Semisynthetic Aminoglycoside Antibacterials. Part 10.[1,2] Synthesis of Novel 1-*N*-Aminoalkoxycarbonyl and 1-*N*-Aminoalkylcarboxamido Derivatives of Sisomicin, Gentamicin B, Gentamicin C_{1a}, and Kanamycin A

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Suitably protected derivatives of sisomicin, 5-*epi*-sisomicin, gentamicin B, gentamicin C_{1a}, and kanamycin A have been converted into a series of 1-*N*-alkoxycarbonyl, 1-*N*-aminoalkoxycarbonyl, 1-*N*-carboxamido, 1-*N*-alkylcarboxamido, and 1-*N*-aminoalkylcarboxamido derivatives. Representative thio-analogues have also been prepared. ^{13}C N.m.r. studies have revealed that these novel semisynthetic aminoglycosides have different solution conformations about the C-6–O glycosidic bond relative to the parent aminoglycosides from which they are derived.

The discovery of amikacin (1) [3] and netilmicin (9) [4,5] gave a considerable impetus to the search for other novel 1-*N*-substituted aminoglycosides that hopefully would exhibit an improved spectrum of antibacterial activity as well as reduced toxicity, relative to the parent aminoglycosides. Thus the synthesis of a number of 1-*N*-amino-acid analogues of butirosin [6] and sisomicin (29) [7] were reported. The synthesis of 1-*N*-[(*S*)-4-amino-2-hydroxybutyryl (HABA), 1-*N*-[(*S*)-3-amino-2-hydroxypropionyl] (HAPA), and a variety of simple 1-*N*-acyl derivatives of gentamicin C_1,[8] gentamicin C_{1a} (89),[9], gentamicin B (62),[10] sisomicin (29),[9] verdamicin,[9] 5-*epi*-gentamicin B,[11] 5-*epi*-sisomicin (49),[12] kamanycin A (8),[13] and 5-*epi*-kanamycin A [11] have also been reported. Butikacin (UK 18, 898) (2), a reduced analogue of amikacin (1), has also been synthesized [14] and a number of other 1-*N*-alkyl [15,16] and 1-*N*-acyl [16–18] derivatives of aminoglycosides have been described in the patent literature. Novel 1-deamino-1-hydroxy, 1-deamino-1-*epi*-hydroxy-, and 1-*epi*-amino-derivatives of aminoglycosides have also been described.[1]

We describe here the synthesis of a novel series of 1-*N*-alkoxycarbonyl, 1-*N*-aminoalkoxycarbonyl, 1-*N*-carboxamido, 1-*N*-alkylcarboxamido, and 1-*N*-aminoalkylcarboxamido derivatives of sisomicin (29), 5-*epi*-sisomicin (49), gentamicin B (62), gentamicin C_{1a} (89), and kanamycin A(8). These derivatives were chosen in view of their close structural similarity to the 1-*N*-HABA side-chain of amikacin (1). It was predicted that these compounds might be expected to be potent, broad-spectrum antibacterials and this was indeed found to be the case. After completion of these studies a patent from Bayer AG appeared [19] describing similar derivatives.

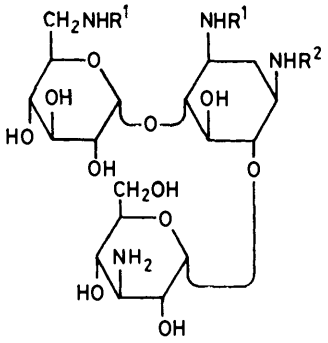
We selected 2-aminoethanol (30) as the first choice of an intermediate for the synthesis of the 1-*N*-aminoalkoxycarbonyl derivatives as this would lead to derivatives in which the terminal amino-group in the side-chain was at approximately the same distance from the cyclitol ring as in the HABA side chain of amikacin (1). Thus 2-aminoethanol (30) was converted into 2-(2,2,2-trichloroethoxycarbonylamino)ethanol (31) which on treatment with phosgene in the presence of triethylamine and *N*-hydroxysuccinimide afforded the active ester (32). The 2-aminoethanol (30) was also converted into the 4-methoxybenzyloxycarbonyl derivative (33) using 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate, and the protected derivative (33) was then converted into the active ester (34). The active ester (34) was treated with 3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)sisomicin (10) [20,*] in methanol–water at 25 °C to give the protected trisaccharide (11) which on treatment with zinc in aqueous acetic acid afforded 1-*N*-(2-aminoethoxycarbonyl)sisomicin (12). The latter was also prepared by condensing the active ester (34) with 3,2′,6′-tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) [20,*] to give the protected sisomicin derivative (14) which was then deprotected by treatment with trifluoroacetic acid. The ^{13}C n.m.r. data for (12) and for the other derivatives prepared in this study are given in Table 1 and it is clear from the protonation shifts that the substituent is located on the 1-amino-group in each case.

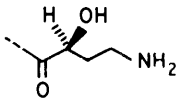
In order to prepare an *N*-alkyl analogue of (12), 2-ethylaminoethanol (35) was converted into the 4-methoxybenzyloxycarbonyl derivative (36) which was then converted into the active ester (37) and condensed with 3,2′,6′-tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) to give 1-*N*-(2-ethylaminoethoxycarbonyl)-sisomicin (15) after deprotection with trifluoroacetic acid.

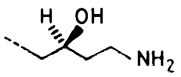
Two unsubstituted alkyloxycarbonyl derivatives of sisomicin were prepared next. *N*-(Methoxycarbonyloxy)succinimide (40) was prepared by treating *N*-hydroxysuccinimide with methyl chloroformate in the presence of pyridine and it was then condensed with 3,2′,6′-tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) in methanol–water to afford, after deprotection with trifluoroacetic acid, 1-*N*-methoxycarbonylsisomicin (16). In a similar manner *N*-(ethoxycarbonyloxy)succinimide (41) was treated with (13) to give 1-*N*-ethoxycarbonylsisomicin (17).

In all the above instances using succinimide active esters, condensation occurred exclusively at the 1-

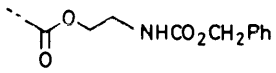
* Full experimental details for the preparation of these key *N*-protected aminoglycoside intermediates by the transition-metal complexing procedure of Nagabhushan will be published elsewhere.



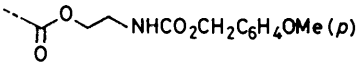
(1) $R^1 = H$, $R^2 =$ 

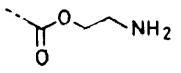
(2) $R^1 = H$, $R^2 =$ 

(3) $R^1 = CO_2CH_2Ph$, $R^2 = H$

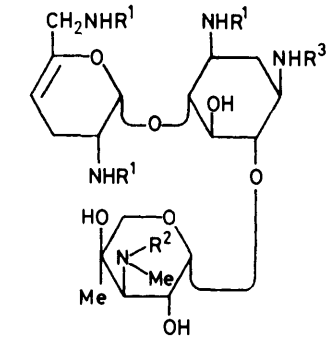
(4) $R^1 = CO_2CH_2C_6H_5$, $R^2 =$ 

(5) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$

(6) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 =$ 

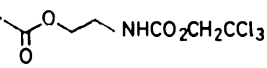
(7) $R^1 = H$, $R^2 =$ 

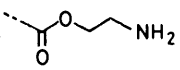
(8) $R^1 = R^2 = H$



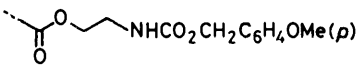
(9) $R^1 = R^2 = H$, $R^3 = Et$

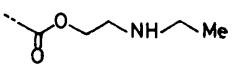
(10) $R^1 = CO_2CH_2CCl_3$, $R^2 = R^3 = H$

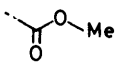
(11) $R^1 = CO_2CH_2CCl_3$, $R^2 = H$, $R^3 =$ 

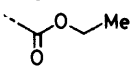
(12) $R^1 = R^2 = H$, $R^3 =$ 

(13) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = R^3 = H$

(14) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$, $R^3 =$ 

(15) $R^1 = R^2 = H$, $R^3 =$ 

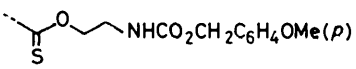
(16) $R^1 = R^2 = H$, $R^3 =$ 

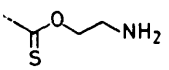
(17) $R^1 = R^2 = H$, $R^3 =$ 

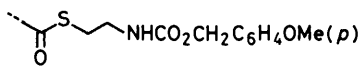
(18) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$, $R^3 = CO_2CH_2CCl_3$

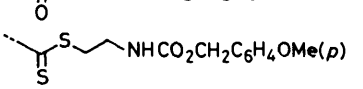
(19) $R^1 = R^2 = CO_2CH_2C_6H_4OMe(p)$, $R^3 = CO_2CH_2CCl_3$

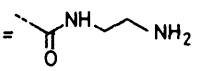
(20) $R^1 = R^2 = CO_2CH_2C_6H_4OMe(p)$, $R^3 = H$

(21) $R^1 = R^2 = CO_2CH_2C_6H_4OMe(p)$, $R^3 =$ 

(22) $R^1 = R^2 = H$, $R^3 =$ 

(23) $R^1 = R^2 = CO_2CH_2C_6H_4OMe(p)$, $R^3 =$ 

(24) $R^1 = R^2 = CO_2CH_2C_6H_4OMe(p)$, $R^3 =$ 

(25) $R^1 = R^2 = H$, $R^3 =$ 

(26) $R^1 = Ac$, $R^2 = R^3 = H$

(27) $R^1 = R^2 = H$, $R^3 = CONH_2$

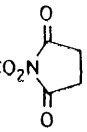
(28) $R^1 = R^2 = H$, $R^3 = CONHMe$

(29) $R^1 = R^2 = R^3 = H$

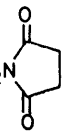
amino-group in spite of the fact that the 3''-amino-group was unprotected. In order to prepare some thio-analogues of (12) we needed to first protect the 3''-amino-group, as imidazole reagents were to be used and these are known usually to react preferentially at the 3''-amino-group, when both the 1- and 3''-amino groups are unprotected.[21] This was accomplished by treating 3,2',6'-tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) with

(30) $R^1 = R^2 = R^3 = H$

(31) $R^1 = CO_2CH_2CCl_3$, $R^2 = R^3 = H$

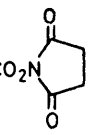
(32) $R^1 = CO_2CH_2CCl_3$, $R^2 = H$, $R^3 =$ 

(33) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = R^3 = H$

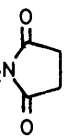
(34) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$, $R^3 =$ 

(35) $R^1 = R^3 = H$, $R^2 = Et$

(36) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = Et$, $R^3 = H$

(37) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = Et$, $R^3 =$ 

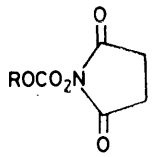
(38) $R^1 = CO_2CH_2Ph$, $R^2 = R^3 = H$

(39) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 =$ 

N-(2,2,2-trichloroethoxycarbonyloxy)succinimide (42) to give the 1-*N*-(2,2,2-trichloroethoxycarbonyl) derivative (18). The latter, on treatment with 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate in dimethyl sulphoxide containing triethylamine, afforded the 3''-*N*-(methoxybenzyloxycarbonyl) derivative (19), which on treatment with zinc in 10% acetic acid–methanol gave the desired 3,2',6',3''-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20).

The conversion of 2-(4-methoxybenzyloxycarbonylamino)ethanol (33) into 1-[2-(4-methoxybenzyloxycarbonylamino)ethoxythiocarbonyl]imidazole (43) was effected smoothly using *NN'*-thiocarbonyldi-imidazole. The imidazole derivative (43) after treatment with triethyloxonium tetrafluoroborate * in dichloromethane was condensed with the tetra-protected sisomicin derivative (20) to give the protected intermediate (21) in good yield. No reaction occurred in the absence of the triethyloxonium tetrafluoroborate. Deprotection of (21) with trifluoroacetic acid afforded 1-*N*-(2-aminoethoxythiocarbonyl)sisomicin (22).

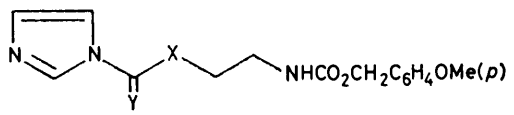
2-Aminoethanethiol hydrochloride was converted into the 4-methoxybenzyloxycarbonyl derivative (46) which on treatment with *NN'*-carbonyldi-imidazole afforded 1-[2-(4-methoxybenzyloxycarbonylamino)ethanethiocarbonyl]imidazole (44). The latter failed to condense with 3,2',6',3''-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) either with or without triethyloxonium tetrafluoroborate. An alternative synthetic route was therefore investigated. The thiol (46) was treated with phosgene in the presence of triethylamine to give the crude thiocarbonyl chloride (47) which was used without further purification to prepare 3,2',6',3''-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-[2-(4-methoxybenzyloxycarbonylamino)ethanethiocarbonyl]sisomicin (23) in the presence of sodium carbonate using aqueous



(40) R = Me

(41) R = Et

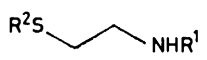
(42) R = CH_2CCl_3



(43) X = O, Y = S

(44) X = S, Y = O

(45) X = Y = S



(46) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$

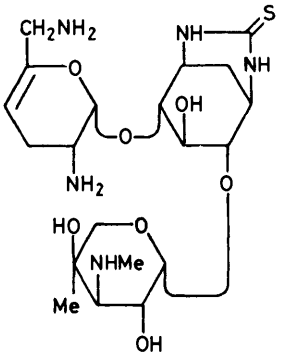
(47) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = COCl$

acetone as the solvent. Attempted deprotection of (23) using trifluoroacetic acid under a variety of conditions led to complex mixtures of products that were not further investigated.

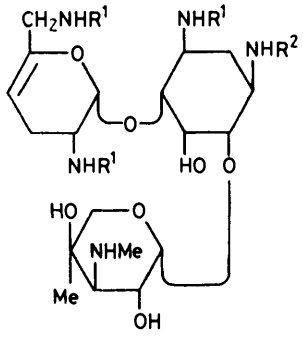
We next attempted to prepare a dithio-analogue of (12) in the following manner. The thiol (46) was treated with *NN'*-thiocarbonyldi-imidazole to give 1-[2-(4-methoxybenzyloxycarbonylamino)ethanethiothiocarbonyl]-imidazole (45). The latter after treatment with triethyloxonium tetrafluoroborate was condensed with 3,2',6',3''-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-sisomicin (20) to give 3,2',6',3''-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-1-N-[2-(4-methoxybenzyloxycar-

* We thank Dr. S. W. McCombie for helpful suggestions.

bonylamino)ethanethiothiocarbonyl]sisomicin (24) in high yield. Deprotection of (24) with trifluoroacetic acid afforded 1-*N* : 3-*N*-thiocarbonylsisomicin (48) as the only isolatable product of the reaction.

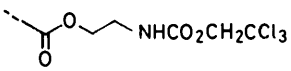


(48)



(49) $R^1 = R^2 = H$

(50) $R^1 = CO_2CH_2CCl_3$, $R^2 = H$

(51) $R^1 = CO_2CH_2CCl_3$, $R^2 =$ 

(52) $R^1 = H$, $R^2 =$ 

It has been demonstrated in these laboratories [22] that epimerization of the 5-hydroxy-group in sisomicin produces an antibacterial derivative (49) that is more potent than sisomicin and which has a vastly improved spectrum of activity against resistant strains of bacteria. The 5-*epi*-analogue of (12) was therefore synthesized as follows. 3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)-5-*epi*-sisomicin (50) on treatment with the succinimide ester (32) afforded the protected derivative (51) which on deprotection with zinc in acetic acid–methanol gave 1-*N*-(2-aminoethoxycarbonyl)-5-*epi*-sisomicin (52).

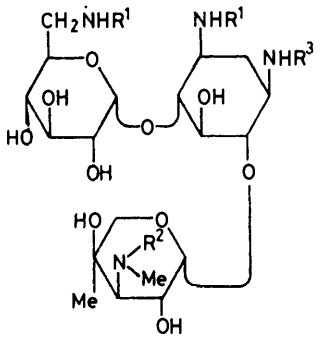
In order to further expand the structure–activity relationships of the 1-*N*-aminoalkyloxycarbonyl derivatives, a variety of such derivatives was prepared using protected gentamicin B derivatives as substrates. 3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) [20,*] on treatment with *N*-(2,2,2-trichloroethoxycarbonyloxy)succinimide (42) gave 3,6′-bis-*N*-benzyloxycarbonyl-1-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin B (54). The latter, on treatment with benzyl chloroformate in the presence of sodium carbonate, gave 3,6′,3″-tris-*N*-benzyloxycarbonyl-1-*N*(2,2,2-trichloroethoxycarbonyl)-gentamicin B (55) which on reduction with zinc in aqueous acetic acid gave 3,6′,3″-tris-*N*-benzyloxycarbonylgentamicin B (56). 2-Benzyloxycarbonylaminoethanol (38) was prepared in the usual way and converted into the succinimide active ester (39). The latter was condensed with each of the protected gentamicin B derivatives (56) and (53) to give the protected derivatives (57) and (58) respectively. Catalytic hydrogenation of the protected derivatives (57) and (58) gave 1-*N*-(2-aminoethoxycarbonyl)gentamicin B (59) in each case.

Several extended-chain derivatives were prepared next. 3-Aminopropanol (73) was converted into 3-benzyloxycarbonylaminopropanol (74) which was in turn converted into the succinimide active ester (75). The latter was condensed with 3,6′,3″-tris-*N*-benzyloxycarbonylgentamicin B (56) in dimethylformamide in the presence of triethylamine to give the protected derivative (60) which on catalytic hydrogenation afforded 1-*N*-(3-aminopropoxycarbonyl)gentamicin B (61). In a similar manner 4-aminobutan-1-ol (76) was converted *via* 4-benzyloxylcarbonylaminobutan-1-ol (77) into the succinimide active ester (78) which was condensed with (53) to give the protected derivative (63). The latter on catalytic hydrogenation afforded 1-*N*-(4-aminobutoxycarbonyl)gentamicin B (64).

Two analogues having the amino-group at the 2-position in the side-chain with an extended alkyl chain were synthesized next. (2*R*)-2-Aminobutan-1-ol (79) was converted into the *N*-benzyloxycarbonyl derivative (80) which was used to synthesize the succinimide active ester (81). Condensation of the latter with (53) gave the protected derivative (65) which on catalytic hydrogenation afforded (2*R*)-1-*N*-(2-aminobutoxycarbonyl)-gentamicin B (66). (2*S*)-2-Amino-4-methylpentan-1-ol (82) was converted into the *N*-benzyloxycarbonyl derivative (83) which was then converted into the succinimide active ester (84). The latter was condensed with (53) to give the protected derivative (67) which on catalytic hydrogenation afforded (2*S*)-1-*N*-(2-amino-4-methylpentyloxycarbonyl)gentamicin B (68).

The 1-*N*-aminoalkoxycarbonyl derivatives of gentamicin C_{1a} and kanamycin A were prepared next. 3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (85) on treatment with the active ester (32) gave (86) which was deprotected with zinc in acetic acid–methanol to give 1-*N*-(2-aminoethoxycarbonyl)gentamicin C_{1a} (87). Similarly 3,6′-bis-*N*-benzyloxycarbonylkanamycin A (3) [20,*] on treatment with the active ester (39) gave (4) which was catalytically hydrogenated to give 1-*N*-(2-aminoethoxycarbonyl)kanamycin A (7). The active ester (34) was also condensed with 3,6′-bis-*N*-(4-methoxybenzyloxycarbonyl)kanamycin A (5) [20,*] to give (6)

* Note as on page 2186.

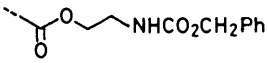


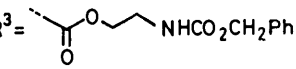
(53) $R^1 = CO_2CH_2Ph$, $R^2 = R^3 = H$

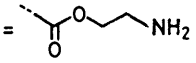
(54) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 = CO_2CH_2CCl_3$

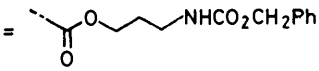
(55) $R^1 = R^2 = CO_2CH_2Ph$, $R^3 = CO_2CH_2CCl_3$

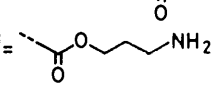
(56) $R^1 = R^2 = CO_2CH_2Ph$, $R^3 = H$

(57) $R^1 = R^2 = CO_2CH_2Ph$, $R^3 =$ 

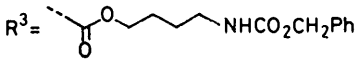
(58) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 =$ 

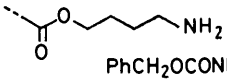
(59) $R^1 = R^2 = H$, $R^3 =$ 

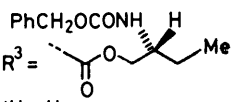
(60) $R^1 = R^2 = CO_2CH_2Ph$, $R^3 =$ 

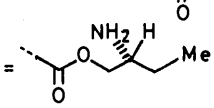
(61) $R^1 = R^2 = H$, $R^3 =$ 

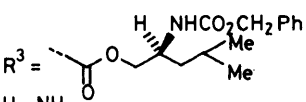
(62) $R^1 = R^2 = R^3 = H$

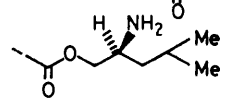
(63) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 =$ 

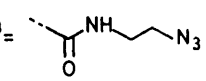
(64) $R^1 = R^2 = H$, $R^3 =$ 

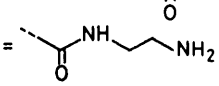
(65) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 =$ 

(66) $R^1 = R^2 = H$, $R^3 =$ 

(67) $R^1 = CO_2CH_2Ph$, $R^2 = H$, $R^3 =$ 

(68) $R^1 = R^2 = H$, $R^3 =$ 

(69) $R^1 = R^2 = CO_2CH_2Ph$, $R^3 =$ 

(70) $R^1 = R^2 = H$, $R^3 =$ 

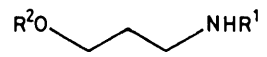
(71) $R^1 = CO_2CMe_3$, $R^2 = R^3 = H$

(72) $R^1 = R^2 = H$, $R^3 = CONH_2$

which on treatment with trifluoroactic acid afforded 1-*N*-(2-aminoethoxycarbonyl)kanamycin A (7).

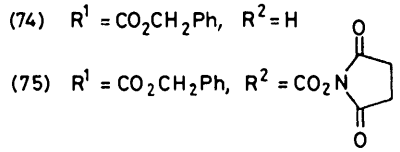
We next turned our attention to the preparation of a series of 1-*N*-carboxamido-analogues. Thus 3,2′,6′,3″-tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) was treated with 2-azidoethyl isocyanate (90) in dimethylformamide containing triethylamine, followed by reduction with triphenylphosphine, hydrolysis with concentrated ammonium hydroxide, and deprotection with trifluoroacetic acid, to give 1-*N*-(2-aminoethylcarboxamido)sisomicin (25). Some 1-*N*-(2-aminoethylcarboxamido)garamine (92) was formed as a by-product in the above reaction. Treatment of 3,2′,6′-tri-*N*-acetylsisomicin (26) [20,*] with silicon tetraisocyanate in dimethylformamide at 0 °C, followed by alkaline hydrolysis afforded 1-*N*-carboxamidosisomicin (27). The use of methyl isocyanate afforded 1-*N*-(methylcarboxamido)-sisomicin (28). Condensation of 2-azidoethyl isocyanate (90), prepared from 2-chloroethyl isocyanate (91), with 3,6′,3″-tris-*N*-benzyloxycarbonylgentamicin B (56) gave the protected derivative (69). Catalytic hydrogenation of (69) gave 1-*N*-(2-aminoethylcarboxamido)gentamicin B (70). Treatment of 3,6′-bis-*N*-t-butoxycarbonylgentamicin B (71) [20,*] with silicon tetraisocyanate, followed by deprotection with trifluoroacetic acid, gave 1-*N*-carboxamidogentamicin B (72). A thiocarboxamido-analogue of gentamicin C_{1a} was also prepared by treatment of 3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (85) [20,*] with ethyl isothiocyanate in tetrahydrofuran, followed by reduction with zinc in acetic acid–methanol to give 1-*N*-(ethylthiocarboxamido)gentamicin C_{1a} (88). From the protonation shifts

* Note as on page 2186.

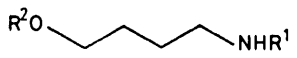


(73) $R^1 = R^2 = H$

(74) $R^1 = CO_2CH_2Ph$, $R^2 = H$

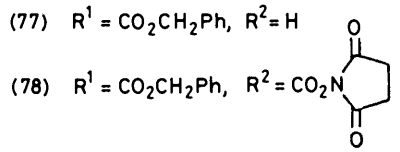


(75) $R^1 = CO_2CH_2Ph$, $R^2 = CO_2N$

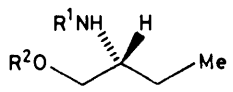


(76) $R^1 = R^2 = H$

(77) $R^1 = CO_2CH_2Ph$, $R^2 = H$

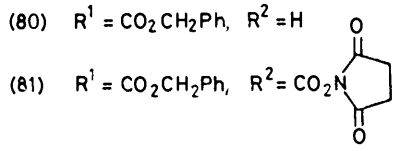


(78) $R^1 = CO_2CH_2Ph$, $R^2 = CO_2N$

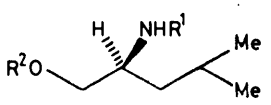


(79) $R^1 = R^2 = H$

(80) $R^1 = CO_2CH_2Ph$, $R^2 = H$

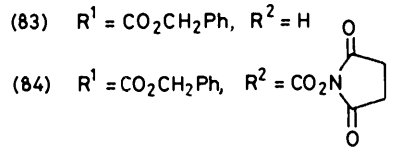


(81) $R^1 = CO_2CH_2Ph$, $R^2 = CO_2N$



(82) $R^1 = R^2 = H$

(83) $R^1 = CO_2CH_2Ph$, $R^2 = H$



(84) $R^1 = CO_2CH_2Ph$, $R^2 = CO_2N$

in the ^{13}C n.m.r. spectrum (Table 1) it is evident that the substituent is located on the 1-amino-group.

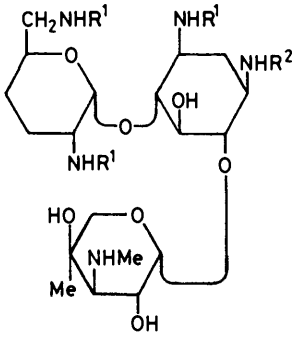
In order to assign unambiguously the ^{13}C n.m.r. data (Table 1) in the case of the gentamicin B derivatives where δ_C for C-1′ and C-1″ are very close, it was necessary to synthesize 1-*N*-(2-aminoethoxycarbonyl)garamine (93) as a model. Thus 3,2′,6′-tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) was treated with the active ester (34), and the crude product (14) was directly hydrolyzed using Amberlite IR 120 (H^+) resin to the corresponding garamine derivative (94). Deprotection of the latter with trifluoroacetic acid gave 1-*N*-(2-aminoethoxycarbonyl)garamine (93). Model 2-deoxystreptamine derivatives were also needed in order to study the solution conformations of these novel 1-*N*-substituted aminoglycosides and these were prepared as follows. 2-Deoxystreptamine (96) was converted into the (±)- mono-*N*-(4-methoxybenzyloxycarbonyl) derivative (97) and the latter on treatment with the active ester (34) followed by deprotection with trifluoroacetic acid, gave (±)-1(3)-*N*-(2-aminoethoxycarbonyl)-2-deoxystreptamine (99). Acetylation of (96) with *N*-acetylimidazole gave (±)-1(3)-*N*-acetyl-2-deoxystreptamine (100). Catalytic hydrogenation of (2*R*)-3,6′-tris-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminobutoxycarbonyl)gentamicin B(65) in the presence of 1M-hydrochloric acid gave (2*R*)-1-*N*-(2-aminobutoxycarbonyl)-2-deoxystreptamine (101). The ^{13}C n.m.r. data for the above model garamine and 2-deoxystreptamine derivatives are given in Table 1.

The ^{13}C n.m.r. data for these novel 1-*N*-alkyloxycarbonyl and 1-*N*-alkylcarboxamido derivatives (Table 1) clearly indicate that the substituents are located on the 1-amino-group in each case. The usual β-protonation shift of −8.0 to −8.3 for C-2 in the unsubstituted aminoglycosides resulting from protonation of both the 1- and 3-amino-groups, has decreased to −4.0 to −4.9 in the 1-NHCOR derivatives, as no protonation is occurring at the 1-amino-group in these derivatives. The normal β-protonation shift of C-6 of −3.5 to −3.9 for the unsubstituted aminoglycosides does not occur as anticipated, in the 1-NHCOR derivatives. Instead C-6 experiences a much smaller shielding in the 1-NHCOR derivatives of 0 to −1.8 which appears to be characteristic of these derivatives, as the 1-NHCOR-2-deoxystreptamine derivatives also exhibit similar shieldings at C-6 at acidic pH (Table 1).

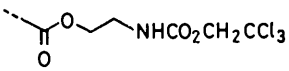
In order to study the rotamer populations about the glycosidic linkages it was necessary to calculate the $\Delta\delta_C$ values in going from the 1-NHCOR-2-deoxystreptamine to the 1-NHCOR-trisaccharides. Selected examples are given in Table 2 along with reference $\Delta\delta_C$ values derived in going from 2-deoxystreptamine to the unsubstituted aminoglycosides used as substrates in this study. In all cases the data refer to fully decarbonated free bases.* Comparison of the chemical shifts of 1-*N*-(2-aminoethoxycarbonyl)-2-deoxystreptamine (99), (±)-1(3)-*N*-acetyl-2-deoxystreptamine (100) and (2*R*)-1-*N*-(2-aminobutoxycarbonyl)-2-deoxystreptamine (101) revealed almost identical chemical shift values for C-2—C-6 in each case. Substituent effects were evident at C-1 in these derivatives as the nature of the side-chain varied and this was to be expected. We were therefore in a position to use the chemical shifts of C-3, C-4, C-5, and C-6 of (101) as base-line data for determining the solution conformations of these 1-NHCOR aminoglycosides without having to consider the nature of R.

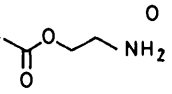
We shall consider first the rotamers about the C-4–O glycosidic bond. From the observed shielding at C-3

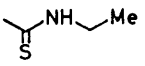
* Obtained by passage of an aqueous solution of the base over Amberlite IRA 401s(OH^-) resin under an inert atmosphere of argon.



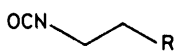
(85) $R^1 = CO_2CH_2CCl_3$, $R^2 = H$

(86) $R^1 = CO_2CH_2CCl_3$, $R^2 =$ 

(87) $R^1 = H$, $R^2 =$ 

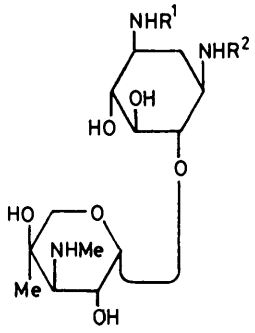
(88) $R^1 = H$, $R^2 =$ 

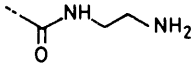
(89) $R^1 = R^2 = H$

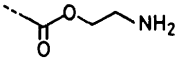


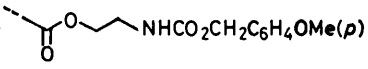
(90) $R = N_3$

(91) $R = Cl$



(92) $R^1 = H$, $R^2 =$ 

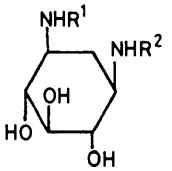
(93) $R^1 = H$, $R^2 =$ 

(94) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 =$ 

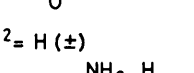
(95) $R^1 = R^2 = H$

(96) $R^1 = R^2 = H$

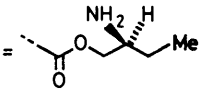
(97) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 = H$ (±)



(98) $R^1 = CO_2CH_2C_6H_4OMe(p)$, $R^2 =$ 

(99) $R^1 = H$ $R^2 =$ 

(100) $R^1 = Ac$, $R^2 = H$ (±)

(101) $R^1 = H$, $R^2 =$ 

and the deshielding of +9.8 to +10.2 at C-4 in gentamicin B (62), gentamicin C_{1a} (89), and kanamycin A (8) it is evident that these derivatives all exhibit rotamer *a* about the C-4–O glycosidic bond, when no substituent is present on the 1-amino-group.[1,23-32] From the data in Tables 1 and 2 it is evident that no change occurs in the rotamer *a* about the C-4–O glycosidic bond in the 1-NHCOR substituted derivatives of gentamicin B (62), gentamicin C_{1a} (89), and kanamycin A (8) upon introduction of the side-chain. In sisomicin (29) the sisosamine adopts a different rotamer in which the sugar has rotated in a clockwise direction about the O–C-4 bond relative to the gentamicins and kanamycin A (8).[32] Shielding is still observed at C-3 while the deshielding at C-4 has been reduced to +6.7. These effects will be discussed in detail in the following paper.[32] For illustrative purposes the conformation may be represented by the approximate rotamer *b*.* Introduction of the 1-NHCOR substituents in the sisomicin series (Tables 1 and 2) resulted in no change in the observed shielding of −1.2 for C-3 and in the observed deshielding of +6.6 for C-4 using (12) as the example, indicating that these derivatives have the same solution conformation about the O–C-4 glycosidic bond as does the parent unsubstituted sisomicin (29). In the case of 5-*epi*-sisomicin (49), epimerization of the 5-hydroxy-group results in a marked clockwise rotation of the sisosamine unit about the O–C-4 glycosidic bond and it is felt that the solution conform-

* It is not possible to define the exact torsion angles in these rotamers from the data available to us, and consequently all rotamers are approximate diagrammatic representations which are compatible with the ^{13}C n.m.r. data and do not imply torsion angles.[32]

TABLE 1

^{13}C N.m.r. chemical shifts (δ_C, p.p.m. downfield from tetramethylsilane in D_2O)

Carbon	(29) [a]	(29)H+ [b,c]	$\Delta\delta_C$(Base→H+)	(12)	(12)H+	$\Delta\delta_C$(Base→H+)	(15)	(51)H+	$\Delta\delta_C$(Base→H+)	(16)	(16)H+	$\Delta\delta_C$(Base→H+)	(17)	(17)H+	$\Delta\delta_C$(Base→H+)	(22)	(22)H+	$\Delta\delta_C$(Base→H+)
C-1	51.7	50.7	−1.0	52.1	51.4	−0.7	52.1	51.3	−0.8	52.1	51.3	−0.8	52.0	51.2	−0.8	55.3	54.4	−0.9
C-2	36.4	28.3	−8.1	35.7	31.3	−4.4	35.6	31.4	−4.2	35.6	31.5	−4.1	35.7	31.5	−4.2	35.0	30.9	−4.1
C-3	50.3	49.0	−1.3	49.9	49.5	−0.4	49.9	49.5	−0.4	49.9	49.5	−0.4	49.9	49.4	−0.5	49.8	49.4	−0.4
C-4	85.2	79.9	−5.3	84.7	80.4	−4.3	84.6	80.5	−4.1	84.7	80.5	−4.2	84.6	80.5	−4.1	84.7	80.4	−4.3
C-5	75.3	74.4	−0.9	75.6	74.5	−1.1	75.6	74.5	−1.1	75.6	74.5	−1.1	75.6	74.5	−1.1	75.8	74.6	−1.2
C-6	87.7	83.9	−3.8	81.7	80.8	−0.9	81.7	80.7	−1.0	81.8	80.5	−1.3	81.7	80.5	−1.2	82.3	80.6	−1.7
C-1′	100.6	97.9	−2.7	100.8	97.9	−2.9	100.8	97.8	−3.0	100.8	97.9	−2.9	100.7	97.8	−2.9	100.8	97.9	−2.9
C-2′	47.5	47.0	−0.5	47.4	47.1	−0.3	47.4	47.1	−0.3	47.4	47.0	−0.4	47.4	47.0	−0.4	47.4	47.1	−0.3
C-3′	25.6	23.9	−1.7	25.5	24.2	−1.3	25.4	24.2	−1.2	25.5	24.1	−1.4	25.5	24.1	−1.4	25.5	24.1	−1.4
C-4′	96.4	101.6	+5.2	97.0	101.5	+4.5	97.2	101.5	+4.3	96.9	101.4	+4.5	97.0	101.4	+4.4	96.7	101.5	+4.8
C-5′	150.2	144.3	−5.9	150.0	144.2	−5.8	149.8	144.2	−5.6	150.2	144.2	−6.0	150.0	144.2	−5.8	150.4	144.2	−6.2
C-6′	43.5	41.5	−2.0	43.6	41.6	−2.0	43.6	41.6	−2.0	43.3	41.5	−1.8	43.3	41.5	−1.8	43.4	41.5	−1.9
C-1″	101.5	101.9	+0.4	99.8	99.3	−0.5	99.8	99.2	−0.6	99.9	99.2	−0.7	99.7	99.1	−0.6	99.8	98.8	−1.0
C-2″	70.0	67.2	−2.8	69.8	66.9	−2.9	69.8	66.9	−2.9	69.8	66.8	−3.0	69.8	66.9	−2.9	70.0	66.9	−3.1
C-3″	64.3	64.2	−0.1	64.1	65.0	+0.9	64.1	65.0	+0.9	64.1	64.9	+0.8	64.2	65.0	+0.8	64.3	65.1	+0.8
C-4″	73.0	70.8	−2.2	73.2	70.8	−2.4	73.1	70.8	−2.3	73.1	70.8	−2.3	73.1	70.7	−2.4	73.1	70.8	−2.3
C-5″	68.5	68.6	+0.1	68.6	67.7	−0.9	68.6	67.7	−0.9	68.5	67.7	−0.8	68.6	67.7	−0.9	68.7	67.8	−0.9
C-6″																		
3-NCH_3	37.9	35.4	−2.5	37.7	36.0	−1.7	37.7	36.0	−1.7	37.7	35.9	−1.8	37.7	36.0	−1.7	37.8	36.0	−1.8
4″-CH_3	22.9	21.8	−1.1	22.5	22.0	−0.5	22.5	22.0	−0.5	22.5	21.9	−0.6	22.5	21.9	−0.6	22.6	22.0	−0.6
C-1‴				158.9	157.7	−1.2	158.7	157.6	−1.1	159.4	159.2	−0.2	159.0	158.8	−0.2	181.0	181.4	+0.4
C-2‴				67.4 [d]	62.3	−5.1	64.9	61.5	−3.4	53.4	53.2	−0.2	62.8	63.0	+0.2	60.8	60.8	
C-3‴				40.5	39.7	−0.8	47.5	46.9	−0.6				14.7	14.7		46.8	46.8	
C-4‴							43.2	44.0	+0.8									
C-5‴							14.1	11.4	−2.7									
C-6‴																		
C-7‴																		

Carbon	(48)	(48)H+	$\Delta\delta_C$(Base→H+)	(52)	(52)H+	$\Delta\delta_C$(Base→H+)	(62) [c]	(62)H+ [c]	$\Delta\delta_C$(Base→H+)	(59)	(59)H+	$\Delta\delta_C$(Base→H+)	(61)	(61)H+	$\Delta\delta_C$(Base→H+)	(64)	(64)H+	$\Delta\delta_C$(Base→H+)
C-1	49.1	49.4	+0.3	48.2	48.6	+0.4	51.5	50.6	−0.9	51.9	51.2	−0.7	51.9	51.2	−0.7	51.9	51.1	−0.8
C-2	16.4	16.8	+0.4	35.9	31.1	−4.8	36.6	28.3	−8.3	35.8	31.5	−4.3	35.7	31.6	−4.1	35.8	31.6	−4.2
C-3	47.3	46.4	−0.9	47.1	48.0	+0.9	49.9	48.4	−1.5	49.5	48.8	−0.7	49.5	48.8	−0.7	49.5	48.7	−0.8
C-4	77.8	77.0	−0.8	80.2	76.6	−3.6	88.7	79.0	−9.7	88.2	79.7	−8.5	87.7	79.8	−7.9	87.2	79.7	−7.5
C-5	70.4	69.7	−0.7	70.2	68.6	−1.6	74.8	73.1	−1.7	75.0	73.0	−2.0	75.1	73.1	−2.0	75.1	73.1	−2.0
C-6	78.5	78.8	+0.3	81.4	79.6	−1.8	87.5	84.6	−2.9	81.5	81.6	+0.1	81.4	81.5	+0.1	81.5	81.3	−0.2
C-1′	99.4	95.3 [f]	−4.1	97.0	93.8	−3.2	100.4	96.6	−3.8	100.9	96.4	−4.5	100.4	96.5	−3.9	100.1	96.4	−3.7
C-2′	47.3	47.2	−0.1	47.0	47.1	+0.1	72.7	71.7	−1.0	72.9	71.7	−1.2	72.6	71.7	−0.9	72.6	71.7	−0.9
C-3′	25.9	22.4	−3.5	25.6	23.8	−1.8	73.8	73.0	−0.8	73.9	73.0	−0.9	73.7	73.1	−0.6	73.6	73.1	−0.5
C-4′	97.1	101.1	+4.0	97.0	101.4	+4.4	71.8	71.6	−0.2	71.9	71.7	−0.2	71.8	71.7	−0.1	71.8	71.7	−0.1
C-5′	149.9	143.4	−6.5	150.1	144.1	−6.0	73.8	69.5	−4.3	73.9	69.4	−4.5	73.7	69.4	−4.3	73.0	69.4	−3.6
C-6′	43.4	41.6	−1.8	43.3	41.5	−1.8	42.7	41.1	−1.6	42.5	41.2	−1.3	42.3	41.2	−1.1	42.2	41.2	−1.0
C-1″	100.1	95.9 [f]	−4.2	101.7	100.8	−0.9	101.0	102.0	+1.0	99.6	99.3	−0.3	99.8	99.1	−0.7	99.5	99.0	−0.5
C-2″	69.6	66.5	−3.1	69.8	66.9	−2.9	70.0	67.2	−2.8	69.8	66.9	−2.9	69.8	66.9	−2.9	69.5	66.9	−2.6
C-3″	64.6	64.8	+0.2	64.1	64.9	+0.8	64.4	64.2	−0.2	64.2	65.0	+0.8	64.2	65.0	+0.8	64.3	65.0	+0.7
C-4″	73.1	70.6	−2.5	73.2	70.8	−2.4	73.0	70.8	−2.2	72.9	70.8	−2.1	73.1	70.8	−2.3	72.9	70.8	−2.1
C-5″	68.7	67.7	−1.0	68.6	67.9	−0.7	68.6	68.6		68.6	67.7	−0.9	68.6	67.7	−0.9	68.5	67.8	−0.7
3″-NCH_3	38.1	35.9	−2.2	37.7	35.9	−1.8	38.0	35.3	−2.7	37.8	36.0	−1.8	37.9	36.0	−1.9	37.0	36.0	−1.6
4″-CH_3	22.5	22.0	−0.5	22.3	21.9	−0.4	23.0	21.7	−1.3	22.5	21.9	−0.6	22.6	21.9	−0.7	22.5	21.9	−0.6
C-1‴	[g]	[g]		158.9	158.1	−0.8				158.8	157.7	−1.1	158.9	158.3	−0.6	158.9	158.7	−0.2
C-2‴				67.8	62.4	−5.4				67.8	62.4	−5.4	64.2	63.6	−0.8	65.8	65.8	
C-3‴				40.5	39.7	−0.8				40.5	39.7	−0.8	30.7	27.1	−3.6	26.2	26.1	−0.1
C-4‴													38.2	37.9	−0.3	25.1 [h]	24.3	−0.8

Carbon	(66)	(66)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(68)	(68)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(89) [a]	(89)H$^+$ [c]	$\Delta\delta_C$(Base→H$^+$)	(87) [h]	(87)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(8)	(88)H$^+$ [e]	$\Delta\delta_C$(Base→H$^+$)	(7)	(7)H$^+$	$\Delta\delta_C$(Base→H$^+$)
C-1	51.9	51.2	−0.7	51.9	51.2	−0.7	51.7	50.5	−1.2	51.9	51.1	−0.8	51.3	50.6	−0.7	51.8	51.1	−0.7
C-2	35.7	31.4	−4.3	35.8	31.5	−4.3	36.7	28.5	−8.2	35.7	31.7	−4.0	36.2	28.2	−8.0	35.7	31.4	−4.3
C-3	49.4	48.8	−0.6	49.5	48.8	−0.7	50.6	49.4	−1.2	49.9	49.9		49.8	48.4	−1.4	49.5	48.8	−0.7
C-4	87.7	79.6	−8.1	87.9	79.6	−8.3	88.3	77.3	−11.0	84.4	77.7	−6.7	88.6	78.8	−9.8	87.7	79.6	−8.1
C-5	75.1	73.0	−2.1	75.1	73.0	−2.1	75.4	75.2	−0.2	75.7	75.2	−0.5	74.9	73.4	−1.5	75.2	73.1	−2.1
C-6	81.5	81.6	+0.1	81.6	81.5	−0.1	87.8	84.3	−3.5	81.9	81.3	−0.6	88.1	84.5	−3.6	82.5	82.3	−0.2
C-1′	100.4	96.3	−4.1	100.5	96.3	−4.2	102.2	95.6	−6.6	98.8	95.3	−3.5	100.3	96.4	−3.9	100.3	96.1	−4.2
C-2′	72.6	71.7	−0.9	72.7	71.6	−1.1	51.0	49.5	−1.5	50.2	49.6	−0.6	72.7	71.7	−1.0	72.7	71.7	−1.0
C-3′	73.7	73.0	−0.7	73.8	73.0	−0.8	27.1	21.2	−5.9	24.5	21.3	−3.2	73.7	72.9	−0.8	73.8	72.6	−1.2
C-4′	71.8	71.7	−0.1	71.8	71.7	−0.1	28.5	26.2	−2.3	27.5	26.3	−1.2	71.8	71.6	−0.2	71.8	71.7	−0.1
C-5′	73.7	69.4	−4.3	73.9	69.4	−4.5	71.5	66.8	−4.7	67.9	66.8	−1.1	73.7	69.4	−4.3	73.8	69.4	−4.4
C-6′	42.4	41.2	−1.2	42.4	41.3	−1.1	46.1	43.5	−2.6	44.3	43.6	−0.7	42.3	41.2	−1.1	42.4	41.3	−1.1
C-1″	99.7	99.3	−0.4	99.6	99.0	−0.6	101.3	101.9	+0.6	99.5	99.1	−0.4	100.8	101.2	+0.4	99.5	99.1	−0.4
C-2″	69.8	66.9	−2.9	69.8	66.9	−2.9	70.2	67.1	−3.1	68.5	66.8	−1.7	72.7	68.9	−3.8	72.7	68.7	−4.0
C-3″	64.2	65.0	+0.8	64.2	65.0	+0.8	64.4	64.1	−0.3	64.5	64.9	+0.4	55.0	55.7	+0.7	54.9	56.1	+1.2
C-4″	73.1	70.8	−2.3	73.1	70.8	−2.3	73.3	70.8	−2.5	72.2	70.8	−1.4	70.1	66.3	−3.8	70.1	66.4	−3.7
C-5″	68.6	67.7	−0.9	68.7	67.7	−1.0	68.7	68.7		68.2	67.7	−0.5	72.9	72.9		72.7	73.1	+0.4
C-6″													61.1	60.9	−0.2	61.1	60.6	−0.5
3″-NCH$_3$	37.8	36.0	−1.8	37.8	36.0	−1.8	38.0	35.5	−2.5	37.0	36.1	−0.9						
4″-CH$_3$	22.5	22.0	−0.5	22.6	22.0	−0.6	23.0	21.9	−1.1	22.2	22.0	−0.2						
C-1‴	158.8	157.6	−1.2	158.8	157.5	−1.3				158.2	157.6	−0.6				158.9	157.7	−1.2
C-2‴	69.8	64.8	−5.0	70.8	65.4	−5.4				64.1	62.3	−1.8				67.7	62.4	−5.3
C-3‴	51.6	53.0	+1.4	48.2	50.2	+2.0				40.0	39.8	−0.2				40.5	39.8	−0.7
C-4‴	26.4	22.9	−3.5	24.9	24.6	−0.3												
C-5‴	10.3	9.9	−0.4	42.8	38.3	−4.5												
C-6‴				22.4	22.6 [b]	+0.2												
C-7‴				23.3	22.3 [b]	−1.0												

Carbon	(25)	(25)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(27)	(28)	(70)	(70)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(72)	(72)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(88) [h]	(88)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(95) [i]	(95)H$^+$ [j]	$\Delta\delta_C$(Base→H$^+$)
C-1	51.1	50.4	−0.7	51.1	51.1	51.0	50.3	−0.7	51.0	50.4	−0.6	55.1	54.1	−1.0	51.7	50.9	−0.8
C-2	36.1	31.8	−4.3	35.7	35.9	36.0	31.9	−4.1	36.0	31.7	−4.9	35.2	31.3	−3.9	36.6	28.5	−8.1
C-3	49.9	49.5	−0.4	49.3	49.8	49.5	48.8	−0.7	49.5	48.8	−0.7	49.8	49.6	−0.2	51.4	50.7	−0.7
C-4	84.9	80.6	−4.3	84.6	84.3	87.0	79.8	−7.2	87.3	79.7	−7.6	85.7	78.2	−7.5	78.8	73.3	−5.5
C-5	75.8	74.6	−1.2	75.7	75.8	75.3	73.2	−2.1	75.1	73.2	−1.9	75.7	75.4	−0.3	75.1	74.6	−0.5
C-6	82.3	80.8	−1.5	82.1	82.2	82.0	81.6	−0.4	81.9	81.5	−0.4	82.1	81.2	−0.9	87.9	84.0	−3.9
C-1′	100.8	97.9	−2.9	100.8	100.6	100.0	96.4	−3.6	100.1	96.4	−3.7	100.5	95.8	−4.7			
C-2′	47.4	47.1	−0.3	47.3	47.2	72.7	71.7	−1.0	72.7	71.7	−1.0	50.4	49.6	−0.8			
C-3′	25.2	24.1	−1.1	25.4	25.3	73.6	73.1	−0.5	73.6	73.1	−0.5	25.8	21.3	−4.5			
C-4′	96.7	101.4	+4.7	97.5	98.6	71.9	71.7	−0.2	71.9	71.7	−0.2	27.9	26.2	−1.7			
C-5′	150.4	144.2	−6.2	*g*	*g*	72.5	69.4	−3.1	72.6	69.4	−3.2	69.4	66.8	−2.6			
C-6′	43.3	41.5	−1.8	43.1	43.1	42.0	41.2	−0.8	42.2	41.2	−1.0	44.9	43.4	−1.5			
C-1″	99.8	99.1	−0.7	99.8	99.9	99.7	99.1	−0.6	99.4	98.9	−0.5	99.4	98.7	−0.7	101.4	101.8	+0.4
C-2″	70.0	67.0	−3.0	69.7	69.6	69.4	67.0	−2.4	69.4	66.8	−2.6	69.2	66.9	−2.3	70.0	67.1	−2.9
C-3″	64.3	65.2	+0.9	64.3	64.3	64.5	65.2	+0.7	64.4	65.0	+0.6	64.4	65.0	+0.6	64.3	64.3	
C-4″	73.1	70.8	−2.3	73.0	72.9	72.7	70.8	−1.9	72.9	70.8	−2.1	72.6	70.7	−1.9	73.2	70.8	−2.4
C-5″	68.6	67.7	−0.9	68.6	68.5	68.3	67.7	−0.6	68.5	67.8	−0.7	68.6	67.9	−0.7	68.5	68.5	
3″-NCH$_3$	37.8	35.9	−1.9	37.5	37.4	37.4	36.0	−1.4	37.5	36.0	−1.5	37.4	35.9	−1.5	38.0	35.4	−2.6
4″-CH$_3$	22.6	21.9	−0.7	22.4	22.3	22.3	21.9	−0.4	22.6	22.0	−0.6	22.4	21.8	−0.6	22.9	21.8	−1.1
C-1‴	160.8	160.4	−0.4	161.7	161.7	160.7	160.4	−0.3	161.5	*g*		*g*	*g*				
C-2‴	43.1	38.2	−4.9		27.1	39.7	38.2	−1.5				39.7	39.7				
C-3‴	41.4	40.8	−0.6			41.0	40.8	−0.2				14.1	14.1				

Carbon	(93)	(93)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(92)	(92)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(96) [a]	(96)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(99)	(99)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(100)	(100)H$^+$	$\Delta\delta_C$(Base→H$^+$)	(101)	(101)H$^+$	$\Delta\delta_C$(Base→H$^+$
C-1	52.1	51.6	−0.5	51.1	50.5	−0.6	51.6	51.0	−0.6	52.4	51.7	−0.7	50.8	50.1	−0.7	51.7	51.7	
C-2	36.2	31.8	−4.4	36.5	32.3	−4.2	37.0	29.0	−8.0	35.6	31.5	−4.1	35.2	31.1	−4.1	35.7	31.5	−4.2
C-3	50.9	51.2	+0.3	50.9	51.2	+0.3	51.6	51.0	−0.6	51.1	51.5	+0.4	51.1	51.5	+0.4	51.1	51.4	+0.3
C-4	78.3	73.7	−4.6	78.4	73.8	−4.6	78.5	73.2	−5.3	78.1	73.5	−4.6	78.1	73.5	−4.6	78.1	73.5	−4.6
C-5	75.2	74.7	−0.5	75.4	74.8	−0.6	76.6	75.5	−1.1	76.6	76.0	−0.6	76.7	76.1	−0.6	76.6	76.1	−0.5
C-6	81.8	80.9	−0.9	82.3	80.9	−1.4	78.5	73.2	−5.3	75.9	74.9	−1.0	75.5	74.6	−0.9	75.8	74.9	−0.9
C-1″	99.7	99.2	−0.5	99.7	99.0	−0.7												
C-2″	69.8	66.9	−2.9	70.0	67.0	−3.0												
C-3″	64.2	65.1	+0.9	64.3	65.2	+0.9												
C-4″	73.2	70.8	−2.4	73.1	70.8	−2.3												
C-5″	68.6	67.7	−0.9	68.6	67.6	−1.0												
3″-NCH$_3$	37.8	36.0	−1.8	37.8	36.0	−1.8												
4″-CH$_3$	22.5	21.9	−0.6	22.6	21.9	−0.7												
C-1‴	159.0	157.8	−1.2	160.8	160.4	−0.4				159.2	158.0	−1.2	174.7	174.9	+0.2	159.1	158.0	−1.1
C-2‴	67.8	62.3	−5.5	43.1	38.2	−4.9				67.6	62.3	−5.3	22.9	22.9		69.8	64.7	−5.1
C-3‴	40.5	39.7	−2.6	41.4	40.8	−0.6				40.5	39.8	−0.7				52.4	53.0	+0.6
C-4‴																26.3	22.9	−3.4
C-5‴																10.3	9.8	−0.5

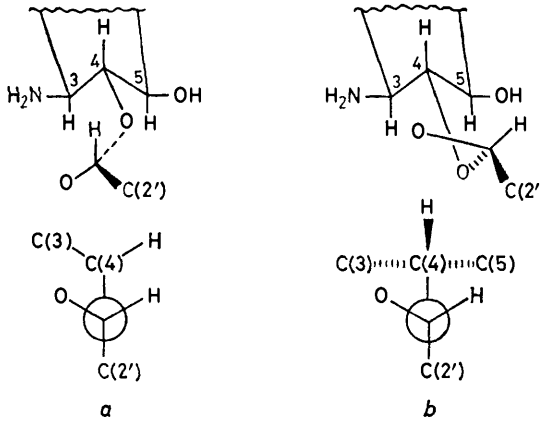
[a] Ref. 24. [b] D. H. Davies, A.K Mallams, J. McGlotten, J. B. Morton, and R. W. Tkach, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1407. [c] P. J. L. Daniels, unpublished observations. [d] Obscured by dioxan reference peak. [e] Ref. 10. [f] May be interchanged in any vertical column. [g] Signal not visible under the conditions employed. [h] Sample partially carbonated. [i] M. Kugelman, A. K. Mallams, and H. Vernay, *J. Chem. Soc., Perkin Trans. 1* 1976, 1113. [j] T. L. Nagabhushan, unpublished observations.

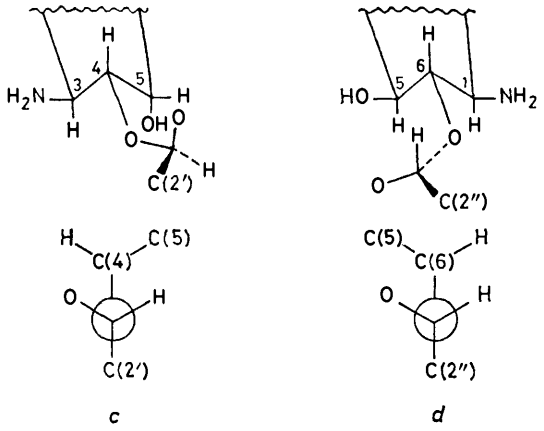
TABLE 2

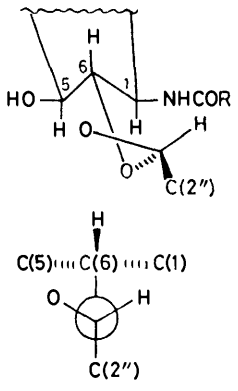
$\Delta\delta_C$ Values for 1-NHCOR-2-deoxystreptamine ⟶ 1-NHCOR-trisaccharide [a]

Carbon	(96) →(29)	(96) →(62)	(96) →(89)	(96) →(8)	(99) →(12)	(99) →(59)	(99) →(7)	(99) →(93)	(101) →(66
C-1	+0.1	−0.1	+0.1	−0.3	+0.4	−0.5	−0.6	−0.3	+0.2
C-2	−0.6	−0.4	−0.3	−0.8	0	+0.2	+0.1	+0.6	0
C-3	−1.3	−1.7	−1.0	−1.8	−1.2	−1.6	−1.6	−0.2	−1.7
C-4	+6.7	+10.2	+9.8	+10.1	+6.6	+10.1	+9.6	+0.2	+9.6
C-5	−1.3	−1.8	−1.2	−1.7	−1.0	−1.6	−1.4	−1.4	−1.5
C-6	+9.2	+9.0	+9.3	+9.6	+5.9	+5.6	+6.6	+5.9	+5.7

[a] All values are for fully decarbonated free bases.







ation is best represented by rotamer *c*.[22,32,33] Although the corresponding 1-*N*-(2-aminoethoxycarbonyl)-2-deoxy-5-*epi*-streptamine was not available for comparison purposes, it is quite clear from the chemical shifts (Table 1) of 1-*N*-(2-aminoethoxycarbonyl)-5-*epi*-sisomicin (52) that it also adopts the same rotamer *c* as 5-*epi*-sisomicin (49), about the C-4–O glycosidic bond. Rotamers *a*, *b*, and *c* all satisfy the requirements of the *exo*-anomeric effect.[34-37]

We shall next consider the rotamers about the C-6–O glycosidic bond where significant differences are observed between the unsubstituted aminoglycosides and their 1-NHCOR derivatives. In sisomicin (29), 5-*epi*-sisomicin (52), gentamicin B (62), gentamicin C_{1a} (89), and kanamycin A (8) the 6-*O*-glycoside adopts rotamer *d* about the C-6–O glycosidic bond [1,22-23] resulting in shielding of C-5 and a deshielding of +9.0 to +9.6 at C-6 (Table 2). Introduction of the 1-NHCOR substituents to any of the above substrates results in a significant clockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond. The solution conformations of these 1-NHCOR derivatives are best represented by the approximate rotamer *e* [32] which results in shielding of C-5 and a significant reduction in the deshielding of C-6 to +5.6 to +6.6 (Table 2). This is best explained by assuming that the normal deshielding of C-6 due to glycosylation at this position is being counteracted by a shielding component due to the clockwise rotation of the 6-*O*-glycoside about the O–C-6 glycosidic bond in these 1-NHCOR derivatives. The net result of this shielding interaction between the C-1″–O-5″ and C-6–H-6 systems is to produce reduced deshielding at C-6. Similarly increased interaction between the C-1–H-1″ and C-6–C-1–NHCOR systems when the sugar rotates in a clockwise direction about the O–C-6 glycosidic bond results in shielding of C-1″ in all of the 1-NHCOR derivatives, relative to the unsubstituted aminoglycosides (Table 1).[32] We feel that conversion of the 1-NH_2 group into a 1-NHCOR group may result in reduced dipolar repulsion between the 1-substituent and the dipole of the C-1″–O-C-6 glycosidic oxygen, leading to the observed clockwise rotation of the 6-*O*-glycoside about the O–C-6 bond in the 1-NHCOR derivatives. Both rotamers *d* and *e* satisfy the requirements of the *exo*-anomeric effect.[34-37]

From ^{13}C n.m.r. data it is evident to us that all 1-*N*-acetyl,[9,32] 1-*N*-HABA, and 1-*N*-HAPA [8,9,10,32,38] derivatives of aminoglycosides also exhibit rotamer *e* about the C-6–O glycosidic bond although this has never been pointed out before. Naito [38] recently showed that

the 'Nagabhushan–Daniels Rule'[39] could not be successfully applied to 1-*N*-HABA and 1-*N*-acetyl derivatives of kanamycin A (8) and the anomalies in these and other *N*-acyl derivatives were ascribed to δ effects produced by acylation of the amino-groups. From the chemical-shift values reported by Naito[38] for kanamycin A (8) it is evident (see Table 1) that his sample of kanamycin A (8) was partially carbonated which renders the $\Delta\delta_C$ values unreliable. Nagabhushan has published ^{13}C n.m.r. data for 1-*N*-HABA and 1-*N*-HAPA derivatives of gentamicin B,[10] but it should be noted that both the samples of gentamicin B (62) and kanamycin A (8) were partially carbonated (see Table 1) and owing to the similarity in the chemical shifts for the anomeric signals for C-1′ and C-1″ in the 1-*N*-HABA and 1-*N*-HAPA derivatives, these could not be unambiguously assigned with the data available at that time, and were in fact misassigned. The Nagabhushan–Daniels Rule[39] merely points to the much larger shielding of C-1′ and C-4 relative to C-1″ and C-6 for the series of compounds that were studied, upon protonation of the amino-groups. However, in the case of these 1-NHCOR derivatives the greater shieldings of C-1′ and C-4 relative to C-1″ and C-6 are still evident, but because these derivatives adopt a different solution conformation about the C-6–O glycosidic bond relative to the parent unsubstituted aminoglycosides,[32] the numerical values of $\Delta\delta_C$ are no longer the same. The factors affecting the Nagabhusan–Daniels Rule as applied to the magnitude of changes in $\Delta\delta_C$ for C-1′, C-4, C-1″, and C-6 will be discussed more fully in the following paper.[32]

It is evident from the studies reported here and from those in the subsequent paper,[32] that careful examination of the ^{13}C n.m.r. data has given us new insights into the solution conformations of 1-NHCOR-substituted aminoglycosides including the clinically important drug amikacin (1). Further discussion of changes in conformation that occur at acidic pH will be found in the following paper in this series.[32]

The novel 1-*N*-aminoalkoxycarbonyl and 1-*N*-aminoalkylcarboxamido derivatives described in this paper are highly potent antibacterials having a greatly improved spectrum of activity against resistant strains of bacteria and their antibacterial activity has been described elsewhere.[2,40-42]

EXPERIMENTAL

Physical data were recorded as described in Part 7.[30]

2-(2,2,2-Trichloroethoxycarbonylamino)ethanol (31).—2-Aminoethanol (30) (20 g) and sodium carbonate (27.8 g) were dissolved in acetone–water (4 : 1 v/v; 500 ml) and 2,2,2-trichloroethyl chloroformate (104.2 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 0 °C for a further 3 h. The solids were filtered off and washed with acetone and the filtrate was evaporated to dryness. The resulting gum was taken up in chloroform (500 ml) and washed with water (3 × 100 ml). The chloroform solution was evaporated to dryness to give *2-(2,2,2-trichloroethoxycarbonylamino)ethanol* (31) (64.5 g, 83%) as a gum (Found: C, 26.25; H, 3.6; Cl, 44.2; N, 6.1. $C_5H_8Cl_3NO_3$ requires C, 25.40; H, 3.4; Cl, 45.0; N, 5.9%), $\nu_{max.}$ (film) 3 300, 2 910, 1 700, 1 520, 1 240, 1 140, and 1 055 cm^{-1}, δ ($CDCl_3$) 3.43 (2 H, t, *J* 7.5 Hz, $HOCH_2CH_2NH$), 3.77 (2 H, t, *J* 7.5 Hz, $HOCH_2CH_2NH$), and 4.77 (2 H, s, $CO_2CH_2CCl_3$).

N-[2-(2,2,2-Trichloroethoxycarbonylamino)ethoxycarbonyloxy]succinimide (32).—2-(2,2,2-Trichloroethoxycarbonylamino)ethanol (31) (10 g) was dissolved in methylene chloride (159 ml) containing phosgene (1 mol equiv.) and triethylamine (5 ml) and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the resulting gum was taken up in ethyl acetate and filtered. The filtrate was added dropwise to a solution of *N*-hydroxysuccinimide (4.83 g) in ethyl acetate (100 ml) containing pyridine (10 ml) and the mixture was stirred at 25 °C for 1 h. The solution was filtered and the filtrate was evaporated to dryness and azeotroped with toluene to afford the *active ester* (32) (14.2 g, 89%) as a viscous gum. A portion was purified by preparative t.l.c. on silica gel using 20% v/v ethyl acetate–methylene chloride as the eluant to give an analytical sample as a waxy solid (Found: C, 31.9; H, 3.3; Cl, 28.1; N, 7.8. $C_{10}H_{11}Cl_3N_2O_7$ requires C, 31.8; H, 2.9; Cl, 28.1; N, 7.4%); $\nu_{max.}$ (film) 3 330, 2 950, 1 820, 1 790, 1 740, 1 725, 1 530, and 1 220 cm^{-1}, δ ($CDCl_3$) 2.83 (4 H, s, $COCH_2CH_2CO$), 3.60 (2 H, m, OCH_2CH_2NH), 4.47 (2 H, t, *J* 7.5 Hz, OCH_2CH_2NH), and 4.78 (2 H, s, $CO_2CH_2CCl_3$).

2-(4-Methoxybenzyloxycarbonylamino)ethanol (33).—2-Aminoethanol (30) (1.5 g) was dissolved in water (200 ml) containing triethylamine (4.83 g) and 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (10.67 g)[43] in dioxan (200 ml) was added to the stirred solution. Stirring was continued for 2 h at 25 °C and the mixture was evaporated to dryness. The residue was dissolved in chloroform and extracted with 0.1M-hydrochloric acid (3 × 25 ml). The chloroform was washed with water, dried ($MgSO_4$), and evaporated to give the (*4-methoxybenzyloxycarbonyl derivative* (33) as a waxy yellow solid (6.39 g, 89%). An analytical sample of (33) was obtained by preparative t.l.c. on silica gel using 5% v/v methanol–chloroform as the eluant, as a yellow crystalline solid, m.p. 75—78 °C (Found: C, 58.75; H, 6.45; N, 6.35. $C_{11}H_{15}NO_4$ requires C, 58.66; H, 6.71; N, 6.22%), $\nu_{max.}$ (Nujol) 3 250, 1 680, 1 535, 1 240, and 1 050 cm^{-1}, δ ($CDCl_3$) 2.40 (1 H, s, OH), 3.38 (2 H, m, $NHCH_2CH_2O$), 3.71 (2 H, t, *J* 5 Hz, $NHCH_2CH_2O$), 3.82 (3 H, s, CH_3O), 5.06 (2 H, s, $CH_3OC_6H_4CH_2OCO$), 6.90 (2 H, d, *J* 9 Hz, $CH_3OC_6H_4CH_2OCO$), and 7.35 (2 H, d, *J* 9 Hz, $CH_3OC_6H_4CH_2OCO$).

N-[2-(4-Methoxybenzyloxycarbonylamino)ethoxycarbonyloxy]succinimide (34).—2-(4-Methoxybenzyloxycarbonylamino)ethanol (33) (5 g) was dissolved in methylene chloride containing phosgene (3 mol equiv.) and triethylamine (2.6 ml) and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the resulting gum was taken up in ethyl acetate and the mixture filtered. The filtrate was added dropwise to a stirred solution of *N*-hydroxysuccinimide (2.55 g) in ethyl acetate (50 ml) containing pyridine (10 ml) and the mixture was stirred at 25 °C for 1 h. The mixture was filtered and the filtrate was evaporated to dryness to give the *active ester* (34) as a gum (7.07 g, 87%). An analytical sample was obtained by preparative t.l.c. on silica gel using 20% v/v ethyl acetate–methylene chloride as the eluant (Found: C, 52.35; H, 5.2; N, 7.6. $C_{16}H_{18}N_2O_8$ requires C, 52.46; H, 4.95; N, 7.65%), $\nu_{max.}$ (film) 3 300, 1 800, 1 780, 1 730, 1 700, 1 500, 1 230, and 1 210 cm^{-1}, δ ($CDCl_3$) 2.76 (4 H, s, $COCH_2CH_2$-

CO), 3.47 (2 H, m, NHCH_2CH$_2$O), 3.78 (3 H, s, CH$_3$O), 4.36 (2 H, t, *J* 5 Hz, NHCH$_2$CH_2O), 5.02 (2 H, s, CH$_3$OC$_6$H$_4$CH_2-OCO), 5.48 (1 H, m, NH), 6.82 (2 H, d, *J* 9 Hz, CH$_3$OC$_6H_4$-CH$_2$OCO), and 7.27 (2 H, d, *J* 9 Hz, CH$_3$OC$_6H_4$CH$_2$OCO).

1-N-[2-(2,2,2-*Trichloroethoxycarbonylamino*)*ethoxycarbonyl*]-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*sisomicin* (11).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)sisomicin (10) (1.7 g) [20,*] was dissolved in methanol–water (1 : 1 v/v) (15 ml) containing *N*-[2-(2,2,2-trichloroethoxycarbonylamino)ethoxycarbonyloxy]succinimide (32) (906 mg) and the mixture was stirred at 25 °C for 3.5 h. The solution was evaporated to dryness and the residue was chromatographed on a silica gel column (15 × 2.5 cm) using 7% v/v methanol–chloroform as the eluant to give the *protected trisaccharide* (11) (1.87 g, 89%) as an amorphous solid (Found: C, 32.8; H, 3.7; Cl, 35.0; N, 6.35. C$_{34}$H$_{46}$-Cl$_{12}$N$_6$O$_{17}$ requires C, 33.0; H, 3.75; Cl, 34.4; N, 6.80%), $[\alpha]_D^{26}$ +77.5° (MeOH), $\nu_{max.}$ (Nujol) 3 350, 2 970, 2 900, 1 740, 1 560, 1 530, 1 250, and 1 050 cm^{-1}, δ (CD$_3$OD) 1.17 (3 H, s, 4″-CH$_3$), 2.53 (3 H, s, 3″-NCH$_3$), and 4.75 (8 H, s, CO$_2$CH_2CCl$_3$).

3,2′,6′-*Tris*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-[2-(4-*methoxybenzyloxycarbonylamino*)*ethoxycarbonyl*]*sisomicin* (14).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) (1 g) [20,*] was dissolved in aqueous methanol (1 : 1 v/v) (15 ml) containing *N*-[2-(4-methoxybenzyloxycarbonylamino)ethoxycarbonyloxy]succinimide (34) (389 mg). The reaction mixture was stirred at 25 °C for 1 h, whereupon additional reagent (34) (39 mg) was added. After stirring for a further 1 h the mixture was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 2.5 cm) using 7% v/v methanol–chloroform as the eluant to give the *protected sisomicin derivative* (14) (807 mg, 64%) as amorphous solid (Found: C, 56.95; H, 6.1; N, 6.75. C$_{58}$H$_{74}$N$_6$O$_{21}$·2H$_2$O requires C, 56.78; H, 6.41; N, 6.85%), $[\alpha]_D^{26}$ +69.3° (DMSO), $\nu_{max.}$ (Nujol) 3 250, 1 690, 1 680, 1 530, 1 510, 1 240, and 1 030 cm^{-1}, δ ([^{2}H$_6$]DMSO) 1.00 (3 H, s, 4″-CH$_3$), 3.75 (12 H, s, C$_6$H$_4$OCH_3), 4.94 (8 H, s, CH_2C$_6$H$_4$OCH$_3$), 6.88 (8 H, m, C$_6H_4$OCH$_3$), and 7.26 (8 H, m, C$_6H_4$OCH$_3$).

1-N-(2-*Aminoethoxycarbonyl*)*sisomicin* (12).—(*a*) 1-*N*-[2-(2,2,2-Trichloroethoxycarbonylamino)ethoxycarbonyl]-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)sisomicin (11) (1.77 g) was dissolved in 90% aqueous acetic acid (50 ml) containing activated zinc powder (1.86 g) and the mixture was stirred at 25 °C for 18 h. The reaction mixture was filtered through a bed of Celite and the filtrate was evaporated to dryness. The residue was dissolved in water (10 ml) and a 10% (w/v) aqueous sodium carbonate was added until the pH reached 10.0. The solids were filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (60 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N-(2-*aminoethoxycarbonyl*)*sisomicin* (12) (110 mg, 14%) as an amorphous solid after passage over Amberlite IRA 40IS (OH$^-$) resin and lyophilization (Found: C, 46.9; H, 8.2; N, 14.7. C$_{22}$H$_{44}$N$_6$O$_9$·2H$_2$O requires C, 46.5; H, 7.80; N, 14.8%), $[\alpha]_D^{26}$ +138.8° (H$_2$O), $\nu_{max.}$ (KBr) 3 350, 3 275, 1 705, 1 680, and 1 045 cm^{-1}, δ (D$_2$O) 1.22 (3 H, s, 4″-CH$_3$), 2.50 (3 H, s, 3″-NCH$_3$), 2.57 (1 H, d, $J_{2'',3''}$ 11 Hz, 3″-H), 2.85 (2 H, t, *J* 6 Hz, CO$_2$CH$_2$CH_2NH$_2$), 3.70 (1 H, dd, $J_{1'',2''}$ 4, $J_{2'',3''}$ 11 Hz, 2″-H), 4.10 (2 H, t, *J* 6 Hz, CO$_2$-CH_2CH$_2$NH$_2$), 4.13 (H, d, $J_{5''eq,5''ax}$ 12 Hz, 5″*eq*-H), 4.90 (1 H, m, 4′-H), 5.11 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.33 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H).

(*b*) 3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-[2-(4-methoxybenzyloxycarbonylamino)ethoxycarbonyl]sisomicin (14) (500 mg) was added to trifluoroacetic acid (2 ml) at 0 °C and the mixture was stirred for 5 min. The solution was added dropwise to diethyl ether (150 ml) and the resulting precipitate was filtered off and chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2:1:1 v/v) as the eluant to give 1-*N*-(2-aminoethoxycarbonyl)sisomicin (12) (91 mg, 41%) as an amorphous solid after passage over Amberlite IRA 40IS (OH$^-$) resin followed by lyophilization. The product was identical with that prepared in (*a*) above.

2-[N-(4-*Methoxybenzyloxycarbonyl*)*ethylamino*]*ethanol* (36).—2-Ethylaminoethanol (35) (10 g) was dissolved in dioxan–water (1 : 1 v/v) (200 ml) containing 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (34.2 g) [43] and the mixture was stirred at 25 °C for 2 h. The solution was evaporated to dryness and the gum was chromatographed on a silica–gel column (60 × 3 cm) using dichloromethane as the eluant to give the *amine* (36) (19 g, 67%) as a waxy solid (Found: C, 61.4; H, 7.55; N, 5.65. C$_{13}$H$_{19}$NO$_4$ requires C, 61.65; H, 7.56; N, 5.53%), $\nu_{max.}$ (film) 3 430, 1 690, and 1 250 cm^{-1}, δ (CDCl$_3$) 1.08 (3 H, t, *J* 6 Hz, CH_3-CH$_2$N), 3.30 (2 H, q, *J* 6 Hz, CH$_3$CH_2N), 3.42 (2 H, m, OCH$_2$CH_2N), 3.62 (2 H, m, OCH_2CH$_2$N), 3.87 (3 H, s, CH_3OC$_6$H$_4$CH$_2$), 5.02 (2 H, s, CH$_3$OC$_6$H$_4$CH_2), and 6.81 and 7.23 (4 H, 2 m, CH$_3$OC$_6H_4$CH$_2$).

N-{2-[N-(4-*Methoxybenzyloxycarbonyl*)*ethylamino*]*ethoxycarbonyloxy*}*succinimide* (37).—2-[*N*-(4-Methoxybenzyloxycarbonyl)ethylamino]ethanol (36) (13 g) was dissolved in dichloromethane (200 ml) containing phosgene (3 mol equiv.). Triethylamine (7 ml) was added dropwise over 0.5 h and the mixture was then stirred at 25 °C for 2 h. The solution was evaporated to dryness and the residue was dissolved in ethyl acetate and filtered. The filtrate was added dropwise to a solution of *N*-hydroxysuccinimide (5.9 g) in ethyl acetate (150 ml) containing pyridine (25 ml). The mixture was stirred at 25 °C for 1 h and then filtered. The filtrate was evaporated to dryness to give the *active ester* (37) (18.3 g, 90%) as a gum. An analytical sample was prepared by preparative t.l.c. on silica gel using 20% v/v ethyl acetate–dichloromethane as the eluant (Found: C, 54.7; H, 5.4; N, 6.85. C$_{18}$H$_{22}$N$_2$O$_8$ requires C, 54.83; H, 5.62; N, 7.10%), $\nu_{max.}$ (film) 1 740 and 1 220 cm^{-1}, δ (CDCl$_3$) 1.32 (3 H, t, *J* 6 Hz, CH_3CH$_2$N), 2.88 (4 H, s, COCH$_2$CH$_2$-CO), 3.45 (2 H, q, *J* 6 Hz, CH$_3$CH_2N), 3.73 (2 H, t, *J* 6 Hz, OCH$_2$CH_2N), 3.86 (3 H, s, CH_3OC$_6$H$_4$CH$_2$), 4.50 (2 H, t, *J* 6 Hz, OCH_2CH$_2$N), 5.14 (2 H, s, CH$_3$OC$_6$H$_4$CH_2), and 6.92 and 7.39 (4 H, m, CH$_3$OC$_6H_4$CH$_2$).

1-N-(2-*Ethylaminoethoxycarbonyl*)*sisomicin* (15).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl(sisomicin (13) (2 g) was dissolved in methanol–water (1 : 1 v/v) (10 ml) containing *N*-{2-[*N*-(4-methoxybenzyloxycarbonyl)ethylamino]-ethoxycarbonyloxy}succinimide (37) (922 mg) and the mixture was stirred at 25 °C for 18 h. The solution was evaporated to dryness and the residue was dissolved in trifluoroacetic acid (30 ml). After 0.5 h at 25 °C, the solution was evaporated to dryness and the residue was chromatographed on an Amberlite CG-50 (NH$_2$) resin column (60 × 5 cm) using gradient elution with aqueous ammonium hydroxide (0.01—0.35M) to give 1-N-(2-*ethylaminoethoxycarbonyl*)*sisomicin* (15) (263 mg, 22%) as an amorphous

* Note as on page 2186.

solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.95; H, 7.1; N, 12.95. $C_{24}H_{46}N_6O_9 \cdot 2CO_2$ requires C, 47.99; H, 7.13; N, 12.91%), $[\alpha]_D^{26}$ +135.2° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 590, 1 050, and 1 000 cm^{-1}, δ (D_2O) 1.09 (3 H, t, *J* 7 Hz, CH_3CH_2N), 1.22 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 2.54 (2 H, q, *J* 7 Hz, CH_3CH_2N), 2.82 (2 H, t, *J* 6 Hz, OCH_2CH_2N), 4.13 (2 H, t, *J* 6 Hz, OCH_2CH_2N), 4.83 (1 H, m, 4′-H), 5.09 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.25 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H).

N-(*Methoxycarbonyloxy*)*succinimide* (40).—Methyl chloroformate (10 g) was added dropwise to a stirred solution of *N*-hydroxysuccinimide (12.2 g) dissolved in ethyl acetate (100 ml) containing pyridine (10 ml) at 0 °C. After the addition was completed the reaction was stirred at 25 °C for 2 h. The solution was evaporated to dryness to give N-(*methoxycarbonyloxy*)*succinimide* (40) (12.8 g, 55%) which crystallized, m.p. 84—86 °C (Found: C, 41.65; H, 3.95; N, 8.35. $C_6H_7NO_5$ requires C, 41.63; H, 4.08; N, 8.09%), $\nu_{max.}$ ($CHCl_3$) 1 805, 1 780, 1 735, and 1 020 cm^{-1} δ ($CDCl_3$) 2.80 (4 H, s, $COCH_2CH_2CO$) and 3.93 (3 H, s, CH_3OCO).

1-N-*Methoxycarbonylsisomicin* (16).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) (5 g) was dissolved in methanol–water (1 : 1 v/v) (50 ml) containing *N*-(methoxycarbonyloxy)succinimide (40) (920 mg) and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and azeotroped with toluene. The residue was dissolved in trifluoroacetic acid (10 ml). After 5 min at 25 °C, the solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2 cm) using chloroform–methanol–3% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant. The product was rechromatographed on a silica-gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give 1-N-*methoxycarbonylsisomicin* (16) (616 mg, 23%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 46.9; H, 7.75; N, 13.1. $C_{21}H_{39}N_5O_9$ requires C, 46.56; H, 8.00; N, 12.93%), $[\alpha]_D^{26}$ +152.3° (H_2O), $\nu_{max.}$ 3 350, 1 710, 1 530, and 1 000 cm^{-1}), δ (D_2O) 1.10 (3 H, s, 4″-CH_3), 2.40 (3 H, s, 3″-NCH_3), 2.46 (1 H, d, $J_{2''ax,3''ax}$ 10.5 Hz, 3″*ax*-H), 3.18 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 3.56 (3 H, s, CH_3OCO), 3.60 (1 H, dd, $J_{1''eq,2''ax}$ 4, $J_{2''ax,3''ax}$ 10.5 Hz, 2″*ax*-H), 4.01 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*eq*-H), 4.79 (1 H, m, 4′-H), 5.01 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.25 (1 H, d, $J_{1'eq,2'ax}$ 3 Hz, 1′*eq*-H).

N-(*Ethoxycarbonyloxy*)*succinimide* (41).—Ethyl chloroformate (10 g) was added dropwise to a stirred solution of *N*-hydroxysuccinimide (10.6 g) dissolved in ethyl acetate (100 ml) containing pyridine (10 ml) at 0 °C. After the addition was completed the reaction was stirred at 25 °C for 2 h. The solution was evaporated to dryness to give N-(*ethoxycarbonyloxy*)*succinimide* (41) (14.6 g, 67%) which crystallized, m.p. 47—51 °C (Found: C, 44.95; H, 4.85; N, 7.8. $C_7H_9NO_5$ requires C, 44.92; H, 4.85; N, 7.48%), $\nu_{max.}$ ($CHCl_3$) 1 808, 1 785, 1 740, and 1 020 cm^{-1}, δ ($CDCl_3$) 1.38 (3 H, t, *J* 7 Hz, CH_3CH_2O), 2.80 (4 H, s, $COCH_2CH_2CO$), and 4.33 (2 H, *q*, *J* 7 Hz, CH_3CH_2O).

1-N-*Ethoxycarbonylsisomicin* (17).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) (5 g) was dissolved in methanol–water (1 : 1 v/v) (50 ml) containing *N*-(ethoxycarbonyloxy)succinimide (41) (995 mg) and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and azeotroped with toluene. The residue was dissolved in trifluoroacetic acid (10 ml). After 5 min at 25 °C, the solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2 cm) using chloroform–methanol–3% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant. The product was rechromatographed on a silica-gel column (110 × 2.5 (cm using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N-*ethoxycarbonylsisomicin* (17) (582 mg, 21%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 49.9; H, 7.9; N, 13.4. $C_{22}H_{41}N_5O_9$ requires C, 50.21; H, 7.85; N, 13.31%), $[\alpha]_D^{26}$ +136.2° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 535, and 1 050 cm^{-1}, δ (D_2O) 1.09 (3 H, s, 4″-CH_3), 1.12 (3 H, t, *J* 7 Hz, CH_3CH_2O), 2.39 (3 H, s, 3″-NCH_3), 2.45 (1 H, d, $J_{2''ax,3''ax}$ 10.5 Hz, 3″*ax*-H), 3.18 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 3.59 (1 H, dd, $J_{1''eq,2''ax}$ 4, $J_{2''ax,3''ax}$ 10.5 Hz, 2″*ax*-H), 3.99 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*eq*-H), 4.00 (2 H, q, *J* 7 Hz, CH_3CH_2O), 4.78 (1 H, m, 4′-H), 5.01 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.24 (1 H, d, $J_{1''eq,2''ax}$ 3 Hz, 1′*eq*-H).

3,2′,6′-*Tris*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-(2,2,2-*trichloroethoxycarbonyl*)*sisomicin* (18).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (13) (4 g) was dissolved in methanol–water (1 : 1 v/v) (100 ml) containing *N*-(2,2,2-trichloroethoxycarbonyloxy)succinimide (42) (1.35 g) and the mixture was stirred at 25 °C for 2 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 3 cm) using 3% methanol–chloroform as the eluant to give the 1-(*trichloroethoxycarbonyl*) *derivative* (18) (4.1 g, 86%) as an amorphous solid (Found: C, 51.0; H, 5.5; Cl, 9.0; N, 5.9. $C_{49}H_{62}Cl_3N_5O_{18} \cdot 2H_2O$ requires C, 51.09; H, 5.77; Cl, 9.23; N, 6.08%), $[\alpha]_D^{26}$ + 71.3° (DMSO), $\nu_{max.}$ (KBr) 3 330, 1 705, 1 690, 1 515, 1 245, and 1 035 cm^{-1}, δ ($CDCl_3$) 1.13 (3 H, s, 4″-CH_3), 2.61 (3 H, s, 3″-NCH_3), 3.77, 3.79, and 3.81 (9 H, s, $CH_2C_6H_4OCH_3$), 4.67 (2 H, s, CH_2CCl_3), 4.99 (6 H, s, $CH_2C_6H_4OCH_3$), and 6.85 and 7.22 (12 H, m, $CH_2C_6H_4OCH_3$).

3,2′,6′,3″-*Tetrakis*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-(2,2,2-*trichloroethoxycarbonyl*)*sisomicin* (19).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-(2,2,2-trichloroethoxycarbonyl)sisomicin (18) (0.5 g) was dissolved in dimethyl sulphoxide (50 ml) containing triethylamine (46 mg) and 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (150 mg) [43] and the mixture was stirred at 25 °C for 18 h. The mixture was washed with ether and the residual gum was chromatographed on a silica-gel column (30 × 2.5 cm) using 6% methanol in chloroform as the eluant to give the 3″-N-(4-*methoxybenzyloxycarbonyl*) *derivative* (19) (418 mg, 73%) as an amorphous solid (Found: C, 53.65; H, 5.7; Cl, 7.3; N, 5.3. $C_{58}H_{70}Cl_3N_5O_{21} \cdot H_2O$ requires C, 53.71; H, 5.60; N, 5.40; Cl, 8.20%), $[\alpha]_D^{26}$ +71.2° (DMSO), $\nu_{max.}$ (KBr) 3 325, 1 710, 1 690, 1 535, 1 515, 1 240, and 1 030 cm^{-1}, δ ($CDCl_3$) 1.08 (3 H, s, 4″-CH_3), 3.01 (3 H, s, 3″-NCH_3), 3.77 and 3.80 (12 H, 2 s, $CH_2C_6H_4OCH_3$), 4.68 (2 H, s, CH_2CCl_3), 4.95 and 5.03 (8 H, s, $CH_2C_6H_4OCH_3$), and 6.83 and 7.23 (16 H, m, $CH_2C_6H_4OCH_3$).

3,2′,6′,3″-*Tetrakis*-N-(4-*methoxybenzyloxycarbonyl*)*sisomicin* (20).—3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-(2,2,2-trichloroethoxycarbonyl)sisomicin (19) (200 mg) was dissolved in 10% acetic acid in methanol (2 ml) and activated zinc (100 mg) was added. The mixture was stirred at 25 °C for 2 h and it was then filtered and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (30 × 2.5 cm) using 6% v/v

methanol–chloroform as the eluant to give the acetate salt of (21) (119 mg). The latter was dissolved in chloroform (5 ml) and the solution was stirred with 14% ammonium hydroxide solution (10 ml) at 25 °C for 18 h. The chloroform layer was separated, dried ($MgSO_4$), filtered, and evaporated to a volume of *ca.* 1 ml. The latter was added dropwise to diethyl ether (10 ml) and the precipitate was filtered off to afford the *tetra-protected sisomicin derivative* (20) (89 mg, 51%) as an amorphous solid (Found: C, 59.5; H, 6.35; N, 6.05. $C_{55}H_{69}N_5O_{19}$ requires C, 59.85; H, 6.30; N, 6.30%), $[\alpha]_D^{26}$ + 92.9° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 360, 1 715, 1 690, 1 510, 1 240, and 1 030 cm^{-1}, δ ($CDCl_3$) 1.07 (3 H, s, 4″-CH_3), 3.03 (3 H, s, 3″-NCH_3), 3.77 and 3.79 (12 H, 2 s, $CH_2C_6H_4OCH_3$), 4.99 (8 H, s, $CH_2C_6H_4OCH_3$), and 6.83 and 7.24 (16 H, m, $CH_2C_6H_4OCH_3$).

1-[2-(4-*Methoxybenzyloxycarbonylamino*)*ethoxythiocarbonyl*]*imidazole* (43).—2-(4-Methoxybenzyloxycarbonylamino)ethanol (33) (2 g) was dissolved in dry tetrahydrofuran (10 ml) containing *NN*′-thiocarbonyldi-imidazole (1.58 g) and the mixture was heated under reflux for 2 h. The reaction mixture was evaporated to dryness and then taken up in dichloromethane (100 ml) and washed with 5% tartaric acid solution (3 × 25 ml). The dichloromethane extract was evaporated to dryness to afford 1-N-[2-(4-*methoxybenzyloxycarbonylamino*)*ethoxythiocarbonyl*]*imidazole* (43) (2.53 g, 85%) as a waxy solid (Found: C, 53.9; H, 5.05; N, 12.7; S, 9.85. $C_{15}H_{17}N_3O_4S$ requires C, 53.73; H, 5.11; N, 12.51; S, 9.56%), $\nu_{max.}$ ($CHCl_3$) 3 450, 1 725, 1 615, 1 515, and 1 250 cm^{-1}, δ ($CDCl_3$) 3.67 (2 H, t, *J* 6 Hz, $NHCH_2CH_2O$), 3.80 (3 H, s, $CH_3OC_6H_4CH_2$), 4.69 (2 H, t, *J* 6 Hz, $NHCH_2CH_2O$), 5.05 (2 H, s, $CH_3OC_6H_4CH_2$), 6.87 and 7.29 (4 H, m, $CH_3OC_6H_4CH_2$), 7.01 (1 H, m, 4′-CH=), 7.60 (1 H, m, 5′-CH=), and 8.33 (1 H, s, 2′-CH=N).

3,2′,6′,3″-*Tetrakis*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-[2-(*methoxybenzyloxycarbonylamino*)*ethoxythiocarbonyl*]-*sisomicin* (21).—1-*N*-[2-(4-Methoxybenzyloxycarbonylamino)ethoxythiocarbonyl]imidazole (43) (2.4 g) was dissolved in dry dichloromethane (10 ml) containing triethyloxonium tetrafluoroborate (870 mg) and the mixture was stirred at 25 °C for 0.5 h. 3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) (2 g) was added and the mixture was stirred at 25 °C for 18 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2.5 cm) using 2% v/v methanol–chloroform as the eluant to give the *tetra-protected intermediate* (21) (2.2 g, 75%) as an amorphous solid (Found: C, 56.25; H, 5.95; N, 6.65; S, 3.0. $C_{67}H_{82}N_6O_{23}\cdot 3H_2O$ requires C, 56.41; H, 6.22; N, 5.89; S, 2.25%), $[\alpha]_D^{26}$ + 64.5° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 515, 1 250, and 1 030 cm^{-1}, δ ($CDCl_3$) 1.08br (3 H, s, 4″-CH_3), 2.98br (3 H, s, 3″-NCH_3), 3.80 (15 H, s, $CH_3OC_6H_4CH_2$), 4.98 (10 H, s, $CH_3OC_6H_4CH_2$), and 6.84 and 7.27 (20 H, 2 m, $CH_3OC_6H_4CH_2$).

1-N-(2-*Aminoethoxythiocarbonyl*)*sisomicin* (22).—3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-[2-(4-methoxybenzyloxycarbonylamino)ethoxythiocarbonyl]sisomicin (21) (2.1 g) was dissolved in trifluoroacetic acid (10 ml) and, after 3 min at 25 °C, the solution was evaporated to dryness. The residue was chromatographed on a silica-gel column (120 × 2 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-*N*-(2-*aminoethoxythiocarbonyl*)*sisomicin* (22) (143 mg, 14%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 41.1; H, 6.8; N, 12.35; S, 4.25. $C_{22}H_{42}N_6O_8S\cdot CO_2\cdot 2H_2O$ requires C, 41.43; H, 6.95; N, 12.60; S, 4.80%), $[\alpha]_D^{26}$ + 102.9° (H_2O), $\nu_{max.}$ (KBr) 3 280, 1 690, 1 550, and 1 050 cm^{-1}, δ (D_2O) 1.26 (3 H, s, 4″-CH_3), 2.63 (3 H, s, 3″-NCH_3), 2.80 (1 H, d, $J_{2''ax,3''ax}$ 11 Hz, 3″*ax*-H), 3.30 (1 H, d, $J_{5''ax,5''eq}$ 12 Hz, 5″*ax*-H), *ca.* 3.68 (4 H, m, $OCH_2CH_2NH_2$), 3.79 (1 H, dd, $J_{1''eq,2''ax}$ 4, $J_{2''ax,3''ax}$ 11 Hz, 2″*ax*-H), 4.07 (1 H, d, $J_{5''ax,5''eq}$ 12 Hz, 5″*eq*-H), 5.01 (1 H, m, 4′-H), 5.07 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.39 (1 H, d, $J_{1'eq,2'ax}$ 3 Hz, 1′*eq*-H).

2-(4-*Methoxybenzyloxycarbonylamino*)*ethanethiol* (46).—2-Aminoethanethiol hydrochloride (10 g) was dissolved in dichloromethane (200 ml) containing 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (26.75 g).[43] Triethylamine (8.88 g) was added dropwise over 0.5 h and the reaction was then stirred at 25 °C for 3 h. The solution was evaporated to dryness and the residue was dissolved in ethyl acetate (200 ml) and filtered. The filtrate was evaporated and the residue was chromatographed on a silica-gel column (30 × 5 cm) using dichloromethane as the eluant to give the *thiol* (46) (20 g, 94%) as a pale yellow solid (Found: C, 55.0; H, 6.25; N, 5.85; S, 13.05. $C_{11}H_{15}NO_3S$ requires C, 54.75; H, 6.27; N, 5.80; S, 13.29%), $\nu_{max.}$ (Nujol) 3 320, 1 690, and 1 240 cm^{-1}, δ ($CDCl_3$) 1.38 (1 H, t, *J* 5 Hz, CH_2SH), 2.62 (2 H, dt, *J* 6 and 8 Hz, $NHCH_2CH_2SH$), 3.38 (2 H, dt, *J* 6 and 6 Hz, $NHCH_2CH_2SH$), 3.82 (3 H, s, $CH_3OC_6H_4CH_2$), 5.06 (2 H, s, $CH_3OC_6H_4CH_2$), and 6.92 and 7.37 (4 H, m, $CH_3OC_6H_4CH_2$).

1-[2-(4-*Methoxybenzyloxycarbonylamino*)*ethanethiocarbonyl*]*imidazole* (44).—2-(4-Methoxybenzyloxycarbonylamino)ethanethiol (46) (3 g) was dissolved in dry tetrahydrofuran (200 ml) containing *NN*′-carbonyldi-imidazole (6 g). The reaction mixture was gradually heated to reflux over 2 h and then evaporated to dryness. The residue was dissolved in dichloromethane (200 ml) and extracted with 5% tartaric acid solution (3 × 50 ml). The dichloromethane layer was dried ($MgSO_4$), filtered, and evaporated to give 1-[2-(4-methoxybenzyloxycarbonylamino)ethanethiocarbonyl]imidazole (44) (3.45 g, 83%) as a solid (Found: C, 54.15; H, 5.35; N, 12.4; S, 9.55. $C_{15}H_{17}N_3O_4S$ requires C, 53.73; H, 5.11; N, 12.51; S, 9.56%), $\nu_{max.}$ ($CHCl_3$) 3 470, 1 725, 1 615, 1 515, and 1 250 cm^{-1}, δ ($CDCl_3$) 3.20—3.50 (4 H, m, $NHCH_2CH_2S$), 3.80 (3 H, s, $CH_3OC_6H_4CH_2$), 5.05 (2 H, s, $CH_3OC_6H_4CH_2$), 6.82 and 7.29 (4 H, m, $CH_3OC_6H_4CH_2$), 7.08 (1 H, m, 4′-CH=), 7.42 (1 H, m, 5′-CH=), and 8.17 (1 H, m, 2′-CH=).

3,2′,6′,3″-*Tetrakis*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-[2-(4-*methoxybenzyloxycarbonylamino*)*ethanethiocarbonyl*]-*sisomicin* (23).—2-(4-Methoxybenzyloxycarbonylamino)-ethanethiol (46) (1 g) was dissolved in 0.9M phosgene in dichloromethane (30 ml) and triethylamine (1.1 ml) was added dropwise over 0.5 h. The mixture was stirred at 25 °C for 1 h and then evaporated to afford 2-(4-methoxybenzyloxycarbonylamino)ethanethiocarbonyl chloride (47) as a yellow gum which was used without further purification.

3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-sisomicin (20) (2 g) was dissolved in acetone–water (3 : 1 v/v) (20 ml) containing sodium carbonate (954 mg) and 2-(4-methoxybenzyloxycarbonylamino)ethanethiocarbonyl chloride (47) (1.64 g) was added. The reaction mixture was stirred at 25 °C for 1 h. The solution was evaporated to dryness and chromatographed on a silica-gel column (120 × 2 cm) using 5% v/v methanol–chloroform as the eluant to give the *tetra-protected sisomicin derivative* (23) (777 mg, 37%) as an amorphous solid (C, 57.1; H, 5.85; N, 6.75; S, 1.55. $C_{58}H_{74}N_6O_{20}S$ requires: C, 57.7; H, 6.2; N,

6.95; S, 2.7%), $[\alpha]_D^{26}$ +68.7° (CH_3OH), $\nu_{max.}$ (KBr) 3 325, 1 695, 1 505, 1 240, and 1 025 cm^{-1}, δ (CD_3OD) 0.97br (3 H, s, 4″-CH_3), 2.98br (3 H, s, 3″-NCH_3), 3.76 (15 H, s, CH_3O-$C_6H_4CH_2$), 4.97 (10 H, s, $CH_3OC_6H_4CH_2$), and 6.83 and 7.24 (20 H, m, $CH_3OC_6H_4CH_2$).

1-[2-(4-*Methoxybenzyloxycarbonylamino*)*ethanethiothiocarbonyl*]*imidazole* (45).—2-(4-Methoxybenzyloxycarbonylamino)ethanethiol (46) (3 g) was dissolved in dry tetrahydrofuran (200 ml) containing *NN*′-thiocarbonyldi-imidazole (6.6 g). The reaction mixture was gradually heated to reflux over 2 h and then evaporated to dryness. The residue was dissolved in dichloromethane (200 ml) and extracted with 5% tartaric acid solution (3 × 50 ml). The dichloromethane layer was dried ($MgSO_4$), filtered, and evaporated to give the *thiocarbonylimidazole* (45) (3.79 g, 87%) as a waxy solid (Found: C, 51.95; H, 4.8; N, 11.95; S, 16.5. $C_{15}H_{17}N_3O_3S_2$ requires C, 51.26; H, 4.88; N, 12.00; S, 18.24%), $\nu_{max.}$ ($CHCl_3$) 3 480, 1 725, 1 615, 1 515, and 1 250 cm^{-1}, δ ($CDCl_3$) 3.76 (4 H, m, $NHCH_2CH_2S$), 3.97 (3 H, s, $CH_3OC_6H_4CH_2$), 5.28 (2 H, s, $CH_3OC_6H_4CH_2$), 7.26 and 7.62 (4 H, 2 m, $CH_3OC_6H_4CH_2$), 7.41 (1 H, m, 4′-CH=), 7.60 (1 H, m, 5′-CH=), and 8.10 (1 H, m, 2′-CH=).

3,2′,6′,3″-*Tetrakis*-N-(4-*methoxybenzyloxycarbonyl*)-1-N-[2-(4-*methoxybenzyloxycarbonylamino*)*ethanethiothiocarbonyl*]-*sisomicin* (24).—1-[2-(4-Methoxybenzyloxycarbonylamino)-ethanethiothiocarbonyl]imidazole (45) (3 g) was dissolved in dry dichloromethane (10 ml) containing triethyloxonium tetrafluoroborate (984 mg) and the mixture was stirred at 25 °C for 0.5 h. 3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) (2 g) was added and the reaction was stirred at 25 °C for 18 h. The solution was concentrated to dryness and the residue was chromatographed on a silica-gel column (120 × 2.5 cm) using 2% v/v methanol–chloroform as the eluant to give the *protected thiocarbonylsisomicin derivative* (24) (1.84 g, 73%) as an amorphous solid (Found: C, 56.5; H, 5.65; N, 6.55; S, 4.55. $C_{67}H_{82}N_6O_{22}S_2$ requires C, 58.02; H, 5.96; N, 6.06; S, 4.62%), $[\alpha]_D^{26}$ +67.1° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 325, 1 690, 1 510, 1 240, and 1 025 cm^{-1}, δ ($CDCl_3$) 1.08br (3 H, s, 4″-CH_3), 2.99br (3 H, s, 3″-NCH_3), 3.79 (15 H, s, $CH_3OC_6H_4CH_2$), 5.00 (10 H, s, $CH_3OC_6H_4CH_2$), and 6.86 and 7.28 (20 H, 2 m, CH_3O-$C_6H_4CH_2$).

1-N:3-N-*Thiocarbonylsisomicin* (48).—3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)-1-*N*-[2-(4-methoxybenzyloxycarbonylamino)ethanethiothiocarbonyl]sisomicin (24) (1.74 g) was dissolved in trifluoroacetic acid (2 ml). After 3 min at 25 °C, the solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (30 × 2.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N:3-N-*thiocarbonylsisomicin* (48) (47 mg, 8%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.1; H, 7.45; N, 14.0; S, 5.7. $C_{20}H_{35}N_5O_7S{\cdot}H_2O$ requires: C, 47.32; H, 7.35; N, 13.80; S, 6.32%), $[\alpha]_D^{26}$ +142.9° (H_2O), $\nu_{max.}$ (KBr) 3 300, 1 680, 1 530, and 1 050 cm^{-1}, δ (D_2O) 1.19 (3 H, s, 4″-CH_3), 2.33 (1 H, d, $J_{2''ax,3''ax}$ 10.5 Hz, 3″*ax*-H), 2.49 (3 H, s, 3″-NCH_3), 3.37 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 4.84 (1 H, m, 4′-H), 4.97 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz. 1″*eq*-H), and 5.11 (1 H, d, $J_{1'eq,2'ax}$ 3 Hz, 1′*eq*-H).

1-N-[2-(2,2,2-*Trichloroethoxycarbonylamino*)*ethoxycarbonyl*]-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)-5-epi-*sisomicin* (51).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)-5-*epi*-sisomicin (50) (1.58 g) * was dissolved in methanol–water (1 : 1 v/v) (15 ml). *N*-[2-(2,2,2-Trichloroethoxycarbonylamino)ethoxycarbonyloxy]succinimide (32) (597 mg) was added and the mixture was stirred at 25 °C for 1 h, whereupon additional reagent (32) (60 mg) was added. After a total of 2 h the mixture was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 2.5 cm) using 7% methanol–chloroform as the eluant to give the *protected* 5-epi-*sisomicin* (51) (1.53 g, 76%) as an amorphous solid (Found: C, 33.5; H, 3.9; Cl, 33.6; N, 6.85. $C_{34}H_{46}Cl_{12}N_6O_{17}$ requires C, 33.04; H, 3.75; Cl, 34.42; N, 6.80%), $[\alpha]_D^{26}$ +74.3° ($CHCl_3$), $\nu_{max.}$ (Nujol) 3 300, 1 710, 1 680, 1 520, and 1 040 cm^{-1}, δ ([$^2H_6$]DMSO) 4.78 (8 H, s, CH_2CCl_3).

1-N-(2-*Aminoethoxycarbonyl*)-5-epi-*sisomicin* (52).—1-*N*-[2-(2,2,2-Trichloroethoxycarbonylamino)ethoxycarbonyl]-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)-5-*epi*-sisomicin (51) (1.52 g) was dissolved in acetic acid–methanol (1 : 4 v/v) (50 ml) containing activated zinc powder (0.78 g) and the mixture was stirred at 25 °C for 18 h. Additional zinc powder (0.39 g) was added and the mixture was stirred for a further 4 h. The mixture was filtered through Celite and the filtrate and washings were concentrated and then dissolved in methanol (10 ml). Concentrated ammonium hydroxide was added until the pH reached 7.0. The mixture was evaporated to dryness and the residue was taken up in water (2 ml) and chromatographed on an Amberlite CG-50 (NH_2) resin column (30 × 3.5 cm). The column was initially eluted with water (1 l) and the aqueous eluant was saved. Elution with 0.1M-, 0.15M-, 0.2M-, 0.25M-, 0.3M-, and 0.35M-ammonium hydroxide (500 ml each) gave the product in the latter fraction (22 mg). The initial aqueous eluant was evaporated to dryness and the residue was dissolved in water (5 ml). A 5% aqueous sodium hydrogen carbonate solution was added until the pH reached 7.0 and the mixture was filtered. The filtrate was evaporated to dryness and the solids were stirred with ethanol-free chloroform and refiltered. The filtrate was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the product (52) (80 mg). The combined fractions (102 mg, 16%) of 1-N-(2-*aminoethoxycarbonyl*)-5-epi-*sisomicin* (52) were obtained as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.45; H, 8.2; N, 15.2. $C_{22}H_{46}N_6O_{10}{\cdot}H_2O$ requires C, 47.64, H, 8.36; N, 15.15%), $[\alpha]_D^{26}$ +148.5° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 680, and 1 040 cm^{-1}, δ (D_2O) 1.21 (3 H, s, 4″-CH_3), 2.49 (3 H, s, 3″-NCH_3), 2.59 (1 H, d, $J_{2''ax,3''ax}$ 11 Hz, 3″*ax*-H), 2.84 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 4.08 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 4.31 (1 H, m, 5*eq*-H), 4.87 (1 H, m, 4′-H), 5.01 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.10 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H).

3,6′-*Bis*-N-*benzyloxycarbonyl*-1-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin B* (54).—3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) (10.73 g) [20]† was dissolved in dry dimethylformamide (800 ml). *N*-(2,2,2-Trichloroethoxycarbonyloxy)succinimide (42) (3.6 g) was added and the mixture was stirred at 25 °C for 2 h with exclusion of moisture. The mixture was evaporated to dryness and the gum was chromatographed on a silica-gel column (120 × 5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution

* Kindly provided by Dr. D. F. Rane.

† Note as on page 2186.

(2 : 1 : 1 v/v) as the eluant to give 3,6′-bis-*N*-benzyloxycarbonyl-1-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin B (54) (8.2 g, 62%) as an amorphous solid (Found: C, 49.55; H, 5.9; Cl, 10.25; N, 6.0. $C_{38}H_{51}Cl_3N_4O_{16}$ requires C, 49.29; H, 5.55; Cl, 11.49; N, 6.05%), $[\alpha]_D^{26}$ +70.8° (MeOH), $\nu_{max.}$ (KBr) 3 375, 1 705, 1 520, and 1 040 cm^{-1}, δ ($CDCl_3$) 0.94 (3 H, s, 4″-CH_3), 2.35 (3 H, s, 3″-NCH_3), 4.88 (6 H, s, $CH_2C_6H_5$ and CH_2CCl_3), and 7.15 (10 H, s, $CH_2C_6H_5$).

3,6′3″-Tris-N-benzyloxycarbonyl-1-N-(2,2,2-trichloroethoxycarbonyl)gentamicin B (55).—3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin B (54) (8.0 g) and sodium carbonate (4.55 g) were dissolved in acetone–water (1 : 1 v/v) (400 ml). Benzyl chloroformate (4.4 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 25 °C for 18 h, and it was then concentrated and extracted with chloroform. The chloroform extracts were washed with water and evaporated to dryness. The resulting solid was chromatographed on a silica gel column (30 × 2 cm) using 7% v/v methanol–chloroform as the eluant to give the *3,6′,3″-tri-N-benzyloxycarbonyl derivative* (55) (8.5 g, 93%) as an amorphous solid. (Found: C, 52.3; H, 5.5; Cl, 9.65; N, 5.15. $C_{46}H_{57}Cl_3N_4O_{18}$ requires C, 52.10; H, 5.42; Cl, 10.03; N, 5.28%), $[\alpha]_D^{26}$ +74.7° (MeOH), $\nu_{max.}$ (KBr) 3 410, 1 700, 1 520, and 1 050 cm^{-1}, δ ($CDCl_3$) 1.00br (3 H, s, 4″-CH_3), 3.00br (3 H, s, 3″-NCH_3), 5.03br (8 H, s, $CH_2C_6H_5$ and CH_2CCl_3), and 7.29br (15 H, s, $CH_2C_6H_5$).

3,6′,3″-Tris-N-benzyloxycarbonylgentamicin B (56).—3,6′,3″-Tris-*N*-benzyloxycarbonyl-1-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin B (55) (8.0 g) was dissolved in acetic acid–water (9 : 1 v/v) (500 ml) and activated zinc (10.41 g) was added. The mixture was stirred at 25 °C for 7 h whereupon additional activated zinc (10.41 g) was added. The mixture was stirred at 25 °C for a further 16 h. Additional activated zinc (20.82 g) was added and the reaction was continued for a further 25 h. The mixture was concentrated and after addition of methanol, it was filtered. The solids were washed with methanol and the combined filtrates were evaporated and the residue was chromatographed on a silica-gel column (160 × 5 cm) using the lower phase of a chloroform–methanol–7% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 3,6′,3″-tris-*N*-benzyloxycarbonylgentamicin B (56) (3.8 g, 57%) as an amorphous solid (Found: C, 55.55; H, 6.15; N, 5.8. $C_{43}H_{56}N_4O_{16}\cdot CO_2\cdot H_2O$ requires C, 55.81; H, 6.17; N, 5.92%), $[\alpha]_D^{26}$ +83.3° (MeOH), $\nu_{max.}$ (KBr) 3 400, 1 700, 1 520, and 1 050 cm^{-1}, δ ($CDCl_3$) 1.03br (3 H. s, 4″-CH_3), 3.02br (3 H, s, 3″-NCH_3), 5.05br (6 H, s, $CH_2C_6H_5$), and 7.25br (15 H, s, $CH_2C_6H_5$).

2-Benzyloxycarbonylaminoethanol (38).[44]—2-Aminoethanol (30) (20 g) and sodium carbonate (84.8 g) were dissolved in acetone–water (1 : 1 v/v) (500 ml) and benzyl chloroformate (83.9 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 0 °C for a further 2.5 h. The solids were filtered off and washed with acetone and the filtrate was evaporated to dryness. The resulting gum was taken up in chloroform and filtered. The filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (140 × 5 cm) using chloroform and then 10% v/v methanol–chloroform as the eluant to give *2-benzyloxycarbonylaminoethanol* (38) (37.7 g, 59%) which crystallized from hexane as needles, m.p. 53—55 °C (Found: C, 60.15; H, 6.7; N, 7.55. $C_{10}H_{13}NO_3$ requires C, 61.53; H, 6.71; N, 7.18%), $\nu_{max.}$ (CCl_4) 3 450, 3 325, 1 720, 1 510, and 1 245 cm^{-1}, δ ($CDCl_3$) 3.29 (2 H, m, $HOCH_2CH_2$-NH), 3.57 (2 H, m, $HOCH_2CH_2NH$), 5.07 (2 H, s, $CH_2C_6H_5$), and 7.31 (5 H, s, $CH_2C_6H_5$).

N-(2-Benzyloxycarbonylaminoethoxycarbonyloxy)succinimide (39).—2-Benzyloxycarbonylaminoethanol (38) (10 g) was dissolved in methylene chloride (200 ml) containing phosgene (15.2 g) and triethylamine (5 ml) and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the resulting gum was taken up in ethyl acetate and filtered. The filtrate (200 ml) was added in portions to a solution of *N*-hydroxysuccinimide (5.9 g) in ethyl acetate (100 ml) containing pyridine (10 ml) and the mixture was stirred at 25 °C for 1 h. The solution was filtered and the filtrate was evaporated to dryness and azeotroped with toluene. The gum was chromatographed rapidly on a silica-gel column (110 × 5 cm) using 10% increasing to 15% v/v ethyl acetate–methylene chloride as the eluant to give the *active ester* (39) (6.1 g, 35%) as a gum (Found: C, 53.65; H, 5.15; N, 7.95. $C_{15}H_{16}N_2O_7$ requires C, 53.57; H, 4.80; N, 8.33%), $\nu_{max.}$ ($CHCl_3$) 3 430, 3 005, 1 750, 1 510, and 1 220 cm^{-1}, δ ($CDCl_3$) 2.75 (4 H, s, $COCH_2$-CH_2CO), 3.50 (2 H, m, OCH_2CH_2NH), 4.38 (2 H, m, OCH_2-CH_2NH), 5.12 (2 H, s, $CH_2C_6H_5$), and 7.35 (5 H, s, $CH_2C_6H_5$).

3,6′,3″-Tris-N-benzyloxycarbonyl-1-N-(2-benzyloxycarbonylaminoethoxycarbonyl)gentamicin B (57).—3,6′,3″-Tris-*N*-benzyloxycarbonylgentamicin B (56) (2.0 g) was dissolved in dry dimethylformamide (100 ml), and triethylamine (228 mg) and *N*-(2-benzyloxycarbonylaminoethoxycarbonyloxy)succinimide (39) (759 mg) were added and the mixture was stirred under dry argon at 25 °C for 3 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–7% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected gentamicin B derivative* (57) (1.94 g, 78%) as an amorphous solid (Found: C, 58.25; H, 6.0; N, 6.1. $C_{54}H_{67}N_5O_{20}$ requires C, 58.63; H, 6.11; N, 6.33%), $[\alpha]_D^{26}$ +60.7° (MeOH), $\nu_{max.}$ (KBr) 3 350, 1 695, 1 515, and 1 040 cm^{-1}, δ ($CDCl_3$) 1.00br (3 H, s, 4″-CH_3), 2.95br (3 H, s, 3″-NCH_3), 5.00br (8 H, s, $CH_2C_6H_5$), and 7.25 br(20 H, s, $CH_2C_6H_5$).

3,6′-Bis-N-benzyloxycarbonyl-1-N-(2-benzyloxycarbonylaminoethoxycarbonyl)gentamicin B (58).—3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) (200 mg) was dissolved in methanol–water (1 : 1 v/v) (5 ml) and triethylamine was added until the mixture reached pH 10. *N*-(2-Benzyloxycarbonylaminoethoxycarbonyloxy)succinimide (39) (98 mg) was added and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2 cm) using the lower phase of a chloroform–methanol–7% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected derivative* (58) (181 mg, 70%) as an amorphous solid (Found: C, 54.85; H, 6.35; N, 7.3. $C_{46}H_{61}N_5O_{18}\cdot 3H_2O$ requires C, 54.70; H, 6.69; N, 6.93%). $[\alpha]_D^{26}$ +63.4° (MeOH), $\nu_{max.}$ (KBr) 3 310, 1 700, 1 540, and 1 045 cm^{-1}, δ ([$^2H_6$]DMSO) 1.00br (3 H, s, 4″-CH_3), 2.50br (3 H, s, 3″-NCH_3), 5.00br (6 H, s, $CH_2C_6H_5$), and 7.30br (15 H, s, $CH_2C_6H_5$).

1-N-(2-Aminoethoxycarbonyl)gentamicin B (59).—(*a*) 3,6′,3″-Tris-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminoethoxycarbonyl)gentamicin B (57) (1.84 g) was dissolved in methanol (50 ml) and 10% palladium–carbon (1.28 g) was added. The mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 18 h. The catalyst was filtered off and

washed with methanol, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (120 × 2 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give 1-*N*-(2-*aminoethoxycarbonyl*)*gentamicin B* (59) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (773 mg, 82%) (Found: C, 41.95; H, 6.75; N, 10.3. $C_{22}H_{43}N_5O_{12}\cdot 2CO_2\cdot 2H_2O$ requires C, 41.57; H, 6.83; N, 10.10%), $[\alpha]_D^{26}$ +130.4° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 535, and 1 045 cm^{-1}, δ (D_2O), 1.20 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 2.88 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 4.11 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 5.10 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.33 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H).

(*b*) 3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminoethoxycarbonyl)gentamicin B (58) (50 mg) was dissolved in methanol (10 ml) and 10% palladium–carbon (30 mg) was added. Dry hydrogen chloride (4 mol equiv.) in methanol was added and the mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 18 h. The reaction was worked up as in (*a*) above to afford 1-*N*-(2-aminoethoxycarbonyl)gentamicin B (59).

3-*Benzyloxycarbonylaminopropanol* (74).—3-Aminopropanol (73) (20 g) and sodium carbonate (22.6 g) were dissolved in acetone–water (4 : 1 v/v) (500 ml) and benzyl chloroformate (68.3 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 0 °C for a further 2.5 h. The solids were filtered off and washed with acetone, and the filtrate was evaporated to dryness. The resulting gum was taken up in chloroform and washed with water. The chloroform solution was evaporated and the residue was triturated with hexane to give 3-*benzyloxycarbonylaminopropanol* (74) (47.5 g, 85%) as crystals, m.p. 47—49 °C (Found: C, 63.12, H, 6.9; N, 6.7. $C_{11}H_{15}NO_3$ requires C, 63.14; H, 7.23; N, 6.69%), *m/e* 209 ($M^{+\cdot}$), $\nu_{max.}$ (KBr) 3 320, 1 680, and 1 535 cm^{-1}, δ ($CDCl_3$) 1.67 (2 H, m, *J* 7.5 Hz, 2-CH_2) 3.28 (2 H, dt, *J* 7.5 Hz, 3-CH_2), 3.62 (2 H, t, *J* 7.5 Hz, 1-CH_2), 5.08 (2 H, s, $CH_2C_6H_5$), and 7.34 (5 H, s, $CH_2C_6H_5$).

N-(3-*Benzyloxycarbonylaminopropyloxycarbonyloxy*)*succinimide* (75).—3-Benzyloxycarbonylaminopropanol (74) (10 g) was dissolved in methylene chloride (178 ml) containing phosgene (14.2 g) and triethylamine (5 ml), and the mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the resulting gum was taken up in ethyl acetate and filtered. The filtrate was added dropwise to a solution of *N*-hydroxysuccinimide (5.5 g) in ethyl acetate (100 ml) containing pyridine (10 ml), and the mixture was stirred at 25 °C for 1 h. The solution was filtered and the filtrate was evaporated to dryness and azeotroped with toluene to afford the *active ester* (75) (15.9 g, 95%). A portion (500 mg) of the material was subjected to preparative t.l.c. on silica gel using 20% v/v ethyl acetate–methylene chloride as the eluant to afford an analytical sample (Found: C, 54.3; H, 5.5; N, 8.35. $C_{16}H_{18}N_2O_7$ requires C, 54.85; H, 5.18; N, 8.00%), *m/e* 350 ($M^{+\cdot}$), $\nu_{max.}$ (film) 3 300, 1 780, 1 750, 1 740, 1 710, 1 520, and 1 210 cm^{-1}, δ ($CDCl_3$) 1.92 (2 H, m, *J* 7.5 Hz, 2-CH_2), 2.73 (4 H, s, $COCH_2CH_2CO$), 3.30 (2 H, dt, *J* 7.5 Hz, 3-CH_2), 4.35 (2 H, t, *J* 7.5 Hz, 1-CH_2), 5.10 (2 H, s, $CH_2C_6H_5$), and 7.36 (5 H, s, $CH_2C_6H_5$).

3,6′,3″-*Tris*-N-(*benzyloxycarbonylaminopropyloxycarbonyl*)*gentamicin B* (60).—3,6′,3″-Tris-*N*-benzyloxycarbonylgentamicin B (56) (1.8 g) was dissolved in dry dimethylformamide (100 ml), and triethylamine (210 mg) and *N*-(3-benzyloxycarbonylaminopropyloxy)succinimide (75) (780 mg) were added and the mixture was stirred at 25 °C for 18 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (110 × 3.5 cm) using chloroform–methanol–7% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected gentamicin B derivative* (60) (1.56 g, 68%) as an amorphous solid (Found: C, 58.8; H, 6.2; N, 6.3. $C_{55}H_{69}N_5O_{20}$ requires C, 59.0; H, 6.2; N, 6.25%). $[\alpha]_D^{26}$ +47.2° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 350, 1 700, and 1 050 cm^{-1}, δ ($CDCl_3$) 1.00br (3 H, s, 4″-CH_3), 2.95br (3 H, s, 3″-CH_3), 5.01br (8 H, s, $CH_2C_6H_5$), and 7.25br (20 H, s, $CH_2C_6H_5$).

1-N-(3-*Aminopropyloxycarbonyl*)*gentamicin B* (61).—3,6′,3″-Tris-*N*-benzyloxycarbonyl-1-*N*-(3-benzyloxycarbonylaminopropyloxycarbonyl)gentamicin B (60) (1.47 g) was dissolved in methanol (50 ml) and 10% palladium–carbon (880 mg) was added. The mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 18 h. Additional 10% palladium–carbon (880 mg) was added and the hydrogenation was continued for a further 18 h. The catalyst was filtered off and washed with methanol, and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (120 × 2 cm) using chloroform–methanol–concentrated ammonium hydroxide (2 : 1 : 1 v/v) as the eluant to give 1-N-(3-*aminopropyloxycarbonyl*)-*gentamicin B* (61) (421 mg, 35%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 44.75; H, 7.45; N, 11.2. $C_{23}H_{45}N_5O_{12}\cdot CO_2\cdot H_2O$ requires C, 44.65; H, 7.34; N, 10.85%), $[\alpha]_D^{26}$ +125.7° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, and 1 050 cm^{-1}, δ (D_2O) 1.18 (3 H, s, 4″-CH_3), 1.73 (2 H, m, *J* 7.5 Hz, 2‴-CH_2), 2.47 (3 H, s, 3″-NCH_3), 4.09 (2 H, t, *J* 7.5 Hz, 1‴-CH_2), 5.06 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.28 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H). The more polar fractions from the column contained gentamicin B (62) (226 mg, 23%).

4-*Benzyloxycarbonylaminobutan*-1-*ol* (77).—4-Aminobutan-1-ol (76) (20 g) was dissolved in acetone–water (4 : 1 v/v) (500 ml) and sodium carbonate (19.3 g) was added. Benzyl chloroformate (58.1 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 0 °C for 3 h and was then filtered and the filtrate was evaporated to give 4-*benzyloxycarbonylaminobutan*-1-*ol* (77) (42.6 g, 85%) as an amorphous solid, m.p. 80—81 °C (Found: C, 64.45; H, 8.05; N, 6.1. $C_{12}H_{17}NO_3$ requires C, 64.56; H, 7.67; N, 6.27%), *m/e* 223 ($M^{+\cdot}$), $\nu_{max.}$ (KBr) 3 325, 1 690, 1 545, and 1 060 cm^{-1}, δ ($CDCl_3$) 1.56 (4 H, m, $ZNHCH_2[CH_2]_2CH_2OH$), 1.73br (1H, s, OH), 3.18 (2 H, m, $ZNHCH_2[CH_2]_2CH_2OH$), 3.60 (2 H, m, $ZNHCH_2[CH_2]_2CH_2OH$), 5.09 (2 H, s, $C_6H_5CH_2$), and 7.38 (5 H, s, $C_6H_5CH_2$).

N-(4-*Benzyloxycarbonylaminobutyloxycarbonyloxy*)*succinimide* (78).—4-Benzyloxycarbonylaminobutan-1-ol (77) (10 g) was dissolved in a solution of phosgene (3 mol equiv.) in dichloromethane (2.0 ml). Triethylamine (5 ml) was added dropwise to the solution and the mixture was stirred at 25 °C for 3 h. The excess of phosgene was removed with a stream of dry nitrogen and the solution was evaporated to dryness. The gum was taken up in ethyl acetate (100 ml), filtered, and then added dropwise to *N*-hydroxysuccinimide (5.2 g) in ethyl acetate (100 ml) containing pyridine (10 ml). After 1 h at 25 °C the mixture was filtered, the filtrate was evaporated, and the residue was azeotroped with toluene to give the *active ester* (78) (15.3 g, 93%) as a gum. Preparative t.l.c. chromatography on silica gel using 20% v/v ethyl acetate–dichloromethane as the eluant afforded an analytical

sample (Found: C, 55.9; H, 5.95; N, 7.2. $C_{17}H_{20}N_2O_7$ requires C, 56.05; H, 5.53; N, 7.69%), *m/e* 364 ($M^{+\cdot}$), $\nu_{max.}$ (film) 3 350, 1 830, 1 780, 1 720, 1 530, and 1 220 cm^{-1}, δ ($CDCl_3$) 1.68 (4 H, m, $ZNHCH_2[CH_2]_2CH_2O$), 2.74 (4 H, s, $COCH_2CH_2CO$), 3.23 (2 H, m, $ZNHCH_2$), 4.36 (2 H, t, *J* 7.5 Hz, CH_2O), 5.07 (2 H, s, $C_6H_5CH_2$), and 7.40 (5 H, s, $C_6H_5CH_2$).

3,6′-*Bis*-N-*benzyloxycarbonyl*-1-N-(4-*benzyloxycarbonylaminobutyloxycarbonyl*)*gentamicin B* (63).—3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) (1.5 g) [20,*] was dissolved in methanol–water (1 : 1 v/v) (100 ml) and triethylamine was added until the pH reached 9.0. *N*-(4-Benzyloxycarbonylaminobutyloxycarbonyloxy)succinimide (78) (729 mg) was added and the mixture was stirred at 25 °C for 3 h. Additional succinimide reagent (78) (729 mg) was added and the mixture was stirred at 25 °C for 18 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 3.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected gentamicin B derivative* (63) (1.58 g, 79%) as an amorphous solid (Found: C, 57.35; H, 6.45; N, 6.95. $C_{48}H_{65}N_5O_{18}$ requires C, 57.65; H, 6.55; N, 7.00%), $[\alpha]_D^{26}$ + 51.8° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 320, 1 700, 1 530, and 1 050 cm^{-1}, δ ($CDCl_3$) 1.09br (3 H, s, 4″-CH_3), 1.50br (4 H, m, $ZNHCH_2[CH_2]_2CH_2O$), 2.49br (3 H, s, 3″-NCH_3), 5.01br (6 H, s, $C_6H_5CH_2$), and 7.15—7.28 (15 H, s, $C_6H_5CH_2$).

1-N-(4-*Aminobutyloxycarbonyl*)*gentamicin B* (64).—3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(4-benzyloxycarbonylaminobutyloxycarbonyl)gentamicin B (63) (1.48 g) was dissolved in methanol–water (1 : 1 v/v) (50 ml) containing 1M-hydrochloric acid (6.0 ml) and 10% palladium–carbon (0.89 g) was added. The mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 2 h. Additional 10% palladium–carbon (0.89 g) was added and the hydrogenation was continued for a further 16 h. The catalyst was filtered off and washed with aqueous methanol. The combined filtrates were treated with Amberlite IRA 40IS (OH^-) resin until pH 11.0 and then filtered. The filtrate was concentrated and the residue was chromatographed first on a silica-gel column (60 × 2.5 cm) and then on a column (110 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant in each case to give 1-N-(4-*aminobutyloxycarbonyl*)*gentamicin B* (64) (456 mg, 52%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) followed by lyophilization (Found: C, 44.65; H, 7.5; N, 10.45. $C_{24}H_{47}N_5O_{12}\cdot 2H_2O$ requires C, 44.36; H, 7.91; N, 10.78%), $[\alpha]_D^{26}$ +124.8° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 550, and 1 045 cm^{-1}, δ (D_2O) 1.21 (3 H, s, 4″-CH_3), 1.60 (4 H, m, $OCH_2[CH_2]_2CH_2NH_2$), 2.50 (3 H, s, 3″-NCH_3), 5.08 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.29 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H).

(2R)-2-*Benzyloxycarbonylaminobutan*-1-*ol* (80).—(2*R*)-2-Aminobutan-1-ol (79) (20 g) was dissolved in acetone–water (4 : 1 v/v) (500 ml) and sodium carbonate (18.7 g) was added. Benzyl chloroformate (56.1 g) was added dropwise to the stirred solution at 0 °C over 0.5 h. The mixture was stirred at 0 °C for 3 h and was then filtered and the filtrate was evaporated to dryness to give the *benzyloxycarbonyl derivative* (80) (42 g, 84%) as a waxy solid. An aliquot (200 mg) was subjected to preparative t.l.c. on silica gel using 5% v/v methanol–chloroform as the eluant (Found: C, 64.9; H, 7.7; N, 6.65. $C_{12}H_{17}NO_3$ requires C, 64.56; H, 7.67; N, 6.27%), *m/e* 192 ($M^{+\cdot}$ − 31), $[\alpha]_D^{26}$ +1.90° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 400, 3 250, 1 700, and 1 540 cm^{-1}, δ ($CDCl_3$) 0.90 (3 H, t, *J* 8 Hz, 4-CH_3), 1.48 (2 H, m, 3-CH_2), 3.05 (1 H, m, 2-CH), 3.55 (2 H, m, 1-CH_2), 5.08 (2 H, s, $CH_2C_6H_5$), and 7.34 (5 H, s, $CH_2C_6H_5$).

(2R)-N-(2-*Benzyloxycarbonylaminobutyloxycarbonyloxy*)-*succinimide* (81).—(2*R*)-2-Benzyloxycarbonylaminobutan-1-ol (80) (5 g) was dissolved in a 0.9M-solution of phosgene in dichloromethane (75 ml). Triethylamine (3.4 g) was added dropwise to the stirred solution at 25 °C over 0.5 h and the mixture was stirred under dry nitrogen for 3 h. The excess of phosgene was removed with a stream of dry nitrogen and the solution was evaporated to dryness. The gum was taken up in ethyl acetate (100 ml), filtered, and then added dropwise to *N*-hydroxysuccinimide (2.59 g) in ethyl acetate (100 ml) containing pyridine (5 ml). After 1 h at 25 °C the mixture was filtered, the filtrate was evaporated, and the residue was azeotroped with toluene to give the succinimide *active ester* (81) (7.5 g, 51%) as a yellow gum. Preparative t.l.c. on silica gel using 20% v/v ethyl acetate–dichloromethane as the eluant afforded an analytical sample (Found: C, 55.9; H, 5.6; N, 7.5. $C_{17}H_{20}N_2O_7$ requires C, 56.04; H, 5.53; N, 7.69%). *m/e* 364 ($M^{+\cdot}$) $[\alpha]_D^{26}$ +20.8° ($CHCl_3$), $\nu_{max.}$ (liquid film) 3 300, 1 830, 1 780, 1 720, 1 530, and 1 220 cm^{-1}, δ ($CDCl_3$) 0.95 (3 H, t, *J* 6 Hz, CH_3CH_2). 1.51 (2 H, dq, *J* 6 Hz, CH_3CH_2) 2.58br (1 H, s, NH), 2.79 (4 H, s, $COCH_2CH_2CO$), 3.69 (1 H, m, >CHNHZ), 4.35 (2 H, d, *J* 4 Hz, CH_2O), 5.13 (2 H, s, $C_6H_5CH_2$), and 7.20 (5 H, s, $C_6H_5CH_2$).

(2R)-3,6′-*Bis*-N-*benzyloxycarbonyl*-1-N-(2-*benzyloxycarbonylaminobutyloxycarbonyl*)*gentamicin B* (65).—3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) (1.5 g) was dissolved in methanol–water (1 : 1 v/v) (100 ml) and triethylamine was added until the pH reached 9.0. (2*R*)-*N*-(2-Benzyloxycarbonylaminobutyloxycarbonyloxy)succinimide (81) (728 mg) was added and the mixture was stirred at 25 °C for 3 h. The mixture was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 2 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected gentamicin B derivative* (65) (1.7 g, 85%) as an amorphous solid (Found: C, 56.7; H, 6.5; N, 6.75. $C_{48}H_{65}N_5O_{18}\cdot H_2O$ requires C, 56.61; H, 6.63; N, 6.87%), $[\alpha]_D^{26}$ +66.1° (DMSO), $\nu_{max.}$ (KBr) 3 320, 1 695, 1 540, and 1 050 cm^{-1}, δ ([$^2H_6$]DMSO) 0.83 (3 H, t, *J* 6 Hz, CH_3CH_2), 1.19 (3 H, s, 4″-CH_3), 5.00 (6 H, s, C_6H_5-CH_2), 7.29 (5 H, s, $C_6H_5CH_2$), and 7.33 (10 H, s, $C_6H_5CH_2$).

(2R)-1-N-(2-*Aminobutyloxycarbonyl*)*gentamicin B* (66).—(2*R*)-3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminobutyloxycarbonyl)gentamicin B (65) (1.6 g) was dissolved in methanol–water (1 : 1 v/v) (50 ml) containing 1M-hydrochloric acid (6.4 ml), and 10% palladium–carbon (0.96 g) was added. The mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 2 h. Additional 10% palladium–carbon (0.96 g) was added and the hydrogenation was continued for a further 16 h. The catalyst was filtered off and washed with aqueous methanol. The combined filtrates were treated with Amberlite IRA 40IS (OH^-) resin until pH 11.0 and then filtered. The filtrate was concentrated to a gum and chromatographed on a silica-gel column (60 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give (2R)-1-N-(2-*aminobutyloxycarbonyl*)-*gentamicin B* (66) (375 mg, 31%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed

* Note as on page 2186.

by lyophilization (Found: C, 44.4; H, 7.85; N, 10.5. $C_{24}H_{47}N_5O_{12}\cdot 3H_2O$ requires C, 44.22; H, 8.19; N, 10.74%), $[\alpha]_D^{26}$ +115.5° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 560, and 1 050 cm^{-1}, δ (D_2O) 0.96 (3 H, t, *J* 7 Hz, CH_3CH_2), 1.24 (3 H, s, 4″-CH_3), 2.57 (3 H, s, 3″-NCH_3), 5.12 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.36 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H).

(2S)-2-*Benzyloxycarbonylamino*-4-*methylpentan*-1-*ol* (83).—(2*S*)-2-Amino-4-methylpentan-1-ol (L-leucinol) (82) (20 g) was dissolved in acetone–water (4 : 1 v/v) (500 ml) and sodium carbonate (14.5 g) was added. Benzyl chloroformate (44.4 g) was added dropwise to the stirred solution at 0 °C over a period of 0.5 h. The mixture was stirred at 0 °C for 3 h and was then filtered, and the filtrate was evaporated to dryness. The resulting gum was chromatographed on a *silica-gel* column (60 × 3 cm) using chloroform as the eluant to give the *benzyloxycarbonyl derivative* (83) (38.5 g, 90%) as a waxy solid (Found: C, 66.85; H, 8.8; N, 5.55. $C_{14}H_{21}NO_3$ requires C, 66.91; H, 8.42; N, 5.57%), *m/e* 251 ($M^{+\cdot}$), $[\alpha]_D^{26}$ −27.6° ($CHCl_3$), $\nu_{max.}$ (KBr) 3 400, 3 325, 1 695, and 1 530 cm^{-1}, δ ($CDCl_3$) 0.91 [6 H, d, *J* 8 Hz, $(CH_3)_2$], 5.08 (2 H, s, $CH_2C_6H_5$), and 7.34 (5 H, s, $CH_2C_6H_5$).

(2S)-N-(2-*Benzyloxycarbonylamino*-4-*methylpentyloxycarbonyloxy*)*succinimide* (84).—(2*S*)-2-Benzyloxycarbonylamino-4-methylpentan-1-ol (83) (5 g) was dissolved in a 0.9M-solution of phosgene in dichloromethane (66 ml). Triethylamine (3.01 g) was added dropwise to the stirred solution at 25 °C over 0.5 h and the mixture was stirred under dry nitrogen for 3 h. The excess of phosgene was removed with a stream of dry nitrogen and the solution was evaporated to dryness. The gum was taken up in ethyl acetate (100 ml), filtered, and then added dropwise to *N*-hydroxysuccinimide (2.29 g) in ethyl acetate (50 ml) containing pyridine (5 ml). After 1.5 h at 25 °C the mixture was filtered and the filtrate was evaporated and the residue was azeotroped with toluene to give the *succinimide active ester* (84) (6.55 g, 84%) as a gum. Preparative t.l.c. on silica-gel using 20% v/v ethyl acetate–dichloromethane as the eluant afforded an analytical sample (Found: C, 57.95; H, 6.25; N, 6.85. $C_{19}H_{24}N_2O_7$ requires C, 58.16; H, 6.17; N, 7.14%), *m/e* 392 ($M^{+\cdot}$), $[\alpha]_D^{26}$ −28.3° ($CHCl_3$), $\nu_{max.}$ (liquid film) 3 300, 1 810, 1 780, 1 720, 1 520, and 1 210 cm^{-1}, δ ($CDCl_3$) 0.93 [3 H, d, *J* 6 Hz, $(CH_3)_2CH$], 0.95 [3 H, d, *J* 6 Hz, $(CH_3)_2CH$], 1.20—1.70 [3 H, m, $(CH_3)_2CHCH_2$], 2.58br (1 H, s, NH), 2.75 (4 H, s, $COCH_2CH_2CO$), 4.03 (1 H, m, $CHNHZ$), 4.30 (2 H, m, OCH_2), 5.10 (2 H, s, $C_6H_5CH_2O$), and 7.37 (5 H, s, $C_6H_5CH_2$).

(2S)-3,6′-*Bis*-N-*benzyloxycarbonyl*-1-N-(2-*benzyloxycarbonylamino*-4-*methylpentyloxycarbonyl*)*gentamicin B* (67).—3,6′-Bis-*N*-benzyloxycarbonylgentamicin B (53) (4 g) [20,*] was dissolved in methanol–water (1 : 1 v/v) (100 ml) and triethylamine was added until the pH reached 9.0. (2*S*)-*N*-(2-Benzyloxycarbonylamino-4-methylpentyloxycarbonyloxy)succinimide (84) (2.09 g) was added and the mixture was stirred at 25 °C for 3 h. Additional (2*S*)-*N*-(2-benzyloxycarbonylamino-4-methylpentyloxycarbonyloxy)succinimide (84) (2.09 g) was added and the mixture was stirred at 25 °C for a further 18 h. The mixture was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the *protected gentamicin B derivative* (67) (4.45 g, 81%) as a solid (Found: C, 57.8; H, 6.8; N, 6.85. $C_{50}H_{69}N_5O_{18}\cdot H_2O$ requires C, 57.41; H, 6.84; N, 6.69%), $[\alpha]_D^{26}$ +62.0° (DMSO), $\nu_{max.}$ (KBr) 3 500, 3 350, 1 700, 1 680, 1 540, 1 520, and 1 050 cm^{-1}, δ ([$^2H_6$]DMSO) 0.84 [6 H, d, *J* 6 Hz, $(CH_3)_2CH$], 1.23 (3 H, s, 4″-CH_3), 5.01 (6 H, s, $C_6H_5CH_2$), 7.30 (5 H, s, $C_6H_5CH_2$), and 7.33 (10 H, s, $C_6H_5CH_2$).

(2S)-1-N-(2-*Amino*-4-*methylpentyloxycarbonyl*)*gentamicin B* (68).—(2*S*)-3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylamino-4-methylpentyloxycarbonyl)gentamicin B (67) (4.25 g) was dissolved in methanol–water (1 : 1 v/v) (150 ml) containing 1M-hydrochloric acid (4.3 ml) and, 10% palladium–carbon (2.7 g) was added. The mixture was hydrogenated at 55 lbf in^{-2} at 25 °C for 18 h. The catalyst was filtered off and washed with aqueous methanol. The combined filtrates were evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 3 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give (2*S*)-1N-(2-*amino*-4-*methylpentyloxycarbonyl*)*gentamicin B* (68) (640 mg, 25%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 44.2; H, 7.0; N, 9.8. $C_{26}H_{51}N_5O_{12}\cdot 4H_2O\cdot CO_2$ requires C, 43.7; H, 8.0; N, 9.8%), $[\alpha]_D^{26}$ +109.5° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 750, 1 540, and 1 050 cm^{-1}, δ (D_2O) 0.90 [3 H, d, *J* 6 Hz, $(CH_3)_2CH$], 0.92 [3 H, d, *J* 6 Hz, $(CH_3)_2CH$], 1.20 (3 H, s, 4″-CH_3), 5.10 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.31 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H). The more polar fractions from the column contained gentamicin B (62) (274 mg, 14%).

1-N-[2-(2,2,2-*Trichloroethoxycarbonylamino*)*ethoxycarbonyl*]-3,2′,6′-*tris*-N-(2,2,2-*trichloroethoxycarbonyl*)*gentamicin* C_{1a} (86).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (85) (2 g) † was dissolved in methanol–water (1 : 1 v/v) (40 ml) containing *N*-[2-(2,2,2-trichloroethoxycarbonylamino)ethoxycarbonyloxy]succinimide (32) (755 mg) and the mixture was stirred at 25 °C for 1 h. Additional reagent (151 mg) was added and the stirring was continued for 2 h. The solution was evaporated to dryness and chromatographed on a silica-gel column (60 × 2.5 cm) using 7% v/v methanol–chloroform as the eluant to give the *protected gentamicin* C_{1a} *derivative* (86) (1.54 g, 62%) as an amorphous solid (Found: C, 33.6; H, 4.0; Cl, 33.4; N, 7.3. $C_{34}H_{48}Cl_{12}N_6O_{17}$ requires C, 33.00; H, 3.9; Cl, 34.4; N, 6.8%), $[\alpha]_D^{26}$ +58.0° (MeOH), $\nu_{max.}$ (Nujol) 3 350, 2 920, 1 730, 1 560, 1 470, and 1 045 cm^{-1}, δ ([$^2H_6$]DMSO) 1.04 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), and 4.78 (8 H, s, $CO_2CH_2CCl_3$).

1-N-(2-*Aminoethoxycarbonyl*)*gentamicin* C_{1a} (87).—1-*N*-[2-(2,2,2-Trichloroethoxycarbonylamino)ethoxycarbonyl]-3,2′,6′-tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (86) (1.5 g) was dissolved in 20% v/v acetic acid–methanol (50 ml) containing activated zinc powder (780 mg) and the mixture was stirred at 25 °C for 18 h. Additional zinc powder (390 mg) was added and the stirring was continued for a further 4 h. The reaction mixture was filtered through a bed of Celite and the filtrate was evaporated to dryness. The residue was dissolved in water (20 ml) and a 20% aqueous sodium carbonate solution (w/v) was added until the pH reached 10.0. The solids were filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (60 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N-(2-*aminoethoxycarbonyl*)*gentamicin* C_{1a} (87) (107 mg, 16%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found:

† Kindly provided by C. E. Luce.

* Note as on page 2186.

C, 46.8; H, 8.7; N, 14.5. $C_{22}H_{44}N_6O_9 \cdot 1.5H_2O$ requires C, 46.9; H, 8.40; N, 14.9%), $[\alpha]_D^{26}$ +107.3° (H_2O), $\nu_{max.}$ (KBr) 3 350, 3 280, 1 705, and 1 050 cm^{-1}, δ (D_2O) 1.25 (3 H, s, 4″-CH_3), 2.57 (3 H, s, 3″-NCH_3), 4.18 (2 H, t, $CO_2CH_2CH_2NH_2$), 5.14 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.29 (1 H, d, $J_{1',2'}$ 4 Hz, 1′-H).

3,6′-Bis-N-benzyloxycarbonyl-1-N-(2-benzyloxycarbonylaminoethoxycarbonyl)kanamycin A (4).—3,6′-Bis-*N*-benzyloxycarbonylkanamycin A (3) (1 g) [20,*] was dissolved in a mixture of water (10 ml) and dioxan (5 ml). Triethylamine was added until the pH reached 9. *N*-(2-Benzyloxycarbonylaminoethoxycarbonyloxy)succinimide (39) (427 mg) in dioxan (10 ml) was added dropwise over 0.5 h. The reaction mixture was then stirred at 25 °C for 3 h. The solution was evaporated to dryness and a portion was chromatographed on a silica-gel column (110 × 1.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give an analytical sample of 3,6′-bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminoethoxycarbonyl)-kanamycin A (4) as an amorphous solid (Found: C, 53.45; H, 5.9; N, 7.3. $C_{45}H_{59}N_5O_{19} \cdot 2H_2O$ requires C, 53.51; H, 6.29; N, 6.93%), $[\alpha]_D^{26}$ +49.7° (MeOH–H_2O) (1 : 1 v/v), $\nu_{max.}$ (KBr) 3 325, 1 680, 1 535, and 1 045 cm^{-1}, δ ([$^2H_6$]-DMSO) 5.02br (6 H, s, $CH_2C_6H_5$) and 7.37br (15 H, s, $CH_2C_6H_5$).

1-N-(2-Aminoethoxycarbonyl)kanamycin A (7).—(*a*) Crude 3,6′-bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminoethoxycarbonyl)kanamycin A (4) (from the previous preparation) was dissolved in methanol–dioxan–water (2 : 1 : 1 v/v) (20 ml) and 1M-hydrochloric acid (5.3 ml) and 10% palladium–carbon (840 mg) were added. The mixture was hydrogenated at 25 °C at 55 lbf in^{-2} and after 2 h additional catalyst (840 mg) was added. The hydrogenation was allowed to proceed for an additional 16 h. The catalyst was filtered off and Amberlite IRA 40IS (OH^-) resin was added to the filtrate until the pH reached 10. The mixture was filtered and evaporated to dryness, and the residue was chromatographed on a silica-gel column (120 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give 1-*N*-(2-*aminoethoxycarbonyl*)*kanamycin A* (7) (132 mg, 17%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 40.05; H, 6.7; N, 10.15. $C_{21}H_{41}N_5O_{13} \cdot 2CO_2 \cdot 2H_2O$ requires C, 39.72; H, 6.52; N, 10.07%, $[\alpha]_D^{26}$ +87.0° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 690, 1 585, 1 540, and 1 030 cm^{-1}, δ (D_2O–DCl, pH 3) 4.18 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 5.07 (1 H, d, $J_{1'',2''}$ 3.5 Hz, 1″-H), and 5.39 (1 H, d, $J_{1',2'}$ 3 Hz, 1′-H).

(*b*) 3,6′-Bis-*N*-(4-methoxybenzyloxycarbonyl)kanamycin A (5) (4 g) [20,*] was dissolved in a mixture of dioxan (20 ml) and water (40 ml). Triethylamine was added until the pH reached 9.0. *N*-[2-(4-Methoxybenzyloxycarbonylamino)-ethoxycarbonyloxy]succinimide (34) (2.04 g) dissolved in dioxan (10 ml) was added dropwise over a period of 0.5 h. After 2 h at 25 °C the solution was evaporated to dryness to give (6). The residue was dissolved in trifluoroacetic acid (3 ml) and after 3 min at 25 °C, the solution was evaporated to dryness *in vacuo* and the residue was azeotroped with toluene. The product was chromatographed on a silica-gel column (160 × 3 cm) using chloroform–methanol–concentrated ammonium hydroxide solution (3 : 4 : 2 v/v) as the eluant to give 1-*N*-(2-aminoethoxycarbonyl)kanamycin A (7) (908 mg, 32%), identical with that prepared in (*a*) above.

1-*N*-(2-*Aminoethylcarboxamido*)*sisomicin* (25).—3,2′,6′,3″-Tetrakis-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) (2 g) was dissolved in dry dimethylformamide (10 ml) containing 2-azidoethyl isocyanate (90) (1 ml) and triethylamine (20 mg) and the reaction mixture was stirred at 25 °C for 18 h. The solution was added dropwise to diethyl ether (500 ml) and the resulting precipitate was filtered off and dissolved in dry pyridine (30 ml) containing triphenylphosphine (500 ml). The mixture was stirred at 25 °C for 1 h. Concentrated ammonium hydroxide (2 ml) was added and after stirring at 25 °C for 1 h, the solution was evaporated to dryness and the residue was azeotroped with toluene (3 × 20 ml). The resulting solid was dissolved in trifluoroacetic acid (5 ml) and after 3 min at 25 °C the solution was evaporated to dryness. After trituration with ether, the residue was dissolved in the minimum volume of tetrahydrofuran and chromatographed on an Amberlite CG-50 (NH_4) resin column (30 × 2 cm) using gradient elution with 0.1—0.3M-ammonium hydroxide as the eluant to give 1-*N*-(2-aminoethylcarboxamido)sisomicin (25) (39 mg, 4%) as an amorphous solid after passage over Amberlite IR 40IS (OH^-) resin followed by lyophilization, $[\alpha]_D^{26}$ +92.5° (H_2O), $\nu_{max.}$ (KBr) 3 340, 1 650, 1 560, and 1 050 cm^{-1}, δ (D_2O) 1.14 (3 H, s, 4″-CH_3), 2.41 (3 H, s, 3″-NCH_3), 2.58 (2 H, t, *J* 6 Hz, $CONHCH_2CH_2NH_2$), 4.78 (1 H, m, 4′-H), 5.03 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.25 (1 H, d, $J_{1'eq,2'ax}$ 3 Hz, 1′*eq*-H), and 1-*N*-(2-*aminoethylcarboxamido*)*garamine* (92) (134 mg, 18%) as an amorphous solid after passage over Amberlite IR 40IS (OH^-) resin followed by lyophilization (Found: C, 44.6; H, 7.85; N, 15.7. $C_{16}H_{33}N_5O_7 \cdot 1.5H_2O$ requires C, 44.25; H, 8.35; N, 16.12%), $[\alpha]_D^{26}$ 91.4° (H_2O), $\nu_{max.}$ (KBr) 3 340, 1 650, 1 560, and 1 050 cm^{-1}, δ (D_2O) 1.19 (3 H, s, 4″-CH_3), 2.47 (3 H, s, 3′-NCH_3), 2.63 (2 H, t, *J* 6.5 Hz, $CONHCH_2CH_2NH_2$), 3.12 (2 H, t, *J* 6.5 Hz, $CONHCH_2CH_2NH_2$), and 5.07 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H).

1-*N*-*Carboxamidosisomicin* (27).—3,2′,6′-Tri-*N*-acetylsisomicin (26) (400 mg) [20,*] was dissolved in dry dimethylformamide (40 ml) and the solution was cooled to 0 °C under nitrogen. A solution of silicon tetraisocyanate (172 mg) in dry dimethylformamide (2.36 ml) was added using a syringe and the mixture was stirred at 0 °C under nitrogen for 24 h. The solution was evaporated to dryness and the residue was heated under reflux with 5% aqueous sodium hydroxide (60 ml) under nitrogen for 16 h. The solution was neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water (800 ml) and then eluted with 7% aqueous ammonium hydroxide (1.2 l). The latter eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-*N*-*carboxamidosisomicin* (27) (80 mg, 23%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.75; H, 7.45; N, 16.8. $C_{20}H_{38}N_6O_8 \cdot 0.5H_2O$ requires C, 48.08; H, 7.86; N, 16.82%), $[\alpha]_D^{26}$ +152.0° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 665, 1 600, 1 355, and 1 050 cm^{-1}, δ_H (D_2O) 1.23 (3 H, s, 4″-CH_3), 2.52 (3 H, s, 3″-NCH_3), 4.90 (1 H, m, 4′-H), 5.16 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.36 (1 H, d, $J_{1'eq,2'ax}$ 2 Hz, 1′*eq*-H).

1-*N*-(*Methylcarboxamido*)*sisomicin* (28).—3,2′,6′-Tri-*N*-acetylsisomicin (26) (400 mg) [20,*] was dissolved in ethanol–water (1 : 1 v/v) (30 ml) and methyl isocyanate (0.046 ml)

* Note as on page 2186.

was added and the mixture was heated under reflux for 16 h. The solution was evaporated to dryness and the residue was heated under reflux with 5% aqueous sodium hydroxide (60 ml) under argon for 7 h. The solution was neutralized with Amberlite IRC 50 (H^+) resin. The resin was washed with water (1 l) and then eluted with 7% aqueous ammonium hydroxide solution (1.5 l). The latter eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 0.18 v/v) as the eluant to give 1-N-(*methylcarboxamido*)*sisomicin* (28) (81 mg, 23%) as an amorphous solid after treatment with Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 47.15; H, 7.5; N, 14.8. $C_{21}H_{40}N_6O_8{\cdot}CO_2{\cdot}H_2O$ requires C, 46.62; H, 7.47; N, 14.83%), $[\alpha]_D^{26}$ +137.6° (H_2O), $\nu_{max.}$ (KBr) 3 300, 1 650, 1 570, and 1 060 cm^{-1}, δ (D_2O) 1.19 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 2.65 (3 H, s, NHCONHCH_3), 4.90 (1 H, m, 4′-H), 5.11 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″-*eq*H), and 5.34 (1 H, d, $J_{1'eq,2'ax}$ 2.5 Hz, 1′*eq*-H).

3,6′,3″-*Tris*-N-*benzyloxycarbonyl*-1-N-(2-*azidoethylcarboxamido*)*gentamicin B* (69).—2-Chloroethyl isocyanate (91) (584 mg) and sodium iodide (834 mg) were dissolved in dry dimethylformamide (10 ml) and the mixture was heated at 60° C for 18 h. The solution was cooled to 25 °C and sodium azide (361 mg) was added and the mixture was stirred at 25 °C for 18 h. The resulting crude solution of 2-azidoethyl isocyanate (90) was used without further purification.

3,6′,3″-Tris-*N*-benzyloxycarbonylgentamicin B (56) (500 mg) was dissolved in dry dimethylformamide (5 ml) and the solution of 2-azidoethyl isocyanate (90) (2 ml) was added. The mixture was stirred at 25 °C for 3 h under dry argon. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 3 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the protected gentamicin B derivative (69) (159 mg, 28%) as an amorphous solid, $[\alpha]_D^{26}$ +75.7° (MeOH), $\nu_{max.}$ (CCl_4) 3 700, 3 350, 2 950, 2 110, 1 730, 1 690, 1 550, and 1 060 cm^{-1}, δ ($CDCl_3$) 1.15br (3 H, m, 4″-CH_3), 3.00br (3 H, m, 3″-NCH_3), 5.00br (6 H, m, $CH_2C_6H_6$), and 7.27 (15 H, s, $CH_2C_6H_5$).

1-N-(2-*Aminoethylcarboxamido*)*gentamicin B* (70).—1-*N*-(2-Azidoethylcarboxamido)-3,6′,3″-tris-*N*-benzyloxycarbonylgentamicin B (69) (139 mg) was dissolved in methanol–water (1 : 1 v/v) (5 ml) containing 1M-hydrochloric acid (0.5 ml), and 10% palladium–carbon (84 mg) was added. The mixture was hydrogenated at 25 °C and 55 lbf in^{-2} for 2 h. Additional catalyst (84 mg) was added and the hydrogenation was allowed to proceed for a further 16 h. The catalyst was filtered off and the filtrate was treated with Amberlite IRA 40IS (OH^-) resin until the pH reached 10. The mixture was filtered and concentrated to dryness. The residue was chromatographed on a silica-gel column (15 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give 1-*N*-(2-aminoethylcarboxamido)-gentamicin B (70) (39 mg, 49%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 41.4; H, 7.5; N, 11.8. $C_{22}H_{44}N_6O_{11}{\cdot}2CO_2{\cdot}2H_2O$ requires C, 41.6, H, 7.00; N, 12.1%), $[\alpha]_D^{26}$ +99.4° (H_2O), $\nu_{max.}$ (KBr) 3 330, 2 920, 1 655, 1 565, and 1 050 cm^{-1}, δ (D_2O) 1.23 (3 H, s, 4″-CH_3), 2.51 (3 H, s, 3″-NCH_3), 4.09 (1 H, d, $J_{5''eq,5''ax}$ 12 Hz, 5″*eq*-H), 5.13 (1 H, d, $J_{1'',2''}$ 4 Hz, 1″-H), and 5.32 (1 H, d, $J_{1',2'}$ 3.5 Hz, 1′-H).

1-N-*Carboxamidogentamicin B* (72).—3,6′-Bis-*N*-t-butoxycarbonylgentamicin B (71) (750 mg) [20,*] was dissolved in dry dimethylformamide (70 ml). Silicon tetraisocyanate (52 mg) in dry dimethylformamide (0.9 ml) was added and the mixture was stirred under dry argon at 25 °C for 24 h. The solution was evaporated to dryness and the residue was dissolved in trifluoroacetic acid (5 ml). After 3 min at 25 °C, the solution was evaporated to dryness. The residue was taken up in water and passed over Amberlite IRA 40IS (OH^-) resin. The aqueous eluate was evaporated to dryness and the residue was chromatographed on a silica-gel column (160 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (1 : 1 : 1 v/v) as the eluant to give 1-N-*carboxamidogentamicin B* (72) (84 mg, 15%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 44.8; H, 7.35; N, 12.9. $C_{20}H_{39}N_5O_{11}{\cdot}0.5H_2O$ requires C, 44.93; H, 7.54; N, 13.10%), $[\alpha]_D^{26}$ +117.7° (H_2O), $\nu_{max.}$ (KBr) 3 370, 1 660, 1 550, and 1 050 cm^{-1}, δ (D_2O), 1.20 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 4.06 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 5.08 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.33 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H).

1-N-(*Ethylthiocarboxamido*)*gentamicin* C_{1a} (88).—3,2′,6′-Tris-*N*-(2,2,2-trichloroethoxycarbonyl)gentamicin C_{1a} (85) (1 g)† was dissolved in tetrahydrofuran (100 ml) and ethyl isothiocyanate (104 mg) in tetrahydrofuran (5 ml) was added over 20 min. The reaction mixture was heated under reflux for 3 h. The solution was evaporated to dryness and the residue was dissolved in 10% acetic acid in methanol (15 ml). Zinc powder (254 mg) was added and the mixture was heated under reflux for 3 h. The mixture was filtered and the filtrate was evaporated to dryness and azeotroped with toluene. 2M-Hydrochloric acid was added until the pH reached 3 and the solution was then evaporated to dryness. The residue was dissolved in water and passed over Amberlite IRA 40IS (OH^-) resin and the aqueous eluate was evaporated to dryness. The residue was chromatographed on a silica-gel column (160 × 1.5 cm) using chloroform–methanol–3% ammonium hydroxide solution (1 : 2 : 1 v/v) as the eluant to give 1-N-(*ethylthiocarboxamido*)*gentamicin* C_{1a} (88) (72 mg, 13%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 49.1; H, 8.0; N, 14.75; S, 5.05. $C_{22}H_{44}N_6O_7S$ requires C, 49.23; H, 8.26; N, 15.66; S, 5.97%), $[\alpha]_D^{26}$ +104.1° (H_2O), $\nu_{max.}$ (KBr) 3 350, 3 275, 1 650, 1 050, and 1 020 cm^{-1}, δ (D_2O) 1.18 (3 H, t, *J* 6.5 Hz, CH_3CH_2NH), 1.21 (3 H, s, 4″-CH_3), 2.50 (3 H, s, 3″-NCH_3), 2.58 (1 H, d, $J_{2''ax,3''ax}$ 10.5 Hz, 3″*ax*-H), 3.27 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*ax*-H), 3.41 (2 H, q, *J* 6.5 Hz, CH_3CH_2NH), 4.08 (1 H, d, $J_{5''ax,5''eq}$ 12.5 Hz, 5″*eq*-H), 5.10 (1 H, d, $J_{1''eq,2''ax}$ 4 Hz, 1″*eq*-H), and 5.19 (1 H, d, $J_{1'eq,2'ax}$ 3.5 Hz, 1′*eq*-H).

3-N-(4-*Methoxybenzyloxycarbonyl*)-1-N-[2-(4-*methoxybenzyloxycarbonylamino*)*ethoxycarbonyl*]*garamine* (94).—3,2′,6′-Tris-*N*-(4-methoxybenzyloxycarbonyl)sisomicin (20) (2 g) was dissolved in methanol–water (1 : 1 v/v) (20 ml) containing *N*-[2-(4-methoxybenzyloxycarbonylamino)-ethoxycarbonyloxy]succinimide (34) (776 mg) and the reaction mixture was stirred at 25 °C for 3 h. The mixture was evaporated to dryness and the residue was dissolved in dry tetrahydrofuran (20 ml) and Amberlite IR 120 (H^+) resin (30 g) was added. The reaction was stirred at 25 °C for 7 h. The resin was filtered off and washed with methanol.

* Note as on page 2186. † Note as on page 2204.

The combined filtrates were evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 2.5 cm) using a chloroform–methanol–concentrated ammonium hydroxide solution (5 : 1 : 1 : 0.1 v/v) as the eluant to give the *garamine derivative* (94) (936 mg, 60%) as an amorphous solid (Found: C, 55.2; H, 6.35; N, 7.55. $C_{34}H_{48}N_4O_{14}$ requires C, 55.42; H, 6.57; N, 7.60%), $[\alpha]_D^{26}$ +74.0° (MeOH), $\nu_{max.}$ (KBr) 3 330, 1 700, 1 508, 1 240, and 1 025 cm^{-1}, δ (CD_3OD) 1.10 (3 H, s, 4′-CH_3), 2.52 (3 H, s, 3′-NCH_3), 3.75 (6 H, s, $CH_3OC_6H_4CH_2$), 4.98 (4 H, s, $CH_3OC_6H_4CH_2$), and 6.80 and 7.22 (8 H, 2 m, $CH_3OC_6H_4CH_2$).

1-N-(2-*Aminoethoxycarbonyl*)*garamine* (93).—3-*N*-(4-Methoxybenzyloxycarbonyl)-1-*N*-[2-(4-methoxybenzyloxycarbonylamino)ethoxycarbonyl]garamine (94) (600 mg) was dissolved in trifluoroacetic acid (3 ml) and the solution was kept at 25 °C for 3 min. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (30 × 2.5 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give 1-N-(2-*aminoethoxycarbonyl*)*garamine* (93) (143 mg, 43%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 44.9; H, 7.7; N, 12.95. $C_{16}H_{32}N_4O_8 \cdot H_2O$ requires C, 45.07; H, 8.04; N, 13.14%), $[\alpha]_D^{26}$ +98.0° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 530, 1 270, and 1 050 cm^{-1}, δ (D_2O) 1.20 (3 H, s, 4′-CH_3), 2.49 (3 H, s, 3′-NCH_3), 2.52 (1 H, d, $J_{2'ax,3'ax}$ 11 Hz, 3′*ax*-H), 2.82 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 3.24 (1 H, d, $J_{5'ax,5'eq}$ 12.5 Hz, 5′*ax*-H), 3.65 (1 H, dd, $J_{1'eq,2'ax}$ 4, $J_{2'ax,3'ax}$ 11 Hz, 2′*ax*-H), 4.05 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), 4.07 (1 H, d, $J_{5'ax,5'eq}$ 12.5 Hz, 5′*eq*-H), and 5.07 (1 H, d, $J_{1'eq,2'ax}$ 4 Hz, 1′*eq*-H).

(±)-1(3)-N-(4-*Methoxybenzyloxycarbonyl*)-2-*deoxystreptamine* (97).—2-Deoxystreptamine (96) (10 g) was dissolved in methanol–water (1 : 1 v/v) (200 ml) containing 4-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (9.38 g) [43] and the mixture was stirred at 25 °C for 1 h. Additional reagent (4.7 g) was added and the mixture was stirred for a further 1 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 3 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the (±)-*mono*-N-(4-*methoxybenzyloxycarbonyl*) *derivative* (97) (14 g, 70%) as an amorphous solid (Found: C, 53.9; H, 6.75; N, 8.45. $C_{15}H_{22}N_2O_6 \cdot 0.5H_2O$ requires C, 53.64; H, 7.05; N, 8.34%), $\nu_{max.}$ (KBr) 3 320, 1 670, 1 240, and 1 035 cm^{-1}, δ ([$^2H_6$]-DMSO) 4.00 (3 H, s, $CH_3OC_6H_4CH_2$), 5.07 (2 H, s, $CH_3OC_6H_4CH_2$), and 6.98 and 7.38 (4 H, m, $CH_3OC_6H_4CH_2$).

(±)-1(3)-N-(2-*Aminoethoxycarbonyl*)-2-*deoxystreptamine* (99).—(±)-1(3)-*N*-(4-Methoxybenzyloxycarbonyl)-2-deoxystreptamine (97) (500 mg) was dissolved in methanol–water (1 : 1 v/v) (5 ml) containing *N*-[2-(4-methoxybenzyloxycarbonylamino)ethoxycarbonyloxy]succinimide (34) (420 mg) and the reaction mixture was stirred at 25 °C for 3 h. The solution was evaporated to dryness and the residue was dissolved in trifluoroacetic acid (2 ml). After 3 min at 25 °C, the solution was evaporated to dryness. The residual gum was extracted with diethyl ether and the insoluble residue was dissolved in water and Amberlite IRA 40IS (OH^-) resin was added until the pH reached 10. The resin was filtered off and washed with water. The combined filtrates were evaporated to dryness and the residue was chromatographed on a silica-gel column (60 × 2 cm) using the lower phase of a chloroform–methanol–concentrated ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give the (±)-N-(2-*aminoethoxycarbonyl*)*deoxystreptamine* (99) (91 mg, 24%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 58.65; H, 5.15; N, 10.6. $C_9H_{19}N_3O_5 \cdot H_2O$ requires C, 58.90; H, 5.46; N, 10.85%), $\nu_{max.}$ (KBr) 3 330, 1 690, 1 540, and 1 020 cm^{-1}, δ (D_2O) 1.21 (1 H, ddd, $J_{1ax,2ax} = J_{2ax,2eq} = J_{2ax,3ax}$ = 12 Hz, 2*ax*-H), 1.95 (1 H, ddd, $J_{1ax,2eq} = J_{2eq,3ax}$ = 4 Hz, $J_{2ax,2eq}$ = 12 Hz, 2*eq*-H), 2.88 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$), and 4.07 (2 H, t, *J* 6 Hz, $OCH_2CH_2NH_2$).

(±)-1(3)-N-*Acetyl*-2-*deoxystreptamine* (100).—2-Deoxystreptamine (96) (500 mg) was dissolved in methanol–water (1 : 1 v/v) (5 ml) containing *N*-acetoxysuccinimide (410 mg) and the mixture was stirred at 25 °C for 6 h. The solution was evaporated to dryness and the residue was chromatographed on a silica-gel column (120 × 2.5 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant to give (±)-1(3)-N-*acetyl*-2-*deoxystreptamine* (100) (330 mg, 52%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 40.15; H, 8.2; N, 11.6. $C_8H_{16}N_2O_4 \cdot 2H_2O$ requires C, 39.99; H, 8.39; N, 11.66%), $\nu_{max.}$ (KBr) 3 300, 1 640, and 1 550 cm^{-1}, δ (D_2O) 1.23 (1 H, ddd, $J_{1ax,2ax} = J_{2ax,2eq} = J_{2ax,3ax}$ = 12 Hz, 2*ax*-H), 1.97 (1 H, ddd, $J_{1ax,2eq} = J_{2eq,3ax}$ = 4 Hz, $J_{2ax,2eq}$ 12 Hz, 2*eq*-H), and 2.00 (3 H, s, NAc).

(2R)-1-N-(2-*Aminobutoxycarbonyl*)-2-*deoxystreptamine* (101).—(2*R*)-3,6′-Bis-*N*-benzyloxycarbonyl-1-*N*-(2-benzyloxycarbonylaminobutoxycarbonyl)gentamicin B (65) (3.9 g) was dissolved in methanol–water (150 ml). 10% Palladium–carbon (2.3 g) and 1M-hydrochloric acid (3.9 ml) were added and the mixture was hydrogenated at 25 °C a 55 lbf in^{-2} for 18 h. Additional 10% palladium–carbon (2.3 g) was added and the hydrogenation was continued for a further 18 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was chromatographed on a silica-gel column (120 × 3 cm) using the lower phase of a chloroform–methanol–14% ammonium hydroxide solution (2 : 1 : 1 v/v) as the eluant. The principal product was rechromatographed on a silica-gel column (160 × 1.5 cm) using chloroform–methanol–concentrated ammonium hydroxide solution (3 : 4 : 2 v/v) as the eluant to give (2R)-1-N-(2-*aminobutoxycarbonyl*)-2-*deoxystreptamine* (101) (137 mg, 13%) as an amorphous solid after passage over Amberlite IRA 40IS (OH^-) resin followed by lyophilization (Found: C, 45.05; H, 7.85; N, 13.2. $C_{11}H_{23}N_3O_5 \cdot CO_2$ requires C, 44.85; H, 7.22; N, 13.08%), $[\alpha]_D^{26}$ +13.8° (H_2O), $\nu_{max.}$ (KBr) 3 350, 1 700, 1 540, 1 280, and 1 040 cm^{-1}, δ (D_2O) 0.93 (3 H, t, *J* 6.5 Hz, CH_3CH_2) and 2.00 (1 H, ddd, $J_{1ax,2eq} = J_{2eq,3ax}$ = 4, $J_{2ax,2eq}$ 12 Hz, 2*eq*-H).

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