Synthesis of 5-*O*- and 6-*O*-Methyldihydrostreptomycin

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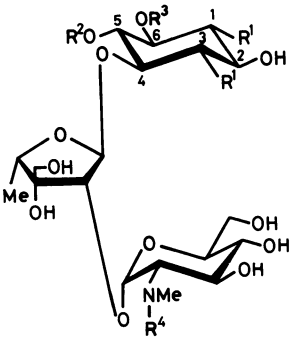
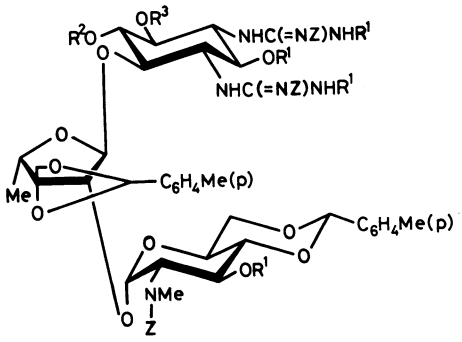
5-*O*- and 6-*O*-Methyldihydrostreptomycin have been prepared from dihydrostreptomycin by a sequence of reactions involving methylation of a selectively protected derivative (**4**) with diazomethane in the presence of tin(II) chloride. The structures of the final products were confirmed by their ^{13}C-NMR spectra and the TACu method.

Streptomycin is inactivated by resistant bacteria[1,2,3] producing inactivating enzymes: AAD(3″) or APH(3″) which adenylylates or phosphorylates the 3″-hydroxyl group, and AAD(6) or APH(6) which adenylylates or phosphorylates the 6-hydroxyl group of streptomycin and dihydrostreptomycin† to give the corresponding *O*-adenylyl or *O*-phosphoryl compounds. To counteract the action of the enzymes AAD(3″) or APH(3″), 3″-deoxydihydrostreptomycin[4,5,6] and 3″-epidihydrostreptomycin[7,8] had been prepared. These compounds showed satisfactory activities against the resistant bacteria owing to the lack of the equatorial 3″-hydroxyl group to be modified. Another device for the enzymes AAD(6) or APH(6), however, was unsuccessful. Chemically prepared 6-deoxydihydrostreptomycin[9] showed only very weak antibacterial activity, suggesting that the 6-hydroxyl group plays an important role in the mechanism of antibacterial action. In connection with our interest in the structure-activity relationships, in this paper, we have described the synthesis of 5-*O*- and 6-*O*-methyldihydrostreptomycin (**9** and **10**). The former derivative (**9**) was prepared to determine the steric influence of the neighboring substituent on the 6-modifying mechanism.

Tris(N^G,N^G,*N*-benzyloxycarbonyl)dihydrostreptomycin (**1**),[10] the starting compound, was treated with *p*-tolualdehyde dimethyl acetal in the presence of acid catalyst under strictly dry conditions. Tris (N^G,N^G,*N*-benzyloxycarbonyl)-5,6:3′,3′a:4″,6″-tris(*O*-*p*-methylbenzylidene)dihydrostreptomycin (**2**) was obtained with slight accompanied mono-, di-, and overacetalated derivatives. Chromatographic separation gave the pure **2**. In this acetal-exchange reaction, formations of N^G,*O*-acetals such as 3,3′a:4″, 6″-bis(*O*-*p*-methylbenzylidene)-N^G,*O*-(*p*-methylbenzylidene) derivatives bearing cyclic bridges between 1-N^G,2-*O*, 2-*O*,3-N^G, or 1-N^G,6-*O*, were also suspected. Formation of such type of compounds was found[11] in the reaction of 2-guanidino-1-cyclohexanols with 1,1-dimethoxycyclohexane (in *N*,*N*-dimethylformamide in the presence of *p*-toluenesulfonic acid). In the above case, however, they were successfully removed in the purification step.



	R^1	R^2	R^3	R^4
DHSM	NHC(=NH)NH_2	H	H	H
1	NHC(=NZ)NH_2[19]	H	H	Z
9	NHC(=NH)NH_2	Me	H	H
10	NHC(=NH)NH_2	H	Me	H
11	NH_2	Me	H	H
12	NH_2	H	Me	H
13	NH_2	H	H	H



	R^1	R^2	R^3
2	H	$CHC_6H_4Me(p)$	
3	Ac	$CHC_6H_4Me(p)$	
4	Ac	H	H
5	Ac	Me	H
6	Ac	H	Me
7	H	Me	H
8	H	H	Me

Z = $C_6H_5CH_2OCO$-

† As the name dihydrostreptomycin has been widely used, we have used this trivial name.

Acetylation of **2** with acetic anhydride in pyridine gave the tetraacetate (**3**), the positions of the acetyl groups being estimated 1,3-N^G,N^G and 2,3″-*O*,*O*. Selective deacetalation of **3** in a medium containing acetic acid gave the corresponding 5,6-*O*-deacetalated derivative (**4**) in a moderate yield. Careful methylation of **4** with diazomethane in the presence[12] of tin(II) chloride gave a mixture of 5-*O* and 6-*O*-methyl derivatives (**5** and **6**), which, without purification, deacetylated (to give **7** and **8**). Treatment of the product mixture with sodium in liquid ammonia (to remove the *p*-methylbenzylidene and benzyloxycarbonyl groups) followed by repeated chromatography gave 5-*O*- and 6-*O*-methyldihydrostreptomycin (**9** and **10**).

The structures of the final products were determined by the ^{13}C-NMR spectra (Table 1). Since dihydrostreptomycin gave well-separated signals at pD≈6, the spectra of the minor (**9**) and the major (**10**) products were measured at the same pD. The ^{13}C signals of **9** and **10** except for C-5,6 were both readily assigned on the basis of comparison of the corresponding chemical shifts of dihydrostreptomycin.[13] The remaining resonances of the minor product (**9**) were those at δ 83.8 and 71.9, and of the major (**10**), at δ 73.7 and 82.5. Since C-5 and C-6 of dihydrostreptomycin resonated[13] at δ 74.2 and 72.4 at pD≈6, respectively, the signals at δ 71.9 of **9** and δ 73.7 of **10** were assigned to C-6 and C-5 of the respective compounds. The signals of 80′(δ) of **9** and **10** were, therefore, assigned to the resonances of C-5 and C-6 carrying the OMe groups of the respective compounds, the chemical shifts being typical[14] for the carbons carrying an ether group. Furthermore, as the results of the negative shift effect of *O*-methylation at the neighboring positions,[14] the signals of C-4 and C-6 of **9**, and C-1 and C-5 of **10** were respectively shifted upfield, compared to the chemical shifts of the corresponding carbons of dihydrostreptomycin. Thus, the major (**10**) and the minor (**9**) methylated products were determined 6-*O*- and 5-*O*-methyldihydrostreptomycin, respectively. These results, in turn, supported that **3** had 5,6-*O*- and not N^G,*O*-benzylidene structure, as already described.

Table 1. The ^{13}C chemical shifts[a] of **9**, **10** and dihydrostreptomycin dissolved in D_2O at pD 6[b]

Carbon	**9**	**10**	Dihydrostreptomycin[13]
C-1	59.6	58.7	59.8
C-2	71.4	71.3	71.5
C-3	59.4	59.1	59.1
C-4	76.0	77.9	78.7
C-5	83.8	73.7	74.2
C-6	71.9	82.5	72.4
C-1′	106.7	106.3	106.7
C-2′	84.4	85.2	84.9
C-3′	81.6	81.5	81.7
C-3′a	64.4	64.3	64.2
C-4′	79.0	78.8	78.5
C-5′	13.6	13.5	13.5
C-1″	94.1	94.6	94.4
C-2″	62.0	62.0	62.1
C-3″	70.3	70.3	70.3
C-4″	70.1	70.1	70.3
C-5″	73.6	73.6	73.6
C-6″	61.3	61.3	61.3
C(1)NH*C*(=NH)NH_2	159.0	158.8	159.2
C(3)NH*C*(=NH)NH_2	158.3	158.5	158.6
NCH_3	32.7	32.7	32.8
OCH_3	60.9	61.2	

a) In ppm downfield from TMS calculated as $\delta^{TMS} = \delta^{dioxane} + 67.4$ ppm. b) Adjusted by addition of DCl.

The structures of **9** and **10** were confirmed by the tetraamminecopper(II) (TACu)§ method[15,16] on their deamidino derivatives (Table 2). Deamidination of **9**, **10** and dihydrostreptomycin according to Polglase[17] gave the corresponding 1,3-diamino derivatives (**11**, **12**, and **13**[17]). 1,3-Di(deamidino)-5-*O*-methyldihydrostreptomycin (**11**) and **13** showed similar $\Delta[M]_{TACu}$ and $\Delta[M]_{TACu\text{-}NH_3}$ values owing to the lack of participation for complexing including the functional groups at C-5, although, in **11**, the proportion of the complexing like B (see Fig. 1) seemed to be slightly less than that in **13**, In 1,3-di(deamidino)-6-*O*-methyldihydrostreptomycin (**12**), the degree of the A-type complex in TACu solution was considered to be essentially the same with the complexings of the other compounds (**11** and **13**). However, after addition of ammonia, complexing could occur both at NH_2(1)–OH(2)(sign−) and OH(2)–NH_2(3)(sign+) competitively, thus showing the value as expected from the apparent complexing formed only at $NHCH_3$(2″)–OH(3″)(see Fig. 2).

The antibacterial spectra of **9** and **10** showed that they had only very slight activities, suggesting that

§ TACu can form complex only with a pair of vicinal amino and hydroxyl groups having relative special orientations of ≈60° dihedral angle and the $\Delta[M]_{TACu}$(= $[M]_{TACu} - [M]_{H_2O}$) shows a value of ±900°, the sign being decided by counterclockwise (positive) or clockwise (negative) orientation in the Newman projection of -(*NH*$_2$)CH-CH(*OH*)-. In the case of **13**, TACu forms complexes mainly at NHMe(2″)–OH(3″)(sign+) and NH_2(1)–OH(2)-(sign−) to give roughly cancelled value (+340[16]), and by slight addition of ammonia to the TACu solution,[16] the latter complex almost disappeared and, instead, two new complexes at OH(2)–NH_2(3)(sign+) and NH_2(1)–OH(6)-(sign+) appeared, to result in giving a large positive value of $\Delta[M]_{TACu\text{-}NH_3}$(+2530[16]). This phenomenon was interpreted by the assumption that TACu made complexes in streptamine portion of **13** in a state of equilibrium as depicted in Fig 1.[16]

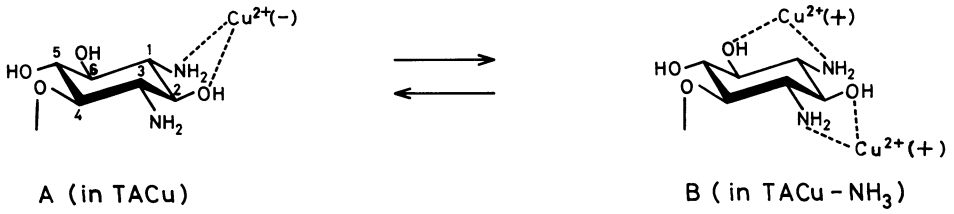
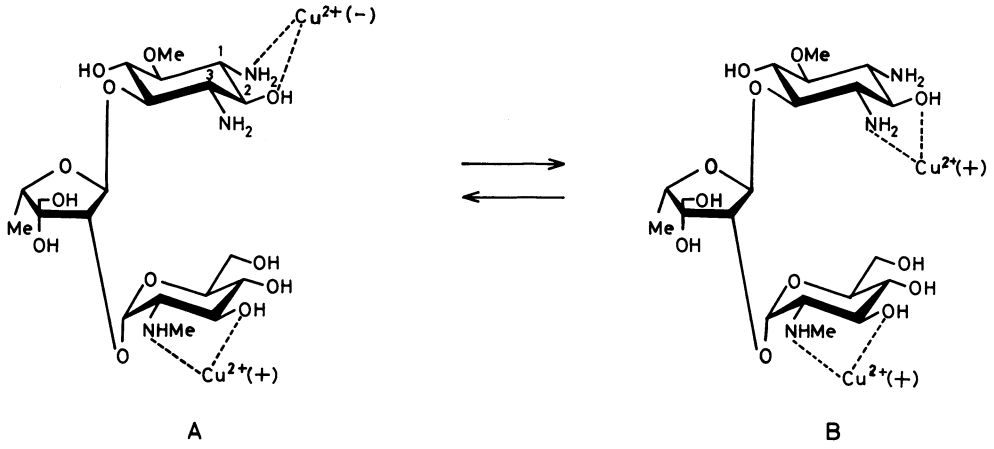


Fig. 1.



In TACu a mixture of 1,2-(type A, ≈73%) and 2,3-complexes(type B, ≈27%) was expected, and in TACu-NH_3, 1,2-(≈46%) and 2,3-complexes(≈54%).

Fig. 2.

Table 2. $\Delta[M]_{TACu}$ values of **11**, **12**, and **13**[17] before and after addition of ammonia

	$[M]_{H_2O}$	$[M]_{TACu}$[a]	$\Delta[M]_{TACu}$	$[M]_{TACu\text{-}NH_3}$[b]	$\Delta[M]_{TACu\text{-}NH_3}$
11	−1110	−760	+350	+920	+2030
12	−1410	−930	+480	−440	+970
13	−1260	−1000	+260	+1380	+2600

a) 0.01 mmol of each sample (as the hydrogencarbonate) was dissolved in 1.0 ml of 0.16 mmol TACu solution and the rotation of the solution was measured in 0.1 dm tube at 436 nm.[16] b) After measuring the $[M]_{TACu}$, 0.001 ml each of 15 mol dm^{-3} ammonium hydroxide solution was added to the solution (0.55 mol) and the rotation of the solution was again measured. The procedure was repeated until the rotation reached to a constant value.[16]

masking of the hydroxyl at C-5 or C-6 both greatly influences the binding of the antibiotic to the bacterial ribosome.

Experimental

General. ^{1}H-NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer except for **9** and **10**, the spectra of which being recorded at 250 MHz in the FT mode with a Bruker WM 250 spectrometer. ^{13}C-NMR spectra were recorded in the FT mode with a Bruker WM 250 spectrometer operating at 62.5 MHz. Thin-Layer chromatography (TLC) was performed on Wakogel B5. For column chromatography, silica gel (Wakogel C-200) was used.

1,3,2″-Tris(N^G,N^G,N-*benzyloxycarbonyl*)-*5,6:3′,3′a:4″,6″-tris*(O-p-*methylbenzylidene*)*dihydrostreptomycin* (**2**). To a solution of **1** (6.0 g) in dry *N,N*-dimethylformamide (120 ml) were added *p*-tolualdehyde dimethyl acetal (24 ml) and molecular sieves 5A (6 g) and, after stirring for 30 min, was added anhydrous *p*-toluenesulfonic acid (3 g), and the mixture was stirred at 60 °C for 5 h under reduced pressure (≈15 Torr (1 Torr=133.322 Pa); to remove the methanol liberated). The solution was poured into a saturated aqueous sodium hydrogencarbonate solution (27 ml) and the precipitates were filtered off with aid of methanol. (If the addition of the reaction mixture to the alkaline solution is reversed, yield of **2** sometimes greatly decreased

owing to removal of the 5,6-acetal group.) The filtrates and washings combined were concentrated to give a syrup, that was extracted with chloroform. The organic solution was washed with saturated aqueous sodium chloride, dried (Na_2SO_4), and concentrated. Addition of hexane to the concentrate gave a solid (≈6 g). Purification of the solid by silica-gel column chromatography with benzene–ethanol–triethylamine (30:1:0.1) as the developer gave an amorphous solid of **2**, 3.18 g (41%), $[\alpha]_D^{25}$ −64° (*c* 1, chloroform). ^{1}H-NMR ($CDCl_3$) δ=1.2—1.4 [3H, CH_3(5′)], 2.3 (9H br s, 3C*H*$_3$C$_6$H$_4$–).

Found: C, 64.12; H, 6.01; N, 7.59%. Calcd for $C_{69}H_{77}N_7O_{18}$: C, 64.19; H, 6.01; N, 7.40%.

*1,3-Di-*N^G*-acetyl-2,3″-di-*O*-acetyl-1,3,2″-tris(*N^G,N^G,N*-benzyloxycarbonyl)-5,6:3′,3′a:4″,6″-*tris(O-p-*methylbenzylidene)dihydrostreptomycin (3).* A mixture of **2** (900 mg) and acetic anhydride (4.8 ml) in pyridine (13.5 ml) was kept at room temperature overnight. Pouring the solution into ice water (100 ml) gave precipitates, that was thoroughly washed with water, then dried to give a solid of **3**, 1.00 g (99%), TLC (benzene–ethanol=15:1): R_f=0.75 (*cf.* **2**: 0.2), $[\alpha]_D^{20}$ −37° (*c* 1, chloroform).

^{1}H-NMR ($CDCl_3$) δ=1.2—1.4 [3H, CH_3(5′)], 1.9—2.2 (12H, mainly 4 singlets with different strength, 4Ac), 2.4 (9H, s, 3C*H*$_3$C$_6$H$_4$–), 2.74 (3H, s, NCH_3).

Found: C, 63.06; H, 5.95; N, 6.46%. Calcd for $C_{77}H_{85}N_7O_{22}$; C, 63.32; H, 5.86; N, 6.71%.

*1,3-Di-*N^G*-acetyl-2,3″-di-*O*-acetyl-1,3,2″-tris(*N^G,N^G,N*-benzyloxycarbonyl)-3′,3′a:4″,6″-bis(*O-p-*methylbenzylidene)dihydrostreptomycin (4).* A solution of **3** (1062 mg) in a mixture of 1,4-dioxane (25 ml) and 50% aqueous acetic acid (25 ml) was allowed to stand at room temperature for 4 h. The solution was poured, with careful agitation, into water (100 ml) containing sodium hydrogencarbonate (20 g) and the mixture extracted with chloroform. The extract, after dried (Na_2SO_4), was concentrated to give a residue, that contained three products, on checking by TLC (benzene–ethanol=15:1), having R_f 0.25 (slight), 0.17 (**4**, major), and 0.12 (slight). Separation with silica-gel column chromatography with benzene–ethanol–triethylamine (30:1:0.05) gave a solid of **4**, 634 mg (64%), $[\alpha]_D^{20}$ −38° (*c* 1, chloroform).

^{1}H-NMR ($CDCl_3$) δ=≈1.3 [3H, CH_3(5′)], 1.9—2.2 (12H, mainly 3 peaks, including unresolved, small strength, 4Ac), 2.4 (6H s, 2C*H*$_3$C$_6$H$_4$–), 2.74 (3H br s, NCH_3).

Found: C, 60.77; H, 6.02; N, 7.06%. Calcd for $C_{69}H_{79}N_7O_{22}$: C, 61.01; H, 5.86; N, 7.22%.

Reaction of 4 with Diazomethane. To an ice-cold solution of **4** (240 mg) in methanol (2.7 ml) containing $SnCl_2 \cdot 2H_2O$ (8 mg, 0.2 mol equiv for **4**) was added diazomethane in ether[18] in small portions, and the reaction was monitored by TLC (benzene–ethanol=7:1). After 20 ml addition of the ether solution, the starting **4** (R_f= 0.37) almost disappeared and the products (**5**, **6**, R_f= 0.43) appeared. Concentration gave a residue, that was extracted with chloroform. The extract was washed thoroughly with water, dried (Na_2SO_4), and concentrated to give a solid (232 mg). The ^{1}H-NMR ($CDCl_3$) spectrum of the products gave a clear peak at δ=3.6 (OMe).

5-O- and 6-O-Methyldihydrostreptomycin (9 and 10). A crude mixture of **5** and **6** (220 mg) dissolved in methanol (22 ml) containing 15 M aqueous ammonia (0.44 ml)(1 M=1 mol dm^{-3}) was kept at room temperature for 1 h. Concentration gave a residue (190 mg), that was purified by silica-gel column chromatography with benzene–ethanol (15:1) to give a mixture of **7** and **8** (125 mg). In the ^{1}H-NMR spectrum, no peak was observed near δ=2 (Ac). A solution of the mixture (115 mg) in oxolane (1.5 ml) was gradually poured into a solution of sodium (≈60 mg) in liquid ammonia (15 ml) maintained at −60 °C, and the blue solution was kept at the temperature for 1.5 h. Addition of methanol until the color disappeared was followed by raising the temperature until 30 °C in order to evaporate the ammonia. The residue was then neutralized with 1 M aqueous hydrochloric acid and charged on a column of Amberlite CG 50 (NH_4^+ form, 20 ml). After washing the column with water, the products were eluted by aqueous ammonium carbonate solution (l→5%, the concentration was gradually changed) and the portion positive for biacetyl color test (indication for the presence of guanidino group) was collected and repeatedly concentrated with several additions of water to remove ammonium carbonate to give a solid (a mixture of **9** and **10**, 32 mg). The mixture was dissolved in 50% aqueous acetic acid (0.6 ml) and separated by column chromatography of microcrystalline cellulose powder (Avicel SF, 15 g, Funakoshi Co., Tokyo) with 1-butanol–pyridine–water–acetic acid (6:4:3:1). Fractions containing **9** and **10** were, respectively, concentrated, and the residue was purified by a column of Amberlite CG 50 (NH_4^+ form) with 5% aqueous ammonium carbonate. The fractions positive for biacetyl were repeatedly concentrated with several additions of water (to remove $(NH_4)_2CO_3$) to give, after drying, solids of **9**, 6.5 mg (5% based on **4**) and **10**, 18.5 mg (15% based on **4**), each as the carbonate.

9: $[\alpha]_D^{20}$ −79° (*c* 0.8, water).

^{1}H-NMR (D_2O, the hydrogencarbonate salt) δ=1.25 [3H d, *J*=7 Hz, CH_3(5′)], 2.43 (3H s, NCH_3), 2.62 (1H dd, H-2″), 3.33 (1H unresolved t, H-5) 3.62 (3H s, OCH_3), 3.83 (1H dd, *J*=4.8 and 12 Hz) and 3.92 (1H dd, *J*=2 and 12 Hz)(H-6,6′), 4.225 (1H d, H-2′), 4.27 (1H q, *J*=7 Hz, H-4′), 5.26 (1H d, H-1″), and 5.29 (1H d, H-1′); $J_{1',2'}$=1, $J_{1'',2''}$=3, and $J_{2'',3''}$=11 Hz.

^{1}H-NMR (D_2O+DCl; pD<1) δ=1.24 [3H d, CH_3(5′)], 2.89 (3H s, NCH_3), 3.32 (1H, H-5), 3.35 (1H dd, H-2″), 3.60 (3H s, OCH_3), 4.25 (1H q, H-4′), 4.305 (1H d, H-2′), 5.28 (1H d, H-1′), and 5.57 (1H d, H-1″); *J* values were the same with those of the hydrogencarbonate.

Found: C, 39.95; H, 6.67; N, 13.55%. Calcd for $C_{22}H_{43}N_{27}O_{12} \cdot 2H_2CO_3$: C, 39.94; H, 6.56; N, 13.59%.

10: $[\alpha]_D^{20}$ −88° (*c* 1, water).

^{1}H-NMR (D_2O, the hydrogencarbonate salt) δ=1.24 [3H d, *J*=7 Hz, CH_3(5′)], 2.41 (3H s, NCH_3), 2.61 (1H dd, H-2″), 3.24 (1H unresolved t, H-6), 3.59 (3H s, OCH_3), 3.83 (1H dd, *J*=4.8 and 12 Hz) and 3.93 (1H dd, *J*=2 and 12 Hz)(H-6,6′), 4.205 (1H d, H-2′), 4.30 (1H q, *J*=7 Hz, H-4′), 5.23 (1H d, H-1″), and 5.29 (1H d, H-1′); $J_{1',2'}$=1, $J_{1'',2''}$=3, and $J_{2'',3''}$=11 Hz.

^{1}H-NMR (D_2O+DCl, pD>1) δ=1.24 [3H d, CH_3(5′)], 2.90 (3H s, NCH_3), 3.25 (1H unresolved t, *J*=≈8 Hz, H-6), 3.36 (1H dd, H-2″), 3.60 (3H s, OCH_3), 4.30 (1H q, H-4′), 4.32 (1H d, H-2′), 5.31 (1H d, H-1′), and 5.58 (1H d, H-1″).

Found: C, 37.65; H, 6.52; N, 12.91%. Calcd for $C_{22}H_{43}N_{27}O_{12} \cdot 2H_2CO_3 \cdot 2H_2O$: C, 37.50; H, 6.84; N, 13.04%.

Alkaline Degradation[17] of 9, 10, and Dihydrostreptomycin. The hydrogencarbonate salts of **9**, **10**, and dihydostrepto-

mycin (20 mg each) were respectively treated in the same manner as reported[17] (1 ml of 90% saturated aqueous barium hydroxide, refluxing for 28 h), and the products were purified by a Dowex 1×2 (OH^- form) resin column with water to give chromatographycally homogeneous (TLC, with chloroform–ethanol-17% aqueous ammonia= 2:2:1), ninhydrin positive, diacetyl and Ehrlich negative, solids of **11** (16 mg#, R_f=0.52), **12** (15 mg#, R_f=0.65) and **13**[17] (16 mg, R_f=0.31).

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The elemental analyses fluctuated in every sample possibly on account of the difference of uptake of CO_2 from the air, but the data showed no much deviation from the values as the monocarbonate.