SYNTHESIS OF 6″-CHLORO-6″-DEOXYAMIKACIN AND SOME ITS ANALOGS

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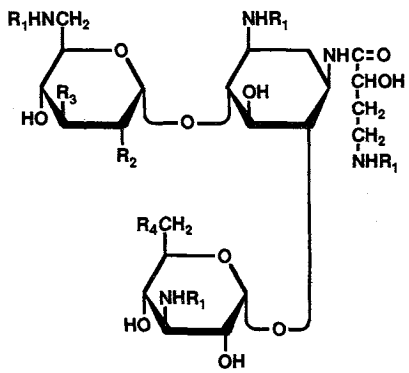
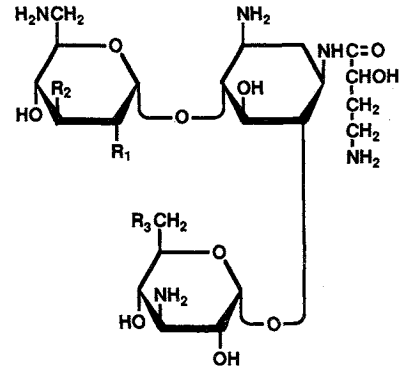
Amikacin[1] (**1**) is a typical semi-synthetic aminoglycoside antibiotic widely used clinically. To examine the antibacterial activity with change of the substituent at C-6″ of this antibiotic, we have undertaken to introduce a halogen atom at this position. As reported, 6″-chloro-6″-deoxykanamycin[2] is more active compared to kanamycin while 6″-deoxy-6″-fluoroamikacin[3] has decreased activity compared to amikacin. This result prompted us to prepare 6″-chloro-6″-deoxyamikacin (**6**) and its analogs.

Tetrakis(*N-tert*-butoxycarbonyl)amikacin[4] (**2**) was directly chlorinated with carbon tetrachloride —triphenylphosphine[5] to give the 6″-chloro-6″-deoxy derivative (**3**) in a moderate yield, meaning that the primary hydroxyl group of **1** at C-6″ is fairly selectively replaced by a chlorine atom in the presence of seven secondary hydrxyl groups. Removal of the *N*-protecting group of **3** gave 6″-chloro-6″-deoxyamikacin (**4**). By a similar route, 6″-deoxy-6″-iodoamikacin (**6**) was prepared from **2** *via* the 6″-deoxy-6″-iodo derivative (**5**). The compound **6** was found to be unstable and gradually degraded on storage. In a similar manner, 6″-chloro-3′,6″-dideoxyamikacin (**10**) was prepared from 3′-deoxyamikacin[6] (**7**) by *N-tert*-butoxycarbonylation (to give **8**) followed by deoxychlorination (to give **9**) and deprotection. 6″-Chloro-2′,3′,6″-trideoxyamikacin (**14**) was also prepared from 2′,3′-di deoxyamikacin[7] (**11**). *N*-Benzyloxycarbonylation of **11** gave **12**, which was then deoxychlorinated as before to give the 6″-chloro-6″-deoxy derivative (**13**). Catalytic hydrogenolysis of the *N*-protecting groups gave **14** without cleavage of the 6″-chloro atom.

Structures of these semi-synthetic products, **4**, **6**, **10**, and **14** were confirmed by their ^{13}C NMR spectra, in which, C-6″ of **4**, **10**, and **14** resonated at ~44.6 ppm (see Table 1), and that of **6** at 8.1 ppm, indicating that the halogen atoms were introduced at C-6″.

Antibacterial spectra of these products were shown in Table 2. 6″-Chloro-3′,6″-dideoxyamikacin was most effective, and it could be roughly concluded that introduction of a chlorine at C-6″ of amikacin (**1**) or its analogs enhances the antibacterial activity against all bacteria, except *Pseudomonas aeruginosa*.





	R_1	R_2	R_3	R_4
2	Boc	OH	OH	OH
3	Boc	OH	OH	Cl
5	Boc	OH	OH	I
8	Boc	OH	H	OH
9	Boc	OH	H	Cl
12	Z	H	H	OH
13	Z.	H	H	Cl

	R_1	R_2	R_3
1	OH	OH	OH
4	OH	OH	Cl
6	OH	OH	I
7	OH	H	OH
10	OH	H	Cl
11	H	H	OH
14	H	H	Cl

Boc: $COOCMe_3$, Z: $COOCH_2C_6H_5$.

Table 1. ^{13}C NMR spectra of amikacin (**1**), **4**, 3′-deoxyamikacin (**7**), **10**, 2′,3′-dideoxyamikacin (**11**), and **14** in D_2O at pD 5.0[a].

Carbon	1[b]	4	7[b]	10	11[b]	14
1	49.6	49.6	49.6	49.7	49.9[c]	49.9[d]
2	31.0	30.9	30.9	31.0	31.0	31.1
3	48.7	48.7	49.0	48.9	49.7[c]	49.8[d]
4	80.1	80.1	79.4	80.3	79.2	79.8
5	73.3	73.3	74.0	73.8	75.4	75.0
6	81.1	81.1	80.9	81.0	80.6	80.8
1′	96.4	96.5	95.6	95.8	98.0	98.4
2′	72.8	73.1	70.3	70.3	28.2	28.2
3′	71.6	71.6	34.6	34.7	25.8	25.8
4′	71.6	71.6	66.3	66.2	72.4	72.4
5′	69.5	69.5	66.8	66.8	66.9	66.8[e]
6′	41.2	41.2	41.2	41.1	41.0	40.9
1″	98.8	98.9	98.8	99.0	98.9	99.1
2″	68.9	68.8	68.8	68.8	68.8	68.8
3″	56.1	55.9	56.1	55.9	56.1	55.9
4″	66.4	67.0	66.5	67.0	66.3	66.7[e]
5″	72.7	71.4	72.7	71.4	72.7	71.3
6″	60.6	44.6	60.5	44.6	60.3	44.5
1‴	176.3	176.3	176.3	176.3	176.3	176.3
2‴	70.4	70.4	70.4	70.4	70.4	70.4
3‴	31.6	31.6	31.6	31.6	31.6	31.6
4‴	37.8	37.8	37.8	37.8	37.7	37.8

[a] Adjusted by DCl. In ppm downfield from Me_4Si.
[b] Shift-values were confirmed by the ^1H-^{13}C shift-correlated 2D spectrum.
[c~e] Could be reversed, respectively.

Table 2. Antibacterial activity (MIC, μg/ml) of compounds synthesized and amikacin (**1**).

Test organisms[a]	1	4	6	10	14
Staphylococcus aureus FDA 209P	1.56	0.78	1.56	0.39	0.78
S. aureus Smith	0.78	0.78	0.78	<0.2	0.39
S. aureus Ap01	3.12	3.12	3.12	0.78	1.56
Bacillus subtilis PCI 219	1.56	0.78	1.56	0.2	0.78
Escherichia coli K-12	0.78	<0.2	0.39	0.39	0.39
E. coli K-12 ML 1629	1.56	0.78	1.56	1.56	1.56
E. coli K-12 LA 290 R 55	1.56	0.78	1.56	0.78	1.56
E. coli W 677	0.78	0.39	0.78	0.78	0.78
E. coli JR 66/W 677	3.12	1.56	6.25	1.56	3.12
E. coli JR 225	0.78	0.39	0.78	0.39	0.78
Mycobacterium smegmatis ATCC 607[b]	0.78	0.78	0.78	0.78	0.78
Klebsiella pneumoniae PCI 602	1.56	0.78	1.56	1.56	0.78
K. pneumoniae 22 No. 3038	3.12	1.56	3.12	1.56	1.56
Proteus vulgaris OX 19	1.56	1.56	1.56	1.56	1.56
Providencia rettgeri GN 311	0.78	0.78	1.56	1.56	0.78
Serratia marcescens	6.25	3.12	12.5	25	6.25
Providencia sp. Pv 16	0.78	0.78	3.12	1.56	0.78
Pseudomonas aeruginosa A3	1.56	1.56	0.78	6.25	12.5
P. aeruginosa TI 13	3.12	3.12	12.5	12.5	25
P. aeruginosa GN 315	25	50	>100	25	50

[a] Agar dilution streak method (Mueller-Hinton agar, 17 hours, 37°C).
[b] 48 hours.

Experimental

Optical rotations were measured with a Perkin-Elmer 241 polarimeter. TLC was carried out on Wakogel B-5 silica gel, and column chromatography, on Wakogel C-200 unless otherwise stated. ^1H and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz with a Bruker WM 250 spectrometer, and chemical shifts are recorded downfield from internal Me_4Si.

3,6',3'',4'''-Tetrakis(*N-tert*-butoxycarbonyl)-6''-chloro-6''-deoxyamikacin (**3**)

A mixture of **2** (500 mg), triphenylphosphine (200 mg), and carbon tetrachloride (0.3 ml) in pyridine (10 ml) was heated at 65°C for 30 minutes. Evaporation followed by column chromatography with chloroform-methanol (20:1 → 10:1) gave a solid of **3**, 219 mg (43%): $[\alpha]_D^{20}$ +38° (*c* 1.7, MeOH).

Anal Calcd for $C_{42}H_{74}ClN_5O_{20}$:
C 50.22, H 7.43, Cl 3.53, N 6.97.
Found: C 50.25, H 7.18, Cl 3.20, N 6.73.

6''-Chloro-6''-deoxyamikacin (**4**)

A solution of **3** (285 mg) in TFA (1 ml) was heated at 40°C for 30 minutes. Concentration gave a residue, that was dissoved in aqueous 5% sodium hydrogen carbonate (1 ml). The solution was poured into a column of CM-Sephadex C-25 (10 ml) and the product was eluted with aqueous 0.2 M ammonia to give a solid of **4**, 144 mg (79%): $[\alpha]_D^{20}$ +85° (*c* 1.7, H_2O); ^1H NMR (20% ND_3 in D_2O) δ 5.05 (1H, d, J=3.7 Hz, 1''-H), 5.28 (1H, d, J=4.0 Hz, 1'-H).

Anal Calcd for $C_{22}H_{42}ClN_5O_{12}\cdot 2H_2O$:
C 41.28, H 6.93, Cl 5.53, N 10.94.
Found: C 41.62, H 7.04, Cl 5.48, N 10.74.

3,6',3'',4'''-Tetrakis(*N-tert*-butoxycarbonyl)-6''-deoxy-6''-iodoamikacin (**5**)

To a solution of **2** (100 mg) in oxolane (3 ml) were added triphenylphosphine (57 mg), iodine (51 mg), and imidazole (30 mg), and the mixture was heated in a pressure tube at 65°C for 4 hours. TLC of the solution with $CHCl_3$-MeOH-aq 28% NH_3 (5:1:0.1) gave spots of Rf 0.45 and 0.5 (trace) with disappearance of the spot (Rf 0.15) of **2**. Concentration gave a residue, that was dissolved in chloroform and the solution was washed with aqueous 5% sodium thiosulfate and water, dried (Na_2SO_4), and concentrated. Column chromatography of the residue with $CHCl_3$-MeOH (1:0→20:1) gave a solid of **5**, 51.2 mg (47%): $[\alpha]_D^{20}$ +43° (*c* 1.7, MeOH).

Anal Calcd for $C_{42}H_{74}IN_5O_{20}$:
C 46.03, H 6.81, I 11.58, N 6.39.
Found: C 46.29, H 6.62, I 11.76, N 6.84.

6''-Deoxy-6''-iodoamikacin (**6**)

A solution of **5** (100 mg) in TFA (0.4 ml) was heated at 40°C for 30 minutes. Concentration gave a residue, that was dissolved in 0.1 M hydrochloric acid (1 ml) and again concentrated to give a solid of **6**, 73.5 mg as the hydrochloride. $[\alpha]_D^{20}$ +48° (*c* 1.7, H_2O). TLC of the solid with $CHCl_3$-BuOH-EtOH-aq 17% NH_3 (2:4:7:7) gave a main spot at Rf 0.22, but attempted column chromatography with silica gel or resin degraded the product giving no pure sample of **6**. The product was unstable especially in an alkaline medium, but moderately stable in a neutral or acidic media. ^1H NMR (D_2O-DCl, pD 3) δ 5.15 (1H, d, J=4 Hz, 1''-H), 5.57 (1H, d, J=4 Hz, 1'-H).

3,6',3'',4'''-Tetrakis(*N-tert*-butoxycarbonyl)-3'-deoxyamikacin (**8**)

To a solution of **7**[6] (358 mg) in DMF-water (1:1, 10.5 ml) were added *tert*-butyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate[8] (Boc-S, 1.4 g) and triethylamine (2 ml), and the mixture was heated at 70°C for 6 hours. Addition of aqueous 28% ammonia followed by concentration gave a residue, that was thoroughly washed with water and then with diethyl ether to give a solid of **8**, 444 mg (73%): $[\alpha]_D^{20}$ +98° (*c* 0.9, MeOH).

Anal Calcd for $C_{42}H_{75}N_5O_{20}$:
C 52.00, H 7.79, N 7.21.
Found: C 51.67, H 7.93, N 7.23.

3,6',3'',4'''-Tetrakis(*N-tert*-butoxycarbonyl)-6''-chloro-3',6''-dideoxyamikacin (**9**)

Compound **8** (500 mg) was treated in the same manner as described for **3** to give a solid of **9**, 242 mg (47%): $[\alpha]_D^{21}$ +60° (*c* 1, MeOH).

Anal Calcd for $C_{42}H_{74}ClN_5O_{19}$:
C 51.03, H 7.55, Cl 3.59, N 7.09.
Found: C 50.77, H 7.66, Cl 3.70, N 7.34.

6''-Chloro-3',6''-dideoxyamikacin (**10**)

Compound **9** (32.5 mg) was treated with TFA (0.3 ml) similarly as described for **4** to give a solid of **10**, 15.5 mg (72%): $[\alpha]_D^{22}$ +58° (*c* 0.5, H_2O); ^1H NMR (20% ND_3 in D_2O) δ 5.05 (1H, d, J=4 Hz, 1''-H), 5.19 (1H, d, J=4 Hz, 1'-H).

Anal Calcd for $C_{22}H_{42}ClN_5O_{11}\cdot H_2CO_3$:
C 42.49, H 6.82, Cl 5.45, N 10.77.
Found: C 42.48, H 6.83, Cl 5.64, N 10.33.

3,6',3'',4'''-Tetrakis(*N*-benzyloxycarbonyl)-2',3'-dideoxyamikacin (**12**)

To a solution **11**[7] (monosulfate, 300 mg) and sodium carbonate (450 mg) in 1,4-dioxane-water (1:1, 8 ml) was added benzyl chloroformate (0.5 ml) at 0°C, and the mixture was kept for 1 hour at room temperature. After concentration, water was added and the resulting precipitates were filtered, washed with water and then diethyl ether, and dried to give a solid of **12**, 441 mg (87%): $[\alpha]_D^{22}$ +42° (*c* 0.7, pyridine).

Anal Calcd for $C_{54}H_{67}N_5O_{19}\cdot\frac{1}{2}H_2O$:
C 59.01, H 6.23, N 6.37.
Found: C 59.12, H 6.09, N 5.98.

3,6',3'',4'''-Tetrakis(*N*-benzyloxycarbonyl)-6''-chloro-2',3',6''-trideoxyamikacin (**13**)

A mixture of **12** (200 mg), triphenylphosphine (90 mg) and carbon tetrachloride (0.1 ml) in pyridine (4 ml) was heated at 65°C for 30 minutes. TLC of the solution with $CHCl_3$-MeOH-aq 28% NH_3 (5:1:0.1) gave a main spot at Rf 0.55 with several minor faster-moving spots. MeOH (0.5 ml) was added and the solution was concentrated. Column chromatography of the residue with $CHCl_3$-MeOH (1:0→50:1) gave a solid of **13**, 96.8 mg (47%): $[\alpha]_D^{22}$ +49° (*c* 0.5, pyridine).

Anal Calcd for $C_{54}H_{66}ClN_5O_{18}\cdot H_2O$:
C 57.57, H 6.08, Cl 3.15, N 6.22.
Found: C 57.80, H 6.01, Cl 3.26, N 5.90.

6''-Chloro-2',3',6''-trideoxyamikacin (**14**)

A solution of **13** (53 mg) in a mixture of 1,4-dioxane-water-acetic acid (2:1.1:0.02, 3 ml) was hydrogenated with Pd-black under atmospheric pressure of hydrogen for 3 hours at room temperature. Concentration gave a residue, that was chromatographed on a column of CM-Sephadex C-25 (3 ml) with aqueous 0.2 M ammonia to give a solid of **14**, 26.6 mg (76%): $[\alpha]_D^{20}$ +73° (*c* 1.1, pyridine); ^1H NMR (20% ND_3 in D_2O) δ 5.06 (1H, d, J=4 Hz, 1''-H), 5.29 (1H, br s, 1'-H).

Anal Calcd for $C_{22}H_{42}ClN_5O_{10}\cdot\frac{1}{2}H_2O\cdot 1\frac{1}{2}H_2CO_3$:
C 41.87, H 6.88, Cl 5.26, N 10.39.
Found: C 41.77, H 6.59, Cl 5.36, N 10.07.

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