

SPECTINOMYCIN MODIFICATION

II. SPECTINOMYCIN C-3'-MODIFICATION
VIA DIAZOKETONE INTERMEDIATES

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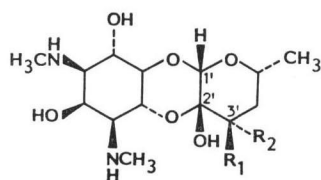
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The C-3'-carbonyl group of *N*-protected spectinomycin is efficiently converted into a diazo group *via* base treatment of the corresponding tosylhydrazone. The diazo group imparts a new synthetically useful reactivity pattern on the sugar ring of the molecule. The synthesis of C-3'-deoxo-, monohalo- and dihalospectinomycins *via* the intermediacy of these diazo compounds is described. The reduced bioactivity of these analogs as compared to the parent and the C-3'-dihydro and aminospectinomycins established the need for hydrogen bonding groups in this region of the molecule for good activity, further refining the structure activity relationships in the spectinomycin series.

One of the fundamental questions regarding spectinomycin structure-activity relationships is the role played by the C-3' substituent. In aqueous solution, spectinomycin (**1a**) exists as the C-3'-ketone hydrate (**1b**)¹. It is not known whether **1a** or **1b** is in fact the actual form of the antibiotic acting at the ribosomal level. Only a limited number of analogs having modifications at C-3' have been reported in the literature. The dihydrospectinomycins², **1c** and **1d**, retain from 0 to 100% of the *in vitro* bioactivity of the parent, depending on the organisms being tested, with the 3'-*R*-isomer **1c** being the more potent. The corresponding 3'-aminospectinomycins **1e** and **1f** have been reported³, with the 3'-*R*-isomer being more potent than the parent whereas the 3'-*S*-isomer is inactive.

A number of possibilities need to be considered in an attempt to rationalize this data. The observed differences in bioactivity could be due to differing electronegativity of the C-3' substituent. This would account for differences between the alcohols and amines and the di-substituted ketone hydrate. A second, more plausible explanation is the opportunity for hydrogen bonding, inherent in the hydroxyl and amino groups, either as hydrogen bond donors or acceptors. Such an explanation could account



- 1a** $R_1, R_2 = O$
1b $R_1 = R_2 = OH$
1c $R_1 = OH, R_2 = H$
1d $R_1 = H, R_2 = OH$
1e $R_1 = NH_2, R_2 = H$
1f $R_1 = H, R_2 = NH_2$

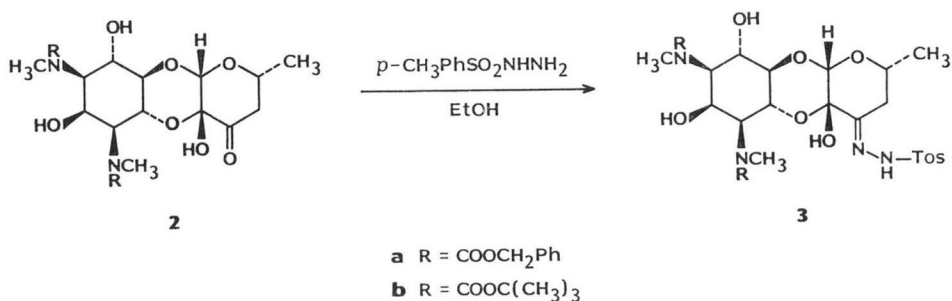
for the difference in activity seen in the epimeric dihydro-analogs where the orientation of the substituents would allow interaction at different points on the receptor site. A third possibility is the involvement of the C-3' substituents in covalent interactions at the receptor site. While this is an attractive choice for the parent **1a** with its extremely electrophilic C-3' carbonyl group, this rationale seems less likely for the dihydro-compounds.

We have been actively pursuing the synthesis of C-3' modified analogs of spectinomycin to

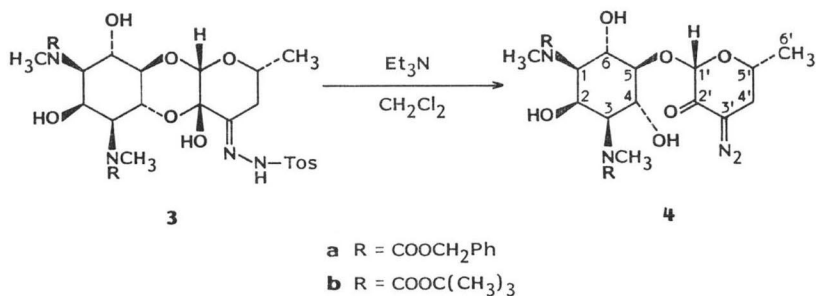
further evaluate the structure-activity relationships in this series. In this and the accompanying paper we describe the synthesis of a number of C-3' analogs *via* the intermediacy of novel spectinomycin derived diazoketones. In subsequent papers we will detail other, more efficient approaches to the most active of these analogs.

Results and Discussion

The sensitivity of spectinomycin to both acid and base has been an important consideration in developing methodology for modification of this antibiotic. In the present study, advantage has been taken of the formation of tosylhydrazone derivatives⁴⁾ of *N,N'*-dibenzoyloxycarbonylspectinomycin (**2a**) and *N,N'*-di-*tert*-butoxycarbonylspectinomycin (**2b**). Thus treatment of either **2a** or **2b** with one equivalent of *p*-toluenesulfonylhydrazide results in the quantitative formation of the corresponding tosylhydrazones **3a** and **3b**. The success of this carbonyl derivatization reaction is somewhat surprising in light of the known facile rearrangement of **2**, especially in the presence of base and nucleophiles^{1,5)}. The formation and reduction of some related oxime derivatives serves as the basis for the synthesis of the 3'-aminospectinomycins **1e** and **1f**⁶⁾.

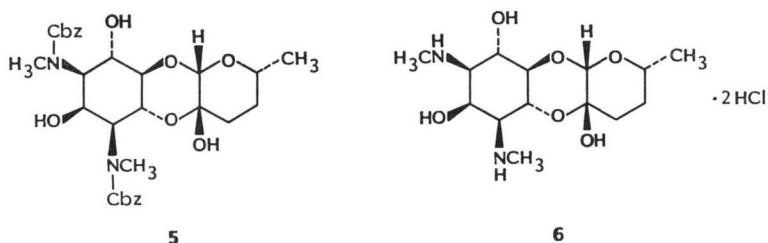


Base catalyzed α -elimination of toluenesulfinic acid⁶⁾ from either tosylhydrazone gives good yields of diazoketones **4**. These compounds are bright yellow solids which are stable for months when stored at 0°C. Spectral evidence clearly establishes the open 2'-keto form for the diazoketones. Thus the ¹³C NMR spectrum shows the 2'-carbonyl carbon and the equivalence of C-1 and C-3 in the actinamine ring. This is the first spectinomycin derivative known which exists exclusively in the 2'-keto form. The preference of these compounds to exist as the *seco* structures is due to the decrease in electrophilicity of the C-2' carbonyl due to resonance interaction with the diazo group.



The exchange of the diazo group for the C-3' carbonyl group of spectinomycin offers the potential to explore new methods of chemical modification of the sugar ring. The usual highly electrophilic nature of the 3'-carbon atom in the keto compound is transformed into nucleophilic character in the diazoketone, thus permitting reaction with a variety of electrophilic reagents. The ready availability of these diazoketones and the choice of protecting groups makes these compounds valuable intermediates for analog synthesis.

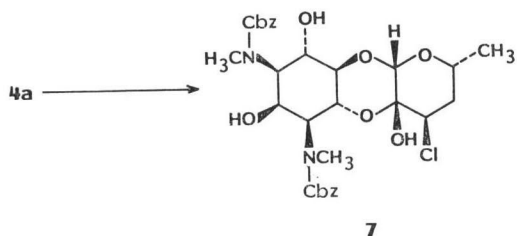
Treatment of **4a** with zinc dust in aqueous acetic acid produces two major products in approximately equal amounts. One product was identified as *N,N'*-dibenzoyloxycarbonylspectinomycin (**2a**) by comparison with an authentic sample. The other product was identified as *N,N'*-dibenzoyloxycarbonyl-3'-deoxospectinomycin (**5**). The formation of ketone **2a** is presumed to occur *via* reduction of the diazo group to the corresponding hydrazone⁷⁾ followed by hydrolysis. The isolation of **2a** confirms the fact that no unanticipated rearrangements of the sugar moiety had occurred during the preparation of the diazoketone. The deoxo compound **5** arises directly from the diazo compound and not from reduction of **2a** since the latter was shown to be stable to the reaction conditions. *N,N'*-Dibenzoyloxycarbonyl-3'-deoxospectinomycin was identified by ¹³C NMR; in particular by the presence in the off-resonance spectrum of a new triplet due to C-3'. Like the parent structure, **5** exists in the closed C-2' hemiketal form. Deprotection of **5** *via* catalytic hydrogenolysis affords 3'-deoxospectinomycin dihydrochloride (**6**).



With the fully unsubstituted analog **6** in hand, we next turned our attention to the preparation of spectinomycin analogs with 3'-halogen substitution to explore the effect of non-hydrogen bonding electronegative substituents on bioactivity. The C-3'-chloro, bromo and dichlorospectinomycins provide an interesting test of the relative importance of electronegativity and hydrogen bonding of the substituents. In particular, the C-3'-*gem*-dichloro compound is a good mimic for the normal spectinomycin carbonyl hydrate in terms of steric bulk and electronegativity.

Numerous examples of the conversion of diazo compounds into mono and dihalo compounds, by reaction with hydrogen halides or halogens, appear in the literature⁸⁾. These reactions are generally facile, high yielding processes and work well with diazoketones and esters. In the case at hand, however, the nature of the substrates **4a** and **4b** places special requirements on these reactions and on further transformation of the products. The most serious of these problems is the propensity of these spectinomycin derived diazoketones to undergo rearrangement under the influence of heat, electrophiles or metal ion catalysis (*vide infra*).

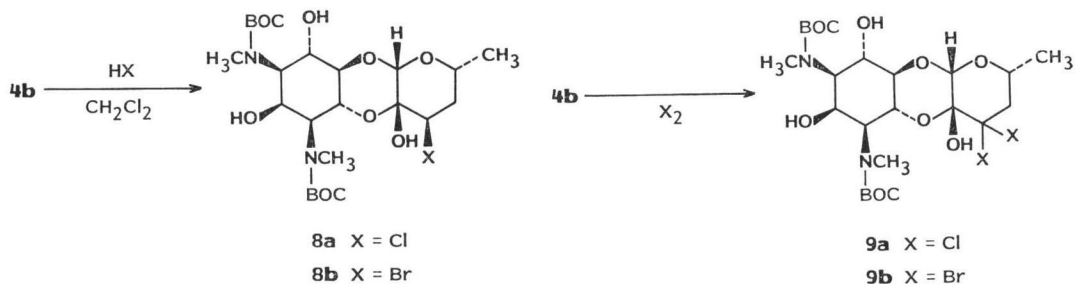
The first of these reactions to be explored was the preparation of the benzyloxycarbonyl (Cbz) protected monochloride **7**. Reaction of **4a** with hydrogen chloride in methylene chloride resulted in immediate loss of the yellow color with evolution of nitrogen gas. Chromatography of the reaction mixture gave the C-3'-chloride **7**. Attempted removal of the protecting groups by catalytic hydrogenolysis was accompanied



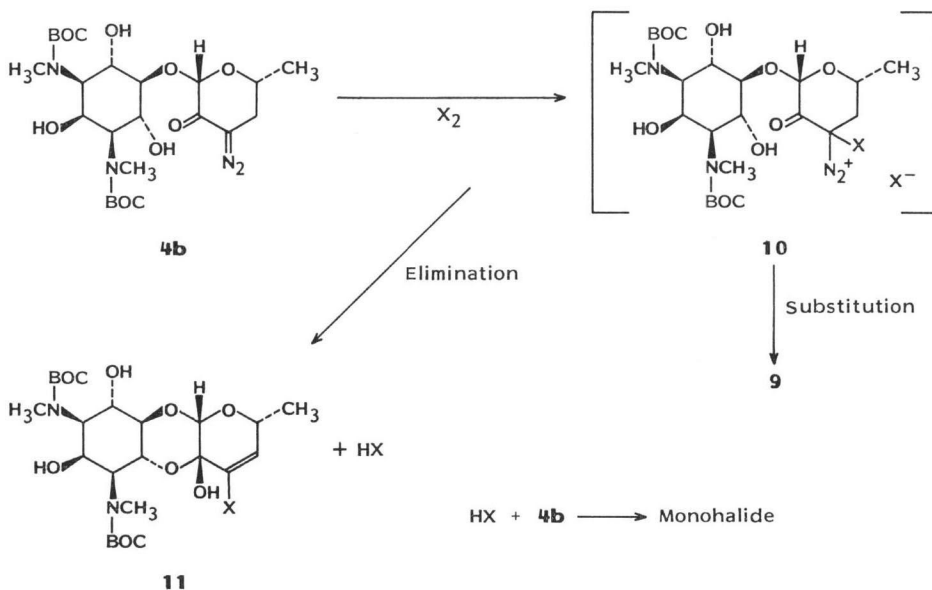
by reductive dehalogenation giving the methylene compound **6** rather than the desired chloride. Clearly, a protecting group other than one removable by hydrogenation was needed.

Our successful experience with the *tert*-butoxycarbonyl (*t*-BOC) protecting group in other spectinomycin chemistry led us to explore this mode of protection. The fundamental ques-

tion to be answered was the relative reactivity of the *t*-BOC and diazo groups towards HCl and HBr since the conditions for reaction of diazo groups with HCl are precisely those conditions we utilize for *t*-BOC removal. In the event, it was found that reaction of the diazoketone functionality with HCl and HBr was sufficiently faster than *t*-BOC removal to allow the preparation and isolation of the *t*-BOC protected monohalides **8a** and **8b**. Competitive removal of the protecting groups did occur to some extent, but no attempt was made to reprotect that portion of the crude product that had suffered deprotection. The highest selectivity, and therefore best yields, were obtained by treatment with one equivalent of the hydrogen halide at low temperature. The products, isolated by chromatography,



Scheme 1.

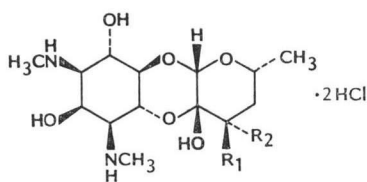


were assigned the 3'-*R* configuration on the basis of NMR studies on the related Cbz protected compounds. Attempts to extend the reaction to the preparation of the corresponding fluoride were unsuccessful.

The reaction of diazoketone **4b**, with chlorine and bromine gave the desired C-3'-dihalospectinomycins **9a** and **9b**. An undesired competitive reaction pathway was encountered in these cases, resulting in the formation of the C-3'-monohalides and the *Δ*-3',4'-vinylhalides. The rationale that best explains the production of these products is shown in Scheme 1. Reaction of diazoketone with the electrophilic halogen generates an intermediate ion pair **10**. Substitution affords the desired *gem*-dihalide **9**. Competitive elimination generates vinyl halide **11** and an equivalent of HX. Reaction of the starting material with the liberated HX produces the monohalide. Although the conditions have not been optimized, low temperature and an excess of the halogen appear to favor the formation of the dihalides.

With the protected mono- and dihalides **8a**, **8b**, **9a** and **9b** in hand, attention was turned to their de-

protection and bioassay. The *t*-BOC protecting groups were removed in excellent yield by treatment with gaseous HCl in methylene chloride to afford the hydrochloride salts **12a~d**. Compounds **12a~c** were stable products whose structure was established by spectral means and gas chromatography-mass spectrometry (GC-MS). Compound **12d** was shown to be the desired dibromide by GC-MS. Bioautography showed a new bioactive product but revealed that **12d** was



12a R₁ = Cl, R₂ = H

12b R₁ = Br, R₂ = H

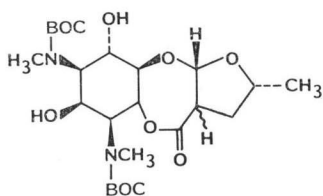
12c R₁ = R₂ = Cl

12d R₁ = R₂ = Br

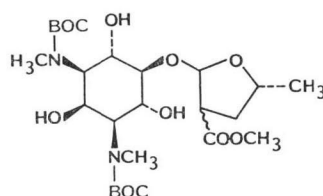
unstable and hydrolyzed to give the parent spectinomycin.

As mentioned previously, one property of the spectinomycin derived diazoketones which has hampered their use in synthesis is their propensity towards rearrangement. Rearrangement has been caused by treatment with acid, metals, other electrophiles and heat. Our initial attempts at extending the successful results described in this paper were frustrated by this rearrangement problem. These experiments are described below. We have subsequently achieved good results in a number of other systems which we will describe in subsequent manuscripts.

The successful conversion of diazo compounds to fluorides by treatment with pyridinium polyhydrogen fluoride has been reported by OLAH⁹⁾. Attempted preparation of a 3'-fluorospectinomycin analog by treatment of diazoketone **4b** with pyridine·(HF)_x complex or HF in methylene chloride was unsuccessful. The only product characterized was the rearranged lactone **13**. Rearrangement of the protonated intermediate with intramolecular capture by the actinamine hydroxyl group was faster than capture by fluoride ion.



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Rearrangement was also observed in the attempted preparation of 3'-alkoxy analogs. Neutral methanolic solutions of diazoketone **4b** were found to be stable. Addition of non-nucleophilic acids, CuI^{10} , BF_3^{11} , or rhodium catalysts¹² led to rearrangement giving methyl ester **14**. Even the rhodium catalysts which are claimed to be useful in rearrangement-prone systems were unsuccessful in the present instance.

Clearly, the intermediate formed on reaction of the diazoketone with electrophilic species is very prone to rearrangement and will do so unless captured efficiently by a nucleophile. The successful preparation of the halo compounds described herein may rely on reaction of the diazo group with HX or X_2 to give an intimate ion pair which collapses to product faster than it rearranges.

Biological Assay

The results of *in vitro* bioassay for the new spectinomycin analogs are shown in Table 1. The results clearly show diminished activity for all of the analogs tested relative to the parent, with the monohalo compounds **12a** and **12b** having the best activity of the four new compounds. Especially interesting is the poor activity of dichloride **12c**, the ketone hydrate analog. The weak activity of this analog supports the hypothesis that the key function of the C-3' substituents in the parent is that of hydrogen bonding groups. The low activity of **12c** also serves to establish the integrity of the geminal dichloro group as being stable and not suffering hydrolysis to generate the parent ketone.

In light of the ease of preparation of these compounds, we decided to proceed with testing *in vivo*. It has been known for some time that spectinomycin has greater *in vivo* potency than would be predicted based on *in vitro* MIC values. The MIC's for the halo analogs **12a~c** vs. *Klebsiella pneumoniae*, while not equal to the parent, are low enough to predict observable *in vivo* activity. This prediction was not verified experimentally. All three halo analogs were inactive at 200 mg/kg, the highest level tested. Trobicin (spectinomycin dihydrochloride pentahydrate) had a CD_{50} of 10.9 mg/kg when tested in *K. pneumoniae* infected mice. The present data does not reveal the reason for this lack of activity, whether it is intrinsic poor activity, poor absorption or metabolic degradation to inactive compounds.

Conclusion

The synthesis of the spectinomycin derived diazoketones **4a** and **4b** has made possible the synthesis of new C-3'-modified spectinomycin analogs as a result of the new chemical reactivity pattern imparted by the diazo group. The marginal antibacterial activity found for the 3'-deoxo- and 3'-halospectino-

Table 1. Minimum inhibitory concentration ($\mu\text{g/ml}$).

Organism	Spectinomycin $2\text{HCl} \cdot 5\text{H}_2\text{O}$	6	12a	12b	12c
<i>Staphylococcus aureus</i> UC 76	7.8	250	125	62.5	250
<i>Streptococcus faecalis</i> UC 694	31.2	>1,000	>1,000	>1,000	1,000
<i>Escherichia coli</i> UC 45	3.9	62.5	62.5	15.6	125
<i>Klebsiella pneumoniae</i> UC 58	2.0	31.2	15.6	7.8	31.2
<i>Pseudomonas aeruginosa</i> UC 95	31.2	500	1,000	1,000	500
<i>Proteus vulgaris</i> UC 93	7.8	250	250	250	125
<i>P. mirabilis</i> UC 6671	7.8	250	125	62.5	125
<i>Salmonella flexneri</i> UC 143	7.8	62.5	62.5	31.2	125
<i>S. typhi</i> UC 215	7.8	250	500	250	125
<i>Serratia marcescens</i> UC 131	3.9	31.2	62.5	62.5	125

mycins lends further support to the hypothesis which requires the presence of hydrogen bonding substituents at C-3' for good bioactivity. Based on this hypothesis, the diazoketones have been utilized for the synthesis of C-3' branched chain spectinomycin analogs which contain one or more hydrogen bonding groups in this critical position of the molecule. The preparation of the first examples of these analogs is the subject of the accompanying manuscript.

Experimental

^{13}C NMR spectra were recorded on a Varian CFT-20 or FT80A spectrometer in the indicated solvents using Me_4Si or CH_3CN (for D_2O solutions) as internal standards. Chemical shifts are reported in parts per million downfield from Me_4Si . ^1H NMR spectra were recorded on a Varian EM 390 spectrometer in the indicated solvent using Me_4Si as an internal standard. IR spectra were obtained using a Perkin Elmer 298 infrared spectrophotometer equipped with a 3,600 data station. Mass spectra, optical rotations, UV spectra and melting points were measured by the Physical and Analytical Chemistry Unit of The Upjohn Company.

The minimum inhibitory concentration (MIC) vs. various bacteria was determined by a microplate broth dilution technique. Serial two-fold dilutions of the antibiotic were prepared in 50 μl of modified brain-heart infusion broth medium¹³⁾ in the wells of a microplate. Each well was then inoculated with 50 μl of standardized cell suspension to yield a final concentration of $\sim 10^5$ viable cells per ml of drug supplemented medium. The microplates were incubated at 37°C for 20 hours and the MIC was read as the lowest concentration of drug that inhibited visible growth of the organism.

N,N'-Dibenzoyloxycarbonylspectinomycin Tosylhydrazone (3a)

In 40 ml of absolute ethanol was dissolved 2.5 g (4.2 mmol) of *N,N'*-di-Cbz-spectinomycin¹⁾ (2a) and 0.77 g (4.2 mmol) of *p*-toluenesulfonylhydrazide. The reaction was stirred for 2 hours at room temperature under N_2 . At this time TLC showed no remaining 2a. Removal of the solvent *in vacuo* afforded 3.13 g (98%) of a white solid foam, which was used without further purification: ^{13}C NMR (acetone- d_6) δ 167.6, 153.4, 144.6, 137.9, 136.4, 130.1, 129.9, 129.0, 128.3, 97.3, 89.9, 74.7, 69.0, 67.2, 66.4, 66.0, 57.70, 57.5, 53.5, 33.2, 31.7, 31.4, 31.2 and 21.3.

N,N'-Dibenzoyloxycarbonyl-3'-deoxo-3'-diazospectinomycin (4a)

In a 500-ml round-bottomed flask was dissolved 13.16 g (17.1 mmol) of *N,N'*-di-Cbz-spectinomycin tosylhydrazone (3a) in 300 ml of CH_2Cl_2 . To this solution was then added 2.4 ml (17.1 mmol) of Et_3N . The solution immediately turned yellow and was stirred under N_2 for 5 hours. Removal of the solvent *in vacuo* afforded 14.25 g of yellow, brittle foam. The product was chromatographed on 1 kg of silica gel, slurry packed with EtOAc. The column was eluted with EtOAc. The elution sequence went as follows: 750 ml EtOAc, nil; 2,450 ml EtOAc, 4.75 g of starting tosylhydrazone; 4,900 ml EtOAc, 6.29 g of diazoketone. The yield of diazoketone based on recovered starting material was 94.2%. The product was obtained as a yellow solid: mp 84~90°C (dec); IR (KBr) 3450, 2950 (m), 2100 (s) and 1675 (s) cm^{-1} ; ^1H NMR (CDCl_3) δ 1.38 (CH_3CH), 2.7, 3.1 (CH_2), 3.1 (CH_3N) 3.2 (CHN , CHO), 5.1 (anomeric, $\text{OCH}_2\text{C}_6\text{H}_5$) and 7.3 (aromatic); ^{13}C NMR (acetone- d_6) δ 189.5, 157.5, 138.0, 129.0, 128.2, 102.1, 92.2, 74.5, 73.8, 69.2, 67.9, 67.11, 64.2, 60.1, 59.8, 59.5, 59.3, 31.8, 31.7, 31.5, 31.3, 30.7, 29.8, 29.2, 28.8 and 20.9; $[\alpha]_D^{25}$ -25° (c 0.7495, CHCl_3); UV (CH_3CN) λ_{max} 297 nm (ϵ 8,181); MS no M^+ observed.

When this reaction was repeated using 2 equivalents of triethylamine 4a was isolated in 75% yield.

N,N'-Di-*tert*-butoxycarbonylspectinomycin (2b)

In 700 ml of H_2O contained in a 3-liter Morton flask with overhead stirrer and addition funnel was dissolved 200 g (0.40 mol) of spectinomycin sulfate tetrahydrate. To this solution was carefully added 64 g (0.80 mol) of NaHCO_3 in batches. After stirring for 0.5 hour, 700 ml of *t*-BuOH was added followed by the addition of 176.6 g (0.81 mol) of di-*tert*-butyldicarbonate over a period of 15 minutes. The reaction was then stirred for 65 hours at room temperature. The reaction was then transferred to a 4-liter separatory funnel and the aqueous phase was separated from the *t*-BuOH. The *t*-BuOH was

removed *in vacuo*. The residue consisted of a gum which was taken up in 500 ml of EtOAc. The aqueous phase was concentrated and extracted with EtOAc (2 × 300 ml). All of the EtOAc solutions were combined and washed with H₂O (1 × 500 ml). An emulsion formed which was broken up with the addition of 500 ml of brine. The aqueous wash was back-washed with 200 ml of EtOAc and the combined organics were washed with brine (2 × 500 ml) and dried over Na₂SO₄. The solvent was removed *in vacuo* and the product was placed under high vacuum overnight to afford 131.4 g (62%) of a white solid. No residual *t*-BuOH was seen by ¹³C NMR. The product was used without further purification: IR (CHCl₃) 3420, 1740 (s), 1675 (s), 1365 (s), 1255 (s), 1160 (s), 1130 (s), 1060 (s) and 885 cm⁻¹; ¹H NMR (CDCl₃) δ 1.4 ((CH₃)₃CO), 1.4 (CH₂CH), 2.0~3.0 (CH₂CO, CHN), 2.9, 3.0 (CH₃N) and 3.1~5.0 (CHO); ¹³C NMR δ 97.3, 91.8, 79.5, 75.1, 68.1, 66.3, 65.6, 60.4, 30.8, 29.8, 28.8, 28.5, 27.9 and 21.5; [α]_D²⁵ -2° (c 0.904, CHCl₃). Exact mass calcd for C₃₃H₆₄N₂O₁₁Si₃: 748.3818, found: 748.3772.

N,N'-Di-*tert*-butoxycarbonylspectinomycin Tosylhydrazone (3b)

A solution containing 100 g (0.19 mol) of *N,N'*-di-*t*-BOC-spectinomycin and 35 g (0.19 mol) of *p*-toluenesulfonylhydrazide in 475 ml of absolute ethanol was stirred for 6.5 hours under N₂ at room temperature. The solvent was removed *in vacuo* and the product was placed under high vacuum overnight. This afforded 131 g (100%) of a white solid which was used without further purification. IR (KBr) 3500 (s), 2950 (s), 1650 (s), 1340 (s, br), 1120 (s, br), 1090 (s, br), 925 (s) and 855 (s) cm⁻¹; [α]_D²⁵ -2° (c 0.982, CHCl₃); ¹H NMR (CDCl₃) δ 1.4 (*t*-butyl), 2.8~3.0 (CH₃N), 3.4~4.8 (CHO), 7.3, 7.8 (C₆H₅CH₃); ¹³C NMR (acetone-*d*₆) δ 153.7, 130.2, 129.8, 128.6, 97.4, 90.0, 79.6, 74.9, 69.1, 66.4, 57.7, 30.8, 30.7, 29.8, 28.9, 28.5, 27.9, 21.3 and 18.6.

N,N'-Di-*tert*-butoxycarbonyl-3'-deoxo-3'-diazospectinomycin (4b)

To a solution containing 50 g (71.3 mmol) of *N,N'*-di-*t*-BOC-spectinomycin tosylhydrazone (3b) in 500 ml of CH₂Cl₂ was added 15 ml (107.6 mmol) of Et₃N. The solution turned yellow/orange and was stirred at room temperature under N₂. During the course of the reaction and additional 10 ml (71.3 mmol) of Et₃N was added. After stirring for 8 hours at room temperature, the reaction was stored in the freezer overnight. The next morning the reaction was concentrated to a volume of 100 ml and chromatographed on 1.2 kg of silica gel which had been slurry packed in CH₂Cl₂. The compound was washed onto the column with CH₂Cl₂ and then the column was eluted with EtOAc. Chromatography afforded 33.0 g (85%) of *N,N'*-di-*t*-BOC-3'-deoxo-3'-diazospectinomycin as a yellow solid; IR (KBr) 3350 (s) 2850 (s), 2050 (s), 1625 (s), 1400 (s), 1325 (s), 1220 (s), 1125 (s), 1030 (s) and 885 (s) cm⁻¹; ¹³C NMR (acetone-*d*₆) δ 189.3, 102.2, 92.7, 79.3, 74.3, 69.1, 68.0, 67.1, 64.0, 59.7, 59.6, 59.2, 30.8, 30.6, 29.9, 29.2, 28.9, 28.6, 27.9 and 21.0; [α]_D²⁵ -26° (c 1.095, CHCl₃).

N,N'-Dicarbobenzyloxy-3'-deoxospectinomycin (5)

In a 500-ml round-bottomed flask was placed 29 g of zinc dust and 30 ml of H₂O - acetic acid, 60:40. To this stirred zinc suspension was added 7.02 g (11.6 mmol) of diazoketone (4a). The reaction was stirred for 1 hour and was filtered. The filtrate was concentrated *in vacuo* to a thick liquid which was then partitioned between 100 ml of CHCl₃ and 50 ml of H₂O. The CHCl₃ phase was separated and combined with 2, 50 ml CHCl₃ extracts of the aqueous phase. The combined CHCl₃ extracts were washed with H₂O (2 × 50 ml) and saturated aqueous NaHCO₃ (1 × 100 ml). The aqueous washes were back-washed with 50 ml of CHCl₃ and the combined organics were washed with 100 ml of brine and dried over Na₂SO₄. Removal of the solvent *in vacuo* left 6.54 g of a white solid. Chromatography on 600 g of silica gel eluting with EtOAc afforded 2.51 g of *N,N'*-di-Cbz-spectinomycin and 3.03 g of a mixture containing the desired product. The mixture was rechromatographed on 100 g of silica gel and eluted with MeOH - CHCl₃ gradient. This afforded 1.03 g of pure *N,N'*-di-Cbz-3'-deoxospectinomycin (15%): mp 126~130°C (dec); IR (KBr) 3402 (s), 2950 (s), 1680 (s), 1450 (s), 1350 (s), 1170 (s), 1060 (s), 890 (m), 780 (m) and 710 (m) cm⁻¹; ¹³C NMR (acetone-*d*₆) δ 157.5, 157.4, 156.8, 138.1, 129.1, 128.3, 97.0, 90.7, 74.5, 72.6, 67.2, 66.4, 65.1, 60.2, 57.4, 36.2, 31.5, 30.7 and 21.4; ¹H NMR (CDCl₃) δ 1.2 (CH₃CH), 1.4~2.0 (CH₂CH₂), 3.0 (CH₃N), 3.5~4.0 (CHO), 5.1 (anomeric) and 7.25 (aromatic); [α]_D²⁵ +26° (c 0.44, CHCl₃).

3'-Deoxospectinomycin Dihydrochloride (6)

A 500 mg (0.85 mmol) sample of *N,N'*-di-Cbz-3'-deoxospectinomycin (5) dissolved in 30 ml of

i-PrOH was hydrogenated in the presence of 517 mg of 10% Pd on BaSO₄ for 5 hours at a pressure of 2 kg/cm². A TLC showed remaining **5**. An additional 237 mg of 10% Pd on BaSO₄ was added and the hydrogenation was continued at 7 kg/cm² overnight. The following morning, TLC showed only a trace of **5**. The reaction was filtered twice through Celite and the filtrate was acidified with 21 ml of 0.1 N HCl - *i*-PrOH solution. Removal of the solvent *in vacuo* afforded 255 mg of a light brown solid. The product was dissolved in H₂O, filtered through a cotton plug and recrystallized from aqueous acetone to give 85 mg (25.5%) of a white crystalline solid: mp 195~198°C (dec); IR (KBr) 3420 (s), 1450 (m), 1380 (m), 1175 (m), 1100 (m) and 1040 (s) cm⁻¹; ¹³C NMR (acetone-*d*₆) δ 96.2 (d), 91.4 (s), 74.2 (d), 70.9 (d), 67.1 (d), 66.1 (d), 62.5 (d), 61.0 (d), 59.5 (d), 35.1 (t), 30.9 (q), 30.0 (t) and 21.1 (q). Exact mass calcd for C₂₉H₆₆N₂O₉Si₅ (penta-*O*-TMS): 678.3767, found: 678.3723.

N,N'-Di-*tert*-butoxycarbonyl-3'-chloro-3'-deoxospectinomycin (**8a**)

In 100 ml of CH₂Cl₂ was dissolved 10.2 g (18.6 mmol) of diazoketone (**4b**). The solution was cooled to -70°C (dry ice/*i*-PrOH) under N₂ and was treated with 143 ml of a freshly titrated 0.13 M HCl - CH₂Cl₂ solution. The addition took 15 minutes and N₂ was evolved. The solution was warmed to room temperature and an additional 20 ml of HCl - CH₂Cl₂ solution was added. After warming, the solvent was removed *in vacuo* to afford 9.29 g of an off-white solid. The material was dissolved in ether and chromatographed on 300 g of silica gel packed in Et₂O. The column was eluted with 2 liters of Et₂O followed by 2 liters of EtOAc. This afforded 2.64 g (25%) of *N,N'*-di-*t*-BOC-3'-chloro-3'-deoxospectinomycin as a white solid. A ¹³C NMR showed the presence of only one isomer: IR (KBr) 3300 (s), 2900 (s), 1625 (s), 1440 (s), 1410 (s), 1330 (s), 1225 (s), 1125 (s), 1050 (s, br), 870 (s) and 600 (s) cm⁻¹; ¹³C NMR (acetone-*d*₆) δ 93.8, 90.5, 79.4, 74.9, 74.5, 74.4, 66.6, 66.4, 63.0, 60.6, 60.4, 39.2, 28.6 and 20.8; [α]_D²⁵ +18° (c 1.006, CHCl₃); ¹H NMR (acetone-*d*₆) δ 1.3 (CH₃), 1.45 (*t*-butyl), 1.7 (CH₂), 3.0 (CH₃N), 3.2~4.7 (CHO) and 4.96 (CHO₂). Exact mass calcd for C₃₃H₆₅N₂O₁₀ClSi₃ (tri-*O*-TMS): 768.3635, found: 768.3679.

Analysis of 500 MHz ¹H NMR data obtained on the corresponding benzyloxy carbonyl derivative **7** revealed coupling constants for the 3'-proton to the axial and equatorial 4'-protons to be 2 and 3 Hz respectively, thus confirming the axial orientation of the C-3' chlorine atom.

N,N'-Di-*tert*-butoxycarbonyl-3'-bromo-3'-deoxospectinomycin (**8b**)

In 50 ml of CH₂Cl₂ was dissolved 10.2 g (18.6 mmol) of diazoketone (**4b**). The solution was cooled to 0°C in an ice bath and treated with 210 ml of a freshly titrated 0.087 M HBr - CH₂Cl₂ solution (18.6 mmol). Nitrogen was evolved during the addition. At the completion of the addition, the reaction was stirred for 2 minutes, until the evolution of N₂ had ceased and the solvent was immediately removed *in vacuo* to afford 10.1 g of an off-white solid. The product was dissolved in MeOH and adsorbed on silica gel. The dry silica gel was then placed on top of a column which had been slurry packed in Et₂O. The column was then eluted with Et₂O and afforded 4.78 g (43%) of *N,N'*-di-*t*-BOC-3'-bromo-3'-deoxospectinomycin as a white solid. A ¹³C NMR showed the presence of only one isomer. IR (KBr) 3400 (s), 2900 (m), 1640 (s), 1420 (s), 1350 (s), 1230 (s), 1130 (s, br), 1050 (s), 880 (s) and 770 (m) cm⁻¹; ¹³C NMR (acetone-*d*₆) δ 93.4, 89.4, 79.0, 74.2, 74.0, 73.8, 67.1, 66.1, 65.9, 60.0, 57.1, 57.0, 56.7, 56.6, 56.4, 55.9, 39.1, 28.0 and 27.3; [α]_D²⁵ +8° (c 0.984, CHCl₃). Exact mass calcd for C₃₃H₆₅N₂O₁₀BrSi₃: 812.3131, found: 812.3159.

N,N'-Di-*tert*-butoxycarbonyl-3'-dichloro-3'-deoxospectinomycin (**9a**)

In a 1-liter, three-necked round-bottom flask equipped with addition funnel and thermometer was placed 150 ml of a 0.24 M Cl₂ solution in CCl₄ (36 mmol) and 180 ml of CH₂Cl₂. The solution was cooled to -70°C (dry ice/*i*-PrOH) and 10 g (18.4 mmol) of *N,N'*-di-*t*-BOC-3'-deoxo-3'-diazospectinomycin (**4b**) in 150 ml of CH₂Cl₂ was added rapidly. Nitrogen evolved during the addition. The reaction was stirred for 5 minutes after completion of the addition and the solvent was removed *in vacuo* immediately. This afforded 11.16 g of a white solid. The product was dissolved in Et₂O - CH₂Cl₂ and chromatographed on 250 g of silica gel slurry packed in Et₂O. The column was eluted with Et₂O and afforded 4.31 g of a white solid which still contained a trace impurity. The product was triturated with Et₂O to afford 1.77 g of pure material. The mother liquors were concentrated and chromatographed on 100 g of silica gel eluting with 1% MeOH in CHCl₃ to afford an additional 2.08 g of pure material. This

was combined with the triturated material to give 3.85 g of *N,N'*-di-*t*-BOC-3',3'-dichloro-3'-deoxospectinomycin as a white solid (35%); IR (KBr) 3350 (s), 2900 (s), 1625 (s), 1450~965 (s, br), 915 (s), 870 (s), 795 (s) and 760 (s) cm^{-1} ; ^{13}C NMR (acetone- d_6) δ 94.6, 92.5, 79.5, 74.5, 68.4, 67.0, 66.6, 66.5, 60.8, 50.4, 28.7 and 20.32; $[\alpha]_D^{25} +10^\circ$ (*c* 1.05, CHCl_3). Exact mass calcd for $\text{C}_{35}\text{H}_{64}\text{N}_2\text{O}_{10}\text{Cl}_2\text{Si}_3$: 802.3245, found; 802.3270.

3'-Chloro-3'-deoxospectinomycin Dihydrochloride (12a)

In 1 liter of CH_2Cl_2 was dissolved 2.0 g (3.6 mmol) of monochloride (8a). The solution was cooled to 0°C in an ice bath and anhydrous HCl was bubbled through the solution for 1 minute. The reaction turned cloudy and was stirred for 1 hour at 0°C . Removal of the solvent *in vacuo* afforded 1.82 g of a light yellow solid. The product was recrystallized from aqueous acetone to afford 586 mg of a white crystalline solid. The mother liquors were lyophilized and the residue crystallized from aqueous acetone to afford an additional 302 mg of product. The total yield of crystallized product was 888 mg (58%): mp $211\sim 220^\circ\text{C}$ (dec); IR (KBr) 3500 (s), 1600 (m, br), 1450 (m), 1380 (m), 1175 (s), 1075 (s), 1040 (s) and 925 (m) cm^{-1} ; ^{13}C NMR (acetone- d_6) δ 95.9, 93.6, 73.6, 70.9, 69.8, 69.6, 65.2, 64.2, 63.8, 62.0, 41.0, 34.5 and 23.2; $[\alpha]_D^{25} +11^\circ$ (*c* 0.828, H_2O). Exact mass calcd for $\text{C}_{29}\text{H}_{65}\text{N}_2\text{O}_6\text{ClSi}_5$; 712.3377, found: 712.3360.

3'-Bromo-3'-deoxospectinomycin Dihydrochloride (12b)

In 1.2 liters of CH_2Cl_2 was dissolved 3.0 g (5.0 mmol) of monobromide (8b). The solution was cooled to 0°C and anhydrous HCl was bubbled through for 1 minute. The reaction turned cloudy and was stirred for 1 hour and 10 minutes at 0°C . Removal of the solvent *in vacuo* left 2.71 g of a white solid. Several attempts were made to crystallize the product, all without success. Finally the mother liquors all were lyophilized to afford 1.56 g of a white fluffy solid: ^{13}C NMR (acetone- d_6) δ 93.5, 90.6, 71.0, 69.34, 67.5, 67.0, 62.6, 61.2, 59.5, 54.3, 38.9, 31.9, 31.8 and 20.5; $[\alpha]_D^{25} +7^\circ$ (*c* 0.59, H_2O).

3',3'-Dichloro-3'-deoxospectinomycin Dihydrochloride (12c)

In 700 ml of CH_2Cl_2 was dissolved 3.0 g (5.1 mmol) of dichloride (9a). The solution was cooled to 0°C in an ice bath and anhydrous HCl gas was bubbled through for 1 minute. The reaction mixture turned cloudy and was stirred for 1 hour at 0°C . Removal of the solvent *in vacuo* left 2.44 g of a white solid. The product was dissolved in 150 ml of H_2O and was crystallized by the addition of 1,400 ml of acetone. The solution was cooled for 2 hours and the product collected by vacuum filtration. The first crop amounted to 856 mg of a white crystalline solid. The mother liquors were lyophilized and the lyophilized material was recrystallized from acetone- H_2O to afford an additional 430 mg of product. Finally, the mother liquors were lyophilized to afford 625 mg of a white solid. The total yield of crystalline product was 1.286 g (55%): mp $240\sim 250^\circ\text{C}$ (dec); IR (KBr) 3500 (s), 3000 (s), 1610 (m), 1450 (m), 1380 (m), 1170 (s), 1120 (s), 1060 (s), 930 (m), 805 (m) and 765 (m) cm^{-1} ; ^{13}C NMR (acetone- d_6) δ 93.8, 92.7, 91.8, 70.5, 69.7, 68.5, 66.8, 62.4, 59.3, 49.3, 33.3, 31.9 and 20.1; $[\alpha]_D^{25} +10^\circ$ (*c* 0.9465, H_2O). Exact mass calcd for $\text{C}_{29}\text{H}_{64}\text{N}_2\text{O}_6\text{Cl}_2\text{Si}_5$ (penta-*O*-TMS): 746.2988, found: 746.3044.

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