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SYNTHESES OF GRAMICIDIN S ANALOGS CONTAINING D-SERINE OR A DEHYDROALANINE RESIDUE, AND ASYMMETRIC HYDROGENATION OF THE DEHYDROALANINE

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Abstract: Gramicidin S (GS) analogs, [D-Ser(Bzl)$^{4,4'}$]-GS (17), [D-Ser$^{4,4'}$]-GS (18), and [L-Orn(Boc)$^{2,2'}$,D-Ser$^{4,4'}$]-GS (19) were synthesized and 17 showed high antibacterial activity. One residue of D-Ser in 19 was converted asymmetrically to D-Ala via α,β-dehydroalanine.

Gramicidin S (GS, Fig. 1) is a cyclic decapeptide antibiotic produced by a strain of *Bacillus brevis*.[1] In order to study structure-activity relationships of GS, we synthesized various analogs in which constituent amino acids in GS were replaced by different residues.[2] Here we report the syntheses of GS analogs containing D-Ser and related residues in place of D-Phe at 4,4' positions. The purpose of this study is to evaluate the role of the hydroxymethyl side chain in D-Ser on the biological activity, and to ascertain whether or not a protected precursor [D-Ser(Bzl)$^{4,4'}$]-GS containing a bulky benzyl group has high activity. An additional purpose is to prepare an analog containing an α,β-dehydroalanine (ΔAla) residue and successive hydrogenation of the residue. Izumiya *et al.* reported highly asymmetric hydrogenation of cyclic dipeptides containing several Δamino acids.[3] Meyer *et al.* reported that the *N*-Me-ΔPhe residue in natural tentoxin was hydrogenated to *N*-Me-D-Phe asymmetrically.[4] In case of AM-toxin I, however, hydrogenation of the constituent ΔAla residue yielded nearly racemic product.[5] Therefore, we were interested in whether asymmetric hydrogenation of the ΔAla residue in the GS analog occurs or not.

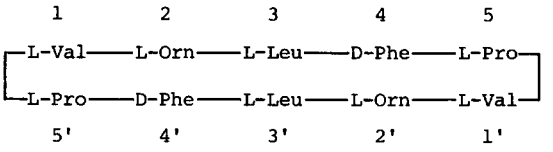
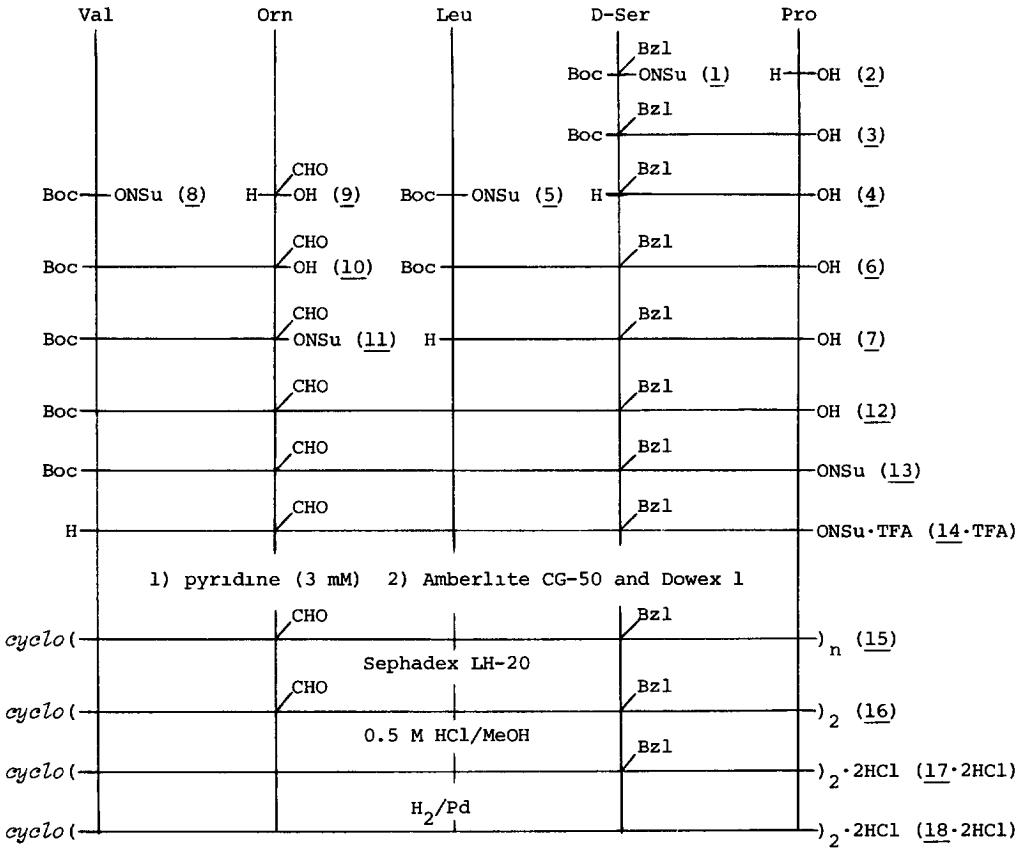


Fig. 1. Structure and numbering of gramicidin S.
(Hereafter a symbol of L will be omitted.)



Scheme 1. Syntheses of [D-Ser(Bzl)$^{4,4'}$]-GS (17) and [D-Ser$^{4,4'}$]-GS (18).

Two analogs, [D-Ser(Bzl)$^{4,4'}$]-GS and [D-Ser$^{4,4'}$]-GS, were synthesized as shown in Scheme 1.[6] Boc-D-Ser(Bzl)-Pro-OH (3) (83%, mp 132-134°C, $[\alpha]_D^{19}$ -31 1 (*c* 1, MeOH)), prepared from Boc-D-Ser=(Bzl)-ONSu (1) and L-proline (2), was treated with TFA to yield H-D-Ser(Bzl)-Pro-OH (4) (94%, mp 97-98°C). Compound 4 was coupled with Boc-Leu-ONSu (5) to give Boc-tripeptide (6) (86%, mp 111-112°C, $[\alpha]_D^{21}$ -39.6 (*c* 1, MeOH)), which was treated with TFA to give 7 (94%, mp 156-157°C, $[\alpha]_D^{20}$ -4.0 (*c* 0 5, H_2O)) Compound 10 (10·DCHA, 73%, mp 143-144°C, $[\alpha]_D^{20}$ -4.9 (*c* 1, EtOH)) was synthesized in a similar manner from Boc-Val-ONSu (8) and δ-formyl-L-ornithine (9). Compound 7 was coupled with Boc-Val-Orn(CHO)-ONSu (11), which was prepared from 10, and yielded Boc-pentapeptide (12) (55%, mp 172-173°C, $[\alpha]_D^{21}$ -58.6 (*c* 0 5, MeOH)). This was converted to ONSu ester (13) (94%) by use of HONSu and EDC, and the ester 13 was treated with TFA to give 14·TFA (98%).

Cyclization of the pentapeptide active ester 14 yielded a mixture (15, 78%) of cyclic dimer and monomer in a weight ratio of 85.15, which was determined by carboxymethylcellulose column chromatography of the deformylated product obtained from 15. The mixture 15 was subjected to Sephadex LH-20 column chromatography to give pure dimer (16) (52% from 13, mp 177-180°C, $[\alpha]_D^{25}$ -211 (*c* 0.1, MeOH), MW 1269 by Hitachi Osmometer Type 115 with MeOH, calcd 1257 5). This was

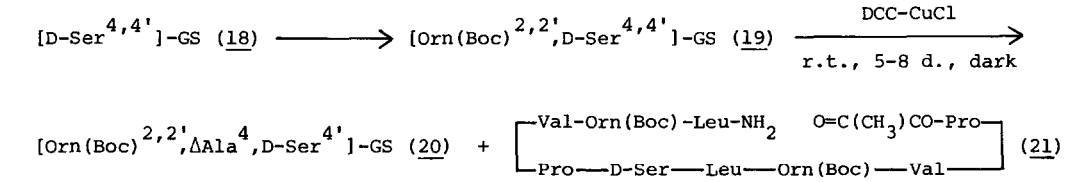
treated with 0.5 M HCl/MeOH for 3 days to give 17·2HCl (82%, mp 250-251°C (d), $[\alpha]_D^{30}$ -154 (*c* 0.1, MeOH)). Successive hydrogenation of 17·2HCl yielded 18·2HCl (86%, mp 251-253°C (d), $[\alpha]_D^{27}$ -209 (*c* 0.1, MeOH)). Each of the ORD spectra of 17 and 18 in EtOH and in 6 M urea showed an intense negative trough ([α] ca -12000) at 234 nm suggesting these analogs possess a rigid conformation similar to that of GS. Table 1 shows the antimicrobial activities of 17, 18 and related

Table 1. Minimum inhibitory concentration (μg/ml) of GS and analogs

	GS	[D-Ser(Bzl)$^{4,4'}$]-GS (17)	[D-Ser$^{4,4'}$]-GS (18)	[D-Ala$^{4,4'}$]-GS[7]
Staphylococcus aureus	6.25	3.13	50	50
Bacillus subtilis	3.13	3.13	12.5	25
Escherichia coli	100	100	>100	>100
Shigella flexneri	6.25	6.25	12.5	-

compounds. Activity of 17 is the same as that of GS. The benzyloxymethyl side chain in D-Ser=(Bzl) is big enough to exhibit full activity. On the other hand, low activity of 18 seems to be due to the small size of the side chain. A similar analog [D-Ala$^{4,4'}$]-GS showed like activity.[7] Thus, the hydrophylic hydroxymethyl group in 18 does not play any particular role in providing the activity.

In order to dehydrate the D-Ser residue in the analog, [Orn(Boc)$^{2,2'}$,D-Ser$^{4,4'}$]-GS (19) (85%, mp 276-278°C (d), $[\alpha]_D^{20}$ -252 (*c* 0.1, MeOH)) was prepared from 18 and it was subjected to (1) tosylation followed by β-elimination using a base,[8] (2) mesylation and similar β-elimination,[9] and (3) direct dehydration with carbodiimide-CuCl.[10] In the last procedure, diisopropylcarbodiimide, DCC or EDC was used as dehydrating reagent. Only by use of DCC-CuCl as shown in Scheme 2, we could dehydrate one D-Ser residue in 19 to give [Orn(Boc)$^{2,2'}$,ΔAla^{4},D-Ser$^{4'}$]-GS (20) (5 8%,



Scheme 2. Dehydration of GS analog.

mp 165-167°C, $[\alpha]_{250\ nm}^{20}$ -4800 (*c* 0.07, EtOH), UV_{max} (MeOH) 205 nm (ε 18600) and 221 nm (shoulder, ε 12400)). After catalytic hydrogenation of 20 and successive acid hydrolysis, amino acid ratio of the hydrolyzate was determined to be Ser 0.44, Pro 1.12, Ala 0.42, Val 1.00, Leu 1.09, Orn 0.87. The ORD spectra of 20 showed a weak and slightly red-shifted trough at 237 nm. This suggests a change in molecular conformation by the introduction of ΔAla. A by-product (21) (21%, mp 188-191°C, $[\alpha]_{250\ nm}^{20}$ -2300 (*c* 0.07, EtOH)) was obtained from the reaction mixture, amino acid

ratio determined upon hydrogenation and acid hydrolysis was as follows, Ser 0.51, Pro 1.14, Val 1 00, Leu 1.08, Orn 1.11. Pyruvic acid was detected from 21 (IR, 1745, 1355, and 1149 cm^{-1}) and from acid hydrolyzate of 21 by dinitrophenylhydrazine test. Some 25% of starting material 19 was recovered unchanged from the reaction mixture. Prolonged reaction caused decomposition of 20 and increasing yield of 21.

We tried catalytic hydrogenation of 20 in MeOH in the presence of Pd black at 25°C. The ΔAla residue in 20 was hydrogenated, and the content of D-Ala was determined by the modified Manning procedure.[11] We found an interesting result chiral induction to produce D-Ala was 96.2%

The mechanism of asymmetric hydrogenation of diketopiperazine derivatives was already reported [3] The side chain of a L (or D)-amino acid restricts the side of the molecule approaching to the catalyst. A possible conformation of 20 favoring asymmetric hydrogenation to produce D-Ala from ΔAla could be constructed using CPK models. This fact suggests that the mode of the reaction is similar to that proposed for diketopiperazine derivatives. Further investigation is in progress to clarify the mechanism of asymmetric hydrogenation

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6) Abbreviations according to IUPAC-IUB Commission, *J. Biol. Chem.*, 247, 977 (1972), are used throughout. Other abbreviation DCC, *N,N*-dicyclohexylcarbodiimide, EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, TFA, trifluoroacetic acid. Satisfactory results of elemental analyses were obtained for all crystalline products.

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