Synthesis of Di-*N*-acetyl-di-*N*-benzyloxycarbonyl-*O*-cyclohexylidenestreptidine[1)]

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Reaction of streptidine with excess benzyloxycarbonyl chloride, followed by partial alkaline hydrolysis, gave di-*N*-benzyloxycarbonylstreptidine (**1**), which, by treatment with 3,4-dihydro-2*H*-pyran, gave a tetra-*O*-tetrahydropyranyl derivative (**3**). Acetylation of **3** then gave a di-*N*-acetyl derivative (**4**), which was, after removal of the tetrahydropyranyl groups, allowed to react with 1,1-dimethoxycyclohexane to furnish di-*N*-acetyl-di-*N*-benzyloxycarbonyl-*O*-cyclohexylidenestreptidine (**6**). This is the first preparation of a useful intermediate for the synthesis of streptidine glycosides

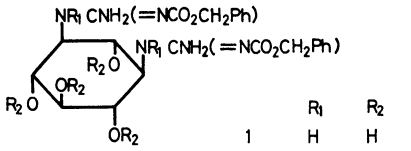
Streptidine[2)] is the aglycone component of streptomycin and related antibiotics. The gross structure of this strongly basic compound was degradatively established by Carter, Folkers, Wintersteiner, and their coworkers,[3)] and, its all-*trans* configuration was confirmed by the total synthesis by Wolfrom *et al.*[4)]

For the total synthesis of antibiotics of streptomycin series, glycosidation of streptidine is an inevitable process, however, it has been hindered by the extremely low solubility of streptidine and its ordinary derivatives in organic solvents. Thus, a suitable derivative of streptidine for the Koenigs-Knorr condensation was investigated. In the initial stage of our studies, we prepared *O*-cyclohexylidene-di-*N*-nitro-streptidine* from streptamine by the reaction with *S*-methyl-*N*-nitroisothiourea followed by cyclohexylidenation with 1,1-dimethoxycyclohexane, however, the derivative still showed limited solubility in organic solvents, giving poor yields of glycosides when condensed with some glycosyl halides such as tetra-*O*-benzyl-glucopyranosyl chloride. After some attempts, di-*N*-acetyl-di-*N*-benzyloxycarbonyl-*O*-cyclohexylidenestreptidine (**6**) was found to be useful for the glycosylation. The present paper deals with its synthesis.

Reaction of streptidine sulfate with excess benzyloxycarbonyl chloride and 2 M sodium hydroxide in aqueous dioxane gave a mixture of per-*N*,*O*-benzyloxycarbonylated products, which however gave the di-*N*-benzyloxycarbonylstreptidine (**1**) in a 51% yield on prolonged treatment with a mixture of 2 M sodium hydroxide in dioxane at room temperature. In the formula of **1**, the positions of the benzyloxycarbonyl groups attached to nitrogens remain to be established and thus arbitrarily assigned.

Attempts to prepare tetra-*N*-benzyloxycarbonylstreptidine were unsuccessful because the benzyloxycarbonyl groups further introduced to the guanidino groups were unusually unstable to be hydrolyzed together with *O*-benzyloxycarbonyl groups by weak alkaline treatment. Acetylation of **1** with acetic anhydride and pyridine gave the hexaacetyl derivative (**2**). Its structure was confirmed by the NMR spectrum except for the positions of acetyl and benzyloxycarbonyl groups attached to the nitrogens which had been arbitrarily assigned. All attempts to convert **2** into di-*N*-acetyl-di-*N*-benzyloxycarbonylstreptidine (**5**) by alkaline treatment were unsuccessful.

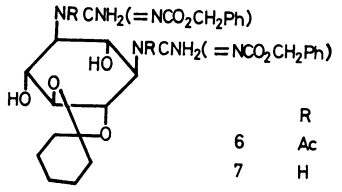
* Mp 195 °C (decomp.). The details will be published elsewhere.



	R_1	R_2
1	H	H
2	Ac	Ac
3	H	THP
4	Ac	THP
5	Ac	H

The following route successfully led to **5**. **1** was converted into the tetra-*O*-tetrahydropyranyl derivative (**3**) in a yield of 70% by the treatment with 3,4-dihydro-2*H*-pyran and *p*-toluenesulfonic acid in DMF. Acetylation of **3** with acetic anhydride and pyridine then gave the di-*N*-acetyl derivative (**4**) quantitatively. Removal of the tetrahydropyranyl groups by the treatment with 50% acetic acid at room temperature for 2.5 hr gave **5** in a yield of 56%. This yield was variable according to the reaction conditions and prolonged reaction lowered the yield.

Finally, treatment of the *N*-protected streptidine with 1,1-dimethoxycyclohexane in the presence of *p*-toluenesulfonic acid in DMF furnished the cyclohexylidene derivative (**6**) as a racemate in a yield of 70%.



	R
6	Ac
7	H

An alternative simplified route to **6** from di-*N*-benzyloxycarbonylstreptidine (**1**) involving the reaction sequence of cyclohexylidenation, per-*N*,*O*-acetylation, and selective removal of the *O*-acetyl group was unsuccessful owing to the difficulty of the last selective hydrolysis.

Compound **6** was successfully used for the synthesis of dihydrostreptomycin as described in the successive paper. In this stage we tested for deblocking; on treatment of **6** under weakly alkaline condition, de-*N*-acetylation proceeded smoothly to give **7**, whose benzyloxycarbonyl groups remained intact. Further treatment of **7** with 50% acetic acid followed by hydrogenolysis with palladium black gave streptidine. It should be noted that the first introduced benzyloxycar-

bonyl groups at the guanidino portions are relatively stable to weak alkali, while the next introduced *N*-acetyls are easily removed by weakly alkaline hydrolysis. We were pleased with this demonstration, because, if the *N*-acetyl groups are normally resistant to weak alkali, removal of the benzyloxycarbonyl groups would simultaneously occur and the guanidino groups would be further transformed into ureido groups.

Experimental

Thin layer (TLC) and column chromatography were carried out on silica gel of Silica Rider 5B (Daiichi Pure Chemicals Co., Ltd., Tokyo) and Wakogel C-200 (Wako Pure Chemicals Co., Ltd., Osaka) unless otherwise stated, respectively, and the spots on tlc were visualized with sulfuric acid.

Di-N-*benzyloxycarbonylstreptidine (**1**).* To a suspension of streptidine sulfate (10 g) in aqueous dioxane (2 : 1, 750 ml), 2M sodium hydroxide solution (28 ml) was added under stirring. The mixture was cooled in an ice bath and 2M sodium hydroxide solution (68 ml) and benzyl chloroformate (19 ml) were added all at once under vigorous stirring, and stirring was continued for 10 min under cooling. Another aliquot of 2M sodium hydroxide solution (68 ml) and benzyl chloroformate (19 ml) were added and stirring was continued for 10 min. After neutralization with hydrochloric acid, the reaction mixture was evaporated and the residue was extracted with dimethylformamide (DMF) (100 ml×2). Evaporation of the solvent gave a syrup. On tlc with benzene–methanol (10 : 1), the syrup gave at least seven spots ranging in R_f 0.05—0.9.

To a suspension of the syrup in dioxane (200 ml), 2M sodium hydroxide (17 ml×5) was added at intervals. After 120 hr, the mixture gave, on TLC with benzene–methanol (6 : 1), two spots of R_f 0.05 (**1**) and 0.56 (benzyl-alcohol). The mixture was neutralized with hydrochloric acid and evaporated to give a residue, which was extracted with DMF. The solution was concentrated and, to the concentrate, ether was added to give a yellow solid. The solid was chromatographed on a column of Amberlite CG 50 (NH_4 form) with dioxane–water–1M ammonia (1 : 3 : 0.2) as eluent. The fraction containing **1** was evaporated to give a colorless solid (7.55 g, 51%), mp 216—217 °C (decomp.), R_f 0.46 (tlc with chloroform–ethanol–17% NH_3 (20 : 10 : 1)); IR (KBr): 1530—1680 cm^{-1} (five peaks).

Found: C, 54.00; H, 5.70; N, 15.77%. Calcd for $C_{24}H_{30}N_6O_8$: C, 54.33; H, 5.70; N, 15.84%.

NMR (in DMSO-d_6; the sample was treated three times with D_2O): τ 7—6 (6H, skeleton protons), 4.95 (4H s, $C\underline{H}_2C_6H_5$), 2.6 (10H s, C_6H_5).

Di-N-*acetyl-tetra*-O-*acetyl-di*-N-*benzyloxycarbonylstreptidine (**2**).* To a suspension of **1** (150 mg) in pyridine (1.5 ml), acetic anhydride (0.43 ml) was added and the mixture was stirred at room temperature overnight. Two drops of water were added and the solution was evaporated. Addition of water gave a solid, which was washed throughly with water, 170 mg (77%), mp 210—211 °C; IR(KBr): 1550, 1635 (s), 1710 (w), 1760 cm^{-1}.

Found: C, 55.01; H, 5.45; N, 10.74%. Calcd for $C_{36}H_{42}N_6O_{14}$: C, 55.24; H, 5.41; N, 10.74%.

NMR (in $CDCl_3$): τ 8.07, 8.03, 7.96, 7.81 (3, 6, 3, 6H s respectively, Ac), 5.5—4.9 (2H, C$\underline{H}$NAc (?)), 4.75 (4H s, $C\underline{H}_2C_6H_5$), 4.85—4.45 (4H, C$\underline{H}$OAc (?)), 2.6—2.46 (10H m, C_6H_5).

Di-N-*benzyloxycarbonyl-tetra*-O-*tetrahydropyranylstreptidine (**3**).* A solution of **1** (720 mg), 3,4-dihydro-2*H*-pyran (1.83 ml) and fuse dried *p*-toluenesulfonic acid (600 mg) in DMF (9 ml) was heated at 50 °C overnight. Triethylamine (1.5 ml) was added and the solution was evaporated. A chloroform solution of the residue was washed with sodium hydrogen carbonate solution, and then with water, dried (Na_2SO_4) and concentrated. Petroleum ether was added to give a solid (980 mg), which was chromatographed in a short column of silica gel with chloroform–ethanol–17% ammonia (400 : 32 : 3)), affording a colorless solid, 830 mg (70%), mp 118—119.5 °C, R_f 0.46 (with chloroform–ethanol–17% ammonia (100 : 8 : 1)); IR (KBr): 1540—1660 cm^{-1}.

Found: C, 60.53; H, 7.10; N, 9.31%. Calcd for $C_{44}H_{62}N_6O_{12}$: C, 60.95; H, 7.21; N, 9.69%.

NMR (in $CDCl_3$): τ 8.8—8.1 (24H, $(-CH_2-)_3$ of THP), 6.8—5.8 (14H, $-OCH_2-$ of THP and skeleton protons), 5.4—4.9 (4H, -OCHO- of THP), 4.82 (4H s, $C\underline{H}_2C_6H_5$), 3.5—2.7 (4H, disappeared on deuteration), ~2.6 (10H, C_6H_5).

Di-N-*acetyl-di*-N-*benzyloxycarbonyl-tetra*-O-*tetrahydropyranylstreptidine (**4**).* To a solution of **3** (200 mg) in pyridine (2 ml), acetic anhydride (0.086 ml) was added and the solution was allowed to stand overnight. After addition of a drop of water, the solution was evaporated and the residue was poured into water to give a solid, 214 mg (98%), mp 82—84 °C, R_f 0.41 (tlc with benzene–acetone (8 : 1)); IR (KBr): 1570, 1645 (with fine slight shoulders), 1705(w) cm^{-1}.

Found: C, 60.61; H, 7.04; N, 8.64%. Calcd for $C_{48}H_{66}N_6O_{14}$: C, 60.62; H, 7.00; N, 8.84%.

NMR (in $CDCl_3$): τ 7.80 (6H s, NAc).

Di-N-*acetyl-di*-N-*benzyloxycarbonylstreptidine (**5**).* A solution of **4** (50 mg) in 50% aqueous acetic acid (0.5 ml) was allowed to stand for 2.5 hr. Sodium hydrogen carbonate (360 mg) was added with stirring and water (20 ml) was then added to give colorless solid (27 mg). The solid was chromatographed on a column of silica gel with ethyl acetate–methyl ethyl ketone (4 : 1), affording a solid, 18 mg (56%), mp 114—116 °C, R_f 0.26 (**4** : 0.9) (tlc with chloroform–ethanol (15 : 1)); IR (KBr): 1570, 1640, 1660, 1705(w) cm^{-1}.

Found: C, 55.04; H, 5.68; N, 13.33%. Calcd for $C_{28}H_{34}N_6O_{10}$: C, 54.72; H, 5.58; N, 13.67%.

NMR (in $CDCl_3$): τ 7.84 (6H s, NAc).

Di-N-*acetyl-di*-N-*benzyloxycarbonyl-4,5 (and 5,6)*-O-*cyclohexylidenestreptidine (**6**).* To a solution of **5** (1.50 g) in DMF (30 ml), 1,1-dimethoxycyclohexane (5 ml) and fuse dried *p*-toluenesulfonic acid (230 mg) were added and the solution was heated at 50 °C under reduced pressure (~30 Torr) for 3 hr. Triethylamine (0.5 ml) was added and the solution was evaporated and the residue was dissolved in chloroform. The solution was washed with sodium hydrogen carbonate solution, dried (Na_2SO_4) and evaporated. The residue was chromatographed on a short column of silica gel with benzene–ethyl acetate (2 : 1) and the fraction containing **6** was evaporated and the solid was reprecipitated from chloroform–petroleum ether, 1.18 g (70%), mp 117—118 °C; IR (KBr): 1570, 1640, 1660, 1705 (w) cm^{-1}.

Found: C, 58.41; H, 5.98; N, 11.83%. Calcd for $C_{34}H_{42}N_6O_{10}$: C, 58.78; H, 6.09; N, 12.10%.

NMR (in $CDCl_3$): τ 8.7—8.2 (10H, cyclohexylidene), 7.79 and 7.75 (each 3H s, NAc), 4.84 (4H s, $C\underline{H}_2C_6H_5$), 2.59 (10H s, $CH_2C_6\underline{H}_5$).

Di-N-*benzyloxycarbonyl-4,5 (and 5,6)*-O-*cyclohexylidenestreptidine (**7**).* *(a) From **6**:* To a solution of **6** (45 mg) in acetone (2 ml), 15M aqueous ammonia (1 ml) was added and the solution was allowed to stand for 1 hr. Evaporation of the solution gave a residue, which was dissolved in chloroform. The solution was washed with sodium hydrogen carbonate solution, dried over sodium sulfate and the solvent

was evaporated to give a solid, 31 mg (78%), mp 147—148.5 °C, R_f 0.33 (tlc with benzene–ethanol (10 : 1)).

Found: C, 58.70; H, 6.35; N, 13.55. Calcd for $C_{30}H_{38}N_6O_8$: C, 59.01; H, 6.27; N, 13.76.

NMR (in $CDCl_3$): No peaks assignable to Ac were observed.

(b) From ***1***: Compound **1** was treated similarly as described for the preparation of **6** from **5**. Yield of **7** was 95%.

Recovery of Streptidine from ***7***. A mixture of **7** (35 mg) and 50% acetic acid (1 ml) was heated at 60 °C for 1 hr. The solution was concentrated and ether was added to give a solid, 30 mg, which was dissolved in aqueous dioxane (1 : 1, 3 ml) and, after addition of two drops of acetic acid, the solution was treated with palladium black and hydrogen in a usual manner. The mixture was filtered, concentrated, and an aqueous solution of the residue was passed through a short column of Dowex 1-X2 (Cl form) with water to afford streptidine dihydrochloride (18 mg).

References

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