Design and Synthesis of Aminoglycoside Antibiotics to Selectively Target 16S Ribosomal RNA Position 1408

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The glucopyranosyl moiety (ring I) of paromomycin was modified in a search for novel aminoglycoside antibiotics. The key intermediates were the 4′,6′-*O*-benzylidenated *N*-Boc derivative **3** and the azido analogue **18**. The bromobenzoates **4** and **19** were prepared by treating the benzylidene acetals **3** and **18**, respectively, with *N*-bromosuccinimide (NBS), and the diol **8** was obtained by hydrogenolysis of **3**. The *C(6′)*-deoxy derivative **5** was obtained from **4** by treatment with Bu_3SnH. Selective fluorodehydroxylation of **8** gave the fluoro derivative **9**. The pseudotrisaccharide **13** was obtained by reductive fragmentaion of the iodo compound **12** obtained from the bromobenzoate **4**. The 3′,6′-anhydro derivative **20** was obtained upon deacetylation of **19**. Standard deprotection gave the *C(6′)*-deoxy compound **7**, the fluoro compound **11**, the pseudotrisaccharide **15**, and the 3′,6′-anhydro-paromomycin **22**. As compared to paromomycin, the *C(6′)*-deoxy and fluorodeoxy derivatives **7** and **11** showed a lower activity against both wild type 1408A and 1408G mutant ribosomes. A lower activity was also found for the 3′,6′-anhydro derivative **22** and for the pseudotrisaccharide **15**.

Introduction. – Aminoglycoside antibiotics (AGA), such as kanamycin A and B, tobramycin, gentamicin C1, C2, C1a, and neomycin B, interact with functional sites of ribosomal RNA (rRNA) [1–4]. The cross-species conservation of these functional sites of rRNA limits the selectivity of aminoglycoside antibiotics and leads to toxicity [5]. The search for AGAs that exploit the subtle differences between prokaryotic and eukaryotic rRNA is thus of crucial importance [5][6], and the recently determined crystal structure of some aminoglycoside antibiotics in complex with the eubacterial decoding site of rRNA [7–10] provides useful information for the design of aminoglycoside antibiotics that may selectively recognise prokaryotic rRNA.

Paromomycin is a representative 4,5-disubstituted 2-deoxystreptamine aminoglycoside antibiotic (*Fig. 1*). It is not administrated systemically because of its poor therapeutic index [11]. According to previous studies, rings I and II of paromomycin are mainly responsible for drug binding [8][9][12].

The crystal structure of the bacterial decoding site in complex with paromomycin shows that the glucopyranosyl ring I of paromomycin intercalates into the bulge formed by A1408, A1492, A1493, and the base pair C1409–G1491 [9] (*Fig. 2*). Thus, ring I is stacked on top of the purine ring of G1491. This orientation allows ring I to form a pseudo base pair with A1408 characterised by H-bonds from C(6′)–OH to N(1) of A1408, and from N(6) of A1408, to C(5′)–O (*Fig. 3*). In addition, C(3′)–OH and C(4′)–OH form H-bonds to the phosphate groups of the two flipped-out adenosines 1492 and 1493, and thereby stabilise the location of ring I. Ring III and ring IV of paromomycin reach down the stem towards the base pairs 1409–1491 and 1410–1490, allowing C(5″)–OH of ring III to form a H-bond with N(7) of G1491.

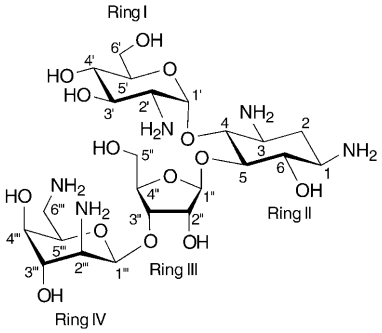

Fig. 1. *Paromomycin*: *numbering of the rings and the atoms*

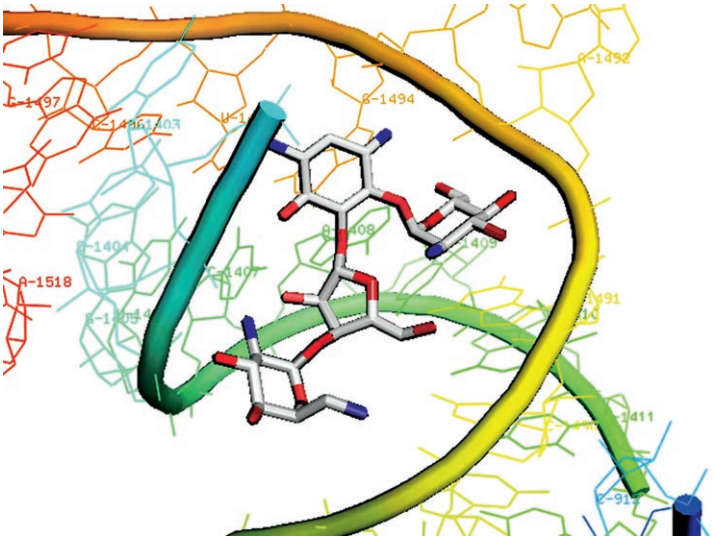

Fig. 2. *Three-dimensional structure of the bacterial decoding site in complex with paromomycin*

We assume that the toxicity of AGAs is due to the structural similarity between eukaryotic mitochondrial and bacterial ribosomes, both possessing an adenosine base at position 1408 [5]. This assumption is based on the observation *i*) that alteration of 1408 adenine to guanine confers high-level drug resistance to AGAs in bacteria [13][14], and *ii*) that cytoplasmic ribosomes of eukaryotes, which are naturally resistant to AGAs, carry a 1408 guanine [5][15]. The H-bond of C(6′)–OH of ring I of paromomycin (and presumably similarly of C(6′)–NH_3^+ of neomycin) to A1408, as suggested by the above mentioned crystal structure, may well be mainly responsible for the interaction of paromomycin with this base. This consideration suggested to modify ring I and particularly to deoxygenate C(6′) of paromomycin. As the removal of C(6′)–OH is expected to reduce the activity of paromomycin, this loss of activity will have to be compensated by introducing new binding sites. We planned to replace the C(6′)–OH group by a H-atom and also by a F-atom. These substitutions will affect the two important interactions of ring I with A1408, *viz*., the H-bond between C(6′)–OH

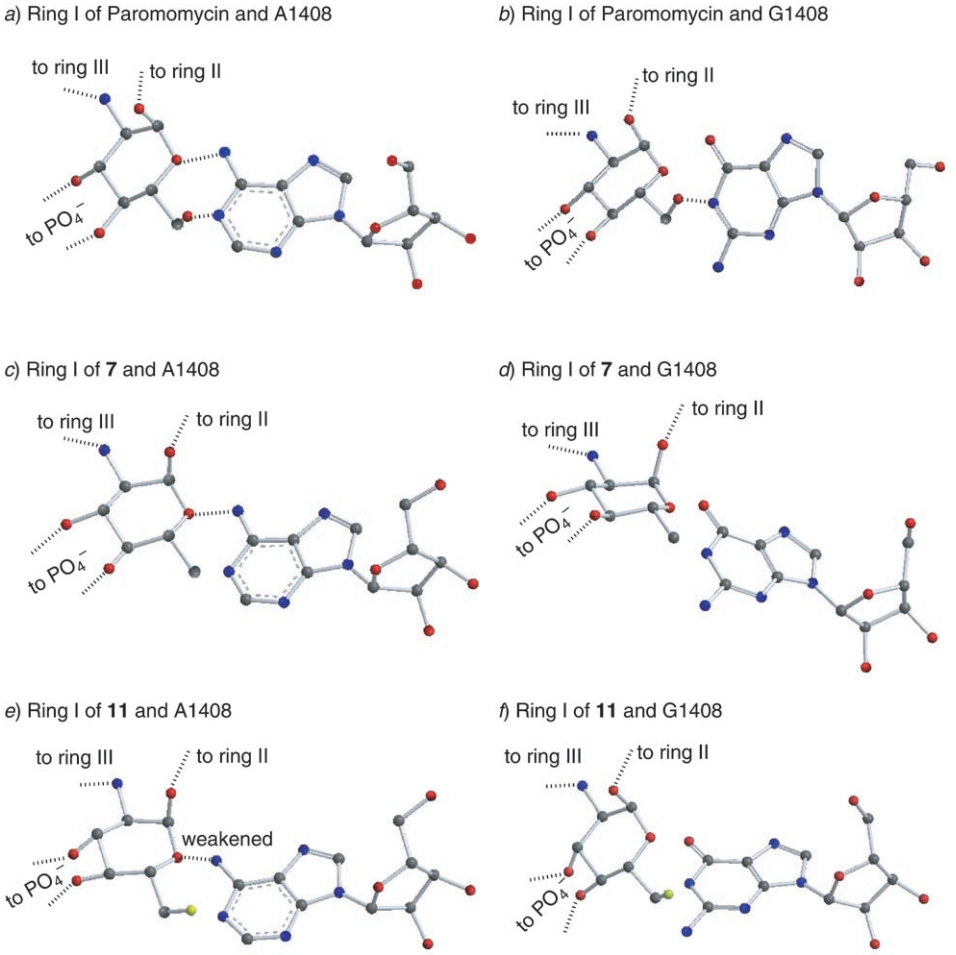

Fig. 3. *H-Bonding interactions* (hashed bonds): a) *between Ring I and A1408* (as seen in the crystal structure); b) *between Ring I and G1408* (presumed if the position of paromomycin remains unchanged); c)–f) *between Ring I of derivative* **7** *and* **11**, *and A1408 and G1408* (presumed if the position of derivatives remains the same as paromomycin in the decoding site)

and N(1) of A1408, and the H-bond from H–N(6) of A1408 to C(5′)–O. Reductive deoxygenation of C(6′)–OH will abolish the interaction of paromomycin with N(1) of A1408; removing the σ-acceptor OH substituent will also make C(5′)–O a better H-bond acceptor for H–N(6) of A1408. Replacement of OH at C(6′) by F will also abolish the H-bond to N(1)–A1408; it will, however, also tend to weaken the H-bond from N(6) of A1408 to C(5′)–O. To further evaluate these and additional interactions of ring I with the 16S decoding site, we also planned to invert the conformation of ring I by forming the 3′,6′-anhydro derivative, and to completely remove ring I. *Hanessian et al.* [16] removed ring I by periodate cleavage, followed by β-elimination. The moderate yield (46%) and the time required (2 days) for this transformation

prompted us to evaluate a new method. On the basis of the above reasoning, we expected the deoxy and fluorodeoxy analogues to be less active, and to preferentially bind to 1408A *vs.* 1408G ribosomes, while the pseudotrisaccharide and the anhydro derivative should be even less active but conceivably differentiate between 1408A and 1408G.

Results and Discussion. – Treatment of paromomycin monohydrate monosulfate (**1**) with $(Boc)_2O$ and Et_3N yielded 64% of the pentacarbamate **2**, that was benzylidenated and acetylated to give the fully protected derivative **3** (79%; *Scheme 1*) [16]. The 1,3-dioxane ring of **3** was cleaved by treatment with NBS [17], and the resulting bromobenzoate **4** (55%) was reductively debrominated with Bu_3SnH in the presence of 1,1′-azobis[cyclohexanecarbonitrile] to provide the *C(6′)*-deoxy derivative **5** (72%) that was deacetylated (0.02N MeONa in MeOH), and then the Boc groups were removed with CF_3COOH/anisole/H_2O [18][1]). These conditions led to 77% of the *C(6′)*-deoxy paromomycin **7** as the pentakis(trifluoroacetate).

Hydrogenolytic debenzylidenation of **3** led to the diol **8** (68%) that was regioselectively fluorinated at C(6′) by treatment with diethylaminosulfur trifluoride (DAST; *Scheme 2*). The resulting fluoro alcohol **9** (67%) was deacetylated to **10** (92%), and then the Boc groups were removed, similarly as described above for the transformation of **6** into **7**. This sequence provided **11** as the pentakis(trifluoroacetate) (65%).

Ring I of paronomycin was removed by reductive fragmentation [19] (*Scheme 3*). The intermediate bromobenzoate **4** was transformed into the iodo derivative **12** that was treated with activated Zn in i-PrOH/H_2O 95:5 at 90° to provide the pseudotrisaccharide **13** (76% from consumed **4**; 73% conversion). Deprotection of **13** *via* **14** was accomplished similarly as described above for **7** and **11** to yield 63% of **15** after conversion to the free base and then to the tetraacetate salt.

The difficulties associated with a thorough interpretation of the NMR spectra of the Boc-protected derivatives prompted us to study some of the above transformations with the corresponding azido derivatives.

The diazo transfer to the paromomycin sulfate **1** (TfN_3, $CuSO_4 \cdot 5\ H_2O$) [20][21] in the presence of Et_3N, $EtN(i\text{-}Pr)_2$, or pyridine led to the pentaazide **16** in at best 33% yield; the yield reported for the analogous transformation of neomycin B sulfate (82%) [22] was never reached (*Scheme 4*). However, diazo transfer (TfN_3, $CuSO_4 \cdot 5\ H_2O$ and Et_3N) to paromomycin (free base) proceeded in a higher yield, providing 53% of **16**. A similar yield (*ca.* 49%) was obtained by azidation of paromomycin sulfate **1** (and of paromomycin) in the presence of *t*-BuOK. The pentaazide **16** was benzylidenated to **17** (75%). Acetylation of **17** gave the fully protected hexaacetate **18** (79%). Regioselective opening of the 1,3-dioxanyl ring of **18** with NBS in CCl_4 in the presence of $BaCO_3$ gave the bromobenzoate **19** (67%). As expected [23], reductive fragmentation to remove ring I of the azide **19** failed, while the 3′,6′-anhydro derivative **20** was readily formed upon deacetylation with K_2CO_3 in aqueous MeOH. The anhydro derivative **20** was further characterised by transformation into the hexaacetate **21**. A sample of

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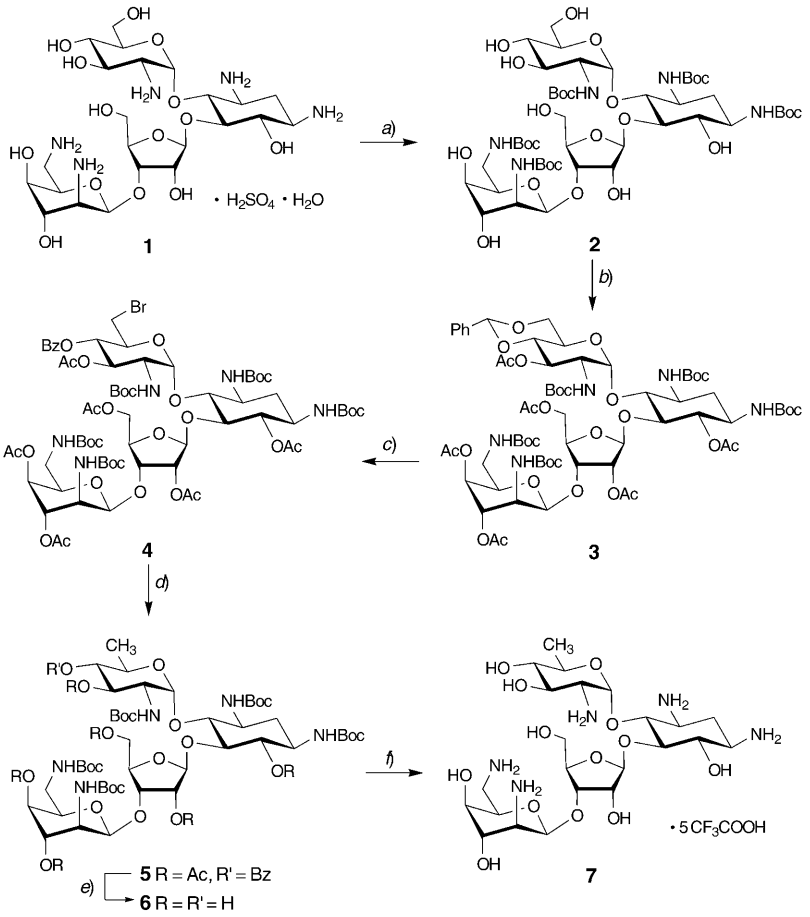
[1]) Several standard conditions that were tried for the removal of the *N*-Boc groups such as treatment of **6** with 2N HCl resulted in partial glycoside cleavage.

Scheme 1

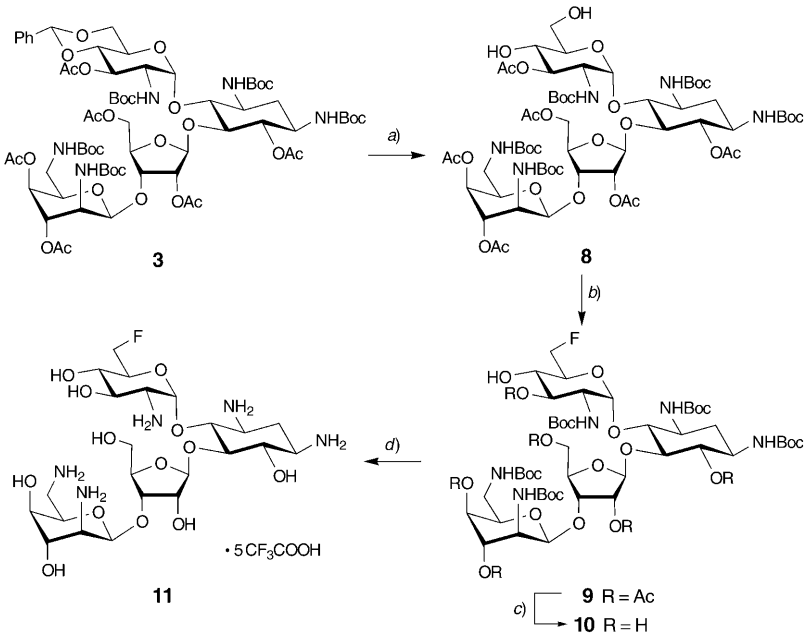


a) $(Boc_2)O$, Et_3N, $MeOH/H_2O$ 1:1, 26°; 64%. *b*) PhCHO, HCOOH, −5 to 0°; then Ac_2O, pyridine, 4-(dimethylamino)pyridine (DMAP), 26°; 79% from **2**. *c*) $BaCO_3$, *N*-bromosuccinimide (NBS), CCl_4, 80°; 55%. *d*) Bu_3SnH, 1,1′-azobis[cyclohexanecarbonitrile], toluene, 80°; 72%. *e*) 0.02N MeONa, MeOH, 26°; 88%. *f*) CF_3COOH/anisole/H_2O 90:3:7, 26°; 77%.

20, obtained by deacetylation of **21**, was hydrogenated in the presence of 10% Pd/C in AcOH to yield, after column chromatography, 47% of **22** as the pentaacetate.

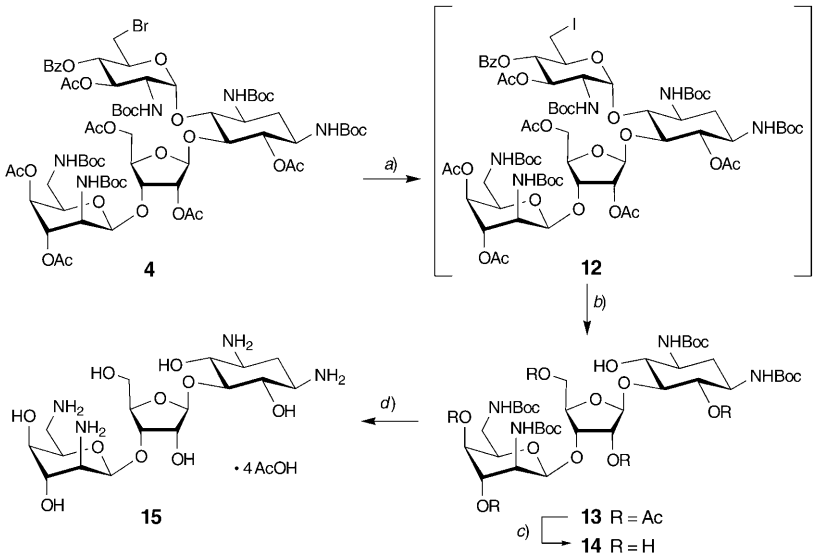
A comparison of the ^{1}H-NMR spectra of the *N*-Boc-protected derivatives, the azides, and the free amines showed that the ribofuranose ring consistently prefers the (*N*)-conformation ($J(1'',2'')=0-3.4$, $J(3'',4'')=4.8-5.2$ Hz, see *Tables* 2–5 in the *Exper. Part*). The idopyranosyl ring of all compounds adopts predominantly the $^4C_1$ conformation ($J(2''',3''')=J(3''',4''')<3.5$ Hz). The unambiguous assignment of H–C(4‴) is based on a DQFCOSY spectrum of **11** showing a *W*-coupling ($^4J(4''',2''')=1.5$ Hz) that confirms the $^4C_1$ conformation of the idopyranosyl ring. The Me(6′) group of **5** was evidenced by a *d* at 1.15 ppm ($J=6.2$ Hz), and confirmed by the ^{13}C(6′) signal at 17.66 ppm and the disappearance of the CH_2 signal at 32.54 ppm. The ^{19}F-NMR spectrum of the fluoro alcohol **9** showed a signal at −233.52 ppm characteristic of CH_2F. The CH_2F group was further evidenced by a *dd* at 82.11 ppm ($^1J(C,F)=172.1$ Hz) in the ^{13}C-NMR spectrum. The ^{1}H-NMR spectrum of the

Scheme 2



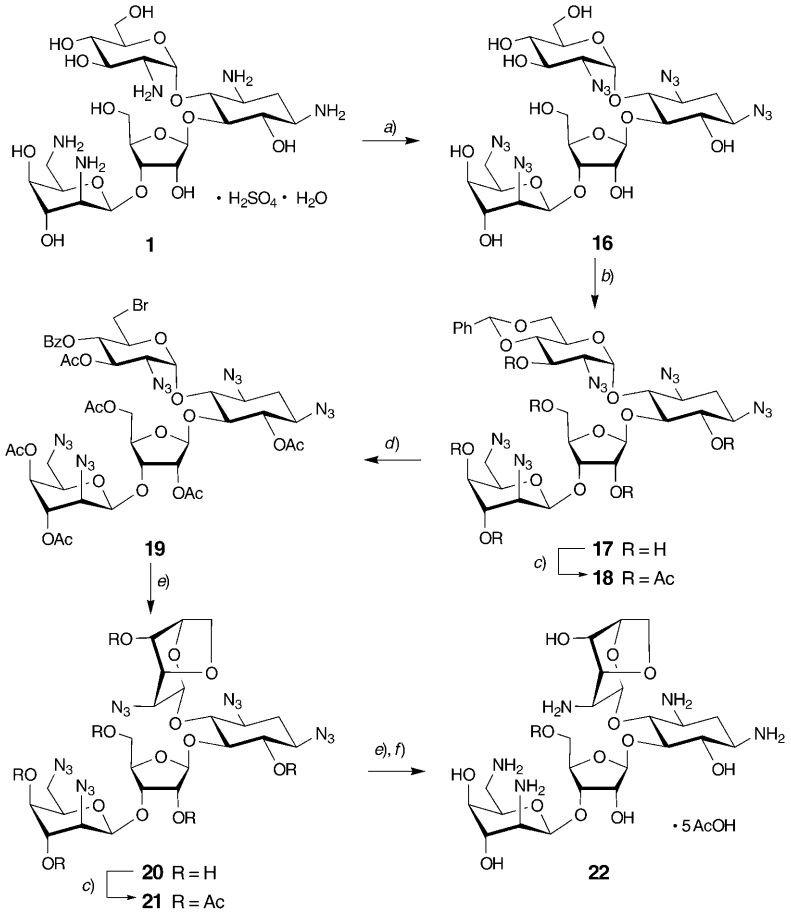
a) H_2, 10% Pd/C, 80% aq. AcOH, 26°; 68%. *b*) Diethylaminosulfur trifluoride (DAST), CH_2Cl_2, −55 to 26°; 67%. *c*) 0.02N MeONa, MeOH, 26°; 92%. *d*) CF_3COOH/anisole/H_2O 90:3:7, 26°; 65%.

Scheme 3



a) NaI, butan-2-one, 80°. *b*) Zn, i-PrOH/H_2O 95:5, 90°; 56% from **4** (73% conversion). *c*) 0.02N MeONa, MeOH, 26°; 78%. *d*) 1. CF_3COOH/anisole/H_2O 90:3:7, 26°; 92%; 2. 50% aq. AcOH; 88%.

Scheme 4



a) TfN_3, *t*-BuOK, *t*-BuOH/H_2O 2:1, 26°; 49%. *b*) PhCHO, HCOOH, −5 to 0°; 75%. *c*) Ac_2O, pyridine, DMAP, 26°; 79%. *d*) $BaCO_3$, NBS, CCl_4, 80°; 67%. *e*) K_2CO_3, MeOH, 26°; 100%. *f*) H_2, 10% Pd/C, 1,4-dioxane/H_2O/AcOH 2:2:0.1, 26°; 47%.

deacetylated fluoro alcohol **10** in (D_6)acetone clearly showed $^2J(H,F) = 49.5$ Hz for the H−C(6′) signal at 4.64 ppm. The CH_2F group of the deprotected pentakis(trifluoroacetate) **11** is evidenced by a *td* in the H-coupled ^{19}F-NMR spectrum at −230.70 ppm ($J = 50.1$ and 26.4 Hz). The $^1C_4$ conformation of the 3′,6′-anhydro derivative **21** is evidenced by the small $J(4',5') = 2.6$, $J(5',6'a) = 2.7$, and $J(5',6'b) = 0.0$ Hz in the ^{1}H-NMR spectrum (*cf.* [24]).

Biological Studies. – The experimental model used to investigate ribosomal drug susceptibility is a single rRNA allelic derivative of the *Gram*-positive eubacterium *Mycobacterium smegmatis* [13]. Genetic manipulations of its single rRNA operon using site-directed mutagenesis and recA-mediated gene conversion result in homogeneous populations of mutant ribosomes [25][26]. Ribosomal drug susceptibility was studied by determination of minimal inhibitory concentrations as described in detail in [26].

The deoxy and fluorodeoxy derivatives **7** and **11** proved to be 16–32 times less active than paromomycin (see *Table 1*). Unexpectedly, their activity against *M. smegmatis* 1408A → G mutant ribosomes also proved lower (16 times) than the one of paromomycin. The loss of activity towards the wild type and the A1408G mutant may be rationalised by postulating a H-bond interaction of C(6′)–OH with A1408 and G1408, *viz.* a H-bond from C(6′)–OH to N(1) of A1408 (wild type), and one from H–N(1) of G1408 to C(6′)–O. A second H-bond may be formed from H–N(6) of A1408 to C(5′)–O. Acetals are known as relatively poor H-bond acceptors [27], but the partial deprotonation of C(6′)–OH by N(1) of A1408 should strengthen the H-bond of N(6) of A1408 to C(5′)–O. No similar H-bond can be formed to G1408 as long as positioning of the aminoglycoside and the relevant nucleobases remains unchanged. This consideration means that there should be a noticeable difference between the deoxy derivative **7** and the fluorodeoxy derivative **11** with respect to interaction with 1408A *vs.* 1408G ribosomes, with **7** forming a stronger H-bond to H–N(6) of A1408 than **11**. That this is not the case suggests that this H-bond is weak and/or that the position of the aminoglycosides is affected by the substitution of C(6)–OH.

Table 1. *Ribosomal Drug Susceptibility*[a])

	Wild type 1408A	Mutant 1408A → G
Paromomycin	1	32–64
Compound **7**	32	512–1024
Compound **11**	16–32	512
Compound **15**	>512	>512
Compound **22**	>512	>512

[a]) Ribosomal drug susceptibility is given as determination of minimal inhibitory concentrations (*MIC* [μg/ml]).

The still lower activity of the 3′,6′-anhydro derivative **22** (512 times less active than paromomycin) indicates that the interaction of H_2N–C(2′), HO–C(3′), and HO–C(4′) of paromomycin is significant and may prove important for the selectivity.

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Experimental Part

General. Solvents were distilled: THF from Na and benzophenone, CH_2Cl_2, MeOH, pyridine, Et_3N from CaH_2. Reactions were carried out under N_2, unless stated otherwise. Qual. TLC: precoated silica-gel glass plates (*Merck* silica gel *60* F_{254}); detection by heating with 'mostain' (400 ml of 10% H_2SO_4 soln., 20 g of $(NH_4)_6Mo_7O_{24}\cdot 6\,H_2O$, 0.4 g of $Ce(SO_4)_2$). Flash chromatography (FC): silica gel *Fluka 60* (0.04–0.063 mm). M.p.: uncorrected. Optical rotations: 1-dm cell at 25°, 589 nm. FT-IR Spectra: KBr or *ca.* 2% soln. in $CHCl_3/CH_2Cl_2$ or ATR, absorption in cm^{-1}. ^1H- and ^{13}C-NMR spectra: chemical shifts δ in ppm rel. to TMS as external standard, and coupling constants *J* in Hz. HR-ES-MS or HR-MALDI-MS: in gentisic acid (=2,5-dihydroxybenzoic acid, DHB) matrix.

*1,3,2′,2‴,6‴-Pentakis{*N*-[(*tert*-butoxy)carbonyl]}paromomycin*[2]) (**2**). A soln. of **1** (*Fluka*; 1 g, 1.40 mmol) in H_2O (20 ml), was treated dropwise with MeOH (20 ml) and then with Et_3N (1.9 ml, 13.6 mmol) and di(*tert*-butyl) dicarbonate (2.4 ml, 10.4 mmol), and stirred at 26° for 18 h. After evaporation at 40°/70 Torr, a soln. of the residue in AcOEt (25 ml) was washed with brine (2 × 10 ml), dried ($MgSO_4$), filtered, and evaporated. FC ($CHCl_3$/AcOEt/MeOH 10:17.5:4) gave **2** (1.1 g, 64%). White solid. M.p. 190–193.5°. R_f ($CHCl_3$/AcOEt/MeOH 4:2.5:0.7) 0.35. $[\alpha]_D^{25} = +40.4$ ($c = 0.08$, MeOH). IR (KBr): 3411*s*, 2978*m*, 2933*m*, 1691*s*, 1517*s*, 1456*m*, 1393*m*, 1368*s*, 1284*m*, 1251*m*, 1167*s*, 1043*s*, 949*w*, 862*w*, 781*w*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz, at 100°): 6.28 (*d*, $J = 8.3$), 6.16 (br. *t*, $w_{1/2} \approx 13.0$), 6.07 (*d*, $J = 7.1$), 5.91 (*d*, $J = 9.6$), 5.68 (*d*, $J = 9.7$) (5 NH); 5.03 (*d*, $J = 3.2$, H–C(1′)); 4.96 (*d*, $J = 4.5$), 4.88 (*d*, $J = 4.7$), 4.45 (*d*, $J = 4.8$), 4.34 (*d*, $J = 4.6$), 4.32–4.26 (br. *s*), 4.27 (*d*, $J = 4.9$), 4.10 (*d*, $J = 3.9$) (7 OH); 4.99 (*d*, $J = 2.7$, H–C(1″)); 4.76 (*d*, $J = 2.0$, H–C(1‴)); 4.04–3.96 (*m*, 2 CH, 1 OH)); 3.82 (*td*, $J \approx 5.5$, 3.6, H–C(4″)); 3.75 (*td*, $J = 6.8$, 1.4, H–C(5‴)); 3.72–3.67 (*m*, 2 H); 3.63–3.50 (*m*, 5 H); 3.48–3.42 (*m*, 3 H); 3.33–3.15 (*m*, 5 H); 3.11 (br. *t*, $J = 6.5$, 4 H); 1.77 (*dt*, $J = 12.8$, 4.2, H_{ax}–C(2)); 1.35–1.24 (*m*, H_{eq}–C(2)); 1.35 (*s*, 9 H), 1.34 (*s*, 9 H), 1.333 (*s*, 9 H), 1.330 (*s*, 18 H) (5 *Me*$_3$C). ^{13}C-NMR (CD_3OD, 75 MHz): see *Table 2*; additionally, 157.77, 157.35, 157.28, 157.06, 156.86 (5*s*, 5 C=O); 79.60 (2 C), 79.37 (2 C), 79.12 (3*s*, 5 Me$_3$*C*); 27.82, 27.70, 27.66 (3*q*, 5 *Me*$_3$C). HR-MALDI-MS: 1138.5423 (35, $[M+Na]^+$, $C_{48}H_{85}N_5NaO_{24}^+$; calc. 1138.5483), 1038.4916 (73, $[M-CO_2CMe_3+H+Na]^+$, $C_{43}H_{77}N_5NaO_{22}^+$; calc. 1038.4958), 938.4379 (99.7, $[M-2\,CO_2CMe_3+2\,H+Na]^+$, $C_{38}H_{69}N_5NaO_{20}^+$; calc. 938.4434), 838.3862 (100, $[M-3\,CO_2$-

Table 2. *^{13}C-NMR Chemical Shifts* [ppm] *of Compounds* **2**–**6**, **8**–**10**, **13**, *and* **14**

	2	**3**	**4**	**5**	**6**	**8**	**9**	**10**	**13**	**14**
Solvent	CD_3OD	$CDCl_3$	$CDCl_3$	$CDCl_3$	CD_3OD	$CDCl_3$	$CDCl_3$	(D$_6$)acetone	$CDCl_3$	CD_3OD
C(1′)	97.96	98.48	97.45	98.46	99.30	98.80	98.47	100.15	–	–
C(2′)	55.88	53.48	52.73	53.27	57.14	53.10	52.56	56.89	–	–
C(3′)	71.67	70.57	70.94	70.93	72.31	73.13	71.54	72.47[a])	–	–
C(4′)	70.93	80.24	69.25	74.39[b])	74.07[b])	70.36	68.58[d])	70.28[e])	–	–
C(5′)	73.41[b])	63.51	71.89	66.74	68.56	74.21[b])	74.37[d])	75.57[e])	–	–
C(6′)	61.81	68.59	32.54	17.66	18.10	62.74	82.11[d])	82.53[e])	–	–
C(1)[a])	52.35	50.45	49.94	50.77	53.21	51.14[b])	50.64	53.22	51.19	53.28
C(2)	34.68	34.41	34.84	34.42	35.47	34.26	34.49	35.70	34.15	35.38
C(3)[a])	51.07	49.12	49.20	48.97	52.01	49.18	49.16	51.76	49.25	52.20
C(4)	77.05[c])	76.15[b])	76.02[b])	81.23	79.80	75.94[b])	79.50	79.42	73.03[b])	74.34
C(5)	86.39	82.46	84.44	82.82	87.43	81.82	82.97	87.80	85.14	83.32
C(6)	73.23	74.59[b])	73.92[b])	76.58[b])	77.24[b])	75.68[a])	76.09[b])	72.65[b])	73.08[b])	74.34
C(1″)	109.22	108.21	108.23	106.67	110.41	106.40	107.04	110.81	107.52	109.18
C(2″)	74.45	74.26[b])	74.36[b])	74.40[b])	75.62[b])	75.06[b])	75.77[b])	72.62[b])	74.48	75.19
C(3″)	78.29[c])	77.27	78.61	74.09[b])	77.93	77.27	77.24	80.97	76.99	77.56
C(4″)	82.29	79.63	79.57	79.58	83.11	80.67	80.26	83.72	79.50	82.87
C(5″)	62.18	62.23	61.38	62.17	63.34	62.74	62.20	63.49	62.14	60.92
C(1‴)	99.15	99.82	97.84	99.65	99.98	99.37	98.91	100.85	97.80	99.57
C(2‴)[a])	50.53	48.95	48.76	48.97	51.08	49.07	48.94	51.76	48.89	52.20
C(3‴)	70.43	69.35	69.25	69.27	71.25	69.27	69.31	70.92	69.30	71.30
C(4‴)	67.72	66.68	66.60	66.59	69.53	66.58	66.60	68.19	66.57	68.67
C(5‴)	74.45[b])	73.16	73.11	73.16	75.22[b])	73.13	73.12	73.60	74.15	76.12
C(6‴)	41.02	40.69	40.67	40.72	41.49	40.71	40.70	41.02	40.48	41.49

[a]) Assignments of C(1), C(3), and C(2‴) may be interchanged. [b])[c]) Assignments may be interchanged. [d]) $^3J(4',F) = 6.7$, $^2J(5',F) = 18.2$, $^1J(6',F) = 172.1$ Hz. [e]) $^3J(4',F) \approx 6.0$, $^2J(5',F) = 21.9$, $^1J(6',F) = 177.8$ Hz.

[2]) For convenience, trivial names are used. The systematic name for paromomycin is 2,6-diamino-2,6-dideoxy-*β*-D-idopyranosyl-(1 → 3)-*β*-D-ribofuranosyl-(1 → 5)-[2-amino-2-deoxy-*α*-D-glucopyranosyl-(1 → 4)-1L-(1,3,5/2,4)-1,5-diaminocyclohexane-2,3,4-triol].

CMe_3 + 3 H + Na]$^+$, $C_{33}H_{61}N_5NaO_{18}^+$; calc. 838.3910), 738.3337 (81, [*M* − 4 CO_2CMe_3 + 4 H + Na]$^+$, $C_{28}H_{53}N_5NaO_{16}^+$; calc. 738.3385), 638.2817 (48, [*M* − 5 CO_2CMe_3 + 5 H + Na]$^+$, $C_{23}H_{45}N_5NaO_{14}^+$; calc. 638.2861). Anal. calc. for $C_{48}H_{85}N_5O_{24}$ (1116. 21): C 51.65, H 7.68, N 6.27; found: C 51.37, H 7.75, N 6.07.

6,3′,2″,5″,3‴,4‴-Hexa-O-*acetyl-4′,6′*-O-*benzylidene-1,3,2′,2‴,6‴-pentakis[*N-*[(*tert-*butoxy)carbonyl]]paromomycin* (**3**). Under Ar, a soln. of **2** (3.01 g, 2.7 mmol) in freshly distilled PhCHO (39 ml, 0.38 mol) was cooled to 0°, treated portionwise with HCOOH (5.1 ml, 0.13 mol) during 10 min, and stirred at −5 to 0° for 24 h (incomplete conversion even on prolonged reaction time). The mixture was neutralized at 0° by portionwise addition of sat. $NaHCO_3$ soln. and then extracted with AcOEt (5 × 30 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), and evaporated. FC (60 g of silica gel; 200 ml of hexane and 300 ml of hexane/AcOEt 9 : 1 → elution of excess PhCHO; 500 ml of AcOEt gave the benzylidene acetal of **2**). It was dissolved in pyridine (8.4 ml, 0.10 mol), treated with Ac_2O (4.9 ml, 0.05 mol) and 4-(dimethylamino)pyridine (DMAP; 354 mg, 2.9 mmol), stirred at r.t. for 18 h, diluted with CH_2Cl_2 (20 ml), and washed with cold 0.5N HCl (10 ml). The aq. layer was again extracted with CH_2Cl_2 (3 × 10 ml). The combined CH_2Cl_2 layers were washed with sat. $NaHCO_3$ soln. (10 ml), brine (10 ml), dried ($MgSO_4$), and evaporated. FC (AcOEt/cyclohexane 9 : 11) gave **3** (3.1 g, 79%). White solid. M.p. 168.6–173.9°. R_f (AcOEt/cyclohexane 2 : 1) 0.66. $[\alpha]_D^{25}$ = +39.5 (*c* = 0.29, MeOH). IR (CH_2Cl_2): 3438*w*, 3054*m*, 2984*w*, 1746*s*, 1716*s*, 1507*s*, 1421*m*, 1368*s*, 1234*s*, 1163*s*, 1041*s*, 896*m*, 861*w*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz, at 100°): 7.39–7.36 (*m*, 2 arom. H); 7.33–7.30 (*m*, 3 arom. H); 6.48 (*d*, *J* = 8.5), 6.32 (br. *t*, $w_{1/2}$ = 15.0), 6.27 (*d*, *J* = 8.9), 5.69 (*d*, *J* = 10.1), 5.15 (*d*, *J* = 9.3) (5 NH); 5.59 (*s*, PhC*H*); 5.47 (*d*, *J* = 4.0, H–C(1′)); 5.20 (*d*, *J* = 2.4, H–C(1″)); 5.01 (*t*, *J* = 10.0, H–C(6)); 4.92 (*dd*, *J* = 5.1, 2.4, H–C(2″)); 4.91 (*t*, *J* = 3.2, H–C(3‴)); 4.69 (*d*, *J* = 2.3, H–C(1‴)); 4.67 (*t*, *J* ≈ 10.0, H–C(3′)); 4.65 (br. *s*, H–C(4‴)); 4.32 (*dd*, *J* = 9.9, 5.0, H_{eq}–C(6′))); 4.26 (*t*, *J* ≈ 5.0, H–C(3″)); 4.25 (*dd*, *J* = 12.0, 3.8, H_a–C(5″)); 4.10 (*dd*, *J* = 11.8, 5.1, H_b–C(5″)); 4.08 (*t*, *J* ≈ 10.4, H–C(4)); 4.05–3.93 (*m*, H–C(5′), H–C(4″), H–C(2‴), H–C(5‴)); 3.85 (*td*, *J* = 10.1, 4.1, H–C(2′)); 3.70 (*t*, *J* = 9.7, H–C(5)); 3.66 (*t*, *J* = 10.1, H_{ax}–C(6′)); 3.64–3.48 (*m*, H–C(1), H–C(3), H–C(4′)); 3.21 (*dt*, *J* = 13.7, 7.0, H_a–C(6‴)); 3.06 (*ddd*, *J* = 13.5, 6.2, 4.5, H_b–C(6‴)); 2.07, 2.06, 2.042, 2.04, 2.03, 2.02 (6*s*, 6 AcO); 1.75 (*dt*, *J* = 13.2, 4.4, H_{eq}–C(2)); 1.63 (*q*, *J* ≈ 12.2, H_{ax}–C(2)); 1.39, 1.38, 1.37, 1.368, 1.33 (5*s*, 5 *Me*$_3$C). ^{13}C-NMR ($CDCl_3$, 100 MHz): see *Table 2*; additionally, 171.31, 170.96, 170.48, 170.32, 169.19, 168.47 (6*s*, 6 Me*C*=O); 156.04, 155.40, 155.23, 154.95, 154.87 (5*s*, 5 NHC=O); 137.12 (*s*); 128.88 (*d*, 2 C); 128.03 (*d*, 2 C); 126.27 (*d*); 101.41 (*d*, Ph*C*H); 28.43 (3 C), 28.32 (3 C), 28.28 (6 C), 28.22 (3 C) (4*q*, 5 *Me*$_3$C); 21.05, 20.95, 20.84, 20.74 (2 C), 20.38 (5*q*, 6 *Me*C=O). HR-MALDI-MS: 1478.6397 (50, [*M* + Na]$^+$, $C_{67}H_{101}N_5NaO_{30}^+$; calc. 1478.6429), 1378.5932 (62, [*M* − CO_2CMe_3 + H + Na]$^+$, $C_{62}H_{93}N_5NaO_{28}^+$; calc. 1378.5905), 1278.5291 (83, [*M* − 2 CO_2CMe_3 + 2 H + Na]$^+$, $C_{57}H_{85}N_5NaO_{26}^+$; calc. 1278.5380), 1178.4927 (100, [*M* − 3 $COOC(CH_3)_3$ + 3 H + Na]$^+$, $C_{52}H_{77}N_5NaO_{24}^+$; calc. 1178.4856), 1078.4325 (54, [*M* − 4 $COOC(CH_3)_3$ + 4 H + Na]$^+$, $C_{47}H_{69}N_5NaO_{22}^+$; calc. 1078.4332), 978.3800 (24, [*M* − 5 $COOC(CH_3)_3$ + 5 H + Na]$^+$, $C_{42}H_{61}N_5NaO_{20}^+$; calc. 978.3808). Anal. calc. for $C_{67}H_{101}N_5O_{30}$ (1456. 55): C 55.25, H 6.99, N 4.81; found: C 55.51, H 6.86, N 4.73.

6,3′,2″,5″,3‴,4‴-Hexa-O-*acetyl-4′*-O-*benzoyl-6′-bromo-1,3,2′,2‴,6‴-pentakis[*N-*[(*tert-*butoxy)carbonyl]]-6′-deoxyparomomycin* (**4**). Under N_2, a soln. of **3** (200 mg, 0.14 mmol) in dry CCl_4 (3 ml, dried over P_2O_5 and stored over 3-Å molecular sieves) was treated with $BaCO_3$ (157 mg, 0.79 mmol; *Fluka*) and NBS (38.5 mg, 0.22 mmol, freshly crystallized from H_2O) and heated to 80° for 25 min. Upon increasing the temp., the colour of the mixture changed from colourless to off-white, pale yellow, orange, and again to pale yellow. The mixture was cooled to 26° and evaporated (40°/220 Torr). The residue was suspended in AcOEt, and the insoluble material was filtered off. The filtrate was washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 9 : 11) gave **4** (117 mg, 55%). White solid, which was recrystallized in Et_2O. M.p. 145.3–151.9°. R_f (AcOEt/cyclohexane/$CHCl_3$ 4 : 3 : 1) 0.35. $[\alpha]_D^{25}$ = +20.5 (*c* = 0.19, $CHCl_3$). IR (CH_2Cl_2): 3437*w*, 3059*w*, 2982*w*, 2927*w*, 1745*s*, 1718*s*, 1601*w*, 1507*s*, 1454*w*, 1421*w*, 1368*s*, 1278*w*, 1267*w*, 1234*s*, 1164*s*, 1041*s*, 861*w*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz, at 100°): 7.88 (*dd*, *J* = 8.4, 1.3, 2 arom. H); 7.64 (*tt*, *J* = 7.4, 1.3, 1 arom. H); 7.48 (br. *t*, *J* = 8.1, 2 arom. H); 6.42 (*d*, *J* = 8.4), 6.31 (br. *s*, 3 H), 5.82 (*d*, *J* = 10.0) (5 NH); 5.57 (*d*, *J* = 4.1, H–C(1′)); 5.23 (*d*, *J* = 2.4, H–C(1″)); 5.19 (*t*, *J* = 9.2, H–C(4′)); 5.14 (*t*, *J* = 9.8, H–C(3′)); 4.93 (*dd*, *J* = 5.2, 2.4, H–C(2″)); 4.91 (*t*, *J* = 3.6, H–C(3‴)); 4.70 (*d*, *J* = 2.0, H–C(1‴)); 4.69 (*t*, *J* ≈ 9.8, H–C(6)); 4.67 (*t*, $w_{1/2}$ = 6.6, H–C(4‴)); 4.36 (*dt*, *J* ≈ 9.2, 2.0, H–C(2‴)); 4.29 (*dd*, *J* ≈ 6.0, 5.2, H–C(3″)); 4.26 (*dd*, *J* ≈ 11.8, 4.0, H_a–C(5″)); 4.11 (*dd*, *J* = 12.0, 4.8, H_b–C(5″)); 4.09 (*t*, *J* ≈ 9.2, H–C(4)); 4.02 (*td*, *J* ≈ 7.2, 1.2, H–C(5‴)); 3.97 (*dt*, *J* = 6.4, 4.0, H–C(4″)); 3.88 (*td*, *J* = 9.8, 4.4, H–C(2′)); 3.83 (*dd*, *J* = 11.6, 3.2, H_a–C(6′)); 3.69–3.54 (*m*, H–C(1), H–C(3), H–C(5′)); 3.66 (*t*, *J* ≈ 10.0, H–C(5)); 3.51 (*dd*, *J* = 11.6, 3.2, H_b–C(6′)); 3.21 (*dt*, *J* = 13.7, 6.9, H_a–C(6‴)); 3.06 (*ddd*, *J* = 13.5, 6.2, 4.5, H_b–C(6‴)); 2.08, 2.07, 2.05, 2.04, 2.03, 1.78 (6*s*, 6 *Me*CO); 1.75 (*dt*, *J* = 13.2, 4.4, H_{eq}–C(2)); 1.63 (*q*, *J* = 12.2, H_{ax}–C(2)); 1.39, 1.385 (2*s*, 2 *Me*$_3$C); 1.377 (*s*, 2 *Me*$_3$C); 1.37 (*s*, *Me*$_3$C). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 2*; additionally, 171.14, 170.76, 170.14 (2 C), 168.99, 168.27 (5*s*, 6 Me*C*=O);

165.23 (*s*, Ph*C*=O); 155.80, 155.06, 154.88, 154.69 (2 C) (4*s*, 5 NHC=O); 135.53 (*d*); 129.78 (*d*, 2 C); 128.57 (*s*); 128.36 (*d*, 2 C); 79.80, 79.57, 79.44, 79.33, 79.13 (5*s*, 5 Me_3C); 28.48, 28.43 (2*q*, 2 *Me*$_3$C); 28.30 (*q*, 2 *Me*$_3$C); 26.93 (*q*, *Me*$_3$C); 21.02 (2 C), 20.89, 20.80, 20.52, 20.46 (5*q*, 6 *Me*C=O). HR-MALDI-MS: 1558.5542 (100, $[M+Na]^+$, $C_{67}H_{100}{}^{81}BrN_5NaO_{30}^+$; calc. 1558.5514), 1556.5547 (76, $[M+Na]^+$, $C_{67}H_{100}{}^{79}BrN_5NaO_{30}^+$; calc. 1556.5534), 1458.5097 (53, $[M-CO_2CMe_3+H+Na]^+$, $C_{62}H_{92}{}^{81}BrN_5NaO_{28}^+$; calc. 1458.4989), 1456.5103 (35, $[M-CO_2CMe_3+H+Na]^+$, $C_{62}H_{92}{}^{79}BrN_5NaO_{28}^+$; calc. 1456.5010), 1358.4577 (31, $[M-2\,CO_2CMe_3+2\,H+Na]^+$, $C_{57}H_{84}{}^{81}BrN_5NaO_{26}^+$; calc. 1358.4465), 1356.4583 (26, $[M-2\,CO_2CMe_3+2\,H+Na]^+$, $C_{57}H_{84}{}^{79}BrN_5NaO_{26}^+$; calc. 1356.4486), 1258.3954 (61, $[M-3\,CO_2CMe_3+3\,H+Na]^+$, $C_{52}H_{76}{}^{81}BrN_5NaO_{24}^+$; calc. 1258.3941), 1256.3967 (61, $[M-3\,CO_2CMe_3+3\,H+Na]^+$, $C_{52}H_{76}{}^{79}BrN_5NaO_{24}^+$; calc. 1256.3961). Anal. calc. for $C_{67}H_{100}BrN_5O_{30}\cdot H_2O$ (1553.43): C 51.80, H 6.62, Br 5.14, N 4.51; found: C 51.55, H 6.32, Br 4.73, N 4.51.

*6,3′,2″,5″,3‴,4‴-Hexa-*O*-acetyl-4′-*O*-benzoyl-1,3,2′,2‴,6‴-pentakis[*N*-[(*tert*-butoxy)carbonyl]]-6′-deoxy-paromomycin* (**5**). Under N_2, a soln. of **4** (117 mg, 0.076 mmol) in toluene (0.5 ml) was treated with Bu_3SnH (45.4 µl, 0.17 mmol) and 1,1′-azobis[cyclohexanecarbonitrile] (3 mg, 0.012 mmol) in succession. The mixture was heated to 80° for 1 h (complete conversion of **4**), cooled to 26°, diluted with hexane (10 ml), and extracted with MeCN (3×10 ml). The combined MeCN layers were washed with hexane (10 ml) and evaporated. FC (AcOEt/hexane 9:11) gave **5** (80 mg, 72%). White solid. M.p. 155–157.4°. R_f (AcOEt/cyclohexane/$CHCl_3$ 4:3:1) 0.28. $[\alpha]_D^{25} = +18.7$ (c=0.18, $CHCl_3$). IR ($CHCl_3$): 3442*w*, 3369*w*, 3020*s*, 2982*m*, 2935*w*, 1743*s*, 1712*s*, 1602*w*, 1507*s*, 1454*m*, 1392*m*, 1368*s*, 1303*m*, 1235*s*, 1228*s*, 1163*s*, 1116*m*, 1041*s*, 908*w*. ^{1}H-NMR ((D_6)DMSO, 400 MHz, at 100°): 7.88 (*dd*, J=8.4, 1.3, 2 arom. H); 7.63 (*tt*, J=7.4, 1.3, 2 arom. H); 7.48 (br. *t*, J=8.0, 1 arom. H); 6.43 (*d*, J=8.6), 6.33 (br. *t*, $w_{1/2}\approx$15.0), 6.29 (*d*, J=9.4), 5.74 (*d*, J=9.7), 5.16 (*d*, J=9.6) (5 NH); 5.47 (*d*, J=3.9, H–C(1′)); 5.23 (*d*, J=2.4, H–C(1″)); 5.11 (*t*, J=9.7, H–C(6)); 4.94 (*dd*, J=5.1, 2.5, H–C(2″)); 4.91 (*t*, $J\approx$3.3, H–C(3‴)); 4.85 (*t*, J=9.7, H–C(4′)); 4.71 (*d*, J=2.0, H–C(1‴)); 4.68 (*t*, $J\approx$9.5, H–C(3′)); 4.65 (*t*, $w_{1/2}$=6.6, H–C(4‴)); 4.29 (*t*, $J\approx$5.0, H–C(3″)); 4.27 (*dd*, $J\approx$12.3, 3.9, H_a–C(5″)); 4.19 (*dq* J=9.9, 6.0, H–C(5′)); 4.11 (*dd*, J=11.8, 5.0, H_b–C(5″)); 4.08 (*t*, J=9.2, H–C(5)); 4.08–4.02 (*m*, H–C(4″), H–C(2‴)); 3.97 (*td*, $J\approx$5.8, 4.6, H–C(5‴)); 3.87 (*td*, J=10.2, 4.1, H–C(2′)); 3.67–3.50 (*m*, H–C(1), H–C(3), H–C(4)); 3.22 (*dt*, J=13.7, 6.9, H_a–C(6‴)); 3.07 (*ddd*, J=13.5, 6.2, 4.9, H_b–C(6‴)); 2.072, 2.07, 2.05, 2.047, 2.04, 2.038 (6*s*, 6 *Me*C=O); 1.75 (*dt*, J=13.2, 4.4, H_{ax}–C(2)); 1.63 (*q*, $J\approx$12.2, H_{eq}–C(2)); 1.39, 1.38 (2*s*, 2 Me_3C); 1.377, 1.36 (2*s*, 3 Me_3C); 1.15 (*d*, J=6.2, 3 H–C(6′)). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 2*; additionally, 170.71, 170.29 (2 C), 168.98, 168.28 (5*s*, 6 Me*C*=O); 165.24 (*s*, Ph*C*=O); 155.85, 155.093, 155.091, 154.78 (2 C) (4*s*, 5 NHC=O); 133.20 (*d*); 129.70 (*d*, 2 C); 129.17 (*s*); 128.29 (*d*, 2 C); 80.13, 79.84 (2 C), 79.50, 79.16 (4*s*, 5 Me_3C); 28.39 (*q*, 3 *Me*$_3$C); 28.32 (*q*, 2 *Me*$_3$C); 21.16, 21.01, 20.93, 20.80, 20.56, 20.42 (6*q*, 6 *Me*C=O); 17.66 (*q*, C(6′)). HR-MALDI-MS: 1478.6397 (100, $[M+Na]^+$, $C_{67}H_{101}N_5NaO_{30}^+$; calc. 1478.6429). Anal. calc. for $C_{67}H_{101}N_5O_{30}\cdot H_2O$ (1474.55): C 54.57, H 7.04, N 4.75; found: C 54.39, H 6.72, N 4.67.

*1,3,2′,2‴,6‴-Pentakis[*N*-[(*tert*-butoxy)carbonyl]]-6′-deoxyparomomycin* (**6**). Under N_2, a soln. of **5** (80 mg, 0.055 mmol) in 0.02N MeONa in MeOH (2 ml) was stirred at 26° for 12 h, and neutralized with *Amberlite-IR120* (H^+ form). Filtration, evaporation, and FC ($CHCl_3$/AcOEt/MeOH 10:17.5:2) gave **6** (54 mg, 88%). White solid. M.p. 188.9–195.8°. R_f ($CHCl_3$/AcOEt/MeOH 4:9:1) 0.40. $[\alpha]_D^{25} = +41.2$ (c=0.27, MeOH). IR (KBr): 3418*s*, 2979*m*, 2934*w*, 1743*s*, 1693*s*, 1515*s*, 1456*w*, 1393*m*, 1368*s*, 1283*w*, 1251*m*, 1168*s*, 1044*s*, 861*w*, 781*w*. ^{1}H-NMR ((D_6)DMSO, 300 MHz, at 100°): 6.31 (br. *d*, J=7.5, 2 H), 6.20 (*d*, J≈8.1), 6.05 (*d*, J=9.6), 5.78 (*d*, J=9.9) (5 NH); 5.17 (br. *s*, $w_{1/2}$=7.9), 5.02 (*d*, $J\approx$4.8), 4.50 (*d*, $J\approx$4.5), 4.46 (*d*, $J\approx$4.5), 4.38 (*d*, $J\approx$4.5), 4.20 (br. *s*, $w_{1/2}$=9.0), 4.07 (br. *s*, $w_{1/2}$=6.0) (7 OH); 5.05 (br. *s*, $w_{1/2}$=5.3, H–C(1′)); 4.95 (*d*, J=3.3, H–C(1″)); 4.82 (*d*, $w_{1/2}$=4.4, H–C(1‴)); 4.07 (br. *s*, $w_{1/2}$=6.0, 1 H); 3.88–3.70 (*m*, 3 H)); 3.67–3.61 (*m*, 3 H); 3.51–2.80 (*m*, 13 H); 1.84 (*dt*, $J\approx$13.5, 4.2, H_{ax}–C(2)); 1.29–1.33 (*m*, H_{eq}–C(2)); 1.42, 1.41 (2*s*, 2 Me_3C); 1.399, 1.39 (2*s*, 3 Me_3C); 1.15 (*d*, J=5.7, 3 H–C(6′)). ^{13}C-NMR (CD_3OD, 75 MHz): see *Table 2*; additionally, 158.39, 157.98 (2 C), 157.68, 157.32 (4*s*, 5 NHC=O); 80.33 (*s*, 2 Me_3C); 80.05 (*s*, 3 Me_3C); 28.72 (*q*, 2 *Me*$_3$C); 28.65 (*q*, 2 *Me*$_3$C); 28.54 (*q*, *Me*$_3$C); 18.10 (*q*, C(6′)). HR-MALDI-MS: 1122.5509 (100, $[M+Na]^+$, $C_{48}H_{85}N_5NaO_{23}^+$; calc. 1122.5533). Anal. calc. for $C_{48}H_{85}N_5O_{23}\cdot H_2O$ (1118.22): C 51.56, H 7.84, N 6.26; found: C 51.12, H 7.48, N 6.15.

1,3,2′,2‴,6‴-Pentaammonium-6′-deoxyparomomycin Pentakis(trifluoroacetate) (**7**). Under Ar, a soln. of **6** (50 mg, 0.045 mmol) in CF_3COOH/anisole/H_2O 100:3:7 (3 ml) was stirred at *ca.* 26° for 1 h and evaporated. The residue was dissolved H_2O and washed with AcOEt. The aq. layer was evaporated to give pure **7** (50 mg, 77%). M.p. 123° (dec.). R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:4:3) 0.39. $[\alpha]_D^{25} = +27.6$ (c=0.09, H_2O). IR (KBr): 3346*w*, 2909*w*, 1669*s*, 1625*m*, 1520*w*, 1433*w*, 1189*s*, 1127*s*, 1037*s*, 840*m*, 798*m*, 723*m*. ^{1}H-NMR (D_2O, 300 MHz): 5.87 (*d*, J=4.2, H–C(1′)); 5.40 (*d*, J=1.8, H–C(1″)); 5.30 (*d*, J=1.8, H–C(1‴)); 4.54 (*dd*, J=6.5, 5.1, H–C(3″)); 4.41 (*dd*, J=4.8, 1.8, H–C(2″)); 4.32 (br. *t*, $J\approx$4.9, H–C(5‴)); 4.24 (*t*, J=2.7, H–C(3‴)); 4.20–4.23 (*m*, H–C(4″)); 4.03–3.66 (*m*, H–C(3′), H–C(4′), H–C(5′), H–C(5), 2 H–C(5″), H–C(4‴)); 3.73 (*t*, J=10.8, H–C(4)); 3.60 (br. *s*, H–C(2‴)); 3.33–3.58 (*m*, H–C(1), H–C(3), H–C(2′), 2 H–C(6‴)); 3.29 (*t*,

Table 3. *^{13}C-NMR Chemical Shifts* [ppm] *of the Ammonium Salts* **7**, **11**, **15**, *and* **22** *in* D_2O

	7	**11**[a])	**15**	**22**
C(1′)	95.16	98.37	–	94.40
C(2′)	53.91	56.33	–	49.60
C(3′)	67.55	71.37	–	69.24[c])
C(4′)	68.46	70.73[b])	–	67.56[c])
C(5′)	73.37	75.15[b])	–	70.30[c])
C(6′)	16.58	84.73[b])	–	69.24
C(1)	48.57	52.40	49.95	49.51
C(2)	34.56	30.90	28.35	27.94
C(3)	49.77	51.34	49.94	48.27
C(4)	75.70	79.46	67.81[c])	75.64
C(5)	81.18	87.15	81.26	81.16
C(6)	69.82	75.14	67.32[c])	67.12[c])
C(1″)	110.15	112.81	108.56	110.18
C(2″)	74.97	76.17	72.90	75.96
C(3″)	74.23	77.80	75.30	79.10
C(4″)	84.61	83.97	82.71	83.46
C(5″)	61.90	62.73	59.98	61.20
C(1‴)	95.16	98.01	95.22	95.33
C(2‴)	50.78	53.52	50.95	50.76
C(3‴)	70.09	70.29	71.27	71.60
C(4‴)	67.27	69.95	70.39	70.80
C(5‴)	72.37	72.86	72.36	73.12
C(6‴)	40.37	43.08	40.51	40.29

[a]) Assignments based on a DQFCOSY and a HSQC spectrum. [b]) $^3J(4',F) = 6.5$, $^2J(5',F) = 17.2$, $^1J(6',F) = 167.7$ Hz. [c]) Assignments may be interchanged.

$J = 9.0$, H–C(6)); 2.50 (*dt*, $J = 12.6, 3.6$, H_{eq}–C(2)); 1.86 (*q*, $J = 12.2$, H_{ax}–C(2)); 1.33 (*d*, $J = 6.3$, 3 H–C(6′)). ^{13}C-NMR (D_2O, 75 MHz): see *Table 3*; additionally, 163.01 (*q*, ^2J(C,F) = 37.5, 5 C=O); 119.00 (*q*, ^1J(C,F) = 291.78, 5 CF_3). HR-ESI-MS: 622.2925 (18.3, $[M+Na]^+$, $C_{23}H_{45}N_5NaO_{13}^+$; calc. 622.2912), 600.3094 (99.8, $[M+H]^+$, $C_{23}H_{46}N_5O_{13}^+$; calc. 600.3092). Anal. calc. for $C_{23}H_{45}N_5O_{13} \cdot 7\ CF_3COOH$ (1397.80): C 31.79, H 3.75, N 5.01; found: C 31.99, H 3.84, N 5.37.

6,3′,2″,5″,3‴,4‴-Hexa-O-*acetyl-1,3,2′,2‴,6‴-pentakis{*N-*[(*tert-*butoxy)carbonyl]}paromomycin* (**8**). H_2 was passed into a suspension of **3** (2.8 g, 1.9 mmol) in 80% aq. AcOH (50 ml) and 10% Pd/C (1.15 g) for 7 h at 26° and atmospheric pressure. Filtration through a pad of *Celite*, evaporation, and FC (AcOEt/hexane 3 : 1) gave **8** (1.8 g, 68%). White solid. M.p. 147.8–150.0°. R_f (AcOEt/cyclohexane 3 : 1) 0.21. $[\alpha]_D^{25} = +45.7$ ($c = 0.22$, MeOH). IR (CH_2Cl_2): 3433*m*, 3375*w*, 3052*w*, 3004*w*, 2977*m*, 1744*s*, 1714*s*, 1605*m*, 1509*s*, 1454*m*, 1392*m*, 1368*s*, 1236*s*, 1165*s*, 1041*s*, 861*m*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz, at 100°): 6.26 (br. *t*, $w_{1/2} = 15$), 6.20 (*d*, $J = 12.0$), 6.17 (*d*, $J = 8.6$), 5.46 (*d*, $J = 10.1$), 5.11 (*d*, $J = 9.4$) (5 NH); 6.20 (*d*, $J = 3.4$), 5.69 (br. *t*, $w_{1/2} = 6.1$) (2 OH); 5.24 (*d*, $J = 3.9$, H–C(1′)); 5.16 (*d*, $J = 2.6$, H–C(1″)); 4.87 (*dd*, $J = 5.2, 2.7$, H–C(2″)); 4.86 (*t*, $J \approx 3.2$, H–C(3‴)); 4.75 (*dd*, $J = 10.7, 9.1$, H–C(3′)); 4.75 (br. *s*, H–C(4‴)); 4.64 (*d*, $J = 2.2$, H–C(1‴)); 4.63 (*t*, $J = 9.4$, H–C(6)); 4.59 (*t*, $J \approx 2.0$, H–C(2‴)); 4.20 (*t*, $J \approx 5.2$, H–C(3″)); 4.19 (*dd*, $J \approx 11.7, 4.2$, H_a–C(5″)); 4.05 (*dd*, $J = 11.7, 5.1$, H_b–C(5″)); 3.96–3.91 (*m*, H–C(5′), H–C(4″)); 3.87 (*t*, $J = 8.5$, H–C(5)); 3.73–3.38 (*m*, H–C(2′), H–C(4′), H_a–C(6′), H–C(1), H–C(3), H–C(4), H–C(5‴)); 3.61 (*dd*, $J \approx 12.0, 4.1$, H_b–C(6′)); 3.17 (*dt*, $J = 13.7, 6.9$, H_a–C(6‴)); 3.00 (*ddd*, $J = 13.5, 6.2, 4.9$, H_b–C(6‴)); 2.01, 2.00, 1.99, 1.98, 1.91, 1.88 (6*s*, 6 *Me*C=O); 1.71 (*dt*, $J = 13.0, 4.5$, H_{eq}–C(2)); 1.54 (*q*, $J \approx 12.2$, H_{ax}–C(2)); 1.33, 1.326, 1.322, 1.32, 1.315 (5*s*, 5 Me_3C). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 2*; additionally, 172.27, 171.21, 170.71, 170.34, 169.01, 168.36 (6*s*, 6 Me*C*=O); 155.87, 155.35, 155.00 (3 C) (3*s*, 5 NHC=O); 79.79 (2 C), 79.59 (2 C), 79.37 (3*s*, 5 Me_3C); 28.46 (*q*, 2 Me_3C); 28.35 (*q*, 2 Me_3C); 26.97 (*q*, Me_3C); 21.18, 21.08, 20.96 (2 C), 20.83, 20.45 (5*q*, 6 *Me*C=O). HR-MALDI-MS: 1390.6126 (59, $[M+Na]^+$, $C_{60}H_{97}N_5NaO_{30}^+$; calc. 1390.6116), 1290.5593 (84,

$[M-CO_2CMe_3+Na]^+$, $C_{55}H_{89}N_5NaO_{28}^+$; calc. 1290.5593), 1190.5107 (100, $[M-2\,CO_2CMe_3+Na]^+$, $C_{50}H_{81}N_5NaO_{26}^+$; calc. 1190.5067). Anal. calc. for $C_{60}H_{97}N_5O_{30}$ (1368.44): C 52.66, H 7.14, N 5.12; found: C 52.79, H 7.16, N 4.99.

6,3′,2″,5″,3‴,4‴-Hexa-O-*acetyl-1,3,2′,2‴,6‴-pentakis[*N-*[(*tert-*butoxy)carbonyl]]-6′-deoxy-6′-fluoroparomomycin* (**9**). Under Ar, a soln. of **8** (200 mg, 0.15 mmol) in CH_2Cl_2 (4 ml) was cooled to −55°, treated with freshly distilled DAST (28 µl, 0.21 mmol), stirred at −55° for 15 min and at 26° for 7 h (complete consumption of **9**), cooled to −18°, treated dropwise with MeOH (6 ml), stirred for 10 min, and evaporated. FC (AcOEt/cyclohexane 2:3) gave **9** (133 mg, 67%). White solid. M.p. 159.2–162.4°. R_f (AcOEt/cyclohexane 5:1) 0.71. $[\alpha]_D^{25}$ = +51.0 (*c* = 0.15, MeOH). IR (KBr): 3373*w* (br.), 2977*w*, 2929*w*, 1741*m*, 1712*s*, 1694*s*, 1510*s*, 1455*w*, 1395*m*, 1366*s*, 1232*s*, 1162*s*, 1115*m*, 1039*s*, 1002*m*, 861*m*. ^{1}H-NMR ((D$_6$)DMSO, 400 MHz, at 100°): 6.31–6.27 (*m*, 2 H), 6.28 (*d*, *J* = 8.6), 5.05 (*d*, *J* = 9.9) (4 NH), 5.18–5.15 (*m*, NH, OH); 5.36 (*d*, *J* = 3.3, H–C(1′)); 5.20 (*d*, *J* = 2.7, H–C(1″)); 4.92 (*t*, *J* ≈ 3.3, H–C(3‴)); 4.92–4.91 (*m*, H–C(2‴)); 4.82 (*dd*, *J* = 10.1, 9.6, H–C(3′)); 4.69 (*d*, *J* = 2.4, H–C(1‴)); 4.67 (*t*, *J* = 10.3, H–C(6)); 4.65–4.64 (*m*, H–C(3‴)); 4.55 (br. *s*, H–C(4‴)); 4.27 (*t*, *J* ≈ 5.3, H–C(3″)); 4.18 (*ddd*, *J* = 59.7, 12.0, 5.1, H_a–C(6′)); 4.17 (*ddd*, *J* = 59.7, 12.0, 3.1, H_b–C(6′)); 4.02–3.95 (*m*, H–C(5′), H–C(4″), H–C(2‴), H–C(5‴)); 3.90–3.87 (*m*, H–C(2′)); 3.82–3.79 (*m*, H–C(4′)); 3.66 (*dd*, *J* = 10.5, 3.7, H_a–C(5″)); 3.63–3.59 (*m*, H–C(4), H_b–C(5″)); 3.55–3.50 (*m*, H–C(1), H–C(3), H–C(5)); 3.20 (*dt*, *J* = 13.6, 7.0, H_a–C(6‴)); 3.05 (*ddd*, *J* = 13.5, 6.2, 4.5, H_b–C(6‴)); 2.06, 2.04, 2.038, 2.03, 2.025, 1.93 (6*s*, 6 *Me*C=O); 1.71 (*dt*, *J* = 12.9, 4.5, H_{eq}–C(2)); 1.61 (*q*, *J* ≈ 12.7, H_{ax}–C(2)); 1.38, 1.375, 1.37 (3*s*, 5 Me_3C). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 2*; additionally, 172.51, 171.32, 170.73, 170.28, 169.03, 168.35 (6*s*, 6 Me*C*=O); 155.88, 155.16, 155.06, 154.85 (2 C) (4*s*, 5 NHC=O); 80.09, 79.85, 79.73, 79.51, 79.33 (5*s*, 5 Me_3C); 28.48 (*q*, 2 Me_3C); 28.43 (*q*, 2 Me_3C); 28.34 (*q*, Me_3C); 21.16 (2 C), 20.99, 20.84 (2 C), 20.48 (4*q*, 6 *Me*C=O). HR-MALDI-MS: 1392.6083 (81, $[M+Na]^+$, $C_{60}H_{96}FN_5NaO_{29}^+$; calc. 1392.6073), 1292.5477 (100, $[M-CO_2CMe_3+Na]^+$, $C_{55}H_{88}FN_5NaO_{27}^+$; calc. 1292.5548), 1192.5024 (71, $[M-2\,CO_2CMe_3+Na]^+$, $C_{50}H_{80}FN_5NaO_{27}^+$; calc. 1192.5024). Anal. calc. for $C_{60}H_{96}FN_5O_{29}$ (1370.43): C 52.59, H 7.06, N 5.11; found: C 52.00, H 7.32, N 4.98.

*1,3,2′,2‴,6‴-Pentakis[*N-*[(*tert-*butoxy)carbonyl]]-6′-deoxy-6′-fluoroparomomycin* (**10**). Under N_2, a soln. of **9** (102 mg, 0.07 mmol) in 0.02N MeONa in MeOH (2 ml) was stirred at 26° for 12 h, neutralized with *Amberlite-IR120* (H^+ form), filtered, and evaporated. FC ($CHCl_3$/AcOEt/MeOH 10:17.5:2) gave **10** (77 mg, 92%). White solid. M.p. 101.9–109.2°. R_f ($CHCl_3$/AcOEt/MeOH 4:9:1) 0.40. $[\alpha]_D^{25}$ = +30.0 (*c* = 0.08, MeOH). IR (KBr): 3354*m*, 2977*w*, 2929*w*, 1682*s*, 1513*s*, 1454*w*, 1392*m*, 1366*s*, 1283*m*, 1248*s*, 1161*s*, 1041*s*, 1020*s*, 996*s*, 859*m*, 780*m*. ^{1}H-NMR ((D$_6$)acetone, 300 MHz): 6.38 (*d*, *J* = 11.4, 2 H), 5.99 (*d*, *J* = 9.3, 2 H), 5.89 (br. *s*) (5 NH); 5.16–5.14 (*m*, 2 H); 4.93 (br. *s*, 2 H); 4.64 (*ddd*, *J* = 2.1, 9.9, 49.5, H_a–C(6′)); 4.61 (*ddd*, *J* = 1.2, 10.5, 49.5, H_b–C(6′)); 4.20 (*dd*, *J* = 5.6, 2.4, 2 H); 4.15 (br. *s*, 1 H); 4.08–4.01 (*m*, 3 H); 3.91–3.72 (*m*, 5 H); 3.65–3.39 (*m*, 7 H); 3.22 (*m*, 1 H); 3.01–2.98 (br. *s*, 3 H); 2.09–2.04 (*m*, H_{eq}–C(2)); 1.45, 1.433, 1.43, 1.42, 1.41 (5*s*, 5 Me_3C); 1.41–1.43 (*m*, H_{ax}–C(2)). ^{13}C-NMR ((D$_6$)acetone, 75 MHz): see *Table 2*; additionally, 157.96, 157.21, 156.90, 156.51, 155.75 (5*s*, 5 NHC=O); 79.69, 79.56 (2 C), 79.10, 78.84 (4*s*, 5 Me_3C); 28.84, 28.75, 28.71, 28.64, 28.61 (5*q*, 5 Me_3C). ^{19}F-NMR ((D$_6$)acetone, 282 MHz, H-decoupled): −235.82 (*s*, F–C(6′)). HR-MALDI-MS: 1140.5417 (100, $[M+Na]^+$, $C_{48}H_{84}FN_5NaO_{23}^+$; calc. 1140.5439). Anal. calc. for $C_{48}H_{84}FN_5O_{23}$ (1118.21): C 51.56, H 7.57, F 1.70, N 6.26; found: C 51.67, H 7.50, F 1.66, N 6.11.

1,3,2′,2‴,6‴-Pentaammonium-6′-deoxy-6′-fluoroparomomycin Pentakis(trifluoroacetate) (**11**). Under Ar, a soln. of **10** (190 mg, 0.17 mmol) in CF_3COOH/anisole/H_2O 90:3:7 (10 ml) was stirred at 26° for 1 h (complete disappearance of **10** on TLC) and evaporated. A soln. of the residue in H_2O was washed with AcOEt and evaporated to afford pure **11** (132 mg, 65%). M.p. 127° (dec.). R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:4:3) 0.39. $[\alpha]_D^{25}$ = +33.2 (*c* = 0.23, H_2O). IR (KBr): 3418*s* (br.), 1682*s*, 1524*m*, 1433*m*, 1401*m*, 1205*s*, 1137*s*, 1053*m*, 1018*m*, 936*w*, 841*m*, 801*m*, 724*m*. ^{1}H-NMR (D_2O, 500 MHz, assignment based on a DQFCOSY and a HSQC spectrum): 5.91 (*d*, *J* = 4.1, H–C(1′)); 5.42 (*d*, *J* = 2.3, H–C(1″)); 5.31 (*d*, *J* = 1.7 H–C(1‴)); 4.76 (br. *dd*, *J* ≈ 3.0, ^1J(6′,F) ≈ 48.0, 2 H–C(6′)); 4.54 (*dd*, *J* = 6.8, 4.9, H–C(3″)); 4.41 (*dd*, *J* = 4.9, 2.3, H–C(2‴)); 4.33 (*ddd*, *J* = 6.5, 4.0, 1.5, H–C(5‴)); 4.25 (*t*, *J* = 3.1, H–C(3‴)); 4.23 (*ddd*, *J* = 6.8, 4.9, 3.0, H–C(4‴)); 4.03 (*t*, *J* = 9.8, H–C(4)); 3.99–3.90 (*m*, H–C(5′)); 3.98 (*dd*, *J* = 10.7, 9.3, H–C(3′)); 3.94 (*dd*, *J* ≈ 12.2, 3.3, H_a–C(5″)); 3.93 (*t*, *J* = 9.3, H–C(5)); 3.85 (*dt*, *J* = 3.3, 1.2, H–C(4‴)); 3.78 (*dd*, *J* = 12.4, 4.8, H_b–C(5″)); 3.71 (*dd*, *J* = 10.4, 9.1, H–C(6)); 3.62 (*t*, *J* = 9.5, H–C(4′)); 3.61 (*dt*, *J* = 3.0, 1.5, H–C(2‴)); 3.55 (*ddd*, *J* = 12.4, 10.3, 4.0, H–C(3)); 3.47 (*dd*, *J* = 10.7, 4.1, H–C(2′)); 3.45 (*dd*, *J* = 13.7, 6.5, H_a–C(6‴)); 3.39 (*dd*, *J* = 13.7, 4.0, H_b–C(6‴)); 3.37 (*ddd*, *J* = 12.4, 10.7, 4.3, H–C(1)); 2.50 (*dt*, *J* = 12.5, 4.2, H_{ax}–C(2)); 1.86 (*q*, *J* ≈ 12.6, H_{eq}–C(2)). ^{13}C-NMR (D_2O, 100 MHz): see *Table 3*; additionally, 165.62 (*q*, ^2J(C,F) = 35.4, 5 C=O); 119.02 (*q*, ^1J(C,F) = 291.7, 5 CF_3). ^{19}F-NMR (D_2O, 282 MHz): −73.05, −73.44, −73.52, −73.57, −74.08 (5*s*, 5 CF_3); −230.70

(*td*, J = 50.1, 26.4, F–C(6′)). HR-ESI-MS: 618.3009 (100, $[M+H]^+$, $C_{23}H_{45}FN_5O_{13}^+$; calc. 618.2998). Anal. calc. for $C_{23}H_{44}FN_5O_{13}\cdot 5\,CF_3COOH$ (1187.74): C 33.37, H 4.16, F 25.59, N 5.90,; found: C 33.51, H 4.25, F 25.54, N 6.01.

6,2″,5″,3‴,4‴-Penta-O-*acetylde(2-amino-2-deoxy-α*-D-*glucopyranosyl)-1,3,2‴,6‴-tetrakis[*N-*[(tert-butoxy)-carbonyl]]paromomycin* (**13**). Under N_2, a soln. of **4** (386 mg, 0.25 mmol) in butan-2-one (10 ml) was treated with NaI (375 mg, 2.5 mmol), heated to 80° for 12 h, cooled to r.t., diluted with AcOEt (15 ml), washed with brine, dried ($MgSO_4$), and evaporated. A soln. of the residue (crude **12**) in Me_2CHOH/H_2O 95 : 5 (18 ml) was treated with activated Zn powder (227 mg, 3.47 mmol), heated to 90° for 3 h, cooled to r.t., diluted with AcOEt (10 ml), and filtered over a *Celite* pad. The filtrate was washed with 0.5M aq. H_2SO_4, brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/cyclohexane 3 : 2) gave **12** (100 mg, 27%) and **13** (150 mg, 56%). M.p. 134.0–136.1°. R_f (AcOEt/cyclohexane 2 : 1) 0.42. $[\alpha]_D^{25} = +22.3$ (c = 0.24, $CHCl_3$). IR ($CHCl_3$): 3516*w*, 3441*w*, 3028*w*, 3013*w*, 2982*w*, 2948*w*, 2927*w*, 1744*s*, 1712*s*, 1601*w*, 1505*s*, 1454*w*, 1388*w*, 1369*s*, 1303*m*, 1225*s*, 1164*m*, 1116*m*, 1079*w*, 1042*m*, 859*w*. ^{1}H-NMR ((D_6)DMSO, 300 MHz, at 100°): 6.42 (br. *s*), 6.35 (br. *s*), 6.32 (br. *s*), 5.22 (*d*, J = 9.6) (4 NH); 5.17 (*s*, H–C(1″)); 5.00 (*d*, J = 4.8, H–C(2″)); 4.93 (*t*, $w_{1/2}$ = 6.0, H–C(4‴)); 4.78 (br. *s*, H–C(1‴)); 4.71–4.66 (*m*, 2 H); 4.40 (*t*, $J \approx 5.4$, H–C(3″)); 4.33 (*dd*, $J \approx 11.1$, 2.7, H_a–C(5″)); 4.20–4.05 (*m*, 4 H); 3.67 (*d*, $w_{1/2}$ = 19.2, 1 H); 3.50 (br. *s*, 2 H)); 3.29–2.73 (*m*, 3 H); 2.09 (*s*, AcO); 2.06 (*s*, 2 AcO); 1.98, 1.91 (2*s*, 2 AcO); 1.82 (br. *d*, J = 13.2, H_{eq}–C(2)); 1.40 (*s*, Me_3C); 1.39 (*s*, 2 Me_3C); 1.37 (*s*, Me_3C); 1.40–1.35 (*m*, H_{ax}–C(2)). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 2*; additionally, 170.83 (2 C), 170.07, 169.13, 168.39 (4*s*, 5 Me*C*=O); 155.71, 155.45, 155.04, 154.80 (4*s*, 4 NHC=O); 28.49, 28.40, 28.32, 26.98 (4*q*, 4 *Me*$_3$C); 20.92 (2 C), 20.76 (2 C), 20.55 (3*q*, 5 *Me*C=O). HR-MALDI-MS: 1087.4773 (63, $[M+Na]^+$, $C_{47}H_{76}N_4NaO_{23}^+$; calc. 1087.4798), 965.4409 (100, $[M-CO_2CMe_3+2\,H]^+$, $C_{42}H_{69}N_4O_{21}^+$; calc. 965.4454). Anal. calc. for $C_{47}H_{76}N_4O_{23}$ (1065.12): C 53.00, H 7.19, N 5.26; found: C 52.76, H 7.12, N 5.12.

De(2-amino-2-deoxy-α-D-*glucopyranosyl)-1,3,2‴,6‴-tetrakis[*N-*[(*tert-*butoxy)carbonyl]]paromomycin* (**14**). Under N_2, a soln. of **13** (107 mg, 0.10 mmol) in 0.02N MeONa in MeOH (2 ml) was stirred at 26° for 12 h (complete disappearance of **13**), neutralized with *Amberlite-IR-120* (H^+ form), filtered, and evaporated. FC ($CHCl_3$/AcOEt/MeOH 10 : 17.5 : 2) gave **15** (67 mg, 78%). White solid. M.p. 184.1–193.0°. R_f ($CHCl_3$/AcOEt/MeOH 4 : 9 : 1) 0.50. IR (KBr): 3415*s* (br.), 2979*m*, 2933*m*, 2526*w*, 1689*s*, 1517*s*, 1454*m*, 1393*m*, 1368*s*, 1286*m*, 1251*m*, 1169*s*, 1090*m*, 1043*s*, 940*w*, 857*w*, 781*w*. ^{1}H-NMR ((D_6)DMSO, 300 MHz, at 100°): 6.35 (br. *s*), 6.12 (br. *s*, 2 H), 5.77 (*d*, $J \approx 12$) (4 NH); 5.18 (*s*, H–C(1″)); 5.17 (br. *s*), 5.07 (br. *s*) (6 OH); 4.82 (br. *s*, H–C(1‴)); 4.57–4.52 (*m*, 3 H); 4.33 (*t*, $J \approx 5.1$, H–C(3″)); 4.21 (br. *s*, H–C(3‴)); 4.04 (*t*, $w_{1/2}$ = 6.0, H–C(4‴)); 3.92–3.90 (*m*, H–C(5‴)); 3.82 (*t*, $J \approx 6.3$, 1 H); 3.77 (br. *s*, 1 H); 3.64 (br. *t*, $J \approx 12.6$, 3 H)); 3.53 (*m*, 1 H); 3.37 (br. *s*, 1 H); 3.17 (br. *s*, 3 H); 1.90 (br. *d*, J = 11.4, H_{ax}–C(2)); 1.40, 1.395 (2*s*, 2 Me_3C); 1.39 (*s*, 2 Me_3C); 1.40–1.39 (*m*, H_{eq}–C(2)). ^{13}C-NMR (CD_3OD, 75 MHz): see *Table 2*; additionally, 158.44, 158.00, 157.66 (2 C) (3*s*, 4 NHC=O); 80.30 (2 C), 80.10, 79.73 (3*s*, 4 Me_3C); 28.49 (*q*, 4 *Me*$_3$C). HR-MALDI-MS: 877.4249 (100, $[M+Na]^+$, $C_{37}H_{66}N_4NaO_{18}^+$; calc. 877.4270). Anal. calc. for $C_{37}H_{66}N_4O_{18}\cdot H_2O$ (872.95): C 50.91, H 7.85, N 6.42; found: C 50.87, H 7.58, N 6.27.

1,3,2‴,6‴-Tetraammoniumde(2-amino-2-deoxy-α-D-*glucopyranosyl)paromomycin Tetraacetate* (**15**). Under Ar, a soln. of **14** (42 mg, 0.05 mmol) in CF_3COOH/anisole/H_2O 80 : 3 : 7 (5 ml) was stirred at 26° for 1 h (complete disppearance of **14**), and evaporated. A soln. of the residue in H_2O was washed with AcOEt. The aq. layer was evaporated to afford the trifluoroacetate corresponding to **15** (39 mg, 92%), which was converted to the free base by passing through *CG-50* (H^+ form) and was then converted to **15** (30 mg, 88%) by dissolution of the base in AcOH/H_2O 1 : 1, followed by evaporation and lyophilisation. M.p. 124.3° (dec.). R_f ($CHCl_3$/MeOH/25% aq. NH_3 1 : 4 : 3) 0.39. $[\alpha]_D^{25} = +7.3$ (c = 0.15, H_2O). IR (KBr): 3418*s* (br.), 1682*s*, 1524*m*, 1433*m*, 1401*m*, 1205*s*, 1137*s*, 1053*m*, 1018*m*, 936*w*, 841*m,* 801*m,* 724*m*. ^{1}H-NMR (D_2O, 500 MHz): 5.26 (*s*, H–C(1″), H–C(1‴)); 4.66 (*dd*, J = 4.6, 7.3, H–C(3″)); 4.39 (*d*, J = 4.4, H–C(2″)); 4.30 (*ddd*, $J \approx 7.0$, 3.0, 1.0, H–C(5‴)); 4.21 (*t,* $J \approx 3.2$, H–C(3‴); 4.20 (*dt*, $J \approx 7.3$, 3.1, H–C(4″)); 3.87 (*dd*, J = 12.7, 2.8, H_a–C(5″)); 3.80 (*t*, J = 1.5, H–C(4‴)); 3.76 (*dd*, J = 12.6, 3.4, H_b–C(5″)); 3.59–3.49 (*m*, H–C(4), H–C(5), H–C(6), H–C(2‴)); 3.42 (*dd*, J = 13.6, 7.0, H_a–C(6‴)); 3.34 (*dd*, J = 13.6, 3.7, H_b–C(6‴)); 3.28 (*td*, $J \approx 13.0$, 4.2, H–C(1)); 3.28 (*td*, $J \approx 13.0$, 4.2, H–C(3)); 2.39 (*dt*, J = 12.6, 4.2, H_{ax}–C(2)); 1.89 (*s*, 4 AcO); 1.71 (*q*, $J \approx 12.5$, H_{eq}–C(2)). ^{13}C-NMR (D_2O, 75 MHz): see *Table 3*; additionally, 181.44 (*s,* 4 MeC=O); 23.31 (*q*, 4 Me). HR-ESI-MS: 477.2152 (100, $[M+Na]^+$, $C_{17}H_{34}N_4NaO_{10}^+$; calc. 477.2173). Anal. calc. for $C_{17}H_{34}N_4O_{10}\cdot 4\,CH_3COOH\cdot 2\,H_2O$ (730.72): C 41.09, H 7.44, N 7.66 found: C 41.28, H 7.54, N 7.50.

1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazidoparomomycin (**16**). A soln. of **1** (7.0 g, 9.6 mmol) in H_2O (220 ml) was treated with *t*-BuOK (8.05 g, 71.7 mmol) and $CuSO_4\cdot 5\,H_2O$ (154 mg, 0.62 mmol), and dropwise with 0.5M TfN_3 in CH_2Cl_2 (220 ml, 110 mmol; addition within 40 min). The colour of the mixture changed from blue to dark green during the course of the reaction. The mixture was stirred at 26° for additional 24 h

and evaporated (40°/70 Torr). A soln. of the residue in AcOEt (100 ml) was washed with brine (2×30 ml), dried ($MgSO_4$), and evaporated. The crude product was acetylated, purified, and deacetylated to afford pure **16** (3.5 g, 49%). R_f ($CHCl_3$/AcOEt/MeOH 8:5:1.4) 0.35. M.p. 161.7–165.2°. $[\alpha]_D^{25} = +131.3$ ($c = 0.07$, MeOH). IR (KBr): 3420*s*, 2929*m*, 2109*s*, 1633*m*, 1384*m*, 1333*m*, 1262*m*, 1036*s*. ^{1}H-NMR (CD_3OD, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 4*. ^{13}C-NMR (CD_3OD, 125 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 5*. HR-ESI-MS: 768.2368 (100, $[M+Na]^+$, $C_{23}H_{35}N_{15}NaO_{14}^+$; calc. 768.2386). Anal. calc. for $C_{23}H_{35}N_{15}O_{14}$ (745.62): C 37.05, H 4.73, N 28.18, O 30.04; found: C 36.97, H 4.79, N 27.65, O 30.41.

*1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylideneparomomycin* (**17**). Under Ar, a soln of **16** (3.0 g, 4.0 mmol) in freshly distilled PhCHO (39 ml, 0.38 mol) was cooled to 0°, treated dropwise with HCOOH (9 ml, 0.23 mol) during 10 min, and stirred at −5 to 0° for 24 h. The mixture was neutralized with sat. $NaHCO_3$ soln. and extracted with AcOEt (5×30 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), and evaporated. FC (60 g of silica gel; 200 ml of hexane and 300 ml of hexane/AcOEt 9:1 (→ elution of excess PhCHO, $CHCl_3$/AcOEt 20:35) yielded **17** (2.5 g, 75%). R_f ($CHCl_3$/AcOEt/MeOH 8:5:1.4) 0.55. $[\alpha]_D^{25} = +93.0$ ($c = 0.34$, MeOH). IR (ATR): 3400*m*, 2934*w*, 2876*w*, 2099*s*, 1700*m*, 1601*w*, 1581*w*, 1496*w*, 1453*m*, 1374*m*, 1255*m*, 1024*m*. ^{1}H-NMR (CD_3OD, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 4*; additionally, 7.50–7.48 (*m*, 2 arom. H); 7.36–7.34 (*m*, 3 arom. H); 5.59 (*s*, PhC*H*). ^{13}C-NMR (CD_3OD, 125 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 5*; additionally, 139.15 (*s*); 129.11 (*d*, 2 C); 127.60 (*d*, 3 C); 103.15 (*d*, Ph*C*H). HR-ESI-MS: 856.2682 (30.7, $[M+Na]^+$, $C_{30}H_{39}N_{15}NaO_{14}^+$; calc. 856.2699). Anal. calc. for $C_{30}H_{39}N_{15}O_{14}\cdot CH_3OH$ (865.76): C 43.01, H 5.01, N 24.27; found: C 43.28, H 4.97, N 23.82.

*6,3′,2″,5″,3‴,4‴-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylideneparomomycin* (**18**). Under N_2, a soln. of **17** (3.49 g, 2.9 mmol) in pyridine (8.4 ml, 0.10 mol) was treated with Ac_2O (4.9 ml, 0.05 mol) and DMAP (354 mg, 2.9 mmol) at 26° for 18 h, diluted with CH_2Cl_2 (20 ml), and washed with cold 0.5N HCl soln. (10 ml). The aq. layer was extracted with CH_2Cl_2 (3×10 ml). The combined CH_2Cl_2 layers were washed with sat. $NaHCO_3$ soln. (10 ml) and brine (10 ml), dried ($MgSO_4$), and evaporated, FC (AcOEt/cyclohexane 7:13) gave **3** (3.1 g, 79%). White solid. M.p. 94.1–98.6°. R_f (AcOEt/cyclohexane 1:1) 0.36. $[\alpha]_D^{25} = +91.5$ ($c = 0.54$, MeOH). IR (ATR): 2930*w*, 2851*w*, 2101*s*, 1742*s*, 1451*w*, 1429*w*, 1370*m*, 1334*w*, 1217*s*, 1174*w*, 1136*w*, 1125*w*, 1094*w*, 1029*s*, 988*m*, 892*w*, 869*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.45–7.42 (*m*, 2 arom. H); 7.38–7.34 (*m*, 3 arom. H); 5.49 (*s*, PhC*H*); 2.17, 2.16, 2.15, 2.12, 2.11, 2.10 (6*s*, 6 AcO). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 170.54, 169.94, 169.82, 169.59, 169.48, 168.33 (6*s*, 6 Me*C*=O); 136.78 (*s*); 129.03 (*d*, 2 C); 128.13 (*d*, 2 C); 126.14 (*d*); 101.60 (*d*, Ph*C*H); 20.97, 20.88, 20.78, 20.66 (4*q*, 6 Me). HR-ESI-MS: 1108.3344 (100, $[M+Na]^+$, $C_{42}H_{51}N_{15}NaO_{20}^+$; calc. 1108.3332). Anal. calc. for $C_{42}H_{51}N_{15}O_{20}\cdot CH_3OH$ (1117.98): C 46.20, H 4.96, N 18.79 found: C 46.26, H 4.88, N 18.48.

*6,3′,2″,5″,3‴,4‴-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′-*O*-benzoyl-6′-bromo-6′-deoxyparomomycin* (**19**). Under N_2, a soln. of **18** (575 mg, 0.53 mmol) in dry CCl_4 (20 ml; dried over P_2O_5 and stored over 3-Å molecular sieves) was treated with $BaCO_3$ (586 mg, 2.97 mmol) and NBS (135.2 mg, 0.76 mmol, freshly crystallized from H_2O), and heated to 80° for 25 min. Upon increasing the temp., the mixture changed from colourless to off-white, pale yellow, orange, and again to pale yellow. The mixture was cooled to 26° and evaporated at 40°/220 Torr. The residue was suspended in AcOEt and filtered. The filtrate was washed with brine, dried ($MgSO_4$), and evaporated. FC (AcOEt/cyclohexane 3:7) yielded **19** (415 mg, 67%). M.p. 155.8–156.4°. R_f (AcOEt/cyclohexane 1:1) 0.35. $[\alpha]_D^{25} = +93.7$ ($c = 0.16$, MeOH). IR (KBr): 2108*s*, 1748*s*, 1637*m*, 1374*m*, 1227*m*, 1044*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.98 (*dd*, $J = 8.3, 1.5$, 2 arom. H); 7.59 (*tt*, $J = 7.5, 1.2$, 2 arom. H); 7.45 (*t*, $J = 7.8$, 1 arom. H); 2.17, 2.168, 2.157, 2.12 (4*s*, 6 AcO). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 171.01, 170.35, 170.22, 170.03, 169.84, 168.78 (6*s*, 6 Me*C*=O); 165.47 (*s*, PhC=O); 134.00 (*d*); 130.17 (*d*, 2 C); 128.89 (*s*); 128.89 (*d*, 2 C); 21.12, 21.03, 20.92 (2 C), 20.83, 20.76 (5*q*, 6 Me). HR-ESI-MS: 1188.2258 (100, $[M+Na]^+$, $C_{42}H_{50}{}^{81}BrN_{15}NaO_{20}^+$; calc. 1188.2417), 1186.2413 (75, $[M+Na]^+$, $C_{42}H_{50}{}^{79}BrN_{15}NaO_{20}^+$; calc. 1186.2438). Anal. calc. for $C_{42}H_{50}BrN_{15}O_{20}$ (1164.84): C 43.31, H 4.33, N 18.04, Br 6.86; found: C 43.48, H 4.34, N 17.84, Br 6.58.

1,3,2′,2‴,6‴-Pentadeamino-3′,6′-anhydro-1,3,2′,2‴,6‴-pentaazidoparomomycin (**20**). A soln. of **19** (160 mg, 0.22 mmol) in MeOH (5 ml) and H_2O (0.02 ml) was treated with K_2CO_3 (425 mg, 3.08 mmol), stirred for 12 h at 25°, and evaporated. The residue was suspended in AcOEt and filtered. The filtrate was washed with brine, dried ($MgSO_4$), and evaporated. FC ($CHCl_3$/AcOEt/MeOH 5:10:1) yielded **20** (161 mg, quant.). M.p. 119.4–122.6°. R_f ($CHCl_3$/AcOEt/MeOH 4:9:1) 0.58. $[\alpha]_D^{25} = +84.3$ ($c = 0.53$, MeOH). IR (KBr): 3435*s*, 2935*m*, 2510*w*, 2107*s*, 1729*w*, 1708*w*, 1633*w*, 1374*m*, 1333*m*, 1265*s*, 1172*m*, 1118*s*, 1066*s*, 1043*s*, 953*w*, 916*w*, 899*w*, 839*w*. ^{1}H-NMR

Table 4. *^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of Compounds* **16**–**21**

	16[a]) CD_3OD	**17**[a]) CD_3OD	**18** $CDCl_3$	**19** $CDCl_3$	**20** CD_3OD	**21**[a]) $CDCl_3$
H–C(1′)	5.80	5.83	5.85	6.08	5.73	5.54
H–C(2′)	3.08	3.25	3.08	3.17	4.26	4.11–4.07
H–C(3′)	3.93	4.11	5.57	5.72	4.05–3.90	4.44
H–C(4′)	3.40	3.55	3.75–3.60	5.24	4.05–3.90	4.64
H–C(5′)	3.96–3.92	4.17–4.07	4.32–4.25	4.59	4.05–3.90	4.54
H_a–C(6′)	3.83	4.22	4.32–4.25	3.61–3.40	3.86	4.11
H_b–C(6′)	3.74	3.76	3.75–3.60	3.61–3.40	3.60	3.91
H–C(1)	3.45–3.33	3.44–3.38	3.53–3.38	3.61–3.40	3.58–3.54	3.43
H_{ax}–C(2)	1.38	1.41	1.60	1.59	1.21	1.39
H_{eq}–C(2)	2.18	2.21	2.38	2.36	2.06	2.24–2.21
H–C(3)	3.51	3.54–3.45	3.53–3.38	3.61–3.40	3.58–3.54	3.47
H–C(4)	3.75–3.67	3.72–3.63	3.67	3.93	3.68	3.76
H–C(5)	3.75–3.67	3.72–3.63	3.89	3.81	3.54	3.67
H–C(6)	3.49	3.54–3.40	4.93	4.97	3.81	4.92
H–C(1″)	5.40	5.39	5.34	5.37	5.20	5.14
H–C(2″)	4.30	4.32	4.91	4.87	4.30	4.75
H–C(3″)	4.45	4.43	4.45–4.39	5.03	4.38	4.31
H–C(4″)	4.14	4.17–4.07	4.32–4.25	4.35	4.14	4.26
H_a–C(5″)	3.83	3.84	4.42	4.46	4.14	4.43
H_b–C(5″)	3.75–3.67	3.72–3.63	4.22	4.28	3.71	4.31
H–C(1‴)	5.13	5.14	4.88	4.87	5.14	4.84
H–C(2‴)	3.68–3.67	3.72–3.63	3.31	3.33	3.43–3.38	3.32
H–C(3‴)	3.94	3.94	5.02	5.03	3.58–3.54	5.04
H–C(4‴)	3.44	3.44	4.70	4.69	3.43–3.38	4.71
H–C(5‴)	4.02	4.02	4.15–4.08	4.09	4.24	4.11–4.07
H_a–C(6‴)	3.65	3.72–3.63	3.60	3.59	3.43–3.38	3.62
H_b–C(6‴)	3.40	3.38	3.25	3.29	3.35	3.31
J(1′,2′)	3.7	3.9	3.6	3.6	2.7	3.6
J(2′,3′)	10.5	10.2	10.4	10.8	2.1	4.9
J(3′,4′)	8.7	9.5	[b])	9.3	[b])	5.1
J(4′,5′)	9.9	9.4	[b])	9.6	[b])	2.7
J(5′,6′a)	2.3	4.9	[b])	5.2	2.4	2.0
J(5′,6′b)	5.1	10.1	[b])	2.4	[b])	3.0
J((6′a,6′b)	11.9	10.1	[b])	[b])	11.9	10.7
J(1,2_{ax})	12.2	12.5	12.5	12.5	12.3	12.6
J(1,2_{eq})	4.3	4.8	4.5	3.6	4.8	4.5
J(1,6)	[b])	10.1	9.6	10.2	9.3	10.0
J(2_{ax},2_{eq})	12.8	12.9	13.2	13.5	13.2	13.5
J(2_{ax}, 3)	12.5	12.5	12.5	12.5	12.3	12.6
J(2_{eq}, 3)	4.3	4.2	4.5	3.6	4.8	4.5
J(3,4)	9.1	[b])	9.6	9.3	10.5	9.5
J(4,5)	[b])	[b])	9.6	9.3	10.5	9.4
J(5,6)	9.4	10.1	9.6	9.6	9.8	9.9
J(1″,2″)	1.7	1.9	1.8	3.0	1.8	3.4
J(2″,3″)	4.6	4.5	5.1	5.1	[b])	5.4
J(3″,4″)	6.7	6.6	[b])	[b])	4.8	[b])
J(4″,5″a)	2.7	2.8	4.8	4.2	[b])	1.9
J(4″,5″b)	5.2	[b])	5.1	2.1	3.9	5.8
J(5″a,5″b)	11.9	11.9	11.8	11.7	13.5	12.2
J(1‴,2‴)	1.7	1.8	2.1	3.6	1.5	1.9
J(2‴,3‴)	3.5	3.4	2.4	1.8	[b])	2.8

Table 4 (cont.)

	16[a]) CD_3OD	**17**[a]) CD_3OD	**18** $CDCl_3$	**19** $CDCl_3$	**20** CD_3OD	**21**[a]) $CDCl_3$
$J(3''',4''')$	3.5	3.4	2.4	2.7	[b])	2.8
$J(4''',5''')$	1.9	2.0	[b])	2.1	2.4	1.9
$J(5''',6'''a)$	8.4	8.5	8.1	8.0	4.5	8.0
$J(5''',6'''b)$	4.7	4.5	4.2	4.4	3.9	4.6
$J(6'''a,6'''b)$	12.9	12.9	12.9	12.9	13.2	12.9

[a]) Assignments based on a DQFCOSY and a HSQC spectrum. [b]) Not assigned.

Table 5. *^{13}C-NMR Chemical Shifts* [ppm] *of Compounds* **16**–**21**

	16[a]) CD_3OD	**17**[a]) CD_3OD	**18** $CDCl_3$	**19** $CDCl_3$	**20** CD_3OD	**21**[a]) $CDCl_3$
C(1′)	98.03	99.18	97.56	96.42	97.69	97.80
C(2′)	64.66	65.20	61.53	60.92	59.45	58.56
C(3′)	72.22	69.63	68.69	69.73[b])	71.61[b])	70.90
C(4′)	71.93	82.97	78.92	69.28[b])	69.94[b])	70.53
C(5′)	74.14	64.59	63.28	71.40	73.86	73.42[b])
C(6′)	62.48	69.85	68.96	32.20	68.43	68.22
C(1)	61.83	61.89	58.02	58.33	60.49[c])	58.40
C(2)	33.06	33.01	31.44	33.20	32.00	32.11
C(3)	61.53	61.29	58.95	59.61	59.86[c])	59.11
C(4)	76.31	77.29[b])	77.00	75.85[c])	81.33	81.78
C(5)	85.30	85.25	82.19	82.46[d])	85.12	82.48
C(6)	77.01	77.77[b])	75.75	75.56[c])	76.22[d])	74.83
C(1″)	108.95	109.73	107.04	107.21	110.67	106.98
C(2″)	75.21	75.14	74.73	75.31[c])	75.71[d])	74.43
C(3″)	77.11	77.27	75.75	76.37[c])	76.32[d])	75.64
C(4″)	83.50	83.46	78.92	80.05[d])	82.22	79.33
C(5″)	63.28	63.70	63.62	63.49	63.20	63.78
C(1‴)	99.79	99.80	99.02	99.70	98.67	99.52
C(2‴)	61.85	61.82	56.46	56.84	60.81	56.59
C(3‴)	71.17	71.16	68.69	68.88	71.72[b])	68.70
C(4‴)	69.60	69.59	65.69	65.86	69.94[b])	65.64
C(5‴)	75.56	75.66	73.57	73.55	74.57	73.45[b])
C(6‴)	52.44	52.50	50.66	50.83	51.41	50.54

[a]) Assignments based on a DQFCOSY and a HSQC spectrum. [b])[c])[d]) Assignments may be interchanged.

(CD_3OD, 300 MHz): see *Table 4*. ^{13}C-NMR (CD_3OD, 75 MHz): see *Table 5*. HR-ESI-MS: 750.2278 (100, $[M+Na]^+$, $C_{23}H_{33}N_{15}NaO_{13}^+$; calc. 750.2280). Anal. calc. for $C_{23}H_{33}N_{15}O_{13}\cdot 1.5\,CH_3OH$ (775.66): C 37.74, H 5.07, N 27.09; found: C 38.09, H 4.74, N 27.05.

*6,4′,2″,5″,3‴,4‴-Hexa-*O*-acetyl-1,3,2′,2‴,6‴-pentadeamino-3′,6′-anyhdro-1,3,2′,2‴,6‴-pentaazidoparomomycin* (**21**). Under N_2, a soln. of **20** (100 mg, 0.14 mmol) in pyridine (1.7 ml, 21 mmol) was treated with Ac_2O (1.0 ml, 10.2 mmol) and DMAP (219.6 mg, 1.8 mmol), stirred at 26° for 18 h, diluted with CH_2Cl_2 (10 ml), and washed with cold 0.5N HCl soln. (10 ml). The aq. layer was extracted with CH_2Cl_2 (3 × 10 ml). The combined CH_2Cl_2 layers were washed with sat. $NaHCO_3$ soln. (10 ml) and brine (10 ml), dried ($MgSO_4$), and evaporated. FC (AcOEt/cyclohexane 7:13) gave **21** (120 mg, 88%). R_f (AcOEt/cyclohexane 1:1) 0.42. $[\alpha]_D^{25} = +61.7$ ($c = 0.32$, $CHCl_3$). IR (CH_2Cl_2): 3052*m*, 2987*m*, 2107*s*, 1747*s*, 1664*w*, 1605*s*, 1446*w*, 1372*w*, 1234*s*, 1167*w*, 1114*w*, 1046*m*. ^{1}H-NMR ($CDCl_3$, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table*

4; additionally, 2.19, 2.18, 2.15, 2.13, 2.12, 2.10 (6*s*, 6 AcO). ^{13}C-NMR ($CDCl_3$, 125 MHz, assignments based on a DQFCOSY and a HSQC spectrum): see *Table 5*; additionally, 171.52, 170.69, 170.32, 169.78, 169.34, 168.49 (6*s*, 6 Me*C*=O); 20.95, 20.80, 20.78, 20.75, 20.70, 20.55 (*6q*, 6 Me). HR-MALDI-MS: 1002.2891 (100, $[M+Na]^+$), $C_{35}H_{45}N_{15}NaO_{19}^+$; calc. 1002.2914).

3′,6′-Anhydroparomomycin 1,3,2′,2‴,6‴-pentaammonium Pentaacetate (**22**). H_2 was passed into a soln. of **20** (117 mg, 0.16 mmol) in 1,4-dioxane/H_2O/AcOH 20 : 20 : 1 (5 ml) containing 10% Pd/C (50 mg) for 12 h at 26° and atmospheric pressure. After filtration through a *Celite* pad, evaporation of the filtrate and FC over silica gel (MeOH/25% aq. NH_3 8 : 3) yielded the pentaamine. It was then dissolved in 50% aq. AcOH (3 ml) evaporated, and then lyophilized to afford **22** (67 mg, 47%). M.p. 120° (dec.). R_f ($CHCl_3$/MeOH/25% aq. NH_3 1 : 4 : 3) 0.39. $[\alpha]_D^{25} = +19.7$ ($c = 0.30$, H_2O). IR (KBr): 3412*s*, 3195*s*, 2923*s*, 1560*s*, 1406*s*, 1340*m*, 1160*m*, 1110*m*, 1053*m*, 1017*m*, 932*w*, 916*w*, 895*w*, 866*w*. ^{1}H-NMR (D_2O, 300 MHz): 5.54 (*d*, $J = 3.3$, H–C(1′)); 5.27 (br. *s*, H–C(1″)); 5.17 (*d*, $J = 2.1$, H–C(1‴)); 4.70 (*s*, 1 H); 4.51–4.48 (*m*, H–C(5′), H–C(2″)); 4.36–4.15 (*m*, 4 H); 4.17–4.13 (*m*, H–C(5‴)); 4.05 (*t*, $J = 11.7$, 1 H); 4.02 (*t*, $J \approx 9.3$, H–C(5)); 3.94–3.91 (*m*, 1 H); 3.85 (*dd*, $J = 12.0$, 3.6, H_a–C(5″)); 3.81 (br. *s*, H–C(4′)); 3.76–3.73 (*m*, H_b–C(5″)); 3.70 (*t*, $J = 9.3$, H–C(4)); 3.62 (*t*, $J \approx 9.3$, H–C(6)); 3.59–3.56 (*m*, 1 H); 3.48–3.27 (*m*, 3 H); 2.46 (*dt*, $J = 12.0$, 3.6, H_{eq}–C(2)); 1.91, 1.90 (*s*, *Me*COOH); 1.79 (*q*, $J \approx 12.6$, H_{ax}–C(2)). ^{13}C-NMR (D_2O, 75 MHz): see *Table 3*; additionally, 180.99 (*s*, 5 OC=O); 23.19 (*q*, 5 Me). HR-MALDI-MS: 598 (100, $[M+H]^+$, $C_{23}H_{44}N_5O_{13}^+$; calc. 598.2936), 620.3 ($[M+Na]^+$, $C_{23}H_{43}N_5NaO_{13}^+$; calc. 620.2755). Anal. calc. for $C_{23}H_{43}N_5O_{13} \cdot 5$ AcOH (897.39): C 44.14, H 7.07, N 7.80; found: C 43.97, H 6.99, N 7.68.

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