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Synthesis and Evaluation of Antibacterial Activity of Bottromycins

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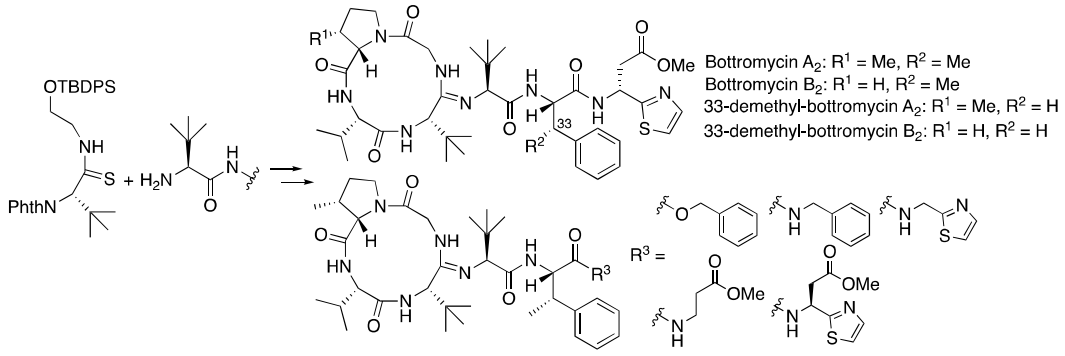


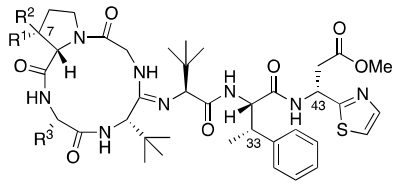
Abstract

Total synthesis of bottromycin A_2 can be accomplished through a diastereoselective Mannich reaction of a chiral sulfinamide, mercury-mediated intermolecular amidination, and cyclization of a constrained tetracyclic peptide. Exploitation of this process allowed the synthesis of several novel bottromycin analogs. The antimicrobial activity of these analogs was evaluated in vitro against Gram-positive bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *enterococci* (VRE). Structure activity relationships were explored taking into consideration the unique three-dimensional structure of the compounds. Notably, one of the new analogs devoid of a methyl ester, which is known to lower the in vivo efficacy of bottromycin, exhibited antibacterial bioactivity comparable to that of vancomycin.

Introduction

Antibiotic-resistant microbes, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE), represent a serious and increasing worldwide problem. Although *Staphylococcus aureus* and the *enterococci* are not always pathogenic, pathogenic strains often infect immunocompromised patients and can cause a variety of potentially life-threatening infections, such as sepsis and endocarditis. Since MRSA and VRE are multidrug resistant strains, infections with such bacteria can be almost impossible to treat. Therefore, the development of new antibacterial drugs, especially those with novel modes of action, is becoming of utmost importance if modern medicine is to overcome the growing antibacterial resistance problem.

Our research group has long been investigating the development of new medicines from microbial metabolite origins.[1] In the course of our research, we have been screening for new anti-MRSA and anti-VRE agents from our natural product library and previously discovered that bottromycin A_2 (**1**) possessed antibacterial activity against MRSA and VRE strains (Figure 1).[2]



Bottromycin A_2 (**1**): R^1 = Me, R^2 = H, R^3 = *i*-Pr
Bottromycin B_2 (**2**): R^1 = H, R^2 = H, R^3 = *i*-Pr
Bottromycin C_2 (**3**): R^1 = Me, R^2 = Me, R^3 = *i*-Pr
Bottromycin D (**4**): R^1 = Me, R^2 = H, R^3 = Me

Figure 1. Bottromycin A_2, B_2, C_2 and D.

Bottromycin was first isolated from the culture broth of *Streptomyces bottropensis* by Waisvisz and co-workers in 1957.[3] Several years later, Umezawa's group isolated bottromycin A_2 (**1**), B_2 (**2**), and C_2 (**3**) from a culture broth of *Streptomyces* No. 3668-L2.[4] Through a series of intensive structural studies the planar

structure of **1** was proposed.[5] The absolute structure of the compound was finally elucidated by our pioneering total synthesis of **1**.[2] Bottromycins are heptapeptides containing a cyclic tetrapeptide and a tripeptide side chain bounded to the skeletal ring via an amidine functional group. In addition, five of the seven amino acid residues of **1** are unusual amino acids, namely, (*R*)-3-(thiazol-2-yl)-β-alanine (Thia-β-Ala-OMe), *erythro*-β-methyl-L-phenylalanine, two L-*tert*-leucines (*t*-Leu) and *cis*-3-methyl-L-proline. Instead of the *cis*-3-methyl-L-proline amino acid residue of **1**, bottromycins B_2 (**2**) and C_2 (**3**) possess L-proline and 3,3-dimethyl-L-proline, respectively. In 2012, Bugni and co-workers reported the isolation of a new analog, bottromycin D (**4**), which contains an L-alanine residue instead of the L-valine of **1**.[6] Through our synthetic studies of bottromycins, we discovered that **1** has an unusual feature in that the three C-terminal residues fold back on the 12-membered cyclic skeleton made by the four *N*-terminal residues (Figure 2).[7] Due to the unique structure of this unprecedented macrocyclic amidine, the rare β-methylated amino acid residues, a terminal (43*R*)-Thia-β-Ala-OMe[8], and its three-dimensional structure, the biosynthesis of bottromycins has latterly been studied in depth.[9]

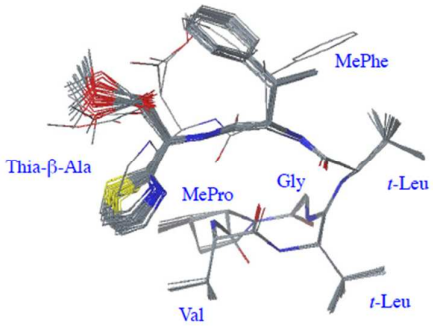


Figure 2. Three-dimensional structure of bottromycin A_2, which was determined in $CDCl_3$.[7]

The bottromycins, especially bottromycin A_2 (**1**), display potent antibacterial activity against Gram-positive bacteria[3,4] and mycoplasma.[10] In 1991, Yokoyama and co-workers reported the anti-MRSA activity of **1**.[11] We also found that bottromycin A_2 (**1**) possesses potent antibacterial activity against various

antimicrobial-resistant bacteria, such as MRSA and VRE, with minimum inhibitory concentration (MIC) values of less than 2.0 μg/mL.[2]

The mode of action of **1** has been identified, bottromycin inhibiting bacterial protein synthesis by interfering with the binding of aminoacyl-tRNA to the A site on the 50S ribosome. But it does not inhibit peptide bond formation and translocation steps.[12] This is a novel mechanism, different from that seen in commonly used antibiotics.

Although **1** has attracted much attention from bioorganic and organic chemists, structure activity relationship (SAR) studies have proven difficult due to the problem of chemical synthesis and derivatization of naturally-occurring **1**. Indeed, modification of the terminal methyl ester has only been reported by three groups, including ours.[13,14] In addition, several synthetic studies of **1** have been published,[15,16] but our total synthesis is the only fully successful method reported to date.[2]

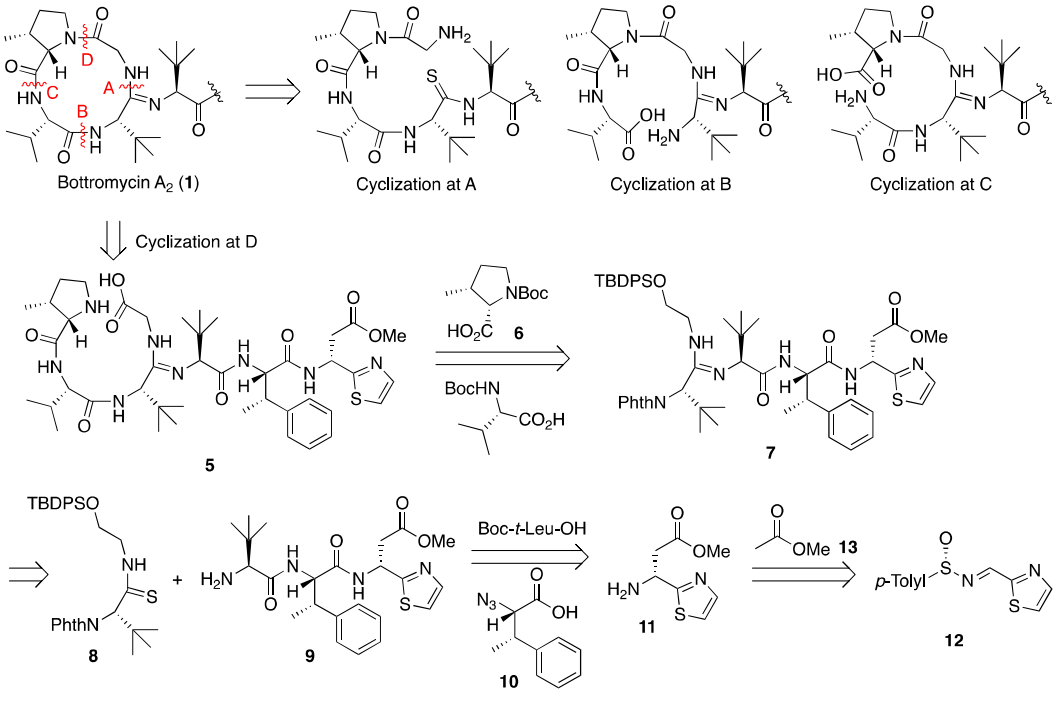
After we discovered the potent anti-MRSA and anti-VRE activity of **1**, we started efforts to develop a new antibacterial agent using **1** as a lead compound. In this manuscript, we describe the detailed total synthesis of bottromycin A_2 (**1**) and B_2 (**2**), together with creation of 33-demethyl analogs[17], along with bottromycin analogs devoid of Thia-β-Ala-OMe, to study SAR focussing on the unnatural amino acid residues. All the new analogs were subjected to an in vitro evaluation against Gram-positive bacteria, including some drug-resistant strains.

Results and Discussion

Bottromycins contain a cyclic tetrapeptide which is linked to a tripeptide side chain at the amidine. A cyclic tetrapeptide is one of the most difficult cyclic systems to prepare by organic chemical synthesis due to its highly constrained three-dimensional structure, in which the planarity of the amide bonds is usually twisted.[18] In addition, the central amidine acts as a nucleophile to attack the internal electrophilic moiety.

Therefore, the construction of a tetracyclic skeleton containing an amidine group is a major obstacle and only Kazmier's group has accomplished that construction to date.[16] There are four possible positions A-D to construct a cyclic tetrapeptide by intramolecular condensation (Scheme 1). We initially considered closing a tetrapeptide ring at the A bond, namely macroamidination. Even though numerous reaction conditions were examined, **1** was not detected.[19] We next examined cyclization at B. However, the cyclization failed due to the steric bulkiness.[19] Cyclization at C was also unsuccessful due to the undesired dominant cyclization of the amidine group.[19] We eventually decided to cyclize at D, via condensation of the *N*-terminal proline and the C-terminal glycine. The corresponding glycinol compound, which the glycine moiety of **5** was reduced, is identical with the degradation product,[2] thus the structure of the synthetic substrate **5** can be confirmed prior to total synthesis. In addition, it is not necessary to take into consideration the epimerization of the C-terminal amino acid.

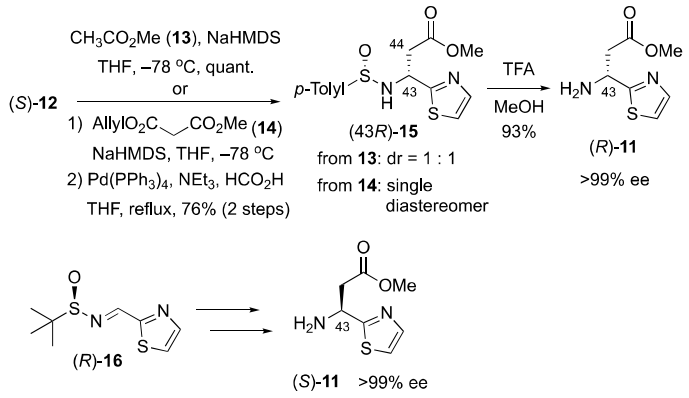
Scheme 1. Retrosynthetic analysis



The cyclization precursor **5** can be obtained through peptide synthesis from amidine **7** with Boc-Val-OH and *cis*-3-methyl-L-proline **6**. Compound **6** is prepared using Sasaki's method.[20] Sauvé and Rao have previously reported the synthesis of acyclic amidines from a thioamide and a primary amine, such as hydroxyl amine, hydrazine, and cyanamide, in the presence of $Hg(OAc)_2$ and NEt_3 in THF.[21] Therefore, we envisioned the construction of an amidine moiety from the thioamide **8**[2] and tripeptide **9** in the presence of an appropriate mercury reagent. The tripeptide **9** would be prepared by condensation with Boc-*t*-Leu-OH, (2*S*, 3*S*)-2-azido-3-phenylbutanoic acid **10**,[22] and (*R*)-Thia-β-Ala-OMe **11**. We anticipated that the compound **11** would arise via a Mannich reaction of Ellman chiral sulfinimine **12** with methyl acetate (**13**).[23]

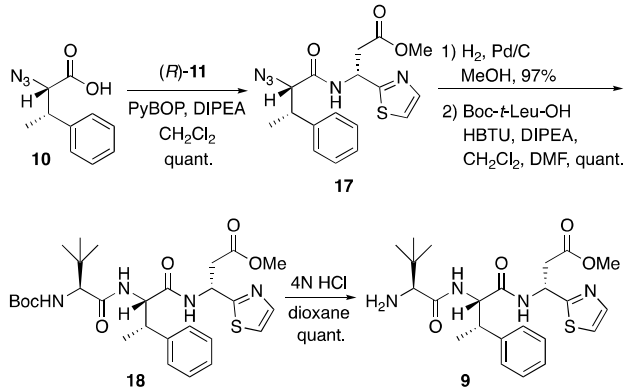
We synthesized the optically active (*R*)-Thia-β-Ala-OMe **11** using a diastereoselective Mannich reaction as shown in Scheme 2. Chiral sulfinimine (*S*)-**12**[2] was treated with methyl acetate (**13**) in the presence of sodium hexamethyldisilazane (NaHMDS) at –78 °C. The reaction proceeded smoothly, providing the corresponding Mannich reaction product **15** in excellent yield without diastereoselectivity, dr = 1 : 1. After examining several nucleophiles, we found that the more sterically bulky malonate-type nucleophiles produced good diastereoselectivity at the C-43 stereogenic center.[17] Indeed, treatment of chiral sulfinimine (*S*)-**12** with allyl methyl malonate **14** in the presence of NaHMDS in THF at –78 °C afforded the corresponding adduct in quantitative yield. Although the C-44 stereogenic center was not controlled, the C-43 stereogenic center was obtained only as the (*R*)-isomer. Subsequently, decarboxylation of the allyl ester was performed by treating with a catalytic amount of $Pd(PPh_3)_4$ in the presence of formic acid and NEt_3 in THF, to provide the methyl ester **15** in 76% yield as a single diastereomer.[24] The chiral auxiliary was removed by treatment with TFA in MeOH to provide (*R*)-Thia-β-Ala-OMe **11** with >99% ee. The optical purity was confirmed by chiral HPLC analysis. The enantiomer of Thia-β-Ala-OMe (*S*)-**11** could be prepared in the same manner from the *tert*-butyl sulfinimine **16**.

Scheme 2. Synthesis of optically active Thia-β-Ala-OMe (11)



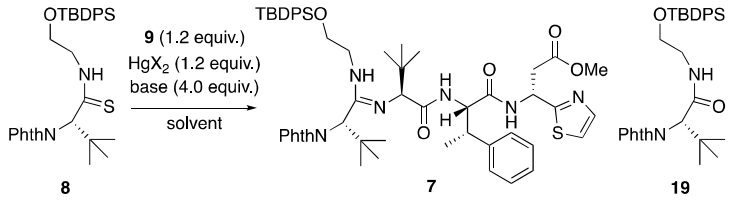
Thia-β-Ala-OMe (*R*)-**11** was then condensed with (2*S*, 3*S*)-2-azido-3-phenylbutanoic acid **10** using 1H-benzotriazol-1-yloxy-tri(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) as a condensation reagent to provide the dipeptide **17** in quantitative yield (Scheme 3). The azide group in **17** was reduced to an amine under hydrogenation conditions followed by condensation with Boc-*t*-Leu-OH provided the tripeptide **18** in quantitative yield. Removal of the Boc group under acidic conditions afforded an amine **9**.

Scheme 3. Synthesis of tripeptide 9



Next, we examined the intermolecular amidination reaction. Thioamide **8**[2] was treated with tripeptide **9** in the presence of NEt_3 in THF (Table 1, entry 1). The reaction did not proceed, even under reflux conditions. The reaction in the presence of $Hg(OAc)_2$ did not give the desired amidine product **7**, instead producing the corresponding amide **19** (entry 2).[21] Using $HgCl_2$ as a Lewis acid instead of $Hg(OAc)_2$ afforded **7** in 67% yield with **19** and the starting thioamide **8** in 8% and 18% yields, respectively (entry 3). The existence of the geometrical tautomer of amidine was suggested by its ^{1}H-NMR spectra. Thioamide **8** was consumed when the more electrophilic $Hg(OTf)_2$ was used as the Lewis acid, although the yield of **7** was not improved (entry 4). We next examined two other solvents, CH_2Cl_2 and MeCN, for the amidination reaction (entry 5, 6). It was found that the polar solvent, MeCN, provided the amidine **7** in a slightly higher yield, 77% (entry 6). Use of 2,6-lutidine as a base instead of NEt_3 improved the yield of **7** to 96% (entry 7).[25]

Table 1. Amidination of thioamide 8 with tripeptide 9



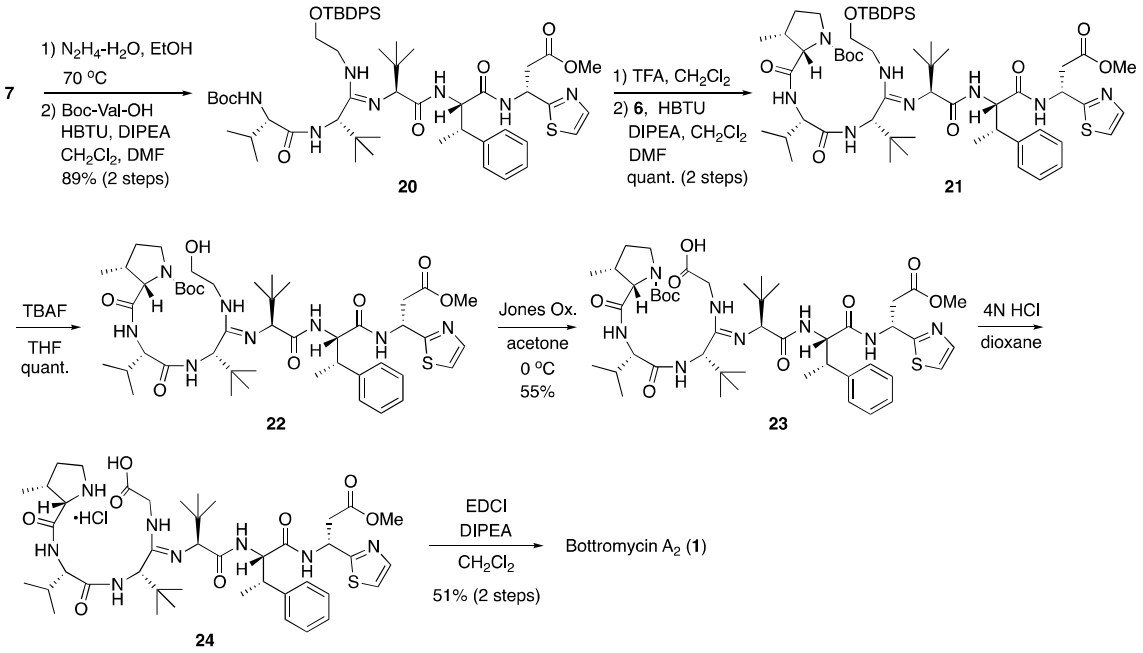
8 **7** **19**

Entry	HgX_2	Base	Solvent	Yield (%)[a]		
				7	**8**	**19**
1[b]	none	NEt_3	THF	No reaction		
2	$Hg(OAc)_2$	NEt_3	THF	n.d.[c]	n.d.[c]	100
3	$HgCl_2$	NEt_3	THF	67	18	8
4	$Hg(OTf)_2$	NEt_3	THF	70	n.d.[c]	29
5	$Hg(OTf)_2$	NEt_3	CH_2Cl_2	71	n.d.[c]	28
6	$Hg(OTf)_2$	NEt_3	MeCN	77	n.d.[c]	23
7	$Hg(OTf)_2$	2,6-lutidine	MeCN	96	n.d.[c]	trace

[a] Isolation yield. [b] Run at reflux condition. [c] n.d. = not detected.

Our next effort was directed toward the elongation of the peptide chain. The Phth group was removed by treatment with hydrazine in EtOH at 60 °C to provide the corresponding amine (Scheme 4). The amine was then condensed with Boc-Val-OH to afford the hexapeptide **20** in 89% yield (2 steps). If the aminoethanol part was oxidized to an ester prior to the removal of the Phth group, an undesired diketopiperazine was formed quickly. Removal of the Boc protecting group of **20**, followed by condensation with 3-methylproline **6**[20], provided the heptapeptide **21** in excellent yields. Thus, all amino acid residues constituted in **1** were connected. The TBDPS group of **21** was removed by treating with TBAF in THF to provide the primary alcohol **22** in 90% yield.[26] The oxidation of **22** to carboxylic acid **23** was problematic due to the nucleophilicity of the internal amidine. Indeed, all oxidation reactions through a generation of aldehyde did not provide the carboxylic acid **23**. The aldehyde intermediate was trapped by the internal amidine to provide the corresponding imidazole. Only Jones oxidation successfully provided the corresponding carboxylic acid **23** in moderate yield (55%). The Boc group on the proline part of **23** was removed by treating with 4N HCl in dioxane. Crude **24** was used for the final cyclization reaction. In this cyclization event, the nucleophilic intramolecular cyclization from amidine was also a problem. Through examination of numerous reaction conditions, we eventually found that the treatment of **24** with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and *i*-Pr_2NEt in CH_2Cl_2 provided bottromycin A_2 (**1**) in moderate yield.[27] All spectra data ($[\alpha]_D$, ^1H and ^{13}C NMR, IR and HRMS) were identical to those of the natural product.[28] Thus, we achieved the first total synthesis of bottromycin A_2 (**1**) and determined its absolute structure.

Scheme 4. Total syntheses of bottromycin A_2 (1)



To study the SAR, we focused on the influence of the unnatural amino acids, such as *cis*-3-methyl-L-proline, *erythro*-β-methyl-L-phenylalanine and Thia-β-Ala-OMe, on antibacterial activity (Figure 3). We chose bottromycin B_2 (**2**), a naturally occurring demethyl analog at C-7, C-33-demethyl-bottromycin A_2 (**25**) and C-33-demethyl-bottromycin B_2 (**26**)[17] as initial targets to study antibacterial impact (Scheme 5, 6).

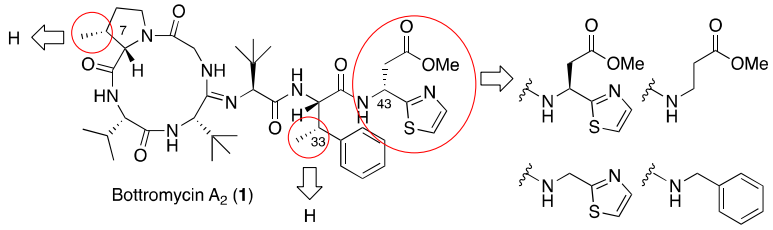
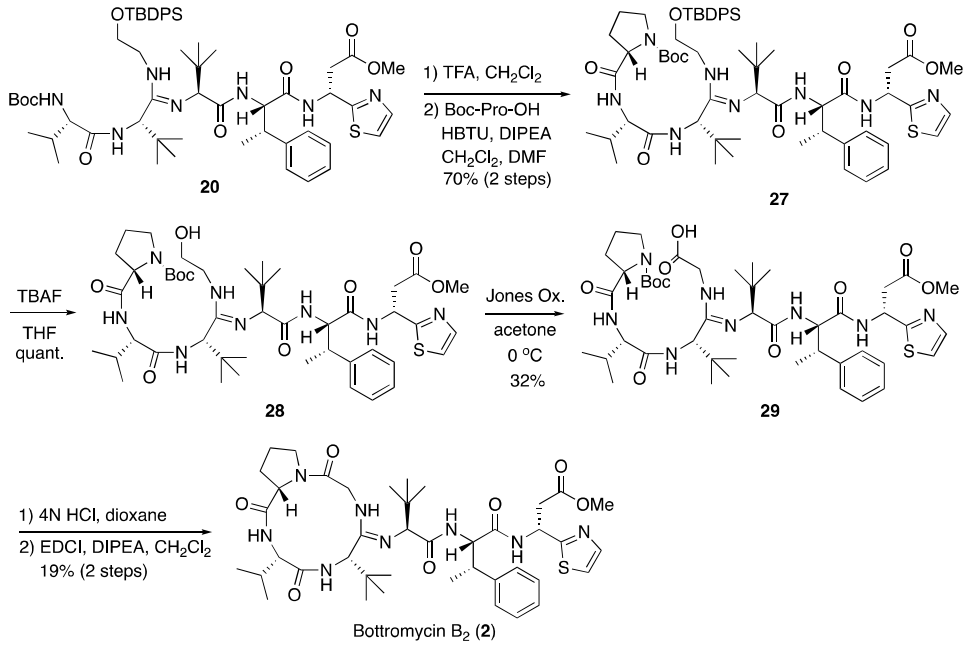


Figure 3. Envisioned new bottromycin analogs.

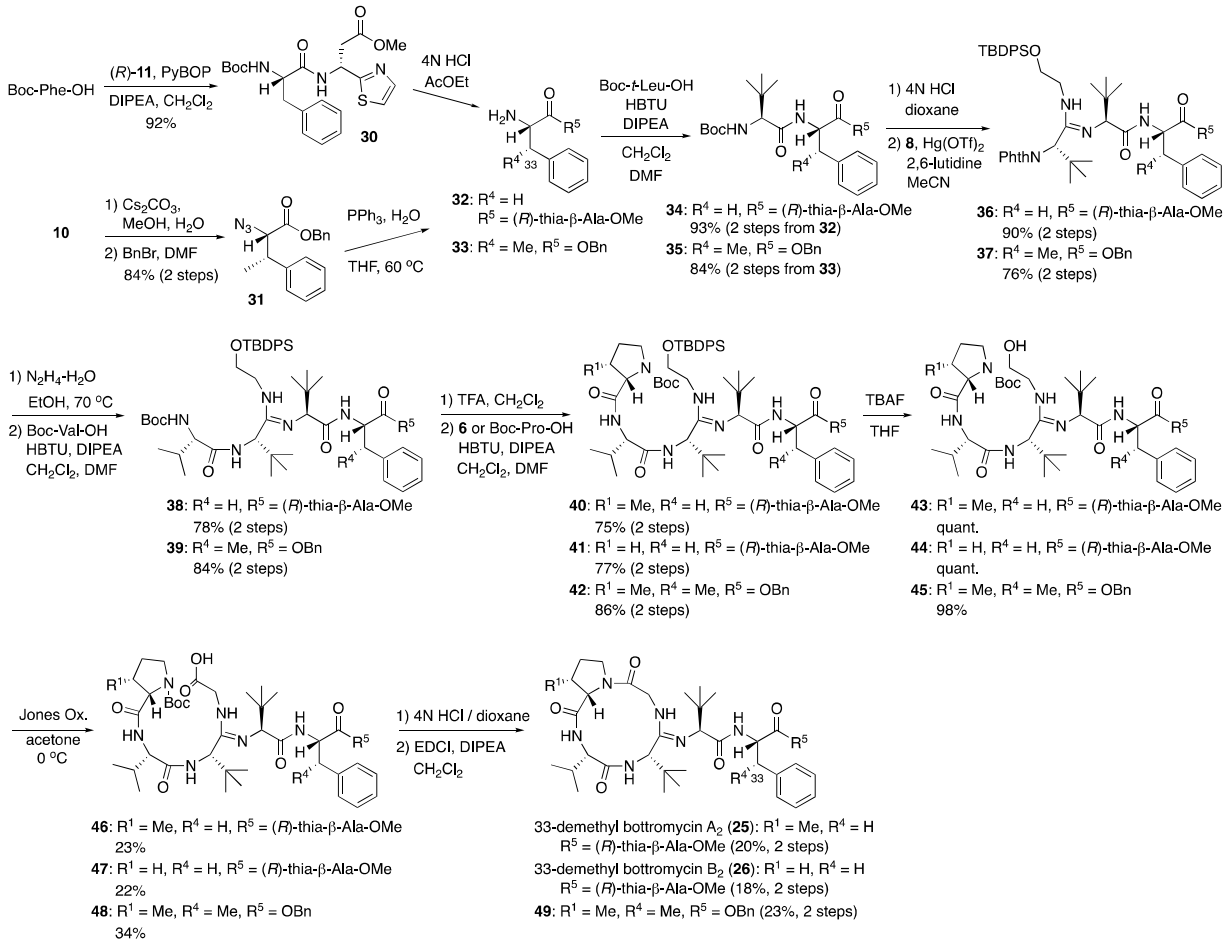
Condensation of pentapeptide **20** with Boc-Pro-OH using 1-[bis(dimethylamino)methylene]-1-*H*-benzotriazolium 3-oxide hexafluorophosphate (HBTU) as a condensation reagent provided heptapeptide **27** in good yield (Scheme 5). Removal of the TBDPS group followed by oxidation of the resulting alcohol provided carboxylic acid **29**. After the removal of the Boc group, the carboxylic acid **29** was cyclized using EDCI as a condensation reagent to provide bottromycin B_2 (**2**) in 19% yield (2 steps) in a similar manner as the synthesis of **1**. The ^{1}H NMR spectra of synthetic **2** was slightly inconsistent with the reported chemical shifts of naturally occurring **2**. This was believed to arise because the chemical shifts being influenced by salt contaminant and/or substrate concentration. All other spectral data, including the ^{13}C NMR spectra, IR spectra and melting point, were in good agreement with reported values.[4a, 5c, 29] Thus, taking into consideration our total synthesis of bottromycin A_2 and its ^{1}H NMR behavior[28], the synthetic compound was strongly suggested to be bottromycin B_2.

Scheme 5. Total synthesis of bottromycin B_2 (2)



The next focus was on the C-33-demethyl analogs of **1** and **2** (Scheme 6). The optically active Thia-β-Ala-OMe (*R*)-**11** was condensed with commercially available Boc-Phe-OH using PyBOP as a condensation reagent. The resultant dipeptide **30** was condensed with Boc-*t*-Leu-OH, after the removal of the Boc group under acidic conditions. Tripeptide **34** was treated with 4N HCl in dioxane, and then reacted with thioamide **8** in the presence of $Hg(OTf)_2$ and 2,6-lutidine in MeCN, affording the corresponding amidine **36** in 90% yield in 2 steps. The **36** was then condensed with Boc-Val-OH and 3-methylproline **6** to provide a heptapeptide **40** in good yield. The silyl ether **40** was transformed to carboxylic acid **46** through removal of the silyl group and Jones oxidation of the primary alcohol. Final cyclization was performed using EDCI as a condensation reagent to provide C-33-demethyl-bottromycin A_2 (**25**). C-33-Demethyl-bottromycin B_2 (**26**) was prepared from **38** with Boc-Pro-OH in a manner similar to the synthesis of the bottromycins. The ^1H NMR spectra of **25** and **26** were complicated because of the existence of conformers, as seen in bottromycin D.[6] This observation suggested the C-33 methyl group is important for the control of three-dimensional structure of the bottromycins.

Scheme 6. Synthesis of C-33-demethyl-bottromycin A_2 (25), C-33-demethyl-bottromycin B_2 (26) and a benzyl ester analog 49



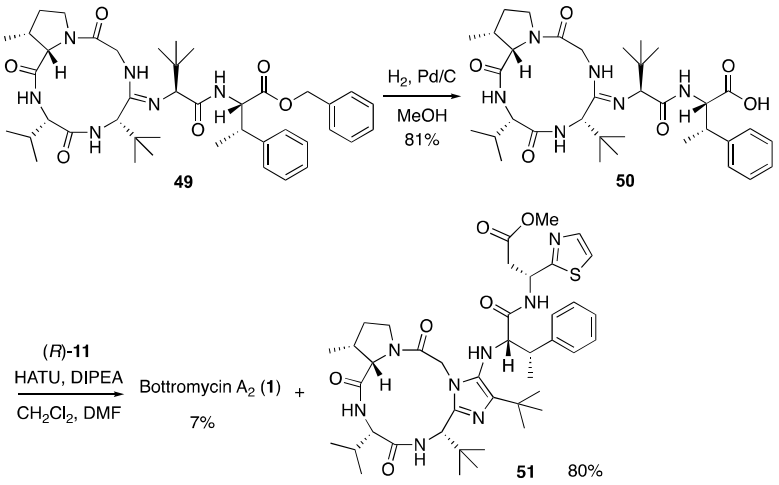
We next decided to focus on the SAR of Thia-β-Ala-OMe. The methyl ester moiety of Thia-β-Ala-OMe is known to affect the antibacterial activity both in vitro and in vivo.[14] Therefore, the efficient synthetic route to access bottromycin analogs containing various functions instead of Thia-β-Ala-OMe was useful for finding lead compounds for use in the development of new antibacterial agents. The synthesis of bottromycin analogs described above started from the condensation of (*R*)-**11**. Thus, the synthesis of bottromycin analogs devoid of Thia-β-Ala-OMe was a problematic undertaking. Consequently, we envisioned the introduction of a C-terminal residue at the end of the analog synthesis.

2-Azido-3-phenylbutanoic acid **10** was treated with Cs_2CO_3 followed by reaction with BnBr to provide the benzyl ester **31** in 84% yield (Scheme 6). The azide **31** was reduced under Staudinger reaction conditions to provide the corresponding primary amine **33** in good yield. The **33** was successfully converted to the benzyl ester analog **49** through an intermolecular amidination of the dipeptide **35** with thioamide **8** and cyclization of the hexapeptide **48**, as described in the above syntheses.

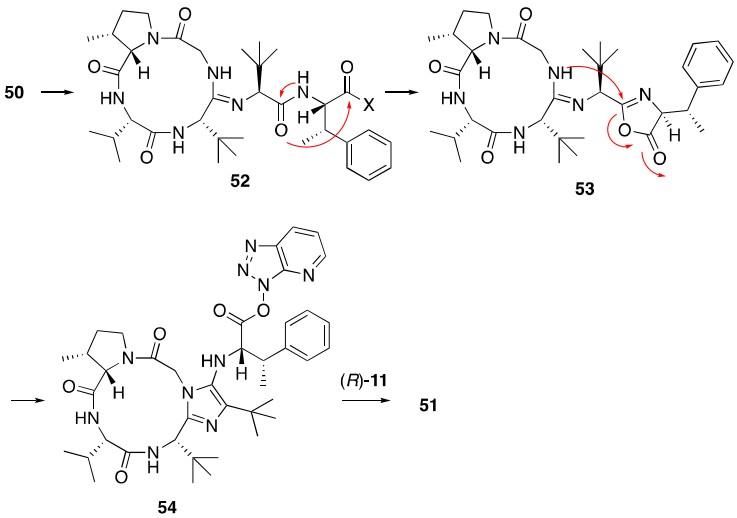
The benzyl ester in **49** could be removed under hydrogenation conditions in the presence of $Pd(OH)_2$ to afford the carboxylic acid analog **50**, which is a key intermediate in the preparation of bottromycin analogs containing various functions at the C-terminal instead of Thia-β-Ala-OMe, in 81% yield (Scheme 7). We set bottromycin A_2 (**1**) as the initial synthetic target to confirm the absolute structure of **50**. The carboxylic acid **50** was treated with (*R*)-**11** in the presence of 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) and DIPEA in a mixed solvent (CH_2Cl_2 : DMF = 3 : 1). The reaction proceeded smoothly, providing **1** together with an unexpected bicyclic product **51** in 7% and 80% yield, respectively. Although the yield of **1** was low, the absolute structure of **50** was confirmed as described. To study the reaction mechanism of the final condensation reaction, **1** was treated under the same condensation conditions. As expected, the reaction did not proceed and **1** was recovered quantitatively. Carboxylic acid **50** was treated under the same condensation condition without Thia-β-Ala-OMe (*R*)-**11**, the reaction being analysed by LC-MS. As a result, starting material **50** was completely consumed and a mass peak identical to the dehydrated activating ester **54** was detected as a major component after stirring for 3 min. This result suggested that the reaction mechanism was as described in Scheme 8. In this mechanism, the starting carboxylic acid **50** was converted to the activated ester **52**, which was trapped by the internal amide moiety to afford an azlactone **53**. The azlactone in **53** was also attacked by internal nucleophilic amidine, followed by isomerization to the more stable imidazole

to provide a dehydrated product **54**. The bicyclic product **51** was obtained following condensation with (*R*)-**11**.

Scheme 7. Total synthesis of bottromycin A_2 (1) from a benzyl ester 49



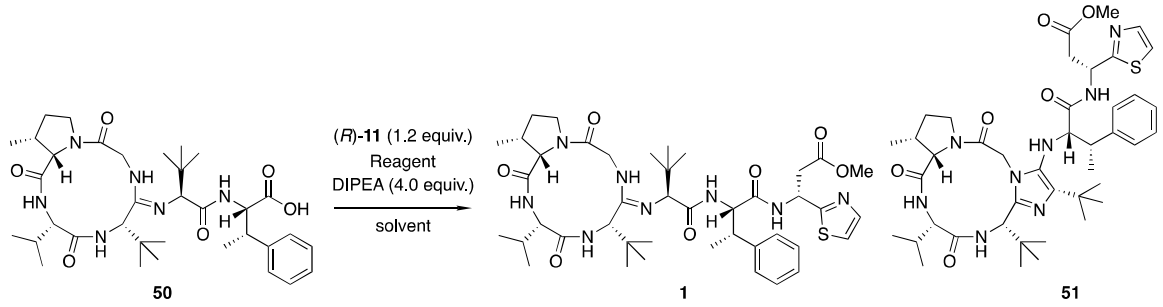
Scheme 8. Plausible reaction mechanism to generate bicyclic product 51



We examined various condensation conditions (Table 2). Use of carbodiimide-type reagents, such as DCC and EDCI, did not improve the yields (entry 2, 3). Common condensation reagents, such as PyBOP,

diphenylphosphoryl azide (DPPA), and propylphosphoric acid anhydride (T3P), were not effective and resulted in a complex mixture (entry 4-6). We next considered using a protic polar solvent, which may change the conformation of **50** to prevent its undesired cyclization. Thus, Kunishima's condensation condition,[30] reaction with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) in methanol, was tried (entry 7). Unfortunately, the yield of **1** was not improved and the bicyclic product **51** was obtained in 80% yield. We also tried azeotropic conditions in the presence of boric acid in toluene (entry 8).[31] The reaction did not proceed at all and the starting material **50** was recovered. We considered that preventing the formation of the azlactone **53** would increase the yield of **1**. Therefore, 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)dimethylaminomorpholino)]uranium hexafluorophosphate (COMU) was used as the condensation reagent (entry 9).[32] However, **1** was not detected at all. Eventually, we found that the use of an excess amounts of 1-hydroxy-7-azabenzotriazole (HOAt) with HATU was the most effective way of generating **1** in 22% yield together with **51** in 75% yield (entry 10).

Table 2. Condensation of 50 with (*R*)-11



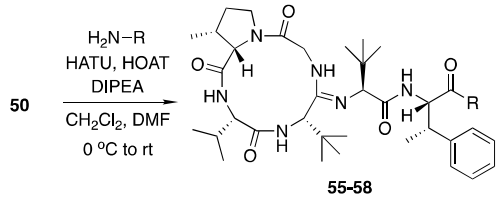
Entry	Reagent	Solvent	**1**[a]	**51**[a]
1	HATU	CH_2Cl_2, DMF	7%	80%
2	DCC, DMAP, CSA	CH_2Cl_2	No reaction	
3	EDCI	CH_2Cl_2	8%	Trace
4	PyBOP	CH_2Cl_2	n.d.[b]	34%

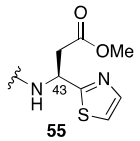
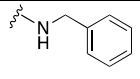
5	DPPA	CH_2Cl_2	Trace	33%
6	T3P	CH_2Cl_2, DMF	7%	Trace
7	DMT-MM	MeOH	7%	80%
8	$B(OH)_3$	Toluene, 120 °C	n.d.[b]	n.d.[b]
9	COMU	CH_2Cl_2, DMF	n.d.[b]	97%
10	HATU, HOAt	CH_2Cl_2, DMF	22%	75%

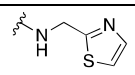
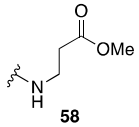
[a] Isolation yield. [b] n.d. = not detected.

Under the optimized reaction conditions, several appropriate amines were condensed with carboxylic acid **50** to clarify the requirement for Thia-β-Ala-OMe, *R*-configuration, C-acetyl group and thiazole group at C-43 for a preliminary SAR study (Table 3). Condensation with (*S*)-Thia-β-Ala-OMe **11**, which was prepared from (*R*)-*t*-butylsulfinimine **16** (Scheme 2), successfully provided the corresponding 43-*epi*-bottromycin A_2 (**55**), a postulated biosynthetic intermediate of **1**.[9] Other amines such as benzylamine, 2-aminomethylthiazole, and β-alanine methyl ester also provided the corresponding analogs **56-58**, in a manner similar to the synthesis of **1**. In each case, a bicyclic product was also generated.

Table 3. Condensation of 50 with amines



Entry	R	Yield (%)
1	 **55**	27
2	 **56**	42

3	 **57**	40
4	 **58**	32

All synthetic bottromycins and their analogs were evaluated in vitro for their antibacterial activity against five Gram-positive bacteria [*S. aureus* FDA209P, *S. aureus* Smith, MRSA70, MRSA 92-1191, VRE (*Enterococcus faecalis* NCTC12201)], using standard serial-dilution techniques specified by National Committee for Clinical Laboratory Standards (Table 4).[33] Synthetic bottromycin A_2 (**1**) displayed potent anti-MRSA and anti-VRE activity, as did naturally occurring **1** (MIC 1-2 μg/mL). Synthetic bottromycin B_2 (**2**), a natural demethyl analog at C-7, revealed slightly reduced antibacterial activity (MIC 4 μg/mL). The antibacterial activity of the C-33-demethyl analogs **25** and **26** was dramatically reduced (MIC >32 μg/mL). The C-33 methyl group is likely to be important in the controlling the three-dimensional structure, as suggested by the ^{1}H NMR described above (Figure 2). The bicyclic analog **51** and a synthetic intermediate[19], which did not contain a cyclic peptide, did not show notable antibacterial activity, probably due to the difference of three-dimensional conformation compared with **1**. A carboxylic acid analog **50**, which did not contain a C-terminal residue, similarly did not demonstrate any antibacterial properties. It has been reported that a Thia-β-Ala-OH analog is devoid of antibacterial activity.[13a,14] Therefore, it might be a decrease of hydrophobicity which led to the reduced antibacterial activity. Unexpectedly, the 43-*epi*-bottromycin A_2 (**55**) revealed comparable antibacterial activity to **1** (MIC 2 μg/mL). Surprisingly, although the benzylamine analog **56** showed comparable antibacterial activity to **1** (MIC 2 μg/mL against MRSA and VRE strains), the benzyl ester analog **49** was not very effective (MIC >32 μg/mL). The C-43-deacetyl analog **57** and C-43-dethiazolyl analog **58** revealed slightly weaker antibacterial activity than **1** (MIC 2-4 μg/mL for **57**, MIC 8 μg/mL for **58**). The promising antibacterial activity of the benzyl amine analog **56** and dethiazolyl analog **58** indicates that the

thiazole moiety of **1** is not essential for the anti-MRSA and anti-VRE activity. However, the aromatic substituent at C-43 seems to be responsible for potent antibacterial activity. All of the thiazolyl modified analogs likely adopted the folded structure, as described above, indicated by the characteristic upfield shift of the valine α-proton in ^{1}H NMR spectra.[2,7] SAR studies are summarized in figure 4. Changing substituents on proline, valine and Thia-β-Ala-OMe has a corresponding impact on the antibacterial activity of **1**. These residues locate on the same side of the three-dimensional structure (Figure 2). Therefore, the binding site of **1** to the 50S-ribosome may be on the side constituted of proline, valine and Thia-β-Ala-OMe. Two sterically bulky *t*-leucines on the opposite side may not bind to the key receptor but are also important in determining the folded three-dimensional structure.

Table 4. Minimum inhibitory concentrations (MICs) of the bottromycin derivatives (μg/mL)

	S. aureus FDA209P[a]	*S. aureus* Smith[a]	MRSA70[b]	MRSA 92-1191[b]	VRE NCTC12201[c]
1[d]	1	1	1	2	1
1[e]	1	1	2	2	1
2[e]	4	4	4	4	4
25	32	>32	>32	>32	32
26	>32	>32	>32	>32	>32
49	>32	32	32	>32	32
50	>32	>32	>32	>32	>32
51	>32	>32	>32	>32	>32
55	2	2	2	2	2
56	2	4	2	2	2
57	4	4	4	4	2

58	8	8	8	8	8
VCM[f]	1	2	0.5	2	>128

[a] *Staphylococcus aureus* FDA209P and Smith: susceptible strains. [b] MRSA70 and 92-1191: MRSA strains isolated from clinical patients. [c] *Enterococcus faecalis* NCTC12201: encoded by *van* A gene. [d] Naturally occurring compound. [e] Synthetic compounds. [f] Vancomycin.

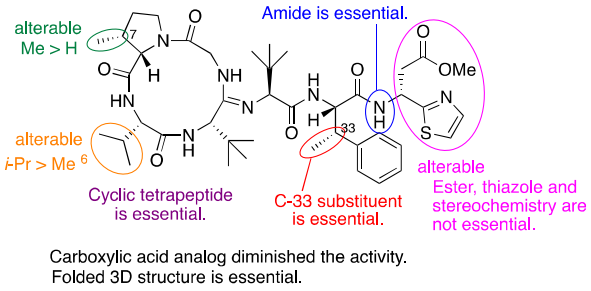


Figure 4. Summary of SAR of 1.

Conclusions

In conclusion, we achieved the first total synthesis of bottromycin A_2 (**1**) through a diastereoselective Mannich reaction, mercury-mediated amidination of thioamide and amine, and cyclization of the tetrapeptide including amidine. Based on this synthetic process, we synthesized bottromycin B_2 (**2**), C-33-demethyl-bottromycin A_2 (**25**), and C-33-demethyl-bottromycin B_2 (**26**). In addition, we developed a new synthetic route to introduce a C-terminal residue at the end of the analog synthesis. This method allowed us to prepare several bottromycin analogs containing several functions instead of Thia-β-Ala-OMe. All synthetic bottromycins and related analogs were subjected to in vitro evaluation of their antibacterial characteristics. It was found that the featured three-dimensional structure and C-33-methyl group of bottromycins was essential for their antibacterial activity. The Thia-β-Ala-OMe moiety could be replaced with other aromatic compounds without losing the antibacterial activity. Notably, a new analog devoid of the methyl ester, which is known to lower in vivo efficacy of bottromycins, showed comparable activity to that of

vancomycin. Therefore, the newly developed synthetic route will likely be important for further SAR studies focused on the C-terminal residue. These findings offer the possibility of accelerating the development of a new class of antibiotics, particularly with potential to combat multidrug-resistant bacteria.

EXPERIMENTAL SECTION

General Information

All reagents and solvents were purchased from commercial suppliers and were used without further purification. Flash chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical Co., Inc., silica gel 60N, spherical neutral, 0.040-0.050 mm). Preparative thin-layer chromatography (Prep. TLC) was carried out with pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254 0.25 or 0.50 mm, Merck KGaA). Nuclear magnetic resonance (NMR) spectra were determined using the JEOL JNM-ECA-500 (^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz)) spectrometer. The chemical shifts are expressed in ppm referenced to the residual solvent peaks of $CDCl_3$ (7.26 ppm, ^{1}H NMR) and CD_3OD (3.31 ppm, ^{1}H NMR) and coupling constant (*J* values) are given in Hertz. Chemical shifts for ^{13}C-NMR were reported in ppm relative to the center line at 77.0 ppm ($CDCl_3$) and 49.0 ppm (CD_3OD). All infrared (IR) spectra were measured on a Horiba FT-210 spectrometer and were reported in wavenumbers (cm^{-1}). Optical rotations were measured with a Jasco P1010 polarimeter. Melting points were measured using the micro melting point apparatus (Yanaco New Science Inc., MP-S3). High-resolution mass spectra (HRMS) were measured on a Micromass LCT spectrometer with a time-of flight (TOF) analyzer.

(R)-N-{N-tert-butoxycarbonyl-L-prolyl-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-tert-leucyl-(erythro-3-methyl-L-phenylalanyl)}-3-amino-3-(thiazol-2-yl)-propanoic acid methyl ester (27). To a solution of **20** (368 mg, 0.35 mmol) in CH_2Cl_2 (4.0 mL) was added TFA (2.5 mL) at 0 ^oC under an argon atmosphere. After being stirred at room temperature for 2 h, the reaction mixture was concentrated

under reduced pressure. The residue was dissolved in ethyl acetate, then basified by 30% aqueous NH_3 at 0 ^oC, after which the mixture was extracted with ethyl acetate (10 mL x 3). The combined organic extracts were washed with brine (30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was used for the next reaction without further purification. To a solution of crude mixture and Boc-Pro-OH (83.0 mg, 0.39 mmol) in CH_2Cl_2 (3.5 mL) was added DIPEA (183 μL, 1.05 mmol) and HBTU (172 mg, 0.46 mmol) in DMF (1.8 mL) at 0 ^oC under an argon atmosphere. After being stirred at room temperature for 10 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (20 mL), followed by washing with 10% aqueous citric acid (20 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (40 mL), then dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (Hexane/$CHCl_3$ = 2/1 to 2/3) to give heptapeptide **27** (282 mg, 70% in 2 steps) as white solid. The ^1H and ^{13}C NMR signals of **27** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **27**. The actual NMR spectra are shown in Supporting Information S-7 and S-8, respectively. IR (Diamond prism) 3278, 2954, 2870, 1651, 1512, 1396, 1257, 1165 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{62}H_{91}N_8O_9S_1Si]^+$ 1151.6399, found 1151.6406; $[\alpha]_D^{24}$ −70.3 (*c* 1.0, $CHCl_3$); mp 167 ^oC.

(R)-N-{N-tert-butoxycarbonyl-L-prolyl-L-valyl-[L-(2-hydroxyethyl)imino-tert-leucyl]-L-tert-leucyl -(erythro-3-methyl-L-phenylalanyl)}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (28). To a solution of **27** (261 mg, 0.226 mmol) in THF (2.3 mL) was added TBAF (1.0 M in THF, 454 μL, 0.454 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 3 h, the reaction mixture was quenched with saturated aqueous NH_4Cl (6.0 mL). The mixture was extracted with ethyl acetate (10 mL) and washed with brine (20 mL), then dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 9/1) to give **28** (205 mg, 99%) as a white solid. The ^1H and ^{13}C NMR signals of **28** were broad and complicated due to the

existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **28**. The actual NMR spectra are shown in Supporting Information S-9 and S-10, respectively. IR (Diamond prism) 3302, 2962, 2360, 1658, 1512, 1396, 1165, 702 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{46}H_{73}N_8O_9S_1]^+$ 913.5221, found 913.5220; $[\alpha]_D^{23}$ −67.5 (*c* 1.0, $CHCl_3$); mp 128-130 °C.

(R)-N-[N-tert-Butoxycarbonyl-L-prolyl-L-valyl-(L-carboxymethylimino-tert-leucyl)-L-tert-leucyl-(erythro-3-methyl-L-phenylalanyl)]-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (29). To a solution of **28** (50.0 mg, 54.8 μmol) in acetone (550 μL) was added dropwise Jones reagent (2.8 M, 824 μL) at 0 °C. After being stirred at the same temperature for 25 min, the reaction was quenched with 2-propanol (0.3 mL) and neutralized with saturated aqueous $NaHCO_3$ (8 mL). The resulting mixture was extracted with ethyl acetate (10 mL x 3) and washed with brine (10 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH/AcOH = 9:1:0.1, elution: $CHCl_3$/MeOH = 9/1) to give carboxylic acid **29** (16.3 mg, 32%) as a white solid: ^1H NMR (500 MHz, CD_3OD) δ 7.72 (d, *J* = 3.3 Hz, 1H), 7.51 (d, *J* = 3.3 Hz, 1H), 7.30-7.15 (4H), 7.10 (m, 1H), 5.77 (m, 1H), 4.87 (m, 1H), 4.57 (d, *J* = 11.0 Hz, 1H), 4.44 (m, 1H), 4.37 (brs, 1H), 4.27 (dd, *J* = 8.3, 3.3 Hz, 1H), 4.07 (s, 1H), 3.70 (s, 3H), 3.70-3.63 (1H), 3.55-3.43 (2H), 3.38 (m, 1H), 3.27 (dd, *J* = 16.4, 4.8 Hz, 1H), 3.20-3.10 (2H), 3.02 (dd, *J* = 16.4, 9.5 Hz, 1H), 2.24-1.77 (4H), 1.48-1.34 (6H), 1.26 (d, *J* = 6.5 Hz, 3H), 0.98 (s, 9H), 0.91 (s, 9H); The ^{13}C NMR signals of **29** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, ^{13}C NMR data assignments are not given for **29**. The actual ^{13}C-NMR spectra are shown in Supporting Information S-12. IR (Diamond prism) 3302, 2962, 2330, 1651, 1520, 1381, 1165, 756 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{46}H_{70}N_8NaO_{10}S_1]^+$ 949.4833, found 949.4831; $[\alpha]_D^{23}$ −55.4 (*c* 1.3, $CHCl_3$); mp 119 °C.

Bottromycin B₂ (2). A solution of **29** (21 mg, 22.6 μmol) in 4 M HCl/dioxane (1.0 mL) was stirred at room temperature under a nitrogen atmosphere for 1 h. The reaction mixture was concentrated under

reduced pressure and the residue was azeotroped with toluene to afford the crude product. To a solution of crude mixture in CH_2Cl_2 (12 mL) was added DIPEA (16 μL, 92 μmol) and EDCI•HCl (44 mg, 0.230 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 10 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL). The solution was washed with H_2O (5 mL) and brine (5 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH /30% aqueous NH_3 = 20/1/0.1, elution: $CHCl_3$/MeOH = 10/1) to give **2** (3.5 mg, 19%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 3.3 Hz, 1H), 7.47 (d, *J* = 6.0 Hz, 1H), 7.36 (d, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.5 Hz, 2H), 7.24 (d, *J* = 3.3 Hz, 1H), 7.21 (m, 1H), 7.10 (d, *J* = 10.5 Hz, 1H), 6.92 (d, *J* = 8.1 Hz, 1H), 6.73 (m, 1H), 5.61 (m, 1H), 4.87 (dd, *J* = 8.1, 4.2 Hz, 1H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.21 (dd, *J* = 9.5, 2.1 Hz, 1H), 4.05 (app t, 1H), 3.91 (s, 1H), 3.83 (dd, *J* = 12.2, 4.1 Hz, 1H), 3.68 (s, 3H), 3.75-3.59 (2H), 3.40 (m, 1H), 3.01 (dd, *J* = 16.9, 6.5 Hz, 1H), 2.86 (dd, *J* = 16.9, 5.5 Hz, 1H), 2.78 (m, 1H), 2.42 (m, 1H), 2.31 (m, 1H), 2.02-1.91 (2H), 1.34 (d, *J* = 7.1 Hz, 3H), 0.99 (s, 9H), 0.95 (s, 9H), 0.75 (d, *J* = 6.5 Hz, 3H), 0.73 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 172.6, 171.7, 171.1, 170.5, 169.6, 169.1, 157.2, 142.7, 141.3, 128.6 (2C), 128.1 (2C), 127.1, 119.9, 70.3, 68.4, 61.1, 57.5, 54.0, 52.1, 48.1, 48.0, 47.5, 41.6, 39.2, 35.4, 32.9 (2C), 27.6 (6C), 26.9, 22.8, 19.5 (2C), 15.4; IR (Diamond prism) 3286, 2962, 1735, 1643, 1504, 1365, 1226, 1164 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{41}H_{60}N_8NaO_7S_1]^+$ 831.4203, found 831.4214; $[\alpha]_D^{23}$ −57.7 (*c* 0.20, $CHCl_3$); mp 140-143 °C.

(R)-N-(N-tert-Butoxycarbonyl-phenylalanyl)-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (30). To a solution of Thia-β-Ala-OH (*R*)-**11** (760 mg, 4.09 mmol) and Boc-Phe-OH (1.08 g, 4.41 mmol) in CH_2Cl_2 (41 mL) was added DIPEA (2.20 mL, 12.3 mmol) and PyBOP (2.76 g, 5.31 mmol) at 0 °C under an argon atmosphere. After being stirred at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL). The solution was washed with 10%

aqueous citric acid (40 mL), saturated aqueous $NaHCO_3$ (50 mL), H_2O (50 mL) and brine (50 mL), then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc = 2/1 to 1/1) to give **30** (1.77 g, quant.) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (d, *J* = 3.5 Hz, 1H), 7.31-7.26 (2H), 7.25-7.19 (4H), 5.62 (dd, *J* = 5.2, 4.3 Hz, 1H), 4.41 (m, 1H), 3.59 (s, 3H), 3.15 (dd, *J* = 17.0, 4.3 Hz, 1H), 3.15 (m, 1H), 3.05 (dd, *J* = 14.0, 7.5 Hz, 1H), 2.67 (dd, *J* = 17.0, 5.2 Hz, 1H), 1.41 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 170.6, 170.0, 155.2, 142.4, 136.5, 129.3 (2C), 128.7 (2C), 127.0, 119.5, 80.2, 55.9, 51.8, 47.1, 38.4, 37.5, 28.2 (3C); IR (Diamond prism) 3262, 2946, 1743, 1697, 1658, 1519, 1241, 1164 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{21}H_{27}N_3NaO_5S_1]^+$ 456.1569, found 456.1599; $[\alpha]_D^{17}$ −18.8 (*c* 0.68, $CHCl_3$). mp: 110-112 °C.

(R)-N-[(N-tert-Butoxycarbonyl-L-tert-leucyl)-L-phenylalanyl]-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (34). To a solution of **30** (1.30 g, 3.00 mmol) in EtOAc (5.0 mL) was added 4 M HCl/EtOAc (15 mL) at room temperature. After being stirred at the same temperature for 30 min, the reaction mixture was concentrated under reduced pressure. The residue was azeotroped with toluene. The crude product was used for the next reaction without further purification. To a solution of crude product and Boc-*t*-Leu-OH (760 mg, 3.00 mmol) in CH_2Cl_2 (15 mL) was added DIPEA (1.00 mL, 5.98 mmol) and HBTU (1.46 g, 3.88 mmol) in DMF (15 mL) at 0 °C under an argon atomosphere. After being stirred at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (50 mL). The solution was washed with 10% aqueous citric acid (30 mL), saturated aqueous $NaHCO_3$ (30 mL), H_2O (30 mL) and brine (30 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel (hexane/EtOAc = 2/1) to give **34** (1.51 g, 93% in 2 steps) as a white amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 (d, *J* = 3.0 Hz, 1H), 7.29-7.24 (2H), 7.23-7.18 (4H), 7.11 (brd, *J* = 8.0 Hz, 1H), 6.48 (m, 1H), 5.59 (m, 1H), 5.19 (brd, *J* = 7.5 Hz, 1H), 4.69 (m, 1H), 3.84 (d, *J* = 8.5 Hz, 1H), 3.59 (s, 3H), 3.16 (dd, *J* =

13.5, 6.0 Hz, 1H), 3.07 (dd, *J* = 17.0, 4.8 Hz, 1H), 3.00 (dd, *J* = 13.5, 8.8 Hz, 1H), 2.63 (dd, *J* = 17.0, 5.5 Hz, 1H), 1.39 (s, 9H), 0.94 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.6, 170.0, 169.8, 155.8, 142.3, 136.3, 129.2 (2C), 128.7 (2C), 127.0, 119.5, 79.8, 62.6, 54.6, 51.8, 47.2, 38.5, 37.4, 34.4, 28.3 (3C), 26.5 (3C); IR (Diamond prism) 3286, 2962, 1735, 1643, 1504, 1365, 1226, 1164 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{27}H_{38}N_4NaO_6S_1]^+$ 569.2409, found 569.2425; $[\alpha]_D^{23}$ −23.1 (*c* 1.0, $CHCl_3$).

(R)-N-{[N-Phthaloyl-L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (36). To a solution of **34** (1.14 g, 2.09 mmol) in dioxane (3.0 mL) was added 4 M HCl/dioxane (15 mL) at room temperature. After being stirred at the same temperature for 1 h, the reaction mixture was diluted with EtOAc basified by 30% aqueous NH_3 at 0 °C. The mixture was extracted with EtOAc (10 mL x 3) washed with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was used for the next reaction without further purification. To a solution of crude product and thioamide **8** (970 mg, 1.74 mmol) in CH_3CN (20 mL) was added 2,6-lutidine (810 μL, 6.96 mmol) and $Hg(OTf)_2$ (1.03 g, 2.09 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 1 h, the reaction was quenched with 5% aqueous $Na_2S_2O_3$ (8.0 mL), filtered through a pad of Celite. The filter cake was washed with EtOAc (40 mL) and the filtrate was washed with 1 M aqueous HCl (20 mL), saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 100/1 to 60/1) to give **36** (1.74 g, 90%) as a colorless amorphous solid. The ^{1}H and ^{13}C NMR signals of **36** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **36**. The actual NMR spectra are shown in Supporting Information S-19 and S-20, respectively. IR (neat) 3382, 3070, 2958, 2860, 1712, 1497, 1384, 1110 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{54}H_{66}N_6NaO_7S_1Si]^+$ 993.4381, found 993.4366; $[\alpha]_D^{25}$ −96.2 (*c* 1.00, $CHCl_3$).

(R)-N-{N-tert-Butoxycarbonyl-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (38). To a solution of **36** (970 mg, 1.00 mmol) in EtOH (10 mL) was added N_2H_4•H_2O (145 μL, 3.00 mmol) at room temperature under an argon atmosphere. After being stirred at 70 °C for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL) then the solution was washed with 15% aqueous NH_3 (20 mL), H_2O (20 mL), and brine (20 mL) then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To a solution of the crude mixture (800 mg, 0.951 mmol) and Boc-Val-OH (227 mg, 1.04 mmol) in CH_2Cl_2 (5.0 mL) was added DIPEA (415 μL, 2.37 mmol) and HBTU (467 mg, 1.23 mmol) in DMF (5.0 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL). The solution was washed with 10% aqueous citric acid (20 mL), a saturated aqueous $NaHCO_3$ (20 mL) and brine (40 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 150/1 to 80/1) to give **38** (791 mg, 78% in 2 steps) as a white amorphous solid. The ^1H and ^{13}C NMR signals of **38** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **38**. The actual NMR spectra are shown in Supporting Information S-21 and S-22, respectively. IR (Diamond prism) 2954, 2861, 1735, 1635, 1504, 1241, 1164, 1103 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{56}H_{81}N_7NaO_8S_1Si]^+$ 1062.5534, found 1062.5515; $[\alpha]_D^{24}$ −52.3 (*c* 1.00, $CHCl_3$).

(R)-N-{N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-tert-leucyl-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (40). To a solution of **38** (320 mg, 0.308 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (300 μL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL), then neutralized with 10% aqueous NH_3 (10

mL) and the organic layer separated. The aqueous phase was extracted with EtOAc (10 mL x 3). The combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To a solution of the crude product and Boc-*cis*-3-methylproline **6** (78.0 mg, 0.339 mmol) in CH_2Cl_2 (1.5 mL) was added DIPEA (160 μL, 0.921 mmol) and HBTU (151 mg, 0.401 mmol) in DMF (1.5 mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (20 mL). The solution was washed with 10% aqueous citric acid (20 mL), a saturated aqueous $NaHCO_3$ (20 mL) and brine (40 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 100/1 to 60/1) to give **40** (265 mg, 75% in 2 steps) as a white solid. The ^1H and ^{13}C NMR signals of **40** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **40**. The actual NMR spectra are shown in Supporting Information S-23 and S-24, respectively. IR (Diamond prism) 3301, 2962, 2877, 1743, 1666, 1511, 1365, 1110 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{62}H_{90}N_8NaO_9S_1Si]^+$ 1173.6218, found 1173.6226; $[\alpha]_D^{25}$ −44.1 (*c* 1.00, $CHCl_3$); mp 90-92 °C.

(R)-N-{N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-[L-(2-hydroxyethyl)imino-tert-leucyl]-L-tert-leucyl-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (43). To a solution of **40** (330 mg, 0.286 mmol) in THF (3.0 mL) was added TBAF (1.0 M in THF, 573 μL, 0.573 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (6.0 mL). The mixture was extracted with EtOAc (10 mL) and washed with brine (20 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 80/1 to 10/1) to give **43** (243 mg, 93%) as white solid. The ^1H and ^{13}C NMR signals of **43** were broad and complicated due to the

existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **43**. The actual NMR spectra are shown in Supporting Information S-25 and S-26, respectively. IR (Diamond prism) 3278, 2962, 2884, 1735, 1650, 1519, 1365, 1218 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{46}H_{72}N_8Na_1O_9S_1]^+$ 935.5041, found 935.5017; $[\alpha]_D^{25}$ −55.5 (*c* 1.00, $CHCl_3$); mp 116-118 °C.

(R)-N-[N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-(L-carboxymethylimino-tert-leucyl)-L-tert-leucyl-L-phenylalanyl]-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (46). To a solution of **43** (30.0 mg, 32.8 μmol) in acetone (320 μL) was added dropwise Jones reagent (2.8 M, 123 μL) at 0 °C. After being stirred at the same temperature for 6 h, the reaction was quenched with 2-propanol (0.3 mL) and neutralized with saturated aqueous $NaHCO_3$ (8 mL). The filtrate was concentrated under reduced pressure, diluted with EtOAc (10 mL) and washed with brine (10 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 80/1, then $CHCl_3$/MeOH/30% aqueous NH_3 = 100/10/1, elution: $CHCl_3$/MeOH = 6/1) to give heptapeptide **46** (7.00 mg, 23%) as a white amorphous solid. The ^1H and ^{13}C NMR signals of **46** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **46**. The actual NMR spectra are shown in Supporting Information S-27 and S-28, respectively. IR (Diamond prism) 3301, 2962, 1735, 1650, 1527, 1373, 1218 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{46}H_{70}N_8NaO_{10}S_1]^+$ 949.4833, found 949.4843; $[\alpha]_D^{27}$ −39.8 (*c* 1.0, $CHCl_3$).

C-33-Demethyl-bottromycin A₂ (25). A solution of **46** (15.1 mg, 16.3 μmol) in 4 M HCl/dioxane (1.0 mL) was stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction mixture was concentrated under reduced pressure and the residue was washed with Et_2O (20 mL), azeotroped with toluene to afford the crude product. To a solution of the crude product in CH_2Cl_2 (8.0 mL) was added DIPEA (14.0 μL, 73.6 μmol) and EDCI•HCl (31.6 mg, 0.184 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 4 h, the reaction mixture was concentrated under reduced pressure.

The residue was dissolved in EtOAc (5 mL). The solution was washed with H_2O (5 mL) and brine (5 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH /30% aqueous NH_3 = 20/1/0.1, elution: $CHCl_3$/MeOH = 10/1) to give C-33-demethyl-bottromycin A_2 (**25**) (3.0 mg, 20%) as a white amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (d, *J* = 2.5 Hz, 1H), 7.43-7.17 (8H), 6.95 (d, *J* = 10.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 5.48 (m, 1H), 4.93 (m, 1H), 4.63 (d, *J* = 10.0 Hz, 1H), 4.06 (d, *J* = 8.0 Hz, 1H), 3.98 (brm, 1H), 3.91 (s, 1H), 3.82 (dd, *J* = 12.5, 4.5 Hz, 1H), 3.76 (m, 1H), 3.65 (s, 3H), 3.56 (m, 1H), 3.51 (m, 1H), 3.33 (dd, *J* = 14.0, 4.5 Hz, 1H), 3.12 (dd, *J* = 17.0, 6.0 Hz, 1H), 2.86-2.76 (3H), 2.59 (m, 1H), 2.52 (m, 1H), 2.02 (m, 1H), 1.68 (m, 1H), 1.14 (d, *J* = 6.5 Hz, 3H), 0.99 (s, 9H), 0.98 (s, 9H), 0.81 (d, *J* = 6.5 Hz, 3H), 0.75 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3, 172.5 (2C), 171.0, 170.9, 169.9, 169.1, 157.0, 142.6, 136.7, 129.7 (2C), 128.7 (2C), 126.9, 119.8, 70.0, 68.8, 65.7, 54.3, 53.0, 52.0, 48.1, 48.0, 47.1, 39.5, 38.5, 38.2, 35.6, 32.9, 30.3, 27.6 (6C), 26.9, 20.0, 19.6, 15.6; IR (Diamond prism) 3286, 2962, 1735, 1643, 1504, 1365, 1226, 1164 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{41}H_{60}N_8NaO_7S_1]^+$ 831.4203, found 831.4197; $[\alpha]_D^{25}$ −16.6 (*c* 0.13, $CHCl_3$).

(R)-N-{N-tert-Butoxycarbonyl-L-prolyl-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-tert-leucyl-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (41). To a solution of **38** (220 mg, 0.211 mmol) in CH_2Cl_2 (1.2 mL) was added TFA (210 μL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL), then neutralized with 10% aqueous NH_3 (10 mL) and the organic layer separated. The aqueous phase was extracted with EtOAc (10 mL x 3). The combined organic extracts were washed with brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To a solution of crude product and Boc-Pro-OH (55.0 mg, 0.255 mmol) in CH_2Cl_2 (1.0 mL) was added DIPEA (111 μL, 0.638 mmol) and HBTU (105 mg, 0.276 mmol) in DMF (1.0

mL) at 0 °C under an argon atmosphere. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL). The solution was washed with 10% aqueous citric acid (10 mL), a saturated aqueous $NaHCO_3$ (10 mL) and brine (20 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 100/1 to 60/1) to give **41** (186 mg, 77% in 2 steps) as a white solid. The ^1H and ^{13}C NMR signals of **41** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **41**. The actual NMR spectra are shown in Supporting Information S-31 and S-32, respectively. IR (Diamond prism) 3316, 2962, 2869, 1735, 1650, 1511, 1388, 1110 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{61}H_{88}N_8NaO_9S_1Si]^+$ 1159.6062, found 1159.6064; $[\alpha]_D^{25}$ −85.6 (*c* 1.00, $CHCl_3$); mp 80-81 °C.

(R)-N-{N-tert-Butoxycarbonyl-L-prolyl-L-valyl-[L-(2-hydroxyethyl)imino-tert-leucyl]-L-tert-leucyl-L-phenylalanyl}-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (44). To a solution of **41** (110 mg, 96.7 μmol) in THF (1.0 mL) was added TBAF (1.0 M in THF, 191 μL, 191 μmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (3.0 mL). The mixture was extracted with EtOAc (5 mL), washed with brine (5 mL), then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 60/1 to 20/1) to give **44** (79.1 mg, 91%) as a white amorphous solid. The ^1H and ^{13}C NMR signals of **44** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **44**. The actual NMR spectra are shown in Supporting Information S-33 and S-34, respectively. IR (Diamond prism) 3286, 2962, 2877, 1735, 1650, 1519, 1365, 1218 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{45}H_{70}N_8Na_1O_9S_1]^+$ 921.4884, found 921.4882; $[\alpha]_D^{24}$ −85.1 (*c* 1.00, $CHCl_3$).

(R)-N-[N-tert-Butoxycarbonyl-L-prolyl-L-valyl-(L-carboxymethylimino-tert-leucyl)-L-tert-leucyl-

L-phenylalanyl]-3-amino-3-(thiazol-2-yl)propanoic acid methyl ester (47). To a solution of **44** (30.0 mg, 33.3 μmol) in acetone (333 μL) was added dropwise Jones reagent (2.8 M, 125 μL) at 0 °C. After being stirred at the same temperature for 6 h, the reaction was quenched with 2-propanol (0.3 mL) and neutralized with saturated aqueous $NaHCO_3$ (8 mL). The filtrate was concentrated under reduced pressure, diluted with EtOAc (10 mL) and washed with brine (10 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 80/1, then $CHCl_3$/MeOH/30% aqueous NH_3 = 100/10/1, elution: $CHCl_3$/MeOH = 6/1) to give heptapeptide **47** (6.60 mg, 22%) as a white amorphous solid. The ^1H and ^{13}C NMR signals of **47** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **47**. The actual NMR spectra are shown in Supporting Information S-35 and S-36, respectively. IR (Diamond prism) 2962, 1735, 1650, 1519, 1373, 1218, 1164, 1118 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+2Na]^{2+}$ calcd for $[C_{45}H_{67}N_8Na_2O_{10}S_1]^{2+}$ 478.7248, found 478.7251; $[\alpha]_D^{25}$ −39.7 (*c* 1.0, $CHCl_3$).

C-33-Demethyl-bottromycin B₂ (26). A solution of **47** (19.1 mg, 20.9 μmol) in 4 M HCl/dioxane (1.0 mL) was stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction mixture was concentrated under reduced pressure and the residue was washed with Et_2O (20 mL), azeotroped with toluene to afford the crude product. To a solution of the crude product in CH_2Cl_2 (10 mL) was added DIPEA (15.0 μL, 83.6 μmol) and EDCI•HCl (35.8 mg, 0.209 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL). The solution was washed with H_2O (5 mL) and brine (5 mL) then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH /30% aqueous NH_3 = 20/1/0.1, elution: $CHCl_3$/MeOH = 10/1) to give C-33-demethyl-bottromycin B_2 (**26**) (2.8 mg, 18%) as white amorphous solid: ^1H NMR (500 MHz, $CDCl_3$) δ 7.65 (d, *J* = 3.0 Hz, 1H), 7.30-7.05 (9H), 6.80 (d, *J* = 8.0 Hz, 1H), 5.56 (m, 1H), 4.86 (m, 1H), 4.63 (d, *J* =

10.5 Hz, 1H), 4.22 (m, 1H), 4.10 (brm, 1H), 3.92 (s, 1H), 3.82 (dd, *J* = 12.8, 4.8 Hz, 1H), 3.71 (m, 1H), 3.66 (m, 1H), 3.65 (s, 3H), 3.46 (m, 1H), 3.38 (dd, *J* = 14.0, 4.0 Hz, 1H), 3.08 (dd, *J* = 17.3, 5.8 Hz, 1H), 2.82-2.74 (3H), 2.59 (m, 1H), 2.29 (m, 1H), 2.08-1.93 (2H), 1.63 (m, 1H), 1.00 (s, 9H), 0.98 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 3H), 0.76 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.5, 172.6, 172.2, 171.0, 170.4, 170.2, 169.0, 157.0, 142.3, 136.7, 129.8 (2C), 128.7 (2C), 126.8, 119.9, 70.0, 68.1, 61.1, 54.3, 53.4, 53.1, 48.1, 47.9, 47.4, 39.3, 38.5, 35.6, 32.9, 32.8, 27.6 (6C), 27.0, 22.8, 19.5, 19.4; IR (Diamond prism) 2962, 1735, 1650, 1519, 1373, 1218, 1164, 1118 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{40}H_{58}N_8NaO_7S_1]^+$ 817.4047, found 817.4047; $[\alpha]_D^{24}$ −23.7 (*c* 0.07, $CHCl_3$).

(2S, 3S)-2-Azido-3-phenylbutylic acid benzyl ester (31). To a stirred solution of **10** (8.48 g, 41.3 mmol) in MeOH (230 mL) was added Cs_2CO_3 (8.08 g, 24.8 mmol) in H_2O (50 mL) at room temperature. After being stirred for 30 min, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in DMF (150 mL) and BnBr (5.15 mL, 43.4 mmol) was added to the solution at room temperature. After being stirred for 2 h, the reaction mixture was diluted with Et_2O (200 mL) then the solution was washed with H_2O (100 mL x 3) and brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 20/1 to 10/1) to afford the benzyl ester **31** (11.0 g, 90%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.20 (10 H), 5.22 (d, *J* = 12.0 Hz, 1H), 5.19 (d, *J* = 12.0 Hz, 1H), 3.99 (d, *J* = 8.0 Hz, 1H), 3.31 (dq, *J* = 8.0, 7.0 Hz, 1H), 1.31 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 141.0, 134.9, 128.6 (7C), 127.6 (2C), 127.3, 67.6, 67.5, 41.8, 18.5; IR (Diamond prism) 3062 2969, 2105, 1735, 1257, 1172, 971, 748, 694 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{17}H_{17}N_3Na_1O_2]^+$ 318.1219, found 318.1213; $[\alpha]_D^{27}$ –57.8 (*c* 2.0, $CHCl_3$).

erythro-N-(N-tert-Butoxycarbonyl-L-tert-leucyl)-3-methyl-L-phenylalanine benzyl ester (35). To a stirred solution of **31** (12.2 g, 41.1 mmol) in THF (410 mL) was added H_2O (6 mL) and Ph_3P (18.4 g, 64.9

mmol) at room temperature under a nitrogen atmosphere. After being stirred at 60 °C for 4 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL). The solution was washed with brine (150 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To a solution of the crude product and Boc-*t*-Leu-OH (10.5 g, 42.3 mmol) in CH_2Cl_2 (410 mL) was added DIPEA (21.5 mL, 123.4 mmol) and PyBOP (27.8 g, 53.5 mmol) at 0 °C under a nitrogen atmosphere. After being stirred for 30 min, the reaction mixture was allowed to reach room temperature and stirred for 15 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (300 mL). The solution was washed with 10% aqueous citric acid (100 mL), saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 10/1 to 5/1) to afford the dipeptide **35** (16.7 g, 84%) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38-7.33 (3H), 7.31-7.20 (5H), 7.10-7.05 (2H), 5.83 (d, *J* = 8.5 Hz, 1H), 5.17 (d, *J* = 9.5 Hz, 1H), 5.14 (d, *J* = 12.0 Hz, 1H), 5.04 (d, *J* = 12.0 Hz, 1H), 4.89 (dd, *J* = 8.5, 4.5 Hz, 1H), 3.75 (d, *J* = 9.0 Hz, 1H), 3.43 (m, 1H), 1.44 (s, 9H), 1.30 (d, *J* = 6.5 Hz, 3H), 0.90 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 170.7, 155.8, 140.1, 135.0, 128.8 (2C), 128.7 (2C), 128.6 (2C), 128.5, 127.6 (2C), 127.4, 79.6, 67.2, 62.7, 57.0, 41.9, 34.2, 28.3 (3C), 26.4 (3C), 17.3; IR (Diamond prism) 2970, 1712, 1666, 1496, 1365, 1249, 1165, 748, 694 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{28}H_{38}N_2Na_1O_5]^+$ 505.2678, found 505.2674; $[\alpha]_D^{27}$ +4.0 (*c* 2.0, $CHCl_3$).

***erythro*-N-[N-Phthaloyl-L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-3-methyl-L-phenylalanine benzyl ester (37).** To a solution of **35** (1.98 g, 4.10 mmol) in 4 M HCl/dioxane (31 mL) was stirred at room temperature under a nitrogen atmosphere. After being stirred at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (30 mL) and neutralized with 30% aqueous NH_3 the organic layer was then separated. The aqueous phase was extracted

with EtOAc (20 mL x 3). The combined organic extracts were washed with brine (60 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product (1.62 g). To a solution of the crude product and thioamide **8** (1.97 g, 3.52 mmol) in CH_3CN (33 mL) was added 2,6-lutidine (1.6 mL, 14.1 mmol) and $Hg(OTf)_2$ (2.12 g, 8.24 mmol) at room temperature under a nitrogen atmosphere. After being stirred at room temperature for 1 h, the reaction was quenched with 5% aqueous $Na_2S_2O_3$ (10 mL) and filtered through a pad of Celite. The filter cake was washed with EtOAc (40 mL) and the filtrate was washed with 1 M aqueous HCl (20 mL), saturated aqueous $NaHCO_3$ (30 mL) and brine (30 mL). The organic solution was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 5/1 to 2/1) to afford an amidine **37** (2.41 g, 76%) as an amorphous solid. The ^1H and ^{13}C NMR signals of **37** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **37**. The actual NMR spectra are shown in Supporting Information S-43 and S-44, respectively. IR (Diamond prism) 2960, 1711, 1493, 1327, 1111, 1084, 750, 700, 613, 532, 503 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{55}H_{67}N_4O_6Si_1]^+$ 907.4830, found 907.4815; $[\alpha]_D^{27}$ –44.6 (*c* 0.5, $CHCl_3$).

erythro-N-{N-tert-Butoxycarbonyl-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-3-methyl-L-phenylalanine benzyl ester (39). To a solution of **37** (38.7 g, 42.7 mmol) in EtOH (213 mL) was added $N_2H_4 \bullet H_2O$ (8.3 mL, 171 mmol) at room temperature under a nitrogen atmosphere. After being stirred at 70 °C for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) then the solution was washed with 10% aqueous NH_3 (50 mL x 2), brine (80 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To a solution of crude product and Boc-Val-OH (12.1 g, 55.5 mmol) in CH_2Cl_2 (424 mL) was added DIPEA (26.0 mL, 149 mmol) and HBTU (24.3 g, 58.0 mmol) in DMF (150 mL) at 0 °C under a nitrogen atmosphere. After being stirred for 30 min, the reaction mixture was allowed to reach room temperature and stirred for 15 h. The reaction mixture

was concentrated under reduced pressure. The residue was dissolved in Et_2O (300 mL). The solution was washed with 10% aqueous citric acid (200 mL), a saturated aqueous $NaHCO_3$ (200 mL) and brine (200 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$ = 100%, then hexane/EtOAc = 2/1) to afford the pentapeptide **39** (16.7 g, 84%) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, *J* = 9.0 Hz, 1H), 7.64-7.60 (4H), 7.45-7.35 (11H), 7.22-7.17 (3H), 7.09-7.05 (2H), 6.89 (d, *J* = 9.5 Hz, 1H), 5.28 (d, *J* = 12.0 Hz, 1H), 5.18 (d, *J* = 12.0 Hz, 1H), 5.18 (m, 1H), 5.04 (dd, *J* = 9.5, 4.3 Hz, 1H), 4.90 (m, 1H), 4.59 (d, *J* = 9.5 Hz, 1H), 4.10 (dd, *J* = 8.8, 6.8 Hz, 1H), 3.70 (ddd, *J* = 10.8, 6.0, 3.8 Hz, 1H), 3.64 (ddd, *J* = 10.8, 7.0, 3.3 Hz, 1H), 3.56 (s, 1H), 3.49 (m, 1H), 3.40 (m, 1H), 3.26 (m, 1H), 1.78 (m, 1H), 1.39 (s, 9H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.06 (s, 9H), 1.06 (s, 9H), 0.98 (s, 9H), 0.76 (d, *J* = 7.0 Hz, 3H), 0.73 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 172.8, 171.6, 157.4, 155.5, 140.7, 135.5 (4C), 134.9, 133.0, 132.8, 129.9 (2C), 128.7 (2C), 128.6 (2C), 128.5 (3C), 127.8 (4C), 127.6 (2C), 127.1, 79.1, 69.8, 67.7, 63.1, 59.2, 56.5, 54.7, 45.4, 42.3, 36.4, 35.8 (2C), 31.6, 28.3 (3C), 27.9 (3C), 26.9 (3C), 26.8 (3C), 19.2, 19.1, 17.9; IR (Diamond prism) 3370, 3062, 2962, 1720, 1666, 1650, 1496, 1365, 1241, 1172, 1103, 748, 701, 501 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{57}H_{82}N_5O_7Si_1]^+$ 976.5984, found 976.5963; $[\alpha]_D^{27}$ –59.1 (*c* 1.0, $CHCl_3$).

N-{N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-[L-(2-tert-butyldiphenylsilyloxyethyl)imino-tert-leucyl]-L-tert-leucyl}-erythro-3-methyl-L-phenylalanine benzyl ester (42). To a solution of **39** (977 mg, 1.00 mmol) in CH_2Cl_2 (8 mL) was added TFA (5.3 mL) at 0 °C under a nitrogen atmosphere. After being stirred at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (15 mL) then neutralized with 10% aqueous NH_3 (10 mL) and the organic layer separated. The aqueous phase was extracted with EtOAc (20 mL x 3). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude product (874 mg). To a solution of the crude product and **6** (274 mg, 1.20 mmol) in CH_2Cl_2 (8.7 mL)

was added DIPEA (0.5 mL, 2.99 mmol) and HBTU (567 mg, 1.50 mmol) in DMF (4.4 mL) at 0 °C under a nitrogen atmosphere. After being stirred for 30 min, the reaction mixture was allowed to reach room temperature and stirred for 15 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in Et_2O (20 mL). The solution was washed with 10% aqueous citric acid (15 mL), saturated aqueous $NaHCO_3$ (15 mL) and brine (15 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH = 200/1, then hexane/EtOAc = 3.5/1) to afford the hexapeptide **42** (935 mg, 86%) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89-7.78 (m, 1H), 7.65-7.58 (4H), 7.47-7.32 (11H), 7.23-7.16 (3H), 7.05 (2H), 7.00 (brd, *J* = 9.7 Hz, 1H), 6.43 (m, 1H), 5.33-5.22 (2H), 5.03 (m, 1H), 4.89 (brs, 1H), 4.59 (m, 1H), 4.51 (m, 1H), 4.03 (m, 1H), 3.69 (m, 1H), 3.65-3.53 (3H), 3.52 (m, 1H), 3.40 (m, 1H), 3.34 (m, 1H), 3.26 (m, 1H), 2.38 (m, 1H), 1.90 (m, 1H), 1.82-1.63 (2H), 1.43-1.34 (3H), 1.37 (s, 9H), 1.07 (brs, 9H), 1.06 (brs, 9H), 0.97 (brd, *J* = 6.0 Hz, 3H), 0.93 (brs, 9H), 0.72 (d, *J* = 7.0 Hz, 3H), 0.67 (m, 3H); The ^{13}C NMR signals of **42** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, ^{13}C NMR data assignments are not given for **42**. The actual ^{13}C NMR spectra are shown in Supporting Information S-48. IR (Diamond prism) 3363, 3062, 2962, 1651, 1496, 1389, 1111, 740, 701, 501 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{63}H_{91}N_6O_8Si_1]^+$ 1087.6668, found 1087.6651; $[\alpha]_D^{27}$ –61.8 (*c* 0.5, $CHCl_3$).

N-{N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-[L-(2-hydroxyethyl)imino-tert-leucyl]-L-tert-leucyl}-erythro-3-methyl-L-phenylalanine benzyl ester (45). To a stirred solution of **42** (527 mg, 485 μmol) in THF (3.5 mL) was added TBAF (1.0 M in THF, 0.9 mL, 921 μmol) at 0 °C under a nitrogen atmosphere. After being stirred at room temperature for 3 h, the reaction was quenched with saturated aqueous NH_4Cl (7 mL) and extracted with EtOAc (20 mL x 3). The combined organic extracts were washed with brine (20 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel ($CHCl_3$/MeOH/30% aqueous NH_3 = 10/1/0.1) to afford the alcohol

45 (405 mg, 98%) as an amorphous solid. The ^{1}H and ^{13}C NMR signals of **45** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **45**. The actual NMR spectra are shown in Supporting Information S-49 and S-50, respectively. IR (Diamond prism) 2956, 1710, 1678, 1493, 1327, 1107, 1084, 746, 700, 611, 501 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{47}H_{73}N_6O_8]^+$ 849.5490, found 849.5477; $[\alpha]_D^{27}$ –64.5 (*c* 0.5, $CHCl_3$).

N-[N-tert-Butoxycarbonyl-(cis-3-methyl-L-prolyl)-L-valyl-(L-carboxymethylimino-tert-leucyl)-L-tert-leucyl]-erythro-3-methyl-L-phenylalanine benzyl ester (48). To a stirred solution of **45** (47.3 mg, 55.7 μmol) in acetone (0.55 mL) was added dropwise Jones reagent (2.8 M, 0.3 mL) at 0 °C. After being stirred at 0 °C for 3 h, the reaction was quenched with 2-propanol (0.5 mL) and neutralized with saturated aqueous $NaHCO_3$ (5 mL). The mixture was filtered through a pad of Celite and washed with EtOAc. The filtrate was extracted with EtOAc (20 mL x 3). The combined organic extracts were washed with brine (20 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to afford **48** (405 mg, 34%) as an amorphous solid. The ^{1}H and ^{13}C NMR signals of **48** were broad and complicated due to the existence of amidine tautomers and rotamers. Therefore, NMR data assignments are not given for **48**. The actual NMR spectra are shown in Supporting Information S-51 and S-52, respectively. IR (Diamond prism) 2968, 2881, 1741, 1652, 1514, 1462, 1379, 1221, 1165, 995, 910, 750. 698, 592. 458 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{47}H_{71}N_6O_9]^+$ 863.5283, found 863.5261; $[\alpha]_D^{25}$ +13.8 (*c* 3.0, $CHCl_3$).

Bottromycin A_2 benzyl ester analog (49). A solution of **48** (17.1 mg, 19.8 μmol) in 4 M HCl/dioxane (0.5 mL) was stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction mixture was concentrated under reduced pressure to afford the crude product. To a solution of the crude product in CH_2Cl_2 (9.9 mL) was added DIPEA (13.8 μL, 79.2 μmol) at room temperature under a nitrogen atmosphere. After being stirred for 10 min, EDCI•HCl (37.9 mg, 198 μmol) was added to the mixture and

stirring continued for 15 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL). The solution was washed with H_2O (3 mL x 2) and brine (3 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH = 10/1) to afford bottromycin benzylester analog **49** (3.0 mg, 23%) as an amorphous solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42-7.28 (5H), 7.25-7.15 (2H), 7.14-7.06 (2H), 6.99 (brd, *J* = 7.5 Hz, 2H), 6.93 (d, *J* = 11.0 Hz, 1H), 6.60 (brd, *J* = 9.5 Hz, 1H), 5.11 (s, 2H), 5.11 (m, 1H), 4.58 (d, *J* = 10.0 Hz, 1H), 3.95 (1H, s), 3.85 (m, 1H), 3.70 (m, 1H), 3.64 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.51 (m, 1H), 3.47 (d, *J* = 8.0 Hz, 1H), 3.38 (m, 1H), 3.26 (brdd, *J* = 12.0, 2.5 Hz, 1H), 2.82 (m, 1H), 2.53 (dd, *J* = 11.5, 5.5 Hz, 1H), 2.36 (m, 1H), 1.99 (m, 1H), 1.64 (m, 1H), 1.38 (d, *J* = 7.0 Hz, 3H), 1.10 (d, *J* = 6.0 Hz, 3H), 1.01 (s, 9H), 0.97 (s, 9H), 0.85 (d, *J* = 6.5 Hz, 3H), 0.80 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.9 (2C), 172.5, 170.6, 168.9, 156.8, 140.5, 134.7, 128.8 (2C), 128.6, 128.1 (2C), 127.9 (2C), 127.7 (2C), 126.8, 70.7, 69.2, 66.8, 65.6, 55.8, 53.6, 47.9, 46.8, 41.9, 38.3, 35.2, 32.9, 30.1, 27.9 (3C), 27.7 (3C), 26.9, 20.1, 19.5, 17.4, 15.5; IR (Diamond prism) 3356, 2962, 1736, 1643, 1497, 1365, 1203, 1141, 1026, 748, 701, 540, 455 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{42}H_{60}N_6Na_1O_6]^+$ 767.4472, found 767.4460; $[\alpha]_D^{27}$ –15.4 (*c* 0.5, $CHCl_3$).

Bottromycin A$_2$ carboxylic acid analog (50). To a stirred solution of **49** (3.0 mg, 4.03 μmol) in MeOH (2.4 mL) was added 10% Pd/C (6.0 mg, Pd: 5.66 μmol) at room temperature under a nitrogen atmosphere. Then the reaction mixture was stirred under hydrogen atmosphere for 2.5 h. The reaction mixture was filtered through a pad of Celite and washed with MeOH. The filtrate was concentrated under reduced pressure to afford the crude product **50** (1.8 mg, 81%). Crude product was used for the next reaction without any purification. Spectra data were collected after purification by preparative TLC ($CHCl_3$/MeOH =8/1) to obtain pure carboxylic acid as a colorless solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (m, 1H), 7.39-7.00 (6H), 6.64 (m, 1H), 4.65-4.34 (2H), 4.00-3.13 (8H), 2.95 (m, 1H), 2.72-2.23 (2H), 1.99 (m, 1H), 1.64 (m, 1H), 1.35 (m, 3H), 1.20 (m, 3H), 1.05-0.86 (18H), 0.64 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.5, 175.5, 174.6,

174.0, 168.7, 156.5, 142.5, 128.4 (2C), 127.8 (2C), 126.7, 71.7, 68.6, 65.4, 60.5, 53.2, 47.7, 46.9, 41.2, 38.4, 34.5, 32.9, 30.3, 27.6 (3C), 27.2 (3C), 27.0, 20.3, 19.6 (2C), 15.6; IR (Diamond prism) 2964, 1740, 1643, 1510, 1454, 1369, 1219, 633, 519, 420 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{35}H_{55}N_6O_6]^+$ 655.4183, found 655.4174; $[\alpha]_D^{27}$ –3.3 (*c* 0.5, $CHCl_3$); mp 196-197 °C.

General procedure for the synthesis of bottromycin A₂ (1) and analogs (55-58) from carboxylic acid 50. To a solution of **50** (1 equiv.) and an amine (20 equiv.) in CH_2Cl_2 (8 μM) were added DIPEA (4 equiv.) and a solution of HATU (3 equiv.) and HOAt (30 equiv.) in DMF (8 μM) at 0 □ under a nitrogen atmosphere. After being stirred for 15 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (10 mL). The solution was washed with H_2O (7 mL) and brine (7 mL), then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$/MeOH/30% NH_3 aq. = 20/1/0.1) to afford the condensation product.

Bicyclic product 51: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 3.0 Hz, 1H), 7.34-7.24 (3H), 7.22 (m, 2H), 7.15 (d, *J* = 3.0 Hz, 1H), 6.57 (brd, *J* = 8.0 Hz, 1H), 6.05 (d, *J* = 10.5 Hz, 1H), 5.38 (m, 1H), 5.13 (d, *J* = 16.5 Hz, 1H), 4.70 (dd, *J* = 9.5, 7.5 Hz, 1H), 4.44 (d, *J* = 9.5 Hz, 1H), 4.25 (d, *J* = 16.5 Hz, 1H), 4.25 (m, 1H), 3.83 (d, *J* = 10.5 Hz, 1H), 3.77-3.66 (2H), 3.59 (s, 3H), 3.39-3.26 (2H), 3.02 (dd, *J* = 17.0, 4.0 Hz, 1H), 2.58 (m, 1H), 2.52 (dd, *J* = 17.0, 6.0 Hz, 1H), 2.14 (m, 1H), 2.00 (m, 1H), 1.52 (m, 1H), 1.38 (d, *J* = 7.5 Hz, 3H), 1.26 (s, 9H), 1.15 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 6.0 Hz, 3H), 0.89 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7, 171.1, 170.9, 169.9, 169.3, 167.5, 142.7, 142.4, 140.0, 139.7, 129.5, 128.6 (2C), 127.7 (2C), 126.9, 119.4, 70.1, 65.7, 55.1, 54.8, 51.9, 47.2, 46.4, 43.5, 42.9, 37.9, 37.8, 37.5, 32.4, 30.5, 30.3 (3C), 29.3, 26.1 (3C), 20.3, 18.6, 14.8, 14.6; IR (KBr) 2958, 2364, 1651, 1508, 1446, 1122, 1018, 768, 660 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+H]^+$ calcd for $[C_{42}H_{61}N_8O_6S_1]^+$ 805.4435, found 805.4404; $[\alpha]_D^{23}$ +50.4 (*c* 0.6, $CHCl_3$); mp 116 °C.

***43-epi-Bottromycin A_2 (55)*:** Yield: 27%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (brm, 1H), 7.70 (d, *J* = 3.0 Hz, 1H), 7.32 (d, *J* = 2.9 Hz, 1H), 7.28-7.14 (5H), 7.01 (d, *J* = 10.8 Hz, 1H), 6.66 (d, *J* = 9.0 Hz, 1H), 5.71 (ddd, *J* = 8.9, 8.9, 4.4 Hz, 1H), 4.94 (brm, 1H), 4.63 (d, *J* = 10.8 Hz, 1H), 4.01 (d, *J* = 7.5 Hz, 1H), 3.99 (brm, 1H), 3.84-3.75 (2H), 3.78 (s, 1H), 3.64-3.53 (2H), 3.59 (s. 3H), 3.44 (m, 1H), 3.22-3.14 (2H), 2.99-2.87 (2H), 2.55 (m, 1H), 2.04 (m, 1H), 1.71 (m, 1H), 1.31 (d, *J* = 7.5 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 5.5 Hz, 3H), 0.99 (s, 9H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.70 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2, 172.7, 171.6, 171.3, 171.0, 169.9, 169.0, 157.1, 142.5, 141.8, 128.5 (2C), 128.1 (2C), 126.9, 119.5, 70.1, 69.3, 65.9, 57.1, 53.5, 51.7, 48.1, 47.3, 47.1, 42.1, 38.6, 38.0, 35.1, 33.0, 30.3, 27.6 (3C), 27.4 (3C), 27.2, 20.0, 19.3, 18.1, 15.7; IR (Diamond prism) 3263, 2964, 1732, 1689, 1641, 1537, 1498, 1435, 1369, 1254, 1225, 1178, 754, 705, 459 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{42}H_{62}N_8Na_1O_7S_1]^+$ 845.4360, found 845.4343; $[\alpha]_D^{26}$ –29.0 (*c* 0.3, $CHCl_3$); mp 125-126 °C.

***Bottromycin A_2 benzylamide analog (56)*:** Yield: 42%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.88 (brm, 1H), 7.33 (m, 2H), 7.30-7.19 (7H), 7.16 (m, 1H), 7.02 (brd, *J* = 10.5 Hz, 1H), 6.87 (d, *J* = 9.5 Hz, 1H), 5.06 (brm, 1H), 4.60 (d, *J* = 11.0 Hz, 1H), 4.45 (dd, *J* = 14.5, 6.5 Hz, 1H), 4.29 (dd, *J* = 14.5, 4.3 Hz, 1H), 4.17 (m, 1H), 3.93 (m, 1H), 3.88 (s, 1H), 3.80-3.68 (3H), 3.57 (ddd, *J* = 11.5, 11.5, 7.0 Hz, 1H), 3.28 (m, 1H), 2.85 (m, 1H), 2.57-2.46 (2H), 2.02 (ddd, *J* = 12.5, 6.3, 6.3 Hz, 1H), 1.69 (m, 1H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.16 (d, *J* = 7.0 Hz, 3H), 0.97 (s, 9H), 0.90 (s, 9H), 0.81 (d, *J* = 6.5 Hz, 3H), 0.74 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.4, 172.7, 172.3, 171.4, 169.1, 157.1, 141.6, 137.5, 128.8 (2C), 128.4 (2C), 128.2 (2C), 127.7, 127.5 (2C), 126.9, 70.4, 69.3, 65.6, 57.3, 53.8, 48.1, 47.0, 43.3, 42.4, 38.5, 35.2, 32.9, 30.3, 27.6 (6C), 26.9, 20.1, 19.5, 16.0, 15.6; IR (Diamond prism) 3263, 3072, 2964, 1687, 1641, 1545, 1498, 1460, 1254, 1032, 754, 704, 598, 759, 403 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{42}H_{61}N_7Na_1O_5]^+$ 766.4632, found 766.4628; $[\alpha]_D^{26}$ –3.8 (*c* 0.3, $CHCl_3$); mp 112-114 °C.

***Bottromycin A_2 deacethyl analog (57)*:** Yield: 40%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.12 (brs, 1H), 7.71 (d, *J* = 3.5 Hz, 1H), 7.36 (m, 1H), 7.22 (m, 1H), 7.18-7.05 (6H), 6.68 (d, *J* = 8.0 Hz, 1H), 4.88 (m, 1H), 4.78 (dd, *J* = 15.5, 5.5 Hz, 1H), 4.57 (m, 1H), 4.55 (d, *J* = 10.0 Hz, 1H), 3.98 (brs, 1H), 3.92 (s, 1H), 3.91 (d, *J* = 8.5 Hz, 1H), 3.79 (m, 1H), 3.74 (dd, *J* = 12.0, 5.0 Hz, 1H), 3.63-3.53 (2H), 3.06 (m, 1H), 2.93-2.80 (2H), 2.56 (m, 1H), 2.04 (m, 1H), 1.70 (m, 1H), 1.37 (d, *J* = 7.5 Hz, 3H), 1.21 (d, *J* = 6.5 Hz, 3H), 0.99 (s, 9H), 0.93 (s, 9H), 0.91 (d, *J* = 6.0 Hz, 3H), 0.86 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.9, 174.2, 173.4, 172.4, 171.5, 169.0, 156.8, 142.7, 142.2, 128.4 (2C), 128.2 (2C), 126.5, 119.7, 70.6, 68.9, 65.5, 57.8, 54.1, 48.0, 47.1, 40.8, 40.6, 38.4, 35.3, 33.0, 30.5, 27.9 (3C), 27.8 (3C), 27.0, 20.1, 19.7, 17.1, 15.9; IR (KBr) 3273, 3072, 2964, 2879, 1645, 1537, 1500, 1311, 1255, 1182, 1140, 754, 704, 662, 598, 411 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{39}H_{58}N_8O_5S_1]^+$ 773.4149, found 773.4132; $[\alpha]_D^{26}$ –56.4 (*c* 0.3, $CHCl_3$); mp 158-159 °C.

***Bottromycin A_2 dethiazolyl analog (58)*:** Yield: 32%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (brm, 1H), 7.33-7.27 (4H), 7.22 (m, 1H), 7.03 (d, *J* = 10.0 Hz, 1H), 6.79 (d, *J* = 8.5 Hz, 1H), 6.30 (m, 1H), 4.83 (m, 1H), 4.64 (d, *J* = 10.5 Hz, 1H), 4.20 (d, *J* = 8.0 Hz, 1H), 3.94 (m, 1H), 3.85 (s, 1H), 3.85-3.74 (3H), 3.67 (s, 3H), 3.58 (ddd, *J* = 11.4, 11.4, 7.0 Hz, 1H), 3.49 (ddd, *J* = 13.0, 12.8, 6.3 Hz, 1H), 3.33 (ddd, *J* = 13.1, 13.0, 6.4 Hz, 1H), 3.26 (qd, *J* = 7.0, 6.0 Hz, 1H), 2.95 (m, 1H), 2.77 (dd, *J* = 11.8, 5.3 Hz, 1H), 2.60-2.51 (2H), 2.44 (m, 1H), 2.04 (m, 1H), 1.71 (m, 1H), 1.28 (d, *J* = 7.0 Hz, 3H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.00 (s, 9H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.88 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.5, 172.5, 172.2, 171.7, 171.4, 169.2, 157.1, 141.8, 128.6 (2C), 128.0 (2C), 127.0, 70.4, 69.9, 65.8, 57.6, 54.0 51.9, 48.0, 47.0, 42.0, 38.5, 35.3, 34.8, 33.5, 32.9, 30.3, 27.6 (3C), 27.5 (3C), 27.0, 20.4, 19.7, 15.7, 15.6; IR (Diamond prism) 3273, 2964, 1739, 1641, 1543, 1500, 1442, 1369, 1255, 1184, 754, 706, 451 cm^{-1}; HRMS (ESI-TOF) *m/z* $[M+Na]^+$ calcd for $[C_{39}H_{61}N_7Na_1O_7]^+$ 762.4530, found 762.4528; $[\alpha]_D^{28}$ –52.7 (*c* 0.3, $CHCl_3$); mp 126-128 °C.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publication website at DOI: acs.joc.xxxxxxx. ^{1}H and ^{13}C NMR spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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26) The absolute structure of **22** was confirmed by comparison with the degradation product[2] obtained after

the removal of the Boc group. All spectra data were identical.

27) We examined several other condensation reagents such as HATU, TBTU, PyBOP, DPPA, BOP-Cl, PyBroP, DIC as well.

28) The ^{1}H NMR spectra of **1** was easy to shift by the existing mineral. Indeed, the purification method was very important to obtain clean spectra. When a small amount of **1**, (<10 mg) was charged on preparative TLC silica gel plates (60F-254, 0.50 mm, cat. No. 1.05744.0001) purchased from Merck, the ^{1}H-NMR spectra was messy.

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