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Synthesis, Conformational Analysis and Biological Studies of Cyclic Cationic Antimicrobial Peptides Containing Sugar Amino Acids

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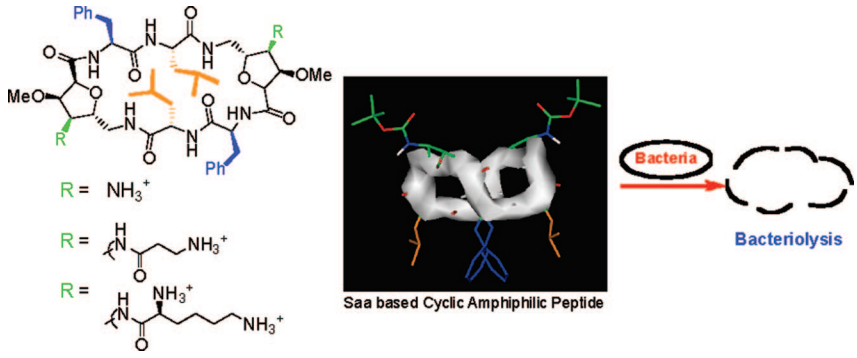

Sugar amino acid based 24-membered macrocyclic C_2-symmetric cationic peptides were designed and synthesized. The cationic group was introduced in the sugar amino acids. The conformation of these cyclic compounds was ascertained through NMR techniques, which proved they were amphipathic in nature. All the compounds were bacteriolytic, showed good activity against the Gr^{+ve} and Gr^{-ve} bacteria, and exhibited low hemolytic activity.

Introduction

In our continuous combat against virulent pathogenic bacteria, development of new antibiotics with novel modes of action assumes great significance today.[1] Easily curable bacterial diseases are nowadays becoming life-threatening owing to the increasing resistance of the pathogens to the established drugs. Cationic antimicrobial peptides (CAPs) are long considered as potential alternative antibiotics.[2] Both linear and cyclic cationic peptides have been found as part of the innate immune response of many vertebrates including humans.[3] These compounds exhibit a primary defense system of the host and are believed to be fighting against bacteria by disrupting their cell membrane through pore formation.[4] CAPs have either α-helical (e.g., magainins, mellitin, etc.)[5] or β-sheet (e.g., gramicidin S, tachyplesins, etc.)[6] structures and are amphiphilic in nature. However, as a result of the hemolytic activity of these natural antibiotics toward human blood cells, their uses as therapeutic agents are restricted.[7] Medicinal chemists are thus very keen to have new CAP antibiotics[8] either with new scaffolds or by mimicking their natural counterparts. Gellman et al. reported[9] a series of compounds with unnatural β-amino acids to obtain helical cationic peptides whose bactericidal activities are comparable to that of magainin. Reports of Overhand[10] and Wipf[11] on the synthesis of cyclic CAPs as gramicidin S mimics

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(1) Chu, D. T. W.; Plattner, J. J.; Katz, L. *J. Med. Chem.* **1996**, *39*, 3853–3874.
(2) (a) Pereira, H. A. *Curr. Pharm. Biotechnol.* **2006**, *7*, 229–234. (b) Brown, K. L.; Hancock, R. E. W. *Curr. Opin. Immunol.* **2006**, *18*, 24–30. (c) Zasloff, M. *Nature* **2002**, *415*, 389–395. (d) Hancock, R. E. W.; Lehrer, R. *TIBTECH* **1998**, *16*, 82–87.
(3) (a) Mookherjee, N.; Hancock, R. E. W. *Cell. Mol. Life Sci.* **2007**, *64*, 922–933. (b) Brogden, K. A. *Nat. Rev. Microbiol.* **2005**, *3*, 238–250. (c) Hancock, R. E. W.; Falla, T.; Brown, M. H. *Adv. Microb. Physiol.* **1995**, *37*, 133–175. (d) Boman, H. G.; Hultmark, D. *Annu. Rev. Microbiol.* **1987**, *41*, 103–126.
(4) (a) Christensen, B.; Fink, J.; Merrifield, R. B.; Mauzerall, D. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5072–5076. (b) Kagan, B. L.; Selsted, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 210–216.
(5) (a) Zasloff, M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5449–5453. (b) Bechinger, B.; Zasloff, M.; Opella, S. J. *Biophys. J.* **1992**, *62*, 12–14.
(6) (a) Hull, S. E.; Karlsson, R.; Main, P.; woolfson, M. M.; Dodson, E. J. *Nature* **1978**, *275*, 206–207. (b) Nakamura, T.; Furunaka, H.; Miyata, T.; Tokunaga, F.; Muta, T.; Iwanaga, S.; Niwa, M.; Takao, T.; Shimonishi, Y. *J. Biol. Chem.* **1988**, *263*, 16709–16713.
(7) Kondejewski, L. H.; Farmer, S. W.; Wishart, D. S.; Hancock, R. E. W.; Hodges, R. S. *Int. J. Peptide Protein Res.* **1996**, *47*, 460–466.

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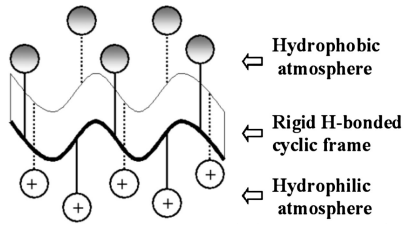
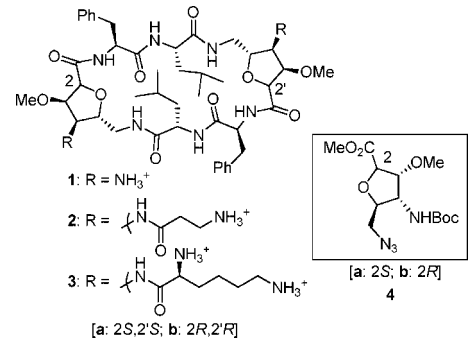


FIGURE 1. Graphical representation of a cyclic CAP molecule in amphiphilic conformation showing segregation of hydrophobic and hydrophilic residues on separate surfaces of an ordered cyclic frame.

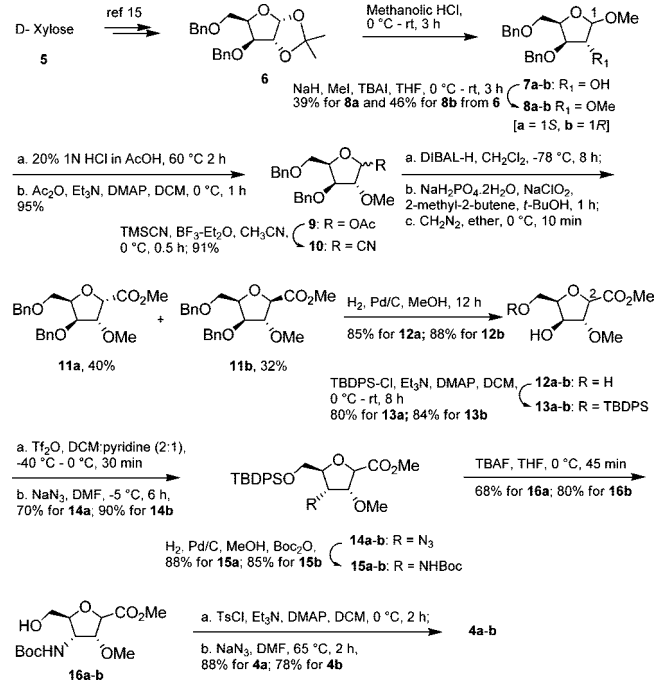
also encouraged us to use suitably functionalized unnatural amino acids in creating new antibacterial compounds.

Sugar amino acids (Saa), an important class of peptidomimetic scaffolds, have been exploited by us and others to create ordered structures.[12] In our initial approach,[13] we synthesized well-defined intramolecularly hydrogen-bonded tetrahydrofuram amino acid containing rigid cyclic peptides as basic framework. Our next target was to impose amphiphilic nature to those secondary structured cyclic peptides by distributing the hydrophobic and hydrophilic residues onto separate surfaces of the molecules (Figure 1), which is a prerequisite for peptides to act as CAPs.[14] Knowing the conformational features of our earlier reported cyclic peptides,[13] we anticipated that tuning the sugar amino acids with suitable functionalities could lead to the generation of novel amphiphilic properties in our cyclic compounds. We report here the synthesis and conformational analysis of a set of novel cyclic peptides (**1**−**3**) that are potentially active against bacteria.

SCHEME 1. Cyclic CAPs (1−3) Containing Sugar Amino Acid 4



SCHEME 2. Synthesis of Sugar Amino Acids 4a and 4b



It was envisaged that placing cationic charges on the tetrahydrofuran rings that occupy one side of the "tennis ball seam" type structure[13] of the cyclic framework would ensure that the hydrophilic side chains remain opposite to the other side chains of hydrophobic Phe, Leu residues.

The functionally tuned building blocks **4a** and **4b** were used to synthesize these cyclic compounds. Introduction of an extra amino group in the C-4 position of Saa ring was our interest from a synthetic point of view. We were also interested to devise a common strategy to synthesize these two diastereomeric sugar amino acids from the same starting material.

Results and Discussion

Synthesis of Compounds 1−3. Synthesis of the building block **4** started from **6**, which was prepared from D-xylose (**5**) following literature procedure,[15] as shown in Scheme 2. The 1,2-*O*-isopropylidene group of **6** was treated with dry HCl in MeOH to yield both the anomers of methyl-3,5-di-*O*-benzyl-D-xylofuranoside **7a,b** in a ratio of 2:3, respectively. The C-2 hydroxyl groups of **7a,b** were etherified using NaH, MeI, and

(8) (a) Chongsiriwatana, N. P.; Patch, J. A.; Czyzewski, A. M.; Dohm, M. T.; Ivankin, A.; Gidalevitz, D.; Zuckermann, R. N.; Barron, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2794–2799. (b) Yang, Z.; Liang, G.; Guo, Z.; Guo, Z.; Xu, B. *Angew. Chem., Int. Ed.* **2007**, *46*, 8216–8219. (c) Ishitsuka, Y.; Arnt, L.; Majewski, J.; Frey, S.; Ratajczek, M.; Kjaer, K.; Tew, G. N.; Lee, K. Y. C. *J. Am. Chem. Soc.* **2006**, *128*, 13123–13129. (d) Liu, Z.; Deshazer, H.; Rice, A. J.; Chen, K.; Zhou, C.; Kallenbach, N. R. *J. Med. Chem.* **2006**, *49*, 3436–3439. (e) Rennie, J.; Arnt, L.; Tang, H.; Nüsslein, K.; Tew, G. N. *J. Ind. Microbiol. Biotechnol.* **2005**, *32*, 296–300. (f) Ilker, M. F.; Nüsslein, K.; Tew, G. N.; Coughlin, E. B. *J. Am. Chem. Soc.* **2004**, *126*, 15870–15875. (g) Fernandez-Lopez, S.; Kim, H. S.; Choi, E. C.; Delgado, M.; Granja, J. R.; Khasanov, A.; Kraehenbuehl, K.; Long, G.; Weinberger, D. A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature* **2001**, *412*, 452–455. (h) Li, C.; Peters, A. S.; Meredith, E. L.; Allman, G. W.; Savage, P. B. *J. Am. Chem. Soc.* **1998**, *120*, 2961–2962.

(9) (a) Porter, E. A.; Wang, X.; Lee, H.-S.; Weisblum, B.; Gellman, S. H. *Nature* **2000**, *404*, 565. (b) Raguse, T. L.; Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 12774–12785. (c) Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2002**, *124*, 7324–7330. (d) Schmitt, M. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 417–428. (e) Mowery, B. P.; Lee, S. E.; Kissounko, D. A.; Epand, R. F.; Epand, R. M.; Weisblum, B.; Stahl, S. S.; Gellman, S. H. *J. Am. Chem. Soc.* **2007**, *129*, 15474–15476.

(10) (a) Grotenbreg, G. M.; Timmer, M. S. M.; Llamas-Saiz, A. L.; Verdoes, M.; van der Marel, G. A.; van Raaij, M. J.; Overkleeft, H. S.; Overhand, M. *J. Am. Chem. Soc.* **2004**, *126*, 3444–3446. (b) Grotenbreg, G. M.; Buizert, A. E. M.; Llamas-Saiz, A. L.; Spalburg, E.; van Hooft, P. A. V.; de Neeling, A. J.; Noort, D.; van Raaij, M. J.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. *J. Am. Chem. Soc.* **2006**, *128*, 7559–7565.

(11) Xiao, J.; Weisblum, B.; Wipf, P. *Org. Lett.* **2006**, *8*, 4731–4734.

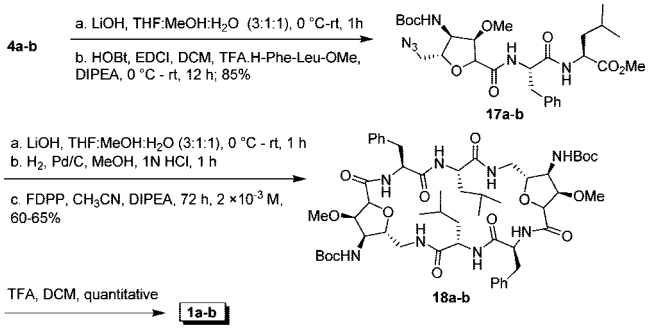
(12) For some review articles on sugar amino acids, see: (a) Risseeuw, M. D. P.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. *Tetrahedron Asymmetry* **2007**, *18*, 2001–2010. (b) Jensen, K. J.; Brask, J. *Peptide Sci.* **2005**, *80*, 747–761. (c) Chakraborty, T. K.; Srinivasu, P.; Tapadar, S.; Mohan, B. K. *J. Chem. Sci.* **2004**, *116*, 187–207. (d) Gruner, S. A. W.; Locardi, E.; Lohof, E.; Kessler, H. *Chem. Rev.* **2002**, *102*, 491–514. (e) Schweizer, F. *Angew. Chem., Int. Ed.* **2002**, *41*, 230–253. (f) Gervay-Hague, J.; Weathers, T. M. In *Pyranosyl Sugar Amino Acid Conjugates: Their Biological Origins, Synthetic Preparations and Structural Characterisation*; Dekker: New York, 2001.

(13) Chakraborty, T. K.; Roy, S.; Koley, D.; Dutta, S. K.; Kunwar, A. C. *J. Org. Chem.* **2006**, *71*, 6240–6243.

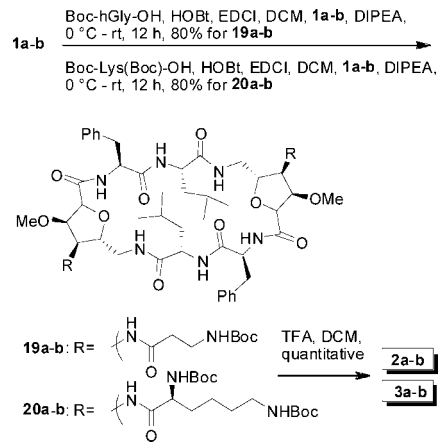
(14) (a) Langham, A. A.; Ahmad, A. S.; Kaznessis, Y. N. *J. Am. Chem. Soc.* **2008**, *130*, 4338–4346. (b) Leontiadou, H.; Mark, A. E.; Marrink, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 12156–12161. (c) Straus, S. K.; Hancock, R. E. W. *Biochim. Biophys. Acta* **2006**, *1758*, 1215–1223.

(15) Dyatkina, N. B.; Azhayev, A. V. *Synthesis* **1984**, 961–963.

SCHEME 3. Synthesis of Cyclic CAPs 1a and 1b



SCHEME 4. Synthesis of Cyclic CAPs 2a,b and 3a,b



a catalytic amount of TBAI. Next, the resulting mixture of anomers **8a,b** was treated with 20% 1N HCl in AcOH and heated at 60 °C for 2 h. The intermediate hemiacetal was acylated to **9** using Ac_2O and Et_3N. The inseparable anomers were treated with TMSCN[16] in presence of BF_3-Et_2O to furnish **10** as an anomeric mixture. Conversion of CN to aldehyde using DIBAL-H,[17] oxidation and treatment with CH_2N_2 resulted in the formation of **11a,b** (5:4) in 72% yield. The anomers could easily be separated at this stage through silica gel column chromatography. The next reactions of these isomers were performed separately.

Complete debenzylation followed by selective TBDPS protection of the primary hydroxyl group yielded **13a,b**. Our next target was to introduce an azido group in the C-4 center. Following Fleet's procedure,[18] the C-4 hydroxyl group was reacted with Tf_2O. The resulting triflate, after flash chromatography, was treated with 6 equiv of NaN_3 in dry DMF at −5 °C to furnish the γ-azido ester **14a,b**. The azido group was hydrogenated in the subsequent step and the resulting amino group was protected in situ using Boc_2O to yield **15a,b**.

Little elimination was observed in the α-anomer that was inseparable from **14a**. However, the eliminated product could be separated in 8% yield in its saturated form in the next step. Primary silyl deprotection using TBAF afforded **16a,b**. Tosylation of the primary hydroxyl group and heating of the tosylated intermediate with NaN_3 in DMF at 65 °C yielded the Saa's **4a,b**.

Synthesis of the cyclic framework of the target molecules is described in Scheme 3. Saponification of Saa's **4a,b** followed by coupling with TFA·H-Phe-Leu-OMe using EDCI and HOBt in the presence of DIPEA afforded **17a,b** in 85% yield. Stepwise saponification of **17a,b** followed by hydrogenation of the azido group to amine yielded the crude tripeptide, which on the subsequent step were cyclodimerized using FDPP[19] in CH_3CN under dilute condition (2×10^{-3} M) to furnish **18a,b** in 60−65% yield. Similar reactions were carried out by us earlier in the cyclodimerization of sugar amino acid containing peptides,[13] in the cyclooligomerization of sugar amino acids,[20] and in the cyclotrimerization of furan-amino acids.[21] One-pot cyclotrimerization of five-membered oxazole and thiazole ring containing amino acids using various reagents has also been reported by others.[22] Theoretical calculations were carried out by us to explain the preferential formation of cyclic trimers of furan amino acids.[23] However, this has still remained a trial and error method in our hands, and we are not yet able to predict the success of similar cyclodimerization reactions with other peptides. Finally, removal of the Boc-protection using TFA in DCM afforded **1a,b** in quantitative yield.

Compounds **1a,b** were separately coupled with Boc-hGly-OH and Boc-Lys(Boc)-OH to furnish **19a,b** and **20a,b**, respectively, following the procedure stated earlier as shown in Scheme 4. Finally, global Boc-deprotection of each compound furnished **2a,b** and **3a,b** in quantitative yields.

Conformational Analysis of 18−20 in $CDCl_3$. For all of the protected cyclic peptides **18**−**20**, NMR studies were carried out in $CDCl_3$ using 5−10 mM solutions in a 500 MHz spectrometer. While peptides **18a**, **18b**, and **20b** were investigated at 300 K, **19a**, **20a**, and **19b** were studied at 303 K. ^{1}H NMR spectra of peptides **18a**, **19a**, and **20a** were very well resolved. The ^{1}H NMR chemical shifts and coupling constants are given in Tables 1−6. For all of the peptides, the presence of only one set of peaks for each type of amino acid residues indicates 2-fold molecular symmetry in the NMR time scale. For peptide **18a**, amide proton chemical shifts (δ) > 7 ppm for the Saa (7.31 ppm) and Phe (8.56 ppm) residues suggest their participation in H-bonding. This was further confirmed by the solvent titration studies, when sequential addition of 300 μL of DMSO-d_6 to 600 μL of $CDCl_3$ solution of the peptide produced a very insignificant change of <0.13 ppm in their chemical shifts ($\Delta\delta$) (see Supporting Information). Similar observations and subsequent conclusions, regarding H-bonding of the Saa and Phe amide protons, were made for peptides **19a** and **20a**. The coupling constants and the nuclear Overhauser effect (NOE) correlation involving the C_εH protons allowed stereospecific assignments for

(16) Koert, U.; Stein, M.; Harms, K. *Tetrahedron Lett.* **1993**, *34*, 2299–2302.
(17) Kabota, I.; Takamura, H.; Yamamoto, Y. *J. Am. Chem. Soc.* **2004**, *126*, 14374–14376.
(18) Hungerford, N. L.; Fleet, G. W. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3680–3685.
(19) (a) Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711–6714. (b) Leonard, M. S.; Joullie, M. M. *Encyclopedia of Reagents for Organic Synthesis*; John Wiley & Sons Ltd.: New York, 2002.
(20) Chakraborty, T. K.; Srinivasu, P.; Bikshapathy, E.; Nagaraj, R.; Vairamani, M.; Kumar, S. K.; Kunwar, A. C. *J. Org. Chem.* **2003**, *68*, 6257–6263.
(21) (a) Chakraborty, T. K.; Tapadar, S.; Raju, T. V.; Annapurna, J.; Singh, H. *Synlett* **2004**, 2484–2488. (b) Chakraborty, T. K.; Tapadar, S.; Kumar, S. K. *Tetrahedron Lett.* **2002**, *43*, 1317–1320.
(22) For some representative works, see:(a) Haberhauer, G.; Rominger, F. *Tetrahedron Lett.* **2002**, *43*, 6335–6338. (b) Somogyi, L.; Haberhauer, G. H.; Rebek, J., Jr. *Tetrahedron* **2001**, *57*, 1699–1708. (c) Bertram, A.; Hannam, J. S.; Jolliffe, K. A.; de Turiso, F. G.-L.; Pattenden, G. *Synlett* **1999**, 1723–1726. (d) Sokolenko, N.; Abbenante, G.; Scanlon, M. J.; Jones, A.; Gahan, L. R.; Hanson, G. R.; Fairlie, D. P. *J. Am. Chem. Soc.* **1999**, *121*, 2603–2604. (e) Wipf, P.; Miller, C. P. *J. Am. Chem. Soc.* **1992**, *114*, 10975–10977. (f) Chen, S.; Xu, J. *Tetrahedron Lett.* **1991**, *32*, 6711–6714.
(23) (a) Sharma, M.; Kumar, P.; Singh, H.; Chakraborty, T. K. *J. Mol. Struct.: THEOCHEM* **2006**, *764*, 111–117. (b) Singh, H.; Arora, K. S.; Tapadar, S.; Chakraborty, T. K. *J. Theor. Comput. Chem.* **2004**, *3*, 555–566.

TABLE 1. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 18a (500 MHz, $CDCl_3$, 300 K) and 1a (600 MHz, H_2O/D_2O (9:1), 303 K)

	residues					
	18a in $CDCl_3$ solution			**1a** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.31 (m)	8.57 (d) J = 6.4	6.40 (d) J = 7.1	7.62 (dd) J = 4.8, 8.0	8.55 (d) J = 6.6	8.79 (d) J = 7.1
$C_\alpha H$	4.45 (d) J = 4.8	4.30 (ddd) J = 6.2, 6.4, 10.5	3.44 (ddd) J = 4.2, 7.1, 10.5	4.60 (d) J = 4.2	4.59 (ddd) J = 6.6, 7.1, 9.6	3.78 (ddd) J = 4.6, 7.1, 10.6
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	4.02 (t) J = 4.8	3.59 (dd) J = 10.5, 13.7	1.78(ddd) J = 4.2, 9.7, 14.3	4.35 (dd) J = 4.2, 4.5	3.40 (dd) J = 9.6, 13.7	1.48 (ddd) J = 4.6, 9.1, 13.7
$C_\beta H_{(pro\text{-}R)}$		3.27 (dd) J = 6.2, 13.7	1.84 (ddd) J = 4.4, 10.5, 14.3		3.29 (dd) J = 7.1, 13.7	1.67 (ddd) J = 3.2, 10.6, 13.7
$C_\gamma H$	3.73 (ddd) J = 4.8, 9.5, 11.2		0.78 (m)	3.60 (dd) J = 4.5, 10.1		0.58 (m)
$C_\delta H$	3.93 (m)		0.74 (d) J = 6.2	4.14 (ddd) J = 2.1, 9.4, 10.1		0.69 (m)
$C_\delta' H$			0.63 (d) J = 6.2			0.61 (m)
$C_\varepsilon H_{(pro\text{-}S)}$	3.93 (m)			3.89 (ddd) J = 2.1, 8.0, 14.5		
$C_\varepsilon H_{(pro\text{-}R)}$	2.57 (ddd) J = 4.5,10.0, 14.0			2.85 (ddd) J = 4.8, 9.4, 14.5		
others	7.31−7.24 (m, 5H, phenyl), 4.92 (d, J = 9.5, 1H, Saa-C_γNH), 3.37 (s, 3H, OCH_3), 1.45 (s, 9H, Boc).			7.35−7.29 (m, 5H, phenyl), 3.37 (s, 3H, OCH_3).		

TABLE 2. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 19a (500 MHz, $CDCl_3$, 303 K) and 2a (600 MHz, H_2O:D_2O (9:1), 303 K)

	residues					
	19a in $CDCl_3$ solution			**2a** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.36 (dd) J = 4.6, 8.2	8.58 (d) J = 6.6	6.37 (m)	7.53 (dd) J = 5.2, 8.3	8.55 (d) J = 6.6	8.80 (d) J = 7.3
$C_\alpha H$	4.51 (d) J = 4.4	4.31 (ddd) J = 6.4, 6.6, 10.0	3.45 (m)	4.66 (d) J = 4.4	4.55 (ddd) J = 6.1, 6.6, 11.0	3.68 (ddd) J = 4.1, 7.3, 11.7
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	4.05 (m)	3.59 (dd) J = 10.0, 13.7	1.77 (ddd) J = 4.3, 9.8, 14.2	4.20 (dd) J = 4.5, 4.6	3.48 (dd) J = 11.0, 13.6	1.43 (m) J = 4.1, 11.2, 13.8
$C_\beta H_{(pro\text{-}R)}$		3.27 (dd) J = 6.4, 13.7	1.84 (ddd) J = 4.4, 10.6, 14.2		3.32 (dd) J = 6.1, 13.6	1.61 (ddd) J = 4.1, 11.7, 13.8
$C_\gamma H$	4.06 (m)		0.79 (m)	4.09 (ddd) J = 4.5, 8.8, 10.1		0.39 (m)
$C_\delta H$	3.97 (m)		0.73 (d) J = 6.7	3.92 (ddd) J = 1.6, 10.1, 11.1		0.64 (d) J = 6.9
$C_\delta' H$			0.63 (d) J = 6.7			0.54 (d) J = 6.5
$C_\varepsilon H_{(pro\text{-}S)}$	3.84 (dd) J = 8.2, 13.6			3.70 (m)		
$C_\varepsilon H_{(pro\text{-}R)}$	2.60 (ddd) J = 4.6, 10.1, 13.6			2.74 (m)		
others	7.33−7.24 (m, 5H, phenyl), 6.09 (d, J = 7.9, Saa-C_γNH), 5.48 (m, 1H, h-Gly-NH), 3.42 (m, 1H, h-Gly-C_βH), 3.37 (m, 1H, h-Gly-C_β'H), 3.37 (s, -OCH_3), 2.50 (m, 1H, h-Gly-C_αH), 2.41(m, 1H, h-Gly-C_αH), 1.44 (s, Boc)			8.30 (d, J = 8.8, Saa-C_γNH), 7.65 (m, 2H, NH_2.TFA), 7.35−7.29 (m, 5H, phenyl), 3.26 (m, 2H, h-Gly-C_βH), 3.38 (s, -OCH_3), 2.74 (m, 2H, h-Gly-C_αH)		

the methylene protons. The assignments are consistent with $^3J_{NH\text{-}C_\varepsilon H(pro\text{-}S)} \approx 8.2$, $^3J_{NH\text{-}C_\varepsilon H(pro\text{-}R)} \approx 4.6$, $^3J_{C_\varepsilon H(pro\text{-}S)\text{-}C_\delta H} \approx 0$, and $^3J_{C_\varepsilon H(pro\text{-}R)\text{-}C_\delta H} \approx 10.0$ Hz and allowed us to recognize the proximity of $C_\varepsilon H$(*pro-R*) with $C_\alpha H$. $^3J_{NH\text{-}C_\varepsilon H(pro\text{-}S)} \approx 8.2$ Hz suggests that the NH and the $C_\varepsilon H$(*pro-S*) protons are *anti-periplanar*, with dihedral angle C(O)−N−C_ε−C_δ (ϕ_{sugar}) ≈ 120°, which falls in the β-region of the Ramachandran plot. The values of $^3J_{NH\text{-}C_\alpha H} \approx 6.6$ and 7.1 Hz for Phe and Leu, respectively, are not distinctive and could arise either due to averaging between several conformations or from a single structure. In either case it is not possible apriori to get unique information on dihedral angle C(O)−N−C_α−C(O) (ϕ) from these couplings.

In view of the stabilization of the structure by involvement of four of the amide protons in H-bonding and additionally the presence of two sugar rings having several constrained backbone dihedral angles, we believe that the structure of the 24-membered macrocycle is fairly rigid and the couplings involving the backbone protons arise from predominantly a single rigid conformation. Due to the presence of 2-fold symmetry, only few medium range characteristic NOE correlations such as NH(Phe)/C_δH(Saa) and NH(Saa)/NH(Leu) were available for deducing the structure of the molecule. The restraint MD calculations were carried out using constraints on the dihedral angles and distances derived from the ROESY data (Supporting Information). The lowest energy MD structures of peptides **18a**−**20a** are shown in Figure 2. The resulting structures show that four H-bonds, PheNH-Phe′CO (Phe′NH-PheCO) and SaaNH-Saa′CO (Saa′NH-SaaCO), stabilize the structure of the 24-membered macrocycle. The PheNH-Phe′CO (Phe′NH-

TABLE 3. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 20a (500 MHz, $CDCl_3$, 303 K) and 3a (600 MHz, H_2O/D_2O (9:1), 303 K)

	residues					
	20a in $CDCl_3$ solution			**3a** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.34 (dd) J = 4.6, 8.2	8.59 (d) J = 6.6	6.37 (m)	7.56 (dd) J = 4.7, 8.2	8.57 (d) J = 6.5	8.85 (d) J = 7.3
$C_\alpha H$	4.51 (d) J = 4.7	4.31 (ddd) J = 6.1, 6.6, 10.5	3.44 (m)	4.70 (d) J = 4.6	4.58 (ddd) J = 6.1, 6.5, 11.1	3.67 (ddd) J = 4.1, 7.3, 11.7
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	4.03 (m)	3.60 (dd) J = 10.5, 13.5	1.82 (m)	4.23 (t) J = 4.6	3.50 (dd) J = 11.1, 13.5	1.41 (ddd) J = 4.1, 11.2, 13.9
$C_\beta H_{(pro\text{-}R)}$		3.27 (dd) J = 6.1, 13.5	1.82 (m)		3.36 (dd) J = 6.1, 13.5	1.64 (ddd) J = 4.0, 11.7, 13.9
$C_\gamma H$	4.08 (m)		0.74 (m)	4.17 (ddd) J = 4.6, 8.8, 10.6		0.38 (m)
$C_\delta H$	4.01 (m)		0.73 (d) J = 6.6	3.93 (ddd) J = 2.0, 10.6, 10.2		0.64 (d) J = 6.5
C_δ′H			0.62 (d) J = 6.6			0.55 (d) J = 6.5
$C_\varepsilon H_{(pro\text{-}S)}$	3.79 (dd) J = 8.2, 13.6			3.64 (ddd) J = 2.0, 8.2, 14.7		
$C_\varepsilon H_{(pro\text{-}R)}$	2.60 (ddd) J = 4.6, 9.9, 13.6			2.75 (ddd) J = 4.7, 10.2, 14.7		
others	7.32−7.24 (m, 5H, phenyl), 6.24 (d, J = 8.9 Hz, 1H, Saa-C_γNH), 5.29 (t, J = 6.0 Hz, 1H, Lys-C_εNH), 5.08 (d, J = 8.3 Hz, 1H, Lys-C_αNH), 4.04 (m, 1H, Lys-C_αH), 3.41 (s, 3H, -OCH_3), 3.10 (m, 2H, Lys-C_εH, C_ε′H), 1.82 (m, 1H, Lys-C_βH), 1.62 (m, 1H, Lys-C_β′H), 1.52 (m, 2H, Lys-C_δH, C_δ′H), 1.43, 1.41 (s, 18H, Boc), 1.40 (m, 2H, Lys-C_γH, C_γ′H)			8.64 (d, J = 8.8, 1H, Saa-C_γNH), 7.48 (bs, 4H, NH_2.TFA), 7.37−7.30 (m, 5H, phenyl), 4.05 (d, J = 8.3, 1H, Lys-C_αNH), 3.39 (s, 3H, -OCH_3), 2.98 (m, 2H, Lys-C_εH, C_ε′H), 1.93 (m, 2H, Lys-C_βH, C_β′H), 1.70 (m, 2H, Lys-C_δH, C_δ′H), 1.45 (m, 2H, Lys-C_γH, C_γ′H)		

TABLE 4. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 18b (500 MHz, $CDCl_3$, 300 K) and 1b (600 MHz, H_2O/D_2O (9:1), 303 K)

	residues					
	18b in $CDCl_3$solution			**1b** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.39 (m)	7.56 (d) J = 8.7	6.67 (m)	8.05 (m)	8.46 (d) J = 8.7	8.43 (d) J = 6.8
$C_\alpha H$	4.27 (m)	4.73 (dt) J = 6.1, 8.7	4.63 (m)	4.61 (m)	4.89 (ddd) J = 5.4, 8.6, 8.7	4.37 (ddd) J = 6.8, 7.6, 7.7
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	3.85 (d) J = 4.3	3.26 (dd) J = 6.1, 14.2	1.66 (td) J = 6.3, 13.5	4.50 (m)	3.32 (dd) J = 5.4, 14.5	1.77 (td) J = 7.7, 13.4
$C_\beta H_{(pro\text{-}R)}$		3.10 (dd) J = 8.7, 14.2	1.45 (m)		3.10 (dd) J = 8.6, 14.5	1.60 (td) J = 7.6, 13.4
$C_\gamma H$	3.90 (m)		1.52 (m)	3.45 (m)		1.55 (m)
$C_\delta H$	3.90 (m)		0.89 (d) J = 6.4	3.97 (m)		0.96 (d) J = 6.7
C_δ′H			0.87 (d) J = 6.4			0.91 (d) J = 7.0
$C_\varepsilon H_{(pro\text{-}S)}$	4.05 (m)			3.82 (m)		
$C_\varepsilon H_{(pro\text{-}R)}$	3.17 (m)			3.54 (m)		
others	7.30−7.20 (m, 5H, phenyl), 5.07 (d, J = 7.4, 1H, Saa-C_γNH), 3.42 (s, 3H, OCH_3), 1.43 (s, 9H, Boc)			7.45−7.26 (m, 5H, phenyl), 3.50 (s, 3H, OCH_3)		

PheCO) H-bond involves a 13-membered pseudo ring, while the SaaNH-Saa′CO (Saa′NH-SaaCO) turn, a pseudo β-turn, contains a 10-membered H-bonded ring around the Phe-Leu residues. Also the dihedral angles ϕ for the Phe and Leu residues are about −66° and 56°, which are consistent with $^3J_{NH\text{-}C_\alpha H} \approx$ 7 Hz for these residues, justifying the consideration that a single rotamer about the N−C_α is responsible for these couplings as well as highly constrained molecular geometry of the macrocycle. It was interesting to note that for the 13-membered pseudo ring, ϕ and Ψ values for the first residue (Leu) are about 56° and 42° respectively, whereas for the second residue, a sugar moiety, being a dipeptide isostere, the corresponding angles are C(O)−N−C_ε−C_δ (ϕ') and N−C_ε−C_δ−O (θ_1), with values of 69° and 55°, respectively. Thus the three residue turn appears very similar to an α-turn[24] (ϕ = −57°, Ψ = −47°) with the signs of the angles reversed, usually referred to as an α′-turn. Similarly, for the pseudo β-turn containing Phe-Leu motif, the backbone ϕ and Ψ dihedral angles for the two residues are −66°, 116° and 56°, 42°, which are close to those for a type II β-turn for the first residue, while for the second residue they resemble those for the α′-turn. The backbone, with four H-bonds, resembles the tennis ball seam, corresponding to a distorted "β-β corner" motif.[13]

The structure for these peptides is very similar to that observed for cyclic hexapeptides containing *t*-tetrahydrofuran amino acids without substituents at the C_β and C_γ.[13] The MD

(24) Nataraj, D. V.; Srinivasan, N.; Saudhamini, R.; Ramakrishnan, C. *Curr. Sci.* **1995**, *69*, 434–447.

TABLE 5. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 19b (500 MHz, $CDCl_3$, 303 K) and 2b (600 MHz, H_2O/D_2O (9:1), 313 K)

	residues					
	19b in $CDCl_3$ solution			**2b** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.47 (dd) J = 4.4, 7.4	7.44 (d) J = 8.4	6.97 (m)	7.93 (m)	8.39 (m)	8.46 (m)
$C_\alpha H$	4.22 (m)	4.71 (ddd) J = 6.0, 8.3, 8.4	4.48 (m)	4.19 (m)	4.76 (m)	4.22 (m)
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	3.85 (d) J = 6.0	3.23 (dd) J = 6.0, 14.1	1.77 (td) J = 6.8, 13.7	3.88 (m)	3.21 (m)	1.71 (td) J = 6.9, 13.9
$C_\beta H_{(pro\text{-}R)}$		3.12 (dd) J = 8.3, 14.1	1.44 (m)		2.93 (dd) J = 7.1, 14.6	1.46 (m)
$C_\gamma H$	4.16 (ddd) J = 6.0, 7.9, 9.6		1.57 (m)	4.19 (m)		1.40 (m)
$C_\delta H$	3.85 (m)		0.90 (d) J = 6.6	3.76 (m)		0.84 (d) J = 7.0
$C_\delta'H$			0.88 (d) J = 6.6			0.75 (d) J = 6.8
$C_\varepsilon H_{(pro\text{-}S)}$	3.97 (dd) J = 7.4, 14.8			3.69 (m)		
$C_\varepsilon H_{(pro\text{-}R)}$	3.13 (m)			3.33 (m)		
others	7.29−7.19 (m, 5H, phenyl), 6.07 (d, J = 7.9, Saa-C_γNH), 5.30 (t, J = 6.4, 1H, h-GlyNH), 3.42 (s, -OCH_3), 3.39 (m, 2H, h-Gly-$C_\beta H$, $C_\beta'H$), 2.40 (m, 2H, h-Gly-$C_\alpha H$, $C_\alpha H$), 1.42 (s, Boc)			7.29−7.04 (m, 5H, phenyl), 8.16 (m, Saa-C_γNH), 3.39 (s, -OCH_3), 3.22 (m, 2H, h-Gly-$C_\beta H$, $C_\beta'H$), 2.68 (m, 2H, h-Gly-$C_\alpha H$, $C_\alpha H$)		

TABLE 6. ^{1}H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 20b (500 MHz, $CDCl_3$, 300 K) and 3b (600 MHz, H_2O/D_2O (9:1), 303 K)

	residues					
	20b in $CDCl_3$ solution			**3b** in H_2O/D_2O (9:1) solution		
protons	Saa	Phe	Leu	Saa	Phe	Leu
NH	7.95 (d) J = 10.4	7.63 (d) J = 8.3	9.62 (d) J = 8.3	8.64 (m)	8.13 (d) J = 8.0	8.70 (d) J = 7.1
$C_\alpha H$	4.38 (s)	4.81 (ddd) J = 4.7, 6.2, 8.3	4.76 (m)	4.26 (m)	4.74 (ddd) J = 4.4, 7.0, 8.0	4.19 (m)
$C_\beta H_{(pro\text{-}S)}/C_\beta H$	4.13 (d) J = 4.7	3.20 (dd) J = 4.7, 14.1	1.77 (ddd) J = 6.2, 7.9, 13.8	3.34 (m)	3.19 (dd) J = 4.4, 14.5	1.71 (m)
$C_\beta H_{(pro\text{-}R)}$		3.13 (dd) J = 6.2, 14.1	1.54 (m)		2.93 (dd) J = 7.0, 14.5	1.36 (m)
$C_\gamma H$	4.02 (m)		1.38 (m)	3.82 (m)		1.36 (m)
$C_\delta H$	4.13 (m)		0.86 (d) J = 6.5	4.23 (m)		0.84 (d) J = 6.2
$C_\delta'H$			0.78 (d) J = 6.5			0.72 (d) J = 5.9
$C_\varepsilon H_{(pro\text{-}S)}$	4.15 (m)			3.92 (m)		
$C_\varepsilon H_{(pro\text{-}R)}$	3.27 (m)			3.80 (m)		
others	7.44 (dd, J = 2.5, 7.5, 1H, Lys-C_εNH), 7.18−7.07 (m, 5H, phenyl), 5.89 (d, J = 8.8, 1H, Saa-C_γNH), 5.16 (d, J = 8.2, 1H, Lys-C_αNH), 4.05 (ddd, J = 3.4, 8.2, 8.9, 1H, Lys-$C_\alpha H$), 3.38 (s, 3H, -OCH_3), 3.32 (m, 1H, Lys-$C_\varepsilon H$), 2.95 (m, 1H, Lys-$C_\varepsilon'H$), 1.85 (m, 1H, Lys-$C_\beta H$), 1.52 (m, 1H, Lys-$C_\delta H$), 1.50 (m, 1H, Lys-$C_\beta'H$), 1.47, 1.42 (s, 18H, Boc), 1.38 (m, 1H, Lys-$C_\delta'H$), 1.23 (m, 2H, Lys-$C_\gamma H$, $C_\gamma'H$)			7.53 (bs, 4H, NH_2·TFA), 7.30−7.04 (m, 5H, phenyl), 7.57 (m, 1H Saa-C_γNH), 3.98 (m, 1H, Lys-$C_\alpha H$), 3.37 (s, 3H, -OCH_3), 3.02 (m, 2H, Lys-$C_\varepsilon H$, $C_\varepsilon'H$), 1.87 (m, 2H, Lys-$C_\beta H$, $C_\beta'H$), 1.71 (m, 2H, Lys-$C_\delta H$, $C_\delta'H$), 1.41 (m, 2H, Lys-$C_\gamma H$, $C_\gamma'H$)		

TABLE 7. Antibacterial Activity (in Lethal Concentration) against Gram-Positive and Gram-Negative Bacteria and Hemolytic Activity of the CAPs

	lethal concentration (μM)				
	Gram-negative		Gram-positive		
compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	% of RBC lysis at 80 μM
1a	35	80	23	23	0
2a	10	40	3	3	67
3a	18	35	9	9	20
1b	460	925	70	70	0
2b	30	60	20	20	0
3b	18	25	18	9	25

pictures, for these cyclic peptides do show very clearly the amphiphilic nature of the macrocycle, with the Leu and Phe side chains on one side of the ring providing a hydrophobic surface, while the polar chains occupy the hydrophilic face of the ring.

These molecules interestingly display very restricted rotations of the side chains. For Phe in peptide **18a**, $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}S)}$ = 10.5 and $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}R)}$ = 6.2 Hz, suggesting large populations of rotamers with χ^1 (N−C_α−C_β−C_{Phe}) ≈ 180° (t) with significant populations of rotamers with $\chi^1 \approx 60°$ (g^+) and −60° (g^-). Using the relations from the literature,[25] we found populations of t, g^+ and g^- isomers to be about 77%, 13% and 10%, respectively. For **19a** and **20a**, the couplings are very similar to those for **18a** and thus provide the t and g^- populations about χ^1 as 72% (t) and 12% (g^-) for **19a** and 77% (t) and 10% (g^-) for **20a**, respectively. For Leu residue in peptide **18a**

(25) Demarco, A.; Llinas, M.; Wüthrich, K. *Biopolymers* **1978**, *17*, 617–636.

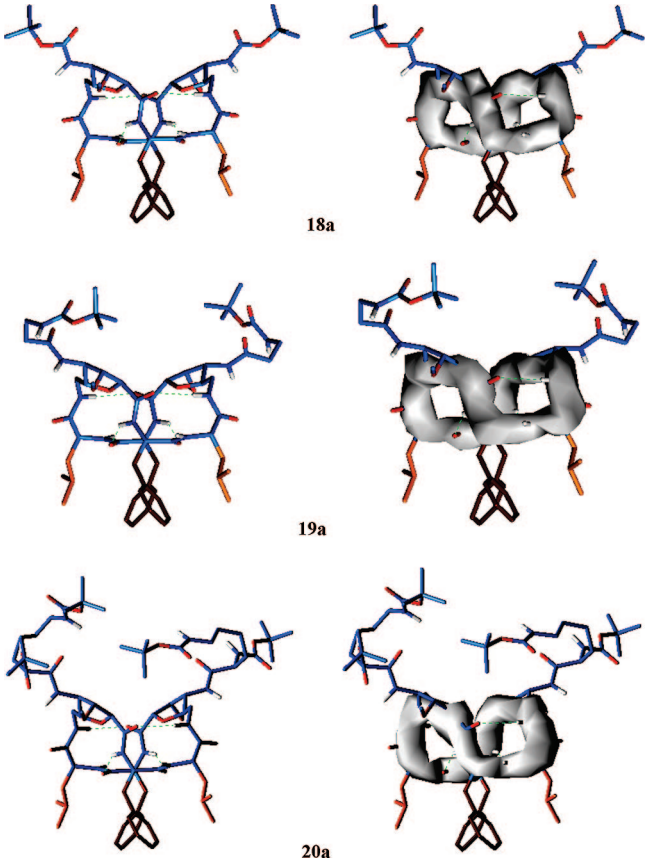


FIGURE 2. Lowest energy MD structure (left) and its backbone highlighted with pseudo-Connolly surface using Insight II (right) of peptides **18a**, **19a**, and **20a**.

and **19a**, $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}R)} \approx 10.5$ and $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}S)} \approx 4.2$ Hz again represent the large populations of rotamers with χ^1 ($N-C_\alpha-C_\beta-C_\gamma$) $\approx -60°$ (g^-) with substantial populations of rotamers with $\chi^1 \approx 180°$ (t). The calculated populations of g^- and t are about 77% and 17%, respectively. It was also interesting to note that for both these peptides, there are significant restrictions even about χ^2, with $^3J_{C_\beta H(pro\text{-}R)\text{-}C\gamma H} \approx 4.4$ and $^3J_{C_\beta H(pro\text{-}S)\text{-}C\gamma H} \approx 9.7$ Hz, which corresponds to a predominance of a rotamer with $C_\beta H_{(pro\text{-}S)}$ in *trans* disposition with respect to $C_\gamma H$. For **20a**, due to overlap of C_β and C_β' protons of Leu residue, it was not possible to obtain the information on χ^1 and χ^2. We also observed that $C_\gamma H$ resonances have unusually small δ values of about 0.7−0.8 ppm for peptides **18a**, **19a**, and **20a**. MD studies very clearly bring out the fact that the Leu residues are located just above the plane of aromatic ring of Phe, which results in a substantial upfield shift for $C_\gamma H$.

The peptides **18b**, **19b**, and **20b** containing a *cis* orientation of the 2,5-substituents in the furanoside ring also display 2-fold symmetry. Though for **18b** and **19b** the amide proton chemical shifts for Saa and Phe residues are >7 ppm, a large value of $\Delta\delta$ (~ 0.7 ppm) in the solvent titration studies seems to disfavor their involvement in H-bonding. In addition, the absence of distinctive couplings involving the backbone protons as well as very few unique medium range NOEs, due to 2-fold molecular symmetry, did not permit us to characterize the geometry of the macrocycle. For **20b**, all of the backbone amide protons appear to be H-bonded, with large δ and small $\Delta\delta$ values, and the couplings involving amide protons are >8.3 Hz. However, a unique structure satisfying these observations and the experimental NOEs, could not be derived.

Conformational Analysis of 1−3 in Water. NMR studies of all the deprotected peptides **1**−**3** were carried out at 303 K (**2b** at 313 K) in H_2O-D_2O (9:1) solutions (5−10 mM) at 500 and 600 MHz. The ^{1}H NMR chemical shifts and coupling constants are given in Tables 1−6. It was found that for **1a**−**3a** the spectral parameters (coupling constants), H-bonding information, and medium range NOE correlations (Supporting Information) are very similar to those obtained for their Boc-protected precursors in $CDCl_3$. In peptide **1a**−**3a**, the temperature coefficients of the amide proton chemical shifts ($\Delta\delta/\Delta T$) for the Phe and Saa residues are much smaller (<4.8 ppb/K) than that for Leu resideue (>10.8 ppb/K), confirming their participation in H-bonding. The stereospecific assignments for methylene protons were obtained like those for **18a**−**20a**. For **1a** and **3a**, the values of $^3J_{NH\text{-}C_\varepsilon H(pro\text{-}S)} \approx 8.0$, $^3J_{NH\text{-}C_\varepsilon H(pro\text{-}R)} \approx 4.7$, $^3J_{C_\varepsilon H(pro\text{-}S)\text{-}C_\delta H} \approx 2.1$, and $^3J_{C_\varepsilon H(pro\text{-}R)\text{-}C_\delta H} \approx 10.0$ Hz, as well as the $C_\varepsilon H(pro\text{-}R)/C_\alpha H$ NOE correlation, are consistent with the assignments. These data further suggest that the NH and the $C_\varepsilon H(pro\text{-}S)$ protons are *antiperiplanar*, with dihedral angle $C(O)-N-C_\varepsilon-C_\delta$ (ϕ_{sugar}) $\approx 120°$. For **2a**, due to overlap, some of the couplings could not be obtained, and therefore the stereospecific assignments were made on the basis of the similarity of the chemical shifts of the methylene protons in this family of peptides. The presence of four H-bonds and two sugar rings renders the macrocycle fairly rigid. Like in **18a**−**20a**, only few medium range NOE correlations such as NH(Phe)/$C_\delta H$(Saa) and NH(Saa)/NH(Leu) were available for deducing the structure. The restraint MD calculations were carried out using dihedral angles constraints and distance constraints derived from the ROESY data (Supporting Information). The resulting lowest energy structures are shown in Figure 3. The structures are very similar to those for **18a**−**20a** in $CDCl_3$, generating a distorted "β-β corner" motif. The rigidity of 24-membered cyclic frameworks of these peptides may be responsible for the similarity of the structure in both nonpolar and polar environments. The amphiphilic nature of the macrocycle of these cyclic peptides, with the Leu and Phe side chains providing a hydrophobic surface on one side of the ring, and the polar chains on the other side forming the hydrophilic face of the ring, is very vividly displayed in the structures (Figure 3).

These molecules also display very restricted rotations of the side chains. For Phe, $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}S)} \approx 10$ and $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}R)} \approx 6$ Hz suggest large populations of t rotamers. It was found that the populations of t and g^+ isomers for **1a**−**3a** are about 69% and 13%, 82% and 17%, and 82% and 18%, respectively. For Leu residue in peptide **1a**−**3a**, $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}R)} \approx 11$ and $^3J_{C_\alpha H\text{-}C_\beta H(pro\text{-}S)} \approx 4$ Hz represent large populations of rotamers with χ^1 ($N-C_\alpha-C_\beta-C_\gamma$) $\approx -60°$ (g^-). The calculated populations of g^- and t are about 78% and 21%, 84% and 16%, and 79% and 16%, respectively, while the populations of g^+ rotamers are very small. As seen earlier for **18a** and **19a** (not well resolved in **20a**), in **1a**−**3a** also there are significant restrictions even about χ^2, with $^3J_{C_\beta H(pro\text{-}R)\text{-}C\gamma H} \approx 4$ and $^3J_{C_\beta H(pro\text{-}S)\text{-}C\gamma H} \approx 10$ Hz, which corresponds to a predominance of a rotamer with $C_\beta H_{(pro\text{-}S)}$ in *trans* disposition with respect to $C_\gamma H$. It was also noticed that $C_\gamma H$ resonances of Leu residues have unusually small δ values of about 0.4−0.6 ppm for these peptides, which is further supported by the MD studies, which show that the Leu residues are located just above the plane of aromatic ring of Phe, resulting in substantial upfield shifts.

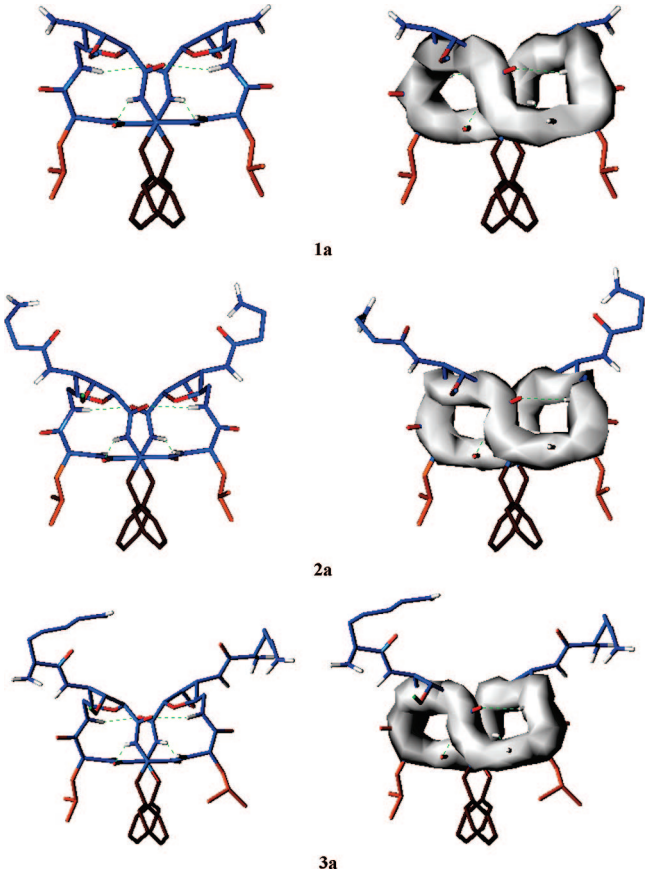


FIGURE 3. Lowest energy MD structure (left) and its backbone highlighted with pseudo-Connolly surface using Insight II (right) of peptides **1a**, **2a**, and **3a**.

Just like their Boc-protected precursors, the spectra of the 2,5-*cis*-compounds **1b**−**3b** in water failed to provide any substantial structural information.

Circular Dichroism Studies of Peptides 1−3. The structures of the cyclic molecules were examined by circular dichroism (CD) studies in 5 mM HEPES buffer (pH 7.4) and TFE, which provides a membrane-like environment and has been used extensively to delineate conformations of peptides in the membrane environment. The characteristics of the CD spectra of sugar amino acid containing peptides have not been documented yet. They can only be compared with the spectra of natural amino acid containing peptides. The CD spectra of compounds **1a**−**3a**, where the tetrahydrofuran rings have 2,5-*trans* orientation, showed minima at ∼204 nm both in buffer and TFE (Supporting Information). The spectra suggest the population of a distorted turn structure[26] in both polar and nonpolar medium in agreement with what was observed in NMR studies.

The CD studies of **1b**−**3b** in TFE and buffer show spectra characteristic of β conformation with a rigid structure in both solvents.

Biological Studies. As expected, all of our designed amphipathic peptides, with the exception of **1b**, showed pronounced membranolytic activity against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive (*S. aureus* and *B. subtilis*) bacteria (Table 7). Among Gram-negative bacteria, activity is more pronounced against *E. coli*. They exhibited greater activity against Gram-positive bacteria as compared to Gram-negative bacteria. Even the antibacterial activity of the least active compound **1b** appears to be more specific toward Gram-positive bacteria. Compound **2a** was the most active against both the Gram-positive and Gram-negative strains, whereas **2b** was less potent than the former though the net cationic charges (+2) and hydrophobic residues are same for the two. Compounds **3a** and **3b** exhibited comparable activity against *E. coli* and *B. subtilis*, while **3b** was more active than **3a** against *P. aeruginosa* and less active against *S. aureus*. Interestingly, **3a** was less active than **2a**, though the increase in net positive charge was expected to contribute to a greater extent toward activity. On the contrary, **3b** had better activity than **2b**. Compound **1a** also displayed far better activity than **1b**. Thus, it indicates that the relative hydrophobicity/hydrophilicity produced by the change in conformation of the sugar ring has an important role for the activity. From the results described here, it should be noted that the 2,5-*trans* sugar amino acid residues containing cyclic compounds were more active than their *cis* congeners.

The compound exhibiting greatest antimicrobial activity (**2a**) showed hemolytic activity of 67% at a concentration ∼8-fold greater than the lethal concentration for *E. coli*. At equivalent concentrations, **3a** and **3b** showed only ∼25% hemolysis. Others did not show any hemolytic activity at concentrations at which they exhibited antibacterial activity.

Conclusion

In summary, we have demonstrated the design and synthesis of sugar amino acid based CAPs. Structurally, these peptides were found to resemble the dumbbell-shaped loloatin cyclopeptides[27] more than the typical β-sheet structures of gramicidin S[6a] or tachyplesins.[6b,28] The encouraging biological activities of these compounds may be useful to generate new CAP-based drugs. Experimental manipulations through the syntheses of various sugar amino acids and their incorporation to these interesting cyclic frames, varying in the ratio of hydrophobicity versus hydrophilicity, and finally their biological performance could be the prospect of tomorrow's antibiotic.

Experimental Section

Synthesis of 7a and 7b. To a stirred solution of dry MeOH (125 mL) at 0 °C was added acetyl chloride (20 mL, 270 mmol) slowly in dropwise manner under nitrogen atmosphere. After 30 min, compound **6** (25 g, 67.5 mmol), dissolved in dry MeOH (50 mL), was added to the reaction mixture at room temperature and stirred for 3 h. The reaction mixture was quenched at 0 °C with liquor ammonia. MeOH was evaporated under reduced pressure, and the product was extracted with EtOAc, washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 18−25% EtOAc in petroleum ether eluant) afforded compound **7a** (9.5 g, 41%) and compound **7b** (11.5 g, 50%) as colorless oils. **Data for 7a** (α anomer): R_f = 0.5 (silica gel, 30% EtOAc in petroleum ether); $[\alpha]^{29}_D$ = +39.63 (*c* 2.4, $CHCl_3$); IR (neat): ν_{max} 2923, 2867, 1452, 1046, 698 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.30−7.22 (m, 10H, Ar*H*), 4.93 (d, J = 4.4 Hz, 1H, C_1-*H*), 4.76−4.45 (m, 4H, two -OCH_2Ph),

(26) Perczel, A.; Hollosi, M. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1996; pp 285−380.

(27) Krachkovskii, S. A.; Sobol, A. G.; Ovchinnikova, T. V.; Tagaev, A. A.; Yakimenko, Z. A.; Azizbekyan, R. R.; Kuznetsova, N. I.; Shamshina, T. N.; Arseniev, A. S. *Russ. J. Bioorg. Chem* **2002**, *28*, 269–273.

(28) Kawano, K.; Yoneya, T.; Miyata, T.; Yoshikawa, K.; Tokunaga, F.; Terada, Y.; Iwanaga, S. *J. Biol. Chem.* **1990**, *265*, 15365–15367.

4.32 (ddd, J = 5.9, 5.8, 4.4 Hz, 1H, C_4-*H*), 4.20 (dd, J = 4.4, 3.6 Hz, 1H, C_2-*H*), 3.94 (dd, J = 5.8, 3.6 Hz, 1H, C_3-*H*), 3.70 (dd, J = 10.2, 4.4 Hz, 1H, C_5-*H′*), 3.58 (dd, J = 10.2, 5.9 Hz, 1H, C_5-*H″*), 3.48 (s, 3H, -OC*H*$_3$), 2.58 (d, J = 7.3 Hz, 1H, C_2-O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.1, 137.9, 128.3, 128.2, 127.7, 127.6, 127.5, 127.4, 101.7, 83.5, 77.3, 76.9, 73.4, 71.8, 69.0, 55.7; MS (ESI): *m*/*z* (%) 345 (5) $[M + H]^+$, 362 (10) $[M + NH_4]^+$, 367 (100) $[M + Na]^+$. HRMS (ESI): calcd for $C_{20}H_{24}O_5Na$ $[M + Na]^+$ 367.1521, found 367.1509. **Data for 7b** (β anomer): R_f = 0.45 (SiO_2, 30% EtOAc in petroleum ether); $[\alpha]^{28}_D = -44.6$ (*c* 2.9, $CHCl_3$); IR (neat): ν_{max} 2924, 2866, 1453, 1054, 699 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.20 (m, 10H, Ar*H*), 4.72 (d, J = 2.3 Hz, 1H, C_1-*H*), 4.63−4.49 (m, 4H, two -OC*H*$_2$Ph), 4.4 (ddd, J = 6.7, 6.0, 5.2 Hz, 1H, C_4-*H*), 4.13 (dd, J = 3.0, 2.3 Hz, 1H, C_2-*H*), 3.9 (dd, J = 6.0, 3.0 Hz, 1H, C_3-*H*), 3.73 (dd, J = 10.5, 5.2 Hz, 1H, C_5-*H′*), 3.63 (dd, J = 10.5, 6.7 Hz, 1H, C_5-*H″*), 3.36 (s, 3H, -OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.0, 137.7, 128.3, 128.2, 127.7, 127.6, 127.5, 109.4, 83.2, 79.9, 79.3, 73.3, 72.2, 69.8, 55.6; MS (ESI): *m*/*z* (%) 345 (5) $[M + H]^+$, 362 (95) $[M + NH_4]^+$, 367 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{20}H_{24}O_5Na$ $[M + Na]^+$ 367.1521, found 367.1521.

Synthesis of 8a and 8b. Sodium hydride (1.8 g, 60% dispersion in oil, 46 mmol) was added portionwise to a stirred solution of **7a** (8 g, 23 mmol) in dry THF (60 mL) at 0 °C under nitrogen atmosphere. After the additions were completed, the reaction mixture was stirred at 0 °C for 15 min. Then MeI (3 mL, 46 mmol) was added slowly to the stirred reaction mixture followed by the addition of TBAI (848 mg, 2.3 mmol). After stirring for 2 h at room temperature, the reaction mixture was quenched at 0 °C by slow addition of saturated aqueous NH_4Cl solution. THF was removed under reduced pressure, and the product was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 16−18% EtOAc in petroleum ether eluant) afforded compound **8a** (8 g, 96%) as a colorless oil. (α-anomer): R_f = 0.5 (SiO_2, 25% EtOAc in petroleum ether); $[\alpha]^{28}_D = +88.0$ (*c* 1.6, $CHCl_3$); IR (neat): ν_{max} 2918, 1453, 1033, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33−7.22 (m, 10H, Ar*H*), 4.91 (d, J = 4.5 Hz, 1H, C_1-*H*), 4.64 (d, J = 12.0 Hz, 1H, H_A of -OCH_2Ph), 4.57 (d, J = 12.0 Hz, 1H, H_B of -OCH_2Ph), 4.53 (d, J = 12.0 Hz, 1H, H_A of another -OCH_2Ph), 4.50 (d, J = 12.0 Hz, 1H, H_B of another -OCH_2Ph), 4.32 (ddd, J = 6.7, 6.0, 4.5 Hz, 1H, C_4-*H*), 4.19 (dd, J = 6.7, 6.0 Hz, 1H, C_3-*H*), 3.82 (dd, J = 6.0, 4.5 Hz, 1H, C_2-*H*), 3.68 (dd, J = 10.5, 4.5 Hz, 1H, C_5-*H′*), 3.55 (dd, J = 10.5, 6.7 Hz, 1H, C_5-*H″*), 3.43 (s, 3H, -OC*H*$_3$), 3.36 (s, 3H, -OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.1, 137.9, 128.3, 128.2, 127.7, 127.6, 127.5, 100.2, 86.3, 81.3, 76.1, 73.4, 72.6, 69.2, 58.2, 55.2; MS (ESI): *m*/*z* (%) 359 (25) $[M + H]^+$, 376 (96) $[M + NH_4]^+$, 381 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{21}H_{26}O_5Na$ $[M + Na]^+$ 381.1677, found 381.1674. Compound **8b** (9.5 g, 91%) was prepared as colorless oil from **7b** (10 g, 29 mmol) following the same procedure as described for the synthesis of **8a**. (β-anomer): R_f = 0.45 (SiO_2, 20% EtOAc in petroleum ether); $[\alpha]^{28}_D = -70.38$ (*c* 1.8, $CHCl_3$); IR (neat): ν_{max} 2920, 1450, 1059, 668 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.22 (m, 10H, Ar*H*), 4.76 (d, J = 1.5 Hz, 1H, C_1-*H*), 4.60 (d, J = 12.0 Hz, 1H, H_A of -OCH_2Ph), 4.57 (d, J = 12.0 Hz, 1H, H_B of -OCH_2Ph), 4.52 (d, J = 12.0 Hz, 1H, H_A of another -OCH_2Ph), 4.51 (d, J = 12.0 Hz, 1H, H_B of another -OCH_2Ph), 4.34 (ddd, J = 6.7, 6.0, 5.2 Hz, 1H, C_5-*H*), 3.94 (dd, J = 6.0, 3.0 Hz, 1H, C_4-*H*), 3.75 (dd, J = 9.8, 5.2 Hz, 1H, C_6-*H′*), 3.69 (dd, J = 3.0, 1.5 Hz, 1H, C_3-*H*), 3.64 (dd, J = 9.8, 6.7 Hz, 1H, C_6-*H″*), 3.38 (s, 3H, -OC*H*$_3$), 3.31 (s, 3H, -OC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.2, 137.6, 128.4, 128.3, 127.7, 127.5, 107.8, 88.8, 81.1, 80.0, 73.4, 72.2, 69.6, 57.6, 55.6; MS (ESI): *m*/*z* (%) 359 (4) $[M + H]^+$ 376 (100) $[M + NH_4]^+$, 381 (20) $[M + Na]^+$. HRMS (ESI): calcd for $C_{21}H_{26}O_5Na$ $[M + Na]^+$ 381.1677, found 381.1673.

Synthesis of 9. To a stirring solution of compound **8a** (7 g, 19.5 mmol) in acetic acid (50 mL) was added 1 N HCl (10 mL) at room temperature. The reaction mixture was then stirred at 65 °C for 2 h. Acetic acid was evaporated under reduced pressure. The reaction mixture was quenched at 0 °C by slow addition of saturated aqueous $NaHCO_3$ solution (100 mL). The product was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo to obtain the hemiacetal, which was used for the next reaction without further purification.

To a stirred solution of the crude hemiacetal in dry dichloromethane (50 mL) at 0 °C was added dry Et_3N (8.1 mL, 58.5 mmol). After 5 min, dry acetic anhydride (2.2 mL, 23.4 mmol) was added slowly to the stirred reaction mixture at 0 °C followed by the addition of DMAP (238 mg, 1.95 mmol). After stirring for 5 min at room temperature, the reaction mixture was quenched at 0 °C by slow addition of saturated aqueous NH_4Cl solution (10 mL). The reaction mixture was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 14−16% EtOAc in petroleum ether eluant) afforded compound **9** (7 g, 95%) as an inseparable mixture of anomers. Following the same procedure, compound **9** was also prepared from **8b**. R_f = 0.45 (SiO_2, 25% EtOAc in petroleum ether); IR (neat): ν_{max} 2929, 2867, 1738, 1094, 697 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31−7.19 (m, total 20H), 6.31 (d, J = 4.5 Hz, 1H), 6.04 (s, 1H), 4.70−4.37 (m, total 9H), 4.15 (dd, J = 6.8, 6.0 Hz, 1H), 3.99−3.93 (m, total 2H), 3.83−3.75 (m, total 4H), 3.71−3.64 (m, 1H), 3.56 (dd, J = 10.6, 5.3 Hz, 1H), 3.37 (s, 3H), 3.35 (s, 3H), 2.09 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (mixture of diastereomers) (75 MHz, $CDCl_3$): δ 169.9, 138.0, 137.9, 137.7, 137.6, 128.3, 128.2, 127.5, 127.4, 127.3, 99.8, 93.8, 87.2, 85.2, 81.8, 80.4, 77.9, 73.3, 73.2, 72.5, 72.0, 68.6, 58.8, 57.6, 21.1, 21.0; MS (ESI): *m*/*z* (%) 410 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{22}H_{26}O_6Na$ $[M + Na]^+$ 409.1627, found 409.1621.

Synthesis of 10. To a stirred solution of **9** (10 g, 25.9 mmol) in dry acetonitrile (70 mL) was added TMSCN (5.2 mL, 38.8 mmol) was added at 0 °C followed by $BF_3 \cdot Et_2O$ (3 mL) and stirring was continued for 30 min at room temperature. The reaction mixture was quenched at 0 °C by slow addition of saturated aqueous $NaHCO_3$ solution (10 mL). Acetonitrile was evaporated under reduced pressure, and the reaction mixture was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 10−12% EtOAc in petroleum ether eluant) afforded compound **10** (8.3 g, 91%) as colorless oil and as a mixture of anomers. R_f = 0.45 (SiO_2, 20% EtOAc in petroleum ether). IR (neat): ν_{max} 2931, 1791, 1244, 1071, 678 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34−7.21 (m, total 20H), 4.83 (d, J = 4.5 Hz, 1H), 4.70−4.46 (m, total 9H), 4.35−4.29 (m, 1H), 4.23−4.17 (m, 1H), 4.10−4.08 (m, 1H), 4.04 (dd, J = 3.8, 2.3 Hz, 1H), 3.98 (dd, J = 3.8, 1.5 Hz, 1H), 3.89 (dd, J = 4.5, 2.3 Hz, 1H), 3.76 (dd, J = 9.8, 6.0 Hz, 1H), 3.70 (dd, J = 6.0, 1.5 Hz, 1H), 3.68−3.50 (m, total 2H), 3.41 (s, 3H), 3.32 (s, 3H); ^{13}C NMR (mixture of diastereomers) (75 MHz, $CDCl_3$): δ 137.7, 137.0, 129.6, 128.5, 128.4, 128.3, 128.1, 127.9, 127.7, 127.4, 117.2, 115.6, 87.4, 84.5, 81.7, 80.5, 80.2, 73.4, 72.6, 72.2, 69.6, 67.7, 67.3, 58.6, 57.8; MS (ESI): *m*/*z* (%) 354 (5) $[M + H]^+$ 376 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{21}H_{23}NO_4Na$ $[M + Na]^+$ 376.1524, found 376.1508.

Synthesis of 11a and 11b. To a stirring solution of compound **10** (8 g, 22.6 mmol) in dry dichloromethane (130 mL) at −78 °C was added DIBAL-H (1.4 M, 16.1 mL) very slowly in a dropwise manner under nitrogen atmosphere. After being stirred for 8 h at −78 °C, the reaction mixture was quenched with saturated aqueous solution of sodium potassium tartrate (50 mL). The reaction mixture was stirred at room temperature until two layers got separated (1 h). Dichloromethane was decanted and the remaining aqueous layer was extracted with EtOAc. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), filtered, concentrated in vacuo and used for the next reaction without further purification.

To a solution of the crude aldehyde, R_f = 0.3 (SiO_2, 30% EtOAc in petroleum ether), in *t*-BuOH/2-methyl-2-butene (2.5:0.3, 63 mL) at room temperature were added$NaClO_2$ (6.1 g, 67.8 mmol) and NaH_2PO_4 (10.5 g, 67.8 mmol) by dissolving in a minimum amount of water.

After being stirred for 1 h, the solvent was removed in rotary evaporator, 1 N HCl was added to the mixture at room temperature to raise the pH up to 5, and the residue was extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated in vacuo. To the resulting crude acid in ether was added diazomethane dropwise at 0 °C until the acid was converted to the methyl ester (as shown by TLC). The solvent was concentrated and silica gel column chromatographic purification (SiO_2, 12−14% EtOAc in petroleum ether eluant) afforded compound **11a** (3.6 g, 40%) and **11b** (2.9 g, 32%) as liquids. **Data for 11a**: $R_f = 0.40$ (SiO_2, 20% EtOAc in petroleum ether); $[\alpha]^{28}_D = -30.52$ (*c* 5.1, $CHCl_3$); IR (neat): ν_{max} 2925, 2859, 1738, 1090, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.30−7.22 (m, 10H, Ar*H*), 4.70 (d, $J = 4.5$ Hz, 1H, C_2-*H*), 4.60−4.48 (m, 4H, two -OCH_2Ph), 4.40 (ddd, $J = 6.8$, 5.2, 3.8 Hz, 1H, C_5-*H*), 4.01 (dd, $J = 4.5$, 2.3 Hz, 1H, C_3-*H*), 3.98 (dd, $J = 5.2$, 2.3 Hz, 1H, C_4-*H*), 3.76−3.63 (m, 2H, C_6-*H′*, C_6-*H″*), 3.73 (s, 3H, -COOCH_3) 3.26 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 138.1, 137.5, 128.4, 128.3, 127.9, 127.7, 127.6, 127.5, 85.3, 80.1, 79.9, 79.8, 73.4, 72.5, 67.4, 58.4, 51.9; MS (ESI): *m/z* (%) 409 (10) [M + Na]$^+$, 795 (100) [2 M + Na]$^+$; HRMS (ESI): calcd for $C_{22}H_{26}O_6Na$ [M + Na]$^+$ 409.1627, found 409.1620. **Data for 11b**: $R_f = 0.45$ (SiO_2, 20% EtOAc in petroleum ether); $[\alpha]^{28}_D = -36.11$ (*c* 1.6, $CHCl_3$); IR (neat): ν_{max} 2928, 2858, 1727, 1098, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.34−7.22 (m, 10H, Ar*H*), 4.60 (d, $J = 12.0$ Hz, 2H, -OCH_2Ph) 4.54 (s, 1H, C_2-*H*), 4.48 (ABq, $J = 11.5$ Hz, 2H, -OCH_2Ph), 4.35 (ddd, $J = 6.0$, 5.8, 4.1 Hz, 1H, C_5-*H*), 4.17 (t, $J = 1.5$ Hz, 1H, C_3-*H*), 3.96 (dd, $J = 4.1$, 1.5 Hz, 1H, C_4-*H*), 3.83 (s, 1H, C_6-*H′*), 3.81 (s, 1H, C_6-*H″*), 3.66 (s, 3H, -COOCH_3) 3.39 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.0, 138.1, 137.5, 128.3, 128.2, 127.5, 127.4, 127.3, 87.1, 81.1, 80.8, 80.3, 73.3, 71.7, 67.9, 57.2, 52.1; MS (ESI): *m/z* (%) 409 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{22}H_{27}O_6$ [M + H]$^+$ 387.1807, found 387.1795.

Synthesis of 12a. To a solution of **11a** (3 g, 7.4 mmol) in MeOH (15 mL) was added Pd on C (10%, 600 mg) and the mixture was hydrogenated under atmospheric pressure using a H_2 balloon for 12 h. The reaction mixture was then filtered through a short pad of Celite, and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 90% EtOAc in petroleum ether eluant) afforded compound **12a** (1.3 g, 85%) as colorless gummy liquid. $R_f = 0.35$ (silica gel, EtOAc). $[\alpha]^{28}_D = -18.8$ (*c* 0.7, $CHCl_3$); IR (neat): ν_{max} 2924, 2855, 1742, 1085, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 4.89 (d, $J = 4.9$ Hz, 1H, C_2-*H*), 4.42−4.40 (m, 1H), 4.27−4.23 (m, 1H), 4.17 (dd, $J = 12.4$, 3.8 Hz, 1H), 4.04−3.98 (m, 2H), 3.78 (s, 3H, -COOCH_3), 3.40 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 87.9, 80.0, 79.9, 75.9, 61.6, 58.7, 52.0; MS (ESI): *m/z* (%) 207 (20) [M + H]$^+$, 224 (100) [M + NH_4]$^+$; HRMS (ESI): calcd for $C_8H_{14}O_6Na$ [M + Na]$^+$ 229.0688, found 229.0680.

Synthesis of 13a. Et_3N (1.62 mL, 11.6 mmol) and TBDPS-Cl (1.7 mL, 6.38 mmol) were added sequentially to a solution of compound **12a** (1.2 g, 5.82 mmol) in dry CH_2Cl_2 (14 mL) at 0 °C. After stirring for 5 min at the same temperature, DMAP (71 mg, 0.58 mmol) was added to the reaction mixture. After being stirred for 8 h at room temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 10−12% EtOAc in petroleum ether eluant) afforded compound **13a** (2.1 g, 80%) as colorless oil. $R_f = 0.5$ (SiO_2, 33% EtOAc in petroleum ether); $[\alpha]^{28}_D = -12.96$ (*c* 6.4, $CHCl_3$); IR (neat): ν_{max} 3206, 2952, 2842, 1647, 1015 cm^{-1};^1H NMR (200 MHz, $CDCl_3$): δ 7.71−7.61 (m, 4H), 7.44−7.34 (m, 6H), 4.86 (d, $J = 5.5$ Hz, 1H, C_2-*H*), 4.43−4.38 (m, 1H, C_5-*H*), 4.24−4.16 (m, 1H, C_6-*H′*), 4.15−4.05 (m, 2H, C_6-*H″*, C_3-*H*), 4.03−3.98 (m, 1H, C_4-*H*), 3.76 (s, 3H, -COOCH_3), 3.43 (s, 3H, -OCH_3), 1.07 (s, 9H, ^tBu); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 135.5, 135.3, 132.2, 131.8, 129.9, 127.8, 87.8, 79.9, 79.7, 75.4, 63.0, 58.5, 51.8, 26.6, 18.9; MS (ESI): *m/z* (%) 462 (66) [M + NH_4]$^+$, 911 (100) [2 M + Na]$^+$; HRMS (ESI): calcd for $C_{24}H_{32}O_6NaSi$ [M + Na]$^+$ 467.1865, found 467.1871.

Synthesis of 14a. To a stirring solution of compound **13a** (935 mg, 2.1 mmol) in dry dichloromethane (5 mL) at −40 °C was added dry pyridine (4 mL). After 5 min, triflic anhydride (0.52 mL, 3.15 mmol) was added to the reaction mixture. After being stirred for 30 min at 0 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc, washed with water, saturated aqueous cupric sulfate solution, water, and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Flash column chromatography afforded quantitative yield of the triflate intermediate, which was used for the next reaction without further characterization.

To a stirring solution of the triflate in dry DMF (5 mL) at −5 °C was added sodium azide (819 mg, 12.6 mmol). After being stirred for 6 h at −5 °C, the reaction mixture was quenched with water (3 mL) and extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 8−10% EtOAc in petroleum ether eluant) afforded compound **14a** (690 mg, 70%) as a colorless oil. $R_f = 0.45$ (SiO_2, 15% EtOAc in petroleum ether); $[\alpha]^{32}_D = +20.2$ (*c* 0.5, $CHCl_3$); IR (neat): ν_{max} 2932, 2859, 2105, 1739, 1104, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.69−7.64 (m, 4H, Ar*H*), 7.47−7.35 (m, 6H, Ar*H*), 4.73 (d, $J = 5.2$ Hz, 1H, C_2-*H*), 4.31 (td, $J = 8.3$, 3.0 Hz, 1H, C_5-*H*), 4.24 (dd, $J = 5.2$, 4.5 Hz, 1H, C_3-*H*), 4.02−3.95 (m, 2H, C_4-*H* and C_6-*H′*), 3.82 (s, 3H, -COOCH_3), 3.79 (dd, $J = 11.3$, 3.0 Hz, 1H, C_6-*H″*), 3.56 (s, 3H, -OCH_3), 1.05 (s, 9H, ^tBu); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.4, 135.6, 135.5, 133.0, 132.8, 129.9, 129.8, 127.8, 127.7, 83.4, 81.0, 79.9, 62.8, 61.0, 60.5, 52.1, 26.8, 19.3; MS (ESI): *m/z* (%) 493 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{24}H_{31}N_3O_5NaSi$ [M + Na]$^+$ 492.1930, found 492.1927.

Synthesis of 15a. To a solution of **14a** (500 mg, 1.06 mmol) in MeOH (3 mL) was added Pd on C (10%, 60 mg) and the mixture was hydrogenated under atmospheric pressure using a H_2 balloon for 15 min. The reaction mixture was then filtered through a short pad of Celite, and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo and were used for the next reaction without further purification.

To a stirring solution of the crude amine in dry dichloromethane (3 mL) was added Boc_2O (0.38 mL, 1.58 mmol) at room temperature. After being stirred for 1 h, the solvent was evaporated in vacuo. Purification by silica gel column chromatography (SiO_2, 8−10% EtOAc in petroleum ether eluant) afforded compound **15a** (510 mg, 88%) as colorless gummy liquid. $R_f = 0.3$ (SiO_2, 25% EtOAc in petroleum ether). $[\alpha]^{28}_D = +28.97$ (*c* 1.4, $CHCl_3$); IR (neat): ν_{max} 2950, 2855, 1745, 1710, 1097, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.68−7.64 (m, 4H, Ar*H*), 7.39−7.32 (m, 6H, Ar*H*), 5.12 (d, $J = 9.0$ Hz, 1H, N*H*Boc) 4.69 (d, $J = 6.0$ Hz, 1H, C_2-*H*), 4.57−4.48 (m, 1H, C_5-*H*), 4.21 (t, $J = 6.0$ Hz, 1H, C_3-*H*), 4.06 (ddd, $J = 9.0$, 6.0, 3.8 Hz, 1H, C_4-*H*), 3.83 (dd, $J = 11.3$, 2.3 Hz, 1H, C_6-*H′*), 3.80 (s, 3H, -COOCH_3), 3.73 (dd, $J = 11.3$, 2.3 Hz, 1H, C_6-*H″*), 3.44 (s, 3H, -OCH_3), 1.46 (s, 9H, ^tBu of Boc), 1.06 (s, 9H, ^tBu of -OTBDPS); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 155.4, 135.7, 135.6, 133.0, 129.7, 129.6, 127.6, 84.1, 81.7, 80.2, 79.7, 63.9, 60.2, 53.5, 52.0, 28.3, 26.8, 19.2; MS (ESI): *m/z* (%) 544 (70) [M + H]$^+$, 561 (100) [M + NH_4]$^+$, 566 (60) [M + Na]$^+$; HRMS (ESI): calcd for $C_{29}H_{41}NO_7NaSi$ [M + Na]$^+$ 566.2550, found 566.2538.

Synthesis of 16a. To a stirring solution of compound **15a** (400 mg, 0.74 mmol) in dry THF (2 mL) at 0 °C was added TBAF in THF (1 M, 1.1 mL, 1.1 mmol). After being stirred for 1 h at 0 °C, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 30−32% EtOAc in petroleum ether eluant) afforded compound **16a** (154 mg, 68%). $R_f = 0.35$ (SiO_2, 60% EtOAc in petroleum ether); $[\alpha]^{28}_D = +20.9$ (*c* 1.8, $CHCl_3$); IR (neat): ν_{max} 3509, 3361, 2946, 1744, 1682, 1520, 1068 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 5.07 (d, $J = 7.9$ Hz, 1H, C_4−N*H*Boc)

4.78 (d, J = 4.7 Hz, 1H, C_2-*H*), 4.24−4.12 (m, 2H, C_3-*H* and C_5-*H*), 3.94−3.84 (m, 2H, C_4-*H* and C_6-*H′*), 3.79 (s, 3H, -COOCH_3), 3.70−3.63 (m, 1H, C_6-*H″*), 3.46 (s, 3H, -OCH_3), 1.45 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.9, 155.9, 82.9, 81.7, 80.3, 79.9, 61.3, 60.6, 52.7, 51.8, 28.1; MS (ESI): *m/z* (%) 206 (45) [M − Boc + H]$^+$, 328 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{13}H_{23}NO_7Na$ [M + Na]$^+$ 328.1372, found 328.1379.

Synthesis of 4a. To a stirring solution of compound **16a** (150 mg, 0.49 mmol) in dry dichloromethane (2 mL) at 0 °C was added dry Et_3N (0.2 mL, 1.47 mmol). After 5 min, TsCl (187 mg, 0.98 mmol) was added to the reaction mixture followed by the addition of DMAP (6 mg, 0.05 mmol). After being stirred for 2 h at room temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl, extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The crude product was used for the next reaction without further purification.

To a stirring solution of the crude product in dry DMF (2 mL) at room temperature was added sodium azide (96 mg, 1.47 mmol). After being stirred for 2 h at 65 °C, the reaction mixture was quenched with water (3 mL) and extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 18−20% EtOAc in petroleum ether eluant) afforded compound **4a** (142 mg, 88%). R_f = 0.5 (silica gel, 35% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = +87.7 (*c* 1.6, $CHCl_3$); IR (neat): ν_{max} 3363, 2978, 2938, 2101, 1763, 1708, 1099 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.98 (d, J = 8.3 Hz 1H, C_4−N*H*Boc), 4.75 (d, J = 5.2 Hz, 1H, C_2-*H*), 4.24−4.16 (m, 1H, C_5-*H*), 4.11−4.03 (m, 2H, C_3-*H* and C_4-*H*), 3.78 (s, 3H, -COOCH_3), 3.68−3.60 (m, 1H, C_6-*H′*), 3.45 (s, 3H, -OCH_3), 3.32 (dd, J = 12.8, 2.3 Hz, 1H, C_6-*H″*) 1.45 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.7, 155.3, 81.7, 81.4, 80.0, 60.6, 53.6, 52.0, 51.8, 28.2; MS (ESI): *m/z* (%) 353 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{13}H_{22}N_4O_6Na$ [M + Na]$^+$ 353.1437, found 353.1442.

Synthesis of 12b. Compound **11b** (2.5 g, 6.2 mmol) was converted into compound **12b** (1.1 g, 88%) following the same procedure as described for the synthesis of **12a**. R_f = 0.35 (SiO_2, EtOAc); $[\alpha]^{28}_D$ = −28.42 (*c* 0.7, $CHCl_3$); IR (neat): ν_{max} 2925, 2859, 1738, 1090, 699 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.62 (d, J = 4.3 Hz, 1H, C_2-*H*), 4.51(s, 1H), 4.35−4.31 (m, 1H), 4.28−4.12 (m, 2H), 4.05−3.97 (m, 1H), 3.79 (s, 3H, -COOCH_3), 3.44 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 90.8, 81.2, 80.2, 76.4, 61.5, 57.5, 52.5; MS (ESI): *m/z* (%) 207 (90) [M + H]$^+$, 224 (100) [M + NH_4]$^+$, 229 (35) [M + Na]$^+$; HRMS (ESI): calcd for $C_8H_{14}O_6Na$ [M + Na]$^+$ 229.0688, found 229.0691.

Synthesis of 13b. Compound **12b** (900 mg, 4.3 mmol) was transformed into compound **13b** (1.6 g, 84%) following the same procedure as described for the synthesis of **13a**. R_f = 0.5 (SiO_2, 30% EtOAc in petroleum ether). $[\alpha]^{28}_D$ = −5.2 (*c* 2.3, $CHCl_3$); IR (neat): ν_{max} 3206, 2952, 1650, 1109, 669 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.79−7.67 (m, 4H, Ar*H*), 7.44−7.34 (m, 6H, Ar*H*), 4.39 (d, J = 1.5 Hz, 1H, C_2-*H*), 4.22 (bs, 1H), 4.15−3.98 (m, 3H), 3.93 (d, J = 1.5 Hz, 1H, C_3-*H*), 3.75 (s, 3H, -COOCH_3), 3.45 (s, 3H, -OCH_3), 1.07 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.6, 135.7, 135.5, 132.6, 132.3, 129.9, 129.8, 127.8, 127.7, 90.1, 81.3, 81.1, 75.4, 62.6, 57.5, 52.3, 26.6, 19.0; MS (ESI): *m/z* (%) 445 (5) [M + H]$^+$, 467 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{24}H_{32}O_6NaSi$ [M + Na]$^+$ 467.1865, found 467.1846.

Synthesis of 14b. Compound **13b** (800 mg, 1.8 mmol) was transformed into compound **14b** (750 mg, 90%) following the same procedure as described for the synthesis of **14a**. R_f = 0.5 (SiO_2, 15% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = +15.76 (*c* 4.8, $CHCl_3$); IR (neat): ν_{max} 2929, 2860, 2108, 1736, 1113, 705 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74−7.65 (m, 4H, Ar*H*), 7.43−7.33 (m, 6H, Ar*H*), 4.47 (d, J = 3.6 Hz, 1H, C_2-*H*), 4.15−4.11 (m, 2H, C_3-*H* and C_5-*H*), 3.92 (dd, J = 6.3, 5.4 Hz, 1H, C_4-*H*), 3.85 (dd, J = 11.8, 3.6 Hz, 1H, C_6-*H′*), 3.80 (dd, J = 11.8, 3.8 Hz, 1H, C_6-*H″*), 3.73 (s, 3H, -COOCH_3), 3.52 (s, 3H, -OCH_3), 1.06 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 135.6, 135.5, 132.8, 132.7, 129.8, 129.7, 127.7, 127.6, 84.2, 82.0, 80.0, 63.4, 61.1, 58.5, 52.3, 26.7, 19.1; MS (ESI): *m/z* (%) 470 (3) [M + H]$^+$, 487 (55) [M + NH_4]$^+$, 492 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{24}H_{31}N_3O_5NaSi$ [M + Na]$^+$ 492.1930, found 492.1930.

Synthesis of 15b. Compound **14b** (400 mg, 0.85 mmol) was transformed into compound **15b** (390 mg, 85%) following the same procedure as described for the synthesis of **15a**. R_f = 0.35 (SiO_2, 25% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = +8.47 (*c* 1.7, $CHCl_3$); IR (neat): ν_{max} 2933, 2860, 1740, 1714, 1093, 703 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73−7.69 (m, 4H, Ar*H*), 7.43−7.33 (m, 6H, Ar*H*), 4.93 (d, J = 6.7 Hz, 1H, N*H*Boc), 4.52 (d, J = 1.5 Hz, 1H, C_2-*H*), 4.16−4.08 (m, 1H, C_5-*H*), 3.99−3.92 (m, 2H, C_3-*H* and C_4-*H*), 3.89−3.85 (m, 2H, C_6-*H′* and C_6-*H″*), 3.69 (s, 3H, -COOCH_3), 3.45 (s, 3H, -OCH_3), 1.40 (s, 9H, ^{t}*Bu* of Boc), 1.05 (s, 9H, ^{t}*Bu* of -OTBDPS); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 155.2, 135.7, 135.6, 133.4, 133.2, 129.6, 127.6, 83.4, 83.0, 80.4, 79.7, 64.7, 57.8, 53.2, 52.2, 28.2, 26.7, 19.2; MS (ESI): *m/z* (%) 544 (20) [M + H]$^+$, 561 (93) [M + NH_4]$^+$, 566 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{29}H_{41}NO_7NaSi$ 566.2550, found 566.2545.

Synthesis of 16b. Compound **15b** (350 mg, 0.64 mmol) was transformed into compound **16b** (180 mg, 80%) following the same procedure as described for the synthesis of **16a**. R_f = 0.35 (silica gel, 40% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = −1.07 (*c* 2.1, $CHCl_3$); IR (neat): ν_{max} 2927, 1740, 1011 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.09 (d, J = 7.5 Hz, 1H, C_4-N*H*Boc), 4.53 (s, 1H, C_2-*H*), 4.29−4.21 (m, 1H, C_5-*H*), 3.93−3.85 (m, 3H, C_3-*H*, C_4-*H* and C_6-*H′*), 3.73 (s, 3H, -COOCH_3), 3.70−3.63 (m, 1H, C_6-*H″*), 3.45 (s, 3H, -OCH_3), 1.42 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.4, 155.5, 83.6, 83.2, 80.0, 79.5, 61.2, 57.5, 52.5, 51.6, 28.0; MS (ESI): *m/z* (%) 306 (68) [M + H]$^+$, 323 (100) [M + NH_4]$^+$, 328 (66) [M + Na]$^+$; HRMS (ESI): calcd for $C_{13}H_{23}NO_7Na$ 328.1372, found 328.1374.

Synthesis of 4b. Compound **16b** (150 mg, 0.49 mmol) was transformed into compound **4b** (121 mg, 78%) following the same procedure as described for the synthesis of **4a**. R_f = 0.5 (SiO_2, 35% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = +80.51 (*c* 2.8, $CHCl_3$); IR (neat): ν_{max} 2934, 2103, 1749, 1711, 1094, 751 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.98 (d, J = 8.3 Hz, 1H, C_4−N*H*Boc), 4.53 (d, J = 0.7 Hz, 1H, C_2-*H*), 4.15−4.02 (m, 1H, C_5-*H*), 3.93−3.85 (m, 2H, C_3-*H*, C_4-*H*), 3.78 (s, 3H, -COOCH_3), 3.53−3.50 (m, 2H, C_6-*H′* and C_6-*H″*), 3.48 (s, 3H, -OCH_3), 1.44 (s, 9H, ^{t}*Bu*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 155.2, 83.1, 81.9, 80.3, 80.1, 57.6, 53.5, 52.8, 52.5, 28.0; MS (ESI): MS (ESI): *m/z* (%) 331 (5) [M + H]$^+$, 354 (100) [M + Na]$^+$; HRMS (ESI): calcd for $C_{13}H_{22}N_4O_6Na$ [M + Na]$^+$ 353.1437, found 353.1431.

Synthesis of 17a. To a solution of **4a** (113 mg, 0.34 mmol) in THF/MeOH/H_2O (1.5:0.5:0.5 mL) at 0 °C was added LiOH·H_2O (36 mg, 0.85 mmol) and this mixture was stirred at room temperature for 1 h. The mixture was then acidified to pH 2 with 1 N HCl. The reaction mixture was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo to obtain the acid. The crude acid was used for the next reaction without further purification.

To a stirred solution of Boc-Phe-Leu-OMe (200 mg, 0.51 mmol) in dry dichloromethane (2 mL) at 0 °C was added trifluoroacetic acid (1 mL) and the mixture was stirred for 2 h at room temperature. The reaction mixture was then concentrated in vacuo to obtain the trifluoroacetate salt.

To a stirring solution of the crude acid in dry dichloromethane (3 mL) at 0 °C were sequentially added HOBt·H_2O (69 mg, 0.51 mmol) and EDCI (98 mg, 0.51 mmol). After 10 min, the above-prepared trifluoroacetate salt was dissolved in dichloromethane (3 mL) and added to the reaction mixture followed by the addition of DIPEA (0.16 mL, 0.9 mmol). After stirring for 12 h at room temperature, the reaction mixture was diluted with EtOAc, washed with 1 N HCl solution, saturated $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 35−37% EtOAc in petroleum ether eluant) afforded compound **17a** (172 mg, 85%).

R_f = 0.4 (SiO_2, 50% EtOAc in petroleum ether); $[\alpha]^{28}_D$ = −5.41 (*c* 1.3, $CHCl_3$); IR (neat): ν_{max} 2958, 2103, 1742, 1707, 1676, 1518, 1167 cm^{-1}; ^1H MNR (300 MHz, $CDCl_3$): δ 7.27−7.19 (m, 5H, Ar*H*), 7.11 (d, *J* = 8.3 Hz, 1H, PheN*H*), 6.17 (d, *J* = 7.5 Hz, 1H, LeuN*H*), 4.84 (d, *J* = 8.3 Hz, 1H, C_4-N*H*Boc), 4.64 (td, *J* = 8.3, 6.7 Hz, 1H, Pheα*H*), 4.52 (d, *J* = 3.8 Hz, 1H, C_2-*H*), 4.50−4.42 (m, 1H, Leuα*H*), 4.21−4.12 (m, 1H, C_5-*H*), 3.98 (t, *J* = 3.8 Hz, 1H, C_3-*H*), 3.82 (ddd, *J* = 8.3, 4.5, 3.8 Hz, 1H, C_4-*H*), 3.63 (s, 3H, -COOC*H*$_3$), 3.56−3.51 (m, 1H, C_6-*H*′), 3.30−3.22 (m, 1H, C_6-*H*″), 3.27 (s, 3H, -OC*H*$_3$), 3.11 (dd, *J* = 13.5, 6.7 Hz, 1H, -C*H*′HPh), 2.99 (dd, *J* = 13.5, 6.7 Hz, 1H, -CH*H*″Ph), 1.52−1.41 (m, 3H, Leu$_\beta$*H*′H, Leu$_\beta$H*H*″and Leu$_\gamma$*H*), 1.40 (s, 9H, ^{t}*Bu* of Boc), 0.81 (d, *J* = 2.3 Hz, 3H, one LeuC*H*$_3$), 0.80 (d, *J* = 2.3 Hz, 3H, another LeuC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 169.9, 168.4, 155, 136.3, 129.4, 128.5, 126.9, 81.8, 81.7, 81.6, 80.4, 60.9, 54.5, 53.7, 52.2, 50.8, 41.3, 37.9, 28.2, 24.7, 22.6, 21.8; MS (ESI): *m*/*z* (%) 491 (10) $[M - Boc + H]^+$, 591 (3) $[M + H]^+$, 613 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{28}H_{42}N_6O_8Na$ $[M + Na]^+$ 613.2961, found 613.2963.

Synthesis of 18a. To a stirring solution of **17a** (113 mg, 0.19 mmol) in THF/MeOH/H_2O (1.5:0.5:0.5 mL) at 0 °C was added LiOH.H_2O (20 mg, 0.47 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was then acidified to pH 2 with 1 N HCl. The reaction mixture was extracted with EtOAc, washed with water and brine, dried (Na_2SO_4), filtered and concentrated in vacuo to obtain the crude acid, which was used for the next reaction without further purification.

To a solution of the acid in MeOH (2 mL) were added Pd on C (10%, 30 mg) and 1 N HCl (0.23 mL), and the mixture was hydrogenated under atmospheric pressure using a a H_2 balloon for 1 h. The reaction mixture was then filtered through a short pad of Celite and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo to obtain the hydrochloride salt, which was used in the next step without further purification.

To a stirring solution of the hydrochloride salt in dry acetonitrile (38 mL, 5×10^{-3} M) at 0 °C was added FDPP (110 mg, 0.29 mmol). After 15 min, DIPEA (0.1 mL, 0.57 mmol) was added to the reaction mixture. After being stirred for 72 h at room temperature, the solvent was evaporated under reduced pressure, and the product was dissolved in EtOAc, washed with 1 N NaOH, water, and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 30−32% Acetone in petroleum ether eluant) afforded compound **18a** (64 mg, 63%). R_f = 0.5 (SiO_2, 50% acetone in petroleum ether); IR (neat): ν_{max} 3298, 2956, 2931, 1679, 1644, 1533, 1089, 750 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.4, 172.2, 171.3, 155.4, 136.8, 129.2, 128.3, 127.0, 81.6, 81.3, 80.0, 79.2, 61.0, 58.0, 55.8, 54.1, 43.0, 36.7, 36.0, 28.3, 24.1, 23.5, 20.6; MS (ESI): *m*/*z* (%) 1087 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{54}H_{80}N_8O_{14}Na$ $[M + Na]^+$ 1087.5691, found 1087.5668.

Synthesis of 17b. Compound **4b** (100 mg, 0.3 mmol) was transformed into compound **17b** (135 mg, 75%) following the same procedure as described for the synthesis of **17a**. R_f = 0.4 (SiO_2, 50% EtOAc in petroleum ether); $[\alpha]_D^{30}$ = +14.94 (*c* 1.8, $CHCl_3$); IR (neat): ν_{max} 3314, 2958, 2103, 1742, 1659, 1084, 699 cm^{-1}; ^1H MNR (300 MHz, $CDCl_3$): δ 7.56 (d, *J* = 7.9 Hz, 1H, PheN*H*), 7.31−7.19 (m, 5H, Ar*H*), 6.26 (d, *J* = 7.9 Hz, 1H, LeuN*H*), 5.0 (d, *J* = 8.3 Hz, 1H, C_4-N*H*Boc), 4.63−4.50 (m, 2H, Pheα*H*, Leuα*H*), 4.34 (s, 1H, C_2-*H*), 3.98 (ddd, *J* = 9.6, 8.3, 3.4,1H, C_5-*H*), 3.87−3.81 (m, 2H, C_3-*H*, C_4-*H*), 3.71 (dd, *J* = 13.0, 9.6 Hz, 1H, C_6-*H*′) 3.70 (s, 3H, -COOC*H*$_3$), 3.53 (dd, *J* = 13.0, 3.4 Hz, 1H, C_6-*H*″), 3.49 (s, 3H, -OC*H*$_3$), 3.17 (dd, *J* = 13.9, 6.7 Hz, 1H, -C*H*′HPh), 3.06 (dd, *J* = 13.9, 6.6 Hz, 1H, -CH*H*″Ph), 1.60−1.45 (m, 3H, Leu$_\beta$*H*′H, Leu$_\beta$H*H*″and Leu$_\gamma$*H*), 1.44 (s, 9H, ^{t}*Bu* of Boc), 0.94 (d, *J* = 6.0 Hz, 3H, one LeuC*H*$_3$), 0.90 (d, *J* = 6.0 Hz, 3H, another LeuC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 169.8, 169.5, 154.9, 136.1, 129.2, 128.5, 126.9, 83.4, 81.7, 81.1, 79.9, 57.2, 54.4, 52.1, 51.6, 50.6, 41.3, 37.6, 28.1, 22.5, 21.8; MS (ESI): *m*/*z* (%) 592 (8) $[M + H]^+$, 614 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{28}H_{42}N_6O_8Na$ $[M + Na]^+$ 613.2961, found 613.2951.

Synthesis of 18b. Compound **17b** (140 mg, 0.23 mmol) was transformed into compound **18b** (75 mg, 61%) following the same procedure as described for the synthesis of **18a**. R_f = 0.5 (silica gel, 40% acetone in petroleum ether); IR (neat): ν_{max} 3285, 2925, 2856, 1649, 1502, 1460, 1089, 752, 666 cm^{-1}; ^1H NMR, see Table 4; ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.0, 170.5, 169.7, 155.7, 136.9, 129.1, 128.6, 126.8, 83.4, 81.2, 80.6, 80.3, 57.2, 54.0, 52.1, 51.7, 41.9, 38.7, 37.5, 28.9, 24.8, 22.8, 22.3; MS (ESI): *m*/*z* (%) 1087 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{54}H_{80}N_8O_{14}Na$ $[M + Na]^+$ 1087.5691, found 1087.5721.

Synthesis of 1a. To a stirred solution of **18a** (20 mg, 0.018 mmol) in dry dichloromethane (2 mL) at 0 °C was added trifluoroacetic acid (1 mL) and the mixture was stirred for 12 h at room temperature. The reaction mixture was then poured into dry diethyl ether (30 mL). White precipitate came out of the solution. Centrifugation and filtration gave white amorphous solid **1a** in quantitative yield. IR (neat): ν_{max} 3285, 3062, 2957, 1646, 1533, 1196, 1130, 1051, 699 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 9.10 (bs, 1H), 8.68 (bs, 1H), 8.38 (bs, 3H), 7.46 (bs, 1H), 7.35−7.19 (m, 5H), 4.63−4.50 (m, 2H), 4.20−4.12 (m, 1H), 4.07−3.96 (m, 1H), 3.93−3.81 (m, 1H), 3.16−3.07 (m, 1H), 1.84−1.72 (m, 1H), 1.61−1.48 (m, 1H), 0.92−0.76 (m, 1H), 0.68 (d, *J* = 5.3 Hz, 3H), 0.63 (d, *J* = 5.3 Hz, 3H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.3, 172.1, 171.0, 137.2, 129.4, 128.7, 126.8, 81.4, 80.6, 77.5, 60.6, 56.0, 54.0, 53.0, 42.0, 36.9, 35.5, 24.0, 21.1; MS (ESI): *m*/*z* (%) 433 (10) $[H + M + H]^{2+}$, 866 (100) $[M + H]^+$; HRMS (ESI): calcd for $C_{44}H_{65}N_8O_{10}$ $[M + H]^+$ 865.4823, found 865.4851.

Synthesis of 1b. Compound **1b** was prepared in quantitative yield from compound **18b** following the same procedure as described for the synthesis of **1a**. IR (neat): ν_{max} 3283, 2960, 1741, 1675, 1658, 1136 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 8.95 (d, *J* = 8.1 Hz, 1H), 8.33 (s, 3H), 8.08 (bs, 1H), 7.95 (bs, 1H), 7.33−7.16 (m, 5H), 4.54 (m, 1H), 4.46−4.35 (m, 2H), 4.13−4.05 (m, 1H), 3.67−3.51 (m, 2H), 3.45−3.31 (m, 2H), 3.30 (s, 3H), 3.24−3.17 (m, 1H), 3.02−2.92 (m, 1H), 1.66−1.45 (m, 3H), 0.92 (d, *J* = 5.7 Hz, 3H), 0.89 (d, *J* = 5.7 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.4, 170.4, 169.5, 138.1, 129.0, 127.0, 126.3, 82.5, 80.6, 79.5, 57.3, 53.9, 52.6, 51.0, 41.8, 41.0, 36.6, 24.3, 22.9, 21.9; MS (ESI): *m*/*z* (%) 434 (60) $[H + M + H]^{2+}$, 866 (100) $[M + H]^+$; HRMS (ESI): calcd for $C_{44}H_{65}N_8O_{10}$ $[M + H]^+$ 865.4823, found 865.4809.

Synthesis of 19a. To a stirring solution of Boc-(h-Gly)-OH (28 mg, 0.15 mmol) in dry dichloromethane (3 mL) at 0 °C was sequentially added HOBt·H_2O (20 mg, 0.15 mmol) and EDCI (29 mg, 0.15 mmol). After 10 min, TFA-salt **1a** (20 mg, 0.018 mmol) was added to the reaction mixture followed by the addition of DIPEA (0.015 mL, 0.09 mmol). After stirring for 24 h at room temperature, the reaction mixture was diluted with dichloromethane, washed with 1 N HCl solution, water, saturated $NaHCO_3$ solution, water, and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by silica gel column chromatography (SiO_2, 4.7% MeOH in dichloromethane eluant) afforded compound **19a** (18 mg, 80%). R_f = 0.5 (silica gel, 10% MeOH in dichloromethane); IR (neat): ν_{max} 3303, 2931, 1648, 1532, 1062, 752 cm^{-1}; ^1H NMR, see Table 2; ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 171.9, 171.3, 156.0, 136.7, 129.2, 128.9, 126.9, 81.6, 79.6, 60.9, 58.0, 54.1, 43.1, 37.1, 36.6, 36.4, 36.1, 28.5, 24.1, 23.5, 20.7; MS (ESI): *m*/*z* (%) 1229 (100) $[M + Na]^+$; HRMS (ESI): calcd for $C_{60}H_{90}N_{10}O_{16}Na$ $[M + Na]^+$ 1229.6433, found 1229.6440.

Synthesis of 20a. To a stirring solution of Boc-Lys(Boc)-OH (52 mg, 0.15 mmol) in dry dichloromethane (3 mL) at 0 °C was sequentially added HOBt·H_2O (20 mg, 0.15 mmol) and EDCI (29 mg, 0.15 mmol). After 10 min, TFA-salt **1a** was added to the reaction mixture followed by the addition of DIPEA (0.015 mL, 0.09 mmol). After stirring for 24 h at room temperature, the reaction mixture was diluted with dichloromethane, washed with 1 N HCl solution, water, saturated $NaHCO_3$ solution, water, and brine, dried

(Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 5.0% MeOH in dichloromethane eluant) afforded compound **20a** (22 mg, 80%). R_f = 0.3 (silica gel, 6% MeOH in dichloromethane); IR (neat): ν_{max} 3305, 2933, 1672, 1657, 1518, 1099, 751 cm^{-1}; ^1H NMR, see Table 3; ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 172.2, 171.9, 171.4, 156.0, 155.2, 136.8, 129.2, 128.8, 126.9, 81.7, 81.6, 79.6, 79.4, 61.0, 58.0, 54.6, 54.0, 53.8, 43.2, 40.0, 36.6, 36.1, 32.6, 28.7, 28.5, 24.2, 23.7, 23.0, 20.8; MS (ESI): *m/z* (%) 784 (100) $[Na + M + Na]^{2+}$; HRMS (ESI): calcd for $C_{76}H_{120}N_{12}O_{20}Na_2$ $[Na + M + Na]^{2+}$ 783.4263, found 783.4260.

Synthesis of 2a. Compound **2a** was prepared in quantitative yield from compound **19a** following the same procedure as described for the synthesis of **1a**. IR (neat): ν_{max} 3284, 2957, 1645, 1531. 1196, 1132, 1066 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 8.98 (bs, 1H), 8.67−8.48 (m, 1H), 8.17 (d, J = 8.4 Hz, 1H), 7.76 (bs, 3H), 7.46 (bs, 1H), 7.30−7.19 (m, 5H), 4.68−4.58 (m, 1H), 4.55−4.48 (m, 1H), 4.07−3.99 (m, 1H), 3.94−3.83 (m, 2H), 3.59−3.51 (m, 1H), 3.21 (s, 3H), 3.16−3.10 (m, 1H), 3.04−2.97 (m, 3H), 2.59−2.53 (m, 2H) 1.84−1.76 (m, 1H), 1.61−1.53 (m, 1H), 0.92−0.77 (m, 1H), 0.69 (d, J = 5.6 Hz, 3H), 0.63 (d, J = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.8, 171.3, 171.2, 169.8, 136.8, 128.9, 128.0, 126.2, 81.4, 80.5, 77.6, 73.6, 59.9, 55.4, 54.0, 52.5, 42.4, 36.5, 35.0, 32.2, 23.4, 20.6; MS (ESI): *m/z* (%) 505 (100) $[H + M + H]^{2+}$, 1008 (20) $[M + H]^+$. HRMS (ESI) $C_{50}H_{76}N_{10}O_{12}$ $[H + M + H]^{+2}$ 504.2816, found 504.2828.

Synthesis of 3a. Compound **3a** was prepared in quantitative yield from compound **20a** following the same procedure as described for the synthesis of **1a**. IR (neat): ν_{max} 3283, 2959, 1675, 1533, 1198, 1135 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.06 (bd, J = 5.6 Hz, 1H), 8.69 (bs, 1H), 8.42 (d, J = 8.4 Hz, 1H), 8.21 (bs, 3H), 7.77 (bs, 3H), 7.56−7.51 (m, 1H), 7.31−7.20 (m, 5H), 4.60(m, 1H), 4.57−4.51 (m, 1H), 4.14−4.07 (m, 1H), 3.96−3.88 (m, 2H), 3.86−3.80 (m, 1H), 3.51−3.44 (m, 1H), 3.42−3.35 (m, 1H), 3.30 (s, 3H) 3.17−3.11 (m, 1H), 2.86−2.78 (m, 2H), 2.50−2.42 (m, 2H) 1.89−1.81 (m, 1H), 1.76−1.70 (m, 2H), 1.59−1.51 (m, 3H), 1.44−1.36 (m, 2H), 0.83−0.76 (m, 1H), 0.68 (d, J = 6.3 Hz, 3H), 0.61 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.0, 171.9, 171.8, 168.9, 136.7, 128.9, 128.1, 126.3, 81.5, 80.9, 78.3, 60.1, 55.7, 53.7, 52.7, 51.6, 42.6, 38.4, 36.2, 30.8, 26.7, 23.5, 23.4, 21.1, 20.5; MS (ESI): *m/z* (%) 562 (100) $[H + M + H]^{2+}$, 1122 (5) $[M + H]^+$; HRMS (ESI): calcd for $C_{56}H_{90}N_{12}O_{12}$ $[H + M + H]^{2+}$ 561.3395, found 561.3388.

Synthesis of 19b. Coupling of **1b** (20 mg, 0.018 mmol) with Boc-(h-Gly)-OH (28 mg, 0.15 mmol) using EDCI (29 mg, 0.15 mmol), HOBt.H_2O (20 mg, 0.15 mmol), and DIPEA (0.015 mL, 0.09 mmol), following the same procedure as described for the synthesis of **19a**, gave compound **19b** (20 mg, 80%). R_f = 0.4 (silica gel, 6% MeOH in dichloromethane); IR (neat): ν_{max} 3303, 2925, 2856, 1741, 1651, 1519, 1461, 1088 cm^{-1}; ^1H NMR, see Table 5; ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.9, 171.9, 170.9, 169.7, 156.0, 136.9, 129.3, 128.7, 126.9, 83.0, 81.7, 81.3, 79.4, 57.2, 54.6, 52.4, 50.6, 40.8, 39.1, 37.9, 36.9, 36.4, 28.4, 24.9, 22.6, 22.5; (ESI): *m/z* (%) 1229 (85) $[M + Na]^+$; HRMS (ESI): calcd for $C_{60}H_{90}N_{10}O_{16}Na$ $[M + Na]^+$ 1229.6433, found 1229.6445.

Synthesis of 2b. Compound **2b** was prepared in quantitative yield from compound **19b** following the same procedure as described for the synthesis of **1a**. IR (neat): ν_{max} 3270, 2959, 1680, 1650, 1533, 1132 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.58 (bs, 1H), 8.61 (d, J = 7.6 Hz, 1H), 8.06 (bs, 1H), 7.90 (bs, 1H), 7.75 (s, 3H), 7.28−7.16 (m, 5H), 4.59−4.47 (m, 1H), 4.46−4.35 (m, 1H), 4.30 (s, 1H), 4.04−3.95 (m, 1H), 3.93−3.85 (m, 1H), 3.60−3.35 (m, 4H), 3.22 (s, 3H), 3.21−3.09 (m, 2H), 3.03−2.90 (m, 3H), 1.65−1.50 (m, 2H), 1.47−1.35 (m, 1H), 0.86 (d, J = 5.3 Hz, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.2, 170.4, 169.8 (two carbons), 137.9, 129.2, 128.0, 126.4, 82.4, 80.9, 79.8, 79.3, 57.0, 53.3, 52.1, 51.1, 40.9, 36.8, 35.2, 32.0, 24.1, 22.5, 22.2; MS (ESI): *m/z* (%) 505 (100) $[H + M + H]^{2+}$, 1008 (10) $[M + H]^+$; HRMS (ESI): calcd for $C_{50}H_{76}N_{10}O_{12}$ $[H + M + H]^{2+}$ 504.2816, found 504.2799.

Synthesis of 20b. Coupling of **1b** (20 mg, 0.018 mmol) with Boc-Lys(Boc)-OH (52 mg, 0.15 mmol) using EDCI (29 mg, 0.15 mmol), HOBt·H_2O (20 mg, 0.15 mmol), and DIPEA (0.015 mL, 0.09 mmol), following the same procedure as described for the synthesis of **20a**, gave compound **20b** (22 mg, 80%). R_f = 0.4 (silica gel, 4% MeOH in dichloromethane); IR (neat): ν_{max} 2986, 1688, 1648, 1523, 1470, 1003 cm^{-1}; ^1H NMR, see Table 6; ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 171.4, 170.8, 167.8, 157.0, 155.2, 136.0, 129.8, 128.1, 126.7, 82.3, 81.8, 80.3, 79.8, 79.4, 57.7, 54.1, 52.6, 51.9, 49.8, 42.0, 40.1, 37.7, 36.9, 33.3, 30.0, 28.4, 28.3, 23.3, 22.7, 21.4; MS (ESI): *m/z* (%) 1544 (100) $[M+ Na]^+$; HRMS (ESI): calcd for $C_{76}H_{120}N_{12}O_{20}Na_2$ $[Na + M + Na]^{2+}$ 783.4263, found 783.4260.

Synthesis of 3b. Compound **3b** was prepared in quantitative yield from compound **20b** following the same procedure as described for the synthesis of **1a**. IR (neat): ν_{max} 3269, 3067, 2959, 1674, 1532, 1463, 1198 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 8.56 (d, J = 6.8 Hz, 1H), 8.46−8.29 (m, 2H), 8.21 (s, 3H), 7.99 (s, 3H), 7.81−7.70 (bs, 1H), 7.28−7.14 (m, 5H), 4.60−4.50 (m, 1H), 4.44−4.33 (m, 2H), 4.06−3.87 (m, 2H), 3.86−3.74 (m, 1H), 3.74.3.53 (m, 2H), 3.27 (s, 3H), 3.18−2.93 (m, 3H), 2.87−2.74 (m, 2H), 1.74−1.25 (m, 9H), 0.98−0.77 (m, 6H); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.1, 170.4, 169.3, 168.8, 137.3, 129.3, 128.0, 126.4, 82.4, 81.0, 79.6, 57.1, 54.9, 52.8, 51.8, 51.4, 40.6, 38.5, 37.1, 30.7, 26.7, 24.1, 22.5, 22.0, 21.0; MS (ESI): *m/z* (%) 562 (100) $[H + M + H]^{2+}$; HRMS (ESI): calcd for $C_{56}H_{90}N_{12}O_{12}$ $[H + M + H]^{2+}$ 561.3395, found 561.3370.

Circular Dichroism Studies. CD spectra were recorded in 5 mM HEPES buffer (pH 7.4) and trifluoroethanol (TFE) on a JASCO J-815 automatic recording spectropolarimeter at 25 °C using a quartz cell of 1 mm path length. The spectra are shown in Supporting Information. Data are represented as molar ellipticities. Sample concentrations were 200 μM in buffer and TFE.

Antibacterial Activity. Bacterial strains used were *Escherichia coli* (MG1655), *Pseudomonas aeruginosa* (NCTC 6750), *Staphyloccoccus aureus* (NCTC 8530), and *Bacillus subtilis* (CCMB collection). The antibacterial activity of the compounds was examined in sterile 96-well plates in a final volume of 100 μL as follows:[29] Bacteria were grown in nutrient broth (Bacto Difco nutrient broth) to mid-log phase and diluted to 10^6 colony-forming units (cfu)/mL in 10 mM sodium phosphate buffer (pH 7.4). Bacteria were incubated with different concentrations of samples for 2 h at 37 °C, and suitably diluted aliquots were plated on nutrient agar plates. After the plates were incubated at 37 °C for 18 h, colonies formed were counted. The concentration of the sample at which no viable colonies were formed was taken as lethal concentration (LC). The average of three independent experiments done in duplicate was determined for LC.

Hemolytic Activity. The hemolytic activities of the compounds were determined using rat red blood cells (RBCs). The RBCs were prepared as follows: 2−3 mL of rat blood was drawn into 12 mL of heparinsed 5 mM HEPES buffer pH 7.4 containing 150 mM NaCl and centrifuged at 4000 rpm for 5 min. The cell pellet was washed three times with the buffer, and the buffy coat was removed. A dilute stock of RBCs was made by diluting the pellet 0.5 to 15 mL with the buffer. Different volumes of each compound were taken in 0.5 mL of 5 mM HEPES buffer pH 7.4 containing 150 mM NaCl. Diluted stock of RBCs containing 1×10^7 cells/mL were added, and the tubes were incubated at 37 °C for 30 min in a gentle shaking water bath. After incubation, the samples were centrifuged at 4000 rpm for 5 min to remove unhemolysed cells. The absorbance of the supernatant was measured at 540 nm. Buffer containing RBCs suspension was taken as buffer blank (A_0). The lysis obtained with 0.1% of Triton X-100 was taken for 100% lysis (A_{100}). The percentage hemolysis was calculated using the following equation: $\%H = (A_{sample} - A_0) \times 100/(A_{100} - A_0)$, where A_{100} and

(29) Krishnakumari, V.; Singh, S.; Nagaraj, R. *Peptides* **2006**, *27*, 2607–2613.

A_0 are the absorbance of 100% and 0% hemolysed cells, respectively. The average of three independent experiments done in duplicate was taken for calculations.

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Supporting Information Available: General experimental procedures, detailed protocols for NMR and MD studies, NOESY/ROESY, TOCSY spectra of **18a,b**−**20a,b** and **1a,b**−**3a,b** and copies of ^{1}H and ^{13}C NMR spectra for all compounds, and CD spectra of **1a,b**−**3a,b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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