MODIFICATION OF SPECTINOMYCIN

1. SYNTHESIS OF 4-AMINOSPECTINOMYCINS

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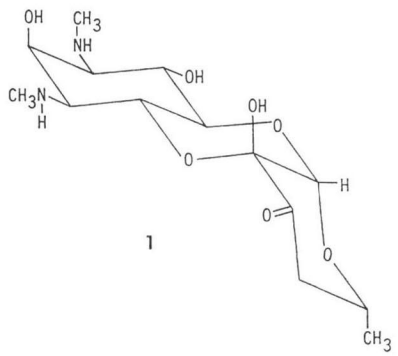
Preparation of 4-dihydro-4-deoxy-4(*R*)-aminospectinomycin (**4a**) and 4-dihydro-4-deoxy-4(*S*)-aminospectinomycin (**4b**) is described. **4a** shows antibiotic activity comparable to spectinomycin, **4b** is devoid of activity.

Spectinomycin (**1**) is an aminocyclitol antibiotic with a unique tricyclic structure[1,2]. It possesses a broad spectrum of activity against Gram-positive and Gram-negative bacteria[3]. The mechanism of action is inhibition of protein synthesis by interaction with the 30 S ribosomal subunit[4]. Spectinomycin is an attractive starting material for chemical modification because it lacks the undesirable nephro- and ototoxic properties of the aminoglycosides.



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Reduction of spectinomycin leads to the epimeric 4-dihydrospectinomycins with antibacterial activity[5]. This finding proves that sp^2 configuration at C-4 is not essential for biological activity and prompted us to synthesize the epimeric 4-amino compounds.

Synthesis

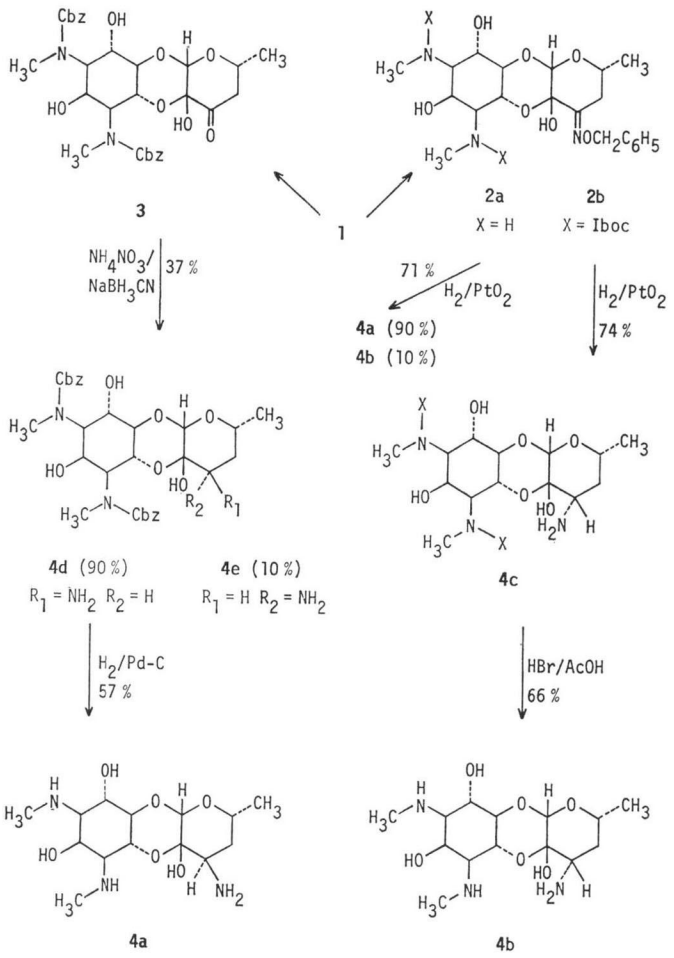
According to Scheme 1 **4a** and **4b** were prepared. In the first route, spectinomycin (**1**) was transformed to the oximes **2a** and **2b**. Catalytic hydrogenation of **2a** in ethanolic hydrogen chloride yielded a mixture of **4a** and **4b**, consisting of about 90% of **4a**. The stereochemical course of the hydrogenation could be changed by introduction of bulky isobornyloxycarbonyl (Iboc) groups into the spectinomycin molecule. Compound **2b** yielded **4c** as sole product, which was converted to **4b**.

The second route to the 4-aminospectinomycins was the reductive amination with sodium cyanoborohydride[7]. Treatment of 6,8-bis(N-benzyloxycarbonyl)spectinomycin (**3**)[8] with ammonium nitrate and sodium cyanoborohydride yielded 37% of a mixture of **4d** (90%) and **4e**. Compound **4d** was transformed into **4a** by cleavage of the carbobenzoxy groups.

Structure of the 4-Aminospectinomycins

The structures of **4a** and **4b** were confirmed by PMR data. The 250 MHz PMR spectra of **4a** and **4b** in D_2O at room temperature are shown in Figs. 1 and 2, respectively. Chemical shifts and coupling constants are listed in Table 1.

Scheme 1. Synthesis of 4-aminospectinomycins.



The assignment of the six actinamine protons was accomplished by spin decoupling experiments. The coupling pattern of these protons is identical with that of spectinomycin and therefore confirms the stereochemistry of the actinamine moiety.

The stereochemistry of the 4-amino group could be derived from $J_{3eq,4}$ and $J_{3ax,4}$, both obtained from the H-4 peaks near 3.7 ppm.

For **4b** we found values of 11.8 and 4.9 Hz for $J_{3ax,4}$ and $J_{3eq,4}$, respectively, confirming the presence of an axial H-4.

On the other hand, the values of 3.9 Hz and 2.4 Hz for $J_{3ax,4}$ and $J_{3eq,4}$, determined by decoupling experiments and computer spectra simulation of the whole proton sequence at C-2/C-3/C-4, confirm the equatorial H-4 in **4a**.

Antimicrobial Properties

Whereas **4b** was inactive *in vitro* up to 160 μg/ml, **4a** showed *in vitro* antibacterial activity comparable to spectinomycin when tested against a number of Gram-positive and Gram-negative bacteria. This great difference between the epimers is surprising when compared with the corresponding epi-

Table 1. PMR-Parameters of 4-dihydro-4-deoxy-4(*R*)-aminospectinomycin (**4a**) and 4-dihydro-4-deoxy-4(*S*)-aminospectinomycin (**4b**). 250 MHz spectra in D_2O at room temperature.

4a				**4b**			
Chemical shifts (ppm)		Coupling constants (Hz)		Chemical shifts (ppm)		Coupling constants (Hz)	
Ring C				Ring C			
H-2	4.12	$J_{CH_3,2}$	6.2	H-2	3.95	$J_{CH_3,2}$	6.2
H-3ax	2.06	$J_{2,3ax}$	7.8	H-3ax	1.76	$J_{2,3ax}$	11.1
H-3eq	1.96	$J_{2,3eq}$	2.1	H-3eq	2.04	$J_{2,3eq}$	2.8
H-4	3.69	$J_{3ax,3eq}$	15.7	H-4	3.67	$J_{3ax,3eq}$	12.8
H-10a	5.06	$J_{3ax,4}$	3.9	H-10a	4.91	$J_{3ax,4}$	11.8
2-CH_3	1.31	$J_{3eq,4}$	2.4	2-CH_3	1.26	$J_{3eq,4}$	4.9
Ring A				Ring A			
H-5a or 9	3.99			H-5 or 9	4.02		
H-6 or 8	3.30			H-6 or 8	3.32		
H-7	4.79			H-7	4.80		
H-8 or 6	3.57			H-8 or 6	3.59		
H-9 or 5a	4.37			H-9 or 5a	4.35		
H-9a	4.07			H-9a	4.09		
N-CH_3	2.88			N-CH_3	2.86, 2.88		

Fig. 1. 250 MHz PMR spectrum of 4-dihydro-4-deoxy-4(*R*)-aminospectinomycin (**4a**).

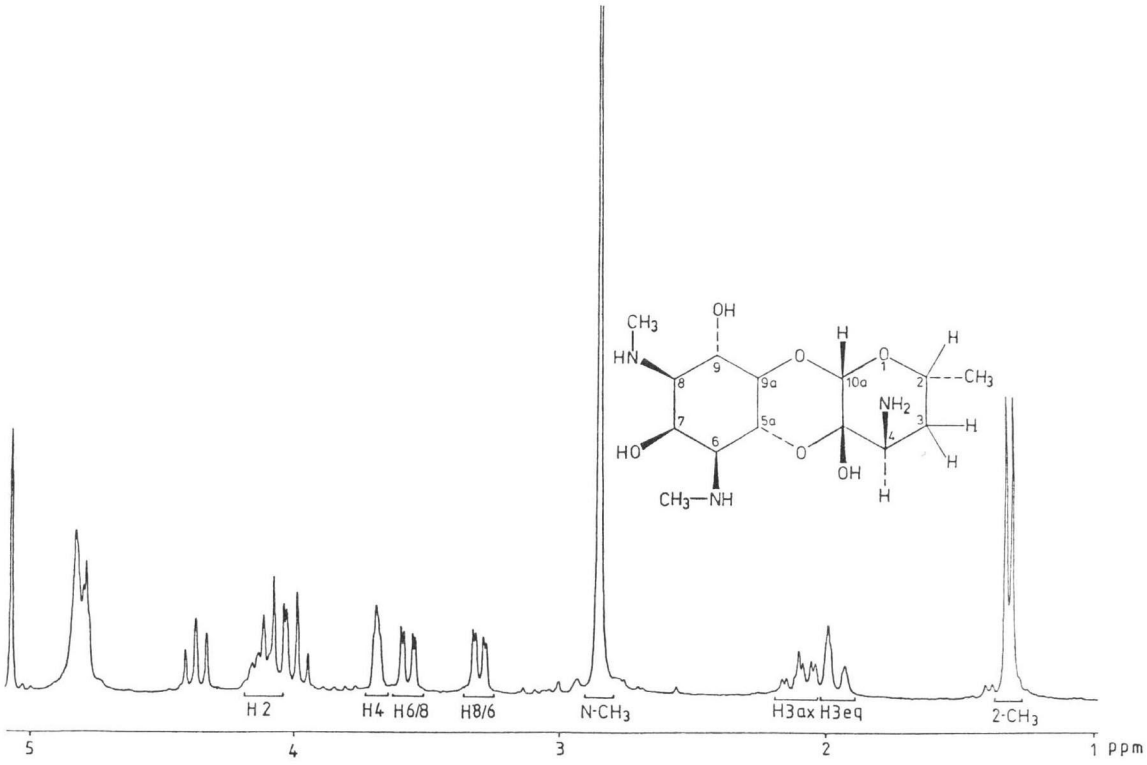
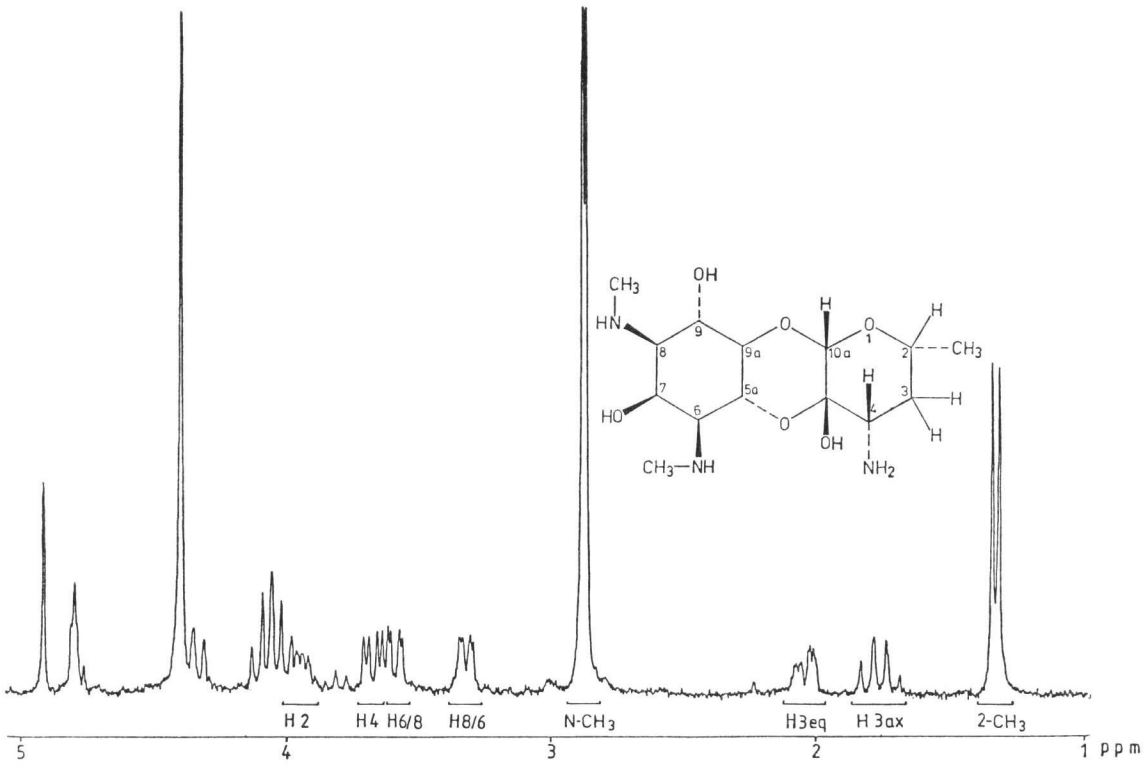


Fig. 2. 250 MHz PMR spectrum of 4-dihydro-4-deoxy-4(*S*)-aminospectinomycin (**4b**).



meric dihydrospectinomycins which show reduced but similar activities (40% and 30% of spectinomycin activity, respectively)[5].

The *in vivo* activity was tested in mice infected with *Escherichia coli* ATCC 11775. In this test **4a** was slightly less active than spectinomycin.

Experimental Section

Melting points were taken with a Büchi 510 instrument and are uncorrected; 80 MHz PMR spectra were measured on a Bruker WP-80 instrument, 90 MHz PMR and 22.63 MHz ^{13}C NMR spectra on a Bruker HX-90 instrument and 250 MHz PMR spectra were obtained on a Bruker WM-250 instrument. Chemical shifts are reported in ppm downfield from internal tetramethylsilane. Mass spectra (MS) of the silylated compounds were obtained on a Varian MAT CH 5 instrument. N,N-Bis-trimethylsilyl-trifluoracetamide was used for introduction of the trimethylsilyl (TMSi) groups.

LiChroprep Si 60 (63~125 μm, size C) and plates precoated with silica gel 60-F 254 or F-254 (all from Merck, Darmstadt) were used for column, thin-layer (TLC) or preparative thin-layer chromatography, respectively.

Spectinomycin-4-O-benzyloxime Dihydrochloride (**2a**)

Spectinomycin dihydrochloride pentahydrate (6 g, 12 mmole) and O-benzylhydroxylamine (3 g, 24 mmole) in 50% aqueous CH_3OH (50 ml) were stirred overnight, concentrated, the residue dissolved in a small amount of dry C_2H_5OH and precipitated with ether to give **2a** (5.0 g, 83%) as a white powder: m.p. 175°C (dec.); PMR (CD_3OD, 90 MHz) δ 1.35 (d, 3H, C-2 CH_3), 1.90~2.20 (dd, 1H, H-3ax), 2.85, 2.90 (2s, 6H, NCH_3), 4.70 (s, 1H, H-10a), 5.17 (s, 2H, CH_2-Ph), 7.35 (s, 5H, aromatic); ^{13}C NMR (D_2O) 158.06 ppm (C-4).

6,8-Bis(N-isobornyloxycarbonyl)spectinomycin-4-O-benzyloxime (**2b**)

Isobornyl chloroformate (2.17 g, 10 mmole) in 20 ml of acetone was added to a stirred solution of **2a** (2.19 g, 5 mmole) in 35 ml of water and 15 ml of acetone (2 hours, 6°C, pH 9.4 by addition of 2 N NaOH). After stirring for one hour at room temperature and addition of 40 ml of a saturated NaCl solution, the mixture was extracted with ethyl acetate. Drying and concentration of the extract gave a residue, purified by dissolving in 10 ml of ether and addition of ligroine (60 ml, b.p. 60~90°C) to yield 2.93 g (69%) of **2b**.

Anal. Calcd. for $C_{43}H_{63}N_3O_{11}$: C, 64.72; H, 7.96; N, 5.27.
Found: C, 64.00; H, 8.01; N, 5.11.

4-Dihydro-4-deoxy-4(*R*)-aminospectinomycin Trihydrochloride (**4a**) and 4-Dihydro-4-deoxy-4(*S*)-aminospectinomycin Trihydrochloride (**4b**)

Compound **2a** (20 g, 39.2 mmole) in 1.8 liters of 3% HCl - C_2H_5OH was hydrogenated (20 g PtO_2, 24 hours, room temperature, 5 atm.). Concentration to 100 ml (0°C) yielded 12.3 g (71%) of a white powder: m.p. 189~194°C; PMR (D_2O, 90 MHz, 60°C) δ 1.3 (d, 3H, C-2 CH_3), 1.9~2.1 (m, 2H, H-3), 2.85 (s, 6H, NCH_3), 5.03 (s, 1H, H-10a); MS *m/z* 693, 621, ($M^+ + 4 \sim 5$TMSi). TLC ($CHCl_3$ - CH_3OH - aq.NH_3, 40: 40: 15) showed two compounds: Rf 0.5 (about 90%), identical with **4a** obtained from **4d** and Rf 0.4, identical with **4b** obtained from **4c**.

6,8-Bis(N-isobornyloxycarbonyl)-4-dihydro-4-deoxy-4(*S*)-aminospectinomycin (**4c**)

Catalytic hydrogenation of **2b** (2.8 g, 3.5 mmole) in 60 ml of 3% HCl - EtOH (2.8 g PtO_2, 60 hours, room temperature, 5 atm.) yielded **4c** (1.8 g, 74%) as a white amorphous powder: PMR ($CDCl_3$ - CD_3OD, 90 MHz) δ 0.8~1.9 (aliphatic), 3.0 (s, 6H, NCH_3), 4.7 (s, 1H, H-10a); MS *m/z* 693 (M^+), 837, 909 ($M^+ + 2 \sim 3$TMSi).

4-Dihydro-4-deoxy-4(*S*)-aminospectinomycin Trihydrobromide (**4b**)

Finely divided **4c** (1.5 g, 2.1 mmole) and freshly prepared 20% HBr - CH_3COOH were stirred vigorously for 2 minutes at room temperature and poured into 200 ml of ether to give **4b** (0.8 g, 66%) as a white amorphous powder: m.p. 230°C (dec.); PMR (D_2O, 90 MHz, 60°C) δ 1.3 (d, 3H, C2-CH_3), 1.9~2.15 (m, 2H, H-3), 2.8, 2.82 (2s, 6H, NCH_3), 4.9 (s, 1H, H-10a); MS *m/z* 621, 549 ($M^+ + 3 \sim 4$TMSi). The 250 MHz PMR data are given in Table 1.

6,8-Bis(N-benzyloxycarbonyl)-4-dihydro-4-deoxy-4(*R*)-aminospectinomycin (**4d**) and 6,8-Bis(N-benzyloxycarbonyl)-4-dihydro-4-deoxy-4(*S*)-aminospectinomycin (**4e**)

One hundred g (0.176 mole) of 6,8-bis(N-benzyloxycarbonyl)spectinomycin (**3**)[6] and 128 g (0.16 mole) NH_4NO_3 in 1 liter of methanol were stirred to clear solution (3 hours, 40°C), cooled to 20°C and 10.3 g (0.167 mole) $NaBH_3CN$ added in one portion. The mixture was stirred for one hour at room temperature and the solvent evaporated (30°C).

The residue was stirred in 1.2 liters of water, 200 ml of 2 N hydrochloric acid and 1.2 liters of methylene chloride, the organic layer separated (centrifugation), the aqueous layer extracted two times with 600 ml of methylene chloride and the combined organic extracts evaporated to dryness. After storing overnight at −30°C the residue was dissolved in 1.3 liters of water and extracted three times with ethyl acetate (670, 350, 350 ml). The combined organic extracts were reextracted two times with dilute acid (600 ml of water and 10 ml of 2 N hydrochloric acid, 300 ml of water and 5 ml of 2 N hydrochloric acid), the aqueous layer combined with the aqueous layer of the first ethyl acetate extractions, washed with 500 ml of ether, adjusted to pH 4 (4 N NaOH), extracted with methylene chloride (discarded) and adjusted to pH 8.5. Extraction with methylene chloride (three times, 1.2 liters) drying and evaporating the solvent gave 37 g (37%) of a white powder, consisting of about 90% of **4d** and 10% of **4e**.

One portion of the mixture was separated into the epimers by column chromatography (CH_3CN - CH_3OH, 6: 1). **4d**: Rf 0.35 (CH_3CN - CH_3OH, 4: 1); PMR ($CDCl_3$, 90 MHz, 60°C) δ 1.22 (d, 3H, C-2CH_3), 1.5~1.8 (m, 2H, H-3), 3.05, 3.08 (2s, 6H, N-CH_3), 4.75 (s, 1H, H-10a), 5.1 (s, 4H, CH_2-Ph), 7.3 (s, 10H, aromatic); MS *m/z* 745,817 ($M^+ + 2 \sim 3$ TMSi). **4e**: Rf 0.23 (CH_3CN - CH_3OH, 4: 1); PMR ($CDCl_3$ - D_2O, 80 MHz) δ 1.22 (d, 3H, C-2CH_3), 1.35~1.7 (m, 2H, H-3), 3.0, 3.02 (2s, 6H, N-CH_3),

4.6 (s, 1H, H-10a), 5.1 (s, 4H, CH_2-Ph), 7.3 (s, 10H, aromatic); MS *m/z* 745,817 ($M^+ + 2 \sim 3$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-aminospectinomycin Trihydrochloride (**4a**)

Compound **4d** (0.8 g, 1.3 mmole) in 50 ml of 3% HCl - C_2H_5OH was hydrogenated (0.8 g Pd-C, 3 hours, 20°C, atmospheric pressure). Removal of catalyst and concentration to 20 ml (15°C) yielded **4a** (320 mg, 57%) as a white powder, washed thoroughly with ether: PMR (D_2O, 90 MHz, 60°C) δ 1.3 (d, 3H, C-2 CH_3), 1.9~2.1 (m, 2H, H-3), 2.85 (s, 6H, NCH_3), 5.05 (s, 1H, H-10a); MS *m/z* 477, 549, 621, 693 ($M^+ + 2 \sim 5$ TMSi). The 250 MHz PMR data are given in Table 1.

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