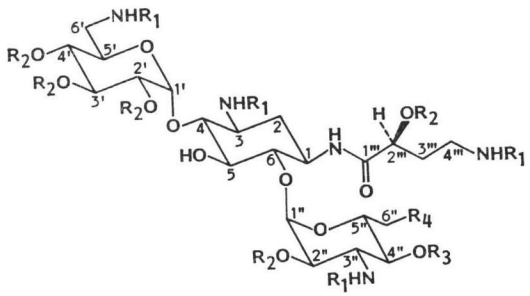
CHEMICAL MODIFICATION OF AMIKACIN AT C-4″ WITH INVERSION OF CONFIGURATION

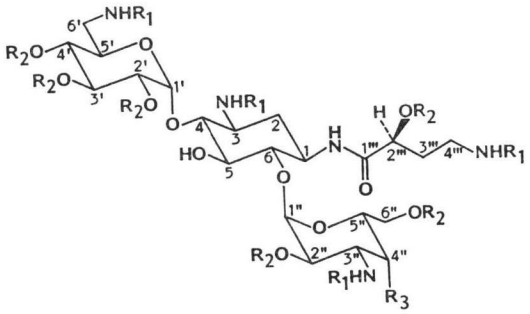
Sir:

With the appearance of an aminoglycoside 4′, 4″-nucleotidyl transferase,[1] selective modification of the clinical important amikacin (**1**) at these positions became of interest. Although enzymatic deactivation involving hydroxyl groups usually is overcome by "deoxygenation",[2] our efforts are focussed on the introduction of a substituent, which lacks ability to participate in a resistance mechanism but preserves the original polarity.

At this time we wish to report on the synthesis and antibacterial activity of 4″-azido-4″-deoxy-4″-*epi*-amikacin (**13**) and 4″-deoxy-4″-fluoro-4″-*epi*-amikacin (**14**). To trace the effect of the configurational change itself as well as that of fluorination, 4″-*epi*-amikacin (**15**) and 6″-deoxy-6″-fluoroamikacin (**9**) were also prepared.

Starting from **1**, protection of the amino functions with the *tert*-butoxycarbonyl (Boc) group by reaction with di-*tert*-butyl dicarbonate in DMSO - H_2O (95: 5) for 6 hours at 60°C gave 3,6′,3″,4‴-tetra-*N*-Boc-amikacin[3] (**2**) in 96% yield. Its treatment with chlorotriphenylmethane





1 $R_1=R_2=R_3=H$, $R_4=OH$
2 $R_1=Boc$, $R_2=R_3=H$, $R_4=OH$
3 $R_1=Boc$, $R_2=R_3=Ac$, $R_4=OTr$
4 $R_1=Boc$, $R_2=R_3=Ac$, $R_4=OH$
5 $R_1=Boc$, $R_2=Ac$, $R_3=H$, $R_4=OAc$
6 $R_1=Boc$, $R_2=Ac$, $R_3=tf$, $R_4=OAc$
7 $R_1=Boc$, $R_2=R_3=Ac$, $R_4=Otf$
8 $R_1=Boc$, $R_2=R_3=Ac$, $R_4=F$
9 $R_1=R_2=R_3=H$, $R_4=F$

10 $R_1=Boc$, $R_2=Ac$, $R_3=N_3$
11 $R_1=Boc$, $R_2=Ac$, $R_3=F$
12 $R_1=Boc$, $R_2=Ac$, $R_3=OAc$
13 $R_1=R_2=H$, $R_3=N_3$
14 $R_1=R_2=H$, $R_3=F$
15 $R_1=R_2=H$, $R_3=OH$

Boc=$-COOC(CH_3)_3$ Ac=$-COCH_3$
Tr=$-CPh_3$ tf=$-SO_2CF_3$

Table 1. ^{13}C NMR data (22.62 MHz, D_2O, 1,4-dioxane (67.4 ppm) as internal standard; δ in ppm; $J_{F,C}$ in Hz*).

C	**1**		**15**		**13**		**14**		**9**	
	pD 11	pD 2	pD 11	pD 2	pD 11	pD 2	pD 11	pD 2	pD 11	pD 2
1″	100.3	98.7	100.3	99.1	100.6	99.1	100.6	99.1	100.2	99.1
2″	72.4	68.8	70.2	66.2	70.4	66.0	70.6	66.1	72.3	68.8
3″	54.9	56.1	51.9	53.1	52.1	52.0	50.2 (17.6)	51.3 (17.6)	54.9	56.0
4″	70.1	66.4	69.5	66.2	64.6	60.4	92.0 (176.5)	87.7 (179.4)	69.0 (5.9)	65.7 (7.3)
5″	72.8	72.7	72.3	71.3	72.7	70.5	nd	nd	nd	71.8 (17.6)
6″	61.1	60.6	61.6	61.2	61.5	60.8	61.0 (5.9)	61.2 (5.9)	83.1 (169.1)	82.7 (167.6)

* Resolution: 1.5 Hz per data point.
nd=not detected.
In parenthesis are indicated *J* values.

Table 2. MIC in Mueller-Hinton broth (μg/ml).

Test organism	1	15	13	14	9
Staphylococcus aureus 162	0.001	0.25	0.125	0.125	0.125
〃 〃 56	0.001	0.25	0.25	0.25	0.125
〃 〃 1328[a,b]	12.5	50	50	50	6.25
〃 〃 1571[b]	6.2	50	50	25	6.25
S. epidermidis 1570[a,c]	6.2	50	50	100	25
Streptococcus faecalis 76	1.0	2.5	2.5	1.25	0.5
Escherichia coli 120	0.25	1.0	0.5	0.5	0.25
〃 〃 2	0.25	1.0	1.0	2.5	1.0
〃 〃 71	0.125	0.5	0.5	1.25	0.5
〃 〃 118	0.25	1.0	0.5	2.5	0.625
〃 〃 170	0.5	1.0	1.0	1.0	0.5
〃 〃 1321[d]	0.3	1.0	0.5	0.39	0.156
〃 〃 1322[b,e,f]	0.8	3.12	1.56	1.56	0.39
〃 〃 1323[g]	0.4	1.56	1.0	0.78	0.625
〃 〃 1324[d,h]	12.5	12.5	12.5	50	3.12
〃 〃 1325[b,d]	0.4	0.5	0.5	0.78	0.312
〃 〃 1329[b,e]	0.2	1.0	0.5	0.39	0.19
〃 〃 1569[b,e]	0.4	0.78	1.0	1.25	0.19
〃 〃 1572[h]	25	50	50	100	12.5
〃 〃 2309[e]	nt	1.56	1.0	1.56	0.5
〃 〃 2310[g]	nt	3.12	1.0	0.78	0.39
〃 〃 2311[h]	nt	12.5	25	25	3.12
Salmonella typhimurium 119	0.25	1.56	1.0	2.5	1.0
Klebsiella pneumoniae 33	0.5	0.78	1.0	2.5	2.5
〃 〃 62	0.125	0.063	0.063	1.0	0.5
〃 〃 678	0.125	0.5	0.25	1.0	0.625
〃 〃 217	0.25	0.25	0.5	1.0	1.0
〃 〃 1133	0.125	1.0	0.25	1.0	0.625
Enterobacter cloacae 220	1.0	3.125	1.563	5.0	2.5
〃 〃 221	0.5	0.781	1.0	2.5	1.0
〃 〃 964	0.5	1.0	1.0	2.5	1.0
E. agglomerans 581	0.001	0.049	0.049	3.12	0.156
Serratia marcescens 218	0.5	0.5	0.5	2.5	1.0
〃 〃 219	0.5	1.0	1.563	2.5	2.5
〃 〃 82	1.563	3.125	3.125	10	10
〃 〃 1577[h]	12.5	25	50	50	25
Proteus mirabilis 89	0.5	1.0	1.0	1.25	0.5
〃 〃 644	1.0	1.563	0.25	5.0	5.0
〃 〃 1438	1.0	3.125	3.125	5.0	2.5
〃 〃 1578[b,h]	0.31	1.0	1.56	1.56	0.78
P. vulgaris 8	0.125	0.125	0.125	1.0	0.312
〃 〃 116	0.5	0.5	0.5	2.5	1.0
P. morganii 90	1.0	6.25	6.25	5.0	10
Pseudomonas aeruginosa 12	0.5	1.0	5.0	1.25	1.0
〃 〃 92	0.25	0.25	1.563	1.0	0.625
〃 〃 493	0.5	1.0	3.125	1.25	0.625
〃 〃 1471[b]	0.78	1.56	12.5	3.12	0.78
〃 〃 1472[h]	3.12	50	50	50	5.0
〃 〃 1473[h]	0.78	6.25	50	6.25	1.56
〃 〃 1474[g]	0.156	3.12	6.25	0.78	0.39
〃 〃 1475[b]	1.56	3.12	6.25	3.12	1.56
〃 〃 1476[g]	1.25	3.12	12.5	3.12	1.56
〃 〃 1579[b,h]	0.39	3.12	12.5	3.12	0.625
〃 〃 1580[h]	5.0	50	50	50	5.0
Providencia stuartii 2312[i]	nt	50	50	50	50

Resistance: [a] ANT 4′, [b] APH 3′, [c] ANT 4″, [d] AAD 3″, [e] ANT 2″, [f] APH 3″, [g] AAC 3, [h] AAC 6′, [i] AAC 2′. nt=Not tested.

in pyridine for 24 hours at 50°C followed by acetylation with acetic anhydride led to 2′,3′,4′,2″,4″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-6″-*O*-triphenylmethylamikacin (**3**, 83%; mp 133~137°C (dec); $[\alpha]_D^{25}$ +69.8° (*c* 1.1, $CHCl_3$)). Detritylation using boron trifluoride - methanol[4] afforded 2′,3′,4′,2″,4″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-amikacin (**4**, 94%; mp 150~154°C (dec); $[\alpha]_D^{25}$ +68.0° (*c* 1.45, $CHCl_3$); Rf* 0.41), which on storing in pyridine - H_2O (1: 1) at room temp for 2 days formed 2′,3′,4′,2″,6″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-amikacin (**5**, 96%; mp 148~151°C (dec); $[\alpha]_D^{25}$ +64.4° (*c* 0.41, $CHCl_3$); Rf* 0.60) through *O*-4″→6″ acetyl migration.[5] Reaction of **5** with 1.5 equivalents to trifluoromethane sulfonic anhydride in dichloromethane - pyridine (20: 1) at 0°C for 15 minutes gave 2′,3′,4′,2″,6″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-4″-*O*-trifluoromethylsulfonylamikacin (**6**, Rf* 0.86), which was treated with sodium azide in DMF - dichloromethane (10: 1) at room temp for 3 hours to yield 2′,3′,4′,2″,6″,2‴-hexa-*O*-acetyl-4″-azido-3,6′,3″,4‴-tetra-*N*-Boc-4″-deoxy-4″-*epi*-amikacin (**10**, 77%; mp 135~138°C (dec); $[\alpha]_D^{25}$ +69.7° (*c* 1.49, $CHCl_3$); Rf* 0.84). Reaction of **6**, with sodium acetate under the same conditions led to 2′,3′,4′,2″,4″,6″,2‴-hepta-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-4″-*epi*-amikacin (**12**, 79%; mp 150~153°C (dec); $[\alpha]_D^{25}$ +78.2° (*c* 2.43, $CHCl_3$); Rf* 0.71). Treatment of **6** with tetra-*n*-butylammonium fluoride** in acetonitrile at room temp for 3 hours gave 2′,3′,4′,2″,6″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-4″-deoxy-4″-fluoro-4″-*epi*-amikacin (**11**, 73%; mp 140~143°C (dec); $[\alpha]_D^{20}$ +63.2° (*c* 0.82, $CHCl_3$); Rf* 0.82).

In all these cases, as previously observed with derivatives of kanamycin A,[6] a small proportion of the 3″-*N*,4″-*epi*-*O*-cyclic carbamate (Rf* 0.20) was also formed.

Trifluoromethanesulfonylation of **4** gave the 6″-triflate **7** (Rf* 0.80); its reaction with tetra-*n*-butylammonium fluoride** within half an hour led to 2′,3′,4′,2″,4″,2‴-hexa-*O*-acetyl-3,6′,3″,4‴-tetra-*N*-Boc-6″-deoxy-6″-fluoroamikacin (**8**, 76%; mp 150~153°C (dec); $[\alpha]_D^{20}$ +65.5° (*c* 0.96, $CHCl_3$); Rf* 0.72).

Zemplén de-*O*-acetylation of **10**, **11**, **12** and **8**, respectively, followed by treatment with TFA gave the deprotected amikacin derivatives, which were purified by chromatography on Amberlite CG 50 (NH_4^+) with 0.1~0.3 N ammonia:

4″-Azido-4″-deoxy-4″-*epi*-amikacin (**13**), 81%; $[\alpha]_D^{25}$ +84.9° (*c* 0.88, H_2O); Rf*** 0.39; IR (KBr) ν_{N_3} 2125 cm^{-1}.

4″-Deoxy-4″-fluoro-4″-*epi*-amikacin (**14**), 82%, $[\alpha]_D^{20}$ +103.3° (*c* 0.12, H_2O), Rf*** 0.29; ^1H NMR (250 MHz, D_2O) δ 3.14 (1H, ddd, $J_{F,3''}$=31.5 Hz, $J_{2'',3''}$=10.5 Hz, $J_{3'',4''}$=2 Hz, 3″-H), 4.86 (1H, dd, $J_{F,4''}$=50 Hz, 4″-H), 4.32 (1H, dt, $J_{F,5''}$=32.5 Hz, $J_{5'',6''}$=6.5 Hz, 5″-H).

4″-*epi*-Amikacin (**15**), 83%; $[\alpha]_D^{25}$ +79.2° (*c* 1.42, H_2O); Rf*** 0.20; ^1H NMR (250 MHz, D_2O) δ 4.19 (1H, dd, $J_{3'',4''}$=3 Hz, $J_{4'',5''}$=1 Hz, 4″-H).

6″-Deoxy-6″-fluoroamikacin (**9**), 77%; $[\alpha]_D^{25}$ +80.1° (*c* 1.05, H_2O); Rf*** 0.29.

As in their verification by ^{13}C NMR spectroscopy all these structural modifications only affected the X″-resonances, the latter only — in comparison with those of the parent amikacin[8] (**1**) — are given in Table 1. Proof for the position of modification results from the ^{19}F-^{13}C couplings, whereas the stereochemistry is evident from the chemical shift of C-3″.[9]

MIC values were determined in Mueller-Hinton broth (Merck 10923) in a microsystem (Dynatech MIC 200, vol 50 μl). Bacterial strains (Sandoz Research Institute culture collection) were stored in liquid nitrogen; the inoculum was prepared immediately after thawing by adjusting the size to 10^5~10^6 cfu/ml Mueller-Hinton broth. The compounds were dissolved in aqua dest and further diluted by log 2 steps; concentrations are given as the free bases.

The MIC values, summarized in Table 2, show that 6″-fluorination (**9**) decreased the activity of amikacin against some strains of *Enterobacteriaceae* by the factor of 4 to 10. The 4″-derivatives of amikacin, **13**, **14** and **15**, were remarkably less active than amikacin, in particular against strains provided with the enzymatic resistance mechanism of ANT 4′, ANT 4″ and AAC 6′.

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* Silica gel 60 F_{254} precoated plates (Merck 5554); EtOAc as eluant.

** According to SHARMA and FRY[7] the product obtained by drying of tetra-*n*-butylammonium fluoride trihydrate consists of tetra-*n*-butylammonium hydrogendifluoride mainly.

*** $CHCl_3$ - MeOH - NH_4OH (25%), 1: 2: 2 as eluant; Rf **1**: 0.20.

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RAINER ALBERT
KARL DAX
ARNOLD E. STÜTZ
Institute of Organic Chemistry,
Technical University Graz,
Stremayrgasse 16, A-8010 Graz,
Austria

JOHANNES HILDEBRANDT
Sandoz Research Institute,
Brunnerstraße 59, A-1235 Vienna,
Austria

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