

TOTAL SYNTHESIS OF MACROCYCLIC ANTIBIOTIC, MICROCOCCIN P₁

Kazuo Okumura, Akinori Ito, Dai Yoshioka, and Chung-gi Shin*

Laboratory of Organic Chemistry, Faculty of Technology, Kanagawa University,
Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan

Abstract —Total synthesis of a macrocyclic antibiotic, micrococcin P₁ (**1**), constructed from four segments called Fragments A, B, C, and D, was achieved. The synthesis of a central 2,3,6-trithiazole-substituted pyridine moiety [Fragment A with C (A-C)], followed by coupling with Fragments B and D synthesized independently gave the protected Fragment A-B-C-D. Final deprotection of all the protecting groups and cyclization gave synthetic **1**.

Micrococcin P₁ (**1**),¹ isolated from the culture of *Bacillus pumilus*, is a unique macrocyclic antibiotic. So far, although many similar antibiotics have been isolated from various kinds of strains,² the synthesis of any such antibiotics has not been reported except micrococcin P (**2**).^{3,4} The antibiotic (**1**) includes characteristic structures, a central 2,3,6-trithiazole-substituted pyridine skeleton called Fragment A-C (**13**) and a thiazole-dehydropeptide moiety similar to **2**. However, the two sites of Fragments A and D of **1** are structurally different from those of **2**, as shown in Figure 1. That is, the P₁ (**1**) is constructed from a 2-[(*R*)-2-(1-amino-2-hydroxypropyl)thiazolyl-4] segment in Fragment A and 2-amino-2-butenic acid (Δ Abu) residue in Fragment D, whereas the P (**2**) is comprised of a 2-[(*Z*)-2-(1-amino-1-propen-1-yl)thiazolyl-4]

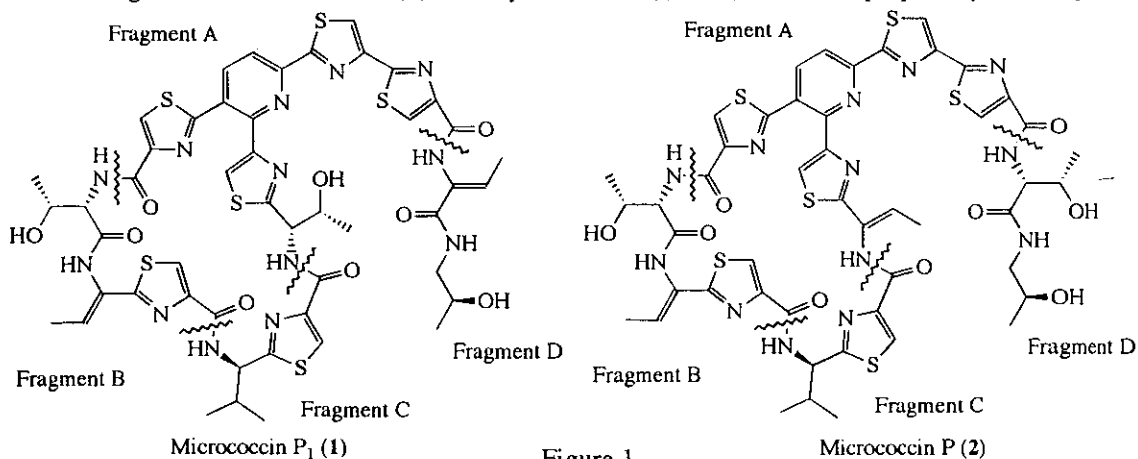
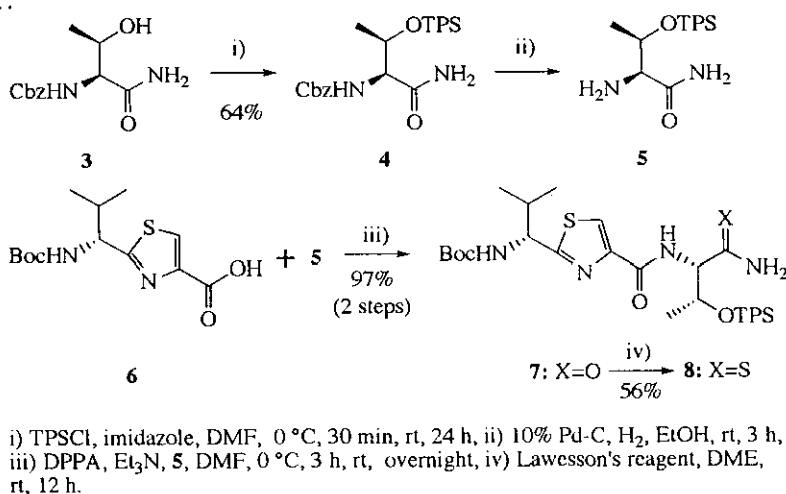


Figure 1.

segment and L-threonine residue. Unfortunately, however, the chemical and physical constants of **1** have not yet been reported except for the ^1H and ^{13}C NMR spectral data.¹ The interesting structure as well as the bioactivity of **1**, which exhibits inhibitory action of bacterial protein synthesis, attracted us to investigate its synthesis and structure-bioactivity relationship. Recently,⁴ we have reported briefly the total synthesis of **2** from versatile 2-bromoacetyl-3-[(4-ethoxycarbonyl)thiazolyl-2]-6-dimethoxymethylpyridine (**9**).⁵ Here, we would like to report on the total synthesis of **1** from **9** via cyclization of Fragment A-B-C-D, after convenient syntheses and couplings of Fragments A, B, C, and D.

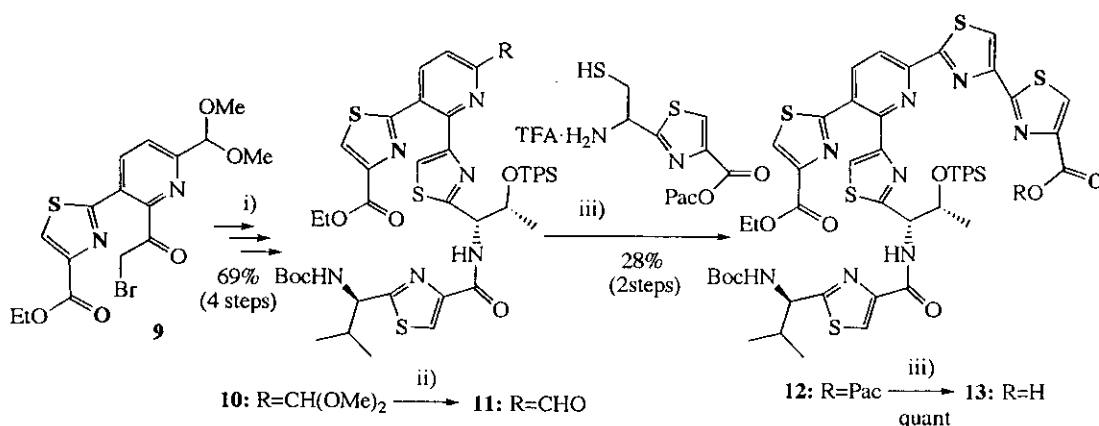
First of all, to synthesize the Fragment A-C so as not to dehydrate the hydroxy group in Fragment A, the *tert*-butyldiphenylsilyl (TPS) group as the protecting group was chosen. Similarly to the case of the synthesis of Fragment A of **2**,⁴ the hydroxyl group on *N*-benzyloxycarbonyl (Cbz)-L-Thr- NH_2 (**3**) was protected with TPSCl to give Cbz-Thr(OTPS)- NH_2 (**4**), the Cbz group of which was hydrogenolyzed with 10% Pd-C to give H-Thr(OTPS)- NH_2 (**5**). The formed **5** was coupled with 2-[(*R*)-2-(*N*-Boc)amino-2-(2-methyl)propyl]thiazole-4-carboxylic acid (**6**; Fragment C)⁶ using diphenylphosphonic azide (DPPA) to give the corresponding dipeptide derivative (**7**). Furthermore, thioamidation of **7** with Lawesson's reagent gave the expected dipeptide thioamide derivative (**8**)⁷ as the precursor for the synthesis of Fragment A-C, as shown in Scheme 1.



Scheme 1.

Subsequently, to construct the Fragment A-C skeleton, thiazolation of **9**⁴ with **8** by using successive KHCO_3 , trifluoroacetic anhydride (TFAA), and 28% NH_3 gave the corresponding 2,3-dithiazolyl-substituted pyridine derivative (**10**). Furthermore, after formylation of **10** with 2M HCl, the immediate thiazolation of the obtained 6-formylpyridine derivative (**11**) with phenacyl (Pac) 2-[(*S*)-(1-amino-2-thiol)propyl]thiazole-4-carboxylate⁴ using trifluoroacetic acid (TFA) and then oxidation with MnO_2 gave the expected 2,3-dithiazolyl-6-bithiazolylpyridine derivative (**12**), by the Shioiri method.⁸ Finally, Pac ester hydrolysis of **12** with 1M LiOH gave the corresponding 6-[4-(carboxy)thiazolyl-2-(4-thiazolyl-2)]pyridine derivative (**13**)⁹ almost quantitatively, as shown in Scheme 2.

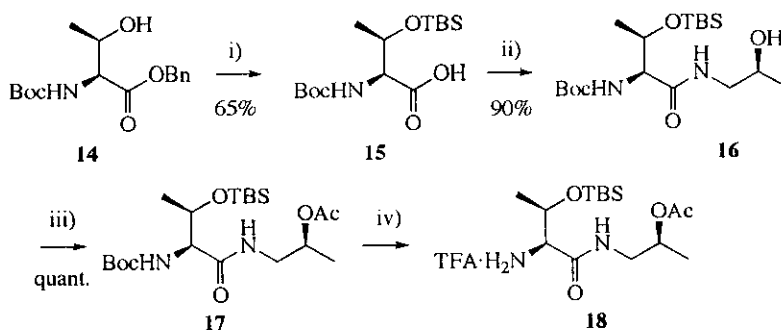
On the other hand, to synthesize the protected Fragment D, first, the protection of Boc-L-Thr-OBn (**14**) with *tert*-butyldibutylsilyl chloride (TBSCl) gave the corresponding Thr(TBS) derivative, the benzyl (Bn) ester of



i) a) **8**, KHCO₃, 0 °C, 30 min, rt, overnight, b) TFAA, pyridine, 0 °C, 2 h, c) 28% NH₃, ethyl acetate, 0 °C, 15 min, ii) 2M HCl-THF, rt, 12 h, iii) a) toluene, rt, 15 min, b) MnO₂, toluene, overnight, iv) 1M LiOH, THF, 0 °C, 2 h.

Scheme 2.

which was hydrogenolyzed with 10% Pd-C to give Boc-L-Thr(TBS)-OH (**15**). Next, coupling of **15** with *S*-(+)-1-aminopropanol using BOP¹⁰ as condensing agent gave Boc-L-Thr(TBS)-NH-(*S*)-(2-hydroxy)propane (**16**), the hydroxyl group of which was acetylated with acetic anhydride (Ac₂O) to give the corresponding (2-acetoxy)propane derivative (**17**) almost quantitatively. Then, deprotection of the Boc group of **17** with TFA in the presence of MS4A (molecular sieves) proceeded smoothly to give the expected *N*-free Thr(TBS)-NH-(2-acetoxy)propane (**18**), as shown in Scheme 3.

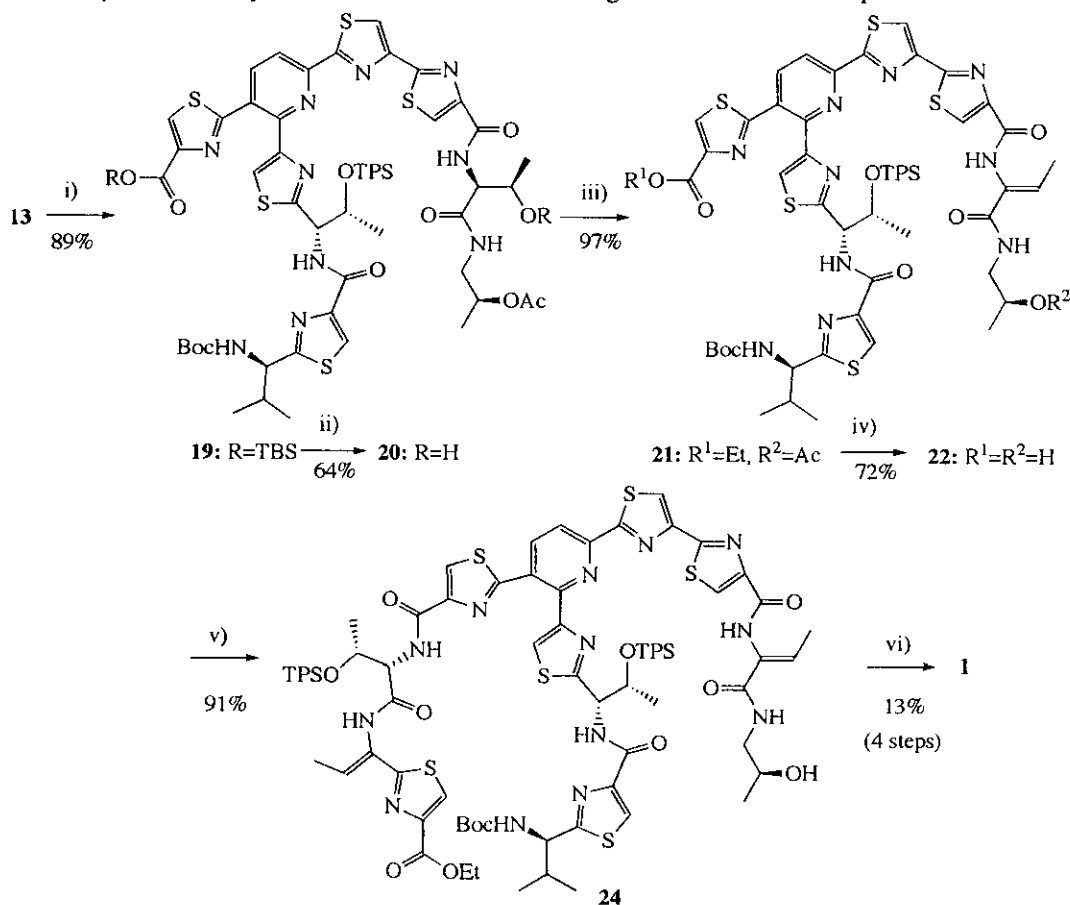


i) a) TBSCl, imidazole, CH₂Cl₂, 0 °C, 30 min, rt, overnight, b) 10%Pd-C, H₂, EtOH, rt, 3 h, ii) (*S*)-(+)-1-aminopropanol, BOP, (*i*-Pr)₂NEt, CH₃CN, 0 °C, 30 min, rt, overnight, iii) Ac₂O, pyridine, rt, 2 h, iv) TFA / CH₂Cl₂, rt, 1 h, MS4A.

Scheme 3.

Finally, the protected Fragment D (**18**) as the precursor was subjected to the coupling with **13** and then β -elimination. Fragment condensation of **13** with **18** by the BOP method gave the protected Fragment A-C derivative (**19**). Selective deprotection of only the TBS group of the side chain of **19** with 70% AcOH was tried successfully to give the corresponding Fragment A-C(**20**)¹¹ containing Thr residue, the hydroxy group of which was mesylated with methanesulfonyl chloride (MsCl) and then β -eliminated with 1,8-diazabicyclo[5.4.0]undecene-7 (DBU) to give the protected Fragment A-C-D (**21**). Then, both ester and acetyl hydrolyses of **21** with 1M LiOH were carried out to give the corresponding 3-[4-carboxythiazolyl-2]pyridine derivative (**22**).¹² Furthermore, condensation of the carboxyl group in **22**

with ethyl 2-[(Z)-1-(*O*-TPS-L-Thr)amino-1-propen-1-yl]thiazole-4-carboxylate (**23**)¹³ by the BOP method gave the protected Fragment A-B-C-D (**24**).¹⁴ After ester hydrolysis of **24** using 1M LiOH, consecutive deprotections of the both PTS and Boc groups with a mixture of TFA and CH₂Cl₂ (4 : 6 v/v), and cyclization by the BOP method gave a crude micrococcin P₁, as shown in Scheme 4. The obtained reddish syrup was purified on a silica gel column using a mixture of CHCl₃ and MeOH (15 : 1 v/v) as the eluent to give crude crystals. Recrystallization from MeOH-EtOAc gave **1**¹⁵ as a colorless powder.



i) **18**, BOP, (*i*-Pr)₂NEt, DMF, 0 °C, 30 min, rt, overnight, ii) 70%AcOH-THF, rt, 72 h, iii) a) MsCl, Et₃N, CH₂Cl₂, 0 °C, 10 min, b) DBU, CH₂Cl₂, 0 °C, 30 min, rt, overnight, iv) 1M LiOH, THF, 0 °C, 3 h, v) **23**, BOP, (*i*-Pr)₂NEt, DMF, 0 °C, 30 min, rt, overnight, iv) (a) 1M LiOH, THF, 0 °C, 1 h, rt, 6 h, (b) TFA:CH₂Cl₂=40:60, rt, 3 h, (c) BOP, (*i*-Pr)₂NEt, DMF, 0 °C, 1 h, rt, overnight, d) TBAF, THF, rt, 30 min.

Scheme 4.

The chemical and physical constants of the synthetic **1** {mp 215-245 °C, [α]_D²⁴ +40.0° (c 0.50, 90% EtOH, λ_{max} 324.6 nm)} were first obtained. Furthermore, it was found that the ¹H and ¹³C NMR spectral data were identical with all of those of the natural **1**. Accordingly, the configurational structure of **1** could be clearly confirmed by the identification of the physical constants (NMR) as well as satisfactory elemental analysis.

In conclusion, the total synthesis of **1** was achieved by the effective selections of the protecting groups and the cyclization conditions as well as the useful synthesis of the promising 2,3,6-trithiazole-substituted

pyridine skeleton.

ACKNOWLEDGEMENT

This work was supported in part by Grant-in-Aid for Scientific Research No. 08640698 from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. B. W. Bycroft and M. S. Gowland, *J. Chem. Soc., Chem. Commun.*, **1978**, 256.
2. For examples: M. Debono, R. M. Molly, J. L. Occlowitz, J. W. Paschel, A. H. Hunt, K. H. Michel, and J. W. Martin, *J. Org. Chem.*, 1992, **57**, 5200.
3. a) P. Brooks, A. T. Fuller, and J. Walker, *J. Chem. Soc.*, **1957**, 689. b) J. Walker, A. Olesker, L. Valente, R. Rabanal, and G. Lukacs, *J. Chem. Soc., Chem. Commun.*, **1977**, 706.
4. C. Shin, K. Okumura, M. Shigekuni, and Y. Nakamura, *Chem. Lett.*, **1998**, 139.
5. K. Okumura, M. Shigekuni, Y. Nakamura, and C. Shin, *Chem. Lett.*, **1996**, 1025.
6. U. Schmidt, P. Gleich, and H. Griesser, *Synthesis*, **1986**, 992.
7. **8**: Colorless powder. mp 95-96 °C. $[\alpha]_D^{24}$ -26.6° (c 0.58, MeOH). IR (KBr): 3400, 2926, 2230, 1896, 1716 cm^{-1} . ^1H NMR (CDCl_3): δ 0.87 and 0.88 (each d, 3H x 2, Ip's CH_3 x 2, $J=6.4$ Hz), 0.90 (d, 3H, Thr's CH_3 , $J=6.7$ Hz), 0.95 (s, 9H, TPS's CH_3 x 3), 1.40 (s, 9H, Boc), 2.25-2.288 (m, 1H, Ip's CH), 4.40-4.60 (m, 1H, Thr's β -H), 4.67-4.70 (m, 2H, Thr's α -H and $\text{CHNH}(\text{Boc})$), 7.36-7.64 (m, 10H, TPS's Ph x 2), 7.74 (br d, 1H, NH, $J=8.2$ Hz), 8.21 (s, 1H, Thiazole ring H), 8.25 (br d, 1H, NH, $J=9.1$ Hz), 9.52 (br s, 1H, NH), 9.85 (br s, 1H, NH).
8. Y. Hamada, M. Shibata, T. Sugiura, S. Kato, and T. Shioiri, *J. Org. Chem.*, 1987, **52**, 1252.
9. **13**: Pale yellow powder. mp 169-170 °C. $[\alpha]_D^{24}$ -20.0° (c 0.03, MeOH). IR (KBr): 3394, 3106, 2986, 1884, 1782, 1680, 1611 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$): δ 0.84-0.96 (m, 9H, Ip's CH_3 x 2 and $\text{CH}(\text{CH}_3)\text{OTPS}$), 0.87 (s, 9H, TPS's CH_3 x 3), 1.24 (t, 3H, Et's CH_3 , $J=7.3$ Hz), 1.41 (s, 9H, Boc), 2.20-2.30 (m, 1H, Ip's CH), 4.24 (q, 2H, Et's CH_2 , $J=7.3$ Hz), 4.35-4.43 (m, 1H, $\text{CH}(\text{CH}_3)\text{OTPS}$), 4.60-4.70 (m, 1H, BocNHCH), 5.15-5.28 (m, 1H, NHCH), 7.17-7.53 (m, 11H, TPS's Ph x 2 and Pyridine ring H), 7.77 (br d, 1H, NH, $J=8.2$ Hz), 8.05, 8.19, 8.28, 8.29, 8.36 (s x 5, 5H, Thiazole ring H x 5), 8.32 (d, 1H, Py's H, $J=8.2$ Hz), 8.47 (br d, 1H, NH, $J=8.6$ Hz), 8.73 (br s, 1H, COOH).
10. BOP=Benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate.
11. **20**: Pale yellow powder. mp 116-117.5 °C. $[\alpha]_D^{24}$ +35.9° (c 0.69, MeOH). IR (KBr): 3718, 3406, 3094, 2938, 2242, 1983, 1788, 1653 cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$): δ 0.86-0.90 (m, 18H, TPS's CH_3 x 3, $\text{CH}(\text{CH}_3)\text{OTPS}$ and Ip's CH_3 x 2), 1.11 (d, 3H, Thr's CH_3 , $J=6.4$ Hz), 1.13 (d, 3H, Aminopropanol's CH_3 , $J=6.4$ Hz), 1.25 (t, 3H, Et's CH_3 , $J=7.0$ Hz), 1.41 (s, 9H, Boc), 1.97 (s, 3H, COCH_3), 2.20-2.30 (m, 1H, Ip's CH), 3.19-3.36 (m, 2H, Aminopropanol's CH_2), 4.08-4.13 (m, 1H, Thr's β -H), 4.25 (q, 2H, Et's CH_2 , $J=7.0$ Hz), 4.37-4.40 (m, 2H, Thr's α -H and $\text{CH}(\text{CH}_3)\text{OTPS}$), 4.66-4.69 (m, 1H, BocNHCH), 4.81-4.86 (m, 1H, Aminopropanol's CH), 5.10 (d, 1H, OH, $J=5.2$ Hz), 5.22-5.24 (m, 1H, NHCH), 7.20-7.55 (m, 10H, TPS's Ph x 2), 7.79 (br d, 1H,

- NH, $J=8.6$ Hz), 8.06 (br d, 1H, NH, $J=8.6$ Hz), 8.14 (br t, 1H, NH, $J=5.8$ Hz), 8.25, 8.30, 8.30, 8.42, 8.61 (s x 5, 5H, Thiazole ring H x 5), 8.31-8.40 (m, 1H, Pyridine ring H), 8.39 (br d, 1H, NH, $J=8.5$ Hz), 8.51 (d, 1H, Pyridine ring H, $J=8.2$ Hz).
12. **22**: Pale yellow powder. mp 204-205 °C. $[\alpha]_D^{24}$ -0.6° (c 0.33, MeOH). IR (KBr): 3376, 2956, 1995, 1887, 1656 cm^{-1} . ^1H NMR (CDCl_3): δ 0.85-0.89 (m, 9H, $\text{CH}(\text{CH}_3)\text{OTPS}$ and Ip's CH_3 x 2), 0.89 (s, 9H, TPS's CH_3 x 3), 1.06 (d, 3H, Aminopropanol's CH_3 , $J=6.4$ Hz), 1.40 (s, 9H, Boc), 1.68 (d, 3H, ΔAbu 's CH_3 , $J=7.0$ Hz), 2.20-2.30 (m, 1H, Ip's CH), 3.02-3.12 (m, 2H, Aminopropanol's CH_2), 3.69-3.72 (m, 1H, Aminopropanol's CH), 4.30-4.40 (m, 1H, $\text{CH}(\text{CH}_3)\text{OTPS}$), 4.60-4.75 (m, 2H, OH and BocNHCH), 5.10-5.25 (m, 1H, NHCH), 6.50 (q, 1H, ΔAbu 's $\text{CH}=\text{}$, $J=7.0$ Hz), 7.15-7.55 (m, 11H, TPS's Ph x 2 and Thiazole ring H), 7.80 (br d, 1H, NH, $J=7.9$ Hz), 7.90-8.00 (m, 2H, NH and Pyridine ring H), 8.11, 8.33, 8.44, 8.58 (s x 4, 4H, Thiazole ring H x 4), 8.20-8.70 (m, 1H, COOH), 8.30-8.40 (m, 1H, Pyridine ring H), 8.52 (br d, 1H, NH, $J=8.2$ Hz), 9.50 (br s, 1H, NH).
13. Y. Nakamura, C. Shin, K. Umemura, and J. Yoshimura, *Chem. Lett.*, **1992**, 1005.
14. **24**: Colorless powder. mp 132.0-133.5 °C. $[\alpha]_D^{24}$ +11.0° (c 0.49, MeOH). IR (KBr): 3370, 3064, 2932, 2248, 1962, 1659 cm^{-1} . ^1H NMR (CDCl_3): δ 0.85-0.91 (m, 12H, TPS's CH_3 x 3 and $\text{CH}(\text{CH}_3)\text{OTPS}$), 0.87 (d, 6H, Ip's CH_3 , $J=6.4$ Hz), 0.97 (s, 9H, TPS's CH_3 x 3), 1.01-1.03 (m, 6H, Thr's CH_3 and Aminopropanol's CH_3), 1.26 (t, 3H, Et's CH_3 , $J=7.3$ Hz), 1.41 (s, 9H, Boc), 1.66 (d, 3H, ΔAbu 's CH_3 , $J=7.0$ Hz), 1.69 (d, 3H, $\text{CH}(\text{CH}_3)\text{OTPS}$, $J=7.0$ Hz), 2.20-2.30 (m, 1H, Ip's CH), 3.03-3.13 (m, 2H, Aminopropanol's CH_2), 3.69-3.73 (m, 1H, Aminopropanol's CH), 4.27 (q, 2H, Et's CH_2 , $J=7.3$ Hz), 4.53-4.57 (m, 2H, $\text{CH}(\text{CH}_3)\text{OTPS}$ and Thr's $\beta\text{-H}$), 4.62 (d, 1H, OH, $J=4.4$ Hz), 4.65-4.75 (m, 2H, Thr's $\alpha\text{-H}$ and BocNHCH), 5.27-5.29 (m, 1H, NHCH), 6.50 (q, 1H, ΔAbu 's $\text{CH}=\text{}$, $J=7.0$ Hz), 6.59 (q, 1H, $\text{CH}(\text{CH}_3)\text{OTPS}$, $J=7.0$ Hz), 7.16-7.66 (m, 20H, TPS's Ph x 4), 7.77 (br d, 1H, NH, $J=7.6$ Hz), 7.89 (br t, 1H, NH, $J=5.5$ Hz), 8.21, 8.26, 8.33, 8.36, 8.45, 8.60 (s x 6, 6H, Thiazole ring H x 6), 8.25-8.30 (m, 2H, NH and Pyridine ring H), 8.50 (br d, 1H, NH, $J=8.2$ Hz), 8.54 (d, 1H, Pyridine ring H, $J=8.2$ Hz), 9.47 (br s, 1H, NH), 9.89 (br s, 1H, NH).
15. Micrococcin P₁ (**1**): Colorless powder. mp 215-245 °C. $[\alpha]_D^{24}$ +40.0° (c 0.5, 90% EtOH). λ_{max} (nm) 324.6. IR (KBr): 3414, 1653, 1527 cm^{-1} . ^1H NMR ($\text{CDCl}_3\text{-CD}_3\text{OD}$): δ 0.78 (d, 3H, $\text{CH}(\text{CH}_3)\text{OTPS}$, $J=6.5$ Hz), 1.15 (d, 3H, Aminopropanol's CH_3 , $J=6.5$ Hz), 1.19 (d, 3H, Ip's CH_3 , $J=7.0$ Hz), 1.31 (d, 3H, Thr's CH_3 , $J=7.0$ Hz), 1.34 (d, 3H, Thr's CH_3 , $J=6.5$ Hz), 1.84 (d, 3H, ΔAbu 's CH_3 , $J=7.0$ Hz), 1.85 (d, 3H, ΔAbu 's CH_3 , $J=7.0$ Hz), 2.82-2.92 (m, 1H, Ip's CH), 3.14-3.37 (m, 2H, Aminopropanol's CH_2), 3.88-3.91 (m, 1H, Aminopropanol's CH), 4.09-4.11 (m, 1H, $\text{CH}(\text{CH}_3)\text{OTPS}$), 4.52-4.53 (m, 1H, $\text{CH}(\text{CH}_3)\text{OTPS}$), 4.54-4.56 (m, 1H, Thr's $\beta\text{-H}$), 4.71-4.77 (m, 1H, Thr's $\alpha\text{-H}$), 5.18 (d, 1H, Ip's CH, $J=9.0$ Hz), 6.57 (q, 1H, $\text{CH}=\text{}$, $J=7.0$ Hz), 6.71 (q, 1H, $\text{CH}=\text{}$, $J=7.0$ Hz), 7.78, 7.98, 8.08, 8.25, 8.29, 8.41 (each s, 6H, Thiazole ring H x 6), 8.17 and 8.34 (d, 1H, Pyridine ring H, $J=8.5$ Hz). ^{13}C NMR ($\text{CDCl}_3\text{-CD}_3\text{OD}$): δ 12.1, 14.0, 19.2, 20.6, 20.7, 20.7, 21.2, 33.1, 47.8, 55.1, 59.2, 60.7, 67.0, 67.3, 69.2, 119.1, 121.6, 122.5, 124.4, 125.0, 126.1, 126.6, 129.4, 130.3, 130.5, 131.6, 131.6, 140.9, 148.6, 149.7, 150.1, 150.6, 150.8, 151.6, 152.1, 153.2, 161.5, 162.1, 162.8, 163.3, 163.6, 166.8, 167.2, 167.5, 169.3, 169.7, 172.3, 172.7.