

## A SYNTHESIS OF DIHYDROSTREPTOMYCIN

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A key protected streptidine derivative (**3**) useful for the synthesis of antibiotics of streptomycin series was prepared by hydrolysis of an acylated dihydrostreptomycin (DHSM) derivative (**2**), and it was condensed with a protected dihydrostreptobiosaminy chloride (**5**) to give two condensation products (**6** and **7**). By deblocking, **6** was led to DHSM and **7** to a biologically inactive isomer (**8**) of DHSM. From the PMR spectrum of 4-*O*-mesyl derivative (**4**) of **3**, the benzyloxycarbonyl and acetyl groups were concluded to be attached to the end nitrogens of the guanidine groups.

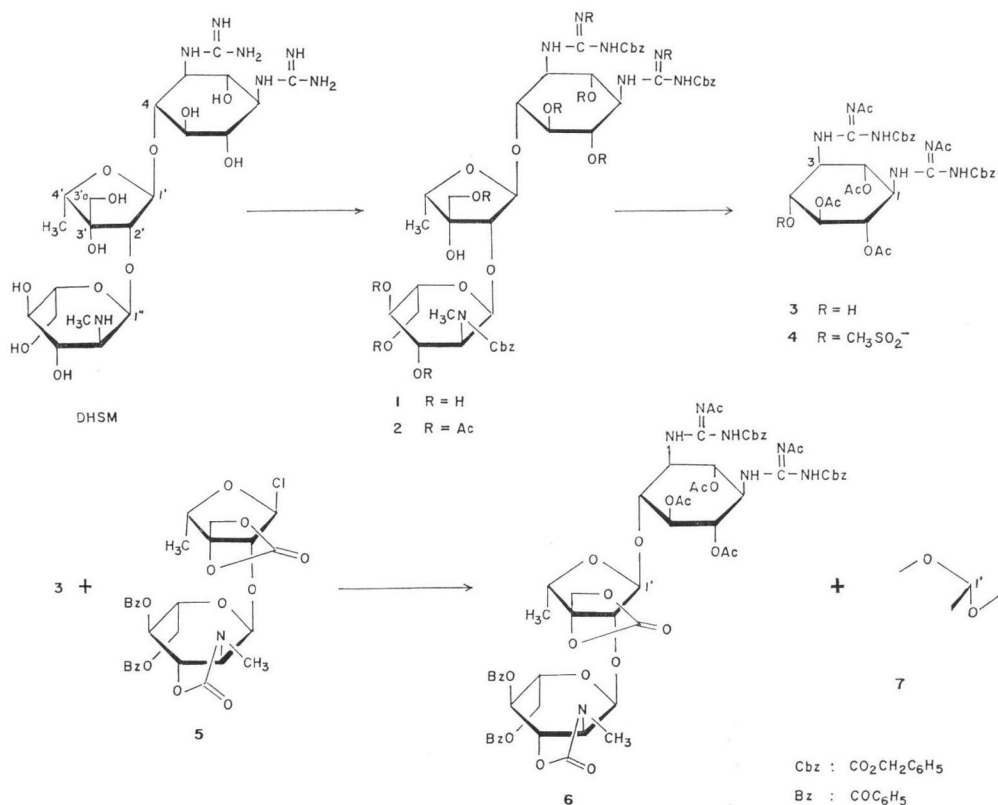
Streptomycin is the first useful streptomyces antibiotic discovered by WAKSMAN<sup>1)</sup> in 1944. Dihydrostreptomycin (DHSM) is produced chemically<sup>2,3)</sup> from streptomycin or by fermentation.<sup>4)</sup> The structure of streptomycin was established<sup>5)</sup> by 1948 except for the glycosidic linkage between streptose and streptidine, which was finally decided to be  $\alpha$ -L<sup>6,7)</sup> in 1965. Total synthesis of DHSM had been accomplished by us<sup>8~11)</sup> in 1974 by a sequence of reactions which comprises synthesis of a protected dihydrostreptobiosaminy chloride and its condensation with a protected streptidine. DHSM was converted to streptomycin by oxidation<sup>12)</sup>, thus completing the total synthesis of streptomycin. Recently, PAULSEN *et al.*<sup>13~15)</sup> reported the synthesis of streptobiosamine. In this paper we describe another synthesis of DHSM.

In our previous synthesis of DHSM a racemate of protected streptidine<sup>10)</sup> was used. In this paper we describe the preparation of an optically active streptidine derivative (**3**) by hydrolysis of a protected DHSM and regiospecific condensation of **3** with a dihydrostreptobiosamine derivative to give dihydrostreptomycin.

Treatment of dihydrostreptomycin with excess benzyl chloroformate and sodium hydroxide in aqueous acetone-chloroform gave a mixture of perbenzyloxycarbonyl products. However, partial deprotection with sodium hydroxide in aqueous dioxane successfully gave **1**, 3, 2''-tris (*N*-benzyloxycarbonyl) dihydrostreptomycin (**1**) in 75% yield from DHSM. The neutral product was negative for diacetyl reagent, indicating that both of the guanidine groups are protected. Acetylation of **1** with acetic anhydride in pyridine gave nonaacetyl derivative (**2**) in 78% yield. Since the tertiary hydroxyl group at C-3 in the dihydrostreptose moiety is expected not to be acetylated, the 9 acetyl groups introduced can be assigned to be attached to the other 7 hydroxyl and the 2 guanidine groups. Hydrolysis of **2** with hydrochloric acid in acetic acid successfully gave optically active **1**, 3-di-*N*-acetyl-2, 5, 6-tri-*O*-acetyl-1, 3-bis (*N*-benzyloxycarbonyl)streptidine (**3**), a key intermediate for the synthesis of antibiotics of streptomycin series, in 68% yield.

To confirm the structure, **3** was mesylated to give optically active 4-*O*-mesyl derivative (**4**). Inspection of its PMR spectrum confirmed the positions of the benzyloxycarbonyl and the acetyl groups attached to the guanidine groups. On irradiation at  $\delta$  5.0 (1- and 3-CH) (when measured in DMSO-*d*<sub>6</sub>), 2 doublets at  $\delta$  9.0 and 9.15 assignable to NH of guanidine groups (the separation of the shift-values indi-

Scheme 1.



cates non-equivalence of the 2 guanidine groups) collapsed to singlets, respectively. This indicates the presence of vicinal protons of  $-\underline{\text{H}}\text{C}-\underline{\text{N}}\text{H}$  at C<sub>1</sub> and C<sub>3</sub>, and that the benzyloxycarbonyl and acetyl groups are attached to end-nitrogens in the guanidine groups. This conclusion is in accord with that reported<sup>14)</sup> by PAULSEN *et al.* for heptaacetyl streptidine.

Condensation of **3** with 2-*O*-[4, 6-di-*O*-benzoyl-2-*N*:3-*O*-carbonyl-2-deoxy-2-(methylamino)- $\alpha$ -*L*-glucopyranosyl]-3, 3a-*O*-carbonyldihydrostreptosyl chloride<sup>11)</sup> (**5**) in dry benzene in the presence of silver carbonate and silver perchlorate in a similar manner as reported<sup>11)</sup> gave 2 major condensation products (**6** and **7**), which were separated by column chromatography. The product **6** of faster mobility was obtained in 16% yield based on **3** and removal of its protecting groups gave DHSM. The optical rotation, PMR, IR and antibacterial spectrum were in accord with those from natural origin. The product **7** of slower mobility was obtained in 12% yield based on **3** and deblocking gave an isomer (**8**) of DHSM, which had no antibacterial activity. The linkage between dihydrostreptose and streptidine in **8** is presumed to be  $\beta$ -*L* from the regiospecific condensation, though the coupling constant  $J_{1,2}$  in the dihydrostreptosyl portion was not clearcut in the PMR spectrum.

### Experimental

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

1, 3, 2''-Tris(*N*-benzyloxycarbonyl)dihydrostreptomycin (1)

To a cold (ice-water) suspension of dihydrostreptomycin sesquisulfate (18.3 g) in aqueous acetone (1: 1,500 ml), benzyl chloroformate (15.4 g) and 2M aqueous sodium hydroxide (90 ml) were added and the mixture was stirred for 30 minutes. Chloroform (100 ml), acetone (100 ml), benzyl chloroformate (42.3 g) and 2 M aqueous sodium hydroxide (125 ml) were added and the mixture was stirred at room temperature overnight. The upper layer which separated after standing for a while was washed with chloroform (100 ml  $\times$  5). The combined lower layer and the chloroform extracts were washed with water, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to give a syrup. The syrup showed, on TLC with  $\text{CHCl}_3$  - EtOH - 17%  $\text{NH}_4\text{OH}$  (20: 10: 1), a mixture of spots larger than Rf 0.9. To a solution of the syrup in dioxane (500 ml), 2 M sodium hydroxide (50 ml  $\times$  3) was added at intervals of 24 hours and the solution was kept at room temperature. The solution showed, on TLC, a single spot (Rf 0.9) after 24 hours, two spots (Rf 0.9 and 0.3) after 48 hours and finally a single spot of Rf 0.3 (1). Addition of acetic acid ( $\sim$  10 ml) followed by concentration of the solution gave a syrup, which was triturated with ether. The syrup was chromatographed on a column of silica gel (600 g) with  $\text{CHCl}_3$  - EtOH - 17%  $\text{NH}_4\text{OH}$  (10: 10: 1) and the fractions containing 1 were concentrated to give a solid, 18.3 g (75%), mp\* 159~160°C (decomp.),  $[\alpha]_D^{25}$  -35° (c 1, EtOH). IR (KBr): 1640  $\text{cm}^{-1}$  (broad). PMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  1.28 (3H d, 5'- $\text{CH}_3$ ), 3.10 (3H s,  $\text{NCH}_3$ ), 7.48 (15H s, phenyl).

Found\*: C 53.24, H 6.02, N 9.54%. Calcd. for  $\text{C}_{45}\text{H}_{59}\text{N}_7\text{O}_{18} \cdot 1.5\text{H}_2\text{O}$ : C 53.35, H 6.16, N 9.68%.

1, 3-Di-*N*-acetyl-2, 5, 6, 3'a, 3'', 4'', 6''-hepta-*O*-acetyl-1, 3, 2''-tris(*N*-benzyloxycarbonyl)dihydrostreptomycin (2)

To a solution of 1 (5.00 g) in pyridine (100 ml), acetic anhydride (50 ml) was added and the solution was kept at 60°C for 4 hours. After gradual addition of water (9 ml) with ice-cooling followed by standing the solution at room temperature for 1 hour, the solution was poured into a large volume of ice-water. Resulting precipitate was filtered and washed with water. Purification by short column chromatography with  $\text{C}_6\text{H}_6$ -EtOAc (10: 1) gave a solid of 2, 5.40 g (78%), mp 111~113°C,  $[\alpha]_D^{25}$  -65° (c 1,  $\text{CHCl}_3$ ). IR (KBr): 1235, 1625 (sh), 1650, 1715 (w), 1760  $\text{cm}^{-1}$ . PMR ( $\text{CDCl}_3$ ):  $\delta$  1.15 (3H d, 5'- $\text{CH}_3$ ); 1.89 (3H), 1.95 (6H), 2.01 (3H), 2.06 (3H), 2.11 (3H), 2.19 (6H) and 2.21 (3H) (each s, Ac); 2.79 (3H s,  $\text{NCH}_3$ ).

Found: C 55.44, H 5.78, N 7.27%. Calcd. for  $\text{C}_{63}\text{H}_{77}\text{N}_7\text{O}_{27}$ : C 55.46, H 5.69, N 7.19%.

1, 3-Di-*N*-acetyl-2, 5, 6-tri-*O*-acetyl-bis(*N*-benzyloxycarbonyl)streptidine (3)

To a suspension of 2 (680 mg) in glacial acetic acid (3 ml), 2 M hydrochloric acid in glacial acetic acid (1 ml) was added and the resulting solution was kept at room temperature overnight. The solution showed, on TLC with  $\text{C}_6\text{H}_6$  -  $\text{CHCl}_3$  - EtOH - 17%  $\text{NH}_4\text{OH}$  (50: 50: 4: 0.5), spots of Rf 0.3 (minor, 2), 0.23 (major, 3), 0.13 (major, acetylated dihydrostreptobiosamine derivative?), 0.08 (minor) and 0.04 (minor). Anhydrous sodium acetate (0.3 g) was added and the mixture was concentrated by addition of toluene. The residue was chromatographed on silica gel (40 g) with  $\text{C}_6\text{H}_6$  -  $\text{CHCl}_3$  - EtOH - 17%  $\text{NH}_4\text{OH}$  (50: 50: 4: 0.5) to give a solid of 3, 254 mg (68%), mp 113~116°C,  $[\alpha]_D^{25}$  +27° (c 1,  $\text{CHCl}_3$ ). IR (KBr): 1230, 1650, 1715 (w), 1760 (sharp)  $\text{cm}^{-1}$ . PMR ( $\text{CDCl}_3$ ):  $\delta$  1.91, 1.92, 2.05, 2.15, 2.16 (each 3H s, Ac); 7.37 (10H s, phenyl); 9.01, 9.18 (each 1H d,  $J \sim 8$  Hz, disappeared on deuteration).

Found: C 55.10, H 5.61, N 11.48%. Calcd. for  $\text{C}_{34}\text{H}_{40}\text{N}_6\text{O}_{18}$ : C 55.13, H 5.44, N 11.35%.

1, 3-Di-*N*-acetyl-2, 5, 6-tri-*O*-acetyl-bis(*N*-benzyloxycarbonyl)-4-*O*-mesylstreptidine (4)

To a cold (0°C) solution of 3 (148 mg) in pyridine (3 ml), methanesulfonyl chloride (0.1 ml) was added and the solution was kept at room temperature overnight. Addition of water (0.1 ml) followed by concentration of the solution gave a syrup. The chloroform solution of the syrup was washed successively with 0.4 M potassium hydrogensulfate, saturated aqueous sodium hydrogencarbonate and water, dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to give a syrup. Purification by short column chromatography with  $\text{C}_6\text{H}_6$  - EtOAc (1: 1) gave a solid of 4, 117 mg (72%), mp 191~193°C (decomp.),  $[\alpha]_D^{25}$  +11° (c 1,  $\text{CHCl}_3$ ). PMR( $\text{CD}_3$ )<sub>2</sub>SO):  $\delta$  1.80 (3H), 1.90 (3H), 2.00 (3H), and 2.20 (6H) (each s, Ac); 3.01 (3H s, Ms), 7.43 (10H, s, phenyl); 9.0 and 9.15 (each 1H d,  $J=9$  Hz; disappeared on deuteration, 1- and 3-NH). Irradiation at  $\delta$  5.0 the doublets at  $\delta$  9.0 and 9.15 collapsed to singlets, respectively. PMR

\* Samples for mp, optical rotation measurements and microanalysis were dried at 60°C *in vacuo* for 2 hours.

(pyridine- $d_5$ ):  $\delta$  1.92 (3H), 1.98 (3H), 2.01 (3H), 2.02 (3H), 2.14 (3H) (each s, Ac); 3.22 (3H s, Ms); 9.83 and 10.00 (each 1H d,  $J=9$  Hz; disappeared on deuteration, 1- and 3-NH). Irradiation at  $\delta$  5.9 the doublets at  $\delta$  9.83 and 10.00 collapsed to singlets, respectively.

Found: C 50.98, H 5.14, N 9.93, S 3.71%. Calcd. for  $C_{35}H_{42}N_6O_{15}S$ : C 51.34, H 5.17, N 10.26, S 3.92%.

#### Condensation of **3** and **5**

A mixture of **3** (150 mg), **5** (150 mg) and Molecular Sieves 3A (400 mg) in dry benzene (3 ml) was kept at room temperature overnight in order to remove humidity, if any. The solution showed, on TLC with  $CHCl_3$  - EtOH - 17%  $NH_4OH$  (50: 4: 0.5), two spots of **3** (Rf 0.47) and **5** (Rf 0.43). Freshly prepared and well dried silver carbonate (400 mg) and silver perchlorate (40 mg) were added and the mixture was stirred at 50°C for 45 hours. Chloroform (10 ml) was added, filtered, and the solution was concentrated to give a yellow-brown syrup. The syrup showed, on TLC with  $CHCl_3$  - EtOH - 17%  $NH_4OH$  (50: 4: 0.5), five spots of Rf 0.26 (minor), 0.29 (minor), 0.38 (**7**), 0.47 (**3**) and 0.52 (**6**). Separation of the products was carried out by silica gel (50 g) column chromatography with the above-described solvent system to give **6** (75~110 ml fractions, 75 mg, 28%), **3** (110~116 ml, 35 mg), a mixture of **3** and **7** (116~125 ml, 57 mg), and **7** containing slight impurity (125~155 ml, 75 mg). Further chromatography of **6** with  $C_6H_6$ -80% aqueous EtOH (25: 1) gave pure **6**, 43 mg (16%). A sample was reprecipitated from benzene - *n*-hexane, mp 150~152°C (decomp.),  $[\alpha]_D^{25} -40^\circ$  (c 1,  $CHCl_3$ ). IR(KBr): 1230 (acetyl ester), 1650, 1740 (sh), 1760, 1780 (sh, cyclic carbamate), 1820 (carbonate)  $cm^{-1}$ . PMR( $CDCl_3$ ):  $\delta$  1.28 (1H d,  $J=6.5$  Hz, 5'- $CH_3$ ); 1.90 (3H), 1.97 (3H), 2.07 (3H) and 2.20 (6H) (each s, Ac); 2.81 (3H s,  $NCH_3$ ), 7.43 (10H s,  $CO_2CH_2C_6H_5$ ); 7.35~7.8 (6H) and 8.0~8.25 (4H) (benzoyl).

Found: C 57.35, H 5.32, N 7.18%. Calcd. for  $C_{63}H_{67}N_7O_{25}$ : C 57.22, H 5.11, N 7.42%.

The solid from 116~125 ml fractions described above was rechromatographed with  $C_6H_6$  -  $CHCl_3$  - EtOH - 17%  $NH_4OH$  (50: 50: 4: 0.5) and the fractions containing mainly **7** were concentrated to give a solid (24 mg). The solid and the solid from 125~155 ml fractions were combined (99 mg) and again chromatographed with the above-described solvent system to give pure **7**, 31 mg (12%). A sample was reprecipitated from benzene-*n*-hexane, mp 152°C (decomp.),  $[\alpha]_D^{25} -38^\circ$  (c 1,  $CHCl_3$ ). IR(KBr): 1230, 1650, 1735, 1760, 1780 (sh), 1825  $cm^{-1}$ . PMR( $CDCl_3$ ):  $\delta$  1.30 (1H d, 5'- $CH_3$ ); 1.95 (3H), 1.99 (6H), and 2.20 (6H) (each s, Ac); 2.94 (3H s,  $NCH_3$ ), 7.35~7.7 (16H), 8.0~8.25 (4H).

Found: C 57.48, H 5.14, N 7.13%. Calcd. for  $C_{63}H_{67}N_7O_{25}$ : C 57.22, H 5.11, N 7.42%.

#### Deblocking of **6**

To a hot (60°C) solution of **6** (46 mg) in dioxane (5 ml), 0.05 M aqueous barium hydroxide (1 ml  $\times$  5) was added at 20-minute intervals and the solution was kept at the temperature. Ammonium carbonate (50 mg) was added and the resulting precipitate was filtered and washed with aqueous dioxane (1: 1). Concentration of the filtrate and the washings combined gave a solid of bis(*N*-benzyloxycarbonyl) dihydrostreptomycin, Rf 0.12 (TLC with  $CHCl_3$  - EtOH - 17%  $NH_4OH=20: 10: 1$ ). A solution of the solid in 50% aqueous dioxane (3 ml) was treated with palladium black under hydrogen (4 kg/ $cm^2$ ) at room temperature overnight. The reaction mixture was filtered and the filtrate was evaporated. The aqueous solution of the residue was neutralized to pH 7 with 1 M hydrochloric acid and the solution was chromatographed on a column of Amberlite CG50 ( $NH_4$  form) with 5% ammonium carbonate. The diacetyl-positive fractions were collected and concentrated with several additions of water to remove excess ammonium carbonate. An aqueous solution of the residue (7 mg) was passed through a column of Dowex 1  $\times$  2 (OH form, 2 ml) with water and the diacetyl-positive fractions were neutralized to pH 4 with 1 M hydrochloric acid. The solution was concentrated to give a solid of DHSM trihydrochloride, 7 mg (30%),  $[\alpha]_D^{25} -93^\circ$  (c 0.5,  $H_2O$ ) [natural origin DHSM  $\cdot 3HCl$ :  $[\alpha]_D^{25} -93^\circ$  (c 1,  $H_2O$ )]. The PMR spectrum of the solid [in  $D_2O$ ,  $\delta$  1.2 (3H, d,  $CCH_3$ ), 2.88 (3H s,  $NCH_3$ ), 5.30 (1H d,  $H-1'$ ), 5.53 (1H d,  $H-1''$ )] was superimposable with that of the natural trihydrochloride. Antibacterial spectrum: *Staphylococcus aureus* FDA 209P, 3.12 (natural, 3.12); *Bacillus agri*, >100 (>100); *Escherichia coli* K-12, 1.56 (0.78); *Pseudomonas aeruginosa* A3, 12.5 (12.5) mcg/ml.

#### Deblocking of **7**

A sample of **7** (36mg) was treated similarly as described for deblocking of **6** to give a solid of diacetyl-

positive **8** trihydrochloride, 7 mg (38%),  $R_{f_{DHSM}}$  1.0 (paper chromatogram with 1-BuOH - pyridine -  $H_2O$  - AcOH=6:4:3:1, descending),  $[\alpha]_D^{25} - 34^\circ$  (c 0.5,  $H_2O$ ). PMR( $D_2O$ ):  $\delta$  1.16 (3H d,  $CCH_3$ ), 2.89 (3H s,  $NCH_3$ ), 5.50 (1H d,  $J=4$  Hz,  $H-1'$ ).

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