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SYNTHESIS OF (±)-NEGAMYCIN AND OF (±)-EPINEGAMYCIN

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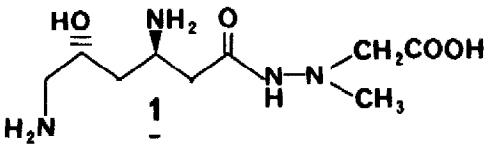
Summary : The antibiotic negamycin and its diastereomer have both been synthesized in racemic form. The latter has been found to possess antibacterial activity, but at a lower level than negamycin itself.

Negamycin (2-(3R,5R)-3,6-diamino-5-hydroxyhexanoyl -1-methylhydrazinoacetic acid) 1 is a bactericidal antibiotic of low acute toxicity, and possesses good Gram negative and anti-



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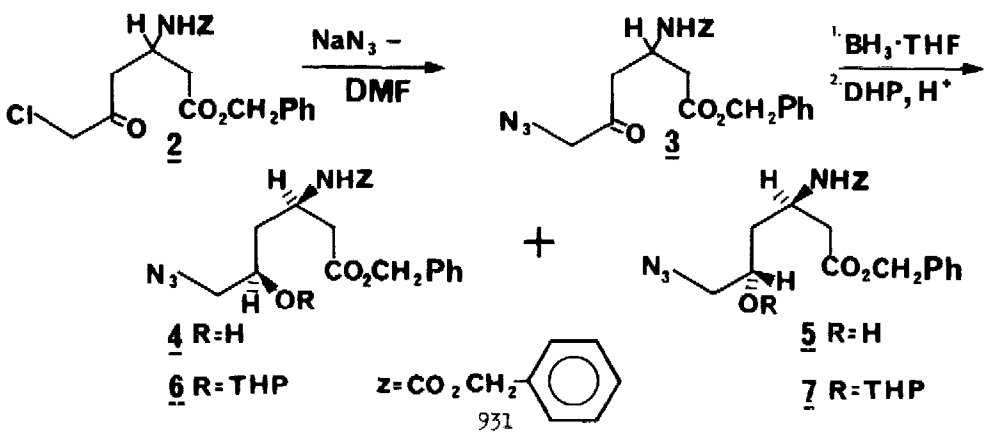
Staphylococcal activity both *in vitro* and *in vivo*[1,2]. Its antibacterial spectrum includes *Pseudomonas* as well as enteric bacteria carrying plasmid-mediated (R-factor) drug resistance. As part of the structure determination[6] and proof of absolute configuration, negamycin was synthesized from D-galacturonic acid and its enantiomer was synthesized from 3-amino-3-deoxy-D-glucose[7].

We report here a synthesis of (±)-negamycin and its diastereomer (*epi*negamycin) via a route adapted to the preparation of analogues. The synthetic equivalents of the amino-acid part (δ-hydroxy-β-lysine) 6 of negamycin and of its diastereomer 7, in which each function may be selectively deprotected for further elaboration, were prepared as shown in Scheme I.

Scheme I

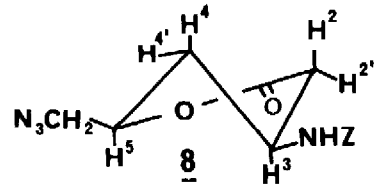


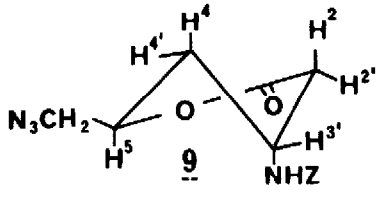
Benzyl N-carbobenzoxy-dl-3-amino-5-oxo-6-chlorohexanoate[8] 2 was converted to the azido-ketone[9a,b] 3 m.p. 74-5°C (Et_2O) in 95 % yield by reaction with sodium azide in DMF at 5°C. Reduction of 3 with 50 % excess borane-THF at room temperature and separation of the isomers by dry column chromatography[10] on deactivated silica gel (15 % water w/w) eluted with diethyl ether-methylene chloride (3:17) gave the azido-alcohol 4[9a,b] m.p. 52-5°C (29 % yield), rf 0.47 (SiO_2, $CHCl_3$-EtOAc, 4:1) and 5[9a,b] oil (36 % yield), rf 0.31 (SiO_2, $CHCl_3$-EtOAc, 4:1). Reaction of 4 and 5 with dihydropyran (2 equiv., CH_2Cl_2, anhyd. TsOH cat.) gave the fully protected derivatives[9a] 6 and 7.

In order to determine the relative configurations of 4 and 5, they were saponified to the free acids which were then, using dicyclohexylcarbodiimide, converted to the corresponding lactones[9a,b] 8 m.p. 53-5°C and 9 m.p. 104°C. *Cis*-1,3- and *trans*-1,3-disubstituted δ-lactone structures could be assigned unequivocally on the basis of their 100 MHz pmr spectra (Table).

Table. p.m.r. Coupling constants.
Approximate dihedral angles predicted from Dreiding models.

	Lactone 8			Lactone 9	
J	Hz	Angle	J	Hz	Angle
4,5	12.0	180°	4,5	9.5	180°
4',5	3.0	50°	4',5	4.5	50°
4,4'	14.0	geminal			
3,4	11.5	180°	3',4	4.0	55°
3,4'	4.0	45°	3',4'	4.0	55°
2,3	9.8	160°	2,3'	5.5	55°
2',3	6.2	30°	2',3'	5.0	55°
2,2'	18.0	geminal	2,2'	17.8	geminal
2',4'	1.5	planar W			

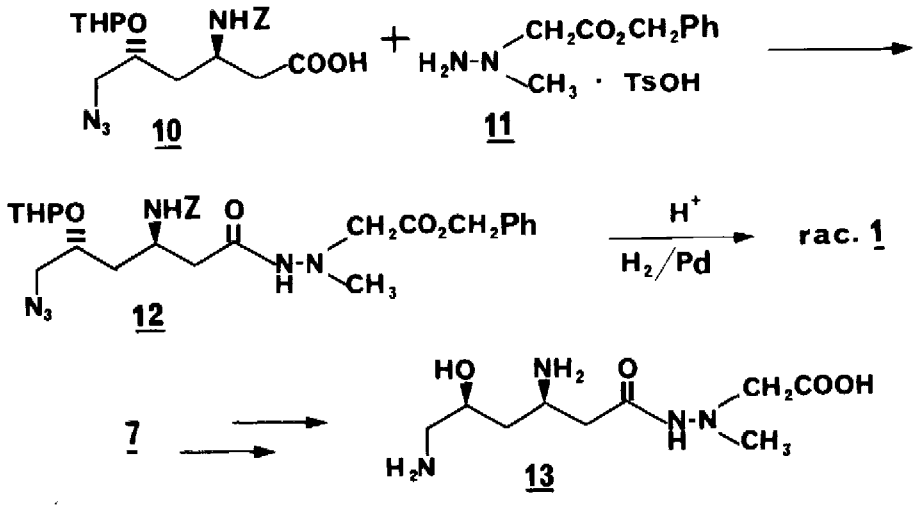




Lactone 8 must exist in the half-chair (envelope) conformation to enable H2' and H4' to be in a planar W conformation ; in this conformation only *cis* substituents, in the more favorable equatorial positions, will give rise to the observed vicinal coupling constants[11]. It therefore follows that 4 has the R,R/S,S configuration and 5 has the R,S/S,R configuration[12].

Completion of the synthesis is illustrated in Scheme II. Saponification of the benzyl ester 6 gave the free acid 10[9a] m.p. 59-60° (i-Pr_2O) which was coupled with α-methylhydrazinoacetic acid protected as the benzyl ester[13], using the mixed anhydride method. Fully protected (±)-negamycin[9a] 12 was obtained as a thick oil. Cleavage of the tetrahydropyranyl ether was effected with 50 % aqueous acetic acid (35°C, 90 min.). Hydrogenation, also in aqueous acetic acid, over 5 % palladium on charcoal yielded a crude product which was placed on Amberlite CG 50 (NH_4^+) and eluted with 0.1 % NH_4OH. Fractions showing a single spot (ninhydrin) when examined by electrophoresis on silica gel plates (acetic acid/formic acid/water : 75/25/900 v/v 900 volts ; rf 1.02, lysine = 1.00) were combined and lyophilised to yield pure (±)-negamycin[9a,b] 1 as a white hygroscopic powder identical to natural (+)-negamycin[14] by electrophoresis in a variety of buffers and by IR and pmr spectroscopy.

Scheme II



Treatment of 1 with benzyl S-(4,6-dimethylpyrimidin-2-yl) thiolcarbonate gave the N,N-biscarbobenzoxy derivative[9a] m.p. 103-5°C which on treatment with diazomethane yielded the methyl ester[9a,b] m.p. 90°C. The yield of the final deprotection and purification was 42 % and the overall yield of (±)-negamycin from 2 was 7.1 %.

Using the same synthetic sequence (±)-epinegamycin 13[9a,b] m.p. 150-180°C dec. was obtained in 10.5 % overall yield. Its N,N-biscarbobenzyoxy derivative[9] had m.p. 110-118° C and the methyl ester[9a,b] m.p. 125-127°C.

In *in vivo* mouse protection tests[15] over a 7 day period (±)-1 was as active as (+)-negamycin against *Salmonella dublin* and more active[16] against *Staphylococcus aureus* and *Pseudomonas aeruginosa* at 12.5 mg/kg. *Epi*negamycin (±)-13 was inactive against *Ps. aeruginosa* but was as active as (±)-1 and penicillin G (50 mg/kg) against *S. dublin* and more active than (±)-1 against *S. aureus*.

References and Notes

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2. Negamycin was isolated from culture filtrates of three strains of *Streptomyces* related to *S. purpeofuscus*[1]. A biosynthetic precursor, L-leucylnegamycin was also isolated from one of these strains[3]. *Epi*-deoxynegamycin has been found in broth filtrates of both *Streptomyces*[4] and *Micromonospora*[5] species.

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9. a) Satisfactory proton magnetic resonance and infrared spectra were obtained for this compound.
b) Correct elemental analysis was obtained for this compound.

10. For a description of this technique, see B. LOEV and M.M. GOODMAN, Chemistry and Industry (London), 2026 (1967).

11. This data compares very favourably with that reported by F.I. CARROLL and J.T. BLACKWELL (Tetrahedron Letters, 48, 4173 (1970)) for *cis* and *trans*-3,5-dimethylvalerolactones. In their *cis* isomer they observe a long-range coupling ($J_{2',4'}$ = 1.9 Hz). Another useful observation is the geminal coupling $J_{2,2'}$ = 18.0 Hz. According to M. BARFIELD and D.M. GRANT (J. Amer. Chem. Soc., 85, 1899 (1963)) J_{gem} is dependent upon the dihedral angle between the methylene group and the π lobes of adjacent π bonds. Assuming this relationship to exist in 8 and 9, the large $J_{2,2'}$ can only be explained by a conformation in which the C=O group bisects the C-5 methylene group. This is seen to be the case in the Dreiding model.

12. Racemic 9 m.p. 102-105°C has recently been prepared from (2RS, 4SR, 6RS)-6-azidomethyl-4-benzyloxycarbonylamino-2-ethoxytetrahydropyran and converted to racemic *epi*negamycin : W. STREICHER, H. REINSHAGEN and F. TURNOWSKY, J. Antibiotics, 31, 725 (1978).

13. W. STREICHER and H. REINSHAGEN, Chem. Ber., 108, 813 (1975).

14. We thank Professor Hameo Umezawa of the Institute of Microbial Chemistry, Tokyo, Japan, for his gift of natural negamycin to ICI Pharmaceuticals Division.

15. We thank Dr. T.D. Hennessey of ICI Pharmaceuticals Division, U.K., for biological testing.

16. The antipode of (+)-negamycin is known[7] to possess some antibacterial activity.

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