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Helical Structures of Cyclopentene-based α,α-Disubstituted α-Amino Acid Homopeptides

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Abstract: The cyclopentene-based α,α-disubstituted α-amino acid $Ac_5c^=$ and its homopeptides, up to nonapeptides, were synthesized. The side-chain cyclopentene was expected to become symmetric, the C^α-carbon to be puckered, and other C^β, $C^{\beta'}$, C^γ, $C^{\gamma'}$-carbons to be coplanar. As expected, side-chain cyclopentene conformations became symmetric and C^α-carbons were puckered. Conformational studies using FT-IR absorption, ^{1}H NMR spectra, and X-ray crystallographic analyses revealed that $Ac_5c^=$ homopeptides did not form a planar conformation, but assumed a 3_{10}-helical structure, similar to cyclopentane-based α,α-disubstituted α-amino acid homopeptides.

Keywords: Conformation · Cyclopentene · α,α-Disubstituted α-amino acid · Helix · Peptide

Masakazu Tanaka graduated with a BSc degree in Pharmaceutical Sciences from Kyushu University in 1986, and then received an MSc degree from the same university. He became a Research Fellow at the Osaka Branch of the National Institute of Health Sciences Japan in 1988. In 1990, he was appointed as a Research Associate at Kyushu University. After receiving his PhD degree under the supervision of Professor Kiyoshi Sakai, he performed postdoctoral work with Professor Dieter Seebach's group in 1994 at ETH in Switzerland. He became an Associate Professor at the Graduate School of Pharmaceutical Sciences, Kyushu University (Professor Hiroshi Suemune's group) in 1997. Since 2008, he has been a Professor at the Graduate School of Biomedical Sciences, Nagasaki University. He received the Yamanouchi Seiyaku Award in Synthetic Organic Chemistry, Japan (1993), the Inoue Research Award for Young Scientists (1994), and the Pharmaceutical Society of Japan Award for Divisional Scientific Promotion (2006). His current research interests involve foldamers, asymmetric catalysts, and cell-penetrating peptides.

1. Introduction

α,α-Disubstituted α-amino acids (dAAs) are non-coded amino acids that have an α-alkyl substituent instead of an α-hydrogen atom.[1–3] dAA homopeptides have been reported to form stable secondary structures, such as α-helices, 3_{10}-helices, and extended planar structures.[4,5] For example, α-aminoisobutyric acid (Aib)-containing homopeptides preferentially form 3_{10}-helical structures,[6] while diethylglycine (Deg)-containing homopeptides adopt extended planar conformations.[7,8] Furthermore, cyclic 1-aminocycloalkanecarboxylic acid (Ac_nc; n = ring size)-containing homopeptides are known to preferentially assume 3_{10}-helical structures.[9] For example, 1-aminocyclopentanecarboxylic acid (Ac_5c) homopeptides were found to preferentially form 3_{10}-helical structures.[10] The side-chain cyclopentane ring formed an envelope conformation, and the puckered carbon was scrambled at the C^α, C^β, and C^γ carbons (Fig. 1). On the other hand, the cyclopentene ring is flatter than the cyclopentane ring, and four carbons may be coplanar in the cyclopentene ring, while the other carbon is puckered. We designed an achiral 1-aminocyclopent-3-enecarboxylic acid ($Ac_5c^=$),[11] in which the C^α atom may be puckered and four other carbons (C^β, $C^{\beta'}$, C^γ, $C^{\gamma'}$) are coplanar. We anticipated whether $Ac_5c^=$ homopeptides, when constructed, form a symmetric planar conformation because the cyclopentene ring may be symmetric and a rigid structure. In the case that $Ac_5c^=$ homopeptides form helical structures, a comparison of helical structures between cyclopentane-amino acid Ac_5c and cyclopentene-amino acid $Ac_5c^=$ peptides may be of interest because Ac_5c-containing peptides may be used as cell-penetrating peptides[12] and helical chiral catalysts.[13]

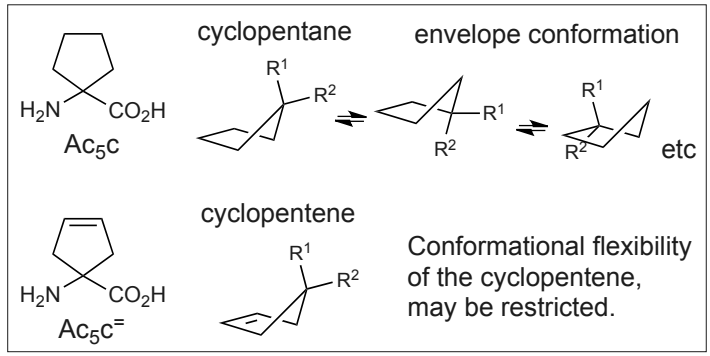

Fig. 1. Conformational flexibilities of the cyclopentane-based amino acid Ac_5c and cyclopentene-based amino acid $Ac_5c^=$.

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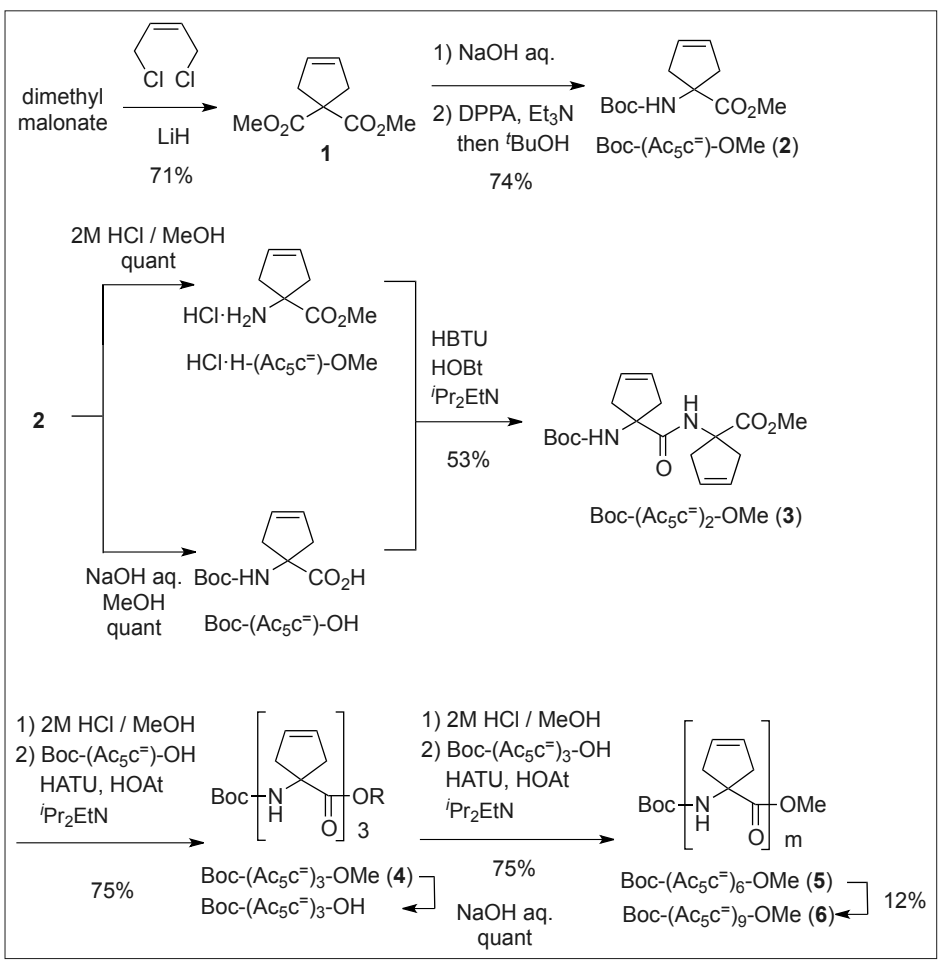
We herein synthesized the cyclopentene-amino acid $Ac_5c^=$, prepared its homopeptides, up to nonapeptides, and investigated their conformations in solution and in the crystalline state.

2. Synthesis of the Cyclopentene-based α,α-Disubstituted α-Amino Acid $Ac_5c^=$ and its Homopeptides

The cyclic amino acid $Ac_5c^=$ was synthesized as described previously[11,14] (Scheme 1). Dimethyl malonate was dialkylated with *cis*-1,4-dichloro-2-butene by LiH to give the cyclic diester **1** in 71% yield.[14] The monohydrolysis of diester **1** with aqueous NaOH, followed by the Curtius rearrangement with diphenyl phosphoryl azide (DPPA) and work-up with ^{t}BuOH afforded the cyclopentene-amino acid Boc-($Ac_5c^=$)-OMe (**2**) in 74% yield. The hydrolysis of **2** with aqueous NaOH gave the *C*-terminal free amino acid Boc-($Ac_5c^=$)-OH in quantitative yield, and deprotection of Boc-protecting group in **2** with 2 M methanolic HCl gave an *N*-terminal free amino acid H-($Ac_5c^=$)-OMe in quantitative yield. Dipeptide (**3**) was prepared by coupling between Boc-($Ac_5c^=$)-OH and H-($Ac_5c^=$)-OMe using 1-[bis(dimethylamino) methylene]-1*H*-benzotriazolium 3-oxide hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt) in 53% yield. Deprotection of Boc-protecting group in dipeptide **3**, and the resulting dipeptide amine was coupled with Boc-($Ac_5c^=$)-OH using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) to give a tripeptide Boc-($Ac_5c^=$)$_3$-OMe (**4**) in 75% yield. Hexapeptide Boc-($Ac_5c^=$)$_6$-OMe (**5**) was prepared by the fragment coupling between tripeptide-carboxylic acid and tripeptide-amine in 75% yield. Similarly, nonapeptide Boc-($Ac_5c^=$)$_9$-OMe (**6**) was prepared by the coupling between hexapeptide-amine and tripeptide carboxylic acid in 12% yield.

3. Conformational Analysis of Homopeptides in Solution

The FT-IR absorption spectra of $Ac_5c^=$ homopeptides Boc-($Ac_5c^=$)$_m$-OMe (m = 3, 6, 9) in $CDCl_3$ showed weak bands in the 3420~3430 cm^{-1} region, which were assigned as hydrogen bond-free, solvated N–H groups, and strong bands in the 3330~3360 cm^{-1} region, which were assigned as hydrogen-bonded N–H groups. The relative intensity of the low-frequency band to the high-frequency band increased as the main-chain length increased (Fig. 2). These FT-IR absorption spectra were very similar to those of the saturated Ac_5c homopeptides.[10] $Ac_5c^=$ homopeptides do not have an α-hydrogen atom, and, thus, it was not possible to apply nuclear Overhauser effect (NOE) correlations using α-hydrogen. We measured correlations between N(n)-H and N(n+1)-H in NOESY NMR spectra. The complete series of sequential d_{NN} correlations between N(n)-H and N(n+1)-H (n = 1~5) were observed in the NOESY NMR spectrum of hexapeptide **5**, and sequential d_{NN} correlations between N(n)-H and N(n+1)-H (n = 1~8) in nonapeptide **6** were observed, except for the case of n = 4 at which signals overlapped (Fig. 3). These results suggested the formation of a helical conformation. Furthermore, ^{1}H NMR measurements were performed following the addition of the strong hydrogen bond acceptor solvent DMSO-d_6 or the paramagnetic free radical 2,2,6,6-tetramethylpiperidin-1-yloxyl (TEMPO) to $CDCl_3$ solution. The addition of DMSO-d_6 affected chemical shifts in two N–H protons, and two N–H peaks shifted to lower magnetic fields with an increase in DMSO-d_6. Furthermore, the addition of the TEMPO radical broadened the peak width of two N–H protons (Fig. 4). These two N–H protons were solvated, and not intramolecularly hydrogen-bonded, suggesting the formation of helical structures in homopeptides **5** and **6**.[10]



Scheme 1. Synthesis of the five-membered ring amino acid $Ac_5c^=$ and its homopeptides.

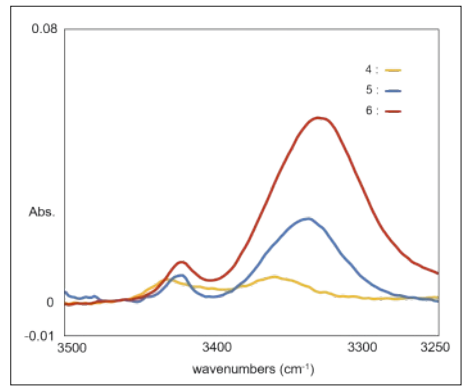


Fig. 2. FT-IR absorption spectra of Boc-($Ac_5c^=$)$_m$-OMe **4** (m = 3), **5** (m = 6), and **6** (m = 9) in $CDCl_3$. A cell with 0.1-mm path length was used. Peptide concentration: 5.0 mM.

4. Secondary Structural Analysis in the Crystalline State

The $Ac_5c^=$ tripeptide **4** yielded a suitable crystal for X-ray crystallographic analysis by the slow evaporation of a mixture of $CHCl_3$ and MeOH.[15] In the asymmetric unit, there was a β-turn structure. The structure was solved in the monoclinic centrosymmetric $P2_1/n$ space group.

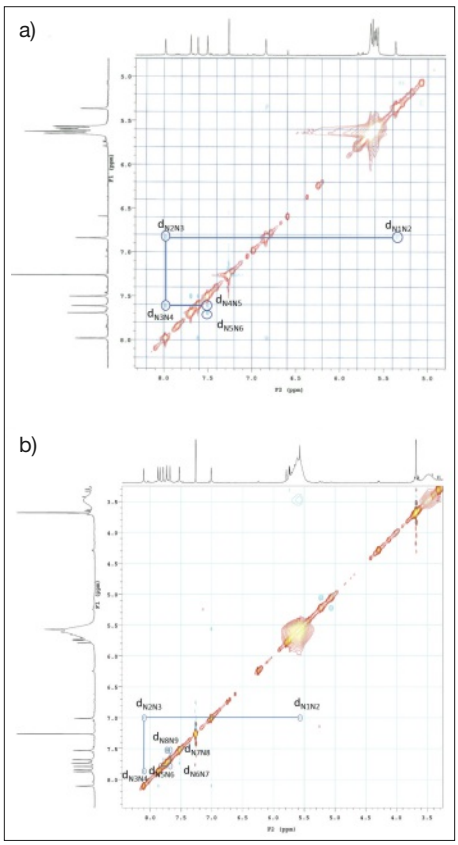


Fig. 3. NOESY NMR spectra of Boc-$(Ac_5c^{=})_m$-OMe a) **5** (m = 6) and b) **6** (m = 9) in $CDCl_3$.

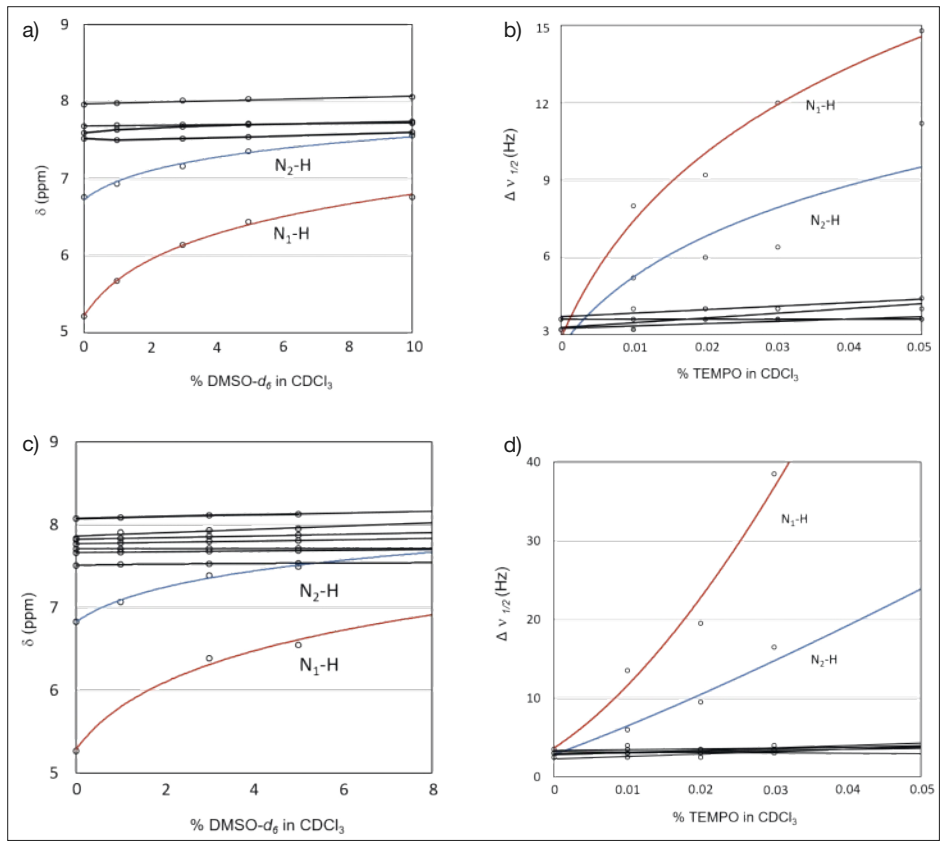


Fig. 4. Plots of N-H chemical shifts in the ^{1}H NMR spectra of **5** (a) and **6** (c) as a function of an increasing percentage of DMSO-d_6 added to the $CDCl_3$ solution, and plots of the bandwidth of the N-H protons of **5** (b) and **6** (d) as a function of an increasing percentage of TEMPO added to the $CDCl_3$ solution.

Thus, the mirror image, right-handed and left-handed turn conformers, existed in the crystalline state. The ϕ and ψ torsion angles were ±60.6 and ±36.3 in residue (1) and ∓55.5 and ∓33.3 in residue (2), respectively, whereas those of residue (3) had the opposite signs of ±49.2 and ±43.8, respectively. This kind of reversal of C-terminal torsion angles to those of the preceding residues was also observed in achiral Ac_5c homopeptides.[10] An intramolecular hydrogen bond of the i←i+3 type was observed between the oxygen of urethane C(0)=O(0) and peptide N(3)-H (Fig. 5).

Recrystallization of the $Ac_5c^{=}$ hexapeptide **5** from DMF afforded crystals suitable for an X-ray crystallographic analysis.[15] The crystal structure was solved in the $P2_1/n$ space group to give a 3_{10}-helical structure with two DMF molecules in the asymmetric unit (Fig. 6). The space group $P2_1/n$ is centrosymmetric, and, thus, right-handed and left-handed helical structures (mirror image) both exist. The average ϕ and ψ torsion angles of residues (1~5) were ±59.0 and ±23.7, accompanied by the reversal of the C-terminal torsion angles (∓58.4 and ∓38.2). The i←i+3 type intramolecular hydrogen bonds, which corresponded to the 3_{10}-helical conformation, were formed between the oxygen of carbonyl C(i)=O(i) and peptide N(i+3)-H (i = 0~3). The formyl oxygen of solvents DMF (A and B) were hydrogen-bonded to peptides N(1)-H and N(2)-H, respectively. Table 4 shows the distance between the $C^{\alpha}(i)$ atom and plane defined by $C^{\beta}(i)$, $C^{\gamma}(i)$, $C^{\gamma'}(i)$, and $C^{\beta'}(i)$ atoms. These distances were shorter than those of the cyclopentane-based amino acid (>0.55 Å),[10] with the side-chain cyclopentene rings on $Ac_5c^{=}$ residues (3), (4), (5), and (6) in particular becoming flatter. The cyclopentene rings were symmetric, and the C^{α}-carbons were puckered. The superimposed structures of Boc-$(Ac_5c^{=})_6$-OMe (**5**) and Cbz-$(Ac_5c)_6$-O^{t}Bu from CCDC-1264125 (X-ray crystallographic analysis by C. Toniolo and coworkers[10]) is shown in Fig. 7. The peptide backbone structure of $Ac_5c^{=}$ hexapeptide **5** matched that of the reported Ac_5c hexapeptide except for C-terminal residue, whereas the conformation of the side-chain cyclopentene in $Ac_5c^{=}$ differed from that of the cyclopentane in Ac_5c. (Tables 1–4)

5. Conclusion

Homopeptides composed of cyclopentene-based dAA; $Ac_5c^{=}$, up to nonapep-

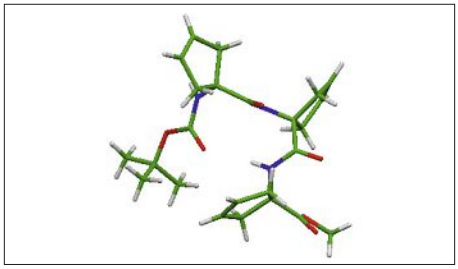


Fig. 5. A β-turn structure of Boc-$(Ac_5c^{=})_3$-OMe (**4**) as elucidated by X-ray crystallographic analysis.

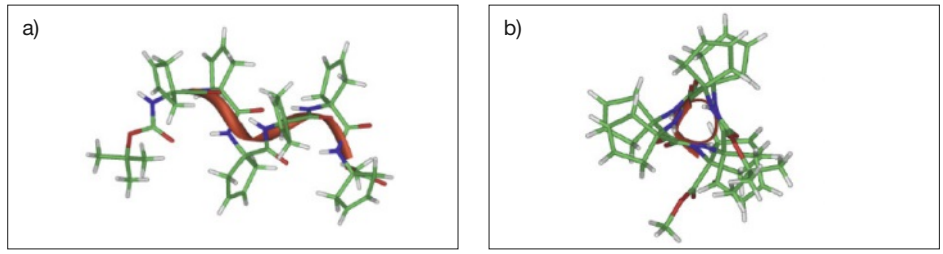


Fig. 6. A 3_{10}-helical secondary structure of Boc-$(Ac_5c^{=})_6$-OMe (**5**) as elucidated by an X-ray crystallographic analysis. a) View perpendicular to the helical axis and b) view along the helical axis.

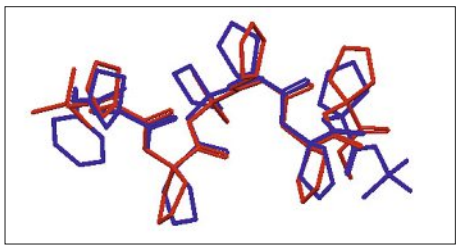


Fig. 7. Superimposed structures of Boc-($Ac_5c^=$)$_6$-OMe (**5**; red) and Cbz-(Ac_5c)$_6$-O^{t}Bu (CCDC-1264125; blue) reported by Toniolo and coworkers.[10]

tides, were synthesized. A conformational analysis using FT-IR absorption, and ^{1}H NMR spectra in $CDCl_3$ solution revealed that $Ac_5c^=$ homopeptides preferentially formed helical structures. An X-ray crystallographic analysis unequivocally showed that the $Ac_5c^=$ hexapeptide formed a 3_{10}-helical structure, but not a planar conformation, and the side-chain cyclopentene ring in $Ac_5c^=$ homopeptides became flatter and C^α-carbons were puckered in the five-membered rings. The olefin in the cyclopentene-based amino acid $Ac_5c^=$ and its peptides may be easily converted into several functional groups.[16]

Acknowledgments

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Table 1. Crystal and diffraction parameters of Boc-($Ac_5c^=$)$_3$-OMe (**4**) and Boc-($Ac_5c^=$)$_6$-OMe (**5**).

	Boc-($Ac_5c^=$)$_3$-OMe (**4**)	Boc-($Ac_5c^=$)$_6$-OMe (**5**)
Empirical formula	$C_{24}H_{33}N_3O_6$	$C_{42}H_{54}N_6O_9{\cdot}2(C_3H_7NO)$
Molecular weight *Mr*	459.53	933.10
Crystal dimensions [mm]	0.10 × 0.09 × 0.02	0.24 × 0.23 × 0.20
Data collection temp. [K]	93	93
Crystal system	monoclinic	monoclinic
Lattice parameters		
a, *b*, *c* (Å)	9.555, 18.267, 14.054	16.608, 17.917, 17.868
α, β, γ (°)	90, 90.266, 90	90, 111.743, 90
V (Å^3)	2453.1	4938.6
Space group	$P2_1/n$	$P2_1/n$
Z value	4	4
D_{calc} [g/cm^3]	1.244	1.255
μ (MoKα) [mm^{-1}]	0.090	0.090
No. of observations	2398	7700
No. of variables	298	604
R_1 ($I > 2\sigma$), wR_2	0.0993, 0.1362	0.0877, 0.2342
Crystallizing solvent	MeOH/$CHCl_3$	DMF

Table 2. Selected torsion angles of Boc-($Ac_5c^=$)$_3$-OMe (**4**) and Boc-($Ac_5c^=$)$_6$-OMe (**5**).[a]

Torsion Angle	Boc-($Ac_5c^=$)$_3$-OMe (**4**)	Boc-($Ac_5c^=$)$_6$-OMe (**5**)
ω0	163.9(3)	169.6(2)
ϕ1	60.6(5)	60.6(3)
ψ1	36.3(5)	27.9(4)
ω1	176.1(3)	175.7(2)
ϕ2	55.6(5)	57.7(3)
ψ2	33.3(5)	22.6(4)
ω2	178.4(4)	179.5(2)
ϕ3	−49.2(5)	57.7(3)
ψ3	−43.8(5)	22.9(4)
ω3	178.9(3)	−178.7(2)
ϕ4	---	57.4(3)
ψ4	---	24.7(4)
ω4	---	−179.4(2)
ϕ5	---	61.8(3)
ψ5	---	20.5(4)
ω5	---	179.3(3)
ϕ6	---	−58.4(4)
ψ6	---	−38.2(3)
ω6	---	−176.6(3)

[a]The number of amino acid residues begins at the *N* terminus of the peptide chain.

Table 3. Intra- and intermolecular H-bond parameters for Boc-$(Ac_5c^{=})_3$-OMe (**4**) and Boc-$(Ac_5c^{=})_6$-OMe (**5**).

Peptide	Donor D–H	Acceptor A	Distance [Å] D···A	Angle [°] D–H···A	Symmetry operations
Boc-$(Ac_5c^{=})_3$-OMe (**4**)					
	N_3-H	O_0	2.967(4)	147.4(2)	x,y,z
	N_1-H	$O_{2'}$	2.842(4)	154.7(2)	−1/2+x,1/2−y,1/2+z
Boc-$(Ac_5c^{=})_6$-OMe (**5**)					
	N_3-H	O_0	3.133(3)	168.5(2)	x,y,z
	N_4-H	O_1	3.037(4)	170.2(2)	x,y,z
	N_5-H	O_2	2.972(4)	162.0(2)	x,y,z
	N_6-H	O_3	2.962(3)	166.0(2)	x,y,z
	N_1-H	$O_{DMF\text{-}A}$	2.924(4)	160.5(2)	x,y,z
	N_2-H	$O_{DMF\text{-}B}$	2.878(4)	163.4(2)	x,y,z

Table 4. Distances between the $C^{\alpha}(i)$ atom and plane defined by $C^{\beta}(i)$, $C^{\gamma}(i)$, $C^{\gamma'}(i)$, and $C^{\beta'}(i)$.[a]

Residue number	Boc-$(Ac_5c^{=})_3$-OMe (**4**) (Å)	Boc-$(Ac_5c^{=})_6$-OMe (**5**) (Å)
Residue 1	0.404	0.387
Residue 2	0.311	0.372
Residue 3	0.196	0.081
Residue 4	–	0.094
Residue 5	–	0.147
Residue 6	–	0.113

[a]The number of amino acid residues begins at the *N* terminus of the peptide chain.

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