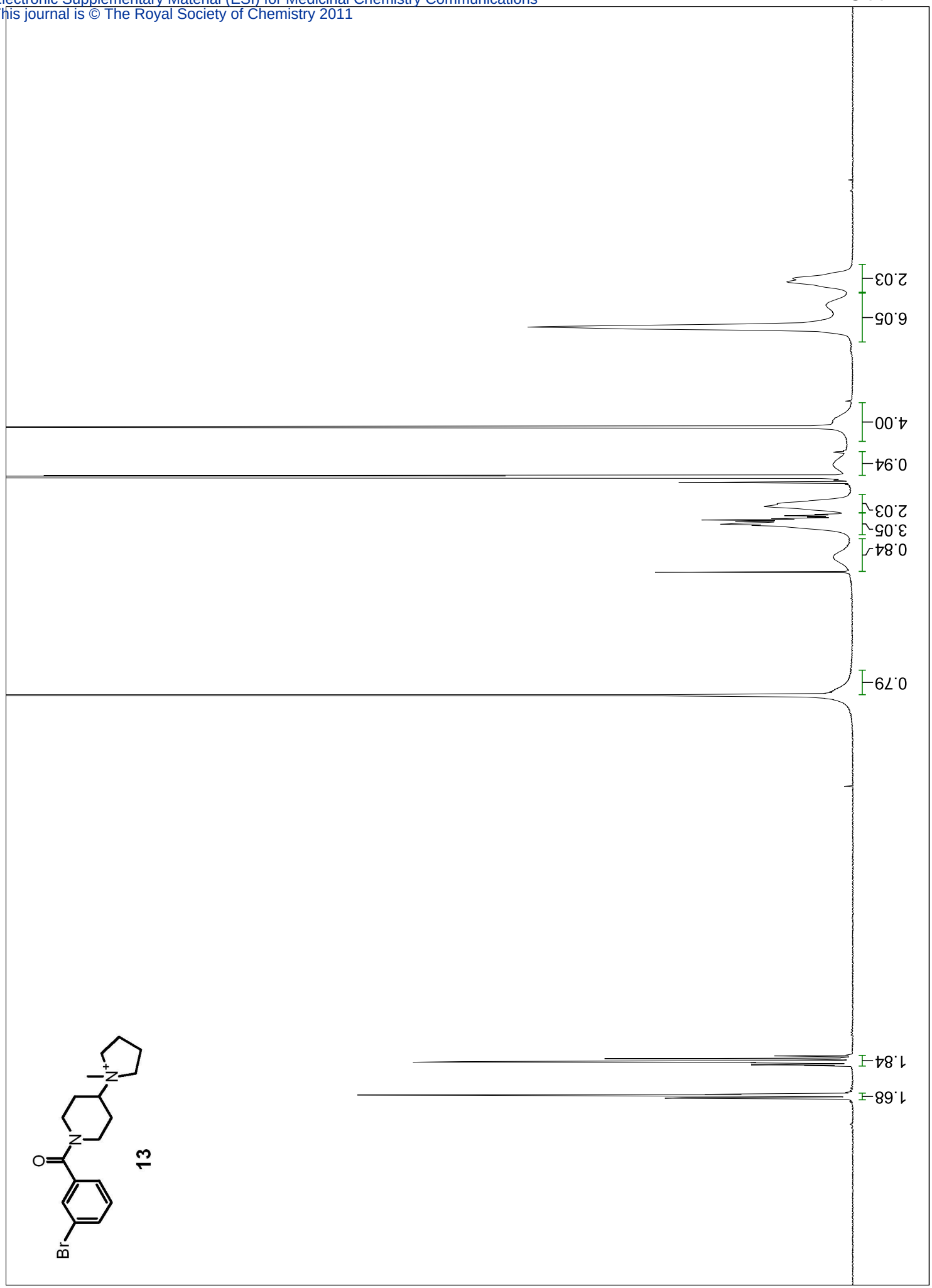

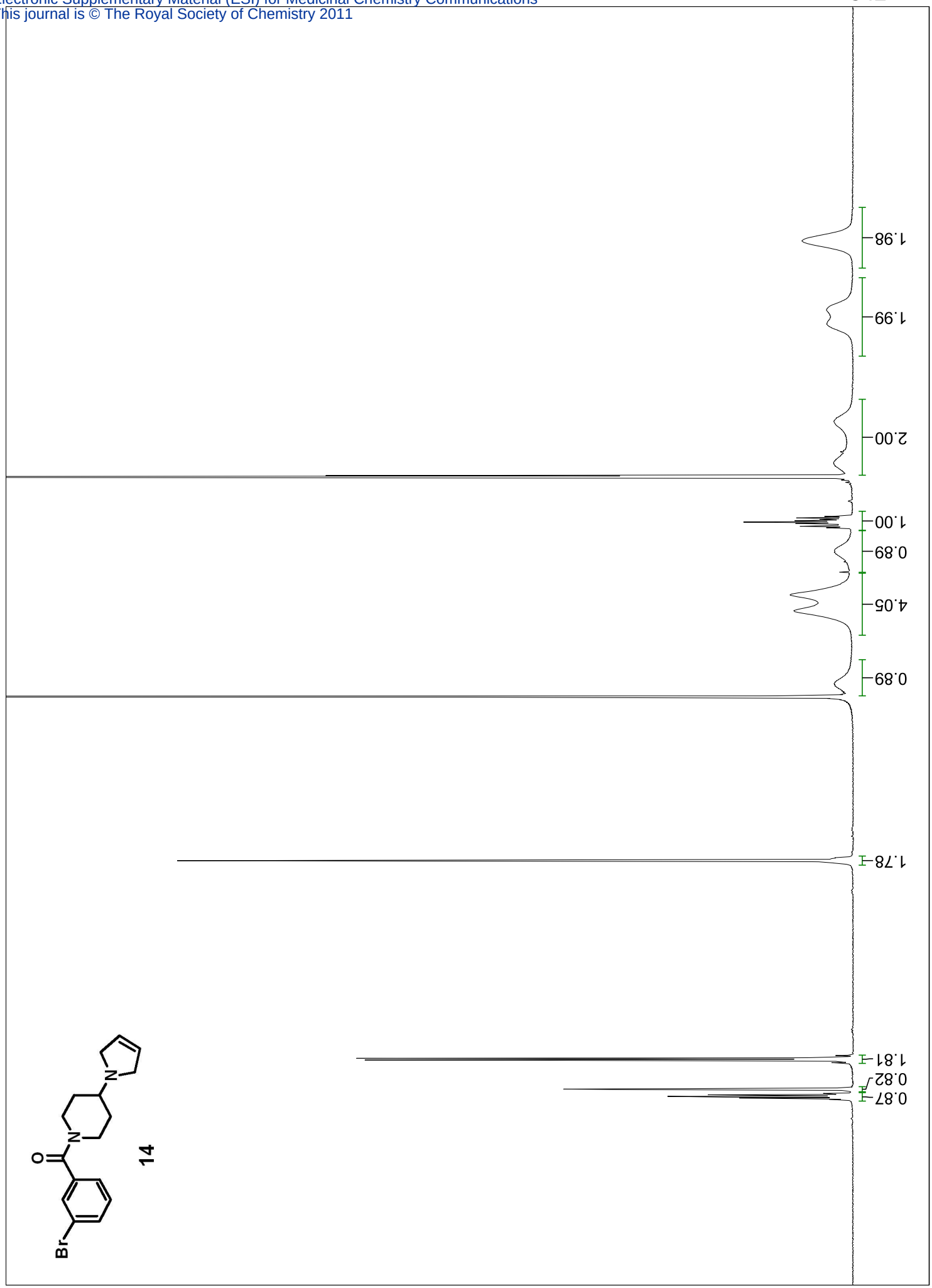

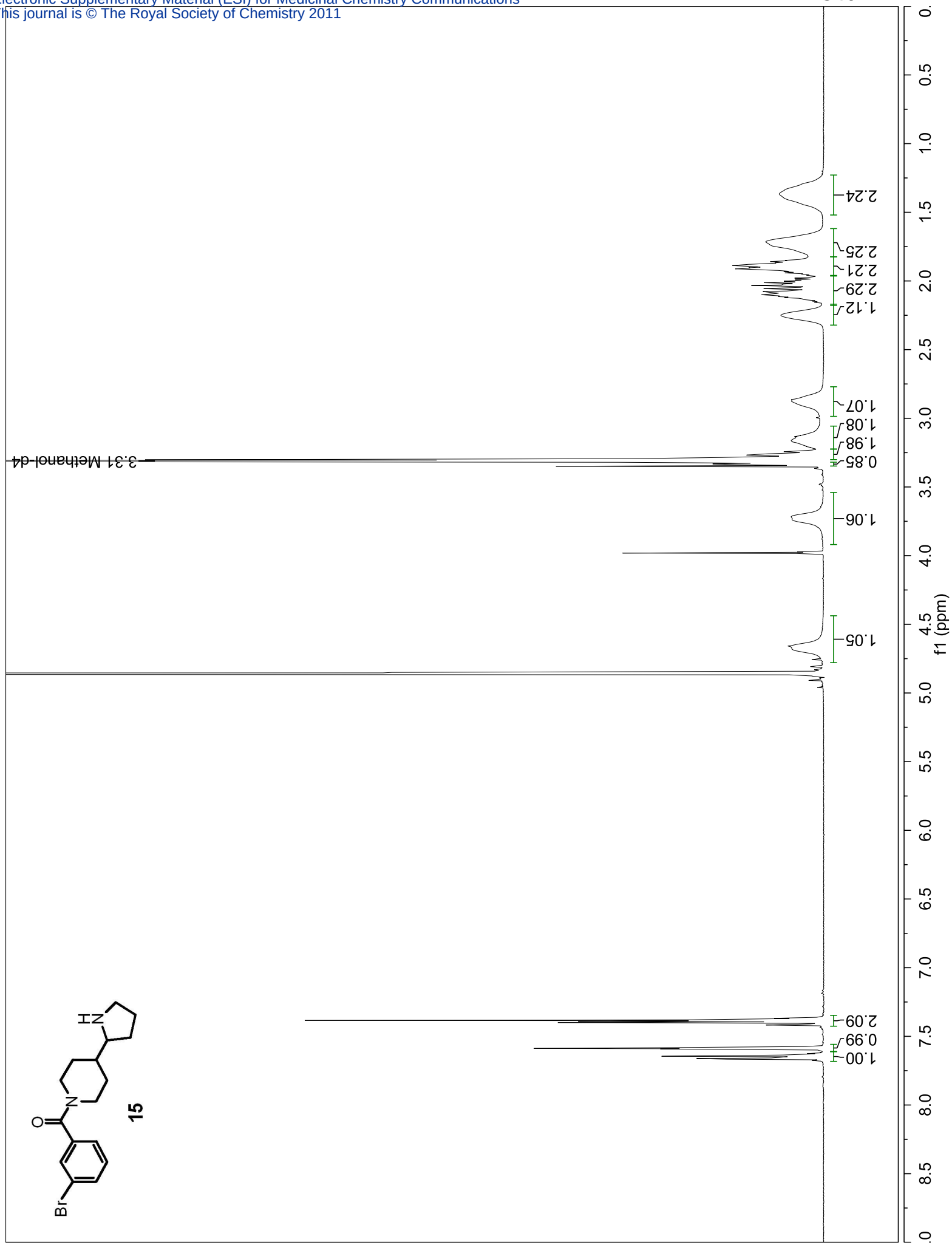

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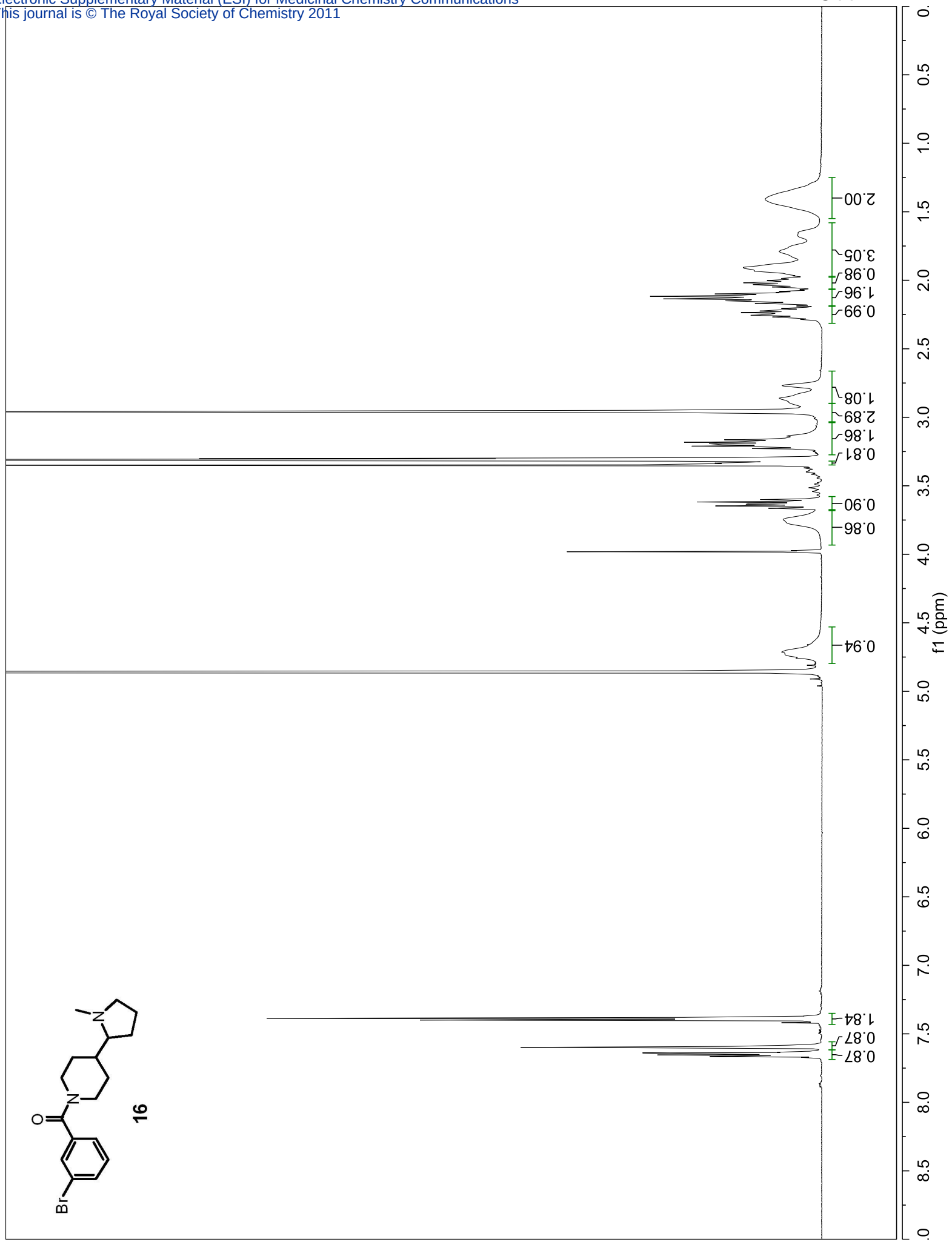

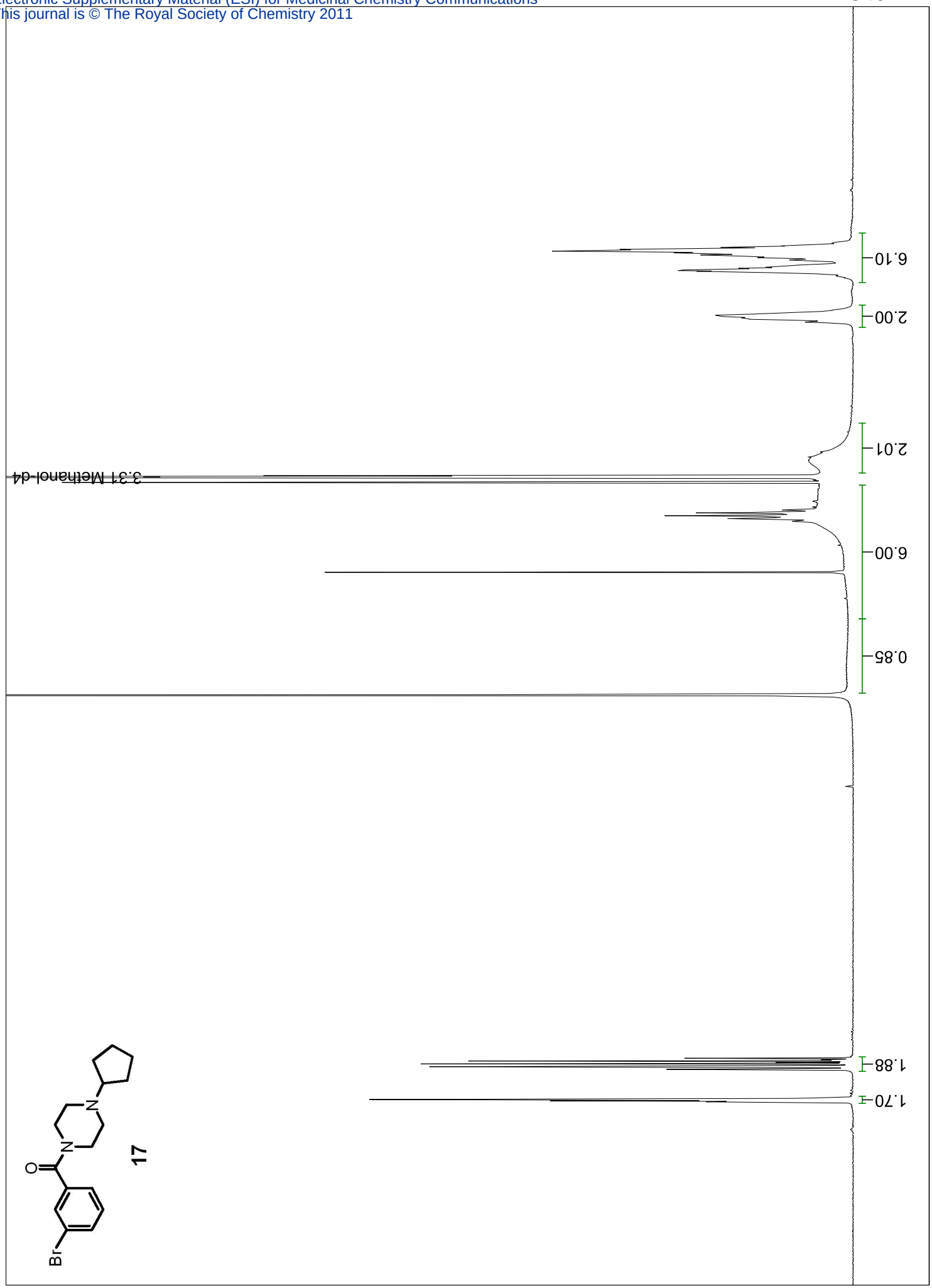

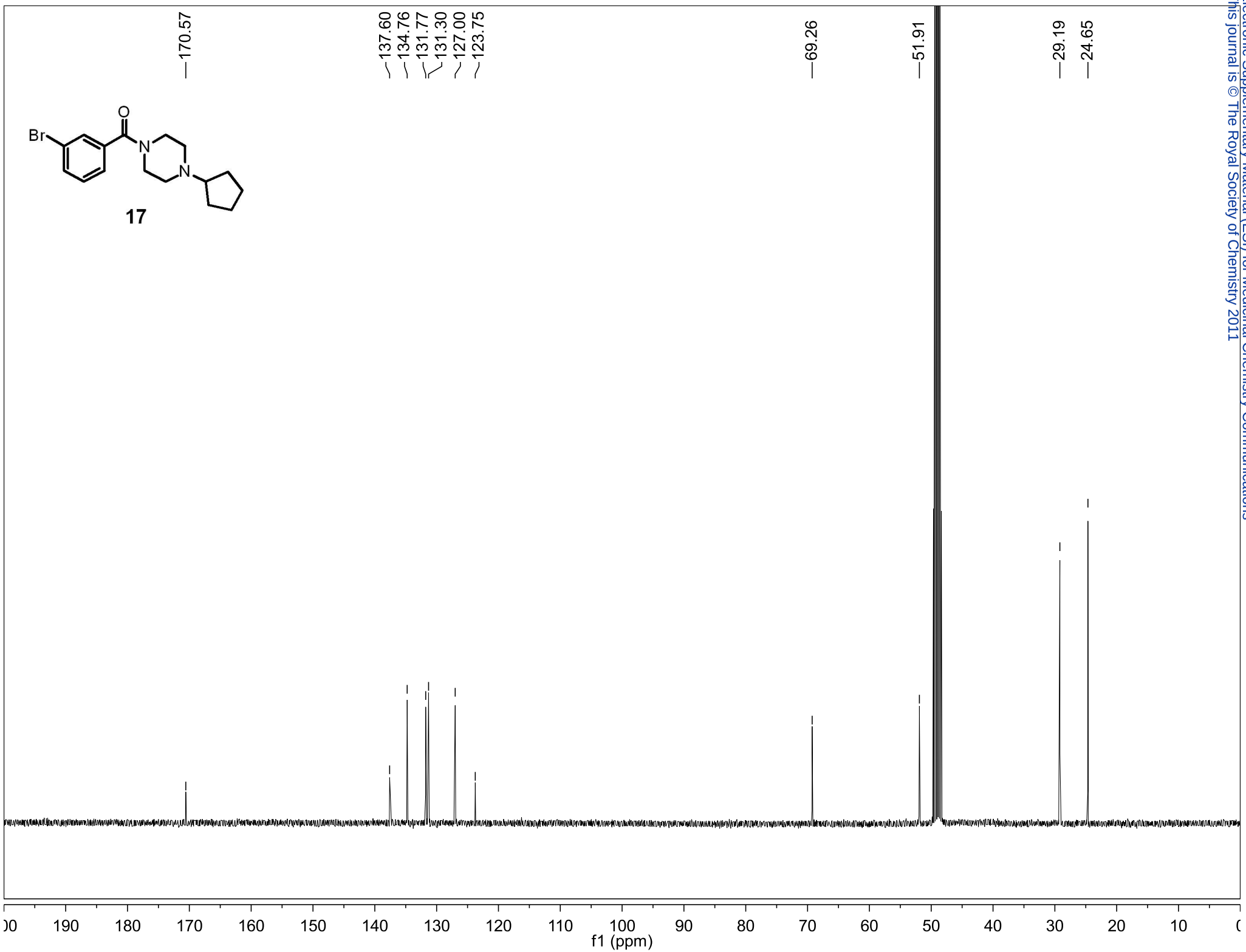

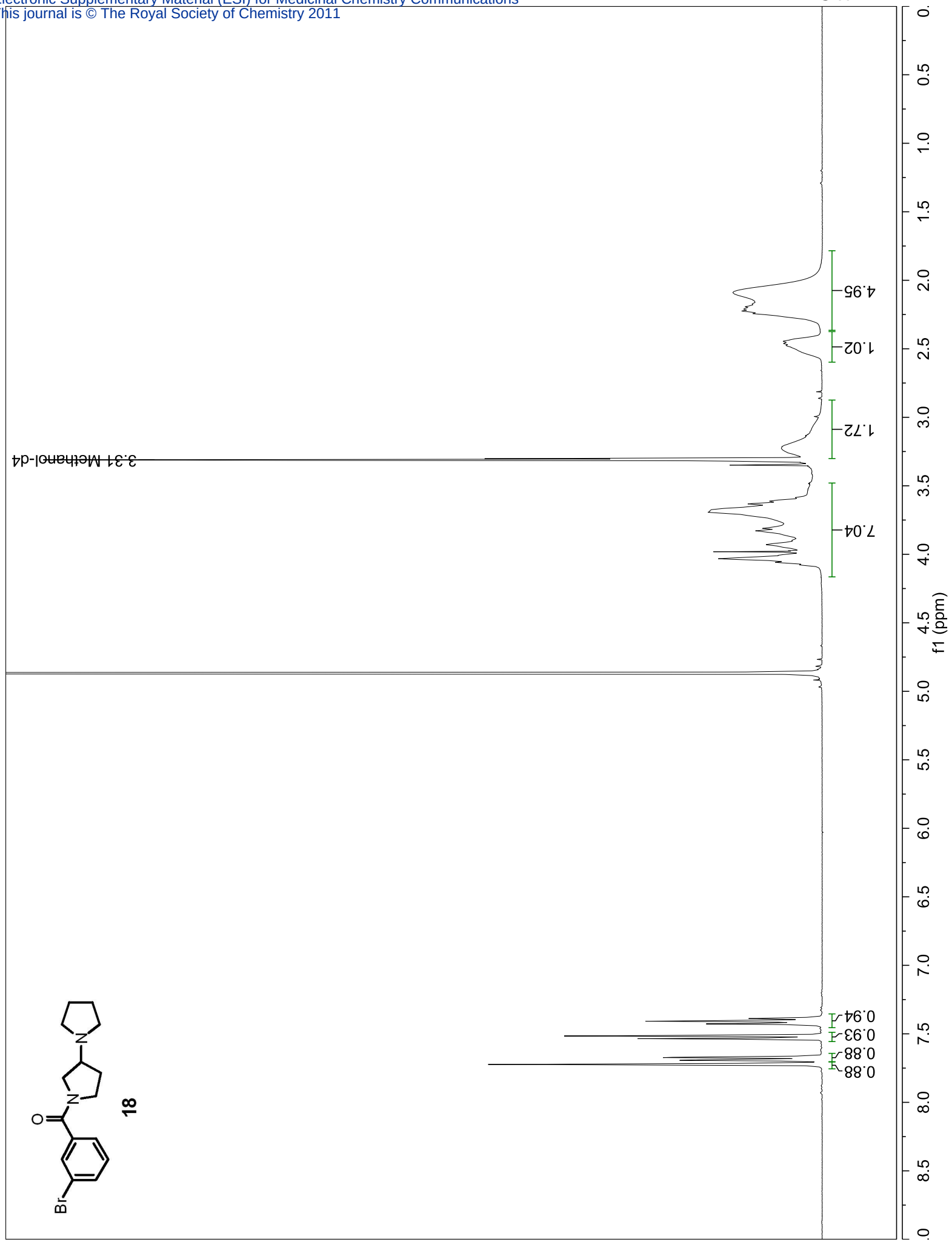

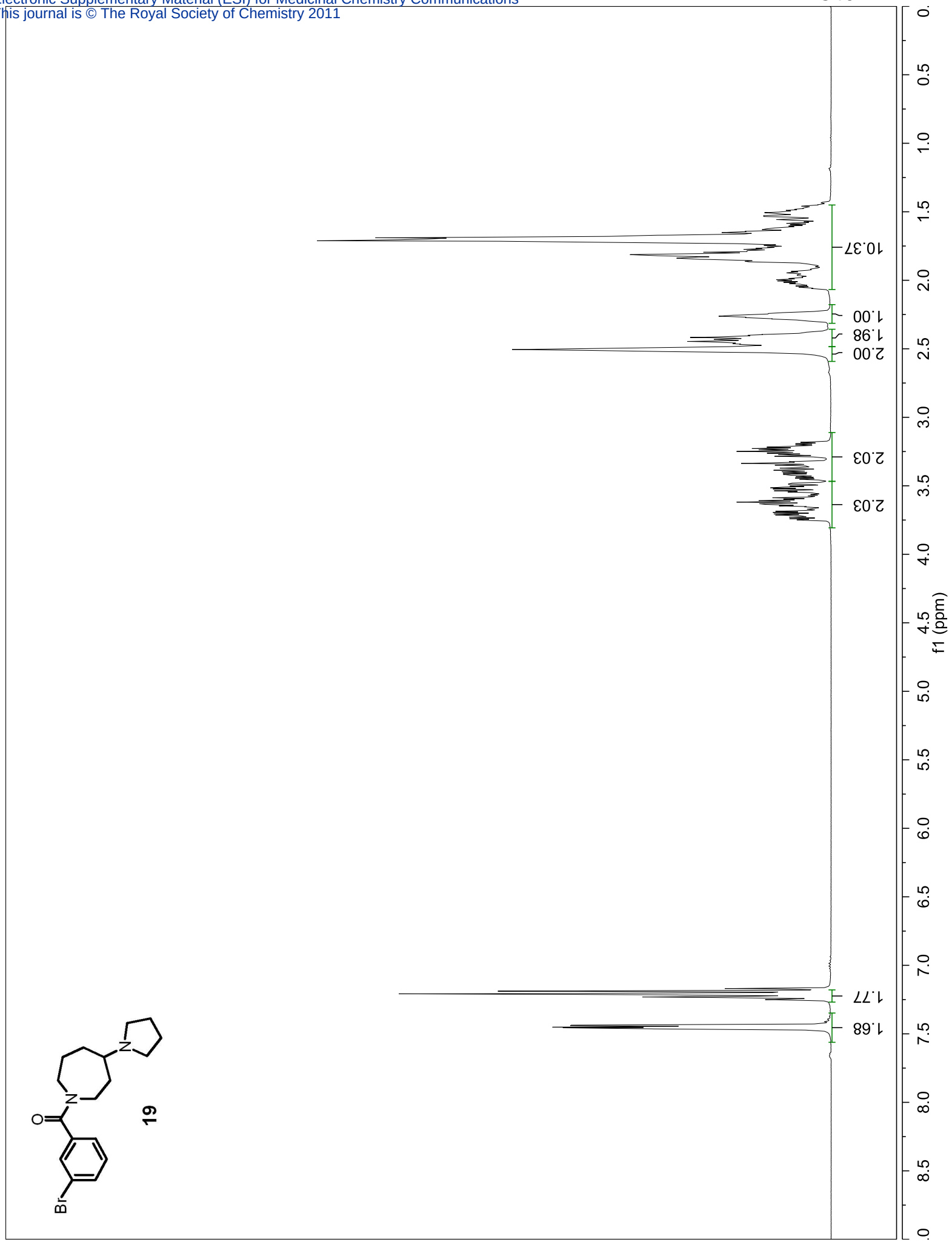

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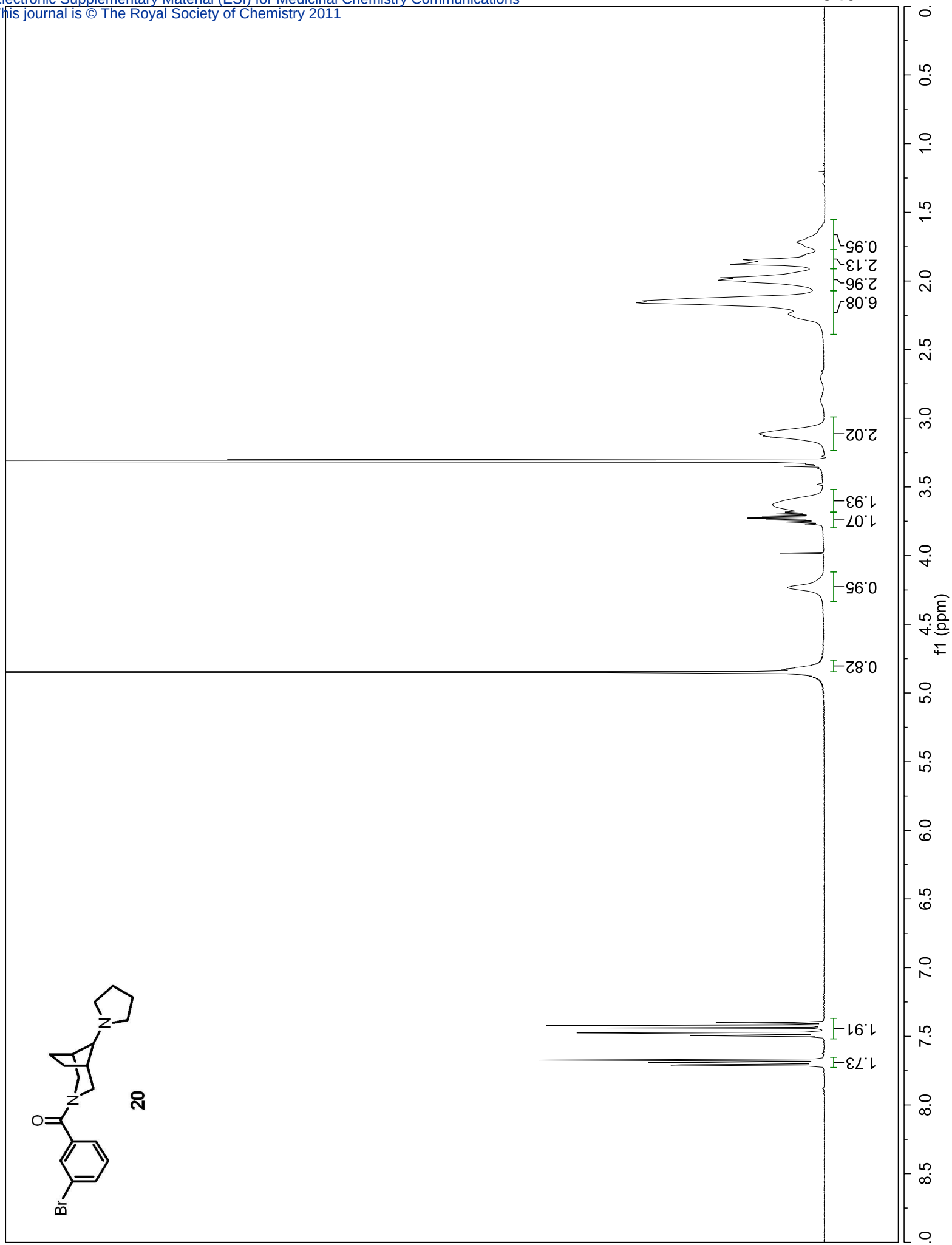

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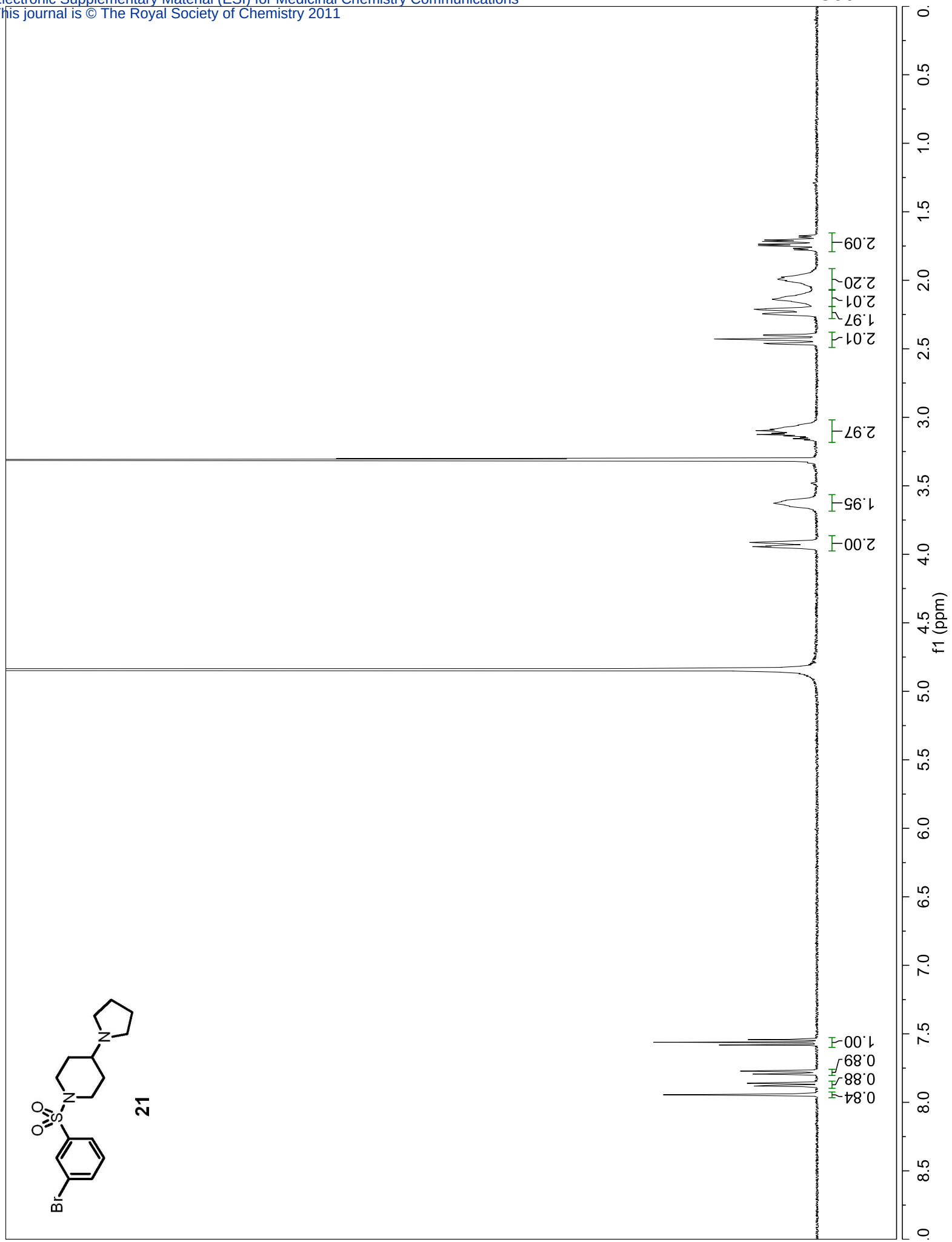

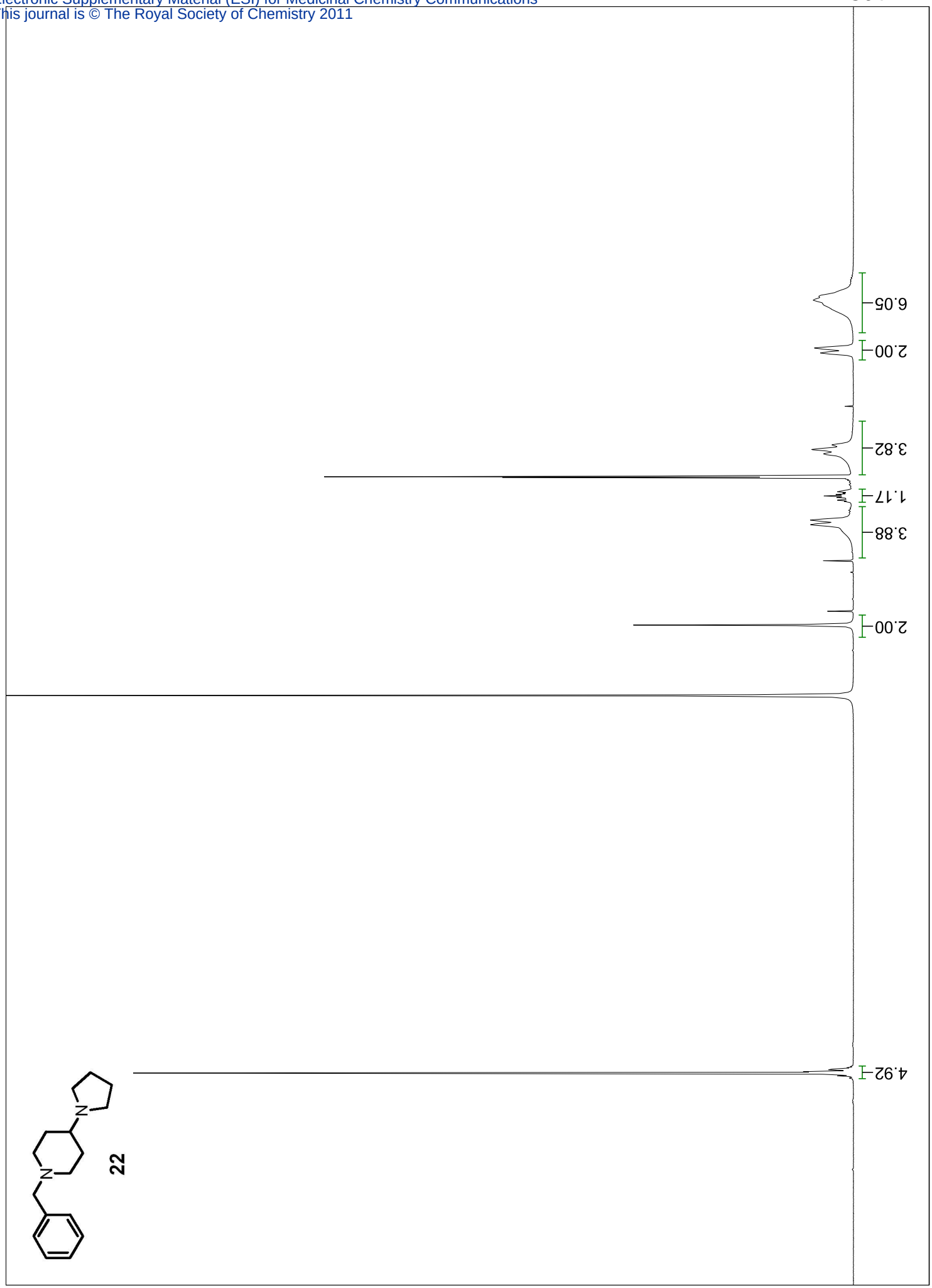

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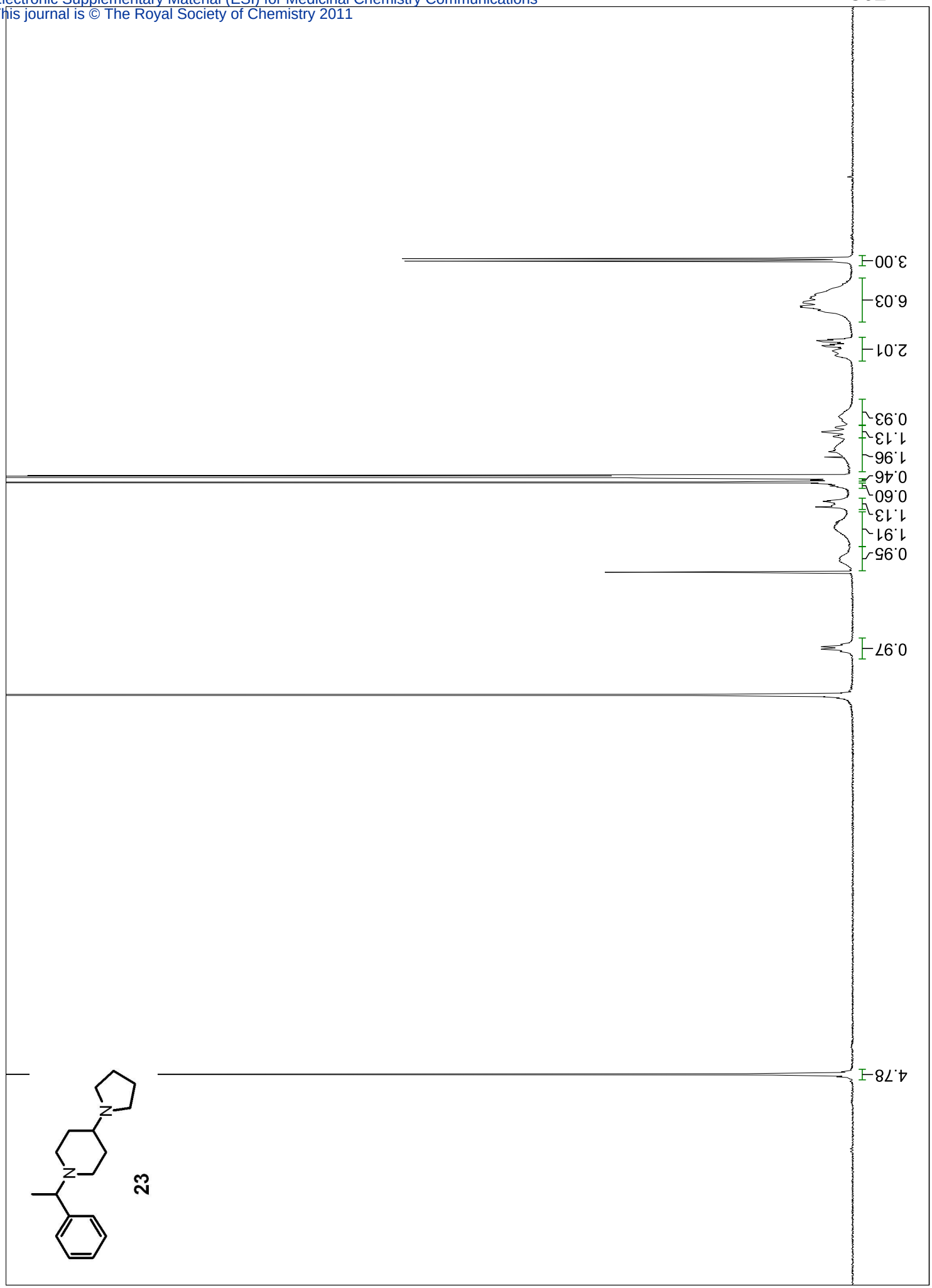

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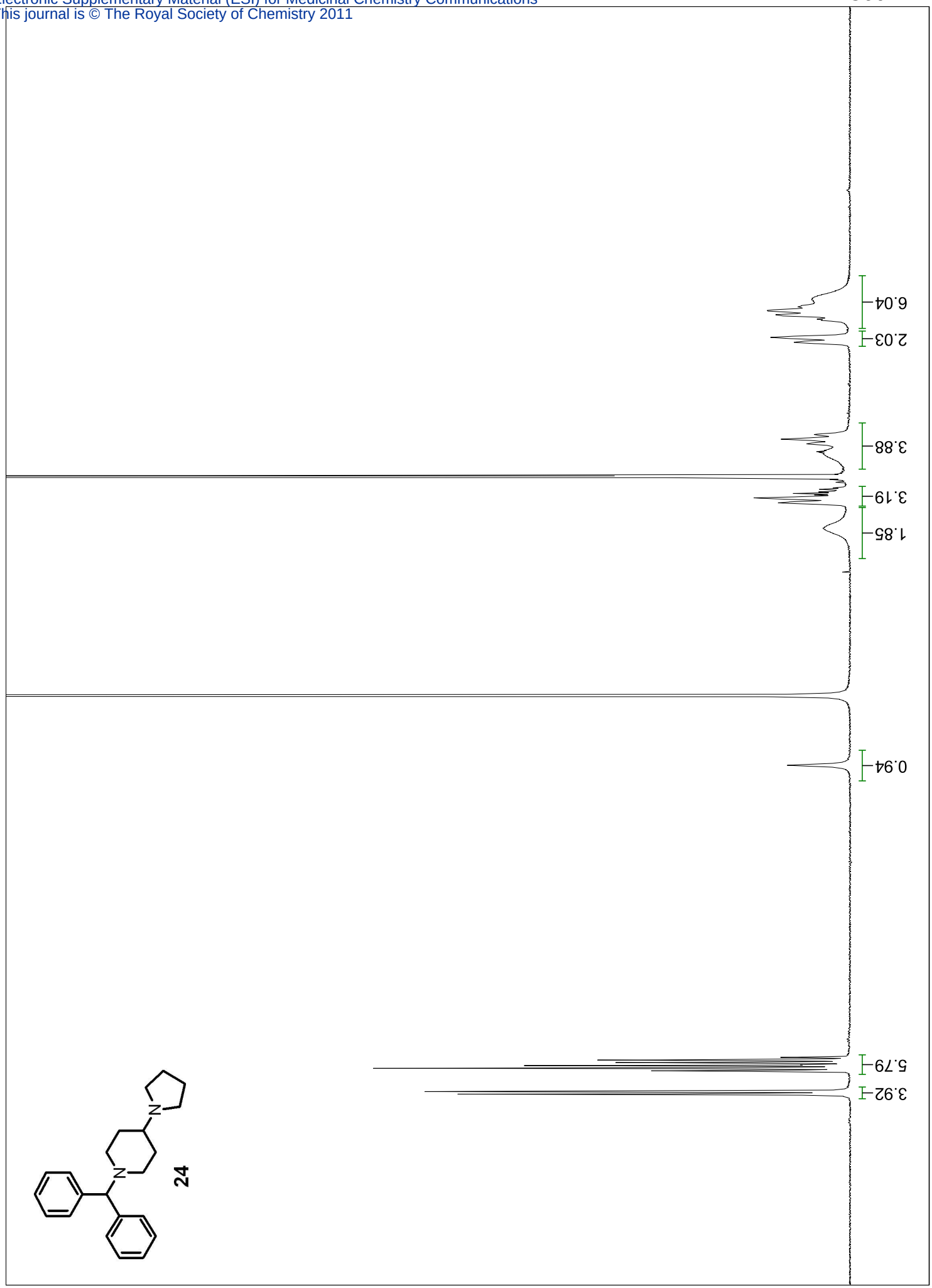

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