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FACILE SYNTHESIS OF (2*R*,3*R*)-PHENYLALANINE-2,3-d_2 AND ITS APPLICATION TO CONFORMATIONAL ANALYSIS OF GRAMICIDIN S

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Abstract: (2*R*,3*R*)-Phenylalanine-2,3-d_2 was synthesized in a good yield through catalytic reduction of *cyclo*(-2,3-dehydrophenylalanyl-D-alanyl-) under an atmosphere of $^2H_2$ and successive acid hydrolysis, and the amino acid was used to conformational analyses of gramicidin S and its derivatives.

Selectively deuterated amino acid is a useful tool for studying conformation and structure-activity relationships of biologically active peptides and proteins. Kirby and Michael[2] synthesized 3-deuterated aromatic amino acids by catalytic hydrogenation of *trans*-2-acylaminocinnamic acid-3-*d* derivatives in the presence of Pd carbon and subsequent deacylation with acylase. They described that the hydrogenation proceeded *cis* insensitive to variation of substituents and catalysts.[2] Kainosho and Ajisaka[3] synthesized selectively deuterated L-amino acids by use of enzymes and studied conformations of the amino acids in aqueous solutions. Nagai and Kobayashi[4] used α-chymotrypsin instead of acylase for resolution of synthesized amino acid derivatives and obtained both diastereomers, (2*S*,3*R*)-*N*-acetyl-phenylalanine-3-*d* and (2*R*,3*R*)-*N*-acetyl-phenylalanine-3-*d* ethyl ester, in the yields of 6% each.

Izumiya *et al.*[5,6] developed a new method for synthesis of optically active amino acids, that is, catalytic hydrogenation of dipeptide anhydride containing a dehydroamino acid residue and an L (or D) amino acid residue. They observed that the condensation of *cyclo*(-*N*-acetylglycyl-*N*-acetyl-L-alanyl-) with benzaldehyde exclusively yielded *cyclo*(-2,3-dehydrophenylalanyl-*N*-acetyl-L-alanyl-) of *Z* configuration. Deacetylation with hydrazine, catalytic hydrogenation in the presence of Pd black, and successive acid hydrolysis yielded L-phenylalanine; chiral induction, 97.0%.[7]

Here we report the synthesis of (2*R*,3*R*)-phenylalanine-2,3-d_2 as an example of facile synthesis of the selectively deuterated and optically active aromatic amino acid. Synthesis of gramicidin S containing two residues of the deuterated D-phenylalanine and reversal of chemical shifts of β methylene protons in the phenylalanine residues in gramicidin S and in its precursor bis(benzyloxycarbonyl)gramicidin S are also described.

cyclo(-Gly-D-Ala-)[8] (1) was obtained from Z-Gly-D-Ala-OMe[9] through hydrogenolysis in a mixture of MeOH and AcOH, followed by evaporation and subsequent refluxing in 0.1 M AcOH in *s*-BuOH for 1.5 h; yield, 96%; mp 241-242°C (decomp); $[\alpha]_D^{21}$ +21.4° (*c* 1, DMF). Five mmol (641 mg) of 1 was converted to *cyclo*(-2,3-dehydrophenylalanyl-D-alanyl-) (2); 655 mg (61%); mp 241-245°C

(decomp); $[\alpha]_D^{21}$ +18.0° (*c* 1, DMF); according to the procedure described for an L-isomer.[7] Catalytic reduction of 2 (864 mg, 4 mmol) in DMF (144 ml) in the presence of Pd black under an atmosphere of $^2H_2$ at 0°C for 3 d yielded *cyclo*(-(2*R*,3*R*)-phenylalanyl-2,3-d_2-D-alanyl-) (3), 845 mg (97%); chiral induction, 98.9%.[10] Stereochemistry of position 3 was assigned as *R* according to the literature.[2] A portion (437 mg, 2 mmol) of 3 was hydrolyzed in 6 M HCl (7 ml) at 110°C for 4.5 h, evaporated, the residue was diluted with water (100 ml), and the solution was treated with charcoal (8.6 g) for 90 min at room temperature. The charcoal filtered was extracted with 5% phenol and 20% AcOH (100 ml each), and the extraction procedure was repeated twice more. Combined extract was washed with ether and the aqueous solution was evaporated. The residue was diluted with water and the solution was applied to a column of Dowex 50 (H^+ form) (0.9 × 8 cm). The column was washed with water, eluted with 1 M NH_4OH, and the eluate was evaporated. The residue, (2*R*,3*R*)-phenylalanine-2,3-d_2, was crystallized from hot water-acetone; yield, 264 mg (80%). Chiral induction was determined as 96.2% by the modified Manning-Moore procedure.[5,6] Content of deuterium was estimated to be 90%±10% based on ^1H NMR.

Figure 1 shows the route for synthesis of gramicidin S containing the deuterated D-phenylalanine obtained. The reaction sequence was chosen to afford desired cyclic decapeptide in high yield and to economize the deuterated D-phenylalanine. Boc-L-Orn(Z)-L-Leu-OMe, derived from Boc-L-Orn(Z)-OH and H-L-Leu-OMe by the DCC-HOBt method in 72% yield; mp 84-87°C; $[\alpha]_D^{21}$ -12.4° (*c* 1, DMF), was treated with TFA to give trifluoroacetate (83%), which was coupled with Boc-L-Val-ONSu to yield Boc-tripeptide-OMe (4), 85%; mp 147-149°C; $[\alpha]_D^{20}$ -16.0° (*c* 1, DMF). Compound 4 was converted to Boc-tripeptide-hydrazide (5); 79%; mp 199-200°C; $[\alpha]_D^{20}$ -15.2° (*c* 1, DMF). (2*R*,3*R*)-Phenylalanine-2,3-d_2 (D-Phe*) (150 mg, 0.9 mmol) was treated with $(Boc)_2O$[11] and then DCHA to give Boc-D-Phe*-OH·DCHA (97%); mp 210-215°C (decomp); $[\alpha]_D^{26}$ -22.5° (*c* 1, MeOH), which was coupled with HONSu by use of DCC to give Boc-D-Phe*-ONSu (69%); mp 138-141°C. This was coupled with L-proline to afford Boc-D-Phe*-L-Pro-OH; 177 mg (74%); mp 167-170°C, which was treated with 3.6 M HCl in dioxane to give H-D-Phe*-L-Pro-OH·HCl (6·HCl) (146 mg). Azide coupling[12] of compounds 5 and 6 afforded a crude mixture, which was subjected to silica gel column chromatography to give

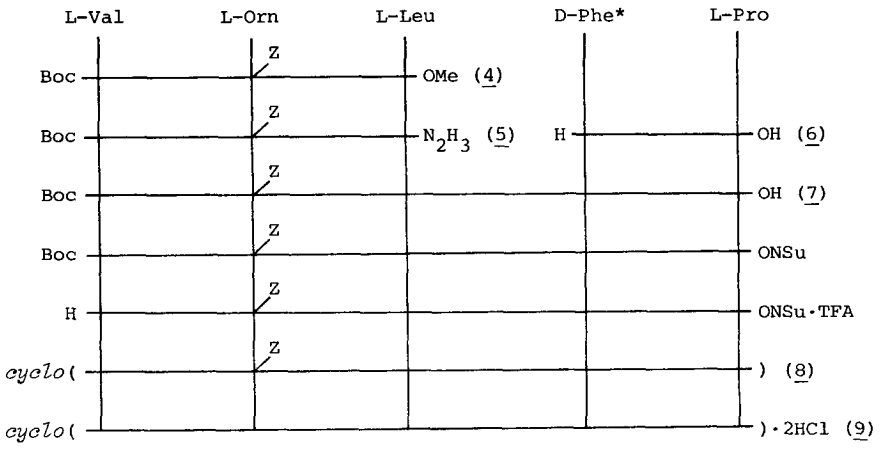

Fig. 1. Synthesis of gramicidin S.

protected pentapeptide 7 (20% from 5); R_f[13] 0.82, lit.[14] 0.82. Formation of a by-product supposedly a tripeptide amide derivative in this step resulted in unexpected lowering of the yield. Compound 7 (105 mg, 0.13 mmol) was coupled with HONSu by use of EDC·HCl, resultant active ester was treated with TFA, and the product was subjected to cyclization in pyridine (40 ml) for 3 d as described before.[15] After evaporation, the residue was put on Amberlyst 15 and A-27 columns (1.8 × 3.5 cm each) and eluted with MeOH. Crude cyclic peptide (56 mg) was purified by Sephadex LH-20 column chromatography (2 × 84 cm) to give [L-Orn(Z)$^{2,2'}$,(2*R*,3*R*)-Phe-2,3-$d_2$$^{4,4'}$]= gramicidin S ($Z_2$GS*) (8), 25.5 mg (28%); R_f 0.96, lit.[16] 0.96. Two mg of 8 was hydrogenolized in MeOH containing 10 µl of 0.3 M HCl in dioxane to give [(2*R*,3*R*)-Phe-2,3-$d_2$$^{4,4'}$]gramicidin S·2HCl (GS*·2HCl) (9); R_f 0.96, lit.[16] 0.96.

^{1}H NMR Spectra were measured on a Jeol FX-90Q spectrometer at 29°C in DMSO-d_6 solutions; tetramethylsilane being used as the internal standard. Sample concentration was 5 mg/0.4 ml except for 9. In this case, 3 mg/0.4 ml. Figure 2 shows ^{1}H NMR spectra of bis(benzyloxycarbonyl)gramicidin S (Z_2GS),[16] 8, gramicidin S·2HCl (GS·2HCl), and 9. In Fig. 2, (a) the signals of L-Orn(Z) δCH_2 and D-Phe βCH_2 are overlapped and appear as broad multiplets around 2.9-3.0 ppm. (b) A sharp singlet of 3*S*-proton of D-Phe* appears at 2.90 ppm superposed on the upfield side of L-Orn(Z) δCH_2 signals. (c) The L-Orn δCH_2 and D-Phe βCH_2 signals resonate around 2.9-3.0 ppm as broad multiplets. The shape of the multiplets differs from that of (a), suggesting some conformation change between (a) and (c). (d) A sharp singlet of 3*S*-proton of D-Phe* appears at 2.98 ppm superposed on the downfield peak of L-Orn δCH_2 signals. Although clear assignments of βCH_2 and δCH_2 protons in D-Phe and L-Orn are difficult because of signal overlaps, comparison of the four spectra shown in Fig. 2 allows us to assess that the signals of *pro-R* proton in Z_2GS

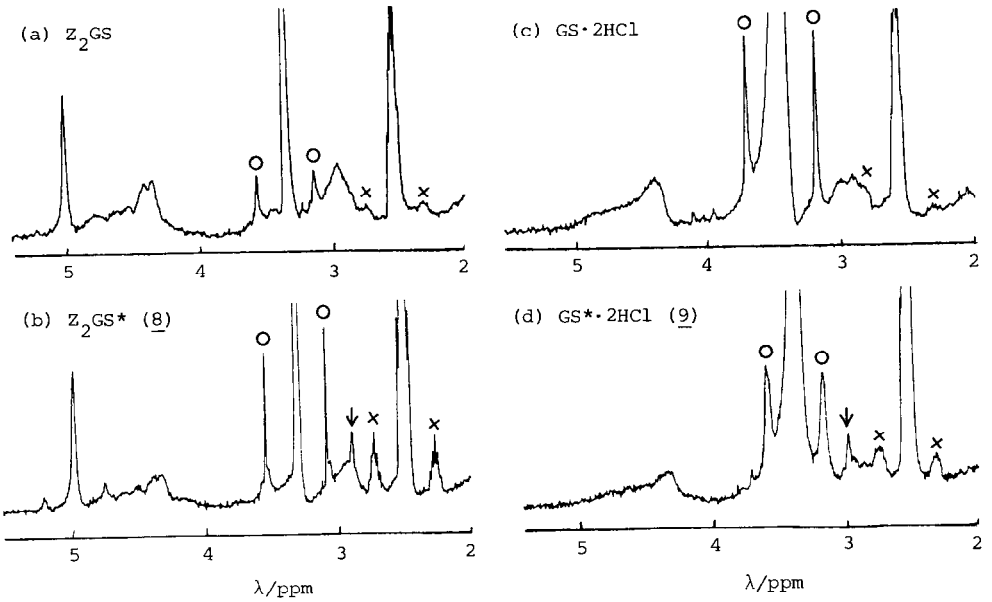

Fig. 2. ^{1}H NMR Spectra of gramicidin S and derivatives. Satelites of HDO (O); satelites of DMSO (×). Arrows in (b) and (d) indicate 3*S*-proton signals.

resonate at about 3.0 ppm and *pro-S* proton at 2.9 ppm; in contrast, *pro-S* proton signals in GS resonate at 3.0 ppm, and *pro-R* about 2.9 ppm.

This chemical shift reversal can be explained in terms of the change in rotamer populations.[17] In regard to the conformation of gramicidin S, the presence of intramolecular hydrogen bonds between L-Orn NH_3^+ and D-Phe CO was proposed.[18] Proximity of aromatic side chains in D-Phe to the pyrrolidine rings in L-Pro was also shown.[19] In case of Z_2GS, however, this conformation should cause steric crowding around D-Phe-L-Pro peptide bonds because bulky benzyloxycarbonyl groups occupy the same loci. The aromatic side chains in D-Phe, therefore, rotate to avoid this steric effect, resulting in the reversal of chemical shifts of βCH_2 signals.

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8) Abbreviations used are according to IUPAC-IUB Commission, *Pure Appl. Chem.*, 40, 317 (1974). Other abbreviations: DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDC·HCl, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; HONSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid.

9) mp 91-93°C; $[\alpha]_D^{23}$ +20.6° (*c* 1, DMF). All crystalline compounds gave satisfactory elemental analyses.

10) Determined with high performance liquid chromatography; column, LiChrosorb RP-18 (4 × 150 cm); solvent, water-CH_3CN (8:1); flow rate, 1.2 ml/min; pressure, 180 kg/cm^2; elution time, 7.5 min for *cyclo*(-L-Phe-D-Ala-) and 10.2 min for *cyclo*(-D-Phe-D-Ala-).

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13) Solvent system used for thin-layer chromatography; R_f, *n*-BuOH-AcOH-pyridine-water (4:1:1:2, v/v).

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