# Synthetic Studies on Quinoxaline Antibiotics. III.[1] Synthesis of Nortriostin A, a Triostin A Analog Lacking *N*-Methyl Groups on the Cystine and Valine Residues

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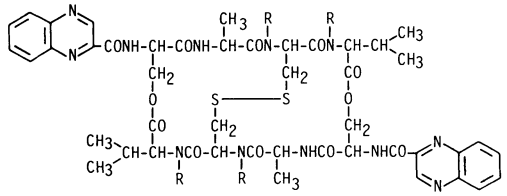
Nortriostin A, which is an analog of a cyclic octadepsipeptide antibiotic triostin A and contains one cystine and two valine residues substituted for the *N,N'*-dimethylcystine and *N*-methylvaline residues of the antibiotic, was synthesized with Z-D-Ser[Boc-Ala-Cys(MeBzl)-Val]-OH and Z-D-Ser[H-Ala-Cys(MeBzl)-Val]-OTce as key intermediates.††† The synthetic analog showed no antimicrobial activity at a concentration of 100 μg/ml and was found to exist as a single structure in solution as examined by $^1$H-NMR spectroscopy. The latter observation suggests that the conformer equilibrium known to occur with triostin A is a consequence of the presence of *N*-methyl peptide bonds in the antibiotic molecule.

In the preceding paper we described the total synthesis of triostin A, a member of the triostin family of the quinoxaline antibiotics, with *S,S'*-dibenzyldihydrotriostin A as intermediate.[1] Triostin A is known to have two interconvertible conformations in solution and their occurrence has been explained by two possibilities: 1) the reversal of the chirality around the disulfide bond,[2] and 2) the *cis-trans* isomerization of the *N*-methyl peptide bonds.[3] The first possibility has recently been ruled out, because the presence of the two conformers was also shown with *S,S'*-dibenzyldihydrotriostin A which lacks the disulfide bond.[1,4] In the present work the des-*N*-tetramethyl analog of triostin A (we propose the name "nortriostin A" for des-N-tetramethyltriostin A.[5] For structure, see Fig. 1) was synthesized and its NMR pattern was compared with that of triostin A to elucidate the second possibility. The synthesis of nortriostin A was carried out in essentially the same manner as described for triostin A,[1] except that the *N*-methyl-L-cysteine and *N*-methyl-L-valine as starting materials were replaced by cysteine and valine, respectively, and the 4-methylbenzyl group[6] in place of the benzyl group was employed for the mercapto-group protection.

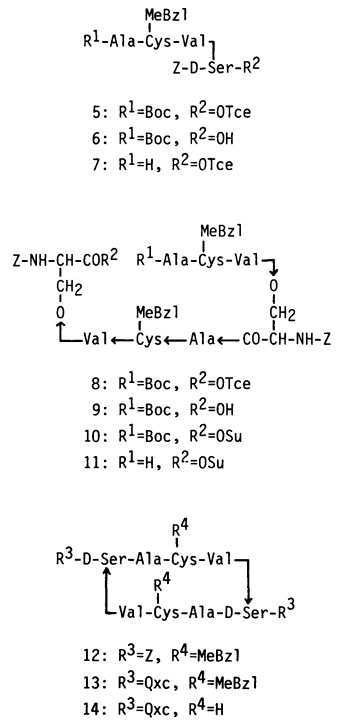


Triostin A: R=CH$_3$

Nortriostin A: R=H

Fig. 1. Triostin A and nortriostin A.

Z-D-Ser-OTce[1] was allowed to react in pyridine in the presence of HOBt with the symmetrical anhydride $(Boc\text{-}Val)_2O$, which had been prepared in advance from Boc-Val-OH by the action of DCC, to give a depsipeptide Z-D-Ser(Boc-Val)-OTce (**3**) in a 90% yield. Compound **3** could also be prepared by the direct coupling of Boc-Val-OH and Z-D-Ser-OTce with DCC in the presence of HOBt, but the yield was as low as 66%. Compound **3** was treated with TFA to remove the Boc group and then coupled with Boc-Cys (MeBzl)-OH (**2**) by the DCC-HOBt method[7] to give Z-D-Ser[Boc-Cys(MeBzl)-Val]-OTce (**4**) in 78% yield. Compound **4** was treated with TFA and the resulting product was coupled with the previously prepared



5: R$^1$=Boc, R$^2$=OTce
6: R$^1$=Boc, R$^2$=OH
7: R$^1$=H, R$^2$=OTce

8: R$^1$=Boc, R$^2$=OTce
9: R$^1$=Boc, R$^2$=OH
10: R$^1$=Boc, R$^2$=OSu
11: R$^1$=H, R$^2$=OSu

12: R$^3$=Z, R$^4$=MeBzl
13: R$^3$=Qxc, R$^4$=MeBzl
14: R$^3$=Qxc, R$^4$=H

Fig. 2. Intermediates involved in the synthesis of nortriostin A.

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††† Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*Biochemistry*, **11**, 1726 (1972)) and include: MeCys, *N*-methyl-L-cysteine; MeVal, *N*-methyl-L-valine; Boc, *t*-butoxycarbonyl; Boc-SDP, *O-t*-butyl *S*-(4,6-dimethyl-2-pyrimidinyl) thiocarbonate; Qxc, 2-quinoxalylcarbonyl; Z, benzyloxycarbonyl; MeBzl, 4-methylbenzyl; Bzl, benzyl; Tce, 2,2,2-trichloroethyl; HOBt, 1-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; DCC, dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; AcOH, acetic acid; TFA, trifluoroacetic acid; DIEA, *N,N*-diisopropylethylamine; TEA, triethylamine; DMF, *N,N*-dimethylformamide; and THF, tetrahydrofuran.

$(Boc\text{-}Ala)_2O$ in the presence of DIEA to produce tetradepsipeptide **5** (for structures of compounds **5**—**14**, see Fig. 2) in 66% yield.

One portion of the key intermediate **5** was treated with zinc in 90% acetic acid to remove the Tce ester giving **6** in a yield of 89%. Another portion of **5** was treated with TFA to remove the Boc group giving **7**. Compounds **6** and **7** were then coupled with DCC-HOBt to obtain the linear octadepsipeptide **8** in 72% yield. The treatment of **8** with zinc in 90% acetic acid at 0 °C for 3 h resulted in complete removal of the Tce ester giving **9** in 84% yield. Compound **9** was coupled to HOSu with DCC and the resulting active ester (**10**) was treated with TFA to give **11** as the TFA salt. For cyclization a DMF solution of **11** was diluted with ethyl acetate to a concentration of 0.2 mmol/l and neutralized with DIEA to initiate the reaction. The cyclic octadepsipeptide (**12**) was thus obtained in 48% yield. The $^1$H-NMR spectrum and field desorption mass spectrum of **12** were consistent with the proposed structure.

The selective removal of the Z group from **12** by the TFA-thioanisole treatment[1,8] and the subsequent acylation with Qxc-Cl gave **13** as amorphous solid in 40% yield. The MeBzl groups on the Cys residues showed no sign of cleavage during the TFA-thioanisole treatment, but they were readily removed by the treatment with HF at 0 °C for 60 min in the presence of anisole to furnish dihydronortriostin A (**14**), which was, upon treatment with iodine in methanol[9] followed by silica-gel column chromatography and recrystallization, converted into nortriostin A (Fig. 1) in a yield of 40%.

Nortriostin A thus obtained was homogeneous in TLC in different solvent systems. The sufficient purity was also shown by HPLC on a μ-Porasil column in acetonitrile-2-propanol (95:5, v/v) and by reversed-phase HPLC on a Nucleosil $5C_{18}$ column in acetonitrile-10 mM (1M=1 mol $dm^{-3}$) phosphoric acid (50:50, v/v). The $^1$H-NMR spectrum was consistent with the proposed structure when analyzed with reference to the spectra of natural[10] and synthetic[1] preparations of triostin A. As assayed for the inhibition of growth of *Bacillus subtilis* PCI-219, nortriostin A was found to be inactive even at a concentration of 100 μg/ml in contrast to triostin A, which was highly active at a level of 0.5 μg/ml.

The first synthesis of nortriostin A was described by Olsen and his associates in 1978,[5] in which they used the depsipeptide of the type Z-D-Ser($R^1$)-Ala-$OR^2$ ($R^1$=Boc (or H)-Cys(Acm)-Val, $R^2$=H or Tce, Acm=acetamidomethyl) as intermediate. In this synthesis a problem was the occurrence of some racemization at the Ala residue involved in the fragment coupling and the subsequent cyclization reactions. In their recent report Olsen and his collaborators have synthesized nortriostin A from the depsipeptide of the type Z-D-Ser($R^1$)-$OR^2$ ($R^1$=Boc (or H)-Ala-Cys(Acm)-Val, $R^2$=H or Bpa, Bpa=*p*-bromophenacyl).[11] Independently, we also used the latter type of intermediates in our synthesis of triostin A itself,[1] and in the present synthesis of nortriostin A as described above. In these syntheses the coupling of the two tetradepsipeptide intermediates and the subsequent cyclization were performed by activation of the carboxyl function of the Z-D-Ser-OH moiety. Therefore, little or no racemization was expected to occur at the Ser residue under ordinary coupling conditions. However, Olsen and his collaborators suggested the possibility of racemization of the D-Ser residues, based on the findings that the carbodiimide-mediated acylation of Z-D-Ser-OBpa with Boc-Val-OH in the presence of 4-(dimethylamino)pyridine and the acidolytic removal of the Boc group from Z-D-Ser[Boc-Ala-Cys(Acm)-Val]-OBpa followed by neutralization with sodium hydrogencarbonate were both accompanied by some formation of a dehydroalanine derivative Z-ΔAla-OBpa.[11] The Bpa ester was introduced in place of the Tce ester, which had been used in the form Z-D-Ser($R^1$)-Ala-OTce in their previous synthesis of nortriostin A, because they were not able to prepare Z-D-Ser-OTce from Z-D-Ser-OH and HOTce by the DCC procedure. We also failed in this attempt but found that the esterification was readily achieved by using Z-D-Ser($Bu^t$)-OH in place of Z-D-Ser-OH to give Z-D-Ser($Bu^t$)-OTce, from which Z-D-Ser-OTce was derived by the TFA treatment.[1] Z-D-Ser-OTce was used as starting material in our synthesis of triostin A[1] and in the present synthesis of nortriostin A as described. Although the Tce group seems to be more favorable than the Bpa group because of the general trend that the former group is cleaved more readily than the latter under the reductive conditions, there is no available information as to whether the Tce group differs from the Bpa group regard to the effect on the dehydroalanine formation. At least we have no positive evidence for the formation of any dehydroalanine derivatives and for the occurrence of recemization.

The $^1$H-NMR spectrum of nortriostin A was measured in deuteriochloroform to show that the hydrogens at position 3 of the two quinoxaline rings gave a single peak, indicating that nortriostin A, which differs from triostin A only by the absence of the *N*-methyl groups, exists as a single structure in solution. On the other hand, we have demonstrated that *S,S'*-dibenzyl-dihydrotriostin A, a derivative of triostin A which lacks the disulfide linkage but has the *N*-methyl peptide bonds, exists as two conformers.[1,4] These observations strongly support the view that the conformer equilibrium, which usually occurs with triostin A, is a consequence of the *cis-trans* isomerization of the *N*-methyl peptide bonds.[12]

## Experimental

Thin-layer chromatography (TLC) was performed on silica-gel plates (precoated Kieselgel $60F_{254}$, Merck) with the following solvent systems (ratios by volume): A, chloroform-methanol (95:5); B, chloroform-methanol (90:10); C, chloroform-methanol-acetic acid (95:5:3); D, benzene-ethyl acetate (7:3). For detection the plates were subjected to ninhydrin staining or to sulfuric acid charring. Column chromatography was performed on prepacked columns (Lobar, size B or C, Merck) or home-made columns (Kieselgel 60, Merck). High-performance liquid chromatography (HPLC) was carried out on a Waters Associates Model 6000A solvent delivery system, equipped with a Waters U6K injector and a Japan Spectroscopic UVIDEC-100-II variable

wavelength UV detector. Proton NMR spectra were measured on a Bruker WM 360 wb NMR spectrometer operated at 360 MHz.

*S-4-Methylbenzyl-cysteine (1).* This compound was synthesized according to the literature;[6] mp 212—214 °C dec, $[\alpha]_D^{22}$ −4.1±1.4°, $[\alpha]_{365}^{22}$ +9.8±1.6° (*c* 0.3, M hydrochloric acid), $[\alpha]_D^{22}$ +23.7±0.9°, $[\alpha]_{365}^{22}$ +96.1±1.3° (*c* 1.0, M sodium hydroxide). Lit,[6] mp 209—211 °C dec.

*Boc-Cys(MeBzl)-OH (2).* To a solution of compound **1** (7.89 g, 35 mmol) and TEA (11 ml, 79 mmol) in dioxane-water (1:1, 40 ml) was added Boc-SDP (12.62 g, 53 mmol) and the mixture was stirred at 30 °C for 15 h. After addition of M sodium hydroxide (60 ml) at 0 °C the mixture was extracted twice with ether. The aqueous phase was then acidified with ice-cold M hydrochloric acid. The resulting oily precipitate was taken into ethyl acetate and the solution was washed with water, dried over magnesium sulfate and evaporated *in vacuo* to give an oil which was solidified by the addition of petroleum ether. Recrystallization from ethyl acetate-petroleum ether gave **2** as needles; 10.8 g (95%), mp 76—77 °C, $[\alpha]_D^{19}$−41.7±0.8 (*c* 1.0, acetic acid). Found: C, 58.89; H, 6.98; N, 4.25; S, 9.83%. Calcd for $C_{16}H_{23}NO_4S$: C, 59.05; H, 7.12; N, 4.30; S, 9.85%.

*Z-D-Ser(Boc-Val)-OTce (3).* *a) With (Boc-Val)$_2$O*: Boc-Val-OH (5.87 g, 27 mmol) was treated with DCC (5.57 g, 27 mmol) in pyridine at 0 °C for 60 min. To this was added a solution of Z-D-Ser-OTce (5.00 g, 13.5 mmol)[1] and HOBt (2.74 g, 20 mmol) in pyridine (10 ml) and the mixture was stirred at 5 °C for 20 h. The DCU which had formed was filtered off and the filtrate was evaporated *in vacuo* to give a residue, which was dissolved in ethyl acetate, and washed with ice-cold M hydrochloric acid, 5% sodium hydrogencarbonate and water. The solution was, after being dried over magnesium sulfate, evaporated *in vacuo* to give a crude preparation of **3** as an oil. The crude product was subjected to chromatography on a silica-gel column with chloroform-methanol (98:2) as solvent. The fractions containing the desired material were pooled and evaporated *in vacuo*. The residue was rechromatographed twice on a Lobar column (size B) with benzene-ethyl acetate (95:5) as solvent. Evaporation of the combined fractions containing the desired material as a single component gave a pure preparation of **3** as an oil; 7.46 g (97%), $[\alpha]_D^{21}$ +3.8±0.4° (*c* 1.0, methanol). TLC: homogeneous in systems A and D. Found: C, 48.77; H, 5.65; N, 4.85; Cl, 18.56%. Calcd for $C_{23}H_{31}N_2O_8Cl_3$: C, 48.48; H, 5.48; N, 4.92; Cl, 18.66%.

*b) By the DCC-HOBt method.* To an ice-cold solution of Boc-Val-OH (1.05 g, 4.8 mmol), Z-D-Ser-OTce (1.63 g, 4.4 mmol) and HOBt (0.65 g, 4.8 mmol) in pyridine (13 ml) was added DCC (1.00 g, 4.8 mmol) and the mixture was stirred at 5 °C overnight. The DCU which had formed was filtered off and the filtrate was evaporated *in vacuo*. The resulting oil was dissolved in ethyl acetate and this was washed with ice-cold M hydrochloric acid and 5% sodium hydrogencarbonate, dried over magnesium sulfate and evaporated *in vacuo*. The crude product thus obtained was purified on silica-gel columns as described above in a) to afford **3** as an oil; 1.65 g (66%), $[\alpha]_D^{23}$ +2.0±0.4° (*c* 1.0, methanol).

*Z-D-Ser[Boc-Cys(MeBzl)-Val]-OTce (4).* Compound **3** (6.84 g, 12 mmol) was treated with TFA (36 ml) at 25 °C for 40 min, followed by evaporation *in vacuo*. The resulting oil was dissolved in ethyl acetate and the solution was washed with 5% sodium hydrogencarbonate, dried over magnesium sulfate and evaporated *in vacuo*. The residue was dissolved in THF (70 ml) together with Boc-Cys(MeBzl)-OH (4.69 g, 14 mmol) and HOBt (1.95 g, 14 mmol). After addition of DCC (2.97 g, 14 mmol) at 0 °C, the mixture was stirred at the same temperature for 3 h and at 25 °C for 1 h. The reaction mixture was worked up in the usual manner to give a crude product which was repeatedly chromatographed on Lobar columns (size C) with chloroform-methanol (99:1) and benzene-ethyl acetate (8:2) as solvents to give **4** as amorphous solid; 7.31 g (78%) $[\alpha]_D^{22}$ −12.0±0.5° (*c* 1.0, methanol). TLC: homogeneous in systems A and D. Found: C, 52.38; H, 5.79; N, 5.19; S, 4.37; Cl, 13.13%. Calcd for $C_{34}H_{44}N_3O_9Cl_3S$: C, 52.55; H, 5.71; N, 5.41; S, 4.13; Cl, 13.69%.

*Z-D-Ser[Boc-Ala-Cys(MeBzl)-Val]-OTce (5).* Boc-Ala-OH (5.11 g, 27 mmol) was allowed to react with DCC (2.79 g, 13.5 mmol) in THF (30 ml) at 0 °C for 2 h. Removal of the separated DCU by filtration afforded a solution of (Boc-Ala)$_2$O. On the other hand, compound **4** (6.99 g, 9 mmol) was treated with TFA (27 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The oily residue was, after being dried over sodium hydroxide pellets *in vacuo*, dissolved in THF (20 ml). The ice-cold solution was then combined with the (Boc-Ala)$_2$O obtained above and this was neutralized by the addition of DIEA. The mixture was then stirred at 5 °C overnight and worked up in the usual manner. The crude product was subjected to chromatography on a silica-gel column (Kieselgel 60, 6×33 cm) with chloroform-methanol (99:1) as solvent. The fractions containing the desired material were collected and evaporated *in vacuo*. Repeated rechromatography on a Lobar column (size C) with chloroform-methanol (99:1) and benzene-ethyl acetate (85:15 and 50:50) as solvents afforded a pure preparation of **5** as amorphous solid; 5.05 g (66%), $[\alpha]_D^{22}$ −25.1±0.7° (*c* 1.0, methanol). TLC: homogeneous in systems A, C, and D. Found: C, 52.46; H, 6.00; N, 6.80; S, 4.05; Cl, 11.82%. Calcd for $C_{37}H_{49}N_4O_{10}SCl_3$: C, 52.39; H, 5.82; N, 6.61; S, 3.78; Cl, 12.54%.

*Z-D-Ser[Boc-Ala-Cys(MeBzl)-Val]-OH (6).* Compound **5** (3.82 g, 4.5 mmol) in 90% acetic acid (135 ml) was treated with zinc powder (14.7 g) at 0 °C for 3 h. After the excess zinc had been filtered off, the filtrate was evaporated *in vacuo* and the residue dissolved in ethyl acetate was washed with ice-cold M hydrochloric acid and water, dried over magnesium sulfate and evaporated *in vacuo*. The resulting material was chromatographed on silica-gel columns (Kieselgel 60) with chloroform-methanol-acetic acid systems (95:5:0.5 and 98:2:1) as solvent to give a pure preparation of **6** as amorphous solid; 2.91 g (89%), $[\alpha]_D^{22}$−36.0±0.7° (*c* 1.0, methanol). TLC: homogeneous in system C. Found: C, 57.76; H, 6.79; N, 7.53; S, 4.53%. Calcd for $C_{35}H_{48}N_4O_{10}S\cdot 1/2H_2O$: C, 57.91; H, 6.80; N, 7.72; S, 4.42%.

*Z-D-Ser{Z-D-Ser[Boc-Ala-Cys(MeBzl)-Val]-Ala-Cys(MeBzl)-Val}-OTce (8).* Copound **5** (2.38 g, 2.8 mmol) was treated with TFA (28 ml) at 25 °C for 30 min, followed by evaporation *in vacuo*. The oily residue was dissolved in ethyl acetate and the solution was shaken with ice-cold 50% potassium carbonate, dried over magnesium sulfate and evaporated *in vacuo* to produce Z-D-Ser[H-Ala-Cys(MeBzl)-Val]-OTce (**7**) as an oil. This was then dissolved in THF (56 ml) together with compound **6** (2.03 g, 2.8 mmol) and HOBt (0.57 g, 4.2 mmol). The mixture was chilled in an ice-bath and, after addition of DCC (0.87 g, 4.2 mmol), stirred for 5 h at 0 °C and 1 h at 25 °C. The DCU which had formed was filtered off and the solvent was evaporated *in vacuo*. The solid residue was chromatographed on silica-gel columns (Kieselgel 60 and Kieselgel H) with chloroform-methanol (98:2) as solvent, followed by precipitation from methanol-ether to give a pure preparation of **8** as amorphous solid; 2.90 g (72%), mp 178—179 C°, $[\alpha]_D^{23}$ −41.4±0.8° (*c* 1.0, methanol). TLC: homogeneous in system B and in ethyl acetate. Found: C, 56.04; H, 6.11; N, 7.94; S, 4.68; Cl, 6.55%. Calcd for $C_{67}H_{87}N_8O_{17}S_2Cl_3$: C, 55.61; H, 6.06; N, 7.74; S, 4.43; Cl, 7.35%.

*Z-D-Ser{Z-D-Ser[Boc-Ala-Cys(MeBzl)-Val]-Ala-Cys(MeBzl)-Val}-OH (9).* Compound **8** (2.60 g, 1.8 mmol) was treated with zinc (5.88 g) in 90% acetic acid (280 ml) at 0 °C

for 3 h. After removal of the excess zinc by filtration the solvent was evaporated *in vacuo*. The residue was dissolved in chloroform-methanol (98:2) and the solution was washed with ice-cold M hydrochloric acid and water, dried over magnesium sulfate, and evaporated *in vacuo*. The crude product (2.34 g) was chromatographed on a silica-gel column (Kieselgel 60, 3.2×28 cm) with chloroform-methanol-acetic acid (98:2:0.5 and 95:5:0.5) as solvent to give **9** as glassy mass; 2.00 g (84%), mp 176—178 °C, $[\alpha]_D^{23}$ −62.7±1.0° (*c* 1.0, chloroform). Found: C, 58.98; H, 6.51; N, 8.31; S, 5.04%. Calcd for $C_{65}H_{86}N_8O_{17}S_2$: C, 59.34; H, 6.59; N, 8.52; S, 4.87%.

*{Z-D-Ser[Z-D-Ser-Ala-Cys(MeBzl)-Val]-Ala-Cys(MeBzl)-Val}-Dilactone, N,N'-$Z_2$-S,S'-$MeBzl_2$-dihydroaponortriostin A (12).* Compound **9** (0.132 g, 0.1 mmol) and HOSu (0.115 g, 1 mmol) were dissolved in DMF (1 ml). To this solution was added DCC (0.206 g, 1 mmol) at 0 °C and the mixture was stirred at 0 °C for 3 h and then at 25 °C for 2 h. The precipitates which formed upon addition of ether were filtered off and dried *in vacuo* to give the HOSu ester (**10**, Fig. 2). Compound **10** was treated with TFA (5 ml) at 25 °C for 30 min and the resulting **11** was precipitated by the addition of ether. These precipitates were dissolved in DMF (5 ml) and the solution was diluted with ethyl acetate (500 ml). To this was added DIEA until the solution became neutral and the mixture was stirred at 25 °C for 5 h. After removal of the solvent by evaporation *in vacuo*, the residue was dissolved in chloroform-methanol (98:2) and this was washed with M hydrochloric acid and 5% sodium hydrogencarbonate, dried over magnesium sulfate and evaporated *in vacuo*. The curde product was repeatedly chromatographed on a Lobar column (size A) with chloroform-methanol (98:2). The fractions containing the desired product as single component were pooled and evaporated *in vacuo* to afford **12** as amorphous solid; 58 mg (48%), mp 246—248 °C, $[\alpha]_D^{23}$ −76.3±1.2° (*c* 1.0, chloroform). NMR ($CDCl_3$, 360 MHz, 37 °C) δ=0.82 (d, 6H, Val γ- and γ'-$CH_3$), 0.84 (d, 6H, Val γ- and γ'-$CH_3$), 1.14 (d, 6H, Ala β-$CH_3$), 2.25 (m, 2H, Val β-CH), 2.28 (s, 6H, MeBzl-$CH_3$), 2.78 (m, 4H, Cys β- and β'-$CH_2$), 3.60 (s, 4H, MeBzl-$CH_2$), 4.23 (m, 2H, Ser β-$CH_2$), 4.27 (m, 2H, Ala α-CH), 4.44 (m, 2H, Ser α-CH), 4.56 (m, 2H, Ser β'-$CH_2$), 4.60 (m, 2H, Val α-CH), 4.63 (m, 2H, Cys α-CH), 5.12 (q, 4H, Bzl-$CH_2$), 6.28 (d, 2H, Ser NH), 6.30 (d, 2H, Cys NH), 6.96 (s, 2H, Ala NH), 7.11—7.33 (m, 18H, aromatic), and 7.87 (d, 2H, Val NH). Found: C, 59.80; H, 6.48; N, 9.14; S, 5.56%. Calcd for $C_{60}H_{76}N_8O_{14}S_2$: C, 60.18; H, 6.40; N, 9.36; S, 5.36%.

*{Qxc-D-Ser[Qxc-D-Ser-Ala-Cys(MeBzl)-Val]-Ala-Cys(MeBzl)-Val}Dilactone, S,S'-$MeBzl_2$-dihydronortriostin A (13).* Compound **12** (0.240 g, 0.2 mmol) and thioanisole (2.5 ml) were dissolved in TFA (25 ml) and the mixture was kept at 25 °C for 5 h, followed by evaporation *in vacuo*. The residue was triturated with ether to give amorphous precipitates, which were filtered off and dried *in vacuo* (0.28 g). These precipitates were dissolved in DMF (2 ml). To the stirred ice-cold solution were added 2-quinoxalinylcarbonyl chloride (Qxc-Cl, 0.077 g, 0.4 mmol) and TEA (0.14 ml, 1 mmol) and the reaction was allowed to proceed at 0 °C for 3 h and at 25 °C for 1 h. The solvent was evaporated *in vacuo*. The residue was dissolved in chloroform-methanol (98:2) and the solution was washed with M hydrochloric acid, dried over magnesium sulfate and evaporated *in vacuo*. The resulting material was chromatographed three times on silica-gel columns (Kieselgel H) with chloroform-methanol (98:2) to give **13** as amorphous solid; 102 mg (40%), mp 221—224 °C, $[\alpha]_D^{23.5}$ −113.8±3.8° (*c* 0.25, chloroform-methanol (98:2)). $\lambda_{max}^{CH_3CN}$ 242.5 nm (ε 77 300), 316 nm (ε 12 100), 325 nm (ε 11 900). NMR ($CDCl_3$, 360 MHz, 37 °C) δ=0.94 (m, 12H, Val γ- and γ'-$CH_3$), 1.41 (d, 6H, Ala β-$CH_3$), 2.26 (d, 6H, MeBzl $CH_3$), 2.37 (m, 2H, Val β-CH), 2.81 (m, 4H, Cys β- and β'-$CH_2$), 3.67 (m, 4H, MeBzl $CH_2$), 4.40 (m, 2H, Ala α-CH), 4.42 (m, 2H, Ser β'-$CH_2$), 4.62 (m, 2H, Ser β'-$CH_2$), 4.82 (m, 2H, Ser α-CH), 4.85 (m, 2H, Val α-CH), 5.20 (m, 2H, Cys α-CH), 6.56 (m, 2H, Cys NH), 7.01 (d, 2H, Ala NH), 7.12, 7.25 (m, 8H, aromatic), 7.87, 8.21 (m, 8H, Qxc), 8.12 (m, 2H, Val NH), 8.80 (d, 2H, Ser NH), and 9.63 (s, 2H, Qxc 3-CH), TLC: homogeneous in system B. Found: C, 58.04; H, 5.84; N, 12.99; S, 5.40%. Calcd for $C_{62}H_{72}N_{12}O_{12}S_2 \cdot 2H_2O$: C, 58.29; H, 5.68; N, 13.16; S, 5.02%.

*Nortriostin A.* Compound **13** (62 mg, 0.05 mmol) was treated with HF (10 ml) in the presence of anisole (0.5 ml) at 0 °C for 60 min. After removal of the HF by evaporation, the residue was dissolved in methanol (50 ml) and the solution was neutralized with TEA. To this was added a solution of iodine (66 mg, 0.25 mmol) in methanol and the mixture was stirred at 25 °C for 2 h. The excess iodine was discharged with 10% ascorbic acid and the solvent was removed by evaporation *in vacuo*. The residue was dissolved in chloroform-methanol (98:2) and the solution was washed with water, dried, and evaporated *in vacuo*. The resulting material was chromatographed two times on silica-gel columns (Kieselgel H) with chloroform-methanol (95:5). The fractions containing the desired product were collected and evaporated *in vacuo*. The residue was dissolved in chloroform-methanol (98:2, 1 ml) and to this was added ether (5 ml) to separate crystalline precipitates, which were filtered off, washed with ether, and dried *in vacuo* to give nortriostin A in pure state; 20 mg (39%), mp 213—215 °C, $[\alpha]_D^{20}$ −50.3±4.9° (*c* 0.1, chloroform). $\lambda_{max}^{CH_3CN}$ 242.5 nm (ε 89 600), 316 nm (ε 14 500), 326 nm (ε 14 200). NMR ($CDCl_3$ 360 MHz) δ=1.12 (d, 6H, Val γ- or γ'-$CH_3$), 1.16 (d, 6H, Val γ- or γ'-$CH_3$), 1.38 (d, 6H, Ala β-$CH_3$), 2.54 (m, 2H, Val β-CH), 2.93 (m, 4H, Cys β- and β'-$CH_2$), 4.45 (m, 2H, Ala α-CH), 4.65 (d, 2H, Ser β-$CH_2$), 4.84 (m, 2H, Val α-CH), 4.88 (m, 2H, Ser α-CH), 5.00 (d, 2H, Ser β'-$CH_2$), 5.68 (m, 2H, Cys α-CH), 6.30 (d, 2H, Ala NH), 6.54 (d, 2H, Cys NH), 7.92 (m, Qxc), 8.11 (d, Qxc), 8.25 (d, Qxc), 8.56 (d, 2H, Val NH), 8.79 (d, 2H, Ser NH), and 9.67 (s, 2H, Qxc 3-CH). Amino acid ratios in acid hydrolysate: Ser 0.92, Ala 1.06, Val 1.00, Cys not determined. Found: C, 50.40; H, 5.20; N, 15.15; S. 6.41%. Calcd for $C_{46}H_{54}N_{12}O_{12}S_2 \cdot 3.5H_2O$: C, 50.49; H, 5.62; N, 15.36; S, 5.86%.

## References

1) Part II: M. Shin, K. Inouye, and H. Otsuka, *Bull. Chem. Soc. Jpn.*, **57**, 2203 (1984); M. Shin, K. Inouye, H. Otsuka, N. Higuchi, and Y. Kyogoku, "Peptide Chemistry 1980," Proceedings of the 18th Symposium on Peptide Chemistry, Nishinomiya, 1980, ed by K. Okawa, Protein Research Foundation (1981), p. 207.

2) J. R. Kalman, T. J. Blake, D. H. Williams, J. Feeney, and G. G. K. Roberts, *J. Chem Soc. Perkin 1*, **1979**, 1313.

3) K. Kawano, N. Higuchi, and Y. Kyogoku, "Peptide Chemistry 1976," Proceedings of the 14th Symposium on Peptide Chemistry, Hiroshima, 1976, ed by T. Nakajima, Protein Research Foundation (1977), p. 93.

4) Y. Kyogoku, N. Higuchi, M. Watanabe, and K. Kawano, *Biopolymers*, **20**, 1959 (1981).

5) T. L. Ciardelli, P. K. Chakravarty, and R. K. Olsen, *J. Am. Chem. Soc.*, **100**, 7684 (1978).

6) B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3750 (1973).

7) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

8) Y. Kiso, K. Ukawa, and T. Akita, *J. Chem. Soc. Chem. Commun*, **1980**, 101.

9) B. Kamber, *Helv. Chim. Acta*, **54**, 927 (1971); U. Ludescher and R. Schwyzer, *ibid.*, **55**, 2052 (1972).

10) H. Otsuka, J. Shoji, K. Kawano, and Y. Kyogoku, *J.*

*Antibiot.*, **29**, 107 (1976).
11) M. K. Dhaon, J. H. Gardner, and R. K. Olsen, *Tetrahedron*, **38**, 57 (1982).
12) N. Higuchi, Y. Kyogoku, M. Shin, and K. Inouye, *Int. J. Peptide Protein Res.*, **21**, 541 (1983).