Chemistry of spectinomycin: its total synthesis, stereocontrolled rearrangement, and analogs[1]

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The total stereocontrolled synthesis of the antibiotic spectinomycin is described, based on the regiospecific functionalization and manipulation of appropriate starting materials. The tertiary ketol rearrangement of the antibiotic and its derivatives was studied and the stereochemical identity of spectinoic acid was established by chemical correlation. Dihydrospectinomycin derivatives undergo unusual solvolysis reactions.

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La synthèse totale et stéréocontrôllée de l'antibiotique spectinomycine est décrite en se basant sur une stratégie de fonctionnalisation et manipulation spécifique de produits de départs appropriés. Le réarrangement des α-cétols tertiaires de la spectinomycine et ses dérivés a été étudié et l'identité stéréochimique de l'acide spectinoïque a été établie par des corrélations chimiques. Les dérivés de la dihydrospectinomycine donnent lieu à des réactions de solvolyses intéressantes.

Spectinomycin is a structurally unique antibiotic which is formally classified among the aminocyclitol group (1). Isolated from several fermentation broths in the early sixties, it was originally given the name actinospectacin (2; for a recent review on the chemistry of spectinomycin, see ref. 3) and is presently used in the veterinary area. It is also widely used in human medicine because of its potent activity against some strains of *Neisseria gonorrhoeae*. It may be the drug of choice against penicillin resistant strains of *Neisseria gonorrhoeae* (4) or for patients allergic to β-lactam antibiotics. Another interesting feature concerning the biological profile of spectinomycin is the fact that it exhibits a greater degree of therapeutic effect in infected animals than might be expected on the basis of its in vitro activities. Extensive studies concerned with structure modification in order to induce greater antibiotic activity were carried out in several laboratories (5, 6). Unfortunately, except for the recently announced 4-amino-4-(*R*)-dihydro derivative (7*a*), and some alkyl spectinomycin analogs (7*b*), efforts to improve the biological profile of spectinomycin have been somewhat disappointing.

The constitutional structure (8, 9), stereochemistry, and absolute configuration (10), as well as the biosynthesis (11) of spectinomycin have been the subject of elegant studies over the years. Its unique structural, conformational, and functional features are depicted in Scheme 1. The tricyclic structure harbors nine chiral carbon atoms, each of which bears at least one hetero atom. Stereoelectronic factors are dominant in view of the presence of three contiguous acetal type linkages (12) and the fused ring systems provide classical cases of the manifestation of natural anomeric stereoselection (13). This feature may be appreciated by considering the conformational depiction of spectinomycin in Scheme 1 where it can be seen that, in the solid state structure (10), one of the oxygen atoms at C4a and C10a has an axial disposition with respect to the other on the same carbon atom (*gauche* orientation), as dictated by the stereoelectronic requirements of the anomeric effect (for a discussion of the anomeric effect, see ref. 14). Deslongchamps, Descotes and their respective groups (15) have found that bicyclic acetals having an axial orientation of alkoxy anomeric substituents are stabilized by at least 1.5 kcal/mol compared to the alternate anomer.

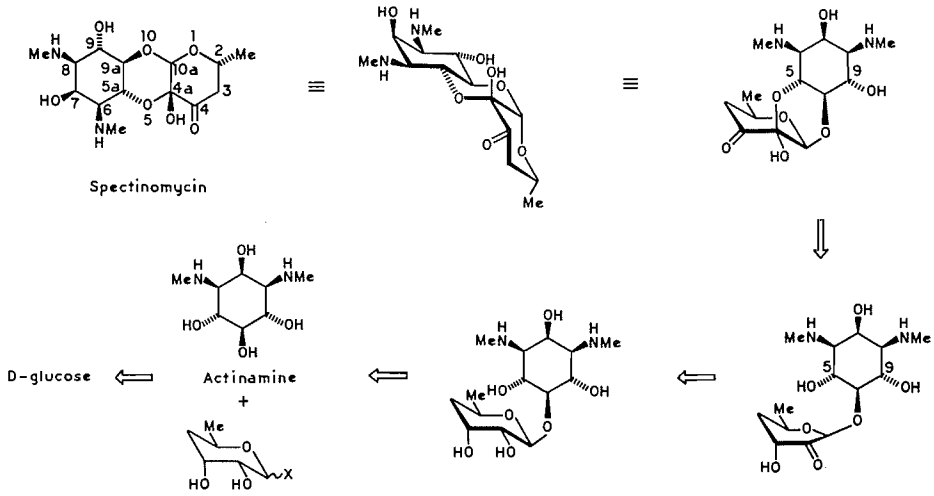
Spectinomycin adopts a "bent" shape in the solid state as well as in solution, and the resulting topological features in terms of functional groups, sidedness, and electronic properties may have an important bearing on its mode of action in inhibiting protein biosynthesis at the ribosomal level.

Surprisingly, little if any effort was reported on the total synthesis of spectinomycin prior to 1977. Thus, Suami *et al.* (16) reported on the synthesis of a tetrahydrospectinomycin derivative by glycosylation of a suitably protected actinamine. Several syntheses of actinamine, the cyclitol portion, were already available at the time (17; actinamine is also easily accessible from spectinomycin, see ref. 8) although by far the quickest access to this compound is by acidic hydrolysis of the antibiotic (9). In 1979, two independent and conceptually different total syntheses of spectinomycin were announced. The Upjohn synthesis (18) utilized L-glucose as starting material and involved a series of unique transformations. Our own synthesis, announced in preliminary form (13*b*, 19), was based on the systematic chemical manipulation of D-glucose and took advantage of some reactions anticipated to occur with a high degree of regiocontrol. It also relied in its final stages on a biomimetically-related process. Our strategy called for the synthesis of one of the four possible tetrahydrospectinomycins (20), namely, the 4*R*,4a*R* isomer (Scheme 1), which, by virtue of a predisposed arrangement of hydroxyl groups was expected to provide the necessary regio- and stereochemical control in subsequent operations leading to a pivotal 4a-keto derivative, and eventually to spectinomycin. With such a precursor in hand, we further expected the critical intramolecular ketalization to be diastereoselective because of the stereoelectronic requirements (12) of the anomeric effect (14).

In this paper, we give a full account of this synthesis, and we report on subsequent work (21) dealing with a unique stereocontrolled rearrangement of spectinomycin. The retrosynthetic analysis shown in Scheme 1 calls for the availability of a derivative of a 4,6-dideoxyhexose, which represents the chiral tetrahydropyran portion of the molecule. The fortuitous D-nature of the corresponding sugar directed our attention to D-glucose as a precursor. An initial objective, therefore, was to

[1] Taken in part from the Ph.D. thesis of René Roy, Université de Montréal, 1981.

[2] NSERCC Predoctoral fellow 1978–1981.

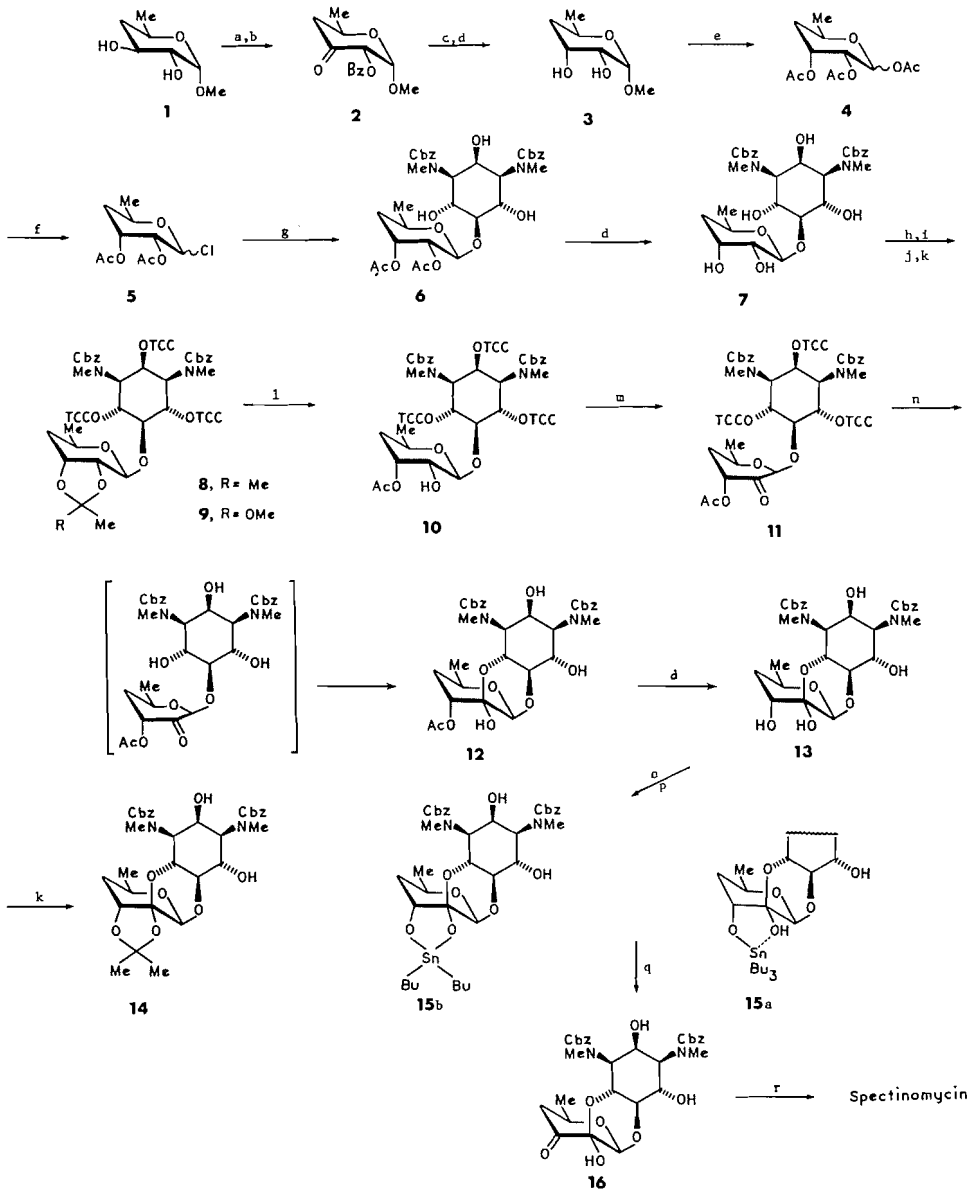


SCHEME 1

find expedient and practical methods to convert this readily available sugar into a dideoxy derivative with a predisposed orientation of hydroxyl groups at C-2 and C-3, as expressed in the structure of the glycoside **3** (Scheme 2). Note that the α-orientation of the *cis*-diol unit commits the synthetic scheme to proceed via a specific tetrahydrospectinomycin as dictated by our original strategy (Scheme 1). The availability of a two-step deoxygenation process (22) at C-4 and C-6 of methyl α-D-glucopyranoside via the intermediacy of the corresponding 4,6-dichloro derivative allowed us to have substantial quantities of the desired intermediate **1**. Attainment of the *cis*-diol orientation necessitated an inversion of configuration at C-3 and the most practical way was a two-step process involving oxidation at C-3 and reduction of the corresponding ketone. Thus, we were faced with the task of selective protection of the C-2 hydroxyl group, a process which was easily accomplished by taking advantage of the nucleophilic enhancement of oxygen atoms in trialkyltin ethers and their selective reaction with electrophiles (23, 24). Upon treatment of **1** with bis(tributyltin) oxide, the intermediate 2-*O*-tributlytin ether was formed selectively due to coordination with the anomeric methoxyl group (24). Reaction with benzoyl chloride gave the known 2-benzoate derivative (25) in 94% yield. Oxidation then gave the crystalline derivative **2** (25), which was reduced to **3**. The outcome of the reduction was predictable because of the α-orientation to the aglycone. Debenzoylation, acetolysis, and transformation into the glycosyl chloride were done in high-yielding steps to provide the reactive intermediate **5**. We were now faced with a stereocontrolled glycosidation with a suitably protected actinamine derivative, and the first of a series of protecting groups that would be compatible with the remainder of the synthetic sequence had to be chosen. Moreover, inspection of the structure of the intended glycoside intermediate placed some stringent requirements with regard to regio and stereocontrol. Thus, only the central hydroxyl group in the symmetrical actinamine molecule must be considered, in a condensation that should lead to a 1,2-*trans* orientation of substituents in the resulting glycoside. Such stereoselectivity can be expected by anticipating the intermediacy of 1,2-acyloxonium ions in the glycoside-forming step (for some reviews on glycoside synthesis, see ref. 26). The regioselectivity, on the other hand, could be achieved by appropriate protection–deprotection procedures so as to free the required hydroxyl group. Clearly this would unduly lengthen the synthesis. It was reasoned that by placing reasonably bulky substituents on the amino groups, the order of reactivity would greatly favor the hydroxyl group in question since it would be the least congested. Such was the case when *N,N'*-[(benzyloxy)carbonyl]-actinamine (16) was allowed to react with **5** in the presence of silver trifluoromethanesulfonate in dichloromethane at 40°C. Selective glycoside formation took place to give the expected product **6** in 60–65% yield. The choice of catalyst was evidently important (27), since a related glycosidation was accomplished in less than 10% yield when silver carbonate was used under typical Koenigs–Knorr conditions (16). Deacetylation of **6** gave the known *N,N'*-[(benzyloxy)carbonyl]-4(*R*),4a(*R*)-tetrahydrospectinomycin **7** (20).

Having thus secured the stereochemical identity of glycosidic linkage in our synthetic sample **6**, we considered various options for manipulating the hydroxyl groups en route to our next target, namely dihydrospectinomycin. To achieve this, it was necessary to set the stage for preferential oxidation at C-2 of the sugar and for intramolecular ketalization. The *cis*-diol unit in 7, comprising an axial–equatorial orientation of hydroxyl groups, proved to be a judicious choice for the synthetic operations to be performed. These called for the formation of a cyclic orthoester **9***b* which was expected to undergo a site-selective solvolysis to give the axial ester – equatorial alcohol **1***a* (28). At the same time the interplay between protecting groups and reaction conditions became, once again, an important issue. In order to achieve selective protection of the actinamine hydroxyl groups, **7** was first transformed into the corresponding isopropylidene derivative and a search for O-protecting groups was initiated, bearing in mind that the resulting derivative should withstand the rigors of aqueous acid,[3] while being susceptible to mild conditions of deprotection. The seldom used trichloroethoxycarbonate group (see, for example, ref. 29) was found to be ideal for these transformations. With these considerations in mind, we set out to test their outcome in the laboratory. The desired derivative **9** was easily prepared from the diol **7** by an uneventful sequence involving the isopropylidene derivative **8**, which upon acid hydrolysis gave the selectively protected diol. The latter was then transformed into

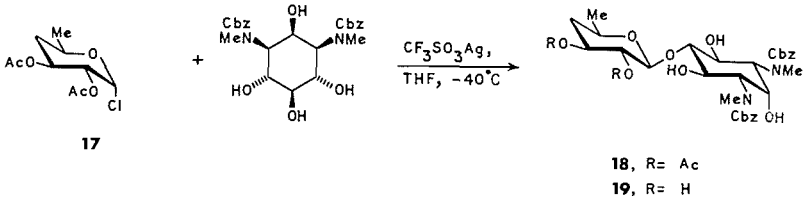
[3] In our original publication, we had used acetates as protecting groups. However, unless carefully controlled, their hydrolysis was accompanied by some intramolecular cyclic carbamate formation, ref. 19.



SCHEME 2. a: $(Bu_3Sn)_2O$, then BzCl; b: PCC; c: $NaBH_4$; d: NaOMe; e: Ac_2O, BF_3; f: HCl, ether; g: Cbz-actinamine, silver triflate, THF, −40°C; h: $Me_2C(OMe)_2$, TsOH; i: $ClCH_2CH_2OCOCl$, pyr., DMAP; j: aq. TsOH, H_2O–MeOH; k: $MeC(OMe)_3$, TsOH; l: aq. AcOH; m: PCC, benzene, reflux; n: Zn dust, AcOH; o: Bu_2SnO, MeOH reflux; p: $(Bu_3Sn)_2O$, benzene; q: NBS or Br_2; r: Pd/C H_2.

the orthoester **9**. Treatment of **9** with dilute acetic acid gave the amorphous monoacetate **10**, in which the acetate group was axial. Thus, the anticipated regiochemistry in the solvolysis of the orthoester ring had been indeed attained. Oxidation of **10** by a recent modification (30) of the Corey–Suggs procedure (31) gave the unstable keto derivative **11** as a syrup. Treatment of **11** with zinc dust in tetrahydrofuran cleanly removed the trichloroethoxycarbonyl groups and regenerated the triol system in the actinamine portion, with concomitant intramolecular ketalization, to give the monoacetyl derivative **12** in high yield. Mild base treatment then gave *N,N'*-[(benzyloxy)carbonyl]-4(*R*)-dihydrospectinomycin **13**, identical in all respects with authentic material. The corresponding acetonide **14** (13*a*, 32) was also easily prepared. Thus, the intermediate keto polyol underwent stereoselective ketalization with only one of the two possible, symmetrically disposed hydroxyl groups in the actinamine portion. This preference can be logically rationalized on stereoelectronic grounds (12, 14), much in the same way as for the free antibiotic itself (13, 18), and it is an anticipated event in this sequence.

Having reached this penultimate stage in our synthesis, we were in a position to address the next critical step in the sequence, namely, the oxidation of **13** to *N,N'*-[(benzyloxy)-carbonyl]spectinomycin **16**. The challenge that we faced was once more one of selectivity. How could one effect preferential oxidation of the 4(*R*)-hydroxyl group in the presence of a pair of axially (C-7) and equatorially (C-9) disposed hydroxyl groups, while maintaining the structural integrity of the molecule? Having explored a number of possible solutions to this problem, we turned once again to organotin chemistry where a unique opportunity for achieving a selective oxidation became evident, particularly in view of the *cis*-disposition of the vicinal diol unit. The oxidation of tributyltin ethers to carbonyl compounds (33) and of dibutyltin acetals to α-hydroxyketones (34) in the presence of bromine were known in the literature (23). This reaction appeared to be admirably suited to our needs,



17

18, R= Ac
19, R= H

SCHEME 3

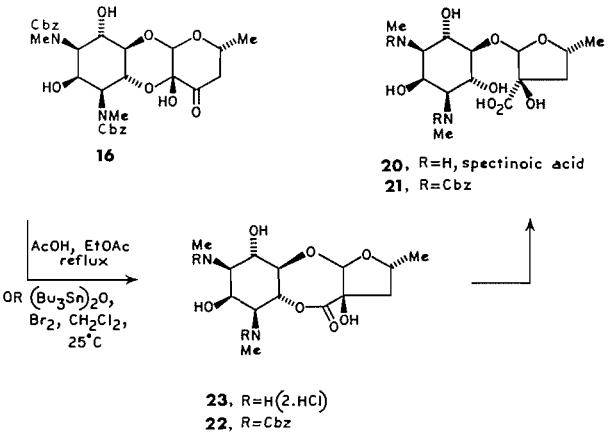
although its adaptation to acetals originating from tertiary and secondary hydroxyl groups was yet to be tested. Treatment of **13** with bis(tri-n-butyltin) oxide, followed by NBS, smoothly led to the desired *N,N'*-[(benzyloxy)carbonyl]spectinomycin **16** in high yield, presumably via the intermediacy of an intramolecularly coordinated tributyltin ether such as **15***a*. Alternatively, **13** could be transformed into the stannylidene acetal **15***b*, then treated with bromine to give the desired products. The former method was found to give cleaner products and could be better controlled, particularly in view of the tendency for the product to over-oxidize to give a by-product, which will be discussed later.

Vital to this successful oxidation was the original choice of a 4(*R*)-axial alcohol since, by its very nature the intermediate tin ether or acetal could only be oxidized at C-4, which has a favorably situated equatorial hydrogen atom.[4] Finally, hydrogenation of **16** under carefully monitored conditions led to the intended target, isolated as the crystalline dihydrochloride pentahydrate in high yield. This product was found to be identical to the natural product in all respects.

During the course of our studies, we also prepared the 4-epi tetrahydro *N,N'*-[(benzyloxy)carbonyl]tetrahydro-4(*S*),4a(*R*)-spectinomycin **19** (Scheme 3). Thus, acetolysis of **1** and glycosyl halide formation led to 2,3-di-*O*-acetyl-4,6-dideoxy-α-D-*xylo*-hexopyranosyl chloride **17** which, when condensed with *N,N'*-[(benzyloxy)carbonyl]actinamine as previously described, gave, after purification, the crystalline derivative **18** in 67% yield. Deacetylation at pH 8.5 gave the crystalline tetrol **19** (16), mp 260–265°C; $[\alpha]_D$ −3.2° ($CHCl_3$).

Stereocontrolled rearrangements

During the course of our oxidation studies of **13** in the presence of bis(dibutyltin) oxide and bromine, we had observed the formation of a secondary product which migrated close to the desired *N,N'*-[(benzyloxy)carbonyl]spectinomycin **16** (21). The same product was also formed when samples of **16** were treated with silica gel, or on prolonged standing in ethyl acetate. The spectroscopic properties of this new product were sufficiently different from those of **16** to warrant further inquiry, and it soon became apparent that a new entity was produced. The constitutional identity of this product was secured from chemical evidence and eventual correlation with a known degradation product of spectinomycin itself. Thus, treatment with sodium ethoxide in tetrahydrofuran led to a methyl ester, while treatment with aqueous sodium hydroxide followed by hydrogenolysis gave the known crystalline spectinoic acid dihydrochloride **20** (Scheme 4) (8). It was therefore evident that the new product was the seven-membered lactone **22**, which presumably arose by a process related to an α-ketol



16

20, R=H, spectinoic acid
21, R=Cbz

23, R=H(2.HCl)
22, R=Cbz

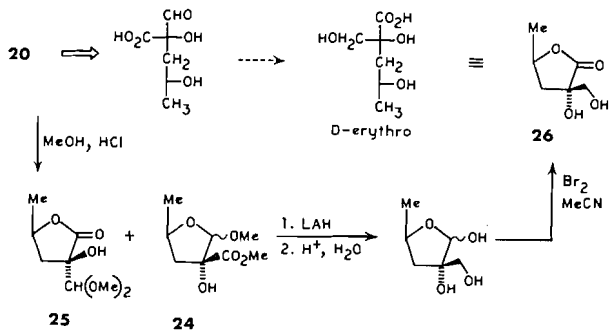
SCHEME 4

rearrangement (for a review, see ref. 35). Such rearrangements are well known to occur under acidic, basic, and solvolytic conditions and may be reversible processes (see, for example, ref. 36). However, many attempts to induce reversibility in our case were unsuccessful, possibly because of an unfavorable alignment of bonds in the lactone. The forward rearrangement process, however, can be nicely visualized to occur by activation of the C-4 carbonyl oxygen atom by an electrophile, and migration of the antiperiplanar C-4a—C-10 bond. The rearrangement did not occur with bis(dibutyltin) oxide or bromine alone, or when the substrate was converted to the 4,4-dimethylacetal derivative. It is therefore possible that the reaction is caused by initial formation of Bu_3SnOBr (33), which can supply a base as well as an electrophile. That a tertiary ketol rearrangement was indeed operative was also demonstrated when the same lactone was formed from **16** in the presence of 10% acetic acid in refluxing ethyl acetate.

Hydrogenolysis of the lactone **22** led to the corresponding amino derivative **23**, which was tested and found to be devoid of activity as an antibacterial agent. It is of interest to note in this regard that **23** is isomeric with spectinomycin and it can be considered as a variant in which the central ring has been expanded and the original α-ketol functionality transposed.

When spectinomycin was treated with strong base, there was formed an acidic degradation product which was called actinospectinoic acid (spectinoic acid) (9) and its formation was explained based on a tertiary ketol rearrangement. Although this substance was crystalline and a new asymmetric center was created at the carbon atom bearing the carboxyl group, no effort was made over the years to secure its configurational identity. The discovery of the acid-catalyzed rearrangement of **16** into the lactone **22** and its correlation with spectinoic acid **20** prompted us to establish the configurational identity of the latter. It had already been known (9) that methanolysis of spectinoic acid gave a mixture of methyl glycosides **24** and the lactone **25** (of unknown absolute configuration at C-2) (Scheme

[4] Vicinal *trans*-disposed stannylidene acetals are also subject to oxidation with bromine (ref. 34). It is also possible that we might be dealing with oligomeric ethers or acetals, rather than discrete derivatives such as **15***a*, *b*.



SCHEME 5

5). Thus if either product were successfully correlated with a known substance, then the task of assigning an absolute stereochemistry would be greatly simplified. We recognized the structural resemblance of α-D-isosaccharino-1,4-lactone to either methanolysis product **24** or **25**. In the hope of achieving structural and stereochemical convergence, we proceeded with a chemical modification program on two fronts. Thus, **24** was converted in three steps into the lactone **26** by standard methodology. The conversion of lactone **27**[5] derived from lactose (37) into our target required a deoxygenation at the terminal primary hydroxymethyl group, which was achieved in straightforward manner by a series of reactions shown in Scheme 6. Comparison of the physical properties of this crystalline *p*-nitrobenzoate ester **29**, derived from both the synthetic and degradation-derived lactone, established their complete convergence. The tertiary ketol rearrangement of spectinomycin therefore produces a single acid, whose absolute configuration at the newly formed asymmetric center is *R*. This, in fact, is the anticipated stereochemical result, based on a stereocontrolled migration of the C-4a—C-10 bond. With the establishment of the stereochemical identity of spectinoic acid some 23 years after its isolation, the last remaining structural problem concerning the parent antibiotic can be considered fully addressed.

In the course of our synthetic studies, we had become intrigued by the prospects of effecting a number of stereocontrolled fragmentation and substitution reactions, particularly with the unique anti-periplanar arrangement of bonds in 4-substituted dihydrospectinomycins (Scheme 7) (for examples of Grob-type fragmentations, see ref. 38). It was therefore of interest to attempt the experiments with the mesylate **31** derived from the 4-(*S*)-dihydro isomer **30** (Scheme 8). At first, heating the mesylate to its melting point did not result in the formation of new products. Consequently solvolytic studies were undertaken. Treatment of **31** with silver carbonate (39) in aqueous acetone gave the 4-(*R*)-dihydro isomer **13** in 47% yield, accompanied by *N,N'*-[(benzyloxy)carbonyl]actinamine. Clearly, solvolysis with net inversion has taken place under these conditions. When the same mesylate was treated with sodium acetate in anhydrous trifluoromethanol (40) at reflux temperature, there was formed the inverted acetate derivative **12** in 38% yield, accompanied by two unidentified side-products. Once again, apparent inversion of configuration had occurred in this reaction. However, when **31** was treated with sodium azide in 1.5% aqueous trifluoromethanol, the corresponding 4-(*S*)-azido derivative **32** was isolated in 30% yield, along with starting material and two unknown by-products. Thus, in this case a net retention of configuration was observed. Catalytic hydrogenation of **32** led to 4-amino-4-(*S*)-dihydrospectinomycin **33**, which was found to be devoid of antibacterial activity,[6] unlike its 4-(*R*) epimer **34** (7). Thus, what appeared to have been an alternative entry into the biologically active series was thwarted by the unexpected. Inspection of molecular models clearly indicates a severly restricted backside approach at C-4 in the conformationally rigid tricyclic system. Two possibilities can be considered for the displacement reactions. The mesylate could, in fact, react in the α-ketomesylate rather than the ketol form in which a much higher order of reactivity is expected (41) to give inverted products. In the case of the α-azido (Scheme 9) derivative, a subsequent base-catalyzed epimerization and subsequent intramolecular ketalization could take place to give the observed product. Alternatively, an intermediate epoxide can be envisaged to be transiently formed and attacked by the nucleophile, regiospecifically, with net retention of configuration. The results in the case of acetate ion and silver carbonate can be rationalized, based on attack of the α-keto mesylate followed by ketalization (without epimerization, or by another mechanism via initial attack on the carbonyl group, followed by an intramolecular reaction leading to the ejection of the mesylate and the formation of an intermediate acetoxonium ion).

These reactions were unsuccessful when the corresponding 4a-methoxy derivative was used, which strongly implies the involvement of the carbonyl group in the open form, or the tertiary hydroxyl group in a cyclic form.

Experimental

Melting points are uncorrected. Optical rotations were measured on a Perkin–Elmer automatic spectropolarimeter, model 141. The ^{1}H nmr spectra were recorded on Varian EM-360 and Bruker WH-90 and 400-MHz instruments using deuterochloroform as solvent unless otherwise stated. The ^{13}C nmr spectra were recorded at 22.6 MHz. Mass spectra were recorded on an AE1-902 mass spectrometer at low resolution unless otherwise indicated. Column chromatography was done using silica gel G254 with application of moderate suction or by the flash technique. Work-up with usual processing signifies extraction with an appropriate organic solvent, washing, drying the organic phase with magnesium sulfate, and evaporation.

*Methyl 2-O-benzoyl-4,6-dideoxy-α-D-*erythro*-hexopyranosid-3-ulose, 2*

A solution of **1** (22) (3.88 g, 24 mmol) in 100 mL of anhydrous toluene containing 9 mL (18 mmol, 0.75 equiv.) of bis(tributyltin) oxide was heated under reflux with a Dean–Stark trap. After 3 h, the solution was cooled to room temperature and benzoyl chloride (3.4 mL, 28 mmol) was added. After stirring 1 h, methanol was added and the solvent evaporated to give a residue which crystallized upon trituration with petroleum ether to give 6 g (94%) of the known 2-benzoate (25), mp 103–105°C; $[\alpha]_D$ +158.6 (*c* 1.21, $CHCl_3$).

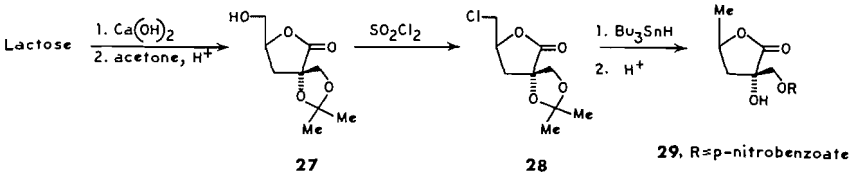
A solution of this product (1.06 g, 4 mmol) was added to a suspension of pyridinium chlorochromate (16 mmol) and sodium acetate (656 mg, 8 mmol) in 50 mL of dichloromethane. After stirring for 15 h, ether was added to the dark suspension, the salts were filtered, and the filtrate was evaporated to a syrup (1.18 g; ~quantitative); $[\alpha]_D$ +65.2° (*c* 0.15, $CHCl_3$) (lit. (25) $[\alpha]_D$ +66°).

*Methyl 4,6-dideoxy-α-D-*ribo*-hexopyranoside, 3*

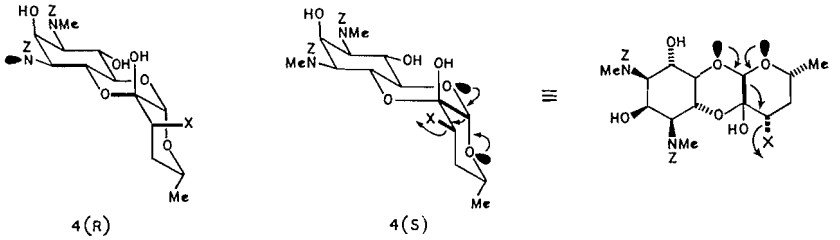
The preceding product (2.9 g, 11 mmol) in 100 mL of methanol was treated with sodium borohydride (492 mg, 13 mmol) and the solution was stirred for 10 min. Excess hydride was destroyed by addition of

[5] We thank Prof. R. B. Yeats, Bishop's University, Lennoxville, Quebec for a generous sample of **27**.

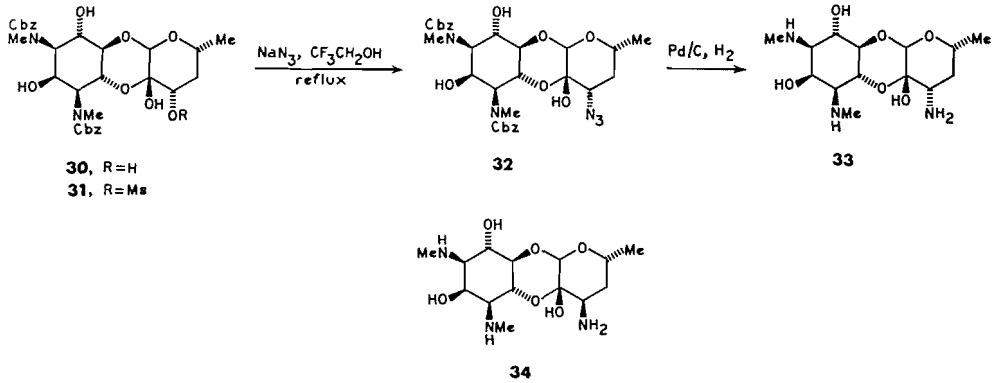
[6] Courtesy of Abbott Laboratories, North Chicago, Illinois.



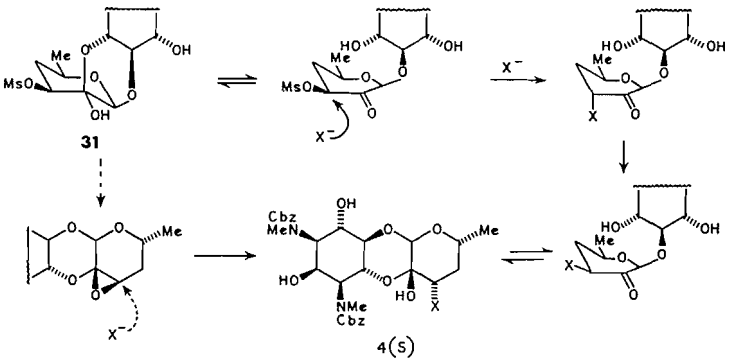
SCHEME 6



SCHEME 7



SCHEME 8



SCHEME 9

aqueous acetic acid to pH 6, the solution was evaporated to dryness, and the residue was extracted with ether. Usual processing gave a syrup which was dissolved in methanol and treated with sodium methoxide. After 24 h, the solution was treated with Dowex-50 (H^+), filtered, and the filtrate was evaporated to dryness. Flash column chromatography gave the pure product **3** as a colorless syrup in almost quantitative yield; $[\alpha]_D$ +112° (*c* 0.56, $CHCl_3$); ^1H nmr (60 MHz, ppm): 1.19 (d, Me, $J_{5,6}$ = 6 Hz), 1.37–2.21 (m, H-4′4′, *J* = 13 Hz), 3.36 (OH), 3.42 (s, OMe), 3.50–4.30 (m, H2, H3, H5), 4.70 (d, H-1, $J_{1,2}$ = 1.3 Hz); ^{13}C nmr (22.6 MHz, ppm): 101.5, 68.8, 68.1, 58.8, 55.9, 39.06, 20.6.

1,2,3-Tri-O-*acetyl-4,6-dideoxy*-α-D-ribo-*hexopyranose*, *4*

The preceding diol (840 mg, 5.2 mmol), acetic anhydride (5 mL), and $BF_3 \cdot Et_2O$ (0.3 mL) were stirred at 0°C for 24 h. The solution was poured into aqueous saturated sodium bicarbonate, and the solution extracted with chloroform and processed as usual to give a syrup which crystallized upon trituration with ethanol to give 563 mg (39%) of the crystalline β-anomeric acetate, mp 131.5–133°C (EtOH); $[\alpha]_D$ −53.3° (*c* 3.32, $CHCl_3$); ^1H nmr (60 MHz, ppm): 1.21 (d, C-Me, $J_{5,6}$ = 6 Hz), 1.77 (m, H-4,4′, *J* = 13 Hz); 2.00, 2.07 (OAc); 4.14 (m, H5), 4.80 (dd, H-2, $J_{1,2}$ = 8 Hz; $J_{2,3}$ = 8 Hz), 5.44 (m, H-3), 6.01 (d, H-1); ms *m/e*: 215 (M^+ − OAc). The mother liquors were chromatographed on silica (hexanes – 10% ethyl acetate) to give 1.07 g (75%) of an anomeric mixture of acetates. *Anal.* calcd. for $C_{12}H_{18}O_7$: C 52.55, H 6.61; found: C 52.80, H 6.63.

2,3-Di-O-*acetyl-4,6-dideoxy*-α,β-D-ribo-*hexopyranosyl chloride*, *5*

The mixture of anomeric triacetates **4** (500 mg, 1.82 mmol) was dried overnight under vacuum (desiccator). It was dissolved in 50 mL

of ether (freshly distilled, LAH), cooled to 0°C, and a stream of HCl gas was bubbled through the solution during 2 h. The solvent was evaporated and the residue was repeatedly coevaporated with dry toluene. This product was kept under argon in THF solution. An aliquot was analyzed by ^{1}H nmr (60 MHz, ppm): 1.20 and 1.35 (d, C-Me, two anomers); 2.09, 2.13, 2.15, 2.17 (OAc, 2 anomers); 5.53 (d, H-1, $J_{1,2}$ = 8 Hz, β-anomer), 6.09 (d, H-1, $J_{1,2}$ = 4 Hz, α-anomer).

4,4a-Di-O-*acetyl*-N,N′-*[(benzyloxy)carbonyl]-4(*R*),4a(*R*)-tetrahydrospectinomycin, **6***

The chloride **5** prepared from 1 g (3.65 mmol) of triacetate **4** was transferred via a double-tip needle into a dry separatory funnel in a total volume of 40 mL of THF. A solution containing *N,N′*-[(benzyloxy)carbonyl]actinamine (2.6 g, 5.46 mmol, 1.5 equiv., previously dried from THF via Dean–Stark) and silver trifluoromethanesulfonate (1.4 g, 5.46 mmol, 1.5 equiv. (Fluka)), in 50 mL of dry THF was stirred at 0°C under argon. The temperature was lowered to −40°C, and the chloride added dropwise over a period of 1 h. The reaction mixture was stirred at −40°C for 3 h, then 1 g of sodium bicarbonate followed by 5 mL of aqueous saturated sodium chloride were added. The mixture was diluted with 150 mL of chloroform, filtered over Celite, and the organic layer was processed as usual to give a foam which was chromatographed over silica (hexanes–EtOAc, 3:7). Those fractions which showed a positive uv absorption test and a negative benzidine–periodate test were combined and processed to give 1.56 g (62%) of the title compound as an amorphous solid; $[\alpha]_D$ −8.2° (*c* 0.11, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3480 (OH), 1750 (OAc), 1685 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.26 (d, C-Me, *J* = 6 Hz), 1.78 (m, H-3,3′); 1.99, 2.05 (OAc); 3.09 (s, NMe_2), 4.81 (dd, H-4a, $J_{4a,10a}$ = 8 Hz, $J_{4a,4}$ = 3 Hz), 4.91 (d, H-10a, $J_{10a,4a}$ = 8 Hz), 5.15 (s, CH_2Ph), 5.52 (m, H-4), 7.35 (s, OH, Ph), etc. *Anal.* calcd. for $C_{34}H_{44}O_{13}$: C 59.29, H 6.44, N 4.07; found: C 58.98, H 6.39, N 3.98.

N,N′-*[(benzyloxy)carbonyl]-4(*R*),4a(*R*)-tetrahydrospectinomycin, **7***

A solution of **6** (700 mg, 1.02 mmol) in 10 mL of methanol was cooled to 0°C and treated with a small chip of sodium metal to give a pH ~ 8.5. After 15 min at 25°C, Dowex-50 (H^+) was added, the mixture filtered, and the filtrate was evaporated to dryness. Purification by chromatography (EtOAc) gave a solid which was recrystallized from acetone to give the title compound, (~quantitative), identical in all respects to authentic material (20) (mp, mixture mp, X-ray powder diffraction diagram, $[\alpha]_D$, ir, nmr); mp 189–192°C; $[\alpha]_D$ −31.3° (*c* 0.53, MeOH). *Anal.* calcd. for $C_{30}H_{40}N_2O_{11}$: C 59.59, H 6.67, N 4.63; found: C 59.55, H 6.78, N 4.46.

7,9-Tri-O-*(2,2,2-trichloroethoxycarbonyl)-4,4a*-O-*isopropylidene*-N,N′-*[(benzyloxy)carbonyl]-4(*R*),4a(*R*)-tetrahydrospectinomycin, **8***

The preceding compound (600 mg, 1 mmol) was suspended in 10 mL of benzene, 1 mL of 2,2-dimethoxypropane and 10 mg of *p*-TsOH were added, and the homogenous solution was stirred at 25°C for 1 h. The acid was neutralized with Dowex-1 (OH^-), the suspension filtered, and the filtrate was evaporated to dryness. Purification by silica gel chromatography (hexanes–EtOAc, 4:1, then 1:1) gave the expected acetonide derivative as colorless crystals (526 mg, 78%); mp 189–193°C; $[\alpha]_D$ −46.1° (*c* 0.94, $CHCl_3$); ir ν_{max} (KBr): 3450 (OH), 1680 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.26 (d, C-Me); 1.37, 1.51 (s, CMe_2); 1.79 (dd, H-3,3′), 2.14 (d, H-3 eq.), 3.09 (s, NMe_2), 4.51 (d, H-10a, $J_{10a,4a}$ = 7 Hz), 5.14 (s, CH_2Ph), 7.35 (OH, Ph); ms *m/e*: 644 (M^+), 626 ($M^+ - H_2O$), etc. *Anal.* calcd. for $C_{33}H_{44}N_2O_{11}$: C 61.48, H 6.88, N 4.34; found: C 60.92, H 6.82, N 4.19.

A solution of the preceding compound (315 mg, 0.47 mmol) in 20 mL of dry pyridine containing 30 mg of *N,N*-dimethylaminopyridine was treated at 0°C with trichloromethyl chloroformate (1 mL, 7 mmol). The solution was left at 25°C overnight, excess reagent was destroyed by adding ice-water (30 min), and the mixture was evaporated in the presence of toluene to eliminate the pyridine. The residue was extracted with ether, the latter was washed successively with aqueous ammonium chloride, then water, and the organic phase was processed as usual. Chromatography over silica (hexanes–EtOAc, 7:3) gave the title compound as a glassy semi-crystalline solid (434 mg, 67%); mp 100–105°C; $[\alpha]_D$ −16.9° (*c* 1.16, $CHCl_3$); ir ν_{max} (KBr): 1765 (OCO_2), 1700 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.22 (d, C-Me); 1.34, 1.49 (s, CMe_2); 2.80 (s, NMe_2), 4.48 (d, H-10a, $J_{10a,4a}$ = 7 Hz), 4.75 (s, CH_2CCl_3), 5.13 (s, CH_2Ph), etc. *Anal.* calcd. for $C_{42}H_{43}Cl_9N_2O_{17}$: C 43.23, H 3.71, Cl 27.34, N 2.40; found: C 43.78, H 4.27, Cl 27.19, N 2.38.

4-O-*acetyl-7,9*-O-*(2,2,2-trichloroethoxycarbonyl)*-N,N′-*[(benzyloxy)carbonyl]-4(*R*),4a(*R*)-tetrahydrospectinomycin, **10***

Compound **8** (300 mg, 0.22 mmol) was dissolved in a mixture of THF (5 mL) and 9:3 methanol–water (10 mL) containing 40 mg of *p*-TsOH. After stirring for 48 h the acid was neutralized with Dowex-1 (OH^-), the mixture was filtered, and the filtrate was evaporated to dryness to given an amorphous solid (280 mg); $[\alpha]_D$ −7.9° (*c* 0.26, $CHCl_3$); ir ν_{max} (KBr): 3450 (OH), 1765 (OCO_2), 1705 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.19 (d, C-Me), 2.82 (s, NMe_2), 5.15 (s, CH_2Ph), 7.34 (s, Ph), etc.

The preceding compound (280 mg, 0.248 mmol), in 10 mL of benzene, was treated with 0.5 mL of trimethylorthoacetate and 10 mg of *p*-TsOH. The solution was stirred at 25°C for 1 h, the acid was neutralized with dry Dowex-1 (OH^-), and the suspension was filtered. Evaporation of the filtrate gave a syrup consisting of **9**, which was dissolved in 10 mL of 80% aqueous acetic acid, and the solution was stirred for 30 min. The solvent was evaporated and the residue treated with toluene several times (with evaporation), to give a syrup that was chromatographed (hexane–EtOAc, 7:3). The desired acetate was obtained as an amorphous solid (hexanes) (270 mg, ~quantitative); $[\alpha]_D$ −21.0° (*c* 0.63, $CHCl_3$); ir ν_{max} (KBr): 3500 (OH), 1770 (OCO_2), 1740 (OAc), 1710 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.20 (d, C-Me), 2.11 (s, OAc), 2.82 (s, NMe_2), 3.41 (dd, H-4a, $J_{4a,10a}$ = 8 Hz, $J_{4a,4}$ = 4 Hz), 4.75 (s, CH_2CCl_3), 5.14 (s, CH_2Ph), 7.34 (s, OH, Ph). *Anal.* calcd. for $C_{41}H_{45}Cl_9N_2O_{13}$: C 41.99, H 3.87, N 2.39; found: C 41.53, H 3.85, N 2.31.

4-O-*acetyl*-N,N′-*[(benzyloxy)carbonyl]-4(*R*)-dihydrospectinomycin, **12***

(a) *From **10***

The preceding (160 mg, 0.14 mmol) was oxidized in the presence of PCC (90 mg, 6 equiv.) in refluxing benzene (10 mL) for 1 h. Excess ether was added, the suspension was filtered over "Florisil", and the filtrates and washing were processed as usual to give a syrup that was chromatographed (hexanes–EtOAc, 7:5). The unstable intermediate 4a-keto derivative **11** was collected in appropriate fractions, and immediately used in the next step. Thus, the resulting syrup was dissolved in 10 mL of glacial acetic acid, 130 mg of zinc dust (freshly activated by washing with 1 *N* HCl), and the mixture was stirred for 30 h. The reaction mixture was poured into aqueous saturated sodium bicarbonate – EtOAc, and the organic extracts were washed and processed as usual to give the title compound. This was purified by preparative thick layer chromatography on silica gel (EtOAc) (syrup, 60 mg, 64%), and was identical in all respects with material prepared from the natural product.

(b) *From* N,N′-*[(benzyloxy)carbonyl]-4(*R*)-dihydrospectinomycin*

The title compound (600 mg, 1 mmol), readily available from spectinomycin (13a, 32), was suspended in 15 mL of benzene, 1 mL of trimethylorthoacetate and 10 mg of *p*-TsOH were added, and the mixture was stirred for 1 h. The homogeneous solution was then treated with Dowex-1 (OH^-) and filtered. Processing the filtrate gave a syrup which was dissolved in 20 mL of 80% aqueous acetic acid (30 min). Evaporation and chromatography as previously described gave the 4-*O*-acetyl derivative **12** as a syrup (494 mg, 77%); $[\alpha]_D$ +27.2° (*c* 1.30, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3600, 3640 (OH); 1730 (OAc), 1690 (Cbz) cm^{-1}; ^{1}H nmr (90 MHz, ppm): 1.26 (d, C-Me), 1.69 (m, H-3,3′), 2.14 (s, OAc); 3.04, 3.07 (s, NMe_2); 4.82 (s, H-10a), 4.95 (t, H-4), 5.15 (s, CH_2Ph), 7.34 (s, Ph).

N,N′-*[(benzyloxy)carbonyl]-4(*R*)-dihydrospectinomycin, **13***

The acetate **12** (100 mg, 0.155 mmol) was dissolved in 5 mL of

methanol containing 0.05 mL of a 1 *N* sodium methoxide solution (pH ~8) and the solution was stirred at 25°C for 15 min. The base was neutralized with Dowex-50 (H^+), the mixture was filtered, and the filtrate was processed as usual to give an amorphous solid (93 mg, ~quantitative), mp 157–160°C; $[\alpha]_D$ +27.5° (*c* 0.50, $CHCl_3$); ir ν_{max} (KBr): 3420 (OH), 1680 (Cbz) cm^{-1}; ^1H nmr (90 MHz, ppm): 1.22 (d, C-Me), 1.69 (m, H-3,3′); 3.02, 3.07 (s, NMe_2); 4.84 (s, H-10a), 5.13 (s, CH_2Ph), 7.33 (OH, Ph).

This product was found to be identical with a sample prepared from spectinomycin (13*a*, 32) (tlc, $[\alpha]_D$, ir, nmr, ms).

Treatment of **13** (30 mg) with 2,2-dimethoxypropane in benzene containing *p*-TsOH gave the corresponding 4,4a-acetonide derivative **14** in 96% yield, identical with material prepared from spectinomycin; glassy amorphous solid, $[\alpha]_D$ +29.6° (*c* 0.27, $CHCl_3$) (lit. (13*a*, 32) $[\alpha]$ +31.17 ($CHCl_3$).

N,N′-*[(benzyloxy)carbonyl]spectinomycin (16)*

A solution containing 298 mg (1 equiv.) of bis(tri-n-butyltin) oxide and **13** (300 mg, 0.5 mmol) in 20 mL of benzene was stirred at 25°C for 1 h, then treated with NBS (134 mg, 1.5 equiv.). After stirring for 10 h, hexanes were added to precipitate the title compound in almost pure form as an amorphous solid (240 mg, 80%). Chromatography on silica gel (hexanes–EtOAc, 2:3) gave pure product, identical with a sample prepared from the antibiotic; $[\alpha]_D$ −4.6° (*c* 0.28, $CHCl_3$); ir ν_{max} (KBr): 3460 (OH), 1685 (Cbz) cm^{-1}; ^1H nmr (90 MHz, ppm): 1.41 (d, C-Me), 1.51 (m, H-3e), 1.65 (m, H-3a); 2.98, 3.08 (NMe_2); 4.70 (s, H-10a), 5.14 (s, CH_2Ph), 7.34 (OH, Ph), etc; ms *m/e*: 600 (M^+), 492 (M^+ − $PhCH_2OH$), 4.65 (M^+ − $PhCH_2OCO$), etc.

The oxidation of **13** (200 mg, 0.33 mmol) was also done in the presence of di-n-butyltin oxide (90 mg, 1.1 equiv.). The mixture was refluxed in methanol (10 mL) for 1 h, evaporated, benzene added, and the solution evaporated again to give a syrup. The stannylidene derivative was dissolved in 5 mL of dichloromethane containing 0.12 mL (1.1 equiv.) of tri-n-butyltin methoxide (24) and the solution was cooled to 0°C. This was treated with a solution of bromine (1.1 equiv., 2% in CH_2Cl_2) dropwise, over a period of 1 h. Residual bromine was destroyed with cyclohexene and the crude product was isolated by precipitation with hexanes to give an amorphous solid (184 mg, 92%). Chromatography as described above gave 139 mg (70%) of pure product.

(+)-Spectinomycin dihydrochloride

A solution of the previous compound (140 mg, 0.23 mmol) in a mixture of 2-propanol and water (~1:1, 5 mL) was hydrogenated in the presence of 100 mg of 5% palladium-on-charcoal, while monitoring the effluent gas for cessation of CO_2 evolution (aqueous $Ca(OH)_2$). The catalyst was filtered, the filtrate was evaporated, and the residue was dissolved in water (3 mL) and acidified with HCl to pH 3. Repeated evaporation with toluene gave a solid which was recrystallized from aqueous acetone (~5 mL). After standing at 0°C overnight, beautiful colorless needles of (+)-spectinomycin dihydrochloride·$5H_2O$ were obtained (104 mg, 90%), mp 205–207°C (dec.); $[\alpha]_D$ +14.8° (*c* 0.42, H_2O), identical to the authentic natural product (9); ir ν_{max} (KBr): 3300 (OH), 1740 (C=O, weak) cm^{-1}, etc.; ^1H nmr (90 MHz, ppm, D_2O): 1.25 (d, C-Me); 2.79, 2.82 (s, NMe_2); 4.86 (s, H-10a); ^{13}C nmr (22.6 MHz, ppm, D_2O): 94.4 (C4a, C10a), 92.6 (C4), 70.6 (C9a), 69.2 (C2), 66.9 (C9), 66.5 (C5a), 62.3 (C8), 60.3 (C7), 59.4 (C6), 42.6 (C3), 31.5 (N8-Me), 31.1 (N6-Me), 20.5 (C2-Me); see also ref. 11; X-ray Cu-powder diffraction diagram (mm distance, m, medium, s, strong): 16.1 m, 24.5 ms, 28 m, 29.2 m, 31.8 s, 42 m, 44.7 m, 51.0 m, 52.9 mm, 54.8 mm, 59.7.

N,N′-*[(benzyloxy)carbonyl]spectinoic acid, 21*

A solution of **16** (1 g, 1.67 mmol) in 25 mL of THF and 2.5 mL of *N* NaOH was stirred for 1 h, then the solution was neutralized with Dowex-50 (H^+). The resin was filtered, and the filtrate was evaporated to a syrup which was dissolved in ethyl acetate. Addition of hexanes gave the title compound as an amorphous solid (950 mg, 92%); $[\alpha]_D$ −47.3° (*c* 2.06, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3440 (OH, CO_2H), 1730 (CO_2H), 1685 (Cbz) cm^{-1}; ^1H nmr (90 MHz, ppm): 1.36 (d, C-Me), 2.19 (m, H-4, 4′); 2.9, 2.04 (s, NMe_2); 5.30 (s, H-2), 5.10 (s, CH_2Ph), 7.30 (Ph), etc.; ms *m/e*: 600 (M^+ − H_2O).

Esterification of **21** with diazomethane in a mixture of ether and THF gave the corresponding methyl ester, isolated as an amorphous solid; $[\alpha]_D$ −48.2 (*c* 0.71, $CHCl_3$); ms *m/e*: 632 (M^+).

Alternatively, treatment of **16** (1 g, 1.67 mmol) with a solution of sodium methoxide (20 mL, pH 8.5, 30 min), followed by neutralization with Dowex-50 (H^+) and processing, gave the ester (787 mg, 75%) as an amorphous solid.

N,N′-*[(benzyloxy)carbonyl]spectinoic acid lactone, 22*

(a) *From **16** under acid catalysis*

A solution containing **16** (600 mg, 1 mmol) in 25 mL of EtOAc and 2 mL of glacial acetic acid was refluxed overnight. A new product was formed with a slightly higher tlc mobility ($CHCl_3$–MeOH, 9:1). The solution was evaporated to dryness in the presence of toluene and the residue was chromatographed over silica gel (hexanes–EtOAc, 2:3) to give 457 mg (76%) of the title lactone as an amorphous solid; $[\alpha]_D$ +67° (*c* 1.45, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3360, 3320 (OH); 1750 (lactone), 1685 (Cbz) cm^{-1}; ^1H nmr (90 MHz, ppm, DMSO-d_6): 1.25 (d, C-Me), 2.20 (m, H-4,4′); 2.95, 299 (s, NMe_2); 5.35 (s, H-2); 5.13, 5.16 (s, CH_2Ph); 7.37 (s, Ph), etc.; ms *m/e*: 600 (M^+), 601 (M^+ + 1), etc.

(b) *From **16** in presence of $(Bu_3Sn)_2O$ and Br_2*

A mixture containing bis(tri-n-butyltin) oxide (596 mg, 1 mmol, 1 equiv.) and **16** (400 mg, 0.67 mmol) in 10 mL of dichloromethane was stirred for 2–3 min, then treated dropwise with 0.05 mL of bromine in 1 mL of dichloromethane under argon. The desired product was formed after 4 h at 25°C. The solution was evaporated to a small volume and the product was precipitated with hexanes, leaving the tin by-products in solution. The solid thus obtained was chromatographed to give 330 mg (83%) of lactone **22**.

(c) *From **13** in presence of bis(tri-n-butyltin) oxide and bromine*

A solution of **13** (200 mg, 0.33 mmol) and 258 mg (0.43 mmol, 1.3 equiv.) of the tin derivative in 10 mL of dichloromethane was treated with bromine (0.02 mL, 1.3 equiv. in 1 mL of dichloromethane) dropwise under argon. After similar treatment as described above, the desired lactone was obtained (170 mg, 85%).

Chemical transformations of the lactone 22

(a) *Methanolysis*

Treatment of **22** (105 mg, 0.18 mmol) in 15 mL of methanol containing 50 mg of potassium carbonate (or sodium methoxide), followed by neutralization with Dowex-50 (H^+), after 10 min gave the methyl ester of **21** (see above) (118 mg, ~quantitative); $[\alpha]_D$ −48.2° (*c* 0.48, $CHCl_3$).

(b) *Base hydrolysis*

A solution of the lactone **22** (500 mg, 0.67 mmol) in 10 mL of THF was cooled to 0°C and treated with *N* NaOH (1 mL, 1.5 equiv.); neutralization with Dowex-50 (H^+), followed by processing, gave **21** (413 mg, ~quantitative) as an amorphous solid.

Spectinoic acid lactone dihydrochloride 23

A solution of **22** (400 mg, 0.67 mmol) in 10 mL of 2-propanol containing 1 mL of water and 1.6 mL *N* HCl was hydrogenated in the presence of 10% palladium-on-charcoal (100 mg). After 1 h the catalyst was filtered, and the filtrate was processed as usual by co-evaporation of the solvent with toluene to give an amorphous solid (225 mg, 83%); $[\alpha]_D$ −64.3° (*c* 1.12, H_2O); ir ν_{max} (KBr): 1720 (C=O) cm^{-1}; ^{13}C nmr (22.6 MHz, ppm, D_2O): 21.8, 31.7, 41.0, 60.1, 61.9, 68.1, 69.4, 77.8, 81.1, 86.9, 108.8, 174.9.

Methanolysis of spectinoic acid. Isolation of 24 and 25

This experiment was done essentially according to a literature procedure (8). A solution containing 10 g of spectinomycin free base (liberated from the salt with Dowex-1) and 20 g of barium hydroxide in 500 mL of water was stirred at room temperature for 24 h. The suspension was filtered, excess barium ions were precipitated with *N* H_2SO_4 until pH ~ 5.5, and the mixture was filtered over Celite. The filtrate was evaporated in the presence of methanol and the resulting

spectinoic acid **20** was used in the next step. The residue was then dissolved in methanol (600 mL) containing 95 mL of acetyl chloride (**caution!**). After 48 h of stirring, ether was added to precipitate actinamine dihydrochloride (7 g). The filtrate was neutralized with sodium methoxide until pH 6–7, the salts were filtered off, and the filtrate was evaporated to dryness. The residue was extracted with ethyl acetate, washed with water, and the organic phase was processed as usual to give 3.06 g (58%) of a colorless syrup. Flash chromatography (hexanes–EtOAc, 3:2) gave an anomeric mixture of glycosides **24** (2.31 g, 75%) and the lactone **25** (0.77 g, 25%). The mixture of glycosides **24** (showed α_D −67.4° $CHCl_3$) (lit. (8) $[\alpha]_D$ −65.5° for a product distilled at 51–54°C (0.05 Torr; 1 Torr = 133.3 Pa)). The lactone **25** showed $[\alpha]_D$ +3.3° (*c* 0.55, $CHCl_3$); ir ν_{max} (film): 3400 (OH), 1770 (γ-lactone) cm^{-1}; ^1H nmr (90 MHz, ppm): 1.43 (d, C-Me); 1.85, 2.79 (dd, H-3, J_{gem} = 13 Hz, J_{vic} = 7.9 Hz); 3.53, 3.63 (s, OMe); 3.55 (s, OH), 4.40 (s, $CH(OMe)_2$), 4.63 (m, H4). The crystalline *p*-nitrobenzoate derivative (DMAP, *p*-nitrobenzoyl chloride) showed mp 123.5–125°C (ether–hexanes); $[\alpha]_D$ −20.0° (*c* 0.19, $CHCl_3$).

*Transformation of **24** into the lactone **26***

A solution containing the mixture of glycosides **24** (210 mg, 1.06 mmol) in 10 mL of ether was reduced with lithium aluminium hydride (130 mg, 2.67 mmol). After 30 min, water was carefully added to the cooled reaction mixture, then excess ether. The organic phase was separated and processed as usual to give a syrup (170 mg, 93%). This product was hydrolyzed in a mixture of THF and *N* HCl (10 mL, 1:1) by refluxing for 1.5 h. After neutralization with Dowex-1 (OH^-), filtration, and evaporation, a syrup was obtained which was purified by flash column chromatography ($CHCl_3$–MeOH, 95%) to give 130 mg (85%) of a chromatographically homogeneous product. Oxidation of a portion (90 mg, 0.60 mmol) in 7 mL acetonitrile, containing 166 mg (0.84 mmol) of barium carbonate, with 0.04 mL (0.72 mmol) of bromine diluted in 1 mL of acetonitrile gave, after 4 h, the desired lactone. Addition of cyclohexene, filtration, and evaporation gave a syrup corresponding to the lactone **26**. The crystalline *p*-nitrobenzoyl derivative **29** was prepared in the usual manner, mp 127–129°C (ether–hexanes); $[\alpha]_D$ +20.9° (*c* 0.87, $CHCl_3$); ir ν_{max} (KBr): 3500 (OH), 1745 (γ-lactone), 1729 (ester); 1520, 1340 (NO_2) cm^{-1}; ^1H nmr (90 MHz, ppm): 1.49 (d, C-Me); 2.02, 2.57 (dd, H-3,3′, J_{gem} = 14 Hz, J_{vic} = 6, 8 Hz); 3.03 (s, OH), 4.59 (d, CH_2OH), 4.88 (m, H-4), 8.26 (m, arom); ms *m/e*: 295 (M^+). *Anal.* calcd. for $C_{13}H_{13}NO_7$: C 52.86, H 4.44, N 4.74; found: C 52.66, H 4.54, N 4.59.

*α-D-Isosaccharino-1,4-lactone acetonide, **27***

α-D-Isosaccharino-1,4-lactone (37) (324 mg, 2 mmol) was dissolved in a 1:5 mixture of 2,2-dimethoxypropane and acetone, and 15 mg of *p*-TsOH was added. After stirring 1 h, the acid was neutralized with Dowex-15 (OH^-), the mixture filtered, and the filtrate was processed as usual to give the title compound as colorless crystals (398 mg, 99%), mp 53–55°C (ether–hexanes); $[\alpha]_D$ +41.5° (*c* 0.46, $CHCl_3$) (lit. (42) mp 56–57°C).

*5-Chloro-5-deoxy-α-D-isosaccharino-1,4-lactone acetonide, **28***

The preceding compound (311 mg, 1.54 mmol) was dissolved in 2 mL of $CHCl_3$ and 2 mL of pyridine, then heated with 0.2 mL of sulfuryl chloride at 0°C. After stirring overnight, the solution was diluted with water, then chloroform, and the organic phase was separated and processed. Evaporation gave a pale yellow syrup which was purified by column chromatography (hexanes–EtOAc, 7:3) to give 292 mg (86%) of the title product as a colorless syrup, $[\alpha]_D$ +28.8° (*c* 0.72, $CHCl_3$).

*Transformation of **28** into lactone **26***

An amount of the chloro derivative **28** (687 mg, 3.1 mmol) was dissolved in 30 mL of toluene and 3.27 mL (12.4 mmol) of tri-n-butyltin hydride was added, followed by 20 mg of azobisisobutyronitrile (AIBN). The mixture was refluxed for 4 h, cooled, the solvents evaporated, and the resulting oily residue was triturated with hexanes to remove a large portion of tin by-products. The residue was dissolved in acetonitrile, hexane added to deposit an oil, and the upper phase decanted. Evaporation of excess solvent gave the 5-deoxy derivative as a syrup (547 mg, 95%); $[\alpha]_D$ +31.7° (*c* 1.03, $CHCl_3$). Hydrolysis of the acetonide (547 mg, 2.94 mmol) was done with 80% aqueous acetic acid at 80°C for 30 min. Evaporation of the solvent and column chromatography gave the desired lactone **26** (430 mg); $[\alpha]_D$ +34.4° (*c* 0.22, $CHCl_3$). Transformation into the *p*-nitrobenzoate derivative **29** was done in the usual manner (DMAP) to give material identical in all respects with a sample obtained from the methanolysis and oxidation of spectinoic acid (mp, mixture mp, $[\alpha]_D$, ir, nmr).

*4-O-methanesulfonyl-N,N′-[(benzyloxy)carbonyl]-4-(S)-dihydrospectinomycin, **31***

To a solution of *N,N′*-[(benzyloxy)carbonyl]-4-(*S*)-dihydrospectinomycin **30**, readily available by catalytic hydrogenation of spectinomycin (43), followed by *N*-benzyloxycarbonylation (600 mg, 1 mmol) in 10 mL of pyridine, was added methanesulfonyl chloride (0.12 mL, 1.3 equiv.) at 0°C. The mixture was stirred at 0°C for 18 h, then ice-water was added, followed by extraction with ethyl acetate. Processing of the organic phase gave a residue which was chromatographed on silica gel ($CHCl_3$–MeOH, 97%) to give 530 mg (79%) of the title compound as an amorphous solid, $[\alpha]_D$ +2.60 (*c* 0.73, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3560, 3390 (OH); 1685 (Cbz); 1340, 1170 (SO_2Me) cm^{-1}; ^1H nmr (90 MHz, ppm, DMSO-d_6): 1.21 (d, C-Me); 2.95, 2.97 (s, NMe_2); 3.20 (s, Ms), 4.62 (s, H-10a), 5.11 (s, CH_2Ph), 7.36 (O, H, Ph), etc.; ms *m/e*: 584 (M^+ − $MeSO_3H$). Calcd.: 584.236; found: 584.236.

*Solvolysis studies with **31***

(a) *With silver carbonate*

A solution of **31** (50 mg, 0.073 mmol) in 10 mL of 1:1 acetone–water mixture was refluxed in the presence of 100 mg of freshly prepared silver carbonate. The suspension was filtered over Celite, the filtrate was evaporated in the presence of 2-propanol, and the residue was chromatographed over thick layer plates (EtOAc). The major product, 21 mg (47%), was found to be *N,N′*-[(benzyloxy)carbonyl]-4-(*R*)-dihydrospectinomycin **13**, identical in all respects with authentic material. Another product (3 mg, 9%) was identified as *N,N′*-[(benzyloxy)carbonyl]actinamine.

(b) *With sodium acetate*

Compound **31** (50 mg, 0.073 mmol) was dissolved in 3 mL of anhydrous trifluoromethanol containing 96 mg (10 equiv.) of anhydrous sodium acetate. After refluxing for 18 h, the solvent was evaporated and the residue was chromatographed on thick layer plates (EtOAc). The major product (18 mg, 38%) was identified as the 4-*O*-acetyl derivative **12** (ir, $[\alpha]_D$, nmr). Deacetylation of this product gave **13**.

(c) *With sodium azide*

A solution of **31** (340 mg, 0.5 mmol) in 20 mL of trifluoromethanol containing 1.5% water was treated with finely powdered sodium azide (4.89 mg, 1.5 equiv.) and the mixture refluxed for 4 h. Filtration and evaporation gave a syrup which was chromatographed on silica (hexanes–EtOAc, 3:7) to give the 4-azido derivative **32** (syrup, 93 mg, 30%) and unreacted starting material (128 mg). The azide showed $[\alpha]_D$ +20.1° (*c* 0.38, $CHCl_3$); ir ν_{max} ($CHCl_3$): 3600, 3200 (OH); 2100 (N_3), 1685 (Cbz) cm^{-1}.

*4-Amino-4-deoxy-(S)-dihydrospectinomycin dihydrochloride, **33***

A solution containing 70 mg (0.11 mmol) of the azide **32** in 7 mL of ethanol and 0.1 mL of aqueous HCl was hydrogenated in the presence of 25 mg of 10% palladium-on-charcoal. After 3 h, the catalyst was filtered, and the filtrate was processed as usual to give a syrup. The latter was taken up in a small volume of ethanol and ether was added to precipitate the product. The hygroscopic solid was separated from the supernatant by decantation, then it was dried in a desiccator to give 45 mg (92%) of the title compound as an amorphous solid, mp 198–200°C (dec.); $[\alpha]_D$ +11.4° (*c* 0.49, H_2O); ^1H nmr (400 MHz, ppm, D_2O): 1.36 (d, C-Me), 1.77 (dd, H-3ax, $J_{3ax,3eq}$ = 12.7 Hz, $J_{3ax,4}$ = 3.9 Hz, $J_{3eq,2}$ = 1.7 Hz), 2.88 (s, NMe_2), 3.32 (dd, H-6, $J_{6,5a}$ = 10.1 Hz, $J_{6,7}$ = 2.9 Hz), 3.61 (dd, H-8, $J_{8,9}$ = 11.0 Hz, $J_{8,7}$ = 2.84 Hz), 3.71 (dd, H-4), 3.95 (m, H-2), 4.01 (dd, H5a, $J_{5a,9a}$ =

10.1 Hz), 4.11 (dd, H-9a, $J_{9a,9}$ = 10.0 Hz), 4.37 (dd, H-9), 4.79 (dd, H-7), 4.96 (s, H-10a); ms *m/e*: 334 ($M^+ + 1$, 315 ($M^+ - H_2O$), etc.

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