A Total Synthesis of Actinomycin D (C_1) via Peptide Cyclization Between Proline and Sarcosine[1]

Syntheses of actinomycin D (C_1)[2] have been reported by BROCKMANN and LACKNER[3], during which the key step, ring closure of the cyclic peptide lactones, was carried out by lactonization with the use of acetylimidazole/acetylchloride.

This communication concerns a synthesis of actinomycin D (Figure 1) during which a *p*-nitrophenyl ester intermediate served to cyclize the peptide lactone rings between proline and sarcosine[4]. The synthetic approach is outlined in Figure 2.

Treatment of the mixed anhydride[5] from benzyloxycarbonyl-L-*N*-methylvaline[6] and isobutylchloroformate with *tert*-butyloxycarbonyl-L-threonine[7-9] and catalytic hydrogenation of the crude product in the presence of palladium black gave the crystalline *β*-di-depsipeptide derivative, *O*-(L-*N*-methylvalyl)-*N*-*tert*-butyloxycarbonyl-L-threonine (I), 49%, m.p. 207°, $[\alpha]_D^{23}$ +57.1° (*c* 1, methanol). Anal. calcd. for $C_{15}H_{28}N_2O_6$ (332.4): C, 54.2; H, 8.49; N, 8.43; O, 28.9. Found: C, 54.2; H, 8.60; N, 8.41; O, 28.9. Condensation of I with benzyloxycarbonylsarcosine[10] by the above mixed anhydride method afforded crystalline *O*-(benzyloxycarbonylsarcosyl-L-*N*-methylvalyl)-*N*-*tert*-butyloxycarbonyl-L-threonine (II), 52%, m.p. 163°, $[\alpha]_D^{25}$ −59.7° (*c* 1, methanol). Anal. calcd. for $C_{26}H_{39}N_3O_{11}$ (537.6): C, 58.1; H, 7.31; N, 7.82; O, 26.8. Found: C, 58.2; H, 7.46; N, 7.69; O, 27.0.

Benzyloxycarbonyl-D-valyl-L-proline-*tert*-butyl ester (III) was obtained as colorless prisms [90%, m.p. 92°, $[\alpha]_D^{19}$ −19.6° (*c* 1, methanol). Anal. calcd. for $C_{22}H_{32}N_2O_5$ (404.5): C, 65.4; H, 7.98; N, 6.93. Found: C, 65.4; H, 8.05; N, 7.06] from a reaction of benzyloxycarbonyl-D-valine-*p*-nitrophenyl ester[11] with *tert*-butyl-L-prolinate[12,13]. Catalytic hydrogenation of III in the presence of palladium black and subsequent reaction with the mixed anhydride from II and isobutylchloroformate afforded the *β*-pentadepsipeptide derivative, *O*-(benzyloxycarbonylsarcosyl-L-*N*-methylvalyl)-*N*-*tert*-butyloxycarbonyl-L-threonyl-D-valyl-L-proline-*tert*-butyl ester (IV), which was purified by counter current distribution in the system: toluene-chloroform-methanol-water (5:5:8:2)[14], and then precipitated from ether/hexane at low temperature to give a colorless amorphous powder, 85%, m.p. 75–80°, $[\alpha]_D^{28}$ −41° ± 1° (*c* 1, methanol). Anal. calcd. for $C_{40}H_{63}N_5O_{11}$ (789.9): C, 60.8; H, 8.04; N, 8.87. Found: C, 60.9; H, 8.08; N, 9.23. A sample for amino acid analysis[15] was hydrolysed with 6*N* HCl at 110° for 24 h. The following molar ratios

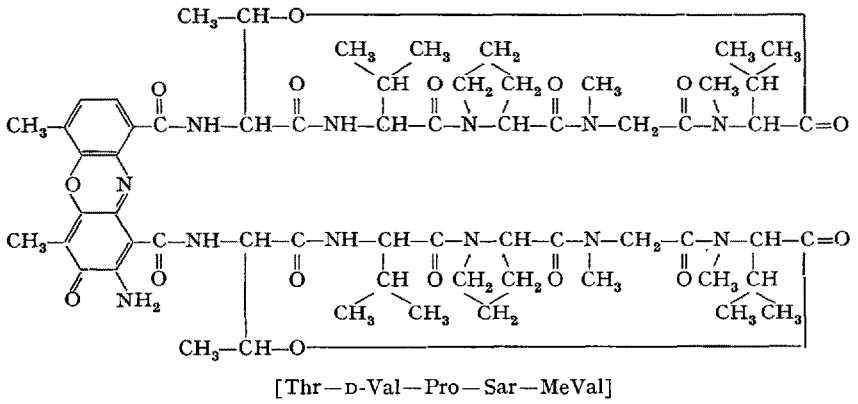

Fig. 1. Structure of actinomycin D (C_1)[2].

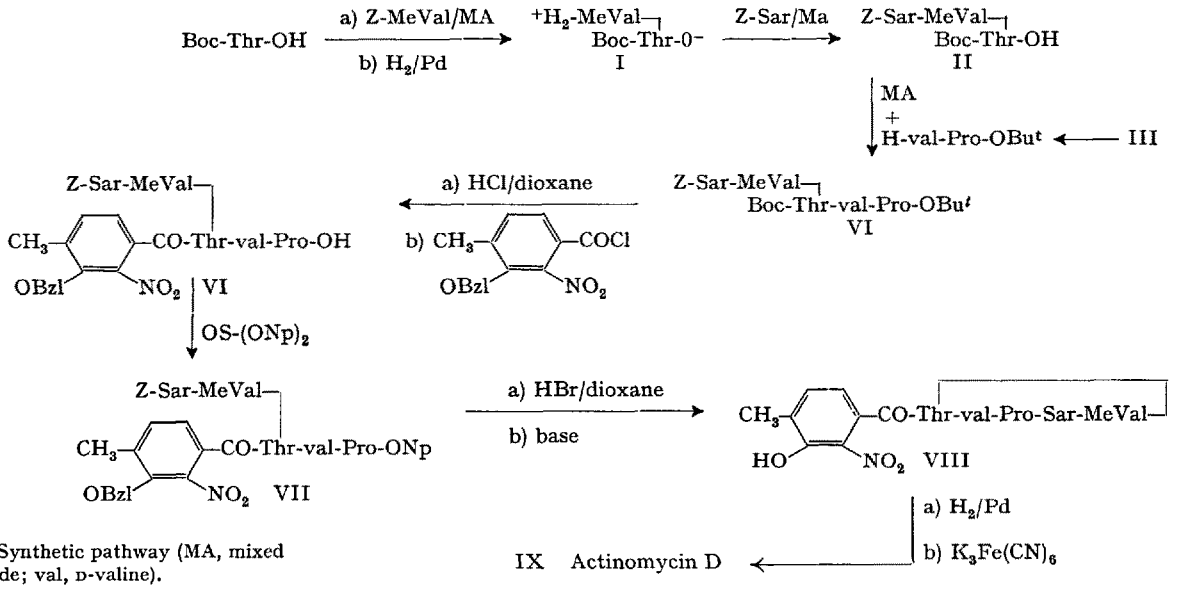

Fig. 2. Synthetic pathway (MA, mixed anhydride; val, D-valine).

were obtained, valine being taken as 1.0: sarcosine 1.0, proline 1.0, threonine 0.9, *N*-methylvaline[16] 1.0. Cleavage by 4*N* HCl in dioxane (30 min at room temperature) of the *tert*-butyloxycarbonyl and *tert*-butyl ester protecting groups gave *O*-(benzyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-threonyl-D-valyl-L-proline hydrochloride (V), 95%, m.p. 160–165°, $[\alpha]_D^{24}$ −45.3° (*c* 1, methanol). Anal. calcd. for $C_{31}H_{48}N_5O_9Cl$ (670.2): C, 55.6; H, 7.22; N, 10.4; Cl, 5.29. Found: C, 55.7; H, 7.25; N, 10.2; Cl, 5.17. V was reacted with 2-nitro-3-benzyloxy-4-methyl-benzoylchloride[17] in the presence of *N*-methylmorpholine. Purification of the crude product by column chromatography on Sephadex LH 20 in methanol afforded *O*-(benzyloxycarbonylsarcosyl-L-*N*-methylvalyl)-*N*-(2-nitro-3-benzyloxy-4-methyl-benzoyl)-L-threonyl-D-valyl-L-proline (VI), 89%, m.p. 115–120°, $[\alpha]_D^{20}$ −4.3° (*c* 0.5, methanol). Anal. calcd. for $C_{46}H_{58}N_6O_{13}$ (903.0): C, 61.2; H, 6.47; N, 9.31. Found: C, 61.2; H, 6.65; N, 9.40. VI was converted to its *p*-nitrophenyl ester derivative (VII) with the use of di-*p*-nitrophenyl sulfite in pyridine[18]. VII was purified by column chromatography on Sephadex LH 20 in ethyl acetate followed by precipitation from benzene into hexane, 88%, m.p. 110–115° (dec.), $[\alpha]_D^{20}$ −21.8° (*c* 0.5, dimethylformamide). Anal. calcd. for $C_{52}H_{61}N_7O_{15}$ (1024.1): C, 61.0; H, 6.00; N, 9.58. Found: C, 61.3; H, 6.59; N, 9.69. Treatment of VII with 4*N* hydrogen bromide in dioxane[19,20] removed the benzyloxycarbonyl group. The cyclization was carried out at high dilution (*c* about 0.05) in pyridine for 6 h at 60°[21]. A crude product was obtained by evaporating the solvent, dissolving the residue in ethyl acetate, successive washing with 1*N* HCl, and water, drying over $MgSO_4$, and evaporating the solvent. Fractionation was carried out by column chromatography on Sephadex LH 20 in methanol. Evaporation of the fractions comprising the first major peak afforded cyclo-(2-nitro-3-hydroxy-4-methyl-benzoyl)-L-threonyl-D-valyl-L-prolyl-sarcosyl-L-*N*-methylvalyl lactone (VIII), which was obtained as an amorphous powder by precipitation from ethyl acetate into hexane, 31%, $[\alpha]_D^{23}$ −14.5° (*c* 0.5, methanol). VIII was without further characterization converted into actinomycin D by catalytic hydrogenation in the presence of palladium black and subsequent oxidation using potassium ferricyanide[22] in a mixture (1:1) of methanol and M/15 phosphate buffer at pH 7.1. Removal of the methanol under reduced pressure, extraction with ethyl acetate, washing of the organic phase with 1*M* $NaHCO_3$, 1*N* HCl, and water, drying over Na_2SO_4, and evaporation gave crude actinomycin (IX), which was recrystallized from ethyl acetate by the addition of hexane. From 100 mg of VIII 76 mg (80%) of IX were obtained, orange red prisms, m.p. 240–242°, $[\alpha]_D^{20}$ −312° ±10° (*c* 0.26, methanol). Anal. calcd. for $C_{62}H_{86}N_{12}O_{16}$ (1255.4): C, 59.3; H, 6.91; N, 13.4. Found: C, 59.3; H, 6.93; N, 13.4. Authentic natural crystalline actinomycin D[23] possessed m.p. 241–243°, $[\alpha]_D^{20}$ −323 ±10° (*c* 0.26, methanol)[24]. Microbiological assays[25], using *Lactobacillus arabinosus* (ATCC 8014) and *L. fermenti* (ATCC 9388) in pantothenate- and thiamine-dependent systems respectively[26], showed that the synthetic material IX is indistinguishable from natural actinomycin D[27].

Zusammenfassung. Es wird eine Totalsynthese von Actinomycin D (C_1) beschrieben, in der die Schlüsselreaktion, nämlich die Zyklisierung der Pentapeptidlaktonringe, durch eine Nitrophenylester-Synthese zwischen Prolin und Sarkosin ausgeführt wurde. Die Estergruppe zwischen der Carboxylgruppe des *N*-Methylvalins und der β-Hydroxylgruppe des Threonins wurde durch Reaktion von Boc-Thr-OH mit dem gemischten Anhydrid aus Z-MeVal-OH und Chlorameisensäure-isobutylester hergestellt.

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[1] Abbreviations follow the rules of the IUPAC-IUB Commission on Biochemical Nomenclature, in Biochemistry *5*, 1445, 2485 (1966); *6*, 362 (1967); J. biol. Chem. *241*, 2491 (1966).

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