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ORIGINAL ARTICLE

# Cyclic α,β-peptoid octamers with differing side chain patterns: synthesis and conformational investigation

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**Abstract** The solution-phase synthesis and cyclisation of three α,β-peptoid octamers with differing side chain patterns is reported. One of these, compound **C**, showed a significantly greater resolution by NMR relative to the other two structurally related octamers. This observation was studied in detail by circular dichroism at a synchrotron light source to facilitate the correlation between the side chain patterns and conformational preference of these three peptoids. The X-ray crystal structure of cyclic octamer **C**, the first high-resolution structure for the α,β-peptoid backbone, was also obtained from methanol. Combined solid- and solution-phase studies allowed the identification of the *N*-2-(benzyloxy)ethyl side chain on the β-residue of the heterogeneous backbone as a key structural feature driving the increased conformational stability for octamer **C**.

**Keywords** Peptidomimetics · α,β-peptoids · Circular dichroism · X-ray crystallography

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**Abbreviations**

| | |
|---|---|
| CD | Circular dichroism |
| COSY | Proton–proton correlation experiment |
| DMAP | 4-Dimethylaminopyridine |
| DMF | *N,N*-Dimethylformamide |
| EDC | 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride |
| HATU | *O*-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate |
| HFIP | 1,1,1,3,3,3-Hexafluoroisopropanol |
| HMBC | Heteronuclear multiple bond correlation experiment |
| HRMS | High-resolution mass spectroscopy |
| HSQC | Heteronuclear single quantum correlation experiment |
| IR | Infrared |
| NMR | Nuclear magnetic resonance |
| rt | Room temperature |
| SI | Supporting information |
| SRCD | Synchrotron radiation circular dichroism |
| TFA | Trifluoroacetic acid |
| TFE | 2,2,2-trifluoroethanol |
| THF | Tetrahydrofuran |
| TLC | Thin layer chromatography |
| UV | Ultraviolet |

## Introduction

α-Peptoids, usually termed peptoids, are bio-inspired oligoamides composed of *N*-substituted glycines (Simon et al. 1992). They are particularly suited for the development of peptidomimetic libraries with vast potential for therapeutic application (Patch et al. 2004; Yoo and Kirshenbaum 2008; Fowler and Blackwell 2009; Zuckermann and Kodadek 2009). Peptoids, compared to peptides, have key advantages such as resistance to enzymatic hydrolysis (Simon et al. 1992; Miller et al. 1994) and improved cell permeability (Kwon and Kodadek 2007). Furthermore, peptoids are not immunogenic (Astle et al. 2008) which is vital for the successful development of therapeutics. The peptoid concept has been further extended to other types of amino acid building blocks. For example, *β*-peptoids (reminiscent of *β*-peptides) are composed of *N*-substituted *β*-alanines (Hamper et al. 1998) and α,*β*-peptoids have been generated from alternating *N*-substituted glycine and *β*-alanine residues (Hjelmgaard et al. 2009; Caumes et al. 2010). Peptoids based on aromatic building blocks (Combs and Lokey 2007; Campbell et al. 2010) and peptide-peptoid hybrids have also been reported (Olsen 2010). In all peptoid backbones, the residues are linked by tertiary amide bonds which can be created from pre-synthesised *N*-substituted amino acid building blocks using standard peptide chemistry or by the unique "submonomer" method where the peptoid chain is elongated in an iterative, stepwise manner, thereby allowing facile access to diversity.

Peptoid backbones are inherently more flexible than peptides since (1) the tertiary amide bonds are devoid of H-bonding donors and (2) they have a decreased energy barrier for *cis*/*trans* isomerisation relative to peptides (Moehle and Hofmann 1996). Despite these structural features, peptoids with bulky *N*-α-chiral side chains (Armand et al. 1997; Kirshenbaum et al. 1998; Wu et al. 2001; Lee et al. 2005) or *N*-aryl side chains (Shah et al. 2008; Seo et al. 2010) have been shown to adopt secondary structures. Stabilisation of the peptoid backbone has been achieved by controlling *cis*/*trans* isomerisation (Sui et al. 2007) of the amide via stereo-electronic effects (Gorske and Blackwell 2006; Gorske et al. 2007, 2009; Fowler et al. 2009) and by introduction of side chain structural features which facilitate hydrogen bonding between the backbone and side chains (Stringer et al. 2010). Despite efforts to reduce the conformational heterogeneity of linear oligopeptoids, only one high-resolution X-ray structure has been reported, corresponding to a PPI-like conformation for a linear pentamer (Wu et al. 2003). Two other secondary structures, a PPII-like conformation (Shah et al. 2008) and a threaded-loop conformation (Huang et al. 2006), have been elucidated by molecular modelling and NMR studies, respectively. In contrast, the conformational investigation for linear *β*-peptoids has been limited to circular dichroism (CD) studies (Olsen et al. 2008; Norgren et al. 2006)—presumably due to increased flexibility arising from the additional methylene unit in the backbone. Cyclisation is an attractive way to impose conformational constraints to peptoids (Hioki et al. 2004), an approach rationalised to α-peptoids by Kirshenbaum (Shin et al. 2007) and to *β*-peptoids by our group (Roy et al. 2008). This approach has been successful—as demonstrated by the significant number of X-ray crystal structures of cyclic α- and *β*-peptoids described since 2007 (Maulucci et al. 2008; Shah et al. 2008; Yoo et al. 2010; Roy et al. 2008).

The first synthesis and preliminary CD study of a novel α,*β*-peptoid backbone were recently reported by us (Hjelmgaard et al. 2009) where the backbone contained 50% of α-chiral aromatic side chains [(*S*)-*N*-(1-phenylethyl)] located on the *β*-peptoid residues and 50% of *N*-2-(benzyloxy)ethyl achiral side chains on the α-peptoid residues. In particular, the communication included a series of cyclic compounds of varying chain length (up to ten residues) where the octameric peptoid (compound **A**, Fig. 1) showed the greatest molar ellipticity per residue by CD. Herein, we report the synthesis and conformational investigation of three octameric α,*β*-peptoid macrocycles differing by their side chain sequence patterns. These macrocycles have been investigated by NMR and CD and we also present the first X-ray structure of an α,*β*-alternating peptoid. This work is aimed at the improved understanding of the conformational behaviour of α,*β*-peptoids and the design of peptoid macrocycles as scaffolds for multivalent ligand display (Cecioni et al. 2010).

## Materials and methods

### Synthesis

Chemicals obtained from commercial sources were used without further purification, unless stated otherwise. THF was distilled from potassium/benzophenone and stored over 4Å molecular sieves. $CH_2Cl_2$ and MeOH were distilled from $CaH_2$ and stored over 4Å molecular sieves. DMF, $Et_2O$, $Et_3N$ and $^iPr_2NEt$ were dried over 4Å molecular sieves. Melting points were determined on a Reichert microscope apparatus and are uncorrected. Specific rotations were measured on a Jasco DIP-370 polarimeter using a 10 cm cell. IR spectra were recorded on a Perkin-Elmer 881 spectrometer and *ν* are expressed in $cm^{-1}$. NMR spectra were recorded on a Bruker AC 400 or a Bruker AC 500 spectrometer. Chemical shifts are referenced to the residual solvent peak and *J* values are given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet,

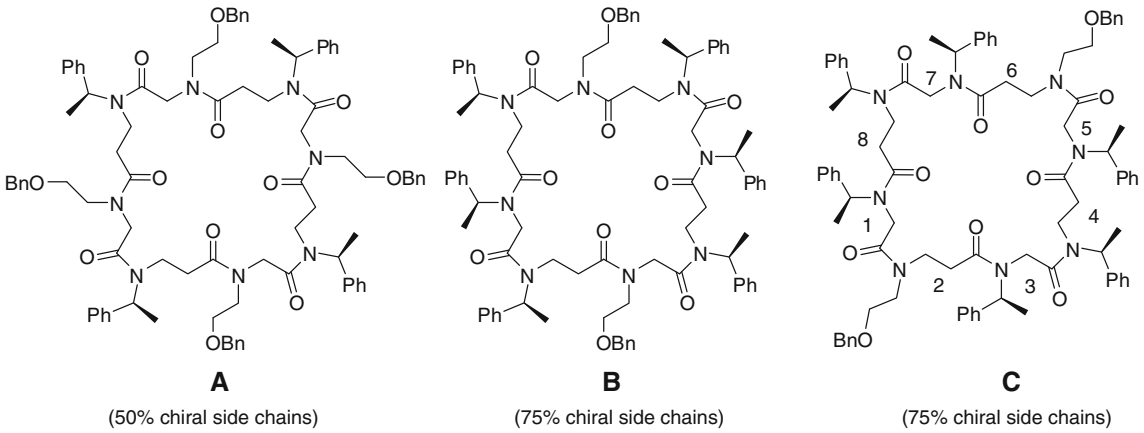



**Fig. 1** Cyclic peptoid octamers **A**, **B** and **C** with different side chain patterns

and (br) broad. Where applicable, assignments were based on COSY, HMBC, HSQC and *J*-mod-experiments. Thin layer chromatography (TLC) was performed on Merck TLC aluminum sheets, silica gel 60, $F_{254}$. Flash chromatography was performed with Merck silica gel 60, 40–63 μm. HRMS was recorded on a Micromass Q-Tof Micro (3,000 V) apparatus.

2-(Benzyloxy)ethyl amine was synthesised following a literature procedure (Hu and Cassady 1995). Synthesis and characterisation of compounds **1a**, **2a**, **3a** and **A** (Scheme 1) were previously reported (Hjelmgaard et al. 2009). Other linear peptoids **1b**, **2b**, **3b**, **1c**, **2c** and **3c** were synthesised following the same procedures (SI: section I).

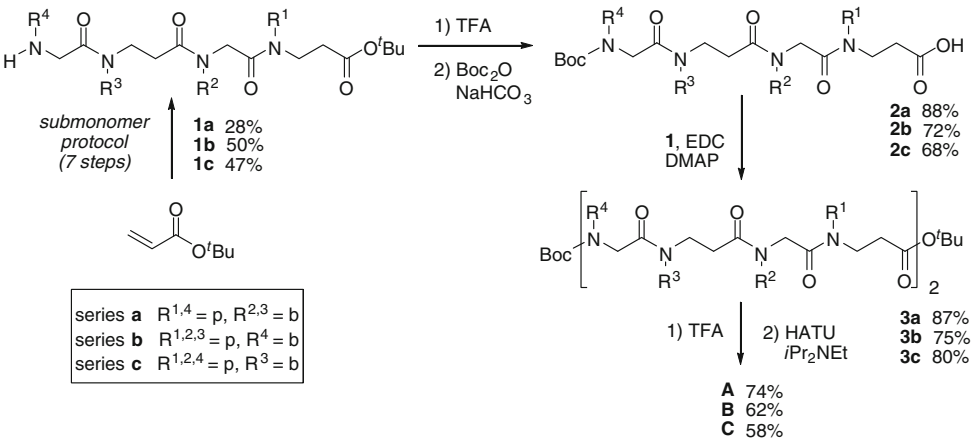
### *Deprotection of linear peptoid octamers **3b-c** and macrocyclisation*

TFA (1 mL per 1 mL $CH_2Cl_2$) was added to a stirred solution of peptoid **3** (1.0 equiv, 0.10 M) in $CH_2Cl_2$ at rt under Ar. The resulting mixture was stirred for 2 h at rt. The solvents were evaporated under reduced pressure and the residue was dried in vacuo, yielding the crude termini deprotected peptoid. To a solution of the crude peptoid (5.0 mM) in $CH_2Cl_2$/DMF (4:1) at 0°C under Ar, enough $^{i}Pr_2NEt$ (approx. 5.0 equiv) was added to turn the mixture slightly basic. HATU (1.2 equiv) was added and the resulting mixture was stirred for 3 days at rt. The solvents were evaporated under reduced pressure and the residue was taken up in EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered and then concentrated under reduced pressure. Flash chromatography of the residue yielded the desired macrocycle.

Characterisation of macrocycles **B** and **C** are described in SI.

### X-ray crystallography

Single-crystal X-ray diffraction experiment was carried out at 100 K on a Bruker Kappa Apex II diffractometer (graphite-monochromated MoKα radiation, $\lambda = 0.71073$ Å). Integration and reduction of the data were performed using the HKL suite (Otwinowski and Minor 1997). The crystal structure was solved by direct methods using Sir92 (Altomare et al. 1993) and refined by full-matrix least




**Scheme 1** Solution-phase synthesis of oligomers and macrocyclisation; p and b are the abbreviations of (*S*)-(1-phenylethyl) and 2-(benzyloxy)ethyl, respectively

squares refinement against $F^2$ using SHELXL-97 (Sheldrick 2008). Due to the lack of any significant anomalous dispersion effects, the absolute configurations of the title compound could not be determined from the diffraction experiments but was known from the method of synthesis. The origin was fixed by floating-origin restraints (Flack and Schwarzenbach 1988). All H atoms were located in difference Fourier maps. The C-bonded H atoms were placed at calculated positions and refined using a riding model, with C–H distances of 0.93–0.98 Å. The H-atom $U_{iso}$ parameters were fixed at 1.2Ueq(C) for methylene groups, methine and aromatic C–H, and at 1.5Ueq(C) for methyl C–H. Relevant crystallographic structure data and refinement details are presented in SI (Table 1). CCDC 814640 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

### Circular dichroism

Circular dichroism spectra were recorded at beamline B23 of the Diamond Light Source, UK using an Olis CD spectropolarimeter (Module B) equipped with a Peltier temperature control system and fitted with a bespoke thermostated cell holder (Jávorfi et al. 2010). A quartz Suprasil cylindrical cell with a path length of 0.01 cm was used. Spectra were recorded at 20°C in acetonitrile (MeCN), methanol (MeOH) or hexafluoroisopropanol (HFIP) supplied by Romil at super-purity grade and 2,2,2-trifluoroethanol (TFE) supplied by Sigma-Aldrich at spectroscopic grade. The following parameters were used: 4 acquisitions, 10 nm/min scan speed, 0.1 nm data interval, 1.0 nm spectral band width, 4 s time constant, 260–175 nm scan range (depending on solvent transparency). A solvent baseline spectrum was recorded in the same cell at proximal time. The spectra were averaged and the solvent baseline subtracted from the resulting spectrum which was normalised for path length and concentration to give the molar ellipticity ([$\theta$], deg cm$^2$/dmol). Temperature experiments were performed by recording two scans at each temperature (using the spectral parameters above) at 5°C intervals from 5°C to a suitable temperature below the solvent boiling point. Spectra were also recorded at 20°C before and after heating; any changes that occurred upon heating were found to be reversible on cooling (data not shown). For the solvent titrations, five samples (for each compound) were prepared at similar concentrations with different solvent combinations and an appropriate solvent background recorded at proximal time for each sample. Samples were prepared volumetrically by dilution from a solution at known concentration to achieve a sample solution of appropriate concentration (SI: Table 6).

## Results and discussion

To assess the influence of the sequence pattern and the ratio of chirality on the conformational stability of cyclic peptoid octamers, two new macrocycles **B** and **C**, both characterised by 75% of chiral side chains were synthesised; in contrast to the previously reported octamer **A** (Fig. 1) (Hjelmgaard et al. 2009). In compound **B**, the *N*-2-(benzyloxy)ethyl substituents are located on the α-residues, like in **A**, and are opposite each other in the ring. The same situation exists in **C** but the two *N*-2-(benzyloxy)ethyl side chains are instead located on the β-residues. This side chain was chosen because it facilitates further chemical manipulation after hydrogenolysis of the benzyl protecting group.

### Synthesis

α,β-Alternating peptoid oligomers were obtained using a previously reported protocol (Hjelmgaard et al. 2009). The different tetramers **2** were synthesised by the submonomer method in solution-phase over seven steps (SI: section I). The octamers **3** were accessed by peptidic homo-coupling of the corresponding tetramers (Scheme 1). Head to tail macrocyclisation of the linear octamers **3** was efficiently accomplished by termini deprotection with TFA then cyclisation at moderate dilution (5 mM) using the benzotriazole-based uronium salt HATU. This coupling reagent was previously demonstrated to be efficient for formation of β-cyclopeptoids (Roy et al. 2008). The three cyclic α,β-peptoid octamers **A–C** were obtained in high purity and good overall yields (58–74%).

### X-ray crystallography

When handling compound **C**, its ability to crystallise in NMR tubes from deuterated methanol was noted. Single crystals suitable for X-ray analysis were obtained by slow evaporation of a methanol solution. Compound **C** crystallised in the space group *P*21 with one molecule in the asymmetric unit. In the crystal structure, the backbone of **C** adopts a $C_2$-symmetrical conformation resembling a bow tie with two loops almost perpendicular to each other (Fig. 2a). This 28-membered ring can also be described as a twisted, tight rectangular form with dimensions approximately 12 Å by 3.5 Å (SI: Fig. 2), where the longer and shorter sides consist of ten and four atoms, respectively. The macrocycle is further characterised by four *cis* and four *trans* amide bonds, present as alternating pairs where two *cis* amides are located at the corners (Fig. 2a).

Similar features have been observed in a cyclic α-peptoid octamer from Kirshenbaum's group which takes on an overall rectangular form, although less twisted and more planar in nature (Shin et al. 2007). Although compound

**C** has a different backbone, the $\phi$ (C(i-1)-N(i)-Cα(i)-C(i)) and $\psi$ (N(i)-Cα(i)-C(i)-N(i+1)) dihedral angles of the α-residues, (residues 1, 3, 5 and 7) which show moderate variations, are comparable to those observed in Kirshenbaum's octamers: the values range from −71° to −94° for the $\phi$ angles and from 169° to 179° for the $\psi$ angles (SI: Table 3). The $\phi$ dihedral angles of the β-residues (C(i-1)-N(i)-Cβ(i)-Cα(i)) of **C** are also close to ±80°. β residues 2 and 6, which are opposite each other in the ring, are connected to α-residues 1 and 5, respectively through *trans* amide bonds. Nevertheless, these residues assume a specific conformation which is different to any helical conformation found for β-peptides (Hill et al. 2001; Seebach and Gardiner 2008). Deviation from planarity of the amide bonds is not significant as observed for other cyclic α-peptoids (Shin et al. 2007). The side chains are displayed in alternating pseudo-axial (residues 2, 4, 6 and 8) and pseudo-equatorial (residues 1, 3, 5 and 7) orientations. Adjacent side chains from each α,β-dimeric unit are located on the same face and an up–down alternation occurs along the sequence. It is of note that only β-peptoid residue side chains adopt a pseudo-axial orientation.

The bent conformation of macrocycle **C** is stabilised through intra and intermolecular weak hydrogen bonds, intermolecular π–π interactions and Van der Waals contacts. Intramolecular CH…O interactions are observed between the carbonyls of residues 1, 4 and 5 and the lateral chains of the residues 5, 6 and 1, respectively (SI: Fig. 1 and Table 2). In the crystal, each molecule is weakly hydrogen bonded to four molecules in a two-dimensional network parallel to the (100) plane. The sheets are held together by aromatic interactions: *N*-2-(benzyloxy)ethyl side chains of residues 2 and 6 fit in a cavity defined by the phenylethyl groups of residues 8, 1, 4 and 5 (Fig. 2a, b).

## Conformational investigation by synchrotron radiation circular dichroism (SRCD) and $^1$H NMR

NMR spectra of oligopeptoids are often very intricate and difficult to interpret because both *cis* and *trans* conformations of the peptoid amides are populated and observable on the NMR time scale. However, the extent of complexity of the $^1$H NMR can provide information regarding the relative conformational stability of peptoid backbones and may depend on solvent environment. Therefore, $^1$H NMR spectra of macrocyclic peptoids **A**, **B** and **C** were recorded in $CDCl_3$, $CD_3CN$ (SI: Figs. 3, 4) and $CD_3OD$ (Fig. 3). Whichever solvent was employed, octamer **C** always gave the sharpest resolution of the $^1$H NMR spectrum; the most resolved being obtained from deuterated methanol and chloroform. For octamer **C**, increased resolution indicated the sampling of a smaller area of conformational space, i.e. less/none of some conformers and increased population of other conformers.

Structural investigation was also carried out by circular dichroism spectroscopy to further explore the observations made by $^1$H NMR and X-ray crystallography. CD is a useful tool for conformational investigation of peptoids due to its sensitivity to subtle changes in the backbone conformation and the limitations of NMR arising from *cis*/*trans* isomerism and lack of hydrogen on the amides to assist interpretation (Sui et al. 2007). The use of synchrotron radiation CD (SRCD) was found to give an extended spectral region compared to the commercial instrument previously used (Hjelmgaard et al. 2009) and thus allowed the identification of distinctive spectral features below 190 nm (SI: Fig. 7). Exposure to an intense synchrotron light source alone may alter the secondary structure (Clarke and Jones 2004; Wien et al. 2005; Jávorfi et al. 2010),

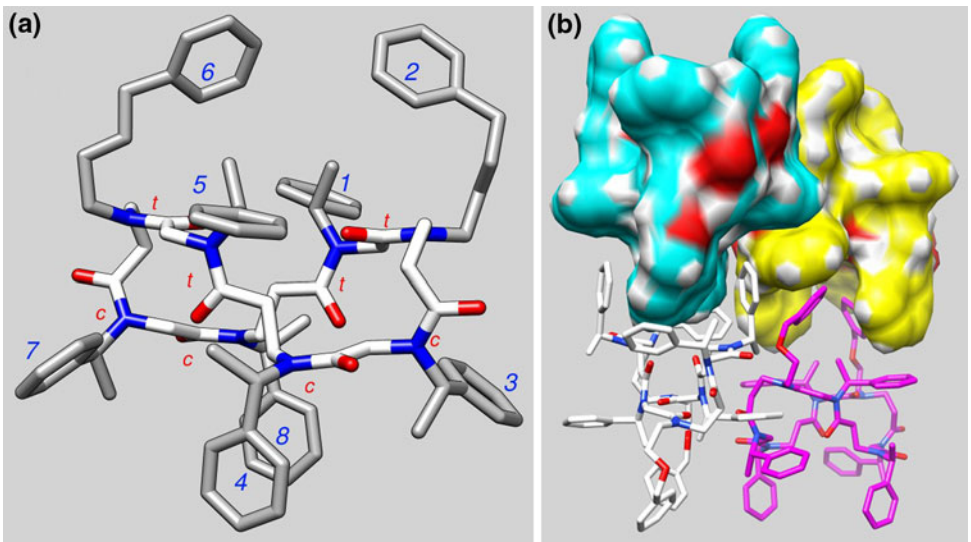



**Fig. 2** X-ray crystal structure of cyclic peptoid **C**. **a** Side view. 1–8: residue number, *c cis*, *t trans*. The longer sides are defined as follow: [$C^3_\alpha$CO-(β)XX$^4$-(α)XX$^5$-N$^6$], [$C^7_\alpha$CO-(β)XX$^8$-(α)XX$^1$-N$^2$]. The shorter sides are [$C^2_\beta C^2_\alpha$CO- N$^3$] and [$C^6_\beta C^6_\alpha$CO-N$^7$]. **b** Part of the crystal packing showing 4 molecules

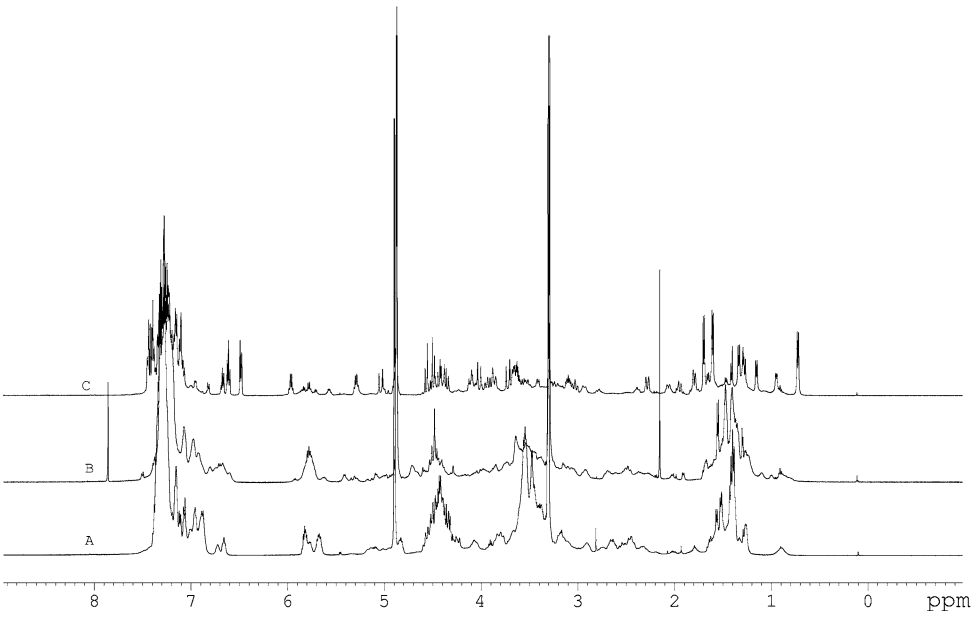



**Fig. 3** $^{1}$H NMR spectra of compounds **A** (*bottom*), **B** (*middle*) and **C** (*top*) in $CD_3OD$ (*ca.* 15 mM, 20°C, 500 MHz)

however, α,β-peptoids were found to be conformationally stable upon irradiation (SI: Fig. 8).

The SRCD of α,β-peptoids **A–C** in MeCN had similar spectral features, with the exception of the positive maximum of **C** being red shifted (Fig. 4). This suggested that the spectral features are not significantly affected by the differences in the side chain sequences. These spectra resemble that reported for a cyclic α-peptoid octamer in the same solvent, where the introduction of a cyclic constraint into a linear peptoid reduced conformational heterogeneity (Holub et al. 2007). The intensity of the spectra is also consistent with that of ordered α-peptoids.

To probe the conformational stability of these systems under different conditions, SRCD spectra were recorded in

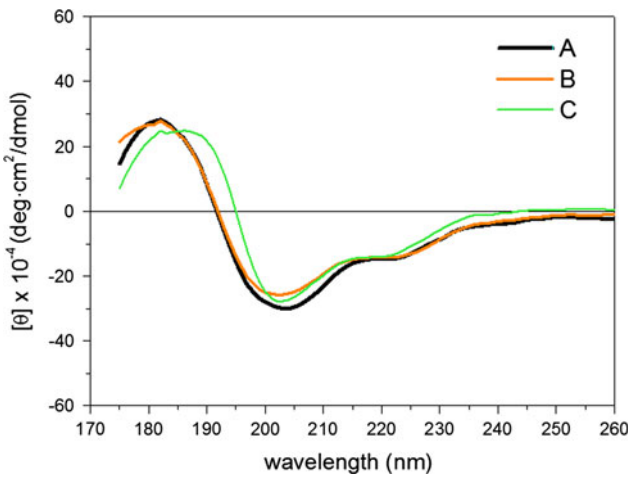



**Fig. 4** SRCD spectra of cyclic octamers (**A–C**) with differing side chain sequences in MeCN. All spectra were recorded at 20°C at known concentrations in the range 224–253 μM

different solvent environments—MeCN, MeOH, TFE and HFIP. The spectral features were altered for the three α,β-peptoids investigated (Fig. 5 and SI: Fig. 9), however, no collapse of secondary structure was observed, as previously reported for a linear β-peptoid (Olsen et al. 2008). This is indicative of the ability of cyclic octamers to sample different areas of conformational space in response to external stimuli, such as the solvent environment. This is perhaps unsurprising given the inherently flexible nature of peptoids (in contrast to peptides) and further flexibility arising from the large ring size and presence of β-residues.

**B** and **C** had comparable positive and negative maxima in MeOH, TFE and MeCN but not in HFIP where distinct spectra were observed (Fig 5 and SI: Fig. 10). By contrast, **A** showed similar spectral features (including ellipticity) to **B** in the four solvents investigated (SI: Fig. 9). This indicated that **A** and **B** respond in a similar manner to different solvents and thus populate similar areas of conformational space regardless of the differences in their side chain patterns.

To explore the effect of differing side chain composition further, a direct comparison can be made between **B** and **C** since they differ only by the positioning of the *N*-2-(benzyloxy)ethyl side chain on an α- or β-residue (respectively). They also have the same percentage of chirality (75%). In TFE and MeOH, **C** showed greater ellipticity than **B**, combined with a red shift of the positive maxima at 190 nm (Fig. 5 and SI: Fig. 10). This indicated the presence of greater conformational order for **C** in TFE and MeOH than for **B**, i.e. the presence of a dominant conformation or an ensemble of closely related conformations. This observation was consistent with the significantly improved resolution observed for **C**, relative to **B**,

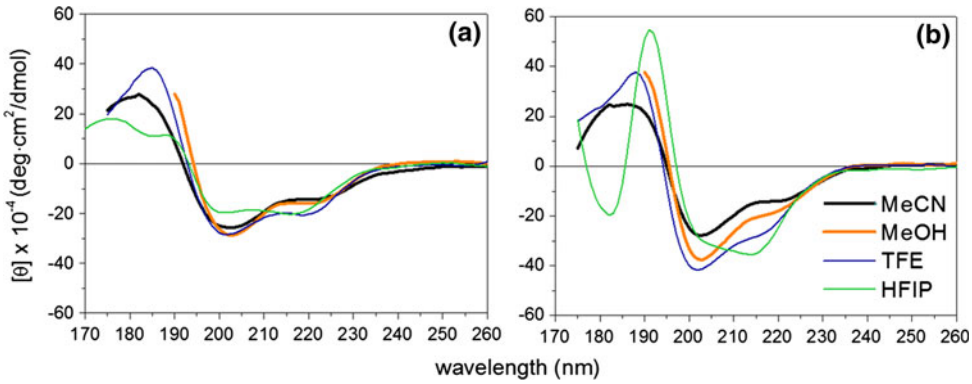



**Fig. 5** SRCD spectra of octamers (**a**) **B** and (**b**) **C** in different solvent environments. All spectra were recorded at 20°C in the solvent stated at known concentrations in the range 210–253 μM

by $^1$H NMR in $CD_3OD$ (Fig. 3). By contrast, a similar ellipticity was observed in MeCN for both **B** and **C**, suggesting a similar conformational preference. [The improved resolution by $^1$H NMR between **B** and **C** in $CD_3CN$ was not as significant as that observed in $CD_3OD$ (SI: Fig. 4).] It is of note that for **C** the ellipticity was greatest in protic solvents. This is indicative of a hydrogen bonding contribution to stabilisation of the backbone by the solvent which is favoured when the *N*-2-(benzyloxy)ethyl side chain is on the *β*-residue (**C**). The similarity of spectra for **A** and **B** in different solvent environments (Fig. 5a and SI: Fig. 9) is consistent with this interpretation as both contain the 2-(benzyloxy)ethyl side chain(s) on the α-residue.

A temperature study of **C** in MeOH by SRCD showed a significant decrease in ellipticity upon heating (Fig. 6a). Temperature studies were also performed for **B** in MeOH and for both **B** and **C** in MeCN (Fig. 6b and SI: Fig. 11–14, Table 5). The change in ellipticity was greatest for **C** in MeOH ($\Delta[\theta]/\Delta T = 2.55 \times 10^{-4}$ deg cm$^2$/dmol T) which confirmed the presence of increased conformational order, i.e. upon heating a greater area of conformational space is sampled giving a more heterogeneous population of conformations and thus lower ellipticity. By contrast, the change upon heating observed for **B** in MeOH and MeCN and **C** in MeCN was less pronounced with $\Delta[\theta]/\Delta T$ in the range 0.74–0.79 $\times 10^{-4}$ deg cm$^2$/dmol T. This was indicative of the greater conformational space sampled by **B** which was not further perturbed by heating and similarly for **C** in MeCN. At the highest temperatures employed, a similar intensity of ellipticity was adopted by both **B** and **C** regardless of solvent. This further validated the enhanced conformational order displayed by **C** in MeOH as a result of the side chain pattern and indicated that the octamers always retain a minimum ellipticity, presumably due to their cyclic constraint.

A temperature study by $^1$H NMR of **C** between 5 and 55°C in $CD_3OD$ was also undertaken and, in particular, focussed on the spectral region corresponding to the benzylic protons of the phenylethyl groups (~5–6 ppm) since their chemical shifts are indicative of amides in a *cis* or *trans* configuration (SI: section II) (Sui et al. 2007). The overall *cis/trans* ratios were estimated by integration of the NC*H*$CH_3$Ph. Only modest variations were observed upon heating with a mean *cis/trans* ratio close to 1; thus giving complementary evidence to the SRCD regarding the conformational stability of **C** since similar maxima are maintained upon heating.

In HFIP, the ellipticity of **C** was significantly greater than that of **B**, however, different spectral features were

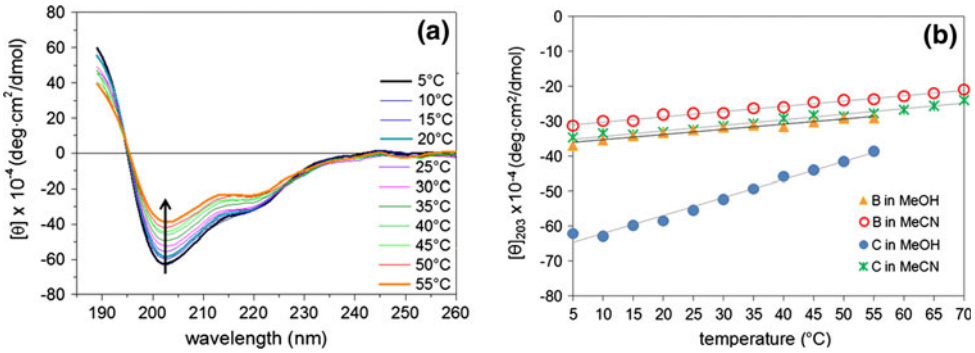



**Fig. 6** **a** Temperature study of compound **C** in MeOH. **b** Change of molar ellipticity at 203 nm versus temperature where $\Delta[\theta]/\Delta T$ ($\times 10^{-4}$ deg cm$^2$/dmol T): **B** in MeOH = 0.74, **B** in MeCN = 0.76, **C** in MeOH = 2.55, **C** in MeCN = 0.79. Spectra were recorded at the temperatures and in the solvents stated at known concentrations in the range 152–235 μM

also observed (Fig. 5); the HFIP spectrum of **B** was consistent with **A** (SI: Fig. 9). This indicated that the backbone conformation in HFIP can be significantly perturbed depending on the position of the *N*-2-(benzyloxy)ethyl side chain. HFIP is known to induce conformational transitions in peptides and proteins under certain circumstances (Hong et al. 1999). However, a direct comparison would not be applicable due to the chemical difference, e.g. the lack of hydrogen bond donors, between the systems investigated herein and peptides. It should be noted that although HFIP is known to induce order in peptides, the effects of HFIP observed for **A** and **B** are much less pronounced than **C** (vide supra), thereby indicating the importance of the side-chain substitution pattern. Temperature studies by SRCD were also performed in TFE and HFIP for both **B** and **C** (SI: Figs. 15–16). In both solvents, a greater decrease of ellipticity was observed for **C** compared to **B**. This was consistent with the presence of a greater conformational order for **C** with respect to **B** in protic solvents—as observed for MeOH. These results further demonstrate that positioning of the *N*-2-(benzyloxy)ethyl side chain on the *β*-residue promoted increased conformational order in protic solvents (SI: Fig. 10).

A surprising observation was made when the solvent effect on conformational preference for **C** was further explored by a solvent titration from MeOH to MeCN (Fig. 7). Rather than the anticipated gradual decrease of ellipticity in MeOH with addition of MeCN, the most intense ellipticity was observed in 1:1 and 1:3 mixtures of MeOH:MeCN. This indicated that the greatest conformational stabilisation occurred in the presence of a mixture of protic/aprotic solvents. $^{1}$H NMR spectra of **C** were obtained in $CD_3OD$, $CD_3CN$ and $CD_3OH$:$CD_3CN$ in a 1:1 ratio (SI: Fig. 5). It was found that similar spectral resolution was obtained in $CD_3OD$ and the mixture of $CD_3OD$/$CD_3CN$. [This may seem inconsistent with SRCD, however, this difference can be rationalised by the limited ability of NMR to distinguish between a group of closely related conformers and a single predominant conformer related to the group of conformers.] This can be explained by an intricate balance of inter (i.e. solvent-peptoid) and intramolecular interactions. For example, the interaction between the peptoid and the solvent could be maximised in 100% MeOH where the contribution from intramolecular interactions is less significant for conformational stabilization. However, when the MeCN is added some of these intermolecular interactions with MeOH may be disrupted promoting intramolecular interaction in the peptoid backbone and/or side chains. This competition between MeOH and MeCN can be regarded as a favourable 'ordered solvation' where the conformational stabilisation is enhanced in a mixture of MeOH and MeCN rather than for each solvent in isolation. Similar findings were also

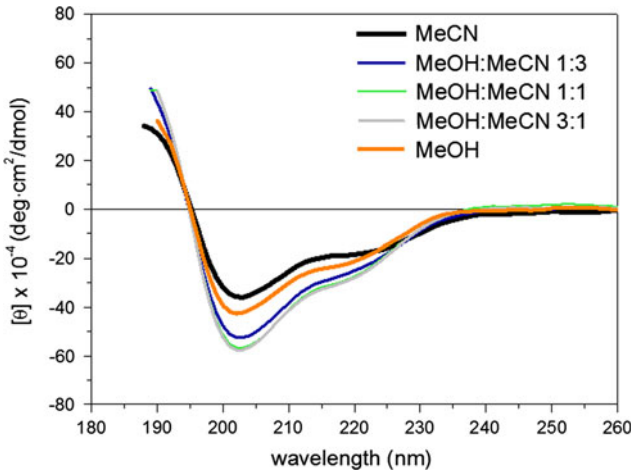



**Fig. 7** Titration of compound **C** in MeOH with increasing percentage of MeCN. All spectra were recorded at 20°C in the solvent stated at the concentration of 229 μM

obtained for **B** (SI: Fig. 17). Solvent titrations with MeCN and HFIP were also undertaken for both **B** and **C** (SI: Fig. 18). It was found that the spectral features of **C** in MeCN were largely maintained until 100% HFIP where a radical conformational change was then observed. By contrast, a more gradual change was observed for **B** upon titration. This is in marked contrast to the observation of MeOH/MeCN mixtures.

## Conclusions

We have synthesised two octameric peptoid macrocycles (**B** and **C**), which are structurally related to **A**, in good overall yields. The different conformational behaviour of **C**, relative to **A** and **B**, was investigated by $^{1}$H NMR and SRCD. Compound **C**, which displayed the sharpest $^{1}$H NMR signals, was easily crystallised from MeOH to obtain the first *α*,*β*-peptoid X-ray structure. Increasing the number of *α*-chiral side chains was found not to be the sole criterion to induce greater conformational order since **B** and **C**, both bearing 75% of chiral side chains, showed different conformational preferences. Overall, **B** retains a conformational flexibility similar to that of **A** (50% chiral). Instead, it was the positioning of the *N*-2-(benzyloxy)ethyl side chain on the *β*-residue (**C** vs. **B**) which was found to increase conformational order in protic solvents for *α*,*β*-peptoids. This observation is vital for understanding the secondary structural preference of these novel systems and how they can be influenced by structural features. Further to this, it has been identified that combinations of protic and aprotic solvents can have a marked conformational effect for peptoids—presumably due to an intricate balance of ordered solvation and backbone and/or side

chain interactions. This work has significantly furthered efforts towards the engineering of compact ordered secondary structures of α,β-peptoids.

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