Electronic Supplementary Material (ESI) for Soft Matter.


# Supplementary information

## The effect of heterocyclic capping group selection on dipeptide hydrogel properties

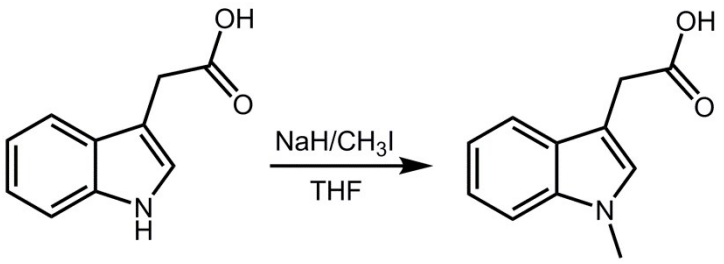
Adam D. Martin*[a], Jonathan P. Wojciechowski[a], Holly Warren[b], Marc in het Panhuis[b] and Pall Thordarson*[a]

[a]School of Chemistry, The Australian Centre for Nanomedicine and the ARC Centre of Excellence for Convergent Bio-Nano Science and Technology The University of New South Wales, Sydney, 2052, NSW, Australia.

[b] Soft Materials Group, School of Chemistry and ARC Centre of Excellence for Electromaterials Science, University of Wollongong, Wollongong, NSW 2522, Australia

E-mails: adam.martin2@unsw.edu.au and p.thordarson@unsw.edu.au; Fax: +61-2-9385-6141; Tel: +61-2-9385-4478.

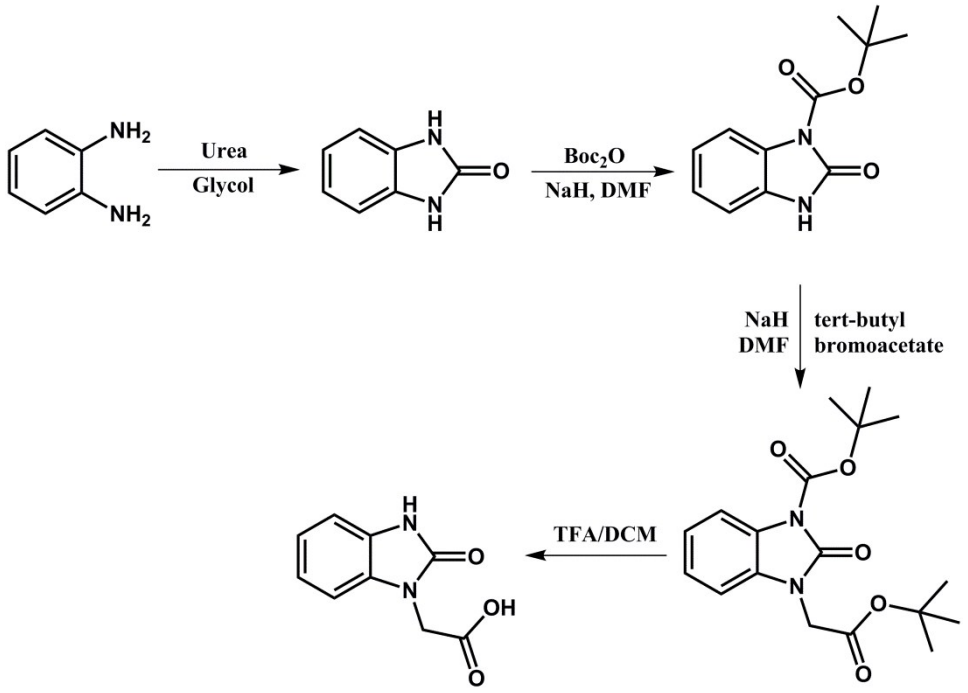
**Contents:**




**Scheme S1** – Methylation of indole-3-acetic acid

**Synthesis of N-methylindole acetic acid**

1 g (8.46 mmol) of benzimidazole and 2.35 g (17 mmol) of $K_2CO_3$ was dissolved in 120 mL of dry acetonitrile. 1.5 mL (10.15 mmol) of *tert*-butyl bromoacetate was added and the mixture refluxed for 4 h. The suspension was filtered and the solvent removed under reduced pressure. The residue was dissolved in dichloromethane (150 mL), washed with water (2 x 100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the resultant oil purified by column chromatography (3:2 hexane:dichloromethane) to give 1.9g (94%) of the *tert*-butyl benzimidazole acetate. Characterisation data was found to be in agreement with that previously reported.[1]




**Scheme S2** – Synthesis of benzimidazolone acetic acid

**Synthesis of benzimidazolone acetic acid**

Benzimidazolone was synthesised according to a previously reported literature procedure, whereupon characterisation data was in good agreement.[2]

165 mg (4.1 mmol) sodium hydride was added to a two-necked flask under nitrogen, and 10 mL of hexane added. This slurry was stirred for one minute, before the sediment was allowed

to settle and the hexane pipette off. This was repeated one more time. 20 mL of dry DMF was then added and the solution cooled to 0 °C. 500 mg (3.73 mmol) of benzimidazolone was then added and the resulting suspension stirred for 30 minutes, upon which time a colour change from grey to green occurred. 815 mg (3.73 mmol) $Boc_2O$ was added and the solution stirred at 0 °C for one hour, before being allowed to warm to room temperature and stirred for a further four hours. The solution was cooled in an ice bath and quenched using methanol, before water (150 mL) was added and the solution extracted with ethyl acetate (100 mL). The organic layer was washed with water (2 x 150 mL) and brine (50 mL), dried over magnesium sulfate and the solvents removed under reduced pressure. Purification by column chromatography (4:1 dichloromethane:ethyl acetate) gave 390 mg (45%) of mono-*Boc*(N1)-benzimidazolone as white crystals.

Characterisation data for **mono-*Boc*(N1)-benzimidazolone**: IR: 3269 (m), 2980 (w), 1770 (s), 1623 (w), 1608 (w), 1480 (m), 1455 (w), 1395 (w), 1372 (m), 1341 (s), 1300 (s), 1284 (m), 1253 (m), 1150 (s), 1117 (s), 1028 (w), 997 (w), 932 (w), 865 (w), 839 (m), 774 (m), 755 (s), 697 (m), 673 (m); $^{1}$H NMR ($CDCl_3$, 400 MHz): 10.33 (s, 1H, N*H*), 7.71 (d, 1H, Ar*H*), 7.09-7.17 (m, 3H, Ar*H*), 1.70 (s, 9H, C$H_3$); $^{13}$C{1H} NMR ($CDCl_3$, 100 MHz): 153.48, 148.74, 127.84, 127.02, 124.30, 122.22, 114.62, 110.06, 85.15, 28.25; HR-MS (ESI): calcd for $C_{12}H_{14}N_2O_3 + Na^+$ : 257.0915, found 257.0897.

75 mg (1.8 mmol) sodium hydride was added to a two-necked flask under nitrogen, and 10 mL of hexane added. This slurry was stirred for one minute, before the sediment was allowed to settle and the hexane pipette off. This was repeated one more time. 20 mL of dry DMF was then added and the solution stirred for one minute. 400 mg (1.71 mmol) of mono-*Boc*(N1)-benzimidazolone was added and the resulting suspension stirred for 30 minutes. 0.25 mL (1.71 mmol) *tert*-butylbromoacetate was added and the solution stirred for two hours. The solution was cooled in an ice bath and quenched using methanol, before water (150 mL) was added and the solution extracted with dichloromethane (60 mL). The organic layer was washed with water (2 x 100 mL) and brine (40 mL), dried over magnesium sulfate and the solvents removed under reduced pressure. Purification by column chromatography (9:1 hexane:ethyl acetate) gave 366 mg (60%) of mono-*Boc*(N1)-*tert*-butyl(N3)-benzimidazolone acetate as a white powder.

Characterisation data for **mono-*Boc*(N1)-*tert*-butyl(N3)-benzimidazolone acetate**: IR: 3067 (w), 2987 (w), 2934 (w), 1774 (w), 1737 (s), 1725 (s), 1698 (m), 1618 (w), 1491 (m), 1455 (w), 1423 (m), 1366 (s), 1324 (s), 1288 (w), 1253 (m), 1228 (m), 1144 (s), 1107 (s), 1058 ms), 1009 (m), 978 (w), 941 (w), 877 (w), 847 (m), 773 (m), 753 (m), 737 (m), 708 (w), 671 (w); $^{1}$H NMR ($CDCl_3$, 400 MHz): 7.82 (d, 1H, *ArH*), 7.13 (quin, 2H, Ar*H*), 6.82 (d, 1H, Ar*H*), 4.47 (s, 2H, C$H_2$), 1.65 (s, 9H, C$H_3$), 1.43 (s, 9H, C$H_3$); $^{13}$C{1H} NMR (DMSO-$d_6$, 100 MHz): 166.24, 151.01, 148.7, 129.20, 126.22, 123.99, 122.51, 114.62, 107.57, 84.77, 82.98,42.72, 28.11, 28.00; HR-MS (ESI): calcd for $C_{18}H_{24}N_2O_5 + Na^+$: 371.1602, found 371.1577.

500 mg (1.43 mmol) mono-*Boc*(N1)-*tert*-butyl(N3)-benzimidazolone acetate was dissolved in a 9:1 mixture of trifluoroacetic acid:dichloromethane (20 mL total volume) and stirred at

room temperature for two hours. The solvents were removed under reduced pressure, the product dissolved in water and lyophilised to give 270 mg (98%) benzimidazolone acetic acid as a white powder.

Characterisation data for **benzimidazolone acetic acid**: IR: 3130 (w), 3062 (w), 2904 (w), 2770 (w), 2603 (w), 1749 (w), 1724 (m), 1682 (s), 1637 (s), 1622 (s), 1489 (s), 1436 (m), 1397 (m), 1364 (w), 1335 (w), 1316 (w), 1273 (m), 1233 (s), 1219 (s), 1167 (m), 1113 (w), 1044 (w), 1011 (w), 966 (w), 894 (m), 796 (w), 758 (s), 752 (s), 699 (s), 665 (w); $^{1}$H NMR (DMSO-$d_6$, 400 MHz): 10.9 (s, 1 H, *CO*O*H*), 7.05 – 7.09 (m, 1H, Ar*H*), 6.96-7.01 (m, 3H, Ar*H*), 4.54 (s, 2H, C$H_2$); $^{13}$C{1H} NMR (DMSO-$d_6$, 100 MHz): 169.69, 154.28, 130.39, 128.29, 121.03, 120.54, 108.76, 107.99, 41.43; HR-MS (ESI): calcd for $C_9H_8N_2O_3$ + $Na^+$: 193.0718, found 193.0608.

**Solid phase peptide synthesis of capped dipeptides**

***Initial amino acid loading***

2-chlorotrityl chloride resin (100-200 mesh; 1% DVB; 1.41 mmol/g) (400 mg, 0.56 mmol) was weighed into a 10 mL polypropylene syringe equipped with a porous polypropylene frit (Torviq SF-1000), which was used as the reaction vessel. The resin was washed with dichloromethane (3 × 5 mL) before being allowed to swell in dichloromethane (5 mL) for at least 0.5 h prior to the loading of the first amino acid.

A solution of Fmoc-AA-OH (3 equiv.) was dissolved in dry dichloromethane (4 mL) and *N,N*-diisopropylethylamine (DIPEA) (0.8 mL, 8 equiv.) and taken up into the syringe with resin and stirred overnight using an orbital shaker. The resin was then washed with dichloromethane (3 x 4mL) and *N,N*-dimethylformamide (DMF) (3 × 4 mL).

***N-terminal Fmoc deprotection***

A solution of 20% (v/v) piperidine in DMF (2 × 4 mL) was added to the resin once for 5 mins, then a fresh aliquot was taken up again and left for 10 mins. The solution was subsequently expelled and the resin washed with DMF (5 × 4 mL). The resulting resin-bound amine was used immediately in the next peptide coupling step.

***Amino acid coupling***

Fmoc-AA-OH (655 mg, 3 equiv.) was dissolved in a 0.45 M solution of 1-hydroxybenzotriazole hydrate (HOBt·$H_2O$)/*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (3.4 mL, 3 equiv.) and DIPEA (0.6 mL, 6 equiv.) and this coupling solution added to the resin and stirred for 45 mins using an orbital shaker. The solution was expelled and the resin washed with DMF (5 x 4 mL).

After another *N*-terminal Fmoc deprotection, iterative couplings were performed in order to build up the required peptide sequence. The synthesis of indole and benzimidazole acetic acid, and their characterisation data, has previously been reported elesewhere.[3]

***N-methylindoleacetic acid coupling***

The N-methylindole capping group was introduced to the peptide in the same manner as for the amino acids. *N*-methylindoleacetic acid (320 mg, 3 equiv.) was dissolved in a 0.5M solution of 1-hydroxybenzotriazole hydrate (HOBt·$H_2O$)/ *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (3.4 mL, 3 equiv.) and DIPEA (0.6 mL, 6 equiv.) and this coupling solution added to the resin and stirred for 45 mins using an orbital shaker. Coupling was confirmed through the Kaiser test.

***Benzimidazolone acetic acid coupling***

The benzimidazolone capping group was introduced using a DIC-based coupling, whereby benzimidazolone acetic acid (214 mg, 2 equiv.) and HOBt.$H_2O$ (152 mg, 2 equiv.) were dissolved in 4 mL DMF and added to the resin, followed by *N*,*N*'-diisopropylcarbodiimide (180 µL, 2 equiv.) and the resin stirred for 2 hours using an orbital shaker. Coupling was confirmed through the Kaiser test.

***Cleavage of the peptide***

After the final coupling step, the resin was washed with DMF (3 x 4 mL) and dichloromethane (3 x 4 mL). A solution of 7:3 dichloromethane: trifluoroacetic acid was then added to the resin and the resin stirred for 15 minutes using an orbital shaker. The cleavage solution was then expelled, the resin washed with dichloromethane (2 x 4 mL) and the solvents evaporated under a stream of nitrogen. The resulting orange residue was lyophilised and purified by semi-preparative HPLC using an acetonitrile/water gradient, giving a white fluffy solid.

Characterisation data for ***N*-methyl indole-diphenylalanine 2**: IR: 3280 (m), 3269 (m), 1632 (s), 1536 (s), 1496 (m), 1474 (w), 1444 (w), 1422 (w), 1382 (w), 1328 (w), 1307 (w), 1247 (m), 1228 (m), 1193 (w), 1153 (w), 1119 (w), 1089 (w), 1062 (w), 1029 (w), 1013 (m), 946 (m), 912 (w), 797 (m), 749 (m), 736 (s), 720 (m), 697 (s); $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.82 (s, 1H), 8.27 (d, *J* = 7.7 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.34 (dd, *J* = 7.8, 5.0 Hz, 2H), 7.29 – 7.15 (m, 11H), 7.11 (t, *J* = 7.6 Hz, 1H), 6.97 – 6.90 (m, 2H), 4.56 (td, *J* = 9.5, 4.0 Hz, 1H), 4.44 (dd, *J* = 13.5, 8.0 Hz, 1H), 3.69 (s, 3H), 3.44 (q, *J* = 15.0 Hz, 2H), 3.03 (ddd, *J* = 17.8, 13.8, 4.7 Hz, 2H), 2.82 (ddd, *J* = 23.7, 13.8, 9.2 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 171.28, 170.29, 137.78, 137.47, 136.41, 129.26, 129.16, 128.16, 127.93, 127.48, 126.41, 126.16, 120.97, 118.86, 118.35, 109.32, 107.95, 53.59, 37.50, 36.70, 32.21, 32.09, 30.69; HR-MS (ESI): calcd for $C_{29}H_{29}N_3O_4 + H^+$: 484.2212, found 484.4203.

Characterisation data for **benzimidazolone-diphenylalanine 3**: IR: 3263 (s), 1696 (m), 1648 (s), 1552 (m), 1492 (m), 1454 (w), 1430 (w), 1407 (w), 1260 (w), 1229 (w), 1163 (m), 1108 (w), 1088 (w), 1031 (m), 1011 (w), 956 (w), 919 (w), 886 (w), 746 (m), 692 (s); $^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.82 (s, 1H), 8.46 (d, *J* = 8.7 Hz, 1H), 8.37 (d, *J* = 7.7 Hz, 1H), 7.30 – 7.16 (m, 10H), 6.97 – 6.90 (m, 2H), 6.88 – 6.81 (m, 1H), 6.42 (d, *J* = 7.7 Hz, 1H), 4.59 (td, *J* = 9.9, 4.0 Hz, 1H), 4.50 – 4.15 (m, 3H), 3.01 (ddd, *J* = 22.5, 13.9, 6.9 Hz, 2H), 2.88 (ddd, *J* = 23.9, 13.8, 7.1 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 172.72, 170.86, 166.26, 154.28, 137.68, 137.53, 130.32, 129.28, 129.13, 128.19, 128.10, 128.05, 126.40, 126.25,

120.82, 120.42, 108.53, 107.79, 53.63, 42.31, 37.73, 36.66; HR-MS (ESI): calcd for $C_{27}H_{26}N_4O_5 + H^+$: 487.1903, found 487.2276.

**Analytical reverse-phase HPLC**

High Performance Liquid Chromatography (HPLC) was recorded on a Shimadzu Prominence LC-20A HPLC system using a Waters XBridge™ C18 10 µm 150 x 4.60 mm analytical column at a flow rate of 1 mL/min. The mobile phase consisted of eluents: A (water with 0.1% formic acid) and B (acetonitrile with 0.1% formic acid). The HPLC trace was obtained using a gradient from 5% B to 95% B over 20 minutes.

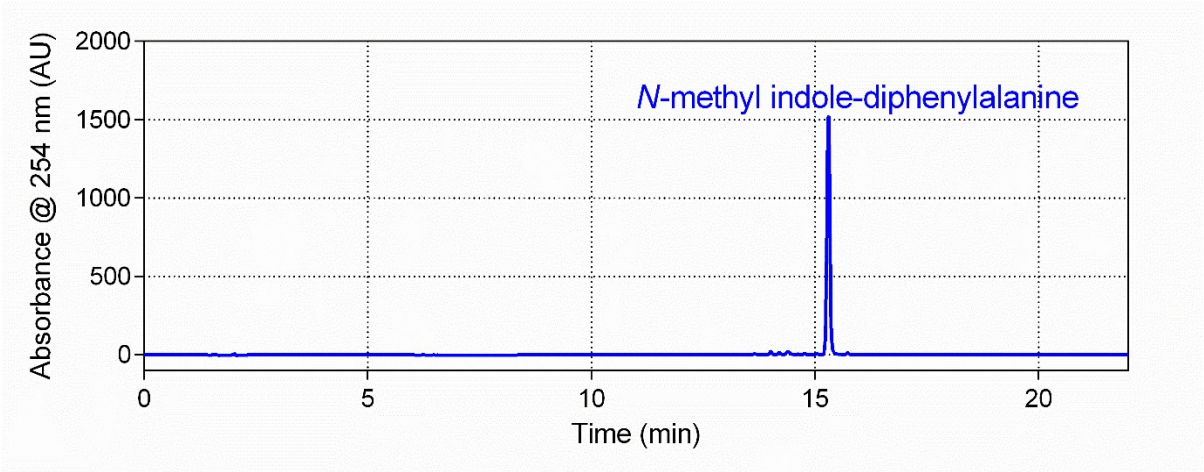



**Figure S1** – HPLC trace of *N*-methylindole-diphenylalanine, **2**.

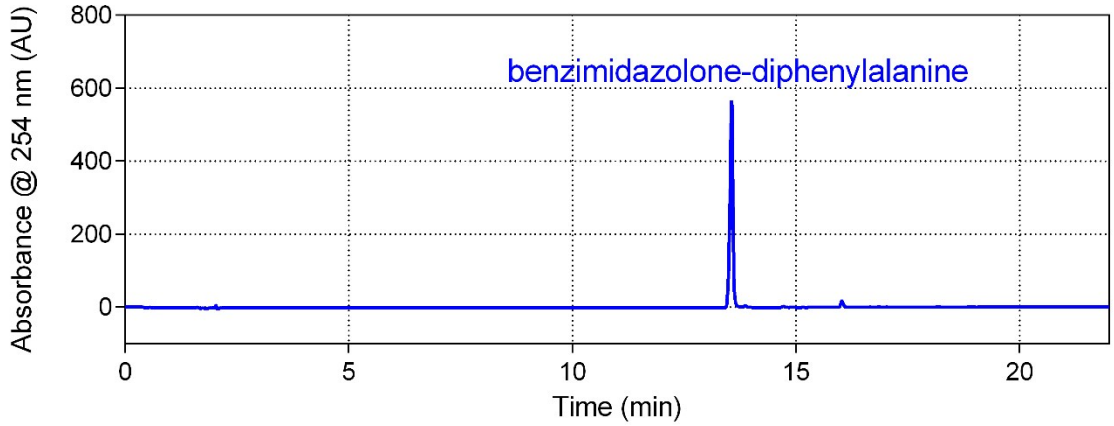



**Figure S2** – HPLC trace of benzimidazolone-diphenylalanine, **3**.

## NMR Spectra

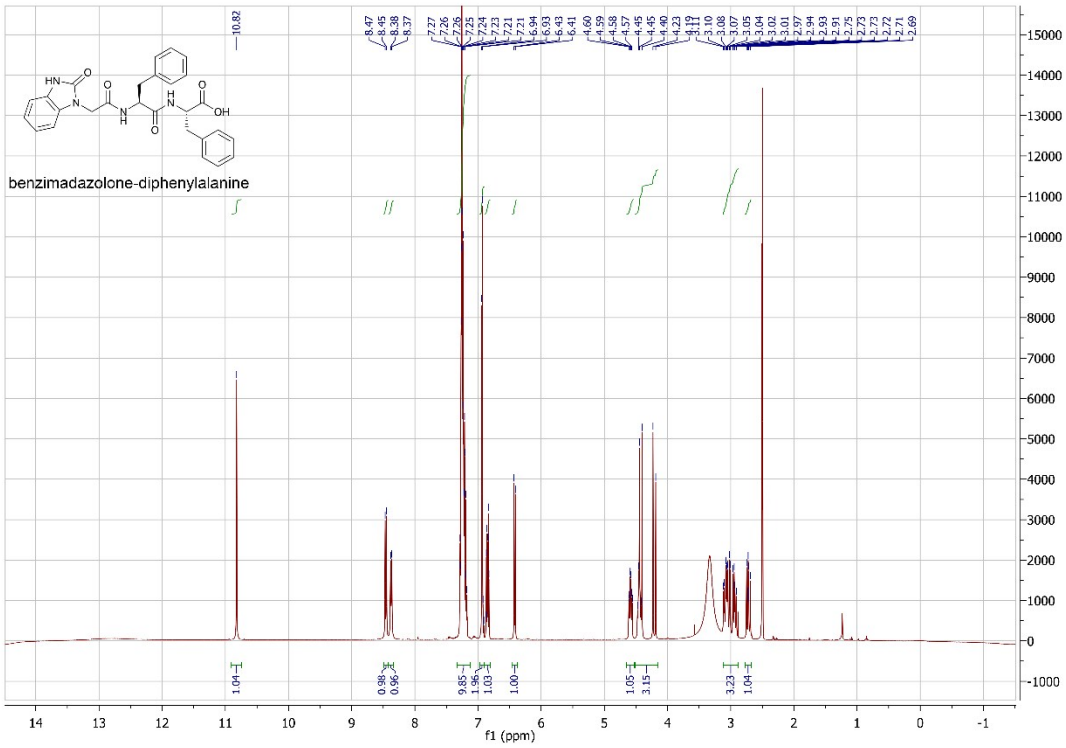



**Figure S3** – $^{1}$H NMR spectra of benzimidazolone-diphenylalanine, **3**.

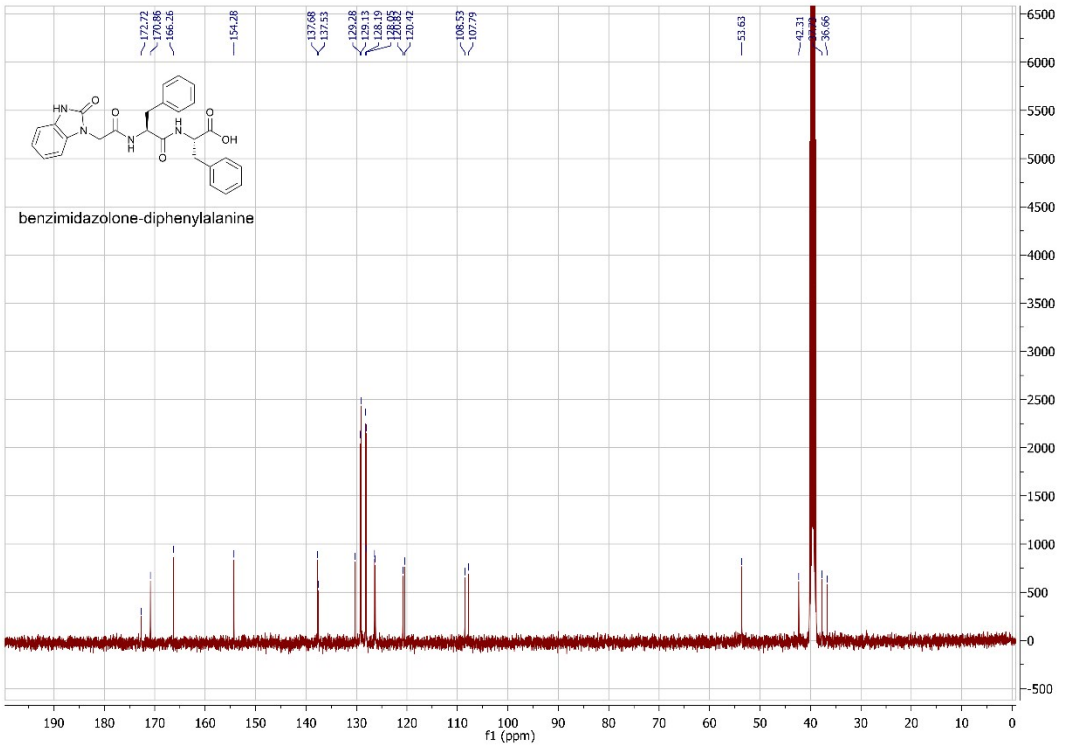



**Figure S4** – $^{13}$C NMR Spectra of benzimidazolone-diphenylalanine, **3**.

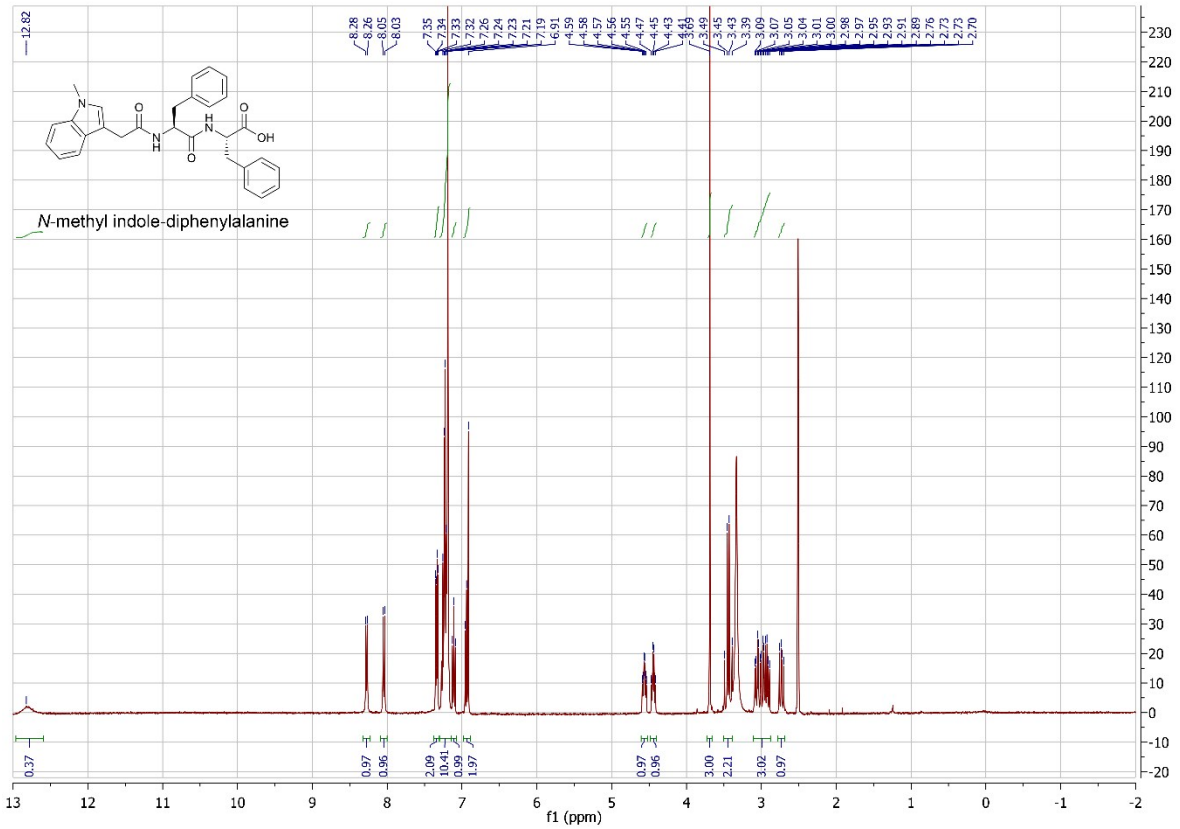



**Figure S5** – $^{1}$H NMR Spectra of *N*-methyl indole-diphenylalanine, **2**.

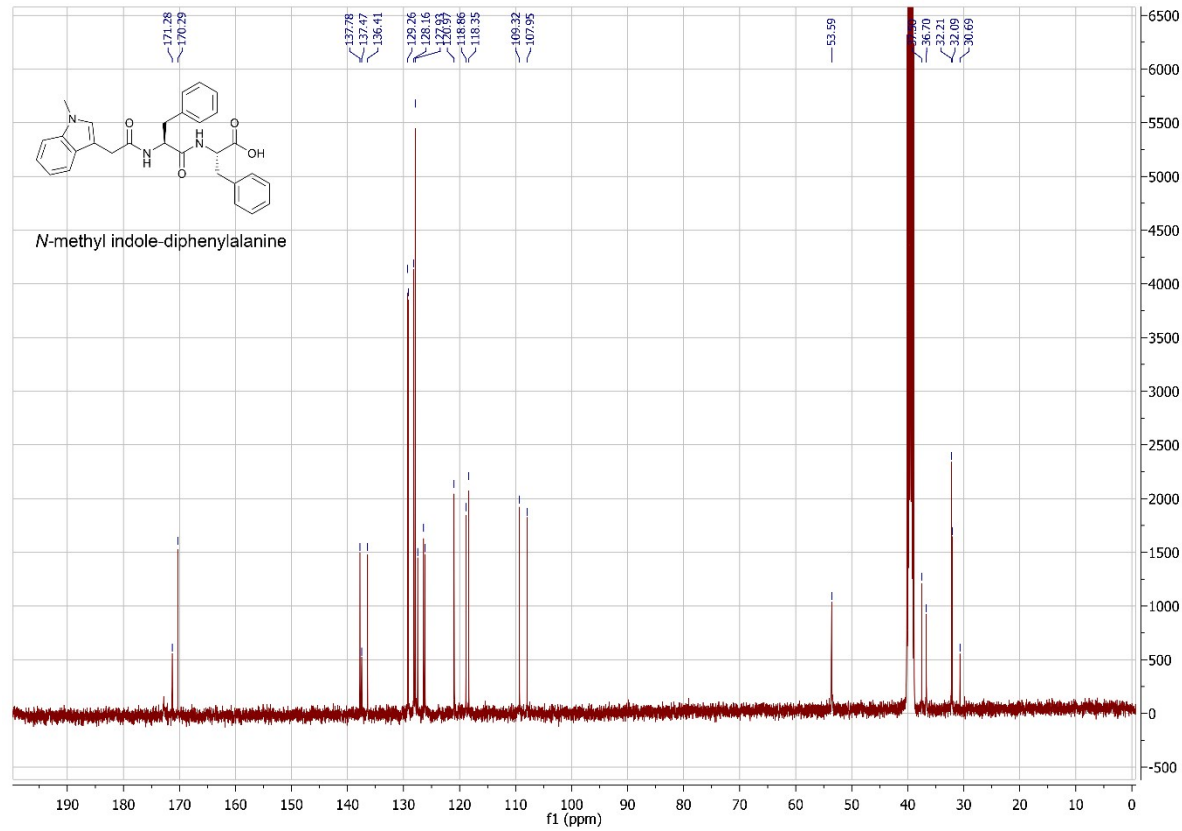



**Figure S6** – $^{1}$H NMR Spectra of *N*-methyl indole-diphenylalanine, **2**.

## Preparation of hydrogels

1.5 equivalents of 0.1 M sodium hydroxide was added to the peptide of interest and milliQ water added to make the suspension up to the required concentration (either 0.5 or 1% (w/v)). This suspension was sonicated until homogenous, upon which time 3 molar equivalents of glucono-δ-lactone was added to lower the pH, resulting in gelation.

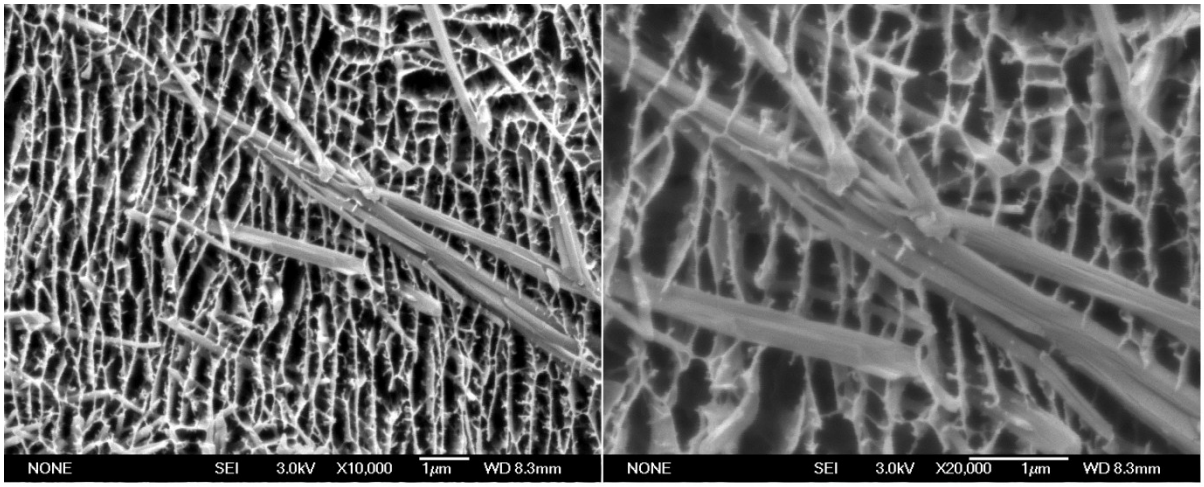



**Figure S7** – Cryo-SEM images of **1**, prepared at 1% (w/v) at two different magnifications.

## Calculation of clogP values

Log P values for each peptide were calculated by first building the structure using ChemDraw, before importing the structure into the molinspiration online interactive log P calculator,[4] which provides accurate values for log P, along with other parameters such as polar surface area.

## References

1. S. R. Kandukuri, L. Y. Jiao, A. B. Machotta and M. Oestreich, *Adv. Synth. Catal.*, 2014, **356**, 1597.
2. Z. Yang, J. Wang, L. Li, C. Ye and H. Liu, *J. Heterocyclic Chem.*, 2009, **46**, 788.
3. A. D. Martin, J. P. Wojciechowski, M. M. Bhadbhade and P. Thordarson, *Langmuir*, 2016, submitted.
4. www.molinspiration.com/services/logp.html