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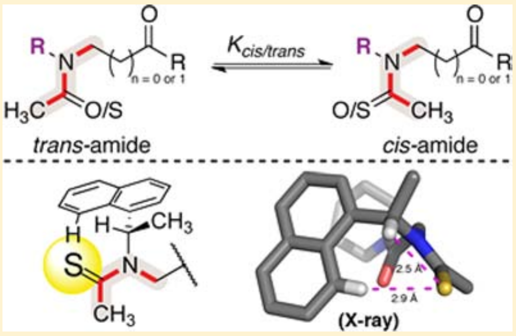
Cis−*Trans* Amide Bond Rotamers in β-Peptoids and Peptoids: Evaluation of Stereoelectronic Effects in Backbone and Side Chains

Jonas S. Laursen, Jens Engel-Andreasen, Peter Fristrup, Pernille Harris, and Christian A. Olsen*

Department of Chemistry, Technical University of Denmark, Kemitorvet 207, DK-2800, Kongens Lyngby, Denmark

Supporting Information

ABSTRACT: Non-natural peptide analogs have significant potential for the development of new materials and pharmacologically active ligands. One such architecture, the β-peptoids (N-alkyl-β-alanines), has found use in a variety of biologically active compounds but has been sparsely studied with respect to folding propensity. Thus, we here report an investigation of the effect of structural variations on the *cis*−*trans* amide bond rotamer equilibria in a selection of monomer model systems. In addition to various side chain effects, which correlated well with previous studies of α-peptoids, we present the synthesis and investigation of *cis*−*trans* isomerism in the first examples of peptoids and β-peptoids containing thioamide bonds as well as trifluoroacetylated peptoids and β-peptoids. These systems revealed an increase in the preference for *cis*-amides as compared to their parent compounds and thus provide novel strategies for affecting the folding of peptoid constructs. By using NMR spectroscopy, X-ray crystallographic analysis, and density functional theory calculations, we present evidence for the presence of thioamide−aromatic interactions through C_{sp^2}−H···S_{amide} hydrogen bonding, which stabilize certain peptoid conformations.



■ INTRODUCTION

The 20 canonical α-amino acids constitute the fundamental set of building blocks necessary for human ribosomal synthesis of the major class of biopolymers comprised of proteins and peptides. In traditional medicinal chemistry, this class of compounds has not been considered suitable for drug development, due to susceptibility to proteolytic degradation in cellular environments and often poor cell permeability properties. Nevertheless, recent tendencies in the pharmaceutical industry have revealed an increased interest in the development of so-called biologics. This may, at least in part, be due to the successful approval and marketing of several monoclonal antibodies as therapeutics during the past decade. In order to circumvent the inherent stability problems, however, extensive research in the field of peptidomimetic designs has been undertaken. In addition to the nature of the functional groups themselves, bioactive α-peptides realize their high potency and selectivity due to stabilized secondary structure formation, which displays these functionalities accurately in three-dimensional space. Non-natural compounds that are capable of adopting stabilized three-dimensional structures mimicking or complementing those found in nature are therefore of great interest, and as a class of compounds, these various chemotypes have been coined “foldamers”.[1] A wide variety of foldamers have been developed and extensively studied,[2] with some of the prominent peptidomimetic examples being β-peptides[3] and peptoids (N-alkylglycines)[4] (Figure 1A).

The tertiary amide backbone architecture in peptoids renders them unable to stabilize putative folded structures by forming intramolecular hydrogen-bond networks. Furthermore, the presence of tertiary backbone amide bonds gives rise to increased flexibility due to a low-energy barrier between *cis* and *trans* configurations. Thus, a high degree of *cis*-amide bonds may occur in peptoids, which is almost exclusively observed at proline in natural peptides and proteins (Figure 1B)[5] and have been enhanced by introduction of synthetic proline derivatives.[6] The effect of various N-alkyl side chain functionalities on this *cis*−*trans* equilibrium in peptoids has been studied by NMR spectroscopy.[7−10] Despite the inherent flexibility of peptoids, secondary structures of oligomeric and cyclic peptoids have been studied in some detail in solution by NMR spectroscopy[11−13] and in the solid state by X-ray crystallography, and some requirements for the formation of secondary peptoid structure have been identified.[13−16] For instance, the handedness of a helical conformation depends on the enantiomeric nature of α-chiral N-alkyl side chains, and the helix formation is favored by the presence of bulky and aromatic substituents.[11,16−18] Electronic n → π^* interactions[19] have also been proposed to take part in the stabilization of secondary structures of peptoids.[8,9] These interactions involve donation of a lone pair from a carbonyl oxygen atom into an empty π^* orbital of carbon atom of another carbonyl or an aromatic ring (Figure 1C)[20] and are optimal when mimicking the Bürgi−Dunitz trajectory for nucleophilic attack.[21] The β-peptides (Figure 1A), on the other hand, retain the capability to form intramolecular hydrogen-bond networks to stabilize secondary

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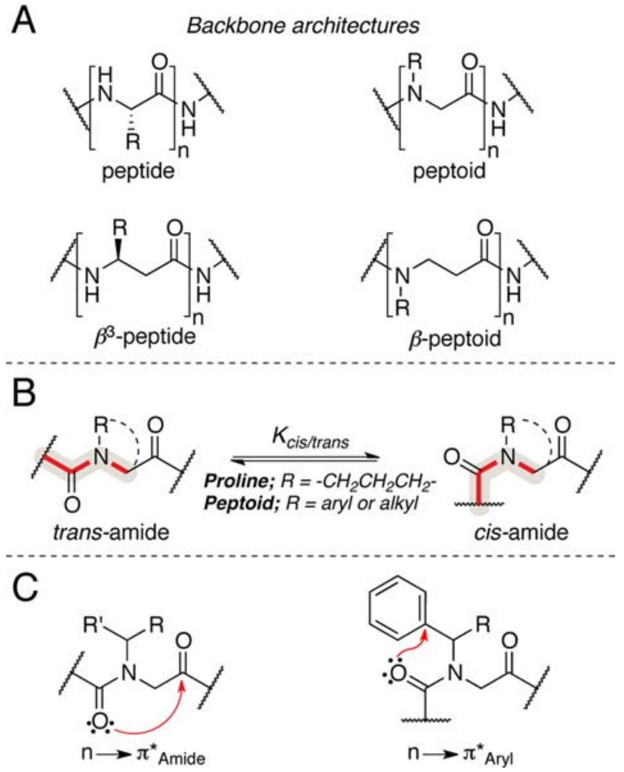


Figure 1. (A) Generic structures of the backbone architectures of peptides (i.e., α-peptides), peptoids (i.e., α-peptoids), β^3-peptides (β^2- and disubstituted β-peptides are not shown), and β-peptoids. (B) Depiction of the equilibrium of *trans*- and *cis*-amide conformations in proline and peptoid residues. (C) Examples of $n \rightarrow \pi^*$ interactions previously reported to exist in peptoids.[8]

structures, while the geometry of known helices is unlikely to be stabilized by $n \rightarrow \pi^*$ interactions.[2,3]

By combining the features of β-peptides and peptoids, the ensembles of available foldameric scaffolds may be expanded with β-peptoids, and several examples of biologically active compounds containing this motif have been reported.[22] The structural properties of compounds with a β-peptoid backbone architecture, however, have been studied to a far lesser extent than its parent compounds since the first examples were reported by Hamper et al. in 1998.[23] The first three-dimensional structure of a β-peptoid, which was achieved for a cyclic tetramer, was thus reported by Taillefumier and co-workers in 2008.[24] Computational studies of linear oligomeric β-peptoids have predicted several possible helical conformations,[25] containing both the *cis*- and *trans*-amides, but studies based on circular dichroism (CD) spectroscopy have been inconclusive.[26] To obtain experimental data regarding the folding propensity of these molecules, we decided to prepare a series of β-peptoid monomers and evaluate the structural influence on *cis*–*trans* amide bond isomerization by NMR spectroscopy under various conditions. Our collection of model compounds was designed to investigate how stereoelectronic effects and substituent bulk affect the conformational preferences of β-peptoid monomers.

■ RESULTS AND DISCUSSION

Design and Synthesis. All our model compounds were based on acylated β-peptoid monomers. This minimal design was chosen to mimic the local interactions of a residue within an oligomer structure. In this way the effect of side chains may be investigated with respect to steric and stereoelectronic interactions. Furthermore, it was the scope of this work to assess whether changes in the electronic properties of the backbone would alter the conformational preferences of the residues.

The first array of monomers was designed to include a structurally diverse set of N-alkyl side chains accommodating variations in steric bulk, α-branching, aromatic vs saturated substituents and finally including an N-aryl substituent (phenyl). The chosen set of eight different side chains (**a**–**h**) was installed in two different monomer series: (1) **3a**–**h** containing a C-terminal ester functionality and (2) **4a**–**h** containing a C-terminal tertiary amide functionality thought to better mimic the local environment of a single residue within an oligomeric structure (Scheme 1A). The *tert*-butylester series was prepared since these can be readily deprotected, which allows for further coupling reactions as well as installation of additional C-teminal functionalities. To probe the effects of the various side chains on the rotameric preference of the β-peptoid amide bond (the *cis*–*trans* equilibrium), acetyl groups were installed to give N-terminal tertiary amides, as also investigated in α-peptoid model systems.[8−10] Analogous to those studies, the *trans*–*cis* isomerism in our compounds could then be determined by integration of the ^{1}H NMR peaks assigned to each rotamer.

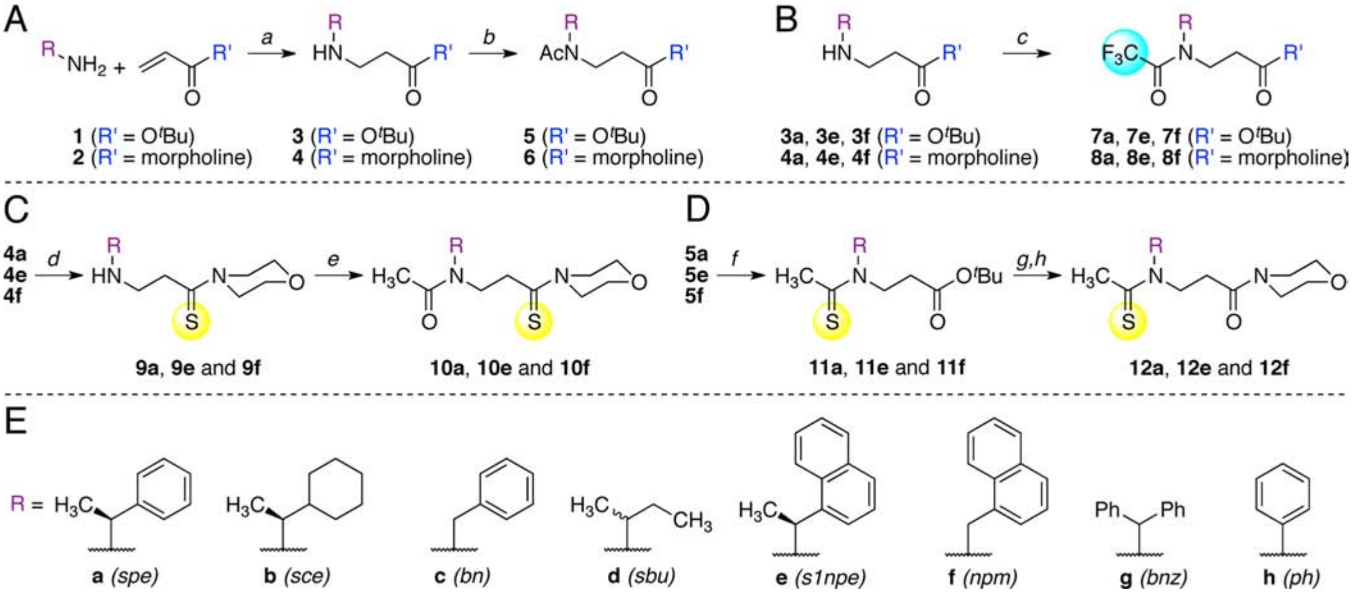
Syntheses of the β-peptoid monomers were achieved by aza Michael addition of a primary amine to acrylester (**1**) or acrylamide (**2**) in MeOH,[24] which has turned out to be an ideal solvent for this tranformation as opposed to the originally reported reactions in DMSO (Scheme 1A).[23] This was followed by acetylation to give the two series of monomer model compounds (**5a**–**h** and **6a**–**h**) for evaluation by NMR spectroscopy.

In addition to the various N-alkyl side chains and differences in C-terminal functionality, we were also interested in probing the possibility of local $n \rightarrow \pi^*$ interactions by altering the electronic properties of amide carbonyls. The N-terminal amides were therefore modified by introduction of trifluoroacetyl groups in place of the acetyl groups in selected compounds (Scheme 1B). These were readily prepared from **3a**,**e**,**f** and **4a**,**e**,**f** by treatment with trifluoroacetic anhydride (Scheme 1B).

Finally, we substituted carbonyl oxygen atoms with sulfur in a selection of compounds to achieve introduction of minimal peptide bond surrogates with altered electronic properties.[27] Both amides in compounds **6a**,**e**,**f** were individually mutated to thioamides to give **10a**,**e**,**f** and **12a**,**e**,**f**, respectively (Scheme 1C,D). For their preparation, we utilized Lawesson's reagent,[28] which selectively converts amides to thioamides in the presence of esters. Preparation of the C-terminal thioamides **10a**,**e**,**f**, were achieved by treating precursors **4a**,**e**,**f** with Lawesson's reagent to give **9a**,**e**,**f**, which were then acetylated to give the target compounds (Scheme 1C). The N-terminal thioamides **12a**,**e**, **f**, on the other hand, were synthesized by treating **5a**,**e**,**f** with Lawesson's reagent to give **11a**,**e**,**f**, followed by *tert*-butylester cleavage and coupling to morpholine to yield the target compounds (Scheme 1D). These changes were thus quite efficiently introduced from common precursors to alter the donor and acceptor capabilities of the two carbonyl groups.

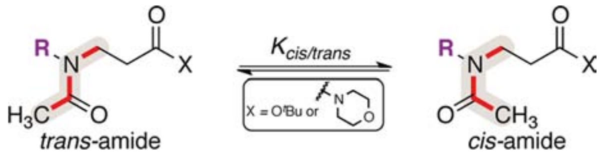
NMR Spectroscopy of Acetylated Monomers. In order to take possible solvent effects into consideration in our evaluation of the monomers, we recorded NMR spectra in six

Scheme 1. Synthesis of β-Peptoid Model Compounds[a]



[a](A) Acetylated monomers **5a–h** and **6a–h**. Reagents and conditions: (a) MeOH, 50 °C, 16 h; (b) (i) for esters Ac_2O (2 equiv), pyridine (2 equiv), DMF, 0 °C → rt, 4 h or (ii) for amides AcCl (2 equiv), pyridine (2 equiv), DMF, CH_2Cl_2, 0 °C, 1 h. (B) Synthesis of trifluoroacetylated monomers **7a,e,f** and **8a,e,f**. Reagents and conditions: (c) Trifluoroacetic anhydride (2 equiv), pyridine (2 equiv), CH_2Cl_2, 0 °C, 4 h. (C and D) Synthesis of thioamide-containing β-peptoid monomeric model compounds. Reagents and conditions: (d) Lawesson's reagent (1.5 equiv), toluene, 110 °C, 3 h; (e) AcCl (2 equiv), *i*-Pr_2NEt (2 equiv), CH_2Cl_2, 0 °C, 3 h; (f) Lawesson's reagent (0.6 equiv), toluene, 110 °C, 1 h; (g) 1 M $LiOH_{aq}$–DMF 1:1, rt, 16 h; (h) morpholine (2 equiv), HBTU (2 equiv), *i*-Pr_2NEt (2 equiv) CH_2Cl_2, rt, 16 h. (E) Abbreviations for N-alkyl side chains used are as follows: spe = (*S*)-1-phenylethyl, sce = (*S*)-1-cyclohexylethyl, bn = benzyl, sbu = *sec*-butyl, s1npe = (*S*)-1-(1-naphthyl)ethyl, npm = 1-naphthylmethyl, bnz = benzhydryl, ph = phenyl.

Table 1. Rotamer Equilibrium Constants ($K_{cis/trans}$) for Acetylated β-Peptoid Monomers in Various Solvents[a] and Their Corresponding Differences in Free Energy (ΔG values given in kJ × mol^{-1})[b]



		D_2O		DMSO-d_6		CD_3OD		CD_3CN		$CDCl_3$		C_6D_6	
compd	side chain	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG
						C-terminal esters							
5a	spe	0.8	0.5	0.8	0.5	0.7	0.9	0.8	0.5	0.9	0.3	0.8	0.5
5b	sce	0.5	1.7	0.6	1.2	0.4	2.2	0.5	1.7	0.4	2.2	0.5	1.7
5c	bn	1.0	0	0.9	0.3	0.9	0.3	0.9	0.3	0.7	0.9	0.7	0.9
5d	sbu	0.5	1.7	0.5	1.7	0.4	2.2	0.4	2.2	0.5	1.7	0.4	2.2
5e	s1npe	n.s.[c]	–	3.6	–3.1	5.6	–4.2	3.6	–3.1	5.3	–4.1	6.3	–4.5
5f	npm	n.s.[c]	–	0.7	0.9	0.9	0.3	0.7	0.9	0.9	0.3	0.9	0.3
5g	bnz	n.s.[c]	–	0.9	0.3	0.6	1.2	0.9	0.3	0.7	0.9	0.5	1.7
5h	ph	0.2	3.9	all *trans*	–	all *trans*	–	all *trans*	–	all *trans*	–	all *trans*	–
						C-terminal amides							
6a	spe	0.7	0.9	0.8	0.5	0.5	1.7	0.8	0.5	0.4	2.2	0.4	2.2
6b	sce	0.3	2.9	0.5	1.7	0.3	2.9	0.4	2.2	0.4	2.2	0.3	2.9
6c	bn	1.0	0	0.9	0.3	0.7	0.9	0.9	0.3	0.3	2.9	0.3	2.9
6d	sbu	0.2	3.9	0.5	1.7	0.2	3.9	0.4	2.2	0.1	5.6	0.1	5.6
6e	s1npe	2.9	–2.6	3.0	–2.7	3.0	–2.7	3.1	–2.8	2.9	–2.6	3.5	–3.1
6f	npm	0.9	0.3	0.6	1.2	0.9	0.3	0.8	0.5	0.4	2.2	0.5	1.7
6g	bnz	0.8	0.5	0.9	0.3	0.5	1.7	0.9	0.3	0.4	2.2	0.5	1.7
6h	ph	n.s.[c]	–	all *trans*	–	all *trans*	–	all *trans*	–	all *trans*	–	all *trans*	–

[a]Determined by integration of ^1H NMR spectra of 12 mM compound solutions at ambient temperature. [b]$\Delta G = -RT \times \ln(K_{cis/trans})$. [c]Not soluble.

different deuterated solvents of varying polarities (Table 1). First, we looked at compound **5a** containing the (*S*)-1-phenylethyl side chain, which is one of the most well-studied functionalities with respect to folding propensity of α-

Table 2. Rotamer Equilibrium Constants ($K_{cis/trans}$) for Trifluoroacetylated β-Peptoid Monomers in Various Solvents[a] and Their Corresponding Differences in Free Energy (ΔG values given in kJ × mol^{-1})[b]

		D_2O		DMSO-d_6		CD_3OD		CD_3CN		$CDCl_3$		C_6D_6	
compd	side chain	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG
C-terminal esters													
7a	spe	n.s.[c]	–	0.4	2.2	0.3	2.9	0.4	2.2	0.3	2.9	0.3	2.9
7e	s1npe	n.s.[c]	–	6.8	−4.7	6.3	−4.3	6.6	−4.6	6.3	−4.3	6.3	−4.3
7f	npm	n.s.[c]	–	0.8	0.5	0.8	0.5	0.8	0.5	0.9	0.3	1.0	0
C-terminal amides													
8a	spe	0.4	2.2	0.4	2.2	0.3	2.9	0.4	2.2	0.2	3.9	0.2	3.9
8e	s1npe	n.s.[c]	–	5.6	−4.2	5.0	−3.9	5.5	−4.1	4.8	−3.8	4.5	−3.7
8f	npm	n.s.[c]	–	0.8	0.5	1.0	0	0.8	0.5	0.7	0.9	0.6	1.2

[a] Determined by integration of ^1H NMR spectra of 12 mM compound solutions at ambient temperature. [b] $\Delta G = -RT \times \ln(K_{cis/trans})$. [c] Not soluble.

peptoids[17] and has been studied briefly by CD spectroscopy in β-peptoids.[26] This model β-peptoid exhibited a slight preference for the *trans*-amide configuration without any notable solvent effect, and expectedly, we further showed that the chiral identity had no influence on the conformational distribution, by synthesizing the corresponding racemate and the (*R*)-enantiomer (see Figure S1Supporting Information). As no significant effects of the concentration on the $K_{cis/trans}$ had previously been reported for peptoids,[9] we gratifyingly found that to be true for β-peptoid solutions in $CDCl_3$ between 6−200 mM as well (Figure S2). Finally, we performed rotating frame Overhauser effect spectroscopy (ROESY) experiments on selected compounds (e.g., see Figure S3) in order to show that the methyne group exhibiting the most downfield chemical shift arises from the *cis*-amide conformation, which is in agreement with previously published peptoid studies.[8−10]

Next, we compared the $K_{cis/trans}$ data for **5a** with those obtained for **5b**, which is a nonaromatic, fully saturated version of **5a**. The loss of aromaticity with only a slight increase in steric bulk gave rise to an increase in the preference for the *trans* conformation. This, in turn, indicates that the aromatic moiety may cause a slight shift toward the *cis*-amide, which is consistent with previous findings reported by Blackwell and coworkers for peptoids.[9] Likewise, this was also the case when we decreased the steric congestion of the side chain by introducing a benzyl group (**5c**), as the recorded $K_{cis/trans}$ values were comparable to those of **5a** in all the tested solvents. We then evaluated a combination of decrease in steric bulk further and removal of aromaticity using the *sec*-butyl side chain (**5d**). Similar equilibrium constants were observed for **5b** and **5d**, which was in accordance with α-peptoid findings,[9] as was the significant preference for *cis*-amide configuration induced by the (*S*)-1-(1-naphthyl)ethyl side chain (**5e**).[15]

Interestingly, introduction of a 1-naphtylmethyl side chain (**5f**) resulted in approximately a 1:1 mixture of rotamers as observed for **5a** and **5b**, showing that the naphthyl group itself is not sufficient to induce a predominant amount of the *cis*-amide. Thus, it would seem that the lack of α-branching enables the naphthyl group to avoid structure inducing steric interactions. Furthermore, we altered the bulk of the side chain by introducing the disubstituted benzhydryl group (**5g**), which, perhaps somewhat suprisingly, also gave rise to similar $K_{cis/trans}$ values as found for compound **5a**. This shows that α-branching only in combination with a very bulky group will promote induction of the *cis*-conformation, which apparently is uniquely well represented in the (*S*)-1-(1-naphthyl)-ethyl side chain. However, alternative constructs, taking advantage of a putative $n \rightarrow \pi^*_{aryl}$ interaction (Figure 1C) by introducing electron-deficient aromatic substituents instead of increasing the steric bulk, have been reported for peptoids as well.[9,29]

Finally, peptoid studies have also shown that direct attachment of a phenyl group to the nitrogen atom (i.e., prepared from aniline subunits) leads to a very strong preference for *trans*-amides.[13] A single β-peptoid model system of this type (**5h**) was evaluated and exhibited the expected selectivity, by virtually giving rise to single sets of signals in all tested solvents when analyzed by ^1H NMR. As mentioned, we also evaluated the entire series of side chains **a−h** in model systems having C-terminal amides (**6a−6h**) instead of esters, to mimic the environment of a β-peptoid residue within an oligomer more appropriately. The data are shown in the lower panel of Table 1, and inspection of the results reveals the same trends as discussed for the C-terminal *tert*-butylesters.

Taken together, our side chain investigations indicate that there may be a slight intrinsic preference for the *trans*-amide rotamer in β-peptoid model systems (**5d** and **6d**), which is in agreement with results from peptoids containing methyl or ethyl side chains.[9] The equilibrium then shifts toward the *cis*-amide rotamer to approximately 1:1 mixtures when adding aromatic functionalities as substituents in the α-position of the side chains. Based on the results of **5a** vs **5b** as well as **6a** vs **6b** (in the polar solvents), it seems plausible that an $n \rightarrow \pi^*_{aryl}$ interaction could play a role. Though, this interaction, in the case of a phenyl or naphthyl group, is too weak to induce the *cis*-amide as the preferred conformation. However, it is not possible to unambiguously attribute this effect of the aromaticity on $K_{cis/trans}$ to an $n \rightarrow \pi^*_{aryl}$ interaction based on our side chain experiments alone. Furthermore, the only examples of a strong preference for the *cis*-amide required quite specific steric properties of the side chain (**5e** and **6e**). In an attempt to gain further insight regarding possible stereoelectronic effects on $K_{cis/trans}$ in β-peptoids, we turned our attention to model systems containing carbonyls with altered electronic properties.

NMR Spectroscopy of Trifluoroacetylated Analogs. As we found that the β-peptoid model systems display the same behavior as peptoids upon side chain substitutions, we turned our attention to backbone modifications. Examples of such investigations have been reported for proline but have not, to the best of our knowledge, been utilized for interrogation of peptoid structure and conformational preference.

Noncovalent $n \rightarrow \pi^*_{amide}$ (Figure 1C) interactions contribute to stabilization of protein secondary structures[30] and have been studied extensively in relation to collagen polyproline type-II helical conformations.[31] The presence of this type of interaction has also been suggested in certain peptoid model systems.[8]

Table 3. Rotamer Equilibrium Constants ($K_{cis/trans}$) for Thioamide-Containing β-Peptoid Monomers in Various Solvents[a] and Their Corresponding Differences in Free Energy (ΔG values given in kJ × mol^{-1})[b]

		D_2O		DMSO-d_6		CD_3OD		CD_3CN		$CDCl_3$		C_6D_6	
compd	side chain	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG	$K_{cis/trans}$	ΔG
10a	spe	0.4	2.2	0.5	1.7	0.5	1.7	0.5	1.7	0.2	3.9	0.2	3.9
10e	s1npe	n.s.[c]	–	2.0	−1.7	3.4	−3.0	2.8	−2.5	2.2	−1.9	2.6	−2.3
10f	npm	n.s.[c]	–	0.9	0.3	0.8	0.5	0.9	0.3	0.3	2.9	0.3	2.9
12a	spe	0.7	0.9	0.9	0.3	0.4	2.2	0.7	0.9	0.5	1.7	0.5	1.7
12e	s1npe	n.s.[c]	–	5.4	−4.1	4.7	−3.8	4.5	−3.7	3.3	−2.9	5.2	−4.0
12f	npm	n.s.[c]	–	1.0	0	0.6	1.2	0.9	0.3	0.3	2.9	0.3	2.9

[a]Determined by integration of ^{1}H NMR spectra of 12 mM compound solutions at ambient temperature. [b]$\Delta G = -RT \times \ln(K_{cis/trans})$. [c]Not soluble.

Although such interactions would not be expected to have a stabilizing effect on β-peptoid secondary structure due to unfavorable geometry,[25] we were interested in testing whether the $K_{cis/trans}$ values in our model systems were sensitive to this type of interaction.

First we reasoned that substitution of the N-terminal acetyl group for a trifluoroacetyl group would significantly alter the electronic properties of the carbonyl through the strong inductive electron-withdrawing effect of fluorine. This should thus decrease the electronegativity of the N-terminal carbonyl, which would render this position weaker as donor of a lone pair from oxygen, whereas the carbonyl carbon atom would become a better acceptor. Since the alkyl side chains exhibited disfavoring of the *cis*-conformer, we chose to investigate trifluoroacetylated analogs containing aromatic side chains exclusively. We thus evaluated model compounds containing (*S*)-1-phenylethyl (**7a**), (*S*)-1-(1-napthyl)-ethyl (**7e**), and naphtylmethyl (**7f**) side chains (Table 2).

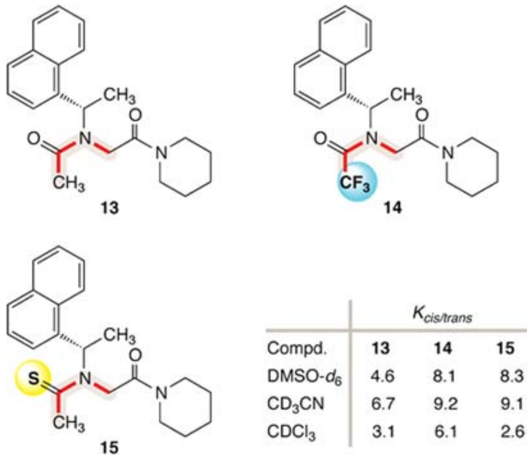
Surprisingly, at first glance, opposite effects were observed for **7a** vs **5a** and **7e** vs **5e** with a decrease and an increase in $K_{cis/trans}$, respectively, while compounds **7f** and **5f** behaved alike each other. Retrospectively, however, we hypothesize that a decrease of the *cis*-amide fraction in the (*S*)-1-phenylethyl system may in fact be explained by a weakened $n \rightarrow \pi^*_{aryl}$ interaction, whereas the opposite trend in the (*S*)-1-(1-napthyl)ethyl system is most likely of entirely steric nature. The trifluoromethyl group is more sterically demanding than the methyl, which may indeed be of particular significance in the already congested amide bond of **7e**. In support of this hypothesis, we recently became aware of a study by Raines and co-workers, in which it was shown that the rotamer equilibrium of a trifluoroacetylated proline derivative was governed by sterics, while the corresponding mono- and difluorinated analogs were affected by the electron-withdrawing inductive effect of fluorine.[29] It was also suggested by Raines and co-workers that fluorine may act as donor of an electron pair to an antibonding π^* orbital of the adjacent carbonyl, which would then results in the opposite of the anticipated inductive effect. Such interactions are indeed precedented in the literature, for example, by using molecular torsion balance double mutant systems.[32]

We also tested the morpholine analogs (**8a**,**e**,**f**), and again these exhibited trends that were similar to the *tert*-butylesters. Thus, it seems unlikely that the C-terminal carbonyl should be involved in the stabilization of monomer conformations. Although we are not able to propose unequivocal guidelines for the effects of introducing fluorine atoms in peptoid or peptide backbones, we believe that this could prove to be a useful addition to the arsenal of strategies for future design of peptide mimics.

NMR Spectroscopy of Thioamide Analogs. Inspired by another study of prolines by Raines and co-workers,[27] we next altered the carbonyl-donor capabilities by individually substituting the oxygen atoms with sulfur to increase the "nucleophilicity". If any carbonyl–carbonyl interactions (in the N → C or C → N directionality) were to be playing a significant role on the β-peptoid conforms, these sulfur substitutions should give rise to differences in the $K_{cis/trans}$ values as compared to the corresponding oxygen-containing compounds. Evaluating first the thioacetylated compounds (**12a**,**e**,**f**), we found that they behaved similar to the acetylated compounds. The only difference was observed in the (*S*)-1-(1-napthyl)ethyl system (**12e**), which showed increased fractions of the *cis*-amide. This would indicate that the sulfur is interacting with the aromatic ring rather than the C-terminal carbonyl. On the other hand, substitution of the C-terminal oxygen atom with sulfur (**10a**,**e**,**f**) resulted in $K_{cis/trans}$ values very similar to those recorded for their acetylated parent monomers (**6a**,**e**,**f**) in all cases (Table 3). This indicates that an $n \rightarrow \pi^*_{amide}$ interaction in the C → N directionality, which in theory should stabilize the *cis* configuration, is highly unlikely. These are the first examples of thioamides in peptoids, and our results show that this minimal amide bond surrogate may be valuable for interrogation of higher oligomers and possibly also in N-alkylglycine-based peptoids.

Peptoids. To address the effects of fluorination or thioamide introduction in peptoids as well, we finally prepared compounds **13**–**15** (Chart 1). These syntheses were achieved by applying published methods for solution-phase peptoid

Chart 1. Structures and $K_{cis/trans}$ Values for the Investigated N-Alkylglycine Peptoids



	$K_{cis/trans}$		
Compd.	13	14	15
DMSO-d_6	4.6	8.1	8.3
CD_3CN	6.7	9.2	9.1
$CDCl_3$	3.1	6.1	2.6

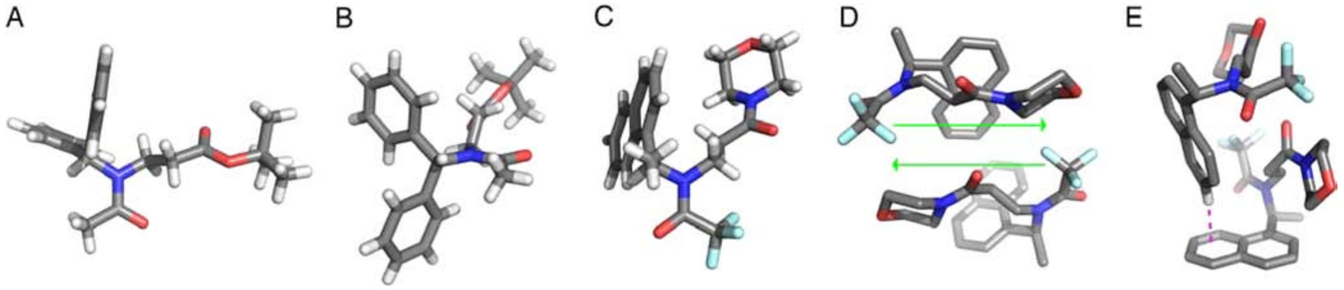


Figure 2. Solid-state structures of compound **5g** (A and B) and compound **8e** (C−E) determined by X-ray crystallography. Atom coloring: gray, carbon; white, hydrogen; red, oxygen; blue, nitrogen; and turquoise, fluorine. Green arrows indicate N → C directionality (D), and the dashed magenta colored line indicates an edge to face aromatic interaction (E). The hydrogen atoms have been removed for clarity in D and E.

synthesis[33] in combination with the protocols described for β-peptoid functionalization *vide supra* (Scheme S1).

Compound **13**, which has been investigated previously, exhibited the same $K_{\text{cis/trans}}$ values as reported in CD$_3$CN and CDCl$_3$ [9] and an intermediate value in DMSO-d_6, suggesting the presence of a solvent effect in this system. Comparing these values to the ones obtained for β-peptoid **8e** revealed a similarly lowered $K_{\text{cis/trans}}$ value in CD$_3$CN as compared to the other tested solvents (Table 1). For compound **14**, an even higher preference for the *cis*-amide conformation was observed, and this was affected to a much lesser extent by a change in the solvent polarity. In analogy to the arguments presented for the trifluoroacetylated β-peptoids, we hypothesize that this equilibrium is primarily dictated by sterics, but also note that the additional stabilization of the *cis*-amide conformation in the peptoid (e.g., $^{\text{DMSO}}K_{\text{cis/trans}}$ for **14** vs **8e** = 7.1 and 5.6, respectively) may involve the aforementioned possibility of an interaction between fluorine and the C-terminal carbonyl. However, compelling evidence for the latter point would require further experimentation.

Finally, the thioamide analog **15**, like β-peptoid **12e**, exhibited higher $K_{\text{cis/trans}}$ values than its oxoamide analog (**13**) in polar solvents, and a significant decrease in the *cis*-amide fraction in CDCl$_3$ (Chart 1). This again indicates that there is an interaction between the sulfur and the aromatic residue, which results in favoring of the *cis*-amide conformation.

X-ray Crystallography. We were able to obtain diffraction quality crystals for two β-peptoids, **5g** and **8e**, by slow evaporation of chloroform solutions as well as the peptoid **15** by slow evaporation from an AcOEt solution. Thus, the solid-state crystal structures of these model compounds were solved by X-ray structure determination. The structure of **5g** revealed an extended backbone conformation (Figure 2A) with *trans*-amide configuration, which is consistent with the obtained $K_{\text{cis/trans}} = 0.7$ in CDCl$_3$. Notably, the two phenyl groups adopt a periplanar relationship and are both pointed away from the acetyl CH$_3$ group (Figure 2B), which may also explain the relatively high *trans*-amide ratio observed in solution despite its significant steric bulk.

Compound **8e** adopted the *cis*-amide conformation in the solid state (Figure 2C), as would also be expected judging from its $K_{\text{cis/trans}}$ values. We had suspected that the attenuated electron lone-pair donor capabilities of the N-terminal carbonyl in the trifluoroacetylated compounds compared to acetylated analogs would result in a decreased $n \rightarrow \pi^*_{\text{aryl}}$ effect. The crystal structure of **8e** shows no such interaction, and there are no signs of fluorine−carbonyl interactions either. However, due to the very dense crystal packing with antiparallel β-peptoid backbones (Figure 2D) and edge to face aromatic π−π interactions (Figure 2E), the presence of $n \rightarrow \pi^*_{\text{aryl}}$ interactions in solution cannot be definitively excluded.

The X-ray crystal structure of the N-terminal thioamide peptoid analog **15** also revealed the presence of a *cis*-amide configuration, as would be expected from the NMR data (Figure 3). The distance between the C-terminal carbonyl and the carbon of the thioamide is consistent with the presence of an $n \rightarrow \pi^*_{\text{amide}}$ interaction (Figure 3A),[19,30] which may explain the higher $K_{\text{cis/trans}}$ values recorded for the glycine-based peptoids compared to the β-alanine-based peptoids. As was also the case for compound **8e**, the solid-state structure did not provide any evidence of an $n \rightarrow \pi^*_{\text{aryl}}$ interaction. Interestingly, however, the distance between one of the naphthyl hydrogen atoms and the sulfur shown in Figure 3B (2.9 Å) is consistent with an overlap of their orbitals to give rise to an aromatic C−H···S$_{\text{amide}}$ interaction. This could offer an alternative explanation of the stabilizing effect on the *cis*-amide conformation obtained by introduction of the N-terminal thioamide functionality. In order to shed more light on the identity of the putative noncovalent carbonyl−aryl interaction in this system, we performed density functional theory (DFT) calculations on selected compounds (*vide infra*). We also

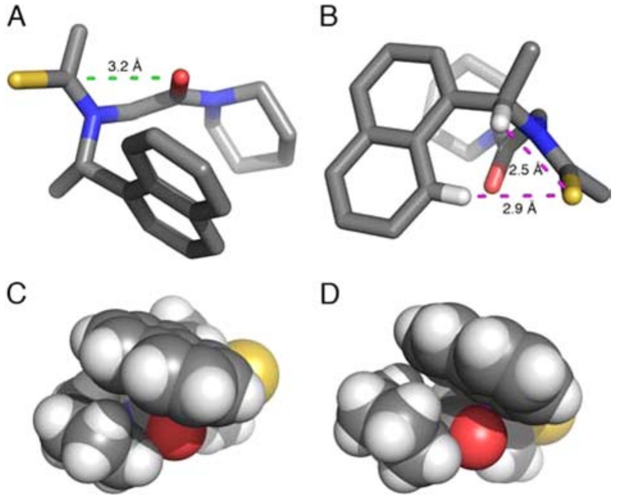


Figure 3. Solid-state structure of compound **15** determined by X-ray crystallography. Stick representations showing the C=O$_{i+1}$···C$_i$=S distance in green (A) and the distances between sulfur and its two closest hydrogen atoms in magenta (B). Space-filling representations showing hydrophobic packing of the naphthyl and the piperidine groups (C) and (D). The hydrogen atoms have been removed for clarity in A and B (except for the two hydrogens in close proximity to sulfur).

note that the proximity of the side chain methyne hydrogen, and the carbonyl in this crystal structure (2.5 Å) as well as in the structure of **8e** described above are consistent with the downfield shift observed in ^{1}H NMR for this proton in the *cis*-amide conformations.

Evidence for Aromatic C−H···S$_{amide}$ Interactions. To gain further insight into the molecular features responsible for a C−H···S$_{amide}$ interaction and its effect on the observed preference for the *cis*-amide configuration in the presence of the (*S*)-1-(1-napthyl)ethyl side chain, a computational study was carried out. Initially, the peptoids (**6e**, **8e**, **12e**, **13**−**15**) were built in either the *cis* or the *trans* configuration and subjected to a conformational search running 1000 steps using the OPLS-2005 force-field[34] and a GB/SA solvation model[35] for water as incorporated in Macromodel version 9.6.[36] The *cis*- or *trans*-amide conformations were retained by applying a constraint of 100 kJ × mol^{-1} × radian^{-2} to those particular dihedral angles. Furthermore, to prevent irrelevant rotamers of the morpholine headgroup to appear in the conformational search, additional dihedral constraints were applied to the N-terminal part of the molecules. The conformational search was carried out using a combination of Monte Carlo multiple minimum (MCMM) algorithm[37,38] and the "Low-Mode" search algorithm,[39] with an energy window of 21 kJ × mol^{-1}. After this initial conformational search all of the generated conformations were submitted to a further optimization with DFT using the B3LYP functional.[40] We used the 6-31G* basis set[41] along with the polarized continuum solvent model (PCM-SCRF)[42] with parameters suitable for water.

The lowest energy conformations of both **6e** and **12e** contained the *cis*-amide configuration in agreement with our $K_{cis/trans}$ data from NMR as well as the X-ray diffraction data (Figure 4). Notably, when visualizing the ensemble of conformations with energies within 21 kJ × mol^{-1} (Figure 4B,D), the more homogeneous positioning of the N-alkyl side chain in the thioamide analog indicates that there may be a stabilizing interaction between the sulfur and the naphthyl group. This is again consistent with the trends of $K_{cis/trans}$ observed by NMR, and the preferred geometry is the same as we found in the solid-state for compound **15** revealing close proximity of the proton in position eight of the naphthyl functionality with the carbonyl (Figure 4A,C).

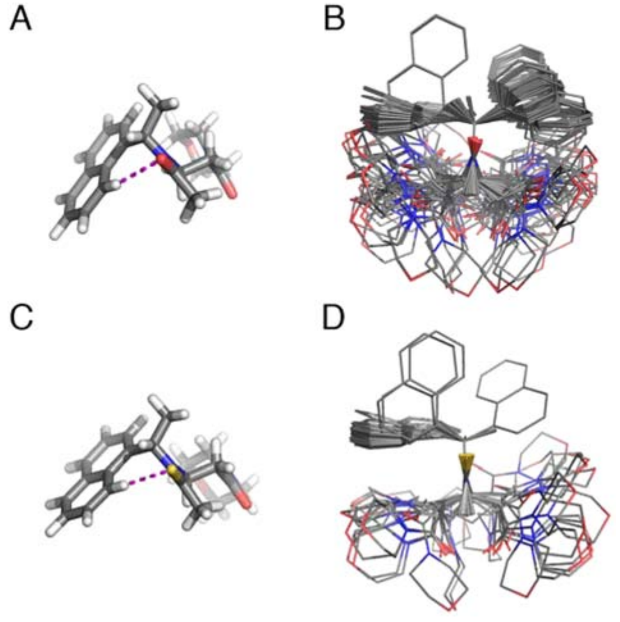


Figure 4. Calculated structures of compounds **6e** (A and B) and **12e** (C and D). All structures within 21 kJ × mol^{-1} of the global minimum were superimposed.

To further investigate the electronic properties responsible for the observed *cis*-amide preference in the thioamide series, we carried out natural bond order (NBO) analyses.[43] By inclusion of the trifluoroacetylated compounds **8e** and **14** we would be able to pinpoint the effect of this substitution in both peptoid and *β*-peptoid backbones. For this purpose, superimposable, low-energy conformations of both *cis*- and *trans*-isomers of **6e**, **8e**, **12e**, **13**−**15** were selected. When comparing the two *cis*-conformations of **6e** and **12e**, it is notable that while the longer C=S compared to C=O (1.7 vs 1.2 Å) caused the distance to the hydrogen of the naphtyl group to increase from 2.9 to 3.2 Å, the NBO analysis clearly showed that the interaction is stronger in the thioamide case.

First of all, the natural charge on the aromatic hydrogen in the thioamide (**12e**) is lower than in the amide compound (0.2436 au for **12e** vs 0.2455 au for **6e**), although both hydrogens are more electron deficient than their neighboring hydrogen, which does not have such intramolecular interactions (0.2503 au for **12e** and 0.2487 au for **6e**). In addition, second-order perturbation analyses of **12e** and **6e** revealed calculated stabilizing energies of this interaction to be 0.86 kcal × mol^{-1} and below the 0.5 kcal × mol^{-1} threshold, respectively.

In the trifluoroacetylated compound **8e**, the amide oxygen is less negatively charged as expected (−0.657 au in **8e** vs −0.716 au in **6e**). As a consequence, the electrostatic interaction with the naphthyl hydrogen is expected to be even smaller than for **6e**, however, in this case it is also below the threshold of 0.5 kcal × mol^{-1}. This suggests that the increased *cis*−*trans* ratio upon change of methyl to trifluoromethyl likely is caused by the increased steric congestion of the larger fluorine atoms rather than arising from an increased electrophilicity of the amide carbonyl carbon. Finally, the three *trans* configured structures featured a fully extended backbone with neither $n \rightarrow \pi^*_{amide}$ nor electrostatic C−H···S$_{amide}$ interactions.

Next, we turned our attention to the peptoid series (**13**−**15**) where the closer proximity of the other carbonyl group may allow for the possibility of $n \rightarrow \pi^*_{amide}$ interactions in addition to the electrostatic C−H···S$_{amide}$ interaction. For all of these compounds, the C−H···S$_{amide}$ interaction shows up in the second-order perturbation analysis part of the NBO analysis, and it is only slightly stronger for the thioamide **15** (0.63 kcal × mol^{-1}) compared to the amide **13** (0.58 kcal × mol^{-1}). For the trifluoroacetylated peptoid **14**, the value is even higher at 0.65 kcal × mol^{-1}, but the small energies considered, these differences may well be within the inaccuracy of the method. These effects on peptoid structure are currently under further investigation in our laboratories.

A comparison of the chemical shifts assigned to the naphthyl H-8 hydrogen in the *cis*-amide conformations of compounds with altered electronic properties of the carbonyl support the presence of the proposed interaction in solution as well (Figure 5). Thus, attenuation of the electron density of the oxygen by introduction of fluorine atoms should render the hydrogen less shielded and cause an upfield shift of the signal, which was indeed what the spectra showed (**6e** vs **8e**). Substitution of oxygen with sulfur (**6e** vs **12e**) should in principle affect this putative interaction in the same manner. However, the opposite

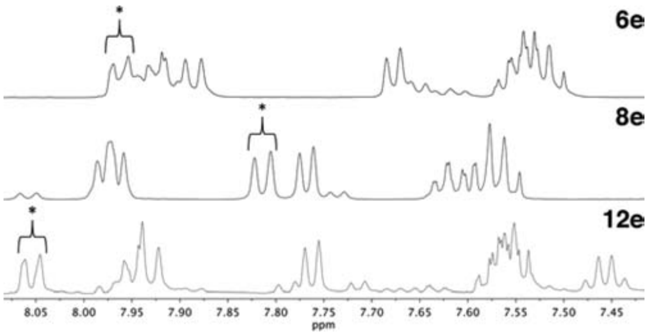


Figure 5. Aromatic region of the ^{1}H NMR spectra of **6e**, **8e**, and **12e** recorded in CD_3CN. The asterisk denotes the signal of the position eight proton of the naphthyl group (see, Figure S5 for signal assignments by COSY and HMBC NMR). The same trend was observed when comparing compounds **13**−**15** (data not shown).

effect was observed with a downfield shift of the signal (Figure 5), which gratifyingly is consistent with the calculated ensembles and the $K_{cis/trans}$ values that indicate a stronger interaction for sulfur. Although hydrogen bonds to oxoamides should be stronger than thioamides, we speculate that the geometric restraint required for formation of the eight-membered ring in our system does not allow for an optimal hydrogen-bond distance, and therefore the larger radius of the sulfur enables a higher degree of orbital overlap than the oxygen. This is supported by the NBO analysis on the β-peptoids **6e** and **12e** (see above). Additionally, the difference in polarizability of thioamides as compared to oxoamides may play a role[44] and could also provide arguments to help explain the solvent effects observed on $K_{cis/trans}$ for some thioamide compounds (*vide supra*).[44a]

CONCLUSIONS

To get a better understanding of the amide bond isomerization in peptoids, we have synthesized and evaluated several series of monomer β-peptoid model systems with varying electronic and steric properties as well as two novel N-alkylglycine (peptoid) model compounds containing a trifluoroacetyl group or an N-terminal thioamide, respectively. Our studies show that some of the trends found in peptoids are directly applicable to β-peptoids. As such, the (*S*)-1-(1-napthyl)ethyl side chain strongly induces the *cis*-amide conformation, while N-aryl gives rise to *trans*. We thus found that a bulky substituent like naphthyl in combination with α-branching is required for a *cis*-amide preference, as a diphenyl-substituted benzhydryl side chain was not sufficiently sterically demanding. In addition to the investigation of various side chain effects, we prepared model systems containing trifluoroacetyl groups as well as thioamides to probe the electronic effects of the carbonyl donor−acceptor capabilities. The NMR-based studies of these compounds provided evidence for an interaction of the N-terminal carbonyl/thiocarbonyl lone pair with the aromatic side chain, but we saw no evidence for conformational stabilization through noncovalent carbonyl−carbonyl interactions. The X-ray crystal structures of two β-peptoid model compounds were solved, which revealed one *trans*- and one *cis*-amide, respectively. Those rotamer conformations were both in agreement with the NMR experiments.

Furthermore, the X-ray crystal structure of a thioamide-containing peptoid model compound was solved, and supported by DFT calculations and NMR chemical shift analysis, this structure indicated the presence of a stabilizing effect through thioamide−aromatic interactions by C_{sp^2}−H···S_{amide} "hydrogen bonds". Whereas aromatic−sulfur interactions have been described for proteins as well as in other systems,[45] the present work, to the best of our knowledge, provides evidence for the first examples of intramolecular conformation-stabilizing effects by introduction of thioamides, which is in contrast to the destabilizing effect of thioamide introduction in α-helical peptides.[46]

Importantly, this work shows that minimal peptide bond surrogates like thioamides as well as fluorinated backbone analogs are useful for investigation of peptoid and β-peptoid structure. These modifications should therefore be considered valuable for other types of peptidomimetics as well. Thioamides, in particular, have recently found use in peptide ligands and have been site-specifically introduced into proteins to probe folding.[47] We envision that the straight forward methodology presented herein may encourage further studies of thioamide-containing peptoid and β-peptoid oligomeric systems.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary figures , experimental methods, charaterization data, ^{1}H NMR and ^{13}C NMR spectra for all synthesized compounds, selected 2D NMR spectra, and crystallographic data (CIF). Coordinates (*X*, *Y*, *Z*) and solution phase SCF energies for global minimum found in each conformational search along with most favorable structure calculated using DFT/B3LYP, and tables of data from the NBO analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

cao@kemi.dtu.dk

Notes

The authors declare no competing financial interest.

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