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Bull. Chem. Soc. Jpn., 72, 1561—1569 (1999)

Total Synthesis of a Macrocyclic Antibiotic, Micrococcin P

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(Received January 27, 1999)

The first total synthesis of a macrocyclic antibiotic, micrococcin P (**1**), was accomplished. After constructing the central 2,3,6-tristhiazolyl-substituted pyridine skeleton [Fragment A—C segment] (**21**) from ethyl 2-(2-bromoacetyl-6-dimethoxymethyl-3-pyridyl)thiazole-4-carboxylate (**15**) in 11 steps, successive fragment condensations of **21** with ethyl 2-[(*Z*)-1-(*O*-*t*-butyldiphenylsilyl-L-threonylamino)-1-propenyl]thiazole-4-carboxylate (**29**) and (*S*)-1-(*O*-methoxymethyl-L-threonylamino)-2-(*O*-methoxymethyl)-2-propanol (**25**) [the protected Fragments B and D moieties, respectively] gave the protected Fragment A–B–C–D segment **34**. Subsequent deprotection of all the protecting groups of **34** with trifluoroacetic acid and then cyclization by using BOP as condensing agent under high dilution conditions gave the expected micrococcin P (**1**). The synthetic **1** was identical with the natural **1** with respect to the chemical and physical properties.

Micrococcin P (**1**),[1] isolated from the culture of *Bacillus pumilus*, is a very unique macrocyclic antibiotic. So far, many similar antibiotics have also been isolated from various kinds of strains.[2] The antibiotic (**1**) includes a characteristic main structure, a central 2,3,6-tristhiazolyl-substituted pyridine skeleton called Fragment A—C (**21**) composed of multithiazole-substituted pyridine (pyridine; Py) and thiazole (thiazole; Tz) amino acid moieties, as shown in Fig. 1. Not only the interesting structure but also the bioactivity of **1**, which exhibits inhibitory action of bacterial protein synthesis, attracted and prompted us to investigate the total synthesis and structure-bioactivity relationship.

So far, the total synthesis of any such macrocyclic antibiotics has not been reported. More recently,[3,4] however, we have reported briefly on the total syntheses of **1** and its homologous micrococcin P_1 from **21**,[5] derived from 6-dimethoxymethyl-1,2-dihydro-2-oxo-3-pyridine carbonitrile[6] via ethyl 2-(2-bromoacetyl-6-dimethoxymethyl-3-pyridyl)thiazole-4-carboxylate (**15**). Furthermore, a similar central tristhiazolyl-substituted pyridine skeleton[7] of a macrocyclic antibiotic, GE 2270A,[8] could be also synthesized from **15**. Accordingly, the intermediate **15** is thought to be a very promising key compound for the total synthesis of various kinds of similar macrocyclic antibiotics, such as GE 37468 A,[9] thiocillins I—III,[10] and nosiheptide.[11] On the other hand, besides the central pyridine skeleton above mentioned, another main substructure, a dehydropeptide segment called Fragment B–C, was also already synthesized by the fragment condensation of ethyl 2-[(*Z*)-1-(*O*-*t*-butyldiphenylsilyl-L-threonylamino)-1-propenyl]thiazole-4-carboxylate (**29**) [Fragment B moiety] with 2-[(*S*)-(1-*t*-butoxycarbonylamino-2-methyl)propyl]thiazole-4-carboxylic acid (**7**) [Fragment C moiety], according to the methods reported earlier.[12,13]

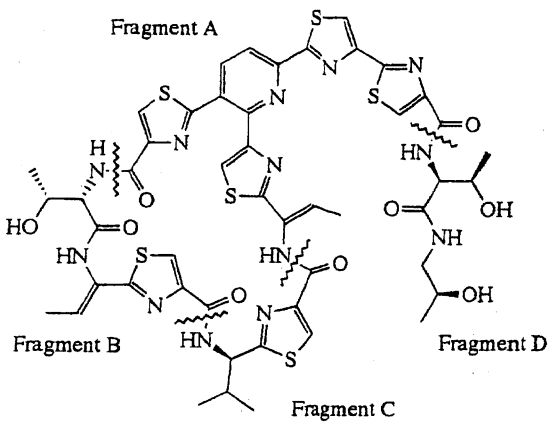


Fig. 1. Micrococcin P (**1**).

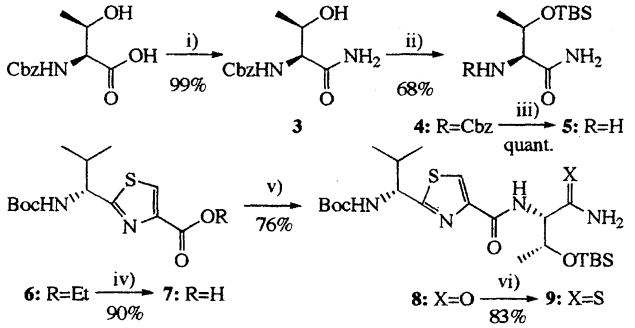
In this paper, we wish to report in detail the first total synthesis of **1** by all the deprotections and final cyclization of the protected Fragment A–B–C–D **34**, derived by the consecutive fragment condensations of the two terminal thiazole carboxylic acid groups of Fragment A–C with Fragment B and (*S*)-1-(*O*-methoxymethyl-L-threonylamino)-2-(*O*-methoxymethyl)-2-propanol (**25**) [Fragment D moiety] prepared independently.

Results and Discussion

The novel syntheses and the various useful couplings of the four Fragments A, B, C, and D derivatives were accomplished successfully as follows. First of all, to synthesize the precursor of the partial skeleton constructing the Fragment A—C segment, the desired dipeptide **9** containing the Fragment C moiety **7** was prepared. Initially, amidation of benzyloxycarbonyl (Cbz)–L-Thr–OH with ClCOOEt and then with 28% aqueous NH_3 gave the corresponding Thr–NH_2 derivative **3**. The hydroxy group was protected with *t*-butyldimethylsilyl chloride (TBSCl) in the presence of imidazole to give Cbz–L-Thr(TBS)–NH_2 (**4**). Subsequent

hydrogenolytic deprotection of the Cbz group of **4** with 10% Pd–C in EtOH gave H–Thr(TBS)–NH_2 (**5**) as an amine (*N*-) component almost quantitatively. On the other hand, ester hydrolysis of ethyl 2-[(*S*)-1-(*t*-butoxycarbonylamino)-2-methylpropyl]thiazole-4-carboxylate (**6**)[14,15] called the Fragment C derivative, with 1 M LiOH (1 M = 1 mol dm^{-3}) gave the corresponding thiazole-4-carboxylic acid derivative **7** as a carboxy (*C*-) component. Then, the usual coupling of **7** with **5** using diphenylphosphoric azide (DPPA) as condensing agent was performed to give the corresponding thiazole-4-carbonyl dipeptide amide derivative **8**. Furthermore, final thioamidation of **8** with Lawesson's reagent in 1,2-dimethoxyethane (DME) gave the expected dipeptide thioamide derivative **9** in 83% yield, as shown in Scheme 1.

Subsequently, to synthesize the precursor of the bithiazole skeleton in Fragment A, preparation of (*R*)-3-*t*-butoxycarbonyl-2,2-dimethylthiazolidine-4-carboxylic acid (**10**)[16] by one-pot protection of L-Cys–OH with acetone and then with di-*t*-butyl dicarbonate (Boc_2O), followed by amidation of the corresponding activated ester with 28% aqueous NH_3 gave the corresponding Cys–NH_2 derivative **11**. Similarly to the case of **9**, after thioamidating, **11** was converted into cystein thioamide **12**, which was further thiazolated by treatment with $BrCH_2COCOOH$ in the presence of $KHCO_3$, according to the Hanztsch method.[17] Then, the formed thiazole-4-carboxylic acid intermediate was esterified in situ with phenacyl bromide (PacBr) to give the expected phenacyl 2-substituted thiazole-4-carboxylate derivative **13** in 56% yield in two steps, as shown in Scheme 2. The two partial precursors (**9** and **13**) thus obtained were utilized for the formation of the Fragment A—C.

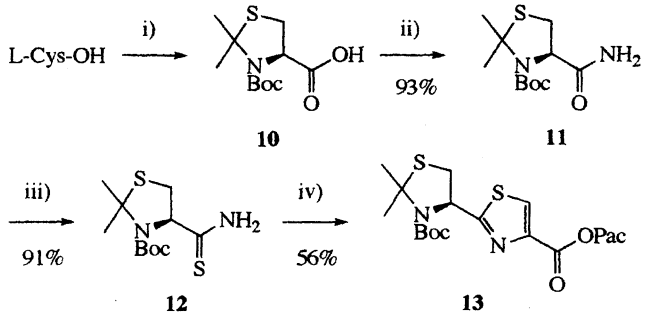


i) (a) ClCOOEt, Et_3N, THF, 0 °C, 20 min, (b) 28% NH_3, THF, 0 °C, 3 h, ii) TBSCl, imidazole, DMF, 0 °C, 30 min, r.t., 24 h, iii) 10% Pd-C, H_2, EtOH, r.t., 3 h, iv) 1M LiOH, H_2O/dioxane, 0 °C, 30 min, r.t., 4 h, v) DPPA, Et_3N, **5**, DMF, 0 °C, 3 h, r.t., overnight, vi) Lawesson's reagent, DME, r.t., 4 h.

Scheme 1.

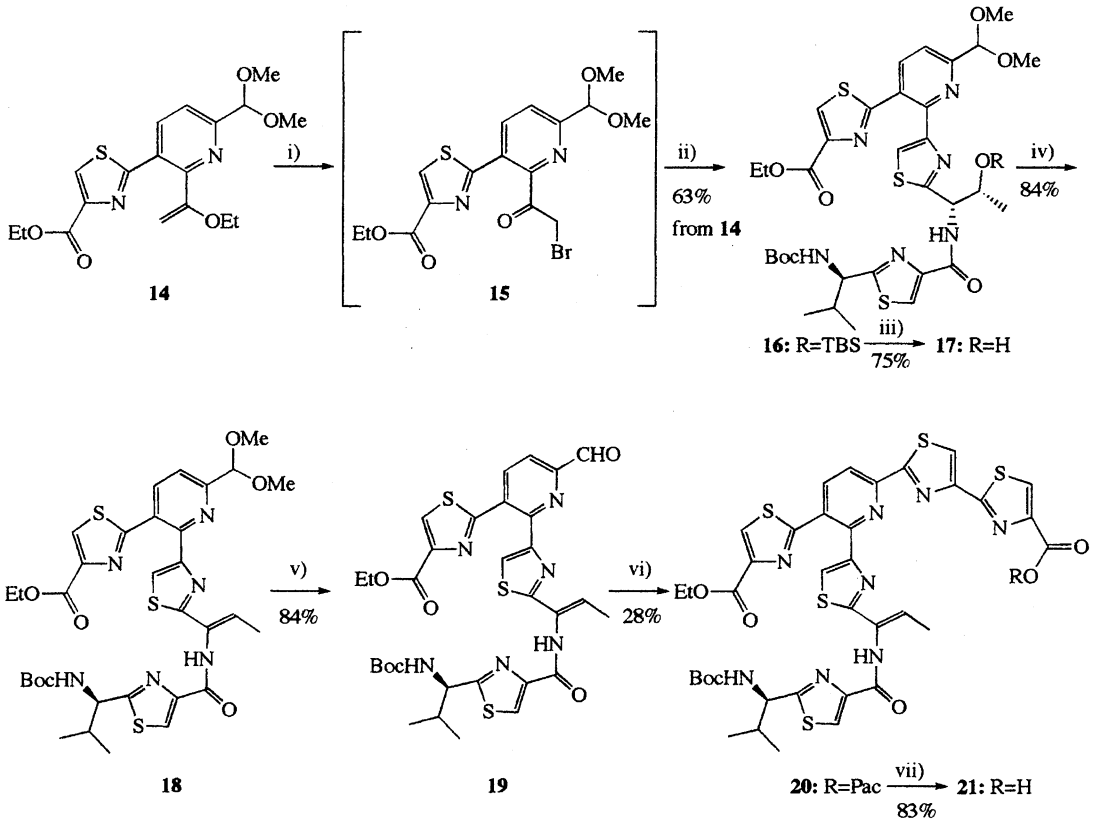
Thus, in order to construct the main Fragment A—C segment, firstly, the promising central 2,3,6-trisubstituted pyridine skeleton **15** was synthesized by the treatment of ethyl 2-[6-dimethoxymethyl-2-(1-ethoxyvinyl)-3-pyridyl]thiazole-4-carboxylate (**14**)[3,7] with *N*-bromosuccinimide (NBS) in aqueous THF. Subsequent one-pot thiazolation of **15** with **9** by using consecutive $KHCO_3$, trifluoroacetic anhydride (TFAA), and 28% aqueous NH_3 was effected successfully to give the corresponding 2,3-di(thiazolyl)-substituted pyridine derivative **16** in 63% yield from **14**. Secondly, deprotection of the TBS group of **16** with tetrabutylammonium fluoride (TBAF) gave the corresponding 2,3-di(thiazolyl)-pyridine derivative **17**, the hydroxy group of which was mesylated with methanesulfonyl chloride (MsCl) and then the mesyloxy group was β-eliminated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to give the corresponding 2-[2-(1-propenyl)thiazol-4-yl]pyridine derivative **18** in 84% yield. Thirdly, after transformation of the 6-dimethoxymethyl group into a formyl group of **19** with 2 M HCl, the immediate thiazolation of the obtained 5,6-di(thiazolyl)pyridine-2-carbaldehyde derivative **19** with **13** by using trifluoroacetic acid (TFA), followed by oxidation with MnO_2[15] gave the expected 2-{2-[(*Z*)-1-{2-(*R*)-1-*t*-butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-3-(4-ethoxycarbonylthiazol-2-yl)-6-[4-(4-phenacyloxycarbonylthiazol-2-yl)thiazol-2-yl]pyridine (**20**). Lastly, Pac ester hydrolysis of **20** with 1 M LiOH gave the corresponding 2-[6-(bithiazol-2-yl)-3-pyridyl]thiazole-4-carboxylic acid derivative **21** [Fragment A—C] in 83% yield, as shown in Scheme 3.

On the other hand, to obtain the protected Fragment D segment **24**, first, the coupling of 3-benzyloxycarbonyl-2,2,5-trimethyloxazolidine-4-carboxylic acid (**22**) with (*S*)-(+)-



i) (a) Acetone, reflux, 16 h, (b) Boc_2O, Et_3N, DMF, 0 °C, 30 min, r.t., overnight, ii) (a) DCC, HOSu, THF, 0 °C, 3 h, (b) 28% NH_3, EtOAc, 0 °C, 30 min., iii) Lawesson's reagent, DME, r.t., 12 h, iv) (a) $BrCH_2COCOOH$, $KHCO_3$, THF, 0 °C, 30 min, r.t., 24 h, (b) PacBr, Et_3N, CH_2Cl_2, 0 °C, 30 min, r.t., 6 h, (c) TFAA, pyridine, 0 °C, 1 h,

Scheme 2.



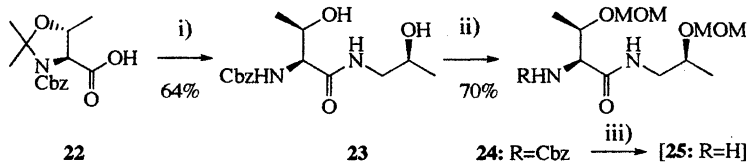
i) NBS, THF / H_2O, r.t., 5 min, ii) (a) **9**, $KHCO_3$, DME, r.t., overnight, (b) TFAA, pyridine, 0 °C, 30 min, r.t., 3 h, (c) 28% NH_3, ethyl acetate, r.t., 30 min, iii) TBAF, THF, 0 °C, 30 h, iv) (a) MsCl, Et_3N, DMSO, r.t., 30 min, (b) DBU, DMSO, r.t., 3 h, v) 2M HCl-THF, r.t., 12 h, vi) (a) **13**, TFA, CH_2Cl_2, r.t., 1 h, (b) toluene, r.t., 15 min, (c) MnO_2, toluene, overnight, vii) 1M LiOH, THF, 0 °C, 2 h.

Scheme 3.

1-amino-2-propanol by the BOP method,[18] followed by the protection of the two hydroxy groups of the obtained (*S*)-Cbz-L-Thr-amino-2-propanol (**23**) with methoxymethyl chloride (MOMCl) in the presence of (*i*-Pr)$_2$NEt gave (*S*)-Cbz-L-Thr(MOM)amino-2-(*O*-MOM)propanol (**24**), as shown in Scheme 4. After deprotection of the Cbz group by the usual method, the formed Fragment D segment **25** was later subjected to the next coupling with the 4-carboxyl group of the 6-(bithiazol-2-yl)pyridine skeleton of **21**.

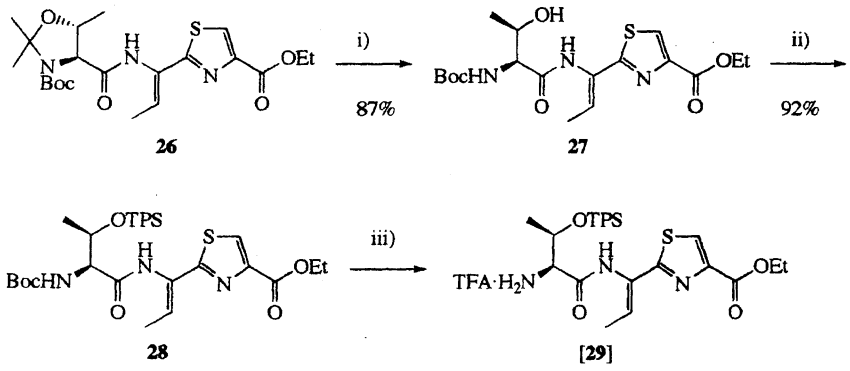
In addition, another building block called Fragment B segment **29** was synthesized in the following manner. Deprotection of the isopropylidene group of ethyl (*S*)-2-[(*Z*)-1-(3-*t*-butoxycarbonyl-2,2,5-trimethyloxazolidine-4-carbonyl-amino-1-propenyl)]thiazole-4-carboxylate (**26**)[13] with TFA gave the product **27** in 87% yield. Furthermore, the protection of the hydroxy group of **27** with *t*-butyldiphenylsilyl chloride (TPSCl) in the presence of imidazole and then deprotection of the Boc group with TFA in the presence of MS4A (molecular sieves) gave the expected ethyl 2-[(*Z*)-1-L-Thr(TPS)amino-(1-propenyl)]thiazole-4-carboxylate (**29**) as an unstable syrup, as shown in Scheme 5. Subsequently, the Fragment B segment **29** thus obtained was also submitted successively to the coupling with the 4-carboxyl group of the 2-thiazolylpyridine skeleton, derived from **21**.

Finally, to accomplish the synthesis of **1**, firstly, after hydrogenolytic deprotection of the Cbz group of **24** with 10% Pd–C, the formed intermediate **25** as an *N*-component was coupled in situ with **21** as a *C*-component using BOP and (*i*-Pr)$_2$NEt in DME to give the corresponding protected Fragment A–C–D segment **32**. Secondly, the ester hydrolysis of



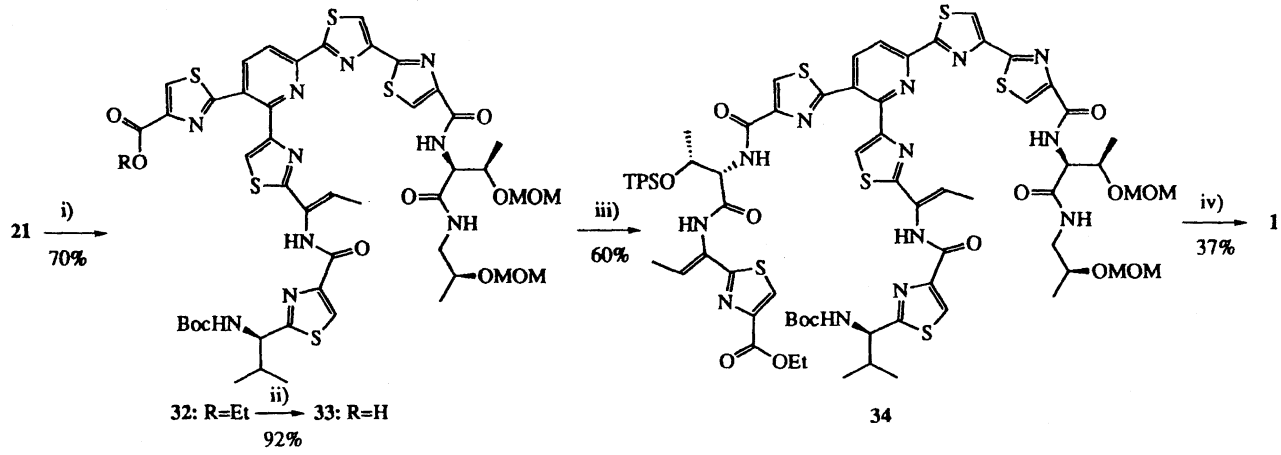
i) (a) (*S*)-(+)-1-Amino-2-propanol, BOP, (*i*-Pr)$_2$NEt, CH_3CN, 0 °C, 30 min, r.t., overnight, (b) 3M HCl, 50 °C, overnight, ii) MOMCl, (*i*-Pr)$_2$NEt, CH_2Cl_2, 0 °C, 30 min, r.t., 24 h, iii) 10%Pd-C, H_2, EtOH, r.t., 30 min.

Scheme 4.



i) TFA:CH_2Cl_2=4:96, r.t., 1 h, ii) TPSCl, imidazole, DMF, 0 °C, 30 min, r.t., overnight, iii) TFA / CH_2Cl_2, r.t., 1 h, MS4A.

Scheme 5.



i) 25, BOP, (i-Pr)$_2$NEt, DMF, 0 °C, 30 min, r.t., 90 min, ii) 1M LiOH, THF, 0 °C, 3 h, iii) 29, BOP, (i-Pr)$_2$NEt, DMF, 0 °C, 30 min, r.t., overnight, iv) (a) 1M LiOH, THF, 0 °C, 1 h, r.t., 6 h, (b) TFA:CH_2Cl_2=40:60, r.t., 3 h, (c) BOP, (i-Pr)$_2$NEt, DMF, 0 °C, 1 h, r.t., overnight.

Scheme 6.

32 with 1 M LiOH, followed by coupling of the obtained hydrolyzate **33** as a *C*-component with **29** as an *N*-component by the BOP method gave the protected linear and final precursor (Fragment A–B–C–D) **34** of micrococcin P in 60% yield. Lastly, one-pot ester hydrolysis of **34** with 1 M LiOH and then deprotection of the Boc, MOM, and TPS groups of **34** with TFA, followed by intramolecular cyclization using BOP and (i-Pr)$_2$NEt in DMF under high dilution conditions at room temperature gave a crude micrococcin P in comparatively good yield, as shown in Scheme 6.

The obtained crude crystals were purified by HPLC using a mixture of hexane and EtOAc (1 : 1 v/v) as the eluent to give **1** as colorless crystals in 34% yield.

Consequently, all of the chemical and physical properties of synthetic micrococcin P (**1**) were almost identical with those of the naturally occurring **1**, as summarized in Table 1. Although the ^{1}H NMR spectral data of the natural **1**, except for ^{13}C NMR, have not been reported, all of the proton and carbon signals of the synthesized **1** could be assigned satisfactorily. In particular, from the ^{1}H NMR spectrum of synthetic **1**, the appearance of the chemical shifts of the 4,5-vicinal protons of the pyridine ring at δ = 8.31 and 8.44—8.47 (d, J = 7.6 Hz) as doublets and the six thiazole ring protons at δ = 7.76, 8.22, 8.23, 8.31, 8.42, and 8.61 as a singlets supports further the formation of **1**, in addition to the agreement of the melting points and the specific rotations.

Table 1. The Chemical and Physical Data of Micrococcin P

	Synthetic	Natural
Mp (°C)	234—248	232—252
$[\alpha]_D^{21}$ (c 1.19, EtOH : H_2O = 9 : 1)	+68.7°	+63.7°
λ_{max} (nm)	344.5	345

In conclusion, the first total synthesis of **1** was accomplished by the useful synthesis of various dehydropeptide derivatives and their various thiazolation as well as the effective synthesis of the 2,3,6-trisubstituted pyridine skeleton. Furthermore, the methodology developed here was found to be applicable effectively to the synthesis of similar macrocyclic antibiotics based on the total synthesis of micrococcin P$_1$[4] and partial syntheses of GE 2270A.[7]

Experimental

The melting points were measured using a Yamato (Model Mp-21) micromelting point apparatus, and are uncorrected. The IR

spectra were recorded using a Hitachi EPI-G2 spectrometer in KBr. The ^{1}H NMR and ^{13}C NMR spectra were measured with JEOL EX 90, FX 200, and JNE 500 spectrometers in $CDCl_3$, DMSO-d_6, C_6D_6, or CD_3OD solutions with tetramethylsilane used as the internal standard. The specific rotations were measured in a 0.5 dm tube using a JASCO DIP-4 polarimeter in MeOH or H_2O. Thin-layer chromatography (TLC) was done with Merck silica gel 60 Art 5554 plates, and column chromatography was carried out with Merck silica gel 60 or Wako gel C-300. High-pressure liquid chromatography (HPLC) analyses and separations were done on the following columns using a mixture of MeOH and $CHCl_3$ (5 : 95 v/v) with a flow rate of 2.0 ml min^{-1} by detecting UV (254 nm) absorption: TSK gel Silica-60 (7.8 mmIDE×60 cmL).

Starting Materials. L-Thr–OH, L-Cys–OH, and D-Val–OH, (*S*)-(+)-1-amino-2-propanol were purchased from Nippon Rikagaku Yakuhin Co. Ltd., Merck K GaA Co., Ltd., and Sigma-Aldrich Japan K. K., respectively.

***N*-Benzyloxycarbonyl-L-threoninamide (3).** To a solution of Cbz–L-Thr–OH (9.55 g, 39.70 mmol) in THF (500 ml) were added, with stirring, Et_3N (6.10 ml, 43.90 mmol) and ethyl chlorocromate (4.18 ml, 43.9 mmol) under cooling. After the mixture was stirred for 1.5 h, 28% aqueous NH_3 (3.99 ml, 59.90 mmol) was further added, with stirring, to the resulting solution for 30 min. The reaction mixture was mixed with saturated NH_4Cl aqueous solution (100 ml) and then concentrated in vacuo. The aqueous residue was extracted with EtOAc (30 ml×2) and the combined extracts were washed with brine (30 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using EtOAc to give colorless crystals. Recrystallization from hexane–EtOAc gave **3** as colorless crystals. Yield 99% (9.48 g). Mp 96—97 °C. $[\alpha]_D^{24}$ +5.5° (*c* 0.73, MeOH). IR 3490, 3334, 2980, 2254, 1956, 1658, 1581, 1527 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 1.05 (d, 3H, CH_3, J = 6.2 Hz), 3.78—4.00 (m, 2H, β-H and OH), 4.73—4.84 (m, 1H, α-H), 5.01 (s, 2H, Cbz's CH_2), 6.74—7.57 (m, 8H, NH_2, NH and Ph). Found: C, 56.23; H, 6.40; N, 10.92%. Calcd for $C_{12}H_{16}N_2O_4 \cdot 1/4H_2O$: C, 56.13; H, 6.47; N, 10.91%.

***N*-Benzyloxycarbonyl-L-3-*O*-(*t*-butyldimethylsilyl)threoninamide (4).** To a solution of **3** (9.48 g, 39.70 mmol) in DMF (200 ml) were added, with stirring, TBSCl (6.20 g, 41.10 mmol) and imidazole (2.80 g, 41.10 mmol) at 0 °C. After the mixture was stirred for 1 h at 0 °C, the resulting solution was stirred continuously at room temperature for 5 h. The reaction mixture was poured into water (150 ml) and the resultant solution was extracted with EtOAc (80 ml×3). The combined extracts were washed with brine (50 ml×3) and dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (1 : 1 v/v) to give **4** as a colorless syrup. Yield 68% (9.54 g). $[\alpha]_D^{24}$ +16.4° (*c* 0.5, MeOH). IR 3928, 3808, 3694, 3580, 3442, 3340, 3214, 2932, 2854, 2254, 1683, 1590 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 0.28 (s, 6H, TBS's CH_3×2), 0.84 (s, 9H, TBS's CH_3×3), 1.06 (d, 3H, CH_3, J = 5.9 Hz), 3.95—4.21 (m, 2H, α-H and β-H), 5.05 (s, 2H, Cbz's CH_2), 6.51 (br d, 1H, CHN*H*, J = 9.6 Hz), 7.17—7.34 (m, 7H, NH_2 and Ph). Found: C, 58.66; H, 8.33; N, 7.17%. Calcd for $C_{18}H_{30}N_2O_4Si$: C, 58.98; H, 8.25; N, 7.61%.

2-*N*-{2-[(*R*)-1-*N*-*t*-Butoxycarbonylamino-2-methylpropyl]-thiazole-4-carbonyl}-L-3-*O*-(*t*-butyldimethylsilyl)threoninamide (8). A suspension of **4** (5.85 g, 16.60 mmol) and 10% Pd–C (0.6 g) in EtOH (150 ml) was stirred under H_2 gas stream at room temperature for 4 h. After removal of Pd–C, the filtrate was concentrated in vacuo. The obtained residue was dissolved in DMF (100 ml). To the resulting solution were added consecutively, with stirring, **7** (5.05g, 16.60 mmol), DPPA (3.96 ml, 18.30 mmol), and Et_3N (2.56 ml, 18.30 mmol) under cooling for 1 h. The reaction mixture was continuously stirred at 0 °C for 1 h and at room temperature overnight, poured into water (100 ml), and then extracted with EtOAc (80 ml×3). The combined extracts were washed with brine (50 ml×3), with 10% citric acid (50 ml×2), and with saturated $NaHCO_3$ aqueous solution (50 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (1 : 1 v/v) to give **8** as a colorless syrup. Yield 68% (5.42 g). $[\alpha]_D^{24}$ +33.0° (*c* 1.0, MeOH). IR 3400, 2962, 2860, 1665, 1529 cm^{-1}. ^{1}H NMR δ = 0.06 (s, 6H, TBS's CH_3×2), 0.84 (s, 9H, TBS's CH_3×3), 0.88 and 0.90 (each d, 6H, CH(CH_3)$_2$, J = 6.3 Hz), 1.08 (d, 3H, CH_3, J = 6.3 Hz), 1.41 (s, 9H, Boc), 2.27—2.29 (m, 1H, C*H*(CH_3)$_2$), 4.30—4.33 (m, 2H, α-H and β-H), 4.52—4.63 (m, 1H, NHC*H*), 7.22 (br s, 1H, NH), 7.51 (br s, 1H, NH), 7.75 (br d, 1H, N*H*CH, J = 8.3 Hz), 7.90 (br d, 1H, N*H*CH, J = 9.2 Hz), 8.18 (s, 1H, Tz's H). Found: C, 53.50; H, 8.50; N, 10.42%. Calcd for $C_{23}H_{42}N_4O_5SSi$: C, 53.67; H, 8.22; N, 10.88%.

2-*N*-{2-[(*R*)-1-*N*-*t*-Butoxycarbonylamino-2-methylpropyl]-thiazole-4-carbonyl}-L-3-*O*-(*t*-butyldimethylsilyl)threoninethioamide (9). A solution of **8** (5.14 g, 9.99 mmol) and Lawesson reagent (2.24 g, 5.99 mmol) in DME (250 ml) was stirred at room temperature for 4 h. Concentration of the reaction mixture in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (3 : 2, v/v) to give residual crystals. Recrystallization from hexane–EtOAc gave **9** as colorless crystals. Yield 83% (4.40 g). Mp 153—154 °C. $[\alpha]_D^{24}$ +59.3° (*c* 0.9, MeOH). IR 3304, 3208, 2962, 2248, 1758, 1668 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ = 0.06 (s, 6H, TBS's CH_3×2), 0.78 (s, 9H, TBS's CH_3×3), 0.84 and 0.87 (each d, 6H, CH(CH_3)$_2$, J = 6.3 Hz), 1.08 (d, 3H, CH_3, J = 6.3 Hz), 1.37 (s, 9H, Boc), 2.18—2.25 (m, 1H, C*H*(CH_3)$_2$), 4.30—4.37 (m, 1H, β-H), 4.52—4.63 (m, 2H, α-H and NHC*H*), 7.71 (br d, 1H, N*H*CH, J = 9.3 Hz), 8.01 (br d, 1H, N*H*CH, J = 9.2 Hz), 8.14 (s, 1H, Tz's H), 9.58 (br s, 1H, NH), 9.76 (br s, 1H, NH). Found: C, 51.66; H, 8.07; N, 10.26%. Calcd for $C_{23}H_{42}N_4O_4S_2Si$: C, 52.04; H, 7.98; N, 10.26%.

(*R*)-3-*t*-Butoxycarbonyl-2,2-dimethylthiazolidine-4-carboxamide (11). To a stirred solution of **10** (3.00 g, 11.50 mmol) in THF (150 ml) were added a solution of DCC (2.85 g, 13.80 mmol) and HOSu (1.45 g, 13.80 mmol) in THF (50 ml) at room temperature. After the mixture was stirred for 3 h, the *N*,*N*′-dicyclohexylurea deposited was filtered off, and the filtrate was concentrated in vacuo. The obtained residue was dissolved in EtOAc (200 ml) and then treated with 28% aqueous NH_3 (1.15 ml, 17.25 mmol) at 0 °C for 30 min. The reaction mixture was washed with saturated $NaHCO_3$ aqueous solution (100 ml) and brine (100 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave crude crystals, which were recrystallized from a hexane–EtOAc to give **11** as colorless needles. Yield 93% (2.78 g). Mp 114—116 °C. $[\alpha]_D^{24}$ −68.5° (*c* 0.5, MeOH). IR 3196, 3008, 1756, 1680 cm^{-1}. ^{1}H NMR δ = 1.48 (s, 9H, Boc), 1.78 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 3.04—3.22 (m, 2H, CHCH_2), 4.74 (m, 1H, C*H*CH_2), 6.19 (br s, 2H, NH). Found: C, 50.77; H, 7.82; N, 10.66%. Calcd for $C_{11}H_{20}N_2O_3S$: C, 50.75; H, 7.74; N, 10.76%.

(*R*)-3-*t*-Butoxycarbonyl-2,2-dimethylthiazolidine-4-carbothioamide (12). A solution of **11** (2.20 g, 8.45 mmol) and Lawesson reagent (1.70 g, 0.42 mmol) in DME (150 ml) was stirred at room temperature for 12 h. Concentration of the reaction mixture in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (3 : 1 v/v) to give

colorless crystals. Recrystallization from a hexane–EtOAc gave **12** as colorless needles. Yield 91% (2.12 g). Mp 138—139 °C. $[\alpha]_D^{24}$ −83.8° (*c* 1.0, MeOH). IR 3428, 3156, 2932, 1698, 1620, 1554 cm^{-1}. ^1HNMR δ = 1.46 (s, 9H, Boc), 1.78 (s, 3H, CH_3), 1.91 (s, 3H, CH_3), 3.39—3.51 (m, 2H, $CHCH_2$), 5.14 (m, 1H, $CHCH_2$), 7.55 (br s, 1H, NH), 7.96 (br s, 1H, NH). Found: 48.05; H, 7.28; N, 10.31%. Calcd for $C_{11}H_{20}N_2O_2S_2$: C, 47.80; H, 7.29; N, 10.13%.

Phenacyl (*R*)-2-(3-*t*-Butoxycarbonyl-2,2-dimethylthiazolidin-4-yl)thiazole-4-carboxylate (13). To a solution of **12** (1.70 g, 6.10 mmol) in DME (100 ml) in the presence of $KHCO_3$ (5.13 g, 5.13 mmol) was added, with stirring, a solution of $BrCH_2COCOOH$ (3.05 g, 18.20 mmol) in DME (100 ml) at 0 °C for 30 min. After the mixture was stirred at room temperature for 24 h, the resulting solution was concentrated in vacuo to give a residue, which was dissolved in EtOAc (150 ml), washed with H_2O (100 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residual substance, which was dissolved in CH_2Cl_2 (100 ml). To the solution were added, with stirring, PacBr (1.82 g, 9.14 mmol) and Et_3N (0.94 ml, 6.81 mmol) at 0 °C. After the mixture was stirred for 6 h, the reaction mixture was further combined with diethyl ether (100 ml) and washed with saturated $NaHCO_3$ aqueous solution (100 ml×3), brine (100 ml), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was again dissolved in DME (100 ml). To the resulting solution was added, with stirring, a solution of TFAA (1.30 ml, 9.38 mmol) and pyridine (1.50 ml, 18.60 mmol) in DME (10 ml) at 0 °C for 1 h. The reaction mixture was concentrated in vacuo to give a residue, which was dissolved in EtOAc (100 ml), washed with 10% citric acid (100 ml×2), and with saturated $NaHCO_3$ aqueous solution (100 ml×2), and with brine (100 ml), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a crude residual substance, which was purified on a silica-gel column using a mixture of hexane and EtOAc (2 : 1 v/v) to give crude crystals. Recrystallization from hexane–EtOAc gave **13** as colorless crystals. Yield 56% (1.64 g). Mp 153—154 °C. $[\alpha]_D^{24}$ −41.6° (*c* 1.0, MeOH). IR 2936, 1689, 1620 cm^{-1}. ^1HNMR δ = 1.38 (s, 9H, Boc), 1.83 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 3.45—3.56 (m, 2H, SCH_2), 5.61 (s, 2H, $COCH_2$), 5.67—5.75 (m, 1H, $CHCH_2$), 7.48—7.99 (m, 5H, Ph), 8.25 (s, 1H, Tz's H). Found: C, 57.03; H, 5.73; N, 5.86%. Calcd for $C_{22}H_{26}N_2O_5S_2$: C, 57.12; H, 5.67; N, 6.06%.

2-{2-[(1*S*,2*R*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-2-*t*-butyldimethylsilyloxypropyl]thiazol-4-yl}-6-dimethoxymethyl-3-(4-ethoxycarbonylthiazol-2-yl)pyridine (16). A solution of **14**[7] (3.00 g, 7.99 mmol) and NBS (2.13 g, 12.98 mmol) in THF–H_2O (50 ml, 1 : 1 v/v) was stirred in the presence of $CaCO_3$ (1.20 g, 12.98 mmol) at room temperature for 5 min. The reaction mixture was extracted with diethyl ether (20 ml×2) and the combined extracts were washed with H_2O (10 ml×2), and dried over anhydrous Na_2SO_4. Concentration in vacuo under 10 °C gave the expected bromoacetyl derivative (**15**) as an intermediate. A solution of **15** in DME (30 ml) was added to a chilled solution of **9** (4.24 g, 7.99 mmol) and $KHCO_3$ (4.00 g, 39.95 mmol) in DME (100 ml) under cooling. After the mixture was stirred for 30 min and at room temperature overnight, the reaction mixture was concentrated in vacuo to give a residue, and the solution in $CHCl_3$ (50 ml) was washed with brine (30 ml×3), and dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residual substance, which was dissolved in DME (100 ml). To the resulting solution were added, with stirring, TFAA (2.21 ml, 15.98 mmol) and pyridine (3.22 ml, 39.95 mmol) under cooling. After the mixture was further stirred for 2 h, the resultant solution was made slightly alkaline with Et_3N and then concentrated in vacuo. The obtained residue was dissolved in EtOAc (100 ml) and washed with brine (50 ml×2). The EtOAc solution was treated with 28% aqueous NH_3 (1.69 ml, 23.97 mmol) and stirred at 0 °C for 30 min. The solution was washed with brine (50 ml×2) and dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residiue, which was finally purified on a silica-gel column using a mixture of hexane and EtOAc (3 : 2 v/v) to give **16** as colorless amorphous material. Yield 63% (4.34 g). IR 3400, 3112, 2956, 2440, 2152, 1713 cm^{-1}. ^1HNMR δ = 0.02 (s, 6H, TBS's CH_3×2), 0.86 (s, 9H, TBS's CH_3×3), 0.95 and 1.00 (d×2, 3H×2, $CH(CH_3)_2$, *J* = 6.6 Hz), 1.12 (d, 3H, CH_3, *J* = 6.6 Hz), 1.41 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.47 (s, 9H, Boc), 2.35—2.42 (m, 1H, $CHCH_3$), 3.45 (s, 6H, OCH_3×2), 4.35—4.46 (m, 1H, NHC*H*), 4.42 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.85—4.95 (m, 1H, $CH(CH_3)_2$), 5.15—5.24 (m, 2H, NHC*H* and N*H*CH), 5.44 (s, 1H, $CH(OCH_3)_2$), 7.64 (d, 1H, Py's H, *J* = 7.9 Hz), 7.80, 8.06 and 8.25 (each s, 3H, Tz's H), 8.17 (d, 1H, Py's H, *J* = 7.9 Hz), 8.25 (s, 1H, Th's H), 8.29 (br d, 1H, CHN*H*, *J* = 8.2 Hz). Found: C, 54.22; H, 6.62; N, 9.69%. Calcd for $C_{39}H_{56}N_6O_8S_3Si$: C, 54.39; H, 6.56; N, 9.76%.

2-{2-[(1*S*,2*R*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-2-hydroxypropyl]thiazol-4-yl}-6-dimethoxymethyl-3-(4-ethoxycarbonylthiazol-2-yl)pyridine (17). To a solution of **16** (6.00 g, 6.97 mmol) in THF (150 ml) was added, with stirring, 1 M-solution of TBAF in THF (10.45 ml, 10.45 mmol) at 0 °C for 30 min. Concentration of the reaction mixture in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (1 : 2 v/v) to give **17** as colorless amorphous material. Yield 75% (3.95 g). IR 3400, 3112, 2968, 2830, 2272, 1713 cm^{-1}. ^1HNMR δ = 0.92 and 0.97 (each d, 6H, $CH(CH_3)_2$, *J* = 6.6 Hz), 1.20 (d, 3H, CH_3, *J* = 6.6 Hz), 1.39 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.44 (s, 9H, Boc), 2.06 (br s, 1H, OH), 2.29—2.38 (m, 1H, $CH(CH_3)_2$), 3.44 (s, 6H, OCH_3×2), 4.28 (dq, 1H, $CHCH_3$, *J* = 6.6 and 7.3 Hz), 4.42 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.65—4.84 (m, 1H, NHC*H*), 5.19—5.27 (m, 2H, CHN*H* and NHC*H*), 5.43 (s, 1H, $CH(OCH_3)_2$), 7.98 (br d, 1H, CHN*H*, *J* = 9.2 Hz), 7.90, 8.05, and 8.25 (each s, 3H, Tz's H×3), 7.66 and 8.15 (each d, 2H, Py's H×2, *J* = 7.9 Hz). Found: C, 52.40; H, 5.80; N, 10.66%. Calcd for $C_{33}H_{42}N_6O_8S_3 \cdot 1/2H_2O$: C, 52.43; H, 5.73; N, 11.12%.

2-{2-[(*Z*)-1-{2-{(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazol-4-carbonylamino}-1-propenyl]thiazol-4-yl}-6-dimethoxymethyl-3-(4-ethoxycarbonylthiazol-2-yl)pyridine (18). To a solution of **17** (3.50 g, 4.63 mmol) and Et_3N (1.90 ml, 13.90 mmol) in DMSO (100 ml) was added, with stirring, MsCl (0.54 ml, 6.95 mmol) at 0 °C for 2 h. DBU (1.04 ml, 6.95 mmol) was added, and was stirred overnight. The reaction mixture was poured into EtOAc (150 ml), and the resulting solution was washed with 10% citric acid (100 ml×2), with saturated $NaHCO_3$ aqueous solution (100 ml×2), and with brine (100 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (2 : 3 v/v) to give **18** as pale yellowish amorphous material. Yield 84% (2.8 g). ^1HNMR δ = 0.95 and 1.00 (each d, 6H, $CH(CH_3)_2$, *J* = 6.9 Hz), 1.39 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.46 (s, 9H, Boc), 1.83 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.33—2.40 (m, 1H, $CH(CH_3)_2$), 3.44 and 3.45 (each s, 6H, OCH_3×2), 4.40 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.91 (m, 1H, NHC*H*), 5.21 (br d, 1H, CHN*H*, *J* = 9.2 Hz), 5.44 (s, 1H, $CH(OCH_3)_2$), 6.44 (q, 1H, CH_3CH=, *J* = 7.3 Hz), 7.65 and 8.22 (each d, 2H, Py's H×2, *J* = 7.9 Hz), 7.77, 8.01, and 8.10 (each s, 3H, Tz's H×3), 8.61 (s, 1H, NHC=).

6-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-5-(4-

ethoxycarbonylthiazol-2-yl)pyridine-2-carbaldehyde (19). A solution of **18** (3.08 g, 4.12 mmol) in THF–1 M HCl (100 ml, 3 : 1) was stirred at room temperature for 12 h. Concentration of the reaction mixture in vacuo gave an aqueous residue, which was extracted with EtOAc (30 ml×3). The combined extracts were washed with brine (100 ml×2) and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residual substance, which was purified on a silica-gel column using a mixture of hexane and EtOAc (2 : 3 v/v) to give **19** as pale yellowish amorphous material. Yield 95% (2.67 g). $[\alpha]_D^{24}$ +20.50° (*c* 0.40, MeOH). IR 3364, 3312, 2968, 1710 cm^{-1}. ^1HNMR δ = 0.94 and 1.10 (each d, 6H, CH(CH_3)$_2$, *J* = 6.9 Hz), 1.40 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.46 (s, 9H, Boc), 1.86 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.32—2.37 (m, 1H, CH(CH_3)$_2$), 4.42 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.91 (m, 1H, NCH), 5.19 (br d, 1H, CHN*H*, *J* = 8.3 Hz), 6.51 (q, 1H, CH_3C*H*=, *J* = 7.3 Hz), 7.83, 8.08, and 8.11 (each s, 3H, Tz's H×3), 8.02 and 8.42 (each d, 2H, Py's H×2, *J* = 8.3 Hz), 8.64 (s, 1H, NHC=), 10.16 (s, 1H, CHO). Found: C, 54.08; H, 5.43; N, 11.81%. Calcd for $C_{31}H_{34}N_6O_6S_3 \cdot 1/2H_2O$: C, 53.83; H, 5.10; N, 12.15%.

2-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-3-(4-ethoxycarbonylthiazol-2-yl)-6-[4-(4-phenacyloxycarbonylthiazol-2-yl)thiazol-2-yl]pyridine (20). A solution of **13** (3.05 g, 6.59 mmol) in CH_2Cl_2–TFA (100 ml, 3 : 1 v/v) was stirred at room temperature for 1 h and then concentrated in vacuo to half volume. The resulting solution was adjusted to pH 7 with saturated $NaHCO_3$ aqueous solution and then extracted with EtOAc (20 ml×3). The combined extracts were washed with brine (30 ml×2) and dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was dissolved in toluene (50 ml). The resulting solution was added to a solution of **19** (3.00 g, 4.39 mmol) in toluene (100 ml) at room temperature. After the mixture was stirred for 15 min, the reaction mixture was washed with 10% citric acid (50 ml×2), with saturated $NaHCO_3$ aqueous solution (50 ml×2), and with brine (50 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was again dissolved in toluene (150 ml). To the resulting solution was added an activated MnO_2 (7.64 g, 87.87 mmol) at room temperature. After the mixture was stirred for 12 h and filtered off with MnO_2, the filtrate was concentrated in vacuo to give crude syrupy material. Purification on a silica-gel column using a mixture of $CHCl_3$ and acetone (15 : 1 v/v) gave crude crystals. Recrystallization from hexane–EtOAc gave **20** as pale yellowish crystals. Yield 28% (1.21 g). Mp 143—145 °C. $[\alpha]_D^{24}$ +15.9° (*c* 0.34, MeOH). IR 3448, 3118, 2974, 1701 cm^{-1}. ^1HNMR (DMSO-d_6) δ = 0.88—0.92 (m, 6H, CH(CH_3)$_2$), 1.32 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.41 (s, 9H, Boc), 1.69 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.24—2.31 (m, 1H, NHC*H*), 4.33 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.60—4.75 (m, 1H, C*H*(CH_3)$_2$), 5.80 (s, 2H, $COCH_2$), 6.34 (q, 1H, CH_3C*H*=, *J* = 7.3 Hz), 7.56—8.04 (m, 6H, Ph and CHN*H*), 8.21, 8.30, 8.57, 8.61, and 8.74 (each s, 5H, Tz's H×5), 8.34 and 8.48 (each d, 2H, Py's H×2, *J* = 7.9 Hz), 9.79 (br s, 1H, NHC=). Found: C, 54.68; H, 4.42; N, 11.16%. Calcd for $C_{45}H_{42}N_8O_8S_5$: C, 54.97; H, 4.31; N, 11.40%.

2-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-6-[4-(4-carboxylthiazol-2-yl)thiazol-2-yl]-3-(4-ethoxycarbonylthiazol-2-yl)pyridine (21). To a solution of **20** (1.00 g, 1.02 mmol) in THF (50 ml) was added, with stirring, 1 M LiOH (1.02 ml, 1.05 mmol) at 0 °C. After the mixture was stirred for 1 h, the reaction mixture was adjusted to pH 7 with Dowex 50 H^+ and then concentrated in vacuo to give a residue. Purification on a silica-gel column using a mixture of $CHCl_3$ and MeOH (4 : 1 v/v) gave crude crystals. Recrystallization from a hexane–EtOAc gave **21** as pale yellowish crystals. Yield 83% (0.73 g). Mp 198.5—204.5 °C. $[\alpha]_D^{24}$ +24.0° (*c* 0.3, MeOH). IR 3430, 3112, 2968, 2074, 1710, 1587 cm^{-1}. ^1HNMR (DMSO-d_6) δ = 0.87—0.91 (m, 6H, CH(CH_3)$_2$), 1.31 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.41 (s, 9H, Boc), 1.68 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.45 (m, 1H, C*H*(CH_3)$_2$), 4.32 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.71 (m, 1H, NHC*H*), 6.32 (q, 1H, CH_3C*H*=, *J* = 7.3 Hz), 7.73 (d, 1H, CHN*H*, *J* = 8.3 Hz), 8.13, 8.16, 8.29, 8.56, and 8.67 (each s, 5H, Tz's H×5), 8.30 and 8.45 (each d, 2H, Py's H×2, *J* = 8.3 Hz), 9.80 (br s, 1H, NHC=), 12.50 (br s, 1H, COOH). Found: C, 48.46; H, 4.12; N, 12.19%. Calcd for $C_{37}H_{36}N_8O_7S_5 \cdot 1/4H_2O$: C, 48.83; H, 4.54; N, 12.31%.

1-(*N*-Benzyloxycarbonyl-L-threonylamino)-2-propanol (23). To a solution of (*S*)-(+)-1-amino-2-propanol (3.00 mg, 3.99 mmol) and **22** (1.29 g, 4.39 mmol) in CH_3CN (100 ml) were added, with stirring, BOP (1.94 g, 4.39 mmol) and (*i*-Pr)$_2$NEt (0.75 ml, 4.39 mmol) at 0 °C. After the mixture was stirred for 30 min and at room temperature overnight, the reaction mixture was concentrated in vacuo to give a residue, which was dissolved in EtOAc (100 ml). The resulting solution was washed with brine (30 ml×3), with 10% citric acid (30 ml×2), and with saturated $NaHCO_3$ aqueous solution (30 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was dissolved in 70% AcOH (50 ml) and 3 M HCl (10 ml). After the mixture was stirred at 50 °C for 6 h, azeotropical distillation with toluene in vacuo gave a residue, which was purified on a silica-gel column using a mixture of MeOH and EtOAc (1 : 20 v/v) to give crude crystals. Recrystallization from hexane–EtOAc gave **23** as colorless needles. Yield 64% (589 mg). Mp 113—114 °C. $[\alpha]_D^{24}$ −3.5° (*c* 0.64, MeOH). IR 3304, 2962, 1692, 1641, 1541 cm^{-1}. ^1HNMR δ = 1.16 (d, 3H, OCHCH_3, *J* = 6.3 Hz), 1.18 (d, 3H, Thr's CH_3, *J* = 6.6 Hz), 2.81—2.84 (m, 1H, Thr's OH), 2.99—3.09 (m, 1H, CH_2's H), 3.42—3.52 (m, 1H, CH_2's H), 3.57 (br s, 1H, OH), 3.84—3.92 (m, 1H, OC*H*CH_3), 3.85—3.89 (m, 1H, Thr's α-H), 4.34—4.39 (m, 1H, Thr's β-H), 5.13 (s, 2H, Cbz's CH_2), 5.90 (br d, 1H, CHN*H*, *J* = 8.3 Hz), 6.87—6.92 (m, 1H, CH_2N*H*), 7.35 (s, 5H, Ph). Found: C, 58.14; H, 7.43; N, 9.22%. Calcd for $C_{15}H_{22}N_2O_5$: C, 58.05; H, 7.15; N, 9.03%.

1-(*N*-Benzyloxycarbonyl-*O*-methoxymethyl-L-threonylamino)-2-(*O*-methoxymethyl)propanol (24). To a solution of **23** (0.60 g, 1.93 mmol) in CH_2Cl_2 (50 ml) were added, with stirring, MOMCl (0.29 ml, 5.80 mmol) and (*i*-Pr)$_2$NEt (0.75 ml, 4.39 mmol) at 0 °C. After the mixture was stirred for 1 h, MOMCl (0.58 ml, 11.60 mmol) and (*i*-Pr)$_2$NEt (1.50 ml, 8.78 mmol) were further added to the resulting solution. After the mixture was stirred at room temperature overnight, the reaction mixture was added to diethyl ether (50 ml) and washed with brine (30 ml×3), 10% citric acid (30 ml×2), and saturated $NaHCO_3$ aqueous solution (30 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using EtOAc to give crude crystals. Recrystallization from hexane–EtOAc gave **24** as colorless crystals. Yield 70% (0.54 g) Mp 76—77 °C. $[\alpha]_D^{24}$ +3.0° (*c* 0.4, MeOH). IR 3802, 2932, 2890, 1689, 1650, 1533 cm^{-1}. ^1HNMR δ = 1.18 (d, 3H, Thr's CH_3, *J* = 6.3 Hz), 1.19 (d, 3H, OCHCH_3, *J* = 6.3 Hz), 3.10—3.62 (m, 2H, CH_2), 3.36 (s, 6H, MOM's CH_3×2), 3.72—3.78 (m, 1H, OC*H*CH_3), 4.28—4.33 (m, 2H, Thr's α-H and β-H), 4.62—4.73 (m, 4H, MOM's CH_2×2), 5.15 (s, 2H, Cbz's CH_2), 5.76 (br d, 1H, CHN*H*, *J* = 6.6 Hz), 6.90—7.00 (m, 1H, CH_2N*H*), 7.37 (s, 5H, Ph). Found: C, 57.26; H, 7.79; N, 7.03%. Calcd for $C_{19}H_{30}N_2O_7$: C, 57.27; H, 7.59; N, 7.03%.

Ethyl 2-[1-(*N*-*t*-Butoxycarbonyl-L-threonylamino)-(*Z*)-1-propenyl]thiazole-4-carboxylate (27). A solution of **26** (3.74 g, 8.28 mol) in TFA–CH_2Cl_2 (250 ml, 4 : 96 v/v) was stirred at room

temperature for 1 h. The reaction mixture was neutralized with saturated $NaHCO_3$ aqueous solution and then poured into diethyl ether (200 ml). The resulting solution was washed with brine (30 ml×3) and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (1 : 2 v/v) to give **27** as a colorless viscous syrup. Yield 87% (2.97 g). $[\alpha]_D^{24}$ −33.1° (*c* 1.0, MeOH). IR 3364, 2974, 1710 cm^{-1}. ^1H NMR δ = 1.17—1.38 (m, 6H, Et's CH_3 and Thr's CH_3), 1.47 (s, 9H, Boc), 1.88 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.17 (br s, 1H, OH), 4.25—4.48 (m, 2H, Thr's α-H and β-H), 4.43 (q, 2H, Et's CH_2, *J* = 7.0 Hz), 5.71 (br d, 1H, CHN*H*, *J* = 7.0 Hz), 6.58 (q, 1H, CH_3CH=, *J* = 7.3 Hz), 7.94 (br s, 1H, NHC=), 8.00 (s, 1H, Tz's H). Found: C, 51.81; H, 6.80; N, 9.67%. Calcd for $C_{18}H_{27}N_3O_6S$: C, 52.29; H, 6.58; N, 10.16%.

Ethyl 2-[1-(*N*-*t*-Butoxycarbonyl-*O*-*t*-butyldiphenylsilyl-L-threonylamino)-(*Z*)-1-propenyl]thiazole-4-carboxylate (28). To a chilled solution of **27** (2.69 g, 6.54 mmol) in CH_2Cl_2 (50 ml) were added, with stirring, imidazole (1.78 g, 26.20 mmol) and TPSCl (2.52 ml, 9.81 mmol) under cooling. After the mixture was stirred for 30 min and overnight at room temperature, the reaction mixture was combined with diethyl ether (30 ml). The resulting solution was washed with brine (30 ml×3) and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of hexane and EtOAc (3 : 1 v/v) to give **28** as a colorless viscous syrup. Yield 92% (3.91 g). $[\alpha]_D^{24}$ −7.3° (*c* 3.8, MeOH). IR 3350, 2932, 2850, 1701 cm^{-1}. ^1H NMR δ = 1.03 (s, 9H, TPS's CH_3×3), 1.13 (d, 3H, Thr's CH_3, *J* = 6.4 Hz), 1.36 (t, 3H, Et's CH_3, *J* = 7.0 Hz), 1.44 (s, 9H, Boc), 1.84 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 4.26—4.57 (m, 2H, Thr's α-H and β-H), 4.41 (q, 2H, Et's CH_2, *J* = 7.0 Hz), 5.48 (d, 1H, CHN*H*, *J* = 7.3 Hz), 6.69 (q, 1H, CH_3CH=, *J* = 7.3 Hz), 7.34—7.73 (m, 10H, Ph×2), 8.05 (s, 1H, Tz's H), 8.37 (br s, 1H, NHC=). Found: C, 61.79; H, 7.02; N, 6.35%. Calcd for $C_{35}H_{45}N_3O_8SSi$: C, 61.99; H, 7.45; N, 6.15%.

2-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-3-(4-ethoxycarbonylthiazol-2-yl)-6-[4-(4-{(1*S*,2*R*)-2-(methoxymethoxy)-1-[(*S*)-2-(methoxymethoxy)propylcarbamoyl]propylcarbamoyl}thiazol-2-yl)thiazol-2-yl]pyridine (32). A solution of **24** (0.39 g, 0.97 mmol) in EtOH (50 ml) was stirred in the presence of 10% Pd–C (0.05 g) under H_2 gas stream at room temperature for 1 h. After removal of Pd–C, the filtrate was concentrated in vacuo to give a residue (**25**) as a reaction intermediate, which was dissolved in DMF (50 ml). To the resulting solution was added **21** (0.70 g, 0.81 mmol). After cooling the solution, BOP (0.54 g, 1.21 mmol) and Et_3N (0.17 ml, 1.21 mmol) were added. After the mixture was stirred for 1 h and at room temperature overnight, the reaction mixture was put together with water (50 ml) and extracted with EtOAc (50 ml×3). The combined extracts were washed with brine (30 ml×3), with 10% citric acid (30 ml×2), and with saturated $NaHCO_3$ aqueous solution (30 ml×2), and then dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (4 : 1 v/v) to give colorless crystals. Recrystallization from hexane–EtOAc gave **32** as colorless crystals. Yield 70% (0.63 g). Mp 106—116 °C. $[\alpha]_D^{24}$ +51.0° (*c* 0.4, MeOH). IR 3370, 3094, 2968, 1758, 1662, 1524 cm^{-1}. ^1H NMR δ = 0.82—0.92 (m, 6H, $CH(CH_3)_2$), 1.09 and 1.16 (each d, 6H, Thr's CH_3 and O–CHCH_3, *J* = 6.3 and 6.3 Hz), 1.32 (t, 3H, Et's CH_3, *J* = 7.3 Hz), 1.41 (s, 9H, Boc), 1.69 (d, 3H, CH_3CH=, *J* = 7.3 Hz), 2.20—2.40 (m, 1H, C*H*$(CH_3)_2$), 3.15—3.19 (m, 2H, NHCH_2CH–O), 3.25 and 3.26 (each s, 6H, MOM's CH_3×2), 3.68—3.74 (m, 1H, NHCH_2C*H*–O), 4.18—4.22 (m, 1H, Thr's β-H), 4.33 (q, 2H, Et's CH_2, *J* = 7.3 Hz), 4.56—4.75 (m, 6H, MOM's CH_2×2, NHC*H*, Thr's α-H), 6.34 (q, 1H, CH_3CH=, *J* = 7.3 Hz), 7.74 (br d, 1H, BocN*H*CH, *J* = 8.6 Hz), 8.04 (br d, 1H, CHN*H*, *J* = 8.9 Hz), 8.22—8.35 (m, 4H, Th's H×2, Py's H and CH_2N*H*), 8.48 (d, 1H, Py's H, *J* = 8.9 Hz), 8.45, 8.58, and 8.59 (each s, 3H, Tz's H×3), 9.80 (s, 1H, NHC=). Found: C, 51.68; H, 5.75; N, 12.18%. Calcd for $C_{48}H_{58}N_{10}O_{11}S$: C, 51.88; H, 5.26; N, 12.60%.

2-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-3-(4-carboxythiazol-2-yl)-6-[4-(4-{(1*S*,2*R*)-2-(methoxymethoxy)-1-[(*S*)-2-(methoxymethoxy)propylcarbamoyl]propylcarbamoyl}thiazol-2-yl)thiazol-2-yl]pyridine (33). To a solution of **32** (0.50 g, 0.45 mmol) in THF–H_2O (40 ml, 3 : 1 v/v) was added 1 M LiOH (0.90 ml, 0.90 mmol), and the mixture was stirred at 0 °C for 3 h. The resulting solution was adjusted to pH 7 with Dowex $50H^+$ and, after removal of the ion exchange resin, the filtrate was concentrated in vacuo to give a residue. The obtained residue was purified on a silica-gel column using a mixture of $CHCl_3$ and MeOH (7 : 1 v/v) to give colorless crystals. Recrystallization from hexane–EtOAc gave **33** as colorless crystals. Yield 92% (0.45 g). Mp 162—168 °C. $[\alpha]_D^{24}$ +48.0° (*c* 0.95, MeOH). IR 3364, 3100, 2962, 1662, 1527 cm^{-1}. ^1H NMR δ = 0.82—0.89 (m, 6H, $CH(CH_3)_2$), 1.08 and 1.15 (each d, 6H, Thr's CH_3 and O–CHCH_3, *J* = 6.3 and 6.3 Hz), 1.38 (s, 9H, Boc), 1.66 (d, 3H, CH_3CH=, *J* = 6.7 Hz), 2.20—2.30 (m, 1H, C*H*$(CH_3)_2$), 3.13—3.19 (m, 2H, NHCH_2CH–O), 3.24 and 3.25 (each s, 6H, MOM's CH_3×2), 3.65—3.75 (m, 1H, NHCH_2C*H*–O), 4.15—4.22 (m, 1H, Thr's β-H), 4.53—4.75 (m, 6H, MOM's CH_2×2, NHC*H*, Thr's α-H), 6.35—6.45 (m, 1H, CH_3CH=), 7.66 (br d, 1H, BocN*H*CH, *J* = 7.9 Hz), 7.90—8.55 (m, 9H, Tz's H×5, Py's H×2, NHC=, and N*H*CH), 9.82 (s, 1H, COOH). Found: C, 49.41; H, 4.93; N, 12.64%. Calcd for $C_{46}H_{53}N_{10}O_{11}S_5 \cdot 2H_2O$: C, 49.36; H, 5.22; N, 12.51%.

2-{2-[(*Z*)-1-{2-[(*R*)-1-*t*-Butoxycarbonylamino-2-methylpropyl]thiazole-4-carbonylamino}-1-propenyl]thiazol-4-yl}-3-(4-{(1*S*,2*R*)-2-*t*-butyldiphenylsilyloxy-1-[(*Z*)-1-(4-ethoxycarbonylthiazol-2-yl)-1-propenylcarbamoyl]propylcarbamoyl}thiazol-2-yl-6-[4-(4-{(1*S*, 2*R*)-2-(methoxymethoxy-1-[(*S*)-2-(methoxymethoxy)propylcarbamoyl]propylcarbamoyl}thiazol-2-yl)-thiazol-2-yl]pyridine (34). A suspension of **28** (0.51 g, 0.74 mmol) and MS4A (0.5 g) in CH_2Cl_2 (30 ml) was stirred at room temperature for 30 min. After adding TFA (15 ml) and stirring under cooling for 1 h, the reaction mixture was made slightly alkaline with Et_3N. The precipitates were filtered off and the filtrate was combined with diethyl ether (30 ml) and then washed with brine (20 ml×2) and finally dried over anhydrous Na_2SO_4. Concentration in vacuo gave ethyl 2-{(*Z*)-1-[3-(*O*-*t*-butyldiphenylsilyl)-L-threonylamino]-1-propenyl}thiazole-4-carboxylate (**29**) as an intermediate, which was dissolved in DMF (20 ml) and then cooled. To the DMF solution were added, with stirring, **33** (0.42 g, 1.40 mmol), BOP (0.25 g, 0.55 mmol), and (*i*-Pr)$_2$NEt (0.01 ml, 0.55 mmol). The resulting solution was stirred for 30 min and then overnight at room temperature. The reaction mixture was put together with water (50 ml) and then extracted with EtOAc (20 ml×3). The combined extracts were washed with 10% citric acid (10 ml×2), with saturated $NaHCO_3$ aqueous solution (10 ml×2), and with brine (10 ml×3) and finally dried over anhydrous Na_2SO_4. Concentration in vacuo gave a residue, which was purified on a silica-gel column using a mixture of $CHCl_3$ and acetone (4 : 1 v/v) to give colorless crystals. Recrystallization from hexane–EtOAc gave **34** as colorless crystals. Yield 60% (0.36 g). Mp 116—118 °C. $[\alpha]_D^{24}$

+15.4° (*c* 0.96, MeOH). IR 3400, 3106, 1962, 1668, 1527 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 0.88 and 0.90 (each d, 6H, $CH(CH_3)_2$, J = 6.9 Hz), 0.96 (s, 9H, TPS's $CH_3\times3$), 1.02, 1.07, and 1.14 (each d, 9H, Thr's $CH_3\times2$, O–$CHCH_3$, J = 6.1 Hz), 1.26 (t, 3H, Et's CH_3, J = 7.3 Hz), 1.40 (s, 9H, Boc), 1.66 and 1.67 (each d, 6H, $CH_3CH=\times2$, J = 7.3 Hz), 2.20—2.32 (m, 1H, $CH(CH_3)_2$), 3.14—3.16 (m, 2H, $NHCH_2CH$–O), 3.23 and 3.24 (each s, 6H, MOM's $CH_3\times2$), 3.68—3.71 (m, 1H, $NHCH_2CH$–O), 4.17—4.25 (m, 1H, Thr's β-H), 4.27 (q, 2H, Et's CH_2, J = 7.3 Hz), 4.55—4.60 (m, 6H, MOM's $CH_2\times2$, Thr's α-H and β-H), 4.71—4.73 (m, 2H, NHC*H*, Thr's α-H), 6.43 and 6.60 (each q, 2H, $CH_3CH=\times2$, J = 7.3 Hz), 7.31—7.33 (m, 11H, TPS's Ph×2, NH), 8.02 (br d, 1H, CHN*H*, J = 8.5 Hz), 8.17—8.31 (m, 6H, CHN*H*×3, Tz's H×2, Py's H), 8.38, 8.43, and 8.47 (each s, 3H, Th's H×3), 8.48 (d, 1H, Py's H, J = 8.3 Hz), 9.84 and 9.92 (each br s, 2H, NHC=×2). Found: C, 55.22; H, 5.51; N, 11.05%. Calcd for $C_{75}H_{89}N_{13}O_{14}S_6Si$: C, 55.71; H, 5.55; N, 11.26%.

Micrococcin P (1). To a solution of **34** (0.16 g, 0.11 mmol) in THF–H_2O (24 ml, 3 : 1 v/v) was added 1M LiOH (0.33 ml, 0.33 mmol), and the mixture was stirred at 0 °C for 30 min. After the mixture was stirred overnight at room temperature, the reaction mixture was adjusted to around neutrality with Dowex $50H^+$ and the ion exchange resin was filtered off. The filtrate was concentrated in vacuo to give a residue, which was stirred in CH_2Cl_2–TFA (30 ml, 3 : 1 v/v) at room temperature for 3 h. Concentration in vacuo gave a residue, which was again dissolved in DMF (100 ml). To the DMF solution were added, with stirring, BOP (57.0 mg, 0.13 mmol) and $(i\text{-Pr})_2$NEt (0.01 ml, 0.33 mmol) under cooling. After the mixture was stirred for 1 h at 0 °C and at room temperature overnight, the reaction mixture was concentrated in vacuo to give crude crystals. The obtained colorless crystals were purified by HPLC using a mixture of hexane and EtOAc (1 : 1 v/v) as the eluent under flow rate 7.6 ml min^{-1} at 40 °C by detecting UV (254 nm) absorption to give **1** as colorless crystals. Yield 37% (0.05 g). IR 3880, 3772, 3718, 3472, 3370, 3070, 2920, 2710, 2566, 2494, 2260, 2032, 1953, 1905, 1857, 1824, 1764, 1722, 1650, 1536 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 0.99 and 1.07 (d, 6H, $CH(CH_3)_2$, J = 6.7 Hz), 1.03 (d, 3H, O–$CHCH_3$, J = 6.1 Hz), 1.11 (d, 3H, Thr's CH_3, J = 6.4 Hz), 1.17 (d, 3H, Thr's CH_3, J = 6.1 Hz), 1.71 and 1.80 (d×2, 6H, $CH_3CH=\times2$, J = 7.0 Hz), 2.59—2.63 (m, 1H, O–$CHCH_3$), 3.05—3.08 (m, 2H, $NHCH_2CH$–O), 3.66—3.70 (m, 1H, $NHCH_2CH$–O), 4.12—4.53 (m, 4H, Thr's α-H×2 and Thr's β-H×2), 4.67 (d, 1H, $HOCHCH_3$, J = 4.6 Hz), 5.13 (d, 1H, Thr's OH, J = 5.2 Hz), 5.27 (dd, 1H, $NHCHCH(CH_3)_2$, J = 7.6 and 9.8 Hz), 5.34 (d, 1H, Thr's OH, J = 5.2 Hz), 6.31—6.42 (m, 2H, $CH_3CH=\times2$), 7.76, 8.22, 8.31, 8.42, and 8.61 (each s, 6H, Tz's H×6), 7.93 (d, 1H, CHN*H*, J = 7.6 Hz), 8.00 (t, 1H, NH, J = 6.1 Hz), 8.08 (d, 1H, NH, J = 8.5 Hz), 8.31 (d, 1H, Py's H, J = 7.6 Hz), 8.44—8.47 (m, 2H, CHN*H* and Py's H), 9.38 and 9.46 (each s, 2H, NHC=×2). ^{13}C NMR (DMSO-d_6) δ = 13.4, 13.8, 18.6, 19.7, 19.9, 20.1, 20.4, 31.8, 46.4, 55.8, 58.1, 58.8, 65.0, 66.6, 66.7, 118.4, 121.8, 122.0, 123.9, 125.1, 125.4, 126.0, 127.2, 128.2, 129.8, 129.9, 131.5, 140.8, 148.4, 148.6, 148.7, 149.1, 149.7, 150.2, 150.4, 152.2, 158.8, 160.0, 160.4, 160.4, 161.7, 163.7, 166.2, 166.5, 168.4, 168.8, 169.6, 170.6. FAB HRMS Found: *m/z* 1252.710. Calcd for $C_{48}H_{49}N_{13}O_9S_6Ag$: $(M+Ag)^+$, 1252.386.

This work was supported in part by Grants-in-Aid for Scientific Research Nos. 08640698 and 10640532 from the Ministry of Education, Science, Sports and Culture.

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