Supplementary Information

Effect of regio- and stereoisomerism on antifouling 2,5-diketopiperazines

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List of supporting information.

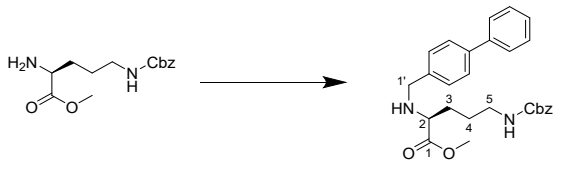
Table 1. Macrofouling organisms used to assess antifouling potency. Key assay parameters are listed.

Scientific Name	Common Name	Assay format (volume)	Test organisms	Test concs .
Ciona savignyi	Transparent sea squirt	12-well plate (7.1 mL)	3 ± 1 larvae/mL	0, 0.05, 0.1, 0.5, 1, 2, 5, 10, 20 µg/mL
Mytilus galloprovincialis	Blue mussel	12-well plate (5 mL)	5 ± 1 larvae/mL	
Spirobranchus cariniferus	Blue tube worm	12-well plate (5 mL)	5 ± 1 larvae/mL	
Undaria pinnatifida	Asian kelp	24-well plate (2 mL)	200 ± 5 gametophyte/mL	

General Experimental Methods

Retention times and retention factors were recorded on a dionex ultimate 3000 HPLC with a VWD set to 210 nm, 220 nm, 254 nm, and 280 nm and a Phenomenex Gemini C18-Si column (150 mm × 4.6 mm, 5 µm) with an eluent system of either A) a gradient of 100:0 A:B to 0:100 A:B over 10 min at 1 mL/min; where solvent A was water (+0.1% v/v trifluoroacetic acid) and solvent B was acetonitrile (+0.1% v/v trifluoroacetic acid); or B) isocratic system of 26:74 A:B over 90 min at 1 mL/min; where solvent A was water (+0.1% v/v trifluoroacetic acid) and solvent B was acetonitrile (+0.1% v/v trifluoroacetic acid), unless stated otherwise.

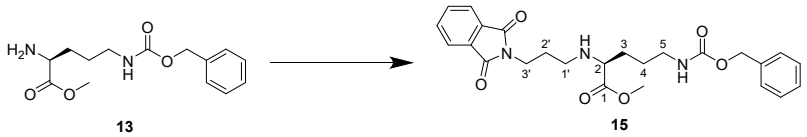
***N*-Bip-L-Orn(Z)-OMe (14)**



13 14

A solution of L-Orn(Z)-OMe.HCl **13** (1.0 g, 3.57 mmol), DIPEA (1.24 mL, 7.13 mmol) and 4-phenylbenzaldehyde (0.65 g, 3.57 mmol) in THF (50 mL) was stirred at rt for 1 h. To this mixture was then added $NaBH_3CN$ (0.67 g, 10.7 mmol), MeOH (5 mL), and AcOH (2.5 mL), and the mixture stirred at rt for a further 4 h. The mixture was then concentrated *in vacuo* and the resulting residue resuspended in EtOAc (20 mL). The organic layer was then washed with NaOH (1 M, 1 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (Pet. Ether-EtOAc 1:1) afforded *title compound* **14** (0.72 g, 45%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.60 – 7.30 (m, 14H, H-Ar), 5.15 – 5.10 (m, 3H, H-NH, H-Cbz), 3.86 (ABq, J = 72.3, 12.9 Hz 2H, H-1′), 3.74 (s, 3H, H-OC$\underline{H}_3$), 3.32 (t, J = 5.8 Hz, 1H, H-2), 3.23 – 3.17 (m, 2H, H-5), 1.76 – 1.60 (m, 4H, H-3, H-4); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 175.8 (C, C-1), 156.5 (C, C-Cbz), 141.0 (C, C-Ar), 140.2 (C, C-Ar), 138.8 (C, C-Ar), 136.7 (C, C-Ar), 128.83 (2 × CH, C-Ar), 128.82 (2 × CH, C-Ar), 128.6 (2 × CH, C-Ar), 128.2 (2 × CH, C-Ar), 128.16 (CH, C-Ar), 127.3 (CH, C-Ar), 127.2 (2 × CH, C-Ar), 127.1 (2 × CH, C-Ar), 66.7 (CH_2, C-Cbz), 60.5 (CH, C-2), 52.0 (CH_2, C-1′), 51.9 (CH_3, C-O$\underline{C}H_3$), 40.9 (CH_2, C-5), 30.8 (CH_2, C-3), 26.5 (CH_2, C-4); **HRMS** (ESI) *m/z*: $[M + H]^+$ cald for $C_{27}H_{31}N_2O_4$, 447.2278; found, 447.2285.

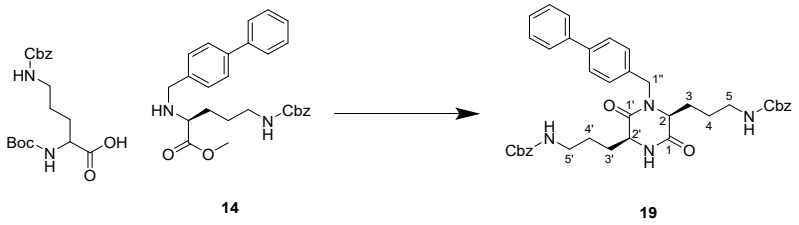
***N*-(*N*-phthaloylpropylamine)-L-Orn(Z)-OMe (15)**



13 15

To a solution of L-Orn(Z)-OMe **15** (0.3 g, 0.83 mmol) and *N*-(3-bromopropyl)phthalimide (0.24 g, 0.91 mmol) in DMF (8 mL) over 4 Å mol sieves (0.3 g) was added dropwise over 12 h TEA (0.12 mL, 0.83 mmol), and the mixture stirred for a further 5 h. The mixture was then poured into H_2O (20 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered, and then concentrated *in vacuo*. Purification *via* flash chromatography (Pet. Ether-EtOAc 1:1) afforded *title compound* **15** (0.29 g, 73%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.82 – 7.78 (m, 2H, H-Phth), 7.70 – 7.65 (m, 2H, H-Phth), 7.32 – 7.26 (m, 5H, H-Cbz), 5.45 (br, 1H, H-NH), 5.07 (s, 2H, H-Cbz), 3.78 – 3.70 (m, 2H, H-3′), 3.67 (s, 3H, OC$\underline{H}_3$), 3.21 – 3.18 (m, 3H, H-2, H-5), 2.70 – 2.64 (m, 1H, H_a-1′), 2.47 – 2.40 (m, 1H, H_b-1′), 1.83 (p, J = 6.9 Hz, 2H, H-2′), 1.70 – 1.52 (m, 4H, H-3, H-4); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 175.5 (C, C-1), 168.3 (2 × C, C-Phth), 156.4 (C, C-Cbz), 136.7 (C, C-Cbz), 133.8 (2 × CH, C-Phth), 132.0 (2 × C, C-Phth), 128.3 (2 × CH, C-Cbz), 128.0 (2 × CH, C-Cbz), 127.9 (CH, C-Cbz), 123.1 (2 × CH, C-Phth), 66.3 (CH_2, C-Cbz), 61.0 (CH, C-2), 51.6 (C-O$\underline{C}H_3$), 45.2 (CH_2, C-1′), 40.7 (CH_2, C-5), 35.8 (CH_2, C-3′), 30.6 (CH_2, C-3), 28.8 (CH_2, C-2′), 26.2 (CH_2, C-4); **HRMS** (ESI) *m/z*: $[M + H]^+$ cald for $C_{25}H_{30}N_3O_6$, 468.2129; found, 468.2113.

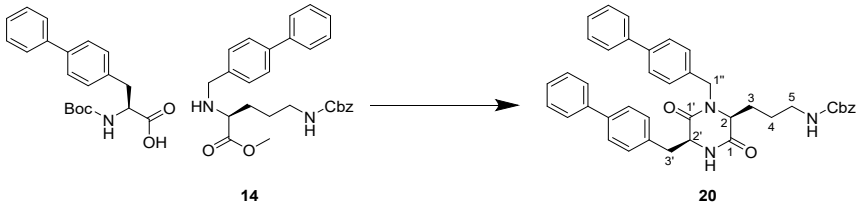
Cyclo(L-Orn(Z)-*N*-Bip-L-Orn(Z)) (19)



To a solution of Boc-L-Orn(Z)-OH (0.49 g, 1.34 mmol) and DIC (0.21 mL, 1.34 mmol) in THF (5 mL) was added DIPEA (0.36 mL, 2.02 mmol), and the mixture stirred at rt for 1 min. A solution of amino acid **14** (0.30 g, 0.67 mmol) in THF (40 mL) was then added, and the mixture stirred at rt for a further 16 h. The solvent was then removed *in vacuo*, and the resulting residue redissolved in EtOAc (10 mL). The organic layer was then washed with HCl (1 M, 10 mL), sat. aq. $NaHCO_3$ (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to afford intermediate **16** (0.23 g, 42%) as a colourless solid.

To a solution of dipeptide **16** (0.23 g, 0.28 mmol) in DCM (3 mL) was added TFA (3 mL), and the mixture stirred at rt for 1 h. The mixture was then concentrated *in vacuo* and the resulting residue taken up in *n*-BuOH (0.1 M AcOH, 30 mL). To this mixture (at 120 °C) was added NMM (0.031 mL, 0.28 mmol), and the mixture heated at reflux for 16 h. The mixture was then allowed to cool to rt and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (10 mL), brine (10 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (DCM-MeOH 19:1) afforded *title compound* **21** (0.12 g, 64%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 8.10 (s, 1H, H-NH), 7.54 – 7.24 (m, 19H, H-Ar), 5.43 – 5.40 (m, 1H, H-NH), 5.32 – 5.29 (m, 1H, H-NH), 5.04 (s, 4H, H-Cbz), 4.00 – 3.95 (m, 3H, H-1″, H-2′), 3.86 – 3.83 (m, 1H, H-2), 3.17 – 3.09 (m, 4H, H-5, H-5′), 2.04 – 1.53 (m, 8H, H-3, H-3′, H-4, H-4′); **HRMS** (ESI +) *m/z*: $[M + Na]^+$ cald for $C_{39}H_{42}N_4NaO_6$, 685.2997; found, 685.2991.

Cyclo(L-Bip-*N*-Bip-L-Orn(Z)) (20)

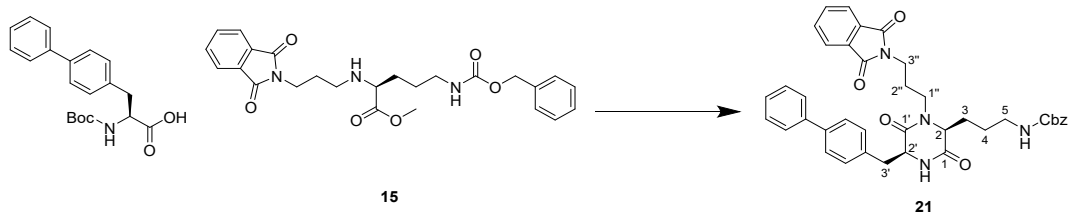


14 **20**

A similar method to that described for compound **16** was followed using Boc-L-Bip-OH (0.31 g, 0.90 mmol), amino acid **14** (0.20 g, 0.45 mmol), DIC (0.14 mL, 0.90 mmol), DIPEA (0.24 mL, 1.34 mmol) and THF (10 mL). Concentration *in vacuo* afforded intermediate **17** (0.31 g, 90 %) as a colourless solid.

A similar method to that described for compound **19** was followed using dipeptide **17** (0.31 g, 0.40 mmol), DCM (5 mL) and TFA (5 mL), then *n*-BuOH (0.1 M AcOH, 10 mL) and NMM (0.044 mL, 0.40 mmol). Purification *via* flash chromatography (Pet. Ether-EtOAc 1:1) afforded *title compound* **20** (0.20 g, 73%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.54 – 7.24 (m, 23H, H-Ar), 6.76 (s, 1H, H-NH), 5.25 (d, J = 14.7 Hz, 1H, H_a-1″), 5.01 (s, 2H, H-Cbz), 4.84 (brt, 1H, H-NH), 4.35 – 4.33 (m, 1H, H-2′), 4.04 (d, J = 14.7 Hz, 1H, H_b-1″), 3.85 – 3.81 (m, 1H, H-2), 3.31 (ABX, J = 13.5, 2.8 Hz, 1H, H_a-3′), 3.16 (ABX, J = 13.5, 7.9 Hz, 1H, H_b-3′), 3.04 – 2.94 (m, 2H, H-5), 1.79 – 1.71 (m, 1H, H_a-3), 1.36 – 1.28 (m, 3H, H_b-3, H-4); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 167.4 (C, C-1), 165.5 (C, C-1′), 156.4 (C, C-Cbz), 141.1 (C, C-Ar), 140.5 (C, C-Ar), 140.4 (C, C-Ar), 140.3 (C, C-Ar), 136.6 (C, C-Ar), 134.7 (C, C-Ar), 134.5 (C, C-Ar), 130.5 (2 × CH, C-Ar), 129.0 (2 × CH, C-Ar), 128.93 (2 × CH, C-Ar), 128.90 (2 × CH, C-Ar), 128.6 (2 × CH, C-Ar), 128.2 (CH, C-Ar), 128.17 (CH, C-Ar), 127.7 (2 × CH, C-Ar), 127.6 (5 × CH, C-Ar), 127.2 (2 × CH, C-Ar), 127.0 (2 × CH, C-Ar), 66.7 (CH_2, C-Cbz), 58.2 (CH, C-2), 57.0 (CH, C-2′), 47.2 (CH_2, C-1″), 40.8 (CH_2, C-3′), 40.4 (CH_2, C-5), 29.1 (CH_2, C-3), 25.2 (CH_2, C-4); **HRMS** (ESI+) *m/z*: $[M + Na]^+$ cald for $C_{41}H_{39}N_3NaO_4$, 660.2833; found, 660.2835.

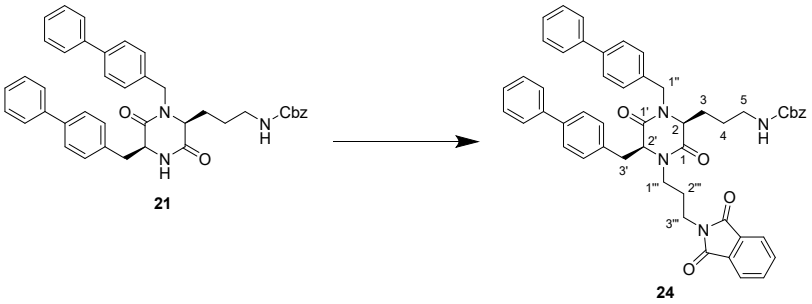
Cyclo(L-Bip-*N*-(*N*-phthyolylpropylamine)-L-Orn(Z)) (21)



A similar method to that described for compound **16** was followed using Boc-L-Bip-OH (0.58 g, 1.71 mmol), amino acid **15** (0.20 g, 0.43 mmol), DIC (0.27 mL, 1.71 mmol), DIPEA (0.30 mL, 1.71 mmol) and THF (10 mL). Concentration *in vacuo* afforded intermediate **18** (0.18 g, 53 %) as a colourless solid.

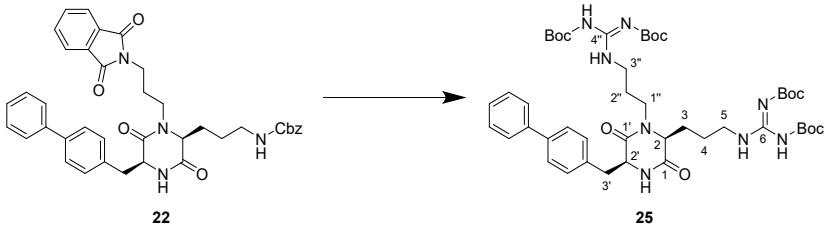
A similar method to that described for compound **19** was followed using dipeptide **18** (0.18 g, 0.23 mmol), DCM (2 mL) and TFA (2 mL), then *n*-BuOH (0.1 M AcOH, 5 mL) and NMM (0.025 mL, 0.23 mmol). Purification *via* flash chromatography (Pet. Ether-EtOAc 3:7) afforded *title compound* **21** (0.13 g, 87%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.68 – 7.23 (m, 18H, H-Ar), 6.73 (s, 1H, H-NH), 5.14 (brt, 1H, H-NH), 5.00 (s, 2H, H-Cbz), 4.26 – 4.24 (m, 1H, H-2′), 3.91 – 3.90 (m, 1H, H-2), 3.74 – 3.67 (m, 3H, H_a-1″, H-3″), 3.22 – 3.00 (m, 5H, H-3′,H-5, H_b-1″), 2.06 – 1.98 (m, 1H, H_a-2″), 1.95 – 1.86 (m, 1H, H_b-2″), 1.75 – 1.72 (m, 1H, H_a-3), 1.39 – 1.36 (m, 2H, H-4), 1.22 – 1.17 (m, 1H, H_b-3); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 168.4 (2 × C, C-Phth), 167.2 (C, C-1), 165.4 (C, C-1′), 156.5 (C, Cbz), 140.4 (C, C-Ar), 140.3 (C, C-Ar), 136.7 (C, C-Ar), 134.8 (C, C-Ar), 134.1 (2 × CH, C-Phth) 132.0 (2 × C, C-Phth), 130.5 (2 × CH, C-Ar), 130.0 (2 × CH, C-Ar), 128.5 (2 × CH, C-Ar), 128.1 (3 × CH, C-Ar), 127.5 (3 × CH, C-Ar), 127.0 (2 × CH, C-Ar), 123.4 (2 × CH, C-Phth), 66.6 (CH_2, C-Cbz), 59.5 (CH, C-2), 56.9 (CH, C-2′), 43.0 (CH_2, C-1″), 40.6 (CH_2, C-3′), 40.3 (CH_2, C-5), 35.7 (CH_2, C-3″), 29.9 (CH_2, C-3), 26.6 (CH_2, C-2″), 25.4 (CH_2, C-4); **HRMS** (ESI) *m/z*: $[M + Na]^+$ cald for $C_{39}H_{38}N_4NaO_6$, 681.2684; found, 681.2679.

Cyclo(*N*-(*N*-phthaloylpropylamine)-L-Bip-*N*-Bip-L-Orn(Z)) (24)



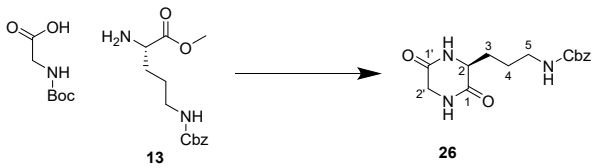
To a solution of DKP **21** (0.2 g, 0.31 mmol) in DMF (5 mL) under nitrogen at -40 °C was added dropwise KHMDS (0.9 M, 0.52 mL, 0.47 mmol), and the mixture stirred for 1 h. To this mixture was added a solution of *N*-(3-bromopropyl)phthalimide (0.17 g, 0.63 mmol) in DMF (3 mL), and the mixture stirred at -40 °C for a further 16 h. The mixture was then poured into sat. aq. NH_4Cl (20 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Purification *via* flash column chromatography (Pet. Ether-EtOAc 1:1) afforded *title compound* **24** (0.07 g, 27%) as pale yellow solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.78 – 7.75 (m, 2H, H-Phth), 7.66 – 7.64 (m, 2H, H-Phth), 7.55 – 7.20 (m, 23H, H-Ar), 5.20 (d, J = 14.9 Hz, 1H, H_a-1″), 5.04 – 4.98 (m, 2H, H-Cbz), 4.75 – 4.72 (m, 1H, H-NH), 4.42 (t, J = 4.8 Hz, 1H, H-2′), 4.01 – 3.91 (m, 2H, H_b-1″, H_a1‴), 3.72 – 3.63 (m, 3H, H-2, H-3‴), 3.33 – 3.23 (m, 2H, H-3′), 2.97 – 2.80 (m, 3H, H_b-1‴, H-5), 2.02 – 1.94 (m, 2H, H-2‴), 1.39 – 1.32 (m, 3H, H_a-3, H-4), 0.65 – 0.63 (m, 1H, H_b-3); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 168.3 (2 × C, C-Phth), 166.2 (C, C-1), 165.6 (C, C-1′), 156.4 (C, C-Cbz), 141.1 (C, C-Ar), 140.6 (C, C-Ar), 140.5 (C, C-Ar), 140.3 (C, C-Ar), 136.7 (C, C-Ar), 134.8 (C, C-Ar), 134.6 (C, C-Ar), 134.1 (2 × CH, C-Phth), 132.0 (2 × C, C-Ar), 130.5 (2 × CH, C-Ar), 129.0 (2 × CH, C-Ar), 128.9 (4 × CH, C-Ar), 128.6 (2 × CH, C-Ar), 128.2 (2 × CH, C-Ar), 127.7 (2 × CH, C-Ar), 127.6 (CH, C-Ar), 127.54 (2 × CH, C-Ar), 127.48 (2 × CH, C-Ar), 127.2 (2 × CH, C-Ar), 127.0 (2 × CH, C-Ar), 123.4 (2 ×CH, C-Phth), 66.6 (CH_2, C-Cbz), 61.7 (CH, C-2′), 58.5 (CH, C-2), 47.4 (CH_2, C-1″), 42.8 (CH_2, C-1‴), 40.3 (CH_2, C-5), 38.3 (CH_2, C-3′), 35.6 (CH_2, C-3‴), 30.2 (CH_2, C-3), 26.9 (CH_2, C-4), 26.3 (CH_2, C-2‴); **HRMS** (ESI +) *m/z*: $[M + H]^+$ cald for $C_{52}H_{48}N_4NaO_6$, 847.3466; found, 847.3473.

Cyclo(L-Bip-*N*-(*N*,*N*′-di-Boc-propylguanidine)-L-Arg-*N*,*N*′-di-Boc) (25)



To a refluxing solution of DKP **22** (0.13 g, 0.20 mmol) in EtOH (2 mL) was added hydrazine hydrate (7 μL, 0.22 mmol), and the mixture stirred at reflux for 16 h. The mixture was then allowed to cool to rt and filtered. The filtrate was concentrated *in vacuo* and the resulting residue redissolved in MeOH (2 mL). To this mixture was added Pd/C (0.02 g, 0.02 mmol 10% wt/wt), and the mixture stirred under H_2 at rt for 16 h. The mixture was then filtered through Celite® and the filtrate concentrated *in vacuo.* The residue was redissolved in H_2O (0.5 mL) and to this mixture was added a solution of *N*,*N*′-di-boc-1*H*-pyrazole-1-carboxamidine (0.13 g, 0.43 mmol) and TEA (0.081 mL, 0.59 mmol) in MeCN (4.5 mL), and the mixture stirred at rt for 6 h. The resulting precipitate was collected *via* vacuum filtration and dried *in vacuo*. Purification *via* flash column chromatography (neat EtOAc) afforded *title compound* **25** (0.094 g, 55%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 11.49 (s, 1H, H-NH), 11.44 (s, 1H, H-NH), 8.50 (t, J = 5.6 Hz, 1H, H-NH), 8.30 (t, J = 5.3 Hz, 1H, H-NH), 7.59 – 7.28 (m, 9H, H-Ar), 6.35 (d, J = 2.7 Hz, 1H, H-NH), 4.31 (m, 1H, H-2′), 3.94 – 3.87 (m, 2H, H-2, H_a-1″), 3.59 – 3.51 (m, 1H, H_a-3″), 3.41 – 3.28 (m, 4H, H-5, H_a-3′, H_b-3″), 3.05 – 2.94 (m, 2H, H_b-3′, H_b-1″), 1.99 – 1.76 (m, 3H, H-2″, H_a-3), 1.63 – 1.38 (m, 39H, H_b-3, H-4, H-Boc); **^{13}C NMR** (100 MHz; $CDCl_3$): δ 166.9 (C, C-1), 165.6 (C, C-1′), 163.63 (C, C-Boc), 163.60 (C, C-Boc), 156.3 (C, C-6 or 4″), 156.2 (C-6 or 4″), 153.4 (C, C-Boc), 153.3 (C, C-Boc), 140.5 (C, C-Ar), 140.4 (C, C-Ar), 134.8 (C, C-Ar), 130.3 (2 × CH, C-Ar), 128.9 (2 × CH, C-Ar), 127.7 (2 × CH, C-Ar), 127.5 (CH, C-Ar), 127.0 (2 × CH, C-Ar), 83.22 (C, C-Boc), 83.19 (C, C-Boc), 79.3 (2 × C, C-Boc), 59.4 (CH, C-2), 57.0 (CH, C-2′), 42.7 (CH_2, C-1″), 41.0 (CH_2, C-3′), 40.1 (CH_2, C-5), 38.2 (CH_2, C-3″), 30.2 (CH_2, C-3), 28.4 (6 × CH_3, C-Boc), 28.2 (3 × CH_3, C-Boc), 28.1 (3 × CH_3, C-Boc), 27.0 (CH_2, C-2″), 24.9 (CH_2, C-4).

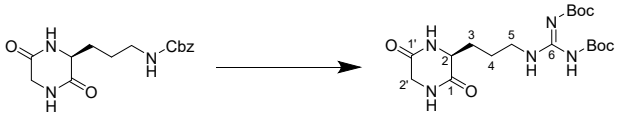
Cyclo(Gly-L-Orn(Z)) (26)



To a solution of Boc-Gly (0.37 g, 2.14 mmol), HATU (0.81 g, 2.14 mmol) and 6-Cl-HOBt (0.36 g, 2.14 mmol) in DMF (20 mL) was added DIPEA (1.14 mL, 6.42 mmol), and the mixture stirred at rt for 5 min. L-Orn(Z)-OMe.HCl (**13**) (0.60 g, 2.14 mmol), was then added, and the mixture stirred at rt for a further 5 h. The mixture was then poured into H_2O (150 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were washed with H_2O (3 × 60 mL), brine (2 × 60 mL), dried over anhydrous $MgSO_4$, filtered, then concentrated *in vacuo* to afford dipeptide **25** (0.9 g, 96%) as a colourless solid which was used without further purification.

To a solution of dipeptide **25** (0.9 g, 2.06 mmol) in DCM (10 mL) was added TFA (5 mL), and the mixture stirred at rt for 1 h. The mixture was then concentrated and the resulting residue taken up in *s*-BuOH (0.1 M AcOH, 50 mL). To this mixture (at 120 °C) was added NMM (0.23 mL, 2.06 mmol), and the mixture heated at reflux for 16 h. The mixture was then cooled to 0 °C and the resulting precipitate collected *via* vacuum filtration. The solid was washed with cold MeOH (2 × 20 mL) and dried *in vacuo* to afford *title compound* **26** (0.28 g, 45%) as a colourless solid which was used without further purification. **^{1}H NMR** (400 MHz; DMSO): δ 8.16 (s, 1H, H-NH), 7.99 (s, 1H, H-NH), 7.38 – 7.29 (m, 5H, H-Cbz), 7.27 (brt, 5.3 Hz, 1H, H-NH), 5.00 (s, 2H, H-Cbz), 3.79 – 3.64 (m, 3H, H-2, H-2′), 3.01 – 2.96 (m, 2H, H-5), 1.67 – 1.63 (m, 2H, H-3), 1.46 – 1.43 (m, 2H, H-4); **^{13}C NMR** (100 MHz; DMSO): δ 167.8 (C, C-1), 166.0 (C, C-1′), 156.1 (C, C-Cbz), 137.2 (C, C-Cbz), 128.3 (2 × CH, C-Cbz), 127.7 (3 × CH, C-Cbz), 65.1 (CH_2, C-Cbz), 54.0 (CH, C-2), 44.2 (CH_2, C-2′), 40.1 (CH_2, C-5), 30.2 (CH_2, C-3), 24.8 (CH_2, C-4).

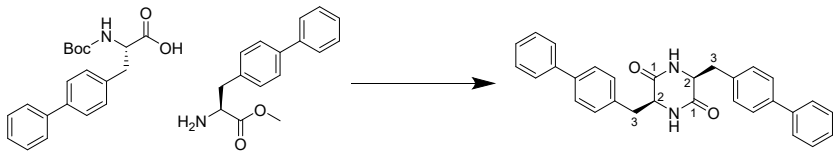
Cyclo(Gly-L-Arg-*N,N′*-di-Boc) (27)



26 **27**

A similar method to that described for compound **4a** was followed using DKP **26** (0.25 g, 0.82 mmol), Pd/C (0.01 g, 0.01 mmol, 10% wt/wt) and DCM:AcOH (1:1, 6 mL), then H_2O (1 mL), *N*,*N*′-di-Boc-1*H*-pyrazole-1-carboxamidine (0.23 g, 0.90 mmol), TEA (0.34 mL, 2.45 mmol) and MeCN (9 mL). Purification *via* flash chromatography (Pet. Ether-EtOAc 3:7) afforded *title compound* **27** (0.12 g, 81%) as a colourless solid. **^{1}H NMR** (400 MHz; DMSO): δ 11.49 (s, 1H, H-NH), 8.33 (t, J = 5.5 Hz, 1H, H-NH), 8.18 (d, J = 2.0 Hz, 1H, H-NH), 8.00 (s, 1H, H-NH), 3.83 – 3.63 (m, 3H, H-2, H-2′) 3.35 – 3.21 (m, 2H, H-5), 1.70 – 1.54 (m, 4H, H-3, H-4), 1.47 (s, 9H, H-Boc), 1.39 (s, 9H, H-Boc); **^{13}C NMR** (100 MHz; DMSO): δ 167.8 (C, C-1), 166.0 (C, C-1′), 163.1 (C, C-Boc), 155.2 (C, C-6), 152.0 (C, C-Boc), 82.8 (C, C-Boc), 78.1 (C, C-Boc), 53.8 (CH, C-2), 44.2 (CH_2, C-2′), 39.5 (CH_2, C-5), 30.0 (CH_2, C-3), 28.0 (3 × CH_3, C-Boc), 27.6 (3 × CH_3, C-Boc), 24.1 (CH_2, C-4).

Cyclo(L-Bip-L-Bip) (29)

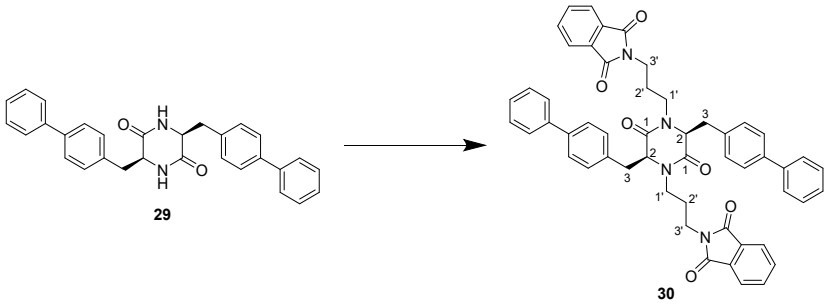


29

To a solution of Boc-L-Bip-OH (1.0 g, 2.93 mmol), HATU (1.11 g, 2.93 mmol) and 6-Cl-HOBt (0.50 g, 2.93 mmol) in DMF (30 mL) was added DIPEA (1.53 mL, 8.79 mmol), and the mixture stirred at rt for 5 min. L-Bip-OMe.HCl (0.75 g, 2.93 mmol) was then added, and the mixture stirred at rt for a further 5 h. The mixture was then poured into H_2O (150 mL) and extracted with DCM (3 × 20 mL). The combined organic layers were washed with H_2O (3 × 60 mL), brine (2 × 60 mL), dried over anhydrous $MgSO_4$, filtered, then concentrated *in vacuo* to afford dipeptide **28** (1.69 g, 99%) as a colourless solid which was used without further purification.

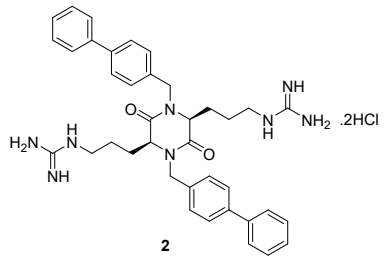
To a solution of dipeptide **28** (1.69 g, 2.92 mmol) in DCM (25 mL) was added TFA (25 mL), and the mixture stirred at rt for 1 h. The mixture was then concentrated and the resulting residue taken up in *s*-BuOH (0.1 M AcOH, 50 mL). To this mixture (at 120 °C) was added NMM (0.30 mL, 2.92 mmol), and the mixture heated at reflux for 16 h. The mixture was then cooled to 0 °C and the resulting precipitate collected *via* vacuum filtration. The solid was washed with cold MeOH (2 × 20 mL) and dried *in vacuo* to afford *title compound* **29** (1.08 g, 83%) as a colourless solid which was used without further purification. **^{1}H NMR** (400 MHz; DMSO): δ 8.03 (d, 2H, H-NH), 7.60 – 7.12 (m, 18H, H-Ar), 4.08 – 4.05 (m, 2H, H-2), 2.70 (dd, J = 13.6, 5.0 Hz, 2H, H_a-3), 2.46 (dd, J = 13.6, 5.9 Hz, 2H, H_b-3); **^{13}C NMR** (100 MHz; DMSO): δ 166.3 (2 × C, C-1), 139.9 (2 × C, C-Ar), 138.3 (2 × C, C-Ar), 135.9 (2 × C, C-Ar), 130.4 (4 × CH, C-Ar), 128.9 (4 × CH, C-Ar), 127.2 (2 × CH, C-Ar), 126.4 (8 × CH, C-Ar), 55.3 (2 × CH, C-2), 38.7 (2 × CH_2, C-3); **HRMS** (ESI) *m/z*: $[M + Na]^+$ cald for $C_{30}H_{26}N_2NaO_2$, 469.1889; found, 469.1886.

Cyclo(*N*-(*N*-phthaloylpropylamine)-L-Bip-*N*-(*N*-phthaloylpropylamine)-L-Bip) (30)



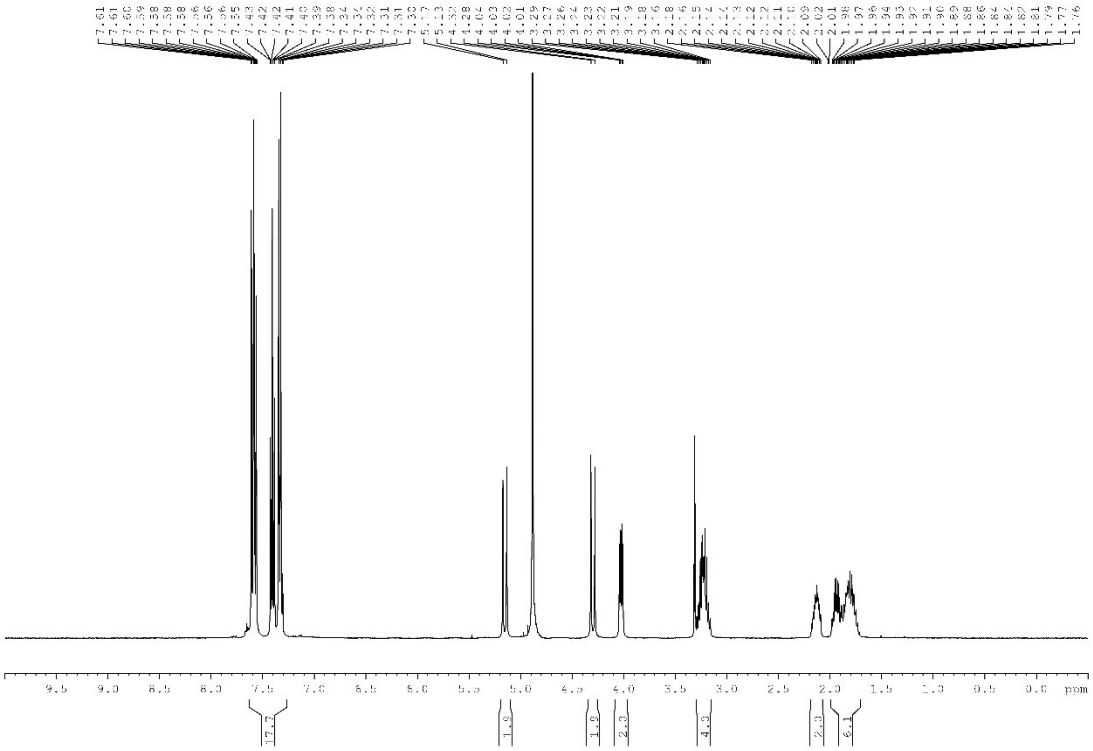
To a solution of DKP **29** (0.2 g, 0.45 mmol) in DMF (8 mL) under nitrogen at 0 °C was added NaH (0.06 g, 1.34 mmol, 60% immersed in mineral oil), and the mixture stirred at 0 °C for 1 h. To this mixture was then added a solution of *N*-(3-bromopropyl)phthalimide (0.6 g, 2.24 mmol) in DMF (2 mL), and the mixture heated at 80 °C for a further 16 h. The mixture was then poured into a solution of sat. aq. NH_4Cl (20 mL) and extracted with DCM (3 × 5 mL). The combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. Purification *via* flash chromatography (Pet. Ether-EtOAc 1:4) afforded *title compound* **30** (0.12 g, 33%) as a colourless solid. **^{1}H NMR** (400 MHz; $CDCl_3$): δ 7.73 – 7.71 (m, 4H, H-Phth), 7.62 – 7.60 (m, 4H, H-Phth), 7.49 – 7.29 (m, 14H, H-Ar), 7.11 (d, 4H, H-Ar), 4.25 (AB<u>X</u>, J = 6.6, 4.5 Hz, 2H, H-2), 3.92 – 3.85 (m, 2H, Ha-1′), 3.69 – 3.58 (m, 4H, H-3′), 2.89 (<u>A</u>BX, J = 14.3, 4.5 Hz, 2H, Ha-2), 2.72 (2H, Hb-1′), 2.40 (A<u>B</u>X, J = 14.3, 6.6 Hz, 2H, Hb-2), 2.07 (m, 4H, H-2′); **^{13}C NMR** (100 MHz; $CDCl_3$): *δ* 168.3 (4 × C, C-Phth), 165.8 (2 × C, C-1), 140.6 (2 × C, C-Ar), 140.3 (2 × C, C-Ar), 135.8 (2 × C, C-Ar), 134.0 (4 × CH, C-Ar), 132.0 (4 × C, C-Phth), 129.9 (4 × CH, C-Ar), 128.8 (4 × CH, C-Ar), 127.6 (4 × CH, C-Ar), 127.4 (2 × CH, C-Ar), 127.1 (4 × CH, C-Ar), 123.3 (4 × CH, C-Phth) 61.7 (2 × CH, C-2), 42.9 (2 × CH_2, C-1′), 38.9 (2 × CH_2, C-3), 35.5 (2 × CH_2, C-3′), 25.9 (2 × CH_2, C-2′); **HRMS** (ESI) *m/z*: $[M + Na]^+$ cald for $C_{52}H_{44}N_4NaO_6$, 843.3153; found, 843.3123.

Cyclo(*N*-Bip-L-Arg-*N*-Bip-L-Arg).2HCl (2)

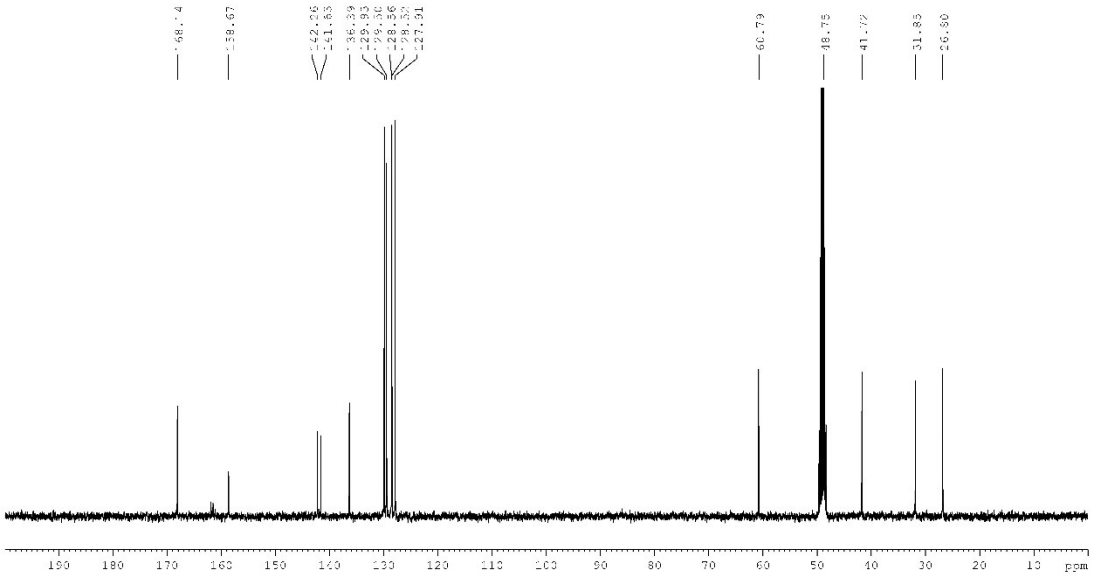


2

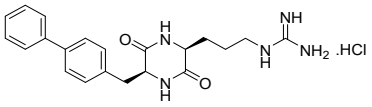
^{1}H NMR (400 MHz, MeOD):



^{13}C NMR (100 MHz, MeOD):

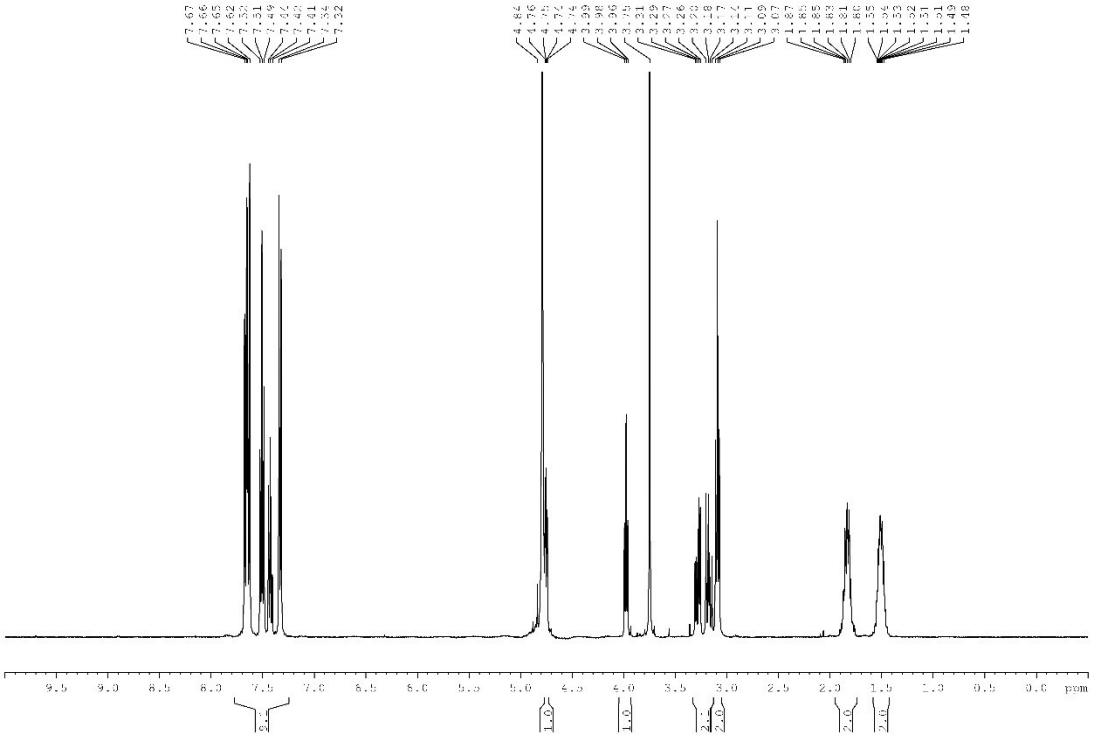


Cyclo(L-Bip-L-Arg).HCl (3)

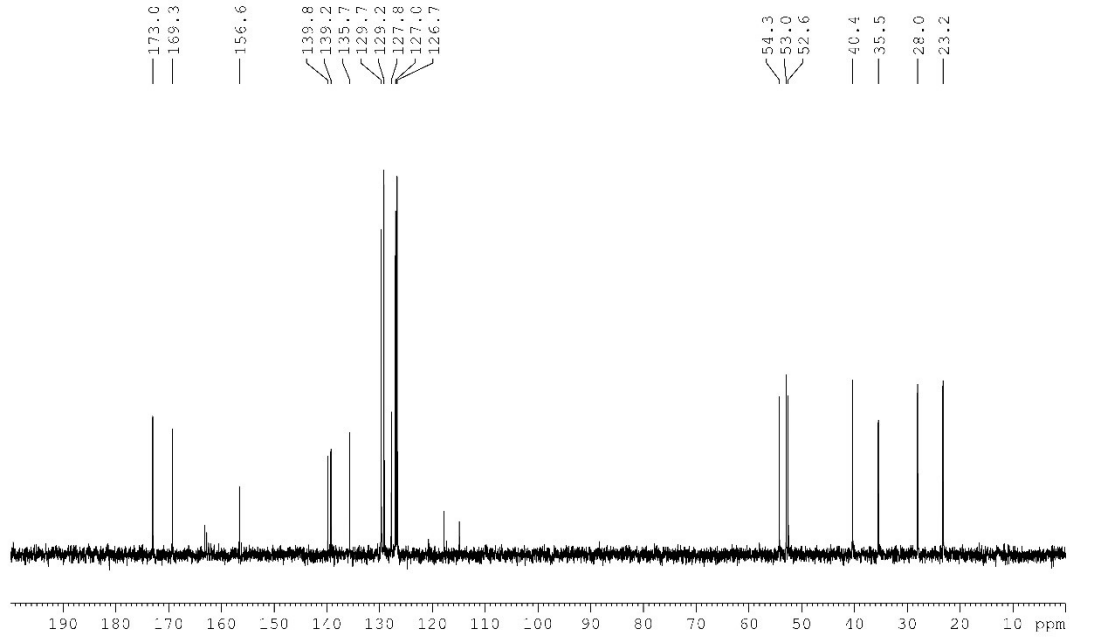


3

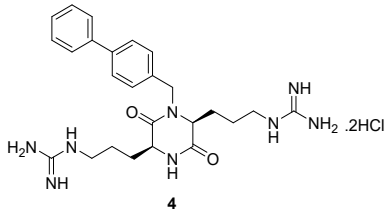
[1]H NMR (400 MHz, D_2O):



[13]C NMR (100 MHz, D_2O):

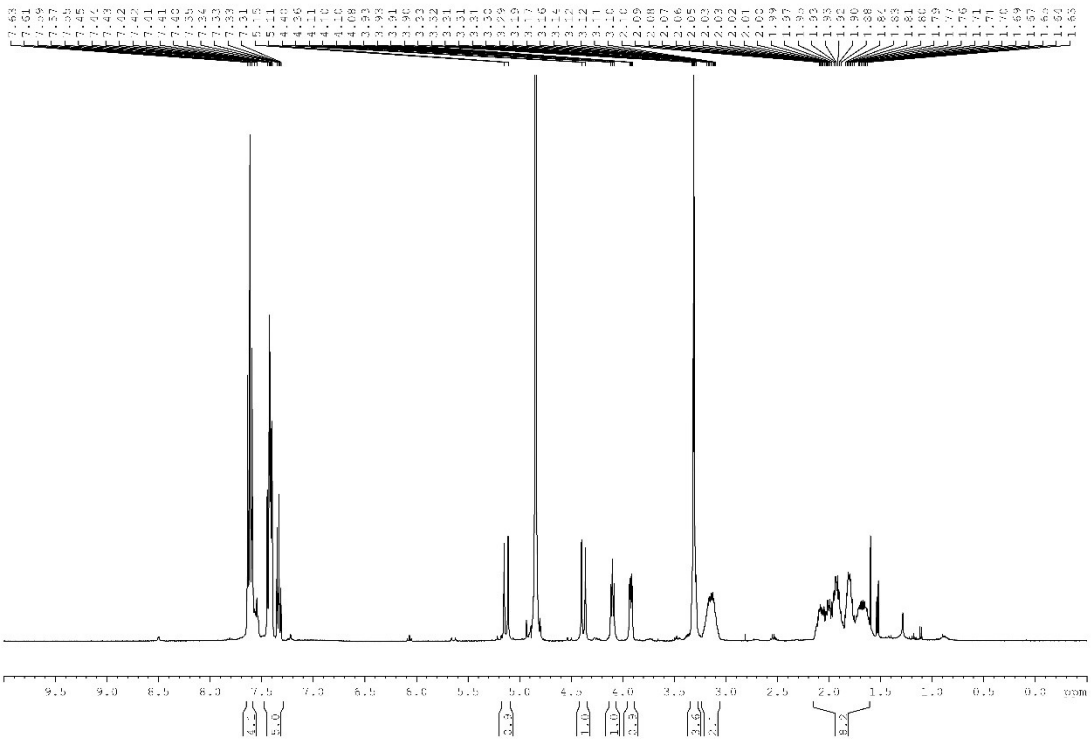


Cyclo(*N*-Bip-L-Arg-*N*-H-L-Arg).2HCl (4)

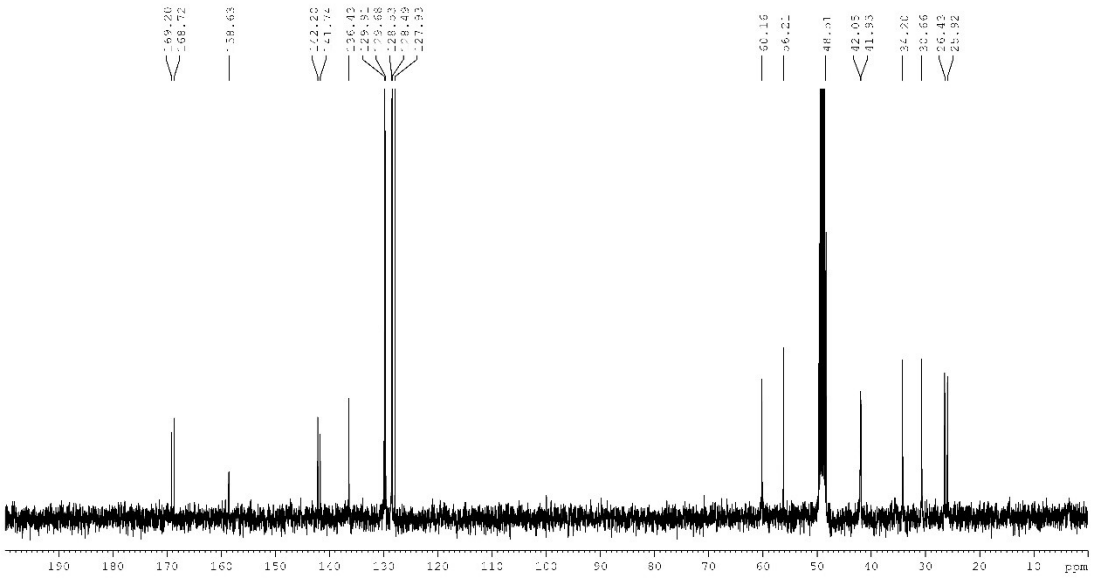


4

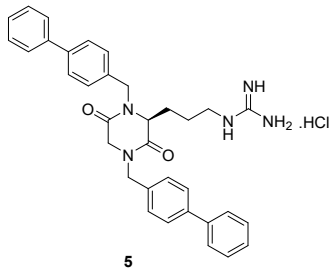
[1]H NMR (400 MHz, MeOD):



[13]C NMR (100 MHz, MeOD):

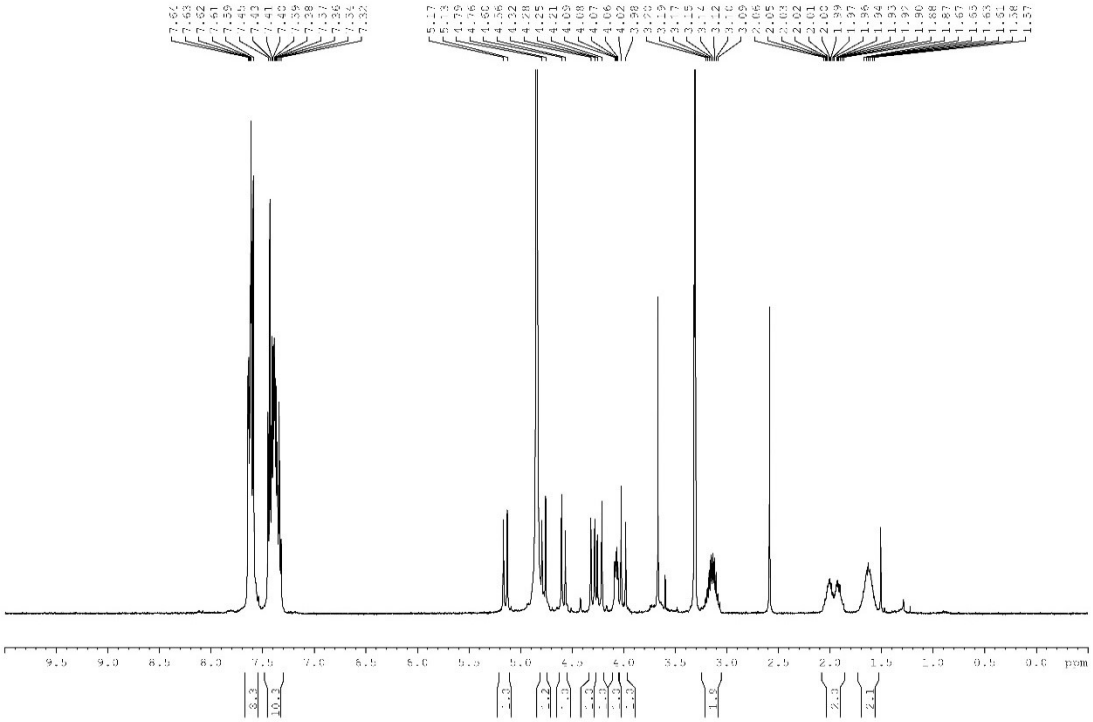


Cyclo(*N*-Bip-Gly-*N*-Bip-L-Arg).HCl (5)

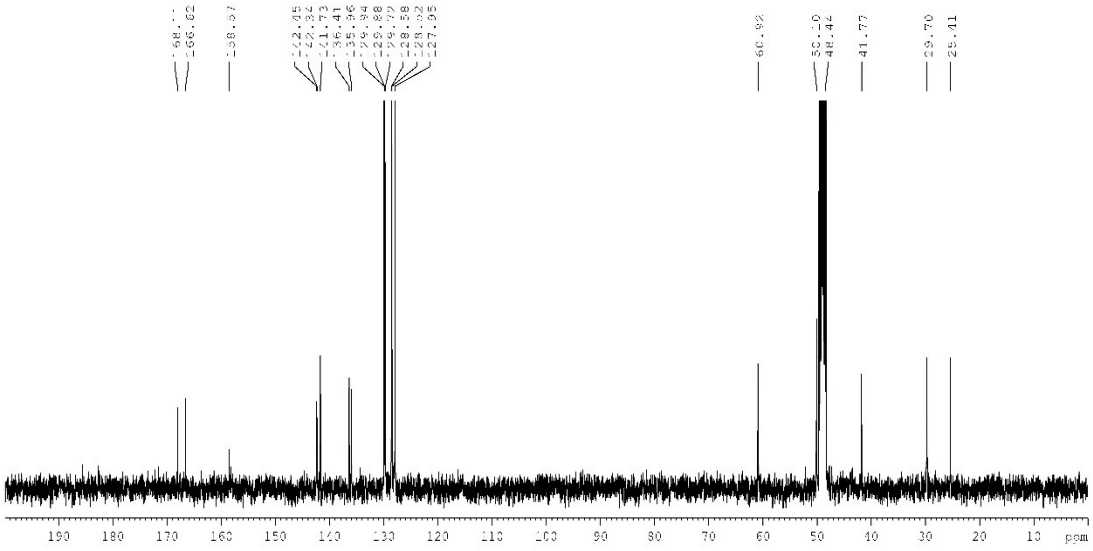


5

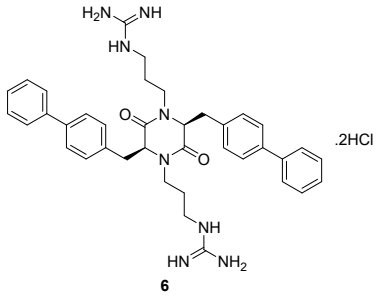
[1]H NMR (400 MHz, MeOD):



[13]C NMR (100 MHz, MeOD):

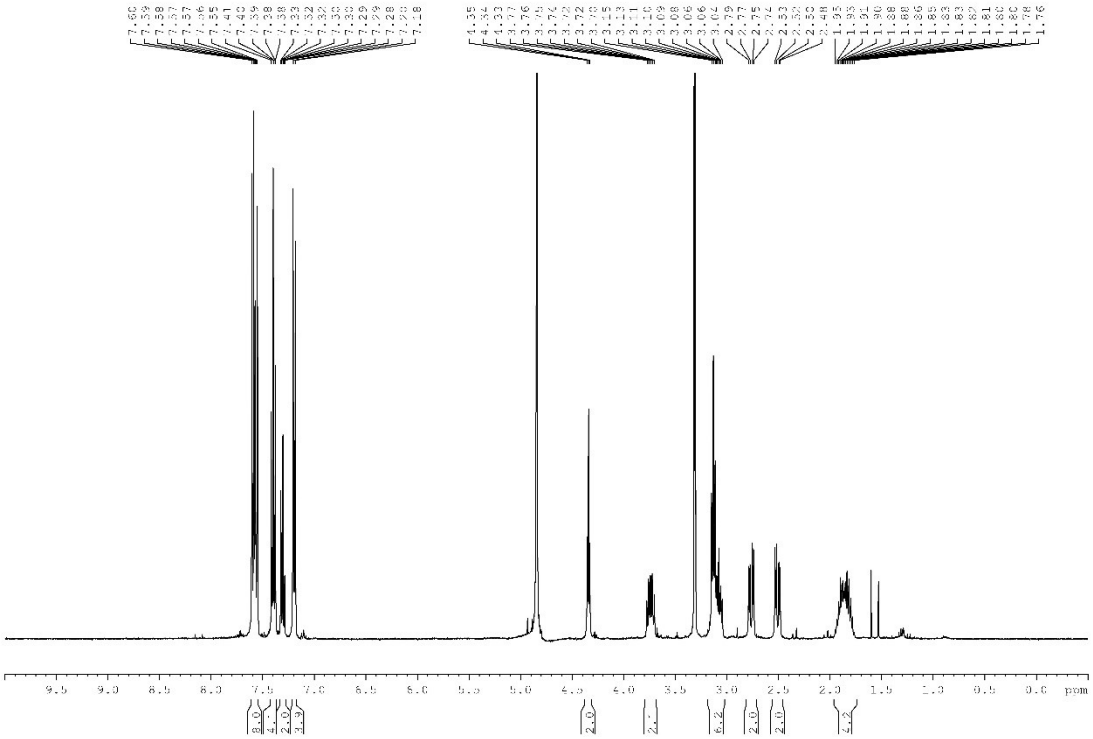


Cyclo(*N*-(propylguanidine)-L-Bip-*N*-(propylguanidine)-L-Bip).2HCl (6)

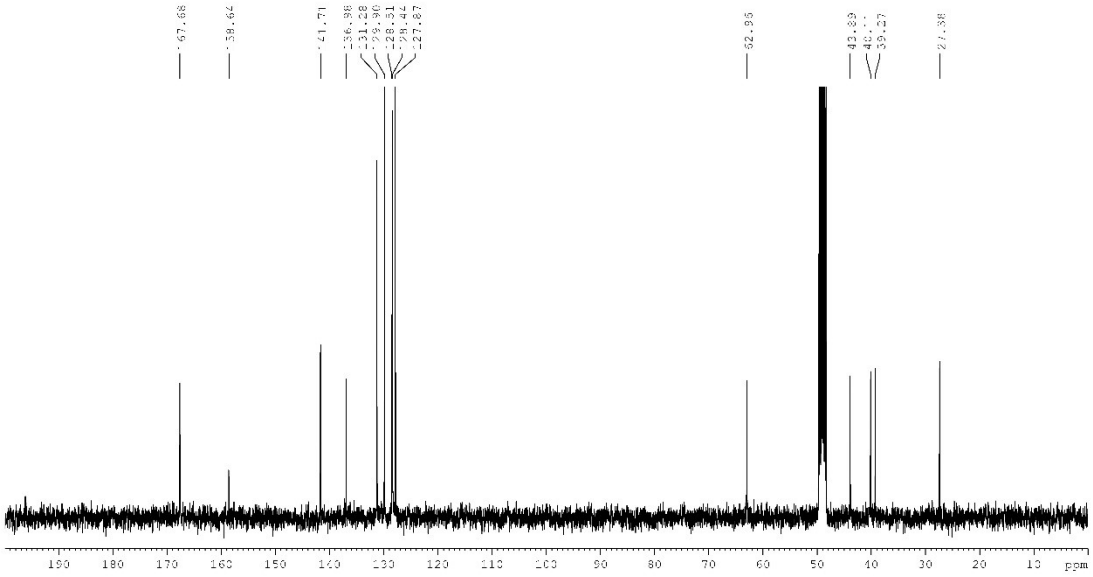


6

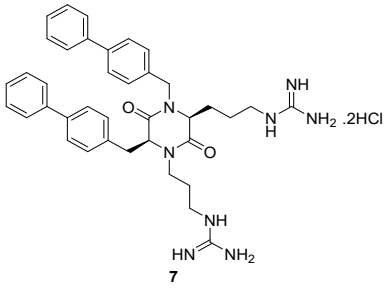
[1]H NMR (400 MHz, MeOD):



[13]C NMR (100 MHz, MeOD):

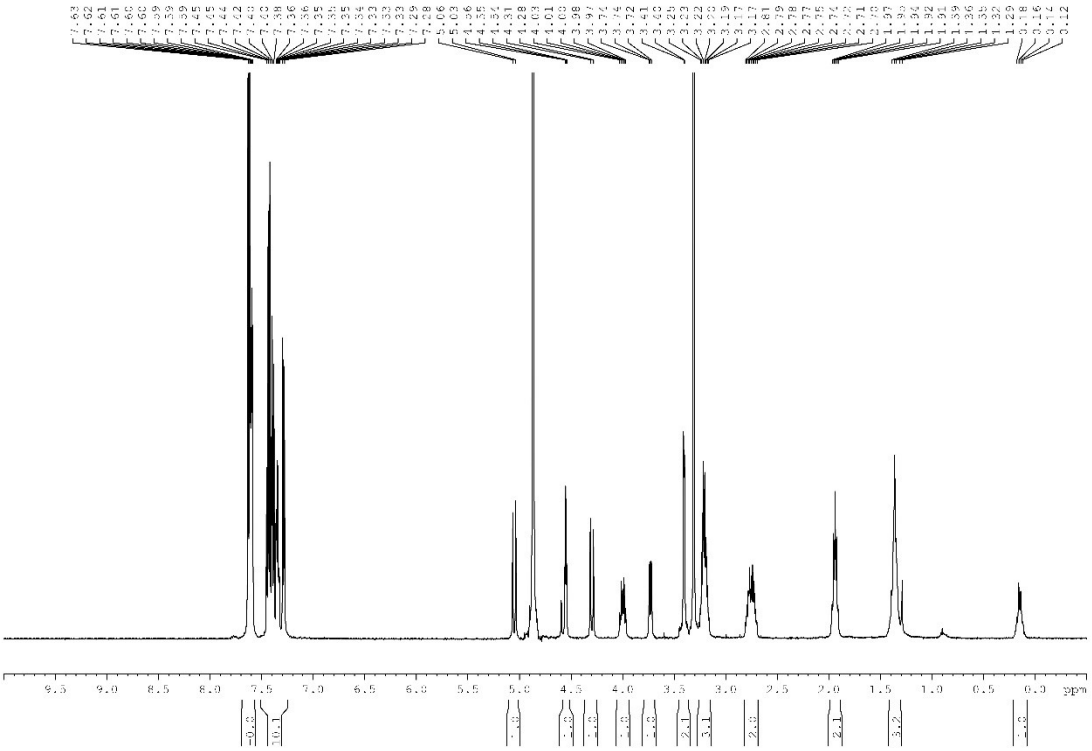


Cyclo(*N*-(propylguanidine)-L-Bip-*N*-Bip-L-Arg).2HCl (7)

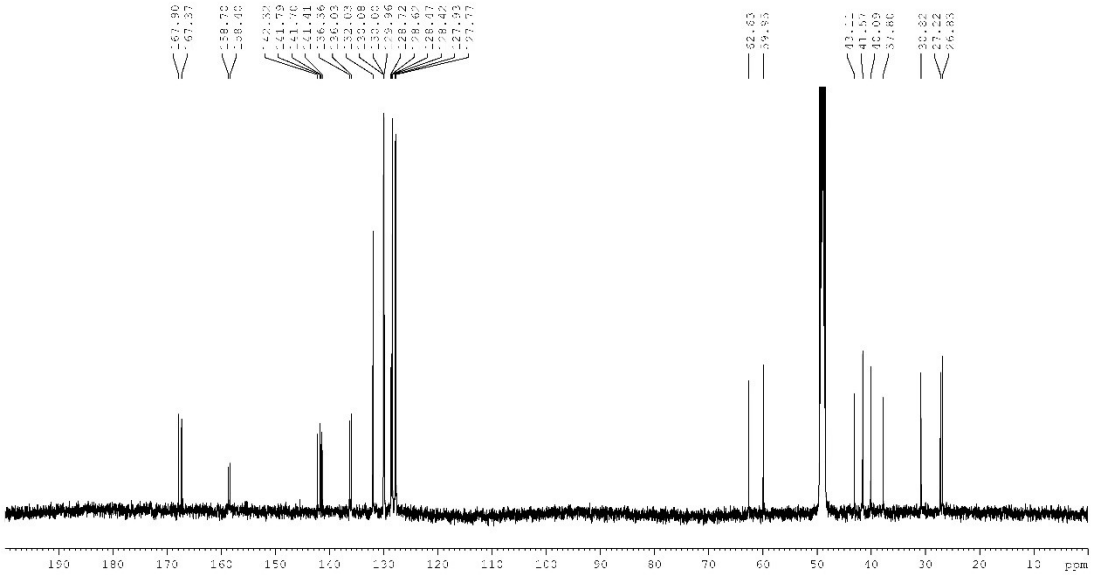


7

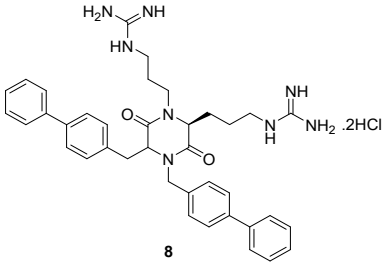
[1]H NMR (500 MHz, MeOD):



[13]C NMR (125 MHz, MeOD):

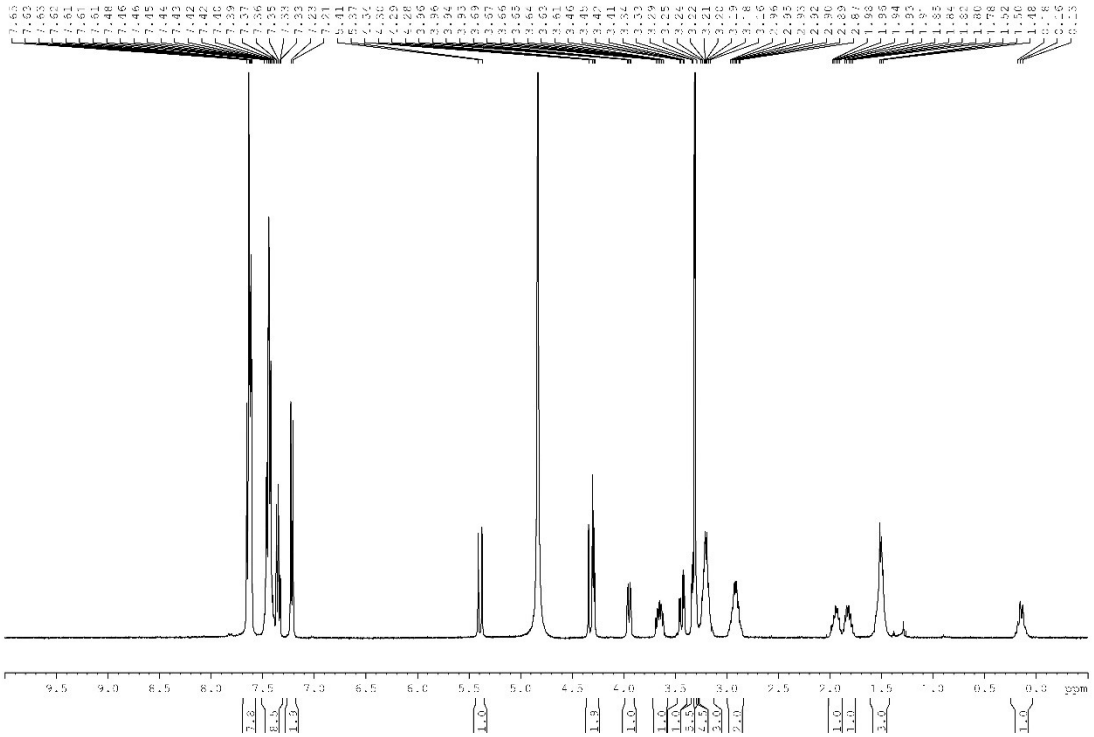


Cyclo(*N*-Bip-L-Bip-*N*-(propylguanidine)-L-Arg).2HCl (8)

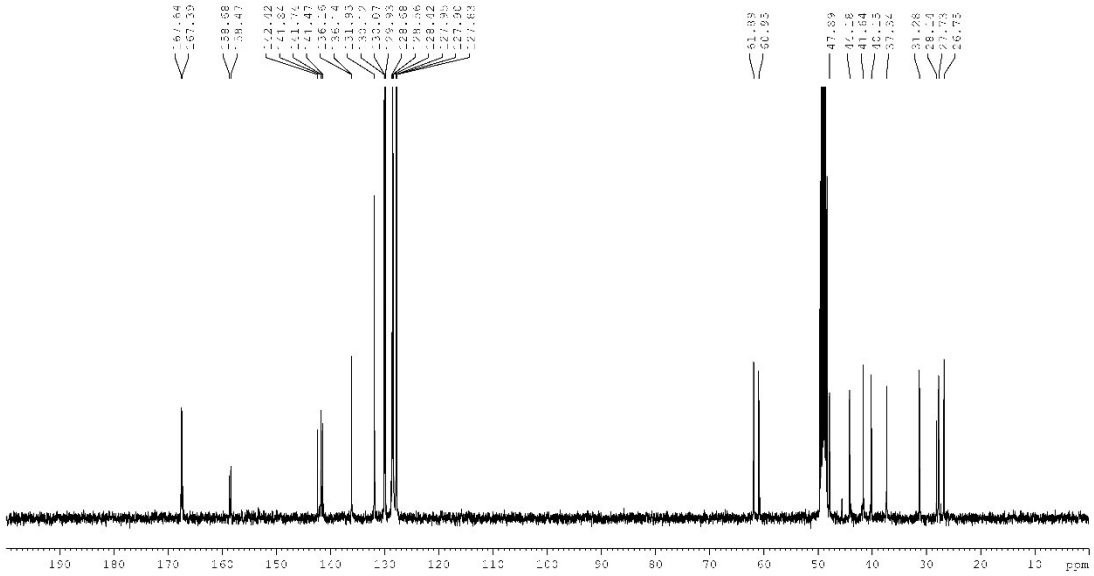


8

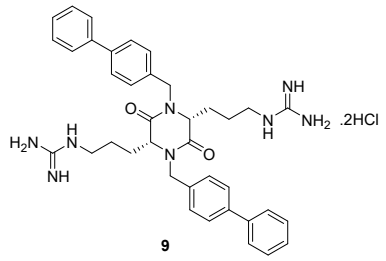
[1]H NMR (400 MHz, MeOD):



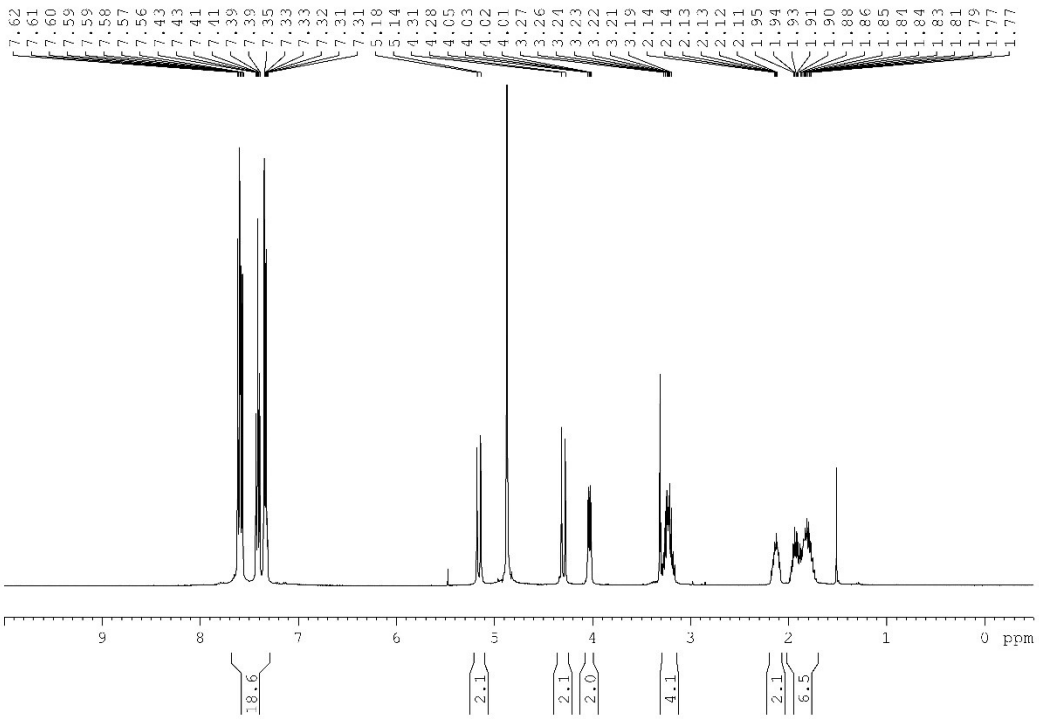
[13]C NMR (100 MHz, MeOD):



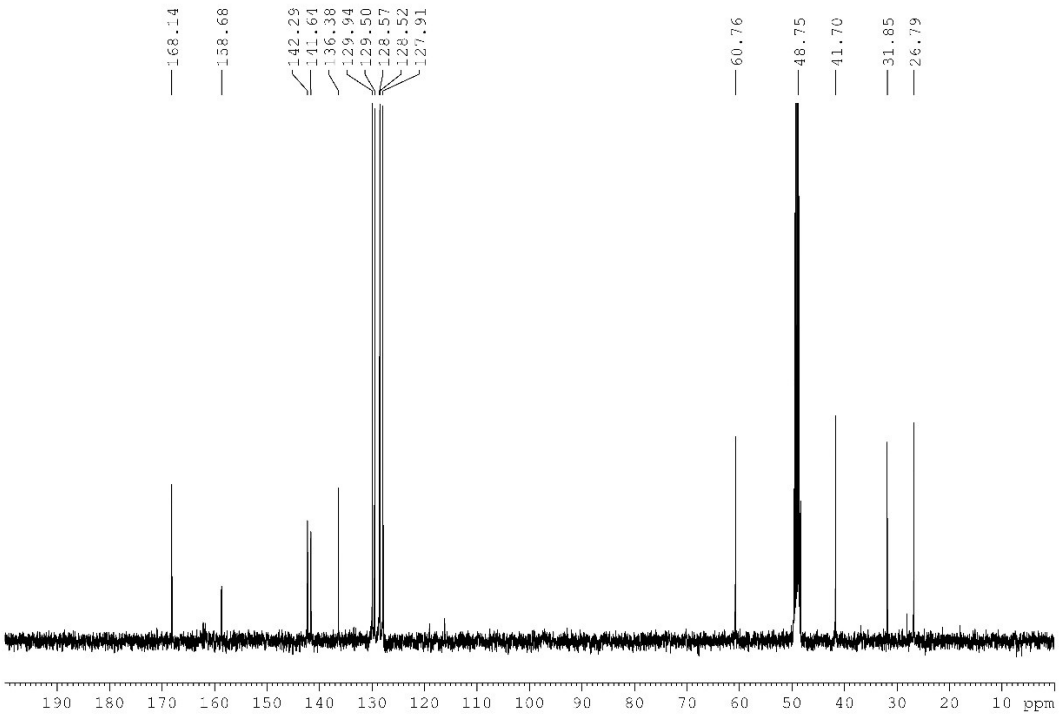
Cyclo(*N*-Bip-D-Arg-*N*-Bip-D-Arg).2HCl (9)



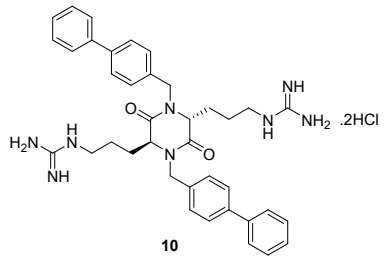
^{1}H NMR (400 MHz, MeOD):



^{13}C NMR (100 MHz, MeOD):

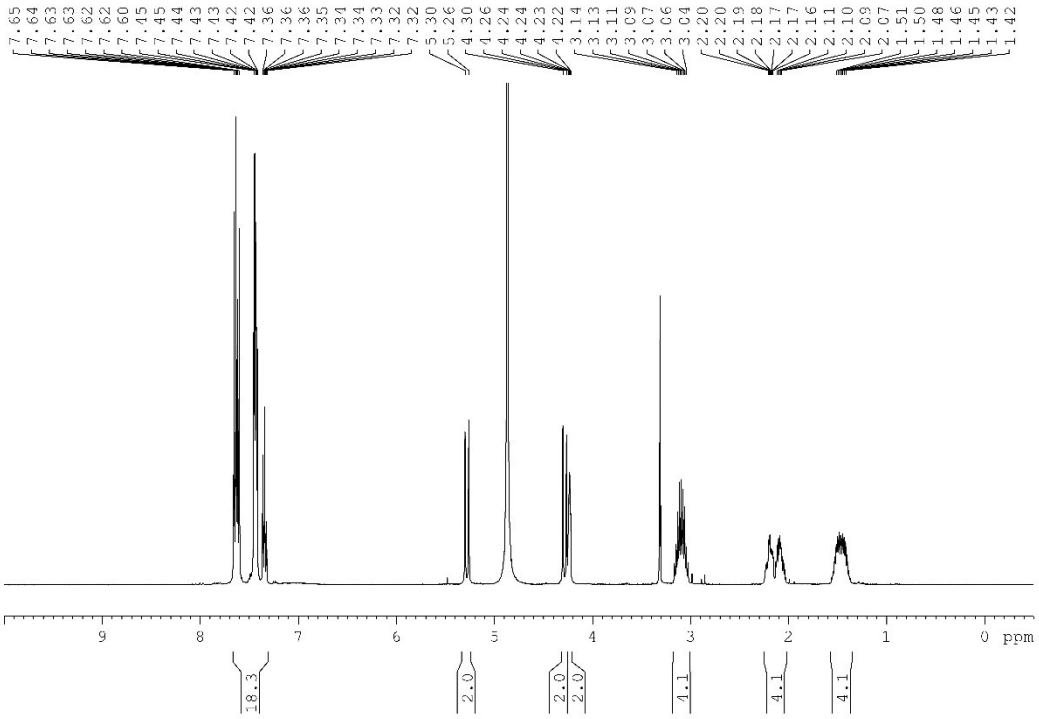


Cyclo(*N*-Bip-L-Arg-*N*-Bip-D-Arg).2HCl (10)

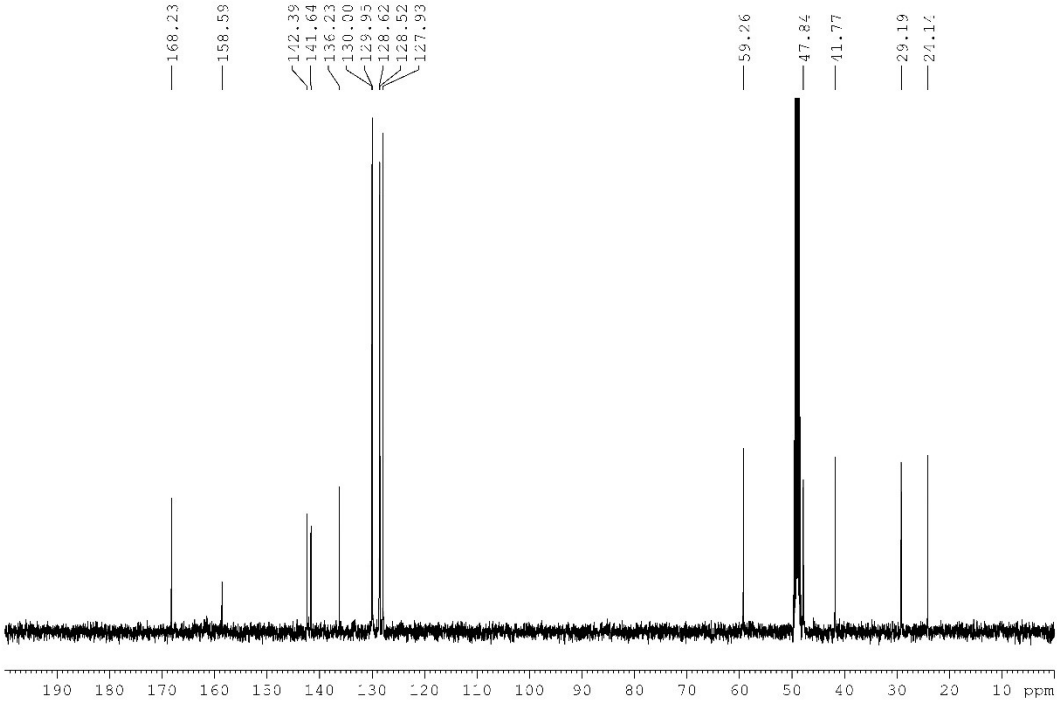


10

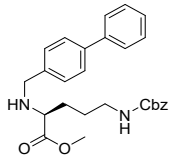
[1]H NMR (400 MHz, MeOD):



[13]C NMR (100 MHz, MeOD):



N-Bip-L-Orn(Z)-OMe (14)

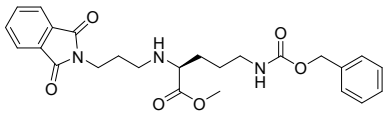


16

^{1}H NMR (400 MHz, $CDCl_3$):

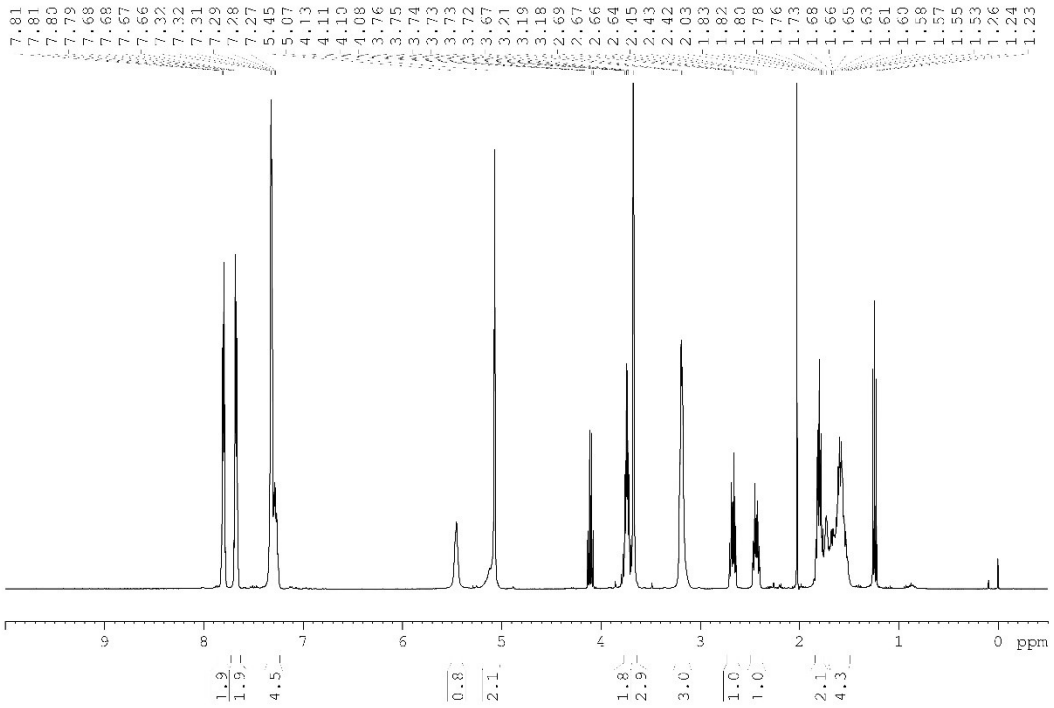
^{13}C NMR (100 MHz, $CDCl_3$):

N-(*N*-phthaloylpropylamine)-L-Orn(Z)-OMe (15)

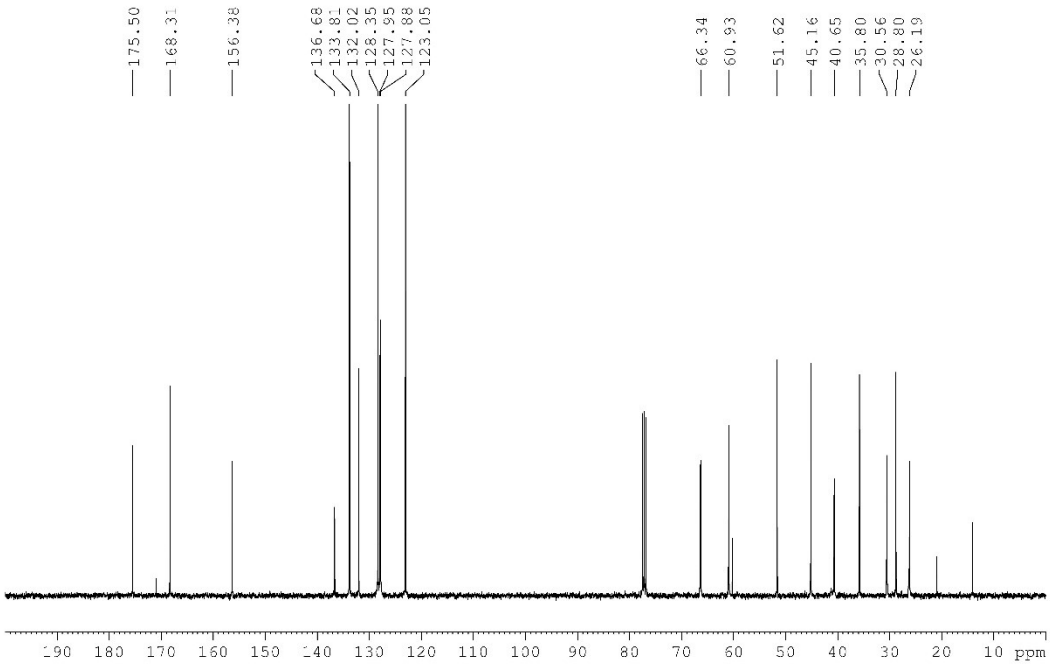


17

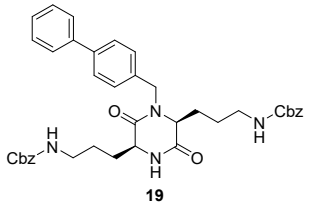
^{1}H NMR (400 MHz, $CDCl_3$):



^{13}C NMR (100 MHz, $CDCl_3$):

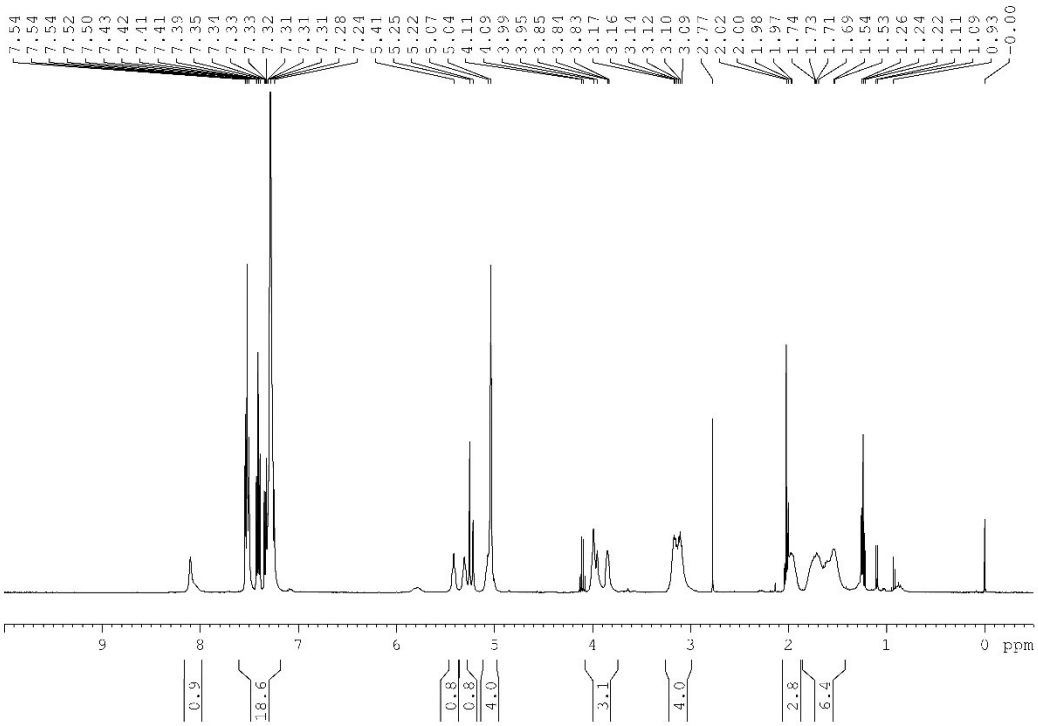


Cyclo(L-Orn(Z)-*N*-Bip-L-Orn(Z)) (19)

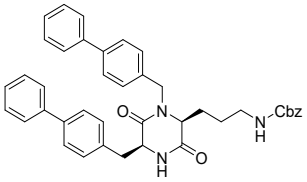


19

[1]H NMR (400 MHz, DMSO):

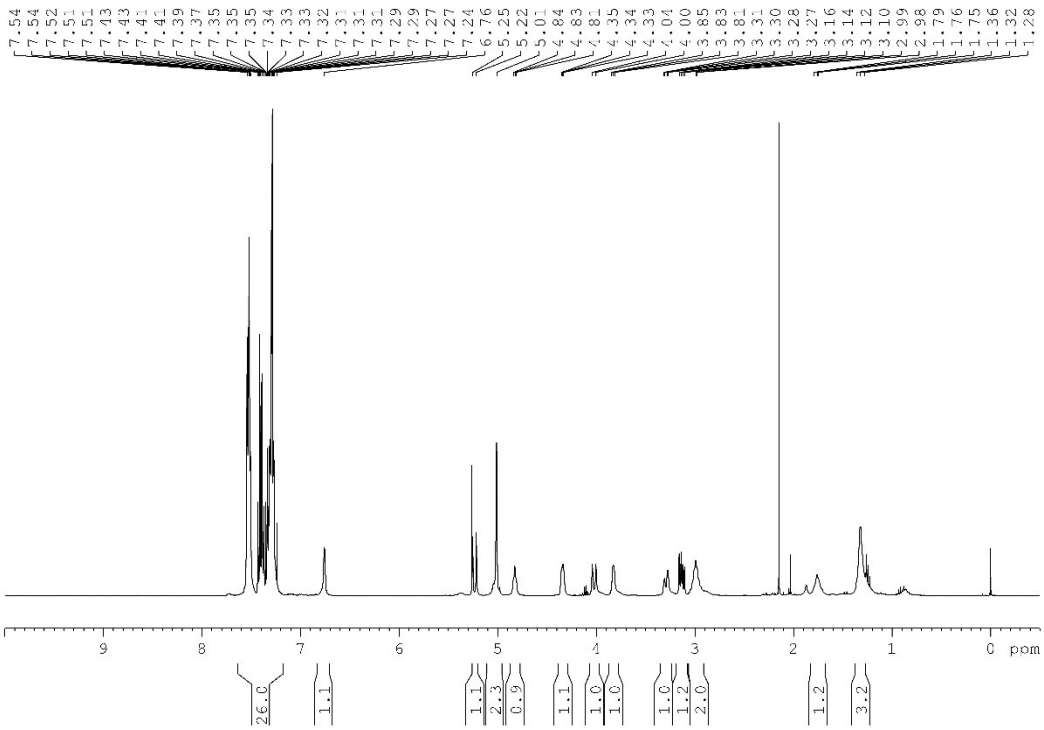


Cyclo(L-Bip-*N*-Bip-L-Orn(Z)) (20)

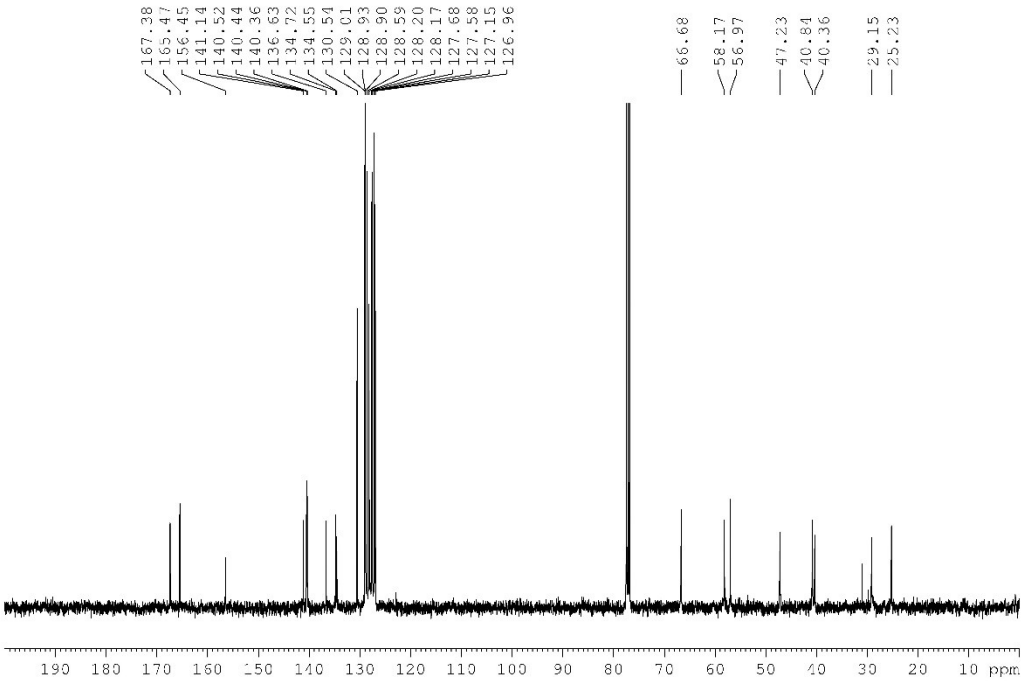


20

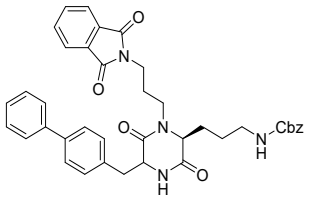
[1]H NMR (400 MHz, $CDCl_3$):



[13]C NMR (100 MHz, $CDCl_3$):

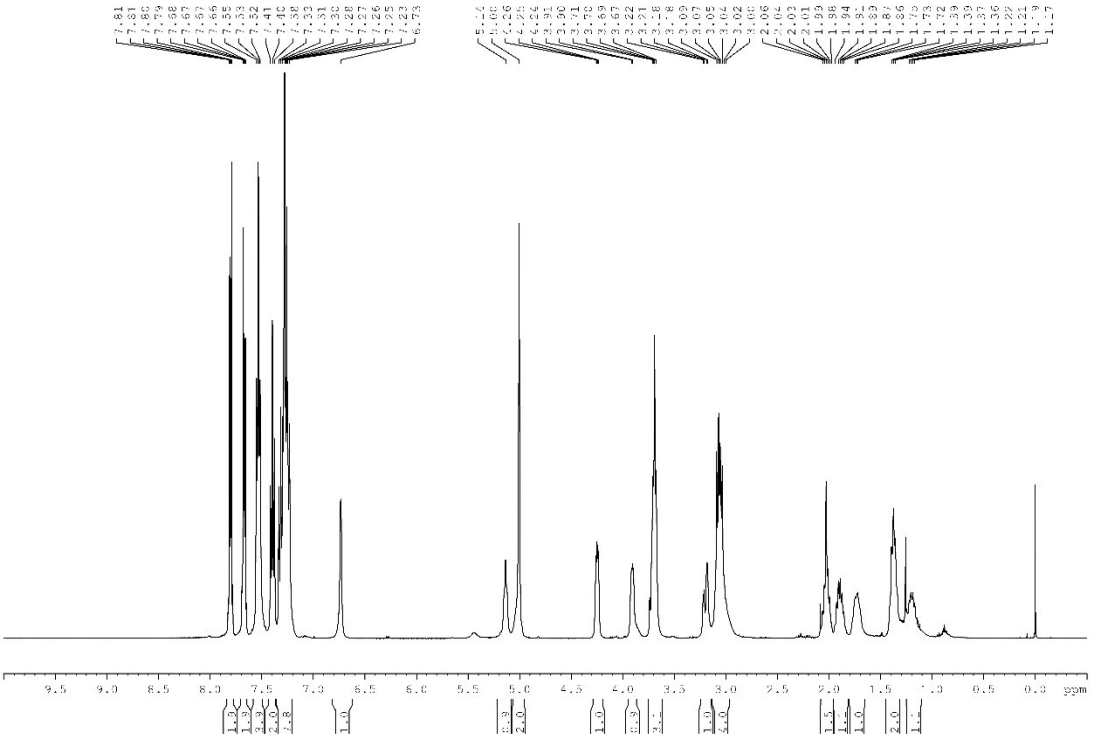


Cyclo(L-Bip-*N*-(*N*-phthyolylpropylamine)-L-Orn(Z)) (22)

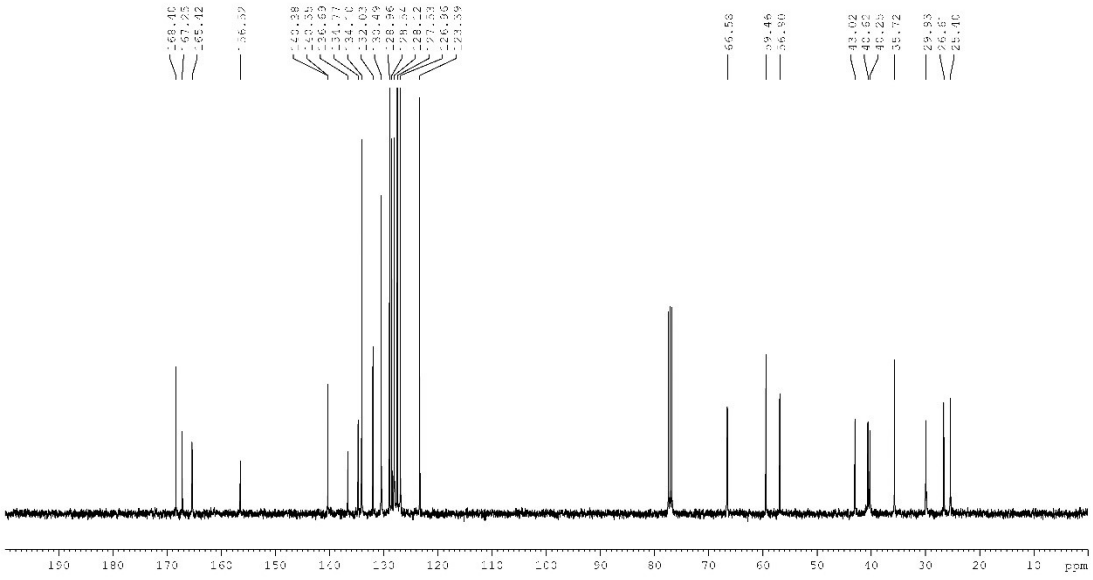


21

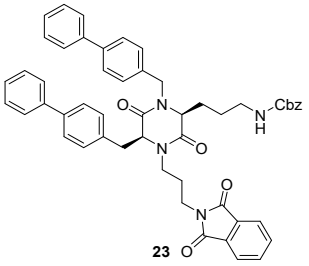
^{1}H NMR (400 MHz, $CDCl_3$):



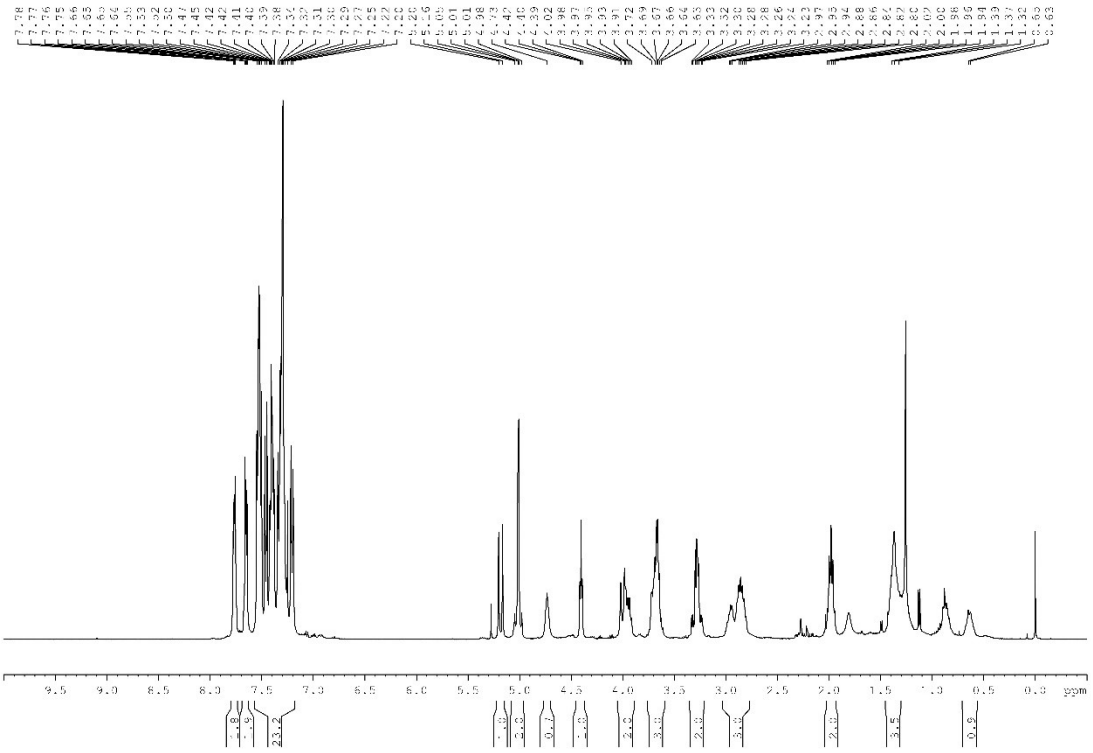
^{13}C NMR (100 MHz, $CDCl_3$):



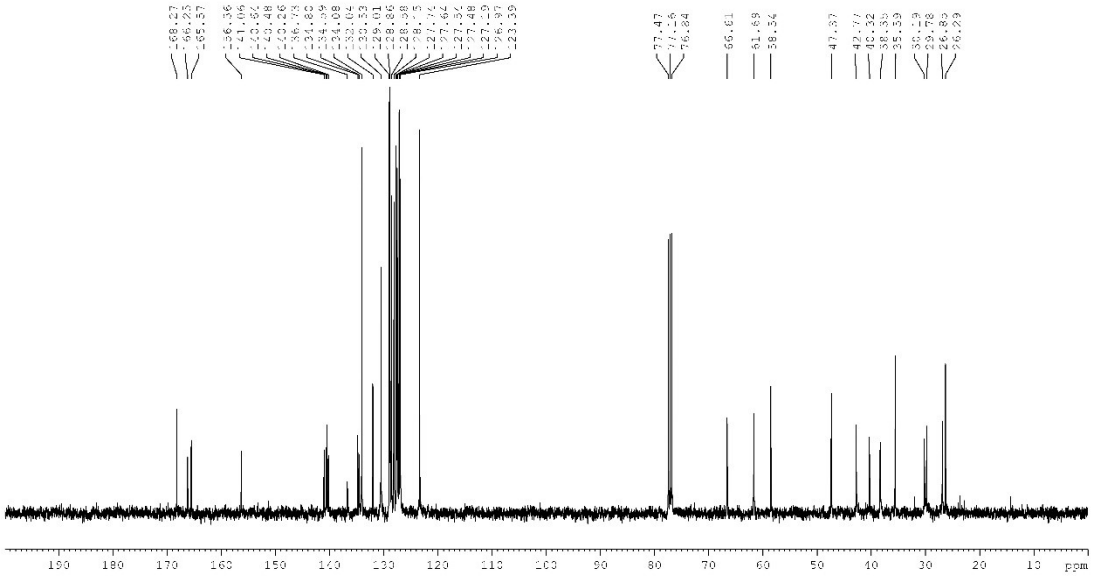
Cyclo(*N*-(*N*-phthaloylpropylamine)-L-Bip-*N*-Bip-L-Orn(Z)) (23)



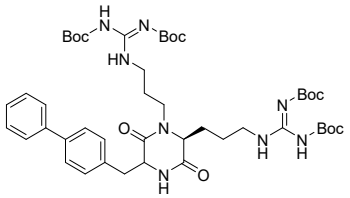
^{1}H NMR (400 MHz, $CDCl_3$):



^{13}C NMR (100 MHz, $CDCl_3$):

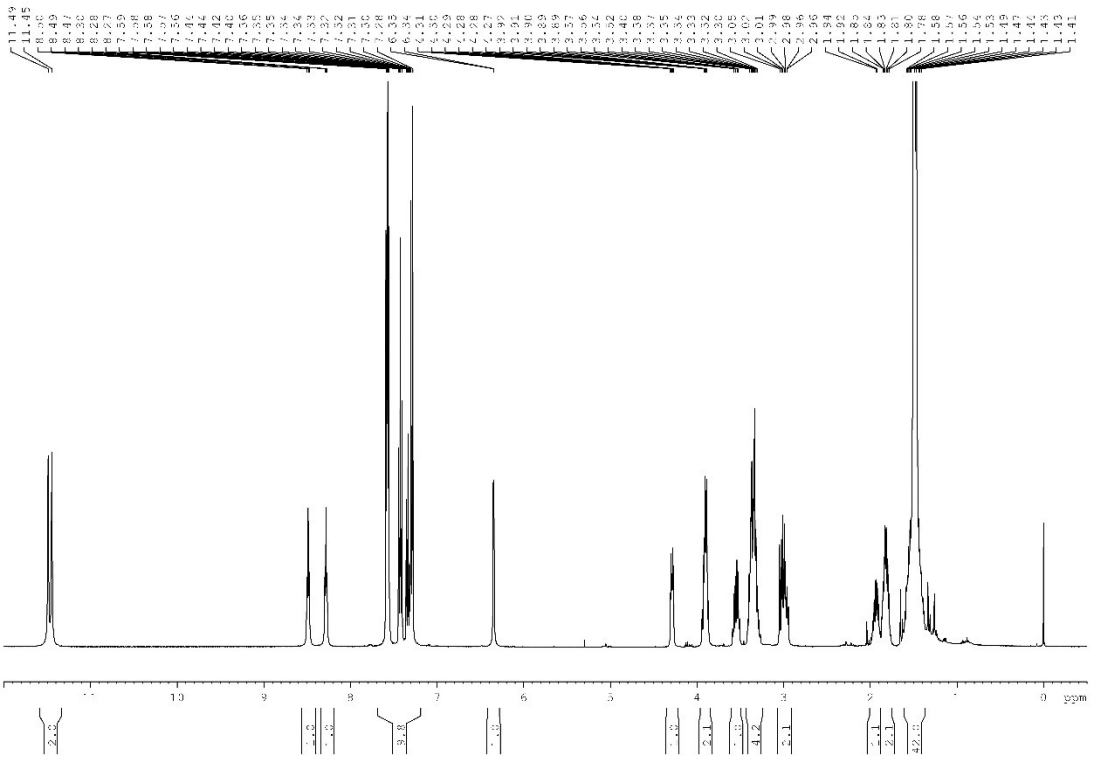


Cyclo(L-Bip-*N*-(*N*,*N*′-di-Boc-propylguanidine)-L-Arg-*N*,*N*′-di-Boc) (24)

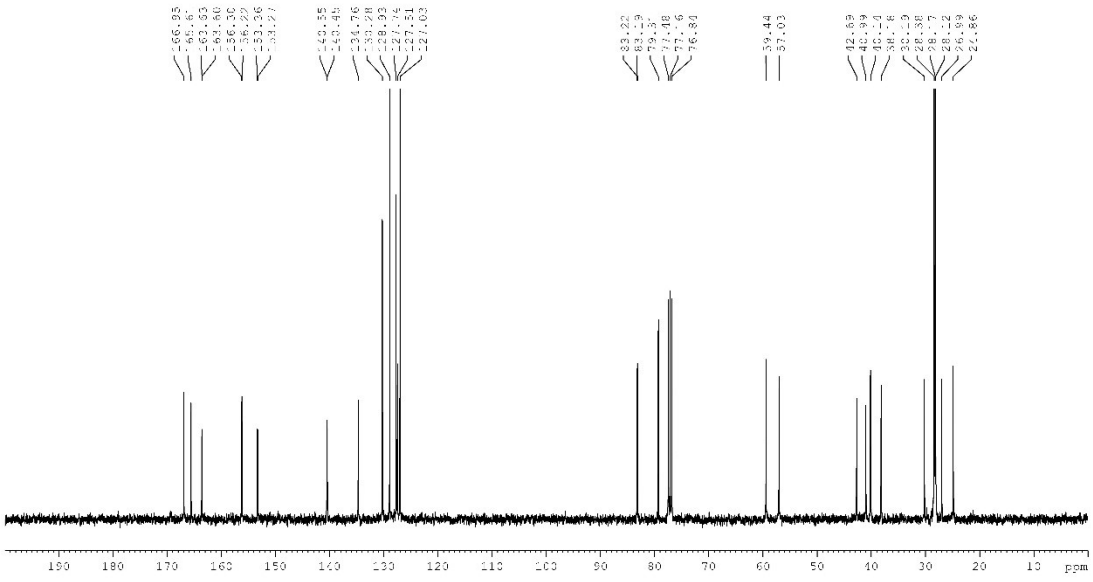


24

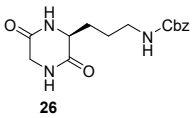
^{1}H NMR (400 MHz, $CDCl_3$):



^{13}C NMR (100 MHz, $CDCl_3$):

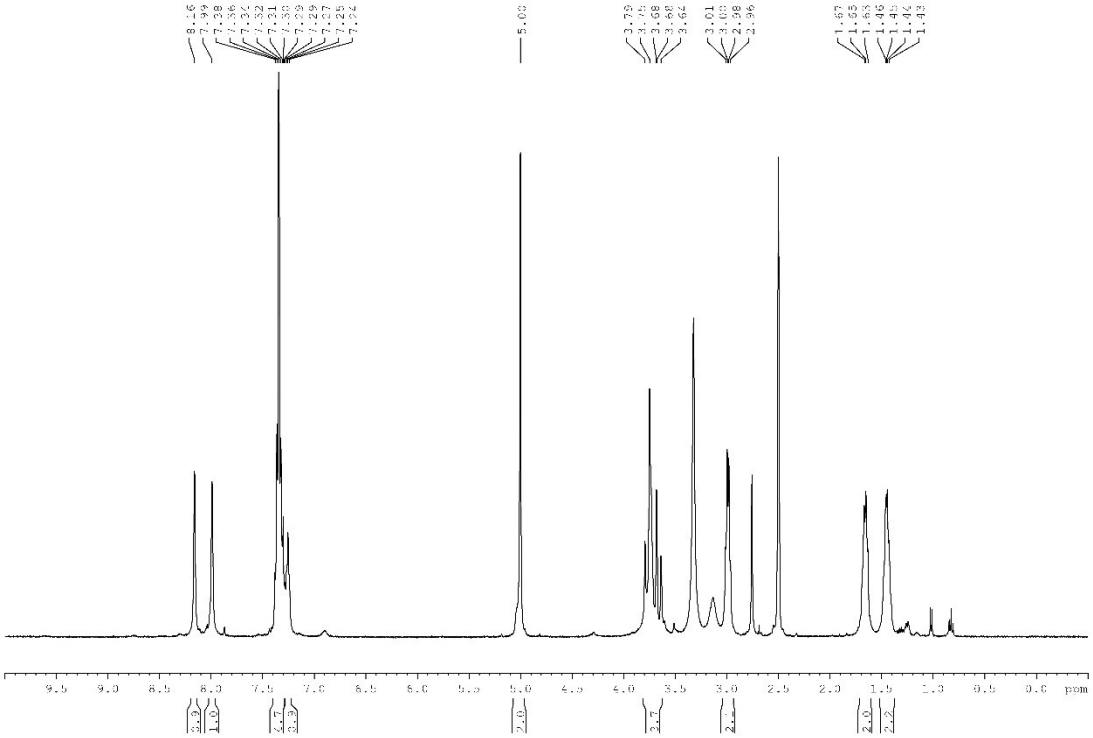


Cyclo(Gly-L-Orn(Z)) (26)

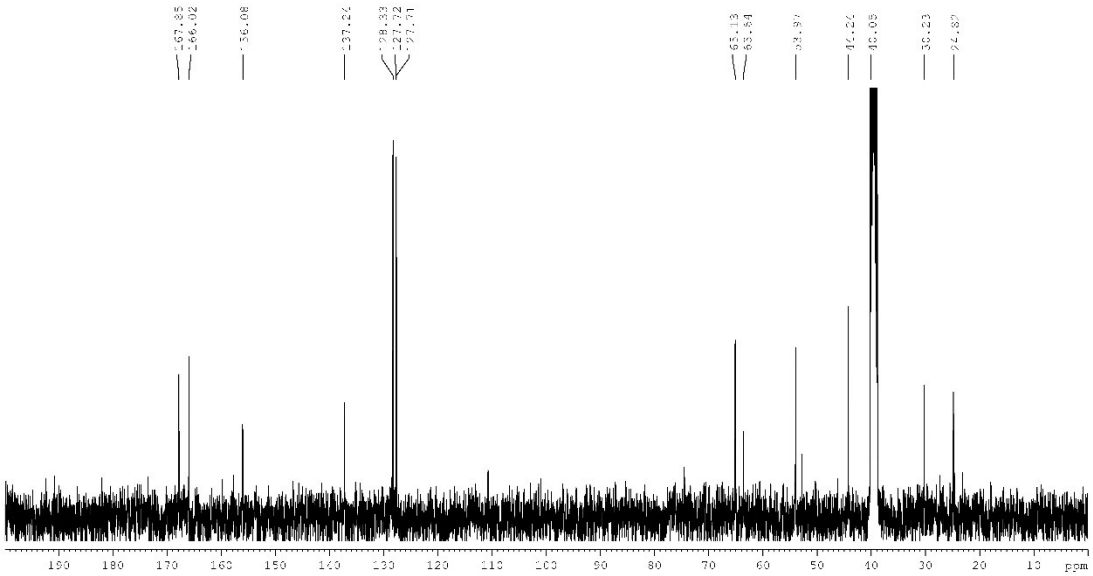


26

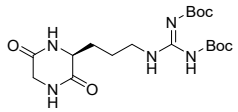
[1]H NMR (400 MHz, DMSO):



[13]C NMR (100 MHz, DMSO):

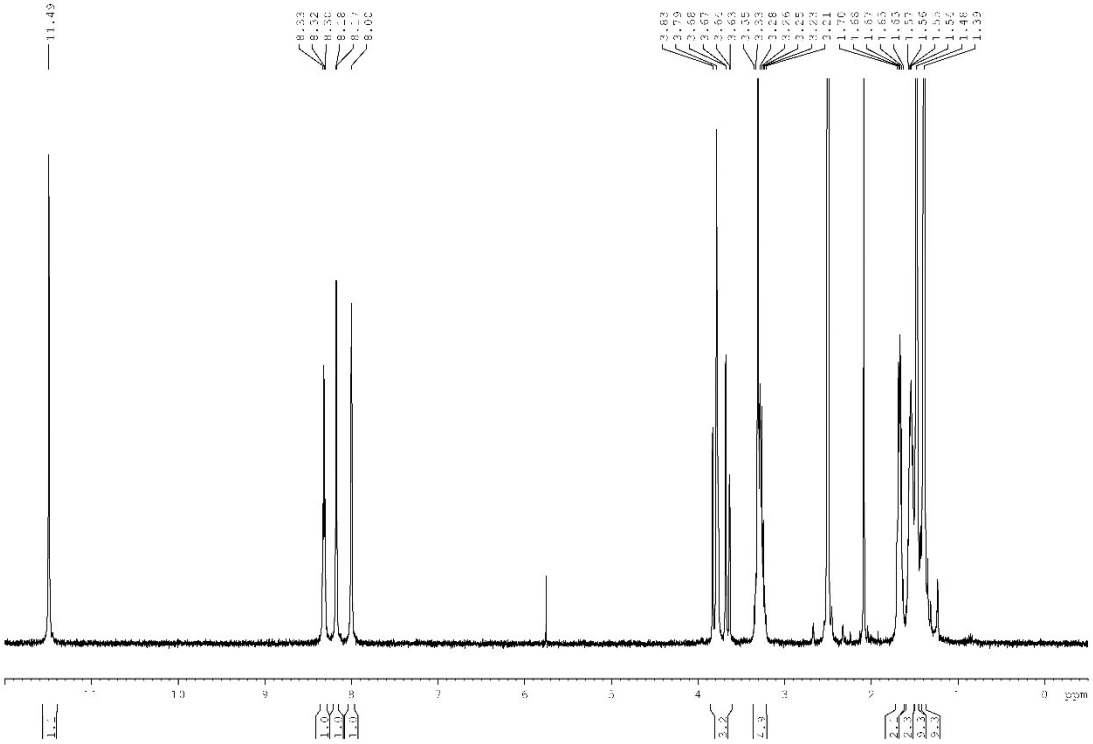


Cyclo(Gly-L-Arg-*N,N'-di*-Boc) (27)

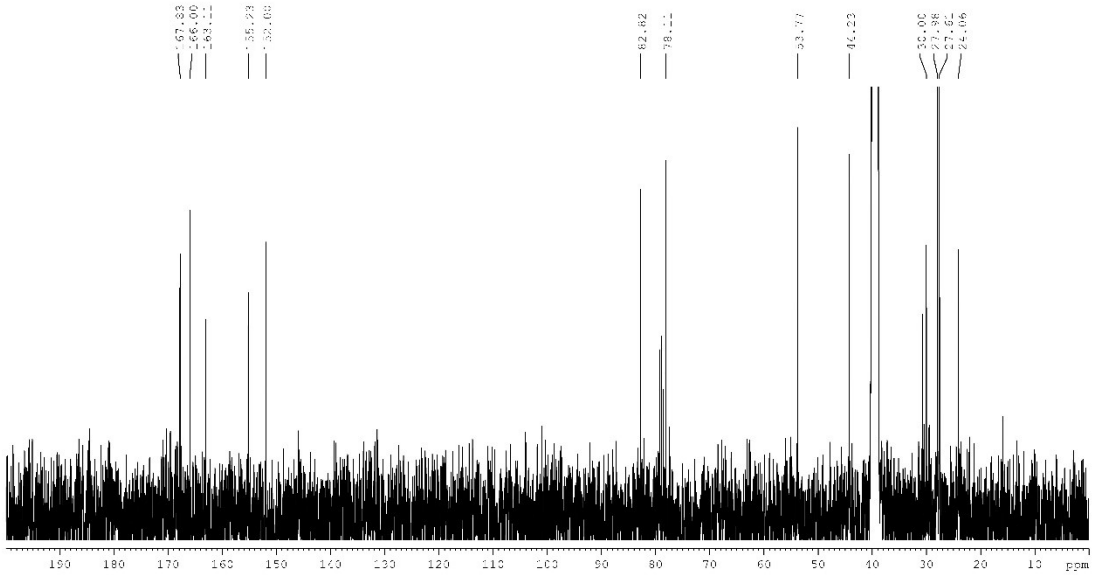


27

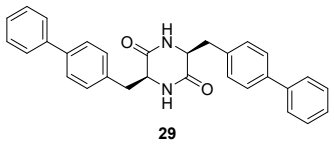
[1]H NMR (400 MHz, DMSO):



[13]C NMR (100 MHz, DMSO):

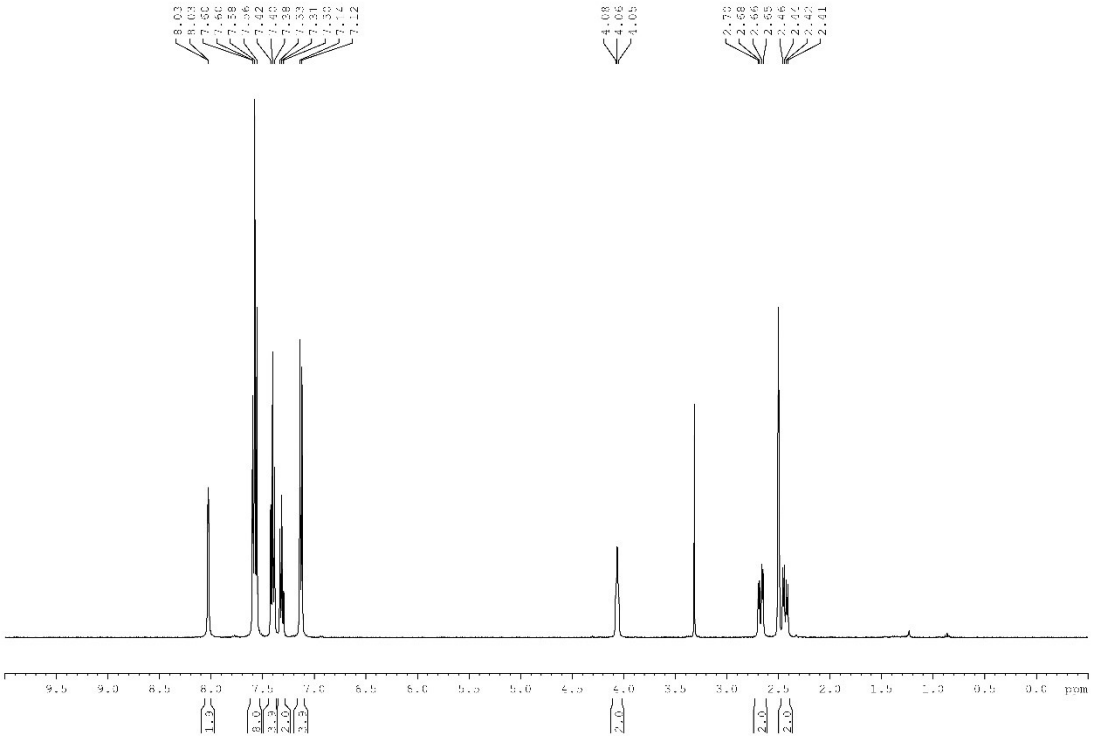


Cyclo(L-Bip-L-Bip) (29)

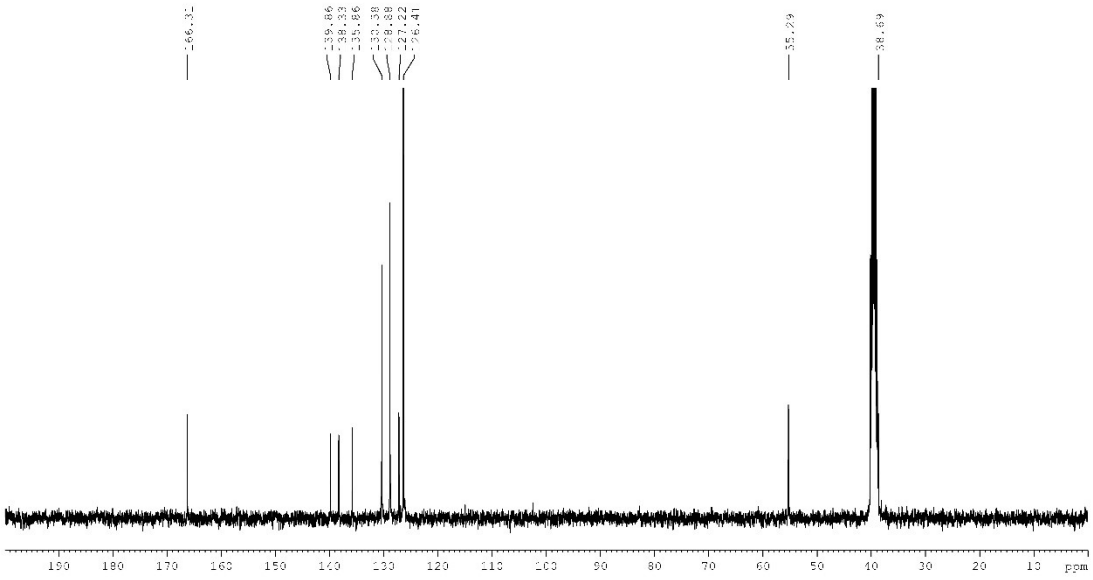


29

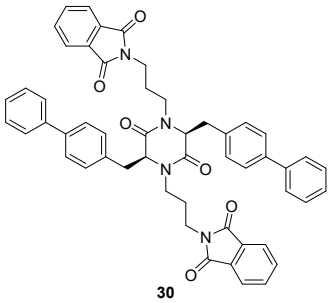
^{1}H NMR (400 MHz, DMSO):



^{13}C NMR (100 MHz, DMSO):

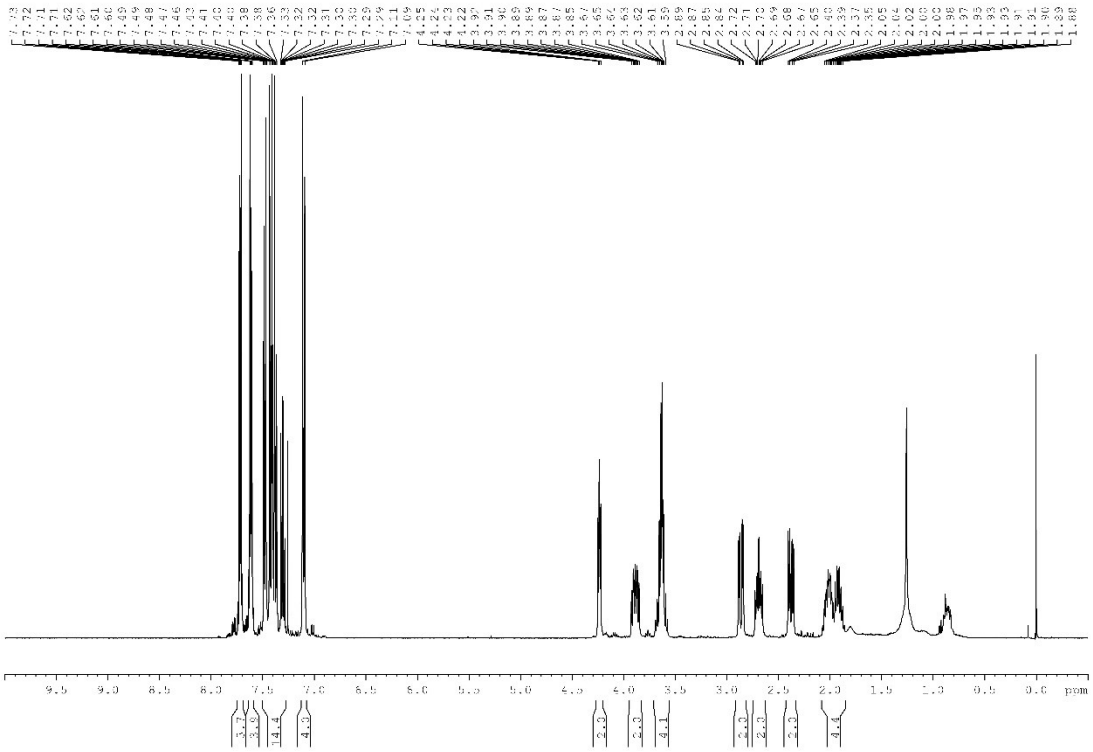


Cyclo(*N*-(*N*-phthaloylpropylamine)-L-Bip-*N*-(*N*-phthaloylpropylamine)-L-Bip) (30)

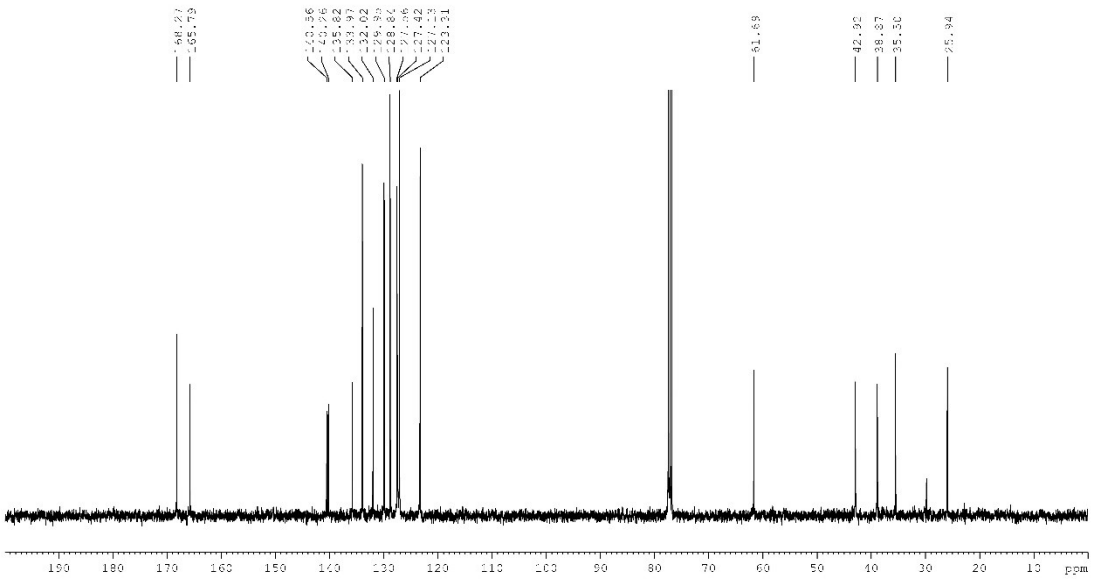


30

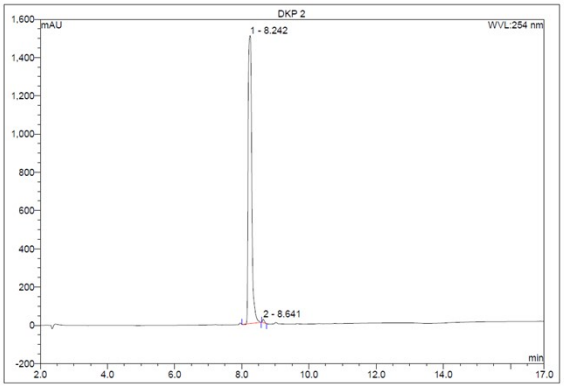
^{1}H NMR (400 MHz, $CDCl_3$):



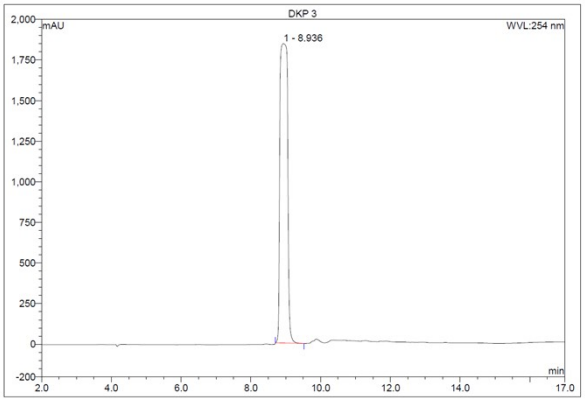
^{13}C NMR (100 MHz, $CDCl_3$):



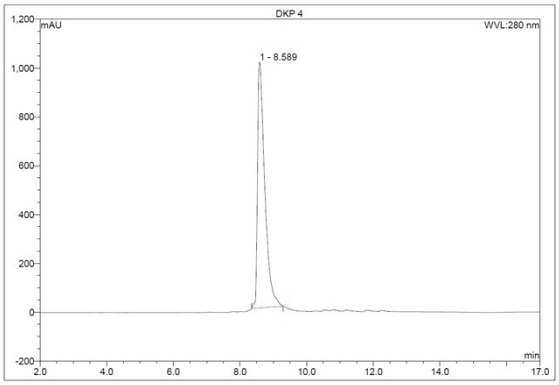
Analytical RP-chromatograms of DKPs 2-10



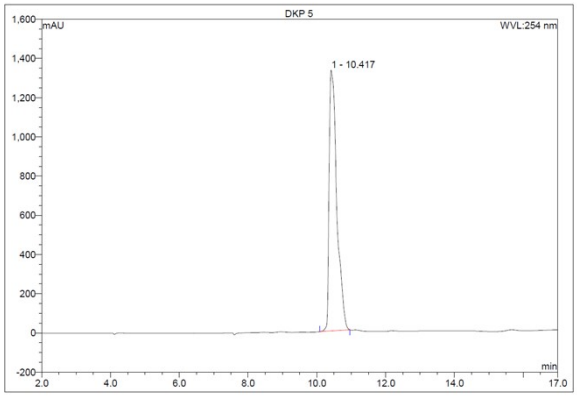
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8.24	n.a.	1505.125	185.256	99.35	n.a.	BMB*
2	8.64	n.a.	17.688	1.212	0.65	n.a.	BMB*
Total:			1522.813	186.469	100.00	0.000	



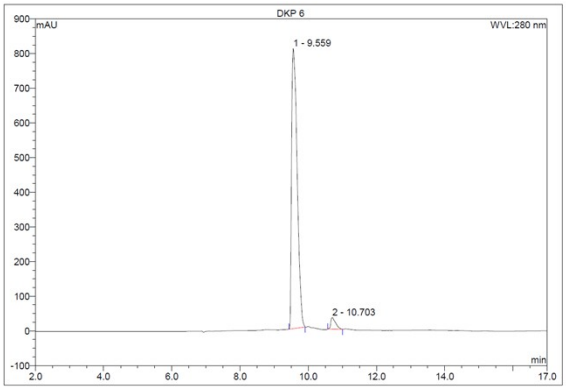
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8.94	n.a.	1843.292	470.428	100.00	n.a.	BMB*
Total:			1843.292	470.428	100.00	0.000	



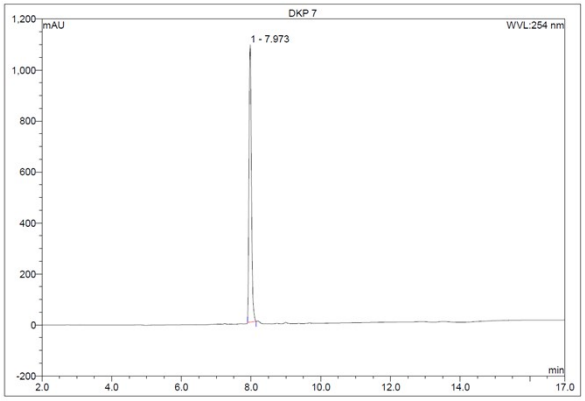
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8.59	n.a.	1005.664	253.550	100.00	n.a.	BMB*
Total:			1005.664	253.550	100.00	0.000	



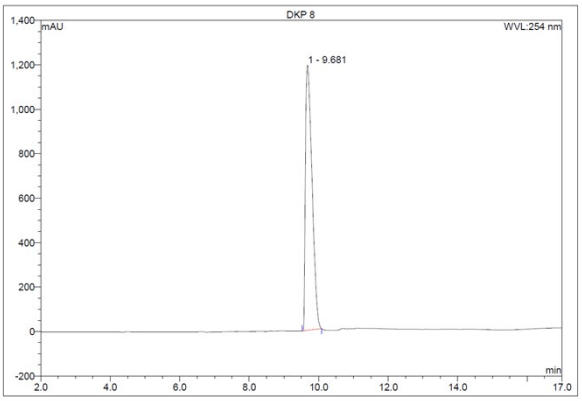
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	10.42	n.a.	1330.950	344.342	100.00	n.a.	BMB*
Total:			1330.950	344.342	100.00	0.000	



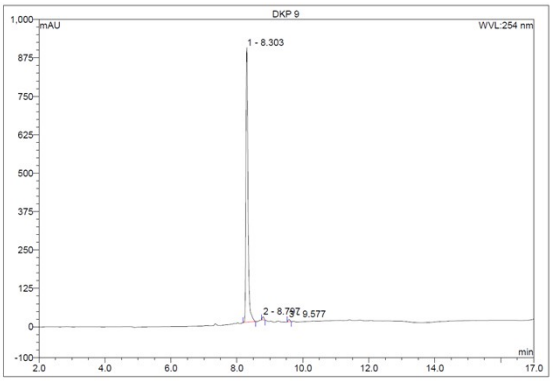
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.56	n.a.	808.128	152.951	96.53	n.a.	BMB*
2	10.70	n.a.	33.266	5.500	3.47	n.a.	BMB*
Total:			841.394	158.451	100.00	0.000	



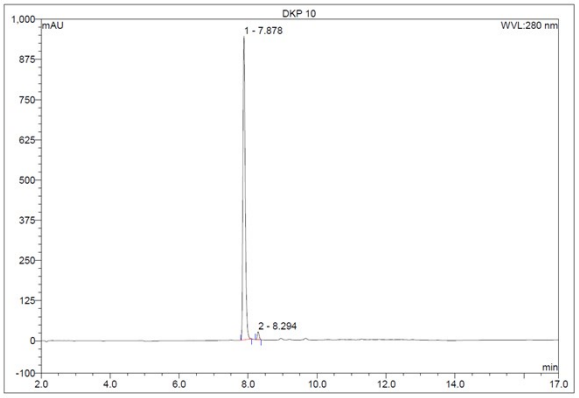
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7.97	n.a.	1088.240	87.260	100.00	n.a.	BMB*
Total:			1088.240	87.260	100.00	0.000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	9.68	n.a.	1192.724	263.461	100.00	n.a.	BMB*
Total:			1192.724	263.461	100.00	0.000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	8.30	n.a.	893.885	64.242	98.34	n.a.	BMB*
2	8.80	n.a.	10.517	0.630	0.96	n.a.	BMB*
3	9.58	n.a.	7.512	0.455	0.70	n.a.	BMB*
Total:			911.914	65.327	100.00	0.000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	7.88	n.a.	944.496	73.051	98.06	n.a.	BMB*
2	8.29	n.a.	24.464	1.448	1.94	n.a.	BMB*
Total:			968.961	74.499	100.00	0.000	