Control of Conformation in α-Helix Mimicking Aromatic Oligoamide Foldamers Through Interactions Between Adjacent Side-Chains

Irene Arrata,[a,b] Claire M. Grison,[a,b] Heather Coubrough, [a,b] Panchami Prabhakaran, [a,b] Mark A. Little,[a] Darren C. Tomlinson,[b,c] Michael E. Webb [a,b] and Andrew J. Wilson [a,b,*]

[a] *School of Chemistry, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK*

[b] *Astbury Centre For Structural Molecular Biology, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, UK*

[c] *School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK*

Supporting Information

Contents

Generic Methods for Monomer Synthesis

a) Procedure for *O*-alkylation

The phenol (1 eq.), alkyl halide (1.5 eq.), and potassium carbonate (3 eq.) were stirred in *N*,*N*-dimethylformamide (4 mL/mmol) and heated to 50°C for 16 hours. The mixture was cooled down to

room temperature and poured into water (3 mL/mmol), extracted with ethyl acetate (3 mL/mmol) and the organic layer was then washed with brine (40 mL/mmol). The organic layer was dried over magnesium sulfate, filtered, and the filtrate was evaporated under vacuum and purified by column chromatography as required.

b) Procedure for nitro reduction – hydrogenation

The nitro compound (1 eq.) was dissolved in methanol:tetrahydrofuran or methanol:ethyl acetate (1:1, 5 mL/mmol) and the solution was bubbled with nitrogen for 5 minutes. Palladium 10% on carbon (10%w) was added and the solution was further bubbled with nitrogen for 5 minutes. Hydrogen was passed through the reaction mixture at room temperature for 16 hours, using a balloon. The mixture was filtered through a pad of celite, washed with methanol, and the filtrate was evaporated.

c) Procedure for nitro reduction – tin chloride

Tin (II) chloride dihydrate (5 eq.) was added to a solution of nitro compound (1 eq.) in ethyl acetate (12 mL/mmol), and the reaction mixture was stirred at 50^0C for 16 hours under a nitrogen atmosphere and with a drying tube. The solution was poured into iced water (4 mL/mmol) and basified with sodium hydrogen carbonate. The product was extracted with ethyl acetate (4 mL/mmol) and the organic layer was washed with sodium hydroxide 1M (2 mL/mmol), water (2 mL/mmol) and brine (2 mL/mmol). The organic layer was dried over magnesium sulfate, filtered, and the filtrate was evaporated under vacuum.

d) Procedure for ester hydrolysis

Sodium hydroxide (6 eq., 10% aqueous solution) was added to a solution of ester (1 eq.) in a 1:1 mixture of methanol and tetrahydrofuran (4 mL/mmol), and the solution was stirred at room temperature for 16 hours. The mixture was poured into dichloromethane (3 mL/mmol) and the organic layer was washed with water (5 mL/mmol). The aqueous layer was acidified to pH 4 with a 1M aqueous hydrochloric acid solution and extracted with dichloromethane (9 mL/mmol). The organic layer was dried over magnesium sulfate, filtered, and the filtrate was concentrated under vacuum.

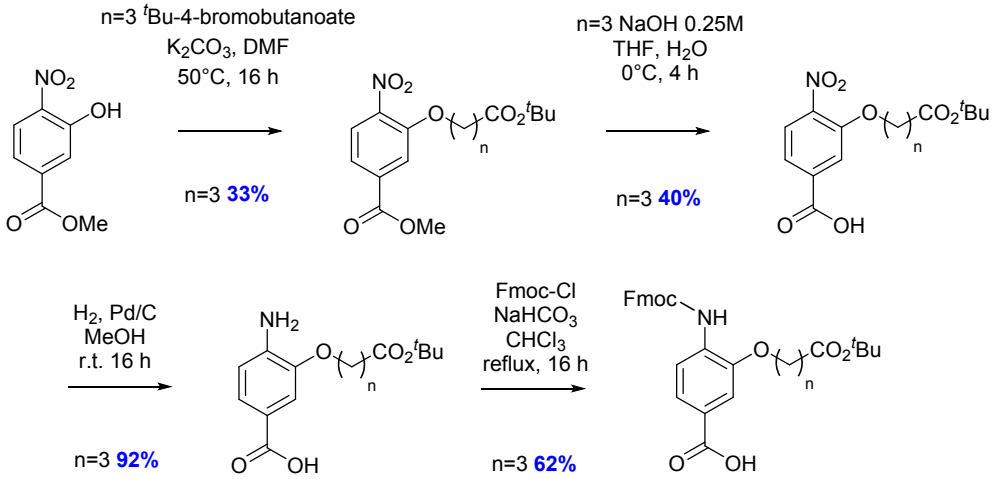
e) Procedure for reductive amination

The amino benzoic acid (1 eq.), aldehyde (1 eq.) and picoline borane (1.2 eq.) were stirred in methanol (2 mL/mmol) at room temperature for 3-16 hours. The solvent was removed and the residue diluted in ethyl acetate (4 mL/mmol), and washed with a 1M aqueous HCl solution (4 mL/mmol). The organic layer was dried over magnesium sulphate, filtered, and the filtrate was concentrated under vacuum.

f) Procedure for Fmoc protection

The amine (1 eq.) and fluorenylmethyl chloroformate (1 eq.) and sodium carbonate (2 eq. for Boc-protected groups) or sodium hydrogen carbonate (1.1 eq. for *tert*-butyl esters) were refluxed for 16 hours in anhydrous chloroform (4 mL/mmol). The residue was evaporated and purified either by precipitation or column chromatography.

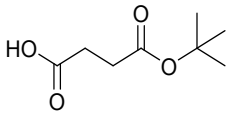
Monomer Synthesis and Characterisation



O-alkylated acid functionalised monomer

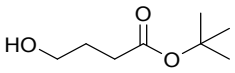
Scheme ESI 1. Synthesis of O-alkylated acid functionalised monomer building block used in this study

C4-(*tert*-butoxy)-4-oxobutanoic acid[1]



Succinic anhydride (5.0 g, 50.0 mmol), *N*-hydroxysuccinimide (1.7 g, 15.0 mmol) and 4-dimethylaminopyridine (610 mg, 5.0 mmol) were dissolved in toluene (25 mL). *tert*-Butanol (5.9 mL, 62.5 mmol) and triethylamine (2.1 mL, 15 mmol) were added sequentially and the mixture was refluxed for 16 hours. The solution was cooled down to room temperature and poured into ethyl acetate (25 mL) and washed with a 10%w citric acid solution (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate, filtered, and the filtrate was concentrated and purified by column chromatography (eluent: dichloromethane). C4-(*tert*-butoxy)-4-oxobutanoic acid was collected as a white solid (3.67 g, 42%). δ_H ($CDCl_3$, 500 MHz) 2.65 (t, 2H, J = 6.8 Hz, -CH_2CO_2H), 2.56 (t, 2H, J = 6.8 Hz, -$CH_2CO_2C(CH_3)_3$), 1.45 (s, 9H, - $CO_2C(CH_3)_3$). δ_C ($CDCl_3$, 125 MHz): 178.0, 171.4, 81.0, 30.0, 29.1, 27.9.

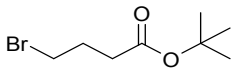
***tert*-Butyl 4-hydroxybutanoate[1]**



4-(*tert*-butoxy)-4-oxobutanoic acid (2.1 g, 11.8 mmol) was dissolved in anhydrous tetrahydrofuran (20 mL) and borane dimethylsulfide complex 2 M in tetrahydrofuran (6.5 mL, 13.0 mmol) was added

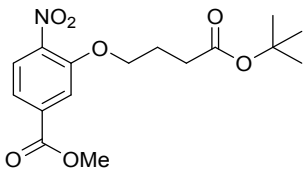
dropwise to the mixture. The solution was stirred at room temperature for 16 hours, and poured into ethyl acetate (100 mL), then washed with water (50 mL) and brine (100 mL). The organic layer was dried over magnesium sulphate, filtered, and the filtrate was concentrated under vacuum and purified by column chromatography (eluent: dichloromethane/methanol: 9.5/0.5), *tert*-butyl 4-hydroxybutanoate was collected as a colourless oil (862 mg, 46%). δ_H ($CDCl_3$, 500 MHz) 3.66 (t, 2H, J = 6.2, -CH_2OH), 2.35 (t, 2H, J = 7.1 Hz, -CH_2CO$_2$C(CH$_3$)$_3$), 2.20 (s, 1H, -O*H*), 1.85 (quint., 2H, J = 6.2 Hz –CH_2-), 1.43 (s, 9H, -CO$_2$C(CH_3)$_3$). δ_C ($CDCl_3$, 125 MHz): 173.4, 80.4, 62.1, 32.4, 28.0, 27.8.

tert-Butyl 4-bromobutanoate[2]



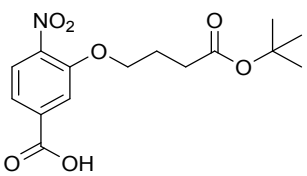
Tert-Butyl 4-hydroxybutanoate (250 mg, 1.6 mmol) and carbon tetrabromide (1.0 g, 3.1 mmol) were dissolved in tetrahydrofuran (5 mL), in an ice bath. A solution of triphenylphosphine (817 mg, 3.1 mmol) in tetrahydrofuran (5 mL) was slowly added and the mixture was allowed to reach room temperature and then stirred for 16 hours. The residue was filtered off, the solvent was removed under vacuum, and the obtained oil was purified by column chromatography (eluent: ethyl acetate/hexane: 5/95) to give *tert*-butyl 4-hydroxybutanoate as a yellow oil (247 mg, 71%). δ_H ($CDCl_3$, 500 MHz) 3.47 (t, 2H, J = 6.8 Hz, -CH_2Br), 2.42 (t, 2H, J = 6.8 Hz, -CH_2CO$_2$C(CH$_3$)$_3$), 2.17 (quint., 2H, J = 6.8 Hz, –CH_2-), 1.44 (s, 9H, -CO$_2$C(CH_3)$_3$).

Methyl 3-(4-(*tert*-butoxy)-4-oxobutoxy)-4-nitrobenzoate



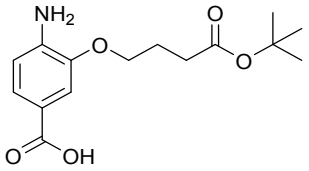
Using the general procedure for *O*-alkylation a). Methyl-4-nitro-3-hydroxybenzoate (180 mg, 0.9 mmol); *tert*-butyl 4-bromobutanoate (247 mg, 1.1 mmol); potassium carbonate (380 mg, 2.76 mmol); *N*,*N*-dimethylformamide. White solid (104 mg, 33%). δ_H ($CDCl_3$, 500 MHz) 7.84 (d, 1H, J = 8.3 Hz, Ar-*H* (H5)), 7.74 (d, 1H, J = 1.5 Hz, Ar-*H* (H2)), 7.70 (dd, 1H, J = 8.3, 1.5 Hz, Ar-*H* (H6)), 4.24 (t, 2H, J = 6.0, -OCH_2-), 3.97 (s, 3H, -OCH_3), 2.49 (t, 2H, J = 7.3 Hz, -CH_2CO$_2$C(CH$_3$)$_3$), 2.16 (quint., 2H, J = 6.3 Hz -CH_2-), 1.46 (s, 9H, - CO$_2$C(CH_3)$_3$). δ_C ($CDCl_3$, 125 MHz) 172.3, 165.2, 151.7, 142.5, 134.8, 125.3, 121.4, 115.6, 80.6, 68.8, 52.8, 31.4, 28.1, 24.3. ν_{max}/cm^{-1} (solid state) = 2985, 2952, 1720, 1530, 1293. HRMS: Calcd. [M+Na]$^+$ ($C_{16}H_{21}NNaO_7$) *m/z* = 362.1216. Found [M+Na]$^+$ *m/z* = 362.1217.

3-(4-(*tert*-butoxy)-4-oxobutoxy)-4-nitrobenzoic acid



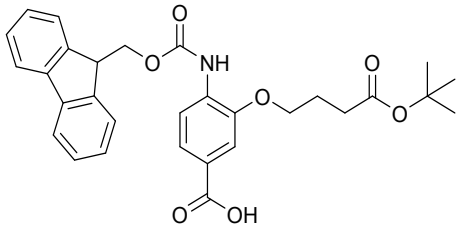
Methyl 3-(4-(*tert*-butoxy)-4-oxobutoxy)-4-nitrobenzoate (650 mg, 1.9 mmol) was dissolved in tetrahydrofuran (25 mL) and water (25 mL), in an ice bath. A 0.25M aqueous solution of sodium hydroxide was added and the mixture was stirred for 4 hours at 0°C. The mixture was then diluted with water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with brine (50 mL) and the solvent was evaporated. The residue was purified by column chromatography (eluent: dichloromethane/methanol 10/0 to 9/1) to give 3-(4-(*tert*-butoxy)-4-oxobutoxy)-4-nitrobenzoic acid as a pale yellow solid (406 mg, 66%). δ_H (MeOD, 500 MHz) 7.82-7.80 (m, 2H, Ar-*H* (H2, H5)), 7.69 (dd, 1H, *J* = 8.3, 1.3 Hz, , Ar-*H* (H6)), 4.23 (t, 2H, *J* = 6.0, -OC*H*$_2$-), 2.47 (t, 2H, *J* = 7.3 Hz, -C*H*$_2$CO$_2$C(CH$_3$)$_3$), 2.10 (quint., 2H, *J* = 6.4 Hz, -C*H*$_2$-), 1.44 (s, 9H, - CO$_2$C(C*H*$_3$)$_3$). δ_C (MeOD, 125 MHz) 199.3, 174.1, 152.6, 143.6, 140.0 125.6, 122.6, 116.9, 81.6, 69.9, 32.5, 28.2, 25.6. ν_{max}/cm^{-1} (solid state) = 2981, 2931, 1735, 1520, 1287. HRMS: Calcd. $[M+Na]^+$ ($C_{15}H_{19}NNaO_7$) *m/z* = 348.1059. Found $[M+Na]^+$ *m/z* = 348.1059.

4-amino-3-(4-(*tert*-butoxy)-4-oxobutoxy)benzoic acid



Using the general procedure for nitro reduction b). 3-(4-(*tert*-butoxy)-4-oxobutoxy)-4-nitrobenzoic acid (782 mg, 2.4 mmol); methanol (20 mL); palladium 10% on carbon (78 mg). Brown powder (651 mg, 92%). δ_H (MeOD, 500 MHz) 7.51-7.47 (m, 2H, Ar-*H* (H2, H6)), 6.70 (d, 1H, *J* = 8.0, Ar-*H* (H5)), 4.07 (t, 2H, *J* = 6.2 Hz, -OC*H*$_2$-), 2.48 (t, 2H, *J* = 7.3 Hz, -C*H*$_2$CO$_2$C(CH$_3$)$_3$), 2.09 (quint., 2H, *J* = 6.4 Hz, -C*H*$_2$-), 1.45 (s, 9H, - CO$_2$C(C*H*$_3$)$_3$). δ_C (DMSO-d$_6$, 125 MHz): 172.6, 144.6, 124.3, 112.5, 80.1, 67.3, 31.9, 28.3, 24.9 (4 x C missing). ν_{max}/cm^{-1} (solid state) = 3362, 2925, 1716, 1146, 751. HRMS: Calcd. $[M+Na]^+$ ($C_{15}H_{21}NNaO_5$) *m/z* = 318.1317. Found $[M+Na]^+$ *m/z* = 318.1318.

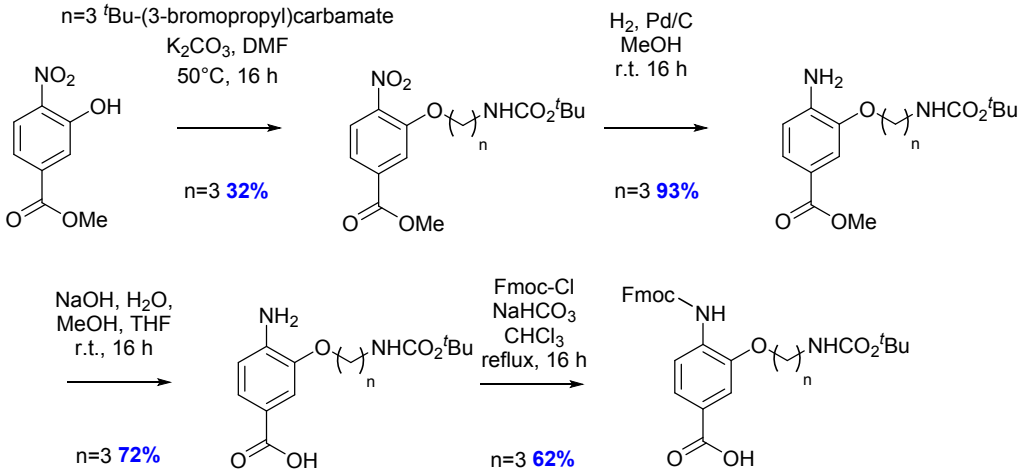
4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(*tert*-butoxy)-4-oxobutoxy)benzoic acid



Using the general procedure for Fmoc protection f). 4-amino-3-(4-(*tert*-butoxy)-4-oxobutoxy)-benzoic acid (1.6 g, 5.4 mmol); fluorenylmethyl chloroformate (1.4 g, 5.4 mmol), sodium hydrogen carbonate (498 mg, 5.9 mmol); anhydrous chloroform (50 mL). The residue was purified by flash chromatography (eluent: dichloromethane/methanol: 10/0 to 9/1) and the product was collected as a white powder (1.73 g, 62%). δ_H ($CDCl_3$, 500 MHz) 8.18 (s broad, 1H, -N*H*), 7.81 (d, 2H, *J* = 7.5 Hz, Ar-*H* (H5)), 7.78-7.76 (m, 1H, Ar-*H*(Fmoc)), 7.69 (dd, 1H, *J* = 7.5, 1.5 Hz, Ar-*H* (H6)), 7.65-7.62 (m, 2H, Ar-*H*

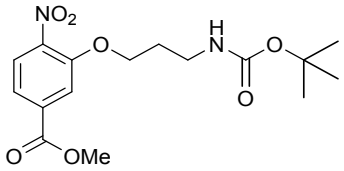
(Fmoc)), 7.59 (d, 1H, *J* = 1.5 Hz, Ar-*H* (H2))), 7.45 (t, 2H, *J* = 7.6 Hz, Ar-*H*(Fmoc)), 7.37 (t, 2H, *J* = 7.6 Hz, Ar-*H*(Fmoc)), 4.55 (d, 2H, *J* = 7.1 Hz, -C*H*$_2$(Fmoc)), 4.36 (t, 1H, *J* = 7.1 Hz, -C*H*(Fmoc)), 4.20 (t, 2H, *J* = 6.2 Hz, -OC*H*$_2$-), 2.50 (t, 2H, *J* = 7.1 Hz, -C*H*$_2$CO$_2$C(CH$_3$)$_3$), 2.23 (quint., 2H, *J* = 6.6 Hz, -C*H*$_2$-), 1.48 (s, 9H, -CO$_2$C(C*H*$_3$)$_3$). δ_C ($CDCl_3$, 125 MHz) 172.2, 171.0, 153.1, 146.3, 143.7, 141.4, 133.0, 127.9, 127.2, 125.1, 124.5, 123.3, 120.1, 117.4, 112.1, 80.8, 68.3, 67.6, 47.1, 32.2, 28.1, 24.5. ν_{max}/cm^{-1} (solid state) = 3426, 2971, 1715, 1681,1195. HRMS: Calcd. $[M+Na]^+$ ($C_{30}H_{31}NNaO_7$) *m/z* = 540.1998. Found $[M+Na]^+$ *m/z* = 540.1996.

O-alkylated amine functionalised monomer



Scheme ESI 2. Synthesis of novel O-alkylated amine functionalised monomer building block used in this study.

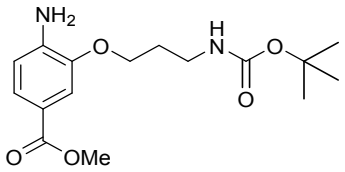
Methyl 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoate



Using the general procedure for *O*-alkylation a). Methyl-4-nitro-3-hydroxybenzoate (5.2 g, 26.4 mmol); *tert*-butyl (3-bromopropyl)carbamate (9.4 g, 39.6 mmol); potassium carbonate (10.9 g, 79.2 mmol); *N*,*N*-dimethylformamide (100 mL). The residue was purified by column chromatography (eluent: hexane/ethyl acetate: 7/3) to give methyl 3-(3-((tert-butoxycarbonyl)amino)propoxy)-4-nitrobenzoate as a yellow solid (3.03 g, 32%). δ_H ($CDCl_3$, 500 MHz) 7.86 (d, 1H, *J* = 8.3 Hz, Ar-*H* (H5)), 7.74 (s, 1H, Ar-*H* (H2)), 7.70 (dd, 1H, *J* = 8.3 Hz, *J* = 0.7 Hz, Ar-*H* (H6)), 4.98 (s br., 1H, -N*H*), 4.26 (t, 2H, *J* =

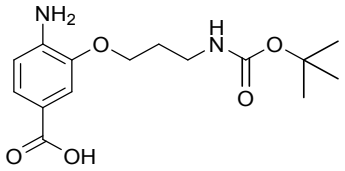
5.9 Hz, -OC*H*$_2$), 3.96 (s, 3H, -OC*H*$_3$), 3.38 (q, 2H, *J* = 5.9 Hz, -C*H*$_2$-NH), 2.08 (quint., 2H, *J* = 5.9 Hz, -C*H*$_2$-), 1.44 (s, 9H, -$CO_2C(CH_3)_3$).

Methyl 4-amino-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzoate



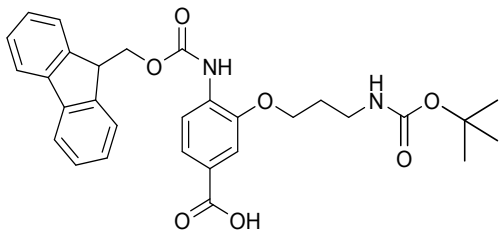
Using the general procedure for nitro reduction b). Methyl 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoate (3.03 g, 8.55 mmol); methanol:ethyl acetate 1:1 (40 mL); palladium 10% on carbon (300 mg). White solid (2.55 g, 93%). δ_H (MeOD, 500 Mz) 7.47 (dd, 1H, *J* = 8.3 Hz, *J* = 1.8 Hz, Ar-*H* (H6)), 7.41 (d, 1H, *J* = 1.8 Hz, Ar-*H* (H2)), 6.70 (d, 1H, *J* = 8.3 Hz, Ar-*H* (H5)), 4.08 (t, 2H, *J* = 5.9 Hz, -OC*H*$_2$-), 3.82 (s, 3H, -OC*H*$_3$), 3.28 (t, 2H, *J* = 6.6 Hz, -C*H*$_2$-NH), 2.01 (quint., 2H, *J* = 6.4 Hz, -C*H*$_2$-), 1.43 (s, 9H, -$CO_2C(CH_3)_3$).

4-amino-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzoic acid



Using the general procedure for ester hydrolysis d). Methyl 4-amino-3-(3-((*tert*-butoxycarbonyl)-amino)propoxy)benzoate (2.55 g, 7.9 mmol); sodium hydroxide solution (20.0 mL, 54.0 mmol); methanol: tetrahydrofuran (30 mL). Hygroscopic brown powder (1.76 g, 72%). δ_H ($CDCl_3$, 500 MHz) 7.64 (d, 1H, *J* = 8.3 Hz, Ar-*H* (H5)), 7.49 (s br., 1H, Ar-*H* (H2)), 6.69 (d, 1H, *J* = 8.3 Hz, Ar-*H* (H6)), 4.73 (s br., 1H, N*H*), 4.15 (t, 2H, *J* = 6.4 Hz, O-C*H*$_2$) 3.38-3.34 (m, 2H, C*H*$_2$-NH), 2.05 (t, 2H, *J* = 6.4 Hz, -C*H*$_2$-), 1.45 (s, 9H, -$CO_2C(CH_3)_3$).

4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzoic acid

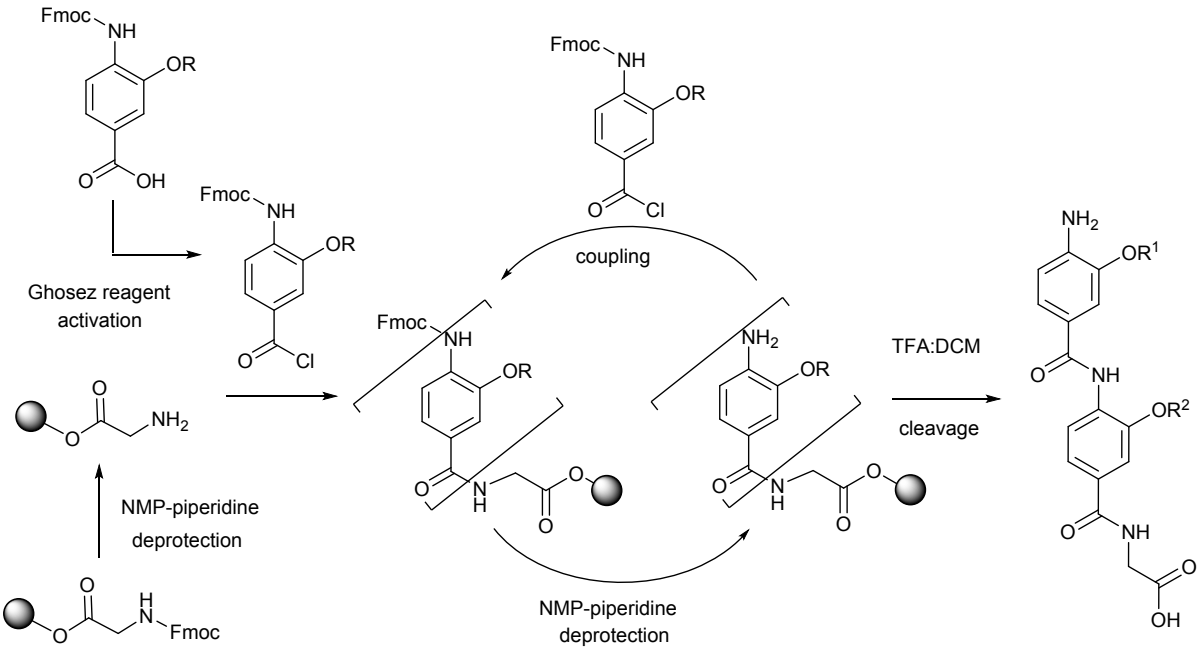


Using the general procedure for Fmoc protection f). 4-amino-3-(3-((*tert*-butoxy-carbonyl)amino)propoxy)benzoic acid (1.76 g, 5.7 mmol); fluorenylmethyl chloroformate (1.76 g, 6.8 mmol); sodium carbonate (1.50 g, 14.2 mmol); anhydrous chloroform (300 mL). The residue was purified by flash chromatography (eluent: dichloromethane/methanol: 10/0 to 0/10) to give the desired product (1.87 g,

62%) as a white powder. δ_H ($CDCl_3$, 500 MHz) 8.14 (s br., 1H, N*H*), 7.80 (d, 2H, *J* = 7.6 Hz, Ar-*H* (H5)), 7.75 (d, 1H, *J* = 6.9 Hz, Ar-*H* (Fmoc)), 7.66 (m, 3H, , Ar-*H* (Fmoc & H6), 7.58 (s, 1H, Ar-*H* (H2)), 7.44 (t, 2H, *J* = 7.6 Hz, Ar-*H* (Fmoc)), 7.35 (t, 2H, *J* = 7.10 Hz, Ar-*H* (Fmoc)), 4.70 (s br., 1H, N*H*), 4.54 (d, 2H, *J* = 6.9 Hz, C*H*$_2$(Fmoc)), 4.35 (t, 1H, J = 6.9 Hz, C*H*(Fmoc)), 4.19 (s br., 2H, O-C*H*$_2$), 3.39 (s br., 2H, C*H*$_2$-NH), 2.09 (t, 2H, *J* = 6.2 Hz, -C*H*$_2$-), 1.44 (s, 9H, -$CO_2C(CH_3)_3$).

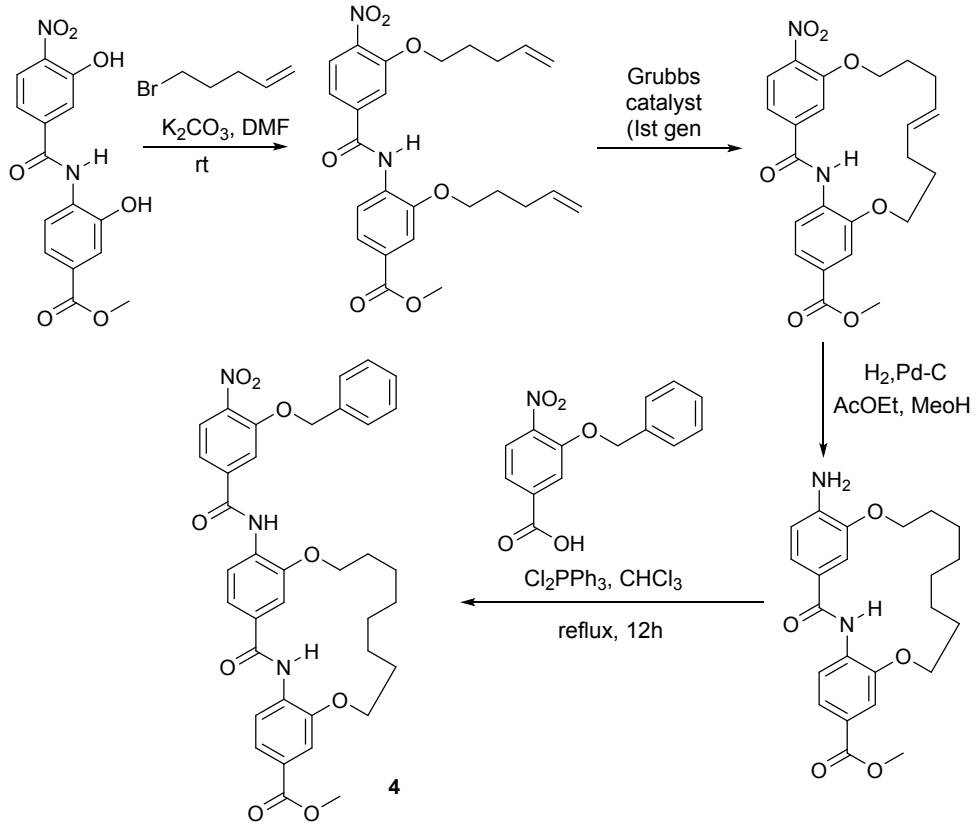
O-alkylated alkyl functionalised monomer**4-((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-3-(isobutoxy)benzoic acid** was synthesized as described previously[3]

Generic Method for O-alkylated Dimers 1-3 Synthesis using Solid-supported Synthesis



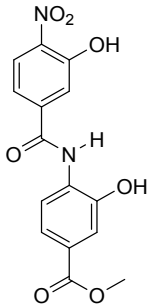
Scheme ESI 3. Overview of approach for solid-supported synthesis of oligoamide dimers **1-3**.

Covalently Constrained Trimer 4 Synthesis and Characterisation of Intermediates



Scheme ESI 4. Synthesis of covalently constrained helix mimetics by "stapling".

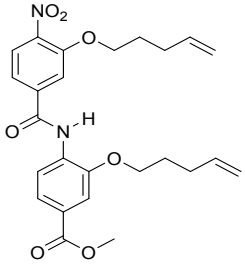
Methyl 3-hydroxy-4-(3-hydroxy-4-nitrobenzamido)benzoate



A solution of methyl 4-amino-3-hydroxybenzoate (1 equiv.) and **4**-nitro-3 hydroxybenzoic acid (1 equiv.) taken in dry acetonitrile was cooled to 0 ^{0}C. To this, was added DIPEA (2 equiv.) followed by PyBOP (1.1 equiv.). The reaction was then allowed to warm to room temperature and then stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the product extracted into ethyl acetate by partition with water. Orange coloured crystals were isolated from a solution of compound in ethyl acetate. M.p: 234 ^{0}C; δ_H (DMSO-d_6, 500 MHz) 11.38 (s, 1H, -O*H*), 10.43 (s, 1H, -

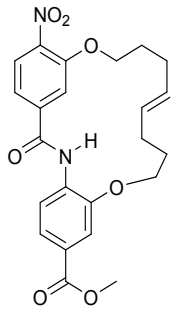
O*H*), 9.67 (s, 1H, -N*H*), 8.00-7.98 (m, 2H, Ar-*H* (1-H5, 2-H5)), 7.63 (s, 1H, Ar-*H* (1-H2)), 7.52-7.41 (m, 3H, Ar-*H* (1-H6, 2-H6, 2-H2)), 3.84 (s, 3H, -OCH_3). δ_C (DMSO-d_6, 125 MHz) 165.8, 163.6, 151.5, 148.6, 139.7, 138.9, 130.1, 126.2, 125.4, 122.8, 120.4, 118.3, 117.8, 115.6, 52.0. ν_{max}/cm^{-1} (solid state) = 3418, 3114, 3091, 2963, 1701, 1668, 1606, 1582, 1508, 1478, 1440, 1422, 1327, 1294, 1267, 1216, 1109, 1096. HRMS Calcd. $[M+H]^+$ $C_{15}H_{13}N_2O_7$ *m/z* = 333.0723. Found: $[M+H]^+$ *m/z* = 333.0717.

Methyl 3-pent-4-enyloxy-4-(3-pent-4enyloxy-4-nitrobenzamido)benzoate



With a slight modification to the general procedure A for *O*-alkylation (reaction was performed at room temperature), the title compound was synthesized. Methyl 3-hydroxy-4-(3-hydroxy-4-nitrobenzamido) benzoate (1.20 g, 3.6 mmol), 5-bromo-1-pentene (0.94 ml, 7.95 mmolK_2CO_3 (2.49 g, 18 mmol). The crude material obtained after the work-up could not be purified by column due to the very close R_f of an *N*-alkylated byproduct. The product was insoluble in methanol whereas the *N*-alkylated byproduct was not. Thus, the product was isolated by crystallization from a solution of methanol and minimum amount of dichloromethane. (893 mg, 53%). Mp: 108 ^oC ; δ_H ($CDCl_3$, 500 MHz) 8.74 (s, 1H, -N*H*), 8.58 (d, 1H, *J* = 8.5 Hz, Ar-*H* (1-H5)), 7.91 (d, 1H, *J* = 8.3 Hz, Ar-*H* (2-H5)), 7.75 (d, 1H, *J* = 8.5 Hz, Ar-*H* (1-H6)), 7.68 (s, 1H, Ar-*H* (1-H2)), 7.60 (s, 1H, Ar-*H* (1-H6)), 7.37 (d, 1H, *J* = 8.3 Hz, Ar-*H* (2-H6)), 5.90-5.79 (m, 2H, -C*H*CH_2-), 5.09-5.01 (m, 4H, -CHCH_2-), 4.22-4.18(m, 4H, -OCH_2-), 3.92 (s, 3H, -OCH_3), 2.30-2.26 (m, 4H, -CH_2-), 2.03-1.94 (m, 4H, -CH_2-). δ_C ($CDCl_3$, 125 MHz) 163.1, 147.0, 141.8, 139.6, 137.1, 137.0, 131.4, 125.9, 125.8, 123.4 118.8, 117.3, 115.9, 115.8, 114.2, 111.7, 69.1, 68.4, 52.2, 30.2, 29.8, 28.3, 27.9. ν_{max}/cm^{-1} (solid state) = 3425, 3076, 2949, 1716, 1681, 1599, 1529, 1424, 1388, 1350, 1284, 1125, 1110. HRMS: Calcd. $[M-H]^-$ ($C_{25}H_{27}N_2O_7$) *m/z* = 467.1818. Found $[M-H]^-$ *m/z* = 467.1818.

Cross-linked dimer



To Methyl 3-pent-4-enyloxy-4-(3-pent-4enyloxy-4-nitrobenzamido)benzoate (0.80 g, 1.7 mmol) in dry and degassed dichloromethane (200 ml) was added Grubbs catalyst-Ist generation (1 equiv.) and the

reaction mixture was refluxed at 40 ^{o}C. LC-MS of the reaction mixture after 12 h indicated complete conversion after which, the reaction mixture was concentrated (737 mg, 98%) and used in the enxt step without further purification. Mp: 233 ^{o}C. δ_H ($CDCl_3$, 500 MHz) 8.74$_{minor}$ (bs, 0.4H, -N*H*), 8.72$_{major}$ (s, 0.6H, -N*H*), 8.66-8.63 (m, 1H, Ar-*H* (1-H5)), 7.95-7.93 $_{major}$ (d, *J* = 8.4 Hz, 0.6H, Ar-*H* (2-H5)), 7.91-7.89 (d, *J* = 8.4 Hz, 0.4H, Ar-*H* (2-H5)), 7.78-7.75 $_{major}$ (m, 1.4H, 1-H6, Ar-*H* (2-H6)), 7.73-7.7 $_{minor}$ (dd, J = 1,7 Hz, 8.4 Hz, 0.6 H, Ar-*H* (1-H6, 2-H6)), 7.63-7.62 $_{major}$ (d, *J* = 1.7Hz, 0.6 H, Ar-*H* (1-H2)), 7.62-7.61 $_{minor}$ (d, 1.7Hz, 0.4 H, Ar-*H* (1-H2)), 7.63 (s, 0.6H, Ar-*H* (2-H2)) $_{major}$, 7.40 $_{minor}$ (s, 0.4H, Ar-*H* (2-H2)), 5.73-5.68 $_{minor}$ (m, 0.4H, -C*H*CH_2-), 5.60-5.48 $_{major}$ (m, 1.6H, -C*H*CH_2-), 4.31-4.23 (m, 4H, -OC*H*$_2$$CH_2$-), 3.93 $_{major}$ (s, 1.9H, -OC*H*$_3$), 3.93 $_{minor}$ (s, 1.1H, -OC*H*$_3$), 2.41-2.39 $_{minor}$ (m, 1.3H, -CHC*H*$_2$-), 2.37-2.31 $_{major}$ (m, 2.7H, -CHC*H*$_2$-), 2.07-1.92 (m, 4H, -$CHCH_2$C*H*$_2$-). ν_{max}/cm^{-1} (solid state) = 3425, 3088, 2948, 1714, 1681, 1597, 1520, 1346, 1285, 1220, 1129, 1043. HRMS: Calcd. $[M+Na]^+$ ($C2_3H_{24}N_2NaO_7$) *m/z* = 463.1481. Found $[M+Na]^+$ *m/z* = 463.1475. Elemental analysis calculated for $C_{23}H_{24}N_2NaO_7$: C, 62.72; H, 5.49, N, 6.36; Found: C, 62.60; H, 5.45, N, 6.20.

Full NMR Spectra for Dimers 1-3 and Trimer 4

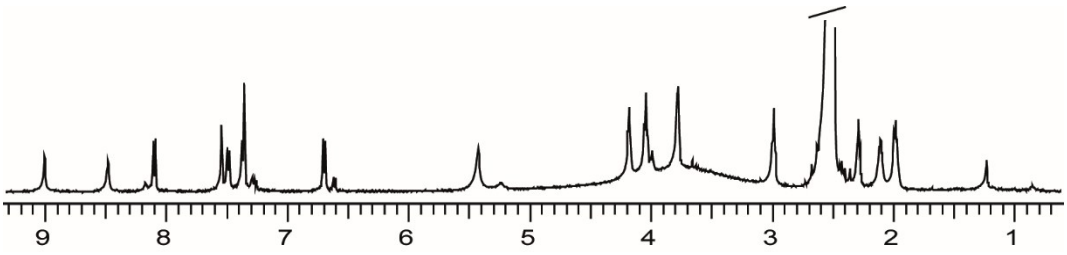


Figure ESI 1. ^{1}H NMR Dimer **1**

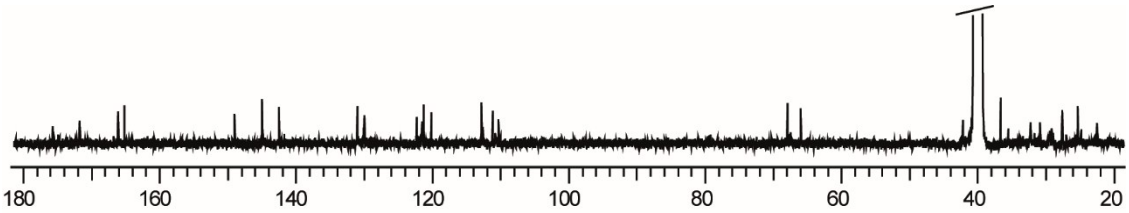


Figure ESI 2. ^{13}C NMR Dimer **1**

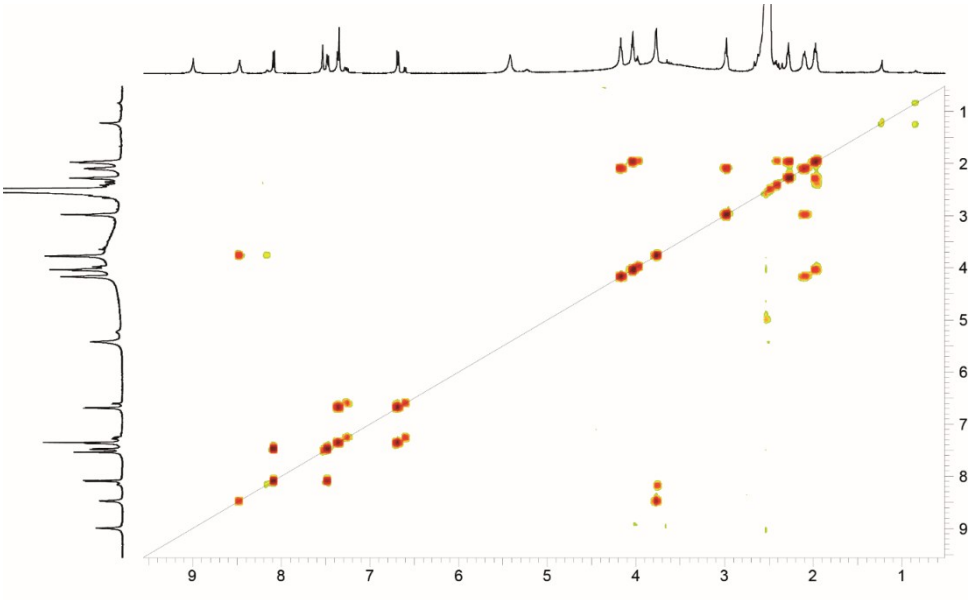


Figure ESI 3. COSY NMR Dimer 1

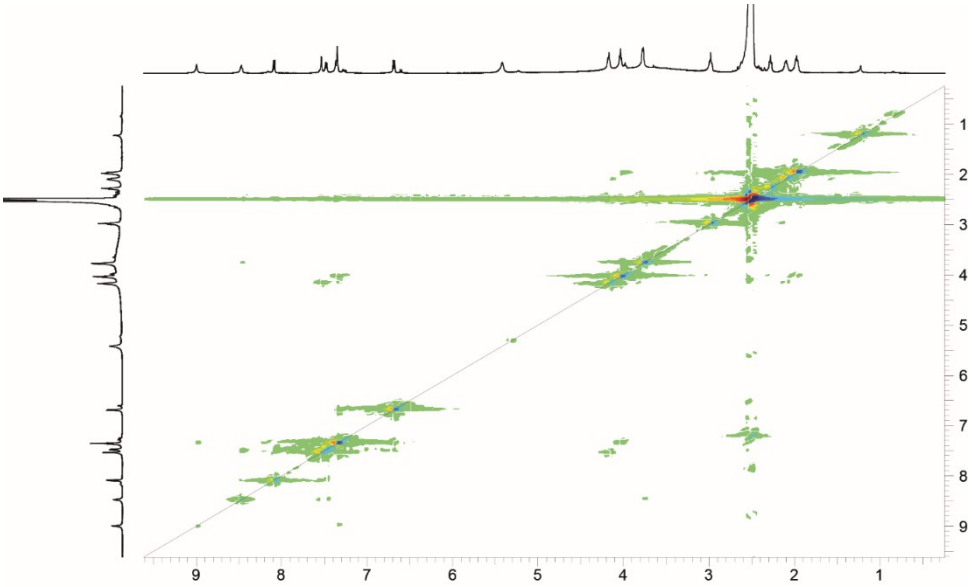


Figure ESI 4. NOESY NMR Dimer 1

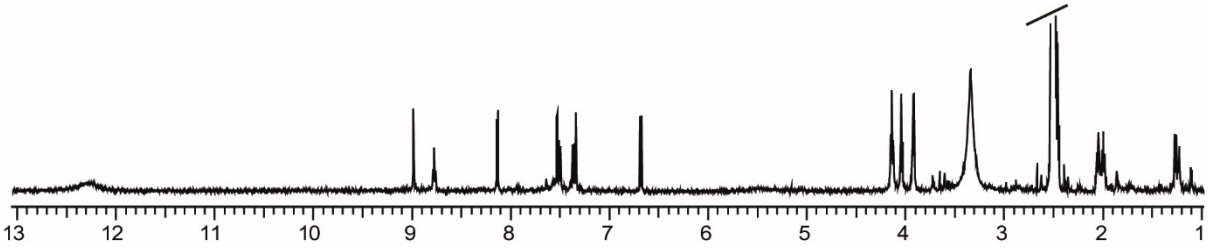


Figure ESI 5. ^{1}H NMR Dimer **2**

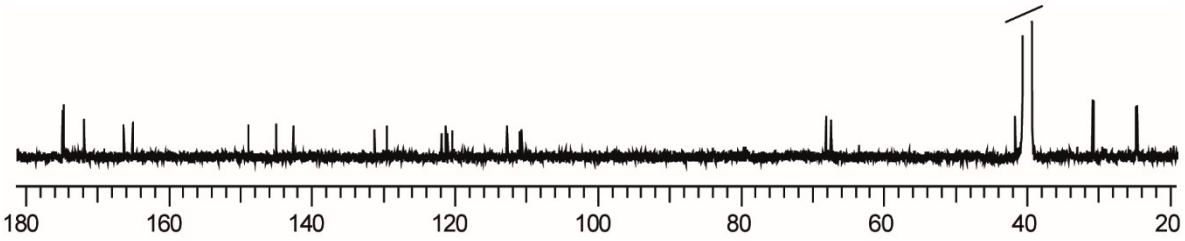


Figure ESI 6. ^{13}C NMR Dimer **2**

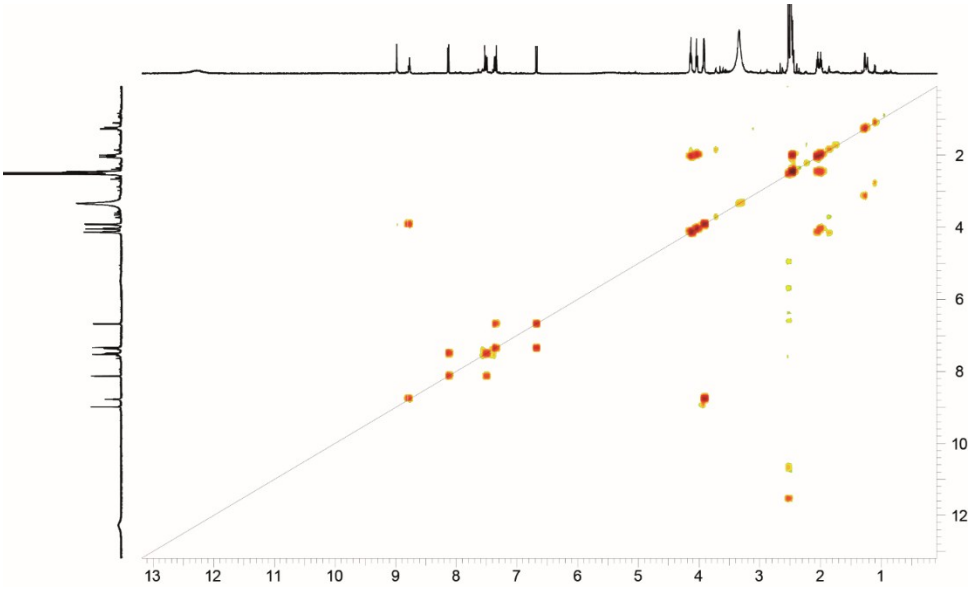


Figure ESI 7. COSY NMR Dimer **2**

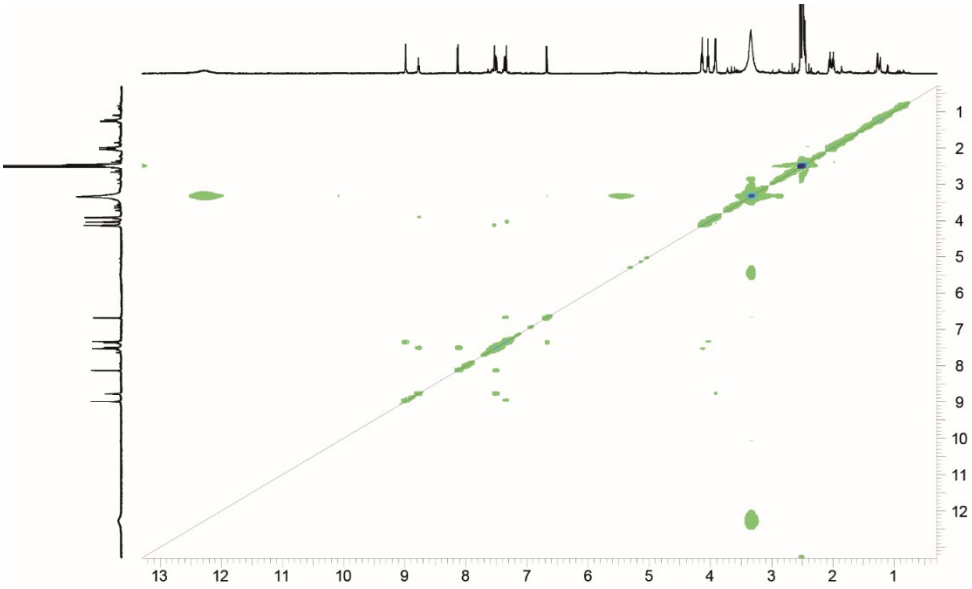


Figure ESI 8. NOESY NMR Dimer **2**

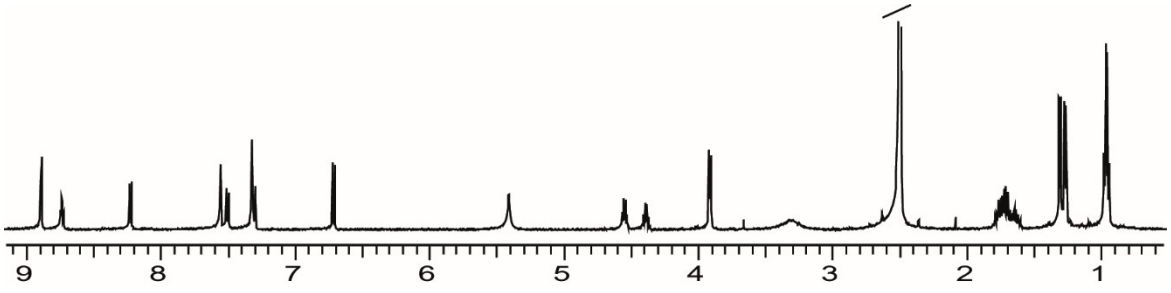


Figure ESI 9. ^{1}H NMR Dimer **3**

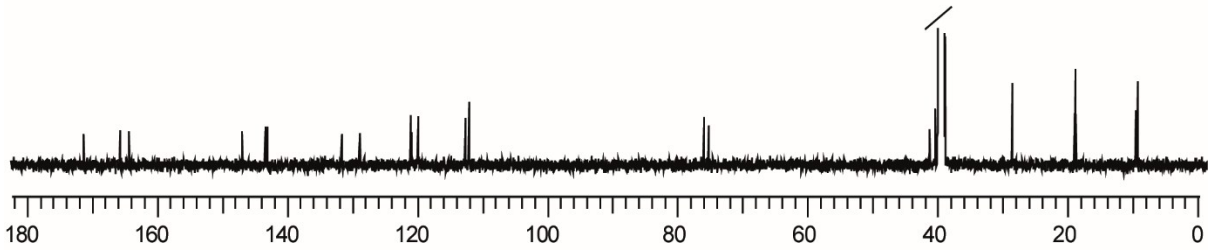


Figure ESI 10. ^{13}C NMR Dimer **3**

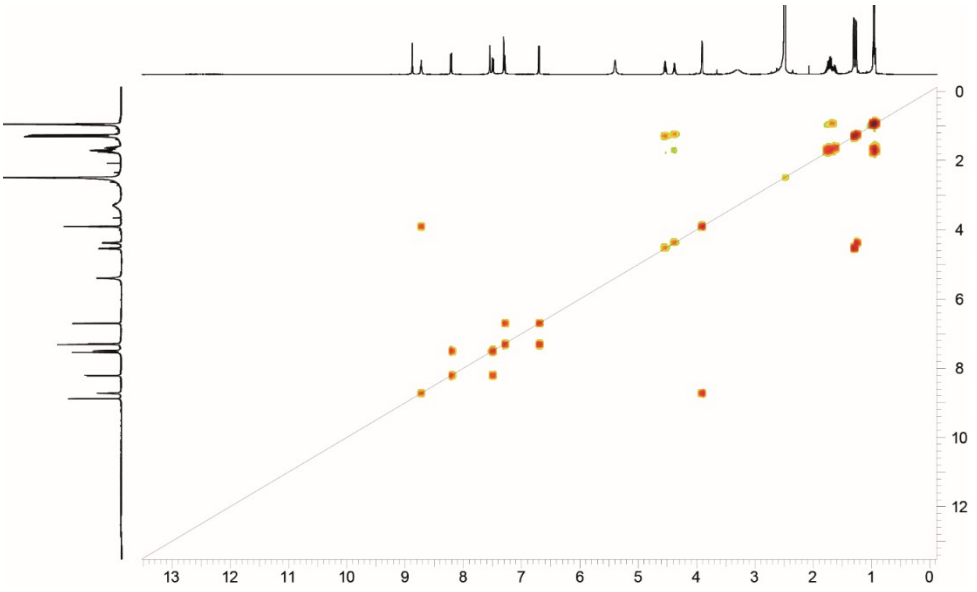


Figure ESI 11. COSY NMR Dimer 3

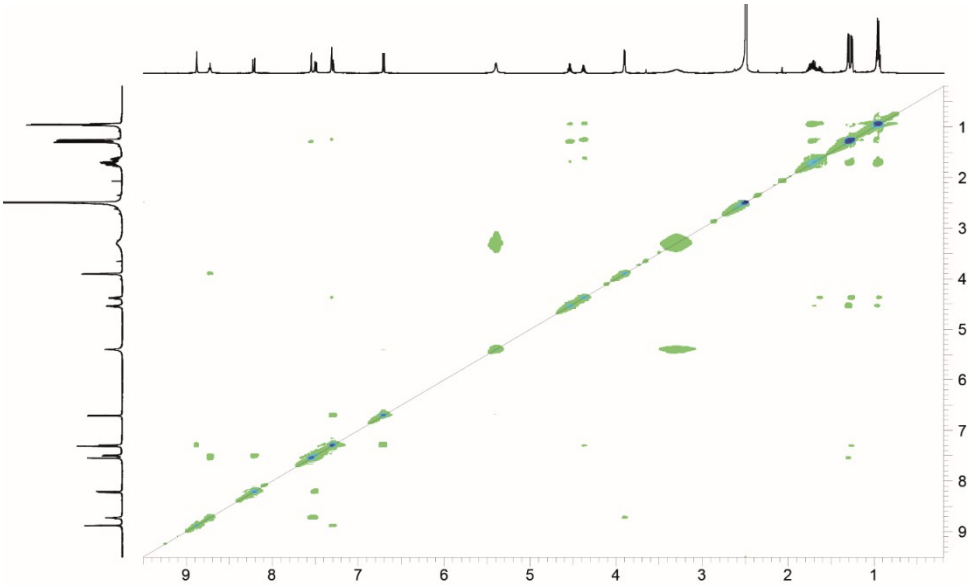


Figure ESI 12. NOESY NMR Dimer **3**

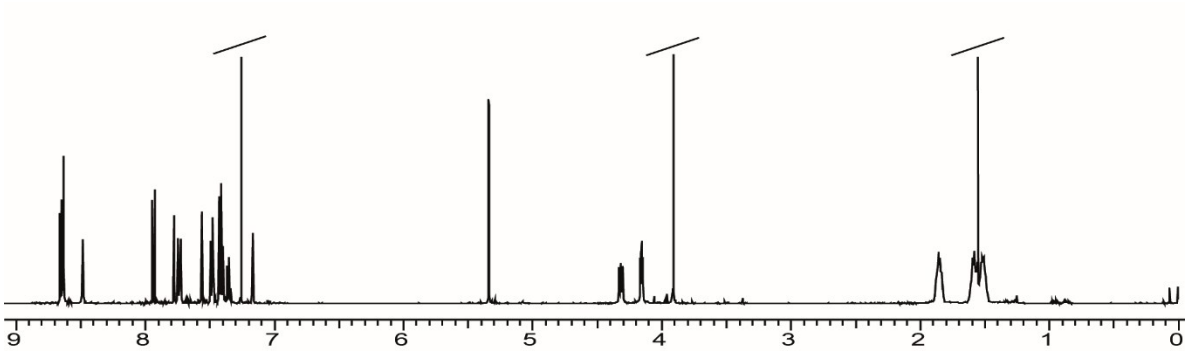


Figure ESI 13. ^{1}H NMR Trimer **4**

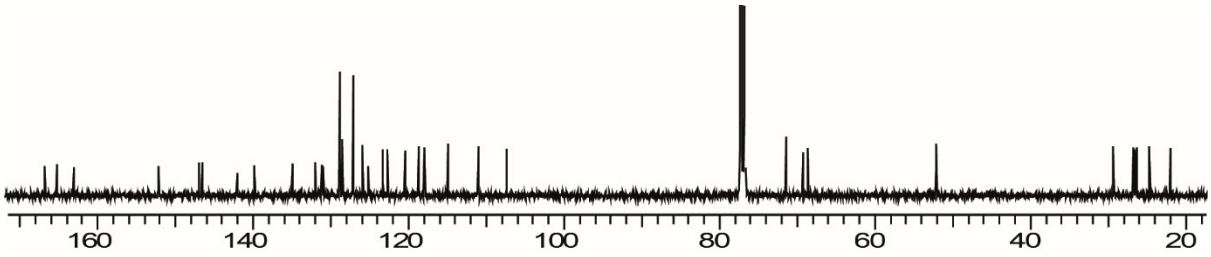


Figure ESI 14. ^{13}C NMR Trimer **4**

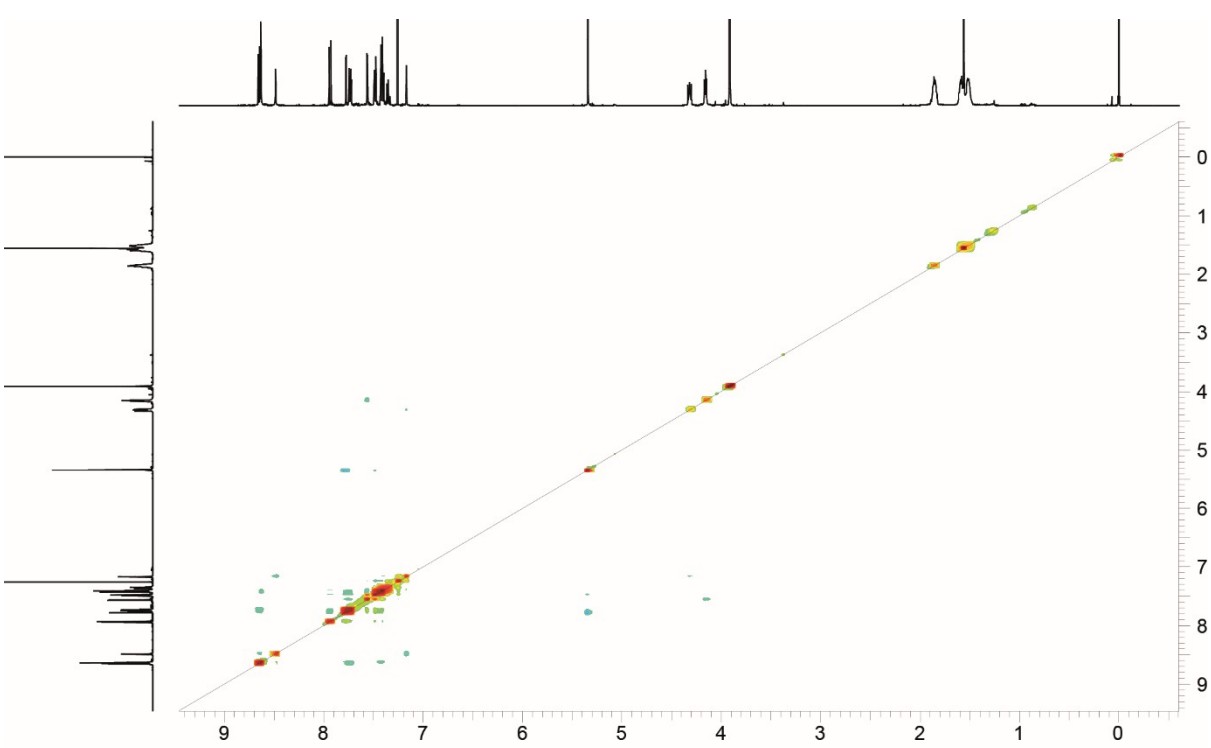
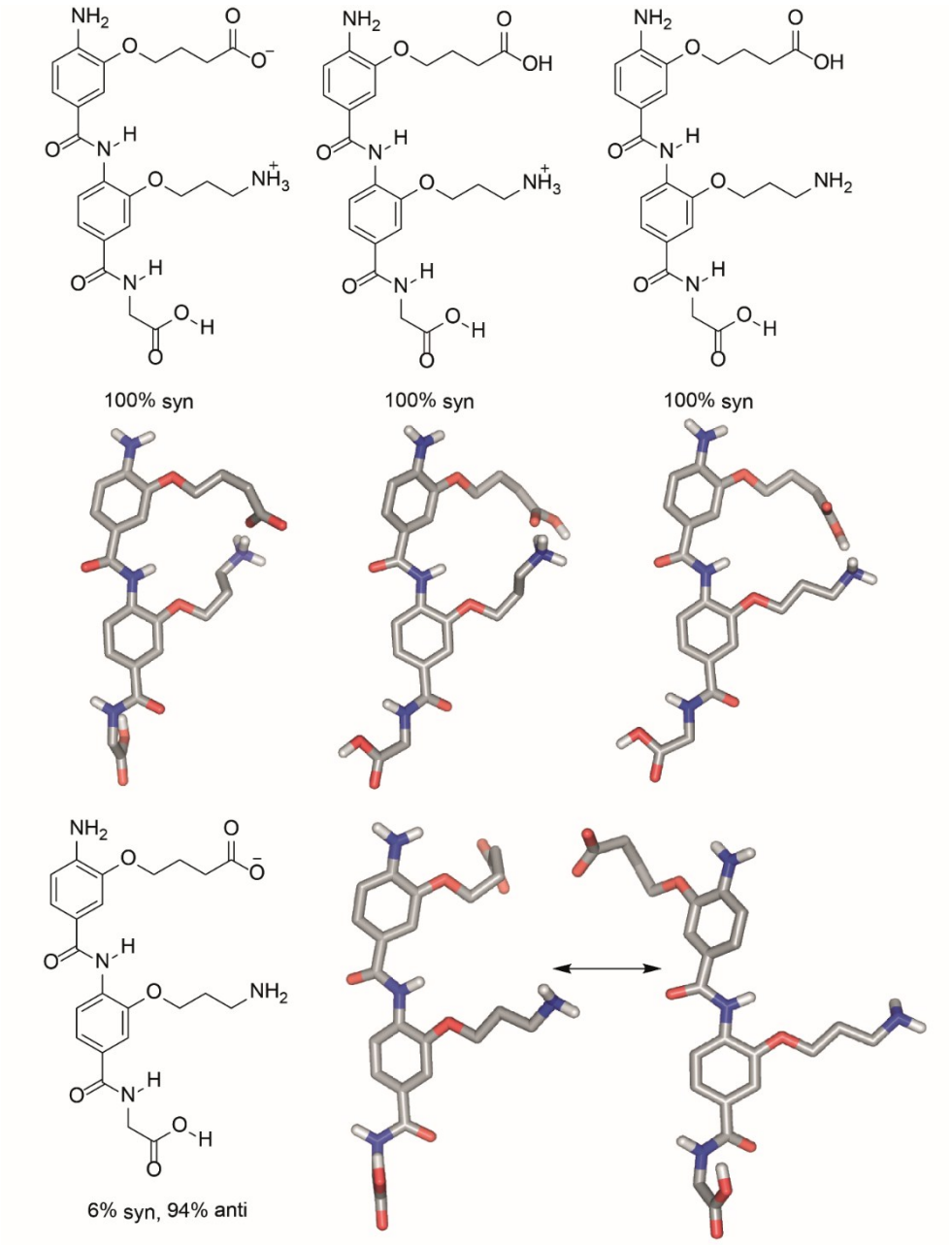
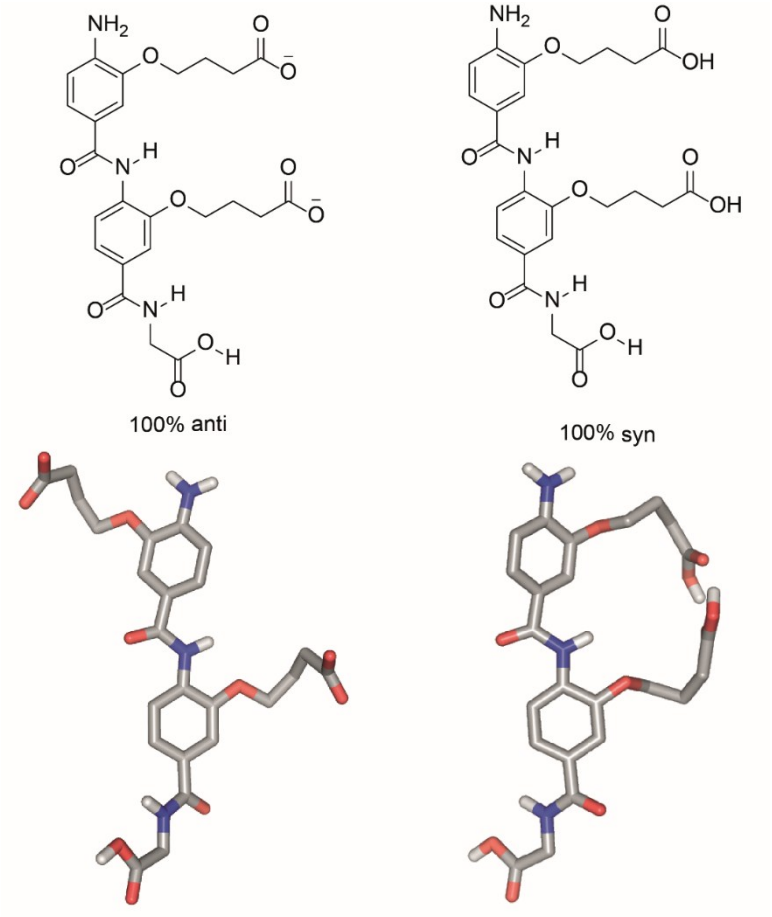


Figure ESI 15. NOESY NMR Trimer **4**



Additional Modelling at Different Ionisation States

Figure ESI 16. Molecular Modelling Analyses for Dimer **1**



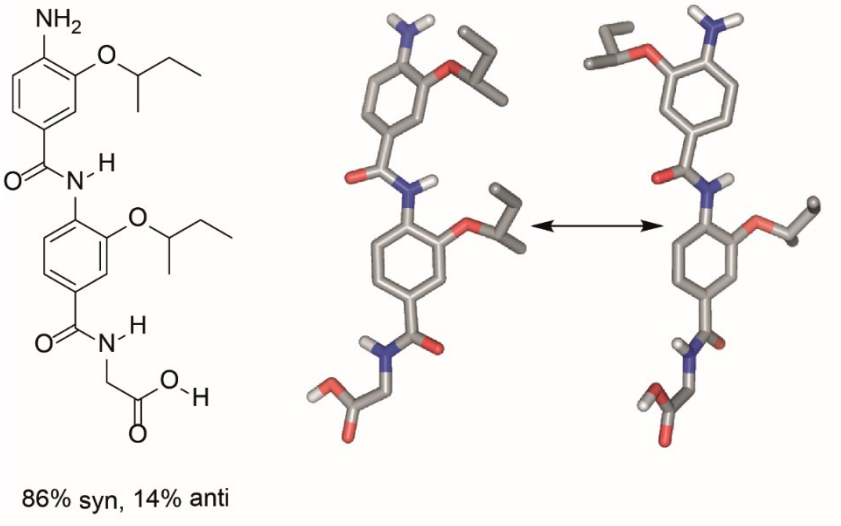


Figure ESI 17. Molecular Modelling Analyses for Dimer **2**

Figure ESI 18. Molecular Modelling Analyses for Dimer **3**

References

1. A. Nortcliffe, I. N. Fleming, N. P. Botting and D. O'Hagan, *Tetrahedron*, 2014, **70**, 8343-8347.
2. S. Machida, N. Kato, K. Harada and J. Ohkanda, *J. Am. Chem. Soc.*, 2011, **133**, 958-963.

3. N. Murphy, P. Prabhakaran, V. Azzarito, J. Plante, M. Hardie, C. Kilner, S. Warriner and A. Wilson, *Chem. Eur. J.*, 2013, **19**, 5546-5550.