Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 5623–5627
Tetrahedron Letters

Synthesis of 3,4-di-*O*-acylated glucose-derived furanoid sugar amino acids (Gaa): conformational analysis of a Leu-enkephalin analog containing di-*O*-myristoylated Gaa

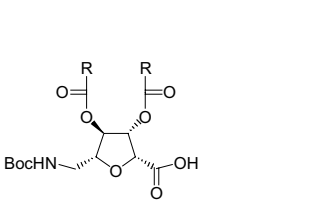
T. K. Chakraborty,* B. Krishna Mohan, S. Uday Kumar, A. Prabhakar and B. Jagadeesh*

Indian Institute of Chemical Technology, Hyderabad 500 007, India

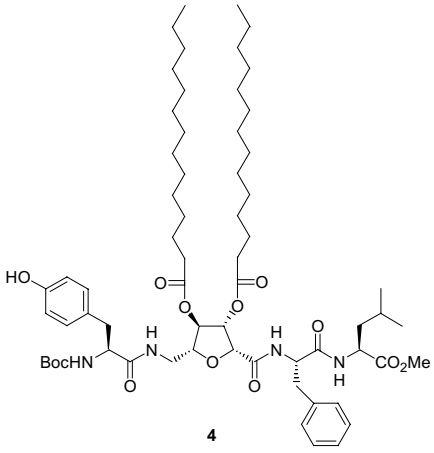
Received 10 May 2004; revised 21 May 2004; accepted 25 May 2004
Available online 11 June 2004

Abstract—3,4-Di-*O*-acylated derivatives **1**–**3** of a glucose-derived furanoid sugar amino acid (Gaa) were synthesized as novel peptide building blocks to study their effects on peptide conformation. Structural analysis of the di-*O*-myristoylated Gaa **3**-containing Leu-enkephalin analog **4** by various NMR techniques and constrained molecular dynamics (MD) simulation studies established a well-defined β-turn structure in DMSO-d_6 with an intramolecular hydrogen bond between PheNH → TyrCO.

For the development of peptides as novel therapeutic agents, it is essential to deliver them efficiently to their specific sites of action.[1] In this connection, the transport of peptides across cell membranes through hydrophobic barriers assumes great importance and has attracted considerable attention in recent years.[2] Peptides with low membrane permeability have been modified covalently by attaching fatty acid moieties to their *C*- or *N*-termini to increase their abilities to penetrate the cells' lipid membranes.[3] As part of our ongoing project on sugar amino acid based molecular designs,[4] we were interested in developing *O*-acylated furanoid sugar amino acids as novel peptide building blocks to find out their effects on peptide conformation, which play important roles in getting them delivered into cells.[5] The advantages of sugar amino acids as building blocks are largely due to their protected/unprotected ring hydroxyl groups that can influence the hydrophobic/hydrophilic nature of the peptides derived from them. Acylation of the hydroxyl groups of a glucose-derived furanoid sugar amino acid (Gaa) with *n*-butanoyl, *n*-octanoyl, and myristoyl groups furnished compounds **1**–**3**, respectively. Insertion of one of these acylated Gaa's **3** into the Gly-Gly segment of Leu-enkephalin led to the di-*O*-myristoylated analog **4**. Conformational studies of furanoid sugar amino acids containing analogs of Leu-enkephalin have been carried out before by us in detail.[6] Herein we describe the synthesis of the acylated derivatives **1**–**3** of Gaa as novel peptide building blocks[7] and the detailed conformational analysis of the di-*O*-myristoylated Gaa **3** containing Leu-enkephalin analog **4** by various NMR techniques and constrained molecular dynamics (MD) simulation studies that established a well-defined β-turn structure in DMSO-d_6 with an intramolecular hydrogen bond between PheNH → TyrC═O.



1: R = *n*-C_3H_7;
2: R = *n*-C_7H_{15};
3: R = *n*-$C_{13}H_{27}$

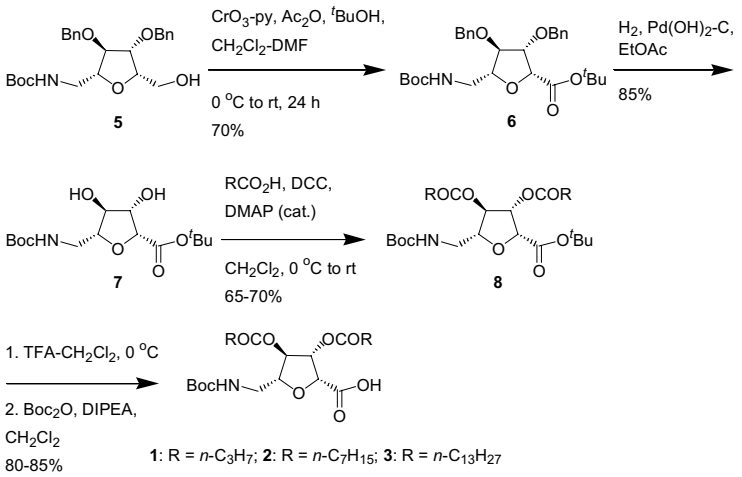


4

Keywords: Di-*O*-acylated furanoid sugar amino acids; Leu-enkephalin; Conformation; NMR.

* Corresponding authors. Tel.: +914027193154; fax: +914027193108; e-mail: chakraborty@iict.res.in

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.05.118



Scheme 1. Synthesis of 3,4-di-*O*-acylated Gaa **1**–**3**.

Scheme 1 outlines the synthesis of the 3,4-di-*O*-acylated derivatives of Gaa **1**–**3**. The starting material was *N*-Boc-6-amino-2,5-anhydro-3,4-di-*O*-benzyl-6-deoxy-D-glucitol **5** whose synthesis from D-glucose was reported by us earlier.[8] Oxidation of **5** with CrO_3-py in the presence of acetic anhydride and *t*-butanol provided the *t*-butyl ester of the D-gluconic acid **6** in 70% yield.[9] Removal of the Bn-protective groups was achieved by hydrogenation using $Pd(OH)_2$-C as catalyst to give diol intermediate **7** in 85% yield. The diol **7** was acylated by reacting with *n*-butanoic acid in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) to give the diacylated product **8** in 65% yield. Treatment of **8** with trifluoroacetic acid (TFA) deprotected both the *C*- and N-termini and the N-terminal was again protected using Boc_2O to furnish the desired product **1** in 80% yield.[10] The same protocol was used to prepare the di-*O*-octanoyl derivative **2** and the di-*O*-myristoylated product **3**.[10]

Next the di-*O*-myristoylated derivative of Gaa **3** was used in the synthesis of the Leu-enkephalin analog **4** following standard solution phase peptide synthesis methods[11] using 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry DMF and/or CH_2Cl_2 as solvents. While the *tert*-butoxycarbonyl (Boc) group was used for *N*-protection, the C-terminal was protected as a methyl ester (OMe). Deprotection of the former was done in TFA-CH_2Cl_2 (1:1). In the racemization free fragment condensation strategy that was followed, compound **3** was first coupled with the dipeptide H-Phe-Leu-OMe as efficiently as with any normal amino acid using the reagents mentioned above to give the tripeptide Boc-Gaa(Myr_2)-Phe-Leu-OMe (Myr = myristoyl) in 90% yield. After removal of the Boc-protection, the resulting tripeptide, H-Gaa(Myr_2)-Phe-Leu-OMe, was reacted with Boc-Tyr(Br-Z)-OH and subsequently hydrogenated using $Pd(OH)_2$-C in EtOAc to furnish the final peptide **4** in 85% yield.[12] The final product **4** was purified by silica gel column chromatography and fully characterized by spectroscopic methods before using it in the conformational studies.

The NMR studies of peptide **4** were carried out in approximately 5 mM solution in DMSO-d_6. The spectrum was very well resolved, and most of the spectral parameters could be obtained and are reported in Table 1. While the assignments were carried out with the help of total correlation spectroscopy (TOCSY),[13] rotating frame nuclear Overhauser effect spectroscopy (RO-

Table 1. ^{1}H Chemical shifts (δ in ppm), coupling constants (J in Hz) and, temperature coefficients ($\Delta\delta/\Delta T$ in ppb/K) of **4** in DMSO-d_6 at 500 MHz

Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Tyr	6.70 (d)	4.04 (ddd)	2.84[a] (dd, J = 13.8, 4.1)			7.00, 6.61 (Ph),	−9.8
	(J = 8.3)		2.61[b] (dd, J = 13.8, 9.6)			9.09 (OH), 1.22 (s, ^{t}Bu)	
Phe	7.85 (d)	4.59 (ddd)	3.02[a] (dd, J = 13.7, 4.7)			7.15–7.26 (m, ArH)	−3.7
	(J = 8.7)		2.94[b] (dd, J = 13.7, 9.3)				
Leu	8.45 (d)	4.29 (ddd)	1.51[a] (m, J = 12.3, 6.3)	1.49 (m)	0.87 (d)		−5.5
	(J = 7.4)		1.60[b] (m, J = 12.3, 9.0)		0.81 (d)	3.60 (s, CO_2CH_3)	

Gaa: NH (8.17, t, J = 6.0), H-6 (pro-*S*) (3.26, m), H-6 (pro-*R*) (3.47, ddd, $J_{NH\text{-}\beta}$ = 6.0, $J_{6,6'}$ = 13.7, $J_{5,6}$ = 8.9), H-5 (3.91, ddd, $J_{4,5}$ = 6.2, $J_{5,6}$ = 8.9, $J_{5,6'}$ = 2.0), H-4 (4.95, m), H-3 (5.32, dd, $J_{2,3}$ = 4.7, $J_{3,4}$ = 1.0), H-2 (4.45, d, $J_{2,3}$ = 4.7), –OCOCH_2– (2.15–2.30, m, 4H), –OCOCH$_2$CH_2– (1.39–1.47, m, 4H), other CH_2s (1.20, m, 40H), terminal CH_3 (0.84–0.85, m, 6H); $\Delta\delta/\Delta T$ = −5.2.

[a] (pro-*S*).

[b] (pro-*R*).

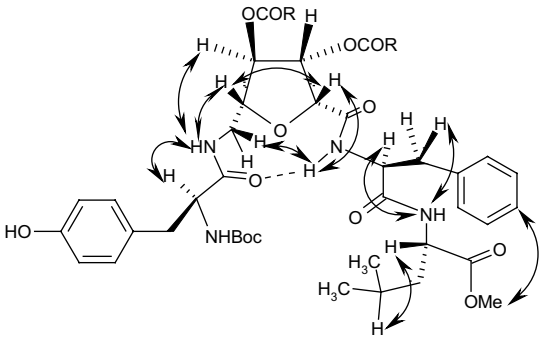


Figure 1. Schematic representation of the proposed structure of **4** with some of the prominent long-range ROEs seen in its ROESY spectrum.

ESY)[14] experiments provided information on the proximity of the protons. Variable temperature studies were carried out to measure the temperature coefficients of the amide proton chemical shifts ($\Delta\delta/\Delta T$), which provided information about their involvement in intramolecular hydrogen bonds.[15] The intensities of the cross-peaks in the ROESY spectrum, shown schematically in Figure 1, were used for obtaining the restraints in the simulated molecular dynamics (MD) calculations.[16]

The $^{3}J_{\mathrm{NH-C\alpha H}}$ values were large (>8 Hz) for Phe and Tyr indicating that the values of φ for these residues were in the vicinity of −120°. The populations of side-chain conformations about Cα-Cβ ($\chi 1$) for all the amide protons could be estimated from the $J_{\alpha-\beta}$ values shown in Table 1. The $J_{\alpha-\beta(\mathrm{pro\text{-}R})}$ and $J_{\alpha-\beta(\mathrm{pro\text{-}S})}$ are 9.6 and 4.1 Hz, respectively, for Tyr and 9.3 and 4.7 Hz, respectively, for Phe. The ^{3}J values suggest that for both these residues the *g* rotamer populations about $\chi 1$ are more than 70%. The predominance of the g^{-} rotamers about Cα-Cβ is further supported by strong ROESY cross-peaks between NH ↔ CβH(pro-*R*) and NH ↔ CβH(pro-*S*) in these residues. The value of $J_{2,3} = 4.7$, $J_{3,4} = 1.0$, $J_{4,5} = 6.2$ Hz in Gaa and the ROESY cross-peak between GaaC2H ↔ C5H support an envelope (C2-*exo* or C3-*endo*) conformer for the sugar ring. The NOE between PheNH → GaaC6H(pro-*R*) and the magnitude of $(\Delta\delta/\Delta T) = -3.7$ ppb/K support the presence of an intramolecular H-bond between PheNH → TyrC=O leading to a 10-membered β-turn-like ring structure. There is no indication of other amide protons participating in H-bonding as is evident from the relatively large magnitude of $\Delta\delta/\Delta T$s. The molecular structure is further supported by the observed ROE cross-peaks between PheNH ↔ GaaC2H, GaaNH ↔ GaaC5H, and GaaNH ↔ GaaC4H.

The cross-peak intensities in the ROESY spectrum were used for obtaining the restraints in the MD calculations using a simulated annealing protocol.[17] The long-range distance constraints (more than three bonds away) were derived based on a two-spin approximation by taking the distance between the Tyr β-protons (1.8 Å) as an internal standard. The long-range distance constraints shown in Table 2, torsional restraints only for amide bonds (*trans*, 180°, force constant 5 kcal/Å) and H-bonding restraint (1.8–2.2 Å, force constant 30 kcal/Å) were used in the MD calculations. Twenty structures were sampled during the constrained MD simulations carried out for a duration of 120 ps using 20 cycles, each of 6 ps periods, of the simulated annealing protocol. The sample structures were subsequently energy minimized and the superimposition of 12 structures from those sampled is shown in Figure 2. The alignment of the hydrogen bonded parts clearly revealed that the possible conformation of this compound involved an intramolecular hydrogen bond between PheNH → TyrC=O.

Table 2. The list of distance constraints (more than three bonds away) used in the MD simulation studies

ROEs	Distance (Å)
1. LeuNH ↔ PheNH	2.5–3.0
2. LeuNH ↔ PheCαH	1.8–2.3
3. LeuNH ↔ LeuCδH	2.4–2.9
4. LeuNH ↔ PheCβH	2.6–3.2
5. PheNH ↔ Gaa2-H	2.3–2.9
6. PheNH ↔ Gaa6-H	2.7–3.3
7. PheNH ↔ PheCβH	3.1–3.8
8. PheNH ↔ PheCβH	2.3–2.8
9. GaaNH ↔ TyrNH	2.0–2.4
10. GaaNH ↔ Gaa5-H	2.2–2.8
11. GaaNH ↔ Gaa4-H	2.4–3.0
12. GaaNH ↔ TyrNH	2.4–3.0
13. GaaNH ↔ PheNH	2.7–3.3
14. LeuαH ↔ TyrCβH	2.0–2.5
15. LeuαH ↔ TyrCβH′	2.3–2.9

The constraints were derived based on a two-spin approximation by taking the distance between the Tyr β-protons (1.8 Å) as an internal standard.

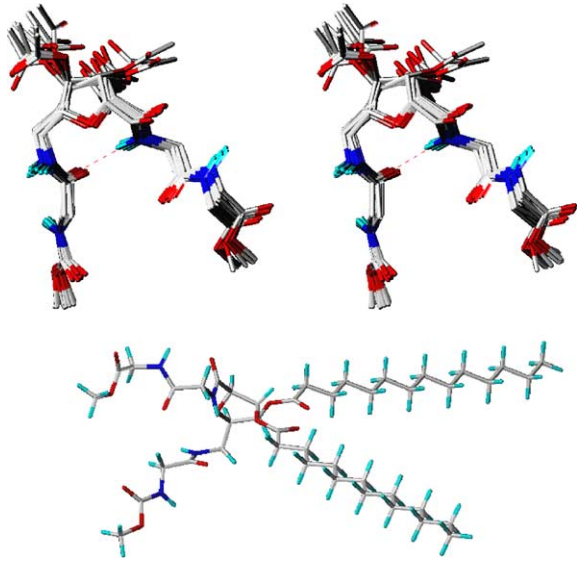


Figure 2. Top: Stereoview of the 12 backbone-superimposed energy-minimized structures of **4**, sampled during 20 cycles of the 120 ps constrained MD simulations following the simulated annealing protocol. For clarity in viewing, only the backbones are shown here omitting the amino acid side chains, fatty acid chains, and all hydrogens except the amide protons; bottom: full view of one of the energy-minimized structures sampled during MD studies.

It is worth noting here that unlike in sugars with free hydroxyls, where an unusual pseudo β-turn with a 9-membered H-bond was observed between AA^{i+2}-NH → AA^{i}-C3OH (AA^{i} is Gaa),[6] in the fatty acylated furanoid sugar amino acid-containing peptide **4**, we observed a 10-membered H-bond between AA^{i+1}-NH → AA^{i-1}-CO (AA^{i} is Gaa). This feature is similar to those reported by us earlier in benzyl-protected Gaa oligomers[18] and by Fleet et al. in acetate- and acetonide-protected examples.[19] Further work on these fatty acylated furanoid sugar amino acids is in progress.

Acknowledgements

The authors wish to thank DST, New Delhi for financial support (T.K.C.) and CSIR, New Delhi for research fellowships (B.K.M. and S.U.K.).

References and notes

1. (a) Green, I.; Christison, R.; Voyce, C. J.; Bundell, K. R.; Lindsay, M. A. *Trends Pharmacol. Sci.* **2003**, *24*, 213–215; (b) Kueltzo, L. A.; Middaugh, C. R. *J. Pharm. Sci.* **2003**, *92*, 1754–1772; (c) Schwarze, S. R.; Hruska, K. A.; Dowdy, S. F. *Trends Cell Biol.* **2000**, *10*, 290–295; (d) Lindgren, M.; Hallbrink, M.; Prochiantz, A.; Langel, U. *Trends Pharmacol. Sci.* **2000**, *21*, 99–103; (e) Schwarze, S. R.; Dowdy, S. F. *Trends Pharmacol. Sci.* **2000**, *21*, 45–48; (f) Stein, W. D. *Transport and Diffusion Across Cell Membranes*; Academic: San Diego, 1986.
2. (a) Pantarotto, D.; Briand, J.-P.; Prato, M.; Bianco, A. *Chem. Commun.* **2004**, 16–17; (b) Malkia, A.; Liljeroth, P.; Kontturi, K. *Chem. Commun.* **2003**, 1430–1431; (c) Boguslavsky, V.; Hruby, V. J.; O'Brien, D. F.; Misicka, A.; Lipkowski, A. W. *J. Peptide Res.* **2003**, *61*, 287–297; (d) Savic, R.; Luo, L.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615–618.
3. For some representative works see: (a) Löwik, D. W. P. M.; Linhardt, J. G.; Adams, P. J. H. M.; van Hest, J. C. M. *Org. Biomol. Chem.* **2003**, *1*, 1827–1829; (b) Foldvari, M.; Baca-Estrada, M. E.; He, Z.; Hu, J.; Attah-Poku, S.; King, M. *Biotechnol. Appl. Biochem.* **1999**, *30*, 129–137; (c) Foldavari, M.; Attah-Poku, S.; Hu, J.; Li, Q.; Hughes, H.; Babiuk, L. A.; Kruger, S. *J. Pharm. Sci.* **1998**, *87*, 1203–1208; (d) Sankaram, M. B. *Biophys. J.* **1994**, *67*, 105–112; (e) Muranishi, S.; Sakai, A.; Yamada, K.; Murakami, M.; Takada, K.; Kiso, Y. *Pharm. Res.* **1991**, *8*, 649–652; (f) Hashimoto, M.; Takada, K.; Kiso, Y.; Muranishi, S. *Pharm. Res.* **1989**, *6*, 171–176.
4. For reviews on sugar amino acids see: (a) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514; (b) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S. *Curr. Med. Chem.* **2002**, *9*, 421–435; (c) Chakraborty, T. K.; Jayaprakash, S.; Ghosh, S. *Comb. Chem. High Throughput Screening* **2002**, *5*, 373–387; (d) Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230–253; (e) Peri, F.; Cipolla, L.; Forni, E.; La Ferla, B.; Nicotra, F. *Chemtracts Org. Chem.* **2001**, *14*, 481–499.
5. For some relevant references see: (a) Kloosterman, D. A.; Goodwin, J. T.; Burton, P. S.; Condari, R. A.; Scahill, T. A.; Blinn, J. R. *Biopolymers* **2000**, *53*, 396–410; (b) Gentry, C. L.; Egleton, R. D.; Gillespie, T.; Abbruscato, T. J.; Bechowski, H. B.; Hriby, V. J.; Davis, T. P. *Peptides* **1999**, *20*, 1229–1238; (c) Romanowski, M.; Zhu, X.; Misicka, A.; Lipkowski, A. W.; Hruby, V. J.; O'Brien, D. F. *Biochim. Biophys. Acta* **1997**, *1329*, 245–258; (d) Burton, P. S.; Condari, R. A.; Hilgers, N. F. H.; Borchardt, R. T. *J. Pharm. Sci.* **1996**, *85*, 1336–1340.
6. (a) Chakraborty, T. K.; Ghosh, S.; Jayaprakash, S.; Sarma, J. A. R. P.; Ravikanth, V.; Diwan, P. V.; Nagaraj, R.; Kunwar, A. C. *J. Org. Chem.* **2000**, *65*, 6441–6457; (b) Chakraborty, T. K.; Jayaprakash, S.; Diwan, P. V.; Nagaraj, R.; Jampani, S. R. B.; Kunwar, A. C. *J. Am. Chem. Soc.* **1998**, *120*, 12962–12963.
7. Simple di-*O*-acetyl furanoid sugar amino acids have been prepared by Fleet's group earlier: Smith, M. D.; Long, D. D.; Marquess, D. G.; Claridge, T. D. W.; Fleet, G. W. J. *Chem. Commun.* **1998**, 2039–2040.
8. Chakraborty, T. K.; Ghosh, S. *J. Indian Inst. Sci.* **2001**, *81*, 117–123.
9. Corey, E. J.; Samuelsson, B. *J. Org. Chem.* **1984**, *49*, 4735.
10. Selected physical data of **1**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.02 (br t, $J = 6.4$ Hz, 1H, N*H*Boc), 5.28 (m, 1H, C3-*H*), 4.97 (m, 1H, C4-*H*), 4.48 (d, $J = 4.8$ Hz, 1H, C2-*H*), 3.84 (ddd, $J = 9.1$, 6.1, 1.8 Hz, 1H, C5-*H*), 3.26 (m, 2H, C6-H_2), 2.35–2.20 (m, 4H, –OCOCH_2–), 1.58–1.20 (m, 4H, –OCOCH$_2$CH_2–), 1.38 (s, 9H, Boc), 0.86 (m, 6H, methyls); MS (ESI) m/z (%) 416 (100) [M−H]$^+$. Selected physical data of **2**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.37 (br t, $J = 6.3$ Hz, 1H, N*H*Boc), 4.80 (m, 1H, C3-*H*), 4.09 (m, 1H, C4-*H*), 4.05 (br d, $J = 4.8$ Hz, 1H, C2-*H*), 3.76 (ddd, $J = 8.8$, 6.3, 1.8 Hz, 1H, C5-*H*), 3.26 (m, 2H, C6-H_2), 2.28–2.06 (m, 4H, –OCOCH_2–), 1.48–1.28 (m, 20H, –CH_2–), 1.24 (s, 9H, Boc), 0.84 (m, 6H, methyls); MS (ESI) m/z (%) 452 (100) [M+Na−Boc]$^+$. Selected physical data of **3**: $[\alpha]_D^{26}$ 10.45 (c 0.0034, $CHCl_3$); IR (KBr) ν_{max} 2925, 2854, 1745, 1515, 1219, 1164, 772 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 7.00 (t, $J = 6.4$, 1H, N*H*Boc), 5.3 (m, 1H, C3-*H*), 5.00 (m, 1H, C4-*H*), 4.6 (d, $J = 4.8$ Hz, 1H, C2-*H*), 3.88 (ddd, $J = 8.8$, 6.4, and 1.9 Hz, 1H, C5-*H*), 3.16 (m, 2H, C6-H_2), 2.30–2.15 (m, 4H, –OCOCH_2–), 1.47–1.39 (m, 4H, –OCOCH$_2$CH_2–), 1.4 (s, 9H, Boc), 1.19–1.39 (m, 40 H, –CH_2–), 0.83 (t, $J = 5.7$ Hz, 6H, methyls); MS (LSIMS) m/z (%) 721 (15) [M+H+Na]$^+$.
11. (a) Bodanszky, M.; Bodanszky, A. *The Practices of Peptide Synthesis*; Springer: New York, 1984; (b) Grant, G. A. *Synthetic Peptides: A User's Guide*; W.H. Freeman: New York, 1992; (c) Bodanszky, M. *Peptide Chemistry: A Practical Textbook*; Springer: Berlin, 1993.
12. Selected physical data of **4**: $[\alpha]_D^{26}$ 14.16 (c 0.0037, $CHCl_3$); IR (KBr) ν_{max} 3414, 2926, 1656, 1219, 771 cm^{-1}; ^{1}H NMR (DMSO-d_6, 200 MHz) listed in Table 1; MS (LSIMS) m/z (%) 1135 (30) [M+H]$^+$.
13. (a) Cavanagh, J.; Fairbrother, W. J.; Palmer, A. G., III; Skelton, N. J. *Protein NMR Spectroscopy*; Academic: San Diego, 1996; (b) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.
14. Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1992**, *114*, 3157–3159.
15. (a) Adams, P. D.; Chen, Y.; Ma, K.; Zagorski, M. G.; Sönnichsen, F. D.; McLaughlin, M. L.; Barkley, M. D. *J. Am. Chem. Soc.* **2002**, *124*, 9278–9286; (b) Kessler, H.; Bats, J. W.; Griesinger, C.; Koll, S.; Will, M.; Wagner, K. *J. Am. Chem. Soc.* **1988**, *110*, 1033–1049; (c) Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512–523.
16. Kessler, H.; Griesinger, C.; Lautz, J.; Müller, A.; van Gunsteren, F. W.; Berendsen, H. J. C. *J. Am. Chem. Soc.* **1988**, *110*, 3393–3396.
17. Molecular mechanics/dynamics calculations were carried out using the Sybyl 6.8 program on a Silicon Graphics O2

workstation. The Tripos force field, with default parameters, was used throughout the simulations. For detailed protocol for MD studies see Supporting information in: Chakraborty, T. K.; Ghosh, A.; Kumar, S. K.; Kunwar, A. C. *J. Org. Chem.* **2003**, *68*, 6459–6462.

18. Chakraborty, T. K.; Srinivasu, P.; Madhavendra, S. S.; Kumar, S. K.; Kunwar, A. C. *Tetrahedron Lett.* **2004**, *45*, 3573–3577.
19. Smith, M. D.; Claridge, T. D. W.; Sansom, M. S. P.; Fleet, G. W. J. *Org. Biomol. Chem.* **2003**, *1*, 3647–3655.