SYNTHESES OF 1-*N*-(2-AMINOETHOXYCARBONYL)KANAMYCIN A AND 1-*N*-(3-AMINOPROPOXYCARBONYL)KANAMYCIN A

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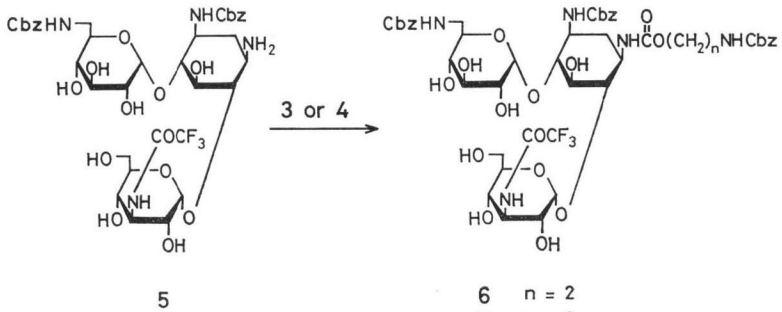
The titled compounds have been prepared and their antibacterial activities were described.

In the course of our synthetic studies of kanamycin derivatives, we were interested in preparing the derivatives which have urethane-type side residues attached at the C-1. This paper describes the syntheses and antibacterial activities of 1-*N*-(2-aminoethoxycarbonyl)kanamycin A (**8**) and 1-*N*-(3-aminopropoxycarbonyl)kanamycin A (**9**).

We selected 3,6′-bis(*N*-benzyloxycarbonyl)-3″-*N*-(trifluoroacetyl)kanamycin A[1] (**5**) as the starting

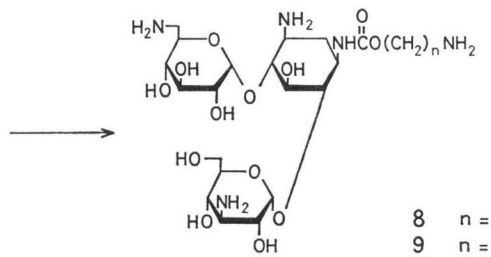
1 n = 2
2 n = 3

3 n = 2
4 n = 3



5

6 n = 2
7 n = 3



8 n = 2
9 n = 3

material. This compound has a single free amino group at C-1 and is conveniently prepared from kanamycin A by zinc chelation method and selective 3′-*N*-trifluoroacetylation[1]. The reagents (**3**, **4**) for the introduction of side-chains of **8** and **9** were prepared from 2-(benzyloxycarbonylamino)ethanol[2] (**1**) and 3-(benzyloxycarbonylamino)propanol[3] (**2**), respectively, by treatment of **1** and **2** with phosgene and then with *N*-hydroxysuccinimide to give *N*-[2-(benzyloxycarbonylamino)ethoxycarbonyloxy]succinimide (**3**) and *N*-[3-(benzyloxycarbonylamino)propoxycarbonyloxy] succinimide (**4**). Couplings of **3** and **4** to the 1-amino group of **5** were successfully carried out to give high yields of the protected 1-*N*-acyl derivatives

Table 1. Minimal inhibitory concentration (mcg/ml) of **8**, **9** and amikacin.

Test organism	**8**	**9**	Amikacin
Staph. aureus FDA 209P	0.78	1.56	1.56
Staph. aureus Smith	<0.2	<0.2	<0.2
Staph. Ap01	6.25	3.12	1.56
Staph. epidermidis 109	6.25	1.56	0.78
Micrococcus flavus FDA16	50	12.5	3.12
Sarcina lutea PCI1001	6.25	12.5	0.78
B. anthracis	<0.2	<0.2	<0.2
B. subtilis PCI219	<0.2	<0.2	<0.2
B. subtilis NRRL B-558	<0.2	<0.2	<0.2
B. cereus ATCC 10702	1.56	3.12	1.56
Corynebact. bovis 1810	12.5	25	1.56
E. coli NIHJ	0.78	0.78	0.78
E. coli K-12	0.78	0.78	0.78
E. coli K-12 R5	>100	>100	100
E. coli K-12 R388	0.78	1.56	0.78
E. coli K-12 J5R11-2	1.56	3.12	1.56
E. coli K-12 ML1629	0.78	1.56	0.78
E. coli K-12 ML1410	3.12	3.12	6.25
E. coli K-12 ML1410 R81	1.56	1.56	1.56
E. coli K-12 LA290 R55	1.56	3.12	1.56
E. coli K-12 LA290 R56	3.12	3.12	1.56
E. coli K-12 LA290 R64	1.56	1.56	1.56
E. coli W677	0.39	1.56	0.78
E. coli JR66/W677	6.25	6.25	1.56
E. coli K-12 C600 R135	0.78	1.56	0.78
E. coli JR225	0.78	1.56	0.39
Mycobacterium smegmatis ATCC 607	0.39	0.2	0.2
Kl. pneumoniae PCI602	0.39	0.78	0.39
Kl. pneumoniae 22 #3038	3.12	3.12	1.56
Sh. dysenteriae JS11910	6.25	12.5	6.25
Sh. flexneri 4b JS11811	6.25	6.25	3.12
Sh. sonnei JS11746	6.25	12.5	6.25
Sal. typhi T-63	0.78	3.12	1.56
Sal. enteritidis 1891	3.12	3.12	3.12
Proteus vulgaris OX19	0.78	0.78	0.78
Proteus rettgeri GN311	25	25	25
Proteus rettgeri GN466	6.25	6.25	6.25
Serratia marcescens	50	50	25
Serratia sp. SOU	>100	>100	100
Serratia sp. 4	6.25	25	12.5
Providencia sp. Pv 16	6.25	12.5	12.5
Providencia sp. 2991	25	12.5	6.25
Ps. aeruginosa A3	0.78	1.56	0.78
Ps. aeruginosa No. 12	3.12	6.25	6.25
Ps. aeruginosa H9	3.12	12.5	6.25
Ps. aeruginosa H11	6.25	12.5	6.25
Ps. aeruginosa TI-13	1.56	3.12	1.56
Ps. aeruginosa GN315	>100	>100	50
Ps. aeruginosa 99	6.25	12.5	6.25
Ps. aeruginosa B-13	25	25	25
Ps. aeruginosa 21-75	>100	>100	6.25
Ps. aeruginosa PSTI	12.5	50	25
Ps. aeruginosa ROS134/PU21	>100	>100	100
Ps. aeruginosa K-Ps102	3.12	6.25	3.12
Mean MIC[4]	3.41	4.60	2.61

(**6** and **7**), which gave, after deblocking, the desired products **8** and **9**, respectively.

Antibacterial activities of **8** and **9** are shown in Table 1. The results indicate that **8** has a similar degree of the activity as amikacin and **9** has a weaker activity. It is noteworthy that the replacement of (*S*)-4-amino-2-hydroxybutyryl residue of amikacin with such a simple residue as aminoalkyloxycarbonyl still gave compounds having not much less activity than amikacin.

After this work was completed, we found a report by MALLAMS *et al.*[5] describing the 1-ureido derivative of kanamycin A (**8**) prepared by a different route.

Experimental

PMR spectra were recorded at 90 MHz with Varian EM-390 spectrometer with TMS as an internal and external (in the case of D_2O) standard. IR spectra were recorded with Hitachi-285 grating infrared spectrophotometer. Optical rotations were determined with Perkin-Elmer 241 polarimeter. Thin-layer chromatography (TLC) was performed on Wakogel B-5 and Kieselgel 60 (E. Merck) and spots were detected with sulfuric acid or 0.5% ninhydrin in pyridine.

N-[2-(Benzyloxycarbonylamino)ethoxycarbonyloxy]succinimide (**3**)

To liquid phosgene (2 ml), a solution of **1** (3.0 g) in dry dichloromethane (9 ml) was added dropwise over a period of 0.5 hour under stirring at −40°C, whereupon a precipitate was observed. The reaction mixture was kept at −20°C for 1 hour and the clear solution was kept at 0°C for 1 hour and at room temperature overnight. After nitrogen was bubbled through the solution for a while, the solution was evaporated under reduced pressure to give 2-(benzyloxycarbonylamino)-ethoxycarbonylchloride as a syrup, 3.9 g; IR (liq. film) 1780 (COCl), 1720, 1530 cm^{-1}; PMR ($CDCl_3$): δ 3.54 (2H q, $NHCH_2CH_2$, J=6 Hz), 4.40 (2H t, OCH_2CH_2, J=6 Hz), 5.17 (2H s, $C_6H_5CH_2O$), 7.44 (5H s, aromatic). To a solution of *N*-hydroxysuccinimide (1.46 g) and sodium hydroxide (0.57 g) in aqueous tetrahydrofuran (3: 2, 55 ml), a solution of the above syrup (3.3 g) in tetrahydrofuran (11 ml) was added dropwise with water-cooling and the mixture was stirred at room temperature for 1 hour. The reaction mixture showed, on TLC with chloroform - ethanol (15: 1), spots of Rf 0.59 (trace), 0.47 (major, **3**), 0.29 (trace), 0.22 (**1**, trace). The mixture was concentrated and the residue was extracted with chloroform (200 ml×4). The chloroform solution was washed with water, dried over sodium sulfate and concentrated to give a syrup, which was washed with hexane to yield **3**, 3.71 g (87%), IR (liq. film) 1800 (broad), 1720, 1520 cm^{-1}; PMR ($CDCl_3$): δ 2.80 (4H s, $COCH_2CH_2CO$), 3.53 (2H q, $NHCH_2CH_2$, J=6 Hz), 4.38 (1H t, OCH_2CH_2, J=6 Hz), 5.15 (2H s, $C_6H_5CH_2O$), 7.42 (5H s, aromatic).

Anal. Calcd. for $C_{15}H_{16}N_2O_7$: C, 53.57; H, 4.80; N, 8.33%
Found: C, 53.86; H, 4.98; N, 8.35%

N-[3-(Benzyloxycarbonylamino)propoxycarbonyloxy]succinimide (**4**)

Compound **2** (3.3 g) was treated with phosgene (3 ml) in a similar manner as described above to give a syrup, 4.2 g; IR (liq. film) 1780, 1710, 1530 cm^{-1}; PMR ($CDCl_3$): δ 1.93 (2H quintet, $CH_2CH_2CH_2$, J=6 Hz), 3.32 (1H q, $NHCH_2CH_2$), 4.40 (1H t, OCH_2CH_2, J=6 Hz), 5.15 (2H s, $C_6H_5CH_2O$), 7.43 (5H s, aromatic). The syrup (2.17 g) was treated with *N*-hydroxysuccinimide (0.92 g) and sodium hydroxide (0.44 g) in a similar manner as described for **3** to give a syrup, 2.6 g (92%); IR (liq. film) 1810, 1790, 1720, 1520 cm^{-1}; PMR ($CDCl_3$): δ 1.95 (2H quintet, $CH_2CH_2CH_2$, J=6 Hz), 2.80 (4H s, $COCH_2CH_2CO$), 3.31 (2H, $NHCH_2CH_2$, J=6 Hz), 4.40 (2H t, OCH_2CH_2, J=6 Hz), 5.13 (2H s, $C_6H_5CH_2O$), 7.42 (5H s, aromatic).

Anal. Calcd. for $C_{16}H_{18}N_2O_7$: C, 54.86; H, 5.18; N, 8.00%
Found: C, 55.06; H, 5.25; N, 7.78%

3,6′-Bis(*N*-benzyloxycarbonyl)-1-*N*-[2-(benzyloxycarbonylamino)ethoxycarbonyl]-3′′-*N*-(trifluoroacetyl)kanamycin A (**6**)

To a solution of **5** (1.55 g) and sodium carbonate (0.15 g) in aqueous tetrahydrofuran (1: 1, 46 ml), a solution of **3** (0.81 g) in tetrahydrofuran (23 ml) was added dropwise and the solution was kept at room temperature for 2 hours. The solution showed, on TLC with chloroform - methanol - 20%

acetic acid (1: 1: 1, lower layer), spots of Rf 0.33 (slight) and 0.27 (major, **6**). Concentration to a small volume gave a precipitate, which was filtered and washed successively with water and ether, and dried to give a solid of **6**, 1.81 g (93%). Since the solid was slightly contaminated with an impurity of Rf 0.33, it was purified by washing with dioxane and reprecipitated from dioxane - water, $[\alpha]_D^{25}+84°$ (*c* 1, pyridine); IR (KBr) 1700, 1530 cm^{-1}.

Anal. Calcd. for $C_{47}H_{58}N_5O_{20}F_3$: C, 52.76; H, 5.46; N, 6.55; F, 5.33%
Found: C, 52.93; H, 5.56; N, 6.22; F, 5.55%

3,6′-Bis(*N*-benzyloxycarbonyl)-1-*N*-[3-(benzyloxycarbonylamino)propoxycarbonyl]-3′′-*N*-(trifluoroacetyl)kanamycin A (**7**)

Compound **5** (417 mg) was treated with **4** (213 mg) and sodium carbonate (37 mg) in a similar manner as described for **6** to give a solid of **7**, 460 mg (86%). Analytical sample was obtained by the similar purification as described for **6**, $[\alpha]_D^{25}+74°$ (*c* 1, pyridine); IR (KBr) 1690, 1530 cm^{-1}; PMR (pyridine-d_5-D_2O = 7: 1, 80°C): δ 1.93 (1H quintet, $CH_2CH_2CH_2$, J=6 Hz and 1H, H-2_{ax}).

Anal. Calcd. for $C_{48}H_{60}N_5O_{20}F_3$: C, 53.18; H, 5.58; N, 6.46; F, 5.26%
Found: C, 53.06; H, 5.89; N, 6.09; F, 4.85%

1-*N*-(2-Aminoethoxycarbonyl)kanamycin A (**8**)

A solution of **6** (1.06 g) dissolved in 1 M ammonia in aqueous dioxane (2: 3, 100 ml) was kept at room temperature for 15 hours, whereupon precipitate was observed. After addition of 1 M ammonia in aqueous pyridine (2: 27, 37.6 ml) (the precipitate was dissolved) the solution was kept for further 30 hours. The solution showed, on TLC with chloroform - methanol - 20% acetic acid (1: 1: 1, lower layer), spots of Rf 0.27 (trace, **6**) and 0.10 (major). Concentration with occasional additions of toluene gave a solid (1.08 g). To the solid suspended in dioxane (40 ml), acetic acid (10 ml) and water (3 ml) were added and the mixture was hydrogenated in the presence of palladium black at room temperature for 2 hours. The solution showed, on TLC with chloroform - methanol - 17% ammonia - water (1: 4: 2: 1), spots of Rf 0.50 (slight) and 0.34 (major, **8**). Filtration followed by concentration with additions of toluene gave a syrup, which was chromatographed over CM-Sephadex C-25 (NH_4 form). After washing the column with water, the product was eluted with ammonia with gradual increase in concentration (0→0.5 M). Concentration of the fractions containing **8** to a small volume followed by addition of ethanol gave a solid of **8** as hemicarbonate, 427 mg (72%); $[\alpha]_D^{25}+91°$ (*c* 1, water); IR (KBr) 1700, 1560 cm^{-1}.

Anal. Calcd. for $C_{21}H_{41}N_5O_{13}\cdot\frac{1}{2}H_2CO_3$: C, 42.85; H, 7.02; N, 11.63%
Found: C, 42.89; H, 7.13; N, 11.39%

1-*N*-(3-Aminopropoxycarbonyl)kanamycin A (**9**)

Compound **7** (327 mg) was detrifluoroacetylated and hydrogenated in a similar manner as described for **8** to give a solid of **9** as $\frac{3}{4}$ carbonate, 131 mg (68%), $[\alpha]_D^{25}+89°$ (*c* 1, water); IR (KBr) 1690, 1560 cm^{-1}; PMR (D_2O); δ 2.15~2.75 (3H, H-2_{eq} and $CH_2CH_2CH_2$).

Anal. Calcd. for $C_{22}H_{43}N_5O_{13}\cdot\frac{3}{4}H_2CO_3$: C, 43.22; H, 7.10; N, 11.08%
Found: C, 43.31; H, 7.05; N, 10.83%

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