Supporting Information

Cation-Halide Transport through Peptide Pores containing Aminopicolinic Acid

Debajyoti Basak[†], Sucheta Sridhar[‡], Amal K. Bera*[‡] and Nandita Madhavan*[†]

[†] Department of Chemistry and [‡]Department of Biotechnology Indian Institute of Technology Madras, Chennai – 600036, Tamil Nadu, India.

amal@iitm.ac.in, nanditam@iitm.ac.in

Table of Contents

General Methods

All reagents for synthesis were purchased from commercial suppliers and used without further purification unless stated otherwise. All air-sensitive reactions were performed using oven-dried glassware in an inert atmosphere of nitrogen. Syringe or cannula was used to transfer air-sensitive solvents and solutions. Dichloromethane and *N*,*N*-diisopropylethylamine (DIEA) were distilled over calcium hydride, methanol was distilled over magnesium oxide and *N*,*N*-dimethylformamide (DMF) was dried over 4 Å molecular sieves. All dry solvents were stored over 4 Å molecular sieves prior to use. All peptides were synthesized in solution using *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-teramethyluronium hexafluorophosphate (HCTU) as a coupling reagent and *N*,*N*-diisopropylethylamine (DIEA) as base. Egg yolk phosphatidyl choline (EYPC) and 8-hydroxypyrene-1,3,6 trisulfonic acid, trisodium salt (HPTS) and *N*,*N*'-dimethyl-9,9'-biacridinium dinitrate (Lucigenin) were purchased from Sigma-Aldrich and used without further purification.

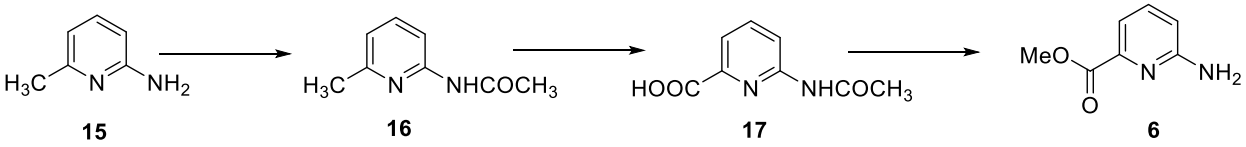
Analytical thin layer chromatography (TLC) was performed on MERCK precoated silica gel 60 F_{254}TLC plates. Eluting solvents are reported as volume percents. Compounds were visualized using UV light and ninhydrin. Flash column chromatography was performed using silica gel from Acme chemicals and active aluminium oxide from Fischer Scientific. All NMR spectra were recorded on Bruker 500 spectrometers using $CDCl_3$, or DMSO-d_6 as solvent. The NMR spectra were referenced using residual solvent peaks as the standard. Chemical shifts are denoted in parts per million (δ), coupling constants (*J*) are reported in Hertz (Hz), and spin multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), apparent quintet (app. quint.), multiplet (m). High resolution mass spectra (HRMS) were recorded on the MICRO-Q-TOF mass spectrometer using the ESI technique. FT-IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. All IR spectra were recorded in the form of KBr pellet. IR spectra peaks are reported in wave numbers (cm^{-1}) as strong (s) and broad (br).

Vesicles were prepared from EYPC lipids using Mini extruder from Avanti Polar Lipids. Conductivity water was used for the preparation of buffers and vesicles. Vesicles were prepared at room temperature in a suitable buffer for the HPTS assay and the buffer pH was adjusted to 7.2 with concentrated NaOH solution. The pH was checked with ^{L1}613 pH meter from Vanira Instruments. Vesicles were prepared in an aqueous solution of 225 mM $NaNO_3$ for the lucigenin assays. Extrusion of vesicles was carried out through 0.1 μm polycarbonate membranes

(Whatman) using the mini excruider from Avanti Polar Lipid. Size exclusion chromatography was carried out using Sephadex (G-50) resin. Fluorescence spectra were recorded on a JASCO FP-6300 fluorescence spectroflurometer using a 3 mL quartz cuvette. A small magnetic stir plate was placed beside the cuvette holder to ensure that the cuvette solutions could be stirred. All graphs were plotted and fitted using Origin Pro8 software. Dynamic light scattering data was recorded on a Malvern Zetasizer ZS instrument using 3 mL quartz cells. For absorbance measurement, A JascoV-650 spectrophotometer equipped with a 1 cm path length quartz cell was used. PMT voltage was kept at 700 V and scans speed at 1,000 nm min^{-1} with the band pass of 2 nm.

Synthesis of Aminopicoline derivative 6:[1]

Scheme S1



N-acetyl 6-aminopicoline 16: To a solution of 6-aminopicoline **15** (15.0 g, 139.0 mmol, 1.0 equiv) in chloroform (75 mL) was slowly added acetic anhydride (19.7 mL, 208.0 mmol, 1.5 equiv) and the reaction mixture was heated to 80 °C for 1 h. The reaction mixture was diluted with chloroform (15 mL) and washed sequentially with water (2 × 25 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL) and water (1 × 25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The crude residue was recrystallized using hexane to afford 18.5 g (89%) of *N*-acetyl 6-aminopicoline **16** as a white solid. TLC R_f = 0.20 (10% ethyl acetate/ hexane).

^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 8.66 (s, 1H; NH_{Ar}), 7.98 (d, *J* = 8.0 Hz, 1H; H_{Ar}),7.57 (t, *J* = 7.5 Hz, 1H; H_{Ar}), 6.86 (d, *J* = 7.5, 1H; H_{Ar}), 2.42 (s, 3H; C$H_{3(Ar)}$), 2.14 (s, 3H; CH_3); **^{13}C NMR** (125 MHz, $CDCl_3$, 25 °C) δ 168.9, 156.3, 150.7, 139.1, 119.3, 111.2, 24.7, 23.8; **IR** (KBr pellet): υ 3243 (b), 3061 (s), 1666 (b), 1545 (b), 1453(s), 1368 (s), 1305 (b), 1156 (s) cm^{-1}; **HRMS**: (ESI$^+$) calcd for $C_8H_{11}N_2O$ $(MH)^+$ 151.0871, found 151.0877.

N-acetyl-6-aminopicolinic acid 17: $KMnO_4$ (28.5 g, 183.0 mmol, 2.5 equiv) was added in portions to a suspension of *N*-acetyl-6-aminopicoline **16** (11.0 g, 73.2 mmol, 1.0 equiv) in water. The reaction mixture was heated to 80 °C for 5 h, following which it was filtered through celite. The precipitate was washed with hot water (2 × 25 mL). The filtrate was concentrated to one third of its original volume and cooled in an ice bath. *N*-acetyl-6-aminopicolinic acid **17** (8.5 g, 65%) was obtained as a white precipitate after slow addition of hydrochloric acid (12 M). TLC R_f = 0.07 (10% ethyl acetate/ methanol). The white solid was used for the next step without further purification.

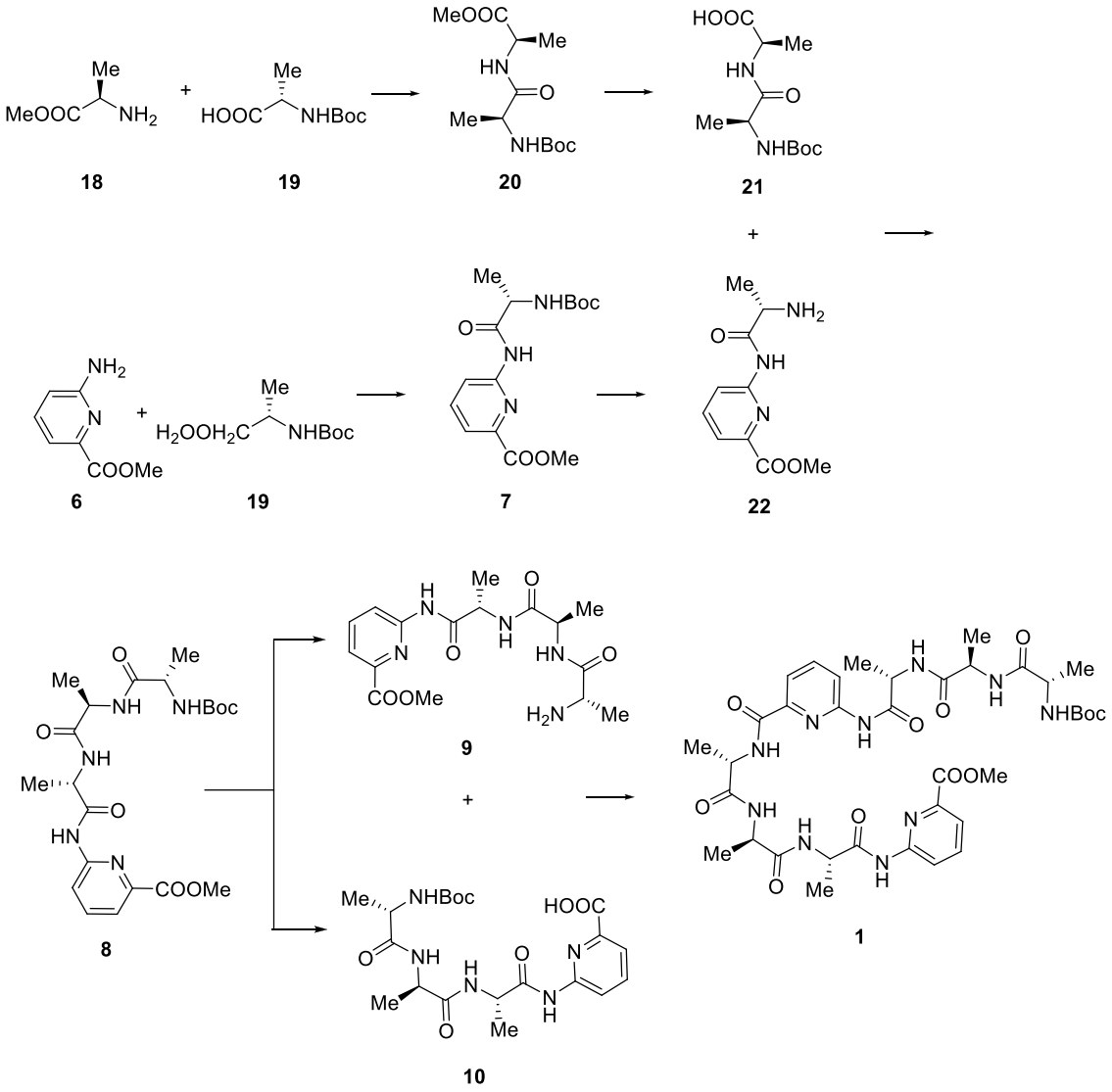
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.91 (s, 1H; NH_{Ar}), 8.24 (d, *J* = 8.0 Hz, 1H; H_{Ar}), 7.95 (t, *J* = 8.0 Hz, 1H; H_{Ar}), 7.73 (dd, *J* = 7.5 Hz, 1H; H_{Ar}), 5.30 (s, 1H; O*H*), 2.11 (s, 3H; CH_3); **^{13}C NMR** (125 MHz, $CDCl_3$, 25 °C) overlapping peaks δ 170.1, 165.7, 151.9, 139.8, 120.2, 117.0, 23.9; **IR** (KBr pellet): υ 3340 (b), 2923 (b), 1947 (b), 1728 (s), 1632(s), 1575 (s), 1487 (s), 1430 (s), 1290 (s), 1236 (s), 772(s) cm^{-1}; **HRMS**: (ESI$^+$) calcd for $C_8H_8N_2O_3Na$ $(MNa)^+$ 203.0433, found 203.0430.

Methyl ester 6: To a solution of *N*-acetyl-6-aminopicolinic acid **17** (0.3 g, 1.8 mmol, 1 equiv) in methanol (25.0 mL, 0.61 mol, 320.0 equiv) was slowly added hydrochloric acid (12 M, 7.5 mL). The reaction mixture was allowed to reflux for 72 h. The residue was neutralized with saturated aqueous $NaHCO_3$ (30 mL), diluted with water (25 mL) and extracted with ethyl acetate (4 × 25 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purification by column chromatography (50% ethyl acetate/ hexane) afforded 0.2 g (77%) of methyl ester **6** as a pale yellow solid. TLC R_f = 0.30 (50% ethyl acetate/ hexane).

^{1}H NMR (500 MHz, $CDCl_3$, 25 °C) δ 7.57 (t, *J* = 8.0 Hz, 1H; H_{Ar}), 7.44 (d, *J* = 7.5 Hz, 1H; H_{Ar}), 6.84 (d, *J* = 8.5 Hz, 1H; H_{Ar}), 3.96 (s, 3H; CH_3) **^{13}C NMR** (125 MHz, $CDCl_3$, 25 °C) δ 165.1, 158.0, 144.0, 139.1, 115.6, 114.2, 53.1; **IR** (KBr pellet): υ 3471 (s), 3362 (s), 1716(s), 1608 (s), 1468 (s), 1351 (s), 1267 (s) cm^{-1}. **HRMS**: (ESI$^+$) calcd for $C_7H_9N_2O_2$ $(MH)^+$ 153.0664, found 153.0666.

Synthesis of octapeptide 1[2]

Scheme S2



Boc-L-Ala- D-Ala-OMe 20: To a solution of Boc-L-Alanine **19** (0.6 g, 3.1 mmol, 1.0 equiv) and D-Ala-OMe **18** (0.4 g, 3.1 mmol, 1.0 equiv) in CH_2Cl_2 (7.0 mL) was added HCTU (1.5 g, 3.6 mmol, 1.2 equiv). DIEA (2.1 mL, 9.2 mmol, 3.0 equiv) was slowly added and the reaction mixture was allowed to stir at RT for 6 h. The solvent was evaporated in vacuo and the residue

was diluted with ethyl acetate (15 mL). The solution was sequentially washed with water (2 × 15 mL), 5% HCl (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (2 × 15 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo. Purification by column chromatography (30 % ethyl acetate/ hexane) afforded 0.7 g (84%) of dipeptide **20** as a white solid. TLC R_f = 0.15 (10% ethyl acetate/ hexane).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 8.15 (d, J = 7.5, 1H; NH_{Ala}), 6.82 (s, 1H; N*H*Boc), 4.26 (app quint, 1H; CH_{Ala}), 4.0 (app quint, 1 H; CH_{Ala}), 3.62 (s, 3H; OCH_3), 1.37 (s, 9H; (CH_3)$_3$C), 1.26 (d, J = 7.5 Hz, 3H; C$H_{3(Ala)}$), 1.16 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 173.0, 172.6, 154.9, 78.0, 51.9, 49.5, 47.5, 28.2, 18.4, 17.2; **IR** (KBr pellet): υ 3368 (s), 3290 (b), 2983 (s), 1751(s), 1699 (s), 1644 (b), 1542 (s), 1507 (s), 1454 (s), 1366 (s), 1299 (s), 1251 (s), 1162 (b) cm^{-1}; **HRMS (ESI$^+$):** calcd for $C_{12}H_{22}N_2O_5Na$ $(MNa)^+$ 297.1426; found 297.1439.

Boc-L-Ala- D-Ala-OH 21: To a solution of dipeptide **20** (0.6 g, 2.2 mmol, 1.0 equiv) in THF (6 mL) were added LiOH (0.2 g, 5.5 mmol, 2.5 equiv) and water (4 mL). The reaction mixture was allowed to stir at RT for 4 h, following which it was diluted with water (10 mL) and washed with ethyl acetate (10 mL). The aqueous layer was acidified with dil HCl to a pH of 1 and was extracted with ethyl acetate (4 × 15 mL). The organic layer was dried over anhydrous sodium sulphate and concentrating in vacuo to afford 0.5 g (93%) of dipeptide **21** as a white solid. TLC R_f = 0.25 (100% ethyl acetate).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.55 (s, 1H; O*H*), 7.98 (d, J = 7.5 Hz, 1H; NH_{Ala}), 6.82 (s, 1H; N*H*Boc), 4.19 (app. quint, 1 H; C$H_{(Ala)}$), 4.0 (app. quint, 1 H; C$H_{(Ala)}$), 1.37 (s, 9H; (CH_3)$_3$C), 1.25 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$) 1.16 (d, J = 7.5 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6) δ 174.0, 172.4, 154.9, 78.1, 49.5, 47.4, 28.2, 18.5, 17.5; **IR** (KBr pellet): υ 3327 (b), 3270 (b), 2982 (s), 1720 (s), 1682 (s), 1638 (S), 1564 (s), 1378 (s), 1257 (s), 1170 (s) cm^{-1}; **HRMS:**(ESI$^+$) calcd for $C_{11}H_{21}N_2O_5$ $(MH)^+$ 261.1450; found 261.1443.

OMe-AP-L-Ala-Boc 7: To a solution of methyl 6-aminopicolinate **6** (0.4 g, 2.9 mmol, 1.0 equiv) and Boc-L-Alanine (0.6 g, 3.2 mmol, 1.1 equiv) in CH_2Cl_2 (7.0 mL) was added HCTU (1.3 g, 3.2 mmol, 1.1 equiv). DIEA (2.0 mL, 8.7 mmol, 3.0 equiv) was slowly added and the reaction mixture was allowed to stir at RT for 12 h. The solvent was evaporated in vacuo, following which the residue was diluted with ethyl acetate (15 mL). The solution was washed sequentially with water (2 × 15 mL), 5% HCl (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (15 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purification by column chromatography (30% ethyl acetate/ hexane) afforded 0.7 g (75%) of dipeptide **7** as a white solid. TLC R_f = 0.30 (40% ethyl acetate/ hexane).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 10.81 (s, 1H; NH_{Ar}), 8.31 (d, J = 8.5 Hz, 1H; H_{Ar}), 7.98 (t, J = 8.5 Hz, 1H; H_{Ar}), 7.77 (d, J = 7.5 Hz, H_{Ar}), 7.14 (d, J = 7.5 Hz, 1H; NHBoc), 4.26 (app. quint, 1H; CH_{Ala}), 3.87 (s, 3H; OCH_3), 1.37 (s, 9H; (CH_3)$_3$C), 1.25 (d, J = 7.5 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 173.5, 164.9, 155.2, 152.0, 145.8, 139.7, 120.4, 117.4, 78.1, 52.4, 50.3, 28.2, 17.8; **IR** (KBr pellet): υ 3298 (b), 2979 (s), 1713 (s), 1680 (s), 1539 (s), 1449 (s), 1307 (s), 1166 (s), 771 (s) cm^{-1}; **HRMS** (ESI$^+$): calcd for $C_{15}H_{22}N_3O_5$ $(MH)^+$ 324.1559, found 324.1553.

NH$_2$-L-Ala-AP-OMe 22: To a solution of dipeptide **7** (0.3 g, 0.9 mmol, 1.0 equiv) in DCM (7 mL) was added trifluoroacetic acid (0.6 mL, 8.3 mmol, 10.0 equiv) and the reaction mixture was allowed to stir for 3 h at RT. The solvent was evaporated in vacuo. The residue was dissolved in water and lyophilized to afford 0.3 g (95%) of dipeptide **22** as a pale yellow solid. TLC R_f = 0.10 (10% MeOH/ DCM).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 11.40 (s, 1H; NH_{Ar}), 8.32 (s, 3H; NH_2, H_{Ar}), 8.06 (t, J = 7.5 Hz, 1H; H_{Ar}), 7.84 (d, J = 7.5 Hz, 1H; H_{Ar}), 4.19 – 4.09 (m, 1H; C$H_{(Ala)}$), 3.88 (s, 3H; OCH_3), 1.45 (d, J = 6.5 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 169.7, 164.7, 151.3, 145.9, 140.2, 121.1, 117.5, 52.5, 49.0, 17.1; **IR** (KBr pellet): υ 3237 (b), 3045 (b), 1673 (b), 1536 (s), 1447 (s), 1314 (s), 1198 (b), 1136 (s) cm^{-1}. **HRMS:** (ESI$^+$) calcd for $C_{10}H_{14}N_3O_3$ $(MH)^+$ 224.1035; found 224.1026.

OMe-AP-L-Ala- D-Ala--L-Ala-Boc 8: To a solution of dipeptide **21** (0.24 g, 0.90 mmol, 1.0 equiv) and dipeptide **22** (0.3 g, 0.9 mmol, 1.0 equiv) in dry DCM (7 mL) was added HCTU (0.42 g, 1.03 mmol, 1.2 equiv). DIEA (0.44 mL, 2.6 mmol, 3.0 equiv) was slowly added to the reaction mixture and the solution was allowed to stir for 12 h at RT. The solvent was evaporated in vacuo and the residue was diluted with ethyl acetate (15 mL). The solution was sequentially washed with water (2 × 15 mL), 5% HCl (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (15 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purified by column chromatography (silica gel; 30% - 60% ethyl acetate in hexane, *v/v*) to afford 0.29 g (70%) of tetrapeptide **8** as a white solid. TLC R_f = 0.2 (50% ethyl acetate/ hexane).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.90 (t, *J* = 14.5 Hz, 1H; NH_{Ar}), 8.29 (t, *J* = 9.5 Hz, 1H; H_{Ar}), 8.15-8.05 (m (rotamers observed), 1H; NH_{Ala}), 7.98 (t, *J* = 7.5 Hz, 1H; H_{Ar}), 7.93 (t, *J* = 6.0 Hz, 1H; NH_{Ala}), 7.78 (dd, *J* = 8.0 Hz, 1.5 Hz; H_{Ar}), 7.0-6.91 (m, 1H; N*H*Boc), 4.58 – 4.41 (m (rotamers observed), 1H; C$H_{(Ala)}$), 4.35 – 4.25 (m, 1H; C$H_{(Ala)}$), 3.95 (app. quint; C$H_{(Ala)}$), 3.87 (s, 3H; OCH_3), 1.38- 1.27 (12H; (CH_3)$_3$C and C$H_{3(Ala)}$), 1.22- 1.19 (m, 3H; C$H_{3(Ala)}$), 1.15 (d, *J* = 7 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d6) δ 172.6, 172.4, 171.9, 164.8, 155.1, 151.9, 145.8, 139.6, 120.4, 117.4, 78.1, 52.4, 49.8, 49.0, 48.0, 28.2, 18.5, 18.1, 17.9; **IR** (KBr pellet): υ 3347 (b), 1703 (b), 1529 (s), 1453 (s), 1377 (s), 1310 (s), 1166 (b). **HRMS:** (ESI^+) calcd for $C_{21}H_{32}N_5O_7$ $(MH)^+$ 466.2302; found 466.2294.

OMe-AP-L-Ala- D-Ala--L-Ala-NH$_2$ 9: To a solution of tetrapeptide **8** (0.18 g, 0.37 mmol, 1.0 equiv) in CH_2Cl_2 (7 mL) was slowly added trifluoroacetic acid (0.2 mL, 3.7 mmol, 10.0 equiv) over the period of 5 minutes using a syringe. The reaction mixture was allowed to stir for 4 h at RT. The solvent was evaporated in vacuo and the resultant salt was dissolved in water (2 mL) and lyophilized to afford 0.18 gm (99%) of product **9** as a pale yellow solid. TLC R_f = 0.10 (15% MeOH/ DCM).

^{1}H **NMR** (500 MHz, DMSO-d_6, 25 °C) δ 10.92 (s, 1H; NH_{Ar} (small peak at 10. 86 due to rotamer)), 8.60 (d, *J* = 5.5 Hz, 1H; NH_{Ala}), 8.4- 8.31 (m, 1H; NH_{Ala}), 8.28 (d, *J* = 8.5 Hz, 1H;

H_{Ar}), 8.10 (s, 3H; NH_3^+), 7.98 (t, J = 8.0 Hz, 1H; H_{Ar}), 7.78 (d, J = 7.5 Hz, 1H; H_{Ar}), 4.6- 4.36 (3H; 3 C$H_{(Ala)}$), 3.87 (s, 3H; OCH_3), 1.35- 1.28 (6H; 2 C$H_{3(Ala)}$), 1.24 (d, J = 7.0 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 172.7, 171.6, 169.1, 164.9, 152.0, 145.9, 139.7, 120.6, 117.4, 69.8, 52.5, 49.0, 48.2, 18.9 18.3, 18.0, 17.9, 17.8, 17.32, 17.26; **IR** (KBr pellet): υ 3315(b), 2980(s), 2933(s), 1657(b), 1532(b), 1456(s), 1250(s), 1168(s). **HRMS:** (ESI$^+$) calcd for $C_{16}H_{24}N_5O_5$ $(M+H)^+$ 366.1777; found 366.1795.

OH-AP-L-Ala- D-Ala--L-Ala-Boc 10: A solution of tetrapeptide **8** (0.05 g, 0.11 mmol, 1.0 equiv) in methanol/ water (2:1) (6 mL) was treated with LiOH (0.02 g, 0.57 mmol, 5.0 equiv) at RT. The solution was allowed to stir for 6 h, following which methanol was evaporated in vacuo. The aqueous layer was diluted with water (5 mL), washed with ethyl acetate (10 mL), acidified with 5% aq. HCl and extracted with ethyl acetate (3 × 10 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo to afford 0.04 g (80%) of tetrapeptide **10** as a white solid. The white solid was used for the next step without further purification. TLC R_f = 0.10 (100% ethyl acetate)

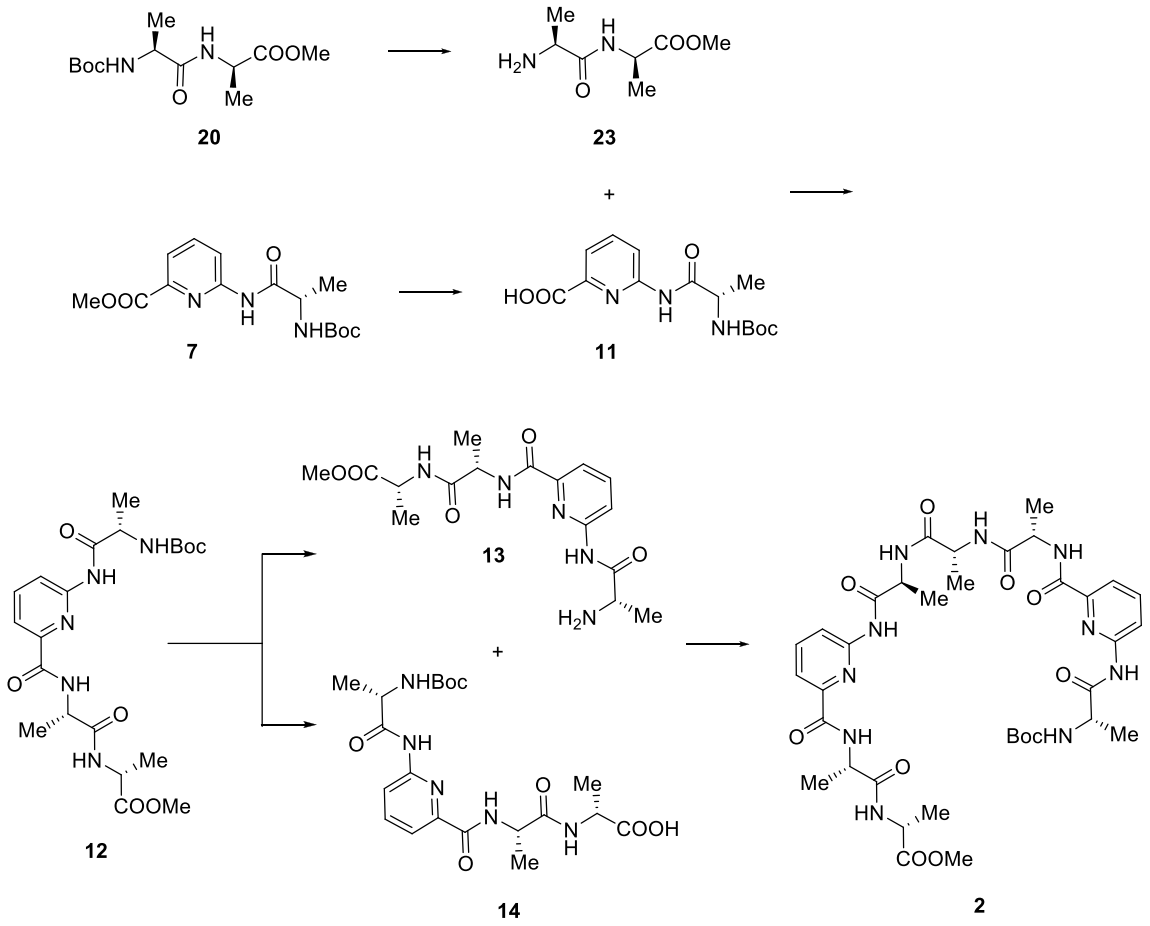
IR (KBr pellet): υ 3239(b), 3087 (b), 1676 (b), 1542(s), 1456(s), 1313(s), 1252 (s), 1203(s), 1136 (s); **HRMS:** (ESI$^+$) calcd for $C_{20}H_{30}N_5O_7$ $(MH)^+$ 452.2145; found 452.2123.

OMe-AP-L-Ala- D-Ala--L-Ala-AP-L-Ala- D-Ala--L-Ala-Boc 1: To a solution of Boc deprotected tetrapeptide **9** (0.10 g, 0.21 mmol, 1.0 equiv) and ester deprotected tetrapeptide **10** (0.10 g, 0. 22 mmol, 1.00 equiv) in CH_2Cl_2 (10 mL) was added HCTU (0.12 gm, 0.23 mmol, 1.10 equiv). DIEA (0.12 mL, 0.64 mol, 3.0 equiv) was slowly added to the reaction mixture and the solution was allowed to stir for 20 h at room temperature. The solvent was evaporated in vacuo and the residue was diluted with ethyl acetate (15 mL). The solution was washed with water (4 × 15 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purification by column chromatography (neutral alumina; 50% – 80% ethyl acetate in hexane, v/v) afforded 0.14 g (77%) of octapeptide **1** as a pale yellow solid. TLC R_f = 0.20 (75% ethyl acetate/ hexane); $[\alpha]^{33}_D$ +6.75 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 10.82 (s, 1H; NH_{Ar}), 10.64 (s, 1H; NH_{Ar}), 8.70 (d, J = 7.0 Hz, 1H; NH), 8.50 (d, J = 8.0 Hz, 1H; NH), 8.41- 8.32 (2H; NH), 8.25- 8.15 (3H; NH, 2 H_{Ar}), 8.0- 7.94 (2H; H_{Ar}), 7.78-7.65 (2H; H_{Ar}), 7.17 (d, J = 6.5 Hz, 1H; NHBoc), 4.65 – 4.5 (3H; 3 C$H_{(Ala)}$), 4.32-4.20 (2H; 2 C$H_{(Ala)}$), 4.12 (app. q, 1H; C$H_{(Ala)}$), 3.61 (s, 3H; OCH_3), 1.37 (s, 9H; (CH_3)$_3$C), 1.35-1.28 (9H; 3 C$H_{3(Ala)}$), 1.26 - 1.22 (9H; 3 C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) multiple peaks δ, 173.4, 173.0, 172.6, 172.1, 172.0, 171.9, 162.8, 162.5, 155.4, 151.0, 150.9, 148.0, 147.9, 140.0, 117.4, 117.3, 116.6, 78.3, 52.1, 50.9, 50.4, 49.1, 48.5, 48.0, 31.7, 29.2, 28.4, 24.5, 23.3, 21.3, 19.8, 18.1; **IR** (KBr pellet): υ 3459 (br), 3282 (s), 2926(s), 1634 (s), 1528 (s), 1453 (s), 1166 (s) cm^{-1}; **HRMS**: (ESI$^+$) Calculated for $C_{36}H_{50}N_{10}O_{11}Na$ $(MNa)^+$ 821.3558, found 821.3568.

Synthesis of octapeptide 2[2]

Scheme S3



NH₂ L-Ala- D-Ala-OMe 23: To a solution of dipeptide **20** (0.3 g, 1.1 mmol, 1.0 equiv) in DCM (7.0 mL) was added trifluoroacetic acid (1.3 g, 10.9 mmol, 10.0 equiv) and the reaction mixture was allowed to stir for 3h at RT. The solvent was removed in vacuo. The resultant salt was dissolved in water and lyophilized to afford 0.3 g (99%) of dipeptide **23** as a pale yellow solid. TLC R_f = 0.10 (7% MeOH/ DCM).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 8.90 (d, J = 6.5 Hz, 1H; NH_{Ala}), 8.22 (s, 3H; NH_3^+), 4.32 (app. quin, 1H; C$H_{(Ala)}$), 3.92-3.82 (m, 1H; C$H_{(Ala)}$), 3.63 (s, 3H; OCH_3), 1.35 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$), 1.30 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 172.7, 169.6, 52.1, 52.0, 48.2, 47.9, 17.3, 17.03, 17.01; **IR** (KBr pellet): υ 3068 (b), 2622 (b), 1736 (b),

1558 (s), 1458 (s), 1140 (b), 838 (s), 722 (s) cm^{-1}. **HRMS:** (ESI^+) calcd for $C_7H_{15}N_2O_3$ $(MH)^+$ 175.1083; found 175.1091.

Boc-L-Ala-AP-OH 11: To a solution of dipeptide **7** (0.4 g, 1.3 mmol, 1.0 equiv) in THF (7.0 mL) at RT were added LiOH (0.1 g, 3.3 mmol, 2.5 equiv) and water (5.0 mL). The reaction mixture was allowed to stir for 4 h. Subsequently the reaction mixture was diluted with water (10 mL) and washed with ethyl acetate (1 × 15 mL). The aqueous layer was acidified with dil HCl to pH 1 and was extracted with ethyl acetate (4 × 15 mL). The combined organic layers were dried over anhydrous sodium sulphate and concentrated in vacuo to afford 0.37 g of Boc-L-Ala-AP-OH dipeptide **11** (92%) as a white solid. This white solid was used for the next reaction without further purification. TLC R_f = 0.15 (100% ethyl acetate).

IR (KBr pellet): υ 3437 (b), 2926 (s), 2361 (s), 1716 (b), 1628 (s), 1539 (s), 1460 (s), 1379 (s) cm^{-1}; **HRMS:**(ESI^+) calcd for $C_{14}H_{20}N_3O_5$ $(MH)^+$310.1403; found 310.1411.

Boc-L-Ala-AP-L-Ala- D-Ala-OMe 12: To a solution of L-Ala- D-Ala-OMe dipeptide **23** (0.18 g, 0.62 mmol, 1.0 equiv) and Boc-L-Ala-AP-OH dipeptide **11** (0.19 g, 0.62 mmol, 1.0 equiv) in dry DCM (7 mL) was added HCTU (0.31 g, 7.40 mmol, 1.20 equiv). DIEA (0.38 mL, 2.20 mmol, 3.60 equiv) was slowly added to the reaction mixture and the solution was allowed to stir for 12 h at RT. The solvent was evaporated in vacuo, following which the residue was diluted with ethyl acetate (15 mL). The solution was sequentially washed with water (2 × 15 mL), 5% HCl (2 × 15 mL), saturated aqueous $NaHCO_3$ (2 × 15 mL) and water (15 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purification by column chromatography (silica gel; 30% - 50% ethyl acetate in hexane, *v/v*) afforded 0.24 g (84%) of tetrapeptide **12** as a white solid. TLC R_f = 0.25 (50% ethyl acetate/ hexane)

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.65 (s, 1H; NH_{Ar}), 8.69 (s, 1H; NH_{Ala}), 8.37 (d, *J* = 8.5 Hz, 1H; NH_{Ala}), 8.25 (d, *J* = 8.5 Hz, 1H; H_{Ar}), 7.98 (t, *J* = 8.0 Hz, 1H; H_{Ar}), 7.75 (d, *J* = 7.5 Hz, 1H; H_{Ar}), 7.17 (d, *J* = 6.5 Hz, 1H; N*H*Boc), 4.65 – 4.59 (m, 1H; C$H_{(Ala)}$), 4.32 – 4.24 (m, 2H; C$H_{(Ala)}$), 3.62 (s, 3H; OCH_3), 1.38 – 1.23 (m, 18H; (CH_3)$_3$C, C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz,

DMSO-d6) δ 172.9, 171.7, 162.4, 155.3, 150.8, 147.8, 140.0, 117.2, 116.5, 78.2, 67.4, 52.0, 50.4, 47.8, 28.2, 21.5, 19.6, 17.7, 16.9; **IR** (KBr pellet): υ 3391 (b), 2925 (s), 2857 (s), 2360 (b), 1677 (b), 1531 (b), 1450 (s), 1059 (b). **HRMS:** (ESI^+) calcd for $C_{21}H_{32}N_5O_7$ $(MH)^+$ 466.2302; found 466.2321.

H_2N-L-Ala-AP-L-Ala- D-Ala-OMe 13: To a solution of tetrapeptide **12** (0.10 g, 0.21 mmol, 1.0 equiv) in DCM (7 mL) was slowly added trifluoro acetic acid (0.20 mL, 2.10 mmol, 10.0 equiv) over a period of 5 minutes using a syringe. The reaction mixture was allowed to stir at room temperature for 3 h, following which the solvent was removed in vacuo. The product was dissolved in water (5 mL) and lyophilized to afford 0.10 g (99%) of tetrapeptide **13** as a pale yellow solid. This yellow solid was used for the next step without further purification. TLC R_f = 0.10 (15% MeOH/ DCM)

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 11.22 (s, 1H; NH_{Ar}), 8.73 (d, J = 7 Hz, 1H; NH_{Ala}), 8.37 (d, J = 8.0 Hz, 1H; H_{Ar}), 8.28 (d, J = 4.0 Hz, 3H; NH_3^+), 8.06 (t, J = 8.5 Hz, 1H; H_{Ar}), 7.82 (dd, J = 7.5, 0.5 Hz, H_{Ar}), 4.63 (app quint, 1H; C$H_{(Ala)}$), 4.28 (app quint, 2H; C$H_{(Ala}$), 3.62 (s, 3H; OCH_3), 1.48 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$), 1.34 (d, J = 7 Hz, 3H; C$H_{3(Ala)}$), 1.30 (d, J =7.5 Hz, 3H; C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) δ 172.9, 171.7, 169.5, 162.2, 158.3, 158.0, 150.1, 148.0, 140.4, 118.0, 116.7, 52.0, 49.0, 47.8, 47.7, 19.6, 17.1, 16.9; **IR** (KBr pellet): υ 3439(b), 2926(s), 1676(s), 1455(s), 1314 (s), 1202(s), 1026(s). **HRMS:** (ESI^+) calcd for $C_{16}H_{24}N_5O_5$ (M^+) 366.1777; found 366.1761.

Boc-L-Ala-AP-L-Ala- D-Ala-OH 14: A solution of tetrapeptide **12** (0.24 g, 0.52 mmol, 1.00 equiv) in methanol-water (2:1, 12 mL) was treated with LiOH (0.03 g, 1.30 mmol, 2.5 equiv) at RT. The solution was allowed to stir for 6 h, following which methanol was evaporated in vacuo. The mixture was diluted with water (10 mL) and subsequently washed with ethyl acetate (2 × 15 mL). The aqueous layer was acidified with 5% aq. HCl and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were dried over anhydrous sodium sulphate, filtered and

concentrated in vacuo to afford 0.17 g of tetrapeptide **14** (71%) as a white solid. This white solid was used for the next step without further purification. TLC R_f = 0.10 (100 ethyl acetate).

IR (KBr pellet): υ 3429(b), 2926(s), 1662(b), 1456(s), 1338(b), 1167(s), 1026(b). **HRMS:** (ESI^+) calcd for $C_{20}H_{30}N_5O_7$ $(MH)^+$ 452.2145; found 452.2127.

Boc-L-Ala-AP-L-Ala- D-Ala- L-Ala-AP-L-Ala- D-Ala-OMe 2: To a solution of Boc deprotected tetrapeptide **13** (0.06 g, 0.12 mmol, 1.0 equiv) and ester deprotected tetrapeptide **14** (0.06 g, 0.12 mmol, 1.0 equiv) in CH_2Cl_2 (7 mL) was added HCTU (0.06 g, 0.15 mmol, 1.2 equiv). DIEA (0.10 mL, 4.40 mmol, 3.0 equiv) was slowly added to the reaction mixture and the solution was allowed to stir for 12 h at room temperature. The solvent was evaporated and the residue was diluted with ethyl acetate (15 mL). The solution was washed with water (4 × 15 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purification by column chromatography (alumina; 50% – 80% ethyl acetate in hexane, v/v) afforded 0.065 g (67%) of octapeptide **2** as a pale yellow solid. TLC R_f = 0.20 (70% ethyl acetate/ hexane); $[\alpha]^{33}_D$ +7.84 (*c* 1.0, $CHCl_3$).

^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) δ 10.84 (s, 1H; NH_{Ar}), 10.68 (s, 1H; NH_{Ar}), 8.78 (t, *J* = 7.0 Hz, 1H; N*H*), 8.58 (d, *J* = 7.5 Hz, 1H; N*H*), 8.41 (d, *J* = 8.0 Hz, 2H; N*H*), 8.29 - 8.21 (3H; N*H*, H_{Ar}), 8.0 - 7.91 (2H; H_{Ar}), 7.75-7.69 (2H; H_{Ar}), 7.2 – 7.18 (m, 1H; N*H*Boc), 4.64 – 4.51 (m, 3H; C$H_{(Ala)}$), 4.39-4.31 (m, 1H; C$H_{(Ala)}$), 4.30-4.21 (2H; C$H_{(Ala)}$), 3.61 (s, 3H; OCH_3), 1.37 (s, 9H; (CH_3)$_3$C), 1.35- 1.21 (18H; 6 C$H_{3(Ala)}$); **^{13}C NMR** (125 MHz, DMSO-d_6, 25 °C) Overlapping peaks δ 172.5, 172.3, 172.0, 171.8, 170.3, 164.8, 162.7, 151.9, 150.6, 147.8, 145.8, 139.8, 139.5, 120.4, 117.4, 117.2, 116.5, 78.1, 59.7, 52.3, 49.9, 48.9, 48.6, 48.3, 48.1, 28.9, 28.1, 20.7, 18.9, 18.3, 17.9, 17.7; **IR** (KBr pellet): υ 3389 (b), 2926(s), 1672 (s), 1532 (s), 1450 (s), 1168 (s) 1027 (b) cm^{-1}; **HRMS**: (ESI^+) Calculated for $C_{36}H_{50}N_{10}O_{11}Na$ $(M+ Na)^+$ 821.3558, found 821.3579.

Vesicle based fluorescence experiments.

HPTS assay in HEPES-NaCl buffer[2.]

Preparation of vesicles

Cholesterol (1.6 mg, 4.1 µmol, 1 equiv) was added to a solution of EYPC lipids (28.4 mg, 36.9 µmol, 9 equiv) in chloroform (0.284 mL). Chloroform was removed under a stream of nitrogen and further in vacuo for 5 h at 0 ºC to give a lipid film. The lipid film was hydrated with 1 mL of a buffer solution containing HPTS dye (0.1 mM HPTS, 10 mM NaCl, 100 mM, HEPES) at pH 7.2. The resulting suspension was allowed to stir at room temperature for 1 h. and then subjected to five freeze-thaw cycles. The vesicle solution was placed in an ice bath and subsequently sonicated in a bath sonicator at 0-4 °C for a total time of 2 minutes (30 s on & 15 s off in degas mode). The vesicles were allowed to anneal for one hour in the refrigerator, following which they were extruded 20 times through 100 nm polycarbonate membranes using a mini-extruder from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: HEPES buffer at pH 7.2 (10 mM NaCl, 100 mM HEPES). The milky white solution containing vesicles were collected and used for further study.

Assay Procedure

Vesicle solution (100 µL), HEPES, NaCl buffer (2.9 mL) and an appropriate amount of peptide in methanol (0.5% DMSO)[3] were added to a cuvette equipped with a magnetic stir bar. The solution in the cuvette was stirred for 2 minutes before the fluorescence experiment was started. After 50 s, a solution of NaOH (20 µL, 0.5 N) was added to the vesicles. 5% Triton X (50 µL) solution was added at 250 s to achieve final equilibration of the dye. Fluorescence measurements were done at an emission wavelength of 510 nm and an excitation wavelength of 460 nm.

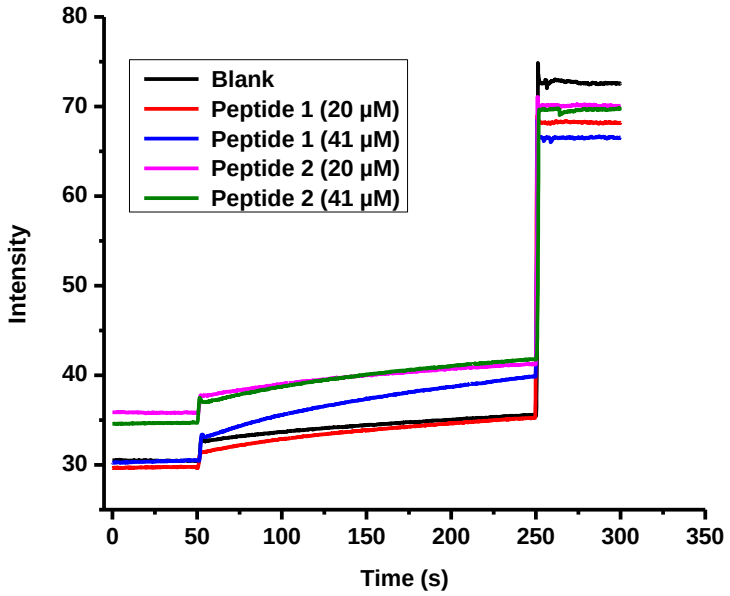


Figure S1. Raw fluorescence vs. time plots for HPTS assay with HEPES-NaCl buffer

Rate constant calculations

For each experiment, the curve obtained from t = 50 s (after addition of NaOH) to t = 250 s (before addition of Triton X) was fitted to Equation 1, where $(1/t_1) = k_{\text{peptide}}$. The curves were fitted using OriginPro 8.5 software.

$$y = y_0 + A_1 e^{-x/t_1} \qquad (1)$$

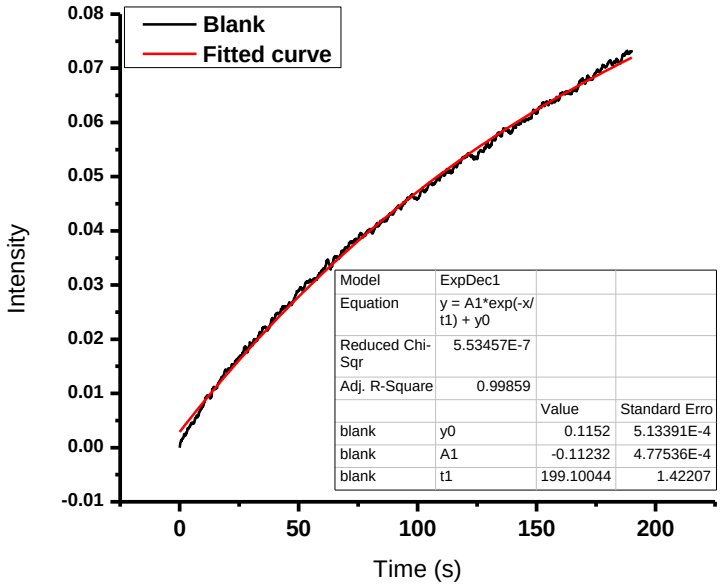


Figure S2. Fitting of fluorescence versus time plot for HPTS assay with MeOH. Inset table shows fitting results.

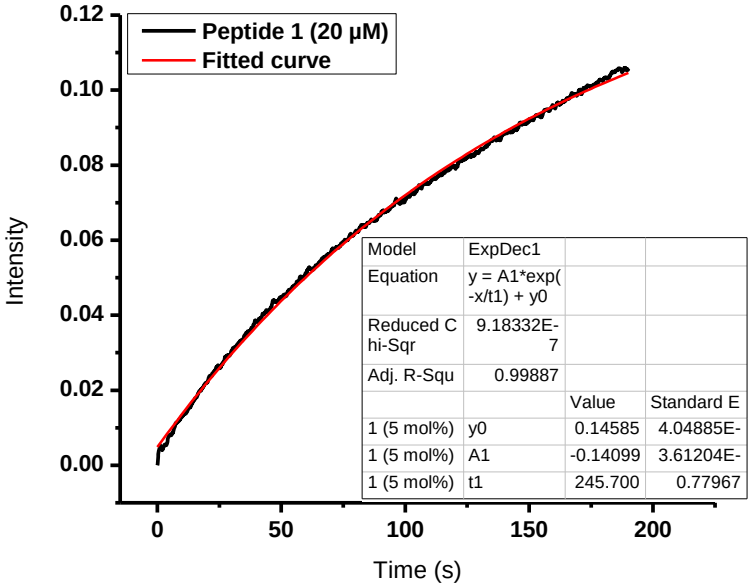


Figure S3. Fitting of fluorescence versus time plot for HPTS assay with 20 μM of peptide **1**. Inset table shows fitting results.

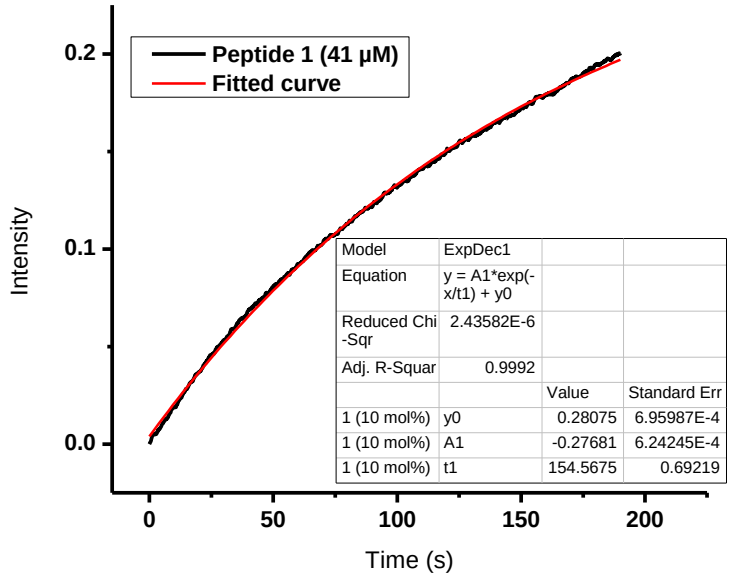


Figure S4. Fitting of fluorescence versus time plot for HPTS assay with 41 μM of peptide **1**. Inset table shows fitting results.

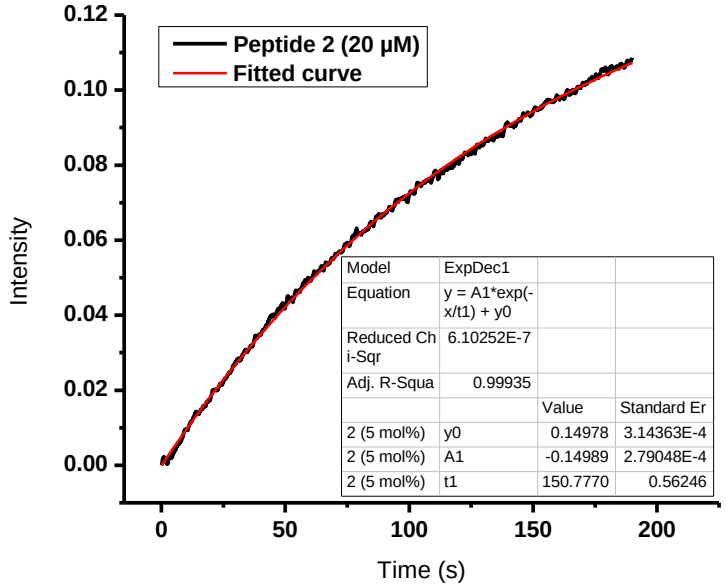


Figure S5. Fitting of fluorescence versus time plot for HPTS assay with 20 μM of peptide **2**. Inset table shows fitting results.

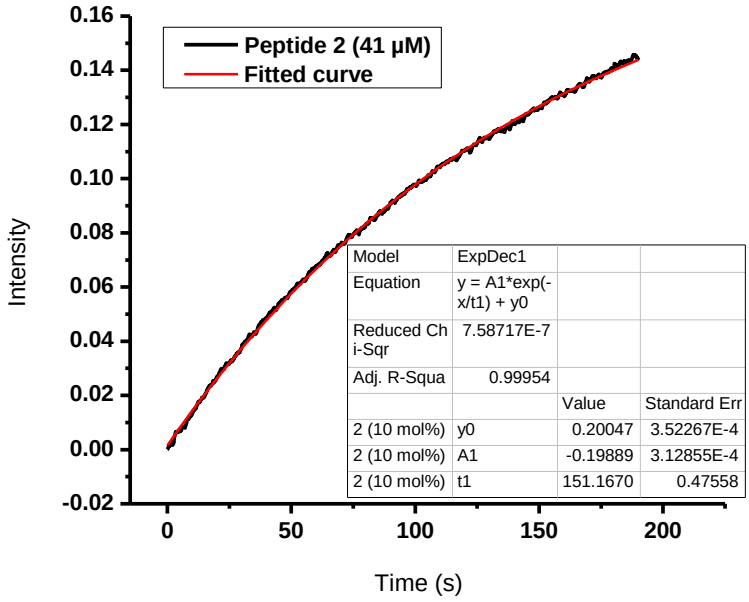


Figure S6. Fitting of fluorescence versus time plot for HPTS assay with 41 µM of peptide **2**. Inset table shows fitting results.

Lucigenin assay with sodium halides[4, 5]

Preparation of vesicles

Cholesterol (1.6 mg, 4.1 µmol, 1 equiv) was added to a solution of EYPC lipids (28.4 mg, 36.9 µmol, 9 equiv) in chloroform. Chloroform was removed under a stream of nitrogen and further in vacuo at 0 °C for 2 h to give a lipid film. The lipid film was hydrated with 1 mL of a solution containing Lucigenin dye (1.0 mM Lucigenin, 225 mM $NaNO_3$). The resulting suspension was allowed to stir at room temperature for 5 minutes and then subjected to five freeze-thaw cycles. The vesicle solution was placed in an ice bath and subsequently sonicated in a bath sonicator at 0-4 °C for a total time of 2 minutes (30 s on & 15 s off in degas mode), following which they were extruded 20 times through 400 nm polycarbonate membranes using a mini-extruder from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography (SEC) using Sephadex G-50 (eluent: 225 mM $NaNO_3$). (Note: Carrying out the SEC twice ensured removal of majority of the extra-vesicular dye). The milky white solution containing vesicles were collected and used for further study.

Assay procedure

Vesicle solution (100 µL) and $NaNO_3$ (2.9 mL) was added to a cuvette equipped with a magnetic stir bar. The solution in the cuvette was stirred for 2 minutes before the fluorescence experiment was started. After 50 s, a solution of 2.0 N NaX (35 µL), where X is Cl^-, Br^- or I^- was added to the vesicles. At 100 s, an appropriate concentration of peptide in 0.5 % DMSO-MeOH was added.[3] 5 % Triton X (50 µL) solution was added at 350 s to achieve final equilibration of the dye. Fluorescence measurements were done at an emission wavelength of 505 nm and an excitation wavelength of 455 nm.

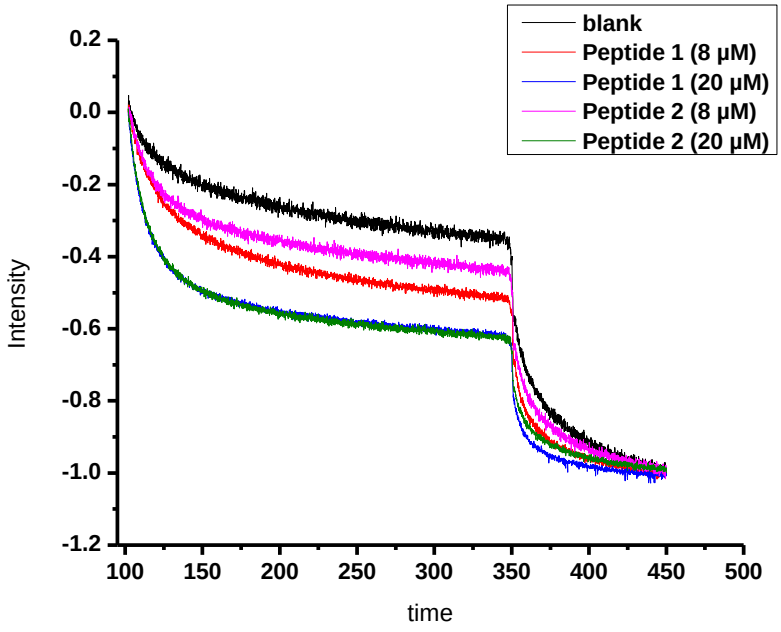


Figure S7. Normalized fluorescence vs. time plots for Lucigenin assay with peptide **1** and **2** in the presence of I^- ions.

Lucigenin assay with metal chlorides

Peptide added after addition of salt

The vesicles were prepared as described above. Vesicle solution (100 µL) and the appropriate metal nitrate solution (2.9 mL) were added to a cuvette equipped with a magnetic stir bar. The solution in the cuvette was stirred for 2 minutes before the fluorescence experiment was started. After 50s, a solution of the appropriate metal chloride (2.0 N, 35 µL), where M is Na^+, K^+, Co^{2+} or Ni^{2+} was added to the vesicles. At 100s, an appropriate concentration of peptide in 0.5% DMSO-methanol was added.[3] 5% Triton X (50 µL) solution was added at 350 s to achieve the final equilibration of the dye. Fluorescence measurements were done at an emission wavelength of 505 nm and an excitation wavelength of 455 nm.

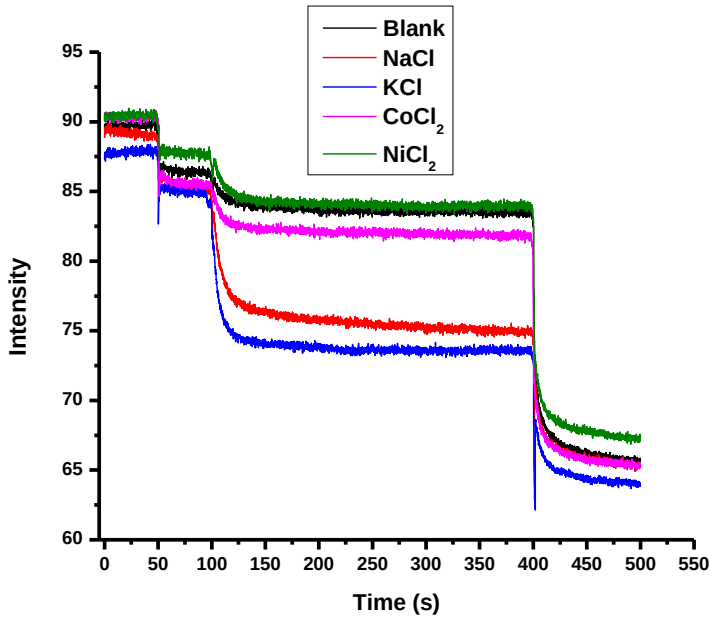


Figure S8. Raw fluorescence vs. time plots for lucigenin assay with with peptide **1** (20 µM) in the presence of different metal chlorides.

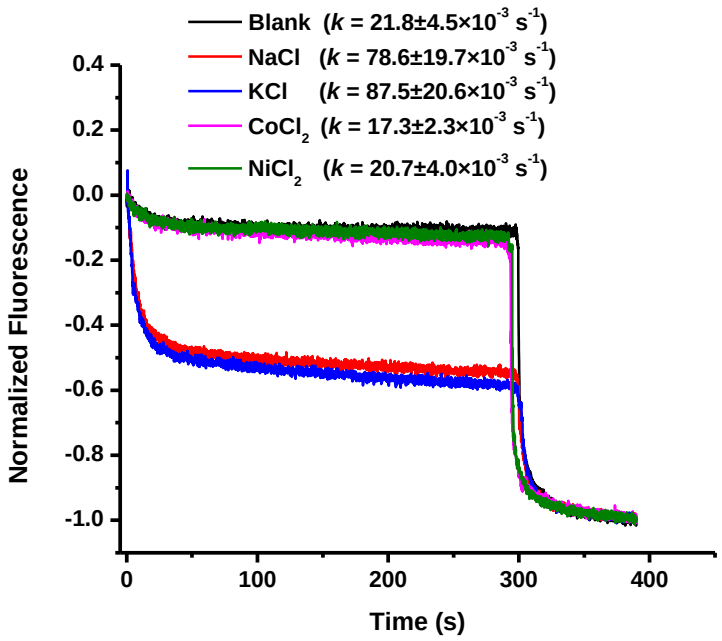


Figure S9. Normalized Fluorescence vs. time plots for lucigenin assay with with peptide **2** (20 μM) in the presence of different metal chlorides.

Rate constant calculations:

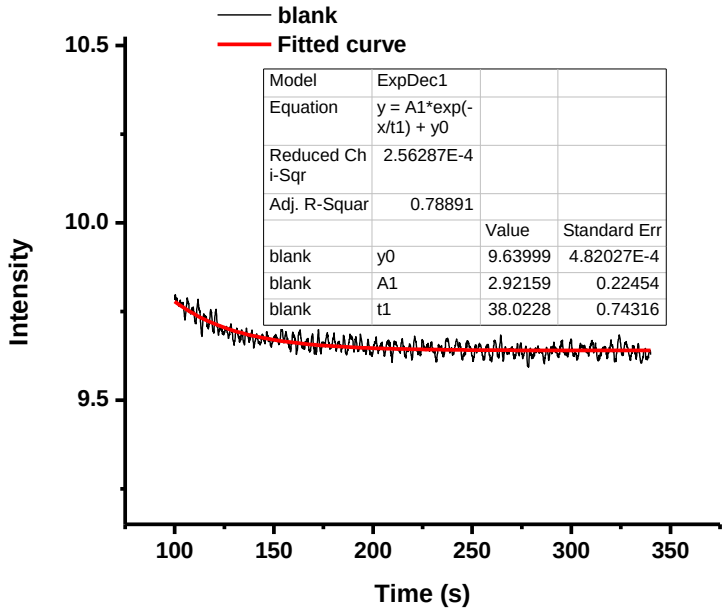
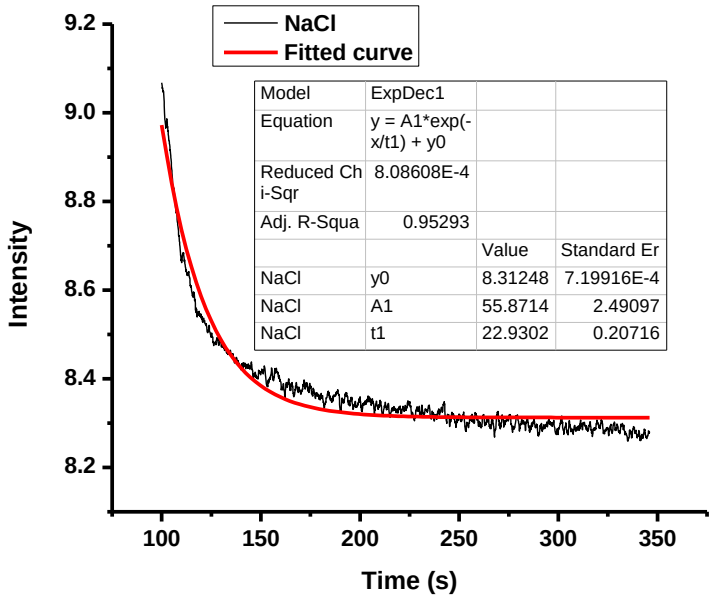
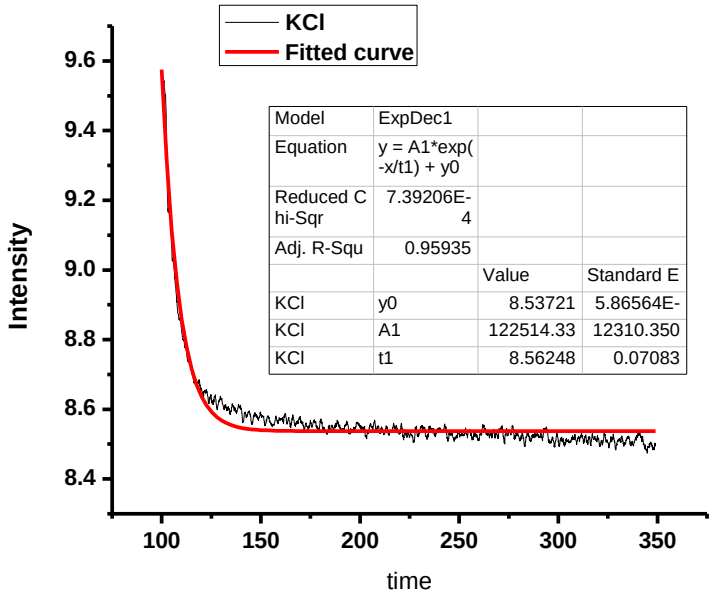


Figure S10. Fitting of fluorescence versus time plot for Lucigenin entrapped vesicles with MeOH using Origin Pro 8.5 Inset table shows fitting results.



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	8.08608E-4		
Adj. R-Squa	0.95293		
		Value	Standard Er
NaCl	y0	8.31248	7.19916E-4
NaCl	A1	55.8714	2.49097
NaCl	t1	22.9302	0.20716

Figure S11. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **1** and NaCl. Inset table shows fitting results.



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	7.39206E-4		
Adj. R-Squ	0.95935		
		Value	Standard E
KCl	y0	8.53721	5.86564E-
KCl	A1	122514.33	12310.350
KCl	t1	8.56248	0.07083

Figure S12. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **1** and KCl. Inset table shows fitting results.

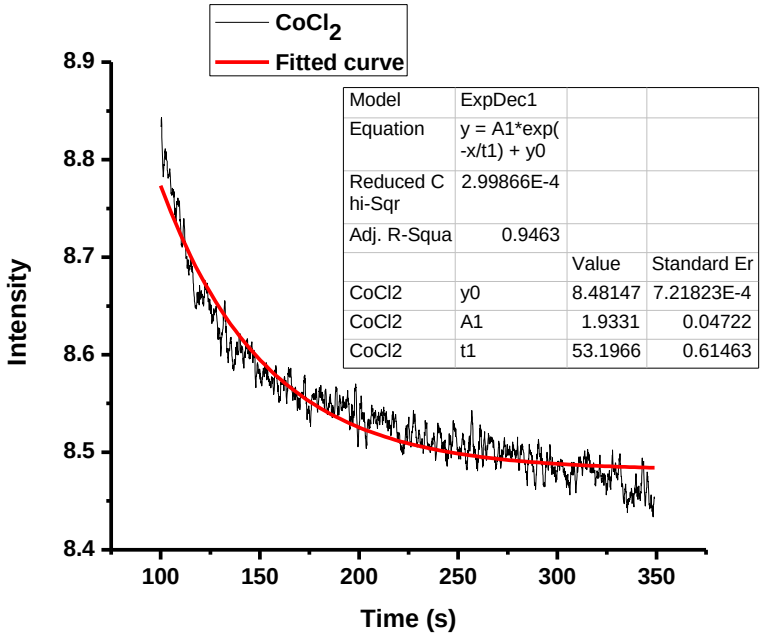


Figure S13. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **1** and $CoCl_2$. Inset table shows fitting results.

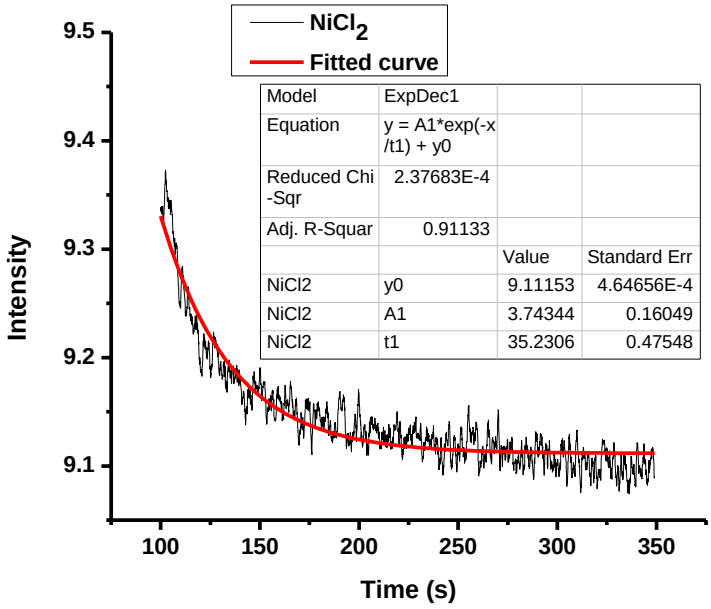
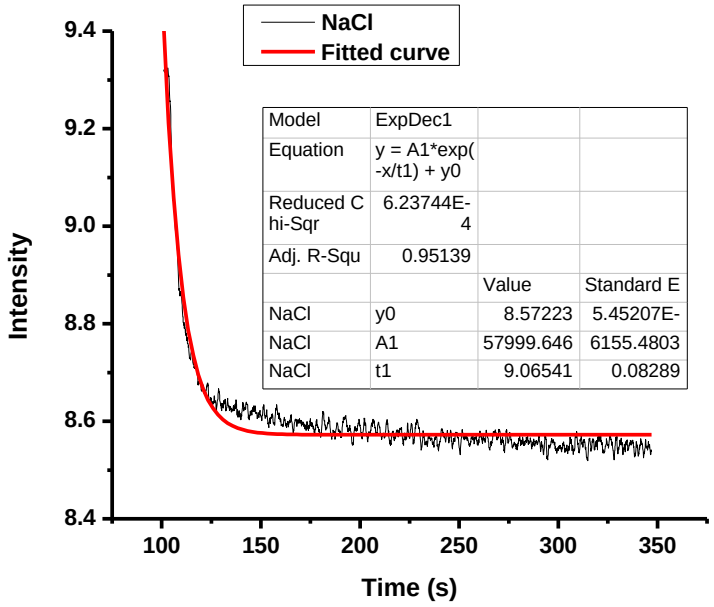
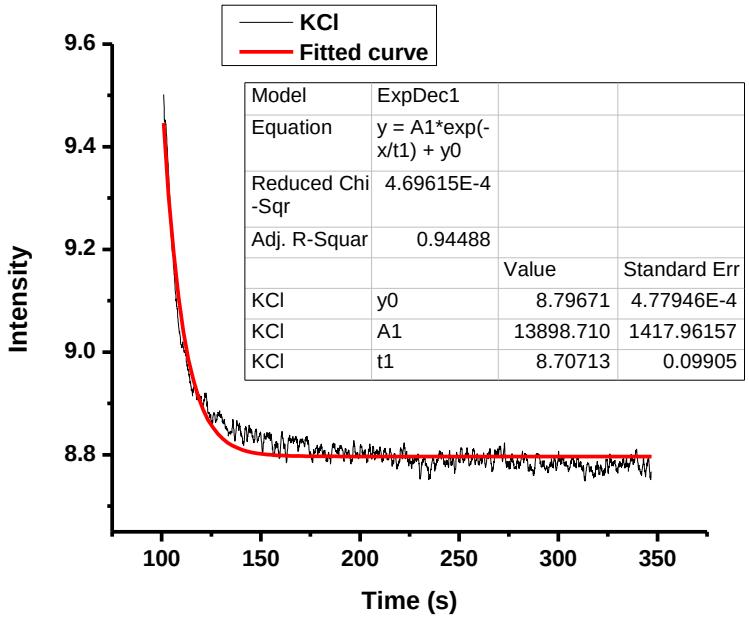


Figure S14. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **1** and $NiCl_2$. Inset table shows fitting results



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	6.23744E-4		
Adj. R-Squ	0.95139		
		Value	Standard E
NaCl	y0	8.57223	5.45207E-
NaCl	A1	57999.646	6155.4803
NaCl	t1	9.06541	0.08289

Figure S15. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** and NaCl. Inset table shows fitting results



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	4.69615E-4		
Adj. R-Squar	0.94488		
		Value	Standard Err
KCl	y0	8.79671	4.77946E-4
KCl	A1	13898.710	1417.96157
KCl	t1	8.70713	0.09905

Figure S16. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** and KCl. Inset table shows fitting results

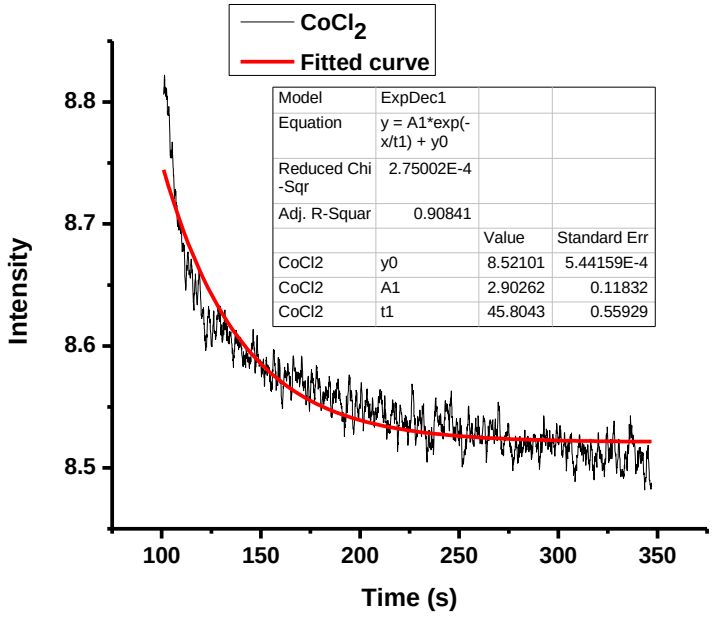


Figure S17. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **2** and $CoCl_2$. Inset table shows fitting results

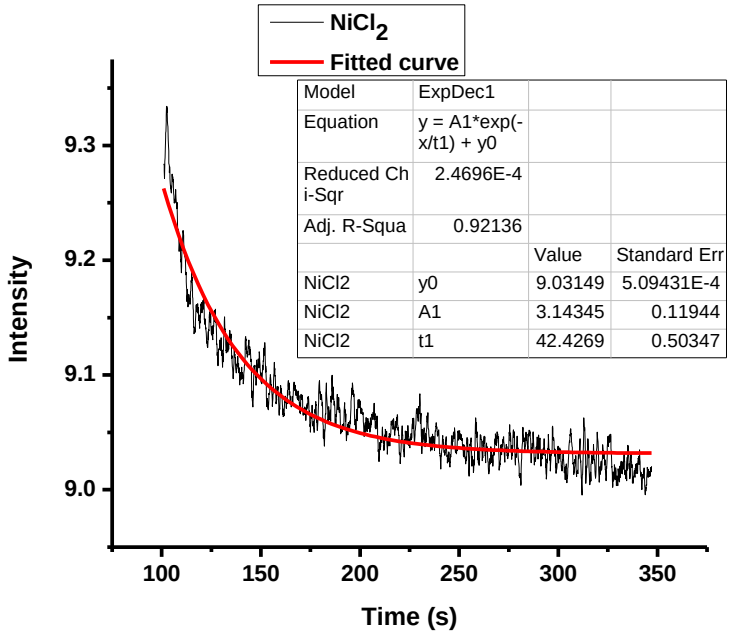


Figure S18. Fitting of fluorescence versus time plot for Lucigenin assay with 20 µM of peptide **2** and $NiCl_2$. Inset table shows fitting results

Peptide premixed with vesicles

The vesicles were prepared as described above. Vesicle solution (100 µL) and the appropriate metal nitrate solution (2.9 mL) and an appropriate amount of peptide in methanol (0.5% DMSO)[3] were added to a cuvette equipped with a magnetic stir bar. The solution in the cuvette was stirred for 2 minutes before the fluorescence experiment was started. After 100s, a solution of the appropriate metal chloride (2.0 N, 35 µL), where M is Na^+, K^+, Co^{2+} or Ni^{2+} was added to the vesicles. 5% Triton X (50 µL) solution was added at 400 s to achieve the final equilibration of the dye. Fluorescence measurements were done at an emission wavelength of 505 nm and an excitation wavelength of 455 nm.

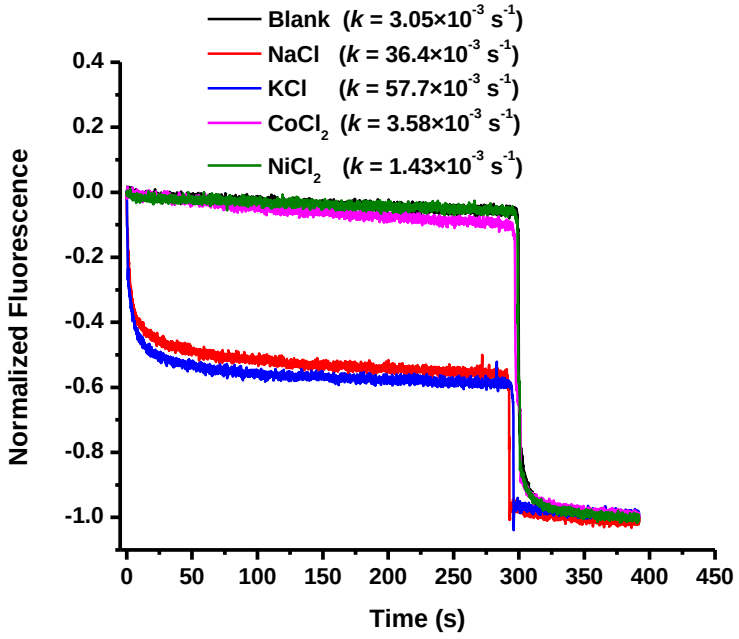


Figure S19. Normalized Fluorescence vs. time plots for lucigenin assay with with peptide **1** (20 µM) premixed in the presence of different metal chlorides.

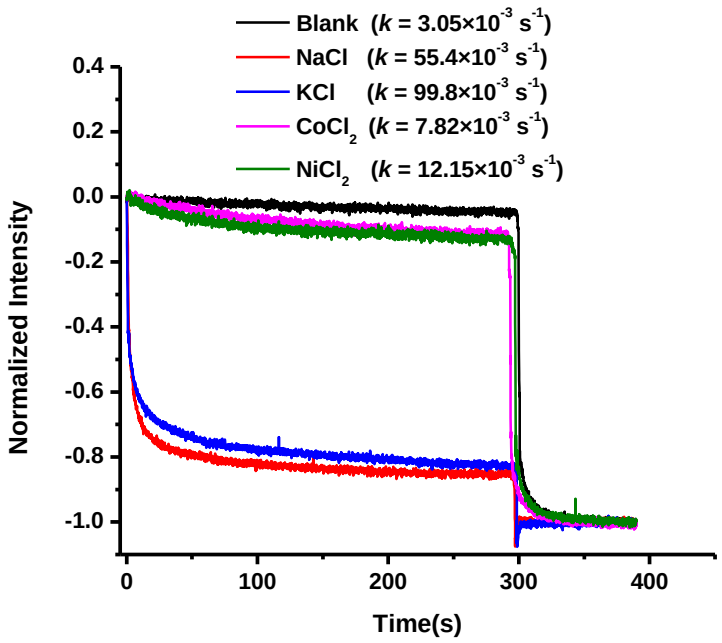


Figure S20. Normalized Fluorescence vs. time plots for lucigenin assay with with peptide **2** (20 μM) premixed in the presence of different metal chlorides.

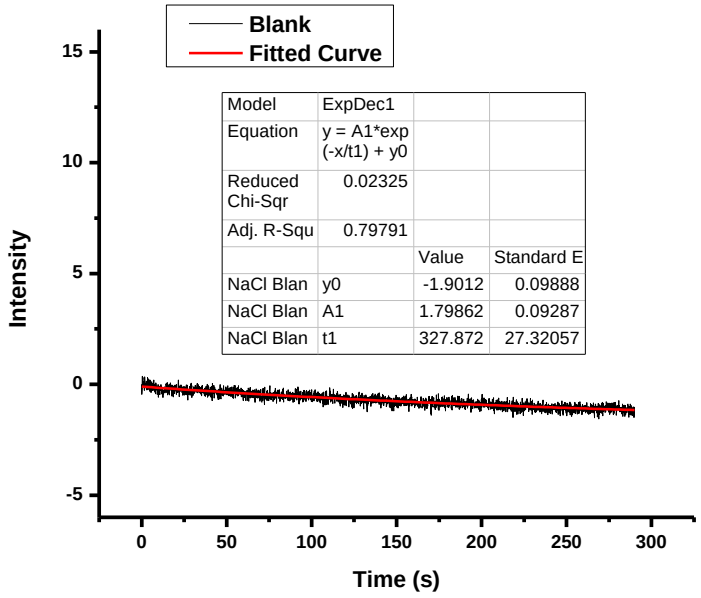


Figure S21. Fitting of fluorescence versus time plot for Lucigenin entrapped vesicles with MeOH using Origin Pro 8.5 Inset table shows fitting results.

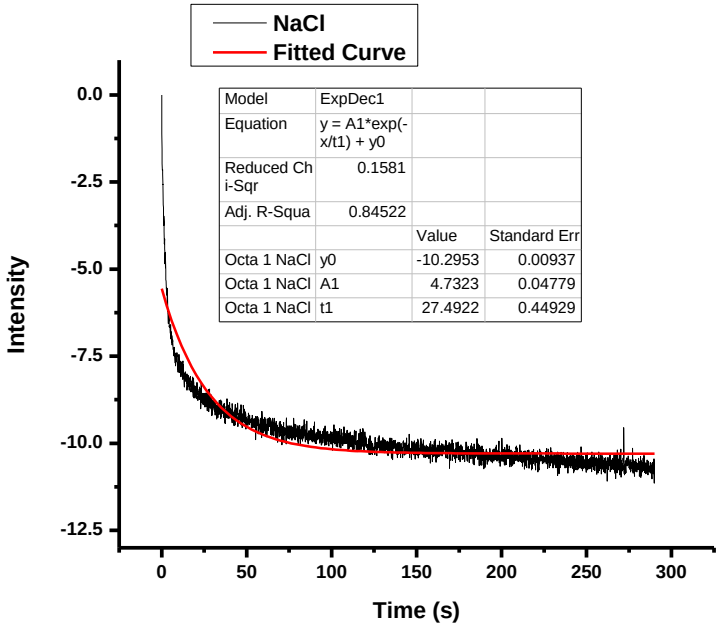


Figure S22. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **1** (premixed) and NaCl. Inset table shows fitting results

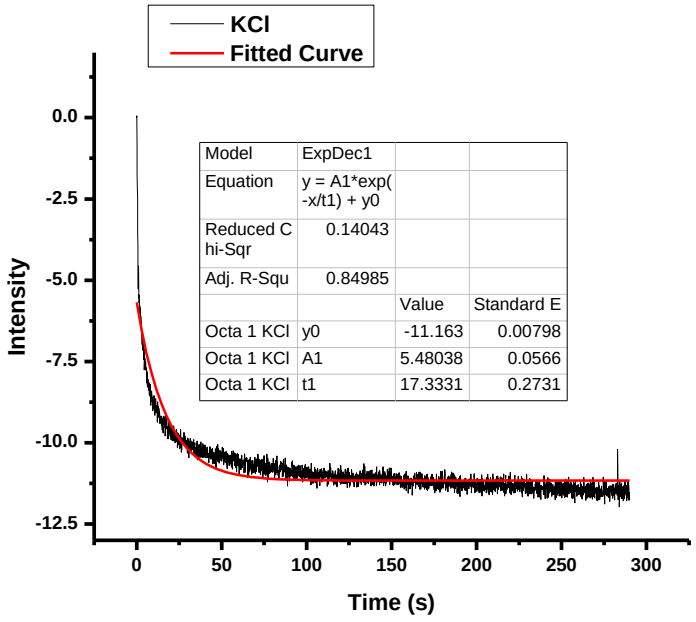


Figure S23. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **1** (premixed) and KCl. Inset table shows fitting results

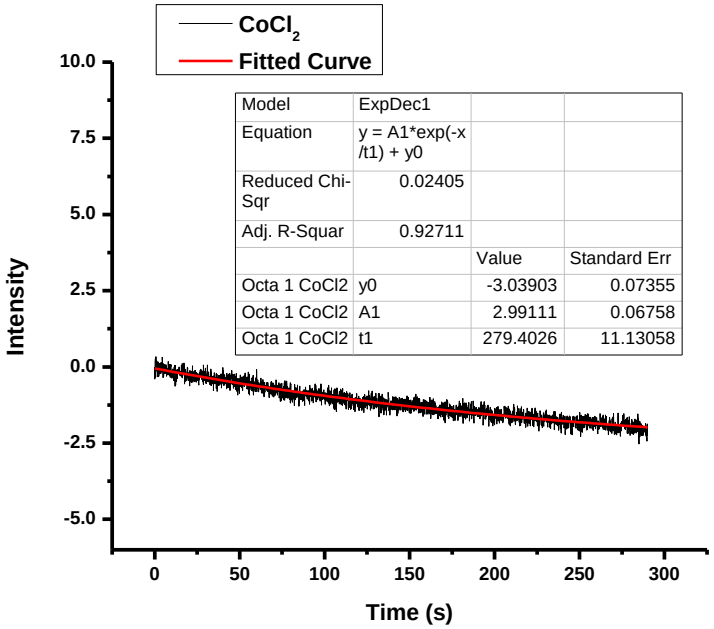


Figure S24. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **1** (premixed) and $CoCl_2$. Inset table shows fitting results

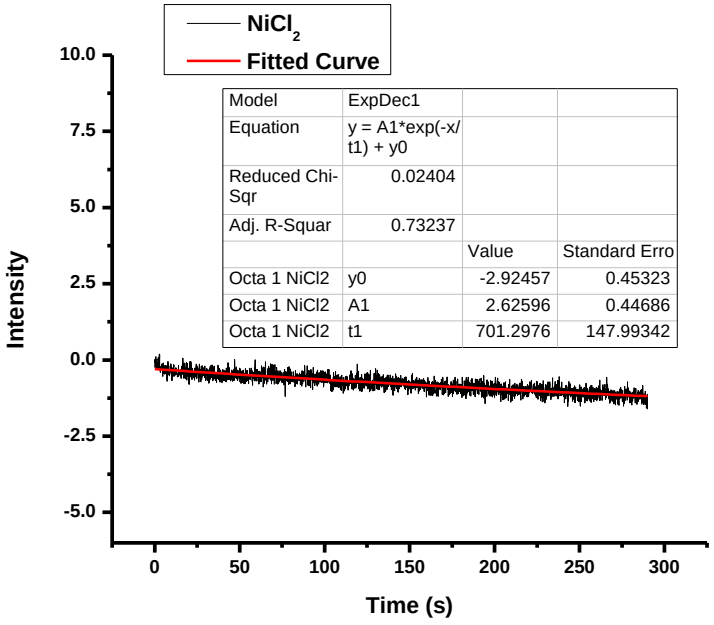
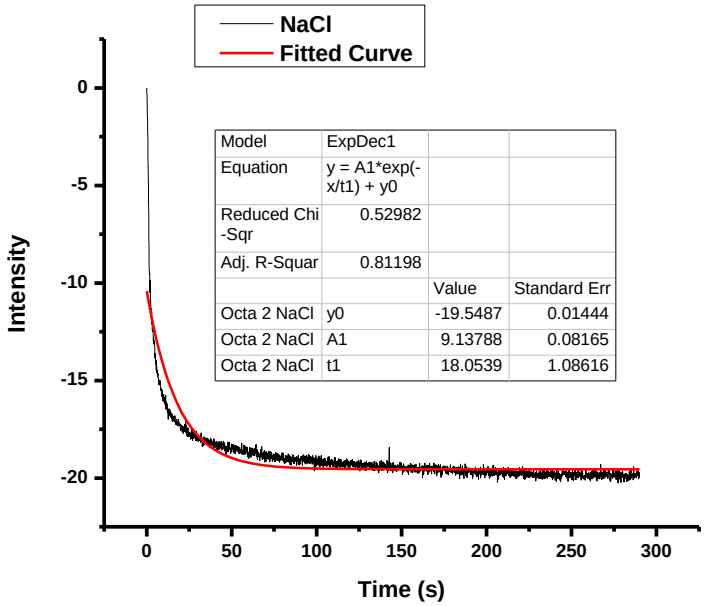
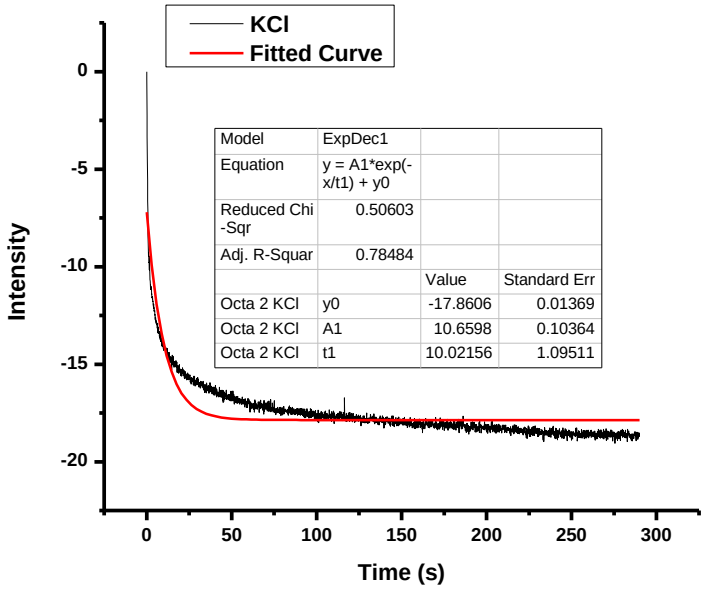


Figure S25. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **1** (premixed) and $NiCl_2$. Inset table shows fitting results.



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	0.52982		
Adj. R-Squar	0.81198		
		Value	Standard Err
Octa 2 NaCl	y0	-19.5487	0.01444
Octa 2 NaCl	A1	9.13788	0.08165
Octa 2 NaCl	t1	18.0539	1.08616

Figure S26. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** (premixed) and NaCl. Inset table shows fitting results.



Model	ExpDec1		
Equation	y = A1*exp(-x/t1) + y0		
Reduced Chi-Sqr	0.50603		
Adj. R-Squar	0.78484		
		Value	Standard Err
Octa 2 KCl	y0	-17.8606	0.01369
Octa 2 KCl	A1	10.6598	0.10364
Octa 2 KCl	t1	10.02156	1.09511

Figure S27. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** (premixed) and KCl. Inset table shows fitting results.

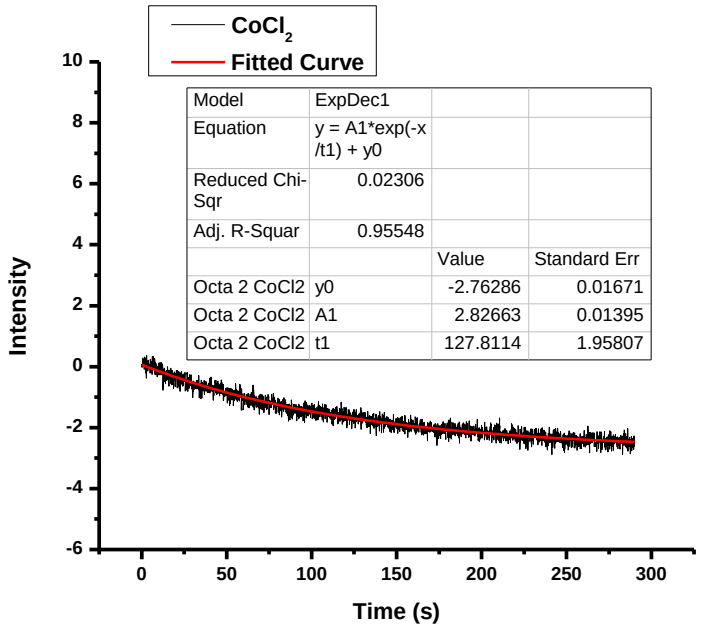


Figure S28. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** (premixed) and $CoCl_2$. Inset table shows fitting results.

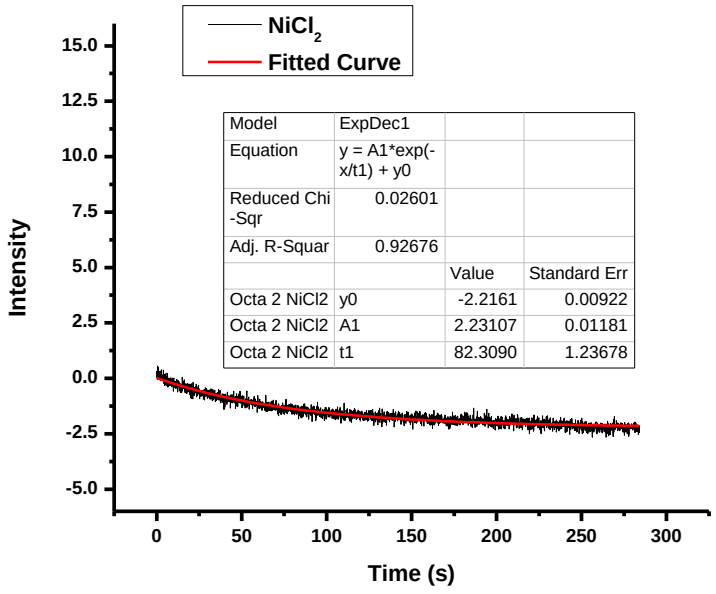


Figure S29. Fitting of fluorescence versus time plot for Lucigenin assay with 20 μM of peptide **2** (premixed) and $NiCl_2$. Inset table shows fitting results.

Dynamic light scattering studies

For the blank readings, 20 µL of MeOH was added to vesicle solutions (100 µL) diluted with HEPES buffer (2.9 mL). For measurement with peptides, an appropriate concentration of peptide in MeOH was added to the vesicle solution.

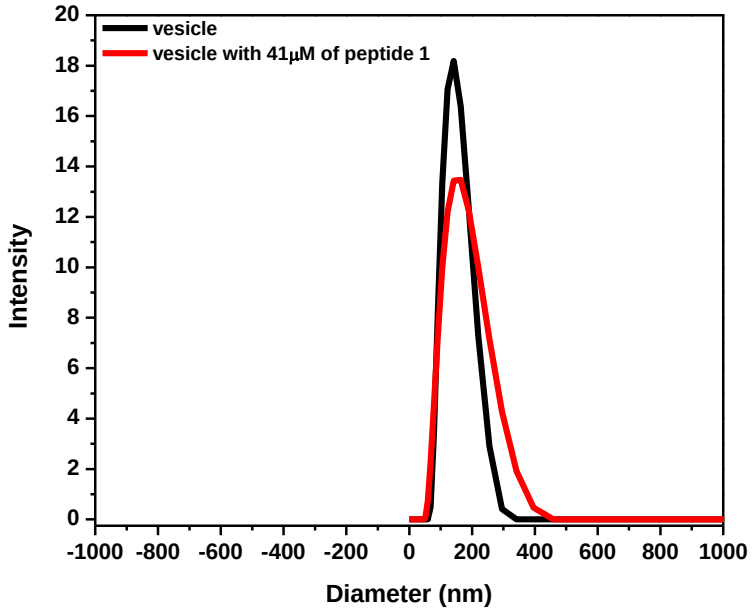


Figure S30. Dynamic light scattering study with octapeptide **1** (41 µM) and vesicles

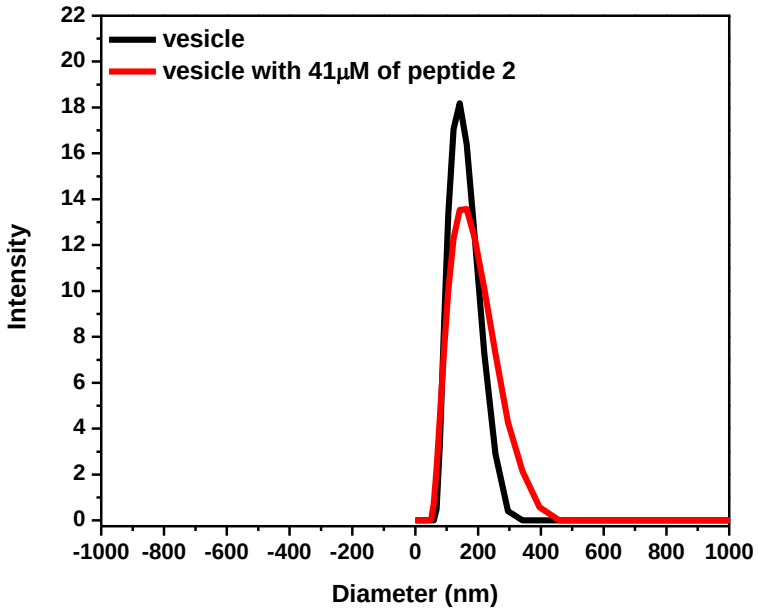


Figure S31. Dynamic light scattering study with octapeptide **2** (41 μM) and vesicles

TEM studies

Sample preparation

A solution of peptide (3.2 mg/mL) in 1% DMSO-MeOH was kept for 48 h at 0 °C. A small amount of this solution (5 μL) was placed on a carbon-coated Copper grid using a micropipette. The loaded support was dried in vacuo for 6 h before TEM images were collected.

Planar Lipid Bilayer Experiment

The ion transport through the peptide channels was also investigated with Planar lipid bilayer (PLB) recording. Synthetic lipid 1,2-diphytanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids, Alabaster, AL) was dissolved in n-decane at a concentration of 20 mg/ml and used to make the lipid bilayer. Briefly, the lipid was painted on to the aperture (pore diameter of 150 μm) of a polystyrene bilayer cuvette (Warner Instruments, USA). Both chambers of the cuvette

contained symmetrical solution (1 M KCl). The cis chamber was held at virtual ground and the trans chamber connected to a recording amplifier via a PC501A head-stage (Warner instrument). Ag-AgCl electrodes were used. Currents were low pass filtered at 1 kHz and digitized at 5 kHz using the Digidata 1440A (Axon Instruments). Once a stable bilayer was obtained, the synthetic peptide was added to the cis chamber and mixed with a magnetic stirrer. Membrane currents were frequently checked to monitor channel incorporation. After stable insertion of the peptides in bilayer, membrane current was recorded at different positive and negative voltages. For measuring reversal potential, the solution of the cis chamber was replaced with 0.5 M KCl while the trans chamber had 1 M KCl. Theoretical reversal potential was estimated using the Nernst Equation and compared with the experimentally derived value.

Absorbance Measurements of Peptides with $CoCl_2$ and $NiCl_2$:

For complexation studies, peptide and chloride salt were mixed at a ratio of 1: 5.4×10^3 which is exactly the same as the peptide to salt ratio used for vesicle bound fluorescence assay.

a) b)

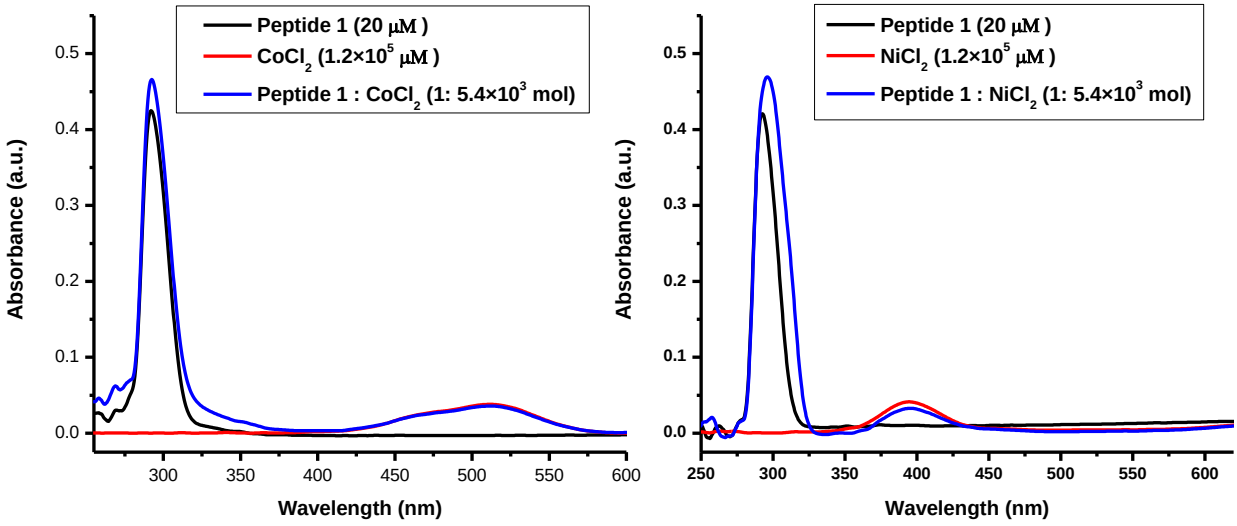


Figure S32. Absorbance spectra to check binding of peptide **1** with a) $CoCl_2$ and b) $NiCl_2$.

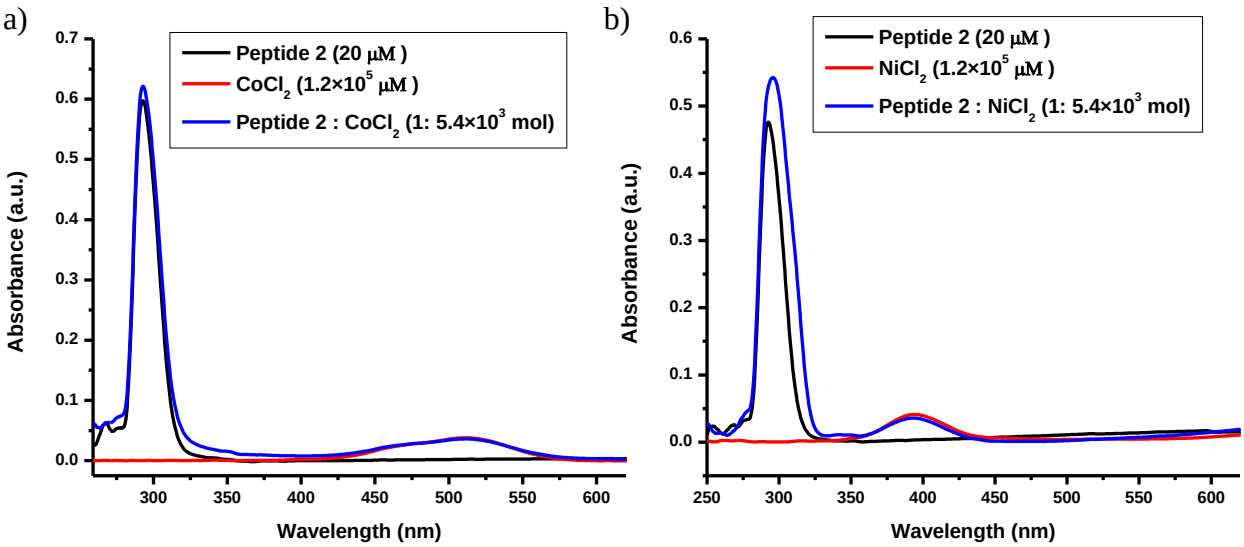


Figure S33. Absorbance spectra to check binding of peptide **2** with a) $CoCl_2$ and b) $NiCl_2$

References and notes:

1) M. Jonathan Fray; A. T. Gillmore; M. S. Slossop; D. J. McManus; I. B. Moses and C. F.B. Praquin, *Org. Proc. Res. Develop.* 2010, **14**, 263.

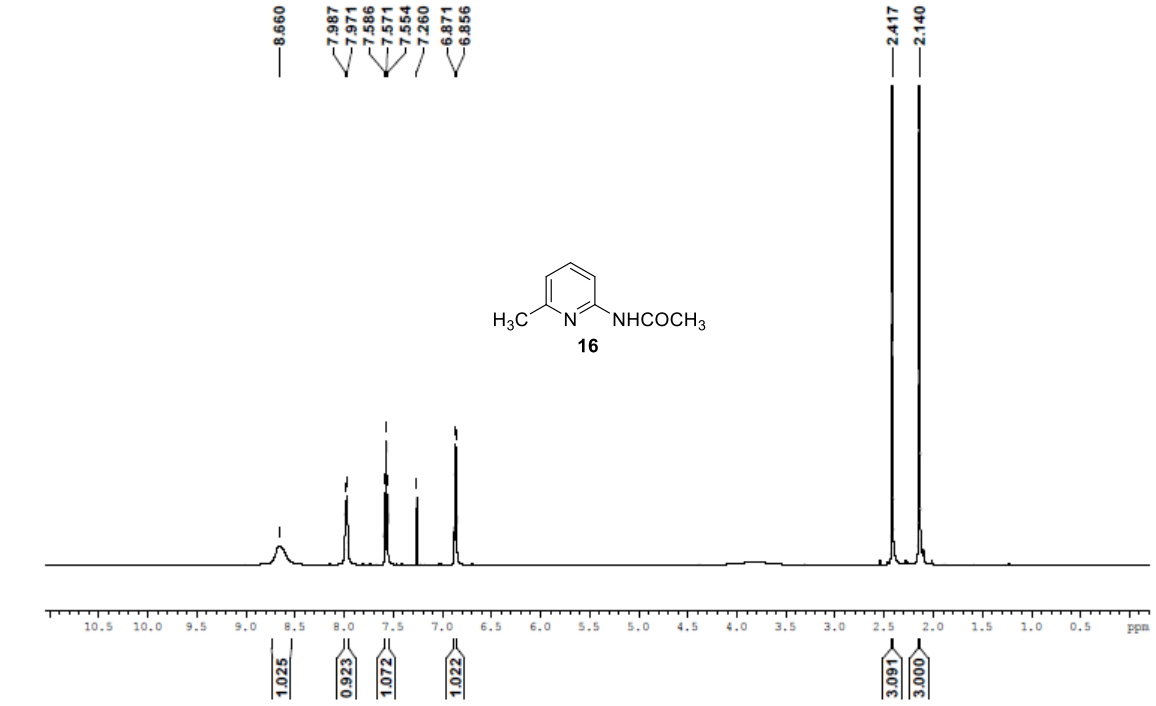
2) B. P. Benke and N. Madhavan, *Chem. Commun.* 2013, **49**, 7340.

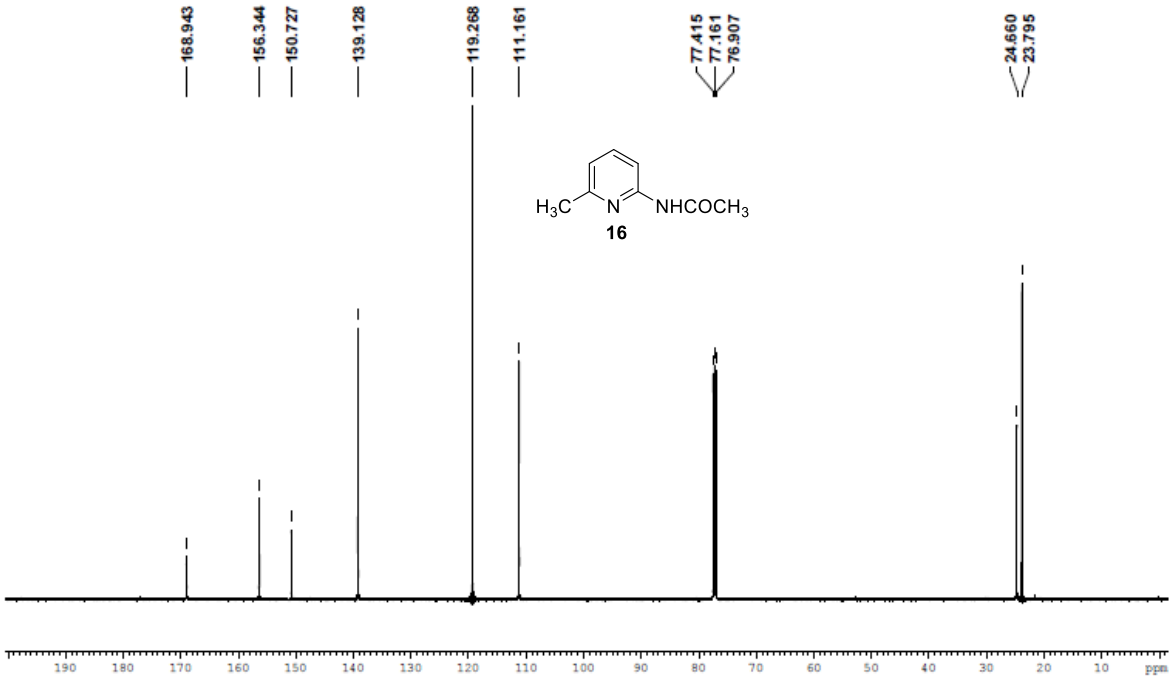
3) Peptide solution was made 3 days before experiment.

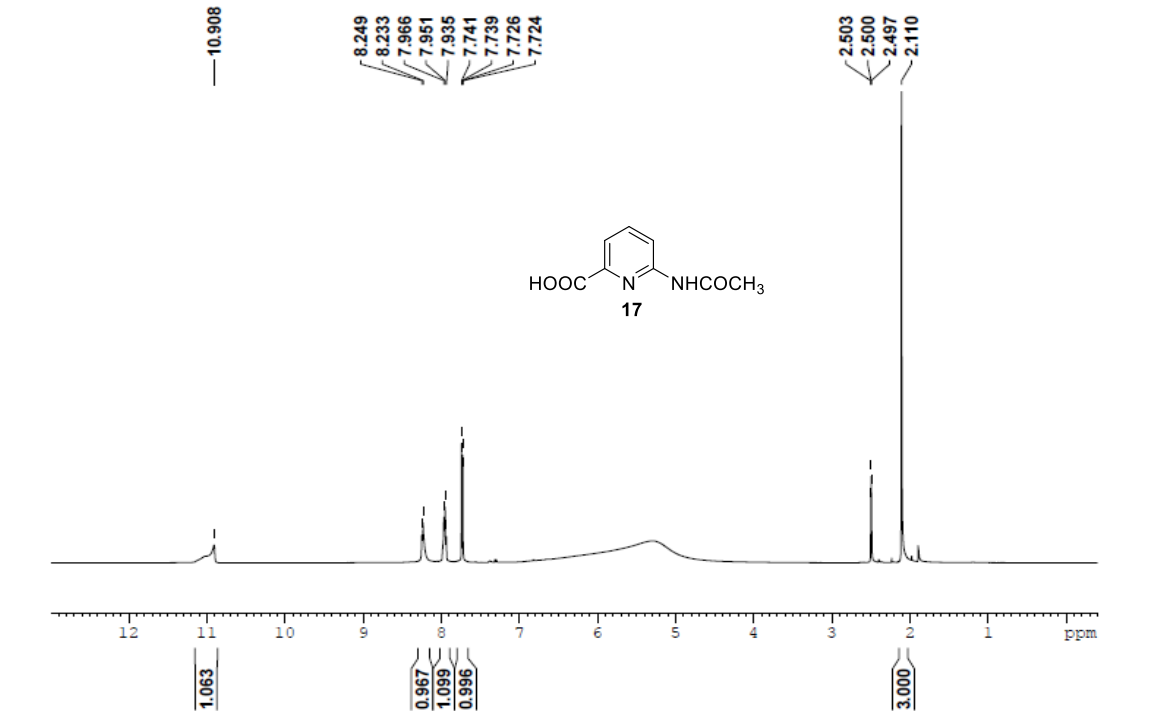
4) B. A. McNally, A. V. Koulov, B. D. Smith, J.-B. Joos and A. P. Davis, *Chem. Commun.*, 2005, 1087.

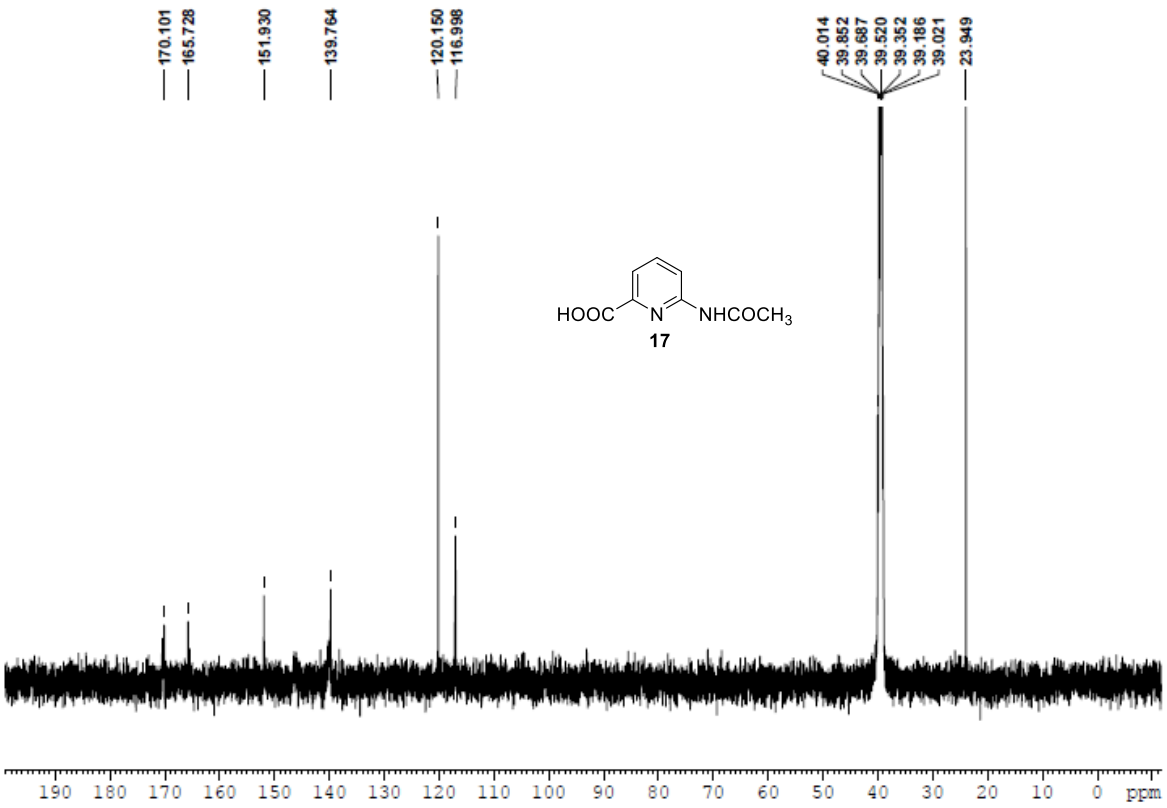
5) N. Madhavan and M. S. Gin, *ChemBioChem.* 2007, **8**, 1834.

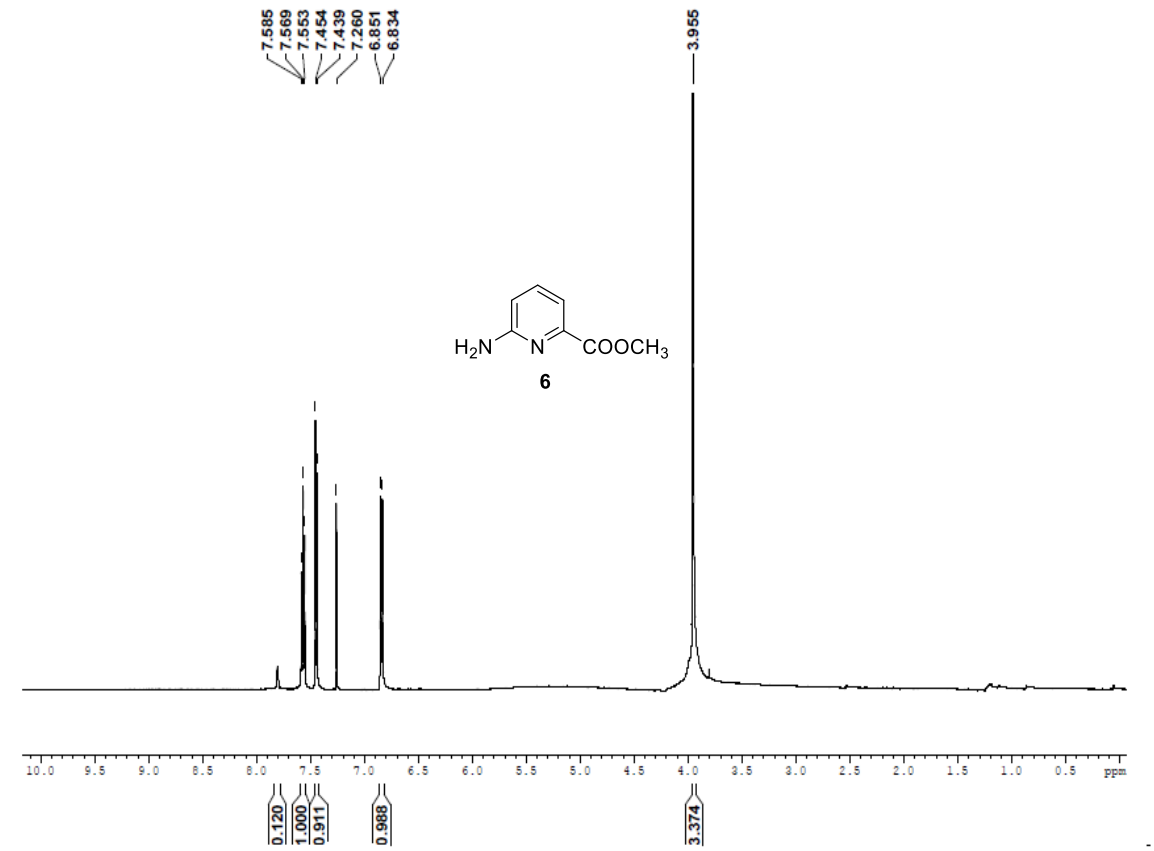
Spectra of compounds

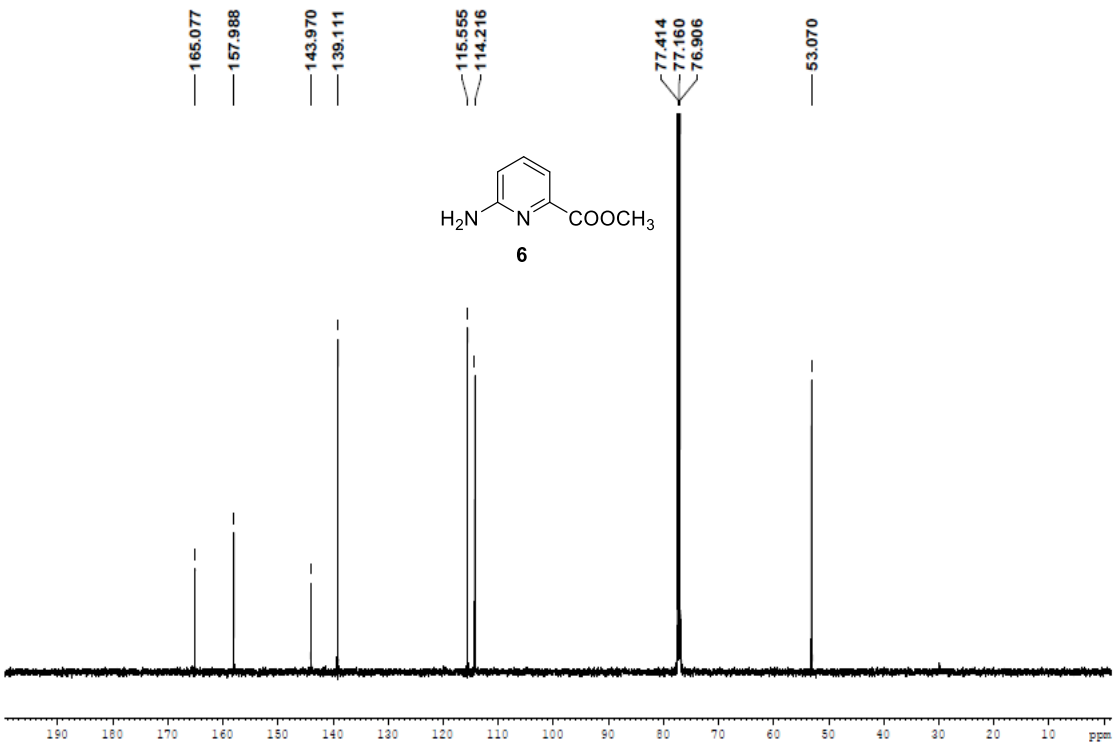


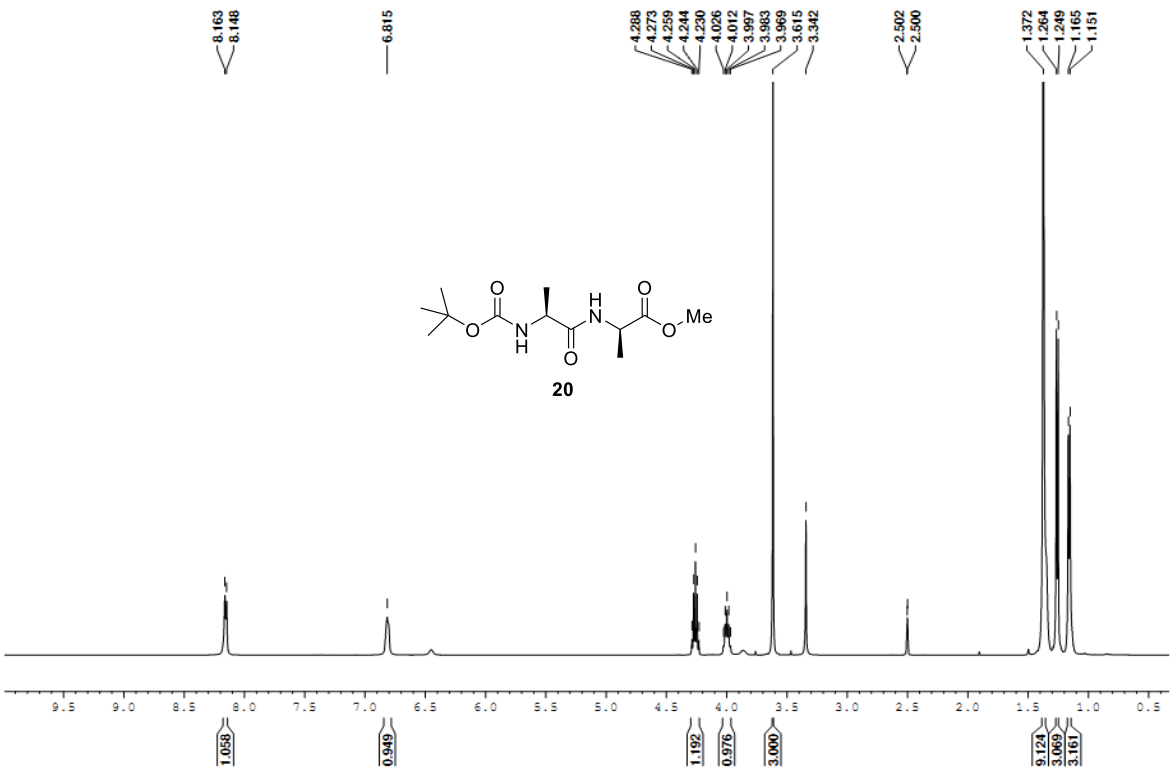


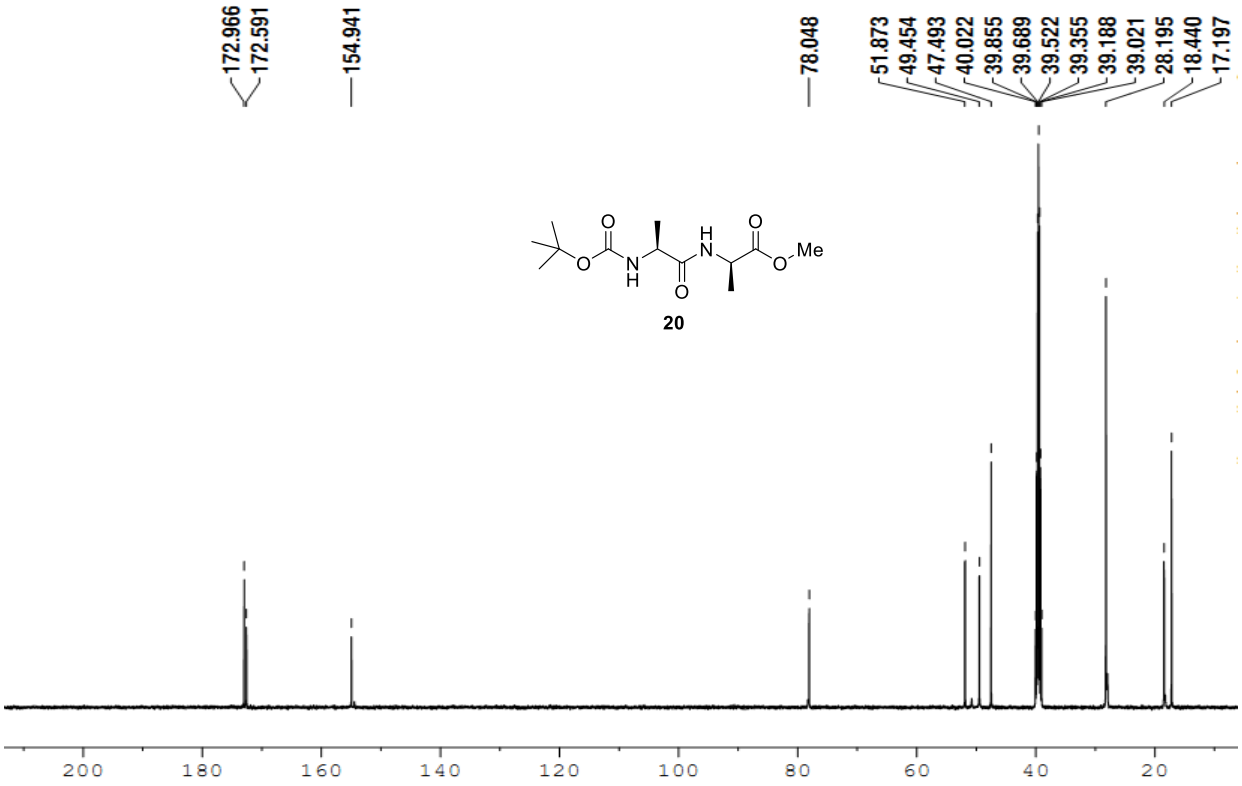

10.908
8.249
8.233
7.966
7.951
7.935
7.741
7.739
7.726
7.724
2.503
2.500
2.497
2.110
HOOC
N
NHCOCH3
17
12
11
10
9
8
7
6
5
4
3
2
1
ppm
1.063
0.967
1.099
0.996
3.000

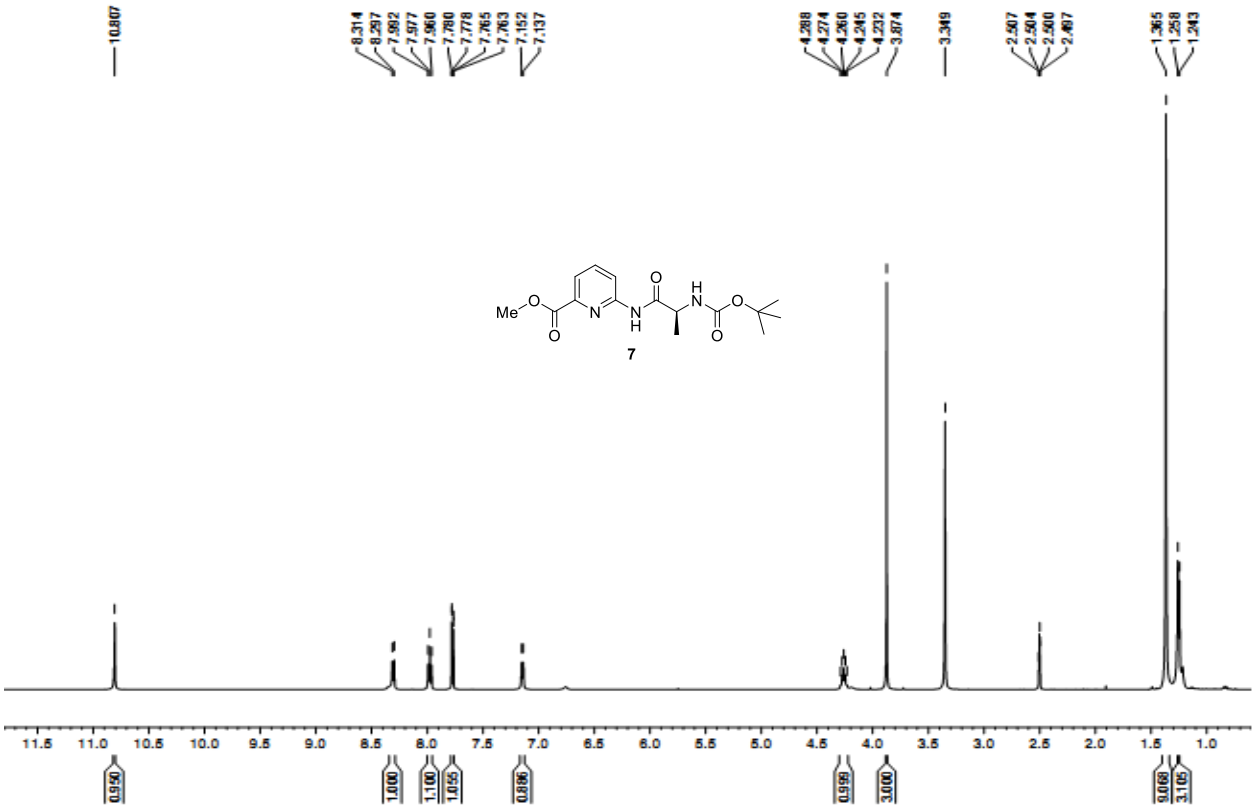

170.101
165.728
151.930
139.764
120.150
116.998
40.014
39.852
39.687
39.520
39.352
39.186
39.021
23.949
HOOC
N
NHCOCH3
17
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

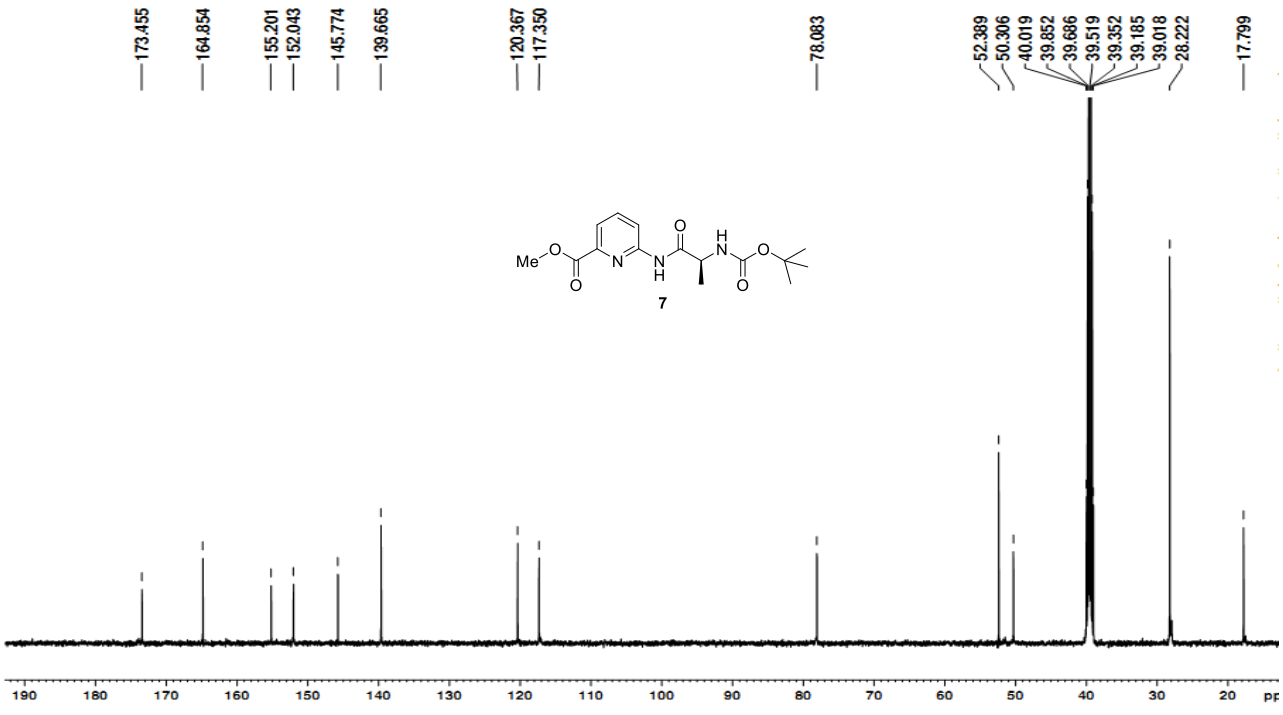

7.585
7.569
7.553
7.454
7.439
7.260
6.851
6.834
3.955
H2N
N
COOCH3
6
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.120
1.000
0.911
0.988
3.374

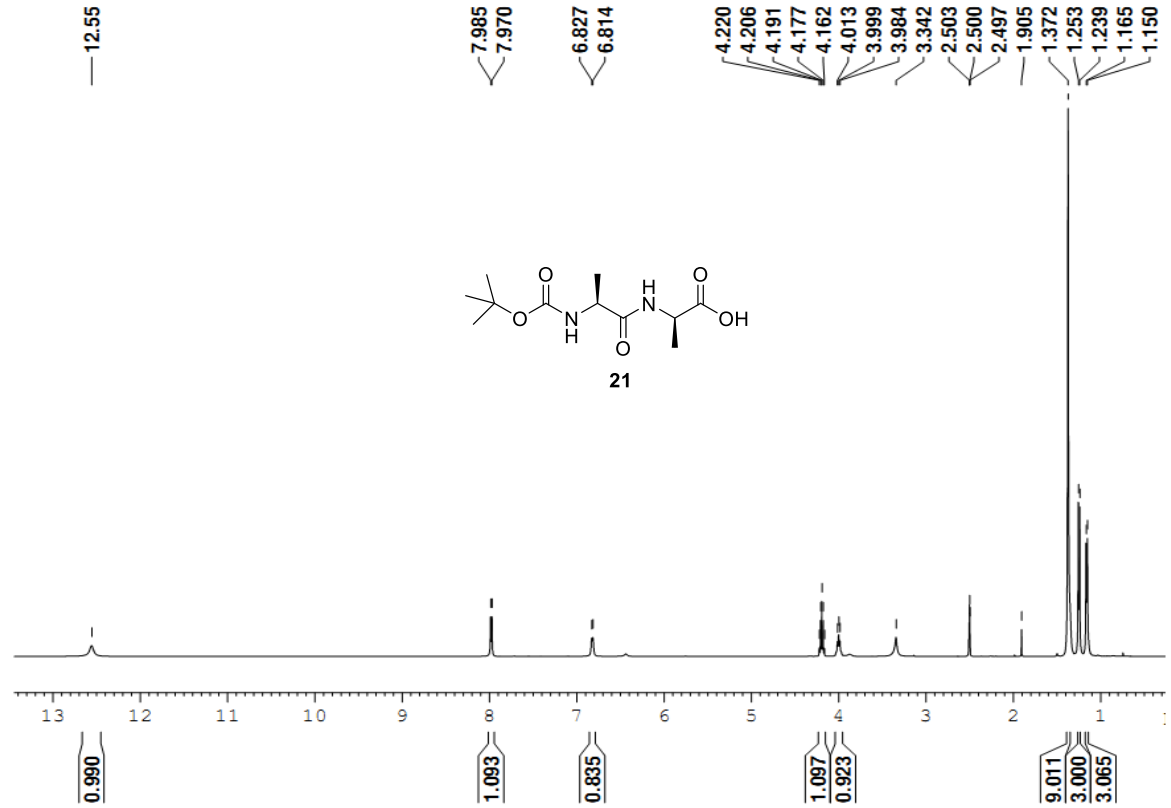

165.077
157.988
143.970
139.111
115.555
114.216
77.414
77.160
76.906
53.070
H2N
N
COOCH3
6
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

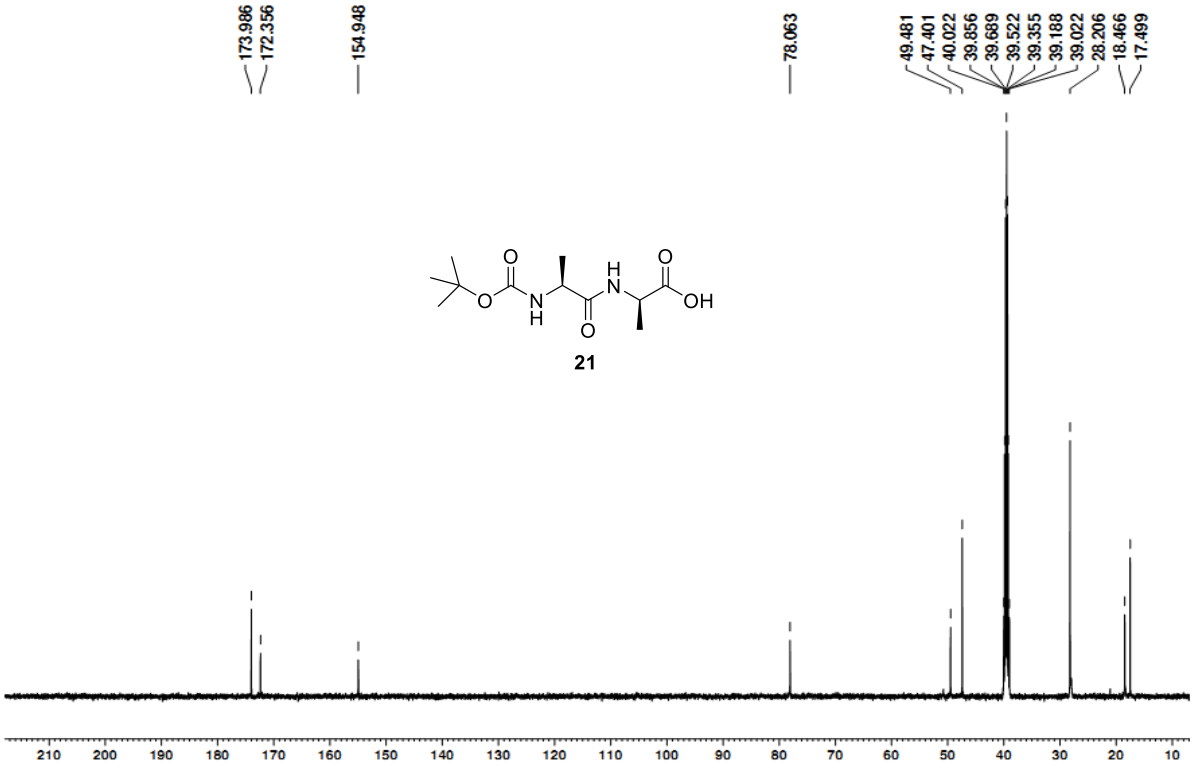

8.163
8.148
6.815
4.288
4.273
4.259
4.244
4.230
4.026
4.012
3.997
3.983
3.969
3.615
3.342
2.502
2.500
1.372
1.264
1.249
1.165
1.151
Me
20
1.058
0.949
1.192
0.976
3.000
9.124
3.069
3.161

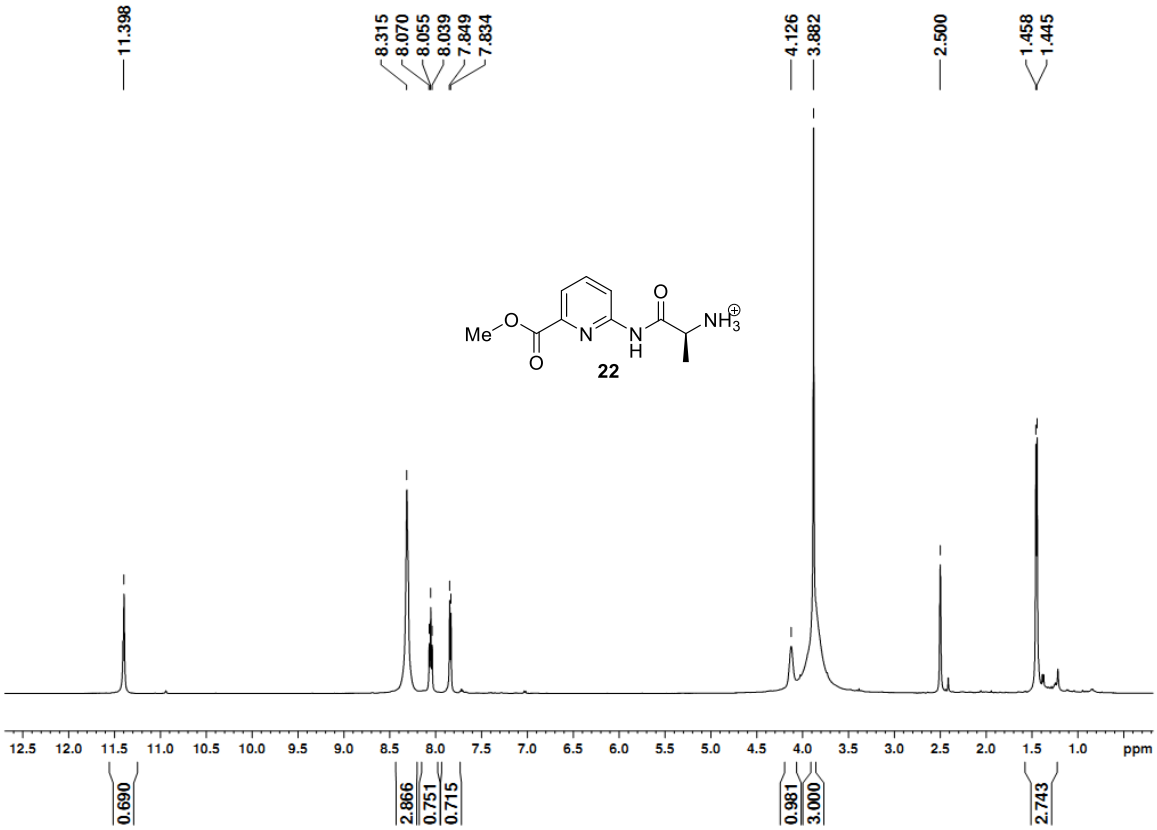

172.966
172.591
154.941
78.048
51.873
49.454
47.493
40.022
39.855
39.689
39.522
39.355
39.188
39.021
28.195
18.440
17.197
Me
20

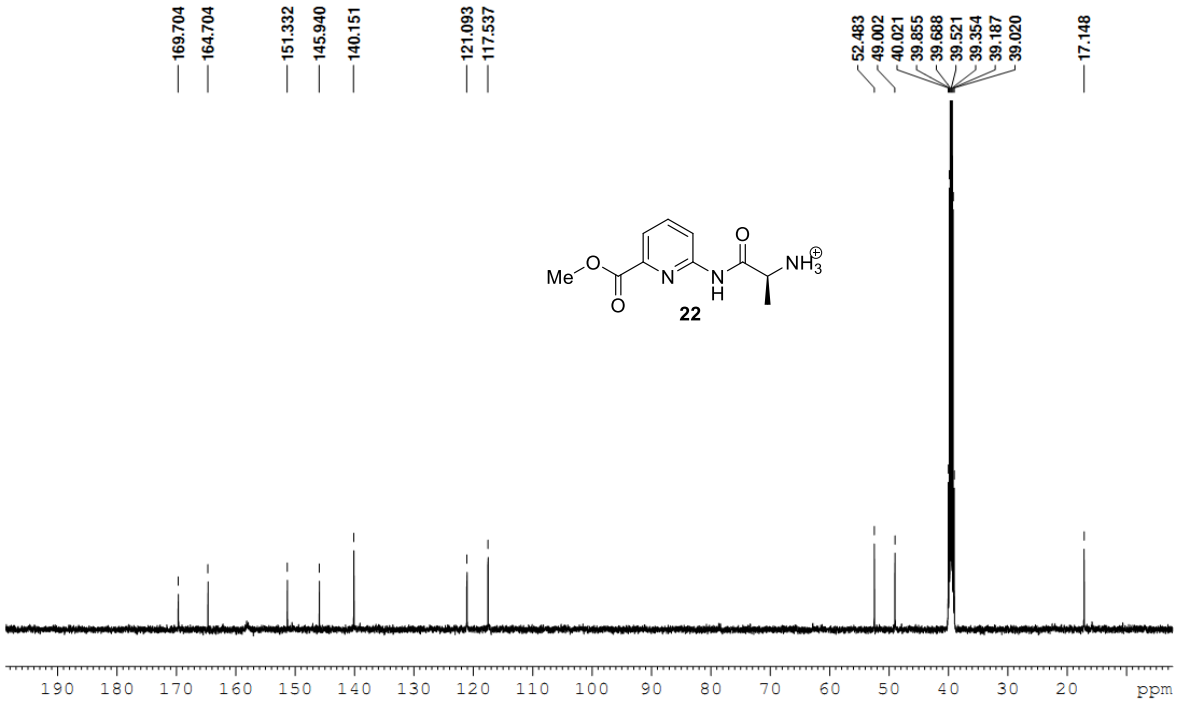

10.807
8.314
8.297
7.992
7.977
7.960
7.780
7.778
7.765
7.763
7.152
7.137
4.288
4.274
4.260
4.245
4.232
3.874
3.349
2.507
2.504
2.500
2.497
1.365
1.258
1.243
Me
O
O
N
H
N
H
O
O
O
7
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.950
1.000
1.100
1.055
0.886
0.999
3.000
9.068
3.105

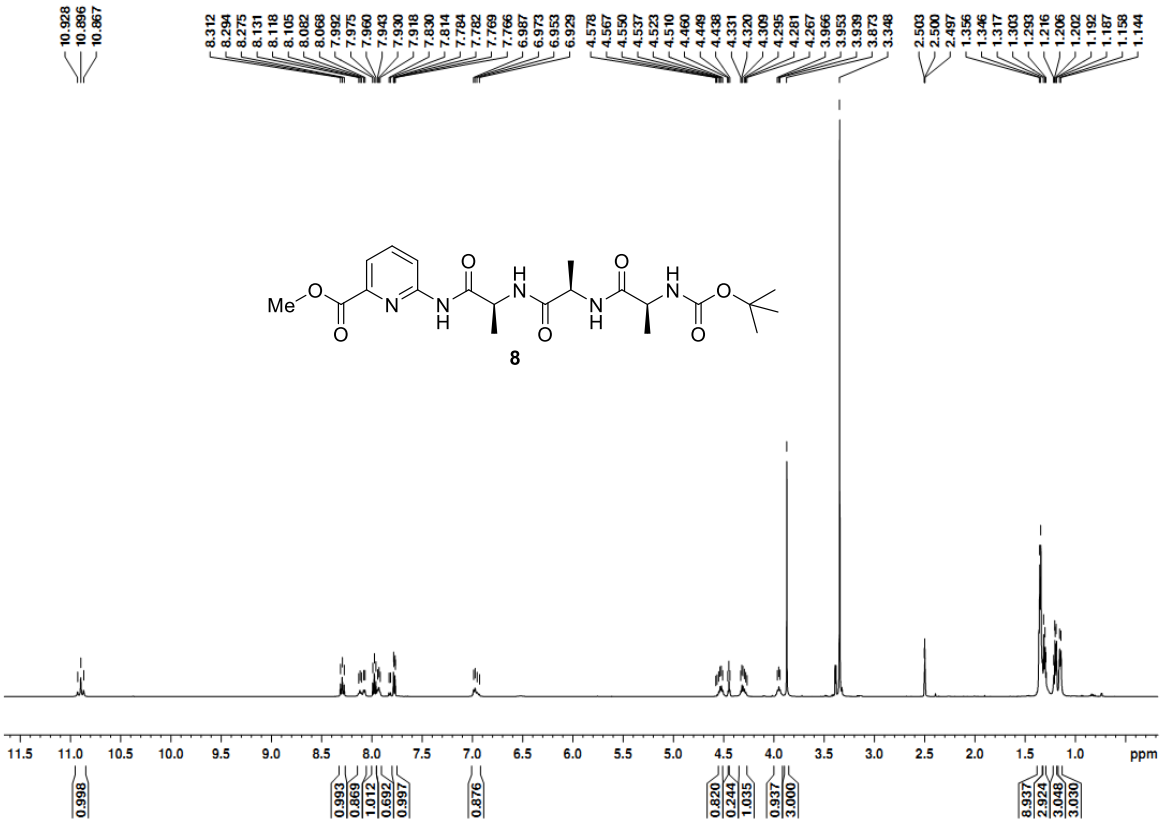

173.455
164.854
155.201
152.043
145.774
139.665
120.367
117.350
78.083
52.389
50.306
40.019
39.852
39.686
39.519
39.352
39.185
39.018
28.222
17.799
Me
O
O
N
H
N
H
O
O
O
7
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

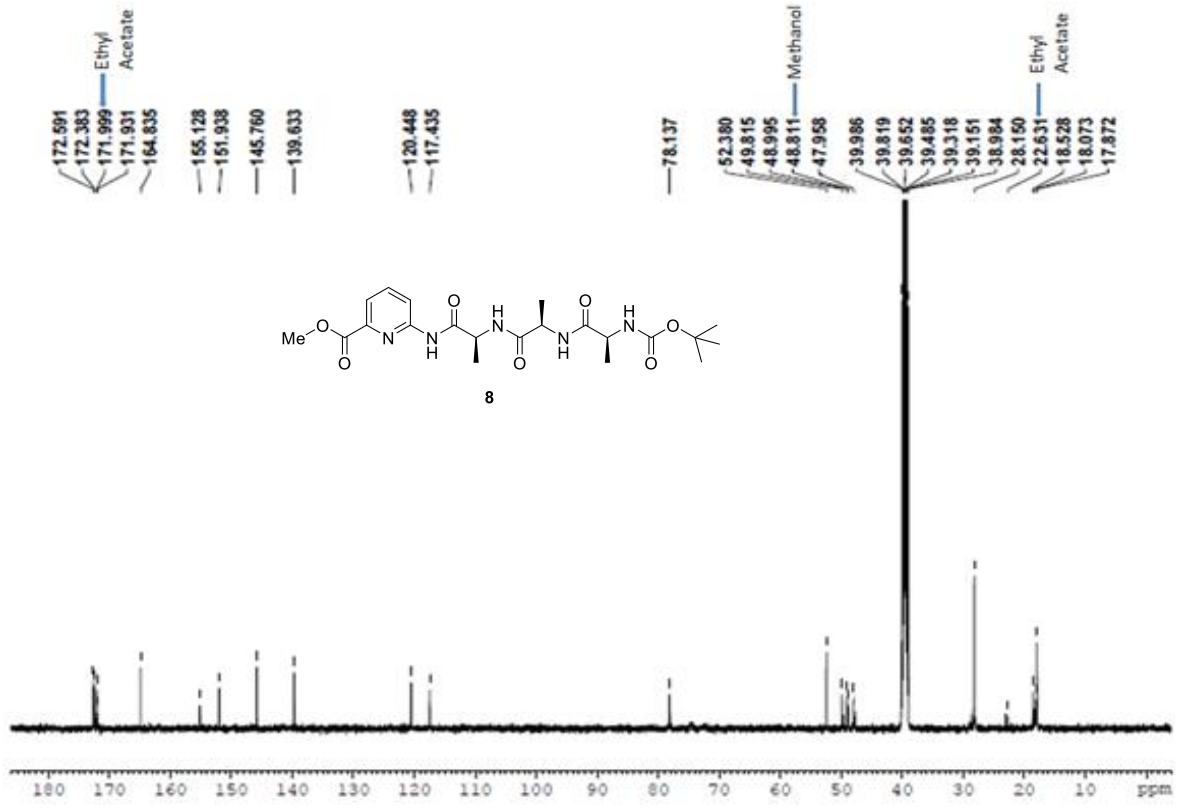

12.55
7.985
7.970
6.827
6.814
4.220
4.206
4.191
4.177
4.162
4.013
3.999
3.984
3.342
2.503
2.500
2.497
1.905
1.372
1.253
1.239
1.165
1.150
21
0.990
1.093
0.835
1.097
0.923
9.011
3.000
3.065

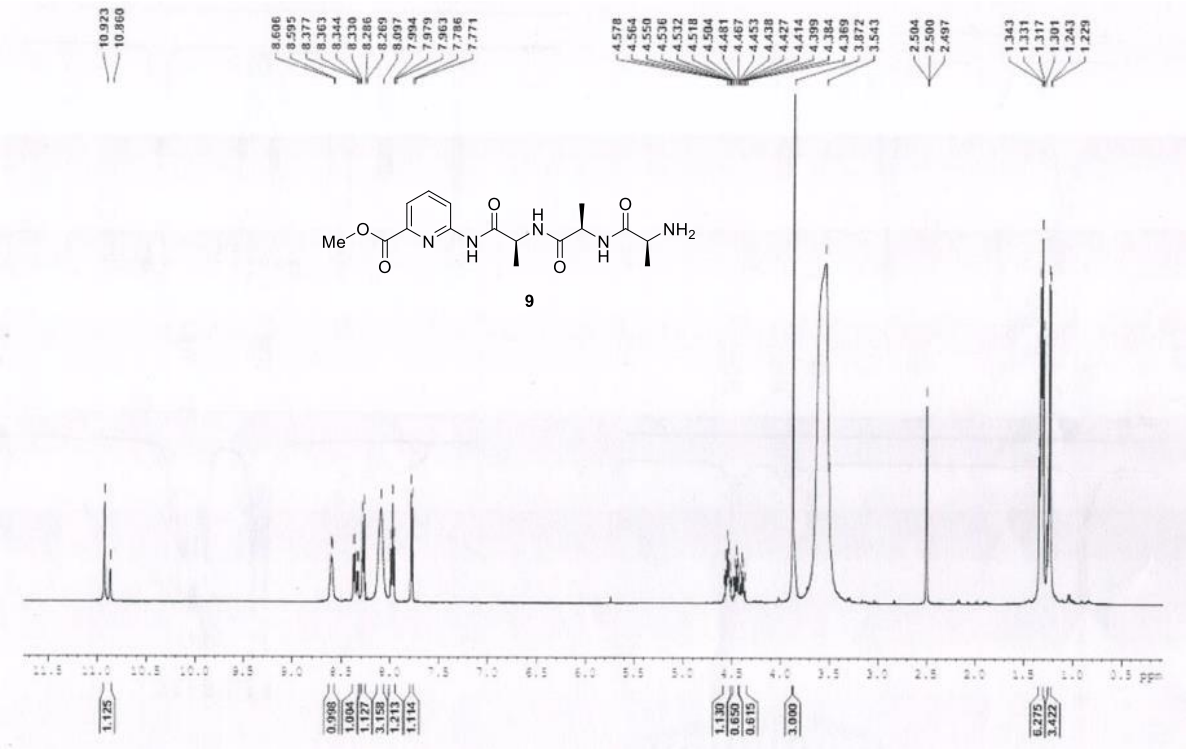

173.986
172.356
154.948
78.063
49.481
47.401
40.022
39.856
39.689
39.522
39.355
39.188
39.022
28.206
18.466
17.499
21

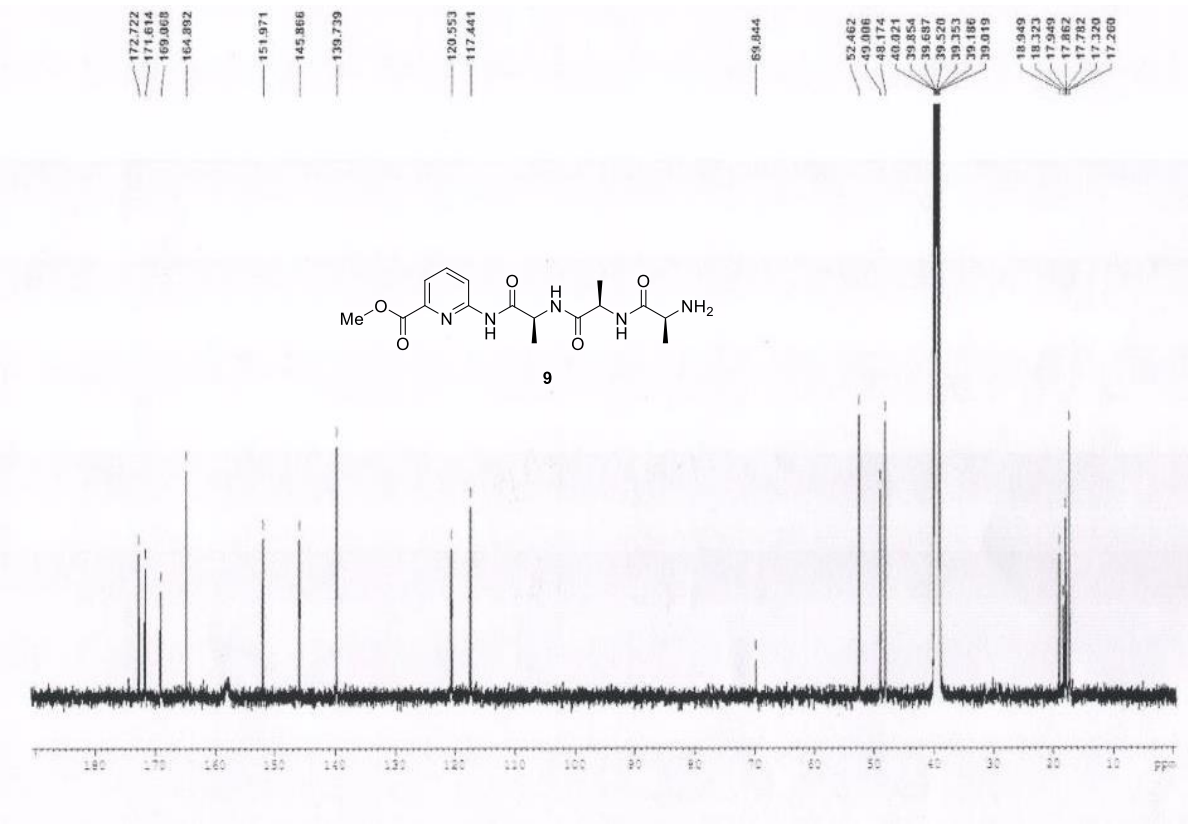

11.398
8.315
8.070
8.055
8.039
7.849
7.834
4.126
3.882
2.500
1.458
1.445
Me
O
N
H
NH3
22
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
0.690
2.866
0.751
0.715
0.981
3.000
2.743

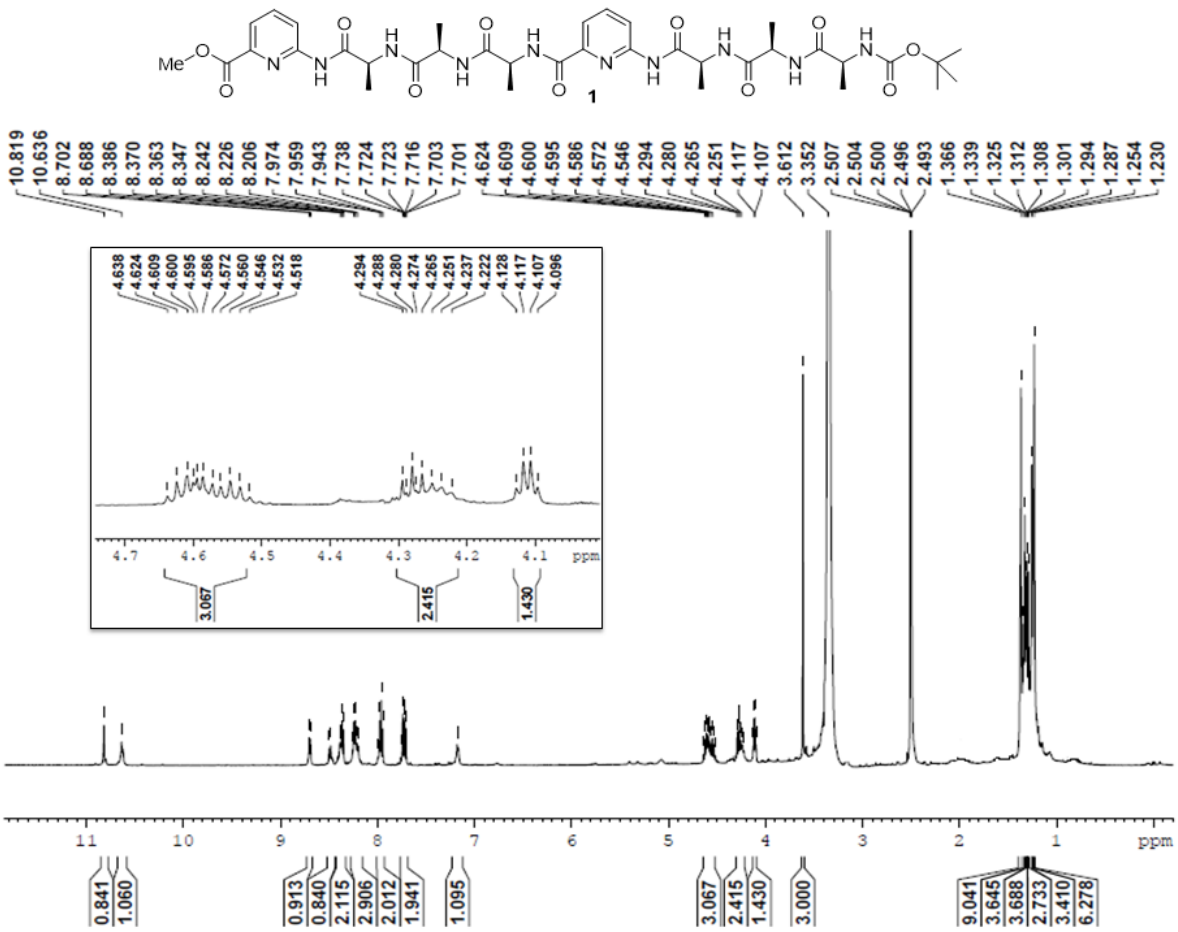

169.704
164.704
151.332
145.940
140.151
121.093
117.537
52.483
49.002
40.021
39.855
39.688
39.521
39.354
39.187
39.020
17.148
Me
O
N
H
NH3
22
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm


10.928
10.896
10.867
8.312
8.294
8.275
8.131
8.118
8.105
8.082
8.068
7.992
7.975
7.960
7.943
7.930
7.918
7.830
7.814
7.784
7.782
7.769
7.766
6.987
6.973
6.953
6.929
4.578
4.567
4.550
4.537
4.523
4.510
4.460
4.449
4.438
4.331
4.320
4.309
4.295
4.281
4.267
3.966
3.953
3.939
3.873
3.348
2.503
2.500
2.497
1.356
1.346
1.317
1.303
1.293
1.216
1.206
1.202
1.192
1.187
1.158
1.144
Me
O
N
H
8
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.998
0.993
0.869
1.012
0.692
0.997
0.876
0.820
0.244
1.035
0.937
3.000
8.937
2.924
3.048
3.030

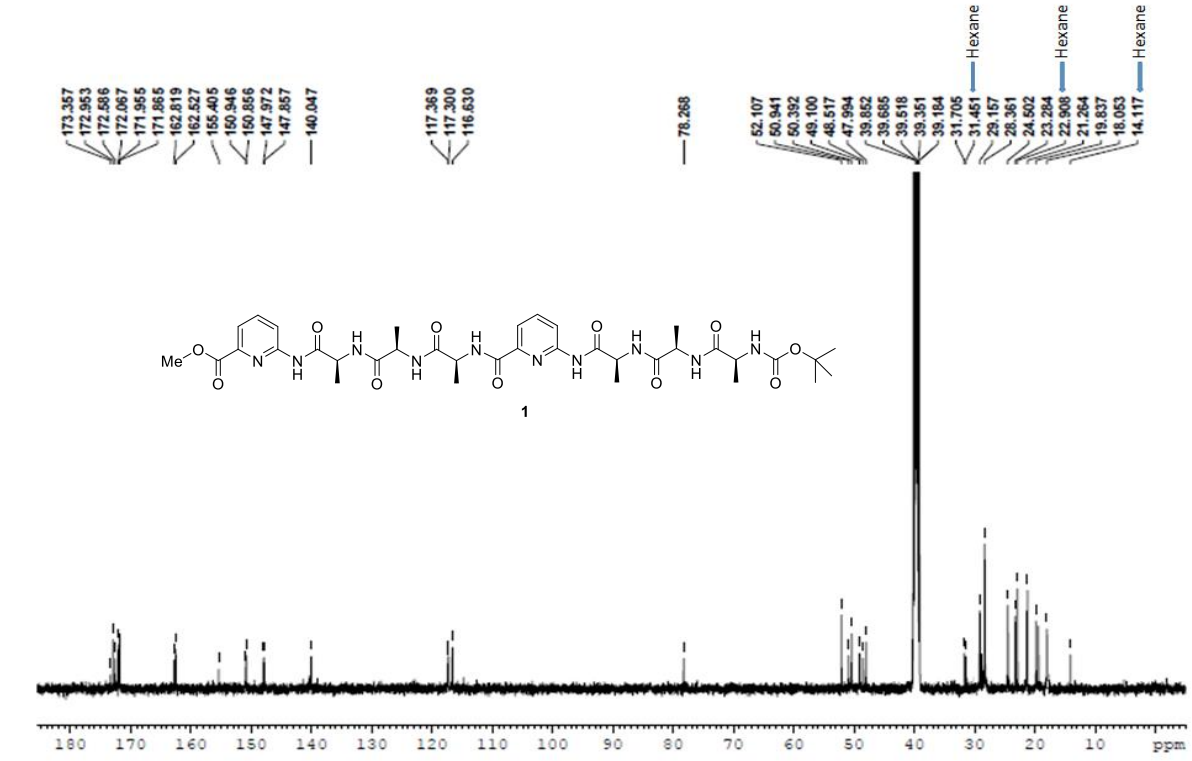

Ethyl Acetate
Methanol
Ethyl Acetate
172.591
172.383
171.999
171.931
164.835
155.128
151.938
145.760
139.633
120.448
117.435
78.137
52.380
49.815
48.995
48.811
47.958
39.986
39.819
39.652
39.485
39.318
39.151
38.984
28.150
22.631
18.528
18.073
17.872
Me
O
N
H
8
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

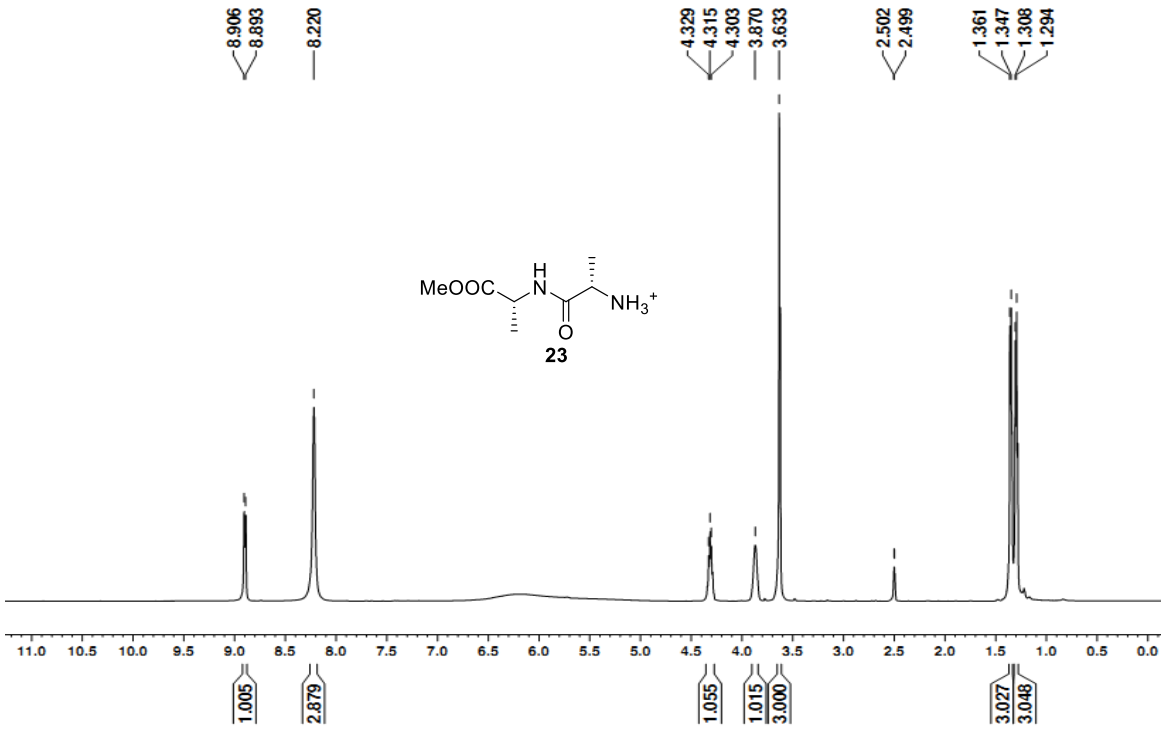

10.923
10.860
8.606
8.595
8.377
8.363
8.344
8.330
8.286
8.269
8.097
7.994
7.979
7.963
7.786
7.771
4.578
4.564
4.550
4.536
4.532
4.518
4.504
4.481
4.467
4.453
4.438
4.427
4.414
4.399
4.384
4.369
3.872
3.543
2.504
2.500
2.497
1.343
1.331
1.317
1.301
1.243
1.229
Me
O
N
H
NH2
9
1.125
0.998
1.004
1.127
3.158
1.211
1.114
1.130
0.650
0.615
3.000
6.275
3.422
ppm

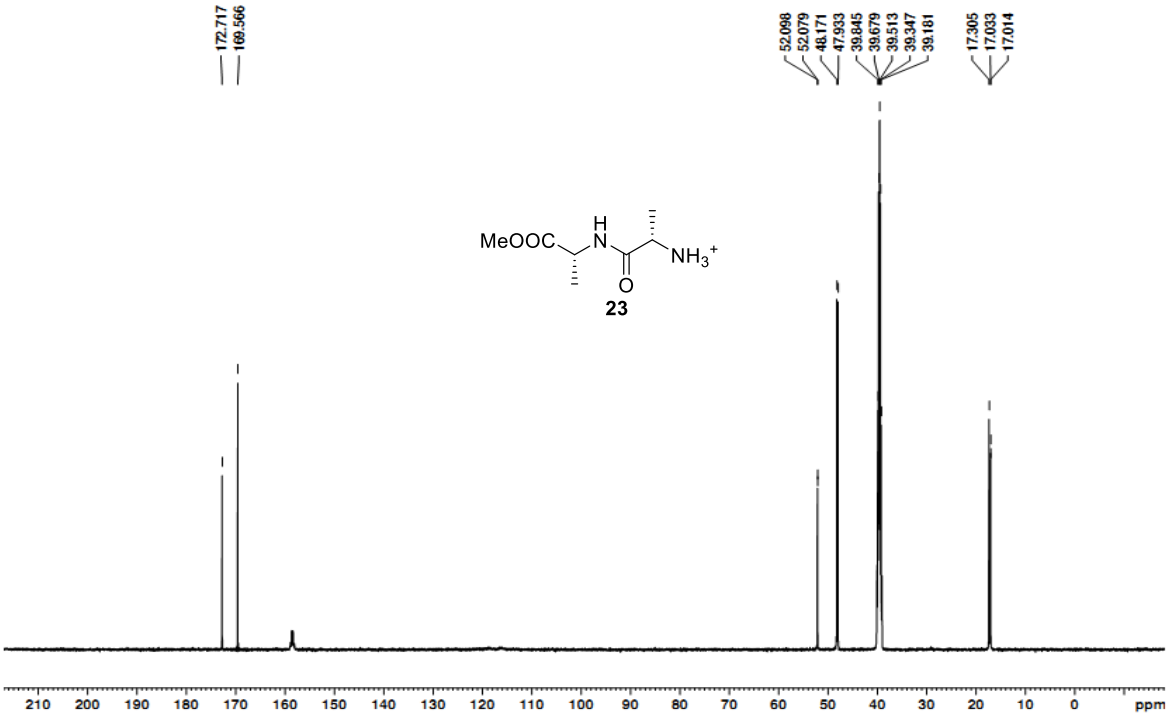

172.722
171.614
169.068
164.892
151.971
145.866
139.739
120.553
117.441
69.844
52.462
49.006
48.174
40.021
39.854
39.687
39.520
39.353
39.186
39.019
18.949
18.323
17.949
17.862
17.782
17.320
17.260
Me
O
N
H
NH2
9
ppm

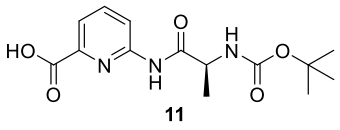


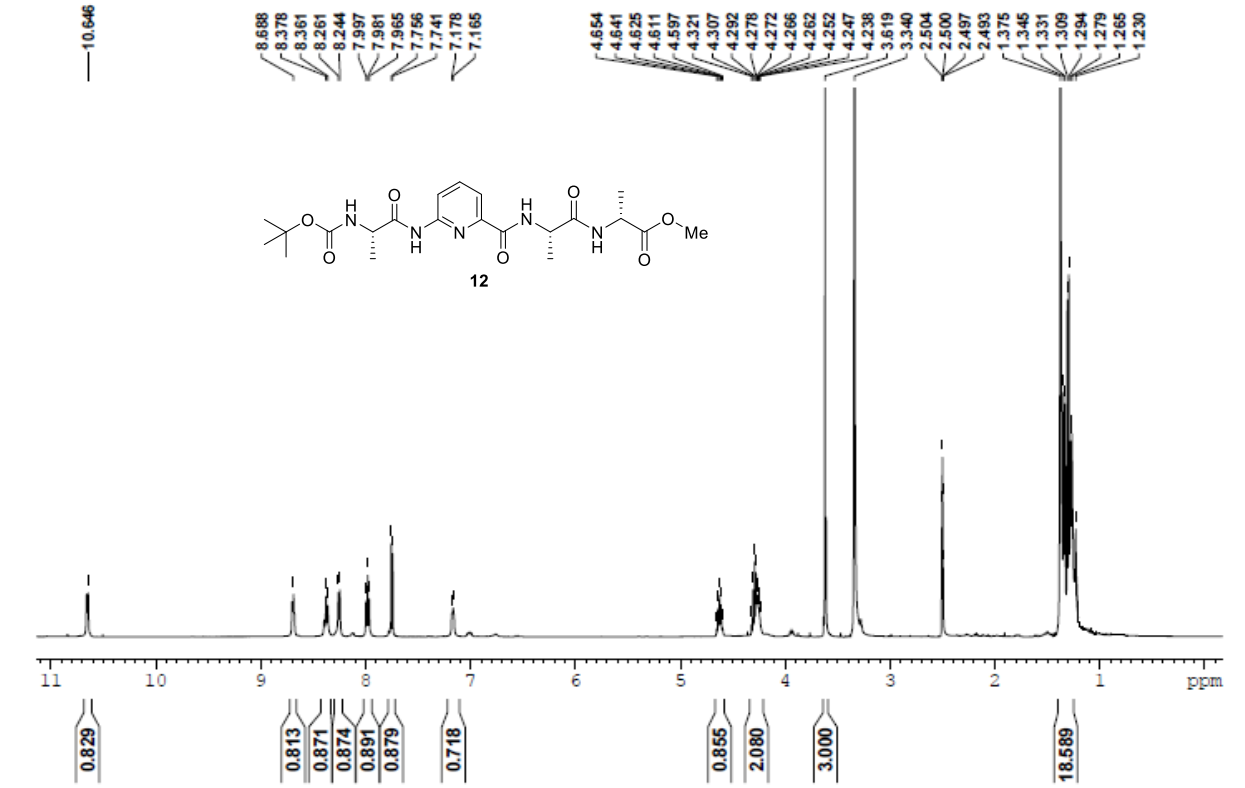
1

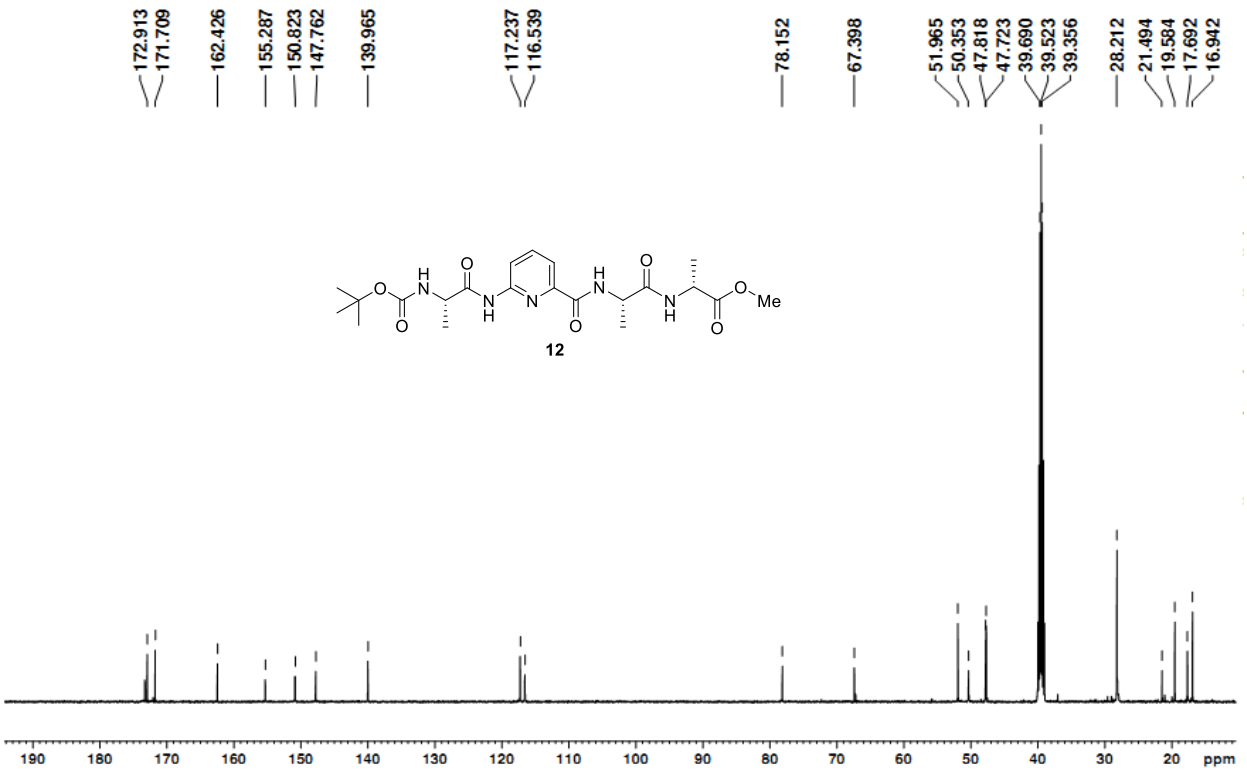

Hexane
Hexane
Hexane
Me
1

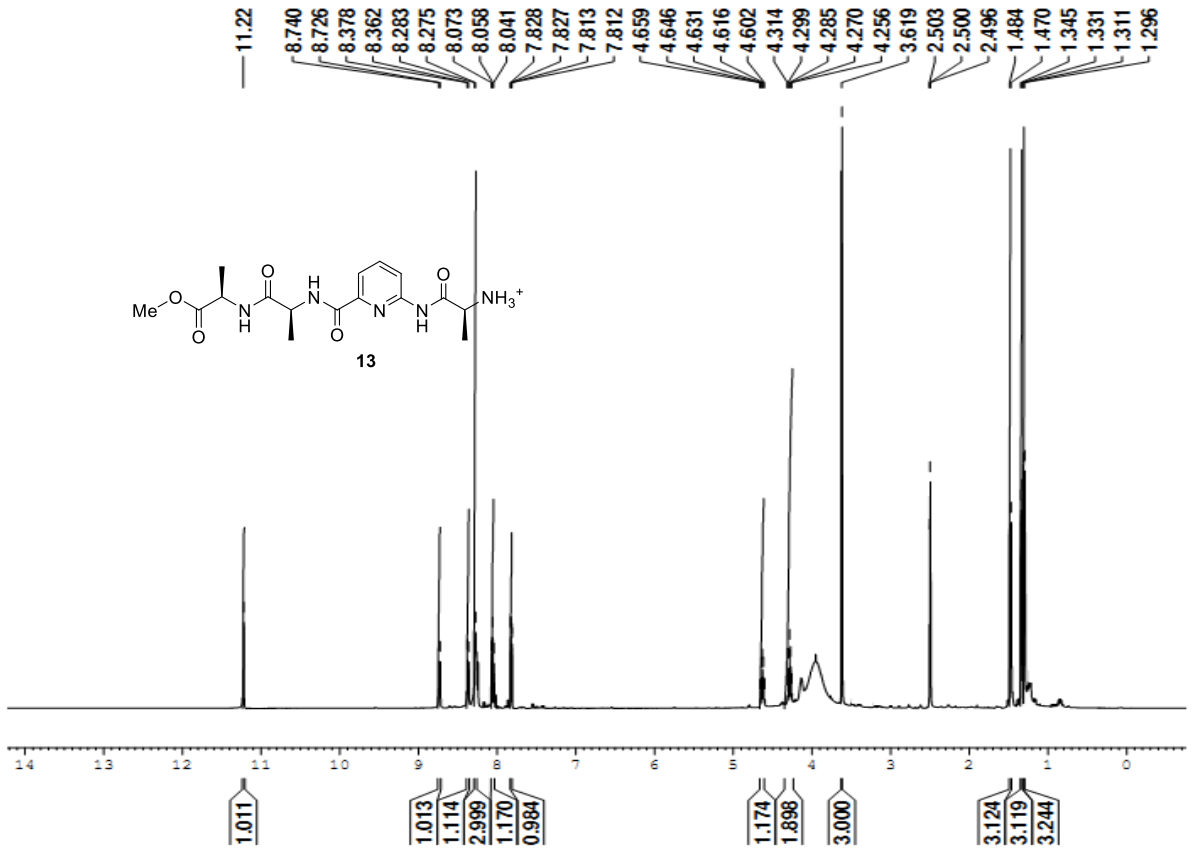

MeOOC
NH3+
23

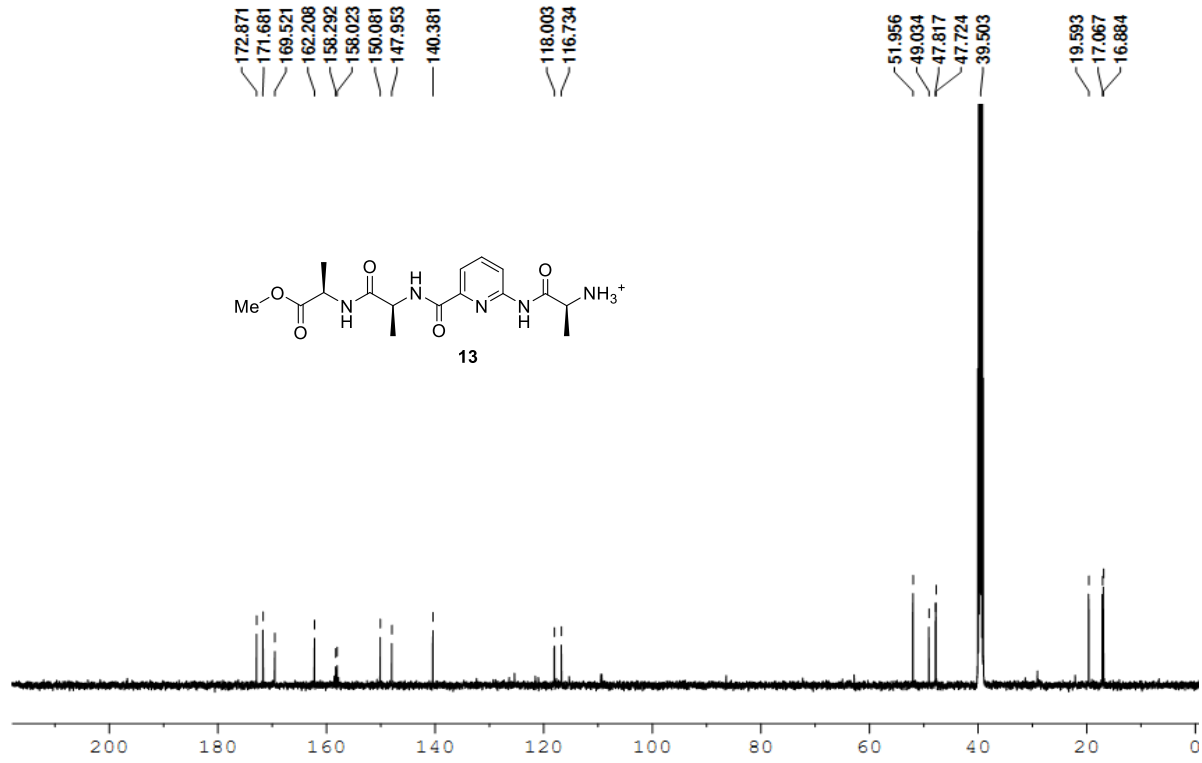

172.717
169.566
52.098
52.079
48.171
47.933
39.845
39.679
39.513
39.347
39.181
17.305
17.033
17.014
MeOOC
H
N
O
NH3+
23
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

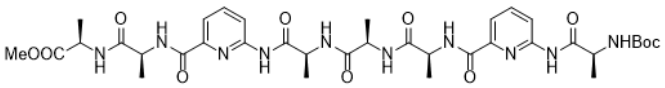

HO
O
N
N
H
O
H
N
O
O
11


10.646
8.688
8.378
8.361
8.261
8.244
7.997
7.981
7.965
7.756
7.741
7.178
7.165
4.654
4.641
4.625
4.611
4.597
4.321
4.307
4.292
4.278
4.272
4.266
4.262
4.252
4.247
4.238
3.619
3.340
2.504
2.500
2.497
2.493
1.375
1.345
1.331
1.309
1.294
1.279
1.265
1.230
Me
12
11
10
9
8
7
6
5
4
3
2
1
ppm
0.829
0.813
0.871
0.874
0.891
0.879
0.718
0.855
2.080
3.000
18.589

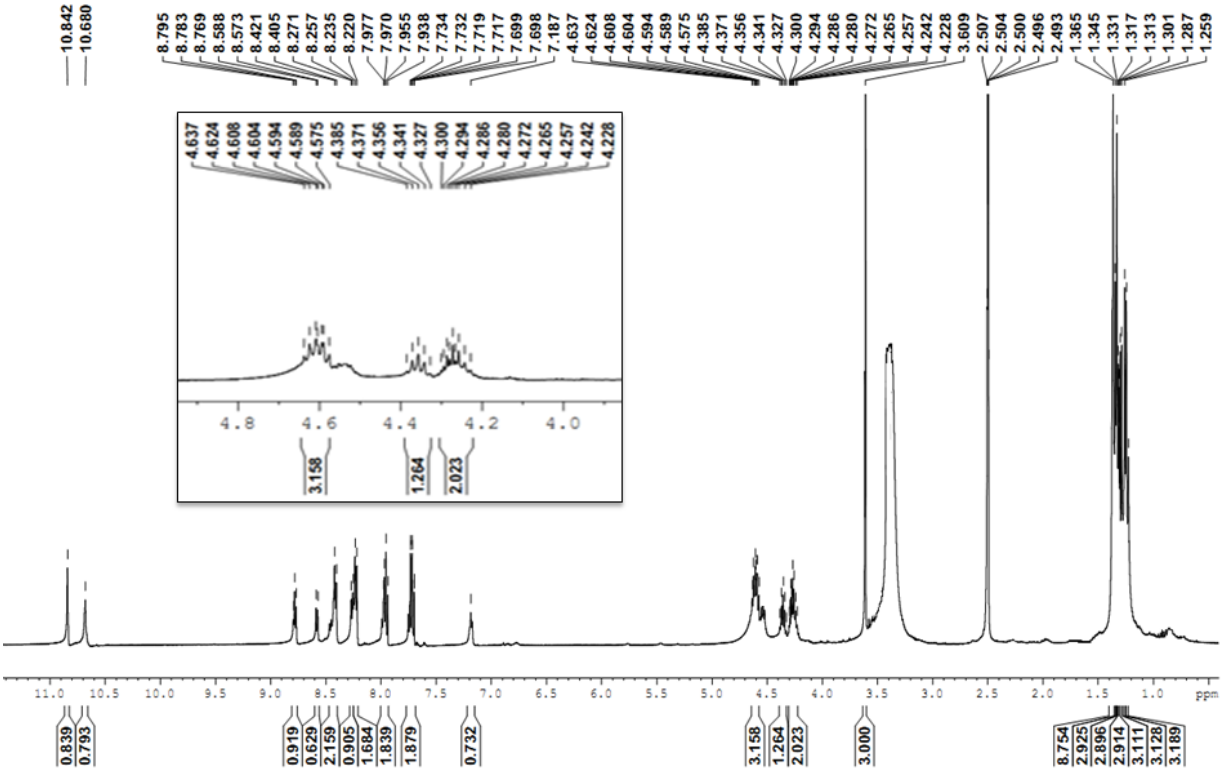

172.913
171.709
162.426
155.287
150.823
147.762
139.965
117.237
116.539
78.152
67.398
51.965
50.353
47.818
47.723
39.690
39.523
39.356
28.212
21.494
19.584
17.692
16.942
Me
12
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

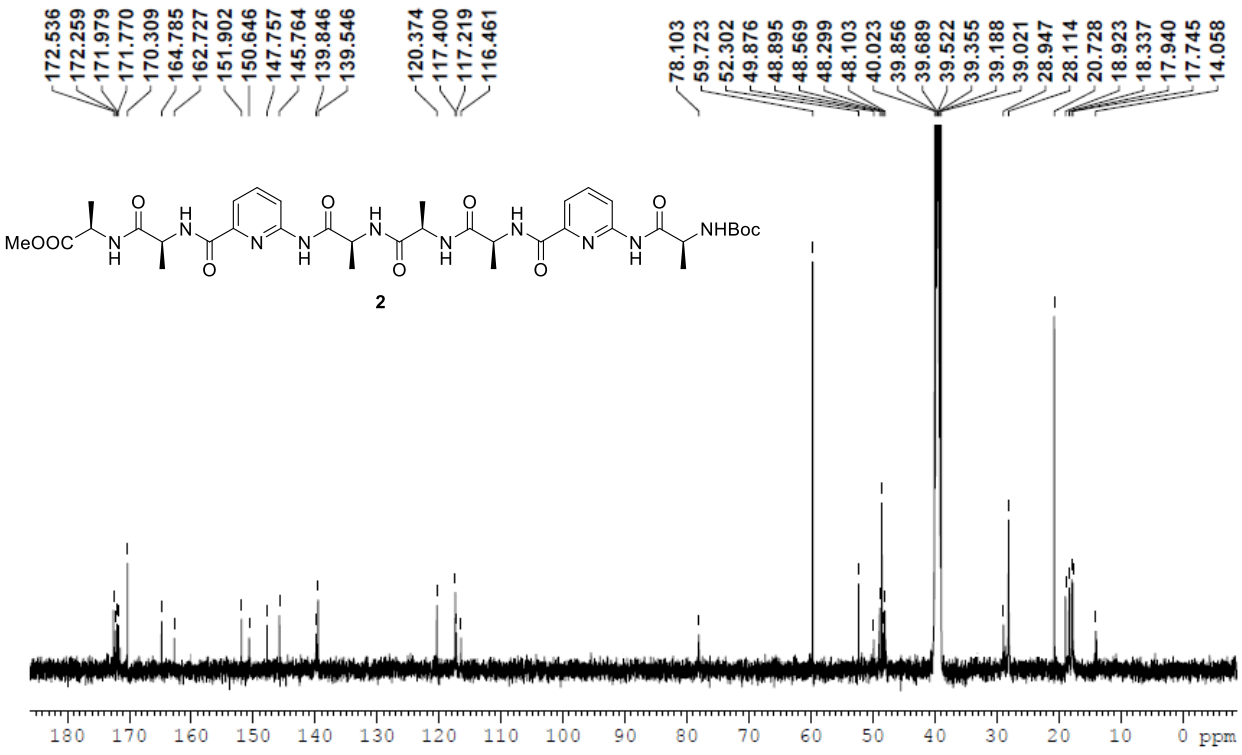

11.22
8.740
8.726
8.378
8.362
8.283
8.275
8.073
8.058
8.041
7.828
7.827
7.813
7.812
4.659
4.646
4.631
4.616
4.602
4.314
4.299
4.285
4.270
4.256
3.619
2.503
2.500
2.496
1.484
1.470
1.345
1.331
1.311
1.296
Me
NH3+
13
1.011
1.013
1.114
2.999
1.170
0.984
1.174
1.898
3.000
3.124
3.119
3.244


172.871
171.681
169.521
162.208
158.292
158.023
150.081
147.953
140.381
118.003
116.734
51.956
49.034
47.817
47.724
39.503
19.593
17.067
16.884
Me
NH3+
13


MeOOC
NHBoc

2


10.842
10.680
8.795
8.783
8.769
8.588
8.573
8.421
8.405
8.271
8.257
8.235
8.220
7.977
7.970
7.955
7.938
7.734
7.732
7.719
7.717
7.699
7.698
7.187
4.637
4.624
4.608
4.604
4.594
4.589
4.575
4.385
4.371
4.356
4.341
4.327
4.300
4.294
4.286
4.280
4.272
4.265
4.257
4.242
4.228
3.609
2.507
2.504
2.500
2.496
2.493
1.365
1.345
1.331
1.317
1.313
1.301
1.287
1.259
3.158
1.264
2.023
ppm
0.839
0.793
0.919
0.629
2.159
0.905
1.684
1.839
1.879
0.732
3.158
1.264
2.023
3.000
8.754
2.925
2.896
2.914
3.111
3.128
3.189


172.536
172.259
171.979
171.770
170.309
164.785
162.727
151.902
150.646
147.757
145.764
139.846
139.546
120.374
117.400
117.219
116.461
78.103
59.723
52.302
49.876
48.895
48.569
48.299
48.103
40.023
39.856
39.689
39.522
39.355
39.188
39.021
28.947
28.114
20.728
18.923
18.337
17.940
17.745
14.058
MeOOC
NHBoc
2
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm