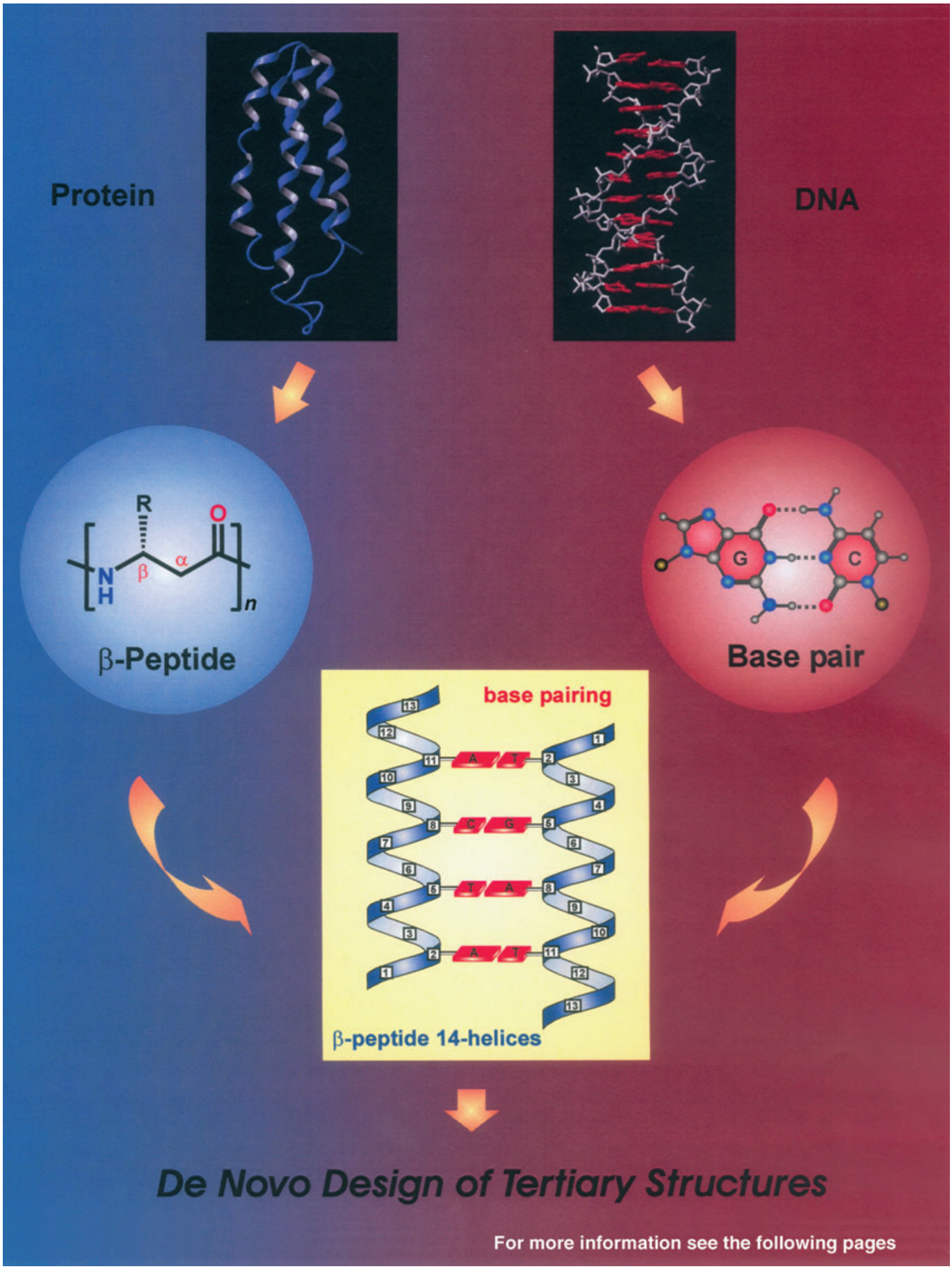

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Three-Dimensional Organization of Helices: Design Principles for Nucleobase-Functionalized β-Peptides

Pradip Chakraborty and Ulf Diederichsen*[a]

Dedicated to Professor Horst Kessler on the occasion of his 65th birthday

Abstract: The construction and molecular recognition of various three-dimensional biomimetic structures is based on the predictable de novo design of artificial molecules. In this regard β-peptides are especially interesting, since stable secondary structures are obtained already with short sequences; one of them is the 14-helix in which every third residue has the same orientation. The covalent functionalization of every third 14-helix side chain with nucleobases was used for a reversible organization of two helices based on nucleobase pairing. A series of β-peptides with various nucleobase sequences was synthesized and the stability of double strand formation was investigated. As few as four nucleobases are sufficient for considerable duplex stability. The stability of base pairing was examined by temperature-dependent UV spectroscopy and the formation of the 14-helix was confirmed by circular dichroism (CD) spectroscopy. The preferred strand orientation of complementary-nucleobase-modified β-peptide helices was investigated as well as the influence of helix content on the duplex stability. The preorganization of a 14-helix in regard to double-strand recognition was tuned by the sequential order of polar β-amino acids or by the amount of 2-aminocyclohexanecarboxylic acid units incorporated, which are known to facilitate 14-helix formation, respectively.

Keywords: base pairing · helical structures · molecular recognition · peptides · self-assembly

Introduction

Protein folding into well-defined three-dimensional structures is essential for various biological processes in living organisms.[1] In this regard, the spatial organization of secondary structures is especially interesting, since it is decisive for the respective functionality. The knowledge of biological activities, functions and mechanisms on the molecular level is often limited partially due to the complexity of protein structures. Therefore, de novo protein design provides an opportunity to investigate the structure–activity relationship between proteins or between proteins and nucleic acids.[2,3] Furthermore, de novo design of artificial molecules capable of forming structurally well-defined and predictable structures offers new possibilities in the field of molecular recognition, catalysis, and protein folding.[4,5] In recent years, considerable effort has been devoted to the construction of artificial tertiary structures.[6] With this contribution we introduce selective and reversible organization into β-peptide helical secondary structures based on nucleobase recognition.

It has been shown that next to proteins, other biomolecules like RNAs are able to fold and adopt predictable secondary structures.[7] In recent years, β-peptides, in particular, have attracted interest due to their ability to form stable helical conformations, differing in helix sense, radius, and side-chain orientation.[8,9] As few as six β-amino acids are sufficient to form stable secondary structures, whereas α-peptides only show significant helix propensity with a minimum of 14 or 15 amino acids.[10] In addition, β-peptide helices are stable in water and organic solvents as well as being resistant towards enzymatic degradation.[11] The most prominent helical secondary structure in β-peptides is the 14-helix with three amino acids per turn orienting every third side chain (i and $i+3$) on the same side of the helix.[12–15] This helix is obtained with β-amino acids that have lateral substituents in β-position. Nevertheless, the helix propensity can be even improved by incorporation of conformationally

[a] P. Chakraborty, Prof. Dr. U. Diederichsen
Institut für Organische und Biomolekulare Chemie
Georg August Universität Göttingen
Tammannstrasse 2, 37077 Göttingen (Germany)
Fax: (+49) 551-392-944
E-mail: udieder@gwdg.de

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constrained cyclic amino acids like *trans*-(1*R*,2*R*)-2-aminocyclohexanecarboxylic acid (ACHC).[12] The combination of stable helix formation and the uniform orientation of every third amino acid side chain offers the unique opportunity to use β-peptides for molecular architecture with peptide helical secondary structures (Figure 1).

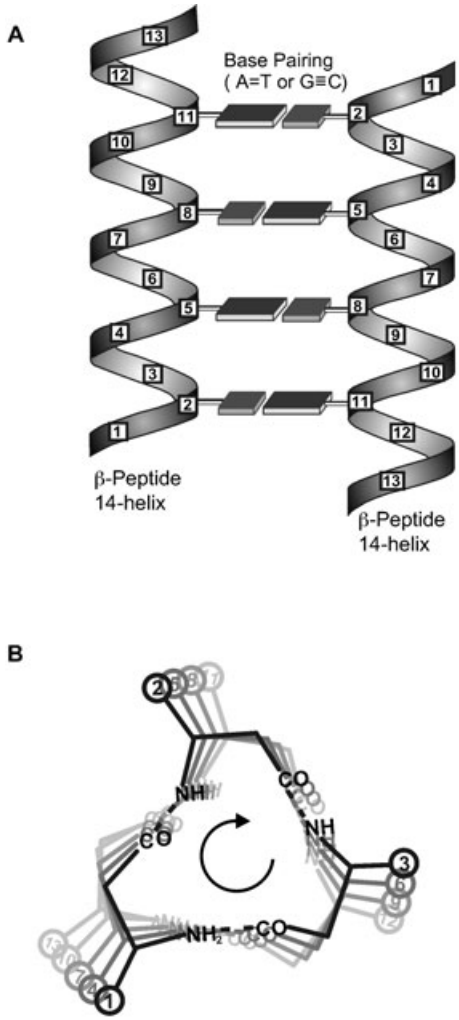


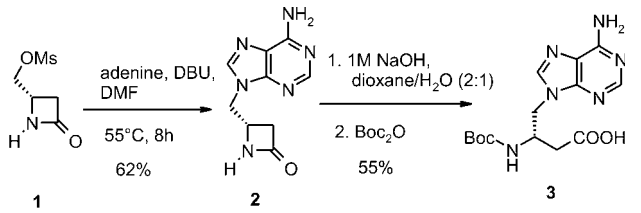
Figure 1. A) Model of antiparallel β-peptide helix association mediated by nucleobase pairing; B) top view of the right-handed 14-helix.

As one possibility for using β-peptide helices for molecular architecture, the self-association of amphiphilic β-peptide 14-helices driven by hydrophobic interactions has already been reported.[16] In addition, specific helix interactions might be obtained by using the recognition potential of hydrogen bonding. The nucleobase pair recognition known from oligonucleotides seems especially well suited for specificity and stable dimerization, since stacking interactions add to the overall stability. As reported already for α-peptides, the modification of amino acid side chains with nucleobases can be used for specific and reversible helix recognition and three-dimensional organization.[17] Furthermore, nucleobase-functionalized α-helices can recognize DNA[18] or a complementary α-helix.[19]

Base pairing provides an excellent driving force and selectivity for self-assembling β-peptide 14-helices. The principle design and first evidence for specific recognition of nucleobase-functionalized β-peptides with respect to tertiary structure formation has been reported.[20] This study contains the synthesis and recognition of a variety of β-peptide helices in order to investigate the stability of double-strand formation by comparison of the A–T and G–C pairing contributions. Furthermore, geometrical parameters like the preferred strand orientation, the positioning of β-homolysine within the β-peptide helix, and the influence of the helix content on duplex formation are evaluated.

Results and Discussion

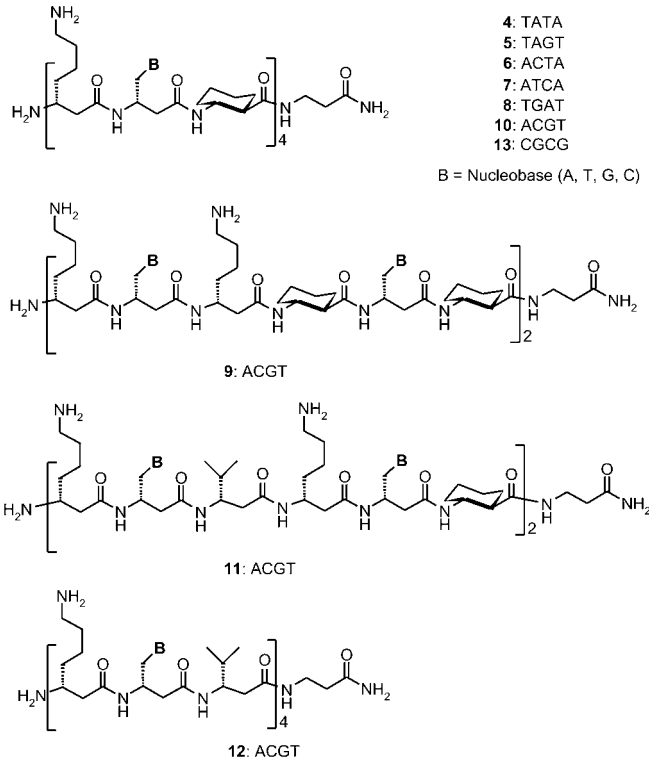
Synthesis of nucleo-β^3-amino acids: *tert*-Butyloxycarbonyl (Boc)-protected nucleo-β^3-amino acids were synthesized as building blocks for the oligomerization of desired β-peptides. The synthesis of β-amino acids Boc-β-HalG-OH, Boc-β-HalC-OH, Boc-β-HalA-OH, and Boc-β-HalT-OH were described previously.[21,22] Nevertheless, Boc-β-HalA-OH was obtained with low optical purity and partial N7-alkylation. Therefore, we report an improved synthesis using a β-lactam as key precursor of enantiomerically pure nucleo-β-amino acids (Scheme 1).[23] β-Lactam mesylate **1**, easily accessible from L-aspartic acid,[24] was alkylated with adenine in presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) providing nucleo-β-lactam **2** exclusively as its N9-regioisomer in 62% yield. By using NaH or K_2CO_3 as base a mixture of N9 and N7 regioisomers was obtained. The desired adeninyl-β-amino acid **3** was obtained in a yield of 55% after hydrolysis of nucleo-β-lactam **2** with 1 M NaOH followed by Boc-protection with Boc_2O under basic conditions. Very high optical purity (*ee* >99%) was indicated by HPLC analysis of the diastereoisomers obtained by Boc-deprotection with trifluoroacetic acid (TFA), followed by coupling with Boc-(*S*)-Ala-OSu or Boc-(*R*)-Ala-OSu, respectively.



Scheme 1. Synthesis of adeninyl-nucleo-β-amino acid **3** from β-lactam **1**.

Design of nucleobase-functionalized β-peptides: For this study we were interested in β-peptides with a right-handed 14-helix secondary structure. Since three residues form one turn, every third amino acid side chain is oriented alike on

the same face of the helix as shown in Figure 1. Previously we have shown that the functionalization of one helical face with three nucleobases already leads to very stable complexes in case of G–C recognition,[20] whereas A–T pairing β-peptides with the same oligomer length are significantly less stable (see the Supporting Information). Since higher aggregation seems likely for oligomers with high G–C content, we decided to investigate β-peptide helices with four nucleobases in order to obtain defined double strands based on the four canonical pairing possibilities A–T, T–A, G–C, and C–G. β-Peptides **4**–**13**, which each contain 13 amino acids, were synthesized with nucleo-β^3-amino acids incorporated in every third position as a recognition unit. For the



remaining two positions per turn, β-homolysine was incorporated to increase the solubility in aqueous media and ACHC was introduced to achieve conformationally stable 14-helices. To evaluate which influence the helix-inducing ACHC has on double-strand formation, the conformationally more flexible β-homovaline was also used instead of ACHC. All oligomers synthesized within this study contain a terminal β-homoglycine residue as a result of synthetic considerations, thereby eliminating the problem of racemization of the resin-bound amino acid.

A–T and G–C pairing in β-peptide recognition: Temperature-dependent UV spectroscopy was applied to determine the thermal stability of the duplexes formed by two complementary-nucleobase-functionalized β-peptide helices; this procedure has also been used in the past for the investigation of nucleobase pairing in DNA, RNA, and other nucleobase-functionalized oligomers.[25] The melting process was considered as a two state model, which assumes that two single strands are only in equilibrium with their corresponding base-paired duplex structure and that no intermediates or partially base-paired structures are involved in the melting process.[25,26] The melting temperatures were obtained from the sigmoidal-shaped curves, obtained from the plot of hyperchromicity (A_{rel}) as a function of temperature.

The A–T base pairing initially was investigated for the two complementary β-peptide oligomers with the sequences ATA and TAT. Since double-strand formation could hardly be detected ($T_m < 5\,°C$, 8 μM),[27] the nucleobase-modified β-peptide helix with the sequence TATA **4** was prepared and provided base pairing with a stability of $T_m = 37\,°C$ (8 μM) as indicated by UV and circular dichrosim (CD) measurements (Figure 2). The CD spectra gave also evidence for the right-handed β-peptide 14-helix conformation of oligomer **4** with a maximum at 215 nm.[28]

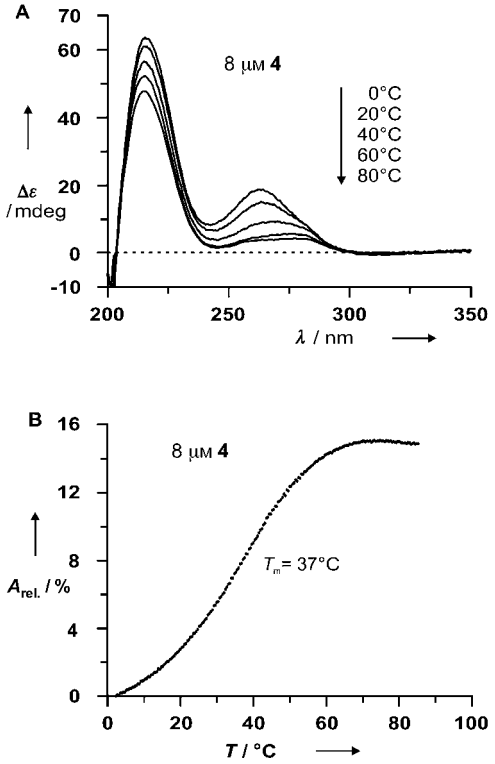


Figure 2. Temperature-dependent CD and UV spectra of A–T pairing β-peptide **4**.

A significant difference between A–T and G–C pairing β-peptide helices was indicated by a comparison of the A/T trimers and tetramer **4** with the respective G/C sequences. The stability of the self-aggregating β-peptide with the sequence CGCG in **13** is too high to be even detected by UV-measurements ($T_m > 80\,°C$, 8 μM). Also aggregates of peptide helices with only three G/C nucleobases are far more stable than expected for double-strand formation (GCG + CGC:

$T_m = 48\,°C$, 8 μM).[20] The contribution of a G–C base pair to the overall stability of the pairing complex can be estimated by comparison of β-peptide tetramers with increasing C–G content (Figure 3). One substitution of a A–T pair for a G–C base pair resulted in an increase of stability of 6 °C (ACTA (**6**) + TAGT (**5**): $T_m = 43\,°C$, 8 μM) or 7 °C (ATCA (**7**) + TGAT (**8**): $T_m = 44\,°C$, 8 μM). However, a second G–C pair leads to an enormous stabilization (ACGT (**10**): $T_m = 72\,°C$, 8 μM). In particular, for the β-peptide sequences that only contain G and C, higher aggregation needs to be considered as it is indicated for the G/C-tetramer **13**.

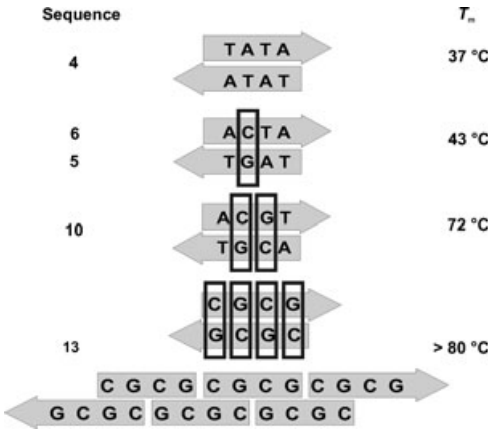


Figure 3. Antiparallel-pairing complexes of nucleobase-modified β-peptide helices with increasing G–C base pair content.

The enormous difference in stability of the A–T and G–C pairing oligomers in the series of β-peptide helices is quite remarkable. Whereas the stability of pairing complexes suggests double-strand formation for oligomers **4**, **5** + **6**, and **7** + **8**, higher aggregates in addition to regular duplex structures cannot be excluded for β-peptide **10** and, especially, oligomer **13**. In the last case, bandlike aggregation needs to be considered (Figure 3) as well as aggregation over the Hoogsteen site of guanine.

Helix orientation in base-pair-mediated double strands: For the design of artificial protein domains and the spatial arrangement of secondary structure elements it is of importance to know about the relative orientation of β-peptide helices organized in a duplex by nucleobase recognition. Therefore, the strand orientation was investigated with respect to a general preference that might result from the β-peptide 14-helix topology. In addition, it would be of interest to know if the proper choice of nucleobase sequence might also allow the formation of the less-preferred helix orientation. The experimental proof for an antiparallel or parallel preference of the 14-helix orientation within a base-pair-mediated double strand was derived from a set of β-peptide oligomers, which, in principle, would have equal chances to form antiparallel or parallel double strands (Figure 4).

Nucleobase-modified β-helices with the sequence TAGT **5** and ACTA **6** or ATCA **7** and TGAT **8** are two pairs of oligomers that are complementary with antiparallel-strand orientation. In contrast, oligomers TAGT **5** and ATCA **7** or ACTA **6** and TGAT **8** are two complementary pairs designed to form a heterodimer if parallel-strand orientation is preferred. The stabilities determined for the double strands with antiparallel orientation of the respective pairing complexes **5** and **6** ($T_m = 43\,°C$, Figure 5) and **7** and **8** ($T_m = 44\,°C$)[20] turned out to be similar as expected for three A–T and one G–C base pairs each. The respective parallel pairing complexes between β-peptides TAGT **5** and ATCA **7** ($T_m = < 5\,°C$) or ACTA **6** and TGAT **8** ($T_m = 29\,°C$) were not observed. The stability detected for the equimolar mixture of oligomers **6** and **8** does not indicate a heterodimer, but rather represents the self-aggregation of β-peptide **8** ($T_m = 28\,°C$).

Only for oligomer **8** (TGAT) this self-pairing was detected; this is surprising especially with respect to the low tendency of oligomer **5** (TAGT) for self-recognition ($T_m = < 5\,°C$), since the sequences only differ in the order of the central nucleobases.[29] Also self-association of oligomers **6** ($T_m = < 0\,°C$) and **7** ($T_m = < 5\,°C$) was negligible. For all oligomers **5**–**8** and the respective equimolar mixtures of β-

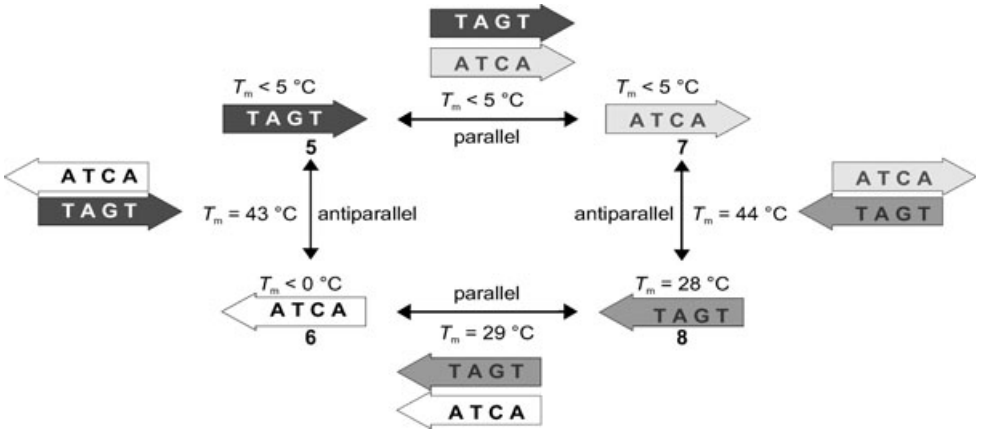


Figure 4. Nucleobase-modified β-peptide sequences designed to prove a preference for antiparallel or parallel helix orientation.

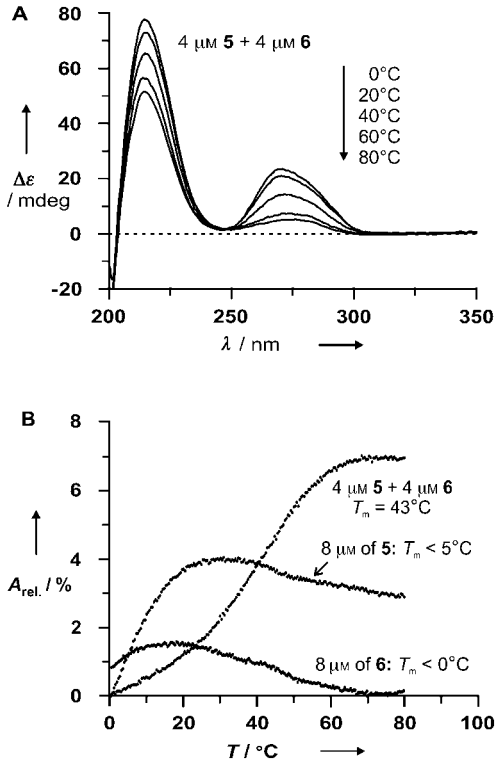


Figure 5. Temperature-dependent CD and UV spectra of an equimolar mixture of β-peptides **5** and **6**. Furthermore, the UV melting curves of self-associated oligomers **5** and **6** are given.

peptides, the right-handed 14-helices were formed as indicated by the positive Cotton effect at 215 nm. Furthermore, the UV melting curves were confirmed by the decrease of CD intensity in the nucleobase absorption range. All thermal denaturation processes have proven to be reversible. Overall, we can conclude that there is a clear preference of nucleobase-modified β-peptide helices to undergo base-pair-mediated organization with antiparallel-helix orientation. Parallel-helix orientation cannot be realized with nucleobase-functionalized β-peptides.

Influence of the helical content on duplex stability: Despite possible higher aggregation phenomena the stability of double strands formed by nucleobase-substituted β-peptide helices is surprisingly high. For comparison, tetrameric DNA-pairing complexes with similar nucleobase sequences are hardly stable enough to be detected by temperature-dependent UV spectroscopy.[30] The β-peptide backbone topology based on the 14-helix is likely to be responsible for the high pairing stabilities. The single-stranded helices are already highly preorganized for double-strand formation. The influence of the helical preorganization was investigated by the synthesis of nucleo-β-peptides with varying amount of ACHC amino acids, since ACHC is known to induce the 14-helix conformation.[12] The self-complementary oligomers **10**–**12** were investigated; they all have the same length and ACGT nucleobase sequence, but different numbers of ACHC units: four in **10**, two in **11**, and none in **12**. For the cyclic and conformationally restricted ACHC β-amino acid, the more flexible β-homovaline was used as substitute.

The CD spectra of β-peptides **10**–**12** were measured at 20 °C (Figure 6A) in Tris·HCl (Tris = tris(hydroxymethyl)aminomethane) buffer. For all three oligomers the strong positive Cotton effect at 215 nm confirms the right-handed 14-helix. Oligomer **10** with four ACHC units shows highest 14-helix propensity, whereas the helix content was reduced with decreasing amounts of the ACHC amino acid. Also the thermal stability of the double strands followed this trend (Figure 6B,C). The decrease in stability from oligomer **10** (T_m = 72 °C) with four ACHC units over peptide **11** (T_m = 62 °C) to sequence **12** (T_m = 54 °C) with no ACHC can be correlated with an increase in β-helix flexibility and, therefore, with lower preorganization with respect to double-strand formation.

Thermodynamic data were derived for three β-peptide-pairing complexes (Table 1) based on the concentration dependency of the T_m values as described by Breslauer for the treatment of DNA double strands (van't Hoff plots are

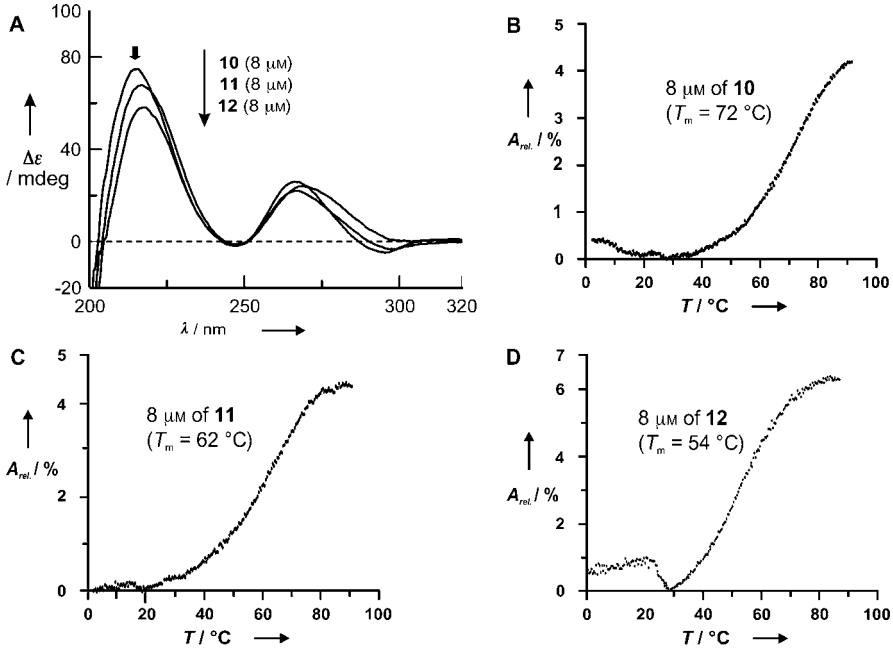


Figure 6. A) CD spectra of oligomers **10**–**12** at 20 °C; B)–D) Temperature-dependent UV spectra of oligomers **10**–**12**.

Table 1. Thermodynamic parameters for the pairing complexes of oligomers **4**, **5** + **6**, **10**, **11** and an oligonucleotide for comparison.

Oligomers	ACHC units	$\Delta G°$ [kcal mol^{-1}]	$\Delta H°$ [kcal mol^{-1}]	$\Delta S°$ [cal mol^{-1} K^{-1}]
4 (TATA)	4	−7.3	−21.1	−46.0
6 (ACTA) + **5** (TAGT)	4	−9.2	−30.7	−72.3
10 (ACGT)	4	−11.3	−33.7	−75.1
11 (ACGT)	2	−12.2	−39.2	−90.6
DNA oligomer CCGG[30]	–	−4.4	−34.2	−95.6

given in the Supporting Information).[31] Especially the entropy values for the nucleobase-modified β-peptide double strands are remarkable in comparison to a self-complementary DNA tetramer. Preorganization of the nucleobases in the stable 14-helix seems to be the key stabilizing factor regarding the formation of nucleobase-modified β-peptide double strands. Lower helix content due to an exchange of two β-homovaline residues for ACHC β-amino acids (oligomers **10** versus **11**) diminishes the preorganization of the nucleobases with respect to double-strand formation.

In addition, interstrand charge repulsion of the phosphodiester anions is lowering the double strand stability in oligonucleotides.[32] The β-peptides carry a similar amount of positive charges since the terminal primary amine and the lysine side chains are protonated under physiological conditions. Nevertheless, the charges of two 14-helical nucleo-β-peptides are clearly oriented apart and should not influence each other in the process of double strand formation.

Positioning of β-homolysine in the 14-helix: The design of the self-recognizing nucleobase-modified β-peptide helices requires the orientation of all the nucleobase modifications on one side of the β-peptide 14-helix. For the remaining two positions per turn β-homolysine and ACHC were used. For the oligomers investigated so far, the orientation of the β-homolysine side chains and ACHC residues is the same as that for oligomer **10** shown in Figure 7A. The relevance of this setup for double-strand formation was investigated by comparison of oligomer **10** with β-peptide **9**, which has the same amino acid content but differs in the sequential order of β-homolysine and ACHC (Figure 7B).

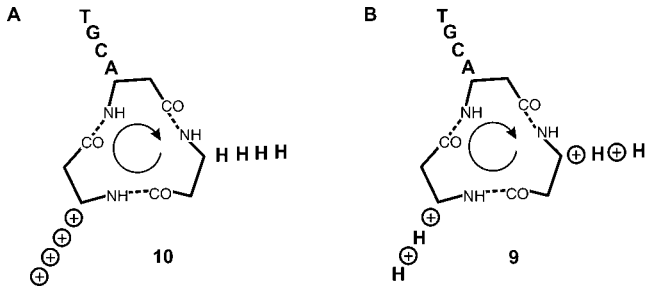


Figure 7. Top views of right-handed 14-helices: schematic representation of the distribution of the β-amino acid side chains. Positively charged β-homolysine side chains are designated by + and the hydrophobic side chain of ACHC and β-homovaline are designated by H. A) Representation of the β-peptide **10** with a high degree of intramolecular charge repulsion by β-homolysine side chains; B) β-peptide **9** with positively charged β-homolysine side chains separated; this leads to a lower degree of charge repulsion.

The double-strand stability of the self-pairing complex of β-peptide **9** was too high to be determined ($T_m > 85\,°C$, Figure 8B). This significant stabilization relative to oligomer **10** ($T_m = 72\,°C$) is likely to result from an effective charge separation by positioning the ACHC amino acids in between two β-homolysine residues. The equal distribution of positively charged side chain residues along two sides of the 14-helix not only resulted in a higher double strand stability, but also the helix content increased with respect to oligomers with positive charges located along one side of the 14-helical backbone as indicated by CD spectroscopy (Figure 8A versus Figure 6A).

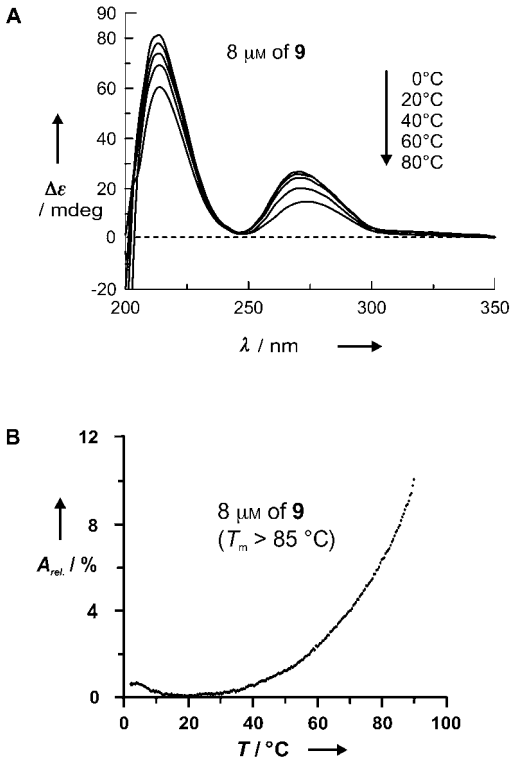


Figure 8. Temperature-dependent CD spectra and UV-melting curve of oligomer **9**.

Conclusion

The specific and reversible three-dimensional organization of peptide helices is of interest with respect to the formation of artificial protein structures and as functional mimics. A series of 14-helices formed by β-peptides were functionalized with nucleobases by using base-pair recognition for specific organization of the duplexes. Four base pairs are sufficient to obtain very stable double strands. Duplexes were

formed specifically with antiparallel strand orientation. The double-strand stability was very much dependent on the nucleobase sequence, but also the influence of the helical content on the preorganization of a single helix for recognition was indicated. Finally, the distribution of charges on the helix surface also turned out to be significant for double-strand stabilization. As a result of this study, the design principles of β-peptide 14-helix recognition mediated by base pairing are well understood in order to be used for molecular architecture with peptide helices.

Experimental Section

General: All reagents were of analytical grade and used without further purification. Solvents were of the highest grade available. Dry solvents were purchased from Fluka and stored over 4 Å molecular sieves. Boc-(*R*,*R*)-ACHC-OH was prepared as described in literature.[33] Boc-β-HVal-OH and Boc-β-HLys(*Z*)-OH were obtained by the Arndt–Eistert homologation of the respective α-amino acids.[34,35] 4-Methylbenzhydrylamine polystyrene (MBHA-PS) resin was obtained from Novabiochem. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) and detection was done under UV light or by coloring with ninhydrin solution (3% in ethanol). Flash chromatography (FC) was performed by using silica gel 60 (0.040–0.063 mm, Merck). Melting points were obtained with a Büchi-501 melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Perkin–Elmer 1600 Series FT-IR with KBr pellets. NMR spectra were recorded with a Varian Unity 300 instrument. Chemical shifts are referenced to the residual solvent peaks of DMSO (^{1}H: $\delta = 2.49$ ppm, ^{13}C: $\delta = 39.5$ ppm). Mass spectra were recorded with a LCG Finnigan spectrometer. High-resolution mass spectra were recorded with a Bruker APEX-IV FT ICR mass spectrometer. HPLC analysis and purification of the oligomers was performed on a Pharmacia Äkta basic system (pump type P-900, variable wavelength detector of 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 purified on a YMC J'sphere column ODS-H80, RP-C18; 250×4.6 mm, 5 μm, 120 Å at a flow rate of 1 mL min^{-1} for analytical samples and 250×20 mm, 5 μm, 120 Å at a flow rate of 10 mL min^{-1} for preparative samples. CD spectra were recorded on a JASCO J-810 spectrometer equipped with a JASCO ETC-505S/PTC-423S temperature controller. All CD measurements were carried out in 10 mM Tris·HCl buffer (pH 7.5) with a quartz cell of 1 cm path length. Spectra represent the average of 5 scans after baseline correction. Temperature-dependent UV spectra were measured with a JASCO V-550 UV/Vis spectrometer equipped with JASCO ETC-505S/ETC-505T temperature controller. All measurements were carried out in Tris·HCl buffer of pH 7.5. The data were collected at 260 nm with a heating rate of 0.5 °C min^{-1} in a quartz cell of 1 cm path length. The oligomer concentration was determined based on the absorption at 260 nm measured at 80 °C considering nucleobases to be completely destacked. The extinction coefficient of each oligomer was assumed as the sum of the extinction coefficients of the contained nucleobases.[27]

(*S*)-4-[(Adenine-9-yl)methyl]azetidin-2-one (2): Under dry conditions β-lactam mesylate **1** (500 mg, 2.79 mmol) and adenine (754 mg, 5.58 mmol) were suspended in dry DMF (25 mL). DBU (0.834 mL, 5.58 mmol) was added to this mixture and stirred at 55 °C for 8 h under argon. DMF was removed under reduced pressure and the residue was purified by flash chromatography (silica gel; EtOAc/MeOH 4:1, 0.5% AcOH) to afford **2** (378 mg, 62%) as a white solid. $R_f = 0.37$ (EtOAc/MeOH 1:1); m.p. 195–196 °C; $[\alpha]_D^{20} = +12.0$ ($c = 0.15$ in methanol); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 2.64$–2.69 (m, 1H; α-CH_2), 2.91–2.98 (m, 1H; α-CH_2), 3.94–3.99 (m, 1H; β-CH_2), 4.32 (d, $^3J_{H,H} = 6$ Hz, 2H; γ-CH_2), 7.19 (s, 2H; NH_2), 8.03 (s, 1H; NH), 8.11 (s, 1H; C8), 8.15 ppm (s, 1H; C2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 41.0$ (Cα), 45.4 (Cβ), 46.7 (Cγ), 118.5 (C5), 140.9 (C8), 149.6 (C4), 152.4 (C2), 155.9 (C3), 166.4 ppm (C=O); IR (KBr): $\tilde{\nu} = 3403$ (s), 3186 (m), 1735 (s, C=O), 1652 (m), 1606 (m), 1574 (m), 1419 (m), 1332 (w), 1304 (w), 1205 (m), 1066 cm^{-1} (m); MS (ESI): *m/z* (%): 219.3 (100) $[M+H]^+$, 241.3 (55) $[M+Na]^+$, 459.3 (62) $[2M+Na]^+$; HRMS(EI): *m/z* calcd for $C_9H_{10}N_6O$: 218.0916; found 218.0916 $[M+H]^+$.

(*S*)-*N*-(*tert*-Butoxycarbonyl)-γ-(adenin-9-yl)-β-homoalanine (3): Compound **2** (327 mg, 1.5 mmol) was dissolved in dioxane/water 2:1 (30 mL). NaOH (1 M, 4.5 mL, 4.5 mmol) was added and stirred at room temperature overnight. The reaction mixture was treated with Boc_2O (820 mg, 3.75 mmol) and pH 9.0 was maintained for three days by repeated addition of 1 M NaOH. The resultant mixture was washed with Et_2O and the aqueous layer was dried. The residue was purified by flash chromatography (silica gel; EtOAc/MeOH 4:1, 0.5% AcOH) followed by reverse phase chromatography (RP-C18 silica gel, 20% MeOH in H_2O). After freeze drying nucleo amino acid **3** (276 mg, 55%) was provided as a white solid. $R_f = 0.44$ (EtOAc/MeOH 1:1); m.p. 214–216 °C; $[\alpha]_D^{20} = +36.6$ ($c = 0.12$ in methanol); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 0.99$ (s, 1.5H; *t*Bu), 1.23 (s, 7.5H; *t*Bu), 2.40–2.44 (m, 2H; α-CH_2), 4.08–4.18 (m, 2H; γ-CH_2), 4.22–4.28 (m, 1H; β-CH), 6.44 (br s, 0.2H; BocNH), 6.84 (d, $^3J_{H,H} = 7.2$ Hz, 0.8H; BocNH), 7.11 (s, 2H; NH_2), 7.91 (s, 1H; H8), 8.12 ppm (s, 1H; H2); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 28.0$ (*t*Bu), 37.7 (Cα), 46.1 (Cγ), 47.7 (Cβ), 77.7 (*t*Bu), 118.5 (C5), 141.0 (C8), 149.8 (C3), 152.2 (C2), 154.7, 155.8, 173.2 ppm (COOH); IR (KBr): $\tilde{\nu} =$ 3415 (s), 2928 (w), 1659 (m), 1498 (w), 1289 (w), 1171 cm^{-1} (w); MS (ESI): *m/z* (%): 337.4 (100) $[M+H]^+$, 359.4 (42) $[M+Na]^+$, 695.3 (43) $[2M+Na]^+$; HRMS(ESI): *m/z* calcd for $C_{14}H_{21}N_6O_4$: 337.1619; found 337.1617 $[M+H]^+$.

General procedure for solid-phase β-peptide synthesis: Oligomers were prepared by manual solid-phase peptide synthesis in a small fritted glass column using a 4-methylbenzhydrylamine polystyrene (MBHA-PS) resin with a loading capacity of 0.62 mmol g^{-1}. For oligomer syntheses a resin preloaded with H-β-HGly-OH (20.0 mg resin, 12.4 μmol homoglycine amide) was used. For peptide bond formation double coupling of the amino acids at 50 °C was required. First, an excess of 5 equiv amino acid (62.0 μmol) was used, activated by *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU; 21.2 mg, 55.8 μmol, 4.5 equiv), 1-hydroxy-7-azabenzotriazole (HOAt; 124 μL, 62.0 μmol, 5 equiv of a 0.5 M solution in DMF), and *N*,*N*-diisopropylethylamine (DIPEA; 30.3 μL,174 μmol, 14 equiv) in DMF (400 μL); the second coupling was performed with 3 equiv of amino acid (37.2 μmol) and activation with HATU (12.7 mg, 33.5 μmol, 2.7 equiv), HOAt/DMF (74.4 μL, 37.2 μmol), and DIPEA (19.5 μL, 112 μmol, 9 equiv) in DMF (400 μL). After swelling the loaded resin for 2 h in CH_2Cl_2 (2 mL), the following procedure was followed for each coupling step: 1) deprotection twice, for 3 min with TFA/*m*-cresol (95:5, 2 mL); 2) washing first five times with CH_2Cl_2/DMF (1:1, 2 mL) and then five times with pyridine (2 mL); 3) double coupling steps, each 1 h gentle moving at 50 °C; 4) washing with CH_2Cl_2/DMF (1:1, 3×2 mL), DMF/piperidine (95:5, 3×2 mL), and CH_2Cl_2/DMF (1:1, 3×2 mL), 5) 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), dried overnight in vacuo. The resin was then 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 reaction mixture was cooled to −20 °C. Trifluoromethanesulfonic acid (TFMSA; 200 μL) was added dropwise with stirring. The mixture was allowed to warm to room temperature within 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. The crude peptide was dissolved in water/acetonitrile and purified by preparative HPLC.

H-(β-HLys-β-HalT-ACHC-β-HLys-β-HalA-ACHC-β-HLys-β-HalT-ACHC-β-HLys-β-HalA-ACHC-β-HGly)-NH_2 (4): Analytical RP-HPLC: $t_R = 26.03$ min, gradient: 20 to 45% eluent B in 30 min; MS (ESI): *m/z* (%): 672.0 (50) $[M+3H]^{3+}$, 1007.2 (100) $[M+2H]^{2+}$, 1341.8 (12) $[2M+3H]^{3+}$; HRMS(ESI): *m/z* calcd for $C_{95}H_{152}N_{32}O_{17}$ $[M+3H]^{3+}$: 671.4025; found 671.4025.

H-(β-HLys-β-HalT-ACHC-β-HLys-β-HalA-ACHC-β-HLys-β-HalG-ACHC-β-HLys-β-HalT-ACHC-β-HGly)-NH_2 (5): Analytical RP-HPLC: $t_R = 21.64$ min, gradient: 22 to 42% B in 30 min; MS (ESI): *m/z* (%): 677.3 (22) $[M+3H]^{3+}$, 1015.0 (100) $[M+2H]^{2+}$, 1352.5 (18) $[2M+3H]^{3+}$; HRMS(ESI): *m/z* calcd for $C_{95}H_{151}N_{32}O_{18}$: 1014.5975; found 1014.5979 $[M+2H]^{2+}$.

H-(β-HLys-β-HalA-ACHC-β-HLys-β-HalC-ACHC-β-HLys-β-HalT-ACHC-β-HLys-β-HalA-ACHC-β-HGly)-NH_2 (6): Analytical RP-HPLC: $t_R = 15.46$ min, gradient: 23 to 45% B in 30 min; MS (ESI): *m/z* (%): 666.8 (32) $[M+3H]^{3+}$, 999.5 (100) $[M+2H]^{2+}$, 1331.8 (20) $[2M+3H]^{3+}$, 1997.0 (7) $[M+H]^{+}$; HRMS(ESI): *m/z* calcd for $C_{94}H_{152}N_{33}O_{16}$: 999.1002; found: 999.1012 $[M+2H]^{2+}$.

H-(β-HLys-β-HalA-β-HLys-ACHC-β-HalC-ACHC-β-HLys-β-HalG-ACHC-β-HLys-β-HalT-ACHC-β-HGly)-NH_2 (9): Analytical RP-HPLC: $t_R = 13.49$ min, gradient: 20 to 30% B in 30 min; MS (ESI): *m/z* (%): 672.3 (100) $[M+3H]^{3+}$, 1007.4 (48) $[M+2H]^{2+}$; HRMS(ESI): *m/z* calcd for $C_{94}H_{152}N_{33}O_{17}$: 671.7342; found 671.7338 $[M+3H]^{3+}$.

H-(β-HLys-β-HalA-ACHC-β-HLys-β-HalC-ACHC-β-HLys-β-HalG-ACHC-β-HLys-β-HalT-ACHC-β-HGly)-NH_2 (10): Analytical RP-HPLC: $t_R = 24.75$ min, gradient: 25 to 45% B in 30 min; MS (ESI): *m/z* (%): 672.4 (52) $[M+3H]^{3+}$, 1007.7 (100) $[M+2H]^{2+}$, 1342.7 (26) $[2M+3H]^{3+}$; HRMS(ESI): *m/z* calcd for $C_{94}H_{152}N_{33}O_{17}$: 671.7342; found 671.7342 $[M+3H]^{3+}$.

H-(β-HLys-β-HalA-β-HVal-β-HLys-β-HalC-ACHC-β-HLys-β-HalG-β-HVal-β-HLys-β-HalT-ACHC-β-HGly)-NH_2 (11): Analytical RP-HPLC: $t_R = 20.72$ min, gradient: 25 to 55% B in 30 min; MS (ESI): *m/z* (%): 995.5 (100) $[M+2H]^{2+}$, 1990.0 (12) $[M+H]^{+}$; HRMS(ESI): *m/z* calcd for $C_{92}H_{151}N_{33}O_{17}$: 995.0982; found 995.0977 $[M+2H]^{2+}$.

H-(β-HLys-β-HalA-β-HVal-β-HLys-β-HalC-β-HVal-β-HLys-β-HalG-β-HVal-β-HLys-β-HalT-β-HVal-β-HGly)-NH_2 (12): Analytical RP-HPLC: $t_R = 25.12$ min, gradient: 22 to 45% B in 30 min; MS (ESI): *m/z* (%): 656.2 (15) $[M+3H]^{3+}$, 983.5 (100) $[M+2H]^{2+}$, 1965.9 (11) $[M+H]^{+}$; HRMS(ESI): *m/z* calcd for $C_{90}H_{151}N_{33}O_{17}$: 983.0982; found 983.0968 $[M+2H]^{2+}$.

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