Communications to the editor

TOTAL SYNTHESIS OF *RAC.* NEGAMYCIN AND OF NEGAMYCIN ANALOGS

Sir:

Negamycin, an antibiotic produced by *Streptomyces purpeofuscus*, inhibits bacterial growth and is especially interesting by virtue of its action on Gram-negative bacteria including *Pseudomonas* and multiple-drug-resistant enteric bacteria[1]. It has been shown to inhibit protein synthesis and to cause miscoding[2~7]. Negamycin has a unique structure[8], [2-{(3R,5R)-3,6-diamino-5-hydroxyhexanoyl}-1-methylhydrazino] acetic acid, which has been confirmed by total synthesis starting from a sugar derivative[9].

Negamycin

The antipode of negamycin, also synthesised from a carbohydrate precursor[9], retained definite antibacterial activity, although much less than that of natural negamycin. Leucylnegamycin[10], a precursor in the biosynthesis of negamycin, showed 42% of the activity of the latter against *Escherichia coli* K12. Recently, H. UMEZAWA and coworkers[11] isolated 3-*epi*-deoxynegamycin and leucyl-3-*epi*-deoxynegamycin from *Streptomyces goshikiensis* and synthesised two leucyl-isomers, all of which compounds had markedly reduced antibacterial activity compared to negamycin.

3-Azaanalogs of negamycin, which we have described in a previous paper[12], were inactive against bacteria. Recently H. UMEZAWA and coworkers[13] reported the synthesis of several negamycin analogs modified in the δ-hydroxy-β-lysine moiety. Of these derivatives, only O-methylnegamycin, deoxynegamycin, and, to a lesser extent, 3-*epi*-deoxynegamycin had antibacterial activity, and all to a lesser degree than negamycin. In general, there was a good correlation between antimicrobial activities of negamycin analogs and their activities in cell-free systems[14]. In the present communication we describe a new total synthesis of *rac.* negamycin, its diastereomeric analog and of several negamycin analogs, mostly modified in the hydrazinoacetic acid part.

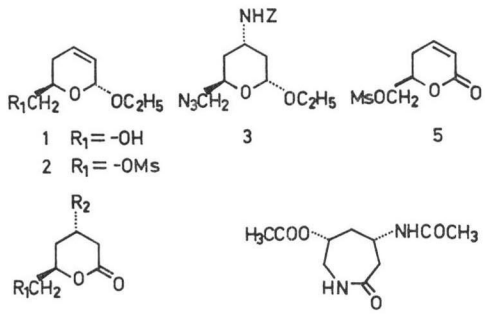
Two key intermediates, **4** and **7**, were prepared from the known[15] (2RS, 6RS) 5,6-dihydro-2-ethoxy-6-hydroxymethyl-2*H*-pyran (**1**). Mesylation of **1** yielded (2RS, 6RS)-5,6-dihydro-2-ethoxy-6-methanesulfonyloxymethyl-2*H*-pyran (**2**) in quantitative yield. Addition of carbamic acid benzyl ester catalysed by BF_3-etherate in dichloromethane at 0°C proceeded regio- and stereospecifically. The product thus obtained was converted directly to azide **3** by reaction with sodium azide in hexamethyl phosphoric triamide (HMPTA) followed by chromatographic purification on silica gel, using chloroform as the eluant, in 78% overall yield. Structure and stereochemistry of **3** as (2RS, 4SR, 6RS)-6-azidomethyl-4-benzyloxycarbonylamino-2-ethoxytetrahydropyran was easily confirmed by comparison of its NMR-spectrum with the spectra of known[16] stereoisomeric 6-acetoxymethyl-2,4-diacetoxytetrahydropyranes. Coupling constants of the protons at C-2, C-4, and C-6 showed that the substituents at C-2 and C-4 occupy axial positions, whereas the substituent at C-6 occupies an equatorial position. Mild hydrolysis of **3** with 50% aqueous acetic acid at 100°C for 1 hour and subsequent oxidation with JONES reagent in acetone gave lactone **4** as a crystalline solid, mp. 102~105°C, in 67% yield.

Mild, acidic acetal cleavage of **2** (10% acetic acid at 20°C) and subsequent oxidation with JONES reagent gave crystalline 5,6-dihydro-6-methanesulfonyloxymethyl-2-oxo-2*H*-pyran (**5**), mp. 40~42°C, in 55% yield. MICHAEL addition of O-benzylhydroxylamine to **5** in ethanol at room temperature yielded crystalline (4RS, 6SR)-4-benzyloxyamino-6-methanesulfonyloxymethyl-2-oxo-2*H*-pyran (**6**), mp. 92~95°C (67% yield), which was converted to azide **7** (100%) by reaction with sodium azide in HMPTA. With regard to the relative configuration of the new asymmetric centres in **6** and **7**, NMR spectra did not permit an unambiguous assignment to be made. Catalytic hydrogenation of **4** and subsequent acetylation yielded (3RS,5SR)-3-acetamido-5-acetoxy-6-aminohexanoic acid ε-lactam (**8**), the structure of which was determined by IR-, NMR- and mass spectra. Reduction and acetylation of **7** in the same manner yielded a product which

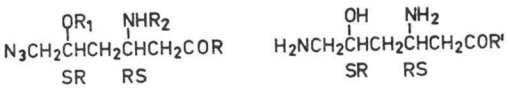
was identical with the one obtained from **4**, thus proving that the tetrahydropyranes, **4** and **7**, have the same stereochemistry.

Treatment of lactones **4** and **7** with various amines, hydrazines, aminoacid benzyl esters and hydrazino acid benzyl esters in toluene under reflux gave amides and hydrazides **9** and **10** (10~45% yield). Removal of protecting groups and conversion of azido- and hydroxylamino-groups into amino groups was carried out in one step by catalytic hydrogenation over palladium black in a mixture of methanol, acetic acid and water to yield the negamycin analogs, **11a~11i**, with the 3RS,5SR-configuration of the hexanoyl part. These compounds were purified by column chromatography on Amberlite CG-50 (NH_4^+) resin, using water and 0.5% aqueous ammonia as the eluant.

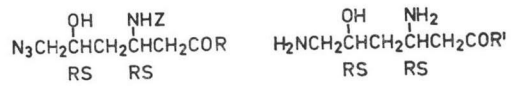
To obtain *rac.* negamycin and negamycin analogs with the 3RS,5RS-configuration of the hexanoyl moiety, the intermediates **9a~9g** were converted to their diastereomers by mesylation of the hydroxyl group to **12a~12g**, and inversion of the configuration at C-5 by treatment with sodium acetate in moist dimethylsulfoxide. Compounds **13** obtained by this procedure gave NMR-spectra very similar to those of **9** but showed slightly different Rf-values on silica gel with chloroform-ethanol-mixtures of various compositions. Catalytic hydrogenation of **13a~13g**, and purification on ion-exchange resin yielded *rac.* negamycin (**14a**) (10% overall yield from **4**) and negamycin analogs with the 3RS,5RS-configuration (**14b~14g**).



1 R_1 = -OH
2 R_1 = -OMs
3
5
4 R_1 = $-N_3$ R_2 = -NHZ
6 R_1 = -OMs R_2 = -NHOBn
7 R_1 = $-N_3$ R_2 = -NHOBn
8

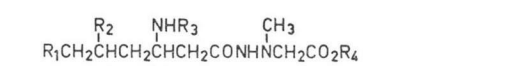


9a-9h R_1 = -H, R_2 = -Z
10a,10g,10i R_1 = -H, R_2 = -OBn
12a-12g R_1 = -Ms, R_2 = -Z
11a-11g



13a-13g
14a-14g

9-14: a R = $-NHNCH_2CO_2Bn$, R' = $-NHNCH_2CO_2H$
b R = $-NHCHCH_2CO_2Bn$, R' = $-NHCHCH_2CO_2H$
c R = $-NHCH_2CH_2CO_2Bn$, R' = $-NHCH_2CH_2CO_2H$
d R = R' = $-NHCH_2CH_2N(C_2H_5)_2$
e R = R' = $-NHCH_2CH_2CH_2CH_3$
f R = R' = $-NH_2$
g R = R' = $-NHN(CH_3)_2$
h R = R' = $-N(C_2H_5)_2$
i R = R' = $-NHNH_2$



15 R_1 = R_2 = $-N_3$, R_3 = -Z, R_4 = -Bn
16 R_1 = R_2 = $-NH_2$, R_3 = R_4 = -H

Z = $-CO_2Bn$, Bn = $-CH_2C_6H_5$, Ms = $-SO_2CH_3$

Table 1. Properties of negamycin analogs

Compound	Rf on TLC[a]	Activity (%)[b]
Negamycin	0.10	100
11a	0.12	25
11b	0.18	<6.25
11c	0.16	<6.25
11d	0.52	6.25
11e	0.56	12.5
11f	0.11	<6.25
11g	0.38	<6.25
11h	0.64	<6.25
11i	0.15	<6.25
14a	0.10	50
14b	0.15	<6.25
14c	0.12	6.25
14d	0.49	<6.25
14e	0.50	<6.25
14f	0.10	<6.25
14g	0.29	<6.25
16	0.37	<6.25

a) Thin-layer chromatography on silica gel G (Merck) developed with chloroform - methanol - 25% aqueous ammonia (2 : 2 : 1).

b) Activities were compared with negamycin (100%) by the method described in Table 2 by using *Staphylococcus aureus* at pH 8.5.

Table 2. Antimicrobial spectra of negamycin, *rac.* negamycin (**14a**) and *rac. epi*-negamycin (**11a**)

Test organisms	SFI-code	Minimum inhibitory concentrations (mcg/ml)*					
		Negamycin		**14a**		**11a**	
		pH 7.0	pH 8.5	pH 7.0	pH 8.5	pH 7.0	pH 8.5
Staphylococcus aureus FDA 209 P	△ 54	10	6.25	25	12.5	75	25
Escherichia coli K12	△ 127	25	2.5	25	6.25	>200	75
Escherichia coli K12	△ 169	10	3.12	50	9.37	>200	75
Escherichia coli K12	△ 230	6.25	1.25	18.75	4.68	150	25
Proteus vulgaris ATCC 13315	△ 116	10	2.5	12.5	3.12	>200	75
Pseudomonas aeruginosa NCIB 950	△ 92	25	10	75	18.75	>200	150
Klebsiella pneumoniae ATCC 10031	△ 217	10	0.78	18.75	2.34	150	12.5
Salmonella typhimurium ATCC 13311	△ 109	10	2.5	37.5	9.37	200	50

* Minimum inhibitory concentrations were determined in 0.5% peptone water (Polypeptone BBL) by incubation at 37°C for 16 hours.

Reaction of mesylate **12a** with sodium azide in HMPTA gave the 5-azido compound (**15**) (probably with inversion of configuration at C-5 to the 3RS,5RS-compound). Catalytic hydrogenation followed by ion-exchange chromatography yielded *rac.* 5-amino-5-deoxynegamycin (**16**).

Properties of negamycin analogs are summarized in Table 1. Among these negamycin analogs, only **11a**, the diastereomeric analog of negamycin, possessed weak antibacterial activity, amounting to *ca.* 5~10% of that of negamycin, while all other derivatives were inactive. *Rac.* negamycin, as expected, displayed *ca.* 50% of the activity of an authentic sample of negamycin (Table 2).

These results indicate that the (1-methylhydrazino)acetic acid part of the negamycin molecule is essential for antibacterial activity. Furthermore, it is noteworthy that the hydroxyl group in C-5 may not be replaced by an amino group (as in **16**) without loss of antibacterial activity.

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