1-*N*-ACYLATION OF GENTAMICIN C_{1a} BY A CYCLIC, CHIRAL γ-AMINO-α-HYDROXY ACID RELATED TO THE (*S*)-4-AMINO-2-HYDROXYBUTYRIC ACID

M. PHILIPPE, A. M. SEPULCHRE, S. D. GERO

Institut de Chimie des Substances Naturelles, C.N.R.S.
91190–Gif-sur-Yvette, France

H. LOIBNER, W. STREICHER and P. STUTZ

SANDOZ Forschungsinstitut,
A–123 Wien, Brunnerstraße 59, Austria

(Received for publication July 12, 1982)

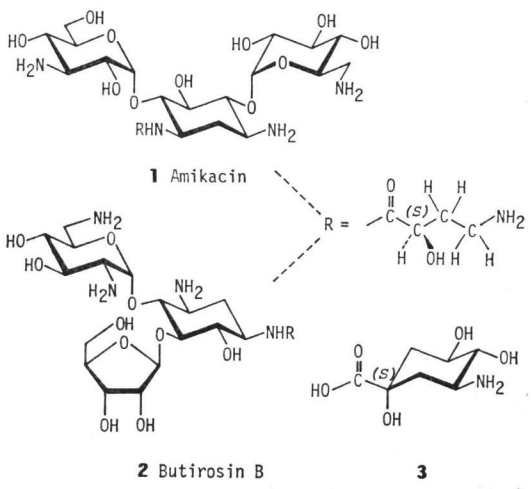
A semisynthetic aminoglycoside antibiotic **15**, containing a cyclic γ-amino-α-hydroxy acid, related to the 1-*N*-4-amino-2-hydroxybutyric acid (AHBA) side chain of butirosins and amikacin, has been prepared. Conveniently protected 3,2′,6′-tris-*N*-*tert*-butoxycarbonylgentamicin C_{1a} (**12**) was condensed with the phtalimido active ester **10** to give after catalytic reduction and deprotection, the hitherto unknown 1-*N*-substituted gentamicin C_{1a} **15**. The requisite side chain was synthesized from the readily available D-(−)-quinic acid. The antibacterial properties of **15** are given.

The design of the novel semisynthetic aminoglycoside amikacin (**1**) as a consequence of the discovery of the naturally occurring antibiotic butirosin B (**2**) (Fig. 1) is well documented[1~3]. The discovery of this clinically useful drug constitutes a breakthrough in the fight against aminoglycoside resistant strains[4].

In general, the hitherto most successful strategy to improve the chemotherapeutic properties of naturally occurring aminoglycoside antibiotics has been the selective acylation or alkylation of the 1-amino group at the 2-deoxystreptamine ring[5~11].

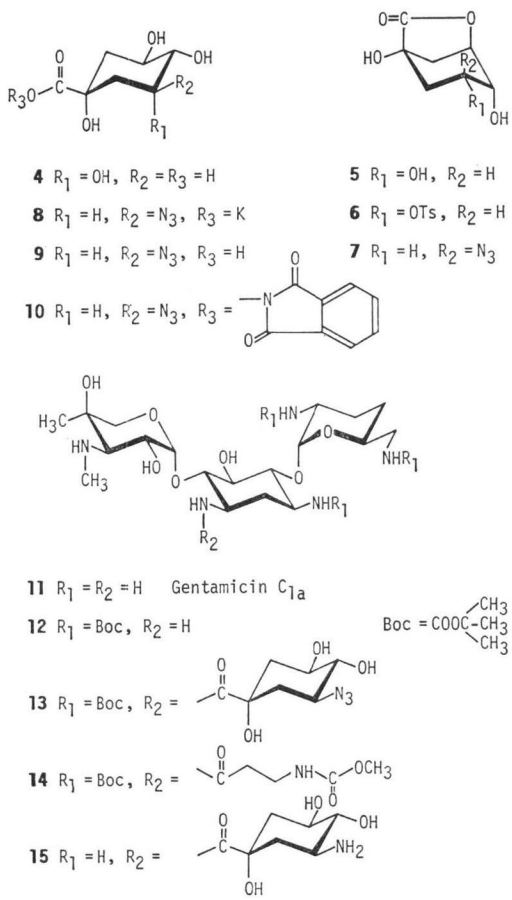
A recent report[12] claiming that the AHBA side chain of butirosin B (**2**) and amikacin (**1**) could also be replaced by cyclic-γ-amino-α-hydroxy acids led us to investigate the usefulness of 1L-1(OH), 4/3,5-3-amino-1,4,5-trihydroxycyclohexanecarboxylic acid (**3**) as a substitute. We report herein the synthesis and biological evaluation of the corresponding 1-substituted gentamicin C_{1a} derivative (**15**). The target aminohydroxy acid **3** was obtained in few steps from the commercially available D-(−)-quinic acid (**4**) (Fig. 2).

Fig. 1



Thus, D-(−)-quinic acid (**4**), when refluxed in *N*,*N*-dimethylformamide, in the presence of Amberlite IR-120 (H^+) resin, under azeotropic removal of water, was readily transformed into the corresponding lactone[13] **5** in 80% yield. Selective tosylation of the latter **5**, at the equatorial

Fig. 2



hydroxyl group, was achieved in excellent yield, using 1.1 equivalent of *p*-toluenesulfonyl chloride in dry pyridine at low temperature. The structure of the monotosylate **6** was confirmed by ^{13}C NMR spectroscopy. Azidolysis of **6** in *N*,*N*-dimethylformamide at 120°C over 24 hours, furnished the azido-lactone **7**. The latter, on treatment with potassium hydroxide in a methanol-water mixture, was transformed into its potassium salt **8** from which, the parent acid **9**, precursor of the desired 1-*N*-acyl side chain, was easily regenerated using a cation exchange resin.

Although numerous published methods have been reported for the selective *N*-acylation of aminoglycosides[14~18], we preferred to use the appropriately protected gentamicin C_{1a} derivative **12** having only the hindered 3″-methylamino and the 1-amino group free for acylation. Selective protection of gentamicin C_{1a} (**11**) was carried out under conditions similar to those described by NAGABHUSHAN[19].

When the coupling reaction of **9** and **12**, was performed using *N*-hydroxysuccinimide-dicyclohexylcarbodiimide in one pot, we obtained the β-alanine derivative **14**, instead of the desired product **13**. It is known that *N*-hydroxysuccinimide and dicyclohexylcarbodiimide can form the *N*-hydroxysuccinimide ester of 3-isocyanopropionic acid[20] which obviously then, reacted with **12** and the methanol used as solvent, to yield **14**.

The azido acid **9** was therefore converted first into its phthalimide ester **10** and then, condensed *in situ* with the 3,2′,6′-tris-*N*-Boc-gentamicin C_{1a} (**12**), to give the desired intermediate **13** in 85% yield. Reaction occurred exclusively, as anticipated, with the 1-amino group. Catalytic hydrogenation of the azide **13**, followed by deprotection using trifluoroacetic acid, led to the target molecule **15**, isolated as the sulfate.

Confirmation of the structure of the desired new 1-*N*-acylgentamicin C_{1a} (**15**) was obtained from a study of its chemical ionization mass and ^{13}C NMR spectra. The mass spectrum of the per-*N*-acetylated derivative of **15** obtained by chemical ionization using isobutane[21], exhibited a molecular ion peak (MH^+) of very weak intensity at *m*/*z* 833 and structurally significant peaks corresponding to the fragment ions A, B and C at *m*/*z* 417, 213 and 205 respectively (Fig. 3).

The ^{13}C NMR spectra of the derivative **15** and the parent gentamicin C_{1a} (**11**) were measured under acidic conditions. The chemical shifts (Table 1) were assigned by comparison with those reported in the literature for gentamicin C_{1a} (free base)[22] taking into account both the known β-protonation shift and *N*-acylation effects[23~25].

Table 1. ^{13}C NMR data of **11** and **15** in D_2O at acidic pH with dioxane as an internal reference ($\delta_{TMS} = \delta_{diox.} + 67.4$).

	11	**15**		**11**	**15**
C-1	49.5[a]	50.2[a]	C-1′	95.5	94.4
C-2	28.5	31.8	C-2′	50.5[a]	50.2[a]
C-3	49.3[a]	50.2[a]	C-3′	21.7[b]	22.5
C-4	77.5	78.8	C-4′	26.2	26.8
C-5	75.2	76.1[b]	C-5′	66.1[c]	67.4 (diox.)
C-6	84.4	80.8	C-6′	43.4	44.1
C-1″	102	99.4	C-1‴		177.8
C-2″	67.1[c]	67.4 (diox.)	C-2‴		75.8
C-3″	64.1	65.8	C-3‴		37.1
C-4″	70.7	71.4	C-4‴		52[a]
C-5″	68.7	68.5	C-5‴		76.4[b]
C-CH_3	21.2[b]	21.9	C-6‴		70.6
N-CH_3	35.3	36.6	C-7‴		40.6

[a,b,c] Assignments may be reversed.

Fig. 3

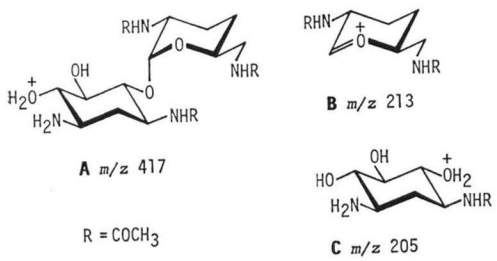


Table 2. *In vitro* comparison of minimal inhibitory concentration (μg/ml) of gentamicin C_{1a} (**11**) and the 1-*N*-substituted gentamicin C_{1a} derivative **15**.

	11	**15**
S. aureus ATCC 10832	0.078	0.062
S. aureus ATCC 29067	0.625	0.625
S. faecalis NCIB 775	1.25	10
P. aeruginosa ATCC 29511	3.12	50
E. coli	1.56	5
P. mirabilis NCIB 60	1.56	10
E. coli (ANT 2″) JR 66	100	10
E. coli (AAC 3) JR 89	250	10
P. mirabilis (AAC 6′)	250	50

Significant chemical shift differences, between **15** and **11**, are observed only for carbons C-6 and C-2 in the 2-deoxystreptamine ring. This observation clearly indicated that the side chain in **15**, is located on the 1-amino group. As shown by the value of the C-6 chemical shift in gentamicin C_{1a} (**11**) (84.4 ppm) and in its *N*-acyl derivative **15** (80.8 ppm), the resulting substituent effect (−3.6 ppm) under acidic conditions, is less marked than reported for the free base (−7 ppm)[24]. This is compatible with no β-protonation shift observed for C-6, in the case of 1-*N*-acylation[24].

Additional corroborative evidence for structure **15** is also provided by its C-2 chemical shift. As expected C-2, in **15** (31.8 ppm) resonates at lower field than in gentamicin C_{1a} (**11**) (28.5 ppm). This is principally due to the fact that, in the 1-*N*-acyl derivatives, the usual β-protonation shift (−8 ppm) observed for C-2 in unsubstituted aminoglycosides, decreases to approximatively −4.5 ppm (protonation of the 3-amino group only). The 1-*N*-acylation effect exerted on C-2 is reported as a moderate upfield shift (−1 ppm)[24].

The *in vitro* microbiological properties of 1-*N*-[1 L-1(OH), 4/3,5-3-amino-1,4,5-trihydroxycyclohexanecarbonyl]gentamicin C_{1a} (**15**) and of the parent gentamicin C_{1a} (**11**) are indicated in Table 2.

The novel 1-*N*-acyl derivative **15** is less active against gentamicin sensitive Gram-negative microor-

ganisms than gentamicin C_{1a}, but has some improvements against gentamicin resistant bacteria. These results are in good agreement with previously well established data[5~11] on the 1-*N*-substitution of aminocyclitol antibiotics, in terms of their resistance toward modifying enzymes.

Experimental

Melting points were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured on a "Quick" Roussel and Jouan polarimeter. IR spectra were obtained on a Perkin-Elmer 297 spectrometer. ^{13}C NMR spectra were obtained on a Bruker WP-60 (15.08 MHz) or HX-90 (22.63 MHz) instruments. Chemical shifts (δ) are reported with reference to tetramethylsilane. Mass spectra were recorded on either an A.E.I. MS-9 or an A.E.I. MS-50 spectrometer. TLC was performed on Schleicher and Schüll plastic backed silica gel plates (F 1500 S 254); the plates were initially examined under UV light (254 and 366 nm) then developed with appropriate spray reagents. Column chromatography was effected, under low pressure, using Merck Kieselgel (Type 60) and the eluant given in parentheses. Evaporations were carried out at below 40°C using a Büchi rotary evaporator.

The microanalyses were performed in the analytical department of the I.C.S.N., Gif sur Yvette.

1L-1(OH),3,4/5-Tetrahydroxycyclohexanecarboxylic 1,5-Lactone (**5**)

A mixture of D-(−)-quinic acid (50 g), Amberlite 1R-120 (H^+), benzene (50 ml) and *N*,*N*-dimethylformamide (200 ml) was heated under reflux with azeotropic removal of water (Dean-Stark apparatus) for 15 hours with stirring. The resin was filtered off; the filtrate was evaporated to dryness, to give the lactone (**5**): (44 g, 90% yield) mp 187°C, $[\alpha]_D^{23}$ +18° (*c* 1, H_2O), IR 1760 cm^{-1} (lactone)[13]. ^{13}C NMR (D_2O) 76.6 (C-1), 41.1 (C-2), 71 (C-3), 67.3 (C-4), 75.8 (C-5), 37.7 (C-6), 178.8 (C-7).

1L-1(OH),3,4/5-1,4,5-Trihydroxy-3-tosyloxycyclohexanecarboxylic 1,5-Lactone (**6**)

A solution of *p*-toluenesulfonyl chloride (12 g, 1.1 equiv.) in pyridine (20 ml) was added dropwise, to a solution of lactone **5** (10 g) in dry pyridine (50 ml), at 0°C. After the addition was complete, the mixture was kept over 10 hours at 0°C and then poured into ice-water containing 1 M hydrochloric acid and extracted with ethyl acetate. The organic phase was dried with magnesium sulfate and evaporated *in vacuo* to dryness. The residue was chromatographed on a silica gel column, using chloroform - ethanol (95: 5 v/v) as the eluant, to give **6** as a solid (15 g, 78% yield) which crystallized from ethyl acetate - hexane (2: 1), mp 120~121°C, $[\alpha]_D^{23}$ −31° (*c* 1, ethanol), IR 1600 cm^{-1} (tosyl), 1760 cm^{-1} (lactone). ^{13}C NMR ($CDCl_3$-CD_3OD) 71.7 (C-1), 36.3 (C-2), 76.2 (C-3), 64.3 (C-4), 76.2 (C-5), 36.3 (C-6), 177.2 (C-7).

Anal. Calcd. for $C_{14}H_{16}O_7S$: C 51.22, H 4.91, S 9.76.
Found: C 51.47, H 4.96, S 9.86.

1L-1(OH),4/3,5-3-Azido-1,4,5-trihydroxycyclohexanecarboxylic 1,5-Lactone (**7**)

A stirred mixture of the tosylate **6** (6 g), sodium azide (5 g, 4 equiv.) and *N*,*N*-dimethylformamide (100 ml) was heated at 120°C for 24 hours. The solvent was evaporated *in vacuo* and the residue then poured into ice-water and extracted with ethyl acetate. The organic layer was evaporated and the solid residue was recrystallized from ethyl acetate - hexane (2: 1) to give the azide **7** (2.7 g, 75% yield), mp 173~174°C, $[\alpha]_D^{23}$ +26° (*c* 1, CH_2Cl_2), IR 2100 cm^{-1} (azide), 1760 cm^{-1} (lactone). ^{13}C NMR (D_2O) 72.4 (C-1), 37.4 or 36.9 (C-2), 60.8 (C-3), 66.1 (C-4), 78.2 (C-5), 36.9 or 37.4 (C-6), 180.9 (C-7).

Anal. Calcd. for $C_7H_9N_3O_4$: C 42.21, H 4.55, N 21.00
Found: C 42.31, H 4.73, N 20.98

1L-1(OH),4/3,5-3-Azido-1,4,5-trihydroxycyclohexanecarboxylic Acid (**9**)

A mixture of the azido-lactone **7** (3 g) and aqueous potassium hydroxide (30 ml, 1.1 equiv.) was stirred at room temperature; after 10 minutes, Amberlite IR-120(H^+) (6 g) was added to convert the potassium salt **8** into the free acid **9** and the stirring was continued at 20°C for 30 minutes. The resin was filtered off and the aqueous filtrate was lyophilized to give the pure title compound **9** (3.2 g, 98% yield), mp 218~220°C, $[\alpha]_D^{23}$ +16° (*c* 1, H_2O). ^{13}C NMR of **8** (D_2O) 75.5 (C-1), 38.9 (C-2), 62.3 (C-3), 78.9 (C-4), 70.6 (C-5), 41.2 (C-6), 181.7 (C-7).

Anal. Calcd. for $C_7H_{11}N_3O_5 \cdot 2H_2O$: C 33.20, H 5.92, N 16.60
Found: C 33.06, H 5.90, N 16.45.

3,2′,6′-Tris-*N*-*tert*-butoxycarbonyl-1-*N*-[1L-1(OH),4/3,5-3-azido 1,4,5-trihydroxycyclohexanecarbonyl]gentamicin C_{1a} (**13**)

To an ice cooled solution of the azide **9** (1 g) in *N*,*N*-dimethylformamide (20 ml) were added *N*-hydroxyphtalimide (0.83 g) and dicyclohexylcarbodiimide (1.2 g). After stirring for 3 hours, to the mixture, was added a solution of **13** (3.5 g) in *N*,*N*-dimethylformamide and the stirring was continued for an additional 3 hours. The precipitated dicyclohexylurea was filtered off and the filtrate was evaporated to dryness. Column chromatography on silica gel using chloroform - methanol - conc. ammonium hydroxide (15: 4: 1) as the eluant, afforded the protected-1-*N*-acylated gentamicin C_{1a} **13** which was recrystallized from chloroform-ether (3.8 g, 85% yield), mp 182~184°C, $[\alpha]_D^{23}$ +38° (*c* 1, CH_2Cl_2).

Anal. Calcd. for $C_{41}H_{72}N_8O_{17}\cdot 2H_2O$: C 49.98, H 7.77, N 11.37
Found: C 49.70, H 7.72, N 11.20

1-*N*-[1L-1(OH),4/3,5-3-amino-1,4,5-trihydroxycyclohexanecarbonyl]gentamicin C_{1a} (**15**)

A solution of the azide (**13**) (0.4 g) in methanol (12 ml) was hydrogenated in the presence of Adams catalyst (50 mg) overnight. After removal of the catalyst, the filtrate was concentrated to dryness (400 mg) and the residue was dissolved in trifluroacetic acid (3 ml). After 2 minutes at 25°C, the trifluoroacetate salt of **15** was precipitated with ether and isolated by filtration. The target compound **15** was obtained as an amorphous solid, after passage over Amberlite IR-45 (OH^-) resin followed by lyophilization.

The latter free base (250 mg) was dissolved in dry methanol (15 ml) and 1N sulfuric acid was added until the pH reached 3. Then the solution was carefully poured into ice-cold, vigorously stirred ether (30 ml) and the stirring continued over 15 minutes.

Final isolation of the precipitated sulfate salt of **15** was achieved by centrifugation (0.32 g, 90%), mp 150~152°C, $[\alpha]_D^{23}$ +95° (*c* 1.2, H_2O).

Anal. Calcd. for $C_{26}H_{58}N_6O_{23}S_3$: C 34.06, H 6.17, O 40.12
Found: C 34.25, H 6.47, O 40.04

Acknowledgment

We thank H. HILDEBRANDT for the evaluation of biological data.

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