Electronic Supplementary Information

Construction of a polyproline structure with hydrophobic exterior using octahydroindole-2-carboxylic acid

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Index

Peptide synthesis S2
NMR experiments S4
Miscellaneous experimental descriptions S6
Rotational rates in Ac-Oic-OMe in deuterium oxide S7
Analytical data of the peptides S8
Copies of the NMR spectra S10
NMR spectra of the peptides S16
References S37
Peptide synthesis

Chlorotrytil resin was pre-loaded with Fmoc-Oic using the original procedure. Chlorotrytil chloride resin (1 g) was swollen in dichloromethane (10 ml). Fmoc-Oic (525 mg) in dichloromethane (2 ml) was added following addition of diisopropylethylamine (DIPEA, 440 μl). The mixture was gently stirred at the room temperature for 75 min, then DIPEA (0.5 ml) and methanol (3 ml) were added and the stirring was continued for the next 25 min. The resin was filtered, washed with dichloromethane, DMF, methanol and diethyl ether, then dried in vacuum. 1.287 g of the resin was obtained as the result. This corresponds to the loading of 0.8-0.9 mmol g⁻¹.

The synthesis of oligopeptides Ac(Oic)₆ was performed manually on the Fmoc-Oic pre-loaded 2-chlorotrityl resin, DMF was used as a solvent. The coupling step was performed with 2 equiv. of Fmoc-Oic, 1.95 equiv. of TBTU, 2 equiv. of HOBT and 4 equiv. of DIPEA all pre-mixed in DMF before addition to the resin. The N-terminal amino acid was installed using Ac-Oic and the coupling reagents. The coupling time was 1.5-1.5 hours. Fmoc-removal was performed by treatment with piperazine:DBU:DMF 5:2:93 (w/w/w) mixture for 20-25 min. After the coupling of Ac-Oic the resin was washed with multiple solvents, and dried in vacuum. Peptide cleavage was done by treatment with hexafluoroisopropanol - dichloromethane (1:3, v/v) mixture for about 20 min. The solvents were removed by nitrogen stream, the residue was taken up in water - acetonitrile mixture and freeze-dried. The primary NMR studies were performed using crude peptide samples. Further purification was accomplished by silica gel column chromatography using ethyl acetate - methanol to dichloromethane - methanol gradient elution. Ac(Oic)₆OMe was prepared by treatment of the crude peptide with acidic methanol (see procedure for Ac-Oic-OMe). Subsequent purification was done using ethyl acetate - methanol (19:1) elution (R_f = 0.5).

para-Bromobenzylated hexapeptide was synthesized in analogous procedure starting from Fmoc-Rink Amide AM resin. The last acetylation step was done with 5 equiv. para-bromobenzoic acid, 4.8 equiv. TBTU, 4 equiv HOBT and 10 equiv. DIPEA, pre-mixed in DMF, and shaken for 1 hour with the hexapeptide-resin. The cleavage was done by treatment with 95 vol % TFA for 2 hours. The crude peptide was lyophilized from acetonitrile - water mixture. Purification was done on silica gel column using ethyl acetate - methanol (20:1) mixture as an eluent (R_f = 0.3). Resulting peptide was glaceous material, which could be crystallized from its methanol solution.

GGXGG peptides were synthesised on Rink Amide resin using similar procedure. For amino acid coupling 2 equiv. of Fmoc-Oic, and 4 equiv. for other amino acids were used. The coupling conditions were same: TBTU:HOBT:DIPEA (0.98:1:2 molar ratio to the Fmoc-amino acid), pre-mixed with Fmoc-amino acid in DMF, coupling time 1-1.5 hours. The cleavage was done by treatment with 95 vol % TFA for 2 hours. Crude peptides were

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purified by reverse phase HPLC on standard preparative C18 column using methanol-water gradient with 0.05 % TFA. The peptides were obtained as glaceous material.
NMR experiments

The NMR spectra were acquired at Bruker Avance III 700 and 500 MHz proton frequency machines equipped with z-gradient TXI and BBFO probes, respectively. Temperature unit calibration was made by measurements of methanol standards. The one-dimensional $^1$H NMR spectra were acquired in 90-degree pulse experiments in one scans in order to avoid signal distortion due to relaxation differences. The proton 90-degree pulse was always calibrated prior the NMR measurements. The $^1$H{$^{15}$N} sofast-HMQC spectra were recorded as described.

$pK_a$ measurements. 5-10 mg of an analyte and 5 mg of potassium dihydrogenphosphate were dissolved in 10 ml of water. This solution was titrated by potassium hydroxide or hydrogen chloride solutions to different pH values read by a pre-calibrated pH-meter. 500 μl aliquots were taken to NMR tubes and 55 μl of deuterium oxide containing minimal amount of sodium 3-(trimethylsilyl)propane-1-sulfonate (TPS) were added to each sample. For samples with pH < 2 a rag wet with some drops of methanol was placed close to the titration vessel. This created enough background methanol vapours to be absorbed by the samples during titration procedure. The $^1$H NMR spectra were acquired in Watergate W5 pulse sequence at 298 K. TPS (δ = 0.000 ppm) or methanol (δ = 3.349 ppm) resonances were used for referencing. The chemical shifts were plotted against pH, fitted to Boltzman fits, and 1st order derivatives of the fit curves gave the $pK_a$s as the extremum points. The measurement errors were obtained by comparing the $pK_a$ values delivered by different resonances and added to the Instrumental error 0.05. The final error was 0.05 – 0.10 $pK_a$ units.

NMR samples in deuterated solvents were at 20 ± 5 g l$^{-1}$ concentration of the analytes. The Ac-AA-OMe samples were measured in pure deuterium oxide. The Ac-AA-OH acid samples in deuterium oxide were prepared from mixtures of Ac-AA-OH and potassium hydrogensulphate pre-lyophilised from deuterium oxide. The KHSO$_4$ concentration in the samples was 20 g l$^{-1}$. The salt samples were prepared as following. Analyte was dissolved in water with potassium dihydrogenphosphate and potassium hydroxide was added to reach pH about 7 (by pH electrode or pH paper). Resulting solution was freeze-dried and subsequently freeze-dried from deuterium oxide, then dissolved in deuterium oxide for measurements. The phosphate concentration in the samples was about 10 g l$^{-1}$. The samples of oligomeric peptides Ac(Oic)$_n$O$^-$ were prepared analogously at about 20 g l$^{-1}$ mass concentrations, except of the highest oligomer with N = 6, for which ~ 10 g l$^{-1}$ concentration was reached in a saturated solution. Deuteromethanol samples were prepared by dissolving the analyte in deuteromethanol. The samples of oligomeric peptides Ac(Oic)$_n$OH were prepared at 20 g l$^{-1}$ concentration. The Ac(Oic)$_n$O$^-$ sample in deuteromethanol was of the same concentration, and was prepared by titrating the peptide with equivalent amount of potassium hydroxide solution, lyophilizing from acetonitrile - water mixture and solving in deuteromethanol.
The cross-relaxation measurements were performed by NOESY with gradients of ROESY pulse sequences. The time domain was inset to give 2-5 Hz resolution in direct, and 10-20 Hz in indirect dimensions, respectively. The indirect dimension was later linearly predicted and zero filled to reach the direct dimension resolution. Standard processing was done in Topspin 3.2 (Bruker). The cross-relaxation spectra were assisted with 1D inversion recovery spectra for estimation of the $T_1$ relaxation times.

For $^1$H EXSY spectra the total relaxation times (acquisition+recycling) were inset to be ≥3·$T_1$ for the analysed resonances. 5 ms mixing time spectra were used for referencing, and at least two exchange spectra with mixing time 250 ms – 3 s were acquired for detection of exchange. Exchange rate matrices were obtained with EXSYCalc (Mestrec) tool. Exchange errors were obtained by analysing different exchanged resonances in the EXSY spectra and do not account for the formal temperature calibration error. Activation energies were calculated using Eyring equation (eqn. S1).

$$E^* = RT(-\ln \frac{k_{\text{exp}}}{T} + 23.76)$$

(eqn. S1)

The $^1$H NOESY spectra were recorded with mixing time 300 – 750 ms, and $^1$H ROESY was acquired with continuous wave spin-lock 500 ms. The NOE values were obtained by integration of the NOESY spectra and calibrated to the diagonal elements. The errors were obtained by taking RMSD of the cross-peak integrals and these were combined with the errors of the diagonal element integrals to give the final error.

The diffusion measurements were conducted by stimulated echo with bipolar gradient pulses on proton spectrum at 298 K. A standard spoil gradient of 600-750 μs was also applied. The diffusion time ($\Delta$) was 50 ms, and the gradient pulse ($\delta/2$) was 1 ms for deuteromethanol and 1.5 ms for deuterium oxide samples. The array spectra were acquired in 128 steps of a linear gradient (2 to 98 %). The processing was done using the standard program provided with the spectrometer. Conversion to molecular sizes for deuterium oxide samples was done using eqn. 3 by taking literature value of the dynamic viscosity $\eta = 1.1\cdot10^{-3}$ Pa s$^{-1}$. For deuteromethanol however we used $\eta$ for pure methanol $5.43\cdot10^{-4}$ Pa s$^{-1}$ that produced a more rational interpretation of the data. The depression of the viscosity value in deuteromethanol samples could have occurred due to the presence of some residual water in commercial deuteromethanol and in the peptide solutes. The measurement error is the error of the logD read-out from the screen.
Miscellaneous experimental descriptions

Molecular modelling

Molecular modelling was done using MOPAC package from Scigress Modeling (Fujitsu). PM6-water algorithm, which accounts for water dielectric constant, was normally applied. This was used in particular for calculating COSMO volumes of the oligomeric peptides.

Crystall structures

Ac-Oic-OMe was crystallized from dichloromethane. p-BrBz-(Oic)$_6$-NH$_2$ peptide was crystallised from methanol. The X-ray diffraction was performed at 150 K at analytical facility of the Institute of Chemistry (TU Berlin). Both compounds produced crystals with P2$_1$2$_1$2$_1$ space groups. Likewise in analogous proline structure the Oic$^3$ residue was close to the solvent molecule, therefore the cyclohexane ring could not be completely resolved in the structure. The coordinate files are deposited in Cambridge Crystallographic Data Centre under the deposition numbers:

Ac-Oic-OMe            CCDC 1510246
p-BrBz-(Oic)$_6$-NH$_2$   CCDC 1510247
Rotational rates in Ac-Oic-OMe in deuterium oxide

Table S1. Rotational rates of Ac-Oic-OMe in deuterium oxide solution as recorded by $^1$H EXSY NMR.

| calibrated temperature, K | rotation constant, s$^{-1}$ |  
|--------------------------|-----------------------------|  
|                           | cis-to-trans                 | trans-to-cis                  |
| 303.3                    | 0.046 ± 0.014                | 0.003 ± 0.001                |
| 309.7                    | 0.074 ± 0.013                | 0.008 ± 0.001                |
| 313.9                    | 0.121 ± 0.018                | 0.013 ± 0.001                |
| 324.6                    | 0.341 ± 0.016                | 0.048 ± 0.003                |
| 329.9                    | 0.627 ± 0.005                | 0.080 ± 0.002                |
| 335.3                    | 1.042 ± 0.064                | 0.146 ± 0.003                |
Properties of the peptides

Table S2. Mass spectra recorded by ESI-MS.

| peptide                  | Theoretical mass, Da | Experimental mass, Th | assignment       | RP-HPLC retention time, min |
|--------------------------|----------------------|-----------------------|------------------|-----------------------------|
| AcGlyGlyProGlyGlyNH$_{2}$ | 384.18               | 385.18                | [M+H]$^+$        | 0.79                        |
|                          |                      | 407.16                | [M+Na]$^+$       |                             |
| AcGlyGlyOicGlyGlyNH$_{2}$| 438.22               | 439.23                | [M+H]$^+$        | 1.04                        |
| Ac(Oic)$_{2}$OH          | 211.12               | 212.13                | [M+H]$^+$        | 2.08                        |
|                          |                      | 234.11                | [M+Na]$^+$       |                             |
| Ac(Oic)$_{2}$OH          | 362.22               | 363.23                | [M+H]$^+$        | 6.52                        |
| Ac(Oic)$_{3}$OH          | 513.32               | 514.33                | [M+H]$^+$        | 7.41                        |
| Ac(Oic)$_{4}$OH          | 664.42               | 665.43                | [M+H]$^+$        | 8.69                        |
| Ac(Oic)$_{5}$OH          | 815.52               | 816.53                | [M+H]$^+$        | 9.47                        |
| Ac(Oic)$_{6}$OH          | 966.62               | 967.62                | [M+H]$^+$        | 9.96                        |
| Ac(Oic)$_{6}$OMe         | 980.64               | 981.64                | [M+H]$^+$        | 11.18                       |
| pBrBz-(Oic)$_{6}$-NH$_{2}$| 1105.56              | 1106.57               | [M+H]$^+$ for $^{79}$Br | 11.10                      |
|                          |                      | 1108.57               | and [M+H]$^+$ for $^{81}$Br |                   |
|                          |                      | 1128.55               | [M+Na]$^+$ for $^{79}$Br |                   |
|                          |                      | 1130.55               | and [M+Na]$^+$ for $^{81}$Br |                   |

RP-HPLC:

Column: Grom-Sil-120-ODS-4-HE (Grace), length 50 mm, ID 2 mm, 3 µm. Gradient using eluent 1 water + 0.1 % HCO$_2$H and eluent 2 acetonitrile + 0.1 % HCO$_2$H. The gradient:

0 - 10 min: Eluent 2: from 20 % to 100 %

10 - 13 min: Eluent 2: hold 100 %

13 - 17 min: Eluent 2: hold 20 %

flow rate: 0.3 ml min$^{-1}$
Table S3. Properties of Ac(Oic)$_n$OH/O$^-$ at 298 K and 700 MHz $^1$H frequency.

| N  | MW, theor. deuteromethanol | MW, deuterium oxide (Ac(Oic)$_n$O$^-$) | -logD | volume, Å$^3$ | -logD | volume, Å$^3$ | $\tau_c$, ns | pK$_a$   |
|----|---------------------------|----------------------------------------|-------|----------------|-------|----------------|--------------|-----------|
| 1  | 211.3 266                 | 8.95                                   | 192   | 9.239 ± 0.005  | 171   | 6              | 0.0586 ± 0.0021 | 3.84 ± 0.05 |
| 2  | 362.4 450                 | 9.06                                   | 410   | 9.350 ± 0.005  | 368   | 13             | 0.126 ± 0.010  | 4.06 ± 0.08 |
| 3  | 513.7 637                 | 9.12                                   | 623   | 9.412 ± 0.009  | 565   | 36             | 0.194 ± 0.012  | 4.04 ± 0.09 |
| 4  | 664.9 839                 | 9.17                                   | 877   | 9.460 ± 0.009  | 787   | 50             | 0.270 ± 0.017  | 4.13 ± 0.07 |
| 5  | 816.1 1015                | 9.21                                   | 1160  | 9.502 ± 0.019  | 1052  | 147            | 0.361 ± 0.050  | 4.08 ± 0.07 |
| 6  | 967.3 1181                | 9.23                                   | 1328  | 9.532 ± 0.032  | 1294  | 320            | 0.444 ± 0.110  | 4.11 ± 0.07 |

Table S4. $\alpha$-CH resonances of all-trans-Ac(Oic)$_n$OH in deuteromethanol (298 K, 700 MHz $^1$H frequency).

| N  | Oic$^1$ | Oic$^2$ | Oic$^3$ | Oic$^4$ | Oic$^5$ | Oic$^6$ |
|----|---------|---------|---------|---------|---------|---------|
| 1  | 4.35 dd, $J = 10.3$, 8.2 Hz | -       | -       | -       | -       | -       |
| 2  | 4.62 dd, $J = 10.0$, 7.5 Hz | 4.42 dd, $J = 10.5$, 8.0 Hz | -       | -       | -       | -       |
| 3  | 4.57 dd, $J = 10.0$, 7.4 Hz | 4.46 dd, $J = 10.4$, 8.0 Hz | -       | -       | -       | -       |
| 4  | 4.81 dd, $J = 10.1$, 7.5 Hz; 4.61 dd, $J = 10.5$, 7.1 Hz; 4.58 dd, $J = 10.0$, 7.2 Hz | 4.47 dd, $J = 10.3$, 7.9 Hz | 4.75 dd, $J = 10.1$, 7.4 Hz; 4.62 dd, $J = 10.4$, 7.2 Hz; 4.59 dd, $J = 10.1$, 7.4 Hz | 4.47 dd, $J = 10.5$, 8.0 Hz | 4.47 dd, $J = 10.5$, 8.0 Hz | 4.47 dd, $J = 10.5$, 8.0 Hz |
| 5  | 4.75 dd, $J = 10.1$, 7.5 Hz; 4.71 dd*, $J = 10.5$, 7.2 Hz; 4.70 dd*, $J = 10.4$, 7.1 Hz; 4.62 dd, $J = 10.4$, 7.3 Hz; 4.59 dd, $J = 10.0$, 7.2 Hz; 4.59 dd, $J = 10.1$, 7.4 Hz | 4.47 dd, $J = 10.5$, 8.0 Hz | 4.48 dd, $J = 10.4$, 8.0 Hz | 4.48 dd, $J = 10.4$, 8.0 Hz | 4.48 dd, $J = 10.4$, 8.0 Hz | 4.48 dd, $J = 10.4$, 8.0 Hz |

* - multiplicity was read out using $^1$H J-resolved experiment.
Copies of the NMR spectra

Ac-Oic-O⁻: ¹H NMR spectrum in buffered deuterium oxide (pH 7) at 700 MHz
Ac-Oic-O: $^{13}$C\{H\} NMR spectrum in buffered deuterium oxide (pH 7) at 126 MHz
Ac-Oic-OMe: $^1$H NMR spectrum in buffered deuterium oxide (pH 7) at 700 MHz
Ac-Oic-OMe: $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum in buffered deuterium oxide (pH 7) at 126 MHz
Fmoc-Oic: $^1$H NMR spectrum in deuteromethanol at 700 MHz
Fmoc-Oic: $^{13}$C{$^{1}$H} NMR spectrum in deuteromethanol at 126 MHz
NMR spectra of the peptides

\( p\text{BrBz-(Oic)}_6-\text{NH}_2 : \) \(^1\text{H}\) NMR spectrum in deuteromethanol at 700 MHz
$^1$H ROESY spectrum (spin-lock 500 ms):
$^3$H NOESY spectrum (mixing time 500 ms):
Ac-GlyGlyOicGlyGly-NH$_2$: $^1$H NMR spectrum in deuteromethanol at 700 MHz
$^3$H NOESY spectrum (mixing time 1 s):
$^1$H NMR spectra of crude Ac(Oic)$_n$OH peptides in deuteromethanol at 700 MHz:

(HFIP – residual hexafluoroisopropanol)
$^3$H NMR spectra of crude Ac(Oic)$_N$O\textsuperscript{-} peptides in buffered deuterium oxide at 700 MHz:
$^3$H NOESY spectrum of Ac(Oic)$_2$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:
$^1$H NOESY spectrum of Ac(Oic)$_2$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:
$\text{^3H NOESY spectrum of Ac(Oic)$_3$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:}$
$^1$H NOESY spectrum of Ac(Oic)$_4$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:
$^1$H NOESY spectrum of Ac(Oic)$_5$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:
$^3$H NOESY spectrum of Ac(Oic)$_6$O$^-$ in buffered deuterium oxide at 700 MHz, mixing time 500 ms:
$^1$H NOESY spectrum of Ac(Oic)$_6$OH in deuteromethanol at 700 MHz, mixing time 750 ms:
$^1$H NOESY spectrum of Ac(Oic)$_6$OMe in deuteromethanol at 700 MHz, mixing time 750 ms:
$^1$H NOESY (EXSY) spectrum of Ac(Oic)$_6$O$^-$ in deuteromethanol at 330 K and 500 MHz, mixing time 1 s:
$^3$H NOESY (EXSY) spectrum of $\text{Ac(Oic)}_6\text{OMe}$ in deuteromethanol at 330 K and 500 MHz, mixing time 1 s:
$^{1}H^{13}C$ HSQC spectrum of Ac(Oic)$_6$O$^{-}$ in deuteromethanol at 298 K and 700&176 MHz frequency:

![Three CH3-resonances](image)

Minor α-CH
$^1$H$^{13}$C HSQC spectrum of Ac(Oic)$_6$OMe in deuteromethanol at 298 K and 700&176 MHz frequency:
$^1$H$^{13}$C) HSQC spectrum of Ac(Oic)$_6$O$^-$ in buffered deuterium oxide at 298 K and 700&176 MHz frequency:
$^1{}^3\text{C}$ HSQC spectrum of Ac(Oic)$_6$OMe in buffered deuterium oxide at 298 K and 700 MHz frequency:
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