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## Note

# An improved synthesis of 3′,4′-dideoxykanamycin B*

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3′,4′-Dideoxykanamycin B (dibekacin) was prepared in ~50% net yield from kanamycin B by sequential 4″,6″-selective protection, *N*,*O*-benzylsulfonylation, 3′,4′-unsaturation, and hydrogenation. The benzylsulfonyl group was introduced both as an amino protective group and as the 3′,4′-di-*O*-sulfonyl group. Removal of the benzylsulfonyl group by metal in liquid ammonia was optimized.

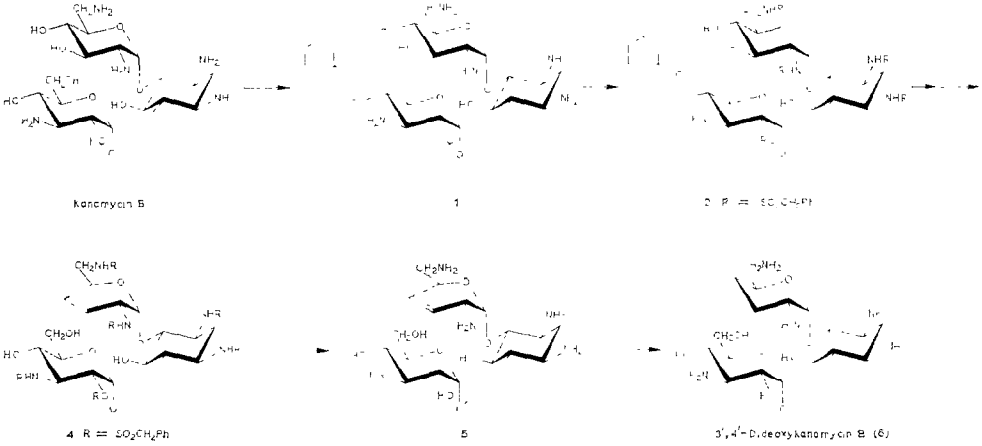
Dibekacin (**6**) was designed on the basis of the enzymic mechanism of resistance to aminoglycoside antibiotics and first synthesized by Umezawa *et al.*[1] in 1972. Because of its remarkable antimicrobial activities against both sensitive and resistant bacteria, including *Pseudomonas aeruginosa*, **6** is one of the most clinically useful semi-synthetic aminoglycosides at present. Such clinical importance of Dibekacin (**6**) prompted us to exploit an improved synthesis for the industrial-scale production of **6**. We now report synthesis of **6** by a shorter and more economical sequence of reactions, also giving higher overall yields, than the method reported previously[2].

## RESULTS AND DISCUSSION

In the present synthesis, 4″,6″-*O*-cyclohexylidenekanamycin B (**1**) was readily prepared from kanamycin B without isolation of kanamycin B 1,3,2′,6′,3″-penta-*p*-toluenesulfonate[3]. The crude **1** was purified by column chromatography on Amberlite CG-50 ($NH_4^+$) to give 4″,6″-*O*-cyclohexylidenekanamycin B (**1**) in 82% yield. Umezawa *et al.*[4] reported that treatment of 3′,4′,2″-tri-*O*-benzylsulfonyl-4″,6″-*O*-cyclohexylidene-1,3,2′,6′,3″-penta-*N*-*p*-tolylsulfonylkanamycin B with sodium iodide

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

afforded the 3′,4′-unsaturated derivative in good yield. Based on these findings, we evaluated the benzylsulfonyl group also as amino protective group. Treatment of **1** with an excess of benzylsulfonyl chloride in pyridine afforded **2** in only poor yield. When **1** was treated with benzylsulfonyl chloride in pyridine in the presence of 4-*N*,*N*-dimethylaminopyridine for 30 min at between −5 and 0°, 1,3,2′,6′,3″-penta-*N*-benzylsulfonyl-3′,4′,2″-tri-*O*-benzylsulfonyl-4″,6″-*O*-cyclohexylidene-kanamycin B



(**2**) was obtained in quantitative yield. Treatment of **2** with sodium iodide in *N*,*N*-dimethylformamide (DMF) readily gave the 3′,4′-unsaturated derivative (**3**) in 97% yield. The $^1$H-n.m.r. spectrum of **3** showed a characteristic signal at $\delta$ 5.45 assigned to 3′,4′-alkenic protons[2]. Hydrolysis of **3** in 80% aqueous acetic acid gave **4**. Removal of the remaining *N*- and *O*-benzylsulfonyl groups of **4** was next studied. Several examples of the reductive cleavage of benzyl[5], benzyloxycarbonyl[4], and tosyl[6] protective groups for amine, alcohol, and thiol groups, using metal in liquid ammonia, are reported in carbohydrate chemistry. Umezawa *et al.*[3] reported reductive cleavage of the sulfonamide and sulfonate groups of 3′,4′-dideoxy-2″-*O*-(methylsulfonyl)-1,3,2′,6′,3″-penta-*N*-tosylkanamycin B sodium in liquid ammonia–ethylamine. We likewise achieved reductive desulfonylation of 1,3,2′,6′,3″-penta-*N*-benzylsulfonyl 2″-*O*-benzylsulfonyl-3′,4′-dideoxy-kanamycin B-3′-ene (**4**) under Birch-reduction conditions, affording **5** in good yield. Reductive cleavage of the *N*,*O*-benzylsulfonyl derivative **4** was readily effected by using sodium or lithium below −45°, and the yield of **5** was dependent to the amount of metal added to the mixture (Tables I and II). When the reaction was performed in liquid ammonia in the absence of ethanol, **5** was obtained in only low yield. With lithium, 44 molar equivalents of metal was needed to obtain the maximal yield of **5**, but the yield of **5** was still lower than that when sodium was used. The maximum yield (84%) was obtained when 33 molar equivalents of sodium was added between −65 and −60°.

TABLE I

EFFECT OF VARYING THE CONCENTRATION OF SODIUM ON THE REDUCTIVE DESULFONYLATION OF COMPOUND **4**[a]

| *Entry* | *Sodium/**4*** *(Molar equiv. ratio)* | *Reaction temperature (°C)* | *Yield of **5*** *(%)* |
|---|---|---|---|
| 1 | 21 | −70 to −65 | 20[b] |
| 2 | 22 | | 54 |
| 3 | 25 | | 61 |
| 4 | 27 | | 65 |
| 5 | 30 | | 66 |
| 6 | 33 | | 67 |
| 7 | 30 | −60 to −65 | 75 |
| 8 | 33 | | 84 |
| 9 | 36 | | 65 |
| 10 | 30 | −50 to −45 | 59 |
| 11 | 33 | | 76 |
| 12 | 36 | | 71 |

[a]All reactions were performed in liquid ammonia (25 mL for 1 g of **4**) containing ethanol (2.5 mL for 1 g of **4**) as a proton source. [b]No ethanol was added in this reaction.

TABLE II

EFFECT OF VARYING THE CONCENTRATION OF LITHIUM ON THE REDUCTIVE DESULFONYLATION OF **4**

| *Entry* | *Lithium/**4*** *(Molar equiv. ratio)* | *Reaction temperature (°C)* | *Yield of **5*** *(%)* |
|---|---|---|---|
| 1 | 36 | −65 to −60 | 57 |
| 2 | 40 | | 69 |
| 3 | 44 | | 68 |
| 4 | 48 | | 60 |
| 5 | 40 | −70 to −65 | 66 |
| 6 | 44 | | 76 |
| 7 | 48 | | 61 |

All reactions were performed in liquid ammonia (25 mL for 1 g of **4**).

Catalytic hydrogenation of **5** gave 3′,4′-dideoxykanamycin B (**6**) in 98% yield. The physical constants, i.r. and $^1$H-n.m.r. spectral data, and antimicrobial activity of **6** were identical with those of authentic Dibekacin. The overall yield of **6** from kanamycin B was >50%.

## EXPERIMENTAL

*General methods.* — Melting points were determined in capillary tubes in a liquid

bath and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 241 polarimeter. I.r. spectra were recorded for potassium bromide disks with a Jasco IR-G instrument. $^1$H-N.m.r. spectra were recorded at 100 MHz with a Varian XL-100, and at 80 MHz with an FT-80A spectrometer. Thin-layer chromatography was performed on Merck silica gel plates No. 5714.

*4″,6″-O-Cyclohexylidenekanamycin B* (**1**). — To a suspension of kanamycin B (4.84 g, 10 mmol) in dry DMF (48 mL) was added anhydrous *p*-toluenesulfonic acid (10.2 g)*, and the mixture was stirred for 2 h at room temperature. 1,1-Dimethoxycyclohexane (20.2 mL) was added and the resulting solution was kept overnight at room temperature. The solution was then made neutral with triethylamine and evaporated to a syrup that was dissolved in water (500 mL). The solution was charged onto a column of Amberlite CG-50 (NHM, 60 mL) which was washed with water (120 mL) and the product was developed with 0.25M aqueous ammonia. The eluate containing **1** was evaporated to give a colorless solid (4.59 g, 82%), m.p. 145° (dec), $[\alpha]_D^{25}$ +120° (*c* 1.0, water); $^1$H-n.m.r. ($D_2O$): $\delta$ 1.0–2.2 (12H, H-2 and cyclohexylidene).

*Anal.* Calc. for $C_{24}H_{45}N_5O_{10} \cdot 0.5\ H_2O$: C, 50.33; H, 8.09; N, 12.23. Found: C, 50.40; H, 8.31; N, 12.20.

*1,3,2′,6′,3″-Penta-*N*-benzylsulfonyl-3′,4′,2″-tri-*O*-benzylsulfonyl-4″,6″-*O*-cyclohexylidene kanamycin B* (**2**). — To a solution of **1** (2.81 g, 5 mmol) in dry pyridine (100 mL), were added benzylsulfonyl chloride (9.88 g, 52 mmol) and 4-*N*,*N*-dimethylaminopyridine (8.22 g, 67 mmol). The mixture was stirred for 30 min at −5 to 0°. Water (2 mL) was added and the solution was kept for 1 h between −5 and 0°, and then the solution was evaporated. The resulting syrup was dissolved in chloroform (80 mL) and the solution washed with water (50 mL, twice), 10% aqueous sodium hydrogencarbonate solution (50 mL), and water (50 mL, twice). The organic layer was evaporated to give a solid that was reprecipitated from methanol–water to afford **2** as a colorless powder (9.03 g, quantitative), m.p. 157 °C (dec), $[\alpha]_D^{25}$ +28.1° (*c* 1.0, chloroform).

*Anal.* Calc. for $C_{80}H_{93}N_5O_{26}S_8$: C, 53.46; H, 5.22; N, 3.92; S, 14.27. Found: C, 52.93; H, 5.18; N, 3.88; S, 14.52.

*1,3,2′,6′,3″-Penta-*N*-benzylsulfonyl-2″-*O*-benzylsulfonyl-4″,6″-*O*-cyclohexylidene-3′,4′-dideoxykanamycin B-3′-ene* (**3**). — A mixture of **2** (7 g) and sodium iodide (70 g) in dry DMF (105 mL) was heated for 30 min at 100°. The resulting solution was shaken with chloroform (105 mL) and water (210 mL), and the chloroform layer was washed with 10% aqueous sodium thiosulfate (100 mL, twice) and water (100 mL), and dried over anhydrous sodium sulfate. The chloroform solution was evaporated to a pale-yellow solid that was chromatographed on a column of silica gel with 50:1 chloroform–methanol to give **3** (5.3 g, 97%), m.p. 143° (dec.); $[\alpha]_D^{25}$ +12.4° (*c* 1.0,

*5.5 Equiv. of PTSA was required to dissolve kanamycin B in DMF and give 4″,6″-*O*-cyclohexylidene kanamycin B successively.

chloroform); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 5.45 (s, 2H, H-3′ and 4′), 1.1–1.9 (12H, H-2 and cyclohexylidene), and 4.12 (12H, $SO_2CH_2C_6H_5$).

*Anal.* Calc. for $C_{66}H_{79}N_5O_{20}S_6$: C, 54.49; H, 5.47; N, 4.81; S, 13.22. Found: C, 54.02; H, 5.32; N, 4.44; S, 13.55.

*1,3,2′,6′,3″-Penta-*N*-benzylsulfonyl-2″-*O*-benzylsulfonyl-3′,4′-dideoxykanamycin B-3′-ene* (**4**). — Water (6.6 mL) was added to a solution of **3** (2.2 g) in acetic acid (22 mL), and the mixture was stirred for 30 min at 90°. The mixture was evaporated to a syrup and the residue was dissolved in ethyl acetate (30 mL). The solution was washed with water (20 mL), 5% aqueous sodium hydrogencarbonate (20 mL, twice), and water (20 mL, twice). The organic layer was evaporated to give a solid (2.0 g). The solid (1 g) was chromatographed on silica gel with 50:1 chloroform–methanol to give **5** as a colorless solid (600 mg), m.p. 140° (dec), $[\alpha]_D^{25}$ +6° (*c* 1.0, chloroform); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 4.15 (12H, $SO_2CH_2C_6H_5$) and 5.42 (s, 2H, H-3′ and 4′).

*Anal.* Calc. for $C_{60}H_{71}N_5O_{20}S_6$: C, 52.42; H, 5.20; N, 5.09; S, 13.99. Found: C, 52.22; H, 5.00; N, 4.77; S, 14.66.

*3′,4′-Dideoxykanamycin B-3′-ene* (**5**). — To a solution of **4** (1 g, 0.73 mmol) in liquid ammonia containing ethanol (2.5 mL) was added fresh sodium (0.4 g, 24 mmol) in four portions during 30 min at a temperature between −60 and −55°. The solution was kept for 10 min between −60 and −55°, and ammonia was then evaporated off by gradual warming to room temperature. Water (50 mL) was added to the resulting residue, and the solution made neutral with 6M hydrochloric acid. The solution was charged onto a column of Amberlite CG-50 ($NH_4^+$, 40 mL) resin, which was washed with water (80 mL), and 0.1M ammonium hydroxide (80 mL), and developed with 0.3M ammonium hydroxide. The eluate containing **5** was evaporated to give a colorless solid (274 mg, 84%), $[\alpha]_D^{25}$ +49° (*c* 1.0, water) (lit.[2] $[\alpha]_D$ +48.8°).

The product was confirmed to be identical with an authentic sample[2] of 3′,4′-dideoxykanamycin B-3′-ene in all respects.

*3′,4′-Dideoxykanamycin B* (**6**). — Compound **5** (450 mg) in water (10 mL), was hydrogenated in the presence of platinum oxide (30 mg) under atmospheric pressure overnight. The catalyst was removed and the solution was charged onto a column of Amberlite CG-50 ($NH_4^+$, 12 mL). The column was washed with water and then eluted with 0.3M ammonium hydroxide to give **6** as a colorless solid (443 mg, 98%). The product was identical with an authentic sample[2] of 3′,4′-dideoxykanamycin B in all respects, including biological activity.

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