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Uniformly Nucleobase-Functionalized β-Peptide Helices: Watson–Crick Pairing or Nonspecific Aggregation

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The organization and architecture of helices is fundamental in folding of protein tertiary structures. Therefore, stable β-peptide helices are used as models for the selective organization of secondary structures. Nucleobases are already established as recognition elements to organize two β-peptide helices in antiparallel orientation. The investigation of β-peptide helices uniformly functionalized with one type of nucleobases provided further insight in the recognition mode and requirements for specific interaction within the linear and very rigid helical backbone topology. Specific helix interaction based on base pair recognition is predominant as soon as Watson–Crick pairing is allowed. If the hydrogen bonding donor/acceptor pattern prohibits the Watson–Crick geometry, a quite stable nonspecific interaction was found based on aromatic interactions or on a nonspecific hydrogen bonding network. The latter aggregation was also confirmed with tyrosine side chains.

Introduction

The interest in peptide secondary structures is mainly due to their essential role in protein folding, tertiary structure organization, and function as recognition element in protein binding sites, between proteins or in the interaction of proteins with oligonucleotides.[1] α-Helices, β-sheets, loops, and turns can be stabilized within folded proteins. Nevertheless, for the investigation of stable isolated secondary structures about 15 amino acids are required for an α-peptide with significant helix propensity.[2] In β-peptides the stability of helices is significantly enhanced since only six amino acids are sufficient to obtain stable helices. Furthermore, β-peptide secondary structures can be designed with respect to the α,β-side chain pattern and respective configuration.[3,4] β-Peptide helices are resistant against enzymatic degradation[5] and can be assigned in radius, helical sense, side chain orientation, and overall dipole. They provide a powerful tool for molecular architecture and as mimic for α-peptide helices investigating recognition, structural stabilization, and folding.

The 14-helix is one of the most prominent β-peptide secondary structures derived from β^3-amino acids with lateral side chain configuration. Three amino acids form one turn, thus orienting every third side chain (*i* and *i* + 3) uniformly along one side of the helix.[6] The helix propensity can be further improved by incorporation of conformationally constrained cyclic amino acids like *trans*-(1*R*,2*R*)-2-aminocyclohexanecarboxylic acid (ACHC).[7] An amphiphilic helix design has been shown to be useful for the organization of β-peptides.[8]

In an alternative approach every third amino acid side chain is functionalized with recognition units like nucleobases providing a linear backbone topology with a uniformly oriented hydrogen bonding pattern (Figure 1).[9] Previously, it was established that recognition of three or four nucleobases is already sufficient for a defined complementarity and formation of antiparallel duplexes with extraordinary stability.[10] With this respect, base pairing is decisive for complex formation. Duplex stability can be correlated to the helix propensity and, therefore, to preorganization of the nucleobases by the rigid β-peptide 14-helix. Establishing this specific duplex formation, so far the use of an unambiguous nucleobase sequence was intended to avoid an experimental setup with several pairing possibilities. Therefore, the 14-helical recognition side was functionalized with two or even four different kinds of nucleobases ensuring Watson–Crick pairing geometry throughout the whole sequence as most likely possibility for all base pairs.

From other DNA and PNA oligomers with linear backbone topology it is evident that oligomers with a uniform nucleobase sequence are generally open for all pairing geometries like Watson–Crick, Hoogsteen, the respective reverse pairing modes, and in addition simultaneous pairing

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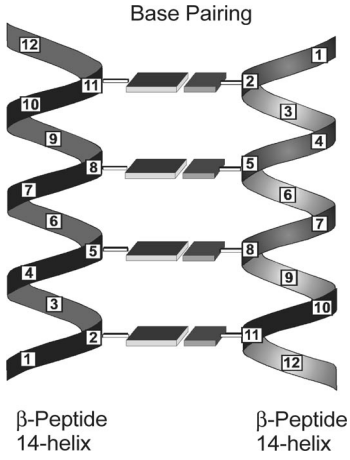
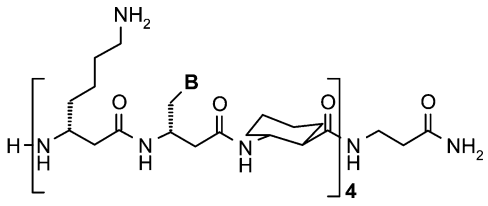


Figure 1. Model of antiparallel base pairing β-peptide helices uniformly functionalized with nucleobases in every third position.

over the Watson–Crick and the Hoogsteen site.[11] In order to fully evaluate the consequences of the linear backbone topology in β-peptide 14-helices on helix recognition, 14-helices were synthesized with four identical nucleobases covalently attached to one side of the helix as β^3-amino acid side chains. Self-aggregation and interaction between these uniformly functionalized helices were investigated in order to evaluate the recognition potential of nucleobases within the well preorganized β-peptide topology.

Oligomers **1**–**4** were synthesized (Figure 2) containing four identical nucleobases on one flank of the 14-helix. Therefore, the design of the β-peptide oligomer required a β-nucleo amino acid in every third position. The remaining positions of a turn were filled with homolysine providing positive charges for solubility under physiological conditions and ACHC for increased helix propensity. Oligomers that allow specific recognition by Watson–Crick pairing geometry were identified by UV and CD spectroscopy. Also β-peptides were found to aggregate based on nonspecific aromatic interactions or hydrogen bonding networks. The nonspecific interaction of nucleobase substituted 14-helices



1: AAAA
2: GGGG
3: CCCC
4: TTTT
5: YYYY

B = nucleobase (A, C, T, G) or phenol side chain (Y)

Figure 2. β-Peptides **1**–**5** uniformly functionalized with nucleobases (A, G, C, T) or with a tyrosine (Y) side chain.

was confirmed by the investigation of the homotyrosine containing β-peptide **5** that provided comparable aggregation phenomena.

Results and Discussion

Synthesis of the β-peptide oligomers was provided as solid phase peptide synthesis at 50 °C on a 4-methylbenzhydrylamine-polystyrene (MBHA) resin preloaded with homoglycine, using 1-[bis(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine 3-oxide hexafluorophosphate (HATU) for activation of the respective Boc-protected nucleo β-amino acid.[12] Purity and constitutional integrity of the oligomers were proven by HPLC and high resolution mass spectrometry. Double-strand formation and aggregation of oligomers **1**–**5** were investigated by temperature-dependent UV spectroscopy. As known from oligonucleotides, PNAs, and previous studies of nucleobase substituted β-peptide 14-helices the separation of base-pair-mediated double strands can be detected as an increase of absorption resulting in sigmoidal shaped curves.[10] A self-pairing β-peptide oligomer with mixed A–T sequence forms duplexes based on specific base pair recognition. An A–T pairing β-peptide oligomer with the general constitution shown in Figure 2 has a remarkable duplex stability of T_m = 37 °C indicated by a sigmoidal hyperchromicity curve (H = 15%).[10] CD spectroscopy serves to indicate the helical secondary structure and orientation of nucleobases with respect to the backbone when involved in base pairing. Based on UV and CD spectroscopy, first the self-aggregation of oligomers **1**–**4** was investigated followed by the respective 1:1 combination of these β-peptides.

Adenine–Adenine Interaction

In general, within linear backbone topologies the A-A base pairing can be realized in the Hoogsteen, reverse Hoogsteen, or reverse Watson–Crick mode forming two hydrogen bonds each. The temperature-dependent UV curve obtained for oligomer **1** was quite surprising showing a sigmoidal decrease of absorption (hypochromicity 5%) with a remarkable transition of T_m = 55 °C (Figure 3) unlikely to result from duplex formation based on A-A base pairing. Hypochromicity effects[13] are known for the formation of higher aggregates like guanine tetrads.[14] Considering the helix is only functionalized by four nucleobases the transition also points to higher aggregating complexes. Intermolecular aggregation is supported by a concentration dependency of the transitions (T_m = 53 °C with 4 μM **1**, T_m = 55 °C with 8 μM **1**, and $T_m > 70$ °C with 16 μM **1**). Formation of a right handed conformation of a β-peptide 14-helix was indicated by a maximum in the CD spectra at 215 nm.[1b,15] The intensity of the Cotton effect around 270 nm usually indicating a preferred conformational orientation of the nucleobases is rather low in the case of oligomer **1** (Figure 3). Base pairing seems not to be involved in aggregate formation.

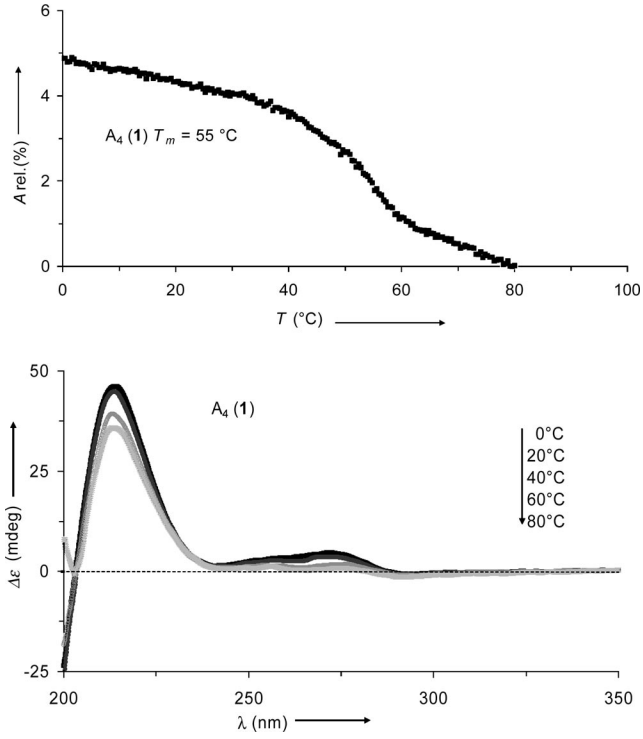


Figure 3. Temperature-dependent UV curve and CD spectra of self-aggregating adeninyl β-peptide **1** (8 μM, 10 mM Tris·HCl buffer, pH 7.5).

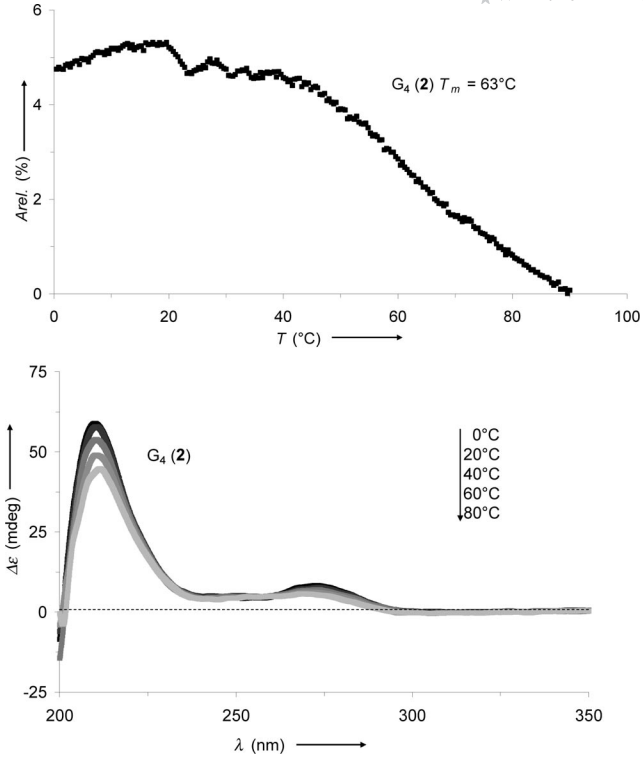


Figure 4. Temperature-dependent UV curve and CD spectra of self-aggregating guaninyl β-peptide **2** (8 μM, 10 mM Tris·HCl buffer, pH 7.5).

Guanine–Guanine Interaction

The results obtained for the self-aggregation of guaninyl β-peptide **2** turned out to be essentially similar to the self-association of the adenine oligomer **1** (Figure 4): temperature-dependent measurements of the UV absorption also lead to a hypochromicity effect (5%), however, with slightly higher transition of T_m = 63 °C and a less pronounced sigmoidal shape of the melting curve (Figure 4). CD spectra measured at various temperatures clearly indicate the 14-helical secondary structure and a Cotton effect with low intensity in the nucleobase absorption region. Also for the guaninyl sequence **2** a higher aggregate seems to be preferred over specific double strand formation. Aggregation in a G-quadruplex or band like structures are known motifs especially for guanine containing oligomers.[14] Indeed, a β-peptide 14-helix with the sequence TGGG (T_m = 55 °C, 11% hypochromicity)[16] provided UV melting curves with a shape and stability (see Supporting Information) that correlates well with the tetrad formation of aminoethylglycine PNA described in literature.[17] Tetrad formation or band like oligomerization are possible aggregation modes for oligomer **2**, even though we have not been able to detect higher aggregates than duplexes for the guaninyl β-peptide **2** by ESI mass spectrometry.

Tyrosine–Tyrosine Interaction

Experimental evidence for nonspecific aromatic interactions or hydrogen bonding networks that are not based on base pair recognition came from self-aggregating adeninyl and guaninyl β-peptides. The β-peptide helix **5** containing tyrosine side chains instead of the purines was investigated as a control oligomer for comparison with nucleobase containing oligomers. The homotyrosine β-peptide helix provides aromaticity, a similar polarity, and a lacking donor/acceptor pattern for specific hydrogen-bond recognition. UV data also indicated a hypochromicity curve (4%) with a T_m = 51 °C (Figure 5). Therefore, aggregation of uniformly substituted β-peptide helices is also possible due to aromatic interactions or a nonspecific hydrogen bonding network. The latter indeed has been identified in a crystal structure of α-tyrosine helices with alternating configura-

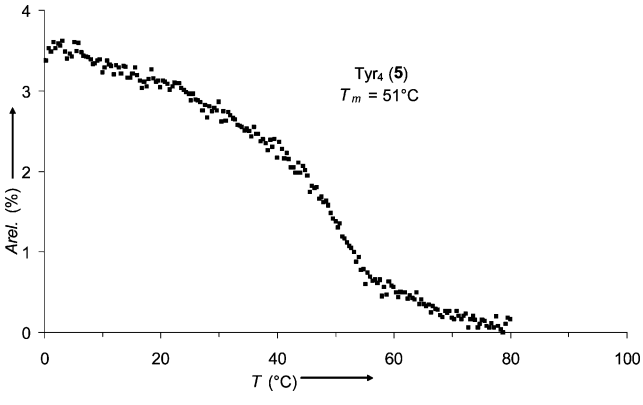


Figure 5. UV melting curve of oligomer **5** (8 μmol, 10 mM Tris·HCl buffer at pH 7.5).

tion.[18] It should be stated, that the aggregation found for tyrosine oligomer **5** does not exclude quadruplex formation in case of β-peptides **1** and **2**. Further, the aggregation phenomena are particular for uniformly substituted 14-helices. As soon as mixed nucleobase sequences are predominant, clear base pair complementarity is provided leading to defined duplex formation.[10]

Cytosine–Cytosine Interaction

The aggregation observed for cytosine β-peptide **3** also turned out to be similar to the complex formation of purinyl oligomers **1** and **2** (Figure 6). The temperature-dependent UV spectrum of oligomer **3** in 10 mM Tris·HCl buffer (pH 7.5) gave an inverted sigmoidal curve with a transition of T_m = 50 °C (6% hypochromicity). CD spectra of **3** measured at various temperatures also showed a strong positive Cotton effect at 215 nm indicating the 14-helix and a Cotton effect at 270 nm with low intensity at lower temperatures. This result is very much reflecting the conclusion drawn for oligomers **1**, **2** and **5** even cytosine has the potential for specific base pairing with another cytosine nucleobase in the reverse Watson–Crick mode with two hydrogen bonds (Figure 7).

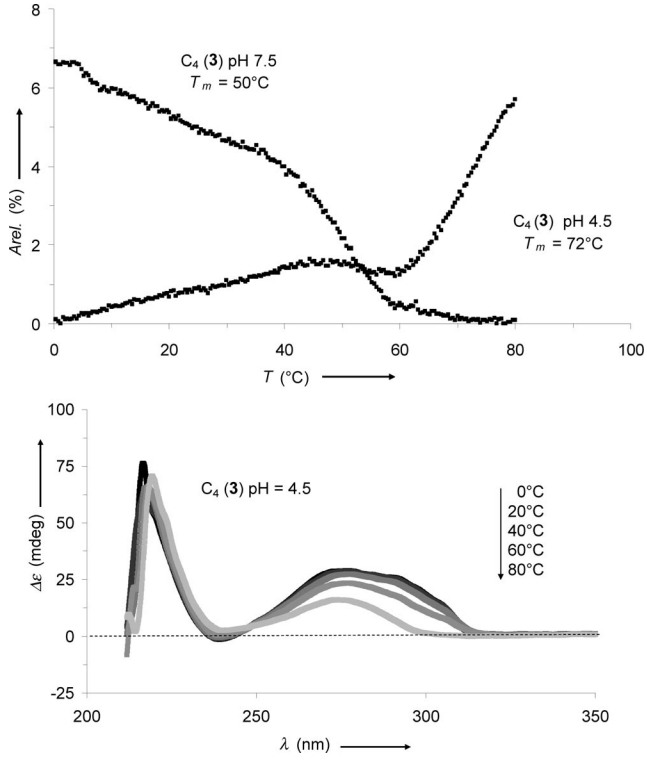


Figure 6. UV and CD spectra of cytosinyl peptide **3** (8 μmol, 10 mM Tris·HCl buffer, pH 7.5 or 100 mmol NaCl, 80 mmol Tris acetate buffer at pH 4.5).

At pH 4.5 half the cytosine nucleobases are protonated allowing the formation of charged base pairs with three hydrogen bonds in the reverse Watson–Crick mode (Figure 7). This recognition under slightly acidic conditions is decisive for oligonucleotides in the i-motif, a tetrad based on interpenetrating, intercalating, C–C$^+$-pairing double strands.[19] Also alanyl-PNA oligomers can be aggregated by C–C$^+$-pairing indicating that oligomers with linear backbone topology are in a position to form an i-motif at pH 4.5.[20] In case of the β-peptide 14-helices it was not expected to obtain an i-motif analogue even at pH 4.5 since the distance of 5 Å for neighbouring nucleobases is too short to allow interpenetration of base pairs. Nevertheless, also a double strand based on C–C$^+$-pairing should be significantly stabilized. Next to the reverse Watson–Crick mode with three hydrogen bonds also a C–C$^+$ Watson–Crick pairing is possible as it seems to be required for the specific interaction of β-peptide helices. Especially, dipole interactions of charged stacking base pairs should contribute to the overall double strand stability. Indeed, UV melting curves obtained with oligomer **3** at pH 4.5 provided a sigmoidal increase of the absorption (6% hyperchromicity) with a transition of T_m = 72 °C (Figure 6). This result is likely to be based on specific C–C$^+$ duplex formation. CD spectra of β-peptide **3** measured at various temperatures support the UV data. Next to a strong Cotton effect indicating the 14-helical conformation the organization of nucleobases up to 60 °C is indicated between 250 and 310 nm (Figure 6).

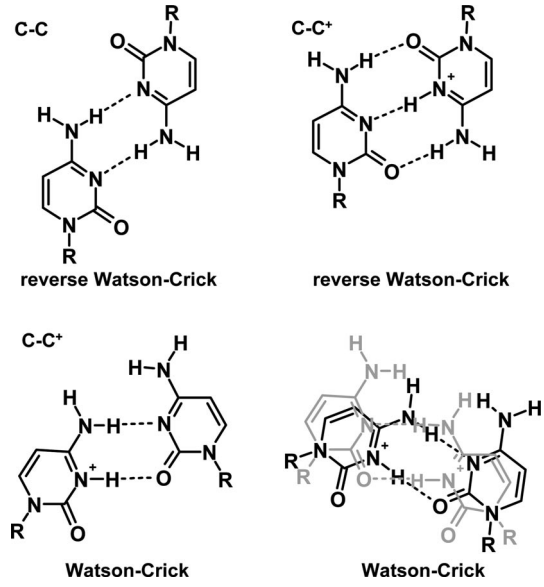


Figure 7. C–C base pairing: semi-protonation at pH 4.5 allows formation of a tridentate reverse Watson Crick mode next to a twodentate Watson–Crick base pair.

Duplex formation of C–C$^+$-pairing β-peptide helices was also indicated comparing ESI-MS experiments at neutral and acidic pH (see Supporting Information).[21] In both cases dimer formation was observed.

Thymine–Thymine Interaction

Whereas the β-peptide oligomers uniformly substituted with adenine, guanine or cytosine provided nonspecific aggregation based on aromatic interactions or hydrogen bonding networks, a completely different recognition pref-

erence was observed for the 14-helix **4** with the sequence TTTT. The temperature-dependent UV spectrum measured with β-peptide **4** under the same conditions as used for oligomers **1**–**3** (8 μM) provided a melting curve with a very low hyperchromicity. This might result from a double strand with low transition ($T_m < 0$ °C). Increasing the β-peptide concentration to 16 μM formation of a pairing complex seems likely with a transition of $T_m = 22$ °C and a hyperchromicity of 6% (Figure 8). For specific recognition between thymine nucleobases two different Watson–Crick and two reverse Watson–Crick modes are possible. CD measurements were in agreement with the UV results. The helical content represented by the Cotton effect at 215 nm was dependent on the oligomer concentration. Especially, the signal between 250 and 300 nm clearly indicated a defined nucleobase orientation as usual for base paired duplexes. Also the duplex stability deduced from UV spectroscopy was verified by CD spectroscopy at different temperatures.

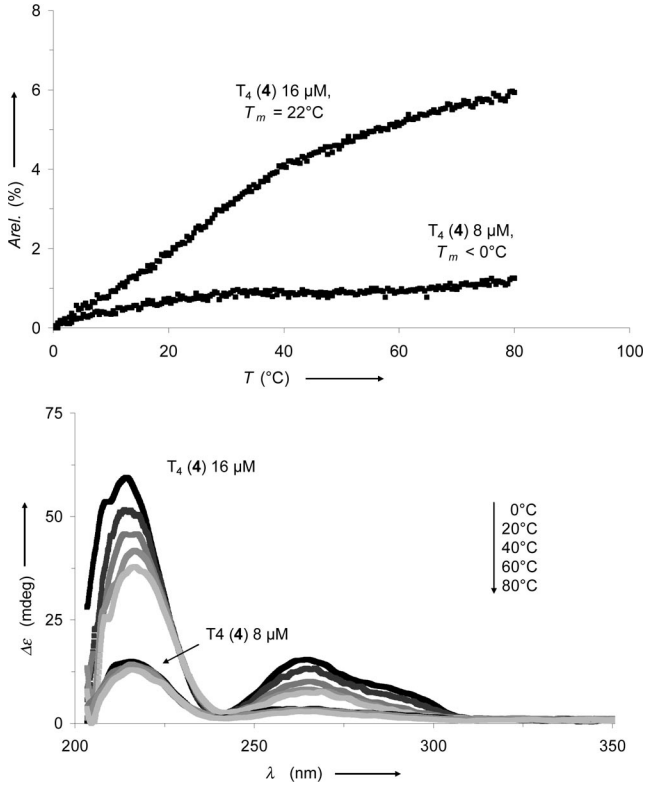


Figure 8. Temperature-dependent UV melting curve and CD spectra of self-aggregating thyminyl β-peptide **4** (8 μM and 16 μM, 10 mM Tris·HCl buffer, pH 7.5).

With the thymine oligomer **4** and the C–C$^+$-pairing cytosine oligomer **3** only two of the uniformly nucleobase substituted β-peptide oligomers provide hyperchromicity derived from base pair recognition whereas self-aggregation of the adeninyl (**1**), guaninyl (**2**), and cytosinyl (**3** at neutral pH) oligomers lead to nonspecific interactions. A plausible explanation might be that the Watson–Crick pairing mode is required for base pair recognition in the β-peptide 14-helix series. This pairing mode is possible for oligomers **3** (at pH 4.5) and **4** but cannot be formed for base pairs A-A, G-G or C–C (at pH 7). Nonspecific interaction seems to become relevant if specific Watson–Crick pairing is prohibited. This interpretation is in agreement with our previous results since all the nucleobase mediated interactions between β-peptide helices investigated so far rely on specific Watson–Crick base pair recognition.[10]

Adenine–Thymine Interaction

Next to self-aggregation of uniformly nucleobase substituted β-peptide helices the recognition of heterodimers was investigated. If double strand formation in the Watson–Crick mode is possible, specific pairing should compete with nonspecific aggregation. First, A–T pairing was investigated considering an equimolar mixture of oligomers **1** and **4** (Figure 9). As expected, double strand formation was indicated by a hyperchromicity curve (7%) and a transition of $T_m = 61$ °C. Specific recognition of both oligomers is supported by a cooperative increase of absorption and a higher stability of the equimolar mixture compared to the values obtained for self-aggregation of β-peptides **1** (T_m = 55 °C) and **4** ($T_m < 0$ °C). Heterodimer formation is also supported by CD spectroscopy showing a strong Cotton effect in the absorption range of the nucleobases. Comparing the 1:1 complex of oligomers **1** and **4** with previously studied self-pairing of the corresponding β-peptide with the sequence TATA ($T_m = 37$ °C, 8 μM)[10] the double strand formed by an equimolar mixture of the uniformly substi-

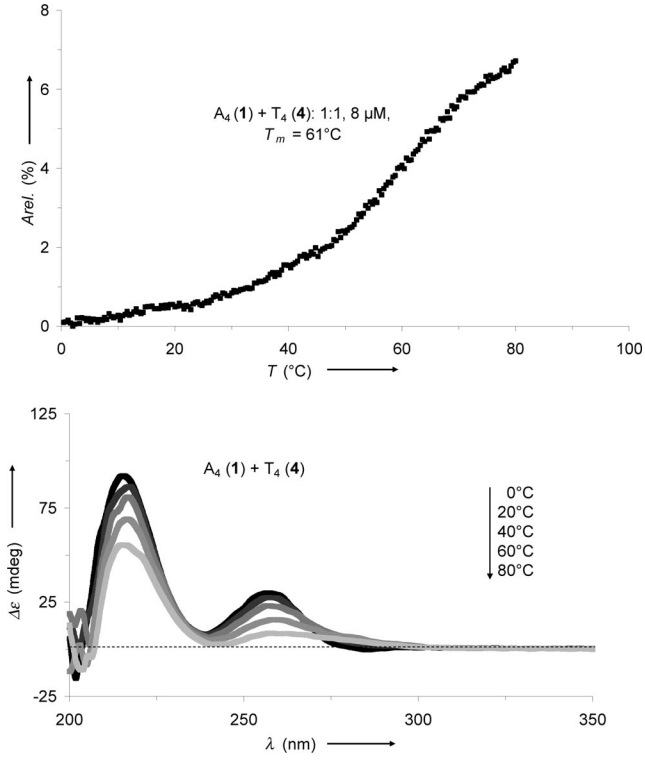


Figure 9. UV melting curve and CD spectra of an equimolar mixture of oligomers **1** and **4** (4 μM each, 10 mM Tris·HCl buffer, pH 7.5).

tuted oligomers provides a significantly higher stability. Even additional aggregation cannot be excluded from UV spectroscopy, the CD spectra of the equimolar mixture of oligomers **1** and **4** and of self-pairing oligomer TATA are very much alike (Figure 9 and ref.[10]) indicating a well defined A–T duplex in both cases. Duplex formation was further supported by ESI mass spectrometry (see Supporting Information).

Thymine–Cytosine and Thymine–Guanine Interaction

In general, base pairing in the Watson–Crick mode is also possible for T–C and T–G recognition. Temperature-dependent UV spectra (Figure 10) provided double strand formation with low stabilities ($T_m < 0$ °C for the equimolar mixture of oligomers **3** + **4** and for the 1:1 mixture of oligomers **2** + **4**). In both cases the UV spectra showed no hypochromicity and, therefore, no indication for nonspecific interactions. Further support comes from CD spectroscopy of both equimolar mixtures (Figure 11) showing signals for the typical 14-helix next to a significant Cotton effect in the nucleobase absorption region of 250–300 nm. The results obtained for T–C and T–G nicely reflect our conclusion from the self-aggregation of uniformly substituted β-peptide helices that Watson–Crick base pairing is formed prior to nonspecific interactions. In general, melting curves obtained for base pairing oligomers in the β-peptide series showed hyperchromicity with complete reversibility, whereas for aggregation indicated by hypochromicity a significant hysteresis was found (see Supporting Information) suggesting that aggregate formation is kinetically hampered.

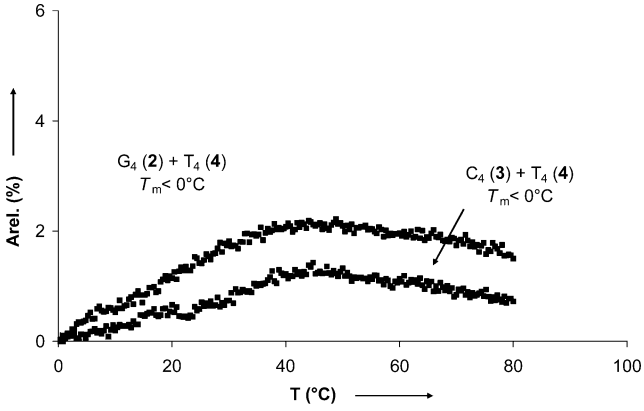


Figure 10. UV melting curves of equimolar mixtures of oligomers **3**+**4** and **2**+**4** (4 μM each, 10 mM Tris·HCl buffer, pH 7.5).

Adenine–Cytosine Interaction

In general, base pairing between adenine and cytosine can be expected in the reverse Watson–Crick and the Hoogsteen mode. Nevertheless, the temperature-depended UV curve and the CD spectra of the equimolar mixture of oligomers **1** and **3** (Figure 12) provided similar results as

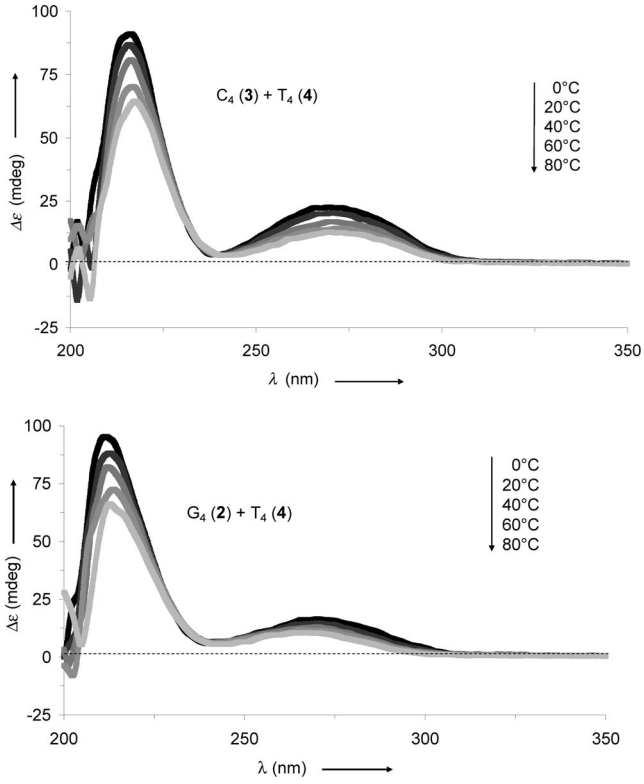


Figure 11. CD spectra of equimolar mixtures of β-peptides **3**+**4** and **2**+**4** (4 μM each, 10 mM Tris·HCl buffer, pH 7.5).

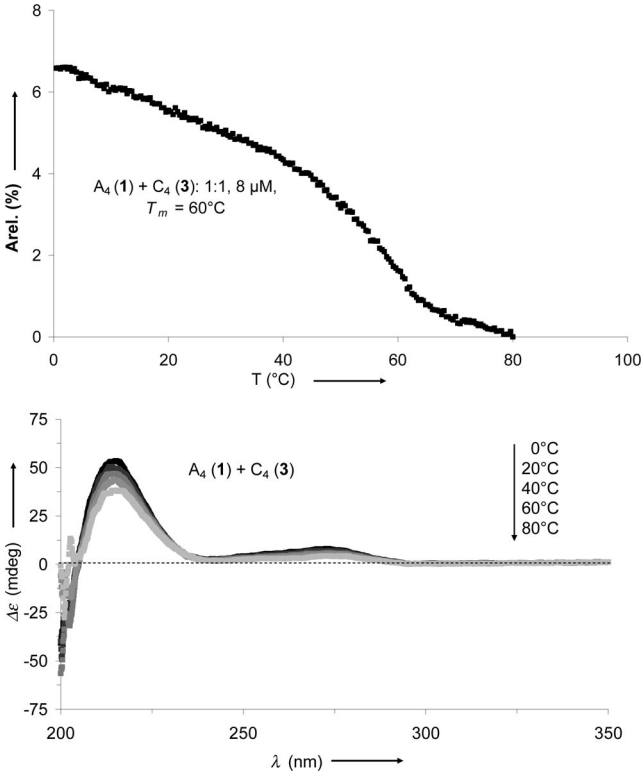


Figure 12. UV melting curve and CD spectra of an equimolar mixture of oligomers **1** and **3** (4 μM each, 10 mM Tris·HCl buffer, pH 7.5).

the self-aggregating β-peptide helices. The intensity of the Cotton effect in the absorption area of nucleobases is low and the aggregate is based on a hypochromicity effect (5%) with a transition of T_m = 60 °C. Solely, the higher stability of the equimolar mixture of oligomers compared to the complex of individual helices points to an interaction between these β-peptides that is of nonspecific nature not involving base pair recognition.

Guanine–Cytosine and Guanine–Adenine Interaction

The interpretation of the experimental results derived from equimolar mixtures of β-peptide helices involving the guaninyl 14-helix **2** is more uncertain. This might be due to the possibility of guanine for higher aggregation. As previously shown, β-peptides containing guanine provide an extraordinary stabilization.[10] The self-aggregation of the guaninyl β-peptide **2** (T_m = 63 °C) competes with heterodimer formation **1** + **2** (T_m = 53 °C) and **2** + **3** (T_m = 57 °C). There is no indication that heteromeric complexes are formed. The slightly lower stabilities determined for the equimolar mixtures of β-peptides **1** + **2** and **2** + **3** are likely due to the lower concentration of the respective self-aggregating oligomers in the mixture (8 μM vs. 4 μM each). Stabilities obtained for complexes varying the ratio of oligomers **2** and **3** from 2:1 (T_m = 62 °C), 1:1 (T_m = 57 °C), to 1:2 (T_m = 53 °C) support the homomeric aggregation. Recognition between with uniformly substituted guaninyl and cytosinyl 14-helices was not observed even the stability of a GCGC self-pairing β-peptide is quite high ($T_m > 80$ °C).[10] Despite the general possibility of A-G and C-G base pairs for Watson–Crick pairing, both complexes resulted in temperature-dependent UV curves with hypochromicity (Figure 13). From the CD spectra of oligomer mixtures of **1** + **2** and **2** + **3** that are not distinctive in the absorption area of the nucleobases (see Supporting Information) we conclude that these β-peptide aggregates exist in parallel arrangements.

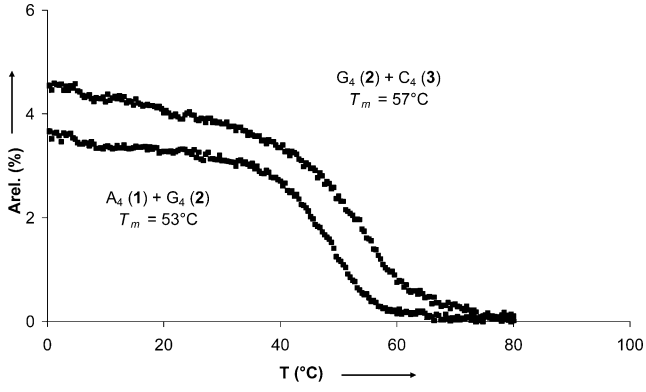


Figure 13. Equimolar mixtures of oligomers **1** + **2** and **2** + **3** (4 μM each, 10 mM Tris·HCl buffer, pH 7.5).

Conclusions

β-Peptide 14-helices were synthesized uniformly functionalized with the canonical nucleobases on one side of the helix. This uniform functionalization provided insight into pairing of oligomers with 14-helical backbone topology. The preference for the Watson–Crick mode for specific recognition of helices became evident, but also a nonspecific mode of interaction with remarkable stability was identified and supported by a β-peptide tyrosinyl-oligomer that provided quite similar aggregates as the nucleobase functionalized oligomers. The specificity for the Watson–Crick pairing mode is remarkable since linear backbone topology should allow all pairing modes that are possible for the respective base pair. The restriction to the Watson–Crick mode is an indication of β-peptide helices differing from the idealized linear backbone topology in order to provide base-pair-specific recognition. The experimental results obtained for all combinations of β-peptide 14-helices uniformly functionalized with all canonical nucleobases are summarized in Table 1. Three cases of interaction can be differentiated: (a) nonspecific interactions were found for the combinations A–A, C–C, G–G, A–C; (b) specific recognition seems accessible in case of the Watson–Crick pairing combinations A–T, T–T, C–C^+, T–C, and T–G (c) the uniformly guanine functionalized oligomer is self-aggregating in a mode that does not allow other oligomers like the cytosinyl or adeninyl β-peptides to compete.

Table 1. Equimolar mixtures of β-peptides **1**–**4** in 10 mM Tris·HCl buffer, pH 7.5, 8 μM oligomer concentration.

	A	**C**	**G**	**T**
A	55 °C	60 °C	53 °C	61 °C
C		50 °C[a]	57 °C	<0 °C
G			63 °C	<0 °C
T				<0 °C[b]

[a] At pH 4.5 C–C^+ pairing is observed providing a transition of T_m = 72 °C. [b] T_m = 22 °C for an oligomer concentration of 16 μM.

Experimental Section

General Remarks: All reagents were of analytical grade and used without further purification. Solvents were of the highest grade available. Dry solvents were stored over molecular sieves (4 Å). (1*R*,2*R*)-Boc-ACHC-OH was prepared as described in literature.[22] Boc-β-HLys(*Z*)-OH and Boc-β-HTyr(Bn)-OH were obtained by Arndt–Eistert homologation of the corresponding α-amino acids. Nucleo-β-amino acids Boc-β-HalA-OH, Boc-β-HalT-OH, Boc-β-HalG-OH, and Boc-β-HalC-OH were synthesized as described in the literature.[10,23] The 4-methylbenzhydrylamine-polystyrene (MBHA-PS) resin was obtained from Novabiochem. HPLC analysis and purification of the oligomers was performed on a Pharmacia Äkta basic (pump type P-900, variable wavelength detector type UV-900) with a linear gradient of A (0.1% TFA in H_2O) to B (MeCN/H_2O, 9:1 + 0.1% TFA). Oligomers were analysed using a YMC J'sphere column ODS-H80, RP-C18; 250 × 4.6 mm, 4 μm, 80 Å with a flow rate of 1 mL min^{-1} and J'sphere column ODS-H80, RP-C18; 150 × 4.6 mm, 4 μm, 80 Å with a flow rate of 1 mL of min^{-1}. For preparative purification YMC J'sphere column ODS-

H80, RP-C18; 250 × 20 mm, 4 μm, 80 Å with a flow rate of 10 mL min^{-1} and J'sphere column ODS-H80, RP-C18; 150 × 10 mm, 4 μm, 80 Å with a flow rate of 3 mL of min^{-1} were used. Mass spectra were recorded with a Finnigan LCQ spectrometer. High-resolution mass spectra were recorded with a Bruker APEX-IV FT-ICR mass spectrometer. CD spectra were recorded with a JASCO J-810 spectrometer equipped with a JASCO ETC-505S/PTC-423S temperature controller. All CD-measurements were carried out in Tris·HCl buffer (pH 7.5) or 100 mmol NaCl, 80 mmol Tris acetate buffer (pH 4.5) in a quartz cell of 1 cm path length. Spectra represent the average of four scans after baseline correction. Temperature-dependent UV spectra were measured with a JASCO V-550 UV/Vis spectrometer equipped with a JASCO ETC-505S/ETC-505T temperature controller. All measurements were carried out in Tris·HCl buffer at pH 7.5 or 100 mmol NaCl, 80 mmol Tris acetate buffer at pH 4.5. The data were collected at 260 nm at a heating rate of 0.5 °C min^{-1} in a quartz cell of 1 cm path length. The oligomer concentrations were determined based on the absorption at 260 nm. The extinction coefficients of the oligomers were calculated as the sum of the extinction coefficients of the contained nucleobases.[24]

General Procedure for Solid-Phase β-Peptide Synthesis: β-Peptide oligomers were synthesized by manual solid-phase peptide synthesis in a small fritted glass column (ø = 1.5 cm). 4-Methylbenzhydrylamine-polystyrene (MBHA-PS) resin was used preloaded with Boc-β-HGly-OH. Oligomers were synthesized on a 16.75 μmol scale using *N*-Boc-β-HGly-MBHA-PS resin (25.0 mg, 0.67 mmol/g loading); peptide coupling was performed at 50 °C. For each coupling reaction an excess of 5.0 equiv. amino acid (83.75 μmol) was used, preactivated with 1-[bis(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine 3-oxide hexafluorophosphate (HATU) (4.5 equiv.), 1-hydroxy-7-azabenzotriazole (HOAt) (5.0 equiv. of a 0.5 M solution in DMF), and DIEA (14 equiv.) in dry DMF (400 μL). After swelling the loaded resin for 2 h in CH_2Cl_2 (2 mL), the following procedure was applied for each coupling step: i. deprotection twice for 3 min with TFA/*m*-cresol (95:5, 2 mL); ii. washing three times with CH_2Cl_2/DMF (1:1, 2 mL), then five times with pyridine (2 mL); iii. coupling steps, 2 h gentle movement at 50 °C; iv. washing with CH_2Cl_2/DMF (1:1, 3 × 2 mL), DMF/piperidine (95:5, 3 × 2 mL), and then CH_2Cl_2/DMF (1:1, 3 × 2 mL); v. capping twice for 3 min with DMF/Ac_2O/DIEA (8:1:1, 2 mL). After the final coupling step the resin was washed with TFA (3 × 2 mL) and CH_2Cl_2 (5 × 2 mL), and dried overnight in vacuo. The resin was transferred into a small glass vessel and suspended in *m*-cresol/thioanisole/ethanedithiol (2:2:1, 500 μL). After stirring for 30 min at room temperature, TFA (2 mL) was added and the mixture cooled to –20 °C. Trifluoromethanesulfonic acid (TFMSA) (200 μL) was added dropwise with vigorous stirring. The mixture was warmed to room temperature over 1.5 h and stirring continued for another 2 h. The mixture was filtered through a fritted glass funnel and TFA was removed under reduced pressure. The crude oligomer was isolated by precipitation from cold diethyl ether (–15 °C) and dried in vacuo. The crude peptide was dissolved in water/CH_3CN, filtered and purified by HPLC. The synthesis of guaninyl oligomer H-(β-HLys-β-HalG-ACHC)$_4$-β-HGly-NH_2 (**2**) has already been described.[10]

H-(β-HLys-β-HalA-ACHC)$_4$-β-HGly)-NH_2 (1): Analytical PR-HPLC: t_R = 20.1 min (gradient: 20–43% B in 30 min). MS (ESI): *m*/*z* (%) = 1015.6 (100) $[M + 2H]^{2+}$. HRMS (ESI): calcd. for $C_{95}H_{148}N_{38}O_{13}$: 1015.61168; found 1015.41001 $[M + 2H]^{2+}$.

H-(β-HLys-β-HalC-ACHC)$_4$-β-HGly)-NH_2 (3): Analytical PR-HPLC: t_R = 20.2 min (gradient: 15–50% B in 30 min). MS (ESI): *m*/*z* (%) = 967.6 (100) $[M + 2H]^{2+}$. HRMS (ESI): calcd. for $C_{91}H_{148}N_{30}O_{17}$: 967.58921; found 967.58894 $[M + 2H]^{2+}$.

H-(β-HLys-β-HalT-ACHC)$_4$-β-HGly)-NH_2 (4): Analytical PR-HPLC: t_R = 18.9 min (gradient: 23–55% B in 30 min). MS (ESI): *m*/*z* (%) = 997.6 (100) $[M + 2H]^{2+}$. HRMS (ESI): calcd. for $C_{95}H_{152}N_{26}O_{21}$: 997.58854; found 997.58811 $[M + 2H]^{2+}$.

H-(β-HLys-β-HalY-ACHC)$_4$-β-HGly)-NH_2 (5): Analytical PR-HPLC: t_R = 19.3 min (gradient: 45–90% B in 30 min). MS (ESI): *m*/*z* (%) = 933.8 (100) $[M + 2H]^{2+}$. HRMS (ESI): calcd. for $C_{99}H_{152}N_{18}O_{17}$: 622.72670; found 622.72678 $[M + 3H]^{3+}$.

Supporting Information (see also the footnote on the first page of this article): CD spectra of oligomers **1**, **5**, **1**+**2**, and **2**+**3**. UV and CD spectra of the β-peptide with sequence TGGG. HR mass spectra of all oligomers; Mass spectra of self-aggregating oligomers **1**, **2**, **3** and of dimer complex of **1**+**4**.

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