Supporting information

Simple Acyclic Dipeptides with Aromatic Units as Anion Carriers

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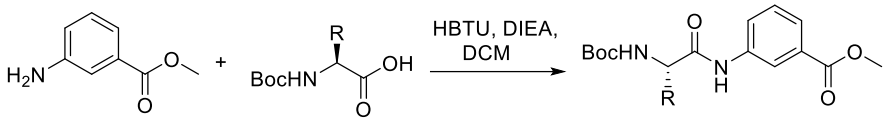
General methods

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 and methanol was distilled over magnesium oxide. All dry solvents were stored over 4 Å molecular sieves prior to use. All peptides were synthesized in solution using HBTU (2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethylisouronium) as a coupling reagent and *N*,*N*-diisopropylethylamine (DIEA) as base. Trisodium salt (HPTS) and *N*,*N*'-dimethyl-9,9'-biacridinium dinitrate (Lucigenin) were purchased from Sigma-Aldrich and used without further purification. Egg yolk phosphatidylcholine (EYPC) was purchased from Avanti Polar Lipids. Analytical thin layer chromatography (TLC) was performed on MERCK pre-coated silica gel 60 F254TLC plates. Eluting solvents are reported as volume percents. Compounds were visualized using UV light and ninhydrin. Flash column chromatography was performed using silica gel. 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), 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 as a thin film. IR spectra peaks are reported in wave numbers (cm^{-1}) as strong (s) and broad (b). Vesicles were prepared from EYPC (DPPC wherever stated) lipids using the mini-extruder set from Avanti polar lipids. MilliQ 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 with the respective base MOH (M = Li, Na, K, Cs) solution. The pH was checked with L1613 pH meter from Vanira Instruments. Vesicles were prepared in an aqueous solution of $NaNO_3$ (225 mM) for lucigenin assays. Extrusion of vesicles was carried out using the mini-extruder set from Avanti Polar Lipid through 0.1 μm and 0.2 μm polycarbonate membranes (Whatman) for EYPC and DPPC assays, respectively. Size exclusion chromatography was carried out using Sephadex (G-50) resin. Fluorescence spectra were recorded on a Horiba Fluoromax-4 fluorescence spectrofluorometer with stirring

capability and an injection port. The studies were carried out in 3 mL quartz cuvettes. All graphs were plotted and fitted using Origin 2019b 64Bit software.

General procedure for synthesis of dipeptides 4 & 5



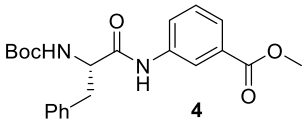
1

2; R = CH2Ph
3; R = Ph

4; R = CH_2Ph; 65%
5; R = Ph; 80%

To a solution of methyl *m*-amino benzoate ester **1**[1] (2.6 mmol, 1 equiv) and boc protected amino acid i.e. phenylalanine **2**[2] or phenylglycine **3**[3] (2.6 mmol, 1 equiv) at 0 °C in DCM was added. HBTU (3.12 mmol, 1.2 equiv) and DIEA (7.8 mmol, 3 equiv). The reaction was allowed to stir at room temperature for 7 h. After the completion of the reaction as monitored by TLC, the mixture was concentrated *in vacuo* and extracted with ethyl acetate. The organic layer was collected and washed with 5% HCl and saturated sodium bicarbonate solution. The organic layer was dried over sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography to afford dipeptide.

Dipeptide 4:

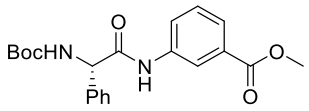


4

The general procedure above was followed and methyl *m*-amino benzoate ester **1**[1] (0.67 g), boc protected phenylalanine **2**[2] (0.7 g), HBTU (1.18 g), DIEA (1.01 g) and DCM (25 mL) were used. The reaction mixture after removal of solvents was extracted with ethyl acetate (2 x 20 mL), washed with 5% HCl (2 x 20 mL) and aqueous saturated sodium bicarbonate (2 x 20 mL). Purification by flash column chromatography (10-25% ethyl acetate/pet. ether) afforded 0.68 g of dipeptide **4** (65%) as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.36 (bs, 1H, CON*H*), 7.94 (d, *J* = 1.2 Hz 1H, Ar*H*), 7.73 (d, *J* = 7.2 Hz, 1H, Ar*H*), 7.66 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.34 – 7.20 (7H, 5 H_{phe}, 1 *H*Ar & $CDCl_3$), 5.31 (bs, 1H, NH_{Boc}), 4.55 (bs, 1H, CH_{phe}), 3.87 (s, 3H, OCH_3), 3.20 – 3.06 (2H, CH_{2ph}), 1.40 (s, 9H, 3 C$H_{3(Boc)}$); **^{13}C NMR** (125 MHz, $CDCl_3$, 25°C): δ = 170.1, 166.7, 156.1, 137.7, 136.6, 130.9, 129.4, 129.1, 128.9, 127.2, 125.6, 124.5, 121, 80.9, 56.8, 52.3, 38.5, 28.4; **IR** (thin film): υ = 3304 (m), 1725 (s), 1673 (s), 1556 (m), 1294 (m), 1169 (m), 755 (m) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{22}H_{26}N_2NaO_5$ (MNa^+) 421.1734, found 421.1736.

Dipeptide 5:

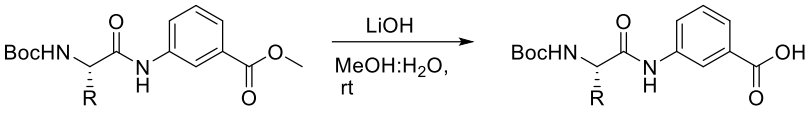


5

The general procedure above was followed and methyl *m*-amino benzoate ester **1**[1] (0.21 g), boc protected phenylglycine **3**[3] (0.26 g), HBTU (0.46 g), DIEA (0.39 g) and DCM (10 mL) were used. The reaction mixture after removal of solvents was extracted with ethyl acetate (2 x 20 mL), washed with 5% HCl (2 x 15 mL) and aqueous saturated sodium bicarbonate (2 x 15 mL). Purification by flash column chromatography (10-25% ethyl acetate/pet. ether) afforded 0.31 g of dipeptide **5** (80%) as a white powder.

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 8.73 (s, 1H, CON*H*), 7.95 (s, 1H, Ar*H*), 7.69 (bs, 2H, Ar*H* & H_{ph}), 7.47 (d, *J* = 7 Hz, 2H, Ar*H*), 7.34 – 7.23 (m, 4H, Ph*H*), 5.95 (bs, 1H, NH_{Boc}), 5.54 (bs, 1H, CH_{pg}), 3.88 – 3.83 (3H, OCH_3), 1.4 (s, 9H, 3 C$H_{3(Boc)}$). **^{13}C NMR** (125 MHz, $CDCl_3$, 25°C): δ = 169.1, 166.7, 155.8, 137.9, 137.3, 130.8, 129.2, 129, 128.7, 127.4, 125.5, 124.4, 120.9, 80.8, 59.3, 52.3, 28.5; **IR** (thin film): υ = 3734 (b), 2950 (m), 2345 (s), 1693 (b), 1522 (m), 1294 (m), 753 (m) cm^{-1}; **HRMS (ESI$^+$)**: calcd. for $C_{21}H_{24}N_2NaO_5$ (MNa^+) 407.1577 found 407.1576.

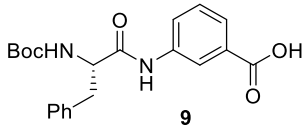
General procedure for synthesis of dipeptide acids



4; R = CH_2Ph
5; R = Ph

9; R = CH_2Ph
10; R = Ph

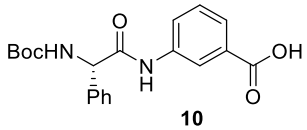
A solution of dipeptide either phe-dipeptide **4** or phg-dipeptide **5** (0.55 mmol, 1 equiv) in 2:1 MeOH:H_2O, was treated with LiOH.H_2O (2.22 mmol, 4 equiv) at room temperature. The reaction mixture was allowed to stir at room temperature for 4 h. The resultant reaction mixture was concentrated *in vacuo* and washed with ethyl acetate (2 x 15). The aqueous layer was acidified to pH 2 with 5% HCl (2 x 15 mL) and extracted with ethyl acetate (2 x 15 mL). The organic layer was dried over sodium sulphate, filtered and concentrated *in vacuo* to afford dipeptide-acid. The acid was used for the next step without further purification.



9

The general procedure was followed and phe-dipeptide **4** (0.22 g) and LiOH.H_2O (0.09) in 2:1 MeOH:H_2O (15 mL), were used for the reaction to afford 0.211 g of methyl ester deprotected phe-dipeptide **9** (99%) as a white solid.

^{1}H NMR (400 MHz, MeOD, 25°C): δ = 8.15 (s, 1H, Ar*H*), 7.74 (app t, *J* = 7.2 Hz 2H, Ar*H*), 7.39 (t, *J* = 8 Hz, 1H, Ar*H*), 7.27 (d, *J* = 4 Hz, 4H, Ph*H*), 7.21 – 7.18 (m, 1H, Ph*H*), 4.44 (t, 1H, *J* = 7.2 Hz, CH_{phe}), 3.12 (dd, *J* = 13.6 Hz, 7.2 Hz, 1H, HCH_{ph}), 2.95 (dd, *J* = 13.6, 8.4 Hz, 1H, HCH_{ph}), 1.39 (s, 9H, 3 C$H_{3(Boc)}$); **^{13}C NMR** (100 MHz, MeOD, 25°C): δ = 172.9, 169.5, 157.6, 139.6, 138.3, 132.6, 130.4, 129.9, 129.4, 127.8, 126.5, 125.8, 122.6, 80.7, 58.1, 39.6, 28.6; **IR** (thin film): *υ* = 3322 (b), 2940 (m), 2441 (m), 1683 (s), 1419 (m), 745 (m) cm^{-1}; **HRMS (ESI$^+$)**: calcd. for $C_{21}H_{24}N_2NaO_5$ (MNa^+) 407.1577 found 407.1579.

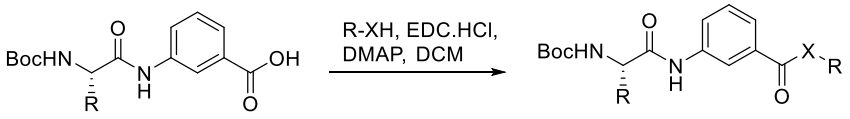


10

The general procedure was followed and phg-dipeptide **5** (0.20 g) and LiOH.H_2O (0.07 g in 2:1 MeOH:H_2O (18 mL), were used for the reaction, to afford 0.159 g of methyl ester deprotected dipeptide **10** (81%) as a white solid.

^{1}H NMR (500 MHz, MeOD, 25°C): δ = 8.20 (s, 1H, Ar*H*), 7.80 (d, *J* = 8 Hz, 1H, Ar*H*), 7.74 (d, *J* = 8 Hz, 1H, Ar*H*), 7.49 (d, *J* = 7.5 Hz, 2H, Ph*H*), 7.42 – 7.30 (4H, Ph*H* & Ar*H*), 5.31 (s, 1H, CH_{pg}), 1.45 (s, 9H, 3 C$H_{3(Boc)}$); **^{13}C NMR** (125 MHz, MeOD, 25°C): δ = 171.6, 169.4, 157.4, 139.9, 138.9, 132.6, 129.9, 129.9, 129.4, 128.6, 126.5, 125.5, 122.4, 80.9, 60.5, 28.7; **IR** (thin film): υ = 3649 (b), 2950 (m), 2342 (m), 1680 (s), 1477 (m), 745 (m) cm^{-1}; **HRMS (ESI$^+$)**: calcd. for $C_{20}H_{23}N_2O_5$ (MH^+) 371.1601 found 371.1604.

General procedure for synthesis of hydrophobic dipeptides 6 and 7



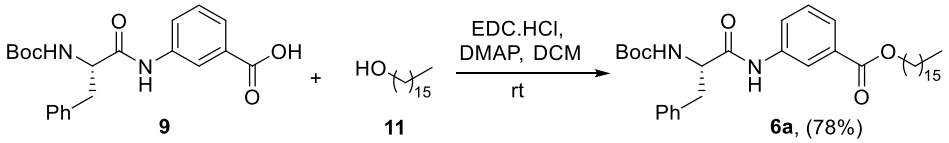
9; R = CH_2Ph
10; R = Ph

6; R = CH_2Ph
7; R = Ph

To a solution of either phenylalanine dipeptide acid **9** or phenylglycine dipeptide acid **10** (1.82 mmol, 1 equiv) and the requisite hydrophobic alcohol or amine (2.18 mmol, 1.2 equiv) in DCM was added EDC.HCl (4.55 mmol, 2.5 equiv) and DMAP (0.728 mmol, 0.4 equiv). The reaction

was allowed to stir at room temperature. After the completion of the reaction as monitored by TLC, the mixture was concentrated *in vacuo* and extracted with ethyl acetate (3 x 50 mL). The organic layer was dried over sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography.

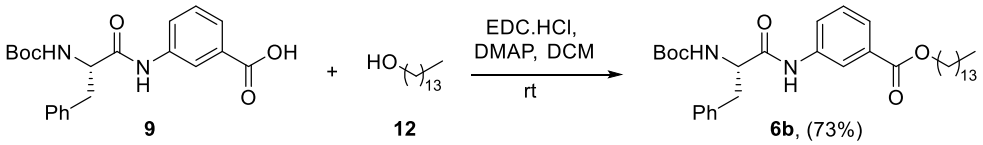
Hexadecane-ester phenylalanine dipeptide 6a:



The general procedure was followed and phenylalanine dipeptide acid **9** (0.1 g), hexadecanol **11** (0.08 g), EDC.HCl (0.12 g), DMAP (0.012 g) and DCM (3 mL) were used. The reaction took 20 h for completion. Purification by flash column chromatography (5 - 15% ethyl acetate/pet. ether) afforded 0.123 g of monomer **6a** (78%) as a white powder.

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): δ = 7.87 (bs, 1H, N*H*$_{(amide)}$), 7.83 (s, 1H, Ar*H*), 7.77 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.37 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.34 – 7.21 (6H, *H*$_{(phe)}$ & $CDCl_3$), 5.12 (bs, 1H, N*H*$_{(Boc)}$), 4.47 (bs, 1H, C*H*$_{phe}$), 4.29 (t, *J* = 7 Hz 2H, OC*H*$_{2(hexdec)}$), 3.15 (d, *J* = 7 Hz, 2H, C*H*$_{2\,(phe)}$), 1.79 – 1.72 (m, 2H, C*H*$_{2(hexdec)}$), 1.65 – 1.60 (6H, C*H*$_{2(hexdec)}$), 1.43 (s, 9H, 3 C*H*$_{3(Boc)}$), 1.28 -1.24 (20H, C*H*$_{2(hexdec)}$), 0.88 (t, *J* = 6.5 Hz, 3H, C*H*$_{3(hexdec)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 169.9, 166.2, 155.9, 137.5, 137.1, 136.5, 131.2, 129.3, 129, 128.8, 127.1, 125.5, 124.5, 120.9, 80.7, 65.3, 63.1, 56.7, 38.4, 31.9, 29.71, 29.68, 29.63, 29.56, 29.5, 29.4, 29.3, 28.7, 28.3, 26, 25.8, 22.7, 14.1; **IR** (thin film): *υ* = 3293 (b), 2924 (s), 2854 (s), 1715 (s), 1682 (s), 1556 (m), 1285 (m), 1171 (m), 754 (s) cm^{-1}; **HRMS (ESI^{+}):** calcd. for $C_{37}H_{57}N_2O_5$ (MH^{+}) 609.4258 found 609.4258.

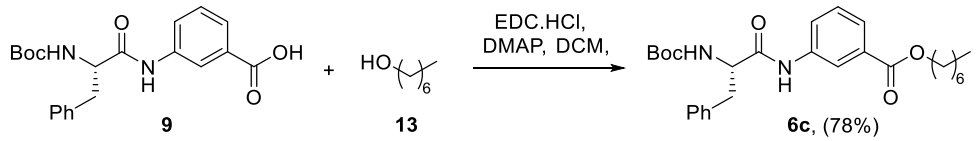
Tetradecane-ester phenylalanine dipeptide 6b



The general procedure was followed and phenylalanine dipeptide acid **9** (0.1 g), tetradecanol **13** (0.066 g), EDC.HCl (0.124 g), DMAP (0.012 g) and DCM (3 mL) were used. The reaction took 6 h for completion. Purification by flash column chromatography (5 - 15% ethyl acetate/pet. ether) afforded 0.110 g of monomer **6b** (73%) as a white powder.

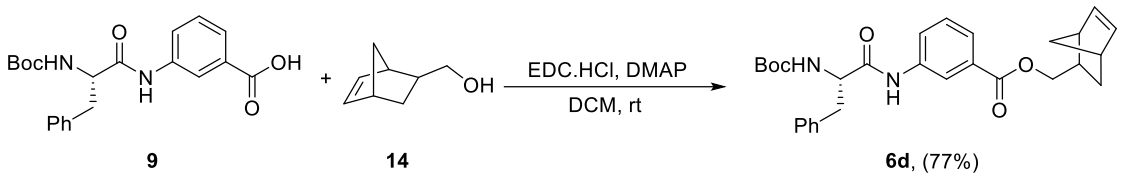
^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 8.07 (bs, 1H, N*H*$_{(amide)}$), 7.9 (s, 1H, Ar*H*), 7.76 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.39 – 7.20 (m, 6H, *H*$_{(phe)}$ & Ar*H*), 5.21 (bs, 1H, N*H*$_{(Boc)}$), 4.50 (bs, 1H, C*H*$_{phe}$), 4.29 (t, *J* = 6.8 Hz 2H, OC*H*$_{2(tetradec)}$), 3.21 – 3.06 (2H, C*H*$_{2\ (phe)}$), 1.82 – 1.70 (4H, C*H*$_{2(tetradec)}$), 1.42 (s, 9H, 3 C*H*$_{3(Boc)}$), 1.35 – 1.20 (20H, C*H*$_{2(tetradec)}$), 0.88 (t, *J* = 6.4 Hz, 3H, C*H*$_{3(tetradec\ \&\ grease)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 170.0, 166.3, 155.9, 137.6, 136.6, 131.4, 129.4, 129.2, 129.0, 127.3, 125.7, 124.6, 121.0, 80.9, 65.5, 38.5, 32.1, 29.82, 29.81, 29.78, 29.74, 29.67, 29.5, 29.4, 28.8, 28.4, 26.1, 22.8, 14.3; **IR** (thin film): *υ* = 3817 (m), 3671 (m), 3293 (m), 2924 (s), 2859 (s), 2342 (m), 1691 (s), 1443 (m), 1283 (m), 1170 (m), 1030 (m), 753 (m) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{35}H_{52}N_2O_5Na$ (MNa^+) 603.3758 found 603.3758.

Heptane-ester phenylalanine dipeptide 6c



The general procedure was followed and phenylalanine dipeptide acid **9** (0.100 g), heptanol **13** (0.044 g), EDC.HCl (0.124 g), DMAP (0.012 g) and DCM (3 mL) were used. The reaction took 6 h for completion. Purification by flash column chromatography (5 - 15% ethyl acetate/pet. ether) afforded 0.097 g of dipeptide **6c** (78%) as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) extra peaks due to rotamers: δ = 8.19 (bs, 1H, N*H*$_{(amide)}$), 7.87 (s, 1H, Ar*H*), 7.75 (d, *J* = 7.2 Hz, 2H, Ar*H*), 7.40 – 7.21 (m, 6H, *H*$_{(phe)}$ & Ar*H*), 5.26 (bs, 1H, N*H*$_{(Boc)}$), 4.53 (bs, 1H, C*H*$_{phe}$), 4.29 (t, *J* = 6.8 Hz, 2H, OC*H*$_{2(hep)}$), 3.21 – 3.05 (m, 2H, C*H*$_{2\ (phe)}$), 1.75 (app. quint., *J* = 7.2 Hz, 2H, C*H*$_{2(hep)}$), 1.45 – 1.22 (17H, C*H*$_{3(Boc)}$, C*H*$_{2(hep)}$), 0.89 (t, *J* = 6.8 Hz, 3H, C*H*$_{3(hep)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 170.0, 166.3, 156.0, 137.6, 136.6, 131.3, 129.4, 129.1, 128.9, 127.2, 125.6, 124.6, 121.0, 80.8, 65.5, 56.8, 38.5, 31.9, 29.8, 29.1, 28.8, 28.4, 26.1, 22.7, 14.2; **IR** (thin film): *υ* = 3957 (m), 3993 (m), 3291 (s), 2933 (s), 1695 (b), 1286 (m), 1038 (m), 753 (s) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{28}H_{39}N_2O_5$ (MH^+) 483.2857 found 483.2857.

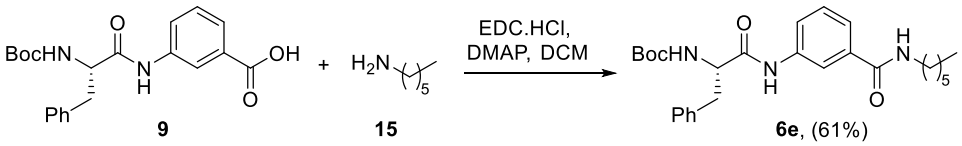


The general procedure was followed and phenylalanine dipeptide acid **9** (0.70 g), exo norbornene alcohol **14** (0.27 g), EDC.HCl (0.872 g), DMAP (0.09 g) and DCM (20 mL) were

used. The reaction took 12 h for completion. Purification by flash column chromatography (10 - 20% ethyl acetate/pet. ether) afforded 0.75 g of dipeptide **6d** (85%) as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) extra peaks due to rotamers: δ = 8.14 (app d. 1H, N*H*$_{(amide)}$), 7.88 (s, 1H, Ar*H*), 7.78 (d, *J* = 6.4 Hz, 2H, Ar*H*), 7.41 – 7.2 (6H, Ar*H*, *H*$_{(phe)}$), 6.16 – 6.06 (2H, C*H* = C*H*), 5.25 (bs, 1H, N*H*$_{(Boc)}$), 4.52 (bs, 1H, C*H*$_{phe}$), 4.39 (dd, *J* = 10.8, 6.4 Hz, 1H, HC*H*$_{(NB)}$), 4.19 (t, *J* = 10 Hz, 1H, HC*H*$_{(NB)}$), 3.21 – 3.07 (m, 2H, C*H*$_{2\,(phe)}$), 2.87 (s, 1H, C*H*$_{(NB)}$), 2.8 (s, 1H, C*H*$_{(NB)}$), 1.92 – 1.74 (2H, C*H*$_{(NB)}$), 1.42 (s, 9H, 3 C*H*$_{3(Boc)}$), 1.36 -1.22 (3H, C*H*$_{2(NB)}$, C*H*$_{(NB)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 170.1, 166.3, 156, 137.6, 137.1, 136.6, 136.4, 131.3, 129.4, 129.2, 128.9, 127.3, 125.7, 124.7, 121.1, 116.3, 80.8, 69.3, 56.8, 45.1, 43.8, 41.7, 38.5, 38.1, 29.7, 28.4 (171.4, 60.5, 21.2, 14.3 correspond to ethyl acetate); **IR** (KBr pellet): υ = 3292 (m), 2971 (m), 1711 (s), 1680 (s), 1545 (m), 1165 (m), 697 (m) cm^{-1}; **HRMS (ESI$^+$)**: calcd. for $C_{29}H_{34}KN_2O_5$ (MK^+) 529.2099 found 529.2099.

Hexyl-amide phenylalanine dipeptide 6e

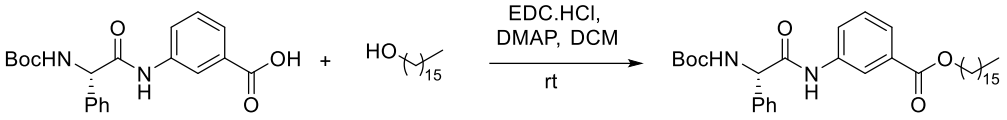


The general procedure was followed and phenylalanine dipeptide acid **9** (0.100 g), hexyl amine **15** (0.024 g), EDC.HCl (0.124 g), DMAP (0.012 g) and DCM (4 mL) were used. The reaction took 6 h for completion. Purification by flash column chromatography (10 - 30% ethyl acetate/pet. ether) afforded 0.074 g of monomer **6e** (61%) as a white powder.

^{1}H NMR (400 MHz, DMSO-d_6, 25°C): δ = 10.22 – 10.13 (1H, N*H*$_{(amide)}$), 8.43 (t, *J* = 5.6 Hz, 1H, Ar*H*), 7.98 (s, 1H, Ar*H*), 7.76 (d, *J* = 8.1 Hz, 1H, Ar*H*), 7.48 (d, *J* = 7.76 Hz, 1H, Ar*H*), 7.41 – 7.25 (m, 5H, Ar*H*$_{(phe)}$), 7.23 – 7.10 (2H, N*H*$_{(Hex\text{-}amide\ \&\ Boc)}$), 4.36 – 4.26 (m, 1H, C*H*$_{phe}$), 3.23 (app. q, *J* = 6.76 Hz, 2H, NHC*H*$_{2(hexamide)}$), 2.99 (dd, *J* = 13.6, 4.4 Hz, 1H, HC*H*$_{(phe)}$), 2.83 (dd, *J* = 13.2, 10 Hz, 1H, HC*H*$_{(phe)}$), 1.55 – 1.46 (m, 2H, C*H*$_{2(hexamide)}$), 1.35 – 1.19 (15H, C*H*$_{2(hexamide)}$, C*H*$_{3(Boc)}$), 0.86 (t, *J* = 6.4, 3H, C*H*$_{3(hexamide)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 171.1, 166.1, 155.5, 139.0, 137.9, 135.6, 129.3, 128.6, 128.1, 126.4, 121.7, 121.6, 118.6, 78.2, 56.6, 37.4, 31.1, 29.1, 28.2, 26.2, 22.1, 13.9; **IR** (thin film): *υ* = 3871 (b), 3735 (b), 3291 (s), 2934 (m), 2341 (m), 1700 (s), 1673 (s), 1543 (m), 1170 (m), 1031 (m), 746 (m) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{27}H_{37}N_3O_4Na$ (MNa^+) 490.2672 found 490.2671.

^{1}H NMR (400 MHz, DMSO-d_6, 100°C): δ = 9.73 (bs, 1H, N$H_{(amide)}$), 8.04 – 7.97 (m, 1H, Ar*H*), 7.95 (t, *J* = 1.7 Hz, 1H, Ar*H*), 7.73 – 7.69 (m, 1H, Ar*H*), 7.51 – 7.47 (m, 1H, Ar*H*), 7.42 – 7.24 (m, 5H, Ar*H*), 7.23 – 7.16 (m, 1H, N$H_{(Hex\text{-}amide}$), 6.46 (d, 1H, *J* = 6 Hz N$H_{(Boc)}$), 4.44 – 4.36 (m, 1H, CH_{phe}), 3.27 (app q., *J* = 5.8 Hz, 2H, NHC$H_{2(hexamide)}$), 3.09 (dd, *J* = 14, 5.4 Hz, 1H, HC*H* $_{(phe)}$), 2.92 (dd, *J* = 14 , 8.8 Hz, 1H, HC*H* $_{(phe)}$), 1.62 – 1.51 (m, 2H, C$H_{2(hexamide)}$), 1.39 – 1.28 (15H, C$H_{2(hexamide)}$, C$H_{3(Boc)}$), 0.89 (t, *J* = 6.8 Hz 3H, C$H_{3(hexamide)}$).

Hexadecane-ester phenylglycine dipeptide 7a



10 **11** **7a**, (72%)

The general procedure was followed and phenylglycine dipeptide acid **10** (0.100 g), hexadecanol **11** (0.078 g), EDC.HCl (0.129 g), DMAP (0.013 g) and DCM (3 mL) were used. The reaction took 12 h for completion. Purification by flash column chromatography (5 - 15% ethyl acetate/pet. ether) afforded 0.110 g of dipeptide **7a** (72%) as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 7.91 (s, 1H, Ar*H*), 7.84 (d, *J* = 8 Hz, 1H, Ar*H*), 7.75 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.44 (d, *J* = 6.8 Hz, 2H, $H_{(pg)}$), 7.40 – 7.32 (4H, $H_{(pg)}$ & Ar*H*), 5.78 (bs, 1H, N$H_{(Boc)}$), 5.35 (bs, 1H, CH_{pg}), 4.28 (t, *J* = 6.4 Hz, 2H, OC$H_{2(hexdec)}$), 1.74 (app. Quint., *J* = 6.8 Hz, 3H, C$H_{2(hexdec)}$), 1.65 – 1.58 (5H, C$H_{2(hexdec)}$), 1.44 (s, 9H, 3 C$H_{3(Boc)}$), 1.32 – 1.20 (20H, C$H_{2(hexdec)}$), 0.88 (t, *J* = 6.8 Hz, 3H, C$H_{3(hexdec)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 169.0, 166.3, 155.8, 137.8, 137.5, 131.2, 129.2, 129.0, 128.7, 127.4, 125.6, 124.5, 120.9, 80.8, 65.4, 59.2, 32.0, 29.8, 29.8, 29.77, 29.72, 29.65, 29.5, 29.4, 28.8, 28.5, 26.1, 22.8, 14.2; **IR** (thin film): *v* = 3817 (m), 3671 (m), 3293 (s), 2924 (s), 2859 (s), 2342 (m), 1691 (s), 1283 (m), 1170 (m), 1030 (m), 753 (m) cm^{-1}; **HRMS (ESI$^+$):** calcd. for $C_{36}H_{55}N_2O_5$ (MH^+) 595.4102 found 595.4101.

10 **14** **7b**, (77%)

Phenylglycine norbornene conjugates 7b

The general procedure was followed and henylglycine dipeptide acid **5** (0.16 g), norbornene alcohol **14** (0.06 g), EDC.HCl (0.2 g), DMAP (0.02 g) and DCM (5 mL) were used and the

reaction took 12 h for completion. Purification by flash column chromatography (10 - 20% ethyl acetate/pet. ether) afforded 0.157 g of dipeptide **7b** (77%) as a white powder.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C) extra peaks and splitting due to minor diastereomer: δ = 8.21 (bs, 1H, CON*H*), 7.93 (s, 1H, Ar*H*), 7.82 (d, *J* = 7.7 Hz, 1H, Ar*H*), 7.76 (d, *J* = 7.7 Hz, 1H, Ar*H*), 7.48 – 7.43 (2H, Ar*H* & H_{ph}), 7.39 – 7.31 (4H, H_{ph}), 6.15 – 6.05 (2H, C*H* = CH_{NB}), 5.85 (bs, 1H, NH_{Boc}), 5.42 (bs, 1H, CH_{phg}), 4.41 – 4.34 (m, 1H, OHCH_{NB}), 4.22 – 4.14 (m,1H, OHCH_{NB}), 2.86 (bs, 1H, CH_{NB}), 2.77 (bs, 1H, CH_{NB}), 1.92 – 1.80 (m, 1H, CH_{NB}), 1.74 – 1.67 (2H, CH_{2NB}), 1.43 (s, 9H, 3 C$H_{3Boc,}$) 1.33 - 1.28 (m, 1H, HCH_{NB}), 1.26 – 1.21 (m, 1H, HCH_{NB}); **^{13}C NMR** (100 MHz, CDCl3, 25°C): δ = 168.7, 166.2, 155.4, 137.6, 137.4, 137.0, 136.2, 129.2, 129.1, 128.7, 127.4, 125.6, 124.4, 120.8, 80.7, 69.2, 68.6, 59.3, 49.4, 45.0, 44.0, 43.7, 41.6, 38.1, 29.6, 28.3; **IR** (KBr pellet): υ = 3310 (s), 2973 (m), 1722 (s), 1557 (s), 1530 (s), 1296 (m), 1168 (s), 761 (m), 714 (m) cm^{-1}; **HRMS (ESI$^+$)**: calcd. for $C_{28}H_{33}N_2NaO_5$ (MNa^+) 477.2384 found 477.2381.

Synthesis of Peptide 16

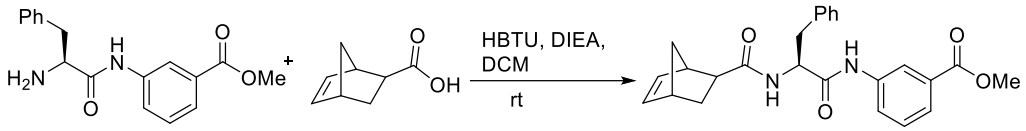
L-phenylalanine-m-aminobenzoic acid-OMe

4 **16**

To a solution of compound **4** (0.2 g, 0.5 mmol, 1 equiv) in DCM (10 mL) at 0 °C, TFA (0.58 mL, 7.5 mmol, 15 equiv) was added. The reaction mixture was allowed to stir for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* in the presence of a secondary trap to afford the ester **16** (0.210 g, 99% yield) as a white solid. The compound was used in the next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6 , 25°C): δ = 10.77 (s, 1H, NH_{amide}), 8.49 (s, 2H, NH_2), 8.18 (s, 1H, Ar*H*), 7.76 (d, *J* = 8 Hz, 1H, Ar*H*), 7.69 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.49 (t, *J* = 8, 1H, Ar*H*), 7.33-7.21 (m, 5H, H_{ph}), 4.22 (app bs, 1H, CH_{phe}), 3.85 (s, 3H, OCH_3), 3.24-3.07 (2H, C$HH_{(phe)}$); **^{13}C NMR** (100 MHz, DMSO-d_6): δ = 167.1, 166.0, 138.3, 134.8, 130.4, 129.5, 128.7, 127.4, 124.9,124.2, 120.2, 54.4, 52.4, 37.1; **IR** (thin film): *v*= 3442 (s), 3360 (s), 2980 (m), 2302 (m), 1697 (s), 1619 (s), 1540 (m), 1430(m), 1360 (m), 1303 (m), 1170 (s), 1112(m), 915(s), 860 (s), 742(m) cm^{-1}; **HRMS (ESI$^+$)** calcd for $C_{17}H_{19}N_2O_3$(MH^+) 299.1390, found 299.1376.

Norbornene amide phenylalanine dipeptide 8a:

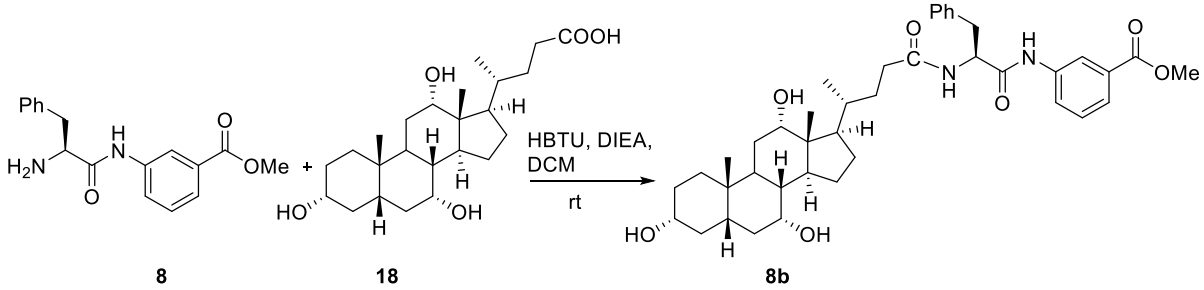


8 **17** **8a**

To a solution of compound **8** (0.2 g, 0.49 mmol, 1 equiv) and exo-NB-COOH **17** (0.067 g, 0.49 mmol, 1equiv) in dry DCM (12 mL) was added HBTU (0.22 g, 0.58 mmol, 1.2 equiv) and DIEA (0.34 mL, 1.9 mmol, 4.0 equiv). The reaction mixture was allowed to stir at RT for 7 h, following which DCM was removed *in vacuo.* The reaction mixture was diluted with ethyl acetate (200 mL) and sequentially washed with 5% aqueous HCl (3 × 50 mL), 0.1N aqueous $NaHCO_3$ (3 × 50 mL) and saturated aqueous NaCl (2 × 30mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (4 - 25% ethyl acetate/pet. ether) to afford 0.19 g of compound **8a** (68% yield) as a white crystalline solid.

^{1}H NMR (500 MHz, $CDCl_3$, 25°C): **^{1}H NMR** (500 MHz, $CDCl_3$, 25°C): δ = 9.12 (d, *J* = 13.5 Hz, 1H, N*H*$_{amide}$), 8.00 (s ,1H, Ar*H*), 7.72 (d, *J* = 8 Hz, 1H, Ar*H*), 7.64 (d, *J* = 8 Hz, 1H, Ar*H*), 7.32-7.17 (m, 6H, Ar*H*), 6.8 – 6.55 (m, 1H, N*H*$_{amide,}$ diastereomers), 6.13-6.08 (m, 1H, C*H*=C*H*$_{nb}$), 6.06-6.01 (m, 1H, C*H*=C*H*$_{nb}$), 5.08-4.98 (m, 1H, C*H*), 3.88-3.82 (2 s, 3H, OC*H*$_3$, diastereomers), 3.25-3.15 (m, 1H, C*H*$_{2(Bn)}$), 3.14-3.08 (m, 1H, C*H*$_{2(Bn)}$), 2.90 (s, 1H, C*H*$_{(nb)}$), 2.86 (s, 1H, C*H*$_{(nb)}$), 2.75 (s, 1H, C*H*$_{(nb)}$), 2.11-2.04 (m, 1H, C*H*$_{2(nb)}$), 1.90-1.83 (m, 1H, C*H*$_{2(nb)}$), 1.77-1.70 (m,1H, C*H*$_{2\ (nb)}$), 1.59-1.52 (m, 1H, $CH_{2(nb)}$); **^{13}C NMR** (125 MHz, $CDCl_3$, 25°C): extra peaks as diastereomers δ = 176.7, 176.6, 170.0, 166.7, 138.5, 138.3, 137.8, 136.6, 136.1, 135.9, 131.0, 129.4, 129.1, 128.9, 127.3, 125.7, 124.6, 121.1, 55.6, 55.5, 52.3, 47.4, 47.0, 46.5, 46.4, 44.8, 44.7, 41.7, 38.4, 30.8, 30.5; **IR**(thin film): ν = 3277 (b), 3152 (s), 3063 (s), 2971 (s), 2949 (s), 2871 (s), 2099 (m), 1725 (s), 1641 (s), 1594 (s), 1439 (s), 1289 (s), 1223 (s), 1107(m), 1082 (m), 975 (m), 902 (m), 802 (m), 753 (s), 727 (s), 699 (s), 497 (s); **HRMS(ESI$^+$)** calcd for $C_{25}H_{26}N_2O_4Na$ (MNa^+) 441.1785, found 441.1785.

Cholic acid amide phenylalanine dipeptide 8b:

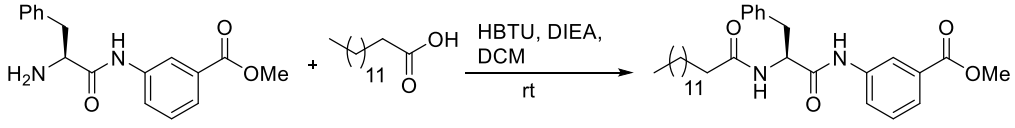


8 **18** **8b**

To a solution of compound **8** (0.20 g, 0.67 mmol, 1 equiv) and cholic acid **18** (0.067 g, 0.49 mmol, 1equiv) in dry DCM (12 mL) was added HBTU (0.22 g, 0.58 mmol, 1.2 equiv) and DIEA (0.34 mL, 1.9 mmol, 4.0 equiv). The reaction mixture was allowed to stir at RT for 12 h, following which DCM was removed *in vacuo.* The reaction mixture was diluted with ethyl acetate (200 mL) and sequentially washed with 5% aqueous HCl (3 × 50 mL), 0.1N aqueous $NaHCO_3$ (3 × 50 mL) and saturated aqueous NaCl (2 × 30 ml). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (3 - 20% ethyl acetate/pet ether) to afford 0.19 g of compound **8b** (63% yield) as a white crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.03 (s, 1H, NH_{amide}), 8.01 (s, 1H, Ar*H*), 7.83-7.69 (2H, Ar*H*), 7.33 (t, *J* = 8, 1Hz, Ar*H*), 7.29-7.19 (5H, Ar*H*), 7.03 (d, *J* = 7.6 Hz, 1H, NH_{amide}), 4.93-4.84 (m, 1H, CH_{Phe}), 4.68-4.43 (bs, 1H, OH_{Cholic}), 3.98-3.91 (bs, 1H, OH_{Cholic}), 3.89 (s, 3H, OCH_3), 3.85-3.80 (bs, 1H, OH_{Cholic}), 3.47-3.03 (5H, CHOH$_{Cholicd}$, C$H_{2(Bn)}$), 2.21 (s, 2H, CH_2CO), 1.96-0.86 (39H, CH, CH_2, CH_3), 0.62 (s, 3H, CH_3); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ = 175.0, 170.3, 167.0, 138.1, 136.7, 130.8, 129.5, 129.2, 128.8, 127.2, 125.6 124.8, 121.2, 73.1, 72.1, 68.7, 68.4, 55.4, 52.4, 46.6, 46.5, 46.0, 41.8, 41.6, 41.3, 39.8, 39.6, 38.3, 35.5, 35.3, 35.0, 32.6, 31.4, 30.6, 28.2, 27.7, 26.7 ; **IR** (thin film): ν= 3391 (b), 2936 (s), 2869 (s), 2248 (m), 1710 (s), 1651 (s), 1595 (m), 1553 (m), 1488 (m), 1443 (s), 1378 (s), 1378 (m), 1293 (s), 1197 (s), 1077 (s), 1042 (m), 949 (m), 911 (s), 856 (m), 733 (s); **LCMS QTOF (ESI$^+$):** Calcd for $C_{41}H_{56}N_2O_7$ (MH^+) 711.3977, found 711.3977.

Myristic acid amide phenylalanine dipeptide 8c:



8 **19** **8c**

To a solution of compound **8** (0.20 g, 0.67 mmol, 1equiv) and myristic acid **19** (0.15 g, 0.67 mmol, 1equiv) in dry DCM (12 mL) was added HBTU (0.305 g, 0.80 mmol,1.2 equiv) and DIEA (0.34 mL, 2.68 mmol, 4.0 equiv). The reaction mixture was allowed to stir at RT for 12 h, following which DCM removed *in vacuo*. The reaction mixture was diluted with ethyl acetate (200 mL) and sequentially washed with 5% aqueous HCl (3 × 50 mL), 0.1 N aqueous $NaHCO_3$ (3 × 50 mL) and saturated aqueous NaCl (2 × 30mL). The organic layer was dried over anhydrous sodium sulphate, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (5 – 50% ethyl acetate/pet ether) to afford 0.19 g of compound **8c** (70% yield) as a white crystalline solid.

^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ = 9.17 (bs, 1H, NH_{amide}), 8.05 (s, 1H, Ar*H*), 7.76 (app. t, 2H, Ar*H*), 7.34 (t, *J* = 8.0 Hz, 1H, Ar*H*), 7.31-7.20 (m, 5H, $H_{(phe)}$), 6.65 (bs, 1H, NH_{amide}), 5.07 (dd, *J* = 13.9, 6.6 Hz, 1H, CH_{Phe}), 3.88 (s, 3H, OCH_3), 3.23 (dd, *J* = 13.9, 6.8 Hz, 1H, C$H_{2(phe)}$), 3.14 (dd, *J* = 7.4 Hz, *J* = 13.8, 1H, C$H_{2(phe)}$) 2.25 (t, *J* = 7.2 Hz, 2H, CH_2), 1.65-1.52 (m, 2H, CH_2), 1.34-1.18 (20 H, C$H_{2(alkyl)}$), 0.90 (t, *J* = 6.8, 3H, C$H_{3(alkyl)}$); **^{13}C NMR** (100 MHz, $CDCl_3$, 25°C): δ= 174.1, 170.1, 166.7, 138.1, 136.5, 130.7, 129.5, 129.1, 128.7, 127.2, 125.5, 124.6, 121.1, 55.3, 52.3, 38.7, 36.7, 32.0, 29.81, 29.78, 29.76, 29.6, 29.5, 29.3, 25.8, 22.8, 14.2; **IR** (thin film): ν = 3281 (b), 3077 (m), 2975 (s), 2855 (s), 1729 (s), 1644 (s), 1555 (s), 1443 (m), 1290 (s), 1107 (m), 909 (s), 735 (s); **HRMS (ESI$^+$)**: Calcd. for $C_{31}H_{45}N_2O_4$ (MH^+) 509.3374, found 509.3379.

Ion transport activity of peptides 4-8 using HPTS assay

Vesicle preparation[4]

EYPC lipid in chloroform (25 mg in 1 mL) was taken in a 10 mL round bottom flask. Chloroform was removed using a stream of nitrogen and further kept *in vacuo* for 3 h at 0 ºC to form a thin film of the lipid. The lipid film was hydrated by a solution of HPTS dye (1 mL of 0.5 mM HPTS, 100 mM NaCl, 10 mM HEPES in water) at pH 7. The resulting mixture was allowed to stir at room temperature for 1 h and was then subjected to three freeze-thaw cycles. The suspension was extruded 19 times through 0.1 μm polycarbonate membranes using a mini-extruder obtained from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: HEPES-NaCl buffer at pH 7.0 (100 mM NaCl, 10 mM HEPES)). The milky white vesicle solution was collected and diluted to 3.2 mL to get 10 mM lipid stock solution.

HPTS assay

30 µL of HPTS containing vesicle was added to 1970 µL of NaCl: HEPES buffer (10 mM HEPES, 100 mM NaCl, pH 8) in a fluorescence cuvette to generate a pH gradient across lipid bilayer. This cuvette was placed in a spectrofluorometer equipped with a magnetic stirrer. For the fluorescence measurement, excitation and emission wavelength used were 460 nm and 510 nm, respectively. A solution of peptide **4-8** (7.55 µM, 5 mol% with respect to lipid) in THF (10 µL) was added at t = 50 s. At t = 250 s, Triton-X (20%, 20 µL) was added to lyse the vesicle and achieve the maximum fluorescence intensity. The maximum intensity obtained was used to normalize the fluorescence intensity (Figure S1). The time (x-axis) was normalized to 0 sec when peptide was added and the intensity was normalized based on the final equilibrated value obtained after addition of Triton X.

a) b)

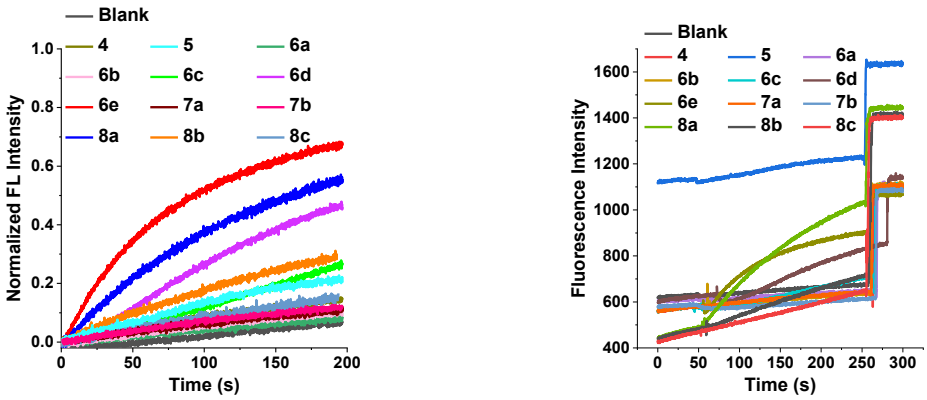


Figure S1. Transport activity of amino acid dipeptides **6 – 8** using HPTS assay with pH gradient in NaCl environment a) normalized FL intensity plot (time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition) b) raw data (peptide concentration = 5 mol% with respect to lipid, 10 µL solution of peptide in THF was used).

Determination of chloride transport using lucigenin assay

Vesicle preparation

EYPC lipid in chloroform (25 mg in 1 mL) was taken in a 10 mL round bottom flask. Chloroform was removed using a stream of nitrogen and further kept *in vacuo* for 3 h at 0 ºC to form a thin lipid film. The lipid film was hydrated by a solution of lucigenin dye (1 mL of 1 mM lucigenin, 225 mM $NaNO_3$). The resulting mixture was allowed to stir at room temperature for 1 h and was then subjected to three freeze-thaw cycles. The suspension was extruded 19

times through 0.1 µm polycarbonate membranes using a mini-extruder obtained from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: 225 mM $NaNO_3$). The milky white vesicle solution was collected and diluted to 3.2 mL to get 10 mM lipid stock solution.

Assay to determine chloride ion transport activity of dipeptides 6-8

30 µL of lucigenin containing vesicle was added to 1970 µL aqueous NaCl solution (225 mM) in a fluorescence cuvette to generate a chloride gradient across lipid bilayer. This cuvette was placed inside the spectrofluorometer equipped with magnetic stirrer. For the fluorescence measurement, excitation and emission wavelength used were 455 nm and 505 nm, respectively. A solution of dipeptide in THF (0.3 mM, 10 µL) was added at t = 50 s. At t = 250 s, Triton-X (20%, 20 µL) was added to lyse the vesicle and achieve the maximum fluorescence quenching. The maximum quenching intensity obtained was used to normalize the fluorescence intensity (Figure S2). (time t = 0 s in the normalized graphs (Figure S2 a) corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition)

b) b)

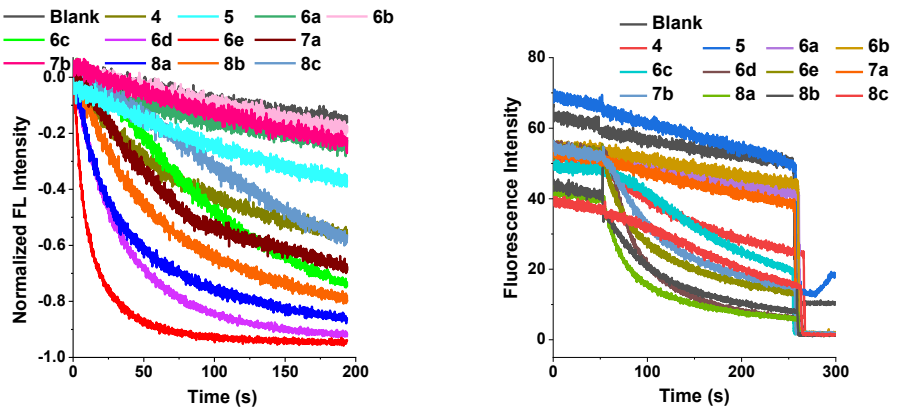


Figure S2. Chloride transport activity of amino acid dipeptide **6 – 8** using Lucigenin assay a) normalized FL intensity plot (time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time just before Triton-X addition) b) raw data. (peptide concentration = 5 mol% with respect to lipid, 10 µL solution of peptide in THF was used).

Determination of sodium transport using HPTS assay

Vesicle preparation[4]

EYPC lipid in chloroform (25 mg in 1mL) was taken in a 10 mL round bottom flask. Chloroform was removed using a stream of nitrogen and further kept *in vacuo* for 3 h at 0 ºC to form a thin lipid film. The lipid film was hydrated by a solution of HPTS dye (1 mL of 0.5 mM HPTS, 100 mM Na_2SO_4, 10 mM HEPES in water) at pH 7. The resulting mixture was allowed to stir at room temperature for 1 h and was then subjected to three freeze-thaw cycles. The suspension was extruded 19 times through 0.1 µm polycarbonate membranes using a mini-extruder obtained from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: HEPES- Na_2SO_4 buffer at pH 7.0 (100 mM Na_2SO_4, 10 mM HEPES)). The milky white vesicle solution was collected and diluted to 3.2 mL to get the 10 mM lipid stock solution.

Assay to study chloride selectivity

30 µL of HPTS containing vesicle was added to 1970 µL of Na_2SO_4:HEPES buffer (10 mM HEPES, 100 mM Na_2SO_4, pH 8) in a fluorescence cuvette to generate a pH gradient across lipid bilayer (Figure S3). This cuvette was placed inside the spectrofluorometer equipped with a magnetic stirrer. For the fluorescence measurement, excitation and emission wavelength used were 460 nm and 510 nm, respectively. A solution of dipeptide **6d**, **6e** or **8a** (0.3 mM) in THF (10 µL) was added at t = 50 s. At t = 250 s. Triton-X (20%, 20 µL) was added to lyse the vesicle and achieve the maximum fluorescence intensity. The time t = 0 s in the normalized graphs (Figure S3a) corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

a) b)

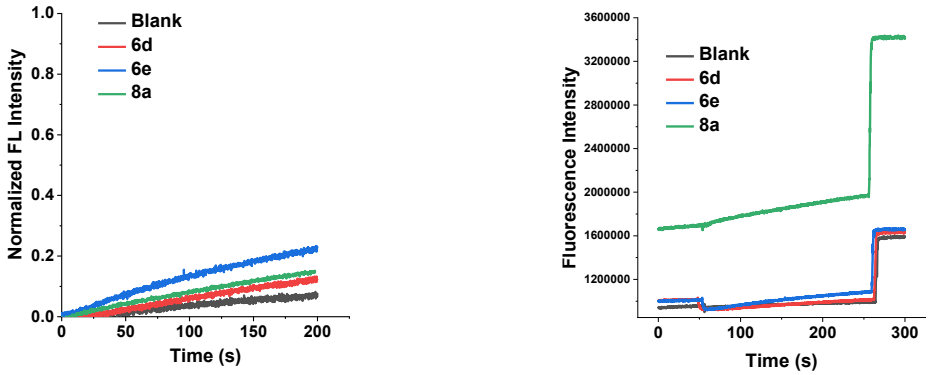


Figure S3. HPTS assay in an Na_2SO_4 environment to determine sodium transport by phenylalanine dipeptide **6d, 6e** & **8a** a) normalized plot (time t = 0 s in the normalized graphs corresponds to the

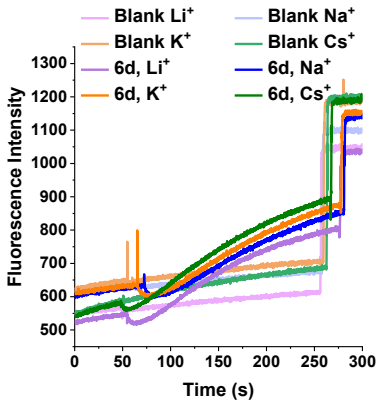
time of peptide addition and t = 200 s in the normalized plot corresponds to the time of before Triton-X addition) b) raw data (peptide **6/8** = 7.55 µM; 5 mol% with respect to lipid in THF).

HPTS assay for ion selectivity based on dual gradient

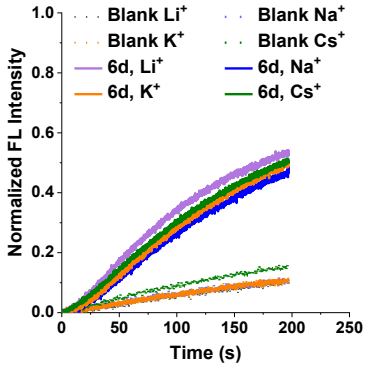
Assay to study effect of external cation

The vesicle preparation and assay procedure described above (page no. S13) was carried out with the only change that 100 mM MCl buffer (10 mM HEPES, 100 mM MCl, (M = Li^+, Na^+, K^+, Cs^+), pH 8) was used in the extravesicular buffer instead of NaCl.

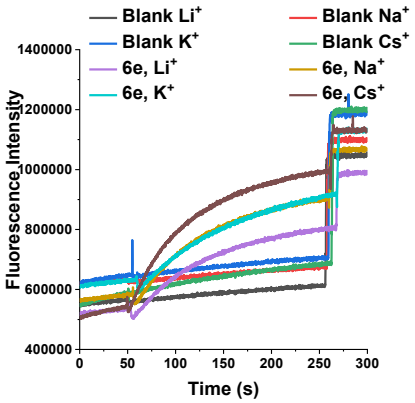
a)



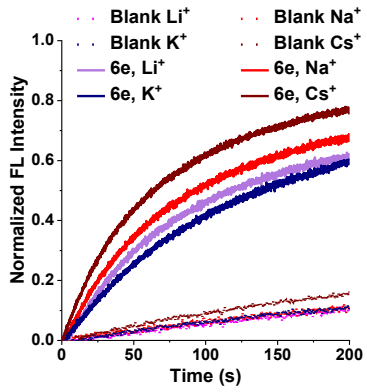
b)



c)

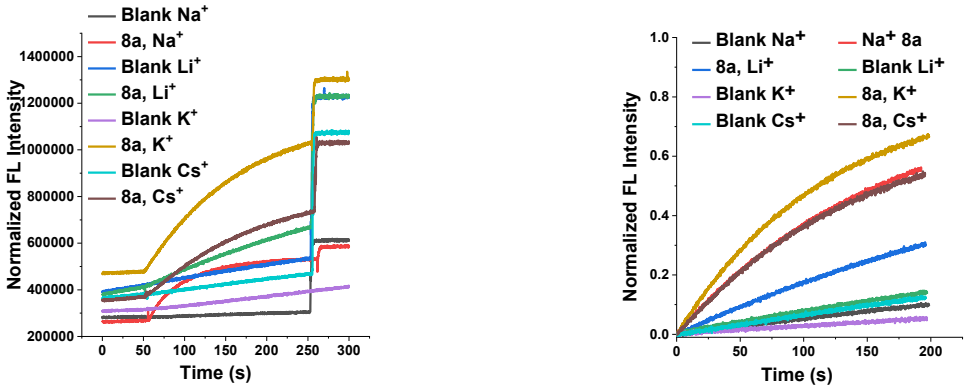


d)



e)

f)



g)

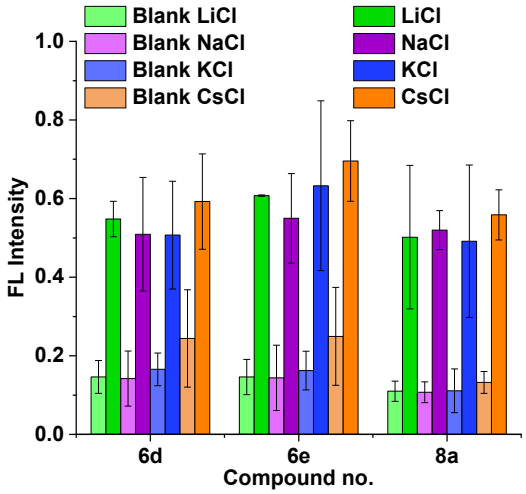


Figure S4. Cation transport activity using dual gradient HPTS assay a) raw data and b) normalized plots for experiments with **6d** c) raw data and d) normalized plot for experiments with **6e** e) raw data and f) normalized plot for experiments with **8a** g) comparison of average bar plot of maximum fluorescence intensity of **6d, 6e** & **8a**, using HPTS assay in presence of MCl, (M = Li^+, Na^+, K^+, Cs^+), pH 8), (dipeptide = 7.55 μM; 5 mol% with respect to lipid, THF solution, Experiment was repeated three times). Time t = 0 s in the normalized graphs (b, d and f) corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

Assay to study effect of external anion

The assay procedure described above (page no. S17) was carried out with the only change that 100 mM NaX (X = F^-, Cl^-, Br^-, NO_2^-, NO_3^-, pH = 8.0) was used in the extravesicular buffer instead of MCl.

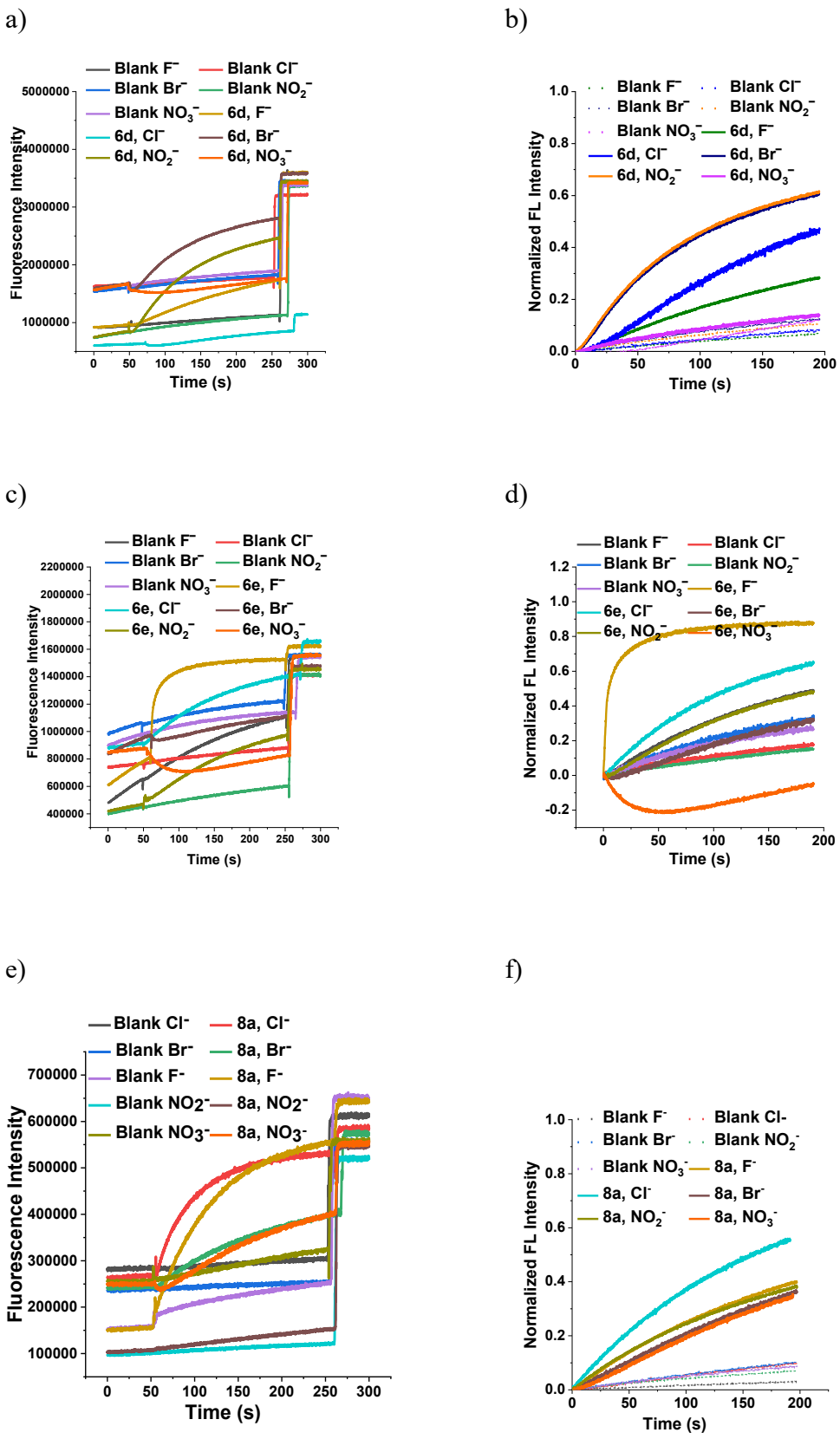


Figure S5. Anion transport activity using dual gradient HPTS assay a) raw data of **6d** b) normalized plot of **6d** c) raw data of **6e** d) normalized plot of **6e** e) raw data of **8a** f) normalized plot of **8a,** in presence of extravesicular NaX (X = F^-, Cl^-, Br^-, NO_2^-, NO_3^-, pH = 8.0), (dipeptide = 7.55 μM,

5 mol% with respect to lipid, Experiment was repeated three times). Time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

HPTS assay for ion selectivity based on anion/cation gradient (single gradient)

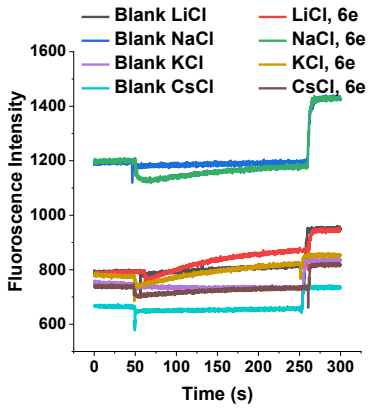
Assay to study cation transport

The vesicle preparation and assay procedure described above (page no. S17) was carried out with 100 mM MCl buffer (10 mM HEPES, 100 mM MCl, (M = Li^+, Na^+, K^+, Cs^+)), with the only change that pH 7 was used in the extravesicular buffer instead of pH 8 and 7.55 µM (5 mol% with respect to lipid) dipeptide concentration used.

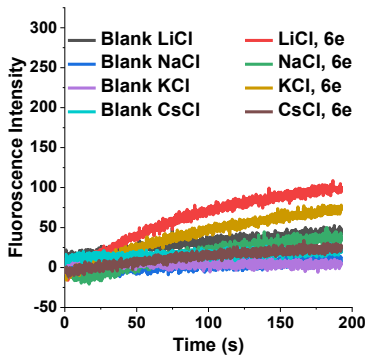
Assay to study anion transport

The assay procedure described above (page no. S20) was carried out with the only change that 100 mM NaX (X = F^-, Cl^-, Br^-, NO_2^-, NO_3^-, pH = 7.0) was used in the extravesicular buffer instead of MCl.

a)



b)



c)

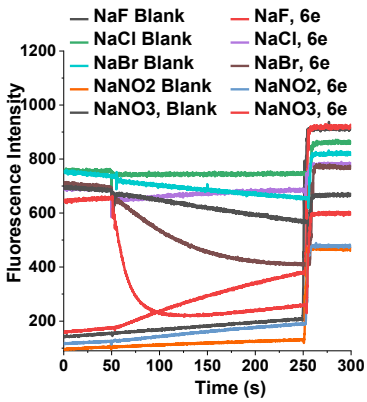


Figure S6. Cation transport activity using HPTS assay in presence of extravesicular MCl buffer, (M = Li^+, Na^+, K^+, Cs^+) of pH 7, a) raw data of **6e** b) normalized fluorescence plot of **6e** c) raw data of anion transport activity of **6e** in presence of extravesicular NaX buffer, (X = F^-, Cl^-, Br^-, NO_2^-, NO_3^-) of pH 7. Time t = 0 s in the normalized graphs corresponds to the time of peptide addition

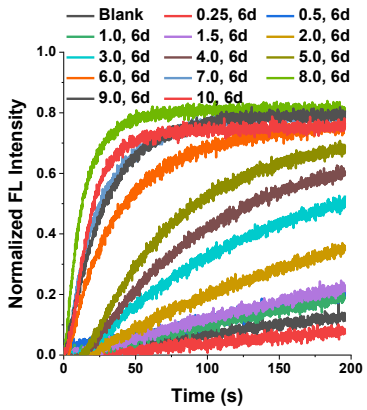
Hill analyses using HPTS assay

To determine the EC_{50} and hill coefficient value of the most efficient peptides **6d, 6e** and **8a**, HPTS assay as described earlier (page no. S13) with extravesicular NaCl buffer of pH 8 was carried out**.** A control experiment was carried out in absence of peptides **6d, 6e** and **8a**, by using the same volume of THF used to solubilize the peptides. The concentration of peptide was varied from 0 to 10 mol% with respect to lipid. Peptides were added at t = 50 s and Triton-X was added at t = 250 s. In the obtained results peptide addition time was normalized to zero and intensity maxima after addition of Triton-X was normalized to 1. Intensity maxima before addition of Triton-X was plotted against the respective peptide concentration to get the Hill plot (Figure S4). Obtained Hill plots were fitted to the Hill equation (Equation S1).

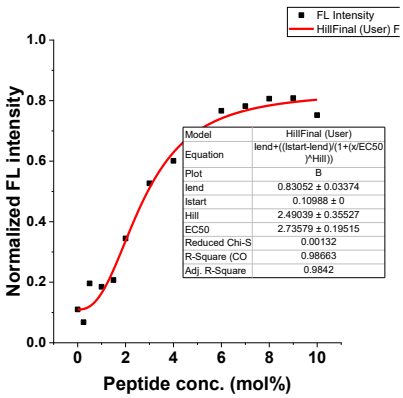
$$I = I_\infty + ((I_0 - I_\infty)/(1+(c/EC_{50})^p)) \quad \text{(S1)}$$

Here, I corresponds to the normalizad intensity, I_∞ and I_0 correspond to the normalized intensity with presence and absence of peptide, respectively. The term EC_{50} represents the peptide concentration required to get half of maximum fluorescence intensity. The term p is Hill coefficient and corresponds to number of peptide molecules associated with a single ion.

a)



b)



c)

d)

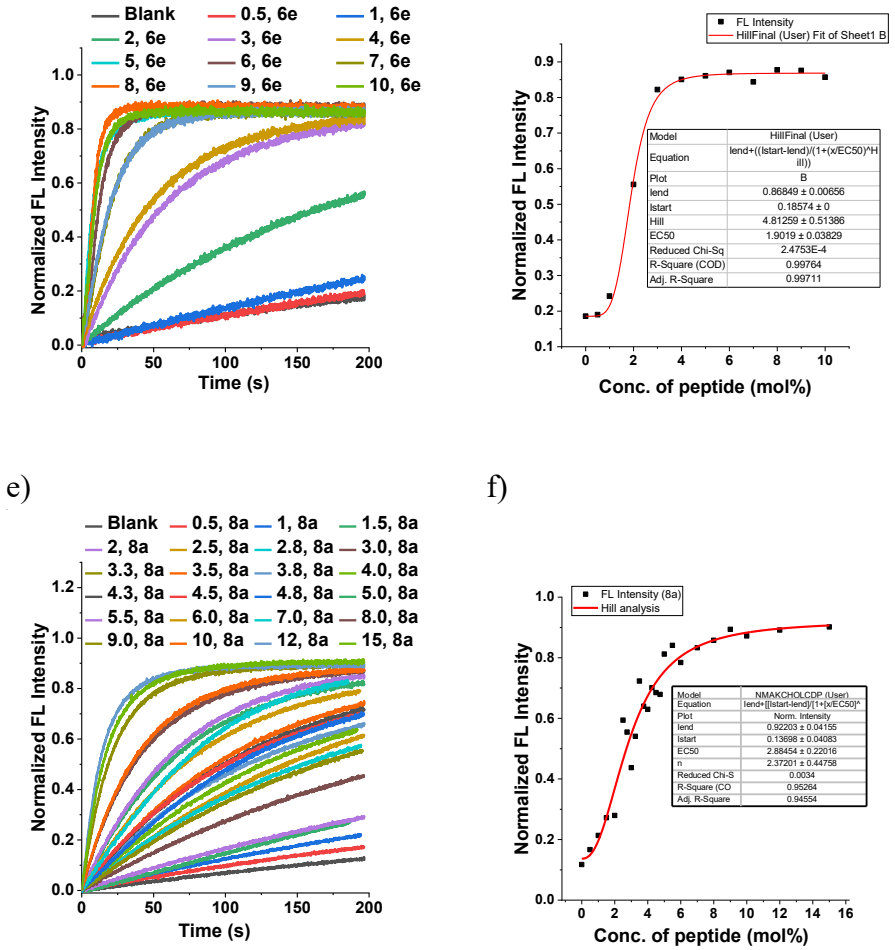


Figure S7. a) Concentration dependent normalized fluorescence intensity plot of peptide **6d** using HPTS assay b) Hill plot of peptide **6d** using HPTS assay c) Concentration dependent normalized fluorescence intensity of peptide **6e** using HPTS assay d) Hill plot of peptide **6e** using HPTS assay e) Concentration dependent normalized fluorescence intensity of peptide **8a** using HPTS assay f) Hill plot of peptide **8a** using HPTS assay**.** The EC_{50} reported in the main paper is the average value of 2 experiments. Time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before addition of Triton-X)

Hill analyses using lucigenin assay

Similar procedure was followed as described earlier (page no. S14) for the lucigenin assay with various concentrations of peptide instead of 5 mol%.

a) b)

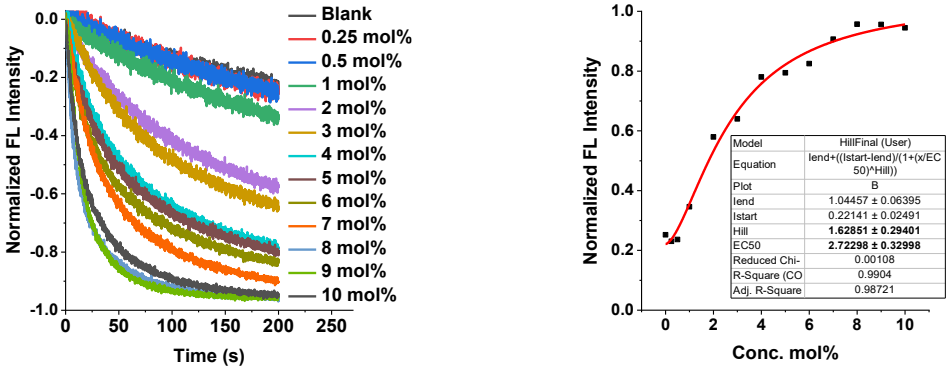


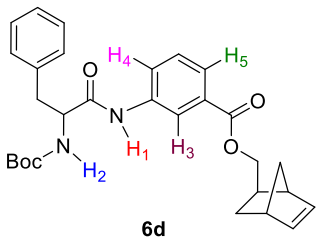
Figure S8. a) Concentration dependent normalized fluorescence intensity plot of peptide **6e** using lucigenin assay b) Hill plot of peptide **6e** using lucigenin assay The EC_{50} reported in the main paper is the average value of 2 experiments. Time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

Anion binding by ^{1}H NMR

NMR binding studies were carried out to probe the anion binding with pheneylalanine scaffold **6d, 6e** and **8a** (Host) using the respective anion source (guest). Concentration of pheneylalanine scaffold (2 mM, $CDCl_3$) was kept constant throughout the experiment whereas the concentration of anion source was varied. A solution of anion source in $CDCl_3$ was prepared under nitrogen atmosphere in a glove box. A solution of pheneylalanine scaffold (300 µL, 3.33 mM) in $CDCl_3$ were added to each NMR tube. Increasing amounts of respective anion source (0 – 200 µL, 0-20 eq. in $CDCl_3$) were added to NMR tubes with host solution (300 µL, 3.33 mM). Final volume of all samples was made up to 500 µL by adding the required amount of $CDCl_3$, so that the concentration of host remained constant (2 mM) throughout the experiment. The tube was sealed with Teflon tape to avoid solvent evaporation.

Chloride binding studies with 6d

Tetrabutylammonium chloride used as anion source. To check the chloride binding 0 -18 equivalents of tetrabutylammonium chloride were added to the the norbornene ester pheneylalanine scaffold **6d**.



6d

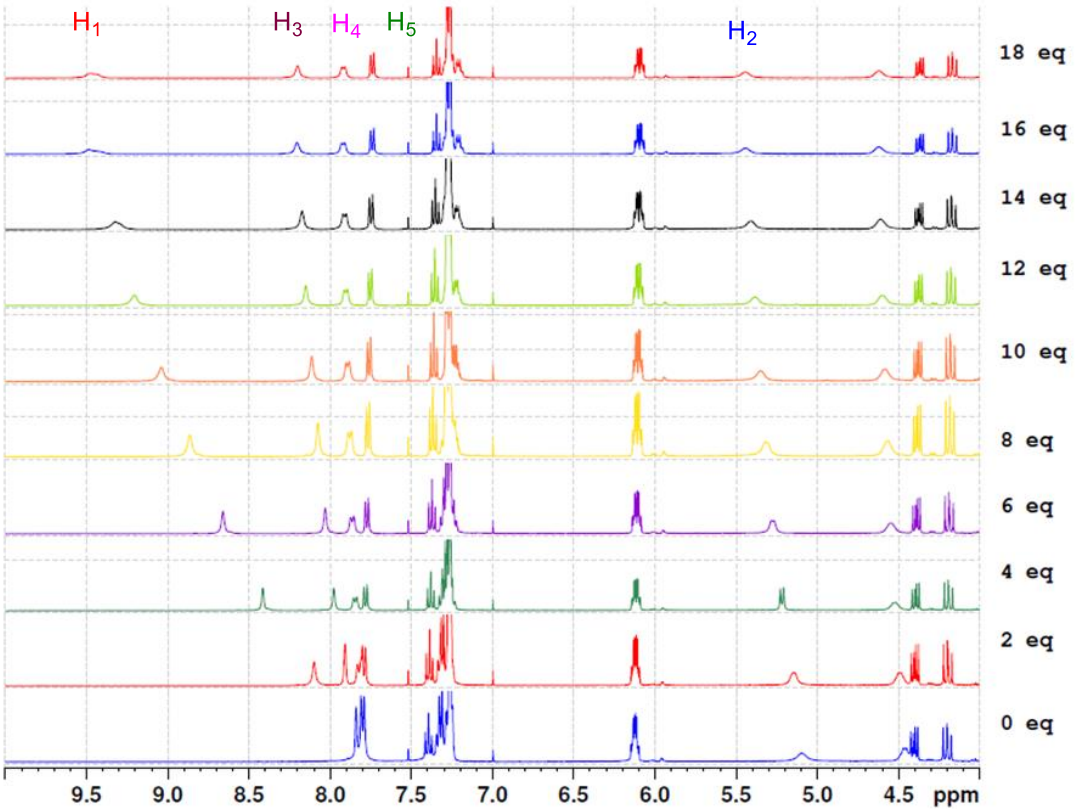


Figure S9. Stacked ^{1}H NMR plot for titration of peptide **6d** (2 mM) with TBACl (equiv. indicated on stacked plot) in $CDCl_3$

Table S1. Representative data obtained for NMR titration of peptide **6d** with TBACl

Conc. 6d (M)	Conc. Cl^- (M)	Equiv. guest	proton H1 (ppm)	proton H2 (ppm)	proton H3 (ppm)	proton H4 (ppm)	proton H5 (ppm)
0.002	0	0	7.8417	5.0953	7.8385	7.8087	7.7889
0.002	0.004	2	8.098	5.147	7.909	7.8202	7.7917
0.002	0.008	4	8.415	5.217	7.978	7.8451	7.7826
0.002	0.012	6	8.66	5.2744	8.031	7.8628	7.7734
0.002	0.016	8	8.865	5.3118	8.076	7.8783	7.7659
0.002	0.02	10	9.041	5.346	8.114	7.8908	7.7595
0.002	0.024	12	9.204	5.381	8.151	7.9028	7.7525
0.002	0.028	14	9.325	5.4065	8.174	7.9099	7.7472
0.002	0.032	16	9.443	5.4415	8.205	7.9199	7.7406
0.002	0.036	18	9.458	5.4447	8.202	7.9191	7.7402

Table S2. Representative comparison of association constants obtained with different binding models using Bindfit Software.

Host: Guest	***K*/*K*$_{11}$ (M^{-1})**	**Error (%)**	***K*$_{12}$/ *K*$_{21}$ (M^{-1})**	**Error (%)**	**δHG (ppm)**	**δHG$_2$ / δH$_2$G (ppm)**	**SSR**	**RMS**	**Covariance**
1:1[a]	27.05	± 1.55			11.292		1.27 E-02	3.32 E-02	3.70 E-03
1:2 (Full)[b]	0.13	± 1.35	5389.53	± 4.97	525.95	8.60	3.32 E-03	1.61 E-02	8.84 E-04
1:2 (Additive)[c]	57.03	± 7.02	69.26	± 6.6	8.97	10.09	4.04 E-03	1.68 E-02	9.73 E-04
1:2 (Non-cooperative)	Fit Failed								
1:2 (Statistical)	Fit Failed								
2:1 (Full)[d]	89.67	± 3.31	-86.70	± -4.99	10.35	13.14	3.63 E-03	1.74 E-02	1.04 E-03
2:1 (Additive)[e]	89.14	± 3.33	-90.45	± -5.09	10.35	12.86	4.23 E-03	1.74 E-02	1.04 E-03
2:1 (Non-cooperative)	Fit Failed								
2:1 (Non-cooperative)	Fit Failed								

[a] http://app.supramolecular.org/bindfit/view/b365fb3f-4c7d-4b0a-b750-7ea3f3c1c897
[b] http://app.supramolecular.org/bindfit/view/63e6a369-8f06-4cf6-acfe-996b31f03f51
[c] http://app.supramolecular.org/bindfit/view/8887866a-b70d-4818-a523-672ef7215f48
[d] http://app.supramolecular.org/bindfit/view/070ae36a-58b2-439c-9763-00dd084def5f
[e] http://app.supramolecular.org/bindfit/view/6b27c08c-621b-44eb-a750-1157a5ea67b1

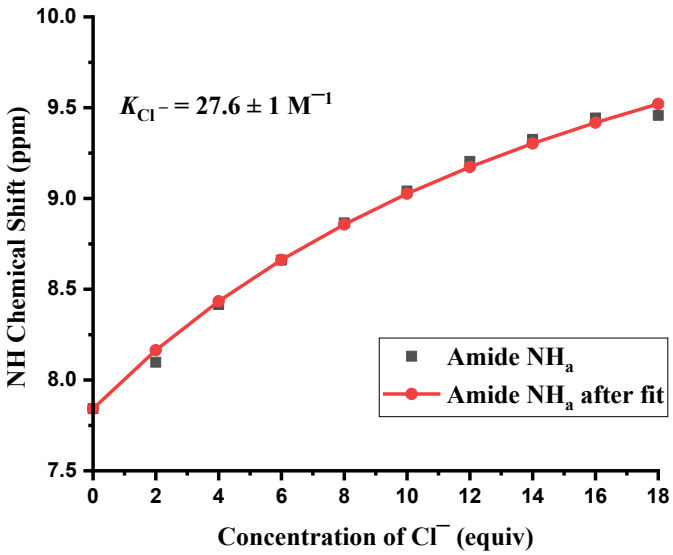
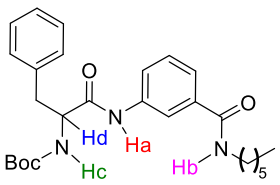


Figure S10. Comparison of experimental and theoretical amide NH shift of **6d** obtained upon titration with TBACl. Data fit to 1:1model. The average association constant obtained after two experiments is showed as inset.

Chloride binding studies with 6e

Tetrabutylammonium chloride used as anion source. To check the chloride binding 0 -18 equivalents of tetrabutylammonium chloride were added to the the hexyl amide pheneylalanine scaffold **6e**.



6e

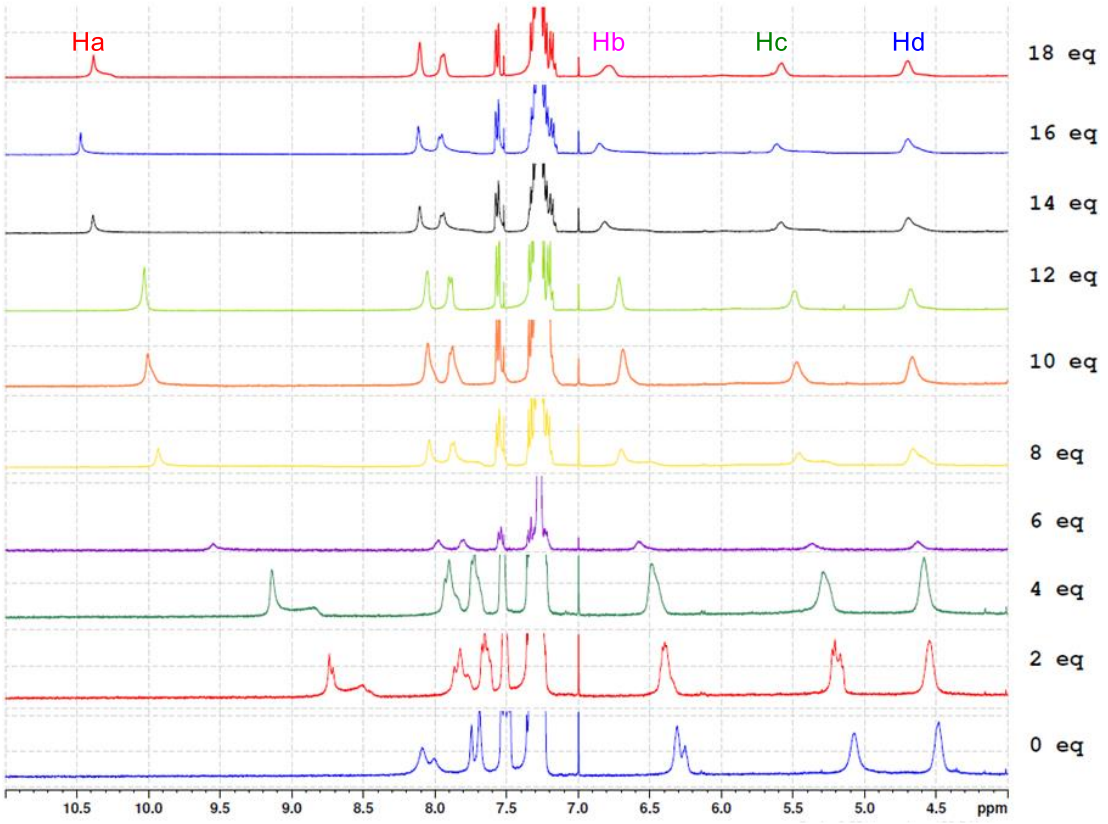


Figure S11. Stacked ^{1}H NMR plot for titration of peptide **6e** (2 mM) with TBACl (equiv. indicated on stacked plot) in $CDCl_3$.

Table S3. Representative data obtained for NMR titration of macrocycle **6e** with TBACl

Conc. 6e (M)	Conc. Cl^- (M)	Equiv. guest	proton Ha (ppm)	proton Hb (ppm)	proton Hc (ppm)	proton Hd (ppm)
0.002	0	0	8.043	6.282	5.076	4.485
0.002	0.004	2	8.723	6.394	5.189	4.544
0.002	0.008	4	9.136	6.489	5.283	4.586
0.002	0.012	6	9.55	6.571	5.366	4.627
0.002	0.016	8	9.934	6.696	5.455	4.663
0.002	0.02	10	10.005	6.684	5.472	4.669
0.002	0.024	12	10.029	6.713	5.49	4.674
0.002	0.028	14	10.389	6.82	5.59	4.692
0.002	0.032	16	10.472	6.849	5.608	4.698
0.002	0.036	18	10.383	6.784	5.579	4.698

Table S4. Representative comparison of association constants obtained with different binding models using Bindfit Software.

Host: Guest	K/K_{11} (M^{-1})	Error (%)	K_{12}/K_{21} (M^{-1})	Error (%)	δHG (ppm)	δHG_2 / δH_2G (ppm)	SSR	RMS	Covariance

1:1[a]	66.81	± 4.24			11.55		0.0871	0.0812	1.13 E-02
1:2 (Full)[b]	64.47	± 13.7	47.62	± 44.58	11.19	10.68	0.0737	0.0774	1.03 E-02
1:2 (additive)[c]	140.61	± 22.90	67.27	± 17.43	9.57	11.1	0.08	0.078	1.05 E-02
1:2 (Non-cooperative)	Fit failed								
1:2 (statistical)	Fit failed								
2:1(Full)[d]	76.84	± 6.86	-149.55	± -18.90	11.05	8.82	0.0738	0.0776	1.03 E-02
2:1 (additive)[e]	122.99	± -16.81	-62.06	± -42.07	11.24	14.44	0.0815	0.0784	1.05 E-02
2:1 (Non-cooperative)	Fit failed								
2:1 (statistical)	Fit failed								

[a] http://app.supramolecular.org/bindfit/view/2cb8f5ed-e7c0-4853-a1a1-2074401578a8
[b] http://app.supramolecular.org/bindfit/view/79d0d024-b38b-4274-bde4-42194b04127a
[c] http://app.supramolecular.org/bindfit/view/0a5cec07-f83a-4e6c-91de-f500b3f9c6e6
[d] http://app.supramolecular.org/bindfit/view/1e7c0fa5-ac6c-42eb-90a9-54924acec09f
[e] http://app.supramolecular.org/bindfit/view/2c2457df-9273-4fc8-8318-9edbf83f0762

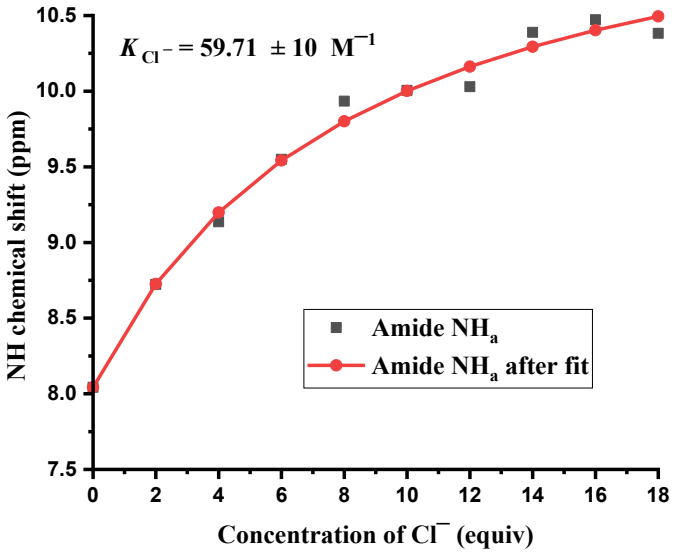
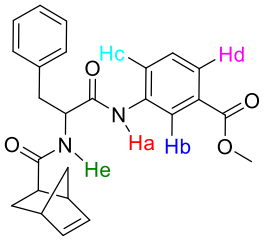


Figure S12. Comparison of experimental and theoretical amide NH shift of **6e** obtained upon titration with TBACl. Data fit to 1:1model. The average association constant obtained after two experiments is showed as inset.

Chloride binding studies with 8a

Tetrabutylammonium chloride used as anion source. To check the chloride binding 0 -20 equivalents of tetrabutylammonium chloride were added to the the norborneneamide pheneylalanine scaffold **8a**.



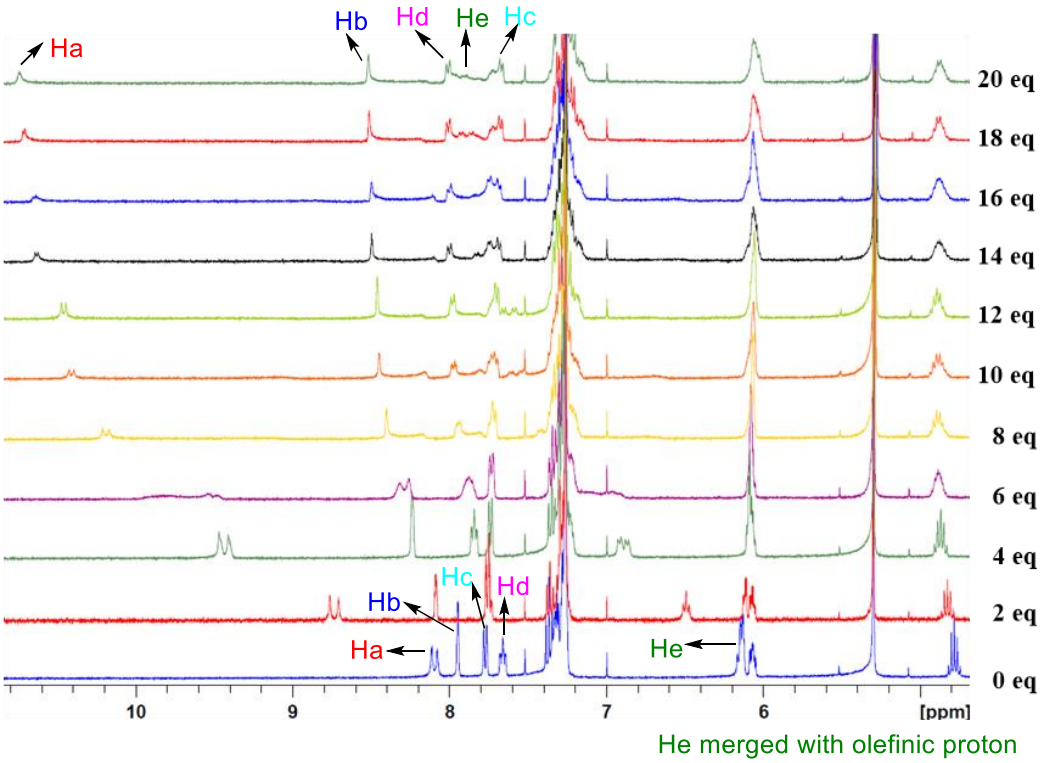


Figure S13. Stacked ^{1}H NMR plot for titration of peptide **8a** (2 mM) with TBACl (equiv. indicated on stacked plot) in $CDCl_3$.

Table S5. Representative data obtained for NMR titration of peptide **8a** with TBACl

Conc. 8a (M)	Conc. Cl^- (M)	Equiv. guest	proton Ha (ppm)	proton Hb (ppm)	proton Hc (ppm)	proton Hd (ppm)	Proton He (ppm)
0.002	0	0	8.102	7.947	7.771	7.6603	6.069
0.002	0.004	2	8.735	8.0857	7.759	7.7478	6.491
0.002	0.008	4	9.435	8.237	7.739	7.841	6.888
0.002	0.012	6	9.509	8.292	7.733	7.8752	7.236
0.002	0.016	8	10.191	8.4	7.715	7.946	7.39
0.002	0.020	10	10.414	8.447	7.705	7.973	7.575
0.002	0.024	12	10.461	8.461	7.698	7.9806	7.618
0.002	0.028	14	10.633	8.495	7.688	7.999	7.79
0.002	0.032	16	10.65	8.497	7.684	8	7.843
0.002	0.036	18	10.714	8.512	7.674	8.0078	7.889
0.002	0.040	20	10.744	8.516	7.67	8.0093	7.94

Table S6. Representative comparison of association constants obtained with different binding models using Bindfit Software.

Host: Guest	***K*/*K*$_{11}$ (M^{-1})**	**Error (%)**	***K*$_{12}$/ *K*$_{21}$ (M^{-1})**	**Error (%)**	**δHG (ppm)**	**δHG$_2$ / δH$_2$G (ppm)**	**SSR**	**RMS**	**Covariance**
1:1[a]	69.11 M^{-1}	± 3.8372 %			11.8911		0.18 06	0.1185	1.9143e-2
1:2 (Full)[b]	20.65 M^{-1}	± 4.0284 %	69.97 M^{-1}	± 11.9800 %	16.9984	9.6608	0.1111	0.0938	1.2014e-2
1:2 (non-cooperative)[c]	426.79 M^{-1}	± 4.0983 %			8.5661	11.4263	0.1269	0.1030	1.4496e-2
1:2 (additive)[d]	101.82 M^{-1}	22.6229 %	127.45 M^{-1}	17.4078 %	9.6474	11.1927	0.1219	0.0992	1.3448e-2
2:1 (Full)[e]	41.73 M^{-1}	± 6.0658 %	- 180.3 M^{-1}	- 2.4294 %	10.3915	5.1003	0.1100	0.0934	1.1909e-2
2:1 (additive)[f]	170.15 M^{-1}	9.9484 %	- 93.72 M^{-1}	- 16.6052 %	11.4319	14.7619	0.1290	0.1030	1.4490e-2
1:2 (statistical)	Fit Failed								
2:1 (non-cooperative)	Fit Failed								
2:1(statistical)	Fit Failed								

[a]http://app.supramolecular.org/bindfit/view/3a63af93-3268-43d5-86f5-76c40a82e583
[b]http://app.supramolecular.org/bindfit/view/04ffe578-f65a-4cd3-8809-842f872f7fbd
[c]http://app.supramolecular.org/bindfit/view/7a4b6d0c-150e-4001-81a7-31a0f4599ac4
[d]http://app.supramolecular.org/bindfit/view/70de32b8-2777-4170-a3b1-39632f48de58
[e]http://app.supramolecular.org/bindfit/view/d444d319-6501-465a-907e-6783707f9971
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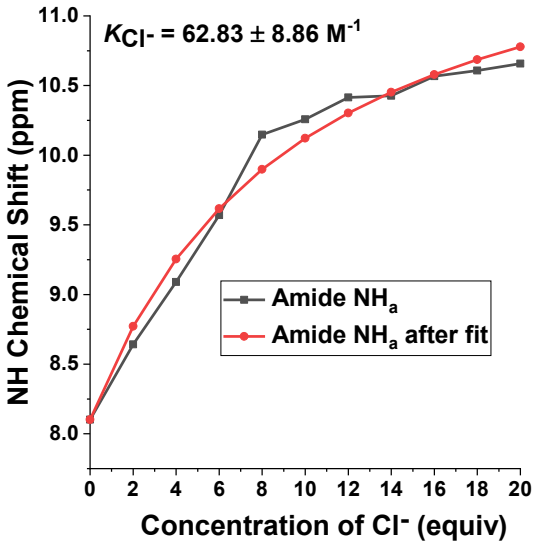
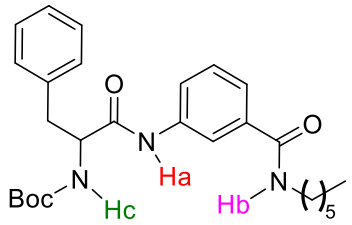


Figure S14. Comparison of experimental and theoretical amide NH shift of **8a** obtained upon titration with TBACl. Data fit to 1:1model. The average association constant obtained after two experiments is showed as inset.

Nitrate binding studies with 6e

Tetrabutylammonium nitrate used as anion source. To check the nitrate binding 0 -18 equivalents of tetrabutylammonium chloride were added to the the hexyl amide pheneylalanine scaffold **6e**.



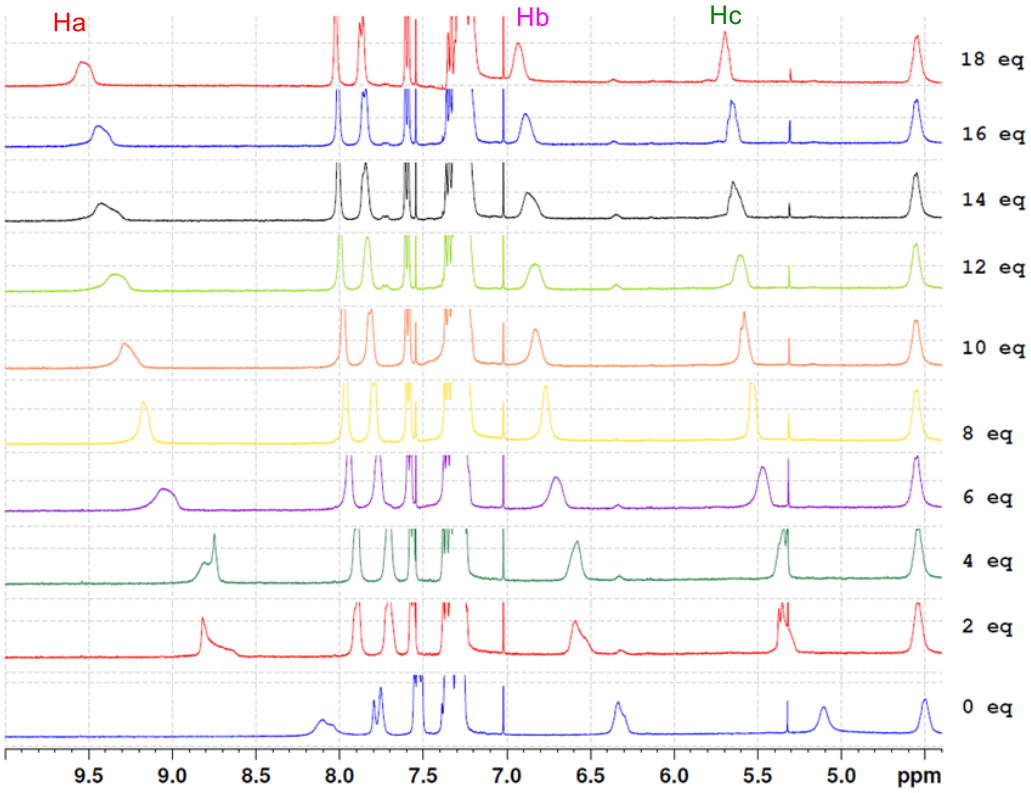


Figure S15. Stacked ^{1}H NMR plot for titration of peptide **6e** (2 mM) with $TBANO_3$ (equiv. indicated on stacked plot) in $CDCl_3$.

Table S7. Representative data obtained for NMR titration of macrocycle **6e** with $TBANO_3$

Conc. 6e (M)	Conc. NO_3^- (M)	Equiv. guest	proton Ha (ppm)	proton Hb (ppm)	proton Hc (ppm)
0.002	0	0	8.074	6.321	5.108
0.002	0.004	2	8.728	6.546	5.337
0.002	0.008	4	8.791	6.581	5.346
0.002	0.012	6	9.044	6.78	5.469
0.002	0.016	8	9.166	6.771	5.536
0.002	0.02	10	9.272	6.828	5.589
0.002	0.024	12	9.329	6.837	5.682
0.002	0.028	14	9.37	6.863	5.633
0.002	0.032	16	9.427	6.89	5.65
0.002	0.040	20	9.525	6.934	5.694

Table S8. Representative comparison of association constants obtained with different binding models using Bindfit Software.

Host: Guest	K/K_{11} (M^{-1})	Error (%)	K_{12}/K_{21} (M^{-1})	Error (%)	δHG (ppm)	δHG_2 / δH_2G (ppm)	SSR	RMS	Covariance
1:1[a]	118.49	± 7.84			9.81		0.0523	0.0553	1.75 E-02

1:2 (Full)[b]	477 E 14	± 398 E 08	43.25	± 7.08	8.57	10.16	3.12 E-02	3.53 E-02	7.19 E-03
2:1[c]	642.78	± 348.33	19246. 74	± - 365.52	11.32	8.27		3.89 E-02	8.78 E-03
	[a] http://app.supramolecular.org/bindfit/view/62318897-9983-418e-af3b-9e98faa02ef0 [b] http://app.supramolecular.org/bindfit/view/0c2d13ca-5ba7-410f-aeb1-3eb4a4a3b6f1 [c] http://app.supramolecular.org/bindfit/view/e5cf1895-0813-464b-9377-9a868709199a								

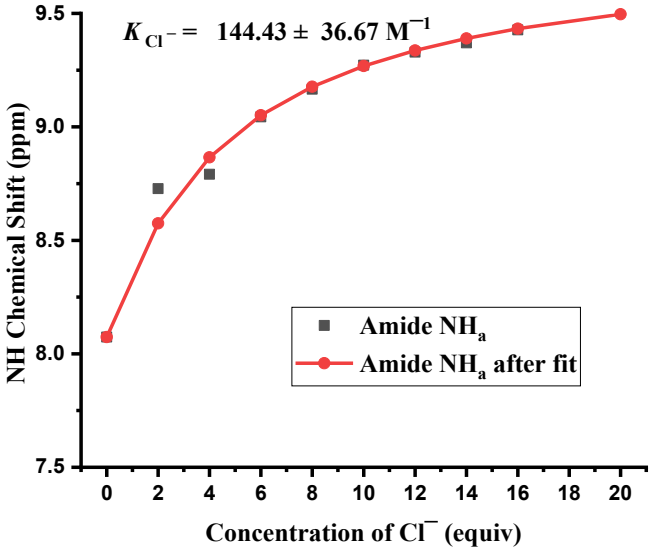
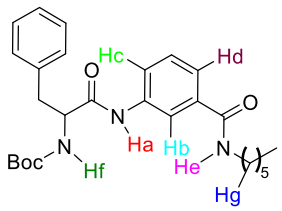


Figure S16. Comparison of experimental and theoretical amide NH shift of **6e** obtained upon titration with $TBANO_3$. Data fit to 1:1model. The average association constant obtained after two experiments is showed as inset.

Phosphate binding studies with 6e

Tetrabutylammonium phosphate used as anion source. To check the phosphate binding 0 to 5 equivalents of tetrabutylammonium phosphate were added to the the hexyl amide pheneylalanine scaffold **6e**.



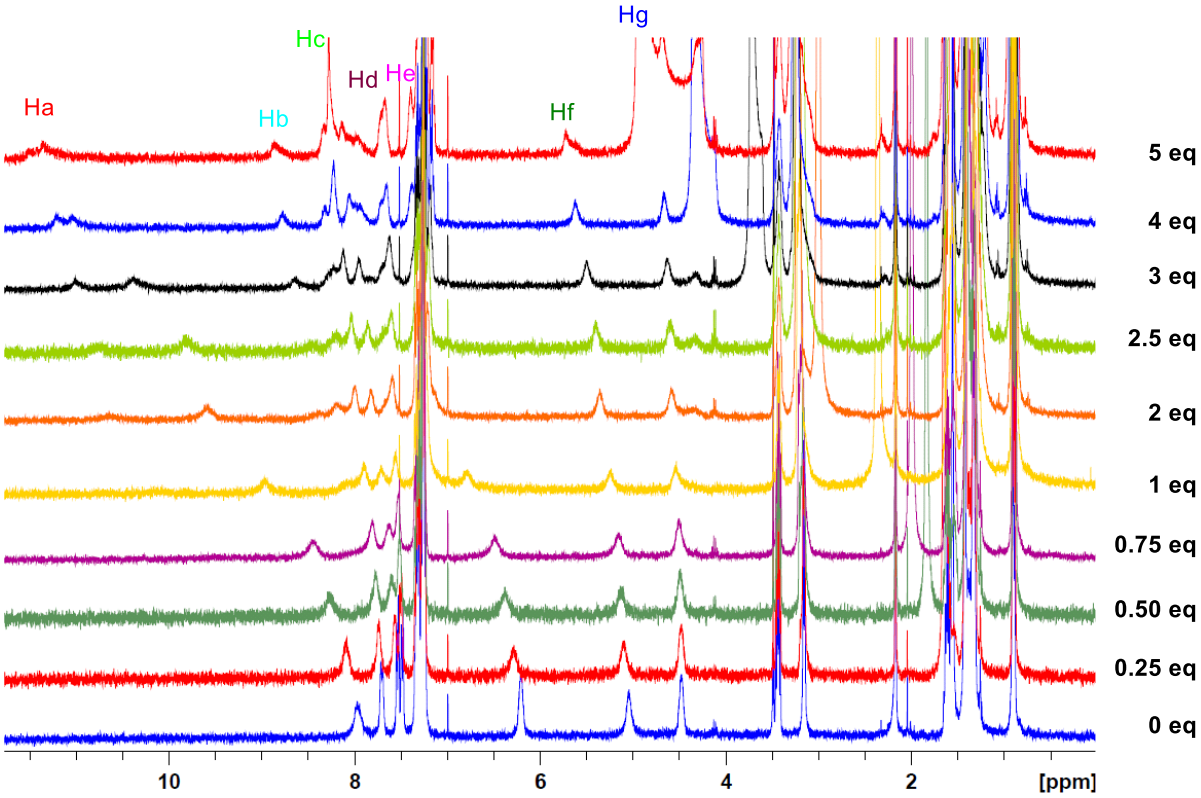


Figure S17. Stacked ^{1}H NMR plot for titration of peptide **6e** (2 mM) with $TBAH_2PO_4$ (equiv. indicated on stacked plot) in $CDCl_3$.

Table S9. Representative data obtained for NMR titration of peptide **6e** with $TBAH_2PO_4$

Conc. 6e (M)	Conc. PO_4^{3-} (M)	Equiv. guest	Proton Ha	Proton Hb	Proton Hc	Proton Hd	Proton He	Proton Hf	Proton Hg
0.002	0	0	7.969	7.078	7.54	7.487	6.212	5.048	1.556
0.002	0.0005	0.25	8.093	7.742	7.567	7.501	6.29	5.102	1.65
0.002	0.001	0.50	8.265	7.774	7.606	7.519	6.382	5.127	1.83
0.002	0.0015	0.75	8.45	7.805	7.634	7.528	6.496	5.154	1.997
0.002	0.002	1.0	8.968	7.897	7.713	7.56	6.786	5.237	2.37
0.002	0.004	2.0	9.588	7.996	8.003	7.591	7.213	5.354	3.009
0.002	0.005	2.5	9.806	8.032	7.854	7.601	7.59	5.398	3.206
0.002	0.006	3.0	10.371	8.124	7.955	7.627	7.863	5.502	3.726
0.002	0.008	4.0	11.103	8.226	8.059	7.655	7.951	5.527	4.345
0.002	0.01	5.0	11.426	8.282	8.138	7.701	8.062	5.725	4.946

Table S10. Representative comparison of association constants obtained with different binding models using Bindfit Software.

Host: Guest	***K*/*K*$_{11}$ (M^{-1})**	**Error (%)**	***K*$_{12}$/*K*$_{21}$ (M^{-1})**	**Error (%)**	**δHG (ppm)**	**δHG$_2$ / δH$_2$G (ppm)**	**SSR**	**RMS**	**Covariance**
1:1[a]	43.78 M^{-1}	± 4.8117 %			19.8866		1.6323	0.1287	1.0550e-2
1:2 (Full)[b]	3971.85 M^{-1}	± 73.4974 %	71.03 M^{-1}	± 6.5290 %	8.7740	16.8974	0.6237	0.1104	8.5031e-3
1:2 (non-cooperative)[c]	607.44 M^{-1}	± 4.7765 %			9.0424	14.3762	0.6450	0.1088	8.2916e-3
1:2 (additive)[d]	172.70 M^{-1}	± 28.7129 %	97.44 M^{-1}	± 40.5013 %	11.3578	14.7465	1.6214	0.1218	9.6234e-3
2:1 (Full)[e]	0.18 M^{-1}	± 13.9342 %	3091936.81 M^{-1}	± 14.7896 %	13557.6175	6.8023	0.4609	0.1072	8.0508e-3
2:1 (non-cooperative)	Fit failed								
2:1 (additive)[f]	136.06 M^{-1}	± 26.8036 %	-60.52 M^{-1}	± -47.9707 %	15.6804	23.3918	1.6221	0.1222	9.6846e-3
1:2 (statistical)	Fit Failed								
2:1(statistical)	Fit Failed								

[a]http://app.supramolecular.org/bindfit/view/be684518-37c8-4fec-9165-c3717260e77f

[b]http://app.supramolecular.org/bindfit/view/b2eba636-4

[c]http://app.supramolecular.org/bindfit/view/b2eba636-4 a7a-45da-ab75-782005fdc37c

[d]http://app.supramolecular.org/bindfit/view/b2eba636-4 a7a-45da-ab75-782005fdc37c

[e]http://app.supramolecular.org/bindfit/view/b2eba636-4 a7a-45da-ab75-782005fdc37c

[f]http://app.supramolecular.org/bindfit/view/b2eba636-4

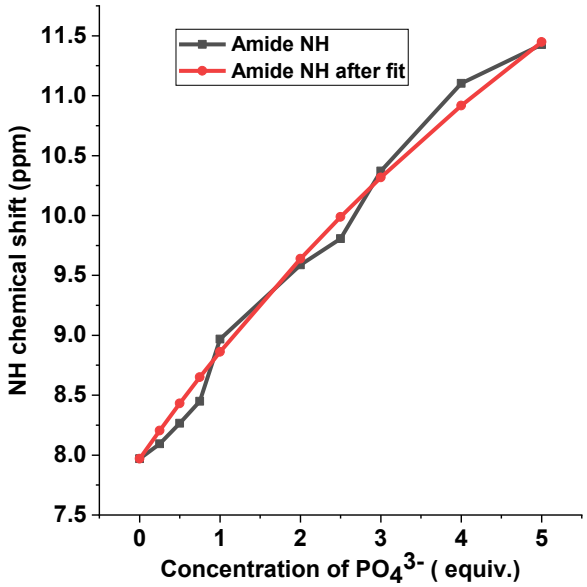


Figure S18. Comparison of experimental and theoretical amide NH shift of **6e** obtained upon titration with $TBAH_2PO_4$. Data fit to 1:1model. The average association constant obtained after two experiments is showed as inset.

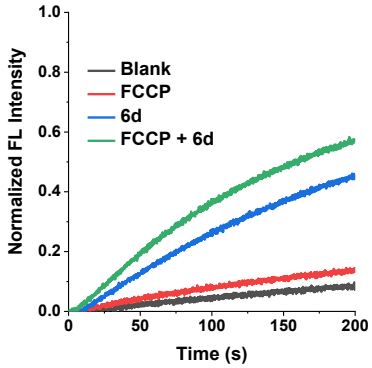
HPTS assays for mechanistic studies

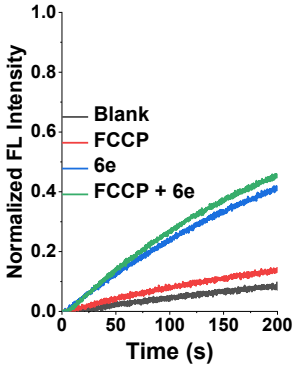
Assay in the presence of FCCP

Vesicles were prepared as described above (page no. S13). The external buffer used was NaCl. For the assay, a solution of FCCP (10 µL, 30 nM) in DMSO was added at t = 50 s and then a solution of dipeptide **6d, 6e** and **8a** (3 mol% with respect to lipid, 10 µL) in THF was added at t = 100 s. Triton-X (20 µL, 20 %) was added at 300 s to lyse the vesicle and get maximum fluorescence intensity (maximum deprotonated HPTS concentration) (Figure S19). Time t =

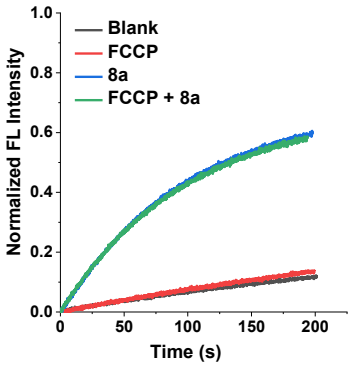
100 s was normalized to 0 which is time of peptide addition and t = 350 s was normalized to 1 which is corresponds to intensity maxima after addition of Triton-X addition.

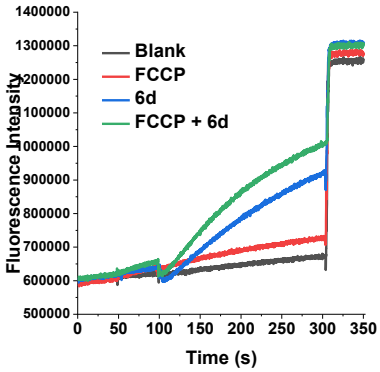
a) b)



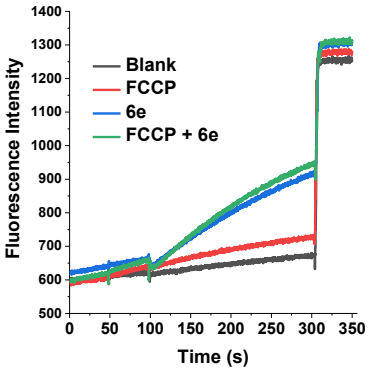


c) d)





e) f)



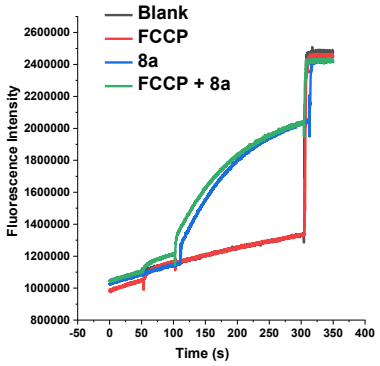


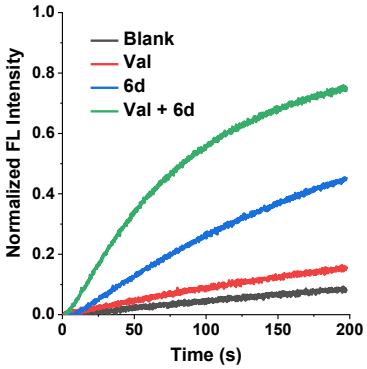
Figure S19. Ion transport activity in presence of proton transporter FCCP a) normalized plot of dipeptide **6d** b) normalized plot of dipeptide **8a** c) raw data of dipeptide **6d** d) raw data of dipeptide **6e** e) raw data of dipeptide **8a**), (dipeptide = 4.53 µM, 3 mol% with respect to lipid, Experiment was

repeated two times in given condition) Time t = 0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to time before Triton-X addition. Peptide was added as a solution in THF and FCCP was added as a solution in DMSO.

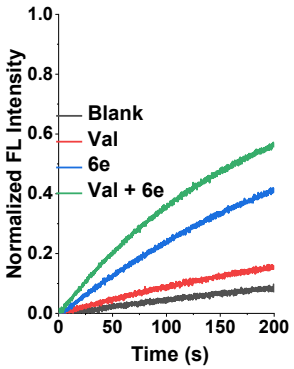
Assay in the presence of Valinomycin

The assay procedure described above for FCCP was carried out, the only change that addition of valinomycin (10 µL, 1 nM) at *t* = 50 s and extravesicular KCl buffer instead of FCCP and NaCl buffer. Time t = 100 s was normalized to 0 which is time of peptide addition and t = 350 s was normalized to 1 which is corresponds to intensity maxima after addition of Triton-X addition.

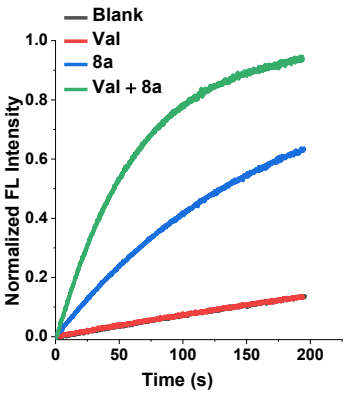
a)



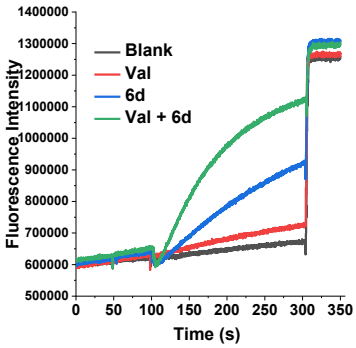
b)



c)



d)



e)

f)

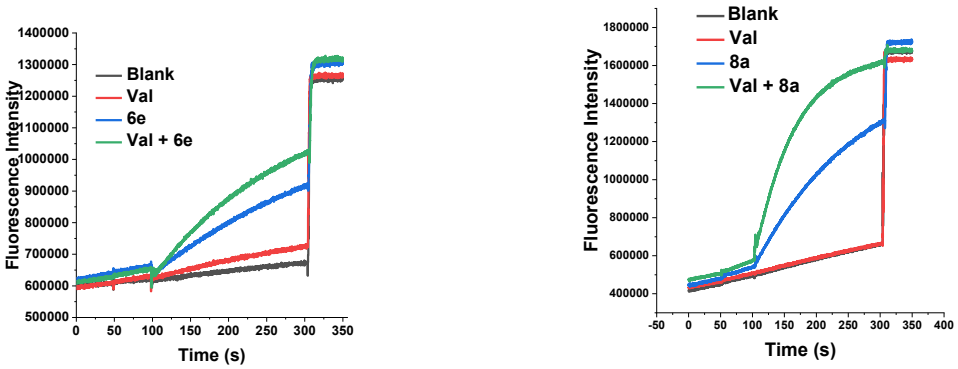


Figure S20. Ion transport activity in presence of K^+ transporter valinomycin a) Normalized plot of dipeptide **6d** b) normalized plot of dipeptide **8a** c) raw data of dipeptide **6d** d) raw data of dipeptide **6e** e) raw data of dipeptide **8a,** (dipeptide = 4.53 µM, 3 mol% with respect to lipid, Experiment was repeated two times in given condition). Time t =0 s in the normalized graphs corresponds to the time of peptide addition and t = 200 s in the normalized plot corresponds to intensity maxima before Triton-X addition. Peptide was added as a solution in THF and FCCP was added as a solution in DMSO.

DPPC assay to determine carrier mechanism[5]

Using Lucigenin Dye

Vesicle preparation

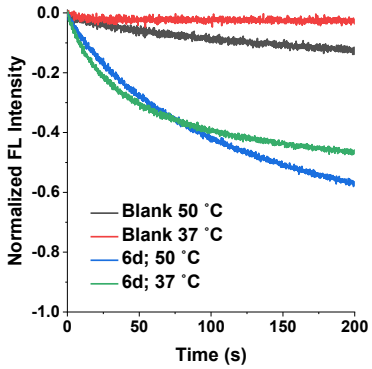
DPPC lipid in chloroform (25 mg in 1 mL) was taken in a 10 mL round bottom flask. Chloroform was removed using a stream of nitrogen and further kept *in vacuo* for 3 h at 0 ºC to form a thin lipid film. The lipid film was hydrated by a solution of lucigenin dye (1 mL of 1 mM lucigenin, 10 mM sodium phosphate (pH 6.4), 100 mM $NaNO_3$). The resulting mixture was allowed to stir at room temperature for 1 h at 45 ºC and was then subjected to seven freeze-thaw cycles. The suspension was extruded 19 times through 0.2 µm polycarbonate membranes at 45 ºC using a mini-extruder obtained from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: 10 mM sodium phosphate (pH 6.4), 100 mM $NaNO_3$ buffer). The milky white vesicle solution was collected and diluted to 3.1 mL to get the 11 mM of lipid stock solution, assuming 100 % collection of lipid during the size exclusion chromatography.

Assay

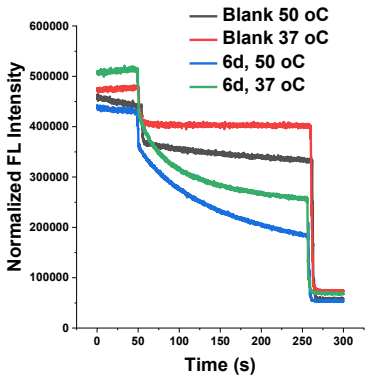
A solution of dipeptide in THF (5 mol% with respect to lipid, 10 µL) and 100 µL of lucigenin containing vesicle was added to 1900 µL aqueous sodium phosphate and sodium nitrate

solution (10 mM, 100 mM respectively) in a fluorescence cuvette to generate chloride gradient across lipid bilayer. This cuvette was placed inside the spectrofluorometer equipped with magnetic stirrer 50 °C for 2 min to allowed to equilibrate to ensure peptide insertion prior to starting the experiment. The experiment was carried out at 50 °C or 20 °C to check fluidity dependant ion transport activity. For the fluorescence measurement, excitation and emission wavelength used were 455 nm and 505 nm, respectively. An aqueous solution of NaCl (20 µL, 4 M) was added at t = 50 s. At t = 250 s, Triton-X (20%, 20 µL) was added to lyse the vesicle and achieve the maximum fluorescence quenching. The maximum quenching intensity obtained was used to normalize the fluorescence intensity (Figure S11). The time t = 0 s in the normalized graphs (Figure S11 a) corresponds to the time of NaCl addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

a)



b)



c)

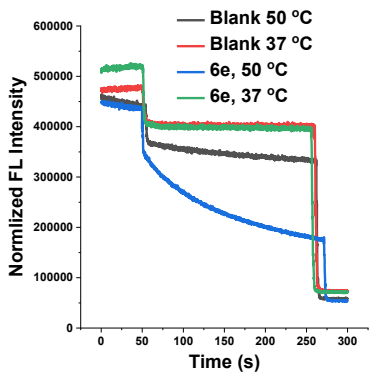


Figure S21. Effect of temperature on lucigenin assay with dipeptide in DPPC lipid membrane (Tc = 41 °C) to confirm carrier mechanism. a) Normalized plot of **6d** b) raw data of **6d** c) raw data of **6e,** Time t = 0 s in the normalized graphs corresponds to the time of NaCl addition and

t = 200 s in the normalized plot corresponds to time before Triton-X addition. Dipeptide = 5 mol%, THF was used to dissolve the peptide.

Using HPTS Dye

Vesicle preparation

DPPC lipid in chloroform (25 mg in 1 mL) was taken in a 10 mL round bottom flask. Chloroform was removed using a stream of nitrogen and further kept *in vacuo* for 3 h at 0 ºC to form a thin lipid film. The lipid film was hydrated by a solution of lucigenin dye (1 mL of 0.5 mM, 10 mM HEPES (pH=7.0), 100 mM NaCl). The resulting mixture was allowed to stir at room temperature for 1 h at 45 ºC and was then subjected to ten freeze-thaw cycles. The suspension was extruded 21 times through 0.2 µm polycarbonate membranes at 45 ºC using a mini-extruder obtained from Avanti Polar Lipids. The extra-vesicular dye was removed by size exclusion chromatography using Sephadex G-50 (eluent: 10 mM HEPES (pH=6.4), 100 mM NaCl buffer). The milky white vesicle solution was collected and diluted to 3.2 mL.

Assay

A solution of dipeptide in THF (5 mol% with respect to lipid, 10 µL) and 30 µL of HPTS containing vesicle was added to 1970 µL sodium chloride- HEPES (10 mM HEPES, 100 mM NaCl) in a fluorescence cuvette to generate chloride gradient across lipid bilayer. This cuvette was placed inside the spectrofluorometer equipped with magnetic stirrer 50 ºC for 2 min to allowed to equilibrate to ensure peptide insertion prior to starting the experiment. The experiment was carried out at 50 ºC or 20 ºC to check fluidity dependant ion transport activity. For the fluorescence measurement, excitation and emission wavelength used were 455 nm and 505 nm, respectively. An aqueous solution of NaCl (20 µL, 0.5 M) was added at t = 50 s. At t = 250 s, Triton-X (20%, 20 µL) was added to lyse the vesicle and achieve the maximum fluorescence quenching. The maximum quenching intensity obtained was used to normalize the fluorescence intensity (Figure S12). The time t = 0 s in the normalized graphs (Figure S12 a) corresponds to the time of NaCl addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition.

a) b)

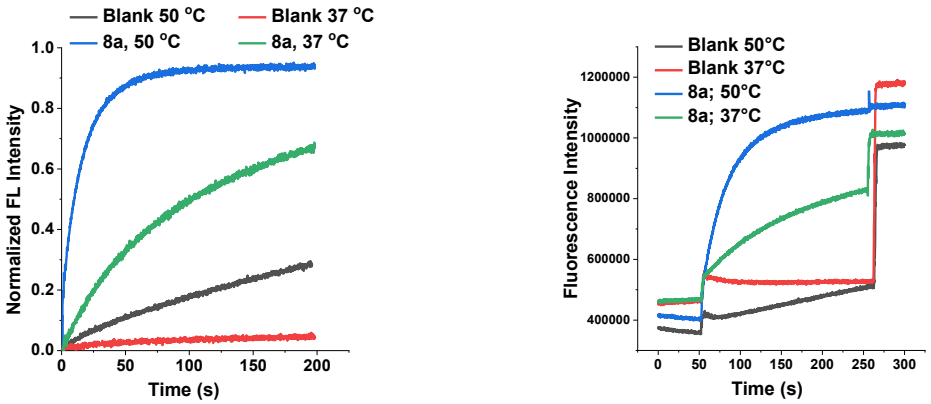


Figure S22. Effect of temperature on HPTS assay with dipeptide in DPPC lipid membrane (T_c = 41 °C) to confirm carrier mechanism. a) Normalized plot of **8a** b) raw data of **8a.** Time t = 0 s in the normalized graphs corresponds to the time of NaCl addition and t = 200 s in the normalized plot corresponds to the time before Triton-X addition. Dipeptide = 5 mol% with respect to lipid, THF was used to dissolve the peptide.

U-tube experiment to determine carrier mechanism

A solution of peptide **6e** in dichloromethane (1 mM,10 mL) was added to a glass U-tube in such a way that the solution height is above the bent portion of the U-tube. In this experiment, the organic phase mimics the role of a lipid membrane and separates the two aqueous phases. An aqueous solution of NaCl (100 mM, 8 mL) was added to one arm of the tube, which is labelled as source and water (8 mL) was added to the other arm labelled as the receiver arm. Aliquots (0.2 mL) were collected from the receiver arm at different time intervals and the concentration of Cl^- was measured using a chloride electrode. Similar experiment carried out using dichloromethane instead of peptide **6e** solution in dichloromethane to check the background transport.

Chloride detection through ion selective electrode

Collected aliquots (0.2 mL) were diluted to 2 mL using deionised water. Ionic strength adjuster (ISA, 40 µL) was added to the resultant mixture under continuous stirring. The chloride concentration was determined using the ISE. Same procedure was repeated for every aliquot at time periods as indicated in Figure S13b.

a) b)

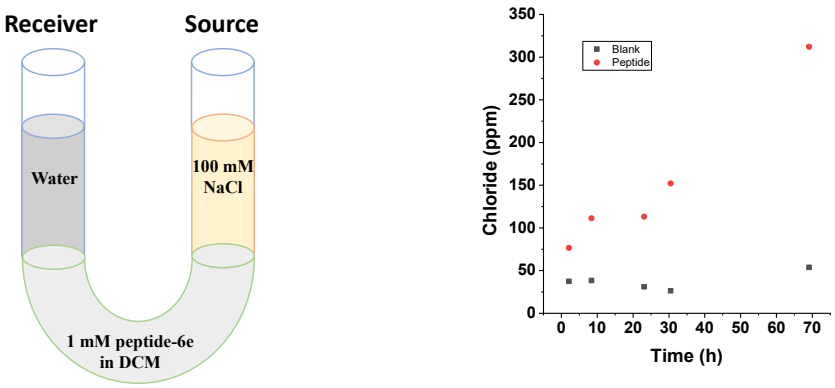
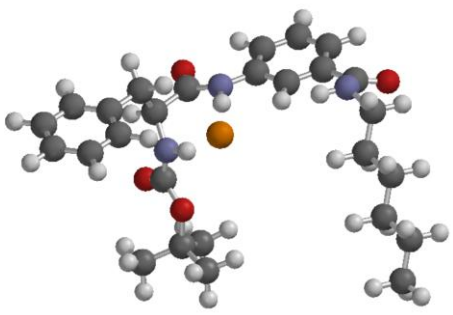


Figure S23. a) Schematic representation of U-tube experiment b) concentration of chloride with time in receiver arm

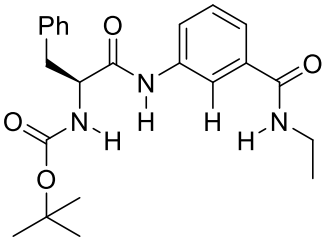
Computational studies to understand anion-complexation of 6e

Geometry optimization was first carried out with the uncomplexed **6e** using MMFF/STO-3G method using PC SPARTAN PRO program. The minimized structure was used to obtained the structure of **6e** complexed with chloride using PC SPARTAN PRO program. Geometry optimization was first done using molecular mechanics (MMFF), following which Hartree-Fock (STO-3G) method was used (Figure S24a). The energy minimum was confirmed by ensuring the absence of imaginary frequencies. The x, y, z coordinates are given in Table S11 and the list of vibrational frequencies (first 50 lines) is given in Table S12. A similar analysis was done for binding of nitrate ion, with the exception that a truncated molecule (Figure S24b) was used to get the optimized geometry of the complex (Figure S24c) using MMFF/PM3/STO-3G method. The energy minimum was confirmed by ensuring the absence of imaginary frequencies. The x, y, z coordinates are given in Table S13 and the list of vibrational frequencies (first 50 lines) is given in Table S14.

a)



b) c)



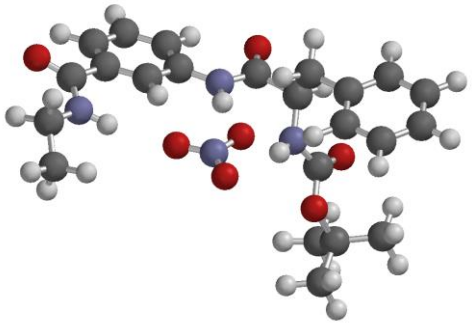


Figure S24. a) Equilibrium geometry of **6e**-chloride complex; b) Model compound used to study NO_3^- binding. c) Equilibrium geometry of **model peptide**-nitrate complex

Table S11. x, y, z coordinates for **6e**:chloride complex.

Number	Label	x	y	z
1	H	-0.446	-5.307	-4.011
2	C	-0.567	-4.631	-3.158
3	C	-0.879	-2.914	-0.998
4	C	-1.828	-4.442	-2.608
5	C	0.547	-3.977	-2.646
6	C	0.4	-3.108	-1.557
7	C	-1.984	-3.58	-1.52
8	H	-2.7	-4.958	-3.026
9	H	1.529	-4.145	-3.107
10	H	-0.994	-2.227	-0.135
11	C	-3.337	-3.359	-0.934
12	O	-4.384	-3.511	-1.553
13	N	-3.393	-3.036	0.458
14	C	-4.573	-2.35	1.022
15	H	-4.613	-2.636	2.092
16	H	-5.494	-2.752	0.554
17	C	-4.519	-0.83	0.883
18	H	-3.582	-0.43	1.323
19	H	-4.492	-0.542	-0.188
20	N	1.457	-2.409	-0.909
21	C	3.612	-1.15	-0.603
22	C	2.752	-2.161	-1.405
23	O	3.203	-2.747	-2.384
24	N	2.91	0.144	-0.403
25	C	3.064	1.156	-1.354
26	O	3.896	1.226	-2.256
27	O	2.152	2.134	-1.072
28	C	2.109	3.37	-1.809
29	C	1.771	3.153	-3.287

30	H	1.563	4.113	-3.777
31	H	2.596	2.676	-3.832
32	H	0.883	2.519	-3.406
33	C	3.401	4.18	-1.664
34	H	3.269	5.188	-2.078
35	H	3.691	4.288	-0.611
36	H	4.241	3.712	-2.195
37	C	0.955	4.13	-1.132
38	H	0.807	5.109	-1.607
39	H	0.009	3.578	-1.204
40	H	1.155	4.304	-0.066
41	H	-2.529	-2.644	0.791
42	H	4.56	-0.962	-1.168
43	C	3.953	-1.785	0.757
44	H	4.518	-2.721	0.577
45	H	3.022	-2.079	1.293
46	C	4.745	-0.878	1.641
47	C	6.199	0.785	3.34
48	C	6.136	-0.835	1.557
49	C	4.08	-0.078	2.575
50	C	4.81	0.749	3.42
51	C	6.86	-0.004	2.406
52	H	6.656	-1.452	0.816
53	H	2.977	-0.1	2.613
54	H	4.288	1.378	4.148
55	H	7.951	0.029	2.336
56	H	6.77	1.438	4.007
57	H	2.051	0.077	0.22
58	H	1.137	-1.808	-0.091
59	C	-5.72	-0.188	1.557
60	H	-5.754	-0.478	2.627
61	H	-6.658	-0.579	1.113
62	C	-5.678	1.326	1.432
63	H	-4.739	1.717	1.875
64	H	-5.644	1.617	0.363
65	C	-6.877	1.972	2.107
66	H	-6.91	1.682	3.176
67	H	-7.815	1.581	1.665
68	C	-6.837	3.479	1.983
69	H	-5.934	3.898	2.446
70	H	-7.703	3.943	2.472
71	H	-6.842	3.799	0.933
72	Cl	0.737	-0.657	1.175

Table S12. List of vibrational frequencies obtained for **6e**:chloride complex.

S.No	Frequency	Type

1	11.53	A
2	15.16	A
3	19.12	A
4	25.82	A
5	28.69	A
6	37.42	A
7	44.75	A
8	51.32	A
9	52.31	A
10	54.99	A
11	61.34	A
12	64.68	A
13	75.43	A
14	86.07	A
15	90.03	A
16	91.05	A
17	112.78	A
18	115.91	A
19	119.10	A
20	125.92	A
21	130.80	A
22	133.63	A
23	142.73	A
24	178.45	A
25	189.72	A
26	193.51	A
27	202.85	A
28	209.36	A
29	220.12	A
30	221.52	A
31	228.86	A
32	248.95	A
33	261.73	A
34	279.32	A
35	293.21	A
36	306.82	A
37	309.45	A
38	332.33	A
39	340.01	A
40	340.89	A
41	356.42	A
42	360.78	A
43	367.10	A
44	395.82	A
45	403.78	A
46	412.11	A

47	437.52	A
48	457.95	A
49	473.40	A
50	478.97	A

Table S13. x, y, z coordinates for **model-system**:nitrate complex.

Number	Label	x	y	z
1	H	4.245	-3.156	-3.434
2	C	3.937	-2.646	-2.51
3	C	3.156	-1.379	-0.155
4	C	4.894	-2.306	-1.56
5	C	2.589	-2.348	-2.317
6	C	2.18	-1.699	-1.135
7	C	4.499	-1.662	-0.378
8	H	5.958	-2.54	-1.715
9	H	1.849	-2.621	-3.086
10	C	5.555	-1.304	0.615
11	O	6.682	-1.856	0.586
12	N	5.279	-0.323	1.539
13	C	6.22	0.028	2.569
14	H	6.147	-0.723	3.411
15	H	7.263	-0.048	2.145
16	C	5.995	1.421	3.137
17	H	4.957	1.531	3.537
18	H	6.156	2.202	2.356
19	N	0.859	-1.319	-0.861
20	C	-1.617	-1.07	-1.018
21	C	-0.277	-1.649	-1.564
22	O	-0.299	-2.387	-2.568
23	H	6.721	1.59	3.967
24	N	-1.553	0.361	-0.865
25	C	-2.398	1.175	-1.585
26	O	-3.122	0.865	-2.55
27	O	-2.345	2.48	-1.14
28	C	-3.057	3.506	-1.867
29	C	-2.563	3.604	-3.301
30	H	-2.99	4.516	-3.781
31	H	-2.876	2.703	-3.883
32	H	-1.449	3.671	-3.317
33	C	-4.558	3.267	-1.81
34	H	-5.092	4.151	-2.229
35	H	-4.878	3.114	-0.752
36	H	-4.829	2.359	-2.402

37	C	-2.696	4.777	-1.096
38	H	-3.23	5.643	-1.549
39	H	-1.595	4.952	-1.142
40	H	-2.994	4.672	-0.026
41	H	4.363	0.067	1.609
42	H	-2.403	-1.322	-1.795
43	C	-1.932	-1.825	0.288
44	H	-2.011	-2.914	0.032
45	H	-1.081	-1.696	1.016
46	C	-3.185	-1.353	0.933
47	C	-5.519	-0.474	2.199
48	C	-4.427	-1.887	0.572
49	C	-3.117	-0.372	1.932
50	C	-4.283	0.063	2.561
51	C	-5.589	-1.449	1.204
52	H	-4.483	-2.653	-0.215
53	H	-2.132	0.051	2.213
54	H	-4.223	0.833	3.344
55	H	-6.562	-1.871	0.915
56	H	-6.436	-0.129	2.697
57	H	-1.134	0.721	-0.028
58	H	0.75	-0.752	-0.032
59	H	2.816	-0.899	0.784
60	N	0.963	0.738	2.385
61	O	0.133	0.201	1.646
62	O	0.591	1.411	3.327
63	O	2.155	0.577	2.156

Table S14. List of vibrational frequencies obtained for **model-system**:nitrate complex.

S.No	Frequency	Type
1	17.42	A
2	22.27	A
3	28.63	A
4	34.83	A
5	39.04	A
6	42.56	A
7	47.17	A
8	49.56	A
9	50.43	A
10	56.69	A
11	66.11	A
12	68.34	A
13	73.66	A

14	84.51	A
15	90.63	A
16	99.72	A
17	104.41	A
18	114.11	A
19	119.74	A
20	130.15	A
21	147.60	A
22	150.18	A
23	157.44	A
24	158.73	A
25	181.15	A
26	199.17	A
27	206.60	A
28	210.84	A
29	219.02	A
30	225.70	A
31	260.81	A
32	267.65	A
33	280.04	A
34	325.45	A
35	346.88	A
36	364.58	A
37	368.85	A
38	369.19	A
39	381.32	A
40	383.83	A
41	410.85	A
42	424.03	A
43	450.28	A
44	460.71	A
45	486.34	A
46	490.15	A
47	504.64	A
48	527.12	A
49	548.39	A
50	553.46	A

Spectra of compounds

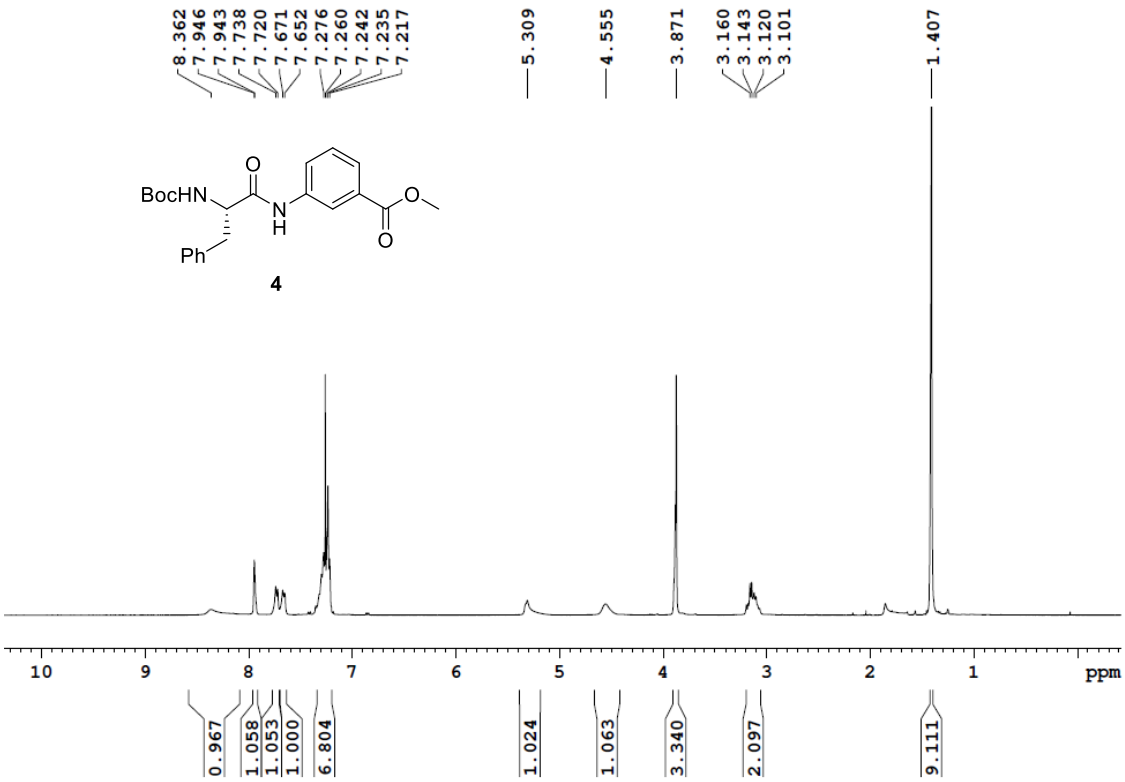


Figure S25 [1]H NMR spectrum of methyl ester phenylalanine dipeptide **4**

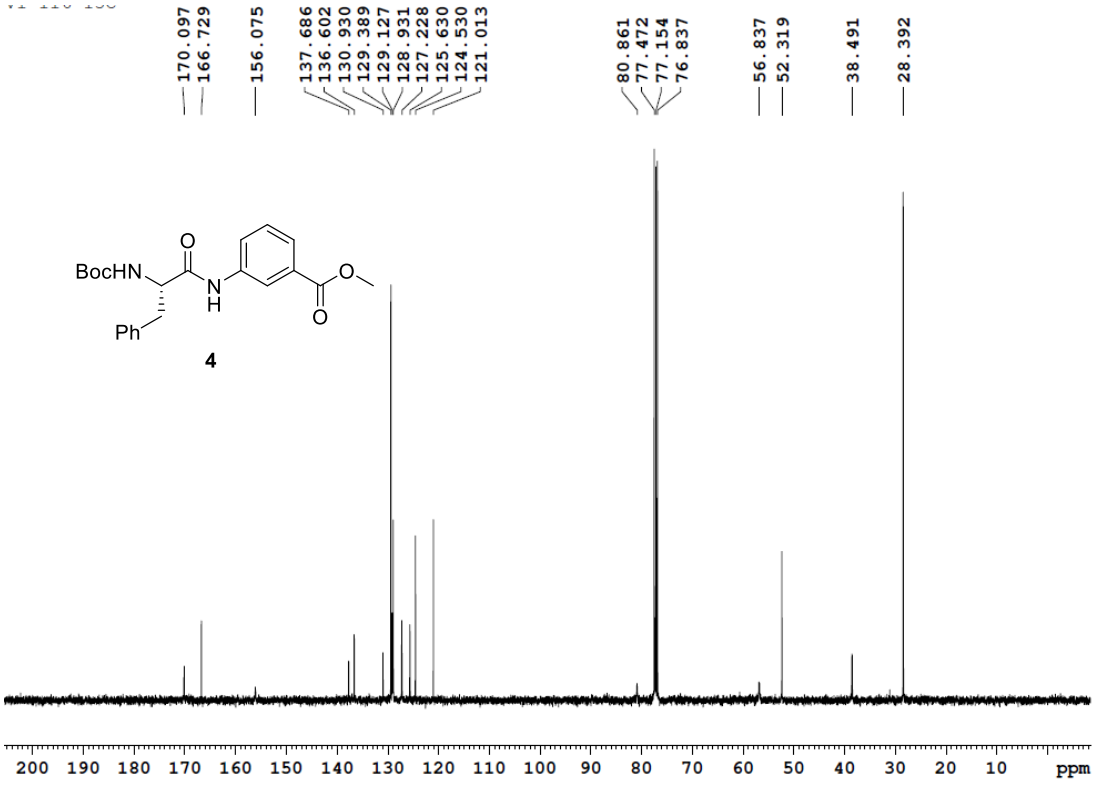


Figure S26. [13]C NMR spectrum of methyl ester phenylalanine dipeptide **4**

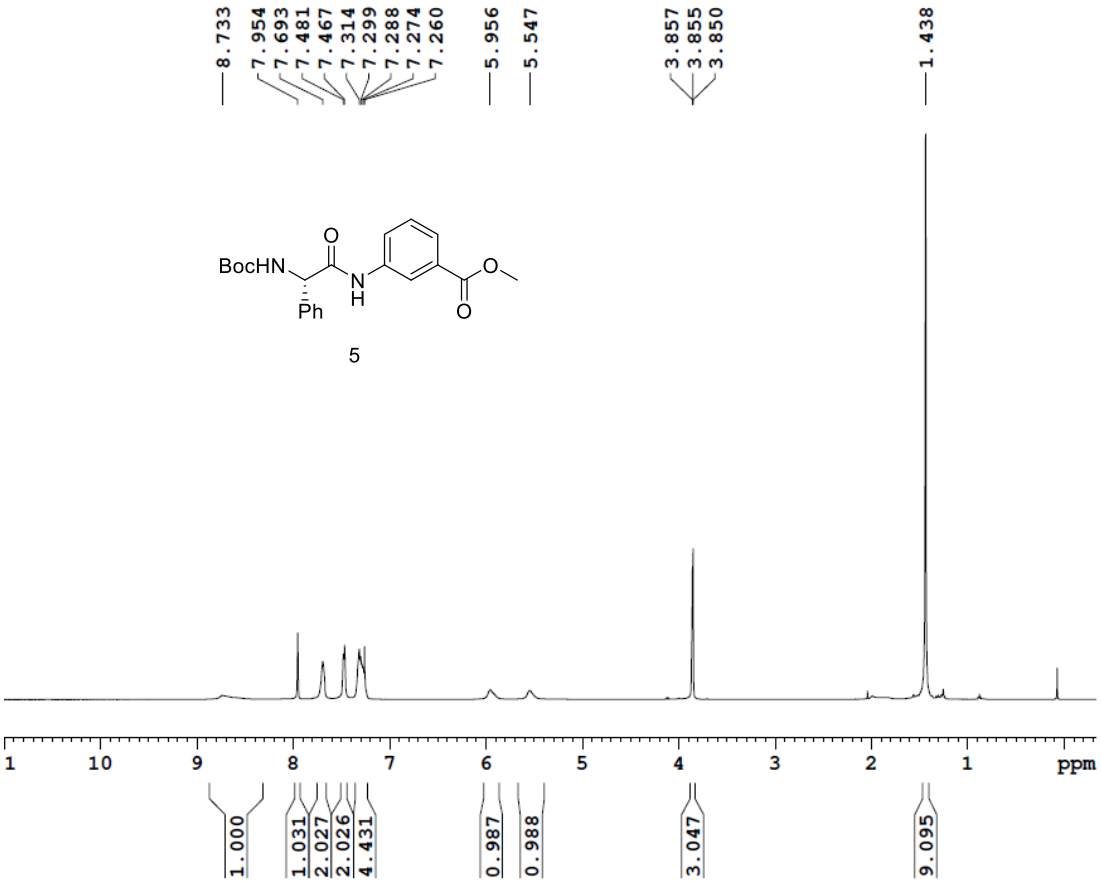


Figure S27. [1]H NMR spectrum of methyl ester phenylglycine dipeptide **5**

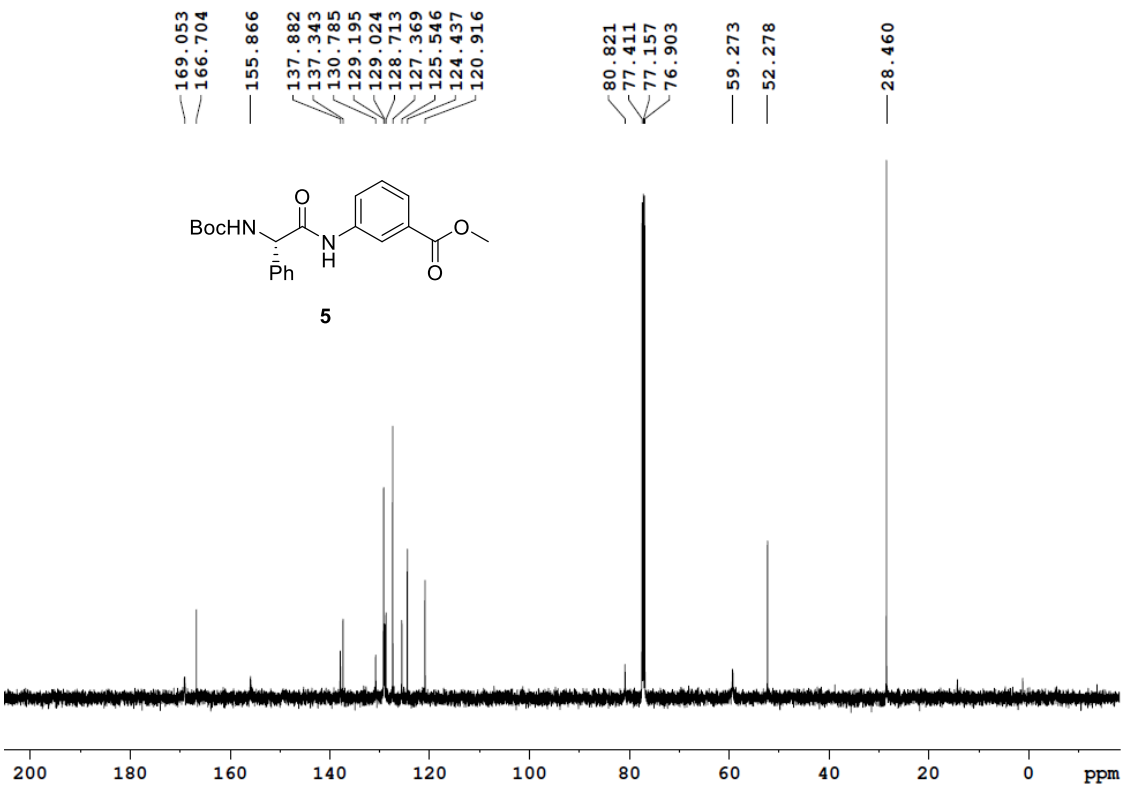


Figure S28. [13]C NMR spectrum of carboxylate phenylglycine dipeptide **5**

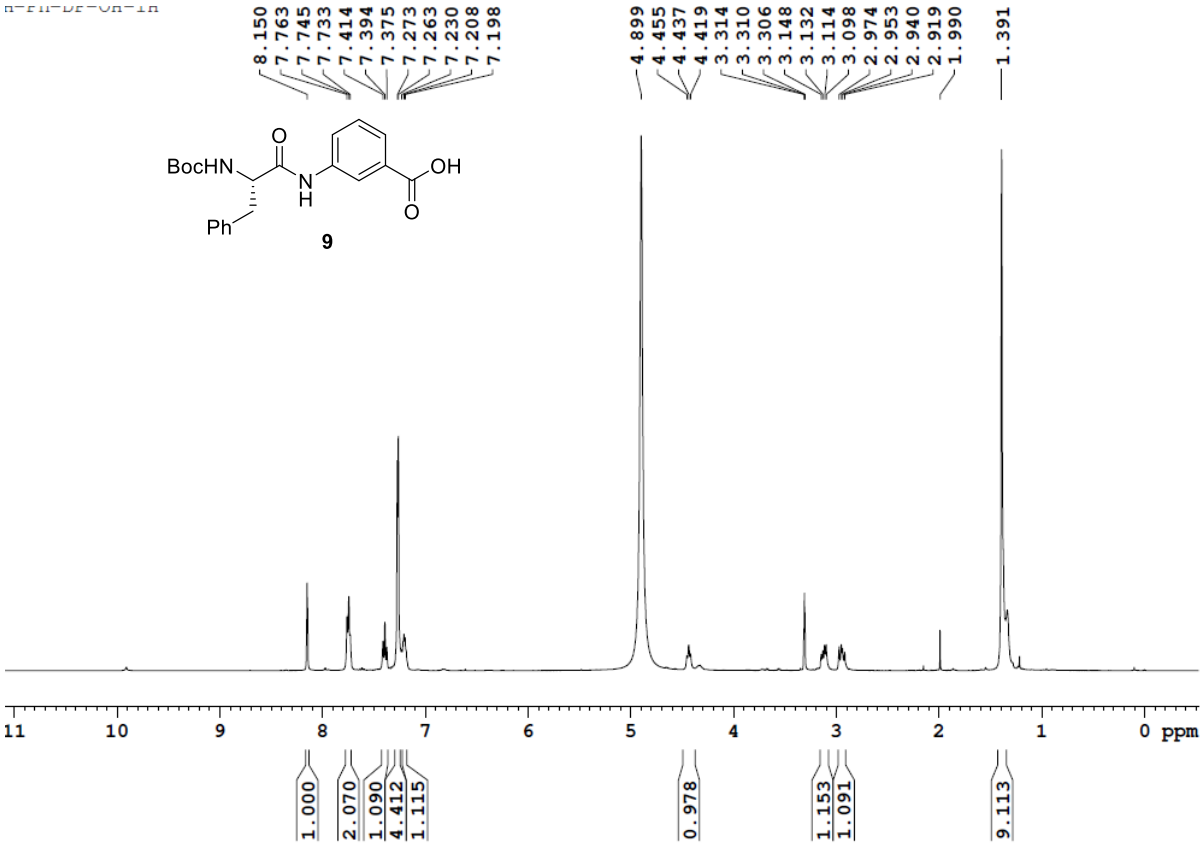


Figure S29. [1]H NMR spectrum of carboxylate phenylalanine dipeptide **9**

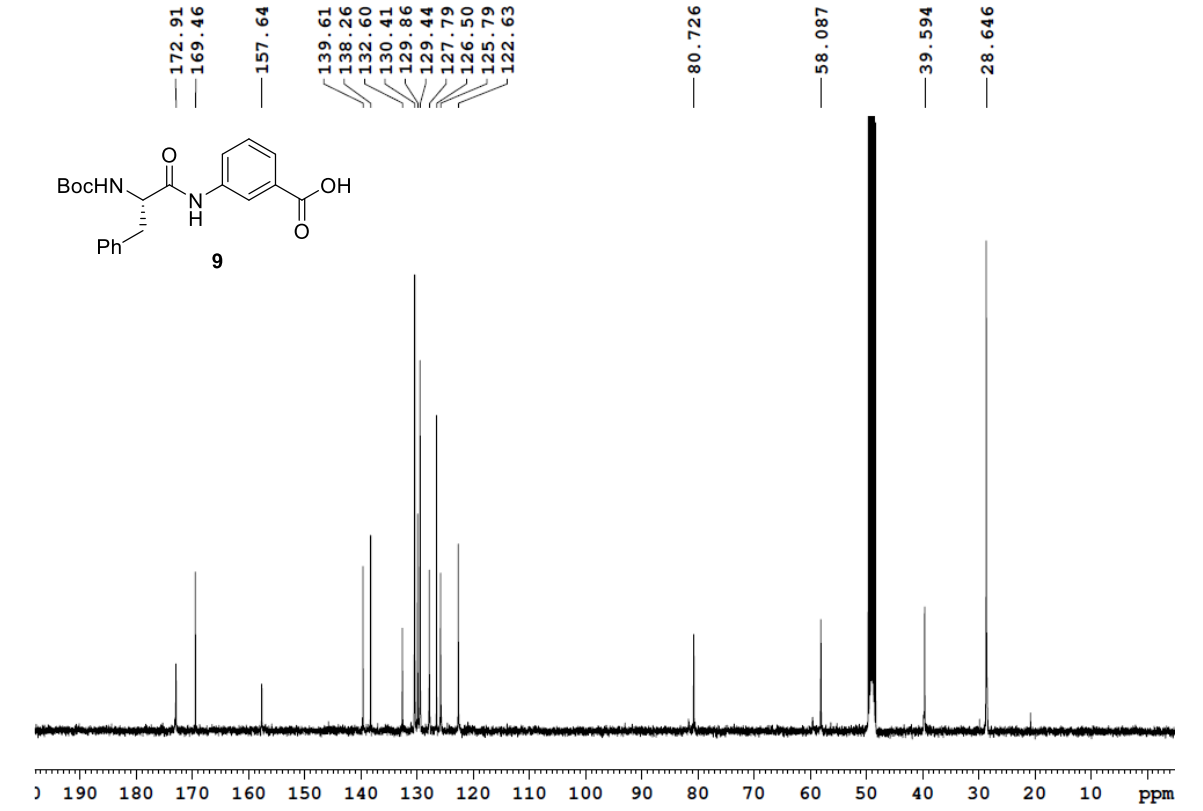


Figure S30. [13]C NMR spectrum of carboxylate phenylalanine dipeptide **9**

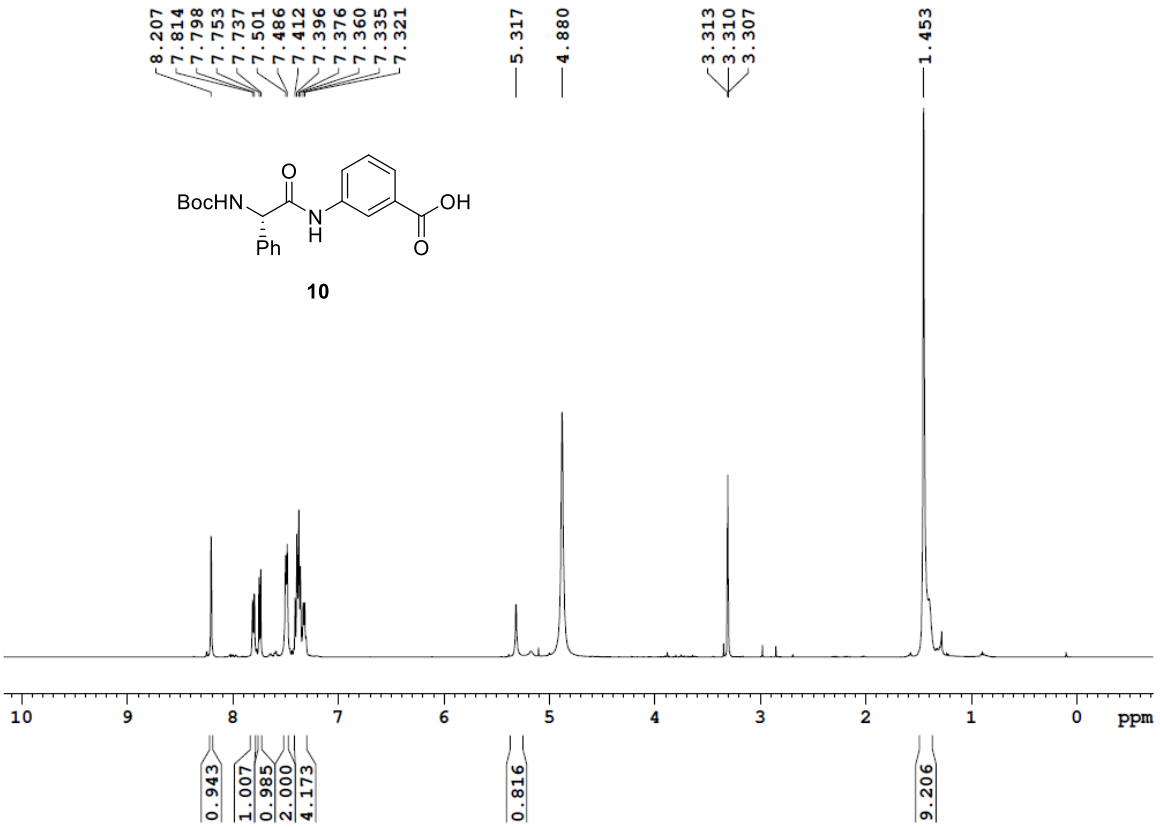


Figure S31. [1]H NMR spectrum of carboxylate phenylglycine dipeptide **10**

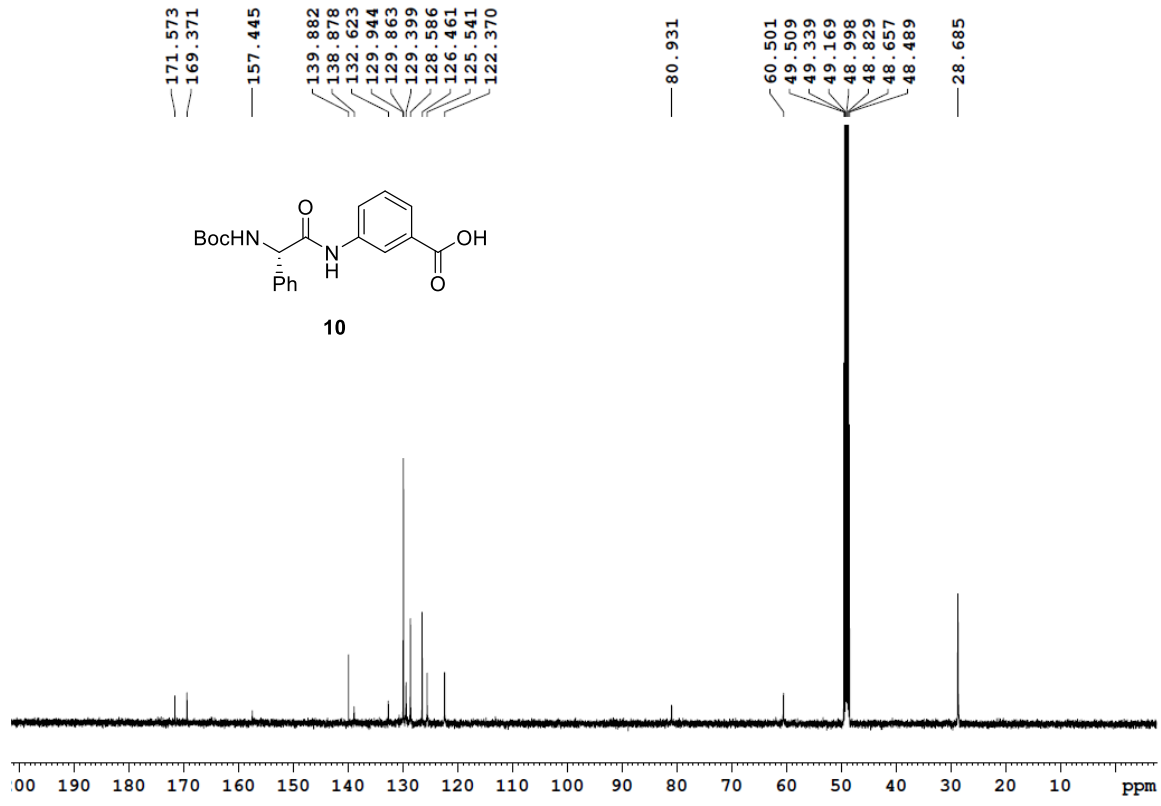


Figure S32. [13]C NMR spectrum of carboxylate phenylglycine dipeptide **10**

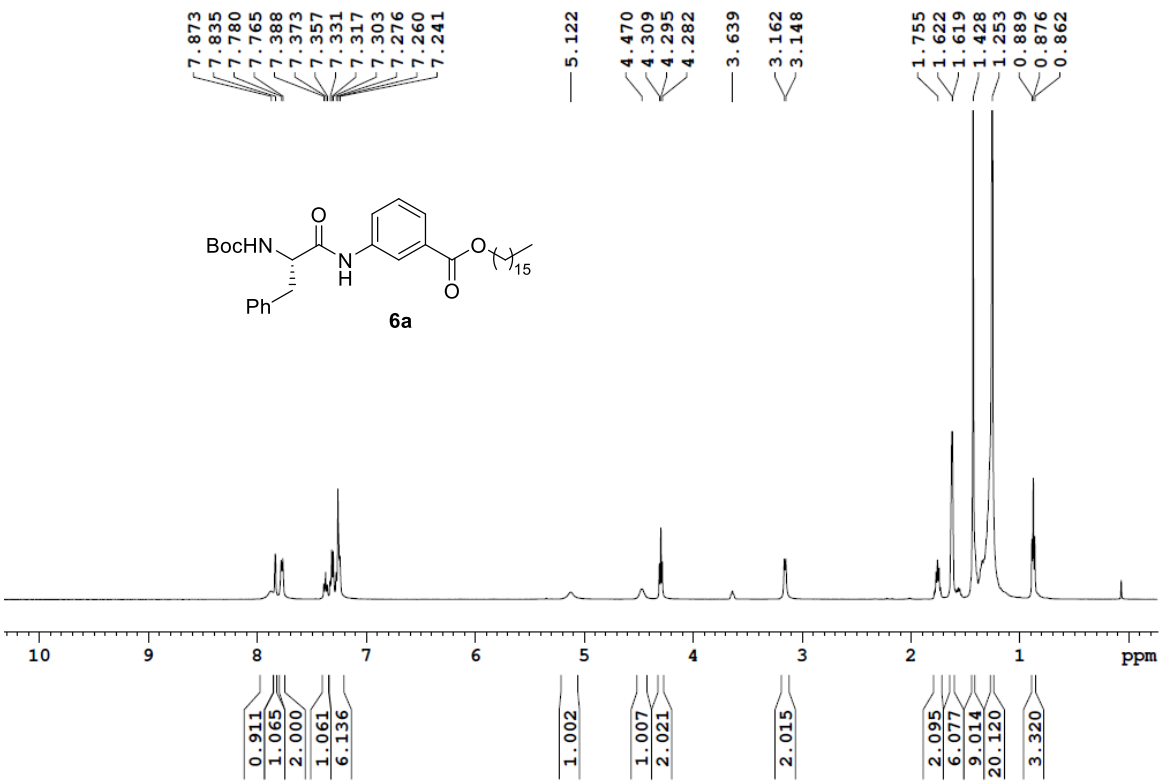


Figure S33. ^{1}H NMR spectrum of hexadecane ester phenylalanine dipeptide **6a**

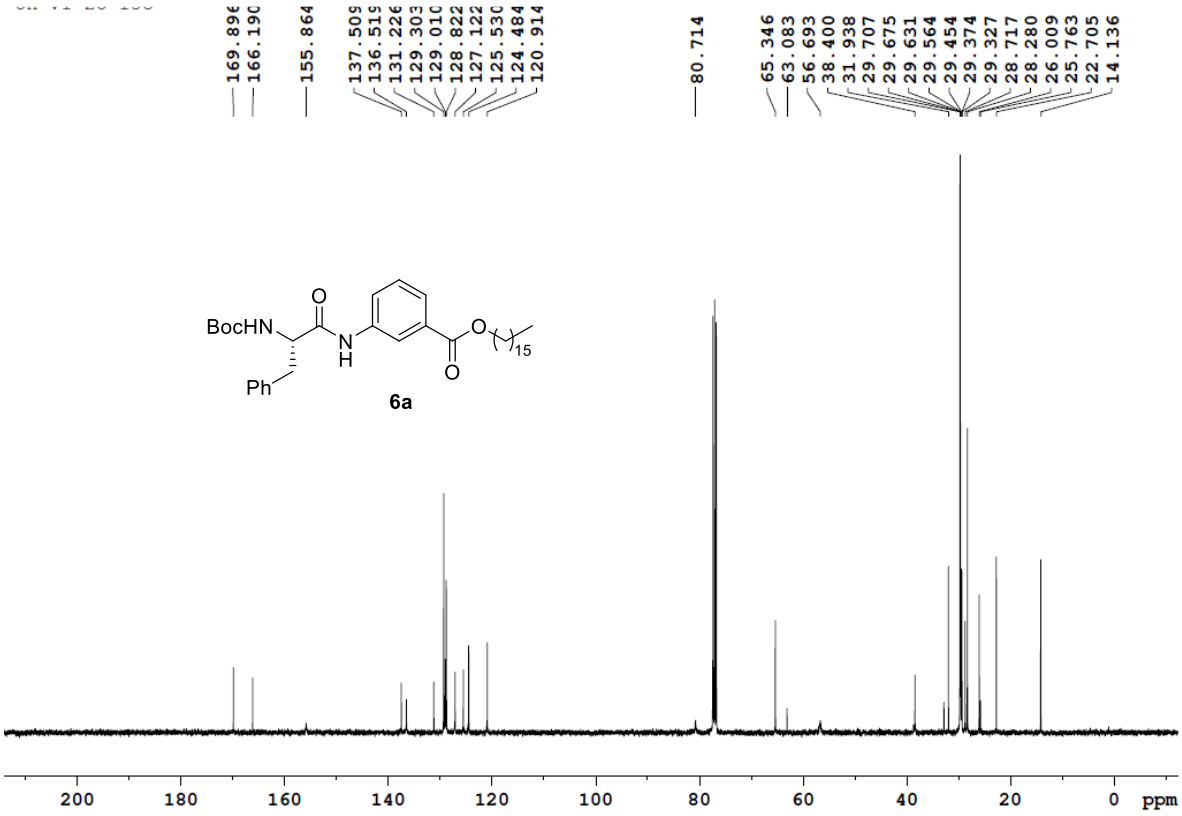


Figure S34. ^{13}C NMR spectrum of hexadecane ester phenylalanine dipeptide **6a**

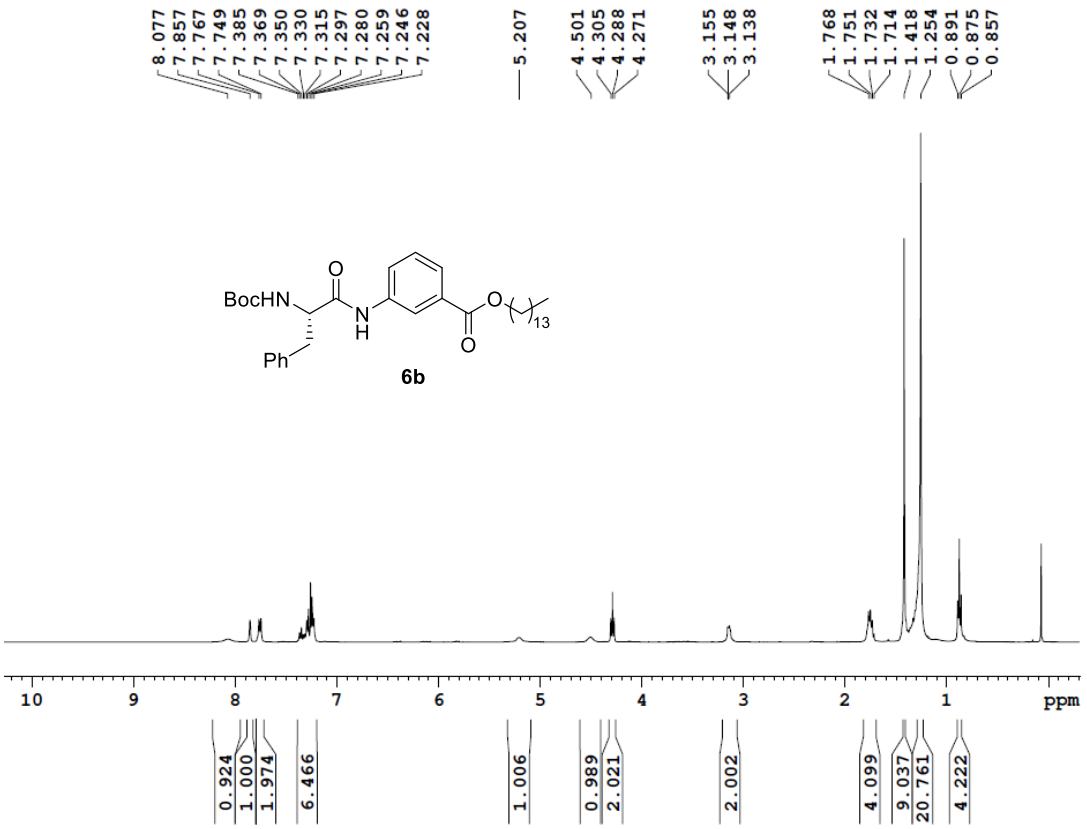


Figure S35. [1]H NMR spectrum of tetradecane ester phenylalanine dipeptide **6b**

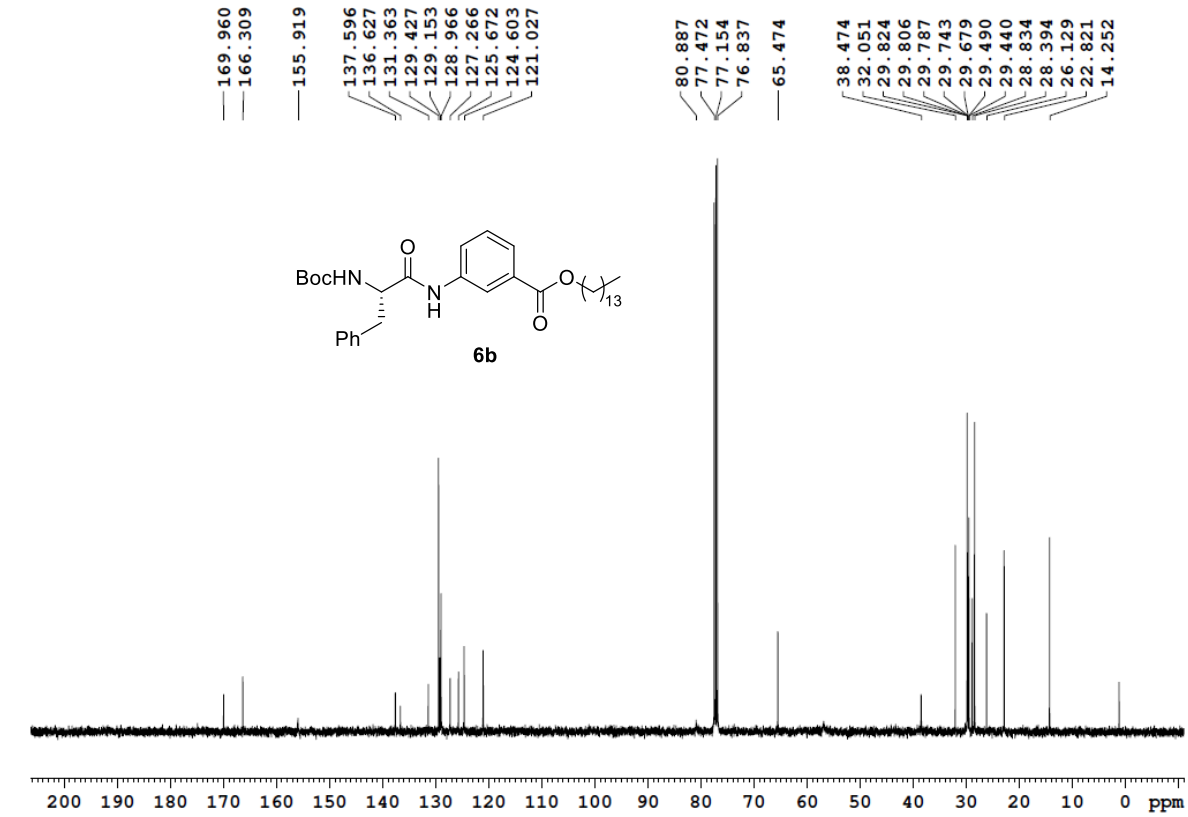


Figure S36. [13]C NMR spectrum of tetradecane ester phenylalanine dipeptide **6b**

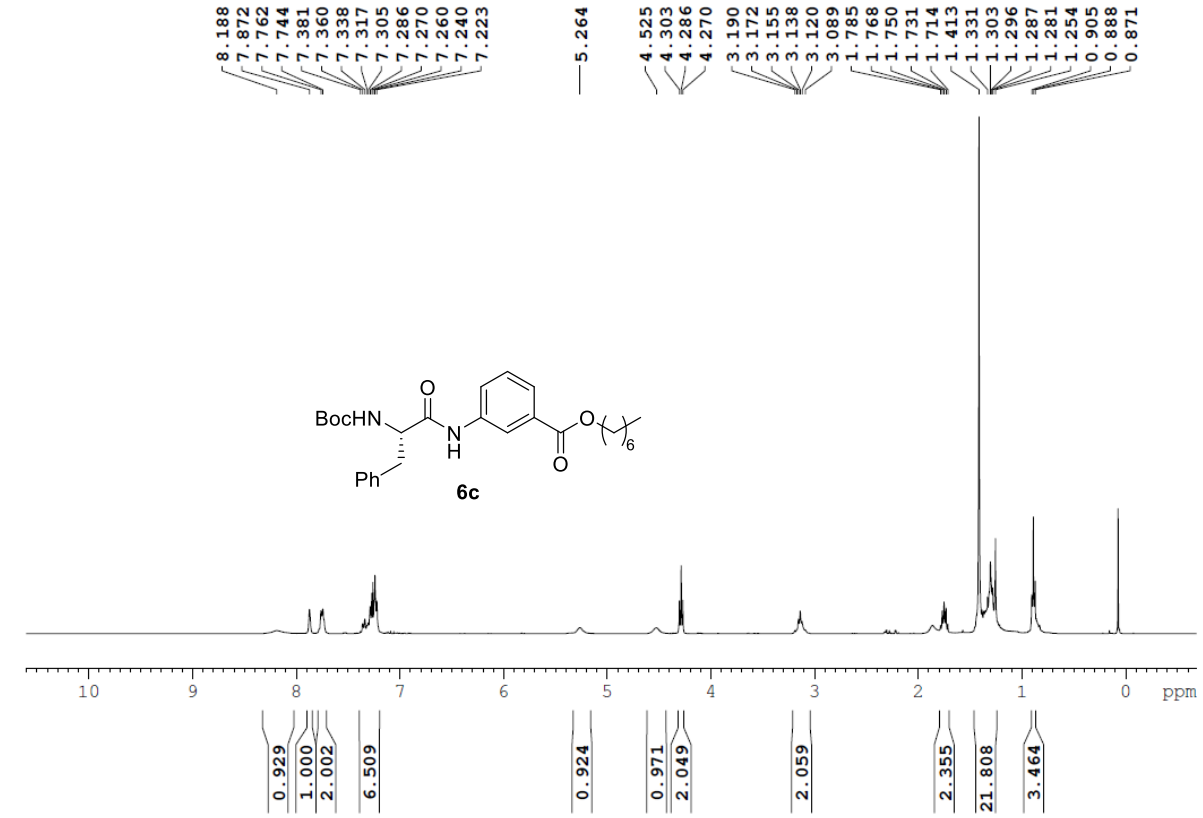


Figure S37. ^{1}H NMR spectrum of heptane ester phenylalanine dipeptide **6c**

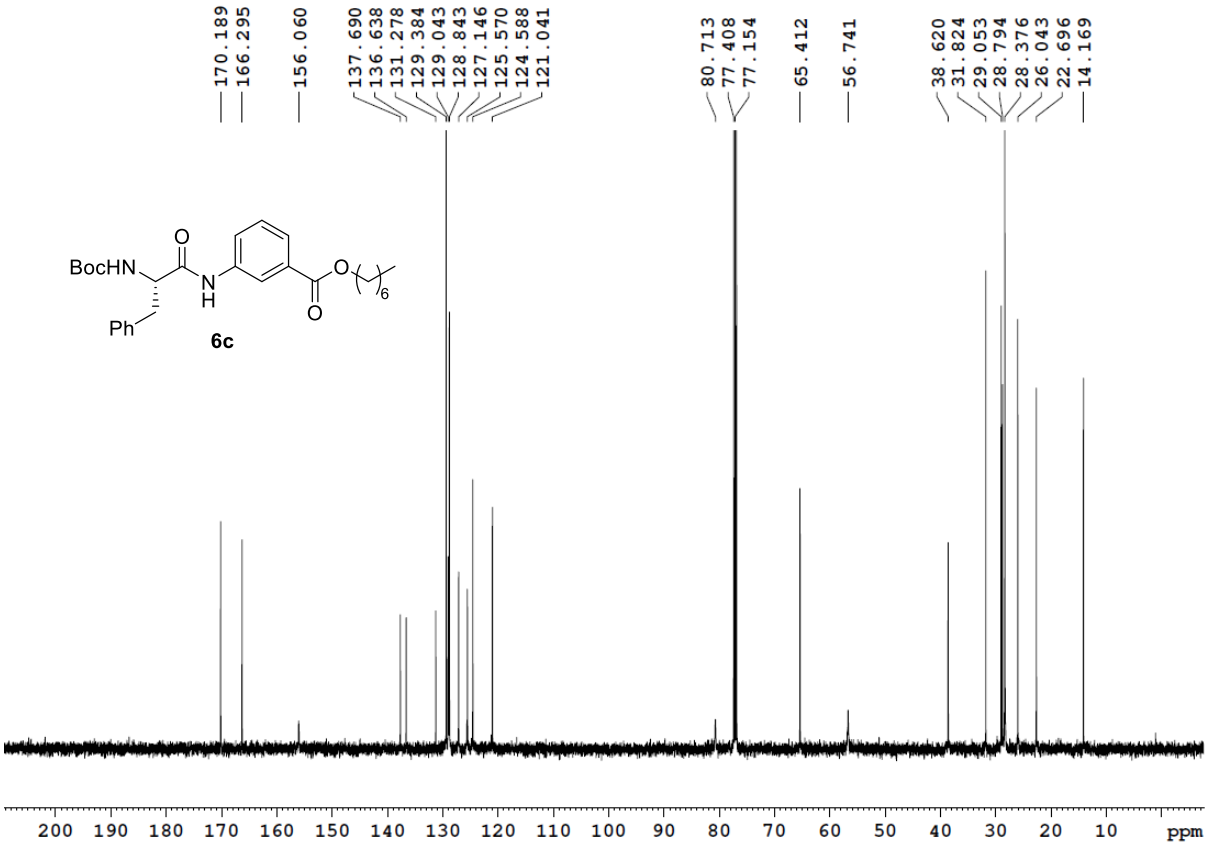


Figure S38. ^{1}H NMR spectrum of heptane ester phenylalanine dipeptide **6c**

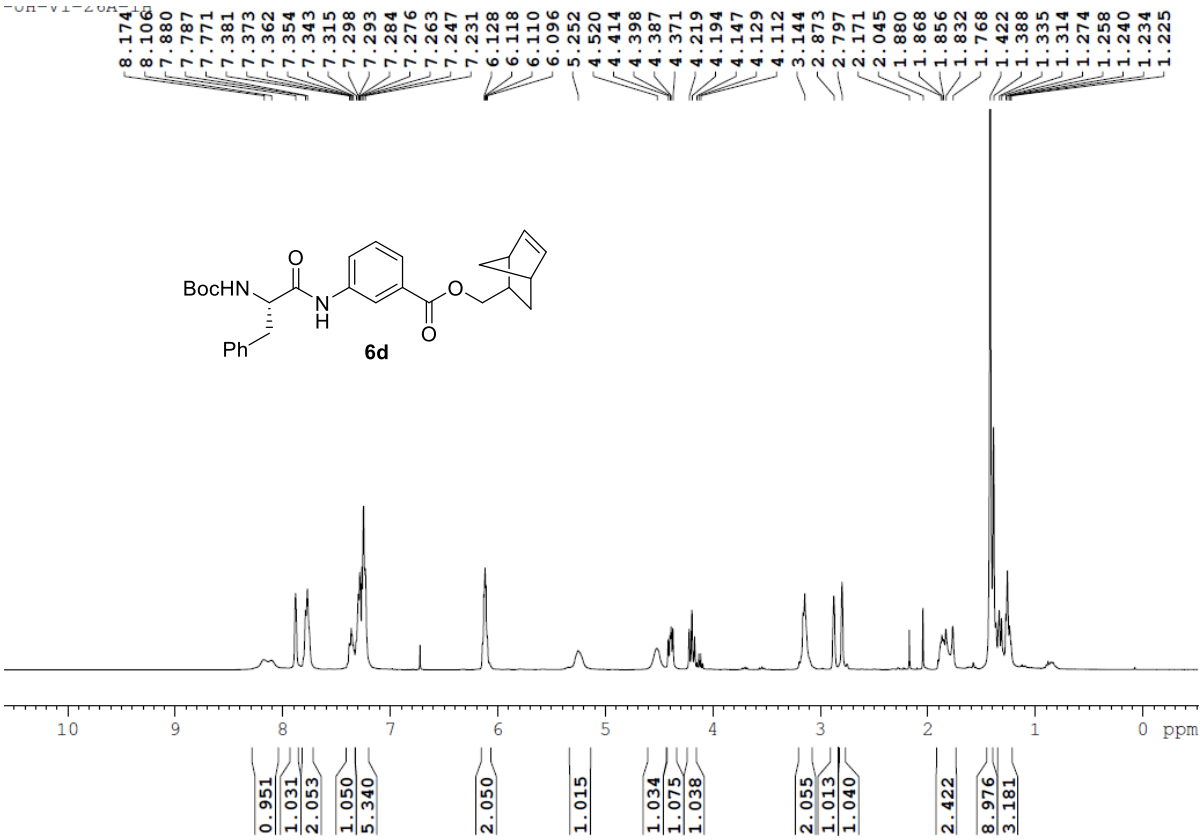


Figure S39. ^{1}H NMR spectrum of norbornene ester phenylalanine dipeptide **6d**

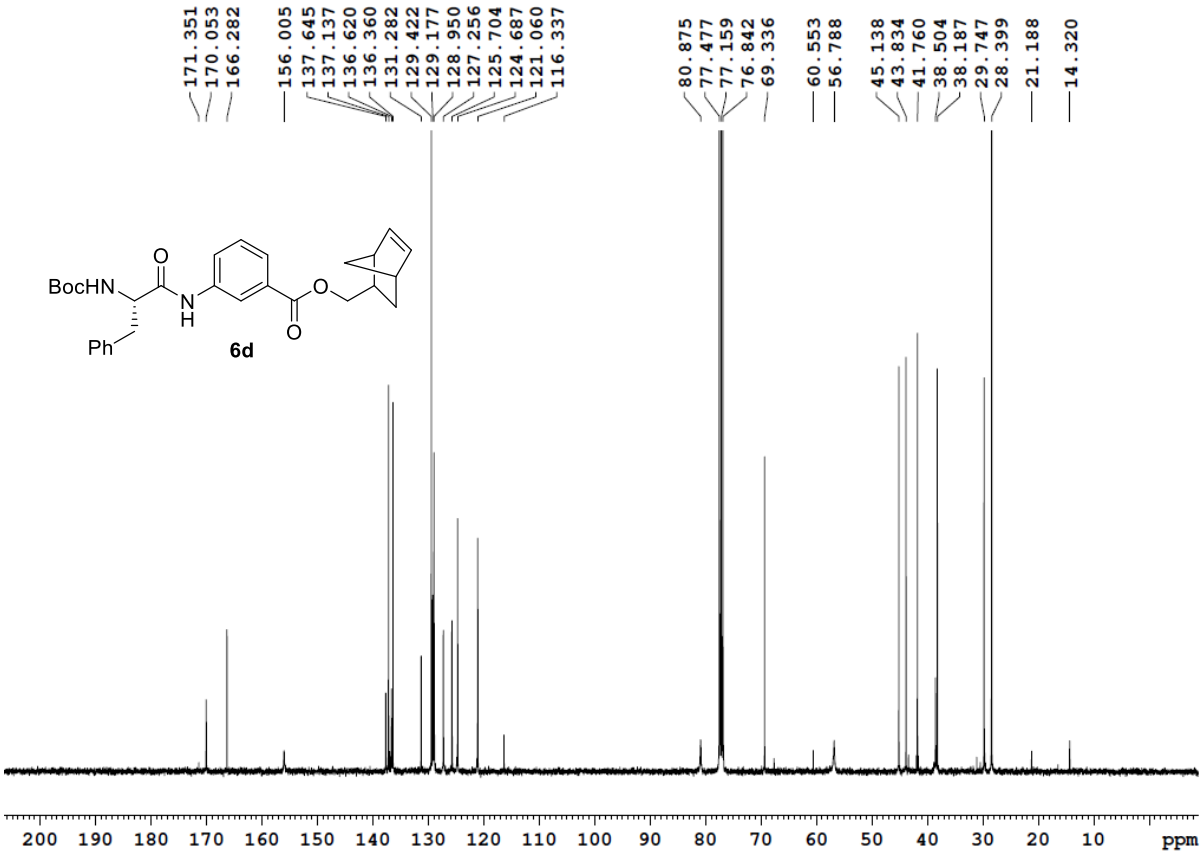


Figure S40. ^{13}C NMR spectrum of norbornene ester phenylalanine dipeptide **6d**

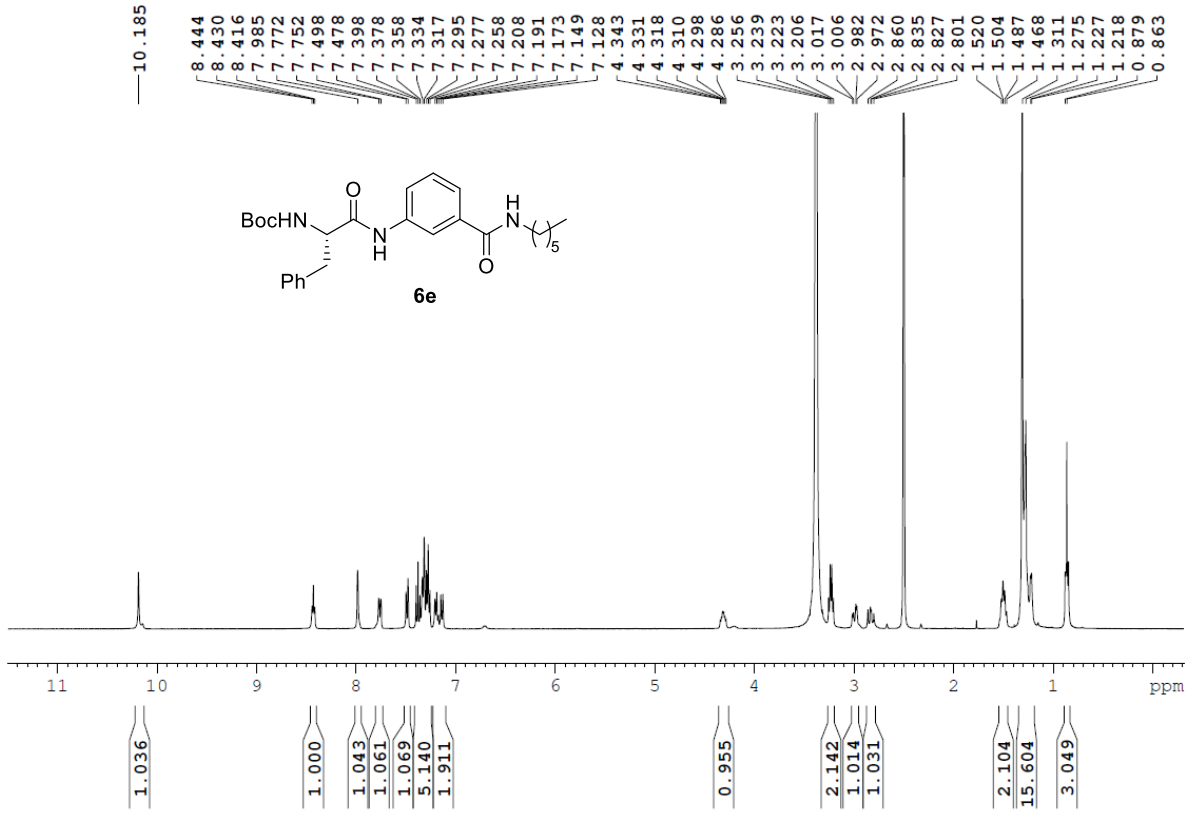


Figure S41. [1]H NMR spectrum of hexyl amide phenylalanine dipeptide **6e** at 25 ˚C

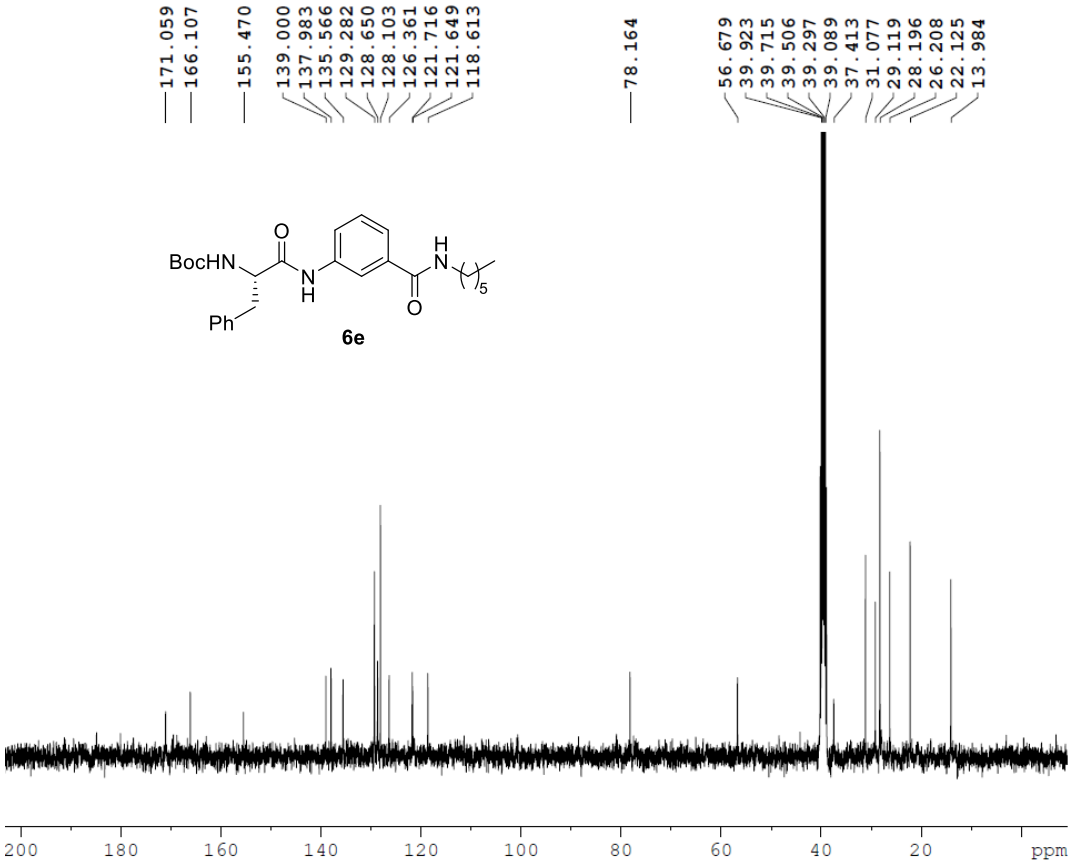


Figure S42. [13]C NMR spectrum of hexyl amide phenylalanine dipeptide **6e** at 25 ˚C

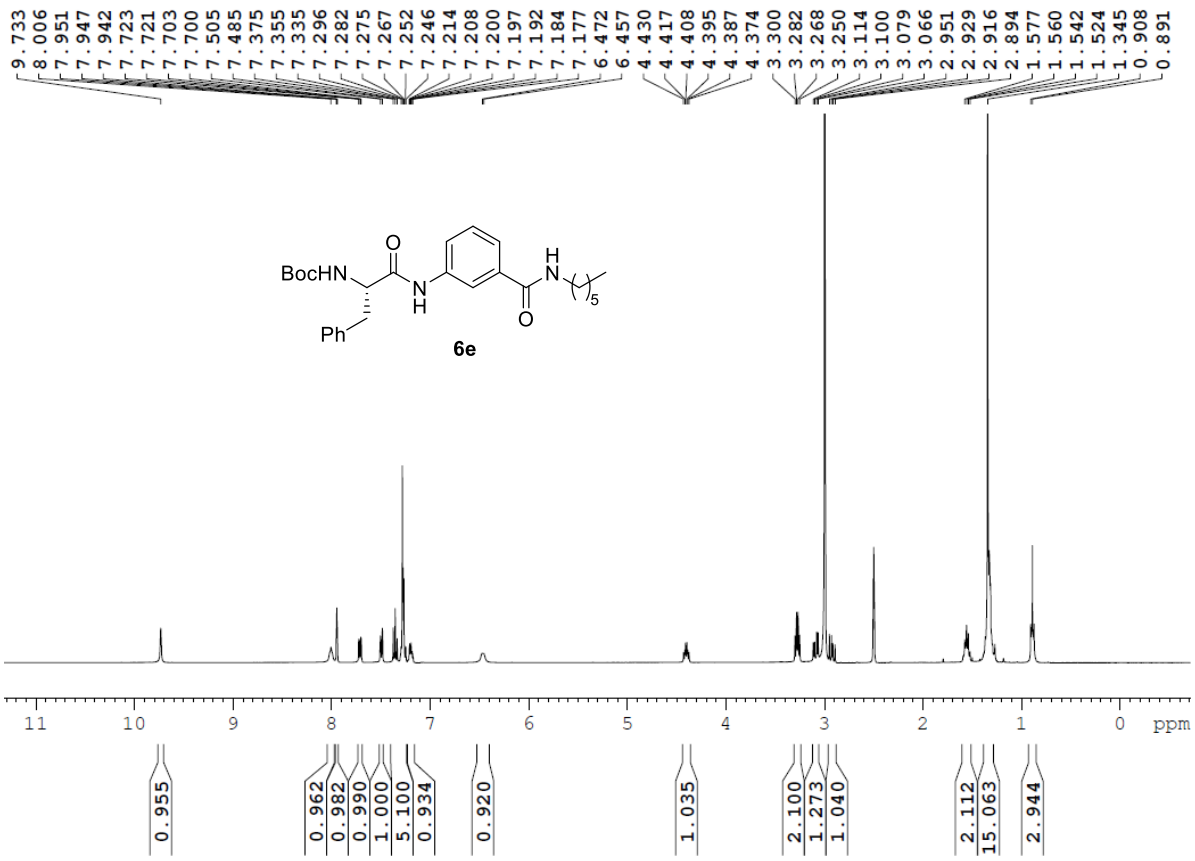


Figure S43. [1]H NMR spectrum of hexyl amide phenylalanine dipeptide **6e** at 100 ˚C

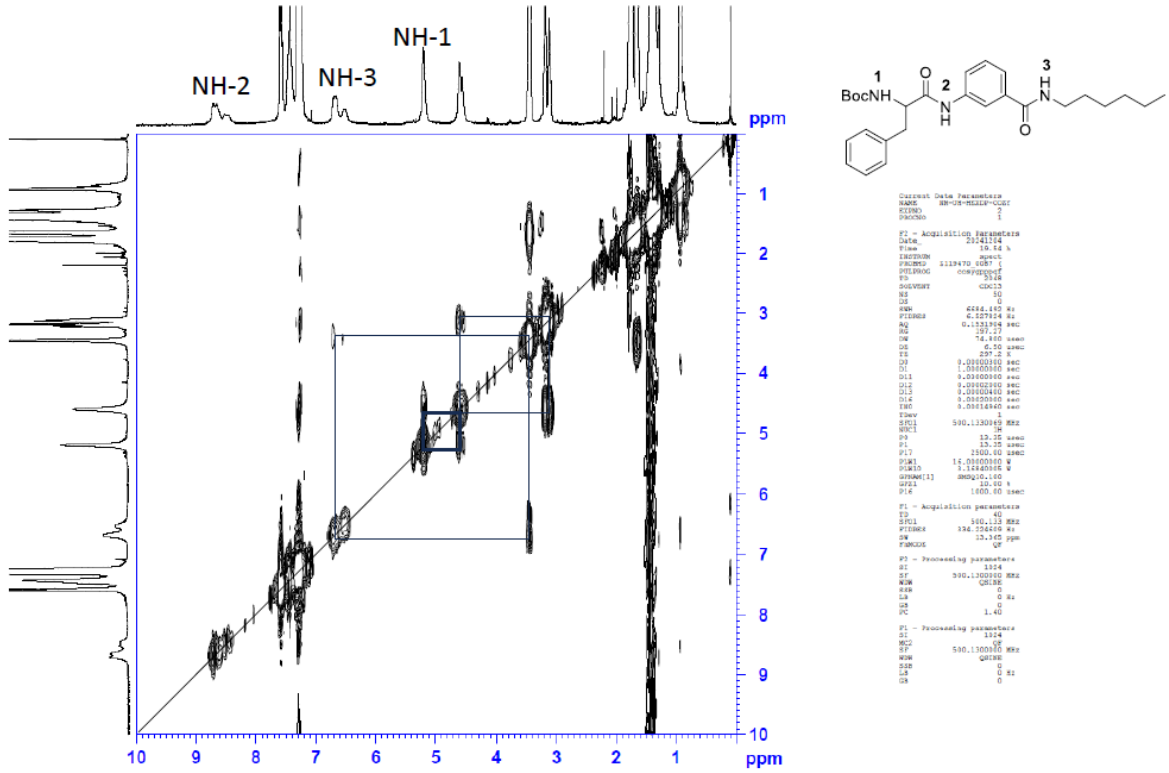


Figure S44. Cozy spectrum of hexyl amide phenylalanine dipeptide **6e**

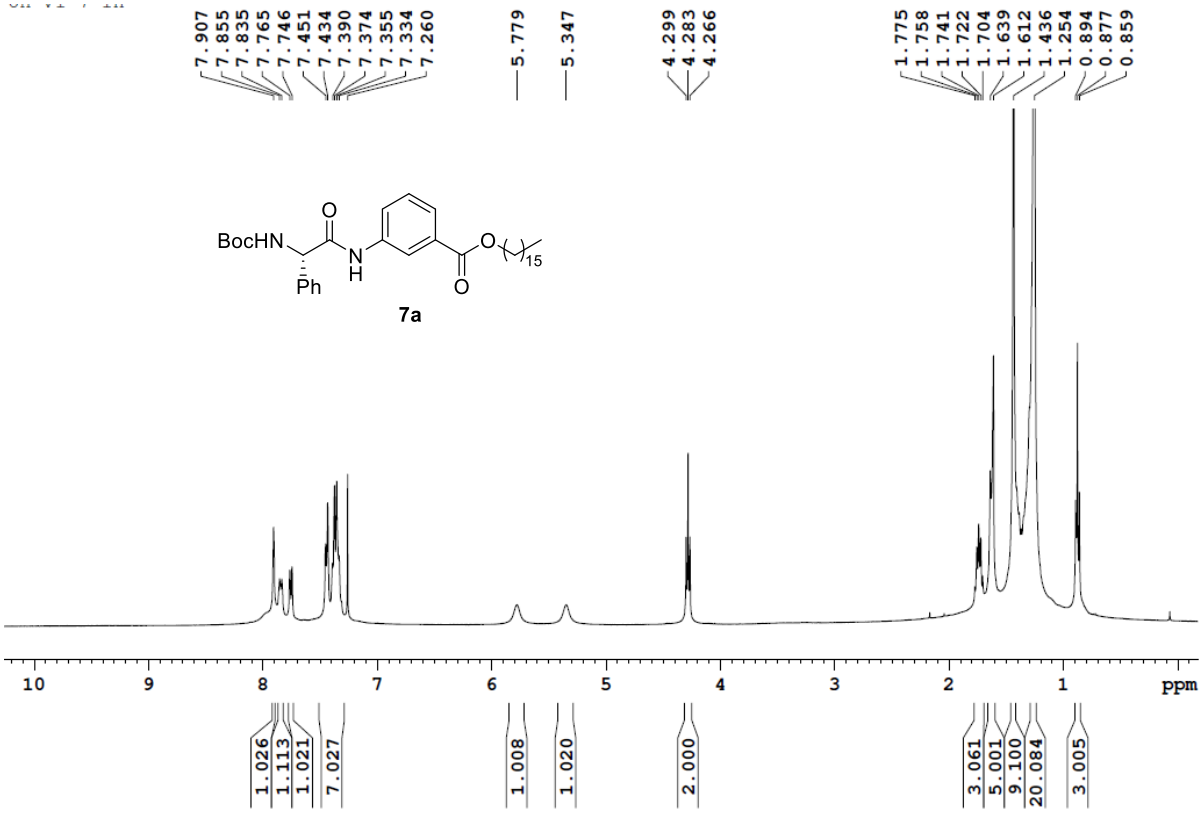


Figure S45. [1]H NMR spectrum of hexadecane ester phenylglycine dipeptide **7a**

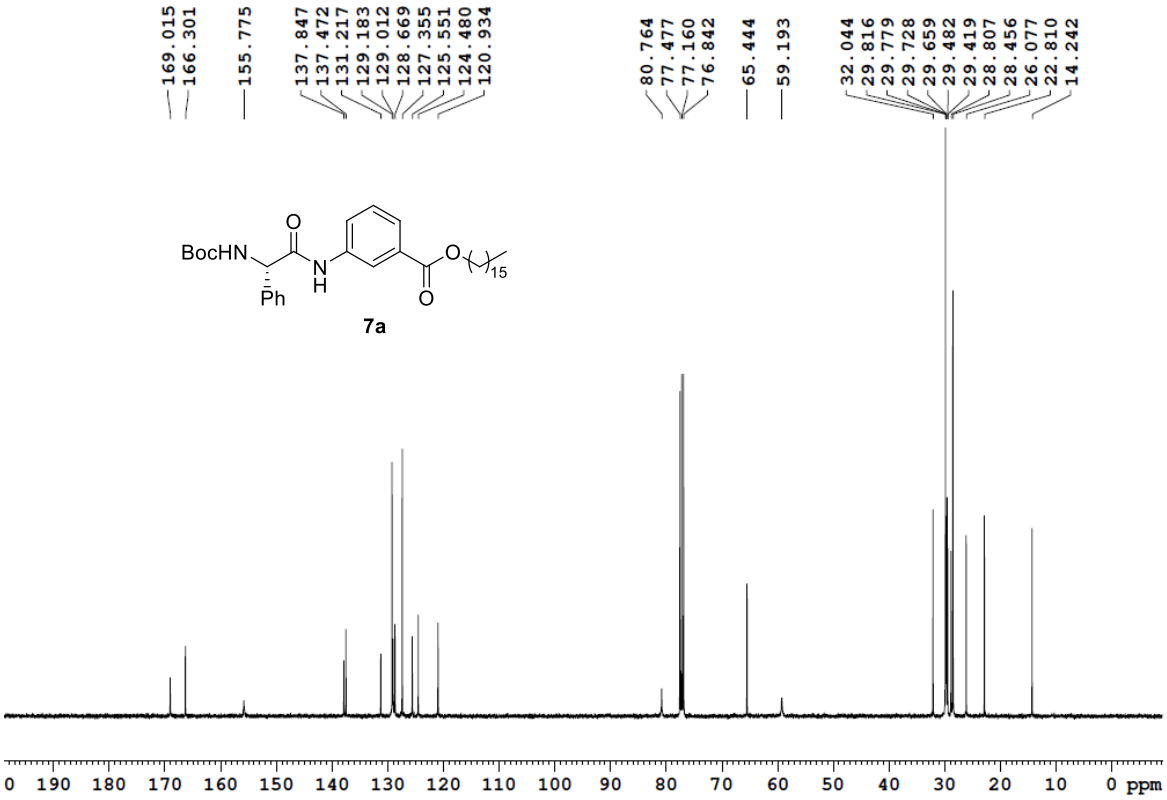


Figure S46. [13]C NMR spectrum of hexadecane ester phenylglycine dipeptide **7a**

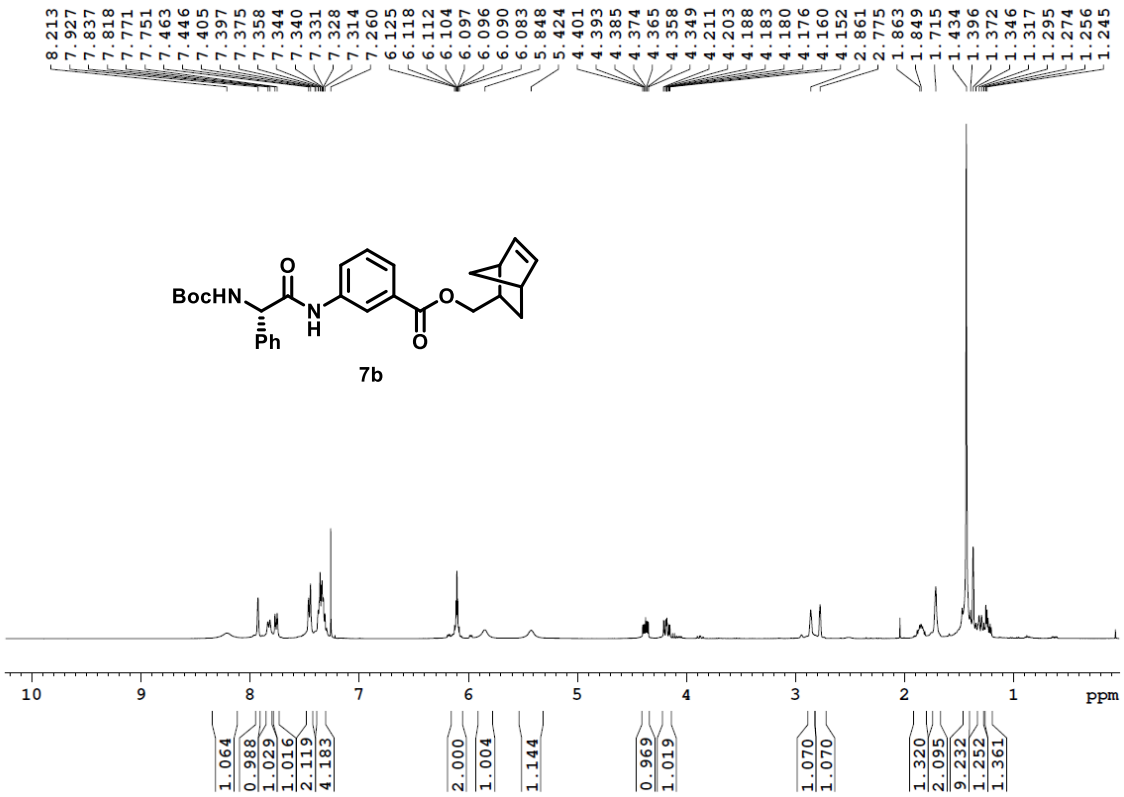


Figure S47. ^{1}H NMR spectrum of norbornene ester phenylglycine dipeptide **7b**

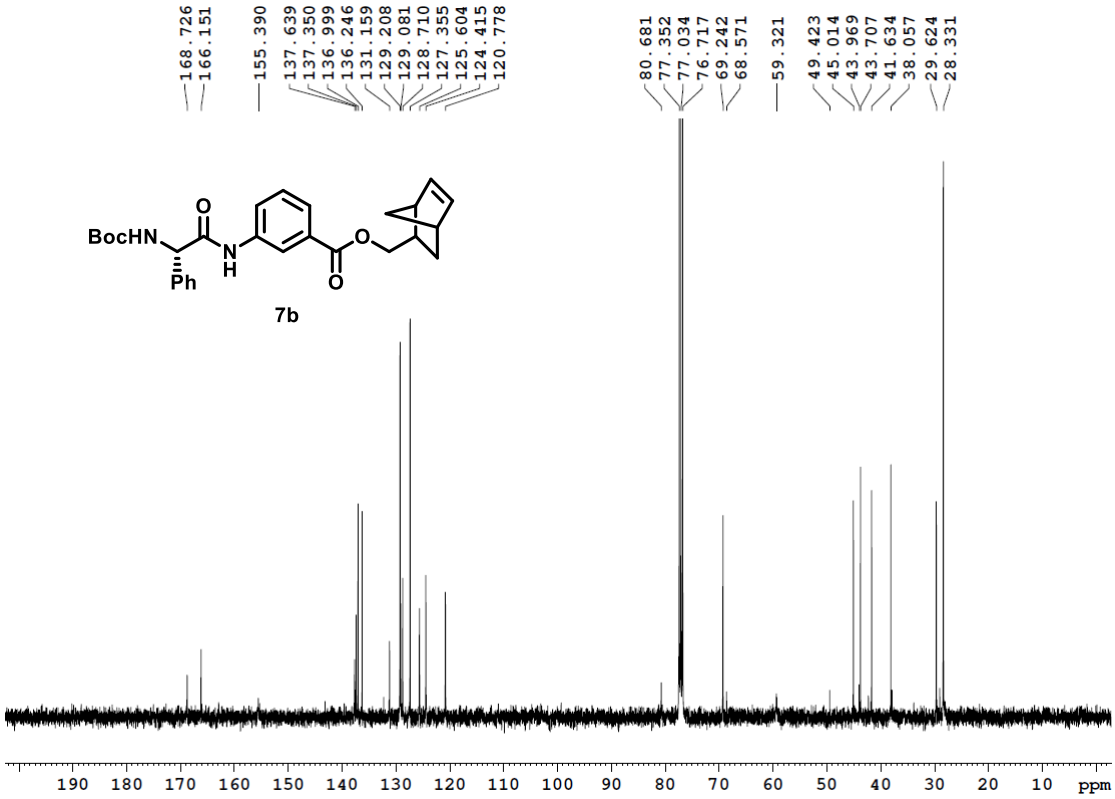


Figure S48. ^{13}C NMR spectrum of norbornene ester phenylglycine dipeptide **7b**

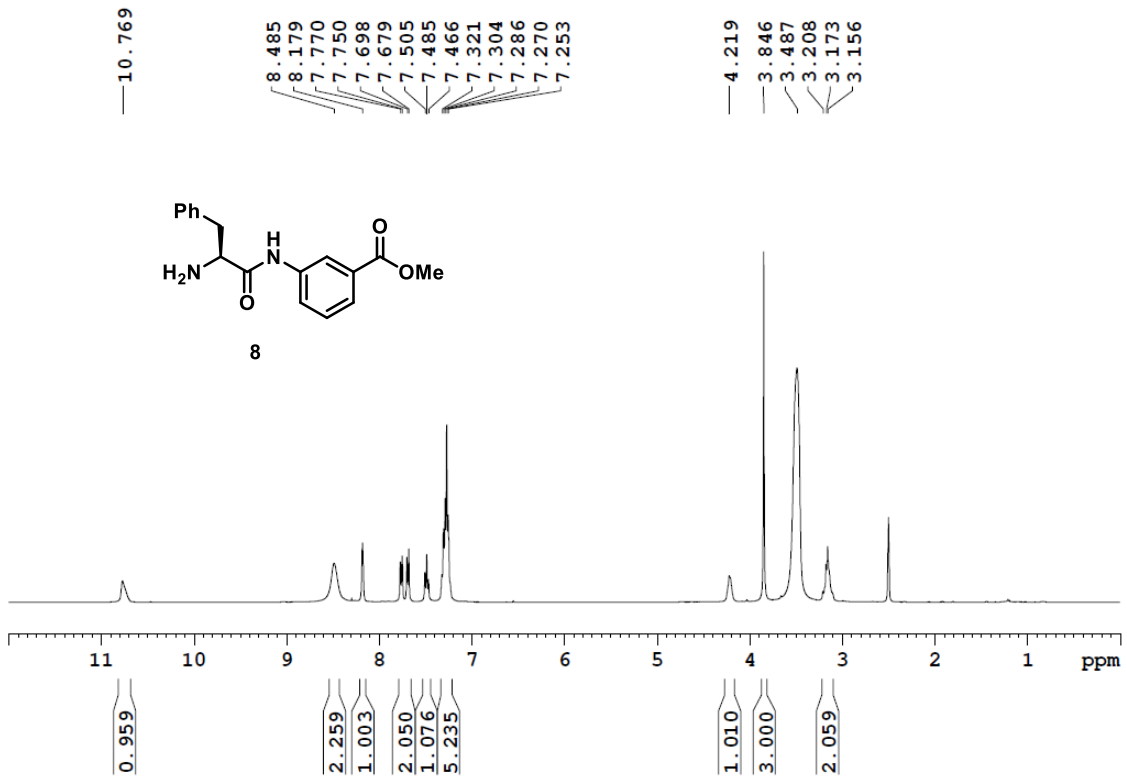


Figure S49. ^{1}H NMR spectrum of dipeptide **8**

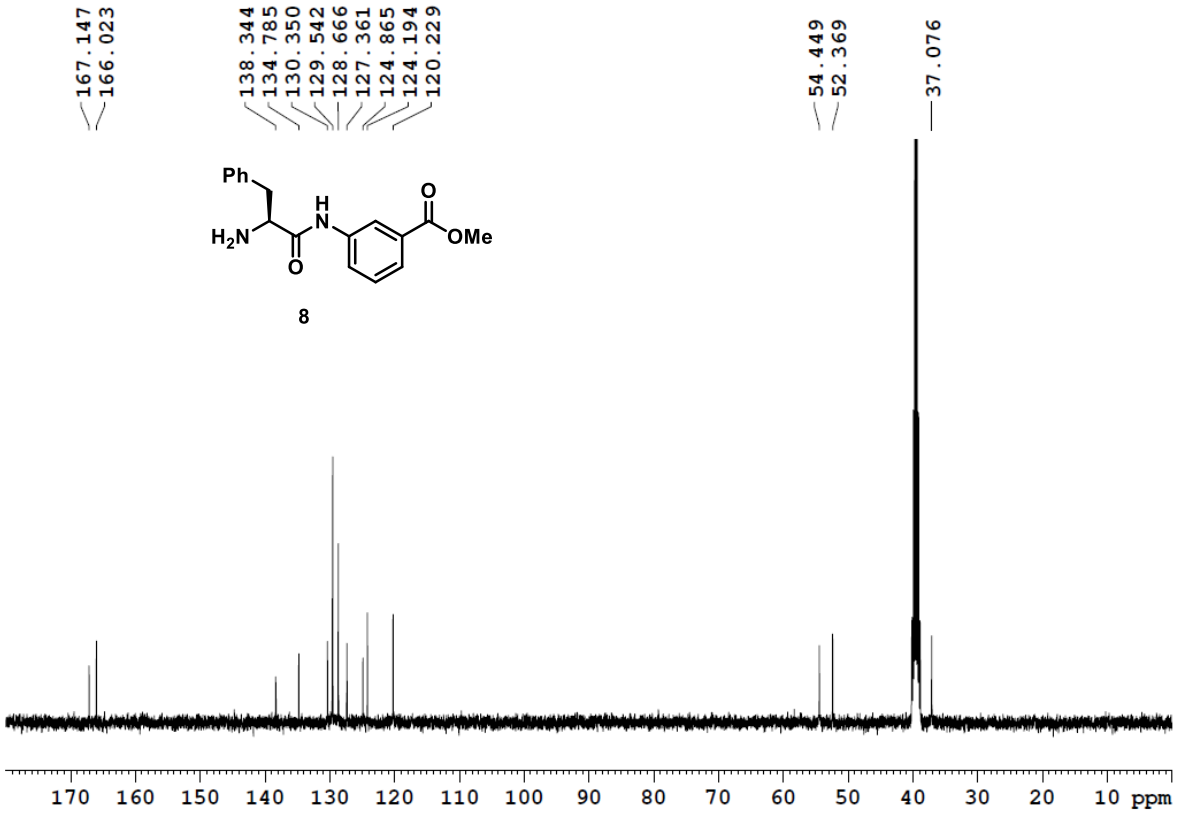


Figure S50. ^{13}C NMR spectrum of dipeptide **8**

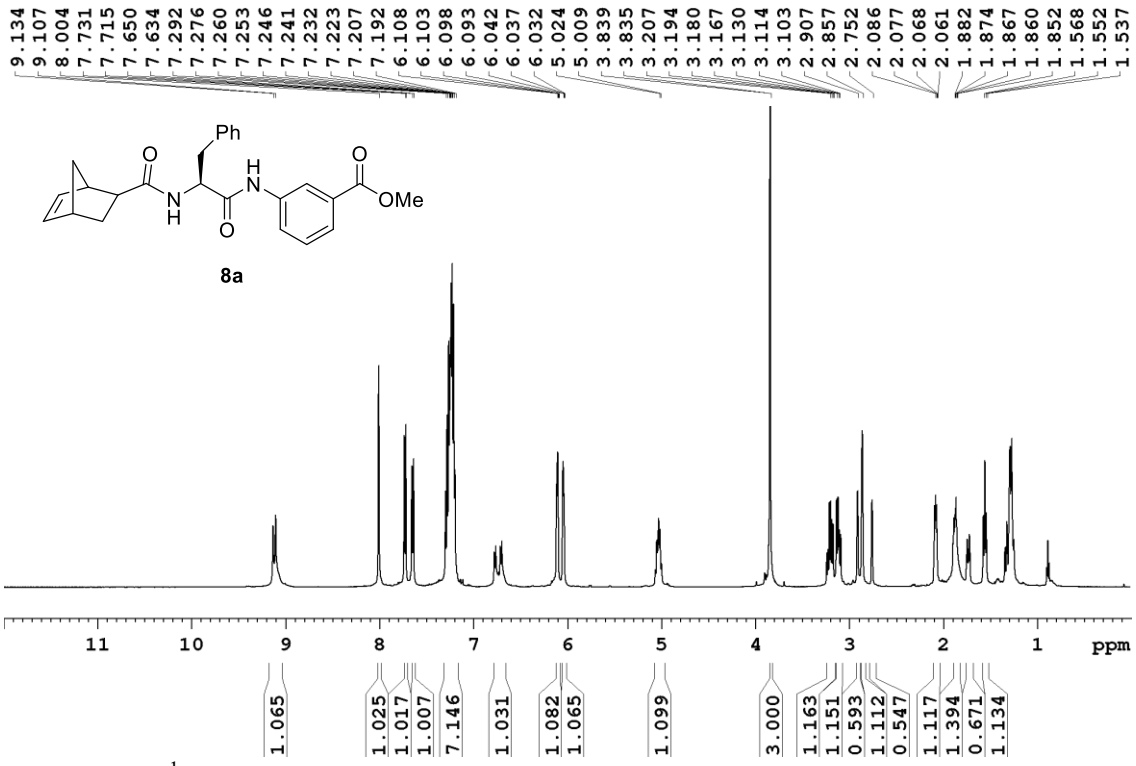


Figure S51. [1]H NMR spectrum of norbornene amide phenylalanine dipeptide **8a**

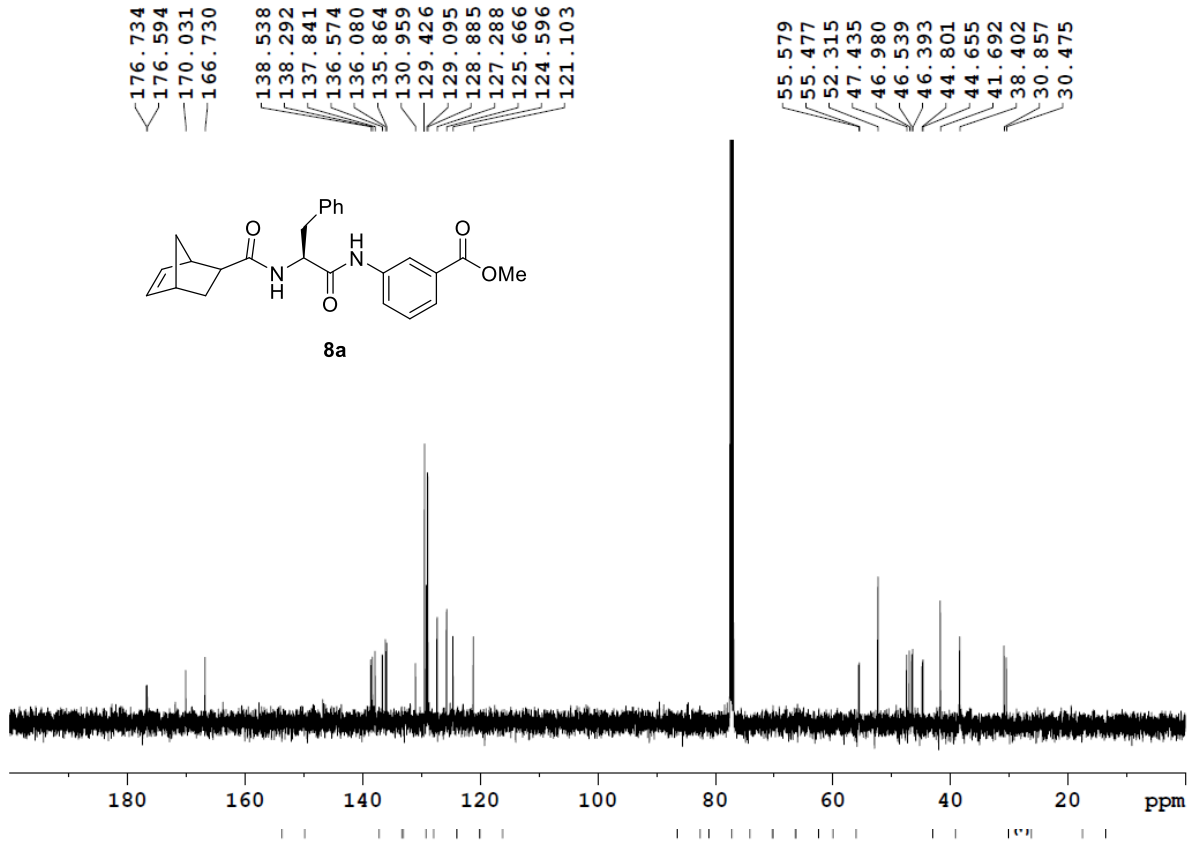


Figure S52. [13]C NMR spectrum of norbornene amide phenylalanine dipeptide **8a**

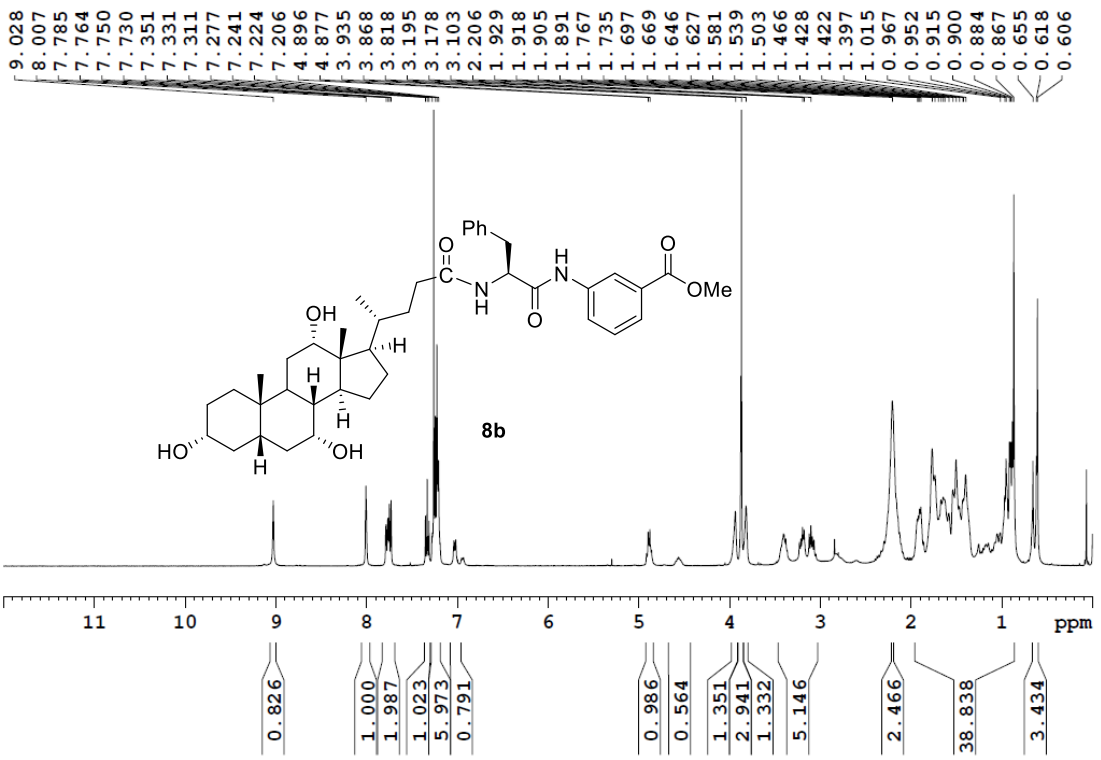


Figure S53. [1]H NMR spectrum of cholic acid amide phenylalanine dipeptide **8b**

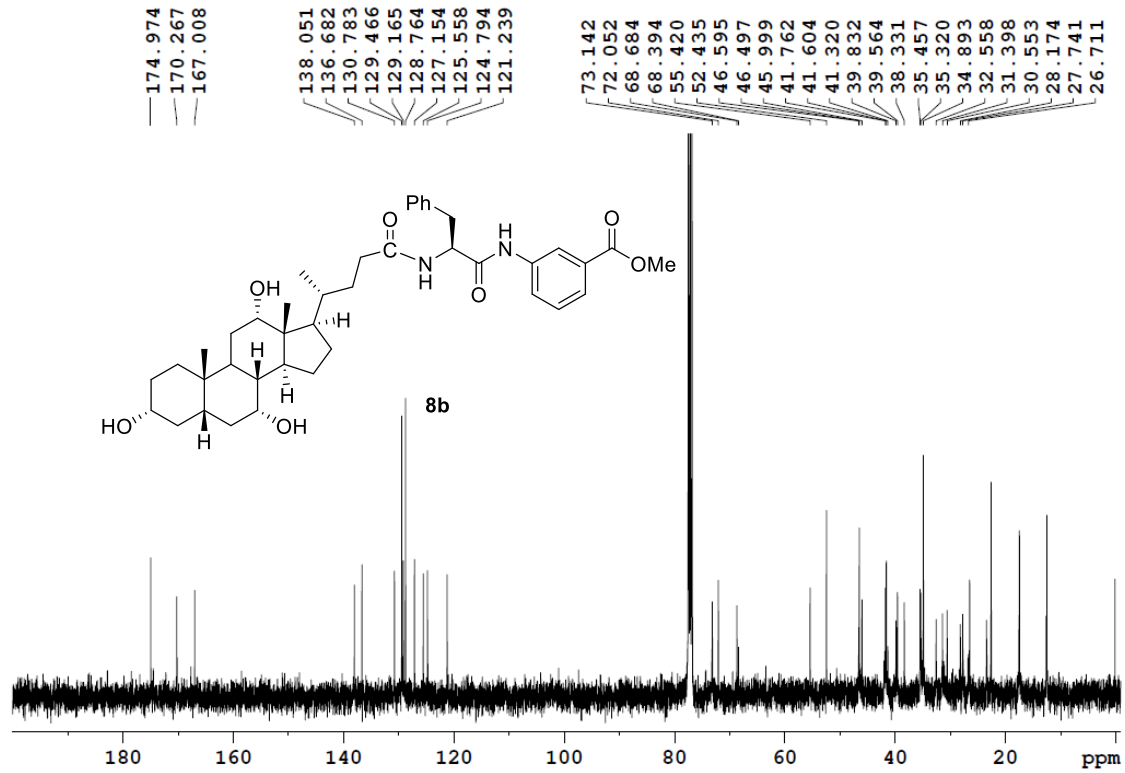


Figure S54. [13]C NMR spectrum of cholic acid amide phenylalanine dipeptide **8b**

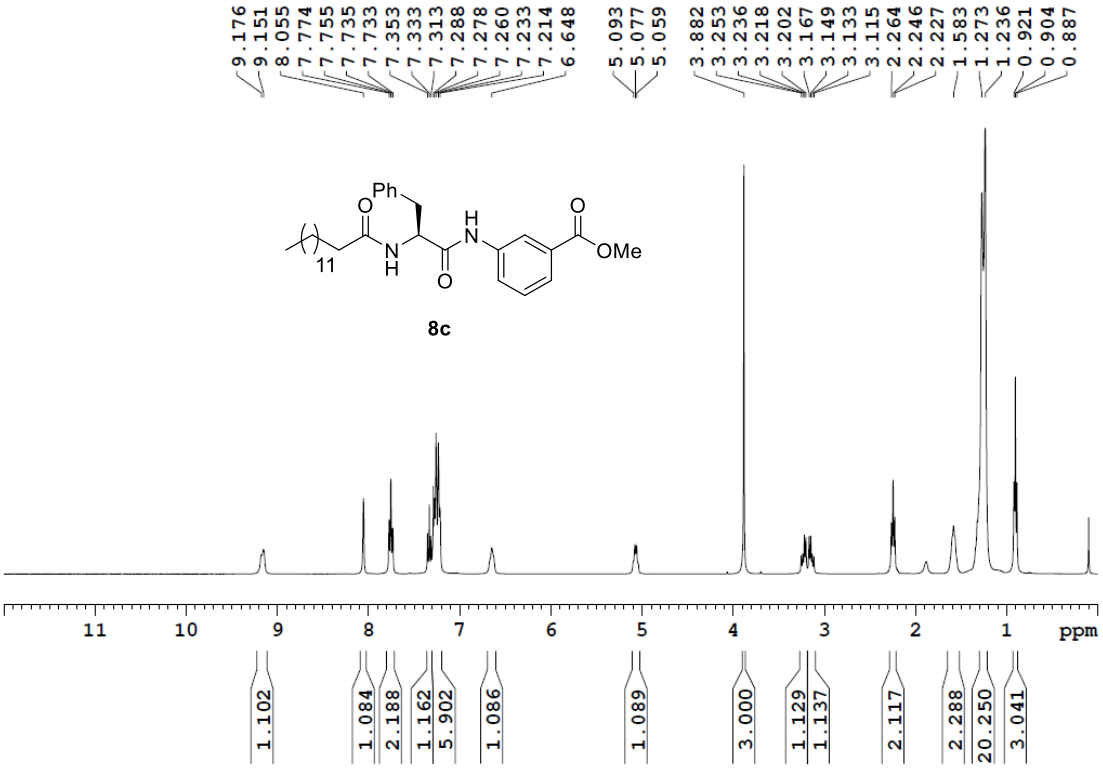


Figure S55. [1]H NMR spectrum of myristic acid amide phenylalanine dipeptide **8c**

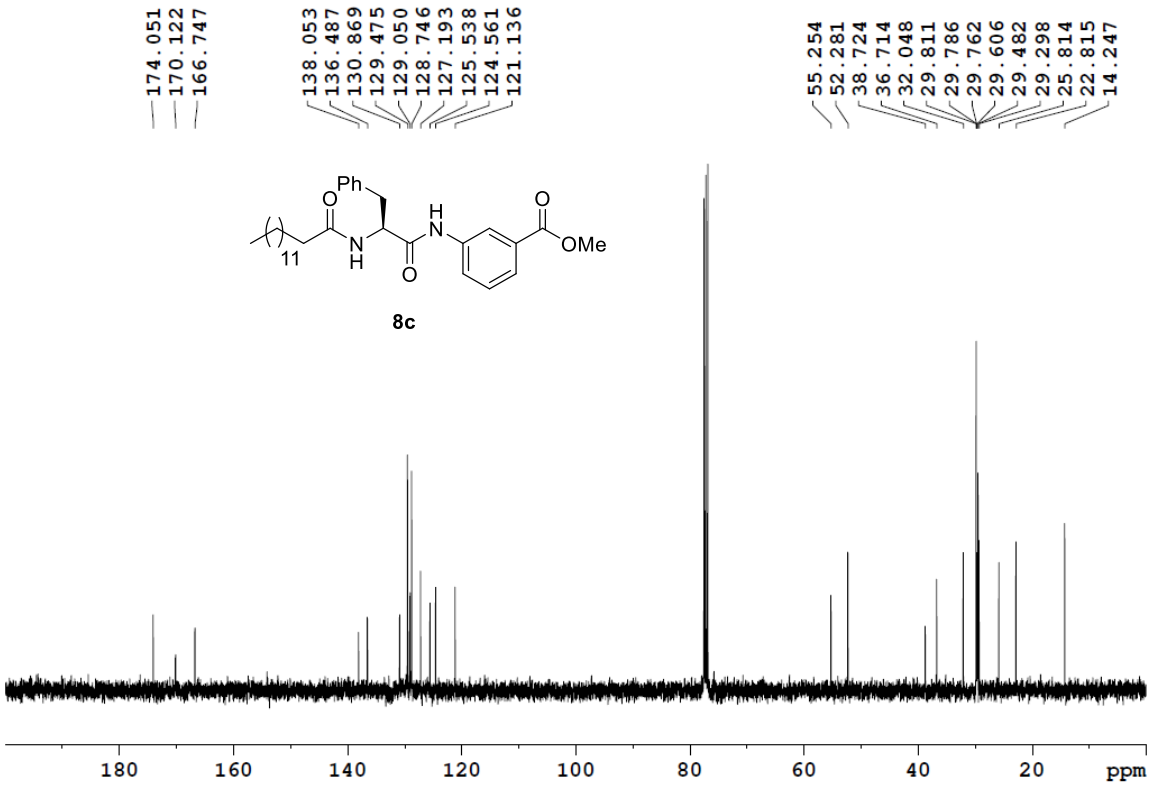


Figure S56. [13]C NMR spectrum of myristic acid amide phenylalanine dipeptide **8c**

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