Total Synthesis of Antibiotic, Micrococcin P, from 2,3,6-Polythiazolesubstituted Pyridine Skeleton [Fragment A-C]

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(Received October 31, 1997; CL-970839)

Total synthesis of an antibiotic, micrococcin P, was first accomplished by the coupling of a 2,3,6-polythiazole-substituted pyridine skeleton [Fragment A-C] with the independently synthesized Fragments B and D, and then deprotection with trifluoroacetic acid and final intramolecular cyclization using BOP as condensing agent.

Micrococcin P (**1**),[1] isolated from the culture of *Bacillus pumilus*, is a unique macrocyclic peptide antibiotic, as shown in Figure 1. Many similar antibiotic peptides have been also isolated from various kinds of strains.[2] The peptide (**1**) includes a characteristic main structure, a 2,3,6-polythiazole-substituted pyridine skeleton called Fragment A-C (**2**) composed of multi-substituted pyridine and thiazole-dehydropeptide moieties. Not only the interesting structure but also the bioactivity of **1**, which exhibits inhibitory action of bacterial protein synthesis, attracted us to investigate its total synthesis and structure-bioactivity relationship.

So far, the total synthesis of any such peptide antibiotics has not been reported. Recently,[3] however, we have reported briefly on the useful synthesis of a very promising precursor Fragment A-C segment (**2**) from 3-cyano-6-dimethoxymethyl-2-pyridone via 2-bromoacetyl-3-[5-ethoxycarbonyl]thiazole-6-dimethoxymethyl-pyridine in twelve steps. Here, after the novel syntheses of the two important residual segments, Fragments B and D, the first total synthesis of **1** was achieved from **2** via the fragment condensations of the two terminal thiazole carboxylic acids with the obtained two Fragments, and subsequent deprotection and final cyclization.

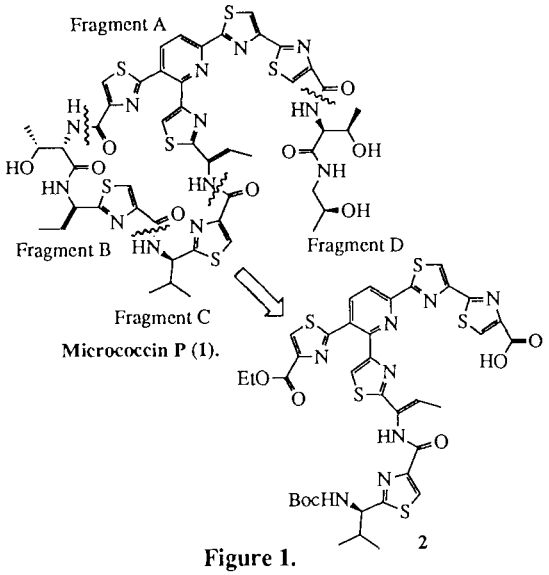
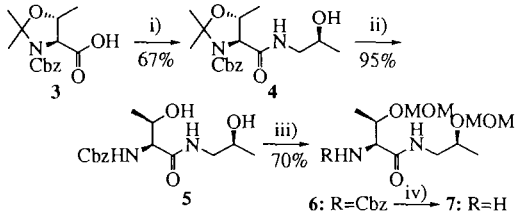


Figure 1.

First of all, in order to synthesize the protected threonine (Thr)-propanolamide called Fragment D (**7**), condensation of *N*-benzyloxycarbonyl (Cbz)-*N*,*O*-isopropylidene (Isop)-Thr-OH (**3**)[4] with (*S*)-(+)-1-aminopropanol using BOP[5] as condensing agent in the presence of $(i\text{-Pr})_2$NEt gave the corresponding Thr-amide derivative (**4**), the Isop group of which was then deprotected with 3M HCl. The obtained *O*-free-Thr-propanolamide (**5**)[6] was again protected with chloromethyl methyl ether (MOMCl) to give the corresponding di-*O*-protected Thr-amide derivative (**6**).[7] The catalytic hydrogenolytic deprotection of the Cbz group with 10% Pd-C under H_2 atmosphere then gave the expected *O*-protected Fragment D (**7**) almost quantitatively, as shown in Scheme 1.

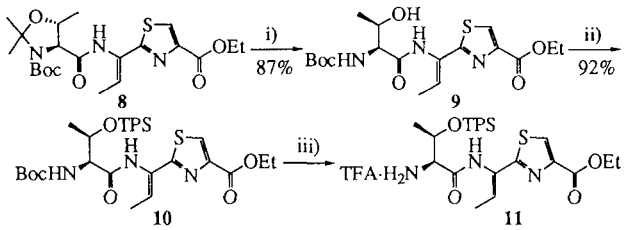


i) (S)-(+)-1-Aminopropanol, BOP, $(i\text{-Pr})_2$NEt, CH_3CN, 0 °C, 30 min., r.t., overnight, ii) 3M HCl, 40 °C, overnight, iii) MOMCl, $(i\text{-Pr})_2$NEt, CH_2Cl_2, 0 °C, 30 min., r.t., 24 h, iv) 10%Pd-C, H_2, EtOH, r.t., 30min.

Scheme 1.

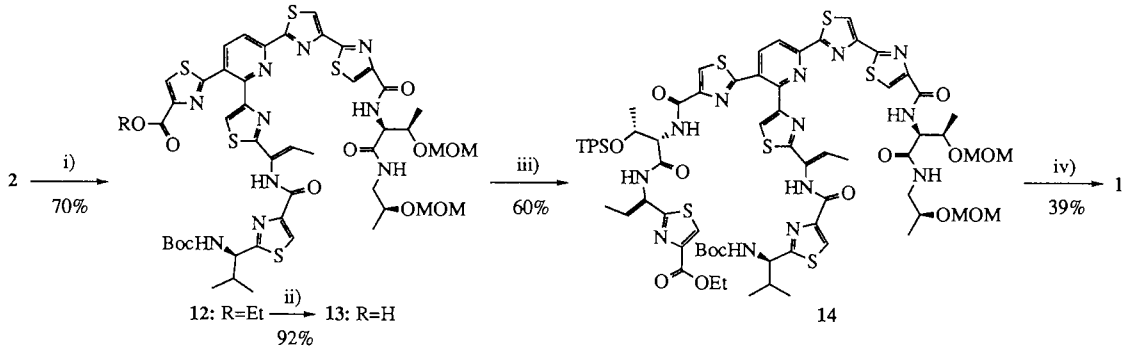
On the other hand, to synthesize the protected Fragment B moiety (**11**), deprotection of the Isop group of ethyl 2-[(*Z*)-(*N*-Boc-*N*,*O*-Isop-L-Thr)amino-1-propen-1-yl]thiazole-4-carboxylate (**8**)[4] with trifluoroacetic acid (TFA) gave the corresponding (*N*-Boc-Thr)aminothiazole-dehydrodipeptide derivative (**9**). The hydroxyl group of **9** was again protected with *t*-butyldiphenylsilyl chloride (TPSCl) in the presence of imidazole to give the corresponding (*N*-Boc-*O*-TPS-Thr)amino-dehydrodipeptide derivative (**10**),[8] the Boc group of which was then deprotected with TFA in CH_2Cl_2 in the presence of 4AMS (molecular sieves) to give (*O*-TPS-Thr)amino-dehydrodipeptide derivative (**11**) as an unstable syrup, as shown in Scheme 2. The obtained Fragments B and D segments were subjected successively to coupling with the two carboxylic acid groups of the 2-thiazole and 6-bithiazole moieties of the pyridine skeleton in **2**, respectively.

Finally, to accomplish the synthesis of **1**, firstly, coupling of **2** with **7** using BOP and $(i\text{-Pr})_2$NEt in DMF at 0 °C for 30 min and



i) TFA:CH_2Cl_2=4:96, r.t., 1 h, ii) TPSCl, Imidazole, CH_2Cl_2, 0 °C, 30 min., r.t., overnight, iii) TFA:CH_2Cl_2=40:60, 4AMS, r.t., 1h.

Scheme 2.



i) 7, BOP, (i-Pr)$_2$NEt, DMF, 0 °C, 30 min., r.t., 90 min., ii) 1M LiOH, THF, 0 °C, 3 h, iii) 11, 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., 3h, (c) BOP, (i-Pr)$_2$NEt, DMF, 0 °C, 1 h, r.t., overnight.

Scheme 3.

then at room temperature (r.t.) for 90 min gave the corresponding Fragment A-C-D segment (**12**). Secondly, after the ester hydrolysis of **12** with 1M LiOH at 0 °C, the obtained hydrolyzate (**13**)[9] was further elongated by the coupling with **11** to give the protected linear precursor (Fragment A-B-C-D) (**14**)[10] of micrococcin P. Subsequently, successive hydrolysis of ethyl ester of **14** with 1M LiOH and deprotection of the Boc, MOM, and TPS groups with TFA, followed by the intramolecular coupling using BOP and (i-Pr)$_2$NEt in one-pot gave a crude micrococcin P, as shown in Scheme 3. The obtained colorless crystals were chromatographed 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**[11] as colorless needles.

The chemical and physical constants of the synthetic **1** {mp 248 °C, $[\alpha]_D^{24}$ +68.7° (c 0.90, 90% EtOH, λmax =344.5 nm} were fully identical with those {mp 252 °C, $[\alpha]_D^{21}$ +63.7° (c 1.19, 90% EtOH), λmax =345 nm} of the natural **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 conclusion, it is worth noting that the first total synthesis of **1** was accomplished by the useful synthesis of various dehydropeptide derivatives and their thiazolation as well as the synthesis of a multi-substituted pyridine skeleton.

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

References and Notes

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5 BOP: Benzotriazole-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate.

6 **5**: Colorless needles. Mp 113-114 °C. $[\alpha]_D^{24}$ +3.4 ° (c 0.64, MeOH). IR (KBr) 3304, 2962, 1692, 1641, 1542 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.15 (d, 3H, Aminopropanol's CH_3, J=6.3 Hz), 1.18 (d, 3H, Thr's CH_3, J=6.6 Hz), 2.84 (br s, 1H, OH), 301-307 (m, 1H, Aminopropanol's CH), 3.42-3.51 (m, 2H, Aminopropanol's CH_2), 3.57 (br s, 1H, OH), 4.10 (dd, 1H, Thr's α-H, J=2.1 and 8.3 Hz), 4.34-4.39 (m, 1H, Thr's β-H), 5.13 (s, 2H, Cbz's CH_2), 5.90 (br d, 1H, NH, J=8.3 Hz), 6.85-7.00 (m, 1H, NH), 7.35 (s,5H, Ph).

7 **6**: Colorless needles. Mp 76-77 °C. $[\alpha]_D^{24}$ +3.0° (c 0.40, MeOH). IR (KBr) 3802, 2932, 2890, 1689, 1650, 1533 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.18 and 1.19 (each d, 6H, Thr's CH_3 and Aminopropanol's CH_3, J=6.3 Hz), 3.10-3.62 (m, 2H, Aminopropanol's CH_2), 3.36 (s, 6H, MOM's CH_3 x 2), 3.72-3.78 (m, 1H, Aminopropanol's CH), 4.28-4.33 (m, 2H, Thr'sα- and β-H), 4.62-4.73 (m, 4H, MOM's CH_2 x 2), 5.15 (s, 2H, Cbz's CH_2), 5.76 (br d, 1H, NH, J=6.6 Hz), 6.90-7.00 (m, 1H, NH), 7.37 (s, 5H, Ph).

8 **10**: Colorless syrup. $[\alpha]_D^{24}$ -7.4° (c 3.80, MeOH). IR (KBr) 2932, 2860, 1701 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=1.03 (s, 9H, TPS's CH_3), 1.13 (d, 3H, Thr's CH_3, J=6.3 Hz), 1.36 (t, 3H, CH_3CH_2, J=7.3 Hz), 1.44 (s, 9H, Boc), 1.84 (d, 3H, CH_3CH=, J=7.3 Hz), 4.37 (q, 2H, CH_3CH_2, J=7.3 Hz), 4.30-4.57 (m, 2H, Thr's α-H and β-H), 5.48 (br d, 1H, NH, J=7.0 Hz), 6.69 (q, 1H, CH_3CH=, J=7.3 Hz), 7.26-7.77 (m, 10H, Ph x 2), 8.05 (s, 1H, Thiazole ring-H), 8.37 (br s, 1H, NH).

9 **13**: Pale yellow powder. Mp 162-168 °C. $[\alpha]_D^{24}$+48.0° (c 0.95, MeOH). IR (KBr) 3364, 3100, 2962, 1662, 1527 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ=0.82-0.89 (m, 6H, Ip's CH_3 x 2), 1.08 and 1.15 (each d, 6H, Thr's CH_3 and Aminopropanol's CH_3, J=6.3 and 6.3 Hz), 1.38 (s, 9H, Boc), 1.66 (d, 3H, CH_3CH=, J=6.7Hz), 2.20-2.30 (m, 1H, Ip's CH), 3.13-3.19 (m, 2H, Aminopropanol's CH_2), 3.24 and 3.25 (each s, 6H, MOM's CH_3 x 2), 3.65-3.75 (m, 1H, Aminopropanol's CH), 4.15-4.22 (m, 1H, Thr'sβ-H), 4.53-4.75 (m, 6H, MOM's CH_2 x 2, BocNHC*H*, Thr's α-H), 6.35-6.45 (m, 1H, CH_3CH =), 7.66 (br d, 1H, BocNH, J =7.9 Hz), 7.90-8.55 (m, 9H, Thiazole ring-H x 5, Pyridine ring-H x 2, and NH x 2), 9.82 (s, 1H, COOH).

10 **14**: Pale yellow powder. Mp 116-118 °C. $[\alpha]_D^{24}$ +15.4° (c 0.96, MeOH). IR 3400, 3106, 1962, 1668, 1527 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ=0.88 and 0.90 (each d, 6H, Ip's CH_3, J=6.9 Hz), 0.96 (s, 9H, TPS's CH_3), 1.02, 1.07, and 1.14 (each d, 9H, CH_3 x 3, J=6.1 Hz), 1.26 (t, 3H, CH_3CH_2, J=7.3 Hz), 1.40 (s, 9H, Boc), 1.66 and 1.67 (each d, 6H, CH_3CH=, J=7.3 Hz), 2.20-2.32 (m, 1H, Ip's CH), 3.14-3.16 (m, 2H, Aminopropanol's CH_2), 3.23 and 3.24 (each s, 6H, MOM's CH_3 x 2), 3.68-3.71 (m, 1H, Aminopropanol's CH), 4.17-4.25 (m, 1H, Thr'sβ-H), 4.27 (q, 2H, CH_3CH_2, J=7.3 Hz), 4.55-4.60 (m, 6H, MOM's CH_2 x 2, Thr's α- and β-H), 4.71-4.73 (m, 2H, BocNHC*H* and Thr's α-H), 6.43 and 6.60 (each q, CH_3CH= x 2, J=7.3 Hz), 7.31-7.33 (m, 11H, TPS' Ph x 2, NH), 8.02 (br d, 1H, NH, J=8.5 Hz), 8.17-8.31 (m, 5H, NH x 2, Thiazole ring-H x 2, Pyridine ring-H), 8.38, 8.43 and 8.47 (each s, 3H, Thiazole ring-H x 3), 8.48 (d, 1H, Pyridine ring-H, J=8.3 Hz), 9.84 and 9.92 (each br s, 2H, NH x 2).

11 Synthetic **1**: ^{1}H NMR (DMSO-d_6) δ=0.99 (d, 3H, Ip's CH_3, J=6,7 Hz), 1.03 (d, 3H, Aminopropanol's CH_3, J=6.1 Hz), 1.07 (d, 3H, Ip's CH_3, J=6.7 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, 3H, CH_3CH=, J=7.0 Hz), 2.59-2.63 (m, 1H, Ip's CH), 3.05-3.08 (m, 2H, Aminopropanol's CH_2), 3.66-3.70 (m, 1H, Aminopropanol's CH), 4.12-4.53 (m, 4H, Thr'sα-H x 2 and Thr's β-H x 2), 4.67 (d, 1H, Aminopropanol's OH, J=4.6 Hz), 5.13 (d, 1H, Thr's OH, J=5.2 Hz), 5.27 (dd, 1H, $(CH_3)_2CHCH$, 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= x 2), 7.76, 8.22, 8.23, 8.31, 8.42, and 8.61 (each s, 6H, Thiazole ring-H x 6), 7.93 (d, 1H, NH, 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, Pyridine ring-H, J=7.6 Hz), 8.44-8.47 (m, 2H, NH and Pyridine ring-H), 9.38 and 9.46 (each s, 2H, NH x 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.