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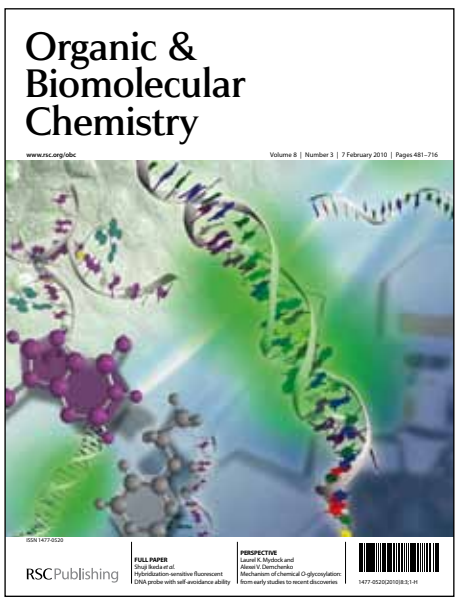

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ARTICLE

Gadolinium-binding cyclic hexapeptoids: synthesis and relaxometric properties

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Two new cyclic hexapeptoids incorporating *N*-carboxylethylglycine and *N*-methoxyethylglycine residues are able to efficiently bind Gd^{3+}. Their thermodynamic stabilities and relaxivities have been assessed by ^{1}H-relaxometric investigations.

Introduction

The chemical and topological diversity of peptidomimetics[1] offers thrilling avenues for the invention of novel scaffolds with unprecedented properties and structural features. Peptoids, or oligomers of *N*-substituted glycines,[2] represent a promising class of biocompatible compounds recapitulating functions and capabilities of natural polypeptides while retaining *in vivo* metabolic stability and remarkable structural diversity.

One of the most intriguing features of peptoids is their capacity to generate cyclic structures for their exalted folding abilites.[3] The properties of cyclic peptoids are multitudinous. Among those, it is worth noting the high affinity for the first group alkali metals ($K_a \sim 10^6$ for Na^+, Li^+ and K^+),[4] which consistently alter the peptoid backbone morphology and dictate highly symmetric conformations.

The intrinsic aptitude of linear[5] and cyclic[4c,d] α-peptoids to perform metal complexation in solution and generate elegant metallated supramolecular assemblies in the solid-state,[4a,d] prompted us to consider Gd^{3+} complexation. The study of the relaxometric behavior of paramagnetic Gd^{3+} complexes is in fact useful to extract information on the chemico-physical and structural features of the metal/ligand systems and could represent a starting point for the identification of new imaging probes.

In this work we report our investigations on the synthesis and the conformational properties displayed by the water-soluble hexa- and tricarboxylated free and Na^+ complexed cyclic hexapeptoids **1** and **2** (Fig. 1). We also describe the formation of Gd^{3+} complexes and their relaxometric characterization.

Results and Discussion

Design and synthesis of the peptoid ligands

The last two decades have witnessed a great interest in the design of Gd^{3+} complexes because of their potential use as MRI contrast agents.[6]

Several chelate complexes have been explored and clear advantages have been observed in the use of macrocyclic vs. linear ligands concerning the thermodynamic and kinetic stabilities. As expected for hard Gd^{3+} ions, carboxylate moieties are particularly suitable ligands to attain stable coordination environments either when they are part of polyaminocarboxylate systems[7] or they are from Asp or Glu residues from peptides[8] or other macromolecules.[9] The carboxamide functionality has also been shown to be a good coordinating moiety for Gd^{3+} ions, in particular when it is part of a macrocyclic ligand.[6,7]

With the aim of extending the knowledge about new frameworks potentially able to coordinate Gd^{3+} ions, two highly polar cyclic peptoids have been synthetized (**1** and **2** in Fig. 1). In addition to the basic peptoid structures, the two ligands are characterized by the presence of six and three carboxyl functionalities, respectively. In **2**, the carboxylate bearing moieties are mixed with ether-bearing arms.

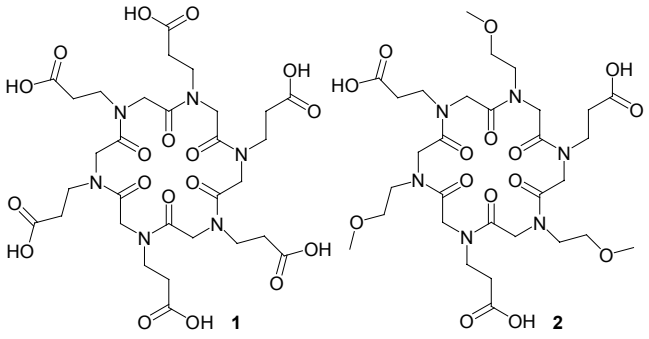


Fig. 1 Structural formulas of cyclic peptoids **1** and **2**.

In principle, ligand **1** and **2** can coordinate Gd^{3+} ions in several ways. Analysis of crystallographic data for $Gd^{3+}L_9$ coordination complexes, in the absence of topological constraints, shows that the D_{3h}-symmetric tricapped trigonal prism (TTP) is the most favorable polytopal form.[10] Moreover, the Gd^{3+} cation is highly oxophilic and the binding with oxygen donors is preferred to the mixture of nitrogen and oxygen donors, offered by aminocarboxylate ligands.[11]

For their peculiar three-dimensional spatial orientation, the amide carbonyls protruding from the eighteen-membered cyclic peptoids scaffold can, in principle, perfectly saturate six orbitals of the idealized TTP geometry as reported in Fig. 2. The coordination sphere of Gd^{3+} cation could be completed by three molecules of water. This hypothesized highly symmetric Gd^{3+} coordination excludes the involvement of the peripheral carboxyl or methoxyl moieties, which retain the sole role of improving the water solubility. The short C_2 chains linking the carboxilates or the methoxy groups to the macrocycle should, in principle, hamper their chelation to the metal centre.

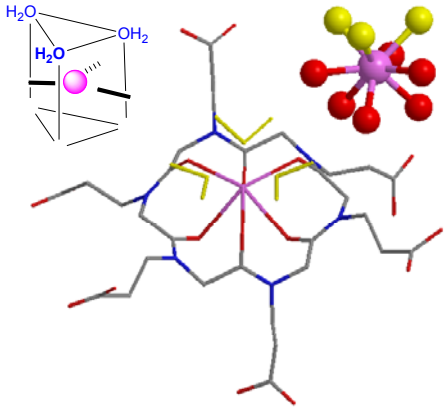
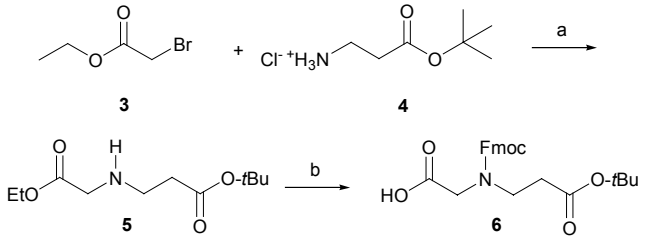


Fig. 2 Centre: idealized model of **1** (hydrogen atoms have been omitted for clarity from **1**, Chem3D® output) complexed with Gd^{3+} including three molecules of water (in yellow). Atom type: C grey, N blue, O red, Gd magenta. Top left: idealized tricapped trigonal prismatic coordination geometry for the Gd^{3+}, including three molecules of H_2O. Top right: geometry of nonacoordinated Gd^{3+}. Ligand type: C=O red, H_2O yellow.

Peptoid **1** and **2** were obtained through solid-phase synthesis. The *N*-fluorenylmethoxycarbonyl,*N*'-carboxymethyl-β-alanine *t*-butyl ester (**6**) building block was easily synthesized through *N*-alkylation and straightforward protecting group processing as shown in Scheme 1 (see ESI†).



Scheme 1. Synthesis of *N*-fluorenylmethoxycarbonyl,*N*'-carboxymethyl-β-alanine **6**. Reagents and conditions: (a) DIPEA; DMF, r.t., 18 h; (b) LiOH, H_2O/1,4-dioxane (1:1), 0 °C, 2h, then $NaHCO_3$, Fmoc-Cl, r.t., 18 h, 65%, three steps.

The on-resin oligomerization was accomplished through a "monomer" and a mixed "submonomer/monomer" approach and gave the linear *N*-*t*-butoxycarbonylethyl/*N*-methoxyethyl glycine oligomer **7** and **8** (Fig. 3, see ESI† for the overall synthetic scheme).

Both the oligomers were prepared with considerable purity (>90%, RP-HPLC) in the presence of the guanidinium- or carbodiimide-type coupling reagents (HATU or DIC, respectively; average coupling yields >98%).

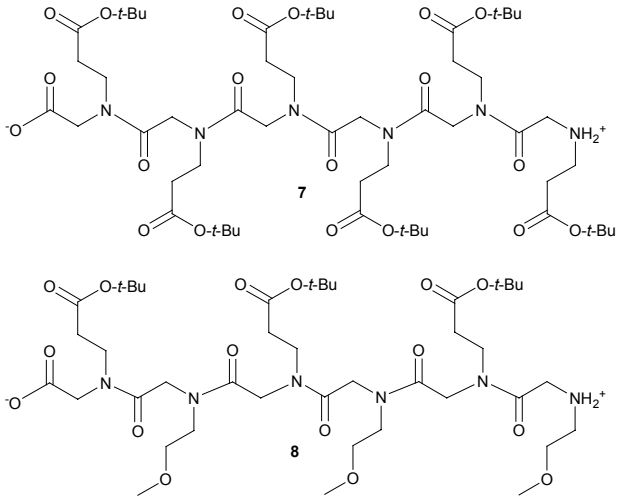


Fig. 3 Linear precursors **7** and **8**.

Linear peptoids **7** and **8** were efficiently cyclized (high dilution conditions: $3.0 \cdot 10^{-3}$ M) with the assistance of HATU to give the protected cyclic peptoids **9** and **10** in good yields (purity >85%, RP-HPLC analysis).

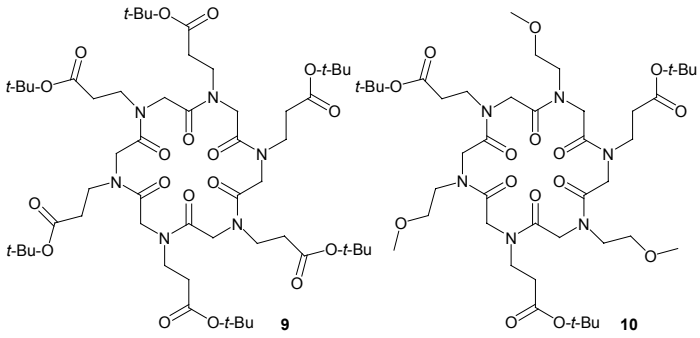


Fig. 4 Structures of cyclic precursors **9** and **10**.

The protected cyclic precursors were then liberated by the *t*-butyl groups with trifluoroacetic acid and *m*-cresol, as cation scavenger, to afford the target compounds **1** and **2**. The complexity of the r.t. ^1H NMR spectrum recorded for the cyclic precursors (**9**/**10**) and target peptoids (**1**/**2**), demonstrated the slow exchange of multiple conformations on the NMR time scale (see ESI†).

In order to evaluate their ability to form metal compexes, complexation studies in the presence of Na^+ cation (Na^+ and Gd^{3+} show similar ionic radius) were performed (^1H NMR

analysis). For all of the studied compounds (**1**,**2** and **9**,**10)**, the addition of an excess amount of sodium picrate induced the formation of complexes with remarkably simplified ^{1}H NMR spectra. In particular, it was evident the formation of a 6-fold symmetric species for the complex with **1**/**9 (**see ESI†), and a 3-fold symmetric species for the complex with **2**/**10 (**see ESI†). Demonstration of efficient Na^+ coordination set the stage for the next steps: the formation of Gd^{3+} chelates and their relaxometric characterization.

Relaxometric properties of Gd^{3+} complexes of 1 and 2

It is well established that information on the solution structures of Gd^{3+} complexes from the acquisition of high resolution ^{1}H and ^{13}C NMR spectra is hampered by the extensive broadening of the ligand resonances. This behavior relies on the large μ_{eff} and the long electronic relaxation time of the Gd^{3+} ion. Therefore, it has become rather customarily to extract structural (and dynamic) information on the Gd^{3+} complexes by investigating the relaxometric properties of their aqueous solutions. Relaxometric studies deal with the measurement of water proton relaxation times that report about the reversible interaction of water molecules in the inner and outer coordination sphere of the paramagnetic Gd^{3+} complexes. The theory of paramagnetic relaxation is well established[12] and it is possible to relate the observed relaxation enhancement of the bulk water protons to structural and dynamic properties of the Gd^{3+} complexes.

To this purpose, we evaluated the ability of **Gd-1** and **Gd-2** to enhance the longitudinal relaxation rate of water protons of the solutions in which they are dissolved. It is possible to follow the complex formation through the relaxometric titration of a given quantity of ligand with increasing amounts of $GdCl_3$. As depicted in Fig. 5S (see ESI†), by measuring the observed relaxation rate (R_1^{obs}= $1/T_1^{obs}$) at 20 MHz (Proton Larmor Frequency) and 25 °C of solutions of ligands **1** and **2** as a function of increasing concentrations of added $GdCl_3$ a straight line is obtained which slope is the relaxivity (r_{1p}) of the readly formed **Gd-1** and **Gd-2** complexes (equation 1):

$$R_1^{obs} = R_{1W} + r_{1p}[Gd\text{-}L] \qquad (1)$$

Where R_{1W} is the diamagnetic contribution of pure water.

After the ligand saturation point, when the added Gd^{3+} is in excess with respect to the ligand, the slope of the line changes to follow the relaxivity of the free Gd^{3+} aqua ion (r_{1p} = 12.98 $mM^{-1}s^{-1}$ at 20 MHz and 25°C). From this experiment relaxivity values (at 20 MHz and 25 °C) of 31.5 $mM^{-1}s^{-1}$ and 27.2 $mM^{-1}s^{-1}$ were determined for **Gd-1** and **Gd-2**, respectively. The relaxivity value is proportional to the efficiency of the paramagnetic complex as relaxation agent and, for the two investigated complexes, they resulted to be significantly higher than those reported for the commercial Gd^{3+} complexes used in the clinical practice (4-6 $mM^{-1}s^{-1}$).[7,13]

A relaxometric procedure was also used in order to estimate the stability constants of **Gd-1** and **Gd-2** complexes. A competition study with another ligand (EDTA) which forms with Gd^{3+} a complex of known K_f (5.01×10^{17})[7] was carried out by measuring the observed longitudinal relaxation rate (at 20 MHz and 25°C) of **Gd-1** and **Gd-2** solutions as a function of the addition of increasing concentrations of EDTA (Fig. 5). The variation in the observed relaxation rate is due to the transfer of the Gd^{3+} ion from ligand **1** (or **2**) to EDTA. By knowing the stability constant of Gd-EDTA and the relaxivities of the Gd^{3+} complexes (**Gd-1** or **Gd-2** and Gd-EDTA / r_{1p} = 7.6 $mM^{-1}s^{-1}$)[14] it is possible to fit these experimental data in order to extract the value of the unknown K_f of the investigated Gd^{3+} complexes (see ESI† for a complete description of the equations involved). Stability constants of $8.84\times10^{15}\pm 5.08\times10^{15}$ ($loK_{Gd\text{-}L}$=15.95±0.38) and $3.65\times10^{14}\pm1.04\times10^{14}$ ($loK_{Gd\text{-}L}$=14.56±0.13) were found for **Gd-1** and **Gd-2**, respectively.

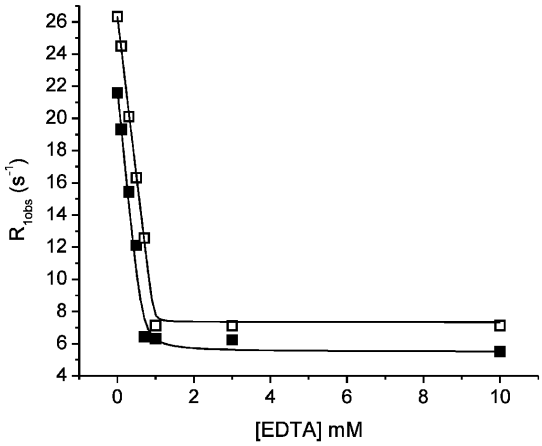


Fig. 5 Variation of the observed longitudinal relaxation rate of solutions of **Gd-1** 0.673 mM (■) and **Gd-2** 0.954 mM (□) as a function of the addition of increasing concentrations of EDTA. Measures were carried out at 25°C, 20 MHz and neutral pH.

For a better understanding of the determinants of **Gd-1** and **Gd-2** relaxometric properties, an analysis of the variation of the relaxivity as a function of the applied magnetic field was carried out. By plotting the relaxivity values as a function of the proton Larmor frequency on a logarithmic scale the so called NMRD (Nuclear Magnetic Resonance Dispersion) profiles are obtained (Fig. 6). Fitting of the experimental data with the equations accounting for the paramagnetic relaxation theory (Solomon-Bloembergen-Morgan equations) it is possible to extract important information on the main parameters governing the relaxation processes (table 1).[15]

The values of the parameters involved in the electronic relaxation (Δ^2 and τ_v) are very similar to each other and to those reported for other Gd^{3+} complexes.[7,14]

The value of the reorientational correlation time (τ_R) depends on the size/molecular weight of the investigated system: the bigger the system, the slower its reorientation. This slowed reorientational motion is the cause of increased relaxivity in the magnetic field range 20-60 MHz.

Table 1 Main relaxometric parameters derived from fitting of NMRD profiles reported in Figure 6.[a]

	Δ^2 (s^{-2})[b]	τ_v[c] (ps)	τ_R[d] (ps)	τ_M[e](s)	q[f]	q_{ss}[g]
Gd-1	2.1×10^{19}	27.5	280	1×10^{-8}	3	15
Gd-2	2.7×10^{19}	22.5	226	1×10^{-8}	3	15

[a] On carrying out the fitting procedure, some parameters were fixed to reasonable values: rGd-H (distance between Gd and protons of the inner sphere water molecule) = 3.1 Å; a (distance of minimum approach of solvent water molecules to Gd ion) = 3.8 Å; D (solvent diffusion coefficient) = $2.2\cdot10^{-5}$ cm^2 s^{-1}. [b] Squared mean transient zero-field splitting (ZFS) energy. [c] Correlation time for the collision-related modulation of the ZFS Hamiltonian. [d] Reorientational correlation time. [e] Exchange life-time of the coordinated water molecule (fixed). [f] Number of inner sphere water molecules. [g] Number of second sphere water molecules.

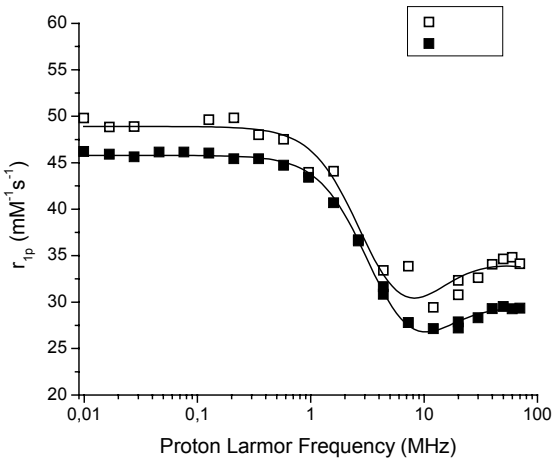


Fig. 6 $1/T_1$ NMRD profiles of **Gd-1** (□), and of **Gd-2** (■), recorded at 25 °C in PBS buffer (pH 7.4). The data refer to 1 mM concentration of the paramagnetic complex.

The difference in τ_R measured for the two complexes reflects the difference in the respective molecular weights. Finally, a good fitting of the experimental data has been found considering three inner sphere (directly coordinated to the Gd^{3+} ion) and fifteen second sphere (kept in the proximity of the paramagnetic ion through hydrogen bonds) water molecules for both **Gd-1** and **Gd-2** complexes. The fact that both **Gd-1** and **Gd-2** display very similar values for q and the other determinants of the observed relaxivities, strongly suggests that the two ligands coordinate the metal in a similar way. Thus, the carboxyl- and ether- bearing arms do not appear to enter the coordination sphere of the Gd^{3+} ion in both complexes. It seems reasonable that the binding involves only the six oxygen donor atoms from the cyclic peptoid leaving three coordination sites available for the coordination of water molecules (as shown in Fig. 2). It is worth noting that such coordination, based only on neutral carboxamide moieties, yields the remarkable value of ca. 10^{16} for the thermodynamic stability of **Gd-1** metal complex.

Conclusions

We have described the design, synthesis, complexing properties and relaxivity values of the two new cyclic hexapeptoids **1** and **2** incorporating *N*-carboxylethylglycine and *N*-methoxyethylglycine residues with Gd^{3+} ions. The measured values for relaxivity in aqueous medium at 20 MHz proton Larmor frequency are particularly high thanks to the conspicuous hydration (q=3) of the complexes. Interestingly, the coordination offered by the cyclic peptoids yields Δ^2 and τ_V values quite similar to the values reported for DOTA-like macroclyclic complexes.

Hence, such cyclopeptoids-based complexes are unique examples of water soluble Gd^{3+} hexadentated chelates demonstrating interesting hydratation numbers, excellent relaxivity values and good stabilities and could be useful as starting point for the identification of new imaging probes.

Attempts to crystallize and solve the solid state structure of **Gd-1** and **Gd-2** are also being made in order to better understand the coordination geometry around the metal. The results will appear in due course.

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Experimental

Reagents purchased from commercial sources were used without further purification. Analytical HPLC analyses were performed on a Jasco PU-2089 quaternary gradient pump equipped with an MD-2010 plus adsorbance detector using C_{18} (Waters, Bondapak, 10 μm, 125Å, 3.9 × 300 mm), reversed phase columns. The NMR spectra were recorded on Bruker DRX 400, (^{1}H at 400.13 MHz, ^{13}C at 100.03 MHz), Bruker DRX 300 (^{1}H at 300.1 MHz, ^{13}C at 75.5 MHz) and Bruker DRX 250 (^{1}H at 250.13 MHz, ^{13}C at 62.89 MHz) spectrometers. Chemical shifts (δ) are reported in ppm relatively to the residual solvent peak ($CHCl_3$, δ = 7.26, $^{13}CDCl_3$, δ: = 77.0; CD_2HCN: δ = 1.98; $^{13}CD_3CN$: δ = 1.80, $CF_3{}^{13}COOD$: δ = 164.0: centre of the quartet; 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; 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. The longitudinal water proton relaxation rate was measured at 25 °C by using a Stelar Spinmaster (Stelar, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery technique. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper constantan thermocouple (uncertainty 0.1 °C). The relaxometric characterization of the field-dependent relaxometry of the paramagnetic Gd^{3+}-probe solutions was carried out through the acquisition of the NMRD profiles. The proton $1/T_1$ NMRD profiles were measured at 25 °C on a fast field-cycling Stelar relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.47 T (corresponding to 0.01-20 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ±1%. Additional data points in the range 20-70 MHz were collected on the Stelar Spinmaster spectrometer

_N_-ethoxycarbonylmethyl-β-alanine _t_-butyl ester (5)

To a stirred solution of the commercially available *tert*-butyl 3-aminopropanoate hydrochloride **4** (3.00 g, 16.5 mmol) and DIPEA (5.74 mL, 33.0 mmol) in DMF dry (15.0 mL), ethyl bromoacetate (0.915 mL, 8.25 mmol) was slowly added. The reaction mixture was stirred overnight, concentrated in vacuo to remove the excess of DMF, dissolved in CH_2Cl_2 (100.0 mL) and washed with brine solution. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated in vacuo to give crude **5** (2.4 g, yellow amorphous solid), which was used in the next step without further purification. Crude **5**: ^{1}H NMR (300.1 MHz, $CDCl_3$) δ 1.31 (3H, t, *J* 7.1 Hz, C$\underline{H}_3$CH$_2$), 1.47 (9H, s, C(C$\underline{H}_3$)$_3$), 2.74 (2H, t, *J* 7.0 Hz, NHCH$_2$C$\underline{H}_2$CO), 3.09 (2H, t, *J* 7.0 Hz, NHC$\underline{H}_2$CH$_2$CO), 3.65 (2H, s, OCC$\underline{H}_2$NH), 4.27 (2H, q, *J* 7.1 Hz, CH$_3$C$\underline{H}_2$); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 28.0, 33.5, 44.3, 49.1, 61.7, 81.4, 168.9, 170.8.

_N_-fluorenylmethoxycarbonyl,_N_'-carboxymethyl-β-alanine _t_-butyl ester (6)

To a stirred solution of crude **5** (2.4 g) in 1,4-dioxane (30.0 mL) at 0 °C, LiOH•H_2O (0.932 g, 22.2 mmol) in H_2O (30.0 mL) was added. After two hours, $NaHCO_3$ (1.52 g, 18.1 mmol) and Fmoc-Cl (3.20 g, 10.4 mmol) were added. The reaction mixture was stirred overnight. Subsequently, $KHSO_4$ was added (until pH=3) and the mixture was concentrated in vacuo to remove the excess of 1,4-dioxane and dissolved in CH_2Cl_2 (100.0 mL). The aqueous layer was extracted with CH_2Cl_2 (three times). The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated in vacuo to give a crude material (4.0 g, yellow amorphous solid). The residue was purified by flash chromatography (CH_2Cl_2/CH_3OH, from: 100/0 to 98/02, 0.1% AcOH) to give **6** (2.28 g, 65%, three steps). ^{1}H NMR (400.13 MHz, $CDCl_3$, mixture of two rotamers) δ 1.44 (4.05H, s, C(C$\underline{H}_3$)$_3$, rotamer b), 1.46 (4.95H, s, C(C$\underline{H}_3$)$_3$, rotamer a), 2.32 (1.1H, t, *J* 6.4 Hz, NCH$_2$C$\underline{H}_2$CO, rot. a) 2.58 (0.9H, t, *J* 6.0 Hz, NCH$_2$C$\underline{H}_2$CO, rot. b), 3.45 (1.1H, t, *J* 6.4 Hz, NC$\underline{H}_2$CH$_2$CO, rot. a), 3.58 (0.9H, t, *J* 6.0 Hz, NC$\underline{H}_2$CH$_2$CO, rot. b), 4.05 (0.9H, s, OCC$\underline{H}_2$N, rot. b), 4.13 (1.1H, s, OCC$\underline{H}_2$N, rot. a), 4.20 (0.45H, t, *J* 6.0 Hz, CH$_2$C$\underline{H}$[Fmoc], rot. b), 4.28 (0.55H, t, *J* 6.0 Hz, CH$_2$C$\underline{H}$[Fmoc], rot. a), 4.44 (0.9H, d, *J* 6.3 Hz, C$\underline{H}_2$CH[Fmoc], rot. b), 4.54 (1.1H, d, *J* 6.0 Hz, C$\underline{H}_2$CH[Fmoc], rot. a), 7.19-7.44 (4H, m, Ar-H[Fmoc]), 7.53 (0.9H, d, *J* 7.3 Hz, Ar-H[Fmoc], rot. b), 7.60 (1.1H, d, *J* 7.3 Hz, Ar-H[Fmoc], rot. a), 7.73 (0.9H, d, *J* 7.3 Hz, Ar-H[Fmoc], rot. b), 7.78 (1.1H, d, *J* 7.3 Hz, Ar-H[Fmoc], rot. a); ^{13}C NMR (62.89 MHz, $CDCl_3$, mixture of two rotamers) δ 27.9, 34.4, 34.6, 44.4, 45.1, 47.0, 49.7, 50.0, 67.6, 80.9, 119.8, 124.7, 125.2, 126.9, 127.0, 127.6, 128.1, 128.9, 141.2, 143.6, 155.6, 156.2, 171.1, 171.6, 174.9, 175.0; HRMS (ES) $[M+H]^+$, *m/z* found 426.1932. $C_{26}H_{32}NO_6^+$ requires 426.1911.

Solid-phase synthesis of peptoid 7 ("monomer" approach)

Linear peptoid **7** was synthesized using the standard manual Fmoc solid-phase peptide synthesis protocol. 0.20 g of 2-chlorotrityl chloride resin (Fluka; 2,α-dichlorobenzhydryl-polystyrene crosslinked with 1% DVB; 100-200 mesh; 1.20 mmol/g) was swelled in dry DCM (2.0 mL) for 45 min and washed twice in dry DCM (2.0 mL). Monomer **6** (0.068 g, 0.16 mmol) in dry DCM (2.0 mL) and DIPEA (0.11 mL, 0.64 mmol) were added on a shaker platform for 1.5 h at room temperature, followed by washing with dry DCM (3.0 mL) then with a mixture of DCM/MeOH/DIPEA (17:2:1) (2 × 3.0 mL) and finally with DMF (3 × 3.0 mL). Resin loaded with the first *N*-Fmoc *N*-alkylated glycine was incubated twice with 20% piperidine/DMF (v/v, 3.0 mL) on a shaker platform for 3 min and 7 min respectively, followed by extensive washes with DMF (3 × 3.0 mL), DCM (3 × 3.0 mL) and DMF (3 × 3.0 mL). The yields of the 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 >95%). After loading of the first monomer all subsequent addition of monomer **6** and Fmoc deprotection steps were performed as follow, until the desired oligomer length was obtained. The resin was incubated with a solution of monomer **6** (0.272 g, 0.64 mmol), HATU (0.236 g, 0.62 mmol), DIPEA (0.222 mL, 1.28 mmol) in dry DMF (2.0 mL) on a shaker platform for 1 h, followed by extensive washes with DMF (3 × 3.0 mL), DCM (3 × 3.0 mL) and DMF (3 × 3.0 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 4.0 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 4.0 mL of 20% HFIP in DCM (v/v) for 5 min, washed twice with DCM (3.0 mL), filtered away and the combined filtrates were concentrated in vacuo. A small amount of the the final product was dissolved in 50% ACN in HPLC grade water and analysed by RP-HPLC for analytical pourposes (eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 μm, 125 Å, 3.9 × 300 mm]). The purified peptoid was subjected to ESI mass spectrometry. Linear peptoid **7** was used in the cyclization step without further purification.

Linear peptoid 7: HPLC retention time: 18.1 min., purity: 91%; HRMS (ES) $[M+H]^+$, *m/z* found 1129.6474 $C_{54}H_{93}N_6O_{19}^+$ requires 1129.6490.

Solid-phase synthesis of peptoid 8 ("monomer/submonomer" approach)

Linear peptoid **8** was synthesized using the standard manual Fmoc solid-phase peptide synthesis protocol. 0.20 g of 2-chlorotrityl chloride resin (Fluka; 2,α-dichlorobenzhydryl-polystyrene crosslinked with 1% DVB; 100-200 mesh; 1.20 mmol/g) was swelled in dry DCM (2.0 mL) for 45 min and washed twice in dry DCM (2.0 mL). Monomer **6** (0.068 g, 0.16 mmol) in dry DCM (2.0 mL) and DIPEA (0.11 mL, 0.64 mmol) were added on a shaker platform for 1.5 h at room temperature, followed by washing with dry DCM (3.0 mL) then with a mixture of DCM/MeOH/DIPEA (17:2:1) (2 × 3.0 mL) and finally with DMF (3 × 3.0 mL). The resin was then incubated twice with 20% piperidine/DMF (v/v, 3.0 mL) on a shaker platform for 3 min and 7 min respectively, followed by extensive washes with DMF (3 × 3.0 mL), DCM (3 × 3.0 mL) and DMF (3 × 3.0 mL). Bromoacetylation reactions were accomplished by reacting the oligomer with a solution of bromoacetic acid (0.222 g, 1.6 mmol) and DIC (0.272 mL, 1.76 mmol) in DMF (3.0 mL) on a shaker platform 40 min at room temperature. Washes with dry DCM (3 × 2.0 mL) and then with DMF (3 × 2.0 mL) followed. To the bromoacetylated resin metoxyethylammine (0.138 mL, 1.6 mmol) in dry DMF (2.0 mL) was added. The mixture was left on a shaker platform for 40 min at room temperature then the resin was washed with DMF (3 × 2.0 mL). The resin was then incubated with a solution of monomer **6** (0.272 g, 0.64 mmol), HATU (0.236 g, 0.62 mmol), DIPEA (0.222 mL, 1.28 mmol) in dry DMF (2.0 mL) on a shaker platform for 1 h, followed by extensive washes with DMF (3 × 3.0 mL), DCM (3 × 3.0 mL) and DMF (3 × 3.0 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 bromoacetylation/amination reactions. The synthesis proceded until the desired oligomer length was obtained. The oligomer on the resin was then cleaved in 4.0 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 4.0 mL of 20% HFIP in DCM (v/v) for 5 min, washed twice with DCM (3.0 mL), filtered away and the combined filtrates were concentrated in vacuo. A small amount of the the final product was dissolved in 50% ACN in HPLC grade water and analysed by RP-HPLC for analytical pourposes (eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 μm, 125 Å, 3.9 × 300 mm]). Linear peptoid **8** was used in the cyclization step without further purification.

Linear peptoid 8: HPLC retention time: 15.1 min., purity: 92%; HRMS (ES) $[M+H]^+$, *m/z* found 919.5248 $C_{42}H_{75}N_6O_{16}^+$ requires 919.5234.

High dilution cyclization. Synthesis of cyclic peptoid 9

To a stirred solution of HATU (0.147 g, 0.388 mmol) and DIPEA (0.105 mL, 0.601 mmol) in dry DMF (39.0 mL) a solution of **7** (0.110 g, 0.097 mmol) in dry DMF (3.3 mL) at r.t., was added by syringe pump in 4 h. After 18 h the resulting mixture was concentrated in vacuo, diluted with DCM (20.0 mL) and a 0.1 N HCl solution (10.0 mL) was added. The mixture was extracted with DCM (3 × 20.0 mL) and the combined organic phases were washed two times with a 0.1 N HCl solution (10.0 mL) and once with water (10.0 mL), dried ($MgSO_4$) and concentrated in vacuo to give crude **9** (0.110 g). A small portion of cyclic peptoid was dissolved in 50% acetonitrile in HPLC grade water and analyzed by RP-HPLC.

Eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 μm, 125 Å, 3.9 × 300 mm]). Purity >85%; HPLC retention time: 20.2 min..

The crude residue was purified by HPLC on a C_{18} reversed-phase preparative column, conditions: 20%-100% B in 40 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 2.0 mL/min, 220 nm. The samples were dried in a falcon tube under low pressure and analyzed with mass spectrometry (zoom scan technique).

Cyclic peptoid 9 (0.068 g, yield: 63%, purity: 98%): ^{1}H NMR (300.1 MHz, $CDCl_3$, complex mixture of conformers) δ 1.44 (54H, b s, $C(C\underline{H}_3)_3$), 2.43-3.30 (24H, b m, $C\underline{H}_2C\underline{H}_2COOt$-Bu), 3.42-4.70 (12H, b m, N-$C\underline{H}_2$-CO); ^{13}C NMR (100.03 MHz, $CDCl_3$, complex mixture of conformers) δ 28.0, 29.6, 33.2, 33.7, 34.0, 34.1, 34.7, 43.8, 44.3, 44.6, 45.0, 45.3, 45.5, 45.7, 45.9, 46.8, 47.6, 47.7, 48.6, 49.0, 49.8, 50.0, 50.4, 51.0, 52.8, 53.2, 53.4, 80.6, 81.4, 167.6, 167.8, 167.9, 168.2, 168.3, 168.6, 168.9, 169.0, 169.1, 169.3, 169.4, 169.5, 169.9, 170.3, 170.4, 170.6, 170.7, 170.8, 170.9, 171.0, 171.1, 171.3, 171.4, 171.6, 171.7, 171.9; HPLC retention time: 20.2 min.; HRMS (ES) $[M+H]^+$, *m/z* found 1111.6395. $C_{54}H_{91}N_6O_{18}^+$ requires 1111.6384.

High dilution cyclization. Synthesis of cyclic peptoid 10

To a stirred solution of HATU (0.304 g, 0.800 mmol) and DIPEA (0.217 mL, 1.24 mmol) in dry DMF (60.0 mL) a

solution of **8** (0.184 g, 0.200 mmol) in dry DMF (7.0 mL) at r.t., was added by syringe pump in 4 h. After 18 h the resulting mixture was concentrated in vacuo, diluted with DCM (20.0 mL) and a 0.1 N HCl solution (10.0 mL) was added. The mixture was extracted with DCM (3 × 20.0 mL) and the combined organic phases were washed two times with a 0.1 N HCl solution (10.0 mL) and once with water (10.0 mL), dried ($MgSO_4$) and concentrated in vacuo to give crude **10** (0.123 g).
A small portion of cyclic peptoid was dissolved in 50% acetonitrile in HPLC grade water and analyzed by RP-HPLC.
Eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 µm, 125 Å, 3.9 × 300 mm]). Purity >85%; HPLC retention time: 17.0 min..
The crude residue was purified by HPLC on a C_{18} reversed-phase preparative column, conditions: 20%-100% B in 40 min [A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile], flow: 2.0 mL/min, 220 nm. The samples were dried in a falcon tube under low pressure and analyzed with mass spectrometry (zoom scan technique).
Cyclic peptoid 10 (0.110 g, yield: 61%, purity: 98%): ^{1}H NMR (400.13 MHz, $CDCl_3$, complex mixture of conformers) δ 1.43-146 (54H, b s, C($C\underline{H}_3$)$_3$), 3.25-3.70 (33H, b m, $C\underline{H}_2C\underline{H}_2COO$*t*-Bu and $C\underline{H}_2C\underline{H}_2OC\underline{H}_3$), 3.94-4.65 (12H, b m, N-$C\underline{H}_2$-CO); ^{13}C NMR (100.03 MHz, $CDCl_3$, complex mixture of conformers) δ 27.7, 27.9, 28.1, 28.3, 33.7, 33.8, 34.2, 34.3, 43.9, 44.4, 44.8, 45.2, 45.4, 46.0, 47.5, 48.4, 48.7, 49.0, 49.3, 49.8, 50.3, 50.7, 52.8, 58.3, 58.7, 59.0, 68.7, 69.5, 70.2, 70.4, 70.6, 71.2, 71.5, 80.5, 80.7, 81.0, 81.4, 168.1, 168.7, 169.2, 169.5, 169.6, 169.9, 170.2, 170.5, 170.6, 170.9, 171.0, 171.2, 171.6, 171.7, 171.8; HRMS (ES) $[M+H]^+$, *m/z* found 901.5138. $C_{42}H_{73}N_6O_{15}^+$ requires 901.5128.

Deprotection. Synthesis of cyclic peptoid 1

Cyclopeptoid **9** (0.058 g, 0.052 mmol) was dissolved in 2.0 mL of TFA/*m*-cresol (95:5), stirred for one hour and concentrated under a flux of nitrogen to one third of the volume. The mixture was then slowly added to 20.0 mL of stirred cold diethyl ether. The white precipitate was centrifuged and washed twice with cold ether (10.0 mL). The white solid was dried in an Eppendorf vial to give **1** (0.040 g, 99% yield), which was used in the complexation with gadolinium without further purification.
A small portion of cyclic peptoids was dissolved in 50% acetonitrile in HPLC grade water and analyzed by RP-HPLC.
Eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 µm, 125 Å, 3.9 × 300 mm]); HPLC retention time: 4.1 min.; purity: >90%.
Cyclic peptoid 1 ^{1}H NMR (400.13 MHz, $CD_3CN/D_2O/CD_3OD$, 6:3:2, complex mixture of conformers) δ 2.46-2.90 (12H, b m, $CH_2C\underline{H}_2COOH$), 3.30-3.68 (12H, m, $C\underline{H}_2CH_2COOH$), 3.70-4.69 (12H, m, N-$C\underline{H}_2$-CO); ^{13}C NMR (100.03 MHz, CF_3COOD, complex mixture of conformers) δ 33.5, 34.0, 34.6, 34.9, 46.4, 46.7, 47.2, 47.6, 48.1, 48.4, 48.5, 48.8, 49.8, 50.5, 51.0, 51.6, 52.3, 52.8, 53.5, 56.3, 172.4, 173.1, 173.2, 173.3, 174.4, 175.3, 176.6, 179.9, 180.0, 180.5, 180.7, 180.9, 181.1, 181.6, 181.8; HPLC retention time: 4.1 min.; HRMS (ES) $[M+H]^+$, *m/z* found 775.2638. $C_{30}H_{43}N_6O_{18}^+$ requires 775.2650.

Deprotection. Synthesis of cyclic peptoid 2

Cyclopeptoid **10** (0.071 g, 0.079 mmol) was dissolved in 2.0 mL of TFA/*m*-cresol (95:5), stirred for one hour and concentrated under a flux of nitrogen to one third of the volume. The mixture was then slowly added to 20.0 mL of stirred cold diethyl ether. The white precipitate was centrifuged and washed twice with cold ether (10.0 mL). The white solid was dried in an Eppendorf vial to give **2** (0.037 g, 64% yield), which was used in the complexation with gadolinium without further purification.
A small portion of cyclic peptoids was dissolved in 50% acetonitrile in HPLC grade water and analyzed by RP-HPLC.
Eluition conditions: 5%-100% B in 30 min [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, Bondapak, 10 µm, 125 Å, 3.9 × 300 mm]); HPLC retention time: 8.2 min.; purity: >90%.
Cyclic peptoid 2 ^{1}H NMR (300.1 MHz, CD_3CN/CD_3OD, 9:1, complex mixture of rotamers) δ 3.24-3.65 (33H, complex signal, $C\underline{H}_2C\underline{H}_2OC\underline{H}_3$, $C\underline{H}_2C\underline{H}_2COOH$), 3.66-4.75 (12H, m, N-$C\underline{H}_2$-CO); ^{13}C NMR (75.5 MHz, CD_3CN/CD_3OD, 40:15, complex mixture of rotamers) δ 33.1, 33.4, 33.7, 33.9, 34.2, 43.8, 44.7, 45.2, 45.3, 45.5, 45.8, 46.0, 46.3, 46.4, 47.0, 50.3, 50.6, 50.8, 51.2, 51.4, 58.8, 59.0, 59.2, 59.5, 70.8, 71.1, 71.3, 71.4, 71.5, 169.7, 170.0, 170.1, 170.4, 170.7, 170.8, 171.1, 171.2, 171.3; HPLC retention time: 8.4 min. HRMS (ES) $[M+H]^+$, *m/z* found 733.3241. $C_{30}H_{49}N_6O_{15}^+$ requires 733.3250.

Complexation of 1 and 2 with $GdCl_3$

Gd-1 and **Gd-2** complexes were synthetized through stepwise addition of 50 µl aliquots of a 11 mM $GdCl_3$ solution to solutions of **1** and **2** 2.13 mM and 2.24 mM, respectively maintaining the pH value at 7 with NaOH 0.1 N. It is possible to follow the complex formation as reported in Fig. 5S (see ESI†).

Notes and references

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† Electronic Supplementary Information (ESI) available: sodium complex formationation for the cyclic peptoids **1**, **2**, **9** and **10** (synthetic schemes, experimental procedure and ^{1}H NMR spectra for the free and complexed ligands), Gd^{3+} complex formation for **1** and **2** (titration

graphs), relaxometric determination of the stability constants of **Gd-1** and **Gd-2** (fitting equations).

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