TOTAL SYNTHESIS OF MACROCYCLIC ANTIBIOTIC, MICROCOCCIN P_1

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Abstract –Total synthesis of a macrocyclic antibiotic, micrococcin P_1 (**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 P_1 (**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 P_1 (**1**) is constructed from a 2-[(*R*)-2-(1-amino-2-hydroxypropyl)thiazolyl-4] segment in Fragment A and 2-amino-2-butenoic acid (ΔAbu) residue in Fragment D, whereas the P (**2**) is comprised of a 2-[(*Z*)-2-(1-amino-1- propen-1-yl)thiazolyl-4]

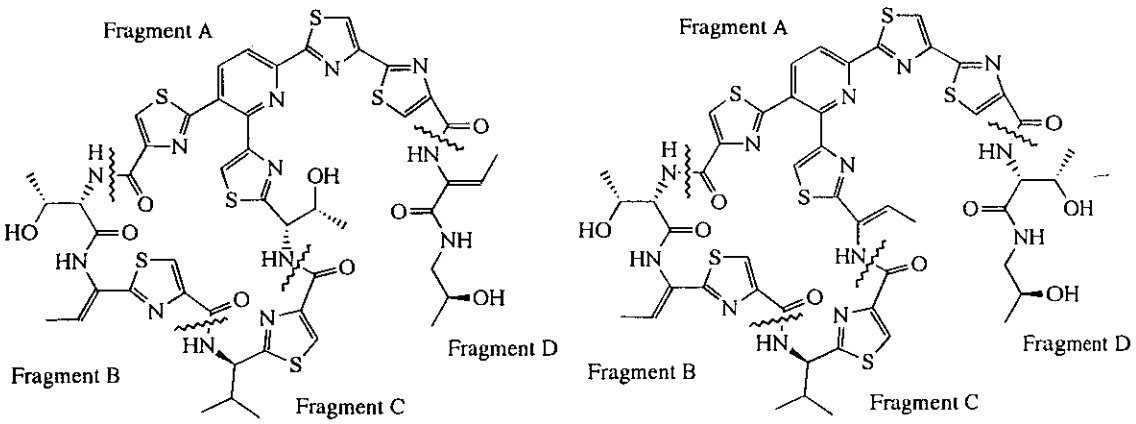
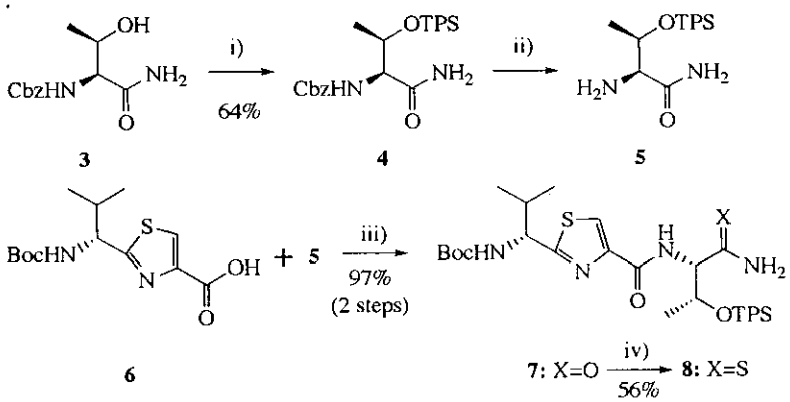


Figure 1.

segment and L-threonine residue. Unfortunately, however, the chemical and physical constants of **1** have not yet been reported except for the ^{1}H 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(TPS)-NH2 (**4**), the Cbz group of which was hydrogenolyzed with 10% Pd-C to give H-Thr(TPS)-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.

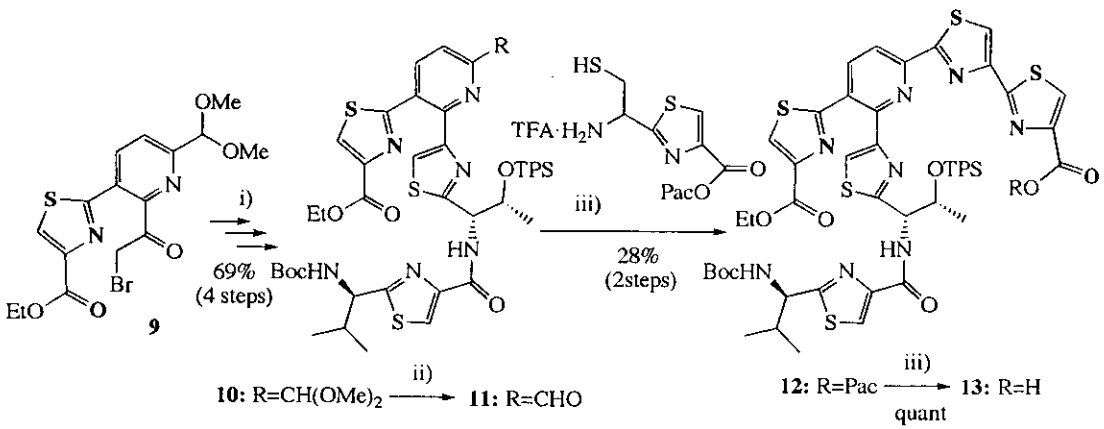


i) TPSCl, imidazole, DMF, 0 °C, 30 min, rt, 24 h, ii) 10% Pd-C, H_2, EtOH, rt, 3 h, iii) DPPA, Et_3N, **5**, DMF, 0 °C, 3 h, rt, overnight, iv) Lawesson's reagent, DME, rt, 12 h.

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 thiazolination of the obtained 6-formylpyridine derivative (**11**) with phenacyl (Pac) 2-[(*S*)-(1-amino-2-thiol)propyl]thiazole-4-carboxylate[4] 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.

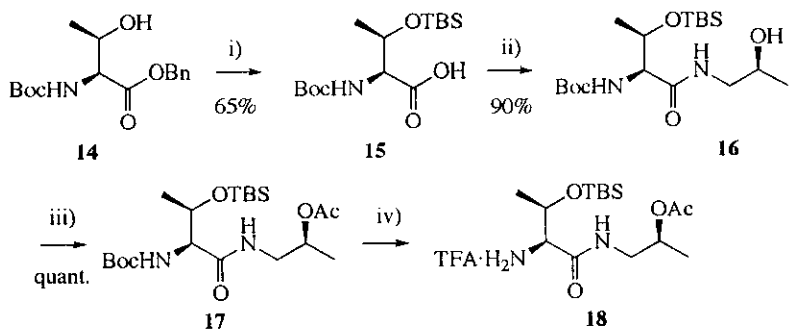
On the other hand, to synthesize the protected Fragment D, first, the protection of Boc-L-Thr-OBn (**14**) with *tert*-butyldibutylsilyl chloride (TBSCl) gave the corresponding Thr(TBS) derivative, the benzyl (Bn) ester of



i) a) **8**, $KHCO_3$, 0 °C, 30 min, rt, overnight, b) TFAA, pyridine, 0 °C, 2 h, c) 28% NH_3, ethyl acetate, 0 °C, 15 min, ii) 2M HCl-THF, rt, 12 h, iii) a) toluene, rt, 15 min, b) MnO_2 , 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 BOP[10] as condensing agent gave Boc-L-Thr(TBS)-NH-(*S*)-(2-hydroxy)propane (**16**), the hydroxyl group of which was acetylated with acetic anhydride (Ac_2O) 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.

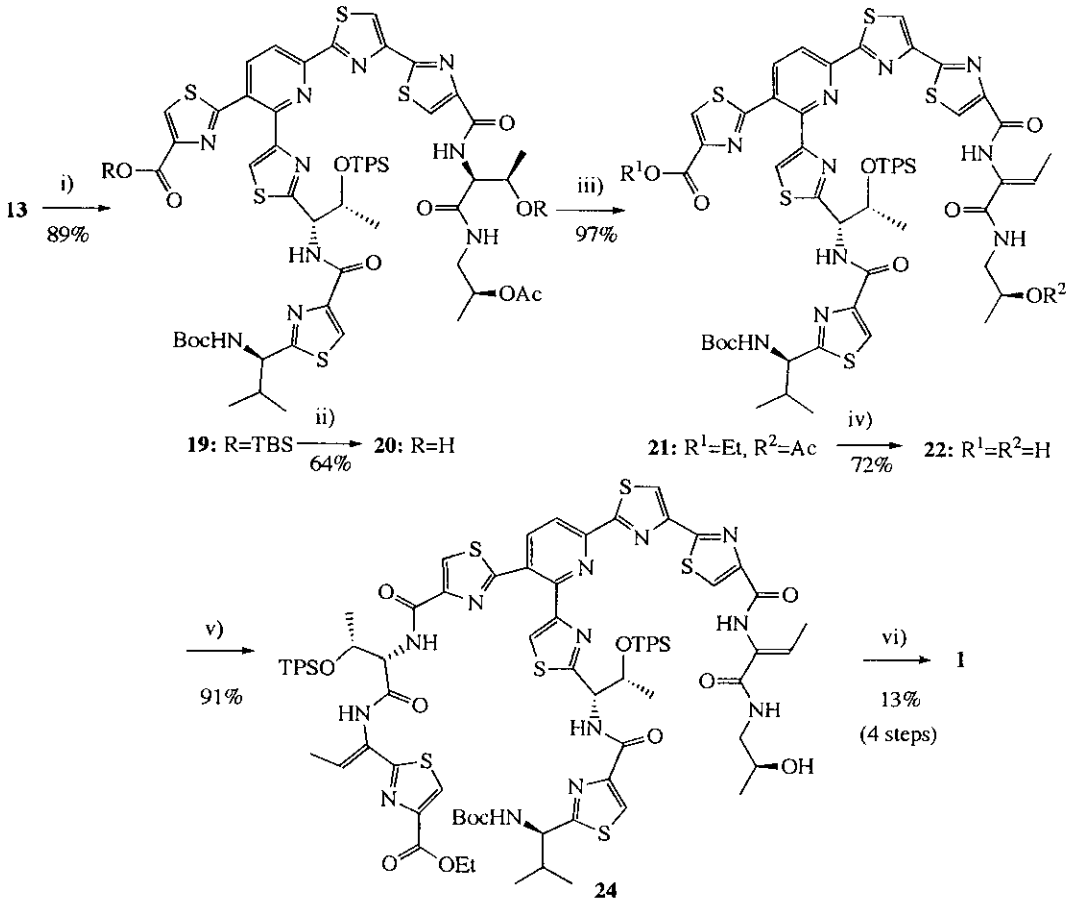


i) a) TBSCl, imidazole, CH_2Cl_2, 0 °C, 30 min, rt, overnight, b) 10%Pd-C, H_2, EtOH, rt, 3 h, ii) (*S*)-(+)-1-aminopropanol, BOP, $(i\text{-Pr})_2NEt$, CH_3CN, 0 °C, 30 min, rt, overnight, iii) Ac_2O, pyridine, rt, 2 h, iv) TFA / CH_2Cl_2, rt, 1 h, MS4A.

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 CH_2Cl_2 (4 : 6 v/v), and cyclization by the BOP method gave a crude micrococcin P_1, as shown in Scheme 4. The obtained reddish syrup was purified on a silica gel column using a mixture of $CHCl_3$ 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)$_2$NEt, DMF, 0 °C, 30 min, rt, overnight, ii) 70%AcOH-THF, rt, 72 h, iii) a) MsCl, Et_3N, CH_2Cl_2, 0 °C, 10 min, b) DBU, CH_2Cl_2, 0 °C, 30 min, rt, overnight, iv) 1M LiOH, THF, 0 °C, 3 h, v) **23**, BOP, (*i*-Pr)$_2$NEt, DMF, 0 °C, 30 min, rt, overnight, iv) (a) 1M LiOH, THF, 0 °C, 1 h, rt, 6 h, (b) TFA:CH_2Cl_2=40:60, rt, 3 h, (c) BOP, (*i*-Pr)$_2$NEt, 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, $[\alpha]_D^{24}$ +40.0° (c 0.50, 90% EtOH, λmax 324.6 nm} were first obtained. Furthermore, it was found that the ^1H and ^{13}C 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

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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 C*H*NHBoc), 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).
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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 (DMSO-d_6): δ 0.84-0.96 (m, 9H, Ip's CH_3 x 2 and CH(C*H*$_3$)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, C*H*(CH$_3$)OTPS), 4.60-4.70 (m, 1H, BocNHC*H*), 5.15-5.28 (m, 1H, NHC*H*), 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 (DMSO-d_6): δ 0.86-0.90 (m, 18H, TPS's CH_3 x 3, CH(C*H*$_3$)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 C*H*(CH$_3$) OTPS), 4.66-4.69 (m, 1H, BocNHC*H*), 4.81-4.86 (m, 1H, Aminopropanol's CH), 5.10 (d, 1H, OH, *J*=5.2 Hz), 5.22-5.24 (m, 1H, NHC*H*), 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 (CDCl3): δ 0.85-0.89 (m, 9H, CH(C*H*$_3$)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, C*H*(CH$_3$)OTPS), 4.60-4.75 (m, 2H, OH and BocNHC*H*), 5.10-5.25 (m, 1H, NHC*H*), 6.50 (q, 1H, ΔAbu's CH=, *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).
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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 CH(C*H*$_3$)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, CH(C*H*$_3$)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, C*H*(CH$_3$)OTPS and Thr's β-H), 4.62 (d, 1H, OH, *J*=4.4 Hz), 4.65-4.75 (m, 2H, Thr's α-H and BocNHC*H*), 5.27-5.29 (m, 1H, NHC*H*), 6.50 (q, 1H, ΔAbu's CH=, *J*=7.0 Hz), 6.59 (q, 1H, C*H*(CH$_3$)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 P$_1$ (**1**): Colorless powder. mp 215-245 °C. $[\alpha]_D^{24}$ +40.0° (c 0.5, 90% EtOH). λmax (nm) 324.6. IR (KBr): 3414, 1653, 1527 cm^{-1}. ^1H NMR ($CDCl_3$-CD_3OD): δ 0.78 (d, 3H, CH(C*H*$_3$)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, C*H*(CH$_3$)OTPS), 4.52-4.53 (m, 1H, C*H*(CH$_3$)OTPS), 4.54-4.56 (m, 1H, Thr's β-H), 4.71-4.77 (m, 1H, Thr's α-H), 5.18 (d, 1H, Ip's CH, *J*=9.0 Hz), 6.57 (q, 1H, CH=, *J*=7.0 Hz), 6.71 (q, 1H, CH=, *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 ($CDCl_3$-CD_3OD): 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.

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