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Preparations of *N*, *N*′-ethylene-bridged dipeptides(eXX) constructed from (S)-methionine, -tryptophan, -tyrosine and -*N*(ε)-benzyloxycarbonyllysine through acid-catalyzed cyclization

Short communication

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Summary. *N*, *N*′-Ethylene-bridged bis-(S)-methionine[(2S, 7S)-2, 7-bis(2-methylthioethyl)-3,6-diazaoctanedioic acid] derived from (S)-methionine and 1,2-dibromoethane was cyclized and esterified simultaneously in boiling ethanol in the presence of an appropriate amount of strong acid such as *p*-toluenesulfonic acid, affording a cyclic compound, *N*, *N*′-ethylene-bridged (S)-methionyl-(S)-methionine ethyl ester {ethyl(2S, 3′S)-4-(methylthio)-2-[2′-oxo-3′-(2-methylthioethyl)-1′-piperazinyl] butanoate}, exclusively in 80–90% yields. It was also found that, by applying this method, 70–80% yields of the other *N*, *N*′-ethylene-bridged dipeptides containing (S)-tryptophan, -tyrosine and -*N*(ε)-benzyloxycarbonyllysine were obtained.

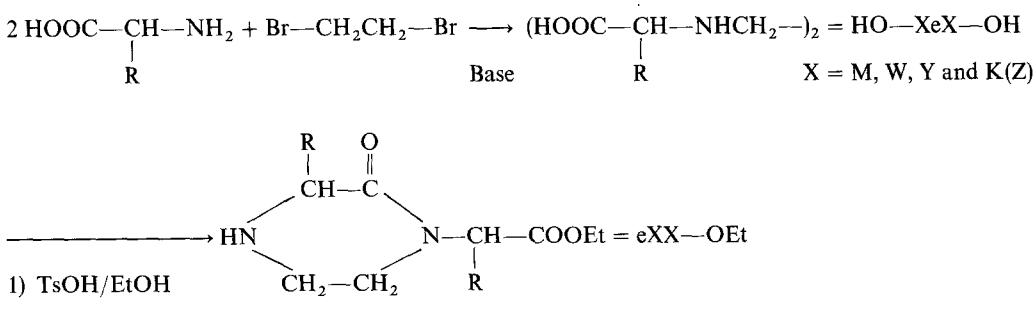
Keywords: Amino acids – Acid-catalyzed cyclization – *N*, *N*′-Ethylene-bridged dipeptides – (S)-Methionine – (S)-Tryptophan – (S)-Tyrosine – (S)-*N*(ε)-Benzyloxy-carbonyllysine

Introduction

Recently, various types of pseudopeptides (Toniolo, 1990) have been prepared through short range cyclizations for studying their functionalities, conformations, biological activities etc. The authors have prepared and used several kinds of *N*, *N*′-ethylene-bridged dipeptides [eXX: X = (S)-alanine(A), -leucine (L), -valine (V) and -phenylalanine(F)] with the relatively stable side chains such as methyl, isobuthyl, isopropyl and benzyl groups. These dipeptides are useful as the units of macrocyclic pseudopeptides, which are lipophilic and have the deep holes to include organic (Kojima et al., 1987) and inorganic (Kojima et al., 1991) substrates, and distinguish (R)- and (S)-enantiomers

(Kojima et al., 1989; Yamashita et al., 1990; Miyake et al., 1990). Moreover, the structures and conformations (Yamashita et al., 1989; Kojima et al., 1991) of these eXX and their derivatives were examined in detail by NMR measurements, X-ray analysis etc. On the other hand, Piercey et al. (1986) reported the use of two kinds of diastereomeric piperazin-2-one(MKP) derivatives [eXX: X = (R)- or (S)-F], which were non-stereoselectively synthesized by the method of Moon (1981, 1986), as the units of substance P antagonist peptides. Also, DiMaio et al. (1989) prepared chiral MKP derivatives which are useful as the units of enkephalin analogs. Our previous method (Kojima et al., 1989; Yamashita et al., 1990) for preparing eXX is the esterification of *N*,*N*′-ethylene-bridged bis α-amino acid (HO-XeX-OH) by the thionylchloride/methanol method (Brenner et al., 1953) and the successive cyclization of the resulting diester (MeO-XeX-OMe) in boiling xylene for several days. However, this method has some problems (poor yield, contamination with by-products etc.) in the preparation of *N*, *N*′-ethylene-bridged (S)-methionyl-(S)-methionine (eMM) whose side chains (thioether) are alkylated and oxidized easily, and unstable at high reaction temperatures, so that should be improved.

This article describes a simple method for preparing eMM-OEt (**1**); the cyclization and esterification of *N*, *N*′-ethylene-bridged bis (S)-methionine (**5** = HO-MeM-OH) proceed at the same time in boiling ethanol using *p*-toluenesulfonic acid monohydrate ($TsOH \cdot H_2O$) as an acid catalyst, giving a desired compound (**1**) in a good yield, exclusively. Moreover, this method is adopted conveniently for the preparations of the other *N*, *N*′-ethylene-bridged dipeptide ethyl esters [**2** = eWW-OEt, **3** = eYY-OEt and **4** = eK(Z)K(Z)-OEt] constructed from (S)-tryptophan (W), -tyrosine (Y) and -*N*(ε)-benzyloxycarbonyllysine[K(Z)], respectively.



1: eMM—OEt(R = $-CH_2CH_2SCH_3$)

2: eWW—OEt(R = $-CH_2-$ indole)

3: eYY—OEt(R = $-CH_2-C_6H_4-OH$)

4: eK(Z)K(Z)—OEt[R = $-(CH_2)_4-NH-COOCH_2-C_6H_5$]

Material and methods

Preparation of eXX-OEt

As a typical example, the preparation of **1** is shown as follows. **5**(13 g, 0.04 mol) was obtained from (S)-methionine (60 g, 0.40 mol) and 1, 2-dibromoethane (37.6 g, 0.20 mol) according to the method similar to that of Schöenberg et al. (1968). mp 270–280°C. $[\alpha]_D = +20°$ (*c* 1.2 in 1*N*-NaOH). Anal. Calcd. for $C_{12}H_{24}N_2O_4S_2$ (324.5): C 44.42, H 7.45, N, 8.63. Found: C 44.19, H 7.49, N 8.62.

5(6.5 g, 0.02 mol) was refluxed with $TsOH \cdot H_2O$ (7.6 g, 0.04 mol) in dry ethanol (300 ml) for 24 hours. The TsOH salt obtained after removal of the solvent was freed by aqueous sodium hydrogen carbonate, and extracted with dichloromethane. A crude oily residue thus obtained was purified by silica-gel column chromatography (chloroform: methanol = 15:1), giving **1** (5.3 g, 0.016 mol) as an oily material in an 80% yield, which proved to be sufficiently pure by TLC, IR, NMR and MS. IR (neat): 1730 (ester C = 0), 1640 (amide C = 0) cm^{-1}. ^1H NMR ($CDCl_3$, TMS as an internal standard): δ(ppm) 1, 27(3H, *t*, ethyl CH_3), 2.10 and 2.11 (3H, *s* and 3H, *s*, SCH_3), 2.04 and 2.28 (4H, *b*, β-CH_2), 2.51 and 2.64 (4H, *b*, γ-CH_2), 3.09 – 3.04 (4H, *m*, ethylene-bridged), 3.66 (1H, *dd*, α-CH on MKP ring), 4.18 (2H, *q*, ethyl CH_2), 5.04 (1H, *dd*, α-CH outside MKP ring). MS m/z: 334 (M^+).

Furthermore, the use of methanesulfonic acid (CH_3SO_3H), sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) as acid catalysts were attempted at the same scale and condition used for $TsOH \cdot H_2O$, giving the results similar to that obtained with $TsOH \cdot H_2O$. In case of H_2SO_4 and HCl, *conc.* H_2SO_4 (4 g, 0.04 mol) and 4*N*-HCl/1, 4-dioxane (10 ml, 0.04 mol) were used, respectively.

2, **3** and **4** were similarly obtained in 70–80% yields as powders by the TsOH/ethanol method described here, and proved to be almostly pure by TLC, IR, NMR and MS.

Table 1 shows the analytical and physical data of 3, 5-dinitrobenzoates (DNB-**1** and –**4**) of **1** and **4**, hydrochloride (HCl · **3**) of **3** and Boc-glycyl-eWW-OEt(BG-**2**).

BG-**2** was obtained by the coupling of **2** with *t*-butoxycarbonylglycine (Boc-G) in tetrahydrofuran according to the DCC (dicyclohexylcarbodiimide)/HOBT(l-hydroxy-benzotriazole) method.

On the other hand, an oily eMM-OMe (**6**) was obtained in a 70% yield in boiling methanol for 24 hours using $TsOH \cdot H_2O$ as an acid catalyst, and was allowed to react with 3, 5-dinitrobenzoylchloride in pyridine at room temperature. The resulting powder was recrystallized from methanol. mp 110–115°C. $[\alpha]_D = +23.6°$ (*c* 1, chloroform). MS m/z: 514 (M^+). Anal. Calcd. for $C_{20}H_{26}N_4O_8S_2$ (514.6): C 46.68, H 5.09, N 10.89. Found: C 46.69, H 5.08, N 10.91.

Table 1. Analytical and physical data of DNB-**1**, BG-**2**, HCl-**3** and DNB-**4**

Compound	Molecular formula	Analysis calcd./found C	H	N	mp (°C)	$[\alpha]_D$ (solvent)	MS m/z (M^+)
DNB-**1**[a]	$C_{21}H_{28}N_4O_8S_2$ (528.6)	47.72 47.65	5.34 5.36	10.60 10.54	112–117	+25.5° ($CHCl_3$)	528
BG-**2**[b]	$C_{33}H_{39}N_5O_6$ (601.7)	65.87 65.78	6.53 6.55	11.64 11.61	223–228	–20.3° (THF)	601
HCl-**3**[a]	$C_{22}H_{27}N_2O_5Cl$ ·1/3 H_2O (440.9)	59.93 59.86	6.32 6.28	6.35 6.24	145–150	–197° (DMF)	398
DNB-**4**[c]	$C_{39}H_{46}N_6O_{12}$ (790.8)	59.23 59.13	5.86 5.85	10.63 10.80	97–102	+18° (EtOH)	790

Recrystallized from [a]ethanol, [b]ethyl acetate, and [c]isopropanol

The dimethyl ester (**7** = MeO-MeM-OMe) of **5** yielded as a minor product was recrystallized as the hydrochloride from methanol. mp 195–203°C. $[\alpha]_D = -21°$ (*c* 0.7, chloroform). MS m/z: 352 (free **7**, M^+). Anal. Calcd. for $C_{14}H_{28}N_2O_4S_2 \cdot 2HCl$ (425.4): C 39.52, H 7.11, N 6.58. Found: C 39.46, H 7.14, N 6.55.

*Preparation of (2S, 3'S)-4-(methylthio)-2-[2'-oxo-3'-(2-methylthioethyl)-1'-piperazinyl] butanoic acid (**8** = eMM-OH)*

5(3.2 g, 0.01 mol) was refluxed in acetonitrile (150 ml) for 24 hours in the presence of $TsOH \cdot H_2O$ (3.8 g, 0.02 mol). The residue obtained after removal of the solvent was washed enoughly with ether and recrystallized from acetonitrile, giving the TsOH salt of **8** in an 80% yield. mp 170–173°C. $[\alpha]_D = -70°$ (*c* 0.7, dimethylformamide). IR (nujol): 2400–2800 (carboxylic acid OH), 1730 (carboxylic acid C = 0), 1640 (amide C = 0) cm^{-1}. ^1H NMR (D_2O, DSS as an internal standard): δ (ppm) 2.10 and 2.11 (3H, *s* and 3H, *s*, SCH_3), 2.10–2.18 (8H, *m*, β-and γ-CH_2), 2.38 (3H, *s*, CH_3), 3.50–3.82 (4H, *m*, ethylene-bridged), 4.32 (1H, *dd*, α-CH on MKP ring), 4.98 (1H, *dd*. α-CH outside MKP ring), 7.36 and 7.68 (4H, *d*, aromatic). MS m/z: 306 (free **8**, M^+), 172 (TsOH). Anal. Calcd. for $C_{19}H_{30}N_2O_6S_3$ (478.7): C 47.68, H 6.32, N 5.85. Found: C 47.60, H 6.35, N 5.91.

Moreover, the TsOH salt of **8** (2.39 g, 5 mmol) was refluxed in dry ethanol (60 ml) for 24 hours in the presence of $TsOH \cdot H_2O$ (0.095 g, 0.5 mmol), providing **1** in a 90% yield, which was identified with **1** obtained from **5** through one-step reaction described in this work.

Measurements

All samples were measured at room temperature using a Jasco IRA-1 (IR spectra), a Jeol GX-400 (NMR) and a Jasco DIP-370 (optical rotation). Their mass spectra were obtained by means of a Jeol D-300.

Results and discussion

In this work, the authors attempted to obtain eMM-OMe (**6**) using HO-MeM-OH (**5**) as a starting material according to our previous method (esterification and successive cyclization). However, it was found that the dimethyl ester (**7**) of **5** was obtained only in a poor yield (20%) by the thionylchloride/methanol method.

Furthermore, the cyclization of the resulting **7** was carried out in boiling xylene for several days, accompanying with decomposition, and gave the desired compound (**6**) in a low yield (below 30%). Therefore, an improved procedure for preparing **6** has been required.

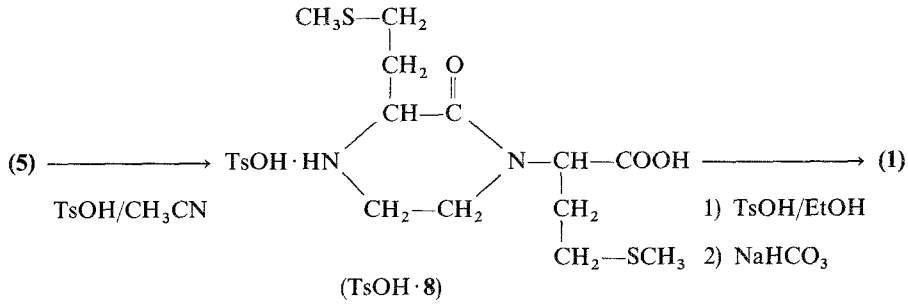
According to the method of Bodanszky (1984) to be useful in the esterification of methionine and tryptophan with the unstable side chains (thioether and indole), the esterification of **5** (13 g, 0.04 mol) was attempted in boiling ethanol (500 ml) for 24 hours with 4 mol equiv. of $TsOH \cdot H_2O$ (30.4 g, 0.16 mol). The residue thus obtained was subjected to silica-gel column chromatography (chloroform:methanol = 15:1), giving two products (major/minor = 3/1). Unexpectedly, IR, NMR and MS measurements revealed that the major product was **1**, and the minor one was the diethyl ester (EtO-MeM-OEt) of **5**. Moreover, it was found that the ratios of **1** to EtO-MeM-OEt were 7/1 or 2/1, respectively, when 3 or 6 mol equiv. of $TsOH \cdot H_2O$ were used for **5**. Also, the use of 1.6 and 2.0

mol equiv. of $TsOH \cdot H_2O$ for **5** provided almostly pure **1** containing negligible amounts of by-products.

These results suggest that the cyclization and the esterification compete each other, and the former proceeds more predominantly than the latter with decreasing the amount of $TsOH \cdot H_2O$ for **5**.

Also, it was found that the use of 2 mol equiv. of CH_3SO_3H, H_2SO_4 and HCl for **5** provided **1** exclusively in 80–90% yields. On the other hand, **5** was entirely recovered when **5** was refluxed in dry ethanol for several days without an acid catalyst. Furthermore, **5** was refluxed in acetonitrile with 2 mol equiv. of $TsOH \cdot H_2O$, giving the TsOH salt of eMM-OH (**8**) in a good yield. This salt was easily converted to 1 with 0.1 mol equiv. of $TsOH \cdot H_2O$ in boiling ethanol.

These results suggest that the cyclization proceeds acid-catalytically, but not thermally.



In order to compare with the previous method (Kojima et al., 1989; Yamashita et al., 1990), the present procedure was adopted for the preparations of eLL-OMe (**9**) and eFF-OMe (**10**). As the result, 80–90% yields of **9** and **10** were obtained.

Furthermore, the spectroscopic and physical data of the hydrochlorides of **9** and **10** obtained here agree well with those of the previous ones.

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