Twice Tied Tight: Enforcing Conformational Order in Bicyclic Peptoid Oligomers

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1 General Experimental Details

Preparative HPLC was performed on a Delta-Pak C18 (Waters, 15 µm, 100 Å, 25 mm x 100 mm) with a linear gradient of 5-95% acetonitrile/water (0.1% TFA) over 40 min with a flow rate of 2.5 mL/min using a Beckman Coulter System Gold instrument.

Analytical HPLC was performed on a C18 reversed-phase analytical RP-HPLC column at room temperature (Peeke Scientific, 5 µm, 120 Å, 2.0 mm x 50 mm) using a Beckman Coulter System Gold instrument. A linear gradient of 5-95% acetonitrile/water (0.1% TFA) over 20 min was used with a flow rate of 0.7 mL/min.

LC-MS was performed on an Agilent 1100 Series LC/ MSD Trap XCT (Agilent Technologies).

MALDI-TOF mass spectra were measured on a Bruker UltrafleXtreme MALDI-TOF/TOF spectrometer using α-Cyano-4-hydroxycinnamic acid as the matrix.

NMR-spectra of peptoids were measured on a Bruker AV 500 high performance digital spectrometer using CDCl₃ and CD₃CN as solvents at 25 °C.

NMR spectra of 3-azidopropan-1-amine were measured on a Bruker AM 400 spectrometer. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) and are referenced to CHCl₃ (7.26 ppm) or CH₃CN (1.94 ppm) as internal standard.

All coupling constants are absolute values and J values are expressed in Hertz (Hz). For assigning signal separation of ¹H NMR spectra the following abbreviations were used: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, m = multiplet, dd = doublet of doublets, Hₘₜ = aromatic proton.

2D-COSY NMR spectra were recorded at room temperature in magnitude mode with 1.5 s relaxation delay before each scan. 2D gradient pulse phase-sensitive NOESY data set were recorded at room temperature with a mixing time of 350 ms, 256 FIDs were recorded for the indirect dimension (F1).

IR (infrared spectroscopy) data were recorded on a FT-IR Bruker IFS 88 as thin films on KBr and are reported as follows: frequency of absorption (cm⁻¹), intensity of absorption (vs = very strong, s = strong, m = medium, w = weak).

EI-MS (electron ionization mass spectrometry) was performed by using a Finnigan MAT 90 (70 eV). The molecular fragments are reported as the ratio between mass and charge (m/z), the intensities are reported as a percentage value relative to the intensity of the base signal (100%).

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2 Experimental Procedures

General. Solvents and reagents purchased from commercial sources were used without further purification. Abbreviations for reagents are as follows: benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP); trifluoroacetic acid (TFA); 1,1,1,3,3,3-hexafluoroisopropyl alcohol (HFIP); dichloromethane (DCM); \textit{N},\textit{N}-dimethylformamide (DMF); \textit{N},\textit{N}-diisopropylcarbodiimide (DIC); diisopropylethylamine (DIPEA); acetonitrile (ACN); methanol (MeOH)

General Synthesis Protocols for peptoid oligomers

Synthesis of the linear peptoid was performed as previously reported\cite{1} via solid phase peptoid synthesis on 2-chlorotrityl chloride resin (NovaBiochem, 1.2 mmol/g, 100–200 mesh) using adjustments in reaction time. Solid phase reactions were performed using fritted 10 mL plastic syringes (Torviq Inc.) filled with the resin. Yields were calculated according to the resin-loading given by NovaBiochem.

Methoxyethylamine (Alfa Aesar) was used as a submonomer for incorporation of \textit{N}-(methoxyethyl)glycine monomer; benzylamine (Alfa Aesar) was used as a submonomer for incorporation of the \textit{N}-(phenylmethyl)glycine monomer; 3-azido-1-aminopropane was synthesized as previously described\cite{2} and used as a submonomer for incorporation of the \textit{N}-(azidopropyl)glycine residue, and aniline (Sigma Aldrich) was used as a submonomer for incorporation of \textit{N}-phenylglycine.

Synthesis of 3-azidopropan-1-amine

The synthesis of 3-azidopropan-1-amine was conducted as previously described\cite{2} and used without further purification. 3-chloropropylamine hydrochloride (1.93 g, 14.8 mmol, 1.00 equiv.) was dissolved in 50 mL water. Sodium azide (2.89 g, 44.4 mmol, 3.00 equiv.) was added and the reaction mixture stirred at 80 °C for 23 h. Approximately 2/3 of the solvent was removed under reduced pressure. It was cooled with an ice bath and 20 mL diethylether were added. KOH (1.89 g, 33.6 mmol, 2.27 equiv.) was added slowly so that the temperature stayed below 10 °C. The organic phase was separated and the aqueous phase was extracted with 20 mL diethylether twice. The combined organic phases were dried over MgSO\textsubscript{4} and the solvent was...
removed under reduced pressure. The colorless liquid could be obtained in 67% yield (993 mg, 9.92 mmol).

$^1$H NMR (400 MHz, CDCl$_3$) δ [ppm]: 1.19 (s, 2 H, NH$_2$), 1.51 (quin., $^3J = 6.8$ Hz, 2 H, CH$_2$CH$_2$CH$_2$), 2.57 (t, $^3J = 6.8$ Hz, 2 H, CH$_2$NH$_3$), 3.16 (t, $^3J = 6.7$ Hz, 2 H, CH$_2$NH$_2$).

$^{13}$C NMR (100 MHz, CDCl$_3$) δ [ppm]: 31.87 (–,CH$_2$CH$_2$CH$_2$), 38.72 (–,CH$_2$NH$_2$), 48.59 (–,CH$_2$N$_3$); – IR (KBr): $\tilde{\nu}$ [cm$^{-1}$] = 3365.8 (m), 2940.2 (m), 2871.5 (m), 2098.6 (s), 1599.0 (w), 1457.8 (w), 1259.5 (m), 1069.3 (w), 849.3 (w), 672.4 (w), 557.9 (vw); – MS (70 eV, EI), m/z (%): 101 (15) [M+H]$^+$, 84 (30) [C$_3$H$_6$N$_3$]$^+$, 70 (36), 57 (80), 44 (100) [C$_2$H$_4$N]$^+$; – HR-MS (70 eV, EI), C$_3$H$_{10}$N$_4$: calc.: 101.0827, found: 101.0828.
Synthesis of linear octamer 3: For the synthesis, 300 mg of 2-chlorotrityl chloride (0.360 mmol, 1.00 equiv.) resin were weighed into a fritted plastic syringe and washed with 3 mL of DCM, followed by swelling in 3 mL of DCM for 5 min. The first submonomer was added by reacting 271 mg of bromoacetic acid (1.95 mmol, 5.40 equiv.) and 321 µL of DIPEA (233 mg, 1.80 mmol 5.00 equiv.) in 3 mL of DCM on a shaker platform for 40 min at room temperature, followed by washes with DCM (three times with 3 mL) and DMF (three times with 3 mL). Bromoacetylated resin was incubated with 3 mL of 1.00 M amine submonomer in DMF (3.00 mmol; 8.33 equiv.) on a shaker platform at room temperature, followed by washes with DMF (three times with 3 mL). The displacement reaction times were 30 min for all alkyl amines. Aniline was allowed to react for 18 h due to the deactivated character of the aryl amine. The subsequent bromoacetylations were carried out by reacting the resin with 500 mg bromoacetic acid (3.60 mmol, 10.0 equiv.) and 600 µL DIC (489 mg, 3.87 mmol, 10.8 equiv.) in 3 mL DMF for 20 min. Coupling steps were continued until the desired peptoid sequence was achieved. After the last displacement step, the resin was washed with DMF and DCM (two times with 3 mL for each solvent). The linear peptoid was cleaved from the resin using 4.50 mL 20% HFIP in DCM (v/v) at room temperature for 30 min. After collecting the cleavage solution, the resin was washed with 3 mL DCM twice and also collected. The solvent was evaporated under a stream of nitrogen gas. After cleavage, 370 mg (0.364 mmol, quant.) of a dark yellow oil were obtained. Cyclization was performed without further purification. Mass calculated: 1015.5 g/mol, mass found: 1016.1 [M+H]^+, 1038.1 [M+Na]^+.
Synthesis of Cyclic Octamer 4: The crude linear peptoid (248 mg, 0.244 mmol, 1.00 equiv.) was dissolved in 100 ml dry, deoxygenated DMF yielding a 2.4 mM solution. To this solution, 250 µL DIPEA (181 mg, 1.40 mmol, 5.74 equiv.) and 375 mg PyBOP (0.721 mmol, 2.95 equiv.) were added. After stirring at room temperature for 30 min, the solvent was removed under reduced pressure. The crude yellow oil was purified using preparative HPLC (30 – 100 % ACN in 40 min, retention time: 21.3 min, detection at 230 nm) After lyophilization, 19.8 mg (19.9 µmol, 8%, purity 99%) of the white powder could be obtained. Mass calculated: 997.4 g/mol, mass found: 998.4 [M+H]⁺, 1020.3 [M+Na]⁺.

1H-NMR (500 MHz, CDCl₃) δ [ppm]: 1.81 (quin., J = 6.9 Hz, 2 H, CH₂CH₂CH₂), 2.20 (t, J = 2.5 Hz, 1 H, C=CH₂), 3.187 (s, 3 H, CH₃), 3.191 (s, 3 H, CH₃), 3.21–3.29 (m, 3 H, 2 × MeOCH₂CH₂H), 3.35–3.37 (m, 6 H, 2 × OCH₂), 3.57–3.71 (m, 8 H, 2 × PhNCCH₂CONPh, HC=CH₂NCCH₂HCO), 3.98–4.02 (m, 3 H, 2 × MeO(CH₂)₂NCH₂CONPh, N(CH₂)₂CH₂HN), 4.44 (d, J = 18.3 Hz, 1 H, N(CH₂)₂CH₂HN), 4.52 (d, J = 18.0 Hz, 2 H, 2 × MeO(CH₂)₂NCH₂CONPh), 4.81 (d, J = 17.7 Hz, 1 H, PhNCH₂COCH₂NPh), 4.85 (d, J = 17.5 Hz, 1 H, PhNCH₂COCH₂NPh), 4.94 (d, J = 17.3 Hz, 1 H, NCH₂COCH₂), 5.23 (d, J = 17.0 Hz, 1 H, PhNCH₂COCH₂NPh), 5.37 (d, J = 17.0 Hz, 1 H, PhNCH₂COCH₂), 7.31–7.42 (m, 16 H, H₆), 7.57–7.53 (m, 4 H, H₆).
Synthesis of triazole-tethered bicyclic octamer 5 and intermolecular crosslinked hexadecamer 6 by Cu-catalyzed 1,3-dipolar cycloaddition: The purified cyclic peptoid 4, 84.0 mg (84.1 µmol, 1.00 equiv.) was dissolved in 170 mL DCM/MeOH 1/1 (v/v) and degassed for 15 min using nitrogen gas, giving a 0.50 mM solution. To this solution, 19.5 µL 2,6-lutidine (18.0 mg, 168 µmol, 2.00 equiv.) and 15.7 mg Cu(CH3CN)4PF6 (42.1 µmol, 0.50 equiv.) were added. The reaction proceeded at room temperature for 48 h. After completion of the reaction (as monitored by analytical HPLC) the solvent was removed under reduced pressure, giving a light-yellow oil. The bicyclic octamer 5 was purified by preparative HPLC (30 – 100 % ACN in 40 min, detector 230 nm, retention time: 14.5 min). After lyophilization, 22.2 mg (22.3 µmol, 27%) of 5 were obtained as a white powder with a purity >99%.

Clear, white, block-like crystals of the bicyclic peptoid 5 could be obtained by slow diffusion of diethyl ether into an ethanolic solution of the bicyclic compound at room temperature.

Mass calculated: 997.4 g/mol, mass found: 998.5 [M+H]+, 1020.4 [M+Na]+.

1H-NMR (500 MHz, CD3CN) δ [ppm]: 1.59–1.67 (m, 1 H, NCH2CH2CH2N), 2.34–2.41 (m, 1 H, NCH2CH2CH2N), 2.44–2.48 (m, 1 H, NCNCH2CH2N), 3.15 (s, 3 H, CH3), 3.18 (s, 3 H, CH3), 3.20–3.24 (m, 2 H, 2 × NCNCH2CH2OMe), 3.30–3.38 (m, 6 H, 2 × CH2OMe, (CH2)3NCOCH2HNPh, PhNCOCH2HN(CH2)2OMe), 3.47 (d, 2J = 16.8 Hz, 1 H, HC=CCH2NCOCH2HNPh), 3.50 (d, 2J = 16.5 Hz, 1 H, PhNCOCH2HNPh), 3.56 (d, 2J = 16.5 Hz, 1 H, PhNCOCH2HNPh), 3.58–3.61 (m, 1 H, PhNCOCH2HN(CH2)2OMe), 3.68–3.75 (m, 3 H, 2 × NCH2CH2OMe, PhNCOCH2HN(CH2)2OMe MeO(CH2)2NCOCH2HN(CH2)2C=CH), 3.82–3.88 (m, 3 H, NCNCH2CH2N, MeO(CH2)2NCOCH2HN(CH2)2), 3.99 (d, 2J = 18.4 Hz, 1 H, MeO(CH2)2NCOCH2HN(CH2)2C=CH), 4.39 (t, 3J = 13.1 Hz, 1 H, NCH2CH2CH2N), 4.48–4.52 (m, 1 H, NNCH2CH2N), 4.69–4.76 (m, 1 H, NNCH2CH2N), 4.90 (d, 2J = 18.7 Hz, 1 H, PhNCOCH2HN(CH2)2OMe), 4.92 (d, 2J = 16.6 Hz, 1 H, PhNCOCH2HNPh), 4.99 (d, 2J = 17.1 Hz, 2 H, PhNCOCH2HNPh, MeO(CH2)2NCOCH2HN(CH2)2), 5.11 (d, 2J = 16.8 Hz, 1 H, HC=CCH2NCOCH2HNPh), 5.32 (d, 2J = 16.6 Hz, 1 H, (CH2)3NCOCH2HNPh), 5.68, (d,
\[ J = 16.0 \text{ Hz}, 1 \text{ H}, \text{NCHC}=\text{CH}, \] 7.18 (d, \( J = 7.0 \text{ Hz}, 2 \text{ H}, H_{ar} \)), 7.29–7.38 (m, 8 \text{ H}, H_{ar} \)), 7.40–4.48 (m, 8 \text{ H}, H_{ar} \)), 7.51 (d, \( J = 7.6 \text{ Hz}, 2 \text{ H} H_{ar} \)), 8.29 (s, 1 \text{ H}, \text{C}=\text{CH}).

Crystallographic data for bicyclic compound 5: Colorless block-like crystals. Crystal size: 0.31 mm × 0.24 mm × 0.21 mm, triclinic, space-group: P -1 (2), cell parameters: 

\[ a = 10.9563(8) \text{ Å}, \quad b = 13.8555(10) \text{ Å}, \quad c = 19.2000(15) \text{ Å}, \quad \alpha = 70.92(0)^\circ, \quad \beta = 84.7^\circ, \quad \gamma = 78.58(0)^\circ, \quad Z = 2, \quad V = 2698.85(111) \text{ Å}^3, \quad \rho_{\text{calc}} = 1.31764 \text{ g/cm}^3. \]

Analytic details for bicyclic peptoid 6:

During the preparative HPLC of the reaction products, the intermolecular crosslinked hexadecamer 6 was also isolated as a separate peak (retention time 25.5 min) and yielded a white powder after lyophilization (6.50 mg, 3.25 µmol, 4%).

The dimeric peptoid 6 was crystalized by slow evaporation from methanol at room temperature


Crystallographic data for dimer 6: Colorless, rectangular crystals. Crystal size: 0.54 × 0.42 × 0.20 mm, monoclinic, space group: I2/a, cell parameters: 

\[ a = 23.1428(13) \text{ Å}, \quad b = 17.9913(10) \text{ Å}, \quad c = 27.126(2) \text{ Å}, \quad \alpha = 90^\circ, \quad \beta = 97.8950(10), \quad \gamma = 90^\circ, \quad Z = 4, \quad V = 11187.3(13) \text{ Å}^3, \quad \rho_{\text{calc}} = 1.185 \text{ g/cm}^3. \]
3 Analytical HPLC traces

Cyclic Octamer 4

Retention time: 7.7 min.

Bridged bicyclic peptoid 5

Retention time: 6.7 min.
Intermolecular dimeric peptoid 6

Retention time: 8.1 min
4 NMR Data

4.1 Cyclic Octamer 4

Spectra taken in CDCl$_3$ at a concentration of 1.05 mM at room temperature.

4.1.1 $^1$H-NMR spectrum
4.1.2 HSQC spectrum
4.1.3 HSQC spectrum, magnified region for backbone and side chain methylene groups
4.1.5 COSY spectrum, magnified region for backbone methylene groups
4.1.6 NOESY spectrum
4.1.7 NOESY spectrum, magnified region of the region for backbone methylene groups
4.2 Bicyclic Octamer 5

Spectra taken in CD$_3$CN at a concentration of 0.78 mM at room temperature.

4.2.1 $^1$H-NMR spectrum
4.2.2 HSQC spectrum
4.2.3 HSQC spectrum, magnified region for backbone and side chain methylene groups
4.2.4 COSY spectrum
4.2.5 COSY spectrum, magnified region for backbone methylene groups
4.2.6 NOESY spectrum

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4.2.7 NOESY spectrum, magnified region for backbone methylene groups
5 Crystal packing of bicyclic octamer 5

Orientation of four bicyclic peptoids within the crystal lattice, depicting the proximity and antiparallel alignment of bridging triazole rings. For clarity, side chains and hydrogen atoms are omitted.

6 NOE signals in bicyclic octamer 5

Structure of the bicyclic peptoid 5. Red arrows indicate observed NOE signals between different backbone CH$_2$ groups at cis amide positions and also between backbone CH$_2$ groups and the tethered side chains. Blue arrows indicate observed NOE signals between backbone- and side chain CH$_2$ groups and the aromatic rings. To enhance clarity, the peptoid macrocycle is shown in an extended linear format with a covalent bond indicated by the waved lines.