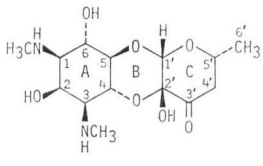
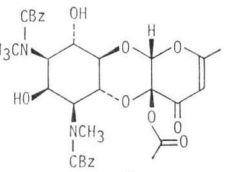
SYNTHESIS AND *IN VITRO* ANTIBACTERIAL PROPERTIES OF ALKYLSPECTINOMYCIN ANALOGS

Sir:

A recent review of spectinomycin chemistry[1] states that all changes in the cyclitol portion (A ring) of the molecule have resulted in loss of activity.



Spectinomycin (**1**)



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Synthetic methods developed in these laboratories[2] have focused on the C ring and have given routes to previously inaccessable 6′-modified spectinomycin analogs. As previously described,[3] these compounds were prepared by Lemieux glycosylation of protected actinamine with "fraudulent" sugars followed by C-3′ carbonyl generation (by an elimination reaction) and deprotection.

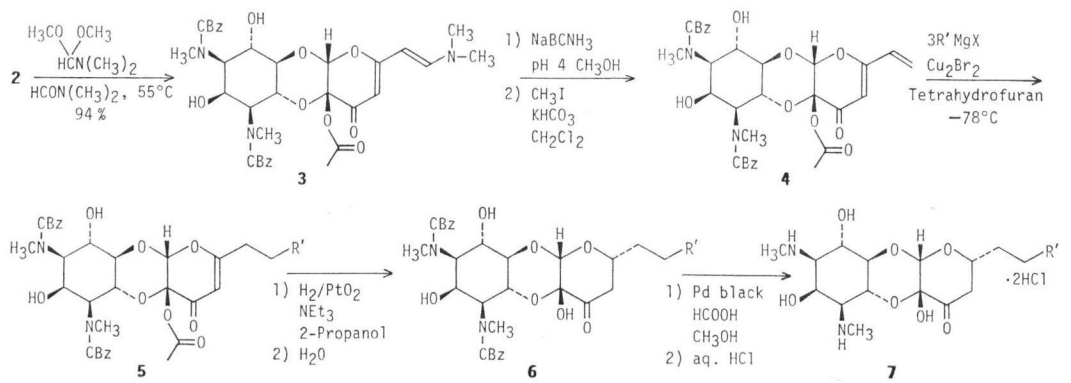
We describe here an alternative method of modification at C-6′ by elaboration of an activated intermediate **2** which we have prepared[2] in three steps from *N,N′*-dibenzyloxycarbonylactinamine and 3,4,6-tri-*O*-acetyl-2-deoxy-2-nitroso-α-L-glucopyranosyl chloride dimer. *In vitro* antibacterial properties of the 6′-alkylspectinomycins will be compared.

The enoneacetate (**2**)[2] reacts cleanly with a mixture of *N,N*-dimethylformamide and *N,N*-dimethylformamide dimethylacetal (55°C, 7 hours) to give 94% yield of enamine (**3**) after a simple silica gel chromatography. A methanolic solution of enamine (**3**) is reduced with sodium cyanoborohydride under acidic conditions (methanolic HCl, methyl orange indicator). After extraction from basic solution, the crude amine (89% yield) is treated with methyl iodide and powdered potassium bicarbonate in methylene chloride to give pure dienoneacetate (**4**) in 61% yield after chromatography.

The versatile dienoneacetate (**4**) is reactive toward a variety of nucleophiles including copper catalyzed Grignard reagents. Three equivalents of Grignard reagent are added to a tetrahydrofuran solution of dienoneacetate (**4**) and catalytic Cu_2Br_2 at −78°C. The solution is quenched by pouring onto ethyl acetate containing acetic acid and the resulting solution washed with water and brine. Chromatography on silica gel gives purified enones (**5**) (yield is 85% when R′ is *n*-C_7H_{15}).

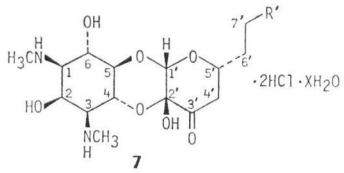
Reduction of the olefinic double bond in **5** is accompanied by carbonyl reduction in many methods. Use of PtO_2 in 2-propanol with added triethylamine suppresses carbonyl reduction and also maintains the presence of the benzyloxycarbonyl blocking groups so that final chromatographic purification can be done before complete deprotection. After hydrogenation for about 2 hours at 1.4 kg/cm², the catalyst is filtered and water is added to the filtrate. A period of 16~20 hours at room temperature is sufficient to remove the 2′-*O*-acetyl group. The solvent is removed and the product chromatographed on silica gel giving **6** (30% yield for R′=*n*-C_7H_{15}).

Deprotection of the nitrogen atoms is cleanly accomplished by using $Pd/HCO_2H/CH_3OH$;[4]



R′=CH_3, C_2H_5, *n*-C_4H_9, *n*-C_7H_{15}, *n*-$C_{10}H_{21}$.

Table 1. ^{13}C NMR shifts (ppm) for spectinomycin analogs (7) (D_2O solvent with CH_3CN as internal standard).



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Assignment	R′=CH_3	R′=C_2H_5	R′=n-C_4H_9	R′=n-C_7H_{15}	R′=$C_{10}H_{21}$
N-CH_3's	28.8, 29.4	29.8, 29.4	29.8, 29.3	30.1, 29.5	30.2, 29.6
C-1	60.6	60.6	60.6	60.6	60.5
C-2	58.6	58.6	58.6	58.6	58.7
C-3	57.6	57.6	57.7	57.5	57.6
C-4	64.6	64.6	64.7	64.7	64.9
C-5	68.8	68.7	68.8	68.8	68.9
C-6	65.0	64.9	65.0	65.1	65.1
C-1′	92.7	92.6	92.7	92.6	92.8
C-2′	92.7	92.6	92.7	92.6	92.7
C-3′	91.0	91.0	91.1	91.1	91.2
C-4′	38.5	38.5	38.6	38.2	39.0
C-5′	70.7	70.9	71.0	70.9	70.9
C-6′	35.0	32.5	32.9	33.4	32.6
C-7′	16.6	25.4	23.2	24.2	24.4
C-8′	12.4	21.0	27.5	28.5	28.4
C-9′		12.3	30.1	28.5	28.8
C-10′			21.0	28.2	28.8
C-11′			12.5	28.2	28.8
C-12′				30.7	28.8
C-13′				21.5	28.8
C-14′				12.8	28.8
C-15′					30.9
C-16′					21.6
C-17′					12.8

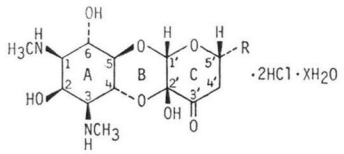
addition of 2 equivalents of HCl to an aqueous solution and lyophilization gives analogs (7) (77% yield for R′=C_7H_{15}). All compounds had satisfactory mass spectra and ^{13}C NMR spectra; the ^{13}C NMR line positions for final products are listed in Table 1.

The lipophilic side chain on ring C is clearly important to the antibacterial potency and spectrum of the spectinomycin molecule. The molecule with no side chain (R=H, made previously)[3] is of very low activity. As the number of carbons increases, a steady improvement in Gram-positive activity (*Staphylococcus aureus, Streptococcus faecalis*) is achieved. Very little change in overall Gram-negative potency is seen for analogs when R is C_1 through C_3. A slight decrease in overall Gram-negative activity is evident when R is C_4 through C_9, followed by a dramatic increase in potency against some Gram-negative species when R is C_{12}. A steady gain in potency against *Pseudomonas aeruginosa* (gentamicin-susceptible and gentamicin-resistant cultures) is achieved when R is C_6 through C_{12}.

The most significant changes in the antibacterial spectrum of the molecule occur when R is C_{12}. This compound has dramatically increased overall Gram-positive and Gram-negative activity, including *P. aeruginosa* activity, but reduced potency against *Serratia marcescens* and *Proteus* species.

The significantly improved Gram-positive activity seen throughout this series of analogs, as well as the *P. aeruginosa* activity exhibited by the longer chain length compounds, make these analogs suitable for further *in vitro* and *in vivo* developmental studies.

Table 2.



Organism	UC#	Minimum inhibitory concentration[c] (μg/ml)								
		R=H[a]	R=CH_3	R=C_2H_5[b]	R=C_3H_7	R=C_4H_9	R=C_5H_{11}	R=C_6H_{13}	R=C_9H_{19}	R=$C_{12}H_{25}$
Staphylococcus aureus	76	62.5	7.8	7.8	3.9	3.9	2.0	1.0	1.0	≤0.25
Streptococcus faecalis	694	>1,000	62.5	62.5			7.8	15.6	3.9	≤0.25
Escherichia coli	45	31.2	3.9	7.8	7.8	31.2	62.5	62.5	15.6	1
Klebsiella pneumoniae	58	7.8	2.0	2.0	2.0	7.8	15.6	15.6	15.6	1
Proteus vulgaris	93	31.2	7.8	7.8	3.9	3.9	15.6	15.6	31.2	62.5
P. mirabilis	6671	62.5	3.9	7.8	15.6	31.2	62.5	62.5	125	62.5
P. morganii	3186		7.8	7.8	7.8	7.8		15.6	62.5	31.2
Shigella flexneri	143	15.6	7.8	7.8	7.8	15.6	31.2	31.2	15.6	1.0
Salmonella typhi	215	62.5	7.8	7.8	7.8	15.6	31.2	62.5	31.2	2.0
Serratia marcescens	131	15.6	3.9	3.9	7.8	31.2	250	250	250	125
Salmonella schottmuelleri	126	125	7.8	15.6	7.8	31.2	125	7.8		
Providencia stuartii	6570	1,000	>250	>250	15.6	15.6	31.2	62.5	62.5	62.5
Enterobacter cloacae	3054		62.5	31.2	62.5	250		250	>250	62.5
Citrobacter freundii	3807		7.8	3.9	7.8	15.6		62.5	31.2	3.9
Pseudomonas aeruginosa	6436[d]		250	250	250	250		250	15.6	7.8
P. aeruginosa	3686		250	250	250	>250		125	7.8	3.9

[a] The preparation of this compound has been described previously.[3)]

[b] The preparation of this compound will be described in another publication.

[c] MIC's were determined using a microplate broth-dilution technique. The test medium was a modified brain heart infusion broth (4.5 g BHI broth (Difco) and 2.0 g glucose per liter distilled water, pH adjusted to 6.7) that provides a sensitive assay for spectinomycin antibiotic activity.

[d] Gentamicin-resistant strain.

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References

1) ROSENBROOK, W., Jr.: Chemistry of spectinomycin. Jap. J. Antibiotics 32 Suppl.: S211~S227, 1979
2) WHITE, D. R.; R. D. BIRKENMEYER, R. C. THOMAS, S. A. MIZSAK & V. H. WILEY: The stereospecific synthesis of spectinomycin. Tetrahedron Lett. 1979: 2737~2740, 1979
3) THOMAS, R. C.; D. R. WHITE, V. H. WILEY & D. A. FORSTER: Synthesis of spectinomycin analogs. *In* "Aminocyclitol Antibiotics." K. L. RINEHART, Jr. & T. SUAMI *eds.*, pp. 121~131, ACS Symposium Series, No. 125, Am. Chem. Soc., 1980
4) ELAMIN, B.; G. M. ANATHARAMAIAH, G. P. ROYER & G. A. MEANS: Removal of benzyl-type protecting groups from peptides by catalytic transfer hydrogenation with formic Acid. J. Org. Chem. 44: 3442~3444, 1979