# SYNTHESIS OF 5″-AMINO-3′,5″-DIDEOXYBUTIROSIN A

ISAMU WATANABE, TSUTOMU TSUCHIYA, FUJIO NAKAMURA, MASA HAMADA* and SUMIO UMEZAWA

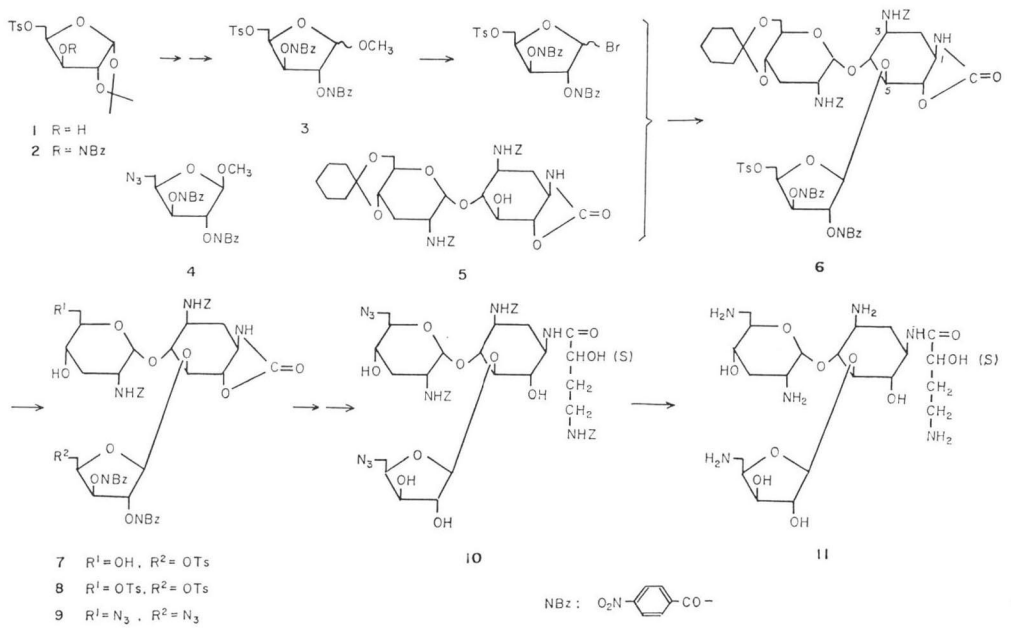
Institute of Bioorganic Chemistry, 1614 Ida, Nakahara-ku, Kawasakishi 211, Japan
*Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

(Received for publication July 7, 1978)

The titled compound was prepared by condensation of 3′-deoxyparomamine derivative (**5**) with 2,3-*O*-bis(*p*-nitrobenzoyl)-5-*O*-tosyl-D-xylofuranosyl bromide followed by 1-*N*-acylation with the active ester of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid. The compound was slightly more active than 3′-deoxybutirosin A against *Pseudomonas*.

CULBERTSON, WATSON and HASKELL[1] have reported that introduction of an amino group at C-5″ of butirosin A, but not B, gave a derivative which had enhanced antibacterial activity relative to the butirosins against some strains including *Pseudomonas*. Since the 5″-hydroxyl group is phosphorylated by resistant bacteria having kanamycin-neomycin phosphotransferases,[2] it is meaningful to displace the hydroxyl group with an amino group to avoid the phosphorylation. Later, WOO[3] and SAEKI *et al.*[4] independently prepared 5″-amino-3′,4′,5″-trideoxybutirosin A and showed that the compound exhibited enhanced activity than 5″-amino-5″-deoxybutirosin A.[1] The additional enhancement in activity is presumably due to the absence of the 3′-hydroxyl group. In this paper we describe the synthesis of 5″-amino-3′,5″-dideoxybutirosin A, which was undertaken since in some cases,[5] a 3′-deoxy compound gave better antibacterial activity than the corresponding 3′,4′-dideoxy compound.

The synthesis was performed *via* a route similar to that reported for 3′-deoxybutirosins.[6] 1,2-*O*-



Isopropylidene-5-*O*-tosyl-D-xylofuranose[7] (**1**) was converted to its 3-*O*-(*p*-nitrobenzoate) (**2**), and, after methanolysis, the deisopropylidene derivative was *p*-nitrobenzoylated to give the 2,3-*O*-bis(*p*-nitrobenzoate) (**3**). Treatment of **3** with hydrogen bromide gave the corresponding 1-bromide, which, without purification, was condensed with 3,2′-bis(*N*-benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-4′,6′-*O*-cyclohexylidene-3′-deoxyparomamine[6] (**5**) in dichloromethane in the presence of mercury(II) cyanide to give the 5-*O*-xylosylparomamine derivative (**6**). In an attempt to use an azido sugar in this synthesis, methyl 5-azido-5-deoxy-2,3-*O*-bis(*p*-nitrobenzoyl)-*β*-D-xylofuranoside (**4**) was treated with hydrogen bromide, however, we found that this reaction cleaved[8] the azido group.

Removal of the cyclohexylidene group of **6** with acid gave the 4′,6′-diol (**7**), and selective tosylation gave 6′-*O*-tosyl derivative (**8**). Treatment of **8** with lithium azide in *N*,*N*-dimethylformamide (DMF) gave 6′-azide-6′-deoxy derivative (**9**). Opening of the 1,6-cyclic carbamate with dilute barium hydroxide followed by 1-*N*-acylation with *N*-hydroxysuccinimide ester[9] of (*S*)-4-benzyloxycarbonylamino-2-hydroxybutyric acid gave **10**. In the above treatment, *p*-nitrobenzoyl groups of **9** were removed simultaneously. Catalytic hydrogenation of **10** removed the benzyloxycarbonyl groups and simultaneously reduced the azido groups to give the desired product (**11**). Its *β*-D-anomeric configuration was deduced from its NMR spectrum in deuterium oxide at pH 1; the H-1″ signal appeared at *δ* 5.47* as a singlet, well separated from other signals confirming the *β*-D-xylofuranosyl structure.

Table 1. Antibacterial spectra of **11** and 3′-deoxybutirosin A

| Test organisms* | Minimal inhibitory concentration (mcg/ml) | |
|---|---|---|
| | **11** | 3′-Deoxybutirosin A |
| *Staphylococcus aureus* FDA 209P | 0.78 | 0.78 |
| *Sarcina lutea* PCI 1001 | 3.12 | 6.25 |
| *Bacillus subtilis* NRRL B-558 | <0.2 | <0.2 |
| *Klebsiella pneumoniae* PCI 602 | 0.78 | 0.39 |
| 〃 type 22 #3038 | 1.56 | 1.56 |
| *Salmonella typhi* T-63 | 0.39 | 0.39 |
| *Escherichia coli* NIHJ | 1.56 | 1.56 |
| 〃 K-12 | 0.78 | 0.78 |
| 〃 〃 R5 | 50 | 100 |
| 〃 〃 ML1629 | 0.78 | 0.78 |
| 〃 〃 ML1630 | 1.56 | 1.56 |
| 〃 〃 LA290 R55 | 1.56 | 1.56 |
| 〃 〃 J5R11-2 | 0.2 | 0.78 |
| 〃 〃 W677 | 0.78 | 0.78 |
| 〃 〃 JR66/W677 | 1.56 | 3.12 |
| *Pseudomonas aeruginosa* A3 | 0.78 | 3.12 |
| 〃 No. 12 | 12.5 | 25 |
| 〃 GN315 | >100 | >100 |
| 〃 TI-13 | 6.25 | 25 |
| 〃 99 | 12.5 | 25 |
| *Proteus rettgeri* GN311 | 6.25 | 6.25 |
| *Mycobacterium smegmatis* ATCC 607** | 0.39 | 0.78 |
| *Serratia* 2019 (Roger Bellon 719) | 12.5 | 6.25 |
| 〃 3 ( 〃 754) | 1.56 | 3.12 |
| *Serratia marcescens* GN 6477 | 3.12 | 1.56 |
| 〃 GN 6502 | 6.25 | 12.5 |
| *Enterobacter* HEN (Roger Bellon 773) | 6.25 | 12.5 |
| *Pyocyanique* ( 〃 743) | 6.25 | 1.56 |
| *Pyocyanique* TAR ( 〃 771) | 6.25 | 25 |

* Agar dilution streak method (nutrient agar, 37°C, 18 hours).
** 42 hours

*In vitro* tests (Table 1) show that 5″-amino-3′,5″-dideoxybutirosin A has slightly enhanced inhibi-

* The H-1″ signals of some of the related compounds are as follows: tetra-*N*-acetylribostamycin: *δ* 5.20, $J_{1'',2''}$ < 1 Hz (AKITA, E.; T. TSURUOKA, N. EZAKI & T. NIIDA: Studies on antibiotic SF-733, a new antibiotic. II. J. Antibiotics 23: 173~183, 1970); neomycin C: *δ* 5.88, $J_{1'',2''}$ = 1 Hz (UMEZAWA, S. & Y. NISHIMURA: Total synthesis of neomycin C. J. Antibiotics 30: 189~191, 1977)

tory activity relative to 3′-deoxybutirosin A against *Pseudomonas aeruginosa* strains.

## Experimental

Thin-layer chromatography (TLC) was performed on Wakogel B-5 with sulfuric acid spray for detection. For column chromatography, silica gel (Wakogel C-200) was used.

1,2-*O*-Isopropylidene-3-*O*-(*p*-nitrobenzoyl)-5-*O*-tosyl-D-xylofuranose (**2**).

A solution of **1** (3.44 g) and *p*-nitrobenzoyl chloride (2.13 g) in a mixture of pyridine (35 ml) and dichloromethane (10 ml) was kept at room temperature for 4 hours. Addition of water (0.2 ml) followed by concentration of the solution gave a residue. The chloroform solution of the residue was washed with aqueous potassium hydrogensulfate, aqueous sodium hydrogencarbonate and water, dried ($Na_2SO_4$), and concentrated to give a solid. Recrystallization from methanol gave needles, 4.25 g (86%), mp 144~145°C, $[\alpha]_D^{25}$ −18° (*c* 1, $CHCl_3$). PMR ($CDCl_3$): $\delta$ 1.33 and 1.53 (each 3H s, $C(CH_3)_2$), 2.40 (3H s, Ts ($CH_3$)), 4.67 (1H d, $J_{1,2}$=4 Hz, H-2), 5.50 (1H d, $J_{3,4}$=3 Hz, H-3), 5.98 (1H d, H-1).

Found: C 53.69, H 4.86, N 2.85, S 6.38%.
Calcd. for $C_{22}H_{23}NO_{10}S$: C 53.54, H 4.70, N 2.84, S 6.50%.

Methyl 2,3-*O*-bis(*p*-nitrobenzoyl)-5-*O*-tosyl-D-xylofuranoside (**3**).

To a solution of **2** (2.22 g) in dichloromethane (40 ml), 0.6 M methanolic hydrochloric acid (85 ml) was added and the solution was kept at 0°C for 3 days. Addition of pyridine (~20 ml) followed by evaporation gave a residue. To an ice-cold solution of the residue in pyridine (44 ml), *p*-nitrobenzoyl chloride (1.05 g) was added and the solution was kept at room temperature for 2.5 hours. Purification as described for **2** gave a syrup, which was chromatographed with benzene - ethyl acetate (30 : 1) to afford a syrup of an anomeric mixture (Rf 0.32 with $C_6H_6$ - EtOAc, 30 : 1), 1.95 g (70%). However, when the earlier fractions of the eluates were collected, pure $\beta$-anomer was obtained, $[\alpha]_D^{25}$ +59° (*c* 1, $CHCl_3$). PMR ($CDCl_3$): $\delta$ 5.12 (1H s, H-1).

Found: C 52.85, H 4.12, N 4.34, S 5.31%.
Calcd. for $C_{27}H_{24}N_2O_{13}S$: C 52.60, H 3.92, N 4.54, S 5.20%.

Methyl 5-azido-5-deoxy-2,3-*O*-bis(*p*-nitrobenzoyl)-$\beta$-D-xylofuranoside (**4**).

A solution of 5-azido-5-deoxy-1,2-*O*-isopropylidene-D-xylofuranose[10] (2.5 g) in 0.4 M methanolic hydrochloric acid (85 ml) was kept at 4°C for 3 days. Addition of pyridine (10 ml) followed by concentration of the solution gave a residue. The solution of the residue in pyridine was treated similarly as described for **3** using 6.6 g of *p*-nitrobenzoyl chloride to give a solid. Trituration with ethyl acetate (15 ml) gave needles, 2.43 g (**4**, 43%). Concentration of the mother liquor followed by chromatography of the residue with benzene - ethyl acetate (50 : 1) gave a mixture (2.28 g, 40%) of **4** and its $\alpha$-anomer. **4**: mp 142~143°C, $[\alpha]_D^{24}$ +117° (*c* 1, $CHCl_3$). IR (KBr): 2100 $cm^{-1}$. PMR ($CDCl_3$): $\delta$ 3.54 (3H s, $OCH_3$), 4.77 (1H q, J=7 Hz, H-4), 5.16 (1H s, H-1), 5.54 (1H broadened s, H-2), 5.76 (1H q, $J_{2,3}$=2 Hz, $J_{3,4}$=7 Hz, H-3).

Found: C 49.00, H 3.58, N 14.30%.
Calcd. for $C_{20}H_{17}N_5O_{10}$: C 49.29, H 3.52, N 14.37%.

3,2′-*N*-Bis(benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-4′,6′-*O*-cyclohexylidene-3′-deoxy-5-*O*-[2,3-*O*-bis-(*p*-nitrobenzoyl)-5-*O*-tosyl-$\beta$-D-xylofuranosyl]paromamine (**6**).

To a cold (with ice-salt) solution of **3** (1.94 g) in dichloromethane (39 ml), hydrogen bromide was introduced until saturation and the solution was kept at 4°C overnight. Evaporation to dryness gave a residue, which was dissolved in dichloromethane (14 ml). To the solution, **5** (536 mg), Drierite (1.94 g) were added and the mixture was stirred for a while. Mercury(II) cyanide (970 mg) was added and the mixture was again stirred vigorously at room temperature overnight. After addition of chloroform (50 ml) and pyridine (0.2 ml) followed by filtration, the solution was washed with aqueous sodium hydrogencarbonate, with water, dried ($Na_2SO_4$) and concentrated. The residue was chromatographed with chloroform - ethanol - triethylamine (100 : 1 : 0.1) to give a chromatographically homogeneous solid (Rf 0.36 with $CHCl_3$-MeOH, 30 : 1), 306 mg (31%), $[\alpha]_D^{25}$ +46° (*c* 1, $CHCl_3$). IR (KBr): 1180

and 1350 ($SO_2$), 1535 ($NO_2$) $cm^{-1}$.

Found: C 57.82, H 5.85, N 3.88, S 2.84%.
Calcd. for $C_{61}H_{63}N_5O_{23}S$: C 58.22, H 5.84, N 3.99, S 3.05%.

3,2′-*N*-Bis(benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-3′-deoxy-5-*O*-[2,3-*O*-bis(*p*-nitrobenzoyl)-5-*O*-tosyl-β-D-xylofuranosyl]paromamine (**7**)

A solution of **6** (453 mg) in a mixture of dioxane (5.4 ml), acetic acid (8.1 ml) and water (2.7 ml) was heated at 60°C for 10 hours. Concentration of the solution gave a residue. The chloroform solution of the residue was washed with aqueous sodium hydrogencarbonate, with water, dried ($Na_2SO_4$) and concentrated. The residue, which contained three components of Rf 0.58 (**6**, slight), 0.39 (slight) and 0.36 (**7**) with $CHCl_3$ - methanol (20 : 1), was chromatographed with chloroform - ethanol (50 : 1→30 : 1) to give a solid of **7**, 285 mg (68%), $[\alpha]_D^{25}+20°$ (*c* 0.5, $CHCl_3$).

Found: C 55.49, H 4.82, N 5.65, S 2.62%.
Calcd. for $C_{55}H_{55}N_5O_{23}S$: C 55.69, H 4.67, N 5.90, S 2.70%.

3,2′-*N*-Bis(benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-3′-deoxy-5-*O*-[2,3-*O*-bis(*p*-nitrobenzoyl)-5-*O*-tosyl-β-D-xylofuranosyl]-6′-*O*-tosylparomamine (**8**).

A solution of **7** (264 mg) and *p*-toluenesulfonyl chloride (170 mg) in pyridine (6.5 ml) was kept at −10°C for 25 hours. Work-up in the usual manner gave a solid, which was chromatographed with chloroform - ethanol (50 : 1) to give a solid of **8** (Rf 0.59 with $CHCl_3$ - MeOH, 15 : 1; **7**: Rf 0.38), 233 mg (78%), $[\alpha]_D^{25}+49°$ (*c* 1, $CHCl_3$). PMR ($CDCl_3$): $\delta$ 2.33 and 2.35 (each 3H s, Ts ($CH_3$)).

Found: C 55.30, H 4.66, N 5.07, S 5.02%.
Calcd. for $C_{62}H_{61}N_5O_{25}S_2$: C 55.56, H 4.59, N 5.23, S 4.78%.

6′-Azido-3,2′-*N*-bis(benzyloxycarbonyl)-1-*N*:6-*O*-carbonyl-3′,6′-dideoxy-5-*O*-[5-azido-5-deoxy-2,3-*O*-bis(*p*-nitrobenzoyl)-β-D-xylofuranosyl]paromamine (**9**).

To a solution of **8** (230 mg) in DMF (4.6 ml), lithium azide (170 mg) was added and the solution was kept at 60°C for 20 hours. After addition of chloroform (50 ml) ,the organic solution was washed with saturated sodium chloride solution, then with water, dried ($Na_2SO_4$), and concentrated to give a residue. Chromatography with chloroform - ethanol (40 : 1) gave a solid of **9** (Rf 0.40 with $CHCl_3$ - MeOH, 20 : 1; **8**: Rf 0.45), 125 mg (67%), $[\alpha]_D^{25}+65°$ (*c* 0.5, $CHCl_3$). IR (KBr): 2100 $cm^{-1}$ ($N_3$); absorption peaks at 1180 and 1350 $cm^{-1}$ disappeared.

Found: C 53.15, H 4.53, N 14.06%.
Calcd. for $C_{48}H_{47}N_{11}O_{19}$: C 53.28, H 4.38, N 14.24%.

6′-Azido-3,2′-*N*-bis(benzyloxycarbonyl)-1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-3′,6′-dideoxy-5-*O*-(5-azido-5-deoxy-β-D-xylofuranosyl)-paromamine (**10**).

To a hot solution (60°C) of **9** (112 mg) in dioxane (5.6 ml), 0.05 M barium hydroxide solution was added with stirring (at first 2.25 ml, 1.7 ml after 30 minutes, and 0.75 ml after additional 15 minutes) and the mixture was stirred for 1 hour at the temperature. After introduction of carbon dioxide, the mixture was concentrated and the residue was extracted with dioxane. The solution showed, on TLC with chloroform - ethanol (2 : 1), a single spot (Rf 0.45) (**9**: Rf 0.9). The solution was concentrated and the residue was dissolved in tetrahydrofuran (1.2 ml) containing triethylamine (4 drops). To the ice-cold solution, the active ester[9] (65.6 mg) was added and the solution was kept at room temperature overnight. The solution was concentrated and the residue was chromatographed with chloroform - ethanol (10 : 1) to give a solid of **10** (Rf 0.53 with $CHCl_3$ - EtOH, 7 : 1). The solid was reprecipitated from chloroform - *n*-hexane, 75 mg (73%), $[\alpha]_D^{25}+21°$ (*c* 0.4, $CHCl_3$). It gave slightly deviated analysis (C: 1.5~2.5%) from the theoretical, caused by the occlusion of chloroform in the solid.

5-*O*-(5-Amino-5-deoxy-β-D-xylofuranosyl)-1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]-3′-deoxyneamine (**11**).

A solution of **10** (61.6 mg) in a mixture of dioxane (1.2 ml), water (0.8 ml) and acetic acid (0.1 ml) was hydrogenated with palladium black under atmospheric pressure of hydrogen. After 2.5 hour, the solution was filtered and concentrated. The residue was chromatographed on a column of CM-Sepha-

dex C-25 ($NH_4$ form) with, after washing with water, aqueous ammonia (0.3→0.45 M) to give a solid of **11** as dicarbonate, 18.7 mg (46%), Rf 0.15 (TLC with $CHCl_3$ - MeOH - 17% $NH_4OH$, 1 : 4 : 3) (3′-deoxybutirosin A[6] Rf 0.24), $[\alpha]_D^{25}+20°$ (*c* 0.2, $H_2O$). IR (KBr): 1570, 1650 $cm^{-1}$. PMR ($D_2O$, at pH 1): $\delta$ 5.47 [1H s, H-1″ ($\beta$-anomer)], 5.81 (1H d, $J_{1',2'}=3.5$ Hz, H-1′).

Found: C 42.11, H 6.76, N 12.60%.
Calcd. for $C_{21}H_{42}N_6O_{10}\cdot 2H_2CO_3$: C 41.69, H 7.00, N 12.68%.

Acknowledgement

The authors wish to express their deep thanks to Prof. HAMAO UMEZAWA, Institute of Microbial Chemistry, for his support and encouragement.

## References

1) CULBERTSON, T. P.; D. R. WATSON & T. H. HASKELL: 5″-Amino-5″-deoxybutirosin, a new semi-synthetic aminoglycoside antibiotic. J. Antibiotics 26: 790～793, 1973
2) UMEZAWA, H.: Biochemical mechanisms of resistance to aminolgycosidic antibiotics. Advan. Carbohyd. Chem. Biochem. 30: 183～225, 1974
UMEZAWA, S.: Structures and syntheses of aminoglycoside antibiotics. Advan. Carbohyd. Chem. Biochem. 30: 111～182, 1974
3) WOO, P. W. K.: 5″-Amino-3′,4′,5″-trideoxybutirosin A, a new semi synthetic aminoglycoside antibiotic. J. Antibiotics 28: 522～529, 1975
4) SAEKI, H.; Y. SHIMADA, E. OHKI & S. SUGAWARA: Synthesis of aminotrideoxybutirosin A, a chemically modified antibiotic active against butirosin-resistant bacteria. J. Antibiotics 28: 530～536, 1975
5) For instance, see IKEDA, D.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 3′-deoxyribostamycin. J. Antibiotics 26: 799～801, 1973
6) WATANABE, I.; T. TSUCHIYA & S. UMEZAWA: Improved syntheses of 3′-deoxybutirosin A and B. Bull. Chem. Soc. Jap. 50: 972～974, 1977
7) LEVENE, P. A. & A. L. RAYMOND: Derivatives of monoacetone xylose. J. Biol. Chem. 102: 317～330, 1933
8) OGAWA, S.; H. FUJIMORI & T. SUAMI: Synthesis of 3,4-di-*O*-acetyl-6-azido-2,6-dideoxy-2-(2′-,4′-dinitroanilino)-α-D-glucopyranosyl chloride. Bull. Chem. Soc. Jap. 49: 2585～2586, 1976
9) KAWAGUCHI, H.; T. NAITO, S. NAKAGAWA & K. FUJISAWA: BB-K8, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 25: 695～708, 1972
10) HANESSIAN, S.: Reductive rearrangement of 5-azido-5-deoxy-pentoses. A new synthesis of sugars containing nitrogen in the ring. Chem. & Ind. 1965: 1296～1297, 1965