CHEMISTRY AND BIOCHEMISTRY OF THE NEOMYCINS. XVII

BIOCONVERSION OF AMINOCYCLITOLS TO AMINOCYCLITOL ANTIBIOTICS

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(Received for publication July 30, 1973)

Twenty-nine analogs of 2-deoxystreptamine have been tested to determine whether they are converted to antibiotics in 2-deoxystreptamine-negative mutants of *Streptomyces fradiae*, *S. rimosus* forma *paromomycinus* and *S. kanamyceticus*. Some analogs substituted at the 2-position of 2-deoxystreptamine undergo bioconversion to active antibiotics.

The 2-deoxystreptamine antibiotics constitute a large group of aminoglycoside antibiotics to which over 20 members have been assigned to date including the paromomycins, the kanamycins, the gentamicins, the nebramycins, ribostamycin, the lividomycins, validomycin and ambutyrosin.[1] We recently reported the development of a new method for preparing semisynthetic aminoglycoside antibiotics[2] using mutants incapable of producing a 2-deoxystreptamine antibiotic in the absence of added 2-deoxystreptamine.

In our application of this method to date we have isolated 2-deoxystreptamine negative (D^-) mutants of *Streptomyces fradiae*,[2] which in unmutated form produces the neomycins, of *S. rimosus* forma *paromomycinus*,[3a,b] which in unmutated form produces the paromomycins, and of *S. kanamyceticus*,[3a,b] which in unmutated form produces the kanamycins. Some of these mutants are capable of converting certain analogs of 2-deoxystreptamine into analogs of the antibiotics produced by the unmutated parent organism. Specifically, we have reported the bioconversion of streptamine to hybrimycins A1 and A2 by the D^- mutant of *S. fradiae*[2] and to hybrimycins C1 and C2 by the D^- mutant of *S. rimosus* forma *paromomycinus*.[3a,b] Also, 2-epistreptamine was bioconverted to hybrimycins B1 and B2 by the D^- mutant of *S. fradiae*.[2]

In order to explore the potential of these systems for the preparation of new semisynthetic antibiotics, we have prepared a large number of analogs of 2-deoxystreptamine and have tested them with the mutants for their ability to undergo bioconversion to active antibiotics. We report here our results with these analogs, shown in Fig. 1. Evaluation of these results suggests guidelines for subsequent synthesis of 2-deoxystreptamine analogs for testing in these systems.

Discussion

The aminocyclitols chosen for testing included representatives of several classes, those in which one primary amino group of deoxystreptamine had been replaced by a hydroxyl, those in which one or both of the primary amino groups had been altered, and those in which one or more of the hydroxyl groups had been modified.

Most of the compounds tested were prepared by methods already described in the chemical literature, a few were obtained from other investigators, and two (**12** and **20**) were prepared by methods developed in our laboratory and summarized in Figs. 2 and 3.

Fig. 1. Aminocyclitols and their derivatives tested in the present study

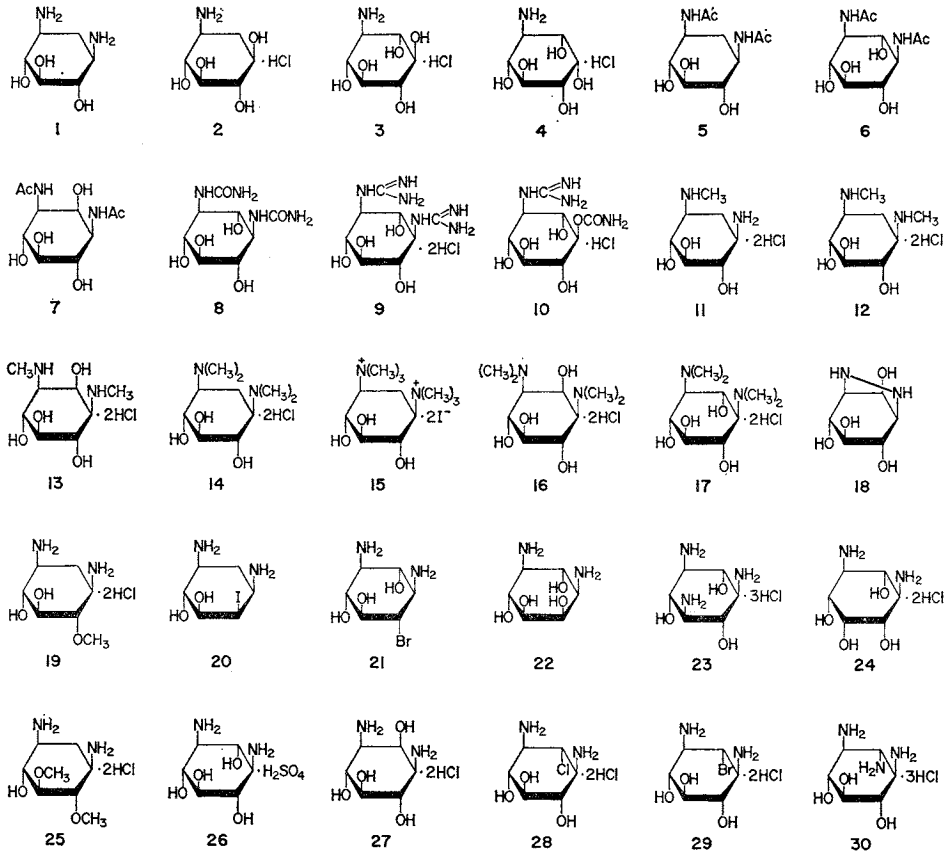
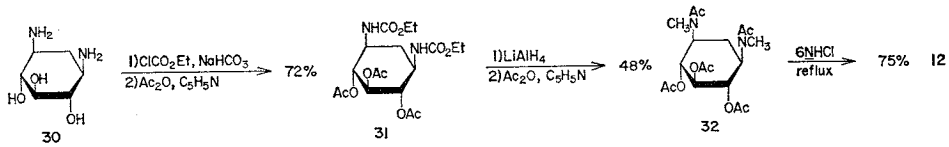


Fig. 2. Synthetic route to N, N′-dimethyl-2-deoxystreptamine (**12**) from 2-deoxystreptamine (**1**)



The results obtained from testing twenty-nine 2-deoxystreptamine analogs are presented in Table 1. Stimulation of bacterial antagonism in the mutant *Streptomyces* colony was scored as +, while no stimulation of bacterial antagonism was scored as —. A strong positive response was observed only for streptamine (**26**), with the D⁻ mutants of *S. fradiae* and *S. rimosus* forma *paromomycinus*, and 2-epistreptamine (**27**), with the D⁻ mutant of *S. fradiae*. 2-Epistreptamine (**26**) stimulates the production of very low levels of an antibacterial substance by the D⁻ mutant of *S. kanamyceticus*.

There are three possible explanations for the failure of any deoxystreptamine analog to be incorporated into an antibiotic—enzyme specificity, cell wall impermeability, and bioinactivity of an antibiotic's analog. No attempt was made to demonstrate that each of the analogs is capable of passing through the cell wall of the mutant organisms, nor were culture filtrates routinely examined for compounds produced which were structural analogs of the antibiotics but biologically inactive; thus, a number of questions remain unanswered. On the other hand, it was felt at the outset that only a limited range of modification of the 2-deoxystreptamine ring would go undetected by the

Fig. 3. Synthetic route to 4-iodo-4-epi-2, 4-dideoxystreptamine (**20**) from 1, 3-dibenzyloxycarbamido-4, 5-di-O-isopropylidene-1, 2, 3-trideoxy-*scyllo*-inositol (**33**).

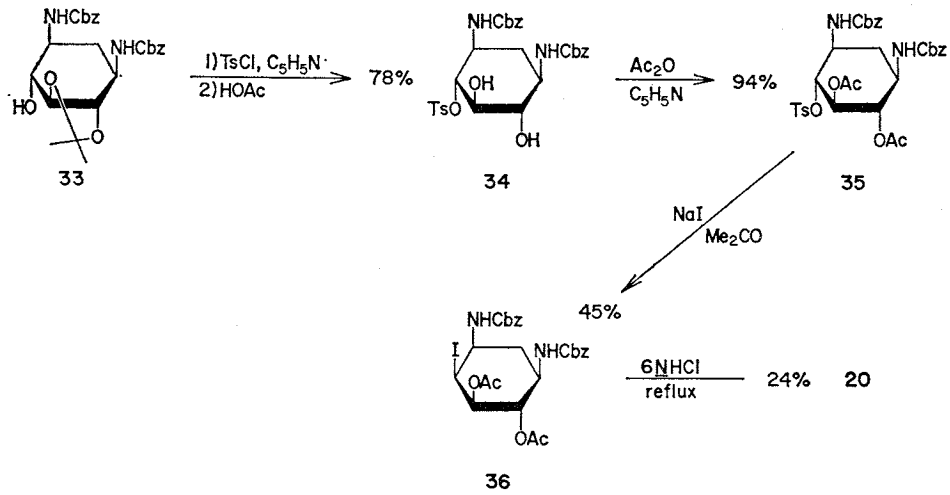


Table 1. Ability of analogs of 2-deoxystreptamine to stimulate antibiotic production by D⁻ mutants of *S. fradiae*, *S. rimosus* forma *paromomycinus*, and *S. kanamyceticus*

Analog[a]	Production of antibiotic by mutant of			Analog[a]	Production of antibiotic by mutant of		
	S. fradiae	*S. rimosus* forma *paromomycinus*	*S. kanamyceticus*		*S. fradiae*	*S. rimosus* forma *paromomycinus*	*S. kanamyceticus*
1	+	+	+	**16**	−	−	−
2	−	−	−	**17**	−	−	−
3	−	−	−	**18**	−	−	−
4	−	−	−	**19**	−	−	−
5	−	−	−	**20**	−	−	−
6	−	−	−	**21**	−	−	−
7	−	−	−	**22**	−	−	−
8	−	−	−	**23**	−	−	−
9	−	−	−	**24**	−	−	−
10	−	−	−	**25**	−	−	−
11	−	−	−	**26**	+	+	−
12	−	−	−	**27**	+	−	±
13	−	−	−	**28**	−	−	−
14	−	−	−	**29**	−	−	−
15	−	−	−	**30**	−	−	−

[a]Structures are found in Fig. 1.

enzyme(s) assembling the subunits of the antibiotics. In any event, analysis of the results of structural modifications can suggest guidelines for subsequent synthesis of new aminocyclitols more likely to be bioconverted into new semisynthetic antibiotics.

The twenty-nine 2-deoxystreptamine analogs tested can be divided into groups on the basis of their structural features which differ from 2-deoxystreptamine. In the following discussion it is assumed that a structural feature which allows bioconversion in one analog (*e.g.* the 2-hydroxyl group) does not prevent bioconversion of another analog bearing an additional modification of the 2-deoxystreptamine structure, but that a structural feature which prevents bioconversion of one

analog will prevent bioconversion of others.

Results from a number of aminocyclitols (**2** through **18**) indicate a rigid requirement for an unsubstituted 1, 3-diamino function. Compounds **2**~**4** represent modifications in which the number of amino groups has been reduced from two to one. As can be seen, no antibiotics were formed. This is probably due to an inability of the enzyme(s) to cope with a mono-amino compound, *i.e.*, due to a lack ef incorporation rather than to formation of an inactive compound. An especially good argument along these lines can be made for bluensamine (**3**), which was shown not to be converted by the D$^-$ mutants into any substance with the expected chromatographic properties, even though it is found in several antibiotics.[22] Compounds **5** through **18** all contain nitrogens at C-1 and C-3 but are substituted on one or both amino groups; no antibiotic was produced from these compounds. Since none underwent bioconversion to antibiotics, the precise effect causing the rejection could not be established. Some of the modifications should lead to inactive antibiotics. This is especially true of the N-acetyl analogs **5**~**7** since neomycin LP (mono-acetylneomycin) is inactive.[22,28] However, it seems nearly certain that if all the compounds (**5**~**18**) had been incorporated into analogs of neomycin, paromomycin or kanamycin some at least would have been antibiotics, since guanido, carbamate and N-methyl groups can be present in biologically active molecules. [Compound **9** (streptidine) is found in streptomycin, compound **10** (bluensidine) in bluensomycin, compound **11** in hygromycin, compound **13** in spectinomycin.]

Since most of the amino group modifications do not involve great changes in polarity, cell wall passage should not be affected. It seems more likely that these deoxystreptamine analogs are simply not incorporated due to rejection by the enzymes involved. In compounds **5** through **8** steric characteristics have been altered and the charge present on the amino groups under physiological conditions removed. Compounds **9** and **10** are more basic than deoxystreptamine and the steric characteristics of 2-deoxystreptamine have been altered as well. In compounds **11**~**14**, **16** and **17** methyl groups have been added onto the nitrogen atoms, altering the steric characteristics of the amino groups markedly but having only a small effect on the charge characteristics and the conformational mobility. Compound **15**, the diquaternary salt, has groups which are not only bulkier but also permanently charged. In compound **18** the ring is expected to be locked in a rigid conformation which differs greatly from that of 2-deoxystreptamine.

The free hydroxyl group on the deoxystreptamine ring of the antibiotic (the 4-hydroxyl in the neomycins and paromomycins, the 5-hydroxyl in the kanamycins) was an attractive site for molecular modification. Compounds **19** through **22** were employed to explore the possibility of using substitutions at the 4-position of 2-deoxystreptamine for modification of neomycin and paromomycin at this site, as well as for shutting the biosynthetic pathway to kanamycin off at an intermediate stage to yield paromamine or neamine analogs. Similarly, **23** and **24** were employed to test the possibility of producing analogs of neamine and paromamine with the D$^-$ mutants of *S. fradiae* and *S. rimosus* forma *paromomycinus*, respectively, and analogs of kanamycin in the D$^-$ mutant of *S. kanamyceticus*. Compound **25** was employed to test for the production of analogs of neamine and paromamine in each of the D$^-$ mutants. In this connection it should be noted that neamine and paromamine are antibiotics in their own right, albeit of lower activity than neomycin and paromomycin. Hence, had neamine or paromamine analogs been formed in appreciable quantities, they should have been detected, unless the variations made had destroyed the bioactivity. The negative results obtained with each of compounds **19** through **25** suggest a requirement for three free hydroxyl groups in the same configuration as found in 2-deoxystreptamine.

The results obtained with compounds **26** through **30** suggest that only limited substitution at the 2-position produces analogs capable of undergoing bioconversion to active antibiotics in these systems. The ineffectiveness of **28** and **29** may have resulted from the bulkiness of the added halogen, although there was some tlc evidence that **28** was converted to an inactive analog by the *S. fradiae* D$^-$ mutant. The addition of an amino group in the 2-position in **30** would be expected to alter dramatically the charge characteristics of the analog relative to 2-deoxystreptamine under physiological conditions.

Experimental

All melting points were determined on a Reichert hot stage microscope. Nmr spectra were measured by Mr. R. THRIFT and his associates on Varian A-60A or HA-100 spectrometers, employing tetramethylsilane as an internal standard. Thin layer chromatography was on silica gel GF_{254} (E. Merck AG, Darmstadt). Spots were detected with iodine vapor. Column chromatography was conducted on Brinkmann silica gel (0.05 to 0.2 mm) or Merck acid washed aluminum oxide.

Paper chromatography (descending) was performed on Whatman No. 1 or 3 MM paper using 1-butanol-pyridine-water, 6:4:3, (BPW 643); 1-butanol-acetic acid-water, 2:2:1, (BAW 221); or 1-butanol-acetic acid-water, 4:1:5, upper phase (BAW 415).

Elemental analyses were performed by Mr. J. NEMETH and associates, on samples dried *in vacuo* over phosphorus pentoxide.

Organisms

The D^- mutants of *S. fradiae* (ATCC 21401),[2] *S. rimosus* forma *paromomycinus* (ATCC 21485)[3] and *S. kanamyceticus* (ATCC 21480)[3] were those described previously. The test organism employed was a strain of *B. subtilis*, isolated at the University of Illinois and shown to be sensitive to all aminoglycosidet antibiotics tested.

Test system

The 2-deoxystreptamine analogs were tested in triplicate against each D^- mutant. A sample of 1~2 mg of an aminocyclitol to be tested for capability of bioconversion to an antibiotic was incorporated into 10 ml of nutrient agar (Baltimore Biological Laboratories) prior to sterilization and pouring into Petri plates. In cases where there was a possibility of degradation during autoclaving, the sample was dissolved in 0.5 ml of 50 % ethanol and this solution was used to flood a nutrient agar plate one day prior to use. Each plate was inoculated with a single streak of spores of a *Streptomyces* mutant, incubated at 26°C for three days and overlaid with 8 ml of melted Bacto streptomycin bioassay agar (Difco) seeded at 48° with a stationary phase culture of *Bacillus subtilis* grown in bioassay broth (Baltimore Biological Laboratories). The plates were stored at 0°C for 1 hour, and incubated at 26°C for 14 hours. Plates containing 2-deoxystreptamine and plates containing no added aminocyclitol were incubated as controls. Each plate was examined for a zone of inhibition around the *Streptomyces* colony.

Aminocyclitols

Samples of 1-amino-1, 2-dideoxy-*scyllo*-inositol hydrochloride[4] (**2**), 4-amino-4-deoxy-*myo*-inositol hydrochloride[5] (**4**), 1, 3, 5-triamino-1, 3, 5-trideoxy-*scyllo*-inositol trihydrochloride[6] (**23**) and 4, 6-diamino-4, 6-dideoxy-*myo*-inositol dihydrochloride[7] (**24**) were obtained from Prof. T. SUAMI, Keio University, Yokohama, as the fully acetylated derivatives. The acetyl groups were removed by heating the derivative in 6 N hydrochloric acid under reflux for 2 hours,[6] and the products were purified by preparative paper chromatography. These conditions give complete deacetylation without causing detectable epimerization of the aminocyclitols. Yields were 83 % of **2** and **23**, 89 % of **4**, and 93 % of **24**.

Bluensamine hydrochloride (**3**) and bluensidine hydrochloride (**10**) were prepared from bluensomycin sulfate (obtained from the Upjohn Co.), employing the procedure of BANNISTER and ARGOUDELIS:[8] **3**, 56 % yield, mp 186~192°C (lit.[8] mp 190~194°C); **10**, 48 % yield (from **3**), mp 312~315°C after darkening at 255~275°C (lit.[8] mp>300°C, darkens at 265°C).

N, N′-Diacetyl-2-deoxystreptamine (**5**) and N, N′-diacetylstreptamine (**6**), prepared in 74 % and 91 % yields, respectively, by acetylation of the free bases of 2-deoxystreptamine (**1**) and streptamine (**26**) with acetic anhydride in methanol gave infrared spectra identical with those of authentic samples of **5** and **6** prepared by Dr. R. F. SCHIMBOR in our laboratory.[24] N, N′-Diacetyl-2-epistreptamine (**7**) was prepared in 71 % yield by acetylation of 2-epistreptamine (**27**) using the method of PAN and DUTCHER:[9] mp 306~308°C (dec.) (lit.[10] mp 310~311°C, dec.). Strepturea (**8**), streptidine dihydrochloride (**9**) and streptamine dihydrosulfate (**26**) were prepared from dihydrostreptomycin (Lilly) by the method of PECK, *et al.*:[11] **8**, 32 % yield, from dihydrostreptomycin,

crystalline, darkened 270~275°, decomposed 290~295°C (lit.[11] darkens 270~275°C, decomposes 290~295°C); **9**, 61% yield, mp 176~200°C (lit.[11] mp 170~210°C); **26**, 31% yield, ir spectrum identical with that of an authentic sample obtained from Dr. H. E. CARTER, University of Illinois.

Hyosamine dihydrochloride (**11**) was prepared in 60% yield by degradation of hygromycin B (Lilly) by the method of WILEY, *et al.*:[12] mp 160~180°C dec. (lit.[12] mp 170~200°C dec.); hyosamine dipicrate, mp 248~251°C dec. (lit.[12] 244~250°C).

Actinamine dihydrochloride (**13**), prepared in 78% yield by degradation of spectinomycin sulfate (Upjohn) by the method of WILEY,[13] had an ir spectrum identical to that of an authentic sample.

N, N, N′, N′-Tetramethyl-2-deoxystreptamine dihydrochloride (**14**), N, N, N′, N′-tetramethyl-2-epistreptamine dihydrochloride (**16**) and N, N, N′, N′-tetramethylstreptamine dihydrochloride (**17**) were prepared from **1**, **27**, and **26**, respectively, by the method of WILEY, *et al.*,[12] except that the samples were isolated as picrate salts: **14**, 21% yield, mp 277~278°C, transition at 172~178°C (lit.[14] mp 277°C); **16**, 19% yield, mp 254~256°C dec. (lit.[12] mp 256~268°C dec.); **17**, 11% yield, mp 281~283°C, transition at 246~248°C (lit.[15] 275~276°C).

N, N, N′ N′-Tetramethyl-2-deoxystreptamine dimethiodide (**15**) was prepared in 69% yield (from **14**) by the method of DALEY, WITKOP, *et al.*;[14] mp 260~265°C (lit.[14] mp 270~273°C).

6, 7-Diazabicyclo[3.2.1]octane-2, 3, 4, 8-tetrol (**18**) and 2-epistreptamine dihydrochloride hemihydrate (**27**) were prepared by the method of SUAMI, *et al.*:[16] **18**, 51% yield, mp 185~195°C dec. (lit.[16] 188~198°C dec.); **27**, 97% yield (from **18**), mp 229~245°C dec. (lit.[16] 219~237°C dec.).

6-O-Methyl-2-deoxystreptamine dihydrochloride (**19**) was prepared by a method described previously in brief.[17] A solution of neomycin B (Upjohn, 7.5 g), dissolved in 100 ml of water, 30 ml of 3 M potassium monohydrogen phosphate solution, and 30 ml of acetic anhydride stood for 30 minutes at room temperature, then was evaporated under reduced pressure. The residue was dissolved in water, evaporated, suspended in ethanol, evaporated again under reduced pressure, extracted with 1% aqueous methanol, and evaporated. Water was added and the solution was deionized with Amberlite MB-3 resin, filtered, concentrated under reduced pressure, and lyophilized to yield 8.4 g (80%) of hexa-N-acetylneomycin B, $[\alpha]_D^{24.2}$ +49.2°C (*c* 0.01, water) [lit.[25] $[\alpha]_D^{25}$ +47.8°C (*c* 0.40, water)]. The material showed only one spot on paper chromatography in BPW 643.

A portion of the resulting hexa-N-acetylneomycin B (4.6 g), 10 g of barium oxide, and 400 mg of barium hydroxide octahydrate were stirred with 30 ml of reagent grade dimethylformamide and 10 ml of methyl iodide. After 10~20 minutes the temperature rose to about 60°C and remained above room temperature while the mixture was stirred (6 hours). The mixture was filtered and evaporated under reduced pressure to a thick syrup, which was diluted with water, acidified with 6 N sulfuric acid until no more barium sulfate precipitated, and neutralized with Dowex-1 ($CO_3^=$). The resin was washed thoroughly with water and the deionized solution was evaporated to a glass, which was dissolved in chloroform and triturated with ether to give 4.87 g of white powder. This powder was gradient eluted (250-ml fractions) with chloroform-methanal from a column containing 150 g of silica gel. The fractions eluted with chloroform - methanol (4:1) shown by tlc ($CHCl_3$-MeOH, 9:1) to contain the product, contaminated with a very small amount of partially methylated hexa-N-acetylneomycin B, were combined and evaporated to yield 2.34 g (46%) of per-O-methyl-hexa-N-acetylneomycin B.

Anal. Calcd. for $C_{23}H_{33}N_6O_6(COCH_3)_6(OCH_3)_7$: OCH_3, 22.51. Found: OCH_3, 20.50.

Per-O-methyl-hexa-N-acetylneomycin B (5.2 g) in 50 ml of 3 N hydrochloric acid was heated at 95°C for 6 hours, cooled, treated with charcoal, filtered, diluted to 300 ml with water, and passed down a column containing 100 ml of Dowex 50-WX8 (H^+). The column was gradient eluted from 0.5~2 N hydrochloric acid in 20-ml fractions. Fractions 64~79 (the first major peak by ninhydrin assay) were combined and evaporated to yield 0.586 g (44%) of **19**. Paper chromatography in BPW 643 showed a major spot plus a trace of 2-deoxystreptamine. A sample of this material was further purified by preparative paper chromatography for testing for bioconversion capability. 6-O-Methyl-

2-deoxystreptamine dihydrochloride (**19**) could be crystallized from aqueous ethanol; $[\alpha]_D^{26}$ −15° (*c* 1.0, H_2O), R_f 0.25 (BAW 221). It was characterized as its N-acetyl derivative, prepared by treatment of 100 mg with Dowex 1 ($CO_3^=$), lyophilization, and acetylation with acetic anhydride in methanol. N, N′-Diacetyl-6-O-methyldeoxystreptamine crystallized with difficulty from methanol: yield 61 mg (58%). It sublimed above 225°C to hair-like crystals which melted 282~284°C (dec.); $[\alpha]_D^{27}$ +12° (*c* 1.0, H_2O); R_f 0.43 (BAW 415). [Lit.[27] mp 280~282°C, $[\alpha]_D^{27}$ +15° (*c* 1.0, H_2O).]

5, 6-Di-O-methyl-2-deoxystreptamine dihydrochloride (**25**) was prepared by the same procedure as **19**. When neamine (free base, 8.3 g), suspended in 150 ml of absolute methanol at 0~5°C, was treated with 10.5 ml of acetic anhydride, the solid dissolved. After 15 minutes the mixture was allowed to warm to room temperature; crystals slowly separated. Recrystallization from hot methanol-acetone yielded 4.67 g (37%) of tetra-N-acetylneamine, $[\alpha]_D^{25}$ +89.5° (*c* 2, water), mp 323°C [lit.[28] $[\alpha]_D^{25}$ +87° (*c* 1.0, water), mp 334~336°C.]

Tetra-N-acetylneamine (2.3 g) was O-methylated with 5.0 g of barium oxide, 200 mg of barium hydroxide octahydrate, and 5 ml of methyl iodide in 15 ml of dimethylformamide according to the procedure described above for the O-methylation of hexa-N-acetylneomycin B. Purification by column chromatography on silica gel yielded fractions containing 2.36 g (92%) of material judged by tlc ($CHCl_3$-MeOH, 9:1) to be per-O-methyl-tetra-N-acetylneamine.

Anal. Calcd. for $C_{12}H_{18}N_4O_2(OCH_3)_4(COCH_3)_4$: C, 52.75; H, 7.74; OCH_3, 22.71,
Found: C, 52.59; H, 8.00; OCH_3, 22.20.

A solution of per-O-methyl-tetra-N-acetylneamine (1.4 g) in 50 ml of 3 N hydrochloric acid was heated at 75°C for 6 hours, then worked up as described above for the preparation of **19**. Ion-exchange column fractions 55~78 were combined and evaporated to yield material that showed three spots on paper chromatography in BAW 415 after acetylation by the phosphate microacetylation method. Crystallization from aqueous ethanol yielded 151 mg (22%) of 5, 6-di-O-methyl-2-deoxystreptamine dihydrochloride (**25**), $[\alpha]_D^{26}$ −11.6° (*c* 1.7, H_2O). The product was neutralized with Dowex 1 ($CO_3^=$) and acetylated with acetic anhydride-methanol. N, N′-Diacetyl-5, 6-di-O-methyldeoxystreptamine was crystallized from ethanol to give colorless needles which sublimed above 260°C without melting; $[\alpha]_D^{26}$ +6.2° (*c* 0.3, H_2O), R_f 0.61 (BAW 415). (Lit.[28] $[\alpha]_D$ +2° (H_2O), R_f 0.62 (BAW 415).]

Anal. Calcd. for $C_6H_{10}N_2O(OCH_3)_2(COCH_3)_2$: C, 52.57; H, 8.05; N, 10.20; OCH_3, 22.62.
Found: C, 52.70; H, 8.24; N, 10.70; OCH_3, 22.60.

2-Bromo-2-deoxystreptamine dihydrochloride (**29**) was obtained in 27% yield (from **27**) by hydrolyzing, as described above for the preparation of **2**, the fully acetylated derivative prepared by the method of SUAMI, *et al.*,[18] [mp 245~247°C (lit.[18] 252~255°C)].

1, 2, 3-Triamino-1, 2, 3-trideoxy-*scyllo*-inositol trihydrochloride (**30**) was prepared in 77% yield from a sample of 4, 5, 6-tri-O-acetyl-1, 3-diacetamido-2-nitro-1, 2, 3-trideoxy-*scyllo*-inositol, obtained from Prof. H. H. BAER, University of Ottawa, by hydrogenation over ADAMS catalyst followed by hydrolysis in 6 N hydrochloric acid and purification by preparative paper chromatography in the solvent system BPW 643. The stereochemistry of the 2-nitro group has not been definitely established, but is presumed to be as shown in Fig. 1.

The preparation of 4-bromo-4-deoxystreptamine hydrosulfate (**21**) and 4-epistreptamine (**22**) has been described elsewhere.[19]

2-Chloro-2-deoxystreptamine dihydrochloride (**28**) was prepared by the procedure used for the synthesis of 2-bromo-2-deoxystreptamine.[18] 2-Epistreptamine dihydrochloride (**27**, 155 mg) and acetyl chloride (2 ml) were heated in a sealed tube for 12 hours at 140~145°C. The tube was cooled, then opened carefully, and the excess acetyl chloride was removed by decantation. The crude crystals were washed thoroughly with ether; yield, 249 mg (99%), mp 230~240°C. The analytical sample of pentaacetyl 2-chloro-2-deoxystreptamine was obtained by recrystallization from ethanol: 174 mg (69%); mp 266.5~268.5°C; nmr (DMSO-d_6) δ 1.94 (s, 9H), 1.80 (s, 6H).

Anal. Calcd. for $C_{16}H_{23}ClN_2O_8$: C, 47.23; H, 5.65; N, 6.89.
Found: C, 47.38; H, 5.76; N, 7.12.

The pentaacetyl derivative (79.5 mg) was heated at reflux with 6 N hydrochloric acid (10 ml) for 90 minutes, then the solution was evaporated under reduced pressure to give a crystalline residue, which was recrystallized from ethanol and water, to give fine plates (50.8 mg, 96%), sintering at 179~188°C and decomposing at 220~222°C,

Anal. Calcd. for $C_6H_{13}ClN_2O_3 \cdot 2HCl$: C, 26.73; H, 5.56; N, 10.39; Cl, 39.46.
Found: C, 26.73; H, 5.82; N, 10.73; Cl, 39.13.

N, N'-Dimethyl-2-deoxystreptamine (**12**) was prepared from 2-deoxystreptamine (**1**), as shown in Fig. 2, by a procedure analogous to that employed by OGAWA, SUAMI, *et al.*, in a synthesis of actinamine (**13**).[10] A solution of **1** (1.0 g), prepared from neomycin (Upjohn) by the method of DYER,[20] and 2.1 g of sodium bicarbonate in 20 ml of water was stirred, and 0.97 ml ofe thyl chloroformate was added dropwise under cooling. The mixture was maintained at approximately pH 9 by adding sodium hydroxide solution. The reaction mixture was allowed to stand at room temperature overnight, then evaporated to dryness under reduced pressure. The residue was treated with 30 ml of acetic anhydride in 30 ml of anhydrous pyridine for 24 hours at room temperature. The insoluble material was removed by filtration, the filtrate was evaporated under reduced pressure, and the residue was treated with 10 ml of acetic anhydride in 10 ml of pyridine at room temperature overnight. The excess acetylating reagent was evaporated under reduced pressure, and the residue was crystallized from ethanol to yield 0.96 g (72%) of 4, 5, 6-triacetyl-1, 3-diethoxycarbamido-1, 2, 3-trideoxy-*scyllo*-inositol (**31**), mp 243~245°C; nmr ($CDCl_3$) δ 4.10 (m, 4, J=7 Hz, $-OCH_2CH_3$), 2.04 (s, 6), 1.98 (s, 3) and 1.23 (t, 6, J=7 Hz, $-CH_2CH_3$).

Anal. Calcd. for $C_{15}H_{20}N_2O_{10}$: C, 50.00; H, 6.53.
Found: C, 49.89; H, 6.50.

A slurry of 4.0 g of lithium aluminum hydride in 100 ml of anhydrous tetrahydrofuran was added dropwise to a cooled, stirred solution of 542 mg of **31** in 60 ml of anhydrous tetrahydrofuran. After addition was complete the mixture was cooled, excess lithium aluminum hydride was destroyed by dropwise addition of acetone, and the mixture was poured into 250 ml of water and ice. The mixture was allowed to stand at room temperature for 24 hours, the precipitate was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was acetylated with 30 ml of acetic anhydride in 30 ml of pyridine at 100°C for 3 hours. The mixture was cooled and filtered, and the filtrate was evaporated under reduced pressure to yield an oily product, which was dissolved in chloroform and applied to a column of 20 g of alumina. The column was eluted with 300 ml of chloroform, the eluate was evaporated, and the residue was dried *in vacuo* and crystallized from ethanol to yield 243 mg (48%) of 4, 5, 6-tri-O-acetyl-1, 3-di-(N-methylacetamido)-1, 2, 3-trideoxy-*scyllo*-inositol (**32**): mp 261~263° with transition at 247°; nmr (DMSO-d_6) δ 2.83 (s, 3), 2.65(s, 3), 2.07(s, 3), 1.93(s, 12).

Anal. Calcd. for $C_{18}H_{28}N_2O_8$: C, 53.99; H, 7.05.
Found: C, 53.92; H, 7.05.

A suspension of **32** (200 mg) in 6 N hydrochloric acid (40 ml) was heated under reflux for 6 hours, then cooled and evaporated under reduced pressure. The residue was dried *in vacuo* over potassium hydroxide pellets aud crystallized from aqueous ethanol to yield 99 mg (75%) of **12**: plates, decomp. point 260~265°C; nmr (D_2O) δ 2.84 (s, 6).

Anal. Calcd. for $C_8H_{18}N_2O_3 \cdot HCl$: C, 36.51; H, 7.66.
Found: C, 36.38; H, 7.58.

4-Iodo-4-*epi*-2, 4-dideoxystreptamine (**20**) was prepared from racemic 1, 3-dibenzyloxycarbamido-4, 5-di-O-isopropylidene-1, 2, 3-trideoxy-*scyllo*-inositol (**33**), prepared by the method of UMEZAWA,

et al.[21] A solution of **33** (600 mg) and *p*-toluenesulfonyl chloride (300 mg) in anhydrous pyridine (10 ml) was allowed to stand at room temperature for 5 days. The mixture was poured into water and extracted with chloroform, and the extracts were washed with dilute hydrochloric acid, then with water, and evaporated under reduced pressure. The residue was dissolved in 100 ml of 80% acetic acid, the solution was stirred for 1 hour at room temperature and filtered, the filtrate was evaporated under reduced pressure, and the residue was dried *in vacuo*. The residue was crystallized from absolute ethanol to yield 584 mg (78%) of rods: mp 194.5~196°C. Recrystallization from the same solvent gave an analytical sample of racemic 1, 3-dibenzyloxycarbamido-4-*p*-toluenesulfonyl-1, 2, 3-trideoxy-*scyllo*-inositol (**34**): mp 195.5~197°C; nmr (DMSO-d_6) δ 7.75 (*d*, 2, J_{ortho}=8 Hz), 7.33 (m, 12), 2.34 (s, 3).

Anal. Calcd. for $C_{29}H_{32}N_2O_8S$: C, 59.57; H, 5.52.
Found: C, 59.72; H, 5.46.

A mixture of **34** (350 mg), acetic anhydride (5 ml), and anhydrous pyridine (5 ml) stood at room temperature overnight, then was evaporated under reduced pressure. The residue was dried *in vacuo* and crystallized from ethanol to yield 379 mg (94%) of fine needles, mp 182~183°C. Recrystallization from the same solvent gave an analytical sample of racemic 5, 6-di-O-acetyl-1, 3-dibenzyloxycarbamido-4-O-*p*-toluenesulfonyl-1, 2, 3-trideoxy-*scyllo*-inositol (**35**): mp 188~189°C; nmr (DMSO-d_6) δ 7.67 (d, 2, J_{ortho}=8 Hz), 7.33 (m, 12), 2.33 (s, 3), 1.84 (s, 3), 1.82 (s, 3)

Anal. Cald. for $C_{33}H_{36}N_2O_{11}S$: C, 59.37; H, 5.47.
Found: C, 59.60; H, 5.57.

A mixture of **35** (1.2 g), sodium iodide (4.2 g), and acetone dried over magnesium sulfate (25 ml) was heated at 110°C for 2.5 hours in a sealed tube, cooled and filtered. The filtrate was evaporated, and the residue was partitioned between water and chloroform. The water layer was washed twice with chloroform, and the combined chloroform extracts were washed with water, sodium thiosulfate solution, and twice with water. The chloroform phase was mixed with benzene and evaporated to dryness under reduced pressure. The residue was treated briefly with 25 ml of acetic anhydride in 25 ml of pyridine at room temperature. The excess acetylating reagent was evaporated, and the residue was dried *in vacuo* and crystallized from ethanol to yield 503 mg (45%) of colorless microcrystals of racemic 5, 6-di-O-acetyl-1, 3-dibenzyloxycarbamido-4-iodo-1, 2, 3, 4-tetradeoxy-*epi*-inositol (**36**): mp 185~186°C; nmr (DMSO-d_6) δ 7.31 (s, 10), 5.05 (s, 2), 5.00 (s, 2), 1.97 (s, 3), 1.80 (s, 3).

Anal. Calcd. for $C_{26}H_{29}IN_2O_8$: C, 50.01; H, 4.68.
Found: C, 49.91; H, 4.62.

Compound **36** was hydrolyzed with 6 N hydrochloric acid, as described above for the preparation of **2**, to give an impure sample of **20** in 24% yield. The glassy compound could not be crystallized and was somewhat unstable.

Anal. Calcd. for $C_6H_{13}IN_2O_2$: C, 26.48; H, 4.82.
Found: C, 29.33; H, 5.95.

Acknowledgements

This work was supported in part by NIH research grant number AI 01278 from the National Institute of Allergy and Infectious Diseases.

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