Total Synthesis of the Anti Methicillin-Resistant *Staphylococcus aureus* Peptide Antibiotics TAN-1057A-D

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Received August 4, 1997[⊗]

Abstract: TAN-1057A−D, dipeptides isolated from bacteria *Flexibacter* sp. PK-74 and PK-176, are new antibiotics with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*. We describe, in detail, the total synthesis of TAN-1057A−D by a convergent route featuring a new method to construct the cyclic amidinourea functional group.

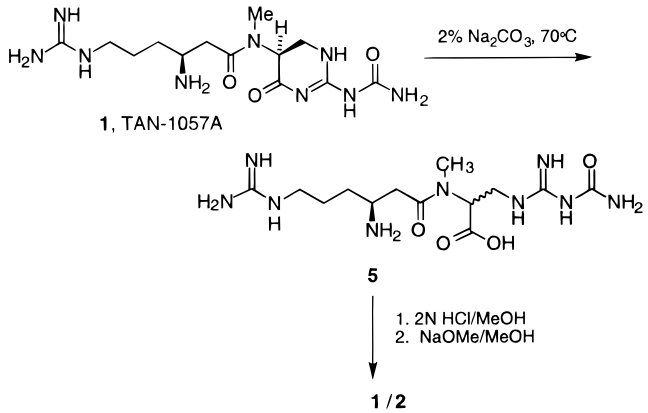
Introduction

Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have become a very serious clinical problem.[1−5] MRSA has developed resistance to most β-lactam antibiotics as well as numerous other antibiotics due to presence of the *mec A* gene.[1] MRSA produces an altered penicillin-binding protein, PBP2a, for which most clinically significant β-lactam antibiotics have low affinity. A desperate search has very recently been initiated to find so-called fourth generation cephalosporins that possess affinity for PBP2a. Screening programs aimed at discovering new structural drug motifs that are efficacious against MRSA have therefore become increasingly significant.

Recently, Takeda Chemical Co., Japan, described the isolation of four new compounds identified as TAN-1057A−D from *Flexibacter* sp. PK-74 and PK-176 (Chart 1).[6,7] These compounds were found to be dipeptide antibiotics with potent activity against MRSA. TAN-1057A−D displayed better activity against Gram-positive bacteria than against Gram-negative bacteria. TAN-1057A and -D, which have the *S*-configuration in the heterocyclic portion of the molecule, were more active than TAN-1057B and -C which possess the *R*-configuration. There was no cross-resistance between TAN-1057 and methicillin, erythromycin, and gentamycin. It is significant to note that TAN-1057A displays potent activity against all of the MRSA strains evaluated and was found to compare very favorably to vancomycin.[6] The Takeda group concluded that the therapeutic effects of TAN-1057A, as determined in mice, were superior to vancomycin and imipenem, especially against MRSA. The preliminary acute toxicity (LD_{50}) data obtained for TAN-1057A was ca. 100 mg/kg upon intraperitoneal injection and 50 mg/kg upon intravenous injection in mice.

TAN-1057A did not inhibit the incorporation of tritiated thymidine and uridine into *S. aureus* FDA 209P or *Escherichia coli* LD-2. However, TAN-1057A inhibited the incorporation of leucine into macromolecules in these organisms at concentrations below the minimal inhibitory concentration (MIC). In addition, poly-A and poly-U-directed protein synthesis was inhibited in an *E. coli* cell-free system at 40 and 10 μg/mL, respectively. TAN-1057A did not inhibit aminoacyl-tRNA synthetase; thus, this drug appears to interfere with protein biosynthesis after the formation of aminoacyl-tRNA. There is no published data concerning the morphological characteristics of susceptible strains treated with TAN-1057; thus, it is not presently known if TAN-1057 inhibits bacterial cell wall protein biosynthesis.

Scheme 1



TAN-1057A and -B are dipeptides consisting of β-homoarginine and a unique heterocyclic amidinourea derivative of 2,3-diaminopropionic acid. TAN-1057A and -B gradually lost their antibacterial activities in basic aqueous solutions due to hydrolytic opening of the six-membered ring system (Scheme 1).[7] Hydrolysis of TAN-1057A occurs in both acidic and basic media, to afford the acyclic form (**5**) with attendant racemization of the α-amino acid stereogenic center. It was also reported[7] that the acyclic form of the molecule can be converted back into the cyclic form via the methyl ester intermediate resulting in a diastereomeric mixture (1:1) of TAN-1057A and -B.

Due to the unique functionality present in these structures and the possibility for the discovery of new therapeutically useful biochemical targets through mechanism of action studies on these substances, we have developed the first synthesis of TAN-1057A−D featuring new methodology for constructing the cyclic amidinourea and report these findings here.

Results and Discussion

A very simple and straightforward retrosynthetic analysis consisting of dissecting the molecule into the β-homoarginine

[⊗] Abstract published in *Advance ACS Abstracts*, December 1, 1997.

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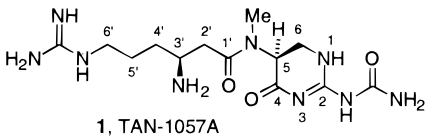
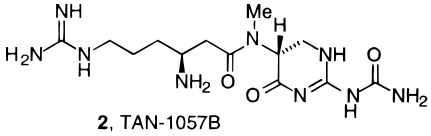
S0002-7863(97)02670-X CCC: $14.00

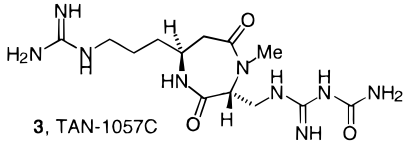
Chart 1



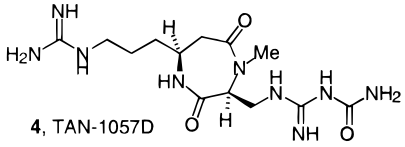
1, TAN-1057A



2, TAN-1057B



3, TAN-1057C



4, TAN-1057D

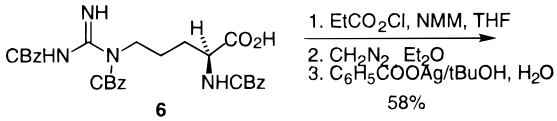
subunit and the 2,3-diaminopropionic acid portion was initially considered. Peptide coupling, followed by heterocycle formation and urea homologation, was expected to give the desired product. Several variants of this approach have been extensively investigated; a successful approach, predicated on new methodology to construct the cyclic amidinourea is described herein.

In planning alternate synthetic routes, the choice of the protecting groups was viewed as being critical to an ultimately successful approach. In addition, the unusual and labile cyclic amidino urea was targeted to be constructed in the late stages of the synthesis. It was deemed crucial to orchestrate the selective assembly of blocking groups such that (1) the guanidino group of the homoarginine moiety could be kept inert, (2) the amino group at C5 could be methylated selectively, and (3) the 3′- and 1-amino groups could be differentially protected and sequentially unmasked. Commercially available tri-*N*-CBZ-L-arginine (**6**) (CBZ = benzyloxycarbonyl) was chosen as the core starting material, since it was expected that catalytic hydrogenation would be compatible with the final unmasking of the fully derivatized, protected structure. The phthalimido group was chosen to block N1, since this group should permit the selective *N*-methylation and be removable under relatively mild conditions.

Synthesis of the *β*-Homoarginine Subunit. Tri-*N*-CBz-L-arginine (**6**) was first converted to the corresponding ethoxycarbonic anhydride in the presence of *N*-methylmorpholine and ethyl chloroformate.[8−10] The mixed anhydride was immediately allowed to react with diazomethane to furnish the corresponding diazoketone. Wolff rearrangement in methanol gave tri-*N*-CBz-*β*-homoarginine methyl ester as a white solid, which was saponified in 2 N LiOH to give the carboxylic acid derivative. However, one *N*-CBz group on the guanidine was lost during saponification and N^{α},N^{γ}-di-*N*-CBz-*β*-homoargine was thus obtained. Alternatively, tri-*N*-CBz-*β*-homoarginine (**7**) was prepared through a modified Arndt−Eistert synthesis with a 1:1 mixture of *tert*-butyl alcohol and water as solvent. This permitted the preparation of the free acid without a saponification step, obviating loss of one *N*-CBz group (Scheme 2).

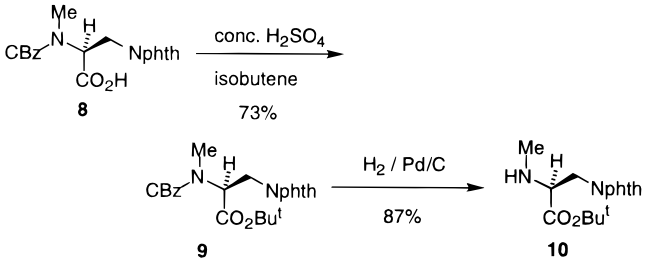
Synthesis of the 2,3-Diaminopropionic Acid Subunit. (2*S*)-N^2-CBz-N^2-methyl-N^3-phthalimido-2,3-diaminopropionic acid (**8**) was prepared according to the literature procedure[11] using L-*N*-CBz-asparagine as starting material. The acid (**8**) was converted into the corresponding *tert*-butyl ester (**9**) using EDCI/*t*-BuOH in excellent yield. However, under these conditions, it was found that complete racemization of the substrate occurred. Optically pure **9** could be obtained by an alternate procedure employing concentrated sulfuric acid and isobutene. Reductive removal of the *N*-CBz group provided the amine component (**10**, Scheme 3).

Scheme 2



6 → **7**: 1. $EtCO_2Cl$, NMM, THF; 2. CH_2N_2, Et_2O; 3. C_6H_5COOAg/tBuOH, H_2O; 58%

Scheme 3



8 → **9**: conc. H_2SO_4, isobutene, 73%; **9** → **10**: H_2 / Pd/C, 87%

Compound **10** proved to be labile to racemization on attempted silica gel column chromatographic purification; this method of purification was thus avoided. As will be discussed below, racemic **10** was ultimately used in the synthesis due to the ubiquitous lability of the α-stereogenic center of this amino acid to epimerization.

Construction of the Amidinourea Subunit. The success of developing a general synthetic approach to this new class of peptide antibiotics relies heavily on an efficient preparation of the unusual, cyclic amidinourea moiety. Only one report in the literature has described this unusual ring system,[12] and recently, a related five-membered cyclic amidinourea was found in an antitumor antibiotic NA22598.[13] All of the published methods, which includes the reaction of guanidines with isocyanates,[14] hydrogenation of 5,3-diamino-1,2,4-oxadiazoles,[15] or the hydrolysis of cyanoguanidines under strongly acidic conditions,[16] are either inefficient, involve harsh reaction conditions, or require numerous steps.[12] We have examined and subsequently determined that none of these methods were suitable for accessing the labile TAN-1057 amidinourea substructure. Recently, we reported[17] a new and efficient method for the preparation of acyclic amidinoureas using *N*-(benzyloxycarbonyl)ureido-*N*′-(benzyloxycarbonyl)-(*S*)-methylisothiourea (**21**) under very mild conditions.[18−20] As a model system,

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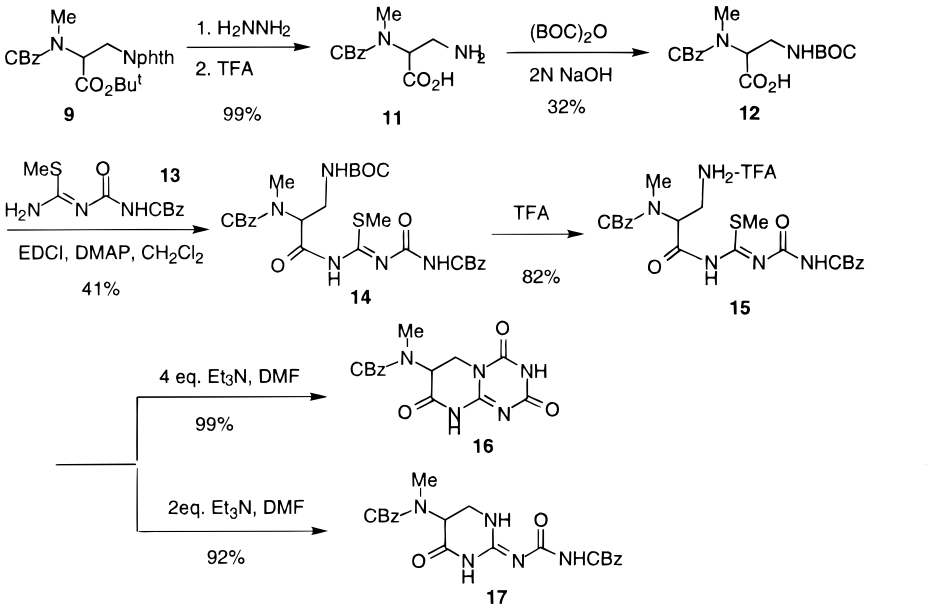
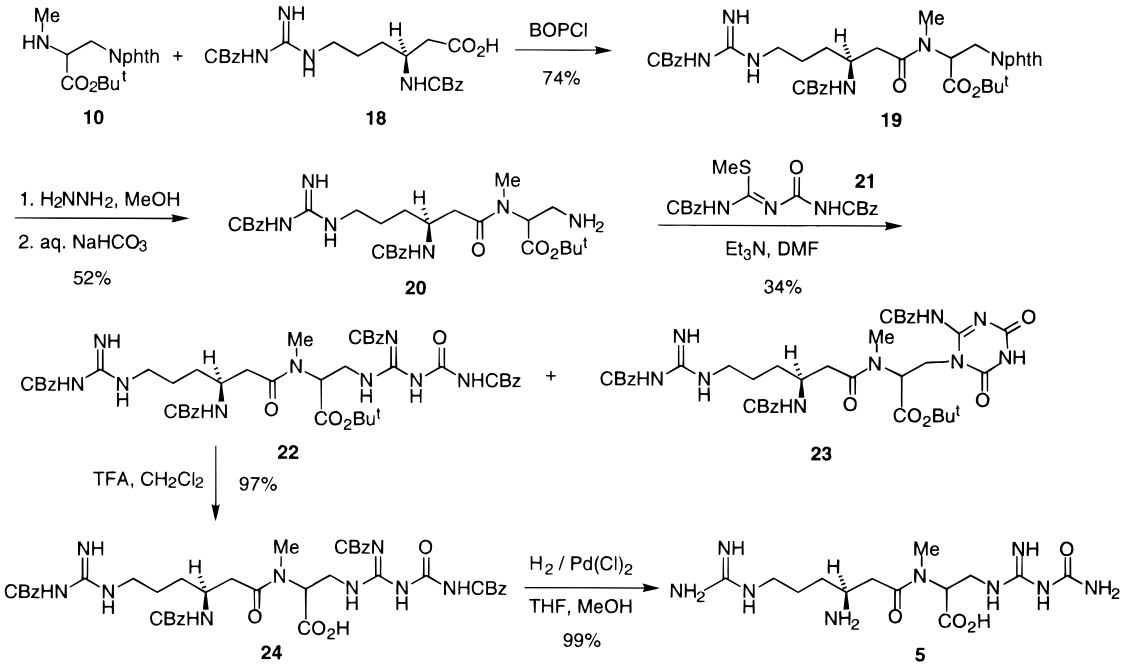
Scheme 4



Scheme 5



the direct preparation of cyclic amidinoureas from peptide substrates was examined, as shown in Scheme 4. *N*-(Benzyloxycarbonyl)ureido-*N*′-(*tert*-butyloxycarbonyl)-(*S*)-methylisothiourea (**35**) was prepared from mono-(*tert*-butyloxycarbonyl)-(*S*)-methylisothiourea and (benzyloxycarbonyl)isocyanate[21] (THF, 100%). Treatment of compound **35** with trifluoroacetic acid (TFA) gave **13**.

Next, carboxylic acid **11** was prepared from protected 2,3-diaminopropionic acid (**9**) following standard protecting group exchange. Coupling of the (*S*)-methylisothiourea derivative **13** with **12** using EDCI in the presence of 4-(dimethylamino)-pyridine (DMAP) gave **14** in 41% yield. Treatment of **14** with TFA removed the *tert*-butyoxycarbonyl (BOC) group to furnish the crude amine−TFA salt **15**. When **15** was treated with 2 mol equiv of triethylamine in DMF, an intramolecular cyclization reaction ensued to furnish the desired amidinourea (**17**) in 92% yield. However, in the presence of 4 mol equiv of triethylamine under the same conditions, the formation of an insoluble substance with spectral characteristics consistent with the bicyclic structure **16** was observed. Careful control of the reaction time and the amount of base added for this cyclization permitted the reproducible construction of the desired monocyclic amidinourea system **17**.

This methodology was first applied to the construction of the fully functionalized acyclic hydrolysis product (**5**) of TAN-1057A, as shown in Scheme 5. The peptide coupling reaction between N^{α},N^{γ}-di-*N*-CBz-β-homoarginine **18** and **10** with BOP-Cl gave the desired peptide **19** in good yield. The phthaloyl group was removed by treatment with hydrazine in methanol to provide the free amine **20**, which was condensed with

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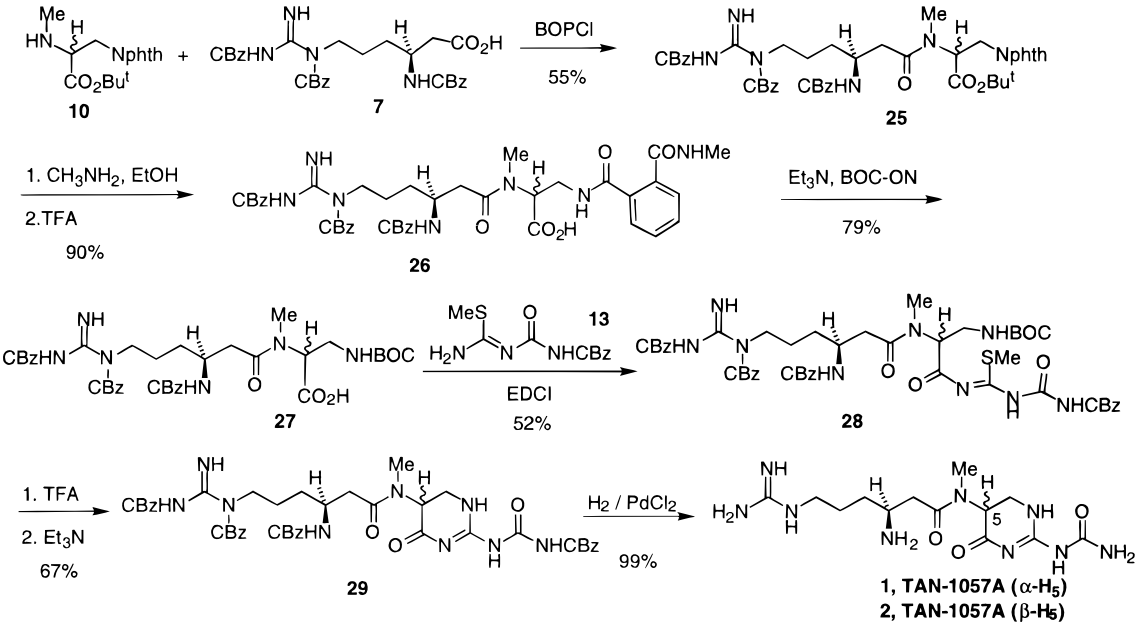
Scheme 6



N-(benzyloxycarbonyl)ureido-*N*′-(benzyloxycarbonyl)-(*S*)-methylisothiourea (**21**) to give the desired product **22** and triazinedione byproduct **23**. The best results were obtained by using 1 equiv of triethylamine and stopping the reaction before completion, where the attendant appearance of the byproduct (**23**) could be minimized (4−5 h, as monitored by TLC).

The tetra-*N*-CBz-protected TAN-1057 precursor **22** was deprotected by catalytic hydrogenation following TFA removal of the *tert*-butyl ester to give the linear compound **5** in excellent yield. Despite extensive effort, we were unable to effect the clean conversion of **5** into TAN-1057A/B.[7] Although this route can technically be considered a formal total synthesis on the basis of the Takeda work,[7] we turned our attention to a reliable and versatile method to construct the biologically active, cyclic amidinourea-containing natural product.

Total Synthesis of TAN-1057A/B. Drawing on the observations from the model study, the approach shown in Scheme 6 was devised. Coupling of tri-N-CBz-homoarginine (**7**) and *d,l*-**10** with BOP-Cl gave the desired peptide **25** in good yield. Attempts to deprotect the phthalimido group with hydrazine in methanol resulted in loss of one of the *N*-CBz groups from the guanidine. An alternative procedure using methylamine in ethanol followed by treatment with TFA gave the corresponding *N*-methylamide (**26**).

When peptide **25** was treated with 30% methylamine in EtOH for more than 5 min, one of the *N*-CBz groups was removed. However, if the reaction was stopped after 5 min., the partially deprotected derivative was obtained without concomitant loss of the *N*-CBz groups. The monoacylated amine was next converted to the corresponding BOC-protected amine **27** by treatment with excess triethylamine in the presence of BOC-ON in dioxane/water.

Initially, we utilized optically pure **10** for coupling with **7**. Under the same conditions, no detectable racemization accompanied this condensation. However, during the removal of the phthalimido group under various conditions, complete epimerization of the C5 stereogenic center was observed. The Takeda group had reported that HPLC-purified TAN-1057A rapidly epimerizes at C5 to a mixture of TAN-1057A + TAN-1057B; as such, it was decided to carry the synthesis forward with racemic **10**. All intermediates from compound **25** forward are therefore 1:1 mixtures of two optically pure diastereomers. Attempted chromatographic separation of each diastereomeric pair proved unsuccessful. Since the lability of this stereogenic center renders the final compounds, in practice, to be handled (and tested biologically) as diastereomeric mixtures, the route chosen turned out to be the most practical and economical.

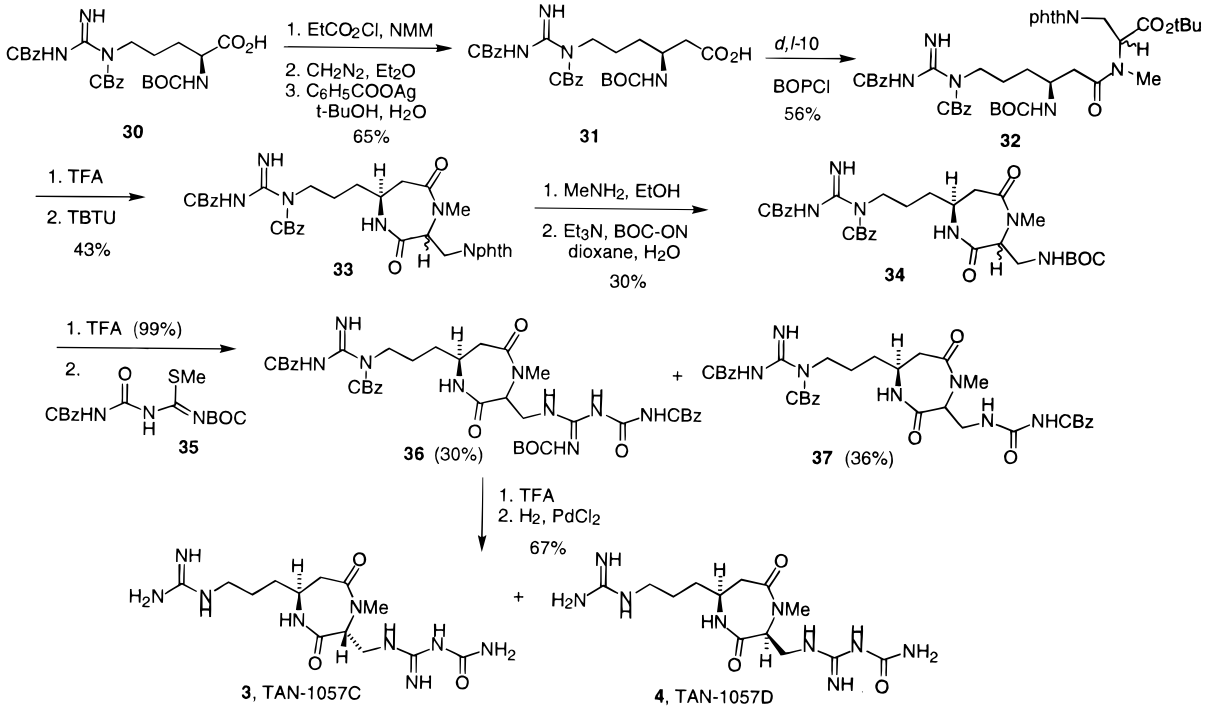
Compound **27** was coupled to *N*-(benzyloxycarbonyl)ureido-(*S*)-methylisothiourea **13** to give **28**[22] in 52% yield. Deprotection of the *N*-*t*-BOC group with TFA produced the corresponding TFA salt. When this substance was treated with triethylamine (2.0 equiv), cyclization ensued to furnish the desired, fully protected TAN-1057A derivative **29**[22] in 67% yield (two steps). This substance proved to be labile undergoing a second cyclization to a substance corresponding to **16** (Scheme 4). Fortunately, this problem can be circumvented by converting **29** into TAN-1057A/B immediately after PTLC purification. The deprotection was very efficient with hydrogen (1 atm) and $PdCl_2$ in methanol for 30 min. Pure TAN-1057A/B was obtained as a ∼1:1 mixture after simple workup. The synthetic material proved to be identical to the natural sample[23] by ^{1}H NMR, mass spectrometric fragmentation (ES^+), IR, mobility by HPLC, and bioassay against *S. aureus*.

Total Synthesis of TAN-1057C/D. TAN-1057C and TAN-1057D are also labile substances that were reported[7] to rapidly convert to a mixture of TAN-1057A/B upon standing in water. Utilizing the methodology developed for the synthesis of TAN-1057A/B reported above, the synthesis of these seven-membered ring isomers was undertaken as shown in Scheme 7. L-N^{α}-*t*-BOC-N^{δ},N^{ω}-di-CBz-*β*-homoarginine (**31**) was prepared from commercially available **30** in the same manner as that used for compound **7**. Coupling of **31** with *d,l*-**10** in the presence of BOP-Cl gave the desired peptide **32** in 56% yield.[22] Treatment of **32** with TFA effected removal of the BOC group and *tert*-butyl ester; cyclization was effected with TBTU furnishing **33**[22] in 43% overall yield. In addition to the desired cyclic peptide, a macrocyclic dimer was also isolated in approximately the same molar ratio to that of **33**; this substance was easily removed by preparative thin layer chromatography. Dilution of the reaction

(22) This material was obtained as an inseparable mixture of diastereomers epimeric at C5.

(23) An authentic sample of natural TAN-1057A/B was kindly furnished by Takeda Pahrmaceutical Co., Japan.

Scheme 7



mixture for the peptide coupling did not, unfortunately, significantly diminish the amount of dimer formed.

Cleavage of the phthaloyl group by the sequential methylamine ring-opening and BOC-ON protocol gave **34** in 30% overall yield. Treatment of this substance with TFA followed by guanidylation with reagent **35** gave the desired, fully protected product **36**[22] in 30% yield accompanied by the unexpected substance **37** (39%). These compounds were separated by chromatography, and sequential deprotection of **36** with TFA followed by catalytic hydrogenation gave TAN-1057C (**3**) and TAN-1057D (**4**) as an inseparable mixture. Further attempted HPLC purification of the synthetic material corroborated the findings of the Takeda group,[7] namely, that rapid conversion of the initial mixture of **3** and **4** to **1** and **2** occurs. Authentication of the synthetic material was provided by comparing ^{1}H NMR, mass spectrometric fragmentation (ES^+), IR, mobility by HPLC, and bioassay against *S. aureus* with that of natural TAN-1057A/B.[23]

The synthetic route devised herein should open the way for a systematic study of the structure/function relationships in this new class of peptide antibiotics. In particular, the synthesis of radiolabeled versions of the natural product for receptor-binding studies is currently being investigated. The cyclization approach described herein to construct the unusual amidinourea moiety provides a general and flexible method for accessing a potentially important generation of anti-MRSA antibiotics. Applications of this methodology toward these goals are presently under active investigation in these laboratories and will be reported in due course.

Experimental Section

General. General procedures and instrumentation have been previously described.[24] Mass spectra were obtained on a 1992 Fisons VG AutoSpec. HPLC analysis of TAN-1057 was carried out using a Waters 6000 pump equipped with a UV detector, utilizing an ODS, YMC Pack A-312 column and 0.1 M phosphate buffer (pH 5.0) as the mobil phase. All of the amino acids used as starting material were purchased from BACHEM Inc. Abbreviations not defined in the text: $(BOC)_2O$ = di-*tert*-butyl dicarbonate; BOC-ON = 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitile; BOP-Cl = bis(2-oxo-3-oxazolidinyl)phosphinic chloride (Aldrich Chemical Co.); EDCI = 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (Aldrich Chemical Co.); NMM = 4-methylmorpholine (Aldrich Chemical Co.); TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (Aldrich Chemical Co.); MNNG = 1-methyl-3-nitro-1-nitrosoguanidine (Aldrich Chemical Co.).

Coupling Product 14. To a solution of **12** (246 mg, 0.7 mmol, 1.0 equiv), DMAP (171 mg, 1.4 mmol, 2.0 equiv), and EDCI (148 mg, 0.77 mmol, 1.1 equiv) in CH_2Cl_2 (2.0 mL) was added **13** (300 mg, 0.84 mmol, 1.2 equiv). The resulting mixture was stirred overnight at room temperature, diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by column chromatography (silica gel, 4:1:0.1 CH_2Cl_2:EtOAc:MeOH) provided 172 mg (41%) of **14** as a semi-solid. ^{1}H NMR (300 MHz, CD_3COOD vs TMS, 333K): δ 1.45 (9H, s), 2.34 (3H, s), 3.06 (3H, s), 3.65 (1H, m), 3.81 (1H, dd, J = 5.4, 5.6 Hz), 4.72 (1H, m), 5.19 (2H, s), 5.26 (2H, s), 7.38 (10H, m). IR (NaCl, film): 3357, 3184, 2976, 1769, 1713, 1534, 1486, 1456, 1403, 1315, 1164, 1066, 1002, 733 cm^{-1}. HRMS: calcd for ($C_{28}H_{35}N_5O_8S$ + H) = 602.2285; found (M + H) = 602.2285.

Cyclization Product 17. Peptide **14** (30 mg, 0.05 mmol) was treated with anisole (0.1 mL) and TFA (2.0 mL) at 0 °C. The mixture was stirred for 5 h at room temperature and concentrated, and the crude **15** was immediately dissolved in THF (1.0 mL). To this solution was added Et_3N (14 μL, 0.1 mmol, 2.0 equiv). The mixture was stirred for 4.5 h at room temperature and poured into CH_2Cl_2/brine. The organic phase was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and triturated with ether to give 21 mg (75%) of **17** as a semi-solid. ^{1}H NMR (300 MHz, DMSO-d_6 vs TMS): δ 2.83/2.88 (3H, s), 3.56 (1H, m), 3.70 (1H, q, J =12.2 Hz), 4.80 (1H, m), 5.11 (4H, s), 7.37 (10H, m). IR (NaCl, film): 3226, 2952, 1766, 1662, 1635, 1542, 1498, 1455, 1390, 1324, 1249, 1198, 1071 cm^{-1}. HRMS: calcd for ($C_{22}H_{23}N_5O_6$ + H) = 454.1727; found (M + H) = 454.1730.

L-N^{α},N^{δ},N^{ω}-Tri-CBz-β-homoarginine Methyl Ester. To a solution of tri-*N*-CBz-L-arginine (BACHEM, Inc.) (1.15 g, 2.0 mmol, 1.0 equiv) in EtOAc (30 mL) was added NMM (250 μL) and ethyl chloroformate (200 μL) at 0 °C. The resulting mixture was stirred for 3 h at 0 °C. The precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG). The solution was stirred overnight at room temperature

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and concentrated to give an oily diazoketone. The diazoketone was dissolved in MeOH (30 mL), and to this solution were added silver benzoate (250 mg, 1 mmol, 0.5 equiv) and triethyl amine (1 mL). The resulting mixture was stirred overnight in the dark at room temperature and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate, and insoluble material was filtered off. The filtrate was washed with saturated sodium bicarbonate solution, saturated sodium chloride solution, 1 M hydrochloric acid, and then finally saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. Purification via column chromatography (silica gel, 5:4:0.2 methylene chloride:EtOAc:MeOH) yielded 873 mg (74%) of L-N^{α},N^{δ},N^{ω}-tri-CBz-β-homoarginine methyl ester as a white solid: mp 139.5−140.5 °C; $[\alpha]_D^{25} = -1.43$ (*c* 0.7, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.49 (2H, m), 1.53 (2H, m), 2.48 (2H, d, $J = 5.40$ Hz), 3.58 (3H, s), 3.93 (1H, m), 5.04 (2H, s), 5.10 (2H, s), 5.19 (2H, s), 5.43 (1H, d, $J = 7.0$ Hz, D_2O exchanged), 7.31 (15H, m), 9.30 (1H, br, D_2O exchanged), 9.46 (1H, br, D_2O exchanged). IR (NaCl, film): 3342, 2936, 1697, 1525, 1455, 1248, 1056, 1024, 667 cm^{-1}. Anal. Calcd for $C_{32}H_{36}N_4O_8$: C, 63.57; H, 6.00; N, 9.27. Found: C, 63.71; H, 5.81; N, 9.20.

L-N^{α},N^{ω}-Di-CBz-β-homoarginine (18). To a solution of L-N^{α},N^{δ},N^{ω}-tri-CBz-β-homoarginine methyl ester (873 mg, 1.44 mmol) in dioxane (16 mL) was added 2 M NaOH (16 mL). The resulting mixture was stirred for 1 h at room temperature, diluted with water, and extracted with ethyl acetate (50 mL). The aqueous layer was adjusted to pH = 5 with 1 M HCl solution and extracted with dichloromethane (2 × 100 mL). The combined organic extract was washed with saturated ammonium chloride, dried over $MgSO_4$, filtered, and concentrated to give a colorless oil. After trituration with anhydrous ethyl ether, 656 mg (100%) of **18** was obtained as a white solid: mp 95−98 °C; $[\alpha]_D^{25} = -14.0$ (*c* 1.0, CH_3OH). ^{1}H NMR (300 MHz, CD_3OD, vs TMS) δ 1.60 (4H, m), 2.37 (2H, m), 3.20 (2H, m), 3.94 (1H, m), 5.07 (2H, s), 5.16 (2H, s), 7.32 (10H, m). IR (NaCl, film): 3314, 2947, 1693, 1613, 1503, 1402, 1259, 1065 cm^{-1}. Anal. Calcd for $C_{23}H_{28}N_4O_6$: C, 60.52; H, 6.18; N, 12.27. Found: C, 60.80; H, 5.80; N, 12.06.

Peptide 19. To a mixture of the acid **18** (540 mg, 1.19 mmol, 1.0 equiv) and NMM (200 μL, 1.43 mmol, 1.2 equiv) in CH_2Cl_2 (2 mL) was added BOP-Cl (456 mg, 1.82 mmol, 1.2 equiv) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C. To the resulting mixture was added amine **10** (360 mg, 1.19 mmol, 1.0 equiv) in CH_2Cl_2 (2 mL). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (200 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc:MeOH, 5:4:0.2) provided 650 mg (74%) of **19** as an oil. ^{1}H NMR (300 MHz, DMSO-d_6, 393K vs TMS): δ 1.43 (13H, m), 2.38 (2H, m), 2.93 (3H, s), 3.05 (1H, m), 3.15 (1H, m), 3.77 (1H, m), 4.04 (2H, m), 5.00 (2H, s), 5.03 (2H, s), 5.15 (1H, m), 7.32 (10H, m), 7.82 (4H, m). IR (NaCl, film): 3396, 2939, 1774, 1716, 1636, 1395, 1287, 1155, 1065 cm^{-1}. HRMS (FAB): calcd for ($C_{39}H_{46}N_6O_9$ + H) = 743.3405; found (M + H) = 743.3439.

Amine 20. To a solution of **19** (700 mg, 0.94 mmol, 1.0 equiv) in MeOH (20 mL) was added hydrazine (0.4 mL, 12.5 mmol, 13 equiv) at 0 °C. The mixture was stirred overnight at room temperature, concentrated, and treated with ethyl acetate (200 mL) and saturated $NaHCO_3$ (50 mL). The organic phase was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated. Purification via column chromatography (silica gel, methylene chloride:MeOH, 10:1−10:4) provided 300 mg (52%) of **20** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$/D_2O vs TMS): δ 1.43 (9H, s), 1.51 (4H, m), 2.56 (2H, m), 2.84 (1H, m), 2.92 (3H, s), 3.06 (1H, m), 3.18 (2H, m), 3.67 (1H, m), 4.85 (1H, m), 5.06 (4H, s), 7.32 (10H, m). IR (NaCl, film): 3374, 3310, 2933, 1718, 1636, 1595, 1285, 1149, 1061 cm^{-1}. HRMS (FAB): calcd for ($C_{31}H_{44}N_6O_7$ + H) = 613.3350; found (M + H) = 613.3366.

Condensation Product 22. To a mixture of **20** (315 mg, 0.79 mmol, 1.5 equiv) in DMF (5 mL) was added **21** (320 mg, 0.52 mmol, 1.0 equiv) and triethylamine (73 μL, 0.52 mmol, 1.0 equiv). The resulting mixture was stirred for 4 h at room temperature. The reaction mixture was concentrated and dried *in vacuo* to give a white solid. The crude product was triturated with EtOAc, and the solid was filtered off. The filtrate was concentrated and separated via column chromatography (silica gel, CH_2Cl_2:ethyl acetate:MeOH, 75:20:5) to afford 167 mg (33.8%) of **22** as a semi-solid. ^{1}HNMR (300 MHz, $CDCl_3$/D_2O vs TMS): δ 1.44 (9H, m), 1.56 (4H, m), 2.56 (1H, m), 2.75 (1H, m), 2.91/2.94 (3H, s), 3.12 (2H, m), 4.11(3H, m), 5.11 (9H, m), 7.31 (20H, m). IR (NaCl, film): 3307, 2946, 1715, 1629, 1449, 1373, 1293, 1218, 1152, 1097, 731, 695 cm^{-1}. HRMS: calcd for ($C_{49}H_{59}N_9O_{12}$ + H) = 966.4361; found (M + H) = 966.4361.

Carboxylic Acid 24. To a mixture of **22** (167 mg, 0.18 mmol, 1.0 equiv) and anisole (0.1 mL) was added TFA (3.0 mL) at 0 °C. The mixture was stirred for 4 h at room temperature, concentrated, and dried *in vacuo*. Trituration with dry ethyl ether provided 153 mg (97%) of **24** as an amorphous powder. ^{1}H NMR (300 MHz, CD_3COOD vs TMS): δ 1.64 (4H, m), 2.70 (2H, m), 3.15 (3H, m), 3.30 (2H, m), 3.96 (3H, m), 5.13 (9H, m), 7.32 (20H, m). IR (NaCl, film): 3310, 2934, 1734, 1700, 1636, 1494, 1454, 1383, 1260, 1187, 1097, 695 cm^{-1}. Anal. Calcd for $C_{45}H_{51}N_9O_{12}H_2O$: C, 58.25; H, 5.76; N, 13.58. Found: C, 58.25; H, 5.76; N, 13.71.

TAN-1057A/B Acyclic Hydrolysis Product 5. To a solution of **24** (110 mg, 0.123 mmol, 1.0 equiv) in MeOH (2 mL)/THF (2 mL) was added $PdCl_2$ (20 mg). The reaction vessel was charged with H_2, and the mixture was hydrogenated at 60 psi for 24 h. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give a 2HCl salt of **5** (46 mg, 100% yield) as an amorphous solid. ^{1}HNMR (300 MHz, D_2O): δ 1.57 (4H, m), 2.66 (1H, m), 2.79 (1H, m), 2.88/2.89 (3H, s), 3.05 (2H, t, $J = 5.7$ Hz), 3.53 (2H, m), 3.73 (1H, m), 4.79 (1H, m). IR (KBr, pellet): 3350, 3179, 2955, 1696, 1622, 1395, 1205, 1135 cm^{-1}. MS (ES$^+$): calcd ($C_{13}H_{28}N_9O_4$ + H) = 374.2; found (M + H) = 374.2, (M − $CONH_2$) = 331.2.

Peptide 25. To a mixture of the acid **7** (1.50 g, 2.54 mmol, 1.0 equiv) and NMM (464 μL, 1.2 mmol, 1.3 equiv) in CH_2Cl_2 (3 mL) was added BOP-Cl (306 mg, 1.2 mmol, 1.2 equiv) at 0 °C. After 10 min of stirring, the amine *d*,*l*-**10** (970 mg, 3.19 mmol, 1.26 equiv) in CH_2Cl_2 (3 mL) was added. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 mL), washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc, 8:2) provided 1.22 g (55%) of **25** as an oil: $[\alpha]_D^{25} = -36.1$ (*c* 0.7, CH_2Cl_2) (this data is for the optically pure diastereomer obtained with (S)-**10**). ^{1}H NMR (300 MHz, CD_3OD): δ 1.20 (2H, m), 1.39 (2H, m), 1.43 (9H, s), 2.21 (1H, m), 2.53 (1H, m), 2.92 (3H, s), 3.72 (3H, m), 4.67 (2H, d, $J = 7.8$ Hz), 4.95 (2H, s), 5.08 (2H, s), 5.20 (2H, s), 5.22 (1H, m), 7.33 (15H, m), 7.64 (2H, m), 7.74 (2H, m). IR (NaCl, film): 3389, 2936, 1774, 1716, 1646, 1609, 1505, 1395, 1370, 1251, 1100, 1008, 722 cm^{-1}. Anal. Calcd for $C_{47}H_{52}N_6O_{11}$: C, 64.37; H, 5.98; N, 9.58. Found: C, 63.38; H, 6.08; N, 9.60.

Peptide 26. To a solution of **25** (500 mg, 0.57 mmol, 1.0 equiv) in CH_2Cl_2 (10 mL) was added 2 N methylamine in MeOH (5.0 mL). The mixture was stirred for 5 min and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc:MeOH, 4:1:0.3) provided 500 mg (97%) of the methylamine adduct as an oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.43 (9H, s), 1.61 (4H, m), 2.55 (1H, m), 2.64 (1H, m), 2.85 (3H, m), 3.03/3.04 (3H, s), 3.70 (1H, m), 3.86 (4H, m), 4.94 (3H, m), 5.09 (2H, s), 5.21 (2H, s), 7.37 (19H, m). IR (NaCl, film): 3383, 3280, 3067, 2935, 1717, 1646, 1507, 1456, 1373, 1252, 1098, 697 cm^{-1}. Anal. Calcd for $C_{48}H_{57}N_7O_{11}$: C, 63.49; H, 6.33; N, 10.80. Found: C, 63.51; H, 6.38; N, 10.81. The crude material obtained above (500 mg, 0.55 mmol, 1.0 equiv) was treated with anisole (0.2 mL) and TFA (5.0 mL) at 0 °C. The resulting mixture was stirred for 1 h at room temperature, concentrated, and triturated in dry ether to give **26** (437 mg, 93%) as an oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.59 (4H, m), 2.53 (1H, m), 2.80 (1H, m), 2.84 (3H, s), 3.03/3.05 (3H, s), 3.87 (5H, m), 4.94 (3H, m), 5.13 (2H, s), 5.22 (2H, s), 7.34 (19H, m). IR (NaCl, film): 3396, 3290, 2945, 1717, 1684, 1615, 1540, 1378, 1254, 1099 cm^{-1}. Anal. Calcd for ($C_{44}H_{49}N_7O_{11}\cdot 3H_2O$): C, 58.33; H, 6.12; N, 10.82. Found: C, 58.22; H, 5.93; N, 10.85.

Carboxylic Acid 27. To a solution of **26** (437 mg, 0.51 mmol, 1.0 equiv) in H_2O/dioxane (8 mL, 1:1) and Et_3N (715 μL, 5.1 mmol, 10 equiv) was added BOC-ON (376 mg, 1.53 mmol, 3.0 equiv). The mixture was stirred overnight and extracted with EtOAc(2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification via column chromatography (silica gel, CH_2-

Cl_2:MeOH, 9:1) provided 320 mg (79%) of **27** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.35 (9H, s), 1.65 (4H, m), 2.50 (2H, m), 2.86 (3H, m), 3.60 (3H, m), 3.95 (3H, m), 5.00 (2H, m), 5.10 (2H, s), 5.24 (2H, s), 7.32 (15H, m). IR (NaCl, film): 3388, 2927, 1714, 1609, 1513, 1454, 1382, 1253, 1173, 1098, 1006, 697 cm^{-1}. HRMS (FAB): calcd for ($C_{40}H_{50}N_6O_{11}$ + H) = 791.3616; found (M + H) = 791.3616.

(*S*)-Methylisothiourea 28. To a solution of **27** (70 mg, 0.066 mmol, 1.0 equiv), DMAP (25 mg, 0.13 mmol, 2.0 equiv), and EDCI·HCl (21 mg, 0.07 mmol, 1.1 equiv) in CH_2Cl_2 (0.5 mL) was added **13** (45 mg, 0.1 mmol, 1.5 equiv). The resulting mixture was stirred overnight at room temperature, diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:1:0.1) provided 36 mg (52%) of **28** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.39 (9H, s), 1.64 (4H, m), 2.28 (3H, s), 2.60 (1H, m), 2.75 (1H, m), 3.05 (3H, m), 3.47 (1H, m), 3.69 (1H, m), 3.91 (3H, m), 4.94 (3H, m), 5.08 (2H, s), 5.20 (2H, s), 5.23 (2H, s), 7.31 (20H, m). IR (NaCl, film): 3388, 2969, 1770, 1713, 1647, 1609, 1499, 1251, 1175, 1099 cm^{-1}. Anal. Calcd for $C_{51}H_{61}N_9O_{13}S$: C, 58.89; H, 5.91; N, 12.12. Found: C, 59.03; H, 6.12; N, 12.19.

Cyclization Product 29. To a mixture of **28** (70 mg, 0.067 mmol, 1.0 equiv) and anisole (0.1 mL) was added TFA (1.0 mL). The resulting mixture was stirred for 15 min at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried *in vacuo* for 2 h and triturated with ethyl ether to give a white solid. This white solid was dissolved in THF (1.5 mL). To this solution was added triethylamine (20 μL, 0.14 mmol, 2.0 equiv). After 10 min of stirring, the solvent was evaporated, and the resulting residue was immediately purified by PTLC (silica gel, CH_2Cl_2:EtOAc: MeOH, 4:1:0.5) to give 40 mg (67%) of **29** as a white solid. This product was unstable with a tendency to form a bicyclic byproduct ($t_{1/2}$ is about 1 day) and was used for next step immediately. ^{1}H NMR (300 MHz, CD_3Cl/D_2O vs TMS): δ 1.62 (4H, m), 2.45 (2H, m), 2.76/ 2.78 (3H, s), 3.32 (1H, m), 3.70 (1H, m), 3.93 (4H, m), 5.17 (6H, m), 7.32 (20H, m). IR (NaCl, film): 3381, 3258, 2934, 1765, 1713, 1646, 1608, 1504, 1452, 1252, 1186, 1096, 1063 cm^{-1}. MS (ES$^+$): calcd for ($C_{45}H_{49}N_9O_{11}$ + H) = 892.4; found (M + H) = 892.4, (M + H − 108) = 784.3.

3(*S*),5′(*S*/*R*)-3-Amino-6-[(aminoiminomethyl)amino]-*N*-[2-[(aminocarbonyl) amino]-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-N-methylhexanamide, TAN-1057A/B. To a solution of **29** (40 mg, 0.045 mmol, 1.0 equiv) in MeOH (1.5 mL)/CH_2Cl_2 (0.5 mL) was added $PdCl_2$ (40 mg). The reaction flask was degassed and charged with H_2 (1 atm). The mixture was stirred for 30 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give a 2HCl salt of TAN-1057A/B (**1/2**) (1:1, 20 mg, 100% yield) as an amorphous solid. This product was identical in mobility by HPLC, NMR, and antibiotic activity by bioassay to authentic TAN-1057A/B (Takeda). ^{1}H NMR (300 MHz, D_2O): δ 1.77 (4H, m), 2.85 (1H, dd, J = 18, 9.3 Hz), 3.00 (1H, dd, J = 18, 4.0 Hz), 3.17 (3H, s), 3.27 (2H, t, J = 6.0 Hz), 3.70 (1H, m), 3.99 (2H, m), 5.14 (1H, dd, J = 12, 8.5 Hz). IR (KBr pellet): 3350, 3179, 2955, 1696, 1622, 1395, 1205, 1135 cm^{-1}. MS (ES$^+$): calcd ($C_{13}H_{25}N_9O_3$ + H) = 356.2; found (M + H) = 356.4.

L-N^{α}-*t*-BOC-N^{δ},N^{ω}-di-CBz-β-homoarginine (31). To a solution of N^{α}-*t*-BOC-di-CBz-L-arginine **30** (BACHEM) (2.72 g, 5.0 mmol, 1.0 equiv) in THF (50 mL) was added NMM (604 μL, 5.5 mmol, 1.1 equiv) and ethyl chloroformate (524 μL, 5.5 mmol, 1.1 equiv) at 0 °C. The resulting mixture was stirred for 3 h at 0 °C. The precipitated amine hydrochloride was rapidly filtered off cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG). The solution was stirred overnight at room temperature and concentrated to give an oily diazoketone. The diazoketone was dissolved in *t*-BuOH/H_2O (60 mL, 1:1), and to this solution were added silver benzoate (1.0 g) and triethylamine (5 mL). The resulting mixture was stirred overnight in the dark at room temperature and then concentrated *in vacuo*. The residue was treated with a ethyl acetate/saturated NaH_2PO_4 aqueous solution. The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:4:0.5) provided 1.80 g (65%) of **31** as a semi-solid: $[\alpha]_D^{25}$ = −1.8 (*c* 2.0, CH_2Cl_2). ^{1}H NMR (300 MHz, CD_3OD): δ 1.39 (9H, s), 1.42 (2H, m), 1.63 (2H, m), 2.36 (2H, m), 3.83 (1H, m), 3.93 (2H, t, J = 7.2 Hz), 5.12 (2H, s), 5.27 (2H, s), 7.36 (10H, m). IR (NaCl, film): 3386, 3286, 2975, 1718, 1610, 1508, 1456, 1380, 1253, 1174, 1098, 1006, 738, 698 cm^{-1}. Anal. Calcd for $C_{28}H_{36}N_4O_8$: C, 60.42; H, 6.52; N, 10.07. Found: C, 60.30; H, 6.62; N, 10.06.

Peptide 32. To the mixture of the acid **31** (1.0 g, 1.79 mmol, 1.0 equiv) and NMM (255 μL, 2.33 mmol, 1.3 equiv) in CH_2Cl_2 (2 mL) was added BOP-Cl (593 mg, 2.33 mmol, 1.3 equiv) at 0 °C. After for 10 min of stirring, *d,l*-**10** (708 mg, 2.33 mmol, 1.3 equiv) in CH_2Cl_2 (3 mL) was added. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Purification via column chromatography (silica gel, methylene chloride:EtOAc, 8:2) provided 838 mg (56%) of **32** as an oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.16 (2H, m), 1.35 (9H, s), 1.43 (2H, m), 1.45 (9H, s), 2.10−2.55 (2H, m), 2.87/2.91 (3H, s), 3.72 (3H, m), 4.08 (1H, m), 5.10 (2H, s), 5.17 (2H, m), 5.24 (2H, m), 7.38 (10H, m), 7.67 (2H, m), 7.74 (2H, m). IR (NaCl, film): 3390, 2974, 1716, 1647, 1609, 1507, 1368, 1252, 1169, 1099, 1006, 719, 696 cm^{-1}. Anal. Calcd for $C_{44}H_{54}N_6O_{11}$: C, 62.70; H, 6.46; N, 9.97. Found: C, 62.60; H, 6.31; N, 9.79.

Cyclic Peptide 33. Compound **32** (179 mg, 0.212 mmol, 1.0 equiv) was treated with anisole (0.1 mL) and TFA (2.0 mL) at 0 °C. The mixture was stirred for 2 h at room temperature, concentrated, and triturated in dry ether to give 140 mg of product as a white solid. This solid was taken up into CH_2Cl_2 (350 mL). To this solution was added TBTU (84 mg, 0.26 mmol, 1.5 equiv) and *N*,*N*-diisopropylethylamine (DIEA) (92 μL, 0.51 mmol, 3.0 equiv). The resulting mixture was stirred 48 h, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification by PTLC (silica gel, CH_2Cl_2:EtOAc: MeOH, 4:1:0.1) provided 50 mg (43%) of **33** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.40 (1H, m), 1.55 H, m), 1.68 (2H, m), 2.64 (1H, m), 2.81/2.86 (3H, s), 3.13 (1H, m), 3.59 (1H, m), 3.93 (2H, m), 4.18 (2H, m), 4.45 (1H, m), 5.09/5.11 (2H, s), 5.26/5.30 (2H, s), 7.34 (10H, m), 7.78 (4H, m). IR (NaCl, film): 3389, 3280, 2948, 1773, 1716, 1659, 1608, 1513, 1394, 1253, 1097, 1006, 910, 724 cm^{-1}. HRMS (FAB): calcd for ($C_{35}H_{36}N_6O_8$ + H) = 669.2672, found (M + H) = 669.2690.

***t*-BOC-Amine 34.** To a solution of **33** (100 mg, 0.15 mmol, 1.0 equiv) in CH_2Cl_2 (2.0 mL) was added 2 N methylamine/MeOH (2.0 mL) at 0 °C. The mixture was stirred for 10 min at 0 °C, concentrated, and separated by column chromatography (silica gel, methylene chloride:EtOAc:MeOH, 4:1:0.3) to give 84 mg of an oil. To a mixture of the oil obtained above in H_2O/dioxane (1 mL, 1:1) and Et_3N (217 μL, 1.5 mmol, 10 equiv) was added BOC-ON (106 mg, 0.45 mmol, 3.0 equiv). The mixture was stirred overnight at room temperature and extracted with EtOAc (2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by column chromatography (silica gel, eluted with CH_2Cl_2:MeOH, 9:1) to give 35 mg (38%) of **34** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.42 (9H, s), 1.47 (2H, m), 1.66 (2H, m), 2.63 (1H, m), 2.85 ($^1/_2$H, m), 2.90/2.93 (3H, s), 3.05 ($^1/_2$H, m), 3.52 (2H, m), 3.72 (1H, m), 3.92 (2H, m), 4.25 (1H, m), 5.11 (2H, s), 5.26/5.27 (2H, s), 7.35 (10H, m). IR (NaCl, film): 3386, 3289, 2934, 1716, 1652, 1609, 1507, 1456, 1366, 1252, 1175, 1095, 1006, 910, 734, 698 cm^{-1}. HRMS (FAB): calcd for ($C_{32}H_{42}N_6O_8$ + H) = 639.3142, found (M + H) = 639.3157.

Condensation Products 36 and 37. Compound **34** (33 mg, 0.052 mmol) was treated with anisole (0.1 mL) and TFA (1.0 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, concentrated, and triturated with dry ether to give 32 mg (99%) of the corresponding amine as an oil. A solution of the crude amine (30 mg, 0.046 mmol, 1.0 equiv), triethylamine (20 μL, 0.14 mmol, 3.0 equiv), and **35** (37 mg, 0.092 mmol, 2.0 equiv) in CH_2Cl_2 (1.0 mL) was stirred for 4 h at room temperature. The resulting mixture was poured into EtOAc, washed with brine, dried over anhydrous Na_2SO_4, filtered, concentrated, and separated on PTLC (silica gel, CH_2Cl_2:EtOAc, 7:3) to give 12 mg (30%) of **36** as a semi-solid and 13 mg (39%) of **37** as an oil. Data for **36**. ^{1}H NMR (300 MHz, CD_3OD): δ 1.42 (2H, m), 1.48 (9H, s), 1.67 (2H, m), 2.70 (1H, m), 2.83/2.89 (3H, s), 2.98 (1H, m), 3.62 (2H, m), 3.91 (3H, m), 4.67 (1H, m), 5.10 (2H, s), 5.14 (2H, s), 5.26 (2H, s), 7.35 (15H, m). IR (NaCl, film): 3384, 3293, 2932, 1716, 1722, 1651, 1491, 1254, 1144, 1008 cm^{-1}. HRMS (FAB): calcd for ($C_{42}H_{51}N_9O_{11}$ + H) = 858.3786; found (M + H) = 858.3763. Data

for **37**. ^{1}H NMR (300 MHz, CD_3OD): δ 1.45 (2H, m), 1.67 (2H, m), 2.73 (1H, m), 2.82 (1H, m), 2.85/2.91 (3H, s), 3.54 (1H, m), 3.70 (1H, m), 3.91 (3H, m), 4.44 (1H, t, J = 9 Hz), 5.10 (2H, m), 5.14 (2H, m), 5.27 (2H, m), 7.35 (15H, m). IR (NaCl, film): 3385, 3272, 2931, 1715, 1651, 1494, 1455, 1379, 1241, 1092 cm^{-1}. HRMS (FAB): calcd for ($C_{36}H_{41}N_7O_9$ + H) = 716.3044; found (M + H) = 716.3021.

[[[[5-[3-[(Aminoiminomethyl)amino]propyl]hexahydro-2(*S*/*R*),5-(*S*)-1-methyl-3,7-dioxo-1*H*-1,4-diazepin-2-yl]methyl]amino]iminomethyl]urea, TAN-1057C/D (3 and 4). Compound **36** (10 mg, 0.012 mmol) was treated with anisole (0.1 mL) and TFA (1.0 mL) at 0 °C. The mixture was stirred for 1 h at room temperature, concentrated, and purified by PTLC (silica gel, CH_2Cl_2:MeOH, 6:1) to give 6.0 mg of a semi-solid. To a solution of this substance (6 mg, 0.0066 mmol, 1.0 equiv) in MeOH (1.0 mL)/CH_2Cl_2 (0.5 mL) was added $PdCl_2$ (8.0 mg). After degassing with N_2, the reaction mixture was charged with H_2 (1 atm) and the mixture was hydrogenated for 10 min. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give the 2HCl salt of **TAN-1057 C/D** (1:1, 3 mg, ~100% yield) as an amorphous solid. ^{1}H NMR (300 MHz, DMSO-d_6 vs TMS): δ 1.51 (4H, m), 2.82 (3H, s), 2.70−2.90 (2H, m), 3.09 (2H, m), 3.43 (1H, m), 3.79 (2H, m), 4.66 ($^1/_2$H, m), 4.75 ($^1/_2$H, m), 7.26 (6H, m), 7.86 (1H, br), 8.06 (1H, br), 8.70 (2H, br), 9.15 (1H, br), 10.35 (1H, br). MS (FAB): calcd for ($C_{13}H_{25}N_9O_3$ + H) = 356.2; found (M + H) = 356.3, (M + 2H)$^{++}$ = 178.7. Upon standing in 0.1 M phosphate buffer at pH = 5, the mixture of **3** and **4** had reverted to a mixture of **1** and **2**.

***N*-(Benzyloxycarbonyl)ureido-*N*′-(*tert*-butyloxycarbonyl)-(*S*)-methylisothiourea (35).** To a solution of (*tert*-butyloxycarbonyl)-(*S*)-methylisothiourea[17,18] (1.69 g, 8.91 mmol, 1.0 equiv) in THF (40 mL) was added *N*-(benzyloxycarbonyl)isocyanate (1.90g, 10.73 mmol, 1.2 equiv). The resulting mixture was stirred for 10 min at room temperature and concentrated. Purification via column chromatography (silica gel, CH_2Cl_2:EtOAc:MeOH, 4:4:0.5) provided 3.25 g (100%) of **35** as a white solid: mp 120−3 °C. ^{1}H NMR (300 MHz, $CDCl_3$ vs TMS): δ 1.48 (9H, s), 2.32 (3H, s), 5.22 (2H, s), 7.37 (5H, m), 7.50 (1H, br, D_2O exchanged), 11.96 (1H, br, D_2O exchanged). IR (NaCl, film): 3270, 2979, 1747, 1664, 1570, 1476, 1370, 1275, 1207, 1138, 1072, 1056 cm^{-1}. Anal. Calcd for $C_{16}H_{21}N_3O_5S$: C, 52.30; H, 5.76; N, 11.44. Found: C, 52.15; H, 5.73; N, 11.38.

***N*-(Benzyloxycarbonyl)ureido-(*S*)-methylisothiourea (13).** Compound **35** (401 mg, 1.0 mmol) was treated with TFA (1.0 mL) and was stirred for 30 min at room temperature, evaporated to dryness, dried under reduced pressure for 2 h, and triturated with anhydrous Et_2O to give 400 mg of **13** as a solid. This crude product was carried on without further purification. ^{1}H NMR (300 MHz, CD_3OD): δ 2.64 (3H, m), 5.21 (2H, m), 7.31 (5H, m). IR (NaCl, CH_2Cl_2): 3252, 2959, 1790, 1682, 1203, 1137, 1025, 778, 722 cm$^{-1.}$

***N*-(Benzyloxycarbonyl)ureido-*N*′-(benzyloxycarbonyl)-(*S*)-methylisothiourea (21).** To a solution of (benzyloxycarbonyl)-(*S*)-methylisothiourea[18] (1.12 g, 5.0 mmol, 1.0 equiv) in THF (20 mL) was added (benzyloxycarbonyl)isocyanate (1.06 mL, 6.0 mmol, 1.2 equiv) at room temperature. After 20 min, the solvent was evaporated and the residue was triturated in anhydrous ethyl ether two times to afford 2.0 g (100%) of **21** as a white solid: mp 165−166 °C (recrystallized CH_2Cl_2/EtOAc). ^{1}HNMR (300 MHz, DMSO-d_6 vs TMS): δ 2.29 (3H, s), 5.15 (2H, s), 5.22 (2H, s), 7.40 (10H, m). IR (NaCl, film): 3226, 3159, 2925, 1749, 1715, 1558, 1469, 1261, 1205 cm^{-1}. Anal. Calcd for $C_{10}H_{12}N_2O_2S$: C, 56.85; H, 4.77; N, 10.47; S, 7.99. Found: C, 57.00; H, 4.97; N, 10.36; S, 7.76.

Acknowledgment. This paper is dedicated to Professor Yoshito Kishi on the occaision of his 60th birthday. This work was supported by the NSF and Microcide Pharmaceutical Co. We thank Takeda Chemical Co., Japan, for the generous gift of an authentic specimen of TAN-1057A/B.

Supporting Information Available: Experimental procedures for the synthesis of compounds **7**, **9**, **10**, and **12** plus HPLC data on the synthetic and natural TAN-1057A/B samples (5 pages). See any current masthead page for ordering and Internet access instructions.

JA972670J