Total Synthesis of Ribostamycin

Harukazu Fukami, Shoji Ikeda, Katsuhiko Kitahara and Minoru Nakajima

Department of Agricultural Chemistry, Kyoto University, Kyoto

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Suitably protected 5-O-β-D-ribofuranosyl-2-deoxystreptamine was condensed with protected 2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl bromide by a modified Königs-Knorr reaction to give three condensation products. One of which was confirmed as a ribostamycin derivative. The others were designated as its 6-O-α- and 6-O-β-isomers by the PMR spectra of their free bases and N-acetyl derivatives and by their chemical reactions.

Ribostamycin (I) produced by Streptomyces ribosidicus has a broad antimicrobial spectrum and low toxicity to mammals.1 It has a structure in which 2,6-diamino-2,6-dideoxy-D-glucose is linked at the C₄ position of 2-deoxystreptamine in the α-form and with D-ribose in the β-form at the C₅ position.2 It may be considered the basic unit of neomycins and butirosin B.3,4 A ribostamycin analog, position, because of steric hindrance from the C₄ glycosyl moiety. In fact, kanamycin derivatives, the 4,6-diglycosides of 2-deoxystreptamine, were successfully synthesized by condensations of the 4-O-glycosyl-2-deoxystreptamine derivatives with the 3-aminoglucosyl halide.6 Ito and his associates have synthesized ribostamycin from a protected neamine derivative and ribofuranosyl chloride with the Königs-Knorr condensation.7 In this case, however, the ribose residue was introduced almost exclusively into the C₅ position. Thus, the reactivity of the C₅ and C₆ hydroxyl groups of 4-O-glycosyl-2-deoxystreptamine in the glycosylation reaction seems to be governed by unpredictable factors. A glycosylation reaction using 5-O-glycosyl-2-deoxystreptamine would be more likely to afford 4,5-disubstituted 2-deoxystreptamine than a reaction using 4-O-glycosyl-2-deoxystreptamine.

We here present an alternative stepwise synthesis of ribostamycin and its positional isomers via the condensation of suitably protected 5-O-β-D-ribofuranosyl-2-deoxystreptamine with 3,4-di-O-acetyl-2,6-dideoxy-2-(‘,4’-dinitroanilino)-6-phthalimido-α-D-glucopyranosyl bromide using a modified Königs-Knorr reaction.

Synthetic methods

i) Preparation of the aglycon (IV). N,N′-Dicarbobenzyloxy-5-O-β-D-ribofuranosyl-2-deoxystreptamine (II)10 was acetonized with
2,2-dimethoxypropane to give the 2', 3'-O-isopropylidene derivative (III) in a quantitative yield. This was selectively pivaloylated at the 5'-primary hydroxyl group with 1.5 mol eq. pivaloyl chloride in pyridine to afford the 5'-pivaloyl derivative (IV) in a 70% yield. To determine the structure, IV was mesylated, then the glycoside bond was cleaved with methanolic hydrogen chloride to yield the optically inactive 2-deoxystreptamine derivative (V) which showed two methyl signals of methanesulfonyl groups at 3.00 ppm as a single peak in its PMR spectra indicating that V was the structurally symmetrical 4,6-di-O-mesyl derivative. Therefore, IV was concluded to have a structure where only the C5' primary hydroxyl group was pivaloylated.

![Chemical structures](image)

ii) Glycoside synthesis. Compound IV was condensed with 2,6-diaminoglucosyl bromide in dry benzene in the presence of Ag2CO3 and AgClO4 at 70°C for 2 hr in the dark. After the usual work up, three products were obtained in a 54% total yield (VIII; 14%, VII; 34%, VI; 6% in order of mobility on TLC). To ascertain the position and configuration of the glycoside linkage, these products were converted to their corresponding free bases, XI, X and IX, respectively; by sequential deacetonization with 80% aqueous acetic acid, by dephthaloylation with n-butylamine in methanol, and finally by deblocking the other protection groups with aqueous barium hydroxide. Among these compounds, only IX had the same degree of antimicrobial activity as authentic ribostamycin (Table I). It was also confirmed to be identical with ribostamycin in respect to its PMR (Fig. 1) and

![PMR Spectra](image)

**TABLE I. ANTIMICROBIAL ACTIVITY**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition diameter (mm)</th>
</tr>
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<tbody>
<tr>
<td>Ribostamycin</td>
<td>22.4</td>
</tr>
<tr>
<td>IX</td>
<td>—</td>
</tr>
<tr>
<td>X</td>
<td>—</td>
</tr>
<tr>
<td>XI</td>
<td>21.2</td>
</tr>
</tbody>
</table>

a) Paper disc method at 100 ppm using S. aureus FDA 209P as a test organism.

b) No activity at 100 ppm.

![Figure 1](image)
Total Synthesis of Ribostamycin 1691

IR spectra as well as its optical rotation.

The PMR spectrum of X showed a doublet signal \((J=4.0 \text{ Hz})\) at 5.37 ppm, which is attributed to the anomeric proton of 2,6-diaminoglucone in the \(\alpha\)-configuration, and a singlet at 5.21 ppm due to the \(\beta\)-D-ribose structure. The \(N\)-acetyl derivative of \(X\) was treated with methanolic hydrogen chloride to give a pseudosaccharide identical with 6-\(O\)-(2,6-diamino-2,6-dideoxy-\(\alpha\)-D-glucopyranosyl)-\(N, N\prime\)-diacetyl-2-deoxystreptamine in its PMR spectra. Thus, \(X\) was designated 6-\(O\)-(2,6-diamino-2,6-dideoxy-\(\alpha\)-D-glucopyranosyl)-5-\(O\)-\(\beta\)-D-ribofuranosyl-2-deoxystreptamine.

The \(N\)-acetyl derivative of XI showed a doublet \((J=7.0 \text{ Hz})\) due to the anomeric proton of the \(\beta\)-glycoside linkage of 2,6-diaminoglucose at 4.62 ppm and a doublet \((J=2.0 \text{ Hz})\) of the same group of \(\text{D-ribose}\) at 5.08 ppm. The condensation product (VIII) was permethylated with methyl iodide and Ag\(_2\)O in DMF. The permethylated derivative was hydrolyzed with 6 N HC\(_1\) in a sealed tube, then acetylated to give the methylated 2-deoxystreptamine derivative (XV). In contrast, hexa-\(N\)-carbobenzyloxy neomycin B gave a methylated 2-deoxystreptamine derivative, 4,5-di-\(O\)-acetyl-\(N, N\prime\)-diacetyl-6-\(O\)-methyl-\(N, N\prime\)-dimethyl-2-deoxystreptamine (XVI) under the same procedures. The PMR and IR spectra of XV were superimposable on those of XVI, whereas the optical rotatory signs of these products were opposite (XV; \(+6.0^\circ\), XVI; \(-8.6^\circ\)). Therefore, XV was identified as 5,6-di-\(O\)-acetyl-\(N, N\prime\)-diacetyl-4-\(O\)-methyl-\(N, N\prime\)-dimethyl-2-deoxystreptamine, an enantiomer of XVI, which showed unequivocally that XI was 6-\(O\)-(2,6-diamino-2,6-dideoxy-\(\beta\)-D-glucopyranosyl)-5-\(O\)-\(\beta\)-D-ribofuranosyl-2-deoxystreptamine.

EXPERIMENTAL

Melting points are uncorrected. PMR spectra were recorded at 90MHz with a Hitachi R-22 spectrometer. IR spectra were recorded with a Shimadzu AR-27G spectrometer. Specific rotations were determined with a Yanagimoto polarimeter OR-50. TLC was conducted using silicagel G (Merck) with two solvent systems (benzene-ethyl acetate and chloroform-methanol). Column chromatography was carried out with silicic acid (Wakogel C-300).

\(N, N\prime\)-Dicarbobenzyloxy-5-\(O\)-(2, 3-\(O\)-isopropylidene-\(\beta\)-d-ribofuranosyl)-2-deoxystreptamine (III)

The solution of \(N, N\prime\)-dicarbobenzyloxy-5-\(O\)-d-ribofuranosyl-2-deoxystreptamine (II) (900 mg), 2, 2-dimethoxypropane (5 ml), \(p\)-toluenesulfonic acid (30 mg) and DMF (10 ml) was stirred at 45°C for 3 hr. The solution was neutralized with Na\(_2\)CO\(_3\) (500 mg) and evaporated in vacuo to a syrup, then extracted with acetone (30 ml). After the removal of inorganic materials by filtration, the filtrate was evaporated to a syrup, then dissolved in acetone (10 ml) and 50% acq. acetic acid (10 ml) after which it was warmed at 45°C for 3 hr. After distillation of the solvent, III (700 mg, 73% yield) was crystallized from ethanol as needles, mp 200°C, \([\alpha]_D^{25}=-52.9^\circ\) (c=0.85, acetone). IR \(\nu_{\text{KBr}}\) cm\(^{-1}\): 3300–3500 (OH, NH) and 1680 (C=O). PMR \(\delta_{\text{DMSO-d6}}\) : 1.22 and 1.33 (singlets, \(=C(\text{CH}_3)\)), 4.95 (4H, Cbz-CH\(_2\)), 5.41 (singlet, H\(_1\)), and 7.27 (10H, Cbz). Anal. Found: C, 59.79; H, 6.40; N, 4.79. Calcd. for C\(_{30}\)H\(_{33}\)N\(_2\)O\(_{11}\): C, 59.79; H, 6.36; N, 4.65%.

\(N, N\prime\)-Dicarbobenzyloxy-5-\(O\)-(2, 3-\(O\)-isopropylidene-5-\(O\)-pivaloyl-\(\beta\)-D-ribofuranosyl)-2-deoxystreptamine (IV)

Pivaloyl chloride (300 mg) was added to the pyridine solution (10 ml) of III (800 mg) and stirred for 10 hr at 15°C while monitored with tlc. Since the reaction was incomplete, more pivaloyl chloride (300 mg) was added. Methanol (10 ml) was added to the solution, then evaporated to a syrup which was dissolved in CHCl\(_3\), washed with \(H_2O\) and dried with \(Na_2SO_4\). After the removal of CHCl\(_3\), the residue scratching in ether-hexane gave needles (700 mg, 77% yield), mp 81°C. \([\alpha]_D^{25}=-32.6^\circ\) (c=0.88, CHCl\(_3\)). IR \(\nu_{\text{KBr}}\) cm\(^{-1}\): 3300–3500 (OH, NH) and 1680–1720 (C=O). PMR \(\delta_{\text{CDCl3}}\) : 1.16 (9H, singlet, pivaloyl), 1.72 and 1.42 (singlets, \(=C(\text{CH}_3)\)), 4.98 (4H, Cbz-CH\(_2\)), 5.41 (singlet, H\(_1\)), and 7.27 (10H, Cbz). Anal. Found: C, 59.79; H, 6.40; N, 4.79. Calcd. for C\(_{35}\)H\(_{46}\)N\(_2\)O\(_{11}\): C, 59.79; H, 6.36; N, 4.65%.

\(N, N\prime\)-Dicarbobenzyloxy-5-\(O\)-(2, 3-\(O\)-isopropylidene-5-\(O\)-pivaloyl-\(\beta\)-d-ribofuranosyl)-2-deoxystreptamine (IV)

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The structural determination of IV\((N, N\prime\)-dicarbobenzyloxy-4, 6-di-\(O\)-methanesulfonyl-2-deoxystreptamine\))

IV (100 mg) was reacted with methanesulfonyl chloride (0.2 ml) in pyridine (5 ml) over night at room temperature. The reaction mixture underwent extraction with CHCl\(_3\), was washed with 0.5 N HCl and water,
and dried with Na2SO4. After evaporation, the syrup was dissolved in 0.6 N methanolic hydrogen chloride and warmed at 50°C for 24 hr. The solution was evaporated and underwent extraction with CHCl3, after which it was washed with saturated aq. NaHCO3 and water, then dried with Na2SO4. The solution was then evaporated to a white residue and crystallization from ethanol-ether gave V (40 mg, 43% yield). The PMR spectrum showed two methyl groups corresponding to methanesulfonyl groups as a single peak, mp 188-9°C, IR νmax cm-1: 3000 (OH), 3350 (NH) and 1690-1700 (C=O). PMR δTMS: 3.00 (6H, singlet, SO3CH3), 4.98 (4H, singlet, Cbz-CH2) and 7.22 (10H, Cbz). Anal. Found: C, 57.49; H, 5.07; N, 6.49. Calcd for C24H30N2S2O11: C, 49.14; H, 5.07; N, 4.78%. 4-O-(3, 4-Di-O-acetyl-2, 6-dideoxy-2′, 4′-dinitroanilino)-6-phthalimido-α-D-glucopyranosyl-(ribsotamycin derivative, VI), 4-O-(3, 4-di-O-acetyl-2, 6-dideoxy-2′-(2′, 4′-dinitroanilino)-6-phthalimido-α-D-glucopyranosyl)-(VII), and 6-O-(3, 4-di-O-acetyl-2, 6-dideoxy-2′-(2′, 4′-dinitroanilino)-6-phthalimido-β-D-glucopyranosyl)-N', N'-di-carbobenzyloxy-5-O-(2, 3-O-isopropylidene-5-O-pivaloyl-β-D-ribofuranosyl)-2-deoxystreptamine (VIII) IV (700 mg) was dissolved in abs. benzene (60 ml) containing Ag2CO3 (1.5 g), AgClO4 (200 mg) and Drierite (7.0 g), then stirred at 70°C for 1 hr in the dark. 2, 6-Diaminoglucosyl bromide was added in four portions (600 mg each) every 30 min. Four hours after the last addition of 2, 6-diaminoglucosyl bromide, inorganic materials were filtered off and washed with CHCl3. The filtrate and washings were combined and washed with water, then dried with Na2SO4. The solvent was removed through evaporation to a syrup, which was chromatographed over sillicic acid (70 g) containing Ag2CO3 (1.5 g), AgClO4 (200 mg) and Drierite (7.0 g), then stirred at 70°C for 1 hr in the dark. 2, 6-Diaminoglucosyl bromide was added in four portions (600 mg each) every 30 min. Four hours after the last addition of 2, 6-diaminoglucosyl bromide, inorganic materials were filtered off and washed with CHCl3. The filtrate and washings were combined and washed with water, then dried with Na2SO4. The solution was evaporated to a syrup which was then stirred with n-butylamine (5 ml) in methanol (8 ml) at 75°C for 17 hr. The solution was evaporated to a syrup which was then stirred with n-butylamine (5 ml) in methanol (8 ml) at 75°C for 17 hr. Then, the syrup obtained by evaporation was reacted with Ba(OH)2 (4 ml) in water (10 ml) and dioxane (5 ml) at 110°C for 30 min. In solution were filtered and the filtrate was neutralized with 3 N H2SO4. After removal of BaSO4 by filtration on a celite 545 pad, the mixture was concentrated and chromatographed on CM-Sephadex C-25 (NH4+) as an amorphous solid, mp 165°C (decomp.). VII (400 mg, 34% yield) was obtained from the 0.1 N fractions on CM-Sephadex C-25 column chromatography, mp 190°C (decomp.), [α]D° +23.9° (c=0.46, H2O). IR νmax cm-1: 3250-3500 (NH, OH), 1570-1590 (NH, bending) and 1000-1050 (C-O-C). PMR δD2O: 5.33 (singlet, H1") and 5.50 (doublet, J=4.0 Hz, H1'). 6-O-(2, 6-Diamino-2, 6-dideoxy-α-D-glucopyranosyl)-5-O-β-D-ribofuranosyl-2-deoxystreptamine (IX; ribostamycin) VI (200 mg) was dissolved in 80% aq. acetic acid (10 ml) and stirred at 75°C for 10 hr. The solution was evaporated to a syrup which was then stirred with n-butylamine (5 ml) in methanol (8 ml) at 75°C for 17 hr. Then, the solution obtained by evaporation was reacted with Ba(OH)2 (8H2O (1.2 g) in water (10 ml) and dioxane (5 ml) at 110°C for 2.5 hr. Insoluble materials were filtered off and the filtrate was neutralized with 3 N H2SO4. After removal of BaSO4 by filtration on a celite 545 pad, the mixture was concentrated and chromatographed on CM-Sephadex C-25 (NH4+) as an amorphous solid, mp 165°C (decomp.). VII (700 mg) was treated as described above. X (100 mg, 39% yield) was obtained from the 0.1 N fractions on CM-Sephadex C-25 column chromatography as an amorphous solid, mp 165°C (decomp.), [α]D° +23.9° (c=0.46, H2O). IR νmax cm-1: 3250-3500 (NH, OH) and 1570 (NH, bending) and 1000-1050 (C-O-C). PMR δD2O: 5.21 (singlet, H1") and 5.37 (doublet, J=4.0 Hz, H1'). 6-O-(2, 6-Diamino-2, 6-dideoxy-α-D-glucopyranosyl)-5-O-β-D-ribofuranosyl-2-deoxystreptamine (XI) VII (700 mg) was treated as described above. X (100 mg, 39% yield) was obtained from the 0.1 N fractions on CM-Sephadex C-25 column chromatography as an amorphous solid, mp 165°C (decomp.), [α]D° +23.9° (c=0.46, H2O). IR νmax cm-1: 3250-3500 (NH, OH) and 1570 (NH, bending). PMR δD2O: 5.21 (singlet, H1") and 5.37 (doublet, J=4.0 Hz, H1'). 6-O-(2, 6-Diamino-2, 6-dideoxy-β-D-glucopyranosyl)-5-O-β-D-ribofuranosyl-2-deoxystreptamine (XI) From VIII (600 mg), XI (90 mg, 41% yield) was obtained as above (0.1 N fractions on CM-Sephadex C-25 column chromatography), mp 190°C (decomp.), [α]D° +57.9° (c=0.38, H2O). IR νmax cm-1: 3250-3500 (NH, OH) and 1570 (NH, bending). PMR δD2O: 5.21 (doublet, J=2.0 Hz, H1") and 5.37 (doublet, J=4.0 Hz, H1').
4-O-(2,6-Diacetamido-2,6-dideoxy-α-D-glucopyranosyl)-N’, N’-diacetyl-5-O-β-D-ribofuranosyl-2-deoxystreptamine (XII)

IX (20 mg) was acetylated with acetic anhydride (3 ml) in aq. methanol (5 ml) at 50°C for 30 min to give XII (15 mg, 55 % yield) as an amorphous solid, mp 190°C, [α]_22D +46.8° (c=0.47, methanol). IR ν_max KBr cm⁻¹: 3250 - 3500 (NH, OH) and 1640 (C=O). PMR (CDCl₃): 1.97, 2.00, 2.02 and 2.05 (singlets, NHCOCH₃), 5.18 (doublet, J=1.0 Hz, H₁) and 5.29 (doublet, J=3.2 Hz, H₁).

6-O-(2,6-Diacetamido-2,6-dideoxy-α-D-glucopyranosyl)-N,N’-diacetyl-5-O-β-D-ribofuranosyl-2-deoxystreptamine (XIII)

By N-acetylation of X (50 mg), XIII (40 mg, 58 % yield) was obtained, mp 195°C, [α]_D +29.9° (c=0.77, methanol), IR ν_max KBr cm⁻¹: 3250-3500 (NH, OH) and 1640 (C=O). PMR (CDCl₃): 1.86, 1.91 and 2.00 TMS (singlets, NHCOCH₃), 5.17 (singlet, H”) and 5.30 (doublet, J=3.0 Hz, H₁).

6-O-(2,6-Diacetamido-2,6-dideoxy-(3-D-glucopyranosyl)-N,N’-diacetyl-5-O-β-D-ribofuranosyl-2-deoxystreptamine (XIV)

By N-acetylation of XI (40 mg), XIV (35 mg, 64 % yield) was obtained. mp 195-7°C. [α]_D -43.5° (c=0.23, methanol). IR ν_max cm⁻¹: 3250-3500 (NH, OH) and 1650 (C=O). PMR (CDCl₃): 1.90, 1.99 and 2.02 (singlets, NHCOCH₃), 4.62 (doublet, J=7.0 Hz, H₁) and 5.08 (doublet, J=2.0 Hz, H₁).

Determination of the glycosidic position on the 2-deoxystreptamine of X

XIII (300 mg) was stirred in 0.6 N methanolic hydrochloride (10 ml) at 40°C for 10 hr. After neutralization with Amberlite IR-400 (OH⁻), the solution was evaporated to a syrup, then crystallized from ethanol to obtain a compound (15 mg, 63 % yield), which was identified as 6-O-(2,6-diacetamido-2,6-dideoxy-α-D-glucopyranosyl)-N,N’-diacetyl-2-deoxystreptamine by comparing its PMR and IR spectra with those of an authentic sample.

Determination of the glycosidic position on the 2-deoxystreptamine of XI

VIII (300 mg) was dissolved in abs. DMF (7 ml) and methyl iodide (2 ml), and Ag₂O was added in four 700 mg portions every 20 min in the dark. After stirring at room temperature for 20 hr, the silver salts were filtered off and washed with DMF. The filtrate and washings were combined and evaporated in vacuo to give a syrup, which was extracted with CHCl₃ and evaporated. The resulting syrup was dissolved in dioxane (2 ml) and 6 N HCl (7 ml) in a sealed tube, and allowed to react at 110°C for 4 hr. The reaction mixture was treated with active charcoal and evaporated to dryness, then it was acetylated with acetic anhydride (3 ml) in pyridine (6 ml). After the reaction, the solvent was removed. The syrup was chromatographed over silicic acid by elution with CHCl₃-methanol (20:2:1). Crystallization from CHCl₃-ether afforded colorless plates of XV (30 mg, 33 % yield). The structure of XV was determined to be 5,6-di-O-acetyl-N,N’-diacetyl-4-O-methyl-N,N’-dimethyl-2-deoxystreptamine by the following data. In its PMR spectrum, the N-methyl, N-acetyl and 0-methyl groups each showed two or three singlet signals instead of only one, which, indicates that they were composed of several conformers, mp 212°C, [α]_D +6.0° (c=0.84, methanol), IR ν_max cm⁻¹: 1740 (ester C=O) and 1630 (amido C=O). PMR (CDCl₃): 1.96-2.15 (12H, COCH₃), 2.72-2.98 (6H, N-CH₃), 3.34 and 3.36 (3H, OCH₃), Anal. Found: C, 54.93; H, 7.61; N, 7.60. Calcd. for C₁₁₇H₂₈O₂N₇: C, 54.82; H, 7.58; N, 7.58. XV was superimposable on 4,5-di-O-acetyl-N,N’-diacetyl-6-O-methyl-N,N’-dimethyl-2-deoxystreptamine which was obtained from hexa-N-carbobenzyloxy-neomycin as above in their PMR and IR spectra mp 211-3°C. [α]_D +6.0° (c=0.84, methanol). Anal. Found: C, 54.70; H, 7.65; N, 7.64 %, but each showed opposite signs in its optical rotation.

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REFERENCES


