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Synthesis of paromomycin derivatives modified at C(5″) to selectively target bacterial rRNA

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Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—The furanosyl moiety (ring III) of C(6′)-deoxyparomomycin and paromomycin was modified in search of aminoglycoside antibiotics with altered selectivity. The key intermediates were the *N*-Boc-protected derivative of C(6′)-deoxyparomomycin and the benzylidene-protected paromomycin. Their $H_2C(5'')$–OH group was oxidised with trichlorocyanuric acid or [bis(acetoxy)iodo]-benzene in the presence of catalytic amounts of TEMPO to yield the corresponding aldehydes and acids, which were transformed into the protected alkoxy imines, amides and the amine. Standard deprotection gave the title compounds derived from C(6′)-deoxy-paromomycin and derived from paromomycin that proved less active than paromomycin and its C(6′)-deoxy analogue.

Keywords: Aminoglycoside antibiotics; Paromomycin; Selectivity; Oxidation; Oxime; Carboxamide; Mycobacterium smegmatis

1. Introduction

Paromomycin, a broad-spectrum aminoglycoside antibiotic,[1,2] binds to the 16S rRNA at the tRNA acceptor A site and impairs the translation process by causing misreading and by hindering translocation.[3–6] The selectivity of paromomycin, that is the discrimination between prokaryotic and eukaryotic ribosomes, and of aminoglycoside antibiotics in general, is significantly limited by the cross-species conservation of the structure of the decoding site of prokaryotic and eukaryotic ribosomes[7,8] with the consequence of significant drug-associated toxicity.[9,10] Experimental results of the interactions between aminoglycosides and mutated A-sites indicate that rRNA sequence differences largely account for the selectivity of aminoglycosides with respect to bacterial and human ribosomes.[11–17] Modifications of strategic structural elements of aminoglycoside antibiotics, or structure based design of new drugs have thus been pursued to obtain antibiotics that discriminate between prokaryotic and eukaryotic rRNA,[18–24] and recent key insights into the crystal structure of the complex formed by paromomycin and the 16S rRNA A-site[25–29] allow for a rational approach to such modifications. Thus, bacterial ribosomes possess a guanine base (G) at position 1491 (numbered according to the *E. coli* sequence), while eukaryotic cytoplasmic ribosomes are characterised by 1491A and mitochondrial ones by 1491C.[11,12,28,30–32] The above mentioned crystal structure shows a distance between C(5″)–O and N(7) of 2.41 Å, indicating that C(5″)–OH of ring III of paromomycin forms a hydrogen bond to N(7) of G1491 in the bacterial ribosome. In eukaryotic cytoplasmic ribosomes, there may be a corresponding hydrogen bond between C(5″)–OH and N(7) of A1491. We planned to impair the (speculative) interaction of paromomycin with N(7) of A1491 by replacing C(5″)–OH by a substituent that cannot act as hydrogen bond donor, and to differentiate between G, A and C on the basis of their different charge density distribution.[33] Aromatic

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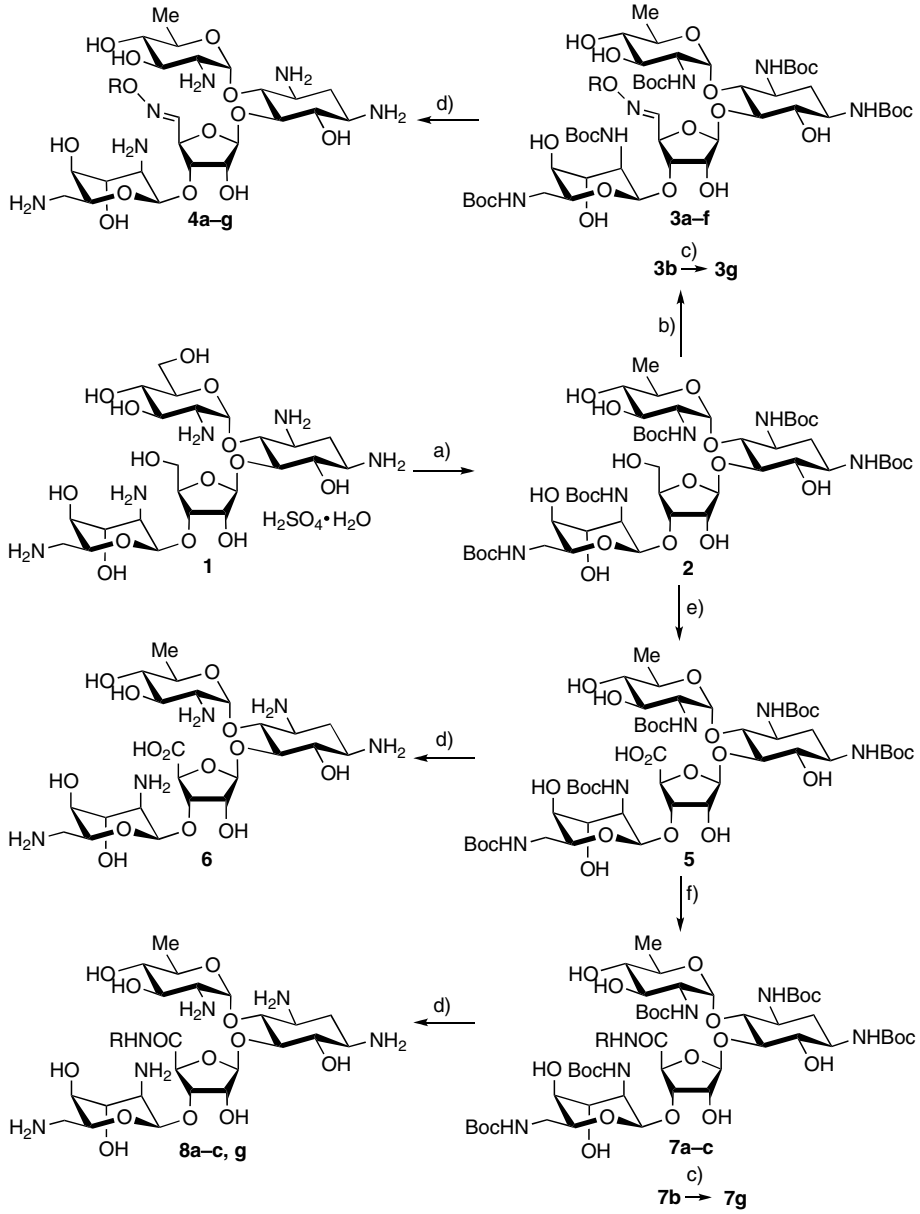
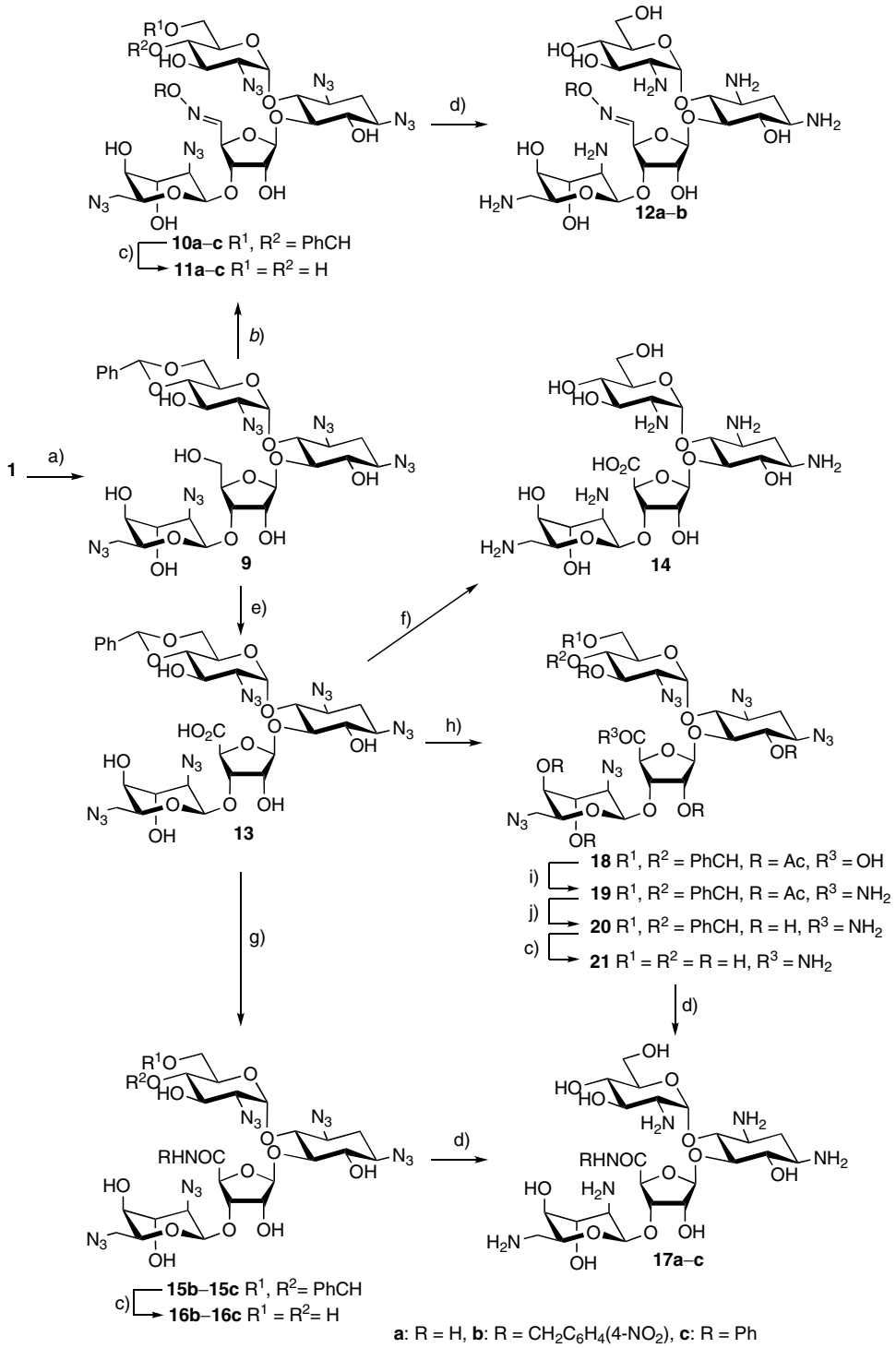
intermolecular interactions (π–π or CH–π interactions[34]) appeared promising, and we speculated that they may also compensate for the partial loss of activity resulting from deoxygenation of C(6′) of paromomycin.[35] Based on similar assumptions, Foloppe et al. screened a library of small molecules to find compounds with good stacking interactions with G1491.[21] We report on the preparation of paromomycin and C(6′)-deoxyparomomycin derivatives bearing either a C(5″)-hydroximo-, *O*-substituted C(5″)-hydroximo-, anilino or a corresponding carboxyl, or aminocarbonyl function and on the influence of these modifications at position C(5″) on the antibiotic activity. This position was already modified by a few research groups. Thus, Baasov et al. prepared a series of branched neomycin (C(6′)=NH_2) derivatives possessing an additional glycosyl moiety at *O*–C(5″)[36,37] to obtain antibiotics resistant towards aminoglycoside phosphotransferase APH(3′) that phosphorylates the C(5″)–OH group. Hanessian et al. prepared C(5″)–NH_2 derivatives of paromomycin and neomycin for the same purpose.[38] The same hydroxymethyl group was also modified to obtain conformationally biased



Scheme 1. Reagents and conditions: (a) six steps, 18% overall yield; (b) TCCA, TEMPO, EtOAc, 25 °C; then $RONH_2 \cdot HCl$, 1:1 pyridine–MeOH, 25 °C; 44–72% from **2**; (c) H_2, Pd/C, MeOH or 80% aq AcOH, 25 °C, 72–88%; (d) 97:3, CF_3 COOH/anisole, 25 °C, 56–91%; (e) BAIB, TEMPO, 1:1 MeCN–water, 23 °C; 54%; (f) EDAC, HOBt, DMF, 0 °C; then DIPEA, RNH_2, 25 °C, 40–45%.

neomycin and paromomycin derivatives. Asensio et al. designed and synthesised neomycin derivatives with a covalent bond between C(5″)–OH and C(2′)–NH_2 to mimic the corresponding hydrogen bond that appears to be formed while interacting with the ribosome, with the intention of overcoming bacterial resistance based



Scheme 2. Reagents and conditions: (a) two steps, 37% overall yield; (b) TCCA, TEMPO, EtOAc, 25 °C, then $RONH_2$·HCl, 1:1 pyridine–MeOH, 25 °C, 46–69% from **9**; (c) TsOH·H_2O, MeOH, 25 °C, 62–89%; (d) $HS(CH_2)_3SH$, MeOH, Et_3N, 25 °C, 71–86%; (e) BAIB, TEMPO, 1:1 MeCN–water, 23 °C, 54%; (f) PMe_3, THF, 1 N NaOH, 50 °C, 84%, then H_2, 10% Pd/C, 80% aq AcOH, 23 °C, 64%; (g) EDAC, HOAt, DIPEA, RNH_2, DMF, 25 °C, 34–64%; (h) Ac_2O, pyridine, 25 °C, 18 h, 76%; (i) *N*,*N*′-carbonyldiimidazole, THF, 25 °C, 2 h, then NH_4OAc, 16 h, 76%; (j) NaOMe, MeOH, CH_2Cl_2, 25 °C, 16 h, 92%.

on the deactivation of the antibiotics by *O*-adenyltransferase ANT(4).[39,40] For the same reason, C(5″)-methyl, propyl or carboxylate derivatives were synthesised.[40] Tor, Hermann et al. studied the selective binding of conformationally constrained paromomycin and neomycin derivatives to A-site and HIV-1 TAR RNA targets.[41,42]

2. Results and discussion

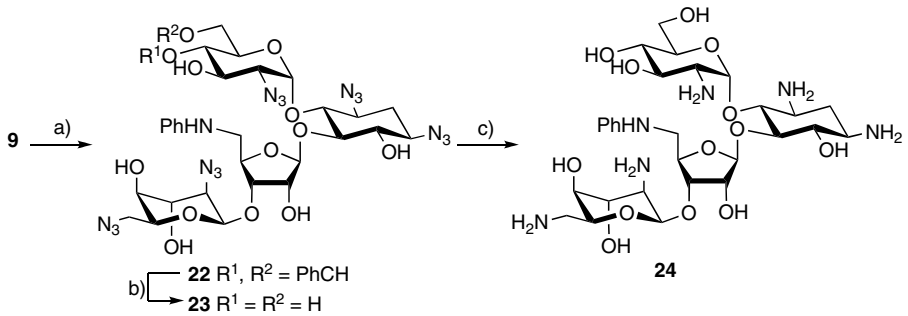
The synthesis of the desired derivatives from the *N*-Boc protected C(6′)-deoxyparomomycin **2**[35] possessing only one primary hydroxyl group appeared straightforward, and was undertaken first (Scheme 1). The alkoxy imines **4a**–**g** were obtained by selective oxidation of the $H_2C(5'')$–OH group of **2** with trichlorocyanuric acid (TCCA) in the presence of catalytic amounts of TEMPO,[43,44] followed by treatment of the crude product (a mixture of aldehyde, hydrate and methyl hemiacetals) with the *O*-substituted hydroxylamines in a mixture of pyridine and methanol, and deprotection of the resulting protected oxime ethers **3a**–**f** that were isolated in yields of 44–72%. The aniline **3g** was obtained in 88% yield by reduction of the nitrophenyl ether **3c** with H_2 in the presence of Pd/C. Minor amounts (< 5%) of the (*Z*) isomers of the alkoxy imines **3a**–**g** were isolated by FC. Removal of the *N*-Boc group of **3a**–**g** with TFA led to the pentakis-trifluoroacetate of the C(6′)-deoxy alkoxy imines **4a**–**g**. The *O*-phenyl oxime **4c** and the *O*-trityl analogue **4f** decomposed in aqueous solution at 25 °C within minutes (**4c**), or days (**4f**).

The *N*-Boc protected C(6′)-deoxy acid **5** (Scheme 1) was prepared in a yield of 54% by regioselective oxidation of the deoxyparomomycin derivative **2** with [bis(acetoxy)iodo]benzene (BAIB) and catalytic amounts of TEMPO.[45] The carboxamides **7a**–**c** were obtained by coupling of the acid **5** using *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (EDAC) in the presence of hydroxybenzotriazole (HOBt). The aniline **7g** was obtained in 72% yield by reduction of the nitrophenyl ether **3c** with H_2 in the presence of Pd/C. Standard deprotection with TFA gave the acid **6** from **5**, and the amides **8a**–**c**,**g** from **7a**–**c**,**g**.

The analogous, paromomycin-derived alkoxy imines **12a**–**b** and the amides **17a**–**c** were obtained by first transforming paromomycin sulfate into the corresponding known pentaazide by diazo transfer from TfN_3 in the presence of $CuSO_4{\cdot}5H_2O$ and *t*-BuOK, followed by benzylidenation according to a known procedure[35] to obtain **9** (Scheme 2). The protected alkoxy imines **10a**–**c** were synthesised similarly as described above for the 6′-deoxy analogues. Selective oxidation of **9** with trichlorocyanuric acid (TCCA) in the presence of a catalytic amount of TEMPO[44] was directly followed by treatment of the resulting crude aldehyde with substituted hydroxylamines in a mixture of pyridine and methanol, to yield 46–69% of the protected *N*-alkoxy imines **10a**–**c**. Debenzylidenation gave the *N*-alkoxy imines **11a** (89%) and **11b** (83%). The *N*-phenoxy imine **11c** was not stable in solution and decomposed within hours at ambient temperature. The pentaazides **11a** and **11b** were reduced with propane-1,3-dithiol[47] to the *N*-alkoxy imines **12a** (76%) and **12b** (71%).

The benzylidene derivative **9** was also transformed into the acid **14**, the amides **17a**–**c**, and the aniline **24** (Schemes 2 and 3). Regioselective oxidation of **9** with [bis(acetoxy)iodo]benzene (BAIB) and catalytic amounts of TEMPO[45] followed by standard Staudinger reaction and debenzylidenation yielded 29% of the acid **14**. The amides **17b**,**c** were prepared by oxidation of **9** to the benzylidene protected acid **13** followed by transformation into the amides **15b**,**c**, using *N*-ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (EDAC) in the presence of 1-hydroxy-7-azabenzotriazole (HOAt) as coupling agent. Hydrolytic cleavage of the phenyl dioxane ring of **15b** and **15c** to provide **16b** and **16c**, and reduction of the azido groups led in yields of 59 and 56% to **17b** and **17c**, respectively.

The primary amide **17a** was prepared in a slightly modified way. Oxidation of **9** followed by acetylation gave the protected acid **18** that was transformed into the amide **19** (76% yield) by treatment with *N*,*N*′-carbonyldiimidazole and ammonium acetate. Standard



Scheme 3. Reagents and conditions: (a) TCCA, TEMPO, EtOAc, 25 °C; then $PhNH_2$, $NaBH_3CN$, 55%; (b) $TsOH{\cdot}H_2O$, MeOH, 25 °C, 91%; (c) PMe_3, THF, 1 N NaOH, 50 °C, 84%.

deacetylation of **19** followed by debenzylidenation gave the pentaazide **21** that was reduced to yield 57% (from **19**) of the amide **17a** (Scheme 2).

The protected aniline **22** was obtained in a yield of 30% by reductive amination with aniline and sodium cyanoborohydride of the aldehyde obtained from **9**,[48] and transformed by standard debenzylidenation and Staudinger reaction/hydrolysis into the desired the aniline **24** (67%) (Scheme 3).

3. Biological studies

The experimental model used to investigate ribosomal drug susceptibility is a single rRNA allelic derivative of the Gram positive eubacterium *Mycobacterium smegmatis*.[46] Genetic manipulations of its single rRNA operon by site directed mutagenesis and recA-mediated gene conversion resulted in homogeneous populations of mutant ribosomes.[14,15,49] Ribosomal drug susceptibility was studied by determining the minimal inhibitory concentrations, as described in detail in Refs. 14 and 15.

All derivatives of paromomycin and (6′)-deoxyparomomycin modified at position C(5′) proved less active against *M. smegmatis* wild type, as compared to the parent compounds (see Table 1). The derivatives **4a**,**b**,**d**–**g**, **6**, **8a**–**c**,**g** were 8–16-fold less active than (6′)-deoxyparomomycin. The relatively most active compound among the paromomycin derivatives is **12a**. It showed a 16 times lower activity than paromomycin. No activity towards ribosomes with a single mutated base at position 1491 (G1491A, G1491C) was found, suggesting that the hydrogen bond between C(5″)–OH and N(7) of G1491 is crucial for binding and activity of paromomycin and its derivatives. Deoxygenation of the $H_2C(5'')$–OH group may also lead to elimination of the intramolecular H-bond to C(2′)–NH_2, which may play a role in stabilising the active conformation of paromomycin. This interpretation is in agreement with observations of Asensio et al.[40] and Tor et al.[41,42] about paromomycin and neomycin derivatives deoxygenated at C(5″). So far, only derivatives of paromomycin and neomycin in which the C(5″) hydroxy group was replaced by an unsubstituted amino group[38] displayed similar antibacterial activities as the parent compounds.

Table 1. Ribosomal drug susceptibility[a]

	Wild type 1491G	Mutant G1491A	Mutant G1491C
Paromomycin	1	64	>512
6′-Deoxyparomomycin	32–64	>512	>512
4a,**c**,**d**	256	512	>512
4b	512	>512	>512
4g	256–512	>512	>512
6, **8a**–**c**,**g**	>512	>512	>512
12a	16–32	>512	>512
12b	64	>512	>512
14	32–64	>512	>512
17a	128	256–512	>512
17b	512	>512	>512
17c	128–56	>512	>512
24	256–512	>512	>512

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

4. Experimental

4.1. General methods

See Ref. 35. ^1H and ^{13}C NMR spectra for Boc-protected compounds (**3a**–**g**, **5**, **7a**–**c**,**g**) are not described; signals were broad, even when the spectra were recorded at 100 °C in Me_2SO-d_6. Minor (*Z*) isomers of alkoxyimines were formed in max. 5% yield. They were separated from the (*E*) isomers but not characterised.

The deoxyparomomycin **2** was prepared from commercially available **1**[50–54] according to a known procedure[35] (Note that the structure of **1** and its derivatives in this paper has to be corrected; the absolute configuration of ring IV should be L).

4.2. General procedure for the transformation of primary alcohols into *N*-alkoxyimino derivatives (GP 1)

Under N_2, a cooled (⩽ 0 °C) 0.05 M soln of the alcohol (1 equiv) in EtOAc was treated with trichloroisocyanuric acid (TCCA; 1.1 equiv) and a catalytic amount of TEMPO (0.05 equiv), allowed to warm to 25 °C, stirred for 25–40 min and basified with 1 N NaOH. After separation of the phases, the aq layer was extracted with EtOAc (3×). The combined org. layers were dried ($MgSO_4$) and evaporated. A 0.05 M soln of the residue (crude aldehyde, its hydrate and methyl hemiacetal) in 1:1 MeOH–pyridine was treated with the appropriate *N*-alkoxyamine, and stirred at 25 °C for 24 h. MeOH was evaporated, the residue was taken up in brine and EtOAc, the phases were separated and the aq layer was extracted with EtOAc (3×). The combined org. layers were dried ($MgSO_4$) and evaporated to give the crude *N*-alkoxyimines.

4.2.1. 5″-(*N*-Benzyloxyimino)-1,3,2′,2‴,6‴-pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxyparomomycin (3a). GP 1

and FC (2:2:0.1 → 2:2:0.3 $CHCl_3$–EtOAc–MeOH) gave **3a** (465 mg, 72%). White solid: mp 173–176 °C; $[\alpha]_D^{25}$ +47.6 (*c* 0.145, MeOH); $R_f = 0.62$ (2:2:0.5, $CHCl_3$–EtOAc–MeOH); IR (ATR); *ν* 3374m, 2979w, 2931w, 2071w, 1682s, 1523w, 1426s, 1393m, 1367s, 1307w, 1254w, 1165s, 1118m, 1041s, 1024s, 975s cm^{-1}; HRESIMS: 1204.6092 (22 $[M+H]^+$, $C_{55}H_{91}N_6NaO_{23}^+$; Calcd 1204.6136), 1226.5926 (100, $[M+Na]^+$,

$C_{55}H_{90}N_6NaO_{23}^+$; Calcd 1226.3193). Anal. Calcd for $C_{55}H_{90}N_6O_{23}$ (1204.32): C, 54.90; H, 7.54; N, 6.98. Found: C, 54.71; H, 7.58; N, 6.72.

4.2.2. 1,3,2′,2‴,6′-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[*N*-(4-nitrobenzyloxy)imino]paromomycin (3b). GP 1 and FC (2:2:0.2 → 3:4:0.5, $CHCl_3$–EtOAc–MeOH) gave **3b** (1406 mg, 62%). White solid: mp 150–153 °C; $[\alpha]_D^{25}$ +41.8 (*c* 0.43, MeOH); $R_f = 0.79$ (2:2:0.75, $CHCl_3$–EtOAc–MeOH); IR (ATR): *v* 3359w, 2977w, 2933w, 1689s, 1603w, 1519s, 1454w, 1392m, 1365m, 1345m, 1283m, 1248m, 1162s, 1041s, 1020s, 993s, 950w, 919w cm^{-1}; HRESIMS: 1270.5785 (100, $[M+Na]^+$, $C_{55}H_{89}N_7NaO_{25}^+$; Calcd 1270.5806). Anal. Calcd for $C_{55}H_{89}N_7O_{25}$ (1248.32): C, 52.92; H, 7.19; N, 7.85. Found: C, 54.00; H, 7.29; N, 7.66.

4.2.3. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[(*N*-phenoxy)imino]paromomycin (3c). GP 1 and FC (3:4:0.1 → 3:4:0.5 $CHCl_3$–Et_2O–MeOH) gave **3c** (226 mg, 70%). White solid: mp 171–173 °C; $[\alpha]_D^{25}$ +11.7 (*c* 0.21, MeOH); $R_f = 0.54$ (3:4:0.75, $CHCl_3$–Et_2O–MeOH); IR (ATR): *v* 3363w, 2979w, 2923w, 1682s, 1593w, 1512m, 1454w, 1392m, 1366m, 1283m, 1248m, 1160s, 1044s, 1033s, 1018s, 922w cm^{-1}; HRESIMS: 1211.5807 (100, $[M+Na]^+$, $C_{54}H_{88}N_6NaO_{23}^+$; Calcd 1211.5799). Anal. Calcd for $C_{54}H_{88}N_6O_{23}$ (1189.3029): C, 54.54; H, 7.46; N, 7.07. Found: C, 54.37; H, 7.50; N, 6.95.

4.2.4. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[*N*-(methoxy)imino]paromomycin (3d). GP 1 and FC (3:4:0.1 → 3:4:0.5, $CHCl_3$–Et_2O–MeOH) gave **3d** (60 mg, 62%). Oil. $[\alpha]_D^{25}$ +34.0 (*c* 1.17, MeOH); $R_f = 0.50$ (3:4:0.75, $CHCl_3$–Et_2O–MeOH); IR (ATR); *v* 3364m, 2978w, 2935w, 2070m, 1681s, 1521m, 1426s, 1393m, 1366s, 1309w, 1254m, 1164s, 1118s, 1040s, 976s cm^{-1}; HRESIMS: 1149.5652 (100, $[M+Na]^+$, $C_{49}H_{86}N_6NaO_{23}^+$; Calcd 1149.5642). Anal. Calcd for $C_{49}H_{86}N_6O_{23}$ (1127.23): C, 52.21; H, 7.69; N, 7.46. Found: C, 52.28; H, 7.64; N, 7.24.

4.2.5. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[*N*-(pentafluorobenzyloxy)imino]paromomycin (3e). GP 1 and FC (2:2:0.1 → 2:2:0.3, $CHCl_3$–EtOAc–MeOH) gave **3e** (284 mg, 49%). White solid: mp 180–184 °C; $[\alpha]_D^{25}$ +134.95 (*c* 0.24, MeOH); $R_f = 0.85$ (2:2:0.5, $CHCl_3$–EtOAc–MeOH); IR (ATR): *v* 3362w, 2978w, 2936w, 1688s, 1505s, 1454w, 1392m, 1366s, 1305m, 1278m, 1248m, 1161s, 1126s, 1037s, 1022s, 959m, 939m cm^{-1}; HRMALDIMS: 1315.5502 (100, $[M+Na]^+$, $C_{55}H_{85}F_5N_6NaO_{23}^+$; Calcd 1315.5484), 1215.4987 (23, $[M-CO_2CMe_3+H+Na]^+$, $C_{50}H_{77}F_5N_6NaO_{21}^+$; Calcd 1215.4960), 1194.5194 (45, $[M-CO_2CMe_3+2H]^+$, $C_{45}H_{69}F_5N_6O_{21}^+$; Calcd 1194.5140). Anal. Calcd for $C_{55}H_{85}F_5N_6O_{23}$ (1294.28): C 51.08, H 6.62, N 6.50. Found: C 51.27, H 6.60, N 6.44.

4.2.6. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[*N*-(trityloxy)imino]paromomycin (3f). GP 1 and FC (2:2:0.1 → 2:2:0.3, $CHCl_3$–EtOAc–MeOH) gave **3f** (162 mg, 44%). White solid: mp 140–142 °C; $[\alpha]_D^{25}$ +35.2 (*c* 0.44, MeOH); $R_f = 0.72$ (2:2:0.7, $CHCl_3$–EtOAc–MeOH); IR (ATR): *v* 3397w, 2977w, 2932w, 1687s, 1506m, 1449w, 1392m, 1366m, 1280m, 1247m, 1159s, 1039s, 1022s, 987s, 961m, 920m cm^{-1}; HRESIMS: 1355.6913 (38, $[M+H]^+$, $C_{67}H_{99}N_6O_{23}^+$; Calcd 1355.6762), 1377.6558 (100, $[M+Na]^+$, $C_{67}H_{98}N_6NaO_{23}^+$; Calcd 1377.6581). Anal. Calcd for $C_{67}H_{98}N_6O_{23}$ (1355.52): C, 59.37; H, 7.29; N, 6.20. Found: C, 59.21; H, 7.13; N, 6.06.

4.2.7. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′,5″-dideoxy-5″-[*N*-(4-aminobenzyloxy)imino]paromomycin (3g). Under H_2, a suspension of **3c** (0.136 mmol, 170 mg) and 10% Pd/C (17 mg) in MeOH (6 mL) was stirred at 25 °C for 12 h, filtered and evaporated. FC (2:2:0.2 → 2:2:0.5, $CHCl_3$–EtOAc–MeOH) gave **3g** (153 mg, 88%). White solid: mp 160–162 °C; $[\alpha]_D^{25}$ +38.3 (*c* 0.36, MeOH); $R_f = 0.46$. (2:2:0.75, $CHCl_3$–EtOAc–MeOH); IR (ATR): *v* 3363m, 2979w, 2932w, 1686s, 1614w, 1520m, 1480w, 1427m, 1393m, 1367s, 1308w, 1281w, 1252m, 1164s, 1117s, 1042s, 974s cm^{-1}; HRESIMS: 1218.6255 (100, $[M+H]^+$, $C_{55}H_{92}N_7O_{23}^+$; Calcd 1218.6245), 1240.6205 (69, $[M+Na]^+$, $C_{55}H_{91}N_7NaO_{23}^+$; Calcd 1240.6064). Anal. Calcd for $C_{55}H_{91}N_7O_{23}$ (1218.34): C, 54.22; H, 7.53; N, 8.05. Found: C, 54.15; H, 7.52; N, 7.89.

4.2.8. 1,3,2′,2‴,6‴-Pentaazido-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-pentadeamino-5″-deoxy-5″-[*N*-(hydroxy)imino]paromomycin (10a). GP 1 and FC (amino phase gel, 9:1, CH_2Cl_2–MeOH) gave **10a** (260 mg, 46%). White solid: mp 130–135 °C; $[\alpha]_D^{25}$ +130.7 (*c* 0.14, MeOH); $R_f = 0.18$ (amino phase gel, 9:1, CH_2Cl_2–MeOH); IR (ATR): *v* 3406w, 2924w, 2099s, 1455w, 1373w, 1333w, 1255m, 1155w, 1089m, 1018s cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 6, additionally, δ 7.50–7.47 (m, 2 arom. H), 7.36–7.32 (m, 3 arom. H), 5.58 (s, C*H*Ph); ^{13}C NMR (125 MHz, CD_3OD) see Table 7, additionally, δ 139.15 (s), 130.02 (d), 129.10 (2d), 127.60 (2d), 104.13 (d, *C*HPh); HRMALDIMS: 429.1026 (100), 869.2661 (79, $[M+Na]^+$, $C_{30}H_{39}N_{16}NaO_{14}^+$; Calcd 869.2651). Anal. Calcd for $C_{30}H_{38}N_{16}O_{14}\cdot MeOH$ (878.76): C, 42.37; H, 4.82, N, 25.50. Found: C, 42.03; H, 4.77; N, 25.60.

4.2.9. 1,3,2′,2‴,6‴-Pentaazido-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-pentadeamino-5″-deoxy-5″-[*N*-(4-nitrobenzyloxy)-imino]paromomycin (10b). GP 1 and FC (4:1 → 4:2, $CHCl_3$–MeCN) gave **10b** (145 mg, 69%). White solid:

mp 131–134 °C; $[\alpha]_D^{28}$ +114.9 (*c* 0.125, MeOH); $R_f = 0.60$ (4:3, $CHCl_3$–MeCN); IR (ATR): *v* 3418w, 2924w, 2099s, 1692w, 1606w, 1520m, 1454w, 1372w, 1346m, 1254m, 1155m, 1097m, 1044m, 1012s cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 6, additionally, *δ* 8.25–8.23 (m, 2 arom. H), 7.62–7.60 (m, 2 arom. H), 7.49–7.47 (m, 2 arom. H), 7.35–7.33 (m, 3 arom. H), 5.55 (s, C*H*Ph), 5.22 (s, CH_2Ph); ^{13}C NMR (125 MHz, CD_3OD) see Table 7, additionally, *δ* 148.98 (s), 147.06 (s), 139.14 (s), 130.04 (d), 129.89, 129.12, 127.62, 124.63 (4 × 2d), 104.20 (d, *C*HPh), 75.68 (t, *C*H_2Ph); HRESIMS: 1004.2979 (100, $[M+Na]^+$, $C_{37}H_{43}N_{17}NaO_{16}^+$; Calcd 1004.2971). Anal. Calcd for $C_{37}H_{43}N_{17}O_{16}$·0.5MeOH (997.86): C, 45.14; H, 4.55; N, 24.86. Found: C, 45.51; H, 4.58; N, 24.32.

4.2.10. 1,3,2′,2‴,6‴-Pentaazido-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-pentadeamino-5″-deoxy-5″-[*N*-(phenoxy)imino]-paromomycin (10c). **GP 1** and FC (2:2:0.1 → 2:2:0.3, $CHCl_3$–EtOAc–MeOH) gave **10c** (378 mg, 69%). White solid: mp 115–118 °C; $[\alpha]_D^{25}$ +85.7 (*c* 0.3, MeOH); $R_f = 0.67$ (2:2:0.5, $CHCl_3$–EtOAc–MeOH); IR (ATR): *v* 3422w, 2105s, 1587w, 1487w, 1474w, 1371w, 1331w, 1255w, 1158w, 1020m cm^{-1}; ^1H NMR (400 MHz, CD_3OD) see Table 6, additionally, *δ* 7.50–7.46 (m, 2 arom. H), 7.35–7.31 (m, 5 arom. H), 7.27–7.15 (m, 2 arom. H), 7.04–7.00 (m, 1 arom. H), 5.52 (s, C*H*Ph); ^{13}C NMR (100 MHz, CD_3OD) see Table 7, additionally, *δ* 160.63 (s), 139.12 (s), 130.02 (d), 130.43, 129.10, 127.59, 124.50 (4 × 2d), 104.13 (d, *C*HPh). Anal. Calcd for $C_{36}H_{42}N_{16}O_{14}$ (922.82): C, 46.85; H, 4.59; N, 24.29. Found: C, 46.74; H, 4.70; N, 24.28.

4.3. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′-deoxy-5″-carboxyparomomycin (5)

A soln of **2** (1.2 mmol, 1.32 g) in 1:1 MeCN–water (14 mL) was treated with bisacetoxyiodobenzene (4.8 mmol, 1.55 g) and TEMPO (0.54 mmol, 84 mg), stirred at 23 °C for 48 h, cooled to 0 °C, treated with 1 N HCl (5 mL) and diluted with AcOEt (50 mL) and H_2O (30 mL). The layers were separated, and the aq layer was extracted with AcOEt (2 × 50 mL). The combined org. layers were washed with brine (80 mL), dried ($MgSO_4$), filtered and evaporated. FC (1:1:0 → 8:8:5, $CHCl_3$–AcOEt–MeOH) gave **5** (722 mg, 54%). White solid: mp 195 °C (dec); $[\alpha]_D^{26}$ +4.0 (*c* 0.285, MeOH); $R_f = 0.41$ (1:1:1, $CHCl_3$–AcOEt–MeOH); IR (ATR): *v* 3359w, 2977w, 2933w, 1682s, 1629w, 1511m, 1455w, 1391m, 1283m, 1367s, 1283w, 1249m, 1161s, 1041s cm^{-1}; HRMALDIMS: 454.0200 (100), 1112.5374 (57, $[M-H]^-$, $C_{48}H_{82}N_5O_{24}^-$; Calcd 1112.5350), 1113.5395 (30, $[M]^-$, $C_{48}H_{83}N_5O_{24}$; Calcd 1113.5428). Anal. Calcd for $C_{48}H_{83}N_5O_{24}$ (1114.20): C, 51.74; H, 7.51; N, 6.29. Found: C, 51.68; H, 7.27; N, 6.18.

4.4. General procedure for the formation of the amides 7a–c (GP 2)

Under N_2, a 0.05 M soln of **5** in DMF was treated at 0 °C with HOBt (2 equiv) and EDAC (2 equiv), and stirred at 0 °C for 30 min. DIPEA (2 equiv) and the corresponding amine (2 equiv) were added, the mixture was stirred at 25 °C for 18 h, cooled to 0 °C, treated with 1 N HCl and diluted with AcOEt and H_2O. The layers were separated and the aq layer was extracted with AcOEt (2×). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered and evaporated.

4.4.1. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′-deoxy-5″-(*N*-benzyl)paromomycincarboxamide (7a). **GP 2** on 49 mg of **5** and FC (1:1:0 → 6:6:0.5, $CHCl_3$–AcOEt–MeOH) gave **7a** (24 mg, 45%). Yellowish solid; $[\alpha]_D^{26}$ +12.8 (*c* 0.23, MeOH); $R_f = 0.36$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); IR (ATR): *v* 3379w, 2970w, 2933w, 1681s, 1512m, 1452m, 1410m, 1391m, 1366s, 1283w, 1246m, 1162s, 1027s, 989s cm^{-1}; HRMALDIMS: 858.4365 (40, $[M-\text{ring I}-CO_2CMe_3+3H]^+$, $C_{39}H_{64}N_5O_{16}^+$; Calcd 858.4348), 1103.5588 (49, $[M-CO_2-CMe_3+2H]^+$, $C_{50}H_{83}N_6O_{21}^+$; Calcd 1103.5616), 1225.5927 (100, $[M+Na]^+$, $C_{55}H_{90}N_6NaO_{23}^+$; Calcd 1225.5955), 1226.5961 (62, $[M+H+Na]^+$, $C_{55}H_{91}N_6NaO_{23}^+$; Calcd 1226.6033). Anal. Calcd for $C_{55}H_{90}N_6O_{23}·H_2O$ (1203.34): C, 54.09; H, 7.59; N, 6.88. Found: C, 54.20; H, 7.23; N, 6.75.

4.4.2. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′-deoxy-5″-[*N*-(4-nitrobenzyl)]paromomycincarboxamide (7b). **GP 2** on 81 mg of **5** and FC (1:1:0 → 9:9:1.2, $CHCl_3$–AcOEt–MeOH) gave **7b** (36 mg, 40%). Yellowish solid; $[\alpha]_D^{27}$ +27.8 (*c* 0.265, MeOH); $R_f = 0.28$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); IR (ATR): *v* 3356w, 2977w, 2933w, 1682s, 1609w, 1515s, 1455w, 1392m, 1366m, 1345m, 1279m, 1248m, 1160s, 1111m, 1034s, 996m cm^{-1}; HRMALDIMS: 803.3705 (36, $[M-\text{ring I}-2CO_2CMe_3+4H]^+$, $C_{34}H_{55}N_6O_{16}^+$; Calcd 803.3675), 903.4260 (33, $[M-\text{ring I}-CO_2CMe_3+3H]^+$, $C_{39}H_{63}N_6O_{18}^+$; Calcd 903.4199), 970.4210 (49, $[M-3CO_2-CMe_3+3H+Na]^+$, $C_{40}H_{65}N_7NaO_{19}^+$; Calcd 970.4233), 1070.4805 (60, $[M-2CO_2CMe_3+2H+Na]^+$, $C_{45}H_{73}N_7NaO_{21}^+$; Calcd 1070.4757), 1170.5469 (73, $[M-CO_2-CMe_3+H+Na]^+$, $C_{50}H_{81}N_7NaO_{23}^+$; Calcd 1170.5282), 1270.5927 (100, $[M+Na]^+$, $C_{55}H_{89}N_7NaO_{25}^+$; Calcd 1270.5806), 1271.5850 (63, $[M+H+Na]^+$, $C_{55}H_9N_7NaO_{25}^+$; Calcd 1271.5884). Anal. Calcd for $C_{55}H_{89}N_7O_{25}·H_2O$ (1266.36): C, 52.17; H, 7.24; N, 7.74. Found: C, 52.07; H, 7.29; N, 7.45.

4.4.3. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′-deoxy-5″-(*N*-phenyl)paromomycincarboxamide (7c). **GP 2** with HOAt instead of HOBt on 46 mg of **5** and FC (1:1:0 → 6:6:0.1, $CHCl_3$–AcOEt–MeOH) gave **7c**

(21 mg, 43%). Yellowish solid: mp 195–200 °C; $[\alpha]_D^{28}$ +5.3 (*c* 0.515, MeOH); $R_f = 0.28$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); IR (ATR): *v* 3364w, 2978w, 2933w, 1682s, 1601w, 1511s, 1446m, 1392m, 1366s, 1345m, 1281m, 1248m, 1161s, 1032s cm^{-1}; HRMALDIMS: 711.3158 (42, $[M-5CO_2CMe_3+5H+Na]^+$, $C_{30}H_{50}N_6NaO_{13}^+$; Calcd 711.3303), 811.3654 (46, $[M-4\ CO_2CMe_3+4H+Na]^+$, $C_{34}H_{56}N_6O_{15}^+$; Calcd 811.3701), 911.4227 (65, $[M-3CO_2CMe_3+3H+Na]^+$, $C_{39}H_{64}N_6NaO_{17}^+$; Calcd 911.4226), 1011.4785 (81, $[M-2CO_2CMe_3+2H+Na]^+$, $C_{44}H_{72}N_6NaO_{19}^+$; Calcd 1011.4750), 1012.4813 (42, $[M-2\ CO_2CMe_3+3H+Na]^+$, $C_{44}H_{73}N_6NaO_{19}^+$; Calcd 1012.4828), 1111.5192 (74, $[M-CO_2CMe_3+H+Na]^+$, $C_{49}H_{80}N_6NaO_{21}^+$; Calcd 1111.5274), 1211.5815 (100, $[M+Na]^+$, $C_{54}H_{88}N_6$-NaO_{23}^+; Calcd 1211.5799), 1212.5840 (60, $[M+H+Na]^+$, $C_{54}H_{89}N_6NaO_{23}^+$; Calcd 1212.5877). Anal. Calcd for $C_{54}H_{88}N_6O_{23}\cdot H_2O$ (1207.32): C, 53.72; H, 7.51; N, 6.96. Found: C, 53.30; H, 7.56; N, 6.69.

4.5. 1,3,2′,2‴,6‴-Pentakis-[*N*-(*tert*-butoxycarbonyl)]-6′-deoxy-5″-[*N*-(4-aminobenzyl)]paromomycincarboxamide (7g)

Under H_2, a suspension of **7b** (0.030 mmol, 44 mg) in 80% aq AcOH (2 mL) and 10% Pd/C (8 mg) was stirred at 23 °C for 18 h, filtered through a pad of *Celite* and evaporated. FC (1:0:0 → 6:6:1, $CHCl_3$–EtOAc–MeOH) gave **7g** (31 mg, 72%). White solid; $[\alpha]_D^{25}$ +23.3 (*c* 0.30, MeOH); $R_f = 0.34$. (3:3:1, $CHCl_3$–EtOAc–MeOH); HRMALDIMS: 1040.5061 (33, $[M-2CO_2CMe_3+2H+Na]^+$, $C_{45}H_{75}N_7NaO_{19}^+$; Calcd 1040.5015), 1118.5717 (46, $[M-CO_2CMe_3+2H]^+$, $C_{50}H_{84}N_7O_{21}^+$; Calcd 1118.5720), 1140.5595 (41, $[M-CO_2CMe_3+H+Na]^+$, $C_{50}H_{83}N_7NaO_{21}^+$; Calcd 1140.5540), 1240.6040 (100, $[M+Na]^+$, $C_{55}H_{91}N_7NaO_{23}^+$; Calcd 1240.6064), 1241.6067 (67, $[M+H+Na]^+$, $C_{55}H_{92}N_7NaO_{23}^+$; Calcd 1241.6142). Anal. Calcd for $C_{55}H_{91}N_7O_{23}\cdot H_2O$ (1236.36): C, 53.43; H, 7.58; N, 7.93. Found: C, 53.48; H, 7.78; N, 7.49.

4.6. General procedure for the de-*N*-bocylation (GP 3)

A soln of the paromomycin derivatives **3a**–**g**, **6**, **8a**–**c**,**g** (1 equiv) in TFA was treated with anisole (6 equiv) and stirred for 1–10 min. After evaporation of the solvent (water bath max. 30 °C), a soln of the residue in H_2O was washed with EtOAc (1×), and taken to dryness to give the crude pentaammonium salts.

4.6.1. 1,3,2′,2‴,6‴-Pentaammonium-6′,5″-dideoxy-5″-(benzyloxyimino)paromomycin pentakis(pentafluoropropionate) (4a·5$C_2F_5CO_2H$). GP 3

FC (RP C-18, 9:1:0.1 → 8:2:0.1, H_2O–MeOH–$C_3F_7CO_2H$) and lyophilisation gave (**4a**·5$C_2F_5CO_2H$) (42 mg, 79%). White solid: mp 140 °C (dec); $[\alpha]_D^{25}$ +38.9 (*c* 0.17, MeOH); $R_f = 0.36$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *v* 3500–2800w, 1667s, 1527w, 1403w, 1335m, 1273w, 1210s, 1158s, 1115s, 1080m, 1038m, 965m, 932m cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 2, additionally, *δ* 7.51–7.43 (m, 5 arom. H), 5.22 (s, CH_2Ph); ^{13}C NMR (126 MHz, D_2O) see Table 3, additionally, *δ* 165.82 (q, $^2J_{C,F}$ 27.5, 5C=O), 139.45 (s), 131.64 (2d), 131.33 (d), 131.04 (2d), 120.16, (dt, $^2J_{C,F}$ 34.8, $^1J_{C,F}$ 285.6, $5CF_2$), 111.18, (td, $^2J_{C,F}$ 39.1, $^1J_{C,F}$ 262.5, $5CF_3$), 78.64 (t, CH_2Ph); HRMALDIMS: 580.2828 (100), 688.3378 (49), 725.3315 (29, $[M+Na]^+$, $C_{30}H_{50}N_6NaO_{13}^+$; Calcd 725.3334). Anal. Calcd for $C_{49}H_{53}N_6F_{35}O_{23}$ (1758.26): C, 34.46; H, 4.04; N, 4.78. Found: C, 34.49; H, 4.05; N, 4.66.

4.6.2. 1,3,2′,2‴,6‴-Pentaammonium-6′,5″-dideoxy-5″-[*N*-(4-nitrobenzyloxy)imino]paromomycin pentakis(trifluoroacetate) (4b·5CF_3CO_2H). GP 3

FC (RP C-18, 9:1 → 8:2, H_2O–MeOH) and lyophilisation gave **4b**·5CF_3CO_2H (48 mg, 91%). White solid: mp 130 °C (dec); $[\alpha]_D^{25}$ +57.5 (*c* 0.135, MeOH); $R_f = 0.30$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_4); IR (ATR): *v* 3372m (br), 1670s, 1519m, 1433w, 1348m, 1188s, 1131s, 1033m, 1013m cm^{-1}; ^1H NMR (600 MHz, D_2O) see Table 2, additionally, *δ* 8.28 (d, *J* 8.7, 2 arom. H), 7.62 (d, *J* 8.7, 2 arom. H), 5.29, 5.28 (2d, *J* 19.3, CH_2Ph); ^{13}C NMR (150 MHz, D_2O) see Table 3, additionally, *δ* 165.64 (q, $^2J_{C,F}$ 292.8, 6C=O), 150.15 (s), 147.66 (s), 131.41 (2d), 126.70 (2d), 119.0 (q, $^1J_{C,F}$ 34.7, $6CF_3$), 77.25 (t, CH_2Ph); HRMALDIMS: 580.2828 (100), 748.3370 (16, $[M+Na]^+$, $C_{30}H_{49}N_7NaO_{15}^+$; Calcd 770.3184). Anal. Calcd for $C_{30}H_{49}N_7O_{15}\cdot 6CF_3CO_2H$ (1335.88): C, 35.23; H, 4.87; N, 6.85; Found: C, 35.20; H, 4.05; N, 6.96.

4.6.3. 1,3,2′,2‴,6‴-Pentaammonium-6′,5″-dideoxy-5″-[*N*-(phenoxy)imino]paromomycin pentakis(trifluoroacetate) (4c·5CF_3CO_2H).

Decomposed at 25 °C within minutes.

4.6.4. 1,3,2′,2‴,6‴-Pentaammonium-6′,5″-dideoxy-5″-[*N*-(methoxy)imino]paromomycin pentakis(trifluoroacetate) (4d·5CF_3CO_2H). GP 3

FC (RP C-18, 9:1 → 8:2, H_2O–MeOH) and lyophilisation gave (**4d**·5CF_3CO_2H) (76 mg, 83%). White solid: mp 140 °C (dec); $[\alpha]_D^{25}$ +38.4 (*c* 0.34, MeOH); $R_f = 0.36$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *v* 3400–2700w, 1668s, 1623m, 1520w, 1432w, 1164s, 1188s, 1128s, 1033s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 2, additionally, *δ* 4.83 (s, OMe); ^{13}C NMR (126 MHz, D_2O) see Table 3, additionally, *δ* 165.67 (q, $^2J_{C,F}$ 35.5, 5C=O), 119.0 (q, $^1J_{C,F}$ 291.8, $5CF_3$), 64.54 (s, OMe); HRMALDIMS: 580.2822 (100), 649.3004 (62, $[M+Na]^+$, $C_{24}H_{46}N_6NaO_{13}^+$; Calcd 649.3021). Anal. Calcd for $C_{34}H_{51}F_{15}N_6O_{23}\cdot H_2O$ (1214.78): C, 34.91; H, 4.38; N, 6.85. Found: C, 34.62; H, 4.40; N, 6.92.

Table 2. ^{1}H NMR chemical shifts [ppm] and coupling constants [Hz] for **4a**–**b**, **4d**–**g** in D_2O

	4a $5C_2F_5CO_2H$	**4b** $5CF_3CO_2H$[a]	**4d** $5CF_3CO_2H$[a]	**4e** $5CF_3CO_2H$[a]	**4f** $5CF_3CO_2H$[a]	**4g**[a]
H-1′	5.88	5.82	5.84	5.93	5.51	5.38
H-2′	3.23–3.19	3.08	3.31–3.24	3.26	2.91	2.72
H-3′	3.94–3.86	3.83	3.99–3.82	3.93	3.80	3.53
H-4′	3.16	3.05	3.22	3.27	3.03	3.13–3.06
H-5′	3.75	3.68	3.69	3.76	3.66	3.84
Me-6′	1.32	1.26	1.26	1.34	1.23	1.27
H-1	3.23–3.19	3.32	3.31–3.24	3.55–3.40	3.78–3.76	2.94–2.82
H_{ax}-2	1.82	1.81	1.80	1.88	1.79	1.30–1.28
H_{eq}-2	2.48–2.45	2.45	2.41	2.50	2.43	2.03–2.00
H-3	3.46–3.35	3.47	3.45	3.54	3.33–3.27	2.94–2.82
H-4	3.94–3.86	3.92	3.82	3.94–3.90	3.40–3.33	3.43
H-5	3.94–3.86	3.87	3.99–3.82	3.92	3.78–3.76	3.73–3.66
H-6	3.68	3.63	3.62	3.69	3.60	3.30
H-1″	5.45	5.42	5.38	5.45	5.36	5.35
H-2″	3.94–3.86	4.36	4.40	4.47	4.00	4.41
H-3″	4.82–4.80	4.62	4.60	4.71	4.49	4.66–4.63
H-4″	4.82	4.82	4.70–4.66	4.80	4.86	4.66–4.63
H-5″	7.82	7.86	7.63	7.79	7.92	7.64
H-1‴	5.23–5.21	5.18	5.25	5.18	5.20	4.98
H-2‴	3.60–3.58	3.55	3.54	3.59	3.55	3.13–3.06
H-3‴	4.21	4.16	4.16	4.21	4.20	4.02
H-4‴	3.79–3.78	3.74	3.77	3.82	3.78–3.76	3.73–3.66
H-5‴	3.94–3.86	3.94–3.91	4.22	4.04–4.00	4.05	4.15–4.05
H_a-6‴	3.30	3.32	3.24	3.40	3.32	3.02
H_b-6‴	3.23–3.19	3.24	3.24	3.33	3.22	3.13–3.06
$J_{1',2'}$	4.1	4.1	4.0	4.1	4.0	3.8
$J_{2',3'}$	[b]	10.4	[b]	10.0	10.4	10.2
$J_{3',4'}$	9.0	9.0	8.9	8.8	9.0	9.3
$J_{4',5'}$	9.0	9.0	8.9	8.9	9.1	9.5
$J_{5',6'}$	6.2	6.2	6.3	6.3	6.2	6.3
$J_{1,2ax}$	2.9	12.7	12.6	12.6	12.5	[b]
$J_{1,2eq}$	[b]	4.2	4.2	4.2	4.2	[b]
$J_{1,6}$	10.3	10.5	10.5	10.6	10.5	9.9
$J_{2ax,2eq}$	12.9	12.7	12.6	12.6	12.5	[b]
$J_{2ax,3}$	12.9	12.7	12.6	12.6	12.5	[b]
$J_{2eq,3}$	[b]	4.2	4.1	4.2	4.1	[b]
$J_{3,4}$	[b]	9.2	10.4	10.2	[b]	9.2
$J_{4,5}$	[b]	8.9	9.1	8.8	[b]	9.4
$J_{5,6}$	8.8	8.9	9.0	9.0	8.9	9.9
$J_{1'',2''}$	2.4	2.9	2.6	2.5	4.2	1.9
$J_{2'',3''}$	4.4	4.5	4.6	4.5	4.3	4.2
$J_{3'',4''}$	5.5	4.9	5.7	5.6	3.4	[b]
$J_{4'',5''}$	5.4	4.7	5.8	5.5	4.2	6.3
$J_{1''',2'''}$	[b]	1.7	1.7	1.7	1.7	1.8
$J_{2''',3'''}$	2.9	3.1	2.9	2.9	2.9	3.3
$J_{2''',4'''}$	[b]	1.4	1.2	1.2	1.1	[b]
$J_{3'''4'''}$	2.9	3.1	3.1	3.1	3.1	3.3
$J_{4''',5'''}$	[b]	1.6	1.5	1.2	1.4	[b]
$J_{5''',6'''a}$	5.2	5.4	5.7	5.3	5.9	[b]
$J_{5''',6'''b}$	[b]	4.2	4.2	4.3	4.2	4.3
$J_{6'''a,6'''b}$	13.7	13.8	13.7	13.7	13.6	13.5

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

4.6.5. 1,3,2′,2‴,6‴-Pentaammonium-6′5″-dideoxy-5″-[*N*-(pentafluorobenzyloxy)imino]paromomycin pentakis(trifluoroacetate) (4e·5CF$_3$CO$_2$H). **GP 3**, FC (RP C-18, 9:1 → 8:2, H_2O–MeOH) and lyophilisation gave **4e**·5CF_3CO_2H (68 mg, 88%). White solid: mp 180 °C (dec); $[\alpha]_D^{25}$ +32.7 (*c* 0.425, MeOH); $R_f = 0.50$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700w, 1667s, 1524m, 1507m, 1434w, 1375w, 1186s, 1126s, 1033s, 1015s, 960m, 939m cm^{-1}; ^{1}H NMR (500 MHz, D_2O) see Table 2, additionally, δ 5.31 (s, PhCH_2); ^{13}C NMR (126 MHz, D_2O): see Table 3, additionally, δ 165.65 (q, $^2J_{C,F}$ 35.5, 5C=O), 149.7–148.7, 147.7–146.7, 141.8–138.9 (3m, 4 arom. H), 130.07 (s, 1 arom. H), 119.01, (q, $^1J_{C,F}$ 291.7, 5CF_3), 65.67 (s,

Table 3. ^{13}C NMR chemical shifts (ppm) for **4a**,**b**,**d**–**g** in D_2O

	4a $5C_2F_5CO_2H$	**4b** $5CF_3CO_2H$[a]	**4d** $5CF_3CO_2H$[a]	**4e** $5CF_3CO_2H$[a]	**4f** $5CF_3CO_2H$[a]	**4g**[a]
C-1′	96.90	96.90	96.83	96.59	96.84	100.76
C-2′	56.28	56.28	56.25	56.22	56.18	57.87
C-3′	71.35	71.21	71.33	71.25	71.35	74.57
C-4′	76.83	76.92	76.73	76.75	76.63	79.39
C-5′	72.58	72.58	72.83	72.83	72.56	77.54
C-6′	19.16	19.21	19.16	19.11	19.11	19.44
C-1	52.43	52.34	52.31	52.31	52.27	52.94
C-2	31.24	30.89	30.74	30.59	31.45	29.25
C-3	51.32	51.28	51.32	51.27	51.27	52.46
C-4	78.56	77.79	77.76	77.49	78.56	80.43
C-5	87.48	88.00	87.73	87.80	88.07	92.42
C-6	75.28	75.21	75.02	75.05	75.49	77.92
C-1″	112.93	112.99	113.03	112.94	112.67	112.17
C-2″	76.61	76.54	76.28	76.63	76.39	77.95
C-3″	81.71	81.64	81.71	81.56	81.42	87.86
C-4″	80.74	80.92	80.71	80.62	81.85	81.47
C-5″	152.95	153.20	151.90	153.24	146.30	155.62
C-1‴	98.86	98.93	98.73	98.91	98.83	101.91
C-2‴	53.41	53.49	53.42	53.43	53.44	54.84
C-3‴	70.27	70.21	70.29	70.17	70.07	71.45
C-4‴	70.30	70.31	70.08	70.36	70.16	72.82
C-5‴	72.31	72.47	72.72	72.41	72.41	76.51
C-6‴	43.14	43.19	43.11	43.07	43.07	43.49

[a] Assignment based on DQFCOSY and HSQC spectrum.

$CH_2C_6F_5$); HRMALDIMS: 580.2835 (100), 794.3057 (21, $[M+H]^+$, $C_{30}H_{46}F_5N_6O^{13+}$; Calcd 794.3043), 815.2845 (56, $[M+Na]^+$, $C_{30}H_{45}F_5N_6NaO_{13}{}^+$; Calcd 815.2862). Anal. Calcd for $C_{40}H_{50}F_{20}N_6O_{23}\cdot H_2O$ (1380.83): C, 34.79; H, 4.80; N, 6.09. Found: C, 34.66; H, 4.55; N, 6.27.

4.6.6. 1,3,2′,2‴,6‴-Pentaammonium-6′,5″-dideoxy-5″-[*N*-(trityloxy)imino]paromomycin pentakis(trifluoroacetate) (4f·5CF$_3$CO$_2$H). GP 3, FC (RP C-18, 9:1 → 8:2, H_2O–MeOH) and lyophilisation gave **4f**·$5CF_3CO_2H$ (70 mg, 84%). White solid: mp 150 °C (dec); $[\alpha]_D^{25}$ +44.9 (*c* 0.17, MeOH); $R_f = 0.70$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): 3500–2700w, 1671s, 1524w, 1592w, 1444w, 1332w, 1198s, 1132s, 1022m, 1018m, 918w cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 2, additionally, δ 7.48-7.38 (m, 15 arom. H); ^{13}C NMR (126 MHz, D_2O) see Table 3, additionally, δ 165.60 (q, $^2J_{C,F}$ 35.6, 5C=O), 152.34 (3s), 131.38 (6d), 130.95 (6d), 130.53 (3d), 116.69, (q, $^1J_{C,F}$ 292.5, $5CF_3$), 94.01 (s, CPh_3); HRMALDIMS: 580.2843 (100), 877.3939 (54, $[M+Na]^+$, $C_{42}H_{58}N_6NaO_{13}{}^+$; Calcd 877.3960).

4.6.7. 6′,5″-Dideoxy-5″-[*N*-(4-aminobenzyloxy)imino]-paromomycin (4g). GP 3, FC (1:4:2, $CHCl_3$–MeOH–25% aq NH_3) and lyophilisation gave **4g** (19 mg, 85%). White solid: mp 160 °C (dec); $R_f = 0.54$ (1:4:3, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700m, 1682m, 1612m, 1544s, 1519s, 1406m, 1335m, 1129m, 1116m, 1044s, 1033s, 1013s, 923m cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 2, additionally, δ 7.28 (d, *J* 8.5, 2 arom. H), 6.86 (d, *J* 8.5, 2 arom. H), 5.05 (s, CH_2Ph); ^{13}C NMR (125 MHz, D_2O) see Table 3, additionally, δ 134.26 (s), 134.02 (2d), 129.76 (s), 118.97 (2d), 78.71 (t, CH_2Ph); HRMALDIMS: 718.3624 (66, $[M+Na]^+$, $C_{49}H_{86}N_6NaO_{23}{}^+$; Calcd 1149.5642).

4.6.8. 1,3,2′,2‴,6‴-Pentaammonium-6′-deoxy-5″-carboxy-paromomycin pentakis(trifluoroacetate) (6·5CF$_3$CO$_2$H). GP 3 on 40 mg of **5**, FC (RP C-18, 1:17, H_2O–MeOH) and lyophilisation gave **6**·$5CF_3CO_2H$ (22 mg, 52%). White solid: mp 145 °C (dec); $[\alpha]_D^{23}$ +28.6 (*c* 0.044, H_2O); $R_f = 0.20$ (7:3, MeOH–25% aq NH_3); IR (ATR): *v* 2917w (br), 1668s, 1627w, 1520m, 1434w, 1334w, 1188s, 1126s, 1035s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 4. ^{13}C NMR (125 MHz, D_2O) see Table 5, additionally δ 163.00 (q, $^2J_{C,F}$ 37.6, 5C=O), 116.37, (q, $^1J_{C,F}$ 291.9, $5CF_3$). HRMALDIMS: 614.2869 (100, $[M+H]^+$, $C_{23}H_{45}N_5O_{14}{}^+$; Calcd 614.2885); 615.2903 (28, $[M+2H]^+$, $C_{23}H_{45}N_5O_{14}{}^+$; Calcd 615.2963). Anal. Calcd for $C_{23}H_{43}N_5O_{14}\cdot 6CF_3CO_2H\cdot H_2O$ (1315.77): C, 31.95; H, 3.91; N, 5.32. Found: C, 31.79; H, 3.90; N 5.32.

4.6.9. 1,3,2′,2‴,6‴-Pentaammonium-6′-deoxy-5″-[*N*-benzyl]paromomycincarboxamide pentakis(trifluoroacetate) (8a·5CF$_3$CO$_2$H). GP 3 on 9 mg of **7a** and lyophilisation gave **8a**·$5CF_3CO_2H$ (8 mg, 84%). White solid; $R_f = 0.30$ (4:1, MeOH–25% aq NH_3); ^1H NMR (500 MHz, D_2O) see Table 4, additionally δ 7.48–7.41 (m, 2 arom. H), 7.40–7.37 (m, 3 arom. H), 4.70 (d, *J* 15.1 Hz, $PhCH_aCH_bNHCO$), 4.38 (d, *J* 15.1 Hz,

$PhCH_aH_bNHCO$); ^{13}C NMR (125 MHz, D_2O) see Table 5, additionally δ 140.18 (s), 131.75 (2d), 130.63 (d), 130.13 (2d), 45.79 (t, $PhCH_2NHCO$); ^{19}F NMR (282 MHz, D_2O) −73.98 (s, CF_3CO_2H); HRMALDI-MS: 558.2714 (100, $[M-\text{ring I}+2H]^+$, $C_{24}H_{40}N_5O_{10}^+$; Calcd 558.2775), 703.3497 (32, $[M+H]^+$, $C_{30}H_{51}N_6O_{13}^+$; Calcd 703.3514). Anal. Calcd for $C_{30}H_{50}N_6O_{13}\cdot 6CF_3CO_2H$ (1386.89): C, 36.37; H, 4.07; N 6.06. Found: C, 36.23; H 3.95; N, 6.18.

4.6.10. 1,3,2′,2‴,6‴-Pentaammonium-6′-deoxy-5″-[*N*-(4-nitrobenzyl)]paromomycincarboxamide pentakis(heptafluorobutanoate) (8b·5C$_3$F$_7$CO$_2$H). **GP 3** on 31 mg of **7b**, FC (RP C-18, 2:3:0.013, H_2O–MeOH–$C_3F_7CO_2H$) and lyophilisation gave **8b**·5$C_3F_7CO_2H$ (25 mg, 55%). White solid: mp 151 °C (dec); $[\alpha]_D^{23}$ +21.1 (*c* 0.04, H_2O); $R_f = 0.28$ (1:4:1, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *v* 2934w (br), 1667s, 1521w, 1403w, 1335m, 1271w, 1211s, 1158s, 1115s, 1080m, 1046m, 1018m,

Table 4. ^1H NMR chemical shifts (ppm) and coupling constants (Hz) for **6**, **8a**–**c**,**g** in D_2O

	6 5CF_3CO_2H[a]	**8a** 5CF_3CO_2H[a]	**8b** 5$C_3F_7CO_2H$[a]	**8c**	**8g**
H-1′	5.73	5.89	5.86	5.67	5.51
H-2′	3.33–3.18	3.35	3.29–3.17	3.20	3.22–3.00
H-3′	3.74	3.88	3.84	3.76	3.71–3.64
H-4′	3.33–3.18	3.24	3.29–3.17	3.17	3.22–3.00
H-5′	3.65–3.60	3.75–3.71	3.77–3.67	3.69–3.52	3.57–3.48
Me-6′	1.21	1.32	1.30	1.27	1.27
H-1	3.33–3.18	3.40–3.34	3.36	3.11–3.01	3.22–3.00
H_{ax}-2	1.76	1.88	1.84	1.45	1.44
H_{eq}-2	2.90	2.51	2.69	2.16	2.13
H-3	3.44	3.56	3.52	3.11–3.01	3.22–3.00
H-4	3.89	4.03	4.00–3.94	3.88–3.73	3.85–3.76
H-5	3.83	3.97	4.00–3.94	3.88–3.75	3.85–3.76
H-6	3.65–3.60	3.75–3.71	3.77–3.67	3.54	3.57–3.48
H-1″	5.39	5.53	5.54	5.50	5.41
H-2″	4.20	4.48	4.51	4.48	4.43–4.41
H-3″	4.46	4.70	4.73	4.72	4.65
H-4″	4.37	4.60	4.65	4.66	4.55
H-1‴	5.20	5.28	5.30	5.08	5.09
H-2‴	3.50	3.61	3.63	3.26	3.22–3.00
H-3‴	4.13	4.23	4.25	4.09	4.09
H-4‴	3.70	3.79	3.80	3.69–3.52	3.71–3.64
H-5‴	4.18	4.24–4.21	4.27	4.08–4.05	4.16–4.14
H_a-6‴	3.33–3.18	3.21–3.20	3.29–3.17	2.95	3.22–3.00
H_b-6‴	3.33–3.18	3.21–3.20	3.29–3.17	2.62	3.22–3.00
$J_{1',2'}$	4.1	4.0	4.0	4.1	3.7
$J_{2',3'}$	11.0	10.6	10.6	10.6	[b]
$J_{3',4'}$	9.2	8.9	8.9	9.3	[b]
$J_{4',5'}$	[b]	9.1	[b]	9.3	[b]
$J_{5',6'}$	6.2	6.3	6.3	6.3	6.1
$J_{1,2ax}$	12.6	12.7	12.4	12.6	12.5
$J_{1,2eq}$	4.3	4.1	4.3	4.0	≈4.9
$J_{1,6}$	[b]	[b]	10.7	9.9	[b]
$J_{2ax,2eq}$	12.6	12.6	12.6	12.7	12.5
$J_{2ax,3}$	12.6	12.7	≈13.0	12.6	12.5
$J_{2eq,3}$	4.3	4.1	4.2	4.0	≈4.9
$J_{3,4}$	10.1	10.1	≈13.0	[b]	[b]
$J_{4,5}$	9.0	8.9	≈13.0	[b]	[b]
$J_{5,6}$	9.0	8.9	[b]	9.9	[b]
$J_{1'',2''}$	3.4	1.7	1.6	[b]	[b]
$J_{2'',3''}$	5.0	4.4	4.4	4.2	5.9
$J_{3'',4''}$	5.2	7.3	7.3	7.8	7.1
$J_{1''',2'''}$	1.6	1.6	1.7	1.6	[b]
$J_{2''',3'''}$	3.2	3.1	3.1	3.1	3.1
$J_{2''',4'''}$	≈1.2	≈1.2	≈1.4	[b]	[b]
$J_{3'''4'''}$	3.2	3.1	3.1	3.1	3.1
$J_{4''',5'''}$	1.6	1.2	1.3	[b]	[b]
$J_{5''',6'''a}$	3.7	[b]	3.6	3.2	[b]
$J_{5''',6'''b}$	7.4	[b]	7.8	8.8	[b]
$J_{6'''a,6'''b}$	[b]	[b]	[b]	13.4	[b]

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

Table 5. ^{13}C NMR chemical shifts (ppm) for **6** and **8a–c** in D_2O

	6 $5CF_3CO_2H$	**8a** $5CF_3CO_2H$[a]	**8b** $5C_3F_7CO_2H$[a]	**8c**
C-1′	96.17	97.81	97.80	100.21
C-2′	54.02	56.57	56.56	57.40
C-3′	68.49	71.16	71.13	71.90
C-4′	74.49	76.85	76.91	77.52
C-5′	72.67	75.04	75.12	76.33
C-6′	16.67	19.20	19.16	19.22
C-1	49.77	52.43	52.50	53.26
C-2	28.08	30.62	30.86	35.15
C-3	48.65	51.27	51.27	51.75
C-4	75.85	77.79	78.18	81.31
C-5	83.74	87.15	87.28	87.80
C-6	69.95	72.76	72.66	72.88
C-1″	109.23	113.30	113.39	112.74
C-2″	73.99	75.64	75.67	77.06
C-3″	80.00	80.52	80.65	82.41
C-4″	81.95	82.11	82.05	83.22
C-5″	176.95	173.68	174.14	173.29
C-1‴	95.87	97.44	97.50	99.32
C-2‴	50.72	53.47	53.51	54.27
C-3‴	67.71	69.97	70.01	71.80
C-4‴	67.23	69.54	69.48	70.48
C-5‴	70.29	73.39	73.49	73.66
C-6‴	40.41	42.96	42.95	42.89

[a] Assignment based on DQFCOSY and HSQC spectrum.

965m, 931m cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 4, additionally δ 8.29 (d, J 8.8 Hz, 2 arom. H), 7.57 (d, J 8.9 Hz, 2 arom. H), 4.70 (d, J 16.0 Hz, $ArCH_aCH_b$-NHCO), 4.54 (d, J 15.9 Hz, $ArCH_aH_bNHCO$); ^{13}C NMR (125 MHz, D_2O) see Table 5, additionally δ 165.89 (t, $^2J_{C,F}$ 24.7, 5C=O), 149.74 (s), 148.07 (s), 130.78 (2d), 126.77 (2d), 120.17 (qt, $^2J_{C,F}$ 33.9, $^1J_{C,F}$ 286.8, $5CF_3CF_2CF_2CO_2H$), 113.5–108.6 (m, $CF_3CF_2CF_2$-CO_2H), 45.31 (t, $PhCH_2NHCO$); ^{19}F NMR (282 MHz, D_2O) −80.76 (t, J 8.3, $CF_3CF_2CF_2CO_2H$); −118.08 (q, J 8.2, $CF_3CF_2CF_2CO_2H$); −127.01 (br s, CF_3CF_2-CF_2CO_2H); HRMALDIMS: 603.2598 (66, [M−ring I+H]$^+$, $C_{24}H_{39}N_6O_{12}^+$; Calcd 603.2626), 748.3345 (100, [M+H]$^+$, $C_{30}H_{50}N_7O_{15}^+$; Calcd 748.3365), 749.3373 (36, [M+2H]$^+$, $C_{30}H_{51}N_7O_{15}^+$; Calcd 749.3443), 770.3134 (37, [M+Na]$^+$, $C_{30}H_{49}N_7NaO_{12}^{15+}$; Calcd 770.3184). Anal. Calcd for $C_{30}H_{49}N_7O_{15}\cdot 7C_3F_7CO_2H$ (2246.02): C, 31.02; H, 2.51; N 4.37. Found: C, 31.07; H 2.26; N, 4.73.

4.6.11. 6′-Deoxy-5″-(*N*-phenyl)paromomycincarboxamide (8c). **GP 3** on 19 mg of **7c**, FC (THF; 1:1, THF–MeOH; 1:0 → 22:3, MeOH–25% aq NH_3) and lyophilisation gave **8c** (11 mg, quant.). White solid; $R_f = 0.41$ (4:1, MeOH–25% aq NH_3); IR (ATR): ν 3187w (br), 2904w, 1670w, 1599w, 1542w, 1496w, 1447w, 1384w, 1322w, 1257w, 1102s, 1038s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 4, additionally δ 7.57–7.54 (m, 2 arom. H), 7.48–7.45 (m, 2 arom. H), 7.32–7.28 (m, arom. H); ^{13}C NMR (125 MHz, D_2O) see Table 5, additionally δ 138.82 (s), 132.14 (2d), 128.86 (d), 124.08 (2d); HRMALDIMS: 689.3341 (100, [M+H]$^+$, $C_{29}H_{49}N_6O_{13}^+$; Calcd 689.3358), 690.3370 (34, [M+2H]$^+$, $C_{29}H_{50}N_6O_{13}^+$; Calcd 690.3436), 711.3196 (42, [M+Na]$^+$, $C_{29}H_{48}N_6NaO_{13}^+$; Calcd 711.3177).

4.6.12. 6′-Deoxy-5″-[*N*-(4-aminobenzyl)]paromomycincarboxamide (8g). **GP 3** on 12 mg of **7g**, FC (THF; 1:1, THF–MeOH; 1:0 → 22:3, MeOH–25% aq NH_3) and lyophilisation gave **8g** (7 mg, 99%). Yellowish solid; $R_f = 0.29$ (4:1, MeOH–25% aq NH_3); IR (ATR): ν 3227w (br), 2920w, 1660w, 1617w, 1519w, 1451w, 1404w, 1338w, 1103s, 1041s cm^{-1}; ^1H NMR (300 MHz, D_2O) see Table 4, additionally δ 7.18 (d, J 8.3 Hz, 2 arom. H), 6.83 (d, J 8.4 Hz, 2 arom. H), 4.46 (d, J 14.9 Hz, $ArCH_aH_bNHCO$), 4.24 (d, J 14.7 Hz, $ArCH_aH_bNHCO$); HRMALDIMS: 263.0540 (100), 718.3616 (95, [M+H]$^+$, $C_{30}H_{52}N_7O_{13}^+$; Calcd 718.3623), 719.3649 (31, [M+2H]$^+$, $C_{30}H_{53}N_7O_{13}^+$; Calcd 719.3701), 740.3432 (76, [M+Na]$^+$, $C_{30}H_{51}N_7$-NaO_{13}^+; Calcd 740.3443).

4.7. 5″-Anilino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-1,3,2′,2‴,6‴-pentadeamino-5″-deoxyparomomycin (22)

A cooled (⩽0 °C) soln of **9** (1.041 g, 1.25 mmol) in EtOAc (10 mL) was treated with trichlorocyanuric acid (TCCA, 304 mg, 1.375 mmol) and TEMPO (25 mg, 0.125 mmol), allowed to warm to 25 °C, stirred for 20 min and basified with 1 N NaOH (5 mL). After separation of the phases, the aq layer was extracted with EtOAc (3 × 5 mL). The combined org. layers were dried ($MgSO_4$) and evaporated. A soln of the residue (crude aldehyde, its hydrate and methyl hemiacetal) in 1:1 THF–$ClCH_2CH_2Cl$ (6 mL) was treated with aniline (109 μL, 1.62 mmol) and $NaBH_3CN$ (80 mg, 1.62 mmol), stirred at 25 °C for 24 h and neutralised with 1 N NaOH. After evaporation of THF, the aq layer was extracted with EtOAc (3 × 5 mL). The combined org. layers were dried ($MgSO_4$) and evaporated. FC (2:2:0.1 → 2:2:0.3, $CHCl_3$–EtOAc–MeOH) gave **22** (343 mg, 30%). White solid: mp 130–140 °C; $[\alpha]_D^{25}$ +131.7 (c 0.28, MeOH); $R_f = 0.62$ (2:2:0.5, $CHCl_3$–EtOAc–MeOH); IR (ATR): ν 3395w, 2927w, 2104s, 1738w, 1603w, 1508w, 1452w, 1366w, 1260w, 1230w, 1125w, 1089m, 1030m cm^{-1}; ^1H NMR (400 MHz, CD_3OD) see Table 7, additionally, δ 7.48–7.46 (m, 2 arom. H); 7.35–7.31 (m, 3 arom. H), 7.15–7.11 (m, 2 arom. H), 6.71–6.64 (m, 3 arom. H), 5.51 (s, C*H*Ph); ^{13}C NMR (100 MHz, CD_3OD) see Table 8, additionally, δ 149.88 (s, C=O), 139.15 (s), 130.21 (2d), 130.00 (s), 129.09 (2d), 127.59 (2d), 118.58 (2d), 114.60 (2d), 104.11 (d, *C*HPh); HRMALDIMS: 420.1610 (100), 909.3363 (81, [M+H]$^+$, $C_{36}H_{45}N_{16}O_{13}^+$; Calcd 909.3352). Anal. Calcd for $C_{36}H_{44}N_{16}O_{13}\cdot CH_3OH$

(940.88): C, 47.23; H, 5.14; N, 24.82. Found: C, 47.08; H, 5.19; N, 24.66.

4.8. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-carboxyparomomycin (13)

A soln of **9** (1.061 g, 1.27 mmol) in 1:1 MeCN–water (24 mL) was treated with bisacetoxyiodobenzene (1.189 g, 3.69 mmol) and TEMPO (91 mg, 0.58 mmol), stirred at 23 °C for 48 h, cooled to 0 °C, treated with 1 N HCl (30 mL) and diluted with AcOEt (50 mL). The layers were separated and the aq layer was extracted with AcOEt (2 × 50 mL). The combined org. layers were washed with brine (100 mL), dried ($MgSO_4$), filtered and evaporated. FC (1:1:0 → 2:2:1, $CHCl_3$–AcOEt–MeOH) gave **13** (585 mg, 54%). White solid: mp 175 °C (dec); $[\alpha]_D^{25}$ +76.0 (*c* 0.265, MeOH); $R_f = 0.20$ (2:2:1, $CHCl_3$–AcOEt–MeOH); IR (ATR): *ν* 3360w, 2934w, 2855w, 2099s, 1602m, 1453w, 1413w, 1375w, 1328w, 1257m, 1142m, 1121m, 1088s, 1026s, 992s, 921m cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 10, additionally *δ* 7.45–7.51 (m, 2 arom. H), 7.32–7.37 (m, 3 arom. H); 5.59 (s, PhC*H*), ^{13}C NMR (125 MHz, CD_3OD) see Table 11, additionally *δ* 139.16 (s), 130.02, 129.11 (3d), 127.61 (2d), 103.17 (d, Ph*C*H); HRMALDIMS: 846.2534 (100, $[M-H]^-$, $C_{30}H_{36}N_{15}O_{15}^-$; Calcd 846.2521). Anal. Calcd for $C_{30}H_{37}N_{15}O_{15}\cdot 2MeOH$ (911.79): C, 42.15; H, 4.97; N, 23.04; found: C, 42.21; H, 4.64; N, 2.54.

4.9. 6,3′,2″,3‴,4‴-Penta-*O*-acetyl-1,3,2′,2‴,6‴-pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-carboxyparomomycin (18)

Under Ar, a soln of **13** (137 mg, 0.161 mmol) in pyridine (6 mL) was treated with Ac_2O (1.7 mL, 17.9 mmol), stirred at 25 °C for 18 h, diluted with AcOEt (30 mL) and washed with cold 1 N HCl (20 mL). The layers were separated and the aq layer was extracted with AcOEt (2 × 30 mL). The combined org. layers were washed with brine (50 mL), dried ($MgSO_4$), filtered and evaporated. FC (7:3, cyclohexane–AcOEt then 3:3:0.05 → 3:3:1, $CHCl_3$–AcOEt–MeOH) gave **18** (131 mg, 76%). Yellowish solid; $R_f = 0.47$ (2:2:1, $CHCl_3$–AcOEt–MeOH); IR (ATR): *ν* 2941w, 2101s, 1744s, 1609m, 1429w, 1370m, 1216s, 1125m, 1093m, 1028s, 970m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 7.47–7.43 (m, 2 arom. H), 7.40–7.30 (m, 3 arom. H), 5.63–5.48 (m, H-3′, H-1″), 5.50 (s, PhC*H*), 5.10–5.06 (m, 1H), 5.00–4.90 (m, 2H), 4.75–4.72 (m, 2H), 4.34–4.25 (m, 2H), 4.11–4.08 (m, 1H), 4.03–3.97 (m, 1H), 3.80–3.30 (m, 9H), 3.20–3.13 (m, H-2′), 2.45–2.35 (m, H_{eq}-2), 2.20, 2.18, 2.16, 2.15, 2.13 (5s, $5CH_3CO$), 1.65 (q, $J_{2ax,2eq} = J_{1,2ax} = J_{3,2ax}$ 12.1 Hz, H_{ax}-2); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 11; additionally *δ* 169.87, 169.52, 168.40 (5s, $5CH_3CO$), 136.77 (s); 129.02 (d), 128.11 (2d); 126.13 (2d); 101.58 (d, Ph*C*H), 75.16, 74.47 and below solvent peak (C-4, C-6, C-2″, C-3″), 21.10, 20.92, 20.74, 20.50 (5q, $5CH_3CO$); HRMALDIMS: 596.1840 (100, $[M-\text{ring III}-\text{ring IV}+Na+H]^+$, $C_{23}H_{27}N_9NaO_9^+$; Calcd 596.1829), 612.1571 (52, $[M-\text{ring III}-\text{ring IV}+K+H]^+$, $C_{23}H_{27}KN_9O_9^+$; Calcd 612.1569), 1080.2995 (94, $[M+Na]^+$, $C_{40}H_{47}N_{15}NaO_{20}^+$; Calcd 1080.3019), 1081.3050 (47, $[M+Na+H]^+$, $C_{40}H_{48}N_{15}NaO_{20}^+$; Calcd 1081.3098), 1096.2719 (77, $[M+K+H]^+$, $C_{40}H_{47}KN_{15}O_{20}^+$; Calcd 1096.2759).

4.10. 6,3′,2″,3‴,4‴-Penta-*O*-acetyl-1,3,2′,2‴,6‴-pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-paromomycincarboxamide (19)

Under Ar, a soln of **18** (98 mg, 0.093 mmol) in THF (5 mL) was treated with *N*,*N*′-carbonyldimidazole (36 mg, 0.22 mmol), stirred at 25 °C for 2 h. The reaction mixture was treated with $AcONH_4$ (32 mg, 0.42 mmol), stirred at 25 °C for 16 h, diluted with AcOEt (12 mL) and washed with H_2O (10 mL). The two layers were separated and the aq layer was extracted with AcOEt (2 × 12 mL). The combined org. layers were washed with brine (20 mL), dried ($MgSO_4$), filtered and evaporated. FC (1:1, cyclohexane–AcOEt) gave **19** (74 mg, 76%). White solid: mp 128–133 °C; $R_f = 0.36$ (4:6, $CHCl_3$–AcOEt); IR (ATR): *ν* 2101s, 1745s, 1698m, 1453w, 1431w, 1370m, 1332 w, 1218s, 1168m, 1130m, 1093m, 1029s, 998s, 968m, 931m cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ* 7.46–7.43 (m, 2 arom. H), 7.37–7.34 (m, 3 arom. H), 6.98 (br s, $CONH_2$), 5.62 (dd, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 9.6 Hz, H-3′), 5.60–5.56 (m, 1H), 5.50 (s, PhC*H*), 5.35 (br s, H-1″), 5.08 (t, $J_{2''',3'''} = J_{3''',4'''}$ 2.9 Hz, H-3‴), 5.05 (d, $J_{1''',2'''}$ 1.9 Hz, H-1‴), 4.99 (dd, $J_{5,6}$ 10.2 Hz, $J_{1,6}$ 9.1 Hz, H-6), 4.78–4.67 (m, 4H), 4.34–4.13 (m, 3H), 4.13–4.03 (m, H-5‴), 4.01 ($J_{4,5} = J_{5,6}$ 9.0 Hz, H-5), 3.80–3.35 (m, 8H), 3.15 (dd, $J_{1',2'}$ 3.9 Hz, $J_{2',3'}$ 10.4 Hz, H-2′), 2.39 (dt, $J_{1,2eq} = J_{2eq3}$ 4.4 Hz, J_{2ax2eq} 13.2 Hz, H_{eq}-2); 2.21, 2.18, 2.16, 2.14, 2.13 (5s, $5CH_3CO$), 1.63 (q, $J_{2ax,2eq} = J_{1,2ax} = J_{3,2ax}$ 12.9 Hz, H_{ax}-2); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 11, additionally *δ* 170.08, 169.87, 169.60, 168.49 (5s, $5CH_3CO$), 136.78 (s), 129.06 (d), 128.15 (2d), 126.14 (2d), 101.58 (d, Ph*C*H), 20.91, 20.77, 20.59, 20.25 (5q, $5CH_3CO$); HRMALDIMS: 1079.3177 (100, $[M+Na]^+$, $C_{40}H_{48}N_{16}NaO_{19}^+$; Calcd 1079.3179), 1080.3236 (50, $[M+Na+H]^+$, $C_{40}H_{49}N_{16}NaO_{19}^+$; Calcd 1080.3258).

4.11. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-paromomycincarboxamide (20)

A soln of **19** (53 mg, 0.05 mmol) in 4:1 MeOH–CH_2Cl_2 was treated with NaOMe (32 mg, 0.60 mmol), stirred at 25 °C for 16 h, neutralised with *Amberlite IR-120* (H^+ form), filtered and evaporated. FC

(1:1:0 → 12:12:0.5, $CHCl_3$–AcOEt–MeOH) gave **20** (39 mg, 92%). White solid; $R_f = 0.18$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); ^1H NMR (300 MHz, CD_3OD) see Table 10, additionally δ 7.51–7.48 (m, 2 arom. H), 7.35–7.33 (m, 3 arom. H), 5.59 (s, PhC*H*).

4.12. General procedure for the formation of the amides 15b–15c (GP 4)

Under N_2, a 0.1 M soln of **13** in DMF was treated with HOAt (1.1 equiv), EDAC (1.1 equiv), DIPEA (2 equiv) and the corresponding amine (1.1 equiv), and the mixture was stirred at 25 °C for 25 h, cooled to 0 °C, treated with 1 N HCl and diluted with AcOEt and H_2O. The two layers were separated and the aq layer was extracted with AcOEt (2×). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered and evaporated.

4.12.1. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-[*N*-(4-nitrobenzyl)]paromomycincarboxamide (15b). GP 4 on 200 mg of **13** and FC (1:1:0 → 6:6:0.05, $CHCl_3$–AcOEt–MeOH) gave **15b** (148 mg, 64%). Yellowish solid: mp 128–132 °C; $[\alpha]_D^{26}$ +85.7 °C (*c* 0.21, MeOH); $R_f = 0.48$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); IR (ATR): *ν* 3403w, 2931w, 2099s, 1666w, 1605w, 1519m, 1455w, 1372w, 1344m, 1256m, 1140m, 1123m, 1087m, 1026s, 992s, 911w cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 10, additionally δ 8.22 (d, *J* 8.8 Hz, 2 arom. H), 7.57 (d, *J* 8.9 Hz, 2 arom. H), 7.50–7.48 (m, 2 arom. H), 7.36–7.32 (m, 3 arom. H), 5.57 (s, PhC*H*), 4.63 (d, *J* 15.8, ArCH_aH_bNHCO), 4.47 (d, *J* 15.8, ArCH$_a$$H_b$NHCO); ^{13}C NMR (125 MHz, CD_3OD) see Table 11, additionally δ 148.70 (s), 147.58 (s), 139.13 (s), 130.03 (d), 129.57 (2d), 129.11 (2d), 127.61 (2d), 124.84 (2d), 103.17 (d, Ph*C*H), 43.34 (t, Ar*C*H$_2$NHCO); HRMALDIMS: 1004.2966 (100, $[M+Na]^+$, $C_{37}H_{43}N_{17}NaO_{16}^+$; Calcd 1004.2971), 1005.3012 (50, $[M+H+Na]^+$, $C_{37}H_{44}N_{17}NaO_{16}^+$; Calcd 1005.3050). Anal. Calcd for $C_{37}H_{43}N_{17}O_{16}\cdot CH_3OH$ (1013.88): C, 45.02; H, 4.67; N 23.49. Found: C, 45.06; H 4.61; N, 23.16.

4.12.2. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-4′,6′-*O*-benzylidene-5″-(*N*-phenyl)paromomycincarboxamide (15c). GP 4 on 95 mg of **13** and FC (1:1:0 → 6:6:0.05, $CHCl_3$–AcOEt–MeOH) gave **15c** (35 mg, 34%). Yellowish solid; $R_f = 0.57$ (3:3:0.5, $CHCl_3$–AcOEt–MeOH); IR (ATR): *ν* 3387w, 2933w, 2877w, 2099s, 1732w, 1659w, 1597w, 1537w, 1495w, 1441w, 1373w, 1315w, 1254m, 1137m, 1122m, 1085m, 1026s, 994s, 918m cm^{-1}; ^1H NMR (300 MHz, CD_3OD) see Table 10, additionally δ 7.65–7.62 (m, 2 arom. H), 7.50–7.47 (m, 2 arom. H), 7.34–7.30 (m, 5 arom. H), 7.14–7.09 (m, arom. H), 5.57 (s, PhC*H*); ^{13}C NMR (75 MHz, CD_3OD) see Table 11, additionally δ 139.33 (s), 139.17 (s), 130.04 (d), 129.89 (2d), 129.89 (2d), 129.13 (2d), 127.62 (2d), 125.67 (d), 121.73 (d), 103.16 (d, Ph*C*H); HRMALDIMS: 945.2941 (100, $[M+Na]^+$, $C_{36}H_{42}N_{16}NaO_{14}^+$; Calcd 945.2964), 946.2972 (45, $[M+H+Na]^+$, $C_{36}H_{43}N_{16}NaO_{14}^+$; Calcd 946.3046). Anal. Calcd for $C_{36}H_{42}N_{16}O_{14}\cdot H_2O$ (940.83): C, 45.96; H, 4.71; N 23.82. Found: C, 46.27; H 4.92; N, 23.32.

4.13. General procedure for the cleavage of 4′,6′-*O*-benzylidene acetals (GP 5)

A 0.05 M soln of 4′,6′-*O*-benzylidene acetals (1 equiv) in MeOH was treated with $TsOH\cdot H_2O$ (1–2 equiv), stirred at 25 °C for 1–6 h, basified with 1 N NaOH or satd $NaHCO_3$ and extracted with EtOAc (3×). The combined org. layers were dried ($MgSO_4$) and evaporated to give the crude product.

4.13.1. 1,3,2′,2‴,6‴-Pentaazido-1,3,2′,2‴,6‴-pentadeamino-5″-deoxy-5″-[*N*-(hydroxy)imino]paromomycin (11a). GP 5 and FC (amino phase gel, 7:3, CH_2Cl_2–MeOH) gave **11a** (79 mg, 89%). White solid: mp 123–125 °C. $R_f = 0.24$ (amino phase gel, 7:3, CH_2Cl_2–MeOH); IR (ATR): *ν* 3351w, 2925w, 2099s, 1633w, 1368w, 1331w, 1259m, 1138m, 1125m, 1024s cm^{-1}; ^1H NMR (500 MHz, CD_3OD) see Table 6; ^{13}C NMR (125 MHz, CD_3OD) see Table 7; HRMALDIMS: 757.2381 (30, $[M-H]^-$, $C_{23}H_{33}N_{16}O_{14}^-$; Calcd 757.2362). Anal. Calcd for $C_{23}H_{34}N_{16}O_{14}$ 2.5MeOH (838.72): C, 36.52; H, 5.29; N, 26.72. Found: C, 36.97; H, 5.24; N, 26.87.

4.13.2. 1,3,2′,2‴,6‴-Pentaazido-1,3,2′,2‴,6‴-pentadeamino-5″-deoxy-5″-[*N*-(4-nitrobenzyloxy)imino]paromomycin (11b). GP 5 and FC (2:2:0.1 → 2:2:0.4, $CHCl_3$–EtOAc–MeOH) gave **11b** (231 mg, 83%). White solid: mp 128–130 °C; $R_f = 0.26$ (2:2:0.5, $CHCl_3$–EtOAc–MeOH); IR (ATR): *ν* 3407w, 2933w, 2104s, 1606w, 1520w, 1346m, 1260w, 1143w, 1030m cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2CO$): δ 8.30–8.20 (m, 2 arom. H), 7.78–7.64 (m, 2 arom. H), 7.75 (d, $J_{4'',5''}$ 4.7 Hz, H-5″), 5.79 (d, $J_{1',2'}$ 4.4 Hz, H-1′), 5.55 (br s, H-1″), 5.26 (s, CH_2Ph), 5.22 (d, $J_{1''',2'''}$ 1.9 Hz, H-1‴), 5.18 (d, *J* 4.35 Hz, 1H), 4.96 (d, *J* 4.05 Hz, 1H), 4.76 (d, *J* 5.0 Hz, 1H), 4.73 (d, *J* 4.0 Hz, 1H), 4.67 (dd, *J* 4.7, 7.2 Hz, 1H), 4.62 (dd, *J* 4.7, 6.8 Hz, 1H), 4.51 (d, *J* 4.7 Hz, 1H), 4.46 (dt, *J* 1.24, 4.8 Hz, 1H), 4.04–4.95 (m, H-5‴), 4.93–4.84 (m, H-5′), 4.84 (t, $J_{5',6'a} = J_{6'a,6'b}$ 9.3 Hz, H-6′a), 4.74 (dd, *J* 8.4, 8.7 Hz, 1H), 4.76–4.71 (m, 1H), 4.68 (dd, $J_{5',6'b}$ 5.0 Hz, $J_{6'a,6'b}$ 9.3 Hz, H-6′b), 4.66–4.57 (m, H-4′,H-3); 4.62 (dd, $J_{5''',6'''a}$ 8.4 Hz, $J_{6a''',6'''b}$ 12.8 Hz, H-6‴a), 4.55–4.48 (m, H-1), 4.44 (dd, $J_{5''',6'''b}$ 4.7 Hz, $J_{6a''',6'''b}$ 12.8 Hz, H-6‴b), 4.08 (dd, $J_{3',2'}$ 10.4 Hz, $J_{1',2'}$ 4.5 Hz, H-2′), 2.21 (dt, $J_{1,2eq} = J_{3,2eq}$ 4.3 Hz, $J_{2ax,2eq}$ 12.4 Hz, H_{eq}-2); 1.45 (q, $J_{2ax,2eq} = J_{1,2ax} = J_{3,2ax}$ 12.4 Hz,

Table 6. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) for **10a**–**c**, **11a**, **22** and **23** in CD_3OD

	10a[a]	**10b**[a]	**10c**[a]	**22**[a]	**11a**	**23**[a]
H-1′	5.85	5.79	5.85	5.88	5.85	5.85
H-2′	3.26	3.16	3.20	3.00	3.10	2.90
H-3′	4.10	4.10	4.11	4.09	4.10	3.93
H-4′	3.55	3.48	3.41	3.32	3.46–3.37	3.30
H-5′	4.13–4.08	4.13	4.15–4.05	4.12–4.08	3.95	3.99
H_a-6′	4.22	4.22	4.18	4.18	3.83	3.80
H_b-6′	3.77	3.73	3.65	3.71–3.65	3.73	3.72–3.67
H-1	3.54–3.50	3.46–3.38	3.41–3.34	3.46–3.39	3.50	3.43–3.38
H_{ax}-2	1.37	1.38	1.37	1.38	1.36	1.38
H_{eq}-2	2.21	2.21	2.21	2.43	2.18	2.19
H-3	3.45–3.40	3.53	3.53	3.55–3.50	3.46–3.37	3.52–3.46
H-4	3.61	3.59	3.61	3.60	3.46–3.37	3.72–3.67
H-5	3.67	3.69	3.72	3.71–3.65	3.68–3.65	3.72–3.67
H-6	3.38	3.38	3.41	3.44	3.46–3.37	3.43–3.38
H-1″	5.44	5.44	5.52	5.36	5.43	5.37
H-2″	4.41	4.38	4.46	4.00	4.39	4.36
H-3″	4.61–4.79	4.61–4.58	4.76	4.45	4.61–4.56	4.41
H-4″	4.61–4.79	4.61–4.58	4.81–4.78	4.29	4.61–4.56	4.28
H-5″a	7.41	7.61	7.88	3.54	7.42	3.52
H-5″b	—	—	—	3.22	—	3.24
H-1‴	5.13	5.11	5.18	5.14	5.12	5.13
H-2‴	3.69–3.65	3.64–3.61	3.72–3.69	3.71–3.65	3.68–3.65	3.72–3.67
H-3‴	4.94	4.92	4.95	4.96	3.93	3.46–3.44
H-4‴	3.46–3.44	3.46–3.38	3.46–3.41	3.46–3.39	3.46–3.45	3.96–3.94
H-5‴	4.02	3.99	4.02	4.04	4.00	4.03
H_a-6‴	3.66	3.64–3.61	3.65	3.67	3.65	3.64
H_b-6‴	3.36	3.32	3.20	3.33	3.37	3.35
$J_{1',2'}$	3.9	3.9	3.9	3.8	3.8	3.7
$J_{2',3'}$	10.1	10.1	10.1	10.2	10.5	10.5
$J_{3',4'}$	9.5	8.8	9.5	9.3	8.9	8.8
$J_{4',5'}$	9.4	9.7	9.5	9.5	10.0	9.9
$J_{5',6'a}$	4.9	5.0	4.9	4.9	2.3	2.4
$J_{5',6'b}$	10.2	10.2	10.0	[b]	4.9	[b]
$J_{6'a,6'b}$	10.1	10.2	10.0	10.0	11.9	11.9
$J_{1,2ax}$	12.6	12.4	12.2	[b]	12.4	12.6
$J_{1,2eq}$	4.2	4.2	4.3	4.2	4.5	4.2
$J_{1,6}$	9.5	9.8	9.5	8.6	9.8	[b]
$J_{2ax,2eq}$	12.6	12.4	12.2	12.5	12.4	12.6
$J_{2ax,3}$	12.6	12.2	12.2	12.5	12.4	12.6
$J_{2eq,3}$	4.2	4.4	4.3	4.2	4.2	4.2
$J_{3,4}$	9.7	9.8	9.8	8.5	[b]	[b]
$J_{4,5}$	8.9	8.8	8.9	9.8	[b]	[b]
$J_{5,6}$	8.1	9.8	8.9	9.8	[b]	[b]
$J_{1'',2''}$	1.4	1.4	1.4	1.3	1.5	1.6
$J_{2'',3''}$	3.5	3.7	3.5	4.5	3.8	4.6
$J_{3'',4''}$	[b]	[b]	1.5/3.9	7.1	[b]	6.9
$J_{4'',5''a}$	6.8	6.3	6.2	3.3	6.3	3.6
$J_{4'',5''b}$	—	—	—	8.1	—	7.7
$J_{5''a,5''b}$	—	—	—	12.9	—	13.0
$J_{1''',2'''}$	1.9	1.9	1.9	1.8	1.9	1.8
$J_{2''',3'''}$	3.8	3.8	3.7	3.3	3.8	[b]
$J_{3'''4'''}$	3.8	3.8	3.7	3.3	3.8	[b]
$J_{4''',5'''}$	2.3	2.4	2.2	2.0	2.3	1.9
$J_{5''',6'''a}$	8.3	8.7	8.6	8.7	10.5	8.5
$J_{5''',6'''b}$	4.6	4.2	4.5	4.1	4.9	4.5
$J_{6'''a,6'''b}$	12.9	13.1	12.9	12.9	12.9	12.9

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

H_{ax}-2); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) see Table 7, additionally, δ 145.95 (2s), 129.04 (2d), 124.58 (2d), 74.64 (t, CH_2Ph); HRESIMS: 916.2664 (100, $[M+Na]^+$, $C_{30}H_{39}N_{17}NaO_{16}^+$; Calcd 916.2658).

4.13.3. 5″-Anilino-1,3,2′,2‴,6‴-pentaazido-1,3,2′,2‴,6‴-pentadeamino-5″-deoxyparomomycin (23). GP 5 and FC (2:2:0.1 → 2:2:0.4, $CHCl_3$–EtOAc–MeOH) gave **23** (126 mg, 92%). White solid: mp 125–127 °C;

Table 7. ^{13}C NMR chemical shifts (ppm) for **10a–c**, **11a**,**b**, **22** and **23** in CD_3OD

	10a[a]	**10b**[a]	**10c**[a]	**22**[a]	**11a**	**11b**[b]	**23**[a]
C-1′	98.66	98.86	98.76	98.81	97.77	96.40	98.26
C-2′	65.15	65.19	64.51	64.89	64.69	63.46	64.36
C-3′	69.96	69.80	69.79	69.38	71.82[c]	71.19[c]	71.92[c]
C-4′	82.88	82.99	82.89	82.85	72.62[c]	71.55[c]	71.98[c]
C-5′	64.54	69.86	64.51	64.58	74.05	73.14	74.07
C-6′	69.83	69.86	69.79	69.82	62.54	61.91	62.59
C-1	61.95	62.00	62.00	62.05	62.16	60.63	62.03
C-2	33.09	33.08	33.10	33.05	33.15	32.09	33.02
C-3	61.38	61.35	61.37	61.42	61.51	60.37	61.38
C-4	77.72	77.79[c]	77.92	77.98	77.59[d]	75.54	77.70
C-5	85.51	85.60	85.56	86.15	85.59	84.34	85.86
C-6	77.65	77.76[c]	77.74	77.90	76.56[d]	74.50	77.01
C-1″	110.98	111.01	111.34	111.86	110.63	107.26	111.22
C-2″	74.80	74.88	74.89	74.86	74.89	74.02	74.92
C-3″	79.53[c]	79.38[d]	79.31[c]	79.06	79.75[e]	78.39	78.93
C-4″	79.78[c]	79.47[d]	79.43[c]	80.70	79.93[e]	79.24	80.65
C-5″	149.80	151.34	153.51	47.40	149.86	150.54	48.36
C-1‴	99.68	99.78	99.85	99.62	99.79	98.74	99.66
C-2‴	62.14	61.94	62.00	61.79	61.98	60.63	61.75
C-3‴	70.97	71.03	71.04	71.24	69.77	68.85	69.57
C-4‴	69.83	69.86	69.76	69.69	70.99	70.01	71.16
C-5‴	75.84	75.92	75.99	75.93	75.72	74.39	75.73
C-6‴	52.35	52.54	52.44	52.60	52.29	51.33	52.45

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Solvent $(CD_3)_2CO$.
[c–e] May be interchanged.

$[\alpha]_D^{25}$ +156.8 (*c* 0.65, MeOH); $R_f = 0.13$ (2:2:0.5, $CHCl_3$–EtOAc–EtOAc); IR (ATR): *ν* 3384w, 2936w, 2098s, 1602w, 1506w, 1373w, 1326w, 1255m, 1123m, 1017s cm^{-1}; ^1H NMR (400 MHz, CD_3OD) see Table 6, additionally, *δ* 7.13–7.07 (m, 2 arom. H), 6.71–6.61 (m, 3 arom. H); ^{13}C NMR (100 MHz, CD_3OD) see Table 7, additionally, *δ* 149.72 (s), 130.09 (2d), 114.60 (2d), 111.22 (d); HRMALDIMS: 844.2868 (100, $[M+Na]^+$, $C_{29}H_{40}N_{16}NaO_{13}^+$; Calcd 844.2858). Anal. Calcd for $C_{29}H_{40}N_{16}O_{13}$ (820.73): C, 42.44; H, 4.91; N, 27.31. Found: C, 42.27; H, 4.98; N, 26.76.

4.13.4. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-5″-[*N*-(4-nitrobenzyl)]paromomycincarboxamide (16b). GP 5 on 86 mg of **15b** and FC (1:1:0 → 6:6:0.5, $CHCl_3$–EtOAc–MeOH) gave **16b** (62 mg, 79%). White solid; $[\alpha]_D^{26}$ +97.9 °C (*c* 0.235, MeOH); $R_f = 0.45$ (2:2:1, $CHCl_3$–EtOAc–MeOH); ^1H NMR (500 MHz, CD_3OD) see Table 10, additionally *δ* 8.21 (d, *J* 8.8 Hz, 2 arom. H), 7.56 (d, *J* 8.9 Hz, 2 arom. H), 4.58 (d, *J* 15.9 Hz, $ArCH_aH_bNHCO$), 4.51 (d, *J* 15.9 Hz, $ArCH_aH_bNHCO$); ^{13}C NMR (125 MHz, CD_3OD) see Table 11, additionally *δ* 148.67 (s), 147.55 (s), 129.51 (2d), 124.76 (2d), 43.38 (t, $ArCH_2NHCO$); HRMALDIMS: 916.2636 (100, $[M+Na]^+$, $C_{30}H_{39}N_{17}NaO_{16}^+$; Calcd 916.2658), 917.2650 (39, $[M+H+Na]^+$, $C_{30}H_{40}N_{17}NaO_{16}^+$; Calcd 917.2737). Anal. Calcd for $C_{30}H_{39}N_{17}O_{16}\cdot CH_3OH$ (927.78): C, 40.22; H, 4.68; N 25.72. Found: C, 40.21; H 4.67; N, 25.44.

4.13.5. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-5″-(*N*-phenyl)paromomycincarboxamide (16c). GP 5 on 86 mg of **15c** and FC (1:1:0 → 6:6:0.4, $CHCl_3$–EtOAc–MeOH) gave **16c** (29 mg, 63%). White solid; $R_f = 0.46$ (2:2:1, $CHCl_3$–EtOAc–MeOH); ^1H NMR (300 MHz, CD_3OD) see Table 10, additionally *δ* 7.65–7.61 (m, 2 arom. H), 7.34–7.28 (m, 2 arom. H), 7.13–7.08 (m, arom. H).

4.13.6. 1,3,2′,2‴,6‴-Pentadeamino-1,3,2′,2‴,6‴-pentaazido-5″-paromomycincarboxamide (21). GP 5 on 35 mg of **20** and FC (1:1:0 → 12:12:1.5, $CHCl_3$–EtOAc–MeOH) gave **21** (24 mg, 72%). White solid; $R_f = 0.41$ (2:2:1, $CHCl_3$–EtOAc–MeOH); ^1H NMR (300 MHz, CD_3OD) see Table 10.

4.14. General procedure for the reduction of azides with propane-1,3-dithiol (GP 6)

A 0.1 M soln of the protected *N*-alkoxyimino or amido azide (1 equiv) in MeOH was treated with Et_3N (25 equiv) and propane-1,3-dithiol (25 equiv), stirred at 25 °C for 24–48 h and evaporated. A soln of residue in H_2O was washed with EtOAc (1×). The aq layer was evaporated to give the crude product.

4.14.1. 1,3,2′,2‴,6‴-Pentaammonium-5″-deoxy-5″-(hydroxyimino)paromomycin pentaacatate (12a·5HOAc). GP 6 FC (9:1 → 7:3, MeOH–25% aq NH_3) and dissolution in 10% aq HOAc, partial evaporation and lyophilisation

gave **12a**·5HOAc (53 mg, 76%). White solid: mp 112 °C (dec); $[\alpha]_D^{25}$ +42.7 (*c* 0.19, H_2O); $R_f = 0.20$ (7:3, MeOH–25 aq NH_3); IR (ATR): *v* 3500–2700m, 1538s, 1399s, 1336m, 1140m, 1118m, 1011s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 8; additionally, δ 1.92 (s, 5OAc); ^{13}C NMR (125 MHz, D_2O) see Table 9, additionally, δ 184.91 (s, 5C=O), 25.78 (q, 5 Me); HRMALDIMS: 596.2136 (100), 651.2797 (14, $[M+Na]^+$, $C_{23}H_{44}N_6NaO_{14}^+$; Calcd 651.2813). Anal. Calcd for $C_{33}H_{64}N_6O_{19}$ (928.89): C, 42.67; H, 6.94; N, 9.05. Found: C, 42.40; H, 6.83; N, 9.34.

Table 8. ^1H NMR chemical shifts (ppm) and coupling constants (Hz) for **12a**,**b** and **24** in D_2O

	12a·5HOAc[a]	**12b**·5HOAc[a]	**24**[a]
H-1′	5.75	5.79	5.34
H-2′	3.34	3.11	2.52
H-3′	3.89	3.87	3.51
H-4′	3.52	3.31	3.19
H-5′	3.84–3.80	3.73–3.70	3.76
H_a-6′	3.89	3.88	3.81
H_b-6′	3.82–3.78	3.71	3.67
H-1	3.31–3.23	3.34–3.30	2.73
H_{ax}-2	1.67	1.80	1.19
H_{eq}-2	2.33	2.44	1.94
H-3	3.31–3.23	3.46	2.87
H-4	3.81	3.98	3.24
H-5	3.89	3.87	3.68–3.61
H-6	3.65	3.65	3.42
H-1″	5.44	5.42	5.31
H-2″	4.46	4.37	4.38
H-3″	4.67	4.68	4.49
H-4″	4.80–4.74	4.81	4.29
H-5″a	7.72	7.86	3.44
H-5″b	—	—	3.35
H-1‴	5.32	5.20	4.94
H-2‴	3.60	3.55	3.02–3.00
H-3‴	4.23	4.17	4.00
H-4‴	3.84–3.80	3.75	3.68–3.61
H-5‴	4.30	3.99–3.97	3.90
H_a-6‴	3.43	3.32	2.98
H_b-6‴	3.38	3.24	2.88
$J_{1',2'}$	4.0	4.0	3.7
$J_{2',3'}$	10.6	10.3	10.3
$J_{3',4'}$	10.7	8.9	9.2
$J_{4',5'}$	9.7	8.9	9.8
$J_{5',6'a}$	6.9	3.3	6.2
$J_{5',6'b}$	[b]	6.4	2.1
$J_{6'a,6'b}$	10.0	10.6	12.1
$J_{1,2ax}$	12.7	12.6	12.1
$J_{1,2eq}$	4.2	4.1	4.1
$J_{1,6}$	9.9	9.1	9.2
$J_{2ax,2eq}$	12.7	12.6	12.1
$J_{2ax,3}$	12.7	12.6	12.1
$J_{2eq,3}$	4.2	4.1	4.3
$J_{3,4}$	8.9	10.1	9.4
$J_{4,5}$	8.8	8.9	9.6
$J_{5,6}$	8.9	9.1	9.2
$J_{1'',2''}$	2.5	3.0	2.2
$J_{2'',3''}$	4.8	4.6	4.8
$J_{3'',4''}$	6.2	5.0	6.3
$J_{4'',5''a}$	5.8	5.0	4.7
$J_{4'',5''b}$	—	—	6.8
$J_{5''a,5''b}$	—	—	13.2
$J_{1''',2'''}$	1.7	1.7	1.8
$J_{2''',3'''}$	3.0	2.9	3.2
$J_{2''',4'''}$	1.1	1.2	[b]
$J_{3'''4'''}$	3.0	3.1	3.2
$J_{4''',5'''}$	1.6	1.7	1.6
$J_{5''',6'''a}$	5.9	5.9	7.8
$J_{5''',6'''b}$	4.1	4.1	4.4
$J_{6'''a,6'''b}$	13.8	13.7	13.5

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

4.14.2. 1,3,2′,2‴,6‴-Pentaammonium-5″-deoxy-5″-[*N*-(4-nitrobenzyloxy)imino]paromomycin pentaacetate (12b·5HOAc). GP 6, FC (9:1 → 7:3, MeOH–25% aq NH_3) and dissolution in 10% aq HOAc, partial evaporation and lyophilisation gave **12b**·5HOAc (76 mg, 71%). White solid: mp 113 °C (dec); $[\alpha]_D^{25}$ +52.1 (*c* 0.165, H_2O); $R_f = 0.26$ (7:3, MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700m, 2920m, 1699w, 1544s, 1518s, 1399s, 1344s, 1103w, 1010s cm^{-1}; ^1H NMR (600 MHz, D_2O) see Table 8, additionally, δ 8.28–8.26 (m, 2 arom. H), 7.63–7.61 (m, 2 arom. H), 5.29 (s, CH_2Ph), 1.94 (s, 5OAc); ^{13}C NMR (150 MHz, D_2O) see Table 9, additionally, δ 182.62 (s, 5C=O), 150.17 (s), 147.60 (s), 131.43 (2d), 126.70 (2d), 77.30 (s,*C*H_2Ph); 25.42 (q, 5 Me); HRMALDIMS: 596.2751 (100), 764.3265 (12, $[M+H]^+$, $C_{30}H_{50}N_7O_{16}^+$; Calcd 764.3314), 786.3115 (23, $[M+Na]^+$, $C_{30}H_{49}N_7NaO_{16}^+$; Calcd 786.3133). Anal. Calcd for $C_{40}H_{69}N_7O_{21}\cdot H_2O$ (1082.02): C, 44.40; H, 6.61; N, 9.06. Found: C, 44.54; H, 6.26; N, 9.04.

Table 9. ^{13}C NMR chemical shifts (ppm) for **12a**,**b** and **24** in D_2O

	12a·5HOAc[a]	**12b**·5HOAc[a]	**24**[a]
C-1′	98.28	97.57	101.57
C-2′	56.40	56.17	57.94
C-3′	72.18	71.54	75.97
C-4′	71.85	71.93	72.65
C-5′	76.24	76.73	75.80
C-6′	62.89	63.10	63.53
C-1	51.68[b]	52.38	53.01
C-2	32.78	32.78	37.72
C-3	52.72[b]	51.47	52.81
C-4	81.54	79.35	85.81
C-5	87.72	87.73	86.90
C-6	75.58	75.15	79.70
C-1″	112.83	112.89	111.93
C-2″	76.24	76.42	75.66
C-3″	81.76	81.60	80.69
C-4″	80.77	80.93	82.03
C-5″	152.37	153.29	49.26
C-1‴	98.87	98.88	101.69
C-2‴	53.46	51.47	53.44
C-3‴	70.41	70.27[b]	73.13
C-4‴	71.36	70.24[b]	71.20
C-5‴	72.76	72.61	76.69
C-6‴	43.15	43.16	43.62

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Assignments can be interchanged.

4.14.3. 5″-Paromomycincarboxamide (17a). **GP 6** on 24 mg of **21**, FC (1:0 → 1:1, THF–MeOH, then 9:1 → 8:2, MeOH–25% aq NH_3), evaporation and lyophilisation gave **17a** (17 mg, 86%). White solid; $[\alpha]_D^{25}$ +33.0 (*c* 0.205, H_2O); $R_f = 0.22$ (7:3, MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700m, 2920m, 1681m, 1579m, 1487w, 1399s, 1336w, 1135s, 1016s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 12; ^{13}C NMR (125 MHz, D_2O) see Table 13; HRMALDIMS: 629.2978 (100, $[M+H]^+$, $C_{23}H_{45}N_6O_{14}^+$; Calcd 629.2994), 651.2797 (32, $[M+Na]^+$, $C_{23}H_{44}N_6NaO_{14}^+$; Calcd 651.2813).

Table 10. ^1H NMR chemical shifts (ppm) and coupling constants (Hz) for **13**, **15b–c**, **16b–c**, **20**, **21** in CD_3OD

	13[a]	**15b**[a]	**15c**	**20**	**16b**[a]	**16c**	**21**
H-1′	5.65	5.58	5.78	5.68	5.65	5.75	5.68
H-2′	3.23	2.97	3.14	3.21	2.94	3.01	3.04
H-3′	4.09	4.06	4.09	4.22–4.00	3.91	3.92	3.92
H-4′	3.59–3.53	3.45	3.83–3.36	3.58–3.42	3.36	3.56–3.32	3.79–3.35
H-5′	4.13	4.15–4.12	4.18–4.08	4.22–4.00	3.94–3.90	3.92–3.82	3.96–3.89
H_a-6′	4.23	4.19	4.21	4.23	3.83	3.90–3.60	3.83
H_b-6′	3.82–3.73	3.76–3.70	3.83–3.36	3.80–3.63	3.77–3.71	3.90–3.60	3.79–3.35
H-1	3.52–3.43	3.44–3.42	3.83–3.36	3.58–3.42	3.44–3.39	3.56–3.32	3.79–3.35
H_{ax}-2	1.49	1.46	1.47	1.46	1.42	1.43	1.41
H_{eq}-2	2.21	2.23	2.22	2.22	2.19	2.19	2.18
H-3	3.59–3.53	3.54–3.49	3.83–3.36	3.58–3.42	3.58–3.47	3.56–3.32	3.79–3.35
H-4	3.86	3.67	3.83–3.36	3.80–3.63	3.77–3.71	3.90–3.60	3.79–3.35
H-5	3.82–3.73	3.74	3.83–3.36	3.80–3.63	3.77–3.71	3.90–3.60	3.79–3.35
H-6	3.82–3.73	3.55	3.83–3.36	3.55	3.60–3.58	3.62	3.79–3.35
H-1″	5.47	5.54	5.52	5.52	5.55	5.54	5.51
H-2″	4.32	4.38	4.42	4.32	4.35	4.40	4.31
H-3″	4.66	4.68	4.73	4.63	4.67	4.72	4.62
H-4″	4.56	4.63	4.68	4.55	4.61	4.66	4.52
H-1‴	5.21	5.20	5.18	5.19	5.18	5.17	5.17
H-2‴	3.86	3.70–3.67	3.83–3.36	3.80–3.63	3.67	3.70–3.68	3.79–3.35
H-3‴	3.99	3.98	3.97	3.97	3.98	3.98	3.96
H-4‴	3.52–3.43	3.48–3.47	3.83–3.36	3.58–3.42	3.58–3.47	3.50–3.42	3.79–3.35
H-5‴	4.02	4.03	4.01–3.96	4.02	4.03	4.01–3.90	4.06–3.99
H_a-6‴	3.63	3.62	3.83–3.36	3.80–3.63	3.61	3.51	3.79–3.35
H_b-6‴	3.47	3.39	3.39	3.42	3.38	3.56–3.32	3.79–3.35
$J_{1',2'}$	3.8	3.8	4.0	3.7	3.7	3.9	3.4
$J_{2',3'}$	10.1	10.1	10.0	10.0	10.6	10.4	10.6
$J_{3',4'}$	2.8	9.3	9.7	[b]	8.8	9.1	9.0
$J_{4',5'}$	9.8	9.4	[b]	[b]	10.0	[b]	[b]
$J_{5',6'a}$	4.9	5.0	4.7	5.0	2.5	[b]	2.2
$J_{5',6'b}$	12.5	[b]	[b]	[b]	[b]	[b]	[b]
$J_{6'a,6'b}$	10.1	9.7	9.7	9.3	11.9	[b]	12.1
$J_{1,2ax}$	12.8	12.6	12.5	12.8	12.6	12.6	12.5
$J_{1,2eq}$	4.4	4.3	4.3	4.4	4.4	4.1	4.4
$J_{1,6}$	[b]	9.6	[b]	9.3	[b]	9.1	[b]
$J_{2ax,2eq}$	12.8	12.8	12.5	12.8	12.6	12.6	12.5
$J_{2ax,3}$	12.8	12.6	12.5	12.8	12.6	12.6	12.5
$J_{2eq,3}$	4.4	4.3	4.3	4.4	4.4	4.1	4.4
$J_{3,4}$	9.4	9.4	[b]	[b]	[b]	[b]	[b]
$J_{4,5}$	9.4	9.3	[b]	[b]	[b]	[b]	[b]
$J_{5,6}$	[b]	9.3	[b]	9.3	[b]	9.1	[b]
$J_{1'',2''}$	2.9	2.7	2.2	2.8	2.2	1.9	2.2
$J_{2'',3''}$	4.3	4.2	3.7	4.0	4.3	4.1	4.0
$J_{3'',4''}$	4.3	5.3	5.7	5.6	5.9	6.1	5.9
$J_{1''',2'''}$	2.0	2.0	1.6	1.9	2.0	1.7	1.9
$J_{2''',3'''}$	3.9	3.8	3.7	3.7	3.8	4.1	3.7
$J_{2''',4'''}$	3.9	[b]	[b]	[b]	≈1.4	[b]	[b]
$J_{3'''4'''}$	3.9	3.8	3.7	3.7	3.8	4.1	3.7
$J_{4''',5'''}$	2.4	2.3	[b]	2.2	2.2	[b]	[b]
$J_{5''',6'''a}$	7.8	8.2	[b]	[b]	8.2	7.8	[b]
$J_{5''',6'''b}$	5.6	4.9	5.6	5.0	4.8	[b]	[b]
$J_{6'''a,6'''b}$	12.8	12.9	[b]	12.8	12.9	12.6	[b]

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

Table 11. ^{13}C NMR chemical shifts (ppm) for **13**, **15b–c**, **16b**, **18** in CD_3OD

	13[a]	15b[a]	15c	16b[a]	18[b]	19[b]
C-1′	99.50	99.92	99.52	98.44	98.17	97.97
C-2′	65.25	65.06	65.30	64.61	61.42	61.30
C-3′	69.40	69.27	69.58	72.02	68.62	68.68
C-4′	83.05	83.01	82.98	72.02	78.78	78.46
C-5′	64.67	64.65	64.67	74.22	63.41	63.22
C-6′	69.87	69.81	69.87	62.47	68.62	68.52
C-1	61.28	61.94	62.02	61.84	58.03	57.90
C-2	32.96	32.80	32.89	32.91	31.23	31.28
C-3	61.34	61.03	61.23	61.41	58.95	58.93
C-4	75.35	78.19	77.84	76.40	[c]	75.96
C-5	85.35	85.56	85.80	85.66	81.65	81.83
C-6	76.25	76.48	76.57	76.19	[c]	74.99
C-1″	105.79	108.90	109.05	108.15	106.05	105.24
C-2″	76.33	75.40	75.48	75.25	[c]	74.00
C-3″	81.43	80.34	80.69	80.23	[c]	78.79
C-4″	83.23	82.21	82.72	82.04	78.78	80.62
C-5″	178.90	172.94	170.74	173.18	181.47	170.80
C-1‴	100.06	100.02	100.29	99.76	100.04	100.13
C-2‴	62.56	62.16	62.23	62.15	57.29	57.07
C-3‴	71.20	71.09	71.10	71.13	68.62	68.52
C-4‴	70.09	69.81	69.87	69.83	65.80	65.50
C-5‴	75.35	75.71	75.77	75.72	73.05	72.99
C-6‴	52.29	52.40	52.09	52.40	50.54	50.44

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Solvent $CDCl_3$.
[c] Not assigned.

4.14.4. 5″-*N*-(4-Nitrobenzyl)paromomycincarboxamide (17b). **GP 6** on 33 mg of **16b**, FC (1:0 → 1:1, THF–MeOH, then 85:15 → 8:2, MeOH–25% aq NH_3), evaporation and lyophilisation gave **17b** (21 mg, 75%). White solid; $[\alpha]_D^{25}$ +22.8 (*c* 0.21, H_2O); $R_f = 0.17$ (8:2, MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700w, 2903w, 1666w, 1602w, 1516m, 1345m, 1108s, 1028s cm^{-1}; ^{1}H NMR (500 MHz, D_2O) see Table 12, additionally, *δ* 8.25 (d, *J* 8.8 Hz, 2 arom. H), 7.56 (d, *J* 8.8 Hz, 2 arom. H), 4.71 (d, $J \approx 16.7$ Hz, ArCH_a-H_bNHCO), 4.47 (d, *J* 15.7 Hz, ArC$H_a H_b$NHCO); ^{13}C NMR (125 MHz, D_2O) see Table 13, additionally, *δ* 149.71 (s), 148.12 (s), 130.95 (2d), 126.73 (2d), 45.22 (t, Ar*C*H_2NHCO); HRMALDIMS: 764.3294 (100, $[M+H]^+$, $C_{30}H_{50}N_7O_{16}^+$; Calcd 764.3314), 765.3321 (36, $[M+2H]^+$, $C_{30}H_{51}N_7O_{16}^+$; Calcd 765.3392), 786.3138 (40, $[M+Na]^+$, $C_{30}H_{49}N_7NaO_{16}^+$; Calcd 786.3133).

4.14.5. 5″-*N*-Phenylparomomycincarboxamide (17c). **GP 6** on 20 mg of **16c**, FC (1:3:0 → 1:3:1, $CHCl_3$–MeOH–25% aq NH_3), evaporation and lyophilisation gave **17c** (15 mg, 89%). Yellowish solid; $[\alpha]_D^{25}$ +23.6 (*c* 0.265, H_2O); $R_f = 0.21$ (8:2, MeOH–25% aq NH_3); IR (ATR): *v* 3500–2700w, 1670w, 1599w, 1492w, 1415m, 1047s cm^{-1}; ^{1}H NMR (500 MHz, D_2O) see Table 12, additionally, *δ* 7.59–7.56 (m, 2 arom. H), 7.49–7.45 (m, 2 arom. H), 7.32–7.29 (m, arom. H); ^{13}C NMR (125 MHz, D_2O) see Table 13, additionally, *δ* 138.79 (s), 132.22 (2d), 128.95 (d), 124.10 (2d); HRMALDIMS: 705.3288 (100, $[M+H]^+$, $C_{29}H_{49}N_6O_{14}^+$; Calcd 705.3307), 706.3320 (34, $[M+2H]^+$, $C_{29}H_{50}N_6O_{14}^+$; Calcd 706.3385), 727.3083 (38, $[M+Na]^+$, $C_{29}H_{48}N_6NaO_{14}^+$; Calcd 727.3126).

Table 12. ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz) for **14**, **17a–c** in D_2O

	14[a]	17a[a]	17b[a]	17c
H-1′	5.78	5.48	5.67	5.92
H-2′	3.45–3.30	3.02	3.12	3.43
H-3′	3.99–3.90	3.55	3.79	3.96
H-4′	3.50	3.71	3.42	3.42
H-5′	3.80–3.72	3.87–3.84	3.80–3.68	3.82–373
H_a-6′	3.99–3.90	3.89	3.85	3.91
H_b-6′	3.80–3.72	3.79	3.80–3.68	3.82–373
H-1	3.45–3.30	3.08–3.01	3.24–3.15	3.39
H_{ax}-2	1.84	1.44	1.59	1.94
H_{eq}-2	2.46	2.14	2.24	2.52
H-3	3.55–3.48	3.08–3.01	3.24–3.15	3.62
H-4	3.99	3.66	3.65	4.04
H-5	3.99–3.90	3.84	3.87	4.14
H-6	3.80–3.72	3.47	3.80–3.68	3.82–373
H-1″	5.48	5.51	5.50	5.56
H-2″	4.36	4.43	4.49	4.58
H-3″	4.58	4.70	4.69	4.80
H-4″	4.45	4.62	4.62	4.73
H-1‴	5.30	5.13	5.17	5.32
H-2‴	3.61	3.25	3.40	3.65
H-3‴	4.24	4.12	4.15	4.25
H-4‴	3.80	3.76–3.75	3.80–3.68	3.74
H-5‴	4.30	4.23	4.24–4.21	4.21
H_a-6‴	3.44–3.40	3.37	3.24–3.15	3.04
H_b-6‴	3.45–3.30	3.30	3.24–3.15	2.80
$J_{1',2'}$	4.0	3.8	3.9	4.0
$J_{2',3'}$	[b]	9.9	10.7	10.7
$J_{3',4'}$	9.4	9.9	9.3	9.1
$J_{4',5'}$	9.4	9.1	9.3	10.7
$J_{5',6'a}$	[b]	2.1	2.1	[b]
$J_{5',6'b}$	[b]	5.4	[b]	[b]
$J_{6'a,6'b}$	[b]	12.0	12.1	[b]
$J_{1,2ax}$	12.5	12.7	12.6	12.7
$J_{1,2eq}$	[b]	4.1	3.9	4.3
$J_{1,6}$	[b]	9.4	[b]	10.7
$J_{2ax,2eq}$	12.5	12.7	12.6	12.7
$J_{2ax,3}$	12.5	12.7	12.6	12.7
$J_{2eq,3}$	[b]	4.1	3.9	4.3
$J_{3,4}$	9.2	9.3	10.1	9.1
$J_{4,5}$	9.2	9.2	9.2	9.1
$J_{5,6}$	[b]	9.2	9.2	10.1
$J_{1'',2''}$	2.9	2.5	1.6	[b]
$J_{2'',3''}$	4.5	4.6	4.5	4.3
$J_{3'',4''}$	5.7	6.0	7.2	7.9
$J_{1''',2'''}$	1.5	1.8	1.6	1.7
$J_{2''',3'''}$	3.1	3.3	3.1	3.1
$J_{3'''4'''}$	3.1	3.3	3.1	3.1
$J_{4''',5'''}$	≈1.2	1.7	[b]	2.0
$J_{5''',6'''a}$	3.3	7.8	[b]	3.3
$J_{5''',6'''b}$	7.5	3.6	[b]	8.2
$J_{6'''a,6'''b}$	[b]	13.6	[b]	13.6

[a] Assignment based on DQFCOSY and HSQC spectrum.
[b] Not determined.

Table 13. ^{13}C NMR chemical shifts (ppm) for **14** and **17a–c** in D_2O

	14[a]	17a[a]	17b[a]	17c
C-1′	99.31	100.77	99.28	98.72
C-2′	56.54	57.51	56.90	56.57
C-3′	71.47	72.27	72.01	71.36
C-4′	71.99	74.54	72.60	76.54
C-5′	75.37	75.81	75.97	74.87
C-6′	63.04	63.14	62.93	62.99
C-1	52.58	53.12	51.74	52.60
C-2	31.31	35.67	33.78	30.64
C-3	51.52	52.26	53.11	51.40
C-4	80.60	84.33	82.57	79.38
C-5	86.44	86.68	87.37	86.88
C-6	76.34	77.05	75.85	71.97
C-1″	112.02	111.06	112.71	113.13
C-2″	76.34	75.92	75.41	76.18
C-3″	82.33	81.54	80.30	81.38
C-4″	84.15	82.66	82.13	82.26
C-5″	180.00	177.41	174.53	172.96
C-1‴	98.29	100.89	98.43	97.93
C-2‴	53.43	54.47	53.94	53.44
C-3‴	70.39	72.27	71.13	70.04
C-4‴	69.82	70.86	69.94	69.47
C-5‴	73.03	73.79	73.62	73.39
C-6‴	43.00	35.67	43.06	42.81

[a] Assignment based on DQFCOSY and HSQC spectrum.

4.15. General procedure for the Staudinger reaction (GP 7)

A 0.02 M soln of the azide (1 equiv) in THF was treated with 1 N NaOH (0.5 mL) and 1 M PMe_3 in THF (6 equiv), stirred for 2–4 h at 50 °C and evaporated. A soln of the residue in H_2O was washed with EtOAc (1×) and evaporated to give the crude amines.

4.15.1. 5″-Anilino-5″-deoxyparomomycin (24). GP 7 and FC (1:4:1 → 1:4:2, $CHCl_3$–MeOH–25% aq NH_3) gave **23** (25 mg, 73%). White solid: mp 153 °C (dec); $[\alpha]_D^{25}$ +58.7 (*c* 0.35, H_2O); $R_f = 0.37$ (1:4:2.5, $CHCl_3$–MeOH–25% aq NH_3); IR (ATR): *ν* 3292m, 2937m, 1647w, 1602m, 1497w, 1456w, 1381w, 1330w, 1257w, 1092w, 1013s cm^{-1}; ^1H NMR (400 MHz, D_2O) see Table 6, additionally, *δ* 7.29–7.25 (m, 2 arom. H), 6.85–6.81 (m, 3 arom. H); ^{13}C NMR (100 MHz, D_2O) see Table 7, additionally, *δ* 150.55 (s), 132.36 (2d), 111.53 (d), 116.71 (2d); HRMALDIMS: 714.3340 (100, $[M+Na]^+$, $C_{29}H_{50}N_6NaO_{13}^+$; Calcd 714.3334). Anal. Calcd for $C_{39}H_{70}N_6O_{18}\cdot 3H_2O$ (1045.04): C, 44.82; H, 7.33; N, 8.04. Found: C, 44.86; H, 6.95; N, 8.24.

4.15.2. Ammonium-5″-carboxyparomomycin monoacetate (14). GP 7 on 65 mg of **13** and FC (1:0 → 1:1, THF–MeOH, then 85:15 → 8:2, MeOH–25% aq NH_3) gave 4′,6′-*O*-benzylidene-5″-carboxyparomomycin (46 mg, 84%). Under H_2, a suspension of 4′,6′-*O*-benzylidene-5″-carboxyparomomycin (0.047 mmol, 34 mg) in 80% aq AcOH (2 mL) and 10% Pd/C (8 mg) was stirred at 23 °C for 20 h, filtered through a pad of *Celite* and evaporated. FC (RP C-18, 1:8:0.05, MeOH–H_2O–AcOH) gave **14** (21 mg, 64%). White solid: mp 227 °C (dec); $R_f = 0.1$. (75:25, MeOH–25% aq NH_3); IR (ATR): *ν* 3041m, 1663s, 1516w, 1433M, 1409m, 1183s, 1124s, 1047s, 1026s cm^{-1}; ^1H NMR (500 MHz, D_2O) see Table 12, additionally, *δ* 1.90 (s, CH_3CO_2H); ^{13}C NMR (125 MHz, D_2O) see Table 13, *δ* 180.14 (s, CH_3CO_2H), 25.78 (q, CH_3CO_2H); HRMALDIMS: 620.2816 (100, $C_{23}H_{44}N_5O_{15}^+$; Calcd 630.2834).

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