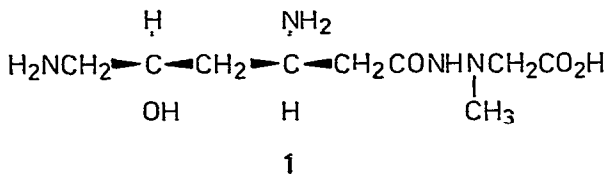
Carbohydrate Research, 83 (1980) 383–388
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

Synthesis of methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside, an intermediate in the synthesis of the antibiotic negamycin

WOLFGANG STREICHER AND HELLMUTH REINSHAGEN
Sandoz Forschungsinstitut Wien, A-1235 Wien 23 (Austria)
(Received August 27th, 1979, accepted for publication, December 2nd, 1979)

Negamycin (**1**) is an antibiotic isolated from *Streptomyces purpeofuscus* and posesses strong growth-inhibitory activity against Gram-negative bacteria[1] The structure of **1** has been determined as {2-[(3*R*,5*R*)-3,6-diamino-5-hydroxyhexanoyl]-1-methylhydrazino}acetic acid, and its total synthesis was first reported by Umezawa

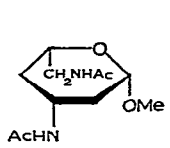


1

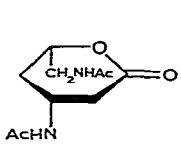
and co-workers[2 3] In an 11-step sequence, D-galacturonic acid was converted into methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-β-L-*threo*-hexopyranoside (**2**) Hydrolysis and oxidation of **2** gave 3,6-diacetamido-2,3,4,6-tetradeoxy-L-*threo*-hexono-1,5-lactone [(3*R*,5*R*)-3,6-diacetamido-5-hexanolide] (**3**), which was identical with a compound previously obtained by degradation of natural negamycin The same paper also described a synthesis of the enantiomer of **3** starting from 3-amino-3-deoxy-D-glucose One of the intermediates obtained in this reaction sequence was methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-α-D-*threo*-hexopyranoside (**4**)

Both syntheses[3] have in common the stepwise elimination of OH-2 and -4 of the respective carbohydrate intermediates For the synthesis of **3**, the configuration at C-3 and -5 was inverted to obtain the desired 3*R*,5*R* configuration. For the synthesis of the enantiomer of **3**, the configuration at C-3 and -5 remained unchanged, leading to the 3*S*,5*S* compound Starting from **3** (or its enantiomer), the *N*-hydroxysuccinimide esters of (3*R*,5*R*)- [or (3*S*,5*S*)-] 3,6-di-*N*-benzyloxycarbonylamino-5-tetrahydropyranyloxyhexanoic acid were prepared[2] Condensation of these active esters with (1-methylhydrazino)acetic acid and subsequent deprotection completed the synthesis of negamycin and its enantiomer

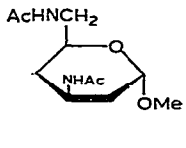
0008-6215/80/0000–0000/$ 02 25,



2



3

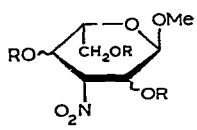


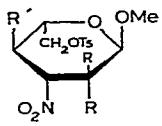
4

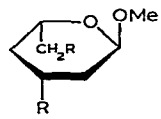
Chmielewski *et al*[4] recently described the synthesis of racemic derivatives of 3,6-diamino-2,3,4,6-tetradeoxy-*threo*-hexopyranoses starting from dihydropyranes Using a similar approach, Streicher *et al*[5] carried out the total synthesis of *rac* negamycin and of negamycin analogs Syntheses of other analogs of negamycin have also been described[6–10]

In the present communication we describe the synthesis of an intermediate for a chiral synthesis of negamycin Baer and Neilson[11] have shown that 2-*O*-acetyl-4,6-*O*-benzylidene-3-deoxy-3-nitrohexopyranosides may be converted in two steps, *via* elimination and hydrogenation, into 4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-hexopyranosides This deacylation–reduction procedure was also performed in one step by the application of sodium borohydride to methyl 3,6-dideoxy-2-*O*-methylsulfonyl-3-nitro-α-D-glucopyranoside (or the 4-*O*-mesyl-isomer) to yield methyl 2,3,6-trideoxy-3-nitro-α-D-*arabino*-hexopyranoside (or the 3,4,6-trideoxy-isomer)[12,13] Our synthetic plan centered on the simultaneous replacement by a hydrogen atom of OH-2 and -4 of a 3-deoxy-3-nitrohexopyranoside For the synthesis starting from a 2,4-di-*O*-acetyl-3-deoxy-3-nitro-L-hexopyranoside, it was anticipated that sodium borohydride would twice eliminate acetic acid and subsequently hydrogenate the nitro-olefin intermediates This would lead to a 2,3,4-trideoxy-3-nitro-L-hexopyranoside having the desired 3*R*,5*R* configuration, provided that the nitro group occupied the equatorial position after the reduction step

Periodate oxidation of methyl α-L-arabinofuranoside[14] and subsequent nitromethane condensation according to the procedure of Baer[15,16] (for the α-D isomer) gave a mixture of isomeric methyl 3-deoxy-3-nitro-α-L-hexopyranosides (**5**) in practically quantitative yield Monotosylation of **5** with *p*-toluenesulfonyl chloride in pyridine gave a mixture of the corresponding 6-*O*-tosyl compounds (**6**) in ~40% yield Chromatographic separation of a small sample of **6** gave two main isomers, which were shown by ^{1}H-n m r analysis to be the α-L-*manno* (**6a**) and α-L-*gluco* (**6b**) compounds However separation of **6** was not required to carry out subsequent steps Boron trifluoride-catalyzed acetylation[17] of **6** gave a mixture of isomeric methyl 2,4-di-*O*-acetyl-3-deoxy-3-nitro-6-*O*-*p*-tolylsulfonyl-α-L-hexopyranosides (**7**) in quantitative yield Treatment of **7** with a large excess of sodium borohydride in ethanol, and subsequent chromatography on silica gel gave, in ~30% yield, a crystalline product, the structure of which was established to be methyl 2,3,4-trideoxy-3-nitro-6-*O*-*p*-tolylsulfonyl-α-L-*threo*-hexopyranoside (**8**) Treatment of **8** with sodium azide in hexamethylphosphoric triamide gave the azide **9** (100%) Catalytic hydrogenation of **9** in the presence of Raney nickel gave the diamino







5 R = R = H
6 R = H R = Ts
7 R = Ac R = Ts

6a R = H R = R = OH
6b R = R = OH R = H
7a R = H R = R = OAc
7b R = R = OAc R = H

8 R = NO_2 R = OTs
9 R = NO_2 R = N_3
10 R = R = NH_2
11 R = R = NHAc

compound **10**, which on acetylation yielded crystalline methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-α-L-*threo*-hexopyranoside (**11**, 70% from **9**) The melting point and optical rotation of **11** are in excellent agreement with the values reported[3] for its enantiomer **4**

The remaining steps (hydrolysis of the glycosidic bond, oxidation to lactone **3**, and opening of this lactone for a total synthesis of negamycin) have been described previously[2,3] The preparation of the intermediate **11**, starting from L-arabinose (6 steps, 6 6% overall yield from methyl α-L-arabinofuranoside), thus provides another synthetic route to negamycin

EXPERIMENTAL

General — Melting points are uncorrected Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter N m r spectra were recorded, for solutions, at 90 or 100 MHz, with tetramethylsilane as internal standard (s, singlet, d, doublet, t, triplet, q, quartet, m, multiplet, b, broad) I r spectra (for potassium bromide discs or chloroform solutions) were recorded with a Perkin–Elmer 421 spectrophotometer T l c was performed on Silica Gel GF_{254} (Merck) plates (the spots being detected with iodine vapor and by visualization under u v light) and column chromatography on silica gel (40–63 μm, Merck)

Methyl 3-deoxy-3-nitro-α-L-hexopyranosides (**5**) — A solution of sodium metaperiodate (61 22 g, 0 29 mol) in water (700 mL) was cooled to 5°, and a solution of methyl α-L-arabinofuranoside[12] (47 g, 0 29 mol) in water (100 mL) was added with stirring over a period of 10 min The mixture was then warmed to 23° and kept for 3 h Ethanol (1 5 L) was added, and the precipitate that formed was removed by filtration and washed with ethanol The filtrate was evaporated to dryness and the residue taken up in ethanol Another crop of inorganic material was filtered off and washed with ethanol The combined ethanol solutions were again evaporated to a syrup The dialdehyde thus obtained was dissolved in dry methanol (500 mL), and nitromethane (18 mL) was added A solution of sodium (7 5 g) in methanol (250 mL) was added during 15 min to the solution cooled to 2° After being rewarmed to 23° for an additional 45 min, the yellow solution was treated with Amberlite IR-120 (H^+) ion-exchange resin (450 mL) and gently stirred for 15 h The solution was decanted from the resin and passed through a column of Amberlite IR-120 (H^+) ion-exchange resin (200 mL) Both batches of resin were washed with methanol, and

the combined solvents were evaporated to give **5** as a syrupy residue (58 5 g, 91%)

*Methyl 3-deoxy-3-nitro-6-*O*-p-tolylsulfonyl-α-*L*-hexopyranosides* (**6**) — To a solution of **5** (36 3 g, 0 16 mol) in freshly distilled pyridine (150 mL) chilled to 0° was added *p*-toluenesulfonyl chloride (34 1 g, 0 18 mol) dissolved in pyridine (50 mL) After being kept for 18 h at room temperature, the solution was poured into 2M hydrochloric acid (1 5 L) The resulting solution was extracted 5 times with chloroform (200 mL each), the combined chloroform layers were washed twice with 2M hydrochloric acid and water, dried (sodium sulfate), and evaporated to yield **6** as a syrup (23 1 g, 38%) The aqueous phase contained large amounts of colored, polar by-products which were not investigated further

Column chromatography (50 1, v/v, chloroform–ethanol) of a sample (2 g) of **6** gave two homogeneous fractions (ratio of **6a** to **6b** ~2 3), both as viscous syrups

*Methyl 3-deoxy-3-nitro-6-*O*-p-tolylsulfonyl-α-*L*-mannopyranoside* (**6a**) — T l c (20 1, v/v, chloroform–ethanol) R_F 0 41, $[\alpha]_D^{20}$ −12 7° (*c* 0 6, chloroform), n m r chloroform-*d*, assignments made after addition of trichloroacetyl isocyanate) δ 4 66 (d, $J_{1,2}$ 2 Hz, H-1), 4 56 (t, $J_{2,1} = J_{2\,3} = 3$ Hz, H-2), 4 45–4 3 (m, $J_{3,4} = J_{4,5} = 9$ Hz, H-3,-4,-6,-6′), 3 74 (td, $J_{5\,6} = J_{5,6} = 3$ Hz, H-5), 3 34 (s, OMe), and 2 44 (s, $C_6H_4CH_3$)

Anal Calc for $C_{14}H_{19}NO_9S$ C, 44 56, H, 5 07, N, 3 71, S, 8 50 Found C, 44.77, H, 5 29, N, 3 41, S, 8 22.

*Methyl 3-deoxy-3-nitro-6-*O*-p-tolylsulfonyl-α-*L*-glucopyranoside* (**6b**) — T l c (20 1, v/v, chloroform–ethanol) R_F 0 34, $[\alpha]_D^{20}$ −98 3° (*c* 0 7, chloroform), n m r (chloroform-*d*, assignments confirmed by INDOR technique) δ 4 73 (t, $J_{3\,2} = J_{3\,4} =$ 10 Hz, H-3), 4 72 (d, $J_{1\,2}$ 4 Hz, H-1), 4 36 (m, H-6, -6′), 4 07 (t, $J_{4\,3} = J_{4,5} = 10$ Hz, H-4), 4 04 (dd, $J_{2,1}$ 4, $J_{2,3}$ 10 Hz, H-2), 3 38 (s, OMe), and 2 43 (s, $C_6H_4CH_3$)

Anal Calc for $C_{14}H_{19}NO_9S$ C, 44 56, H, 5 07, N, 3 71, S, 8 50 Found C, 44 63, H, 5 18, N, 3 52, S, 8 17

*Methyl 2,4-di-*O*-acetyl-3-deoxy-3-nitro-6-*O*-p-tolylsulfonyl-α-*L*-hexopyranosides* (**7**) — A solution of **6** (23 1 g, 61 mmol) in acetic anhydride (75 mL) was treated at 5° with boron trifluoride etherate (1 5 mL) After 15 h at room temperature, the solution was evaporated under diminished pressure The residue was taken up in ethyl acetate, and the solution washed with saturated aqueous sodium hydrogencarbonate and water, dried (sodium sulfate), and evaporated to yield **7** (26 7 g, 95%)

Treatment of **6a** or **6b** in the same manner gave the corresponding diacetates **7a** and **7b**, which were chromatographically homogeneous (t l c , chloroform) and identical with the main spots of the isomeric mixture **7** Compound **7a** was obtained as a viscous syrup, and **7b** crystallized from dichloromethane–isopropyl ether

*Methyl 2,4-di-*O*-acetyl-3-deoxy-3-nitro-6-*O*-p-tolylsulfonyl-α-*L*-mannopyranoside* (**7a**) — $[\alpha]_D^{20}$ −14 5° (*c* 1, chloroform), n m r (chloroform-*d*) δ 5 65 (t, $J_{4\,3} = J_{4\,5} = 10\,5$ Hz, H-4), 5 52 (dd, $J_{2\,3}$ 3 5 Hz, H-2), 4 98 (dd, $J_{3,4}$ 10 5 Hz, H-3), 4 78 (d, $J_{1\,2}$ 2 Hz, H-1), 4 4–3 8 (m, H-5,-6,-6′), 3 41 (s, OMe), 2 48 (s, $C_6H_4CH_3$), 2 08 (s, OAc), and 2 05 (s, OAc)

Anal Calc for $C_{18}H_{23}N_{11}S$ C, 46 85, H, 5 02, N, 3 03, S, 6 94 Found C, 47 56, H, 5 23, N, 2 76, S, 6 60

*Methyl 2,4-di-*O*-acetyl-3-deoxy-3-nitro-6-*O*-*p*-tolylsulfonyl-α-*L*-glucopyranoside* (**7b**) — M p 115–116°, $[\alpha]_D^{20}$ −112 4° (*c* 1, chloroform), n m r (chloroform-*d*, assignments confirmed by INDOR technique) δ 5 30 (t, $J_{3,4} = J_{4,5} = 9$ 5 Hz, H-4), 5 16 (dd, $J_{2\ 3}$ 10 5 Hz, H-2), 4 97 (d, $J_{1,2}$ 3 5 Hz, H-1), 4 93 (dd, $J_{3,4}$ 9 5 Hz, H-3), 3 40 (s, OMe), 2 40 (s, $C_6H_4CH_3$), 2 09 (s, OAc), and 2 06 (s, OAc)

Anal. Calc for $C_{18}H_{23}NO_{11}S$ C, 46 85, H, 5 02, N, 3 03, S, 6 94 Found C, 47 19, H, 5 03, N, 2 92, S, 7 06

*Methyl 2,3,4-trideoxy-3-nitro-6-*O*-*p*-tolylsulfonyl-α-*L*-threo-hexopyranoside* (**8**) — Sodium borohydride (13 4 g) was added, in portions, with stirring, to a solution of **7** (26 7 g, 58 mmol) in ethanol (400 mL) The suspension was stirred for 20 h at 20° and then adjusted to pH 6 by addition of acetic acid After removal of most of the solvents, the residue was taken up in 0 1M hydrochloric acid (300 mL), and the solution extracted several times with chloroform The combined extracts were dried (sodium sulfate) and the solvent was evaporated The residue (16 6 g) was purified by column chromatography, with chloroform as the eluent The fractions that were homogeneous in t l c (chloroform) were combined and evaporated Crystallization from isopropyl ether gave pure **8** (6 g, 30%), m p 76–78°, $[\alpha]_D^{22}$ −78 4° (*c* 1 2, chloroform), ν_{max}^{KBr} 1540 and 1350 cm^{-1} (NO_2), n m r (chloroform-*d*) δ 4 88 (bd, $J_{1\ 2a}$ 2 5 Hz, H-1), 4 82 (tt, $J_{3\ 2a} = J_{3\ 4} = 11\ 7$, $J_{3\ 2e} = J_{3\ 4e} = 4\ 5$ Hz, H-3), 5 15–4 8 (m, H-5,-6,-6′), 3 31 (s, OMe), 2 46 (s, $C_6H_4CH_3$), and 2 5–1 5 (m, H-2a, -2e,-4a,-4e)

Anal Calc for $C_{14}H_{19}NO_7S$ C, 48 70, H, 5 55, N, 4 06 Found C, 48 72, H, 5 58, N, 4 05

Spectroscopic examination of the slower-moving fractions indicated that one component was the corresponding α-*L*-*erythro* isomer of **8** Other by-products were shown to contain additional hydroxyl groups

*Methyl 6-azido-2,3,4,6-tetradeoxy-3-nitro-α-*L*-threo-hexopyranoside* (**9**) — Compound **8** (15 g, 43 mmol) and sodium azide (20 g) were added to hexamethylphosphoric triamide (100 mL), and the mixture was heated at 75° for 1 h with stirring The solution was then poured into water, and the aqueous phase was extracted several times with ether The combined extracts were washed with water and dried (sodium sulfate) After evaporation of the solvent, **9** remained as a chromatographically (20 1, v/v, chloroform–ethanol) homogeneous oil (9 1 g, 97%), $[\alpha]_D^{22}$ −111° (*c* 1 25, methanol), ν_{max}^{film} 2100 (N_3), 1550, and 1360 cm^{-1} (NO_2), n m r (chloroform-*d*) δ 4 97 (bd, $J_{1\ 2a}$ 2 5 Hz, H-1), 4 87 (tt, $J_{3\ 2a} = J_{3,4a} = 12$, $J_{3,2e} = J_{3\ 4e} = 4\ 5$ Hz, H-3), 4 15–3 85 (m, H-5), 3 39 (s, OMe), 3 40–3 20 (m, H-6, H-6′), and 2 6–1 6 (m, H-2a,-2e,-4a,-4e)

Anal Calc for $C_7H_{12}N_4O_4$ C, 38 89, H, 5 59, N, 25 91 Found C, 39 32, H, 5 56, N, 25 45

*Methyl 3,6-diamino-2,3,4,6-tetradeoxy-α-*L*-threo-hexopyranoside* (**10**) — To a solution of **9** (4 g, 18 5 mmol) in methanol (100 mL) was added Raney nickel (0 5 g),

and the suspension was hydrogenated for 15 h at 1 MPa and room temperature The catalyst was filtered off and the filtrate evaporated, to yield **10** (2 3 g, 78%), which was used for the next step without purification

*Methyl 3,6-diacetamido-2,3,4,6-tetradeoxy-α-*L*-threo-hexopyranoside* (**11**) — Crude **10** (1 1 g, 6 9 mmol) was dissolved in methanol (100 mL) and treated with acetic anhydride (20 mL) After being kept for 2 h at room temperature, the solution was evaporated to dryness and the residue was crystallized from methanol–ether to yield **11** (1 5 g, 89%), m p 192–194° Recrystallization from ethyl acetate–hexane raised the m p to 196–197°, $[\alpha]_D^{22\,5}$ −132 6° (*c* 0 7, water) [lit [3] for the enantiomer m p 191–192, $[\alpha]_D^{20}$ +137° (*c* 0 8, water)], ν_{max}^{KBr} 1650 cm^{-1} (NHAc), n m r (chloroform-*d*) δ 4 80 (bd, $J_{1\,2a}$ 3 Hz, H-1), 4 27 (m, on exchange with D_2O, collapsed to tt, $J_{3\,2e} = J_{3\,4e} = 4$, $J_{3\,2a} = J_{3\,4a} = 12$ Hz, H-3), 3 86 (m, H-5), 3 44 (dd, $J_{6\,6}$ 13, $J_{6\,5}$ 4 Hz, H-6), 3 32 (s, OMe), 3 19 (dd, $J_{6\,,6}$ 13, $J_{6\,,5}$ 7 Hz, H-6′), 2 0 (s, OAc), 1 94 (s, OAc), ~2 (m, H-2e,4e), 1 42 (dt, $J_{2a\,1}$ 3, $J_{2a\,2e} = J_{2a\,3} = 12$ Hz, H-2a), and 1 14 (q, $J_{4,3a} = J_{4a,4e} = J_{4a,5} = 12$ Hz, H-4a)

Anal. Calc for $C_{11}H_{20}N_2O_4$ C, 54 08, H, 8 25, N, 11 47 Found C, 54 18, H, 8 30, N, 11 51

ACKNOWLEDGMENTS

The authors thank Dr G Schulz for his assistance in the interpretation of n m r. spectra, and A Pirkner and O Wostrowsky for skilful technical assistance

REFERENCES

1 M Hamada, T Takeuchi, S Kondo, Y Ikeda, H Naganawa, K Maeda, Y Okami, and H Umezawa, *J Antibiot*, 23 (1970) 170–171
2 S Kondo, S Shibahara, S Takahashi, K Maeda, H Umezawa, and M Ohno, *J Am Chem Soc*, 93 (1971) 6305–6306
3 S Shibahara, S Kondo, K Maeda, H Umezawa, and M Ohno, *J Am Chem Soc*, 94 (1972) 4353–4354
4 M Chmielewski, J Jurczak, and A Zamojski, *Tetrahedron*, 34 (1978) 2977–2981
5 W Streicher, H Reinshagen, and F Turnowsky, *J Antibiot*, 31 (1978) 725–728
6 W Streicher and H Reinshagen, *Chem Ber*, 108 (1975) 813–819
7 S Kondo, K Iinuma, K Yoshida, K Yokose, Y Ikeda, M Shimazaki, and H Umezawa, *J Antibiot*, 29 (1976) 208–211
8 Y Uehara, M Hori, S Kondo, M Hamada, and H Umezawa, *J Antibiot*, 29 (1976) 937–943
9 S Kondo, K Yoshida, T Ikeda, K Iinuma, Y Honma, M Hamada, and H Umezawa, *J Antibiot*, 30 (1977) 1137–1139
10 W V Curran and J H Boothe, *J Antibiot*, 31 (1978) 914–918
11 H H Baer and T Neilson, *Can J Chem*, 43 (1965) 840–846
12 H H Baer and F F Z Georges, *Can J Chem*, 55 (1977) 1100–1103
13 H H Baer and F F Z Georges, *Can J Chem*, 55 (1977) 1348–1353
14 R S Wright and H G Khorana, *J Am Chem Soc*, 80 (1958) 1944–1948
15 H H Baer, *Methods Carbohydr Chem*, 6 (1972) 245–249
16 H H Baer, *Can J Chem*, 43 (1965) 3074–3079
17 H H Baer, F Kienzle, and F Rajablee, *Can J Chem*, 46 (1968) 80–84