SPECTINOMYCIN MODIFICATION

III. SPECTINOMYCIN ANALOGS WITH C-3′-BRANCHED CHAIN SUGARS

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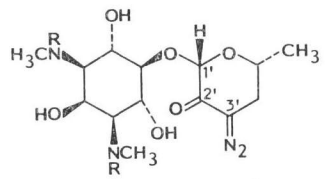
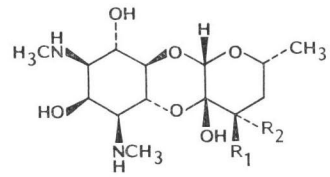
A variety of C-3′-branched chain analogs of spectinomycin has been synthesized *via* the intermediacy of spectinomycin derived diazoketones. *In vitro* antibacterial assay of these compounds has underscored the importance of hydrogen bonding functional groups in this region of the molecule. The most potent of these analogs had activity greater than or equal to the parent.

In the previous paper[1], we reported the synthesis of the spectinomycin derived diazoketones **1a** and **1b**, and described their conversion into 3′-deoxo-, halo- and dihalospectinomycin analogs. The modest antibacterial activity observed for these compounds reinforced our hypothesis that 3′-substituents capable of hydrogen bonding were necessary for good bioactivity. Among the known C-3′-spectinomycin analogs with such substituents, the parent ketone hydrate **2a** is more potent than either of the monosubstituted alcohols **2b** and **2c**, while the *R*-amino analog **2d** is more potent than the alcohols and the *S*-amino compound **2e** is surprisingly inactive.

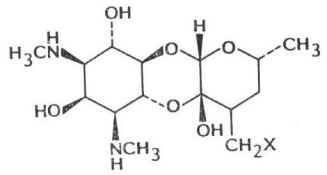


1a R = $PhCH_2OC(=O)$

1b R = $(CH_3)_3COC(=O)$



2a $R_1 = R_2 = OH$
2b $R_1 = OH$, $R_2 = H$
2c $R_1 = H$, $R_2 = OH$
2d $R_1 = NH_2$, $R_2 = H$
2e $R_1 = H$, $R_2 = NH_2$



3 X = OH, NR_2

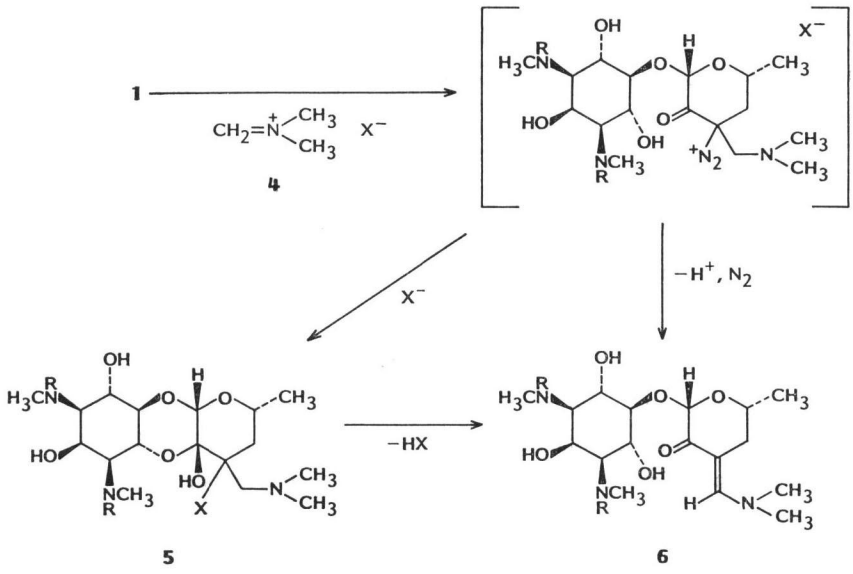
To further delineate the structure-activity relationships in the spectinomycin series, we wished to prepare other analogs with hydrogen bonding functional groups at the C-3′ position. One of our goals was the synthesis of analogs with C-3′-branched chain sugar moieties, such as **3**, which would contain carbon spacers between C-3′ and the heteroatom groups thereby altering their possible interactions at the ribosomal binding site. The nucleophilic character imparted to C-3′ by the incorporation of the diazo group into **1** provides a viable synthetic entry into ring systems such as in **3**, *via* reaction of the diazoketones with

carbon electrophiles. The use of such an approach for the successful synthesis of C-3′-branched chain spectinomycin analogs is the subject of this paper.

Results and Discussion

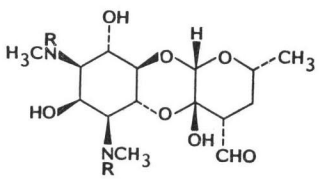
To introduce a one carbon fragment which contained synthetically useful functionality, we envisioned the reaction of the diazoketones with ESCHENMOSER's salt[2] **4** (X=I) to give either the aminohalide **5** or vinylogous amide **6** directly (Scheme 1). While we were unaware of any prior examples of the reaction of this reagent with diazo compounds, we thought that the reaction would succeed since our previously successful reactions with the rearrangement prone[1] diazoketones had all involved reagents which utilized halide ions to trap the intermediate diazonium species.

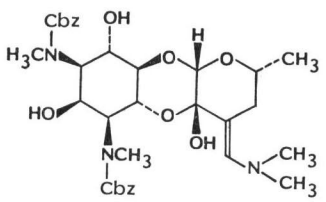
Scheme 1.



a R = Cbz
b R = BOC

In the event, using salt **4** (X=I), reaction with **1** was rapid as evidenced by the evolution of nitrogen, but a multitude of products was formed, presumably due to the reactivity of the iodide. Reaction of **1a** with salt **4** (X=Cl)[3] in dimethylformamide, however, unexpectedly gave aldehyde **7**, presumably arising from hydrolysis of the desired **6** by adventitious water present in the reaction, catalyzed by the





7a R = Cbz
7b R = BOC

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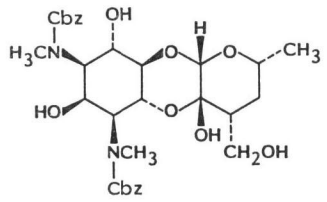
HCl liberated in the condensation. The presence of HCl was also manifested by the formation of the C-3′-chloro compound[1].

The desired preparation of vinylogous amide **6** was finally achieved by reaction of **1** with a suspension of chloride **4** (X=Cl) in dry acetonitrile in the presence of triethylamine as an acid scavenger. The troublesome hydrolysis and chloride formation were avoided by using these conditions. As anticipated, the product was found to exist in the open C-2′-keto form **6** and not in the closed hemiketal form **8** as determined by ^{13}C NMR. The presence of equilibrium amounts of the closed form, with its nonconjugated enamine, could not be excluded, however, and may in fact offer an explanation for the facile hydrolysis of the vinylogous amide functionality.

Having achieved the introduction of a functionalized carbon atom at C-3′ we next explored the utility of **6** for analog synthesis. The facile hydrolysis of the vinylogous amide system could be accomplished on a preparative scale with either **6a** or **6b** to afford aldehyde **7**. Reduction of the aldehyde group with sodium borohydride gave alcohol **9** which afforded **10** on deprotection. Compound **10** is the first example of a spectinomycin analog which contains a branched chain sugar, and is a methylene-spaced analog of 3′-*S*-dihydrospectinomycin (**2c**).



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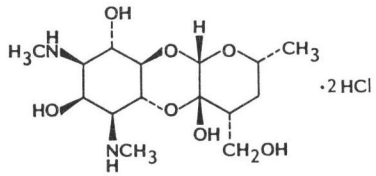

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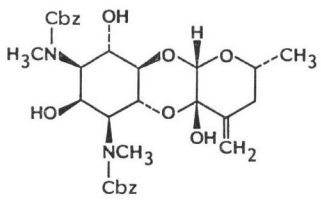
The second reaction sequence to be explored was the reduction of the vinylogous amide system. The problems associated with the reduction of such systems have been reviewed by GREENHILL[4]. This functional group is usually highly resistant to many reducing agents, and depending on the reaction conditions, the products may include the amino ketone, amino alcohol and deaminated products. Treatment of enaminoketone **6a** with either methanolic HCl and sodium cyanoborohydride or sodium bis-(2-methoxyethoxy)aluminum hydride resulted in the formation of the exomethylene compound **11** and amine **12**, along with some products from over-reduction. The ratio of **11** to **12** and the degree of over-reduction was found to be highly pH dependent and nonreproducible, but sufficient quantities of both compounds were obtained for additional studies. Reductive deprotection of **12** gave **13**, a branched chain amino sugar spectinomycin analog.

The methylene group in **11** offered an excellent opportunity to explore the effect of introduction of heteroatoms both at C-3′ and in the branching side chain. Thus osmium tetroxide treatment of **11**



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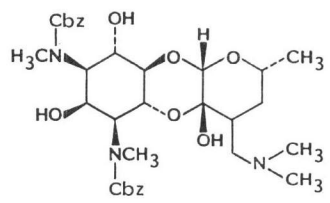

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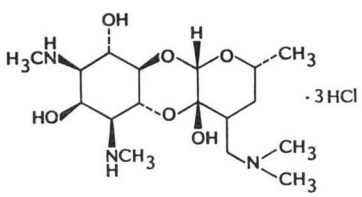

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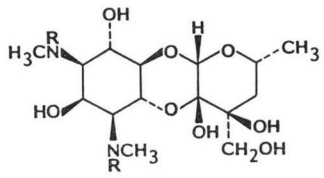
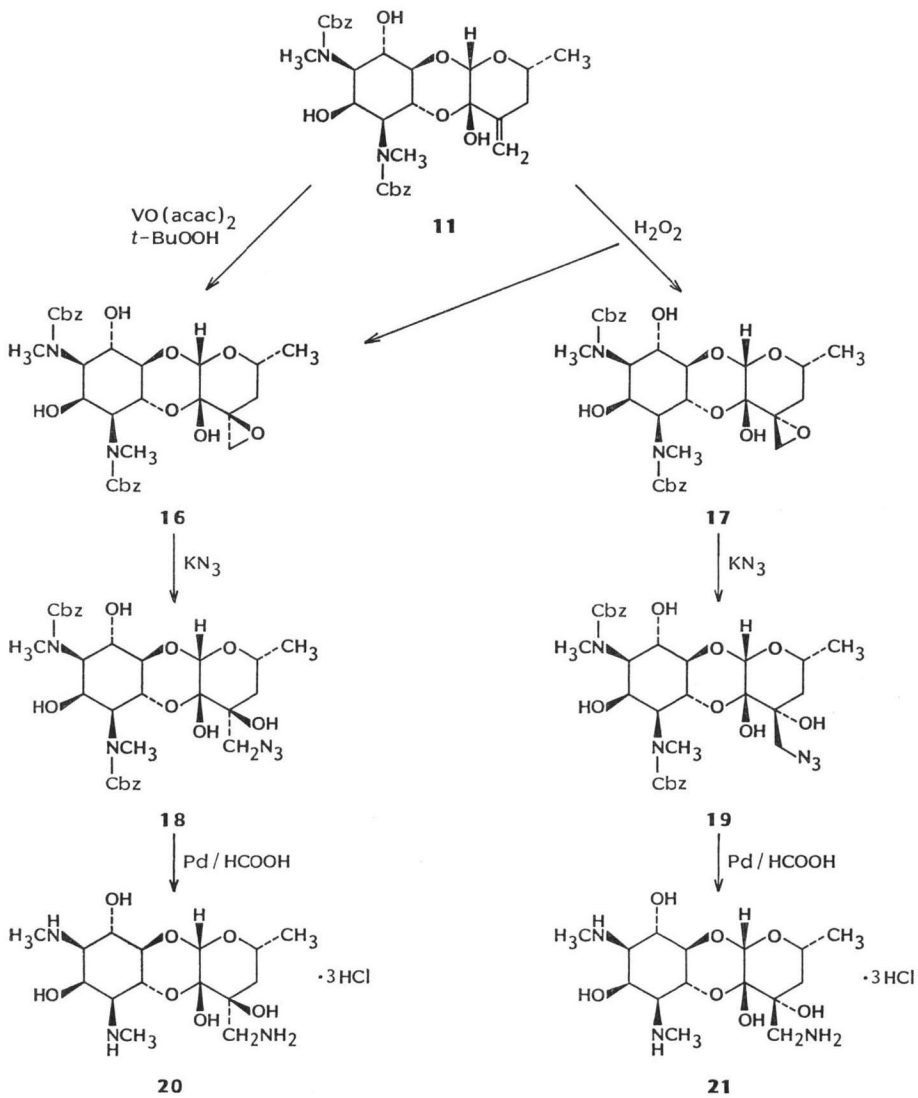


14 R = Cbz

15 R = H, 2HCl salt

gave diol **14** which was deprotected to give **15**, a unique analog of the normal spectinomycin ketone hydrate which cannot equilibrate to a keto form and in which one of the hydroxyl groups is extended further away from the ring system than in the parent. Stereospecific epoxidation of the double bond using the SHARPLESS procedure[5] afforded epoxide **16** whereas treatment with basic hydrogen peroxide gave an

Scheme 2.



11

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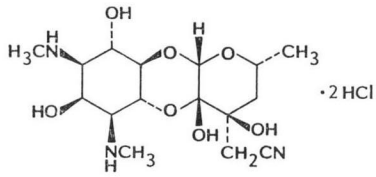
isomeric mixture of epoxides **16** and **17** in a ratio of 2.7: 1 (67% total isolated yield) (Scheme 2). These compounds served as valuable substrates for the synthesis of branched chain analogs bearing C-3′ hydroxyl groups *via* nucleophilic opening of the epoxide ring. Reaction of either epoxide with potassium azide gave the corresponding azidoalcohols **18** and **19** which were deprotected with concomitant azide reduction to yield the epimeric amino alcohols **20** and **21**.

Other nucleophiles were used in the epoxide ring opening methodology to provide new analogs to further evaluate the structure-activity relationships in this series. Thus, reaction of **16** with cyanide ion followed by deprotection gave nitrile **22**. In a similar fashion, using amines as the nucleophilic species, the *N*-substituted aminomethyldihydrospectinomycins **23**, **24** and **25** were synthesized. With a variety of C-3′ branched chain spectinomycin analogs in hand, we proceeded with the biological evaluation of these compounds to test our hypothesis regarding the importance of hydrogen bonding functional groups in this region of the molecule.



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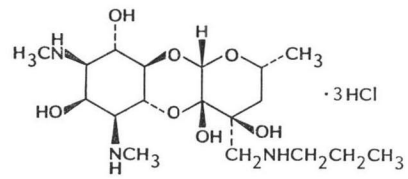

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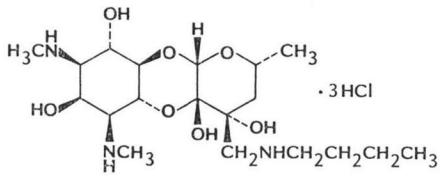

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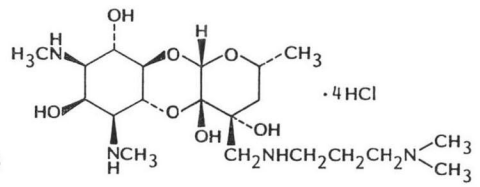

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Biological Evaluation

The results of *in vitro* antibacterial assay for the new spectinomycin analogs are shown in the Table 1. Hydroxymethyl analog **10** with its single heteroatom substituent is seen to be much less active than the parent and is in fact less active than 3′-*S*-dihydrospectinomycin **2c**[6]. Comparison of **10** with diol **15** in which a C-3′ heteroatom has been reintroduced clearly shows the beneficial effect of the additional heteroatom. However, diol **15**, which is an analog of the spectinomycin ketone hydrate (**2a**) in which the α-hydroxyl group is further away from the sugar ring due to the insertion of the methylene group, is significantly less active than the parent compound, establishing the critical role of the spatial orientation of the hydroxyl groups or the importance of the ability of **2a** to equilibrate to the keto form. A comparison of the dimethylamino substituted analog **13** with alcohol **10** shows the enhancement of activity derived from incorporation of a nitrogen atom.

The data described above suggests that the amino-alcohol containing analogs **21**~**25** should be the most active in this series, and this prediction is borne out by the data. The aminomethyl dihydrospectinomycin analogs **20** and **21** are both very active compounds, with activity equal to the parent against most of the organisms tested. The poor activity of the cyano analog **22** establishes the need for a basic nitrogen atom in the branching substituent. The two *N*-alkylated analogs **23** and **24** provide an interest-

Table 1. Minimum inhibitory concentration (μg/ml).

Organism	Spectinomycin $2HCl \cdot 5H_2O$	**10**	**13**	**15**	**20**	**21**	**22**	**23**	**24**	**25**
Staphylococcus aureus UC 76	7.8	>250	62.5	31.2	3.9	3.9	31.2	>250	3.9	1.0
Streptococcus faecalis UC 694	62.5	>250	>250	>250	>250	125	—	>250	31.2	250
Escherichia coli UC 45	3.9	125	31.2	31.2	1.0	1.0	31.2	62.5	2.0	1.0
Klebsiella pneumoniae UC 58	2.0	125	15.6	3.9	1.0	1.0	7.8	31.2	1.0	0.5
Pseudomonas aeruginosa UC 95	31.2	>250	>250	>250	62.5	31.2	250	>250	125	62.5
Proteus vulgaris UC 93	7.8	>250	31.2	62.5	7.8	3.9	125	125	3.9	3.9
P. mirabilis UC 6691	7.8	>250	31.2	62.5	3.9	7.8	125	125	15.6	7.8
Salmonella flexneri UC 143	3.9	125	62.5	15.6	3.9	3.9	15.6	62.5	3.9	2.0
Serratia marcescens UC 131	3.9	250	31.2	15.6	2.0	1.0	31.2	62.5	2.0	0.25
Providencia stuartii UC 6570	>250	>250	>250	>250	>250	15.6	>250	>250	15.6	7.8

ing contrast. Alkylation of the amino group in the C-3′-*S*-series (**23**) results in a decrease in activity relative to the unsubstituted aminomethyl analog (**20**). In the C-3′-*R*-series (**24**) the good activity of the parent (**21**) is maintained. Analog **25** in which a second amino group is incorporated in the branching side chain is seen to be the most potent of all the analogs tested. This compound is significantly more active than spectinomycin against many of the organisms tested.

The results of the *in vitro* bioassay, taken with the data from the previous paper[1] establish the critical role of hydrogen bonding functional groups at or near C-3′ in the spectinomycin skeleton. The aminomethyldihydrospectinomycins and their *N*-substituted analogs constitute the first members of a new family of highly active branched chain spectinomycin analogs. We have pursued this lead through the development of a superior route to the parent aminomethyldihydrospectinomycins and through investigation of a variety of nitrogen substituents. The results of these studies will be the subject of future communications.

Experimental

^{13}C NMR spectra were recorded on a Varian CFT-20 or FT-80A 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 medium[7] 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 milliliter 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.

Preparation of Enamino Ketone **6a**

A 100-ml Morton flask was flame dried under a stream of N_2. After cooling, the flask was charged with 2.0 g (21.4 mmol) of *N*,*N*-dimethylmethylene ammonium chloride and 50 ml of acetonitrile. To this suspension was added 1.7 ml (23.1 mmol) of dry Et_3N followed by 5.0 g (8.2 mmol) of diazoketone (**1a**)[1]. The solution was stirred for 4.5 hours at room temperature under N_2. During this time a slow evolution of N_2 was observed, the yellow color of the diazoketone faded and a white precipitate formed. The reaction mixture was filtered and the filtrate concentrated *in vacuo* to afford 6.73 g of a yellow solid.

The product was taken up in $CHCl_3$ and chromatographed on 250 g of silica gel slurry packed in $CHCl_3$. The column was eluted with an acetone - $CHCl_3$ gradient as follows with 50 ml fractions being collected: 500 ml 10% acetone in $CHCl_3$; 500 ml 20% acetone in $CHCl_3$; 5 liters 30% acetone in $CHCl_3$ and 1 liter 40% acetone in $CHCl_3$. Fractions 50~133 contained pure enamino ketone. These fractions were pooled and the solvent was removed *in vacuo* to afford 3.14 g (60%) of enamino ketone: ^{13}C NMR (acetone-d_6) δ 190.0, 154.1, 138.2, 129.1, 128.3, 128.0, 102.6, 99.4, 91.6, 74.5, 69.7, 68.1, 67.9, 67.2, 60.1, 43.6, 33.3, 31.8 and 21.4; ^1H NMR ($CDCl_3$) δ 1.32 (CH_3CH, d, J=6 Hz), 3.11 (NCH_3), 3.3~4.4 (OCH, NCH), 5.1 (anomeric, $OCH_2C_6H_5$), 7.25 (aromatic) and 7.7 (=$CHN(CH_3)_2$); $[\alpha]_D^{25}$ +64° (c 0.998, $CHCl_3$); UV max (EtOH) 204 (ε 22,150) and 338 (17,500); IR ($CHCl_3$) 3439 (m), 3009 (m), 2905 (m), 1691 (s), 1652 (m), 1539 (s), 1485 (s), 1453 (s), 1421 (s), 1385 (s), 1343 (s), 1268 (s), 1171 (s) and 1024 cm^{-1}; MS (for tristrimethylsilyl ether minus TMSOH) $C_{39}H_{57}N_3O_9Si_2$ requires 767.3633, found 767.3648.

N,N'-Dibenzyloxycarbonyl-3'-deoxo-3'-formylspectinomycin (**7a**)

To a solution containing 1.0 g (1.6 mmol) of enamino ketone (**6a**) in 25 ml of THF was added 1.6 ml of 1 N HCl. The reaction was stirred at room temperature for 2 hours. The THF was then removed *in vacuo* and the residue partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ was separated and combined with a 30-ml $CHCl_3$ extract of the aqueous phase. The combined extracts were washed with 25 ml of brine and dried by filtering through a cone of $MgSO_4$. Removal of the solvent *in vacuo* afforded 806 mg of a white solid. The product was dissolved in CH_2Cl_2 and chromatographed on 40 g of silica gel, slurry packed in EtOAc - hexane, 1: 1. The column was eluted with an EtOAc - hexane gradient as follows with 45 ml fractions being collected: 100 ml of EtOAc - hexane (6: 4), 100 ml EtOAc - hexane (7: 3) and 1 liter of EtOAc - hexane (8: 2). Elution volume 450~630 ml contained 408 mg of pure *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-formylspectinomycin as a white solid: ^{13}C NMR (acetone-d_6) δ 138.3, 129.2, 128.5, 96.4, 92.4, 74.5, 73.8, 73.7, 71.1, 67.3, 66.3, 65.4, 61.0, 60.5, 60.4, 57.9, 57.8, 57.4, 56.2, 31.7, 30.8 and 21.4; ^1H NMR (acetone-d_6) δ 1.25 (CH_3CH), 1.8~1.9 (CH_2, CH), 2.7 ($CHCHO$), 3.1 (CH_3N), 3.2 (CH_3N), 5.1 (anomeric, $OCH_2C_6H_5$), 7.3 (aromatic) and 9.9 (CHO); IR (KBr) 3400 (s), 1650 (s), 1430 (s), 1380 (s), 1330 (s), 1150 (s), 1110 (s) and 1050 (s) cm^{-1}; $[\alpha]_D^{25}$ +23° (c 0.969, $CHCl_3$); MS (for tristrimethylsilyl ether minus CH_3) $C_{39}H_{59}N_2O_{11}Si_3$ requires 815.3426, found 815.344.

N,N'-Dibenzyloxycarbonyl-3'-deoxo-3'-hydroxymethylspectinomycin (**9**)

In 10 ml of MeOH was dissolved 200 mg (0.325 mmol) of aldehyde (**7a**). To this solution was added 4 mg (0.015 mmol) of $NaBH_4$. The reaction was stirred for 40 minutes at room temperature and quenched with 5 ml of 5% aqueous $NaHCO_3$. The MeOH was removed *in vacuo* and the residue partitioned between EtOAc (25 ml) and H_2O (25 ml). The EtOAc was separated and combined with a 15 ml EtOAc extract of the aqueous phase. The combined extracts were washed with 20 ml of brine and dried over Na_2SO_4. After filtering, removal of the solvent *in vacuo* left 212 mg of a white solid. The product was dissolved in CH_2Cl_2 and chromatographed on 40 g of silica gel, slurry packed in EtOAc - hexane, 1: 1. The column was eluted with 1 liter of EtOAc - hexane, 7: 3, to afford in elution volume 315~908 ml, 190 mg (95%) of *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-hydroxymethylspectinomycin as a white solid: ^{13}C NMR (acetone-d_6) δ 129.2, 128.4, 96.9, 92.7, 74.9, 74.6, 74.4, 73.8, 71.6, 67.2, 66.6, 64.8, 62.6, 61.2, 61.1, 60.4, 57.9, 57.5, 45.2, 33.0, 31.6 and 21.5; ^1H NMR (acetone-d_6) δ 1.1 (CH_3CH, d, J=6 Hz), 3.02 (NCH_3), 3.3~4.2 (NCH, OCH), 5.02 (anomeric, $OCH_2C_6H_5$) and 7.25 (aromatic); IR (KBr) 3450 (s), 1650 (s), 1425 (s), 1325 (s), 1150 (s), 1125 (s), 1075 (s), 890 (m), 775 (m), 750 (m) and 700 (m) cm^{-1}; $[\alpha]_D^{25}$ +17° (c 0.700, $CHCl_3$). MS m/z 817 (tri-*O*-TMS minus CH_3), 750, 634, 495, 393, 359, 321, 305, 270, 216, 170, 108, 91 and 73.

3'-Deoxo-3'-hydroxymethylspectinomycin Dihydrochloride (**10**)

In 2 ml of MeOH was dissolved 63 mg (0.102 mmol) of *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-hydroxymethylspectinomycin. To this solution was added 75 mg of Pd black and 40 μl of HCOOH. The mixture was stirred for 7 minutes, filtered and the solvent removed *in vacuo*. This afforded 50 mg of a glass. The material was dissolved in H_2O and 2 ml of 0.1 N HCl was added. The sample was immediately frozen and lyophilized overnight. This afforded 27 mg of a white solid (62%): ^{13}C NMR (D_2O) δ 96.3, 93.2, 73.3, 71.3, 67.2, 66.6, 62.5, 61.7, 59.5, 45.2, 32.8, 32.6, 31.9 and 21.2; MS (for tristrimethylsilyl ether minus CH_3) $C_{23}H_{49}N_2O_7Si_3$ requires 549.2847, found 549.2855.

N,N'-Dibenzyloxycarbonyl-3'-deoxo-3'-methylenespectinomycin (**11**) and *N,N'*-Dibenzyloxycarbonyl-3'-deoxo-3'-(*N,N*-dimethylaminomethyl)spectinomycin (**12**)

In 10 ml of MeOH was dissolved 2.0 g (3.1 mmol) of enamino ketone (**6a**). To this solution was added a trace of bromcresol green indicator in MeOH. The solution was adjusted to a pH $\leq$4 by the addition of 0.1 N methanolic HCl and 65 mg (1.03 mmol) of $NaCNBH_3$ in 1 ml of MeOH was added. The pH was kept near 4 and the reaction stirred for 2.5 hours. The reaction was then quenched by the addition of 5 ml of saturated $NaHCO_3$ and the MeOH was removed *in vacuo*. The residue was partitioned between 20 ml of H_2O and 20 ml of EtOAc. The EtOAc was separated and combined with a 10-ml EtOAc extract of the aqueous phase. The combined extracts were washed with 20 ml of brine and dried over Na_2SO_4. Removal of the solvent *in vacuo* afforded 1.73 g of a blue solid. The product was dissolved in $CHCl_3$ and chromatographed on 200 g of silica gel, slurry packed in $CHCl_3$. The column was eluted with an acetone - $CHCl_3$ gradient as follows: 400 ml 10% acetone in $CHCl_3$; 400 ml 20% acetone in $CHCl_3$; 2 liters 30% acetone in $CHCl_3$; 1 liter acetone - $CHCl_3$ (1: 1). Elution volume 1,750~1,300 ml contained 439 mg of *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-methylenespectinomycin as a white solid. Elution volume 3,500~3,800 ml contained 167 mg of amino compound **12**. The yield of desired methylene compound was 23%: ^{13}C NMR (acetone-d_6) δ 146.0, 138.3, 129.1, 128.3, 112.1, 97.5, 92.1, 74.5, 74.0, 72.9, 67.2, 66.4, 65.2, 60.3, 57.8, 57.7, 57.5, 40.8, 31.6 and 21.3; ^1H NMR ($CDCl_3$) δ 1.2 (CH_3CH, d, J=6 Hz), 2.0~2.5 (CH, CH_2), 2.9 (NCH_3), 3.8~4.4 (CHO, CHN), 4.5 (CH_2=), 5.1 (anomeric, $OCH_2C_6H_5$) and 7.2 (aromatic); IR ($CHCl_3$) 3673 (w), 3587 (m), 3418 (s), 3009 (s), 2952 (s), 1682 (s), 1486 (s), 1454 (s), 1407 (s), 1386 (s), 1346 (s), 1264 (s), 1234 (s), 1167 (s), 1128 (s), 962 (m) and 918 (m) cm^{-1}; $[\alpha]_D^{25}$ $-6°$ (*c* 1.042, $CHCl_3$); MS (for trimethylsilyl ether minus CH_3) $C_{30}H_{57}N_2O_{10}Si_2$ requires 727.3026, found 727.3069.

For the amino compound **12**: ^{13}C NMR (acetone-d_6) δ 138.0, 129.1, 128.4, 96.8, 93.1, 75.0, 74.7, 71.7, 67.2, 66.4, 64.7, 60.1, 57.3, 45.6, 39.9, 34.1, 31.7, 31.6 and 21.5; ^1H NMR (acetone-d_6) δ 1.1 (CH_3-CH, d, J=6 Hz), 2.2 ($N(CH_3)_2$), 3.1 (NCH_3), 3.3~4.7 (OCH, NCH), 5.1 (anomeric, $OCH_2C_6H_5$) and 7.25 (aromatic); IR ($CHCl_3$) 3427 (m), 3363 (m), 3003 (m), 2984 (m), 2955 (m), 2904 (m), 1686 (s), 1454 (s), 1404 (s), 1384 (s), 1343 (s), 1225 (m), 1163 (s), 1125 (s), 1061 (s), 1028 (s), 989 (m), 951 (m), 911 (m) and 694 (m) cm^{-1}; MS (for tristrimethylsilyl ether) $C_{43}H_{69}N_3O_{10}Si_3$ requires 859.4290, found 859.4316.

3'-Deoxo-3'-(*N,N*-dimethylaminomethyl)spectinomycin Trihydrochloride (**13**)

In 5 ml of MeOH was dissolved 100 mg (0.155 mmol) of *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-(*N,N*-dimethylaminomethyl)spectinomycin. To this solution was added 100 mg of Pd black and 62 μl (1.55 mmol) of HCOOH. The reaction was stirred for 1 hour at room temperature, was filtered and the solvent removed *in vacuo* to afford 90 mg of a glass. The material was dissolved in H_2O and 4.6 ml (0.46 mmol) of 0.1 N aqueous HCl was added. The solution was immediately frozen and lyophilized overnight to afford 72 mg (96%) of a white solid: ^{13}C NMR (D_2O) δ 95.6, 92.2, 72.5, 70.8, 67.0, 66.4, 62.3, 61.1, 59.0, 57.5, 45.2, 44.2, 39.7, 33.6, 32.0, 31.9 and 20.9; MS (for tristrimethylsilyl ether) $C_{26}H_{57}N_3O_6Si_3$ requires 591.3555, found 591.3536.

N,N'-Dibenzyloxycarbonyl-3'-hydroxymethyldihydrospectinomycin (**14**)

In 2 ml of acetone was dissolved 178 mg (0.30 mmol) of *N,N'*-dibenzyloxycarbonyl-3'-deoxo-3'-methylenespectinomycin, 30 mg (0.114 mmol) of $Et_4NOAc \cdot 4H_2O$ and 66 μl (0.485 mmol) of 70% aqueous *t*-BuOOH. The solution was cooled to 0°C and 152 μl of 2.5% OsO_4 in *tert*-BuOH was added. The solution was stirred for one hour at 0°C and was warmed to room temperature. The solution was stirred for an additional 4.5 hours and was quenched with the addition of 2 ml of 10% aqueous $NaHSO_3$ and 2 ml of EtOAc. This mixture was stirred for 15 minutes, poured into 20 ml of brine, and extracted with EtOAc (2×20 ml). The combined extracts were washed with 20 ml of brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to afford 171 mg of a brown solid. The product was dissolved in $CHCl_3$ and streaked on two 2000 μ Silica gel preparative TLC plates. The plates were eluted with 10% MeOH - $CHCl_3$ and visualized under short UV light. The more polar UV active band on each plate was collected and eluted with EtOAc. This afforded 46 mg (24%) of the title compound as a white solid: ^{13}C NMR (acetone-d_6) δ 138.2, 129.2, 128.5, 94.4, 93.9, 74.5, 73.9, 73.6, 67.4, 67.0, 66.6, 65.3, 61.3, 61.2, 61.1, 60.5, 57.9, 57.7, 57.5, 38.6, 31.8, 31.6 and 21.1.

3′-Hydroxymethyldihydrospectinomycin Dihydrochloride (**15**)

In 6 ml of MeOH was dissolved 45 mg (0.071 mmol) of *N,N′*-dibenzyloxycarbonyl-3′-(hydroxymethyl)dihydrospectinomycin. To this solution was added 57 mg of Pd black and 30 μl (0.75 mmol) of HCOOH. The reaction was stirred for 12 minutes, filtered and the solvent removed *in vacuo* to afford 32 mg of a glass. The material was dissolved in H_2O and 1.4 ml of 0.1 N HCl was added. The solution was immediately frozen and lyophilized to afford 26 mg (83%) of a white solid: ^{13}C NMR (D_2O) δ 93.8, 93.7, 74.5, 70.9, 68.4, 67.0, 66.8, 65.9, 62.4, 61.6, 59.3, 37.8, 32.5, 31.9 and 20.9; MS *m/z* 724 (M^+-1), 513, 437, 401, 309, 217, 206, 187, 165, 147, 129, 117, 94, 73, 55 and 43.

3′-*R*-*N,N′*-Dibenzyloxycarbonyl-3′-deoxo-3′-methylenespectinomycin Oxide (**16**)

In 10 ml of CH_2Cl_2 was dissolved 1.0 g (1.67 mmol) of *N,N′*-dibenzyloxycarbonyl-3′-deoxo-3′-methylenespectinomycin. To this solution was added 20 mg (0.075 mmol) of vanadium (IV) oxide bis-(2,4-pentanedionate) ($VO(acac)_2$) followed by 2.56 ml of a 0.625 M solution of *t*-BuOOH in CH_2Cl_2 (1.67 mmol). The solution turned deep red and was stirred at room temperature for 22 hours. At this time an additional 1 ml of *t*-BuOOH - CH_2Cl_2 solution was added along with an additional 10 mg of $VO(acac)_2$. The reaction was stirred for 5 hours at which time TLC showed complete reaction. The solution was then poured into 20 ml of 5% aqueous Na_2SO_3. The CH_2Cl_2 was separated and combined with a 20-ml CH_2Cl_2 extract of the aqueous phase. The combined extracts were washed with 20 ml of brine and dried over Na_2SO_4. The solution was filtered and the solvent was removed *in vacuo* to afford 1.07 g of a brown solid. The material was dissolved in CH_2Cl_2 and chromatographed on 10 g of silica gel, slurry packed in EtOAc - hexane, 1: 1. The column was eluted with 1 liter of EtOAc - hexane, 1: 1. In elution volume 200~1,000 ml there was obtained 800 mg (78%) of the title compound as a white solid: ^{13}C NMR (acetone-d_6) δ 138.2, 129.2, 128.5, 96.8, 90.8, 75.0, 74.6, 74.5, 69.5, 67.4, 66.5, 65.8, 61.2, 60.2, 59.3, 57.8, 57.5, 47.5, 38.4, 31.8 and 21.3; ^1H NMR ($CDCl_3$) δ 1.2 (CH_3CH, d, J=6 Hz), 2.0~2.8 (CH_2, CH), 3.1 (NCH_3), 3.4~4.8 (OCH, NCH), 5.2 (anomeric, $OCH_2C_6H_5$) and 7.3 (aromatic); IR ($CHCl_3$) 3412 (m), 3004 (m), 1680 (s), 1477 (m), 1450 (s), 1404 (m), 1384 (m), 1343 (s), 1241 (m), 1164 (s), 1120 (s), 1061 (s) and 886 (m) cm^{-1}; $[\alpha]_D^{25}$ +3° (*c* 0.9265, $CHCl_3$); MS (for tristrimethylsilyl ether minus CH_3) $C_{39}H_{59}N_2O_{11}Si_3$ requires 815.3426, found 815.3443.

3′-*S*-*N,N′*-Dibenzyloxycarbonyl-3′-deoxo-3′-methylenespectinomycin Oxide (**17**)

In 20 ml of MeOH was dissolved 1.09 g (1.67 mmol) of *N,N′*-dibenzyloxycarbonyl-3′-deoxo-3′-methylenespectinomycin and 531 mg (5.0 mmol) of Na_2CO_3. To this solution was added 0.2 ml of 30% H_2O_2. The reaction was stirred at room temperature for 10.5 hours, at which time TLC showed complete reaction. The reaction was poured into 50 ml of H_2O and extracted with EtOAc (2×30 ml). The combined extracts were washed with 30 ml of brine and dried over Na_2SO_4. After filtering, removal of the solvent *in vacuo* afforded 1.027 g of a white solid. The product was taken up in CH_2Cl_2 and chromatographed on 75 g of silica gel, slurry packed in EtOAc - hexane, 1: 1. The column was eluted as follows with 30 ml fractions taken: 700 ml EtOAc - hexane (1: 1), 2 liters EtOAc - hexane, (6: 4) and 500 ml EtOAc - hexane (7: 3). Elution volume 1,830~3,090 ml contained 186 mg of pure epoxide **17** (18% yield): ^{13}C NMR (acetone-d_6) δ 138.2, 129.1, 128.4, 96.4, 91.6, 74.9, 74.5, 69.6, 67.2, 66.4, 65.4, 60.5, 60.2, 57.5, 50.8, 38.1, 31.7 and 21.3; MS (for bistrimethylsilyl ether minus CH_3) $C_{36}H_{51}N_2O_{11}Si_2$ requires 743.3031, found 743.2991.

3′-*S*-*N,N′*-Dibenzyloxycarbonyl-3′-azidomethyldihydrospectinomycin (**18**)

In 30 ml of H_2O - EtOH, 1: 5 was dissolved 1.139 g (1.85 mmol) of epoxide **16**, 1.53 g (18.5 mmol) of KN_3 and 1.0 g (18.6 mmol) of NH_4Cl. The resulting solution was heated at 85°C for 20 minutes, cooled, and concentrated *in vacuo*. The residue was partitioned between 30 ml of EtOAc and 30 ml of H_2O. The EtOAc was separated and combined with a 20-ml EtOAc extract. The combined extracts were washed with 15 ml of brine and dried over Na_2SO_4. The solution was filtered and the solvent removed *in vacuo* to afford 975 mg of a light yellow solid. The product was dissolved in $CHCl_3$ and chromatographed on 40 g of silica gel, slurry packed in $CHCl_3$. The column was eluted as follows: 100 ml 1% MeOH in $CHCl_3$; 1 liter 2% MeOH in $CHCl_3$; 1 liter 10% MeOH in $CHCl_3$. In elution volume 400~700 ml there was 427 mg of the title compound (35%); ^{13}C NMR (acetone-d_6) δ 138.1, 129.2, 128.8, 128.4, 94.5, 92.6, 75.3, 74.4, 73.8, 67.3, 66.4, 65.4, 61.2, 61.0, 60.4, 57.7, 57.6, 57.3, 55.7, 39.2, 31.5 and 21.1; ^1H NMR

(acetone-d_6) δ 1.2 (CH_3CH, d, J=6 Hz), 1.3~1.9 (CH_2), 3.1 (NCH_3), 3.5~4.8 (OCH, NCH), 5.1 (anomeric, $OCH_2C_6H_5$), 5.8 (CH_2N_3) and 7.3 (aromatic); IR ($CHCl_3$) 3563 (m), 3450 (m), 3015 (s), 2978 (m), 2936 (m), 2112 (s), 1690 (s), 1485 (s), 1452 (s), 1406 (s), 1383 (s), 1344 (s), 1205 (s), 1169 (s), 1124 (s), 1108 (s), 1080 (s), 1056 (s) and 723 (s) cm^{-1}; $[\alpha]_D^{25}$ $-7°$ (c 0.994, $CHCl_3$); MS (for tristrimethylsilyl ether minus CH_3) $C_{39}H_{60}N_5O_{11}Si_3$ requires 858.3597, found 858.3578.

3′-*S*-3′-Aminomethyldihydrospectinomycin Trihydrochloride (**20**)

In 5 ml of MeOH was dissolved 100 mg (0.152 mmol) of azide **18**. To this solution was added 100 mg of Pd black and 61 μl (1.52 mmol) of HCOOH. The reaction was stirred for 10 minutes, was filtered and the solvent was removed *in vacuo* to afford 79 mg of a clear glass. The product was taken up in H_2O and 4.6 ml (0.46 mmol) of 0.1 N aqueous HCl was added. The sample was frozen and lyophilized overnight to afford 62 mg (86%) of a white solid: ^{13}C NMR (D_2O) δ 93.5, 92.6, 72.5, 70.6, 68.0, 66.8, 62.3, 61.1, 59.0, 44.4, 38.8, 32.1, 31.8 and 20.6; MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{26}H_{58}N_3O_7Si_4$ requires 636.3352, found 636.3334.

3′-*R*-*N*,*N*′-Dibenzyloxycarbonyl-3′-azidomethyldihydrospectinomycin (**19**)

To a solution of 90 mg (0.146 mmol) of epoxide **17** in 4 ml of absolute ethanol was added 113 mg (1.4 mmol) of KN_3 and 53 mg (1.0 mmol) of NH_4Cl. The solution was stirred 5.5 hours at 50°C under a nitrogen atmosphere, cooled to room temperature, and stirred 16 hours. The EtOH was removed *in vacuo* and the residue was partitioned between 50 ml of EtOAc and 5 ml of H_2O. The EtOAc was dried by brine and $MgSO_4$ and concentrated *in vacuo* to afford 93 mg (0.142 mmol, 97%) of product as a white solid: ^{13}C NMR (acetone-d_6) δ 158.0, 138.6, 129.2, 128.7, 128.4, 95.0, 92.9, 76.6, 74.4, 67.5, 67.3, 66.4, 65.5, 61.0, 57.8, 54.5, 39.9, 31.8 and 21.4; IR ($CHCl_3$) 3580, 3400, 3010, 2940, 2109, 1780, 1500, 1300, 1170 and 1070 cm^{-1}.

3′-*R*-3′-Aminomethyldihydrospectinomycin Trihydrochloride (**21**)

Azide **19** (36 mg, 0.055 mmol) was dissolved in 0.5 ml of MeOH and 36 mg of Pd black was added followed by the addition of 21 μl (0.55 mmol) of HCOOH. The mixture was stirred 10 minutes at room temperature and filtered, rinsing the catalyst 4 times with MeOH. The solution was concentrated *in vacuo*, to give a glass which was dissolved in 5 ml of H_2O, treated with 0.18 ml (0.18 mmol) of 1 N HCl and lyophilized to give 30 mg of a white solid, the hydrate of the product: Rf 0.30 (silica gel, $CHCl_3$ - MeOH - NH_4OH, 3:4:2); ^{13}C NMR (D_2O, CH_3CN internal reference) δ 94.3, 93.6, 73.0, 70.7, 68.4, 67.0, 66.3, 62.6, 60.7, 59.5, 43.4, 40.8, 31.9, 31.6 and 21.1; MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{26}H_{58}N_3O_7Si_4$ requires 636.3352, found 636.3345.

3′-*R*-3′-Cyanomethyldihydrospectinomycin Dihydrochloride (**22**)

A) In 100 ml of H_2O - EtOH, 1:5 is combined 1.0 g (1.62 mmol) of epoxide **16**, 1.05 g (16.2 mmol) of KCN and 866 mg of NH_4Cl. The mixture is stirred for 5 hours and the EtOH is removed *in vacuo*. The residue is partitioned between EtOAc and H_2O and the organic layer is dried with brine and Na_2SO_4. Removal of solvent affords 959 mg of white solid which is chromatographed on 100 g of silica gel, packed with $CHCl_3$. The column is eluted as follows: 1 liter 1% MeOH in $CHCl_3$, 1 liter 2% MeOH in $CHCl_3$, 1 liter 3% MeOH in $CHCl_3$, 1 liter 4% MeOH in $CHCl_3$, 1 liter 5% MeOH in $CHCl_3$, 2 liters 10% MeOH in $CHCl_3$. In elution volume 1,890~2,835 ml there is obtained 254 mg of recovered epoxide. In elution volume 3,510~4,410 ml there is obtained 156 mg (19.7% yield based on recovered epoxide) of the benzyloxycarbonyl (Cbz) derivative of the title compound: ^{13}C NMR (acetone-d_6) δ 138.2, 138.1, 129.2, 128.5, 118.2, 94.5, 92.1, 74.4, 74.2, 73.8, 73.7, 73.3, 67.3, 66.4, 65.7, 61.2, 61.1, 57.8, 57.7, 57.4, 57.3, 40.6, 31.7, 31.6, 24.9 and 20.9; MS m/z 785 (di-TMS derivative, M^+), 512, 455, 299, 276, 247, 199, 149, 131, 116, 97, 85, 73 and 57.

B) In 2 ml of MeOH is dissolved 37 mg (0.057 mmol) of step A product and 50 mg of Pd black and 23 μl of HCOOH are added. The mixture is stirred 2 minutes and is filtered. Removal of solvent *in vacuo* affords 25 mg of a white solid which is dissolved in 10 ml of 0.1 N HCl and lyophilized to afford 28 mg of the title compound: ^{13}C NMR (D_2O) δ 120.3, 94.0, 92.8, 73.7, 71.1, 68.7, 67.2, 62.7, 61.6, 59.4, 40.5, 32.4, 32.0, 25.5 and 20.9; MS (for tetrakistrimethylsilyl ether) $C_{28}H_{59}N_3O_7Si_4$ requires 661.3430, found 661.3407.

3′-*S*-3′-(*N*-Propylaminomethyl)dihydrospectinomycin Trihydrochloride (**23**)

A) In 5 ml of freshly distilled $PrNH_2$ was dissolved 93 mg (0.151 mmol) of epoxide **16**. The resulting solution was refluxed for 2 hours at which time TLC showed complete loss of starting material, with the formation of one more polar, ninhydrin active product. The $PrNH_2$ was removed *in vacuo* and the residue was concentrated twice from EtOAc. The product was then placed on the high-vacuum pump for 2 hours. This afforded 84 mg (83% yield) of a white solid, the Cbz derivative of the title compound. ^{13}C NMR (acetone-d_6) δ 138.2, 129.1, 128.3, 94.2, 74.9, 74.4, 74.0, 73.0, 67.2, 66.7, 66.5, 66.3, 65.1, 64.7, 60.3, 57.5, 57.4, 55.8, 52.5, 40.2, 31.5, 23.4, 21.2 and 11.9.

B) In 5 ml of MeOH was dissolved 80 mg (0.12 mmol) of step A product. To this solution was added 93 mg of Pd black and 46 μl of HCOOH. The reaction was stirred for 30 minutes, filtered, and the solvent removed *in vacuo* to afford 73 mg of a white solid. The product was taken up in 1 ml of H_2O and 4 ml of 0.1 N HCl was added. The sample was frozen and lyophilized to afford 71 mg of a white solid (100%): ^{13}C NMR (D_2O) δ 92.6, 91.7, 71.9, 69.8, 67.0, 66.1, 65.9, 61.5, 60.3, 58.0, 51.4, 50.5, 38.2, 31.2, 19.9, 18.8 and 10.4. MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{29}H_{64}N_3O_7Si_4$ requires 678.3821, found 678.3791.

3′-*R*-3′-(*N*-Butylaminomethyl)dihydrospectinomycin Trihydrochloride (**24**)

A) Epoxide **17** (272 mg, 0.44 mmol) was dissolved in 5 ml of *n*-butylamine and heated for 24 hours at 60°C. The amine solvent was removed *in vacuo* and the residue was dissolved in 20 ml of EtOAc. The solution was extracted with 20 ml of H_2O, adjusted to pH 2 with 10% HCl. The organic layer was further extracted with 10 ml of ~1% HCl. The combined aqueous phases were extracted with 20 ml of EtOAc. The total EtOAc solution was dried with brine and $MgSO_4$ and concentrated *in vacuo* to give 76 mg of a colorless glass which was identified as starting epoxide by TLC. The aqueous extracts were adjusted to pH 10 with 1 N NaOH and extracted with two 20-ml portions of EtOAc. This solution was dried with brine and $MgSO_4$ and concentrated *in vacuo* to afford 202 mg of product as a white solid (0.29 mmol, 67%, 91% based on recovered starting material). Rf 0.43 (silica gel, 10% MeOH in $CHCl_3$); ^{13}C NMR (acetone-d_6) δ 158.0, 138.2, 129.1, 128.3, 128.1, 97.1, 93.8, 74.6, 71.7, 67.1, 66.4, 65.2, 61.0, 57.6, 54.4, 49.3, 44.5, 32.1, 31.6, 21.5, 20.8 and 14.2; MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{46}H_{78}N_3O_{11}Si_4$ requires 960.4713, found 960.4703.

B) The substrate from step A (160 mg, 0.23 mmol) was dissolved in 4 ml of MeOH and 100 mg of Pd black was added followed by the addition of 87 μl (2.3 mmol) of HCOOH. The mixture was stirred 30 minutes at room temperature and filtered, rinsing the catalyst well with MeOH. The solvent was removed *in vacuo* to give a glass which was dissolved in 3 ml of H_2O, treated with 0.75 ml (0.75 mmol) of 1 N HCl and lyophilized to give 126 mg (0.24 mmol, 100%) of product **24** as a white solid: Rf 0.84 (silica gel, $CHCl_3$ - MeOH - NH_4OH, 3:4:2); ^{13}C NMR (D_2O, CH_3CN internal reference) δ 94.1, 93.3, 73.3, 70.5, 68.3, 66.9, 66.2, 62.5, 60.6, 59.4, 51.0, 49.5, 40.9, 31.9, 31.5, 27.9, 21.0, 20.1 and 13.9; MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{30}H_{66}N_3O_7Si_4$ requires 692.3978, found 692.3967.

3′-*R*-3′-[(3-Dimethylaminopropyl)aminomethyl]dihydrospectinomycin Tetrahydrochloride (**25**)

A) Epoxide **17** (600 mg, 0.98 mmol) was dissolved in 5 ml of 3-dimethylaminopropylamine and heated at 60°C for 71 hours. The excess amine was removed *in vacuo* and the residue was dissolved in 75 ml of EtOAc which was extracted with 2×5 ml of H_2O and 25 ml of brine, dried with $MgSO_4$ and concentrated to give 600 mg of pale yellow solid. The product was chromatographed on 30 g of 230~400 mesh silica gel packed with 5% MeOH in $CHCl_3$ and eluted with 500 ml of 5% MeOH in $CHCl_3$ with 0.5% NH_4OH, 1 liter of 10% MeOH in $CHCl_3$ with 1% NH_4OH and the rest 20% MeOH in $CHCl_3$ with 1% NH_4OH collecting 30 ml fractions. Pooling of fractions 29~56 gave 235 mg (0.33 mmol, 33%) of the Cbz derivative of the title compound as an off white solid: Rf 0.22 (silica gel, 20% MeOH in $CHCl_3$ with 1% NH_4OH); ^{13}C NMR (acetone-d_6) δ 157.0, 138.2, 129.1, 128.4, 96.9, 93.8, 74.6, 74.2, 71.8, 67.1, 66.4, 65.2, 60.5, 58.1, 57.6, 54.4, 48.1, 45.4, 44.2, 31.7, 27.3 and 21.5; MS (for tetrakistrimethylsilyl ether minus CH_3) $C_{47}H_{81}N_4O_{11}Si_4$ requires 989.4979, found 989.4968.

B) To a solution of 230 mg (0.32 mmol) of the substrate from step A in 5 ml of MeOH was added 150 mg of Pd black and 0.12 ml (3.2 mmol) of HCOOH. The mixture was stirred 3 hours at room temperature and filtered rinsing the catalyst with MeOH. The solvent was removed *in vacuo* and

the residue was dissolved in H_2O and treated with 1.4 ml (1.4 mmol) of 1 N HCl. Lyophilization gave 200 mg (0.33 mmol, 100%) of product **25** as an off white solid: Rf 0.77 (silica gel, $CHCl_3$ - MeOH - NH_4OH, 3: 4: 2); ^{13}C NMR (D_2O, CH_3CN internal reference) δ 94.1, 93.2, 73.3, 70.5, 68.2, 66.9, 66.2, 62.4, 60.6, 59.3, 55.2, 51.4, 46.4, 43.9, 40.8, 31.9, 31.5, 21.7 and 21.0; MS (hexakistrimethylsilyl derivative) *m/z* 880 (M^+); ($M^+ - CH_3$) $C_{37}H_{85}N_4O_7Si_6$ requires 865.5034, found 865.5037.

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