JOC The Journal of Organic Chemistry
Article
pubs.acs.org/joc

Solvent-Directed Switch of a Left-Handed 10/12-Helix into a Right-Handed 12/10-Helix in Mixed β-Peptides

Prashanth Thodupunuri,[†] Sirisha Katukuri,[‡] Kallaganti V. S. Ramakrishna,[‡] Gangavaram V. M. Sharma,[*,†] Ajit C. Kunwar,[‡] Akella V. S. Sarma,[*,‡] and Hans-Jörg Hofmann[*,§]

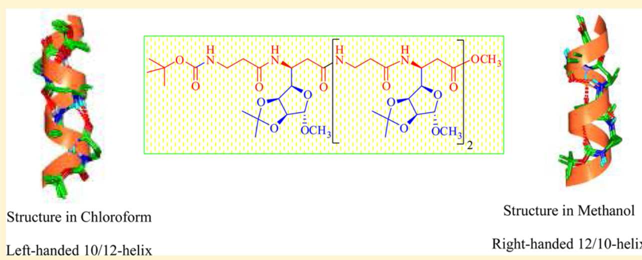
[†]Organic and Biomolecular Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

[‡]Nuclear Magnetic Resonance & Structural Chemistry Division, CSIR-Indian Institute of Chemical Technology, Hyderabad 500 007, India

[§]Institute of Biochemistry, Faculty of Biosciences, University of Leipzig, Brüderstrasse 34, D-04103 Leipzig, Germany

Supporting Information

ABSTRACT: Present study describes the synthesis and conformational analysis of β-peptides from C-linked carbo-β-amino acids [β-Caa$_{(l)}$] with a D-*lyxo* furanoside side chain and β-hGly in 1:1 alternation. NMR and CD investigations on peptides with an (S)-β-Caa$_{(l)}$ monomer at the N-terminus revealed a right-handed 10/12-mixed helix. An unprecedented solvent-directed "switch" both in helical pattern and handedness was observed when the sequence begins with a β-hGly residue instead of a (S)-β-Caa$_{(l)}$ constituent. NMR studies on these peptides in chloroform indicated a left-handed 10/12-helix, while the CD spectrum in methanol inferred a right-handed secondary structure. The NMR data for these peptides in CD_3OH showed the presence of a right-handed 12/10-helix. NMR investigations in acetonitrile indicated the coexistence of both helix types. Quantum chemical studies predicted a small energy difference of 0.3 kcal/mol between the two helix types, which may explain the possibility of solvent influence. Examples for a solvent-directed switch of both the H-bonding pattern and the handedness of foldamer helices are rare so far. A comparable solvent effect was not found in the corresponding peptides with (R)-β-Caa$_{(l)}$ residues, where right-handed 12/10-helices are predominating.



INTRODUCTION

The structural features of peptides and proteins are the basis for their function.[1] However, the application of native peptides for pharmacological and pharmaceutical purposes is often limited by their insufficient stability toward proteases and unfavorable transport properties.[2] To circumvent such problems in peptide design, researchers initiated the use of unnatural amino acids instead of the native α-amino acids. Among the great number of possibilities of amino acid modification, homologous amino acids, in particular β-amino acids, have attracted special attention, at first to substitute single α-amino acid constituents of peptides, later as constituents of peptides completely composed of homologous amino acids.[3] It was a rather surprising and unexpected result to find characteristic secondary structure elements, as for instance helices, in such homologous peptide sequences. The first helices in short sequences of β-amino acids (β-peptides) were reported in 1996[4] and stimulated the field of "foldamers".[5] This field, over a period of time, expanded beyond peptides with homogeneous backbones[6] to peptides with heterogeneous backbones composed of different homologous amino acids, as for instance alternating α- and β-amino acids.[7] Extensive quantum chemical studies were carried out on numerous foldamer classes by several groups.[8] They were complemented by comprehensive molecular dynamics simulations on the conformational and dynamic features of foldamer secondary structures.[8] Experimental and theoretical studies indicate a wide variety of competing secondary structures within the same foldamer class, in particular various helix alternatives with different hydrogen bonding patterns and handedness, but also sheet and turn structures. As known from native α-peptides, several internal and external factors, like steric bulk of the monomers,[9] special side chain interactions,[10] stereochemical patterning,[11] temperature,[12] and solvent polarity as well as concentration,[13] control the secondary structure formation and decide about the final peptide structure.

The first examples of β-peptide helices showed hydrogen-bonded rings of the same size with all hydrogen bonds pointing in backward or, alternatively, in forward direction along the sequence. A novel helix type was presented by Seebach et al.[14] in sequences of alternating β^2- and β^3-amino acids. In these peptides, helices with alternating 12- and 10-membered (12-mr and 10-mr) hydrogen-bonded rings and an alternating change of the hydrogen bond direction appear, which were called "mixed" helices. Since these helices can either begin with a 10-

Received: November 30, 2016
Published: January 17, 2017

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

or a 12-membered hydrogen-bonded ring, two alternative mixed helices are possible, which differ in stability according to theoretical calculations.[8c,f,h−j] The competition between these two structures may be influenced by the above-mentioned structural and external factors.

On the basis of theoretical predictions, we have reported the synthesis of *β*-peptides[15] composed of the "epimeric" (*R*)- and (*S*)-C-linked carbo *β*-amino acids [(*R*)- and (*S*)-*β*-Caa$_{(x)}$][16] in 1:1 alternate order. These amino acids bear a D-*xylo* furanoside side chain. In these peptides, the two above-mentioned mixed helix alternatives were found for the first time as right-handed 12/10- and 10/12-helices, indicating a "switch" in helical conformation caused by the "epimeric" nature of the monomers. In the meantime, diverse amino acids in the design of mixed *β*-peptides[17] and further motifs for a design of 12/10- and 10/12-helices were suggested by us[18] and other authors.[19]

In this context, Kessler et al.[20] obtained a 12/10-helix in *β*-peptides alternately composed of furanoid sugar amino acid (fSAA) and the simplest *β*-amino acid *β*-hGly (**11**), which is achiral. In comparable studies, we combined the above-mentioned (*R*)- and (*S*)-*β*-Caa$_{(x)}$ residues bearing a D-*xylo* furanoside side chain with *β*-hGly in 1:1 alternation. In sequences of (*R*)-*β*-Caa$_{(x)}$ and *β*-hGly, we obtained a right-handed 12/10-helix if the sequence begins with an (*R*)-*β*-Caa$_{(x)}$ constituent, whereas a right-handed 10/12-helix resulted in peptides with *β*-hGly at the N-terminus. In peptide sequences of the corresponding (*S*)-*β*-Caa$_{(x)}$ amino acid and *β*-hGly,[21] a switch of handedness occurred and left-handed 12/10-helices resulted with an (*S*)-*β*-Caa$_{(x)}$ at the N-terminus, but left-handed 10/12-helices in sequences beginning with *β*-hGly. This was the first report on the formation of left-handed 10/12-helices and the results clearly showed the impact of the side chain on helix handedness. It is interesting that stable 12/10-helix structures were obtained in oligomers consisting only of *β*-hGly constituents by capping with a chiral amino acid or a short helical peptide.[21]

The above findings stimulated the present study to investigate the helix formation in mixed *β*-peptides **1**−**8** (Figure 1), prepared from (*S*)- and (*R*)-*β*-Caa$_{(l)}$s **9** and **10** with a D-*lyxo* furanoside side chain and *β*-hGly **11** in 1:1 alternation and to compare the results with the former data reported for the corresponding peptides with D-*xylo* furanoside side chain.[21] The synthesis of peptides composed of *β*-Caa$_{(l)}$s with a D-*lyxo* furanoside side chain was already reported by us[22] in the design of Helix-Turn-Helix (HTH) motifs, using *β*-hGly residues in the turn region.

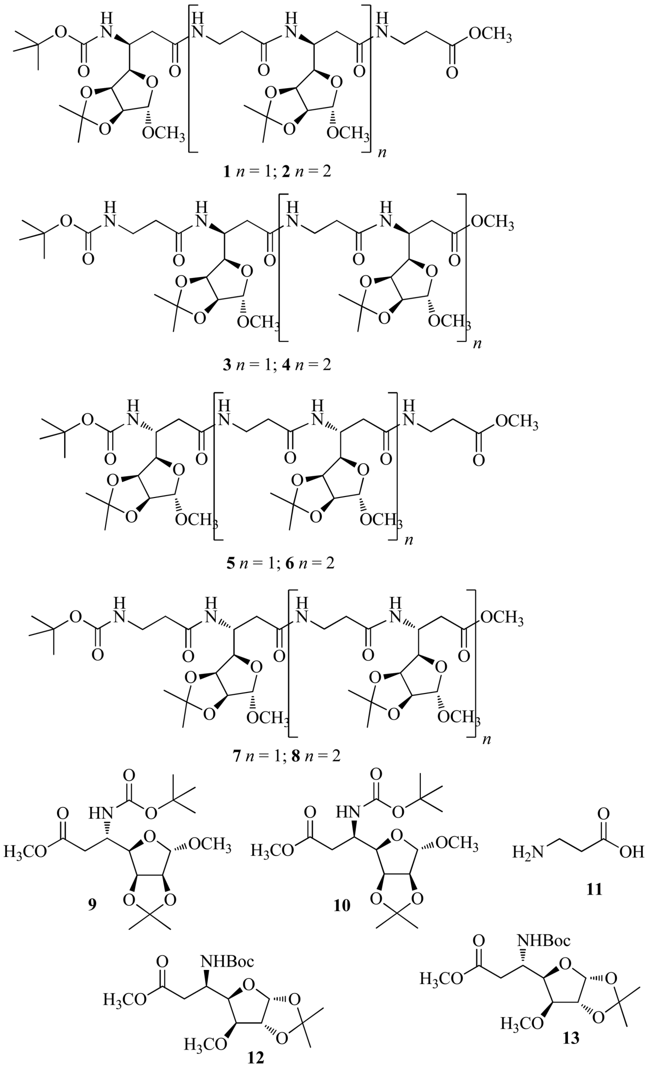


Figure 1. Structures of the peptides **1**−**8** and their *β*-amino acid constituents **9** and **10** with a D-*lyxo* furanoside side chain and *β*-hGly **11** (For comparison with **9** and **10**, the *β*-amino acids **12** and **13** with a D-*xylo* furanoside side chain are given).

■ RESULTS AND DISCUSSION

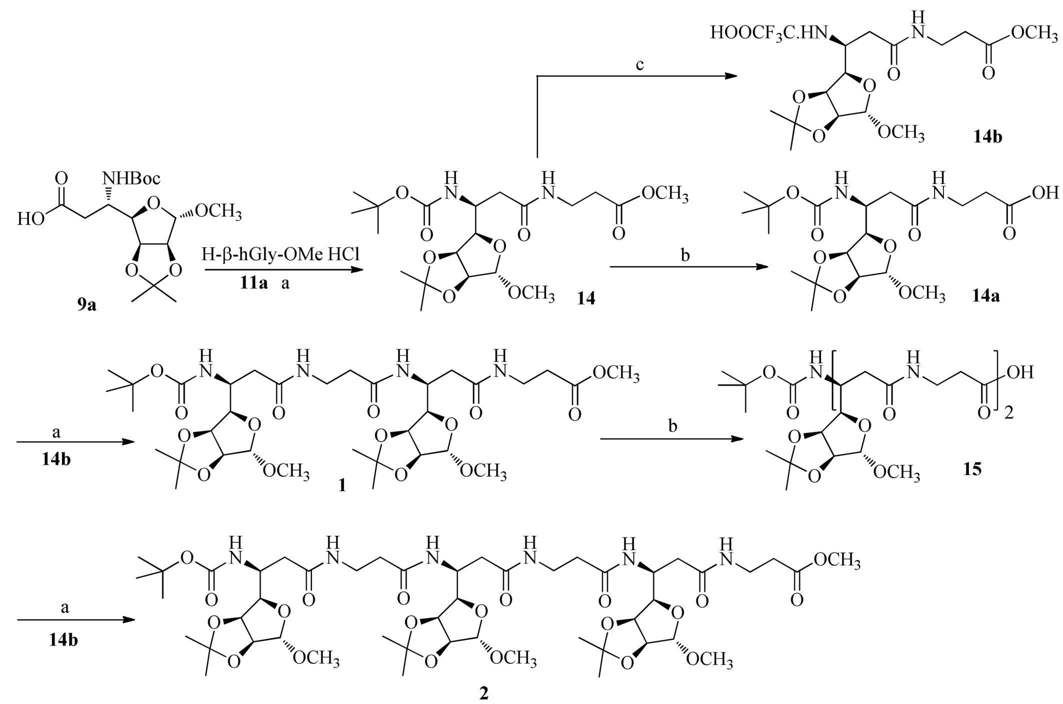
Synthesis of Peptides 1−8. Peptides **1**−**8** were prepared by standard peptide coupling methods,[23] using EDCI, HOBT and DIPEA in solution phase. The synthesis of peptides **1** and **2** was achieved from Boc-(*S*)-*β*-Caa$_{(l)}$-OMe **9** and **11**. Accordingly, acid **9a** (prepared from **9**) on reaction with the salt **11a** (prepared from **11**) in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **14** in 67% yield (Scheme 1). Treatment of peptide **14** with base (LiOH) furnished the acid **14a**, while reaction of **14** with CF_3COOH in CH_2Cl_2 gave the salt **14b**. Acid **14a** on coupling as above with the salt **14b** in CH_2Cl_2 afforded the tetrapeptide **1** in 55% yield. Ester **1** on base hydrolysis with LiOH furnished the acid **15**, which on further coupling with the salt **14b** gave the hexapeptide **2** in 49% yield.

Likewise, for the synthesis of peptides **3** and **4**, ester **9** was treated with CF_3COOH to furnish salt **9b**. Coupling of acid **11b** with salt **9b** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 gave the dipeptide **16** in 57% yield (Scheme 2). Base (aq. LiOH) hydrolysis of **16** afforded **16a**, while **16** on reaction with CF_3COOH in CH_2Cl_2 furnished **16b**. Coupling (EDCI, HOBt, and DIPEA) of acid **16a** with salt **16b** in CH_2Cl_2 provided the tetrapeptide **3** (48%). Base hydrolysis of **3** with LiOH furnished the acid **17**, which on further coupling with the salt **16b** gave the hexapeptide **4** in 45% yield.

Peptides **5** and **6** were synthesized from Boc-(*R*)-*β*-Caa$_{(l)}$-OH **10a** and **11** (Scheme 3). Accordingly, acid **10a** (prepared from **10**) on coupling (EDCI, HOBt, and DIPEA) with the salt **11a** in CH_2Cl_2 afforded the dipeptide **18** in 65% yield. Peptide **18** on base hydrolysis with aq. LiOH furnished **18a**, while treatment of **18** with CF_3COOH in CH_2Cl_2 gave the salt **18b**. Coupling (EDCI, HOBt, and DIPEA) of acid **18a** with the salt **18b** in CH_2Cl_2 provided the tetrapeptide **5** in 52% yield. Ester **5** on base hydrolysis with LiOH afforded the acid **19**, which on further reaction with the salt **18b** furnished hexapeptide **6** in 44% yield.

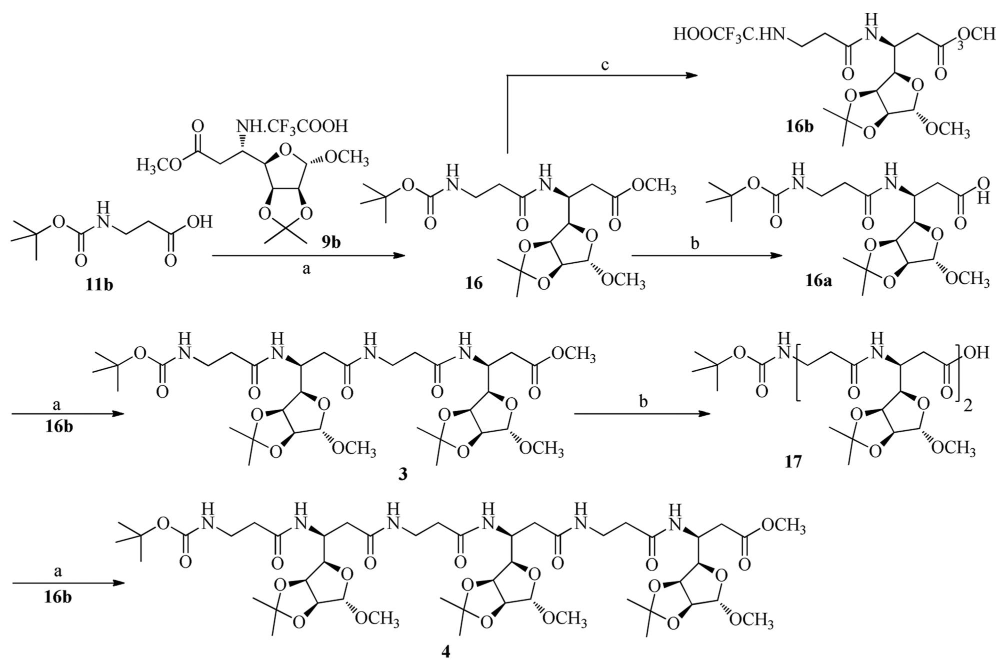
DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

Scheme 1. Synthesis of Hybrid Peptides 1–2[a]



[a]Reagents and conditions: (a) HOBt (1.2 equiv), EDCI (1.2 equiv), D1PEA (2 equiv), dry CH_2Cl_2, 0 °C to rt, 8 h; (b) LiOH, THF:MeOH:H_2O (3:1:1), 0 °C to rt, 1 h; (c) CF_3CO_2H, dry CH_2Cl_2, 2 h.

Scheme 2. Synthesis of Hybrid Peptides 3–4[a]

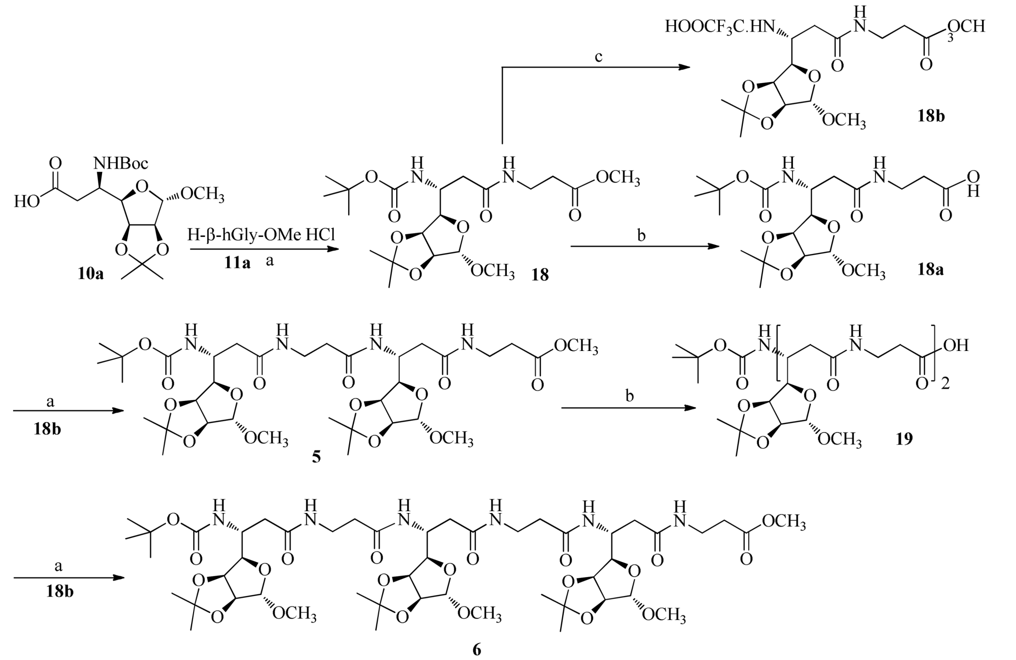


[a]Reagents and conditions: (a) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C to rt, 8 h; (b) EiOH, THE:MeOH:H_2O (3:1:1), 0 °C to rt, 1 h; (c) CF_3CO_2H, dry CH_2Cl_2, 2 h.

Similarly, for the synthesis of peptides **7** and **8**, ester **10** was treated with CF_3COOH to give salt **10b**. Acid **11b** on coupling with **10b** in the presence of EDCI, HOBt, and DIPEA in CH_2Cl_2 afforded the dipeptide **20** in 57% yield (Scheme 4). Peptide **20** on base hydrolysis with LiOH gave **20a**, while reaction of **20** with CF_3COOH in CH_2Cl_2 afforded **20b**. Coupling (EDCI, HOBt, and DIPEA) of acid **20a** with salt **20b** in CH_2Cl_2 furnished the tetrapeptide **7** in 43% yield (Scheme 3). Base hydrolysis of **7** with LiOH gave the acid **21**, which on further coupling with the salt **20b** provided the hexapeptide **8** in 41% yield.

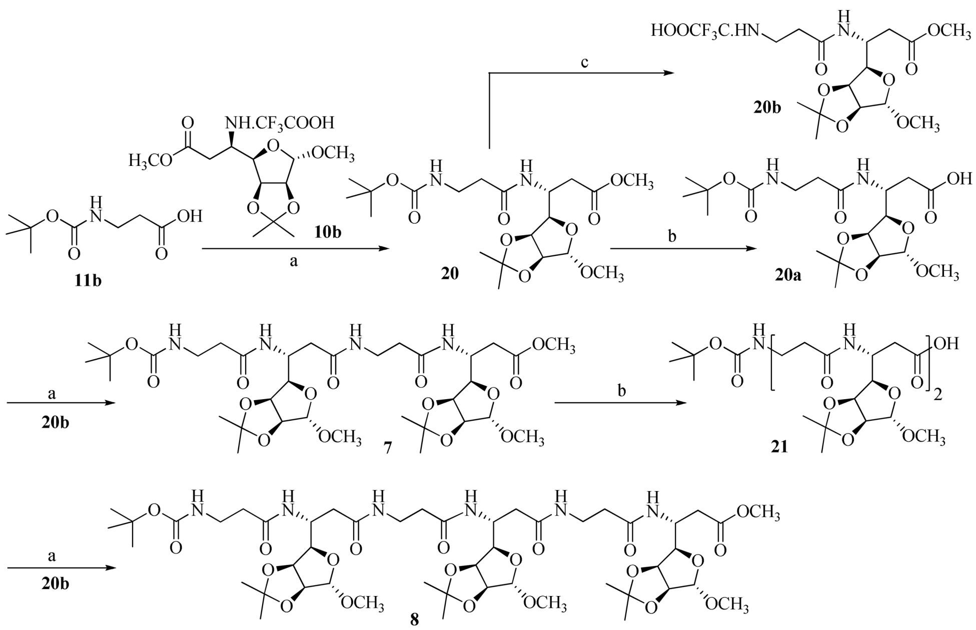
Conformational Analysis. The NMR spectra of peptides **1–8** were recorded as 3–5 mM solutions in $CDCl_3$.[24] The proton NMR spectra of peptides **1** and **2** with (*S*)-*β*-$Caa_{(l)}$ at

Scheme 3. Synthesis of Hybrid Peptides 5–6[a]



[a]Reagents and conditions: (a) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C to rt, 8 h; (b) LiOH, THF:MeOH:H_2O (3:1:l),0 °C to rt, 1 h; (c) CF_3CO_2H, dry CH_2Cl_2, 2 h.

Scheme 4. Synthesis of Hybrid Peptides 7–8[a]



[a]Reagents and conditions: (a) HOBt (1.2 equiv), EDCI (1.2 equiv), DIPEA (2 equiv), dry CH_2Cl_2, 0 °C to rt, 8 h; (b) LiOH, THF:MeOH:H_2O (3:1:1), 0 °C to rt, 1 h; (c) CF_3CO_2H, dry CH_2Cl_2, 2 h.

the N-terminus showed a good dispersion in the amide region. The appearance of NH(1) signals at δ > 5.68 ppm for peptide **1** and δ > 6.14 ppm for peptide **2** as well as the presence of a weak NOE correlation NH(1)/NH(2) suggested the participation of NH(1) in 10-mr hydrogen bonding. Solvent titration studies[25] on peptides **1** and **2** with DMSO-d_6 indicated that, except NH(2), all amide protons, participate in H-bonding.

For peptide **1**, the long-range backbone NOE interactions CβH(2)/NH(4), CβH(2)/CαH$_{(pro\text{-}R)}$(4) and the weak NH(1)/NH(2) and NH(3)/NH(4) NOE correlations provided ample evidence for a right-handed 10/12-helical structure, while the CD profile did not show distinct structural features. However, the hexamer **2** exhibited a definite structure in $CDCl_3$.[24] The presence of CβH(2)/NH(4), CβH(2)/CαH$_{(pro\text{-}R)}$(4), CβH(4)/NH(6), and CβH(4)/CαH$_{(pro\text{-}R)}$(6) NOE correlations in the ROESY spectrum (Figure 2) suggests the existence of 12-mr hydrogen-bonded rings, involving H-bonds from NH(4) to CO(1) and NH(6) to CO(3).

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018–2031

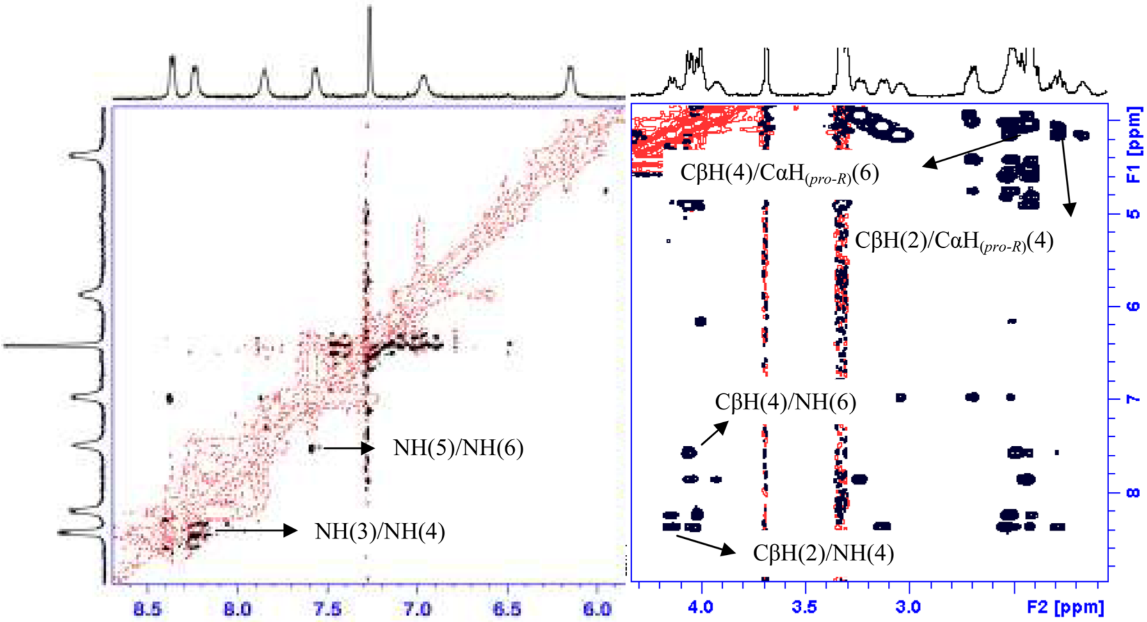


Figure 2. ROESY spectrum of peptide **2** depicting characteristic NOE correlations.

In addition, the weak NH(3)/NH(4) and NH(5)/NH(6) NOE correlations indicate the presence of 10-mr rings with H-bonds between NH(3) and CO(4) as well as NH(5) and CO(6). All the NOE correlations are characteristic for a right-handed 10/12-helical structure in **2**. For all the *β*-Caa residues, a large $^{3}J_{C\beta H/C4H}$ coupling constant implies the preponderance of a structure with $\chi^1 \approx 180°$.

MD simulations on peptide **2** on the basis of the available NMR data revealed the presence of a right-handed 10/12-helix with heavy atom and backbone rmsd values of 0.67 and 0.41 Å, respectively (Figure 3). Although the NH(1)/NH(2) correlation is very weak according to the ROESY spectrum, the MD minimized structures display a distance of 2.5 Å between NH(1) and NH(2), indicating a 10-membered hydrogen-bonded cycle between NH(1) and CO(2). The average backbone dihedral angles for peptide **2** are listed in Table 1.

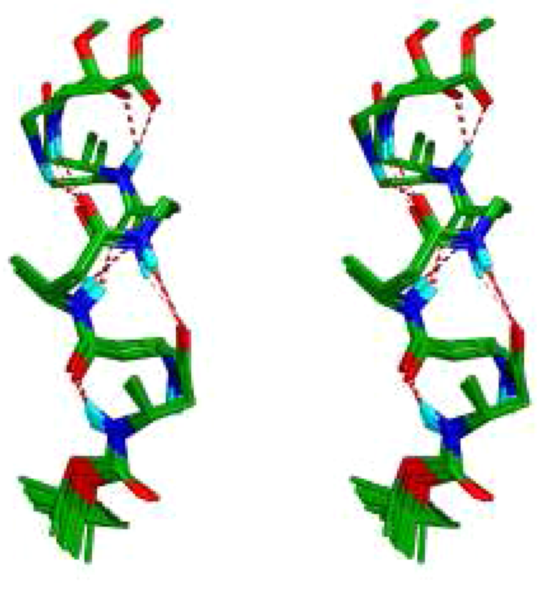


Figure 3. Stereoview of a superimposition of 20 minimum energy structures for peptide hexamer **2** from MD simulations based on NMR data in $CDCl_3$ (hydrogen atoms removed for clarity).

Table 1. Average Backbone Dihedral Angles[a] in the Tetra- and Hexapeptides 1, 2, 3, and 4 from the MD Simulations on the Basis of the NMR Data in $CDCl_3$ and from Quantum Chemical Studies at the B3LYP/6-31G* Level of Density Functional Theory

		β-Caa(l)			*β*-hGly		
peptide[b]	helix type[c]	*φ*	*θ*	*ψ*	*φ*	*θ*	*ψ*
1[d]	*rh*-10/12	86	64	−84	−102	62	84
2[d]	*rh*-10/12	88	65	−100	−105	62	85
2[e]	*rh*-10/12	92.0	61.3	−99.9	−98.6	61.3	91.2
3[d]	*lh*-10/12	98	−67	−76	−83	−65	95
4[d]	*lh*-10/12	100	−66	−83	−80	−60	97
4[e]	*lh*-10/12	100.4	−59.4	−85.0	−89.7	−67.1	105.4

[a]In degrees. [b]See Figure 1; A = *β*-hGly, S = (*S*)-*β*-Caa(l). [c]*rh*: right-handed, *lh*: left-handed. [d]NMR study in $CDCl_3$. [e]DFT//B3LYP/6-31G* level; averaged over residues 2 and 4 and 3 and 5, respectively.

In agreement with the NMR data, the right-handed 10/12-helix of the peptide hexamer **2** is most stable among the four mixed helix alternatives with 10- and 12-mr hydrogen-bonded rings in a systematic quantum chemical conformational analysis at the B3LYP/6-31G* level of density functional theory (DFT) (Table 2; backbone torsion angles in Table 1, for details of the quantum chemical conformational search, see Experimental Section).

Support for this structure comes also from CD studies in methanol. The CD profile of peptide **2** shows a characteristic signature[21] with a positive molar ellipticity at 202 nm that corresponds to a right-handed 10/12-helical structure.[24]

Different from this result, a left-handed 12/10-helix was found in the earlier studies on the corresponding peptides with (*S*)-*β*-Caa(x) bearing a D-*xylo* furanoside side chain.[21] Initial studies on the monomeric (*S*)- and (*R*)-*β*-Caa(l) **9** and **10**

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

Table 2. Relative Energies in kcal/mol of the Mixed 10/12- and 12/10-Helix Alternatives of the Peptides 2, 4, 6 and 8 Consisting of (*S*)- and (*R*)-*β*-Caa$_{(l)}$s Bearing a D-*lyxo* Furanoside Side Chain and *β*-hGly in 1:1 Alternate Order at the B3LYP/6-31G* Level of Density Functional Theory

peptide[a]	*rh*-10/12[b]	*lh*-10/12[b]	*rh*-12/10[b]	*lh*-12/10[b]
2	**0.0**[e]	9.8	–[d]	8.6
4	–[d]	**0.0**[g]	0.3	10.9
6	7.6	**0.0**[c]	0.4	–[d]
8	**0.0**[f]	–[d]	10.3	8.9

[a]See Figure 1. [b]*rh* = right-handed; *lh* = left-handed. [c]E_T = −3784.424222 au. [d]Not possible from steric reasons. [e]E_T = −3784.435426 au. [f]E_T = −3784.430419 au. [g]E_T = −3784.413737 au.

(Figure 1) with a D-*lyxo* furanoside side chain showed a similarly puckered conformation for the side chain with the C4 atom in the ring plane. In contrast, in the monomeric (*S*)- and (*R*)-*β*-Caa$_{(x)}$s **12** and **13** with a D-*xylo* furanoside side chain (Figure 1), the C4 atom is out of plane. The different arrangement of the C4 atom is also confirmed for the peptides **1** and **2** with D-*lyxo* furanoside side chain and the corresponding peptides with xylose side chain,[21] which might be the origin of the differences in helix formation in these two peptide classes.

The study is further extended to peptides **3** and **4** with *β*-hGly at the N-terminus. It is found that both peptides revealed downfield shifts of amide protons with good dispersion in their proton NMR spectra in $CDCl_3$.[24] Solvent titration studies[25] with DMSO-d_6 showed that several amide protons participate in hydrogen bonding, indicated by small chemical shift changes. The solvent titration and NOE correlation data suggest a left-handed 10/12-helix structure for tetrapeptide **3**. To understand the unusual behavior, hexapeptide **4** was studied in detail. The distinctive NOE correlations C*β*H(2)/NH(4) and C*β*H(4)/NH(6) shown in the ROESY spectrum of peptide **4** (Figure 4) confirm the existence of 12-mr hydrogen-bonded rings between CO(1)···NH(4) and CO(3)···NH(6). Further, the correlations NH(3)/NH(4) and NH(5)/NH(6) reveal the presence of 10-mr hydrogen bonding between NH(3) and CO(4) and NH(5) and CO(6), respectively. For the three *β*-Caa residues, a large $^3J_{C\beta H/C4H}$ coupling constant implies the preponderance of a structure with $\chi^1 \approx 180°$. The crucial NOE correlations, C*β*H(2)/C*α*H$_{(pro\text{-}S)}$(4) and C*β*H(4)/C*α*H$_{(pro\text{-}S)}$(6), which determine the handedness of the observed helix, confirm a left-handed helical structure. In addition, the MD simulations on the basis of the NMR information also suggest a left-handed 10/12-helix in $CDCl_3$. The average backbone dihedral angles from the analysis of the NMR data and the quantum chemical calculations are given in the Tables 1 and 3.

Table 3. Average Backbone Dihedral Angles[a] of the Right-Handed 12/10- and the Left-Handed 10/12-Helices of Peptide 4 from MD Simulations on the Basis of the NMR Data in CD_3OH and CD_3CN Solvents in Comparison with Quantum Chemical Data at the B3LYP/6-31G* Level of Density Functional Theory

		β-Caa$_{(l)}$			*β*-hGly		
peptide[b]	helix type[c]	*φ*	*θ*	*ψ*	*φ*	*θ*	*ψ*
4[d]	*rh*-12/10	106	61	−100	−106	60	81
4[e]	*rh*-12/10	90	55	−86	−107	60	92
4[f]	*rh*-12/10	91.8	62.8	−102.7	−100.7	59.6	88.8
4[e]	*lh*-10/12	108	−65	−86	−93	−63	97

[a]In degrees. [b]See Figure 1; A = *β*-hGly, S = (*S*)-*β*-Caa$_{(l)}$. [c]*rh*: right-handed, *lh*: left-handed. [d]NMR study in CD_3OH. [e]NMR study in CD_3CN. [f]DFT//B3LYP/6-31G* level; averaged over residues 2 and 4 and 3 and 5, respectively.

The CD profile of peptide **4** in methanol shows a maximum at 202 nm (Figure 5), which corresponds unequivocally to a

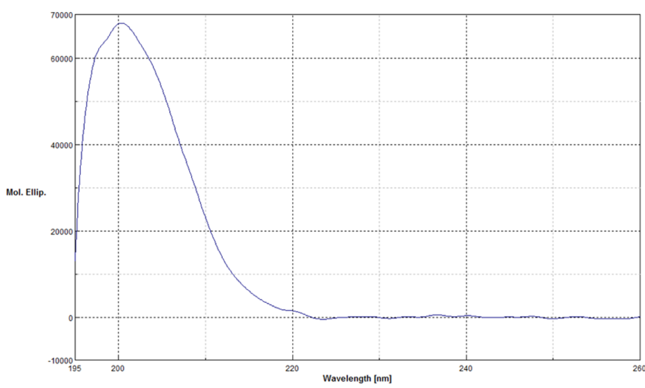


Figure 5. CD spectrum of peptide **4** in methanol showing a positive molar ellipticity.

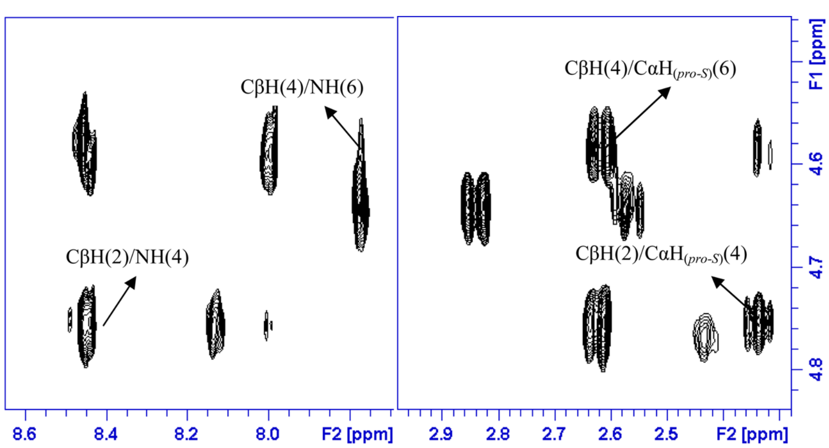


Figure 4. ROESY spectrum of peptide **4** in $CDCl_3$ showing the characteristic correlations for a left-handed 10/12- helix.

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

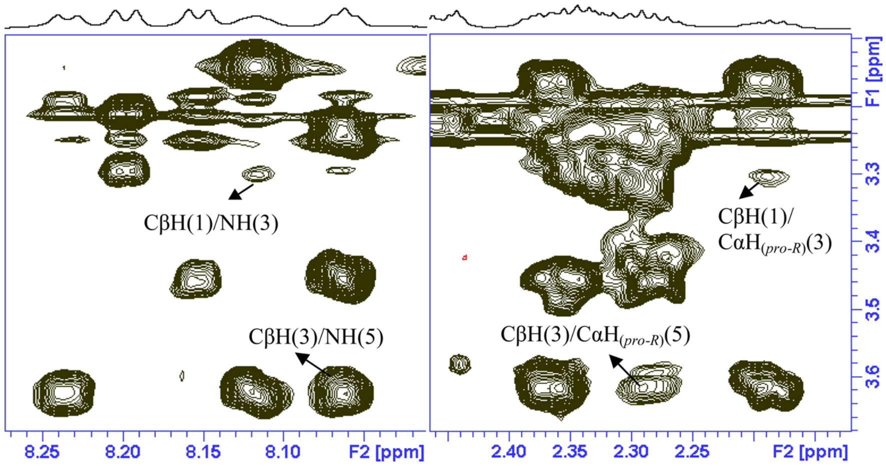


Figure 6. ROESY spectrum of peptide **4** in CD_3OH with the crucial NOE correlations labeled.

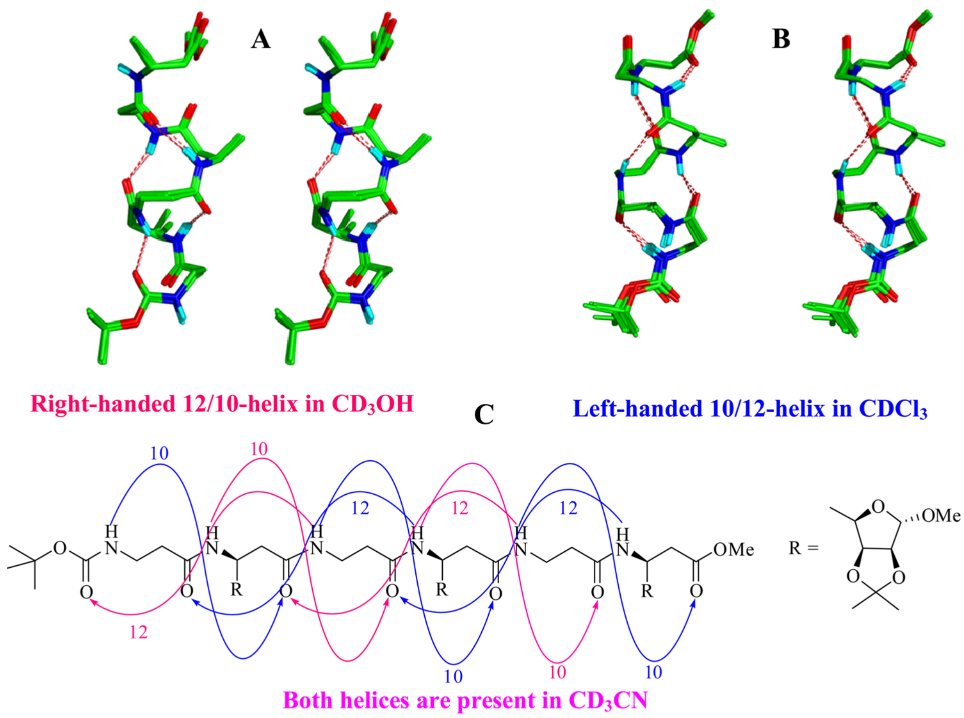


Figure 7. Stereoview of a superimposition of the 20 minimum energy structures of peptide **4**: (A) right-handed 12/10- and (B) left-handed 10/12-helices from the MD simulations on the basis of the available NMR data for CD_3CN (hydrogen atoms removed for clarity); (C) H-bonding interactions of all the helices in different solvents.

right-handed helical structure. This is in contrast to the left-handed 10/12-helix structure found by NMR in the solvent $CDCl_3$. Obviously, a change of the solvent induces a change of handedness.

These unusual findings prompted us to undertake an NMR study on peptide **4** in methanol (CD_3OH), a polar protic solvent, to understand the contradictory CD results in the two solvents. The NOE correlations $C\beta H(1)/C\alpha H_{(pro\text{-}R)}(3)$ and $C\beta H(3)/C\alpha H_{(pro\text{-}R)}(5)$ from the ROESY spectrum of **4** in CD_3OH (Figure 6) clearly support the right-handed structure in agreement with the CD profile. In addition, the correlations $C\beta H(1)/NH(3)$, $C\beta H(3)/NH(5)$, NH(2)/NH(3), and NH(4)/NH(5) support a right-handed 12/10-helix, resembling structures observed earlier.[21] The 12/10-helix remains stable in the MD simulations. Its average backbone torsion angles are given in Table 3.[24]

The theoretical conformational analysis on peptide **4** predicts the left-handed 10/12- and the right-handed 12/10-helices as approximately equivalent in energy (Table 2), suggesting that external factors like solvents may easily influence the structure equilibrium in favor of the one or the other helical alternative.

The observation of a change of the hydrogen bonding pattern and a "switch" in handedness from a left-handed 10/12-helix to a right-handed 12/10-helix by the change of the solvent from $CDCl_3$ to CD_3OH is rather interesting and very unusual. Some relevance for the present work have the results by Fülöp and co-workers,[26] who found a switch from an 18-helix into a 12-helix in β-peptides in the solvent methanol after dilution. Recently, a switch from an M- to a P-helix for poly(quinoxaline-2,3-diyl) copolymers was reported by a change from the solvent benzene to trifluorobenzene.[27]

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

The results inspired us to investigate the behavior of peptide **4** in a solvent with properties different from the other two solvents. Therefore, the NMR study was repeated in the polar aprotic solvent acetonitrile (CD_3CN). Interestingly, the ROESY spectrum of peptide **4** in CD_3CN displays information corresponding to the presence of both mixed helices (see Supporting Information). Thus, the NOE correlations C*β*H(2)/NH(4), C*β*H(4)/NH(6), C*β*H(2)/C*α*$H_{(pro\text{-}S)}$(4), C*β*H(4)/C*α*$H_{(pro\text{-}S)}$(6), NH(3)/NH(4), and NH(5)/NH(6) correspond to a left-handed 10/12-helix, whereas, the NOE correlations C*β*H(1)/NH(3), C*β*H(3)/NH(5), C*β*H(1)/C*α*$H_{(pro\text{-}R)}$(3), C*β*H(3)/C*α*$H_{(pro\text{-}R)}$(5), NH(2)/NH(3), and NH(4)/NH(5) unequivocally indicate the existence of the right-handed 12/10-helix alternative.

The data sets from the analysis of the NMR spectrum in CD_3CN were the basis for MD simulations, which support the presence of both helices in this solvent. The overlays of the minimum energy structures of the left-handed 10/12- and the right-handed 12/10-helices are displayed in Figure 7A and 7B along with various hydrogen bond interactions in different solvents (Figure 7C). The average backbone dihedral angles resulting from the MD simulations based on the NMR data for acetonitrile are listed in Table 3 together with those estimated for methanol and the quantum chemical data.

These results clearly demonstrate the role of solvent−solute interactions for structure formation. The switch of the handedness in peptide **4** going from solvent $CDCl_3$ to solvent CD_3OH is a nice example for the importance of such interactions. Phenomena like this become especially visible when the competing structures are of comparable stability.

On the basis of the results presented above, it seemed to be worthwhile to continue the study with the corresponding peptides composed of (*R*)-*β*-$Caa_{(l)}$ and *β*-h-Gly constituents. A detailed spectral analysis of the peptides **5**−**8** suggests a right-handed 12/10-helix for the peptides **5** and **6** with (*R*)-*β*-$Caa_{(l)}$ at the N-terminus, and a right-handed 10/12-helix for the peptides **7** and **8** with *β*-hGly at the N-terminus. Again, molecular dynamics simulations on the basis of the NMR data[24] confirmed these structures. The average backbone dihedral angles for the preferred mixed helix conformers of the peptides **5**, **6** and **7**, **8** resulting from the MD simulations are given in Table 4.

The right-handed 12/10-helix of the peptide hexamer **6**, indicated by NMR spectroscopy, is only by 0.4 kcal/mol more unstable than the left-handed 10/12-helix alternative, which was predicted as the most stable among the four mixed helix alternatives with 10- and 12-mr hydrogen-bonded rings according to a systematic quantum chemical conformational analysis at the B3LYP/6-31G* level of density functional theory (DFT) (Table 2). Despite the relatively small energy difference between both helix alternatives, a comparable solvent effect as found for the (*S*)-*β*-$Caa_{(l)}$ peptides was not observed. In agreement with the NMR data, the quantum chemical conformational analysis provides the right-handed mixed 10/12-helix as most stable helix conformer for the peptide hexamer **8**. The average backbone torsion angles for the peptides **6** and **8** arising from the quantum chemical geometry optimizations are compared with those from the MD simulations in Table 4.

Table 4. Average Backbone Dihedral Angles[a] in the Tetra- and Hexapeptides 5, 6 and 7, 8 from the MD Simulations on the Basis of the NMR Data in $CDCl_3$ and from Quantum Chemical Studies at the B3LYP/6-31G* Level of Density Functional Theory

		β-$Caa_{(l)}$			*β*-hGly		
peptide[b]	helix type[c]	*φ*	*θ*	*ψ*	*φ*	*θ*	*ψ*
5[d]	*rh*-12/10	−101	65	82	91	65	−103
6[d]	*rh*-12/10	−87	70	96	110	67	−81
6[e]	*rh*-12/10	−94.9	54.5	93.3	85.8	64.3	−93.7
7[d]	*rh*-10/12	−106	65	86	97	75	−110
8[d]	*rh*-10/12	−108	67	85	87	62	−106
8[e]	*rh*-10/12	−99.1	63.5	89.6	86.8	63.5	−104.0

[a]In degrees. [b]See Figure 1; A = *β*-hGly, R = (*R*)-*β*-$Caa_{(l)}$. [c]*rh*: right-handed, *lh*: left-handed. [d]NMR study in $CDCl_3$. [e]DFT//B3LYP/6-31G* level; averaged over residues 2 and 4; 3 and 5, respectively.

The structure data for peptides **5**, **6** and **7**, **8** are similar to those observed for the corresponding peptides with (*R*)-*β*-$Caa_{(x)}$ constituents bearing a D-*xylo* furanoside side chain.[21] Interestingly, the molar ellipticities in the CD profiles of the peptides with D-*lyxo* furanoside side chains are higher than found for the corresponding peptides with a D-*xylo* furanoside side chain, which hints to more stable secondary structures in the peptides with the D-*lyxo* furanoside side chains.

■ CONCLUSIONS

The present study describes the synthesis and conformational analysis of a new series of peptides **1**−**8** composed of *β*-hGly in 1:1 alternation with (*S*)- or (*R*)-*β*-Caa bearing a D-*lyxo* furanoside side chain. The peptides with an (*S*)-*β*-$Caa_{(l)}$ monomer at the N-terminus reveal the presence of a right-handed 10/12-helix, as supported by a theoretical study. Especially interesting are the results for the peptides with a *β*-hGly residue at the N-terminus in alternation with (*S*)-*β*-$Caa_{(l)}$ constituents. The NMR studies show a left-handed 10/12-helix in the solvent $CDCl_3$, while CD spectrum of this peptide in solvent MeOH indicates the opposite handedness. Indeed, the NMR studies in the more polar solvent CD_3OH suggest the presence of a right-handed 12/10-helix. This is an unprecedented "switch" between two helical structures with different hydrogen bonding pattern and different handedness, which is reported in 12/10-helices for the first time. Moreover, the structural features of both helix types were observed in the polar aprotic solvent acetonitrile. The unusual solvent effect can well be understood by looking at the approximately energetic equivalence of both helices according to quantum chemical calculations. To the best of our knowledge, no other examples with such remarkable effects of an excessive change of helix type and handedness by a mere solvent change were found until now.

The peptides composed of (*R*)-*β*-$Caa_{(l)}$ constituents in alternation with *β*-hGly revealed results, that are in agreement with data of former investigations on the corresponding peptides with (*R*)-*β*-$Caa_{(x)}$ residues bearing a D-*xylo* furanoside side chain.

The data of this study demonstrate several possibilities to influence helix type and handedness, as for instance by a different configuration of the amino acid constituents and the variation of their side chains and even by the nature of the solvent. The study shows the wide variety of possibilities to realize special types of secondary structures. Thus, experimental and theoretical methods may efficiently contribute to a rational peptide and protein design in a concerted action.

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

■ EXPERIMENTAL SECTION

All the NMR spectra (1D and 2D experiments) were obtained on 300 MHz, 400 MHz, 500 MHz, −600 MHz, and 700 MHz spectrometers at 278−303° K, sample concentrations were 3−5 mM in $CDCl_3$, CD_3OH, and CD_3CN solvents, using tetramethylsilane (TMS) as internal standard. The chemical shifts are shown in δ scales. All chemical shifts were measured from 1D NMR spectra and the coupling constants were measured with resolution-enhanced 1D spectrum. The proton resonance assignments were carried out by using two-dimensional total correlation spectroscopy (TOCSY) and rotating frame Overhauser effect spectroscopy (ROESY) experiments in phase sensitive mode. The TOCSY experiments were performed with a mixing time of 0.08 s, whereas the ROESY experiments, which provide the spatial proximity of the protons, were performed with mixing times of 0.2−0.4 s. TOCSY and ROESY spectra were acquired with 4096 complex data points in the t2 domain and 192 or 256 increments in the t1 domain containing 16−24 scans with relaxation delay of 1.5 s. Information on the H-bonding in $CDCl_3$ was obtained from solvent titration studies at 298° K by sequentially adding up to 300 μL of DMSO-d_6 in 600 μL of $CDCl_3$ solution of the peptides. The two-dimensional data were processed with shifted sine bell or Gaussian apodization in both dimensions. The acquired free induction decays (FID) were zero-filled during processing to give a final matrix of 2048 × 2048. The CD spectra of peptides in MeOH (0.2 mM) were recorded in quartz cells (2 mm path length) at room-temperature within a scan range of 190−260 nm and a scanning speed of 20 nm/min. All spectra represent one scan, each of 100 ms time constant and are background-corrected and smoothened over 2−5 data points using binomial method.

Model building and restrained molecular dynamics simulations[28] on peptides **1**−**8** were carried out employing the Insight II (97.0)/Discover program.[29] The CVFF force field was used throughout the simulations with default parameters using a distance-dependent dielectric constant with $\varepsilon = 4.8$ for chloroform. The distance restraints were obtained from the volume integrals in the ROESY spectra[30] using a two-spin approximation and a reference distance of 1.8 Å for geminal protons. The dihedral angles were estimated from three-bond proton−proton J-couplings, but were not used in the calculations. The complete sets of NOE distance constraints considered for the structure calculations are given in the Supporting Information. The initial structures were minimized by steepest descent algorithm at first and followed by conjugate gradient method for a maximum of 1000 iterations each or an RMS deviation of 0.001 kcal/mol, whichever was earlier. The energy-minimized structures were the starting point for the MD simulations. A number of interatomic distance restraints at a corresponding temperature were used for the MD runs. The molecules were subjected to a simulated annealing protocol after an initial equilibration for 50 ps. Starting from an initial temperature of 300 K, they were heated to 1500 K in four steps with an increment of 300 K and simulated for 2.5 ps at each step and subsequently cooled back to 300 K in four steps by decreasing the temperature by 300 K in each step, again simulating for 2.5 ps at each step. The structure was saved and the above process repeated 100 times, saving one structure each time. The 100 structures generated were energy-minimized again. From these 100 energy-minimized structures, the 20 lowest energy structures were superimposed for display. For a better visualization, some atoms/atomic groups have been removed from the figures.

The quantum chemical calculations were performed on the hexamers **2**, **4**, **6**, and **8** at the B3LYP/6-31G* level of density functional theory, employing the Gaussian03 program package.[31] Four alternative mixed helix types with 10- and 12-mr hydrogen-bonded cycles can formally be expected: 10/12-left- and right-handed and 12/10-left- and right-handed helices, respectively. Some of the 10/12- and 12/10-helix arrangements can a priori be excluded from steric reasons (Table 2). The starting values for the backbone torsion angles φ, θ, and ψ in the geometry optimizations came from former theoretical studies,[8c,h,i] those for the side chain orientations (χ_1) from the present NMR studies. The side chain torsion angles χ_1 were systematically varied in steps of 60°. In short oligomers like hexamers, the 10/12-helices could be advantaged over the 12/10-helix alternatives since one hydrogen bond more can be formed along the sequence. Deviations from this rule in favor of 12/10-helices may be caused by steric effects or special side chain-backbone interactions. It should be kept in mind that if one of the four possibilities is realized for a special peptide, the helix with the same hydrogen bonding pattern, but opposite handedness must be preferred in the peptide with the opposite configuration of the amino acid constituents, because it represents the mirror image of the original peptide. Since the side chains of the peptides **1**−**8** are chiral themselves and their chirality does not change with that of the constituent, the last condition is only approximately fulfilled and the two peptides represent only approximate mirror images of more or less different stability.

Boc-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-OMe (14). A cooled solution (0 °C) of **9** (0.75 g, 2.0 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.06 g, 2.4 mmol) and stirred at room temperature. After 1 h, pH was adjusted to 2−3 with 1 N HCl solution at 0 °C and extracted with ethyl acetate (2 × 50 mL). The organic layer was dried (Na_2SO_4) and evaporated to give **9a** (0.69 g, 95%) as a white solid, which was used as such for further reaction.

To a stirred suspension of β-hGly **11** (0.4 g, 4.4 mmol) in MeOH (5 mL), dry HCl gas was bubbled at room temperature until the solid was dissolved. MeOH was evaporated and the residual salt **11a** was dried under high vacuum and used as such for the next reaction.

A solution of acid **9a** (0.53 g, 1.46 mmol), HOBt (0.29 g, 2.19 mmol), EDCI (0.42 g, 2.19 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated with the amine salt **11a** and DIPEA (0.37 mL, 4.38 mmol) and stirred at room temperature for 8 h. The reaction mixture was quenched with aq. satd. NH_4Cl solution (10 mL). After 10 min, it was diluted with $CHCl_3$ (2 × 10 mL) and washed with water (10 mL), $NaHCO_3$ solution (10 mL), and brine (10 mL). The organic layers were dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (60−120 mesh silica gel, 60% Ethyl acetate in pet. ether) afforded **14** (0.48 g, 67%) as a colorless syrup; $[\alpha]^{20}_D = +90.0$ (c 0.05, $CHCl_3$); IR (KBr) ν 3746, 2954, 2923, 2851, 2310, 1709, 1692, 1646, 1525, 1219, 1054, 772 cm^{-1}; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 6.40 (b, 1H, NH-2), 5.22 (b, 1H, NH-1), 4.89 (s, 1H, C_1H-1), 4.68 (d, 1H, J = 3.8 Hz, C_3H-1), 4.53 (d, 1H, J = 5.5 Hz, C_βH-1), 4.15 (m, 2H, C_4H-1, C_2H-1), 3.70 (s, 3H, COOMe), 3.52 (m, 2H, C_βH-2, $C_\beta{}'$H-2), 3.30 (s, 3H, OMe), 2.62 (s, 2H, C_αH-1, C_αH-2), 2.54 (t, 2H, J = 6.0 Hz, $C_\alpha{}'$H-1, $C_\alpha{}'$H-2), 1.45 (s, 3H, Ac), 1.44 (s, 9H, Boc), 1.28 (s, 3H, Ac); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 172.7, 170.6, 155.7, 112.5, 106.5, 85.0, 79.5 (2C), 79.3, 54.4, 51.7, 48.0, 38.4, 34.7, 33.7, 28.3 (3C), 25.9, 24.6; HRMS (ESI+) m/z calculated for $C_{20}H_{34}N_2O_9Na(M^+ + Na)$ 469.2156, found 469.2122.

Boc-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-OMe (1). A cooled solution (0 °C) of **14** (0.17 g, 0.38 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.01 g, 0.46 mmol) and stirred at room temperature. Work up as described for **9a** gave **14a** (0.15 g, 90%) as a colorless solid, which was used as such for further reaction.

To a solution of **14a** (0.15 g, 0.34 mmol), HOBt (0.07 g, 0.52 mmol), and EDCI (0.1 g, 0.52 mmol) in CH_2Cl_2 (5 mL), amine salt **14b** [prepared from **14** (0.18 g, 0.4 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (1 mL)] and DIPEA (0.08 g, 0.67 mmol) were added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.6% CH_3OH in $CHCl_3$) afforded **1** (0.15 g, 55%) as a colorless solid; mp 136−138 °C; $[\alpha]^{20}_D = +50.0$ (c 0.05, $CHCl_3$); IR (KBr) ν 3284, 2983, 2931, 1739, 1692, 1650, 1535, 1439, 1369, 1289, 1271, 1106, 1079, 1019, 963, 857, 771, 666 cm^{-1}; ^{1}H NMR (500 MHz, 278 K, $CDCl_3$) δ 7.67 (b, 1H, NH-2), 7.62 (d, 1H, J = 9.5 Hz, NH-3), 7.00 (b, 1H, NH-4), 5.68 (d, 1H, J = 9.8 Hz, NH-1), 4.94 (s, 1H, C_1H-3), 4.93 (s, 1H, C_1H-1), 4.74 (dd, 1H, J = 3.8, 5.9 Hz, C_3H-3), 4.68 (dd, 1H, J = 3.9, 5.9 Hz, C_3H-1), 4.60 (m, 1H, C_βH-3), 4.54 (d, 2H, J = 5.9 Hz, C_2H-1, C_2H-3), 4.44 (m, 1H, C_βH-1), 4.06 (dd, 1H, J = 3.8, 9.5 Hz, C_4H-3), 3.99 (m, 1H, C_βH-2), 3.91 (dd, 1H, J = 3.9, 7.3 Hz, C_4H-1), 3.76 (m, 1H, C_βH-4), 3.71 (s, 3H, COOMe), 3.32 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.12 (m, 1H, $C_\beta{}'$H-2), 2.77 (dd, 1H, J = 3.9, 13.7 Hz, C_αH-1), 2.73 (dddd, 1H, J = 3.8, 3.8, 8.2, 16.5

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

Hz, C_{β}'H-4), 2.54 (m, 1H, C_{α}H-3), 2.50 (m, 1H, C_{α}H-4, C_{α}'H-4), 2.43 (m, 1H, C_{α}'H-1), 2.41 (m, 1H, C_{α}'H-3), 2.28 (m, 2H, C_{α}H-2, C_{α}'H-2), 1.64 (s, 6H, Ac), 1.48 (s, 6H, Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.3, 171.7, 171.1, 170.7, 156.0, 112.7, 112.5, 106.8, 106.5, 85.1, 85.0, 79.9, 79.8, 79.6, 79.5, 54.6, 54.4, 51.8, 48.2, 47.4, 39.7, 38.4, 37.2, 36.2, 35.1, 33.7, 28.4 (2C), 26.0, 25.9, 24.8, 24.6; HRMS (ESI+) *m/z* calculated for $C_{34}H_{56}N_4O_{15}$Na-(M^++Na) 783.3634, found 783.3593.

Boc-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-OMe (2). A cooled solution (0 °C) of **1** (0.05 g, 0.07 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.002 g, 0.1 mmol) and stirred at room temperature. Work up as described for **9a** gave **15** (0.05 g, 95%) as a colorless solid, which was used as such for further reaction.

To a solution of **15** (0.05 g, 0.07 mmol), HOBt (0.01 g, 0.10 mmol) and EDCI (0.02 g, 0.10 mmol) in CH_2Cl_2 (5 mL), amine salt **14b** [prepared from **14** (0.04 g, 0.07 mmol) and CF_3COOH (0.1 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.02 g, 0.13 mmol) were added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.8% CH_3OH in $CHCl_3$) afforded **2** (0.04 g, 49%) as a colorless solid; mp 157−158 °C; $[\alpha]^{20}_D$ = +94.0 (*c* 0.05, $CHCl_3$); IR (KBr) ν 3286, 3019, 2924, 2853, 1692, 1651, 1541, 1441, 1373, 1213, 1105, 1080, 1022, 964, 746, 667 cm^{-1}; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 8.36 (dd, 1H, *J* = 3.5, 9.4 Hz, NH-4), 8.24 (d, 1H, *J* = 7.8 Hz, NH-3), 7.85 (b, 1H, NH-6), 7.56 (d, 1H, *J* = 8.2 Hz, NH-5), 6.94 (b, 1H, NH-2), 6.14 (d, 1H, *J* = 7.8 Hz, NH-1), 4.91 (s, 1H, C_1H-1), 4.87 (s, 2H, C_1H-3, C_1H-5), 4.80 (dd, 1H, *J* = 3.5, 5.9 Hz C_3H-3), 4.74 (dd, 1H, *J* = 3.8, 5.9 Hz, C_3H-1), 4.59 (m, 1H, C_{β}H-3), 4.52 (d, 3H, *J* = 5.9 Hz, C_2H-1, C_2H-3, C_2H-5), 4.50 (m, 1H, C_{β}H-5), 4.40 (m, 1H, C_{β}H-1), 4.14 (m, 1H, C_{β}H-2), 4.06 (dd, 1H, *J* = 3.2, 9.5 Hz, C_4H-5), 4.05 (dd, 1H, *J* = 3.2, 9.5 Hz, C_4H-5), 4.04 (m, 1H, C_{β}H-4), 4.03 (dd, 1H, *J* = 3.8, 9.6 Hz, C_4H-1), 4.01 (dd, 1H, *J* = 3.5, 9.6 Hz, C_4H-3), 3.92 (m, 1H, C_{β}H-6), 3.63 (s, 3H, COOMe), 3.33 (s, 3H, OMe), 3.32 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.23 (m, 1H, C_{β}'H-6) 3.12 (m, 1H, C_{β}'H-4), 3.04 (m, 1H, C_{β}'H-2), 2.70 (m, 1H, C_{α}'H-6), 2.69 (m, 1H, C_{α}H-1), 2.54 (m, 1H, C_{α}H-3), 2.52 (m, 1H, C_{α}'H-2), 2.50 (m, 1H, C_{α}'H-1), 2.47 (m, 1H, C_{α}'H-4), 2.44 (m, 1H, C_{α}H-6), 2.42 (dd, 1H, *J* = 4.2, 9.8 Hz, C_{α}H-5), 2.40 (m, 1H, C_{α}'H-3), 2.38 (dd, 1H, *J* = 5.5, 8.6 Hz, C_{α}'H-5), 2.27 (m, 1H, C_{α}H-4), 2.16 (m, 1H, C_{α}'H-2), 1.45 (s, 3H, Ac), 1.44 (s, 9H, Boc), 1.43 (s, 3H, Ac), 1.41 (s, 3H, Ac), 1.30 (s, 3H, Ac), 1.29 (s, 3H, Ac), 1.28 (s, 3H, Ac); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.8, 171.5, 171.4 (3C), 170.8, 156.1, 112.5, 112.4, 112.4, 106.8, 106.6, 106.1, 85.0, 84.9, 84.8, 79.7, 79.5, 79.5, 79.4, 79.3, 79.1, 54.3, 54.2, 54.2, 54.1, 52.0, 48.1, 46.9, 46.7, 39.9, 38.6, 38.3, 38.2, 37.8, 36.9, 36.8, 35.6, 34.6, 28.5 (3C), 26.0 (2C), 25.9 (2C), 24.7 (2C), 24.6 (2C); HRMS (ESI+) *m/z* calculated for $C_{48}H_{78}N_6O_{21}$Na-(M^++Na) 1097.5112, found 1097.5044.

Boc-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-OMe (16). To a stirred solution of *β*-hGly 11 (0.31 g, 3.5 mmol) in 4 N NaOH (1 mL), $(Boc)_2O$ (0.8 mL, 3.5 mmol) was added slowly for 2 h and stirred at room temperature for 18 h. The reaction mixture was washed with petroleum ether (3 × 2 mL) and washed with aq. sat. $NaHCO_3$ (5 mL). Both the aq. NaOH and aq. sat. $NaHCO_3$ layers were mixed and acidified with aq. sat. $KHSO_4$ (pH = 2) and extracted with ethyl acetate (2 × 5 mL). The organic layer was dried (Na_2SO_4) and evaporated to give **11b** (0.3 g, 94%) as a colorless solid, which was used for the next reactions.

A solution of acid **11b** (0.30 g, 1.93 mmol) [prepared from **11** (0.31 g, 3.5 mmol) in 4N NaOH (1 mL) and $(Boc)_2O$ (0.8 mL, 3.5 mmol)], HOBt (0.32 g, 2.34 mmol), and EDCI (0.46 g, 2.34 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min. Amine salt **9b** [prepared from **9** (0.6 g, 0.16 mmol) and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.40 g, 3.16 mmol) were added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 55% ethyl acetate in pet. ether) afforded **16** (0.60 g, 57%) as a colorless syrup; $[\alpha]^{20}_D$ = +84.0 (*c* 0.05, $CHCl_3$); IR (KBr) ν 3753, 3611, 3355, 2921, 2852, 1741, 1709, 1691, 1549, 1531, 1464, 1377, 1168, 1076, 720, 602 cm^{-1}; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 6.15 (d, 1H, *J* = 7.7 Hz, NH-2), 5.20 (b, 1H, NH-1), 4.89 (s, 1H, C_3H-2), 4.65 (m, 1H, C_{β}H-1), 4.60 (t, 1H, *J* = 7.3 Hz, C_4H-2), 4.55 (d, 1H, *J* = 5.6 Hz, C_2H-1), 4.16 (dd, 1H, *J* = 3.6, 7.3 Hz, C_3H-1), 3.69 (s, 3H, COOMe), 3.41 (m, 2H, C_{β}H-1, C_{β}'H-1), 3.30 (s, 3H, OMe), 2.78 (m, 2H, C_{α}H-1, C_{α}H-2), 2.38 (m, 2H, C_{α}'H-1, C_{α}'H-2), 1.63 (s, 3H, Ac), 1.45 (s, 9H, Boc), 1.29 (s, 3H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$, 298 K) δ 171.9, 171.0, 155.9, 112.6, 106.6, 84.9, 79.4, 79.0, 78.7, 54.6, 51.7, 45.8, 36.6, 36.2, 35.6, 28.3 (3C), 25.9, 24.5; HRMS (ESI+) *m/z* calculated for $C_{20}H_{34}N_2O_9$Na(M^++Na) 469.2156, found 469.2121.

Boc-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-OMe (3). A cooled solution (0 °C) of **16** (0.16 g, 0.34 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.01 g, 0.52 mmol) and stirred at room temperature. Work up as described for **9a** gave **16a** (0.14 g, 90%) as a colorless solid, which was used as such for further reaction.

A solution of **16a** (0.14 g, 0.38 mmol), HOBt (0.06 g, 0.48 mmol), and EDCI (0.09 g, 0.48 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated sequentially with **16b** [prepared from **16** (0.18 g, 0.38 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (1.2 mL) at 0 °C] and DIPEA (0.16 mL, 0.93 mmol) and stirred for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.2% CH_3OH in $CHCl_3$) afforded **3** (0.14 g, 48%) as a colorless solid; mp 142−143 °C; $[\alpha]^{20}_D$ = +90.0 (*c* 0.05, $CHCl_3$); IR (KBr) ν 3743, 3353, 3272, 2952, 2842, 1725, 1708, 1691, 1661, 1645, 1449, 1108, 1013, 723, 699, 639, 606 cm^{-1}; ^{1}H NMR (500 MHz, 268 K, $CDCl_3$) δ 7.22 (d, 1H, *J* = 8.5 Hz, NH-4), 7.18 (dd, 1H, *J* = 4.7, 6.9 Hz, NH-3), 6.68 (d, 1H, *J* = 9.1 Hz, NH-2), 5.74 (t, 1H, *J* = 5.9 Hz, NH-1), 4.91 (s, 2H, C_1H-2, C_1H-4), 4.70 (dd, 1H, *J* = 3.7, 5.8 Hz, C_3H-2), 4.64 (m, 1H, C_{β}H-2), 4.62 (m, 1H, C_{β}H-4), 4.56 (dd, 1H, *J* = 3.4, 5.8 Hz, C_3H-4), 4.55 (d, 2H, *J* = 5.8 Hz, C_2H-2, C_2H-4), 4.11 (dd, 1H, *J* = 3.4, 8.3 Hz, C_4H-4), 4.04 (dd, 1H, *J* = 3.7, 7.5 Hz, C_4H-2), 3.81 (m, 1H, C_{β}H-3), 3.71 (s, 3H, COOMe), 3.48 (m, 1H, C_{β}H-1), 3.38 (m, 1H, C_{β}'H-1), 3.31 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.24 (m, 1H, C_{β}'H-3), 2.82 (dd, 1H, *J* = 4.5, 15.0 Hz, C_{α}H-4), 2.63 (m, 1H, C_{α}'H-4), 2.61 (m, 1H, C_{α}H-2), 2.53 (dd, 1H, *J* = 7.9, 13.8 Hz, C_{α}'H-2), 2.42 (m, 1H, C_{α}H-1), 2.37 (m, 1H, C_{α}H-3), 2.36 (m, 1H, C_{α}'H-1), 2.31 (m, 1H, C_{α}'H-3), 1.42 (s, 6H, Ac), 1.42 (s, 6H, Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.2, 171.6, 171.0, 170.7, 156.0, 112.8, 112.5, 106.6, 106.9, 85.1,79.9, 79.8, 79.6, 79.5, 79.3, 54.6, 54.4, 51.8, 48.3, 47.5, 39.6, 38.4, 37.1, 36.1, 35.1, 28.4 (2C), 26.0, 25.9, 24.8, 24.6; HRMS (ESI+) *m/z* calculated for $C_{34}H_{56}N_4O_{15}$Na(M^++Na) 783.3634, found 783.3593.

Boc-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-*β*-hGly-(*S*)-*β*-Caa$_{(l)}$-OMe (4). A cooled solution (0 °C) of **3** (0.05 g, 0.06 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.003 g, 0.1 mmol) and stirred at room temperature. Work up as described for **9a** gave **17** (0.05 g, 95%) as a colorless solid, which was used as such for further reaction.

A solution of **17** (0.05 g, 0.06 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated sequentially with **16b** [prepared from **16** (0.33 g, 0.07 mmol) and CF_3COOH (0.1 mL) in CH_2Cl_2(1 mL) at 0 °C] and DIPEA (0.02 mL, 0.096 mmol) and stirred for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 4.4% CH_3OH in $CHCl_3$) afforded **4** (0.03 g, 45%) as a colorless solid; mp 126−128 °C; $[\alpha]^{20}_D$ = +60.0 (*c* 0.05, $CHCl_3$); IR (KBr) ν 3745, 3610, 3524, 3395, 2923, 2852, 2314, 1840, 1785, 1693, 1645, 1550, 1514, 1219, 1019, 962, 772, 630, 605, 581 cm^{-1}; ^{1}HNMR (500 MHz, 298 K, $CDCl_3$) δ 8.45 (d, 1H, *J* = 8.3 Hz, NH-4), 8.12 (b, 1H, NH-3), 7.99 (b, 1H, NH-5), 7.76 (d, 1H, *J* = 7.3 Hz, NH-6), 7.07 (d, 1H, *J* = 9.2 Hz, NH-2), 5.75 (b, 1H, NH-1), 4.91 (s, 1H, C_1H-2), 4.89 (s, 1H, C_1H-6), 4.82 (s, 1H, C_1H-4), 4.77 (m, 1H, C_{β}H-2), 4.76 (m, 1H, C_3H-4), 4.74 (dd, 1H, *J* = 3.2, 5.8 Hz, C_3H-2), 4.70 (dd, 1H, *J* = 3.3, 5.8 Hz, C_3H-6), 4.59 (dd, 1H, *J* = 4.6, 7.8 Hz, C_{β}H-4), 4.54 (d, 1H, *J* = 5.8 Hz, C_2H-2), 4.53 (d, 1H, *J* = 5.8 Hz, C_2H-6), 4.52 (d, 1H, *J* = 5.8 Hz, C_2H-4), 4.07 (dd, 1H, *J* = 3.3, 8.2 Hz, C_4H-6), 4.02 (dd, 1H, *J* = 3.2, 9.0 Hz, C_4H-4), 4.01 (m, 2H, C_{β}H-3), 3.98 (m, 1H, C_{β}H-5), 3.93 (m, 1H, C_{β}H-6), 3.71 (s, 3H, COOMe), 3.62 (m, 1H, C_{β}H-1), 3.31 (s, 3H, OMe), 3.30 (s, 3H, OMe), 3.29 (m, 1H, C_{β}'H-1), 3.23 (s, 3H, OMe), 3.07 (m, 1H, C_{β}'H-5), 3.01 (m, 1H, C_{β}'H-3), 2.83 (dd, 1H, *J* =

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

4.3, 14.2 Hz, C_αH-6), 2.62 (dd, 2H, J = 3.9, 12.8 Hz, C_αH-2, C_αH-4), 2.57 (dd, 1H, J = 7.8, 14.2 Hz, C_α'H-6), 2.51 (m, 1H, C_αH-3), 2.46 (m, 1H, C_αH-1), 2.43 (m, 1H, C_α'H-2), 2.41 (m, 1H, C_αH-5), 2.33 (dd, 1H, J = 8.6, 12.8 Hz, C_α'H-4), 2.30 (m, 1H, C_α'H-1), 2.22 (ddd, 1H, J = 2.5, 7.1, 13.2 Hz, C_α'H-5), 2.14 (m, 1H, C_α'H-3), 1.67 (s, 9H, Ac), 1.64 (s, 9H, Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.1, 171.9, 171.8, 171.1, 171.0, 170.9, 156.5, 112.8, 112.5, 112.4, 106.8, 106.7, 106.4, 85.1, 85.0, 84.8, 80.1, 79.9, 79.6, 79.5, 79.4, 79.3, 54.5, 54.3, 52.2, 47.3, 46.6, 46.4, 40.5, 39.1, 37.6, 37.1, 36.8, 36.7, 36.4, 31.9, 29.7, 28.4 (3C), 26.0, 25.9, 25.7, 24.6, 24.5, 22.7; HRMS (ESI+) m/z calculated for $C_{48}H_{78}N_6O_{21}Na(M^+ +Na)$ 1097.5112, found 1097.5042.

Boc-β-hGly-(S)-β-Caa$_{(l)}$-β-hGly-(S)-β-Caa$_{(l)}$-β-hGly-(S)-β-Caa$_{(l)}$-OMe (4) in CD_3OH. ^{1}HNMR (700 MHz, 298 K, CD_3OH) δ 8.23 (d, 1H, J = 9.0 Hz, NH-4), 8.19 (d, 1H, J = 9.3 Hz, NH-2), 8.15 (d, 1H, J = 8.7 Hz, NH-6), 8.11 (b, 1H, NH-3), 8.06 (t, 1H, J = 5.6 Hz, NH-5), 6.44 (t, 1H, J = 6.0 Hz, NH-1), 4.76 (dd, 1H, J = 3.5, 5.8 Hz, C_3H-4), 4.75 (dd, 1H, J = 3.5, 5.8 Hz, C_3H-2), 4.64 (dd, 1H, J = 3.5, 5.8 Hz, C_3H-6), 4.46 (d, 3H, 5.8 Hz, C_2H-2, C_2H-4, C_2H-6), 4.44 (m, 2H, C_βH-2, C_βH-6), 4.37 (m, 1H, C_βH-4), 4.01 (dd, 1H, J = 3.5, 9.0 Hz, C_4H-4), 4.00 (m, 2H, C_4H-2, C_4H-6), 3.60 (m, 1H, C_βH-3), 3.57 (s, 3H, C_1H-2, C_1H-4, C_1H-6), 3.44 (dt, 1H, J = 6.5, 13.2 Hz, C_βH-5), 3.30 (m, 1H, C_βH-1), 3.25 (m, 1H, C_β'H-5), 3.23 (m, 1H, C_β'H-1), 3.22 (s, 3H, OMe), 3.21 (s, 3H, OMe), 3.20 (s, 3H, OMe), 3.15 (m, 1H, C_β'H-3), 2.65 (dd, 1H, J = 4.4, 15.2 Hz, C_αH-6), 2.50 (dd, 1H, J = 7.9, 15.2 Hz, C_α'H-6), 2.46 (t, 1H, J = 4.4 Hz, C_αH-4), 2.45 (t, 1H, J = 4.4 Hz, C_αH-2), 2.37 (m, 1H, C_α'H-2), 2.35 (m, 1H, C_αH-3), 2.34 (m, 1H, C_αH-5), 2.33 (dd, 1H, J = 4.4, 8.3 Hz, C_α'H-4), 2.31 (m, 1H, C_αH-1), 2.27 (m, 1H, C_α'H-5), 2.19 (m, 1H, C_αH-3), 1.34 (s, 6H, Ac), 1.32 (s, 6H, Ac), 1.31 (s, 6H, Ac), 1.20 (s, 9H, Boc); ^{13}C NMR (175 MHz, 298 K, $CDCl_3$) δ 172.2, 171.8, 171.3, 156.9, 112.4, 112.3, 106.8, 106.5, 106.4, 85.0, 79.6, 79.5, 79.4, 79.3, 79.2, 78.7, 53.5, 52.4, 50.9, 46.9, 46.6, 46.5, 37.6, 37.4, 36.9, 36.3, 36.1, 35.5, 29.3, 27.3, 25.0, 23.6, 23.5, 23.4.

Boc-β-hGly-(S)-β-Caa$_{(l)}$-β-hGly-(S)-β-Caa$_{(l)}$-β-hGly-(S)-β-Caa$_{(l)}$-OMe (4) in CD_3CN. ^{1}HNMR (700 MHz, 293 K, CD_3CN) δ 8.10 (d,1H, J = 9.2 Hz, NH-4), 7.90 (t, 1H, J = 5.5 Hz, NH-5), 7.76 (d, 1H, J = 9.8 Hz, NH-2), 7.68 (dd, 1H, J = 2.9, 8.8 Hz, NH-3), 7.32 (d, 1H, J = 8.7 Hz, NH-6), 5.76 (t, 1H, J = 6.1 Hz, NH-1), 4.85 (dd, 1H, J = 3.5, 5.7 Hz, C_3H-4), 4.81 (dd, 1H, J = 3.5, 5.7 Hz, C_3H-2), 4.80 (s, 1H, C_1H-2), 4.79 (s, 1H, C_1H-4), 4.78 (s, 1H, C_1H-6), 4.69 (dd, 1H, J = 3.5, 5.9 Hz, C_3H-6), 4.52 (d, 1H, J = 5.9 Hz, C_2H-6), 4.51 (d, 2H, J = 5.7 Hz, C_2H-2, C_2H-4), 4.46 (m, 1H, C_βH-2), 4.43 (m, 1H, C_βH-6), 4.37 (m, 1H, C_βH-4), 4.01 (dd, 1H, J = 3.5, 9.0 Hz, C_4H-6), 3.97 (dd, 2H, J = 3.5, 9.7 Hz, C_4H-2, C_4H-4), 3.91 (m, 1H, C_βH-3), 3.72 (m, 1H, C_βH-5), 3.63 (s, 3H, COOMe), 3.43 (m, 1H, C_βH-1), 3.27 (m, 1H, C_β'H-1), 3.28 (s, 3H, OMe), 3.27 (s, 3H, OMe), 3.26 (s, 3H, OMe), 3.08 (m, 1H, C_β'H-5), 3.03 (m, 1H, C_β'H-3), 2.67 (dd, 1H, J = 4.6, 14.9 Hz, C_α'H-6), 2.50 (dd, 1H, J = 7.1, 14.9 Hz, C_αH-6), 2.44 (dd, 1H, J = 4.4, 12.7 Hz, C_α'H-2), 2.41 (m, 1H, C_α'H-3), 2.40 (m, 1H, C_α'H-4), 2.38 (m, 2H, C_αH-1, C_αH-5), 2.32 (m, 1H, C_α'H-2), 2.29 (m, 1H, C_α'H-1), 2.22 (m, 1H, C_αH-4), 2.20 (dd, 1H, J = 7.3, 14.9 Hz, C_α'H-5), 2.12 (m, 1H, C_αH-3), 1.39 (s, 6H, Ac), 1.38 (s, 6H, Ac), 1.33 (s, 6H, Ac), 1.26 (s, 9H, Boc); ^{13}C NMR (175 MHz, 298 K, $CDCl_3$) δ 172.8, 171.2, 113.3, 113.1, 107.6, 107.1, 86.0, 80.6, 80.4, 80.3, 54.8, 54.7, 52.5, 47.8, 46.6, 47.2, 39.7, 39.1, 37.8, 37.4, 37.1, 32.7, 30.4, 28.7, 26.4, 24.9, 23.4, 14.4.

Boc-(R)-β-Caa$_{(l)}$-β-hGly-OMe (18). A cooled solution (0 °C) of **10** (0.6 g, 1.6 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.06 g, 2.4 mmol) and stirred at room temperature. Work up as described for **9a** gave **10a** (0.53 g, 93%) as a white solid, which was used as such for further reaction.

A solution of acid **10a** (0.53 g, 1.46 mmol), HOBt (0.296 g, 2.19 mmol), and EDCI (0.42 g, 2.19 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated with amine **11a** [prepared from **11** (0.4 g, 4.4 mmol) in MeOH (5 mL), and dry HCl gas at room temperature] and DIPEA (0.37 mL, 4.38 mmol) and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 55% ethyl acetate in pet. ether) afforded **18** (0.47 g, 65%) as a colorless syrup; $[\alpha]^{20}_D$ = +86.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3743, 2969, 2939, 1692, 1628, 1514, 1466, 1219, 1054, 1032, 1012, 772, 671 cm^{-1}; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 6.55 (b, 1H, NH-2), 5.64 (b, 1H, NH-1), 4.86 (s, 1H, C_1H-1), 4.75 (s, 1H, C_3H-1), 4.53 (d, 1H, J = 6.0 Hz, C_βH-1), 4.26 (t, 1H, J = 6.0 Hz, C_4H-1), 4.08 (s, 1H, C_2H-1), 3.70 (s, 3H, COOMe), 3.51 (m, 2H, C_βH-2, C_β'H-2), 3.28 (s, 3H, OMe), 2.66 (b, 1H, C_αH-1), 2.53 (m, 3H, C_α'H-1, C_αH-2, C_α'H-2), 1.48 (s, 3H, Ac), 1.43 (s, 9H, Boc), 1.30 (s, 3H, Ac); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 172.6, 170.6, 155.8, 112.5, 106.6, 84.7, 79.7, 79.2, 78.6, 54.4, 51.6, 47.6, 38.5, 34.7, 33.7, 28.2 (3C), 25.7, 24.4; HRMS (ESI+) m/z calculated for $C_{20}H_{34}N_2O_9Na(M^+ +Na)$ 469.2156, found 469.2119.

Boc-(R)-β-Caa$_{(l)}$-β-hGly-(R)-β-Caa$_{(l)}$-β-hGly-OMe (5). A cooled solution (0 °C) of **18** (0.16 g, 0.21 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.01 g, 0.2 mmol) and stirred at room temperature. Work up as described for **9a** gave **18a** (0.15 g, 95%) as a white solid, which was used as such for further reaction.

A solution of **18** (0.18 g, 0.4 mmol) and CF_3COOH (0.4 mL) in CH_2Cl_2 (1 mL), was stirred at room temperature for 2 h. Solvent was evaporated, the residual salt **18b** dried under vacuum and used as such for the next reaction.

To a solution of **18a** (0.15 g, 0.34 mmol), HOBt (0.07 g, 0.52 mmol), and EDCI (0.1 g, 0.52 mmol) in dry CH_2Cl_2 (5 mL), amine salt **18b** was added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.6% CH_3OH in $CHCl_3$) afforded **5** (0.14 g, 52%) as a colorless solid; mp 131−133 °C; $[\alpha]^{20}_D$ = +120.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3743, 3681, 3590, 2968, 2921, 2863, 2311, 1708, 1661, 1550, 1514, 1219, 1054, 1032, 1012, 772 cm^{-1}; ^{1}H NMR (500 MHz, 298 K, $CDCl_3$) δ 7.63 (d, 1H, J = 8.6 Hz, NH-3), 7.53 (dd, 1H, J = 4.2, 8.4 Hz, NH-2), 6.62 (t, 1H, J = 6.1 Hz, NH-4), 5.76 (d, 1H, J = 9.6 Hz, NH-1), 4.87 (s, 1H, C_1H-1), 4.77 (dd, 1H, J = 3.5, 5.9 Hz, C_3H-3), 4.73 (dd, 1H, C_3H-1), 4.60 (m, 1H, C_βH-3), 4.52 (d, 2H, J = 5.8 Hz, C_2H-3, C_2H-1), 4.02 (dd, 1H, J = 3.5, 8.2 Hz, C_4H-3), 3.98 (dd, 1H, J = 3.5, 9.3 Hz, C_4H-1), 3.85 (m, 1H, C_βH-2), 3.71 (s, 3H, COOMe), 3.60 (m, 1H, C_βH-4), 3.40 (m, 1H, C_β'H-4), 3.27 (s, 6H, OMe), 3.19 (m, 1H, C_β'H-2), 2.71 (dd, 1H, J = 3.9, 13.9 Hz, C_αH-3), 2.61 (dd, 1H, J = 4.5, 13.9 Hz, C_αH-1), 2.60 (m, 1H, C_αH-4), 2.52 (m, 1H, C_α'H-4), 2.48 (dd, H, J = 9.6, 13.9 Hz, C_αH-1), 2.25 (m, 2H, C_αH-2, C_α'H-2), 1.64 (s, 6H, Ac), 1.48 (s, 6H, Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.1, 171.7, 171.5, 170.5, 156.4, 112.6, 107.0, 106.8, 84.8, 84.9, 80.0, 79.5, 79.3, 78.9, 54.6, 54.5, 51.8, 48.9, 46.2, 39.5, 38.9, 37.2, 36.1, 35.0, 33.5, 29.7, 28.3 (3C), 26.0, 25.7, 24.5, 24.4; HRMS (ESI+) m/z calculated for $C_{34}H_{56}N_4O_{15}Na(M^+ +Na)$ 783.3634, found 783.3587.

Boc-(R)-β-Caa$_{(l)}$-β-hGly-(R)-β-Caa$_{(l)}$-β-hGly-(R)-β-Caa$_{(l)}$-β-hGly-OMe (6). A cooled solution (0 °C) of **5** (0.054 g, 0.07 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.002 g, 0.1 mmol) and stirred at room temperature. Work up as described for **9a** gave **19** (0.05 g, 92%) as a colorless solid, which was used as such for further reaction.

To a solution of **19** (0.05 g, 0.06 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (5 mL), amine salt **18b** [prepared from **18** (0.04 g, 0.07 mmol) and CF_3COOH (0.1 mL) in CH_2Cl_2 (0.5 mL)] and DIPEA (0.02 g, 0.09 mmol) was added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.8% CH_3OH in $CHCl_3$) afforded **6** (0.04 g, 44%) as a colorless solid; mp 138−139 °C; $[\alpha]^{20}_D$ = +140.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3294, 2982, 2932, 1649, 1514, 1440, 1369, 1212, 1166, 1095, 1048, 1018, 996, 751, 666 cm^{-1}; ^{1}H NMR (600 MHz, 298 K, $CDCl_3$) δ 8.46 (dd, 1H, J = 3.5, 9.1 Hz, NH-4), 8.37 (d, 1H, J = 9.6 Hz, NH-3), 8.32 (d, 1H, J = 9.4 Hz, NH-5), 8.24 (dd, 1H, J = 3.6, 9.4 Hz, NH-2), 6.64 (t, 1H, J = 6.2 Hz, NH-6), 5.87 (d, 1H, J = 10.3 Hz, NH-1), 4.89 (s, 1H, C_1H-1), 4.86 (s, 1H, C_1H-5), 4.83 (s, 1H, C_1H-3), 4.77 (dd, 1H, J = 3.5, 5.9 Hz, C_3H-5), 4.76 (m, 1H, C_βH-3), 4.72 (dd, 1H, J = 3.6, 5.9 Hz, C_3H-1), 4.71 (m, 1H, C_βH-1), 4.68 (dd, 1H, J = 3.1, 5.9 Hz, C_3H-3), 4.63 (dq, 1H, J = 3.2, 9.4 Hz, C_βH-5), 4.52 (d, 2H, J = 5.9 Hz, C_2H-1, C_2H-5), 4.47 (d, 1H, J = 5.9 Hz, C_2H-3), 4.00 (m, 1H, C_βH-2), 3.99 (dd, 1H, J = 3.1, 9.3 Hz, C_4H-3), 3.98 (dd, 1H, J = 3.6, 9.3 Hz, C_4H-1), 3.93 (m, 1H, $C_\beta H$-4), 3.92 (dd, 1H, J = 3.5, 9.4 Hz, C_4H-5), 3.71 (s, 3H, COOMe), 3.65 (m, 1H, $C_\beta H$-6), 3.36 (m, 1H, $C_\beta'H$-6), 3.29 (s, 3H, OMe), 3.24 (s, 3H, OMe), 3.23 (s, 3H, OMe), 3.01 (m, 1H, $C_\beta'H$-4), 2.97 (m, 1H, $C_\beta'H$-2), 2.92 (dd, 1H, J = 3.6, 12.7 Hz, $C_\alpha H$-3), 2.74 (dd, 1H, J = 3.2, 13.3 Hz, $C_\alpha H$-5), 2.69 (ddd, 1H, J = 4.8, 8.2, 15.3 Hz, $C_\alpha H$-6), 2.68 (dd, 1H, J = 4.4, 12.7 Hz, $C_\alpha H$-1), 2.53 (m, 1H, $C_\alpha'H$-6), 2.39 (m, 1H, $C_\alpha'H$-1), 2.38 (m, 1H, $C_\alpha'H$-5), 2.26 (m, 2H, $C_\alpha H$-4, $C_\alpha'H$-4), 2.25 (m, 1H, $C_\alpha'H$-3), 2.17 (m, 1H, $C_\alpha'H$-2), 2.14 (m, 1H, $C_\alpha'H$-4), 1.67 (s, 9H, 3 x Ac), 1.64 (s, 9H, 3 x Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (150 MHz, 298 K, $CDCl_3$) δ 173.3, 172.1, 172.0, 171.7, 171.6, 170.7, 156.8, 112.8, 112.6,112.4, 107.6, 107.0, 106.6, 84.9, 84.8, 80.7, 80.5, 80.0, 79.5, 79.3, 78.8, 54.9, 54.6, 54.3, 54.2, 51.8, 49.9, 47.0, 46.7, 41.5, 40.7, 39.9, 38.0, 37.3, 37.0, 36.9, 36.6, 36.3, 35.1, 33.3, 32.7, 31.9, 30.0, 29.7, 29.3 (3C), 28.3, 26.0, 25.7, 24.5, 24.4, 22.7; HRMS (ESI+) m/z calculated for $C_{48}H_{78}N_6O_{21}Na$-(M^++Na) 1097.5112, found 1097.5048.

Boc-β-hGly-(*R*)-β-Caa$_{(l)}$-OMe (20). A solution of acid **11b** (0.30 g, 1.93 mmol) [prepared from **11** (0.31 g, 3.5 mmol) in 4N NaOH (1 mL) and $(Boc)_2O$ (0.8 mL, 3.5 mmol)], HOBt (0.32 g, 2.34 mmol), and EDCI (0.46 g, 2.34 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min. Amine salt **10b** [prepared from **10** (0.6 g, 0.16 mmol)and CF_3COOH (0.2 mL) in CH_2Cl_2 (2 mL)] and DIPEA (0.40 g, 3.16 mmol) were added and stirred at room temperature for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 51% Ethyl acetate in pet. ether) afforded **20** (0.4 g, 57%) as a colorless syrup; $[\alpha]^{20}_D$ = +87.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3727, 2969, 2311, 1692, 1645, 1550, 1514, 1219, 1054, 1032, 1012, 772 cm^{-1}; ^1H NMR (500 MHz, 298 K, $CDCl_3$) δ 6.52 (d, 1H, J = 8.1 Hz, NH-2), 5.20 (b, 1H, NH-1), 4.90 (s, 1H, C_3H-2), 4.73 (m, 1H, $C_\beta H$-1), 4.69 (t, 1H, J = 6.9 Hz, C_4H-2), 4.54 (d, 1H, J = 6.0 Hz, C_2H-1), 4.14 (dd, 1H, J = 3.2, 6.5 Hz, C_3H-1), 3.69 (s, 3H, COOMe), 3.40 (m, 2H, $C_\beta H$-2, $C_\beta'H$-2), 3.29 (s, 3H, OMe), 2.82−2.66 (m, 2H, $C_\alpha H$-1, $C_\alpha H$-2), 2.32 (d, 2H, J = 5.6 Hz, $C_\alpha'H$-1, $C_\alpha'H$-2), 1.66 (s, 3H, Ac), 1.49 (s, 9H, Boc), 1.31 (s, 3H, Ac); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 171.6, 171.0, 155.8, 112.6, 106.5, 84.7, 79.6, 79.0, 77.9, 54.5, 51.7, 45.7, 36.8, 36.6, 35.9, 28.2 (3C), 25.8, 24.3; HRMS (ESI+) m/z calculated for $C_{20}H_{34}N_2O_9Na$(M^++Na) 469.2156, found 469.2120.

Boc-β-hGly-(*R*)-β-Caa$_{(l)}$-β-hGly-(*R*)-β-Caa$_{(l)}$-OMe (7). A cooled solution (0 °C) of **20** (0.16 g, 0.34 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.01 g, 0.52 mmol) and stirred at room temperature. Work up as described for **9a** gave **20a** (0.14 g, 90%) as a colorless solid, which was used as such for further reaction.

A solution of **20a** (0.14 g, 0.38 mmol), HOBt (0.06 g, 0.48 mmol), and EDCI (0.09 g, 0.48 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under N_2 atmosphere for 15 min, treated sequentially with **20b** [prepared from **20** (0.18 g, 0.38 mmol) and CF_3COOH (0.4 mL) in dry CH_2Cl_2 (1.2 mL) at 0 °C] and DIPEA (0.16 mL, 0.93 mmol) and stirred for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 2.2% CH_3OH in $CHCl_3$) afforded **7** (0.11 g, 43%) as a colorless solid; mp 177−179 °C; $[\alpha]^{20}_D$ = +78.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3745, 3611, 3525, 2920, 2851, 1736, 1646, 1550, 1461, 1376, 1272, 1210, 1167, 1096, 966, 721, 609, 581 cm^{-1}; ^1H NMR (500 MHz, 298 K, $CDCl_3$) δ 7.58 (d, 1H, J = 8.8 Hz, NH-4), 7.08 (b, 1H, NH-3), 6.87 (d, 1H, J = 9.3 Hz, NH-2), 5.78 (d, 1H, J = 6.1 Hz, NH-1), 4.91 (s, 1H, C_1H-2), 4.88 (s, 1H, C_1H-4), 4.82 (m, 1H, $C_\beta H$-2), 4.76 (m, 1H, $C_\beta H$-4), 4.75 (dd, 1H, J = 3.4, 5.9 Hz, C_3H-2), 4.70 (dd, 1H, J = 3.4, 5.9 Hz, C_3H-4), 4.54 (d, 1H, J = 5.9 Hz, C_2H-2), 4.53 (d, 1H, J = 5.9 Hz, C_2H-4), 4.03 (dd, 1H, J = 3.4, 7.0 Hz C_4H-2), 3.99 (dd, 1H, J = 3.4, 7.6 Hz, C_4H-4), 3.79 (m, 1H, $C_\beta H$-3), 3.70 (s, 3H, COOMe), 3.41 (m, 1H, $C_\beta H$-1), 3.39 (m, 1H, $C_\beta'H$-1), 3.28 (s, 6H, OMe), 3.23 (m, 1H, $C_\beta'H$-3), 2.86 (dd, 1H, J = 4.3, 14.2 Hz, $C_\alpha H$-4), 2.59 (dd, 1H, J = 3.4, 14.2 Hz, $C_\alpha'H$-4), 2.57 (dd, 1H, J = 3.5, 10.3 Hz, $C_\alpha H$-2), 2.48 (dd, 1H, J = 3.5, 9.3 Hz, $C_\alpha'H$-2), 2.33 (m, 1H, $C_\alpha H$-1), 2.30 (m, 1H, $C_\alpha H$-3), 2.24 (m, 1H, $C_\alpha'H$-3), 1.47 (s, 6H, Ac), 1.44 (s, 6H, Ac), 1.29 (s, 9H, Boc); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 173.3, 171.8, 171.4, 170.4, 156.0, 112.8, 112.7, 107.0, 106.8, 84.9, 79.9, 79.5, 79.4, 79.0, 78.3, 54.6, 54.5, 52.0, 47.3, 46.3, 39.2, 37.4, 37.2, 36.4, 36.2, 31.5, 29.7, 28.4 (3C), 26.0, 25.9, 24.7, 24.3, 22.6; HRMS (ESI+) m/z calculated for $C_{34}H_{56}N_4O_{15}Na$(M^++Na) 783.3634, found 783.3598.

Boc-β-hGly-(*R*)-β-Caa$_{(l)}$-β-hGly-(*R*)-β-Caa$_{(l)}$-β-hGly-(*R*)-β-Caa$_{(l)}$-OMe (8). A cooled solution (0 °C) of **7** (0.05 g, 0.06 mmol) in THF:MeOH:H_2O (3:1:1) (1 mL) was treated with LiOH (0.003 g, 0.1 mmol) and stirred at room temperature. Work up as described above for **9a** gave **21** (0.05 g, 95%) as a colorless solid, which was used as such for further reaction.

A solution of **21** (0.05 g, 0.06 mmol), HOBt (0.01 g, 0.09 mmol), and EDCI (0.02 g, 0.09 mmol) in CH_2Cl_2 (5 mL) was stirred at 0 °C under a N_2 atmosphere for 15 min, treated with **20b** [prepared from **20** (0.33 g, 0.07 mmol) and CF_3COOH (0.1 mL) in dry CH_2Cl_2 (1 mL) at 0 °C] and DIPEA (0.02 mL, 0.09 mmol) and stirred for 8 h. Work up as described for **14** and purification of the residue by column chromatography (60−120 mesh silica gel, 4.4% CH_3OH in $CHCl_3$) afforded **8** (0.02 g, 41%) as a colorless solid; mp 179−180 °C; $[\alpha]^{20}_D$ = +164.0 (c 0.05, $CHCl_3$); IR (KBr) ν 3302, 3080, 2982, 2924, 2852, 1727, 1646, 1538, 1454, 1371, 1271, 1235, 1211, 1164, 1088, 969, 856, 753, 667, 628, 608 cm^{-1}; ^1H NMR (500 MHz, 298 K, $CDCl_3$) δ 8.63 (d, 1H, J = 9.4 Hz, NH-4), 8.32 (d, 1H, J = 9.2 Hz, NH-6), 8.23 (d, 1H, J = 8.8 Hz, NH-3), 7.73 (d, 1H, J = 8.5 Hz, NH-5), 7.00 (d, 1H, J = 9.7 Hz, NH-2), 6.10 (b, 1H, NH-1), 5.06 (m, 1H, $C_\beta H$-2), 4.91 (s, 1H, C_1H-2), 4.88 (s, 1H, C_1H-4), 4.81 (s, 1H, C_1H-6), 4.76 (dd, 1H, J = 3.4, 5.7 Hz, C_3H-2), 4.75 (dd, 1H, J = 3.5, 5.7 Hz, C_3H-6), 4.73 (m, 1H, $C_\beta H$-6), 4.71 (dd, 1H, J = 2.8, 5.8 Hz, C_3H-4), 4.68 (m, 1H, $C_\beta H$-4), 4.54 (d, 2H, J = 5.7 Hz, C_2H-2), 4.53 (d, 2H, J = 5.7 Hz, C_2H-6), 4.50 (d, 1H, J = 5.8 Hz, C_2H-4), 4.04 (dd, 1H, J = 3.4, 8.0 Hz, C_4H-2), 3.96 (m, 1H, $C_\beta H$-3), 3.94 (dd, 1H, J = 3.5, 8.5 Hz, C_4H-6), 3.92 (m, 1H, $C_\beta H$-5), 3.88 (dd, 1H, J = 2.8, 9.4 Hz, C_4H-4), 3.71 (s, 3H, COOMe), 3.45 (m, 2H, $C_\beta H$-1, $C_\beta'H$-1), 3.28 (s, 3H, OMe), 3.25 (s, 3H, OMe), 3.24 (s, 3H, OMe), 3.07 (m, 1H, $C_\beta'H$-5), 2.97 (m, 1H, $C_\beta'H$-3), 2.94 (dd, 1H, J = 2.8, 15.1 Hz, $C_\alpha H$-6), 2.88 (dd, 1H, J = 3.4, 12.8 Hz, $C_\alpha H$-4), 2.71 (dd, 1H, J = 2.0, 12.2 Hz, $C_\alpha H$-2), 2.51 (m, 1H, $C_\alpha'H$-6), 2.35 (m, 1H, $C_\alpha H$-1), 2.34 (m, 1H, $C_\alpha H$-1), 2.31 (m, 1H, $C_\alpha H$-3), 2.26 (dd, 1H, J = 2.6, 11.7 Hz, $C_\alpha H$-5), 2.19 (m, 1H, $C_\alpha'H$-4), 2.15 (m, 1H, $C_\alpha'H$-5), 2.10 (m, 1H, $C_\alpha'H$-3), 1.51 (s, 6H, Ac), 1.47 (s, 6H, Ac), 1.45 (s, 9H, Boc), 1.42 (s, 6H, Ac); ^{13}C NMR (125 MHz, 298 K, $CDCl_3$) δ 176.0, 174.0, 172.0, 171.7, 171.6, 171.2, 155.5, 112.8, 112.7, 112.5, 107.6, 107.4, 106.7, 85.1, 85.0, 84.9, 81.0, 80.1, 79.6, 79.5, 79.2, 78.9, 78.1, 54.8, 54.5, 54.4, 52.3, 46.9, 46.3, 37.9, 37.2, 37.1, 37.0, 36.5, 36.4, 31.9, 31.2, 31.1, 30.1, 30.0, 29.6 (3C), 29.4, 29.3, 29.2, 28.5, 26.1, 26.2, 25.9, 24.9, 24.5, 24.3, 24.1, 22.6; HRMS (ESI+) m/z calculated for $C_{48}H_{78}N_6O_{21}Na$(M^++Na) 1097.5112, found 1097.5046.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02856.

NMR spectra, solvent titration plots, distance constraints used in MD calculations, MD structures, CD spectra and the Cartesian coordinates for conformers (peptides) **2**, **4**, **6**, and **8** obtained by the theoretical calculations (PDF)

PDB files of these conformers (ZIP)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: esmvee@iict.res.in.

*E-mail: ssav@iict.res.in.

*E-mail: hofmann@uni-leipzig.de.

ORCID

Sirisha Katukuri: 0000-0002-4951-8092

Gangavaram V. M. Sharma: 0000-0001-5638-788X

Notes

The authors declare no competing financial interest.

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

ACKNOWLEDGMENTS

The authors are thankful for financial support from CSC-0108 (ORIGIN)/CSC-0406 (AARF), CSIR, New Delhi. TP and KS thank UGC/CSIR, New Delhi, for financial support.

REFERENCES

(1) (a) Richardson, J. S. *Adv. Protein Chem.* **1981**, *34*, 167. (b) Creighton, T. E. *Proteins: Structures and Molecular Principles*, 2nd ed.; Freeman, W. H. & Co.: New York, 1993. (c) Gademann, K.; Hintermann, T.; Schreiber, J. V. *Curr. Med. Chem.* **1999**, *6*, 905. (d) DeGrado, W. F.; Schneider, J. P.; Hamuro, Y. *J. Pept. Res.* **1999**, *54*, 206.

(2) (a) DeGrado, W. F. *Adv. Protein Chem.* **1988**, *39*, 124. (b) Hruby, V. J.; Pettitt, B. M. *Computer-Aided Drug Design: Methods and Applications*; Perum, T. J., Propst, C. L., Eds.; Dekker: New York, 1989; p 405. (c) Kessler, H. In *Trends in Drug Research*; Classen, V., Ed.; Elsevier Sci. Publ.: Amsterdam, 1990; p 73. (d) Schiller, P. W. *Medicinal Chemistry for the 21st Century*; Wermuth, C. G., Ed.; IUPAC Publ. Blackwell Publ.: Oxford 1991. (e) Giannis, A. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (f) Kirshenbaum, K.; Zuckermann, R. N.; Dill, K. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530.

(3) Kiss, L.; Cherepanova, M.; Fülöp, F. *Tetrahedron* **2015**, *71*, 2049.

(4) (a) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071.

(5) (a) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015. (b) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (c) Seebach, D.; Beck, A. K.; Daniel, J. B. *Chem. Biodiversity* **2004**, *1*, 1111. (d) *Foldamers: Structure, Properties and Applications*; Hecht, S., Huc, I., Eds.; Wiley-VCH: Weinheim, Germany, 2007. (e) Bouillère, F.; Thétiot-Laurent, S.; Kouklovsky, C.; Alezra, V. *Amino Acids* **2011**, *41*, 687. (f) Martinek, T. A.; Fülöp, F. *Chem. Soc. Rev.* **2012**, *41*, 687. (g) Sharma, G. V. M.; Kunwar, A. C. *Recent Research Developments in Foldamer Chemistry*, ed. Nageswer, Y. V. D., Nova Science Publishers, Inc.: New York, 2012.

(6) (a) Fülöp, F.; Martinek, T. A.; Toth, G. K. *Chem. Soc. Rev.* **2006**, *35*, 323. (b) Seebach, D.; Gardiner, J. *Acc. Chem. Res.* **2008**, *41*, 1366. (c) Wu, Y. D.; Han, W.; Wang, D. P.; Gao, Y.; Zhao, Y. L. *Acc. Chem. Res.* **2008**, *41*, 1418. (d) Horne, W. S.; Price, J. L.; Gellman, S. H. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 9151.

(7) (a) Hayen, A.; Schmitt, M. A.; Nagassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505. (b) De Pol, S.; Zorn, C.; Klein, C. D.; Reiser, O.; Zerbe, O. *Angew. Chem., Int. Ed.* **2004**, *43*, 511. (c) Sharma, G. V. M; Nagendar, P.; Radha Krishna, P.; Jayaprakash, P.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878. (d) Horne, W. S.; Gellman, S. H. *Acc. Chem. Res.* **2008**, *41*, 1399. (e) Pilsl, L. K. A.; Reiser, O. *Amino Acids* **2011**, *41*, 709. (f) Sharma, G. V. M; Reddy, K. S.; Basha, S. J.; Reddy, K. R.; Sarma, A. V. S. *Org. Biomol. Chem.* **2011**, *9*, 8102.

(8) (a) Wu, Y. D.; Wang, D. P. *J. Am. Chem. Soc.* **1998**, *120*, 13485. (b) Daura, X.; Jaun, B.; Seebach, D.; Van Gunsteren, W. F.; Mark, A. E. *J. Mol. Biol.* **1998**, *280*, 925. (c) Möhle, K.; Günther, R.; Thormann, M.; Sewald, N.; Hofmann, H.-J. *Biopolymers* **1999**, *50*, 167. (d) Seebach, D.; Schreiber, J. V.; Abele, S.; Daura, X.; Van Gunsteren, W. F. *Helv. Chim. Acta* **2000**, *83*, 34. (e) Van Gunsteren, W. F.; Bürgi, R.; Peter, C.; Daura, X. *Angew. Chem., Int. Ed.* **2001**, *40*, 351. (f) Günther, R.; Hofmann, H.-J. *Helv. Chim. Acta* **2002**, *85*, 2149. (g) Doerksen, R. J.; Chen, B.; Yuan, J.; Winkler, J. D.; Klein, M. L. *Chem. Commun.* **2003**, *20*, 2534. (h) Baldauf, C.; Günther, R.; Hofmann, H.-J. *Angew. Chem., Int. Ed.* **2004**, *42*, 1594. (i) Baldauf, C.; Günther, R.; Hofmann, H.-J. *Biopolymers* **2005**, *80*, 675. (j) Baldauf, C.; Hofmann, H.-J. *Helv. Chim. Acta* **2012**, *95*, 2348. (k) Glättli, A.; Daura, X.; Bindschädler, P.; Jaun, B.; Mahajan, Y. R.; Mathad, R. I.; Rueping, M.; Seebach, D.; Van Gunsteren, W. F. *Chem. - Eur. J.* **2005**, *11*, 7276.

(9) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

(10) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 7574. (b) Fernandes, C.; Faure, S.; Pereira, E.; Thery, V.; Declerck, V.; Guillot, R.; Aitken, D. J. *Org. Lett.* **2010**, *12*, 3606.

(11) (a) Gellman, S. H.; Price, J. L.; Horne, W. S. *J. Am. Chem. Soc.* **2007**, *129*, 6376. (b) Mandity, I. M.; Weber, E.; Martinek, T. A.; Olajos, G.; Toth, G. K.; Vass, E.; Fülöp, F. *Angew. Chem., Int. Ed.* **2009**, *48*, 2171. (c) Mandity, I. M.; Fülöp, L.; Vass, E.; Toth, G. K.; Martinek, T. A.; Fülöp, F. *Org. Lett.* **2010**, *12*, 5584.

(12) (a) Jaun, B.; Tanaka, M.; Seiler, P.; Kühnle, F. N. M.; Braun, C.; Seebach, D. *Liebigs Ann./Recueil* **1997**, 1697. (b) Fee, L.; Bravo Rodriguez, K.; Phillips, C.; Seidel, R. W.; Wieberneit, F.; Raphael, S.; Nikos, L. D.; Sanchez Garcia, E.; Sander, W. *J. Phys. Chem. B* **2013**, *117*, 3560.

(13) Gorske, B. C.; Bastian, B. L.; Geske, G. D.; Blackwell, H. E. *J. Am. Chem. Soc.* **2007**, *129*, 8928.

(14) (a) Seebach, D.; Gademann, K.; Schreiber, J. V.; Matthews, J. L.; Hintermann, T.; Jaun, B. *Helv. Chim. Acta* **1997**, *80*, 2033. (b) Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V.; Oberer, L.; Hommel, U.; Idmer, H. *Helv. Chim. Acta* **1998**, *81*, 932.

(15) Sharma, G. V. M; Reddy, K. R.; Krishna, P. R.; Sankar, A. R.; Narsimulu, K.; Kumar, S. K.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc.* **2003**, *125*, 13670.

(16) (a) Sharma, G. V. M; Reddy, V. G.; Chander, A. S.; Reddy, K. R. *Tetrahedron: Asymmetry* **2002**, *13*, 21.

(17) (a) Chandrasekhar, S.; Reddy, S. M.; Babu, B. N.; Jagadeesh, B.; Prabhakar, A.; Jagannadh, B. *J. Am. Chem. Soc.* **2005**, *127*, 9664. (b) Sharma, G. V. M; Reddy, K. S.; Dutta, S. K.; Subash, V.; Narsimulu, K.; Anjaiah, G.; Basha, S. J.; Kunwar, A. C. *Chem. - Eur. J.* **2012**, *18*, 16046. (c) Sharma, G. V. M; Yadav, T. A.; Kanakaraju, M.; Prashanth, T.; Reddy, P. P.; Kunwar, A. C. *Chem. - Asian J.* **2014**, *9*, 3153.

(18) (a) Sharma, G. V. M; Jadhav, V. B.; Ramakrishna, K. V. S.; Jayaprakash, P.; Narsimulu, K.; Subash, V.; Kunwar, A. C. *J. Am. Chem. Soc.* **2006**, *128*, 14657. (b) Sharma, G. V. M; Subash, V.; Narasimulu, K.; Sankar, A. R.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 8207. (c) Sharma, G. V. M; Manohar, V.; Subash, V.; Dutta, S. K.; Kunwar, A. C. *J. Org. Chem.* **2008**, *73*, 3689.

(19) (a) Mándity, I.; Fülöp, L.; Vass, E.; Toth, G. K.; Martinek, T. A.; Fülöp, F. *Org. Lett.* **2010**, *12*, 5584. (b) Mándity, I.; Monsignori, A.; Fülöp, L.; Forro, E.; Fülöp, F. *Chem. - Eur. J.* **2014**, *20*, 4591.

(20) Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stockle, M.; Kessler, H. *Chem. - Eur. J.* **2002**, *8*, 4365.

(21) Sharma, G. V. M; Reddy, K. R.; Krishna, P. R.; Sankar, A. R.; Narsimulu, K.; Kumar, S. K.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3961.

(22) (a) Sharma, G. V. M; Subash, V.; Narsimulu, K.; Ravi Sankar, A.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 8207. (b) Sharma, G. V. M; Prashanth, T.; Sirisha, K.; Basha, S. J.; Reddy, P. G.; Sarma, A. V. S. *J. Org. Chem.* **2014**, *79*, 8614.

(23) (a) Bodanszky, M. *Peptide Chemistry: A Practical Textbook*; Springer: New York, 1988. (b) Chan, L. C.; Cox, G. B. *J. Org. Chem.* **2007**, *72*, 8863.

(24) See Supporting Information.

(25) Solvent titration studies were carried out by sequentially adding up to 33% (v/v) of DMSO-d_6 to 600 μL $CDCl_3$ solutions of the peptides.

(26) Szolnoki, E.; Hetényi, A.; Martinek, T. A.; Szakonyi, Z.; Fülöp, F. *Org. Biomol. Chem.* **2012**, *10*, 255.

(27) (a) Nagata, Y.; Takeda, R.; Suginome, M. *Chem. Commun.* **2015**, *51*, 11182. (b) Suginome, M.; Yamamoto, T.; Nagata, Y. *Yuki Gosei Kagaku Kyokaishi* **2015**, *73*, 1141. (c) Nagata, Y.; Nishikawa, T.; Suginome, M. *ACS Macro Lett.* **2016**, *5*, 519.

(28) (a) Kaptein, R.; Zuiderweg, E. R. P.; Scheek, R. M.; Boelens, R.; van Gunsteren, W. F. *J. Mol. Biol.* **1985**, *182*, 179. (b) Brunger, A. T.; Clore, G. M.; Gronenborn, A. M.; Karplus, M. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 3801. (c) Bassolino-Klimas, D.; Tejero, R.; Krystek, S.

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031

R.; Metzler, W. J.; Montelione, G. T.; Bruccoleri, R. E. *Protein Sci.* **1996**, *5*, 593.

(29) *Discover*, Version 2.98; Biosym Molecular Simulations: San Diego, CA, 1995.

(30) (a) Neuhaus, D.; Williamson, M. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers: New York, 1989. (b) Thomas, P. D.; Basus, V. J.; James, T. L. *Proc. Natl. Acad. Sci. U. S. A.* **1991**, *88*, 1237.

(31) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J.C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, Revision C.02; Gaussian, Inc.: Wallingford, CT, 2004.

DOI: 10.1021/acs.joc.6b02856
J. Org. Chem. 2017, 82, 2018−2031