Supporting Information

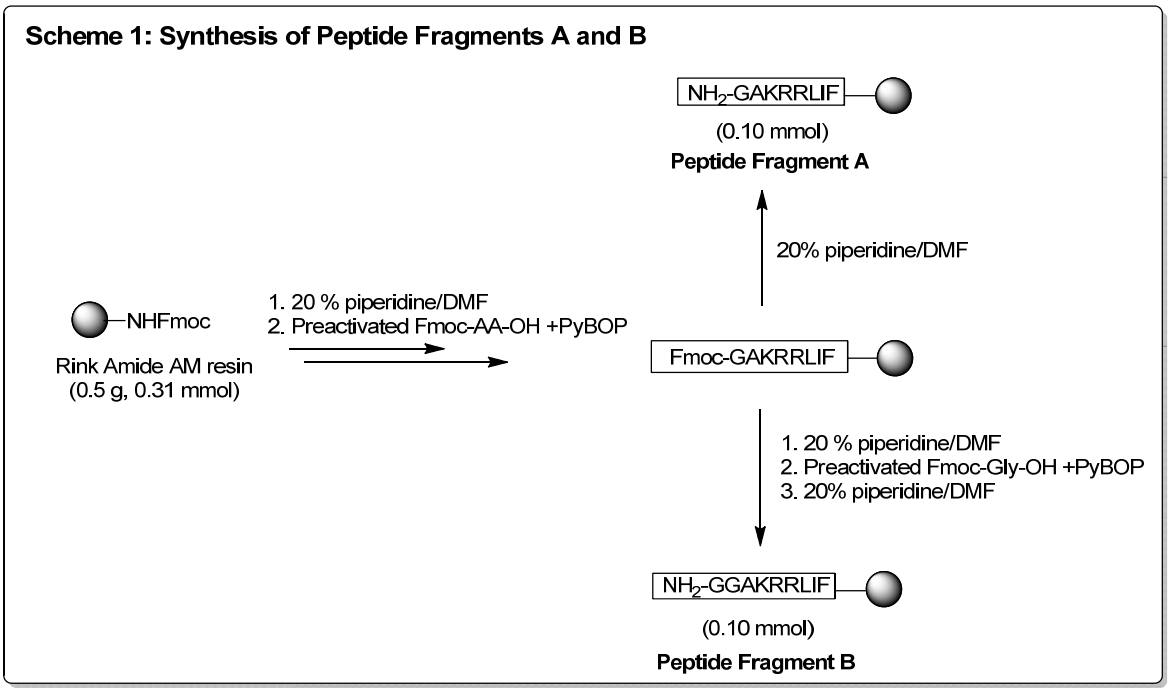
Experimental Section

a) Peptide Synthesis

Rink Amide AM resin (200-400 mesh, 0.62 mmol g-1 loading) was purchased from Nova Biochem. Fmoc-protected amino acids were purchased from Nova Biochem. PyBOP™ was purchased from CEM. N-Methylmorpholine (NMM), dimethyl sulfoxide (DMSO), triisopropylsilane (TIPS) and trifluoroacetic acid (TFA) were purchased from Aldrich and were used without further purification. HPLC grade N,N-Dimethylformamide (DMF) was obtained from Fischer Scientific.

Peptide fragments **A** and **B** were prepared by use of solid phase peptide synthesis (SPPS) on a manually operated CEM microwave as shown in Scheme 1. The Rink Amide AM resin was swollen in DMF for 15 minutes prior to use. The microwave programs used were as follows: Fmoc-deprotection = 3 min, 20 w, 75 ˚C with 20% piperidine in DMF; peptide coupling = 10 min, 20 w, 75 ˚C, using Fmoc-AA, NMM and PyBOP. Analysis of peptide fragments A and B was carried out MALDI-TOF mass spectra recorded on an Applied BiosystemsTM Voyager-DE STR instrument in positive ion mode using an α-cyano-4-hydroxycinnamic acid matrix.

Scheme 1: Synthesis of Peptide Fragments A and B



Supporting Information

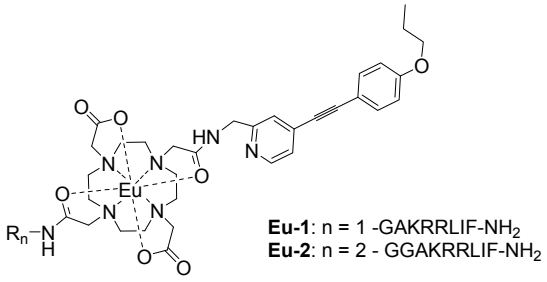
Peptide Fragment A: GAKRRLIF-NH_2

Rink Amide AM resin (0.5 g, 0.31 mmol) was suspended in a 20% solution of piperidine in DMF, stirred for 20 min at room temperature, and washed with DMF prior to use in subsequent steps. The peptide was then obtained by a stepwise elongation of the peptide chain using the microwave programs outlined above. The first amino acid to be coupled, Fmoc-Phe-OH (480 mg, 4 equiv) was dissolved in DMF and coupled to the resin in the presence of PyBOP (645 mg, 4 equiv) and NMM (135 µl, 4 equiv) and the microwave peptide coupling program. The remaining Fmoc amino acid derivatives: Fmoc-Ile-OH (438 mg, 4 equiv), Fmoc-Leu-OH (438 mg, 4 equiv), Fmoc Arg (Pbf)-OH (804 mg, 4 equiv), Fmoc Lys (Boc)-OH (581 mg, 4 equiv), Fmoc-Ala-OH (386 mg, 4 equiv), and Fmoc-Gly-OH (369 mg, 4 equiv) were coupled in an analogous fashion. Removal of the N-terminal Fmoc group from 1/3 of the original resin (0.1 mmol scale) gave peptide fragment A with a free amino group on the N-terminus. Cleavage of a small amount of the peptide from the resin using TFA: TIPS: H_2O (0.9 ml: 0.05 ml: 0.05 ml) gave a sample of the free peptide the identity of which confirmed by MALDI-TOF mass spectra.

Peptide Fragment B: GGAKRRLIF-NH_2

Fmoc-GAKRRLIF-NH_2 (0.10 mmol) previously prepared was Fmoc-deprotected using the microwave procedure described above. Peptide coupling with Fmoc-Gly-OH (123 mg, 4 equiv), PyBOP (215 mg, 4 equiv) and NMM (45 µl, 4 equiv) and the microwave peptide coupling program was then performed. Finally, removal of the N-terminal Fmoc group gave peptide fragment B with a free amino group on the N-terminus. Cleavage of a small amount of the peptide from the resin using TFA: TIPS: H_2O (0.9 ml: 0.05 ml: 0.05 ml) gave a sample of the free peptide the identity of which confirmed by MALDI-TOF mass spectra.

b) Preparation and Characterisation of Complexes Eu-1 and Eu-2



Eu-1: n = 1 -GAKRRLIF-NH_2
Eu-2: n = 2 - GGAKRRLIF-NH_2

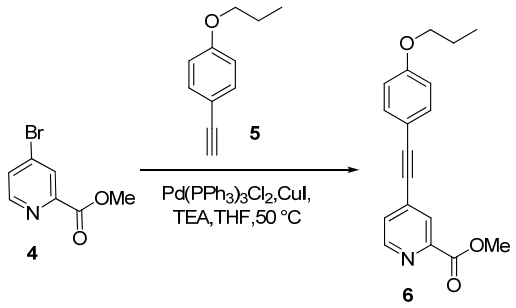
General Information

All air and water sensitive reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. All the chemicals were purchased commercially and used without further purification. Anhydrous THF was distilled

Supporting Information

from sodium-benzophenone, and dichloromethane was distilled from calcium hydride. Yields refer to chromatographically, unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm Tsingdao silica gel plates (60F-254) that were analyzed by UV or staining with KMnO4 (200 mL H_2O of 1.5 g $KMnO_4$, 10 g K_2CO_3 and 1.25 mL of 10% aqueous NaOH). Tsingdao silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. NMR spectra were recorded on either a Brüker Advance 300 (^{1}H: 300 MHz, ^{13}C: 75.5 MHz), or Brüker Advance 500 (^{1}H: 500 MHz, ^{13}C: 125.8 MHz). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectra were obtained from Applied Biosystems (ABI) Q-Star Elite MALDI-TOF Mass Spectrometer.

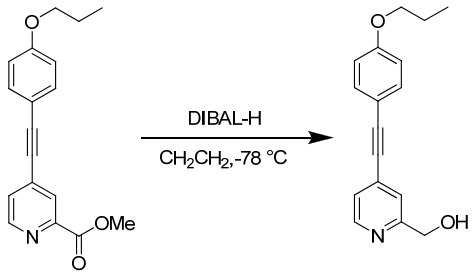
Synthesis of methyl 4-((4-propoxyphenyl)ethynyl)picolinate (6)



To a stirred solution of methyl 4-bromopicolinate (**4**) (2.0 g, 9.26 mmol) in TEA/THF (10ml/30ml) was added $Pd(PPh_3)_3Cl_2$, CuI, and 1-ethynyl-4-propoxybenzene (**5**). The resulting mixture was stirred at 50 ºC for 12 hours and then treated with a saturated aqueous solution of NaCl (40 mL). The aqueous layer was extracted by ethyl acetate (30 mL ×3), and then the combined organic layers were dried over sodium sulfate, filtered and concentrated. Silica gel flash column chromatography (hexanes: ethyl acetate = 5:1) of the residue gave a pale yellow solid (2.64 g, 8.94 mmol, 97%) as the product. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.71 (d, J = 5.0 Hz, 1H), 8.20 (s, 1H), 7.52 (dd, J = 5.1 Hz, 1.5Hz, 1H), 7.51 (d, J = 8.8 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 4.03 (s, 3H), 3.97 (t, J = 6.6 Hz, 2H), 1.86-1.81 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.3, 160.3, 149.7, 148.1, 133.6, 133.3, 128.1, 126.9, 114.8, 113.5, 96.0, 85.0, 69.7, 52.9, 29.6, 22.4, 10.3; HRMS m/z calcd. for $C_{18}H_{18}NO_3$ $(M+H)^+$ 296.1281, found 296.1281, calcd. for $C_{18}H_{17}NO_3$ $(M+Na)^+$ 318.1101, found 318.1113.

Supporting Information

Synthesis of (4-((4-propoxyphenyl)ethynyl)pyridin-2-yl)methanol (7)



6 7

To a stirred solution of 6 (2.6 g, 8.8 mmol) in CH_2Cl_2 (40 mL) at -78 ºC was added DIBAL-H (36 mL of a 1.0 M solution in toluene, 36mmol) dropwise. The resulting mixture was stirred at 0 ºC for 5 hours and then treated with methanol (5 mL) at -78 ºC slowly. The solution was warmed to room temperature and treated with saturated aqueous solution of potassium sodium tartrate (30mL). After stirring for 12 hours, the solution was treated with H_2O (20 mL) and the pH of the solution was brought to neutral by adding a saturated aqueous solution of NH_4Cl. The aqueous layer was extracted with diethyl ether (50 mL ×3). The combined extracts were dried over Na_2SO_4, filtered and concentrated. Silica gel flash column chromatography (hexanes: ethyl acetate = 5:1) of the residue gave an off-white solid (1.1 g, 4.1 mmol, 47%) as the product. The aldehyde intermediate was re-submitted to the above procedures and 85% overall yield of **7** was obtained. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.55 (br, 1H), 7.50 (d, J = 8.7 Hz, 2H), 7.44 (br, 1H), 7.39 (br, 1H), 6.91 (d, J = 8.8 Hz, 2H), 4.83 (br, 1H), 3.97 (t, J = 6.6 Hz, 2H), 1.87-1.80 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) : δ 160.8, 158.1, 145.0, 137.0, 134.0, 125.1, 124.0, 114.9, 112.9, 99.5, 85.2, 69.8, 62.5, 22.4, 10.4; HRMS m/z calcd. for $C_{17}H_{18}NO_2$ $(M+H)^+$ 268.1332 , found 268.1346.

Synthesis of 2-(azidomethyl)-4-((4-propoxyphenyl)ethynyl)pyridine (8)

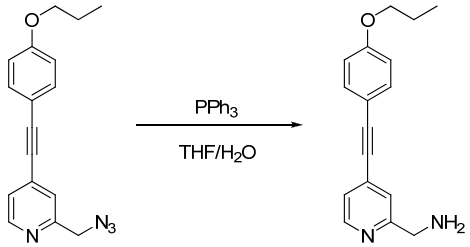
7 8

To a stirred solution of **7** (1.0 g, 3.7 mmol) and TEA (2.7 mL, 18.5 mmol) in CH_2Cl_2 (30ml) at 0 ºC was added MgCl (0.86 mL, 11.1 mmol) slowly. The resulting mixture was stirred at

Supporting Information

room temperature for 0.5 hour and then treated with water (20mL). The aqueous layer was extracted with CH_2Cl_2 (20 mL ×3), and then the combined organic extracts were dried over sodium sulfate, filtered and concentrated. To a stirred solution of the crude product in anhydrous DMF (15 mL) was added TBAI (136 mg, 0.37 mmol) and NaN_3 (355 mg, 5.6 mmol). The resulting mixture was stirred at room temperature for 12 hours and then treated with water (15 mL). The aqueous layer was extracted with ethyl acetate (30 mL ×3), and the combined organic extracts were dried over sodium sulfate, filtered and concentrated. Silica gel flesh column chromatography (hexanes to hexanes: ethyl acetates = 30:1) of the residue gave a colorless liquid (864 mg, 2.96 mmol, 80%) as the product. ^{1}H NMR ($CDCl_3$, 500MHz): δ 8.56 (d, J = 5.1 Hz, 1H), 7.47 (d, J = 8.7 Hz, 2H), 7.42 (s, 1H), 7.30-7.29 (d, J = 5 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 4.49 (s, 2H), 3.95 (t, J = 6.6 Hz, 2H), 1.86-1.78 (m, 2H), 1.05 (t, J = 7.4, 3H); 13C NMR ($CDCl_3$, 125 MHz) : δ 160.1, 155.8, 149.5, 133.5, 133.0, 124.5, 123.5, 114.7, 113.7, 95.0, 85.4, 69.6, 55.5, 22.5, 10.4; HRMS m/z calcd. for $C_{17}H_{17}N_4O$ $(M+H)^+$ 293.1397, found 293.1400.

Synthesis of (4-((4-propoxyphenyl)ethynyl)pyridin-2-yl)methanamine (9)

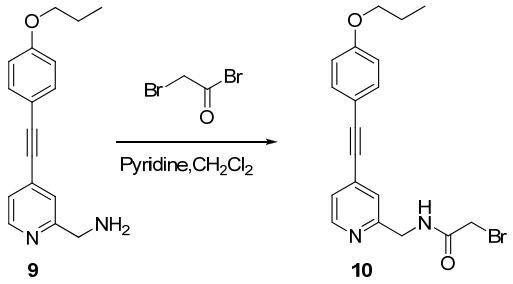


8 **9**

To the solution of **8** (800 mg, 2.74 mmol) in THF/H_2O (5:1, 15 mL) was added PPh_3 (790 mg, 3.0 mmol) at room temperature. The resulting mixture was stirred at room temperature for 12 hours and then treated with water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (20mL × 3) and the combined organic extracts were dried over sodium sulfate, filtered and concentrated. Silica gel flesh column chromatography (CH_2Cl_2: MeOH = 10:1) a white solid (439mg, 1.6 mmol, 60%) as the product. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.53 (d, J = 5.0 Hz, 1H), 7.47 (d, J = 8.7 Hz, 2H), 7.38 (s, 1H), 7.24 (d, J = 5.0 Hz, 1H), 6.89 (d, J = 8.7 Hz, 2H), 3.99 (s, 3H), 3.95 (t, J = 6.6 Hz, 2H), 1.93 (s, 2H), 1.84 (t, J = 7.1 Hz, 3H); ^{13}C NMR (CDCl3, 75 MHz): δ 161.7, 159.9, 149.2, 133.4, 132.4, 123.4, 122.9, 114.6, 113.8, 94.2, 85.7, 69.6, 47.5, 22.5, 10.5; HRMS m/z calcd. for C17H19N2O $(M+H)^+$ 267.1492, found 267.1487.

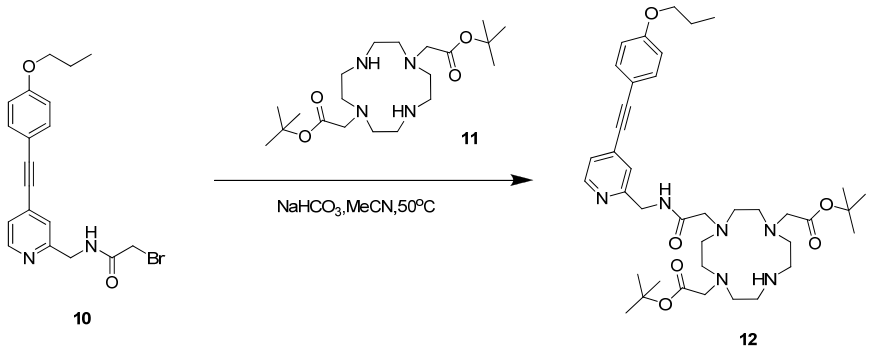
Supporting Information

Synthesis of 2-bromo-N-((4-((4-propoxyphenyl)ethynyl)pyridin-2-yl)methyl)acetamide (10)



To a stirred solution of **9** (400 mg, 1.5 mmol) in CH2Cl2 (10 mL) was added pyridine (0.85mL, 7.5 mmol) and bromoacetated bromide (0.4 mL,4.5 mmol) at 0 ^{o}C. The resulting mixture was stirred at 0^{o}C for 2 hours and then treated with an aqueous saturated $NaHCO_3$ solution (15mL). The aqueous layer was extracted with ethyl acetate (20mL × 3) and the combined organic extracts were dried over sodium sulfate, filtered and concentrated. Silica gel flash column chromatography (ethyl acetate: hexanes = 1:1) of the residue gave a pale yellowed solid (369mg, 0.95 mmol, 68%) as the product. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.53 (d, J = 5.1 Hz, 1H), 7.70 (br, 1H), 7.48 (dd, J = 7.1 Hz, 1.8Hz, 2H), 7.34 (s, 1H), 7.29 (s, 1H), 7.28 (s, 1H), 6.90, (dd, J = 7.1 Hz, 1.8Hz, 2H), 4.60 (d, J = 5 Hz, 2H), 3.97-3.95 (m, 4H), 1.87-1.80 (m, 2H), 1.06 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.6, 160.1, 155.6, 149.0, 133.5, 132.8, 124.2, 123.5, 114.7, 113.7, 94.9, 85.4, 69.6, 44.8, 28.9, 22.5, 10.4; HRMS m/z calcd. for $C_{19}H_{20}N_2O_2Br$ $(M+H)^+$ 387.0703, found 387.0714 .

Synthesis of tert-butyl 2,2'-(4-(2-oxo-2-((4-((4-propoxyphenyl)ethynyl)pyridin-2-yl)methylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (12)

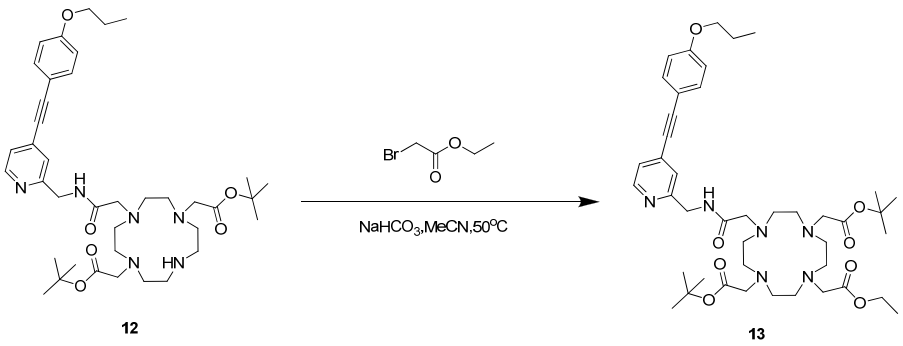


To a stirred solution of tert-butyl 2,2'-(1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (**11**) (188 mg, 0.47 mmol) in anhydrous MeCN was added $NaHCO_3$ (65 mg, 0.78 mmol) and 10 (60 mg, 0.16 mmol). The resulting mixture was stirred at room-temperature for 16 hours. The mixture was then filtered and the filtrate was concentrated. Silica gel flesh column

Supporting Information

chromatography (CH_2Cl_2: MeOH = 20:1) of the residue gave a pale yellow solid (95 mg, 0.13 mmol, 87%) as the product. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.49 (d, J = 5.5 Hz, 1H), 8.35 (t, J = 5 Hz, 1H), 7.47 (d, J = 9 Hz, 2H), 7.38 (s, 1H), 7.30 (d, J = 5 Hz, 1H), 6.89 (d, J = 8.5 Hz, 2H), 4.57 (d, J = 5.5 Hz, 2H), 3.96 (t, J = 6.5 Hz, 2H), 3.26 (s, 2H), 3.14-2.89 (m, 20H), 1.86-1.79 (m, 2H), 1.40 (s, 18H), 1.05 (t, J = 7.5, 3H) ; ^{13}C NMR ($CDCl_3$, 75MHz): δ 171.7, 170.0, 160.0, 156.8, 148.7, 133.4, 133.0, 124.3, 124.1, 114.6, 113.3, 95.3, 85.1, 81.6, 69.5, 57.5, 56.3, 55.3, 52.6, 48.6, 47.2, 44.8, 28.0,22.3,10.4; HRMS m/z calcd. for $C_{39}H_{59}N_6O_6$ $(M+H)^+$ 707.4491, found 707.4641.

Synthesis of tert-butyl 2,2'-(4-(2-ethoxy-2-oxoethyl)-10-(2-oxo-2-((4-((4-propoxyphenyl)ethynyl) pyridine-2-yl)methylamino)ethyl)-1,4,7,10-tetraaza-cyclododecane-1,7-diyl)diacetate (13)

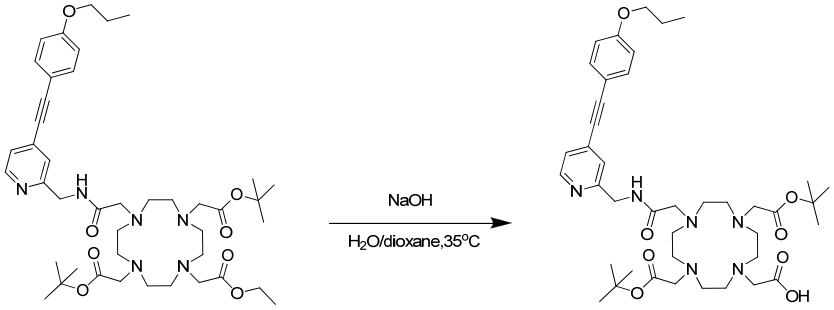


12 13

To a stirred solution of tert-butyl 2,2'-(4-(2-ethoxy-2-oxoethyl)-10-(2-oxo-2-((4-((4-propoxyphenyl)ethynyl) pyridin-2-yl)methylamino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (**12**) (80 mg, 0.11 mmol) in anhydrous MeCN (1.5mL) was added $NaHCO_3$ (48 mg, 0.56 mmol) followed by ethyl 2-bromoacetate (38 µL, 0.34 mmol). The resulting mixture was stirred at 50 ^{o}C for 16 hours. The mixture was then filtered and the filtrate was concentrated. Silica gel flesh column chromatography (CH_2Cl_2: MeOH = 20:1) of the residue gave a pale yellow solid (82 mg, 0.10 mmol, 92%) as the product. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.84 (t, J = 5.8 Hz, 1H), 8.43 (d, J = 5.1 Hz, 1H), 7.46 (d, J = 8.6 Hz, 2H), 7.39 (s, 1H), 7.17 (d, J = 5.1 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 4.56 (br, 2H), 4.12 (m, 2H), 3.95 (t, J = 6.6 Hz, 2H), 3.55 (br, 2H), 3.50-1.90 (m, 22H), 1.86-1.79 (m, 2H), 1.36 (s, 18H), 1.24 (t, J = 7.2 Hz, 3H), 1.05 (t, J = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.0, 172.4, 172.3, 160.0, 158.6, 148.6, 133.4, 132.6, 123.8, 122.7, 114.7, 114.0, 94.3, 85.9, 81.9, 69.7, 61.1, 56.4, 55.7, 55.0, 50.0 (br), 44.4, 27.9, 22.5, 14.1, 10.4; HRMS m/z calcd. for $C_{43}H_{65}N_6O_8$ $(M+H)^+$ 793.4858, found 793.4873.

Supporting Information

Synthesis of 2-(4,10-bis(2-tert-butoxy-2-oxoethyl)-7-(2-oxo-2-((4-((4-propoxyphenyl)-ethy-nyl)pyridin-2-yl)methylamino)ethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid (14)

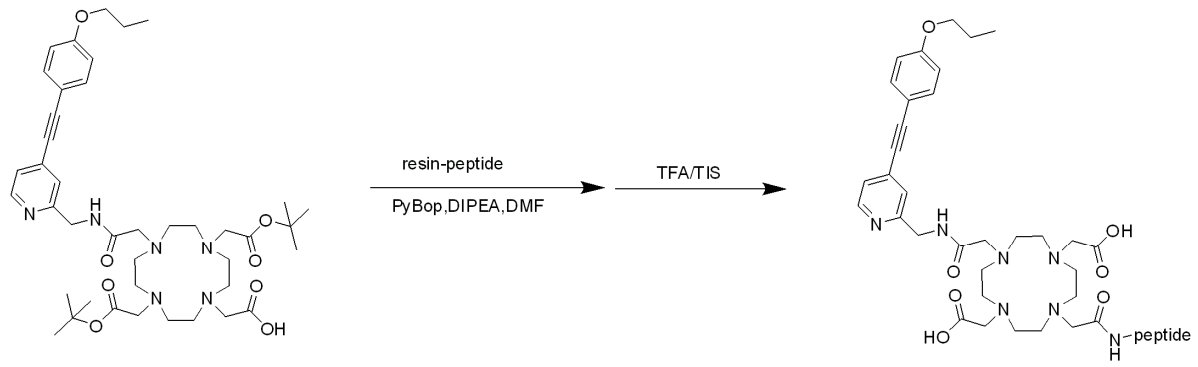


13 14

Tert-butyl 2,2'-(4-(2-ethoxy-2-oxoethyl)-10-(2-oxo-2-((4-((4-propoxyphenyl)ethynyl)pyridin-2-yl)methyl amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (**13**) (80 mg, 0.10mmol) was dissolved in 2mL of a 1:1 (v:v) 1,4-dioxane:NaOH (a 0.4 M aqueous solution). This solution was stirred 36 hours under N_2 at 35°C. 1,4-Dioxane was evaporated under reduced pressure, and water (5 mL) was added. After extracting with CH_2Cl_2 (15mL × 4), the organic phases were combined and washed with water (15 mL) and brine (15 mL), dried with anhydrous Na_2SO_4, and concentrated to give a off-white solid (50 mg, 0.066 mmol, 65%) as the product. ^{1}H NMR ($CDCl_3$, 300MHz): δ 8.43 (d, J = 8.5 Hz, 1H), 8.38 (br, 1H), 7.46 (d, J = 9 Hz, 2H), 7.38 (s, 1H), 7.20 (d, J = 5.1 Hz, 1H), 6.88 (d, J = 8.7 Hz, 2H), 4.53 (d, J = 5.1 Hz, 2H), 3.94 (t, J = 6.6 Hz, 2H), 3.46-2.10 (m, 24H), 1.85-1.78 (m, 2H), 1.34 (s, 18H), 1.031 (t, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$, 125MHz): δ 174.8, 171.8, 171.6, 160.1, 157.7, 148.5, 133.5, 133.0, 123.8, 123.1, 114.8, 113.8, 94.9, 85.7, 81.9, 69.7, 56.7, 56.4, 56.0, 50.6 (br), 44.1, 28.1, 22.5, 14.0, 10.4; HRMS m/z calcd. for $C_{41}H_{61}N_6O_8$ $(M+H)^+$ 765.4545, found 765.4545.

Supporting Information

Synthesis of ligands (1 and 2) with peptides A and B



peptide = -[Gly-Gly-Ala-Lys-Arg-Arg-Arg-Leu-Ile-Phe]-NH_2 (**1**)

-[Gly-Gly-Gly-Ala-Lys-Arg-Arg-Arg-Leu-Ile-Phe]-NH_2 (**2**)

General procedures for peptide coupling: To a stirred solution of acid **14** (26 mg, 0.032 mmol) in anhydrous DMF (2 mL) was added benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBop) (17mg, 0.032mmol), N,N-diisopropylethylamine (DIPEA) (9 µL,0.048 mmol). After 5 minute stirring at room temperature for activation of carboxylate, this solution was added over the resin-bounded peptides (**A** and **B**) (0.016 mmol). N_2 was passed through the resin suspension for 8 hours. The resin was then filtered, washed with DMF (3mL × 3 × 3min).

General procedures for global deprotection and cleavage from the resin: A 3 mL of cleavage cocktail (150 µL of DCM, 75 µL of TIS, and TFA to 3mL) was added to the resin-bounded coupling products. The resulting mixture was passed N_2 and mixed for 8 hours. The resin was then filtered, and the TFA filtrate was concentrated under reduced pressure. The residue was washed with diethyl ether and dried under reduced pressure to give ligands with peptide **A** for ligand **1** and with peptide **B** for ligand **2** as pale solids. **1**: MALDI-MS m/z calcd. for $C_{77}H_{121}N_{22}O_{15}^+$ $(M+H)^+$ 1593.9376, found 1593.9907; **2**: MALDI-MS m/z calcd. for $C_{79}H_{121}N_{22}O_{16}^+$ $(M+H_3O)^+$ 1650.9591, found 1650.9667.

Synthesis of complexes Eu-1 and Eu-2

The Eu-**1** and Eu-**2** were synthesised of with ligand **1** or **2** (0. 01 mmol) and $Ln(CO_3)_3$ (0.013 mmol) were stirred in H_2O (40 ml) at 35 ^oC for 24 hours. Any excess $Ln(CO_3)_3$ was filtered off and the solvent was removed under vacuum.

Supporting Information

Eu-**1** (0.005g, ~75%). MALDI-MS m/z: calced. for $C_{77}H_{118}N_{22}O_{15}Eu^+$ $(M+H)^+$: 1743.8354, found 1743.6483; Elemental Analysis: $C_{77}H_{118}N_{22}O_{15}Eu^+$ Calcd. C: 53.03, H: 6.82, N: 17.67; Found: C: 53.08, H: 6.86, N: 17.63.

Eu-**2** (0.004g, ~71%). MALDI-MS m/z: calced. for $C_{79}H_{121}N_{23}O_{16}Eu^+$ 1800.8568 $(M+H)^+$, found 1800.9763; Elemental Analysis: $C_{79}H_{121}N_{23}O_{16}Eu^+$ Calcd. C: 52.69, H: 6.77, N: 17.89; Found: C: 52.65, H: 6.76, N: 17.86.

Gd-**1** (0.004g, ~71%). MALDI-MS m/z: Calcd. for $C_{77}H_{118}N_{22}O_{15}Gd^+$: 1748.8588 $(M+H)^+$, found 1748.8333; Elemental Analysis: $C_{77}H_{120}N_{22}O_{16}Gd^+$ Calcd. C: 52.87, H: 6.80, N: 17.62; Found: C: 52.85, H: 6.83, N: 17.55.

Gd-**2** (0.004g, ~71%) MALDI-MS m/z: Calcd. for $C_{79}H_{121}N_{23}O_{16}Gd^+$ $(M+H)^+$: 1805.8603 found1805.8832; Elemental Analysis: $C_{79}H_{123}N_{23}O_{17}Gd$ Calcd. C: 52.53, H: 6.75,: 17.84 Found: C: 52.52, H: 6.78, N: 17.82.

References:

1. Sanders, G. M.; Van Dijk, M.; Vand der Plas, H. C. J. Heterocycl. chem. 1982, 19, 7

Supporting Information

c) Photo-physical Properties of complexes Eu-1 and Eu-2 with Cyclin A

UV-Visible absorption spectra in the spectral range 200 to 1100 nm were recorded by an HP UV-8453 spectrophotometer. Single-photon luminescence spectra were recorded using an Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer that was equipped with a red sensitive single photon counting photomultiplier by Peltier Cooled Housing. The spectra were corrected for detector response and stray background light phosphorescence. The quantum yields of the compounds Eu-**1** and Eu-**2** were measured by Demountable 142mm (inner) diameter barium sulphide-coated integrating sphere supplied with two access ports. For multi-photon experiments, the 800 nm pump source was from the fundamental of a femtosecond mode-locked Ti:Sapphire laser system (output beam ~ 150 fs duration and 1 kHz repetition rate). The lasers were focused to spot size ~ 50 μm via an f = 10 cm lens onto the sample. The emitting light was collected with a backscattering configuration into a 0.5 m spectrograph and detected by a liquid nitrogen-cooled CCD detector. A power meter was used to monitor the uniform excitation.

The theoretical framework and experimental protocol for the two-photon cross-section measurement have been outlined by Webb and Xu. (J. Opt. Soc. Am. B, 1996, 13, 481-489) In this approach, the two-photon excitation (TPE) ratios of the reference and sample systems are given by:

$$\frac{\sigma_2^S \cdot \phi^S}{\sigma_2^R \cdot \phi^R} = \frac{C_R \cdot n_S \cdot F^S(\lambda)}{C_S \cdot n_R \cdot F^R(\lambda)}$$

where ϕ is the quantum yield, C is the concentration, n is the refractive index, and F(λ) is the integrated photoluminescent spectrum. In our measurements, we have ensured that the excitation flux and the excitation wavelengths are the same for both the sample and the reference. The two-photon absorption cross-section σ_2 of Eu-**1** and Eu-**2** (also with the addition of Cyclin A) was determined using Rhodamine 6G as reference.

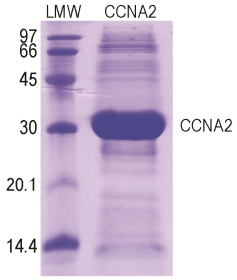
Titration experiments were conducted to investigate the effect of Cyclin A on the two europium complexes. Liquid concentrated stock solution of Cyclin A was added individually and gradually to a solution of the complex concerned. Addition was ceased either when the volume of added Cyclin A totalled 5% of the complex solution or the influence on complex luminescence was saturated. Single-photon luminescence spectra were determined via the aforementioned procedures.

Supporting Information

d) Preparation of Cyclin A and In-Vitro Imaging

Preparation of Cyclin A

Human cyclin A2 protein (CCNA2) (residues 175-432) fused to a hexa-histidine at N-terminus was expressed in E. coli bacterial strain BL21(DE3) using pET-28a-c(+) vector (Novagen, USA). The protein was purified using Ni Sepharose™ 6 Fast Flow column (GE Healthcare Life Sciences, United Kingdom) and concentrated to the final concentration of 0.898mM.



Tissue cultures and In-Vitro microscopy imaging.

Human cervical carcinoma (HeLa) cells were maintained in an RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in 5% CO_2. Cells were passaged every 3–5 days. To study the in-vitro behavior of the lanthanide complexes, experiments were carried out with a commercially available UV and multi-photon confocal microscopy [Leica SP5 (upright configuration)] equipped with Xenon Lamp and ultrafast femto-second laser (Coherent II, tunable from 680 nm to 1050 nm). For the in-vitro imaging, the cells were imaged in the tissue culture chamber (5% CO_2, 37^{o}C). The excitation beam produced by the Xenon Lamp with the power of ~ 6 to 10 mW and focused on coverslip-adherent cells using a 40 x oil immersion or 60 x water immersion objective. Long Pass Filter (LB500) was used. For NIR excitation, the excitation beam prodived by the Ti:sapphire laser. Band-Pass filters were used for the monitoring. MTT viability assay was performed as reported in previous literature. Briefly, three thousand HeLa cells were seeded in 96-well plates 24 hr prior to exposure to the europium complex or DMSO as control. After various exposure time points, 20 µl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/ml) was added to the culture medium in each well and incubated for 5 hours at 37°C. The media was removed, 200 µl DMSO solubilizing reagent was added and incubation was carried out for another hour to dissolve the formazan crystals. The absorbance was measured at 570 nm on a Labsystem Multiskan microplate reader (Merck Eurolab, Switzerland). MTT assays were conducted in triplicate wells, and

repeated twice. Each data point represents the ratio of mean values between the europium versus the DMSO control. (A. P. Wilson, Cytotoxicity and Viability Assays in Animal Cell Culture: A Practical Approach, 3rd ed. (ed. Masters JRW) Oxford University Press: Oxford, 1, (2000))

Supporting Information

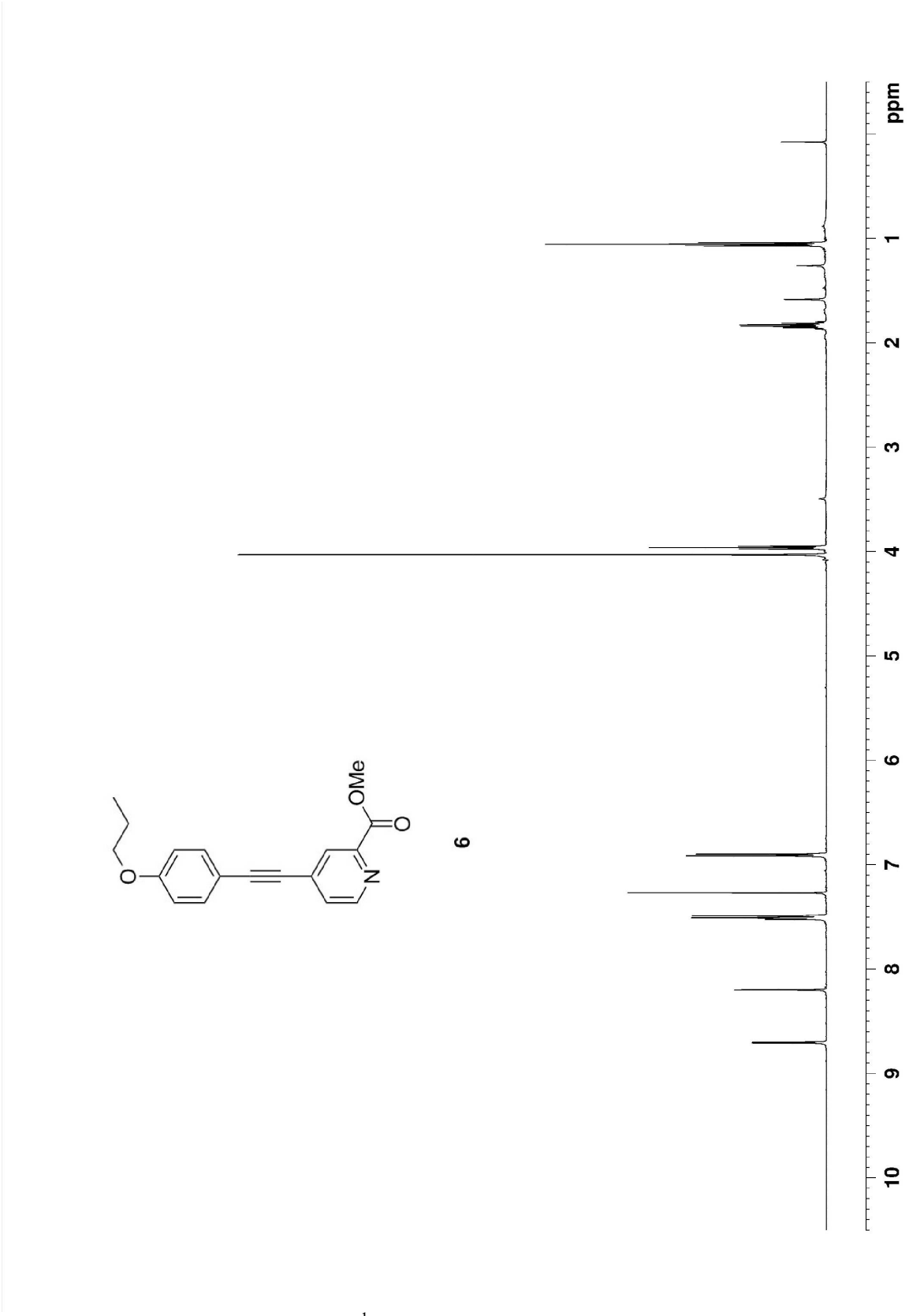


Figure S1. ^{1}H NMR spectrum of compound **6**

Supporting Information

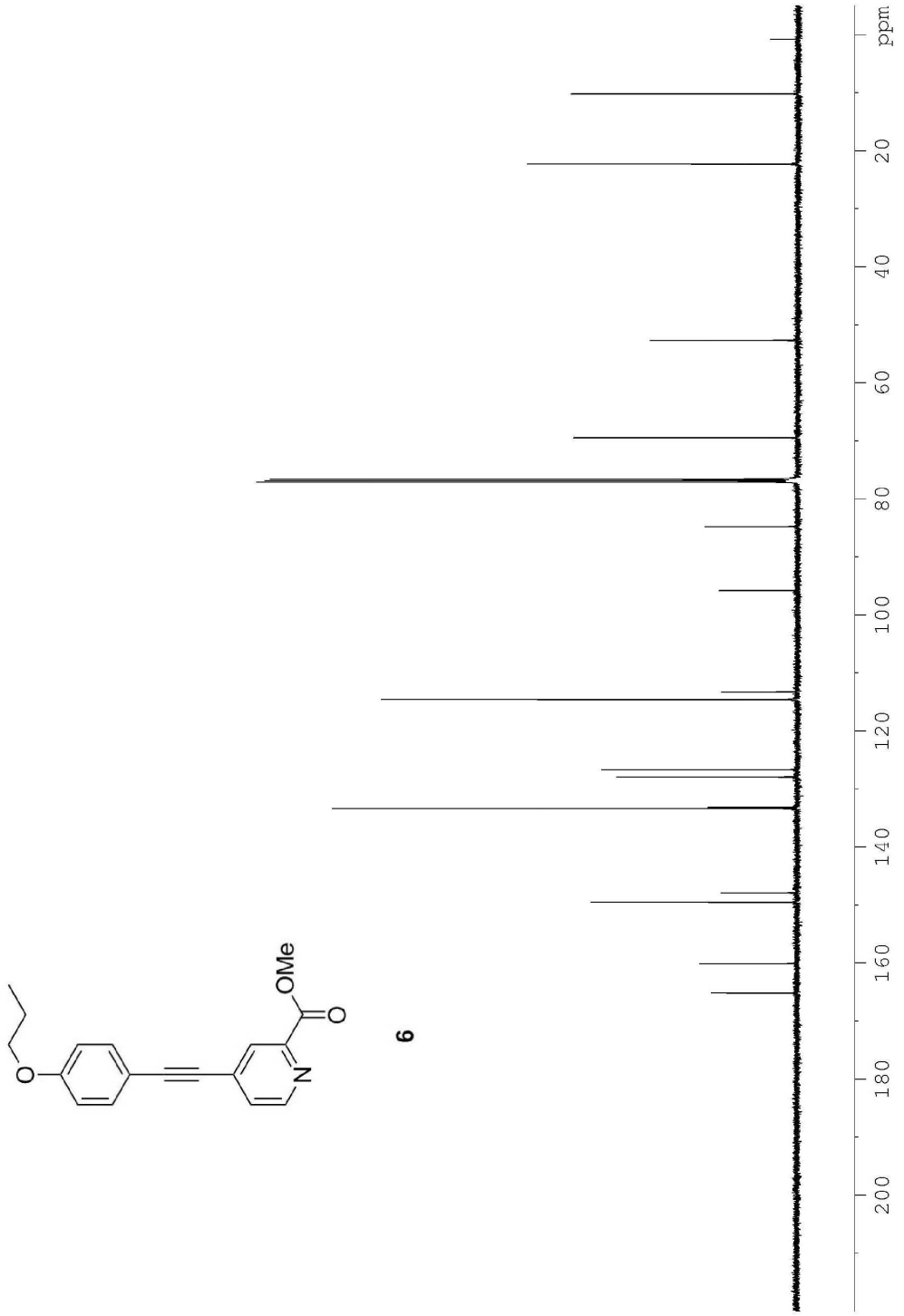


Figure S2. ^{13}C NMR spectrum of compound **6**

Supporting Information

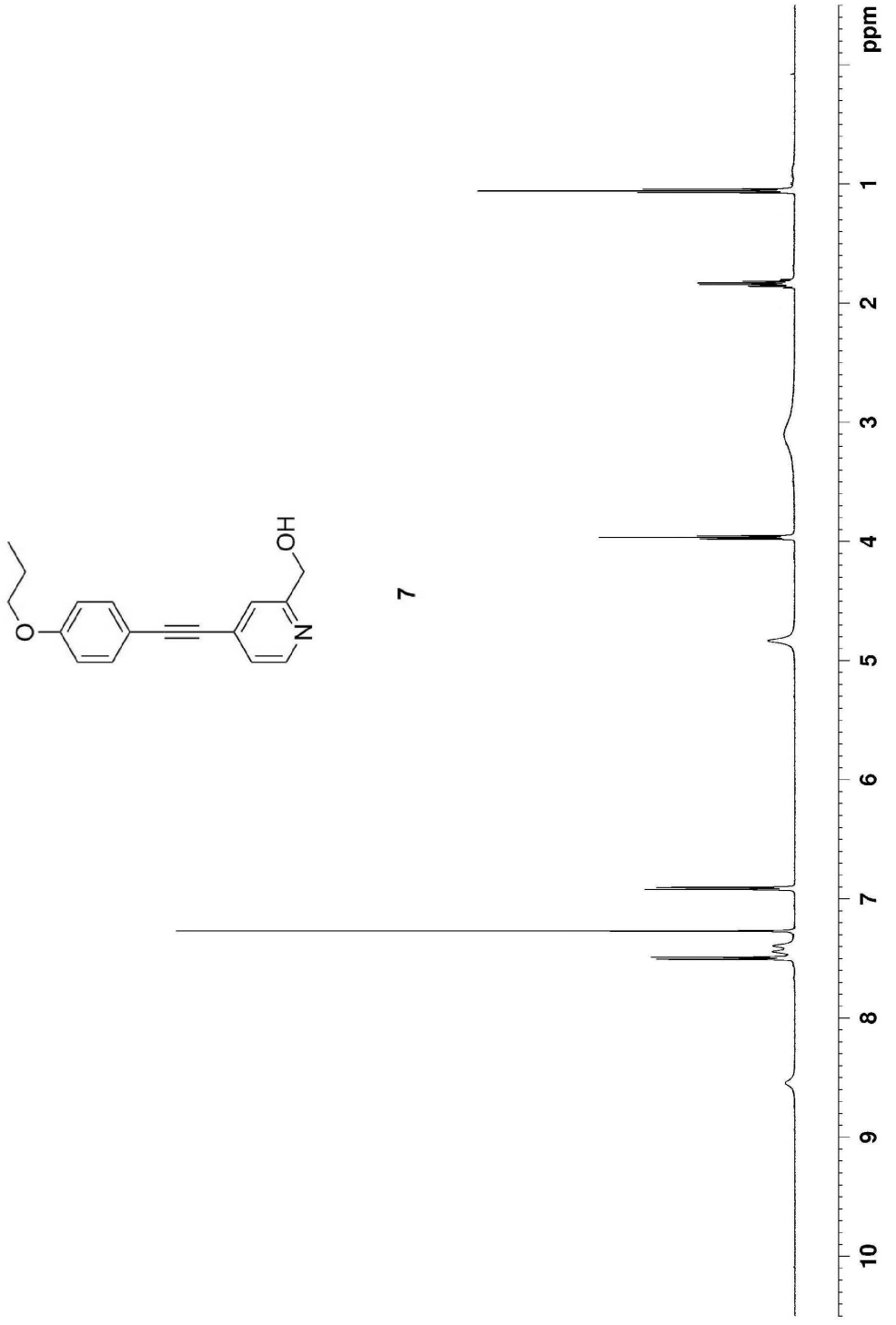


Figure S3. ^{1}H NMR spectrum of compound **7**

Supporting Information

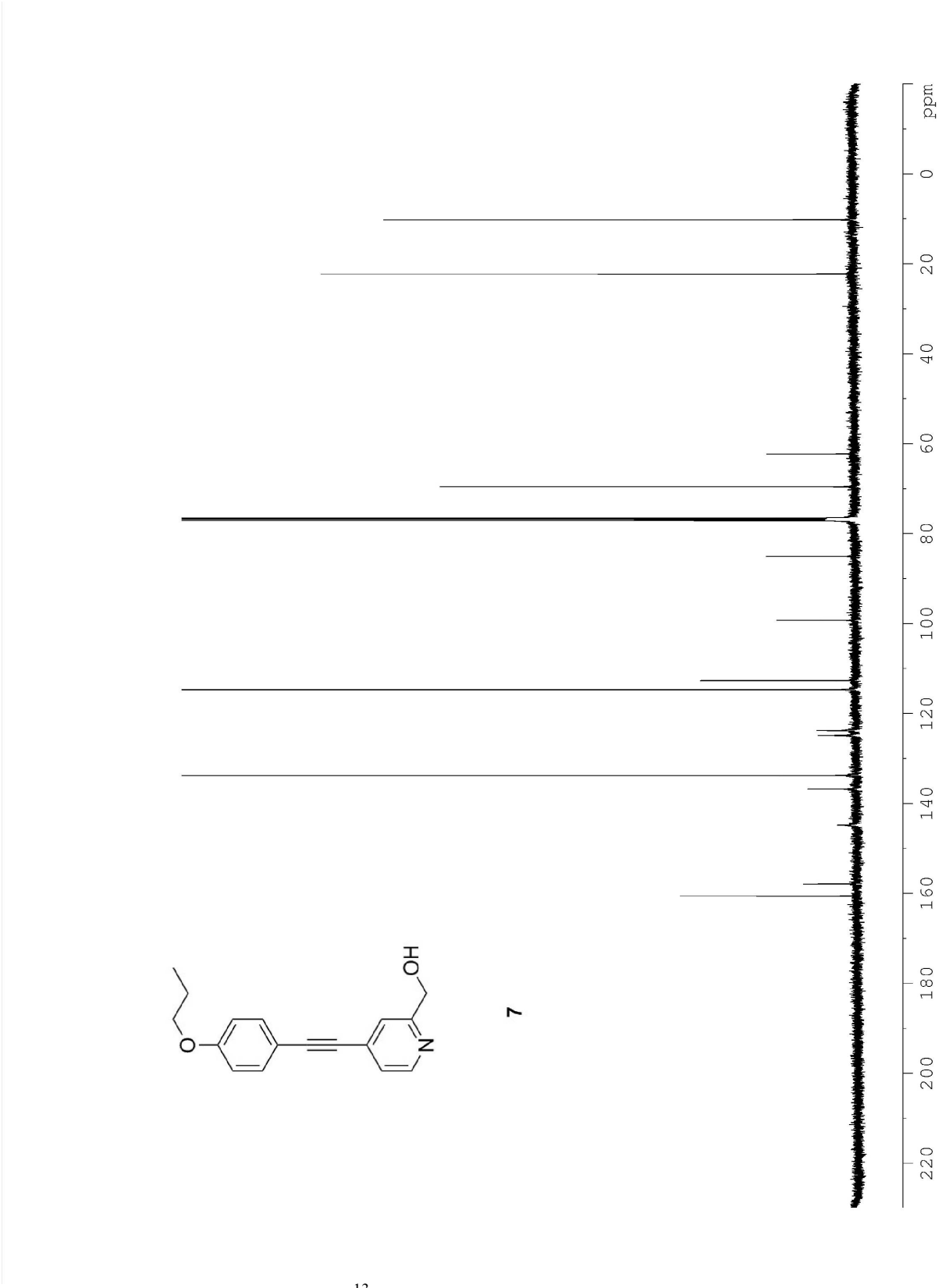


Figure S4. ^{13}C NMR spectrum of compound **7**

Supporting Information

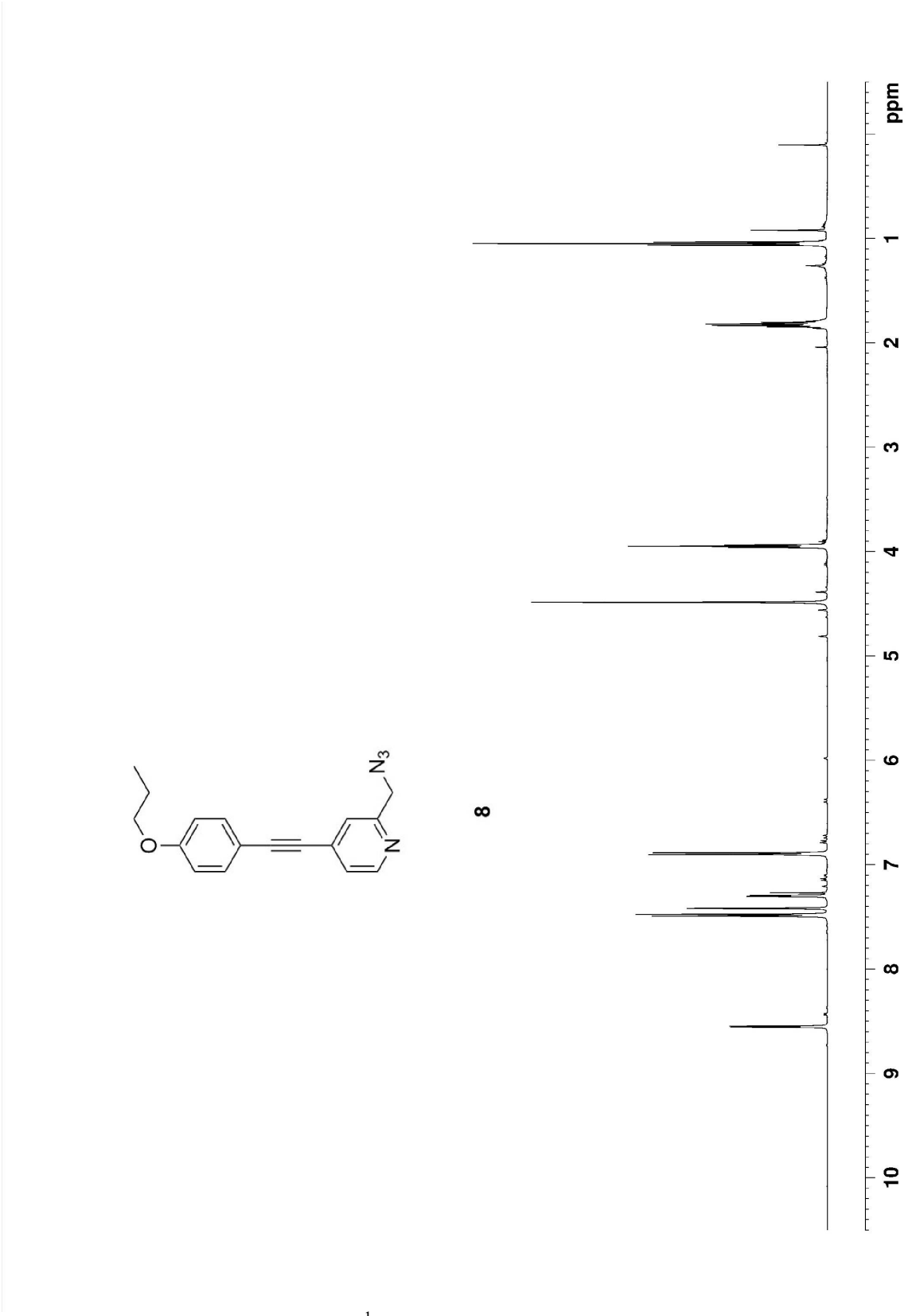


Figure S5. ^{1}H NMR spectrum of compound **8**

Supporting Information

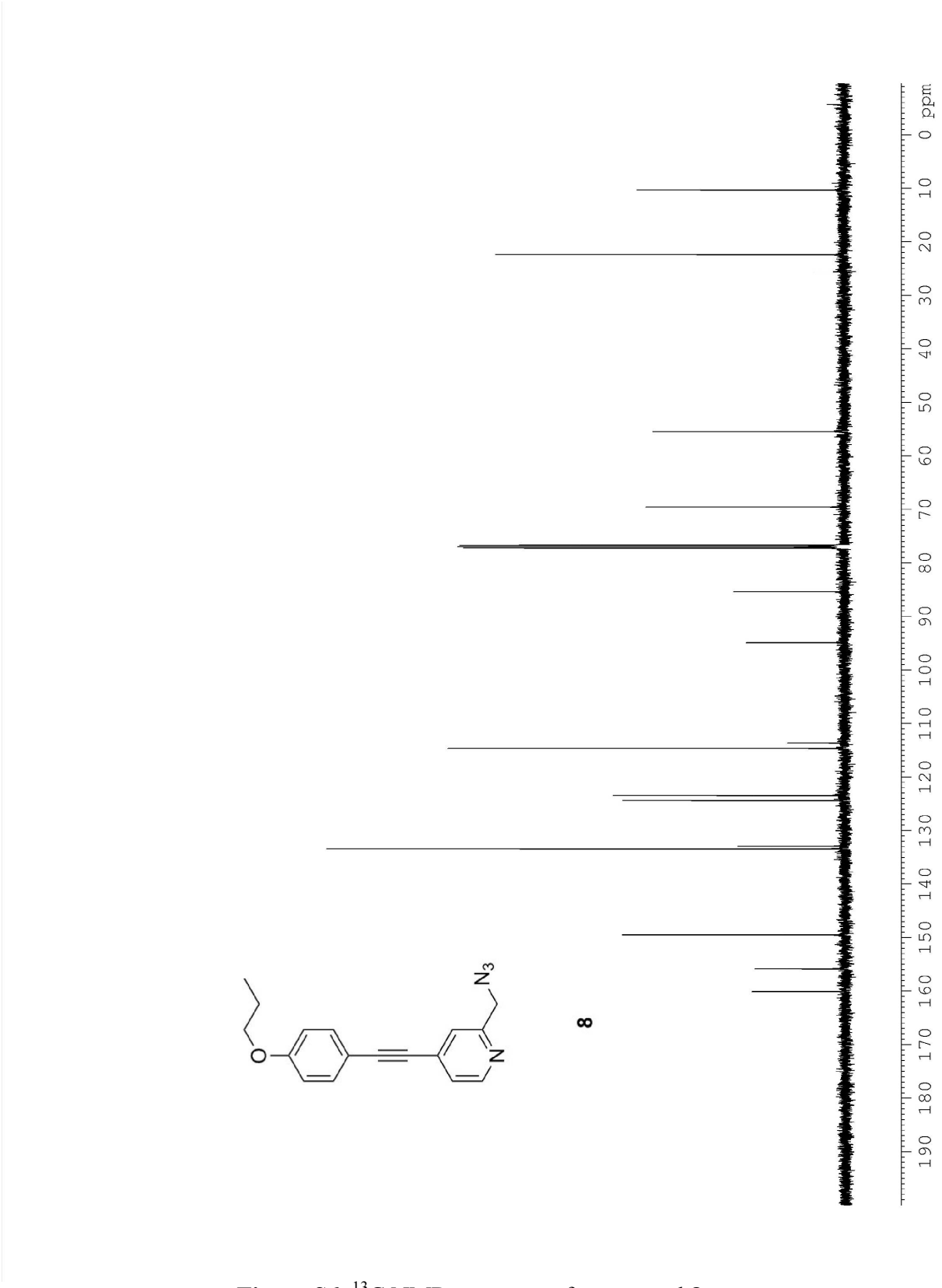


Figure S6. ^{13}C NMR spectrum of compound **8**

Supporting Information

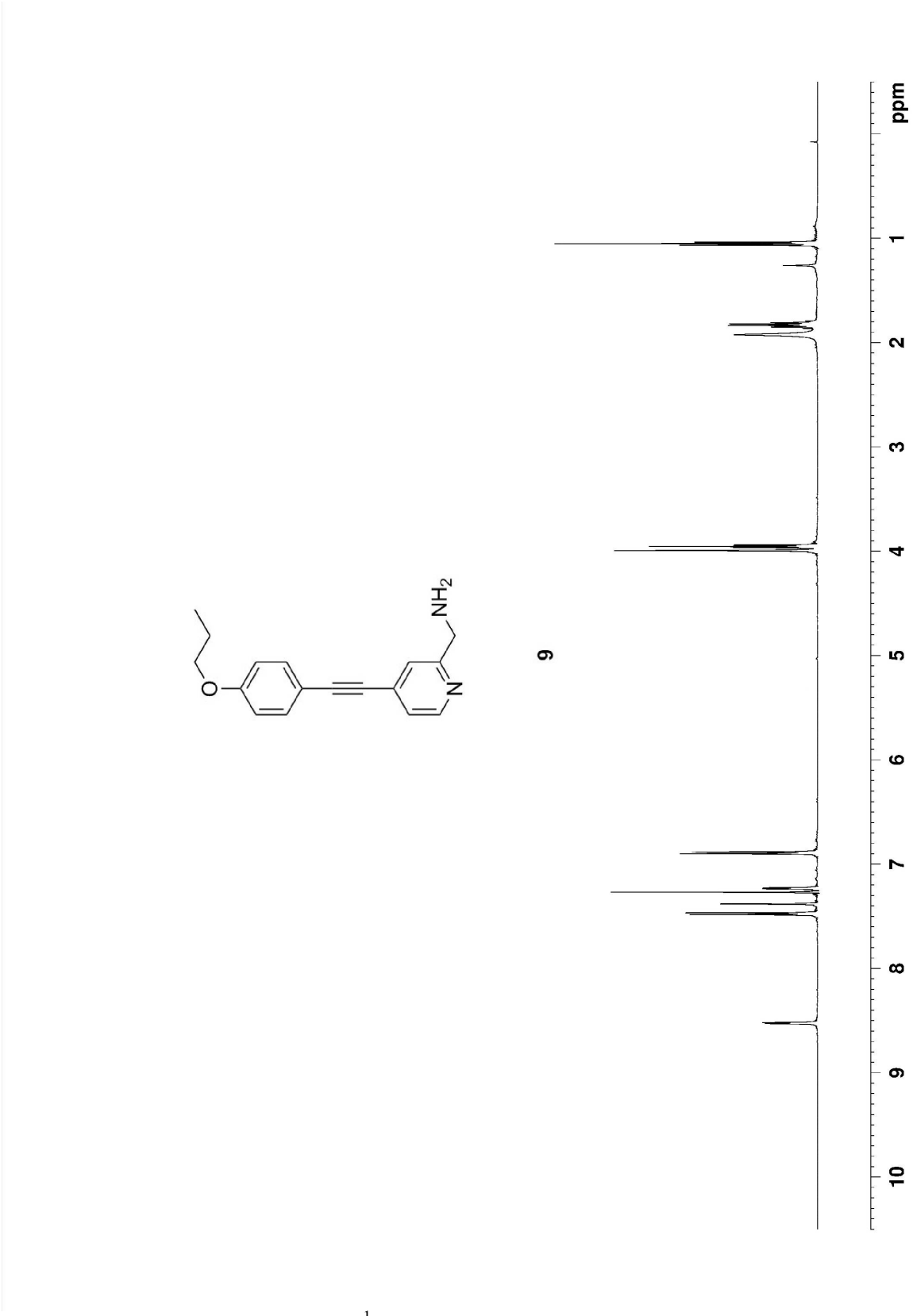


Figure S7. ^{1}H NMR spectrum of compound **9**

Supporting Information

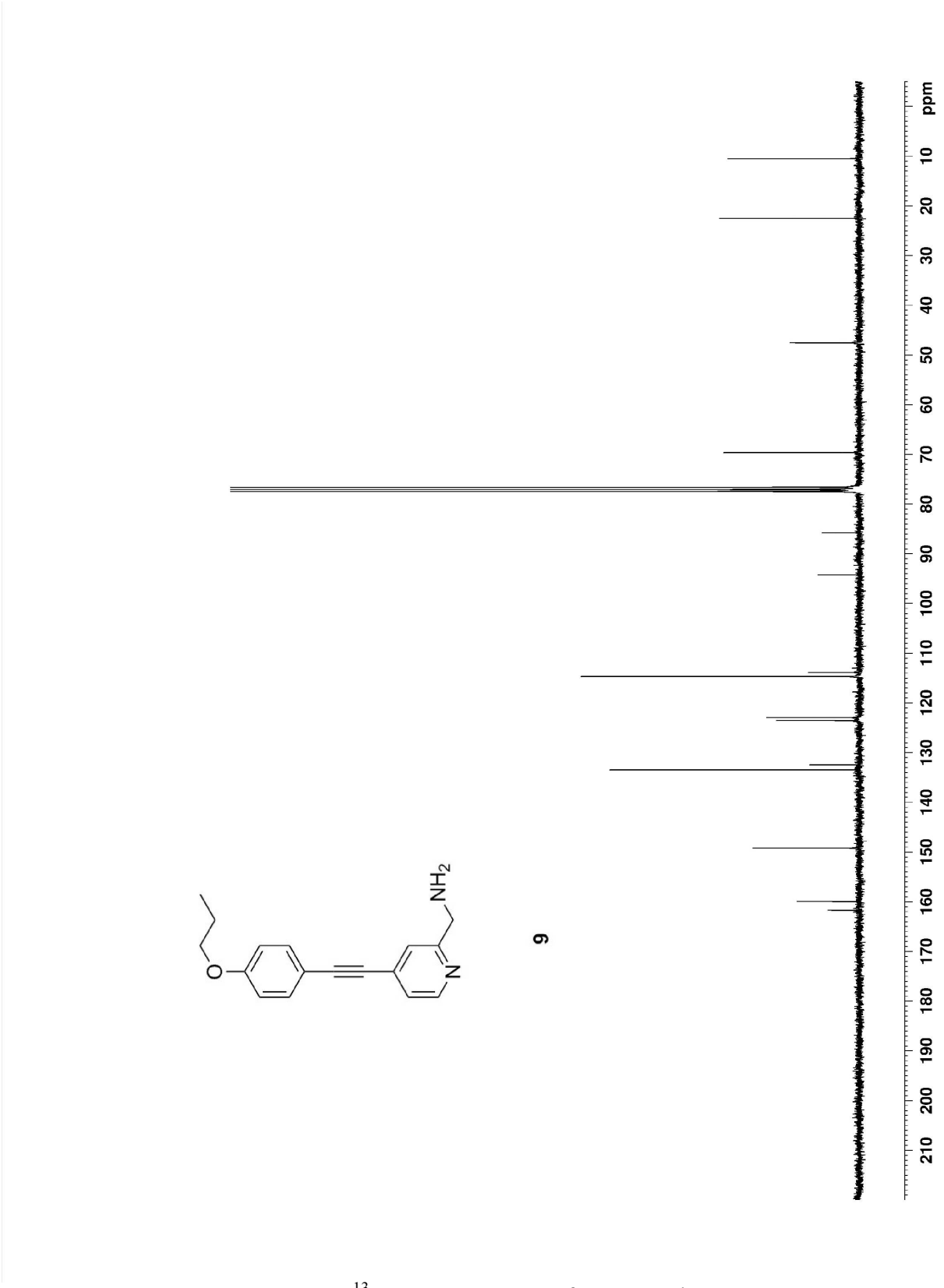


Figure S8. ^{13}C NMR spectrum of compound **9**

Supporting Information

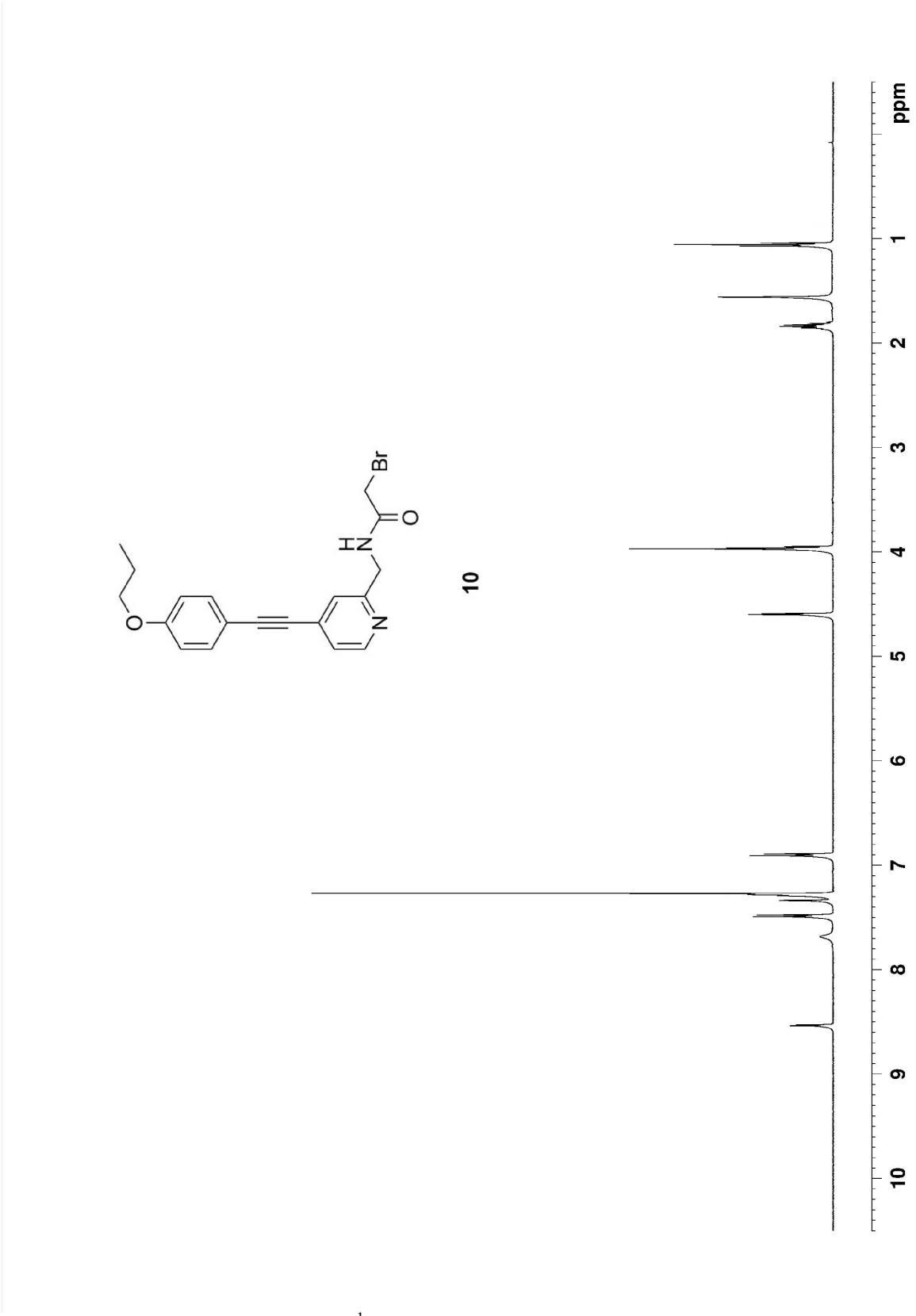


Figure S9. ^{1}H NMR spectrum of compound **10**

Supporting Information

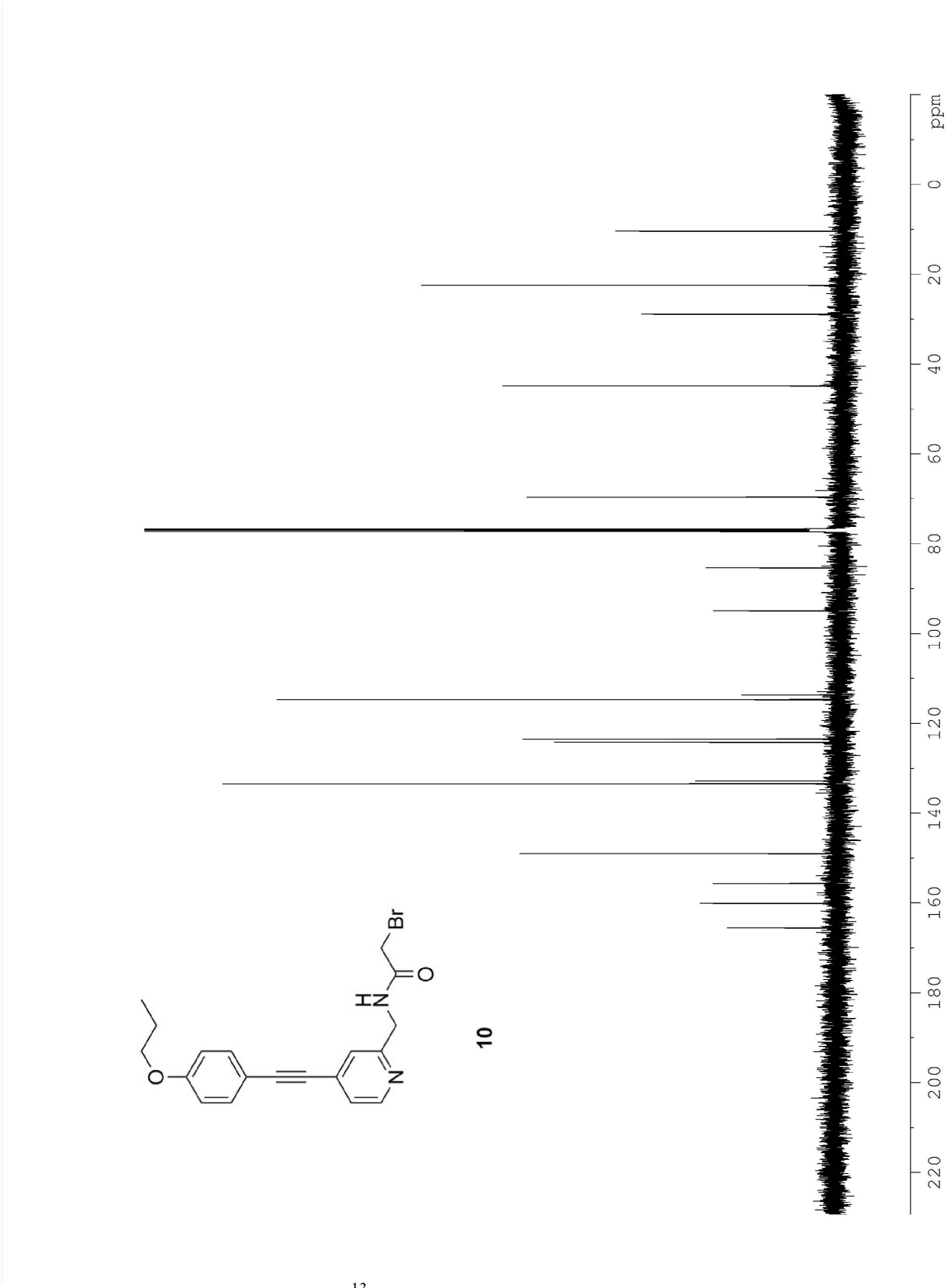


Figure S10. ^{13}C NMR spectrum of compound **10**

Supporting Information

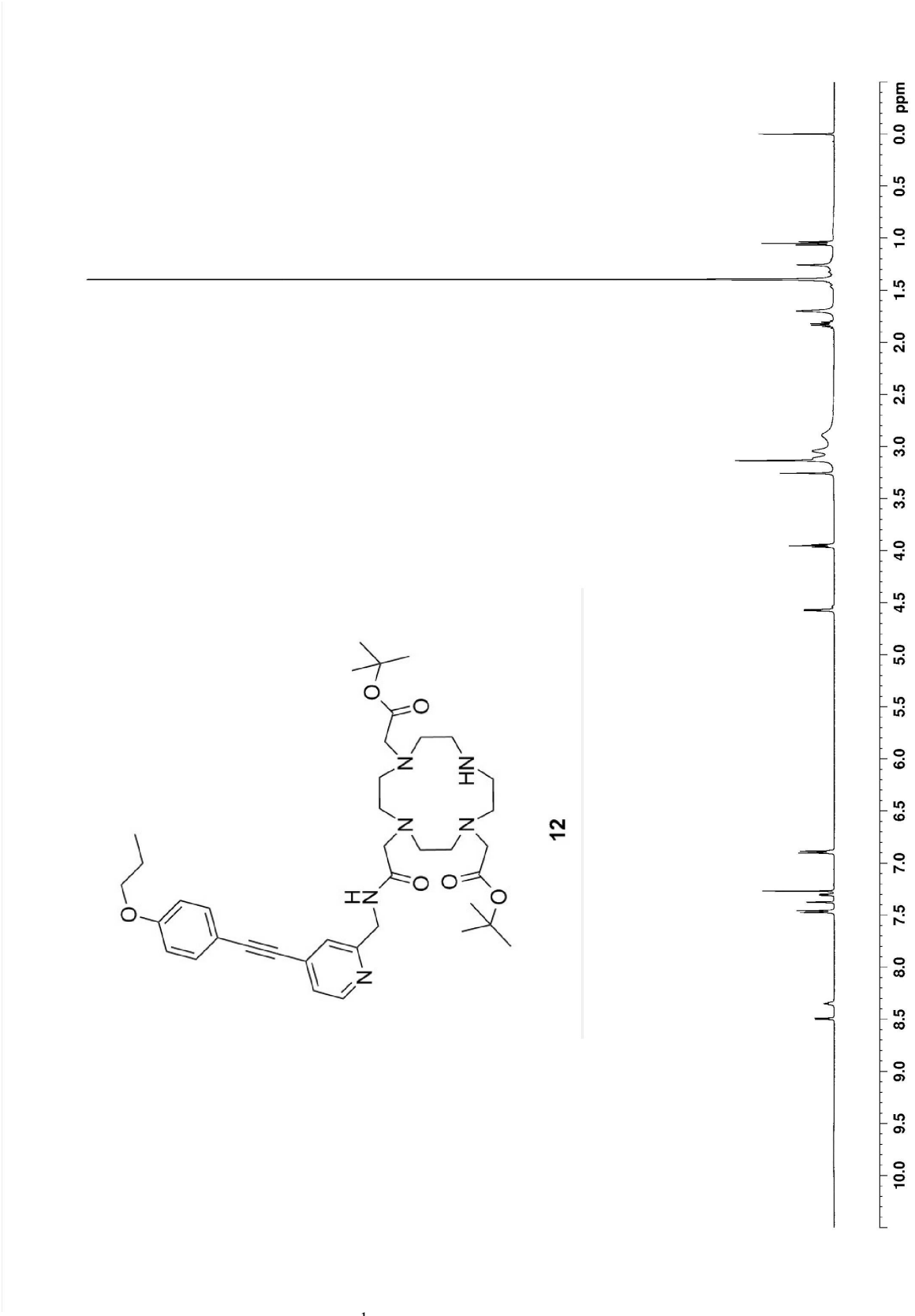


Figure S11. ^{1}H NMR spectrum of compound **12**

Supporting Information

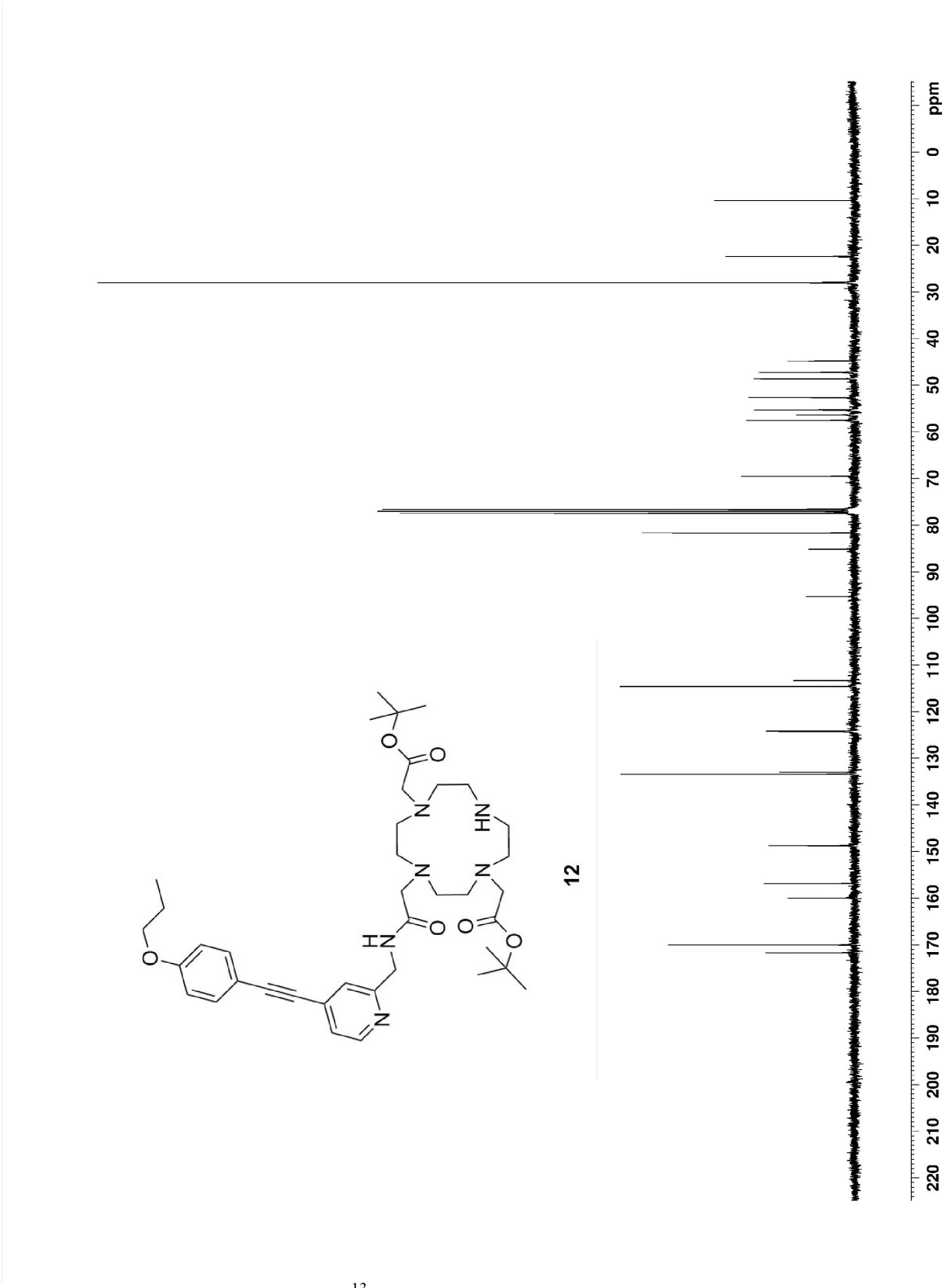


Figure S12. ^{13}C NMR spectrum of compound **12**

Supporting Information

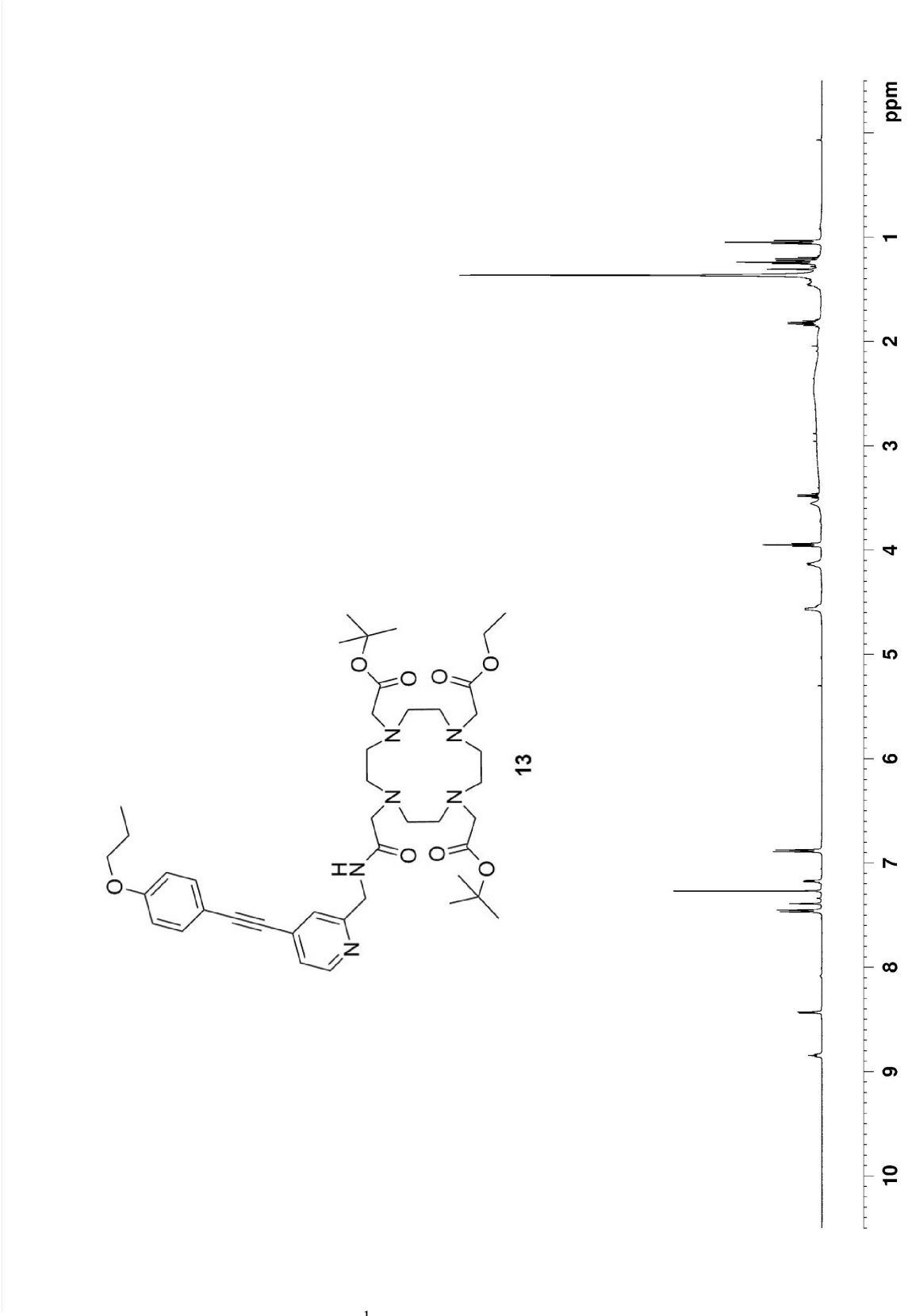


Figure S13. ^{1}H NMR spectrum of compound **13**

Supporting Information

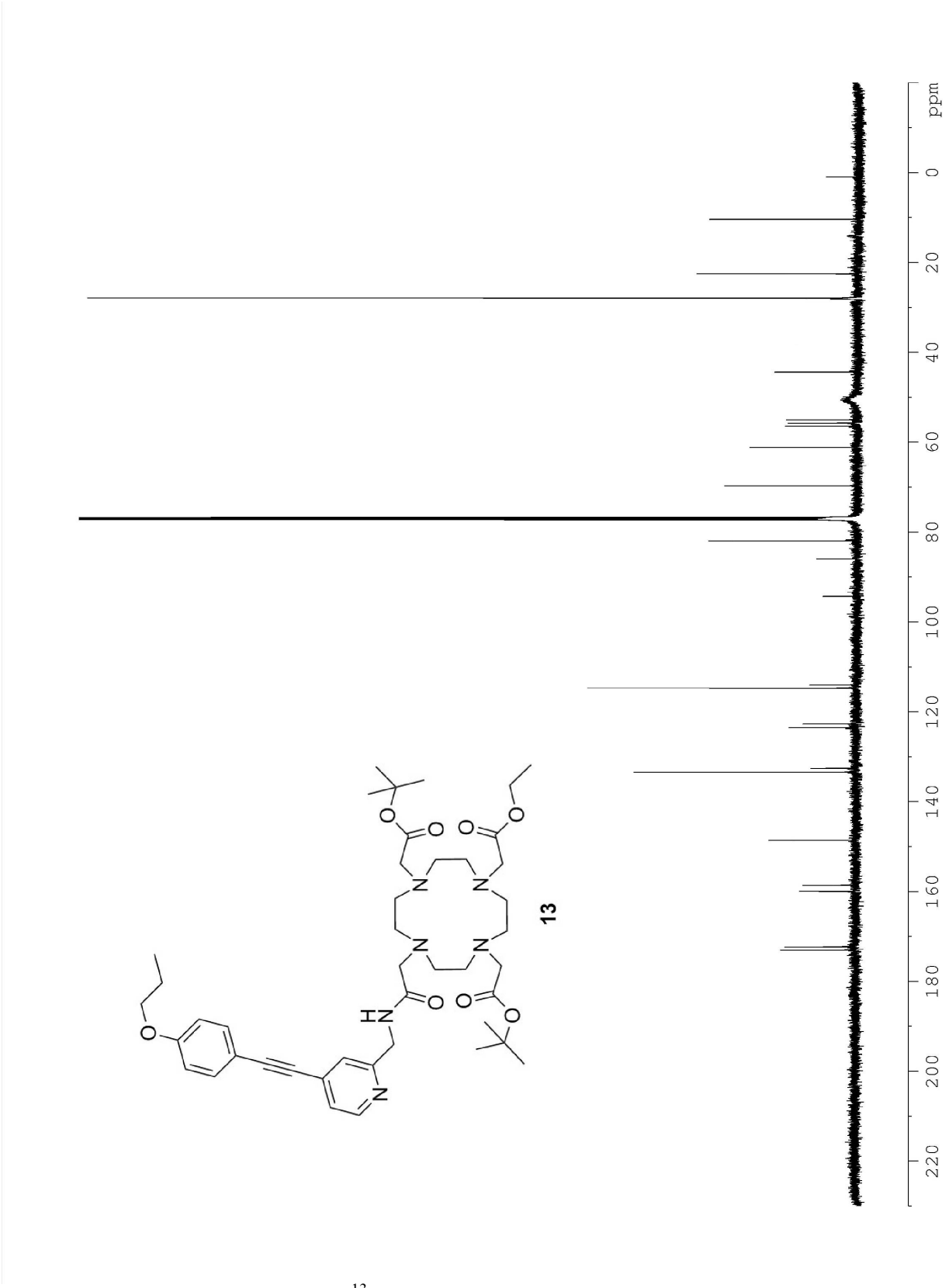


Figure S14. ^{13}C NMR spectrum of compound **13**

Supporting Information

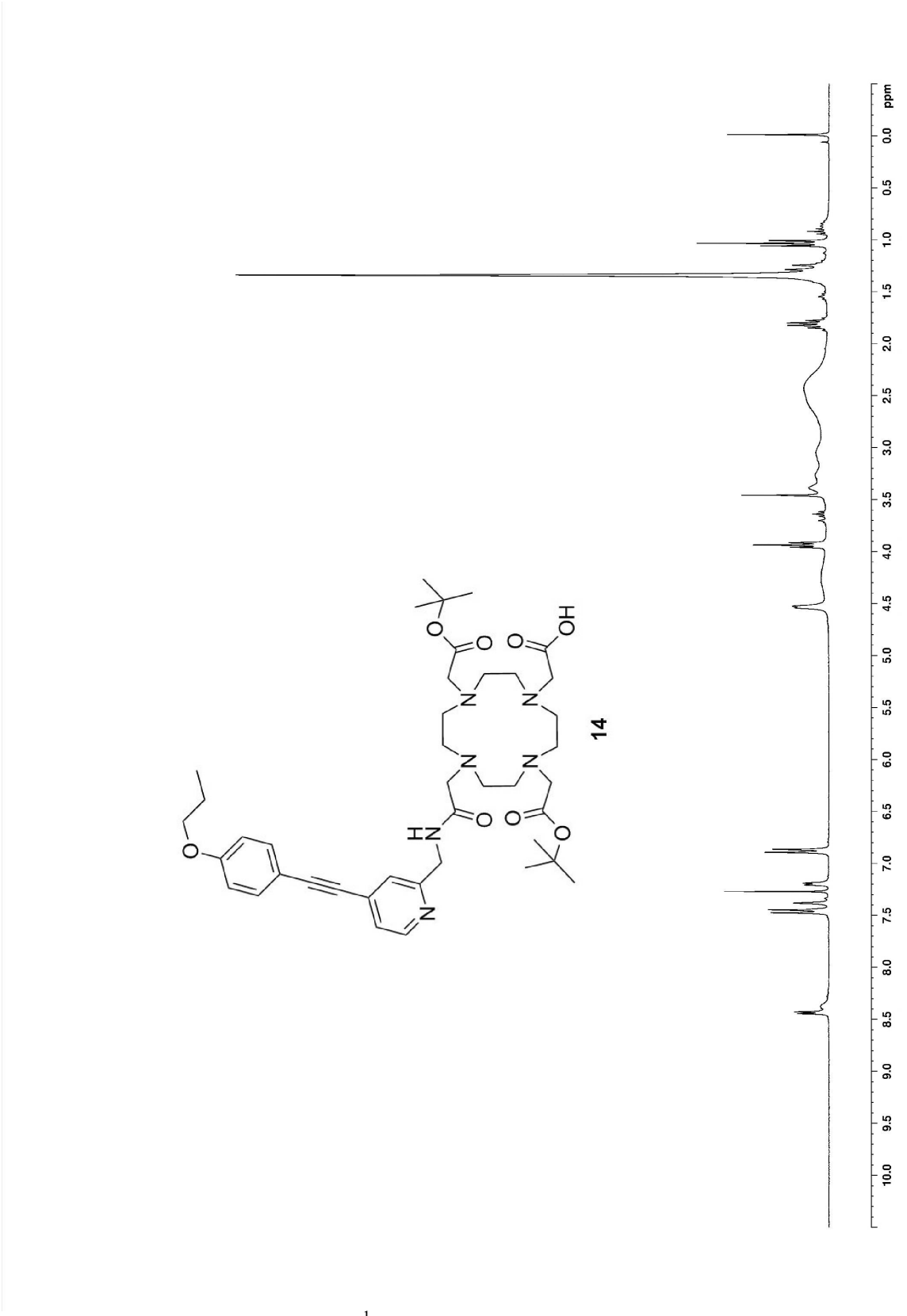


Figure S15. ^{1}H NMR spectrum of compound **14**

Supporting Information

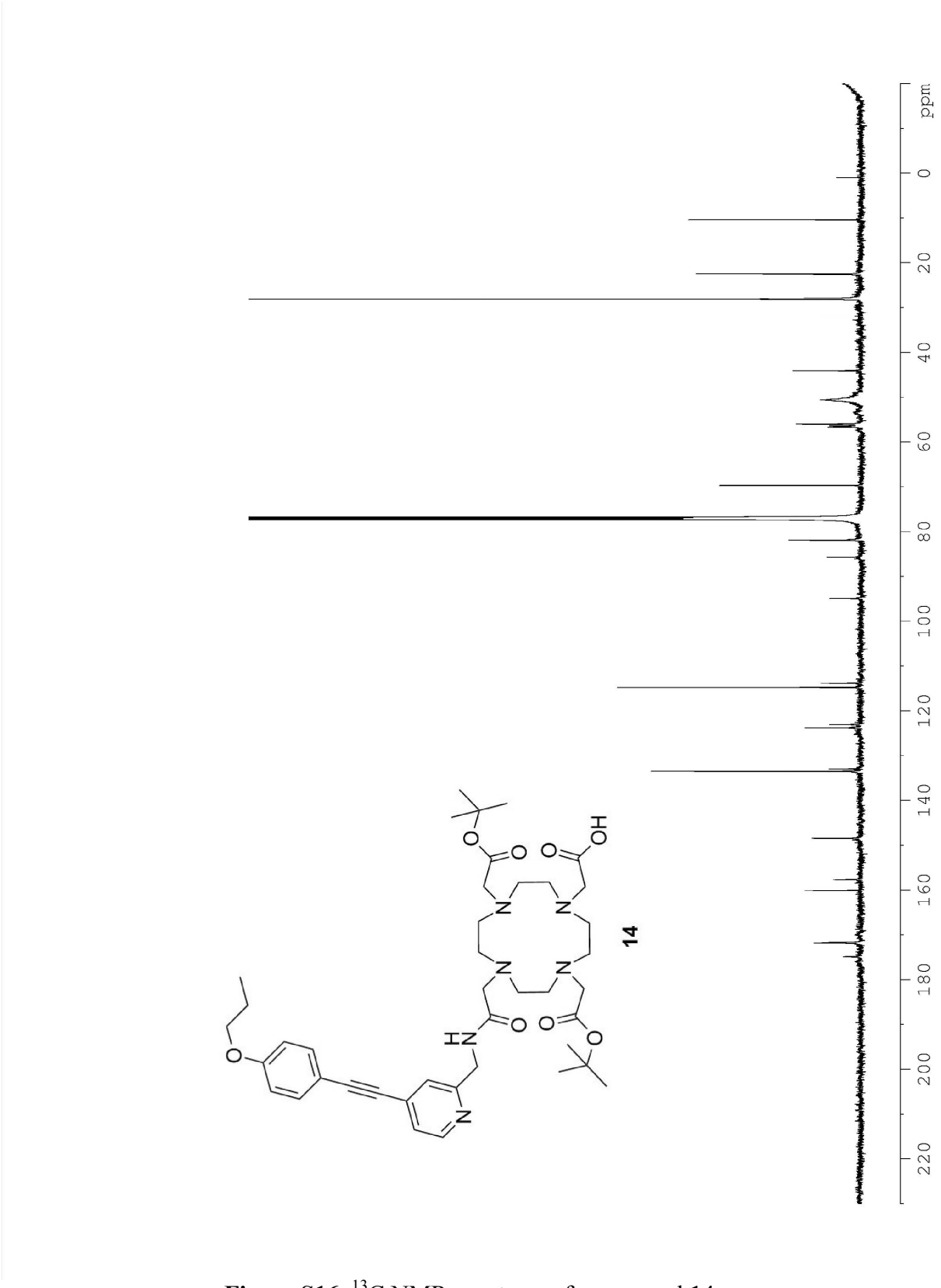


Figure S16. ^{13}C NMR spectrum of compound **14**

Supporting Information

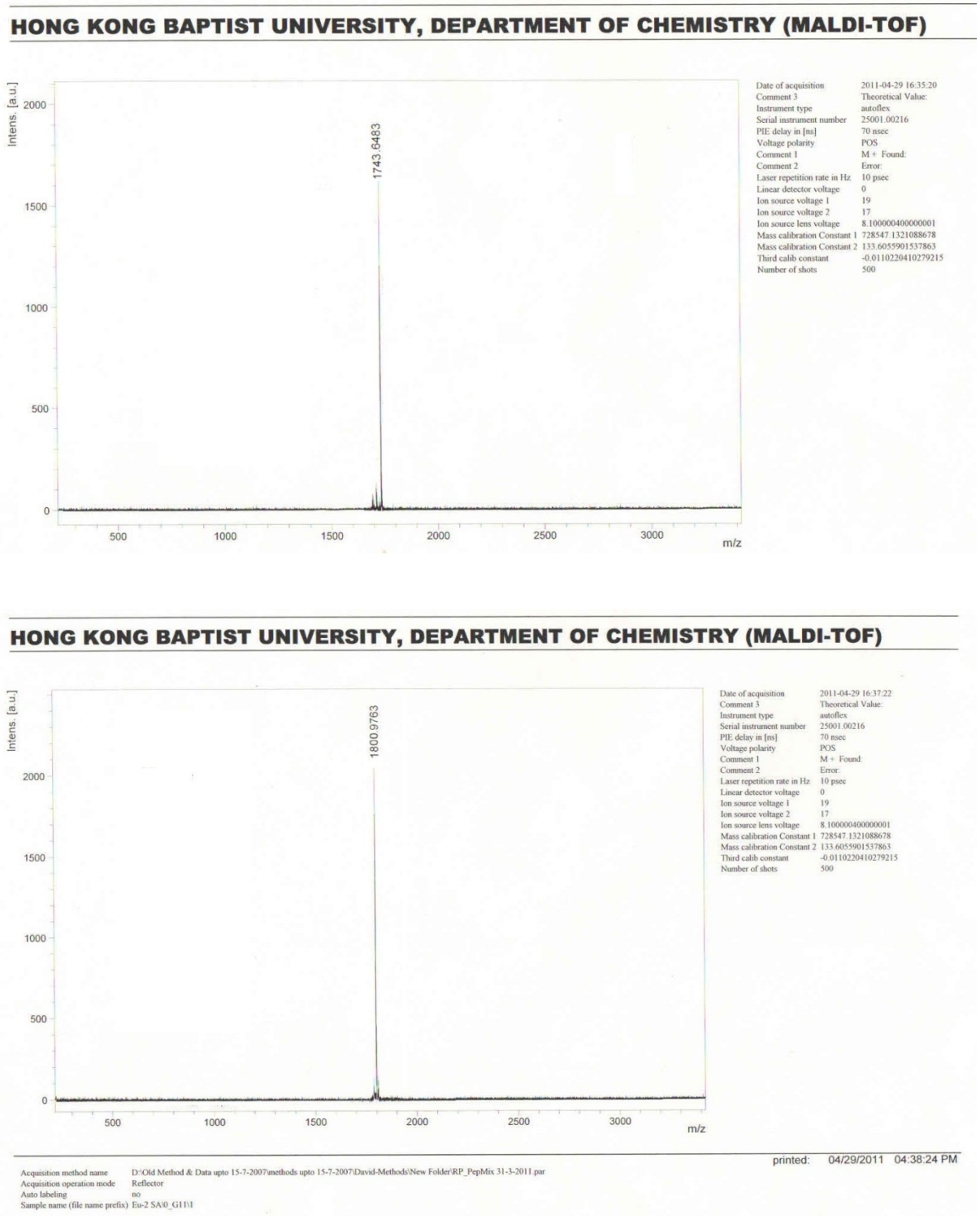


Figure S17. The MALDI-MS spectra of Eu-**1** (upper) and Eu-**2**. (lower)

Supporting Information

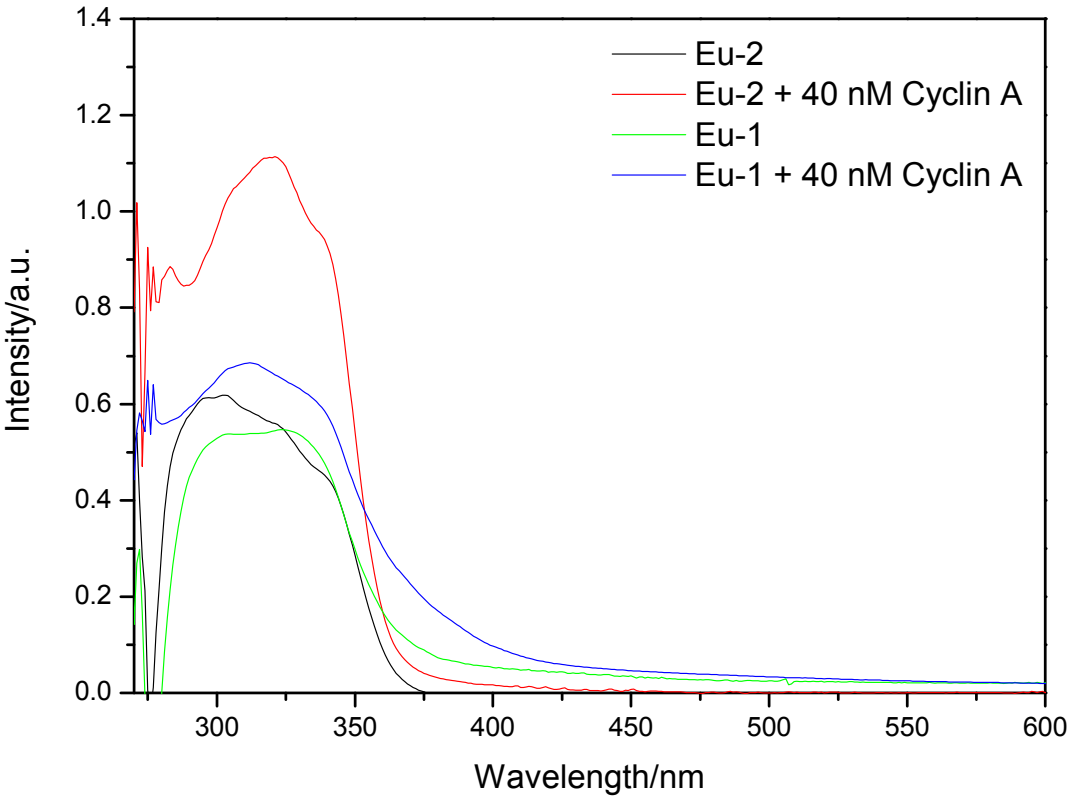


Figure S18. UV-Vis absorption spectra of Eu-**1** and Eu-**2** in the solution of TRIS.

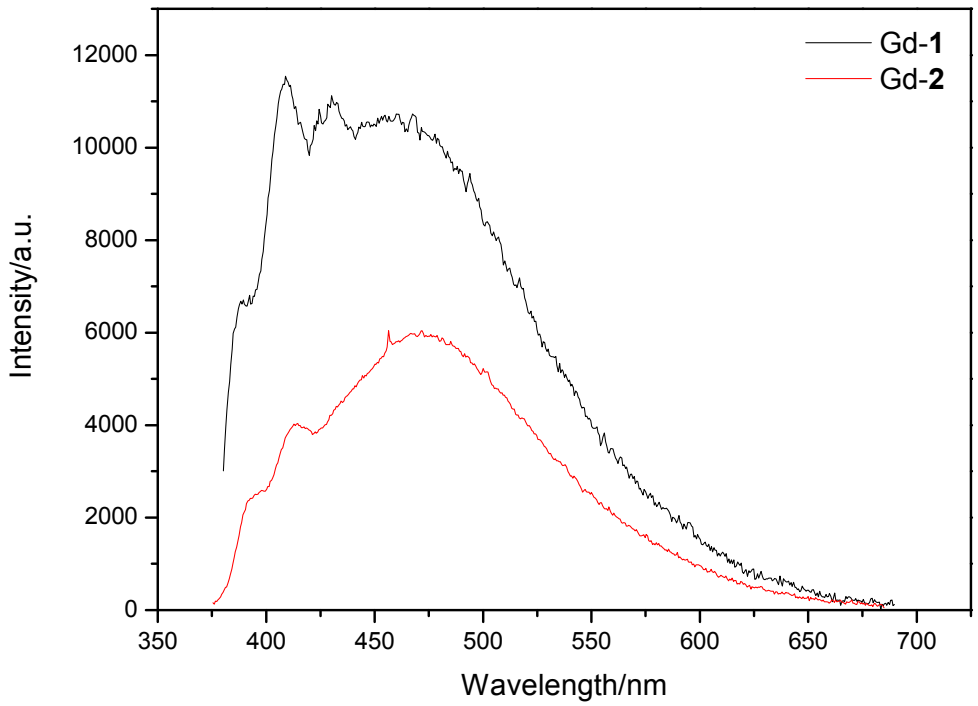


Figure S19. The emission spectra of Gd-**1** and Gd-**2** in the solution of 2-methyl tetrahydrofuran at 77K.