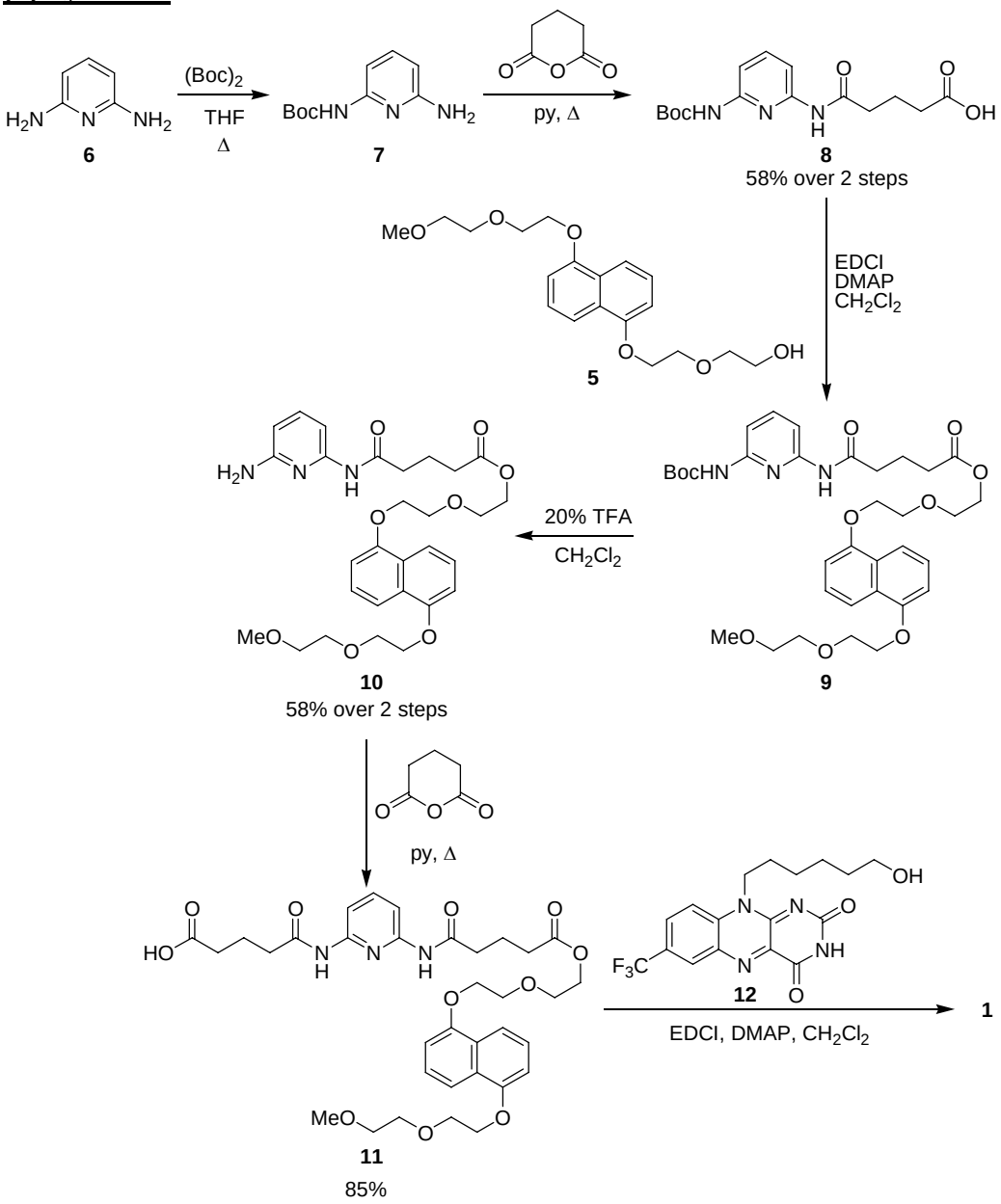
Electronic Supplementary Information

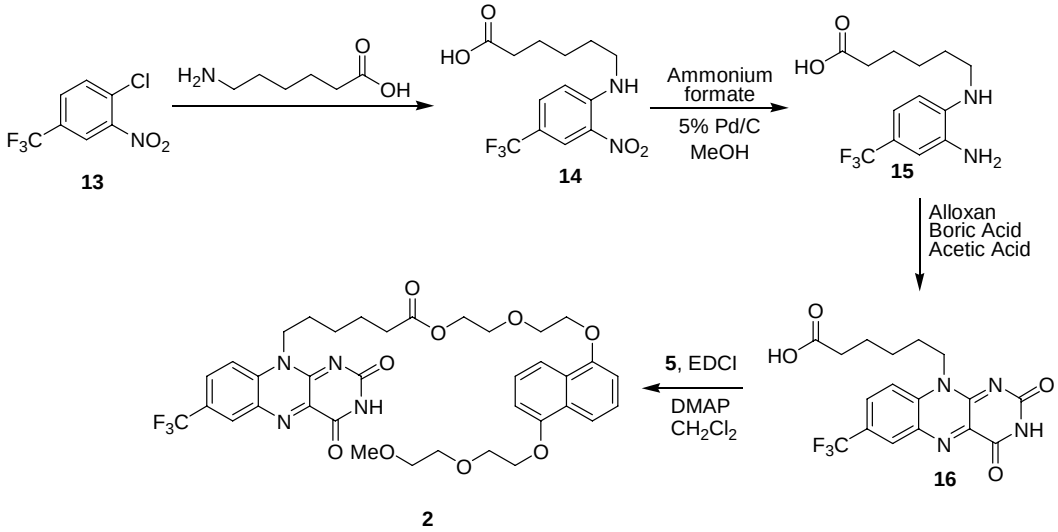
Model systems for flavoenzyme activity: intramolecular self-assembly of a flavin derivative via hydrogen bonding and aromatic interactions

Stuart T. Caldwell,[a] Graeme Cooke,[a*] Shanika G. Hewage,[a] Suhil Mabruk,[a] Gouher Rabani,[a] Vincent Rotello,[b] Brian O. Smith.[c] Chandramouleeswaran Subramani,[b] Patrice Woisel.[d]
(a) Glasgow Centre for Physical Organic Chemistry, WestCHEM, Department of Chemistry, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ, U.K.
(b) Department of Chemistry, University of Massachusetts at Amherst, Amherst, MA 01002, USA
(c) IBLS, University of Glasgow, Glasgow, G12 8QQ, U.K.
(d) Laboratoire de Chimie Organique et Macromoléculaire, Université des Sciences et Technologies de Lille, Villeneuve d'Ascq, 59655, France

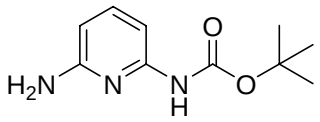
graemec@chem.gla.ac.uk

(a) Synthesis





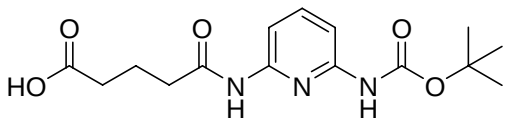
Compound 7: (6-Amino-pyridin-2-yl)-carbamic acid *tert*-butyl ester



Di-*tert*-butylcarbonate (10.0 g, 45.81 mmol, 1.0 equiv) was added to a solution of 2-6 diaminopyridine (5.0 g, 45.81 mmol) in THF (120 ml). The resulting solution was stirred a 60°C overnight. After cooling the solvent was removed under vacuum to give a residue that was used without further purification. The product may be purified by column chromatography on silica eluting with Et_2O:petroleum ether 1:1 to give the carbamate **7** as a white solid foam (9.59 g, 64%). Mpt. 124-126°C.

δ_H (400 MHz; $CDCl_3$) 1.49 (9H, s, $(CH_3)_3$), 4.62 (2H, s, NH_2), 6.12 (1H, d, J = 7.9 Hz, H-3), 7.18 (1H, d, J = 7.5 Hz, H-5), 7.37 (1H, t, J = 7.9 Hz, H-4), 8.45 (1H, broad s, NH). δ_C (100 MHz: $CDCl_3$) 28.19 (3 x CH_3), 80.75 (C), 101.79 (CH), 102.91 (CH), 139.79 (CH), 150.69 (C), 152.64 (C), 157.40 (C). m/z (EI): 209.2 (M^+, 15%), 109.1 (100), 82.9 (100). HRMS found: 209.1163. $C_{10}H_{15}O_2N_3$ requires (M^+), 209.1165.

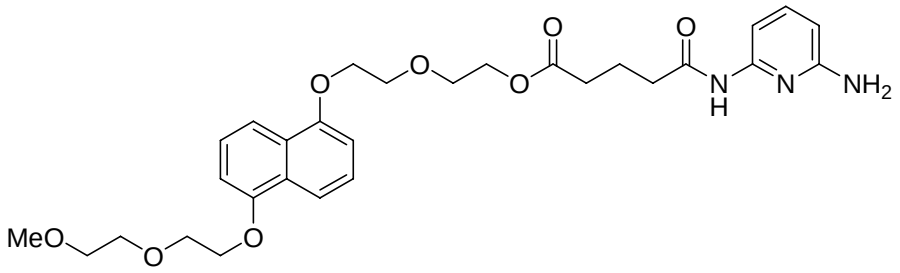
Compound 8: 4-(6-*tert*-Butoxycarbonylamino-pyridin-2-ylcarbamoyl)-butyric acid



Glutaric anhydride (10.5 g, 92.0 mmol, 2.0 equiv) was added to a solution of crude carbamate (from ~46 mmol scale reaction) in pyridine (150 ml). The solution was then heated at ~80°C overnight. After cooling the pyridine was removed under vacuum, the residue was dissolved in EtOAc (150 ml) then washed with water (2 x 200 ml). The organic layer was dried over magnesium sulphate and concentrated under vacuum. The resulting solid was washed with Et_2O to give the acid **8** (8.10 g, 55% over two steps). This material was used without further purification.

δ_H (400 MHz: $CDCl_3$) 1.52 (9H, s, 3 x CH_3), 2.02-2.09 (2H, m, CH_2), 2.55-2.58 (2H, m, CH_2), 2.69 (2H, t, J = 8.0 Hz, CH_2), 6.92 (1H, s, NH), 7.68-7.74 (2H, m, Ar-H), 7.97 (1H, dd, J = 1.0 + 7.5 Hz, Ar-H), 10.31 (1H, s, NH).
δ_C (100 MHz: D-6 DMSO) 20.53 (CH_2), 28.25 (3 x CH_3), 33.19(CH_2), 35.34 (CH_2), 79.82 (C), 108.02 (CH), 108.21 (CH), 139.93 (CH), 150.57 (C), 150.99 (C), 152.85 (C), 171.97 (C), 174.40 (C).

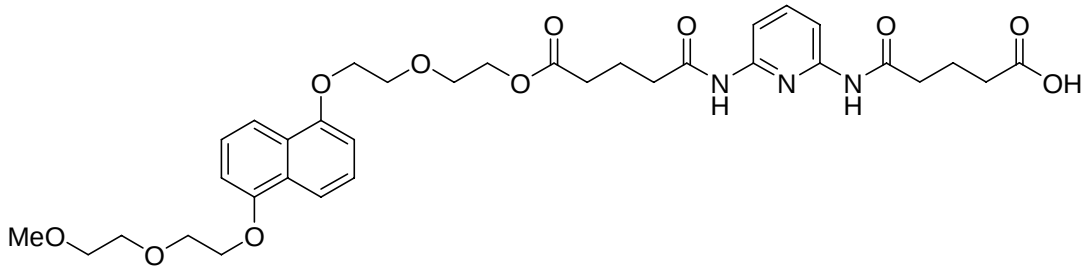
Compound 9: 4-(6-Amino-pyridin-2-ylcarbamoyl)-butyric acid 2-(2-{5-[2-(2-methoxy-ethoxy)-ethoxy]-naphthalen-1-yloxy}-ethoxy)-ethyl ester



EDCI (955 mg, 4.98 mmol, 1.5 equiv) was added to a solution of acid **8** (1.18 g, 4.4 mmol, 1.1 equiv), alcohol **5** (1.16 g, 4.00 mmol) and DMAP (50 mg, 0.4 mmol, 0.1 mmol) in CH_2Cl_2 (20 ml). The solution was stirred at r.t. overnight, diluted with CH_2Cl_2 (50 ml), washed with water (2 x 100 ml) dried over magnesium sulphate and concentrated under vacuum. The resulting residue was then used in the next reaction without further purification.
Trifluoroacetic acid (5 ml) was added to a solution of crude ester **9** in CH_2Cl_2 (20 ml). The solution was stirred at r.t. for 4 h then carefully neutralised to pH 7 using saturated sodium hydrogencarbonate solution. The organic layer was dried over magnesium and concentrated under vacuum. The residue was purified by column chromatography on silica eluting EtOAc to give **10** as pale yellow solid (1.08 g, 58% over two steps).

δ_H (400 MHz: $CDCl_3$): 1.94 (2H, quin, CH_2), 2.29 (2H, t, J = 7.2 Hz, CH_2), 2.35 (2H, t, J = 7.2 Hz, CH_2), 2,74 (1H, broad s, OH), 3.37 (3H, s, OMe), 3.56-3.58 (2H, m, OCH_2), 3.75-3.77 (2H, m, OCH_2), 3.80-3.82 (2H, m, OCH_2), 3.93-3.97 (4H, m, 2 x OCH_2), 4.23-4.27 (6H, m, 3 x OCH_2), 4.42 (2H, s, NH_2), 6.14 (1H, d, J = 8.4 Hz, Ar-H), 6.78 (2H, d, J = 8.0 Hz, 2 x Ar-H), 7.28-7.38 (3H, m, Ar-H), 7.46 (1H, d, J = 8.0 Hz, Ar-H), 7.84 (2H, t, J = 8.5 Hz, Ar-H), 8.20 (1H, s, NH). δ_C (100 MHz: $CDCl_3$): 20.31 (CH_2), 32.91 (CH_2), 35.88 (CH_2), 58.89 (CH_3), 63.38 (CH_2), 67.66 (CH_2), 69.14 (CH_2), 69.51 (CH_2), 69.62 (CH_2), 70.63 (CH_2), 71.79 (CH_2), 102.89 (CH), 102.89 (CH), 104.03 (CH), 105.48 (CH), 105.51 (CH), 114.30 (CH), 114.51 (CH), 124.91 (CH), 125.03 (CH), 126.49 (C), 126.52 (C), 139.83 (CH), 149.66 (C), 154.01 (C), 154.11 (C), 157.07 (C), 170.66 (C), 172.99 (C). m/z (FAB): 556.3 ($(M+H)^+$, 100%), 206.6 (50), 111.6 (55). HRMS found: 556.2660. $C_{29}H_{38}O_8N_3$ requires ($(M+H)^+$, 556.2659.

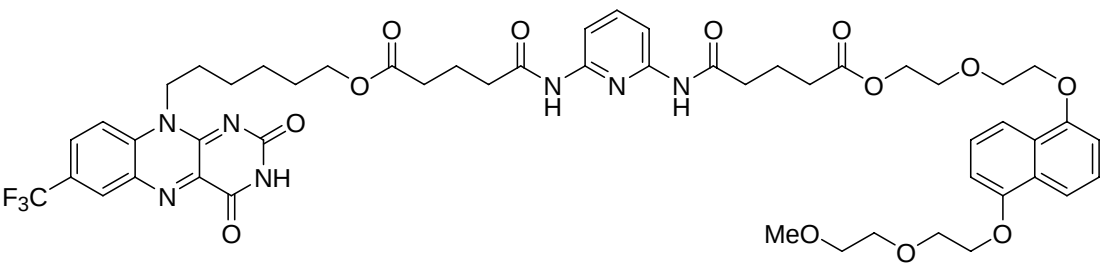
Compound 11: 4-(6-{4-[2-(2-{5-[2-(2-Methoxy-ethoxy)-ethoxy]-naphthalen-1-yloxy}-ethoxy)-ethoxycarbonyl]-butyrylamino}-pyridin-2-ylcarbamoyl)-butyric acid



Glutaric anhydride (443 mg, 3.88 mmol, 2.0 equiv) was added to a solution of **10** (1.08 g, 1.94 mmol) in pyridine (20 ml). The solution was then heated at ~80°C overnight. After cooling the pyridine was removed under vacuum, the residue was dissolved in EtOAc (150 ml) then washed with water (2 x 200 ml). The organic layer was dried over magnesium sulphate and concentrated under vacuum. The resulting mixture was purified by column chromatography on silica eluting acetone:petroleum ether 3:2 to give the acid **11** as an colourless solid (1.08 g, 85%). Mpt. 98-100°C

δ_H (400 MHz: $CDCl_3$): 1.97-2.06 (4H, m, 2xCH_2), 2.04-2.49 (6H, m, 3 x CH_2), 2.55-2.59 (2H, m, CH_2), 3.39 (3H, s, OMe), 3.58-3.60 (2H, m, OCH_2), 3.77-3.80 (2H, m, OCH_2), 3.80-3.86 (2H, m, OCH_2), 3.97-3.99 (4H, m, 2 x OCH_2), 4.26-4.31 (6H, m, 3 x OCH_2), 6.80 (2H, d, J = 7.7 Hz, Ar-H), 7.31 (1H, t, J = 7.9 Hz, Ar-H), 7.32 (1H, t, J = 8.1Hz, Ar-H), 7.71 (1H, t, J = 8.1 Hz, Ar-H), 7.81-7.87 (3H, m, Ar-H), 7.96 (1H, d, J = 8.1 Hz, Ar-H), 8.01 (1H, broad s, NH), 9.53 (1H, broad s, NH). δ_C (100 MHz: $CDCl_3$): 20.20 (2 x CH_2), 32.60 (CH_2), 32.93 (CH_2), 35.98 (CH_2), 36.13 (CH_2), 59.03 (CH_3), 63.55 (CH_2), 67.77 (CH_2), 67.80 (CH_2), 69.32 (CH_2), 69.67 (CH_2), 67.76 (CH_2), 70.78 (CH_2), 71.99 (CH_2), 105.56 (CH), 105.61 (CH), 109.84 (CH), 109.99 (CH), 114.36 (CH), 114.71 (CH), 124.98 (CH), 125.13 (CH), 126.60 (C), 126.65 (C), 141.82 (CH), 148.95 (C), 149.63 (C), 154.11 (C), 154.25 (C), 170.85 (C), 171.69 (C), 173.00 (C), 177.53 (C). Found: C, 60.83; H, 6.65; N, 6.50. $C_{34}H_{43}O_{11}N_3$ requires C, 60.98; H, 6.47; N, 6.27 %.

Compound 1: 4-(6-{4-[6-(1,3-Dioxo-8-trifluoromethyl-2,3-dihydro-1*H*-pyrido[3,4-*b*]quinoxalin-5-yl)-hexyloxycarbonyl]-butyrylamino}-pyridin-2-ylcarbamoyl)-butyric acid 2-(2-{5-[2-(2-methoxy-ethoxy)-ethoxy]-naphthalen-1-yloxy}-ethoxy)-ethyl ester

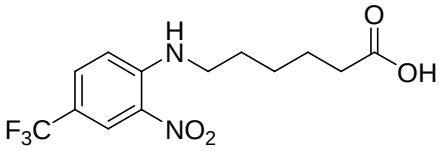


EDCI (232 mg, 1.21 mmol, 2.5 equiv.) was added to a solution of flavin **12** (185 mg, 0.48 mmol), acid **11** (317 mg, 0.48 mmol, 1.0 equiv.) and DMAP (6 mg, 0.05 mmol,

0.1 equiv.) in CH_2Cl_2 (6 ml). The resulting solution was stirred at r.t. for 24 h, diluted with CH_2Cl_2 (30 ml) then washed with water (2 x 50 ml). The organic layer was dried over magnesium sulphate and concentrated under vacuum. The residue was purified by column chromatography on silica eluting with EtOAc. The product was further purified by recrystallisation from EtOAc to give the ester as a yellow solid (73 mg, 15%). Mpt. 133-135^oC.

δ_H (400 MHz: $CDCl_3$): 1.50-1.57 (4H, m, 2 x CH_2), 1.75-1.86 (4H, m, 2 x CH_2), 2.04-2.16 (4H, m, 2 x CH_2), 2.44 (2H, t, J = 6.7 Hz, CH_2), 2.56 (2H, t, J = 7.1 Hz, CH_2), 2.61 (2H, t, J = 7.9 Hz, CH_2), 2.74 (2H, t, J = 7.1 Hz, CH_2), 3.41 (3H, s, OMe), 3.61-3.63 (2H, m, OCH_2), 3.79-3.84 (4H, m, 2 x OCH_2), 3.93-3.95 (2H, m, OCH_2), 3.97-3.98 (2H, m, OCH_2), 4.13 (2H, t, J = 7.2 Hz, OCH_2), 4.17-4.22 (4H, m, 2 x OCH_2), 4.30-4.33 (2H, m, OCH_2), 4.51 (2H, broad s, NCH_2), 6.65 (2H, d, J = 7.7 Hz, 2 x Ar-H), 7.18 (2H, t, J = 8.1 Hz, 2 x Ar-H), 7.62 (2H, d, J = 8.7 Hz, 2 x Ar-H), 7.66 (1H, d, J = 8.5 Hz, Ar-H), 7.74 (1H, t, J = 8.1 Hz, Ar-H), 7.98 (1H, dd, J = 9.0 Hz + 1.9 Hz, Ar-H), 8.02 (1H, d, J = 8.1 Hz, Ar-H), 8.03 (1H, d, J = 8.1 Hz, Ar-H), 8.49 (1H, d, J = 1.9 Hz, Ar-H), 9.81 (1H, s, NH), 10.40 (1H, s, NH), 12.74 (1H, s, NH). δ_C (100 MHz: $CDCl_3$): 20.25 (CH_2), 20.79 (CH_2), 24.40 (CH_2), 25.29 (CH_2), 25.66 (CH_2), 27.37 (CH_2), 33.25 (CH_2), 33.75 (CH_2), 35.51 (CH_2), 36.02 (CH_2), 44.79 (CH_2), 58.94 (CH_3), 63.32 (CH_2), 64.42 (CH_2), 67.63 (CH_2), 67.70 (CH_2), 69.26 (CH_2), 69.44 (CH_2), 69.65 (CH_2), 70.68 (CH_2), 71.88 (CH_2), 105.28 (CH), 105.40 (CH), 110.03 (CH), 110.11 (CH), 114.20 (CH), 114.26 (CH), 116.46 (CH), 122.81 (q, J = 272.5 Hz, CF_3), 124.87 (CH), 125.00 (CH), 126.11 (C), 126.17 (C), 128.50 (q, J = 34.3 Hz, C), 130.57 (q, J = 3.8 Hz, CH), 131.46 (q, J = 3.0 Hz, CH), 133.98 (C), 134.69 (C), 138.09 (C), 140.75 (CH), 150.09 (C), 150.27 (C), 150.31 (C), 153.83 (2 x C), 156.54 (C), 160.58 (C), 171.92 (C), 172.15 (C), 173.07 (C), 173.17 (C).
m/z (FAB): 1034.73 ($(M+H)^+$, 100%), 365.3 (20), 283.3 (20). HRMS found: 1034.4121. $C_{51}H_{59}O_{13}N_7F_3$ requires $(M+H)^+$, 1034.4123.

Compound 14: 6-[2-nitro-4-(trifluoromethyl)anilino]hexanoic acid.

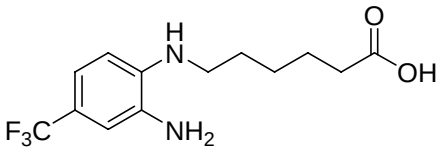


A mixture of 4-chloro-3-nitro-benzotrifluoride (8.00 g, 35.46 mmol), 6-aminocaproic acid (6.97 g, 53.20 mmol) and triethylamine (5.30 mL, 38.02 mmol) was heated at reflux for 4 days in tetrahydrofuran (300 mL). After cooling down and evaporation of the solution to dryness, the resulting mixture was purified by column chromatography (silica gel, dichloromethane then acetone), to afford **14** as a bright yellow solid (1.54 g, 20%). Mpt. 82-83^oC.

δ_H (400 MHz: D-6 DMSO): 1.33-1.40 (2H, m, CH_2), 1.51-1.66 (4H, m, 2 x CH_2), 2.22 (2H, t, J = 7.3 Hz, CH_2), 3.40 (2H, quartet, J = 6.7 Hz, CH_2), 7.21 (1H, d, J = 9.2 Hz, H-5, 7.74 (1H, dd, J = 2.0 Hz + 9.2 Hz, H-4), 8.28 (1H, d, J = 2.0 Hz, H-3), 8.45 (1H, t, J = 5.5 Hz, NH), 11.99 (1H, s, OH). δ_C (100 MHz: D-6 DMSO): 24.10 (CH_2), 25.80 (CH_2), 27.79 (CH_2), 33.49 (CH_2), 44.22 (CH_2), 114.68 (q, J = 33.7 Hz, C),

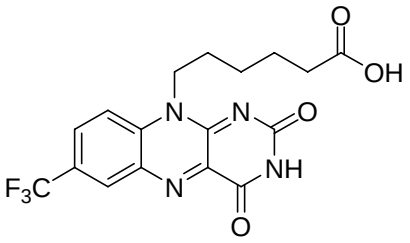
115.81 (CH), 123.79 (q, J = 270.3 Hz, C), 123.98 (q, J = 4.2 Hz, CH), 129.86 (C), 131.79 (q, J = 5.8 Hz, CH), 146.70 (C), 174.34 (C),
IR (NaCl, cm^{-1}): 3386 (OH), 2933 (CH_2), 2873 (CH_2), 1709 (C=O), 1635 (NH), 1535 (NO_2), 1363 (NH aryl.), 1272 (C-N aryl.), 1258 (C(=O)-O), 1147 (CF_3), 1111 (CF_3), 692 (CC arom.); MS (EI): m/z 320 (13%, M^+), 301 (8%), 285 (3%), 219 (100%). Found: C, 48.63; H, 4.65; N, 8.83. $C_{13}H_{15}F_3O_4N_2$ requires C, 48.75; H, 4.72; N, 8.75 %.

Compound 15: 6-[2-amino-4-(trifluoromethyl)anilino]hexanoic acid



A suspension of palladium (0.39 g, 5% wt.% on activated carbon) in methanol (20 mL) was added drop wise to a stirred cold solution of **14** (0.60 g, 1.870 mmol) in methanol (100 mL) under an inert atmosphere in order to perform a hydrogenolysis reaction. Ammonium formate (0.47 g, 7.48 mmol) was added to the reaction mixture and it was allowed to stir for 2 h at room temperature. The catalyst was removed by gravity filtration and the solvent was evaporated to yield the crude material which was dissolved in dichloromethane (100 mL) and washed with water (150 mL). The organic phase was dried over magnesium sulphate, filtered and evaporation of the solvent gave the product **15** as a pale violet solid (0.42 g, 79%). Mpt. 75-76^oC.
δ_H (400 MHz; DMSO-d_6) 6.70 (d, J = 7.6 Hz, 1 H), 6.37 (s, 1 H), 6.06 (d, J = 8 Hz, 1 H), 3.01 (t, J = 6.8 Hz, 2 H), 2.21 (t, J = 7.2 Hz, 2 H), 1.55 (m, 4 H), 1.33 (m, 2 H); δ_C (100 MHz: D-6 DMSO):δ 174.39 (1C, C(=O)O), 143.49 (1C, C(NH_2) arom.), 139.46 (1C, C(CF_3) arom.), 128.53 (1C, C(NH) arom.), 124.45 (1C, CH arom.), 123.09 (1C, CH arom.), 112.67 (1C, CH arom.), 41.85 (1C, CF_3), 33.62 (1C, CH_2-NH), 28.15 (1C, CH_2-CO), 26.27 (1C, CH_2), 26.13 (1C, CH_2), 24.34 (1C, CH_2); IR (NaCl, cm^{-1}): 3385 (OH), 2933 (CH_2), 1707 (C=O), 1636 (NH), 1363 (NH aryl.), 1313 (C(=O)-O), 1237 (C-N aryl.), 1149 (CF_3), 1102 (CF_3), 1051 (CH arom.), 709 (CH arom.); MS (EI): m/z 290 (M^+). Found: C, 53.66; H, 5.99; N, 9.51. $C_{13}H_{17}F_3O_2N_2$ requires C, 53.79; H, 5.90; N, 9.65 %.

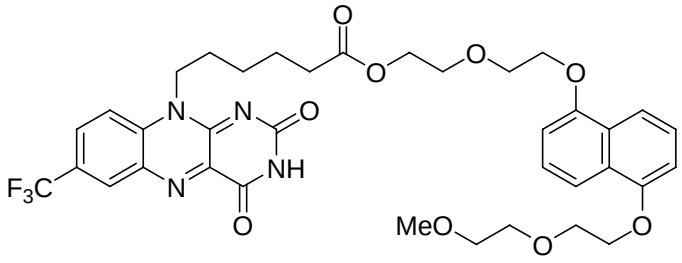
Compound 16: 7-Trifluoromethy-(N(10)-(5-carboxypentyl))isoalloxazine.



Alloxan monohydrate (0.23 g, 1.44 mmol) and boron oxide (0.20 g, 2.88 mmol) were added to a solution of **15** (0.42 g, 1.44 mmol) in glacial acetic acid (50 mL). The resulting mixture was heated under reflux for 2 hours, before allowing the reaction to stir for 2 days at room temperature. The crude residue obtained after evaporating the solvent under vacuum was purified by silica gel chromatography (dichloromethane/acetone (1:1 v/v) as eluent) and precipitation using acetone and light petroleum to afford **16** as a yellow solid (0.35 g, 61%). Sample decomposed around 207°C without melting.

δ_H (400 MHz, DMSO-d_6) δ 12 .04 (s, 1 H), 11.56 (s, 1 H), 8.50 (s, 1 H), 8.21 (d, J = 9.2 Hz, 2 H), 8.16 (d, J = 9.2 Hz, 2 H), 4.58 (t, J = 7.6 Hz, 2 H), 2.25 (t, J = 7.2 Hz, 2 H), 1.73 (m, 2 H), 1.58 (m, 2 H), 1.49 (m, 2 H); δ_C (100 MHz: DMSO-d_6): 206.71 (1C, C(=O)OH), 174.41 (1C, C(=O)), 170.23 (1C, C(=O)), 159.36 (1C, arom.), 155.63 (1C, arom.), 150.87 (1C, arom.), 140.43 (1C, arom.), 134.88 (1C, arom.), 133.94 (1C, arom.), 117.94 (1C, C=N), 68.88 (1C, C=N), 44.26 (1C, CF_3), 33.46 (1C, CH_2), 30.65 (1C, CH_2), 26.04 (1C, CH_2), 25.58 (1C, CH_2), 24.17 (1C, CH_2); IR (KBr, cm^{-1}): 2930 (CH_2), 2841 (CH_2), 2545 (OH), 1730 (C=O acid), 1592 (CC arom.), 1434 (C-O-H), 1262 (C(=O)-O), 1185 (CF_3), 1141 (CF_3), MS (FAB): m/z 397 (100%, $M+H^+$), 379 (16%), 352 (5%), 238 (7%), 75 (3%). Found: C, 51.41; H, 3.76; N, 14.28. $C_{17}H_{15}F_3O_4N_4$ requires C, 51.52; H, 3.81; N, 14.14 %.

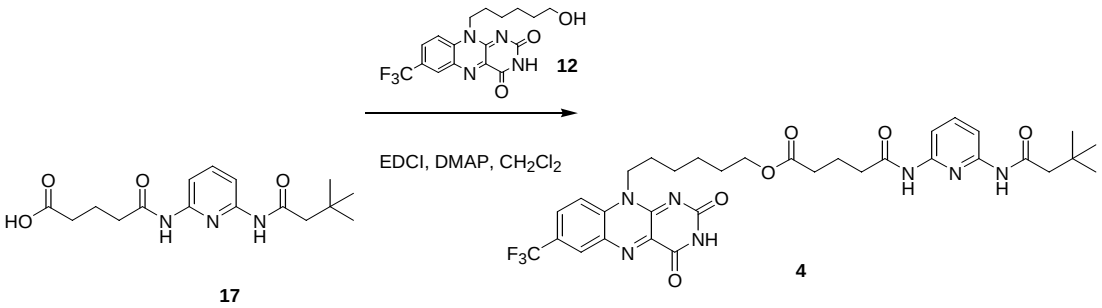
Compound 2: 1-(2-(7-Trifluoromethy-isoalloxazine-N(10)-pentylcarbonyloxyethoxy)ethoxy)-5-(2-(2-methoxyethoxy)ethoxy)naphthalene



7-Trifluoromethy-(N(10)-(5-carboxypentyl))isoalloxazine (0.42g, 1.06 mmol) and 1-(2-(2-methoxyethoxyethoxy))-5-(2-(2-hydroxyethoxyethoxy))naphthalene **5** (0.37g , 1.06 mmol) was added to N,N'-dimethylformamide (60 mL) in the presence of N-(3-dimethylaminopropyl)-N'-ethyl-carbodiimide hydrochloride (0.30 g, 1.59 mmol) and 4-dimethylamino-pyridine (0.19 g, 1.59 mmol) as catalysts. The reaction was stirred at room temperature for 48 h. The solvent was removed under vacuum and the residue was partitioned between dichloromethane (100 mL) and water (100 mL). The organic phase was dried with magnesium sulphate, filtered and concentrated under reduced pressure. Silica gel column chromatography using light petroleum and acetone (7:3 v/v) as eluent afforded **2** as a bright red viscous oil (0.17 g, 23%).

δ_H (400 MHz, $CDCl_3$): 9.35 (s, 1 H), 8.35 (s, 1 H), 7.87 (d, J = 8.4 Hz, 1 H), 7.54 (d, 1+1 H), 7.45 (d, J = 8.8 Hz, 1 H), 7.11 (t, J = 8 Hz, 1 H), 7.00 (t, J = 8 Hz, 1 H), 6.62 (d, J = 7.6 Hz, 1 H), 6.41 (d, J = 7.6 Hz, 1 H), 4.31 (t, J = 4.4 Hz, 2 H), 4.15 (m, 4 H), 4.05 (m, 2 H), 3.92 (m, 4 H), 3.81 (m, 2 H), 3.75 (m, 2 H), 3.62 (m, 2 H), 3.35 (s, 3 H), 2.35 (t, J = 6.8 Hz, 2 H), 1.66 (m, 2 H), 1.55 (m, 2 H), 1.45 (m, 2 H); δ_C (100 MHz: $CDCl_3$): 24.16 (CH_2), 25.51 (CH_2), 25.62 (CH_2), 29.61 (CH_2), 33.60 (CH_2), 44.88 (CH_2), 58.84 (CH_3), 63.50 (CH_2), 67.62 (CH_2), 67.67 (CH_2), 69.40 (CH_2), 69.71 (CH_2), 69.77 (CH_2), 70.55 (CH_2), 71.83 (CH_2), 105.01 (CH), 105.45 (CH), 113.87 (CH), 114.35 (CH), 116.55 (CH), 122.97 (q, J = 272.2 Hz,CF_3), 124.94 (CH), 125.18 (CH), 125.89 (C), 126.03 (C), 128.04 (q, J = 34.3 Hz, C), 130.19 (q, J = 4.1 Hz, CH), 130.88 (q, J = 2.8 Hz, CH), 134.00 (C), 134.34 (C), 138.51 (C), 149.62 (C). IR (NaCl, cm^{-1}): 3060 (CH arom), 2930 (CH_3), 1728 (C=O ester), 1593 (CC arom.), 1454 (CH_3), 1178 (CF_3), 1128 (CF_3), 1079 (C(=O)O-C), 777 (α-sub naph.); MS (FAB): m/z 729 (20%, $M+H^+$), 379 (8%), 307 (6%), 155 (100%), 137 (82%). Found: C, 59.23; H, 5.51; N, 7.55. $C_{36}H_{39}F_3O_9N_4$ requires C, 59.34; H, 5.39; N, 7.69 %.

Compound **4** was synthesised as shown below.

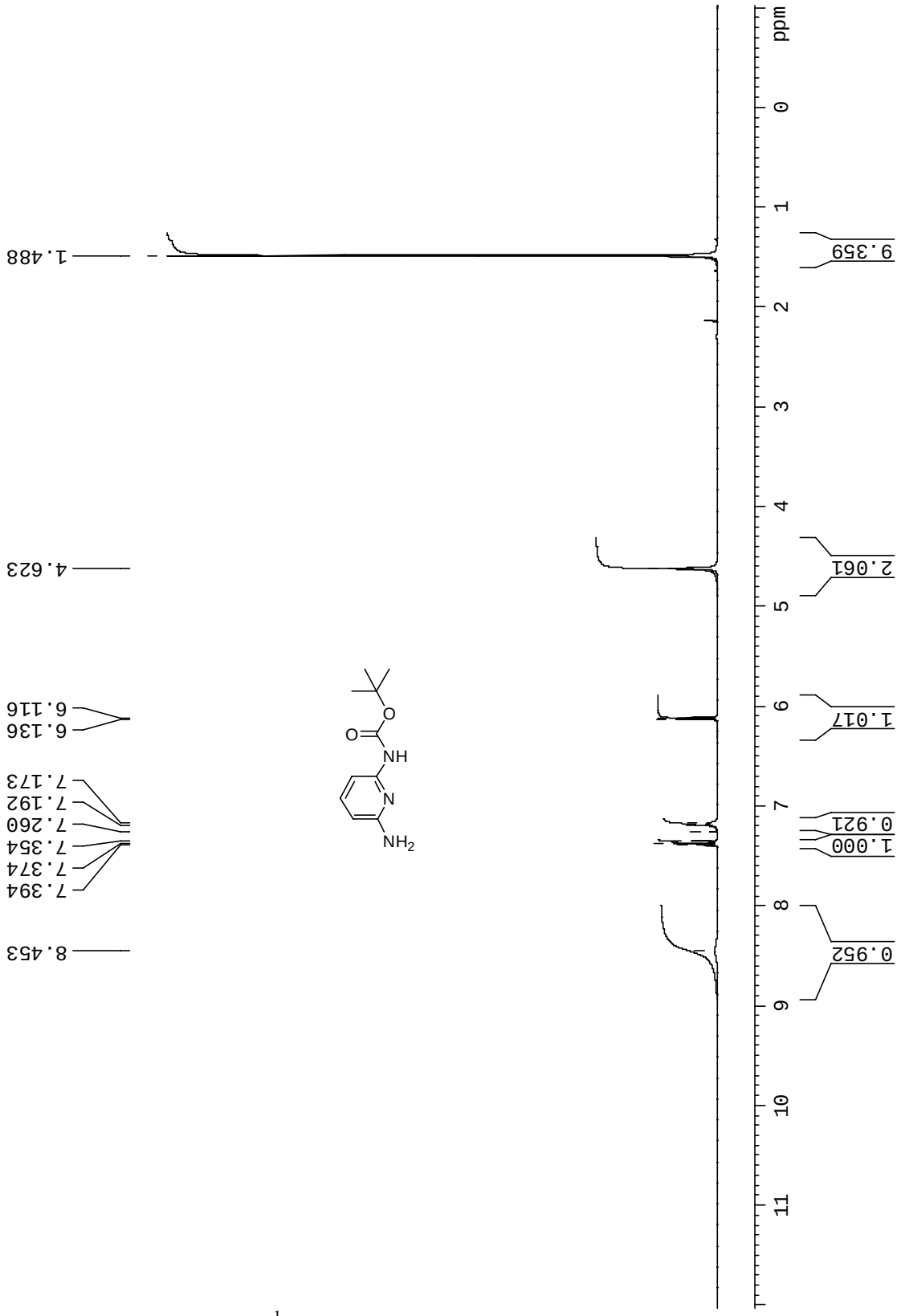


Compound 4: 4-[6-(3,3-Dimethyl-butyrylamino)-pyridin-2-ylcarbamoyl]-butyric acid 6-(1,3-dioxo-8-trifluoromethyl-2,3-dihydro-1*H*-pyrido[3,4-*b*]quinoxalin-5-yl)-hexyl ester

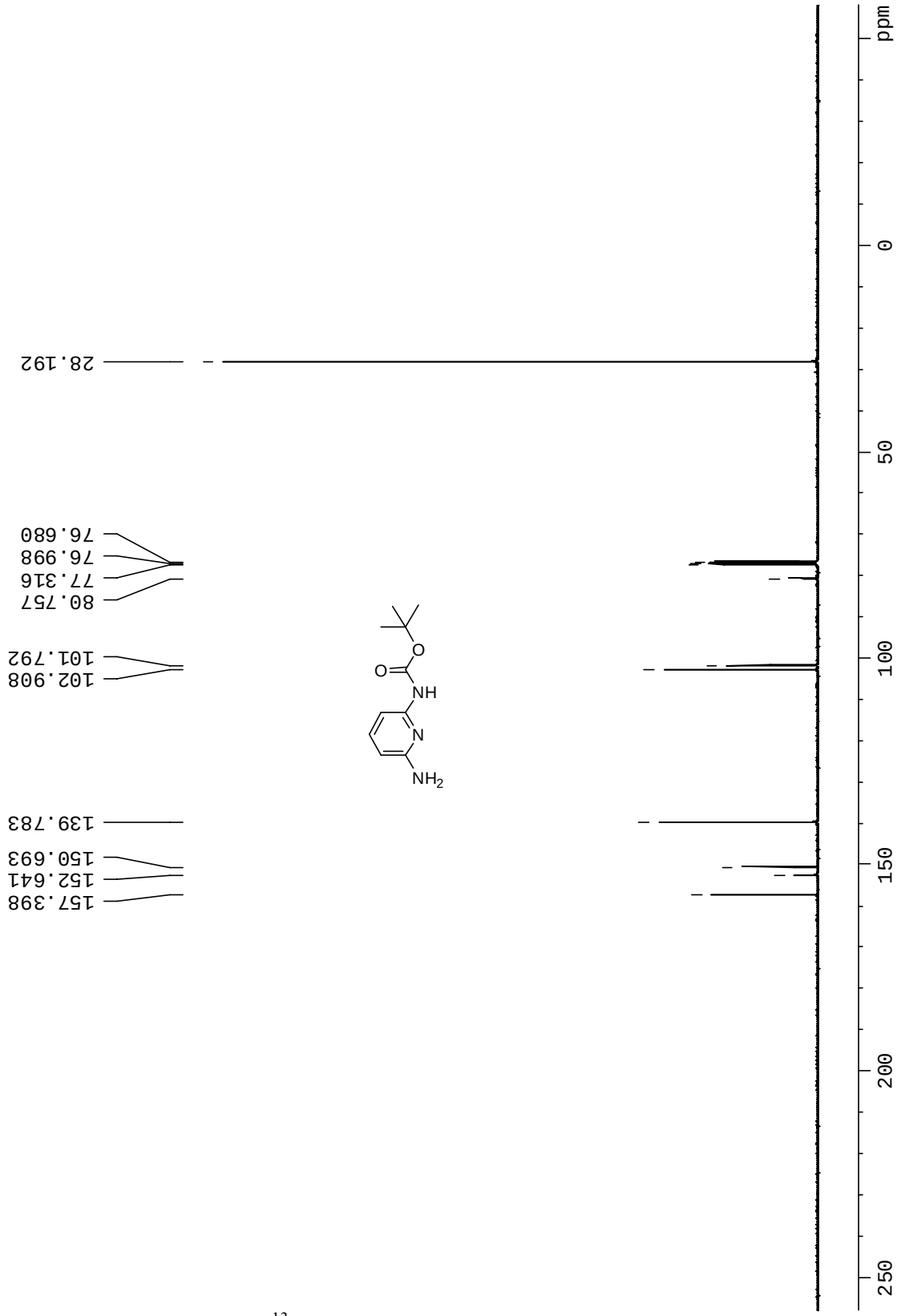
EDCI (187 mg, 0.98 mmol, 1.5 equiv.) was added to a solution of acid **17** (210 mg, 0.65 mmol, 1.0 equiv.), flavin **12** (250 mg, 0.65 mmol, 1.0 equiv.) and DMAP (7 mg, 0.06 mmol, 0.1 equiv.) in CH_2Cl_2 (5 ml). The resulting solution was stirred overnight at r.t., diluted with CH_2Cl_2 (30 ml), washed with water (2 x 100 ml), dried over magnesium sulphate and concentrated under vacuum. The residue was then purified

by column chromatography eluting with EtOAc:CH_2Cl_2 (1:1) to give **4** as a bright yellow solid (220 mg, 49%). Mpt. 230-231^oC.

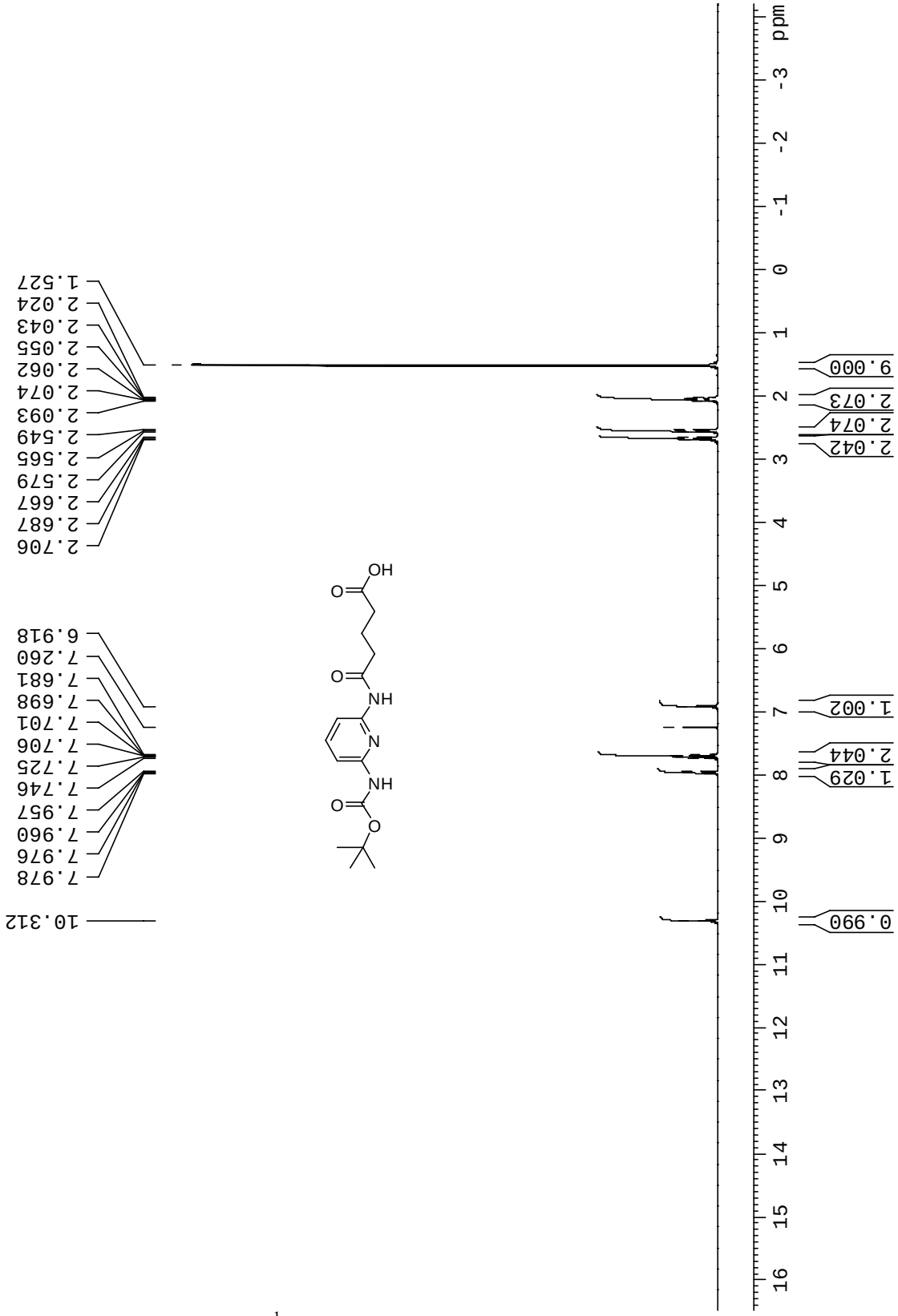
δ_H (400 MHz: $CDCl_3$): 1.10 (9H, s, $(CH_3)_3$), 1.54-1.60 (4H, m, 2 x CH_2), 1.76-1.83 (2H, m, CH_2), 1.91-1.98 (2H, m, CH_2), 2.02-2.09 (2H, m, CH_2), 2.43 (2H, t, J = 6.8 Hz, CH_2), 2.48 (2H, s, CH_2), 2.56-2.60 (2H, m, CH_2), 4.12 (2H, t, J = 7.2 Hz, CH_2), 4.76 (2H, broad s, CH_2), 7.69 (1H, t, J = 8.1 Hz, Ar-H), 7.80 (1H, d, J = 9.0 Hz, Ar-H), 7.97 (1H, d, J = 8.1 Hz, Ar-H), 7.98 (1H, d, J = 8.1 Hz, Ar-H), 8.11 (1H, dd, J = 2.0 Hz + 9.0 Hz, Ar-H), 8.65 (1H, d, J = 2.0 Hz), 9.61 (1H, broad s, NH), 10.24 (1H, broad s, NH), 12.77 (1H, broad s, NH). δ_C (100 MHz: D-6 DMSO): 20.25 (CH_2), 25.17 (CH_2), 25.68 (CH_2), 26.21 (CH_2), 27.99 (CH_2), 29.50 (CH_3), 30.84 (C), 32.81 (CH_2), 34.99 (CH_2), 44.27 (CH_2), 48.98 (CH_2), 63.72 (CH_2), 108.96 (CH), 109.02 (CH), 119.92 (CH), 123.49 (q, J = 272 Hz, CF_3), 125.72 (q, J = 33.1 Hz, C), 128.89 (q, J = 4.0 Hz, CH), 130.04 (q, J = 3.0 Hz, CH), 133.93 (C), 134.86 (C), 139.75 (CH), 140.48 (C), 150.20 (2 x C), 150.86 (C), 155.62 (C), 159.35 (C), 170.81 (C), 171.49 (C), 172.56 (C). m/z (FAB): 686.4 ($(M+H)^+$, 100%), 629.3 (20), 365.3 (40). HRMS found: 686.2916. $C_{33}H_{39}O_6N_7F_3$ requires ($(M+H)^+$, 686.2914.



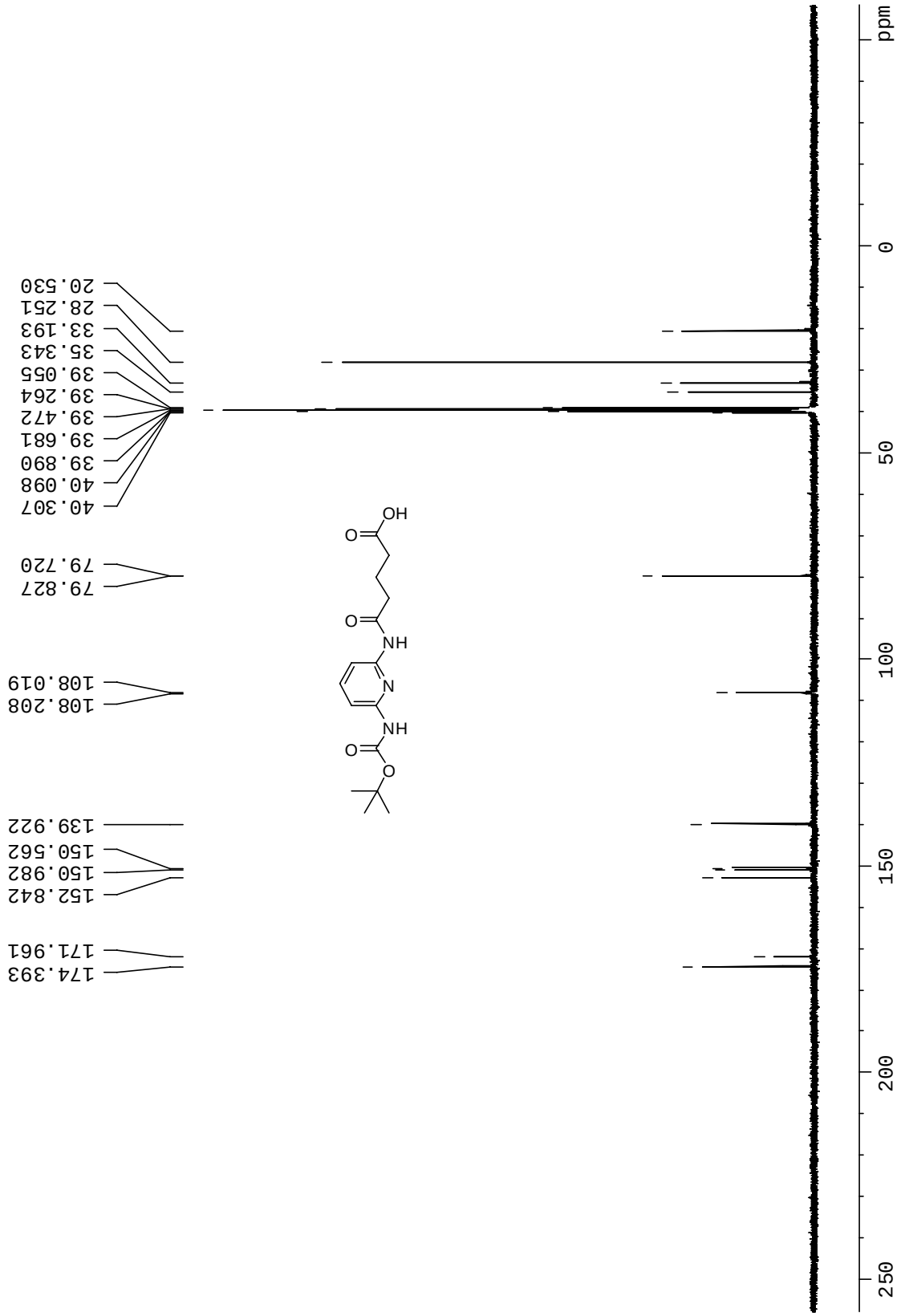
^{1}H NMR spectrum of compound **7**



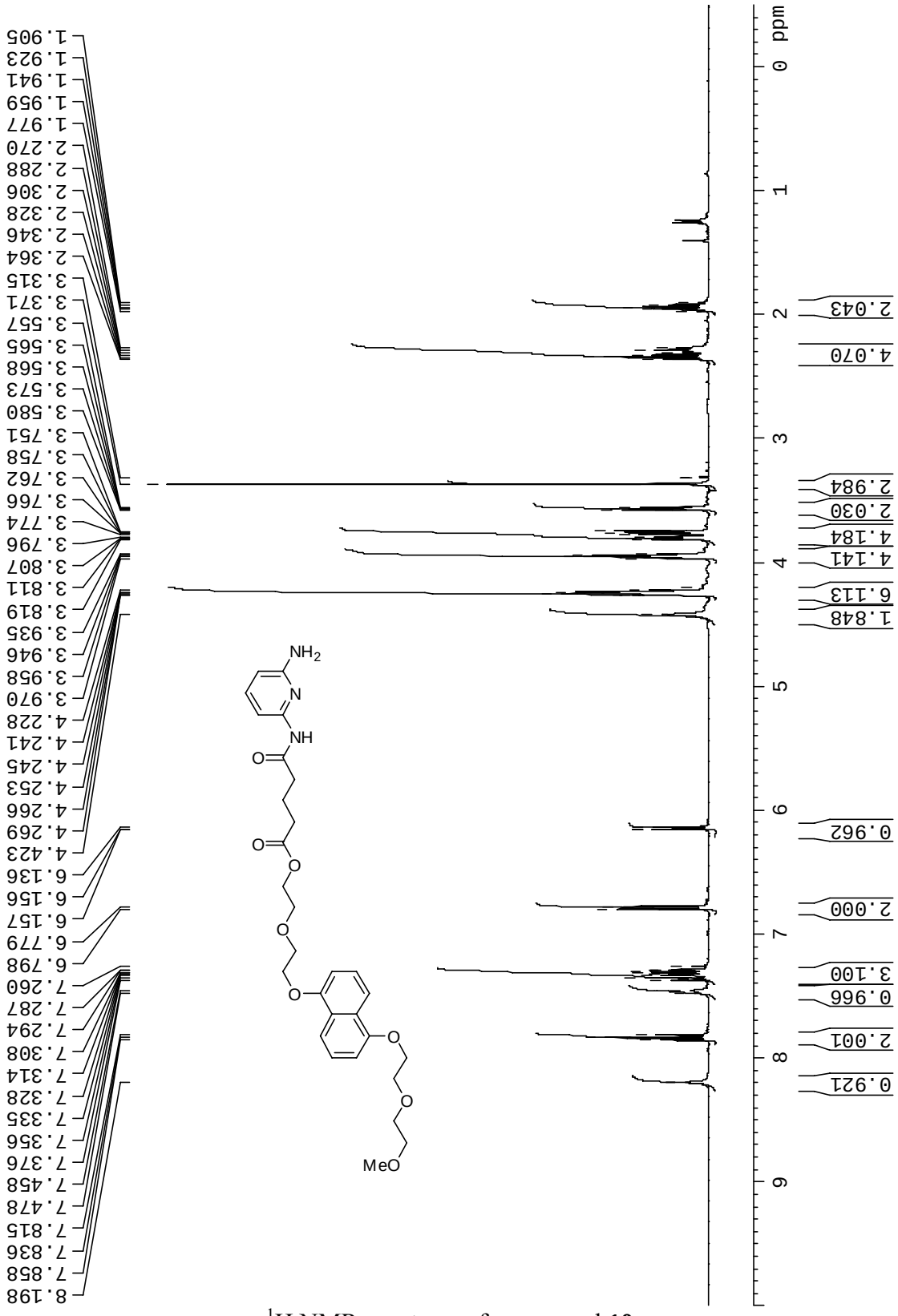
^{13}C NMR spectrum of compound **7**



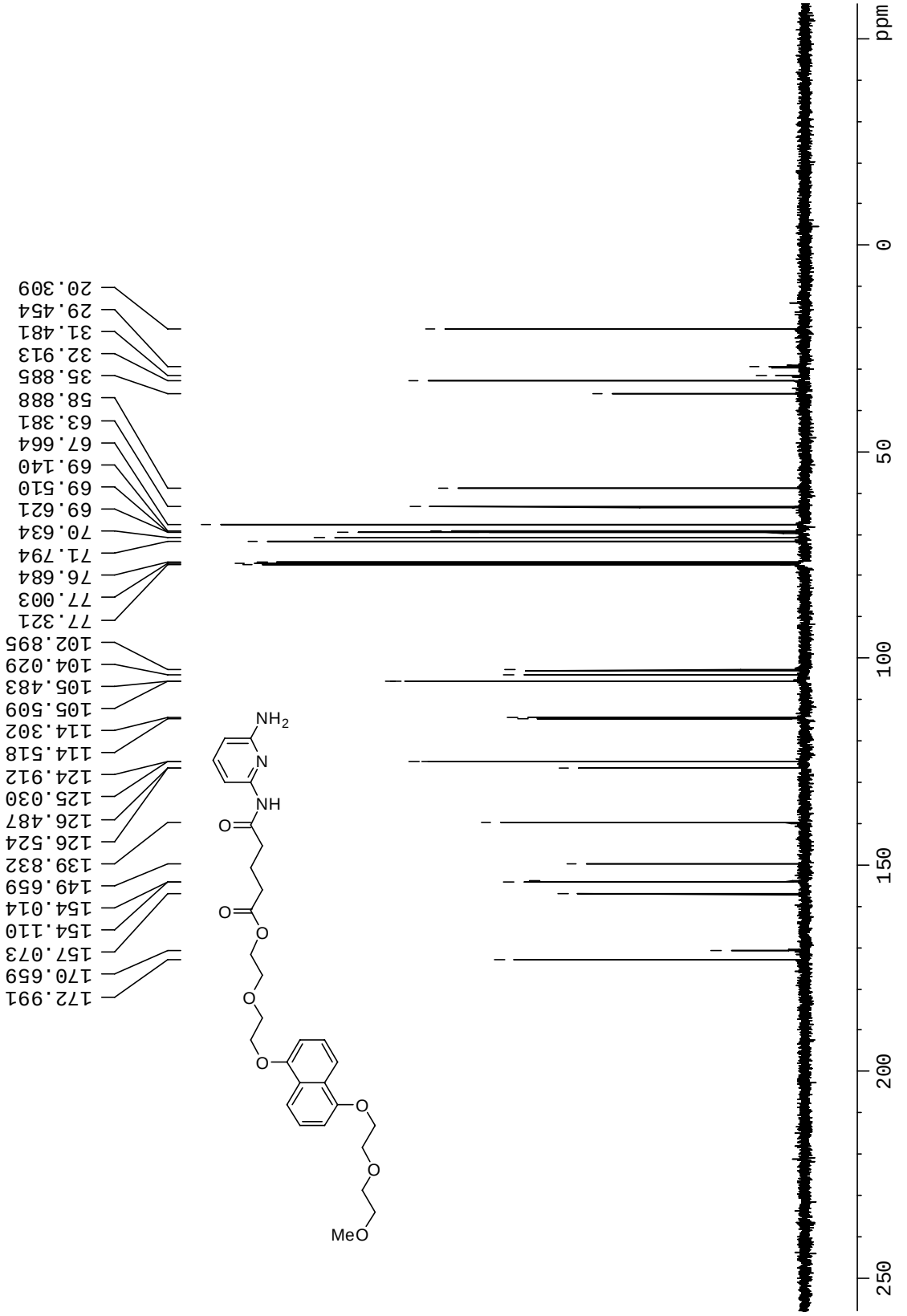
^{1}H NMR spectrum of compound **8**



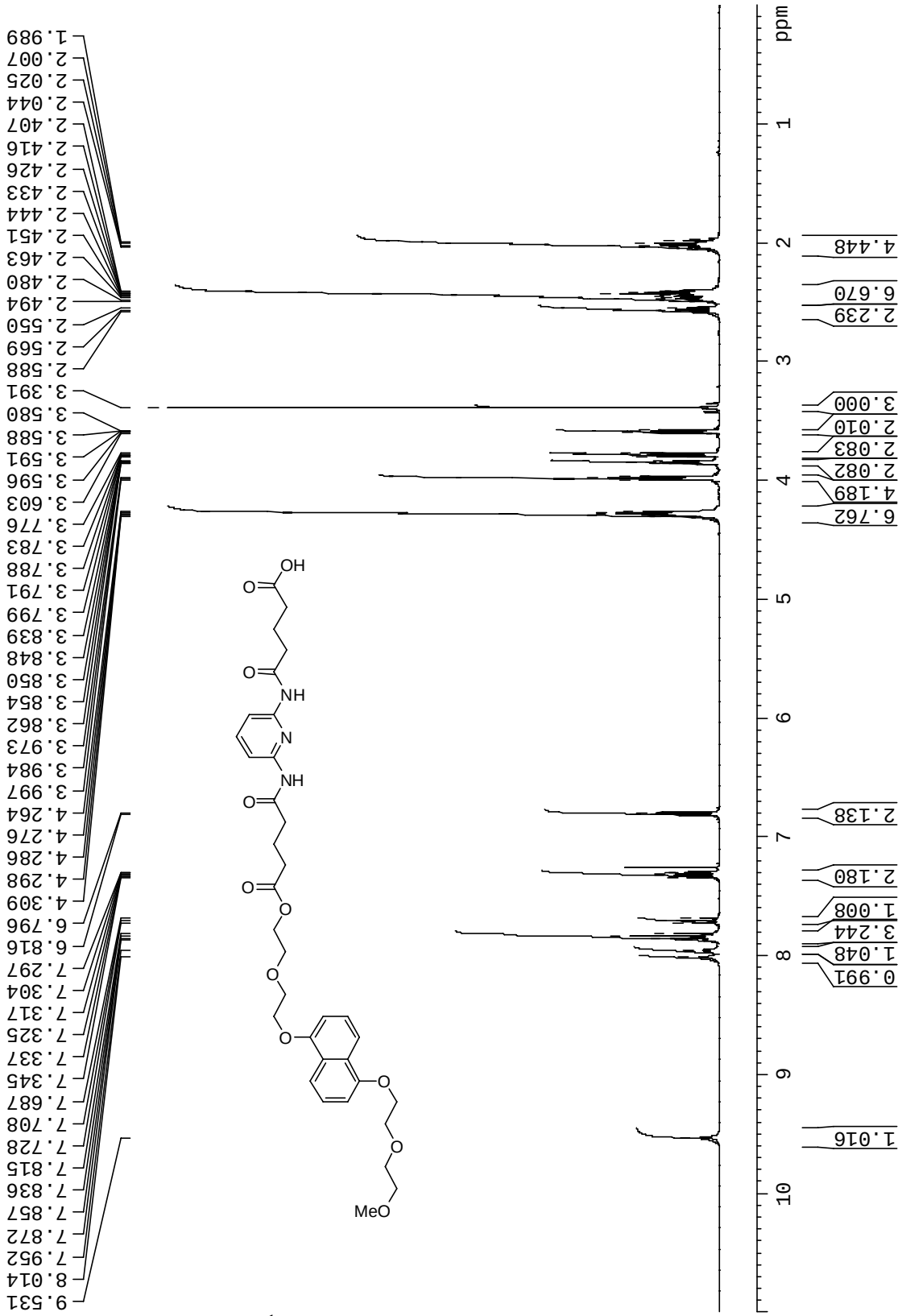
^{13}C NMR spectrum of compound **8**



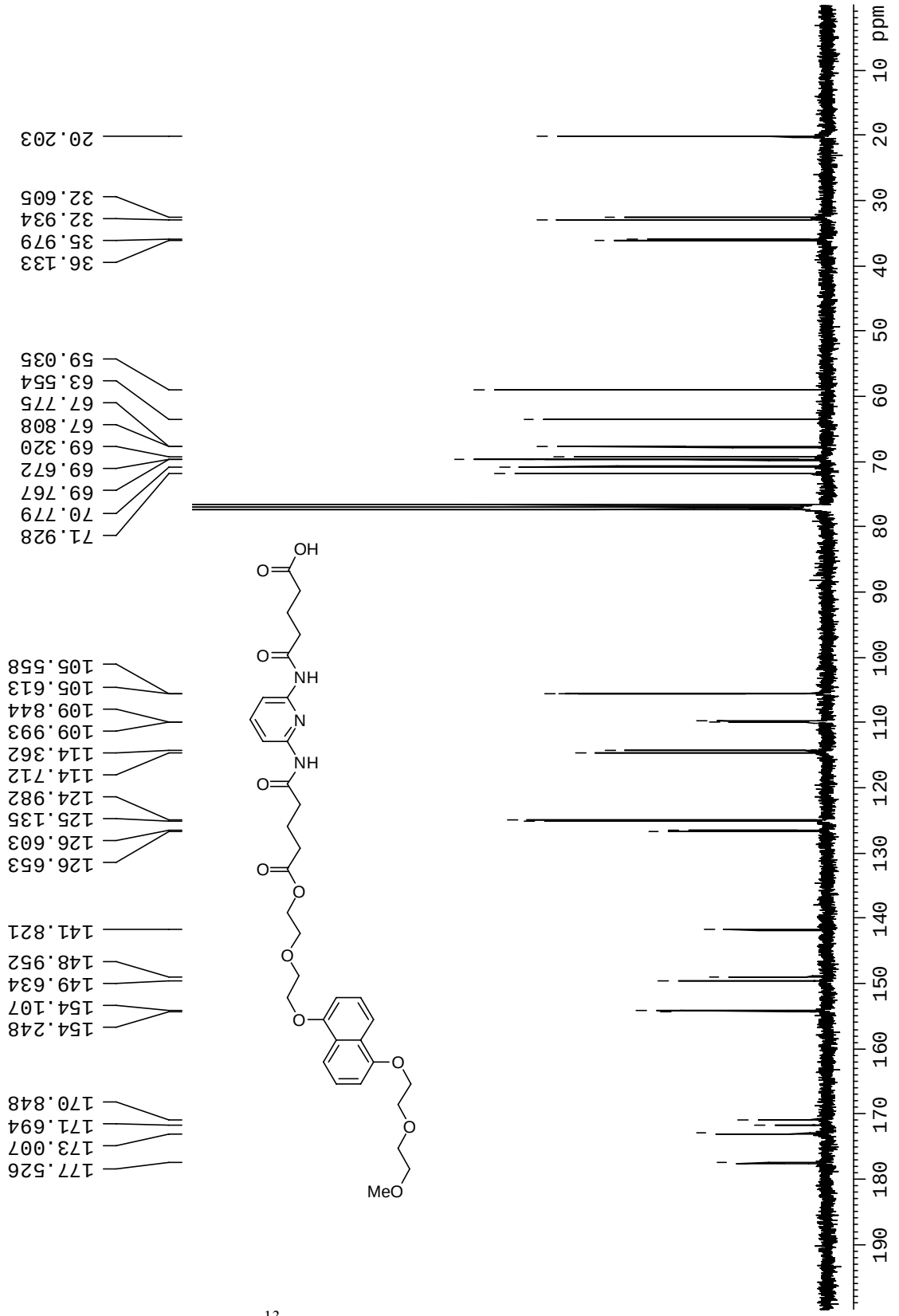
^{1}H NMR spectrum of compound **10**



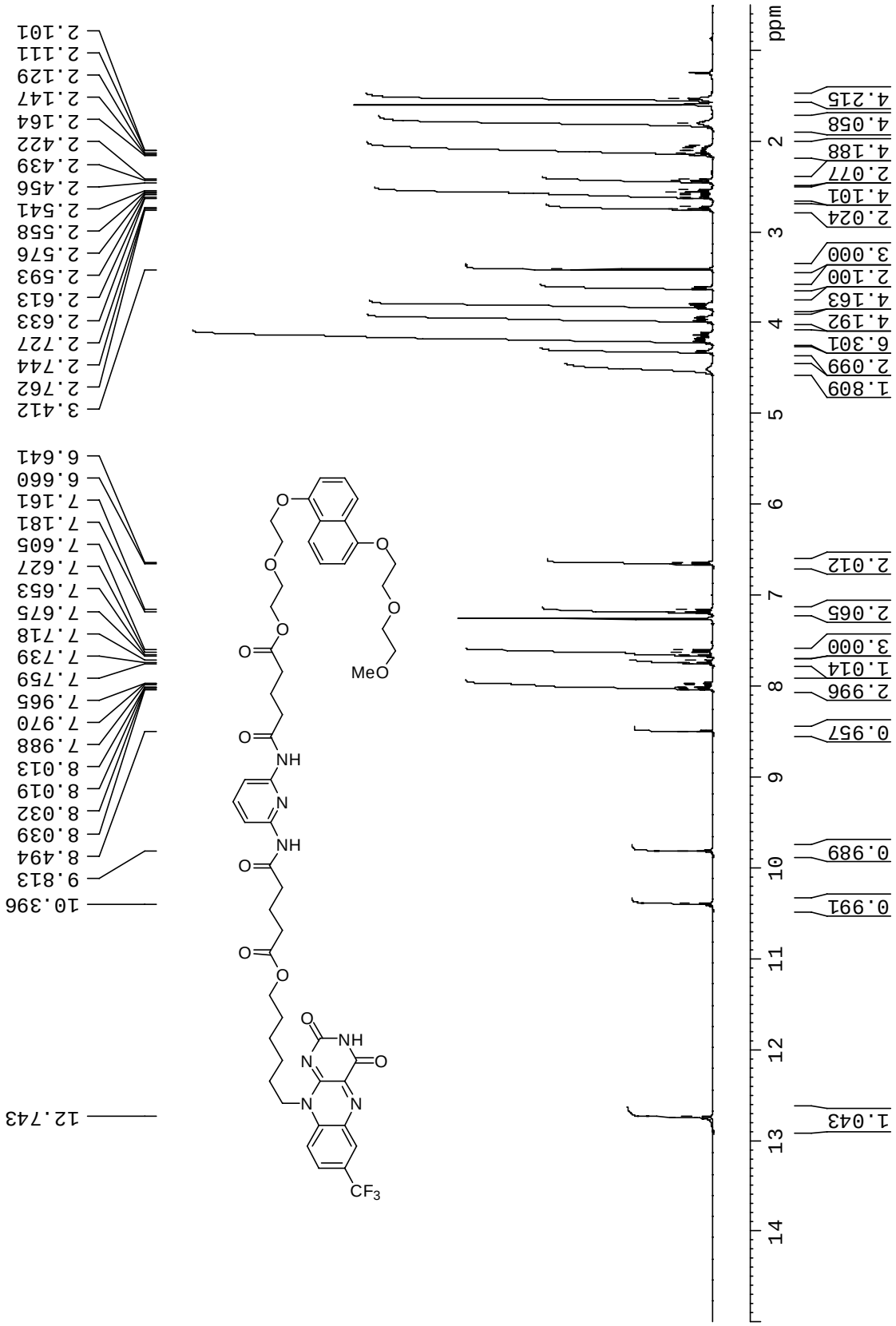
^{13}C NMR spectrum of compound **10**



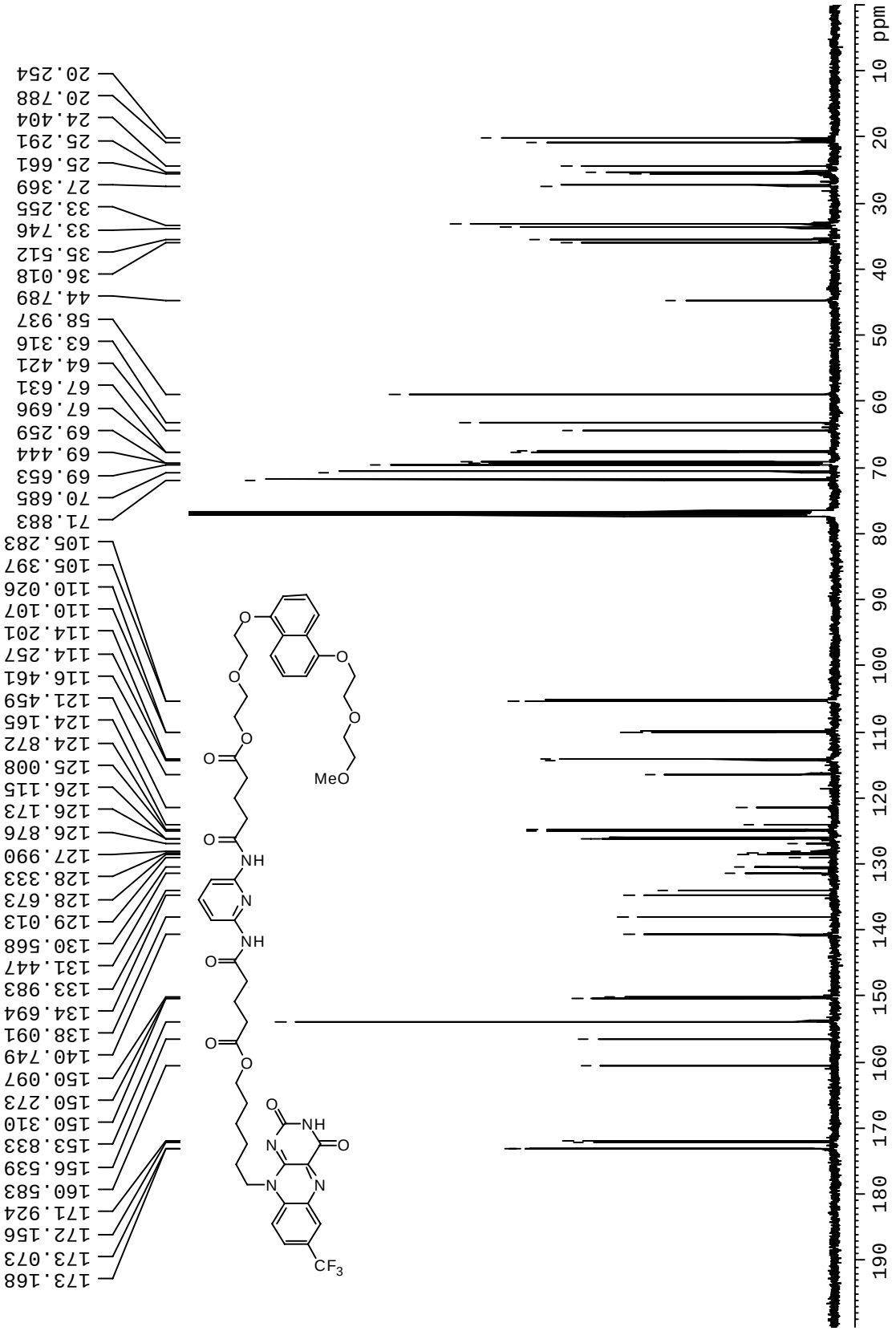
^{1}H NMR spectrum of compound **11**



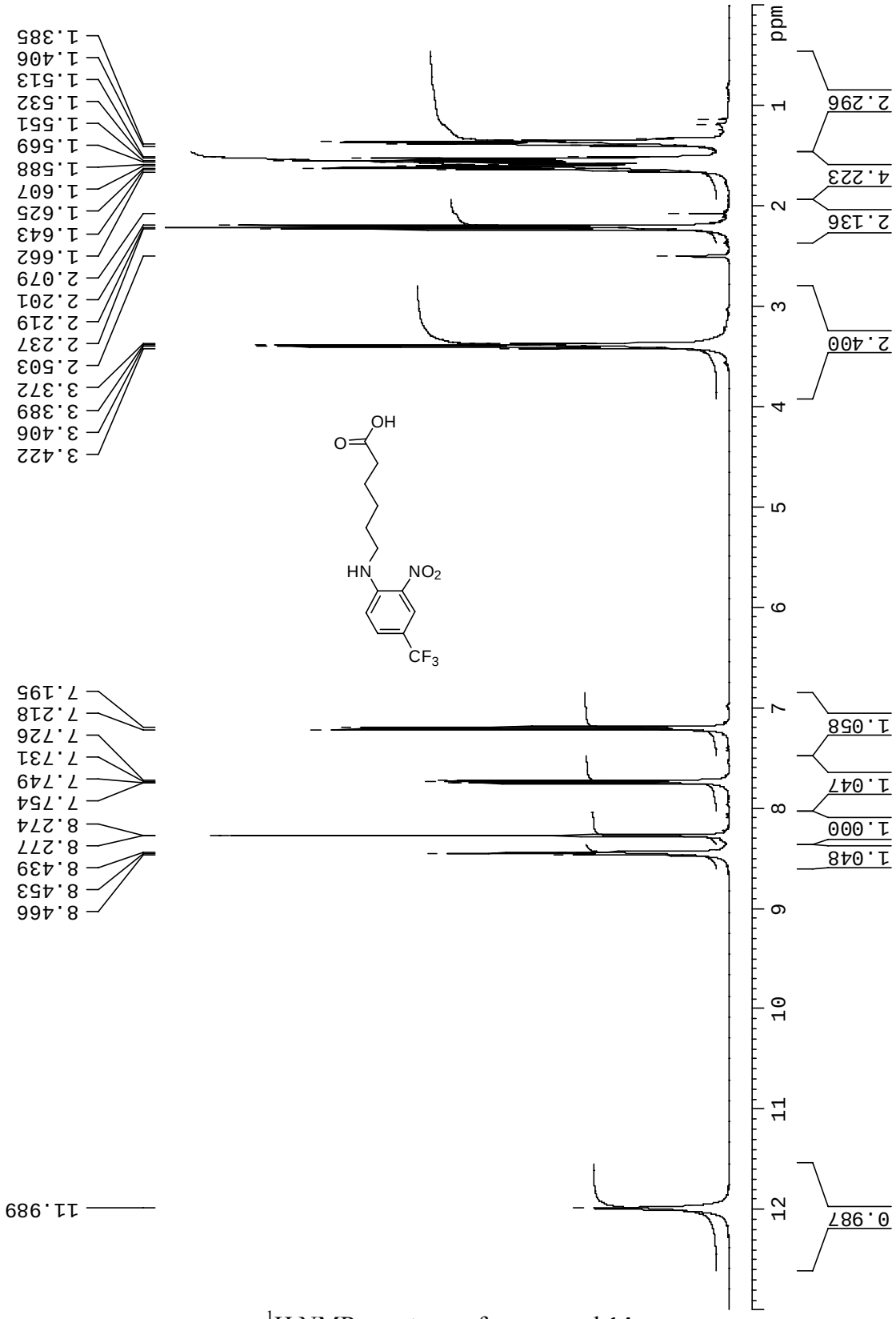
^{13}C NMR spectrum of compound **11**



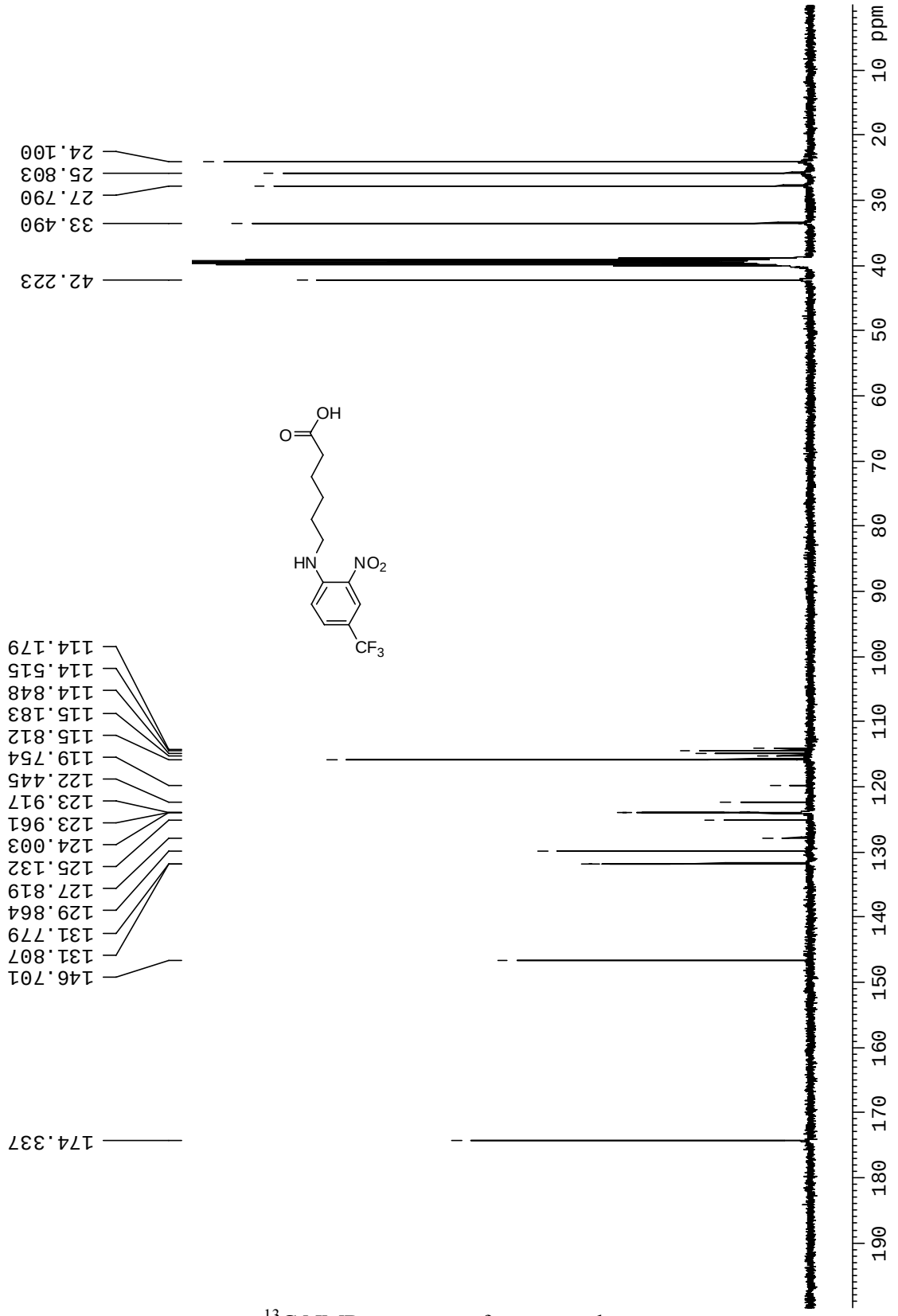
^{1}H NMR spectrum of compound **1**



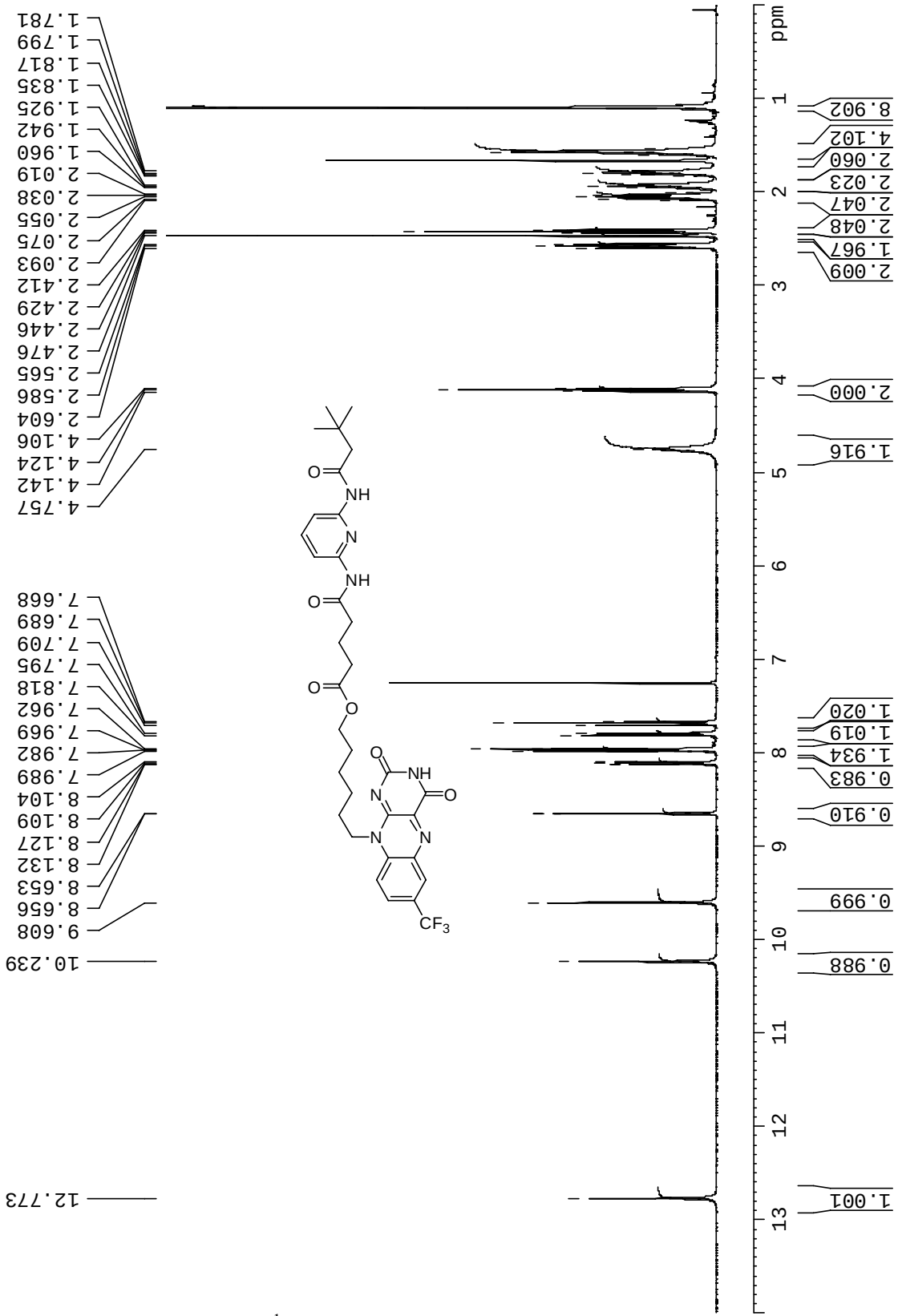
^{13}C NMR spectrum of compound **1**



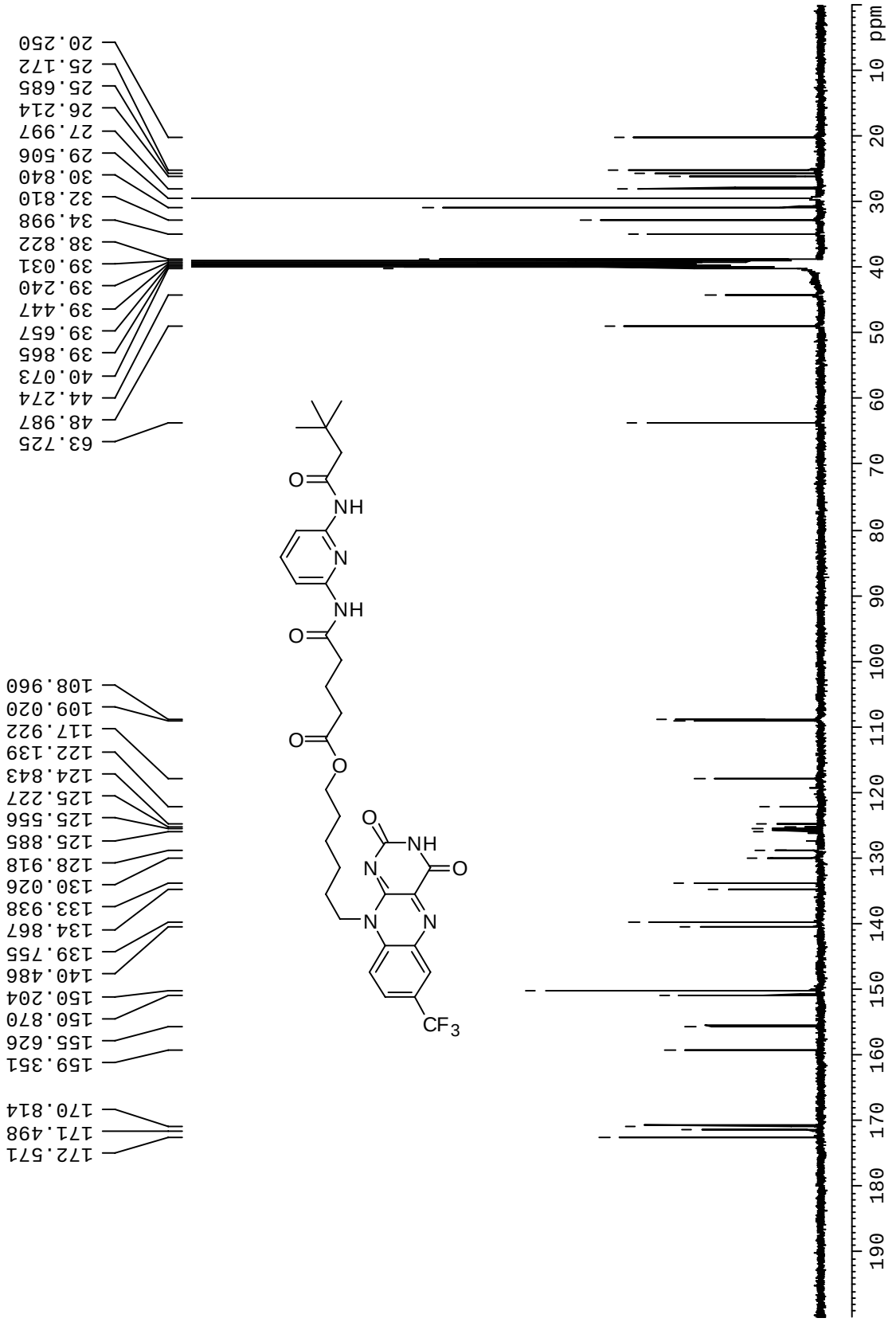
^{1}H NMR spectrum of compound **14**



^{13}C NMR spectrum of compound **14**



^{1}H NMR spectrum of compound **4**



^{13}C NMR spectrum of compound **4**

The synthesis of compounds **3**[1] , **5**[2], **7**[3] , **12**[4] , **17**[5] have been reported previously.

1 Cooke, G.; Garety, J. F.; Jordan, B.; Kryvokhyzha, N.; Parkin, A.; Rabani, G.; Rotello, V. M. *Org. Lett.* **2006**, 8, 2297.
2. Ashton, P. R.; Ballardini, R.; Balzani, V.; Boyd S. E.; Credi, A. *Chem. Eur. J.* **1997**, *3*, 152.
3. Kolomiets, E.; Berl, V.; Lehn, J.-M. *Chem. Eur. J.* **2007**, *13*, 5466.
4. Carroll, J. B.; Cooke, G.; Garety, J. F.; Jordan, B. J.; Mabruk, S.; Rotello, V. M. *Chem. Commun.* 2005, 3838.
5. Boyd, A. S. F.; Carroll, J. B.; Cooke, G.; Garety, J. F.; Jordan, B. J.; Mabruk, S.; Rosair, G.; Rotello, V. M. *Chem. Commun.* **2005**, 2468.

(b) Optical properties

(i)

(ii)

Figure S1:(i) Flavins **3**, **2**, **4**, **1** (solution in DCM) (left to right) viewed under ambient light (ii) Flavins **3**, **2**, **4**, **1** (solution in DCM) (left to right) viewed under irradiation with light of wavelength 365 nm.

UV/vis spectroscopy.
The spectra were recorded on a Perkin-Elmer Lamda 25 spectrometer.

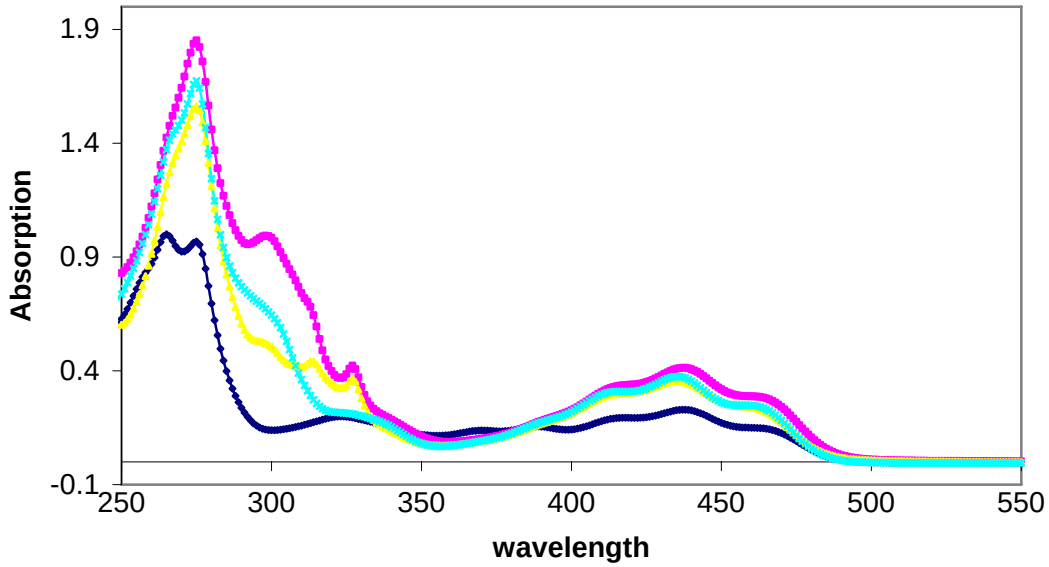


Figure S2: UV/vis spectra for compounds **1 (pink)**, **2 (yellow)**, **3 (dark blue)**, **4 (light blue)**, Spectra recorded at ~4.4x10^{-5}M in chloroform.

Fluorescence Spectroscopy
Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrometer.
All fluorescence experiments were run at ~4.4x10^{-5}M in chloroform except **3** which was run at ~4.4x10^{-6}M. Excitation wavelength = 437 nm for all samples.

(c) Electrochemistry
Cyclic voltammetry experiments were performed using a CH Instruments 440A electrochemical workstation. The electrolyte solution (0.1 M) was prepared from recrystallised Bu_4NPF_6 and dry CH_2Cl_2. A three electrode configuration was used with a platinum disc working electrode, a platinum wire counter electrode and a silver/ silver chloride reference electrode. The solution was purged with nitrogen prior to recording the electrochemical data, and all measurements were recorded under a nitrogen atmosphere.

(d) Modelling

Structure **1** was minimized using the MMFF forcefield as implemented by Macromodel 7.0, with preliminary annealing using molecular dynamics (300K).

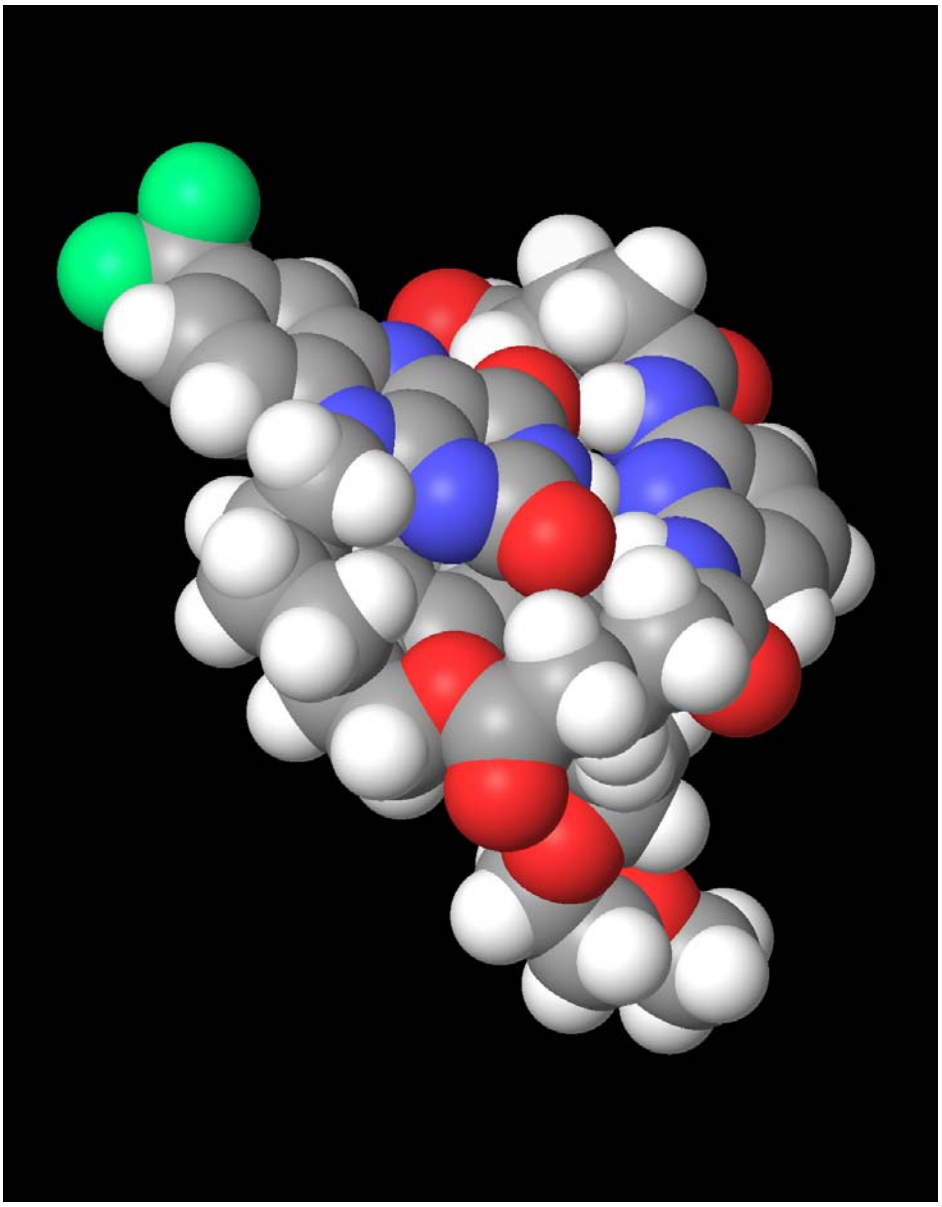


Figure S3: Modelled structure of **1** showing the compact self-assembled structure. Colour schemes: fluorine (green), oxygen (red), nitrogen (blue), carbon (grey), hydrogen (white)