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Synthesis and Mutasynthesis of Pseudosaccharides Related to Aminocyclitol-Glycoside Antibiotics

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The aminocyclitol glycosides enumerated in Table 1, are produced mainly by Streptomyces species but also by Micromonospora, Bacillus and even Pseudomonas species (1, 2, 3). They constitute a very important class of clinically used antibiotics and provide a cover for the pathogens most commonly found in the hospital environment. To varying degrees all the amino-glycosides are toxic (oto and nephrotoxicity) and therefore their administration is strictly controlled.

It was first noticed in 1965 (4) that some pathogenic bacteria became resistant to these antibiotics, and in subsequent years, it was shown that the major resistance mechanism was an R-factor mediated enzymatic inactivation, resulting in O-phosphorylation, O-nucleotidylation or N-acetylation of the antibiotics at different positions (5, 6, 7). The discovery and explanation of these enzymatic inactivations led to an increased effort to find from natural sources (through soil screening programs) and through chemical modification procedures new products effective against resistant organisms.

Chemical modification of already existing naturally occuring antibiotics, either by removal of certain functional groups subject to inactivating enzymes or by substitution (acylation or alkylation) of the 1-amino group of the 2-deoxystreptamine moiety, led to semi-

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TABLE 1 MAJOR DISCOVERIES OF AMINOGLYCOSIDE ANTIBIOTICS (1944-1977)

YEAR	ANTIBIOTIC	PRODUCING ORGANISM
1944	STREPTOMYCIN	S. GRISEUS
1949	NEOMYCIN	S. FRADIAE
1957	KANAMYCIN	S. KANAMYCETICUS
1959	PAROMOMYCIN	S. RIMOSUS F. PAROMOMYCINUS
1961	SPECTINOMYCIN	S. SPECTABILIS
1963	GENTAMICIN C	M. PURPUREA
1965	KASUGAMYCIN	S. KASUGAENSIS
1968	TOBRAMYCIN	S. TENEBRARIUS
1970	RIBOSTAMYCIN	S. RIBOSIDIFICUS
1970	SISOMICIN	M. INYOENSIS
1971	LIVIDOMYCIN	S. LIVIDUS
1971	BUTIROSIN	B. CIRCULANS
1973	APRAMYCIN	S. TENEBRARIUS
1974	MINOSAMINOMYCIN	ACTINOMYCES SP
1975-77	SELDOMYCIN	S. HOFUENSIS
1976	SORBISTIN	P. SORBICINII
1977	FORTIMICIN	M. OLIVOASTEROSPORA

M = Micromonospora ; S = Streptomyces ; B = Bacillus ; P = Pseudomonas.

synthetic derivatives [dibekacin (8), amikacin (9), netilmicin (10), UK 18892 (11) and Sch 21420 (12), Fig. 1], which were active against aminoglycoside resistant bacteria.

In our laboratory we have pursued two different but complementary approaches : mutasynthesis and total chemical synthesis and we will report here our efforts to produce pseudosaccharides related to aminoglycoside antibiotics.

Let us first examine the structural features of the pseudodisaccharide moiety of the aminoglycosides which exhibits antibacterial activity. Except in fortimicin B, in which the aglycone is a novel 1,4-diaminocyclitol named fortamine, the other pseudodisaccharides presented in Figure 2, contain 2-deoxystreptamine which is assymetrically α-glycosylated at position 4 by a variety of aminohexopyranosides which differ from each other by the presence or absence of amino, hydroxyl and double bond functions.

The prerequisite for structure-activity relationship studies is the readily availability of cyclitols or amino-cyclitols and their α-glycosides. They might be obtained by either mutasynthesis or total chemical synthesis.

Meso 2-deoxystreptamine and meso 2,5-dideoxystreptamine can be obtained by hydrolysis of natural antibiotics or by chemical synthesis respectively (13, 14, 15) but they were considered unsuitable for practical chiral synthesis. Quinic acid, on the other hand, possesses functional groups and an absolute configuration amenable to our coveted goals and therefore was chosen as starting material.

In this article we deal briefly with the preparation of 2,6-di- and 2,5,6-trideoxystreptamines and 3,5-

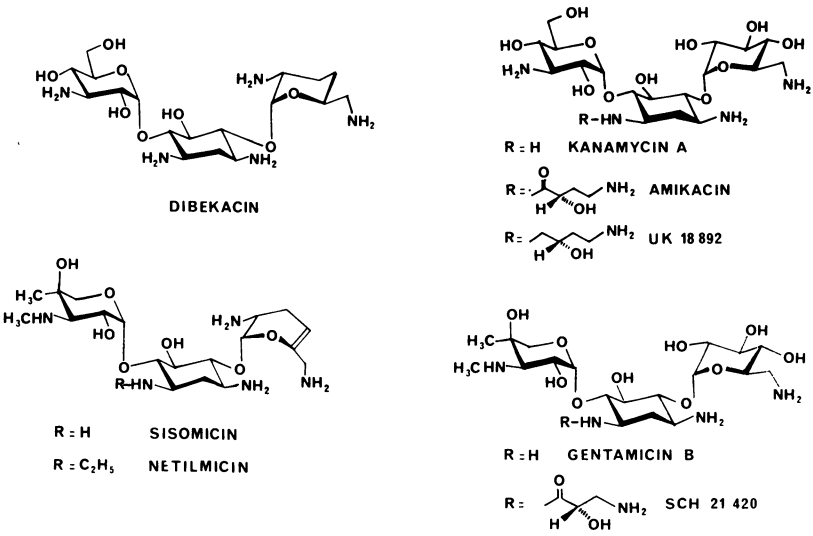


Figure 1.

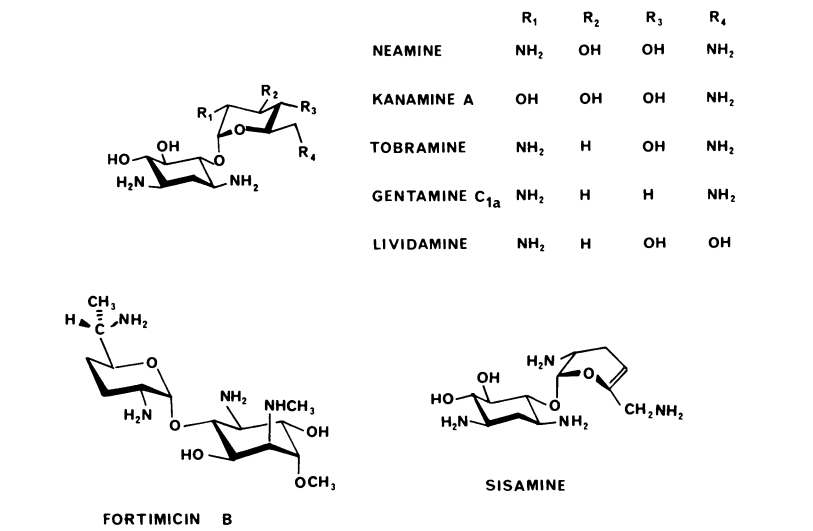


Figure 2.

dideoxyfortamine and an acid catalysed α-glycosylation procedure leading to hitherto unkown pseudodisaccharides.

SYNTHESIS OF CYCLITOL AND AMINOCYCLITOL DERIVATIVES FROM QUINIC ACID

- Preparation of D-2,6-dideoxystreptamine 6 and D-2,5,6-trideoxystreptamine 10.

In view of the importance of preparing analogs of 4-substituted or 4,5-disubstituted 2-deoxystreptamine antibiotics, it seemed attractive to prepare chiral 2,6-dideoxystreptamine, 2,5,6-trideoxystreptamine and their precursors for use in microbial transformation or for total synthesis.

Quinic acid 1 was readily transformed as we described recently (16) in excellent overall yield, to the 3,4-O-cyclohexylidene 3,4/5-trihydroxycyclohexanone 2. The latter was quantitatively reduced with lithium borohydride to a mixture of two epimeric diols 3 and 4. The trans diol (4), on acidic hydrolysis followed by selective tosylation, gave the ditosylate 5, which was transformed by azidolysis followed by hydrogenation using Adam's catalyst into 2,6-dideoxystreptamine 6 (17) (Fig. 3).

The 2,5,6-trideoxystreptamine 10 was also obtained from the ketone 2 in the following way : treatment of the ketone 2 with p-toluenesulfonyl chloride in pyridine gave the α,β unsaturated ketone 7 which was catalytically reduced to the saturated ketone 8. The latter in turn, was regiospecifically converted to a cyclohexanetriol derivative which after deprotection and selective tosylation furnished the ditosylate 9. From 9, the re-

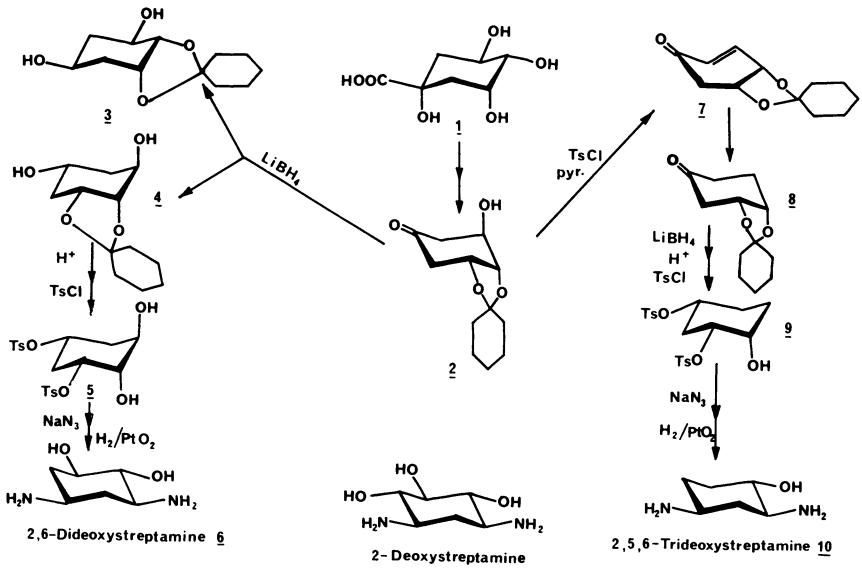


Figure 3.

quired 2,5,6-trideoxystreptamine 10 was obtained by treatment with sodium azide followed by Adam's catalyst reduction of the diazide formed.

A priori, the mode of synthesis of 6 and 10 outlined on Fig. 3, from quinic acid 1, should lead to chiral products possessing the same absolute configuration as the 2-deoxystreptamine aglycone in the natural products. Indeed these compounds exhibited optical rotations and their ^{13}C NMR data were consistent with the structures proposed. Racemic 2,6-dideoxy-streptamine and 2,5,6-trideoxystreptamine have been reported (18).

- Synthesis of 3,5-dideoxyfortamine

Recently, a completely new type of aminocyclitol glycoside antibiotic, fortimicins A and B, have been isolated from Micromonospora species (19). These pseudodisaccharides contain the 6-epi-purpurosamine subunit which is α-linked to the hitherto unknown chiral aglycon fortamine. The isolation of this antibiotic complex is a major event in the development of novel aminoglycoside antibiotics because it contains the chiro-1,4-diaminocyclohexanetetrol and not the usually encountered 1,3-diaminocyclohexanetriol (2-deoxystreptamine). Additionnally, they are comparatively simple molecules being pseudodisaccharides and therefore within easy reach for the synthetic chemist.

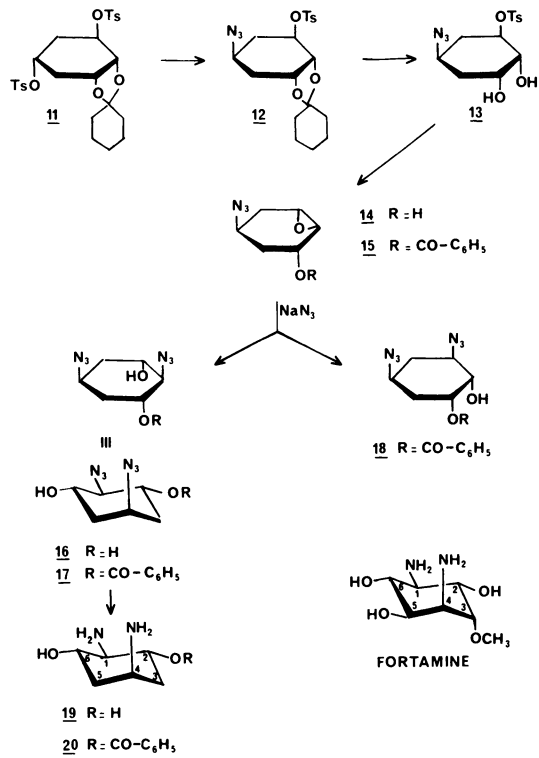
We were interested in 1,4-diaminocyclohexanols for our mutasynthetic and total chemically synthetic studies. The synthesis of the meso and chiral fortamine derivatives utilized ditosyl O-cyclohexylidene cyclohexanetetrol 11 derived also from quinic acid (1) as previously described (16). A very brief exposure of the ditosylate 11 to sodium azide in dimethylformamide gave the monoazide 12. Hydrolysis removed the cyclohexylidene

group and treatment of the diol 13 with sodium methoxide in methanol furnished the epoxide derivative 14 which was readily converted to its benzoate 15 (Fig. 4).

Azidolysis of 14 afforded the meso 1,4-diazido derivative 16 in high yield (85 %) whereas similar treatment of its benzoate 15 gave a mixture of 1,4 and 1,3 diazido compounds 17 and 18 in a ratio of 4:6, respectively. Reduction of the diazides 16 and 17, by the usual method yielded the meso (19) and chiral (20) 3,5-dideoxyfortamine derivatives as was evident from their optical rotation and ^{13}C NMR data. The ^{13}C NMR spectra of the meso 19 and the chiral 20 compounds exhibited four and six signals respectively.

MUTASYNTHESIS

Having in hand this variety of aminocyclitols, we attempted to incorporate them into novel antibiotics using idiotrophs of antibiotic-producing strains (Fig. 5). Using the mutasynthetic method of Rinehart (20) we were succesful in incorporating 2,6-dideoxystreptamine 6 and 2,5,6-trideoxystreptamine 10 into antimicrobial products : 6-deoxyneomycin complex (16) and 5,6-dideoxyneamine (21), using the idiotroph of Streptomyces fradiae (22) as indicated in fig. 5. We were unable to transform 3,5-dideoxy-fortamine 19 into bioactive product using the same mutant. Positive result experienced previously with neamine (23) - using idiotroph of S.rimosus forma paromomycinus (24) - encouraged us to attempt the bioconversion of some of our synthetic pseudodissacharides depicted in Figure 9, but until now, no bioactive products could be obtained.



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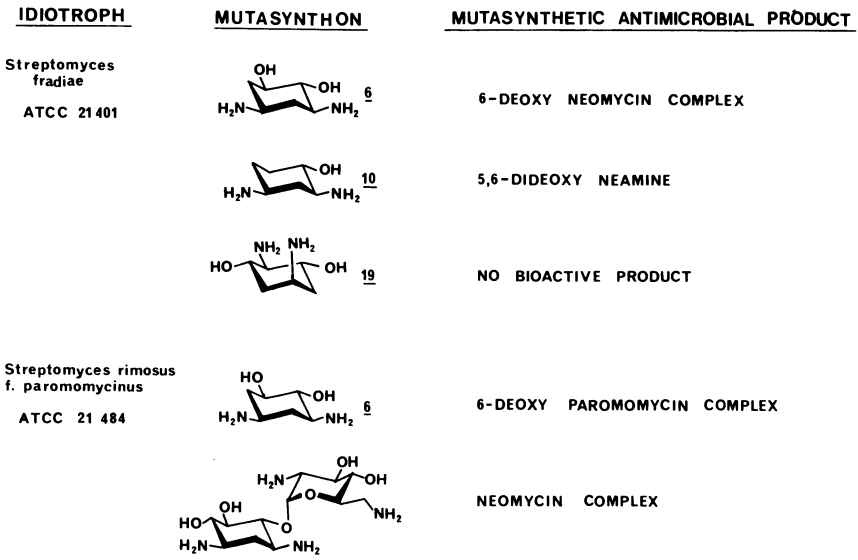

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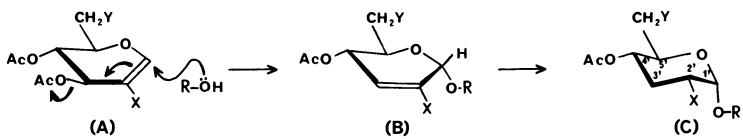


Figure 6.

TOTAL SYNTHESIS OF PSEUDODISACCHARIDES RELATED TO AMINOGLYCOSIDE ANTIBIOTICS

All of the clinically important aminoglycoside antibiotics are produced either by fermentation or by chemical modification of the natural products (Fig.1).

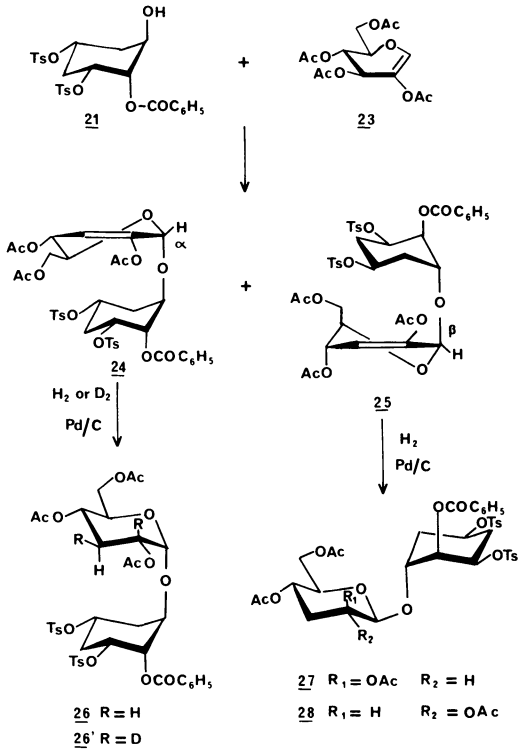
To produce pseudosaccharides differing greatly from the fermentation products, we decided, having a selection of potentially useful aglycon handles, to construct α-glycosides related to pseudosaccharides units of the natural products, by strictly synthetic means.

The long standing problem of 1,2-cis-glycoside synthesis (25, 26, 27, 28) was, at the outset of our work, our major concern. It was essential that any synthetic approach adopted should produce an α-glycoside linkage with high stereospecificity. The method developed in our laboratory and described here not only fulfils this requirement but in addition simultaneously yielded deoxygenated products at the C-3' position, a feature that is necessary for the avoidance of a major pathway of enzymatic inactivation, as well as for enhanced biological activity.

The scheme in figure 6 sets out the basic two step reaction sequence : the first step is a "quasi SN_2" reaction consisting of an acidcatalysed addition of an alcohol (R-OH) to the glycal (A) with allylic rearrangement ; this type of reaction has been extensively studied using simple alcohols (29, 30). The second step involves the hydrogenation of the double bond in compound (B). If (B) could be reduced with high regiospecificity from the β face, the resulting product (C) would be the required 3'-deoxy α-glycoside having the natural D-ribo-configuration.

For our purpose, the group R in the scheme needs to be a suitably protected aminocyclitol unit or some easily modified precursor of such a molecule. The chiral ditosyl-cyclohexanetetrol 5 and ditosyl-cyclohexanetriol 9 are such precursors. In particular 5 can be selectively substituted at either hydroxyl group. The reaction of 5 with benzoyl choride in the presence of imidazole gave the benzoate 21, while with t-butyldimethyl silyl chloride the silyl ether 22 was obtained.

As a typical example, we give here details of the synthesis of 5-O-(3'-deoxy-α-D-*ribo*hexopyranosyl)2,6-dideoxy streptamine 30 (31) using compound 21 and the glycal 23 (Fig. 7 and 8). A dichloroethane solution of compound 23 is added to a dichloroethane solution of 21 (1 equiv.) containing a catalytic amount of boron-trifluoride-ether at - 20°C over 15 min. The reaction mixture is maintained at - 15°C for another 2 h. After extraction a mixture of two products is obtained in 94 % yield. The major component (82 %) was isolated by a single crystallisation from alcohol. ^{1}H NMR data suggested that this compound was the α-glycoside 24 ($J_{1'-3'}$ 0,5, $J_{4'-5'}$ 9 Hz). The β anomer 25 was formed in 12 % yield ($J_{1'-3'}$ 0.6 ; $J_{4'-5'}$ 4.5 Hz). The α-glycoside 24 was regiospecifically hydrogenated or deuteriated in quantitative yield using 10 % palladium on carbon in ethylacetate in the presence of a trace of glacial acetic acid to compounds 26 and 26' respectively. As indicated by the ^{1}H NMR data obtained for 26 ($J_{1'-2'}$ 5 Hz) and especially for the dideuterio compound 26' ($J_{3'-4'}$ 10 Hz), the reduction occured exclusively from the β face of the molecule, there was no evidence for the formation the D-*arabino* isomer. In contrast, catalytic reduction of the β-glycoside 25 using the same conditions, proceeded sluggishly yielding two products in poor yield which were



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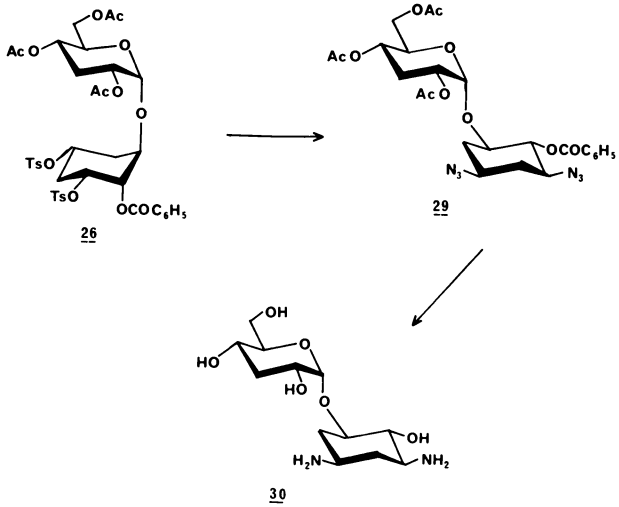

Figure 8.

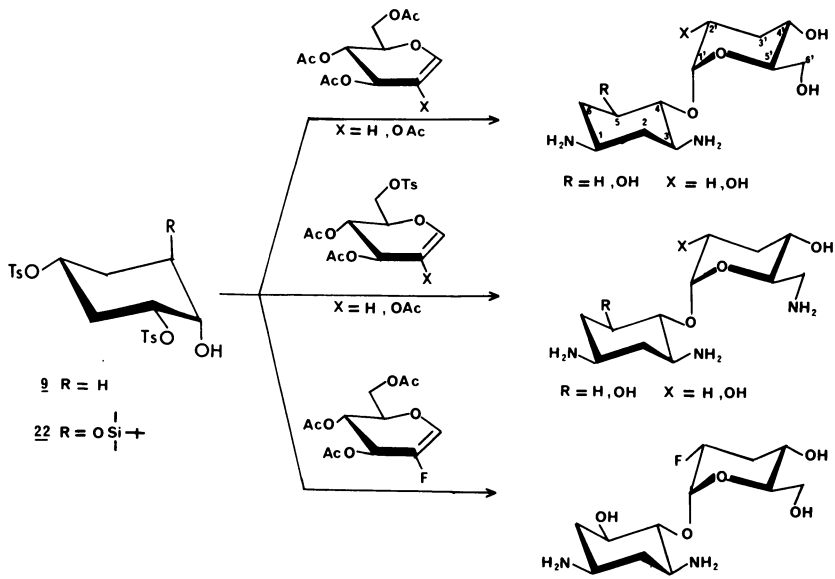

Figure 9.

characterised as compounds 27 (30 %) ($J_{1'-2'}$ 1.5 Hz) and 28 (18 %) ($J_{1'-2'}$ 9 Hz). In addition some hydrogenolysed products were formed which were not examined.

Azidolysis of 26, using sodium azide in N,N-dimethyl formamide at 110°C over 2h gave a mixture of three products in 81 % yield which were separated by silica gel chromatography. The major component (51 %) was identified as 29. The two minorcomponents arose by elimination of toluene-p-sulphonic acid. Saponification of 29 followed by reduction in the presence of PtO_2 in methanol-water (1:1) gave compound 30. (Fig. 8)

Using a variety of glycals and cyclitol derivatives, pseudodisaccharides depicted in Fig. 9 have been prepared. These products are related to the 4-O-substituted 2-deoxystreptamine glycosides (Fig. 2) which represented the minimum requirement for antimicrobial activity. The yield of the α-glycosylation procedure varies between 65-90 % and depends on the nature of the glycals and aglycones used in the reaction. Especially, pseudodisaccharides with a range of groups at the strategic 2' and 6' positions including 2',3' dideoxy and 2'-fluoro pseudodisaccharides have been synthetised (16, 31, 32).

The extension of this α-glycosylation procedure for the synthesis of a pseudotrisaccharide 33 (33) (Fig. 10), related to ribostamycin and the butirosins was also investigated. Since there are many effective antibiotics of this class containing a β-D-ribosyl group at the 5 position ; we first prepared the 5-O-D-ribosyl derivative 31 from compound 5 by condensation with tri-O-benzoyl-β-D-ribofuranosyl chloride in the presence of mercury (II) bromide and molecular sieves (4Å) under reflux over 8 hours (33). The latter on treatment with glycal 23 under the usual conditions

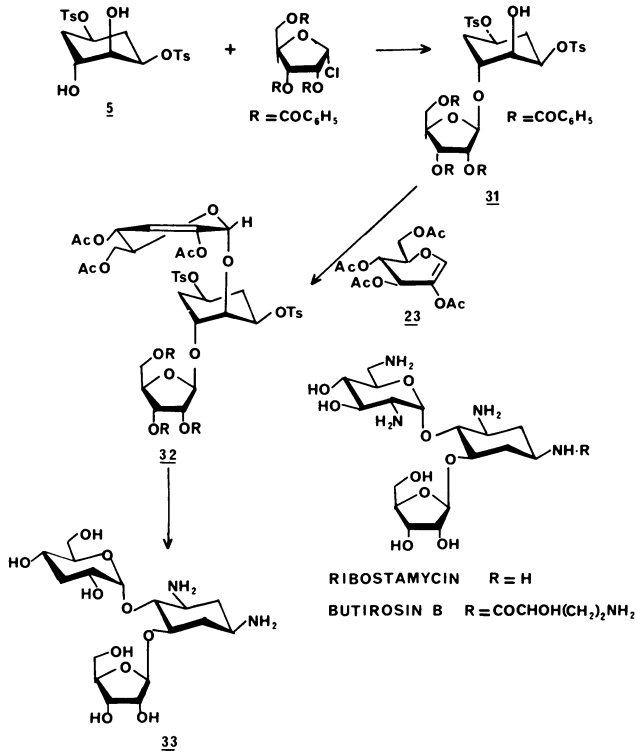

Figure 10.

afforded the unsaturated pseudotrisaccharide 32, in 62 % yield. Using well established methods ; 32 was converted to the trisaccharide 33.

We feel that the discovery of novel and more efficient antibiotics for clinical use might be obtained by the combination of the mutasynthetic and the total synthetic methodology.

Despite that the yield of biotransformation using idiotrophs is extremely low, mutasynthesis might provide a rapid information concerning the impact exerted by the mutasynthon on the antibacterial activity. The results of our studies indicated that the removal of hydroxy groups at C-6 or at C-5 and C-6, does not affect greatly the biological properties. The microbial spectra of 6-deoxyneomycins and 5.6-dideoxyneamine were very similar to that of neomycins and neamine, respectively.

Total chemical synthesis allows the introduction of a variety of functional groups as summarized in Figure 9 and we hope that our methodology will lead to novel type of bioactive substances.

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Literature Cited

1 Umezawa, S. ; Advan.Carbohyd.Chem.Biochem., 1974, 30, 111.

2 Cox, D.A. ; Richardson, K. ; Ross, B.C. ; Topics in Antibiotic Chemistry, Sammes P.G. Ed., Ellis Horwood Limited, England, 1977, 1, 1.

3 Nara, T., Annual Reports on Fermentation Processes, Perlman, D., Academic Press 1977 ; 1, 299.
4 Okamoto, S., Suzuki, Y., Nature, 1965, 108, 1301.
5 Umezawa, H., Advan.Carbohyd.Chem.Biochem., 1974, 30 183.
6 Umezawa, H., Drug Action and Drug Resistance in Bacteria, Mitsuhashi, S., Ed. University Park Press, Tokyo, 1975, 2, 211.
7 Davies, J., Smith, D.J., Ann.Rev. of Microbiol., 1978, 32, 464.
8 Umezawa, H. ; Umezawa, S., Tsuchiya, T., Okazaki, Y., J. Antibiotics, 1971, 24, 485.
9 Kawaguchi, H. ; Naito, T. ; Nakagawa, S., Fujisawa, K., J. Antibiotics, 1972, 25, 695.
1C Wright, J.J. ; J.C.S. Chem. Comm., 1976, 206.
11 Richardson, K.; Jevons, S., Moore ; J.W., Ross ; B.C., Wright, J.R. J.Antibiotics, 1977, 30, 843.
12 Nagabhushan, T.L. ; Cooper, A.B., Tsai, H. ; Daniels P.J.L., Miller G.H. ; J.Antibiotics, 1978, 31, 681
13 Kavadias, G. ; Velkof, S., Belleau, B. ; Can.J.Chem. 1978, 56, 404.
14 Suami, T. ; Ogawa, S. ; Uchino, H. ; Funaki, Y. ; J.Org.Chem., 1975, 40, 456.
15 Ogawa, S., Veda, T., Funaki, Y., Hongo, Y., Kasuga, A., Suami, T., J.Org.Chem., 1977, 42, 3083
16 Cleophax, J. ; Géro, S.D. ; Leboul, J. ; Akhtar, M. ; Barnett, J.E.G. ; Pearce, C.J., J.Amer.Chem.Soc., 1976, 98, 7110.
17 Rolland, A. ; Thesis, University Paris-Sud, Orsay, 1978.
18 Ogawa, S., Hongo, Y., Fujimori, H., Iwata, K., Kasuga, A., Suami, T., Bull.Soc.Chem.Japan, 1978, 51, 2957.

19 Egan, R.S. ; Stanaszek, R.S. ; Cirovic, M. ; Mueller, S.L. ; Tadanier, J. ; Martin, J.R. ; Collum, P. ; Goldstein, A.W. ; De Vault, R.L. ; Sinclair, A.C. ; Fager, E.E. ; Mitscher, L.A. ; J.Antibiotics, 1977, 30, 552.

20 Rinehart, Jr., K.L., Pure Appl.Chem., 1977, 49, 1367.

21 Sepulchre, A.M. ; Quiclet, B. ; Colas, C.; Rolland, A. ; Cleophax, J., Pearce, C.J., Géro, S.D., Abst 18th Int.Conf.Antimicrob.Agents Chemother. Atlanta, 1978, N°172.

22 Shier, W.T. ; Rinehart, Jr., K.L. ; Gottlieb, D., Proc.Nat.Acad.Sci., U.S.A., 1969, 63, 198.

23 Pearce, C.J. ; Barnett, J.E.G. ; Anthony, C. ; Akhtar, M. ; Géro, S.D., Biochem.J., 1976, 159, 601.

24 Shier, W.T. ; Schaefer, P.C. ; Gottlieb, D., Rinehart, Jr., K.L., Biochem., 1974, 13, 5073.

25 Lemieux, R.U., James, K., Nagabhushan, T.L., Can. J.Chem., 1973, 51, 42.

26 Wulff, G., Röhle, G., Angew.Chem.Int.Ed., 1974, 13, 157.

27 Lemieux, R.U., Hendriks, K.B., Stick, R.V., James, K., J.Amer.Chem.Soc., 1975, 97, 4056.

28 Pougny, J.R., Nassr, M.A.M., Naulet, N., Sinaÿ, P., Nouv.J.Chim., 1978, 2, 389.

29 Ferrier, R.J., Advan.Carbohyd.Chem.Biochem., 1969, 24, 199

30 Ferrier, R.J., Fortschr.Chem.Forsch., 1970, 14, 389.

31 Cleophax, J., Do Khac Duc, Delaumény, J.M., Géro, S.D., Rolland, A., J.C.S.Chem.Comm., 1978, 771.

32 Vass, G., Rolland, A., Cleophax, J., Mercier, D., Quiclet, B., Géro, S.D., J.Antibiotics, 1979, 32, 670.

33 Cléophax, J., Delaumény, J.M., Géro, S.D., Rolland, A., Rolland, N., J.C.S.Chem.Comm., 1978, 773.

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