α-Aminoxy Oligopeptides: Synthesis, Secondary Structure, and Cytotoxicity of a New Class of Anticancer Foldamers


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Abstract: a-Aminoxy peptides are peptidomimetic foldamers with high proteolytic and conformational stability. To gain an improved synthetic access to a-aminoxy oligopeptides we used a straightforward combination of solution- and solid-phase-supported methods and obtained oligomers that showed a remarkable anticancer activity against a panel of cancer cell lines. We solved the first X-ray crystal structure of an a-aminoxy peptide with multiple turns around the helical axis. The crystal structure revealed a right-handed 2α-helical conformation with precisely two residues per turn and a helical pitch of 5.8 Å. By 2D ROESY experiments, molecular dynamics simulations, and CD spectroscopy we were able to identify the 2α-helix as the predominant conformation in organic solvents. In aqueous solution, the a-aminoxy peptides exist in the 2α-helical conformation at acidic pH but exhibit remarkable changes in the secondary structure with increasing pH. The most cytotoxic a-aminoxy peptides have an increased propensity to take up a 2α-helical conformation in the presence of a model membrane. This indicates a correlation between the 2α-helical conformation and the membranolytic activity observed in mode of action studies, thereby providing novel insights in the folding properties and the biological activity of a-aminoxy peptides.

Introduction

The use of unnatural amino acids to control secondary structures is a promising strategy to overcome drawbacks of peptide drugs, such as conformational flexibility and poor bioavailability.[1] In recent years, significant efforts have been devoted to the investigation of peptidomimetic foldamers. β-Peptides are the most thoroughly studied class of foldamers, and pioneering research by the groups of Seebach and Gellman revealed that oligopeptides constructed from β-amino acids can adopt helical conformations that are structurally similar to the α-helix.[2,3]

α-Aminoxy peptides, the oxo-analogs of β-peptides, were also found to be promising candidates for foldamers.[4] The aminoxy amide bond is resistant to enzymatic degradation, and peptide analogs constructed from α-amino acids show remarkable conformational stability, which has been demonstrated by Yang and coworkers through quantum chemical calculations, NMR, FT-IR, and CD spectroscopy.[4,5] The preferred secondary structure of homochiral α-aminoxy peptides is stabilized by strong intramolecular hydrogen bonds between the C=O, oxygen and the N–H, proton leading to so-called α-N–O turns.[6] It has been hypothesized that oligomers can adopt a conformation consisting of consecutive α-N–O turns resulting in a 18α-helix.[6] Presumably, in such a 18α-helix, the spatial arrangement of the side chains is similar as in an α-helix in canonical peptides, thereby highlighting the potential of α-aminoxy peptides as mimics for bioactive peptides. However, until now, this helical conformation has only been studied for short oligomers (mainly α-aminoxy dipeptide amides).[6] This is partly due to the limited access to long-chained oligomers. The synthesis of longer α-aminoxy peptide sequences under solution-phase conditions is generally challenging, which also hampers the applicability of α-aminoxy peptides as foldamers for biomedical applications. Even though the field of anticancer peptides (ACPs) is rapidly emerging,[6] no anticancer properties have been reported for α-aminoxy peptides thus far.

Here we present an improved synthesis of α-aminoxy oligopeptides based on a straightforward combination of solution-phase and solid-phase supported methods. We report a remarkable cytotoxic activity, particularly of long-chained oligomers, against a panel of cancer cell lines of different chemosensitivities and tissue origins. X-ray crystallography and circular dichroism (CD) spectroscopy were applied to identify a 2α-helix as the prevalent fold of the α-aminoxy oligopeptides. We compared the results from membrane integrity assays as well as apoptotic assays with the CD data in the presence of liposomes as a membrane model. The CD experiments suggest that the membranolytic properties of the most cytotoxic α-aminoxy peptides are associated with an increased propensity for a 2α-helix formation in the membrane environment.

Results and Discussion

Chemistry

Synthesis of the building blocks: In order to synthesize a mini library of α-aminoxy oligopeptides (1), we first prepared a series of D-α-aminoxy acids (D-OXaa-OH) using literature procedures (Scheme 1).[7] Briefly, phthaloyl-protected monomers 5a-d were synthesized from their respective L-α-amino acids by conversion into the L-α-hydroxy acids 2a-d followed by acetylation, esterification, and deprotection of the acetyl group to yield the tert-butyl protected L-α-hydroxy acids 3a-d. The desired phthaloyl-protected α-α-amino acids 5a-d were obtained through a Mitsunobu reaction with N-hydroxypthal-
imide, followed by deprotection of the tert-butyl ester. The carboxybenzyl-protected \( \alpha \)-aminoxy acids 7a–c were prepared from the respective \( \alpha \)-amino acids by their corresponding \( \alpha \)-bromo acids 6a–c. The subsequent reaction with benzyl N-hydroxycarbamate (CbzNH-OH) in the presence of sodium hydride as a base afforded the desired Cbz-protected monomers 7a–c.

To the best of our knowledge only one solid-phase-supported protocol for the synthesis of \( \alpha \)-aminoxy oligopeptides has been published thus far.\[5\] The method reported by Shin and coworkers allowed the preparation of a series of pentamers.\[5\]

In the initial phase of this project, we attempted to prepare the hexamer 1a (Phth-N\( _\text{H} \)Ile-N\( _\text{H} \)Ile-N\( _\text{H} \)Ile-N\( _\text{H} \)Ile-N\( _\text{H} \)Leu-N\( _\text{H} \)) utilizing this published protocol. However, the desired product was only obtained in low crude yield (20\%) and purity (33\%), which is in line with previous reports about difficulties in the synthesis of peptide sequences containing repetitive aliphatic side chains, such as sec-butyldi.\[8\]

We therefore aimed at the development of a more efficient approach that allows the preparation of longer \( \alpha \)-aminoxy oligopeptides. For this purpose, we decided to use a combination of solution- and solid-phase peptide synthesis. We synthesized a panel of dimeric building blocks in solution by deprotection of the phthaloyl groups of 4b–d using hydrazine hydrate to provide the free aminoxy groups followed by an EDC/HOBt mediated amide coupling reaction with the phthaloyl- or Cbz-protected \( \alpha \)-aminoxy acids 5 or 7 to afford the \( \alpha \)-aminoxy dipeptides 8a–k (Table 1).

**Solid-phase synthesis of \( \alpha \)-aminoxy oligopeptides:** The required N-protected \( \alpha \)-aminoxy dipeptide acids (PG-N\( ^\text{Xaa-N\( ^\text{Xaa-OH} \)) were obtained from the corresponding tert-butyl protected derivatives 8a–k directly before use.\[11\] Preparative conditions for the synthesis of the model hexamer 1a were optimized by investigating several well-established coupling reagents (see Table S1 in the Supporting Information for details). The results from this screening showed significant differences between the investigated coupling reagents. The BOP/HOBt/NEM coupling system gave the highest crude yield (67\%) and purity (57\%) compared with EDCI/HOBt, DIC, DIC/HOBt, HBTU/HOBt/NEM, and PyBOP/HOBt/NEM. Thus, BOP/HOBt/NEM was chosen as the coupling system for all subsequent solid-phase supported reactions.

We next prepared a series of hexamers and decamers (Scheme 2). In all cases, the resin was loaded by the reaction

<table>
<thead>
<tr>
<th>Entry</th>
<th>PG-N( ^\text{H} )</th>
<th>R( ^\text{1} )</th>
<th>R( ^\text{2} )</th>
<th>Product</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{Bu} )</td>
<td>( _\text{Bu} )</td>
<td>8a</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Bu} )</td>
<td>8b</td>
<td>71</td>
</tr>
<tr>
<td>3</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{Bu} )</td>
<td>( _\text{Me} )</td>
<td>8c</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{Bu} )</td>
<td>( _\text{Me} )</td>
<td>8d</td>
<td>54</td>
</tr>
<tr>
<td>5</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Bu} )</td>
<td>8e</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{Bu} )</td>
<td>( _\text{Me} )</td>
<td>8f</td>
<td>63</td>
</tr>
<tr>
<td>7</td>
<td>Phth-N( _\text{H} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Bu} )</td>
<td>8g</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>Cbz-NH( _\text{Me} )</td>
<td>( _\text{Bu} )</td>
<td>( _\text{Me} )</td>
<td>8h</td>
<td>86</td>
</tr>
<tr>
<td>9</td>
<td>Cbz-NH( _\text{Me} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Me} )</td>
<td>8i</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>Cbz-NH( _\text{Me} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Bu} )</td>
<td>8j</td>
<td>64</td>
</tr>
<tr>
<td>11</td>
<td>Cbz-NH( _\text{Me} )</td>
<td>( _\text{sBu} )</td>
<td>( _\text{Bu} )</td>
<td>8k</td>
<td>43</td>
</tr>
</tbody>
</table>

**Table 1.** Solution-phase synthesis of \( \alpha \)-aminoxy dipeptides.

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**Scheme 1.** Synthesis of \( \alpha \)-aminoxy acids.

**Scheme 2.** Synthesis of \( \alpha \)-aminoxy dipeptides.
of the deprotected Fmoc Rink Amide PEG AM resin with three equivalents of the respective phthaloyl-protected α-aminooxy dipeptide acid (Phth-NO2-Xaa-NO2-Xaa-OH) in the presence of the BOP/HOBt/NEM coupling system for 24 h. Iterative cycles of the phthaloyl deprotection (5% hydrazine hydrate in methanol, 2 × 15 min) and the amide coupling (3 equiv of the respective dimer and coupling agents, 24 h) were performed to synthesize the resin-bound oligopeptides. In the case of the final coupling step, we used both phthaloyl- and Cbz-protected dimers to afford oligomers with two different cap groups. After the desired chain length was reached, all products were cleaved from the resin and precipitated with cold diethyl ether to give the crude products (Scheme 2, Table 2). The crude α-aminooxy peptides 1a–i were subsequently purified by semipreparative RP-HPLC to a purity greater than 95%, lyophilized, and characterized by HRMS (Table 2). To the best of our knowledge, the decamers 1h and 1i are the longest α-aminooxy peptides reported so far.

### Biological evaluation

**Anticancer activity**: While no anticancer activities have been reported for α-aminooxy peptides, recent reports regarding the cytotoxic activities of various ACPs[6,10] and ACP mimetics (anticancer foldamers)[6,10] prompted us to investigate the anticancer activity of the α-aminooxy peptides 1a–i. All compounds were first tested for their cytotoxic properties against the human esophageal squamous cancer cell line Kyes510 and its cisplatin resistant subclone Kyes510 CisR. Several compounds exhibited remarkable anticancer activities (Table 3). It is worth noting that the most potent α-aminooxy peptide 1h (IC50 = 1.4 μM) exceeded the potency of the reference compound cisplatin (IC50 = 2.0 μM) against Kyes510 cells and also showed the highest activity against Kyes510 CisR cells (IC50 = 2.6 μM). All compounds were subsequently tested for their anticancer activity against the human ovarian cancer cell lines A2780 and A2780 CisR (Table 3). Remarkably, the most potent α-aminooxy peptides 1h and 1i showed an up to twofold higher activity against the cisplatin resistant subclone A2780 CisR than against the native cell line A2780. Again, the decamer 1h showed the highest activity with IC50 values of 3.3 μM (A2780) and 1.7 μM (A2780 CisR). From the data summarized in Table 3 some preliminary structure–activity relationships can be drawn: 1) Replacement of the phthaloyl by a Cbz protecting group leads to a up to tenfold increase in the anticancer potency (see 1d vs. 1e), 2) decamers are more potent than hexamers (see 1a–g vs. 1h, i), and 3) aromatic side chains increase the anticancer activity (see 1b vs. 1e; and 1a vs. 1d). To probe the selectivity of our α-aminooxy peptides against cancer cells, we investigated the cytotoxicity of 1a–i against human embryonic kidney 293 (HEK293) cells (Table 3). The results showed that the most potent α-aminooxy peptides 1e, h, i are less toxic to normal cells than to cancer cells. In summary, even though the preference for cancer cells still needs to be improved for an increased therapeutic index, α-aminooxy peptides can be considered a new class of anticancer foldamers.

### Conformational analysis

The activity of ACPs and anticancer foldamers is generally associated with a specifically folded bioactive conformation.[6,10] As the folding behavior of long-chained α-aminooxy peptides is still elusive, we analyzed the conformations of representative

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**Table 2.** Yields, purities, and HRMS data of the α-aminooxy peptides 1a–i prepared by solid-phase synthesis.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Peptide[a]</th>
<th>Crude purity [%]</th>
<th>Purity[b] [%]</th>
<th>Isolated yield[c] [%]</th>
<th>m/z[d] [calcd]</th>
<th>m/z[d] [obs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phth-NO2-Ile-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1a</td>
<td>57</td>
<td>96</td>
<td>30</td>
<td>922.5132</td>
<td>922.5135</td>
</tr>
<tr>
<td>2</td>
<td>Cbz-NO2-Ile-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1b</td>
<td>62</td>
<td>96</td>
<td>32</td>
<td>926.5445</td>
<td>926.5447</td>
</tr>
<tr>
<td>3</td>
<td>Cbz-NO2-Ala-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1c</td>
<td>61</td>
<td>96</td>
<td>24</td>
<td>800.4036</td>
<td>800.4028</td>
</tr>
<tr>
<td>4</td>
<td>Phth-NO2-Leu-NO2-Ile-NO2-Ile-NO2-Phe-NO2-Phe-NH2 1d</td>
<td>64</td>
<td>96</td>
<td>24</td>
<td>990.4819</td>
<td>990.4819</td>
</tr>
<tr>
<td>5</td>
<td>Cbz-NO2-Leu-NO2-Ile-NO2-Ile-NO2-Phe-NO2-Phe-NH2 1e</td>
<td>36</td>
<td>97</td>
<td>18</td>
<td>994.5132</td>
<td>994.5131</td>
</tr>
<tr>
<td>6</td>
<td>Cbz-NO2-Ala-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1f</td>
<td>66</td>
<td>98</td>
<td>28</td>
<td>876.4349</td>
<td>876.4354</td>
</tr>
<tr>
<td>7</td>
<td>Cbz-NO2-Ala-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1g</td>
<td>75</td>
<td>97</td>
<td>42</td>
<td>910.4193</td>
<td>910.4200</td>
</tr>
<tr>
<td>8</td>
<td>Cbz-NO2-Ala-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1h</td>
<td>54</td>
<td>96</td>
<td>21</td>
<td>1460.7195</td>
<td>1460.7202</td>
</tr>
<tr>
<td>9</td>
<td>Cbz-NO2-Ala-NO2-Ile-NO2-Ile-NO2-Leu-NO2-Leu-NH2 1i</td>
<td>49</td>
<td>97</td>
<td>22</td>
<td>1494.7039</td>
<td>1494.7029</td>
</tr>
</tbody>
</table>

[a] All abbreviations refer to α-configured α-aminooxy acids. [b] Purity after purification by RP-HPLC. [c] Yields are calculated based on the loading capacity of the resin. [d] Based on HRMS.

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**Table 3.** Cytotoxic activity (MTT) of 1a–i against cancer cell lines and HEK293 cells.[a,b]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kyes510</th>
<th>Kyes510 CisR</th>
<th>A2780</th>
<th>A2780 CisR</th>
<th>HEK293</th>
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</thead>
<tbody>
<tr>
<td>cisplatin hexamers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>&gt;100</td>
<td>66.0 ± 4.0</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1b</td>
<td>55.8 ± 1.4</td>
<td>65.8 ± 6.5</td>
<td>91.6 ± 6.5</td>
<td>&gt;100</td>
<td>17.5 ± 1.1</td>
</tr>
<tr>
<td>1c</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1d</td>
<td>58.0 ± 1.3</td>
<td>54.6 ± 1.2</td>
<td>57.5 ± 3.0</td>
<td>70.0 ± 2.4</td>
<td>20.2 ± 0.8</td>
</tr>
<tr>
<td>1e</td>
<td>5.7 ± 0.1</td>
<td>7.8 ± 0.1</td>
<td>7.5 ± 0.2</td>
<td>8.9 ± 0.6</td>
<td>20.2 ± 0.8</td>
</tr>
<tr>
<td>1f</td>
<td>63.6 ± 1.6</td>
<td>77.3 ± 1.7</td>
<td>81.7 ± 1.4</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1g</td>
<td>40.2 ± 0.8</td>
<td>52.4 ± 1.2</td>
<td>55.0 ± 2.5</td>
<td>60.9 ± 1.5</td>
<td>84.0 ± 2.8</td>
</tr>
<tr>
<td>decamers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1h</td>
<td>1.4 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>9.0 ± 0.6</td>
</tr>
<tr>
<td>1i</td>
<td>2.7 ± 0.1</td>
<td>6.6 ± 0.5</td>
<td>8.0 ± 0.4</td>
<td>3.9 ± 0.1</td>
<td>26.3 ± 1.3</td>
</tr>
</tbody>
</table>

[a] CisR = cisplatin resistant. [b] Data shown are the mean ± SE of at least 3 experiments.
members of this novel class of anticancer foldamers by X-ray crystallography, molecular modeling, NMR spectroscopy, molecular dynamics (MD) simulations, and CD spectroscopy.

**X-ray crystallography:** We obtained diffraction-quality crystals of the α-aminoxy hexapeptide 1b by crystallization from acetonitrile/water. The packing of the molecules in the crystal structure (Figure 1A) is dominated by two types of interactions: 1) Each molecule is head-to-tail connected to neighboring ones by hydrogen bonds resulting in a zig-zag chain; the characteristic angle of this zig-zag chain seems to depend on the steric demand of the terminal side chains (Figure 1A). 2) The side chains of adjacent zig-zag chains interlock with each other (Figure 1A).

The conformation observed in the crystal structure of 1b (Figure 1B) is in several aspects in good agreement with the folding pattern previously predicted for α-aminoxy peptides:[4d] 1) The secondary structure is stabilized by the formation of eight-membered hydrogen-bonded rings leading to multiple highly conserved α N–O turns. 2) The side chains alternate on opposite sides of the helix. This results in the interlocking of neighboring side chains, which possibly contributes to the stability of the molecule (Figure 1A). As a consequence of all these interactions, the backbone of the all-α-configured α-aminoxy hexapeptide forms a right-handed helix. 3) The α-aminoxy peptide helix is rather flat (Figure 1B, bottom) compared to other helices of conventional spiral shape, such as α-helices or 310-helices.[4e]

In comparison to previous conformational studies on α-aminoxy peptides, we observed some subtle but important differences. By quantum mechanical calculations, the amide carbonyl at the i+2 position had been predicted to be twisted by +30–50° relative to the corresponding carbonyl at the i position, thereby leading to a 1.8α-helical conformation.[4] However, the crystal structure of 1b reveals that precisely two residues are necessary to complete a full turn around the helical axis, which demonstrates that α-aminoxy peptides adopt a 2α-helix. The observed helical pitch of 5.8 Å (± 0.1 Å) (Figure 1B) lies in between the helical pitches observed in typical protein secondary structures, such as α-helices (5.4 Å) or 310-helices (6.0 Å), in contrast to the computationally predicted helical pitch of 6.5 Å.[4e]

![Figure 1. X-ray crystal structure of the α-aminoxy hexapeptide 1b.](image-url)

**Figure 1.** X-ray crystal structure of the α-aminoxy hexapeptide 1b. A) Crystal packing of 1b. Arrows denote the orientation of the α-aminoxy hexapeptides (C-terminus → N-terminus). B) Close-up view of 1b in side view (top, C-terminus at the top) and rotated by 90° (bottom, C-terminus oriented towards the viewer). Intramolecular hydrogen bonds are depicted as dashed lines. Overlay of Cα and Cζ atoms of the crystal structure of 1b onto (C) Cα and Cζ atoms of a canonical α-helix and (D) Cα and Cζ atoms of a 310-helix, resulting in a i,i+1 pattern (in which j ranges from one to eight). The Cbz protecting group is oriented towards the helical C-terminus. In the lower panel, the overlays are rotated by 90°, such that the helical C-terminus is oriented towards the viewer. The carbon atoms of the crystal structure of 1b are depicted in green (A–D). The helical structures are depicted as black sticks with transparent cartoon representations (C + D). The magenta spheres highlight the helical reference atoms addressed by the side chains of 1b. The root mean square deviation (RMSD) and average angle deviation η (see text for definition) were used to evaluate the quality of the superimposition.
Molecular modeling: To probe if α-aminoxy peptides can mimic specific features of protein secondary structures, we overlaid the Cα and Cβ atoms of the crystal structure of 1b (Figure 1B) onto respective atoms of a canonical α-helix (backbone dihedrals \( \psi = -48.0^\circ \) and \( \phi = -57.0^\circ \); Figure 1C), and a \( 3_{\omega} \)-helix (\( \psi = -74.0^\circ \) and \( \phi = -4.0^\circ \); Figure 1D). The root mean square deviation (RMSD) of the Cα and Cß atoms of 1b with respect to equivalent atoms of the secondary structures and the average angle deviation (\( \eta \)) of the respective Cα–Cβ bond vectors were used to evaluate the quality of the superimposition (Figure 1C,D).

For the α-helix, amino acid side chains in the \( i,j,i+j,k \) positions (with \( j \) and \( k \) in the range of two to eight) of two helix turns are well addressed by α-aminoxy oligopeptides when their N-terminus is oriented towards the C-terminus of the α-helix. For the substitution pattern \( i,j,i+4,i+8 \) (side chains on one helical face, Figure 1C), we determined a RMSD of roughly 0.5 Å and a \( \eta \) smaller than 25°, indicating similar positions of the Cα and Cß atoms and similar side chain orientations. Similar RMSD and \( \eta \) values were found for the substitution patterns \( i,i+2,i+6 \) (Figure 1C), \( i,i+2,i+4 \), and \( i,i+2,i+4,i+8 \) (side chains on both helical faces, Figure S1 A in the Supporting Information). In contrast, other helical substitution patterns, which have been described for protein–protein interfaces \([11]\) \( i,i+4,i+7 \), Figure 1C; \( i,i+3,i+7 \) and \( i,i+4,i+6 \), Figure S1 A), reveal larger positional and/or angle deviations (up to 1.41 Å or 48.32°).

The substitution pattern \( i,i+3,i+6 \) of a \( 3_{\omega} \)-helix is perfectly addressed by the side chains of the α-aminoxy oligopeptides (RMSD = 0.15 Å, \( \eta = 4.05^\circ \)) when the Cbz protecting group is oriented towards the helical C-terminus (Figure 1D). In addition, the side chains of the α-aminoxy peptides can also mimic a great variety of other \( 3_{\omega} \)-helical side chains patterns \( (i,i+j \) with \( 1 \leq j \leq 8 \)), resulting in a RMSD smaller than 0.7 Å and a \( \eta \) of roughly 30° (Figure 1D). For both secondary structures, larger positional and/or angle deviations are found if the Cbz protecting group of 1b is oriented towards the helical N-terminus (Figure S1 B,C).

Taken together, α-aminoxy oligopeptides represent a new class of helix mimetics that can mimic several substitution patterns of α-helices and \( 3_{\omega} \)-helices. Although there is an increasing number of synthetic scaffolds for the mimicry of side chains on a single face of an α-helix, there are very few examples that can reproduce the side chain projection of two helical faces.\([12]\) Therefore, these findings may enable the structure-based design of α-aminoxide peptide-based one-sided and double-sided helix mimetics with potential applications as inhibitors of protein–protein interactions or as analogs of α-helical ACPs.

Conformational properties in solution: As shown above, the similarity in the fold and helical pitch of the \( 2_{\omega} \)-helix and the secondary structures of canonical peptides, namely α- and \( 3_{\omega} \)-helices, is intriguing and highlights the particular importance of this structural element. Yet to understand its role for biological activity it is indispensable to know the conditions that support the existence of the \( 2_{\omega} \)-helix in solution.

To study the conformational properties of 1b and 1c in solution, we performed 2D rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments in CDCl₃ at room temperature. The \( ^1H \) NMR spectra of 1b and 1c revealed no evidence for the presence of multiple conformations. In agreement with previously published data,\([8,9]\) all internal aminoxy protons of 1b and 1c were clustered in the range of 11.5–12.5 ppm, whereas the N-terminal aminoxy and the C-terminal NH₃ protons were shifted upfield (see the Supporting Information). Key ROE cross-peaks observed in the 2D ROESY spectra of 1b and 1c are illustrated in Figure S2 and S3 (see the Supporting Information). Previously, Yang and coworkers found a characteristic ROE pattern for the typical α N–O turn of a homochiral α-aminoxy peptide with each internal aminoxy proton (NH), showing a strong ROE to its own α-methylene proton (Cα-H) and a weaker ROE cross-peak to the neighboring backbone methine proton (Cα-H).\([10]\) The same ROE pattern was observed in our 2D ROESY analysis of 1c (Figure S2), which indicates the presence of repetitive α N–O turns. While the overlap of some signals in the \( ^1H \) NMR spectrum of 1b hampered the assignment of some of the weaker ROE cross-peaks, the key ROE pattern (Figure S3) was also observed for 1b. Furthermore, the interatomic distances reflect by these ROE signals are in good agreement with the distances observed in the crystal structure of 1b (Figure S4).

To corroborate this observation and to gain insights regarding the preferred conformation of 1b in solution, we performed molecular dynamics (MD) simulations of 1.5 μs length of 1b in CHCl₃. The conformational ensemble was clustered according to the structural similarity of the backbone. In general, for all clusters I–X, the distances measured between the respective hydrogen atoms are in agreement with the occurrence of an ROE pattern that is indicative for repetitive α N–O turns, in particular for ROEs observed in the N-terminal part of the molecule (Figure S4). Representative structures of 1b extracted from the clusters I and II, which contain more than 73% of all conformations (Figure S5), show pronounced movements of the N-terminal Cbz group and the C-terminal α-aminoxide only. For the core region, however, a conformation very similar to the crystal structure is found, with characteristic repetitive α N–O turns forming a rather flat helix with the side chains alternating on the opposite sides of the helix (Figure 2). Based on these observations, we conclude that the \( 2_{\omega} \)-helix consisting of repetitive α N–O turns is retained in a hydrophobic environment such as CDCl₃.

CD spectroscopy: In proteins and peptides, a conformation can be identified from their CD spectra by the assignment of characteristic motifs that correspond to a particular secondary structure. For molecules in which such characteristic CD motifs are not known, CD spectra can be correlated with molecular geometries through the comparison of experimental spectra to spectra obtained from quantum chemical calculations. The recent development of simplified theoretical models allows the simulation of accurate CD spectra of large molecules at comparably low computational cost.\([13,14]\) We calculated a CD spectrum for the crystal structure geometry of 1b at the simplified
time-dependent density functional theory (sTD-DFT) level ($\omega$B97X/TZVPP)\textsuperscript{14} and compared it to experimental CD spectra of solutions of 1b and 1f–i in 2,2,2-trifluoroethanol (TFE) (Figure 3). The good agreement between the calculated and experimental spectrum of 1b suggests that the $\alpha_2$-helix is retained in TFE. The spectral patterns of 1f–i look very similar and are in good agreement with previously reported data on short-chained $\alpha$-aminoxy peptides.\textsuperscript{4} Furthermore, this CD pattern is also reminiscent of a $\beta$-turn secondary structure in canonical peptides, which agrees with the fact that the $\alpha_2$-helix can be interpreted as a motif of consecutive turns. In proteins and peptides, prototypic CD patterns indicate a particular secondary structure, more or less irrespective of the individual amino acid sequence. This is due to the fact that the spectral patterns arise from the dipolar coupling of electronic transitions in the peptidic carbonyls of the backbone, while the contributions from the side chains are often negligible.\textsuperscript{15} Obviously, the same also holds true for $\alpha$-aminoxy peptides. Except for a slight additional positive intensity around 210 nm in 1i that is possibly due to the unique arrangement of the aromatic side chains in this compound (Figure 3, inset), the same overall band pattern is observed in all spectra. This suggests that not only compound 1b, but also $\alpha$-aminoxy peptides 1f–i, take up a $\alpha_2$-helical structure in TFE. The corresponding CD spectra recorded in acetonitrile look very similar, which indicates that the secondary structure is also retained in this solvent (Figure S6). Furthermore, we did not observe significant temperature-dependent changes in the CD spectra of 1f–i recorded in TFE (1–20°C) and acetonitrile (−2–35°C), which highlights the conformational stability of these $\alpha$-aminoxy peptides (Figure S7).

To study the conformational properties in an aqueous environment, we recorded CD spectra of the hexamer 1g, an $\alpha$-aminoxy peptide with satisfactory solubility in water in the range from pH 2.5–11.2 (Figure 4). Below pH 6.5, the water solubility of 1g decreases significantly. The CD spectrum at acidic pH values remains basically unchanged and exhibits the spectral features of a $\alpha_2$-helix (Figure 4A, inset). However, similar to previous studies on $\alpha$-aminoxy dipeptides,\textsuperscript{46} significant changes occur at higher pH values. This suggests the existence of at least one additional conformation that is in a pH-dependent equilibrium with the $\alpha_2$-helix in aqueous solutions. Every pH-dependent spectrum can be viewed as the linear combination of the spectral contributions that correspond to individual pH-dependent species. To determine the number of pH-dependent species, their individual CD spectra, and the $pK_s$ value(s) associated with the pH-dependent conformational transition(s) in the pH range from 6.5–11.2, we performed a matrix least-squares (MLS) global fitting,\textsuperscript{16} a method that we have previ-
Figure 4. A) pH-dependent CD spectra of 1g in the range from pH 6.5–11.2 (3D representation). Inset: pH 2.5–6.0 2D representation. The low pH values have been omitted from the MLS (matrix least-squares) analysis due to the low solubility below pH 6.5. The colors are chosen according to litmus paper color coding from blue (basic) to red (acidic). B) Pure spectra from MLS global fitting that correspond to spectral contributions from the 2α-helical structure and another conformer (left) and the corresponding titration curves (right). The dots represent the coefficient with which the pure spectra contribute to each pH-dependent spectrum. The curves are the theoretical Henderson–Hasselbalch curves assuming a transition at a pKₐ of 8.2.

A behavior could be explained by a lower pKₐ of the terminal aminoxy groups in comparison to the pKₐ of the internal aminoxy groups in which the protons are involved in strong intramolecular hydrogen bonding (Figure 1). Deprotonation of a terminal aminoxy group at around neutral pH would have only little impact on the conformational stability, but would increase the solubility in water. Deprotonation at higher pH, on the other hand, disrupts the intramolecular hydrogen bonds and invokes a conformational change, thereby eventually leading to the loss of the helical secondary structure.

Mode of action studies and CD spectroscopy in a membrane environment

Most known ACPs act through membranolytic and/or apoptotic modes of action.[6] As a first step to elucidate the role of the conformational folding in the cytotoxic activity of α-aminoxy peptides (Table 3), we studied the membrane damage and apoptosis induction of the two decamers 1h and 1i, and the hexamers 1b, 1f, and 1g that are at least 20-fold less cytotoxic than 1h and 1i. Both membraneolysis and late apoptosis eventually result in membrane damage that allows nuclear DNA staining with propidium iodide (PI). However, membranolytic compounds lead to a much earlier cellular uptake of PI than late apoptosis. Early apoptosis does not lead to membrane damage, but instead DNA fragmentation can be detected after cell lysis and PI staining. Flow cytometry was, thus, used to easily distinguish between membraneolysis (direct PI uptake) and apoptosis induction (PI uptake only after cell lysis and DNA fragmentation). Figure 5 shows significant differences in the membrane damage and apoptosis induction for the decamers 1h and 1i compared to the hexamers 1b, 1f, and 1g. Whereas 2 μM 1h (approx. 2×IC₅₀ by MTT) do not show significant differences compared to the untreated control, 5 μM 1h (roughly 3.5×IC₅₀) significantly increased the membrane damage but did not change the number of apoptotic cells (Figure 5A). The second most potent peptide 1i behaved similarly; the membrane damage was significantly increased at 5 and 10 μM without affecting the apoptosis. In contrast, 50 μM of the hexamers 1b, 1f, or 1g (roughly IC₅₀) increased the apoptosis significantly, whereas the membrane damage was not significantly affected. Concerning the membrane damage and apoptosis induction, 1b resembles the DNA damaging agent cisplatin (cDDP, Figure 5A) more than the structurally related peptides 1h and 1i. Figure 5B summarizes the ratios of the membrane damage and apoptosis induction. The decamers 1h and 1i have significantly increased ratios over the untreated control, cisplatin was in the range of the untreated control, and the hexamers 1b, 1f, and 1g displayed reduced ratios (1f and 1g significantly). The membrane damage induced by the decamers 1h and 1i only leads to an increase in apoptosis at incubation times longer than 24 h, while the hexamers 1b, 1f, and 1g, similar to cisplatin, directly increase apoptosis. Hence, the decamers 1h and 1i act through a different mechanism of action compared to the hexamers. This indicates that the chain length contributes to the preferred mode of action of the α-aminoxy peptides.
Many ACPs are unordered in aqueous solution but adopt an active folded conformation upon interaction with hydrophobic surfaces such as cellular membranes. Conversely, ACPs that have a higher propensity for ordered secondary structures also have a higher membrane binding affinity and might therefore act by a membranolytic mechanism of action. To study the putative correlation between the conformational folding and membrane binding, we performed CD measurements of the hexamers $1f$ and $1g$ and the decamers $1h$ and $1i$ in the presence of liposomes (POPC/POPS 50:50, 80 nm diameter) as a model for membranes (Figure 6). The experiments were performed at pH 9, a pH value in which most of the $\alpha$-aminoxy peptides exhibit a good solubility in water and are predominantly present in a disordered conformation. To estimate the relative proportions of the secondary structure in the absence and presence of liposomes from these CD spectra, we performed a spectral fitting using spectra obtained from the CD analysis of $1g$ as spectral standards. As a standard for the disordered conformation we used the high-pH-species pure spectrum from the MLS global fitting (Figure 4B); for the $\beta$-helical conformation we used the low-pH-species pure spectrum (Figure 4B) and an average spectrum of $1g$ recorded at low pH values (Figure 4A, inset). Addition of liposomes induces only marginal changes for the hexamers $1f$ and $1g$ compared to the corresponding CD spectra in the absence of lipids. The coefficients from the linear combination of the spectral standards suggest a ratio of around 30% $\beta$-helix and 70% disordered structure both in the absence and presence of liposomes. In contrast, the decamers $1h$ and $1i$ exhibit significant spectral changes upon addition of liposomes. In $1h$, the amount of $\beta$-helix increases from around 20 to 60%. For $1i$ the liposome-induced disappearance of the positive intensity around 235 nm, which indicates a disordered conformation, suggests a virtually full transition (ca. 90%) to a $\beta$-helical structure when $1i$ binds to lipid membranes. The cytotoxicity of the decamers $1h$ and $1i$ is, in contrast to $1f$–$1g$, at least in parts related to membranolysis (Figure 5). Together with the findings from the CD experiments in the presence of liposomes, this suggests that the membranolytic properties of $1h$ and $1i$ are associated with an increased propensity for a $\beta$-helical conformation in a membrane environment.

**Conclusion**

In this study, we have developed an improved synthesis for long-chained $\alpha$-aminoxy oligopeptides through a combined solution- and solid-phase approach. Several of the novel hexamers and decamers showed promising anticancer activities against the cancer cell lines Kyse510 and A2780, as well as their cisplatin resistant subclones. Decamer $1h$, the most potent oligomer (Kyse510 $IC_{50} = 1.4 \mu M$), was less toxic to normal HEK293 cells than to the cancer cell lines, indicating a moderate preference for cancer cells.

We obtained the first X-ray structure of an $\alpha$-aminoxy peptide consisting of multiple turns around the helical axis and
identified a right-handed $\alpha_2$-helical conformation with precisely two residues per turn and a helical pitch of 5.8 Å. This $\alpha_2$-helix can mimic the spatial arrangement of the peptide side chains in $\alpha$-helices and 3$_{10}$-helices. By 2D ROESY experiments, MD simulations, and CD spectroscopy we identified a $\alpha_2$-helix as the predominant conformation in organic solvents. However, in aqueous solutions, we observed a pH-dependent equilibrium between the $\alpha_2$-helix and another conformation. As CD spectroscopy in the presence of liposomes revealed that long-chained $\alpha$-aminoxy peptides have an increased propensity to take up a $\alpha_2$-helical conformation in a membrane environment, we conclude that this conformation is responsible for the membranolytic activity of the decameric $\alpha$-aminoxy peptides observed in our model of action studies.

In summary, the improved access to $\alpha$-aminoxy peptide hexamers and decamers allowed for a thorough analysis of their anticancer activity, conformational fold, and modes of action of this novel class of bioactive compounds. These findings should improve the understanding of $\alpha$-aminoxy peptides as foldamers and enable the structure-based design of peptide analogs that mimic helical structures such as $\alpha$-helices and 3$_{10}$-helices in the future.

**Experimental Section**

**Chemistry**

Materials and general methods, synthetic protocols for the preparation of the dimeric building blocks, and the compound characterization data for all novel compounds are given in the Supporting Information.

**Solid-phase synthesis of $\alpha$-aminoxy oligopeptides 1a–i:**

The manual peptide synthesis was conducted in fritted PE syringes on a 0.2 mmol scale. After the resin swelling for 60 min in DMF, the Fmoc deprotection of the Rink Amide PEG resin (loading 0.56 mmolg$^{-1}$) was accomplished with 20% piperidine in DMF (2 × 15 min, 2 mL/100 mg of resin) before the resin was sequentially washed with DMF, CH$_2$Cl$_2$, and DMF (3 × 2 mL/100 mg resin, agitation for 15 s and then drained). A solution of the free acid$^{[9]}$ of the respective phthaloyl-protected $\alpha$-aminoxy dipeptide (0.6 mmol, 3.0 equiv), BOP (265.4 mg, 0.6 mmol, 3.0 equiv), and HOBT (81.1 mg, 0.6 mmol, 3.0 equiv) in DMF (5 mL) was agitated for 1 min, NEM (101 µL, 0.8 mmol, 4.0 equiv) was added, and this solution was then added to the resin. The amide coupling was performed for 24 h at room temperature. Afterwards, the resin was sequentially washed with DMF, CH$_2$Cl$_2$, and DMF. Then, the phthaloyl group was removed by treatment with 5% hydrazine monohydrate in methanol for 15 min (2 x) and the resin was sequentilly washed with DMF, MeOH, CH$_2$Cl$_2$, and DMF. After the assembly of the $\alpha$-aminoxy hexamer or decamer by iterative cycles of the phthaloyl deprotection, the amide coupling reaction, and several washing cycles (final washing with CH$_2$Cl$_2$), the crude product was cleaved from the support with TFA/TES (98:2, v/v, 5 mL) for 1.5 h. The filtrate was concentrated in a stream of nitrogen to a volume of smaller than 1 mL. The crude product was precipitated with cold diethyl ether, centrifuged, and the diethyl ether was discarded. This procedure was repeated twice to obtain the crude $\alpha$-aminoxy peptide. For the semipreparative purification, the crude $\alpha$-aminoxy peptides were redissolved in acetonitrile and purified on a Macherey-Nagel Nucleosil C8 RP-HPLC column (VP 250/10 Nucleosil 100–5 C8ec) at a flow rate of 4 mL min$^{-1}$. Fractions containing only the desired $\alpha$-aminoxy peptide were collected and lyophilized from the HPLC solvents, yielding the purified $\alpha$-aminoxy peptides (>95% purity in all cases, for details see Table 2 and the Supporting Information).

**Biological evaluation**

**Cell lines and cell culture:** The human esophagus cell line Kyse510 and human embryonic kidney cells HEK293 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The human ovarian carcinoma cell line A2780 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, United Kingdom). The cisplatin resistant CisR cell lines were generated by exposing the parental cell lines to weekly cycles of cisplatin at an inhibitory concentration of 50% according to Gosepath et al. and Eckstein et al.$^{[16,19]}$. The cell lines were grown at 37°C under humidified air supplemented with 5% CO$_2$ in RPMI 1640 (A2780, Kyse510) or DMEM (HEK293) media containing 10% fetal calf serum, penicillin (120 IU mL$^{-1}$), and streptomycin (120 µg mL$^{-1}$).

**MTT cell viability assay:** MTT assays were performed as previously described.$^{[22]}$ MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Applichem (Darmstadt, Germany). In brief, A2780, Kyse510, and HEK293 cell lines were seeded in 96-well plates. After 24 h, the cells were exposed to increased concentrations of the test compounds. Incubation was ended after additional 72 h, and the cell survival was determined by adding the MTT solution (5 mg mL$^{-1}$ in PBS buffer). The formazan precipitate was dissolved in DMSO and the absorbance was measured at 544 and 690 nm in a FLUOstar microplate reader (BMG LabTech, Offenburg, Germany).

**Analysis of the cell membrane integrity and apoptosis:** To measure the membrane integrity, Kyse510 cells were grown in 6-well plates and treated with the indicated concentrations of the compounds for 24 h. Treatment with 0.2% Triton X-100 for 20 min served as a positive control. After harvesting and washing with PBS, the cells were stained with 20 µg mL$^{-1}$ propidium iodide (PI, Santa Cruz Biotechnology, Heidelberg, Germany) dissolved in 0.9% NaCl for 15 min in the dark at 37°C. Then, the cells were analyzed by flow cytometry (Partec, Münster, Germany). To determine apoptosis, Kyse510 cells were treated in 24-well plates with the indicated concentrations of the compounds for 24 h. 5% DMSO was used as a positive control. After incubation, the cells were lysed over-night in hypotonic staining buffer (0.1% Triton X-100, 0.1% sodium citrate in sterile filtered water) containing 100 µg mL$^{-1}$ PI and analyzed by flow cytometry.

**Data analysis:** Concentration–effect curves were obtained using Prism 4.0 from GraphPad (San Diego, CA, USA) by fitting the pooled data to the four-parameter logistic equation. Flow cytometry data were analyzed using FlowMax 2.82 (Partec, Münster, Germany).

**Crystal structure analysis**

Long rod shaped crystals of the hexamer 1b with an average size of 15 × 15 × 600 µm$^3$ were obtained after one day from acetoniitrile/water (60:40). After overlaying the crystal containing the mother liquid with mineral oil for cryoprotection, the crystals were harvested with lithium loops and flash frozen in liquid nitrogen. A dataset of the hexamer 1b was collected at 100 K at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) on beamline ID30A-3. According to the instrumentation at the beamline, the
resolution was limited to 1.02 Å in the corners of the detector. On the other hand, each hydrogen atom of the structure could be located from the ΔF map. The A alerts in the check cif procedure are solely caused by the low resolution of the experiment at the synchrotron. Crystallographic data: space group P2\(_1\)2\(_1\)2\(_1\), \(a = 5.1627(1)\) Å, \(b = 23.5630(5)\) Å, \(c = 41.5043(10)\) Å, \(24874 \) measured reflections, synchrotron radiation (\(λ = 0.9775\) Å), 3489 unique refinements used for the refinement, \(wR(β) = 0.0836\), \(R(β) = 0.0359\), CCDC 1420774 contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

**Computational methods**

The polyalanine reference α-helix and 3\(_\beta\)-helix were generated using the Maestro suite of programs.\([11]\) Therefore, the backbone dihedrals φ (\(q\)) and psi (\(\psi\)) were set to \(q = -48.0°\) and \(\psi = -57.0°\) for the α-helix and to \(q = -74.0°\) and \(\psi = -4.0°\) for the 3\(_\beta\)-helix.\([21]\) Superimposition of the crystal structure of 1b on C\(_\alpha\) and C\(_\beta\) atoms of the reference structure was performed manually using the pair-fitting option in PyMOL.\([22]\) The average angle deviation (\(q\)) of the respective C\(_\alpha\)–C\(_\beta\) bond vectors was calculated using vector_angle.py by Robert L. Campbell with PyMOL. Details regarding the MD simulations of 1b in chloroform are given in the Supporting Information.

**CD analysis**

**CD experiments:** Circular dichroism (CD) spectra of the α-aminooxy oligopeptides were recorded on a J-180 spectropolarimeter (Jasco, Tokyo, Japan) equipped with a PFD-425S Peltier temperature cell (Jasco) at 20 °C. Solutions of 1b (100 μM), 1f (123 μM), 1g (57 μM), 1h (41 μM), and 1i (38 μM) in TFE (2,2,2-trifluoroethanol) were placed in a 1 mm quartz cell (Hellma, Mullheim, Germany). The initial concentration for the pH titration of 1g was 50 μM in sodium hydroxide solution (pH 11.24). Lower pH values were established by addition of 100 mM hydrochloric acid solution, except for the pH range from 8–3.5, in which 10 mM hydrochloric acid was used, and determined with a pH meter (Metrohm, Herisau, Switzerland). CD spectra were recorded subsequently at pH 11.24, 11.00, 10.42, 10.00, 9.48, 9.01, 8.23, 8.02, 7.48, 6.99, 6.51, 6.51, 5.95, 5.48, 4.96, 4.49, 4.00, 3.52, 3.02, and 2.51 in a 2 mm quartz cell (Hellma). For the liposome-binding analysis we prepared liposomes with an average diameter of 80 nm according to a method described previously.\([23]\) Slow evaporation of the solvent from a chloroform solution of equimolar amounts of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) under nitrogen led to a thin lipid film that was redispersed in water. The aqueous lipid dispersion was extruded 21 times through an 80 nm Nucleopore polycarbonate membrane (Whatman, Dassel, GE, USA) with a LiposFast extruder (Avestin, Ottawa, Canada). CD spectra of the solutions of 1f (142 μM), 1g (56 μM), 1h (46 μM), and 1i (33 μM) in ammonium chloride buffer (pH 9) and of 6:1 (v/v) mixtures of the α-aminooxy peptide solutions and a suspension of POPC/POPS liposomes in ammonium chloride buffer (1.64 μM in respect to POPC) were recorded in a 1 mm cell. All spectra were corrected for the solvent background (TFE, water, ammonium chloride buffer, liposome suspension in ammonium chloride buffer). Spectra recorded in aqueous solvents are shown after smoothing by Fourier filtering (resolution: 4 nm).

**Fitting analyses:** Prior to the matrix-least-squares (MLS) Henderson–Hasselbalch global fitting of the pH-dependent CD spectra of 1g, all spectra were normalized with respect to their corresponding UV/Vis absorbance spectra to correct for concentration errors arising from the addition of the acid solution and from the pH-dependent solubility. The MLS procedure was performed in MATLAB (MathWorks, Natick, MA, USA) in analogy to what has been described elsewhere.\([18]\) In brief, the pH-dependent data set was reduced by singular-value decomposition to obtain a matrix in which common spectral features throughout all pH values were clustered together, and another matrix containing the coefficients with which the features in the first matrix contribute to each observed spectrum. The pK value for the transition from the disordered to the 2\(_\beta\)-helical conformation was determined by least-squares fitting of the coefficient matrix with an algebraic equivalent of the Henderson–Hasselbalch equation. The pure spectra of the conformational species were obtained after matrix inversion and mathematical transformation as described elsewhere.\([23]\) The semi-quantitative analysis of the CD spectra of the α-aminooxy oligopeptides 1f–i in the absence and in the presence of liposomes was done by least-squares fitting of the pure spectra from the MLS global fitting and an average of the (normalized) spectra recorded between pH 6.0–2.5 in the spectral range from 200–204 nm and from 223–300 nm in MATLAB. The relative proportions given in Figure 6 represent the linear coefficients from the spectral fitting normalized to 100 %. Coefficients associated with the low pH species from the MLS global fitting and with the spectra recorded at pH values smaller than 6 were combined and attributed to the 2\(_\beta\)-helical structure.

**Quantum chemical calculation:** A single-point energy calculation of the crystal structure of 1b was performed at the density functional theory (DFT) level in Gaussian 09\([25]\) using the 6-31G* functional and TZVPP\([26]\) (TZVPP basis with discarded f-functions and TZVP basis on hydrogen atoms) as a basis set. CD and absorbance intensities (velocity representation) were calculated at the simplified time-dependent DFT (stD-DFT) level with the stDFA program by Grimme and co-workers.\([18]\) The spectra shown in Figure 3 were constructed with a Gaussian line shape with a half width at the half maximum of 0.3 eV and an energy shift of −0.6 eV.

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