Aminoglycoside-Hybrid Ligands Targeting the Ribosomal Decoding Site

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The bacterial ribosome is the primary target for many classes of antibiotics including the aminoglycosides, tetracyclins, macrolides, and oxazolidinones, all of which interact predominantly with ribosomal RNA (rRNA), thereby interfering with cellular protein synthesis.[1,2] These antibiotics bind selectively to RNA sites that harbor unique sequence signatures that distinguish bacterial from eukaryotic targets. The aminoglycoside antibiotics of the related neomycin B and paromomycin classes, for example, induce translational miscoding by recognizing specifically the bacterial 16S rRNA at the decoding site, which differs by two bases from the eukaryotic sequence (Figure 1).[3,4] In contrast, the aminoglycoside hygromycin B binds to a site that is conserved among bacteria and eukaryotes,[3] inhibiting protein synthesis indiscriminately in organisms from both lineages.[5] Consequently, hygromycin B, which blocks ribosomal translocation without causing significant miscoding,[5,6] is toxic to eukaryotes and thus not used in anti-infective therapy. The binding sites of paromomycin and hygromycin B are located immediately adjacent to each other, within helix 44 of 16S rRNA,[3,7,8] which plays a key role for mRNA decoding[4,8] and has been implicated in movements during translocation.[7,9,10] X-ray crystallographic studies on the whole ribosomal 30S subunit, individual domains of rRNA, and antibiotic complexes thereof have revealed three-dimensional structures of paromomycin and hygromycin B in complex with their RNA targets.[7,8,11] Comparison of the individual aminoglycoside complexes shows that the binding sites of paromomycin and hygromycin B are partially overlapping at the position of the U1406·U1495 base pair (Figure 1b, c). Mutations at these residues conferring resistance to either aminoglycoside, in agreement with the structural data, have been described.[12]

In this report, we outline an approach to develop novel lead structures based on aminoglycoside-hybrid ligands that were conceived to bridge between the paromomycin and hygromycin B binding sites in helix 44 of bacterial rRNA and thereby potentially interfere with ribosomal function. To obtain such bridging RNA binders, we designed compounds that combined the neamine core moiety of neomycin B, which is known to confer bacterial decoding-site-specific RNA binding, along with substituents at the 1- and 6-positions of the 2-deoxystreptamine (2-DOS) ring, which were chosen to project into the hygromycin B binding site (Figure 1c). In the superimposition of the rRNA complexes of paromomycin and hygromycin B, the aminoglycosides overlap at the 2-DOS moieties, which adopt almost identical orientations, shifted by approximately 3 Å along the RNA helix. Ramakrishnan and co-workers have noted that this displacement corresponds exactly to the distance between neighboring residues in the RNA helix.[8] This observation, along with the wide conservation of the 2-DOS moiety among natural aminoglycosides, emphasizes the role of the 2-DOS ring system as a privileged scaffold for RNA recognition.[13]

Structural studies on aminoglycoside–RNA complexes have revealed that the 1- and 3-amino groups of 2-DOS are predominantly involved in RNA base recognition.[7,11,14] The hydroxy groups in the 4-, 5-, and 6-positions are often linked to additional sugar moieties. Many aminoglycosides of the potent neomycin and kanamycin classes of antibiotics carry a glucosamine-based substituent at the 4-position. The minimal aminoglycoside core structure of neamine, consisting of 2-DOS, linked at the 4-position to 2,6-diaminoglucose (Figure 1), interferes with protein synthesis at nanomolar concentration and shows moderate antibacterial potency (Table 1).[15] Removal of the 5-hydroxy group leads to enhanced activity of the resulting 5-deoxyneamine against aminoglycoside-resistant bacteria (Table 1).[16] Since the 2,5-dideoxystreptamine (2,5-dDOS) core of 5-deoxyneamine is readily available, we used it as a starting material for the synthesis of the novel RNA binders described herein (Figure 1d).

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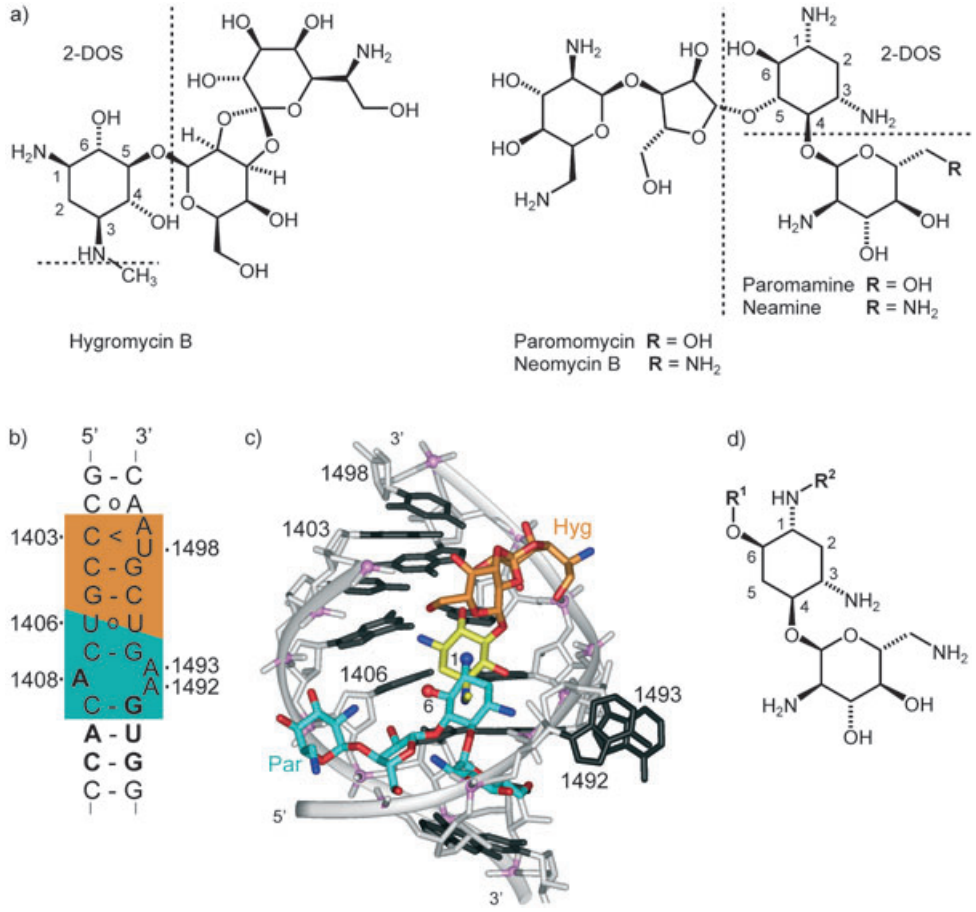


Figure 1. *a) Hygromycin B, a 5-substituted N3-methyl-2-deoxystreptamine aminoglycoside that inhibits translation in bacteria and eukaryotes. Paromomycin and neomycin B are 4,5-disubstituted 2-deoxystreptamine (2-DOS) aminoglycosides that interfere specifically with bacterial protein synthesis. The paromamine and neamine core structures, which are comprised of 2-DOS linked to a glucosamine moiety, are common to many potent aminoglycoside antibiotics. b) Secondary structure of the bacterial decoding-site internal loop and flanking sequences of helix 44 in 16S rRNA. Residues that are specific to the bacterial sequence are shown in bold. Nucleotides involved in aminoglycoside binding sites are in colored boxes (blue: paromomycin/neomycin B binding site; orange: hygromycin B binding site). c) Three-dimensional structure of the bacterial decoding-site RNA in complex with paromomycin[7] (blue sticks) and superimposed with hygromycin B bound to helix 44[8] (yellow and orange sticks). The 2-DOS moiety of hygromycin B (yellow) overlaps with the 2-DOS in paromomycin whose 1- and 6-positions are indicated. RNA bases, dark gray; sugar-phosphate backbone, light gray with phosphate groups in pink. d) 2,5-Dideoxystreptamine (2,5-dDOS) derivatives described here, which contain the 5-deoxyneamine core, were designed to bind in a bridging mode between the paromomycin/neomycin B and hygromycin B binding sites of helix 44.*

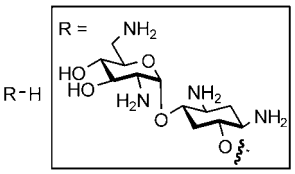
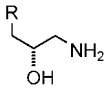
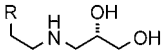
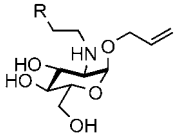
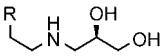
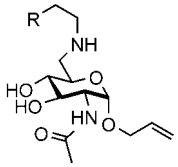
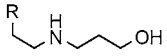
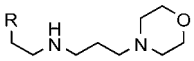
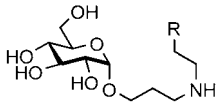
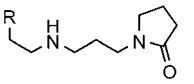
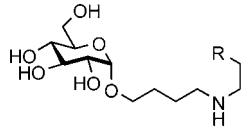
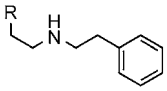
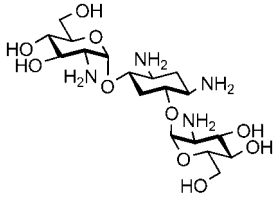
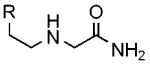
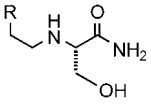
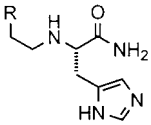
Based on the overlap of the 2-DOS moieties of paromomycin and hygromycin B in the crystal structures,[7,8,11] we designed several series of 2,5-dDOS N1 and O6 derivatives, which were conceived to extend the interactions of the compounds with RNA into the hygromycin B binding site (Figure 1 d). Specifically, we synthesized O6-alkyl, O6-alkylamine, O6-acetamide (Tables 1 and 2), as well as N1-alkyl and N1-amide derivatives (Table 3).

Two different approaches were pursued in parallel to synthesize the desired deoxyneamine analogues. The first route required the independent synthesis of two appropriately functionalized components, the optically active 2,5-dDOS monoacetate **2** and a protected form of the activated glucosamine **3**, as shown in Schemes 1–3, and 6, below. The second approach was a modification of a more linear route, reported by Mobashery and co-workers,[17] that allowed differentiation of the amine moiety at the 1-position for further functionalization (Schemes 4, 5, and 7, below).

Specifically, epoxidation of cyclohexa-1,4-diene with 3-chloroperoxybenzoic acid (*m*CPBA), followed by *syn*-stereoselective epoxide opening with hydrazine, after Pd-catalyzed hydrogenation, produced the desired *meso*-2,5-dDOS (Scheme 1), as previously described.[18] Protection of the amines as the corresponding azides was achieved by the action of triflic azide under $CuSO_4$ catalysis,[19] furnishing diazo diol **1**. Transformation of the two hydroxy groups to acetates, followed by enzymatic resolution with Novozym 435[20] resulted in the formation of monoacetate **2**. The relative stereochemistry of **2** was established by the concurrent synthesis of 5-deoxyneamine **7** via two different routes, the one presented in Scheme 2 (below), as well as by deprotection of the natural neamine-derived advanced intermediate **24** (Scheme 4, below), and direct comparison of these compounds. Coupling[20] of **3** with **1** produced the desired α,α-anomer in 74% yield after chromatographic purification. Staudinger reduction of the azides followed by hydrogenolysis of the benzylic ethers furnished **4** in excellent overall yield (for analytical data see the Supporting Information).

Incorporation of benzylic-type functionalities at the 6-position, which potentially exploit π-stacking interactions at the binding site, dictated a protection strategy not based on reductive cleavage for the 3′- and 4′-hydroxy groups. Treatment of the previously described[21] diol **5** with PMB-Cl (PMB = 4-methoxybenzyl) and sodium hydride in DMF furnished the corresponding di-PMB ether in quantitative yield (Scheme 2). Coupling with **2** followed by saponification of the acetate produced alcohol **6**. The parent aminoglycoside 5-deoxyneamine (**7**) was synthesized by oxidative cleavage of the PMB-ethers in **6** followed by Staudinger reduction of the azides. Comparison of **7** with deprotected **24**, obtained from neamine by a semisynthetic route (Scheme 4, below), provided verification of the absolute stereochemical configuration of **7** and hence **2**. Alkylation of the 6-hydroxy group in **6** was performed by utilizing chlorides (**a**–**d**, inset, Scheme 2), sodium hydride and catalytic

Table 1. *Structure–activity relationships for 2,5-dideoxystreptamine O6-ether derivatives.*

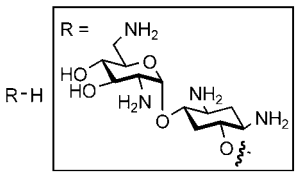
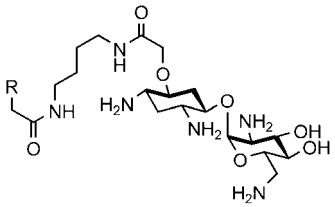
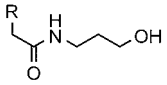
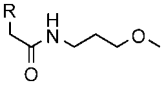
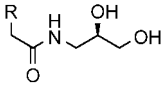
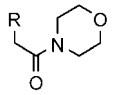
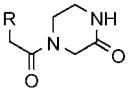
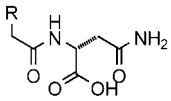
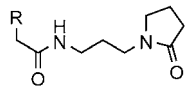
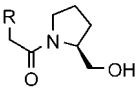
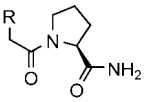
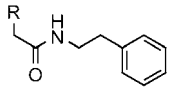
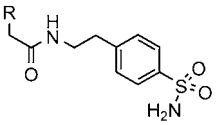
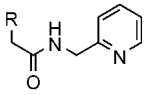
Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure
7 0.25 16/4		**15 f** 1.7 64/64		**18 f** 0.94 64/64	
18 a 1.4 >64/32		**15 e** 1.8 64/32		**18 j** 1.7 64/64	
18 b 1.0 64/32		**18 k** 0.39 32/16		**18 d** 1.0 >64/32	
18 l 4.3 >64/32		**9 b** 13 >64/>64		**18 e** 3.4 >64/64	
18 m 4.5 >64/32		**9 a** 77 >64/>64		**18 g** 0.32 64/16	
4 68 >64/8		**9 c** 180 >64/>64		**18 i** 0.89 32/8	
0.37 16/8	Neamine[b]	**9 d** 44 >64/>64		**18 h** 2.2 64/32	
0.032 1/0.1	Neomycin[b]			**18 c** 0.55 4/16	

[a] $B_{IVT}IC_{50}$: concentration [μM] required for 50% inhibition in a bacterial in vitro transcription assay, calculated as the average of six replicate experiments for each compound (±10%). MIC: minimum inhibitory concentration [μg mL^{-1}], determined as the average of triplicate measurements in serial dilution against *E. coli* (strain ATCC-25922, first value) and *Staphylococcus aureus* (strain ATCC-25923, second value). Assays were performed as previously described.[28] [b] For structures of the natural aminoglycosides see Figure 1a.

tetra-*n*-butylammonium iodide (TBAI), producing the benzylic-type ethers in very good yields. The same two-step deprotection sequence furnished the 5-deoxyneamine analogues **9a**–**d**. The derivatives **15e** and **15f** were conceived to evaluate potential H-bonding interactions in proximity to the 6-hydroxy group. These analogues were obtained by alkylation of alcohol **10**[22] with triflates **11e** and **12f**, synthesized from the corresponding azido alcohols **11** and **12**,[23] followed by removal of the protecting groups.

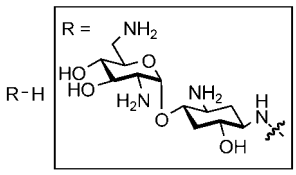
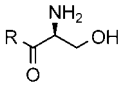
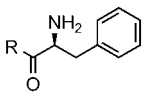
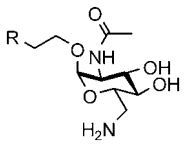
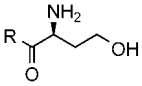
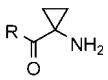
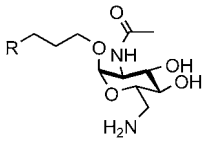
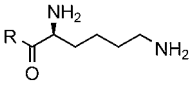
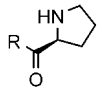
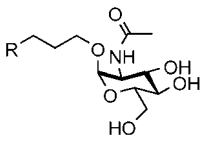
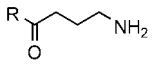
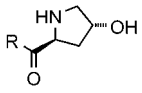
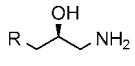
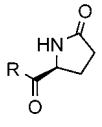
The 5-deoxyneamine derivatives **18a**–**m** (Scheme 3) were synthesized to explore the potential for simultaneous occupation of the paromomycin and hygromycin B binding sites. Allylation of the 6-hydroxy group in **10**[22] followed by ozonolysis and reductive work-up, furnished aldehyde **16** in excellent yield. Reductive amination of **16** with a variety of commercially available (**c**–**e, g**–**i, k**) as well as synthetically accessible (**a**, **b**, **f**, **j**, **L**, **m**, Scheme 3)[24] amines resulted in the formation of analogs **18a**–**m**, after deprotection.

Table 2. *Structure–activity relationships for 2,5-dideoxystreptamine O6-acetamide derivatives.*

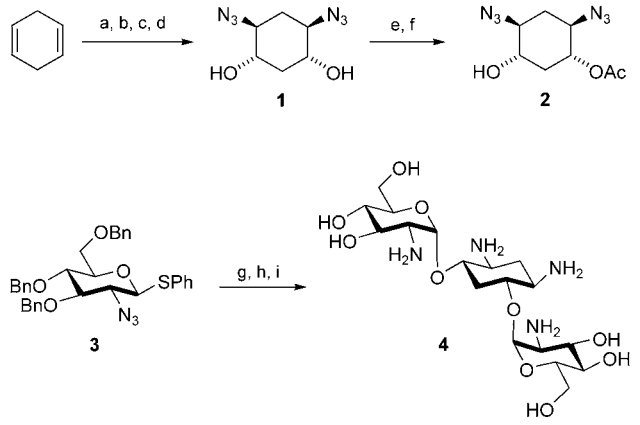
Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure
7 0.25 16/4		**42 f** 2.6 $>$64/8			
42 d 49 n.d.		**42 e** $>$200 n.d.		**42 l** 26 $>$64/$>$64	
42 i $>$200 n.d.		**42 j** 20 $>$64/$>$64		**42 k** $>$200 n.d.	
42 m 32 $>$64/$>$64		**42 h** $>$200 n.d.		**42 g** $>$200 n.d.	
42 b 14 $>$64/$>$64		**42 c** 43 n.d.		**42 a** 76 n.d.	

[a] See footnote to Table 1.

Table 3. *Structure-activity relationships for 2,5-dideoxystreptamine N1-derivatives.*

Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure	Compound $B_{IVT}IC_{50}$ MIC[a]	Structure
7 0.25 16/4		**43 c** 75 n.d.		**43 i** 160 n.d.	
39 100 n.d.		**43 b** 3.3 $>$64/32		**43 d** 87 n.d.	
38 100 n.d.		**43 a** 3.8 64/16		**43 f** 11 64/$>$64	
35 $>$200 n.d.		**43 h** 23 $>$64/$>$64		**43 g** 140 n.d.	
		37 1.9 64/$>$64		**43 e** 60 n.d.	

[a] See footnote to Table 1.

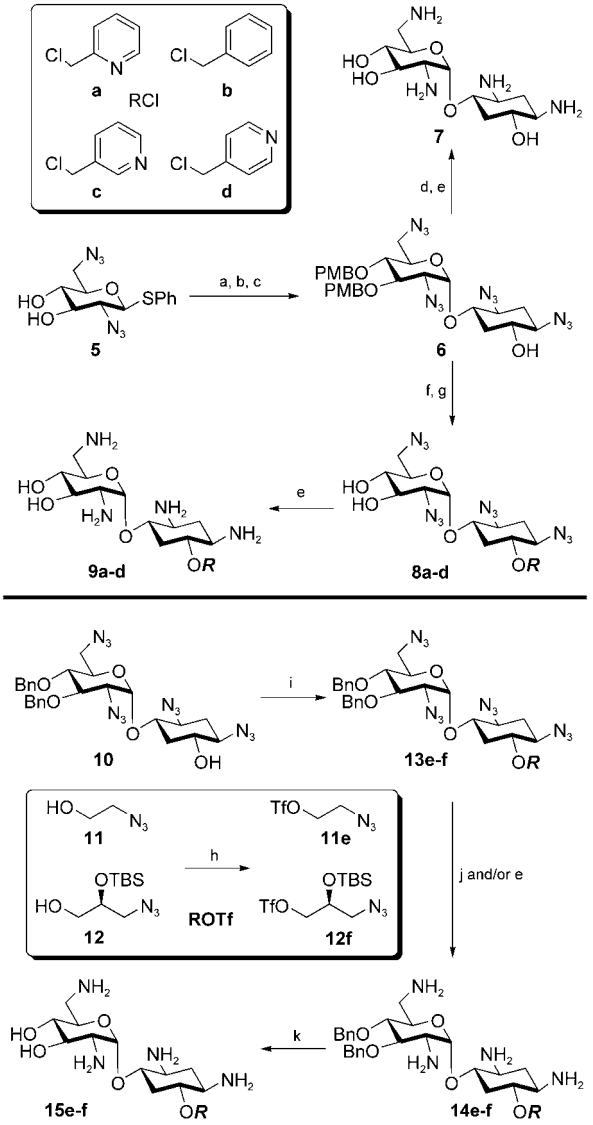


Scheme 1. *Reagents and conditions: a) cyclohexadiene (1 equiv), mCPBA (2.1 equiv), CH_2Cl_2, 8 h at 0°C, 16 h at 23°C, 39% for the* syn *isomer and 9% for the* anti *isomer; b)* syn*-epoxide (1.0 equiv), hydrazine (1.5 equiv), EtOH, reflux, 19 h, 98%; c) diol (1.0 equiv), Pd/C (10%, 0.01 equiv), AcOH/H_2O (1:1), 50 psi, 7 h, 60°C, 89%; d) diamine (1.0 equiv), TfN_3 (4.0 equiv; Tf = trifluoromethanesulfonyl), $CuSO_4 \cdot 5H_2O$ (0.2 equiv), 4-(dimethylamino)pyridine (4-DMAP; 2.0 equiv), MeOH/H_2O (4:1, 0.1 M), 20 h, 23°C, 50%; e)* **1** *(1.0 equiv), Ac_2O (10.0 equiv), 4-DMAP (0.10 equiv), pyridine, 18 h, 23°C, 93%; f) diacetate (1.0 equiv), Novozym 435 (1:1 w/w), toluene/potassium phosphate buffer (pH 6.2), 72 h, 22% for* **2** *(76% recovered* **1***); g)* **3** *(1.0 equiv),* **1** *(4.0 equiv),* N*-iodosuccinimide (NIS; 8.0 equiv), 4 Å molecular sieve, Et_2O/CH_2Cl_2 (4:1, 0.03 M), 2 h, −30–0°C, 74%; h) PMe_3 (1 M in THF, 6.0 equiv), pyridine/NH_4OH (7:1), 3 h, 23°C, 93%; i) benzyl ether (1.0 equiv), $Pd(OH)_2$ (20%/C, 0.01 equiv), AcOH, H_2 (1 atm), 14 h, 23°C, 87%.*

Our molecular-modeling studies indicated that modification of the N1 amino group might provide access to the hygromycin B binding site (Figure 1c). For the synthesis of N1 derivatives, a synthetic strategy developed by Mobashery and coworkers was followed.[17] Compound **19**[17] was first treated with sulfuryl chloride in pyridine to furnish axial chloride **20** in 72% yield, which was reduced with nBu$_3$SnH-AIBN (AIBN = 2,2′-azobisisobutyronitrile) to produce the deoxygenated compound **21** in excellent yield (Scheme 4). Activation of the cyclic carbamate, induced by *tert*-butoxycarbonyl (Boc) protection, followed by basic hydrolysis and final acidic removal of the Boc and the methoxymethyl ether (MOM) protecting groups, resulted in the formation of amino-triol **24** in excellent overall yield.

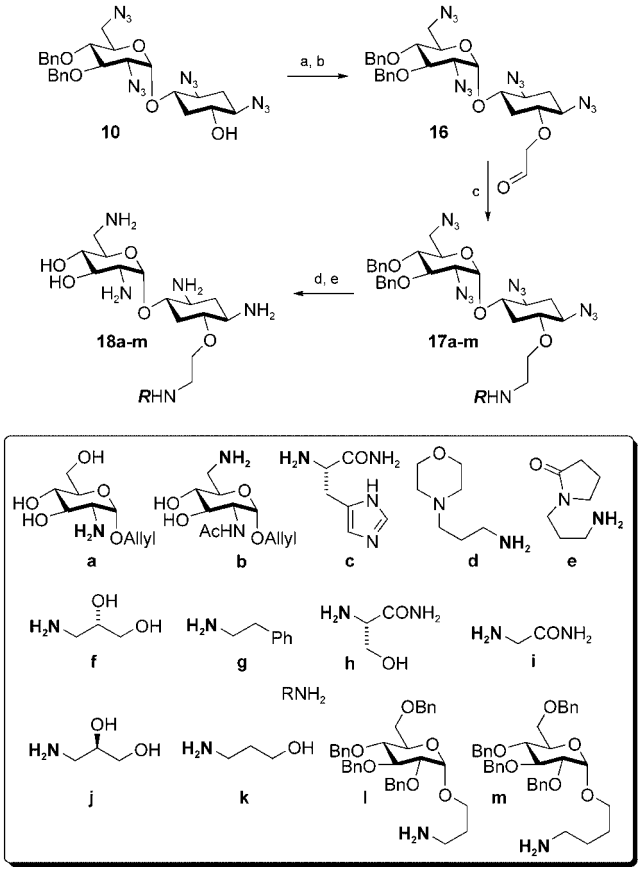
A variety of different α-glycosides with two- or three-carbon linkers were synthesized by known reaction sequences[25] and modified at the 6-position to include both amino- and hydroxy-functionalities. Aldehydes **30**, **31**, **33**, and **34**, resulting from ozonolysis of alkenes **26**–**29** (Scheme 5), were used without further purification. Aldehyde **32**, the product of a Dess–Martin oxidation of the corresponding glycidol-derived analogue, was included to further explore spatial requirements at the aminoglycoside-binding site. Reductive amination between **24** and aldehydes **30**–**34** followed by deprotection resulted in the formation of 5-deoxyneamine analogues **35**–**39**.

In an approach similar to that presented in Scheme 3, the introduction of an amide linker along with a variety of hydrogen-bond donors or acceptors, as well as groups capable of stacking interactions was pursued. Specifically, alcohol **6** was treated with 2-iodoethyl acetate to produce, after ester hydrolysis, carboxylic acid **40** in 94% yield (Scheme 6). Treatment of **40** with Vilsmeier complex gave the acid chloride, which was coupled with amines (**a**–**m**). The resulting amides were deprotected to yield amides **42a**–**m**. In the case of diamine **f**, the corresponding dimer **42f** was isolated (Table 2).

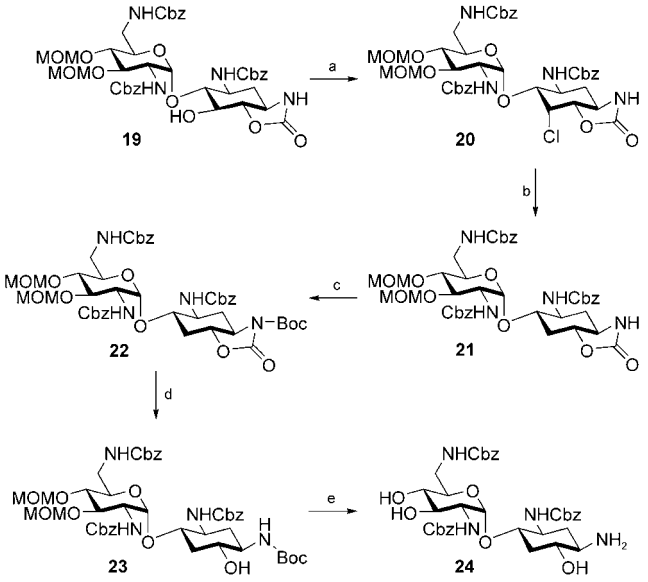


Scheme 2. *Reagents and conditions: a)* **5** *(1.0 equiv), NaH (2.5 equiv), PMB-Cl (2.2 equiv), DMF (0.5 M), 4 h, −5→23°C, quantitative; b) thioglycoside (1.0 equiv),* **2** *(1.5 equiv), NIS (2.0 equiv), 4 Å molecular sieves, Et_2O/CH_2Cl_2 (4:1, 0.03 M), 2 h, −30→0°C, 82%; c) acetate (1.0 equiv), K_2CO_3 (2.0 equiv), MeOH (0.5 M), 2 h, 23°C, quantitative; d)* **6** *(1.0 equiv), ammonium cerium(IV) nitrate (CAN; 3.0 equiv), CH_3CN/H_2O (9:1, 0.3 M), 3 h, 23°C, 89%; e) azide (1.0 equiv), PMe_3 (8.5 equiv, 1 M in THF), NH_4OH/pyridine (1:7), 4 h, 23°C, 84%; f)* **6** *(1.0 equiv), RCl (**a**–**d**; 1.5 equiv), NaH (3.0 equiv), TBAI (0.05 equiv), DMF/THF (3:1, 0.1 M), 5 h, 23°C, 68–87%; g) PMB ethers (1.0 equiv), CAN (3.0 equiv), CH_3CN/H_2O (9:1, 0.3 M), 3 h, 23°C, 81–92% for* **8a**–**d***; h)* **11** *or* **12** *(1.0 equiv), Tf_2O (1.5 equiv), CH_2Cl_2, 3 h, 0→23°C, quantitative; i)* **10** *(1.0 equiv),* **11e** *or* **12f** *(1.2 equiv), NaH (1.5 equiv), DMF (0.5 M), 4 h, 0→23°C, 82% for* **13e***, 85% for* **13f***; j)* **13f** *(1.0 equiv), tetra-*n*-butylammonium fluoride (TBAF; 1.5 equiv), THF, 2 h, 0→23°C, 95%; k)* **14e**–**f** *(1.0 equiv), $Pd(OH)_2$ (20%/C, 0.01 equiv), AcOH, H_2 (1 atm), 14 h, 93% for* **15e** *and 91% for* **15f***. TBS =* tert*-butyldimethylsilyl.*

Finally, the use of acid fluorides[26] (**a**–**i**, Scheme 7) or the synthesis of the corresponding amides provided the required che-



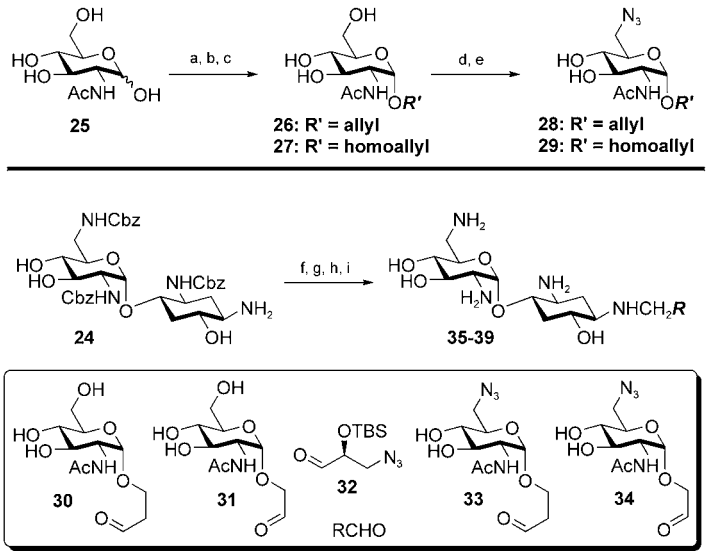
Scheme 3. *Reagents and conditions: a)* ***10*** *(1.0 equiv), allyl bromide (1.5 equiv), NaH (3.0 equiv), DMF (0.1 M), 2 h, 0→23 °C, 87%; b) allyl ether (1.0 equiv), O_3, CH_2Cl_2/MeOH, 10 min, −78 °C; then Me_2S (10.0 equiv), 1 h, −78→23 °C, 93%; c)* ***16*** *(1.0 equiv), RNH_2 (****a–m****; 4.0 equiv), $NaBH_3CN$ (1.0 equiv), MeOH (0.2 M), AcOH to pH 5.0, 12 h, 23 °C, 72–91%; d)* ***17a–m*** *(1.0 equiv), PMe_3 (8.5 equiv, 1 M in THF), NH_4OH/pyridine (1:7), 4 h, 23 °C; e) benzyl ethers (1.0 equiv), $Pd(OH)_2$ (20%/C, 0.01 equiv), AcOH, H_2 (1 atm), 14 h, 8–19% overall yield for* ***18a–m****.*



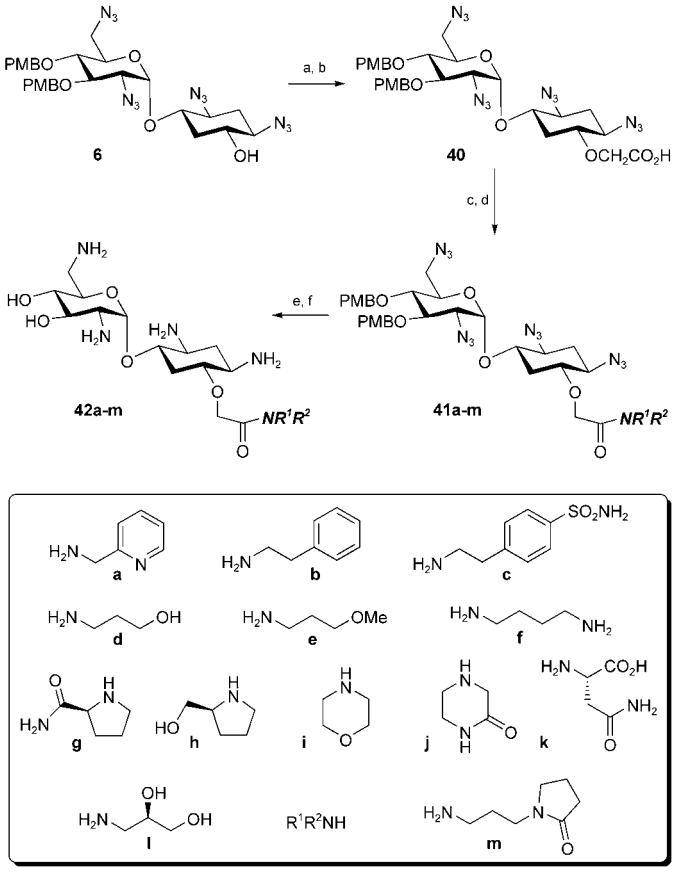
Scheme 4. *Reagents and conditions: a)* ***19*** *(1.0 equiv), SO_2Cl_2 (5.0 equiv), pyridine (0.25 M), 4 h, −20→0 °C, 72%; b)* ***20*** *(1.0 equiv), nBu_3SnH (5.0 equiv), AIBN (0.01 equiv), toluene (0.2 M), 4 h, 90 °C, 93%; c)* ***21*** *(1.0 equiv), 4-DMAP (0.2 equiv), Et_3N (1.3 equiv), Boc_2O (1.3 equiv), THF (0.2 M), 15 h, 23 °C, 85%; d)* ***22*** *(1.0 equiv), LiOH (10.0 equiv), dioxane (0.25 M), 30 min, 23 °C, 96%; e)* ***23*** *(1.0 equiv), HCl (2 M in MeOH)/$CHCl_3$ (1:1), 48 h, 23 °C, 91%. Cbz = benzyloxycarbonyl.*

moselectivity for the preferential reaction of the N1 amine over the three hydroxy functionalities present in **30**. Catalytic hydrogenation and acid treatment accomplished the removal of all protecting groups, furnishing analogues **43a–i**.

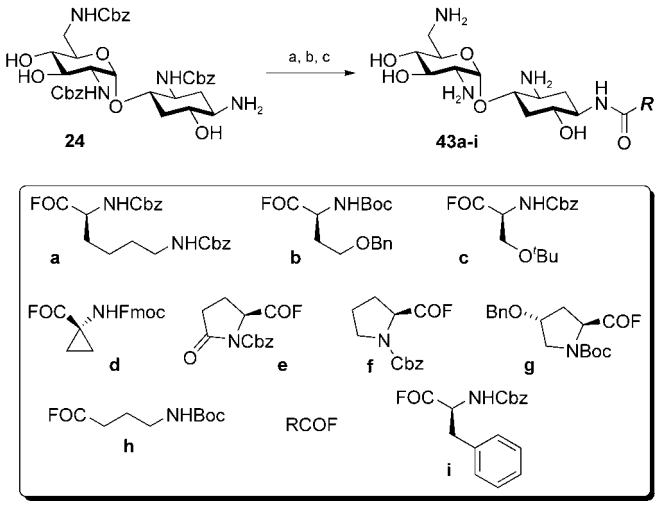
The synthetic N1- and O6-substituted 2,5-dDOS derivatives were tested for their activity as inhibitors of bacterial and eukaryotic in vitro translation, as well as for suppression of bacterial growth and toxicity against eukaryotic cells (Tables 1, 2, and 3). The parent compound 5-deoxyneamine (**7**) was a slightly more potent inhibitor of bacterial in vitro translation than neamine and showed improved activity against *S. aureus* (Table 1). Whereas the O6-linked 2,5-dDOS derivatives were inferior inhibitors of bacterial growth with respect to either neamine or 5-deoxyneamine, several compounds displayed comparable potency in the bacterial translation assay (Table 1). The four glycoside derivatives **18a**, **18b**, **18l**, and **18m**, which were designed to project a flexibly linked sugar moiety into the hygromycin B binding site, were four- to 16-fold less potent inhibitors of bacterial translation and growth compared to 5-deoxyneamine. Binding of these



Scheme 5. *Reagents and conditions: a) BF_3·Et_2O (1.5 equiv), allyl- or homoallyl-alcohol (0.2 M), 4 h, 90 °C; b) Ac_2O (5.0 equiv), pyridine (0.2 M), 14 h, 0→23 °C, 80–82% (α-anomer); c) NaOMe (2.0 equiv), MeOH, 4 h, 23 °C, 90–91%; d) 4-toluenesulfonyl chloride (1.2 equiv), pyridine (0.2 M), 0→23 °C, 14 h; e) NaN_3 (4.0 equiv), DMF (0.5 M), 4 h, 70 °C, average 60% for two steps; f)* ***24*** *(1.0 equiv), RCHO (****30–34****; 2.0 equiv), $NaBH_3CN$ (1.0 equiv), MeOH (0.2 M), AcOH to pH 5.0, 12 h, 23 °C, 74% average yield; g) silyl ether (1.0 equiv), TBAF (1.5 equiv), THF (0.25 M), 1 h, 23 °C, 93%; h) Cbz-protected amines (1.0 equiv), $Pd(OH)_2$ (20%/C), AcOH, H_2 (1 atm), 14 h, 23 °C, 84–87% yield; i) azides (1.0 equiv), PMe_3 (2.5 equiv, 1 M in THF), NH_4OH/pyridine (1:7), 4 h, 23 °C, 91% for* ***37****, 78% for* ***38****, 77% for* ***39****.*



Scheme 6. *Reagents and conditions: a) ICH_2CO_2Et, TBAI (0.2 equiv), toluene, NaOH (12 M, 40 equiv), 14 h, 23 °C, 94 %; b) LiOH·H_2O (3.0 equiv), THF, 60 °C, 14 h, 89 %; c) $(COCl)_2$ (3.0 equiv), DMF (3.0 equiv), CH_2Cl_2, (0.1 M), −20 °C, 1 h; then* ***40*** *in CH_2Cl_2 (0.2 M); d) R^1R^2NH (5.0 equiv), pyridine (5.0 equiv), CH_2Cl_2, 0→ 23 °C, 57–96 %; e) 20 % trifluoroacetic acid in CH_2Cl_2, p-toluenethiol (4.0 equiv), 30 min, 23 °C; f) PMe_3 (6.0 equiv, 1 M in THF), pyridine/NH_4OH (7:1), 3 h, 23 °C, 62–87 %.*



Scheme 7. *Reagents and conditions: a)* ***24*** *(1.0 equiv), RCOF (1.5 equiv),* N,N-*diisopropylethylamine (2.0 equiv), DMF/CH_2Cl_2 (1:1, 0.1 M), 1 h, 23 °C, 80 % average yield; b) $Pd(OH)_2$ (20 %/C), AcOH, H_2 (1 atm), 14 h, 23 °C, 85 % average yield; c) HCl (1.0 M), 30 min, 23 °C, quantitative. Fmoc = 9-fluorenylmethoxycarbonyl.*

synthetic aminoglycosides to the decoding-site RNA was measured by using a fluorescence-based assay that we had previously established.[27,28] The affinity of compounds **18a**, **18b**, **18l**, and **18m** for the decoding site ranged from 1.0–3.5 μM; this was consistent with their potency as inhibitors of bacterial translation.

The antibacterial activity of the synthetic aminoglycoside **4** against *S. aureus* is likely to be due to action on a target other than the ribosome, as indicated by the moderate impact on translation by this compound. Among the strongest inhibitors of translation was the alcohol **18k**, which showed better activity than either of the related vicinal diol diastereomers **18f** and **18j**. While the secondary hydroxy group in the diols provides added potential for hydrogen-bond interactions, it might also restrict the orientation of the terminal alcohol and, thus, prevent a more favorable interaction with the RNA. A similar phenomenon had been observed before for paromamine and the corresponding 6′-diol derivative.[15] The relatively good potency of the phenyl derivative **18g** and the histidine analogue **18c** as translation inhibitors might involve stacking of bases in the RNA target against the phenyl and imidazole groups, which are present in both compounds in a comparable structural context. Despite a slightly more favorable IC_{50} of translation inhibition, the histidine analogue **18c** showed lower activity in bacterial growth inhibition compared to the parental amide **18i**, potentially due to reduced membrane permeation of the more highly charged imidazole derivative. Replacement of the heterocycle by a hydroxy group in **18h** led to further reduction of translation inhibition and antibacterial potency. Interestingly, the O6-linked compounds **18d**, **18e**, **18f**, **18g**, and **18j** displayed eukaryotic cytotoxicity with an IC_{50} of cell proliferation inhibition in the range of 70–100 μM whereas all other 2,5-dDOS derivatives described here, including the related amides **18c**, **18h**, and **18i**, showed no eukaryotic cytotoxicity.[29] It is generally believed that the cationic character of aminoglycosides prevents their penetration into eukaryotic cells,[30] and thus the nature of the O6 substitution may have an impact on the permeation properties of the compounds.

Introduction of an acetamide linker at the O6-position of the 2,5-dDOS moiety, limiting the conformational flexibility of the substituents, yielded inactive compounds that no longer inhibited translation efficiently (Table 2). The single exception was the 5-deoxyneamine dimer **42f**, which retained some potency as an inhibitor of bacterial translation and growth. The antibacterial potency of the dimer **42f** was comparable to other aminoglycoside dimers that have been described in the literature.[31]

The coupling of an additional sugar moiety at the N1-position of 2,5-dDOS resulted in inactive compounds for different sugars and linker lengths (Table 3). Among the synthesized N1-substituted 5-deoxyneamine derivatives, the amines **37**, **43a**, and **43b** showed some activity as translation inhibitors. Shortening of the N1-sidechain by one methylene group in the analogue **43c** resulted in a greater than 20-fold loss of inhibition compared to the alcohol **43b**. The N1-substituent of **43b** was related to the (*S*)-4-amino-2-hydroxybutyryl group at the N1-position of the aminoglycoside antibiotic amikacin, which had

previously been used as a template for the design of novel antibacterial compounds.[17]

In contrast to the individual derivatization of the N1- and O6-positions of 5-deoxyneamine described here, Mobashery and co-workers have synthesized a small series of O6-(ω-aminoalkyl)-substituted neamine derivatives that retained the (*S*)-4-amino-2-hydroxybutyryl group of amikacin at the N1-position. Some of these doubly substituted compounds showed antibacterial activity superior to the parent neamine.[17] In a cocrystal structure determined for one of these synthetic derivatives bound to a decoding-site RNA construct,[32] the N1-linked substituent was oriented toward the hygromycin B binding site, in line with observations from our modeling studies that led to the 5-deoxyneamine series described here.

In conclusion, we have synthesized and tested in biological assays novel aminoglycoside-hybrid ligands designed to target the ribosomal decoding site. The ligands described here were conceived to bridge between the paromomycin and hygromycin B binding sites in helix 44 of bacterial rRNA. Whereas several of the hybrid ligands were active as inhibitors of bacterial translation, none of the compounds showed potency superior to the parental aminoglycosides, once again demonstrating the exquisite sensitivity of the natural ligands to modification. Whether the biological activity of the synthetic ligands was due to simultaneous interaction at the paromomycin and hygromycin binding sites, as suggested by the design concept, cannot be proved by the functional assays described here. In order to answer this question, we will use an affinity assay that is currently being developed based on a similar system that we had previously established for the paromomycin binding site.[27] Results will be reported in a future communication.

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Keywords: aminoglycosides · antibiotics · drug design · medicinal chemistry · RNA recognition

[1] a) E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, M. J. Waring, *The Molecular Basis of Antibiotic Action*, Wiley, London, **1981**; b) C. Walsh, *Antibiotics: Actions, Origins, Resistance*, ASM Press, Washinghton DC, **2003**.

[2] a) V. Ramakrishnan, *Cell* **2002**, *108*, 557–572; b) A. Bashan, R. Zarivach, F. Schluenzen, I. Agmon, J. Harms, T. Auerbach, D. Baram, R. Berisio, H. Bartels, H. A. Hansen, P. Fucini, D. Wilson, M. Peretz, M. Kessler, A. Yonath, *Biopolymers* **2003**, *70*, 19–41.

[3] a) D. Moazed, H. F. Noller, *Nature* **1987**, *327*, 389–394; b) E. Cundliffe in *Structure, Function and Genetics of Ribosomes* (Eds.: B. Hardesty, G. Kramer), Springer, New York, **1986**, pp. 586–604.

[4] J. M. Ogle, A. P. Carter, V. Ramakrishnan, *Trends Biochem. Sci.* **2003**, *28*, 259–266.

[5] a) M. J. Cabanas, D. Vasquez, J. Modolell, *Eur. J. Biochem.* **1978**, *87*, 21–27; b) A. Gonzales, A. Jimenez, D. Vasquez, J. E. Davies, D. Schindler, *Biochim. Biophys. Acta* **1978**, *521*, 459–469.

[6] E. P. Bakker, *J. Gen. Microbiol.* **1992**, *138*, 563–569.

[7] J. M. Ogle, D. E. Brodersen, W. M. Clemons, M. J. Tarry, A. P. Carter, V. Ramakrishnan, *Science* **2001**, *292*, 897–902.

[8] D. E. Brodersen, W. M. Clemons, A. P. Carter, R. J. Morgan-Warren, B. T. Wimberly, V. Ramakrishnan, *Cell* **2000**, *103*, 1143–1154.

[9] J. M. Ogle, F. V. Murphy, M. J. Tarry, A. P. Carter, V. Ramakrishnan, *Cell* **2002**, *111*, 721–732.

[10] J. Frank, R. K. Agrawal, *Nature* **2000**, *406*, 318–322.

[11] Q. Vicens, E. Westhof, *Structure* **2001**, *9*, 647–658.

[12] P. Pfister, S. Hobbie, Q. Vicens, E. C. Bottger, E. Westhof, *ChemBioChem* **2003**, *4*, 1078–1088.

[13] a) T. Hermann, *Biopolymers* **2003**, *70*, 4–18; b) S. J. Sucheck, Y.-K. Shue, *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 462–470; c) Y. Tor, T. Hermann, E. Westhof, *Chem. Biol.* **1998**, *5*, R277–R283; d) D. Vourloumis, M. Takahashi, G. C. Winters, K. B. Simonsen, B. K. Ayida, S. Barluenga, S. Qamar, S. Shandrick, Q. Zhao, T. Hermann, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3367–3372.

[14] a) Q. Vicens, E. Westhof, *J. Mol. Biol.* **2003**, *326*, 1175–1188; b) Q. Vicens, E. Westhof, *Chem. Biol.* **2002**, *9*, 747–755.

[15] K. B. Simonsen, B. K. Ayida, D. Vourloumis, M. Takahashi, G. C. Winters, S. Barluenga, S. Qamar, S. Shandrick, Q. Zhao, T. Hermann, *ChemBioChem* **2002**, *3*, 1223–1228.

[16] T. Suami, S. Nishiyama, Y. Ishikawa, S. Katsura, *Carbohydr. Res.* **1977**, *53*, 239–246.

[17] J. Haddad, L. P. Kotra, B. Llano-Sotelo, C. Kim, E. F. Azucena, Jr., M. Liu, S. B. Vankulenko, C. S. Chow, S. Mobashery, *J. Am. Chem. Soc.* **2002**, *124*, 3229–3237.

[18] G. Kavadias, S. Velkof, B. Belleau, *Can. J. Chem.* **1978**, *56*, 404–409.

[19] a) C. J. Cavender, V. J. Shiner, *J. Org. Chem.* **1972**, *37*, 3567–3569; b) P. B. Alper, S.-C. Hung, C.-H. Wong, *Tetrahedron Lett.* **1996**, *37*, 6029–6032.

[20] W. A. Greenberg, E. S. Priestley, P. S. Sears, P. B. Alper, C. Rosenbohm, M. Hendrix, S.-C. Hung, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 6527–6541.

[21] D. Vourloumis, G. C. Winters, M. Takahashi, K. B. Simonsen, B. K. Ayida, S. Shandrick, Q. Zhao, T. Hermann, *ChemBioChem* **2003**, *4*, 879–885.

[22] Alcohol **10** was obtained analogously to **6** (Scheme 2).

[23] H. R. Pfaendler, V. Weimar, *Synthesis* **1996**, *11*, 1345–1349.

[24] Synthesis of amines **a** and **b** of Scheme 3 is presented in Scheme 5; amines **f** and **j** resulted from regioselective opening of the corresponding epoxide with sodium azide (glycidol (1.0 equiv), NH_4Cl (1.0 equiv), NaN_3 (5.0 equiv), H_2O (0.3 M), 90 °C, 16 h) followed by Staudinger reduction; amines **l** and **m** were obtained from standard glycosylation (NIS, TfOH) of perbenzylated glucose with the corresponding azido alcohols, followed by Staudinger reduction.

[25] A. Vasella, C. Witzig, R. Husi, *Helv. Chim. Acta* **1991**, *74*, 1362–1372.

[26] a) L. A. Carpino, D. Sadat-Aalaee, H. G. Chao, R. H. DeSelms, *J. Am. Chem. Soc.* **1990**, *112*, 9651–9652; b) L. A. Carpino, E.-S. M. E. Mansour, D. Sadat-Aalaee, *J. Org. Chem.* **1991**, *56*, 2611–2614; c) G. Kokotos, C. Noula, *J. Org. Chem.* **1996**, *61*, 6994–6996.

[27] S. Shandrick, Q. Zhao, Q. Han, B. K. Ayida, M. Takahashi, G. C. Winters, K. B. Simonsen, D. Vourloumis, T. Hermann, *Angew. Chem.* **2004**, *116*, 3239–3244; *Angew. Chem. Int. Ed.* **2004**, *43*, 3177–3182.

[28] S. Barluenga, K. B. Simonsen, E. S. Littlefield, B. K. Ayida, D. Vourloumis, G. C. Winters, M. Takahashi, S. Shandrick, Q. Zhao, Q. Han, T. Hermann, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 713–718.

[29] The eukaryotic cytotoxicity of compounds was measured in a cell proliferation assay performed as described in ref. [28].

[30] N. Seiler, A. A. Hardy, J. P. Moulinoux, *Prog. Drug Res.* **1996**, *46*, 183–241.

[31] a) S. J. Sucheck, A. L. Wong, K. M. Koeller, D. D. Boehr, K. A. Draker, P. Sears, G. D. Wright, C.-H. Wong, *J. Am. Chem. Soc.* **2000**, *122*, 5230–5231; b) F. Agnelli, S. J. Sucheck, K. A. Marby, D. Rabuka, S.-L. Yao, P. S. Sears, F.-S. Liang, C.-H. Wong, *Angew. Chem.* **2004**, *116*, 1588–1591; *Angew. Chem. Int. Ed.* **2004**, *43*, 1562–1566.

[32] R. J. M. Russell, J. B. Murray, G. Lentzen, J. Haddad, S. Mobashery, *J. Am. Chem. Soc.* **2003**, *125*, 3410–3411.

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