THE SYNTHESIS OF DEOXYNEGAMYCIN AND SOME RELATED COMPOUNDS

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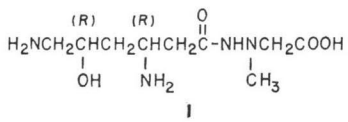
(Received for publication May 13, 1978)

The novel antibiotic negamycin **1** containing a new hydroxyamino acid linked to N-methylhydrazinoacetic acid has been described by UMEZAWA and coworkers.[1,2,3] We report here the synthesis and biological activity of the deoxy analog and its higher homolog and several compounds related to deoxynegamycin wherein the N-methylhydrazinoacetic acid moiety was replaced. Deoxynegamycin and its enantiomer were synthesized and their activities were reported by UMEZAWA and coworkers in 1976.[4,5]



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N,N′-Dibenzyloxycarbonyl-(*R*)-*β*-lysine **6** (n=3) was obtained by ARNDT-EISTERT method.[6] N,N′-Dibenzyloxycarbonyl-(*R*)-ornithine **2** (n=3) was treated with phosphorus pentachloride in ether to afford the acid chloride **3** (n=3) which was converted to the diazoketone **4** (n=3) with ethereal diazomethane. Rearrangement of compound **4** (n=3) with silver benzoate and triethylamine in methanol gave N,N′-dibenzyloxycarbonyl-(*R*)-*β*-lysine methyl ester **5** (n=3). Saponification produced the acid **6** (n=3) which was coupled to ethyl N-methylhydrazinoacetate (**7**)[7] *via* the mixed anhydride procedure utilizing isobutyl chloroformate and N-methylmorpholine in tetrahydrofuran to give the protected derivative **8** (n=3). Hydrolysis in methanolic base gave the acid **9** (n=3), which was treated with hydrogen in the presence of palladium on carbon to afford deoxynegamycin (**10**, n=3). The homolog **10** (n=4) was prepared by the same reaction sequence starting from N,N′-dibenzyloxycarbonyl-(*R*)-lysine (**2**, n=4). These reactions are illustrated on Chart 1.

The *in vitro* activity of deoxynegamycin is approximately one half that of the parent antibiotic as shown on Table 1. The homolog of deoxynegamycin (**10**, n=4) exhibited only marginal *in vitro* activity.

Table 1. *In vitro* activity of negamycin and deoxynegamycin

Culture	Strain designation	MIC (mcg/ml)	
		Deoxynegamycin	Negamycin
Escherichia coli	K-1972-1	16	8
Escherichia coli	K-1972-2	16	8
Enterobacter sp.	K-1972-1	32	16
Klebsiella pneumoniae	K-1972-2	32	16
Proteus mirabilis	K-1972-12	64	32
Proteus mirabilis	K-1972-16	64	32
Proteus vulgaris	K-1972	64	32
Pseudomonas aeruginosa	PA_7	64	32
Pseudomonas aeruginosa	12-4-7	64	32
Salmonella typhimurium	K-1972	32	8
Salmonella enteritidis	K-1972	32	16
Shigella flexneri (2a)	RB	4	2
Shigella flexneri (5)	Citarella	64	32
Serratia marcescens	Finland	64	16
Serratia marcescens	K132	32	16
Herellea vaginicola	Washington 885-863	32	16
Herellea vaginicola	Hughes	32	16
Staphylococcus aureus	Morton #1169	32	8
Staphylococcus aureus	Jackson #4	64	16
Enterococcus (*S. faecalis*)	Isenberg	>128	>128
Escherichia coli	#311	16	8
Proteus mirabilis	#4361	64	16
Klebsiella pneumoniae	AD	4	2

Coupling of N,N′-dibenzyloxycarbonyl-(*R*)-*β*-lysine (**6**, n=3) with the ethyl esters of sarcosine, (±)-3-aminobutyric acid, and N-phenylhydrazinoacetic acid followed by saponification of the ester groups and hydrogenolysis of the carbobenzoxy moieties afforded the three derivatives (**11**, **12** and **13**) shown in Chart 2. In addition the hydrazide of (*R*)-*β*-lysine (**14**) was also prepared. None of these compounds exhibited any interesting antimicrobial activity.

This work and previous work by UMEZAWA and coworkers[4,5] demonstrated the very critical

Chart 1.

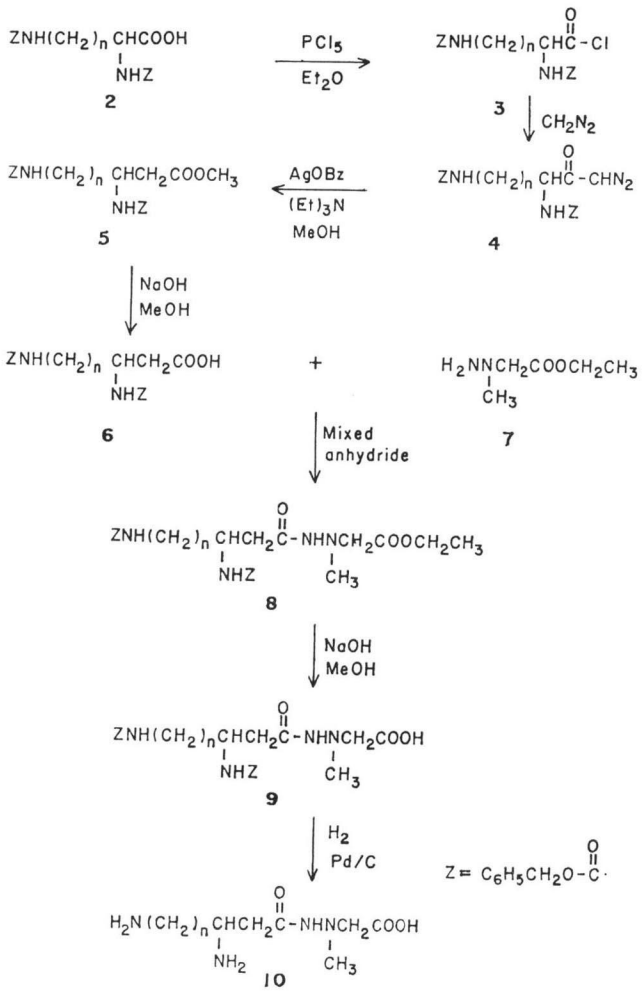
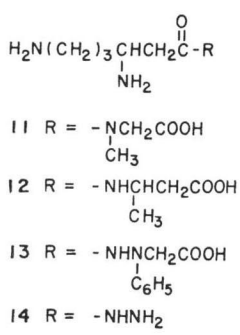


Chart 2.



nature of the kind and positions of the nitrogens with respect to each other in deoxy analogs and presumably in negamycin itself to ensure biological activity.

Experimental

All compounds reported had the expected spectral properties (IR and NMR). Thin-layer chromatography was carried out on Cellulose F using butanol - pyridine - acetic acid - water (15 : 10 : 1 : 12) as solvent system.

N,N′-Dibenzyloxycarbonyl-(*R*)-*β*-lysine methyl ester (**5**, n=3)

N,N′-Dibenzyloxycarbonyl-(*R*)-ornithine (**2**, n=3) (5.0 g, 12.5 mmol) and phosphorus pentachloride (2.75 g, 13.2 mmol) in 100 ml of diethyl ether was stirred in an ice bath for 3.0 hours. Hexane (80 ml) was added and the white crystalline product was collected by filtering. This product was slurried in ether (100 ml) and added to 200 ml of a cold, ethereal solution of diazomethane (prepared from 10 g of N-methyl-N′-nitro-N-nitrosoguanidine). The mixture was allowed to stand in the cold for 1.0 hour, then at room temperature overnight. The excess diazomethane was destroyed with acetic acid and the reaction mixture was evaporated at reduced pressure. The resulting light yellow crystals were dissolved in methanol (50 ml) and a freshly prepared solution of silver benzoate (0.5 g) in triethylamine (10 ml) was added. The mixture was stirred at room temperature for 1.0 hour, heated to boiling, filtered, and evaporated under reduced pressure. The resulting oil was crystallized and recrystallized from ethyl acetate - hexane to give 2.2 g of white crystals, m.p. 100~102°C.

Anal. Calcd. for $C_{23}H_{28}N_2O_6$:
C, 64.47; H, 6.59; N, 6.54.
Found: C, 64.03; H, 6.59; N, 6.54.

N,N′-Dibenzyloxycarbonyl-(*R*)-*β*-lysine (**6**, n=3)

N,N′-Dibenzyloxycarbonyl-(*R*)-*β*-lysine methyl ester (**5**, n=3) (2.0 g, 4.7 mmol) was added to a solution of 20 ml of 1 N sodium hydroxide in methanol - tetrahydrofuran (50 ml : 15 ml) and

the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was acidified with concentrated hydrochloric acid (2 ml), diluted with water (25 ml), and filtered to afford 1.7 g (87%) of crystals, m.p. 152~154°C. (Ref. 8 reports m.p. 152~153°C for the enantiomer): $[\alpha]_D^{25}+5°\pm2$ (c 0.33, MeOH).

Anal. Calcd. for $C_{22}H_{26}N_2O_6$:
C, 63.75; H, 6.32; N, 6.76.
Found: C, 63.88; H, 6.45; N, 6.95.

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoic acid 2-(ethoxycarbonylmethyl)-1-methylhydrazide (**8**, n=3)

Method A

To a cold solution of N,N′-dibenzyloxycarbonyl-(*R*)-β-lysine (**6**, n=3) (1.5 g, 3.62 mmol) and N-methylmorpholine (0.4 ml, 3.62 mmol) dissolved in 25 ml of tetrahydrofuran was added isobutyl chloroformate (0.48 ml, 3.62 mmol). The mixture was stirred in the cold for 15 minutes, then a cold mixture of ethyl 1-methylhydrazinoacetate hydrochloride (0.61 g, 3.63 mmol) and N-methylmorpholine (0.4 ml, 3.62 mmol) in 25 ml of tetrahydrofuran were added. The reaction mixture was stirred in the cold for 15 minutes, at room temperature for 3 hours, then brought to boiling, cooled and poured into water. The aqueous solution was extracted with ethyl acetate and the organic extract was washed with water, dilute hydrochloric acid, saturated sodium bicarbonate, and then dried over magnesium sulfate. Evaporation of the solvent left an oil which was crystallized from ethyl acetate-hexane to afford 1.5 g, (79%), m.p. 118.5~120°C: $[\alpha]_D^{25}+4°\pm3$ (c 0.3, MeOH).

Anal. Calcd. for $C_{27}H_{36}N_4O_7$:
C, 61.35; H, 6.87; N, 10.60
Found: C, 61.53; H, 6.92; N, 10.38.

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoic acid 2-(carboxymethyl)-2-methylhydrazide (**9**, n=3)

Method B

A solution of (*R*)-3,6-dibenzyloxycarbonylaminohexanoic acid 2-(ethoxycarbonylmethyl)-2-methylhydrazide (**8**, n=3) (1.0 g, 1.89 mmol) in 10 ml of methanol and 3 ml of 1 N sodium hydroxide was stirred at room temperature for 20 minutes then diluted with 50 ml of water. The solution was acidified with concentrated hydrochloric acid (0.3 ml) and extracted with ethyl acetate. The ethyl acetate extract was washed with water and brine then dried over magnesium sulfate, evaporated and the resulting oil was crystallized from ethyl acetate-hexane to give 0.65 g (70%) of white crystals, m.p. 113.5~115.5°: $[\alpha]_D^{25}+6°\pm2$ (c 0.51, MeOH).

Anal. Calcd. for $C_{25}H_{32}N_4O_7$:
C, 59.99; H, 6.44; N, 11.19.
Found: C, 60.44; H, 6.61; N, 10.89.

(*R*)-3,6-Diaminohexanoic, acid 2-(carboxymethyl)-2-methylhydrazide (**10**, n=3) (Deoxynegamycin)

Method C

A solution of (*R*)-3,6-dibenzyloxycarbonylaminohexanoic acid 2-(carboxymethy)-2-methylhydrazide (**9**, n=3) (0.5 g, 1.0 mmol) in 50 ml of methanol was hydrogenated at atmospheric pressure over 10% palladium on carbon until the evolution of carbon dioxide ceased. Evaporation of solvent gave the product as a glass-like solid: Rf 0.28, $[\alpha]_D^{25}$ $-6°\pm3$ (c 0.32, H_2O), (Lit[4]; $[\alpha]_D^{23}-5°$).

N,N′-Dibenzyloxycarbonyl-(*R*)-β-homolysine methyl ester (**5**, n=4)

Prepared as described for compound **5** (n=3) starting from N,N′-dibenzyloxycarbonyl-(*R*)-lysine, m.p. 74~77°C.

Anal. Calcd. for $C_{24}H_{30}N_2O_6$:
C, 65.14: H, 6.83; N, 6.33.
Found: C, 65.36; H, 6.84; N, 6.44.

N,N′-Dibenzyloxycarbonyl-(*R*)-β-homolysine (**6**, n=4)

Prepared as described for compound **6** (n=3), m.p. 126~128.5°: $[\alpha]_D^{25}+5°\pm2$ (c 0.54, MeOH).

Anal. Calcd. for $C_{23}H_{28}N_2O_6$:
C, 64.47; H, 6.59; N, 6.54.
Found: C, 64.73; H, 6.73; N, 6.62.

(*R*)-3,7-Dibenzyloxycarbonylaminoheptanoic acid 2-(carboxymethyl)-2-methylhydrazide ethyl ester (**8**, n=4)

Prepared by Method A as described for the corresponding β-lysine analog (**8**, n=3), m.p. 92~95°C, $[\alpha]_D^{25}+5°\pm1$ (c 0.92, MeOH).

Anal. Calcd. for $C_{28}H_{38}N_4O_7$:
C, 61.97; H, 7.06; N, 10.33.
Found: C, 62.30; H, 7.51; N, 9.90.

(*R*)-3,7-Dibenzyloxycarbonylaminoheptanoic acid 2-(carboxymethyl)-2-methylhydrazide (**9**, n=4)

Prepared by Method B as described for the corresponding β-lysine analog (**9**, n=3), m.p. 92~95°C: $[\alpha]_D^{25}$ +7°±2 (*c* 0.52, MeOH).

Anal. Calcd. for $C_{26}H_{34}N_4O_7$:
C, 60.68; H, 6.66; N, 10.89.
Found: C, 60.61; H, 6.71; N, 10.76.

(*R*)-3,7-Diaminoheptanoic acid 2-(carboxymethyl)-2-methylhydrazide (**10**, n=4)

Prepared by Method C as described for deoxynegamycin (**10**, n=3) as a glass-like solid: Rf 0.20, $[\alpha]_D^{25}$ −5°±3 (*c* 0.31, H_2O).

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoylsarcosine ethyl ester

Prepared by Method A using sarcosine ethyl ester, m.p. 72~76°C: $[\alpha]_D^{25}$+6°±3(*c* 0.33, MeOH).

Anal. Calcd. for $C_{27}H_{35}N_3O_7$:
C, 63.14; H, 6.87; N, 8.18.
Found: C, 62.64; H, 6.69; N, 7.71.

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoylsarcosine

Prepared by Method B, m.p. 60~63°C: $[\alpha]_D^{25}$ −6°±3 (*c* 0.31, MeOH).

Anal. Calcd. for $C_{25}H_{31}N_3O_7$:
C, 61.84; H, 6.44; N, 8.66.
Found: C, 61.71; H, 6.55; N, 8.54.

(*R*)-3,6-Diaminohexanoylsarcosine (**11**)

Prepared by Method C. Rf 0.40, $[\alpha]_D^{25}$ −19°±4 (*c* 0.31, H_2O).

Ethyl (±)3-[(*R*)-3,6-dibenzyloxycarbonylaminohexanoylamino]butyrate

Prepared by Method A using ethyl (±)-3-aminobutyrate: m.p. 122~127°C, $[\alpha]_D^{25}$+3°±2 (*c* 0.52, MeOH).

Anal. Calcd. for $C_{28}H_{37}N_3O_7$:
C, 63.74; H, 7.07; N, 7.97.
Found: C, 63.93; H, 7.03; N, 7.94.

(±)3-[(*R*)-3,6-Dibenzyloxycarbonylaminohexanoylamino]-butyric acid

Prepared by Method B: m.p. 139~145°C: $[\alpha]_D^{25}$ 0°±2 (*c* 0.5, MeOH).

Anal. Calcd. for $C_{26}H_{33}N_3O_7$:
C, 62.50; H, 6.66; N, 8.41.
Found: C, 62.33; H, 6.77; N, 8.47.

(±)3-[(*R*)-3,6-Diaminohexanoylamino]-butyric acid (**12**)

Prepared by Method C: Rf 0.3 with small amount of impurity of Rf 0.52, $[\alpha]_D^{25}$ 0°±4 (*c* 0.32, H_2O).

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoic acid 2-(ethoxycarbonylmethyl)-2-phenylhydrazide

Prepared as decribed under Method A using N-phenylhydrazinoacetic acid ethyl ester, m.p. 120~122.5°C: $[\alpha]_D^{25}$ +6°±2 (*c* 0.52, MeOH).

Anal. Calcd. for $C_{32}H_{38}N_4O_7$:
C, 65.07; H, 6.48; N, 9.48.
Found: C, 64.96; H, 6.57; N, 9.23.

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoic acid 2-(carboxymethyl)-2-phenylhydrazide

Prepared as described under Method B: m.p. 143~146°C: $[\alpha]_D^{25}$ +7°±2 (*c* 0.54, MeOH).

Anal. Calcd. for $C_{30}H_{34}N_4O_7$:
C, 64.04; H, 6.09; N, 9.96.
Found: C, 63.66; H, 6.42; N, 9.89.

(*R*)-3,6-Diaminohexanoic acid 2-(carboxymethyl)-2-phenylhydrazide (**13**)

Prepared as described under Method C: Rf 0.50.

(*R*)-3,6-Dibenzyloxycarbonylaminohexanoic acid hydrazide

A solution of N,N′-dibenzyloxycarbonyl-(*R*)-β-lysine methyl ester (**5**, n=3) (2.0 g, 4.67 mmol) and hydrazine hydrate (2.5 ml, 51.5 mmol) in 20 ml of ethanol was refluxed for 16 hours, treated with Norit, filtered and cooled to give 1.0 g (50%) of white crystals: m.p. 168~171°C: $[\alpha]_D^{25}$ +3°±1 (*c* 1.0, MeOH).

Anal. Calcd. for $C_{22}H_{28}N_4O_5$:
C, 61.66; H, 6.59; N, 13.08.
Found: C, 61.51; H, 6.67; N, 13.09.

(*R*)-3,6-Diaminohexanoic acid hydrazide (**14**)

Prepared by Method C and converted to the hydrochloride salt by evaporation of a methanolic solution neutralized to pH 2 with hydrochloric acid: Rf 0.2, $[\alpha]_D^{25}$ −15°±4 (*c* 0.28, H_2O).

Acknowledgement

We wish to thank Mr. W. FULMOR and staff for the spectral data and Mr. L. BRANCONE and staff for the microanalytical information presented herein. Special thanks are also due to Mr. G. REDIN, Mr. A. DORNBUSH and Mrs. N. KUCK for the biological data presented.

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