MODIFICATION OF SPECTINOMYCIN

2. DERIVATIVES OF 4-DIHYDRO-4-DEOXY-4(*R*)-AMINOSPECTINOMYCIN

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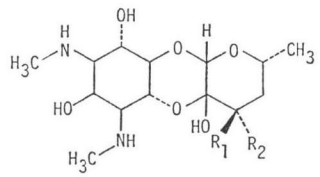
Acyl derivatives **5a**~**j** and alkyl derivatives **7a**~**r** of 4-dihydro-4-deoxy-4(*R*)-aminospectinomycin (**1a**) were prepared and tested for antibacterial activity. Only acyl compounds derived from long chain aliphatic acids or amino acids showed activity *in vitro*, but were inactive when tested *in vivo*. All alkyl derivatives were active *in vitro*. *In vivo* however only the short chain derivatives **7a**~**c** were active. Compound **7b** showed higher activity than spectinomycin.

In the preceding paper synthesis and biological properties of the epimeric 4-aminospectinomycins (**1**) were described[1].

Compound **1a** possesses interesting antibiotic properties and is therefore a suitable starting material for further derivatives. In this paper we describe preparation and biological properties of alkyl and acyl derivatives of **1a**.

Fig. 1.



1a $R_1 = NH_2$ $R_2 = H$
1b $R_1 = H$ $R_2 = NH_2$

Synthesis

The derivatives were prepared according to Scheme 1.

Compound **3** was acylated with acid chlorides, mixed anhydrides with alkyl carbonates or N-hydroxysuccinimide esters to give **4a**~**j** (R_1 see Table 1). In the cases of **4i** and **4j**, the corresponding N-Cbz amino acids were used.

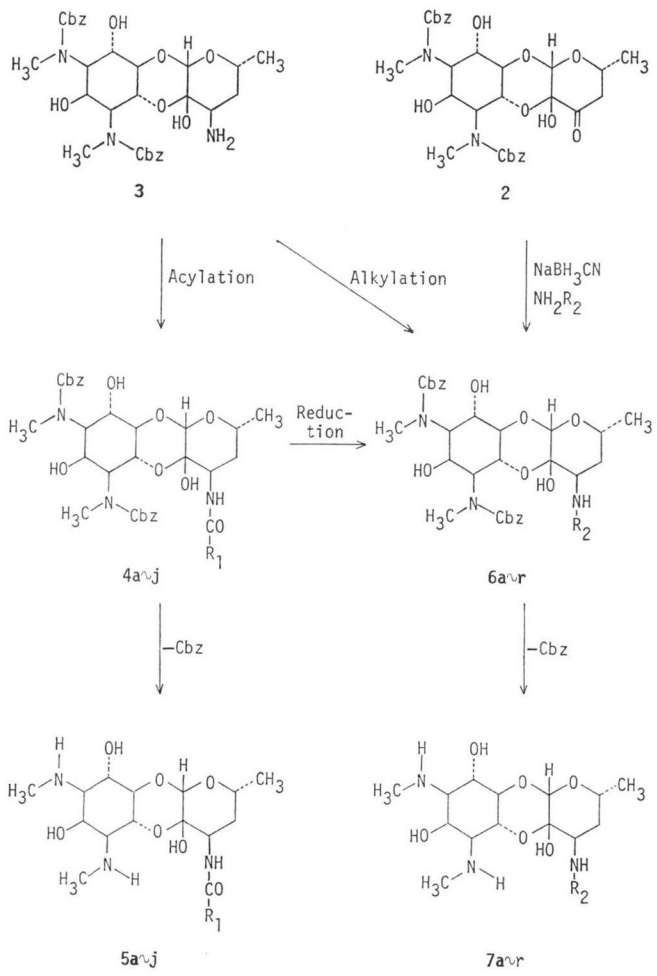
Compounds **6a**~**r** (R_2 see Table 2) were synthesized from **2** by reductive amination (method 1), from **4** by reduction of the amide function (method 2) and from **3** by alkylation with alkyl halides (method 3) or activated olefins (method 4) or by reductive alkylation with carbonyl compounds (method 5). Compounds **6n** and **6o** were prepared by reduction of **6k** and **6l**. Removal of the carbobenzoxy groups by catalytic hydrogenation, in the cases of **4i** and **4j** by catalytic hydrogenation, followed by treatment with boron tristrifluoroacetate gave **5a**~**j** and **7a**~**r**, as listed in Tables 1 and 2. Synthesis of compounds **7** by methods 1 or 2 gave identical products, thus confirming the *R*-configuration of the compounds **7** obtained by method 1.

The disubstituted derivatives **8a** and **8b** were prepared by reductive methylation of **6a** and **6b**, followed by removal of the carbobenzoxy groups.

Structure-Activity Relationships

The *in vitro* data of **5a**~**j** and **7a**~**r** are listed in Tables 1 and 2. Simple acyl derivatives like **5a**,

Scheme 1.



g, **h** were inactive. Activity however could be obtained by increasing the chain length (**5d**, **e**, **f**) or introduction of an amino group (**5i**, **j**). **5d**, **e**, **f** showed remarkably good activity against *E. coli* and *Pseudomonas*.

Short alkyl chains led to compounds with antibacterial activity comparable to spectinomycin (**7a**, **b**, **c**). Increase in chain length led first to a decrease (**7d**, **e**) and then to an increase of activity, especially against *Pseudomonas* (**7f**, **g**).

Introduction of a carbonyl group into the alkyl chain led to enhanced activity (**7k**, **l** compared with **7a**) whereas other substituents like ethoxycarbonyl, hydroxy or anilinocarbonyl led to substances with weaker activity (**7m**, **n**, **o**, **q**, **r**). Three benzyl derivatives were tested. Only **7j** showed activity comparable to spectinomycin.

The dialkyl derivatives **8a** and **b**, not shown in Tables 1 and 2, are devoid of activity *in vitro*.

The *in vitro* activity was tested in mice infected with *E. coli* ATCC 11775. Most of the compounds were inactive at the highest subcutaneous dosage of 40 mg/kg. Only the short chain alkyl derivatives

Table 1. 4-Dihydro-4-deoxy-4(*R*)-acylaminospectinomycins **5**.

		MIC[a] μg/ml						
5	R_1	*St. aur.* SG 511	*Sc. Arons.*	*E. coli* ATCC 11775	*Ps. aer.* Hbg	*Serr. marc.* ATCC 13880	*Klebs.* ATCC 10031	*Prot. mir.*
a	CH_3	>160	>160		>160	>160	>160	>160
b	C_5H_{11}	10	5	>80	>160	>80	>80	>80
c	C_9H_{19}	10	1.2	80	80	>80	>80	>80
d	$C_{11}H_{23}$	2.5	1.2	2.5	10	>80	40	>80
e	$C_{15}H_{31}$	2.5	1.2	2.5	10	20	10	>80
f	$C_{17}H_{35}$	2.5	1.2	5	10	20	10	20
g	C_6H_5	>160	120		>160	>160	>160	>160
h	OC_2H_5	>80	>80	>80	>160	>80	>80	>80
i	$CH_2CH_2NH_2$	2.5	10	5	40	20	5	5
j	$CHOHCH_2NH_2$	5	20	10	80	20	5	20
	Spectinomycin	10	10	20	120	20	20	20
	1a	5	10	20	160	10	5	20

[a] Serial dilution, nutrient broth, inoculum 3×10^4 CFU/ml.

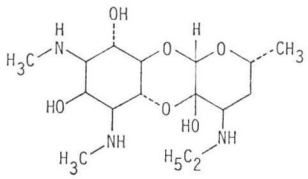
Table 2. 4-Dihydro-4-deoxy-4(*R*)-alkylaminospectinomycins **7**.

			MIC[a] μg/ml						
7	R_2	Method of alkylation	*St. aur.* SG 511	*Sc. Arons.*	*E. coli* ATCC 11775	*Ps. aer.* Hbg	*Serr. marc.* ATCC 13880	*Klebs.* ATCC 10031	*Prot. mir.*
a	CH_3	1	10	10	10	>80	40	20	20
b	C_2H_5	2	5	20	10	40	20	10	5
c	i-C_3H_7	1	10	10	10	>80	40	20	20
d	C_5H_{11}	1	40	10	>80	160	>80	80	40
e	C_9H_{19}	2	10	5	40	80	>80	80	80
f	$C_{12}H_{25}$	2	10	2.5	10	20	40	20	40
g	$C_{18}H_{37}$	2	5	2.5	5	10	10	10	40
h	$CH_2C_6H_5$	5	20	5	40	>80	80	80	80
i	$CH_2C_6H_4OCH_3(p)$	5	10	2.5	40	>80	80	>80	>80
j	$CH_2C_6H_4N(CH_3)_2(p)$	5	5	5	20	120	20	20	10
k	$CH_2CH_2COCH_3$	4	5	10	10	40	5	10	10
l	$CH_2CH_2COC_2H_5$	4	5	10	10	20	10	10	10
m	$CH_2CH_2COOC_2H_5$	4	20	20	40	>160	80	40	80
n	$CH_2CH_2CHOHCH_3$	4	20	40	20	>80	80	40	40
o	$CH_2CH_2CHOHC_2H_5$	4	20	20	80	>80	>80	80	80
p	$CH_2COC_6H_4OCH_3(p)$	3	5	10		80	10	10	10
q	$CH_2COOC_2H_5$	3	40	40		>160	160	80	80
r	$CH_2CONHC_6H_5$	3	20	20		>160	80	40	80

[a] Serial dilution, nutrient broth, inoculum 3×10^4 CFU/ml.

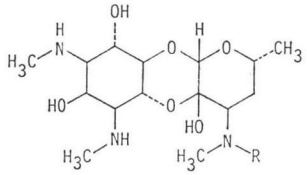
7a~**c** showed activity. The most active compound **7b** is superior to spectinomycin (ED_{50} 7.5 mg/kg, spectinomycin 17.6 mg/kg).

Fig. 2.



7b

Fig. 3.



8a R = CH_3
8b R = C_2H_5

Experimental Section

80 MHz PMR spectra were measured on a Bruker WP-80 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-trimethylsilyltrifluoroacetamide was used for introduction of the trimethylsilyl (TMSi) groups.

The new compounds prepared in this work could not be freed from solvents. Therefore no correct values of elementary analyses could be obtained.

Only one representative example for each synthetic method is described.

4-Dihydro-4-deoxy-4(*R*)-decanoylaminospectinomycin Dihydrochloride (**5c**)

Capric acid (1.25 g, 7.3 mmole) and triethylamine (1.0 ml, 7.3 mmole) were dissolved in 20 ml of CH_2Cl_2, the mixture cooled to $-10°C$ and isobutylchloroformate (0.69 ml, 7.3 mmole) added dropwise. After stirring for another 30 minutes at $-10°C$, **3** (4.0 g, 6.6 mmole) in 20 ml of CH_2Cl_2 was added. The mixture was stirred for one hour without external cooling, extracted with water (2×50 ml) and the organic layer dried (Na_2SO_4) and evaporated. Column chromatography (SiO_2, CH_2Cl_2 - CH_3OH, 18: 1) of the residue yielded 2.4 g (49%) of **4c** as a white powder: MS *m/z* 971 ($M^+ +3$ TMSi).

The product was hydrogenated in 60 ml of 3% HCl-EtOH with 1 g of 10% Pd-C (room temperature, 1 atm., 2 hours). Removal of catalyst, concentration and trituration with ether afforded 1.65 g (93%) of **5c** as a white powder.

PMR (D_2O, 80 MHz) δ 0.8 (t, broad, 3H, CH_3), 1.25 (m, 14H, CH_2, 3H, C-2 CH_3), 1.5~1.8 (m, 2H, H-3), 2.3 (t, 2H, $COCH_2$), 2.9 (s, broad, 6H, NCH_3), 5.1 (s, 1H, H-10a); MS *m/z* 703, 775, 847, 919 ($M^+ +3\sim6$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-benzoylaminospectinomycin Dihydrochloride (**5g**)

To **3** (2.7 g, 4.5 mmole) in 40 ml of dioxane and 3 ml of water benzoylchloride (690 mg, 4.95 mmole) in 20 ml of dioxane was added dropwise (15 minutes, 10°C, pH 8 by addition of 2 N NaOH). After stirring for 30 minutes at 10°C the mixture was treated with 150 ml of a saturated NaCl solution, extracted with ethyl acetate and the organic layer dried ($MgSO_4$) and evaporated. Trituration of the residue with 100 ml of ether gave 1.8 g (56.8%) of **4g** as a white powder: 1.7 g (2.4 mmole) of this product were hydrogenated with 1.5 g of 5% Pd-C as described for **5c** to give 1 g (85%) of **5g** as a white powder: PMR (CD_3OD, 80 MHz) δ 1.28 (d, 3H, C-2 CH_3), 1.90 (m, 2H, H-3), 2.9 (s broad, 6H, NCH_3), 5.15 (s, 1H, H-10a), 7.5~8 (m, 4H, aromatic); MS *m/z* 725, 797, 869 ($M^+ +4\sim6$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-(2-amino)-propionylaminospectinomycin Trihydrochloride (**5i**)

Dicyclohexylcarbodiimide (1.1 g, 5 mmole), dissolved in a small amount of THF was added to N-benzyloxycarbonyl-ß-alanine (1.1 g, 5 mmole) and N-hydroxysuccinimide (0.6 g, 5 mmole) in THF-dioxane. After stirring for 2 hours at 0°C and for 2 hours at room temperature, N,N'-dicyclohexylurea was filtered off. The filtrate was added to a solution of **3** (3.0 g, 5 mmole) in CH_2Cl_2 at 0°C, stirred for 5 hours at 0°C and for 10 hours at room temperature, evaporated and the residue purified by column chromatography (SiO_2, CH_2Cl_2 - CH_3OH, 10: 1) to give 2.3 g (57%) of **4i**: MS *m/z* 1022 ($M^+ +3$ TMSi). Hydrogenation of **4i** (0.8 g, 1 mmole) as described for **5c** removed only the carbobenzoxy groups attached to the methylamino groups to give 0.5 g (92%) of a white powder: MS *m/z* 698 ($M^+ +5$ TMSi). This

product (0.5 g, 0.9 mmole), dissolved in CF_3COOH, was triturated with boron tristrifluoroacetate (3.5 g, 10 mmole) for 3 hours at 0°C, for 4 hours at room temperature and the solvent evaporated. The residue was dissolved repeatedly in CH_3OH, the solvent evaporated, the residue dissolved in 3% HCl-EtOH, again evaporated and the residue triturated with ether to give 0.3 g (80%) of **5i** as a white powder: PMR (D_2O, 80 MHz) δ 1.3 (d, 3H, C-2 CH_3), 1.9 (m, 2H, H-3), 2.9 (m, 6H, NCH_3, 2H, $COCH_2$); MS *m/z* 692, 764, 836, 908 ($M^+ + 4 \sim 7$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-ethylaminospectinomycin Trihydrochloride (**7b**) (method 1)

Dry ethylamine (5.4 g, 120 mmole) in 60 ml of dry CH_3OH was treated with 5 N HCl-CH_3OH (20 ml, 100 mmole), followed by addition of **2** (12 g, 20 mmole) and, after cooling to 5°C, by addition of $NaBH_3CN$ (1.25 g, 20 mmole). The solution was stirred for 2 hours at 5°C, poured into 400 ml of 0.5 N HCl and extracted three times with 200 ml of ethyl acetate. The organic extracts were washed with 3 portions of 300 ml of H_2O and 10 ml of 2 N HCl. The combined aqueous layers were brought to pH 8.5~9 by addition of 2 N NaOH and extracted two times with 300 ml of CH_2Cl_2. After drying (Na_2SO_4) and evaporating, column chromatography (SiO_2, $CHCl_3$ - CH_3OH, 5: 1) gave 5.9 g (47%) of **6c** as a white powder: MS *m/z* 845 ($M^+ + 3$ TMSi). Hydrogenation of 1.9 g (3 mmole) of **6b** as described for **5c** afforded 1.2 g (85%) of **7b** as a white powder: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.2~1.6 (m, 3H, CH_2CH_3, 3H, C-2 CH_3), 1.9 (m, 2H, H-3), 2.9 (s broad, 6H, NCH_3), 5.1 (s, 1H, H-10a); MS *m/z* 577, 649, 721 ($M^+ + 3 \sim 5$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-ethylaminospectinomycin Trihydrochloride (**7b**) (method 2)

To a stirred suspension of $NaBH_4$ (1.13 g, 30 mmole) and **4a** (1.93 g, 3 mmole) in dry dioxane (50ml) was added CF_3COOH (2.3 ml, 30 mmole) in 10 ml of dioxane (10 minutes, room temperature). After the evolution of gas had ceased, the mixture was heated to reflux for 2 hours, cooled, poured into 250 ml of H_2O and extracted three times with 50 ml of CH_2Cl_2. The combined extracts were washed with H_2O, dried (Na_2SO_4) and evaporated to give, after column chromatography (SiO_2, $CHCl_3$ - CH_3OH, 5: 1), 0.96 g (50%) of **6b** as a colorless amorphous solid: MS *m/z* 845 ($M^+ + 3$ TMSi). 0.63 g (1 mmole) of **6b** were hydrogenated as described for **5c** to give 0.35 g (74%) of **7b** as a white powder. PMR, MS and TLC confirm identity with **7b** prepared by method 1: Rf 0.66 (SiO_2, $CHCl_3$ - CH_3OH - aq. conc. NH_3, 1: 1: 0.2).

4-Dihydro-4-deoxy-4(*R*)-*p*-methoxy-phenacylaminospectinomycin Trihydrochloride (**7b**) (method 3)

3 (3.6 g, 6 mmole), *p*-methoxyphenacylbromide (1.47 g, 6.3 mmole) and K_2CO_3 (840 mg, 6 mmole) were stirred in 21 ml of CH_3CN (20 hours, room temperature). The mixture was poured into 50 ml of water, extracted with ethyl acetate, the organic layer dried and evaporated. Column chromatography (SiO_2, toluene - ethyl acetate, 4: 1) gave 1.4 g (30%) of **6p** as a white powder. 700 mg (0.9 mmole) of this product in 300 ml of C_2H_5OH were hydrogenated with 700 mg of 5% Pd-$BaSO_4$ (7 hours, room temperature, 3 atm.). The solvent was evaporated (30°C), the residue dissolved in 30 ml of C_2H_5OH, treated with HCl-EtOH and then with ether to give 300 mg (57%) of **7p**: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.3 (d, 3H, C-2 CH_3), 2.1 (m, 2H, H-3), 2.9 (s, 6H, NCH_3), 3.95 (s, 3H, OCH_3), 5.25 (s, 1H, H-10a), 7.0, 8.1 (q, 4H, aromatic); MS *m/z* 697, 769 ($M^+ + 3 \sim 4$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-(3-oxo)-butylaminospectinomycin Trihydrochloride (**7k**) (method 4)

Methyl vinyl ketone (0.78 ml, 9.5 mmole) in 10 ml of dry CH_3OH was dropped into a stirred solution of **3** (5.7 g, 9.5 mmole) in dry CH_3OH (40 ml, 40°C). The mixture was stirred for 1 hour at 40°C, evaporated, the residue dissolved in 0.1 N HCl (10 ml) and extracted three times with 30 ml of $CHCl_3$. The combined extracts were dried and evaporated to a residue, purified by column chromatography (SiO_2, $CHCl_3$ - CH_3OH, 10 : 1) to give **6k** (4.0 g, 62%) as an amorphous powder: MS *m/z* 887 ($M^+ + 3$ TMSi). 0.67 g (1 mmole) of **6k** were hydrogenated as described for **5c** to give 0.24 g (47%) of **7k** as a white powder: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.2 (d, 3H, C-2 CH_3), 2.0 (m, 2H, H-3), 2.2 (s, 3H, $COCH_3$), 2.5 (t, 2H, CH_2CO), 2.9 (s broad, 6H, NCH_3), 5.1 (s, 1H, H-10a); MS *m/z* 619, 691, 763 ($M^+ + 3 \sim 5$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-(4-dimethylamino)-benzylaminospectinomycin Trihydrochloride (**7j**) (method 5)

3 (3.6 g, 6 mmole) and 4-dimethylaminobenzaldehyde in 30 ml of dry C_2H_5OH were stirred at room temperature for 5 hours. PtO_2 (3.6 g) was added, the mixture hydrogenated (5 atm., 12 hours), filtered and evaporated. Column chromatography (SiO_2, $CHCl_3$ - CH_3OH, 20: 1) yielded **6j** (2.94 g, 66%) as a colorless foam: MS *m/z* 950 ($M^+ + 3$ TMSi). 0.75 g (1.03 mmole) of **6j** were hydrogenated as described for **5c** to give 0.37 g (63%) of **7j** as a white powder: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.3 (d, 3H, C-2 CH_3), 2.0 (m, 2H, H-3), 2.9 (s broad, 6H, NCH_3), 3.3 (s, 6H, NCH_3, aromatic), 5.2 (s, 1H, H-10a); MS *m/z* 682, 754, 826 ($M^+ + 3 \sim 5$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-(3-hydroxy)-butylaminospectinomycin Trihydrochloride (**7n**)

6k (1.3 g, 1.9 mmole) was dissolved in dry EtOH (50 ml), PtO_2 (0.26 g) added and the mixture hydrogenated (5 atm., 12 hours, room temperature). Evaporation gave an amorphous residue of **6n** (1.2 g, 82%): MS *m/z* 889 ($M^+ + 3$ TMSi). Hydrogenation as described for **5c** gave 0.8 g (87%) of **7n** as a white powder: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.3 (m, 3H, C-2 CH_3, 3H, CH_3), 1.9 (m, 2H, H-3, 2H, CH_2-CH(OH)), 2.9 (s, broad, 6N, NCH_3), 5.1 (s, 1H, H-10a); MS *m/z* 693, 765 ($M^+ + 4 \sim 5$ TMSi).

4-Dihydro-4-deoxy-4(*R*)-dimethylaminospectinomycin Trihydrochloride (**8a**)

$NaBH_3CN$ (0.4 g, 6.5 mmole) was added to a solution of **6a** (2.46 g, 4 mmole) and 40% aqueous formaldehyde (1.5 g, 20 mmole) in 15 ml of acetonitrile. After stirring for 15 minutes the mixture was neutralized by dropwise addition of acetic acid, stirred for 45 minutes and evaporated at reduced pressure. 20 ml of 2 N NaOH were added and the mixture extracted with CH_2Cl_2. Drying and evaporating, followed by column chromatography (SiO_2, $CHCl_3$ - CH_3OH, 9: 1) afforded 0.96 g (40%) of a colorless amorphous solid: MS *m/z* 845 ($M^+ + 3$ TMSi). 0.4 g (0.63 mmole) were hydrogenated as described for **5c** to give 0.03 g (10%) of **8a** as a white powder: PMR ($CDCl_3$ - CD_3OD, 80 MHz) δ 1.2 (d, 3H, C-2 CH_3), 1.8 (m, 2H, H-3), 3.0 (s broad, 12H, NCH_3), 5.3 (s, 1H, H-10a); MS *m/z* 577, 649 ($M^+ + 3 \sim 4$ TMSi).

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Reference

1) MAIER, R.; E. WOITUN, A. REUTER, W. REUTER & B. WETZEL: Modification of spectinomycin. 1. Synthesis of 4-aminospectinomycins. J. Antibiotics 34: 16~21, 1981