Design and Synthesis of New Aminoglycoside Antibiotics Containing Neamine as an Optimal Core Structure: Correlation of Antibiotic Activity with in Vitro Inhibition of Translation

William A. Greenberg, E. Scott Priestley, Pamela S. Sears, Phil B. Alper, Christoph Rosenbohm, Martin Hendrix, Shang-Cheng Hung, and Chi-Huey Wong*

Contribution from the Department of Chemistry and Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

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Abstract: The structure and activity of the pseudodisaccharide core found in aminoglycoside antibiotics was probed with a series of synthetic analogues in which the position of amino groups was varied around the glucopyranose ring. The naturally occurring structure neamine was the best in the series according to assays for in vitro RNA binding and antibiotic activity. With this result in hand, neamine was used as a common core structure for the synthesis of new antibiotics, which were evaluated for binding to models of the *Escherichia coli* 16S A-site ribosomal RNA, in vitro protein synthesis inhibition, and antibiotic activity. Analysis of RNA binding revealed some correlation between the relative affinity and specificity of RNA binding and antibacterial efficacy. However, the correlation was not linear. This result led us to develop the in vitro translation assay in an effort to better understand aminoglycoside−RNA interactions. A linear correlation between in vitro translation inhibition and antibiotic activity was observed. In addition, IC$_{50}$s in the protein synthesis assay were typically lower than the K_ds obtained for RNA binding, suggesting that binding of these compounds to intact ribosomes is tighter in these cases than binding to the model RNA oligonucleotides. This reflects possible differences in RNA conformation between intact ribosomes and the free RNA of the model system, or possible high-affinity ribosomal binding sites in addition to the A-site RNA.

Introduction

The aminoglycosides[1] are a group of clinically important antibiotics which function through binding to specific sites in prokaryotic ribosomal RNA and affecting the fidelity of protein synthesis.[2−4] They have served as a paradigm for studying small molecule−RNA interactions in a rapidly growing number of structural[5−7] and chemical[8−11] efforts.

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As is the case for all classes of antibiotics, the emergence of resistance has resulted in a need for new structures with antibiotic activity.[12] Because of the unwieldy size and dense functionality of aminoglycosides such as neomycin (Figure 1), an important concern for the design of new compounds is the identification of smaller, simpler structures which retain the activity of the larger parent structures. Analysis of the structural elements of naturally occurring aminoglycosides reveals that the vast majority contains the *meso*-1,3 diaminocyclitol 2-deoxystreptamine (Figure 1), glycosylated at the 4-position, as well as the 5- (neomycin class) or 6- (kanamycin−gentamicin class) position with a variety of aminosaccharides. Several aminoglycosides (i.e., neomycin, ribostamycin, kanamycin B) share a common pseudodisaccharide core, 4-*O*-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-2-deoxystreptamine, commonly known as neamine (**1**, Figure 1). Many other substitution patterns on the glucopyranose are known, such as 2-amino-2-deoxyglucopyranose in paromomycin, 6-amino-6-deoxyglucopyranose in kanamycin A, 4-amino-4-deoxyglucopyranose in apramycin, and various deoxygenated analogues, such as in tobramycin and gentamicin.

We sought to probe the structure of this pseudodisaccharide core to find the optimal substitution pattern as a starting point for synthesis of new potential antibiotics. A series of pseudodisaccharides with amino groups at variable positions (**1−6**) was synthesized (Figure 2). These compounds were analyzed for binding to models of the *Escherichia coli* 16S A-site ribosomal RNA and for antibacterial activity. The optimal structure, **1**, was then incorporated as a core structure into a series of analogues (**7−15**) appended with various polyamino,

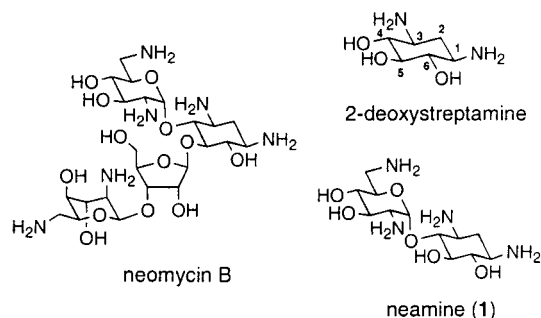(12) Davies, J. *Science* **1994,** *264,* 375−382.

Figure 1. Structures of neomycin B, the diaminocyclitol 2-deoxy-streptamine, which is found in most aminoglycoside antibiotics, and the pseudodisaccharide neamine (**1**).
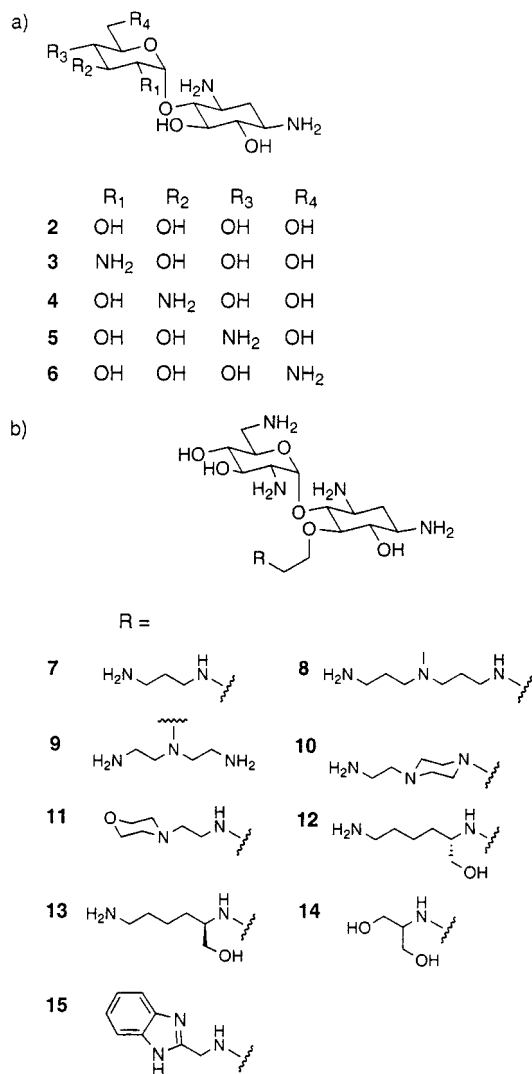

Figure 2. Structures of compounds **2**−**15**.

amino alcohol, or aromatic substitutions which were designed to improve RNA binding affinity and potentially antibacterial activity. These compounds were tested for RNA binding and antibacterial activity, as well as for their ability to inhibit protein synthesis in vitro, using a luciferase-based assay. The results of the latter assay correlate well in a qualitative sense with antibacterial activity.

Results and Discussion

Synthesis of Glycosyl Acceptor. Synthesis of pseudodisaccharides **2**−**6** required desymmetrization of the *meso* compound

Scheme 1. Synthesis of Glycosyl Acceptors **17** and **20**[a]
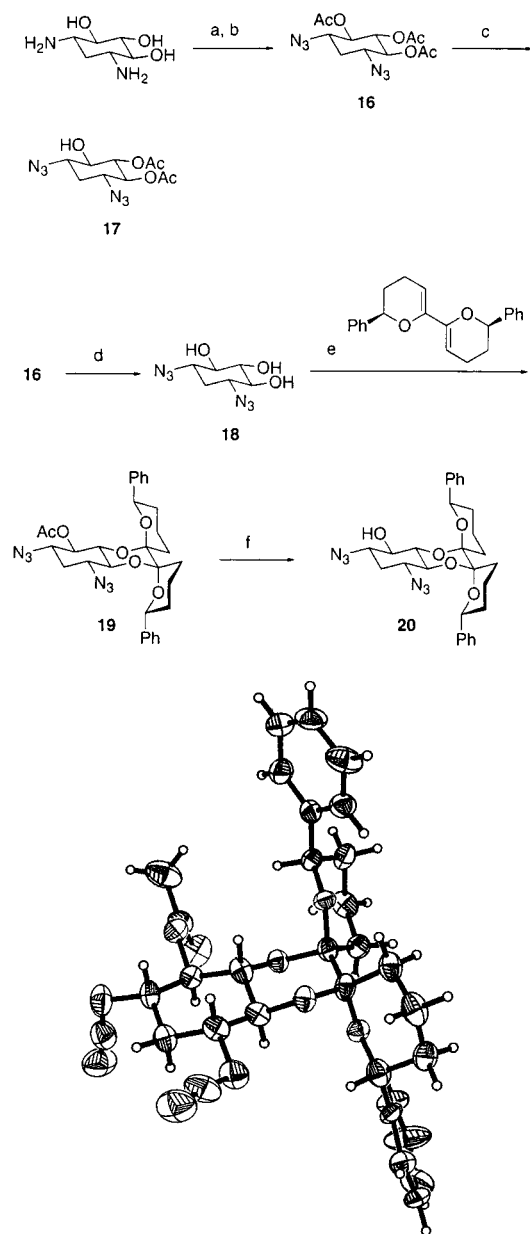
[a] Reagents and conditions: (a) TfN$_3$, Et$_3$N, CuSO$_4$, MeOH/CH$_2$Cl$_2$. (b) Ac$_2$O, pyridine, 75% over two steps. (c) *Candida antarctica* lipase, 1:1 toluene/phosphate buffer, pH 6.2, 71% (95% based on recovered starting material). (d) NaOMe, MeOH, 100%. (e) (i) Ley bis-dihydropyran, CSA, CHCl$_3$; (ii) Ac$_2$O, DMAP, pyridine, 50% over two steps. (f) NaOMe, MeOH/dioxane, 90%.

2-deoxystreptamine, to differentiate the 4-hydroxyl group from the 5- and 6-hydroxyl groups. Two different procedures, one enzymatic and the other chemical, were explored (Scheme 1).

The enzymatic approach relied on an enantioselective deacetylation using a resin-immobilized lipase. 2-Deoxystreptamine[13] was converted in 75% overall yield to the diazido triacetyl derivative **16** by Cu^{2+}-catalyzed azido transfer with triflic azide,[14] followed by treatment with acetic anhydride and 4-DMAP in pyridine. Compound **16** was selectively deacetylated with Novozym 435 (*Candida antarctica* lipase immobilized on a macroporous acrylic resin; Novo Nordisk), providing **17** in

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71% yield (95% based on recovered starting material). The absolute stereochemistry of **17** was confirmed by showing that synthetic paromamine (**3**) derived from **17** was identical in all respects to paromamine obtained from the natural source (see Experimental Section).

The second desymmetrization approach utilized the dispiro-ketal protection/desymmetrization chemistry of Ley and co-workers.[15] Compound **16** was deacetylated (catalytic NaOMe, MeOH, 100%) to provide **18**. When treated with Ley's (2R,2′R)-PDHP reagent[15] and catalytic camphorsulfonic acid in refluxing chloroform, followed by acetylation, **19** was obtained as the major isomer in 50% yield. The structure of **19** was confirmed by solving its crystal structure (Scheme 1). Deacetylation (catalytic NaOMe in MeOH/dioxane, 90%) provided **20**, the substrate for selective glycosylation at the 4-position. However, attempts at glycosylation of **20** provided only the undesired β anomer, likely due to the steric constraints of the dispiroketal protecting group (data not shown). Therefore, glycosyl acceptor **17** was used for the synthesis of **2**–**6**.
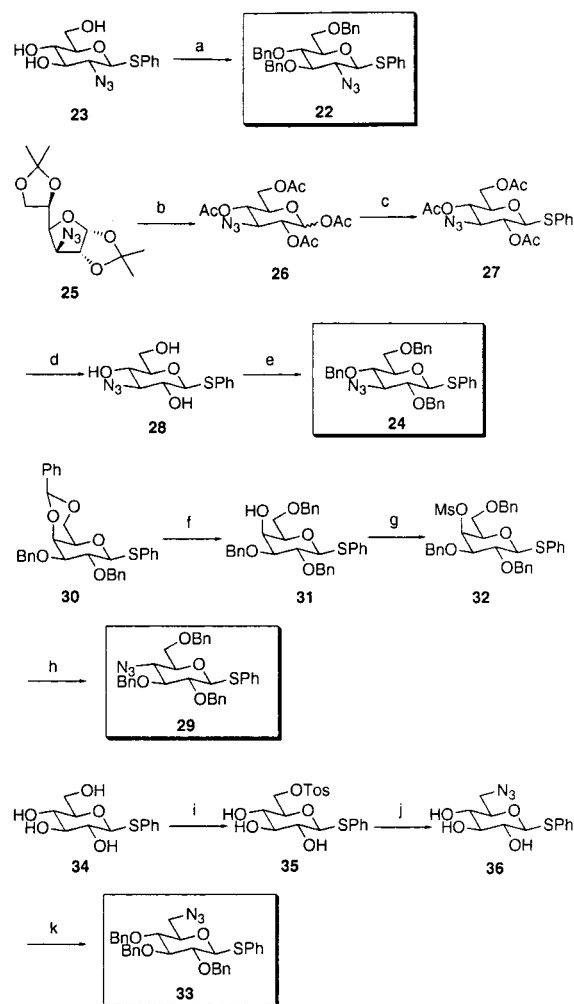
Synthesis of Glycosyl Donors. Scheme 2 outlines the preparation of the glycosyl donors used in synthesizing **2**–**6**. Compound **22**, the donor for preparing **3**, was produced by benzylation (BnBr, NaH, DMF, 60%) of thioglycoside **23**.[16]

The 3-azido donor **24** was synthesized from known diaceto-nide **25**[17] in five steps. Acetonide cleavage (90% TFA/H$_2$O) and peracetylation (Ac$_2$O, NaOAc) provided glucopyranoside **26** in 59% yield. Conversion to the β-phenylthioglycoside **27** (thiophenol, BF$_3$·OEt$_2$, CHCl$_3$, 64%), acetate removal (NaOMe, MeOH, 100%), and benzylation (BnBr, NaH, DMF, 85%) provided donor **24**.

The 4-azido donor **29** was synthesized from galactose benzylidene acetal **30**.[18] Regioselective reduction of the ben-zylidene acetal (BH$_3$·Me$_3$N, AlCl$_3$, THF, 85%)[19] provided **31**, which was mesylated (CH$_3$SO$_2$Cl, pyridine, 73%). The mesylate was displaced with sodium azide in DMF, providing **29** in 84% yield. The 6-azido donor **33** was synthesized from **34**[20] by tosylation of the primary 6-hydroxyl (TsCl, pyridine, 75%), displacement with sodium azide in DMF (90%), and benzylation (BnBr, NaH, DMF, 57%).

Synthesis of Pseudodisaccharides 2–**6.** 2-Deoxystreptamine acceptor **17** was glycosylated with thioglycoside donors[21] **21**, **22**, **24**, **29**, and **33** (*N*-iodosuccinimide, triflic acid, Et$_2$O/CH$_2$Cl$_2$, −15 °C), providing pseudodisaccharides **37**–**41**, respectively, after deacetylation, in 58−100% yield (Scheme 3). The α/β selectivity of the glycosylation reactions was typically in the range of 10:1 for these substrates. The use of diethyl ether as cosolvent was crucial to this selectivity. In dichloromethane at −60 °C, the α/β selectivity was only 2.5:1, and the overall yield of **37** was poor. Reduction of azides and benzyl ethers in a single step proved problematic, but complete deprotection was effected in moderate to excellent yield over two steps. The azides were reduced with hydrazine[22] (H$_2$NNH$_2$,

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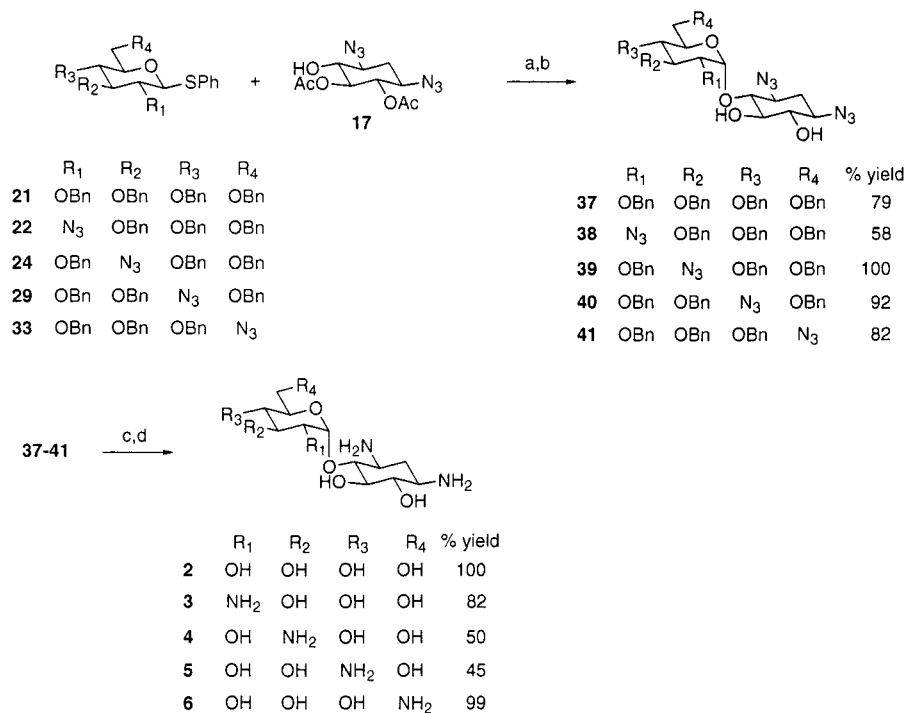
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Scheme 2. Synthesis of Glycosyl Donors **22**, **24**, **29**, and **33**[a]

[a] Reagents and conditions: (a) BnBr, NaH, DMF, 60%. (b) (i) 90% TFA/H$_2$O; (ii) Ac$_2$O, NaOAc, 59% over two steps. (c) Thiophenol, BF$_3$·OEt$_2$, CHCl$_3$, 64%. (d) NaOMe, MeOH, 100%. (e) BnBr, NaH, DMF, 85%. (f) BH$_3$·Me$_3$N, AlCl$_3$, THF, 85%. (g) MsCl, pyridine, 73%. (h) NaN$_3$, DMF, 100 °C, 84%. (i) TsCl, pyridine, 75%. (j) NaN$_3$, DMF, 80 °C, 90%. (k) BnBr, NaH, DMF, 57%.

20% Pd(OH)$_2$/C, Degussa type, MeOH, reflux), followed by benzyl ether cleavage (H$_2$ (1 atm), 20% Pd(OH)$_2$/C, Degussa type, AcOH/H$_2$O), providing **2**–**6**.

Synthesis of Neamine Derivatives 7–**15.** Scheme 4 outlines the synthesis of neamine derivatives **7**–**14**. The best route to the pseudodisaccharide building block **43**, in which the 5-hydroxyl group of 2-deoxystreptamine is differentiated from the other hydroxyl groups, begins from inexpensive neomycin. Protection of the amino groups as azides using triflic azide[14] was followed by peracetylation for the purpose of easier puri-fication from byproducts of the azido transfer reaction and de-acetylation. This material was benzylated (NaH, benzyl bromide, tetrabutylammonium iodide, DMF, 80%), providing protected neomycin **42** in 46% overall yield from neomycin sulfate. The β-ribofuranosyl bond of neomycin was selectively cleaved with 1 N HCl in methanol/dioxane, providing building block **43** in 71% yield after recrystallization. Under these conditions, some cleavage of the β-L-idopyranosyl bond occurs, but little or no cleavage of the key α-L-glucopyranosyl bond occurred. The methyl glycoside of the lower half of neomycin (neobiosamine) was also obtained, albeit in lower yield due to the partial cleavage of the aforementioned β-L-idose glycosidic bond.

Scheme 3. Synthesis of Pseudodisaccharides **2**−**6**[a]

	R$_1$	R$_2$	R$_3$	R$_4$
21	OBn	OBn	OBn	OBn
22	N$_3$	OBn	OBn	OBn
24	OBn	N$_3$	OBn	OBn
29	OBn	OBn	N$_3$	OBn
33	OBn	OBn	OBn	N$_3$

	R$_1$	R$_2$	R$_3$	R$_4$	% yield
37	OBn	OBn	OBn	OBn	79
38	N$_3$	OBn	OBn	OBn	58
39	OBn	N$_3$	OBn	OBn	100
40	OBn	OBn	N$_3$	OBn	92
41	OBn	OBn	OBn	N$_3$	82

	R$_1$	R$_2$	R$_3$	R$_4$	% yield
2	OH	OH	OH	OH	100
3	NH$_2$	OH	OH	OH	82
4	OH	NH$_2$	OH	OH	50
5	OH	OH	NH$_2$	OH	45
6	OH	OH	OH	NH$_2$	99

[a] Reagents and conditions: (a) NIS, TfOH, Et$_2$O/CH$_2$Cl$_2$, −15 °C, 30 min. (b) NaOMe, MeOH. (c) H$_2$NNH$_2$, 20% Pd(OH)$_2$/C, MeOH, reflux. (d) H$_2$, 20% Pd(OH)$_2$/C, AcOH/H$_2$O.

The 5-hydroxyl group of **43** was alkylated (NaH, allyl bromide, tetrabutylammonium iodide, DMF, 99%), giving **44**, which was subjected to ozonolysis and reductive amination of the resultant aldehyde **45**, providing compounds **46**−**53**. For substrates in which more than one amine was available to react during reductive amination, all but one amine was protected as the benzyl carbamate. In the case of **46**, the secondary amine formed in the reductive amination step was protected as a benzyl carbamate before proceeding with the next step. This step was found to be unnecessary and was not performed on the other compounds in this series. One-step deprotection of all protecting groups by catalytic hydrogenation was problematic, as was observed with compounds **2**−**6**. In this case, the best deprotection protocol relied on first reducing the azides by a Staudinger reaction (PMe$_3$, THF/H$_2$O), followed by benzyl ether and benzyl carbamate reduction with sodium in ammonia, providing compounds **7**−**14** after cation-exchange chromatography.

Because the deprotection procedure described in Scheme 4 did not allow introduction of aromatic substituents, a new procedure was sought which would efficiently reduce at least four azides and three benzyl ethers. After much investigation, the conditions shown in Scheme 5 were developed and used to synthesize benzimidazole-containing neamine derivative **15**. Benzimidazole has been used as an arginine mimic.[23] Protected compound **54** was subjected to transfer hydrogenation (H$_2$NNH$_2$, Raney Ni, EtOH),[22] followed by catalytic hydrogenolysis under acidic conditions (H$_2$ (1 atm), Pd(OH)$_2$/C (Degussa type), AcOH/H$_2$O), to provide **15**.

RNA Binding and Antibiotic Activity of 2−6. For several years, we have used surface plasmon resonance (SPR) to probe the interactions of aminoglycosides and synthetic analogues with RNA.[8] In particular, we have been interested in specific binding to prokaryotic 16S ribosomal A-site RNA, the binding site which

is implicated for the antibiotic activity of this class of molecules. By studying the interactions of aminoglycosides with small model RNA sequences, it is expected that a better understanding of the molecular basis of their antibiotic activity will be gained, thereby allowing the design of antibiotics with improved profiles with respect to toxicity and resistance. Compounds **1**−**6** were screened on a streptavidin-modified SPR chip for RNA binding against three related 5′-biotinylated RNA oligonucleotides which have served as A-site model sequences (Figure 3) in our previous studies.[8] AS-wt contains the wild-type sequence from *E. coli*, U1406A contains a single-base mutation which improves binding of aminoglycosides, and U1495A contains a single-base mutation which is deleterious to binding. The improved binding to the U1406A mutant is rationalized by forming an A−U base pair with U1495, which forms a critical hydrogen bond to the aminoglycoside paromomycin according to the NMR structure.[5] In the wild-type sequence, U1495 is mispaired to U1406 and likely is less conformationally well-defined. The U1495 mutation is deleterious to binding because the same U1495 that forms a hydrogen bond to the aminoglycoside has been replaced by an adenine.

Data obtained from the SPR screen are illustrated in Figure 4 and summarized in Table 1. Neamine was found to be the most effective and specific A-site binder, although the 2- and 6-amino derivatives **3** and **6** did have quite good affinity for the U1406A mutant. More importantly, neamine was the most active antibiotic. In fact, of compounds **2**−**6**, only **6** showed any activity in the Kirby−Bauer disk assay[24] against *E. coli*, *Pseudomonas aeruginosa*, or *Staphylococcus aureus*. Its minimum inhibitory concentration (MIC)[25] was less than that of

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Scheme 4. Synthesis of Neamine Derivatives **7**−**14**[a]
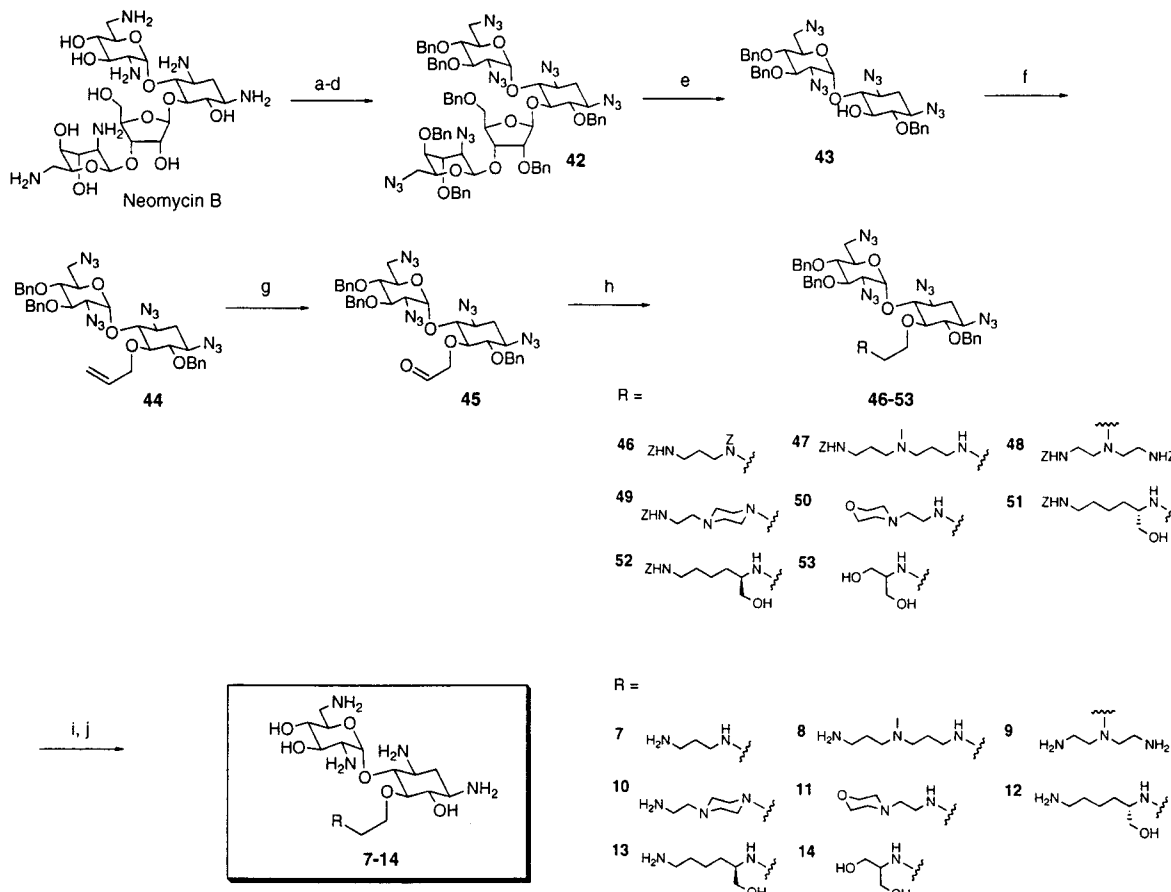
[a] Reagents and conditions: (a) TfN$_3$, Et$_3$N, CuSO$_4$, MeOH/H$_2$O/CH$_2$Cl$_2$. (b) Ac$_2$O, pyridine. (c) NaOMe, MeOH, 58% from neomycin sulfate. (d) BnBr, NaH, Bu$_4$NI, DMF, 80%. (e) 1 M HCl, MeOH/dioxane, 71%. (f) allyl bromide, Bu$_4$NI, DMF, 99%. (g) O$_3$, CH$_2$Cl$_2$/MeOH, then Me$_2$S. (h) RNH$_2$, NaCNBH$_3$, AcOH/MeOH, 33−63% over two steps. (i) PMe$_3$, THF/H$_2$O. (j) Na, NH$_3$/THF, 13−80% over two steps.

Scheme 5. Synthesis of Aromatic Neamine Derivative **15**[a]
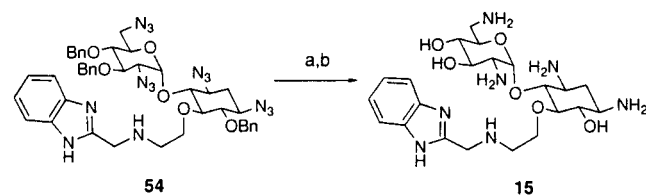
[a] Reagents and conditions: (a) Raney nickel, hydrazine, EtOH. (b) H$_2$ (1 atm), 20% Pd(OH)$_2$/C, AcOH/H$_2$O, 32% over two steps.

neamine (**1**) (Table 1). With these results, it was concluded that neamine (**1**) was the most promising pseudodisaccharide core for further development.

RNA Binding and Antibiotic Activity of 7−15. The diamine, triamine, amino alcohol, and aromatic substitutions on neamine derivatives **7**−**15** were chosen for several reasons. The encouraging results on simplified neomycin analogues[8b] suggested that linear alkyl diamines might be effective replacements for more complex aminosaccharides. We were also interested in probing the effect of neighboring hydroxyl groups, in keeping with the identification of 1,2- and 1,3-amino alcohols as an effective phosphate-binding motif.[26] Finally, the structural simplicity of this series, relative to the natural products and previously synthesized analogues,[8b] meant that their synthesis would be easier and more amenable to rapid development of analogues.

(26) Hendrix, M.; Alper, P. B.; Priestley, E. S.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 95−98.
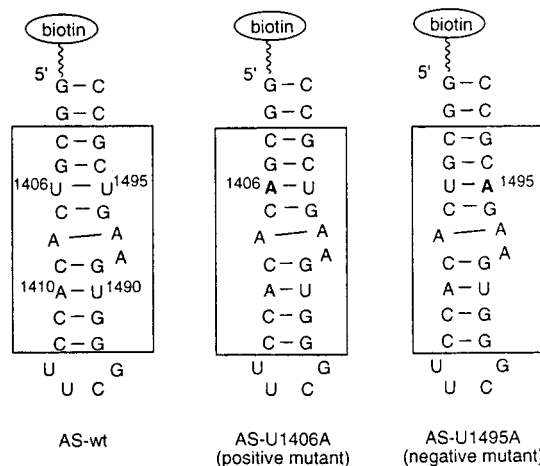

Figure 3. A-Site model RNA sequences screened by surface plasmon resonance for aminoglycoside binding.

RNA binding data obtained by SPR and antibiotic activity for compounds **7**−**15** are summarized in Table 2. As expected, addition of amines to the neamine core typically resulted in a 10−20-fold enhancement in RNA binding affinity compared to the parent pseudodisaccharide. Compounds **7**−**10**, **12**, and **13** all bound in the submicromolar range. Specificity was slightly degraded, being in the 2−3-fold range, compared to 4−6-fold for neamine. There is only a weak correlation between RNA binding affinity and antibacterial activity. Despite binding more strongly than neamine, several analogues were weaker antibiot-
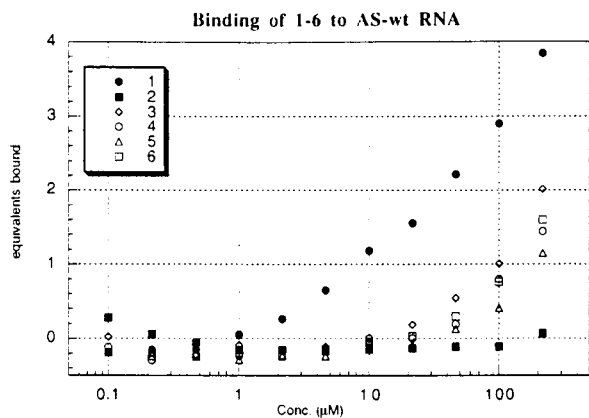
Figure 4. Representative SPR binding data (equivalents bound as a function of concentration) for compounds **1**−**6** to AS-wt RNA.

Table 1. RNA Binding to Variants of the Decoding Region A-Site and Antibacterial Activity for Compounds **1**−**6**[a]

compd	AS-wt	U1406A	U1495A	MIC, *E. coli* ATCC 25922
1	7.8	5.5	31	50
2	>300	>300	>300	no activity
3	50	15	60	no activity
4	80	80	70	no activity
5	140	110	120	no activity
6	80	20	70	>250

[a] Dissociation constants and minimum inhibitory concentrations are in units of micromoles per liter.

Table 2. RNA Binding to Variants of the Decoding Region A-Site and Antibacterial Activity for Compounds **7**−**15**[a]

compd	AS-wt	U1406A	U1495A	MIC, *E. coli* ATCC 25922
7	0.7	0.4	1.5	100
8	0.5	0.4	1.0	50
9	0.8	0.5	1.0	100
10	0.4	0.25	0.7	100
11	7.0	8.0	10	>100
12	0.8	0.7	1.6	25
13	1.0	0.7	1.8	25
14	4.0	4.0	6.0	100
15	4.9	3.1	3.7	>100

[a] Dissociation constants and minimum inhibitory concentrations are in units of micromoles per liter.

ics. Similar antibiotic profiles were observed against *S. aureus*, but none of the synthetic compounds (or neamine) were active against *P. aeruginosa*.

The isomers **12** and **13** present an interesting case. The addition of hydroxymethyl groups to the diaminoalkyl substituent had negligible effect on RNA binding (compare **12** and **13** to **7**) but did have a beneficial effect on antibiotic activity. The simplest explanation for the improvement is that the hydroxyl group improves cell permeability. Another possible factor is that in vitro binding to these model RNA sequences does not exactly mimic the binding event on whole ribosomes in vivo. This issue is discussed in detail in the following section. The fact that the isomers **12** and **13** behaved identically both in RNA binding and in antibiotic activity assays suggests that the substituent at the 5-position of the 2-deoxystreptamine ring remains flexible upon binding; that is, both isomers can adopt productive conformations when bound to RNA.

In Vitro Inhibition of Translation. The lack of a close correlation for these compounds between effectiveness as RNA binders and as antibiotics is in line with similar observations made previously for many of the naturally occurring amino-
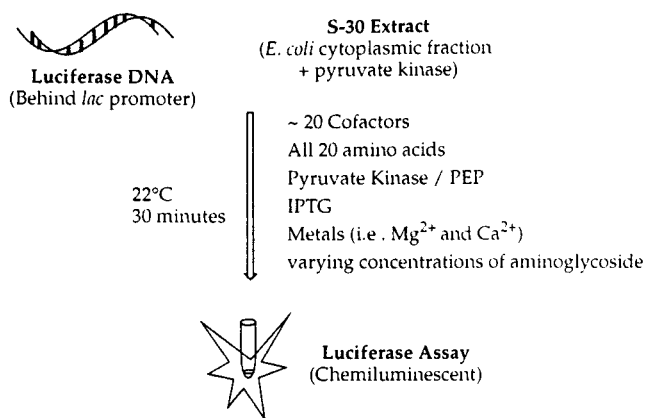

Figure 5. Schematic of the in vitro translation assay. A plasmid-borne luciferase reporter gene is combined with *E. coli* cytoplasmic S-30 extract, containing transcription and translation machinery, and incubated with necessary cofactors and amino acids for 30 min. Reactions are then assayed for production of active luciferase.

glycosides.[8c] Many compounds which are known to be effective antibiotics, such as gentamicin and ribostamycin, appeared to be relatively poor A-site binders with respect to both affinity and specificity. In an effort to understand this discrepancy, we reasoned that studies on inhibition of whole ribosomes, using a cell-free protein synthesis assay, could be informative. Direct study in vitro on the effect of these compounds on translation, the acknowledged mechanism of antibiotic action, should provide a more accurate model than the indirect study solely of RNA binding.

The coupled transcription/protein synthesis assay is illustrated in Figure 5. A cytoplasmic extract of *E. coli* (which includes ribosomes and transcription machinery) is combined with all amino acids and required cofactors, a plasmid containing the reporter gene luciferase, and varying concentrations of the aminoglycoside of interest. After induction with IPTG, the reaction proceeds for 30 min and is then assayed for expression of enzymatically active luciferase.

Protein synthesis inhibition data were collected for a series of compounds of interest, including the aminoglycosides neomycin, gentamicin, and ribostamycin, previously reported synthetic derivatives **55** and **56**,[8b] neamine, and compounds **12** and **13** from this work (Figure 6). Dose-dependent inhibition by these compounds is illustrated in Figure 7. The data are collected and compared to MIC values against *E. coli* and K_d values on the AS-wt RNA in Table 3.

The natural products neomycin and gentamicin are equally potent antibiotics, with measured MICs of 1.6 μM, although the AS-wt binding data are very different (low nanomolar versus low micromolar). When IC$_{50}$ values in the protein synthesis assay are compared, however, the two compounds appear similar. Both inhibit translation and, by inference, bind to the A-site of intact ribosomes at 20−30 nM concentration. The synthetic derivatives **55** and **56** also inhibit translation in this range, which is consistent with their exceptional antibiotic activity, despite their apparently weaker A-site binding compared to neomycin in the SPR assay. The new translation inhibition data explain why the synthetic molecule **56** is as potent an antibiotic as most active naturally occurring aminoglycosides. Ribostamycin curiously has a MIC (12.5 μM) that is lower than its K_d against AS-wt (25 μM). However, it inhibits translation at 100 nM, suggesting that it binds to the intact ribosome at a 250-fold lower concentration than it binds to the A-site model RNA. Likewise, neamine inhibits translation at a 20-fold lower concentration than it binds to AS-wt. Compounds **12** and **13**
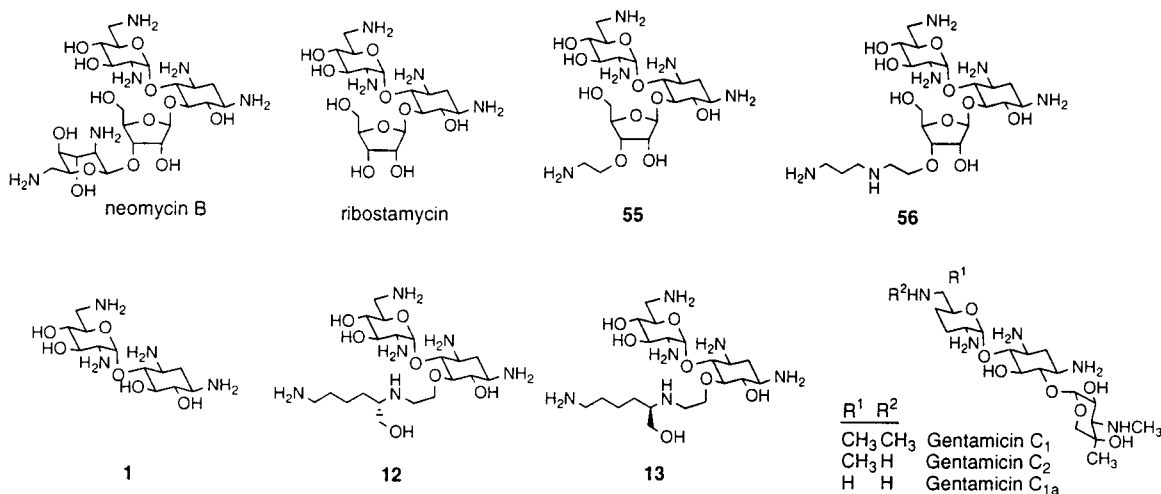
Figure 6. Structures of the aminoglycosides and synthetic derivatives that were tested in the in vitro translation assay. Gentamicin was tested as the commercially available gentamicin C complex.
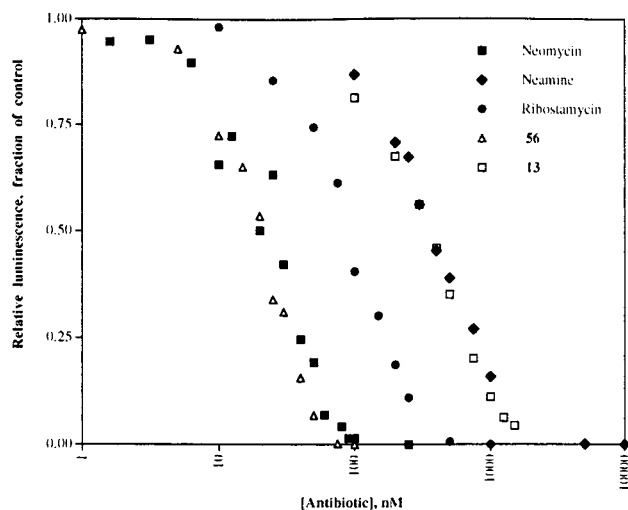

Figure 7. Semilogarithmic plot of in vitro inhibition of translation, measured as the yield of active luciferase as a function of concentration for neomycin, ribostamycin, neamine, and compounds **13** and **56**. The data points represent the average of 1−3 independent experiments.

Table 3. Comparison of Results for in Vitro Translation Inhibition, Minimum Inhibitory Concentration, and A-Site RNA Binding for Aminoglycosides Shown in Figure 6[a]

compd	in vitro translation IC$_{50}$	MIC, *E. coli* ATCC 25922	K_d (AS-wt)
gentamicin	0.020	1.6	1.7
56 (ref 8b)	0.020	1.6	0.26
neomycin	0.028	1.6	0.020
55 (ref 8b)	0.025	3.1	1.7
ribostamycin	0.10	12.5	25
13	0.33	25	1.0
12	0.42	25	0.8
neamine	0.41	50	7.8

[a] All values are concentrations in micromoles per liter.

had an order of magnitude improvement over neamine in AS-wt binding but had very similar IC$_{50}$ values, which is consistent with their similar MICs.

An overall review of Table 3 shows a close qualitative correlation between in vitro translation IC$_{50}$ values and MIC values against *E. coli*. In general, MICs are at about 100-fold higher concentrations than IC$_{50}$s. The differences in these values likely reflect two major factors: cell permeability and high ribosome concentration. The latter is of particular note. Ribo-

somal RNA is the most prevalent RNA in the cell, and at low inhibitor concentrations, a tight ribosome-binding inhibitor is titrating only a fraction of the very large number of ribosomes. Only at higher concentrations are all ribosomes saturated and protein synthesis significantly impaired. More potent ribosome-targeting antibiotics could be envisioned if they were designed to be catalytic inhibitors, as is the case with the extremely toxic proteins ricin[27] and diphtheria toxin.[28]

The apparent differences in aminoglycoside binding affinity for the 16S A-site RNA, whether isolated in a model oligonucleotide or in the context of the ribosome, may be due to a number of factors. It is possible that aminoglycosides have other high-affinity binding sites on the ribosome besides the A-site. However, no such sites were identified in Noller's footprinting experiments on intact ribosomes.[3,4] It is possible that additional stabilizing contacts are made with neighboring ribosomal proteins or with RNA which is distal in primary sequence but proximal in space. It is also possible that neighboring proteins or RNA affect binding indirectly by affecting the conformation of the A-site RNA so that it adopts a different structure, more amenable to aminoglycoside binding, than it does as an isolated system such as the A-site model oligonucleotides. The in vitro translation assay provides a good correlation between in vitro activity and antibiotic activity and represents a reliable new method of understanding the relative efficacy of synthetic aminoglycoside analogues. A clearer understanding of aminoglycoside interactions with the ribosome on a molecular level awaits high-resolution structural data on the ribosome, a formidable challenge due to its size (several megadaltons) and complexity, but one which we hope will be solved soon.[29]

Experimental Section

General. All reactions were carried out in oven-dried glassware under a positive pressure of argon. Tetrahydrofuran and diethyl ether were distilled from sodium metal/benzophenone ketyl. Acetonitrile, toluene, and dichloromethane were distilled from calcium hydride. Reactions were monitored by analytical thin-layer chromatography (TLC) on EM silica gel 60 F$_{254}$ plates (0.25 mm), visualized by ultraviolet light and/or by staining with ceric ammonium molybdate or ninhydrin. ^{1}H NMR spectra were obtained on a Bruker AMX-400

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(400 MHz), AMX-500 (500 MHz), or DRX-600 (600 MHz) spectrometer at ambient temperature. Data were reported as follows: chemical shift on the δ scale (using either TMS or residual protio solvent as internal standard), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) in hertz, and integration. ^{13}C NMR spectra were obtained with proton decoupling on a Bruker AMX-400 (100 MHz), AMX-500 (125 MHz), or DRX-600 (150 MHz) spectrometer and were reported in ppm with residual solvent for internal standard (77.0 for CDCl$_3$).

2-Deoxy-1,3-diazido-4,5,6-tri-*O*-acetylstreptamine (15). Triflic azide solution (ca. 0.5 M in dichloromethane, 80 mL, 40 mmol) was added slowly dropwise to a mixture of 2-deoxystreptamine[13] (3.24 g, 20.0 mmol), triethylamine (29.0 mL, 208 mmol), and CuSO$_4$ (193 mg, 1.21 mmol) in methanol (200 mL). The reaction was stirred for 21 h at room temperature and concentrated under reduced pressure to a green oil. (***Caution: Do not concentrate completely, as triflic azide has been reported to be explosive in the absence of solvent.***) The residue was purified by chromatography on silica gel with a gradient from 80% ethyl acetate/1% triethylamine/19% hexanes to 20% methanol/1% triethylamine/79% ethyl acetate. This material (4.3 g) was dissolved in anhydrous pyridine (40 mL), cooled to 0 °C, and treated with acetic anhydride (8.5 mL, 90 mmol). The reaction was allowed to warm to room temperature and stirrred for 19 h. 4-(Dimethylamino)pyridine (114 mg, 0.933 mmol) was added, followed 3 h later by an additional aliquot of acetic anhydride (3.0 mL, 32 mmol). After 40 min, the reaction was quenched by addition of methanol (20 mL) and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL), washed successively with 1 N HCl, saturated sodium bicarbonate, and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. To remove residual triflic amide, the residue was dissolved in ethyl acetate (100 mL), washed with 1 N NaOH (twice) and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. This material was chromatographed with a gradient of 15 to 30% ethyl acetate in hexanes to give **15** (5.11 g, 75%) as a colorless amorphous solid: R_f 0.31 (25% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.08−5.01 (m, 3H), 3.65−3.51 (m, 2H), 2.33 (ddd, J = 4.5, 4.5, 13.5, 1H), 2.09 (s, 6H), 2.01 (s, 3H), 1.56 (ddd, J = 12.7, 12.7, 13.5, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 169.9, 169.5, 73.5, 71.5, 58.0, 31.8, 20.5, 20.4; HRMS (FAB) *m/e* calcd for C$_{12}$H$_{17}$N$_6$O$_6$ (M + H$^+$) 341.1210, found 341.1217.

2-Deoxy-1,3-diazido-5,6-di-*O*-acetylstreptamine (17). Novozym 435 (*Candida antarctica* lipase immobilized on a macroporous acrylic resin, Novo Nordisk, 0.329 g) was added to **16** (0.340 g, 1.00 mmol) in a well-stirred mixture of toluene (7 mL) and 0.1 M sodium phosphate buffer, pH 6.2 (7 mL), at ambient temperature. After 72 h, TLC indicated no further conversion, and the reaction mixture was filtered and the retained enzyme washed with ethyl acetate and water. The aqueous layer of the combined filtrate and washings was extracted twice with ethyl acetate. The combined organics were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel with 10% acetone in toluene to give **17** (0.211 g, 71%) as a colorless solid, along with recovered **16** (0.085 g, 25%). Compound **17**: R_f 0.17 (10% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 4.99−4.94 (m, 2H), 3.62−3.56 (m, 2H), 3.52−3.46 (m, 1H), 2.82 (br s, 1H, OH), 2.30 (ddd, J = 4.5, 4.5, 13.0, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 1.46 (ddd, J = 12.5, 13.0, 13.0, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.7, 169.9, 74.7, 73.8, 73.5, 60.3, 58.2, 31.8, 20.7, 20.6; HRMS (FAB) *m/e* calcd for C$_{10}$H$_{15}$N$_6$O$_5$ (M + H$^+$) 299.1104, found 299.1115.

2-Deoxy-1,3-diazidostreptamine (18). Sodium methoxide (approximately 1 M solution in methanol) was added dropwise to **16** (2.47 g, 7.24 mmol) in methanol (30 mL) to give a "pH" of 11. After being stirred for 2.5 h at ambient temperature, the reaction was neutralized with Amberlite IR-120 (H$^+$). The solution was concentrated under reduced pressure to give **18** (1.55 g, 100%) as a white solid: R_f 0.24 (75% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CD$_3$OD) δ 3.38−3.29 (m, 2H), 3.23−3.20 (m, 3H), 2.08 (ddd, J = 4.4, 4.4, 13.0, 1H), 1.22 (ddd, J = 12.3, 12.3, 13.0, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 77.6, 77.0, 62.3, 33.4; MS (ESI neg) *m/e* 213 ([M − H$^+$]$^−$).

Acetylated Dispiroketal (19). Camphorsulfonic acid (0.013 g, 0.055 mmol) was added to a solution of **16** (0.109 g, 0.511 mmol) and Ley's

bis-dihydropyran[15] (0.208 g, 0.652 mmol) in CHCl$_3$ (10 mL, distilled from P$_2$O$_5$), and the resulting mixture was refluxed for 18 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate, and washed with saturated aqueous sodium bicarbonate and brine. The aqueous layers were backwashed with ethyl acetate, and the combined organics were dried (MgSO$_4$) and concentrated under reduced pressure. The residue was coevaporated twice from anhydrous toluene. Acetic anhydride (0.140 mL, 1.48 mmol) was added dropwise to a solution of the residue and 4-(dimethylamino)pyridine (3.1 mg, 0.025 mmol) in pyridine (5 mL) at ambient temperature. The reaction mixture was stirred for 24 h and then quenched by addition of methanol (3 mL). The reaction mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate and brine. The aqueous layers were backwashed with ethyl acetate and the combined organics dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was chromatographed on silica gel with a gradient of 0 to 4% ethyl acetate in hexanes to provide **19** (0.146 g, 50%) as a slightly yellow solid: R_f 0.29 (10% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.41−7.34 (m, 8H), 7.32−7.27 (m, 2H), 4.98 (dd, J = 9.5, 9.5, 1H), 4.76 (dd, J = 2.0, 12.0, 1H), 4.62 (dd, J = 2.0, 12.0, 1H), 3.72 (dd, J = 9.5, 9.5, 1H), 3.68 (dd, J = 9.5, 9.5, 1H), 3.52 (ddd, J = 4.5, 10.0, 12.5, 1H), 3.42 (ddd, J = 4.5, 10.0, 12.5, 1H), 2.18 (s, 3H), 2.13 (ddd, J = 4.5, 4.5, 13.0, 1H), 2.09−2.01 (m, 2H), 1.91−1.64 (m, 8H), 1.53−1.45 (m, 2H), 1.36 (ddd, J = 13.0, 13.0, 13.0, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 169.5, 142.8, 142.7, 128.4, 128.2, 127.5, 127.4, 125.9, 98.3, 97.9, 73.0, 72.4, 72.3, 72.0, 68.6, 58.5, 57.2, 33.5, 33.4, 32.4, 28.0, 20.7, 18.9 (x2); HRMS (FAB) *m/e* calcd for C$_{30}$H$_{34}$CsN$_6$O$_6$ (M + Cs$^+$) 707.1594, found 707.1615.

Dispiroketal (20). A suspension of acetylated dispiroketal **19** (0.146 g, 0.254 mmol) in methanol (5 mL) and ether (1 mL) was treated with sufficient sodium methoxide (approximately 1 M in methanol) to give a "pH" of ~10. After the solution was stirred for 21 h at ambient temperature, TLC indicated incomplete reaction. 1,4-Dioxane (3 mL) was added, and all solid dissolved. After 3 h, the reaction was diluted with ethyl acetate and washed with 5% ammonium chloride and brine. The organic phase was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 0 to 10% ethyl acetate in hexanes (loaded as a dichloromethane solution) to provide **20** (0.121 g, 90%) as a colorless solid: R_f 0.22 (10% ethyl acetate in hexanes); ^{1}H NMR (400 MHz, CDCl$_3$) δ 4.76 (dd, J = 2.5, 11.7, 1H), 4.73 (dd, J = 2.5, 11.7, 1H), 3.64−3.50 (m, 4H), 3.38−3.33 (m, 1H), 2.57 (br s, 1H, OH), 2.17−1.99 (m, 5H), 1.92−1.85 (m, 2H), 1.80−1.70 (m, 4H), 1.53−1.47 (m, 2H), 1.25 (app q, J = 10.1, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 142.8 (×2), 128.3, 127.4 (×2), 125.9 (×2), 98.1, 97.9, 73.7, 72.4, 72.2, 71.7, 70.4, 60.1, 57.4, 33.4, 32.4, 28.1, 28.0, 18.9, 18.8; HRMS (FAB) *m/e* calcd for C$_{28}$H$_{32}$CsN$_6$O$_5$ (M + Cs$^+$) 665.1489, found 665.1469.

2-Azido-2-deoxy-3,4,6-tri-*O*-benzyl-1-phenylthio-β-D-glucopyranoside (22). Sodium hydride (0.203 g, 8.46 mmol) was added in one portion to a solution of thioglycoside **23**[15] (0.782 g, 2.63 mmol) and benzyl bromide (1.2 mL, 10 mmol) in dimethylformamide (20 mL) at 0 °C. The reaction was allowed to slowly warm to ambient temperature. After 2 h, the reaction was quenched by addition of methanol (3 mL). This material was combined with an identically processed reaction on 201 mg of thioglycoside **23**. The combined quenched reaction mixtures were concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with saturated sodium bicarbonate and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was recrystallized from ethanol to give **22** (0.895 g, 60%) as colorless needles: R_f 0.62 (25% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.60 (m, 2H), 7.35−7.19 (m, 18H), 4.86 (d, J = 10.5, 1H), 4.83 (d, J = 10.5, 1H), 4.79 (d, J = 11.0, 1H), 4.62 (d, J = 12.0, 1H), 4.58 (d, J = 10.5, 1H), 4.54 (d, J = 12.0, 1H), 4.41 (d, J = 10.0, 1H), 3.78 (dd, J = 2.0, 11.0, 1H), 3.74 (dd, J = 4.0, 11.0, 1H), 3.61 (dd, J = 9.5, 9.5, 1H), 3.51 (dd, J = 9.5, 9.5, 1H), 3.47 (ddd, J = 2.0, 4.0, 9.5, 1H), 3.34 (dd, J = 9.5, 9.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.2, 137.8, 137.6, 133.6, 131.1, 129.0, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.5, 85.9, 85.0, 79.3, 77.5, 75.9, 75.0, 73.4, 68.7, 65.0; HRMS (FAB) *m/e* calcd for C$_{33}$H$_{33}$CsN$_3$O$_4$S (M + Cs$^+$) 700.1246, found 700.1273.

1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-α/β-D-glucopyranoside (26). Diacetonide **25**[17] (0.77 g, 2.7 mmol) was treated with 90% trifluoroacetic acid/water (10 mL). After 1 h, the reaction mixture was concentrated under reduced pressure and coevaporated twice with water. The residue was dried under oil pump vacuum. This material was dissolved in hot acetic anhydride (2 mL) and added dropwise to a suspension of sodium acetate (0.502 g, 6.1 mmol) in refluxing acetic anhydride (2 mL). After 10 min, the reaction mixture was cooled to ambient temperature, poured into a well-stirred dichloromethane/water mixture, and neutralized with sodium carbonate. The organic phase was separated and washed with brine, and the aqueous phases were backwashed with ethyl acetate. The combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 25 to 33% ethyl acetate in hexanes to give **26** (0.596 g, 59%) as a colorless oil: R_f 0.36 (33% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CDCl$_3$, 3:1 mixture of β and α anomers), β-anomer δ 5.68 (d, J = 8.0, 1H), 5.05 (dd, J = 8.0, 10.0, 1H), 5.03 (dd, J = 10.0, 10.0, 1H), 4.25 (d, J = 4.5, 12.5, 1H), 4.10 (d, J = 2.5, 12.5, 1H), 3.79 (ddd, J = 2.5, 4.5, 10.0, 1H), 3.70 (dd, J = 10.0, 10.0, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H), α-anomer δ 6.31 (d, J = 3.5, 1H), 5.03 (m, 1H), 4.96 (dd, J = 4.0, 10.5, 1H), 4.22 (dd, J = 4.0, 13.0, 1H), 4.10 (m, 1H), 4.04 (m, 1H), 3.97 (dd, J = 10.5, 10.5, 1H), 2.19 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$, 3:1 mixture of β and α anomers), β-anomer δ 169.1, 169.0, 168.9, 91.8, 73.5, 70.0, 67.7, 64.2, 61.4, 20.8, 20.7, 20.6 (×2), α-anomer δ 170.6, 169.3, 169.2, 88.7, 70.0, 69.9, 67.7, 61.4, 60.8, 30.9, 20.8, 20.6, 20.4; HRMS (FAB) m/e calcd for C$_{14}$H$_{19}$CsN$_3$O$_9$ (M + Cs$^+$) 506.0176, found 506.0159.

3-Azido-3-deoxy-2,4,6-tri-*O*-acetyl-1-phenylthio-β-D-glucopyranoside (27). Boron trifluoride diethyl etherate (1.0 mL, 7.9 mmol) was added dropwise to a solution of glycosyl acetate **26** (0.593 g, 1.59 mmol) and thiophenol (175 µL, 1.70 mmol) in chloroform (3 mL, distilled from phosphorus pentoxide). The reaction mixture was stirred at ambient temperature for 22 h and then quenched by addition of saturated sodium bicarbonate (several milliliters). The layers were separated, and the aqueous layer was backwashed with dichloromethane. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 3 to 4% acetone in toluene to provide **27** (0.431 g, 64%) as a colorless, amorphous solid: R_f 0.17 (5% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.51–7.47 (m, 2H), 7.33–7.30 (m, 3H), 4.94 (dd, J = 10.0, 10.0, 1H), 4.92 (dd, J = 10.0, 10.0, 1H), 4.66 (d, J = 10.0, 1H), 4.21–4.15 (m, 2H), 3.68 (m, 1H), 3.67 (dd, J = 10.0, 10.0, 1H), 2.18 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 170.6, 169.2, 169.0, 132.8, 131.9, 128.9, 128.3, 86.2, 76.3, 70.0, 68.2, 65.8, 62.2, 20.8, 20.7, 20.6; HRMS (FAB) m/e calcd for C$_{18}$H$_{21}$CsN$_3$O$_7$S (M + Cs$^+$) 556.0155, found 556.0173.

3-Azido-3-deoxy-1-phenylthio-β-D-glucopyranoside (28). Sodium methoxide (0.4 M in methanol, 100 µL, 40 µmol) was added to a suspension of triacetate **27** (0.431 g, 1.02 mmol) in methanol (10 mL). The reaction was stirred for 16 h at ambient temperature. TLC indicated that the reaction was incomplete, and additional sodium methoxide solution (3.0 mL, 1.2 mmol) was added. After 1.5 h, the reaction was quenched with IR-120(H$^+$), filtered, and concentrated under reduced pressure to give **28** (0.303 g, quantitative) as a colorless solid: R_f 0.14 (25% acetone/toluene); ^{1}H NMR (500 MHz, CD$_3$OD) δ 7.56–7.54 (m, 2H), 7.32–7.26 (m, 3H), 4.62 (d, J = 9.5, 1H), 3.84 (dd, J = 2.0, 12.0, 1H), 3.65 (dd, J = 5.8, 12.0, 1H), 3.35 (m, 1H), 3.33 (dd, J = 9.0, 9.0, 1H), 3.27 (dd, J = 9.5, 9.5, 1H), 3.21 (dd, J = 9.5, 9.5, 1H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 134.9, 132.9, 129.9, 128.5, 89.6, 82.4, 73.0, 72.8, 70.0, 62.5; HRMS (FAB) m/e calcd for C$_{12}$H$_{15}$NaN$_3$O$_4$S (M + Na$^+$) 320.0681, found 320.0691.

3-Azido-3-deoxy-2,4,6-tri-*O*-benzyl-1-phenylthio-β-D-glucopyranoside (24). Sodium hydride (0.085 g, 3.5 mmol) was added in one portion to a solution of thioglycoside **28** (0.293 g, 0.985 mmol) and benzyl bromide (420 µL, 3.53 mmol) in dimethylformamide (5 mL) at 0 °C. The reaction was allowed to slowly warm to ambient temperature. After 4 h, the reaction was quenched with methanol (1 mL). The reaction mixture was concentrated under reduced pressure. The residue was

dissolved in ethyl acetate, washed with saturated sodium bicarbonate solution and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 0 to 10% ethyl acetate in hexane (compound was loaded in dichloromethane) to provide **25** (0.476 g, 85%) as a slightly off-white solid: R_f 0.27 (10% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.58–7.56 (m, 2H), 7.47–7.45 (m, 2H), 7.39–7.24 (m, 16H), 4.90 (d, J = 10.0, 1H), 4.80 (d, J = 10.5, 1H), 4.74 (d, J = 10.0, 1H), 4.63 (d, J = 9.5, 1H), 4.62 (d, J = 12.0, 1H), 4.56 (d, J = 9.5, 1H), 4.54 (d, J = 12.0, 1H), 3.75 (dd, J = 2.0, 11.0, 1H), 3.72 (dd, J = 3.5, 11.0, 1H), 3.60 (dd, J = 9.0, 9.0, 1H), 3.49 (dd, J = 9.0, 9.0, 1H), 3.45 (m, 1H), 3.33 (dd, J = 9.5, 9.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.0, 137.4, 137.3, 133.4, 132.0, 129.0, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 87.7, 79.3, 79.2, 76.1, 75.3, 74.9, 73.5, 70.6, 68.6; HRMS (FAB) m/e calcd for C$_{33}$H$_{33}$NaN$_3$O$_4$S (M + Na$^+$) 590.2089, found 590.2073.

2,3,6-Tri-*O*-benzyl-1-phenylthio-β-D-galactopyranoside (31). A solution of acetal **30**[18] (0.203 g, 0.375 mmol) in THF (4 mL) was stirred for 10 min over 4-Å molecular sieves. Borane–trimethylamine complex (0.178 g, 2.44 mmol) was added in one portion, followed by aluminum chloride (0.307 g, 2.30 mmol). After 4.5 h, additional borane–trimethylamine complex (0.111 g, 1.52 mmol) and aluminum chloride (0.158 g, 1.18 mmol) were added, and the reaction was stirred overnight at ambient temperature. The reaction mixture was filtered through Celite, neutralized with IR-120(H$^+$), filtered again through Celite, and concentrated under reduced pressure. The residue was coevaporated four times with methanol, dissolved in ethyl acetate, filtered, and concentrated. This material was purified by chromatography on silica gel with a gradient of 0 to 5% ethyl acetate in toluene to give **31** (173 mg, 85%) as a cloudy oil: R_f 0.16 (5% ethyl acetate/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.58–7.56 (m, 2H), 7.41–7.39 (m, 2H), 7.37–7.28 (m, 12H), 7.25–7.23 (m, 4H), 4.83 (d, J = 10.0, 1H), 4.74 (d, J = 10.5, 1H), 4.72 (d, J = 12.0, 1H), 4.68 (d, J = 12.0, 1H), 4.64 (d, J = 10.0, 1H), 4.56 (s, 2H), 4.10 (m, 1H), 3.82–3.73 (m, 3H), 3.61–3.56 (m, 2H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.1, 137.8, 137.6, 133.8, 131.7, 128.8, 128.4, 128.3(x2), 128.2, 127.9, 128.7, 127.7 (×2), 127.2, 87.6, 82.5, 76.9 (×2), 75.6, 73.6, 72.0, 69.4, 66.8; HRMS (FAB) m/e calcd for C$_{33}$H$_{34}$CsO$_5$S (M + Cs$^+$) 675.1181, found 675.1161.

2,3,6-Tri-*O*-benzyl-4-*O*-methanesulfonyl-1-phenylthio-β-D-galactopyranoside (32). Methanesulfonyl chloride (25 µL, 0.32 mmol) was added dropwise to a solution of alcohol **31** (0.138 g, 0.254 mmol) in pyridine (3 mL) at 0 °C. The reaction was allowed to slowly warm to ambient temperature. Additional portions of methanesulfonyl chloride were added until TLC indicated that the reaction was complete (7 h). The reaction mixture was diluted with ethyl acetate, washed with 1 N HCl, saturated sodium bicarbonate, and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with 14% ethyl acetate in hexanes to provide **32** (116 mg, 73%) as a colorless oil: R_f 0.27 (5% ethyl acetate/hexanes); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.57–7.54 (m, 2H), 7.40–7.23 (m, 18H), 5.34 (d, J = 2.0, 1H), 4.84 (d, J = 10.5, 1H), 4.81 (d, J = 10.3, 1H), 4.75 (d, J = 10.3, 1H), 4.66 (m, 1H), 4.63 (d, J = 11.2, 1H), 4.59 (d, J = 10.7, 1H), 4.48 (d, J = 11.2, 1H), 3.78–3.71 (m, 3H), 3.65–3.62 (m, 2H), 2.98 (s, 3H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.7, 137.5, 137.0, 133.3, 132.1, 128.9, 128.5, 128.4 (×2), 128.2 (×2), 128.1, 127.9 (×2), 127.6, 87.8, 80.9, 76.5, 76.0, 75.7, 75.4, 73.8, 72.9, 67.9, 39.0; HRMS (FAB) m/e calcd for C$_{34}$H$_{36}$CsO$_7$S$_2$ (M + Cs$^+$) 753.0957, found 753.0941.

4-Azido-4-deoxy-2,3,6-tri-*O*-benzyl-1-phenylthio-β-D-glucopyranoside (29). Sodium azide (0.326 g, 5.01 mmol) was added to a solution of mesylate **32** (0.608 g, 0.979 mmol) in dimethylformamide (10 mL). The reaction was stirred at 100 °C for 13 h, cooled to ambient temperature, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate and brine. The aqueous layers were backwashed with ethyl acetate, and the combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel in toluene to give **29** (0.468 g, 84%) as a colorless, amorphous solid: R_f 0.23 (toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.57–7.55 (m, 2H), 7.40–7.23 (m, 18H), 4.91 (d, J = 11.0,

1H), 4.88 (d, J = 11.0, 1H), 4.83 (d, J = 10.5, 1H), 4.71 (d, J = 10.5, 1H), 4.63 (d, J = 11.5, 1H), 4.62 (d, J = 9.0, 1H), 4.57 (d, J = 12.0, 1H), 3.80 (dd, J = 2.0, 10.5, 1H), 3.72 (dd, J = 4.5, 10.5, 1H), 3.66 (dd, J = 9.8, 9.8, 1H), 3.55 (dd, J = 9.0, 9.0, 1H), 3.51 (dd, J = 9.0, 9.0, 1H), 3.32 (ddd, J = 2.0, 4.5, 10.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.0, 137.8, 137.6, 133.4, 132.1, 128.9, 128.5, 128.4, 128.2, 128.0, 127.7, 87.6, 84.9, 80.5, 78.0, 75.8, 75.4, 73.5, 69.2, 61.9; HRMS (FAB) m/e calcd for C$_{33}$H$_{33}$CsN$_3$O$_4$S (M + Cs$^+$) 700.1246, found 700.1271.

1-Phenylthio-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (35). A solution of *p*-toluenesulfonyl chloride in pyridine (0.57 M, 15 mL, 8.6 mmol) was added dropwise to thioglycoside **34** (1.84 g, 6.76 mmol) in pyridine (20 mL) at 0 °C. The transfer was quantitated with pyridine (5 mL). After 4 h at 0 °C, the reaction was allowed to slowly warm to ambient temperature and stirred for an additional 16 h. The reaction was then quenched by addition of methanol (3 mL) and concentrated under reduced pressure. The residue was dissolved in dichloromethane and washed sequentially with saturated sodium bicarbonate, 1 N HCl, and saturated sodium bicarbonate. The aqueous layers were backwashed with dichloromethane. The organic phases were combined, dried (MgSO$_4$), decanted, and concentrated under reduced pressure. The residue was chromatographed on silica gel with a gradient of 50% ethyl acetate/hexanes to 100% ethyl acetate to provide **35** (2.17 g, 75%) as a colorless foam: R_f 0.37 (1% methanol/ethyl acetate); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.79 (m, 1H), 7.77 (m, 1H), 7.44−7.41 (m, 1H), 7.29 (m, 1H), 7.27 (m, 1H), 7.24−7.19 (m, 3H), 4.46 (d, J = 9.5, 1H), 4.26 (m, 2H), 3.84 (br s, 3H, OH), 3.55−3.38 (m, 3H), 3.28 (dd, J = 9.5, 9.0, 1H), 2.38 (s, 3H); HRMS (FAB) m/e calcd for C$_{19}$H$_{22}$NaO$_7$S$_2$ (M + Na$^+$) 449.0705, found 449.0718.

6-Azido-6-deoxy-1-phenylthio-β-D-glucopyranoside (36). Sodium azide (0.397 g, 6.11 mmol) was added to thioglycoside **35** (2.17 g, 5.09 mmol) in dimethylformamide (20 mL). This suspension was placed in an 80 °C oil bath and stirred for 5 h. The reaction mixture was cooled to ambient temperature and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate and brine. The aqueous layers were backwashed with ethyl acetate, and the combined organic phases were dried (Na$_2$SO$_4$), decanted, and concentrated under reduced pressure. The crude material was purified by chromatography on silica gel with ethyl acetate to give **36** (1.36 g, 90%) as a slightly yellow oil: R_f 0.42 (1% methanol/ethyl acetate); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.54−7.52 (m, 2H), 7.31−7.29 (m, 3H), 4.92 (br s, 1H, OH), 4.51 (d, J = 10.0, 1H), 4.27 (br s, 1H, OH), 3.98 (br s, 1H, OH), 3.56−3.37 (m, 5H), 3.34 (dd, J = 9.0, 9.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 133.1, 131.2, 129.1, 128.5, 87.8, 78.4, 77.5, 71.8, 70.1, 51.4; HRMS (FAB) m/e calcd for C$_{12}$H$_{15}$NaN$_3$O$_4$S (M + Na$^+$) 320.0681, found 320.0692.

6-Azido-6-deoxy-2,3,4-tri-*O*-benzyl-1-phenylthio-β-D-glucopyranoside (33). Sodium hydride (0.376 g, 15.7 mmol) was added in one portion to a solution of thioglycoside **36** (1.34 g, 4.5 mmol) and benzyl bromide (1.9 mL, 16 mmol) in dimethylformamide (20 mL) at 0 °C. After 1 h, the reaction mixture was allowed to slowly warm to ambient temperature. After 3 h, additional portions of benzyl bromide (0.55 mL, 4.6 mmol) and sodium hydride (88 mg, 3.7 mmol) were added. TLC indicated no change after an additional 1.5 h, and the reaction was quenched with methanol (3 mL). The reaction was concentrated under reduced pressure. The residue was dissolved in ethyl acetate, washed with 5% ammonium chloride solution and brine, dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 0 to 10% ethyl acetate in hexane (compound was loaded in dichloromethane) to provide **33** (1.47 g, 57%) as a colorless solid (an analytically pure sample was obtained by recrystallization from ethanol): R_f 0.24 (10% ethyl acetate/hexanes); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.59 (m, 2H), 7.42−7.25 (m, 18H), 4.93 (d, J = 10.5, 2H), 4.88 (d, J = 11.0, 1H), 4.85 (d, J = 11.0, 1H), 4.76 (d, J = 10.0, 1H), 4.67 (d, J = 10.0, 1H), 4.61 (d, J = 11.0, 1H), 3.71 (dd, J = 9.0, 9.0, 1H), 3.56−3.51 (m, 3H), 3.46 (m, 1H), 3.35 (dd, J = 13.0, 5.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.1, 137.9, 137.6, 132.9, 132.7, 129.0, 128.5, 128.5, 128.4, 128.2, 128.0 (×2), 127.9 (×2), 127.8 (×2) 87.7, 86.5, 80.8, 78.1, 78.0, 75.8, 75.5, 75.2, 51.4; HRMS (FAB) m/e calcd for C$_{33}$H$_{33}$CsN$_3$O$_4$S (M + Cs$^+$) 700.1246, found 700.1220.

4-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-2-deoxy-streptamine (37). Donor **21** (0.349 g, 0.551 mmol) and acceptor **17** (0.125 g, 0.419 mmol) were coevaporated twice from dry toluene and further dried under high vacuum overnight. Diethyl ether (8.0 mL) and dichloromethane (2.0 mL) were added, and the reaction was cooled to −30 °C. Trifluoromethanesulfonic acid (7.0 μL) was added to a solution of *N*-iodosuccinimide (0.184 g, 0.818 mmol) in diethyl ether (6.0 mL) and dichloromethane (6.0 mL). A portion (8.0 mL) of the resulting faint orange solution was added slowly dropwise to the solution of **21** and **17**, which was maintained at −30 °C. After 30 min, the reaction mixture was diluted with diethyl ether and washed with 10% aqueous sodium bisulfite, saturated sodium bicarbonate, and brine. The aqueous layers were back extracted with diethyl ether. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 2 to 10% ethyl acetate in toluene to provide a slightly yellow oil (0.311 g, R_f 0.14 in 5% ethyl acetate/toluene). This material was dissolved in tetrahydrofuran (1.0 mL) and methanol (9.0 mL), and sodium methoxide (0.5 M in methanol, 200 μL, 0.1 mmol) was added. The reaction mixture was stirred at ambient temperature for 16 h. The solution was neutralized with Amberlite IR-120(H$^+$), filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 5 to 7% acetone in toluene to give **37** (0.244 g, 79%) as a colorless oil: R_f 0.21 (10% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.37−7.26 (m, 18H), 7.14−7.12 (m, 2H), 4.96 (d, J = 4.0, 1H, H1′), 4.93 (m, 2H), 4.89 (d, J = 12.0, 1H), 4.81 (d, J = 10.5, 1H), 4.79 (br s, 1H, OH), 4.74 (d, J = 11.5, 1H), 4.65 (d, J = 12.0, 1H), 4.51 (d, J = 10.5, 1H), 4.47 (d, J = 12.5, 1H), 4.04 (dd, J_1 = J_2 = 9.5, 1H), 4.03 (m, 1H), 3.81 (dd, J = 3.0, 10.5, 1H), 3.76 (dd, J_1 = J_2 = 9.5, 1H), 3.69 (dd, J = 1.8, 10.5, 1H), 3.48−3.37 (m, 3H), 3.24 (m, 1H), 3.18 (dd, J_1 = J_2 = 9.0, 1H), 2.98 (br s, 1H, OH), 2.28 (ddd, J = 4.5, 4.5, 13.5, 1H), 1.46 (ddd, J = 12.0, 12.0, 13.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.3, 138.0, 137.7, 136.8, 128.7, 128.5, 128.4, 128.3, 128.0, 127.8, 127.7, 127.6, 101.3, 85.4, 82.3, 79.1, 77.7, 75.6, 75.3, 75.1, 74.5, 73.5, 71.4, 68.0, 59.6, 59.4, 32.3; HRMS (FAB) m/e calcd for C$_{40}$H$_{44}$CsN$_6$O$_8$ (M + Cs$^+$) 869.2275, found 869.2247.

4-*O*-(2-Azido-2-deoxy-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-2-deoxystreptamine (38). Donor **22** (148 mg, 0.261 mmol) and acceptor **17** (60 mg, 0.201 mmol) were coevaporated twice from dry toluene and further dried under high vacuum overnight. Diethyl ether (4.0 mL) and dichloromethane (1.0 mL) were added, and the reaction was cooled to −10 °C. Trifluoromethanesulfonic acid (5.0 μL, 56 μmol) was added to a solution of *N*-iodosuccinimide (118 mg, 0.524 mmol) in diethyl ether (3.0 mL) and dichloromethane (3.0 mL). A portion (5.5 mL) of the resulting faint orange solution was added slowly dropwise to the solution of **22** and **17**, which was maintained at −10 °C. After 30 min, the reaction mixture was diluted with diethyl ether and washed with 10% aqueous sodium bisulfite, saturated sodium bicarbonate, and brine. The aqueous layers were back extracted with diethyl ether. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with a gradient of 3 to 5% ethyl acetate in toluene to provide a slightly yellow oil (90 mg, R_f 0.15 in 5% acetone/toluene). This material was dissolved in tetrahydrofuran (0.2 mL) and methanol (1.8 mL), and sodium methoxide (0.6 M in methanol, 100 μL, 60 μmol) was added. The reaction mixture was stirred at ambient temperature for 19 h. The solution was neutralized with Amberlite IR-120(H$^+$), filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with 7% acetone in toluene to give **38** (78 mg, 58%) as a colorless oil: R_f 0.10 (10% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.35−7.25 (m, 13H), 7.16−7.14 (m, 2H), 5.15 (d, J = 3.6, 1H, H1′), 4.92 (d, J = 10.5, 1H), 4.85 (d, J = 10.5, 1H), 4.78 (d, J = 10.8, 1H), 4.64 (d, J = 12.0, 1H), 4.54 (d, J = 10.8, 1H), 4.49 (d, J = 12.0, 1H), 4.20 (d, J = 2.0, 1H, OH), 4.09 (m, 1H), 3.98 (dd, J_1 = J_2 = 9.1, 1H), 3.82−3.76 (m, 2H) 3.68 (dd, J = 2.0, 10.8, 1H), 3.64 (dd, J = 3.6, 10.2, 1H), 3.45−3.38 (m, 3H), 3.26−3.24 (m, 2H), 2.94 (d, J = 2.0, 1H, OH), 2.30 (m, 1H), 1.48 (m, 1H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 137.8, 137.7, 137.5, 128.5, 128.4 (×2), 128.0 (×2), 127.9, 127.8 (×2), 127.7, 99.5, 83.9, 81.0, 78.1, 75.8, 75.6, 75.3, 75.0, 73.6, 71.7, 68.0, 64.2, 59.6, 58.7,

32.0; HRMS (FAB) *m/e* calcd for $C_{33}H_{37}CsN_9O_7$ (M + Cs$^+$) 804.1870, found 804.1838.

4-*O*-(3-Azido-3-deoxy-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-2-deoxystreptamine (39). Donor **24** (138 mg, 0.244 mmol) and acceptor **17** (60 mg, 0.202 mmol) were coevaporated twice from dry toluene and further dried under high vacuum overnight. Diethyl ether (4.0 mL) and dichloromethane (1.0 mL) were added, and the reaction was cooled to −15 °C. Trifluoromethanesulfonic acid (5.0 µL, 56 µmol) was added to a solution of *N*-iodosuccinimide (110 mg, 0.489 mmol) in diethyl ether (3.0 mL) and dichloromethane (3.0 mL). A portion (3.0 mL) of the resulting faint orange solution was added slowly dropwise to the solution of **24** and **17**, which was maintained at −15 °C. After 30 min, the reaction mixture was diluted with diethyl ether and washed with 10% aqueous sodium bisulfite, saturated sodium bicarbonate, and brine. The aqueous layers were back extracted with diethyl ether. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with 5% ethyl acetate in toluene to provide a slightly yellow oil (R_f 0.46 in 10% acetone/toluene). This material was dissolved in tetrahydrofuran (0.4 mL) and methanol (1.6 mL), and sodium methoxide (0.5 M in methanol, 100 µL, 50 µmol) was added. The reaction mixture was stirred at ambient temperature for 20 h. An additional portion of sodium methoxide (0.5 M in methanol, 200 µL, 100 µmol) was added. After being stirred a further 1 h, the solution was neutralized with Amberlite IR-120(H$^+$), filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with 10% acetone in toluene to give **39** (139 mg, 100%) as a colorless oil: R_f 0.22 (10% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.35−7.25 (m, 13H), 7.16−7.14 (m, 2H), 5.15 (d, *J* = 3.6, 1H, H1′), 4.92 (d, *J* = 10.6, 1H), 4.85 (d, *J* = 10.6, 1H), 4.78 (d, *J* = 10.8, 1H), 4.64 (d, *J* = 12.0, 1H), 4.54 (d, *J* = 10.8, 1H), 4.49 (d, *J* = 12.0, 1H), 4.20 (d, *J* = 2.3, 1H, OH), 4.09 (m, 1H), 3.98 (dd, $J_1 = J_2$ = 9.1, 1H), 3.82−3.76 (m, 2H) 3.68 (dd, *J* = 1.8, 10.8, 1H), 3.64 (dd, *J* = 3.6, 10.2, 1H), 3.45−3.38 (m, 3H), 3.26−3.24 (m, 2H), 2.94 (d, *J* = 1.8, 1H, OH), 2.30 (m, 1H), 1.48 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 137.5, 137.3, 136.3, 128.8, 128.7, 128.6, 128.4, 128.2 (×2), 128.0, 127.9, 100.2, 85.2, 77.2, 76.2, 75.5, 75.3, 74.9, 74.3, 73.6, 71.0, 67.8, 65.9, 59.5, 59.2, 32.2; HRMS (FAB) *m/e* calcd for $C_{33}H_{37}CsN_9O_7$ (M + Cs$^+$) 804.1870, found 804.1840.

4-*O*-(4-Azido-4-deoxy-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-2-deoxystreptamine (40). Donor **29** (137 mg, 0.241 mmol) and acceptor **17** (60 mg, 0.202 mmol) were coevaporated twice from dry toluene and further dried under high vacuum overnight. Diethyl ether (4.0 mL) and dichloromethane (1.0 mL) were added, and the reaction was cooled to −15 °C. Trifluoromethanesulfonic acid (6.0 µL, 68 µmol) was added to a solution of *N*-iodosuccinimide (112 mg, 0.498 mmol) in diethyl ether (3.0 mL) and dichloromethane (3.0 mL). A portion (3.0 mL) of the resulting faint orange solution was added slowly dropwise to the solution of **29** and **17**, which was maintained at −15 °C. After 30 min, the reaction mixture was diluted with diethyl ether and washed with 10% aqueous sodium bisulfite, saturated sodium bicarbonate, and brine. The aqueous layers were back extracted with diethyl ether. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with 5% ethyl acetate in toluene to provide a colorless oil (154 mg, R_f 0.54 in 10% acetone/toluene). This material was dissolved in tetrahydrofuran (0.4 mL) and methanol (1.6 mL), and sodium methoxide (0.7 M in methanol, 200 µL, 140 µmol) was added. The reaction mixture was stirred at ambient temperature for 19 h, and the solution was then neutralized with Amberlite IR-120(H$^+$), filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with 5% acetone in toluene to give **40** (125 mg, 92%) as a colorless oil: R_f 0.07 (5% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.42−7.26 (m, 15H), 4.95 (d, *J* = 3.5, 1H, H1′), 4.90 (s, 2H), 4.86 (d, *J* = 12.0, 1H), 4.70 (d, *J* = 11.5, 1H), 4.65 (d, *J* = 12.0, 1H), 4.59 (d, *J* = 1.0, 1H, OH), 4.49 (d, *J* = 12.0, 1H), 3.87 (app t, *J* = 9.8, 1H), 3.84 (m, 1H), 3.76−3.70 (m, 2H) 3.66 (dd, *J* = 1.8, 10.8, 1H), 3.60 (dd, *J* = 3.5, 9.5, 1H), 3.47−3.36 (m, 3H), 3.26 (m, 1H), 3.17 (m, 1H), 2.88 (d, *J* = 1.0, 1H, OH), 2.26 (ddd, *J* = 4.4, 4.4, 13.5, 1H), 1.44 (app q, *J* = 12.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 137.6, 136.5, 128.7, 128.5 (×2), 128.4, 128.1, 128.0, 127.8

(×2), 101.1, 85.2, 80.4, 78.9, 75.7, 75.5, 75.4, 74.5, 73.6, 70.6, 68.2, 61.8, 59.6, 59.3, 32.2; HRMS (FAB) *m/e* calcd for $C_{33}H_{37}CsN_9O_7$ (M + Cs$^+$) 804.1870, found 804.1896.

4-*O*-(6-Azido-6-deoxy-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-2-deoxystreptamine (41). Donor **33** (273 mg, 0.481 mmol) and acceptor **17** (60 mg, 0.202 mmol) were coevaporated twice from dry toluene and further dried under high vacuum overnight. Diethyl ether (8.0 mL) and dichloromethane (2.0 mL) were added, and the reaction was cooled to −15 °C. Trifluoromethanesulfonic acid (10.0 µL, 113 µmol) was added to a solution of *N*-iodosuccinimide (216 mg, 0.960 mmol) in diethyl ether (6.0 mL) and dichloromethane (6.0 mL). A portion (6.0 mL) of the resulting faint orange solution was added slowly dropwise to the solution of **33** and **17**, which was maintained at −15 °C. After 30 min, the reaction mixture was diluted with diethyl ether and washed with 10% aqueous sodium bisulfite, saturated sodium bicarbonate, and brine. The aqueous layers were back extracted with diethyl ether. The combined organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with 4% ethyl acetate in toluene to provide a colorless oil (331 mg, R_f 0.55 in 10% acetone/toluene). This material was dissolved in tetrahydrofuran (0.8 mL) and methanol (3.2 mL), and sodium methoxide (0.9 M in methanol, 400 µL, 377 µmol) was added. The reaction mixture was stirred at ambient temperature for 19 h, and the solution was then neutralized with Amberlite IR-120(H$^+$), filtered, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel with 5% acetone in toluene to give **41** (222 mg, 82%) as a colorless oil: R_f 0.30 (10% acetone/toluene); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.35−7.26 (m, 15H), 4.96 (d, *J* = 4.0, 1H, H1′), 4.92 (m, 2H), 4.81 (d, *J* = 11.0, 1H), 4.88 (d, *J* = 12.0, 1H), 4.73 (d, *J* = 11.5, 1H), 4.62 (m, 2H), 4.06 (m, 1H), 4.03 (dd, *J* = 9.2, 9.2, 1H), 3.60−3.54 (m, 3H), 3.45−3.36 (m, 4H), 3.26 (m, 1H), 3.17 (m, 1H), 3.02 (s, 1H), 2.27 (ddd, *J* = 4.5, 4.5, 13.5, 1H), 1.45 (ddd, *J* = 12.5, 12.5, 12.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 138.1, 137.7, 136.7, 128.7, 128.5, 128.4, 127.9, 127.6, 100.8, 85.2, 82.0, 79.3, 78.2, 75.5, 75.3, 75.2, 74.6, 71.0, 59.6, 59.3, 51.0, 32.2; HRMS (FAB) *m/e* calcd for $C_{33}H_{37}CsN_9O_7$ (M + Cs$^+$) 804.1870, found 804.1896.

4-*O*-(α-D-Glucopyranosyl)-2-deoxystreptamine (2). A solution of pseudodisaccharide **37** (91 mg, 0.12 mmol) in methanol (6 mL) was degassed by evacuating the flask and backfilling with argon three times. Hydrazine (25 µL, 0.80 mmol) was added, followed immediately by 20% Pd(OH)$_2$ on carbon (∼10 mg, Degussa type), and the reaction mixture was heated at reflux for 16 h. The solution was cooled to ambient temperature, filtered through Celite, and concentrated under reduced pressure to afford a colorless oil (80 mg). This material was dissolved in a mixture of acetic acid (2.5 mL) and water (2.5 mL), and the solution was degassed by evacuating the flask and backfilling with argon three times. Next, 20% Pd(OH)$_2$ on carbon (∼10 mg, Degussa type) was added, and the solution was purged with hydrogen. The reaction mixture was stirred at ambient temperature under hydrogen gas (∼1 atm, balloon) for 22 h (an additional portion of catalyst was added after 6 h). The solution was filtered through Celite and concentrated under reduced pressure. The residue was purified by chromatography on Amberlite CG-50 (NH$_4$$^+$, 1.5-cm-diameter × 10.5-cm column) with a gradient of 0 to 1.2% concentrated ammonium hydroxide in water. Fractions containing product were pooled and concentrated under reduced pressure. The residue was dissolved in water (several milliliters), acidified with acetic acid, and lyophilized to provide **2** (57 mg, 100%) as its oily diacetate salt: ^{1}H NMR (600 MHz, D$_2$O) δ 5.28 (d, *J* = 4.0, 1H, H1′), 3.80 (dd, *J* = 2.2, 12.0, 1H), 3.71 (m, 1H), 3.67 (app t, *J* = 9.7, 1H), 3.63−3.58 (m, 3H), 3.54 (dd, *J* = 4.0, 10.0, 1H), 3.47−3.42 (m, 2H), 3.29 (app t, *J* = 9.7, 1H), 3.22 (m, 1H), 2.38 (ddd, *J* = 4.2, 4.2, 12.5, 1H), 1.83 (s, 6H), 1.73 (ddd, *J* = 12.5, 12.5, 12.5, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ 181.5, 99.5,81.6, 74.8, 73.9, 73.4, 73.1, 72.0, 70.2, 61.4, 50.5, 49.4, 29.0, 23.6; MS (ESI) 325 (MH$^+$).

Paromamine (3). A solution of pseudodisaccharide **38** (73 mg, 0.11 mmol) in methanol (6 mL) was degassed by evacuating the flask and backfilling with argon three times. Hydrazine (85 µL, 2.7 mmol) was added, followed immediately by 20% Pd(OH)$_2$ on carbon (∼10 mg, Degussa type), and the reaction mixture was heated at reflux for 90 h. The solution was cooled to ambient temperature, filtered through Celite,

and concentrated under reduced pressure to afford a colorless solid (68 mg). This material was dissolved in a mixture of acetic acid (2 mL) and water (2 mL), and the solution was degassed by evacuating the flask and backfilling with argon three times. Next, 20% Pd(OH)$_2$ on carbon (~10 mg, Degussa type) was added, and the solution was purged with hydrogen. The reaction mixture was stirred at ambient temperature under hydrogen gas (~1 atm, balloon) for 21 h (an additional portion of catalyst was added after 5 h). The solution was filtered through Celite and concentrated under reduced pressure. The residue was purified by chromatography on Amberlite CG-50 (NH$_4$$^+$, 1.5-cm-diameter × 10.5-cm column) with a gradient of 0 to 2.5% concentrated ammonium hydroxide in water. Fractions containing product were pooled and concentrated under reduced pressure. The residue was dissolved in water (3 mL), acidified with acetic acid, and lyophilized to provide **3** (45 mg, 82%) as its oily triacetate salt. All spectral data for synthetic and naturally derived paromamine were identical: [α]$_D$ = +68° (c = 1, H$_2$O) (natural paromamine), [α]$_D$ = +67° (c = 1, H$_2$O) (synthetic paromamine); R_f 0.11 (10% concentrated ammonium hydroxide/methanol); ^{1}H NMR (600 MHz, D$_2$O) δ 5.54 (d, J = 3.9, 1H, H1′), 3.85−3.79 (m, 2H), 3.76 (m, 1H), 3.71 (app t, J = 9.5, 1H), 3.66 (dd, J = 6.4, 12.2, 1H), 3.56 (app t, J = 9.2, 1H), 3.47 (app t, J = 9.5, 1H), 3.42−3.36 (m, 2H), 3.33 (dd, J = 3.9, 10.9, 1H), 3.21 (m, 1H) 2.37 (ddd, J = 4.3, 4.3, 12.6, 1H), 1.83 (s, 9H), 1.71 (ddd, J = 12.6, 12.6, 12.6, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ 182.1, 98.0, 82.0, 75.6, 74.4, 73.4, 70.2, 70.0, 61.1, 54.9, 50.6, 49.6, 29.7, 23.9; MS (ESI) 324 (MH$^+$).

4-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-2-deoxystrept-amine (4). A solution of pseudodisaccharide **39** (139 mg, 0.20 mmol) in methanol (10 mL) was degassed by evacuating the flask and backfilling with argon three times. Hydrazine (63 μL, 2.0 mmol) was added, followed immediately by 20% Pd(OH)$_2$ on carbon (~10 mg, Degussa type), and the reaction mixture was heated at reflux for 46 h (an additional portion of catalyst was added after 17 h). The solution was cooled to ambient temperature, filtered through Celite, and concentrated under reduced pressure to afford a colorless solid (130 mg). This material was dissolved in a mixture of acetic acid (2.5 mL) and water (2.5 mL), and the solution was degassed by evacuating the flask and backfilling with argon three times. Next, 20% Pd(OH)$_2$ on carbon (~10 mg, Degussa type) was added, and the solution was purged with hydrogen. The reaction mixture was stirred at ambient temperature under hydrogen gas (~1 atm, balloon) for 46 h (an additional portion of catalyst was added after 23 h). The solution was filtered through Celite and concentrated under reduced pressure. The residue was purified by chromatography on Amberlite CG-50 (NH$_4$$^+$, 1.5-cm-diameter × 10.5-cm column) with a gradient of 0 to 2.5% concentrated ammonium hydroxide in water. Fractions containing product were pooled and concentrated under reduced pressure. The residue was dissolved in water (3 mL), acidified with acetic acid, and lyophilized to provide **4** (52 mg, 50%) as its oily triacetate salt: R_f 0.10 (10% concentrated ammonium hydroxide/methanol); ^{1}H NMR (600 MHz, D$_2$O) δ 5.58 (d, J = 3.9, 1H, H1′), 3.79−3.69 (m, 4H), 3.64−3.58 (m, 2H), 3.54 (app t, J = 10.0, 1H), 3.45−3.39 (m, 2H), 3.36 (app t, J = 10.6, 1H), 3.19 (m, 1H), 2.36 (ddd, J = 4.3, 4.3, 12.6, 1H), 1.80 (s, 9H), 1.71 (ddd, J = 12.6, 12.6, 12.6, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ 182.0, 98.1, 81.0, 74.9, 73.7, 73.4, 68.6, 66.5, 60.8, 55.6, 50.5, 49.4, 29.2, 23.9; MS (ESI) 324 (MH$^+$).

4-*O*-(4-Amino-4-deoxy-α-D-glucopyranosyl)-2-deoxystrept-amine (5). A solution of pseudodisaccharide **40** (124 mg, 0.18 mmol) in methanol (10 mL) was degassed by evacuating the flask and backfilling with argon three times. Hydrazine (30 μL, 0.96 mmol) was added, followed immediately by 20% Pd(OH)$_2$ on carbon (~10 mg, Degussa type), and the reaction mixture was heated at reflux for 21 h (an additional portion of catalyst was added after 17 h). The solution was cooled to ambient temperature, filtered through Celite, and concentrated under reduced pressure to afford a waxy solid (111 mg). This material was dissolved in a mixture of acetic acid (2.5 mL) and water (2.5 mL), and the solution was degassed by evacuating the flask and backfilling with argon three times. Next, 20% Pd(OH)$_2$ on carbon (~20 mg, Degussa type) was added, and the solution was purged with hydrogen. The reaction mixture was stirred at ambient temperature under hydrogen gas (~1 atm, balloon) for 15 h. The solution was filtered through Celite and concentrated under reduced pressure. The residue

was purified by chromatography on Amberlite CG-50 (NH$_4$$^+$, 1.5-cm-diameter × 10.5-cm column) with a gradient of 0 to 2.5% concentrated ammonium hydroxide in water. Fractions containing product were pooled and concentrated under reduced pressure. The residue was dissolved in water (3 mL), acidified with acetic acid, and lyophilized to provide **5** (42 mg, 45%) as its oily triacetate salt: R_f 0.07 (10% concentrated ammonium hydroxide/methanol); ^{1}H NMR (600 MHz, D$_2$O) δ 5.40 (d, J = 3.9, 1H, H1′), 4.04 (m, 1H), 3.82 (app t, J = 10.0, 1H), 3.77 (dd, J = 3.4, 12.3, 1H), 3.72−3.67 (m, 2H), 3.64−3.60 (m, 2H), 3.49−3.43 (m, 2H), 3.21 (m, 1H), 3.14 (app t, J = 10.0, 1H), 2.38 (ddd, J = 4.2, 4.2, 12.5, 1H), 1.83 (s, 9H), 1.73 (ddd, J = 12.5, 12.5, 12.5, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ 181.8, 98.1, 80.9, 74.3, 73.5, 71.9, 70.5, 69.6, 61.1, 52.8, 50.5, 49.0, 29.0, 23.8; MS (ESI) 324 (MH$^+$).

4-*O*-(6-Amino-6-deoxy-α-D-glucopyranosyl)-2-deoxystrept-amine (6). A solution of pseudodisaccharide **41** (106 mg, 0.16 mmol) in methanol (5 mL) was degassed by evacuating the flask and back-filling with argon three times. Hydrazine (19 μL, 0.60 mmol) was added, followed immediately by 20% Pd(OH)$_2$ on carbon (~10 mg, Degussa type), and the reaction mixture was heated at reflux for 19 h. The solution was cooled to ambient temperature, filtered through Celite, and concentrated under reduced pressure to afford a waxy solid (95 mg). This material was dissolved in a mixture of acetic acid (2.5 mL) and water (2.5 mL), and the solution was degassed by evacuating the flask and backfilling with argon three times. Next, 20% Pd(OH)$_2$ on carbon (~20 mg, Degussa type) was added, and the solution was purged with hydrogen. The reaction mixture was stirred at ambient temperature under hydrogen gas (~1 atm, balloon) for 22 h. The solution was filtered through Celite and concentrated under reduced pressure. The residue was purified by chromatography on Amberlite CG-50 (NH$_4$$^+$, 1.5-cm-diameter × 10.5-cm column) with a gradient of 0 to 4% concentrated ammonium hydroxide in water. Fractions containing product were pooled and concentrated under reduced pressure. The residue was dissolved in water (3 mL), acidified with acetic acid, and lyophilized to provide **6** (79 mg, 99%) as its oily triacetate salt: R_f 0.12 (20% concentrated ammonium hydroxide/methanol); ^{1}H NMR (600 MHz, D$_2$O) δ 5.43 (d, J = 3.9, 1H, H1′), 3.90 (m, 1H), 3.74 (dd, J = 10.0, 10.0, 1H), 3.66 (dd, J = 9.5, 9.5, 1H), 3.62 (dd, J = 9.5, 9.5, 1H), 3.55 (dd, J = 3.9, 10.0, 1H), 3.48 (m, 1H), 3.44 (dd, J = 9.5, 9.5, 1H), 3.31 (dd, J = 3.1, 13.3, 1H), 3.26 (dd, J = 9.5, 9.5, 1H), 3.20 (m, 1H), 3.07 (dd, J = 8.0, 13.4, 1H), 2.38 (m, 1H), 1.83 (s, 9H), 1.75 (ddd, J = 12.5, 12.5, 12.5, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ 181.5, 96.5, 79.2, 74.0, 73.8, 73.0, 71.6(x2), 69.5, 50.6, 48.5, 41.1, 28.9, 23.7; MS (ESI) 324 (MH$^+$).

Hexaazido-hepta-*O*-benzyl Neomycin (42). A freshly prepared dichloromethane solution of triflic azide[14] (180 mL, ~1.5 M) was added slowly to a mixture of neomycin trisulfate hydrate (25.0 g, 26.2 mmol), triethylamine (34.5 mL, 250 mmol), and CuSO$_4$·5H$_2$O (411 mg, 1.65 mmol) in 150 mL of H$_2$O plus 400 mL of methanol. The mixture was stirred at room temperature. When TLC analysis showed incomplete reaction after 8 h (R_f = 0.6, EtOAc), an additional portion of triflic azide (90 mL, ~1.5 M in dichloromethane) was added, and the mixture was stirred for 10 h. The solvent was then removed under reduced pressure, and the residue was redissolved in 200 mL of ethyl acetate. The solution was extracted with saturated aqueous NaHCO$_3$ and concentrated to a green foam. For ease of purification, the product was peracetylated: the crude green foam was dissolved in 30 mL of pyridine and 20 mL of acetic anhydride, and 4-(dimethylamino)pyridine (168 mg, 5 mol %) was added. The mixture was stirred for 3 h at room temperature, concentrated under reduced pressure, redissolved in 200 mL of ethyl acetate, and extracted twice with 100 mL of 1 M aqueous HCl. The organic layer was concentrated and passed through a plug of silica, eluting with 2:1 EtOAc/hexanes, and then concentrated to a light yellow gum. This was dissolved in 100 mL of methanol, and sodium methoxide in methanol was added to a pH of 11. The solution was stirred for 2 h and then neutralized with Amberlite IR-120 (H$^+$), filtered, and concentrated under reduced pressure, providing an off-white foam (11.72 g, 15.2 mmol, 58%) which was directly benzylated: the foam was dissolved in 80 mL of DMF, and tetrabutylammonium iodide was added (2.8 g, 7.5 mmol). Benzyl bromide was added (31.6 mL, 266 mmol), followed by sodium hydride (3.65 g, 152 mmol) in ~100-mg

portions every 3−5 min over 2 h. After a total of 12 h, the solvent was removed in vacuo, and the residue was dissolved in 200 mL of ethyl acetate, extracted with 200 mL of water, washed with 100 mL of brine, and concentrated. The product was purified by silica gel chromatography, eluting first with hexanes to remove excess benzyl bromide and then with 4:1 hexanes/EtOAc, providing **42** as a straw-colored gum (17.2 g, 12.1 mmol, 80%, 46% from neomycin sulfate): R_f 0.5 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.15−7.37 (m, 35H), 6.16 (d, J = 3.8, 1H), 5.66 (d, J = 5.5, 1H), 4.69−4.99 (m, 6H), 4.39−4.66 (m, 8H), 4.21−4.34 (m, 2H), 4.12 (m, 1H), 4.03 (dd, J_1 = 11, J_2 = 8, 1H), 3.92−3.99 (m, 2H), 3.74−3.83 (m, 3H), 3.61−3.70 (m, 2H), 3.56 (dd, J_1 = 10, J_2 = 3, 1H), 3.40−3.50 (m, 4H), 3.30−3.38 (m, 2H), 3.20−3.30 (m, 3H), 3.11 (m, 1H), 2.99 (dd, J_1 = 10, J_2 = 3.5, 1H), 2.86 (dd, J_1 = 13.5, J_2 = 3.5, 1H), 2.25 (ddd, J_1 = 12.5, J_2 = J_3 = 4.5, 1H), 1.42 (ddd, J_1 = J_2 = J_3 = 12.5, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 138.3, 137.9, 137.7, 137.0, 136.9, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 127.4, 106.4, 98.6, 95.6, 84.2, 82.5, 82.0, 81.7, 79.9, 78.6, 75.5, 75.3, 75.1, 74.8, 74.4, 73.3, 72.8, 72.3, 71.7, 71.4, 70.9, 70.2, 63.1, 60.3, 59.9, 57.2, 51.3, 51.0, 32.5; HRMS (FAB) for C$_{72}$H$_{76}$N$_{18}$O$_{13}$ (M + Cs$^+$) calcd 1533.4894, found 1533.4998.

1,3,2′,6′-Tetraazido-6,3′,4′-tri-*O*-benzyl Neamine (43). Compound **42** (3.87 g, 2.76 mmol) was dissolved in 120 mL of 1 M HCl in 5:5:1 methanol/dioxane/water and stirred under reflux for 24 h. The acid was quenched with solid NaHCO$_3$, and the solution was filtered and concentrated in vacuo. The residue was chromatographed on silica (75:15:10 hexanes/EtOAc/toluene), providing an oil, which was further purified by recrystallization from 8:1:1 hexanes/EtOAc/toluene, producing **43** as colorless long needles (1.03 g). The mother liquor and remaining column fractions containing starting material were resubjected to the reaction conditions for 16 h, providing an additional 330 mg of **43** after chromatography and recrystallization. Total yield of **43** was 1.36 g (1.96 mmol, 71%): R_f 0.4 (5:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.25−7.43 (m, 15H), 5.25 (d, J = 3.0, 1H), 4.85−4.92 (m, 6H), 4.63 (d, J = 9.5, 1H), 4.14 (m, 1H), 3.98 (dd, J_1 = 8.5, J_2 = 7.5, 1H), 3.70 (br, 1H), 3.47−3.67 (m, 4H), 3.45 (m, 1H), 3.40 (dd, J_1 = 10.5, J_2 = 3.5, 1H), 3.23−3.32 (m, 3H), 2.30 (ddd, J_1 = 10.5, J_2 = J_3 = 4.5, 1H), 1.48 (ddd, J_1 = J_2 = J_3 = 10.5, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.7, 137.6, 137.4, 128.6, 128.5, 128.3, 128.1, 127.8, 99.3, 83.6, 82.9, 80.6, 78.7, 76.7, 75.7, 75.4, 75.2, 71.3, 64.1, 59.8, 58.7, 50.9, 32.3; HRMS (FAB) for C$_{33}$H$_{36}$N$_{12}$O$_6$ (M + Cs$^+$) calcd 829.1935, found 829.1968.

5-*O*-Allyl-1,3,2′,6′-tetraazido-6,3′,4′-tri-*O*-benzyl Neamine (44). Compound **43** (810 mg, 1.21 mmol) was dissolved in 10 mL of DMF. Tetrabutylammonium iodide (425 mg, 1.14 mmol) was added, followed by sodium hydride (44 mg, 1.73 mmol). Allyl bromide was added (303 μL, 3.5 mmol), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with 1 mL of methanol, the solvent was removed under reduced pressure, and the residue was redissolved in 30 mL of ethyl acetate and extracted with brine. After the organic layer was concentrated, the product was purified by silica chromatography (10:1 hexanes/EtOAc), providing **44** as a colorless gum (890 mg, 99%): R_f 0.55 (10:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.26−7.41 (m, 15H), 5.96 (m, 1H) 5.59 (d, J = 4.0, 1H), 5.30 (m, 1H), 5.19 (m, 1H), 4.86−4.90 (m, 3H), 4.81 (m, 2H), 4.63 (m, 1H), 4.45 (m, 1H), 4.38 (m, 1H), 4.27 (ddd, J_1 = 10, J_2 = 4.5, J_3 = 3.0, 1H), 3.99 (dd, J_1 = 10.5, J_2 = 9.0, 1H), 3.32−3.59 (m, 9H), 2.27 (ddd, J_1 = 13.0, J_2 = J_3 = 4.5, 1H), 1.44 (ddd, J_1 = J_2 = J_3 = 13.0, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 137.7, 137.6, 137.3, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 116.4, 97.6, 84.4, 84.1, 80.0, 78.6, 77.5, 75.9, 75.1, 70.9, 63.5, 60.0, 59.1, 50.9, 32.3; HRMS (FAB) for C$_{36}$H$_{40}$N$_{12}$O$_6$ (M + Cs$^+$) calcd 869.2248, found 869.2214.

Aldehyde 45. Compound **44** (290 mg, 0.39 mmol) was dissolved in 15 mL of 1:1 dichloromethane/methanol and cooled to −78 °C. Ozone was bubbled into the solution until it turned blue ($\sim$ 20 s), at which time the solution was flushed with O$_2$. Dimethyl sulfide (300 μL, 4.1 mmol) was added, and the mixture was warmed to room temperature and stirred for 1 h. The solvent was removed under reduced pressure, providing $\sim$290 mg of crude **45** as a colorless oil, which was used without further purification: R_f 0.1 (6:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.55 (s, 1H), 7.26−7.38 (m, 15H), 5.49 (d, J =

4.0, 1H), 4.82−4.93 (m, 4H), 4.64 (app. q, J = 10.5, 1H), 4.50 (m, 2H), 4.27 (ddd, J_1 =10, J_2 =4.0, J_3 =2.5, 1H), 3.94 (dd, J_1 = 10.5, J_2 = 9.0, 1H), 3.52−3.60 (m, 2H), 3.50 (dd, J_1 = 13.0, J_2 = 2.5, 1H), 3.35−3.43 (m, 6H), 2.32 (ddd, J_1 = 12.5, J_2 = J_3 = 4.5, 1H), 1.51 (ddd, J_1 = J_2 = J_3 = 12.5, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 197.8, 137.5, 137.5, 137.0, 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8, 98.1, 85.1, 84.3, 80.0, 79.3, 78.7, 78.3, 75.9, 75.4, 75.1, 71.2, 63.3, 60.2, 58.8, 50.8, 32.0; HRMS (FAB) for C$_{35}$H$_{38}$N$_{12}$O$_7$ (M + Cs$^+$) calcd 871.2041, found 871.2071.

General Procedure for Reductive Aminations (46−54). Crude aldehyde **45** ($\sim$100 mg, 0.14 mmol) was dissolved in 0.90 mL of methanol. Three equivalents of the appropriate protected amine[30] (0.40 mmol) was added as a 1 M solution in methanol (0.40 mL), followed by 0.43 mL of 1 M solution of acetic acid in methanol. The pH was checked and adjusted to about 6 with acetic acid, if necessary. NaCNBH$_3$ was added as a freshly prepared 0.3 M solution in methanol (0.19 mL), and the mixture was stirred at room temperature for 2 h. The reaction was quenched with a few drops of water and concentrated under reduced pressure, and the product was purified by silica chromatography (1−5% MeOH/0.5−1% triethylamine/dichloromethane). In the case of **46**, the secondary amine arising from the reductive amination was protected as a benzyl carbamate (benzyl chloroformate, pyridine) before proceeding to the next step. Reported yields are combined for the ozonolysis and reductive amination steps.

Compound 46: yield 48 mg, 39%; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.25−7.40 (m, 25H), 5.50 (m, 1H), 5.43 (m, 1H), 5.01−5.13 (m, 4H), 4.80−4.87 (m, 4H), 4.73 (m, 1H), 4.60−4.65 (m, 2H), 4.24 (m, 1H), 3.96−4.00 (m, 3H), 3.85−3.89 (m, 1H), 3.05−3.54 (m, 13H), 2.26 (m, 1H), 1.63 (m, 2H), 1.42 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 156.6, 155.8, 137.7, 137.6, 137.4, 136.7, 136.5, 129.6, 129.2, 128.8, 128.4, 128.3, 128.0, 127.9, 127.8, 127.5, 97.6, 84.7, 84.5, 84.4, 79.9, 79.6, 78.6, 75.7, 75.4, 75.2, 75.1, 71.8, 71.1, 67.4, 66.5, 62.7, 60.1, 59.1, 50.9, 47.7, 46.5, 45.3, 44.6, 38.0, 37.4, 32.1, 27.8, 25.4; HRMS (FAB) for C$_{54}$H$_{60}$N$_{14}$O$_{10}$ (M + Cs$^+$) calcd 1197.3671, found 1197.3738.

Compound 47: yield 78 mg, 60%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.25−7.42 (m, 20H), 5.71 (m, 1H), 5.59 (d, J = 2.9, 1H), 5.07 (s, 2H), 4.83−4.88 (m, 5H), 4.61 (m, 1H), 4.26 (m, 1H), 3.90−4.03 (m, 3H), 3.19−3.56 (m, 11H), 2.81 (m, 2H), 2.59−2.73 (m, 2H), 2.38 (m, 4H), 2.26 (m, 1H), 2.15−2.18 (m, 4H), 1.62−1.65 (m, 4H), 1.45 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.6, 137.4, 136.7, 136.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 97.6, 84.8, 84.4, 79.8, 78.7, 77.5, 75.6, 75.4, 75.1, 71.1, 66.5, 63.1, 60.2, 59.1, 56.5, 55.6, 50.9, 49.3, 48.7, 41.6, 39.6, 37.4, 32.1, 26.7; HRMS (FAB) for C$_{50}$H$_{63}$N$_{15}$O$_8$ (M + Cs$^+$) calcd 1134.4038, found 1134.4099.

Compound 48: yield 65 mg, 52%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.25−7.38 (m, 25H), 5.56 (d, J = 3.8, 1H), 5.28 (m, 2H), 5.02−5.09 (m, 4H), 4.81−4.90 (m, 4H), 4.71 (m, 1H), 4.59 (m, 1H), 4.25 (m, 1H), 4.02 (m, 1H), 3.91 (m, 1H), 3.76 (m, 1H), 3.04−3.56 (m, 13H), 2.64 (m, 2H), 2.51 (m, 4H), 2.19 (m, 1H), 1.37 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.5, 137.6, 137.6, 137.6, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 97.6, 84.6, 84.4, 79.5, 78.7, 75.3, 75.1, 71.7, 71.1, 66.6, 63.0, 60.0, 59.2, 54.0, 53.9, 50.9, 45.7, 38.9, 31.9; HRMS (FAB) for C$_{55}$H$_{63}$N$_{15}$O$_{10}$ (M + H$^+$) calcd 1094.4961, found 1094.4917.

Compound 49: yield 81 mg, 63%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27−7.38 (m, 20H), 5.68 (d, J = 3.8, 1H), 5.28 (m, 1H), 5.12 (m, 4H), 4.84−4.91 (m, 4H), 4.63 (m, 1H), 4.28 (m, 1H), 3.88−4.10 (m, 3H), 3.25−3.58 (m, 11H), 2.85 (m, 2H), 2.70 (m, 1H), 2.23−2.54 (m, 9H), 1.44 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 156.3, 137.6, 137.4, 136.8, 136.7, 128.5, 128.5, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 97.7, 84.6, 84.5, 79.7, 78.7, 77.6, 75.7, 75.5, 75.1, 71.0, 67.3, 67.1, 66.7, 63.3, 59.1, 57.5, 57.0, 52.6, 50.9, 49.8, 46.3, 45.8, 43.7, 37.4, 32.2; HRMS (FAB) for C$_{49}$H$_{59}$N$_{15}$O$_8$ (M + Cs$^+$) calcd 1118.3725, found 1118.3789.

Compound 50: yield 61 mg, 53%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.25−7.35 (m, 15H), 5.69 (d, J = 2.9, 1H), 4.83−4.89 (m, 6H), 4.63 (m, 1H), 4.28 (m, 1H), 3.99−4.06 (m, 3H), 3.91 (m, 1H), 3.68 (m,

(30) (a) Atwell, G. J.; Denny, W. A. *Synthesis* **1984**, 1032−1033. (b) Adamczyk, M.; Fishpaugh, J. R.; Heuser, K. J. *Org. Prep. Proced. Int.* **1998**, *30*, 339−348.

4H), 3.33–3.57 (m, 8H), 2.84 (m, 2H), 2.70 (m, 2H), 2.40 (m, 6H), 2.27 (m, 1H), 1.43 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 137.6, 137.4, 136.8, 128.5, 128.5, 128.4, 128.2, 128.0, 128.0, 128.0, 127.9, 127.8, 97.6, 84.6, 79.8, 78.7, 77.5, 75.7, 75.5, 75.1, 73.1, 71.0, 66.9, 63.3, 60.1, 59.2, 58.7, 53.7, 50.9, 50.0, 46.3, 32.2; HRMS (FAB) for C$_{41}$H$_{52}$N$_{14}$O$_7$ (M + Cs$^+$) calcd 985.3198, found 985.3226.

Compound 51: yield 59 mg, 44%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.27–7.41 (m, 20H), 5.63 (d, J = 3.8, 1H), 5.08 (s, 2H), 4.82–4.88 (m, 6H), 4.61 (d, J = 11, 1H), 4.26 (m, 1H), 3.97–4.02 (m, 2H), 3.88 (m, 1H), 3.16–3.59 (m, 13H), 2.86 (m, 1H), 2.74 (m, 1H), 2.56 (m, 1H), 2.28 (m, 1H), 1.27–1.50 (m, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.3, 137.5, 137.4, 128.6, 128.5, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.8, 97.6, 87.8, 84.5, 79.7, 78.7, 77.4, 75.7, 77.1, 77.1, 77.1, 75.7, 75.1, 63.2, 60.1, 59.2, 58.8, 50.9, 46.5, 40.7, 32.2, 31.0, 30.3, 30.1, 23.0; LRMS (ESI) for C$_{49}$H$_{60}$N$_{14}$O$_9$ (M + H$^+$) calcd 990, found 990.

Compound 52: yield 75 mg, 56%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.26–7.41 (m, 20H), 5.65 (d, J = 3.8, 1H), 5.08 (s, 2H), 4.80–4.88 (m, 6H), 4.61 (d, J = 11.4, 1H), 4.27 (m, 1H), 3.97–4.07 (m, 2H), 3.84 (m, 1H), 3.16–3.58 (m, 13H), 2.87 (m, 1H), 2.72 (m, 1H), 2.55 (m, 1H), 2.28 (m, 1H), 1.28–1.50 (m, 6H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.3, 137.6, 137.4, 128.5, 128.5, 128.1, 128.1, 128.0, 128.0, 127.8, 97.7, 84.5, 79.7, 78.7, 77.5, 75.7, 75.5, 75.1, 73.3, 71.1, 66.6, 63.2, 62.5, 60.1, 59.1, 58.8, 50.9, 46.8, 40.7, 32.2, 31.0, 30.3, 30.1, 23.0; HRMS (FAB) for C$_{49}$H$_{60}$N$_{14}$O$_9$ (M + Cs$^+$) calcd 1121.3722, found 1121.3772.

Compound 53: yield 66 mg, 62%; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.26–7.42 (m, 15H), 5.70 (d, J = 3.5, 1H), 4.84–4.90 (m, 6H), 4.63 (m, 1H), 4.26 (m, 1H), 3.99–4.08 (m, 2H), 3.88 (m, 1H), 3.33–3.68 (m, 13H), 2.84 (m, 2H), 2.69 (m, 1H), 2.31 (m, 3H), 1.46 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 137.5, 137.4, 134.6, 128.5, 127.9, 127.8, 97.5, 84.6, 84.5, 79.6, 78.7, 77.5, 75.6, 75.4, 75.1, 73.0, 71.6, 63.8, 62.6, 60.6, 60.0, 59.4, 50.4, 46.9, 46.1, 32.0; HRMS (FAB) for C$_{38}$H$_{47}$N$_{13}$O$_8$ (M + Cs$^+$) calcd 946.2725, found 946.2696.

Compound 54: yield 62 mg, 62%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.20–7.53 (m, 19H), 5.70 (d, J = 3.8, 1H), 4.77–4.91 (m, 6H), 4.62 (m, 1H), 4.29 (m, 1H), 3.99–4.11 (m, 4H), 3.90 (m, 1H), 3.25–3.58 (m, 8H), 2.81 (m, 2H), 2.28 (m, 1H), 1.45 (m, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 153.7, 137.6, 137.6, 137.5, 128.6, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 122.3, 97.7, 84.6, 84.4, 79.4, 78.6, 77.5, 75.7, 75.4, 75.1, 73.3, 71.1, 63.1, 60.1, 59.2, 51.0, 49.5, 47.7, 32.2; HRMS (FAB) for C$_{43}$H$_{47}$N$_{15}$O$_6$ (M + Cs$^+$) calcd 1002.2888, found 1002.2844.

General Procedure for Deprotection of 46–53 (7–14). The starting material was dissolved in 4.5 mL of THF and 0.5 mL of 0.1 M aqueous NaOH. Then, 4.5 equiv of PMe$_3$ was added as a 1 M solution in THF, and the mixture was stirred at room temperature (or 50 °C if the reaction was proceeding slowly) for 2 h. When complete, the mixture was concentrated and passed through a short silica column (10:1 MeOH/NH$_3$(aqueous). The crude amine product was then dissolved in 2 mL of 1:1 EtOH/THF and added in small portions to a blue solution of sodium metal (~80 mg) in liquid ammonia (25 mL) and THF (10 mL). After all starting material had been added, the mixture was stirred until the blue color returned and was stable for 15 min. At this time, the reaction was quenched with ammonium formate (500 mg in 1 mL of water), the solvent was evaporated off, and the product mixture was lyophilized. Products were purified by ion-exchange chromatography (Amberlite CG-50, NH$_4$$^+$ form) with a linear gradient of aqueous ammonia. Gradients of 0–10, 0–15, or 0–20% concentrated aqueous ammonia were used. Fractions (3 mL) were collected with an automated fraction collector, and those containing product were pooled and lyophilized and then converted to their hydrochloride salts by dissolving in an excess of 0.1 M aqueous HCl and lyophilizing again. Yields are reported for the HCl salts, combined for the two-step deprotection.

Compound 7: yield 19 mg, 65%; ^{1}H NMR (600 MHz, D$_2$O) δ 5.69 (d, J = 3.6, 1H), 4.03 (m, 1H), 3.86–3.98 (m, 3H), 3.76 (m, 1H), 3.68 (dd, J_1 = J_2 = 9.0, 1H), 3.44 (dd, J_1 = J_2 = 9.0, 1H), 3.40 (dd, J_1 = 13.5, J_2 = 3.5, 1H), 3.24–3.39 (m, 6H), 3.14 (m, 2H), 3.05 (m, 2H), 2.33 (ddd, J_1 = J_2 = J_3 = 12.5, 2H), 2.02–2.10 (m, 2H), 1.42 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H); ^{13}C NMR (150 MHz, D$_2$O) δ

96.3, 84.6, 76.8, 74.3, 72.0, 71.0, 70.7, 67.7, 55.0, 51.6, 50.3, 49.1, 46.2, 41.6, 38.1, 30.6, 25.2; LRMS (ESI) for C$_{17}$H$_{38}$N$_6$O$_6$ (M + H$^+$) calcd 423, found 423, (M + Cl$^-$), calcd 457, found 457.

Compound 8: yield 34 mg, 58%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.84 (d, J = 3.5, 1H), 4.18–4.24 (m, 2H), 4.04–4.14 (m, 2H), 3.94 (m, 1H), 3.88 (t, J = 9.5, 1H), 3.80 (t, J = 9.5, 1H), 3.30–3.60 (m, 12H), 3.21 (t, J = 7.8, 2H), 3.10 (t, J = 7.8, 2H), 2.94 (s, 3H), 2.46 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 2.11–2.24 (m, 4H), 1.91 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H), 1.64–1.77 (m, 4H), 1.41–1.51 (m, 2H); ^{13}C NMR (100 MHz, D$_2$O) δ 99.0, 87.8, 86.5, 79.0, 77.4, 74.9, 70.7, 59.0, 55.7, 55.5, 55.3, 53.3, 52.3, 51.3, 50.3, 42.4, 42.1, 38.9, 30.6, 29.4, 25.8, 24.5; LRMS (ESI) for C$_{21}$H$_{47}$N$_7$O$_6$ (M + H$^+$) calcd 494, found 494, (M + Cl$^-$) calcd 528, found 528.

Compound 9: yield 39 mg, 44%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.88 (d, J = 3.5, 1H), 4.20 (t, J = 9, 1H), 3.92- -4.14 (m, 4H), 3.77–3.86 (m, 2H), 3.34–3.64 (m, 8H), 3.21 (t, J = 6.5, 4H), 3.03 (m, 4H), 2.49 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.93 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H); ^{13}C NMR (100 MHz, D$_2$O) δ 95.7, 87.9, 85.6, 75.5, 74.9, 72.6, 72.5, 70.6, 55.6, 55.4, 52.9, 52.4, 51.3, 42.5, 38.1, 30.3; LRMS (ESI) for C$_{18}$H$_{41}$N$_7$O$_6$ (M + H$^+$) calcd 452, found 452, (M + Cl$^-$) calcd 486, found 486.

Compound 10: yield 8 mg, 13%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.84 (d, J = 3.8, 1H), 4.21 (m, 1H), 4.04–4.13 (m, 2H), 3.93 (m, 1H), 3.85 (t, J = 9.5, 1H), 3.79 (t, J =9, 1H), 3.26–3.62 (m, 13H), 2.80–2.90 (m, 8H), 2.48 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.91 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H); ^{13}C NMR (100 MHz, D$_2$O) δ 102.7, 87.9, 86.7, 81.0, 73.3, 69.5, 68.2, 56.8, 56.0, 55.5, 54.8, 51.5, 51.0, 50.0, 45.5, 45.2, 35.1, 34.2, 30.3; LRMS (ESI) for C$_{20}$H$_{43}$N$_7$O$_6$ (M + H$^+$) calcd 478, found 478, (M + Cl$^-$) calcd 512, found 512.

Compound 11: yield 34 mg, 68%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.87 (d, J = 3.5, 1H), 4.24 (m, 2H), 4.07–4.16 (m, 2H), 3.79–3.97 (m, 6H), 3.35–3.65 (m, 17H), 2.50 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.94 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H); ^{13}C NMR (100 MHz, D$_2$O) δ 96.0, 87.8, 85.3, 75.9, 73.2, 72.5, 70.5, 68.4, 66.6, 55.5, 54.9, 54.5, 52.3, 51.2, 50.7, 44.1, 42.3, 37.8, 30.4; LRMS (ESI) for C$_{20}$H$_{42}$N$_6$O$_7$ (M + H$^+$) calcd 479, found 479, (M + Cl$^-$) calcd 513, found 513.

Compound 12: yield 21 mg, 60%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.81 (d, J = 3.8, 1H), 4.10–4.23 (m, 3H), 4.03 (t, J = 9, 1H), 3.88–3.96 (m, 2H), 3.71–3.86 (m, 3H), 3.32–3.58 (m, 9H), 2.99 (t, J = 7.8, 2H), 2.45 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.89 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H), 1.64–1.77 (m, 4H), 1.41–1.51 (m, 2H); ^{13}C NMR (100 MHz, D$_2$O) δ 94.0, 87.8, 85.5, 75.0, 72.6, 72.5, 70.6, 68.7, 61.9, 60.4, 55.6, 52.8, 52.3, 51.3, 47.5, 42.4, 41.5, 30.5, 29.1, 29.0, 24.4; LRMS (ESI) for C$_{20}$H$_{44}$N$_6$O$_7$ (M + H$^+$) calcd 481, found 481, (M + Cl$^-$) calcd 515, found 515.

Compound 13: yield 34 mg, 80%; ^{1}H NMR (400 MHz, D$_2$O) δ 5.85 (d, J = 3.5, 1H), 4.24 (m, 2H), 4.05–4.15 (m, 2H), 3.80–3.95 (m, 4H), 3.75 (m, 1H), 3.35–3.65 (m, 9H), 3.00 (t, J = 7.6, 2H), 2.49 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.94 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H), 1.65–1.75 (m, 4H), 1.43–1.49 (m, 2H); ^{13}C NMR (100 MHz, D$_2$O) δ 95.8, 87.9, 85.3, 75.8, 74.8, 72.5, 70.6, 68.4, 61.6, 60.6, 55.6, 52.3, 51.3, 46.9, 42.4, 41.6, 30.3, 29.0, 28.9, 24.4; LRMS (ESI) for C$_{20}$H$_{44}$N$_6$O$_7$ (M + H$^+$) calcd 481, found 481, (M + Cl$^-$) calcd 515, found 515.

Compound 14: yield 11 mg, 76%; ^{1}H NMR (500 MHz, D$_2$O) δ 5.82 (d, J = 3.5, 1H), 4.25 (m, 1H), 4.18 (t, J = 9.2, 1H), 4.13 (m, 1H), 4.04 (t, J = 9.5, 1H), 3.76–3.92 (m, 8H), 3.75 (m, 1H), 3.51–3.59 (m, 2H), 3.38–3.48 (m, 6H), 2.46 (ddd, J_1 = J_2 = J_3 = 12.5, 1H), 1.89 (ddd, J_1 = 12.5, J_2 = J_3 = 4.0, 1H); ^{13}C NMR (100 MHz, D$_2$O) δ 102.5, 87.9, 85.4, 75.1, 72.3, 70.9, 68.6, 62.6, 59.9, 59.8, 58.6, 55.6, 52.3, 51.2, 47.6, 42.3, 32.8; HRMS (FAB) for C$_{17}$H$_{37}$N$_5$O$_8$ (M + H$^+$) calcd. 440.2720, found 440.2704.

Compound 15. Protected compound **54** (60 mg, 0.069 mmol) was dissolved in 2 mL of ethanol. Hydrazine (11 μL, 0.35 mmol) was added, followed by Raney nickel (~30 mg). The mixture was stirred at room temperature for 2 h and then filtered through Celite, concentrated, and redissolved in 2 mL of 1:1 acetic acid/water. Palladium hydroxide (20 mg, 20% on C, Degussa type) was added, and the mixture was stirred under a hydrogen balloon for 12 h. The product was filtered, lyophilized, and purified by ion-exchange chromatography (Amberlite CG-50, NH$_4$$^+$ form) with a linear gradient (0–10%) of aqueous ammonia. Fractions

(3 mL) were collected with an automated fraction collector, and those containing product were pooled and lyophilized and then converted to the hydrochloride salt by dissolving in an excess of 0.1 M aqueous HCl and lyophilizing again: yield 16 mg, 32%; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.83 (m, 2H), 7.61 (m, 2H), 5.86 (d, J = 3.8, 1H), 4.18–4.34 (m, 3H), 4.08 (t, J = 9.8, 1H), 3.81–3.96 (m, 3H), 3.31–3.64 (m, 10H), 2.49 (m, 1H), 1.95 (app q, J = 12.7, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 202.9, 135.1, 129.0, 117.2, 95.9, 85.4, 75.8, 74.9, 72.6, 72.4, 70.5, 68.5, 55.5, 52.3, 51.3, 50.7, 44.4, 42.4, 30.3; HRMS (FAB) for C$_{22}$H$_{37}$N$_7$O$_6$ (M + H$^+$) calcd 496.2884, found 496.2889.

SPR Binding Studies. 5′-Biotinylated A-site RNA was prepared exactly as described previously.[8] Binding experiments and curve fitting were also performed as previously described.[8] Data from 2–4 independent experiments for each compound were averaged to obtain the reported dissociation constants.

Antibiotic Testing. Minimal inhibitory concentrations (MICs) were determined according to published protocols.[25] Briefly, *E. coli* ATCC 25922 was grown in Mueller−Hinton broth (cation-adjusted, BBL Microbiology Systems) to an optical density (OD$_{600}$) of ∼ 0.5, and then diluted to OD$_{600}$= 0.1. Samples of antibiotic were prepared in Mueller−Hinton broth over the desired concentration range. For each concentration, 50 mL of diluted culture was combined with 1 mL of antibiotic sample and incubated at 37 °C for 4−6 h. The control sample (no antibiotic) typically would have an OD$_{600}$ of 1.2−1.5 at this time. The absorbance of each sample was read, and the MIC was considered to be the lowest concentration of antibiotic at which OD$_{600}$ was less than 1% of the control.

In Vitro Translation Assay. A coupled transcription−translation assay was performed with luciferase DNA to determine the extent of translational inhibition in the presence of the various aminoglycosides. The DNA template was constructed by cleaving the luciferase gene from the vector pBestluc (Promega) and placing it into the vector pBluescript SK$^-$ (Stratagene) behind the *lac* promoter. The transcription/translation mixture, or S-30 extract, and the reaction buffers were prepared as recommended by Ellman et al.[31] with slight modifications. Magnesium and calcium concentrations were optimized for the extract.

Final concentrations in the translation mixture were as follows: Tris−acetate (pH 7.4), 70 mM; dithiothreitol, 2.2 mM; ATP (sodium salt), 1.5 mM; UTP, GTP, CTP (sodium salts), 1 mM each; phophoenolpyruvate (potassium salt), 33 mM; PEG-8000, 2.4% (w/v); folinic acid, 42 μg/mL; pyridoxine hydrochloride, 33 μg/mL; NADP, 33 μg/mL; FAD, 33 μg/mL; *p*-aminobenzoic acid, 13.6 μg/mL; tRNA (from *E. coli*, type XXI from Sigma), 0.2 mg/mL; potassium acetate, 88 mM; ammonium acetate, 44 mM; calcium acetate, 4 mM; magnesium acetate, 8 mM; IPTG (isopropylthiogalactoside, induces *lac* expression), 1 mM; amino acids (all 20), 0.1 mM of each; 8 μL of S-30 extract and 1 μg of DNA template per 25 μL of translation solution. The translation assays were performed by mixing all of the reagents, varying amounts of the compounds to be tested, and the DNA template in a RNase-free microcentrifuge tube. The S-30 extract was always added last, and the reaction was maintained at 21 ± 1 °C in a water bath. The reaction was terminated after 30 min by diluting the reaction 10-fold with luciferase dilution reagent (25 mM Tris−phosphate, pH 7.8; 2 mM DTT; 2 mM 1,2-diaminocyclohexane *N,N,N′,N′*-tetraacetate; 10% glycerol; 1% Triton X-100; and 1 mg/mL bovine serum albumin). Translation yield was determined by mixing 10 μL of the diluted reaction mixture with 50 μL of luciferase assay reagent (20 mM Tricine, pH 7.8; 15 mM MgSO$_4$; 0.1 mM EDTA; 33.3 mM DTT; 270 μM coenzyme A; 470 μM luciferin; and 530 μM ATP) and monitoring the luminescence with a Turner Designs luminometer. For each assay, points were collected in duplicate, and the full assays were performed at least three times for each compound.

(31) Ellman, J.; Mendel, D.; Anthony-Cahill, S.; Noren, C. J.; Schultz, P. G. *Methods Enzymol.* **1991**, *202*, 301−337.