An Improved Synthesis of *threo*-3-Methyl-D-cysteine

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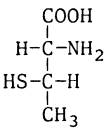
Synopsis. Synthesis of *threo*-3-methyl-D-cysteine, a moiety of β-methyllanthionine as a component of the peptide antibiotics nisin and subtilin, was achieved *via* (2*R*,3*R*)-1-*t*-butoxycarbonyl-3-methyl-2-aziridinecarboxamide derived from D-threonine. An addition of thioacetic acid or thiobenzoic acid to the aziridinecarboxamide gave *S*-acyl-β-mercapto-α-amino acid amide derivatives which were hydrolyzed directly or after the preformation of disulfide bond to afford the desired amino acid.

In our previous paper,[1] we reported the preparation of *threo*-3-methyl-D-cysteine (**1**), *i.e.*, (2*S*,3*S*)-2-amino-3-mercaptobutanoic acid[2] which corresponds to a moiety of β-methyllanthionine (**2**)[3] in the peptide antibiotics



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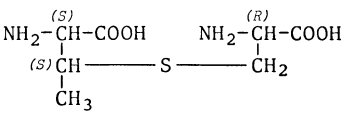

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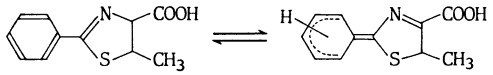
nisin[4] and subtilin,[5] *via* (2*R*,3*R*)-1-benzyloxycarbonyl-3-methyl-2-aziridinecarboxylic acid methyl ester (Z-3-MeAzyOMe) derived from D-threonine.[1] According to this procedure, the addition of thiobenzoic acid to the aziridinecarboxylic ester in the presence of $BF_3 \cdot Et_2O$ gave *threo*-*S*-benzoyl-*N*-benzyloxycarbonyl-3-methyl-D-cysteine methyl ester in less than 60% yield, which was caused by the formation of *N*-benzyloxycarbonyl-*O*-thiobenzoyl-D-threonine methyl ester as a by-product. Certainly, an equilibrium between thiobenzoic *S*-acid (C_6H_5COSH) and *O*-acid (C_6H_5CSOH) resulted in such undesirable side reaction. On the other hand, it was found that the reaction of thiobenzoic acid with 3-methyl-2-aziridinylcarbonyl peptides produced solely *S*-benzoyl-3-methylcysteinyl peptides without the addition of Lewis acid which seemed to enhance the reactivity of *O*-acid relatively.

Based on the above observation and consideration, we attempted to improve the synthetic procedure of **1** by use of aziridinecarboxamide in place of the ester derivative. Furthermore, *t*-butoxycarbonyl (Boc) group was adopted as *N*-protecting group rather than benzyloxycarbonyl (Z) group to prevent an attack of benzyl cation formed during acid hydrolysis to free mercapto group. Thus, (2*R*,3*R*)-1-*t*-butoxycarbonyl-3-methyl-2-aziridinecarboxamide [(2*R*,3*R*)-Boc-3-MeAzyNH$_2$] (**4**) was prepared in the usual way. The addition of thiobenzoic acid to **4** took place smoothly with the sole formation of *S*-benzoyl-*N*-Boc-*threo*-3-methyl-D-cysteinamide (**5a**) as we expected. An overall yield in the introduction of mercapto moiety was now considerably increased, even though the modified procedure required an extra step for the amidation. In addition, the easy precipitation of the product from the reaction mixture made the procedure so simple that we could avoid the tedious extraction step to separate the product from ill-smelling thiocarboxylic acid and also the column chromatographic purification as in the case of ester derivative.

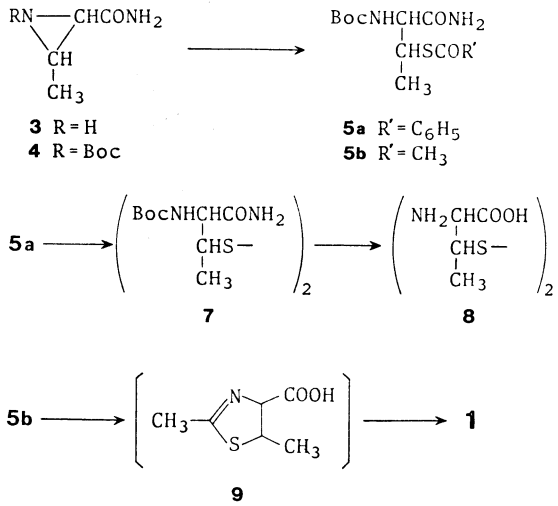
However, although the introduction of mercapto moiety was able to be achieved successfully, acid hydrolysis of **5a** resulted in the unexpected formation of quite stable 5-methyl-2-phenyl-2-thiazoline-4-carboxylic acid (**6**)[6] which gave racemic amino acid conceivably through a tautomerism between **6** and **6′**



6 **6′**

under prolonged heating. Therefore, in order to avoid such an undesirable formation of thiazoline compound, *S*-benzoyl group was first removed to afford a disulfide derivative **7** which was then satisfactorily hydrolyzed to an optically pure *threo*-3,3′-dimethyl-D-cystine (**8**).[7]

Another or even better solution of the problem was arisen from the following consideration. Since the formation of stable thiazoline ring may be due to the stabilization of the molecule by phenyl group, suitable choice of the *S*-acyl moiety seemed to make the hydrolysis easy. Based on such an assumption, use of *S*-acetyl-*N*-Boc-*threo*-3-methyl-D-cysteinamide (**5b**) for this reaction was attempted. The compound **5b** was prepared in a similar manner as in the preparation of **5a**. Indeed, the acid hydrolysis of **5b** under the usual conditions proceeded readily *via* a labile thiazoline **9** as a plausible intermediate to produce an optically pure *threo*-3-methyl-D-cysteine (**1**) in a satisfactory yield. Thus, we succeeded to establish an improved preparative method of **1** which can be utilized as an important component for the syntheses of rings B, C, D, and E in nisin.



3 R = H
4 R = Boc

5a R′ = C_6H_5
5b R′ = CH_3

7 **8**

9

Experimental

(2R,3R)-3-Methyl-2-aziridinecarboxamide [(2R,3R)-3-MeAzyNH$_2$] (3). A solution of (2*R*,3*R*)-3-MeAzyOMe prepared from (2*R*,3*R*)-trityl-3-MeAzyOMe (2.80 g, 7.84 mmol) according to the usual manner[8] in 45 ml of methanol was saturated with liquid ammonia under cooling in an ice-salt bath. The reaction mixture was kept in a pressure bottle at 25 °C for 4 d. Evaporation of the solvent and excess ammonia gave crystalline product in a yield of 740 mg (94%). A crop of the product was recrystallized from methanol and ether: mp 132—133.5 °C; $[\alpha]_D^{18}$ +103° (*c* 0.900, DMF).

Found: C, 48.00; H, 8.01; N, 27.54%. Calcd for $C_4H_8ON_2$: C, 47.99; H, 8.05; N, 27.98%.

(2R,3R)-Boc-3-MeAzyNH$_2$ (4). To a solution of (2*R*,3*R*)-3-MeAzyNH$_2$ (**3**) (7.20 g, 72.0 mmol) in 100 ml of methanol was added di-*t*-butyl dicarbonate (17.3 g, 79.0 mmol). The reaction mixture was concentrated *in vacuo* to afford crystalline product which was recrystallized from ethyl acetate and hexane: yield 12.3 g (85.3%); mp 118—119 °C; $[\alpha]_D^{18}$ +116° (*c* 1.06, $CHCl_3$).

Found: C, 53.96; H, 8.05; N, 13.89%. Calcd for $C_9H_{16}O_3N_2$: C, 53.99; H, 8.05; N, 13.99%.

S-*Benzoyl*-N-t-*butoxycarbonyl*-threo-*3-methyl*-D-*cysteinamide (5a).* Thiobenzoic acid (8.28 g, 60.0 mmol) was added to a solution of **4** (6.00 g, 30.0 mmol) in 60 ml of dichloromethane. A part of the crystalline product precipitated after stirring at room temperature overnight and hexane was added to complete the precipitation, yield 9.62 g (94.8%). An analytical sample was obtained by recrystallization from ethanol and hexane: mp 165.5—167 °C; $[\alpha]_D^{18}$ −41.7° (*c* 0.700, MeOH).

Found: C, 57.01; H, 6.64; N, 8.13; S, 9.37%. Calcd for $C_{16}H_{22}N_2O_4S$: C, 56.79; H, 6.55; N, 8.28; S, 9.47%.

S-*Acetyl*-N-t-*butoxycarbonyl*-threo-*3-methyl*-D-*cysteinamide (5b).* The reaction of thioacetic acid (6.36 g, 84.0 mmol) and **4** (6.00 g, 30.0 mmol) in dichloromethane (60 ml) was carried out in a similar manner as the preparation of **5a** and crystalline product **5b** was obtained in a yield of 7.86 g (94.9%). An analytical sample was prepared by recrystallization from ethyl acetate and hexane: mp 104—104.5 °C; $[\alpha]_D^{20}$ −35.5° (*c* 1.17, MeOH).

Found: C, 48.00; H, 7.29; N, 10.15; S, 11.68%. Calcd for $C_{11}H_{20}N_2O_4S$: C, 47.81; H, 7.29; N, 10.14; S, 11.60%.

N,N′-*Bis*(t-*butoxycarbonyl*)-threo-*3,3′-dimethyl*-D-*cystinediamide (7).* To a solution of **5a** (7.00 g, 20.7 mmol) in 200 ml of methanol was added dropwise 1 M† methylamine in methanol (20.7 ml, 20.7 mmol) for 8 h. After stirring overnight, 0.5 M iodine in methanol was added until the completion of the oxidation. The reaction mixture neutralized with methylamine was concentrated *in vacuo* to about 100 ml. The residue was cooled in an ice bath and the precipitate was filtered. The product was washed with hexane, suspended in water, and again filtered to afford pure material: yield 3.71 g (77.0%); mp 224—226 °C; $[\alpha]_D^{18}$ +82.1° (*c* 0.610, DMF).

Found: C, 46.22; H, 7.32; N, 11.91; S, 13.81%. Calcd for $C_{18}H_{34}N_4O_6S_2$: C, 46.33; H, 7.34; N, 12.01; S, 13.74%.

threo-*3,3′-Dimethyl*-D-*cystine (8).* A suspension of **7** (3.60 g, 7.72 mol) in 70 ml of 6 M HCl was refluxed for 10 h at 110 °C. The hydrolyzate concentrated *in vacuo* was suspended in 80 ml of ethanol to remove insoluble NH_4Cl and the filtrate was concentrated again. A solution of the residue in 25 ml of water and 30 ml of ethanol was neutralized with pyridine and then kept in refrigerator overnight. Crystalline precipitate was filtered, washed with ethanol, and recrystallized from water and ethanol: yield 1.96 g (94.7%); mp 190—196 °C (decomp); $[\alpha]_D^{27}$ −419° (*c* 0.540, 1 M HCl) [lit,[2d]) $[\alpha]_D^{19}$ −414° (*c* 1, 1 M HCl)].

Found: C, 34.65; H, 6.12; N, 10.06; S, 23.51%. Calcd for $C_8H_{16}O_4N_2S_2 \cdot 0.4H_2O$; C, 34.87; H. 6.15; N, 10.17; S, 23.27.

threo-*3-Methyl*-D-*cysteine (1).* A suspension of **5b** (1.00 g, 3.62 mmol) in 20 ml of 6 M HCl was refluxed for 10 h at 110 °C under argon atmosphere. The same work-up as mentioned for the preparation of **8** gave colorless crystalline product: yield 0.40 g (82%); mp 199.5—200 °C; $[\alpha]_D^{23}$ +16.9° (*c* 1.24, 1 M HCl) [lit,[2c]) $[\alpha]_D^{25}$ +17.5° (*c* 1, 1 M HCl)].

Found: C, 35.41; H, 6.82; N, 10.28; S, 23.03%. Calcd for $C_4H_9O_2NS \cdot 0.1H_2O$; C, 35.05; H, 6.77; N, 10.23; S, 23.41%.

† 1 M = 1 mol dm^{-3}.

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5) E. Gross, H. H. Kiltz, *Hoppe-Seyler's Z. Physiol. Chem.*, **354**, 310 (1973).

6) When the acid hydrolysis was stopped within 1—2 h, optically pure **6** was isolated: Yield 81%; mp 154—155.5 °C; $[\alpha]_D^{18}$ −114° (*c* 0.910, MeOH).

7) Dimethylamide derivative corresponding to **7** showed also an extreme resistance against the hydrolysis. A refluxing for a long time caused racemization of the product.

8) K. Nakajima and K. Okawa, *Biopolymers*, **20**, 1811 (1981).