PAPER

Water soluble hydrophobic peptoids *via* a minor backbone modification†

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Peptoids – oligomers of *N*-substituted glycine – are an important class of peptide mimics that are widely used in areas ranging from biology and medicine to metal binding and catalysis. The utility of peptoids, however, especially for applications in aqueous solutions, is often hampered by the hydrophobic nature of their sequences dictated by structural and functional requirements. Herein we describe a simple method to solubilize hydrophobic peptoids in water without modifying their original sequences, *via* the insertion of biocompatible and low cost piperazine or homopiperazine groups at the N- or C-terminus of the peptoid backbone. We show that our method can be applied for the production of α-, β- and azapep-toids, which display high water solubility for long periods of time. Moreover, circular dichroism (CD) spec-troscopy revealed that the incorporation of piperazine groups within α-peptoids, having chiral naphthyl monomers, β-peptoids and unstructured azapeptoids results in peptoids that exhibit high conformational order in water.

Introduction

Peptidomimetic foldamers[1] are often designed to emulate the structure of peptides towards biological applications, mainly because they are both biocompatible and resistant to proteo-lytic degradation. Among peptide mimics, peptoids[2] – *N*-substituted glycine oligomers – are especially interesting due to their ease of synthesis on a solid support,[3] their ability to fold into well-defined secondary structures[4] and their employ-ment in various biological activities including protein–protein interaction,[5] metal binding[6] and catalysis.[7] Moreover, com-pared to peptides, peptoids exhibit improved cell per-meability,[8] proteolytic resistance,[9] and tolerance towards high salt concentration and various pH conditions. Despite these unique properties and advances in the design of peptoids as biomimetic materials,[10–14] their development towards practical utilization is still limited. This is because many sequences that hold potential for useful applications often consist of hydro-phobic monomers, which are necessary for maintaining their secondary structures and/or biological activity,[15] resulting in peptoids that are not soluble in water. It is known that helical structures in peptoids are stabilized by the incorporation of chiral bulky side chains within the peptoid backbone.[4] In the absence of hydrogen bonding, peptoid secondary structures are enforced by steric and electronic interactions giving raise to polyproline type helices, with a pitch of roughly 3 residues per turn. Thus, (*S*)- or (*R*)-phenylethyl *N*-substituents (*N*spe and *N*rpe, respectively), for example, can prompt the formation of peptoid helices provided that they constitute the majority of the side chains along the peptoid scaffold,[4a–d] but these helices are both hydrophobic and water insoluble, and there-fore they are useless for practical applications in water and/or buffer solutions.

Currently, solubilizing hydrophobic peptoids in water is not straightforward and water-soluble peptoids are generated *via* the incorporation of polar hydrophilic pendant groups within the peptoid sequence.[16] This approach, however, requires that at least 66% of the pendant monomers are hydrophilic and are spread evenly along the sequence to ensure water-solubility of the peptoid.[16] These requirements not only complicate the synthesis by adding protection and de-protection steps, but more importantly lead to major alternation and elongation of the original sequences, as well as to a significant decrease in the hydrophobicity of peptoids, which can cause loss of their activity. This is specifically problematic in the case of thera-peutic compounds, which include hydrophobic groups essen-tial for their activity, and in the case of metal chelators, in which the hydrophilic side chains can coordinate to metal ions, thus effecting the metal-biding properties of the peptoid. In light of these limitations, and in order to make significant progress in the development of peptoids as practical materials, it is essential to find new ways to solubilize hydrophobic pep-toids in water while preserving their sequence, structure and function.

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In this article, we report that simply by incorporating one or more piperazine or homopiperazine units within the backbone of various hydrophobic α-peptoid, β-peptoid and azapeptoid oligomers, we can ensure their water-solubility, while maintaining their sequence and structure. Moreover, some of these structured as well as unstructured peptoids show increased conformational order in water, as evident from CD spectroscopy.

Results and discussion

Our preliminary investigations showed that the incorporation of only one polar group, namely a primary amine, an alcohol, a carboxylic acid or a sulfonic acid as a pendent group at the N-terminus of the hydrophobic peptoid hexamer **P1** (peptoids **SP1–SP4**, respectively, Fig. 1A), did not lead to water-soluble peptoids (Fig. 1B).

We therefore decided to explore the possibility of incorporating polar group(s) within the backbone of the peptoid, at either the N- or the C-terminus. Initially, we chose to use piperazine, which is biocompatible, and can be introduced into the peptoid backbone *via* the solid phase "submonomer" method.[3] The incorporation of piperazine and its derivative oxopiperazine within various peptoid backbones has been previously reported by Kodadek's group.[17] As piperazine has two secondary amine units that are equally reactive towards bromoacetic acid, both can react simultaneously to connect between two peptoid oligomers forming a "peptoid dimer" (*e.g.* **PD**, Fig. 2). In order to selectively react only one of the secondary amines towards the formation of a "peptoid monomer" (*e.g.* **PM**, see Fig. 2), *N*-protected piperazine is typically used, and protection and de-protection steps are required in the synthesis.[17] Aiming to avoid these additional synthetic steps, we performed some reaction optimizations on a bromo-acetylated short peptoid trimer containing methyl benzyl synthons (**P**, Fig. 2). Our initial attempts to include piperazine at the N-terminus of **P** using the typical "submonomer" conditions afforded only 60% of the desired **PM** and 40% of **PD** (Fig. 2),
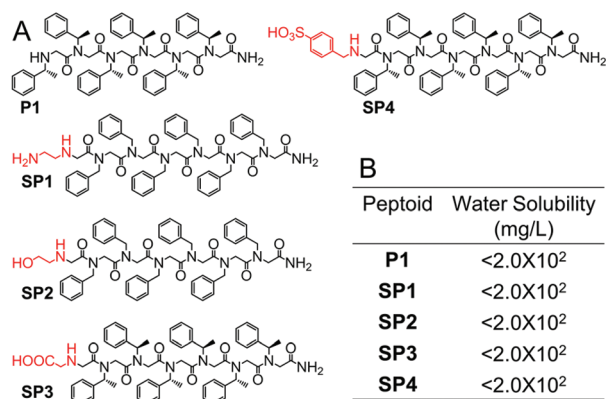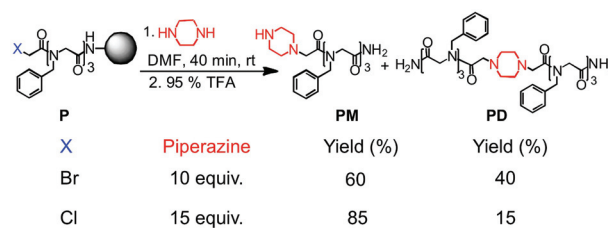

Fig. 2 Insertion of piperazine into a peptoid trimer *via* the submonomer method and reaction optimization.

P	X	Piperazine	PM Yield (%)	PD Yield (%)
	Br	10 equiv.	60	40
	Cl	15 equiv.	85	15

as estimated from HPLC analysis. It is known that replacing bromoacetic acid with chloroacetic acid in peptoid synthesis improves the selectivity and efficiency of the reaction.[18] Indeed, using chloroacetic acid as well as increasing the amount of piperazine from 10 equiv. to 15 equiv. and the reaction time from 20 min to 40 min improved significantly the ratio between the two products affording 85% **PM** and only 15% **PD** (Fig. 2). Increasing the amount of piperazine to 20 and 25 equiv. did not improve this ratio further.

Our attempts to crystalize either **PM** or **PD** were not successful; however, we were able to crystallize a short peptoid "dimer" **PD′** bearing two *N*spe groups, which we synthesized using a solution phase method (see Experimental Section for details). The single crystal X-ray analysis of **PD′** showed that piperazine is in the chair conformation with one *N*spe group above the plane of the piperazine ring and the second *N*spe group below the plane of the piperazine ring (Fig. 3).

Using the optimized conditions for obtaining a "**PM**" type peptoid, we modified oligomer **P1**, a hydrophobic helical peptoid (Fig. 2A), to include piperazine. Thus, peptoids **P2** and **P3** (Fig. 4A) were designed to evaluate the ability of piperazine to solubilize **P1** in water when placed either at its N- or C-terminus, respectively. Peptoid **P4** (Fig. 4A) was designed to test whether water solubility can be achieved when piperazine is incorporated within a cyclic hydrophobic peptoid. The linear peptoids **P1**, **P2** and **P3**, as well as **P-linear** (see the ESI†), incorporating piperazine, *N*spe and chloropropylamine (*N*pl, only **P-linear**) as synthons, were synthesized on a solid support employing the "submonomer" protocol. Peptoid **P4** was prepared by a microwave-assisted cyclization of peptoid **P-linear** on a solid support, following a method that we have recently published.[19] All the peptoids were analyzed and purified by HPLC, and the identity of their sequences was con-

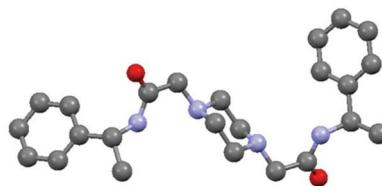**Fig. 1** Sequences of hydrophobic peptoids incorporating one polar monomer (A) and their water solubility data (B).

Peptoid	Water Solubility (mg/L)
P1	$<2.0 \times 10^2$
SP1	$<2.0 \times 10^2$
SP2	$<2.0 \times 10^2$
SP3	$<2.0 \times 10^2$
SP4	$<2.0 \times 10^2$

Fig. 3 Solid-state structure of **PD′** as determined by single crystal X-ray analysis.

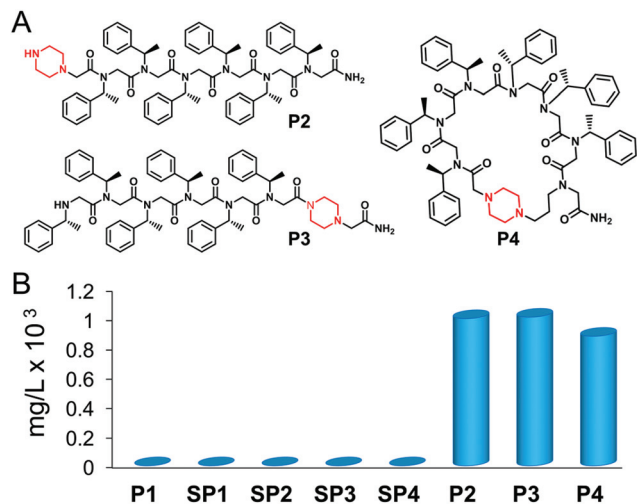This journal is © The Royal Society of Chemistry 2018

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Fig. 4 (A) Sequences of peptoids P1, P2, P3 and P4. (B) Water solubility data of peptoids P1, P2, P3 and P4.

Fig. 5 (A) Sequences of peptoids P5 and P6. (B) Water solubility of peptoids P2, P5 and P6.

firmed by ESI-MS. Water solubility tests were carried out by a gradual addition of 5 μL distilled water to 1 mg peptoid until a clear solution was obtained. This assay was repeated three times and average values are presented in Fig. 4B. The results demonstrate the enormous effect of piperazine on P1; the peptoid became completely water soluble at a concentration of about $(1.00 \pm 0.15) \times 10^3$ mg L^{-1} upon the insertion of only one piperazine at either its N-terminus or C-terminus (P2 and P3, respectively). P4 is also water soluble, albeit at a concentration of about $(8.80 \pm 0.23) \times 10^2$ mg L^{-1}, probably due to its more hydrophobic nature, having no secondary amine available for hydrogen bonding with water. These peptoids are water soluble over time and no precipitation was detected even after several months in undisturbed solutions at room temperature. We also tested the water solubility of P2 in Tris buffer under different pH conditions and found that it exhibits high water solubility in acidic and near natural pH [$(1.66 \pm 0.16) \times 10^3$ mg L^{-1} at pH 4.5 and $(1.28 \pm 0.14) \times 10^3$ mg L^{-1} at pH 7.5], and lower water solubility in basic pH ($<2.0 \times 10^2$ mg L^{-1} at pH 10.5), which is consistent with the water solubility of piperazine at different pH values in the same buffer (Fig. S1A,† inset).

In order to understand the unique ability of piperazine to solubilize P1 in water, specifically in comparison with the disability of the other polar groups that we have tested (Fig. 4B), we note that according to our results piperazine peptoids can be water-soluble even when they are lacking the terminal NH group (i.e. P4, Fig. 4), implying that this group does not impact the peptoid's water solubility. Interestingly, in both P3 and P4, the two piperazine–nitrogen atoms are part of the backbone and are both hydrogen bond acceptors. This is in contrast to the other polar groups that we have tested (see Fig. 1A), which are all peptoid side chains, and have only one atom, which is a hydrogen bond acceptor. We therefore suggest that the presence of hydrogen bond acceptors within the peptoid backbone is an important factor in the ability of piperazine to solubilize
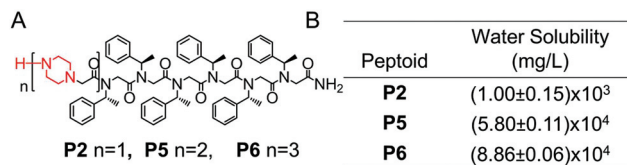
P1. In order to explore this possibility, we synthesized two more peptoids, P5 and P6, having two and three piperazine units, respectively, at their N-terminus (Fig. 5A), which add two and four more hydrogen bond acceptors, respectively, to P2. These peptoids were isolated and their water solubility was tested using the same assay detailed above. The water solubility of P5 was 50 times higher than that of P2, and a further increase was observed in the water solubility of P6 (Fig. 5B). The concentrations of P5 and P6 were also 30 and 106 times, respectively, higher than that of P2 at pH 10.5 (Fig. S1b,† inset). These results imply that the increase in water solubility arises from the addition of nitrogen atoms, capable of hydrogen bonding with water, and support our assumption that the presence of hydrogen bond acceptors within the peptoid backbone is important for solubilizing P1 in water.

To probe this point even further, we wanted to explore the ability of another polar group with two nitrogen atoms to solubilize P1 in water, and chose homopiperazine, which is a seven-membered ring analogous to piperazine. Using our optimized conditions to the solid phase synthesis (see Fig. 2), we prepared peptoids P7 and P8, which have one or two homopiperazine units, respectively, at the N-terminus of P1 (Fig. 6A), and tested their solubility in water. These investigations revealed that the incorporation of homopiperazine results in highly water-soluble peptoids, with P8 being solubilized at a concentration double than that of P7 (Fig. 6B). Overall, these experiments validate that the number of nitrogen atoms is a key factor in the water solubility of P1 when either piperazine or homopiperazine is incorporated within its scaffold.

To demonstrate that our method can be utilized for solubilizing various hydrophobic α-peptides in water, we chose to add piperazine or homopiperazine at the N-terminus of P9, P10 and P11 (Fig. 7). P9, a tetramer bearing four (S)-(−)-1-naphthylethylamine (Ns1npe) groups,[4j] and P10, a pentamer having five (S)-(−)-1-cyclohexylethylamine (Nsch) groups,[4d] have been previously reported and their helical structures have

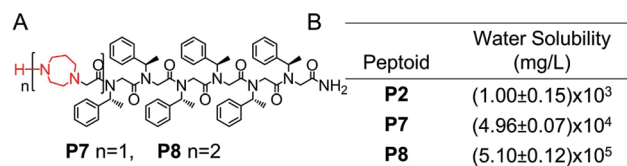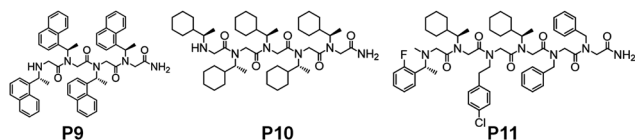Fig. 6 (A) Sequences of peptoids P7 and P8. (B) Water solubility data of peptoids P7 and P8.

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Fig. 7 Sequences of hydrophobic peptoids **P9**, **P10** and **P11**.

Table 1 Sequences and water solubility of peptoids P9–P23

Entry	Peptoid	Sequence	Water solubility (mg L^{-1})
1	**P9**	(Ns1npe)$_4$	$<2.0 \times 10^2$
2	**P10**	(Nsch)$_5$	$<2.0 \times 10^2$
3	**P11**	(Npm)$_2$-Nsch-Ncpe-Nsch-Nfpm	$<2.0 \times 10^2$
4	**P12**	(Ns1npe)$_4$-piperazine	$<2.0 \times 10^2$
5	**P13**	piperazine-(Ns1npe)$_4$	$<2.0 \times 10^2$
6	**P14**	(Ns1npe)$_4$-homopiperazine	$<2.0 \times 10^2$
7	**P15**	(Nsch)$_5$-piperazine	$<2.0 \times 10^2$
8	**P16**	(Nsch)$_5$-homopiperazine	$<2.0 \times 10^2$
9	**P17**	piperazine-(Npm)$_2$-Nsch-Ncpe-Nsch-Nfpm	$<2.0 \times 10^2$
10	**P18**	(Ns1npe)$_4$-(piperazine)$_2$	$(1.33 \pm 0.14) \times 10^4$
11	**P19**	(Nsch)$_5$-(piperazine)$_2$	$(7.52 \pm 0.11) \times 10^3$
12	**P20**	(piperazine)$_2$-(Npm)$_2$-Nsch-Ncpe-Nsch-Nfpm	$(8.44 \pm 0.10) \times 10^4$
13	**P21**	(Ns1npe)$_4$-(piperazine)$_3$	$(1.76 \pm 0.07) \times 10^5$
14	**P22**	(Nsch)$_5$-(piperazine)$_3$	$(7.35 \pm 0.09) \times 10^4$
15	**P23**	(piperazine)$_3$-(Npm)$_2$-Nsch-Ncpe-Nsch-Nfpm	$(1.35 \pm 0.07) \times 10^5$

been determined by X-ray crystallography and solution NMR.[4d,g] **P11** has been designed according to the sequence of a biologically active peptoid recently described by the group of Kodadek.[20]

These three peptoids are not soluble in water (Table 1, entries 1–3). The incorporation of one piperazine or homopiperazine unit at the N-terminus of these oligomers did not enable their water solubility, as it afforded peptoids **P12**–**P17** that are insoluble in water (Table 1, entries 4–9). The insertion of two piperazine units within **P9**–**P11** resulted in the water-soluble peptoids **P18**–**P20** (Table 1, entries 10–12). This water solubility was further increased upon the incorporation of one more piperazine unit (peptoids **P21**–**P23**, Table 1, entries 13–15). These results show that the water solubility of various hydrophobic peptoids could be tuned by including one or two piperazine and/or homopiperazine units within their backbone.

At this point, we wished to investigate whether the two peptoids **P1** and **P9**, both were shown to adopt a polyproline type I (PP-I) helix,[4d,g] maintain their secondary structure in water, after being modified with piperazine or homopiperazine. In contrast to polyproline peptides that are composed of either *cis* or *trans* amide bonds, peptoid monomers can favor both the *cis* and *trans* orientation of the amide bond.[4] This can be easily evidenced from circular dichroism (CD) spectroscopy, which is a key tool for describing the secondary structure of peptoids. Thus, in acetonitrile, **P1** exhibits CD spectra charac-

teristic of peptoids having only phenyl side chains (phenyl peptoids), with a maximum near 190 nm and two minima, one near 200 nm, which has been associated with the *trans*-amide bond conformation, and the second near 220 nm, which is associated with the *cis*-amide bond conformation[4a–d] (Fig. 8A, blue line). The CD spectra of both peptoids **P2** and **P3** in acetonitrile show a slight decrease in the intensity of the band near 200 nm (Fig. 8A red and brown lines, respectively). The CD measurements of **P2** and **P3** in water afforded spectra that are comparable to those obtained in acetonitrile, implying that the helical structure associated with the sequence of **P1** is also maintained in water. Notably, the CD spectra of **P2** in water do not show any significant change when increasing the temperature from 0 °C to 90 °C (Fig. 8B), indicating that the helical structure is stable in water.

CD measurements in Tris buffer at pH 4.5 or 7.5 afforded double minima near 200 and 220 nm, characteristic of the helical structure (Fig. S1a†). These bands were also observed in the CD spectra recorded at pH 10.5, albeit in a much lower intensity (Fig. S1a†). This observation can be attributed to the lower solubility of **P2** in basic solution, as implied by the CD spectra of **P6** (highly soluble at pH 10.5), showing band intensities comparable to those of **P2** as measured in acidic and near natural pH (Fig. S1b†). The CD spectra of peptoids **P7** and **P8** reveal an intense band near 220 nm both in acetonitrile and water (Fig. 8C), and are similar to the spectrum of **P1**, indicating that the helical structure is maintained in water.

As previously reported, peptoid sequences containing only Ns1npe groups (naphthyl peptoids) adopt helical structures with all *cis* orientation of their amide bonds; therefore, their CD spectra lack the double minima discussed above and

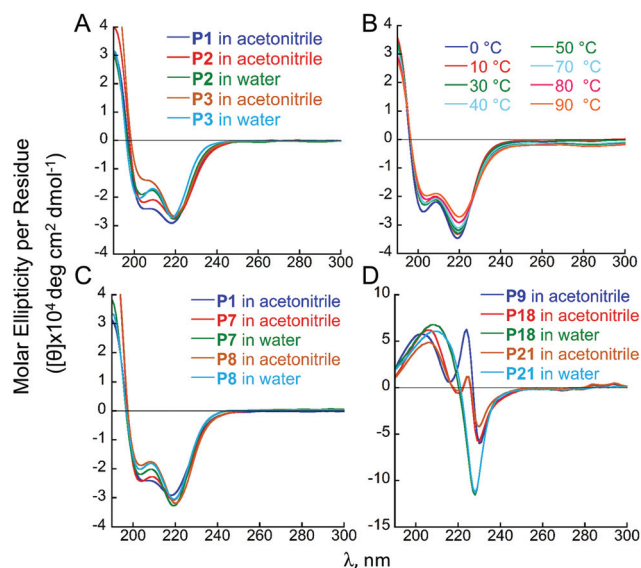**Fig. 8** (A) CD spectra of peptoids **P1**–**P3** (100 µM) in acetonitrile and water. (B) CD spectra of **P2** (100 µM) at different temperatures. (C) CD spectra of **P1** and **P7**–**P8** (100 µM) in acetonitrile and water. (D) CD spectra of **P9**, **P18** and **P21** (100 µM) in acetonitrile and water.

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resemble more the CD spectra of PP-I helices.[4g] Accordingly, the CD spectrum of **P9** in acetonitrile exhibits a broad maximum band near 205 nm, a reduced minimum band near 220 nm and an intense minimum band near 230 nm.[4g] Both bands near 220 and 230 nm in the CD spectra of **P18** and **P21** recorded in acetonitrile show a slight increase in their intensity compared with the bands in the CD spectrum of **P9**, indicating that the conformational order of **P9** is slightly increased upon the insertion of one or two piperazine groups at its N-terminus. The CD spectra of **P18** and **P21** in water, however, changed such that the band near 220 nm disappeared and the intensity of the band near 230 nm doubled. These spectra are actually similar to the ones reported for longer Ns1npe homo-oligomers (in acetonitrile), and the increase in the intensity of this band was associated with higher conformational order and the formation of a robust helical structure.[4g] This observation is remarkable, demonstrating that the addition of piperazine to a helical peptoid can result in a water-soluble hydrophobic peptoid with an extreme helix stability in aqueous solution. This higher conformational order can be attributed to intramolecular hydrophobic interactions between the Ns1npe groups within the same peptoid that are enhanced in the aqueous solution.

Along with intramolecular hydrophobic interactions, intermolecular hydrophobic interactions between the Ns1npe groups from different peptoids can also take place, leading to peptoid aggregates. The aggregation of hydrophilic peptoids containing Ns1npe groups upon their solvation in water has been recently studied and realized by fluorescence spectroscopy.[16c] In order to investigate whether aggregates are being formed while dissolving the piperazine peptoids that contain Ns1npe groups in water, we subjected oligomers **P18** and **P21** to fluorescence measurements (Fig. S2†). In case of aggregation, an excimer band is expected to appear at 392 nm.[16c] According to the spectra we obtained, no band was observed near 392 nm. We also recorded the CD spectra of **P21** at various concentrations ranging from 40 μM to 180 μM in water and recorded some increase in the intensity of the band near 228 nm (Fig. S3a†). This increase can imply that some aggregation occurs in water. As we did not observe the expected excimer band in the fluorescence spectrum, we suggest that only some of the peptoids aggregate and that the intermolecular interactions between the methylnaphthyl groups in the peptoids that do aggregate are not strong enough to affect their emission properties. To further explore the possibility of aggregation in water, we have performed the same CD experiment with the peptoids **P2**, **P5** and **P8**, all having Nspe monomers and one or two piperazine or homopiperazine groups, and their water solubility is similar to or lower than that of **P21**. In these cases, there was almost no increase in the minimum band near 219 nm (Fig. S3b–d†), suggesting that no self-assembly or aggregation of these peptoids occurs in water. Based on both the fluorescence and CD measurements, we can propose that most of the hydrophobic interactions within these peptoids are intramolecular, supporting their high conformational order in water.
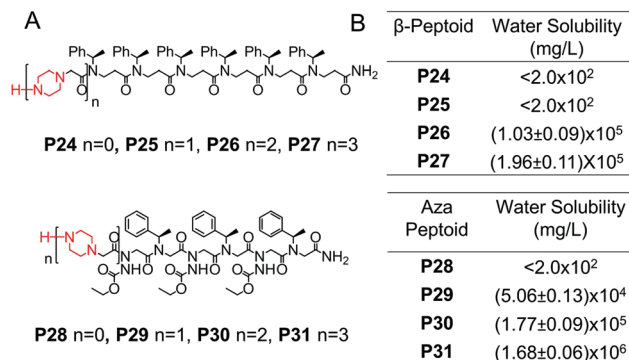

Fig. 9 (A) Sequences of β-peptoids **P24–P27** and azapeptoids **P28–31**. (B) Water solubility data for peptoids **P24–P31**.

After understanding the effect of piperazine on the water solubility of α-peptoids, we wished to explore whether we could broaden the scope of our method by utilizing this polar group to solubilize other types of peptoids in water. To this aim, we have synthesized two more peptoid hexamers, a β-peptoid and an azapeptoid (**P24** and **P28**, respectively, Fig. 9A), both are hydrophobic and water insoluble (Fig. 9B). We have then modified these peptoids by introducing one to three piperazine units at their N-terminus generating the β-peptoids **P25–P27**, respectively, and the azapeptoids **P29–P31**, respectively (Fig. 9A). All peptoids were analyzed and purified by HPLC and their identity was verified by MS analysis (see the ESI†).

Water solubility tests were carried out as described above and the results demonstrate the enormous effect of piperazine on both the β-peptoid **P24** and the azapeptoid **P28**; the peptoids became completely water-soluble after the addition of two piperazine groups to **P24** and only one piperazine group to **P28** (**P26** and **P29**, respectively, Fig. 9B). Although **P25** is not soluble in water, the shorter β-peptoids **P24a** (a tetramer) and **P24b** (a pentamer) could be solubilized in water upon the incorporation of only one piperazine group (Fig. S3†). We therefore concluded that piperazine could be utilized to enable the water solubility not only of α-peptoids, but also of β-peptoids and azapeptoids.

β-Peptoids containing various side chains generally exhibit *cis–trans* isomerization equilibria in solution similar to their peptoid analogues.[21,22] Indeed, the CD spectra of β-peptoids **P24–P27** in acetonitrile show characteristic double minima near 200 and 220 nm, albeit with the latter being less intense than in the case of α-peptoids, in accordance with literature reports on phenyl β-peptoids[21] (Fig. 10A, blue, red, green and orange lines). The CD spectra of water soluble β-peptoids **P26** and **P27**, on the other hand, show a much more intense negative absorbance band near 220 nm, implying that, as in the case of **P9**, intramolecular hydrophobic interactions in water give raise to a much higher conformational order of the helical structure. These results are an exciting demonstration of the secondary structure stabilization of β-peptoids in water by a simple backbone modification.
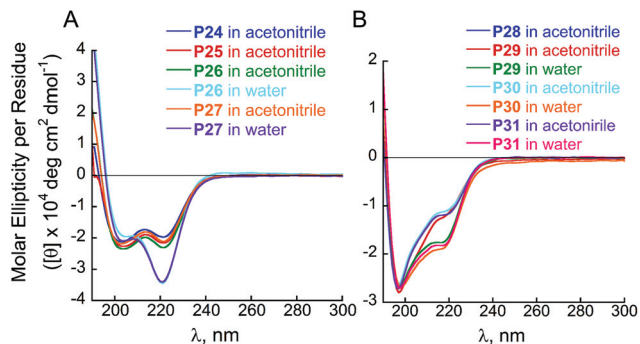
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Fig. 10 CD spectra of 100 μM (A) peptides **P24**–**P27** and (B) **P28**–**P31** in acetonitrile and water.

In contrast to α-peptoids and β-peptoids, in which the tertiary amide bond shows a strong preference for the *cis* geometry, azapeptoids exist almost entirely in the *trans* conformation, as was previously determined by solution ^{1}H NMR measurements and X-ray crystallography.[23] To the best of our knowledge, the CD spectra of azapeptoids were never reported in the literature. Therefore, we initially performed the CD measurements of azapeptoids **P28**–**P31** in acetonitrile, which reveal one intense minimum at 195 nm, suggesting the absence of a secondary structure such that these azapeptoids are not helical (Fig. 10B). In water, however, the CD spectra of azapeptoids **P29**, **P30** and **P31** exhibit an additional absorbance band near 220, indicating an increase in the population of conformers with *cis*-amide bond(s) that results in the initial formation of a helical structure (Fig. 10B). This unique observation suggests, for the first time, that simple incorporation of 1–2 piperazine units within unstructured peptoids can initiate structure formation in water.

Conclusions

In summary, we have developed a unique method for enabling the water solubility of hydrophobic peptoids by simply incorporating piperazine or homopiperazine groups within their scaffolds without alternating their sequences or secondary structures, and in fact while increasing their overall conformational order in water. We demonstrated that the water solubility of various α-, β- and azapeptoids could be tuned by the number and type of the inserted polar group, providing a general method for solubilizing hydrophobic sequences in water. Based on systematic studies by CD spectroscopy, we discovered that the conformational order of all peptoids, and especially that of α-peptoids, having chiral naphthyl monomers, β-peptoids and unstructured azapeptoids, is highly increased in water. These results are unique and represent the first demonstration of stabilizing hydrophobic peptoid helices in aqueous solutions. Piperazine and homopiperazine peptoids are also soluble under various pH conditions and their secondary structures are stable in a wide range of temperatures. Fluorescence spectroscopy as well as variable concen-

trations CD measurements revealed that no self-assembly or aggregation occurs while dissolving the piperazine containing peptoids in water, excluding the possibility of intermolecular hydrophobic interactions between these peptoids. As a result, only intramolecular hydrophobic interactions occur, within the monomers of the same peptoid, providing a good explanation for the high increase in their conformational order in water. Overall, we believe that our findings will open up new horizons in the chemistry of peptoids, by leading to the design of novel functional biomimetic compounds with various possibilities for practical applications in water.

Experimental section

Materials

Rink amide resin and Knorr amide RAM resin were purchased from Novabiochem; (*S*)-1-phenylethylamine (*N*spe), benzylamine (*N*pm), (*S*)-1-cyclohexylethylamine (*N*sch), (*S*)-1-(2-naphthyl)ethylamine (*N*snpe) piperazine, acryloyl chloride, iPr$_2$Net, and homopiperazine were purchased from Acros Organics, Israel; carbazole, *N*,*N*′-diisopropylcarbodiimide (DIC), bromoacetic acid and chloroacetic acid were purchased from Sigma Aldrich. Other reagents and solvents were purchased from commercial sources and used without additional purification. Trifluoroacetic acid (TFA) was purchased from Apollo Scientific Ltd.

Instrumentation

Peptoid oligomers were analyzed by reversed-phase HPLC (analytical C18 column, 5 μm, 100 Å, 2.0 × 50 mm) on a Jasco UV-2075 instrument. A linear gradient of 5–95% acetonitrile (ACN) in water (0.1% TFA) over 10 min was used at a flow rate of 0.7 mL min^{-1}. The detection wavelength was 214 nm. Preparative HPLC was performed using a phenomenex C18 column (15 μm, 100 Å, 21.20 × 100 mm) on a Jasco UV-2075 instrument. Peaks were eluted with a linear gradient of 5–95% ACN in water (0.1% TFA) over 50 min at a flow rate of 5 mL min^{-1}. The detection wavelength was 230 nm. Mass spectrometry was performed on a Waters LCT Premier mass spectrometer and an Advion expression mass spectrometer under electrospray ionization (ESI), direct probe ACN : H$_2$O (70 : 30), and flow rate 0.3 ml min^{-1}. CD measurements were performed using an Applied Photophysics Chirascan circular dichroism spectrometer. ^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument. Coupling constants are given in Hz. The abbreviations used to indicate the multiplicity are: s, singlet; d, doublet; m, multiplet; bs, broad signal. X-ray crystallography was carried out on a Kappa CCD diffractometer at 293 K. Microwave reactions were conducted by using ECM Discover SP microwave.

Preparation of linear peptoid oligomers

Peptoids were synthesized manually on Rink amide resin using the submonomer approach at rt. Typically, 100 mg of resin was allowed to swell in dichloromethane (DCM) for

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40 minutes before initiating oligomer synthesis. The de-protection of resin was performed by the addition of 20% piperidine solution (1.5 ml in dimethylformamide (DMF)) and the reaction was allowed to shake at room temperature for 20 min before piperidine was washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min). Bromoacetylation was completed by the addition of 20 eq. bromoacetic acid (1.2 M in DMF, 8.5 mL g^{-1} resin) and 24 eq. of diisopropylcarbodiimide (DIC) (2 mL g^{-1} resin); this reaction was allowed to shake at room temperature for 20 min. Following the reaction, the bromoacetylation reagents were washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min) and 20 eq. of sub-monomer amine (1.0 M in DMF, 10 mL g^{-1} resin) were added. The amine displacement reaction was allowed to shake at room temperature for 20 min and was followed by multiple washing steps (DMF, 10 mL g^{-1} resin). This two-step addition cycle was modified as follows: to introduce piperazine scaffolds, initially, chloro-acylation was completed by the addition of 20 eq. chloroacetic acid (1.2 M in DMF, 8.5 mL g^{-1} resin) and 24 eq. of DIC (2 mL g^{-1} resin); this reaction was allowed to agitate at room temperature for 35 min. Following the reaction, the chloro-acylation reagents were washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min) and 15 eq. of piperazine (105 mg, 1.23 M in DMF, 10 mL g^{-1} resin after each substitution reaction with piperazine washed with hot DMF, 10 mL g^{-1} resin [3 × 1 min]) was added. The amine displacement reaction was allowed to shake at room temperature for 45 min and was followed by multiple washing steps (DMF, 10 mL g^{-1} resin) (3 × 1 min). Bromoacylations or chloroacylation and amine displacement steps were repeated until the desired peptoids were obtained. To cleave the peptoid oligomers from a solid support for analysis, approximately 5 mg of resin was treated with 95% trifluoroacetic acid (TFA) in water (40 mL g^{-1} resin) for 10 minutes. The cleavage cocktail was evaporated under nitrogen gas and the peptoid oligomers were re-suspended in a 0.5 mL HPLC solvent (1 : 1 HPLC grade acetonitrile : HPLC grade water). To cleave the peptoid oligomers from a solid support for preparative HPLC, the beads were treated with 5 ml of 95% TFA in water for 30 minutes. The cleavage cocktail was evaporated under low pressure, re-suspended in a 2 mL HPLC solvent and lyophilized overnight.

Preparation of linear β-peptoid oligomers

Rink amide resin (100 mg) was allowed to swell in DCM for 40 minutes before initiating oligomer synthesis. The de-protection of resin was performed by the addition of 20% piperidine solution (1.5 ml in DMF) and the reaction was allowed to shake at room temperature for 20 min before piperidine was washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min). A solution of acryloyl chloride (6 equiv.) and i-Pr$_2$NEt (12 equiv.) in DMF (5 ml) was added to a Rink amide resin. After shaking for 3 h, the resin was drained and subsequently washed with DMF (3 × 3 ml), MeOH (3 × 3 ml), and DCM (3 × 3 ml). Then a solution of submonomer amine (6 equiv.) in MeOH (5 ml) was added to the resin that was

shaken at 50 °C for 18 h, and then washed as described above. Then for piperazine insertion initially, chloro-acylation was completed by the addition of 20 eq. chloroacetic acid (1.2 M in DMF, 8.5 mL g^{-1} resin) and 24 eq. of diisopropyl-carbodiimide (2 mL g^{-1} resin); this reaction was allowed to agitate at room temperature for 35 min. Following the reaction, the chloro-acylation reagents were washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min) and 15 equiv. of piperazine (105 mg, 1.23 M in DMF, which is 10 mL solution to 1 g resin) was added. After each substitution reaction with piperazine, the resin was washed with hot DMF (10 mL g^{-1} resin, 3 × 1 min). The amine displacement steps were repeated until the desired peptoids were obtained. Thereafter, the crude product(s) was cleaved from the solid support for analysis. Approximately 5 mg of resin was treated with 50% TFA in DCM (40 mL g^{-1} resin) for 10 minutes. The cleavage cocktail was evaporated under nitrogen gas and the peptoid oligomers were re-suspended in a 0.5 mL HPLC solvent (1 : 1 HPLC grade acetonitrile : HPLC grade water). To cleave the peptoid oligomers from a solid support for preparative HPLC, the beads were treated with 50% TFA in DCM for 2 × 30 min. The cleavage cocktail was evaporated under low pressure, resuspended in a 2 mL HPLC solvent and lyophilized overnight.

Preparation of linear azapeptoid oligomers

For the synthesis of azapeptoids, Knorr amide RAM resin was used. Knorr amide resin (100 mg) in DMF (2 mL) was allowed to swell at room temperature for 1 hour and the fluorenyl-methyloxycarbonyl (Fmoc) protecting group was then removed with 20% piperidine in DMF (2 mL) and subsequently washed with DMF (3 × 3 ml) for 30 min. After the Fmoc group was removed, 2-bromoacetic acid (1 mL, 2 M in DMF) and DIC (1 mL, 3.4 M in DMF) were added. The reaction vessel containing the resin was stirred for about 10 min at 37 °C and subsequently washed with DMF (3 × 3 ml). Then a solution of amine/carbazate (2 mL, 2 M in NMP) was added and stirred at 37 °C for 1 h. Then for piperazine insertion initially, chloro-acylation was completed by the addition of 20 eq. chloroacetic acid (1.2 M in DMF, 8.5 mL g^{-1} resin) and 24 eq. of diisopropylcarbodiimide (2 mL g^{-1} resin); this reaction was allowed to agitate at room temperature for 35 min. Following the reaction, the chloro-acylation reagents were washed off from the resin using DMF (10 mL g^{-1} resin) (3 × 1 min) and 15 eq. of piperazine (105 mg, 1.23 M in DMF, 10 mL g^{-1} resin after each substitution reaction with piperazine washed with hot DMF, 10 mL g^{-1} resin [3 × 1 min]) was added. After amine displacement steps were repeated until the desired peptoids were obtained, then the crude product was cleave the peptoid oligomers from a solid support for analysis. Approximately 5 mg of resin was treated with 95% TFA and 5% water (40 mL g^{-1} resin) for 10 minutes. The cleavage cocktail was evaporated under nitrogen gas and the peptoid oligomers were re-suspended in a 0.5 mL HPLC solvent (1 : 1 HPLC grade acetonitrile : HPLC grade water). The resin was treated with the cleavage cocktail of 95% TFA and 5% water for 1 h at room

temperature. The cleavage cocktail solution was then dried by a stream of argon gas, and cold ether was added to precipitate out the compounds, which were then subjected to reversed phase HPLC to confirm the purity of the products.

Preparation of the cyclic peptoid P4

Rink amide resin-bound **P-linear** (100 mg) and KtBuO (55 mg, 6 equivalents) in a 2 ml 1 : 1 mixture of DMF : tetrahydrofuran were transferred into a microwave reactor. The reactor was subjected to microwave irradiation (dynamic mode) for 60 minutes with 100 watt power at 60 °C. Completion of the reaction was monitored by analytical HPLC. Upon completion of the reaction, the resin was washed with 2% HCL (2 ml), DMF and DCM. To cleave the peptoid oligomers from a solid support for analysis, approximately 5 mg of resin was treated with 95% TFA in water (40 mL g^{-1} resin) for 10 minutes. The cleavage cocktail was evaporated under nitrogen gas and the peptoid oligomers were re-suspended in a 0.5 mL HPLC solvent (1 : 1 HPLC grade acetonitrile : HPLC grade water). To cleave the peptoid oligomers from a solid support for preparative HPLC, the beads were treated with 5 ml of 95% TFA in water for 30 minutes. The cleavage cocktail was evaporated under low pressure, re-suspended in a 2 mL HPLC solvent and lyophilized overnight.

General procedure for the solution phase synthesis of PD′

Nspe (13 mmol) and TEA (15 mmol, 2.092 ml) were added to dry DCM (30 ml) in a flame-dried, three-necked flask with a magnetic bar. The flask was purged with N$_2$, and cooled to −78 °C in a dry ice/acetone bath. Bromoacetyl bromide (14 mmol, 1.219 ml) in DCM (10 ml) was added drop-wise *via* the syringe to the stirring solution under a gentle stream of nitrogen gas. After the addition was completed, the reaction mixture was stirred at −78 °C for 30 minutes, and then removed from the bath and stirred for 6 h at room temperature. Thereafter, the mixture was transferred to a separatory funnel, and washed with 10% w/v equiv. of citric acid and saturated bicarbonate solution. The compound was dried over anhydrous sodium carbonate, the solvent was evaporated and the crude compound was used as such for the next step. The corresponding amide bromide (2 mmol) and K$_2$CO$_3$ (10 mmol) were dissolved in 50 ml acetonitrile followed by the addition of 1 mmol piperazine and the mixture was refluxed for 6 h. The solvent was evaporated and washed with a mixture of water, dichloromethane and brine, and finally dried with anhydrous sodium carbonate. After evaporating DCM, the compound was washed with cold ether three times to obtain the pure compound **PD′**. Note: This strategy does not require column chromatography for purification. **PD′** was crystallized as follows: 100 mg were dissolved in 10 ml diethyl ether/methanol and allowed to evaporate slowly at room temperature. The obtained colorless single crystals were found to be suitable for X-ray crystallography analysis. ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.32 (m, 10H), 5.16 (t, 2H), 3.0 (m, 4H), 2.61 (bs, 8H), 1.52 (d, 6H). ESI-MS: calculated [M + H]$^+$: 408.55; found: 408.59.

Conflicts of interest

There are no conflicts to declare.

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