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AN EFFICIENT METHOD FOR SELECTIVE AMINO ACID PROTECTION OF *MESO*-2,2'-DIAMINODICARBOXYLIC ACID: AN IMPROVED SYNTHESIS OF FK-156

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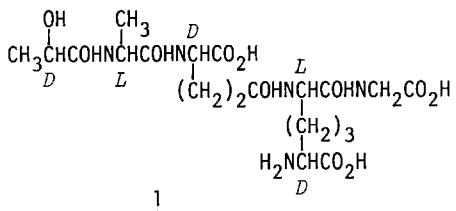
Summary: An efficient method for selective amino acid protection with proper differentiation between the two amino acid groups in *meso*-2,2'-diaminopimelic acid has been achieved [7 → 8 → 9 → 10 → 11] and applied to the synthesis of FK-156 [10 → 12 → 14 → 1].

Considerable attention is currently focused on the peptidoglycan derivatives isolated from bacterial cell-walls owing, particularly, to the interesting biological activities displayed by this group of natural products[1]. FK-156 (1) is a new, immunologically active microbial metabolite[2] with a close resemblance in structure to the cell-wall peptidoglycan peptides. In the connection with a synthetic program on this natural product and the related peptidoglycan peptides, it became necessary to contrive an efficient and convenient method for selective amino acid protection with proper differentiation between the two amino acid moieties in *meso*-2,2'-diaminopimelic acid which constitutes the central part of this class of compounds. In the first total synthesis of 1 which we have reported in the preceding paper[3], this selective protection was performed via an enzymatic



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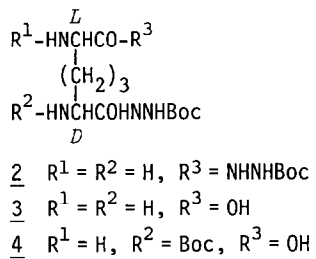


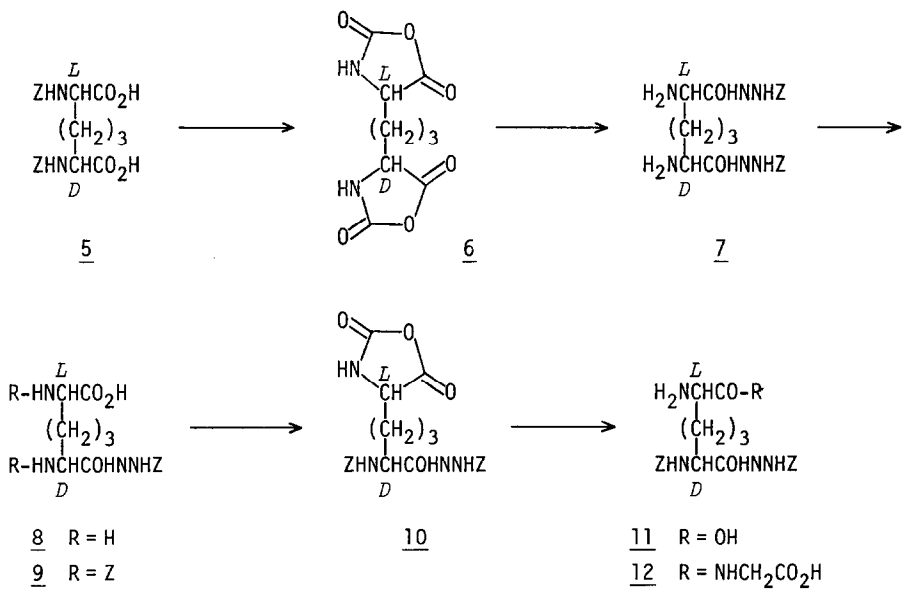
2 $R^1 = R^2 = H$, $R^3 = NHNHBoc$
3 $R^1 = R^2 = H$, $R^3 = OH$
4 $R^1 = H$, $R^2 = Boc$, $R^3 = OH$

hydrolysis [2 → 3] and a subsequent conversion [3 → 4] by a sequence of reactions involving a copper chelate amino protection. We now describe a significantly im-

proved procedure and its application to the synthesis of 1. The key in our new approach is to prepare the *N*-carboxyanhydride 10 which can be readily converted by hydrolysis into the selectively protected amino acid 11 and is also expected to serve well by itself as an active intermediate for condensation with glycine leading to the fragment 12, a key compound for the synthesis of 1. This synthesis of 10 has been accomplished by a two-step reaction sequence [8 → 9 → 10] following an enzymatic hydrolysis [7 → 8] (see Scheme 1).

The starting material was the bis-*N*-carboxyanhydride 6[4], which was allowed to react with benzyl carbazate (2.0 equiv) in the presence of an excess of AcOH in MeCN (10°C, 30 min) to provide the symmetrically substituted bis(Z-hydrazide) 7 [mp 93 - 5°C, R_f 0.40 (A)[5]] in 86 % yield. For differentiation of the two chiral centers in 7, 7 was subjected to the asymmetric hydrolysis using the aminopeptidase readily available from *Streptomyces sapporonensis*[6,7] under somewhat different conditions from those in the case of 2[3] (because of a sparing solubility of 7 in H_2O). Thus, 7 was incubated in 20 % aqueous MeCN in the presence [100 unit/g

Scheme 1



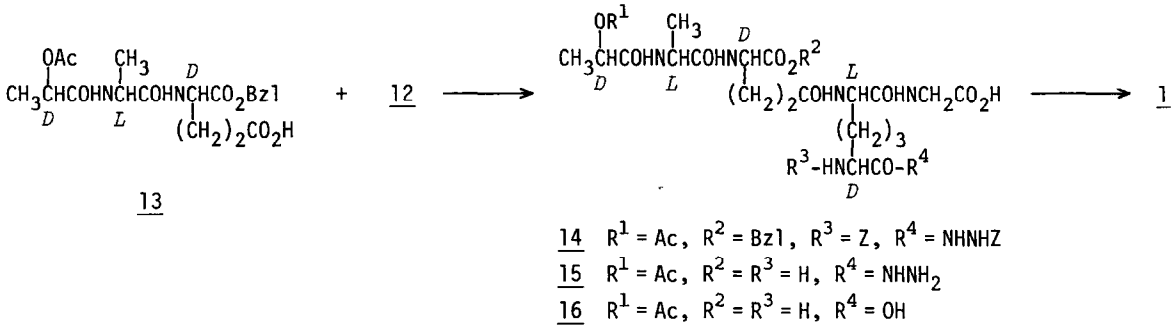
(substrate)] of a crude powder of the enzyme [100 unit/mg (protein)] at 37°C at pH 7.0 - 7.5 and, after 1.5 h, the mixture was purified by column chromatography of Dia-ion HP-20 to afford the product 8 [mp 213 - 7°C(dec), $[\alpha]_D$ -21.3°(c 1.0, AcOH), p*Ka* 2.1, 7.1, 9.5 (H_2O), R_f 0.22 (A)] in 80 % yield. The stereochemistry and the optical purity (~ 100 %) of 8 was confirmed by comparison with the sample

derived by the hydrolysis using the animal-originated leucine aminopeptidase (hog kidney)[8].

The selective protection of the amino group at the D asymmetric center in 8 was achieved *via* 10 as follows. Carbobenzyloxylation of 8 according to the standard manner gave the di-Z derivative 9 [mp 139 - 141°C, $[\alpha]_D$ +17.3°(c 1.0, MeOH), 90 %], which in turn was treated with $SOCl_2$ (2.5 equiv) in CH_2Cl_2 (0°C → room temperature, 1 h) to produce the *N*-carboxyanhydride 10 [viscous oil, ν_{max} ($CHCl_3$) 1850, 1780 cm^{-1}] in quantitative yield. Hydrolysis of 10 with dilute HCl gave the α-amino acid 11 [mp 232 - 3°C(dec), $[\alpha]_D$ +19.2°(c 0.5, MeOH), p*K*a 3.6, 10.1 (50 % Me_2SO)[10], R_f 0.56 (A)], thus providing an extremely simple procedure for the selective amino acid protection with differentiating the chiral centers in *meso*-2,2'-diaminopimelic acid.

We proceeded then to the synthesis of 1. For the preparation of the fragment 12, the use of 10 which is, as well as the precursor of, the active derivative of 11 was examined instead of the use of 11 itself (see Scheme 1). A solution of 10 in MeCN was combined at 0°C with a solution of glycine (3.0 equiv) in H_2O containing Na_2CO_3 (4.0 equiv) and the mixture was stirred at the same temperature for 2 h. Under these conditions, 12 [mp 204 - 7°C(dec), $[\alpha]_D$ +28.2°(c 0.5,

Scheme 2



14 R^1 = Ac, R^2 = Bzl, R^3 = Z, R^4 = NHNHZ
15 R^1 = Ac, R^2 = R^3 = H, R^4 = $NHNH_2$
16 R^1 = Ac, R^2 = R^3 = H, R^4 = OH

AcOH), R_f 0.58 (A)] was obtained in 74 % yield. The terminating steps of the synthesis required the condensation of 12 with the appropriately protected lactoyl dipeptide 13[11] (see Scheme 2). The latter compound 13 was preactivated with isobutyl chloroformate in the same way as described previously[3] and coupled, at -10 to 0°C (2 h), to the silyl ester of 12, prepared *in situ* by treatment with bis(trimethylsilyl)acetamide in CH_2Cl_2-DMF (room temperature, 30 min), affording the condensation product 14 [mp 172 - 5°C, $[\alpha]_D$ -2.5°(c 0.5, MeOH), R_f 0.23 (B)] in 86 % yield. Finally, the protecting groups in 14 were removed by hydrogenolysis [14 → 15] over 10 % Pd-C (AcOH-H_2O), oxidation with Br_2 (2.5 equiv, H_2O) followed

by a spontaneously occurring hydrolysis [15 → 16] and alkaline hydrolysis with aqueous Na_2CO_3 (pH 10) [16 → 1]. Purification by column chromatography using Dia-ion HP-20 provided, in 68 % yield from 14, pure 1 which was identified with the natural product[2] and the sample synthesized previously[3].

This synthesis of FK-156 using the above method for the selective amino acid protection of *meso*-2,2'-diaminopimelic acid is highly efficient and sufficiently adaptable to the preparative purpose for acquisition of the sample necessary for biological testing and may also be applied to the synthesis of analogous compounds related to the peptidoglycan peptides.

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REFERENCES AND NOTES

1 For reviews, see e.g. (a) L. Chedid and E. Lederer, *Biochem. Pharmacol.*, 1978, 27, 2183; (b) P. Duker, L. Tarcsay, and G. Baschang, *Annu. Rep. Med. Chem.*, 1979, 14, 146; (c) E. Lederer, *J. Med. Chem.*, 1980, 23, 819.

2 T. Gotoh, Y. Kuroda, M. Okuhara, T. Tanaka, T. Nishiura, M. Kohsaka, H. Aoki, and H. Imanaka, Abstract paper of *21st Intersci. Conf. Antimicr. Agents Chemother.* Chicago, Ill., 1981, 414.

3 K. Hemmi, H. Takeno, S. Okada, O. Nakaguchi, Y. Kitaura, and M. Hashimoto, *J. Am. Chem. Soc.*, in press.

4 Preparation of 6 from di-Z-meso-2,2'-diaminopimelic acid (5) has been described in our preceding communication[3].

5 Analytical thin-layer chromatography was performed with silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: A, *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper phase); B, AcOEt–AcOH (5 : 1).

6 This microorganism-originated enzyme is inexhaustible and sufficiently adaptable to a large-scale preparation of 8 (see also ref. 3).

7 Isolation and characterization of the enzyme was performed by Imanaka *et al.*, to be published. Regarding the activity of the enzyme, one unit was defined as the quantity capable of hydrolyzing 1.0 μmol of L-leucine *p*-nitroanilide per min at pH 7.0 and 37°C.

8 This hydrolysis was carried out as shown in the case of 2 by Bricas et al.[9], except 30 % aqueous MeCN was used as solvent instead of Tris buffer (pH 8.5) and the pH was controlled by addition of dilute NaOH.

9 P. Dezélée and E. Bricas, *Bull. Soc. Chim. Biol.*, 1967, 44, 1579.

10 Under the same condition, alanine showed p*K*a 3.6 and 10.4.

11 Preparation of 13 has been described in our preceding communication[3].

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