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AN ALTERNATE SYNTHETIC APPROACH TO GOUGEROTIN

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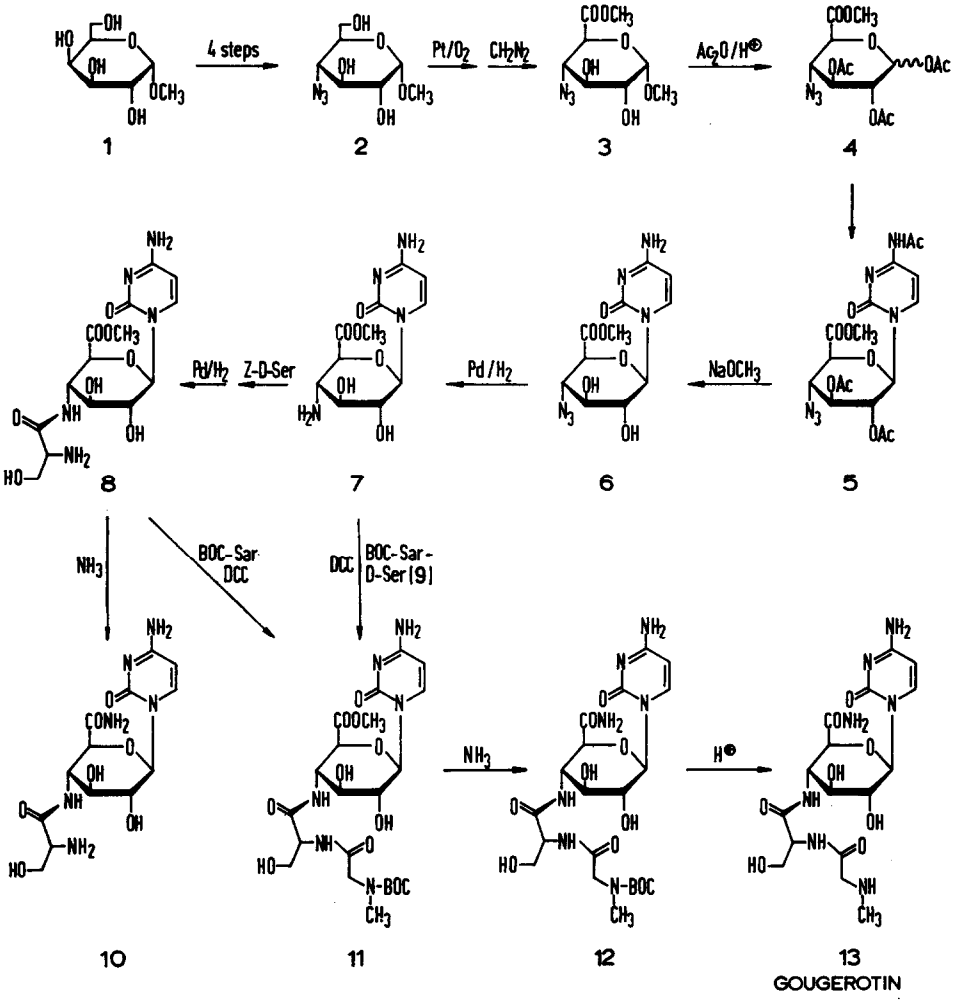
Gougerotin, an aminoacyl-aminohexosyl-cytosine antibiotic [2] elaborated by various streptomycetes [3], has been shown topossess versatile antibacterial [4], acaricidal [5], anti-mycoplasma [4b, 6] and antiviral [7] activities, of which at least the former are due to interference with protein biosynthesis at the peptide chain elongation stage [8]. Its correct structure (13) has only gradually evolved [9] requiring two revisions [9b, d] before being finalized by partial [9e-g] and a first, 17 step total synthesis from methyl α-D-galactoside (1) [9h]. Our approach to the synthesis of gougerotin comprises a total of 13 steps from 1, based on a rather different series of reactions, the salient features being the catalytic oxidation of methyl 4-azido-4-deoxy-α-D-glucoside (2) to the uronic acid, its subsequent acetolysis (3 → 4) [10], the stannic chloride catalyzed glycosidation with bis-trimethylsilyl-N-acetyl-cytosine (4 → 5) [11] and the direct introduction of the peptide portion (7 → 11) by coupling with BOC-blocked sarcosyl-D-serine.

Catalytic oxidation of the primary hydroxyl group in 2, accessible from 1 in four steps in high overall yield (43 % [10a, 12]), was readily accomplished by air over platinum black [13] to give on esterification with diazomethane the known [15] azidouronate 3 in 64 % yield. Treatment of 3 with acetic anhydride containing 2 % conc. sulfuric acid for 12 h at ambient temperature afforded, on elution from a silica gel column [16a] to remove slower moving impurities, a 59 % yield of 4 as a syrup [17], comprising an approximate 1 : 3 α/ß-mixture on the basis of pmr data ($CDCl_3$): δ 5.63 (7 Hz-d, 0.3 H, H-1e) and 6.34 (3 Hz-d, 0.7 H, H-1a). When reacted in dichloroethane with N^4-acetyl-bis(trimethylsilyl)-cytosine in the presence of stannic chloride (12 h, 60°), the highly crystalline azido-nucleoside 5 [17] was obtained in 67 % yield: needles, mp 225 - 227° effervesc., $[\alpha]_D^{22}$ + 38° (c 0.4, $CHCl_3$); relevant pmr peaks ($CDCl_3$) at δ 1.95 and 2.12 (two 3H-s, 2'- and 3'-OAc), 2.26 (3H-s, N-Ac), 3.85 (3H-s, OMe), 6.15 (1H-d, J=9 Hz, H-1'), 7.45 and 7.67 (two 1H-d, J=7 Hz, H-5 and H-6). De-O-acetylation with methanolic sodium methoxide to 6, isolated as the monohydrochloride [mp 189 - 190°, $[\alpha]_D^{22}$ + 39°

(c 0.5, MeOH), 85 % yield], and subsequent hydrogenation over 10 % Pd/C in aqueous methanol containing hydrochloric acid gave 7 in form of the crystalline dihydrochloride monohydrate (87 % yield), identical by mp (220 - 225° dec), uv and ir data with the product prepared [18] by acid hydrolysis of gougerotin and subsequent esterification.



For attachment of the dipeptide unit, N-t-butoxycarbonyl-sarcosyl-D-serine (9) [syrup, $[\alpha]_D^{25}$ - 4° (c 3, MeOH)] was used, readily accessible from its methyl ester [19] in 79 % yield by alkaline hydrolysis (N NaOH/methanol, 0.5 h, 25°) and purification via a cellulose column [16b] Coupling of 7 with 2 equiv. each of 9, triethylamine and dicyclohexylcarbodiimide (DCC) in methanol-acetonitrile followed by brief treatment with a strongly basic ion exchange resin (Merck III) to remove N, N'-diacylated product and subsequent column chromatography on silica gel [16c] afforded the desired dipeptidyl nucleoside 11 in 56 % yield: monohydrate of mp

175 - 178°; $[\alpha]_D^{26}$ + 39° (c 1, H_2O); uv (in 0.1 N H_2SO_4) λ_{max} 276 nm; (in water) λ_{max} 266; (in 0.1 N NaOH) λ_{max} 268; relevant pmr peaks (DMSO-d_6) δ 7.60 and 5.81 (two 7 Hz-d, H-6 and H-5), 5.57 (8 Hz-d, H-1'), 2.80 (3H-s, N-CH_3), 1.40 (9H-s, BOC). In an alternate procedure, 7 was converted into 11 in a 51 % overall yield by DCC-coupling with N-benzyloxycarbonyl-D-serine (Z-D-Ser) [9h], hydrogenolysis, and reaction of the resulting seryl-nucleoside 8 — readily isolated as the stable dihydrochloride, mp 225 - 230° (dec), $[\alpha]_D^{25}$ + 4,2° (c 1, MeOH), yield 74 % from 7 — with N-t-butoxycarbonyl-sarcosine (BOC-Sar) by DCC(triethylamine in acetonitrile/methanol.

The final two steps were performed simply by exposure of 11 to methanolic ammonia (4 h, 25°) to give BOC-gougerotin 12 [mp 213 - 215° (dec), $[\alpha]_D^{25}$ + 52° (c 0.5, H_2O), 78 % yield] and subsequent removal of the protecting group by treatment with trifluoroacetic acid (0.5 h, 25°). Gougerotin was isolated by passing through a small column of a strongly basic ion exchanger (Merck III) and recrystallization from methanol as colorless, needle-shaped crystals in 84 % yield.

The synthetic product was identical with natural gougerotin [20] by thin layer chromatographic [1], ir, rotational and pmr [1] comparison. In addition, the inhibitory activities on protein biosynthesis, as evaluated with the 70S promoted AcLeu-transfer from CACCA-LeuAc to puromycin [21], were also identical for synthetic and S. gougerotii derived 13, the apparent inhibition constant K_i' being 5.0 μM. In the two gougerotin analogs 8 and 10 [22], however, the inhibitory activities are reduced by factors of 252 and 63 as evidenced by K_i' - values of 1260 (8) and 317 μM (10), stressing the importance of the sarcosyl portion in the antibiotic for full biological activity.

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References and Notes

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(3) Its isolation has been recorded from Streptomyces gougerotii [4a], from Streptomyces S-154 [4b], from Streptomyces SF-1238, genealogically close to S. toyocaensis [4c], and from S. toyocaensis var. aspiculamyceticus [4d]. Since the antibiotic elaborated by the latter species, i.e. aspiculamycin, has been shown to be identical with gougerotin [1], the designation Streptomyces toyocaensis var. gougerotii for this strain appears to be more appropriate.

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(15) The data obtained [mp 82 - 83° and $[\alpha]_D^{25}$ + 166° (c 0.8, $CHCl_3$)] correspond well with those reported by Fox et al. [10a], yet are at variance with others [14].

(16) Eluants used: cyclohexane-ethyl acetate 3 : 1 (a), ethyl acetate-methanol-water 10 : 2 : 1 (b) and 5 : 2 : 1 (c).

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(20) A sample, commercially available from Calbiochem. USA, was used for comparison.

(21) (a) One of the most sensitive model systems for evaluating interference with peptide chain elongation; cf. lit. [2a] for specification. (b) We are indebted to Dr. H. M. Menzel of this laboratory for the biological evaluations.

(22) Prepared by treatment of 8. HCl with methanolic ammonia as a monohydrate, mp 243 - 246° (dec), $[\alpha]_D^{25}$ + 48° (c 0.3, H_2O).