■ Peptidomimetics

Structural Investigation of Hybrid Peptide Foldamers Composed of α -Dipeptide Equivalent β -Oxy- δ^5 -amino AcidsRahi M. Reja,^[a] Vivek Kumar,^[a] Gijo George,^[b] Rajat Patel,^[a] DRGKoppalu R. Puneeth Kumar,^[a] Srinivasarao Raghothama,^[b] and Hosahudya N. Gopi^{*[a]}

Abstract: Due to their equivalent lengths, δ -amino acids can serve as surrogates of α -dipeptides. However, δ -amino acids with proteinogenic side chains have not been well studied because of synthetic difficulties and because of their insolubility in organic solvents. Recently we reported the spontaneous supramolecular gelation of δ -peptides composed of β (O)- δ^5 -amino acids. Here, we report the incorporation of β (O)- δ^5 -amino acids as guests into the host α -helix, α,γ -hybrid peptide 12-helix and their single-crystal conformations. In addition, we studied the solution conformations of hybrid peptides composed of 1:1 alternating α and β (O)- δ^5 -amino acids. In contrast to the control α -helix structures, the

crystal structure of peptides with β (O)- δ^5 -amino acids exhibit α -helical conformations consisting of both 13- and 10-membered H-bonds. The α,δ -hybrid peptide adopted mixed 13/11-helix conformation in solution with alternating H-bond directionality. Crystal-structure analysis revealed that the α,γ^4 -hybrid peptide accommodated the guest β (O)- δ^5 -amino acid without significant deviation to the overall helix folding. The results reported here emphasize that β (O)- δ^5 -amino acids with proteinogenic side chains can be accommodated into regular α -helix or 12-helix as guests without much deviation of the overall helix folding of the peptides.

Introduction

Hydrogen bonds play a crucial role in stabilizing protein secondary structures and their mimetics derived from various types of non-natural building blocks.^[1] Among the various non-natural building blocks, peptides derived from β - and γ -amino acids^[2] and the hybrid peptides with the combination of $(\alpha\beta)_n$,^[3] $(\beta\gamma)_n$,^[4] $(\alpha\gamma)_n$ ^[5] have been extensively investigated. The helical structures of β -, γ - and hybrid peptides are classified based on the number of atoms involved in the intramolecular H-bonds.^[6] A variety of helical structures stabilized by 6- to 17-membered H-bonding pseudocycles have been observed in the peptide foldamers.^[7] In the majority of cases, the H-bonds are unidirectional; however, in some cases the helices were also stabilized by H-bonds with alternating directionality (mixed helices).^[8]

In comparison to β -, γ - and their hybrid peptides, foldamers composed of δ -amino acids are scarcely discussed in the literature.^[9] Nevertheless, compared to the β -, γ - and other non-nat-

ural amino acids, δ -amino acids have a particular appeal because they closely resemble the dipeptide segments of α -peptides. This means that the length of the δ -amino acid is nearly equal to that of the α -dipeptide, and that α -dipeptides can be replaced by the δ -amino acids. Based on extensive ab initio calculations, Hofmann and colleagues examined the possible helix types available to the oligomers of unsubstituted δ -amino acids.^[9a] The theoretical analysis revealed that oligomers of δ -amino acids have an ability to adopt a variety of helical structure depending on the directionality and the number of residues involved in the H-bonding.^[9a] The groups of Chakraborty^[9b-e] and Sharma^[9f] reported a number of different hybrid helices from carboamino and furan based δ -amino acids. As observed in the $(\beta\gamma)_n$ hybrid peptides, the sequences of $(\alpha\delta)_n$ are also expected to adopt 13-helices similar to the α -helices. In a continuation, Sharma and co-workers showed by NMR study and theoretical calculations the formation of 11/13 mixed helices from α/δ hybrid peptides composed of sugar amino acids.^[9f] Apart from the pyranose and furanose based δ -amino acids, Huc and co-workers showed helical folding from quinolone-based δ -amino acids in single crystals.^[9g] Furthermore, Balaram and co-workers examined the stability of a designed helix by replacing a Gly-Gly segment with δ -aminovaleic acid (δ -Ava).^[9h] In addition, 2,5-disubstituted δ -amino acids and the corresponding methylene oxy derivatives have been explored as isosteres of peptide bonds in the design of biologically active peptides.^[10] Peptides composed of these δ -amino acids have also been explored for the design self-assembled peptide nanotubes and ion channels.^[11] However, to date, there has been little success in the design of foldamers com-

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posed of δ -amino acids with proteinogenic side-chains. This is probably due to the difficulties in the synthesis of δ^5 -amino acid with proteinogenic side chains. Another important problem associated with these δ -amino acids is their low solubility in organic and aqueous solvents.^[12] A list of δ -amino acids reported to date is shown in Figure 1. We have been working in the area of peptide foldamers composed of various types of non-natural amino acids. We showed stable C_{12} helices,^[5b] mixed helices with alternating H-bond directionality,^[8h] and 15/17-helices from α,γ^4 -hybrid peptide foldamers^[7f] in single crystals. Recently, we examined the hydrogelation properties of hybrid peptides composed of γ - and δ -amino acids.^[12] Compared to dipeptides composed of δ^5 -amino acids with a complete carbon backbone, δ^5 -amino acids with "O" at the β -position was found to be soluble in organic solvents and to form gels in aqueous buffers. The straightforward synthesis and the high solubility of the new δ -amino acids (i.e., $\beta(O)$ - δ^5 -amino acids) coupled with their similarity to α -dipeptides, motivated us to systematically investigate the structural properties of $\beta(O)$ - δ^5 -amino acids by their incorporation as a guest in α -peptides, α,γ^4 -hybrid peptides and as 1:1 alternating α - and $\beta(O)$ - δ^5 -amino acids. Here, we report the structural properties of $\beta(O)$ - δ^5 -amino acids in α -peptides, α,γ^4 -hybrid peptides and α,δ^5 -hybrid peptides both in single crystals and in solution.

Figure 1. Chemical structures of different δ -amino acids reported in the literature.

Results and Discussion

The sequences of the hybrid peptides composed of $\beta(O)$ - δ^5 -amino acid are shown in the Scheme 1. The $\beta(O)$ - δ^5 -amino acids were synthesized from Boc protected amino alcohol as reported previously.^[12,13] All the peptides were synthesized by conventional solid-phase peptide synthesis strategy on Rink Amide resin at 0.2 mmol scale and purified by RP-HPLC using a MeOH/H₂O solvent system. Peptide **P1** was used as a control peptide. In peptide **P2**, we replaced Phe-Gly dipeptide with $\beta(O)$ - δ^5 -Phe in the α -helix. To understand whether α,δ^5 -hybrid peptides with 1:1 alternating α - and $\beta(O)$ - δ^5 -amino acid can give a 13-helical structure similar to the α -peptides, we designed peptide **P3**. Furthermore, to investigate the versatility $\beta(O)$ - δ^5 -amino acid, we designed peptide **P4** by incorporating $\beta(O)$ - δ^5 -amino acid as a guest in the sequence of the α,γ^4 -hybrid peptide. The α,γ^4 hybrid peptides are known to form stable C_{12} helix.^[14] X-ray diffraction structures of peptides **P1** and **P2** are shown in Figure 2. The X-ray diffraction quality single crystals of the control α -helix **P1** were grown from slow evaporation of aqueous methanol solution and its structure is shown in Figure 2A. The helical structure is stabilized by six hydrogen bonds. Except for the terminal Aib, all NHs and carbon-

Scheme 1. Sequences of peptides under investigations.

Figure 2. X-ray structure of peptides A) **P1** and B) **P2**. $\beta(O)$ - δ^5 -Phe is shown in green. C) An overlay of structure of peptides **P1** and **P2** (magenta and green represent peptide **P1** and **P2**, respectively). Top views of the structures of the peptides are shown in the lower panel.

yls involved in the 13-membered H-bond form ($i \leftarrow (i+4)$), with average H—O distance 2 ± 0.2 Å and N—O distance: 2.9 ± 0.1 Å. The average value of the torsion angles $\varphi = -50 \pm 20^\circ$ and $\psi = -50 \pm 20^\circ$. Like in many designed peptides containing Aib residues, the terminal residue does not participate in the canonical helix. Peptide **P1** adopted α -helix conformation in the single crystals.

To understand the effect of the incorporation of the $\beta(O)$ - δ^5 -amino acid on the structures of the α -helices, we synthesized

peptide **P2** in which the central dipeptide Phe-Gly was replaced with $\beta(\text{O})$ - δ^5 -Phe. The conformation of $\beta(\text{O})$ - δ^5 -amino acid can be represented by the backbone torsion angles $\varphi(\text{C}=\text{O}-\text{N}-\text{C}^\delta-\text{C}^\gamma)$, $\theta_1(\text{N}-\text{C}^\delta-\text{C}^\gamma-\text{O}^\beta)$, $\theta_2(\text{C}^\delta-\text{C}^\gamma-\text{O}^\beta-\text{C}^\alpha)$, $\theta_3(\text{C}^\gamma-\text{O}^\beta-\text{C}^\alpha-\text{C}=\text{O})$ and $\psi(\text{O}^\beta-\text{C}^\alpha-\text{C}=\text{O}-\text{N})$ (Scheme 1). The X-ray diffraction quality single crystals of peptide **P2** were obtained with a helical structure as shown in the Figure 2B from the slow evaporation of aqueous methanol. The peptide adopted an uncommon 10/13/16-helical structure (Figure 2B) that was stabilized by eight intramolecular hydrogen bonds. Interestingly, the first three hydrogen bonds are 10-membered pseudocycles between the i and $i+3$ residues. The residues $\text{Ac}(1)\text{CO}\leftarrow\text{HN}\text{Aib}(3)$, $\text{Aib}(1)\text{CO}\leftarrow\text{NH}\text{Leu}(4)$ and $\text{Leu}(2)\text{CO}(i)\leftarrow\text{NH}\delta^5\text{Phe}(5)$ ($i+3$) are involved in the 10-membered H-bonds. The next two H-bonds involve 13-membered pseudocycles: residues $\text{Ala}(3)\text{CO}\leftarrow\text{HN}\text{Leu}(6)$ and $\text{Leu}(4)\text{CO}(i)\leftarrow\text{NH}\text{Ala}(7)$ ($i+3$). The next hydrogen bonds are two 10-membered between $\beta(\text{O})$ - δ^5 -Phe(5) $\text{CO}(i)\leftarrow\text{HN}\text{Leu}(8)$ ($i+3$) and between $\text{Leu}(6)\text{CO}(i)\leftarrow\text{NH}\text{Aib}(9)$ ($i+3$). The terminal amide NH is involved in a 16-membered hydrogen bond between $\text{Leu}(6)\text{CO}(i)\leftarrow\text{CONH}_2$. It is interesting to note that, except for the terminal H-bond, both 10- and 13-membered H-bonds in the peptide **P2** are stabilized by the residues $i\leftarrow i+3$. The torsion angles of amino acid residues in **P2** are tabulated and given in the Supporting Information. The torsion angles of the $\beta(\text{O})$ - δ^5 -Phe were found to be $\varphi=-92.56^\circ$, $\theta_1=52.95^\circ$, $\theta_2=79.20^\circ$, $\theta_3=-158.37^\circ$, and $\psi=7.12^\circ$. The torsion angles of other residues were found to be very similar to those of the residues of **P1**. In comparison to **P1**, incorporation of the $\beta(\text{O})$ - δ^5 -Phe leads to disruption in the H-bonding pattern in **P2**. The first three residues in **P2** adopted 3_{10} -helix conformation, while in **P1** they adopt an α -helix conformation. The H-bonding pattern of both peptides **P1** and $\mathbf{P2}$ are shown in Figure 3. These results also suggest a small energy difference between the two helix types. Probably, the lack of two H-bonds indeed disturbed the H-bonding pattern throughout the helix in peptide **P2**. The overlay structure of peptides **P1** and **P2** is shown in Figure 2C. It is evident that the helical pore of peptide **P2** was a little narrow compared to peptide **P1** due to incorporation of $\beta(\text{O})$ - δ^5 -Phe amino acid in place of the Phe-Gly dipeptide residue.

Figure 3. H-bonding pattern observed in the X-ray structure of peptides **P1** and **P2**.

Recently, Gellman and colleagues demonstrated 13-membered H-bond stabilized β,γ -hybrid helices.^[4c] Hofmann and colleagues have theoretically predicted the formation of 13-helix formation by β,γ -hybrid peptides.^[8e] In addition to the β,γ -hybrid helices, α,δ -hybrid peptides are also expected to adopt a 13-helical conformation. Given that the length of β,γ - and α,δ -hybrid peptides are similar, it is expected that α,δ -hybrid peptides can also form a 13-helix. The structural details of α -peptides, β,γ - and α,δ -hybrid peptides are shown in Scheme 2. To understand how the 1:1 alternating α,δ -hybrid

Scheme 2. Schematic representation of structural analogy of α -, β,γ - and α,δ -peptides.

peptides can adopt 13-helix conformation similar to α -helix, peptide **P3** was designed and synthesized. Unfortunately, this peptide did not give X-ray quality single crystals; therefore, the conformation of the peptide **P3** was analyzed by the 2D NMR analysis. The ^1H NMR of peptide **P3** in CDCl_3 (3 mM) revealed well dispersed NH and C^δH , suggesting a well-defined secondary structure in solution. The amino acid types and sequential connectivity of the residues of **P3** were established based on TOCSY and ROESY spectra. The analysis of ROESY spectrum revealed the sequential NOEs between the $\text{NH}(i)\leftrightarrow\text{NH}(i+1)$. Among them, medium $\text{NH}(3)\leftrightarrow\text{NH}(4)$, $\text{NH}(5)\leftrightarrow\text{NH}(6)$ and weak NOEs such as $\text{NH}(1)\leftrightarrow\text{NH}(2)$, $\text{NH}(2)\leftrightarrow\text{NH}(3)$ were observed. In addition to the $\text{NH}\leftrightarrow\text{NH}$ NOEs, $\text{NH}(3)\leftrightarrow\text{C}^\delta\text{H}(2)$ and $\text{NH}(7)\leftrightarrow\text{C}^\delta\text{H}(6)$ were also observed. Furthermore, $[\text{D}_6]\text{DMSO}$ titration in CDCl_3 indicated that only NH of Aib1 is exposed to solvent. The conformation of peptide **P3** in solution was obtained by using distance restraints in ROESY. The ensemble of NMR structures resulting from the MD simulations based on the H-bond constraints and NOE data is shown in Figure 4A. In contrast to the expected 13-helix, peptide **P3** adopted a 13/11-helix conformation in solution. The structure is stabilized by alternating intramolecular $i\rightarrow i+3$ and $i\rightarrow i-1$ mixed H-bonds. Surprisingly, the H-bonds observed in the peptide **P3** displayed opposite directionality. The 13-membered H-bonds are realized between $i\rightarrow i+3$, whereas 11-membered H-bonds are realized between $i\rightarrow i-1$ residues. Similar type of mixed helices are also observed in the α,β -^[4a] and α,γ -^[8g,h,14c] hybrid peptides; however, we have not observed this type of mixed H-bonding directionality in **P2**. The backbone torsion angles for $\beta(\text{O})$ - δ^5 -amino acid in the peptide **P3** together with other reported δ -amino acids are shown in Table 1.

Both theoretical and experimental investigations suggested that a 12-helix from α,γ -hybrid peptides is one of the most

Figure 4. A) Solution structure of peptide **P3** and B) X-ray structure of peptide **P4**. $\beta(\text{O})$ - δ^5 -Phe and γ^4 -Phe are shown in green and cyan in peptide **P4**. The top view structures of peptides are shown in the lower panel.

Table 1. Backbone torsion angles of different δ -amino acids^[a]

	φ [°]	θ_1 [°]	θ_2 [°]	θ_3 [°]	ψ [°]
P2	−92.5	53	79	−158	7.12
P3	60 ± 3	45 ± 5	96 ± 5	-80 ± 10	60 ± 5
P4	−107	61	80	−154	1.8

[a] Present study.

stable helical structures.^[14] To understand whether the α, γ^4 -hybrid peptide 12-helix can accommodate $\beta(\text{O})$ - δ^5 -amino acids as guests, we designed peptide **P4**. Peptide **P4** was composed of 1:1 alternating α - and γ^4 -amino acids except at position 4, where $\beta(\text{O})$ - δ^5 -Phe was incorporated. The X-ray diffraction structure of peptide **P4** is shown in Figure 4A. The crystal structure analysis of **P4** revealed that $\beta(\text{O})$ - δ^5 -amino acid is well accommodated in the helical structure. The helical structure is stabilized by six intramolecular hydrogen bonds.

The γ^4 -residues in **P4** are involved in 12-membered hydrogen-bonding between $(i) \leftarrow (i+3)$ residues with an average H–O distance of 2.1 ± 0.1 Å and N–O distance 3 ± 0.1 Å. The average torsion angle value of the γ^4 -residue is $\varphi = -130 \pm 20^\circ$, $\theta_1(\text{N}-\text{C}^\gamma-\text{C}^\beta-\text{C}^\alpha) = 55 \pm 1^\circ$, $\theta_2(\text{C}^\gamma-\text{C}^\beta-\text{C}^\alpha-\text{C}) = 58 \pm 1^\circ$ and $\psi = -120 \pm 10^\circ$. The stereochemical analysis of γ^4 -Phe residues in the peptide indicates that they adopted *gauche*⁺, *gauche*⁺ (*g*⁺, *g*⁺, $\theta_1 \approx \theta_2 \approx 60^\circ$) local conformations about the $\text{C}^\beta-\text{C}^\gamma$ and $\text{C}^\alpha-\text{C}^\beta$ bonds, which are concurrent with the reported value.^[14a,b] In addition, the helical structure **P4** is also stabilized by the 13-membered H-bond system involving Aib3(*i*) (CO) and NH of

$\gamma^4\text{Phe6} \leftarrow (i+3)$. The $\beta(\text{O})$ - δ^5 -Phe is accommodated in the 13-membered hydrogen-bond system, with average H–O distance: 2.1 ± 0.1 Å, N–O distance: 3 ± 0.1 Å. The torsion angles of the $\beta(\text{O})$ - δ^5 -Phe are $\varphi = -107.17^\circ$, $\theta_1 = 61.88^\circ$, $\theta_2 = 79.95^\circ$, $\theta_3 = -154.25^\circ$ and $\psi = 1.88^\circ$. The backbone torsion values of $\beta(\text{O})$ - δ^5 -Phe residue observed in **P4** are consistent with the values observed in peptide **P2**. In contrast to peptide **P1**, the terminal Aib residue participates in the canonical helix structure. The H-bond parameters and torsion angles of peptide **P4** are given in the Supporting Information. The H-bonding pattern observed in peptides **P3** and **P4** are shown in Figure 5.

Figure 5. H-bonding pattern observed in the solution structure of peptide **P3** and X-ray structure of **P4**.

Conclusion

We have demonstrated the utilization of α -di-peptide mimetics, $\beta(\text{O})$ - δ^5 -amino acids, in the design of hybrid peptides foldamers. Insertion of each $\beta(\text{O})$ - δ^5 -amino acid leads to the removal of two canonical H-bonds in a α -helix. Although the $\beta(\text{O})$ - δ^5 -amino acid can be accommodated into the α -peptide without deviation from the overall helix folding, the deficiency of the two H-bonds forces the molecule to adopt a different type of helix, stabilized by both 10- and 13-membered H-bonds. In addition, the helical structure was also stabilized by the unusual 16-membered H-bond at the C-terminus. The introduction of $\beta(\text{O})$ - δ^5 -amino acid into a α, γ^4 -hybrid peptide leads to a new helix consisting of both 12- and 13-membered H-bonds. In contrast to the anticipated 13-helix, α, δ^5 -hybrid peptide showed a mixed 13/11-helix. Overall, these studies highlight the versatile nature of the $\beta(\text{O})$ - δ^5 -amino acids and suggest that these amino acids can be further explored in the design of proteolytically stable peptidomimetics.

Experimental Section

Materials and methods

All chemicals, reagents and amino acids were purchased from commercially available sources. Hexane, MeOH, DCM and EtOAc were purchased from commercially available sources and distilled prior

to use. Silica gel (120–200 mesh) was used for column chromatography. All final peptides were purified by reverse-phase HPLC. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using the residual solvent signal as internal standard (CDCl_3). Two-dimensional NMR spectra (ROESY and TOCSY) were recorded at 600 MHz. Chemical shifts (δ) and coupling constants (J) are reported in parts per million (ppm) and Hz, respectively. Mass of pure peptides was confirmed by MALDI-TOF/TOF analysis.

Synthesis of *tert*-butyl *N*-Boc- β (O)- δ^5 -amino acid

N-Boc- β (O)- δ^5 -amino acid was synthesized by the reported protocol from *N*-Boc-amino alcohol.^[12,13] Briefly, to a solution containing water (20 mL) and toluene (1:1), sodium hydroxide (250 mmol) was dissolved under ice-cold conditions. Thereafter, *tert*-butyl bromoacetate (15 mmol) was added slowly to the biphasic reaction mixture at 0 °C. The solution containing *N*-Boc-amino alcohol (10 mmol) in toluene (10 mL) was then added slowly to the reaction mixture at 0 °C. The reaction mixture was stirred for about 3 h at RT. After completion of reaction (confirmed by TLC), the organic layer was extracted with diethyl ether (2 × 30 mL), and the combined organic layer was washed with brine (2 × 30 mL) and dried over anhydrous sodium sulfate. The organic layer was then evaporated under reduced pressure to give a white gummy product. The crude product of *tert*-butyl protected *N*-Boc- β (O)- δ^5 -amino acid was purified by column chromatography using EtOAc/pet ether solvent system.

Solid-phase synthesis of peptides P1–P4

Synthesis of peptides P1–P4 were performed on a solid support of Rink amide resin on a 0.2 mmol scale using standard Fmoc based peptide synthesis chemistry. All the coupling reactions were carried out in NMP solvent using HBTU/HOBt as coupling reagents and DIEA as base. After each coupling, deprotection of the Fmoc group was performed by using 20% piperidine in DMF. The *N*-terminal of the peptides were acylated using acetic anhydride in pyridine (3:5). After completion of synthesis, cleavage of the peptide from the resin was achieved by treatment of the resin with a cocktail mixture of TFA/TIPS/ H_2O /phenol (90:5:3:1) for 3 h. After completion of the reaction, the resin was filtered through sintered glass funnel. The crude reaction mixture was then evaporated under vacuum and precipitated out slowly by adding diethyl ether at 0 °C to give a white gummy product. The precipitated crude peptides were centrifuged and the precipitation was dissolved in HPLC grade MeOH and purified by reverse-phase HPLC on a C18 column using MeOH/ H_2O gradient as a solvent system.

Crystallographic information of peptides P1–P4

Peptide P1 (CCDC 1919640 (P1)) contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre).

Crystals of peptide P1 were obtained from the slow evaporation of a saturated solution of methanol/water. A Bruker AXS SMART APEX CCD diffractometer was used for collection of the X-ray data using $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 100 K temperature, ω -scans ($2\theta = 56.66^\circ$), for a total of 75166 independent reflections. Space group $P1$, $a = 10.2137$, $b = 16.143$, $c = 18.663 \text{ \AA}$; $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 102^\circ$; $V = 3008.64 \text{ \AA}^3$; Triclinic, $Z = 1$ for chemical formula $\text{C}_{104} \text{H}_{177} \text{N}_{22} \text{O}_{24}$; $\rho_{\text{calcd}} = 1.170 \text{ g cm}^{-3}$, $\mu = 0.084 \text{ mm}^{-1}$, $F(000) = 1147$. SHELXS-97.1 was used to obtain the structure using the direct method approach. The final R value was 0.1105 ($wR2 = 0.2662$) with 26099 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1385 variables, $S = 1.145$.

Peptide P2 (CCDC 1919644 (P2)) contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre).

Crystals of peptide P2 were obtained from the slow evaporation of a saturated solution of methanol/water. A Bruker AXS SMART APEX CCD diffractometer was used for the collection of the X-ray data using $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 100 K temperature, ω -scans ($2\theta = 56.89^\circ$), for a total of 117916 independent reflections. Space group $P2_1$, $a = 10.419$, $b = 30.242$, $c = 10.627 \text{ \AA}$; $\alpha = 90^\circ$, $\beta = 113.583^\circ$, $\gamma = 90^\circ$; $V = 3068.82 \text{ \AA}^3$, Monoclinic, $Z = 2$ for chemical formula $\text{C}_{51} \text{H}_{86} \text{N}_{10} \text{O}_{14}$, $\rho_{\text{calcd}} = 1.151 \text{ g cm}^{-3}$, $\mu = 0.084 \text{ mm}^{-1}$, $F(000) = 1148$. SHELXS-97.1 was used to obtain the structure using the direct method approach. The final R value was 0.0811 ($wR2 = 0.2107$) with 15326 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 691 variables, $S = 0.999$.

Peptide P4 (CCDC 1919641 (P4)) contains the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre).

Crystals of peptide P4 were obtained from the slow evaporation of a saturated solution of methanol/water. A single crystal with size dimensions of $0.23 \times 0.15 \times 0.11$ was mounted on a small loop with help of paraffin oil. A Bruker AXS SMART APEX CCD diffractometer was used for the collection of the X-ray data using $\text{MoK}\alpha$ radiation ($\lambda = 0.71073 \text{ \AA}$) at 100 K temperature, ω -scans ($2\theta = 43.20^\circ$), for a total of 6348 independent reflections. Space group $P2_1$, $a = 16.198$, $b = 17.843$, $c = 19.642 \text{ \AA}$; $\alpha = 90^\circ$, $\beta = 106.055^\circ$, $\gamma = 90^\circ$; $V = 5455.53 \text{ \AA}^3$, Monoclinic, $Z = 2$ for chemical formula $\text{C}_{104} \text{H}_{152} \text{N}_{16} \text{O}_{21}$, $\rho_{\text{calcd}} = 1.194 \text{ g cm}^{-3}$, $\mu = 0.084 \text{ mm}^{-1}$, $F(000) = 2112$. SHELXS-97.1 was used to obtain the structure using the direct method approach. The final R value was 0.0678 ($wR2 = 0.1878$) with 25616 observed reflections ($F_0 \geq 4\sigma(|F_0|)$) and 1292 variables, $S = 0.920$.

NMR spectroscopy

NMR spectra of all monomers and peptide P3 were carried out with a 400 and 600 MHz spectrometer, respectively. Two-dimensional NMR spectroscopy was carried out by using a reported protocol.^[7f] The phase-sensitive mode was used to collect two-dimensional NMR spectra (ROESY and TOCSY) with the help of time-proportional phase-incrimination (TPPI) methods. All the contours were assigned based on ROESY and TOCSY analysis. For the t_1 and t_2 dimensions, sets of 512 and 1024 data points were used, respectively. During the TOCSY and ROESY data analysis, 32 and 72 transients were collected, respectively. In both the dimensions, 600 MHz spectral width was used. Spin-lock time of 200 and 250 ms were used to obtain the ROESY spectra. Zero-filling was done which finally resulted in a data set of $2 \text{ K} \times 1 \text{ K}$. Before processing, a shifted square sine bell window was used.

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Conflict of interest

The authors declare no conflict of interest.

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