JOC Article

Synthesis of Gramicidin S and Its Analogues via an On-Resin Macrolactamization Assisted by a Predisposed Conformation of the Linear Precursors

Xianzhang Bu, Xiaoming Wu, Na Lee Joyce Ng, Chun Kit Mak, Chuanguang Qin, and Zhihong Guo*

Department of Chemistry and Biotechnology Research Institute, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, China

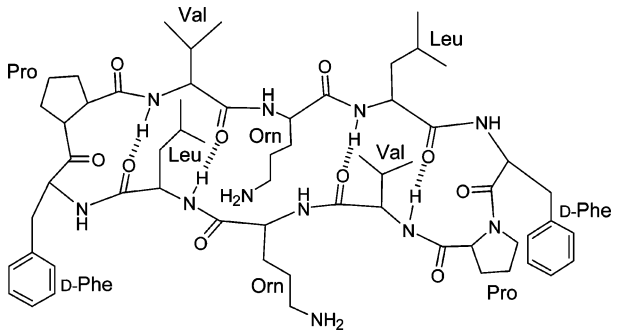
chguo@ust.hk

Received November 21, 2003

A simple and efficient preparation of gramicidin S and its analogues is described. It involves solid-phase peptide synthesis and on-resin macrolactamization without side chain protection, affording cyclic products in high yield and high purity. The high specificity of the cyclization reaction was shown to originate in the formation of a pre-organized conformation of the linear biosynthetic precursor of gramicidin S. This facile method will provide convenient access to the analogues of the natural product for functional optimization to counter microbial resistance.

Introduction

Gramicidin S (**1a**) is a cyclic decapeptide antibiotic with two repetitive pentapeptide units of D-Phe-Pro-Val-Orn-Leu, which is produced by *Bacillus brevis*.[1] In liquid media, it exhibits potent antibiotic activities against a broad spectrum of both Gram-positive and Gram-negative bacteria, as well as several pathogenic fungi.[2] However, it also exhibits high hemolytic activity that restricts the antibiotic to topical application.[3] Under physiological conditions, the antibiotic adopts a preformed rigid antiparallel β-pleated sheet structure that is amphipathic with the polar side chains on one side and the hydrophobic side chains on the other (Figure 1).[4] The natural product's activity is dependent on its interaction with the cell membranes, resulting in disruption of normal barrier functions of the cell walls.[5] Due to this unique mode of action, it is likely to have low risk of provoking bacterial resistance because development of such resistance requires significant alteration of the lipid composition of the cell wall.[6] Therefore, it has become a focal point of interest to optimize this antibiotic's therapeutic index for treatment of microbial resistance that has become an increasing threat to public health.[7]



1a Gramicidin S

FIGURE 1. Antiparallel β-pleated sheet structure of gramicidin S.

Availability of gramicidin S analogues is a major issue in the functional optimization of the natural product. Traditional synthesis of the analogues involves ring closure at high dilution after *C*-terminal activation by *N*-hydroxysuccinimide ester (ONSu) or azide.[1b,8] A number of other methods are also available for cyclic peptide synthesis.[9] Nonetheless, these methods are insufficient to meet the need of providing economic access to large

(1) (a) Gause, G. G.; Brazhnikova, M. G. *Nature* **1944**, *154*, 703. (b) Izumiya, N.; Kato, T.; Aoyagi, H.; Waki, M.; Kondo, M. In *Synthetic Aspects of Biologically Active Cyclic Peptides-Gramicidin and Tyrocidines*; Kodansha Ltd. and Wiley: Tokyo, 1979.

(2) (a) Kondejewski, L. H.; Farmer, S. W.; Wishart, D. S.; Hancock, R. E. W.; Hodges, R. S. *Int. J. Pept. Protein Res.* **1996**, *47*, 460−466. (b) Kondejewski, L. H.; Farmer, S. W.; Wishart, D.; Kay, C. M.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1996**, *271*, 25261−25268.

(3) Lambert, H. P.; O'Grady, F. W. In *Antibiotic and Chemotherapy*, 6th ed.; Churchill Livingstone: Edinburgh, U.K., 1992; pp 232−233.

(4) (a) Stern, A.; Gibbons, W. A.; Craig, L. C. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 734−741. (b) Ohnishi, M.; Urry, D. W. *Biochim. Biophys. Res. Commun.* **1969**, *36*, 194−202.

(5) Prenner, E. J.; Lewis, R. N. A. H.; McElhaney, R. N. *Biochim. Biophys. Acta* **1999**, *1462*, 201−221.

(6) (a) Hancock, R. E. W. *Lancet* **1997**, *349*, 418−422. (b) Kondejewski, L. H.; Jelokhani-Niaraki, M.; Farmer, S. W.; Bruce, L.; Kay, C. M.; Sykes, B. D.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1999**, *274*, 13181−13192.

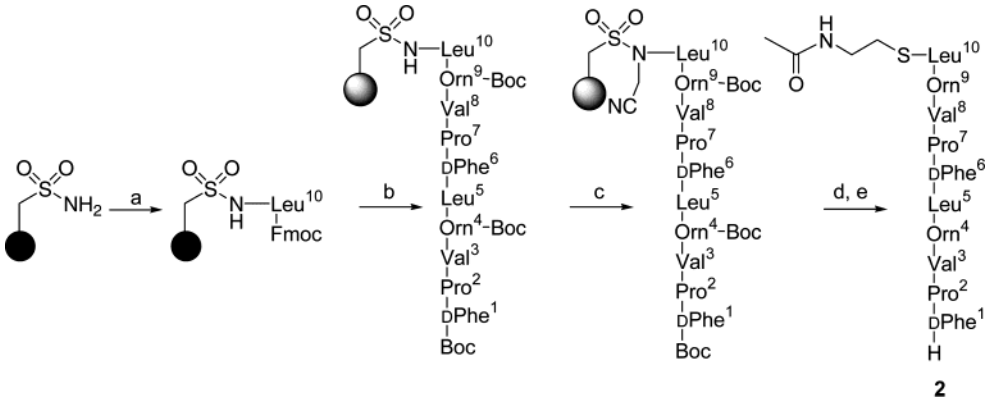
(7) (a) Neu, H. C. *Science* **1992**, *257*, 1064−1073. (b) Cohen, M. L. *Science* **1992**, *257*, 1050−1055.

(8) (a) Kondejewski, L. H.; Farmer, S. W.; Wishart, D. S.; Kay, C. M.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1996**, *271*, 25261−25268. (b) Kondejewski, L. H.; Jelokhani-Niaraki, M.; Farmer, S. W.; Bruce, L.; Kay, C. M.; Sykes, B. D.; Hancock, R. E. W.; Hodges, R. S. *J. Biol. Chem.* **1999**, *274*, 13181−13192. (c) McInnes, C.; Kondejewski, L. H.; Hodges, R. S.; Sykes, B. D. *J. Biol. Chem.* **2000**, *275*, 14287−14294. (d) Lee, D. L.; Hodges, R. S. *Biopolymers* **2003**, *71*, 28−48.

(9) For reviews, see: (a) Lambert, J. N.; Mitchell, J. P.; Roberts, K. D. *J. Chem. Soc., Perkin Trans. 1* **2001**, 471−484. (b) Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243−2266. (c) Wipf, P. *Chem. Rev.* **1995**, *95*, 2115−2134.

10.1021/jo035712x CCC: $27.50
Published on Web 03/17/2004

SCHEME 1. Synthesis of the Linear Biosynthetic Precursor of Gramicidin S (2)[a]



[a] Reagents and conditions: (a) PyBOP, DIPEA, $CHCl_3$, Fmoc-Leu-OH, −20 °C, 8 h, repeat once; (b) standard Fmoc solid-phase peptide synthesis; deprotection: 20% piperidine in DMF; coupling: Fmoc-amino acid (Boc-DPhe-OH for the last residue), DIC, HOBt, 2 h; (c) ICH_2CN, NMP, DIPEA, 24 h; (d) NAC−SH/THF; (e) CF_3COOH/phenol/*i*-Pr_3SiH/H_2O = 88:5:5:2, 1 h.

amount of natural product analogues due to the poor tendency of the linear peptide precursors to cyclize. Recently, we showed that the isolated thioesterase domain in gramicidin synthase can be used to cyclize linear precursors on solid support as a general method for combinatorial synthesis of gramicidin S analogues.[10] However, this method is limited by the enzyme's substrate specificity, and the enzymatic cyclization of the linear precursors is incomplete. For the optimization of gramicidin S therapeutic index, a less restrictive and more convenient synthetic method that is adaptable to combinatorial synthesis is highly desirable.

It has been implicated in the literature that the linear biosynthetic precursor of gramicidin S adopts a preorganized conformation that may be utilized for development of novel facile synthesis of the natural product and analogues.[10,11] In our investigation of enzymatic cyclization of gramicidin S precursor, we found that the carboxy-activated on-resin precursor and its analogues cyclize spontaneously into head-to-tail products in ammonia solution, without protection of active side-chain amine groups of the two ornithine residues.[10] The absence of the hydrolytic or aminolytic or side chain-to-tail cyclization products indicates that the linear precursor adopts a predisposed conformation highly favorably for the head-to-tail cyclization reaction. Similar conformational propensity is also implicated in another report of highly specific and high-yielding synthesis of the natural peptide product.[11] In addition, the linear biosynthetic precursor of tyrocidine A, a distinct but similar decapeptide antibiotic of gramicidin S, has also been demonstrated to possess a similar favorable conformation that has been utilized to develop a facile synthesis of cyclic peptides on the basis of the structural framework of the natural product.[12] Since both tyrocidine A and gramicidin S form antiparallel β-pleated sheet structures,[4,13] we reasoned that the precursor for gramicidin S most likely has a similar conformational preference that can be used for

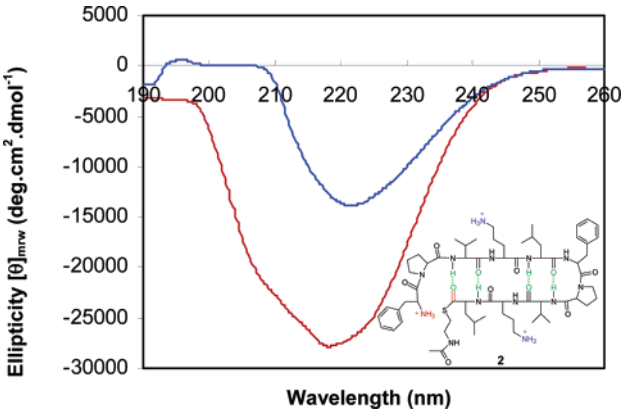


FIGURE 2. Circular dichroism spectra of gramicidin S in 50 mM Tris·HCl buffer (pH 7.0, red line) and its linear precursor in THF (blue line). The inset is the projected structure of the linear precursor (**2**).

synthetic purposes. Here, we explore the probable conformational propensity of the linear precursors to develop a convenient synthetic method for the natural product and its analogues that is readily adaptable to combinatorial synthesis.

Results and Discussions

First, we examined whether the linear biosynthetic precursor of gramicidin S exists in an antiparallel β-sheet structure as the cyclic natural product. The *N*-acetylcysteaminyl (NAC) thioester of the linear precursor (**2**) was synthesized with 4-sulfamylbutyryl AM resin (Scheme 1) and subjected to circular dichroism (CD) analysis. As shown in Figure 2, analysis of the CD spectrum indicated as expected that the precursor possesses a typical β-sheet structure in THF attested by the curvature at around 220 nm. This conformation closely resembles the antiparallel β-pleated sheet structure of the aqueous solution

(10) Wu, X.; Bu, X.; Wong, K. M.; Yan, W.; Guo, Z. *Org. Lett.* **2003**, *5*, 1749−1752.
(11) Wishart, D. S.; Kondjewski, L. H.; Semchuk, P. D.; Sykes, B. D.; Hodges, R. S. *Lett. Pept. Sci.* **1996**, *3*, 53−60.
(12) (a) Bu, X.; Wu, X.; Xie, G.; Guo, Z. *Org. Lett.* **2002**, *4*, 2893−2895. (b) Qin, C.; Bu, X.; Wu, X.; Guo, Z. *J. Comb. Chem.* **2003**, *5*, 353−355.
(13) (a) Kuo, M.-C.; Gibbons, W. A. *Biochemistry* **1979**, *18*, 5855−5867. (b) Kuo, M.-C.; Gibbons, W. A. *J. Biol. Chem.* **1979**, *254*, 6278−6287. (c) Kuo, M.-C.; Drakenberg, T.; Gibbons, W. A. *J. Am. Chem. Soc.* **1980**, *102*, 520−524. (d) Kuo, M.-C.; Gibbons, W. A. *Biophys. J.* **1980**, *32*, 807−836. (e) Zhou, N.; Mascagni, P.; Gibbons, W. A.; Niccolai, N.; Rossi, C.; Wyssbrod, H. *J. Chem. Soc., Perkin Trans. 2* **1985**, *4*, 581−587.

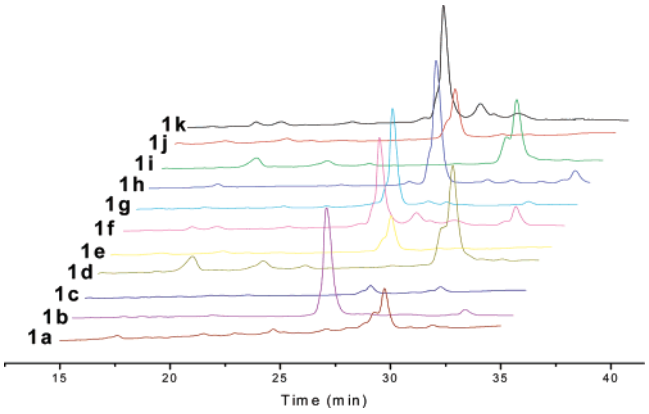


FIGURE 3. HPLC chromatograms of the unpurified cleavage products (**1a**−**k**) of the linear precursors **4a**−**k**. The analyses were performed with a Waters 600E system with a reversed-phase semipreparative XTerra RP18 column, 7 μm, 7.8 × 300 mm. Separation conditions were as follows: 2.0 mL min^{-1} flow rate, a linear gradient of 80 to 20% A in 25 min, 20 to 0% A in another 10 min, washed with 100% B for 10 min, and then calibrated at 80% A for 15 min. Solution A was 0.1% TFA in double-deionized H_2O, and solution B was 0.1% TFA in acetonitrile.

structure of gramicidin S, which is maintained by four interstrand hydrogen bonds,[4] shown by the near-identical shape of CD spectra for the precursor (**2**) and the natural product.

In an attempt to determine whether the β-sheet conformation predisposed the linear precursor of gramicidin S to self-cyclize, **3a** was activated by cyanomethylation, deprotected to remove Boc and *t*-Bu groups, and treated in a basic solution to deprotonate the *N*-terminal ammonium group for cyclization (Scheme 2), similar to the tyrocidine A synthesis.[12b] After the reaction, the product was precipitated with cold ether, dried to obtain a white solid with a crude yield of 25%, and dissolved in DMSO for determination of purity and structure. HPLC analysis showed that only a product (purity >87%, Figure 3) with the same retention time as the authentic gramicidin S was present in the unpurified product mixture. Mass spectroscopic and ^{1}H NMR data indicated that the only product was the expected head-to-tail cyclization product. Moreover, the purified product showed consistent antibacterial activity with the authentic gramicidin S.[2a] Indeed, these results showed that the linear precursor specifically cyclized head-to-tail without interference from the δ-NH_2 of the two ornithine residues, most likely due to the predisposed product-like conformation.

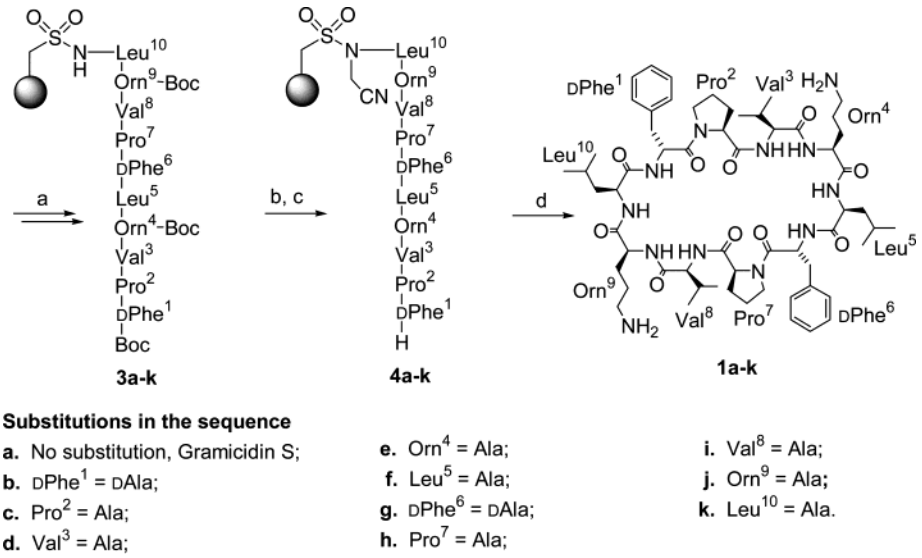
To explore the usefulness of this conformation-dependent method for gramicidin analogue synthesis, we determined the influence of the side chains of the constituent amino acids of the gramicidin S template on the cyclization efficiency by an "alanine-scanning" method used for the tyrocidine A scaffold.[12b] The component amino acids of the gramicidin S precursor were sequentially substituted by alanine. Configuration of individual amino acids was maintained in the side chain swapping since it has been known to affect the conformation of the linear peptides.[12a] The substituted linear precursors were synthesized and cyclized in parallel using Irori's AccuTag-100 Combinatorial Chemistry System,[14] according to the method shown in Scheme 2. Starting from 30 mg of 4-sulfamylbutyryl AM resin (0.8 mmol/g) for each compound, the alanine-substituted products were obtained in good yields and characterized without purification as summarized in Table 1.

As shown in Figure 3, no product was formed when Pro-2 in the linear precursor was substituted. For the other nine substitutions, HPLC chromatograms showed that the unpurified cleavage products predominantly contained one peak with a purity of >80%. It is interesting to note the identical retention time (t_R) for the cleavage products of precursor pairs **4b**/**4g**, **4d**/**4i**, **4e**/**4j**, and **4f**/**4k**, which strongly suggests that the corresponding cleavage products are the expected head-to-tail cyclization product since the symmetric t_R distribution is consistent with the C_2 symmetry of the gramicidin S template. Moreover, mass spectroscopic data showed that each cleavage reaction gave rise to a dominant molecular ion consistent with the cyclic product of the commensurate linear peptide precursor, free from any linear hydrolytic or aminolytic products. Furthermore, after HPLC purification, the alanine-substituted analogues were subjected to ^{1}H NMR structural determination and the cleavage products of the precursor pairs **4b**/**4g**, **4d**/**4i**, **4e**/**4j**, and **4f**/**4k** were indeed found to be identical. In addition, spectrum comparison revealed that the alanine analogues differed from the wild-type gramicidin S only for the substituted residue, indicating that the cyclic products possessed a ring structure closely resembling the antiparallel natural product and that the cyclization of the linear precursors was dominantly head-to-tail with little interference from the unprotected amino groups. These results showed that the individual side chain of the composing amino acid residues of the gramicidin S scaffold (except for that of Pro-2) has little effect on the formation of the pre-organized favorable conformation and thus does not affect the strong tendency of the linear precursors to self-cyclize.

It is interesting to note that Pro-2 is irreplaceable whereas its equivalent at a symmetrical position in the gramicidin S structure, proline-7, can be substituted with an alanine. This difference can be understood on the basis of the projected β-sheet conformation for the linear precursor (**2**) in which Pro-2 facilitates the β-turn formation, a function well-known for proline residues,[15] to bring the two reactive groups in close proximity and thus is indispensable in the ring closure. On the other hand, although Pro-7 is also involved in the β-sheet conformation (**2**), its turn-facilitating capability is much less significant due to the strong driving force of the four inter-strand hydrogen bonds. The insignificance of this proline residue in the β-turn formation is also evidenced by comparing to the similar conformation-assisted synthesis of tyrocidine A analogues in which no proline residue at the corresponding position is required in the sheet formation of the linear precursors.[12b] However, the only proline involved in the ring closure of tyrocidine A precursor, which occupies an equivalent position as the Pro-2 in gramicidin S precursor, is replaceable by an alanine.[12b] This discrepancy might result from subtle difference in the orientation of the *N*- and *C*-termini of

(14) Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289−2291.
(15) Hutchinson, E. G.; Thornton, J. M. *Protein Sci.* **1994**, *3*, 2207−2216.

SCHEME 2. Solid-Phase Synthesis of Gramicidin S and Its Alanine-Substituted Analogues[a]



[a] Reagents and conditions: (a) standard Fmoc solid-phase peptide synthesis, see Scheme 1; (b) ICH_2CN/DIPEA/NMP, 24 h; (c) CF_3COOH/phenol/*i*-Pr_3SiH/H_2O = 88:5:5:2, 1 h; (d) DIPEA/THF.

TABLE 1. Characterization of the Cyclization Reactions of the Linear Decapeptide Precursors 4a−h

product	substituted residue	t_R[a] (min)	calcd mass	$[M + H]^+$	yield[b] (%)	purity[c] (%)
1a (wt)	none	29.7	1140.7	1141.7	25	87
1b	D-Phe^1	26.5	1064.7	1065.8	53	92
1c	Pro^2				0	
1d	Val^3	31.1	1113.4	1113.9	29	82
1e	Orn^4	27.7	1069.7	1070.5	32	85
1f	Leu^5	26.6	1098.6	1099.9	52	89
1g	D-Phe^6	26.6	1064.7	1065.8	49	82
1h	Pro^7	28.0	1114.7	1116.0	45	81
1i	Val^8	31.1	1113.4	1114.7	34	83
1j	Orn^9	27.7	1069.7	1070.8	32	82
1k	Leu^{10}	26.6	1098.6	1099.6	34	85

[a] Retention time of the major product peak from HPLC chromatograms shown in Figure 2. [b] Yield of crude products after ether precipitation. [c] Ratio of the area of the cyclic product peak to the total integration over 15−35 min on the HPLC chromatograms in Figure 2.

the predisposed β-sheet conformations of tyrocidine A and gramicidin S precursors. In tyrocidine A precursor, the termini might be brought closer together for the reactive terminal groups to effectively react with each other without the help of the proline, whereas a proline is needed for the gramicidin S precursor to facilitate the turn formation to bring the two more separated terminal groups close enough together to achieve the ring closure.

Despite the indispensability of Pro-2, the readily exchangeability of the other nine residues of the gramicidin S scaffold still allows a very large structural space to be explored. Although the exact scope of the allowable variation in the gramicidin S scaffold remains to be defined, the pre-organized conformation of the linear precursors should be tolerant to variations in the side chains since the favorable conformation is maintained by four strong backbone hydrogen bonds. Indeed, simultaneous variations at three composing amino acid residues have been successfully incorporated into the cyclic structure without affecting the conformational propensity in a previous investigation.[10]

Conclusions

In summary, we have demonstrated that the predisposed conformation of linear gramicidin S precursor and its analogues can be utilized to design a simple and efficient method for analogue synthesis. Apparently, this facile synthetic method can be readily adapted to combinatorial synthesis. In comparison with the reported chemoenzymatic cyclization method,[10] this chemical approach allows more flexibility in the precursor sequence and thus a much larger structural space to be explored. This method should provide a convenient access to a large amount of gramicidin S analogues to discover new leads for containment of microbial resistance.

Experimental Section

General Methods. ^1H NMR spectra were recorded on a spectrometer and calibrated with the residual undeuterated solvent or TMS. Mass spectra were recorded on a triple-stage quadruple mass spectrometer using fast atom bombardment (FAB) ionization mode. The products were unpurified for the mass spectroscopy, whereas the NMR spectra were recorded with samples purified by HPLC.

Solid-Phase Synthesis of the Linear Biosynthetic Precursor of Gramicidin S (2). Synthesis of the linear biosynthetic precursor of gramicidin S (**2**) was accomplished according to Scheme 1. The synthesis started from 100 mg of 4-sulfamyl butyryl AM resin (0.8 mmol/g) and strictly followed a published procedure up to the cyanomethylation.[12b] Subsequently, the activated resin was suspended in 4 mL of 10% *N*-acetylcysteamine in THF and stirred under N_2 atmosphere at room temperature for 20 h. Most THF in the resulting solution was removed by aspiration, and the product was precipitated by cold ether. The Boc protecting groups were removed by dissolving the linear precursor in 2 mL of the deblocking agent (CF_3COOH/phenol/*i*-Pr_3SiH/H_2O = 88:5:5:2) and stirring for 3 h at room temperature. The crude product was then precipitated by cold ether, dissolved in 0.3 mL of methanol, and purified by HPLC to obtain 21.6 mg of white

solid (21.4% yield). FAB-MS: *m/z* 1260.9 ([M + H]$^+$, calcd *m/z* 1259.8). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 8.70 (t, J = 9.0 Hz, 1H), 8.59 (d, J = 7.5 Hz, 1H), 8.54 (d, J = 8.0 Hz, 1H), 8.34 (s, 1H), 8.24 (t, J = 9.0 Hz, 1H), 8.17 (m, 1H), 8.09 (m, 1H), 7.97−8.03 (m, 2H), 7.77−7.84 (br, 5H), 7.23−7.45 (m, 10H), 5.07 (d, J = 8.5 Hz, 1H), 4.84 (m, 1H), 4.17−4.53 (m, 12H), 3.66 (m, 1H), 3.58 (m, 1H), 3.46 (m, 1H), 3.25 (m, 2H), 3.15 (m, 1H), 3.06 (m, 1H), 2.95 (m, 2H), 2.85 (m, 5H), 2.29 (m, 1H), 2.18 (m, 1H), 2.06 (m, 2H), 1.89 (s, 3H), 1.43−1.88 (m, 16H), 1.24 (m, 1H), 1.11 (m, 1H), 0.72−1.03 (m, 24H).

Solid-Phase Synthesis, Analysis, and Purification of Cyclic Gramicidin S and Its Analogues. Solid-phase synthesis of the linear peptide precursors and their cyclization into gramicidin S and analogues strictly followed a published procedure.[12b] Reactions were carried out in MicroKan reactors with 30 mg of 4-sulfamylbutyryl AM resin (0.8 mmol/g) for each compound. The crude product was precipitated with cold ether, dissolved in 100−300 μL of methanol, and subjected to HPLC analysis which was performed with a reversed-phase semipreparative XTerra RP_{18} column, 7 μm, 7.8 × 300 mm. Separation conditions were: 2.0 mL min^{-1} flow rate, a linear gradient of 80% to 20% A in 25 min, 20 to 0%A in another 10 min, washed with 100% B for 10 min, and then calibrated at 80% A for 15 min. Solution A was 0.1% TFA in double-deionized H_2O and solution B was 0.1% TFA in acetonitrile. For ^{1}H NMR spectroscopic analysis, crude products were purified by HPLC using above gradient and obtained as white solid after evaporating solvent from the collected product fractions.

Wild-type Gramicidin S (1a). FAB-MS: *m/z* 1141.4 ([M + H]$^+$, calcd mass 1140.7). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.06 (s, 1H, NH), 8.70 (d, J = 9.2 Hz, 1H, NH), 8.33 (d, J = 9.2 Hz, 1H, NH), 7.85 (d, J = 7.6 Hz, 2H, NH), 7.74−7.68 (m, 8H), 7.4−7.2 (m, 18H), 7.1 (s, 1H), 6.98 (s, 1H), 4.74−4.04 (m, 10H), 2.95−2.66 (m, 10H), 2.07−2.03 (m, 2H), 1.91−1.19 (m, 24H), 0.81−0.73 (m, 24H).

Ala^1-gramicidin S (1b = 1g). FAB-MS: *m/z* 1065.8 ([M + H]$^+$, calcd mass 1064.7). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.17 (s, 1H), 8.97 (s, 1H), 8.85−8.80 (m, 2H), 8.43 (d, J = 10 Hz, 2H), 7.79−7.73 (m, 6H), 7.49−7.35 (m, 8H), 4.88 (m, 2H), 4.73−4.64 (m, 2H), 4.52−4.41 (m, 6H), 4.03−3.99 (m, 1H), 3.12−3.09 (m, 1H), 2.96−2.81 (m, 6H), 2.27−2.07 (m, 6H), 1.88−1.86 (m, 4H), 1.72−1.31 (m, 24H), 0.98−0.87 (m, 16H).

Ala^3-gramicidin S (1d = 1i). FAB-MS: *m/z* 1113.9 ([M + H]$^+$, calcd mass 1113.4). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.07 (s, 2H), 8.72 (d, J = 10 Hz, 2H), 8.47 (d, J = 10 Hz, 2H), 8.18 (d, J = 10 Hz, 2H), 7.76 (s, 2H), 7.55−7.08 (m, 16H), 5.01−4.98 (m, 1H), 4.79 (m, 1H), 4.68−4.38 (m, 8H), 3.07−2.93 (m, 8H), 2.17−2.00 (m, 4H), 1.87 (m, 1H), 1.74−1.30 (m, 24H), 0.92−0.89 (m, 16H).

Ala^4-gramicidin S (1e = 1j). FAB-MS: *m/z* 1070.5 ([M + H]$^+$, calcd mass 1069.7). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.05 (s, 1H), 8.98 (s, 1H), 8.75 (d, J = 10 Hz, 1H), 8.69 (d, J = 10 Hz, 1H), 8.47 (d, J = 10 Hz, 1H), 8.32 (d, J = 10 Hz, 1H), 7.82 (s, 14H), 7.39−7.33 (m, 13H), 7.26 (d, J = 10 Hz, 1H), 5.00−4.98 (m, 1H), 4.78−4.73 (m, 1H), 4.68−4.55 (m, 3H), 4.49−4.40 (m, 4H), 3.08−2.83 (m, 6H), 2.18−2.15 (m, 3H), 2.04 (s, 2H), 1.91−1.29 (m, 20H), 0.92−0.87 (m, 22H).

Ala^5-gramicidin S (1f = 1k). FAB-MS: *m/z* 1099.9 ([M + H]$^+$, calcd mass 1098.6). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.14 (s, 1H), 9.05 (s, 1H), 8.81 (d, J = 10 Hz, 2H), 8.45 (m, 2H), 7.79 (s, 8H), 7.41−7.08 (m, 18H), 4.82 (s, 2H), 4.68 (s, 2H), 4.51−4.33 (m, 8H), 3.07−2.86 (m, 6H), 2.21−2.06 (m, 2H), 1.86−1.34 (m, 18H), 1.38−1.29 (m, 2H), 0.97−0.89 (m, 14H).

Ala^6-gramicidin S (1g = 1b). FAB-MS: *m/z* 1065.8 ([M + H]$^+$, calcd mass 1064.7).

Ala^7-gramicidin S (1h). FAB-MS: *m/z* 1116.0 ([M + H]$^+$, calcd mass 1114.7). ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C, TMS): δ 9.16 (s, 1H), 8.99 (d, J = 10 Hz, 1H), 8.92 (d, J = 10 Hz, 1H), 8.79 (d, J = 10 Hz, 1H), 8.75 (d, J = 10 Hz, 1H), 8.42 (d, J = 10 Hz, 1H), 8.38 (d, J = 10 Hz, 1H), 7.78 (s, 6H), 7.61 (d, J = 10 Hz, 1H), 7.41−7.31 (m, 14H), 4.86 (m, 2H), 4.69−4.64 (m, 2H), 4.50−4.37 (m, 6H), 4.20−4.18 (m, 1H), 3.07−2.83 (m, 8H), 2.22−2.18 (m, 2H), 2.06 (m, 1H), 1.86−1.34 (m, 20H), 1.15−1.14 (m, 4H), 0.98−0.90 (m, 16H).

Ala^8-gramicidin S (1i = 1d). FAB-MS: *m/z* 1114.7 ([M + H]$^+$, calcd mass 1113.4).

Ala^9-gramicidin S (1j = 1e). FAB-MS: *m/z* 1070.8 ([M + H]$^+$, calcd mass 1069.7).

Ala^{10}-gramicidin S (1k = 1f). FAB-MS: *m/z* 1099.6 ([M + H]$^+$, calcd mass 1098.6).

Acknowledgment. This work was supported in part by the Innovation and Technology Fund (ITS/119/00) and a grant from the Research Grants Council (CERG HKUST601503) of the Government of the Hong Kong Special Administrative Region, China. We also thank the Department of Chemistry at the HKUST for providing postgraduate studentships to X.W. and N.L.J.N.

Supporting Information Available: FAB-MS and ^{1}H NMR spectra of the cyclic peptide products **1a**−**k**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO035712X