Synthesis and Evaluation of Paromomycin Derivatives Modified at C(4′)

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The 2-amino-2-deoxy-α-D-glucopyranosyl moiety (ring I) of paromomycin was replaced by a 2,4-diamino-2,4-dideoxy-α-D-glucopyranosyl, 2,4-diamino-2,4-dideoxy-α-D-galactopyranosyl, 2-amino-2-deoxy-α-D-galactopyranosyl, or 3,4,5-trideoxy-4-aza-α-D-*erythro*-heptoseptanosyl moiety to investigate the effect of the substituent at C(4′) on the interaction with ribosomal RNA. The triflate **6** was prepared from the key intermediate pentaazido 3′,6′-dibenzyl ether **5**, and the hexosulose **10** was obtained by oxidation of **5** with *Dess–Martin*'s periodinane. Stereoselective reduction of **10** with $NaBH_4$ gave the alcohol **11** that was transformed into the triflate **12**. The epimeric hexaazides **7** and **13** were obtained by treating the triflates **6** and **12**, respectively, with tetrabutylammonium azide. Periodate cleavage of glycol **2** yielded the dialdehyde **24** that was reductively aminated with aniline and benzylamine to give the 3,4,5-trideoxy-4-aza-α-D-*erythro*-heptoseptanosides **25** and **26**, respectively. Standard azide reduction and debenzylation yielded **9** (2,4-diamino-2,4-dideoxy-α-D-galactopyranosyl ring I), **13** (2-amino-2-deoxy-α-D-galactopyranosyl ring I), **17** (2,4-diamino-2,4-dideoxy-α-D-glucopyranosyl ring I), and **27** and **28** (3,4,5-trideoxy-4-aza-α-D-*erythro*-heptoseptanosyl ring I). The derivatives **9** and **13** possessing a D-*galacto*-configured ring I were less active than the corresponding D-*gluco*-analogues **17** and paromomycin (**1**), respectively. The *C(4′)*-aminodeoxy derivative **17** (D-*gluco* ring I) and the known 4′-deoxyparomomycin (**23**), prepared by a new route, displayed slightly lower antibacterial activities than paromomycin (**1**). Cell-wall permeability is not responsible for the unexpectedly low activity for **17**, as shown by cell-free translation assays. The results evidence that the orientation of the substituent at C(4′) is more important than its nature for drug binding and activity.

Introduction. – The crystal structures of paromomycin [1][2] and of a few other aminoglycoside antibiotics in complex with the decoding A site of bacterial 16S rRNA show a very similar relative orientation of rings I and II that is responsible for the key interactions that result in drug binding [3–10]; for paromomycin, the interaction of ring I appears to be most important. The base at position 1408 of 16S rRNA is a critical determinant of aminoglycoside selectivity. Drug-susceptible bacterial ribosomes are characterised by an adenine, while drug resistance of eukaryotic cytoplasmic ribosomes is conferred by a guanine at the homologous position [11]. Considering that modifications of ring I may selectively affect the interaction with the bacterial (in contradistinction to the eukaryotic) decoding site, we had replaced HO–C(6′) of paromomycin with either H or F [12]. However, these modifications reduced the activity both against *M. smegmatis* wild-type ribosomes characterized by A1408 and against A1408G (eukaryotic type) mutants. The 3′,6′-anhydro derivative, where ring I adopts an inverted chair conformation, lost any activity.

It appeared of interest to investigate the effect on the biological activity of the somewhat less incisive inversion of the configuration of C(4′) and of the introduction of other substituents. We planned to prepare *galacto*-configured paromomycin analogues, and to replace the C(4′)–OH by a C(4′)–NH_2 group. The crystal structure of paromomycin in complex with the bacterial 16S rRNA decoding site suggests that C(4′)–OH interacts with the phosphate group of A1493, an interaction that might be strengthened by replacing the OH by a ammonium group. For the sake of a better comparison, we also wished to evaluate the activity and selectivity in our test system of the known 4′-deoxyparomomycin (**23**) [13], with the intention of preparing this compound by a different synthetic route. Finally, we planned to also synthesize the (3′,4′-imido)-heptoseptanosyl 4′-azaparomomycin derivatives **27** and **28**, this time opting for a novel, if again more incisive, modification of ring I.

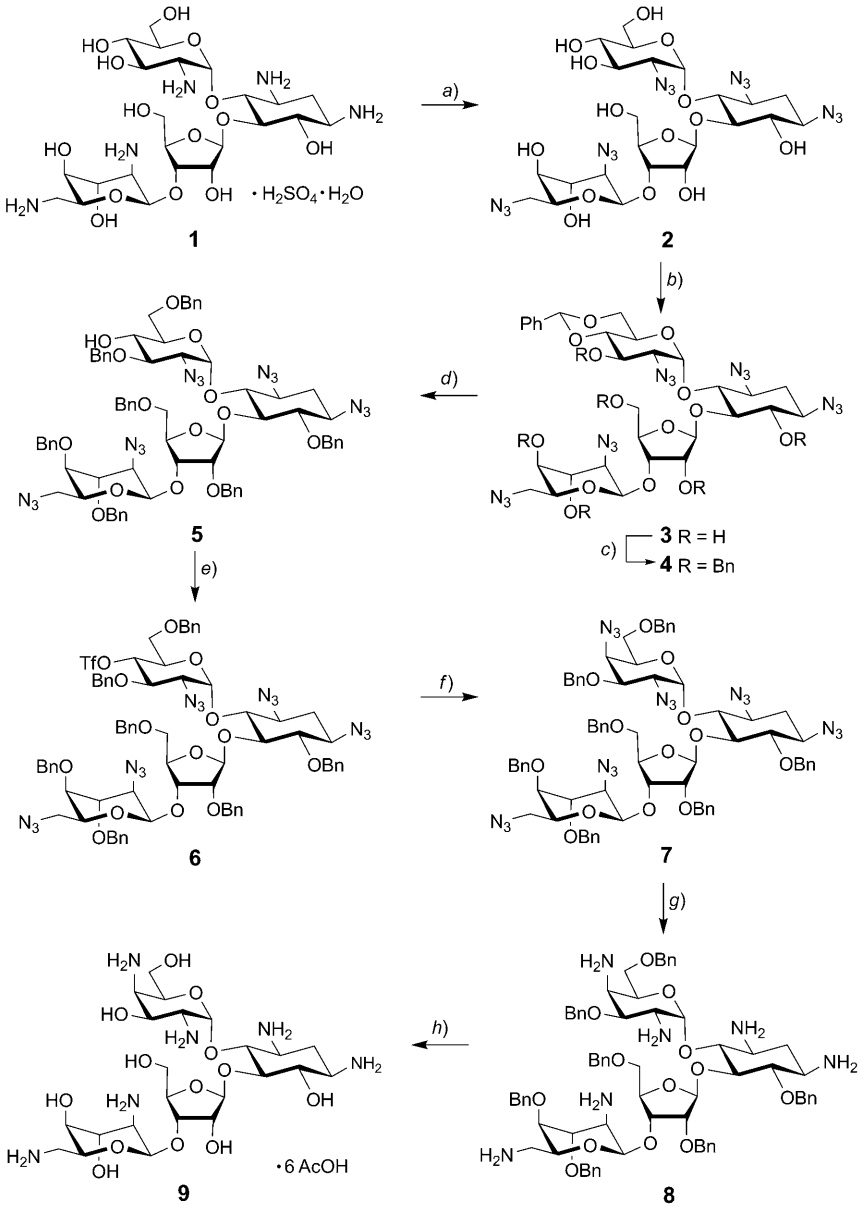
Results and Discussion. – The known pentaazido-paromomycin **2** [12] was prepared from paromomycin sulfate (**1**) by diazo transfer [14–16] from TfN_3 in the presence of $CuSO_4 \cdot 5\ H_2O$ and *t*-BuOK (*Scheme 1*). Benzylidenation of **2** gave 75% of **3** [12] that was *O*-benzylated with BnBr in the presence of NaH in THF to yield 55% of the hexabenzyl ether **4**. Regioselective reductive cleavage of the 1,3-dioxane ring of **4** with $NaCNBH_3$ in the presence of 0.7N HCl [17][18] gave the 6′-*O*-benzyl ether **5** (93%). This common intermediate was transformed into the triflate **6** (62%) and further, by treatment with Bu_4NN_3 [19], to the *galacto*-configured hexaazide **7** (79%). *Staudinger* reduction of **7** and hydrolytic workup gave the hexaamine **8** (71%). Hydrogenolysis of the BnO groups in the presence of $Pd(OH)_2$ in $AcOH/H_2O$ 2:3 yielded the 4′-*epi*-4′-amino-4′-deoxyparomomycin hexaacetate **9** (88%). Remarkably, replacing $AcOH/H_2O$ 2:3 by $AcOH/H_2O/D_2O$ 2:2:1 improved this hydrogenolysis.

Oxidation of the alcohol **5** with *Dess–Martin*'s periodinane gave the hexos-4-uloside **10** (81%; *Scheme 2*) that was reduced with $NaBH_4$ to the pentaazide **11** (77%) possessing a *galacto*-configured ring I. *Staudinger* reduction and hydrolysis of **11** to the pentaamine **12** (65%), followed by hydrogenolysis, gave the 4′-*epi*-paromomycin pentaacetate trihydrate **13** (82%).

To prepare the 4′-amino-4′-deoxyparomomycin analogue **17**, we transformed the pentaazide **11** into the triflate **14** (75%; *Scheme 3*). Displacement of the TfO by an N_3 group yielded the hexaazide **15** (63%), possessing a *gluco*-configured ring I, that was transformed *via* **16** into the hexaammonium acetate **17** (70%), similarly as described above for the preparation of **9** and **13**.

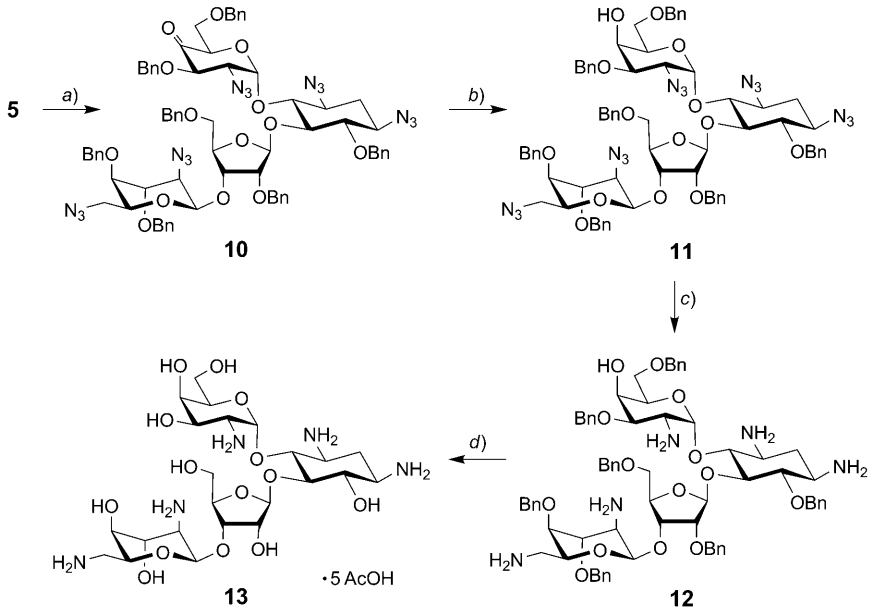
The synthesis of 4′-deoxyparomomycin (**23**) was initiated by cleaving the dioxane ring of **4** with $TsOH \cdot H_2O$ in CH_2Cl_2/MeOH 4:1 to give diol **18** (42%, 59% conversion; *Scheme 4*). *Staudinger* reaction and hydrolysis, followed by (benzyloxy)carbonylation of the amino groups led to the carbamate **19** (80% from **18**) that was transformed into the 6′-*O*-pivaloyl ester **20** (83%). The xanthate **21** was obtained in good yield from **20**, but standard *Barton–McCombie* deoxygenation conditions (Bu_3SnH and AIBN in boiling toluene [20]) did not transform **21** to the desired deoxy derivative **22**. The deoxy derivative was, however, obtained in 90% yield by treating **21** with H_3PO_2, AIBN, and Et_3N in 1,4-dioxane [21]. Reductive deprotection and aminolysis of **22** by treatment with Na in liquid NH_3 yielded 68% of 4′-deoxyparomomycin (**23**).

Scheme 1



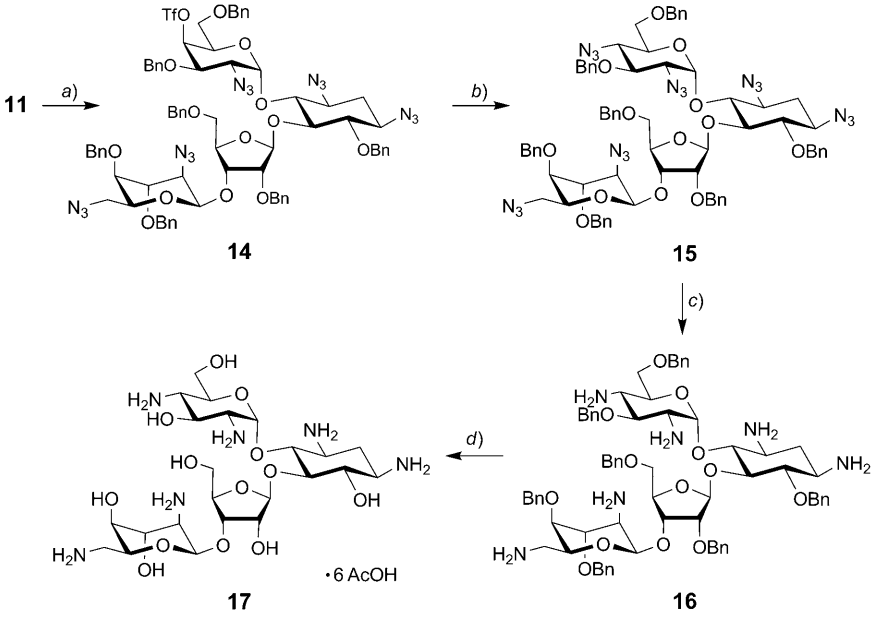
a) TfN_3, *t*-BuOK, *t*-BuOH/H_2O 2:1, 26°; 49%. *b*) PhCHO, HCO_2H, −5 to 0°; 75%. *c*) NaH, BnBr, Bu_4NI, THF, 0 to 26°; 55%. *d*) $NaCNBH_3$, 0.7M HCl in Et_2O, 4-Å mol. sieves, THF, 0°; 93%. *e*) Tf_2O, pyridine, CH_2Cl_2, −10°; 62%. *f*) Bu_4NN_3, MeCN, 26°; 79%. *g*) 0.1M aq. NaOH, 1M PMe_3 in THF, THF, 50°; 71%. *h*) H_2, $Pd(OH)_2$, AcOH/H_2O/D_2O 2:2:1, 6 bar, 26°; 78%.

Scheme 2



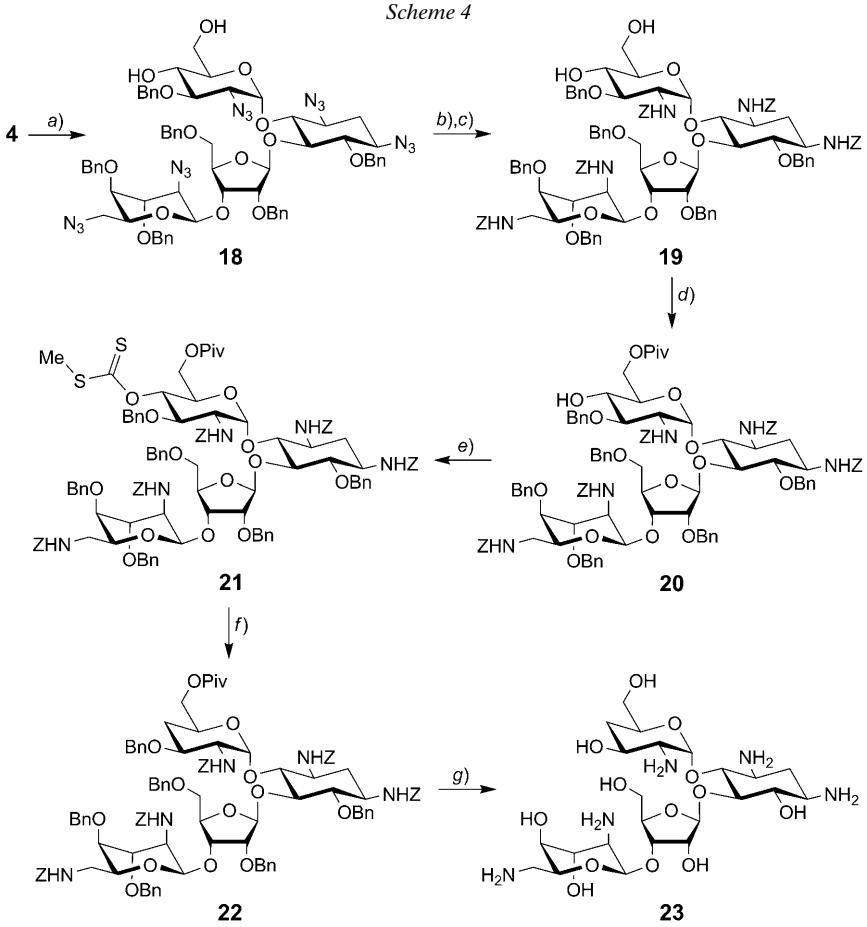
a) *Dess–Martin* periodinane, CH_2Cl_2, 26°; 81%. *b*) $NaBH_4$, THF/H_2O 1:1, 26°; 77%. *c*) 0.1M aq. NaOH, 1M PMe_3 in THF, THF, 50°; 65%. *d*) H_2, $Pd(OH)_2$, AcOH/H_2O/D_2O 2:2:1, 6 bar, 26°; 82%.

Scheme 3



a) Tf_2O, pyridine, CH_2Cl_2, −10 to 20°; 75%. *b*) Bu_4NN_3, MeCN, 26°; 63%. *c*) 0.1M aq. NaOH, 1M PMe_3 in THF, THF, 50°; 80%. *d*) H_2, $Pd(OH)_2$, AcOH/H_2O/D_2O 2:2:1, 6 bar, 26°; 88%.

Scheme 4

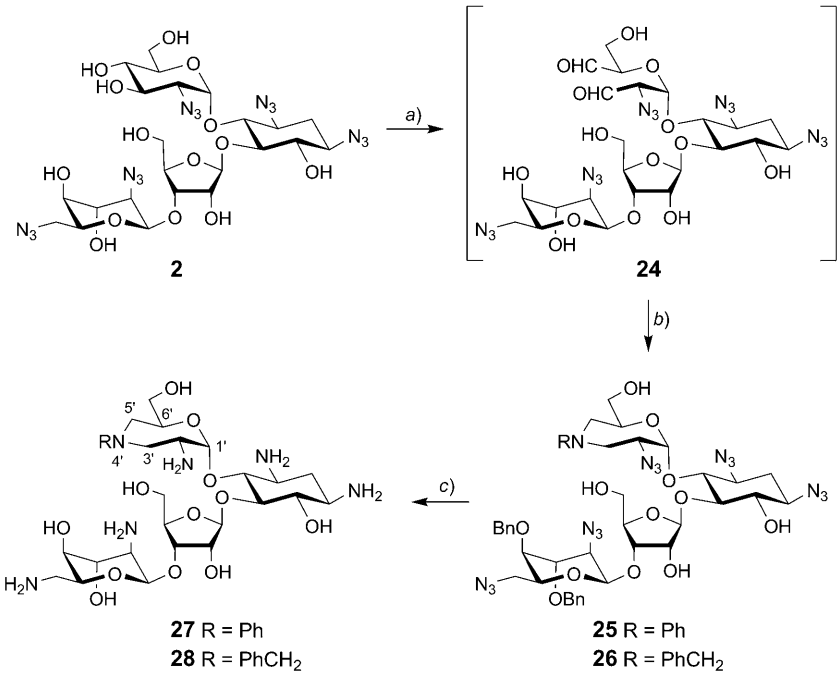


a) $TsOH \cdot H_2O$, CH_2Cl_2/MeOH 4 : 1, r.t.; 42% yield, 59% conversion. *b*) 0.1M aq. NaOH, 1M PMe_3 in THF, THF, 50°. *c*) ZCl (Z = (benzyloxy)carbonyl), Na_2CO_3, MeOH, r.t.; 80% from **18**. *d*) PivCl, pyridine, CH_2Cl_2, r.t.; 83%. *e*) NaH, CS_2, 1*H*-imidazole (cat.), MeI, THF, 0° to r.t.; 84%. *f*) H_3PO_2, 2,2′-azobis(isobutyronitrile) (AIBN), Et_3N, dioxane, reflux; 90%. *g*) Na, liq. NH_3, THF, −78 to r.t.; 68%.

Periodate cleavage of the pentaazide **2** was expected to proceed regioselectively, considering the preferred $^4C_1$ conformation of ring IV [22]. Indeed, treatment of **2** with 1.4 equiv. of H_5IO_6 gave the dialdehyde **24** that was reductively aminated with aniline, or with benzylamine [23][24], to provide the 4-phenylated and 4-benzylated 3,4,5-trideoxy-4-aza-α-D-*erythro*-heptoseptanosides **25** (32%; *Scheme 5*) and **26** (36% **2**), respectively. *Staudinger* reaction/hydrolysis of **25** and **26** gave the hexaamines **27** (66%) and **28** (68%), respectively.

A comparison of the ^{1}H-NMR spectra of the benzylated azides and the *O*-benzylated or debenzylated free amines showed that the ribofuranose ring of the azides prefers consistently if weakly the (*S*) conformation ($J(1'',2'')/J(3'',4'') = 1.05-1.11$) whereas the (*N*) conformation predominates in the free amines ($J(1'',2'')/J(3'',4'') =$

Scheme 5



a) H_5IO_6, 4-Å mol. sieves, THF, 0°. *b*) $NaCNBH_3$, RNH_2, AcOH, MeOH, 26°; from **2** 32% for **25**; 36% for **26**. *c*) 0.1M aq. NaOH, 1M PMe_3 in THF, THF, 50°; 66% for **27**; 68% for **28**.

0.39 – 0.52)[1]). These conformational preferences differ from those of the analogous *O*-Ac protected derivatives [12]. The idopyranosyl ring of all compounds adopts predominantly the $^4C_1$ conformation ($J(2''',3''') = 1.8-3.1$, $J(3''',4''') = 2.7-3.1$). The small $J(3',4')$ value of 3.3 and 2.7 Hz for **7** and **11**, respectively, evidences the *galacto*-configuration of ring I. A comparison of the chemical shift of C(4′) in **7** (59.14 ppm) and **15** (62.23 ppm) confirms the inversion of configuration[2]).

Biological Studies. – The experimental model used to investigate ribosomal drug susceptibility is a single rRNA allelic derivative of the *Gram*-positive eubacterium *Mycobacterium smegmatis* [25]. Genetic manipulations of its single rRNA operon using site-directed mutagenesis and RecA-mediated gene conversion result in homogeneous

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[1]) The range could not be determined precisely for all compounds, but were always >1 for the azides, and <1 for the amines.

[2]) The C(4′)-atom of the *gluco*-configured derivatives resonates at lower field than that of the corresponding *galacto*-configured isomer, as evidenced by the ^{13}C chemical shifts of the hydroxy (71. 7 (**5**) *vs.* 66.8 ppm (**11**)), trifloxy (68.6 (**6**) *vs.* 67.1 ppm (**14**)), azido (62.2 (**15**) *vs.* 59.1 ppm (**7**)), and amino derivatives (57.2 (**16**) *vs.* 52.0 ppm (**8**)). The axial N_3 substituent of **7** leads to an upfield shift of *ca.* 3 ppm for C(3′) and C(5′).

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

	Wild type	Mutant A1408G	Mutant G1491A	Mutant G1491C
Paromomycin (**1**)	1	64	32–64	512
9	128	>512	>512	>512
13	32	>512	512	>512
17	4	256	256	>512
23	2	256	128	>512
27	>512	>512	>512	>512
28	≥512	≥512	>512	>512

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

populations of mutant ribosomes [26–29]. Ribosomal drug susceptibility was assessed by determination of minimal inhibitory concentrations (*MIC*s) [27–29].

All new derivatives proved less active against *M. smegmatis* wild type ribosomes (*Table 1*). The derivatives **9** and **13** possessing a D-*galacto*-configured ring I displayed lower antibacterial activity than the corresponding D-*gluco*-configured analogues **17** and paromomycin (**1**), respectively. This is in agreement with the postulated interaction of a C(4′)–OH (and also of a C(4′)–NH_3^+) group with the phosphate moiety of A1493. The 4′-amino-4′-deoxy derivative **17** possessing a D-*gluco*-configured ring I proved slightly less active than paromomycin. This result is in contradiction to the expectations that were based on a comparison of the assumed interaction of a C(4′)–OH or a C(4′)–NH_3^+ with a phosphate group of A1493. This decrease of activity could be due to the influence of counter ions and of hydration, and/or to an impaired cell wall permeability. Similarly to previously reported results [13], 4′-deoxyparomomycin (**23**) was found to be only slightly less active than the parent antibiotic.

To study the specificity of the interaction of the new paromomycin analogues with prokaryotic ribosomes, we also investigated the drug susceptibility of ribosomes with one of the mutations A1408G, G1491A, or G1491C. These 16S rRNA positions are polymorphic, characteristic for eukaryotic ribosomes, and main determinants of aminoglycoside selectivity [11][30]. As shown in *Table 1*, modification at C(4′) had little, if any, effect on drug selectivity.

To exclude the effect of cell wall permeability, the IC_{50} values for the derivatives **9**, **13**, **17**, and **23** were determined in cell-free translation assays on strains of *M. smegmatis*, *i.e.*, $(UUU)_{12}$-mRNA-directed phenylalanine incorporation and the synthesis of luciferase in coupled transcription–translation reactions [30] (see *Tables 2* and *3*). Higher concentrations of the derivatives **9** and **13** (*galacto*-configured ring I) than those of their analogues **17** and paromomycin (**1**) (*gluco*-configured ring I), respectively, were required to inhibit by 50% the incorporation of [^{14}C]phenylalanine or the synthesis of luciferase. Similarly, compared to paromomycin (**1**), a higher concentration of the 4′-amino-4′-deoxy derivative **17** and of the 4′-deoxy derivative **23** was required for *in-vitro* inhibition of protein synthesis. Thus, the lower antibacterial activity of these derivatives cannot be attributed to differences in cell-wall permeability, but is a direct reflection of their interaction with the ribosome.

The results evidence that the orientation of the substituent at C(4′) is of importance for drug binding and activity, while the nature of this substituent appears to have little

Table 2. *Aminoglycoside-Induced Inhibition of $(UUU)_{12}$-Directed Phenylalanine Incorporation*

	IC_{50} [μM][a])
Paromomycin (**1**)	0.5
9	40.0–47.0
13	6.0–9.0
17	0.5–1.0

[a]) Aminoglycoside concentrations required to inhibit [^{14}C]phenylalanine incorporation to 50% (IC_{50}).

Table 3. *Aminoglycoside-Induced Inhibition of Luciferase Synthesis*

	IC_{50} [μM][a])
Paromomycin (**1**)	0.02
9	1.80
13	0.20
17	0.04
23	0.03

[a]) Aminoglycoside concentrations required to inhibit synthesis of active luciferase to 50% (IC_{50}).

effect on the activity, as no significant difference was observed for paromomycin (**1**), the D-*gluco*-configured 4′-amino-4′-deoxy derivative **17** (both possessing a H-bond donor substituent at C(4′)), and 4′-deoxyparomomycin (**23**). An H-bond donating substituent at C(4′) does not appear to be necessary for drug binding and activity.

We thank Dr. *B. Bernet* for checking the manuscript.

Experimental Part

General. See [12].

1,3,2′,2‴,6‴-Pentaazido-6,3′,2″,5″,3‴,4‴-hexa-O-*benzyl-4′,6′*-O-*benzylidene-1,3,2′,2‴,6‴-pentadeaminoparomomycin* (**4**). Under Ar, a soln. of **3** [12] (1.5 g, 1.8 mmol) in THF (20 ml) was treated with NaH (795 mg, 50–60% suspension in oil, *ca.* 18.2 mmol), BnBr (2.1 ml, 16.2 mmol), Bu_4NI (204 mg, 0.54 mmol), stirred at 0° for 8 h and at 26° for 20 h, cooled to 0°, and diluted portionwise with H_2O. After evaporation of THF, the aq. layer was extracted with AcOEt (5 × 30 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 3:17) gave **4** (1.3 g, 55%). White solid. R_f (AcOEt/cyclohexane 1:3) 0.55. M.p. 62.2–65.7°. $[\alpha]_D^{25} = +71.0$ ($c = 0.14$, $CHCl_3$). IR (ATR): 3091*w*, 3058*w*, 3032*w*, 2933*w*, 2867*w*, 2099*s*, 1607*w*, 1585*w*, 1496*m*, 1454*m*, 1368*m*, 1330*m*, 1261*m*, 1125*m*, 1088*s*, 1069*s*, 1027*s*, 999*s*. ^{1}H-NMR ($CDCl_3$, 400 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 4*; additionally, 7.51–7.12 (*m*, 35 arom. H); 5.50 (*s*, PhC*H*); 4.95 (*d*, $J = 10.7$), 4.90 (*d*, $J = 11.3$), 4.77 (*d*, $J = 11.3$), 4.72 (*d*, $J = 10.7$), 4.62 (*d*, $J = 12.0$), 4.59 (*d*, $J = 11.2$, 2 H), 4.53 (*d*, $J = 12.8$), 4.46 (*d*, $J = 11.8$), 4.44 (*d*, $J = 11.8$), 4.41 (*d*, $J = 12.0$), 4.26 (*d*, $J = 12.0$) (6 PhCH_2). ^{13}C-NMR ($CDCl_3$, 100 MHz; assignments based on a HSQC spectrum): see *Table 5*; additionally, 138.36, 138.10, 137.90, 137.60, 137.43, 137.04, 136.96 (7*s*, 7 arom. C); 128.69–126.11 (several *d*, 35 arom. CH); 101.39 (*d*, PhCH); 75.03, 74.90, 73.26, 73.20, 72.42, 71.77 (6*t*, 6 PhCH_2). HR-ESI-MS: 1256.5186 (21, $[M - 5\,N_2 + Na]^+$, $C_{72}H_{75}N_5NaO_{14}^+$; calc. 1256.5208), 689.2823 (100, $[M - C_{39}H_{41}N_6O_7 - 2\,H + Na]^+$, $C_{33}H_{32}N_9NaO_7^+$; calc. 689.2322). Anal. calc. for $C_{72}H_{75}N_{15}O_{14} \cdot H_2O$ (1392.47): C 62.10, H 5.57, N 15.09; found: C 61.97, H 5.32, N 14.59.

1,3,2′,2‴,6‴-Pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-O-*benzyl-1,3,2′,2‴,6‴-pentadeaminoparomomycin* (**5**). Under N_2, a soln. of **4** (2.25 g, 1.64 mmol) in THF (50 ml) was treated with 4-Å molecular sieves, stirred for 1 h, cooled to 0°, treated with $NaCNBH_3$ (1.79 g, 28.48 mmol), and then dropwise with 0.7M HCl in Et_2O (45 ml, 31.5 mmol; methyl orange was added to indicate the pH), and stirred for 4 h. After neutralization with $NaHCO_3$ soln. and evaporation of THF, the aq. layer was extracted with AcOEt (3 × 30 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 9:41) gave **5** (2.1 g, 93%). White solid. R_f (AcOEt/cyclohexane 1:4) 0.29. M.p. 135°. $[\alpha]_D^{25} = +71.4$ ($c = 0.52$, $CHCl_3$). IR ($CHCl_3$): 3574*w*, 3067*w*, 3007*w*, 2926*w*, 2870*w*, 2106*s*, 1603*w*, 1497*w*, 1454*m*, 1364*w*, 1330*w*, 1262*m*, 1207*m*, 1123*m*, 1074*m*, 1042*m*, 1028*m*, 914*w*. ^{1}H-NMR ($CDCl_3$,

Table 4. *^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Azido Derivatives* **4**–**7**, **10**, **11**, **14**, *and* **15** *in* $CDCl_3$

	4[a])	**5**	**6**	**7**	**10**	**11**	**14**	**15**
H–C(1′)	6.10	6.16	6.17	6.14	6.36	6.18	6.23	6.18
H–C(2′)	3.08	3.00	2.85	3.64–3.57	3.52	3.42	3.42	3.02
H–C(3′)	4.10	3.98–3.87	4.04	4.15	4.47	4.01	4.07	3.93
H–C(4′)	3.44	3.53–3.39	4.77	4.08	–	4.14[b])	5.51	3.54
H–C(5′)	4.08	4.11	3.92–3.88	4.29–4.27	4.84	3.78–3.72	4.28–4.22	3.99
H_a–C(6′)	4.33–4.28	3.81	3.79–3.75	3.77–3.73	4.02–3.97	3.78–3.72	3.76–3.64	3.80–3.74
H_b–C(6′)	3.65	3.77–3.67	3.79–3.75	3.77–3.73	3.85–3.71	3.78–3.72	3.76–3.64	3.80–3.74
H–C(1)	3.47–3.38	3.53–3.39	3.50–3.38	3.44	3.53–3.43	3.48–3.35	3.63–3.53	3.44
H_{ax}–C(2)	1.41	1.35	1.36	1.33	1.39	1.31	1.35	1.33
H_{eq}–C(2)	2.24	2.23	2.24	2.21	2.25	2.19	2.22	2.22
H–C(3)	3.47–3.38	3.53–3.39	3.50–3.38	3.40	3.53–3.43	3.48–3.35	3.45–3.35	3.41
H–C(4)	3.67	3.77–3.67	3.63	3.64–3.57	3.85–3.71	3.69	3.58	3.63
H–C(5)	3.94	3.98–3.87	3.91	3.91	3.99	3.92	3.90	3.91
H–C(6)	3.29	3.25	3.23	3.21	3.29	3.21	3.21	3.24
H–C(1″)	5.66	5.67	5.61	5.63	5.71	5.64	5.63	5.64
H–C(2″)	3.96	3.98–3.87	3.92–3.88	3.92	4.02–3.97	3.94	3.94	4.27–4.23
H–C(3″)	4.33–4.28	4.30–4.24	4.27–4.26	4.23	4.33–4.28	4.23–4.21	4.28–4.22	4.44–4.42
H–C(4″)	4.33–4.28	4.30–4.24	4.27–4.26	4.29–4.27	4.33–4.28	4.23–4.21	4.28–4.22	4.27–4.23
H_a–C(5″)	3.82	3.77–3.67	3.71–3.62	3.64–3.57	3.85–3.71	3.78–3.72	3.76–3.64	3.80–3.74
H_b–C(5″)	3.57	3.58	3.54	3.52	3.60	3.53	3.76–3.64	3.80–3.74
H–C(1‴)	4.88	4.87	4.88	4.83	4.92	4.83	4.83	4.84
H–C(2‴)	3.34[b])	3.35[b])	3.35[b])	3.33[b])	3.40[b])	3.32[b])	3.32[b])	3.33
H–C(3‴)	3.76	3.77–3.67	3.79–3.75	3.75	3.85–3.71	3.78–3.72	3.76–3.64	3.57
H–C(4‴)	3.13[b])	3.13[c])	3.13[b])	3.12[b])	3.17[b])	3.11[b])	3.11[b])	3.12[b])
H–C(5‴)	3.79–3.77	3.77–3.67	3.79–3.75	3.64–3.57	3.85–3.71	3.78–3.72	3.76–3.64	3.80–3.74
H_a–C(6‴)	3.65	3.63	3.71–3.62	3.64–3.57	3.67	3.59	3.76–3.64	3.60
H_b–C(6‴)	2.90	2.90	2.89	2.90	2.93	2.90	2.88	2.90
J(1′,2′)	3.9	3.6	3.6	3.6	3.3	3.6	3.9	3.6
J(2′,3′)	10.1	10.2	10.2	10.5	10.5	10.5	10.5	10.1
J(3′,4′)	9.7	[d])	9.0	3.3	–	2.7	2.4	9.3
J(4′,5′)	9.3	9.6	10.2	1.2	–	[d])	< 1.5	9.6
J(5′,6′a)	4.9	2.1	[d])	[d])	2.4	[d])	[d])	[d])
J(5′,6′b)	10.0	[d])	[d])	[d])	8.7	[d])	[d])	3.0
J((6′a,6′b)	10.0	10.5	[d])	[d])	[d])	[d])	[d])	[d])
J(1,2_{ax})	12.6	12.6	12.9	12.6	12.6	12.6	12.9	12.9
J(1,2_{eq})	4.6	4.8	4.2	4.8	4.2	4.8	4.8	4.5
J(1,6)	9.1	[d])	9.0	10.2	[d])	9.0	8.7	9.3
J(2_{ax},2_{eq})	13.2	13.2	13.2	13.2	13.5	12.6	13.5	12.9
J(2_{ax},3)	12.6	12.6	12.9	12.6	12.6	12.6	12.9	12.9
J(2_{eq},3)	4.6	4.8	4.2	4.5	4.2	4.8	4.8	4.2
J(3,4)	8.6	9.4	9.0	9.3	9.3	9.0	9.0	9.3
J(4,5)	8.6	[d])	9.0	9.3	9.3	8.7	8.7	9.4
J(5,6)	8.7	9.4	8.7	9.3	9.3	9.0	8.7	9.3
J(1″,2″)	5.4	5.7	5.7	5.7	6.0	5.7	6.0	6.0
J(2″,3″)	[d])	[d])	[d])	5.1	[d])	5.1	5.7	[d])
J(3″,4″)	[d])	[d])	[d])	2.1	[d])	[d])	[d])	[d])
J(4″,5″a)	1.8	[d])	[d])	[d])	[d])	[d])	[d])	[d])
J(4″,5″b)	3.1	2.4	3.0	3.6	2.7	3.3	[d])	[d])

Table 4 (cont.)

	4[a])	**5**	**6**	**7**	**10**	**11**	**14**	**15**
J(5″a,5″b)	10.5	10.8	10.5	10.5	10.4	10.2	[d])	[d])
J(1‴,2‴)	1.9	1.8	2.1	1.8	2.1	1.5	1.5	1.8
J(2‴,3‴)	2.0	[d])	[d])	[d])	1.8	[d])	[d])	2.1
J(3‴,4‴)	3.1	[d])	[d])	3.0	[d])	[d])	[d])	2.7
J(4‴,5‴)	[d])	[d])	[d])	[d])	[d])	[d])	[d])	[d])
J(5‴,6‴a)	8.3	8.4	[d])	[d])	8.4	8.4	[d])	8.1
J(5‴,6‴b)	3.9	4.2	3.6	4.2	4.2	3.9	3.9	4.2
J(6‴a,6‴b)	13.0	12.8	12.8	12.8	12.9	12.8	12.6	12.8

[a]) Assignments based on a DQFCOSY and a HSQC spectrum. [b]) $w_{1/2} = 6.0$ Hz. [c]) $w_{1/2} = 6.9$ Hz. [d]) Not assigned.

300 MHz): see *Table 4*; additionally, 7.40–7.19 (*m*, 35 arom. H); 4.97 (*d*, *J* = 10.5), 4.91 (*d*, *J* = 11.7), 4.77 (*d*, *J* = 11.4), 4.69 (*d*, *J* = 10.8), 4.66 (*d*, *J* = 12.0), 4.63 (*d*, *J* = 11.1), 4.59 (*d*, *J* = 11.1), 4.57 (*d*, *J* = 11.8), 4.54 (*d*, *J* = 11.8), 4.47 (*d*, *J* = 12.0), 4.44 (*d*, *J* = 12.0), 4.42 (*d*, *J* = 12.9), 4.32 (*d*, *J* = 11.8), 4.26 (*d*, *J* = 11.8) (7 Ph*C*H$_2$); 2.48 (*d*, *J* = 2.1, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 138.46, 138.40, 138.23, 138.15, 137.78, 137.23, 137.17 (7*s*, 7 arom. C); 130.27–127.61 (several *d*, 35 arom. CH); 75.22 (2 C), 73.63 (2 C), 73.51, 73.08, 72.58 (5*t*, 7 Ph*C*H$_2$). HR-ESI-MS: 1414.5416 (26, $[M + K]^+$, $C_{72}H_{77}KN_{15}O_{14}^+$; calc. 1414.5411), 1398.5698 (100, $[M + Na]^+$, $C_{72}H_{77}N_{15}NaO_{14}^+$; calc. 1398.5672). Anal. calc. for $C_{72}H_{77}N_{15}O_{14}$ (1376.49): C 62.83, H 5.64, N 15.26; found: C 62.65, H 5.72, N 15.05.

1,3,2′,2‴,6‴′-Pentaazido-6,3′,6′,2″,5″,3‴,4‴′-hepta-O-*benzyl-1,3,2′,2‴,6‴′-pentadeamino-4′*-O-*[(trifluoromethyl)sulfonyl]paromomycin* (**6**). Under N_2, a soln. of pyridine (0.12 ml, 1.54 mmol) and Tf_2O (0.26 ml, 1.54 mmol) in CH_2Cl_2 (10 ml) was stirred for 30 min at −10°, treated portionwise with a soln. of **5** (950 mg, 0.69 mmol) over 30 min, and stirred at −10° for 3 h. After neutralization with aq. $NaHCO_3$ and extraction with CH_2Cl_2 (3 × 30 ml), the combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 3:22) gave **6** (648 mg, 62%). White solid. R_f (AcOEt/cyclohexane 1:4) 0.44. M.p. 49–51°. $[\alpha]_D^{25} = +89.0$ ($c = 0.13$, $CHCl_3$). IR ($CHCl_3$): 2927*w*, 2857*w*, 2105*s*, 1602*m*, 1491*w*, 1454*m*, 1410*m*, 1357*w*, 1329*w*, 1243*w*, 1141*m*, 1079*m*, 1037*m*, 1024*m*, 928*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.37–7.17 (*m*, 35 arom. H); 4.93 (*d*, *J* = 10.8), 4.85 (*d*, *J* = 11.7), 4.72 (*d*, *J* = 10.2), 4.71 (*d*, *J* = 10.5), 4.63 (*d*, *J* = 11.7), 4.61 (*d*, *J* = 11.4), 4.55 (*d*, *J* = 12.0), 4.54 (*d*, *J* = 11.1), 4.41 (*d*, *J* ≈ 9.9), 4.41 (*d*, *J* = 11.7, 3 H), 4.32 (*d*, *J* = 11.7), 4.25 (*d*, *J* = 12.3), (7 Ph*C*H$_2$). ^{13}C—NMR ($CDCl_3$, 75 MHz): see *Table 4*; additionally, 137.81, 137.67, 137.61, 137.39, 136.91, 136.84, 136.79 (7*s*, 7 arom. C); 128.58–127.48 (several *d*, 35 arom. CH); 118.26 (*q*, ^1J(C,F) = 316.6, CF_3); 75.13, 74.90, 73.46, 73.38, 73.16, 72.40, 71.73 (7*t*, 7 Ph*C*H$_2$). ^{19}F-NMR ($CDCl_3$, 282 MHz): −74.60. HR-ESI-MS: 1530.5179 (22, $[M + Na]^+$, $C_{73}H_{76}F_3N_{15}NaO_{16}S^+$; calc. 1530.5165), 803.5333 (100, $[M - C_{39}H_{41}N_6O_7 + H]^+$, $C_{34}H_{36}F_3N_9O_9S^+$; calc. 803.2309). Anal. calc. for $C_{73}H_{76}F_3N_{15}O_{16}S$ (1508.54): C 58.12, H 5.08, N 13.93; found: C 58.64, H 5.09, N 12.76.

4′-epi-*1,3,2′,4′,2‴,6‴′-Hexaazido-6,3′,6′,2″,5″,3‴,4‴′-hepta*-O-*benzyl-1,3,2′,2‴,6‴′-pentadeamino-4′-deoxyparomomycin* (**7**). Under N_2, a soln. of **6** (514 mg, 0.34 mmol) in MeCN (10 ml) was treated with Bu_4NN_3 (1 g, 3.52 mmol), stirred for 10 h at r.t., and evaporated. A soln. of the residue in CH_2Cl_2 was washed twice with brine, dried ($MgSO_4$), filtered, and evaporated. FC (amino-phase silica gel; AcOEt/cyclohexane 3:22) gave **7** (376 mg, 79%). White solid. R_f (AcOEt/cyclohexane 1:4) 0.44. M.p. 48.4°. $[\alpha]_D^{25} = +71.0$ ($c = 0.18$, $CHCl_3$). IR ($CHCl_3$): 2928*m*, 2852*w*, 2105*s*, 1603*w*, 1493*w*, 1454*m*, 1364*w*, 1324*w*, 1263*m*, 1090*m*, 1029*m*, 915*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.44–7.13 (*m*, 35 arom. H); 4.94 (*d*, *J* = 10.5), 4.75 (*d*, *J* = 11.4), 4.70 (*d*, *J* = 11.4), 4.65 (*d*, *J* ≈ 9.6, 2 H), 4.62 (*d*, *J* = 12.0, 2 H), 4.57 (*d*, *J* = 11.4, 2 H), 4.55 (*d*, *J* = 11.7), 4.42 (*d*, *J* = 11.7), 4.41 (*d*, *J* = 11.4), 4.31 (*d*, *J* = 12.0), 4.25 (*d*, *J* = 12.0) (7 Ph*C*H$_2$). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 138.07, 137.80 (2 C), 137.45, 137.03, 136.93, 136.86 (6*s*, 7 arom. C); 128.58–127.37 (several *d*, 35 arom. CH); 74.92, 73.28 (3 C), 72.28, 72.271, 71.62 (5*t*, 7 Ph*C*H$_2$). HR-MALDI-MS: 1439.5373 (10, $[M + K]^+$, $C_{72}H_{76}KN_{18}O_{13}^+$; calc.

Table 5. *^{13}C-NMR Chemical Shifts* [ppm] *of the Azido Derivatives* **4**–**7**, **10**, **11**, **14**, *and* **15** *in* $CDCl_3$

	4[a])	**5**	**6**	**7**	**10**	**11**	**14**	**15**
C(1′)	96.50	96.21	95.51	95.72	95.62	95.97	95.68	95.99
C(2′)	62.87	62.76	63.02	59.59	63.80	60.38	60.10	62.93
C(3′)	75.98	79.91	81.53	75.82	79.18	75.92	81.63	78.47
C(4′)	82.53	70.40	68.60	59.14	202.27	66.83	67.07	62.23
C(5′)	62.97	71.66	71.44	67.41	72.93	68.82	72.77	70.13
C(6′)	69.00	70.47	67.84	68.72	68.13	69.76	67.24	69.00
C(1)[b])	60.34	60.58	60.33	60.17	60.37	60.14	59.64	60.35
C(2)	32.39	32.77	32.39	32.29	32.49	32.60	32.08	32.57
C(3)[b])	59.87	60.38	59.88	59.88	60.01	58.78	58.62	60.08
C(4)	75.42	75.00	75.40	74.68	75.52	74.67	74.94	74.79
C(5)	81.85	82.06	81.99	81.64	81.94	81.73	81.99	81.85
C(6)	84.14	84.38	84.08	84.03	84.23	84.16	84.00	84.09
C(1″)	106.25	106.26	106.34	105.92	106.08	105.94	105.98	106.02
C(2″)	82.42	82.62	82.23	82.40	82.60	82.47	82.47	82.30
C(3″)	75.51	75.74	76.75	75.52	75.62	75.67	75.49	75.49
C(4″)	82.11	82.25	82.09	82.08	82.22	82.14	82.18	81.99
C(5″)	70.37	71.93	70.08	69.96	69.98	70.15	69.91	70.02
C(1‴)	98.60	98.87	98.55	98.57	98.76	98.67	98.57	98.61
C(2‴)	57.31	57.49	57.29	57.19	57.37	57.38	57.16	57.29
C(3‴)	72.96	73.40	74.39	72.80	74.03	72.94	72.77	72.86
C(4‴)	71.60	72.31	72.84	71.33	71.47	71.48	71.33	71.41
C(5‴)	74.39	74.50	75.28	74.13	74.39	74.24	74.19	74.23
C(6‴)	51.14	51.25	51.16	50.89	51.17	51.05	50.94	51.04

[a]) Assignments based on a DQFCOSY and a HSQC spectrum. [b]) Assigments for **5**–**7**, **10**, **11**, **14**, and **15** may be interchanged.

1439.5476), 1423.5701 (100, $[M + Na]^+$, $C_{72}H_{76}N_{18}NaO_{13}^+$; calc. 1423.5737), 1373.5812 (22, $[M - N_2 + H]^+$, $C_{72}H_{77}N_{16}O_{13}^+$; calc. 1373.5856). Anal. calc. for $C_{72}H_{76}N_{18}O_{13}$ (1401.49): C 61.70, H 5.47, N 17.99; found: C 61.54, H 5.48, N 17.76.

4′-epi-*4′-Amino-6,3′,6′,2″,5″,3‴,4‴-hepta*-O-*benzyl-4′-deoxyparomomycin* (**8**). A soln. of **7** (198 mg, 0.14 mmol) in THF (20 ml) was treated with 0.1M aq. NaOH. (4.2 ml) and 1M PMe_3 in THF (2.24 ml, 2.24 mmol), stirred for 2 h at 50°, and evaporated. FC (amino phase silica gel; MeOH/cyclohexane 3:97) gave **8** (125 mg, 71%). White solid. R_f (MeOH/$CHCl_3$ 1:19) 0.34. M.p. 45.2–47.2°. $[\alpha]_D^{25} = +37.8$ ($c = 0.37$, $CHCl_3$). IR (ATR): 3373*w*, 3029*w*, 2912*m*, 2865*m*, 1587*m*, 1496*m*, 1453*s*, 1363*m*, 1328*m*, 1207*m*, 1072*s*, 1025*s*, 908*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 6*; additionally, 7.36–7.18 (*m*, 35 arom. H); 4.95 (*d*, $J = 11.4$), 4.64 (*d*, $J = 11.7$), 4.63 (*d*, $J = 11.7$), 4.58 (*d*, $J = 12.3$), 4.51 (*d*, $J \approx 9.0$, 5 H), 4.51 (*d*, $J = 11.7$), 4.49 (*d*, $J = 12.6$), 4.44 (*d*, $J = 12.0$), 4.37 (*d*, $J = 12.0$), 4.24 (*d*, $J = 11.7$) (7 Ph*CH*$_2$); 1.50 (br. *s*, $w_{1/2} = 72.0$, 6 NH_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 7*; additionally, 138.23, 138.19, 138.00, 137.94, 137.67 (2 C), 137.14 (6*s*, 7 arom. C); 128.38–127.39 (several *d*, 35 arom. CH); 75.01, 73.44, 73.02 (3 C), 71.90, 71.33 (5*t*, 7 Ph*CH*$_2$). HR-ESI-MS: 1267.6584 (2, $[M + Na]^+$, $C_{72}H_{88}N_6NaO_{13}^+$; calc. 1267.6301); 1245.6464 (14, $[M + H]^+$, $C_{72}H_{89}N_6O_{13}^+$; calc. 1245.6488), 905.4517 (100, $[M - C_{20}H_{25}N_2O_3 + 2\,H]^+$, $C_{52}H_{65}N_4O_{10}^+$; calc. 905.4701). Anal. calc. for $C_{72}H_{88}N_6O_{13} \cdot 4\,H_2O$ (1317.56): C 65.63, H 7.34, N 6.38; found: C 65.96, H 7.26, N 6.72.

4′-epi-*4′-Amino-4′-deoxyparomomycin Hexaacetate* (**9**). A soln. of **8** (87 mg, 0.07 mmol) in AcOH/H_2O/D_2O 2:2:1 (3 ml) was treated with $Pd(OH)_2$ (65 mg) and stirred for 12 h at r.t. under 6 bar of H_2. Filtration and evaporation gave **9** (53 mg, 78%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:4:3) 0.40. M.p. 125° (dec.). $[\alpha]_D^{25} = +53.1$ ($c = 0.27$, H_2O). ^{1}H-NMR (D_2O, 300 MHz): see *Table 6*; additionally, 1.96 (*s*, 6 AcO). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 7*; additionally, 182.19 (*s*, 6 C=O); 25.77 (*q*,

Table 6. *^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Protected Amines* **8**, **12**, *and* **16** *in $CDCl_3$, and of the Deprotected Ammonium Salts* **9**, **13**, *and* **17** *in D_2O*

	8	**12**	**16**	**9**	**13**[a])	**17**
H–C(1′)	5.45	5.41	5.42	5.78	5.73	5.67
H–C(2′)	3.04	3.17	2.90	3.46	3.56	3.34–3.17
H–C(3′)	3.95–3.92	3.95–3.93	3.69–3.58	4.36	4.08	3.56–3.53
H–C(4′)	3.20[b])	3.99	2.84	3.55–3.47	4.02	2.93
H–C(5′)	4.30–4.22	4.26–4.18	4.28–4.21	4.29	4.09–4.08	3.83–3.63
H_a–C(6′)	3.72–3.60	3.73	3.42–3.35	3.78–3.65	3.77	3.83–3.63
H_b–C(6′)	3.72–3.60	3.73	3.42–3.35	3.78–3.65	3.75	3.83–3.63
H–C(1)	2.78–2.68	2.78–2.66	2.79–2.69	3.35–3.22	3.28	3.34–3.17
H_{ax}–C(2)	1.08	1.11	1.11	1.78	1.71	1.60
H_{eq}–C(2)	1.82	1.84	1.85	2.38	2.37	2.23
H–C(3)	2.78–2.68	2.78–2.66	2.79–2.69	3.35–3.22	3.35–3.32	3.34–3.17
H–C(4)	3.37	3.37	3.04	3.84	3.86	3.83–3.63
H–C(5)	3.55	3.51	3.49	4.00	3.84	3.83–3.63
H–C(6)	3.04	3.05	3.36	3.63	3.64	3.56–3.53
H–C(1″)	5.37	5.39	5.33	5.29	5.37	5.27
H–C(2″)	3.95–3.92	3.95–3.93	3.92[b])	4.22	4.37	4.27
H–C(3″)	4.30–4.22	4.26–4.18	3.97	4.44	4.53	4.40
H–C(4″)	4.30–4.22	4.26–4.18	4.28–4.21	4.12–4.08	4.19	4.19
H_a–C(5″)	3.72–3.60	3.63–3.61	3.70	3.78–3.65	3.91	3.83–3.63
H_b–C(5″)	3.51	3.56	3.61	3.78–3.65	3.78	3.83–3.63
H–C(1‴)	4.56	4.61	4.64	5.18	5.29	5.17[c])
H–C(2‴)	2.94[b])	2.94[d])	2.92	3.47[e])	3.58	3.46[f])
H–C(3‴)	3.72–3.60	3.63–3.61	3.42–3.35	4.12–4.08	4.22	4.11
H–C(4‴)	3.20[b])	3.19[d])	3.19[b])	3.81[b])	3.81	3.83–3.63
H–C(5‴)	3.44	3.63–3.61	3.91–3.84	4.22	4.30	4.10–4.05
H_a–C(6‴)	2.78	2.77	2.76	3.55–3.47	3.42	3.31
H_b–C(6‴)	2.52	2.52	2.51	3.25	3.36	3.25
J(1′,2′)	3.9	3.9	3.3	4.2	4.1	3.7
J(2′,3′)	10.2	10.5	10.5	11.4	11.1	[g])
J(3′,4′)	[g])	2.4	9.6	4.5	2.8	10.2
J(4′,5′)	[g])	< 1.5	9.6	< 0.8	< 1.5	10.2
J(5′,6′a)	[g])	5.6	[g])	2.1	4.3	[g])
J(5′,6′b)	[g])	5.6	[g])	4.8	3.2	[g])
J((6′a,6′b)	[g])	[g])	[g])	[g])	12.0	[g])
J(1,2_{ax})	12.6	12.9	12.9	12.9	12.5	12.1
J(1,2_{eq})	4.2	4.2	4.2	4.2	4.1	4.2
J(1,6)	9.0	9.3	9.6	9.6	10.5	[g])
J(2_{ax},2_{eq})	13.2	12.9	12.9	12.9	12.6	12.9
J(2_{ax},3)	12.6	12.9	13.2	12.9	12.6	12.1
J(2_{eq},3)	4.2	4.2	4.2	4.2	4.3	4.2
J(3,4)	9.3	9.3	9.3	9.3	9.3	[g])
J(4,5)	9.0	9.3	9.6	9.3	9.3	[g])
J(5,6)	9.0	9.3	9.3	9.6	9.1	[g])
J(1″,2″)	3.6	3.9	3.6	2.1	2.5	[g])
J(2″,3″)	[g])	[g])	[g])	5.4	4.8	5.0
J(3″,4″)	[g])	[g])	3.9	6.4	6.8	5.0
J(4″,5″a)	[g])	[g])	2.4	[g])	3.0	[g])
J(4″,5″b)	3.3	3.3	5.4	[g])	4.3	5.0

Table 6 (cont.)

	8	**12**	**16**	**9**	**13**[a])	**17**
J(5″a,5″b)	10.1	10.5	10.5	[g])	12.4	[g])
J(1‴,2‴)	2.1	[g])	2.1	[g])	1.7	[g])
J(2‴,3‴)	[g])	[g])	[g])	[g])	3.1	[g])
J(3‴,4‴)	[g])	[g])	[g])	[g])	3.1	2.9
J(4‴,5‴)	[g])	[g])	[g])	[g])	1.5	[g])
J(5‴,6‴a)	7.5	7.5	7.2	[g])	6.7	6.7
J(5‴,6‴b)	5.1	4.9	5.1	4.5	4.0	4.5
J(6‴a,6‴b)	13.2	13.4	13.7	14.1	13.7	13.5

[a]) Assignment based on a DQFCOSY and a HSQC spectrum. [b]) $w_{1/2} = 6$ Hz. [c]) $w_{1/2} = 5.5$ Hz. [d]) $w_{1/2} = 10$ Hz. [e]) $w_{1/2} = 4.2$ Hz. [f]) $w_{1/2} = 6.5$ Hz. [g]) Not assigned.

6 *Me*C=O). HR-ESI-MS: 637.3024 (36, $[M + Na]^+$, $C_{23}H_{46}N_6NaO_{13}^+$; calc. 637.3021), 615.3204 (17, $[M + H]^+$, $C_{23}H_{47}N_6O_{13}^+$; calc. 615.3201), 455.2325 (100, $[M - C_6H_{13}N_2O_3 + 2\,H]^+$, $C_{17}H_{35}N_4O_{10}^+$; calc. 455.2353).

1,3,2′,2‴,6‴-Pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-O-*benzyl-1,3,2′,2‴,6‴-pentadeamino-4′-deoxy-4′-oxoparomomycin* (**10**). Under N_2, a soln. of **5** (713 mg, 0.52 mmol) in CH_2Cl_2 (10 ml) was treated with a 15 wt-% soln. of *Dess–Martin* periodinane in CH_2Cl_2 (2.21 ml, 0.70 mmol), and stirred for 12 h at 26°. After neutralization with aq. $NaHCO_3$ and extraction with CH_2Cl_2 (3 × 30 ml), the combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 9:41) gave **10** (576 mg, 81%). White sticky solid. R_f (AcOEt/cyclohexane 1:4) 0.29. M.p. *ca.* 55°. $[\alpha]_D^{25} = +93.1$ (c = 0.60, $CHCl_3$). IR (ATR): 3030*w*, 2924*w*, 2864*w*, 2098*s*, 1736*m*, 1496*m*, 1453*m*, 1366*m*, 1328*w*, 1260*m*, 1208*w*, 1026*s*, 914*w*, 819*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.48–7.19 (*m*, 35 arom. H); 5.02 (*d*, J = 10.5), 4.97 (*d*, J = 10.8), 4.73 (*d*, J = 10.5), 4.67 (*d*, J = 10.8), 4.66 (*s*, 2 H), 4.64 (*d*, J = 12.9, 2 H), 4.53 (*d*, J = 11.7), 4.50 (*d*, J = 12.0), 4.46 (*d*, J = 12.3), 4.44 (*d*, J = 12.0), 4.36 (*d*, J = 12.3), 4.30 (*d*, J = 12.3) (7 PhCH_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 138.08, 138.00, 137.85, 137.45, 136.97 (2 C), 136.90 (6*s*, 7 arom. C); 129.03–127.51 (several *d*, 35 arom. CH); 75.19, 73.95, 73.46 (2 C), 73.37, 72.46, 71.78 (6*t*, 7 PhCH_2). HR-MALDI-MS: 1412.5260 (45, $[M + K]^+$, $C_{72}H_{75}KN_{15}O_{14}^+$; calc. 1412.5255), 1396.5536 (100, $[M + Na]^+$, $C_{72}H_{75}N_{15}NaO_{14}^+$; calc. 1396.5516). Anal. calc. for $C_{72}H_{75}N_{15}O_{14}$ (1374.46): C 62.92, H 5.50, N 15.29; found: C 62.66, H 5.59, N 15.09.

4′-epi-*1,3,2′,2‴,6‴-Pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta*-O-*benzyl-1,3,2′,2‴,6‴-pentadeaminoparomomycin* (**11**). Under N_2, a soln. of **10** (376 mg, 0.27 mmol) in THF/H_2O 1:1 (4 ml) was treated with $NaBH_4$ (33 mg, 0.87 mmol) and stirred for 3 h at 26°. After neutralization with 10% aq. AcOH (0.5 ml) and evaporation, a suspension of the residue in H_2O was extracted with CH_2Cl_2 (3 × 15 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 9:41) gave **10** (290 mg, 77%). White solid. R_f (AcOEt/cyclohexane 1:3) 0.26. M.p. 49.6–51.6°. $[\alpha]_D^{25} = +75.9$ (c = 0.30, $CHCl_3$). IR (ATR): 3390*w*, 3081*w*, 3059*w*, 2928*w*, 2105*s*, 1602*w*, 1497*w*, 1454*w*, 1360*w*, 1324*w*, 1262*m*, 1118*m*, 1087*m*, 1039*m*, 956*w*, 906*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.38–7.13 (*m*, 35 arom. H); 4.94 (*d*, J = 10.5), 4.70 (*s*, 2 H), 4.64 (*d*, J = 10.8, 2 H), 4.61 (*d*, $J \approx 9.3$, 2 H), 4.56, (*d*, J = 11.4, 2 H), 4.44 (*d*, J = 10.2), 4.40 (*d*, J = 10.8), 4.30 (*d*, J = 12.0), 4.25 (*d*, J = 11.7, 2 H) (7 PhCH_2); 2.38 (br. *s*, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 138.17, 138.12, 137.87, 137.49, 137.29, 136.94, 136.89 (7*s*, 7 arom. C); 128.62–127.44 (several *d*, 35 arom. CH); 75.06, 73.36 (3 C), 72.41, 71.92, 71.76 (5*t*, 7 PhCH_2). HR-MALDI-MS: 1414.5402 (15, $[M + K]^+$, $C_{72}H_{77}KN_{15}O_{14}^+$; calc. 1414.5411), 1398.5691 (100, $[M + Na]^+$, $C_{72}H_{77}N_{15}NaO_{14}^+$; calc. 1398.5672), 1348.5719 (23, $[M - N_2 + H]^+$, $C_{72}H_{78}N_{13}O_{14}^+$; calc. 1348.5791). Anal. calc. for $C_{72}H_{77}N_{15}O_{14} \cdot 2\,H_2O$ (1412.50): C 61.22, H 5.78, N 14.87; found: C 60.95, H 5.24, N 14.80.

4′-epi-*6,3′,6′,2″,5″,3‴,4‴-Hepta*-O-*benzylparomomycin* (**12**). A soln. of **11** (71 mg, 0.05 mmol) in THF (10 ml) was treated with 0.1M aq. NaOH (1.8 ml) and 1M PMe_3 in THF (0.45 ml, 0.45 mmol), and stirred for 2 h at 50°. Evaporation and FC (amino-phase silica gel; MeOH/$CHCl_3$ 3:97) gave **12** (42 mg,

Table 7. *^{13}C-NMR Chemical Shifts* [ppm] *of the Protected Amino Derivatives* **8**, **12**, *and* **16** *in $CDCl_3$, and of the Deprotected Amino Derivatives* **9**, **13**, *and* **17** *in D_2O*

	8	**12**	**16**	**9**	**13**[a])	**17**
C(1′)	99.87	99.81	99.72	100.86	99.21	98.11
C(2′)	51.60	52.01	53.26	55.12	53.59	57.11
C(3′)	80.71	80.63	80.43	67.67	68.91	69.99
C(4′)	51.96	66.38	56.88	57.17	70.57	55.17
C(5′)	70.11	69.97	73.39	75.13	75.30	74.90
C(6′)	70.11	70.26	70.04	65.51	63.79	62.85
C(1)	51.40[b])	51.73[b])	51.99[b])	54.66[b])	52.72	53.57[b])
C(2)	38.62	38.63	38.49	33.06	32.51	32.66
C(3)	51.04[b])	51.41[b])	51.72[b])	53.81[b])	51.81	52.76[b])
C(4)	76.32	76.36	76.29	82.83	82.12	83.93
C(5)	86.04	86.54	86.55	89.03	87.31	87.30
C(6)	87.27	87.17	86.96	77.17	75.62	75.53
C(1″)	107.00	107.19	107.45	114.83	112.65	112.62
C(2″)	81.74	81.57	83.54	78.37	76.14	76.08
C(3″)	80.28	79.67	81.19	80.06	77.85	77.85
C(4″)	81.16	81.18	81.57	86.42	83.92	81.78
C(5″)	70.71	70.26	70.35	65.09	62.74	63.12
C(1‴)	100.81	101.18	101.65	101.09	98.20	99.12
C(2‴)	49.10	51.30	51.41	55.84[b])	53.62	51.69
C(3‴)	75.64	75.63	75.71	72.61	70.43	70.53
C(4‴)	71.90	73.00	72.95	72.26	70.03	70.39
C(5‴)	74.57	74.61	74.56	73.16	72.99	72.94
C(6‴)	42.46	42.29	42.42	45.44	43.15	43.10

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

65%). White solid. R_f (MeOH/$CHCl_3$ 1:19) 0.21. M.p. 55°. $[\alpha]_D^{25} = +33.4$ ($c = 0.18$, $CHCl_3$). IR ($CHCl_3$): 3375*w* (br.), 3309*w*, 3081*w*, 3054*w*, 2926*m*, 2874*m*, 1601*m*, 1493*m*, 1454*m*, 1366*m*, 1093*m*, 1070*m*, 1025*m*, 917*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 6*; additionally, 7.37–7.19 (*m*, 35 arom. H); 4.94 (*d*, J = 11.4), 4.69 (*d*, J = 11.7), 4.63 (*d*, J = 12.0), 4.59 (*d*, J = 12.9), 4.57–4.52 (*m*, 7 H), 4.50 (*d*, J = 12.3), 4.44 (*d*, J = 12.3), 4.39 (*d*, J = 12.3) (7 Ph*C*H_2); 1.60 (br. *s*, $w_{1/2} \approx 53.0$, 7 NH_2, OH). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 7*; additionally, 138.24, 137.98 (3 C), 137.71 (2 C), 137.18 (4*s*, 7 arom. C); 128.45–127.61 (several *d*, 35 arom. CH); 75.08, 73.62, 73.17, 73.09, 71.96 (2 C), 71.86 (6*t*, 7 Ph*C*H_2). HR-MALDI-MS: 1284.5860 (54, $[M + K]^+$, $C_{72}H_{87}KN_5O_{14}^+$; calc. 1284.5887), 1268.6147 (92, $[M + Na]^+$, $C_{72}H_{87}N_5NaO_{14}^+$; calc. 1268.6147), 1246.6307 (98, $[M + H]^+$, $C_{72}H_{88}N_5O_{14}^+$; calc. 1246.6328), 905.4727 (79, $[M - C_{20}H_{25}N_2O_3 + H]^+$, $C_{52}H_{63}N_3O_{11}^+$; calc. 905.4463), 565.2919 (100, $[M - C_{20}H_{25}N_2O_3 - C_{20}H_{24}NO_4 + 3\,H]^+$, $C_{32}H_{41}N_2O_7^+$; calc. 565.2914). Anal. calc. for $C_{72}H_{87}N_5O_{14} \cdot 3\,H_2O$ (1300.53): C 66.49, H 7.21, N 5.38; found: C 66.69, H 7.15, N 5.48.

4′-epi-*Paromomycin Pentaacetate Trihydrate* (**13**). A soln. of **12** (30 mg, 0.02 mmol) in AcOH/H_2O/D_2O 2:2:1 (3 ml) was treated with $Pd(OH)_2$ (20 mg) and stirred for 36 h at r.t. under 6 bar of H_2. Filtration and evaporation gave **13** (18 mg, 82%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:4:3) 0.44. M.p. 125° (dec.). $[\alpha]_D^{25} = +55.5$ ($c = 0.19$, H_2O). ^{1}H-NMR (D_2O, 600 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 6*; additionally, 1.90 (*s*, 5 AcO). ^{13}C-NMR ($CDCl_3$, 150 MHz; assignments based on a HSQC spectrum): see *Table 7*; additionally, 183.70 (*s*, 5 C=O); 25.72 (*q*, 5 *Me*C=O). HR-ESI-MS: 638.2846 (100, $[M + Na]^+$, $C_{23}H_{45}N_5NaO_{14}^+$; calc. 638.2861), 616.3025 (78, $[M + H]^+$, $C_{23}H_{46}N_5O_{14}^+$; calc. 616.3041), 455.2325 (100, $[M - C_6H_{13}N_2O_3 + 2\,H]^+$, $C_{17}H_{35}N_4O_{10}^+$; calc. 455.2353). Anal. calc. for $C_{23}H_{45}N_5O_{14} \cdot 5\,AcOH \cdot 3\,H_2O$ (969.93): C 40.86, H 7.38, N 7.22; found: C 41.14, H 7.15, N 7.29.

4′-epi-*1,3,2′,2‴,6‴-Pentaazido-6,3′,6′,2″,5″,3‴,4‴-hepta*-O-*benzyl-1,3,2′,2‴,6‴-pentadeamino-4′*-O-*[(trifluoromethyl)sulfonyl]paromomycin* (**14**). Under N_2, a soln. of pyridine (0.03 ml, 0.36 mmol) and Tf_2O (0.06 ml, 0.36 mmol) in CH_2Cl_2 (5 ml) was stirred for 30 min at $-10°$, treated portionwise with a soln. of **11** (206 mg, 0.15 mmol) in CH_2Cl_2 (5 ml) during 30 min, and stirred for 5 h at $-10°$ and for 4 h at 20°. After neutralization with aq. $NaHCO_3$ and extraction with CH_2Cl_2 (3×30 ml), the combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC (AcOEt/cyclohexane 3:22) gave **14** (170 mg, 75%). White solid. R_f (AcOEt/cyclohexane 1:4) 0.44. M.p. 48.0–48.9°. $[\alpha]_D^{25} = +79.1$ ($c = 0.17$, $CHCl_3$). IR ($CHCl_3$): 3054*w*, 2923*w*, 2870*w*, 2104*s*, 1601*w*, 1493*w*, 1454*w*, 1408*m*, 1362*w*, 1333*w*, 1259*w*, 1239*w*, 1141*m*, 1077*w*, 1031*w*, 916*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.42–7.12 (*m*, 35 arom. H); 4.95 (*d*, $J = 10.5$), 4.87 (*d*, $J = 11.4$), 4.66 (*d*, $J = 10.5$), 4.661 (*d*, $J = 11.4$), 4.58 (*d*, $J = 12.0$), 4.54 (*d*, $J = 12.0$), 4.52 (*d*, $J = 12.0$, 2 H), 4.50 (*d*, $J = 11.7$), 4.41 (*d*, $J = 11.7$), 4.39 (*d*, $J = 11.7$), 4.36 (*d*, $J \approx 12.5$), 4.28 (*d*, $J \approx 12.3$), 4.24 (*d*, $J = 12.3$) (7 Ph*C*H_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 137.93, 137.75, 137.46, 137.40, 136.91, 136.84, 136.28 (7*s*, 7 arom. C); 128.58–127.30 (several *d*, 35 arom. CH); 118.26 (*q*, ^1J(C,F) = 316.6, CF_3); 74.94, 73.24, 73.23 (2 C), 72.29, 72.28, 71.60 (6*t*, 7 Ph*C*H_2). ^{19}F-NMR ($CDCl_3$, 282 MHz): −73.85. HR-MALDI-MS: 1530.5119 (100, $[M + Na]^+$, $C_{73}H_{76}F_3N_{15}NaO_{16}S^+$; calc. 1530.5165), 1380.5642 (56, $[M - TfOH + Na]^+$, $C_{72}H_{75}N_{15}NaO_{13}^+$; calc. 1380.5566). Anal. calc. for $C_{73}H_{76}F_3N_{15}O_{16}S$ (1508.54): C 58.12, H 5.08, N 13.93, S 2.13; found: C 58.22, H 5.29, N 13.76, S 2.27.

1,3,2′,4′,2‴,6‴-Hexaazido-6,3′,6′,2″,5″,3‴,4‴-hepta-O-*benzyl-1,3,2′,2‴,6‴-pentadeamino-4′-deoxyparomomycin* (**15**). Under N_2, a soln. of **14** (106 mg, 0.076 mmol) in MeCN (3 ml) was treated with Bu_4NN_3 (238 mg, 0.84 mmol) and stirred for 10 h at r.t. After evaporation, a soln. of the residue in CH_2Cl_2 (10 ml) was washed twice with brine, dried ($MgSO_4$), filtered, and evaporated. FC (amino-phase silica gel; AcOEt/cyclohexane 3:22) gave **15** (62 mg, 63%). Colourless oil. R_f (amino-phase silica gel; AcOEt/cyclohexane 1:3) 0.45. $[\alpha]_D^{25} = +98.1$ ($c = 0.17$, $CHCl_3$). IR ($CHCl_3$): 2868*w*, 2105*s*, 1603*w*, 1495*w*, 1454*w*, 1364*w*, 1322*w*, 1264*m*, 1081*m*, 1027*m*, 919*w*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 4*; additionally, 7.45–7.14 (*m*, 35 arom. H); 4.96 (*d*, $J = 10.5$), 4.85 (*d*, $J = 10.8$), 4.81 (*d*, $J \approx 11.0$), 4.67 (*d*, $J = 12.3$, 2 H), 4.62 (*d*, $J = 12.0$), 4.58 (*d*, $J = 12.0$), 4.56 (*d*, $J = 11.7$, 2 H), 4.49 (*d*, $J = 12.6$), 4.42 (*d*, $J = 12.0$), 4.41 (*d*, $J = 12.0$), 4.31 (*d*, $J = 12.0$), 4.25 (*d*, $J = 12.0$) (7 Ph*C*H_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 5*; additionally, 138.02, 137.82, 137.74, 137.41, 137.21, 136.87, 136.80 (7*s*, 7 arom. C), 128.56–127.31 (several *d*, 35 arom. CH); 75.35, 75.07, 73.40, 73.26, 73.23, 72.36, 71.71 (7*t*, 7 Ph*C*H_2). HR-ESI-MS: 1439.5408 (13, $[M + K]^+$, $C_{72}H_{76}KN_{18}O_{13}^+$; calc. 1439.5476), 1423.5705 (100, $[M + Na]^+$, $C_{72}H_{76}N_{18}NaO_{13}^+$; calc. 1423.5737), 1373.5836 (18, $[M - N_2 + H]^+$, $C_{72}H_{77}N_{16}O_{13}^+$; calc. 1373.5856). Anal. calc. for $C_{72}H_{76}N_{18}O_{13} \cdot H_2O$ (1419.50): C 60.92, H 5.54, N 17.76; found: C 60.70, H 5.31, N 17.33.

4′-Amino-6,3′,6′,2″,5″,3‴,4‴-hepta-O-*benzyl-4′-deoxyparomomycin* (**16**). A soln. of **15** (38 mg, 0.03 mmol) in THF (6 ml) was treated with 0.1M aq. NaOH (1.08 ml) and 1M PMe_3 in THF (0.32 ml, 0.32 mmol), stirred for 2 h at 50°, and evaporated. FC (amino-phase silica gel; MeOH/cyclohexane 3:97) gave **16** (27 mg, 80%). White solid. R_f (MeOH/$CHCl_3$ 1:19) 0.23. M.p. 45.3–47.2°. $[\alpha]_D^{25} = +21.5$ ($c = 0.22$, $CHCl_3$). IR ($CHCl_3$): 3389*w* (br.), 3305*w* (br.), 3059w, 2924*m*, 2865*m*, 1601*m*, 1496*w*, 1454*m*, 1361*w*, 1311*w*, 1094*m*, 1068*m*, 1026*m*, 908*m*. ^{1}H-NMR ($CDCl_3$, 300 MHz): see *Table 6*; additionally, 7.35–7.20 (*m*, 35 arom. H); 4.93 (*d*, $J = 11.1$), 4.89 (*d*, $J = 11.7$), 4.68 (*d*, $J = 11.4$), 4.58 (*d*, $J \approx 13.0$), 4.57 (*d*, $J = 12.3$, 2 H), 4.53 (*d*, $J \approx 13.0$), 4.532 (*d*, $J = 11.1$, 2 H), 4.51 (*d*, $J = 11.7$), 4.44 (*d*, $J = 12.0$, 2 H), 4.42 (*d*, $J = 12.0$), 4.23 (*d*, $J = 11.4$) (7 Ph*C*H_2); 1.53 (br. *s*, $w_{1/2} = 72.0$, 6 NH_2). ^{13}C-NMR ($CDCl_3$, 75 MHz): see *Table 7*; additionally, 138.65, 138.18, 137.98, 137.94, 137.67 (2 C), 137.13 (6*s*, 7 arom. C), 128.44–127.46 (several *d*, 35 arom. CH); 75.12, 74.43, 73.39, 73.21, 73.05, 71.92 (2 C) (6*t*, 7 Ph*C*H_2). Anal. calc. for $C_{72}H_{88}N_6O_{13} \cdot 3\,H_2O$ (1299.55): C 66.54, H 7.29, N 6.47; found: C 66.23, H 7.01, N 6.48.

4′-Amino-4′-deoxyparomomycin Hexaacetate (**17**). A soln. of **16** (16 mg, 0.01 mmol) in AcOH/H_2O/D_2O 2:2:1 (3 ml) was treated with $Pd(OH)_2$ (13 mg) and stirred for 12 h at r.t. under 6 bar of H_2. Filtration and evaporation gave **17** (11 mg, 88%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:4:3) 0.40. M.p. 125° (dec.). $[\alpha]_D^{25} = +44.6$ ($c = 0.21$, H_2O). ^{1}H-NMR (D_2O, 400 MHz): see *Table 6*; additionally, 1.79 (*s*, 6 AcO). ^{13}C-NMR ($CDCl_3$, 100 MHz): see *Table 7*; additionally, 183.89 (*s*, 6 OC=O); 25.77 (*q*, 6 *Me*C=O). HR-ESI-MS: 637.3035 (86, $[M + Na]^+$, $C_{23}H_{46}N_6NaO_{13}^+$; calc. 637.3021), 477.2178 (100, $[M - C_6H_{13}N_2O_3 + Na + H]^+$, $C_{17}H_{34}N_4NaO_{10}^+$; calc. 477.2178).

1,3,2′,2‴,6‴-Pentaazido-6,3′,2″,5″,3‴,4‴-hexa-O-*benzyl-1,3,2′,2‴,6‴-pentadeaminoparomomycin* (**18**). A soln. of **4** (3.37 g, 2.45 mmol) in CH_2Cl_2/MeOH 4:1 (25 ml) was treated at r.t. with $TsOH \cdot H_2O$ (0.70 g, 3.68 mmol), and stirred for 12 h. After evaporation, FC (AcOEt/cyclohexane 2:3) gave **18** (1.33 g, 42%) and **4** (1.4 g, 42%). R_f (AcOEt/cyclohexane 1:1) 0.59. White solid. IR (ATR): 3446*w*, 3030*w*, 2871*w*, 2099*s*, 1605*w*, 1496*w*, 1454*m*, 1361*m*, 1215*m*, 1026*s*, 913*m*. ^{1}H-NMR ($CDCl_3$, 400 MHz; assignments based on a DQFCOSY and a HSQC spectrum): 7.51–7.14 (*m*, 30 arom. H); 6.17 (*d*, $J = 3.6$, H–C(1′)); 5.69 (*d*, $J = 5.8$, H–C(1″)); 4.99 (*d*, $J = 10.5$, PhC*H*); 4.93 (*d*, $J \approx 11.4$, PhC*H*); 4.91 (br. *s*, H–C(1‴)); 4.72 (*d*, $J = 10.7$, PhC*H*); 4.67 (*d*, $J = 11.4$, PhC*H*); 4.63 (*d*, $J = 12.0$, PhC*H*); 4.59 (*d*, $J = 11.8$, PhC*H*); 4.482 (*s*, PhCH_2); 4.475 (*d*, $J = 11.8$, PhC*H*); 4.41 (*d*, $J = 12.0$, PhC*H*); 4.33 (br. *s*, H–C(4″)); 4.32 (br. *s*, H–C(3″)); 4.32 (*d*, $J = 12.0$, PhC*H*); 4.26 (*d*, $J = 12.1$, PhC*H*); 3.99 (*t*, $J = 5.1$, H–C(2″)); 3.97 (*d*, $J = 8.9$, H–C(5)); 3.91–3.82 (*m*, H–C(3′), H–C(5′), H_a–C(5″)); 3.82–3.70 (*m*, 2 H–C(6′), H–C(3‴), H–C(5‴)); 3.70–3.57 (*m*, H–C(4), H_b–C(5″), H_a–C(6‴)); 3.48–3.36 (*m*, H–C(1), H–C(3), H–C(4′)); 3.36 (br. *t*, $J \approx 2.0$, H–C(2‴)); 3.30 (*t*, $J = 9.3$, H–C(6)); 3.13 (br. *t*, $J \approx 1.9$, H–C(4‴)); 2.89 (*dd*, $J = 12.9$, 3.75, H_b–C(6‴)); 2.86 (*dd*, $J = 10.2$, 3.65, H_b–C(2′)); 2.23 (*dt*, $J = 13.2$, 4.5, H_{eq}–C(2)); 2.16 (*d*, $J = 3.3$, HO–C(4′)); 1.67 (*dd*, $J = 8.1$, 4.9, HO–C(6′)); 1.40 (*q*, $J = 12.7$, H_{ax}–C(2)). ^{13}C-NMR ($CDCl_3$, 100 MHz; assignments based on a HSQC spectrum): 138.31, 138.11, 137.93, 137.56, 137.02, 136.95 (6*s*, 6 arom. C); 128.70–127.15 (several *d*, 30 arom. *C*H); 106.17 (*d*, C(1‴)); 98.69 (*d*, C(1‴)); 95.97 (*d*, C(1′)); 84.29 (*d*, C(6)); 82.54 (*d*, C(2″)); 82.19 (*d*, C(4″)); 82.01 (*d*, C(5)); 79.71 (*d*, C(3′)); 75.54 (*d*, C(3″)); 75.09, 74.94 (2*t*, 2 PhCH_2); 74.94 (*d*, C(4)); 74.45 (*d*, C(5‴)); 73.22 (*t*, 2 PhCH_2); 72.93 (*d*, C(3‴)); 72.42, 71.76 (2*t*, 2 PhCH_2); 71.61 (*d*, C(4‴)); 71.56 (*d*, C(5′)); 70.46 (*d*, C(4′)); 70.32 (*t*, C(5″)); 62.66 (*d*, C(2′)); 62.16 (*t*, C(6′)); 60.41, 60.29 (2*d*, C(1), C(3)); 57.28 (*d*, C(2‴)); 51.19 (*t*, C(6‴)); 32.56 (*t*, C(2)). HR-MALDI-MS: 1308.5197 (100, $[M + Na]^+$, $C_{67}H_{71}N_{15}NaO_{14}^+$; calc. 1308.5203).

6,3′,2″,5″,3‴,4‴-Hexa-O-*benzyl-1,3,2′,2‴,6‴-pentakis*-N-*[(benzyloxy)carbonyl]paromomycin* (**19**). A soln. of **18** (1.15 g, 0.89 mmol) in THF (24 ml) was treated with 0.1M aq. NaOH (6 ml) and 1M PMe_3 in THF (6.7 ml, 6.7 mmol), stirred for 2.5 h at 50°, and evaporated. An ice-cold soln. of the crude product and Na_2CO_3 (1.3 g, 12.3 mmol) in MeOH (20 ml) was treated dropwise with CbzCl (0.76 ml, 5.4 mmol), and stirred at r.t. for 6 h. After filtration and evaporation, a soln. of the residue in AcOEt (20 ml) was washed with H_2O and brine, dried (Na_2SO_4), filtered, and evaporated. FC (AcOEt/cyclohexane 2:3) gave **19** (1.3 g, 80%). R_f (AcOEt/cyclohexane 2:1) 0.58. IR (ATR): 3419*w*, 3330*w*, 3063*w*, 3032*w*, 2933*w*, 1704*s*, 1514*s*, 1497*m*, 1454*m*, 1331*m*, 1299*m*, 1216*s*, 1043*s*, 1025*s*, 912*m*. HR-MALDI-MS: 1849.7579 (100, $[M + Na]^+$, $C_{105}H_{111}N_5NaO_{24}^+$; calc. 1849.7550).

6,3′,2″,5″,3‴,4‴-Hexa-O-*benzyl-1,3,2′,2‴,6‴-pentakis*-N-*[(benzyloxy)carbonyl]-6′*-O-*(2,2-dimethylpropanoyl)paromomycin* (**20**). A soln. of **19** (600 mg, 0.47 mmol) in CH_2Cl_2/pyridine 5:3 (16 ml) was treated with pivaloyl chloride (0.3 ml, 2.45 mmol) and stirred for 12 h at r.t. Evaporation and FC (AcOEt/cyclohexane 2:3) gave **20** (523 mg, 83%). R_f (AcOEt/cyclohexane 1:1) 0.63. IR (ATR): 3415*w*, 3036*w*, 2938*w*, 2844*w*, 1715*s*, 1513*m*, 1454*m*, 1212*m*, 1052*s*, 1032*s*, 1016*s*, 910*w*. HR-MALDI-MS: 1933.8059 (100, $[M + Na]^+$, $C_{110}H_{119}N_5NaO_{25}^+$; calc. 1933.8125).

6,3′,2″,5″,3‴,4‴-Hexa-O-*benzyl-1,3,2′,2‴,6‴-pentakis*-N-*[(benzyloxy)carbonyl]-6′*-O-*(2,2-dimethylpropanoyl)-4′*-O-*[(methylsulfanyl)carbothioyl]paromomycin* (**21**). A suspension of NaH (16 mg, 0.41 mmol) in THF (10 ml) at 0° was treated with a soln. of **20** (523 mg, 0.27 mmol) in THF (10 ml), stirred for 10 min, treated with CS_2 (0.3 ml, 5 mmol) and 1*H*-imidazole (10 mg, 0.15 mmol), stirred at r.t. for 2 h, treated with MeI (50 µl, 0.81 mmol), and stirred for 20 min. The mixture was treated with sat. aq. NH_4Cl (10 ml), and extracted with AcOEt (3 × 15 ml). The combined org. layers were washed with brine, dried (Na_2SO_4), filtered, and evaporated. FC (AcOEt/cyclohexane 1:3) gave **21** (460 mg, 84%). R_f (AcOEt/cyclohexane 2:3) 0.50. IR (ATR): 3424*w*, 3334*w*, 3063*w*, 3031*w*, 2932*w*, 1716*s*, 1586*w*, 1513*m*, 1454*m*, 1331*m*, 1212*s*, 1045*s*, 1026*s*, 964*m*, 912*w*. HR-MALDI-MS: 2023.7670 (100, $[M + Na]^+$, $C_{112}H_{121}N_5NaO_{25}S_2^+$; calc. 2023.7723).

6,3′,2″,5″,3‴,4‴-Hexa-O-*benzyl-1,3,2′,2‴,6‴-pentakis*-N-*[(benzyloxy)carbonyl]-4′-deoxy-6′*-O-*(2,2-dimethylpropanoyl)paromomycin* (**22**). A soln. of **21** (355 mg, 0.18 mmol), Et_3N (0.2 ml, 1.44 mmol), and H_3PO_2 (0.2 ml, 3.65 mmol) in 1,4-dioxane (10 ml) was heated to reflux, treated with a soln. of AIBN (300 mg, 1.83 mmol) in 1,4-dioxane (5 ml) during 15 min, and kept at reflux for 24 h. The mixture was cooled to r.t., diluted with AcOEt (20 ml), washed with H_2O (2 × 12 ml) and brine, dried (Na_2SO_4), filtered, and evaporated. FC (AcOEt/cyclohexane 1:3) afforded **22** (303 mg, 84%). R_f (AcOEt/

cyclohexane 2:3) 0.50. IR (ATR): 3424*w*, 3021*w*, 2972*w*, 1715*s*, 1605*w*, 1515*m*, 1454*m*, 1233*m*, 1092*m*, 1047*s*, 1032*s*, 1020*s*, 912*w*. HR-MALDI-MS: 1916.8143 (100, $[M + Na]^+$, $C_{110}H_{119}N_5NaO_{24}^+$; calc. 1917.8176).

4′-Deoxyparomomycin (**23**). A cold (−78°) soln. of condensed NH_3 (*ca.* 20 ml) in THF (5 ml) was treated portionwise with Na (70 mg, 3.04 mmol). The resulting dark blue soln. was treated dropwise with a soln. of **22** (230 mg, 0.12 mmol) in THF (5 ml) and then allowed to reach r.t. over 5 h. After dilution with MeOH (10 ml), the mixture was neutralized with *Amberlite IR-120* (H^+ form), filtered, and evaporated. FC (amino-phase silica gel: $CHCl_3$/MeOH/25% NH_3 15:45:1), evaporation, filtration, and lyophilization gave **23** (51 mg, 68%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:3:2) 0.39. IR (ATR): 3103*m*, 2909*m*, 1543*s*, 1454*w*, 1379*w*, 1344*w*, 1138*w*, 1015*s*, 932*w*. ^{1}H-NMR (D_2O, 400 MHz; assignments based on a DQFCOSY and a HSQC spectrum): 5.25 (*d*, *J* = 3.7, H−C(1′)); 5.23 (*d*, *J* = 2.9, H−C(1″)); 4.83 (*d*, *J* = 1.8, H−C(1‴)); 4.29 (*dd*, *J* = 6.1, 5.2, H−C(3″)); 4.13 (*dd*, *J* = 5.1, 2.9, H−C(2″)); 4.01–3.95 (*m*, H−C(5′), H−C(4″)); 3.88 (*t*, *J* = 3.3, H−C(3‴)); 3.82 (*dt*, *J* ≈ 8.4, 4.0, H−C(5‴)); 3.76–3.67 (*m*, H−C(3′), H_a−C(6′)); 3.59 (*dd*, *J* = 12.4, 5.3, H_b−C(6′)); 3.53 (*dd*, *J* = 12.2, 3.5, H_a−C(5″)); 3.50 (*t*, *J* = 9.3, H−C(5)); 3.50 (*t*, *J* ≈ 3.0, H−C(4‴)); 3.44 (*dd*, *J* = 12.2, 6.4, H_b−C(5″)); 3.29 (*t*, *J* = 9.3, H−C(4)); 3.13 (*t*, *J* = 9.6, H−C(6)); 2.89 (*dd*, *J* = 13.5, 8.5, H_a−C(6‴)); 2.89 (*dd*, *J* = 3.8, 1.8, H−C(2‴)); 2.76 (*dd*, *J* = 13.6, 4.3, H_b−C(6‴)); 2.72 (*ddd*, *J* = 12.4, 9.7, 4.2, H−C(3)); 2.58 (*ddd*, *J* = 12.0, 9.8, 3.8, H−C(1)); 2.50 (*dd*, *J* = 10.1, 3.6, H−C(2′)); 1.86–1.76 (*m*, H_{eq}−C(2), H_{eq}−C(4′)); 1.28 (*q*, *J* = 12.0, H_{ax}−C(4′)); 1.06 (*q*, *J* = 12.4, H_{ax}−C(2)). ^{13}C-NMR (D_2O, 100 MHz; assignments based on a HSQC spectrum): 110.99 (*d*, C(1″)); 102.80 (*d*, C(1′)); 102.08 (*d*, C(1‴)); 86.87 (*d*, C(5)); 85.84 (*d*, C(4)); 84.21 (*d*, C(4″)); 80.11 (*d*, C(6)); 78.64 (*d*, C(3″)); 77.82 (*d*, C(5‴)); 76.08 (*d*, C(2″)); 73.27 (*d*, C(3‴)); 72.63 (*d*, C(5′)); 71.17 (*d*, C(4‴)); 70.42 (*d*, C(3′)); 66.48 (*t*, C(5″)); 63.88 (*t*, C(6′)); 59.64 (*d*, C(2′)); 55.29 (*d*, C(2‴)); 52.92 (*d*, C(1), C(3)); 43.70 (*t*, C(6‴)); 38.14 (*t*, C(2)); 37.10 (*t*, C(4′)). HR-MALDI-MS: 600.3087 (100, $[M + H]^+$, $C_{23}H_{46}N_5O_{13}^+$; calc. 600.3092).

D-*Glyceraldehyde-(2 → 1)-(1*R*)-2-azido-2-deoxy*-D-*glycerodialdehyde-(1 → 4)-1,3,2‴,6‴-tetraazido-1,3,2‴,6‴-tetradeamino-4*-O-*de(2-amino-2-deoxy-α*-D-*glucopyranosyl)paromomycin* (**24**). Under N_2, a soln. of **2** (163 mg, 0.22 mmol) in THF (20 ml) was treated with 4-Å molecular sieves, stirred for 1 h, cooled to 0°, treated with H_5IO_6 (72 mg, 0.32 mmol), stirred for 3 h, neutralized with sat. aq. $NaHCO_3$, and extracted with AcOEt (3 × 20 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated to afford crude **24** (90 mg, *ca.* 55%) that was used as such for further reactions. Yellow oil. R_f ($CHCl_3$/AcOEt/MeOH 4:8:1) 0.42.

1,3,2‴,6‴-Tetraazido-1,3,2‴,6‴-tetradeamino-4-O-*de(2-amino-2-deoxy-α*-D-*glucopyranosyl)-4*-O-*(3,4,5-trideoxy-4*-N-*phenyl-4-aza-α*-D-erythro-*heptoseptanosyl)paromomycin* (**25**). Under N_2, a soln. of crude **24** (300 mg, 0.4 mmol) in MeOH (10 ml) was treated with $NaCNBH_3$ (102 mg, 1.61 mmol) and portionwise, over 1 h, with a soln. of $PhNH_2$ (37 µl, 0.40 mmol) and AcOH (97 µl, 1.7 mmol) in MeOH (3 ml), and stirred at r.t. for 12 h. Et_3N (1 ml) was added, and the mixture was taken to dryness. A suspension of the residue in sat. aq. $NaHCO_3$ was extracted with AcOEt (3 × 20 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC ($CHCl_3$/AcOEt 5:9) gave **25** (90 mg, 32% from **2**). Yellow oil. R_f ($CHCl_3$/AcOEt/MeOH 4:8:1) 0.52. ^{1}H-NMR (CD_3OD, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 8*; additionally, 7.17 (*dt*, *J* = 8.8, 1.6, 2 arom. H); 6.88 (*dd*, *J* = 8.9, 0.9, 2 arom. H); 6.80 (*dt*, *J* = 7.3, 0.9, 1 arom. H). ^{13}C-NMR (CD_3OD, 125 MHz; assignments based on a HSQC spectrum): see *Table 9*; additionally, 151.48 (*s*); 130.13 (*d*, 2 C); 118.65 (*d*); 114.99 (*d*, 2 C). HR-MALDI-MS: 827.2883 (32, $[M + Na]^+$, $C_{29}H_{40}N_{16}NaO_{12}^+$; calc. 827.2909), 805.3070 (100, $[M + H]^+$), $C_{29}H_{41}N_{16}O_{12}^+$; calc. 805.3090), 461.2003 (46, $[M - C_{11}H_{17}N_6O_7 + 2\,H]^+$, $C_{18}H_{25}N_{10}NaO_5^+$; calc. 461.2009).

1,3,2‴,6‴-Tetraazido-1,3,2‴,6‴-tetradeamino-4-O-*de(2-amino-2-deoxy-α*-D-*glucopyranosyl)-4*-O-*(4*-N-*benzyl-3,4,5-trideoxy-4-aza-α*-D-erythro-*heptoseptanosyl)paromomycin* (**26**). Under N_2, a soln. of crude **24** (300 mg, 0.4 mmol) in MeOH (10 ml) was treated with $NaCNBH_3$ (102 mg, 1.61 mmol) and portionwise, during 1 h, with a soln. of $BnNH_2$ (44 µl, 0.40 mmol) and AcOH (97 µl, 1.7 mmol) in MeOH (3 ml), stirred at r.t. for 12 h, basified with Et_3N (1 ml), and evaporated. A suspension of the residue in sat. aq. $NaHCO_3$ was extracted with AcOEt (3 × 20 ml). The combined org. layers were washed with brine, dried ($MgSO_4$), filtered, and evaporated. FC ($CHCl_3$/AcOEt 5:9) gave **26** (100 mg, 36% from **2**). Yellow oil. R_f ($CHCl_3$/AcOEt/MeOH 20:40:7) 0.63. ^{1}H-NMR (CD_3OD, 300 MHz): see *Table 8*;

Table 8. *^{1}H-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Azides* **25** *and* **26** *in CD_3OD, and the Amines* **27** *and* **28** *in D_2O*

	25[a])	**26**	**27**[a])	**28**[a])		**25**	**26**	**27**	**28**
H–C(1′)	5.86	5.62	5.45	5.22	J(1′,2′)	2.3	3.6	1.4	1.9
H–C(2′)	4.04–3.99	3.54–3.29	3.38–3.39	3.22–3.21	J(2′,3′a)	[b])	[b])	5.0	[b])
H_a–C(3′)	4.04–3.99	3.00	3.94	3.01–2.95	J(2′,3′b)	[b])	[b])	3.2	2.3
H_b–C(3′)	3.44–3.40	2.57	3.33	2.53	J(3′a,3′b)	[b])	13.2	14.9	13.4
H_a–C(5′)	3.81	3.06	3.83–3.79	3.01–2.95	J(5′a,5′b)	14.5	12.8	15.3	14.2
H_b–C(5′)	3.18	2.33	3.21	2.33	J(5′a,6′)	10.4	9.9	10.6	10.6
H–C(6′)	4.41	4.36–4.32	4.38	4.04–4.01	J(5′b,6′)	5.3	6.0	7.6	[b])
H_a–C(7′)	3.83	3.78	3.81	3.73	J(6′,7′a)	5.3	2.7	7.0	3.5
H_b–C(7′)	3.65	3.73–3.62	3.74	3.63–3.59	J(6′,7′b)	3.1	[b])	3.1	[b])
					J(7′a,7′b)	11.8	11.9	12.1	13.1
H–C(1)	3.44–3.40	3.54–3.29	2.73	2.68	J(1,2_{ax})	12.5	12.3	12.0	12.0
H_{ax}–C(2)	1.29	1.23	1.21	1.15	J(1,2_{eq})	4.4	4.2	4.2	4.1
H_{eq}–C(2)	2.13	2.10	2.00	1.96	J(1,6)	9.8	[b])	9.9	9.8
H–C(3)	3.50	3.54–3.29	2.83	2.78	J(2_{ax},2_{eq})	13.0	12.6	12.9	13.0
H–C(4)	3.78	3.54–3.29	3.53	3.43	J(2_{ax},3)	12.5	12.3	12.3	12.2
H–C(5)	3.57	3.54–3.29	3.56	3.50	J(2_{eq},3)	4.1	4.2	4.1	4.2
H–C(6)	3.44–3.40	3.54–3.29	3.25	3.19	J(3,4)	9.4	[b])	9.2	9.6
					J(4,5)	9.4	[b])	9.2	9.4
					J(5,6)	9.2	[b])	9.1	9.3
H–C(1″)	5.24	5.19	5.28	5.21	J(1″,2″)	2.7	3.0	2.4	2.4
H–C(2″)	4.25	4.21	4.28	4.24	J(2″,3″)	4.8	4.9	5.0	4.9
H–C(3″)	4.33	4.27	4.43	4.35	J(3″,4″)	5.9	6.0	6.4	6.4
H–C(4″)	4.13	4.15–4.04	4.15	4.07	J(4″,5″a)	2.6	[b])	3.4	3.6
H_a–C(5″)	3.81	3.73–3.62	3.87	3.58	J(4″,5″b)	5.9	6.0	[b])	6.9
H_b–C(5″)	3.66	3.58	3.73	3.50	J(5″a,5″b)	11.5	11.9	12.1	11.7
H–C(1‴)	5.14	5.12	4.97	4.91	J(1‴,2‴)	1.9	1.8	1.9	1.9
H–C(2‴)	3.68	3.54–3.29	3.04	3.01–2.95	J(2‴,3‴)	1.5	3.3	3.3	3.3
H–C(3‴)	6.34	3.93	4.03	4.00	J(3‴,4‴)	3.4	3.3	3.3	3.3
H–C(4‴)	3.44–3.40	3.54–3.29	3.66	3.62	J(4‴,5‴)	[b])	1.8	1.7	1.6
H–C(5‴)	4.04–3.99	4.00	3.98	3.89	J(5‴,6‴a)	5.7	8.7	8.0	8.4
H_a–C(6‴)	3.64	3.54–3.29	3.06	3.01–2.95	J(5‴,6‴b)	4.4	4.2	4.3	4.3
H_b–C(6‴)	3.37	3.54–3.29	2.95	2.85	J(6‴a,6‴b)	13.0	[b])	13.5	13.5

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

additionally, 7.39–7.20 (*m*, 5 arom. H); 3.68 (*s*, PhCH_2). ^{13}C-NMR (CD_3OD, 75 MHz): see *Table 9*; additionally, 138.86 (*s*); 129.87 (*d*, 2 C); 128.88 (*d*, 2 C); 127.83 (*d*); 63.72 (*t*, PhCH_2). HR-MALDI-MS: 841.3097 (12, $[M + Na]^+$), $C_{30}H_{42}N_{16}NaO_{12}^+$; calc. 841.3066), 819.3225 (100, $[M + H]^+$), $C_{30}H_{43}N_{16}O_{12}^+$; calc. 819.3246).

4-O-*De(2-amino-2-deoxy-α*-D-*glucopyranosyl)-4*-O-*(3,4,5-trideoxy-4*-N-*phenyl-4-aza-α*-D-erythro-*heptoseptanosyl)paromomycin* (**27**). A soln. of **25** (90 mg, 0.11 mmol) in THF (10 ml) was treated with 0.1M aq. NaOH (3.1 ml) and 1M PMe_3 in THF (0.84 ml, 0.84 mmol), heated to 50° for 2 h, and evaporated. FC (MeOH/25% aq. NH_3 4:3) gave **27** (50 mg, 66%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:3:4) 0.55. $[\alpha]_D^{25} = +18.6$ ($c = 0.23$, H_2O). ^{1}H-NMR (D_2O, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 8*; additionally, 7.32 (*dt*, $J = 7.3$, 1.5, 2 arom. H); 6.95 (*dd*, $J = 8.1$, 2 arom. H); 6.80 (*t*, $J = 7.3$, 1 arom. H). ^{13}C-NMR (D_2O, 125 MHz; assignments based on a HSQC spectrum): see *Table 9*; additionally, 151.55 (*s*); 132.44 (*d*, 2 C); 119.94 (*d*); 115.10 (*d*, 2 C). HR-MALDI-MS: 697.3384 (50, $[M + Na]^+$), $C_{29}H_{50}N_6NaO_{12}^+$; calc. 697.3384), 675.3551 (100, $[M + H]^+$),

Table 9. *^{13}C-NMR Chemical Shifts* [ppm] *and Coupling Constants* [Hz] *of the Azides* **19** *and* **20** *in CD_3OD, and the Amines* **21** *and* **22** *in D_2O*

	25[a])	**26**	**27**[a])	**28**[a])		**25**[a])	**26**	**27**[a])	**28**[a])
C(1′)	100.48	99.56	101.29	101.08	C(1″)	111.42	111.11	111.65	111.67
C(2′)	64.44	61.26	57.34	55.33	C(2″)	75.17	74.26	75.79	75.64
C(3′)	51.59	55.36	53.96	59.85	C(3″)	77.52	77.16	78.90	78.94
C(5′)	54.26	59.77	53.86	59.29	C(4″)	83.63	83.13	84.08	84.02
C(6′)	73.75	74.76	74.84	75.79	C(5″)	63.92	64.02	64.41	64.41
C(7′)	63.78	63.34	63.78	64.24					
C(1)	61.46	61.38	52.99	53.03	C(1‴)	100.00	100.89	101.94	102.03
C(2)	33.46	33.21	37.48	37.90	C(2‴)	61.77	61.24	55.27	55.25
C(3)	62.03	61.68	52.06	52.06	C(3‴)	71.18	70.74	73.19	73.26
C(4)	78.94	78.70	83.70	86.68	C(4‴)	69.62	69.25	71.15	71.14
C(5)	86.51	86.10	86.44	84.24	C(5‴)	75.71	75.33	77.35	78.10
C(6)	77.38	77.16	79.52	79.76	C(6‴)	52.57	52.24	43.65	43.73

[a]) Assignments based on a DQFCOSY and a HSQC spectrum.

$C_{29}H_{51}N_6O_{12}^+$; calc. 675.3565). Dissolution of **27** in 50% aq. AcOH, followed by evaporation and lyophilization, gave **27**·5 AcOH·H_2O. Anal. calc. for $C_{29}H_{50}N_6O_{12}\cdot 5$ AcOH·H_2O (993.02): C 47.17, H 7.31, N 8.46; found: C 46.80, H 7.27, N 8.46.

4-O-*De(2-amino-2-deoxy-α*-D-*glucopyranosyl)-4*-O-*(4*-N-*benzyl-3,4,5-trideoxy-4-aza-α*-D-erythro-*heptoseptanosyl)paromomycin* (**28**). A soln. of **26** (90 mg, 0.11 mmol) in THF (10 ml) was treated with 0.1M aq. NaOH (3.1 ml) and 1M PMe_3 in THF (0.84 ml, 0.84 mmol), heated to 50° for 2 h, and evaporated. FC (MeOH/25% aq. NH_3 4:3) gave **28** (52 mg, 68%). White solid. R_f ($CHCl_3$/MeOH/25% aq. NH_3 1:3:4) 0.55. $[\alpha]_D^{25} = +24.7$ ($c = 0.22$, H_2O). IR (KBr): 3360*s* (br.), 3287*s* (br.), 2922*s*, 1595*s*, 1491*m*, 1454*m*, 1381*m*, 1356*m*, 1237*w*, 1149*s*, 1028*s*, 930*s*, 856*w*. ^{1}H-NMR (D_2O, 500 MHz; assignments based on a DQFCOSY and a HSQC spectrum): see *Table 8*; additionally, 7.43–7.34 (*m*, 5 arom. H); 3.74 (*s*, PhCH_2). ^{13}C-NMR (D_2O, 125 MHz; assignments based on a HSQC spectrum): see *Table 9*; additionally, 139.60 (*s*); 132.61 (*d*, 2 C); 131.21 (*d*, 2 C); 130.45 (*d*); 65.15 (*t*, PhCH_2). HR-MALDI-MS: 711.3555 (23, $[M + Na]^+$), $C_{30}H_{52}N_6NaO_{12}^+$; calc. 711.3541), 689.3705 (100, $[M + H]^+$), $C_{30}H_{53}N_6O_{12}^+$; calc. 689.3721). Dissolution of **28** in 50% aq. AcOH, followed by evaporation and lyophilization, gave **28**·5 AcOH·H_2O. Anal. calc. for $C_{30}H_{52}N_6O_{12}\cdot 5$ AcOH·H_2O (1007.04): C 47.71, H 7.41, N 8.35; found: C 47.33, H 7.13, N 8.36.

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