Supporting Information

Stereoselective self-sorting in the self-assembly of a Phe-Phe extended guanidiniocarbonyl pyrrole carboxylate zwitterion: Formation of two diastereomeric dimers with significantly different stabilities

Fabian Rodler[a], *Wilhelm Sicking*[a], *and Carsten Schmuck**[a]

[a] *Institut für Organische Chemie, Universität Duisburg-Essen, Universitätsstraße 7, 45141 Essen, Germany. Fax: + 49 202 183 3097; E-Mail: carsten.schmuck@uni-due.de*

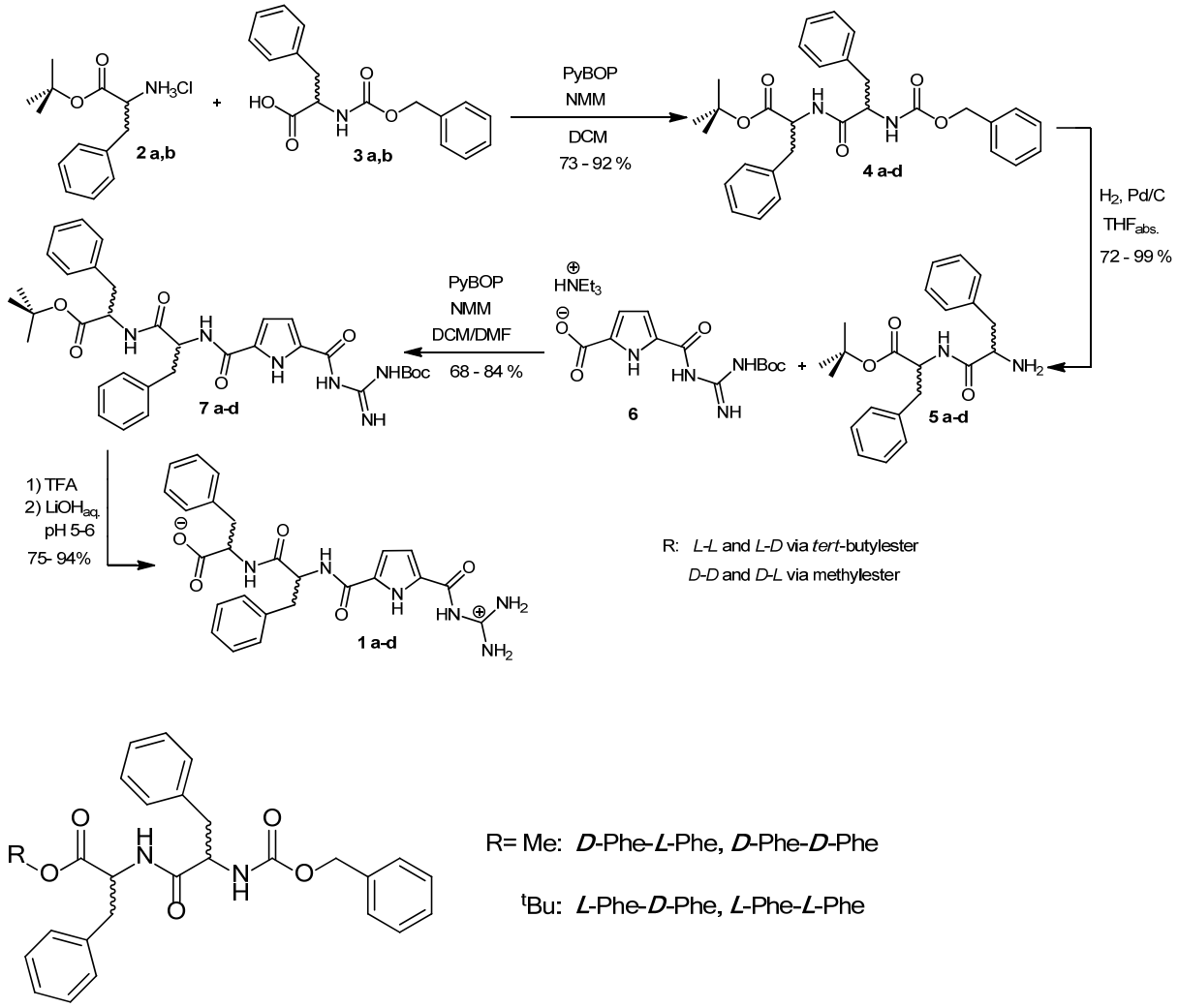
Table of Content

1 General information

All solvents were dried and destilled under nitrogen before use. All other reagents were used as obtained from either Aldrich or Fluka. Reactions were monitored by TLC on silica gel plates (Machery-Nagel POLYGRAM SIL G/UV254). Visualization of the spots was carried out by fluorescence quenching with 254 nm UV light. All melting points were measured with a Büchi Melting-Point B-450 apparatus with open end glass capillary tubes. The melting points are not corrected. The NMR-spectra were recorded at room temperature with Bruker DMX 300 and DRX 500 spectrometer. The ^{1}H-NMR spectra were recorded at 300 and 500, the ^{13}C-NMR spectra at 75 MHz or 125 MHz. The chemical shifts are relative to the signals of the used solvent DMSO-d_6 (δ_{1H} = 2.50 and δ_{13C} = 39.52). The apparent coupling constants are given in Hertz. The description of the fine structure means: s = singulett, br.s = broad singulett, d = dublett, m = multiplett. All IR spectra were measured on a *Jasco* FT/IR-430 spectrometer. The maxima are classified in three intensities: s (strong), m (middle), w (weak) and are reported in cm^{-1}. All mass spectra were recorded with a Bruker BioTOF III spectrometer. UV/Vis spectra were recorded with a JASCO UV spectrophotometer.

2 Synthesis of zwitterion 1a-d

The synthesis of the four stereoisomers of GCP-Phe-Phe-OH **1a-d** followed a standard protocol (Scheme 2). First, the four N-Cbz protected dipeptides N-Cbz-PhePhe-OR **4a-d** (R either Me or tBu) were prepared from the corresponding amino acids using PyBOP in CH_2Cl_2 as the coupling reagent. Then after hydrogenolysis of the Cbz-protecting group with H_2 in the presence of Pd/C, the free amines **5a-d** were coupled with the Boc-protected guanidinocarbonyl pyrrole carboxylic acid **6**[10] again using PyBOP in a mixture of CH_2Cl_2 and DMF as the coupling reagent. Finally, the Boc group and the *tert*-butyl ester in **7a-d** were cleaved off with TFA and the methyl ester was removed with LiOH to yield the four stereoisomeric zwitterions **1a-d**



2.1 Synthesis of the fully protected PhePhe-dipeptides 4a-d

2.1.1 fully protected *L*-Phe-*L*-Phe (4a)

A mixture of ***L***-phenylalanine *tert*-butyl ester hydrochloride (1.29 g, 5.01 mmol, 1.0 eq), Cbz-protected ***L***-phenylalanine (1.50 g, 5.01 mmol, 1.0 eq), PyBOP (2.61 g, 5.01 mmol, 1.0 eq) and NMM (7.5 ml) was stirred in CH_2Cl_2 (25 ml) for 24 h at room temperature. After evaporation of the solvent, the resulting orange oil was dissolved in ethyl acetate (50 ml). The solution was then washed with 1M Na_2CO_3 (20 ml), 1M $NaHSO_4$ (20 ml), water (20 ml) and brine (saturated solution of NaCl in water, 20 ml). The organic phase was dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography

(R_f = 0.91, SiO_2, ethyl acetate/ cyclohexane = 7/3) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 2.31 g (92%);

mp 120 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.37 (d, 1 H, J = 6.0 Hz, N*H*), 7.45 (d, 1 H, J = 9.0 Hz, N*H*), 7.19-7.33 (m, 15 H, CH_{aryl}), 4.92 (s, 2 H, CH_2), 4.25-4.41 (m, 2 H, C*H*), 2.94-2.99 (m, 1 H + 2 H, CH_2), 2.66-2.74 (m, 1 H, CH_2), 1.31 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.6, 170.4, 155.7, 138.0, 136.9, 129.2, 129.1, 128.2, 128.1, 128.0, 127.6, 127.4, 126.5, 126.2, 80.6, 65.2, 55.9, 54.2, 37.5, 36.8, 27.4; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3432 [br], 2985 [m], 1724 [s], [s], 1627 [s], 1542 [s], 1454 [w], 1392 [w], 1265 [w], 1238 [s], 1145 [s], 1047 [w], 964 [m], 939 [m], 842 [m], 754 [m], 698 [m]; ESI-MS (MeOH) m/z 503.26 ([$C_{30}H_{35}N_2O_5$]$^+$, calcd 503.25), 525.24 ([$C_{30}H_{34}N_2O_5Na$]$^+$, calcd 525.24).

2.1.2 fully protected *D*-Phe-*D*-Phe (4b)

A mixture of ***D***-phenylalanine methyl ester hydrochloride (865 mg, 4.01 mmol, 1.0 eq), Cbz-protected ***D***-phenylalanine (1.20 g, 4.01 mmol, 1.0 eq), PyBOP (2.09 g, 4.01 mmol, 1.0 eq) and NMM (4.4 ml) was stirred in CH_2Cl_2 (30 ml) for 24 h at room temperature. After evaporation of the solvent, the resulting orange oil was dissolved in ethyl acetate (50 ml). The solution was then washed with 1M Na_2CO_3 (50 ml), 1M $NaHSO_4$ (50 ml), water (50 ml) and brine (50 ml). The organic phase was dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.76, SiO_2, ethyl acetate/ cyclohexane = 6/4) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 1.38 g (75%);

mp 146 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.46 (d, 1 H, J = 7.5 Hz, N*H*), 7.45 (d, 1 H, J = 9.5 Hz, N*H*), 7.17-7.34 (m, 15 H, CH_{aryl}), 4.93 (s, 2 H, CH_2), 4.50 (ddd, 1 H, C*H*), 4.23 (ddd, 1 H, C*H*), 3.58 (s, 3 H, CH_3), 2.90-3.09 (m, 1 H + 2 H, CH_2), 2.64-2.73 (m, 1 H, CH_2) ; ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.8, 171.7, 155.7, 138.0, 137.0, 129.2, 129.1, 128.2, 128.1, 128.0, 127.6, 127.4, 126.6, 126.2, 65.2, 51.8, 37.4, 36.6; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 1658 [m], 1531 [m], 1288 [m], 1240 [s], 1147 [m], 1035 [m], 1147 [m], 1035 [m], 698 [s]; ESI-MS (MeOH) m/z 461.21 ([$C_{27}H_{29}N_2O_5$]$^+$, calcd 461.21), 483.19 ([$C_{27}H_{28}N_2O_5Na$]$^+$, calcd 483.19).

2.1.3 fully protected *L*-Phe-*D*-Phe (4c)

A mixture of ***L***-phenylalanine *tert* butyl ester hydrochloride (1.04 g, 4.03 mmol, 1.0 eq), Cbz-protected ***D***-phenylalanine (1.21 g, 4.03 mmol, 1.0 eq), PyBOP (2.10 g, 4.03 mmol, 1.0 eq) and NMM (4.6 ml) was stirred in CH_2Cl_2 (40 ml) for 24 h at room temperature. After evaporation of the solvent, the resulting orange oil was dissolved in ethyl acetate (50 ml). The solution was then washed with 1M Na_2CO_3 (50 ml), 1M $NaHSO_4$ (50 ml), water (50 ml) and brine (50 ml). The organic phase was dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.78, SiO_2, ethyl acetate/ cyclohexane = 6/4) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 1.38 g (71%);

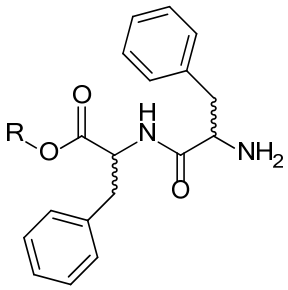
mp 121 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.42 (d, 1 H, J = 8.3 Hz, N*H*), 7.43 (d, 1 H, J = 9.7 Hz, N*H*), 7.18-7.32 (m, 15 H, CH_{aryl}), 4.93 (s, 2 H, CH_2), 4.40 (ddd, 1 H, C*H*), 4.27 (ddd, 1 H, C*H*), 3.02 (dd, 1 H, CH_2),

2.86 (dd, 1 H, CH_2), 2.74 (dd, 1 H, CH_2), 2.54-2.59 (m, 1 H, CH_2), 1.37 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.4, 170.5, 155.7, 138.0, 137.2, 129.3, 129.2, 128.3, 128.1, 128.0, 127.6, 127.4, 126.5, 126.2, 80.8, 65.1, 56.0, 54.0, 37.6, 37.1, 27.5; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3251 [br], 1720 [m], 1644 [s], 1531 [s], 1454 [w], 1367 [w], 1288 [w], 1240 [s], 1145 [s], 1041 [w], 912 [w], 842 [s], 748 [s], 698 [s]; ESI-MS (MeOH) *m/z* 503.32 ([$C_{30}H_{35}N_2O_5$]$^+$, calcd 503.25), 525.29 ([$C_{30}H_{34}N_2O_5Na$]$^+$, calcd 525.24).

2.1.4 fully protected *D*-Phe-*L*-Phe (4d)

A mixture of ***D***-phenylalanine methyl ester hydrochloride (1.23 g, 5.68 mmol, 1.0 eq), Cbz-protected ***L***-phenylalanine (1.70 g, 5.68 mmol, 1.0 eq), PyBOP (2.96 g, 5.68 mmol, 1.0 eq) and NMM (8.5 ml) was stirred in CH_2Cl_2 (30 ml) for 24 h at room temperature. After evaporation of the solvent, the resulting orange oil was dissolved in ethyl acetate (50 ml). The solution was then washed with 1M Na_2CO_3 (50 ml), 1M $NaHSO_4$ (50 ml), water (50 ml) and brine (50 ml). The organic phase was dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.68, SiO_2, ethyl acetate/ cyclohexane = 6/4) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 2.25 g (86%);

mp 145 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.56 (d, 1 H, *J* = 7.9 Hz, N*H*), 7.39 (d, 1 H, *J* = 8.8 Hz, N*H*), 7.17-7.35 (m, 15 H, CH_{aryl}), 4.92 (s, 2 H, CH_2), 4.54 (ddd, 1 H, C*H*), 4.25 (ddd, 1 H, C*H*), 3.64 (s, 3 H, CH_3), 3.07 (dd, 1 H, CH_2), 2.86 (dd, 1 H, CH_2), 2.64 (dd, 1 H, CH_2). 2.44 (m, 1 H, CH_2) ; ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.9, 171.5, 155.7, 138.0, 137.0, 136.9, 129.2, 129.1, 128.3, 128.2, 127.9, 127.6, 127.4, 126.6, 126.2, 65.1, 55.9, 53.3, 51.9, 37.6, 37.5, 37.0; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3282 [br], 1735 [m], 1691 [w], 1654 [m], 1536 [s], 1454 [w], 1369 [w], 1265 [w], 1240 [s], 1145 [s], 1054 [w], 842 [m], 752 [s], 698 [s]; ESI-MS (MeOH) *m/z* 461.21 ([$C_{27}H_{29}N_2O_5$]$^+$, calcd 461.21), 483.19 ([$C_{27}H_{28}N_2O_5Na$]$^+$, calcd 483.19).



R = Me: *D*-Phe-*D*-Phe, *D*-Phe-*L*-Phe

^{t}Bu: *L*-Phe-*L*-Phe, *L*-Phe-*D*-Phe

2.2 Deprotection of the Cbz-group (5a-d)

2.2.1 ^{t}Bu-*L*-Phe-*L*-Phe-NH_2 (5a)

Fully protected ***L***-Phe-***L***-Phe (800 mg, 1.59 mmol, 1 eq) was dissolved in $THF_{abs.}$ (40 ml). A suspension of Pd/C (80 mg) in $THF_{abs.}$ (10 ml) was added under nitrogen atmosphere. The mixture was stirred under H_2-atmosphere at room temperature until TLC control (R_f: 0.18, SiO_2, ethyl acetate/cyclohexane = 5/5 + 1% NEt_3)

showed no more starting material. The resulting black precipitate was filtrated over a celite pad and washed several times with $THF_{abs.}$. The solvent was evaporated and the slightly brown product was dried over silica gel orange in the desiccator. Yield: 568 mg (97%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.21 (d, 1 H, J = 8.0 Hz, N*H*), 7.13-7.29 (m, 10 H, CH_{aryl}), 4.43 (q, 1 H, C*H*), 3.39 (ddd, 1 H, C*H*), 2.88-3.00 (m, 1 H + 2 H, CH_2), 2.55-2.59 (m, 1 H, CH_2), 1.64 (s, 2 H, NH_2), 1.35 (s, 9 H, CH_3).

2.2.2 MeO-D-Phe-D-Phe-NH_2 (5b)

Fully protected ***D***-Phe-***D***-Phe (700 mg, 1.52 mmol, 1 eq) was dissolved in $THF_{abs.}$ (40 ml). A suspension of Pd/C (72 mg) in $THF_{abs.}$ (10 ml) was added under nitrogen atmosphere. The mixture was stirred under H_2-atmosphere at room temperature until TLC control (R_f: 0.08, SiO_2, ethyl acetate/cyclohexane = 5/5 + 1% NEt_3) showed no more starting material. The resulting black precipitate was filtrated over a celite pad and washed several times with $THF_{abs.}$. The solvent was evaporated and the slightly brown product was dried over silica gel orange in the desiccator. Yield: 360 mg (72%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.24 (d, 1 H, J = 7.7 Hz, N*H*), 7.12-7.30 (m, 10 H, CH_{aryl}), 4.55 (q, 1 H, C*H*), 3.60 (s, 3 H, CH_3), 3.37-3.41 (m, 1 H, C*H*), 2.84-3.04 (m, 1 H + 2 H, CH_2), 2.52-2.61 (m, 1 H, CH_2), 1.62 (s, 2 H, CH_2).

2.2.3 ^{t}Bu-***L***-Phe-***D***-Phe-NH_2 (5c)

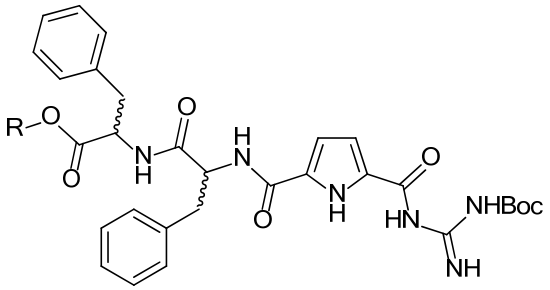
Fully protected ***L***-Phe-***D***-Phe (1.00 g, 1.99 mmol, 1 eq) was dissolved in $THF_{abs.}$ (40 ml). A suspension of Pd/C (90 mg) in $THF_{abs.}$ (10 ml) was added under nitrogen atmosphere. The mixture was stirred under H_2-atmosphere at room temperature until TLC control (R_f: 0.09, SiO_2, ethyl acetate/cyclohexane = 5/5 + 1% NEt_3) showed no more starting material. The resulting black precipitate was filtrated over a celite pad and washed several times with $THF_{abs.}$. The solvent was evaporated and the slightly brown product was dried over silica gel orange in the desiccator. Yield: 631 mg (86%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.19 (d, 1 H, J = 8.0 Hz, N*H*), 7.14-7.30 (m, 10 H, CH_{aryl}), 4.43 (q, 1 H, C*H*), 4.24 (ddd, 1 H, C*H*), 3.41-3.45 (m, 1 H, CH_2), 2.82-3.01 (m, 1 H + 2 H, CH_2), 1.63 (s, 2 H, NH_2), 1.33 (s, 9 H, CH_3).

2.2.4 MeO-***D***-Phe-***L***-Phe-NH_2 (5d)

Fully protected ***D***-Phe-***L***-Phe (826 mg, 1.79 mmol, 1 eq) was dissolved in $THF_{abs.}$ (40 ml). A suspension of Pd/C (85 mg) in $THF_{abs.}$ (10 ml) was added under nitrogen atmosphere. The mixture was stirred under H_2-atmosphere at room temperature until TLC control (R_f: 0.05, SiO_2, ethyl acetate/cyclohexane = 5/5 + 1% NEt_3) showed no more starting material. The resulting black precipitate was filtrated over a celite pad and washed several times with $THF_{abs.}$. The solvent was evaporated and the slightly brown product was dried over silica gel orange in the desiccator. Yield: 580 mg (99%).

^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.29 (d, 1 H, J = 7.2 Hz, N*H*), 7.17-7.30 (m, 10 H, CH_{aryl}), 4.52 (q, 1 H, C*H*), 3.61 (s, 3 H, CH_3), 3.37-3.42 (m, 1 H, C*H*), 2.75-3.06 (m, 1 H + 3 H, CH_2), 2.43-2.47 (m, 1 H, CH_2), 1.67 (s, 2 H, NH_2).



R = Me: *D*-Phe-*D*-Phe, *D*-Phe-*L*-Phe

^{t}Bu: *L*-Phe-*L*-Phe, *L*-Phe-*D*-Phe

2.3 Synthesis of the fully protected Phe-Phe-derived zwitterions 7a-d

2.3.1 Fully protected zwitterion 7a (*L*-Phe-*L*-Phe)

A mixture of the *N*-Boc-protected guanidinocarbonylpyrrol carboxylate triethyl ammonium salt [1] (530 mg, 1.33 mmol, 1.0 eq), PyBOP (697 mg, 1.33 mmol, 1.0 eq), NMM (2.0 ml) and the dipeptide **5a** (541 mg, 1.47 mmol, 1.1 eq) was stirred in CH_2Cl_2/DMF (10/1, 50 ml) for 24 h at room temperature. The resulting yellow solution was hydrolized with water (20 ml). The suspension was then extracted with ethyl acetate (4 x 50 ml), the organic phases were combined, dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.76, SiO_2, ethyl acetate/cyclohexane = 7/3) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 726 mg (84%);

mp 122 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.41 (br.s, 1 H, N*H*), 10.84 (br.s, 1 H, N*H*), 9.31 (br.s, 1 H, N*H*), 8.48-8.60 (m, 3 H, N*H*), 7.12-7.33 (m, 10 H, CH_{aryl}), 6.77 (s, 2 H, pyrrole-C*H*), 4.77 (ddd, 1 H, C*H*), 4.39 (q, 1 H, C*H*), 2.81-3.12 (m, 4 H, CH_2), 1.45 (s, 9 H, CH_3), 1.32 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.5, 170.3, 159.2, 158.4, 138.1, 137.1, 129.2, 129.1, 128.2, 128.0, 126.5, 126.2, 112.6, 80.7, 54.3, 53.7, 37.4, 36.8, 27.8, 27.5; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3260 [br], 2990 [w], 2780 [w], 1755 [s], 1720 [s], 1530 [s], 1270 [s], 1140 [s], 700 [s]; ESI-MS (DMSO) *m*/*z* 647.33 ([$C_{34}H_{43}N_6O_7$]$^+$, calcd 647.32), 669.31 ([$C_{34}H_{42}N_6O_7Na$]$^+$, calcd 669.30).

2.3.2 Fully protected zwitterion 7b (*D*-Phe-*D*-Phe)

A mixture of the *N*-Boc-protected guanidinocarbonylpyrrol carboxylate triethyl ammonium salt [1] (398 mg, 1.00 mmol, 1.0 eq), PyBOP (520 mg, 1.00 mmol, 1.0 eq), NMM (2.0 ml) and the dipeptide **5b** (360 mg, 1.10 mmol, 1.1 eq) was stirred in CH_2Cl_2/DMF (10/1, 50 ml) for 24 h at room temperature. The resulting yellow solution was hydrolized with water (20 ml). The suspension was then extracted with ethyl acetate (4 x 50 ml), the organic phases were combined, dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.63, SiO_2, ethyl acetate/cyclohexane = 7/3) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 330 mg (55%);

[1] Schmuck, C.; Bickert, V; Merschky, M.; Geiger, L.; Rupprecht, D.; Dudaczek, J.; Wich, P.; Rehm, T.; Machon, U. *Eur. J. Org. Chem.* **2008**, 324.

mp 142 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.33 (br.s, 1 H, N*H*), 10.84 (br.s, 1 H, N*H*), 9.34 (br.s, 1 H, N*H*), 8.54-8.59 (m, 3 H, N*H*), 7.13-7.31 (m, 10 H, CH_{aryl}), 6.77 (s, 2 H, pyrrole-C*H*), 4.76 (ddd, 1 H, C*H*), 4.52 (ddd, 1 H, C*H*), 3.59 (s, 3 H, CH_3), 2.79-3.10 (m, 4 H, CH_2), 1.45 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.7, 171.5, 159.3, 158.4, 138.0, 137.0, 129.0, 128.2, 128.0, 126.6, 126.2, 112.6, 53.7, 51.9, 37.3, 36.6, 27.8, 22.4; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 1632 [s], 1540 [s], 1241 [s], 755 [m], 700 [s], 626 [m]; ESI-MS (DMSO) *m*/*z* 605.28 ([$C_{31}H_{37}N_6O_7$]$^+$, calcd 605.27), 627.27 ([$C_{31}H_{36}N_6O_7Na$]$^+$, calcd 627.25).

2.3.3 Fully protected zwitterion 7c (*L*-Phe-*D*-Phe)

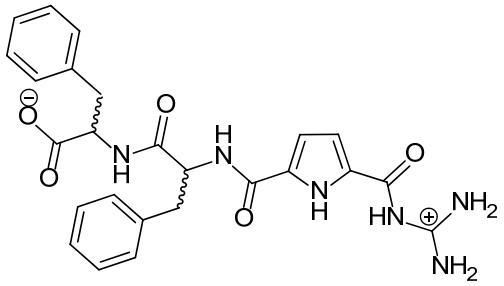
A mixture of the *N*-Boc-protected guanidinocarbonylpyrrol carboxylate triethyl ammonium salt **1** (521 mg, 1.31 mmol, 1.0 eq), PyBOP (681 mg, 1.31 mmol, 1.0 eq), NMM (2.0 ml) and the dipeptide **5c** (539 mg, 1.44 mmol, 1.1 eq) was stirred in CH_2Cl_2/DMF (10/1, 50 ml) for 24 h at room temperature. The resulting yellow solution was hydrolized with water (20 ml). The suspension was then extracted with ethyl acetate (4 x 50 ml), the organic phases were combined, dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.44, SiO_2, ethyl acetate/cyclohexane = 5/5) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 518 mg (57%);

mp 132 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.45 (br.s, 1 H, N*H*), 10.82 (br.s, 1 H, N*H*), 9.33 (br.s, 1 H, N*H*), 8.51-8.54 (m, 3 H, N*H*), 7.10-7.23 (m, 10 H, CH_{aryl}), 6.77 (s, 2 H, pyrrole-C*H*), 4.74 (ddd, 1 H, C*H*), 4.42 (ddd, 1 H, C*H*), 2.61-3.05 (m, 4 H, CH_2), 1.45 (s, 9 H, CH_3), 1.35 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.2, 120.5, 159.2, 158.4, 138.0, 137.2, 129.3, 129.1, 128.1, 128.0, 126.5, 126.2, 113.7, 112.7, 80.9, 53.9, 37.7, 37.1, 27.8, 27.5; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3275 [br], 2863 [w], 1727 [m], 1629 [s], 1540 [s], 1455 [w], 1394 [w], 1369 [w], 1295 [m], 1241 [s], 1149 [s], 1047 [w], 842 [s], 754 [m], 700 [s]; ESI-MS (DMSO) *m*/*z* 647.33 ([$C_{34}H_{43}N_6O_7$]$^+$, calcd 647.32), 669.31 ([$C_{34}H_{42}N_6O_7Na$]$^+$, calcd 669.30).

2.3.4 Fully protected zwitterion 7d (*D*-Phe-*L*-Phe)

A mixture of the *N*-Boc-protected guanidinocarbonylpyrrol carboxylate triethyl ammonium salt **1** (533 mg, 1.34 mmol, 1.0 eq), PyBOP (699 mg, 1.34 mmol, 1.0 eq), NMM (2.0 ml) and the dipeptide **5d** (480 mg, 1.47 mmol, 1.1 eq) was stirred in CH_2Cl_2/DMF (10/1, 50 ml) for 24 h at room temperature. The resulting yellow solution was hydrolized with water (20 ml). The suspension was then extracted with ethyl acetate (4 x 50 ml), the organic phases were combined, dried with magnesium sulphate and evaporated. The resulting orange oil was purified by column chromatography (R_f = 0.59, SiO_2, ethyl acetate/cyclohexane = 7/3) yielding a colourless oil which was dissolved in a mixture of methanol and water. After lyophilisation the pure product was a colourless powder. Yield 550 mg (68%);

mp 143 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.21 (br.s, 1 H, N*H*), 10.82 (br.s, 1 H, N*H*), 9.33 (br.s, 1 H, N*H*), 8.50-8.70 (m, 3 H, N*H*), 7.12-7.24 (m, 10 H, CH_{aryl}), 6.76 (s, 2 H, pyrrole-C*H*), 4.72 (ddd, 1 H, C*H*), 4.56 (ddd, 1 H, C*H*), 3.63 (s, 3 H, CH_3), 2.57-3.11 (m, 4 H, CH_2), 1.45 (s, 9 H, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 171.9, 171.3, 159.2, 158.4, 138.0, 137.1, 129.2, 129.1, 128.2, 128.0, 126.6, 126.2, 113.6, 112.7, 53.9, 52.0, 37.6, 37.0, 27.7; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 1725 [m], 1630 [m], 1533 [m], 1238 [s], 1147 [s], 1029 [w], 845 [m], 698 [w]; ESI-MS (DMSO) *m*/*z* 605.28 ([$C_{31}H_{37}N_6O_7$]$^+$, calcd 605.27), 627.27 ([$C_{31}H_{36}N_6O_7Na$]$^+$, calcd 627.25).



2.4 Deprotection of the Boc group and ester functionality, zwitterions 1a-d

2.4.1 Zwitterion 1a (L-Phe-L-Phe)

A solution of fully protected precursor **7a** (600 mg, 0.93 mmol, 1.0 eq) in TFA (5 ml) was stirred at room temperature until TLC control (SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% triethylamine and SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% TFA) showed no more starting material. The TFA was evaporated *in vacuo* to give a slightly brown solid, which was dissolved in water (30 ml). The zwitterion was produced by adjusting the pH to a value of 5.8 with 1 N aqueous sodium hydroxide. The colourless precipitate was filtered and washed several times with pure water and diethyl ether. The pure zwitterionic product was dried over phosphorous pentoxide in the desiccator. Yield 315 mg (70%);

mp 252 °C (decomposition); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.45 (s, 1 H, N*H*), 12.42 (s, 1 H, N*H*), 10.07 (s, 2 H, N*H*), 8.93 (d, 1 H, J = 7.1 Hz, N*H*), 8.64 (d, 1 H, J = 8.2 Hz, N*H*), 8.00 (s, 2 H, N*H*), 6.77-7.22 (m, 2 H + 10 H, pyrrole-C*H* + CH_{aryl}), 5.44 (s, 1 H, C*H*), 4.39 (ddd, 1 H, C*H*), 2.75-2.93 (m, 4 H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 176.4, 170.2, 160.3, 159.1, 156.3, 137.7, 137.5, 137.1, 129.2, 131.1, 129.2, 128.9, 127.5, 127.3, 125.7, 118.0, 111.2, 55.3, 54.1, 39.8, 38.2; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3301 [br], 2989 [m], 2956 [m], 1633 [s], 1536 [s], 1241 [s], 1145 [m], 1055 [w], 964 [m], 838 [w], 750 [s], 698 [s]; ESI-MS (DMSO) m/z 491.21 ([$C_{25}H_{27}N_6O_5$]$^+$, calcd 491.20), 513.19 ([$C_{25}H_{26}N_6O_5Na$]$^+$, calcd 513.19).

2.4.2 Zwitterion 1b (D-Phe-D-Phe)

A solution of fully protected precursor **7b** (250 mg, 0.41 mmol, 1.0 eq) in TFA (5 ml) was stirred at room temperature until TLC control (SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% TFA) showed no more starting material. The TFA was evaporated *in vacuo* to give a slightly brown oil, which was dissolved in a mixture of THF and water (30 ml, 4/1). After addition of lithium hydroxide monohydrate (168 mg, 4.10 mmol, 10 eq), the reaction mixture was stirred until TLC control (ethyl acetate/cyclohexane = 7/3 + 1% NEt_3) showed no more starting material. After evaporation of the organic solvent, water (20 ml) was added. The zwitterion was produced by adjusting the pH to a value of 5.8 with 1 N aqueous hydrochloric acid. The colourless precipitate was filtered and washed several times with pure water and diethyl ether. The pure zwitterionic product was dried over phosphorous pentoxide in the desiccator. Yield 190 mg (95%);

mp 252 °C (decomposition); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 14.45 (s, 1 H, N*H*), 12.42 (s, 1 H, N*H*), 10.07 (s, 2 H, N*H*), 8.93 (d, 1 H, J = 7.1 Hz, N*H*), 8.64 (d, 1 H, J = 8.2 Hz, N*H*), 8.00 (s, 2 H, N*H*), 6.77-7.22 (m, 2 H + 10 H, pyrrole-C*H* + CH_{aryl}), 5.44 (s, 1 H, C*H*), 4.39 (ddd, 1 H, C*H*), 2.75-2.93 (m, 4 H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 176.4, 170.2, 160.3, 159.1, 156.3, 137.7, 137.5, 137.1, 129.2, 131.1, 129.2, 128.9, 127.5,

127.3, 125.7, 118.0, 111.2, 55.3, 54.1, 39.8, 38.2; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 3301 [br], 2989 [m], 2956 [m], 1633 [s], 1536 [s], 1241 [s], 1145 [m], 1055 [w], 964 [m], 838 [w], 750 [s], 698 [s]; ESI-MS (DMSO) *m*/*z* 491.21 ($[C_{25}H_{27}N_6O_5]^+$, calcd 491.20), 513.19 ($[C_{25}H_{26}N_6O_5Na]^+$, calcd 513.19).

2.4.3 Zwitterion 1c (L-Phe-D-Phe)

A solution of fully protected precursor **7c** (400 mg, 0.62 mmol, 1.0 eq) in TFA (5 ml) was stirred at room temperature until TLC control (SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% triethylamine and SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% TFA) showed no more starting material. The TFA was evaporated *in vacuo* to give a slightly brown solid, which was dissolved in water (30 ml). The zwitterion was produced by adjusting the pH to a value of 5.8 with 1 N aqueous sodium hydroxide. The colourless precipitate was filtered and washed several times with pure water and diethyl ether. The pure zwitterionic product was dried over phosphorous pentoxide in the desiccator. Yield 247 mg (81%);

mp 282 °C (decomposition); ^{1}H-NMR (300 MHz, DMSO-d_6, 30 mM) δ 13.48 (br.s, 1 H, N*H*), 11.99 (s, 1 H, N*H*), 9.60 (br.s, 2 H, N*H*), 8.73 (d, 1 H, *J* = 8.9 Hz, N*H*), 8.44 (d, 1 H, *J* = 8.5 Hz, N*H*), 7.80 (br.s, 2 H, N*H*), 6.83-7.25 (m, 2 H + 10 H, pyrrole-C*H* + CH_{aryl}), 5.44 (br.s, 1 H, C*H*), 4.53 (ddd, 1 H, C*H*), 2.72-2.89 (m, 4 H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6, 30 mM) δ 175.5, 169.3, 158.9, 137.7, 137.5, 130.0, 129.2, 129.0, 128.0, 127.4, 126.3, 125.8, 116.7, 111.8, 54.6, 53.1, 39.8, 38.3; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 2994 [w], 1628 [s], 1537 [s], 1394 [m], 1241 [s], 1146 [m], 838 [m], 752 [s], 698 [s]; ESI-MS (DMSO) *m*/*z* 491.21 ($[C_{25}H_{27}N_6O_5]^+$, calcd 491.20), 513.19 ($[C_{25}H_{26}N_6O_5Na]^+$, calcd 513.19).

2.4.4 Zwitterion 1d (D-Phe-L-Phe)

A solution of fully protected precursor **7d** (400 mg, 0.66 mmol, 1.0 eq) in TFA (5 ml) was stirred at room temperature until TLC control (SiO_2, ethyl acetate/cyclohexane = 7/3 + 1% TFA) showed no more starting material. The TFA was evaporated *in vacuo* to give a slightly brown oil, which was dissolved in a mixture of THF and water (30 ml, 4/1). After addition of lithium hydroxide monohydrate (270 mg, 6.60 mmol, 10 eq), the reaction mixture was stirred until TLC control (ethyl acetate/cyclohexane = 7/3 + 1% NEt_3) showed no more starting material. After evaporation of the organic solvent, water (20 ml) was added. The zwitterion was produced by adjusting the pH to a value of 5.8 with 1 N aqueous hydrochloric acid. The colourless precipitate was filtered and washed several times with pure water and diethyl ether. The pure zwitterionic product was dried over phosphorous pentoxide in the desiccator. Yield 266 mg (82%);

mp 282 °C (decomposition); ^{1}H-NMR (300 MHz, DMSO-d_6, 30 mM) δ 13.48 (br.s, 1 H, N*H*), 11.99 (s, 1 H, N*H*), 9.60 (br.s, 2 H, N*H*), 8.73 (d, 1 H, *J* = 8.9 Hz, N*H*), 8.44 (d, 1 H, *J* = 8.5 Hz, N*H*), 7.80 (br.s, 2 H, N*H*), 6.83-7.25 (m, 2 H + 10 H, pyrrole-C*H* + CH_{aryl}), 5.44 (br.s, 1 H, C*H*), 4.53 (ddd, 1 H, C*H*), 2.72-2.89 (m, 4 H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6, 30 mM) δ 175.5, 169.3, 158.9, 137.7, 137.5, 130.0, 129.2, 129.0, 128.0, 127.4, 126.3, 125.8, 116.7, 111.8, 54.6, 53.1, 39.8, 38.3; FT-IR $\tilde{\nu}$ (ATR) [cm^{-1}] 2994 [w], 1628 [s], 1537 [s], 1394 [m], 1241 [s], 1146 [m], 838 [m], 752 [s], 698 [s]; ESI-MS (DMSO) *m*/*z* 491.21 ($[C_{25}H_{27}N_6O_5]^+$, calcd 491.20), 513.19 ($[C_{25}H_{26}N_6O_5Na]^+$, calcd 513.19).

3 NMR experiments

3.1 NMR dilution studies

Solutions of **1d** with varying concentrations (0.5 to 100 mM) were obtained by diluting aliquots of a concentrated stock solution in DMSO-d_6 to a total volume of 0.60 ml. The chemical shifts of the guanidinio amide NH signal and the pyrrole NH signal were recorded for each sample relative to the deuterated solvent.

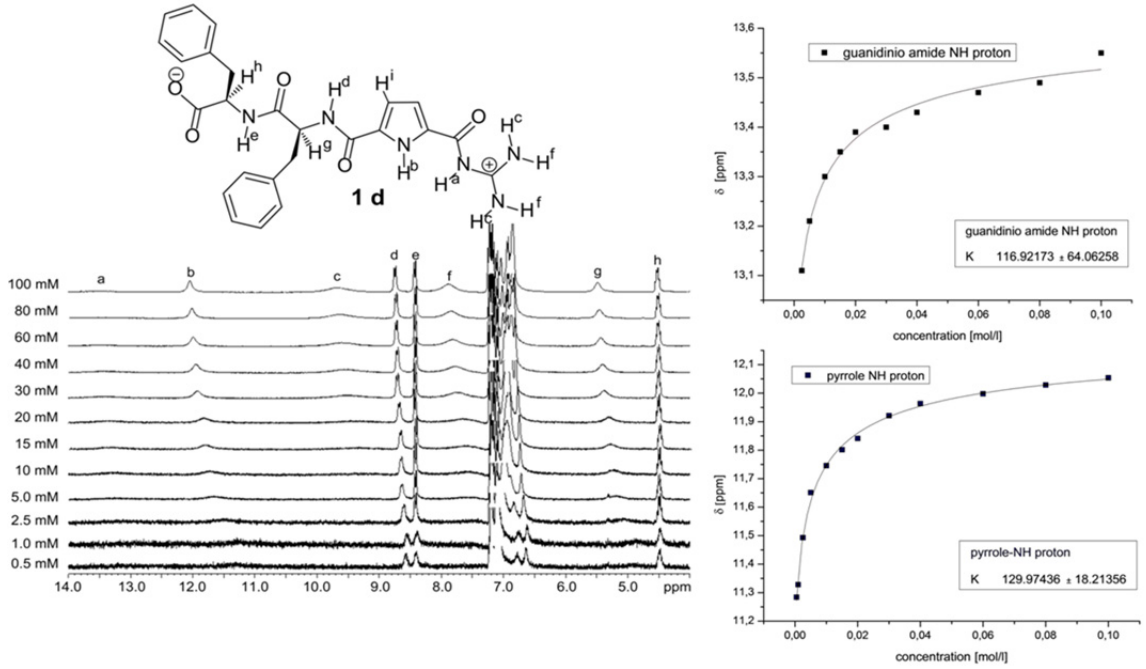


Figure S1: NMR dilution study of **1d** in DMSO-d_6 (300 MHz) and concentration dependent shift of guanidinio amide NH proton *a* and pyrrole NH proton *b*.

Solutions of **1a** with varying concentrations (0.1 to 1 mM) were obtained by diluting aliquots of a concentrated stock solution in DMSO-d_6 to a total volume of 0.60 ml.

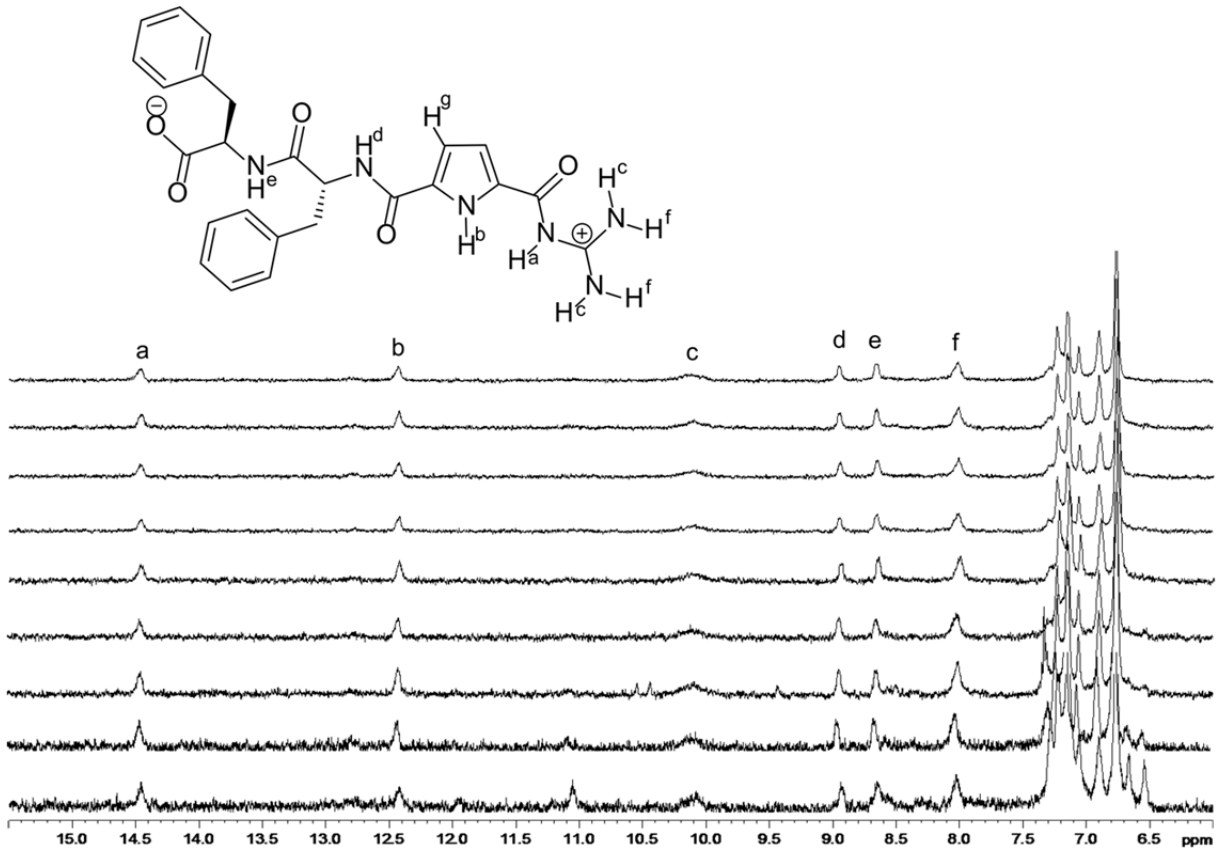


Figure S2: NMR dilution study of **1a** in DMSO-d_6 (0.10 to 1.00 mM, 500 MHz).

3.2 ^{1}H/^{1}H-NOESY

Freshly prepared samples of **1a** and **1d** in DMSO-d_6 (25 mM) were used for the NOESY experiments.

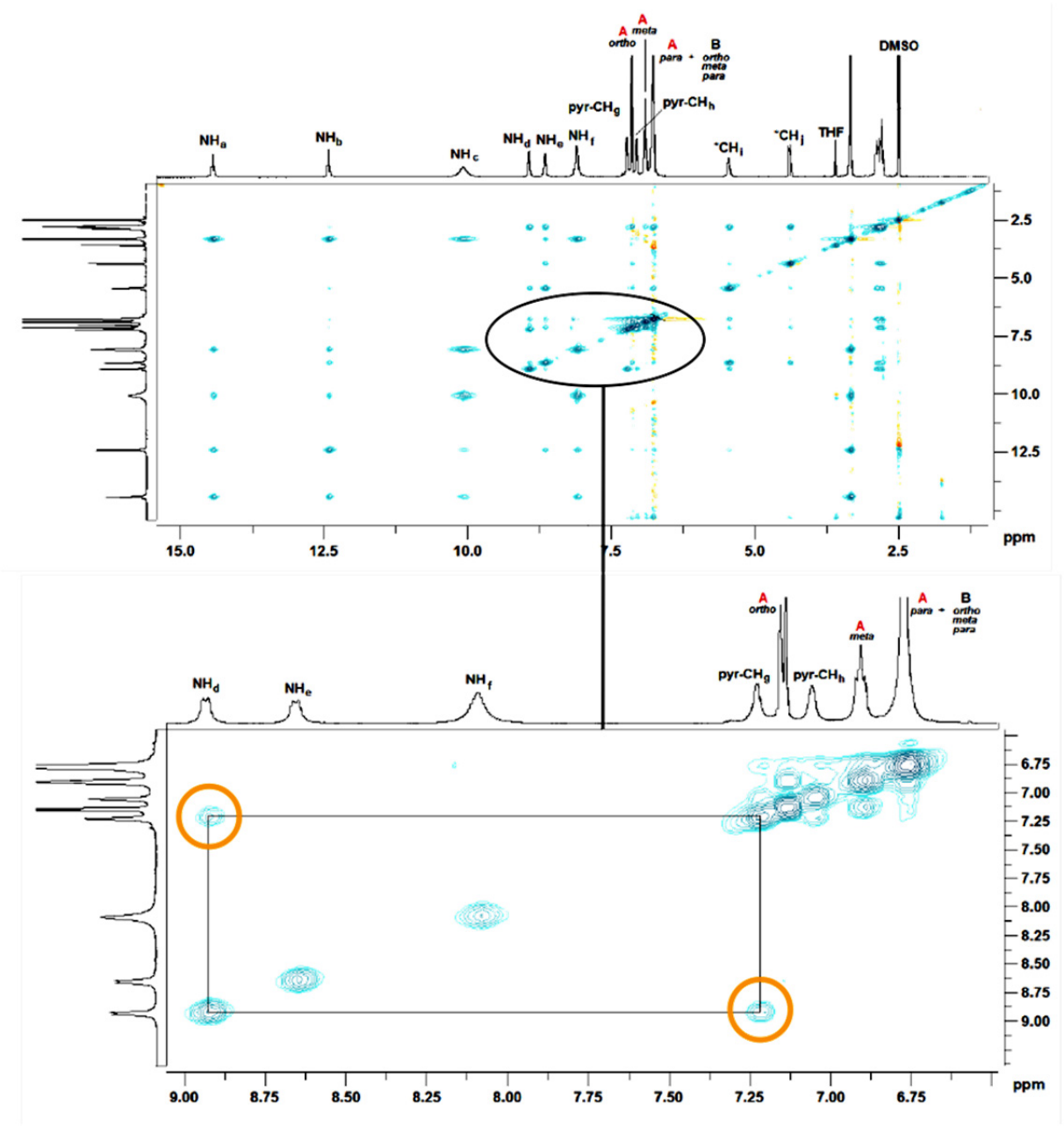


Figure S3: NOESY spectrum of **1a** in DMSO-d_6 (25 mM) with the NOE-contact (orange circles).

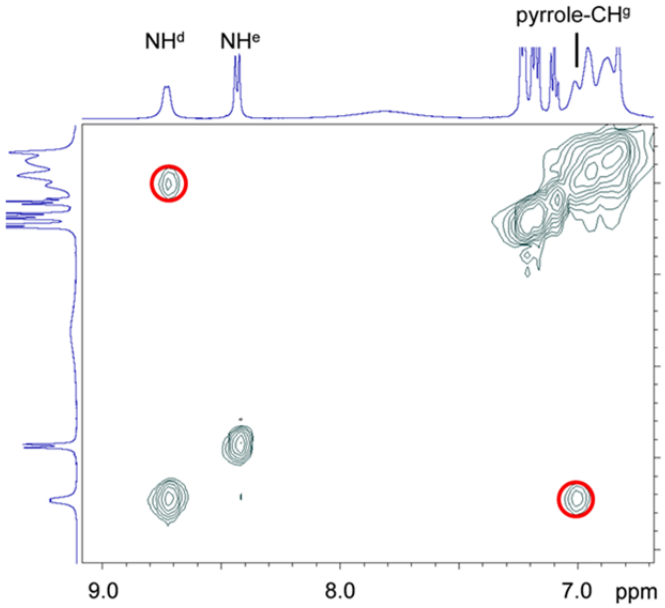


Figure S4: Enhanced part of the NOESY spectrum of zwitterion **1d** in DMSO-d_6 (25 mM) with the NOE-contact (red circles).

3.3 DOSY

For the DOSY experiments freshly prepared samples of **1a** (50 mM), **1a•HCl** (50 mM) and **1d** (30 mM) in DMSO-d_6 were used.

Table S1: Signal dependent diffusion coefficients D of **1a**, **1a•HCl** and **1d**

compound **1a**

δ [ppm]	$D \cdot 10^{-10}$ [m²/s]
14.453	1.217
12.424	1.176
10.073	1.183
8.928	1.196
8.645	1.212
8.002	1.230
7.050	1.205
5.442	1.205
4.387	1.239

compound **1a•HCl**

δ [ppm]	$D \cdot 10^{-10}$ [m²/s]
12.766	1.411
12.432	1.418
11.927	1.408
8.550	1.436
7.460	1.386
7.245	1.382
6.833	1.410
4.776	1.403
4.465	1.382
2.930	1.432

compound **1d**

δ [ppm]	$D \cdot 10^{-10}$ [m²/s]
11.988	1.283
8.738	1.297
8.432	1.299
7.095	1.163
4.518	1.296

3.4 H/D exchange experiments

For the H/D exchange experiments freshly prepared samples of **1b** (10 mM) and **1d** (10 mM) in DMSO-d_6 were used. After addition of 10 µl D_2O, time dependent NMR spectra were recorded.

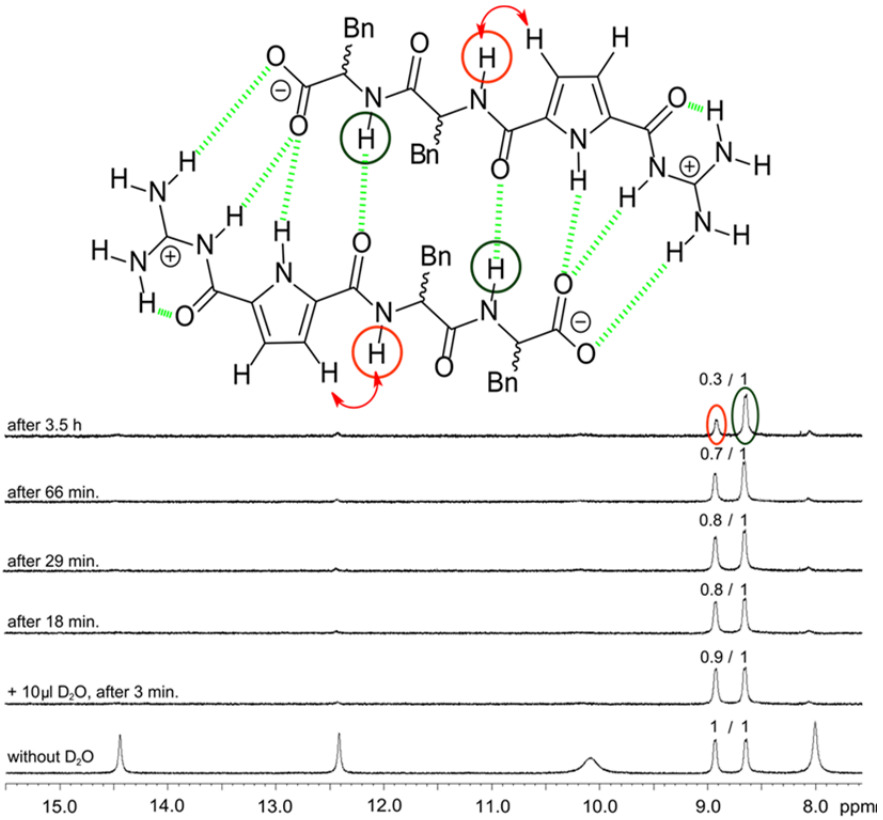


Figure S5: H/D exchange experiment of **1b** (10 mM) in DMSO-d_6.

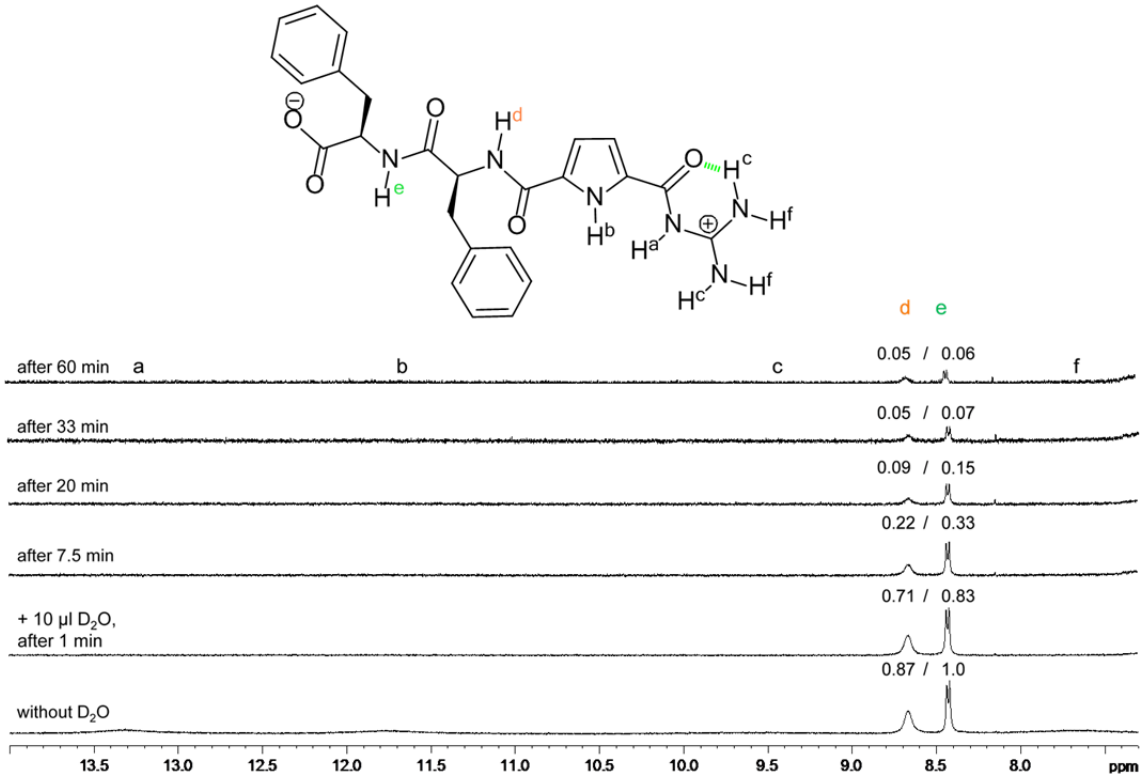


Figure S6: H/D exchange experiment of **1d** (10 mM) in DMSO-d_6.

3.5 NMR spectra of different mixtures of stereoisomers

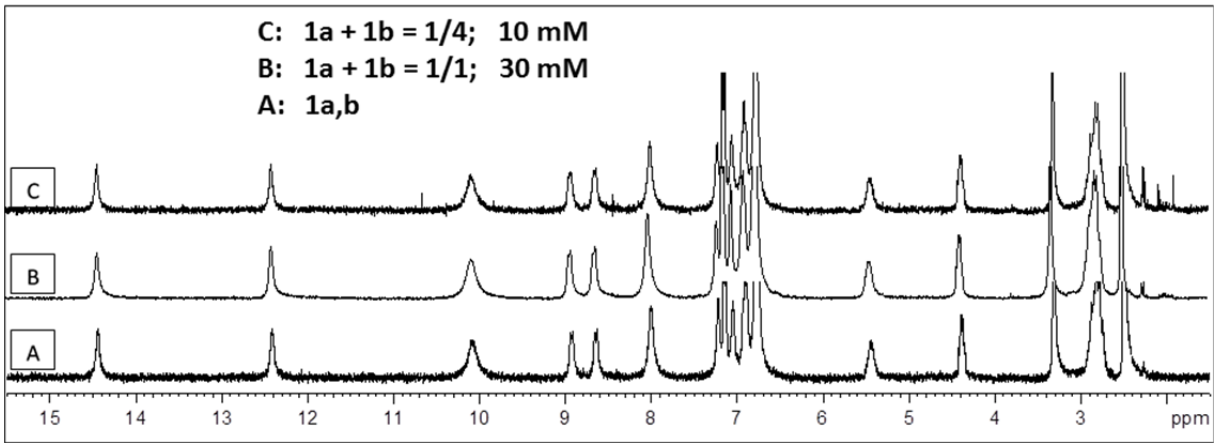


Figure S7: Mixture of the enantiomers **1a** and **1b** in DMSO-d_6.

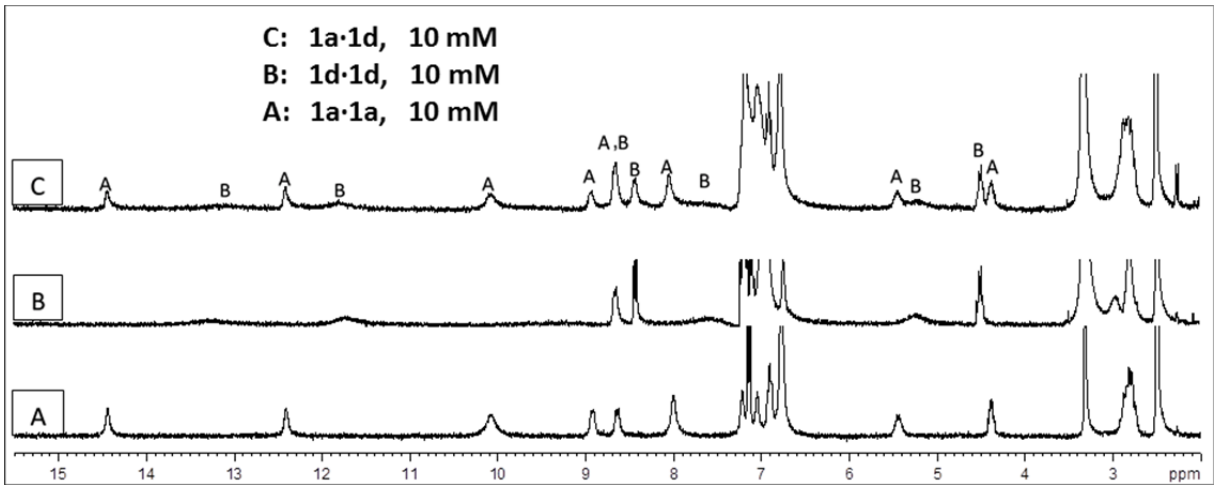


Figure S8: Mixture of the two diastereomers **1a** and **1d** in DMSO-d_6.

4 UV/Vis

Solutions of **1b** with varying concentrations (0.005 to 0.11 mM) were obtained by diluting aliquots of a concentrated stock solution in DMSO-d_6 to a total volume of 1.0 ml.

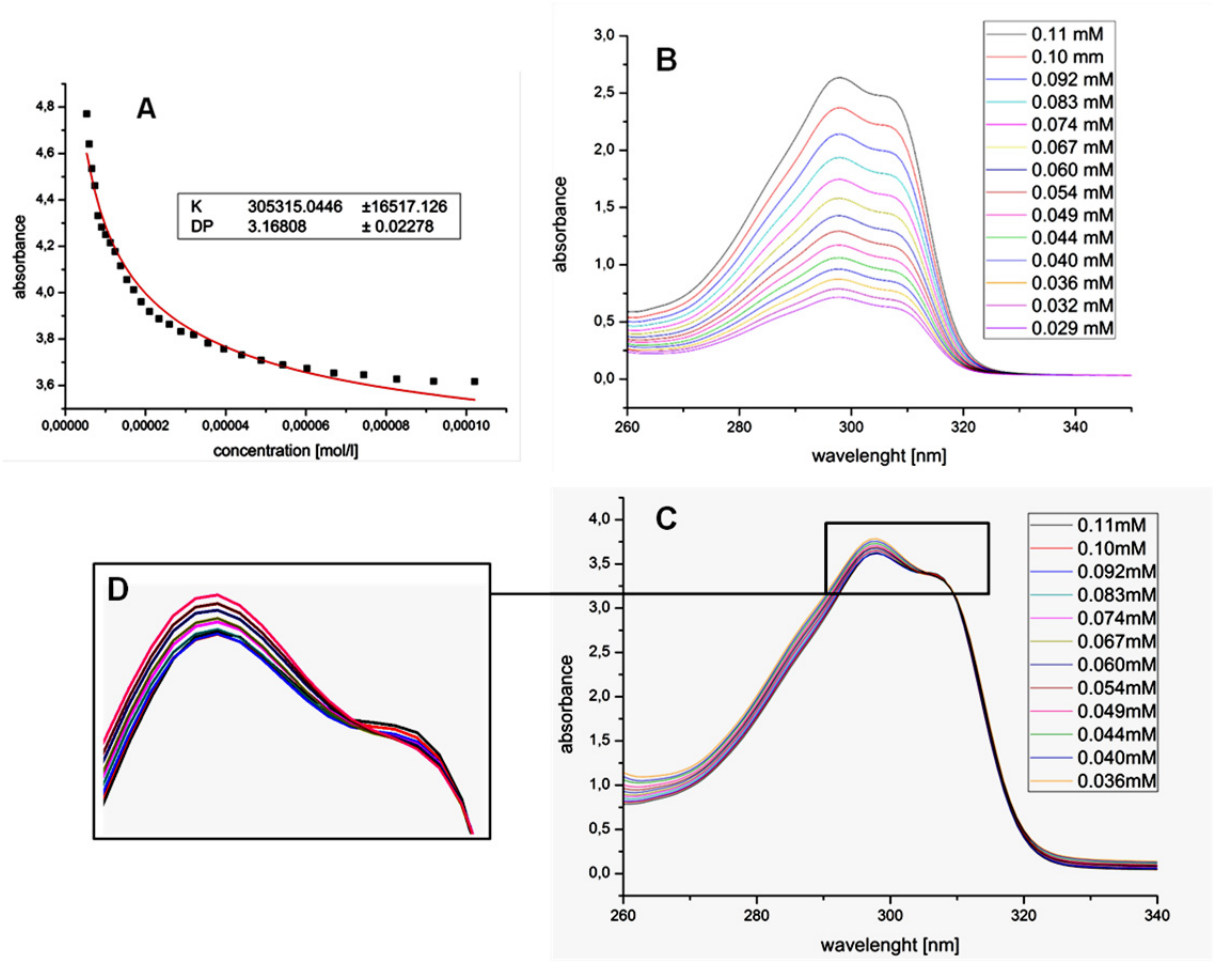


Figure S9: UV/Vis spectra of **1b** in DMSO-d_6. Quantitative analysis of the concentration dependent UV change **A**, UV dilution study **B**, dilution effect normalized UV spectra **C** and extended part of the dilution effect normalized UV spectra **D**.

5 $^{1}H/^{13}C$-NMR spectra of 1a-d

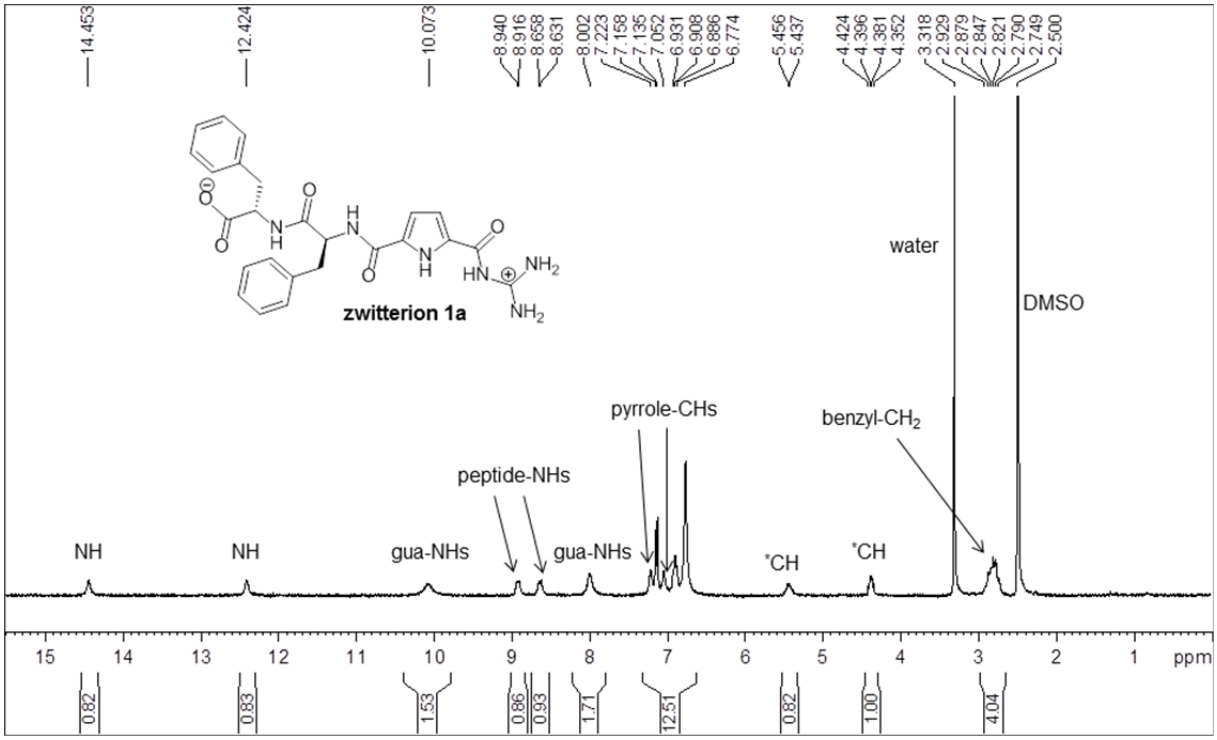


Figure S10: ^{1}H-NMR spectrum of zwitterion **1a** in DMSO-d_6 (300 MHz).

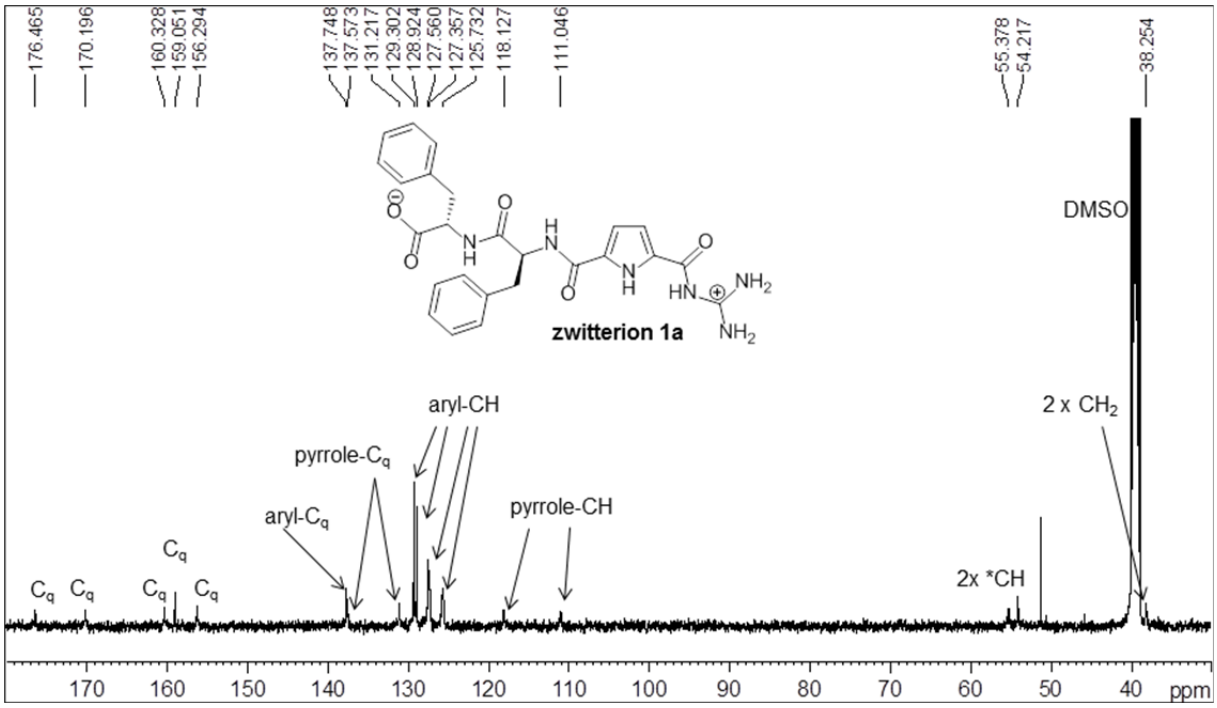


Figure S11: ^{13}C-NMR spectrum of zwitterion **1a** in DMSO-d_6 (125 MHz).

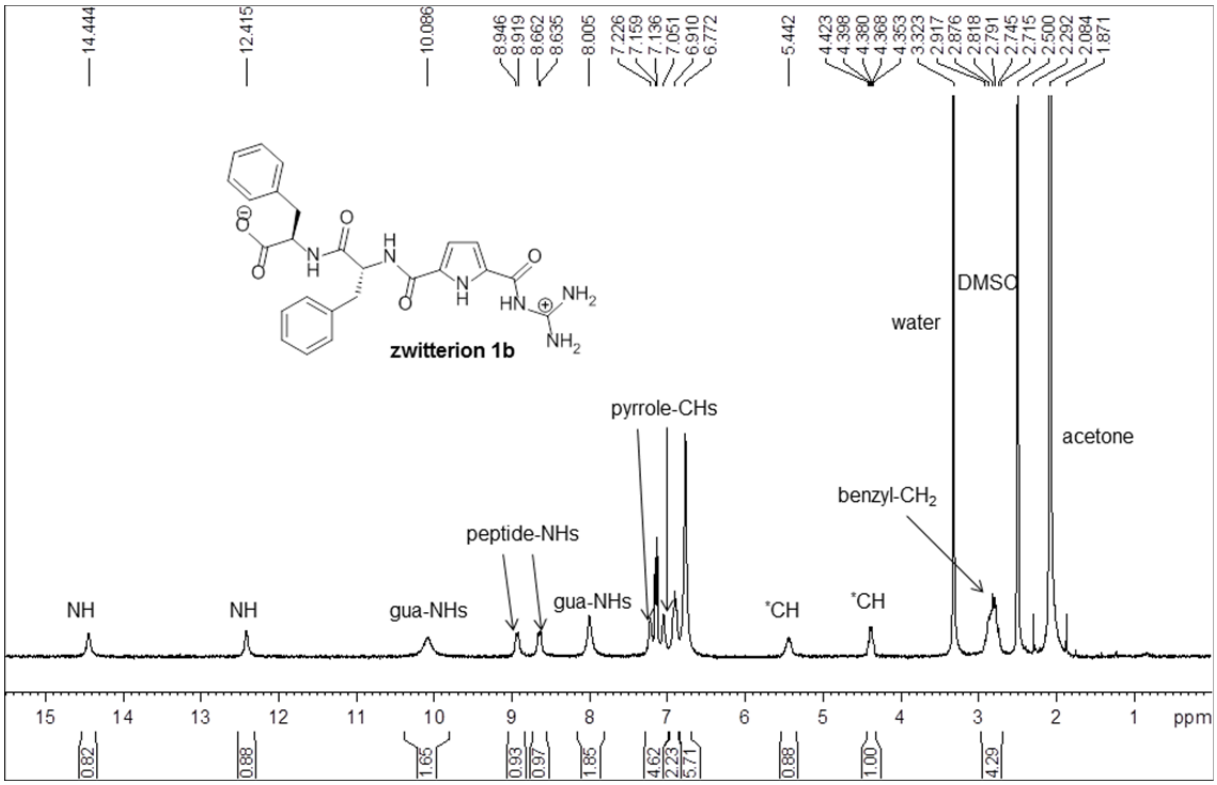


Figure S12: ^{1}H-NMR spectrum of zwitterion **1b** in DMSO-d_6 (300 MHz).

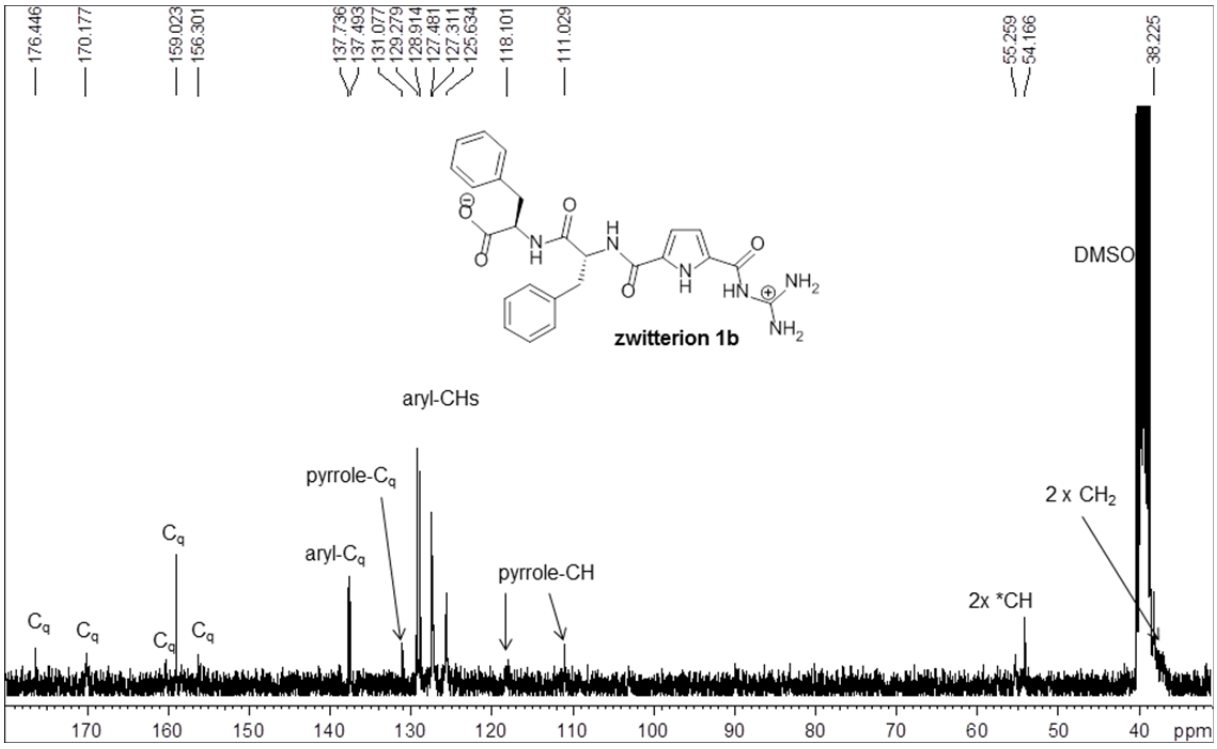


Figure S13: ^{13}C-NMR spectrum of zwitterion **1b** in DMSO-d_6 (75 MHz).

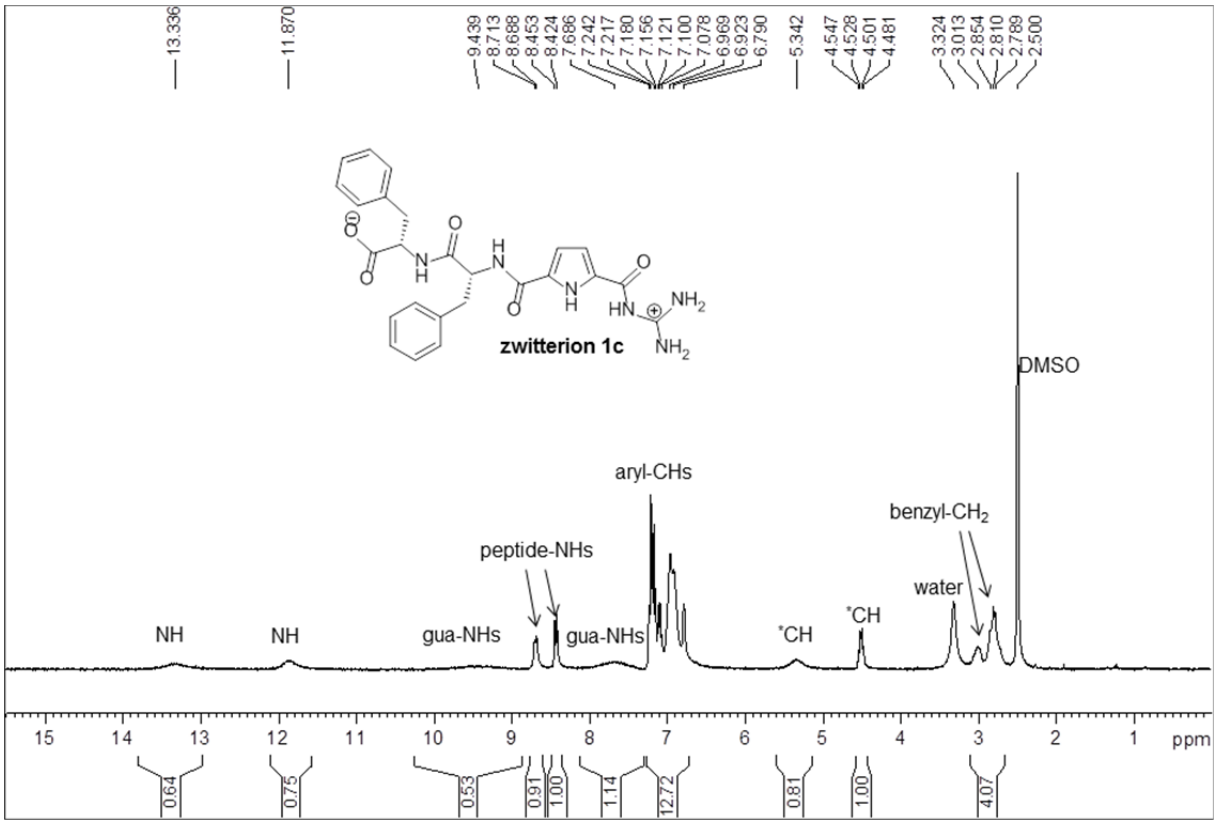


Figure S14: ^{1}H-NMR spectrum of zwitterion **1c** in DMSO-d_6 (300 MHz, 20 mM).

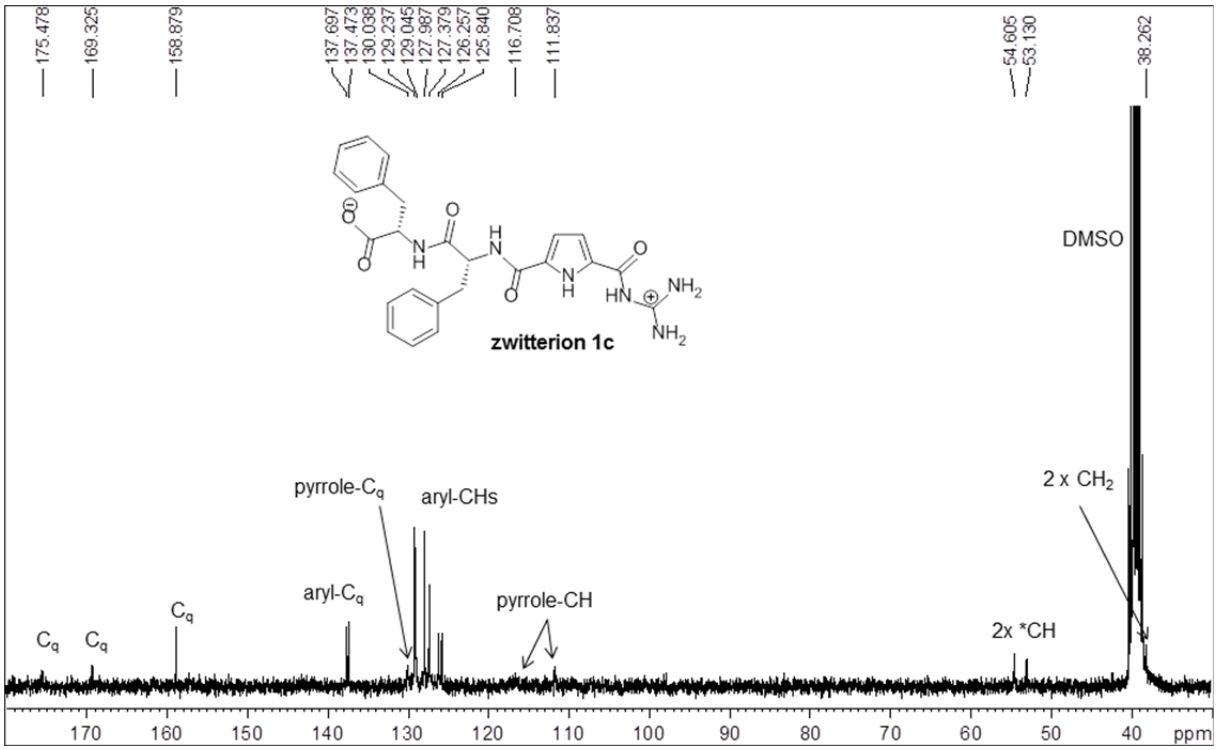


Figure S15: ^{13}C-NMR spectrum of zwitterion **1c** in DMSO-d_6 (75 MHz, 20 mM).

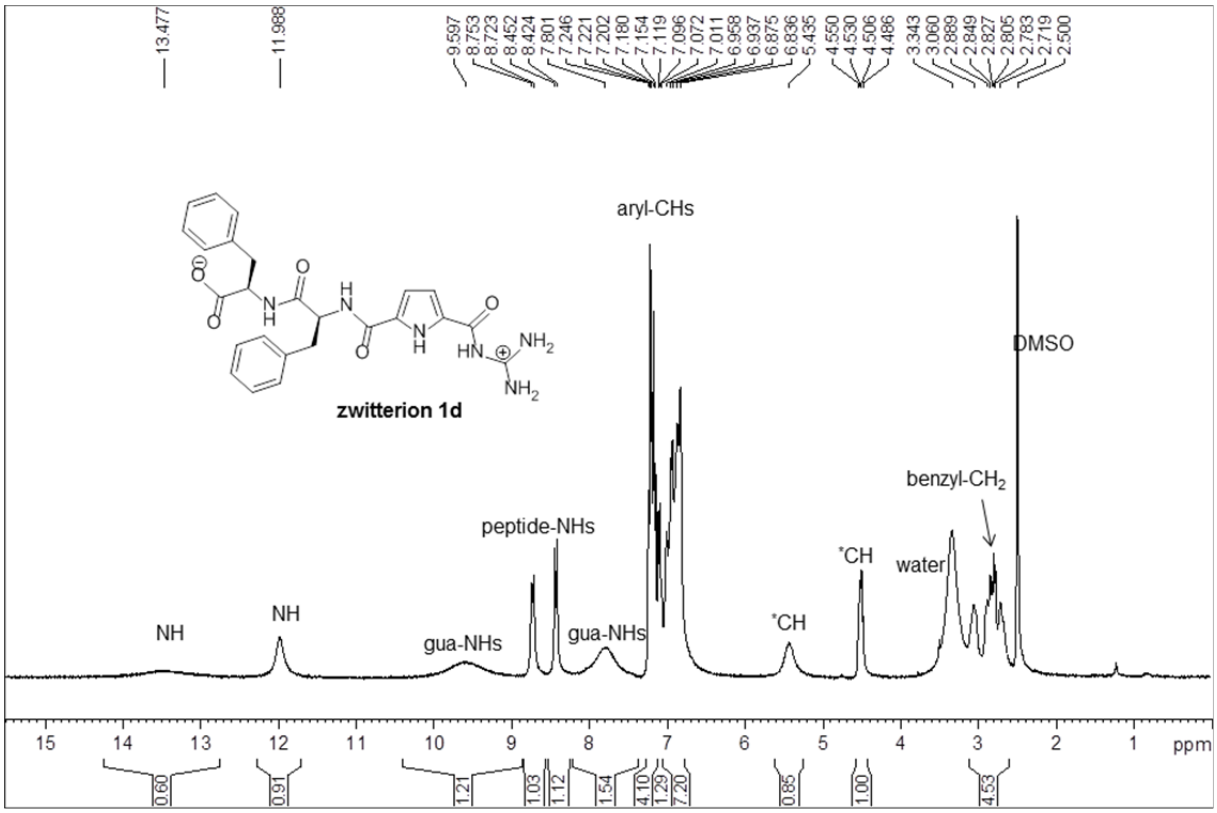


Figure S16: ^{1}H-NMR spectrum of zwitterion **1d** in DMSO-d_6 (300 MHz, 50 mM).

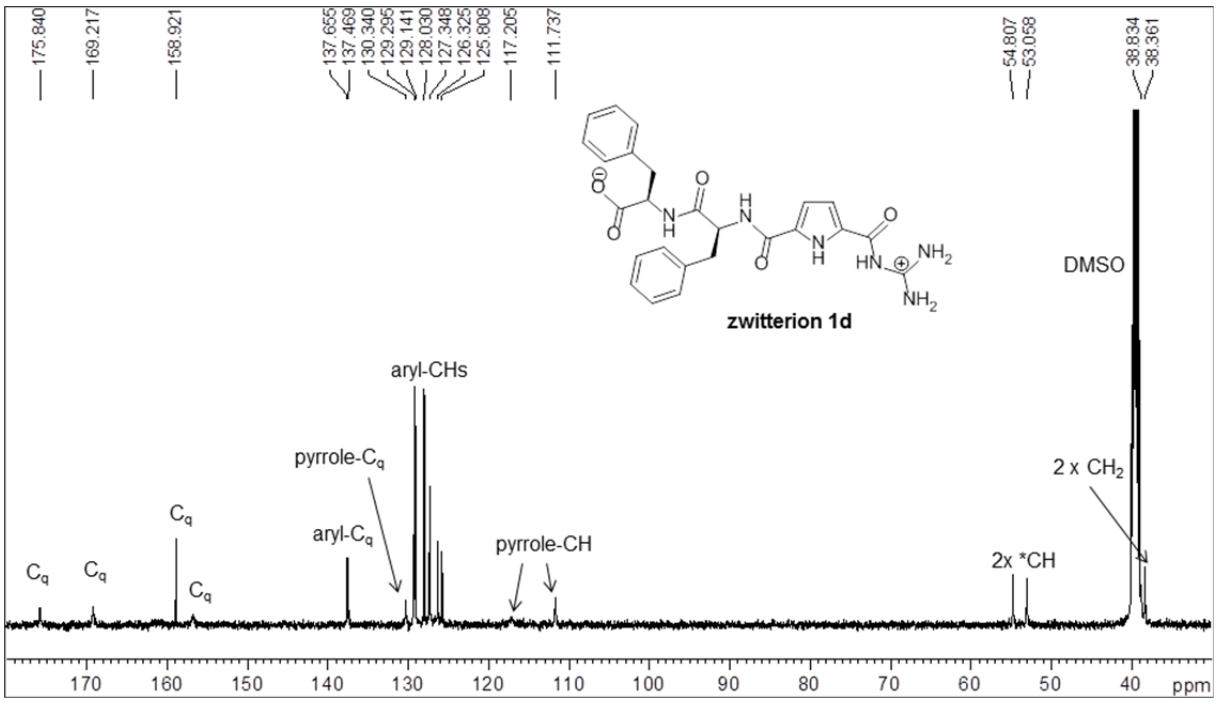


Figure S17: ^{13}C-NMR spectrum of zwitterion 1**d** in DMSO-d_6 (125 MHz, 50 mM).

6 DFT calculations of the two dimers 1a•1a and 1c•1c

Density Functional Theory (DFT) optimizations where performed on the optimized MacroModel conformations (OPLS force field, DMSO solvation model, MC conformational search with 50.000 steps; the resulting minimum was found several times during the conformational search) using Gaussian09 program suite. The functional used was UPBE1PBE, acronym = PBE0, in conjunction with the Pople style 6-31G* basis set. The solvation environment has been taken into account by the SCRF-PCM method (Self Consistent Reaction Field Polarizable Continuum Model). DMSO has been chosen as dielectricum (dielectrical constant ε=46.826) First the extended monomers were calculated and then these structures were relaxed to the optimized ground state conformation of the monomer to obtain information on the charge interaction within the monomer. The energy for dimerization was calculated using the geometry optimized structures of both the monomers and the dimers. Results are given in Hartree and kcal/mol.

Table S2: Geometry optimizations, PBE0/6-31G*.

			L,L				D,L	
	E_{rel}	E_{rel}	E_{SCRF}	μ [D]	E_{rel}	E_{rel}	E_{SCRF}	μ [D]
extended monomer	**0.0**	**0.0**	-1671.18483102	62.7	**0.0**	**+1.5**	-1671.18240477	61.4
			-1671.18483102				-1671.18240477	
	0.0		-3342.36966204		**0.0**		-3342.36480954	
optimized monomer	**-18.7**		-1671.21466508	13.2	**-21.3**		-1671.2164842	12.9
			-1671.21466508				-1671.2164842	
		0.0	-3342.42933016			**0.0**	-3342.4329684	
		↓				↓		
optimized dimer	**-66.3**	**-28.9**	-3342.47528978 0.0	1.9	**-64.7**	**-21.9**	-3342.46786679 +4.6 kcal/mol	19.2

In further steps we performed single point calculations using pbe0 and m05-2x functional in conjugation with an extended basis set to minimize the basis set superposition error (BSSE). The increase of the basis functions reduces BSSE. According to our knowledge the basis set limit is reached even in DFT calculations with the basis set 6-311+G**. The m05-2x functional is claimed to provide especially good results for supramolecular complexes including aromatic and dispersion interactions. Hence we chose this result for the discussion in the main text. However, the pbe0 functional provides the same picture.

Table S3: Single point calculation using PBE0/6-311+G**.

			L,L				D,L	
	E_{rel}	E_{rel}	E_{SCRF}	μ [D]	E_{rel}	E_{rel}	E_{SCRF}	μ [D]
extended monomer	**0.0**	**0.0**	-1671.64112681	63.5	**0.0**	+1.3	-1671.63909781	62.2
			-1671.64112681				-1671.63909781	
	0.0		-3343.2822		**0.0**		-3343.27819562	
optimized monomer	**-10.7**		-1671.65805947	13.5	**-14.3**		-1671.66184274	13.1
			-1671.65805947				-1671.66184274	
		0.0	-3343.31611894			**0.0**	-3343.32368548	
		↓				↓		
optimized dimer	**-50.9**	**-29.7**	-3343.36340881 0.0	1.8	**-48.3**	**-19.7**	-3343.35511668 +5.2 kcal/mol	19.4

Table S4: Single point calculation using M05-2X/6-311+G**.

			L,L				D,L	
	E_{rel}	E_{rel}	E_{SCRF}	μ [D]	E_{rel}	E_{rel}	E_{SCRF}	μ [D]
extended monomer	**0.0**	**0.0**	-1673.37418900	64.0	**0.0**	+0.6	-1673.37317178	62.7
			-1673.37418900				-1673.37317178	
	0.0		-3346.7484		**0.0**		-3346.74634356	
optimized monomer	**-14.2**		-1673.39688400	13.6	**-13.4**		-1673.39456701	13.2
			-1673.39688400				-1673.39456701	
		0.0	-3346.7938			**0.0**	-3346.78913402	
		↓				↓		
optimized dimer	**-58.2**	**-29.6**	-3346.84094207 0.0	1.8	**-53.8**	**-26.9**	-3346.83203433 +5.6 kcal/mol	19.2

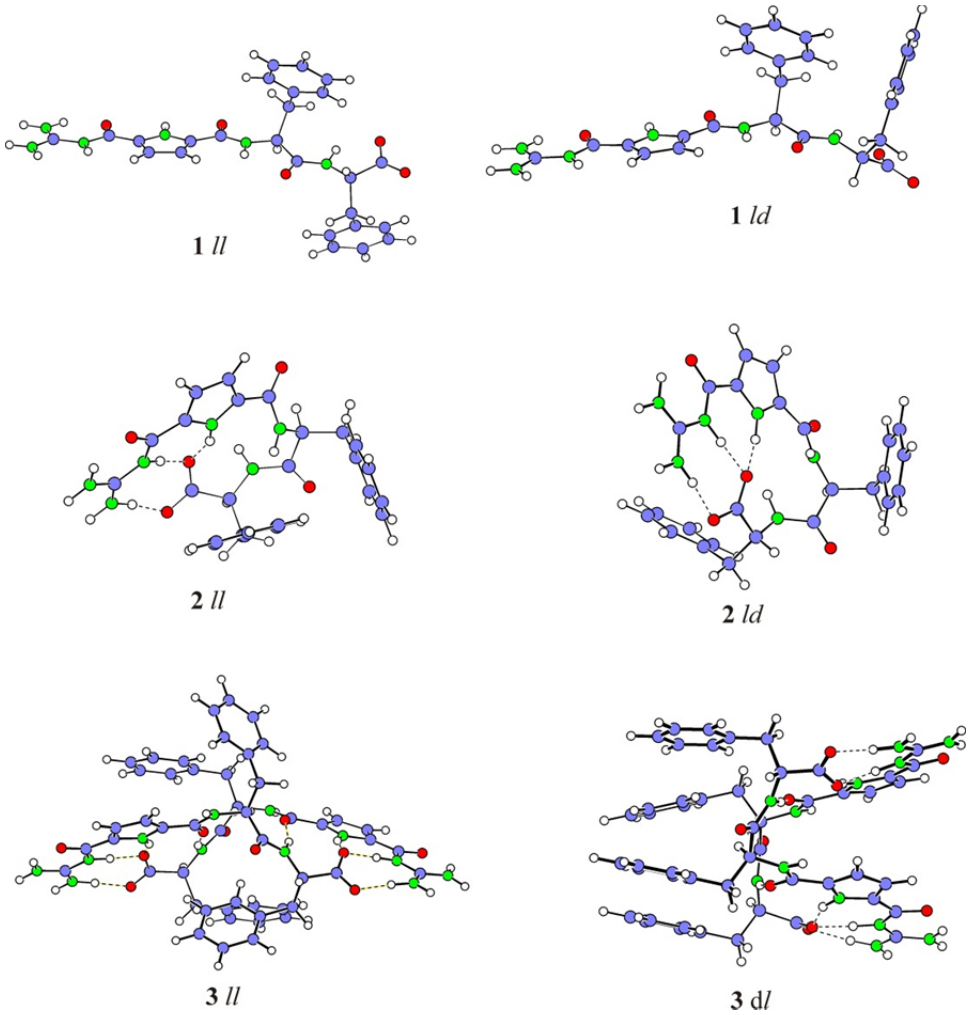


Figure S18: Structures obtained from the energy optimization for the extended monomer **1**, the optimized ground state conformer of the monomer **2** and the dimer **3**.