SELECTIVE *N*-ACYLATION OF GENTAMICIN ANTIBIOTICS— SYNTHESIS OF 1-*N*-ACYL DERIVATIVES

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Treatment of acid addition salts of aminoglycoside-aminocyclitol antibiotics of the gentamicin-sisomicin class, with one equivalent of triethylamine and an acylating agent results in selective formation of 1-*N*-acyl derivatives. This is in contrast to acylation of the antibiotic free bases which results in preferential acylation of other basic centres in the molecule. Origins of the observed selectivity are discussed. *In vitro* antibacterial activities of several new 1-*N*-acyl derivatives of gentamicin, sisomicin and verdamicin are reported.

Methods for selective acylation at the C-1 amino group of aminoglycoside-aminocyclitol antibiotics have assumed great importance since the demonstration that amikacin,[1] the 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl] derivative of kanamycin A, and similar derivatives of other aminoglycoside antibiotics[2-6] are effective against many bacteria resistant to the parent compounds. Published methods for the preparation of 1-*N*-acylaminoglycosides have hitherto required the selective protection, and subsequent deprotection, of those amino groups in the molecule more reactive than the C-1 amino group. We now wish to report a general procedure for the preparation of 1-*N*-acyl derivatives of the gentamicin-sisomicin class of antibiotics by direct, selective acylation of the parent compounds without the need for prior *N*-protection. A related selective 1-*N*-alkylation procedure has been published recently.[7,8]

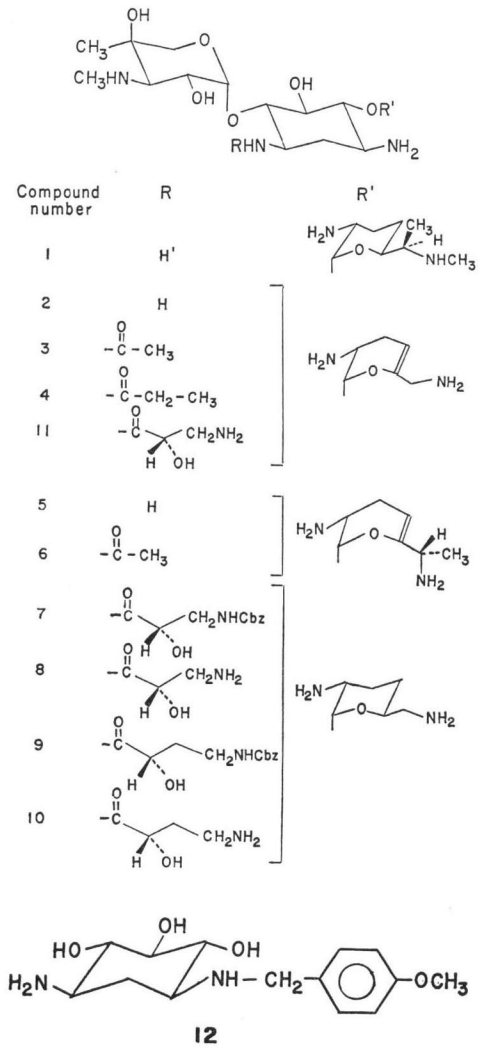
Under conditions normally employed for *N*-acylation, in which the free base of the antibiotic is utilized, acylation occurs preferentially at the primary carbinamine function of those antibiotics in which it is present. Such a preference has been shown for kanamycin[1] and gentamicin C_{1a}.[9] In the case of sisomicin (**2**), in which the primary carbinamine function is also allylic,[10] this preference is even more pronounced in that reaction of sisomicin (**2**) with acetyl-imidazole affords the 6′-*N*-acetyl derivative in >90% yield.[11] On the other hand, gentamicin C_1 (**1**), in which the side-chain at C-5′ is more highly substituted and approach to the C-6′ amino group is correspondingly hindered, undergoes acylation initially at the C-2′ and C-3 amino groups.[6]

We have now shown that this selectivity in the site of acylation is pH-dependent and that it is dramatically altered by choosing reaction conditions in which the amino groups of the antibiotic are almost completely protonated. Under these conditions the C-1 amino group is the most reactive in the molecule towards acylating agents whereas primary carbinamine functions are among the least reactive. Suitable conditions are achieved by the addition of one equivalent of a tertiary amine base to a solution of the fully neutralized acid addition salt or by direct titration of a solution of the substrate to approximately pH 7.

For example, addition of an excess of acetic anhydride to an aqueous methanolic solution of sisomicin sulfate to which one equivalent of triethylamine had been added, gave 1-*N*-acetylsisomicin (**3**) as the major product in 25% yield after chromatography, together with approximately 20% of recovered starting material. The site of acylation was determined according to the method of KAWAGUCHI and co-workers,[1] by borohydride reduction of the tetra-*p*-methoxybenzylidene derivative to form the tetra-*p*-methoxybenzyl analogue followed by acid hydrolysis to give 2-deoxy-3-*N*-*p*-methoxybenzyl-streptamine (**12**), $[\alpha]_D^{26}+46.6°$ (lit.[6] $+44°$).

Using the general method outlined above, the acyl derivatives of gentamicin C_{1a}, sisomicin and verdamicin (**5**) listed in Fig. 1 were prepared. These include both simple alkanoyl compounds as well as the more complex *S*-ω-amino-2-hydroxyacyl derivatives. The latter materials were prepared using suitably *N*-protected active ester derivatives as acylating agents, followed by removal of the *N*-protecting group. In all cases the structures of these derivatives were assigned from their PMR, CMR and mass spectra and by the aforementioned method of KAWAGUCHI.[1]

Fig. 1



CMR titration studies conducted in our laboratories[8] have revealed a wide range of pKa values for the amino groups of the gentamicin antibiotics, and have shown that the C-6′ amino group is the most basic and the C-1 amino group the least basic amino group in the molecule. The selectivity observed in the acylation reaction described reflects the relative rates of reaction of the different amino groups in the molecule. These rates are dependent upon the relative equilibrium concentrations of the free amines under the pH conditions used, that is upon their pKa values, as well as upon steric factors. Some of these steric factors will also be pH-dependent in that protonation of amine groups has been postulated[13,14] to result in conformational changes involving rotation about the C_4-O bond and the adjacent glycosidic linkage.

The selectivity between the two 2-deoxystreptamine amino groups (which have similar pKa values), is presumably largely a consequence of the dissymetric environments of the two groups.

Little selectivity was observed in the acylation of highly hydroxylated antibiotics such as gentamicin B and kanamycin A.

Table 1. *In vitro* antibacterial activities of some 1-*N*-acylaminoglycosides.

Test organism	Minimal inhibitory concentration (mcg/ml)*						
	(2)	(3)	(4)	(6)	(8)	(10)	(11)
Pseudomonas aeruginosa Stone 20	0.3	0.075	3.0	0.075	0.3	0.3	0.3
,, Stone 39	0.3	0.75	17.5	3.0	7.5	3.0	0.75
,, NRRL 3223	0.08	0.75	17.5	3.0	3.0	0.75	0.75
,, Stone 130[a]	17.5	3.0	>25	7.5	17.5	17.5	7.5
,, Travers 1[b]	>25	3.0	>25	3.0	17.5	17.5	7.5
Escherichia coli F14-BK	0.3	0.75	7.5	0.75	3.0	3.0	3.0
,, 1574-1	0.3	0.75	7.5	0.75	3.0	0.75	3.0
,, W677/R55[c]	7.5	0.3	7.5	0.75	3.0	0.3	7.5
,, La 290/R55[c]	17.5	0.75	3.0	0.3	3.0	3.0	3.0
,, JR 88[a]	>25	3.0	>25	3.0	3.0	3.0	0.75
Klebsiella pneumoniae Ad 17[d]	0.3	0.3	3.0	0.75	7.5	0.75	3.0
,, Ad 18[d]	0.3	0.3	3.0	0.75	3.0	0.75	3.0
,, Georgetown 3694[c]	7.5	0.3	17.5	0.75	3.0	0.75	3.0
Providence 164[e]	>25	17.5	>25	>25	>25	17.5	>25
Staphylococcus aureus FDA 209P	0.08	0.3	17.5	0.75	0.3	0.75	0.75
,, Wood	0.03	0.3	7.5	0.3	0.075	0.75	0.3
Bacillus subtilis 6623	<0.05	<0.05	0.3	0.03	<0.01	0.075	0.075

* In MUELLER-HINTON broth pH 7.2

Resistance mechanisms: a) aminoglycoside 3-*N*-acetyltransferase-I, b) aminoglycoside 3-*N*-acetyltransferase-II, c) aminoglycoside 2″-*O*-nucleotidyltransferase, d) aminoglycoside 3′-phosphotransferase-II, e) aminoglycoside 2′-*N*-acetyltransferase.

The *in vitro* antibacterial activities of the 1-*N*-acylaminoglycosides listed in Fig. 1 are shown in Table 1 in comparison with those of sisomicin (**2**). The most surprising feature of these results is the high activity of the 1-*N*-acetyl derivatives of sisomicin and verdamicin (compounds **3** and **6** respectively), which show similar or slightly reduced potency compared with the parent antibiotics against sensitive organisms, but increased activity against sisomicin-resistant organisms possessing 3-*N*-acetylating and 2″-*O*-nucleotidylating enzymes. This is in contrast to the weak activity report for the analogous derivative of kanamycin.[5] 1-*N*-Propionylsisomicin (**4**), on the other hand, is a relatively weak antibiotic. The 1-*N*-[(*S*)-ω-amino-2-hydroxyacyl] derivatives (**8**, **10**, and **11**) show reduced potency compared with the parent antibiotics *versus* sensitive organisms, but have substantial activity against resistant organisms. This appears to be a general phenomenon for this type of derivative of aminoglycoside antibiotics having a C-2′ amine group and has been noted previously for derivatives of gentamicin C_1.[6] This is in contrast to the overall enhancement of activity accompanying the attachment of the *S*-4-amino-2-hydroxybutyryl side-chain to the C-1 amino group of kanamycin A.

Experimental

FOURIER transform NMR spectra were taken in D_2O solution using a Varian XL-100-15 instrument equipped with a 620 L computer. PMR shifts are reported downfield from sodium 2, 2-dimethyl-2-silapentane sulfonate using the HOD line (δ 4.68 ppm) as an internal reference. Optical rotations were recorded using a Bendix Model 143 automatic polarimeter. Mass spectra

were obtained using a Varian MAT CH5 spectrometer at 70 eV with a probe temperature of 150~250°C. Column chromatography was carried out on silica gel (70~230 mesh), E. Merck, Darmstadt, W. Germany.

1-*N*-Acetylsisomicin (**3**)

To a solution of sisomicin sulfate (0.7 g) in water-methanol (3 : 2, 75 ml) was added triethylamine (0.14 ml). After 10 minutes, acetic anhydride (1 ml) was added. After 1 hour, the solution was evaporated to dryness and the residue was dissolved in water and passed through a column of Amberlite IRA-401 S(OH^-) ion-exchange resin. The eluant was lyophilized and the residue was chromatographed on silica gel (25 g) in the lower phase of a chloroform - methanol - ammonium hydroxide (7 % NH_3)(2:1:1) system to give, 1-*N*-acetylsisomicin (110 mg, 23 % yield); $[\alpha]_D^{26}$+159° (*c* 0.3, H_2O); PMR (D_2O) δ 1.22 (3H, s, C-C$\underline{H}_3$); 2.02 (3H, s, -COC$\underline{H}_3$); 2.53 (3H, s, N-C$\underline{H}_3$); 4.88 (1H, m, =C$\underline{H}$-); 5.08 (1H, d, $J_{1'',2''}$=4Hz, H-1''); 5.35 (1H, d, $J_{1',2'}$ =2Hz, H-1'); MS $(M+1)^+$ *m/e* 490, M^+ *m/e* 489:

Found: M^+ 489.27986 $C_{21}H_{39}N_5O_8$.
Requires: M 489.29

Starting material (100 mg) was also obtained by collecting the appropriate column fractions.

1-*N*-Propionylsisomicin (**4**)

Sisomicin sulfate (1.25 g) was converted to 1-*N*-propionylsisomicin (**4**) in 27 % yield by treatment with propionic anhydride (1.5 ml) in the presence of triethylamine (0.125 ml) in a manner similar to that described above. The product had m.p. 125~130°C (decomp.), $[\alpha]_D^{26}$+ 147° (*c* 0.3, H_2O); PMR (D_2O) δ 1.08 (3H, t, J=7.5 Hz, $CH_2C\underline{H}_3$); 1.18 (3H, s, C-C$\underline{H}_3$); 2.25 (2H, m, $CH_2C\underline{H}_3$); 2.48 (3H, s, NHC$\underline{H}_3$); 4.87 (1H, m, H-4'); 5.07 (1H, d, $J_{1',2'}$=4.0Hz, H-1''); 5.34 (1H. d, J=$1_{1'}$,$_2$ 2.0Hz, H-1'); MS $(M+1)^+$ *m/e* 504, M^+ *m/e* 503.

Found: M^+ 503.2908 $C_{22}H_{41}N_5O_8$.
Requires: M 503.2955.

1-*N*-Acetylverdamicin (**6**)

Acetylation of verdamicin sulfate (**5**) (1.25 g) following the procedure described for the preparation of 1-*N*-acetylsisomicin (**3**) from sisomicin sulfate (**2**) afforded **6** in 27 % yield, m.p. 140~145°C (decomp.), $[\alpha]_D^{26}$+131° (*c* 0.3, H_2O); PMR (D_2O) δ 1.12 (3H, d, J=7.5Hz, 6'-C-C$\underline{H}_3$); 1.15 (3H, s, 4''-C-C$\underline{H}_3$); 1.95 (3H, s, COC$\underline{H}_3$); 2.45 (3H, s, 3''-N-C$\underline{H}_3$); 4.83 (1H, m, H-4'); 5.03 (1H, d, $J_{1'',J_{2''}}$=4.0Hz, H-1''); 5.28 (1H, d, $J_{1',2'}$=2.0Hz, H-1'). MS$(M+1)^+$ *m/e* 504, M^+ *m/e* 503.

Found: M^+ 503.2910 $C_{22}H_{41}N_5O_8$.
Requires: M 503.2955.

1-*N*-[(*S*)-3-Benzyloxycarbonylamino-2-hydroxypropionyl]-gentamicin C_{1a} (**7**)

To a stirred solution of *N*-benzyloxycarbonyl - L - isoserine[11] (1.91 g, 8 mmoles) and *N*-hydroxysuccinimide (0.92 g, 8 mmoles) in ethyl acetate (60 ml) maintained at about 5°C was added, in small portions, dicyclohexylcarbodiimide (1.65 g, 8 mmoles). After 3 hours, the reaction mixture was filtered and the combined filtrate and ethyl acetate washings were concentrated to dryness under reduced pressure. The residue was dissolved in dimethylformamide (26.5 ml) and used directly in the next step.

To a solution of gentamicin C_{1a} sulfate (3.71 g, 5.3 mmoles) in water-methanol (3:2, 45 ml) was added, with stirring, triethylamine (0.75 ml). After 10 minutes the solution of the active ester was added dropwise. After 2 hours at room temperature the pH of the solution was adjusted to 8 by treatment with Amberlite 1RA-401 S (OH^-) ion-exchange resin. The resin was removed by filtration and the combined filtrate and washing evaporated to dryness. Chromatography of the residue on a 4 cm by 90 cm column of silica gel in the lower phase of a chloroform - methanol - concentrated ammonium hydroxide (2:1:1) system gave the title compound in 20 % yield (0.7 g), $[\alpha]_D^{26}$+105° (*c* 0.3, H_2O); PMR (D_2O) δ 1.17 (3H, s, C-C$\underline{H}_3$); 2.51 (3H, s, N-C$\underline{H}_3$); 2.59 (1H, d, $J_{2'',3''}$=11 Hz, H-3''); 3.79 (H-2''); 4.2 (1H, dd, J=4.0

and 7.0Hz, H-2‴); 5.03 (2H, m, H-1′ and H-2″, both ~3.5Hz); 5.09 (2H, PhC$\underline{H}_2$-); 7.42 (5H, phenyl).

Anal. Calcd. for $C_{30}H_{50}N_6O_{11}\cdot H_2O$: C, 52.39; H, 7.47; N, 12.22%.
Found: C, 52.12; H, 7.57; N, 12.06%.

1-*N*-[(*S*)-3-Amino-2-hydroxypropionyl]-gentamicin C_{1a} (**8**)

1-*N*-[(*S*)-3-Benzyloxycarbonylamino-2-hydroxypropionyl]-gentamicin C_{1a} (**7**) (0.2 g) was dissolved in water (20 ml) and methanol (8 ml) and hydrogenated in the presence of 5% palladium on carbon (65 mg) at 55 psi for 5 hours at room temperature. The catalyst was removed by filtration through Celite and the eluant was treated with Amberlite IRA-401 S (OH^-) ion-exchange resin and lyophilized to give the title compound (0.16 g); $[\alpha]_D^{26}+115°$ (*c* 0.3, H_2O); PMR (D_2O) δ 1.22 (3H, s, C-C$\underline{H}_3$); 2.59 (3H, s, N-C$\underline{H}_3$); 5.09 (1H, d, $J_{1'',2''}=4.0$ Hz, H-1″); 5.17 (1H, d, $J_{1',2'}=3.5$ Hz, H-1′).

Anal. Calcd. for $C_{22}H_{44}N_6O_9\cdot H_2CO_3$: C, 45.01; H, 7.39; N, 13.70%.
Found: C, 44.98; H, 7.81; N, 13.68%.

1-*N*-[(*S*)-4-Amino-2-hydroxybutyryl]-gentamicin C_{1a} (**10**)

Following the procedure described above for the preparation of **8**, gentamicin C_{1a} sulfate (1.4 g) was reacted with *N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryloxy]-succinimide (1.0 g)[1] and triethylamine (0.28 ml). The 1-*N*-[(*S*)-4-benzyloxycarbonylamino-2-hydroxybutyryl]-gentamicin C_{1a} (**9**) thus obtained (0.28 g) in 20% yield was hydrogenolyzed as mentioned above to give pure **10**, $[\alpha]_D^{26}+100°$ (*c* 0.2, water); PMR (D_2O) δ 1.17 (3H, s, C-C$\underline{H}_3$); 2.48 (3H, s, N-C$\underline{H}_3$); 3.75 (1H, dd, $J_{1'',2''}=3.7$ Hz, $J_{2'',3''}=11$ Hz, H-2″); 4.22 (1H, q, J=9.5 Hz and 4.0 Hz, H-3‴), 5.04 (2H, H-1′ and H-1″).

Anal. Calcd. for $C_{23}H_{46}N_6O_9\cdot CO_2\cdot 4H_2O$: C, 42.25; H, 7.66; N, 11.82%.
Found: C, 42.02; H, 7.49; N, 11.96%.

1-*N*-[(*S*)-3-Amino-2-hydroxypropionyl]-sisomicin (**11**)

A solution of (*S*)-3-benzyloxycarbonylamino-2-hydroxypropionic acid (5 g) in 20% aqueous dioxan (100 ml) was hydrogenated in the presence of 30% palladium-on-carbon (0.2 g) at 50 psi. After 3 hours, the catalyst was removed by filtration and the filtrate evaporated to dryness. The residue was dried thoroughly and then dissolved in ice-cold trifluoroacetic anhydride (30 ml). The mixture was set aside 3 hours at room temperature. The excess reagent was removed by evaporation *in vacuo* and the residue triturated with benzene to a hygroscopic solid which was isolated by filtration, washed with benzene and dried to give 3.84 g (91%). The compound was used in the next step without further purification since the mass and nmr spectra were consistent with the structure for *S*-3-*N*-trifluoroacetamido-2-hydroxypropionic acid [*m/e* 201 (M^+), 202 $(M+1)^+$; NMR (D_2O) δ 3.72 (3H, d, J=5.5 Hz, H-3), 4.48 (1H, t, J=5.5 Hz, H-2)]. 1-*N*-[(*S*)-3-Amino-2-hydroxypropionyl]-sisomicin (**11**) was prepared from sisomicin sulfate and the above amino acid in a manner similar to that described for the preparation of **8** with the exception that prior to chromatographing the crude condensation product, the mixture was treated with 30 ml of ammonium hydroxide for 5 hours to remove the *N*-trifluoroacetamido blocking group. Pure 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl]-sisomicin (**11**) was obtained in 19% yield, $[\alpha]_D^{26}+139°$ (*c* 0.038, water); PMR (D_2O) δ 1.18 (3H, s, C-C$\underline{H}_3$); 2.53 (3H, s, N-C$\underline{H}_3$); 4.0 (1H, d, $J_{5''_{ax,eq}}=13$ Hz, H-5″$_{eq}$); 4.15 (1H, unresolved q, H-2‴); 4.83 (1H, m, =C$\underline{H}$); 5.06 (1H, d, $J_{1'',2''}=4$Hz, H-1″); 5.33 (1H, d, ($J_{1',2'}=2$Hz, H-1′). MS $(M+1)^+$ *m/e* 535, M^+ *m/e* 534.

Anal. Calcd. for $C_{22}H_{42}N_6O_9\cdot(1/2)H_2CO_3$: C, 47.74; H, 7.66; N, 14.85%.
Found: C, 47.39; H, 7.14; N, 15.16%.

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