Synthetic Study on Peptide Antibiotic Nisin. III. Synthesis of Ring C[1)]

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Synopsis. A cyclic sulfide moiety corresponding to ring C in peptide antibiotic nisin was successfully synthesized from a disulfide compound prepared from a heptapeptide involving L-cysteine and *threo*-3-methyl-D-cysteine through desulfurization reaction with hexaethylphosphorus triamide.

A peptide antibiotic nisin has been used as a food preservative widely in Europe because of its antibacterial activity particularly against *Clostridium botulinum*. The unique structural feature of nisin was found in the presence of three dehydro amino acid residues and of five cyclic sulfide parts which are composed of *meso*-lanthionine in ring A and *threo*-methyllanthionine in rings B to E.[2)] Our study on the total synthesis of nisin was motivated not only from a viewpoint of the synthetic interest but also for a confirmation of the proposed structure as shown in Fig. 1.

In order to proceed the synthesis of such compound of complex structure, we first exploited a general method for preparation of lanthionine peptide by desulfurization[3)] from the corresponding disulfide peptide with hexaethylphosphorus triamide

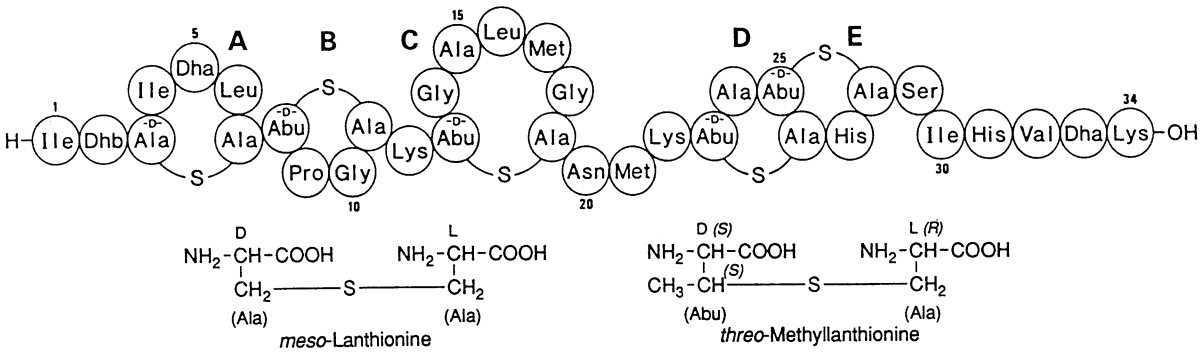

Fig. 1. Structure of nisin. Abu: 2-aminobutyric acid; Dha: dehydroalanine; Dhb: dehydrobutyrine (=3-methyldehydroalanine).

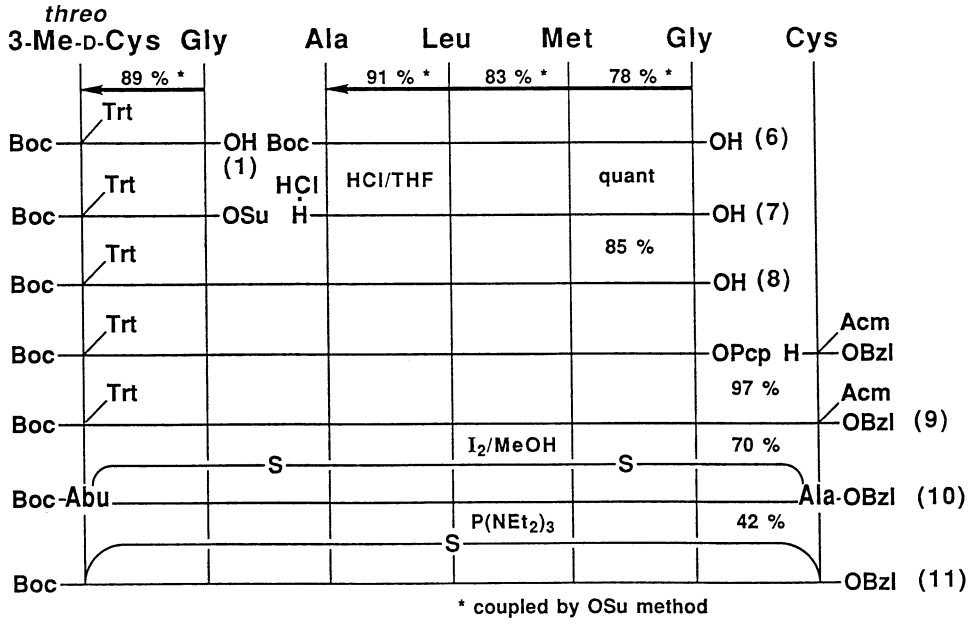

Fig. 2. Synthetic scheme of Boc-(ring C)-OBzl.

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[$P(NEt_2)_3$]. This method was successfully applied to the syntheses of rings A and B in nisin already.[4,5] Hence, the synthesis of third cyclic part, ring C, was also attempted by application of this procedure. Previously, we reported on the preliminary preparation of ring C derivative whose amino and carboxyl groups were protected with *t*-butoxycarbonyl and methyl groups, respectively.[1] However the yield of final desulfurization step did not exceed 9%, even though several reaction conditions were examined and modified. Therefore, we attempted to achieve the planned synthesis of the ring C by replacement of methyl ester with benzyl ester with expectation of use of this product to a total synthesis of nisin itself. Thus, we now present a synthesis of Boc-(ring C)-OBzl (**11**) in this study.

As shown in Fig. 2,[6] tetrapeptide **6** was first prepared by stepwise elongation with *N*-hydroxysuccinimide (HOSu) ester method. Fragment condensation of HOSu ester of *N*-terminal dipeptide **1** with tetrapeptide hydrochloride **7** gave hexapeptide **8** which was coupled with HCl·H-Cys(Acm)-OBzl by pentachlorophenyl ester method to give linear heptapeptide **9**. An iodine oxidation of **9** afforded monomeric disulfide **10** [fast atom bombardment mass spectrometry (FAB-MS), *m/z* 856 $(M+H)^+$]. Finally, the desulfurization reaction of cyclic disulfide peptide **10** was carried out in anhyd THF under high dilution conditions [0.67 mM (1 M=1 mol dm^{-3})]. Two main compounds were obtained by silica-gel column chromatography of the crude product. FAB-MS showed that the one was a desired monomeric sulfide derivative **11** [*m/z* 824 $(M+H)^+$], whereas the other was a dimeric sulfide derivative as assigned for its deprotected product [*m/z* 1267 $(M+H)^+$].[7]

In case of the synthesis of ring B containing *threo*-methyllanthionine residue, we already revealed that the configuration of the β-carbon atom of *threo*-3-methyl-D-cysteine was completely retained through desulfurization reaction, since a hydrolysis of the product gave the natural *threo* form of methyllanthionine, indicating that the desulfurization reaction proceeded in configuration-retaining route out of two possible pathways.[5] The same reaction mechanism may be also applicable to the case of the preparation of ring C. In fact, amino acid analysis of the hydrolyzate of cyclic sulfide peptide **11** unambiguously indicated a sole formation of *threo*-methyllanthionine residue. Thus, we succeeded in the synthesis of ring C part in nisin.

The results of our synthetic studies of rings B and C suggested that the desulfurization reaction of disulfide peptides composed of *threo*-3-methylcysteine and cysteine could be a generally applicable method to the synthesis of *threo*-methyllanthionine-containing peptides. In fact, we utilized this method for the synthesis of conjunctive ring D-E moiety in nisin[8] and accomplished the total synthesis of nisin.[9]

Experimental

All melting points are uncorrected. The FAB-MS spectra were obtained with a JEOL JMS-DX 300 mass spectrometer. Specific rotations were obtained with a Perkin-Elmer 141 polarimeter. Amino acid analysis was carried out with Hitachi 655A type liquid chromatograph system equipped with 655-3410 unit (column: Hitachi custom #2619F, 4×150 mm, 58 °C; buffer: sodium citrate buffer;[10] flow rate: 0.4 ml min^{-1}). Samples for the analysis were hydrolyzed with constant boiling 6M HCl in sealed tubes at 110 °C for 24 h.

Boc-3-Me-D-Cys(Trt)-Gly-OH·DCHA (1). To a soln of Boc-3-Me-D-Cys(Trt)-OSu[5] (6.54 g, 15.7 mmol) in THF (50 ml) was added a soln of Gly (2.36 g, 31.4 mmol) and triethylamine (TEA) (3.18 g, 31.4 mmol) in H_2O (15 ml). The mixture was stirred at room temp overnight and concd in vacuo. The residue was dissolved in EtOAc and 10% aq citric acid. EtOAc layer was washed with saturated NaCl soln, dried over $MgSO_4$, and then concd in vacuo. To an ether soln of the residue were added dicyclohexylamine (DCHA) (2.85 g, 15.7 mmol) and hexane under ice cooling. Cryst product was filtered after thorough cooling: yield 9.98 g (89.1%); mp 101.5—104 °C; $[\alpha]_D^{28}$ −64.5° (*c* 0.954, DMF). Anal. ($C_{42}H_{57}N_3O_5S \cdot 0.25H_2O$) C, H, N, S.

Boc-Met-Gly-OH (2). To a soln of Boc-Met-OSu (24.6 g, 71.0 mmol) in DMF (70 ml) was added a soln of Gly (10.7 g, 142 mmol) and TEA (14.4 g, 142 mmol) in H_2O (50 ml). The mixture was stirred at room temp overnight. The residue obtained by vac concn was applied to silica-gel column [Merck silica gel 60 (70—230 mesh), 400 g, 7×20 cm, $CHCl_3$-MeOH-AcOH=95:5:3] to give **2**, which was crystallized from hexane: yield 17.0 g (78.0%); mp 116—119 °C (decomp); $[\alpha]_D^{16}$ −16.5° (*c* 1.13, MeOH). Anal. ($C_{12}H_{22}N_2O_5S \cdot 0.3H_2O$) C, H, N, S.

HCl·H-Met-Gly-OH (3). Dipeptide **2** (16.6 g, 54.2 mmol) was dissolved in 1.4 M HCl/AcOH (780 ml). The soln was allowed to stand at room temp for 2.5 h and concd in vacuo. An oily residue was triturated with ether to give colorless powder, which was subjected to the following reaction without purification.

Boc-Leu-Met-Gly-OH·DCHA (4). To a soln of **3** (54.2 mmol) in DMF (150 ml) were added Boc-Leu-OSu (17.8 g, 54.2 mmol) and TEA (16.4 g, 162 mmol) under ice cooling. The mixture was stirred at room temp overnight and concd in vacuo. The residue was dissolved in EtOAc and saturated $NaHCO_3$ soln. Aqueous layer was acidified with citric acid and then extracted with EtOAc. EtOAc layer was washed with saturated NaCl soln, dried over $MgSO_4$, and concd in vacuo. To a soln of the residue in EtOAc (100 ml) was added DCHA (9.83 g, 54.2 mmol) under ice cooling. Colorless crystals were filtered and recrystd from EtOH and hexane: yield 26.9 g (82.5%): mp 156—158 °C (decomp); $[\alpha]_D^{16}$ −35.1° (*c* 1.10, MeOH). Anal. ($C_{30}H_{56}N_4O_6S \cdot 0.75H_2O$) C, H, N, S.

HCl·H-Leu-Met-Gly-OH (5). Tripeptide **4** (63.3 g, 105 mmol) was treated with EtOAc and 10% aq citric acid. EtOAc layer was washed with H_2O and dried over $MgSO_4$. The oily residue obtained by vac concn was treated with 1.4 M HCl/AcOH (850 ml) as described for the preparation of **3**. The colorless powder obtained was subjected to the following reaction without purification.

Boc-Ala-Leu-Met-Gly-OH (6). To a soln of **5** (105 mmol) in DMF (350 ml) were added Boc-Ala-OSu (33.1 g, 116 mmol) and TEA (31.9 g, 315 mmol) under ice cooling. The mixture was stirred at room temp overnight and concd in vacuo. The residue was dissolved in EtOAc and saturated $NaHCO_3$ soln. Aqueous layer was acidified with citric acid and then extracted with EtOAc. EtOAc layer was washed with saturated NaCl soln, dried over $MgSO_4$, and concd in vacuo. Cryst residue was recrystd from EtOH and hexane: yield 45.7 g (88.7%); mp 161—164 °C (decomp); $[\alpha]_D^{16}$ −64.8° (*c* 1.02, MeOH). Anal. ($C_{21}H_{38}N_4O_7S$) C, H, N, S.

HCl·H-Ala-Leu-Met-Gly-OH (7). Tetrapeptide **6** (19.9 g, 40.6 mmol) was treated with 1.4 M HCl/AcOH (220 ml) as described for the preparation of **3**. The colorless powder obtained was subjected to the following reaction without purification.

Boc-3-Me-D-Cys(Trt)-Gly-Ala-Leu-Met-Gly-OH (8). Dipeptide **1** (29.1 g, 40.6 mmol) was treated with EtOAc and 10% aq citric acid. EtOAc layer was washed with H_2O, dried over $MgSO_4$, and concd in vacuo. To a soln of the residue in THF (170 ml) were added HOSu (4.90 g, 42.6 mmol) and dicyclohexylcarbodiimide (8.80 g, 42.6 mmol) under ice cooling. The mixture was stirred at 0 °C for 1 h and then at room temp for 3.5 h. The precipitate was filtered off and the filtrate was concd in vacuo. To a soln of the residue in DMF (220 ml) were added **7** (40.6 mmol) and TEA (12.3 g, 122 mmol) under ice cooling. The mixture was stirred at room temp overnight and concd in vacuo. To the residue were added AcOH (40 ml) and H_2O (300 ml). The colorless precipitate was filtered and washed with H_2O and ether: yield 35.1 g (95.3%); mp 197—199 °C (decomp); $[\alpha]_D^{16}$ −72.3° (*c* 1.02, MeOH). Anal. ($C_{46}H_{62}N_6O_9S_2 \cdot H_2O$) C, H, N, S.

Boc-3-Me-D-Cys(Trt)-Gly-Ala-Leu-Met-Gly-Cys(Acm)-OBzl (9). To a soln of **8** (4.07 g, 4.49 mmol) in DMF (16 ml) were added pentachlorophenol (1.32 g, 4.94 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (947 mg, 4.94 mmol). The mixture was stirred at 0 °C for 2.5 h and at room temp for 30 min. To the mixture was added a soln of HCl·H-Cys(Acm)-OBzl (1.72 g, 5.39 mmol) and TEA (545 mg, 5.39 mmol) in DMF (20 ml) under ice cooling. The mixture was stirred at room temp overnight and then diluted with H_2O. The precipitate was filtered and washed with H_2O and ether: yield 5.15 g (97.9%); mp 180—181 °C (decomp); $[\alpha]_D^{20}$ −99.5° (*c* 1.00, DMF). Anal. ($C_{59}H_{78}N_8O_{11}S_3 \cdot 2H_2O$) C, H, N, S.

Boc-3-Me-D-Cys-Gly-Ala-Leu-Met-Gly-Cys-OBzl (10). To a soln of **9** (4.85 g, 4.14 mmol) in MeOH (2.5 l) was added I_2 (3.15 g, 12.4 mmol) in MeOH (250 ml) under vigorous stirring. The oxidation was carried out at room temp for 30 min and quenched by an addition of 0.2 M $Na_2S_2O_3$ soln until a color of I_2 disappeared. The residue obtained by vac concn was purified by silica-gel column chromatography [Merck silica gel 60 (230—400 mesh), 150 g, 3.5×44 cm, $CHCl_3$-MeOH-AcOH=90:5:3] to give **10**, which was crystallized from MeOH and ether: yield 2.48 g (70.0%); mp 160—161 °C (decomp); $[\alpha]_D^{20}$ −13.3° (*c* 1.04, DMF); FAB-MS, *m/z* 856 [$(M+H)^+$]. Anal. ($C_{37}H_{57}N_7O_{10}S_3$) C, H, N, S.

Boc-D-Abu-Gly-Ala-Leu-Met-Gly-Ala-OBzl (11). To a soln of **10** (350 mg, 0.414 mmol) in anhyd THF (620 ml) was added $P(NEt_2)_3$ (1.02 g, 4.14 mmol). The mixture was stirred at 25 °C overnight and concd in vacuo. The residue triturated with hexane was filtered and washed with 10% aq citric acid, H_2O, and ether. The powder was purified by silica-gel column chromatography [Merck silica gel 60 (230—400 mesh), 18 g, 1.3×39 cm, $CHCl_3$-acetone=3:2] to give **11**, which was recrystd from MeOH and ether: yield 142 mg (41.6%); mp 248—250 °C (decomp); $[\alpha]_D^{20}$ −41.8° (*c* 1.03, DMF); FAB-MS, *m/z* 824 [$(M+H)^+$]. Amino acid analysis: Melan (0.93), Gly (1.76), Ala (1.00), Met (1.05), Leu (1.21). Anal. ($C_{37}H_{57}N_7O_{17}S_2 \cdot H_2O$) C, H, N, S.

References

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6) Abbreviations used are as follows: Boc, *t*-butoxycarbonyl; OBzl, benzyloxy; OPcp, pentachlorophenoxy; OSu, succinimidooxy; Trt, trityl; Acm, acetamidomethyl.

7) In order to prevent an unfavorable dimerization, we tried the desulfurization reaction on the solid support. As a result, the depression of dimerization could be achieved effectively, however the yield of desired ring C was not satisfactory (16%). M. Kitazawa, K. Fukase, T. Wakamiya, and T. Shiba, "Peptide Chemistry 1986," ed by T. Miyazawa, Protein Research Foundation, Osaka (1987), p. 317.

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10) The buffer system was slightly different from the standard system in order to separate methyllanthionine from other amino acids: buffer A, sodium citrate dihydrate 7.34 g, NaCl 5.02 g, citric acid monohydrate 20.56 g, EtOH 104 ml, 2,2′-thiobisethanol 5.22 ml, and octanoic acid 0.1 ml in 1000 ml of aq soln; buffer B, sodium citrate dihydrate 26.67 g, NaCl 54.35 g, citric acid monohydrate 6.10 g, EtOH 40 ml, and octanoic acid 0.1 ml in 1000 ml of aq soln; gradient: A 100% (0—12 min), A 85%/B 15% (12—37 min), A 19%/B 81% (37—47 min), B 100% (47—62 min).