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AMINOGLYCOSIDE ANTIBIOTICS: SYNTHESIS OF NEBRAMINE, TOBRAMYCIN AND 4"-EPI-TOBRAMYCIN

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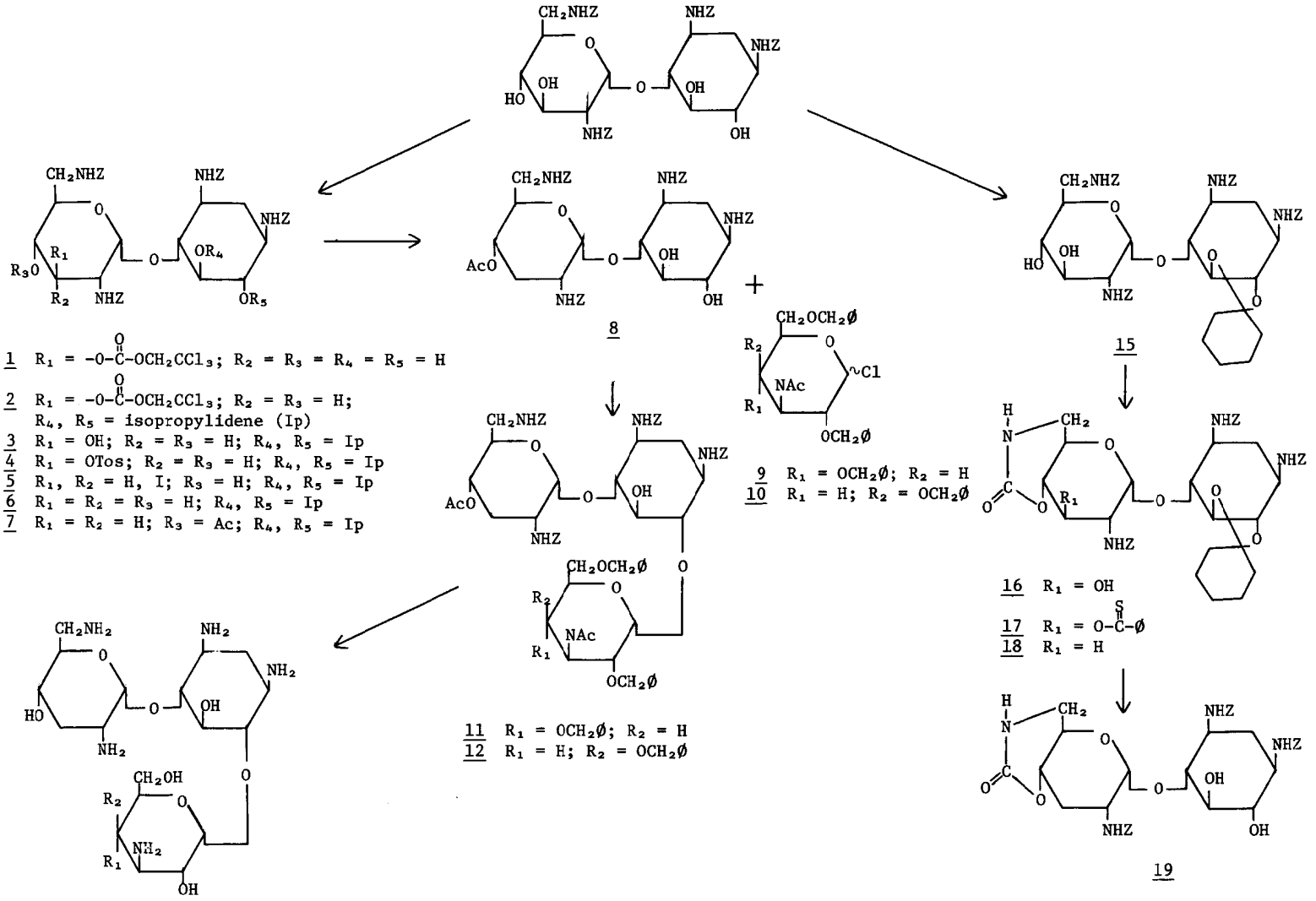
In a study aimed at the partial synthesis of aminoglycoside antibiotics[1] showing an improved spectrum of activity, we have devised practical syntheses of several key intermediates that can be converted to a variety of semi-synthetic aminoglycosides. As an example of this study we have synthesized tobramycin as well as its unnatural 4" epimer. Tobramycin is a naturally occurring aminoglycoside with useful clinical applications.[2]

Our approach starts with the pseudodisaccharide neamine which is easily obtained from naturally occurring aminoglycosides. In our study, two new methods have been developed for the efficient conversion of neamine to the important key intermediate pseudodisaccharide nebramine[3] (3'-deoxyneamine). Nebramine is converted to tobramycin and its analogs by 6α-glycosylation with an appropriate sugar. Hitherto, nebramine has only been available by selective hydrolysis of tobramycin.

In the first method, tetracarbobenzyloxyneamine[4] was selectively esterified at 3' with trichloroethoxycarbonyl chloride (5 equiv.) in pyridine at -5° for 20 hr. Chromatography on silica gel afforded 55% of the 3'-trichloroethyl carbonate 1, m.p. 165-168°; $\alpha]_D$ 33° ($CHCl_3$). This step is the key to this route since it allows for selective reactions at the remaining 5,6 and 4' hydroxyl groups depending upon the reagent used. For instance, subsequent treatment of the monoester 1 with 2,2-dimethoxypropane in DMF with pTSA at 130° gave the 5,6 isopropylidene



1 $R_1 = -O-\overset{O}{\overset{\|}{C}}-OCH_2CCl_3$; $R_2 = R_3 = R_4 = R_5 = H$
2 $R_1 = -O-\overset{O}{\overset{\|}{C}}-OCH_2CCl_3$; $R_2 = R_3 = H$; R_4, R_5 = isopropylidene (Ip)
3 $R_1 = OH$; $R_2 = R_3 = H$; $R_4, R_5 = Ip$
4 $R_1 = OTos$; $R_2 = R_3 = H$; $R_4, R_5 = Ip$
5 $R_1, R_2 = H, I$; $R_3 = H$; $R_4, R_5 = Ip$
6 $R_1 = R_2 = R_3 = H$; $R_4, R_5 = Ip$
7 $R_1 = R_2 = H$; $R_3 = Ac$; $R_4, R_5 = Ip$

8

9 $R_1 = OCH_2\emptyset$; $R_2 = H$
10 $R_1 = H$; $R_2 = OCH_2\emptyset$

11 $R_1 = OCH_2\emptyset$; $R_2 = H$
12 $R_1 = H$; $R_2 = OCH_2\emptyset$

13 $R_1 = OH$; $R_2 = H$; tobramycin
14 $R_1 = H$; $R_2 = OH$; 4''-epitobramicin

15

16 $R_1 = OH$
17 $R_1 = O-\overset{S}{\overset{\|}{C}}-\emptyset$
18 $R_1 = H$

19

intermediate 2 (95%) from which the trichloroethyl carbonate function was selectively removed with ammonia in methanol to afford the isopropylidene derivative 3. This selective 3' esterification method thus affords an improved route to 3, previously prepared by an alternative method,[4] that resulted in a difficultly separable mixture of 5,6 and 3',4' monoisopropylidene derivatives.

Transformation of 3 to a nebramine derivative followed an analogous route described by Umezawa for the conversion of kanamycin B to tobramycin.[5] Selective 3' tosylation of 3 with tosyl chloride in pyridine yielded 3'-tosylate 4, m.p. 99-103°, which was converted to the iodo derivative 5 (65%), m.p. 96-100°C, with sodium iodide in dry DMF at 95-100° for 20 hr. Hydrogenolysis of 5 with Raney nickel in dioxane afforded the 3'-deoxy derivative 6, m.p. 185-190°. Acetylation of 6 gave the 4' acetate 7, m.p. 90-95°. Acetonide hydrolysis with aqueous acetic acid gave the desired selectively blocked 4'-O-acetyl nebramine derivative 8, m.p. 177-180°, which was required for selective 6α-glycosylation reactions to give tobramycin and its analogs.

A second method, the deoxygenation of a 3'-thionobenzoate derivative of neamine to a nebramine derivative, followed the general method developed by Barton and McCombie for the synthesis of deoxy sugar.[6] This deoxygenation procedure gave a higher overall yield (45%) of the nebramine derivative 19, thus being the method of choice for the preparation of nebramine. The known 5,6-cyclohexylidene derivative of tetracarbobenzyloxy neamine[7] 15 was treated with NaH in DMF to give the 4',6'-cyclic carbamate derivative 16. Treatment of 16 with the imidoyl chloride methochloride of N,N-dimethylbenzamide in methylene chloride and THF, followed by in situ treatment with hydrogen sulfide-triethylamine generated the 3'-thionobenzoate 17. Reduction of the thionobenzoate with tributyltin hydride proceeded smoothly in refluxing toluene affording the 3'-deoxy derivative 18, which on hydrolysis with 80% acetic acid gave the desired nebramine derivative 19.

To complete the synthesis of tobramycin and its congeners from the pseudodisaccharide 8, we took advantage of the well established use of pyranosyl halide derivatives with a nonparticipating group at C-2 such as a benzyl ether to insure stereoselective formation of α-anomers.[8] Thus 8 was condensed with the pyranosyl chloride 9[9] in the presence of mercuric cyanide and calcium sulfate in dioxane and toluene (1:2). The resulting mixture was sequentially deblocked, initially with ammonia in methanol to remove the ester function, followed by reductive removal of the N-carbobenzoxy and benzyl blocking groups with sodium and liquid ammonia. The ammonium hydroxide fractions from ion exchange chromatography on Biorex 70-H^+ were deacetylated by brief

treatment with boiling sodium hydroxide in 1:1-dioxane-water to afford a mixture, that consisted chiefly of tobramycin, although minor amounts of the 6β and C-5 isomer were also detected.[10] Silica gel chromatography afforded tobramycin 13 identical with a natural sample in physical properties as well as antibacterial activity.

Similar treatment of 8 with the galactopyranosyl halide 10[11] ultimately gave the 4"-epimer of tobramycin 14, $\alpha]_D$ 78° (H_2O).

Other analogs (e.g. the 3-amino xylo derivative) of tobramycin were prepared by C-6 glycosylation[12] of the nebramine derivative 19.

It is of interest to note that 4"-epi-tobramycin exhibits a similar antibacterial spectrum and potency to that of tobramycin.

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10. We have observed with neamine and gentamine derivatives that when both the C-5 and C-6 hydroxyl groups are available for glycosysation reaction occurs predominantly at C-6.
11. U.S. Patent 3,985,727 (Oct. 12, 1976), P.J.L. Daniels (to Schering-Plough Co.). We thank Dr. P.J.L. Daniels and Mr. C. E. Luce for providing us with this galactopyranoside intermediate.
12. Unpublished results.

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