SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF DISUBSTITUTED AMIKACIN DERIVATIVES

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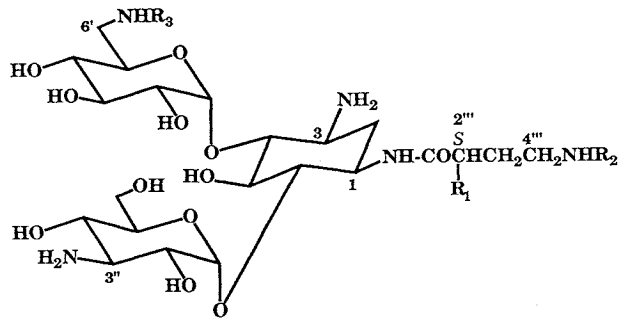
The previously reported (*S*)-2‴-deoxy-2‴-fluoroamikacin (**1**)[1], 4‴-*N*-formimidoylamikacin (**2**)[2] and 4‴-*N*-amidinoamikacin (**3**)[2] showed antibacterial activity and acute lethal toxicity (LD_{50}) comparable to amikacin. These data prompted us to elucidate activity/toxicity profiles of disubstituted amikacin derivatives which have a fluorine atom at the 2‴-position and amidine and/or guanidine groups at the 4‴- and/or 6′-position. This paper describes the synthesis and antibacterial activity of such bis-functionalized derivatives of amikacin (**4**～**9**) (Fig. 1).

Synthesis

The 4‴,6′-di-amidino (**4**) and di-guanidino (**5**) derivatives of amikacin were prepared from 4‴,6′-di-*N*-benzyloxycarbonyl(Cbz)-amikacin[3] (**10**). Reaction of **10** with di-*tert*-butyl dicarbonate in 50% aqueous THF (room temperature, 3 days) followed by hydrogenolysis (H_2/Pd-C in MeOH-THF-H_2O-AcOH (2:1:1:0.1)) gave the key intermediate, 3,3″-di-*N*-*tert*-butoxycarbonyl(Boc)-amikacin (**11**, 97%; mp 240°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1680, 1650, 1530; TLC, Rf 0.30 (silica gel, CH_3CN-H_2O-NH_4OH (12:3:2)); $C_{32}H_{59}N_5O_{17}\cdot 2H_2CO_3$†). Di-*N*-formimidoylation of **11** with a large excess of ethyl formimidate hydrochloride[4] in MeOH (room temperature, 2 days), followed by treatment with 90% TFA gave 4‴,6′-di-*N*-formimidoylamikacin (**4**, 58%; mp 220°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1715, 1650; ^1H NMR (400 MHz, D_2O) δ 5.19 (1H, d, J=4.0 Hz, 1″-H), 5.55 (1H, d, J=3.7 Hz, 1′-H), 7.83, 7.84, 7.87, 7.89 (2H, each s, CH=N)[2]; FAB-MS m/z 639 (M+H); $C_{24}H_{45}N_7O_{13}\cdot 2H_2SO_4\cdot 2H_2O$). Similarly, di-*N*-amidination of **11** with a large excess of *S*-methyl-*N*-nitroisothiourea[5] in DMSO (60°C, 2 hours), followed by treatment with 90% TFA and subsequent hydrogenolysis in the presence of palladium black afforded 4‴,6′-di-

Fig. 1. Mono- and di-substituted derivatives of amikacin.



	R_1	R_2	R_3
Amikacin	OH	H	H
1	F	H	H
2	OH	CH=NH	H
3	OH	C(=NH)−NH_2	H
4	OH	CH=NH	CH=NH
5	OH	C(=NH)−NH_2	C(=NH)−NH_2
6	F	H	CH=NH
7	F	CH=NH	H
8	F	H	C(=NH)−NH_2
9	F	C(=NH)−NH_2	H

† All compounds given a molecular formula gave correct microanalyses.

Table 1. *In vitro* Activity of bis-functionalized derivatives of amikacin.

Compound	Geometric mean of MIC (μg/ml)				
	Gp-S (5 strains)	Gp-R (4)	Gn-S (8)	Gn-R (8)	Pa (7)
Di-substituted derivative					
4	1.6	30	3.1	6.8	25
5	11	>50	39	>50	>50
6	3.1	42	5.7	11	41
7	1.2	50	3.4	9.6	30
8	22	>50	39	>50	>50
9	1.8	>50	4.8	15	41
Mono-substituted derivative					
1	0.60	42	1.6	4.4	7.6
2	0.56	30	1.6	3.3	8.1
3	0.40	28	1.5	4.3	11
Amikacin	0.65	30	1.4	3.7	5.2

Gp-S: Kanamycin A (KM-A)-sensitive *Staphylococcus aureus* (3 strains) and *S. epidermidis* (2), Gp-R: KM-A-resistant *S. aureus* (1), *S. epidermidis* (1), *Enterococcus faecalis* (1) and *E. faecium* (1), Gn-S: KM-A-sensitive *Escherichia coli* (2), *Proteus mirabilis* (1), *P. vulgaris* (1), *Morganella morganii* (1), *Klebsiella pneumoniae* (1), *Citrobacter freundii* (1), and *Serratia marcescens* (1), Gn-R: KM-A-resistant *E. coli* (5), *K. pneumoniae* (1), *S. marscescens* (1), and *Enterobacter cloacae* (1), Pa: *Pseudomonas aeruginosa* (7).

N-amidinoamikacin (**5**, 45%; mp 250°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1670, 1650; ^1H NMR (400 MHz, D_2O) δ 5.26 (1H, d, J=3.5 Hz, 1''-H), 5.60 (1H, d, J=3.5 Hz, 1'-H); FAB-MS m/z 670 (M+H); $C_{24}H_{47}H_9O_{13}\cdot 2H_2SO_4\cdot H_2O$).

For the preparation of the 4'''-(or 6'-)amidino or guanidino derivatives of amikacin with a 2'''-fluoro substituent (**6**~**9**), conventional acylation of 3-*N*-Boc-6'-*N*-Cbz-3''-*N*-trifluoroacetylkanamycin A[6] with (*S*)-4-*N*-Boc-amino-2-fluorobutyric acid and of 3,6'-di-*N*-Boc-3''-*N*-trifluoroacetylkanamycin A[7] with (*S*)-4-*N*-Cbz-amino-2-fluorobutyric acid[1], followed by a series of reactions (i) concd NH_4OH, room temperature, 2 days; ii) $(Boc)_2O$ in 50% aqueous THF, room temperature, 3 days; and iii) H_2/Pd-C, in MeOH-THF-H_2O-AcOH (2:1:1:0.5), 1 hour) gave the key intermediate, (*S*)-2'''-deoxy-2'''-fluoro-3,3'',4'''-tri-*N*-Boc-amikacin (6'-free amino) (**12**, 46%; mp 235~240°C (dec); IR ν_{max} (KBr) cm^{-1} 1690, 1560; TLC, Rf 0.30 (silica gel, $CHCl_3$-EtOH-NH_4OH (2:4:1)); $C_{37}H_{66}FN_5O_{18}\cdot H_2CO_3$) and (*S*)-2'''-deoxy-2'''-fluoro-3,3'',6'-tri-*N*-Boc-amikacin (4'''-free amino) (**13**, 51%; mp 255~260°C (dec); IR ν_{max} (KBr) cm^{-1} 1685, 1540; Rf 0.40 ($CHCl_3$-EtOH-NH_4OH (2:4:1)); $C_{37}H_{66}FN_5O_{18}\cdot H_2CO_3$). Using the procedure described in the preparation of **4**, *N*-formimidoylation of **12** at the 6'-position and that of **13** at the 4'''-position, followed by deprotection gave (*S*)-2'''-deoxy-2'''-fluoro-6'-*N*-formimidoylamikacin (**6**, 72.5%; mp 250°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1715, 1650, 1560; ^1H NMR (400 MHz, D_2O) δ 5.17 (1H, d, J=3.7 Hz, 1''-H), 5.18 (1H, ddd, J=48, 9.2 and 3.7 Hz, CHF), 5.56 (1H, d, J=4.0 Hz, 1'-H), 7.87 and 7.89 (1H, each s, CH=N)[2]; FAB-MS m/z 615 (M+H); $C_{23}H_{43}FN_6O_{12}\cdot 2H_2SO_4\cdot 2H_2O$) and (*S*)-2'''-deoxy-2'''-fluoro-4'''-*N*-formimidoylamikacin (**7**, 74.5%; mp 250°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1715, 1670, 1560; ^1H NMR (400 MHz, D_2O) δ 5.14 (1H, ddd, J=48, 8.2 and 3.3 Hz, CHF), 5.17 (1H, d, J=3.7 Hz, 1''-H), 5.58 (1H, d, J=4.0 Hz, 1'-H), 7.84 and 7.87 (1H, each s, CH=N)[2]; FAB-MS m/z 615 (M+H); $C_{23}H_{43}FN_6O_{12}\cdot 2H_2SO_4\cdot 2\frac{1}{2}H_2O$). Similarly, *N*-amidination of **12** and **13**, followed by deprotection gave (*S*)-6'-*N*-amidino-2'''-deoxy-2'''-fluoroamikacin (**8**, 54%; mp 210°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1680, 1540; ^1H NMR (400 MHz, D_2O) δ 5.17 (1H, d, J=3.7 Hz, 1''-H), 5.18 (1H, ddd, J=48, 8.8 and 3.7 Hz, CHF), 5.52 (1H, d, J=4.0 Hz, 1'-H); FAB-MS m/z 630 (M+H); $C_{23}H_{44}FN_7O_{12}\cdot 2H_2SO_4\cdot 2\frac{1}{2}H_2O$) and (*S*)-4'''-*N*-amidino-2'''-deoxy-2'''-fluoroamikacin (**9**, 71%; mp 220°C (gradually dec); IR ν_{max} (KBr) cm^{-1} 1650, 1540; ^1H NMR (400 MHz, D_2O) δ 5.12 (1H, ddd, J=49, 8.8 and 3.7 Hz, CHF), 5.17 (1H, d, J=4.0 Hz, 1''-H), 5.58 (1H, d, J=3.7 Hz, 1'-H); FAB-MS m/z 630 (M+H); $C_{23}H_{44}FN_7O_{12}\cdot 2H_2SO_4\cdot 4H_2O$).

Antibacterial Activity

The MICs of the aminoglycosides in the present study were determined by a 2-fold serial dilution method in Mueller-Hinton agar against 32 strains of

test organisms, which are classified into 5 groups as shown in the footnote to Table 1. The *in vitro* activity of **1**~**9** was assessed by the geometric mean of MICs of the test organisms belonging to each of the groups.

The amidino derivatives, **4**, **6** and **7**, were more active than the corresponding guanidino derivatives, **5**, **8** and **9**, respectively. Introduction of a guanidino group at the 6′-position (**5** and **8**) was found to cause marked decrease of the activity. All of bis-functionalized derivatives of amikacin prepared in this study were at least 2- to 3-fold less active than the corresponding mono-substituted derivatives and amikacin. This indicates that combined modification at multiple sites of amikacin might not be a good direction for the improvement of its antibacterial activity.

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