May, 1978] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 51 (5), 1501—1506 (1978) 1501

Synthetic Studies on Quinoxaline Antibiotics. Synthesis of Quinoxaline-2-carbonyl Tetradepsipeptide Derivatives Related to Triostin A*

Masaru Shin, Ken Inouye, and Hideo Otsuka
Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553

(Received November 26, 1977)

The synthesis is described of four quinoxaline-2-carbonyl (Qxc) tetradepsipeptide derivatives, Qxc–D-Ser[Z–MeCys(Bzl)–MeVal]–Ala–OH, Qxc–D-Ser[Bpoc–MeCys(Bzl)–MeVal]–Ala–OBu^t (**2**), Qxc–D-Ser(Z–MeVal)–Ala–MeCys(Bzl)–OH, and Qxc–D-Ser(Bpoc–MeVal)–Ala–MeCys(Bzl)–OBu^t, corresponding to the partial structure of a quinoxaline antibiotic, triostin A. The Qxc group was introduced at the dipeptide or tripeptide stage with Qxc–ONp as acylating agent. Qxc–D-Ser–Ala–OBu^t and Qxc–D-Ser–Ala–MeCys(Bzl)–OBu^t were acylated with Z–MeVal–OH by using *p*-toluenesulfonyl chloride as activating agent to give the desired depsipeptides in excellent yields, whereas the acylation with Bpoc–MeVal–OH by the same procedure was unsuccessful. The latter could be achieved by the *N,N'*-carbonyldiimidazole method, though the yields were relatively low. The treatment of compound **2** with an aqueous acetic acid-formic acid mixture for removal of the Bpoc group brought about concomitant formation of *cyclo*[–MeCys(Bzl)–MeVal–].

The quinoxaline antibiotics[1] are produced by various species of streptomycetes including *S. aureus* and reported active against gram-positive bacteria, certain tumors and to inhibit DNA-dependent RNA synthesis. The triostins, a family of the quinoxaline antibiotics, were isolated by Shoji *et al.*[2] and their structure was determined by Otsuka and Shoji[3] to be a quinoxaline-2-carbonyl(Qxc)-peptide lactone with a bridged disulfide linkage (Fig. 1). As a result of the recent NMR and MS studies, the structure of echinomycin[4] (quinomycin A[5]), which is also a member of the quinoxaline antibiotics, has been revised,[6] while that of triostin A has rather been confirmed.[7] Some synthetic studies on these antibiotics have appeared, in which the Qxc derivatives of some amino acids,[8] peptides,[9] and related compounds[10] were prepared. Very recently Ciardelli and Olsen[11] have reported the synthesis of des-*N*-tetramethyltriostin A, an analogue differing from the natural compound by lack of the *N*-methyl groups on the cysteine and valine residues. This desmethyl analogue had no activity toward *Staphylococcus aureus*, however.[11] We wish now to report the synthesis of tetradepsipeptide derivatives, Qxc–D-Ser[Z–MeCys(Bzl)–MeVal]–Ala–OH (**1**), Qxc–D-Ser[Bpoc–MeCys(Bzl)–MeVal]–Ala–OBu^t (**2**), Qxc–D-Ser(Z–MeVal)–Ala–MeCys(Bzl)–OH (**3**), and Qxc–D-Ser(Bpoc–MeVal)–Ala–MeCys(Bzl)–OBu^t (**4**), which has been performed as a step toward the total synthesis of triostin A.

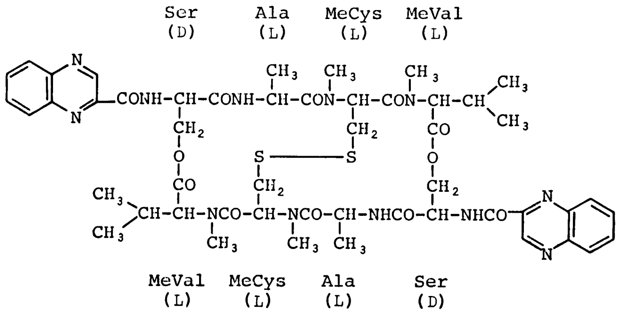

Fig. 1. Structure of triostin A.[3]

As is seen in Fig. 1, the structure of triostin A has the point of symmetry and can be split into two equal portions, each containing one Qxc group and four different amino acid residues, D-Ser, Ala, MeCys, and MeVal. Thus, the formation of the desired octadepsipeptide ring may be accomplished by combining a selected pair of the two halves, and a key to the total synthesis may be the choice of the position at which the ring is closed. The ring-closure by the lactonization between the MeVal and the D-Ser residues seems to be inadequate, because the low reactivity of the hydroxyl group of serine would require the carboxyl component highly activated,[12] and this could be a cause for some side reactions and racemization. The cyclization should, therefore, be carried out at a peptide bond. The synthesis of compounds **1**, **2**, **3**, and **4** was performed along this line.

Quinoxaline-2-carboxylic acid (Qxc–OH) as a starting material was synthesized by the method of Keller-Schierlein and Prelog[1b] from 2-methylquinoxaline *via* 2-styrylquinoxaline as intermediate. *N*-Methyl-L-valine was prepared according to the procedure of Quitt *et al.*[13] and *N*-methyl-*S*-benzyl-L-cysteine was derived from L-thiazolidine-4-carboxylic acid as described by Yamashiro *et al.*[14] As a reagent for introducing the Qxc group quinoxaline-2-carboxylic acid *p*-nitrophenyl ester (Qxc–ONp) was prepared by the treatment of Qxc–OH with *p*-nitrophenyl trifluoroacetate[15] in pyridine. Qxc–ONp was also prepared from Qxc–OH and *p*-nitrophenol by the mixed acid anhydride method using benzenesulfonyl chloride. This crystalline compound is stable, but readily reacts

---

* Abbreviations used in this communication are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochemistry*, **5**, 2585 (1966); *ibid.*, **6**, 362 (1967); *ibid.*, **11**, 1726 (1972)] and include MeCys: *N*-methyl-L-cysteine, MeVal: *N*-methyl-L-valine, Qxc: quinoxaline-2-carbonyl, Z: benzyloxycarbonyl, Boc: *t*-butoxycarbonyl, Bpoc: 2-(4-biphenylyl)-2-propoxycarbonyl, Bzl: benzyl, OBu^t: *t*-butoxy, ONp: *p*-nitrophenoxy, DCC: dicyclohexylcarbodiimide, CDI: *N,N'*-carbonyldiimidazole, CDMI: 2,2'-dimethyl-1,1'-carbonyldiimidazole, Tos-Cl: *p*-toluenesulfonyl chloride, TFA: trifluoroacetic acid, TEA: triethylamine, DCHA: dicyclohexylamine, DMF: *N,N*-dimethylformamide, and THF: tetrahydrofuran.

with amines to give their Qxc derivatives. In view of the general trend that a *p*-nitrophenyl ester is less reactive toward alcohols than the corresponding acid chloride, Qxc–ONp may be more suitable than Qxc–Cl[10)] for the preferential *N*-acylation in the presence of the hydroxyl group of serine residue.

Synthesis of compounds **1** and **2** was performed as illustrated in Fig. 2. Compound **5** was hydrogenolyzed to remove the Z group and the product was acylated with an equimolar amount of Qxc–ONp to give a crystalline Qxc-dipeptide (**6**) in a moderate yield. Compound **6** was also synthesized by Gerchakov and Schultz[9)] from Qxc–D-Ser–OH and H–Ala–OBu^t, but the mp and $[\alpha]_D$ values reported are significantly lower than those of our product. This might be an indication of some racemization which could occur in the coupling reaction with a Qxc–amino acid as carboxyl component. The subsequent acylation of **6** with Bpoc–MeVal–OH at the hydroxyl group of serine residue could be achieved by the CDI[16)] or CDMI[17)] method to give compound **7**, though the yield was relatively low. This contrasts with the finding by Chen *et al.*[18)] that the CDI (and the mixed anhydride) methods are unsuccessful in such depsipeptide bond formation. When the MeVal was introduced by using Z–MeVal–OH[19)] and Tos–Cl in some excess, the desired depsipeptide, Qxc–D-Ser(Z–MeVal)–Ala–OBu^t, was obtained in an excellent yield. However, the same method failed to give componud **7**, probably because the symmetrical anhydride, which could be formed from Bpoc–MeVal–OH by means of Tos–Cl,[20)] was sterically hindered by the bulky Bpoc

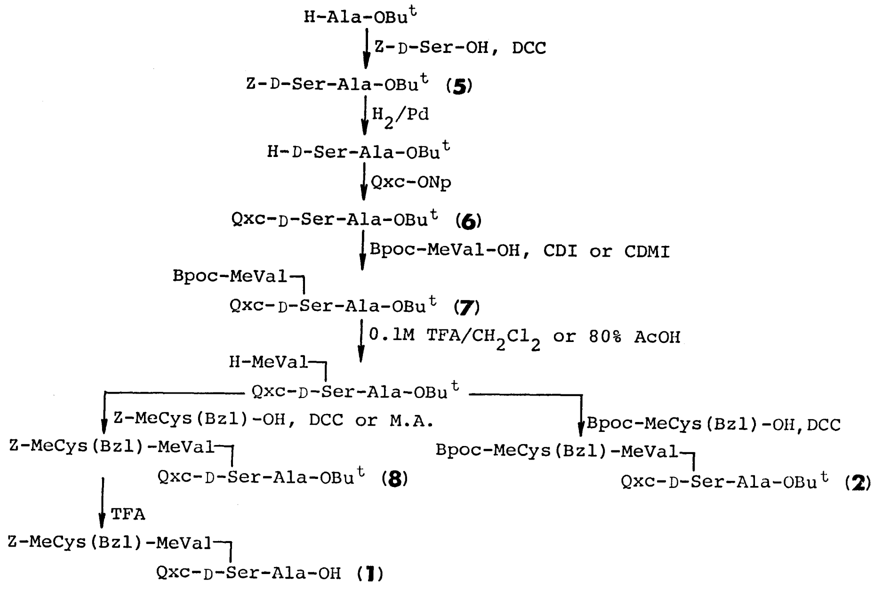

Fig. 2. Synthesis of tetradepsipeptide derivatives related to triostin A (part 1).

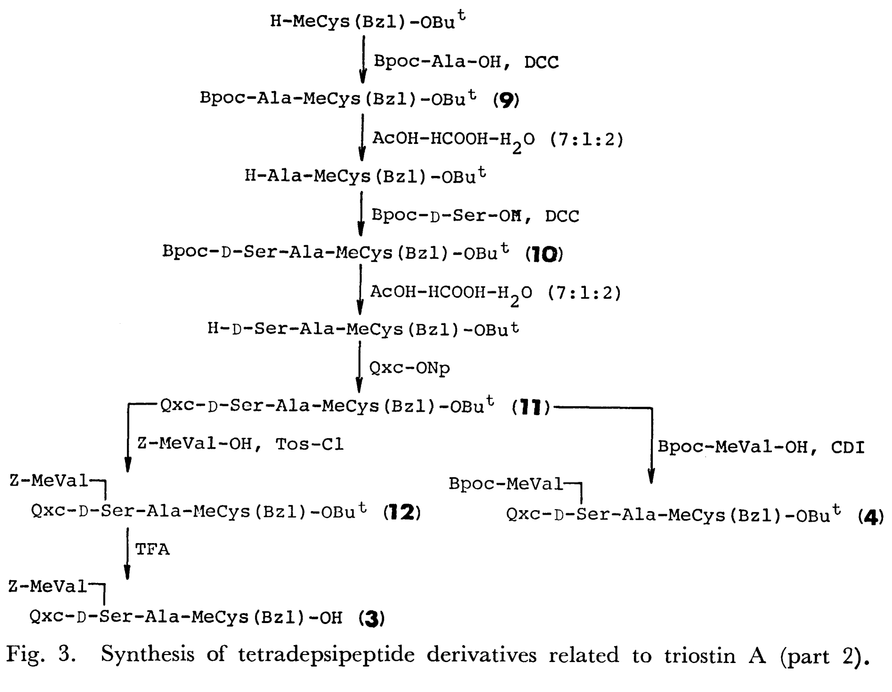

Fig. 3. Synthesis of tetradepsipeptide derivatives related to triostin A (part 2).

groups. Compound **7** was then treated with 0.1 M TFA in dichloromethane at room temperature or with 80% acetic acid at 70 °C to remove the Bpoc group. The resulting compound was coupled with Z–MeCys(Bzl)–OH[14] by the DCC or mixed anhydride method and with Bpoc–MeCys(Bzl)–OH by the DCC method to give **8** and **2**, respectively, in moderate yields. The treatment of **8** with TFA yielded **1** as desired, but the treatment of **2** with an aqueous acetic acid–formic acid mixture[21] yielded not the desired Qxc–D-Ser[H–MeCys(Bzl)–MeVal]–Ala–OBu^t but two major products, which were isolated and identified as *cyclo*[–MeCys(Bzl)–MeVal–] and **6**, respectively. The data of MS supported the structure of the cyclic compound. In view of this easy formation of the cyclic dipeptide by intramolecular aminolysis, compound **2** does not seem to be useful as intermediate for the total synthesis of triostin A. We next tried, therefore, to synthesize **3** and **4** according to the procedure illustrated in Fig. 3.

Bpoc–Ala–OH[22] and H–MeCys(Bzl)–OBu^t prepared according to Roeske[23] were coupled with DCC to give a dipeptide (**9**). The treatment of **9** with an acetic acid–formic acid–water mixture[21] yielded the N^α-free compound, which was coupled with Bpoc–D-Ser–OH by the DCC method. The crystalline tripeptide derivative (**10**) thus obtained was treated with acid as above, followed by acylation with Qxc–ONp to produce compound **11**. In the subsequent step Z–MeVal–OH was introduced by the use of Tos–Cl and Bpoc–MeVal–OH by the CDI method to the hydroxyl group of D-Ser residue of **11** to give **12** and **4**, respectively. As in the synthesis of **7**, Bpoc–MeVal–OH could not be introduced by the Tos–Cl method. The treatment of **12** with TFA gave **3** in a moderate yield. In the synthesis of **1**, **2**, **3**, and **4**, most of the intermediates as well as the final products resisted at crystallization, probably because of the presence of *N*-methylamino acid(s) in their molecules. These amorphous products were purified extensively by means of silica gel column chromatography. The tetradepsipeptide derivatives thus obtained, **3** and **4** in particular, would serve as intermediates for the production of triostin A. The final steps toward this goal are now in progress.

Experimental

Thin-layer chromatography (TLC) was performed on silica gel plates (Kieselgel GF$_{254}$ or precoated Kieselgel 60F$_{254}$, Merck) with the following solvent systems (ratios by volume): A, ethyl acetate; B, benzene–ethyl acetate (1 : 1); C, benzene–ethyl acetate (7 : 3); D, benzene–ethyl acetate–acetic acid (70 : 30 : 3); E, dichloromethane–ethyl acetate (9 : 1); F, dichloromethane–ethyl acetate (8 : 2); G, cyclohexane–ethyl acetate (2 : 8); H, chloroform–methanol (95 : 5); I, chloroform–methanol (8 : 2); J, chloroform–methanol–acetic acid (95 : 5 : 3); K, chloroform–methanol–acetic acid (90 : 10 : 3); L, 1-butanol–acetic acid–water (4 : 1 : 2). For column chromatography Kieselgel 60 (Merck) was used throughout unless otherwise specified.

D-Serine was purchased from Fluka AG, Buchs, Switzerland and 2-methylquinoxaline from Aldrich Chemical Co., Inc., Milwaukee, Wisconsin, U. S. A. *N*-Methyl-L-valine ($[\alpha]^{23}_{D}$ +32.5 ±0.8° (*c* 1.0, 6M HCl); lit,[13] $[\alpha]^{23}_{D}$ +33.1° (*c* 1, 6M HCl)), *N*-methyl-*S*-benzyl-L-cysteine (mp 202—203 °C dec, $[\alpha]^{23.5}_{D}$ +67.2 ±1.2° (*c* 1.0, 1M HCl); lit.[14] mp 205—206 °C dec, $[\alpha]^{20}_{D}$ +67.5° (*c* 1, 1M HCl)), and quinoxaline-2-carboxylic acid (mp 228—229 °C; lit,[4b] mp 211—212 °C) were synthesized according to the literature.

p-*Nitrophenyl Quinoxaline-2-carboxylate* (*Qxc–ONp*). *a*) *With* p-*Nitrophenyl Trifluoroacetate*: To a solution of quinoxaline-2-carboxylic acid (13.93 g, 80 mmol)[4b] in anhydrous pyridine (80 ml) was added *p*-nitrophenyl trifluoroacetate (20.69 g, 88 mmol)[15] and the mixture was stirred at 25 °C for 3.5 h. The desired product was precipitated by the addition of cold water (400 ml). Recrystallization from ethyl acetate gave needles; yield 21.57 g (91%), mp 198—199 °C. Found: C, 61.14; H, 3.13; N, 14.32%. Calcd for $C_{15}H_9N_3O_4$: C, 61.02; H, 3.07; N, 14.23%.

b) *With Benzenesulfonyl Chloride and* p-*Nitrophenol*: Quinoxaline-2-carboxylic acid (0.52 g, 3 mmol) in anhydrous pyridine (20 ml) was allowed to react with benzenesulfonyl chloride (0.77 ml, 6 mmol) at 0 °C for 5 min and to this was added *p*-nitrophenol (0.42 g, 3 mmol). The mixture was stirred at 0 °C for 60 min and then ice-cold water (100 ml) was introduced. The precipitate collected was recrystallized from ethyl acetate; yield 0.42 g (47%), mp 197—198 °C. Found: C, 60.80; H, 3.14; N, 14.35%.

Bpoc–MeVal–OH. *N*-Methyl-L-valine (3.94 g, 30 mmol)[13] was dissolved in 40% benzyltrimethylammonium hydroxide in methanol (Triton B, 13.7 ml) and the solution was evaporated *in vacuo*. The residue was, after evaporation from DMF, dissolved in DMF (15 ml) together with Bpoc–N_3 (11.25 g, 40 mmol)[21] and TEA (11.2 ml), and the mixture was stirred at 40 °C for 60 min. Some water (60 ml) was added and the mixture was washed with ether. The aqueous solution was then acidified with ice-cold M citric acid at 0 °C. The oily precipitate which separated was extracted with ether. The organic extracts were combined, dried (Na_2SO_4) and evaporated to give the desired product, which was crystallized from ethyl acetate–petroleum ether; yield 4.33 g (34%), mp 138.5—139 °C, $[\alpha]^{20.5}_{D}$ −60.1± 1.0° (*c* 1.0, methanol). TLC: homogeneous (H_2SO_4) in system D. Found: C, 71.66; H, 7.40; N, 4.20%. Calcd for $C_{22}H_{27}NO_4$: C, 71.52; H, 7.37; N, 3.79%.

Bpoc–MeCys(Bzl)–OH·DCHA. *N*-Methyl-*S*-benzyl-L-cysteine (9.01 g, 40 mmol)[14] was acylated with Bpoc–N_3 (13.99 g, 50 mmol) in the same manner as described above for Bpoc–MeVal–OH. The oily product was dissolved in ether (20 ml) and DCHA (4.53 g, 25 mmol) was added. The crystalline precipitate which formed was filtered off and recrystallized from methanol–ether; yield 13.55 g (53%), mp 146—148 °C, $[\alpha]^{20}_{D}$ −53.9 ±1.0° (*c* 1.0, methanol). TLC: homogeneous (H_2SO_4) in systems D and J. Found: C, 72.72; H, 8.20; N, 4.51; S, 4.94%. Calcd for $C_{39}H_{52}N_2O_4S$: C, 72.63; H, 8.13; N, 4.34; S, 4.97%.

Bpoc–D-Ser–OH·H_2O. This compound was prepared in the same manner as for Bpoc–MeVal–OH. Crystallization from chloroform–petroleum ether gave the desired product in a 73.5% yield; mp 76—77 °C, $[\alpha]^{22}_{D}$ +7.6 ±0.5° (*c* 1.0, methanol). TLC: homogeneous (H_2SO_4) in system M. Found: C, 63.25; H, 6.91; N, 4.12%. Calcd for $C_{19}H_{21}NO_5 \cdot H_2O$: C, 63.15; H, 6.42; N, 3.88%.

The DCHA salt prepared in the usual manner showed mp 152—154 °C, $[\alpha]^{23}_{D}$ −4.3 ±0.5° (*c* 1.0, methanol).

H–MeCys(Bzl)–OBu^t·HCl. *N*-Methyl-*S*-benzyl-L-cysteine (2.25 g, 10 mmol)[14] was treated with 2-methylpropene in dioxane (15 ml) in the presence of sulfuric acid

(1.5 ml) according to Roeske[23] to give the corresponding *t*-butyl ester, which was isolated as the hydrochloride and recrystallized from dichloromethane-ethyl acetate; yield 1.81 g (57%), mp 153—153.5 °C, $[\alpha]_D^{23.5}$ −11.9 ±0.6° (*c* 1.0, methanol). TLC: homogeneous (H_2SO_4) in system L. Found: C, 56.53; H, 7.62; N, 4.38; S, 10.03; Cl, 11.25%. Calcd for $C_{15}H_{23}NO_2S$: C, 56.68; H, 7.61; N, 4.41; S, 10.09; Cl, 11.15%.

*Z-D-Ser-Ala-OBu^t (**5**).* Z-D-Ser-OH (4.78 g, 20 mmol)[24] and H-Ala-OBu^t (2.90 g, 20 mmol) were coupled with DCC (4.13 g, 20 mmol) in ethyl acetate-dichloromethane (2 : 5, 70 ml) at 5 °C for 20 h. After the dicyclohexylurea which had separated was filtered off, the filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with ice-cold M hydrochloric acid and M sodium hydrogencarbonate, dried (Na_2SO_4), and evaporated *in vacuo* to give the desired product, which was recrystallized from ethyl acetate-petroleum ether; yield 5.12 g (70%), mp 89—90 °C, $[\alpha]_D^{28}$ −14.4 ±0.5° (*c* 1.1, methanol). TLC: homogeneous (ninhydrin, after pretreatment with HBr) in systems A and I. Found: C, 59.32; H, 7.08; N, 7.68%. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.64%.

*Qxc-D-Ser-Ala-OBu^t (**6**).* Compound **5** (7.33 g, 20 mmol) was hydrogenolyzed over palladium in methanol for 2 h to give H-D-Ser-Ala-OBu^t as an oil. This was then allowed to react with Qxc-ONp (5.91 g, 20 mmol) in ethyl acetate (100 ml) at 25 °C for 20 h. The product was purified on a silica gel column with chloroform-methanol (9:1) as solvent. The fractions containing the desired material as a single component, as examined by TLC, were combined and evaporated *in vacuo* to give a crystalline residue. This was recrystallized from benzene-hexane; yield 5.90 g (76%), mp 99—100 °C, $[\alpha]_D^{27}$ −114.2 ±2.9° (*c* 0.5, methanol), −100.4 ±1.7° (*c* 0.9, DMF). λ_{max}^{MeOH} 243 nm (ε 43 700), 320 nm (ε 7 450), 325 nm (ε 7 440). TLC: homogeneous (H_2SO_4) in system A. Lit: mp 94—96 °C, $[\alpha]_D^{27}$ −79.2° (*c* 5, DMF).[9] Found: C, 58.94; H, 6.18; N, 14.47%. Calcd for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.42%.

Qxc-D-Ser(Z-MeVal)-Ala-OBu^t. *a) Carbonyldiimidazole Method*: To a solution of Z-MeVal-OH (0.29 g, 1.1 mmol)[19] in anhydrous THF (4 ml) was added CDMI (0.21 g, 1.1 mmol) and the mixture was stirred at 25 °C for 25 min. To this was added compound **6** (0.43 g, 1 mmol) with some THF as solvent. After the reaction mixture had been kept at 25 °C for 2 days, the product was precipitated by the addition of water and extracted with ethyl acetate. The organic solution was dried (Na_2SO_4) and evaporated *in vacuo* to give a residue, which was purified on a silica gel column with benzene-ethyl acetate (1:1) as solvent; yield 0.19 g (30%), $[\alpha]_D^{25}$ −93.7 ±2.9° (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems A and B. Found: 62.36; H, 6.45; N, 10.87%. Calcd for $C_{33}H_{41}N_5O_8$: C, 62.35; H, 6.50; N, 11.02%.

b) p-Toluenesulfonyl Chloride Method: Z-MeVal-OH (2.65 g, 10 mmol) was allowed to react with Tos-Cl (1.43 g, 7.5 mmol) in dry pyridine (10 ml) at 0 °C for 15 min. To this was added **6** (2.01 g, 5 mmol) and the mixture was stirred at 0 °C for 3 h. The product was precipitated by the addition of water and purified as described above in a); yield 2.88 g (91%), $[\alpha]_D^{25}$ −91.5 ±2.6° (*c* 0.5 methanol). TLC: homogeneous (H_2SO_4) in systems A and B. Found: C, 62.38; H, 6.21; N, 10.89%.

*Qxc-D-Ser(Bpoc-MeVal)-Ala-OBu^t (**7**).* Bpoc-MeVal-OH (3.70 g, 10 mmol) was allowed to react with CDI (1.78 g, 11 mmol) in anhydrous THF at 0 °C. When effervescence ceased compound **6** (3.88 g, 10 mmol) was added. The mixture was stirred at 25 °C for 5 h, after which the product was precipitated with water. Purification on a silica gel column with dichloromethane-ethyl acetate (8 : 2) as solvent afforded **7** as glassy material; yield 2.93 g (40%), $[\alpha]_D^{24.5}$ −62.2 ±2.1° (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems D and F. Found: C, 66.34; H, 6.80; N, 9.85%. Calcd for $C_{41}H_{49}N_5O_8$: C, 66.55; H, 6.75; N, 9.61%.

Compound **7** was also synthesized under the same conditions except for the use of CDMI in place of CDI; yield 28%, $[\alpha]_D^{27}$ −60.9 ±2.1° (*c* 0.5, methanol).

*Qxc-D-Ser[Z-MeCys(Bzl)-MeVal]-Ala-OBu^t (**8**).* *a) Mixed Anhydride Method*: Compound **7** (0.44 g, 0.6 mmol) was treated with 0.1M TFA in dichloromethane (60 ml) at 25 °C for 60 min to give Qxc-D-Ser(H-MeVal)-Ala-OBu^t. The mixture was neutralized with TEA (0.75 ml, 5.4 mmol) and concentrated to *ca.* 5 ml. Z-MeCys(Bzl)-OH, derived from the DCHA salt (0.49 g, 0.9 mmol)[14] by the treatment with Dowex 50W × 8 (H^+ form) in 50% ethanol, and TEA (0.13 ml, 0.9 mmol) were dissolved in dichloromethane (5 ml) and ethyl chloroformate (0.09 ml, 0.9 mmol) was added at −10 °C. The resulting solution of the anhydride was mixed with a solution of the N^α-free tridepsipeptide obtained above and TEA (0.83 ml) was added. The reaction mixture was kept at 5 °C for 16 h. The product precipitated by the addition of water was chromatographed repeatedly on silica gel columns with chloroform-methanol (99 : 1) as solvent; yield 0.26 g (51%), $[\alpha]_D^{25}$ −146.5 ±3.7° (*c* 0.4, methanol). TLC: homogeneous (H_2SO_4) in system K. Found: C, 62.58; H, 6.34; N, 9.98; S, 4.09%. Calcd for $C_{44}H_{54}N_6O_9S$: C, 62.69; H, 6.46; N, 9.97; S, 3.80%.

b) DCC Method: Compound **7** (1.48 g, 2 mmol) was deblocked with 80% acetic acid (50 ml) at 70 °C for 60 min. The solvent was evaporated *in vacuo* and the residue was precipitated from ether-petroleum ether. The oily precipitate separated by decantation was lyophilized from 0.05M hydrochloric acid to give the N^α-free depsitripeptide hydrochloride. This was then coupled with Z-MeCys(Bzl)-OH, derived from the DCHA salt (1.08 g, 2 mmol), by means of DCC (0.41 g, 2 mmol) in dichloromethane in the presence of TEA (0.28 ml, 2 mmol) at 5 °C overnight. The reaction mixture was worked up in the usual manner and the resulting product was purified on a silica gel column with benzene and benzene-ethyl acetate systems (7:3 and 1:1) as solvent. The fractions of the last solvent system were combined and evaporated *in vacuo* to give **8** in pure form; yield 1.19 g (71%), $[\alpha]_D^{22}$ −137.7 ±3.6° (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems B and C. Found: C, 62.56; H, 6.28; N, 9.74; S, 4.02%.

*Qxc-D-Ser[Z-MeCys(Bzl)-MeVal]-Ala-OH (**1**).* Compound **8** (0.84 g) was treated with anhydrous TFA (10 ml) at 25 °C for 60 min. After removal of TFA by evaporation *in vacuo* the residue was dissolved in ethyl acetate and the solution was washed with water, dried (Na_2SO_4) and evaporated *in vacuo* to give glassy material; yield 0.77 g (97%), $[\alpha]_D^{23.5}$ −142.7 ±3.6° (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems B and J. Found: C, 60.81; H, 5.94; N, 10.41; S, 4.52%. Calcd for $C_{40}H_{46}N_6O_9S$: C, 61.05; H, 5.89; N, 10.68; S, 4.07%.

*Qxc-D-Ser[Bpoc-MeCys(Bzl)-MeVal]-Ala-OBu^t (**2**).* Compound **7** (0.74 g, 1 mmol) was deblocked with 80% acetic acid to give the N^α-free tridepsipeptide, which was converted into the hydrochloride as in the synthesis of **8**. This was then coupled with Bpoc-MeCys(Bzl)-OH, derived from the DCHA salt (0.65 g, 1 mmol) by shaking with M citric acid in the presence of dichloromethane, by the DCC method as described above for compound **8**. The product was chromatographed on a silica gel column with benzene–ethyl acetate (7:3) as solvent; yield 0.57 g (60%), $[\alpha]_D^{22}$ $-121.5 \pm 3.6°$ (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems B and D. Found: C, 65.72; H, 6.61; N, 8.78; S, 3.76%. Calcd for $C_{52}H_{62}N_6O_9S$: C, 65.94; H, 6.60; N, 8.87; S, 3.38%.

Treatment of **2** *with Aqueous Acetic Acid–Formic Acid Mixture. Formation of* Cyclo[*–MeCys(Bzl)–MeVal–*]. Compound **2** (0.38 g, 0.4 mmol) was treated with acetic acid–formic acid–water (7:1:2, 4 ml)[21] at 25 °C for 2 h. The solvent was evaporated *in vacuo* and the oily residue was precipitated from ethyl acetate–petroleum ether. The resulting material was subjected to a column of silica gel (26 g) and eluted with benzene–ethyl acetate (7:3, 200 ml) and then with ethyl acetate (150 ml). The ethyl acetate fractions were combined and evaporated *in vacuo*. The residue (0.22 g) was rechromatographed on a silica gel column (25 g, Kieselgel H, Merck) with chloroform–methanol (95:5) as solvent. Tubes 15—17 (5 g/tube) were combined and evaporated *in vacuo* to give an oil (72 mg, 56% as *cyclo*[–MeCys(Bzl)–MeVal–]); $[\alpha]_D^{26}$ $-43.5 \pm 1.7°$ (*c* 0.5, methanol). Found: C, 63.00; H, 7.59; N, 8.42; S, 9.61%. Calcd for $C_{17}H_{24}N_2O_2S$: C, 63.72; H, 7.55; N, 8.74; S, 10.00%. MS, *m/e* 320 (M), 229, 197, 183, 155, and 91.[25]

From tubes 19—24 (5 g/tube) was obtained a crystalline material, which was recrystallized from benzene–hexane (118 mg, 76% as compound **6**); mp 96—98 °C, $[\alpha]_D^{22}$ $-109.0 \pm 0.7°$ (*c* 0.5, methanol). IR, identical with that of an authentic sample of **6**.

Bpoc–Ala–MeCys(Bzl)–OBu^t (**9**). H–MeCys(Bzl)–OBu^t·HCl (6.36 g, 20 mmol) was treated with 50% potassium carbonate at 0 °C in the presence of ether and the organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give the corresponding free base. This was then coupled with Bpoc–Ala–OH (6.55 g, 20 mmol)[22] by means of DCC (4.13 g, 20 mmol) in ethyl acetate (10 ml). The reaction mixture was worked up in the usual manner to give an oily product, which was chromatographed on a silica gel column with benzene–ethyl acetate (8:2) as solvent to give **9** in pure form; yield 11.19 g (95%), $[\alpha]_D^{22}$ $-106.9 \pm 1.5°$ (*c* 1.0, methanol). TLC: homogeneous (H_2SO_4) in systems C and E. Found: C, 69.25; H, 7.24; N, 4.59; S, 5.36%. Calcd for $C_{34}H_{42}N_2O_5S$: C, 69.12; H, 7.17; N, 4.74; S, 5.43%.

*Bpoc–*D*-Ser–Ala–MeCys(Bzl)–OBu*^{t} (**10**). Compound **9** (10.63 g, 18 mmol) was treated with a mixture of acetic acid–formic acid–water (7:1:2; 100 ml) at 25 °C for 2 h. The solvent was evaporated *in vacuo* and the residue was treated with saturated potassium carbonate in the presence of ethyl acetate. The organic solution was washed with saturated sodium chloride, dried (Na_2SO_4) and evaporated *in vacuo* to give H–Ala–MeCys(Bzl)–OBu^t as an oil. This was then coupled with Bpoc–D-Ser–OH (6.18 g, 18 mmol) by the DCC method in dichloromethane in the usual manner. The product was chromatographed on a silica gel column (Kieselgel H, Merck) with ethyl acetate–cyclohexane (8:2) as solvent and crystallized from ethyl acetate–petroleum ether to give fine needles; yield 8.80 g (76%), mp 128—129 °C, $[\alpha]_D^{23}$ $-42.6 \pm 1.6°$ (*c* 0.5, methanol). TLC: homogeneous (ninhydrin, after pretreatment with HCl) in system D. Found: C, 65.39; H, 6.95; N, 6.47; S, 4.74%. Calcd for $C_{37}H_{47}N_3O_7S$: C, 65.66; H, 6.99; N, 6.20; S, 4.73%.

*Qxc–*D*-Ser–Ala–MeCys(Bzl)–OBu*^{t} (**11**). Compound **10** (6.78 g, 10 mmol) was treated with acetic acid–formic acid–water (7:1:2) at 25 °C for 2 h to give the N^α-free tripeptide and this was allowed to react with Qxc–ONp (3.25 g, 11 mmol) in ethyl acetate in the presence of TEA (1.54 ml) for 20 h. The product was purified on a silica gel column with chloroform–methanol (9:1) as solvent; yield 3.90 g (65%), mp 60—70 °C, $[\alpha]_D^{22}$ $-153.6 \pm 3.8°$ (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems A and H. Found: C, 59.99; H, 6.32; N, 11.86; S, 5.29%. Calcd for $C_{30}H_{37}N_5O_6S$: C, 60.49; H, 6.26; N, 11.76; S, 5.38%.

*Qxc–*D*-Ser(Z–MeVal)–Ala–MeCys(Bzl)–OBu*^{t} (**12**). Z–MeVal–OH (0.42 g, 1.6 mmol)[19] was coupled with **11** (0.48 g, 0.8 mmol) by means of Tos–Cl (0.23 g, 1.2 mmol) in pyridine, as in the synthesis of Qxc–D-Ser(Z–MeVal)–Ala–OBu^t. The crude product was purified by repeated chromatography on silica gel columns with chloroform–methanol (95:5) and ethyl acetate–cyclohexane (8:2) as solvent; yield 0.48 g (72%), $[\alpha]_D^{22}$ $-129.8 \pm 3.4°$ (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems G and H. Found: C, 62.47; H, 6.28; N, 9.77; S, 4.03%. Calcd for $C_{44}H_{54}N_6O_9S$: C, 62.69; H, 6.46; N, 9.97; S, 3.80%.

*Qxc–*D*-Ser(Z–MeVal)–Ala–MeCys(Bzl)–OH* (**3**). Compound **12** (0.42 g, 0.5 mmol) was treated with TFA (5 ml) at 25 °C for 30 min. After removal of TFA by evaporation *in vacuo* the residue was dissolved in ethyl acetate and the solution was washed with water, dried (Na_2SO_4) and evaporated *in vacuo* to give a glassy material, which was purified by chromatography on a silica gel column (Kieselgel H, Merck) with chloroform–methanol–acetic acid (90:10:3) as solvent; yield 0.37 g (94%), $[\alpha]_D^{25.5}$ $-126.1 \pm 3.3°$ (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in system K. Found: C, 60.35; H, 5.81; N, 10.42; S, 4.34%. Calcd for $C_{40}H_{46}N_6O_9S$: C, 61.05; H, 5.89; N, 10.68; S, 4.07%.

*Qxc–*D*-Ser(Bpoc–MeVal)–Ala–MeCys(Bzl)–OBu*^{t} (**4**). Bpoc–MeVal–OH (0.55 g, 1.5 mmol) was allowed to react with CDI (0.29 g, 1.8 mmol) in anhydrous THF at 25 °C for 30 min. To this was added a solution of **11** (0.60 g, 1.0 mmol) in THF. The mixture was stirred at 25 °C for 22 h and evaporated *in vacuo*. The residue was, after being washed with water, purified on a silica gel column (Kieselgel H, Merck) with chloroform–methanol (98:2) as solvent; yield 0.20 g (21%), $[\alpha]_D^{26.5}$ $-99.6 \pm 2.6°$ (*c* 0.5, methanol). TLC: homogeneous (H_2SO_4) in systems C, G, and J. Found: C, 65.62; H, 6.58; N, 8.63; S, 3.62%. Calcd for $C_{52}H_{62}N_6O_9S$: C, 65.94; H, 6.60; N, 8.87; S, 3.38%.

References

1) K. Katagiri, T. Yoshida, and K. Sato, "Antibiotics," Vol. 3, ed by J. W. Corcoran and F. E. Hahn, Springer Verlag (1975), p. 234 and references cited therein.

2) J. Shoji and K. Katagiri, *J. Antibiot.*, **A14**, 335 (1961); H. Otsuka and J. Shoji, *ibid.*, **A19**, 128 (1966).

3) H. Otsuka and J. Shoji, *J. Antibiot.*, **A16**, 52 (1963); J. Shoji, K. Tori, and H. Otsuka, *J. Org. Chem.*, **30**, 2772 (1965); H. Otsuka and J. Shoji, *J. Antibiot.*, **A18**, 134 (1965); H. Otsuka and J. Shoji, *Tetrahedron*, **21**, 2931 (1965); *ibid.*, **23**, 1535 (1967).

4) a) R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, L. Neipp, V. Prelog, P. Reusser, and H. Zähner, *Helv. Chim. Acta*, **40**, 199 (1957); b) W. Keller-Schierlein and V. Prelog, *ibid.*, **40**, 205 (1957); c) W. Keller-Schierlein, M. Lj. Mihailović, and V. Prelog, *ibid.*, **42**, 305 (1959).

5) T. Yoshida and K. Katagiri, *J. Antibiot.*, **A14**, 330 (1961).

6) D. G. Martin, S. A. Mizsak, C. Biles, J. C. Stewart, L. Baczynskyj, and P. A. Meulman, *J. Antibiot.*, **28**, 332 (1975); A. Dell, D. H. Williams, H. R. Morris, G. A. Smith,

J. Feeney, and G. C. K. Roberts, *J. Am. Chem. Soc.*, **97**, 2497 (1975).

7) H. Otsuka, J. Shoji, K. Kawano, and Y. Kyogoku, *J. Antibiot.*, **29**, 107 (1976).

8) S. Gerchakov, P. J. Whitman, and H. P. Schultz, *J. Med. Chem.*, **9**, 266 (1966).

9) S. Gerchakov and H. P. Schultz, *J. Med. Chem.*, **12**, 141 (1969).

10) H. C. Koppel, I. L. Honigberg, R. H. Springer, and C. C. Cheng, *J. Org. Chem.*, **28**, 1119 (1963).

11) T. L. Ciardelli and R. K. Olsen, *J. Am. Chem. Soc.*, **99**, 2806 (1977).

12) J. Meienhofer, *J. Am. Chem. Soc.*, **92**, 3771 (1970).

13) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).

14) D. Yamashiro, H. L. Aanning, L. A. Branda, W. D. Cash, V. V. S. Murti, and V. du Vigneaud, *J. Am. Chem. Soc.*, **90**, 4141 (1968).

15) S. Sakakibara and N. Inukai, *Bull. Chem. Soc. Jpn.*, **37**, 1231 (1964); *ibid.*, **38**, 1979 (1965).

16) H. A. Staab, *Justus Liebigs Ann. Chem.*, **609**, 75 (1957); *Angew. Chem.*, **68**, 754 (1956).

17) S. Akabori, S. Sakakibara, and M. Shin, Proceedings of the 2nd Symposium on Peptide Chemistry, Osaka, 1963, Institute for Protein Research, Osaka University (1963), p. 39.

18) W.-Y. Chen, M.-L. Hsu, and R. K. Olsen, *J. Org. Chem.*, **40**, 3110 (1975).

19) Pl. A. Plattner, K. Vogler, R. O. Studer, P. Quitt, and W. Keller-Schierlein, *Helv. Chim. Acta*, **46**, 927 (1963); J. R. McDermott and N. L. Benoiton, *Can. J. Chem.*, **51**, 1915 (1973).

20) J. H. Brewster and C. J. Ciotti, Jr., *J. Am. Chem. Soc.*, **77**, 6214 (1955).

21) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 622 (1968).

22) S. S. Wang and R. B. Merrifield, *Int. J. Pept. Protein Res.*, **1**, 235 (1969); E. Schnabel, G. Schmidt, and E. Klauke, *Justus Liebigs Ann. Chem.*, **743**, 69 (1971).

23) R. Roeske, *J. Org. Chem.*, **28**, 1251 (1963).

24) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 25 (1955); H. Adkins, R. M. Ross, D. C. Schroeder, C. L. Mahoney, and W. W. Gilbert, *J. Am. Chem. Soc.*, **76**, 147 (1954).

25) MS was measured by Dr. Yuzo Nakagawa of this laboratory on a Hitachi mass spectrometer.