Stabilising Peptoid Helices Using Non-Chiral Fluoroalkyl Monomers

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Abstract: Stability towards protease degradation combined with modular synthesis has made peptoids of considerable interest in the fields of chemical biology, medicine, and biomaterials. Given their tertiary amide backbone, peptoids lack the capacity to hydrogen-bond, and as such, controlling secondary structure can be challenging. The incorporation of bulky, charged, or chiral aromatic monomers can be used to control conformation but such building blocks limit applications in many areas. Through NMR and X-ray analysis we demonstrate that non-chiral neutral fluoroalkyl monomers can be used to influence the \( K_{\text{cis}}/K_{\text{trans}} \) equilibria of peptoid amide bonds in model systems. The cis-isomer preference displayed is highly unprecedented given that neither chirality nor charge is used to control the peptoid amide conformation. The application of our fluoroalkyl monomers in the design of a series of linear peptoid oligomers that exhibit stable helical structures is also reported.

Peptoids (Figure 1) are a class of foldamers that are being developed as potential therapeutics,[1] biomaterials,[2] chemical sensors,[3] and organocatalysts.[4] They represent an attractive platform for biological and pharmaceutical applications as they are highly resistant to protease degradation.[5] However, given their tertiary amide backbone, peptoids lack the capacity to form hydrogen bonds so that their secondary structures are dominated by relatively weak interactions. Considerable efforts have been devoted to try and understand the relationships between a peptoid primary sequence and its folded structure.[6–10] The \( \text{cis}/\text{trans} \) isomerization of the tertiary amide bond is the major cause of conformational heterogeneity in peptoid oligomers. Despite this, the groups of Zuckermann and Barron have demonstrated that \( \delta \)-chiral aromatic monomers, such as \( \text{N} \)-Spe (1), can stabilize the \( \text{cis} \) configuration of the peptoid amide bond largely through steric effects (Figure 1b,c).[6,7] Peptoid oligomers of \( \text{N} \)-Spe (1) fold into stable all \( \text{cis} \)-amide helices, structurally similar to that of a peptide PPI helix.[6,7] Gorske and Blackwell also reported.

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Figure 1. a) General structure of an \( \alpha \)-peptoid and the amide \( \text{cis}/\text{trans} \) isomerization process. b) The \( \text{cis} \)-inducing \( \alpha \)-methyl chiral aromatic monomers 1 and 2 and the sterically demanding \( \text{Bu} \) monomer 3. c) Summary of the general sequence requirements for helical secondary structure induction in peptoids. d) The \( \beta \)-fluorine gauche effect. e) Dipole interactions in \( \alpha \)-fluoroamides.

that the synergistic application of steric and non-covalent \( n \rightarrow \pi^* \) interactions (NCIs) in aromatic systems could also be used to design stable \( \text{cis} \)-amide peptoid monomers (e.g., \( \text{N}1\text{ape} \), 2). However, it is not possible to use the aforementioned NCIs to stabilize the \( \text{cis} \)-amide configuration of alkylation peptoid monomers, and thus the design of stable peptoid helices remains dominated by the use of chiral aromatic residues (e.g., 1 and 2).[9] Recently, Faure, Taillefumier, and co-workers exploited steric effects in the design of a non-chiral \( \text{Bu} \) alkyl monomer that has a clear \( \text{cis} \)-amide preference (\( \text{N} \)-\( \text{Bu} \), 3).[10] Whereas 3 offers a route to control peptoid structure that avoids the use of aromatic building blocks, the design of non-chiral but stable \( \text{cis} \)-amide alkyl monomers is an area that is still highly underdeveloped.

It is in this context that we sought to explore the potential application of fluorine incorporation as a tool to modulate the conformational preferences of alkyl peptoid monomers. Fluorine is a relatively small atom, close in size to hydrogen, but a \( \text{H} \) to \( \text{F} \) swap can give rise to significant changes in the electronic and structural properties of a molecule.[11,12] For example, fluorine may engage in stereo electronic hyperconjugative interactions with neighbouring \( \text{C} \)–\( \text{H} \) bonds...
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To determine the nature of the interactions present within 11–13, we next examined how the solvent polarity influenced the cis/trans ratios. When using protic MeOD, the $K_{cis\text{-}trans}$ values observed were collectively lower than those found in CD$_3$CN (Figure 3a,b). However, the general increases in the cis-isomer preference produced upon fluorine incorporation were still clearly maintained. This outcome indicates that hydrogen bonding is not involved in the cis-isomer stabilization observed in 11–13 (Figure 3a–c). The use of CDCl$_3$ also reduced the $K_{cis\text{-}trans}$ values recorded, and this general trend is in good agreement with previous observations reported for other model peptoid systems.[8b–d] Despite this general decrease, the cis-amide preferences of 11 and 12 in CDCl$_3$ were still significantly greater than that of the control 10. Upon moving from no fluorine atoms (10) to either one (11) or two (12), relative changes in the free energy of $–0.81$ kcal mol$^{-1}$ and $–1.13$ kcal mol$^{-1}$, respectively, were seen.

These relative $\Delta G_{cis\text{-}trans}$ changes are in fact larger in CDCl$_3$ (non-polar) than in CD$_3$CN (polar), and this finding supports the hypothesis that an electronic cis-stabilizing effect is occurring. Remarkably, in CDCl$_3$, the N3/Et monomer (7) actually has a greater ability to stabilise a cis-amide preference than the chiral aromatic NRpe monomer (9; $K_{cis\text{-}trans} = 1.28$ vs. 0.94; Figure 3a). The N3/Et-containing peptoid 13 was found to be more affected in CDCl$_3$, and it produced a strong out-of-trend shift to the trans isomer (Figure 3a,b). Given this observation, we hypothesised that the energetic penalty that 13 experiences in the cis conformation may arise from an increased solvation barrier as non-polar solvents are well-known to disfavour structures where large dipoles are.

(\sigma(CH)→\sigma^*(CF)). This ability of fluorine to enforce the preorganisation of its local environment is most keenly observed when fluorine atoms are located β to electron-withdrawing groups. In such an arrangement, the fluorine gauche effect is seen (Figure 1d).[12,13] Notably, the fluorine gauche effect is more pronounced in β-fluorooamides than in other related systems.[12,13] However, in α-fluorooamides, CF/ C=O dipolar interactions dominate, and the fluorine atom adopts a trans-periplanar arrangement (Figure 1c).[14] The peptoid amide bond cis/trans equilibrium in our model systems (Figure 2; 10–14) was analysed by a range of established NMR methods (see the Supporting Information for the synthesis of 10–14).[8b–d] The non-fluorinated peptoid 10 exhibits a cis/trans equilibrium that highly favours the trans isomer (CD$_3$CN; $\Delta G_{cis\text{-}trans} = 0.28$, $K_{cis\text{-}trans} = 0.66$; Figure 3a). Relative to this, all of the fluorinated peptoids (11–13) showed an enhanced preference for the cis-amide conformation (Figure 3). Initial NMR analysis (in CD$_3$CN) revealed that even the introduction of a single fluorine atom β to the amide bond enhanced the cis-amide preference by 0.37 kcal mol$^{-1}$ when compared to 10. Incorporation of a second fluorine atom further increased the cis-amide preference. Indeed, unlike 10 and 11, the difluorinated peptoid 12 shows a highly prominent cis-amide conformation in solution, with $\Delta G_{cis\text{-}trans} = –0.42$ kcal mol$^{-1}$ and $K_{cis\text{-}trans} = 2.05$ (Figure 3a). We were surprised to note that the $K_{cis\text{-}trans}$ value exhibited by 12 is comparable to those seen when cis-inducing chiral aromatic monomers are used (e.g., for 14, $K_{cis\text{-}trans} = 2.08$ in CD$_3$CN). Initial NMR analysis revealed a linear correlation between the $\Delta G_{cis\text{-}trans}$ values observed and the electron-withdrawing character of the C$_n$ carbon substituent when one or two fluorine atoms were incorporated (e.g., 10 to 12; Figure 3b,c). This correlation indicated a clear relationship between the inductive properties of the fluorinated groups and the cis/trans ratios produced ($\phi_1$; Figure 3c,e). An even greater cis-isomer preference was observed when the N3/Et-containing peptoid 13 was analysed (CD$_3$CN; $K_{cis\text{-}trans} = 2.24$).

Figure 2. a) Synthesis of model piperinidyl acetamides 10–14. b) Peptoid monomers used in this study. c) Reference model dipeptoids (10, 14) and novel β-fluoroethyl (11), β-difluoroethyl (12), and β-trifluoroethyl (13) systems.

Figure 3. a) Average $K_{cis\text{-}trans}$ values in model systems 10–14. [a] From each replica, $\Delta G = –RT \ln (K_{cis\text{-}trans})$ at 25 °C. Averages and standard deviation values are given for $n = 6$ or $n = 4$ (†). [b] Average $K_{cis\text{-}trans}$ values vs. the number of fluorine atoms present ($n$). [c] Correlation between $\Delta G_{cis\text{-}trans}$ and C$_n$-substituent field/inductive constants ($\phi_4$). [d] Schematic representation of the proposed peptoid cis-amide isomer stabilization by inductive factors. [e] Inductive constants of C$_n$ groups.[5]
present. As depicted in Figure 3d, the overall dipolar moment within trans-13 is likely to be lower than that within the corresponding cis isomer as the carbonyl and side-chain dipoles are opposed. This solvation effect should be less pronounced in 11 and 12 as they have weaker dipoles (Figure 3e).

Next, we explored the role that fluorine/amide gauche interactions could play in enhancing the cis-isomer preferences. The vicinal (three-bond coupling) $J_{HF}$ coupling constants were thus analysed (Figure 4a).[17,18] In 11, a $J_{HF,cal}$ value of 20.0 Hz was calculated for an ideal fluorine/amide gauche conformation of the side chain (g). A significantly lower value of 8.0 Hz was obtained for the alternative fluorine/amide anti configuration (a). The experimental value found within the predominant cis-11 isomer was $J_{HF,obs} = 25.7$ Hz (CD$_3$CN). This result strongly suggested an overall fluorine/amide gauche orientation within the side chain. Two staggered conformations for 12 were also examined, and the experimental value of $J_{HF,obs} = 14.9$ Hz was in perfect agreement with an anti(gauche) configuration (Figure 4b). This finding indicates that only one F atom may be actually located gauche to the peptoid amide group, and this is contrary to the more intuitive (+/−/−) configuration that would be expected. No significant variations in the experimental $J_{HF,obs}$ values were seen within each cis/trans pair in any of the solvents tested, indicating that the fluorine/amide relative arrangement is retained between conformers. The NMR results suggest that fluorine gauche effects are not solely responsible for the cis-isomer preferences observed in 11 and 12. In 13, fast rotation of the CF$_3$ group was inferred as the experimental $J_{HF,obs}$ value greatly deviated from the calculated value, which assumes a static fluorine/amide arrangement (Figure 4c).

We were able to crystallize dipeptoids 12 and 13 from their EtOAc saturated solutions.[19] The solid-state structures for 12 and 13 and the conformations suggested by NMR analysis were in perfect agreement (Figure 4d,e). It is worth noting that from the crystal structures of 12 and 13, it would appear that neither fluorine–oxygen repulsive interactions nor unfavourable steric clashes contribute substantially to the cis/trans conformation preferences observed in these systems. As shown in Figure 4d,e, the fluorinated groups in 12 and 13 display a well-defined orthogonal orientation relative to the amide bond planes. This orientation minimizes the potential steric clashes and/or electronic repulsion imposed by the CHF$_2$/CF$_3$ groups. Overall, our findings support the hypothesis that the enhanced cis-amide preferences observed in 11–13 arise from the inductive effects imposed by the fluorine atom(s). As the polarization at C$_F$ increases, the peptoid cis-amide preference also increases. We propose that this is due to the fact that the $Δ$4 on C$_F$ can form a syn-periplanar stabilising dipolar interaction with the amide C=O (Figure 3d).

Encouraged by the cis/trans ratios achieved in the model systems (11–13), we then moved to see if the non-chiral fluoroalkyl monomers could be exploited to design stable peptoid helices. To this end, we designed a control 15-mer peptoid, Pep.1, using non-chiral alkyl ethylamine monomers (Figure 5). A single $\text{N}^\text{Spe}$ residue was introduced as a chiral reporter for circular dichroism (CD) spectroscopy. The Pep.1 sequence was then altered by substituting in the various fluorinated monomers (6–8) in place of some, but not all, of the NEt residues (group 1, Pep.2–4). In a second group of fluorinated pepptides, all of the NEt residues present were replaced (group 2, Pep.5–7). We were pleased to see that structural analysis of the peptoid oligomers Pep.2–Pep.7 by CD spectroscopy revealed the presence of stable peptoid helices (Figure 5b,c). In all of the peptoids studied, substitution of the NEt residues by any of the fluorinated monomers clearly enhanced the CD minima at 218 nm ($M_{218}$), which is characteristic of an increase in helicity. When five substitutions (NEt for a fluoroalkyl monomer) were made in non-consecutive positions (group 1, Pep.2–4, $n_1 = 5$), the overall increases in molar ellipticity were found to correlate with the number of fluorine atoms within the side chain. For example, upon going from the non-fluorinated peptoid (Pep.1) to the N1/Et-based analogue (Pep.2), a change in molar ellipticity of $ΔM_{218} = 6660$ deg cm$^2$ dmol$^{-1}$ was observed. Similarly, incorporation of N2/Et (7) and N3/Et (8) produced approximately two- and threefold higher increases in $M_{218}$ (Pep.3, $ΔM_{218} = 12640$; Pep.4, $ΔM_{218} = 17000$ deg cm$^2$ dmol$^{-1}$).

When the more heavily substituted peptoids from group 2 were analysed, higher values of $M_{218}$ were found, indicating that the secondary structure enhancement induced by the incorporation of fluorinated side chains has an overall accumulative behaviour (Pep.5–7; Figure 5d,e). In fact, the

Figure 4. Theoretical versus experimental vicinal $J_{HF}$ coupling constants within a) 11, b) 12, and c) 13 in their preferred cis conformations (CD$_3$CN). Ball-and-stick representations of the crystal structures of cis-amides d) 12 and e) 13.[14]
average increases in $M_{q218}$ per fluorine residue incorporated ($\Delta M_{q218}/n_f$) in peptoid sequences from group 1 (Pep.1–Pep.4, $n_f=5$; parts b, c) and group 2 (Pep.5–Pep.7, $n_f=9$; parts d, e).

average increases in $M_{q218}$ produced by each $N1f$/Et (6) and $N2f$/Et (7) monomer introduced in these sequences were higher than those observed when only five replacements were made ($\Delta M_{q218}/n_f$; Pep.2 vs. Pep.5 and Pep.3 vs. Pep.6; Figure 5c–e). These results revealed a broadly cooperative effect between neighbouring fluorinated side chains. Interestingly, this synergy between consecutive monomers did not occur when $N3f$/Et monomers were used (Pep.4 vs. Pep.7).

Based on our crystal structure data, we could evaluate that the volumes of the CHF$_2$ and CF$_3$ groups are 29.63 and 40.47 Å$^3$ respectively. Based on this, we hypothesise that the behaviour seen for Pep.7 may be related to unfavourable steric and/or repulsive interactions between the CF$_3$ groups of adjacent $N3f$/Et monomers. Overall, the results from the CD studies (Figure 5) are highly unprecedented owing to the fact that none of the fluorinated monomers investigated are either chiral, aromatic, or charged, and yet they can support the formation of stable peptoid helices.

In summary, we have shown that the selective and strategic incorporation of fluorine atom(s) offers a new route to control the amide bond isomerism in peptoids containing alkyl side chains. Through NMR and X-ray analysis we demonstrated that simple non-chiral fluoroalkyl monomers can be used to influence the key $K_{cis/ trans}$ equilibria of a peptoid amide bond and induce a remarkable degree of cis-amide preference. The cis-isomer preference is highly unprecedented given that neither chirality nor charge was being used to control the peptoid amide conformation. The data gathered support the hypothesis that inductive effects imparted by the fluorine atom(s) and not fluorine gauche effects underpin the cis-isomer stabilization observed. The novel fluoroalkyl monomers were also used to prepare a series of peptoid oligomers that exhibited stable helical structures despite only having one chiral aromatic residue. The application of fluorine in the design of alkyl monomers offers a new approach to control amide bond isomerism in peptoid sequences, overcoming the current need for high levels of chiral side chains. Given the lack of alternatives available, the $N1f$/Et, $N2f$/Et, and $N3f$/Et alkyl monomers offer exciting new tools to design structurally stable peptoid systems with applications in a range of areas, including medicine and biomaterials.

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**Conflict of interest**

The authors declare no conflict of interest.

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Figure 5. a) Peptoid oligomers Pep.1–Pep.7. b, d) Average CD spectra and c, e) average absolute increases in $M_{q218}$ per fluorine residue incorporated ($\Delta M_{q218}/n_f$) in peptoid sequences from group 1 (Pep.1–Pep.4, $n_f=5$; parts b, c) and group 2 (Pep.5–Pep.7, $n_f=9$; parts d, e).
On the same side: NMR spectroscopy and X-ray analysis were applied to demonstrate that non-chiral, neutral fluoroalkyl monomers can be used to promote enhanced levels of cis-amide preference in model peptoid systems. These fluoroalkyl monomers were also directly applied to design a series of peptoid oligomers that exhibit stable helical structures, as shown by CD spectroscopy.