Probing the Specificity of Aminoglycoside−Ribosomal RNA Interactions with Designed Synthetic Analogs

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Abstract: The binding of neomycin B and related aminoglycoside antibiotics to the prokaryotic ribosomal RNA decoding region has been investigated using a recently developed surface plasmon resonance assay. A number of naturally occurring aminoglycosides containing a neamine or neamine-like substructure bind specifically to a model of the site of the ribosomal decoding region RNA. This recognition event is the basis of the antibacterial activity of this class of compounds. A series of analogs was designed and synthesized to probe the role of neomycin ring IV (2,6-dideoxy-2,6-diamino-β-L-idopyranose). The binding results indicate that the positive charge presented on the idose ring is necessary for specific binding *in vitro* and cannot be replaced by amines attached *via* flexible linkers. However, the antibiotic activity (minimum inhibitory concentration) of the analog where ring IV is replaced with a diamine tail is the same as neomycin B in a liquid culture assay against *Escherichia coli*.

Introduction

Targeting RNA sequences using small molecule drugs is a topic of significant interest. To this end, the interactions necessary for an RNA recognition event to occur need to be understood at the molecular level. Aminoglycoside antibiotics, as a class, have long been known to bind RNA. They exert their antibacterial effects at least in part by binding to specific target sites in the bacterial ribosome.[1] For the structurally related antibiotics neamine (**1**), ribostamycin (**2**), neomycin B (**3**), and paromomycin (**4**) (Figure 1), the binding site has been localized to the A-site of the prokaryotic 16S ribosomal decoding region RNA,[2] which is shown in Figure 2. Binding of aminoglycosides to this RNA target interferes with the fidelity of mRNA translation and results in miscoding and truncation, leading ultimately to bacterial cell death. The biological properties of naturally occurring and synthetic aminoglycoside antibiotics have been reviewed.[3]

There is also considerable biochemical data describing the interaction of this class of compounds with a variety of other biologically relevant RNA sequences. Apart from 16S ribosomal RNA[2] these include two HIV-1 mRNA regulatory domains, the Rev response element (RRE)[4] and the transactivation response element (TAR),[5] as well as the group I intron[6] and the hammerhead ribozyme.[7] In addition, numerous RNA sequences which bind particular aminoglycosides have been derived from *in vitro* selection experiments.[8] Among aminoglycoside−RNA interactions, the binding of neomycin type antibiotics to 16S RNA stands out because it is linked to the biological activity of this class of compounds. This interesting mode of action of aminoglycosides has prompted investigation of their RNA recognition capabilities in model systems,[9] combinatorial synthesis of derivatives as possible inhibitors of RNA recognition,[10] and semisynthesis of known antibiotics to

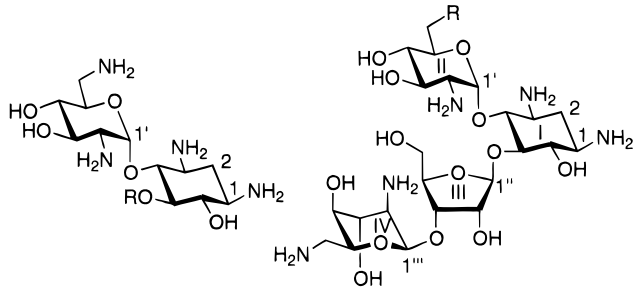

Figure 1. Structures of representative aminoglycoside antibiotics.

(1) (a) Tanaka, N. Mechanism of Action of Aminoglycoside Antibiotics. *Handbook of Experimental Pharmacology*; Springer-Verlag: New York, 1982; Vol. 62, p 221. (b) Cundliffe, E. Recognition Sites for Antibiotics Within rRNA. In *The Ribosome*; Hill, W. E., *et al.*, Eds.; American Society of Microbiology: Washington, DC, 1990; p 479. (c) Noller, H. F. *Annu. Rev. Biochem.* **1991**, *60*, 191.

(2) (a) Moazed, D.; Noller, H. F. *Nature* **1987**, *327*, 389. (b) Puhroit, P.; Stern, S. *Nature* **1994**, *370*, 659. (c) Recht, M. I.; Fourmy, D.; Blanchard, S. C.; Dahlquist, K. D.; Puglisi, J. D. J. *Mol. Biol.* **1996**, *262*, 421. (d) Miyaguchi, H.; Narita, H.; Sakamoto, K.; Yokoyama, S. *Nucleic Acids Res.* **1996**, *24*, 3700.

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(4) (a) Zapp, M. L.; Stern, S.; Green, M. R. *Cell* **1993**, *74*, 969. (b) Werstuck, G.; Zapp, M. L.; Green, M. R. *Chem. Biol.* **1996**, *3*, 129.

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(6) (a) von Ahsen, U.; Davies, J.; Schroeder, R. *Nature* **1991**, *353*, 368. (b) von Ahsen, U.; Schroeder, R. *Nucleic Acids Res.* **1991**, *19* (9), 2261. (c) von Ahsen, U.; Davies, J.; Schroeder, R. J. *Mol. Biol.* **1992**, *226* (4), 935. (d) von Ahsen, U.; Noller, H. *Science* **1993**, *260*, 1500.

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(8) (a) Wang, Y.; Rando, R. R. *Chem. Biol.* **1995**, *2*, 281. (b) Lato, S. M.; Boles, A. R.; Ellington, A. D. *Chem. Biol.* **1995**, *2*, 291. (c) Wallis, M. G.; von Ahsen, U.; Schroeder, R.; Famulok, M. *Chem. Biol.* **1995**, *2*, 543. (d) Famulok, M.; Huttenhofer, A. *Biochemistry* **1996**, *35*, 4265. (e) Wang, Y.; Killian, J.; Hamasaki, K.; Rando, R. R. *Biochemistry* **1996**, *35*, 12338.

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

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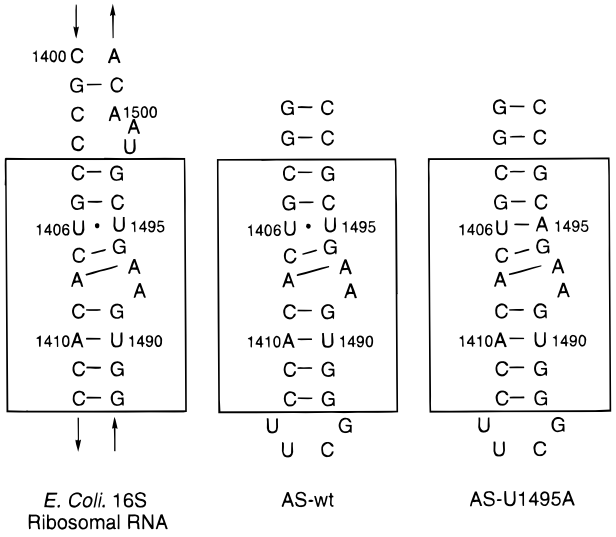

Figure 2. Sequences of the RNA molecules used in this study.

understand their function and improve their activity.[11] Also, other investigators have synthesized non-aminoglycoside molecules that bind RNA.[12]

Recently, it has been shown that a short RNA hairpin containing the residues 1404−1412 and 1488−1497 of the *Escherichia coli* ribosomal RNA (AS-wt, Figure 2) retains the aminoglycoside binding properties of the site embedded in the prokaryotic ribosome.[2c,d] A critical base for specific aminoglycoside binding is U1495 which, in the wild type sequence, is engaged in a noncanonical U:U base pair. Replacement of U1495 with A leads to complete loss of specific aminoglycoside binding.[2c,d] For this reason, AS-U1495A can serve as a negative control for *in vitro* binding studies.

Puglisi and co-workers have determined the solution structure of the AS-wt complex with **4** by NMR.[13] A representation of the pertinent intermolecular contacts is shown in Figure 3. Paromomycin (**4**) rings I and II fit into a pocket created by the asymmetrical bulge. Ring II (2-amino-2-deoxy-D-glucose) stacks against the G1491-C1409 base pair, and ring I (2-deoxystreptamine) spans across the major groove, making hydrogen bonds to N7 of G1494 and O6 of U1495. On the other hand, the structural data indicated that ring IV (2,6-dideoxy-2,6-diamino idose) had an ill-defined position in the complex. The apparent dynamic nature of this residue raised questions regarding its contribution to the binding event. The L-idose moiety either could contribute to the specificity of the interaction or might be merely a platform to present additional charges increasing only the affinity.

We have investigated the specificity of aminoglycoside binding to the A-site RNA with a series of naturally occurring aminoglycosides (**1**−**4**) using our recently developed surface

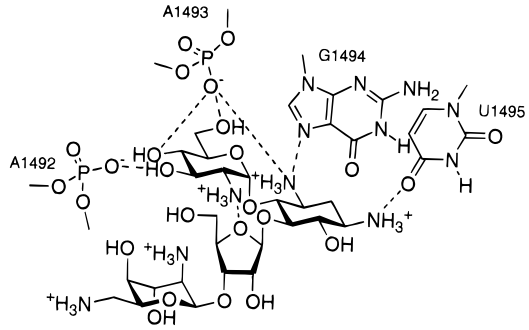

Figure 3. Schematic representation of the binding of paromomycin to the target fragment of 16S ribosomal RNA based on the study reported by Puglisi et al.[13]

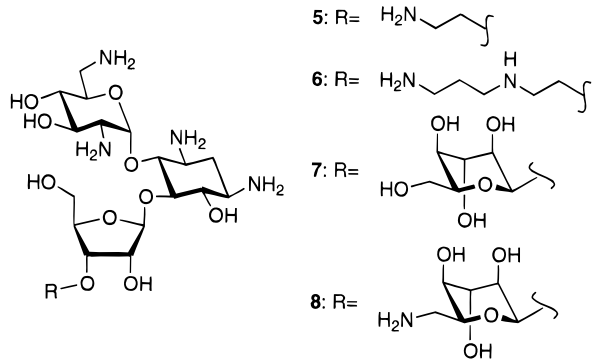

Figure 4. Target molecules to study the role of ring IV of neomycin B.

plasmon resonance based assay[14] and compared RNA binding affinities for AS-wt and AS-U1495A. To address the contribution of ring IV, we synthesized a series of neomycin B derivatives modified in the idose ring. To examine whether the amino groups of ring IV need to be displayed on a rigid platform, the L-idose ring was replaced with an acyclic side chain presenting either one (**5**) or two (**6**) amines (Figure 4). To dissect the role of specific charges while maintaining the native idose ring, either both (**7**) or one (**8**) of the amines was replaced with a hydroxyl group.

We reasoned that these modifications would provide a test for probing the ionic contribution, since hydrogen bond donor abilities, electronegativity, and steric demand are similar for both functional groups. In order to learn how well the model system correlates with *in vivo* activity, we checked the new compounds for antibacterial activity against three bacterial strains using the disk method (Kirby−Bauer technique).[15a] The minimum inhibitory concentrations (MICs) of the compounds were then determined using the broth dilution technique.[15b]

Results

Our retrosynthetic analysis (Scheme 1) led us to fragments **10** and **11** as suitable precursors for the key pseudotrisaccharide unit **9** from which all desired compounds can be constructed.[16]

(10) Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.

(11) (a) Wang, H.; Tor, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1951. (b) Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734.

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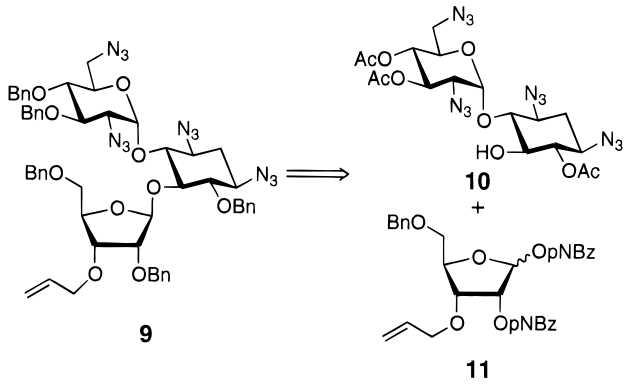
(13) Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367.

(14) Hendrix, M.; Priestley, E. S.; Joyce, G. F.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 3641.

(15) For antibiotic testing protocols, see: (a) Disk testing protocols: Phillips, I.; Williams, D. In *Laboratory Methods in Antimicrobial Chemotherapy*; Garrod, L., Ed.; Churchill Livingstone Press: Edinburgh, 1978; pp 3−30. (b) MIC determination: Waterworth, P. M. In *Laboratory Methods in Antimicrobial Chemotherapy*; Garrod, L., Ed.; Churchill Livingstone Press: Edinburgh, 1978; pp 31−40, Barry, A. L. *The Antimicrobic Susceptibility Test: Principles and Practice*; Lea and Febiger: Philadelphia, 1976. (c) Standards: Lorian, V. *Antibiotics in Laboratory Medicine*, 2nd ed.; Williams and Wilkins: Baltimore, 1986.

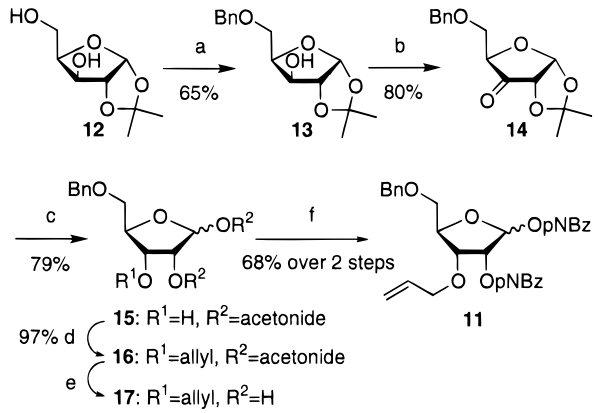
Scheme 1. Retrosynthetic Analysis



Compound **10** can be prepared from **1** which in turn is available by acidic hydrolysis of neomycin B (**3**).[17]

The choice of a suitable nitrogen protecting group was crucial to our effort. Past synthetic work on the aminoglycosides has relied on the use of alkyl carbamates[18] (including benzyloxycarbonyl groups, cyclic carbamates,[16,19] and *tert*-butyloxycarbonyl groups[11a,20]) and trifluoroacetamides[21] for the protection of the various primary amines. In our experience, the presence of multiple Cbz groups makes NMR spectra of the intermediates difficult to interpret, presumably due to the slow interconversion of rotamers. The stability of ethyl and cyclic carbamates can cause severe problems during deprotection, and the solubility characteristics of polycarbamoylated aminoglycosides are not always compatible with the requirements for glycosidation. The lability associated with the trifluoroacetamide and Boc groups made strategies utilizing these groups unattractive. Finally, none of the acyl-type protecting groups address the issue of protecting the acidic NH that is formed upon acylation of a primary amine. These problems can be overcome by the use of azides as nitrogen protecting groups. To this end, we have introduced a metal-catalyzed version[22] of the original diazo transfer protocol[23] which allows the convenient conversion of amines to azides with retention of stereochemistry. Using this protocol, neamine (**1**) was converted into tetraazidoneamine, which was regioselectively acetylated to afford **10**.[22]

Scheme 2[a]



[a] Conditions: (a) (i) Bu_2SnO, toluene, azeotropic H_2O removal, (ii) BnBr, TBAI, 110 °C; (b) Swern oxidation; (c) $NaBH_4$, MeOH; (d) NaH, allyl bromide, DMF; (e) DMF, 1 N HCl, Δ; (f) *p*NBzCl, pyridine.

The next building block, ribose donor **11**, was constructed in a seven-step sequence starting with the 1,2-*O*-isopropylidenexylose (**12**) following a route analogous to that reported by Umezawa (Scheme 2). Stannyl ester activation and subsequent benzylation provided **13**. A two-step oxidation/reduction sequence[24] served to invert the stereochemistry at the 3 position and provide **15**. Alkylation with allyl bromide served to install the allyl group to afford **16**. Finally, the acetonide was removed to afford **17**.

The *p*-nitrobenzoyl ester was installed in the anomeric position to give **11** rather than the acetate which was employed by Umezawa and co-workers.[16] The reason for this was that the attempted condensation of acceptor **10** with the anomeric acetate led to poor conversions, presumably due to the reversible nature of this glycosidation. However, the *p*-nitrobenzoyl group solved this problem since *p*-nitrobenzoic acid precipitates from the reaction mixture and thus shifts the equilibrium in favor of the condensation (Scheme 3).

Reaction of **10** and **11** provided the desired β-linked pseudotrisaccharide **18** in 63% yield along with an additional 18% of the α anomer which could be equilibrated to the desired product by resubjecting it to the glycosidation conditions. The anomeric configuration of **18** was assigned on the basis of the observed coupling constants of the H1″ proton.[25] The protecting groups were subsequently normalized to benzyl ethers to obtain the key pseudotrisaccharide **9**. The allyl group was then used in a dual role. For access to the alkyl amino derivatives (Scheme 4), the allyl group of **9** could be converted to an aldehyde to set up the introduction of nitrogen by reductive amination *(vide infra)*. In order to construct glycosylated derivatives, a mild two-step deallylation[26] of **9** yielded a suitable acceptor for subsequent glycosidation reactions (**20**).

For the preparation of **5** and **6**, compound **9** was cleaved to the key aldehyde **21** by ozonolysis. Compound **21**, in turn, was reductively aminated with the mono-Cbz adduct of 1,3-diaminopropane to give **22**. By contrast, use of unprotected 1,3-diaminopropane resulted in the formation of an aminal, which was not reduced. The deprotection proceeded *via* a two-step sequence to yield the desired **6**. First, the azides were reduced *via* Staudinger reaction, and the six remaining protecting

(16) For the total synthesis of neomycin B, see: (a) Usui, T.; Umezawa, S. *J. Antibiot.* **1987**, 1464. (b) Usui, T.; Umezawa, S. *Carbohydr. Res.* **1987**, 133.

(17) Ford, J. H.; Bergy, M. E.; Brooks, A. A.; Garrett, E. R; Alberti, J.; Dryer, J. R.; Carter, H. E. *J. Am. Chem. Soc.* **1955**, *77*, 5311.

(18) (a) Suami, T.; Nishiama, S.; Ihikawa, Y.; Katsura, S. *Carbohydr. Res.* **1976**, *52*, 187. (b) Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. *Carbohydr. Res.* **1977**, *53*, 239. (c) Suami, T.; Nishiyama, S.; Ishikawa, Y.; Katsura, S. *Carbohydr. Res.* **1977**, *56*, 415. (d) Canas-Rodriguez, A.; Ruiz-Proveda, S. G. *Carbohydr. Res.* **1977**, *58*, 379. (e) Suami; T.; Nishiyama, S; Ihikawa, Y.; Katsura, S. *Carbohydr. Res.* **1978**, *65*, 57. (f) Pfeiffer; F. R.; Schmidt, S. J.; Kinzig; C. M.; Hoover, J. R. E.; Weisbach, J. A. *Carbohydr. Res.* **1979**, *72*, 119.

(19) (a) Umezawa, S.; Koto, S.; Tatsuta, K.; Hineno, H.; Nishimura, Y.; Tsumura, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 537. (b) Umezawa, S.; Takagi, Y.; Tsuchia, T. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1411. (d) Kumar, V.; Remers, W. A. *J. Org. Chem.* **1978**, *43*, 3327. (d) Kumar, V.; Jones, G. S., Jr.; Blacksberg, I.; Remers, W. A.; Misiek, M.; Pursiano, T. A. *J. Med. Chem.* **1980**, *23*, 42. (e) Sharma, M. N.; Kumar, V.; Remers, W. A. *J. Antibiot.* **1982**, *35*, 905.

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(22) Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029.

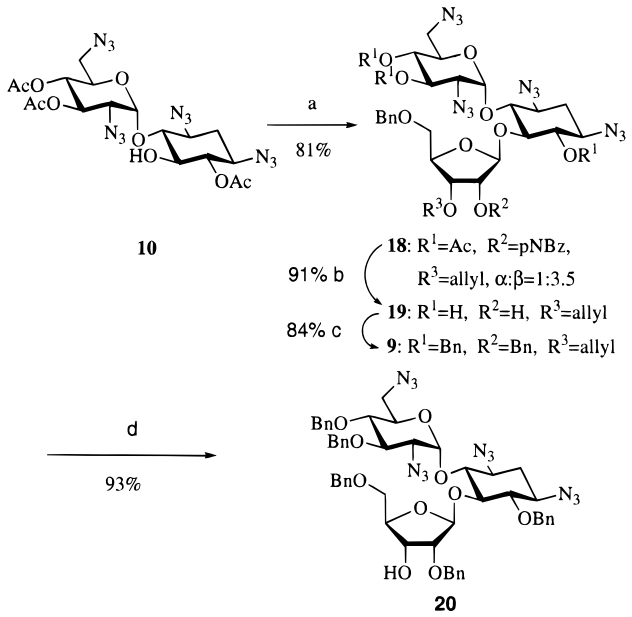
(23) Vasella, A.; Witzig, C.; Chiara, J.-L.; Martin-Lomas, M. *Helv. Chim. Acta* **1991**, *74*, 2073.

(24) (a) Yanagisawa, H.; Kinoshita, M.; Nadaka, S.; Umezawa, S. *Bull. Chem. Soc. Jpn.* **1970**, *43*, 246. (b) Wanatabe, I.; Tsuchia, T.; Takase, T.; Umezawa, S.; Umezawa, H. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 2369.

(25) The desired β anomer featured no coupling to H2″ whereas the α anomer had a coupling constant of 4 Hz.

(26) Lamberth, C.; Bednarski, M. D. *Tetrahedron Lett.* **1991**, *32*, 7369.

Scheme 3[a]



[a] Conditions: (a) **11**, BF_3OEt_2, CH_2Cl_2; (b) LiOH, H_2O, MeOH; (c) BnBr, NaH, DMF; (d) (i) bis(methyldiphenylphosphine)(COD)Ir^IPF_6 activated by hydrogen, THF, (ii) OsO_4, $Me_4NO{\cdot}2H_2O$, CH_2Cl_2.

groups were reductively cleaved using sodium in ammonia. The direct treatment of azido-protected aminoglycosides with Na/NH_3 led to mixtures of products containing deaminated derivatives which presumably arose from the homolysis of the C−N bond following the one-electron reduction of an azide.

Synthesis of **5** proved to be somewhat more challenging. Attempts to use benzylamine as the nitrogen source in order to avoid extra steps led to an unexpected deprotection problem. During attempted dissolving metal reduction, the *N*-benzyl group was reduced to the corresponding inert Birch product. To circumvent this problem, compound **21** was converted to **23** by reductive amination with *p*-methoxybenzylamine followed by carbamoylation with ZOSu. The PMB group was then oxidatively cleaved using CAN to afford **24**, and the standard two-step deprotection protocol was used to obtain the desired analog **5**.

Attention was then focused on the idose synthesis (Scheme 5). A number of approaches to L-*ido* and L-*gulo* configured systems[27] have been described in the literature. However, none of the known methods were concise, with the possible exception of Paulsen's elegant rearrangement of peracetylated D-glucose to D-idose,[27a] but to prepare the L-derivative would require the expensive L-glucose pentaacetate.

A low-temperature hydroboration of **25**[28] with excess borane followed by oxidation of the carbon boron bond yielded a mixture of **26** and **27** which could be separated by chromatography, but separation was achieved much more readily after closure to the anhydrosugar **28**.[29] Compound **28** was remarkably stable, but proved to be labile to a sulfur nucleophile under TMSOTf promotion. The equilibrium was shifted toward the otherwise disfavored open form due to the strength of the resulting O−Si bond. Removal of the TMS group led to a 1:18 mixture of the anomers **29** and **30**. The C6 oxygenated donor was constructed by allylation of **30** at C6 to afford **31**.[30] Attempts to introduce nitrogen at C6 of **30** by activating the 6 position as the mesylate followed by displacement with azide proved to be fruitless due to intramolecular participation of the anomeric methyl sulfide which was followed by attack at the anomeric center to yield a product with sulfur at the 6 position. However, introduction of an amino substituent at C6 was eventually achieved through a chemoselective Swern oxidation followed by reductive amination with allylamine to afford **32**. Subsequent deallylation[31] and Cbz protection led to the desired donor **33**.

The tetrasaccharide core was assembled by glycosidation of **20** with the glycosyl donors **31** and **33** to yield the protected pseudotetrasaccharides **34** and **36**, respectively (Scheme 6). Both reactions proceeded with complete selectivity for the desired β anomer, presumably due to the triaxial conformation of the donor in solution (evident by the small coupling constants of the ring protons). This conformation would lead to a severe (1,3) diaxial interaction if the product was formed in the α configuration. Compound **34** was deallylated[26] to afford **35** and then subjected to our standard two-step deprotection protocol to afford 2‴,6‴-didesamino-2‴,6‴-dihydroxyneomycin B (**7**). Analogous deprotection of **36** yielded 2‴-desamino-2‴-hydroxyneomycin B (**8**).

The RNA binding properties of **1**−**8** were analyzed using a surface plasmon resonance based assay that was recently developed in these laboratories.[14] This assay allows the determination of both affinity and specificity of small molecule−RNA interactions. For the analysis, the compounds were injected over a matrix containing RNA that was immobilized through a biotin tag, and the equilibrium binding values were recorded at various concentrations. A sample of binding curves is shown in Figure 5.

Nonlinear curve fitting was then used to determine the values of the dissociation constants (K_D). The specificity of binding, *i.e.*, the ability to discriminate between different RNA sequences, was evaluated by comparing the binding to the target sequence (AS-wt) and the negative control (AS-U1495A). The ratio of K_D(AS-U1495A) to K_D(AS-wt) was taken to be a representation of the specificity of the binding event. The data are summarized in Table 1.

The compounds were assayed for biological activity against three bacterial reference strains, *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923), using the Kirby−Bauer disk method,[15a] in which paper disks containing known amounts of antibiotic are placed on plates inoculated with bacterial cultures, and the diameters of the zones of inhibition (DZI), apparent as clear regions around the disks, are measured after overnight growth. Figure 6 shows a representative disk assay.

Table 2a gives the zone diameters measured for the three bacterial strains. The zone diameters measured for the strains with known antibiotics are well within the accepted limits.[15a] The minimum inhibitory concentrations were determined via

(27) (a) Paulsen, H. *Methods Cabohydr. Chem.* **1972**, *6*, 142−148. (b) Horton, D.; Tsai, J.-H. *Methods Carbohydr. Chem.* **1980**, *8*, 177. (c) Andrews, G. C.; Crawford, T. C.; Bacon, B. E. *J. Org. Chem.* **1981**, *46*, 2976. (d) Medakovic, D. *Carbohydr. Res.* **1994**, *253*, 299.

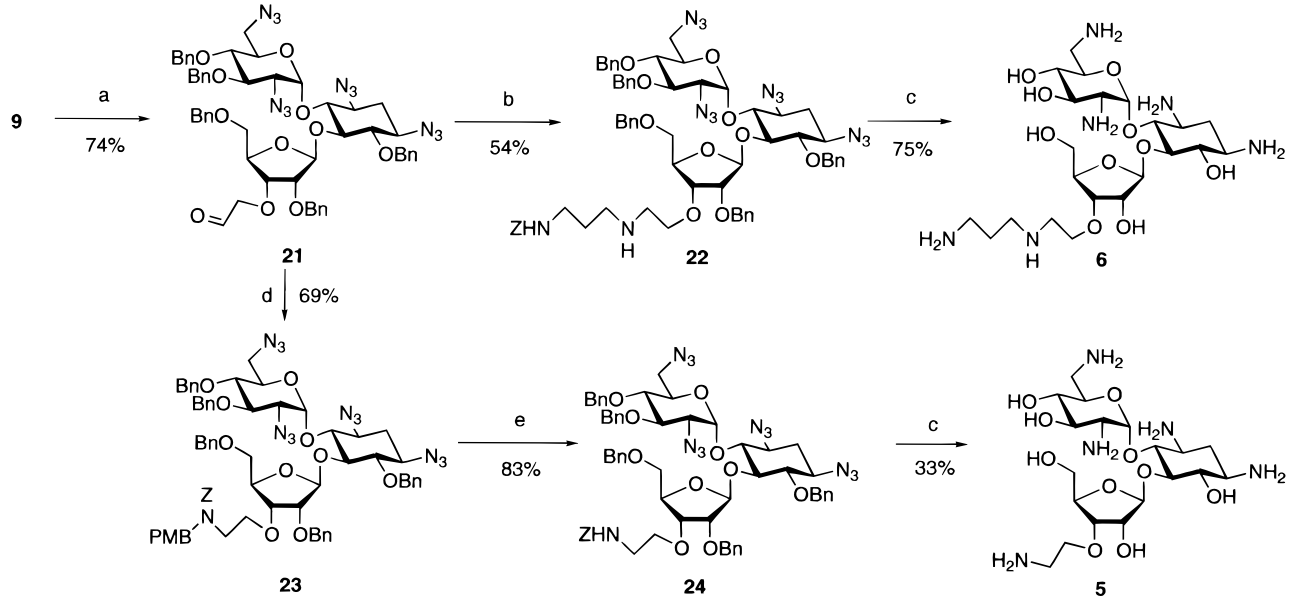
(28) Semeria, D.; Philippe, M.; Delaumeny, J.-M.; Sepulchre, A.-M.; Gero, S. D. *Synthesis* **1983**, 710.

(29) It should be noted that attempts to introduce nitrogen at the 6 position in **27** yielded discouraging results. Introduction of azide *via* activation as the mesylate and displacement was sluggish and low yielding. Subsequent attempts to generate the thioglycoside with TMSOTf and MeSTMS led to ring opening, and attempted hydrolysis by treatment with aqueous H_2SO_4 led to the decomposition of the azide prior to liberation of the anomeric center.

(30) Use of benzyl bromide instead of allyl bromide as an electrophile led to decomposition under conditions necessary for reaction while Lewis acid promoted benzylation with benzyl 2,2,2-trichloroacetimidate led to reclosure to **28**.

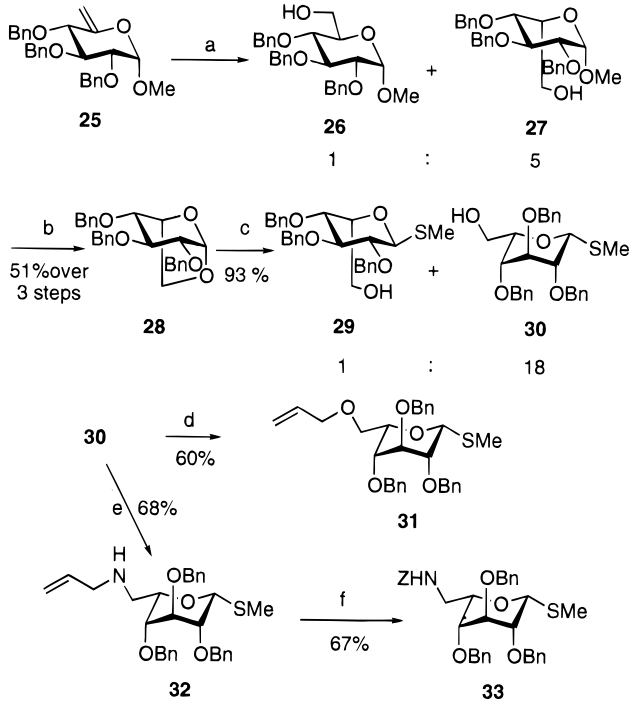
(31) Laguzza, B.; Ganem, B. *Tetrahedron Lett.* **1981**, 22, 1483.

Scheme 4[a]



[a] Conditions: (a) (i) O_3, CH_2Cl_2, (ii) DMS; (b) mono-Cbz-1,3-diaminopropane, AcOH, pH 6, MeOH, $NaBH_3CN$; (c) (i) PMe_3, THF, H_2O, 1 N NaOH, (ii) Na, NH_3, THF, (iii). Amberlite CG-50 cation exchange chromatography; (d) (i) PMB−NH_2, AcOH, pH 6, MeOH, $NaBH_3CN$, (ii) ZOSu, CH_2Cl_2; (e) acetonitrile−H_2O (9:1), CAN.

Scheme 5[a]



[a] Conditions: (a) (i) BH_3·THF, THF, 0 °C, (ii) HOOH, NaOH; (b) AcOH, concentrated HCl, 70 °C; (c) (i) MeSTMS, TMSOTf, CH_2Cl_2, (ii) TBAF; (d) DMF, NaH, allyl bromide; (e) allylamine, AcOH, pH 6, $NaBH_3CN$; (f) (i) Wilkinson's catalyst, acetonitrile−H_2O (84:16), distillation, (ii) Z-OSu, CH_2Cl_2.

the broth dilution technique,[15b] and the results are shown in Table 2b. The results for neomycin are within accepted ranges for sensitive *E. coli* strains.[15c] It is worth noting that some testing protocols recommend overnight growth of the cultures, rather than the minimum time required to obtain good growth of the control. MIC values were tested for several of the control antibiotics using overnight growth, and the MIC values observed were 2−4× greater than those observed for 4−6 h growth.

Discussion

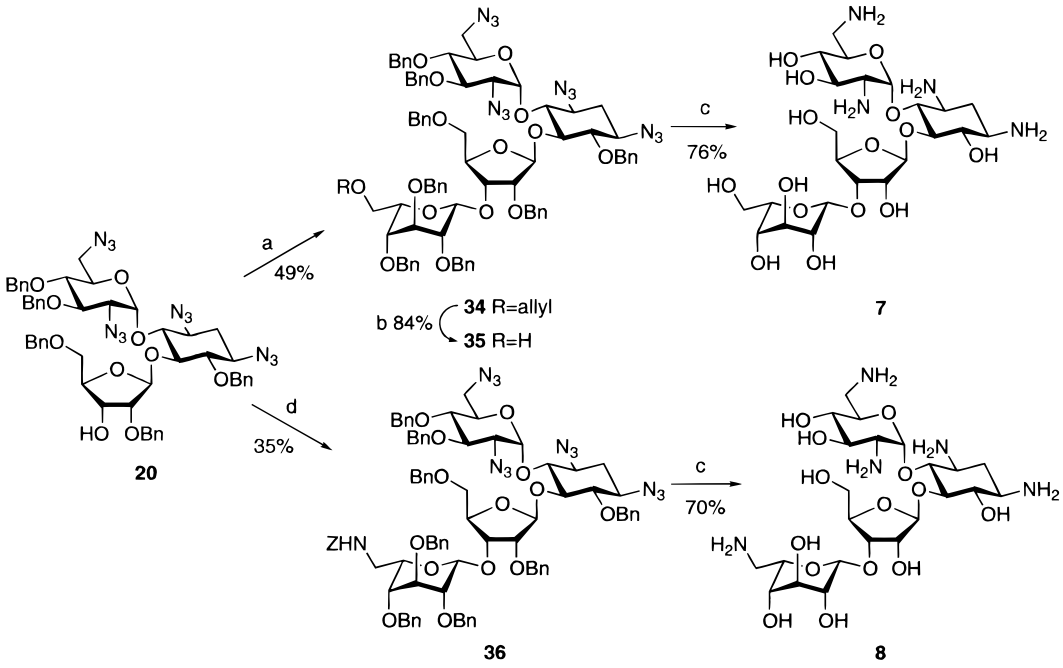
The available NMR structure of the paromomycin−AS-wt complex[13] suggested that the pseudodisaccharide portion of the molecule was mainly responsible for recognition. However, the Biacore data on neamine (**1**), ribostamycin (**2**), and neomycin B (**3**) suggested that the idose ring was responsible for a large portion of both affinity and specificity (Table 1). This observation made a good case for studying the role of this ring in the binding event.

To probe this role, the neomycin B analogs have the idose ring replaced with a flexible monoamine tail (**5**) or a diamine tail (**6**) or with idose analogs containing only hydroxy groups (**7**) or one amine group (**8**). In contrast to previous observations, all of the aminoglycosides tested here using surface plasmon resonance[14] show some degree of specificity in the recognition of AS-wt. As expected, the overall affinity correlates with the net charge of the molecule, neomycin B being the tightest binder.

The binding data for compounds **1**−**8** can be compared by starting with neamine (**1**) and observing the changes in binding that occur as functionality is added to the molecule. Neamine (**1**) binds the AS-wt sequence with a dissociation constant of 7.8 μM and approximately 4-fold specificity relative to the AS-U1495A sequence. Ribostamycin (**2**) adds a ribose ring to this core at the 5 position of 2-deoxystreptamine and has 3-fold lower affinity with specificity similar to that of neamine while retaining the same overall charge. Addition of an uncharged idose platform to ribostamycin (**2**) to generate **7** results in a molecule which has virtually the same RNA binding profile. This result implies that the idose ring by itself (without amines) does not contribute to the affinity or specificity of binding.

Addition of an amine at a defined position on the idose platform to give compound **8** improves affinity by 40-fold in relation to **7** and makes the interaction relatively more specific. Addition of a second amine to this platform to generate the parent agent neomycin B (**3**) results in further improvement of the overall affinity, without affecting specificity. When a single positive charge on a flexible ethyl tether rather than a saccharide

Scheme 6[a]



[a] Conditions: (a) **31**, NIS, AgOTf (cat), 3 Å MS, CH_2Cl_2; (b) (i) bis(methyldiphenylphosphine)(COD)Ir(I)PF_6 activated by hydrogen, THF, (ii) OsO_4, $Me_4NO{\cdot}2H_2O$, CH_2Cl_2; (c) (i) PMe_3, THF, H_2O, 1 N NaOH, (ii) Na, NH_3, THF, EtOH, (iii) Amberlite CG-50 anion exchange chromatography; (d) **33**, NIS, AgOTf (cat), 3 Å MS, CH_2Cl_2.

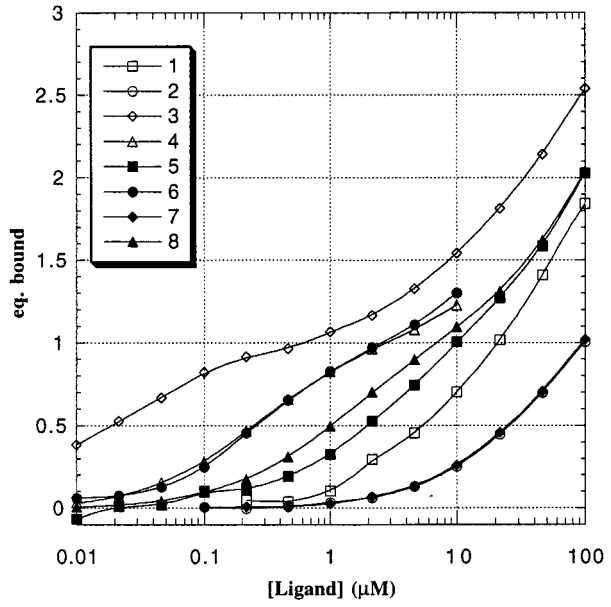


Figure 5. Titration curves for compounds **1**−**8** binding to AS-wt.

platform is attached to ribostamycin (**2**) to give **5**, the binding affinity goes up by an order of magnitude but specificity is unaffected. The addition of another positive charge on an even more flexible linker (**6**) gives diminishing returns on the affinity without affecting the specificity. These results indicate that, within the scope of this model, the rigid scaffold of the idose ring is necessary in order to preserve the specificity exhibited by neomycin B.

Paromomycin (**4**) which differs from neomycin B (**3**) only in position 6′ can best be compared to **8**, which has the same number of charges. Compared to **8**, paromomycin (**4**) shows somewhat higher affinity but lower specificity. The control antibiotic streptomycin was used to demonstrate that unrelated aminoglycoside antibiotics which are not known to bind to this

Table 1. *In Vitro* Binding Data for the Natural Antibiotics and Synthetic Analogs Used in This Study

compd	K_D(AS-wt)[a] (μM)	K_D(AS-U1495A)[a] (μM)	specificity factor[b]
1	7.8	31	4
2	25	90	4
3	0.019	0.38	20
4	0.20	2.7	14
5	1.7	10	6
6	0.26	1.6	6
7	28	123	4
8	0.70	14	19
streptomycin	95	74	1

[a] All K_D values were determined in duplicate except for K_D(AS-wt) of **4** and **8** which were determined in triplicate. The deviation from the mean was <100% in all cases. The standard deviation for K_D(AS-wt) of **4** and **8** was 29% and 49%, respectively. Solution conditions: 150 mM NaCl, 10 mM HEPES (pH 7.4), 3.4 mM EDTA. [b] K_D(AS-U1495A)/K_i(AS-wt).

sequence exhibit a much reduced binding affinity and no specificity for the native sequence over the AS-U1495A mutant.

The MIC data for these compounds in *E. coli* (Table 2b) indicate that the *in vivo* activity does not always correlate well with the *in vitro* binding data. Compounds **5** and **6** both have significantly lower binding affinities toward the AS-wt RNA than neomycin and show considerably higher nonspecific binding to the AS-U1495A species (Table 1), yet have very nearly the same antimicrobial activity as neomycin B (**3**) itself. Neamine, which appears to bind better to AS-wt than ribostamycin, shows inferior antimicrobial activity. These discrepancies may reflect different uptake dynamics of the different compounds, or perhaps a slightly different conformation of the ribosome *in vivo*. Figure 7 shows the published[13] representative structure of paromomycin interacting with AS-wt on the left and a possible structure of **6** interacting with the same model sequence.

The presumption that the binding mode is similar for the two molecules can be justified by the near identity of rings I, II,

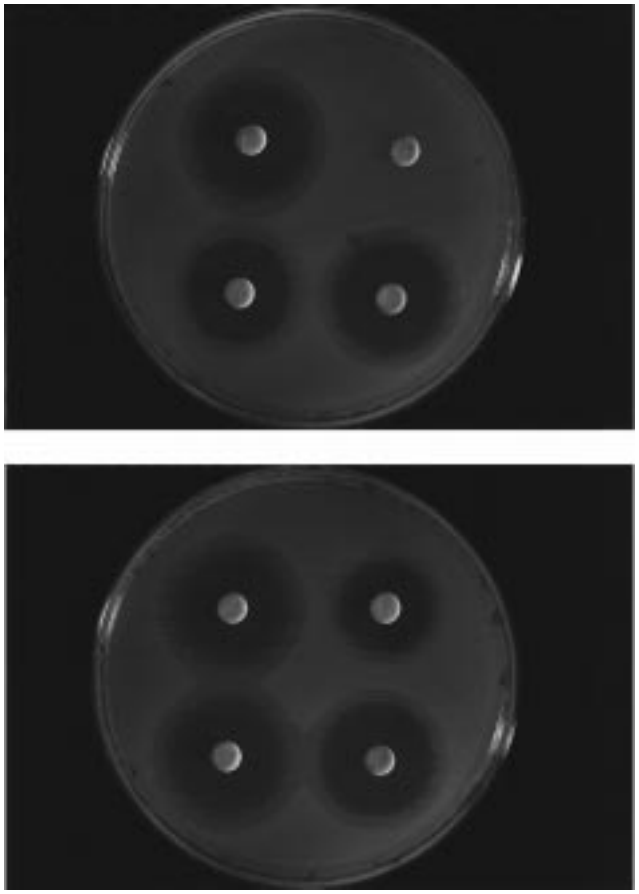


Figure 6. Representative Kirby−Bauer disk assay. (A, top) Clockwise, from top left: neomycin (33 nmol); negative control; compound **6** (33 nmol); neamine (200 nmol). (B, bottom) Clockwise from top left: compound **8** (33 nmol); compound **7** (33 nmol); compound **6** (33 nmol); compound **5** (33 nmol).

and III and the comparison between neomycin B and the synthetic analogs regarding their ^{13}C-NMR shifts and coupling constants (Table 3). The 5′-phosphate of A1493 makes a fairly long range contact with the 6′-OH of paromomycin, but since neomycin-like structures feature a 1,3-hydroxyamine motif[9] between the 4 and 6 positions, this interaction may well be closer for this class of molecules. The structure shows that the diamine tail is in an area rich in potential phosphate contacts, with the 5′-phosphates of U^{1406}, C^{1407}, A^{1408}, G^{1488}, and G^{1489} all being candidates. This may mean that the interaction of a simple doubly positively charged appendage with a highly electronegative major groove of RNA is enough to orient rings I, II, and III into their binding pocket.

The antimicrobial activity of compounds **5** and **6** holds promise for the design of novel antibiotics. It is apparent that gross changes are tolerated in the structure of aminoglycoside antibiotics without significant effect on biological activity. This observation should allow design of structurally simpler molecules which could possibly address issues of drug resistance.

Experimental Section

Unless otherwise stated, all reactions were performed under an Ar atmosphere with the assistance of magnetic stirring. THF and CH_2Cl_2 were distilled under Ar prior to use from benzophenone ketyl and CaH_2, respectively. All other solvents and reagents were purchased anhydrous and used as received. NMR spectra were recorded using either a Bruker AMX-500 or a Bruker DRX-600 instrument. Synthesis of the biotinylated RNA and surface plasmon resonance detected binding

Table 2. Antibacterial Activities of Aminoglycosides

A. Diameters of Zones of Inhibition (DZI), mm[a]

antibiotic	amount (nmol)	*E. coli*	*S. aureus*	*Ps. aeruginosa*
1	200	18.5	18.5	NI
2	33	16.5	14.5	NI
3	33	20.5	21.5	9.5
4	33	18	19.5	NI
5	33	18.5	18.5	NI
6	33	19	21	NI
7	33	16.5	11.5	NI
8	33	19	19.5	NI

B. Minimum Inhibitory Concentrations (MICs) against *E. coli* ATCC 25922[b]

antibiotic	MIC (μM)	MIC (μg/mL)
1	50	26
2	12.5	8
3	1.6	1.5
4	6.25	5.5
5	3.1	2.3
6	1.6	1.4
7	12.5	10
8	3.1	2.6

[a] The zones of inhibition as determined by the Kirby−Bauer disk method are given. In the case of neomycin, 30 μg (33 nmol) of neomycin sulfate was used per disk. For all other compounds, the molar amount was kept constant at 33 nmol, except for the neamine standard, which was increased 6-fold due to its low activity. [b] The minimum inhibitory concentrations are given in both μM and μg/mL. (Weight/mL was calculated on the basis of the predicted molecular weights of the compounds' sulfate salts.) NI = no inhibition.

experiments were performed as described previously.[14] Solution conditions: 150 mM NaCl, 10 mM HEPES (pH 7.4), 3.4 mM EDTA.

***K*$_D$ Determination from the Binding Curves.** K_D values were determined by fitting to the equation

$$\text{equiv} = a\left(\frac{c}{K_D(1)+c} + \frac{c}{K_D(2)+c} + \frac{c}{K_D(3)+c} + ...\right) + b$$

wherein c = concentration, a = adjustment factor to adjust the value of response units considered to be 1 equivalent, b = correction to adjust the baseline to 0, and $K_D(1)$, $K_D(2)$, ... = stepwise dissociation constants.

The fitting routine of the program Kaleidagraph was used for all calculations. The starting values for a and b were set to 1 and 0, respectively. The number of K_D values used in the fitting was adjusted depending on the observed range of equivalents bound but generally varied from 3 to 4.

Antimicrobial Testing. Kirby−Bauer Disk Test. These tests were performed exactly as described.[15a] Reference strains *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *Ps. aeruginosa* ATCC 27853 were obtained as packs of lyophilized pellets (Difco), which were freshly reconstituted every few days. To make the antibiotic disks, paper disks (6 mm diameter, BBL Microbiology Systems) were wetted through with 20 μL of solution containing an appropriate amount (usually 33 nmol) of antibiotic. The wet disks were placed in a desiccator overnight, and used the next day.

Minimal Inhibitory Concentration (MIC) Testing. *E. coli* ATCC 25922 was grown in Mueller−Hinton broth (cation-adjusted, BBL Microbiology Systems) to an optical density of approximately 0.5 (absorbance read at 600 nm), and then diluted to an OD_{600} of 0.1. Samples of antibiotic were prepared in Mueller−Hinton broth, typically a series of 2-fold dilutions from 0.1 mM to <1 μM. A 50 mL sample of the diluted culture was added to 1 mL of each of the antibiotic samples, and the cultures were allowed to grow at 37 °C for 4−6 h, at which point the negative control sample (no antibiotic) typically had an absorbance of 1.2−1.5. The absorbance of each sample was read (λ = 600 nm), and MIC was considered to be the lowest antibiotic concentration at which the absorbance was less than 1% of the control.

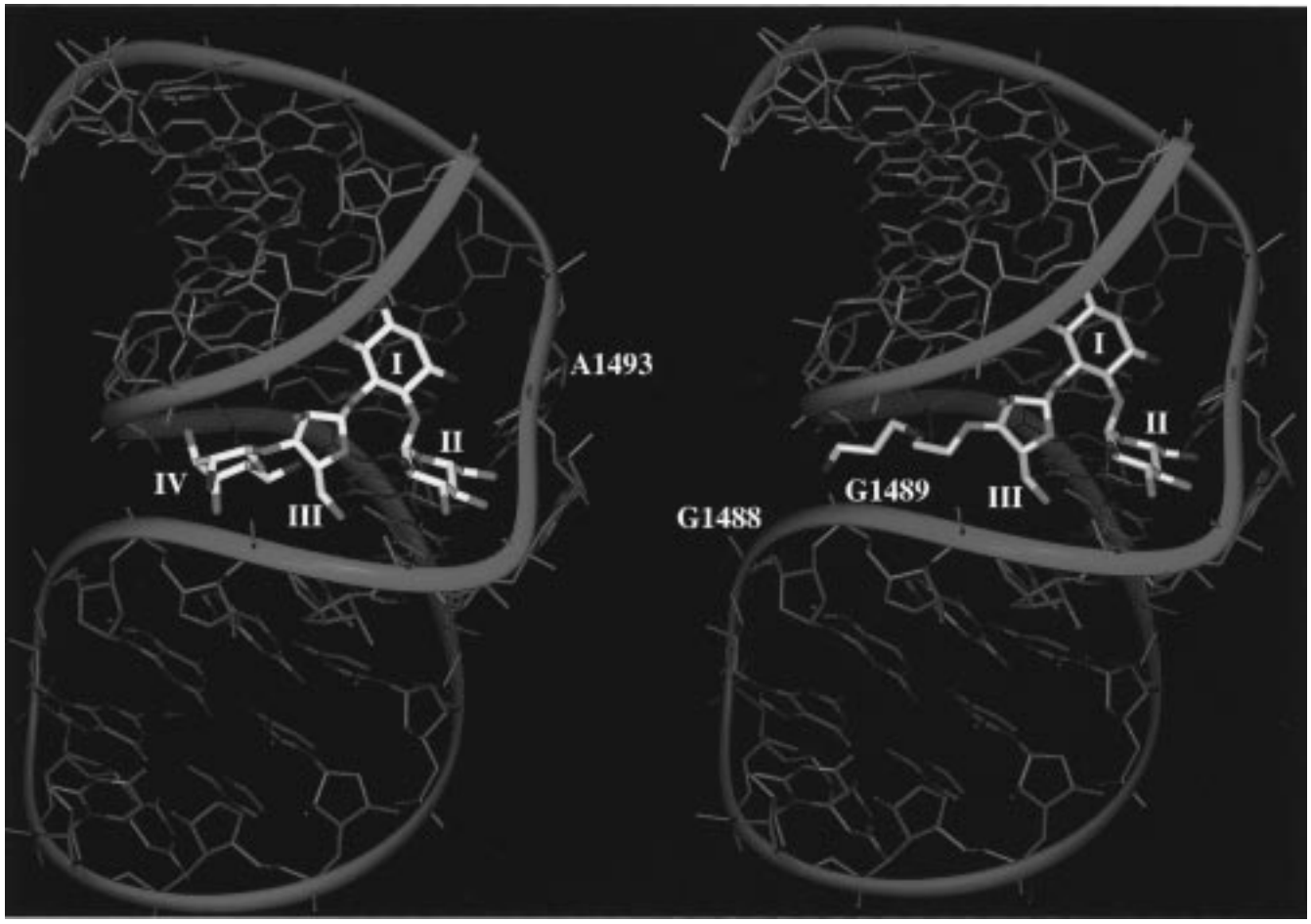


Figure 7. A model of paromomycin (**4**) bound to AS-wt created on the basis of the coordinates from Puglisi[13] (left) and possible representation of **6** bound to AS-wt (right). The *trans*-1,3-hydroxyamine motif of ring II points toward the phosphate group of A1493. The amine tail also interacts with phosphate groups.

5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (13). 1,2-*O*-Isopropylidene-α-D-xylofuranose (**12**) (4.2 g, 22.08 mmol) was dissolved in toluene (120 mL) and treated with Bu_2SnO (5.76 g, 23.19 mmol). The reaction was then refluxed overnight with azeotropic removal of water. The Dean−Stark trap was then removed and replaced with a standard reflux condenser. The reaction was treated with BnBr (5.66 g, 33.12 mmol) and kept at 110 °C for 7 h. Upon addition of EtOAc and water, a solid formed which was filtered. The organic phase was washed with saturated sodium bicarbonate solution and brine and dried over Na_2SO_4. Chromatography of the resulting oil using a gradient of 25% to 30% to 35% EtOAc in hexane afforded 4.01 g (65%) of the title compound as an oil which solidified after standing under vacuum: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.31 (s, 3H, acetonide methyl), 1.48 (s, 3H, acetonide methyl), 3.68 (s, 1H, OH), 3.90 (dd, 2H, J_1 = 11 Hz, J_2 = 4 Hz, H5a), 3.93 (dd, 2H, J_1 = 11 Hz, J_2 = 4 Hz, H5b), 4.25 (dd, 1H, J_1 = 7 Hz, J_2 = 4 Hz, H4), 4.27 (m, 1H, H3), 4.50 (d, 1H, J = 4 Hz, H2), 4.60 (ABq, 2H, J = 12 Hz, $\Delta\nu$ = 29.7 Hz, $PhCH_2O$), 5.97, (d, 1H, J = 4 Hz, H1), 7.25−7.4 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.1, 26.7, 68.1, 74.0, 76.3, 78.0, 85.2, 104.8, 111.5, 127.8, 128.0, 128.5, 137.0; HRMS for $C_{15}H_{20}O_5$ (M + Na) calcd 303.1208, found 303.1201.

5-*O*-Benzyl-3-oxo-1,2-*O*-isopropylidene-α-D-xylofuranose (14). Methylene chloride (100 mL) was cooled to −78 °C, and DMSO (2.79 g, 35.76 mmol) was added, followed by oxalyl chloride (2.18 g, 17.16 mmol). The reaction was allowed to stir for 20 min at this temperature and then treated with a solution of **13** (4.01 g, 14.3 mmol) in 30 mL of CH_2Cl_2. The reaction was allowed to slowly warm to −35 °C and was kept at that temperature for 15 min before the addition of triethylamine (7.24 g, 71.5 mmol). The reaction was allowed to warm to room temperature, extracted with saturated sodium bicarbonate solution and saturated NaCl solution, and dried over Na_2SO_4. Flash chromatography on 200 mL of silica gel using a gradient of 0% to 0.5% to 1% to 1.5% MeOH in $CHCl_3$ afforded 3.2 g (80.4%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.43 (s, 3H, acetonide methyl), 1.46 (s, 3H, acetonide methyl), 3.72−3.75 (m, 2H, H5a and H5b), 4.35 (dd, J_1 = 4 Hz, J_2 = 1 Hz, 1H, H2), 4.45 (m, 1H, H4), 4.51 (ABq, J = 12 Hz, $\Delta\nu$ = 15.75 Hz, $PhCH_2O$), 6.13 (d, J = 4 Hz, H1), 7.2−7.4 (m, 5H, C_6H_5); ^{13}C NMR (125 MHz) δ 27.2, 27.6, 70.0, 73.6, 76.7, 79.8, 103.5, 114.1, 127.4, 127.8, 128.4, 128.5, 137.3; HRMS for $C_{15}H_{18}O_5$ (M + Na) calcd 301.1052, found 303.1043.

5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (15). Compound **14** (3.2 g, 11.5 mmol) was dissolved in 50 mL of anhydrous methanol and treated with $NaBH_4$ (218 mg, 5.75 mmol). The reaction was allowed to stir for 1 h and then quenched with water. The solvent was removed, and the reaction was partitioned between EtOAc and saturated sodium bicarbonate solution. The organic phase was dried with brine and Na_2SO_4. Flash chromatography on 120 mL of silica gel using a gradient of 25% to 30% to 35% to 40% EtOAc in hexane afforded 2.53 g (79%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.37 (s, 3H, acetonide methyl), 1.56 (s, 3H, acetonide methyl), 2.42 (d, 1H, J = 10 Hz, OH), 3.64 (dd, 1H, J_1 = 11 Hz, J_2 = 4.5 Hz, H5a), 3.79 (dd, 1H, J_1 = 11 Hz, J_2 = 2.5 Hz, H5b), 3.92 (m, 1H, H4), 3.3.97 (m, 1H, H3), 4.56 (dd, 1H, J_1 = 4.5 Hz, J_2 = 3.5 Hz, 1H, H2), 4.60 (s, 2H, $PhCH_2O$), 5.84 (d, 1H, J = 3.5 Hz, H1), 7.27−7.37 (m, 4H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.4, 26.5, 68.5, 71.7, 73.5, 78.3, 79.7, 104.1, 112.6, 127.6, 127.7, 128.4, 137.8; HRMS for $C_{15}H_{20}O_5$ (M + Na) calcd 303.1208, found 303.1200.

3-*O*-Allyl-5-*O*-benzyl-1,2-*O*-isopropylidine-α-D-ribofuranose (16). Compound **15** (500 mg, 1.784 mmol) was dissolved in 10 mL of DMF and cooled to ice bath temperature. The reaction was treated with sodium hydride (47 mg, 1.963 mmol) followed by allyl bromide (647 mg, 5.352 mmol). After 20 min, another 20 mg of NaH was added.

Table 3. ^{13}C NMR Shifts and Coupling Constants of Neomycin B and the Synthetic Analogs

	C1	C2	C3	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	C6′
neo B	51.4	29.9	49.9	77.3	86.3	74.0	97.0	55.0	69.6	72.1	70.8	41.6
5	51.3	29.5	49.9	76.8	86.2	74.0	97.1	55.0	69.5	72.0	70.9	41.5
6	51.3	29.5	49.9	76.9	86.2	74.0	97.1	55.0	69.5	72.0	70.9	41.5
7	51.3	29.5	49.9	76.9	86.3	73.9	97.0	54.9	69.5	72.0	70.9	41.6
8	51.3	29.5	49.9	76.7	86.3	73.9	97.0	54.9	69.5	72.0	70.9	41.6

	J (H2ax, H1) (Hz)	*J* (H2eq, H1) (Hz)	*J* (H2ax, H3) (Hz)	*J* (H2eq, H3) (Hz)
neo B	12.6	4.1	12.6	4.1
5	12.6	4.1	12.6	4.1
6	12.6	4.1	12.6	4.1
7	12.6	4.1	12.6	4.1
8	12.6	4.1	12.6	4.1

	J (H2ax, H2eq) (Hz)	*J* (H3, H4) (Hz)	*J*(H4, H5) (Hz)	*J* (H5, H6) (Hz)
neo B	12.6	broad	broad	9.4
5	12.6	10.5	10.1	
6	12.6	10.4	9.9	
7	12.6	10.3	10.3	9.2
8	12.6	10.2	10.2	9.3

	J (H1, H6) (Hz)	*J* (H1′, H2′) (Hz)	*J* (H2′, H3′) (Hz)	*J* (H3′, H4′) (Hz)
neo B	10.4	4.0	10.8	9.2
5	10.7	4.0	10.8	9.3
6	10.6	3.9	10.9	9.5
7	10.6	4.0		9.4
8	10.4	4.0	10.9	9.3

	J (H4′, H5′) (Hz)	*J* (H5′, H6′a) (Hz)	*J* (H6′a, H6′b) (Hz)
neo B		6.7	13.6
5	9.3	6.4	13.6
6	9.5	6.4	13.2
7	9.4	6.3	13.7
8	9.3	6.4	13.7

After all starting material was consumed, the reaction was quenched with AcOH and the solvent was removed. The residue was taken up in EtOAc, washed with water, saturated sodium bicarbonate solution, and brine, and dried over Na_2SO_4. Flash chromatography on 70 mL of silica gel using a gradient of 12% to 15% to 18% to 20% EtOAc in hexane afforded 555 mg (97%) of the title compound: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.36 (s, 3H, acetonide methyl), 1.58 (s, 3H, acetonide methyl), 3.61 (dd, 1H, $J_1 = 11$ Hz, $J_2 = 4$ Hz, H5a), 3.79 (dd, 1H, $J_1 = 11$ Hz, $J_2 = 2$ Hz, H5b), 3.85 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 4.5$ Hz, H3), 4.07 (dddd, 1H, $J_1 = 12.5$ Hz, $J_2 = 6$ Hz, $J_3 = J_4 = 1.5$ Hz, 1H, H4), 4.12−4.17 (m, 2H, CH_2CHCH_2O), 4.60 (ABq, 2H, $J = 12$ Hz, $\Delta\nu = 45$ Hz, $PhCH_2O$), 4.60 (dd, 1H, $J_1 = J_2 = 4$ Hz, H2), 5.21 (ddd, 1H, $J_1 = 11.5$ Hz, $J_2 = J_3 = 1.5$ Hz, CH_2CHCH_2O), 5.28 (ddd, 1H, $J_1 = 17.5$ Hz, $J_2 = J_3 = 1.5$ Hz, CH_2CHCH_2O), 5.78 (d, 1H, $J = 4$ Hz, H1), 5.36−5.46 (m, 1H, CH_2CHCH_2O), 5.27−7.36 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 26.4, 26.7, 67.8, 71.6, 73.5, 77.3, 77.4, 77.8, 103.9, 112.8, 118.0, 127.6, 127.7, 128.3, 134.4, 138.0; HRMS for $C_{18}H_{24}O_5$ (M + Na) calcd 343.1521, found 343.1513.

1,2-*O*-(4-Nitrobenzoyl)-3-*O*-allyl-5-*O*-benzyl-α/*β*-D-ribofuranose (11). Compound **16** (757 mg, 2.36 mmol) was dissolved in 15 mL of dioxane and treated with 5 mL of 1 N HCl solution. The reaction was then warmed to 80 °C for 1.5 h and cooled back to room temperature (RT). The acid was quenched by addition of solid sodium bicarbonate, and the solvent was removed. The residue was partitioned between water and EtOAc. The water layer was further extracted twice with EtOAc, and the combined organic extracts were dried over $MgSO_4$. The solvent was removed, and the residue was treated with pyridine (15 mL), 4-nitrobenzoyl chloride (1.04 g, 5.60), and a few crystals of DMAP. The reaction was stirred overnight, and the solvent was removed. The residue was taken up in EtOAc and washed with water, saturated $CuSO_4$ solution followed by saturated ammonium chloride solution, and brine. The combined organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was chromatographed over 50 mL of silica gel using 10% to 12% to 15% EtOAc in hexane to afford 910 mg (68%) (over two steps) of the product as a chromatographically separable mixture (approximately 4:1) of anomers. Data for the β anomer: ^1H NMR ($CDCl_3$, 500 MHz) δ 3.73 (dd, 1H, $J_1 = 11$ Hz, $J_2 = 3$ Hz, H5a), 3.86 (dd, 1H, $J_1 = 11$ Hz, $J_2 = 2.5$ Hz, H5b), 4.05−4.18 (m, 2H, CH_2CHCH_2O), 4.40 (ddd, 1H, $J_1 = 8$ Hz, $J_2 = J_3 = 3$ Hz, H4), 4.53 (s, 2H, $PhCH_2O$), 4.63 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 4.5$ Hz, H3), 5.15−5.28 (m, 2H, CH_2CHCH_2O), 5.70 (d, 1H, $J = 4.5$ Hz, H2), 5.75−5.86 (m, 1H, CH_2CHCH_2O), 6.56 (s, 1H, H1), 7.20−7.30 (m, 5H, C_6H_5), 8.00−8.35 (m, 8H, $C_6H_4NO_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 68.5, 72.3, 73.5, 75.0, 75.8, 82.1, 99.4, 118.1, 123.5, 123.7, 127.6, 127.8, 128.4, 130.9, 131.0, 133.6, 134.5, 137.7, 150.6, 150.8, 163.0 163.5; HRMS for $C_{29}H_{26}N_2O_{11}$ (M + Na) calcd 601.1434; found 601.1447. Data for the α anomer: ^1H NMR ($CDCl_3$, 500 MHz) δ 3.70 (dd, 2H, $J_1 = 3.5$ Hz, $J_2 = 3$ Hz, 2H, H5a,b), 4.05−4.10 (m, 2H, CH_2CHCH_2O), 3.70 (dd, $J_1 = 6.5$ Hz, $J_2 = 3$ Hz, 1H, H3), 4.55−4.60 (m, 3H, H4 and $PhCH_2O$), 5.22−5.37 (m, 2H, CH_2CHCH_2O), 5.47 (dd, $J_1 = 6$ Hz, $J_2 = 4$ Hz, 1H, H2), 5.77−5.86 (m, 1H, CH_2CHCH_2O), 6.81 (d, $J = 4$ Hz), 7.35−7.42 (m, 5H, C_6H_5), 8.08−8.30 (m, 8H, $C_6H_4NO_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 69.3, 72.1, 73.3, 73.7, 75.6, 84.9, 95.9, 117.3, 123.6, 127.7, 127.9, 128.5, 130.7, 131.0, 134.0, 134.4, 135.1, 137.5, 150.7, 163.5, 163.6; MS for $C_{29}H_{26}N_2O_{11}$ (M + Na) calcd 601, found 601; for $C_{29}H_{26}N_2O_{11}$ (M + Cl^-) calcd 613, found 613.

6,3′,4′-Tri-*O*-acetyl-3″-*O*-allyl-5″-*O*-benzyl-1,3,2′,6′-tetranzidoribostamycin (18). Compound **11** (3.5 g, 6.18 mmol) and compound **10** (1.34 g, 2.43 mmol) were dissolved in 15 mL of CH_2Cl_2 and cooled in an ice bath. Then, $BF_3{\cdot}OEt_2$ (922 mg, 6.5 mmol) was added *via* syringe, and the reaction was allowed to stir for 4.5 h. By this time a large amount of precipitate had formed. The reaction was quenched by addition of triethylamine until the solution became homogeneous. Chloroform was added and the reaction was extracted with saturated $NaHCO_3$ solution and brine and dried over Na_2SO_4. Chromatography over 200 mL of silica gel using a gradient of 5% to 10% to 15% to 20% to 25% to 30% EtOAc in hexane yielded 2.02 g of the donor, 1.47 g of the β anomer (63%) and 0.43 g of the α anomer (18%). Data for the β anomer: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.61 (ddd, 1H, $J_1 = J_2 = J_3 = 13$ Hz, H2 eq), 2.05 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.37 (ddd, 1H, $J_1 = 13$ Hz, $J_2 = J_3 = 4.5$ Hz, H2 ax), 3.10 (dd, 1H, $J_1 = 11$ Hz, $J_2 = 3.5$ Hz, H2′), 3.22 (dd, 1H, $J_1 = 13.5$ Hz, $J_2 = 5.5$ Hz, H6′a), 3.32 (dd, 1H, $J_1 = 13.5$ Hz, $J_2 = 3$ Hz, H6′b), 3.42 (ddd, 1H, $J_1 = 13$ Hz, $J_2 = 10$ Hz, $J_3 = 4.5$ Hz, H1), 3.49

(ddd, 1H, J_1 = 13 Hz, J_2 = 10 Hz, J_3 = 4.5 Hz, H3), 3.58 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4.5 Hz, H5″a), 3.68 (dd, 1H, $J_1 = J_2$ = 10 Hz, H4), 3.82 (dd, 1H, J_1 = 10.5 Hz, J_2 = 2.5 Hz, H5″b), 3.85 (dd, 1H, $J_1 = J_2$ = 10 Hz, H5), 3.90 (dd, 1H, J_1 = 12.5 Hz, J_2 = 6 Hz, CH_2CHCH_2O), 4.00 (dd, 1H, J_1 = 12.5 Hz, J_2 = 5.5 Hz, 1H, CH_2CHCH_2O), 4.16-4.22 (m, 2H, H3″ and H4″), 4.38−4.42 (m, 1H, H5′), 4.58 (ABq, 2H, J = 11.5 Hz, $\Delta\nu$ = 51.2 Hz, $PhCH_2O$), 4.86 (dd, 1H, $J_1 = J_2$ = 10 Hz, H4′), 4.96 (dd, 1H, $J_1 = J_2$ = 10 Hz, H6), 5.09 (dd, 1H, J_1 = 10 Hz, J_2 = 1.5 Hz, CH_2CHCH_2O), 5.16 (dd, J_1 = 17 Hz, J_2 = 1.5 Hz, 1H, CH_2CHCH_2O), 5.29 (d, 1H, J = 3 Hz, H2″), 5.38 (dd, 1H, J_1 = 11 Hz, J_2 = 9.5 Hz, H3′), 5.40 (s, 1H, H1″), 5.63−5.74 (m, 1H, CH_2CHCH_2O), 6.07 (d, 1H, J = 3.5 Hz, H1′), 7.2−7.35 (m, 5H, C_6H_5), 8.15−8.35 (m, $C_6H_4NO_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.7, 20.9, 31.3, 50.9, 58.1, 58.9, 61.0, 69.0, 69.2, 69.4, 70.2, 73.5, 75.1, 75.9, 76.2, 76.6, 80.4, 82.6, 96.1, 107.8, 118.1, 123.7, 127.8, 127.9, 128.6, 130.9, 133.5, 134.7, 137.7, 150.8, 163.5, 169.7, 170.0; HRMS for $C_{40}H_{45}N_{13}O_{16}$ (M + Na) calcd 986.3005. found 986.3035. Data for the α anomer: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.58 (ddd, 1H, $J_1 = J_2 = J_3$ = 13 Hz, H2 eq), 2.04 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.38 (ddd, 1H, J_1 = 13 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.18 (dd, 1H, J_1 = 13.5 Hz, J_2 = 4.5 Hz, H6′a), 3.30−3.37 (m, 2H, H6′b, H2′), 3.43 (ddd, 1H, J_1 = 12 Hz, J_2 = 10 Hz, J_3 = 4.5 Hz, H3), 3.50 (ddd, 1H, J_1 = 12.5 Hz, J_2 = 10 Hz, J_3 = 4.5 Hz, H1), 3.57 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H4), 3.58 (dd, 1H, J_1 = 11 Hz, J_2 = 4 Hz, H5′a), 3.71 (dd, 1H, J_1 = 11 Hz, J_2 = 2.5 Hz, H5′b), 3.80 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H5), 3.88−4.02 (m, 2H, CH_2CHCH_2O), 4.08 (dd, J_1 = 7.5 Hz, J_2 = 5 Hz, 1 H, H3″), 4.22−4.26 (m, 1 H, H4″), 4.42−4.47 (m, 1H, H5′), 4.58 (ABq, 2H, J = 12 Hz, $\Delta\nu$ = 43.5 Hz, $PhCH_2O$), 4.92−4.99 (m, 2H, H6, H4′), 5.08−5.19 (m, 2H, CH_2CHCH_2O), 5.47−5.44 (m, 2H, H1′, H3′), 5.58 (d, 1H, J = 4 Hz, H1″), 5.67 (dd, 1H, $J_1 = J_2$ = 5 Hz, H2″), 5.67−5.76 (m, 1H, CH_2CHCH_2O), 7.28−7.42 (m, 5H, C_6H_5), 8.23−8.35 (m, 2H, $C_6H_4NO_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.57, 20.63, 21.1, 31.5, 50.5, 58.1, 58.6, 61.0, 68.7, 69.0, 69.4, 70.3, 71.5, 72.0, 73.5, 73.6, 75.8, 79.4, 80.0, 82.5, 97.4, 103.0, 118.1, 123.7, 127.8, 128.4, 130.4, 131.1, 133.6, 134.7, 137.6, 150.8, 164.2, 169.6, 169.9, 170.0; HRMS for $C_{40}H_{45}N_{13}O_{16}$ (M + Cs) calcd 1096.2162, found 1096.2119.

3″-*O*-Allyl-5″-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (19). Compound **18** (1.47 g, 1.525 mmol) was dissolved in a mixture of MeOH and dioxane, 1:1 (30 mL). The reaction was then treated with a solution of LiOH (384 mg, 9.151 mmol) in 10 mL of H_2O. The mixture was allowed to stir overnight at room temperature, and the solvent was removed. The reaction was partitioned between EtOAc and saturated $NaHCO_3$ and extracted three times with EtOAc. The combined organic phases were dried over $MgSO_4$ and purified on 100 mL of silica gel using 50% to 55% to 60% EtOAc in hexane to afford 947 mg (93%) of product as a white foam: ^{1}H NMR (CD_3OD, Bruker AMX-500) δ 1.35 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.19 (ddd, J_1 = 12.5 Hz, 1H, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.02 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H2′), 3.27 (dd, 1H, J_1 = 10 Hz, J_2 = 9 Hz, H4′), 3.34−3.45 (m, 3H, H1, H3, H6′a), 3.46−3.54 (m, 1H, H5), 3.50 (dd, 1H, J_1 = 13 Hz, J_2 = 2.5 Hz, H6′b), 3.58 (dd, 1H, J_1 = 11 Hz, J_2 = 5.5 Hz, H5″a), 3.61−3.65 (m, 2H, H4, H6), 3.72 (dd, 1H, J_1 = 11 Hz, J_2 = 3 Hz, H5″b), 3.84 (dd, 1H, J_1 = 10.5 Hz, J_2 = 9 Hz, H3′), 3.98 (dddd, 1H, J_1 = 12.5 Hz, J_2 = 6Hz, $J_3 = J_3$ = 1.5 Hz, CH_2CHCH_2O), 4.01 (dd, 1H, J_1 = 7 Hz, J_2 = 4.5 Hz, H3″), 4.06−4.15 (m, 3H, H5′, H4″, CH_2CHCH_2O), 4.31 (dd, 1H, J_1 = 4.5 Hz, J_2 = 1 Hz, H2″), 4.57 (ABq, 2H, J = 12 Hz, $\Delta\nu$ = 25.3 Hz, $PhCH_2O$), 5.15 (ddd, J_1 = 10.5 Hz, $J_2 = J_3$ = 1.5 Hz, 1H, CH_2CHCH_2O), 5.27 (ddd, 1H, J_1 = 17 Hz, $J_2 = J_3$ = 1.5 Hz, CH_2CHCH_2O), 5.33 (d, 1H, J = 1 Hz, H1″), 5.86−5.94 (m, 1H, CH_2CHCH_2O), 5.91 (d, 1H, J = 4 Hz, H1′), 7.25−7.40 (m, 5H, C_6H_5); ^{13}C NMR (CD_3OD, 125 MHz) δ 33.1, 52.6, 61.3, 61.8, 64.8, 71.6, 72.3, 72.4, 72.6, 73.1, 74.3, 74.5, 77.2, 77.4, 79.1, 81.4, 85.4, 97.9, 110.6, 117.8, 128.7, 129.0, 129.4, 135.9, 139.4; HRMS for $C_{27}H_{36}N_{12}O_{10}$ (M + Cs) calcd 821.1732, found 821.1726.

3″-*O*-Allyl-6,3′,4′,3″,5″-penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (9). Compound **19** (974 mg, 1.414 mmol) was dissolved in 20 mL of DMF and treated with 8 mL of BnBr. The solution was cooled using an ice bath and treated with sodium hydride (204 mg, 8.484 mmol) in one portion. The cooling bath was then removed and the reaction was stirred for 1 h. AcOH was added to quench the NaH, and the solvent was removed. The reaction was picked up in EtOAc and washed with water twice. The organic phases were combined and dried over $MgSO_4$ and purified on 100 mL of silica gel using 10% to 12.5% to 15% EA/H to afford 1.24 g, 84% of product: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.43 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.26 (ddd, 1H, J_1 = 12.5 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.20−3.27 (m, 2H, H5, H2′), 3.30 (dd, 1H, J_1 = 13.5 Hz, J_2 = 5 Hz, H6′a), 3.35−3.45 (m, 3H, H1, H3, H4′), 3.30 (dd, 1H, J_1 = 13.5 Hz, J_2 = 2.5 Hz, H6′b), 3.58 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4.5 Hz, H5″a), 3.60−3.72 (m, 3H, H4, H6, H5″b), 3.72−3.82 (m, 2H, CH_2CHCH_2O), 3.84 (dd, 1H, $J_1 = J_2$ = 5.5 Hz, H3″), 3.92 (dd, 1H, J_1 = 5 Hz, J_2 = 3.5 Hz, H2″), 3.98 (dd, 1H, J_1 = 10 Hz, J_2 = 9 Hz, H3′), 4.15−4.22 (m, 2H, H4″, H5′), 4.42−4.90 (m, 10 H, $PhCH_2O$), 5.12 (ddd, J_1 = 10.5 Hz, $J_2 = J_3$ = 1.5 Hz, 1H, CH_2CHCH_2O), 5.12 (ddd, J_1 = 17 Hz, $J_2 = J_3$ = 1.5 Hz, 1H, CH_2CHCH_2O), 5.12 (d, 1H, J = 3Hz, H1″), 5.75−5.84 (m, 1H, CH_2CHCH_2O), 5.96 (d, 1H, J = 3.5Hz, H1′), 7.2−7.4 (m, 25 H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.2, 51.1, 59.6, 60.4, 63.5, 70.2, 70.9, 71.0, 72.3, 73.3, 74.9, 75.1, 75.5, 76.1, 78.5, 80.1, 80.5, 80.8, 81.2, 83.3, 96.0, 107.3, 116.8, 127.5, 127.8, 127.9, 128.1, 128.3, 128.4, 134.5, 137.4, 137.8, 138.0, 138.2; HRMS for $C_{55}H_{60}N_{12}O_{10}$ (M + Cs) calcd 1181.3610, found 1181.3641.

6,3′,4′,3″,5″-Penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (20). Bis(methyldiphenylphoshine)(1,5-cyclooctadiene)iridium(I) hexafluorophosphate (40 mg, 0.05 mmol) was suspended in THF (5 mL), and H_2 was bubbled through this suspension for 20 min. The resulting clear solution was transferred *via* syringe into a solution of compound **9** (1.24 g, 1.18 mmol) in THF (15 mL). After 1 h, a quantitative conversion to a slightly less polar material was observed by TLC (25% EtOAc in hexane). The solvent was removed, and the residue was corotary evaporated with CH_2Cl_2 several times. The reaction was then taken up in CH_2Cl_2 (30 mL) and treated with trimethylamine *N*-oxide dihydrate (197 mg, 1.77 mmol), and a solution of OsO_4 in tBuOH (enough solution to deliver 3 mg of OsO_4, 0.012 mmol). After the reaction was complete (overnight) the solvent was removed and the residue was purified over 100 mL of silica gel using 20% to 25% to 30% EtOAc in hexane to obtain 1.11 g (93.3%) of the title compound as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.45 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.28 (ddd, J_1 = 12.5 Hz, 1H, $J_2 = J_3$ = 4.5 Hz, H2 ax), 2.35 (d, 1H, J = 4 Hz, OH), 3.21 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H2′), 3.25 (dd, 1H, $J_1 = J_2$ = 9 Hz, H5), 3.21 (dd, 1H, J_1 = 13 Hz, J_2 = 5 Hz, H6′a), 3.35−3.44 (m, 3H, H1, H3, H4′), 3.47 (dd, 1H, J_1 = 13 Hz, J_2 = 2.5 Hz, H6′b), 3.57 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H5″a), 3.61 (dd, 1H, $J_1 = J_2$ = 9 Hz, H4 or H6), 3.65 (dd, 1H, $J_1 = J_2$ = 9 Hz, H4 or H6), 3.72 (dd, 1H, J_1 = 10.5 Hz, J_2 = 3 Hz, H5′b), 3.92 (dd, 1H, J_1 = 4 Hz, J_2 = 3 Hz, H2″), 3.97 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H3′), 4.00−4.06 (m, 2H, H3″, H4″), 4.15−4.20 (m, 1H, H5′), 4.39 (ABq 2H, J = 11.5, $\Delta\nu$ = 23.6 Hz, $PhCH_2O$), 4.52 (d, 1H, J = 12.5 Hz, $PhCH_2O$), 4.60 (dd, 2H, $J_1 = J_2$ = 11 Hz, $PhCH_2O$), 4.76 (d, 1H, J = 11 Hz, $PhCH_2O$), 4.80−4.90 (m, 4H $PhCH_2O$), 5.45 (d, 1H, J = 3 Hz, H1″), 5.98 (d, 1H, J = 4 Hz, H1′), 7.13−7.40 (m, 25H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.3, 51.1, 59.6, 60.6, 63.5, 70.5, 70.6, 70.9, 72.9, 73.3, 74.9, 75.37, 75.41, 76.0, 78.5, 80.1, 81.6, 82.2, 83.0, 83.5, 127.5, 127.6, 127.8, 128.0, 128.1, 128.4, 128.5, 137.1, 137.4, 137.76, 137.78, 138.1; HRMS for $C_{52}H_{56}N_{12}O_{10}$ (M + Cs) calcd 1141.3297, found 1141.3267.

3″-*O*-(2-Oxoethyl)-6,3′,4′,3″,5″-penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (21). Compound **9** (112 mg, 107 μmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to −78 °C. Ozone was passed through the solution until the blue color persisted. Then DMS (66 μL, 1.07 mmol) was added to the reaction, and the mixture was stirred at ambient temperature for 2 days. The solvent was removed, and the residue was chromatographed over 50 mL of silica gel using a 25% to 30% to 35% to 40% gradient of EtOAc in hexane to afford 83 mg (74%) of the title compound as an oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.17 (ddd, 1H, J_1 = 12.5 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.19−3.25 (m, 2H, H5, H2′), 3.30 (dd, 1H, J_1 = 11 Hz, J_2 = 5 Hz, H6′a), 3.36−3.45 (m, 3H, H4′, H1, H3), 3.48 (dd, 1H, J_1 = 11 Hz, J_2 = 2 Hz, H6′b), 3.59 (dd, 1H, J_1 = 10 Hz, J_2 = 4 Hz, H5″a), 3.62−3.78 (m, 5H, H4, H6, H5″b, OCH_2CHO), 3.80 (dd, 1H, $J_1 = J_2$ = 4.5 Hz, H3″), 3.92 (dd, 1H, J_1 = 4.5 Hz, J_2 = 3.5 Hz, H2″), 3.99 (dd, 1H, J_1 = 9.5 Hz, J_2 = 9 Hz, H3′), 4.15−4.22 (m,

2H, H4″, H5′), 4.46−4.90 (m, 10H, PhCH_2O), 5.58 (d, 1H, J_1 = 3.5 Hz, H1″), 5.93 (d, 1H, J_1 = 3.5 Hz, H1′), 7.23−7.37 (m, 25H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.3, 51.1, 59.6, 60.4, 63.5, 69.9, 71.0, 72.7, 73.4, 74.9, 75.0, 75.2, 75.5, 76.0, 78.5, 78.9, 80.0, 80.7, 80.8, 81.0, 83.4, 96.0, 106.7, 127.4, 127.6, 127.7, 127.8, 127.9, 128.1, 128.4, 137.5, 137.6, 137.7, 138.0, 200.4; MS for $C_{54}H_{58}N_{12}O_{11}$ (M + Cs) calcd 1183, found 1183 (the peak was too weak for an exact match).

3″-*O*-(2-*N*-(3-*N*-Cbz-propylamino)-ethylamino-6,3′,4′,3″,5″ -penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (22). Compound **21** (50 mg, 48 μmol) was suspended in MeOH (2 mL). A solution of mono-CBZ-propylenediamine (81 mg, 389 μmol) was made up in MeOH (2 mL) and acidified with glacial acetic acid until pH 6 (pH paper). This solution was then added to the aldehyde mixture, and to this was added THF until homogeneity was achieved. The reaction was treated with an excess of solid $NaCNBH_3$, and the amination was complete in minutes. The reaction was diluted with ethyl acetate and extracted with 1 N NaOH twice. The organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was purified on 50 mL of silica gel using a gradient of 2% to 3% to 4% to 5% MeOH in $CHCL_3$ to afford 32 mg (54%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 1.45−1.53 (m, 2H, NHZCH_2CH_2CH_2NH−), 2.24 (ddd, 1H, J_1 = 12.5 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 2.50−2.58 (m, 2H, NHZCH_2CH_2CH_2NH−), 2.55−2.66 (m, 2H, N-CH_2CH_2-O), 3.09−3.22 (m, 2H, NHZCH_2CH_2CH_2-NH−), 3.18−3.31 (m, 4H, H5, H2′, N-CH_2CH_2-O), 3.30 (dd, 1H, J_1 = 13.5 Hz, J_2 = 5.5 Hz, H6′a), 3.34−3.42 (m, 2H, H1, H3), 3.41 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H4′), 3.48 (dd, 1H, J_1 = 13.5 Hz, J_2 = 2.5 Hz, H6′b), 3.56 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H5″a), 3.59−3.69 (m, 3H, H4, H6, H5″b), 3.78 (dd, 1H, $J_1 = J_2$ = 5 Hz, H3″), 3.93 (dd, 1H, J_1 = 5 Hz, J_2 = 3.5 Hz, H2″), 3.98 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H3′), 4.12−4.21 (m, 2H, H5′, H4″), 4.42−4.55 (m, 3H, PhCH_2O), 4.56−4.63 (m, 2H, PhCH_2O), 4.73−4.90 (m, 5H, PhCH_2O), 5.05−5.10 (m, 2H, PhCH_2O), 5.45−5.50 (m, 1H, N*H*ZCH_2CH_2CH_2NH−), 5.55 (d, 1H, J = 3.5 Hz, H1″), 5.95 (d, 1H, J = 3.5 Hz, H1′), 7.23−7.37 (m, 30 H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.1, 29.7, 32.2, 39.9, 47.5, 49.1, 51.1, 59.6, 60.4, 63.5, 6.4, 69.1, 70.3, 70.9, 72.3, 73.3, 74.9, 75.0, 75.4, 76.2, 78.1, 78.5, 80.1, 80.4, 80.8, 81.1, 83.3, 96.0, 107.1, 127.5, 127.6, 127.7, 127.9, 128.1, 128.3, 128.4, 128.5, 136.8, 137.6, 137.60, 137.83, 138.1, 156.4; HRMS for $C_{65}H_{74}N_{14}O_{12}$ (M + Cs) calcd 1375.4665, found 1375.4709.

3″-*O*-(2-*N*-(3-Propylamino)ethylaminoribostamycin (6). Compound **22** (45 mg, 36 μmol) was dissolved in THF (5 mL) and treated with H_2O (500 μL) and 1 N NaOH (50 μL). A solution of PMe_3 in THF (159 μL of a 1 N solution) was added, and the reaction was allowed to stir for 10 h. The reaction mixture was then loaded onto a 50 mL column of silica gel and eluted with a gradient of 0% to 2.5% to 5% to 10% concentrated NH_3 in MeOH. The product fractions were pooled and coevaporated with THF (three times). THF (7 mL) was added via syringe to a dry three neck flask equipped with a Dewar condenser. Then ammonia (∼20 mL) was condensed into the reaction vessel. A chunk of Na (93 mg, 4 mmol) was then allowed to dissolve in the ammonia for 15 min. Then a solution of the polyamine in a mixture of EtOH and THF (500 μL each) was added in one portion and washed down with THF. The reaction was stirred until the blue color was discharged. Then an aqueous solution of ammonium formate (235 mg, 3.7 mmol) was added, and the ammonia was allowed to evaporate overnight. The remaining solvent was removed *in vacuo*, and the residue was loaded onto a column of Amberlite CG-50 cation exchange resin (0.5 cm × 7 cm) in its NH_4^+ form and eluted with a linear gradient of 0% to 7.5% NH_3 in H_2O (100 mL of each in a gradient maker). After lyophilization, neutralization, and relyophilization, 21.5 mg (75%) of **6**·6HCl salt was obtained: ^{1}H NMR (D_2O, pD 2 with Cl^- as counterinon, 500 MHz) δ 1.95 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.6 Hz, H2 eq), 2.09−2.17 (m, 2H, $NH_2CH_2CH_2CH_2CH_2NH$−), 2.53 (ddd, 1H, J_1 = 12.6 Hz, $J_2 = J_3$ = 4.1 Hz, H2 ax), 3.13 (dd, 2H, $J_1 = J_2$ = 7.9 Hz, NH_2CH$_2$CH$_2$CH_2NH−), 3.23 (dd, 2H, $J_1 = J_2$ = 8.0 Hz, NH_2CH$_2$-CH$_2$CH_2NH−), 3.33 (dd, 1H, J_1 = 13.2 Hz, J_2 = 6.4 Hz, H6′a), 3.32−3.39 (m, 2H, N-CH_2CH$_2$-O), 3.40 (ddd, 1H, J_1 = 12.6 Hz, J_2 = 10.6 Hz, J_3 = 4.1 Hz, H1), 3.44−3.52 (m, 2H, H2′, H6′b), 3.52 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H4′), 3.60 (ddd, 1H, J_1 = 12.6 Hz, J_2 = 10.4 Hz, J_3 = 4.1 Hz, H3), 3.72−3.78 (m, 2H, H6, H5″a), 3.88−4.01 (m, 5H, N-CH$_2$CH_2-O, H5″b, H5′, H5), 4.04 (dd, 1H, J_1 = 10.9 Hz, J_2 = 9.5 Hz, H3′), 4.11 (dd, 1H, J_1 = 7.2 Hz, J_2 = 4.6 Hz, H3″), 4.18 (dd, 1H, J_1 = 10.4 Hz, J_2 = 9.9 Hz, H4), 4.18−4.21 (m, 1H, H4″), 4.48 (dd, 1H, J_1 = 4.6 Hz, J_2 = 1.7 Hz, H2″), 5.45 (d, 1H, J = 1.6 Hz, H1″), 6.06 (d, 1H, J = 3.9 Hz, H1′); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 25.1 ($NH_2CH_2CH_2CH_2NH$−), 29.5 (C2), 38.0 ($NH_2CH_2CH_2CH_2NH$−), 41.5 (C6′), 46.0 ($NH_2CH_2CH_2CH_2NH$−), 48.8 (N-CH_2CH_2-O), 49.9 (C3), 51.3 (C1), 55.0 (C2′), 62.3 (C5″), 66.5 (N-CH$_2$CH_2-O), 69.5 (C3′), 70.9 (C5′), 72.0 (C4′), 74.0 (C6), 76.9 (C4), 78.4 (C3″), 82.6 (C4″), 86.2 (C5), 97.1 (C1′), 112.0 (C1″); MS: for $C_{22}H_{46}N_6O_{10}$ (M + H) calcd 555, found 555; for $C_{23}H_{45}N_5O_{14}$ (M − H) calcd 553, found 553.

3″-*O*-(2-*N*-(*p*-Methoxybenzyl)(Cbz)ethylamino)-6,3′,4′,3″,5″-penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (23). Compound **21** (76 mg, 72 μmol) was suspended in MeOH (2 mL). A solution of *p*-methoxybenzylamine (99 mg, 720 μmol) was made up in MeOH (2 mL) and acidified with glacial acetic acid until pH 6 (pH paper). This solution was then added to the aldehyde mixture, and to this was added THF until homogeneity was achieved. The reaction was treated with an excess of solid $NaCNBH_3$, and the amination was over in a matter of minutes. The reaction was diluted with ethyl acetate and extracted with 1 N NaOH twice. The organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was purified on 50 mL of silica gel using a gradient of 2% to 3% to 4% to 5% MeOH in $CHCL_3$. The resulting amine was then dissolved in CH_2Cl_2 and treated with ZOSu (22 mg, 86 μmol). The reaction mixture was then directly chromatographed on 50 mL of silica gel using a gradient of 5% to 10% to 15% ethyl acetate in hexane to afford 65 mg (69%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.43 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.25 (ddd, 1H, J_1 = 12.5 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.22 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H2′), 3.16−3.35 (m, 4H, N-CH_2CH_2-O), 3.30 (dd, 1H, J_1 = 13.5 Hz, J_2 = 5.5 Hz, H6′a), 3.30−3.44 (m, 3 H, H1, H3, H4′), 3.47 (dd, 1H, J_1 = 13.5 Hz, J_2 = 2.5 Hz, H6′b), 3.45−3.55 (m, 1H, H5″a), 3.58−3.61 (m, 3H, H4, H6, H5″b), 3.62−3.71 (m, 4H, OMe, H3″), 3.83−3.93 (m, 1H, H2″), 3.97 (dd, 1H, J_1 = 10.5 Hz, J_2 = 9 Hz, H3′), 4.03−4.15 (m, 1H, H4″), 4.15−4.21 (m, 1H, H5′), 4.32−4.52 (m, 5H, PhCH_2O), 4.59 (d, J = 12 Hz, 2H, PhCH_2O), 4.71−4.89 (m, 5H, PhCH_2O), 5.14 (s, 2H, PhCH_2O), 5.48−5.52 (m, 1H, H1″), 5.92−5.98 (m, 1H, H1′), 6.76 (dd, J_1 = 17.5 Hz, J_2 = 8 Hz, C_6H_4OMe), 7.04 (dd, J_1 = 61 Hz, J_2 = 8 Hz, 2H, C_6H_4OMe), 7.14−7.37 (m, 30 H, C_6H_5); ^{13}C NMR ($CDCl_3$, Bruker 125 MHz) 32.2, 45.6, 46.5, 50.78, 50.82, 51.1, 55.2, 59.6, 60.5, 63.5, 67.2, 68.8, 70.2, 70.3, 70.9, 72.36, 72.39, 73.3, 74.9, 75.06, 75.10, 75.4, 76.11, 76.15, 78.3, 78.5, 80.1, 80.6, 80.78, 80.84, 81.2, 81.4, 83.3, 96.0, 107.4, 107.5, 113.8, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.3, 128.4, 128.7, 129.4, 129.8, 137.5, 137.8, 138.1; HRMS for $C_{70}H_{75}N_{13}O_{13}$ (M + Cs) calcd 1438.4662, found 1438.4597.

3″-*O*-(2-*N*-Cbz-ethylamino-6,3′,4′,3″,5″-penta-*O*-benzyl-1,3,2′,6′-tetraazidoribostamycin (24). Compound **23** (65 mg, 50 μmol) was dissolved in a mixture of acetonitrile and water (9:1, 4 mL) and treated with CAN (136 mg, 249 μmol). After 4.5 h, the reaction was quenched by addition of a 1 N solution of $Na_2S_2O_4$. The aqueous layer was extracted twice with ethyl acetate, and the pooled organic phases were dried over $MgSO_4$. Chromatography of the residue over 40 mL of silica gel using a gradient of 15% to 20% to 25% to 30% ethyl acetate in hexane afforded 49 mg (83%) of product: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (ddd, 1H, $J_1 = J_2 = J_3$ = 12.5 Hz, H2 eq), 2.26 (ddd, 1H, J_1 = 12.5 Hz, $J_2 = J_3$ = 4.5 Hz, H2 ax), 3.05−3.27 (m, 6H, H5, H2′, NHZCH_2CH$_2$-O), 3.31 (dd, 1H, J_1 = 13.5 Hz, J_2 = 5 Hz, H6′a), 3.34−3.43 (m, 2H, H1, H3), 3.42 (dd, 1H, $J_1 = J_2$ = 9.5 Hz, H4′), 3.48 (dd, 1H, J_1 = 13.5 Hz, J_2 = 2.5 Hz, H6′b), 3.54 (dd, 1H, J_1 = 10.5 Hz, J_2 = 4 Hz, H5″a), 3.56−3.66 (m, 3H, H4, H6, H5″b), 3.71 (dd, 1H, $J_1 = J_2$ = 5 Hz, H3″), 3.89 (dd, 1H, J_1 = 5 Hz, J_2 = 3 Hz, H2″), 3.97 (dd, J_1 = 10 Hz, J_2 = 9.5 Hz, 1H, H3′), 4.07−4.12 (m, 1H, H4″), 4.17−4.22 (m, 1H, H5′), 4.42−4.53 (m, 3H, PhCH_2O), 4.59 (d, J = 12 Hz, 2H, PhCH_2O), 4.73−4.89 (m, 5H, PhCH_2O), 5.06 (s, 2H, PhCH_2O), 5.13−5.18 (m, 1H, N*H*ZCH_2CH$_2$-O), 5.52 (d, 1H, J = 3 Hz, H1″), 5.91 (d, 1H, J = 3.5 Hz, H1′), 7.10−7.7.45 (m, 30H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.7, 32.3, 40.9, 51.1, 59.5, 60.4, 63.5, 66.6, 68.9, 70.2, 70.9, 72.4, 73.3, 74.9, 75.0, 75.5, 76.2, 78.3, 78.5, 80.1, 80.2, 80.7, 81.0, 83.3, 96.0, 107.1, 127.3, 127.6, 127.7, 127.8,

127.9, 128.0, 128.1, 128.3, 128.4, 136.5, 137.5, 137.6, 137.7, 138.0, 156.3; HRMS for $C_{62}H_{67}N_{13}O_{12}$ (M + Cs) calcd 1318.4086, found 1318.4032.

3″-*O*-Ethyl-2-aminoribostamycin (5). The deprotection was carried out starting with compound **24** in the exact manner as the preparation of compound **6** to afford the title substance in 33% yield. It should be noted that this is a result from a single experiment where there was a problem with the reduction of the azides and a better yield can probably be obtained: ^{1}H NMR (D_2O, pD 2 adjusted with DCl, 500 MHz) δ 1.41 (ddd, 1H, $J_1 = J_2 = J_3 = 12.6$ Hz, H2 eq), 2.24 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.1$ Hz, H2 ax), 3.26 (dd, 2H, $J_1 = J_2 = 4.9$ Hz, $NH_2CH_2CH_2$-O), 3.32 (dd, 1H, $J_1 = 13.6$ Hz, $J_2 = 6.4$ Hz, H6′a), 3.41 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = 10.7$ Hz, $J_3 = 4.1$ Hz, H1), 3.44−3.52 (m, 2H, H2′, H6′b), 3.51 (dd, 1H, $J_1 = J_2 = 9.3$ Hz, H4′), 3.60 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = 10.5$ Hz, $J_3 = 4.1$ Hz, H3), 3.71−3.78 (m, 2H, H5″a, H6), 3.83−3.92 (m, 2H, $NH_2CH_2CH_2$-O), 3.93 (dd, 1H, $J_1 = 12.6$ Hz, $J_2 = 2.8$ Hz, H5″b), 3.93−4.00 (m, 1H, H5′), 3.98 (dd, 1H, $J_1 = J_2 = 10.1$ Hz, H5), 4.03 (dd, 1H, $J_1 = 10.8$ Hz, $J_2 = 9.3$ Hz, H3′), 4.10 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 4.5$ Hz, H3″), 4.13−4.20 (m, 2H, H4, H4″), 4.46 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 1.4$ Hz Hz, H2″), 5.44 (dd, 1H, $J_1 = 1.4$ Hz, H1″), 6.05 (dd, 1H, $J_1 = 4$ Hz, H1′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.5 (C2), 40.8 ($NH_2CH_2CH_2$-O), 41.5 (C6′), 49.9 (C3), 51.3 (C1), 55.0 (C2′), 62.2 (C5″), 67.5 ($NH_2CH_2CH_2$-O), 69.5 (C3′), 70.9 (C5′), 72.0 (C4′), 74.0 (C6), 74.9 (C2″), 76.8 (C4), 78.3 (C3″), 82.7 (C4″), 86.2 (C5), 97.1 (C1′), 112.0 (C1″); MS for $C_{19}H_{39}N_5O_{10}$ (M + H) calcd 498, found 498; for $C_{19}H_{39}N_5O_{10}$ (M − H) calcd 496, found 496.

1,6-Anhydro-2,3,4-Tri-*O*-benzylidopyranoside (28). α,*O*-Methyl-2,3,4-*O*-benzyl-5,6-dianhydroglucopyranoside (**25**) (5.62 g, 12.098 mmol) was dissolved in THF (20 mL) and cooled in an ice/water bath. The reaction was then treated with a 1 M solution of BH_3·THF in THF (50.9 mL, 50.9 mmol). The hydroboration was complete after an hour, and the reaction mixture was then slowly dripped into a cooled flask containing concentrated HOOH (18.1 mL) in 1 N NaOH (181 mL). The aqueous layer was extracted three times with EtOAc, and the organic phases were back-extracted with water. The EtOAc solution was dried over $MgSO_4$, and the solvent was removed. The residue was dissolved in 50 mL of AcOH and treated with 10 drops of 12 N HCl. The reaction was warmed to 70 °C and allowed to proceed for 1 h after which time the solvent was removed and the residue was purified by column chromatography over 200 mL of silica gel using 10% to 12.5% to 15% EtOAc in hexane to obtain 2.74 g (51% or 80% per step) of the product as an oil which solidifies upon standing under vacuum.

Methyl 2,3,4-Tri-*O*-benzyl-α-glucopyranoside (26): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.63 (dd, 1H, $J_1 = 7.5$ Hz, $J_2 = 5.5$ Hz, OH), 3.36 (s, 3H, OC*H*$_3$), 3.50 (dd, 1H, $J_1 = 9.5$ Hz, $J_2 = 3.5$ Hz, H2), 3.52 (dd, 1H, $J_1 = J_2 = 9.5$ Hz, H4), 3.62−3.67 (m, 1H, H6a), 3.67−3.72 (m, 1H, H5), 3.74−3.79, (m, 1H, H6b), 4.01 (dd, 1H, $J_1 = J_2 = 9.5$ Hz, H3), 4.56 (d, 1H, $J = 3.5$ Hz, H1), 4.65 (dd, 1H, $J_1 = J_2 = 12$ Hz, PhC*H*$_2$O), 4.85 (dd, 1H, $J_1 = J_2 = 11.5$ Hz, PhC*H*$_2$O), 4.92 (ABq, 2H, $J = 11$ Hz, $\Delta\nu = 49.3$ Hz, PhC*H*$_2$O), 7.25−7.40 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.2, 61.8,70.6, 73.4, 75.0, 75.8, 79.9, 81.9, 98.1, 127.6, 127.9, 128.0, 128.1, 128.4, 128.5, 138.1, 138.7; HRMS for $C_{28}H_{32}O_6$ (M + Cs) calcd 597.1253, found 597.1265.

Methyl 2,3,4-Tri-*O*-benzyl-β-idopyranioside (27): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.74 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 5$ Hz, OH), 3.48 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 3$ Hz, H2), 3.48 (s, 3H, OC*H*$_3$), 3.64 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 5.5$ Hz, H4), 3.80−3.87 (m, 1H, H6a), 3.88−3.94, (m, 1H, H6b), 3.97 (ddd, 1H, $J_1 = J_2 = J_3 = 5.5$ Hz, H5), 4.05 (dd, 1H, $J_1 = J_2 = 8$ Hz, H3), 4.53 (d, 1H, $J = 3$ Hz, H1), 4.54−4.83 (m, 6H, PhC*H*$_2$O), 7.25−7.40 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 56.9, 63.1, 73.7, 73.8, 74.9, 75.0, 76.9, 77.8, 78.2, 99.9, 127.8, 127.9, 128.0, 128.1, 128.4, 128.5, 137.7, 138.2, 138.3; HRMS for $C_{28}H_{32}O_6$ (M + Na) calcd 465.2277, found 487.2108.

1,6-Anhydro-2,3,4-Tri-*O*-benzylidopyranoside (28): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.48 (dd, 1H, $J_1 = 8$ Hz, $J_2 = 1.5$ Hz, H2), 3.66−3.75, (m, 2H, H4, H6a), 3.78 (dd, 1H, $J_1 = J_2 = 8$ Hz, H3), 4.13 (d, 1H, $J = 8$ Hz, H6b), 4.39 (dd, 1H, $J_1 = J_2 = 4.5$ Hz, H5), 4.60−4.88 (m, 6H, PhC*H*$_2$O), 5.30 (d, 1H, J = 1.5 Hz, H1), 7.25−7.40 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 65.5, 73.0, 73.1, 73.2, 75.5, 79.3, 81.8, 82.4, 99.6, 127.6, 127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 137.9, 138.0, 138.6; HRMS for $C_{27}H_{28}O_5$ (M + Cs) calcd 565.0991, found 565.1015.

Methyl 2,3,4-Tri-*O*-benzyl-1-deuxy-β-1-thioidopyranoside (30). Compound **28** (1.31 g, 2.820 mmol) was dissolved in 15 mL of CH_2Cl_2 and treated with (methylthio)trimethylsilane (1.07 g, 8.460 mmol) and trimethylsilyl trifluoromethanesulfonate (1.25 g, 5.640 mmol) and stirred for 40 h. The reaction was then quenched by addition of an excess of triethylamine and was subsequently treated with a 1 M solution of TBAF in THF (15 mL). After the desilylation was complete, the reaction was diluted wtih EtOAc and extracted three times with 1 N NaOH and once with water. The EtOAc solution was dried over $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography over 100 mL of silica gel using 30% to 35% to 40% to 45% EtOAc in hexane to obtain the α anomer first (70 mg, 5%) and then the β anomer (1.20 g, 88.5%).

Methyl 2,3,4-Tri-*O*-benzyl-1-deoxy-α-1-thioidopyranioside (29): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.17 (s, 3H, SC*H*$_3$), 3.51 (dd, 1H, $J_1 = J_2 = 4.5$ Hz, H2), 3.55 (dd, 1H, $J_1 = J_2 = 4.5$ Hz, H3), 3.71 (dd, 1H, $J_1 = 12$ Hz, $J_2 = 4.5$ Hz, H6a), 3.76 (dd, 1H, $J_1 = J_2 = 4.5$ Hz, H3), 3.94 (dd, 1H, $J_1 = 12$ Hz, $J_2 = 7$ Hz, H6b), 4.30−4.35 (m, 1H, H5), 4.40−4.78 (m, 6H, PhC*H*$_2$O), 5.13 (d, 1H, $J = 4$ Hz, H1), 7.20−7.40 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.3, 62.0, 69.6, 72.6, 73.2, 75.4, 75.7, 77.1, 83.6, 127.8, 127.9, 128.1, 128.2, 128.4, 128.5, 137.6, 137.7, 137.8.

Methyl 2,3,4-Tri-*O*-benzyl-1-deoxy-β-1-thioidopyranioside (30): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.89 (dd, 1H, $J_1 = 9.5$ Hz, $J_2 = 3.5$ Hz, OH), 2.24 (s, 3H, SC*H*$_3$), 3.25−3.27 (m, 1H, H4), 3.51−3.57 (m, 2H, H2, H6a), 3.66 (dd, 1H, $J_1 = J_2 = 3$ Hz, H3), 3.83 (ddd, 1H, $J_1 = 8$ Hz, $J_2 = 4$ Hz, $J_3 = 2$ Hz, H6b), 4.00 (ddd, 1H, $J_1 = 11.5$ Hz, $J_2 = 8$ Hz, $J_3 = 3.5$ Hz, H5), 4.22−4.39 (m, 3H, PhC*H*$_2$O), 4.55−4.64 (m, 3H, PhC*H*$_2$O), 4.79 (d, 1H, J = 1.5 Hz, H1), 7.14−7.38 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.7, 62.7, 70.7, 71.7, 71.8, 72.1, 73.2, 75.3, 77.2, 85.1, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 137.4, 137.6, 137.7; HRMS for $C_{28}H_{32}O_5S$ (M + Cs) calcd 613.1025, found 613.1051.

Methyl 2,3,4-Tri-*O*-benzyl-1-deoxy-β-1-thio-6-deoxy-6-(allyloxy)-idopyranoside (31). Compound **30** (245 mg, 510 μmol) was dissolved in 3 mL of DMF and treated with NaH (24 mg, 1.02 mmol) followed by allyl bromide (185 mg, 1.53 mmol). After being stirred overnight, the reaction was quenched by additon of MeOH and the solvent was removed in *vacuo*. The resulting residue was partitioned between EtOAc and H_2O. The organic phases were then dried over $MgSO_4$, and the solvent was removed. Chromatography over 50 mL of silica gel using a gradient of 15% to 20% to 25% EtOAc in hexane afforded 160 mg (60%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.22 (s, 3H, SC*H*$_3$), 3.34−3.47 (m, 1H, H4), 3.45−3.48 (m, 1H, H2), 3.60−3.65 (m, 2H, H3, H6a), 3.71 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 6$ Hz, H6b), 3.92−3.97 (m, 2H, H3, CH_2CHCH_2O), 3.99−4.05 (m, 1H, CH_2-CHC*H*$_2$O), 4.28 (s, 2H, PhC*H*$_2$O), 4.31 (ABq, 2H, $J = 12$ Hz, $\Delta\nu = 49.7$ Hz, PhC*H*$_2$O), 4.51−4.58 (m, 2H, PhC*H*$_2$O), 4.77 (d, 1H, $J = 1.5$ Hz, H1), 5.12−5.28 (m, 2H, C*H*$_2$CHCH$_2$O), 5.82−5.92 (m, 1H, CH_2CHCH_2O), 7.05−7.38 (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, Bruker AMX-500) δ 14.5, 69.5, 71.0, 71.8, 71.9, 72.1, 72.3, 73.0, 75.1, 76.2, 84.9, 1 16.8, 127.7, 127.8, 127.9, 128.2, 128.4, 128.5, 134.8, 137.8, 138.0, 138.1; HRMS for $C_{31}H_{36}O_5S$ (M + Cs) calcd 653.1338, found 653.1366.

Methyl 2,3,4-Tri-*O*-benzyl-1-deoxy-β-thio-6-deoxy-6-(allylamino)-idopyranoside (32). DMSO (1.3 g, 3.39 mmol) was dissolved in CH_2Cl_2 (20 mL), and the solution was cooled to −78 °C. The reaction was treated with 2 M oxalyl chloride in CH_2Cl_2 (2.21 mL, 4.42 mmol), and the reaction was allowed to stir for 15 min. Then, a solution of compound **30** (1.63 g, 3.39 mmol) in CH_2Cl_2 (10 mL) was added dropwise *via* syringe. The reaction was allowed to proceed at −78 °C for 45 min, then triethylamine (1.72 g, 16.96 mmol) was added, and the reaction was allowed to warm to room temperature. The reaction was diluted with EtOAc and extracted twice with water. The organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was dissolved in methanol (15 mL). A solution of allylamine (1.94 g, 33.9 mmol) was neutralized to pH 6 (pH paper) using glacial acetic acid, and this solution was added to the solution of the aldehyde.

The reaction was then treated with $NaCNBH_3$ (213 mg, 3.4 mmol). The transformation was complete within 15 min. The solvent was removed, and the reaction was taken up in EtOAc. The organic phases were dried over $MgSO_4$, and the solvent was removed. The residue was purified by column chromatography over 100 mL of silica gel using 5% to 6% to 7% MeOH in $CHCL_3$ to obtain 1.20 g (68%) of the title compound as an oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.23 (s, 3H, SC*H*$_3$), 2.53 (dd, 1H, J_1 = 12.5 Hz, J_2 = 3.5 Hz, H6a), 3.16 (dd, 1H, J_1 = 12.5 Hz, J_2 = 9 Hz, H6b), 3.18−3.26 (m, 3H, H4 and CH_2CHCH_2O), 3.49−3.51 (m, 1H, H2), 3.66 (dd, 1H, $J_1 = J_2 = 3$ Hz, H3), 3.87−3.92 (m, 1H, H5), 4.26−4.61 (m, 6H, PhCH_2O), 4.78 (d, 1H, J = 1.5 Hz, H1), 5.03−5.17 (m, 2H, CH_2CHCH_2O), 5.78−5.88 (m, 1H, CH_2CHCH_2O), 7.14−7.38, (m, 15H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.7, 49.6, 52.2, 70.8, 71.8, 72.0, 72.6, 73.2, 75.3, 75.9, 85.2, 116.2, 127.7, 127.8, 128.0, 128.3, 128.4, 128.5, 136.5, 137.5, 137.9; HRMS for $C_{31}H_{37}NO_4S$ (M + Na) calcd 542.2341, found 542.2353.

Methyl 2,3,4-Tri-*O*-benzyl-1-deoxy-β-1-thio-6-deoxy-6-(carbobenzyloxyamino)idopyranoside (33). Compound **32** (894 mg, 1.72 mmol) was dissolved in a mixture of acetonitrile and water (84/16) and brought to reflux. A system was set up such that the solvent in the pot was continuously being distilled off while fresh acetonitrile/water mixture was added to replace the distillate. A suspension of Wilkinson's catalyst (300 mg, 1.720 mmol) in the acetonitrile/water mixture was added, and the reaction was allowed to reflux vigorously. The reaction was complete in 2 h, and the solvent was removed. The residue was dissolved in CH_2Cl_2 and cooled with an ice bath. The reaction was then treated with a solution of *N*-(benzyloxycarbonyloxy)succinimide (536 mg, 2.15 mmol) in CH_2Cl_2 (5 mL). The reaction was complete within 15 min. The solvent was removed, and the residue was chromatographed over 100 mL of silica gel using 17.5% to 20% to 22.5% to 25% EtOAc in hexane to afford 706 mg (67%) of the title compound as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.20 (s, 3H, SC*H*$_3$), 3.20−3.23 (m, 1H, H4), 3.34−3.41 (m, 1H, H6a), 3.44−3.52 (m, 2H, H6b, H2), 3.64 (dd, 1H, $J_1 = J_2 = 2.5$ Hz, H3), 3.79−3.84 (m, 1H, H5), 4.20−4.36 (m, 3H, benzillic protons), 4.52−4.61 (m, 3H, PhCH_2O), 4.74 (s, 1H, H1), 4.86−4.91 (m, 1H, CH_2-N*H*Z), 5.02−5.1 (m, 2H, PhCH_2O), 7.13−7.20 (m, 4H, C_6H_5), 7.26−7.37, (m, 16H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 14.6, 41.8, 66.6, 70.4, 71.7, 71.8, 72.0, 73.2, 75.1, 75.3, 85.0, 127.9, 128.0, 128.3, 128.4, 128.5, 136.6, 137.3, 137.5, 137.8, 156.4; HRMS for $C_{36}H_{39}NO_6S$ (M + Cs) calcd 746.1552, found 746.1568.

Compound 34. Compound **20** (69.4 mg, 69 μmol) and 31 (97 mg, 186 μmol) were mixed and dried overnight over P_2O_5. Then CH_2Cl_2 (5 mL) was added *via* syringe. The reaction was cooled to −10 °C using an ice/salt bath, and NIS (46 mg, 20 μmol) was added. The reaction was allowed to stir for 15 min, and then a catalytic amount of AgOTf (∼2 mg) was added. The reaction assumed a purple color and was allowed to proceed for 45 min before being quenched with triethylamine. The reaction was then filtered through a pad of Celite, and the solvent was removed. Chromatography of the residue over 50 mL of silica gel using a gradient of 10% to 15% to 20% to 25% ethyl acetate in hexane afforded 50 mg (49%) of the desired product: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (ddd, 1H, $J_1 = J_2 = J_3 = 12.5$ Hz, H2 eq), 2.24 (ddd, 1H, $J_1 = 12.5$ Hz, $J_2 = J_3 = 4.5$ Hz, H2 ax), 3.10 (dd, 1H, $J_1 = 10.5$ Hz, $J_2 = 4$ Hz), 3.22−3.32 (m, 4H), 3.36−3.48 (m, 4H), 3.10 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 5.5$ Hz), 3.59 (dd, 1H, $J_1 = J_2 =$ 3.5 Hz), 3.61−3.67 (m, 2H), 3.83 (dd, 1H, $J_1 = 10.5$ Hz, $J_2 = 2$ Hz), 3.84−3.97 (m, 5H), 4.00 (dd, 1H, $J_1 = J_2 = 9.5$ Hz), 4.10−4.25 (m, 4 H), 4.34−4.61 (m, 10H), 4.67−4.75 (m, 3H), 4.76−4.89 (m, 3H), 4.93 (d, 1H, J = 11 Hz), 5.11−5.16 (m, 1H), 5.19−5.27 (m, 1H), 5.55 (d, J = 4.5 Hz, 1H, H1″), 5.79−5.89 (m, 1H), 6.14 (d, 1H, J = 4 Hz, H1′), 7.02−7.37 (m, 40H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.4, 51.2, 59.8, 60.4, 63.2, 69.4, 70.2, 70.8, 71.9, 72.1, 72.2, 72.4, 72.6, 73.2, 73.9, 74.0, 74.1, 74.8, 75.2, 75.3, 75.4, 76.5, 78.5, 80.1, 81.8, 82.0, 82.3, 83.9, 95.6, 100.5, 107.0, 116.9, 127.3, 127.4, 127.5, 127.6, 127.7, 127.8, 128.0, 128.1, 128.2, 128.4, 128.5, 134.8, 137.6, 137.7, 137.82, 137.84, 138.0, 138.4, 138.8; HRMS for $C_{82}H_{89}N_{12}O_{15}$ (M + Cs) calcd 1614.5625, found 1614.5539.

Compound 35. Bis(methyldiphenylphoshine)(cyclooctadienyl)-iridium(I) hexafluorophosphate (5 mg, 6 μmol) was suspended in THF (5 mL), and H_2 was bubbled through this suspension for 20 min. The resulting clear solution was transferred via syringe into a solution of compound 34 (50 mg, 34 μmol) in THF (15 mL). After 1 h, a quantitative conversion to a slightly less polar material was observed. The solvent was removed, and the residue was coevaporated with CH_2Cl_2 several times. The reaction was then taken up in CH_2Cl_2 (30 mL) and treated with trimethylamine *N*-oxide dihydrate (19 mg, 0.17 mmol), and a solution of OsO_4 in tBuOH (20 μL of the 2.5 wt % commercial preparation). After the reaction was over (overnight), the solvent was removed and the residue was purified over 50 mL of silica gel using 15% to 20% to 25% to 30% EtOAc in hexane to obtain 41 mg (84%) of the title compound: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.41 (ddd, 1H, $J_1 = J_2 = J_3 = 16$ Hz, H2 eq), 2.24 (ddd, 1H, $J_1 = 16$ Hz, $J_2 = J_3 =$ 5.5 Hz, H2 ax), 2.70−2.82 (m, 1H, OH), 3.15−3.23 m, 2H), 3.25−3.43 (m, 6H), 3.47 (dd, 1H, $J_1 = 16.5$ Hz, $J_2 = 2.5$ Hz), 3.47−3.57 (m, 1H), 3.59 (dd, $J_1 = J_2 = 11.5$ Hz, 1H), 3.63−3.77 (m, 4H), 3.78−3.84 (m, 1H), 3.89−4.03 (m, 3H), 4.15−4.21 (m, 1H), 4.23−4.49 (m, 8H), 4.52−4.72 (m, 7H), 4.78−4.90 (m, 4H), 5.52 (d, 1H, J = 4.5 Hz, H1″), 5.98 (d, 1H, J = 4.5 Hz), 7.06−7.37 (m, 40H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.3, 51.1, 59.6, 60.4, 62.6, 63.3, 69.3, 70.9, 72.1, 72.3, 73.0, 73.6, 74.0, 74.7, 74.9, 75.1, 75.4, 75.8, 76.0, 78.4, 80.0, 81.3, 81.5, 81.9, 83.4, 95.9, 99.9, 107.4, 127.3, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 137.65, 137.69, 137.8, 137.9, 138.0, 138.6; HRMS for $C_{79}H_{85}N_{12}O_{15}$ (M + Cs) calcd 1574.5312, found 1574.5397.

2‴,6‴-Didesamino-2‴,6‴-dihydroxyneomycin B (7). The deprotection of **35** (31 mg, 2.15 μmol) was carried out in the exact manner as the preparation of compound **6** to afford 12.4 mg (76%) of **7**·4HCl: ^{1}H NMR (D_2O, adjusted with DCl, 600 MHz) δ 1.85 (ddd, 1H, $J_1 = J_2 = J_3 = 12.6$ Hz, H2 eq), 2.24 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.1$ Hz, H2 ax), 3.24 (dd, 1H, $J_1 = 13.7$ Hz, $J_2 = 6.3$ Hz, H6′a), 3.32 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = 10.6$ Hz, $J_3 = 4.1$ Hz, H1), 3.37−3.43 (m, 2H, H2′, H6′b), 3.43 (dd, 1H, $J_1 = J_2 = 9.4$ Hz, H4′), 3.51 (ddd, 1H, $J_1 =$ 12.6 Hz, $J_2 = 10.3$ Hz, $J_3 = 4.1$ Hz, H3), 3.57−3.60 (m, 1H, H4‴), 3.65 (dd, 1H, $J_1 = 10.6$ Hz, $J_2 = 9.2$ Hz, H6), 3.72−3.82 (m, 4H, H6‴a, H6‴b, H5″a, H2‴), 3.85−3.97 (m, 5H, H5″b, H5′, H5, H3′, H5‴), 3.99 (dd, 1H, $J_1 = J_2 = 3.7$ Hz, H3‴), 4.06 (dd, 1H, $J_1 = 10.3$ Hz, $J_2 = 9.2$ Hz, H4), 4.14−4.18 (m, 1H, H4″), 4.35 (dd, 1H, $J_1 = 4.7$ Hz, $J_2 = 1.7$ Hz, H2″), 4.42 (dd, 1H, $J_1 = 7.2$ Hz, $J_2 = 4.7$ Hz, H3″), 64.89 (d, 1H, J = 1.3 Hz, H1‴), 5.35 (d, 1H, J = 1.7 Hz, H1″), 5.98 (d, 1H, J = 4 Hz, H1′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.5 (C2), 41.5 (C6′), 49.9 (C3), 51.3 (C1), 55.0 (C2′), 2.8 (C5″ and (C2‴ or C2‴)), 9.46 (C4‴), 9.52 (C3′), 70.7 (C2‴ or C6‴), 70.9 (C5′), 71.1 (C3‴), 72.0 (C4′), 74.0 (C6), 75.3 (C2″), 76.8, 76.9 (C3″, C4, C5‴), 3.0 (C4″), 6.1 (C5), 97.1 (C1′), 100.3 (C1‴), 111.6 (C1″); MS for $C_{23}H_{44}N_4O_{15}$ (M + H) calcd 617, found 617; for $C_{23}H_{45}N_5O_{14}$ (M − H) calcd 615, found 615.

Compound 36. Compound **20** (321 mg, 0.32 mmol) and compound **33** (312 mg, 0.510 mmol) were dried together with 3 Å MS (250 mg) overnight. Then CH_2Cl_2 (5 mL) was added, and the reaction was cooled to −10 °C using an ice/salt bath. After 30 min of stirring, NIS (125 mg, 0.56 mmol) was added, and the reaction was allowed to stir for 15 min. Then, a catalytic amount of AgOTf was added, and the reaction was allowed to stir for 30 min prior to quenching with triethylamine. The reaction was then filtered through a pad of Celite, and the solvent was removed. Chromatography of the residue over 50 mL of silica gel using a gradient of 10% to 15% to 20% to 25% ethyl acetate in hexane afforded 175 mg (35%) of the desired product: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.35 (ddd, 1H, $J_1 = J_2 = J_3 = 12.5$ Hz, H2 eq), 2.17 (ddd, 1H, $J_1 = 12.5$ Hz, $J_2 = J_3 = 4.5$ Hz, H2 ax), 3.12 (dd, J_1 = 10 Hz, $J_2 = 3.5$ Hz, 1H, H2′), 3.15 (dd, $J_1 = J_2 = 9$ Hz, 1H, H3‴), 3.21−3.33 (m, 3H, H1, H3, H4‴), 3.29 (dd, 1H, $J_1 = 13.5$ Hz, $J_2 =$ 4.5 Hz, H6′a), 3.34−3.49 (m, 4H, H5, H4′, H6‴a, H6‴b), 3.47 (dd, 1H, $J_1 = 13.5$ Hz, $J_2 = 2.5$ Hz, H6′b), 3.55−3.72 (m, 5H, H4, H6, H5″a, H5″b, H2‴), 3.77−3.83 (m, 1H, H5‴), 3.95 (dd, 1H, $J_1 = 4.5$ Hz, $J_2 = 4$ Hz, H2″), 4.00 (dd, 1H, $J_1 = 10$ Hz, $J_2 = 9.5$ Hz, H3′), 4.13−4.19 (m, 1H, H5′), 4.19−4.24 (m, 2H, H3″, PhCH_2O), 4.29−4.34 (m, 2H, H4″, PhCH_2O), 4.38−5.12 (m, 17H, PhCH_2O and H1‴), 5.47−5.53 (m, 1H, CbzN*H*), 5.54 (d, 1H, $J_1 = 4$ Hz, H1″), 5.99 (d, 1H, $J_1 = 3.5$ Hz, H1′), 7.02−7.37 (m, 40 H, C_6H_5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 32.2, 41.6, 51.2, 59.6, 60.3, 63.2, 66.5, 69.4, 70.9, 71.7,

72.4, 72.6, 73.3, 73.89, 73.93, 74.2, 74.3, 74.9, 75.1, 75.3, 75.8, 76.6, 78.5, 79.9, 81.3, 81.5, 82.1, 83.4, 95.9, 100.1, 107.2, 127.2, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 136.6, 137.56, 137.60, 137.80, 137.81, 138.1, 138.6, 156.5; HRMS for $C_{87}H_{91}N_{13}O_{16}$ (M + Cs) calcd 1706.5761, found 1706.5849.

2‴-Desamino-2‴-hydroxyneomycin B (8). The deprotection of compound **36** (60.7 mg, 39 μmol) was carried out in the exact manner as the preparation of compound **6** to afford 21.6 mg (70%) of **8**·5HCl: ^{1}H NMR (D_2O, pD 2 adjusted with DCl, Bruker AMX-500) δ 1.95 (ddd, 1H, $J_1 = J_2 = J_3 = 12.6$ Hz, H2 eq), 2.24 (ddd, 1H, $J_1 = 12.6$ Hz, $J_2 = J_3 = 4.1$ Hz, H2 ax), 3.33 (dd, 1H, $J_1 = 13.7$ Hz, $J_2 = 6.4$ Hz, H6′a), 3.35−3.44 (m, 3H, H6‴a, H1, H6‴b), 3.46−3.51 (m, 2H, H2′, H6′b), 3.52 (dd, 1H, $J_1 = J_2 = 9.3$ Hz, H4′), 3.60 (ddd, 1H, $J_1 = 12.8$ Hz, $J_2 = 10.2$ Hz, $J_3 = 4.1$ Hz, H3), 3.71−3.74 (m, H4‴), 3.77 (dd, 1H, $J_1 = 10.4$ Hz, $J_2 = 9.3$ Hz, H6), 3.80 (dd, 1H, $J_1 = 12.4$ Hz, $J_2 = 5.1$ Hz, H5″a), 3.85−3.88 (m, 1H, H2‴), 3.95 (dd, 1H, $J_1 = 12.4$ Hz, $J_2 = 3.0$ Hz, H5″b), 3.94−3.99 (m, 1H, H5′), 3.99 (dd, 1H, $J_1 = 10.2$ Hz, $J_2 = 9.3$ Hz, H5), 4.04 (dd, 1H, $J_1 = 10.9$ Hz, $J_2 = 9.3$ Hz, H3′), 4.11 (dd, 1H, $J_1 = J_2 = 3.5$ Hz, H3‴), 4.18 (dd, 1H, $J_1 = J_2 = 10.2$ Hz, H4), 4.23−4.28 (m, 2H, H4″, H5‴), 4.44 (dd, 1H, $J_1 = 4.8$ Hz, $J_2 = 2.4$ Hz, H2″), 4.52 (dd, 1H, $J_1 = 6.5$ Hz, $J_2 = 4.8$ Hz, H3″), 5.03 (d, 1H, $J = 1.2$ Hz, H1‴), 5.46 (d, 1H, $J = 2.4$ Hz, H1″), 6.09 (d, 1H, $J = 4$ Hz, H1′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 29.5 (C2), 41.6 (C6′), 42.0 (C6‴), 49.9 (C3), 51.3 (C1), 54.9 (C2′), 61.8 (C5″), 69.5 (C3′), 70.0 (C4‴), 70.2 (C2‴), 70.9 (C5′), 71.0 (C3‴), 72.01 (C4′), 72.04 (C5‴), 73.9 (C6), 75.2 (C2″), 76.7 (C4), 76.9 (C3″), 83.1 (C4″), 86.3 (C5), 97.0 (C1′), 100.1 (C1‴), 111.7 (C1″); MS for $C_{23}H_{45}N_5O_{14}$ (M + H) calcd 616, found 616; for $C_{23}H_{45}N_5O_{14}$ (M − H) calcd 614, found 614.

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