SPECTINOMYCIN MODIFICATION

IV. THE SYNTHESIS OF 3′-AMINOMETHYLDIHYDROSPECTINOMYCINS *VIA* SPECTINOMYCIN 3′-CYANOHYDRINS

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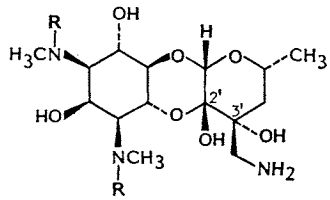
The C-3′-carbonyl group of *N*-protected spectinomycin is converted into the corresponding aminomethylalcohols *via* the intermediacy of cyanohydrins. Methodology for the selective synthesis of either epimer with retention of protection in the aminocyclitol ring provides valuable synthetic intermediates for the preparation of analogs of this important antibiotic. The new methodology provides an efficient synthesis of the highly active 3′-aminomethyldihydrospectinomycins.

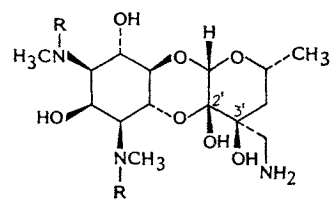
In the previous paper in this series,[1] we described the synthesis of the epimeric 3′-aminomethyldihydrospectinomycins **1** and **2** *via* the intermediacy of spectinomycin-derived diazoketones. The aminomethyldihydrospectinomycins and representative *N*-alkyl analogs were shown to have excellent *in vitro* antibacterial activity, being, in some instances, superior to the parent C-3′-keto-compound, spectinomycin (**3**). On the basis of this exciting lead discovery, we wished to prepare **1** and **2** in large quantities for a more thorough biological evaluation and, in suitably protected form, as precursors for an analog program.

Our efforts were hampered by a number of important considerations. First, the original synthetic route to these compounds required a large number of steps, some of which gave modest yields. Second, the nature of the synthesis of **1** and **2** and the stereochemical bias inherent in the spectinomycin skeleton dictated that only the 3′-*S*-isomer **2** could be obtained in high yield. Ironically, the 3′-*R*-series of analogs derived from **1** were far more potent than the readily available 3′-*S* compounds. Finally, the methodology employed afforded **1** and **2** with no protection remaining on the aminocyclitol nitrogen atoms, thus limiting their utility for further analog work.

In this paper, we report the development of new methodology that solves the problems inherent in the original synthesis, providing 3′-aminomethyldihydrospectinomycins from either epimeric series in quantity by a short, efficient synthetic sequence. The use of the actinamine-protected 3′-amino-



1 R = H
11 R = Cbz



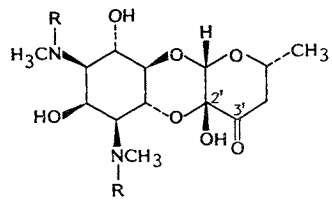
2 R = H
8 R = Cbz

methyldihydrospectinomycins for the synthesis of a variety of bioactive spectinomycin analogs is the subject of the accompanying and subsequent papers.

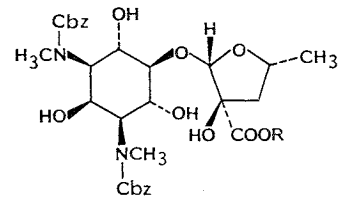
Results and Discussion

The most direct method for the transformation of a ketone to the corresponding aminomethyl-alcohol is *via* conversion to a cyanohydrin derivative followed by reduction of the nitrile group.[2] While this methodology has been employed in carbohydrate derivatives by others (*vide infra*), the inherent chemical instability of spectinomycin derivatives was cause for concern. The parent antibiotic **3** or more organic-soluble derivatives such as *N,N'*-dibenzyloxycarbonylspectinomycin (**4**) undergo facile rearrangement to actinospectinoic acid derivatives (**5**) in the presence of even mild base.[3] In the presence of weak protic or Lewis acids, a similar rearrangement takes place with internal trapping to generate lactone **6**.[4] The conditions that result in rearrangement of spectinomycin are identical to those normally employed for cyanohydrin formation.

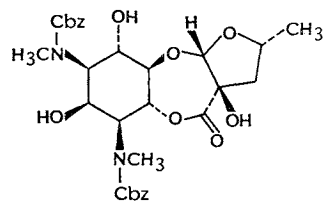
In light of the potential utility of spectinomycin-derived cyanohydrins, we decided to attempt their synthesis in spite of the instability of the substrate. For our initial attempt, we elected to employ



3 R = H
4 R = Cbz



5 R = H
5a R = CH_3



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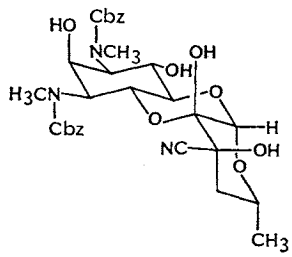

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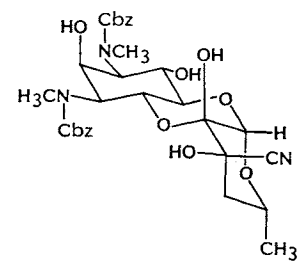

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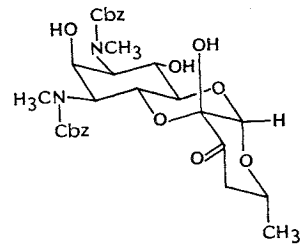
acetone cyanohydrin as a convenient source of hydrogen cyanide, and treated a methanolic solution of **4** with this reagent in the presence of K_2CO_3. From this reaction a single cyanohydrin, subsequently shown to be the 3′-*S*-isomer **7**, was isolated in 75% yield after chromatography. We were pleased to find that whereas in the absence of a source of cyanide ion such conditions would lead to rapid rearrangement, cyanohydrin formation was rapid and effectively protected the substrate from destruction. Only small amounts of the rearranged methyl ester **5a** were observed.



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With the cyanohydrin in hand, we required a method for the selective reduction of the nitrile in the presence of the benzyloxycarbonyl (Cbz) protecting groups. Catalytic hydrogenation employing either platinum or rhodium catalysts[5] resulted in reduction with concomitant deprotection. Transfer hydrogenation[6] with palladium black and formic acid resulted in deprotection without nitrile reduction. After extensive screening of solvent/catalyst combinations, it was discovered that Raney nickel in acetic acid selectively reduced the nitrile group without removing the Cbz protection, affording amine **8**, albeit in a modest 40% isolated yield. Subsequent removal of the Cbz groups by the transfer hydrogenation technique gave **2**, thus establishing the stereochemistry of cyanohydrin formation. This two-step synthesis of **2** is vastly superior to the original route, allowing easy preparation of multigram quantities for *in vivo* evaluation.

The successful preparation of **8** with its free aminomethyl group and protection of the actinamine nitrogens provided new opportunities for analog synthesis, but once again, the synthetic methodology provided the less active stereochemical series, the compounds for which we already had viable routes. We were somewhat surprised by the stereochemical outcome of the cyanide addition reaction, since our earlier work[1,7] provided numerous examples of reactions at C-3′ involving attack from the less hindered β-face (see stereo-structure **9**). LUKACS *et al.*[8] have reported a study on cyanohydrin formation in a series of 2-deoxy-3-keto-sugars. In the cases studied, reaction under either kinetic or thermodynamic control resulted in the opposite stereochemistry at the newly formed asymmetric center. This stereochemistry was independent of the anomeric configuration and directly related to the stereochemistry of the neighboring oxygen substituent at C-4. The authors rationalized the outcome of their kinetic cyanohydrin formation reactions by preferential equatorial attack on the keto sugar, while the thermodynamic products were those which minimized electrostatic interaction of oxygen substituents on neighboring carbon atoms in the product. In a more recent study, BRIMACOMBE and RAHMAN[9] cited further examples of carbohydrate substrates in which the stereochemistry at C-4 has a dominant effect on the kinetic and thermodynamic cyanohydrins of C-3 ketones.

The spectinomycin ring system provides a unique opportunity for studying cyanohydrin formation of 3-uloses. As depicted in structure **9**, the presence of the C-2′-hemiketal locks the sugar ring into a single conformation, with a sterically unencumbered β-face. The present instance, however, is the first case that we are aware of in which the carbon atom α to the carbonyl group bears two oxygen substituents. A comparison of the two epimeric cyanohydrins reveals that **7**, the product of the base-catalyzed reaction has the C-3′-hydroxyl substituent gauche to one of the C-2′-oxygen atoms and

antiperiplanar to the other. The alternative isomer **10** has the C-3′-hydroxyl group gauche to both of the C-2′-oxygens. An extension of the literature precedent cited above suggest that **7** should be the thermodynamically more stable isomer, and that **10**, while predicted to be less stable, could be the product of kinetic attack at the carbonyl from the unencumbered β-face. We were therefore encouraged to explore other conditions for the spectinomycin cyanohydrin forming reaction to search for the desired 3′-*R*-isomer **10**. The weaker base $NaHCO_3$ was used as the basic catalyst for the cyanohydrin exchange between acetone cyanohydrin and Cbz-spectinomycin. At early times during the reaction a new, less polar product was observed, along with **7** and residual ketone **4**. As the reaction progressed, the new product was seen to decrease at the expense of the thermodynamic product. Attempts to increase the amount of the new product by using low temperatures and short reaction times were unsuccessful. In an attempt to provide very mild conditions and a method for halting the reaction with a minimum of workup, the weakly basic ion-exchange resin Amberlite IR-45 was employed. Use of this catalyst in methanol with an excess of acetone cyanohydrin led to the predominance of the kinetic product **10**, as judged by TLC. Removal of the catalyst by filtration halted the cyanohydrin exchange, but isolation of **10** was still a problem. Upon chromatography on silica gel, **10** rapidly equilibrates with starting ketone and the isomeric cyanohydrin **7**, which is a sink for the system. Nevertheless, sufficient amounts of **10** were isolated to allow its characterization as the desired epimeric cyanohydrin. Reduction of **10** with Raney nickel in acetic acid gave **11** which was deprotected to give **1**. This provided the first really viable route to **1** in reasonable quantity.

As a final modification of the synthetic methodology, we hoped to avoid the troublesome isolation of the labile kinetic cyanohydrin by simply taking the crude mixture of **7**, **10** and residual **4**, doing the Raney nickel reduction, and separating the derived amines **8** and **11**. We also foresaw the possibility that the reduction of **10**, with its sterically more accessible nitrile group, might be significantly faster than that of **7**, allowing for selectivity in this step. This idea proved to be readily reduced to practice.

The crude product from the resin-catalyzed cyanohydrin formation was reduced with the Raney nickel system. By careful monitoring of the reaction it was found that the reduction of **10** was much faster than **7** and that the reaction could be halted at an appropriate point to give a mixture of amine **11** along with residual **4** and **7** from which the desired product could be isolated by simple acid - base extraction, leaving the residual ketone for potential recycling. This technique was further streamlined by switching to acid catalysis for the cyanohydrin reaction, and by the observation that the reduction of **10** was sufficiently facile to allow the use of platinum oxide as the catalyst. The latter modification simplifies the workup by avoiding the formation of nickel salts which arise from dissolution of the Raney nickel in the acidic medium. Treatment of ketone **4** with KCN and acetic acid in methanol gave the mixture of cyanohydrins, again enriched in the kinetic product **10**, which on reduction and acid-base workup afforded amine **11** in 48% yield. Thus, a simple, one-pot procedure for the conversion of Cbz-spectinomycin to 3′-*R*-aminomethyldihydro-*N*,*N*′-dibenzyloxycarbonylspectinomycin (**11**) was in hand, providing new opportunities for analog synthesis.

Conclusion

We have established the viability of the cyanohydrin approach to the preparation of aminomethyldihydrospectinomycins even in this rearrangement-prone system. Proper selection of reaction conditions allows the preparation of either the kinetic or thermodynamic cyanohydrin, compounds whose relative stabilities are in accord with other carbohydrate-derived cyanohydrins. Complete equilibra-

tion to the thermodynamic isomer, or selective reduction of the kinetic isomer in mixtures, allows the synthesis of either epimeric series of aminoalcohol. Selective reduction of the nitrile while retaining protection in the aminocyclitol ring provides useful intermediates for further synthesis. The use of **8** and **11** for the preparation of a series of *N*-alkyl and *N*-acyl derivatives as well as for the synthesis of novel ring-expanded spectinomycins *via* the Tiffeneau-Demjanov rearrangement will be described elsewhere.[10]

Experimental

^{13}C NMR spectra were recorded on a Varian CFT-20 or FT80A spectrometer in the indicated solvent using $(CH_3)_4Si$ or CH_3CN (for D_2O solutions) as internal standards. Chemical shifts are reported in ppm downfield from $(CH_3)_4Si$. IR spectra were recorded using a Perkin-Elmer 298 IR spectrometer equipped with a 3600 data station. Mass spectra and optical rotations were measured by the Physical and Analytical Chemistry Unit of The Upjohn Company.

N,N'-Dibenzyloxycarbonyl-3'-(*S*)-spectinomycin Cyanohydrin (**7**)

In 25 ml of MeOH were combined 5.0 g (8.33 mmol) of *N,N'*-dibenzyloxycarbonylspectinomycin and 0.76 ml (8.33 mmol) of acetone cyanohydrin. To this solution was then added 100 mg (0.72 mmol) of K_2CO_3. The reaction was stirred for 4.5 hours and then concentrated *in vacuo*. The residue was taken up in 40 ml of EtOAc, washed once with 50 ml brine and dried over Na_2SO_4. The solution was then filtered and concentrated *in vacuo* to afford 5.0 g of a white solid. The product was taken up in $CHCl_3$ and chromatographed on 200 g of silica, slurry packed in $CHCl_3$. The column was eluted as follows: 1 liter 1% MeOH - $CHCl_3$, 1 liter 2% MeOH - $CHCl_3$, 2 liters 4% MeOH - $CHCl_3$, 2 liters 5% MeOH - $CHCl_3$. In elution volume 3.3 liters to 6.0 liters was found 3.92 g (6.25 mmol) of the title compound (75% yield): ^{13}C NMR (MeOH-d_4) δ 138.1, 138.0, 129.4, 129.2, 128.8, 128.4, 94.4, 91.1, 74.8, 74.6, 74.5, 73.3, 68.2, 66.8, 66.4, 66.2, 60.9, 57.8, 42.1, 31.7, 20.6; $[\alpha]_D$ +4° (*c* 0.8935, $CHCl_3$); MS *m/z* 816, 801, 745, 629, 493, 449, 359, 305, 270, 170, 144, 91, 73.

N,N'-Dibenzyloxycarbonyl-3'-(*S*)-3'-(aminomethyl)dihydrospectinomycin (**8**)

In 10 ml of AcOH was dissolved 3.33 g (5.31 mmol) of *N,N'*-dibenzyloxycarbonylspectinomycin-3'-(*S*)-cyanohydrin. The solution was added to a Parr bottle containing 5.0 g of wet Raney nickel. The solution was then hydrogenated for 3.5 hours at an initial pressure of 1.05 kg/cm^2. The solution was then filtered through Magnesol and the acetic acid was removed *in vacuo*. The residue was partitioned between H_2O and EtOAc. The H_2O was separated and combined with **2**, 100 ml 1 N HCl washes of the EtOAc. The aqueous washes were made alkaline with 1 N NaOH (pH 10) and extracted with EtOAc (2×100 ml). The combined organic extracts were then washed with 100 ml of brine and were dried over $MgSO_4$. After filtering, removal of the solvent *in vacuo* left 1.323 g (40% yield) of the title compound: ^{13}C NMR (MeOH-d_4) δ 158.9, 158.4, 138.1, 129.5, 129.0, 128.7, 94.8, 93.7, 74.7, 73.9, 73.3, 68.7, 68.3, 67.7, 66.8, 65.7, 61.2, 57.9, 45.8, 39.7, 31.8, 21.2; IR (ATR) cm^{-1} 3385 (m), 1680 (s), 1497 (m), 1455 (s), 1407 (m), 1386 (m), 1344 (s), 1267 (m), 1210 (m), 1166 (s), 1079 (s), 1058 (s), 955 (m), 915 (m), 887 (m), 772 (m), 751 (m), 698 (m); exact mass calcd for $C_{31}H_{42}N_3O_{11}$: 632.2819, found: 632.2802.

N,N'-Dibenzyloxycarbonyl-3'-(*R*)-3'-aminomethyldihydrospectinomycin (**10**)

In 100 ml of 1 N acetic acid in methanol was dissolved 1.5 g (25 mmol) of *N,N'*-dibenzyloxycarbonylspectinomycin. To this solution was added 20 ml of a 1 M solution of potassium cyanide in MeOH - water (1 : 1). The reaction was stirred for 30 minutes at room temperature and was concentrated *in vacuo*. The concentrate was partitioned between EtOAc and water. The EtOAc was separated and combined with a second EtOAc extract. The extractions were washed with brine and dried over magnesium sulfate. Removal of the solvent *in vacuo* afforded 1.72 g of a white solid. The product was immediately taken up in 30 ml of 2 N AcOH - MeOH and hydrogenated in a Parr bottle in the presence of 1.5 g of platinum oxide at an initial pressure of 2.8 kg/cm^2 for 30 minutes. The reaction was filtered through Celite and concentrated *in vacuo*. The residue was partitioned between EtOAc and water. The water was separated and combined with a second water wash. The aqueous solution

was made alkaline with concentrated NH_4OH and extracted with EtOAc. The extracts were washed with brine and dried over magnesium sulfate. Removal of the solvent *in vacuo* afforded 764 mg of the title compound as a white solid (48% yield): ^{13}C NMR (MeOH-d_4) δ 158.2, 138.0, 129.4, 128.8, 128.6, 96.3, 94.3, 74.6, 73.8, 68.1, 66.7, 65.5, 60.9, 60.8, 58.0, 45.6, 41.4, 31.8, 21.5; IR (ATR) cm^{-1} 3397 (m), 1685 (s), 1497 (m), 1479 (m), 1455 (m), 1407 (m), 1386 (m), 1345 (s), 1266 (m), 1212 (m), 1163 (s), 1121 (s), 1060 (s), 771 (m), 751 (m), 698 (m); exact mass calcd for $C_{31}H_{42}N_8O_{11}$: 632.2819, found: 632.2824.

3′-(*S*)-Aminomethyldihydrospectinomycin Trihydrochloride (2)

In 10 ml of MeOH were combined 40 mg (0.062 mmol) of *N*,*N*′-dibenzyloxycarbonyl-3′-(*S*)-aminomethyldihydrospectinomycin and 54 mg of palladium black. To this mixture was added 23 μl (0.62 mmol) of formic acid. After 1 hour, an additional 50 mg of palladium black and 73 μl (1.3 mmol) of formic acid were added to complete the reaction. The reaction was filtered and concentrated *in vacuo* to afford 33 mg of a glass, which was taken up in 2 ml of water and treated with 2 ml of 0.1 N HCl. The solution was frozen and lyophilized to afford 28 mg (0.059 mmol, 95.5% yield) of the title compound as a white solid, identical by ^{13}C NMR and TLC with the compound previously reported.[1]

3′-(*R*)-Aminomethyldihydrospectinomycin Trihydrochloride (1)

In 5 ml of MeOH were combined 75 mg (0.12 mmol) of *N*,*N*′-dibenzyloxycarbonyl-3′-(*R*)-aminomethyldihydrospectinomycin and 75 mg of palladium black. To this mixture was added 46 μl (1.2 mmol) of formic acid. After 15 minutes, an additional 46 μl of formic acid were added. After stirring for 30 minutes, TLC showed complete reaction. The reaction was filtered and concentrated to afford 43 mg of a white solid. The product was taken up in H_2O and treated with 0.5 ml of 1 N HCl. The solution was frozen and lyophilized to afford 58 mg (0.12 mmol, 100% yield) of the title compound as white solid, identical by ^{13}C NMR and TLC with the compound previously reported.[1]

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