# SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-*N*-[4-(SUBSTITUTED)AMIDINO AND GUANIDINO-2-HYDROXYBUTYRYL]KANAMYCINS A AND B

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The synthesis and biological properties of 1-*N*-[4-(substituted)amidino and guanidino-2-hydroxybutyryl]kanamycins A and B are described. Reaction of 3,3'',6'-tri-*N*-*tert*-butoxycarbonyl-amikacin with an appropriate amidinating or guanidinating reagent and subsequent deblocking gave a series of amikacin derivatives having an amidino or guanidino group on the 4'''-position. The corresponding kanamycin B analogs were also prepared by a similar procedure. Among these derivatives, 1-*N*-(4-formamidino- and guanidino-2-hydroxybutyryl)kanamycins A (**7a** and **7k**) and B (**11** and **14**) exhibited antibacterial activity similar to the corresponding 4-amino analogs. The nephrotoxic potential of selected compounds is also briefly discussed.

Aminoglycoside antibiotics are widely used in the treatment of serious infections caused by Gram-negative bacteria, although their clinical use is associated with dose-limiting nephrotoxicity[1]. Consequently, decreased nephrotoxicity is an important target for new aminoglycoside antibiotics.

Aminoglycosides have been reported to accumulate in kidney cortex and to induce nephrotoxicity, and their toxic potential to correlate with their renal concentrations[2~4]. On the other hand, no correlation was also observed for some aminoglycosides[5,6]. Recently, TULKENS and his co-workers have intently investigated the mechanism of aminoglycoside-induced nephrotoxicity[7~9], but it is still unclear whether or not renal drug concentration correlates with the degree of the toxicity. Furthermore, any papers have not been reported on the structure-nephrotoxicity relationship serving for exploring compounds having low nephrotoxic potential.

Although no rationale has been elucidated for the relationship between acute toxicity of aminoglycoside antibiotics and their nephrotoxicity, it has been demonstrated in clinical use that the maximum tolerated daily dose is nearly proportional to the $LD_{50}$ value[10]. In terms of acute toxicity, there have been published some papers[11~13] observing enhanced acute toxicity depending on the increased number of amino groups in the molecule. On the other hand, many instances[12,14~19] have been reported that the antibacterial activity was enhanced by introduction of an additional amino group to the molecule.

In searching for improved compounds, we assumed that an increase of basicity of an aminoglycoside molecule by replacing an amino group with a more basic functional group, instead of increasing the number of amino groups, could improve the antibacterial activity without increasing the toxicity. The basicity of an amino group (p*K*a *ca.* 10) could be increased by converting it to an amidino (p*K*a *ca.* 12) or guanidino (p*K*a *ca.* 13) group.

Introduction of an amidino or guanidino group in some aminoglycoside antibiotics has been reported to reduce acute toxicity and/or nephrotoxicity compared to the parent antibiotics. For example, 2'-*N*-formimidoylistamycin B ($LD_{50}$ >300 mg/kg)[20] is less toxic than istamycin (150 mg/kg), while their

antibacterial activities are similar. Another example is the 2'-guanidyl derivative of gentamicin $C_1$ (S86451), which was found at least 2~5 times less toxic than gentamicin $C_1$ in terms of nephrotoxic potential[21]. The third example is streptomycin with two guanidino groups and one amino group in the molecule, which exhibits much lower nephrotoxicity than typical aminoglycosides having four or five amino groups[22].

This paper describes the synthesis and biological activities of 1-*N*-[4-(substituted)amidino- and 4-guanidino-2-hydroxybutyryl]kanamycins A and B.

## Chemistry

Preparation of the key intermediates (**4** and **5**) is summarized in Scheme 1. Di-*N*-*tert*-butoxycarbonyl(Boc)-kanamycin A (**1a**)[23] was converted to di-*N*-Boc-mono-*N*-benzyloxycarbonyl(Cbz)-amikacin (**2a**) by reported procedures[24~26]. The remaining 3''-amino group on **2a** was protected with the Boc group to give the mono-*N*-Cbz-tri-*N*-Boc-amikacin (**3a**). Removal of the Cbz group of **3a** by catalytic hydrogenation afforded the key intermediate, 3,3'',6'-tri-*N*-Boc-amikacin **4**.

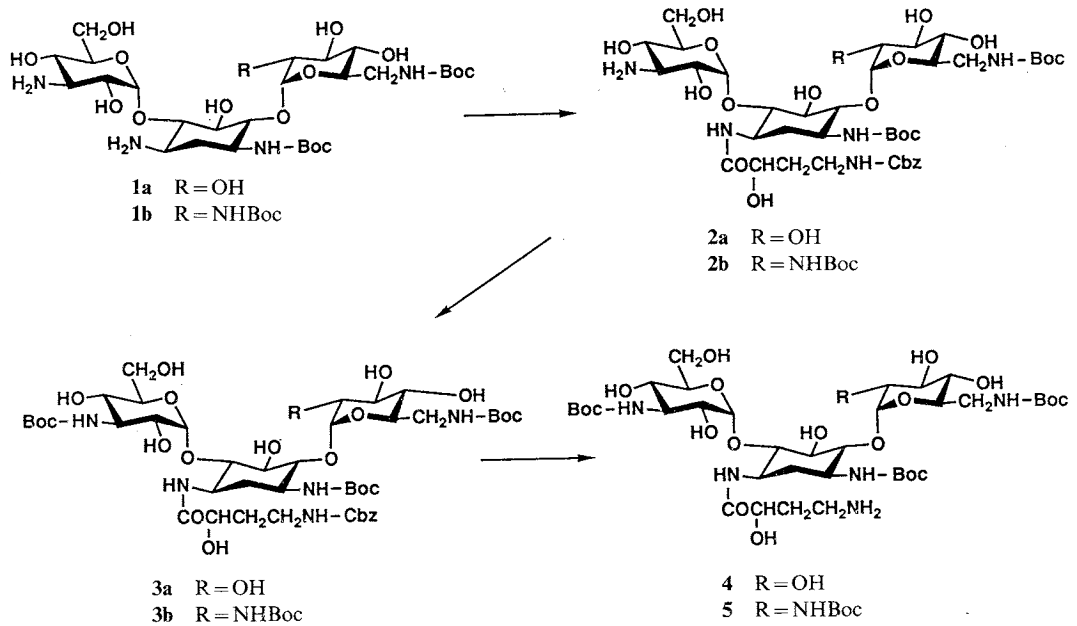
Another key intermediate, 2',3,3'',6'-tetra-*N*-Boc-1-*N*-[4-amino-2-hydroxybutyryl(AHB)]kanamycin B (**5**), was prepared from tri-*N*-Boc-kanamycin B (**1b**)[27] by a method similar to that described above.

Scheme 2 shows the procedures for preparation of the 4'''-amidino or guanidino derivatives. Reaction of **4** with ethyl formimidate hydrochloride[28], followed by deblocking of **6a** with trifluoroacetic acid (TFA) gave 1-*N*-(4-amidino-2-hydroxybutyryl)kanamycin A (**7a**). The acetamidino derivative **7j** was similarly prepared by use of ethyl acetimidate.

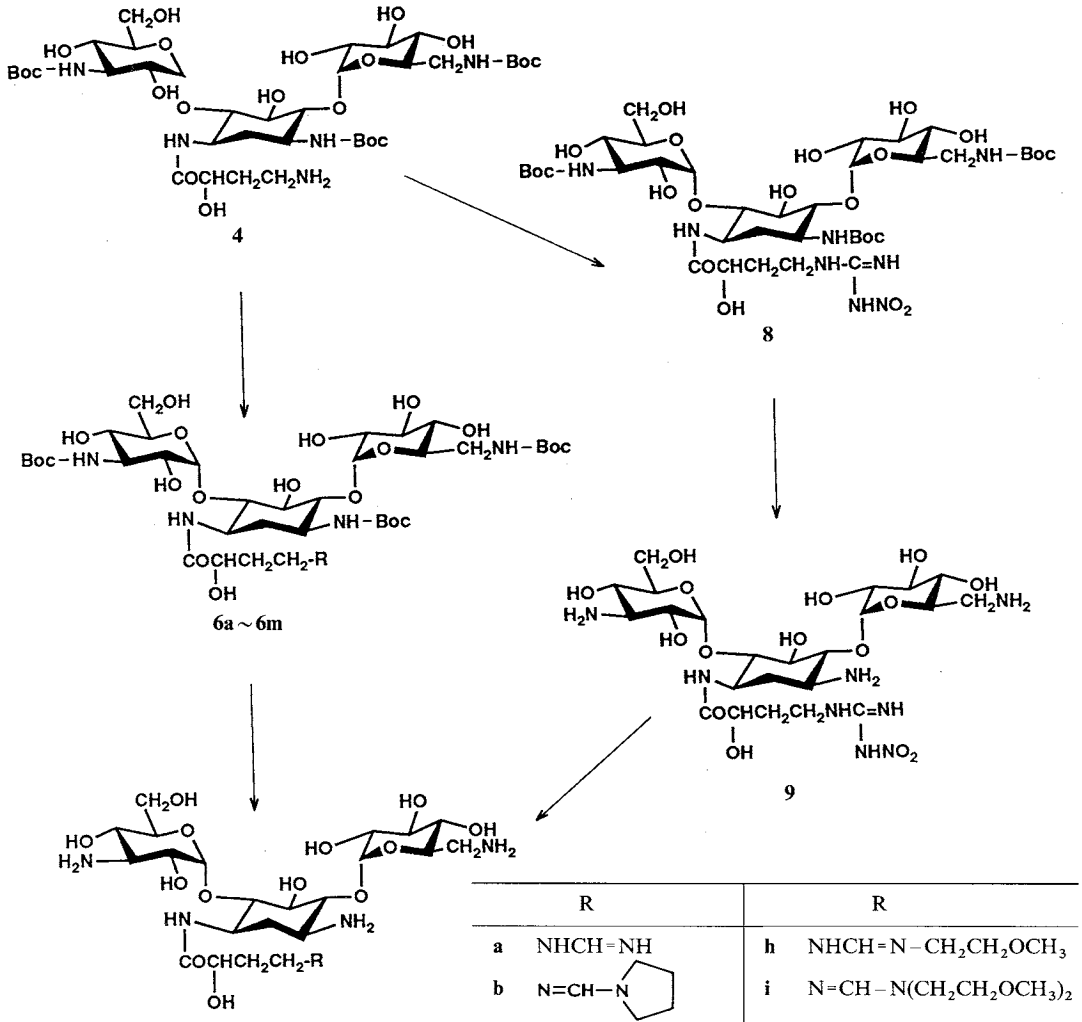
For preparation of the *N*-alkylamidino derivatives (**7b**~**7i**), appropriate *N*-alkylformamides were reacted with dimethyl sulfate to give methoxyimidinium salts which were condensed *in situ* with **4** to give the *N*-substituted imidates, **6b**~**6i**. The Boc groups of **6b**~**6i** were removed with TFA to give **7b**~**7i**.

The morpholino derivative **7e** was also prepared by an alternative route. The 4'''-amino group of **4** was activated by reaction with ethyl *N*-cyanoimidate[29] to give the 4'''-*N*-cyanoformimidate (**6m**) which

Scheme 1. Preparation of key intermediates, **4** and **5**.



Scheme 2. Preparation of 1-*N*-[4-(substituted)amidino- and guanidino-2-hydroxybutyryl]kanamycin A.




| | R | | R |
|---|---|---|---|
| a | NHCH=NH | h | NHCH=N–$CH_2CH_2OCH_3$ |
| b | N=CH–N (pyrrolidine) | i | N=CH–$N(CH_2CH_2OCH_3)_2$ |
| c | N=CH–N (piperidine) | j | NHC(=$NH_2$)–$CH_3$ |
| d | N=CH–N (azepane) | k | NHC(=NH)–$NH_2$ |
| e | N=CH–N (morpholine) | l | NHC(=NH)–COOH |
| f | N=CH–$NMe_2$ | m | NH–CH=N–CN |
| g | NHCH=N–$C_2H_5$ | n | NHCHO |

was then treated with morpholine to afford **6e** and subsequently deblocked to **7e**. This procedure may be applicable to other *N*-amidino derivatives.

For introduction of a guanidino group, **4** was treated with *S*-methyl-*N*-nitroisothiourea[30] to afford the protected *N*-nitroguanidine (**8**), which was reacted with TFA to give the *N*-nitroguanidino derivative (**9**). Catalytic hydrogenation of **9** afforded 1-*N*-(4-guanidino-2-hydroxybutyryl)kanamycin A (**7k**).

Reaction of **4** with benzyl *S*-ethylthiooxaminium tetrafluoroborate[31] (prepared *in situ* by the reaction of benzyl thiooxamate and triethyloxonium tetrafluoroborate) gave the oxamic acid derivative (**6l**), which was deblocked by treatment with TFA to afford the amidinocarboxylic acid (**7l**).

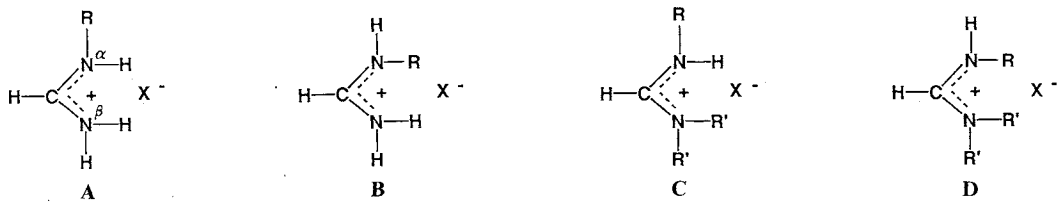
The final products (**7**) were all isolated as the sulfate and characterized principally by $^1$H NMR spectra as shown in the Experimental section.

Table 1. The $^1$H NMR and $^{13}$C NMR spectral data of compounds **7a** and **7k** in comparison with amikacin (AMK) determined at pH 2 in $D_2O$.

| Position | $^{13}$C NMR $\delta$ (ppm) | | | $^1$H NMR | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AMK | **7a** | | | **7k** | | |
| | AMK[33] | **7a** | **7k** | $\delta$ (ppm) | $\delta$ (ppm) | Multiplicity | $J$ (Hz) | $\delta$ (ppm) | Multiplicity | $J$ (Hz) |
| 1 | 49.6 | 49.6 | 49.6 | *ca.* 4.1 | *ca.* 4.1 | m | | *ca.* 4.1 | m | |
| 2 | 30.9 | 30.9 | 31.0 | 1.84 (ax) | 1.85 (ax) | q | 13 | 1.88 (ax) | q | 13 |
| | | | | 2.26 (eq) | 2.23 (eq) | dt | 13, 8 | 2.24 (eq) | ddd | 13, 7, 4 |
| 3 | 48.7 | 48.8 | 49.8 | 3.66 | *ca.* 3.6 | m | | 3.58 | ddd | 13, 10, 4 |
| 4 | 79.8 | 79.7 | 79.5 | *ca.* 3.9 | *ca.* 3.9 | m | | 3.94 | t | 10 |
| 5 | 73.2 | 73.5 | 73.8 | *ca.* 3.9 | *ca.* 3.9 | m | | 3.89[a] | t | 10 |
| 6 | 81.8 | 80.9 | 81.0 | *ca.* 3.9 | *ca.* 3.9 | m | | 3.90[a] | t | 10 |
| 1′ | 96.3 | 96.5 | 96.7 | 5.58 | 5.58 | d | 4 | 5.61 | d | 4 |
| 2′ | 71.6 | 71.6 | 71.7 | 3.71 | 3.68 | dd | 4, 10 | 3.69 | dd | 10, 4 |
| 3′ | 73.1 | 73.0 | 73.0 | *ca.* 3.8 | *ca.* 3.8 | m | | *ca.* 3.8 | m | |
| 4′ | 71.6 | 71.7 | 71.9 | *ca.* 3.4 | 3.44 | m | | 3.38[b] | t | 10 |
| 5′ | 69.5 | 69.5 | 69.6 | 4.07 | 4.03 | m | 3, 8 | 4.03 | ddd | 10, 8, 3 |
| 6′ | 41.2 | 41.2 | 41.3 | *ca.* 3.2 (ax) | 3.18 (ax) | dd | 8, 13 | 3.18 | dd | 13, 8 |
| | | | | *ca.* 3.8 (eq) | 3.5 (eq) | m | | 3.48 | dd | 13, 3 |
| 1″ | 98.8 | 98.7 | 98.8 | 5.20 | 5.18 | d | 4 | 5.19 | d | 4 |
| 2″ | 68.8 | 68.8 | 68.8 | *ca.* 3.8 | 3.77 | dd | 4, 11 | 3.79 | dd | 10, 4 |
| 3″ | 56.1 | 56.1 | 56.1 | *ca.* 3.4 | *ca.* 3.5 | m | | 3.42[b] | t | 10 |
| 4″ | 66.3 | 66.3 | 66.4 | 3.73 | 3.70 | t | 10 | 3.73 | t | 10 |
| 5″ | 72.8 | 72.8 | 72.8 | *ca.* 4.1 | *ca.* 4.1 | m | | *ca.* 4.1 | m | |
| 6″ | 60.5 | 60.6 | 60.6 | *ca.* 3.8 | *ca.* 3.8 | m | (2H) | *ca.* 3.8 | m | (2H) |
| 1‴ | 176.3 | 176.6 | 176.9 | | | | | | | |
| 2‴ | 70.4 | 69.8 | 69.8 | 4.31 | *ca.* 4.2 | m | | 4.23 | dd | 10, 4 |
| 3‴ | 31.6 | 32.1 | 33.3 | 1.99, 2.20 | *ca.* 1.9 | m | (2H) | *ca.* 2.0 | m | (2H) |
| 4‴ | 37.8 | 38.8 | 38.6 | *ca.* 3.2 | *ca.* 3.6 | m | (2H) | *ca.* 3.4 | m | (2H) |
| 5‴ | | 155.2 | 157.8 | | 7.82, 7.83 | s | | | | |

[a,b] Assignments may be interchanged.

Scheme 3. Rotational isomers of **7a** (**A** and **B**) and its *N*,*N*-disubstituted derivatives (**C** and **D**).




R = The residue of 7. $X^- = \frac{1}{2}(SO_4^{=})$. R′ = *N*-Substituent.

The $^{1}H$ and $^{13}C$ NMR spectra of **7a** and **7k** are summarized in Table 1 as compared with those of amikacin. As shown in the table, **7a** and **7k** showed similar $^{1}H$ and $^{13}C$ NMR spectra to those of amikacin, except for signals caused by the formamidino group ($^{1}H$ NMR $\delta$ 7.82 and 7.83, 3:1, total 1H, s; $^{13}C$ NMR $\delta$ 155.2) in **7a** and the guanidino group ($^{13}C$ NMR $\delta$ 157.8) in **7k**.

The formamido proton of **7a**, which occurred as a doublet at room temperature as shown above,

Scheme 4. Preparation of 1-*N*-(4-amidino- and guanidino-2-hydroxybutyryl)kanamycin B.

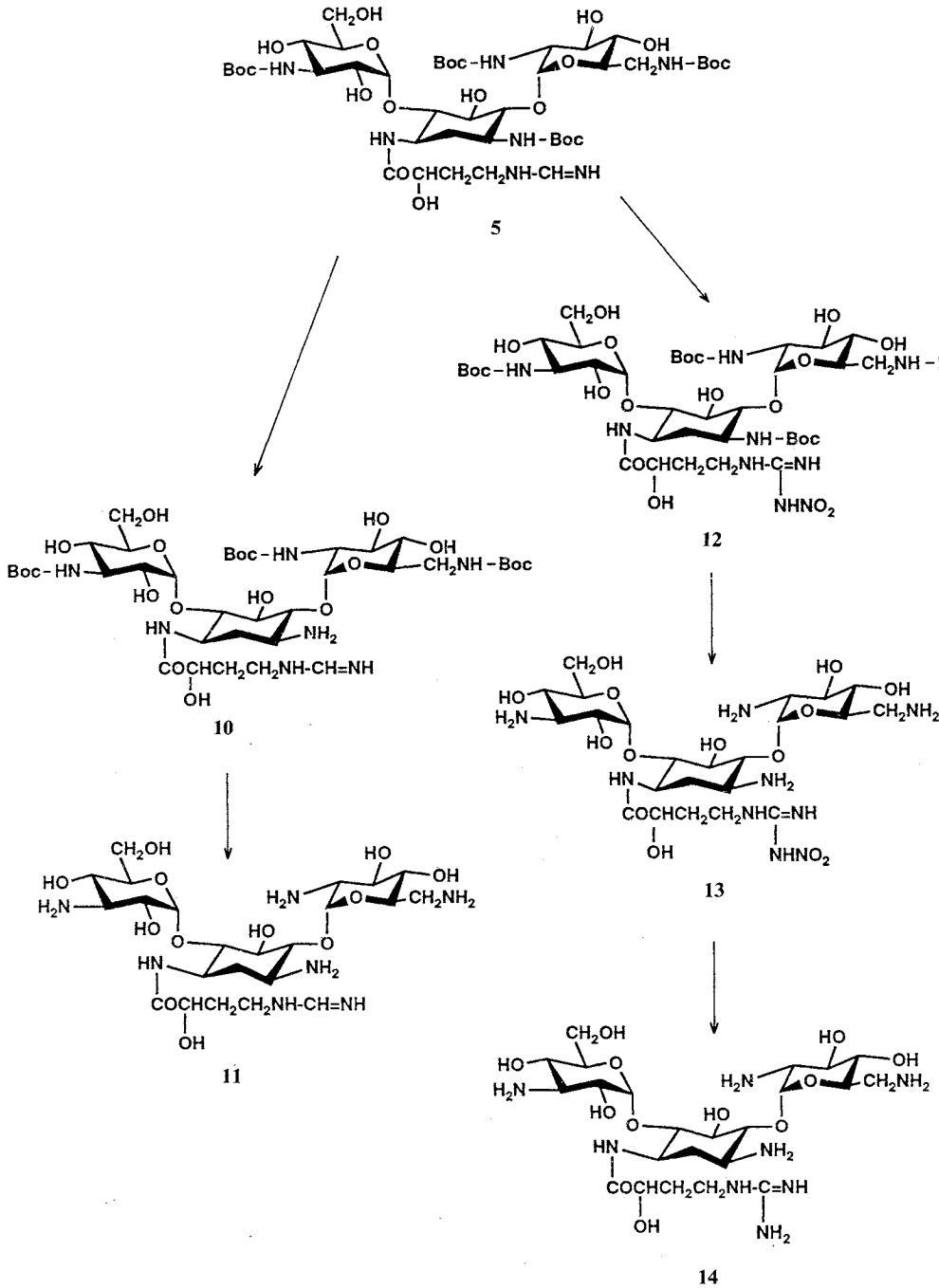


Table 2. *In vitro* antibacterial activity of 1-*N*-(4-amidino- and guanidino-2-hydroxybutyryl)kanamycins A and B, and derivatives.

| Organism[a] | MIC (μg/ml) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **7a** | **7b** | **7c** | **7d** | **7e** | **7f** | **7g** | **7h** | **7i** | **7j** | **7k** | **7l** | **11** | **14** | AMK | AHB-KMB |
| *Staphylococcus aureus* Smith | 0.8 | 3.1 | 1.6 | 1.6 | 0.8 | 3.1 | 1.6 | 1.6 | 12.5 | 1.6 | 0.8 | 50 | 0.8 | 0.4 | 0.8 | 0.4 |
| *Escherichia coli* Juhl A15119 | 3.1 | 12.5 | 12.5 | 12.5 | 6.3 | 12.5 | 6.3 | 12.5 | 50 | 3.1 | 3.1 | >100 | 1.6 | 12.5 | 3.1 | 1.6 |
| *E. coli* ML1630 | 1.6 | 12.5 | 6.3 | 6.3 | 3.1 | 6.3 | 6.3 | 6.3 | 25 | 3.1 | 1.6 | 50 | 1.6 | 1.6 | 1.6 | 1.6 |
| *E. coli* JR66/W677 | 3.1 | 50 | 25 | 50 | 6.3 | 12.5 | 25 | 25 | 100 | 12.5 | 3.1 | >100 | 50 | >50 | 3.1 | 12.5 |
| *Klebsiella pneumoniae* D11 | 0.4 | 1.6 | 0.8 | 1.6 | 0.8 | 1.6 | 1.6 | 3.1 | 6.3 | 1.6 | 0.8 | 12.5 | 0.8 | 0.8 | 0.8 | 0.4 |
| *Enterobacter cloacae* A21006 | 1.6 | 12.5 | 6.3 | 6.3 | 3.1 | 6.3 | 6.3 | 12.5 | 12.5 | 6.3 | 1.6 | 50 | 1.6 | 1.6 | 1.6 | 1.6 |
| *Serratia marcescens* A22302 | 50 | >100 | 100 | >100 | 50 | 100 | >100 | >100 | >100 | >50 | >50 | >100 | 50 | >50 | 25 | 12.5 |
| *Pseudomonas aeruginosa* A9843A | 12.5 | ND | ND | ND | 6.3 | 25 | 25 | 25 | 100 | 12.5 | 6.3 | 100 | 12.5 | 3.1 | 3.1 | 6.3 |
| *P. aeruginosa* A20601 | 3.1 | 50 | 3.1 | 1.6 | 6.3 | 25 | 25 | 25 | 100 | 12.5 | 3.1 | 50 | 6.3 | 3.1 | 3.1 | 3.1 |
| *P. aeruginosa* GN315 | 50 | 100 | 12.5 | 25 | 50 | >100 | >100 | >100 | >100 | >50 | 50 | >100 | 12.5 | 50 | 12.5 | 3.1 |

[a] Resistance mechanism: *E. coli* ML1630, APH(3′)-1; *E. coli* JR66/W677, APH(3′)-II, AAD(2″); *E. cloacae* A21006, APH(3′)-I; *S. marcescens* A22302, AAC(6′)-II; *P. aeruginosa* A20601, AAC(3)-I; *P. aeruginosa* GN315, AAC(6′)-IV. Abbreviation for aminoglycoside-modifying enzymes, see ref 34.

Table 3. *In vivo* antibacterial activity of 4‴-amidino and guanidino derivatives of amikacin (AMK) in mice.

| Test organism | $PD_{50}$ (mg/kg, im) | | | | |
|---|---|---|---|---|---|
| | **7a** | **7e** | **7j** | **7k** | AMK |
| *Staphylococcus aureus* Smith | 0.78 | 1.8 | 3.5 | 0.63 | 1.7 |
| *Escherichia coli* Juhl | 1.2 | 4.2 | 3.5 | 1.1 | 3.1 |
| *Pseudomonas aeruginosa* A9843A | 7.8 | 7.2 | >10 | 9.1 | 7.3 |

coalesced into a singlet ($\delta$ 7.82) in determination at an elevated temperature (about 70°C), whereas the *N*,*N*-disubstituted derivatives (such as **7b**, **7c** *etc.*) gave a singlet even at room temperature. Two peaks in **7a** are probably due to the occurrence of the rotational isomers (**A** and **B**) shown in Scheme 3, in which the C-$N^{\alpha}$ (and also C-$N^{\beta}$) has appreciable double bond character so that rotation around this bond is restricted[32]. In the *N*,*N*-disubstituted derivatives, the isomer **C** is favored very much over the other rotational isomer (**D**) probably as the result of steric repulsion between the substituents R and R′.

Table 4. Acute toxicity and renal accumulation of 4‴-amidino and guanidino derivatives of amikacin (AMK) in mice.

| Compound | $LD_{50}$ (mg/kg, iv) | Renal level[a] ($\mu$g/g-tissue) |
|---|---|---|
| **7a** | 390 | 28.2 |
| **7k** | 224 | 31.8 |
| AMK | 327 | 52.8 |

[a] See the Experimental section.

Generally, the 4‴-*N*-formimidoyl derivatives were stable in acidic solution (pH < 5), but rather unstable in alkaline solution. For example, an attempted purification of 4‴-formimidoylamikacin (**7a**) by chromatography using CM-Sephadex C-25 and diluted ammonium hydroxide afforded biologically inactive 4‴-*N*-formylamikacin (**7n**). On the other hand, the morpholino derivative (**7e**) was rapidly decomposed to amikacin in pH 8 phosphate buffer.

The 4‴-amidino (**11**) and guanidino (**14**) derivatives of 1-*N*-AHB-kanamycin B were prepared from **5** by similar procedures as shown in Scheme 4. Compounds **7k** and **14** showed a positive reaction in the Sakaguchi test.

## Biological Properties

The *in vitro* antibacterial activity of the 4‴-amidino and guanidino derivatives prepared in this study was tested by an agar dilution method with the results shown in Table 2. The 1-*N*-(4-amidino- and 4-guanidino-2-hydroxybutyryl)kanamycins A (**7a** and **7k**) and B (**11** and **14**) were nearly as active as amikacin and 1-*N*-AHB-kanamycin B, respectively. Good activity of the morpholino derivative (**7e**) should be attributed to formation of amikacin under the test conditions (see the chemistry section). The acetimidoyl derivative (**7j**) was 2~4 times less active than amikacin and the amidinocarboxylic acid derivative (**7l**) was marginally active. Other analogs having *N*-substituent(s) showed reduced antibacterial activity.

Table 3 shows the *in vivo* activity of four selected derivatives. As compared to amikacin, **7a** and **7k** were 2~3 times more potent against both *Staphylococcus aureus* Smith and *Escherichia coli* Juhl and showed nearly the same level of activity against *Pseudomonas aeruginosa* A9843A, while **7e** was as active as and **7j** was less active than amikacin against the three infections.

We utilized renal accumulation and acute toxicity for estimating the potential of the selected compounds to cause their undesirable side effects in humans. Table 4 shows renal accumulation and acute toxicity of **7a** and **7k** compared with those of amikacin in mice. The renal levels of **7a** and **7k** were 53% and 60%

that of amikacin, respectively, suggesting a decreased nephrotoxicity potential. Compound **7a** was somewhat less toxic, whereas **7k** was more toxic than amikacin in terms of $LD_{50}$ values.

## Experimental

### General Procedures

#### Analysis

MP's were determined with a Yanagimoto micro hot-stage apparatus and are uncorrected. IR spectra were recorded on an Analect FX-6160 FT-IR spectrometer and NMR spectra were recorded on a Jeol CL 60HL or Jeol GX-400 spectrometer.

#### Biological Evaluation

MICs were determined by the 2-fold serial agar dilution method using Mueller-Hinton agar with an inoculum size of $10^6$ cfu/ml. *In vivo* antibacterial activity was determined in experimental infections in mice. Mice were challenged ip with 100 times the median lethal dose of the pathogen in a 5% suspension of hog gastric mucin. Test materials were administered im immediately after the bacterial challenge to groups of 5 mice at each dose level. The 50% protective dose ($PD_{50}$) was determined 4 days after infection. Acute lethal toxicity ($LD_{50}$) was determined 4 days after iv injection in a group of 5 mice at each dose level. For the determination of renal accumulation, 20 mg/kg of test sample was injected im in a group of 5 mice once a day for 4 days. The mice were sacrificed 24 hours after the last injection and the kidneys were isolated, homogenized with 6 volumes (v/w) of 1/15 M phosphate buffer (pH 7.5) and centrifuged (3,000 rpm, 10 minutes). The amount of antibiotic in the supernatant was determined by bioassay (test organism, *Bacillus subtilis* PCI 219).

#### 4‴-*N*-Cbz-3,6′-di-*N*-Boc-amikacin A (**2a**)

To a suspension of **1a** (3.42 g, 5 mmol) in acetonitrile (50 ml) was added hexamethyldisilazane (7 ml). The reaction mixture was refluxed for 17 hours and concentrated to an oil. To the oil was added dry acetone (50 ml) and the *N*-hydroxysuccinimide ester of 4-Cbz-amino-2-hydroxybutyric acid (1.58 g, 5 mmol). The reaction mixture was stirred at ambient temperature for 3 hours. Water (3 ml) was added and the solution was acidified to pH 2 with 2 N HCl, stirred at room temperature for 15 minutes, neutralized with 1 N $NH_4OH$ and concentrated to a small volume. The residual precipitate was collected by decantation, crystallized from acetone and collected by filtration to give 2.49 g (54%) of **2a**. The mother liquor was concentrated and triturated with a small volume of acetone to afford 2.07 g (45%) of the second crop of **2a** as a powder (total 4.56 g, 99%): MP >250°C; IR (KBr) $cm^{-1}$ 1690, 1535, 1160, 1050; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 1.40 (18H, s, $CH_3$), 7.32 (5H, s, Ph).

*Anal* Calcd for $C_{40}H_{65}N_5O_{19}\cdot 3H_2O$: C 49.32, H 7.35, N 7.19.
Found: C 49.22, H 7.21, N 7.12.

Compound **2a** was also prepared from **1a** by use of ethyl trifluoroacetate and the *N*-hydroxysuccinimide ester of 4-*N*-Cbz-amino-2-hydroxybutyric acid in DMSO (yield 73%)[19].

#### 1-*N*-(4-*N*-Cbz-amino-2-hydroxybutyryl)-2′,3,6′-tri-*N*-Boc-kanamycin B (**2b**)

Acylation of **1b** (1.30 g, 1.64 mmol) with the *N*-hydroxysuccinimide ester of 4-*N*-Cbz-amino-2-hydroxybutyric acid (689 mg, 1.97 mmol) by a procedure similar to that described above gave **2b** (750 mg, 45%): MP 215°C (dec); IR (KBr) $cm^{-1}$ 1680, 1510; TLC, Rf 0.63 (silica gel, $CHCl_3$ - EtOH - 28% $NH_4OH$ (4 : 4 : 1)).

#### 4‴-*N*-Cbz-3,3″,6′-tri-*N*-Boc-amikacin (**3a**)

To a solution of **2a** (4.6 g, 5.0 mmol) in a mixture of THF - $H_2O$ - triethylamine (7 : 3 : 2, 120 ml) was added 2-Boc-thio-4,6-dimethylpyrimidine (2.4 g, 10 mmol). The reaction mixture was stirred at 60°C for 17 hours and concentrated to *ca.* 20 ml. The resulting precipitate was collected by filtration and recrystallized from EtOAc - THF - $H_2O$ to afford 4.3 g (83%) of **3a**: MP 225~230°C (dec); IR (KBr) $cm^{-1}$ 1690, 1530, 1160, 1040; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 1.43 (27H, s, $CH_3$), 7.26 (5H, s, Ph).

*Anal* Calcd for $C_{45}H_{73}N_5O_{21}$: C 52.98, H 7.21, N 6.87.
Found: C 52.70, H 7.38, N 6.65.

1-*N*-(4-*N*-Cbz-amino-2-hydroxybutyryl)-2′,3,3″,6′-tetra-*N*-Boc-kanamycin B (**3b**)

Compound **2b**, (750 mg, 0.76 mmol) was acylated with bis-Boc anhydride (498 mg, 2.28 mmol) by the procedure described in the preparation of **3a** to give **3b** (700 mg, 85%), which was used for the next reaction without purification: MP 235°C (dec); IR (KBr) $cm^{-1}$ 1680, 1510; TLC, Rf 0.85 (silica gel, $CHCl_3$-EtOH-28% $NH_4OH$ (4:4:1)).

3,3″,6′-Tri-*N*-Boc-amikacin (**4**)

A solution of **3a** (15.2 g, 19.3 mmol) in a mixture of THF-MeOH-$H_2O$-AcOH (10:5:5:1, 420 ml) was hydrogenated in the presence of 10% Pd-C (7.6 g). The reaction mixture was worked up as usual and crystallized from MeOH-EtOAc to afford 13.5 g (93%) of **4**: MP 270°C (dec); IR (KBr) $cm^{-1}$ 1680, 1530, 1160, 1040; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 1.45 (27H, s, $CH_3$).

*Anal* Calcd for $C_{37}H_{67}N_5O_{19}\cdot 2H_2O$: C 48.20, H 7.76, N 7.60.
Found: C 48.18, H 7.63, N 7.34.

1-*N*-AHB-2′,3,3″,6′-tetra-*N*-Boc-kanamycin B (**5**)

Compound **3b** (700 mg) was hydrogenated in the presence of 10% Pd-C (350 mg) to give 572 mg (93%) of **5**: MP 220°C (dec); IR (KBr) $cm^{-1}$ 1675, 1520; TLC, Rf 0.18 (silica gel, $CHCl_3$-EtOH-28% $NH_4OH$ (4:4:1)).

1-*N*-(4-Formamidino-2-hydroxybutyryl)kanamycin A (**7a**)

To a solution of **4** (1.00 g, 1.13 mmol) in dry MeOH (30 ml) was added ethyl formimidate hydrochloride (1.00 g, 9.1 mmol). The reaction mixture was adjusted to pH 8 with dil sodium methoxide in MeOH and stirred at room temperature for 17 hours, adjusted to pH 4 with 2 N HCl and filtered. The filtrate was concentrated and the residue chromatographed on silanized silica gel (Merck, 5% $HCOONH_4$-THF (5:1)) and then Sephadex LH-20 (THF-MeOH-$H_2O$ (2:1:1)), to give crude **6a** (1.65 g), which was used in the next reaction without further purification: IR (KBr) $cm^{-1}$ 1630, 1590, 1400, 1350, 1160, 1040; $^1H$ NMR (60 MHz, DMSO-$d_6$+$D_2O$) $\delta$ 1.42 (27H, s, $CH_3$), 8.05 (1H, s, N=CH-N).

Crude **6a** (826 mg) was dissolved in TFA (15 ml); the reaction mixture was kept at room temperature for 40 minutes and then concentrated to dryness. The residue was chromatographed on a column of Sephadex G-10 to afford 420 mg (79%) of **7a** as a TFA salt.

A solution of the TFA salt (335 mg) in cold 2 N $H_2SO_4$ (3.1 ml) was added to stirred and ice-chilled EtOH (62 ml) and the precipitate was collected by filtration to give a powder. The powder was dissolved in water (3 ml) and the solution adjusted to pH 6 with Bio Rad-AG-1X10 ($OH^-$) and filtered. The filtrate was lyophilized to give 239 mg (70% from **4**) of **7a**-sulfate. MP 230°C (dec); IR (KBr) $cm^{-1}$ 1700, 1620, 1510; $^1H$ NMR (400 MHz) and $^{13}C$ NMR (100 MHz), see Table 1.

*Anal* Calcd for $C_{24}H_{46}N_6O_{13}\cdot 2H_2SO_4\cdot 4H_2O$: C 32.21, H 6.53, N 9.39, S 7.17.
Found: C 32.50, H 6.81, N 9.11, S 7.40.

Treatment of crude **6a** (127 mg) by a procedure similar to that described above except for chromatography on CM-Sephadex C-25 ($NH_4^+$, eluting with 0.2 N $NH_4OH$) instead of Sephadex G-10 (eluting with water) afforded 100 mg (92% from **6a**) of 4‴-*N*-formylamikacin (**7n**). IR (KBr) $cm^{-1}$ 1650, 1520, 1120; $^1H$ NMR (60 MHz, $D_2O$) 1.5~2.5 (4H, m, $CH_2$), 5.20, 5.60 (1H each, d, $J$=3.5 Hz, anomeric H), 8.08 (1H, s, CHO).

*Anal* Calcd for $C_{23}H_{43}N_5O_{13}\cdot 1\frac{1}{2}H_2SO_4\cdot 4H_2O$: C 33.82, H 6.66, N 8.57, S 5.89.
Found: C 33.89, H 6.48, N 8.47, S 6.15.

General Procedure for 1-*N*-[2-Hydroxy-4-(3-substituted-formamidino)butyryl]kanamycin A (**7b**~**7i**)

Dimethyl sulfate (1.2 mol equivalents) was added to the *N*-alkylformamide at 60°C for 17 hours to afford an oil. The oil (500 mg) was added to a solution of **4** (500 mg, 0.565 mmol) in the parent formamide (3 ml). The solution was held at room temperature overnight and then chromatographed on a column of Sephadex LH-20 (THF-MeOH-$H_2O$ (2:1:1)) to give a crude product **6b**~**6i**, which was used in the

next step without purification.

The above product was dissolved in 90% TFA (10 ml); the reaction mixture was kept at ambient temperature for 0.5 hour and then concentrated *in vacuo*. The residue was purified by chromatography on a column of CM-Sephadex C-25 ($NH_4^+$, gradient elution with 3~5 N $HCOONH_4$) and Sephadex G-10 (150 ml, water) to afford a powder. The residual powder was dissolved in 2 N $H_2SO_4$ (2 ml) and the solution added dropwise to stirred and ice-chilled EtOH (20 ml). The resulting precipitate was collected by filtration and dissolved in water (2 ml). The solution was adjusted to pH 6 with Dowex 1X2 (OH) and filtered off. The filtrate was lyophilized to give the desired products, **7b**~**7i**.

1-*N*-[2-Hydroxy-4-(1-pyrrolidinomethylideneamino)butyryl]kanamycin A (**7b**)

Yield 47% from **4**; IR (KBr) $cm^{-1}$ 1700, 1630, 1130, 1050; $^1H$ NMR (400 MHz, $D_2O$+DCl, pH 2) $\delta$ 1.83 (1H, q, $J$=13 Hz, 2-$H_{ax}$), 1.8~2.2 (6H, m, $CH_2$), 2.24 (1H, dt, $J$=13 and 4 Hz, 2-$H_{eq}$), 3.18 (1H, dd, $J$=14 and 8 Hz, 2'-H), 5.18 (1H, d, $J$=4 Hz, 1''-H), 5.55 (1H, d, $J$=4 Hz, 1'-H), 8.03 (1H, s, N=CH-N).

*Anal* Calcd for $C_{27}H_{50}N_6O_{13}\cdot 2H_2SO_4\cdot 4H_2O$: C 34.69, H 6.68, N 8.99, S 6.86.
Found: C 34.66, H 6.79, N 8.75, S 7.02.

1-*N*-[2-Hydroxy-4-(1-piperidinomethylideneamino)butyryl]kanamycin A (**7c**)

Yield 73%; IR (KBr) $cm^{-1}$ 1700, 1630, 1130, 1050; $^1H$ NMR (400 MHz, $D_2O$+DCl, pH 2) $\delta$ 1.69 (6H, br s, $CH_2$), 2.24 (1H, dt, $J$=13 and 4 Hz, 2-$H_{eq}$), 3.19 (1H, dd, $J$=13 and 8 Hz, 2'-H), 5.18 (1H, d, $J$=4 Hz, 1''-H), 5.56 (1H, d, $J$=4 Hz, 1'-H), 7.79 (1H, s, N=CH-N).

*Anal* Calcd for $C_{28}H_{52}N_6O_{13}\cdot 2H_2SO_4\cdot 4H_2O$: C 35.44, H 6.80, N 8.86, S 6.76.
Found: C 35.48, H 6.81, N 8.67, S 6.94.

1-*N*-[4-(Hexamethyleneiminomethylideneamino)-2-hydroxybutyryl]kanamycin A (**7d**)

Yield 61%; IR (KBr) $cm^{-1}$ 1690, 1630, 1130; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.5~2.5 (12H, m, $CH_2$), 5.22, 5.63 (1H each, d, $J$=3.5 Hz, anomeric H), 7.94 (1H, S, N=CH-N).

*Anal* Calcd for $C_{29}H_{54}N_6O_{13}\cdot 1\frac{1}{2}H_2SO_4\cdot 8H_2O$: C 35.33, H 7.46, N 8.52, S 4.88.
Found: C 35.60, H 7.22, N 8.53, S 4.53.

1-*N*-[2-Hydroxy-4-(4-morpholinomethylideneamino)butyryl]kanamycin A (**7e**)

Yield 28%; IR (KBr) $cm^{-1}$ 1710, 1620, 1500, 1130, 1050; $^1H$ NMR (60 MHz, $D_2O$) 5.20, 5.62 (1H each, d, $J$=3.5 Hz, anomeric H), 7.85 (1H, s, N=CH-H); Paper chromatography (Whatman No. 50, BuOH - AcOH - $H_2O$ (8:2:5), ninhydrin and bioautography, test organism *B. subtilis* PCI 219), Rf 0.03 (amikacin Rf 0.17).

Alternative preparation of **7e**: To a stirred solution of **4** (500 mg, 0.564 mmol) in dry MeOH (40 ml) was added ethyl cyanoimidate (610 mg, 0.620 mmol)[29] and after 5 hours additional ethyl cyanoimidate (300 mg). The solution was stirred at room temperature for 24 hours, concentrated to dryness and triturated with ether to give crude **6m**. To the solution of crude **6m** in MeOH was added morpholine (5 ml); the reaction mixture was stirred at room temperature for 24 hours, evaporated to dryness and triturated with ether to give crude **6e**. The crude **6e** was treated by the same method as that described in the general procedure to give 223 mg (45%) of **7e**, which was identical to **7e** obtained by the general procedure.

A solution of **7e** (1 mg) in 0.15 M, pH 8, phosphate buffer (1 ml) was kept at ambient temperature for 1 hour. The solution was shown to contain comparable amounts of **7e** (Rf 0.03) and amikacin (0.17) by paper chromatography as described above.

1-*N*-[4-(Dimethylaminomethylideneamino)-2-hydroxybutyryl]kanamycin A (**7f**)

Yield 12%, IR (KBr) $cm^{-1}$ 1705, 1620, 1120, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 3.03, 3.22 (3H each, s, $NCH_3$), 5.15, 5.55 (1H each, d, $J$=3.5 Hz, anomeric H), 7.78 (1H, s, N=CH-N).

1-*N*-[4-(Ethylaminomethylideneamino)-2-hydroxybutyryl]kanamycin A (**7g**)

Yield 9%; IR (KBr) $cm^{-1}$ 1700, 1630, 1120, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.27 (3H, t, $J$=7 Hz, $CH_3$), 1.5~2.5 (4H, m, $CH_2$), 5.18, 5.60 (1H each, d, $J$=3.5 Hz, anomeric H), 7.81 (1H, s, N=CH-N).

1-*N*-[2-Hydroxy-4-(2-methoxyethylaminomethylideneamino)butyryl]kanamycin A (**7h**)

Yield 19%; IR (KBr) $cm^{-1}$ 1700, 1620, 1130, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.5~2.5 (4H, m, $CH_2$), 3.57 (3H, s, $OCH_3$), 5.22, 5.62 (1H each, d, $J$=3.5 Hz, anomeric H), 7.90 (1H, s, N=CH–N).

1-*N*-4-[*N*,*N*-Bis-(2-methoxyethyl)aminomethylideneamino]-2-hydroxybutyryl]kanamycin A (**7i**)

Yield 45%; IR (KBr) $cm^{-1}$ 1690, 1620, 1120, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.5~2.5 (4H, m, $CH_2$), 3.42 (6H, s, $OCH_3$), 5.22, 5.64 (1H each, d, $J$=3.5 Hz, anomeric H), 7.90 (1H, s, N=CH–N).

*Anal* Calcd for $C_{29}H_{56}N_6O_{15}\cdot 2H_2SO_4\cdot 3H_2O$: C 35.57, H 6.79, N 8.58, S 6.55.
Found: C 35.83, H 6.90, N 8.48, S 6.70.

1-*N*-(4-Acetamidino-2-hydroxybutyryl)kanamycin A (**7j**)

By a procedure similar to that used for **7a**, **4** (500 mg) was treated with ethyl acetimidate (500 mg) and sodium methoxide (pH 8) in MeOH (15 ml) at room temperature overnight and subsequently deblocked with TFA to give **7j** *via* **6j**.

Yield 89% from **4**; IR (KBr) $cm^{-1}$ 1630, 1120, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.7~2.5 (4H, m, $CH_2$), 2.30 (3H, s, N=C–$CH_3$), 5.20, 5.62 (1H each, d, $J$=3.5 Hz, anomeric H).

1-*N*-(4-Guanidino-2-hydroxybutyryl)kanamycin A (**7k**)

To a suspension of **4** (300 mg, 0.339 mmol) in dry DMSO (3 ml) was added *S*-methyl-*N*-nitroisothiourea (300 mg, 2.22 mmol)[30]. The reaction mixture was stirred at 55°C for 5 hours, loaded on a column of Sephadex LH-20 (100 ml) and eluted with THF - MeOH - $H_2O$ (1 : 1 : 1) to give **8** (285 mg, 86%): IR (KBr) $cm^{-1}$ 2970, 2920, 1680, 1530, 1370, 1160, 1040; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 1.45 (27H, s, $CH_3$).

Compound **8** (242 mg) was dissolved in 90% TFA (10 ml). The reaction mixture was kept at room temperature for 1 hour and concentrated *in vacuo* to dryness. The residue was chromatographed on a column of CM-Sephadex C-25 ($NH_4$, gradient elution with 0.05~0.2 N $NH_4OH$) to give **9** (138 mg, 79%): IR (KBr) $cm^{-1}$ 1640, 1370, 1280, 1040; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.5~2.5 (4H, m, $CH_2$), 5.25, 5.50 (1H each, d, $J$=3.5 Hz, anomeric H).

*Anal* Calcd for $C_{23}H_{44}N_8O_{15}\cdot 2H_2CO_3$: C 37.69, H 6.07, N 14.06.
Found: C 37.70, H 6.15, N 14.00.

Compound **9** (138 mg) was dissolved in $H_2O$ - AcOH (2 : 1, 6 ml) and hydrogenated in the presence of 10% Pd - C (138 mg) at room temperature under atmospheric pressure overnight. The reaction mixture was filtered and the filtrate concentrated *in vacuo*. The residue was treated as described in the general procedure to give 74 mg (44%) of **7k** as an amorphous powder: IR (KBr) $cm^{-1}$ 1630, 1500, 1120, 1050; $^1H$ and $^{13}C$ NMR: (400 MHz), see Table 1.

*Anal* Calcd for $C_{23}H_{45}N_7O_{13}\cdot 2H_2SO_4\cdot 2H_2O$: C 32.13, H 6.21, N 11.40, S 7.46.
Found: C 32.34, H 6.13, N 11.65, S 7.56.

1-*N*-[2-Hydroxy-4-(oxaloimidoylamino)butyryl]kanamycin A (**7l**)

To a solution of benzyl thiooxamide[31] (1.0 g, 5.1 mmol) in $CH_2Cl_2$ (5 ml) was added 1 M triethyloxonium tetrafluoroborate in $CH_2Cl_2$ (5 ml); the solution was kept at room temperature for 1 hour. To the solution was added a solution of **4** (886 mg, 1 mmol) and triethylamine (405 mg, 4 mmol) in DMSO (5 ml). The reaction mixture was allowed to stand at ambient temperature for 2 days, treated with activated carbon (20 mg), neutralized with AcOH (pH *ca.* 4, 5 drops) and filtered. The filtrate was subjected to chromatography on Sephadex LH-20 (100 ml) eluted with THF - MeOH - $H_2O$ (2 : 1 : 1) to give a colorless powder. The IR and NMR spectra of the powder indicated that the benzyl ester had been cleaved during the work-up procedure to give the desired acid **6l** (664 mg): IR (KBr) $cm^{-1}$ 1680, 1530, 1160, 1040; $^1H$ NMR (60 MHz, DMSO-$d_6$) $\delta$ 1.42 (27H, s, $CH_3$). Compound **6l** (664 mg) was dissolved in 90% TFA (6 ml). The reaction mixture was kept at ambient temperature for 0.5 hour and then concentrated *in vacuo*. The residue was chromatographed on CM-Sephadex C-25 ($NH_4$, gradient elution with water - 1.5 N ammonium formate) and Sephadex G-10 (water), successively, to afford **7l** as a formate (176 mg). The formate was treated as described in the general procedure to give 89 mg (23%) of **7l**: IR (KBr) $cm^{-1}$ 1670, 1520, 1390, 1130, 1050; $^1H$ NMR (60 MHz, $D_2O$) $\delta$ 1.5~2.5 (4H, m, $CH_2$), 5.23, 5.62 (1H each, d, $J$=3.5 Hz, anomeric H); $^{13}C$ NMR (20 MHz, $D_2O$+DCl, pH 2) $\delta$ 30.9, 31.9, 40.1, 41.2, 48.7, 49.6, 56.1,

60.6, 66.4, 68.8, 69.5, 70.0, 71,7, 72.8, 73.1, 73.3, 79.8, 81.1, 96.3, 98.8, 156.2, 159.6, 176.6.

*Anal* Calcd for $C_{24}H_{44}N_6O_{15}\cdot 1\frac{1}{2}H_2SO_4\cdot 5H_2O$: C 31.61, H 6.52, N 9.21, S 5.27.
Found: C 31.81, H 6.33, N 9.19, S 5.87.

1-*N*-(4-Formamidino-2-hydroxybutyryl)kanamycin B (**11**)

By a procedure similar to that described for the preparation of **7a**, **5** (150 mg) was amidinated with ethyl formimidate hydrochloride and deblocked with TFA to afford 159 mg (89%) of **11**: MP 215°C (dec); IR (KBr) $cm^{-1}$ 1713, 1645, 1574; $^1H$ NMR (400 MHz, $D_2O$) $\delta$ 5.21 (1H, d, $J$=3.3 Hz, 1″-H), 6.05 (1H, d, $J$=4 Hz, 1′-H), 7.85 (1H, m, N=CH–N).

*Anal* Calcd for $C_{23}H_{45}N_7O_{12}\cdot 2\frac{1}{2}H_2SO_4\cdot H_2O$: C 30.94, H 6.10, N 10.98, S 8.97.
Found: C 31.20, H 6.19, N 10.59, S 8.68.

1-*N*-(4-Guanidino-2-hydroxybutyryl)kanamycin B (**14**)

To a solution of **5** (257 mg, 0.31 mmol) in dry DMSO (3 ml) were added *S*-methyl-*N*-nitroisothiourea (82 mg, 0.61 mmol) and 5.2 M NaOMe in MeOH (0.06 ml, 0.31 mmol). The reaction mixture was stirred at 60°C for 2 hours and at room temperature for 2 days. It was diluted with EtOAc (50 ml), washed with water (10 ml × 2), concentrated *in vacuo*, and triturated with ether to afford crude **12** (310 mg).

Deblocking of **12** (310 mg) with TFA by a procedure similar to that employed with the kanamycin A analogs gave **13** (53 mg, 23%): MP 220°C (dec); IR (KBr) $cm^{-1}$ 1680, 1620, 1520; TLC, Rf 0.73 ($CHCl_3$ - EtOH - 28% $NH_4OH$, 4 : 4 : 1).

By the procedure used for the preparation of **7i**, **12** (50 mg) afforded **14**, (60 mg, sulfate): MP 235°C (dec); IR (KBr) $cm^{-1}$ 1669, 1654, 1543, 1523; $^1H$ NMR (400 MHz in $D_2O$) $\delta$ 1.8~2.8 (4H, m, $CH_2$), 5.22 (1H, d, $J$=3.7 Hz, 1″-H), 6.07 (1H, d, $J$=3.7 Hz, 1′-H).

*Anal* Calcd for $C_{23}H_{46}N_8O_{12}\cdot 3\frac{1}{2}H_2SO_4H\cdot 4H_2O$: C 26.51, H 5.90, N 10.75, S 10.77.
Found: C 26.21, H 5.69, N 11.06, S 10.24.

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## References

1) BLASER, J.; S. RÜTTIMANN, H. BHEND & R. LÜTHY: Increase of amikacin half-life during therapy in patients with renal insufficiency. Antimicrob. Agents Chemother. 23: 888~891, 1983
2) SAIRIO, E.; A. KASANEN, L. KANGAS, A.-L. NIEMINEN & L. NIEMINEN: The nephrotoxicity and renal accumulation of amikacin. tobramycin and gentamicin. Exp. Path. Bd. 15: S370~375, 1978
3) LUFT, F. C. & S. A. KLEIT: Renal parenchymal accumulation of aminoglycoside antibiotics in rats. J. Infect. Dis. 130: 656~659, 1974
4) GILBERT, D. N.; C. PLAMP, P. STARR, W. H. BENNETT, D. C. HOUGHTON & G. PORTER: Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob. Agents Chemother. 13: 34~40, 1978
5) LUFT, F. C.; M. N. YUM & S. A. KLEIT: Comparative nephrotoxicities of netilmicin and gentamicin in rats. Antimicrob. Agents Chemother. 10: 845~849, 1976
6) PARKER, R. A.; D. N. GILBERT, D. C. HOUGHTON, G. A. PORTER & W. M. BENNETT: Comparative nephrotoxicities of high-dose netilmicin and tobramycin in rats. Antimicrob. Agents Chemother. 18: 346~348, 1980
7) TULKENS, P. M.: Experimental studies on nephrotoxicity of aminoglycosides at low doses; mechanisms and perspectives. Am. J. Med. 80 (Suppl 6B): 105~114, 1986
8) LAURENT, G. & P. M. TULKENS: Aminoglycoside nephrotoxicity: cellular and molecular aspects. ISI Atlas Sci. Pharmacol. 1: 40~44, 1987
9) TULKENS, P. M.: Nephrotoxicity of aminoglycoside antibiotics. Toxicol. Lett. 46: 107~123, 1989
10) PRICE, K. E.: The potential for discovery and development of improved aminoglycosides. Am. J. Med. 80 (Suppl. 6B): 182~189, 1986
11) FUJISAWA, K.; T. HOSHIYA & H. KAWAGUCHI: Aminoglycoside antibiotics. VII. Acute toxicity of aminoglycoside antibiotics. J. Antibiotics 27: 677~681, 1974
12) TODA, S.; S. NAKAGAWA, T. NAITO & H. KAWAGUCHI: Aminoglycoside antibiotics. XV. Chemical conversion of neomycin B to paromomycin I, 6‴-deamino-6‴-hydroxyneomycin B and 6‴-deamino-6‴-hydroxyparomomycin

I. J. Antibiotics 36: 87~91, 1983
13) NARITA, Y.; S. MASUYOSHI, T. YAMASAKI, T. NAITO & H. KAWAGUCHI: Synthesis and activity of butirosin derivatives with 5″-amidino and 5″-guanidino substituents. J. Antibiotics 44: 86~92, 1991
14) NAITO, T.; S. NAKAGAWA & S. TODA (Bristol-Banyu Res. Inst.): 5″-Amino-4′,5″-dideoxybutirosin A. Jpn. Kokai 35132 ('75), Mar. 4, 1975
15) CULBERTON, T. P.; D. R. WATSON & T. H. HASKELL: 5″-Amino-5″-deoxybutirosin, a new semi-synthetic aminoglycoside antibiotic. J. Antibiotics 26: 790~793, 1973
16) WATANABE, I.; T. TSUCHIYA, S. UMEZAWA & H. UMEZAWA: Synthesis of 6′-amino-6′-deoxylividomycin B and 6′-deoxy-6′-methylamino- and 6′-deoxy-6′-(2-hydroxymethylamino)lividomycin B. J. Antibiotics 26: 802~804, 1973
17) WOO, P. W. K.: 5″-Amino-3′,4′,5″-trideoxybutirosin A, a new semisynthetic aminoglycoside antibiotic. J. Antibiotics 28: 522~529, 1975
18) SAEKI, H.; Y. SHIMADA, E. OHKI & S. SUGAWARA: Synthesis of aminotrideoxybutirosin A, a chemically modified antibiotic active against butirosin-resistant bacteria. J. Antibiotics 28: 530~536, 1975
19) KONDO, S.; H. YAMAMOTO, K. IINUMA, K. MAEDA & H. UMEZAWA: Synthesis of 6′,5″,6‴-triamino-6′,5″,6‴-trideoxylividomycin A and 6′,5″-diamino-6′,5″-dideoxylividomycin B. J. Antibiotics 29: 1134~1136, 1976
20) UMEZAWA, H.; Y. OKAMI & S. KONDO (Microb. Chem. Res. Found.): Formimidoyl istamycin B and its process. Jpn. Kokai 150080 ('81), Nov. 29, 1981
21) MALDAGUE, P.; G. LAURENT, M. B. CARLIER & P. TULKENS: A 2′-guanidyl derivative of gentamicin (S86451) with reduced nephrotoxicity: Studies at low and medium dose levels in the rat. Arch. Toxicol. Suppl. 7: 455~458, 1984
22) WILLIAMS, P. D.; D. B. BENNETT, C. R. GLEASON & G. H. HOTTENDORF: Correlation between renal membrane binding and nephrotoxicity of aminoglycosides. Antimicrob. Agents Chemother. 31: 570~574, 1987
23) MATSUDA, K.; N. YASUDA, H. TSUTSUMI & T. TAKAYA: Studies on antiviral agents. II. Synthesis and *in vitro* antiviral activity on new kanamycin derivatives having higher acyl group at *N*-1 position. J. Antibiotics 38: 1050~1060, 1985
24) CRON, M. J.; J. G. KEIL, J.-S. LIN, M. V. RUGGERI & D. WALKER: Selective N-acylation of kanamycin A. J. Chem. Soc. Chem. Commun. 1979: 266~267, 1979
25) DANIELS, P. J. L.; J. WEINSTEIN & T. L. NAGABHUSHAN: The syntheses of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl]gentamicin $C_1$ and 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl]gentamycin $C_1$. J. Antibiotics 27: 889~893, 1974
26) TSUCHIYA, T.; Y. TAKAGI & S. UMEZAWA: 1-*N*-acylation of aminocyclitol antibiotics via zinc chelation and regiospecific *N*-trifluoroacetylation. Tetrahedron Lett. 1979: 4951~4954, 1979
27) MATSUDA, K.; N. YASUDA, H. TSUTSUMI & T. TAKAYA: Studies on antiviral agents. V. Synthesis and *in vitro* antiviral activity of new aminoglycoside derivatives having palmitoyl group. J. Antibiotics 40: 843~854, 1987
28) OHME, R. & E. SCHMITZ: A simple synthesis of alkyl formimidates. Angew. Chem. Int. Ed. Engl. 6: 566, 1976
29) CEREDA, E.; E. BELLORA & A. DONETTI: A new, convenient synthesis of $N^2$-aryl-$N^1$-dialkylformimidates and $N^2$-aryl-$N^1$,$N^1$-dialkylformimidates. Synthesis 1986; 288~291, 1986
30) FISHBEIN, L. & J. A. GALLAGHAN: The preparation and reactions of 2-alkyl-1(or 3)-nitro-2-thiopseudourea. Part I. Reaction with amines. J. Am. Chem. Soc. 76: 1877~1879, 1954
31) NII, Y.; K. OKANO, S. KOBAYASHI & M. OHNO: Synthesis of amidinoformic acids using benzyl cyanoformate as a synthon. Tetrahedron Lett. 1979: 2517~2520, 1979
32) HÄFELINGER, G.: General and theoretical aspects of amidines and imidec acid derivatives. *In* The Chemistry of Amidines and Imidates. *Ed.*, S. PATAI, pp. 1~84, John Wiley & Sons, 1975
33) TODA, S.; S. NAKAGAWA, T. NAITO & H. KAWAGUCHI: Carbon-13 NMR analysis of amikacin and related compounds. Tetrahedron Lett. 1978: 3913~3916, 1978
34) MITSUHASHI, S.: Proposal for a rational nomenclature for phenotype, genotype, and aminoglycoside-aminocyclitol modifying enzymes. *In* Drug Action and Drug Resistance in Bacteria. 2. Aminoglycoside Antibiotics. *Ed.*, S. MITSUHASHI, pp. 269~275, University of Tokyo Press, 1975