Tetrahedron Letters No. 7, 545 - 548, 1976. Pergamon Press. Printed in Great Britain.

# TOTAL SYNTHESIS OF RIBOSTAMYCIN

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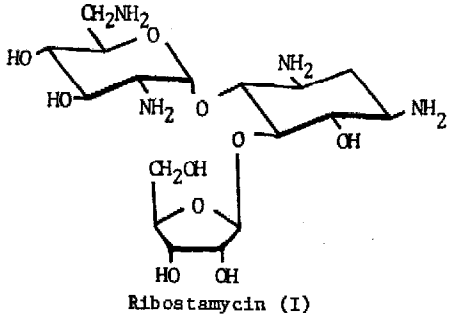
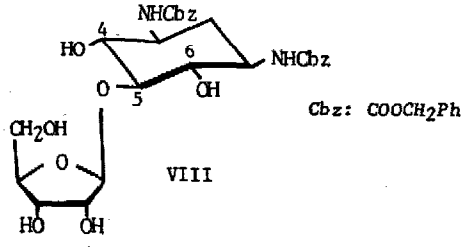
(Received in Japan 5 December 1975; received in UK for publication 2 January 1976)

Ribostamycin (I), an aminoglycoside antibiotic produced by *Streptomyces ribosidificus* has a broad antimicrobial activity[1]. The structure is close to that of neomycin, in which 2,6-diaminohexose is bound to the $C_3$ hydroxyl group of the D-ribose moiety of ribostamycin[2),3)], and is also similar to butirosin B except for the L-γ-amino-α-hydroxybutyric acid side chain attached at the $C_1$ amino group[4)]. Ito et al. reported the synthesis of ribostamycin by condensation of the neamine derivative with 2,3,4-tri-O-benzoyl-D-ribofuranosyl chloride (II) under Königs-Knorr conditions[5)]. In this paper, we report an alternative synthetic route of ribostamycin and related compounds *via* the condensation of the suitably protected 5-O-β-D-ribofuranosyl-2-deoxystreptamine with 3,4-di-O-acetyl-2,6-dideoxy-2-(2',4'-dinitroanilino)-6-phthalimido-α-D-glucopyranosyl bromide (III)[6)] by a modified Königs-Knorr reaction.



Ribostamycin (I)



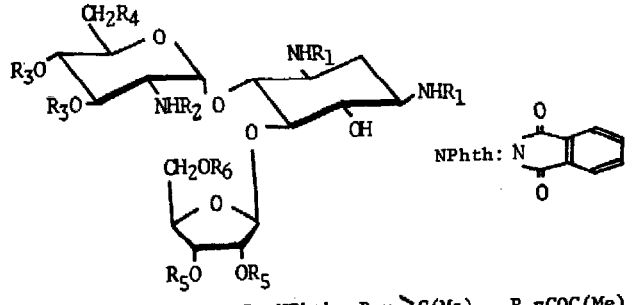
VIII

Cbz: $COOCH_2Ph$

By the condensation ($AgClO_4$-$Ag_2CO_3$, benzene-dioxane (1:1), 75°C, 3 hr) of 4(and/or 6)-O-acetyl-N,N'-dicarbobenzoxy-2-deoxystreptamine[7)] with II[8)], and subsequent chromatographic separation on silicic acid column with $CHCl_3$, we obtained two isomers of 5-O-β-D-ribofuranosyl-2-deoxystreptamine derivatives (IVa; m.p. 208°, $[\alpha]_D^{22}$ +21.6°, c=0.53, $CHCl_3$, IVb; m.p. 191°, $[\alpha]_D^{22}$ +17.6°, c=0.88, $CHCl_3$), together with 4-O-β-D- (V; m.p. 237°, $[\alpha]_D^{22}$ +31.2°, c=0.59,

$CHCl_3$) and 6-O-β-D-ribofuranosyl-2-deoxystreptamine derivatives (VI; m.p. 151°, $[\alpha]_D^{22}$ +6.5°, c=0.62, $CHCl_3$). Total yield was about 60%. Both IVa and IVb gave an identical compound (VII; m.p. 195°, $[\alpha]_D^{22}$ +34.7°, c=0.65, $CHCl_3$) on acetylation. PMR and IR spectra of the deacylated product of IVa and IVb (VIII; m.p. 231°, $[\alpha]_D^{25}$ -37.9°, c=0.58, DMF) were superimposed on that of the authentic sample derived from N-carbobenzoxy neomycin according to the method of Hanessian et al.[9),10)]. The structures of V and VI were determined unambiguously to be the 4-O-isomer and 6-O-isomer, respectively, by comparision of $\Delta[M]_{CuAm}$ values of the corresponding N-acetyl derivatives (IX; m.p. 269-70°, $[\alpha]_D^{25}$ -48.1°, c=0.69, $H_2O$, $\Delta[M]_{CuAm}$ -2920°, X; m.p. 280-3°, $[\alpha]_D^{25}$ -1.5°, c=0.66, $H_2O$, $\Delta[M]_{CuAm}$ +65°). From Reeve's empirical rule[11)], it was concluded that, since the vicinal hydroxyl groups of the 2-deoxystreptamine moiety are anti-clockwise in IX, but clockwise in X, the ribose is bound to $C_4$ of 2-deoxystreptamine in IX and $C_6$ in X.

VIII was acetonated with 2,2-dimethoxypropane in DMF in the presence of p-toluenesulfonic acid at 45°C for 2 hr to give a 2',3'-O-isopropylidene derivative (m.p. 200°, $[\alpha]_D^{22}$ -52.9, c=0.85, acetone). The $C_5'$ hydroxyl group was then pivaloylated selectively with pivaloyl chloride in pyridine[12)] (15°C, 1.5 mol. eq. pivaloyl chloride, 3 hr, 80% yield) to give the corresponding 5'-O-pivaloyl derivative (XI; m.p. 81°, $[\alpha]_D^{25}$ -32.6°, c=0.82, $CHCl_3$). XI was condensed with III by a modified Königs-Knorr reaction ($AgClO_4$-$Ag_2CO_3$, benzene, 75°C, 3 hr). The condensed product was composed of at least three components, XII, XIII and XIV in order of mobility on thin layer chromatogram ($CHCl_3$:methanol=30:1), which were separated on silicic acid column (benzene:ethyl acetate=5:1) and purified by rechromatography (silicic acid, $CHCl_3$:methanol=30:1). XII was obtained in 14% yield (m.p. 184°, $[\alpha]_D^{26}$ -33.8°, c=0.57, $CHCl_3$). Anal. Found: C,57.49; H,5.23; N,6.68. Calcd. for $C_{59}H_{66}N_6O_{23}$: C,57.74; H,5.42; N,6.85%), XIII in




XIV; $R_1$=Cbz, $R_2$=DNP, $R_3$=Ac, $R_4$=NPhth, $R_5$=$>C(Me)_2$, $R_6$=$COC(Me)_3$

XVII; $R_1$=H, $R_2$=H, $R_3$=H, $R_4$=$NH_2$, $R_5$=H, $R_6$=H

a 34% yield (m.p. 117°C, $[\alpha]_D^{26}$ -23.8°, c=1.09, $CHCl_3$). Anal. Found: C, 57.96; H, 5.71; N, 7.15%) and XIV in a 6% yield (m.p. 134°, $[\alpha]_D^{26}$ +3.6°, c=0.55, $CHCl_3$). Anal. Found: C, 57.61; H, 5.46; N, 6.55%).

XII, XIII and XIV gave the corresponding free bases XV, XVI and XVII in 10-20% yield on removing the protecting groups with the following procedures; deacetonation with 50% aq. acetic acid (70°C, 10 hr), dephthaloylation with n-butylamine in abs. methanol (75°C, 16 hr), followed by treatment with $Ba(OH)_2 \cdot 8H_2O$ in water-dioxane (110°C, 2 hr). The reaction products

Table 1 Antibacterial Activity and Optical Rotation

| | 100ppm | $[\alpha]_D^{24}$ |
|---|---|---|
| Ribostamycin free base (I) | 22.4 mm [1] | +51.2° [2] |
| XVII | 21.2 | +55.6° |
| XV | — | +23.9° |
| XVI | — | -57.9° |

1. diameter of inhibition zone by paper disk method using *Staphylococcus aureus* FDA 209P.
2. lit.(ref. 1), $[\alpha]_D$ +42°.

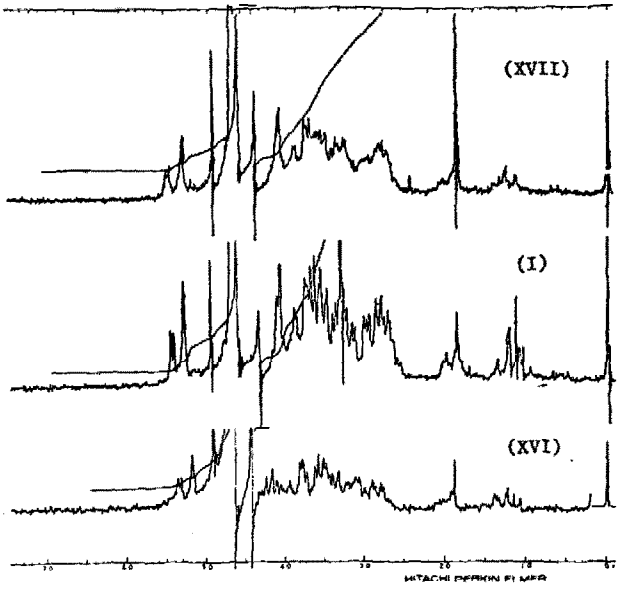



Fig.1 PMR spectra of XVII, I and XVI in $D_2O$ at 90MHz.

were purified by chromatography on CM-Sephadex C-25 ($NH_4^+$ form) (eluent: water, 0.1N-0.3N aq. $NH_3$ successively). XVII was identical with an authentic sample of ribostamycin (I), in such respects as PMR (Fig. 1) and IR spectra, and specific rotation (Table 1). $R_f$ values of thin layer chromatogram ($CHCl_3$:methanol:conc. $NH_3$:$H_2O$=1:4:2:1) and paper chromatogram (n-butanol: pyridine:acetic acid:$H_2O$=6:4:1:3), and biological activity (Table 1). XVI was shown to be the positional isomer of XVII by comparision of the PMR spectra (Fig. 1). This structure was further confirmed by the selective removal of the ribose moiety from its tetra-N-acetyl derivative with mild acid hydrolysis (5% methanolic hydrogen chloride, 50°C, 3 hr) to give 6-O-(2,6-diacetamido-2,6-dideoxy-α-D-glucopyranosyl)-N,N'-diacetyl-2-deoxystreptamine[6]. XV is probably the β-isomer of XVI since in the PMR spectra of its N-acetyl derivative the anomeric proton signal of the ribose moiety appears at 5.15 ppm as a doublet (J=2.0Hz) and that of 2,6-diaminoglucose moiety at 4.75 ppm splitting with 7.5Hz suggesting β-glycosidic linkage.

We are grateful to Professor Koichi Koshimizu and Miss Shigeko Yamashita for the 90MHz NMR measurements.

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