CHEMICAL MODIFICATION OF SOME GENTAMICINS AND SISOMICIN AT THE 3″-POSITION

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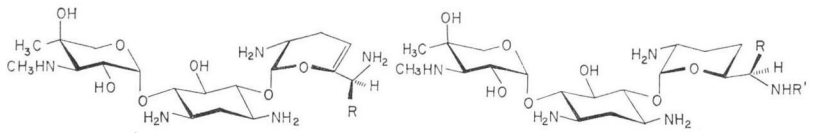
(Received for publication October 18, 1977)

Chemical and photochemical oxidative methods of de-*N*-methylation of some gentamicins and sisomicins at the 3″-position are described. Selective acetylation of gentamicins and sisomicins at the 1, 3, 2′ and 6′ and of gentamicin B at the 1, 3, and 6′ positions are achieved by treatment of the free bases with carbon dioxide prior to acetylation. De-*N*-methylation of the above selectively blocked gentamicins and sisomicins followed by re-alkylation at the 3″-position and de-*N*-protection gives a series of 3″-*N*-alkyl analogues. The *in vitro* antibacterial properties of the new derivatives of gentamicins and sisomicins are given.

Recent chemical and microbiological studies in our laboratories have shown[1] that sisomicin (**1**) can be readily modified at the 6′-amino group by alkylation to yield a number of 6′-*N*-alkylsisomicins which inhibit the growth of sisomicin sensitive organisms and organisms that inactivate sisomicin by 6′-*N*-acetylation.[2,3] Further, we have demonstrated that selective *N*-alkylation of sisomicin, verdamicin (**2**) and some of the gentamicins (**3**～**5**) at the 1-position provides the corresponding 1-*N*-alkyl derivatives which possess a microbiological spectrum of activity superior to that of the parent antibacterials.[4,5] Of particular interest is the finding that alkylation at the 1-position in these antibiotics prevents enzymic adenylylation at the 2″-position by resistant organisms known to inactivate aminoglycoside antibiotics.[5,6] These results and the fact that netilmicin, the 1-*N*-ethyl derivative of sisomicin shows less chronic toxicity[5~7] than sisomicin in animal models prompted us to examine the possibility of replacing the *N*-methyl group at the 3″-position of sisomicin and gentamicin C_2 by other alkyl functions and study its effect on the antimicrobial spectrum. This publication is hence concerned with methods of removal of the 3″-*N*-methyl group in sisomicin and the gentamicins, and the synthesis and microbiological evaluation of a number of their 3″-*N*-alkyl derivatives.



1 Sisomicin R = H
2 Verdamicin R = CH_3

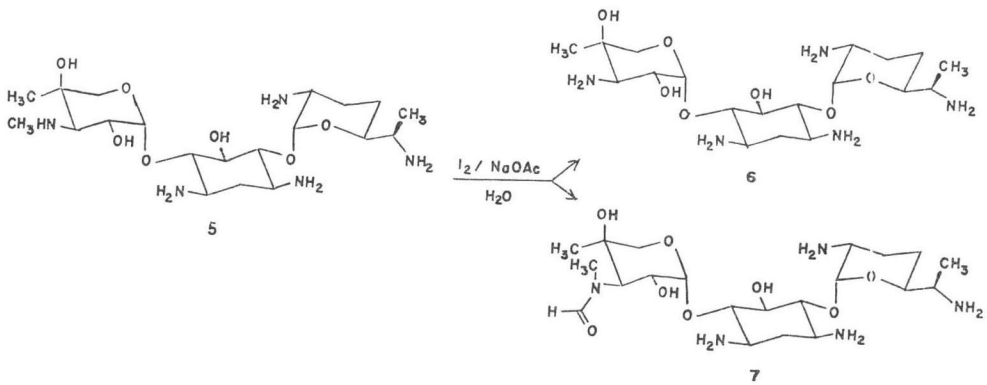
3 Gentamicin C_{1a} R = R′ = H
4 Gentamicin C_1 R = R′ = CH_3
5 Gentamicin C_2 R = CH_3, R′ = H

3″-De-*N*-Methylation

Oxidative removal of the *N*-methyl groups from the 3-dimethylamino moiety of a mycaminose or desosamine residue of macrolide antibiotics using iodine or bromine under suitably buffered conditions has been described by scientists at Abbott Laboratories.[8] Depending mainly upon the amount of

halogen employed, the product is mono or di-de-*N*-methyl derivative. Although it was apparent that under these conditions sisomicin (**1**) and verdamicin (**2**) would be unstable and cleave to give the pseudodisaccharide garamine,[9] the above method appeared attractive for application in the gentamicin class of antibiotics. Thus, treatment of gentamicin C_2 (**5**) with iodine and sodium acetate in aqueous methanol at 45°C for 24 hours afforded a major product (**6**) and a minor product (**7**) as evidenced by tlc examination of the reaction mixture. Some unreacted starting material was also present in the mixture. Compounds **6** and **7** were isolated in pure state by column chromatography.* The most polar component (Compound **6**) had $[\alpha]_D^{26}$ +159.8° (water) and gave elemental analysis consistent with the formula $C_{19}H_{39}N_5O_7$ required by the 3″-de-*N*-methyl-gentamicin C_2 (**6**). The mass spectrum of the compound exhibited a peak at *m/e* 450 attributable to the $(M^+ + 1)$ ion.[11] The presence of deoxystreptamine moiety in the molecule was indicated by the characteristic ions at *m/e* 191, 173, 163 and 145.[11] Also present in the spectrum were the ions at *m/e* 333, 305 and 287 arising from the pseudodisaccharide unit, gentamine C_2.[11] The absence of peaks at *m/e* 160 for the garosamine and at *m/e* 350, 322 and 304 for the pseudodisaccharide, garamine, moieties[11] along with the presence of the corresponding peaks 14 mass units lower for 3″-de-*N*-methylgarosamine and 3″-de-*N*-methyl-garamine units confirmed the structure of the compound to be 3″-de-*N*-methyl-gentamicin C_2 (**6**). The pmr spectrum of the compound was consistent with the structure. Thus, the anomeric hydrogens H-1′ and H-1″ appeared at δ 5.15 and 5.13 ppm, respectively with coupling constants of 4 Hz. The 6′-C-methyl and the 4″-C-methyl protons showed signals at δ 1.09 and 1.21 ppm with the former appearing as a doublet with a spacing of 6.5 Hz and the latter as a singlet. The H-2″ (δ 3.7 ppm, $J_{2'',3''}$ = 10 Hz) and H-5$_a$″ (δ 4.09 ppm, J = 12 Hz) resonances were also readily recognizable in the spectrum. The yield of 3″-de-*N*-methyl-gentamicin C_2 (**6**) was 21%.

The second product (**7**) which was more polar than gentamicin C_2 but less polar than 3″-de-*N*-methyl-gentamicin C_2 (**6**) when examined by tlc had $[\alpha]_D^{26}$ +173.6° (water). The mass spectrum indicated the compound to be 3″-*N*-formyl gentamicin C_2 (**7**). Thus, the molecular ion at *m/e* 491 was in agreement with the composition $C_{21}H_{41}N_5O_8$ found by elemental analysis. The presence of peaks



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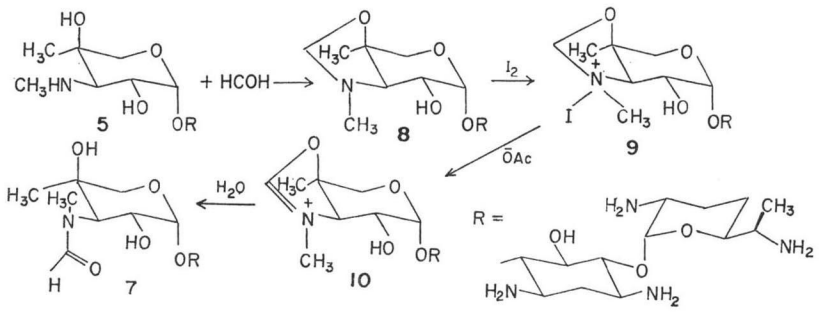
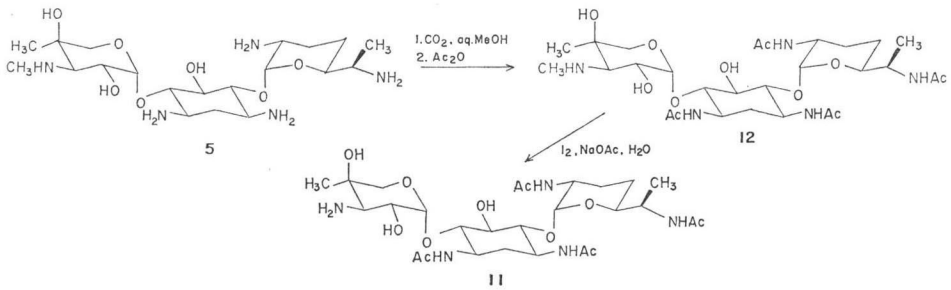
* During the preparation of this manuscript the de-*N*-methylation of antibiotic XK-62-2 appeared in a German patent.[10] In contrast to the methods described in this manuscript, the patented procedures do not lead to selectively *N*-blocked 3″-de-*N*-methyl gentamicins (for *e.g.* 1,3,2′,6′-tetra-*N*-acetyl-3″-de-*N*-methyl-gentamicin C_2) which are the desired intermediates for 3″-*N*-alkylation with other alkyl groups. Furthermore, as will be apparent from this manuscript, the general photochemical oxidative procedure described here is also suitable for the 1,3,2′,6′-tetra-*N*-acetylsisomicin which cannot be made by the iodine-sodium acetate method.



at *m/e* 188 (3″-*N*-formyl-garosaminyl unit) and *m/e* 378, 350, 332 (3″-*N*-formyl-garamine unit) confirmed the presence of the formyl group on the garosamine moiety.[11] The remainder of the spectrum was consistent with the assigned structure and the other characteristic prominent peaks are given in the experimental section. The pmr spectrum of the compound showed the 3″-*N*-methyl resonance at δ 3.0 ppm; 0.5 ppm downfield from the resonance position of the 3″-*N*-methyl protons in gentamicin C_2. This is in accord in view of the deshielding effect of the *N*-formyl group and has been observed in gentamicin A_4[12]. The formyl proton appeared at δ 8.2. The remainder of the pmr parameters were as expected and are given in the experimental section. The yield of **7** was 14%.

The formation of **7** can be readily rationalized. The oxidative de-*N*-methylation produces formaldehyde which can react with gentamicin C_2 (**5**) to give the oxazolidine **8**. Oxidation of **8** with iodine in the presence of base leads to the iminium ion **10**. Hydrolysis of **10** gives rise to 3″-*N*-formyl-gentamicin C_2, presumably *via* initial formation of the *O*-formyl derivative followed by rapid transformylation.

In order to prepare various 3″-*N*-alkyl derivatives of 3″-de-*N*-methyl-gentamicin C_2 (**6**), it was thought that 1, 3, 2′, 6′-tetra-*N*-acetyl 3″-de-*N*-methylgentamicin C_2* (**11**) would be an ideal intermediate which after reductive alkylation at the 3″-position followed by removal of the protecting groups would give the desired products. Compound **11** was prepared from gentamicin C_2 (**5**) as follows. It is known that aqueous solutions of aminoglycoside antibiotics absorb atmospheric carbon dioxide and the resulting carbonated samples often give poorly resolved nmr spectra. It is also known that in the gentamicin-sisomicin class of compounds the chemical shift of the 3″-*N*-methyl proton is highly dependent on the extent of carbonation. For example, the *N*-methyl proton of gentamicin C_2 (**5**) free base in deuterium oxide appeared at δ 2.55 ppm. On treatment of the solution with 2

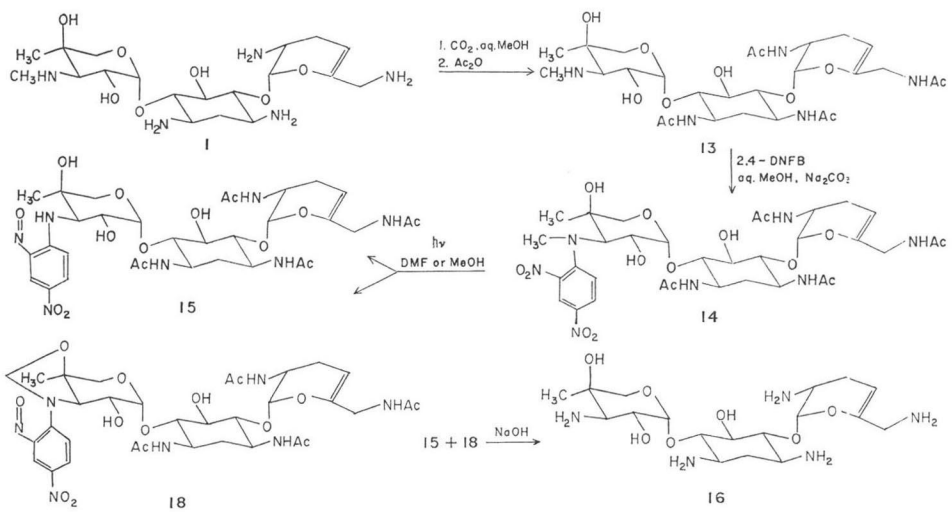


* Protecting groups other than acetyls are plausible.

equivalents of carbon dioxide, the *N*-methyl protons were deshielded to 2.8 ppm. It appeared therefore, that the carbonated secondary amino function, also being next to the tertiary alcoholic center, might be the least reactive of the amino groups in gentamicin C_2 towards acylating agents under these conditions. Thus, when a solution of gentamicin C_2 (**5**) in aqueous methanol was treated with carbon dioxide followed by acetic anhydride, the major product formed was 1,3,2′,6′-tetra-*N*-acetyl-gentamicin C_2 (**12**) which was isolated in 60% yield. The structure was apparent from the pmr spectrum which showed the *N*-methyl resonance at δ 2.49 ppm indicating no acetylation at this site. The four *N*-acetyl protons appeared at δ 2.01 and 1.99 ppm. The mass spectrum showed the ($M^+ + 1$) ion at *m/e* 632 and the garosaminyl fragment at *m/e* 160[11], as required by the assigned structure. The remainder of the pmr and mass spectral data are given in the experimental section.

Treatment of 1,3,2′,6′-tetra-*N*-acetyl-gentamicin C_2 (**12**) with iodine and sodium acetate as before afforded, as expected, the corresponding de-*N*-methyl derivative **11** which was isolated in 37% yield after chromatographic purification.

As mentioned earlier, oxidative removal of the 3″-*N*-methyl group with electrophilic halogens is not applicable to sisomicin type structures (**1** and **2**) which contain a vinyl ether function. Therefore, we thought that if an oxidative process could be carried out selectively at the 3″-position by an intramolecular process involving only the 3″-position, then one might be able to de-*N*-methylate sisomicin type structures without affecting the cyclic vinyl ether linkage. In order to effect such an oxidative cleavage of the *N*-methyl group, we considered the possibility of intramolecular photochemical oxidation of the *N*-methyl group by way of a 3″-*N*-2,4-dinitrophenyl derivative **14**, since it is well established that photochemical removal of *o*-nitrobenzyl groups involves the transfer of an oxygen atom from the nitro group to the benzylic position.[13~15] The selection of the 2,4-dinitrophenyl derivative was based on the ease of reaction of the 2,4-dinitrofluorobenzene with the 3″-amine and the presumed ease of removal of the *o*-nitroso-*p*-nitrophenyl group as compared to *o*-nitroso-phenyl.*



* Although no examples have been reported of photooxidative removal of the *N*-methyl group in aliphatic compounds, the de-*N*-methylation of *N*-methyl-2,6-dinitro-4-trifluoromethyl aniline by this process has been reported by R. E. McMAHON.[16]

Selective tetra-*N*-acetylation of sisomicin (**1**) by the carbon dioxide - acetic anhydride method described above afforded 1,3,2′,6′-tetra-*N*-acetyl-sisomicin (**13**) in 61% yield. 2,4-Dinitrophenylation of **13** by the standard procedure gave 1,3,2′,6′-tetra-*N*-acetyl-3″-*N*-(2,4-dinitrophenyl)-sisomicin (**14**) in near quantitative yield. Photolysis of **14** in dimethylformamide with a 450-watt high pressure mercury lamp (Hanovia) and pyrex filter under nitrogen for 15 hours at room temperature gave two products when examined by tlc which were isolated by column chromatography. The 100 MHz pmr spectrum of the more polar compound **15** in deuterium oxide showed no *N*-methyl protons. The presence of four *N*-acetyl groups (δ 1.84, 1.91, 1.98 and 1.98 ppm), a tertiary *C*-methyl group (δ 0.96 ppm) and two anomeric protons (δ 5.23, H-1″, $J_{1''2''}=3.5$ Hz and δ 5.33 ppm, H-1′, $J_{1'2'}=$ 2.3 Hz) was consistent with the structure for 1,3,2′,6′-tetra-*N*-acetyl-3″-de-*N*-methyl-3″-*N*-(2-nitroso-4-nitrophenyl)-sisomicin (**15**). The aromatic region of the spectrum showed an AB quartet due to the protons H-5 and H-6 of the 2-nitroso-4-nitrophenyl moiety. The third aromatic proton (H-3) of **15** appeared as a singlet at δ 8.75 ppm. Hydrolysis of **15** with 1 N sodium hydroxide at 100°C afforded 3″-de-*N*-methyl-sisomicin (**16**) whose structure was proven by nmr and mass spectral analysis as in the case of the 3″-de-*N*-methyl-gentamicin C_2 described above (see experimental). Mild base hydrolysis of **15** afforded 1,3,2′,6′-tetra-*N*-acetyl-3″-de-*N*-methyl-sisomicin (**17**).

The second product of photolysis, compound **18**, had near coincidental Rf with the starting material in several solvent systems but could be isolated in pure state after multiple chromatography. Base hydrolysis of **18** also gave 3″-de-*N*-methyl-sisomicin (**16**). The pmr spectrum of the compound contained no *N*-methyl protons, had four *N*-acetyl resonances (δ 1.9, 1.97, 2.0 and 2.0 ppm) and a tertiary *C*-methyl signal (δ 1.01 ppm). The anomeric hydrogens H-1′ and H-1″ showed signals at δ 5.44 and δ 5.15 ppm, and the olefinic proton appeared at δ 4.83 ppm. The presence of a 2-proton broadish multiplet at δ 2.8 ppm suggested structure **18** for the compound.

The intermediate **19** formed in the photolysis of **14** can either lose formaldehyde to give **15** or lose hydroxide ion to give the iminium ion **20**. Attack of the 4″-hydroxyl group on the methylene

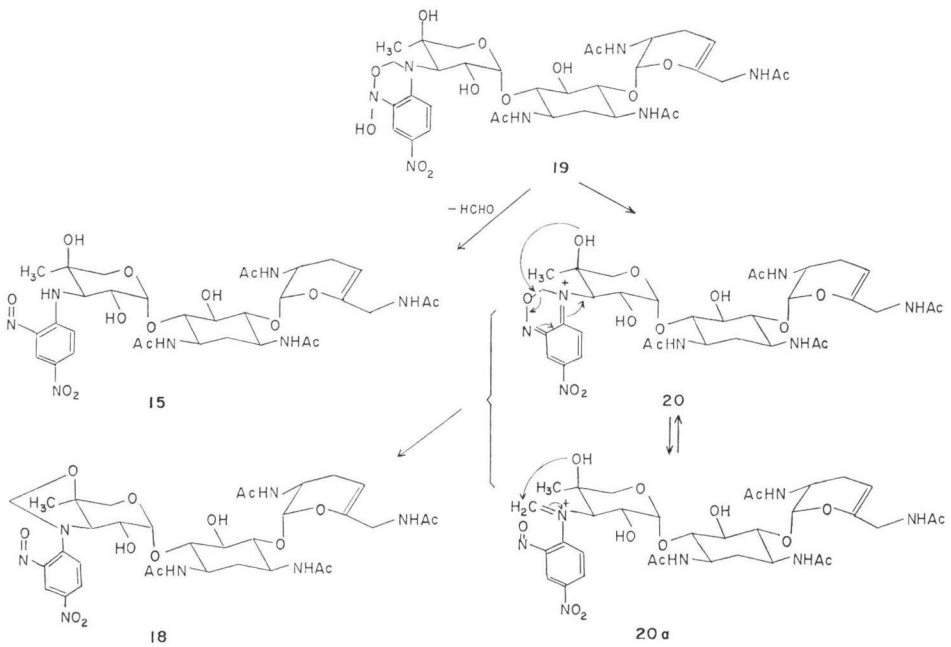


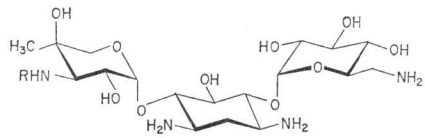
Table 1. Minimum inhibitory concentrations (μg/ml)* of 3″-de-*N*-methyl gentamicin C_2, 3″-de-*N*-methyl sisomicin, 3″-de-*N*-methyl gentamicin B and some 3″-*N*-alkyl-3-de-*N*-methyl derivatives of gentamicin C_2

Organism		Inactivation mechanism	1	16	21	22	5	6	23	24	25
	ATCC 10536		0.075	0.3	0.3	0.75	0.25	0.075	0.075	0.3	0.075
	St. M 1574–1		0.3	0.3	0.75	3.0	0.25	0.3	0.075	0.3	0.75
	St. M F14-BK		0.3	0.3	0.3	0.75	0.25	0.3	0.075	0.3	0.075
Escherichia coli	JR 66	ANT (2″)	17.5	17.5	>25	>25	>16	17.5	>25	>25	>25
	JR 88	AAC (3)	17.5	17.5	0.75	3.0	>16	>25	NG	>25	NG
	JR 90	AAC (3)	17.5	>25	3.0	7.5	>16	>25	>25	>25	>25
	St. M 589	APH (3′)	0.75	7.5	>25	>25	4.0	7.5	7.5	17.5	7.5
	St. M Baker 2	APH (3′)	0.3	3.0	>25	>25	1.0	0.3	0.75	3.0	0.75
	Stone 20		0.75	0.3	0.75	0.75	0.03	0.75	0.03	0.3	0.03
	Stone 39		0.075	0.3	3.0	17.5	0.25	0.075	0.3	3.0	0.3
	St. M 762		0.075	0.3	7.5	17.5	0.25	0.3	0.3	3.0	0.3
	St. M 1395		0.3	0.3	3.0	17.5	0.25	0.3	0.3	7.5	0.3
Pseudomonas	NRRL 3223		0.075	0.3	3.0	>25	0.25	0.075	0.075	3.0	0.075
aeruginosa	Stone 130	AAC (3)	>25	7.5	17.5	>25	>16	>25	>25	—	>25
	Stone 138	AAC (3)	>25	3.0	3.0	>25	>16	>25	>25	—	>25
	Capetown 18	AAC (3)	17.5	3.0	3.0	>25	>16	>25	17.5	—	>25
	Travers I	AAC (3)-II	>25	>25	>25	>25	>16	>25	>25	—	>25
	GN 315	AAC (6′)-I	7.5	>25	>25	>25	0.5	0.3	0.75	>25	0.75
	Ad 17	APH (3′)-II	0.3	0.3	17.5	NG	0.5	0.3	0.75	0.75	0.3
	Ad 18	APH (3′)-II	0.3	0.075	17.5	NG	0.125	0.3	0.75	0.03	0.075
Klebsiella spp.	Georgetown 3694	ANT (2″)	17.5	7.5	>25	>25	>16	>25	>25	>25	>25
	Georgetown 3020	ANT (2″)	17.5	3.0	17.5	>25	>16	17.5	>25	>25	>25
	Oklahoma 6	ANT (2″)	>25	17.5	>25	>25	>16	—	>25	>25	>25
Proteus mirabilis	Harding		0.75	3.0	3.0	7.5	0.5	3.0	0.3	3.0	0.3
Proteus rettgeri	Membel		0.3	17.5	0.3	7.5	2.0	17.5	0.75	>25	0.75

* MUELLER-HINTON Broth, pH 7.2

carbon with subsequent loss of a proton could lead to compound **18**.

The relative amounts of **15** and **18** formed in the photolysis of **14** depends on the solvent. In methanol, for example, compound **15** is the sole product, while in dimethylformamide the proportion of **15** to **18** is 2: 1. The yield of a mixture of **15** and **18** is 56%. The overall yield of 3″-de-*N*-methyl-sisomicin (**16**) from 1,3,2′,6′-tetra-*N*-acetyl-3″-(2,4-dinitrophenyl)-sisomicin (**14**) without isolating the intermediates was 41%. In a manner similar to that described above for the conversion of sisomicin (**1**) to 3″-de-*N*-methyl-sisomicin (**16**) or 1,3,2′,6′-tetra-*N*-acetyl-3″-de-*N*-methyl-sisomicin (**17**), gentamicin B (**21**) and gentamicin C_2 (**5**) were converted to 3″-de-*N*-methyl-gentamicin B (**22**) and 3″-de-*N*-methyl-gentamicin C_2 (**6**), respectively. The 1,3,2′,6′-tetra-*N*-acetyl-3″-de-*N*-methyl-gentamicin C_2 (**11**) was also isolated and characterized as before.



21 Gentamicin B, R = CH_3
22 3"-De-N-methyl gentamicin B, R = H

3″-*N*-Homologues

1,3,2′,6′-Tetra-*N*-acetyl-3″-de-*N*-methyl-gentamicin C_2 (**11**) was subjected to reductive alkylation at pH 4~4.5 using sodium cyanoborohydride and an aldehyde.[4] In each case, the crude product was deblocked using sodium hydroxide and subjected to chromatographic purification. Thus, the 3″-*N*-ethyl (**23**), *n*-propyl (**24**), *n*-butyl (**25**) and ω-aminobutyl (**26**) derivatives of 3″-de-*N*-methyl-gentamicin C_2 (**6**) were prepared. All of the above compounds were characterized from their mass spectrometric fragmentation patterns[11] and pmr spectral properties (see experimental).

Biology

In Table 1, the minimum inhibitory concentrations (MIC's) of the 3″-de-*N*-methyl derivatives of gentamicin C_2 (**6**), gentamicin B (**22**) and sisomicin (**16**) are given along with those of their parent compounds. Also included in the table, are the MIC's of a number of 3″-*N*-alkyl-3″-de-*N*-methyl derivatives. As is apparent from the table all the semisynthetic derivatives have potencies and spectra of antibacterial activity similar to the parent antibiotics from which they are derived.

Experimental

Details of experimental techniques have been published in our earlier papers.[12,17]

3″-De-*N*-methyl-gentamicin C_2 (**6**) by the iodine-sodium acetate method

Gentamicin C_2 (10 g, 21.6 mmol) was dissolved in 50% aqueous methanol (250 ml) and iodine (10 g) and sodium acetate (20 g) were added with stirring. The reaction mixture was maintained at 45°C for 4 hours while the pH of the solution was kept between 8 and 9 by the addition of 1 N sodium hydroxide at hourly intervals. The reaction was allowed to run for an additional 18 hours, after which time additional amounts of iodine (5 g) and sodium acetate (10 g) were added and the pH of the solution adjusted to 9. After a further 6 hours, the solution was decolourized by the addition of aqueous sodium thiosulfate and the mixture was stirred with Amberlite IRC-50 ion-exchange resin in the H^+ form (400 ml) unitl all the aminoglycosides were absorbed. The resin was separated and washed carefully with water followed by 2 N ammonium hydroxide. The ammonia eluate was concentrated *in vacuo* to a residue (8.9 g) which was chromatographed on a column of silica gel (200 g) using the lower phase of a chloroform - methanol - ammonium hydroxide (2: 1: 1) mixture as the eluent. The first compound to be eluted was gentamicin C_2 (1.5 g) which was followed by 3″-*N*-formyl-

gentamicin C_2 (**7**) (1.5 g, 14%), $[\alpha]_D^{26}$ +173.6° (*c* 0.4, water). MS: $(M+1)^+$ 491, *m/e* 378, 350, 332, 188, 333, 305, 287, 191, 173, 163 and 145. PMR (D_2O) δ 1.06 (3H, d, J=6.5 Hz, –CHC<u>H</u>$_3$), 1.1 (3H, s, C–C<u>H</u>$_3$), 3.0 (3H, s, N–C<u>H</u>$_3$), 4.14 (1H, d, J=12 Hz, H-5″ eq), 4.35 (1H, dd, J=4, 11 Hz, H-2″), 5.16 (1H, d, J=4 Hz, H-1′ or H-1″), 5.3 (1H, d, J=4 Hz, H-1′ or H-1″), 8.14, 8.28 (1H, rotamers, C<u>H</u>O).

Anal. Calcd. for $C_{21}H_{41}N_5O_8$: C, 51.3; H, 8.4; N, 14.25%
Found: C, 51.08; H, 8.43; N, 14.07%

The last compound to be eluted from the column was 3″-de-*N*-methyl-gentamicin C_2 (**6**) (2.0 g, 21%), $[\alpha]_D^{26}$ +159.8° (*c* 0.3, water). MS: $(M+1)^+$ 450, *m/e* 336, 333, 318, 305, 308, 290, 287, 275, 272, 191, 173, 163, 146 145, 143. PMR(D_2O) δ 1.09 (3H, d, J=6.5 Hz, –CH–C<u>H</u>$_3$), 1.21 (3H, s, C–C<u>H</u>$_3$), 3.7 (1H, dd, J=4 Hz, 10 Hz, H–2″), 4.09 (1H, d, J=12 Hz, H–5″ eq), 5.13 (1H, d, J=4 Hz, H–1″), 5.15 (1H, d, J=4 Hz, H–1′).

Anal. Calcd. for $C_{19}H_{39}N_5O_7$: C, 50.8; H, 8.7; N, 15.6%
Found: C, 50.7; H, 8.7; N, 15.4%

1,3,2′,6′-Tetra-*N*-acetyl-gentamicin C_2 (**12**)

Dry ice (8.8 g) was added to a stirred solution of gentamicin C_2 (23.2 g, 50 mmol) in 50% aqueous methanol (1.5 liters). After 10 minutes at room temperature, a 1 M solution of acetic anhydride in tetrahydrofuran was added dropwise (200 ml). Ten minutes after completion of the addition, thin-layer chromatography on silica gel using chloroform - methanol - ammonium hydroxide (30: 10: 1) as the solvent system indicated the presence of one major spot and one minor spot in the ratio of about 90: 10. Work-up in the usual manner and isolation of the major product by column chromatography on silica gel (1: 50 ratio) using the above solvent system afforded pure 1,3,2′,6′-tetra-*N*-acetyl-gentamicin C_2 (**12**), $[\alpha]_D^{26}$ +160° (*c* 0.43, water), in 60% yield. MS: $(M+1)^+$ 632, *m/e* 501, 473, 455, 434, 416, 406, 388, 275, 257, 247, 229, 227, and 160. PMR (D_2O) δ 1.13 (3H, d, J=7.0 Hz CHC<u>H</u>$_3$), 1.22 (3H, s, C–C<u>H</u>$_3$), 1.99, 2.01 (12H, NCOC<u>H</u>$_3$), 2.49 (3H, s, *N*–C<u>H</u>$_3$), 5.09 (1H, d, J=4 Hz, H–1″), 5.35 (1H, d, J=3.0 Hz, H–1′).

Anal. Calcd. for $C_{28}H_{49}N_5O_{11}\cdot 0.5\ H_2CO_3$: C, 51.73; H, 7.46; N, 10.58%
Found: C, 51.61; H, 7.35; N, 10.87%

1,3 ,2′,6′-Tetra-*N*-acetyl-3″-de-*N*-methyl-gentamicin C_2 (**11**)

Sodium acetate (1.2 g) and iodine (2.4 g) were added to a solution of tetra-*N*-acetyl-gentamicin C_2 (1.75 g) in 50 % aqueous dioxane (40 ml). The mixture was stirred in a nitrogen atmosphere at 40°C. Amberlite IRA 401S (OH^-) resin was introduced to bring the pH of the solution to 10.4. After 20 minutes, additional amounts of the resin was added to bring the pH of the solution to 8.4. After 21 hours, the solution was filtered and the filtrate treated with Amberlite IR-120 (H^+) resin to absorb the products completely. After washing the resin with water, the resin was washed with 3% ammonium hydroxide solution. The ammonia solution was evaporated *in vacuo* and the residue lyophilized to give crude products which were subjected to chromatography on silica gel (50 g) using chloroform - methanol - ammonium hydroxide (30: 10: 0.5) as the eluant. The pure fractions containing the product were pooled, concentrated and lyophilized to give 0.33 g (19%) of pure **11**, $[\alpha]_D^{26}$ +164.3° (*c* 0.7, water). MS: M^+ 617, $(M^+ +1)$ 618, *m/e* 501, 483, 473, 455, 420, 402, 392, 374, 275, 257, 247, 229, 227, 146. PMR (D_2O): δ 1.18 (3H, s, 4″–C–C<u>H</u>$_3$), 1.05, 1.18 (3H, d, 6′–C–C<u>H</u>$_3$), 1.97, 2.0 (12H, *N*<u>Ac</u>), 5.11 (1H, d, J=4.0 Hz, H–1″), 5.35 (1H, d, J=3.5 Hz, H–1′).

Anal. Calcd. for $C_{27}H_{47}N_5O_{11}\cdot 1.5H_2O$: C, 50.30; H, 7.81; N, 10.86%
Found: C, 50.35; H, 7.42; N, 10.81%

1,3,2′,6′-Tetra-*N*-acetyl-sisomicin (**13**)

This compound, $[\alpha]_D^{26}$ +191° (*c* 0.2, water), was prepared in 65% yield from sisomicin (22.35 g, 50 mmol) in a manner similar to that described above for the preparation of 1,3,2′,6′-tetra-*N*-acetyl gentamicin C_2 (**12**). MS: M^+ 615, *m/e* 592, 550, 485, 467, 457, 439, 434, 416, 406, 388, 275, 257, 247, 229, 211 and 160. PMR (D_2O), δ 1.18 (3H, s, C–C<u>H</u>$_3$), 1.88 ~ 1.98 (14H, 4 acetyls + H2 eq + H2 ax) 2.48 (3H, s, *N*-<u>Me</u>), 2.57 (1H, d, J=10 Hz, H–3″), 4.88 (1H, m, H–4′), 5.07 (1H, d, J=4 Hz, H–1″)

and 5.48 (1H, d, J=3 Hz, H-1′).

Anal. Calcd. for $C_{27}H_{45}N_5O_{11}\cdot H_2O$: C, 51.17; H, 7.47; N, 11.05%
Found: C, 50.95; H, 7.30; N, 11.25%

1,3,2′,6′-Tetra-*N*-acetyl-3″-*N*-(2,4-dinitrophenyl)-sisomicin (**14**)

2,4-Dinitrofluorobenzene (9.4 ml) was added dropwise to a stirred and cooled solution of 1,3,2′,6′-tetra-*N*-acetyl sisomicin (22.8 g) (**13**) and sodium carbonate (7.8 g) in water (40 ml) and methanol (100 ml). The reaction mixture was stirred for 18 hours at room temperature after which time the solids were removed by filtration, washed with methanol and the combined filtrates concentrated *in vacuo*. The concentrate was chromatographed on 900 g of silica gel using a solvent system consisting of chloroform - methanol - ammonium hydroxide (30:10:1). The homogeneous fractions containing the product were pooled and concentrated to dryness to yield 23 g of pure **14**, $[\alpha]_D^{26}$ +329.2° (*c* 0.6, methanol). PMR (D_2O, 100 MHz) δ 1.16 (3H, s, C-CH$_3$), 1.91, 1.96, 1.99 (12H, NCOCH$_3$), 2.87 (3H, s, NCH$_3$), 4.89 (1H, m, H-4′), 5.32 (1H, d, J=4 Hz, H-1′), 5.51 (1H, d, J=4.5 Hz, H-1″), 7.48 (1H, d, J=9.0 Hz, H-6‴), 8.20 (1H, dd, J=2.6 Hz, 9.0 Hz), 8.69 (1H, d, J=2.6 Hz).

Anal. Calcd. for $C_{33}H_{47}N_7O_{15}\cdot 2H_2O$: C, 48.59; H, 6.05; N, 12.67%
Found: C, 48.98; H, 6.22; N, 12.67%

3″-De-*N*-methyl-sisomicin (**16**)

Tetra-*N*-acetyl-3″-*N*-(2,4-dinitrophenyl)-sisomicin (**14**) (2 g) was dissolved in dimethylformamide (300 ml) and the solution irradiated with a 450-watt high pressure mercury lamp (Hanovia) through a pyrex filter while bubbling nitrogen through the solution. After 5 hours, the solvent was removed by evaporation in high vacuum at 50°C and the residue chromatographed on silica gel (200 g) using chloroform - methanol - ammonium hydroxide (30: 10: 1) as the solvent system. Work-up in the usual manner gave two products, fraction A (0.84 g) and fraction B (0.63 g) which were combined, dissolved in 1 N sodium hydroxide (60 ml) and the solution heated under nitrogen and refluxed for 24 hours. The antibiotic was adsorbed on Amberlite 1RC-50 ion-exchange resin in the proton form, the resin was washed with water followed by 7% ammonium hydroxide. The ammonia wash was concentrated and the residue chromatographed on silica gel using chloroform - methanol - ammonium hydroxide (2: 1: 0.35). The pure fractions were pooled, concentrated and lyophilized to give 1.3 g of **16**, $[\alpha]_D^{26}$ 173.4° (*c* 0.6, water). MS: M^+ 433, *m/e* 416, 348, 336, 318, 290, 317, 299, 271, 191, 173, 163, 145, 146, 127. PMR (D_2O, δ), 1.2 (3H, s, C-CH$_3$), 2.78 (1H, d, J=10.5 Hz, H-3″), 4.09 (1H, d, J=12.0 Hz, H-5 eq), 4.91 (1H, m, H-4′), 5.09 (1H, d, J=4 Hz, H-1″), 5.24 (1H, d, J=2.4 Hz, H-1′).

Anal. Calcd. for $C_{18}H_{35}N_5O_7\cdot 0.5H_2O$: C, 48.86; H, 8.20%
Found: C, 48.83; H, 8.1%

Tetra-*N*-acetyl-3″-de-*N*-methyl-3″-*N*-(2-nitroso-4-nitrophenyl)-sisomicin (**15**) and tetra-*N*-acetyl-3″-de-*N*-methyl-3″-*N*-(2-nitroso-4-nitrophenyl)-3″-*N*-4″-*O*-methylene-sisomicin (**18**)

In a separate experiment, fraction B described above was shown to be tetra-*N*-acetyl-3″-de-*N*-methyl-3″-*N*-(2-nitroso-4-nitrophenyl)-sisomicin (**15**), $[\alpha]_D^{26}$ +186° (*c* 0.3, water). PMR (D_2O) δ 0.96 (3H, s, C-CH$_3$), 1.84, 1.91, 1.98 (12H, *N*-Ac), 4.88 (1H, m, H-4′), 5.33 (1H, d, J=3.5 Hz, H-1″), 5.53 (1H, d, J=2.3 Hz, H-1′), 7.98 (1H, d, J=9.0 Hz, H-6‴), 8.46 (1H, dd, H-5‴) and 8.75 (1H, d, H-3‴).

Anal. Calcd. for $C_{33}H_{46}N_7O_{15}\cdot 3.5\ H_2O$: C, 47.17; H, 6.43; N, 12.03%
Found: C, 47.23; H, 6.10; N, 11.75%

Fraction A described above was shown to be tetra-*N*-acetyl-3″-de-*N*-methyl-3″-(2-nitroso-4-nitrophenyl)-3″-*N*-4″-*O*-methylene-sisomicin (**18**), $[\alpha]_D^{26}$ +199° (*c* 0.3, water). PMR (D_2O) δ 1.01 (3H, C-CH$_3$), 1.9, 1.97, 2.0 (12H, *N*-acetyl), 2.8 (2H, m, *N,O*-CH$_2$), 4.83 (1H, m, H-4′), 5.15 (1H, d, J=3 Hz, H-1″), 5.44 (1H, d, J=2 Hz, H-1′), 7.28, 8.08, 8.49 and 9.43 (aromatic protons).

Anal. Calcd. for $C_{33}H_{45}N_7O_{14}\cdot 2.5\ H_2O$: C, 48.00; H, 6.23; N, 11.87%
Found: C, 48.06; H, 6.18; N, 12.16%

3″-De-*N*-methyl-gentamicin B

Gentamicin B (9.64 g) was converted to 1,3,6′-tri-*N*-acetyl-gentamicin B in a manner similar to

that described for the preparation of **12**. The product was converted as in the preparation of **14**, to 1,3,6′-tri-*N*-acetyl-3″-*N*-(2,4-dinitrophenyl)-gentamicin B (**23**) in 55% overall yield from gentamicin B.

The above compound (2 g) was photolyzed in 450 ml methanol as mentioned above for 15 hours. The solvent was removed *in vacuo* and the residue chromatographed on 100 g silica gel using chloroform - methanol - ammonium hydroxide (2: 1: 0.35) as the eluant. The two products of photolysis, fraction A (0.478 g) and fraction B (1.168 g) were pooled together and after removal of the solvent *in vacuo*, the residue was dissolved in 45 ml 1 N sodium hydroxide and heated under reflux in a nitrogen atmosphere for 24 hours. The cooled solution was neutralized to pH 7 with dilute sulfuric acid and concentrated to dryness *in vacuo*. The residue was triturated with a mixture of chloroform - methanol - ammonium hydroxide (3: 4: 2). The solids were removed by filtration, the filtrate concentrated and chromatographed on 200 g silica gel using the same solvent system. Fractions containing the pure product were worked-up in the usual manner to give 0.5 g (42%) of pure 3″-de-*N*-methyl-gentamicin B, $[\alpha]_D^{26}$ +162.1° (*c* 0.5, water). MS: (MH) 469, *m/e* 451, 422, 352, 334, 324, 306, 336, 318, 308, 290, 191, 173, 163, 145, 162, 146. PMR (D_2O) δ, 1.14 (3H, s, C–C<u>H</u>$_3$), 2.8 (1H, d, J=10.5 Hz, H–3″), 4.03 (1H, d, J=12.5 Hz, H–5″ eq), 5.07 (1H, d, J=3.5 Hz, H–1″), 5.34 (1H, d, J=3 Hz, H–1′).

Anal. Calcd. for $C_{18}H_{36}N_4O_{10}\cdot H_2CO_3$: C, 43.02; H, 7.22; N, 10.56%
Found: C, 43.19; H, 7.68; N, 10.63%

3″-De-*N*-methyl-gentamicin C_2 (**6**) by the photolytic method

1,3,2′,6′-Tetra-*N*-acetyl gentamicin C_2 (**12**) (1.89 g) was converted to the corresponding 3″-*N*-(2,4-dinitrophenyl) derivative in 92% yield in a manner similar to the conversion of **13** to **14**. The compound had $[\alpha]_D^{26}$ +270.4° (*c* 0.9, water).

Anal. Calcd. for $C_{33}H_{52}N_7O_{19}\cdot 1.25H_2CO_3$: C, 48.37; H, 6.16; N, 11.20%
Found: C, 48.35; H, 5.94; N, 11.80%

The above compound, 2 g, was photolyzed in 450 ml of methanol as described in the above procedures for 13 hours. The solvent was removed *in vacuo* and the residue chromatographed on 100 g of silica gel using a mixture of chloroform - methanol - ammonium hydroxide (30: 10: 1). The two products of photolysis, fraction A (0.48 g) and fraction B (0.9 g) were combined and dissolved in 45 ml of 1 N sodium hydroxide. The basic solution was refluxed for 24 hours under nitrogen and work-up as in the case of **22** afforded pure 3″-de-*N*-methyl-gentamicin C_2 in 40% yield from gentamicin C_2, identical with the product obtained from the iodine-sodium acetate method.

The 3″-*N*-alkyl derivatives of 3″-de-*N*-methyl-gentamicin C_2 were prepared by the general method given below for the preparation of 3″-*N*-butyl-3″-de-*N*-methyl gentamicin C_2 (**24**).

3″-*N*-Butyl-3″-de-*N*-methyl-gentamicin C_2 (**24**)

1,3,2′,6′-Tetra-*N*-acetyl-3″-de-*N*-methyl gentamicin C_2 (**11**) (630 mg, 1 mmole) was dissolved in 20 ml 90% aqueous methanol and chilled in ice water. Butyraldehyde (0.2 ml) was added and the pH was adjusted to 4.5 with dilute hydrochloric acid. Sodium cyanoborohydride (120 mg) was added to the stirring solution. Tlc after 30 minutes (chloroform - methanol - 28% ammonium hydroxide, 25: 10: 2.2) showed ~60% conversion. The pH was adjusted to 4.5 and additional 0.1 ml aldehyde and sodium cyanoborohydride (60 mg) were added. The above procedure was repeated once again and showed no starting material left. The reaction mixture was evaporated to about 2 ml, diluted in 15 ml 2 N sodium hydroxide and refluxed 18 hours under nitrogen.

The antibiotic was absorbed onto 40 ml Amberlite IR 120 proton form ion-exchange resin, the resin was washed with water and the crude antibiotic eluted with 4 portions of 7% ammonium hydroxide. Freeze drying gave 428 mg of crude product. The product was chromatographed on 45 g of silica gel using chloroform - methanol - ammonium hydroxide (25: 10: 2.2), taking 5-ml fractions, 10 fractions per hour. Fractions 85~103 were pooled and freeze-dried to give 95 mg (0.19 mmoles 19%) of 3″-*N*-butyl-3″-de-*N*-methyl gentamicin C_2. $[\alpha]_D^{26}$ +156° (*c* 0.4, water); MS: (M^+ +1) 506, *m/e* 488, 333, 315, 305, 287, 392, 374, 364, 346, 202, 143. PMR (D_2O): δ 1.36 (3H, t, J=7.0 Hz, 4‴–C–C<u>H</u>$_3$), 1.53 (3H, d, J=7.0 Hz, 6′–C–C<u>H</u>$_3$), 1.67 (3H, s, 4″–C–C<u>H</u>$_3$), 3.30 (1H, d, J=13.0 Hz, H–5″$_{ax}$), 4.01 (1H, d, J=13.0 Hz, H–5″$_{eq}$), 5.06 (1H, d, J=4.0 Hz, H–1″), 5.12 (1H, d,

J=4.0 Hz, H-1').

Anal. Calcd. for $C_{23}H_{47}N_5O_7 \cdot 0.5CO_2$: C, 53.49; H, 8.98; N, 13.27%
Found: C, 53.48; H, 9.32; N, 13.05%

3''-De-*N*-methyl-3''-*N*-propyl-gentamicin C_2 (**23**)

The compound was obtained in 27% yield and had $[\alpha]_D^{26}$ +161.0° (*c* 0.4, water). MS: (M^+ +1) 492, *m/e* 474, 378, 360, 350, 332, 333, 315, 305, 287, 188, 143. PMR (D_2O): δ 0.85 (3H, dd, J=7.0 Hz, J=8.0 Hz, $\underline{CH_3}$–CH_2), 1.02 (3H, d, J=6.5 Hz, 6'–C–$\underline{CH_3}$), 5.05 (1H, d, J=4.0 Hz, H-1''), 5.10 (1H, d, J=4.0 Hz, H-1').

Anal. Calcd. for $C_{22}H_{45}N_5O_7 \cdot 0.5CO_2$: C, 52.61; H, 8.83; N, 13.63%
Found: C, 52.33; H, 8.91; N, 13.29%

3''-*N*-4'''-Aminobutyl-3''-*N*-de-*N*-methyl-gentamicin C_2 (**25**)

4-Acetamidobutyraldehyde was prepared *in situ* by dissolving the corresponding diethyl acetal (1 ml) in 5 ml 1 N hydrochloric acid at room temperature. After 30 minutes, the solution was neutralized by the addition of 1 N sodium hydroxide. The resulting solution of the aldehyde was used as in the above experiments for reductive alkylation with **11**. Compound **25** was obtained in 23% yield, $[\alpha]_D^{26}$ +145.4° (*c* 0.4, water). MS: (M^+ +1) 521, *m/e* 407, 389, 379, 361, 333, 315, 305, 297, 217, 143. PMR (D_2O): δ 1.03 (3H, d, J=7.0 Hz, 6'–C–$\underline{CH_3}$), 1.15 (3H, s, 4''–C–$\underline{CH_3}$), 1.54 (2H, m, C–$\underline{CH_2}$–C), 5.03 (overlapping anomeric protons).

Anal. Calcd. for $C_{23}H_{48}N_6O_7 \cdot H_2CO_3$: C, 49.47; H, 8.64; N, 14.42%
Found: C, 49.63; H, 8.66; N, 14.47%

Acknowledgments

The authors wish to thank the analytical department headed by Mr. J. McGLOTTEN and the microbiology department.

References

1) DANIELS, P. J. L.; T. L. NAGABHUSHAN & W. N. TURNER: The preparation and microbiological properties of some 6'-*N*-alkyl derivatives of sisomicin. in preparation.
2) UMEZAWA, H.; M. OKANISHI, R. UTAHARA, K. MAEDA & S. KONDO: Isolation and structure of kanamycin inactivated by a cell free system of kanamycin-resistant *E. coli*. J. Antibiotics 20: 136~141, 1967
3) BENVENISTE, R. & J. DAVIES: Enzymatic acetylation of aminoglycoside antibiotics by *Escherichia coli* carrying an R factor. Biochemistry 10: 1787~1796, 1971
4) WRIGHT, J. J.: Synthesis of 1-*N*-ethylsisomicin. A broad-spectrum semisynthetic aminoglycoside antibiotic. J. Chem. Soc., Chem. Comm. 1975: 206~208, 1975
5) MILLER, G. H.; G. ARCIERI, M. J. WEINSTEIN & J. A. WAITZ: Biological activity of netilmicin, a broad-spectrum semisynthetic aminoglycoside antibiotic. Antimicr. Agents & Chemoth. 10: 827~836, 1976
6) BENVENISTE, R. & J. DAVIES: R-Factor mediated gentamicin resistance: a new enzyme which modifies aminoglycoside antibiotics. Febs Lett. 14: 293~296, 1971
7) LUFT, F.; N. N. YUM & S. A. KLEIT: Comparative nephrotoxicities of netilmicin and gentamicin in rats. Antimicr. Agents & Chemoth. 10: 845~849, 1976
8) FREIBERG, L. A.: Verfahren zur Demethylierung von 3-Aminomakroliden. German patent 2,154,032, 1971
9) REIMANN, H.; D. J. COOPER, A. K. MALLAMS, R. S. JARET, A. YEHASKEL, M. KUGELMAN, H. F. VERNAY & D. SCHUMACHER: The structure of sisomicin, a novel unsaturated aminocyclitol antibiotic from *Micromonospora inyoensis*. J. Org. Chem. 39: 1451~1457, 1974
10) TOMIOKA, S. M. & Y. K. MORI: Verfahren zur Demethylierung von Aminoglykosidantibiotika. German patent 2,550,168, 1974
11) DANIELS, P. J. L.; M. KUGELMAN, A. K. MALLAMS, R. W. TKACH, H. F. VERNAY, J. WEINSTEIN & A. YEHASKEL: Mass spectral studies on aminocyclitol antibiotics. J. Chem. Soc., Chem. Comm. 1971: 1629~1631, 1971
12) NAGABHUSHAN, T. L.; W. N. TURNER, P. J. L. DANIELS & J. B. MORTON: The gentamicin antibiotics. 7.

Structures of the gentamicin antibiotics A_1, A_3 and A_4. J. Org. Chem. 40: 2830~2834, 1975

13) PATCHORNIK, A.; B. AMIT & R. B. WOODWARD: Photosensitive protecting groups. J. Am. Chem. Soc. 92: 6333~6335, 1970

14) a) ZEHAVI, U.; B. AMIT & A. PATCHORNIK: Light-sensitive glycosides. I. 6-Nitroveratryl-β-D-glucopyranoside and 2-nitrobenzyl-β-D-glucopyranoside. J. Org. Chem. 37: 2281~2285, 1972
b) ZEHAVI, U.; B. AMIT & A. PATCHORNIK: Light sensitive glycosides. II. 2-Nitrobenzyl-6-deoxy-β-L-mannopyranoside and 2-nitrobenzyl-6-deoxy-β-L-galactopyranoside. J. Org. Chem. 37: 2285~2288, 1972

15) Hébert, J. & D. Gravel: *O*-Nitrophenylethylene glycol: A photosensitive protecting group for aldehydes and ketones. Canad. J. Chem. 52: 187~189, 1974

16) MCMAHON, R. E.: The photochemical demethylation of *N*-methyl-2,6-dinitro-4-trifluoromethylaniline: a possible model for enzymatic dealkylation. Tetrahed. Lett. 1966: 2307~2310, 1966

17) WRIGHT, J. J.; A. B. COOPER, P. J. L. DANIELS, T. L. NAGABHUSHAN, D. RANE, W. N. TURNER & J. WEINSTEIN: Selective *N*-acylation of gentamicin antibiotics. Synthesis of 1-*N*-acyl derivatives. J. Antibiotics 29: 714~719, 1976