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α-Aminoxy Peptoids: A Unique Peptoid Backbone with a Preference for *Cis*-Amide Bonds

Viktoria Krieger,[a] Emanuele Ciglia,[a] Roland Thoma,[b] Vera Vasylyeva,[b] Benedikt Frieg,[a] Nader de Sousa Amadeu,[b] Thomas Kurz,[a] Christoph Janiak,[b] Holger Gohlke,[a] and Finn K. Hansen*[a,c]

Abstract: α-Peptoids, or *N*-substituted glycine oligomers, are an important class of peptidomimetic foldamers with proteolytic stability. Nevertheless, the presence of *cis*-/*trans*-amide bond conformers, which contribute to the high flexibility of α-peptoids, is considered as a major drawback. A modified peptoid backbone with an improved control of the amide bond geometry could therefore help to overcome this limitation. Here we have performed the first thorough analysis of the folding propensities of α-aminoxy peptoids (or *N*-substituted 2-aminoxyacetic acid oligomers). To this end, the amide bond geometry and conformational properties of a series of model α-aminoxy peptoids were investigated using 1D and 2D NMR experiments, X-ray crystallography, NBO analysis, CD spectroscopy, and MD simulations revealing a unique preference for *cis*-amide bonds even in the absence of *cis*-directing side chains. The conformational analysis based on the MD simulations revealed that α-aminoxy peptoids can adopt helical conformations that can mimic the spatial arrangement of peptide side chains in a canonical α-helix. Given their ease of synthesis and conformational properties, α-aminoxy peptoids represent a new member of the peptoid family capable of controlling the amide isomerism while maintaining the potential for side-chain diversity.

Introduction

α-Peptoids, or oligomers of *N*-substituted glycine, have several advantages over peptides as potential bioactive compounds including proteolytic stability and increased cell permeability.[1,2] Peptoid libraries have been utilized as protein binding agents and as inhibitors of protein-protein interactions,[3-6] although primary screening hits identified from peptoid libraries have usually not displayed high activity or potency.[7] The major limitation of peptoids is the lack of conformational constraints due to the absence of internal hydrogen bonding, which may reduce their binding affinity to proteins. In contrast to peptides, a high degree of *cis*-amide bonds is observed in α-peptoids.[8] The *cis*- and *trans*-amide conformations are almost isoenergetic for *N*-alkyl α-peptoid monomers, and studies have shown that the nature of the side chain can significantly modulate the ratio of *cis*-/*trans*-amide bond conformers.[9] Nevertheless, α-peptoids can form stable, helical secondary structures when the *cis*-/*trans*-amide isomerism is optimally controlled.

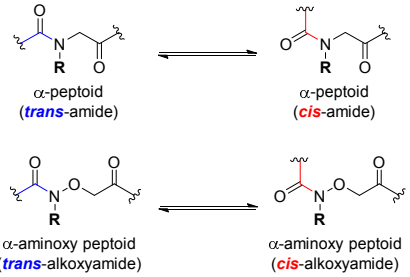

Figure 1. Selected peptoid backbones.

Similarly, it has been hypothesized that β-peptoids (*N*-alkyl β-alanines) can exhibit foldamer properties.[10-14] A recent study by Olsen and co-workers provided a high-resolution X-ray crystal structure of a β-peptoid hexamer containing strongly *cis*-inducing *N*-(*S*)-1-(1-naphthyl)ethyl side chains. The crystal structure disclosed all-*cis* amide bond geometry and a right-handed helical conformation with exactly three residues per turn and a helical pitch of 9.6-9.8 Å between turns.[15] However, it is important to note that the amide bond geometry in β-peptoids is also highly dependent on the nature of the side chain.[16]

Thus far, the control of the folding properties of α- and β-peptoids is mainly achieved by incorporation of specific *cis*- or *trans*-directing side chains. This focus on a small number of side chains comes at the expense of diversity.[7,17] Accordingly, it is worthwhile to address this limitation and to aim at the design of peptoid backbones with an improved control of *cis*-/*trans*-amide isomerism. In 2002, Shin and Park reported the preparation of α-aminoxy peptoid pentamers, but the folding properties were not investigated.[18] The backbone-controlled secondary structures of α-aminoxy peptides[19,20] prompted us to analyze the conformational properties of α-aminoxy peptoids, or oxa-analogues of β-peptoids, in detail. In this work, we report the results of our study revealing a unique *cis*-amide preference of α-aminoxy peptoids in comparison to other peptoid backbones.

Results and Discussion

We first designed and synthesized a series of model α-aminoxy peptoids utilizing an N-terminal acetyl and a C-terminal

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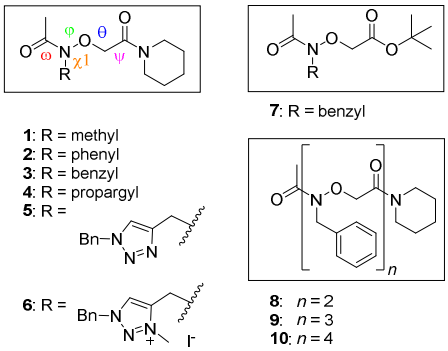

Figure 2. α-Aminoxy peptoids investigated. The relevant backbone dihedral angles are defined: ω [Cα (*i* – 1); C (*i* – 1); N; O], φ [C (*i* – 1); N; O, Cα], θ [N; O; Cα, C], and ψ [O, Cα; C, N (*i* + 1)], χ_1 [NCβ; NCα; N; O].

piperidinyl cap group to investigate the influence of several side chains on the amide conformation (see Supporting Information for synthetic details). To be consistent with peptoid nomenclature, we use the terms "*cis*" and "*trans*" to describe the amide dihedral angle omega referring to the relative position of the backbone atoms (see Figure 1). As a consequence, the *cis*-conformer features an *E*-configured hydroxamate moiety, while the *trans*-conformer corresponds to a *Z*-configured hydroxamate. The definition of all other relevant dihedral angles is outlined in Figure 2. α-Aminoxy peptoids **1**,**3**-**5** were designed to investigate the *cis*-/*trans*-isomerism in the absence of strongly *cis*- or *trans*-directing side chains. Compound **2** was included due to its strongly *trans*-amide inducing phenyl side chain.[21] α-Aminoxy peptoid **6** served as a *cis*-promoting model compound due to the *cis*-directing triazolium side chain.[22] Model peptoid **7** containing a *tert*-butyl ester was incorporated to analyze an alternative C-terminal cap group. α-Aminoxy peptoids **8**-**10** were synthesized as model compounds for oligomeric α-aminoxy peptoids to study conformational preference in oligomers (see Supporting Information for synthetic details).

We first studied the conformational homogeneity of our model systems using standard 1D NMR techniques at room temperature. ^{1}H NMR spectra of peptoid monomers **1-6** were recorded in $CDCl_3$ at room temperature and revealed only a single set of NMR signals. However, some signals appeared broad. We therefore collected ^{1}H spectra of monomers **1-6** in acetonitrile-d_3, methanol-d_4, D_2O and DMSO-d_6 observing again some relatively broad signals in the case of peptoids **1-5** (see Supporting Information). These results prompted us to study the ^{1}H NMR spectra in more detail. We performed a variable temperature (VT)-NMR on α-aminoxy peptoid **3** in $CDCl_3$. Cooling caused the signals of **3** to split into two sets of sharp signals at -20°C and -30°C in ratios of 87:13 and 86:14, respectively (Figure S1). In order to exclude that the second species arises from the piperidinyl group, we repeated the VT-NMR experiment with model α-aminoxy peptoid **7** containing a *tert*-butyl ester as the C-terminal cap. Compound **7** followed the same trend as observed for α-aminoxy peptoid **3** (Figure S2). To study whether the additional NMR signals arise from aggregation, we recorded ^{1}H NMR spectra of **3** at -30°C at varying concentrations (0.15-150 mM, Figure S3) which showed no concentration dependency of the peak ratio. Interestingly, a VT-NMR experiment with the deacetylated analog of α-aminoxy peptoid **3** (2-((benzylamino)oxy)-1-(piperidin-1-yl)ethan-1-one) did not show any additional conformational peaks (Figure S4). Thus, we reasoned the presence of two sets of NMR signals might indicate the coexistence of two well-defined species that equilibrate slowly on the NMR time scale, e.g. *cis*-/*trans*-amide bond rotamers. This hypothesis was supported by detection of EXSY signals in 2D NOESY experiment on **3** in $CDCl_3$ at –30°C (Figure S5).[23] This behavior is typical for protons under significant chemical exchange on the saturation time scale and thus implies the existence of rotamers.[24] Subsequent ^{1}H NMR experiments with compounds **1**,**2**,**4**-**6** at -30°C provided further evidence for *cis*-/*trans*-amide bond conformers. In the case of compounds **1**,**4**, and **5** we detected a similar behavior as observed for **3** with ratios[25] of 84:16 (**1**), 84:16 (**4**), and 82:18 (**5**). Notably, the ^{1}H NMR of peptoid **2** (containing a *trans*-directing side chain) revealed an increased amount of the minor conformer at -30°C in $CDCl_3$ (ratio 65:35), whereas α-aminoxy peptoid **6** (containing a *cis*-directing side chain) showed well-defined peaks with no evidence for a significant population of another conformer. To study the conformational stability of longer peptoid sequences, α-aminoxy peptoid oligomers **8**-**10** were synthesized (see Supporting Information). ^{1}H NMR spectra conducted in $CDCl_3$, acetonitrile-d_3 and methanol-d_4 showed well-defined signals (see Supporting Information for details). ^{1}H NMR spectra measured at -30°C in $CDCl_3$ revealed one major conformer in all cases. However, in α-aminoxy dipeptoid **8** a second conformational isomer was observed (ratio 86:14). In the case of α-aminoxy tripeptoid **9** and α-aminoxy tetrapeptoid **10** only traces of by-peaks were observed.

Since several model monomers and oligomers revealed the presence of two conformers, we performed comprehensive NMR experiments on α-aminoxy peptoid **3** in $CDCl_3$ to determine the Arrhenius activation energy for the rotational barrier. For this purpose, the interconversion rate constants at the respective coalescence temperatures were measured by VT-NMR experiments using different NMR instruments. A plot of the temperature dependence of the interconversion rate constants afforded the Arrhenius activation energy (E_a = 42±2 kJ mol^{-1}, see Supporting Information for details).

In order to investigate whether α-aminoxy peptoid monomers prefer *cis*- or *trans*-configured amide bonds, we decided to study the amide bond geometry of our model compounds in detail. Based on our observed side chain dependency we assumed a *cis*-preference in the case of our model α-aminoxy peptoids. To confirm a preferred *cis*-amide bond configuration in solution, the ω torsion was analyzed by 2D NMR spectroscopy. Compound **6** was chosen for a 2D NOESY NMR spectrum due to the absence of any additional conformational peaks. We observed a strong cross peak between the N-terminal acetyl group and the backbone methylene protons as well as a weak NOE between the acetyl and the side chain methylene protons (Figure S6). We reasoned that a *cis*-configured amide (ω ≈ 0°) would fulfill these distance requirements, whereas a *trans*-configured amide

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(ω ≈ 180°) should show a stronger NOE between the acetyl and the side chain methylene protons. A 2D NOESY spectrum of α-aminoxy peptoid **3** at -30°C revealed for the major conformer a strong cross peak between the N-terminal acetyl group and the backbone methylene protons which indicates *cis*-amide geometry (Figure S7). Notably, our results are in good agreement with data reported from Grel and co-workers who showed that simple alkoxyamides prefer *cis*-amide bonds in the absence of hydrogen bonds.[26] The above mentioned data suggest that the α-aminoxy peptoid monomers **1,3**-**6** possess a strong preference for *cis*-configured amide bonds. In particular, it is worth noting that the model compounds **1,3**-**5** are showing a significant preference for *cis*-amide bonds (> 80%) even in the absence of any *cis*-directing side chains. In contrast, α-peptoids bearing identical cap groups and side chains revealed inhomogeneous mixtures of *cis*/*trans* rotamers or even a preference for *trans*-amide bonds (Figure S8).

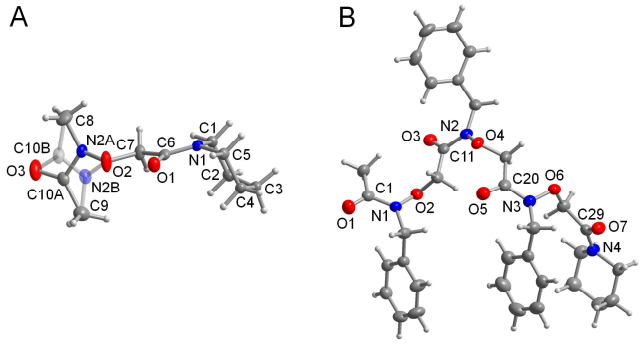

Figure 3. Thermal ellipsoid plots of (A) α-aminoxy peptoid **1** and (B) α-aminoxy tripeptoid **9** (50% probability for **1**, 70% for **9**, H atoms with arbitrary radii). The N and C(=O) atom of the *N*-methylacetamide group in **1** is disordered in a C_2-type (180°) rotation around the O3-O2 connector with equal occupancy (see Supporting Information for further details); one of the disordered positions is shown semi-transparent.

Fortunately, we were able to obtain X-ray quality crystals of α-aminoxy peptoid monomer **1** and tripeptoid **9** by slow evaporation from $CDCl_3$. The X-ray structure analysis of α-aminoxy peptoid **1** proved a *cis*-amide configuration in the solid state. Although the *N*-methylacetamide group in α-aminoxy peptoid **1** was disordered in the crystal structure, it is important to note that the amide is *cis*-configured in both the A and B component (Figure 3). The X-ray structure of α-aminoxy tripeptoid **9** revealed an all *cis*-amide geometry (Figure 3). The observed torsion angles are summarized in Table S1. Interestingly, the N-terminal (*i*) and internal (*i*+1) monomer disclosed almost identical backbone torsion angles, whereas the C-terminal monomer (*i*+2) showed different torsion angles. This phenomenon can be explained by packing effects in the solid state (see Supporting Information for a detailed discussion). The X-ray structure shows the trimer adopting a zig-zag conformation in which the *i* and *i*+2 side chains are oriented on the same side of the backbone, whereas the *i*+1 benzyl group points in the opposite direction.

On the first view the *cis*-preference of α-aminoxy peptoids is somewhat surprising keeping in mind that α-aminoxy peptides preferentially adopt *trans*-amide bonds.[27] However, we, and others, showed that the secondary structure in α-aminoxy peptides is primarily stabilized by eight-membered ring hydrogen bonds between $C{=}O_i$ and $N\text{-}H_{i+2}$ which are not possible in the case of α-aminoxy peptoids.[19,20,27] Thus, in order to further investigate the *cis*-amide preference in α-aminoxy peptoids, we performed a natural bond orbital (NBO) analysis on all-methyl model peptoids in *cis*-amide and *trans*-amide configurations (Figure 4 A+B) as well as the *cis*-amide α-aminoxy tripeptoid **9** (Figure 4 C).

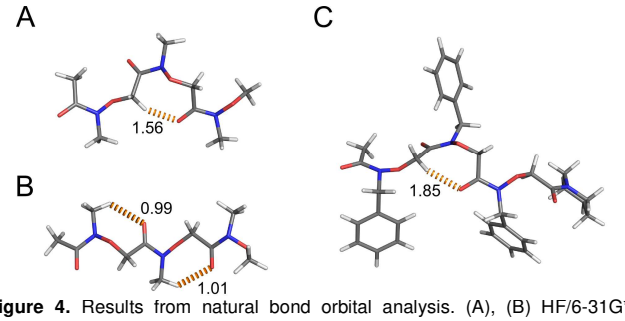

Figure 4. Results from natural bond orbital analysis. (A), (B) HF/6-31G*-optimized structures from an all-methyl model compound in *cis* (A) and *trans* (B) configuration. (C) HF/6-31G*-optimized structure of α-aminoxy tripeptoid **9** in *cis* configuration. In (A – C) the orange dotted lines depict donor-acceptor interactions. The labels depict the donor-acceptor stabilization energies (in kcal mol^{-1}) averaged over the two respective oxygen lone pairs, determined by the second-order perturbation theory analysis.

For the model peptoid in *cis*-amide configuration, the NBO analysis revealed $C_{\alpha,i}\text{–}H\cdots O{=}C_{i+1}$ donor-acceptor interactions, which results in an eight-membered δ turn according to the classification by Toniolo and Benedetti[28] (Figure 4 A); second order perturbation theory analysis in the NBO basis revealed a mean donor-acceptor stabilization energy of 1.56 kcal mol^{-1} (Figure 4 A, Figure S9 A). The C_α hydrogen atom involved in the $C_\alpha\text{–}H\cdots O{=}C$ interaction is more electron deficient than the geminal, non-involved hydrogen (0.2458 au in C_α–**H**···O=C vs. 0.2281 au in C_α–**H**; Figure S9 A), indicating that electrons are delocalized via the $C_\alpha\text{–}H\cdots O{=}C$ interaction. The stabilization energy computed here is close to the absolute value of the lower limit of the electronic association energy D_e of 2.1 kcal mol^{-1} computed for *N*,*N*-dimethylformamide dimers associated via C–H···O=C hydrogen bonds.[29] The C_α ··· O distance of 3.1 Å coincides with expectations of distances of weak hydrogen bonds.[30] As to the *trans* configuration, no $C_\alpha\text{–}H\cdots O{=}C$ interaction was revealed by the NBO analysis but rather a $C\omega_{,i}\text{–}H\cdots O{=}C_i$ interaction within the same residue (Figure 4 B); the latter interaction (stabilization energy: ~1.00 kcal mol^{-1}) is weaker than the $C_{\alpha,i}\text{–}H\cdots O{=}C_{i+1}$ one (Figure 4 A+B; Figure S9 A+B).

In agreement with results from the all-methyl model peptoid in *cis* configuration, NBO analysis also revealed the eight-membered δ turn in the *cis*-amide α-aminoxy tripeptoid **9** (Figure 4 C). Here, the donor-acceptor stabilization energy is stronger than in the model peptoid (1.85 kcal mol^{-1} in **9** vs. 1.56 kcal mol^{-1} in the model peptoid; Figure 4 A+C; Figure S9 A+C). This might be explained by the electron-pushing effect of the benzyl side chains, leading to the amide oxygen being more negatively charged (-0.7269 au for **9** vs. -0.6980 au for the model peptoid; Figure S9 A+C).

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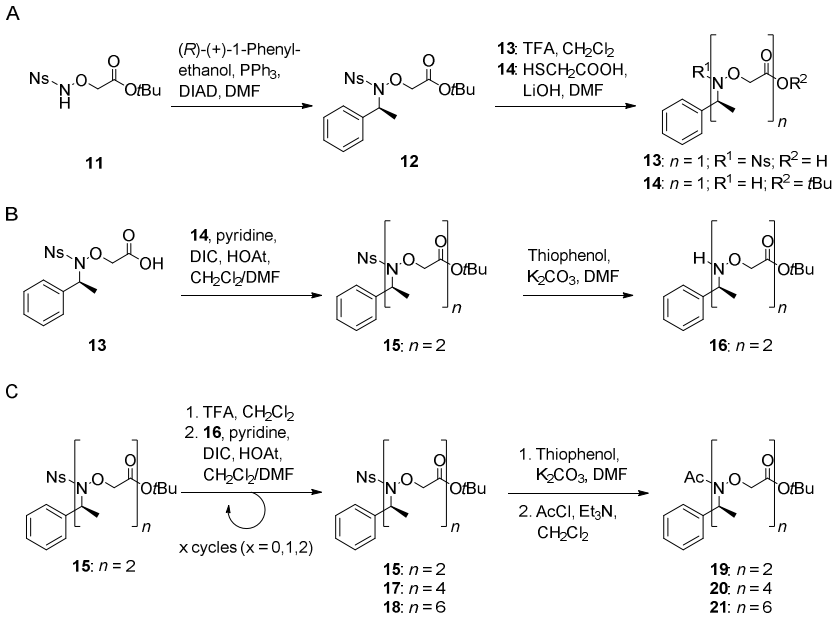

Scheme 1. Synthesis of α-aminoxy peptoids **19**-**21** containing α-chiral aromatic side chains (Ns = 2-nitrobenzenesulfonyl).

Taken together, by means of NBO calculations, we found stabilizing donor-acceptor interactions for both *cis* and *trans* configurations. The formation of an eight-membered δ turn between residues *i*→*i*+1 in the *cis* configuration is accompanied by a favorable stabilization energy for $C_{\alpha,i}$–H···O=C_{i+1} interactions, which could explain the *cis*-amide preference for α-aminoxy peptoids. Note that, to the best of our knowledge, no unambiguous report has become available so far for the occurrence of a δ turn in a linear peptide.[31]

To gain further insight into potential folding propensities of oligomers we decided to synthesize oligomers featuring α-chiral side chains (Scheme 1). Circular dichroism (CD) spectroscopy has been extensively used to study the secondary structure of α-peptoids. Figure 5 shows the CD spectra of the α-aminoxy peptoids **19**-**21** in acetonitrile.

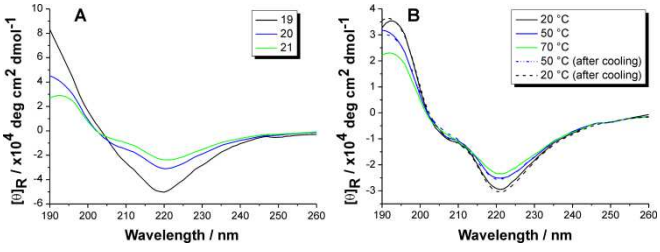

Figure 5. A) CD spectra of compounds **19**-**21** in acetonitrile (50 µM) at 25°C; B) CD spectra of compound **21** at varying temperatures in acetonitrile (50 µM) (Data are expressed in terms of per-residue molar ellipticity (deg cm^2/dmol).

The CD spectra of **19**-**21** reveal a characteristic minimum around 220 nm. Somewhat surprisingly, the intensity of the minimum near 220 nm decreased with increasing chain length of the α-aminoxy peptoids. This phenomenon might indicate that this signal is not caused by secondary structure formation but is related to the *N*-(*S*)-1-phenylethyl-containing amide motif itself.[11,15] To verify this hypothesis we synthesized an acetylated monomer containing the *N*-(*S*)-1-phenylethyl group (see Supporting Information). The CD spectrum of this α-chiral monomer disclosed a strong minimum at around 220 nm (Figure S10). Based on this result it appears unlikely that the minimum at 220 nm is related to secondary structure formation as simple monomers would not be expected to adopt an ordered conformation. On the other hand, starting at the length of a tetramer a shoulder appeared at 209 nm. It could be speculated that this minimum at 209 nm might be indicative of length-dependent secondary-structure formation. Since characteristic CD signals do not necessarily need to be indicative of a folding event,[32] further studies on longer oligomers will be required to investigate the nature of the minimum near 209 nm in more detail. However, to study the thermostability of the observed CD spectral bands, we collected CD spectra of the hexamer **21** at temperatures in the range 20-70°C. As can be seen in Figure 5 B, the hexamer **21** shows only a small reduction in ellipticity and no change in shape until 70°C, denoting thermal stability of the CD spectral bands.

It is not possible to identify the secondary structure of novel peptidomimetics in solution by solely analyzing CD spectra. We therefore designed and synthesized tetramer **28** and hexamer **29** in order to perform additional conformational studies. Based on the X-ray analysis of tripeptoid **9**, we speculated that α-aminoxy peptoids can adopt a conformation in which the *i*, *i*+2 and *i*+4 side chains are located on one side of the peptoid face, whereas the *i*+1, *i*+3 and *i*+5 side chains are located on the opposite side. Thus, we designed the hexamer **29** containing α-chiral, aromatic side chains at the *i+1*, *i*+3 and *i*+5 positions to create an "aromatic face", which is known to stabilize peptoid helices.[33] Three structurally diverse side chains were chosen for the *i*, *i*+2 and *i*+4 in order to minimize overlapping NMR signals.

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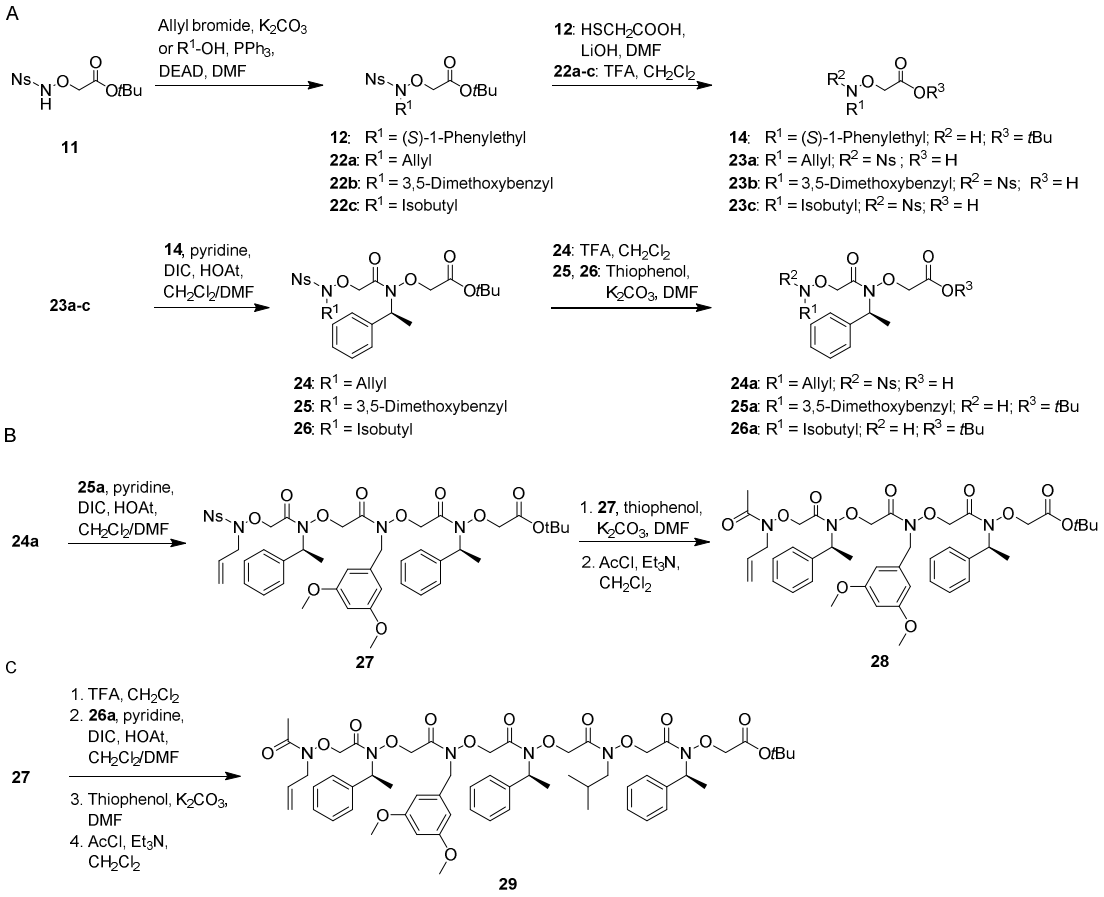
The tetramer **28** represents a simplified and truncated analogue of **29**. The synthesis of the oligomers **28** and **29** is outlined in Scheme 2 (see Supporting Information for synthetic details). Briefly, we prepared a set of dimeric building blocks (Scheme 2 A), which were subsequently assembled to the desired oligomers by amide coupling reactions followed by deprotection and acetylation of the N-terminal aminoxy moiety (Scheme 2 B, C). We conducted 2D NOESY experiments on **28** and **29** to gain further insights into the amide bond geometry and secondary structure of oligomeric α-aminoxy peptoids. When analyzing tetramer **28** we observed a strong cross peak between the N-terminal acetyl group and the backbone methylene group of the N-terminal monomer (*i*) as well as a strong cross peak between the backbone protons of the monomers *i*+2 and *i*+3 indicating *cis*-amide geometry for the C- and N-terminal amide bonds (Figure S11). However, no cross peaks could be observed for the internal amides. Moreover, no further distinct inter-residue NOEs could be detected. In the case of hexamer **29**, a clear NOE was found for the C-terminal amide suggesting a *cis*-amide geometry (Figure S12).

Unfortunately, no inter-residual NOESY cross-peaks could be detected. This issue was further investigated by a proton inversion-recovery experiment (Table S2), revealing significant differences in longitudinal-relaxation times of backbone- and sidechain protons, which can affect cross-relaxation. Notably, a VT-NMR experiment on compound **28** revealed no indications for the presence of *cis/trans*-amide bond rotamers (Figure S13). The CD spectra of **28** and **29** in acetonitrile (Figure S14) look very similar when compared to the spectra of compounds **19-21** (Figure 5).

Since our CD and NMR data are insufficient to verify the presence of distinct secondary structures, we performed comprehensive computational studies to investigate the conformational preferences of α-aminoxy peptoids, and their potential as helix mimetics. The backbone torsion angles φ, θ, ω, and ψ (Figure 2) are critical for an accurate description of the conformational preferences. We parameterized φ and θ for molecular dynamics (MD) simulations at the molecular mechanics level based on *ab initio* calculations (Figure 2 and Figure S15). Fitting of the molecular mechanics energy against the *ab initio* energy for angles φ and θ provided very good correlations ($R^2 \geq 0.95$ (Figure S15)). For the ω and ψ backbone torsion angles (Figure 2), molecular mechanics energies using parameters of the GAFF force field (see Supporting Information) already yielded a very good agreement with *ab initio* energies (R^2 = 0.99 (Figure S16)). The energy minima identified for the torsion potentials of φ, θ, ω, and ψ showed good agreement with the torsion angles found in the crystal structure of α-aminoxy



Scheme 2. Synthesis of tetramer **28** and hexamer **29**.

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peptoids **1** and **9** (Figures S15 and S16, Table S3). Hence, the torsion angle parameters accurately reproduce relative energies of the backbone dihedral angles of α-aminoxy peptoids.

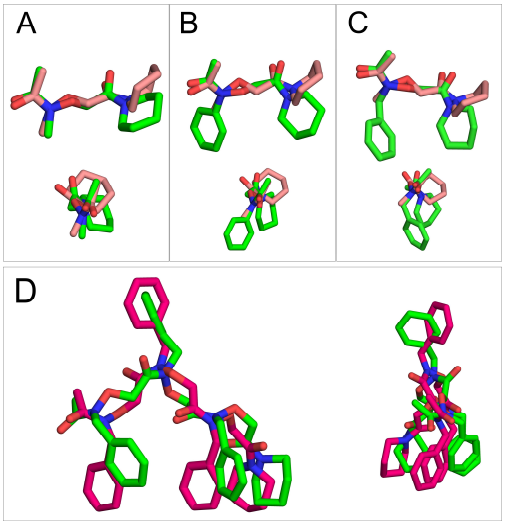

Figure 6. (A), (B), and (C): Overlay of low-energy conformations of α-aminoxy peptoids **1**, **2**, and **3** obtained from MD simulations (green) onto a crystal structure of α-aminoxy peptoid **1** (pink), resulting in RMSD of the backbone atoms of 0.57 Å, 0.60 Å, and 0.59 Å, respectively. In the lower panel, the overlays are rotated by 90°. (D): Overlay of a low-energy conformation of α-aminoxy peptoid **9** obtained from MD simulations (green) onto a crystal structure of α-aminoxy peptoid **9** (magenta), resulting in an RMSD of the backbone atoms of 1.2 Å. In the right panel, the overlay is rotated by 90°.

Subsequently, we performed MD simulations of α-aminoxy peptoids **1** (3.5 μs length), **2** and **3** (3 μs length each), and **9** (3.5 μs length) in explicit chloroform. Choosing this solvent allows for a direct comparison of simulation results with NMR data (see above). The MD simulations reached equilibrium with respect to conformational preferences of φ, θ, and ψ angles, as indicated by highly symmetric torsion angle distributions (Figures S17, S18, S19) and frequent transitions between energetically preferred states (Figures S20, S21, S22). In contrast, for α-aminoxy peptoids **1**,**2**, or **3** started either with a *cis*- or *trans*-amide conformation, a transition in the ω torsion angle between the two conformers was not observed, in line with an energetic barrier separating the two conformers (Figure S16 for the *N*-ethoxyformamide model compound; Figure S23 for α-aminoxy peptoid **30** (*N*,*N*-dimethyl-2-((*N*-methylacetamido)-oxy)acetamide) for probing the effect of an acetyl cap group and an *N*-methyl side chain) and previous work.[34] In addition, our calculations show that the *cis* conformer is preferred over the *trans* conformer by ~2.8 kcal mol^{-1} for peptoid **30**, whereas *cis* and *trans* conformers are virtually isoenergetic for the *N*-ethoxyformamide model compound (Figures S23 and S16, respectively), which is consistent with *cis/trans* energy differences of up to 1.4 kcal mol^{-1} in regular (not α-aminoxy) peptoids, as reported by Yoo and Kirshenbaum.[35] These findings also agree with the above NMR experiments and crystal structure analyses. Finally, the distributions of backbone torsion angles of monomeric α-aminoxy peptoids **1**,**2**, and **3** observed in the MD simulations showed a very good to good agreement with the torsion angle values found in the crystal structures of α-aminoxy peptoids **1** and **9** (Figures S17, S18, S19, and Table S3). Relative free energies of α-aminoxy peptoid **1** as a function of the backbone dihedral angles φ, θ, and ψ computed from the frequency of occurrence of conformations during MD simulations allowed us to identify preferred backbone conformations of α-aminoxy peptoid **1** (Figure S24); given the almost identical torsion angle distributions (Figures S17, S18, S19), similar conformations are expected to be favorable also for other α-aminoxy peptoids. The lack of mirror symmetry in the θ/φ and θ/ψ projections of the relative free energy is compatible with the observation that the amide nitrogen shows a certain degree of pyramidalization, in agreement with Jordan and co-workers, leading to a transient chirality.[34] Superimposing low-energy conformations of α-aminoxy peptoids **1**,**2**, and **3** extracted from the respective MD simulations onto the crystal structure of α-aminoxy peptoid **1** results in backbone atom root mean square deviations (RMSD) ≤ 0.6 Å (Figure 6 A, B and C). Similarly, overlaying a low-energy conformation of α-aminoxy peptoid **9** onto the crystal structure of the same compound results in a backbone atom RMSD = 1.2 Å (Figure 6 D). These results show that the MD simulations yield low-energy conformations of α-aminoxy peptoids that are in very good agreement with experimentally determined structures.

We used the thus validated setup of MD simulations to investigate the conformational properties of the more complex tetrameric (**28**) and hexameric (**29**) α-aminoxy peptoids. MD simulations of **28** (3.5 μs length) and **29** (2 μs length) in explicit chloroform yielded again distributions of backbone torsion angles that are in very good agreement with values of the crystal structures of α-aminoxy peptoids **1** and **9**, and are similar to those obtained with α-aminoxy peptoids **1**,**2**, and **3** (data not shown). The conformational ensembles obtained by the MD simulations of α-aminoxy peptoid **28** and **29** were clustered according to the RMSD. The four most populated clusters that account for 71% (α-aminoxy peptoid **28**) and 64% (α-aminoxy peptoid **29**) of the MD ensembles were further analyzed. Overlaying the C_β atoms of α-aminoxy peptoid **28** onto C_β atoms of a canonical α-helix shows that the α-aminoxy peptoid scaffold can closely mimic the spatial arrangement of peptide side chains at positions *i*, *i* + 1, *i* + 3, *i* + 5. When the N-terminus of the peptoid is oriented toward the helix C-terminus, the RMSD of the coordinates of the respective atom pairs is 0.5 Å (Figure 7 A). When the peptoid is oriented in the opposite direction, the RMSD is 0.7 Å (Figure 7 B). Overlaying the C_β atoms of peptoid **29** with C_β atoms of a canonical α-helix shows that the α-aminoxy peptoid scaffold can also address an *i*, *i* + 2, *i* + 3, *i* + 5, *i* + 7, *i* + 11 amino acid pattern when the N-terminus of the peptoid is oriented toward the helix C-terminus (Figure 7 C), and alternatively an *i*, *i* + 4, *i* + 6, *i* + 8, *i* + 9, *i* + 11 pattern when the peptoid is oriented in the opposite direction (Figure 7 D). The RMSD of the coordinates of the respective atom pairs is 1.1 Å in both cases. In summary, the conformational analysis based on the MD simulations reveals that α-aminoxy peptoids adopt conformations that can mimic *i*, *i* + 1, *i* + 3, *i* + 5; *i*, *i* + 2, *i* + 3, *i* + 5, *i* + 7, *i* + 11; and *i*, *i* + 4, *i* + 6, *i* + 8, *i* + 9, *i* + 11 patterns of substituent orientations with respect to an α-helix.

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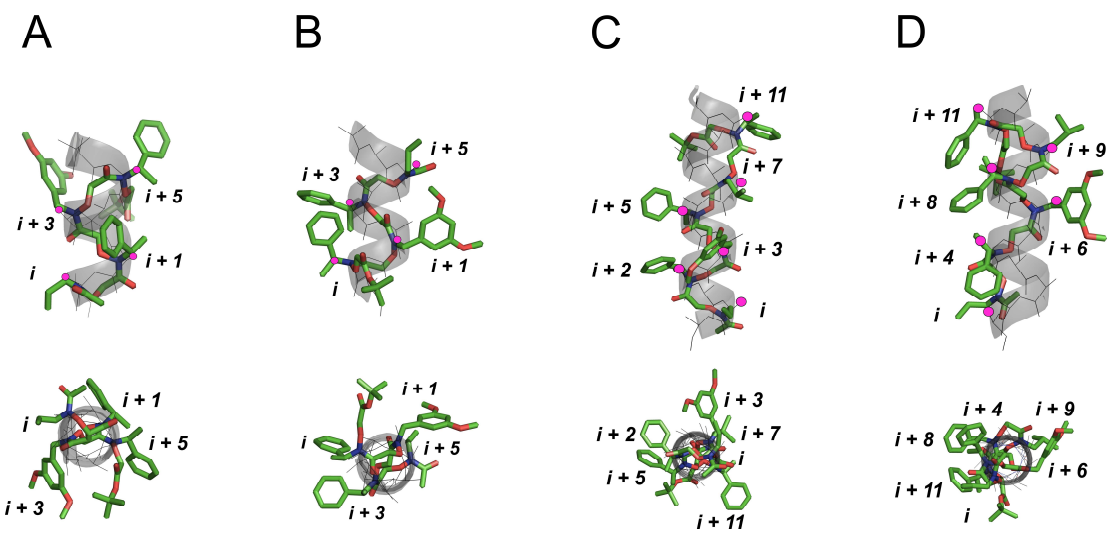

Figure 7. Overlay of C_β atoms of low-energy conformations of α-aminoxy peptoids **28** (A, B) and **29** (C, D) onto C_β atoms of a canonical α-helix (indicated by the pink spheres). In the case of α-aminoxy peptoid **28**, the scaffold can closely mimic the spatial arrangement of peptide side chains at positions *i*, *i* + 1, *i* + 3, *i* + 5, with an RMSD of the coordinates of the respective atom pairs of 0.5 Å when the N-terminus of the α-aminoxy peptoid is oriented toward the helix C-Terminus, and 0.7 Å when the α-aminoxy peptoid is oriented in the opposite direction (A and B, respectively). In the case of α-aminoxy peptoid **29**, the scaffold can closely mimic the spatial arrangement of peptide side chains at positions *i*, *i* + 2, *i* + 3, *i* + 5, *i* + 7, *i* + 11 when the N-terminus of the α-aminoxy peptoid is oriented toward the helix C-terminus (C), and *i*, *i* + 4, *i* + 6, *i* + 8, *i* + 9, *i* + 11 when the α-aminoxy peptoid is oriented in the opposite direction (D), with an RMSD of 1.1 Å in both cases.

Conclusions

Taken together, we have performed the first thorough analysis of the folding propensities of α-aminoxy peptoids. To this end, we designed and synthesized a series of model α-aminoxy peptoids utilizing a well-established cap group to investigate the influence of several side chains on the amide conformation and secondary structure. Interestingly, for α-aminoxy peptoids we observed a unique preference for *cis*-amide bonds in comparison to other peptoid backbones. The formation of an eight-membered δ turn that is stabilized by $C_{\alpha,i}$–H···O=C_{i+1} interactions between residues *i*→*i*+1 in the *cis* configuration might explain the *cis*-amide preference of α-aminoxy peptoids. MD simulations suggest that α-aminoxy oligopeptoids can fold into a distinct secondary structure closely mimicking the spatial arrangement of peptide side chains in a canonical α-helix. It is worth noting that α-aminoxy peptoid monomers can be easily prepared from the readily available starting material **11** either by classical alkylation with alkyl halides or by Mitsunobu reaction. This efficient monomer synthesis may therefore allow for the preparation of α-aminoxy peptoid libraries with a large chemical diversity in the future. Given their ease of synthesis, structural diversity, and conformational properties, α-aminoxy peptoids represent a promising new class of peptidomimetic foldamers that may be highly useful in a variety of novel applications such as material sciences and medicinal chemistry. In conclusion, α-aminoxy peptoids were introduced as a new peptoid backbone capable of controlling the amide isomerism while maintaining the potential for side-chain diversity.

Experimental Section

See the Supporting Information for experimental details. The structural data has been deposited with the Cambridge Crystallographic Data Center (CCDC-No. 1508156 and 1508157).

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Keywords: peptidomimetics • foldamers • peptoids • α-aminoxy peptoids • helix mimetics

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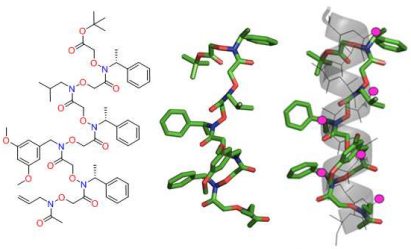

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