Fluorinated Aromatic Monomers as Building Blocks to Control α-Peptoid Conformation and Structure

Diana Gimenez,† Guangfeng Zhou,‡ Matthew F. D. Hurley,† Juan A. Aguilar,† Vincent A. Voelz*,‡ and Steven L. Cobb*,†

† Durham University, Department of Chemistry, South Road, Durham, DH1 3LE, U.K.
‡ Temple University, Department of Chemistry, Philadelphia, Pennsylvania 19122, U.S.

Supporting Information Placeholder

ABSTRACT: Peptoids are peptidomimetics of interest in the fields of drug development and biomaterials. However, obtaining stable secondary structures is challenging and designing these requires effective control of the peptoid tertiary amide cis/trans equilibrium. Herein, we report new fluorine containing aromatic monomers that can control peptoid conformation. Specifically, we demonstrate that a fluoropyridine group can be used to circumvent the need for monomer chirality to control the cis/trans equilibrium. We also show that incorporation of a trifluoro-methyl group (NCF3)† rather than a methyl group (NRpe) at the α-carbon of a monomer gives rise to a 5-fold increase in cis-isomer preference.

α-Peptides (Figure 1a) are resistant to protease degradation† and are thermally stable.‡ They are of interest as therapeutics§-¶ and as biomaterials.¶ However, because a peptoid is composed of N-alkyl amide bonds, there is no capacity to use hydrogen bonding to stabilize folded structures. Accessing and designing stable structures, such as helices,⁵⁻⁶ ribbons,⁶⁻⁷ loops⁸ or sheets⁹⁻¹⁰ relies on utilizing a limited number of peptoid monomers that can predictably restrict the amide bond isomerism. An early advance was reported by Zuckermann and Barron, who showed that monomers with N-α-chiral aromatic side-chains, such as NSpe (2)(Figure 1b), can stabilise all cis-amide polypeptide helices (PPI).¹⁰,¹¹ Gorske and Blackwell explored non-covalent interactions (NCI) including sterics, hydrogen bonding and electronic n→π⁺ effects to control the cis/trans equilibrium in model peptoid systems, through which they identified new chiral peptoid monomers such as NSpe (3) and NSe (4) (Figure 1b), able to impose larger Kcis/trans values than NSpe (2).¹¹⁻¹³ Monomers such as 3 and 4 exert their effects through a synergistic combination of steric factors (e.g. α-methylation) and electronic n→π*Ar interactions. While all of the aforementioned monomers are neutral, pioneering work by Taillufumier and Faure demonstrated that positively charged triazolium-type monomers impart an impressive level of conformational control into a peptoid backbone.¹² Yet, while this approach gives some of the largest Kcis/trans values reported, the use of charged monomers puts restrictions on designed peptoid sequences.

While some work has been carried out to exploit fluorine in the design of new peptoid monomers (e.g. NSe, 4), we sought to investigate the application of perfluoro-heteroaromatics, such as tetrafluoropyridine. We hypothesized that such highly electron-deficient systems would favor even stronger n→π*Ar interactions, overcoming the need for monomer chirality. To investigate this hypothesis, three model di-peptoids based on the non-chiral benzylamine (Npm, 1), pyrindylmethanamine (Npym, 9), and (tetrafluoropyrindyl)methanamine (NSe/pym, 10) were prepared for conformational analysis (Figure 2b).

We recently reported that fluorine atom(s) β to the amide bond nitrogen promote enhanced cis-amide preference in...
non-chiral alkyl type monomers (Figure 1c). This cis-amide preference was found to rely on fluorine induced dipolar interactions. Following this we envisaged that incorporation of a trifluoromethyl (CF₃) group into an aromatic monomer might allow both electronic (dipolar interactions) and steric effects to be utilized in tandem to access systems in which the cis/trans equilibrium completely favors one isomer. To explore this, we prepared the α-trifluoromethyl (NCF₃)Rpe model peptoid 12 (Figure 2c). For comparison, the non-fluorinated reference 11 was also prepared. The synthetic route employed to model peptoids (1, 9-12) is shown in Figure 2a.\(^{20,21}\)

We employed H-NMR and H-H NOESY to evaluate the cis/trans ratios present within the model systems (see SI for further details).\(^{24,20,21}\) Analysis of 1 showed that, as reported, the benzyl side chain induces a solvent dependent conformational preference (Figure 2d).\(^{21}\) In CDCl₃, a trans-amide geometry is favored by 0.79 kcal mol\(^{-1}\), while in more polar solvents no conformation preference was found (e.g. ΔG=0 in both CD₃CN and CD₃OD, Figure 2d). Replacement of the aromatic ring with a hetero-aromatic group caused a significant increase in the cis-isomer preference seen across all of the solvents tested (1 versus 9 in Figure 2d). When the electron-withdrawing character of the aromatic ring was further increased, through the incorporation of a tetrafluoropyridine group (NCF₃pym), an impressive increase in the K\(_{cis\text{-}trans}\) values was seen (10 in Figure 2d and Figure 3). Indeed, 10 showed a 3-fold higher K\(_{cis\text{-}trans}\) value in CDCl₃ than its non-fluorinated analogue 9 (K\(_{cis\text{-}trans}\)= 1.41 vs. 0.47, Figure 3). The conformational preference of 10 was enhanced in polar solvents where highly biased cis-populations were seen (K\(_{cis\text{-}trans}\)(CD₃CN)= 3.22, 76% cis-isomer; K\(_{cis\text{-}trans}\)(CD₃OD)= 2.22, 69% cis-isomer content; Figure 2d). Interestingly, analysis of the NOE correlations within 10 implied that the fluoro-pyridine ring sits facing the N-terminal carbonyl group in the cis-isomer (Figure 3). This result supports a mechanism in which the cis conformation is stabilized by means of fluorine enhanced n→π*ₜₐ interactions. The K\(_{cis\text{-}trans}\) values recorded for 10 are among the highest ever reported for a neutral non-chiral monomer in this type of peptoid model system. What is more remarkable is that despite being a non-chiral monomer, the cis-isomer preferences induced by NCF₃pym are comparable or even greater than those produced by the widely utilized α-chiral monomers such as 2 and 4 (Figure 2b and Figure 2d).

We then turned our attention to the effects imparted by fluorine at the α-methyl position. The results obtained for the reference (11) agreed fully with those previously reported for the (S)-enantiomer (NSpe, 2) (Figure 2d).\(^{21}\) 11 exhibited almost no conformational preference in CDCl₃ (K\(_{cis\text{-}trans}\)= 1.0). Again, in line with the literature, in polar solvents its cis/trans equilibrium shifted in favour of the cis-isomer, particularly in CD₃CN (K\(_{cis\text{-}trans}\)= 2.07).\(^{22}\) In stark contrast, however, the NCF₃Rpe containing di-peptoid 12 displayed a high degree of conformational preference. Both the H and the ²⁷F NMR data of 12 revealed the presence of one isomer in solution with a predominance of 82±4% (Figure 2d). It was not possible to use H-H NOESY correlations within 12 to assign the configuration of the major isomer preferer (e.g. see Fig. S5s). Therefore, to better understand the conformational preference exhibited by 12, computational studies were performed using both ab initio QM and replica exchange molecular dynamics (REMD).

Scans of side-chain and backbone dihedral angles were performed using DFT at the B3LYP/6-31G+(2d,p)//HF/6-31G(p) level of theory (Figure 4). To identify side-chain conformational minima, χ₁ and χ₂ angles were first scanned from 0° to 360° in 30° intervals starting from typical backbone conformations of peptoids: cis-amide α₀ (φ,ψ = -90°, 180°), trans-amide α₀ (φ,ψ = -90°, 180°), and trans-amide C₄ (φ,ψ = 180°, 80°), with all remaining dihedral angles unrestrained during geometry optimization (Figure 4b, Fig. S15). The results showed a preference for χ₁, χ₂ near (-90°, +15°) in cis-amide structures, and a mixture of (-90°, +15°) and (+90°, +15°) preferences in trans-amide structures, consistent with similar work for related molecules.\(^{25}\) Next, full backbone dihedral scans of φ and ψ angles (15° intervals) were performed starting from cis-amide and trans-amide isomers with -90° and +90° χ₁ angles, with all dihedral angles except φ and ψ unrestrained during optimization (Fig. S6f). From these studies, the cis-amide energy minimum was found to be 1.26 kcal mol⁻¹ lower than the trans-amide, in excellent agreement with MD data.

![Figure 2](image1.png)

**Figure 2.** a) Synthesis of model peptoids; b) Non-chiral dipeptides 1, 9-10; c) Chiral di-peptoids 11, 12. d) Average K\(_{cis\text{-}trans}\) values for 1, 9-12. From each replica, ΔG= -RTln(K\(_{cis\text{-}trans}\)) at 25 °C. Averages and SD values given for n=6; |ΔH|=5 and |ΔS|=3. Major isomer assigned as cis, in agreement with MD data.

![Figure 3](image2.png)

**Figure 3.** a) Amide bond geometry in systems 1, 9 and 10. b) Experimental ¹H-¹H NOESY correlations within cis/trans conformers of 10. All K\(_{cis\text{-}trans}\) values as determined in CDCl₃.

![Figure 4](image3.png)

**Figure 4.** a) Side-chain conformational preferences of 12. b) Backbone dihedral preferences of 12.
Figure 4. a) The lowest-energy conformation of 12, annotated with dihedral angle definitions (see SI for details). b) Lowest-energy minima found in φ, ψ backbone dihedral scans started from cis- and trans-amide conformations with side-chain orientations χ1 = -90° and +90°. c) REMD simulations of oligomeric analogues of 12, Ac-[NCF3]Rpe]α-Pip (n=1,2,3,4,5) showing an increasing preference to form right-handed cis-amide helices. d) Space-filling model of the predicted pentamer structure (n=5). e-f) Longitudinal views of a representative frame of the oligomer from the lowest temperature replica (300 K).

agreement with the experimental Kcis/trans values measured (Figure 2d). In comparison, similar calculations for NSpe show a cis/trans energy minima gap of only 0.2 kcal mol⁻¹. These results, in combination with the experiments above, strongly indicated that the single isomer seen experimentally corresponded to the cis-amide conformation.

The computed backbone dihedral (ϕ,ψ) landscape of 12 resembles that of the NSpe monomer, but unlike NSpe, which favors a negative backbone ϕ-angle (near -90°) by -1 kcal mol⁻¹ over the positive angle (near +90°), 12 favors the positive angle by -0.8 kcal mol⁻¹ (Fig. S6). This may partly be due to unfavorable proximity (3.1 Å) of the carboxyl oxygen to the nearest fluorine in the electronegative CF₃ group for the cis-amide negative ϕ-angle conformation (5.2 Å for the positive ϕ-angle conformation).

Comparative analysis of model systems 1, 9, 10, 11 and 12 using natural bond orbital (NBO) analysis (see SI for details)

Figure 5. a) Structure of peptoid oligomers 13-15. b) Main H-NMR parameters of NSpe and NCF₃Rpe residues as analyzed in 15. c) Structure and average CD spectra (n=3) of peptoid hetero-oligomers 13 (shown in red) and 14 (shown in blue). All measurements in CH₃CN.

suggests the presence of n→π* effects, as seen by a decrease in the natural charge of the carbonyl oxygen atom and increase in the total π* occupancy of the aromatic system. The extent of these effects is strongly correlated with the experimental Kcis/trans ratios, and are most significant for system 10. These effects are less significant for system 12, supporting a mechanism of cis-isomer stabilization in 12 in which a combination of inductive and steric factors, rather than n→π* interactions per se, act to achieve such a large Kcis/trans ratio. This result also agrees with the orientation of the carbonyl and aromatic groups in the minimum energy structure of 12.

To predict the conformational preferences of NCF₃Rpe oligomers, we performed replica exchange molecular dynamics (REMD) simulations of 12 as well as the related oligomeric species Ac-[NCF₃]Rpe]α-Pip, for n = 1,2,3,4,5. (Figure 4c). The simulations agreed with QM studies (Table S6), with cis-amide populations above 95% for all residues (Fig. S7). Strikingly, simulations also predicted that larger oligomers are increasingly prone to form stable right-handed helices (e.g. negative ϕ-angle), with NCF₃Rpe pentamers displaying cis-amide helix populations of nearly 100% (Figure 4c-f). These results likely stem from the large Kcis/trans value for 12,
which is additionally rewarded by the excellent side-chain packing achieved in the helical conformation. The difference between the $\phi$-angles seen in the model (12) and the $N^{\varepsilon}$-Rpe oligomers we believe arises due to 12 being unconstrained by the side chain packing that is present within the oligomers.

The REMD simulations were experimentally validated by $\nu^F$-NMR (Fig. S26) and circular dichroism (CD) (Fig. S40) analysis of oligomers 13-15 (Figure 5). By comparison of 13 and 14 it can be seen that increasing the number of sequential $N^{\varepsilon}$-Rpe residues leads to an increase in the conformational homogeneity. Similarly, removal of $N^{\varepsilon}$-Rpe residues from the sequence (e.g. 14 versus 15) leads to a decrease in conformational homogeneity. In addition, HSQC-TOSCY and NOE/SY analysis of peptide 14 demonstrated the enhanced $cis$-$amide$ preference of the $N^{\varepsilon}$-Rpe residues ($K_{cis/trans}$ = 2.3) compared to the $N$-Rpe ($K_{cis/trans}$ = 0.7) (Figure 5b; Figs. S127 and S130). To further explore the application of $N^{\varepsilon}$-Rpe as a tool to stabilize the helical conformation of longer peptides two model hetero-oligomers were prepared (16 and 17, Figure 5c). When the secondary structures of 16 and 17 were analyzed by CD clear differences beyond the opposing helical chirality enforced by both monomers (e.g. left-hand helix for NRpe and a right-handed helix for $N^{\varepsilon}$-Rpe) were seen. Specifically, 17 showed a dramatic 42% increase in the $\alpha$-helix compared to 16. This result provides clear evidence that the substitution of non-fluorinated NRpe residues by their $\alpha$-trifluoromethyl analogues ($N^{\varepsilon}$-Rpe) offers a route to enhance the peptoid secondary helical structure.

In summary, we report the application of fluorine as a tool to design monomers which enhance the conformational stability of $\alpha$-peptoids. The $K_{cis/trans}$ values recorded for the $N^{\varepsilon}$-pym containing di-peptoid 10 are among the largest ever reported for a non-chiral monomer. $N^{\varepsilon}$-pym also represents the first example of a non-chiral, non-charged aromatic monomer that can induce a strong $cis$-$amide$ preference. The $N^{\varepsilon}$-pym residue achieves its high $K_{cis/trans}$ values by pushing the electronic n→n* effects to the limit of what is possible in a neutral system. We recently reported the application of fluorine inductive/ di-polar effects as a new tool to modulate $K_{cis/trans}$ ratios\(^3\) but the $N^{\varepsilon}$-Rpe monomer demonstrates the benefits of combining fluorino induced steric and fluorine induced inductive electronic effects. Indeed, as evidenced by NMR analysis, $ab$ initio and molecular dynamics calculations, the $N^{\varepsilon}$-Rpe monomer has the ability to push the $K_{cis/trans}$ equilibrium to essentially favor one single isomer. REMD simulations of $N^{\varepsilon}$-Rpe oligomers predicted the formation of highly stable right-handed helices and this was experimentally validated via preparation and conformational analysis of a series of $N^{\varepsilon}$-Rpe containing peptoid oligomers. $N^{\varepsilon}$-pym and $N^{\varepsilon}$-Rpe provide a much-needed expansion of the limited tool-box of monomers available for the rational design of conformationally stable peptoids.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website. This material includes: Experimental procedures and characterization data for peptoid monomers 1, 9-12 and oligomers 13-17 (PDF). X-ray crystallographic data for by-product from 12 (CIF).

AUTHOR INFORMATION

Corresponding Authors

* s.l.cobb@durham.ac.uk; voelz@temple.edu

Author Contributions

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