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SYNTHESIS OF N^g-STREPTOLIDYL GULOSAMINIDE — A NEW EVIDENCE FOR THE PROPOSED STRUCTURE OF ANTIBIOTIC STREPTOTHRICIN

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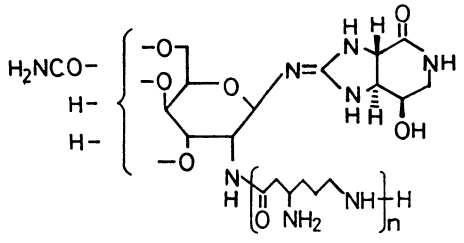
The title compound was synthesized and identified with a partial hydrolysis product from natural streptothricin. This work confirmed the presence of the proposed glycosyl guanidine structure in the antibiotic and established a method for its preparation.

Streptothricin is a classical antibiotic, which shows the potent activity against wide range of bacteria as well as some pathogenic fungi. After its isolation by Waksman and Woodruff in 1942,[1] numerous compounds of the same category with similar structures have been reported, constituting a family of so-called streptothricin group antibiotics.[2] However, none of them has been used clinically because of their inherent toxicity.

Streptothricin is composed of 2-amino-2-deoxy-D-gulose (D-gulosamine), L-β-lysin and a unique guanidino amino acid, streptolidine. Its total structure, except the location of the carbamoyl group was deduced as 1 by van Tamelen and Carter et al. in 1961.[3] In this situation, chemical synthesis seems to be the most effective way to establish the structure and may offer a possibility to generate new and useful antibiotics with less toxicity. We now report a synthesis of N^g-streptolidyl 2-amino-2-deoxy-β-D-gulopyranoside (N^g-streptolidyl gulosaminide)(2) and its identification with the natural specimen obtained by partial hydrolysis of streptothricin.[4] This result gave an unequivocal evidence supporting the presence of a β-glycosidic linkage in the natural antibiotic between 2-amino-2-deoxygulose and the exocyclic nitrogen atom of streptolidine. This was proposed by the above authors[3] but had not been fully established yet. Successful construction of this linkage means an accomplishment of the key step toward the total synthesis of antibiotics of this type.



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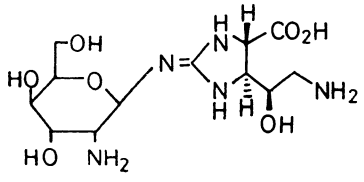
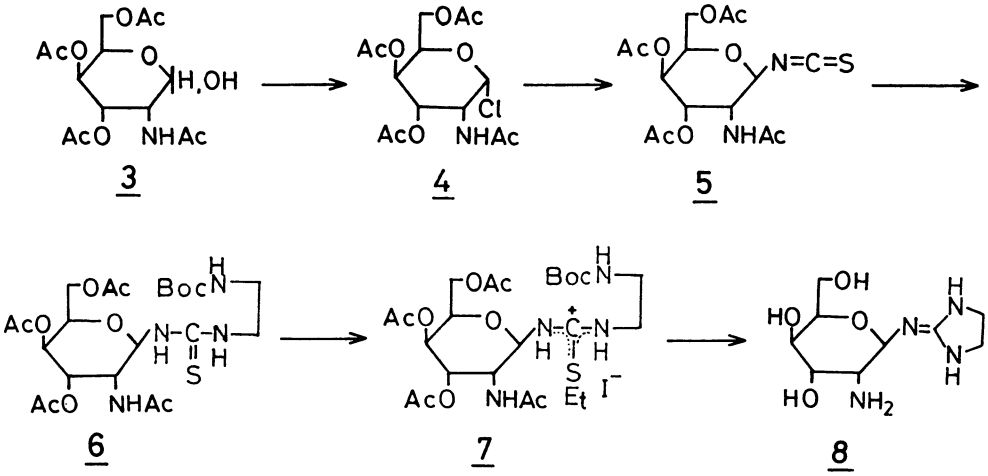


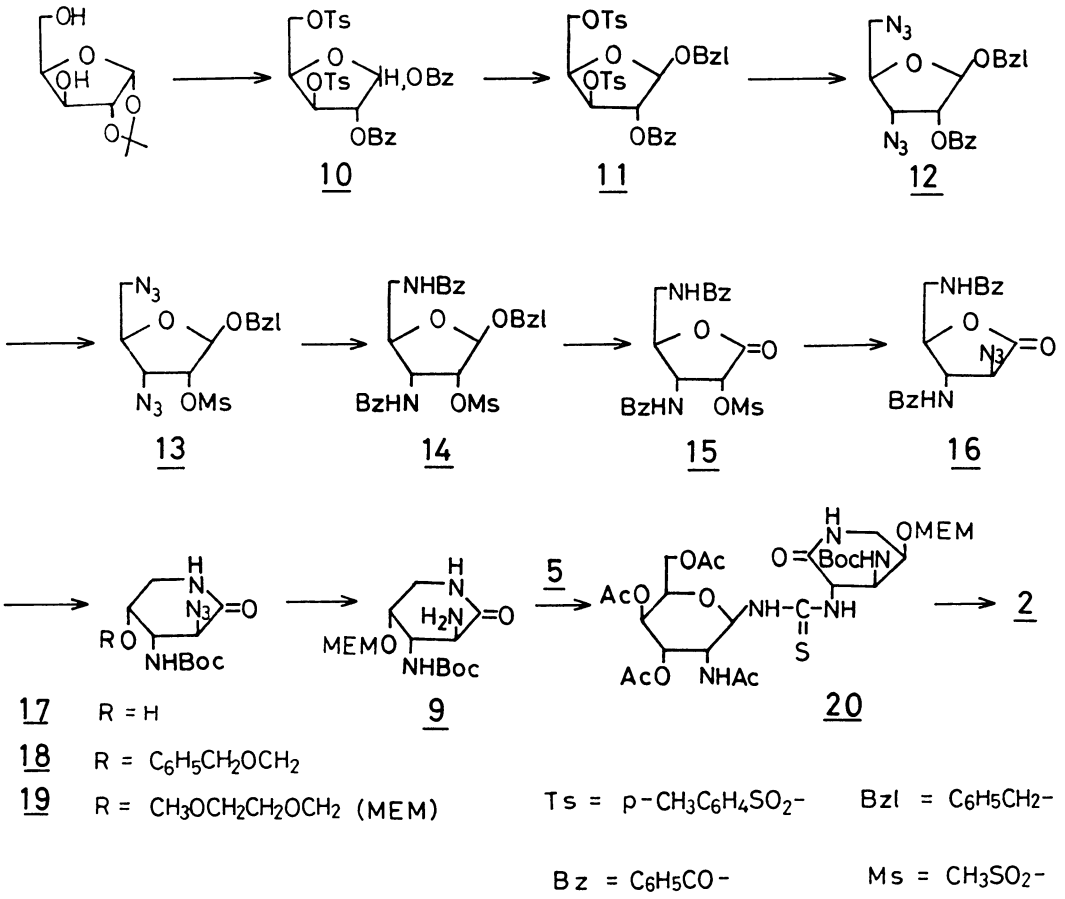
2

In order to build up the desired glycosyl cyclic guanidine structure, an unequivocal route via β-glycosyl isothiocyanate was developed by use of ethylenediamine as a model compound for streptolidine moiety as shown in the scheme. 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-gulopyranose (3), prepared from benzyl 2-amino-2-deoxy-α-D-gulopyranoside,[5] was converted via 1-O-trifluoroacetate into the corresponding glycosyl chloride (4) [i) trifluoroacetic anhydride in CH_2Cl_2 at -60°C for 20 hr, ii) dry HCl in CH_2Cl_2 at -60°C to room temperature].[6] After evaporation of the solvent in vacuo the residual chloride (4) was treated directly with KSCN (in anhydrous acetone at room temperature for 24 hr) to give β-glycosyl isothiocyanate (5) as colorless syrup (40% from 3, NMR: δ5.18 d, J=9.6Hz, H-1).[7,8] Condensation of 5 with mono-N-Boc-ethylenediamine (in THF at room temperature for 20 hr) gave a thiourea derivative (6) (80%, syrup, FD-MS: M^+ m/z 549),[7] which was then alkylated with ethyl iodide (reflux in dry THF under Ar for 6 hr) to afford S-ethylisothiourea derivative (7). After evaporation of the solvent in vacuo, 7 was directly treated with trifluoroacetic acid (TFA) to cleave the Boc group (at room temperature for 15 min). TFA was removed in vacuo and the residue was dissolved in THF. Spontaneous cyclization occurred on neutralization with triethylamine to give a glycosyl guanidine derivative, in which the exocyclic nitrogen atom of a five membered cyclic guanidine derivative is bound to C-1 of 2-amino-2-deoxy-D-gulose moiety. It was deacetylated (reflux in 1N HCl for 3 hr) to give 2-amino-2-deoxy-β-D-gulopyranosyl (N',N''-ethylene)guanidine (8) (56% from 6; bis-4'-hydroxyazobenzenesulfonate: mp 208-210°C, $[\alpha]_D^{17}$ -16.0° (c 0.500, H_2O)).[7]

In order to apply the above procedure to the synthesis of 2, a suitably protected precursor of streptolidine moiety, i.e., 2,5-diamino-3-(Boc-amino)-2,3,5-trideoxy-4-O-methoxyethoxymethyl-D-arabino-δ-lactam (9), was next prepared through multi-step stereospecific conversion from D-xylose.[9] Thus, 1,2-di-O-benzoyl-3,5-di-O-tosyl-D-xylofuranose (10), obtained from known 1,2-O-isopropylidene-D-



Boc = $(CH_3)_3COC(=O)-$



xylofuranose,[10] was converted into benzyl glycoside (11) [i) dry HBr in CH_2Cl_2, ii) benzyl alcohol $Hg(CN)_2$ at room temperature for 15 hr; 67%, mp 101.5-103.5°C).[7] The two tosyloxy groups in 11 were then substituted with azido group (NaN_3 in DMF at 120°C for 5 hr) to give a diazide (12) (57%, syrup).[7] After removal of the benzoyl group on C-2 and subsequent mesylation [i) NaOMe, ii) mesyl chloride-pyridine at 0°C], the resultant 13 was subjected to hydrogenation (10% Pd-C in MeOH at 0°C for 5 hr), and the product was isolated as dibenzamido derivative (14) (81% from 13, mp 199-200°C dec).[7] It was then converted into γ-lactone (15) (65%, mp 198-199°C dec)[7] via hydrogenolytic cleavage of the benzyl glycoside (Pd-black in acetic acid at 8 atm) followed by oxidation of C-1 (CrO_3 in acetic acid at room temperature). Substitution of the 2-mesyloxy function, which is now adjacent to the carboxyl carbon atom, with azido group proceeded smoothly and afforded an azido lactone (16) (47%, mp 192.5-193°C dec),[7] which has the same regio- and stereochemistry of the substituents on C_5-unit as streptolidine.[11] Removal of the two benzoyl groups in 16 [i) $Et_3O \cdot BF_4 - K_2CO_3$ in CH_2Cl_2 – dioxane, ii) dil HCl in dioxane][12] followed by t-butoxycarbonylation under basic condition (BocCl in pyridine at -20°C) afforded 17 (44% from 15, syrup), which was characterized as a 3-O-benzyloxymethyl derivative (18) (mp 177-178°C)[7] or 3-O-methoxyethoxymethyl derivative (19).

After hydrogenation of the azido group in 19, the resulting 9 was coupled

with glycosyl isothiocyanate (5) (in THF at room temperature for 15 hr) to give a thiourea derivative (20), which was then treated as in the model experiment mentioned above [i) EtI in refluxing THF for 2 hr, ii) TFA at room temperature for 30 min, iii) triethylamine in THF at room temperature overnight, iv) reflux in 1N HCl for 3 hr] to afford 2 as hydrochloride (26% from 19, syrup) after purification with column chromatography on Sephadex G-25 (1-BuOH - pyridine - acetic acid - H_2O, 15:10:3:12). It was characterized as crystalline tris-4'-hydroxyazobenzene-sulfonate (mp 205-210°C(dec), $[\alpha]_D^{18}$ -10.0° (c 0.27, H_2O).[7] Synthetic 2 hydrochloride was fully identical in ^1H- and ^{13}C-NMR spectroscopy with the natural one obtained by partial hydrolysis of the antibiotic.

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References

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5) Benzyl 2-amino-2-deoxy-D-guloside was prepared by modification of the method of H.M.Noorzad and P.H.Gross, Carbohyd. Res., 31, 229 (1973).
6) Direct conversion of 2-acetamido-2-deoxy-D-gulose into 4 with acetyl chloride did not give satisfactory result.
7) Syrupy substance was purified with silica gel column chromatography. The corresponding compound gave reasonable NMR spectrum. Satisfactory elemental analyses were obtained for each crystalline compound.
8) Under these reaction conditions, only the desired glycosyl isothiocyanate (5), but no isomeric thiocyanate, was formed as confirmed in our previous model experiments with glucosamine derivatives; S.Imaoka, K.Shima, S.Kusumoto, and T.Shiba, presented at the 41st National Meeting of the Chemical Society of Japan, April 1980, Osaka (Abstract II, p.1112).
9) Our previous synthetic route for streptolidine was not applicable for preparation of a suitably protected precursor such as 9. Cf. S. Kusumoto, S. Tsuji, and T. Shiba, Bull. Chem. Soc. Jpn., 47, 2690 (1974).
10) B.R.Baker, and R.E.Schaub, J. Am. Chem. Soc., 77, 5902 (1955).
11) Hydrogenation of 16 followed by benzoylation afforded tribenzamidolactone which was identical with the authentic sample prepared in the previous work.[9]
12) Although hydrolytic cleavage of the lactone ring occurred to a considerable extent in the debenzoylation step, the hydroxy acid thus formed could easily converted into lactam form with diazomethane via methyl ester.

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