Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION

Size-dependent cation transport by cyclic α-peptoid ion carriers

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List of abbreviations

Bn: benzyl

DCM: dichloromethane

DIPEA: diisopropylethylamine

DMF: dimethylformamide

Fmoc: 9-fluorenylmethoxycarbonyl

HATU: O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

HEPES: 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid

HFIP: hexafluoroisopropanol

PyBOP: benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate

RP HPLC: reversed-phase high-performance liquid chromatography

TFA: trifluoroacetic acid

SUPPORTING INFORMATION

General procedures

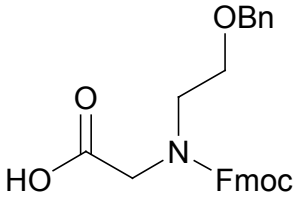
Reagents purchased from commercial sources were used without further purification.

All reactions involving air or moisture sensitive reagents were carried out under a dry argon atmosphere. CH_2Cl_2 was distilled from CaH_2. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light or by spraying with phosphomolybdic acid or ninhydrin solutions and drying. HPLC analysis were performed on a C_{18} reversed-phase analytical and preparative columns (Waters, μBondapak, 10 μm, 125Å 3.9 mm × 300 mm and 7.8 × 300 mm respectively) using an Agilent 1100 series liquid chromatograph (Hewelett-Packard, Palo Alto, CA), equipped with a G-1312A binary pump, a G-1328B rheodyne injector, and a G-1365B multiple wavelength detector set at 220 nm. Yields refer to chromatographically and spectroscopically (^{1}H- and ^{13}C-NMR) pure materials. The NMR spectra were recorded on Bruker DRX 400, (^{1}H at 400.13 MHz, ^{13}C at 100.03 MHz), Bruker DRX 250 (^{1}H at 250.13 MHz, ^{13}C at 62.89 MHz), and Bruker DRX 300 (^{1}H at 300.1 MHz, ^{13}C at 75.5 MHz) spectrometers. Chemical shifts (δ) are reported in ppm relatively to the residual solvent peak ($C\mathit{H}Cl_3$, δ = 7.26, $^{13}\mathit{C}DCl_3$, δ: = 77.0; $CD_2\mathit{H}CN$: δ = 1.98; $^{13}CD_3CN$: δ = 1.39, in the case of solvent mixtures, the considered residual peak was that of the most abundant deuterated solvent) and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet; b, broad. Coupling costants (*J*) are quoted in Hz. Homonuclear decoupling, COSY-45 and DEPT experiments completed the full assignment of each signal. High resolution ESI-MS spectra were performed on a Q-Star Applied Biosystem mass spectrometer. . ESI-MS analysis in positive ion mode was performed using a Finnigan LCQ Deca ion trap mass spectrometer (ThermoFinnigan, San Josè, CA, USA) and the mass spectra were acquired and processed using the Xcalibur software provided by Thermo Finnigan. Samples were dissolved in 1:1 CH_3OH/H_2O, 0.1 % formic acid, and infused in the ESI source by using a syringe pump; the flow rate was 5 μl/min. The capillary voltage was set at 4.0 V, the spray voltage at 5 kV, and the tube lens offset at -40 V. The capillary temperature was 220 °C. Data were acquired in $MS^1$and MS^n scanning modes. Zoom scan was used in these experiments.

1.0 Synthesis

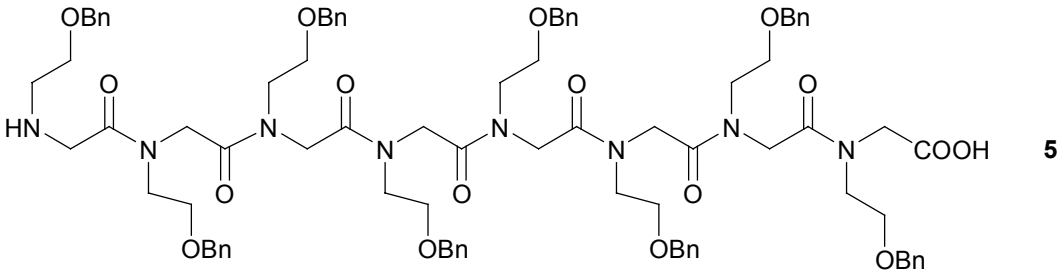
1.1 Solid-phase synthesis of linear precursor 5 and 6

Linear peptide sequence was synthesized using standard manual Fmoc solid-phase peptide synthesis protocols. 0.30 g of 2-chlorotrityl chloride resin (Fluka; 2,α-dichlorobenzhydryl-polystyrene crosslinked with 1% DVB; 100-200 mesh; 1.55 mmol/g) was swelled in dry DMF (3 mL) for 45 min and washed twice in dry DCM (3 mL). The first *N*-Fmoc *N*-benzyloxyethyl glycine (**7**,[1] 0.17 mmol) in dry DCM (3 mL) and DIPEA (0.68 mmol) were added on a shaker platform for 1.5 h at room temperature, followed by washing with dry DCM (3 mL) then twice with a mixture of DCM/MeOH/DIPEA (17:2:1) and finally with DMF (3 × 3 mL).



7

Resin loaded with the first *N*-Fmoc *N*-alkylated glycine was incubated twice with 20% piperidine/DMF (v/v, 3 mL) on a shaker platform for 3 min and 7 min respectively, followed by extensive washes with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). Note that, to avoid the formation of diketopiperazine, the deprotection of the second aminoacid was done incubating twice with 20% piperidine/DMF (v/v, 3 mL) on a shaker platform for 3 min. The yields of loading step and of the following coupling steps were evaluated interpolating the absorptions of dibenzofulvene-piperidine adduct (λ_{max} = 301, ε = 7800 M^{-1} cm^{-1}), obtained in Fmoc deprotection step (the average coupling yield was >96%). After loading of the first monomer all subsequent *N*-Fmoc *N*-benzyloxyethylglycine addition and Fmoc deprotection steps were performed as follow, until the desired oligomer length was obtained. The resin was incubated with a solution of *N*-Fmoc *N*-benzyloxyethylglycine (0.68 mmol), HATU (or PyBOP) (0.66 mmol), DIPEA (1.36 mmol) in dry DMF (2 mL) on a shaker platform for 1 h, followed by extensive washes with DMF (3 × 3 mL), DCM (3 × 3 mL) and DMF (3 × 3 mL). Chloranil test was performed and once the coupling was complete the Fmoc group was deprotected with piperidine as described above and the resin washed again to prepare it for the next coupling. The oligomer-resin was cleaved in 4 mL of 20% HFIP in DCM (v/v). The cleavage was performed on a shaker platform for 30 min at room temperature the resin was then filtered away. The resin was treated again with 4 mL of 20% HFIP in DCM (v/v) for 5 min, washed twice with DCM (3 mL), filtered away and the combined filtrates were concentrated in vacuo. The final products were dissolved in 50% ACN in HPLC grade water and analysed by RP-HPLC (see paragraph 3.1) and ESI mass spectrometry.

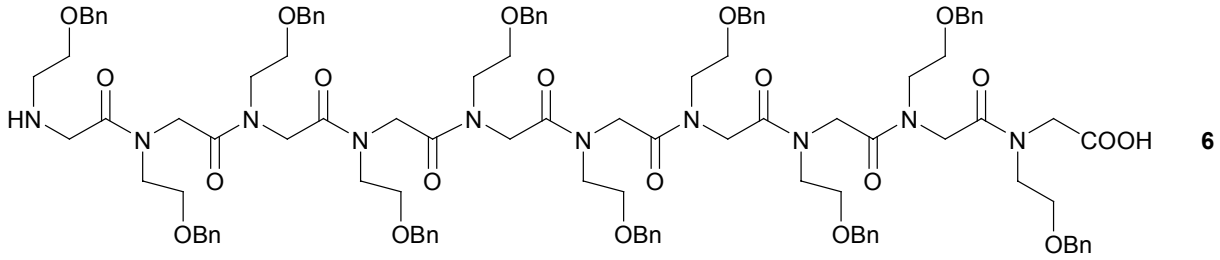


5

5: ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers, broad signals) δ: 3.15-3.90 (34H, m); 3.90-4.20 (6H, m); 4.20-4.70 (24H, m); 7.20-7.36 (40H, bs).

^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers, broad signals) δ: 47.9, 50.0, 68.5, 72.8, 127.6, 128.3, 128.9, 137.6, 169.3.

HR ESI-MS: $[M+H]^+$ m/z 1547.787 (calcd for $C_{88}H_{107}N_8O_{17}$ 1547.775).



6

6: ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers, broad signals) δ: 2.90-3.80 (40H, bs); 3.81-4.70 (40H, bs); 7.10-7.50 (50H, bs).

^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers, broad signals) δ: 47.6, 49.7, 67.8, 72.9, 127.4, 127.5, 128.1, 137.7, 168.9.

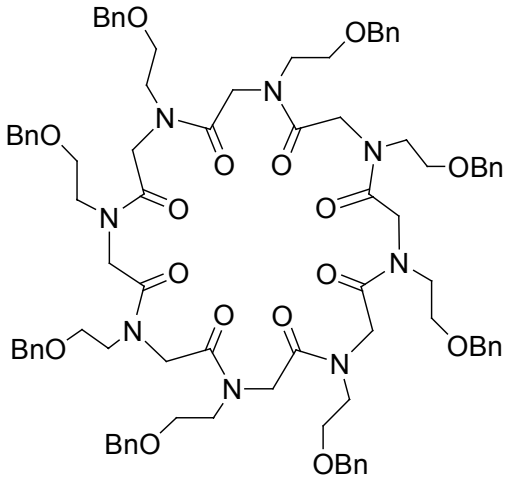
HR ESI-MS: $[M+H]^+$ m/z 1929.953 (calcd for $C_{110}H_{133}N_{10}O_{21}$ 1929.965).

1.2 High dilution cyclization. Synthesis of compound 3 and 4

To a stirred solution of **5** (or **6**) (0.39 mmol) and DIPEA (0.42 mL, 2.40 mmol) in dry DMF (166 mL) at r.t., a solution of PyBOP (0.624 g, 1.20 mmol), in dry DMF (20 mL) was added by syringe pump in 4 h. After 18 h the resulting mixture was concentrated in vacuo, diluted with AcOEt (50 mL) and a 0.5 N HCl solution (50 mL) was added. The mixture was extracted with AcOEt (2 × 50 mL) and the combined organic phases were washed three times with water (25 mL), dried ($MgSO_4$) and concentrated in vacuo. See paragraph 3.2 for the HPLC chromatograms of the cyclizations. The yields were >90%, based on HPLC chromatograms.

The crude residues from the PyBOP-induced cyclizations, were purified by HPLC on a C_{18} reversed-phase preparative column. A linear gradient of 40-100% acetonitrile in water (0.1% TFA) over

18 min was used with a flow rate of 2.0 mL/min, for further 2 min with a flow rate of 1.5 mL/min, and for the last 10 min with a flow rate of 1.0 mL/min. The samples were dried in a falcon tube with a N_2 flow.

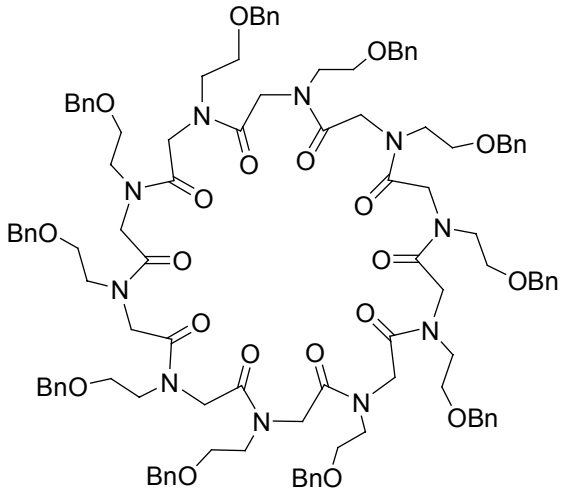


3

3: ^{1}H NMR (400 MHz, CD_3CN:$CDCl_3$ 9:1, mixture of rotamers, Figure S1) δ: 3.15-3.90 (30H, m); 4.20-4.61 (25H, m); 4.62-4.80 (5H, m); 7.26-7.36 (40H, m, Ar-H)

^{13}C NMR (100 MHz, CD_3Cl, mixture of rotamers, Figure S2) δ: 46.1, 46.9, 47.3, 47.7, 48.1, 48.5, 48.8, 49.1, 49.5, 50.0, 50.4, 67.3, 67.6, 67.7, 68.1, 72.8, 73.2, 73.4, 127.5, 127.6, 127.7, 127.9, 128.1, 128.3, 137.7, 167.3, 168.9, 169.1, 169.5, 169.6, 170.4.

HR ESI-MS: $[M+Na]^+$ m/z 1529.770 (calcd for $C_{88}H_{105}N_8O_{16}$ 1529.761).



4

4: ^{1}H NMR (400 MHz, CD_3CN:$CDCl_3$ 9:1, mixture of rotamers, Figure S3) δ: 3.00-3.70 (40H, bs); 4.00-4.65 (40H, bs); 7.00-7.30 (50H, bs, Ar-H).

^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers, broad signals, Figure S4) δ: 48.1, 50.1, 68.4, 69.2, 72.3, 72.9, 73.5, 127.0, 127.6, 128.2, 137.8, 138.2, 168.4, 168.7, 169.5, 169.9, 170.2.

HR ESI-MS: $[M+Na]^+$ m/z 1911.931 (calcd for $C_{110}H_{131}N_{10}O_{20}$ 1911.954).

2.0 ^{1}H- and ^{13}C NMR spectra

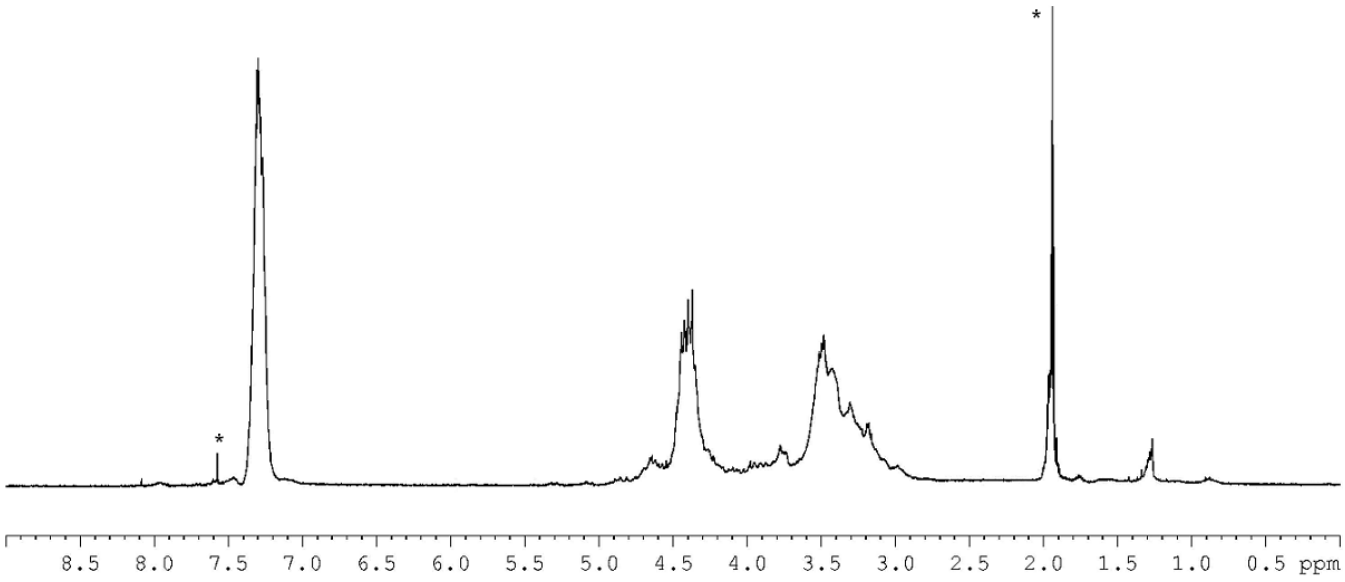


Figure S1. ^{1}H NMR (400 MHz, CD_3CN:$CDCl_3$ 9:1, mixture of rotamers) of **3.** Residual solvent peaks are labelled with *.

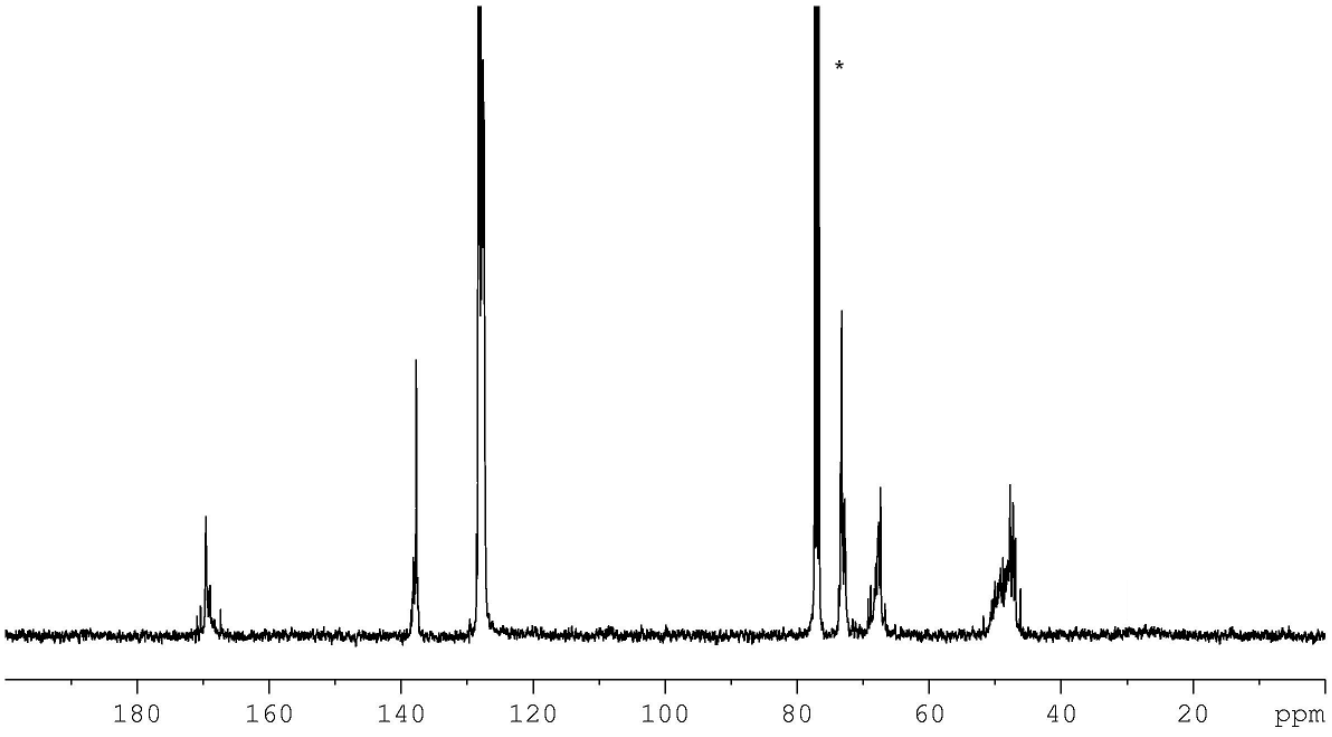


Figure S2. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) of **3.** The residual solvent peak is labelled with *.

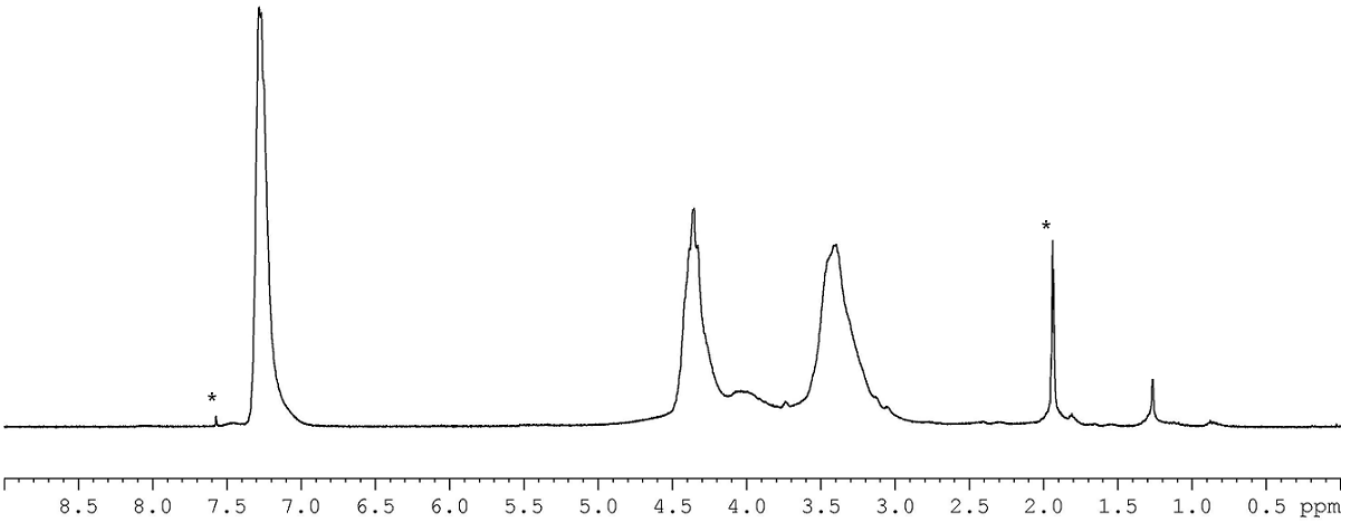


Figure S3. ^{1}H NMR (400 MHz, CD_3CN:$CDCl_3$ 9:1, mixture of rotamers) of **4.** Residual solvent peaks are labelled with *.

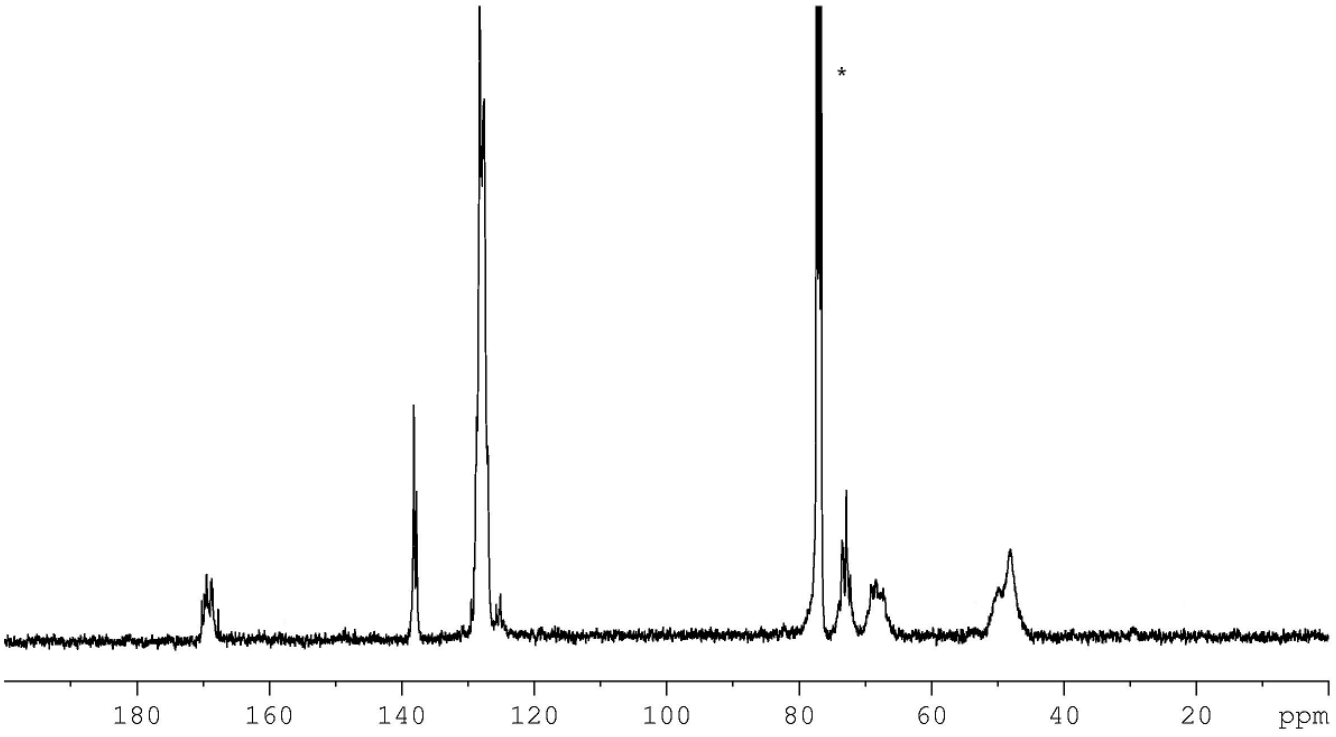


Figure S4. ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) of **4.** The residual solvent peak is labelled with *.

3.0 HPLC analysis

3.1 HPLC chromatograms for linear peptoids 5 (Figure S5) and 6 (Figure S6)

HPLC analysis for linear peptoids **5** and **6** (from solid-phase synthesis).

Conditions: 5 → 100% B in 30 minutes (A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile), flow: 1.0 ml/min, 220 nm. C_{18} reversed-phase analytical column (Waters, μBondapak, 10 μm, 125Å, 3.9 mm × 300 mm)

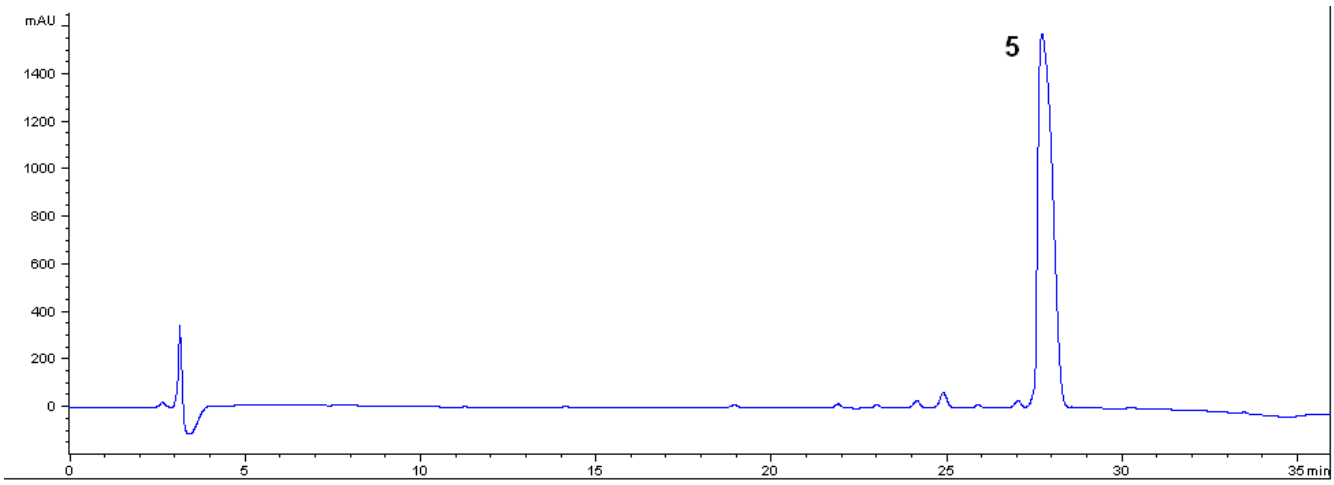


Figure S5. HPLC chromatograms for linear peptoids **5**.

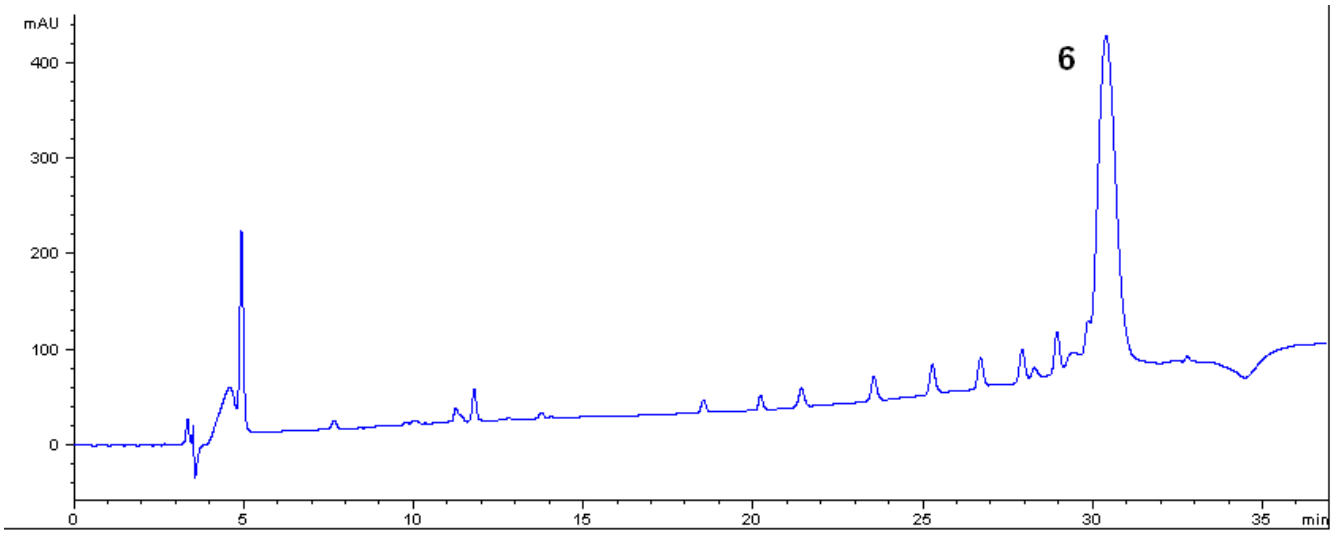


Figure S6. HPLC chromatograms for linear peptoids **5**.

3.2 HPLC chromatograms for cyclization of peptoids 5 (Figure S7) and 6 (Figure S8)

Conditions: 5 → 100% B in 30 minutes (A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile), flow: 1.0 ml/min, 220 nm. C_{18} reversed-phase analytical column (Waters, μBondapak, 10 μm, 125Å, 3.9 mm × 300 mm).

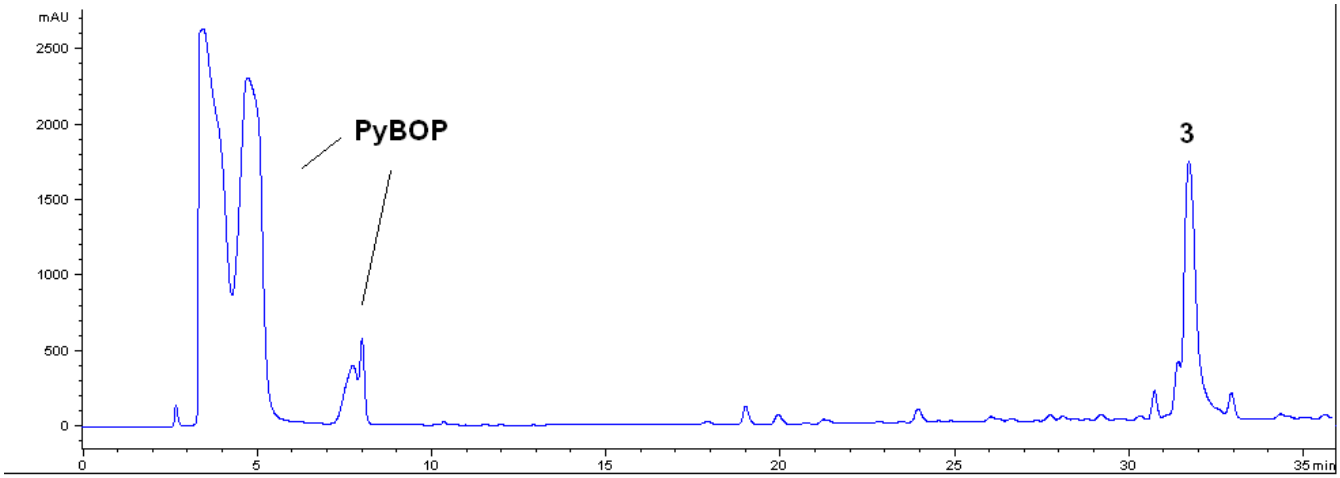


Figure S7. *PyBOP cyclization* of linear peptoid **5** to give cyclic peptoid **3** (10 μL of the reaction mixture reaction were quenched, after 18 hours, with 140 μL of 1:1 mixture H_2O/CH_3CN and injected in the HPLC).

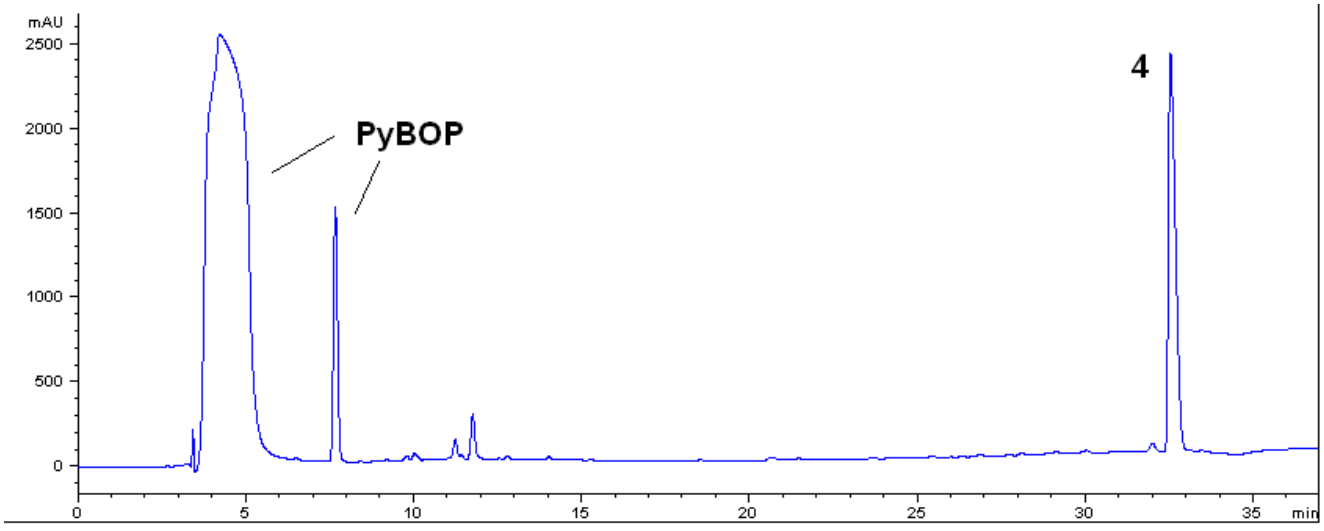


Figure S8. *PyBOP cyclization* of linear peptoid **6** to give cyclic peptoid **4** (10 μL of the reaction mixture reaction were quenched, after 18 hours, with 140 μL of 1:1 mixture H_2O/CH_3CN and injected in the HPLC).

4.0 Complexation studies

4.1 Synthesis of the complexes

To a 4.0 mM solution of **3** in CD_3CN:$CDCl_3$ 9:1 (0.5 mL), were added increasing amounts of sodium picrate. After any addition, the mixture was stirred vigorously for 15 minutes and the spectrum was acquired (see spectral data in the next paragraph).

Small or no variations of the ^{1}H NMR spectrum were observed after addition of Li^+, K^+, Rb^+, Cs^+, and NH_4^+.

The same procedure was used for compound **4**. Small or no variations of the ^{1}H NMR spectrum were observed after addition of Li^+, Na^+, K^+, Rb^+, Cs^+, and NH_4^+.

4.2 Complex sodium picrate-3. Spectral data. (Figure 1 (c) of the paper and top spectrum of figure S9)

^{1}H NMR (400 MHz, CD_3CN:$CDCl_3$ 9:1, 25 °C, 4.0 mM solution, spectra of the 10:1 complex) δ: 2.94 (8H, bd, *J* = 15.6 Hz, -NC*H*HCH$_2$OBn); 3.24-3.60 (24H, m, -NCH*HC*H$_2$OBn); 3.87 (8H, d, *J* = 16.7 Hz, -OCC*H*HN, pseudoequatorial); 4.30 (8H, d, *J* = 11.5 Hz, -OC*H*HAr); 4.36 (8H, d, *J* = 11.5 Hz, -OCH*H*Ar); 4.64 (8H, d, *J* = 16.7 Hz, -OCCH*H*N, pseudoaxial); 7.28-7.37 (40H, m, Ar-H).

^{13}C NMR (100 MHz, CD_3CN:$CDCl_3$ 9:1, 25 °C, 4.0 mM solution, figure S10) δ: 49.1 (× 8), 49.3 (× 8), 68.9 (× 8), 74.3 (× 8), 127.5 (*C*-H; picrate), 128.1 (*o*-position; picrate), 129.1 (× 8), 129.2 (× 16), 129.8 (× 16), 139.7 (× 8), 143.2 (*p*-position; picrate), 163.5 (*ipso*-position; picrate), 171.1 (× 8).

ESI-MS: [M+Na^+] m/z 1530.2

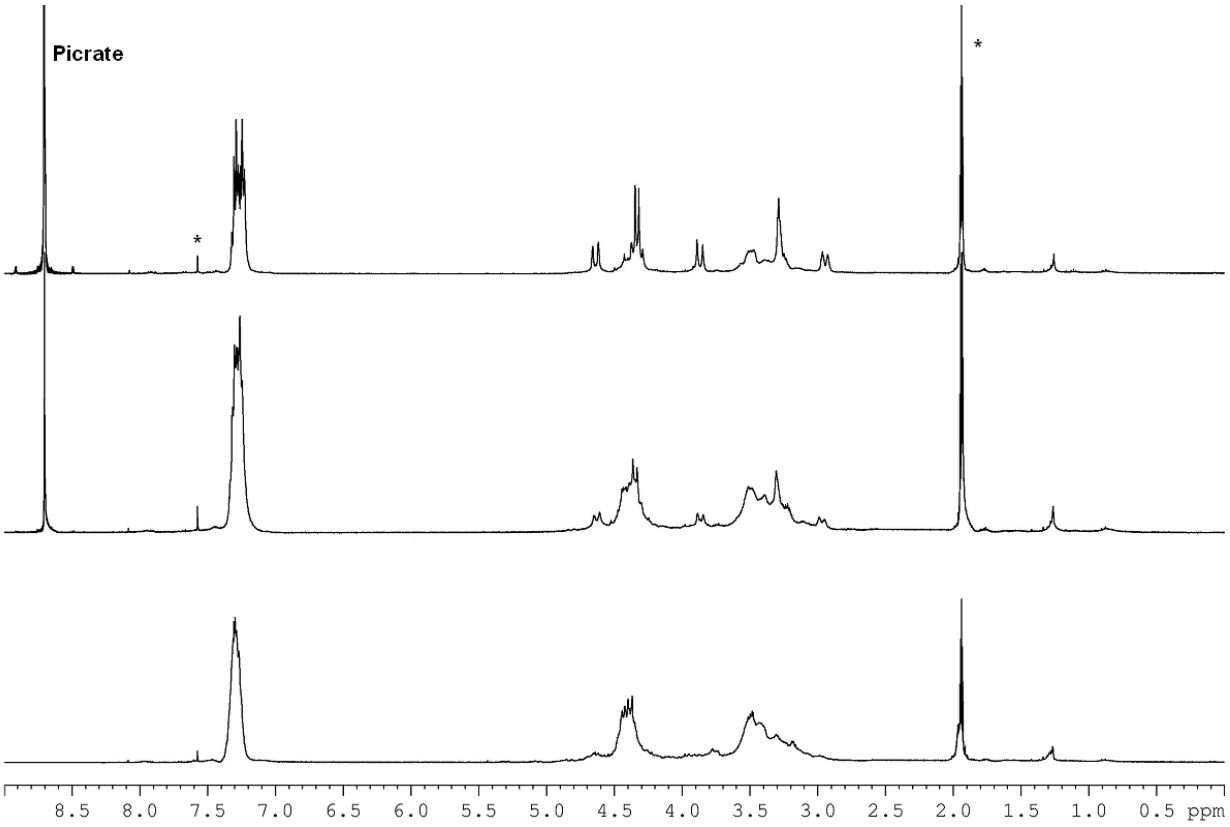


Figure S9. Full (0-9.0 ppm) ^{1}H NMR spectra of free **3** (a) ($CD_3CN/CDCl_3$ 9:1 solution, 25 °C, [**3**] = 4.0 mM, 400 MHz) and in the presence of 2.0 eq. (b) or 10.0 eq. (c) of sodium picrate. Residual solvent peaks are labelled with *.

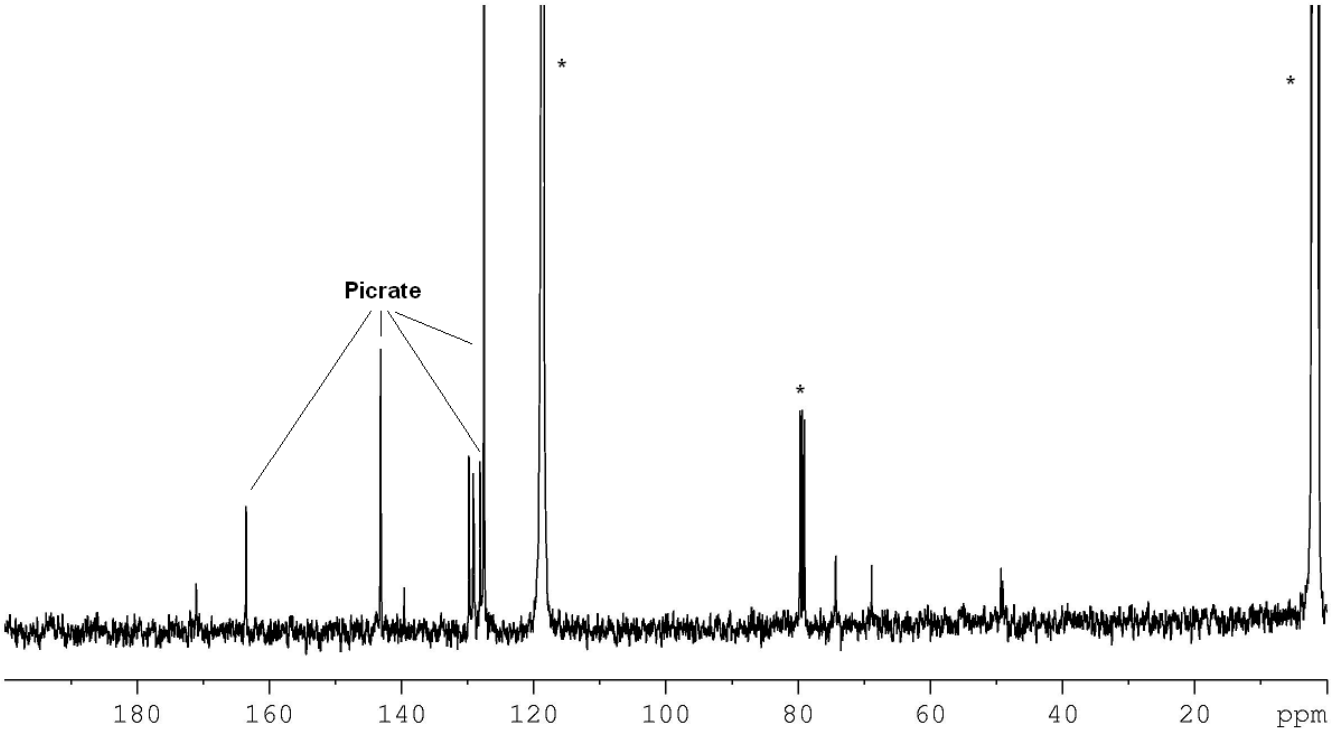


Figure S10. ^{13}C NMR spectra of **3** in the presence of 10.0 eq. (b) of sodium picrate ($CD_3CN/CDCl_3$ 9:1 solution, 25 °C, [**3**] = 4.0 mM, 100 MHz). Residual solvent peaks are labelled with *.

5.0 Computational details

In order to allow a full exploration of the conformational space of the molecules, MM/MD calculations at different temperatures (300 K, 500K, 700K/10 ns) were performed using the AMBER force field (MacroModel software package)[2]. All the so obtained structures (in number of 100) were minimized using the Polak-Ribier Conjugate Gradient algorithm (PRCG, 1000 steps, maximum derivative less than 0.05 kcal/mol). This led to the selection of the lowest energy minimum conformers for the molecules. A final optimization on the S_8 conformation was performed at ONIOM calculation performed with MPW1PW91/6-31G(d) level (Gaussian Software Package)[3] for the cyclic core and HF/3-21G for the side chains, providing the three dimensional model of **3**.

Atom coordinates of compound **3.**

C	3.909584	1.672238	-0.31297
C	3.774239	2.986009	0.46785
C	4.773817	-0.55348	-0.46976
N	4.765231	0.729061	0.182715
C	2.978971	-3.76605	0.460018
N	3.879423	-2.85107	-0.19015
C	3.940736	-1.58026	0.308535
C	-2.97932	3.767646	0.466693
N	-3.88034	2.854632	-0.18543
C	-3.94171	1.582498	0.3097
N	2.859463	3.887474	-0.18119
C	1.586081	3.940809	0.312093
C	0.559332	4.773949	-0.466
C	-0.56006	-4.77049	-0.47326
N	0.723352	-4.76031	0.177394
C	1.664902	-3.90379	-0.31984
N	-0.72381	4.762378	0.185195
C	-1.66574	3.907166	-0.3136
C	-4.77618	0.55822	-0.47045
N	-4.76784	-0.72556	0.17953
C	-3.90985	-1.66672	-0.31599
C	-3.77325	-2.98096	0.46377
N	-2.85917	-3.88186	-0.18699
C	-1.58614	-3.93827	0.306762
O	-1.2642	-3.38455	1.346267
O	-3.29285	-1.50368	-1.35697
O	-3.39031	1.259675	1.350221
O	-1.50371	3.294805	-1.35751
O	1.264572	3.384041	1.350095

O	3.29349	1.511276	-1.35485
O	3.389991	-1.26075	1.350435
O	1.502155	-3.28894	-1.36218
C	4.475342	-3.2783	-1.47011
C	0.841281	-5.48296	1.457916
C	-3.28823	-4.4773	-1.46658
C	-5.49188	-0.84336	1.459316
C	-4.47739	3.285689	-1.46353
C	-0.84136	5.482507	1.46721
C	3.287725	4.484635	-1.46029
C	5.489433	0.845071	1.462528
H	4.752987	3.46125	0.589514
H	3.367587	2.78353	1.460555
H	5.80164	-0.90961	-0.59358
H	4.342195	-0.40598	-1.46166
H	2.776676	-3.35818	1.45229
H	3.455014	-4.74428	0.583015
H	-2.7765	3.357026	1.457718
H	-3.45508	4.745641	0.592661
H	0.413424	4.343607	-1.45873
H	0.914216	5.802451	-0.58789
H	-0.91529	-5.79873	-0.5963
H	-0.41459	-4.3388	-1.46545
H	-4.34541	0.412689	-1.46301
H	-5.80392	0.915153	-0.59254
H	-4.75168	-3.45648	0.586814
H	-3.36504	-2.77881	1.455941
H	4.637706	-2.43712	-2.12251
H	3.787551	-3.93729	-1.97063
H	-0.11157	-5.46219	1.957484
H	1.550295	-5.00267	2.110758
H	-2.44792	-4.64027	-2.11995
H	-3.94736	-3.78906	-1.9664
H	-5.47092	0.109257	1.959328
H	-4.64097	2.446339	-2.118
H	-3.78971	3.945609	-1.96301
H	0.111304	5.459811	1.967016
H	-1.55103	5.001577	2.118851
H	2.44707	4.64766	-2.11319
H	3.947261	3.797456	-1.96105
H	5.464545	-0.1071	1.963199
H	5.013023	1.557164	2.114801
H	-5.01281	-1.55301	2.112368
C	4.002178	5.811852	-1.22181
H	4.867628	5.673556	-0.58232
H	3.338723	6.523933	-0.74203
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H	1.094562	-8.30613	7.132023
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C	5.449897	7.850288	-3.99308
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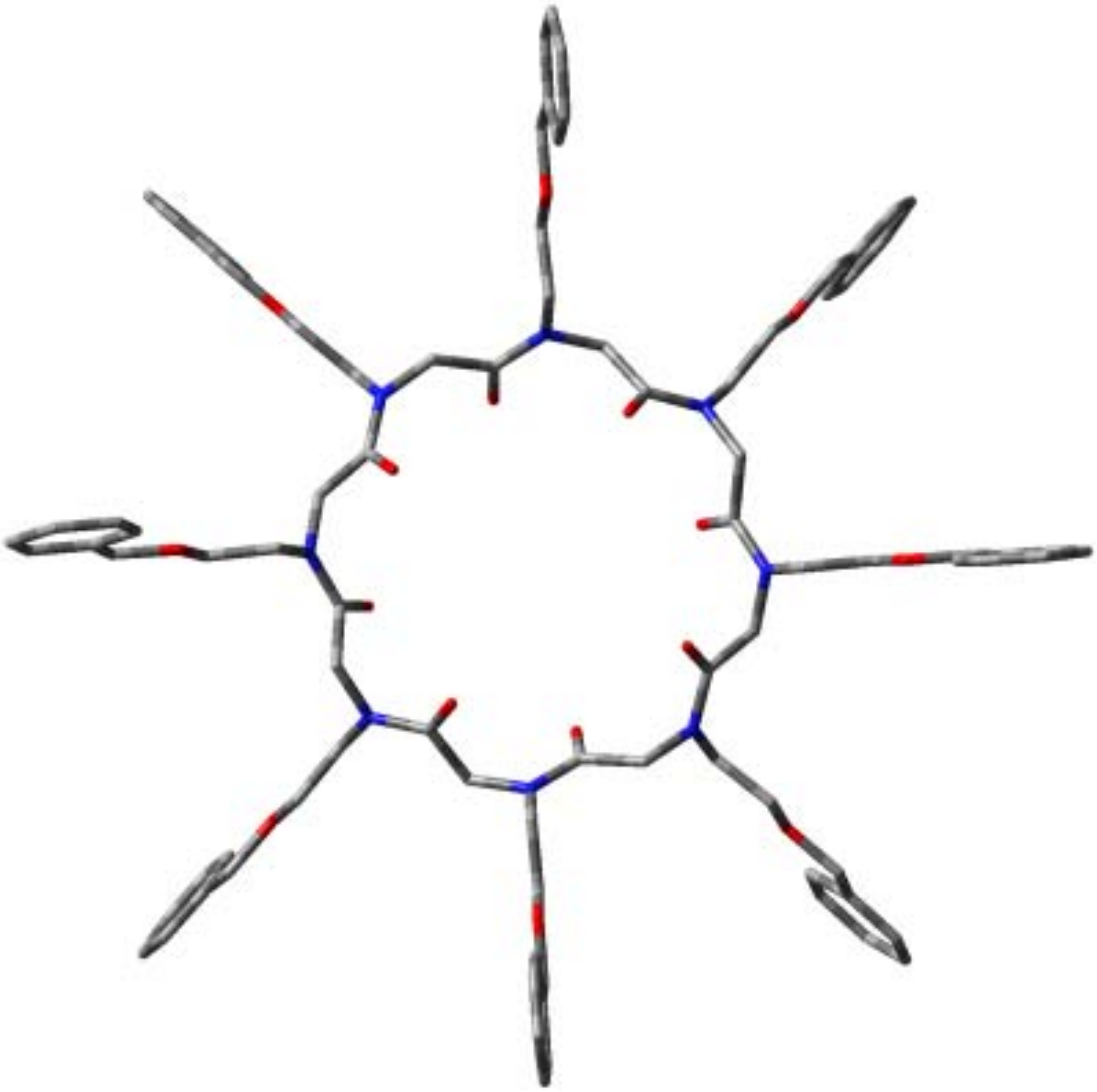


Figure S11. Structure of **3** from computational studies. Hydrogen atoms omitted for clarity.

6.0 Extraction studies

7.1 Determination of binding affinities for compound 3 and 4

Association constants K_{ass} were calculated from the equation $K_{ass} = K_e/K_d$, according to the methodology reported by Cram and coworkers.[4] K_d values, which represents the distribution constants of the picrate salts between water and $CHCl_3$, were previously determined by Cram,[4] while K_e were calculated following the "ultraviolet method" reported by Cram and coworkers.[5] All ultraviolet (UV) measurements were made on a Beckman DU 640 Spectrometer at 380 nm at 24-26 °C, using spectrophotometric grade solvents. The picrate salts were prepared according to literature procedures,[6] and dried under high vacuum before use. 0.015 M for the Li^+, Na^+, K^+, NH_4^+ and 0.010 M for Rb^+ and Cs^+ salts aqueous picrate solutions were prepared. Aliquots of these solutions (250 μL of Li^+, Na^+, K^+, NH_4^+ and 375 μL of Rb^+ and Cs^+ solutions) were introduced in six Eppendorf vials, and to each of these, 250 μL of a solution 0.015 M of the host in $CHCl_3$ was added. The vials were capped (in order to prevent evaporation) and mixed thoroughly, using a Vortex "Maxi Mixer", for five minutes. They were finally centrifuged for 3 min, and after separation of the two phases. Aliquots of 50 μL of aqueous solution were pipetted into 5.0 mL of volumetric flasks which were brought to the mark with CH_3CN. Successively 200 μL of these solutions were diluted in 800 μL of CH_3CN. Aliquots of 100 μL of the organic phase were removed from each phase with a Hamilton syringe and diluted in 5.0 mL of CH_3CN. Successively 200 μL of this solution were diluted in 800 μL of CH_3CN. The absorbance of each sample was then measured against the appropriate blank solution at 380 nm at 25 °C. R, K_e, K_{ass} and $\Delta G°$ were thus calculated in the proper way.[4]

7.0 Ion transport studies

7.1. General Procedures

L-α-phosphatidyl-DL-glycerol sodium salt (EYPG, 20 mg/mL chloroform solution) and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, 20 mg/mL chloroform solution) were purchased from Avanti Polar Lipids; egg yolk phosphatidylcholine (EYPC, 100 mg/mL chloroform solution), and 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) were from Sigma; Triton® X-100 and HEPES buffer were purchased from Fluka; all salts were of the best grade available from Aldrich and were used without further purification.

Size exclusion chromatography (SEC) was performed using pre-packed columns Sephadex™ G-25 M (PD-10) from Amersham Biosciences.

Liposomes were prepared by extrusion using a 10 mL Lipex™ Thermobarrel EXTRUDER (Northern Lipids Inc.) connected to a thermostatic bath (25°C if not otherwise indicated). The 100 nm polycarbonate membranes are Nucleopore Track-Etch Membranes from Whatman.

Fluorescence spectra were recorded on Perkin-Elmer LS-50B fluorimeter and ^{23}Na-NMR-spectra on Jeol GX-270 spectrometer (270 MHz).

Fluorimetric experiments were conducted at 25°C if not otherwise indicated, ^{23}Na-NMR experiments were conducted at 27°C.

The ionophores concentration is given in percent with respect to the total concentration of lipids. Mother solutions of ionophores were prepared in methanol. Control experiments showed that the amount of methanol added to the vesicular suspension in the different experiments (maximum amount 1.0 % in volume) did not affect the permeability of the membrane.

7.2 HPTS assay.[6] A mixture of 225 μL of EYPC chloroform solution (100 mg/mL, 30 μmol) and 60 μL of EYPG chloroform solution (20 mg/mL, 1.5 μmol) was first evaporated with Ar-flux to form a thin film and then dried under high vacuum for 3 h. The lipid cake was hydrated in 1.5 mL of 0.1 mM HPTS solution (HEPES 25 mM, 100 mM NaCl, pH 7) for 30 min at 40°C. The lipid suspension was submitted to 5 freeze-thaw cycles (-196°C/40°C) using liquid nitrogen and a thermostatic bath, and then extruded under nitrogen pressure (15 bar) at room temperature (10 extrusions through a 0.1 μm polycarbonate membrane). The LUV suspension was separated from extravesicular dye by size exclusion chromatography (SEC) (stationary phase: pre-packed column Sephadex™ G-25, mobile phase: HEPES buffer) and diluted with HEPES buffer to give a stock solution with a lipid concentration of 5 mM (assuming 100% of lipids were incorporated into liposomes). 104 μL of the lipid suspension were placed in a fluorimetric cell, diluted to 3040 μL with the same buffer solution used for the liposome preparation and kept under gently stirring. The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of methanolic solution of the ionophore (5-30 μL of the appropriate mother solution in order to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25°C for 30 min. After incubation the time course of fluorescence was recorded for 200 s (λ_{ex} 460 nm, λ_{em} 510 nm) and then 50 μL of 0.5 M NaOH were rapidly added through an injector port and the fluorescence emission was recorded for 1200 s. Maximal changes in dye emission were obtained by final lysis of the liposomes with detergent (40 μL of 5% aqueous solution Triton® X-100). Fluorescence time courses were normalized using the following equation:

$$FI = \frac{(F^t - F^0)}{(F^\infty - F^0)} \cdot 100$$

where F^t is the fluorescence intensity measured at time t, F^0 is the fluorescence intensity at ionophore addition, F^∞ is the fluorescence intensity at saturation after lysis with Triton. The apparent first order

rate constants for the transport process were obtained by non-linear regression analysis of the fluorescence data vs. time. The fit error on the rate constant was always less than 1%.

7.3 Determination of cation and anion selectivity with the HPTS assay (Matile's protocol).[7]

The vesicle suspension (104 μL stock solution, prepared as described above, point 2.1) was placed in a fluorimetric cell and diluted to 3040 μL with the appropriate buffer solution (25 mM HEPES, 100 mM MCl with M = Li^+, Na^+, K^+, Rb^+, Cs^+, or 100 mM NaX with X = Cl^-, Br^-, I^-, NO_3^-, ClO_4^-, SO_4^{2-}, pH 7). The total lipid concentration in the fluorimetric cell was 0.17 mM. An aliquot of methanolic solution of the ionophore (5-30 μL of the appropriate mother solution in order to obtain the desired $mol_{compound}/mol_{lipid}$ ratio) was then added to the lipid suspension and the cell was incubated at 25°C for 30 min. After incubation the time course of fluorescence was recorded for 200 s (λ_{ex} 460 nm, λ_{em} 510 nm) and then 50 μL of 0.5 M MOH (with M = Li^+, Na^+, K^+, Rb^+, Cs^+ depending on the cation present in the extravesicular buffer solution) were rapidly added through an injector port and the fluorescence emission was recorded for 1200 s. Maximal changes in dye emission were obtained by final lysis of the liposomes with detergent (40 μL of 5% aqueous solution Triton® X-100). Fluorescence time courses were normalized as previously described and corrected for the permeation of the anion or cation under investigation in the absence of ionophore by subtracting the relative fluorescence time course.

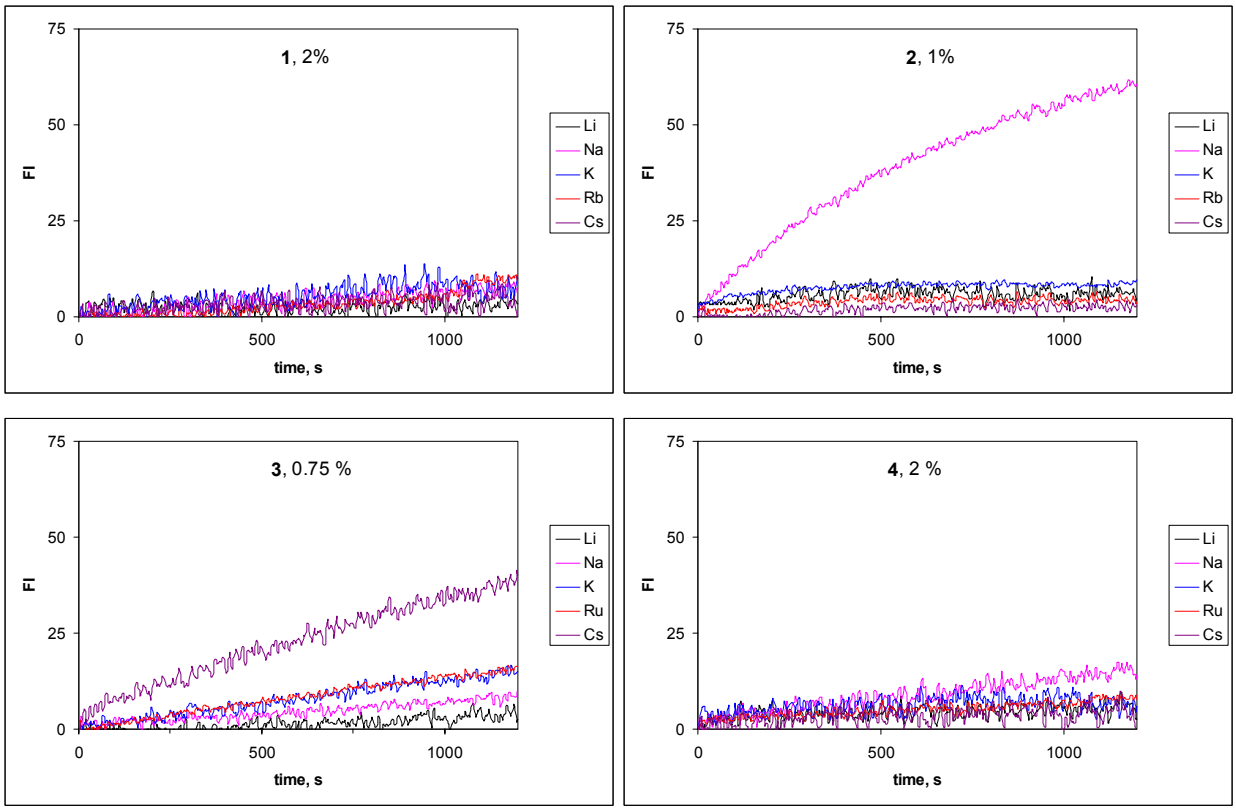
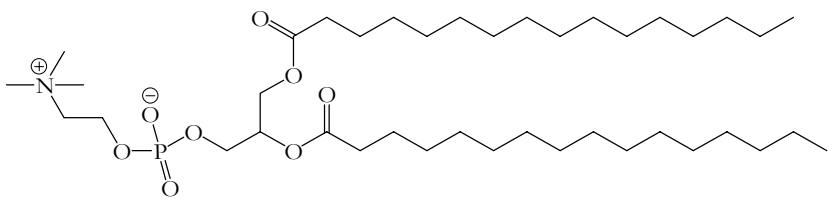


Figure S12. Normalize fluorescence time courses for cyclopeptoids **1-4** in the HPTS assay in the presence of the different alkaline cations (100 mM MCl, pH 7.0, base pulse by addition of 50 µL of 0.5 M MOH). The concentration of cyclopeptoid is given in the Figure. The kinetic traces are corrected for the permeation of the cation under investigation in the absence of ionophore.

7.4 HPTS assay with membranes in gel-phase.[8,9]

The vesicle suspension was prepared as described above (point 7.2) starting from 1155 µL of DPPC (Tc = 41 °C) chloroform solution (20 mg/mL, 31.5 µmol); swelling, thaw and extrusion cycles were performed at 50°C (above T_c). The kinetic traces were registered at 25°C (below Tc) and 45 °C (above Tc) as described above, after incubation of the ionophore in the cell at 50°C for 30 min. Fluorescence time courses were normalized as previously described.



DPPC structure.

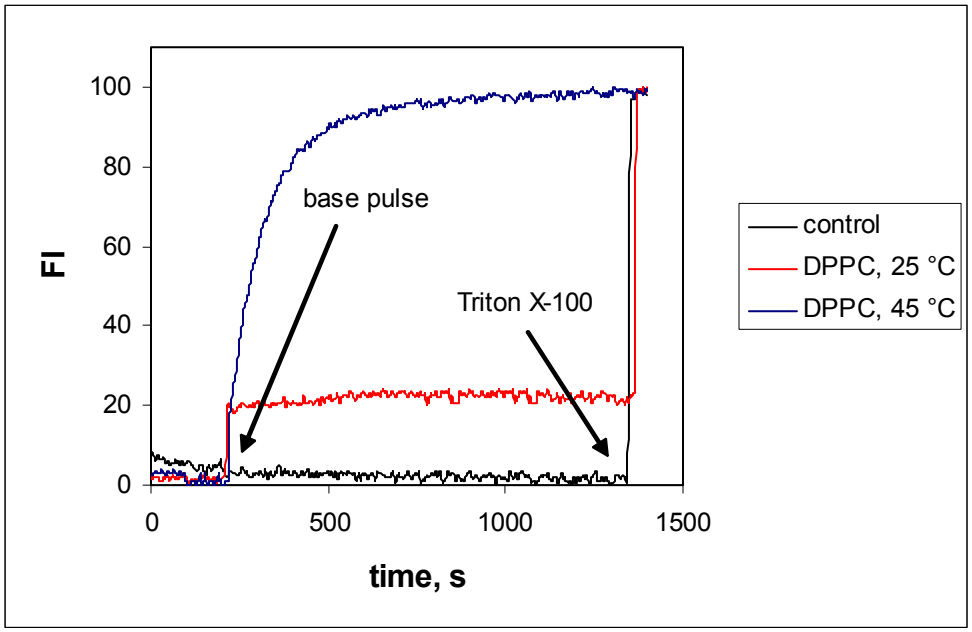


Figure S13. Normalized fluorescence change in HPTS emission (FI, λ_{ex} 460 nm, λ_{em} 510 nm) as a function of time after addition of the base (50 µL of 0.5 M NaOH) to DPPC LUVs loaded with HPTS (0.1 mM HPTS, 0.17 mM total lipid concentration, 25 mM HEPES, 100 mM NaCl, pH 7.0, total volume 3 mL), in the presence of 0.5 % **2**. The experiments have been performed at 25 °C when the membrane is in the gel-phase (red curve) and 45 °C when the membrane is in the fluid-phase (blue curve). The control kinetic trace in the absence of ionophore (black curve) has been recorded at 25 °C. Control experiments have shown that the initial fluorescence jump of about 20 FI units is due to a partial release of HPTS from the liposomes during the incubation of the ionophore at 50 °C. The arrows in Figure show the time of the base pulse (200 s) and of the addition of Triton X-100 (1350 s) to lise the liposome.

7.5. ^{23}Na-NMR transport assays.[10]

A mixture of 150 µL of EYPC chloroform solution (100 mg/mL, 20 µmol), 40 µL of EYPG chloroform solution (20 mg/mL, 1 µmol) and an aliquot of methanolic solution of the desired ionophore was first evaporated with Ar-flux to form a thin film and then dried under high vacuum for 3 h. The lipid film was hydrated in 1 mL of LiCl solution (100 mM in H_2O/D_2O 90:10) for 30 min at 40°C. The lipid suspension was submitted to 5 freeze-thaw cycles (-196°C/40°C) using liquid nitrogen and a thermostatic bath, and then extruded under nitrogen pressure (15 bar) at room temperature (10 extrusions through a 0.1 µm polycarbonate membrane).

350 μL of the lipid dispersion and 350 μL of a *shift reagent* solution (4 mM $DyCl_3 \cdot 6H_2O$, 12 mM $Na_5P_3O_{10}$, 40 mM NaCl in H_2O/D_2O 90:10) were mixed in a 5 mm NMR-tube. During the experiment, spectra were recorded every 30 min over 14 h (accumulation *in continuo*) and the areas of the signals were calculated with Jeol Delta software. The % of Na^+ inside liposomes was from the measured liposome entrapped volume (1.5 %).

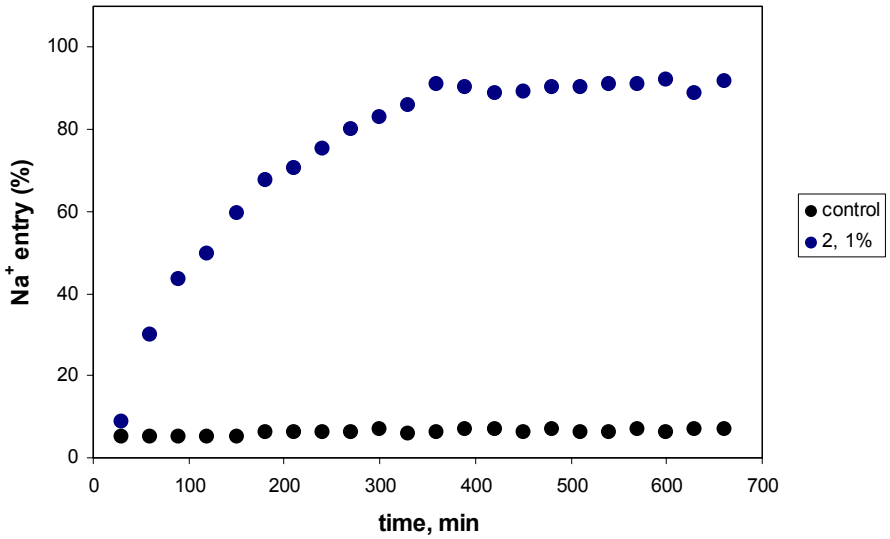


Figure S14. Kinetic profiles for the entry of Na^+ into 95:5 EYPC/EYPG vesicles containing **2** (1%, ●) and without additives (●) at 27 °C. The total concentration of lipids was 10.5 mM. These results show that ionophore **2,** under the reported conditions, is able to transport efficiently Na^+ across the lipid bilayer .

8.0 References

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