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Synthesis and Antibacterial Activity of Propylamycin Derivatives Functionalized at the 5”- and Other Positions with a View to Overcoming Resistance due to Aminoglycoside Modifying Enzymes.

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Abstract

Propylamycin (4’-deoxy-4’-propylparomomycin) is a next generation aminoglycoside antibiotic that displays increased antibacterial potency over the parent, coupled with reduced susceptibility to resistance determinants and reduced ototoxicity in the guinea pig model. Propylamycin nevertheless is inactivated by APH(3’)-Ia, a specific aminoglycoside phosphotransferase isozyme that acts on the primary hydroxy group of the ribofuranosyl moiety (at the 5”-position). To overcome this problem we have prepared and studied the antibacterial and antiribosomal activity of various propylamycin derivatives carrying amino or substituted amino groups at the 5”-position in place of the vulnerable hydroxy group. We find that the introduction of an additional basic amino group at this position, while overcoming the action of the aminoglycoside phosphoryltransferase isozymes acting at the 5”-position as anticipated, results in a significant drop in selectivity for the bacterial over the eukaryotic ribosomes that is predictive of increased ototoxicity. In contrast, 5”-deoxy-5”-formamidopropylamycin retains the excellent across-the-board levels of antibacterial activity of propylamycin itself, while circumventing the action of the offending aminoglycoside phosphotransferase isozymes, and affording even greater selectivity for the bacterial over the eukaryotic ribosomes. Other modifications to address the susceptibility

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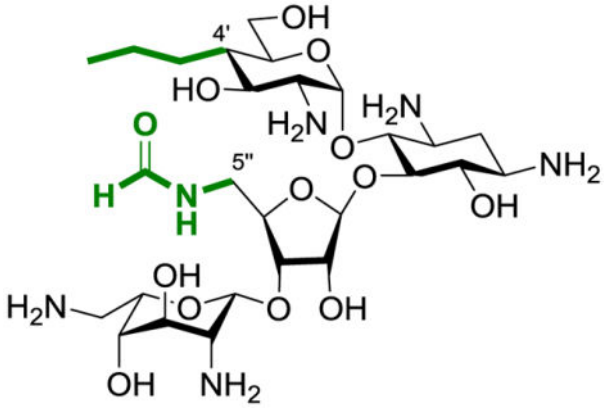
of propylamycin to the APH(3')-Ia isozyme including deoxygenation at the 3'-position and incorporation of a 6',5"-bis(hydroxyethylamino) modification offer no particular advantage.

Graphical Abstract



Synopsis: The TOC graphic shows the structure of the optimal compound with the two key features colored green for emphasis.

Keywords

Aminoglycoside modifying enzymes; ribosomal methyltransferases; antibacterial; antiribosomal; ototoxicity

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We recently described propylamycin **1** (Figure 1),[1] a next generation 2-deoxystreptamine-type (DOS) aminoglycoside antibiotic (AGA)[2–5] derived from the parent paromomycin **2** by substitution of the 4'-hydroxy group in ring I by a propyl group. Propylamycin displays superior in vitro antibacterial activity to the parent against a broad selection of ESKAPE pathogens and improved in vivo activity against *E. coli* in neutropenic mouse thigh and septicemia models. Propylamycin retains full activity against clinical isolates carrying various aminoglycoside modifying enzymes (AMEs)[6–11] that impart resistance against the parent, including the AAC(3), and AAC(6') aminoglycoside acetyltransferases, the aminoglycoside nucleotidyltransferases ANT(4',4"), and aminoglycoside phosphotransferases from the APH(3') family. Additionally, propylamycin retains full activity in the presence of the G1405 ribosomal methyltransferases (RMTs) that completely abrogates the activity of all DOS-type AGAs in current clinical use.[12–15] Finally, at the target level, propylamycin shows enhanced selectivity for inhibition of protein synthesis by the bacterial ribosome over hybrid bacterial ribosomes carrying the complete decoding A site of the human mitochondrial, A1555G mutant mitochondrial, and cytoplasmic ribosomes resulting in reduced ototoxicity in a guinea pig model. In spite of these multiple attributes, we have subsequently found that, like 3'-deoxyparomomycin or lividomycin B **3**,[16] propylamycin is susceptible to inactivation by a subclass of APH(3')s acting at both the 3'- and the 5"-hydroxy groups, namely the APH(3',5")s.[17]

In seeking to overcome the susceptibility of propylamycin toward the APH(3',5")s, we were guided by our efforts on the development of the apralogs, namely 5-*O*-furanosylated analogs of the atypical DOS-type AGA apramycin **4**.[18, 19] Thus, while the simple 5-*O*-β-D-ribofuranosyl apralog **5**, described originally by Japanese scientists and subsequently by Fridman and coworkers,[20, 21] was susceptible to APH(3',5")s, derivatives lacking the primary hydroxy group in the furanosyl ring were protected from the action of these AMEs. In particular, the aminodeoxy derivatives **6** and **7** (Figure 1) were found to exhibit improved in vitro and in vivo antibacterial activity over apramycin itself in the presence of multiple relevant AMEs, including the APH(3',5")s, and RMTs. In addition to displaying improved activity **6** and **7** also showed increased selectivity, as determined by a cell-free ribosomal translation assay and as borne out by reduced cochleotoxicity in ex vivo mouse cochlear ex-plant studies.

Combining the attributes of propylamycin **1** and apralogs **6** and **7**, we designed and report here on the synthesis and evaluation of the antiribosomal and antibacterial activities of the 5"-aminodeoxy analogs **8**–**13** of propylamycin (Figure 2). In order to help distinguish between the 3' and 5"-prongs of the APH(3',5")s, we also report the synthesis and assessment of 3'-deoxypropylamycin **14**. Finally, exploring an alternative avenue to the introduction of amino functionality at the 5"-position, we describe a 6',5"-bis(hydroxyethylamino) analog **15** of propylamycin (Figure 2).

Results and Discussion

Synthesis.

The synthesis of the 5"-aminodeoxy analogs **8**–**13** of propylamycin **1** was based on that developed for propylamycin itself, with suitable modification for functionalization of the primary hydroxy group in the ribofuranoside side chain. Briefly, the paromomycin 4',6'-*O*-benzylidene pentatrifluoroacetamide **16**, obtained from paromomycin as described previously,[1] was converted to the 5"-*O*-silyl ether **17** by standard means in 95% yield. Perbenzoylation with benzoic anhydride in the presence of 4-dimethylaminopyridine in pyridine to give **18** in 92% yield, was followed by desilylation using tetrabutylammonium fluoride under standard conditions to give the mono-ol **19** suitable for introduction of the requisite amino group in 99% yield. Triflation with triflic anhydride in the presence of 2,6-lutidine was followed by displacement with sodium azide giving the azide **20** in 77% yield overall. Staudinger reaction with trimethylphosphine to give amine **21** in 40% yield was followed by conversion to the benzyl carbamate **22** in 87% yield under standard conditions, hydrolysis of the benzylidene acetal with aqueous acetic acid to give diol **23** in 86% yield, and regioselective monobenzoylation with benzoyl cyanide to give **24** in 91% yield, primed for introduction of the propyl chain. To this end triflation of mono-ol **24** was followed by displacement with potassium iodide in acetone affording the galacto-configured axial iodide **25** in 83% yield. Treatment of iodide **25** with allyl phenyl sulfone in 1,2-dichlorobenzene with initiation by triethylborane and oxygen at 0 °C[1] then gave the 4'-deoxy-4'-*C*-propyl derivative **26** in 40% yield (Scheme 1).

In addition to the allylation of **25** by the action of allyl phenyl sulfone with initiation by triethylborane and oxygen, which mirrored the earlier synthesis of propylamycin itself (Scheme 1), we chose to briefly explore the use of photocatalytic initiation for allylation. A variety of transition metal-based photocatalytic systems have been employed to promote the coupling of alkyl iodides with allyl phenyl sulfone and its congeners, including palladium and manganese-based catalysts[22, 23] and iridium-based systems[24] typically in the presence of a tertiary amine as sacrificial electron source, and indeed we have employed one such Ir-based system in our laboratories in the course of a synthesis of bradyrhizose.[25] Here, we elected to examine the metal-free 1,3,4,5-tetra(*N*-carbazolyl)-2,6-dicyanobenzene (4-CzIPN) photocatalyst in combination with triethylamine as overall reductant[26, 27] as applied recently to allylation of alkyl iodides with allyl phenyl sulfone as trap.[28] Working with *N*-*tert*-butyloxycarbonyl-4-iodopiperidine we were able to reproduce, the literature photocatalytic allylation reaction,[28] but were frustrated to find that application of the same conditions to iodide **25** resulted only in the formation of the elimination product **27**, which ultimately was traced to competing elimination of the axial iodide by the triethylamine (Scheme 2).

We therefore prepared the corresponding equatorial iodide **29** by Lattrel-Dax epimerization of the gluco-alcohol **24** to its galacto-epimer **28**,[29–31] followed by triflation and displacement with potassium iodide (Scheme 3). White light irradiation of equatorial iodide **29** in the presence of 5 mol % CzIPN and triethylamine in DMSO afforded a 1:1.2 mixture of the desired allylation product **26** and the reduction product **30-H**, but none of the elimination product **27**, thereby clearly demonstrating the configuration sensitive nature of the elimination reaction. A slight improvement of the **26**:**30-H** ratio to 1:1 was achieved by using an excess (5 equiv) of allyl phenyl sulfone. The hydrogen atom required for the formation of reduction product **30-H** is not derived from the solvent DMSO as was revealed by the use of d_6-DMSO. However, replacement of triethylamine by d_{15}-triethylamine resulted in the formation of 3:1 mixture of **26**:**30-H** with approximately 50% incorporation of deuterium (1:1 **30-H**:**30-D**), suggesting that triethylamine is the ultimate hydrogen atom source for the formation of **30-H**, and that reduction can be at least in part suppressed leading to improved yields of allylation by means of the kinetic isotope effect, as is known for other radical C-C bond forming processes.[32] Overall, while these brief studies were ultimately unsuccessful in providing a practical route to **26**, they do serve to outline some limitations in the application of photocatalytic processes to the complex substrates particularly when tertiary amines are required as overall reductant.

Returning to the synthesis of the target aminodeoxy propylamycin analogs **8**–**13**, hydrogenation of **26** over palladium on charcoal in 80% acetic acid afforded the pivotal amine **31** in quantitative yield. Reductive amination of **31** with a pair of trifluoracetamide substituted aldehydes **32** and **33**, prepared as described in the supporting information, afforded the corresponding amines **34** and **35**, which were immediately protected as the corresponding trifluoracetamides **36** and **37** for ease of purification in moderate overall yield for the two steps. Acylation of amine **31** with benzyloxycarbamoyl chloride, formic acetic anhydride, benzylisocyanate and *N*-trifluoroacetylglycine, the latter with the aid of HATU, afforded the corresponding 5”-amido derivatives **38**–**41** in good to excellent yield. Finally, deprotection of **36**–**41**, yielding **8**–**13** was achieved by a two step protocol, designed to

prevent O→N benzoyl group migration,[1] involving initial removal of the benzoate esters with magnesium methoxide in dry methanol followed by heating with aqueous barium hydroxide in moderate to good overall yield following HPLC purification (Scheme 4).

For the synthesis of 3'-deoxypropylamycin **14** we developed a route based on cleavage of ring I from a suitable protected paromomycin derivative followed by reinstallation of the modified ring I. A suitable glycosyl donor for this purpose **45** was obtained from the 1,6-anhydro pyranose derivative **42**, itself available from 1,6-anhydroglucose via the Cerny epoxide as described previously,[33] by ring opening with *S*-trimethylsilyl thiophenol in the presence of zinc iodide[34] followed by treatment with aqueous methanolic potassium carbonate to give the thioglycoside **43** in 87% yield as a 2.8:1 α:β mixture of anomers. Acetylation of **43** under standard conditions gave **44** in 97% yield and was followed by oxidation with Selectfluor[35] giving the glycosyl sulfoxide **45** in 98% yield as a mixture of isomers. Sulfoxide **45** was coupled to acceptor **46**, prepared as previously described,[36] under the aegis of triflic anhydride and in the presence of the hindered base 2,4,6-tri-*tert*-butylpyrimidine affording **47** as a 12:1 α:β mixture in 48% yield. Hydrogenolysis of **47** over Pearlman's catalyst followed by heating with aqueous barium hydroxide and finally chromatography over Sephadex C25 gave the target compound **14** in 41% yield (Scheme 5).

Finally, exploring an alternative avenue to the introduction of an amino group at the 5"-position, propylamycin **1** was converted in 86% yield to the penta-*N*-benzyloxycarbamoyl derivative **48**. This was followed by selective sulfonylation of the two primary hydroxy groups with 2,4,6-triisopropylbenzene sulfonyl chloride in pyridine giving **49** in 45% yield. Heating to 100 °C with ethanolamine then afforded the doubly substituted derivative **50** in 40% yield, and was followed by hydrogenolysis and chromatography over Sephadex to give **15** in 21% yield (Scheme 6).

Antibacterial Activity Against Wild-type Bacterial Strains

All newly prepared compounds and the comparators propylamycin **1**, paromomycin **2**, and lividomycin B **3** were tested for activity against a panel of ESKAPE pathogens sourced from the diagnostic department of the Institute of Medical Microbiology, at the University of Zurich. This panel was made up of a Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA) strain, and a panel of wild-type Gram negative pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*) (Table 1). Although we have previously demonstrated the parent propylamycin to show excellent activity against *Mycobacteria*,[1] we excluded them from this study because of their low incidence of APH(3')s. The replacement of the hydroxy group at the 5"-position of propylamycin **1** by a primary amine as in **8** resulted in an approximately two-fold reduction in activity across the range of ESKAPE pathogens assayed, with the exception of the *P. aeruginosa* strain for which a two-fold improvement in activity was seen. No advantage was gained in terms of activity by the elaboration of the 5"-amine in **3** into two diamino alkyl chains as in **9** and **10**, rather a gradual drop-off in activity was observed as the chain was lengthened and a further amino group incorporated. Notably, the protection of the 5"-amino group in **3** in the form of the formamide **11** restored activity across the board to levels seen in propylamycin itself, and comparable to those of the clinical AGAs gentamicin

and plazomicin. The corresponding urea and glycinamide modification at the 5”-position, **12** and **13**, were not as effective as the formamide but nevertheless retained good activity. 3’-Deoxypropylamycin **14** was marginally less active than propylamycin itself, but was notably more active than 3’-deoxyparomomycin (lividomycin B) **3** thereby clearly highlighting the general increase in activity conferred by the 4’-deoxy-4’-propyl modification. Finally, the 6’,5”-bis(hydroxyethylamino) derivative **15** was notably less active than propylamycin, consistent with the effects of the analogous modification on paromomycin reported earlier.[37]

Antibacterial Activity Against Resistant Bacterial Strains

To determine the ability of the various modifications to overcome resistance due to the presence of AMEs, particularly the APHs acting at the 3’- and 5”-positions, we turned to a panel of engineered *E. coli* with each member carrying a specific resistance determinant (Table 2). In addition to surveying four APH isoforms, we also investigated the AAC(3)-IV AME known to be problematic in the apramycin series,[18, 19] and two RMTs acting on G1405, which seriously abrogate the activity of all DOS-type AGAs in current clinical use (Table 2). All four APHs included in this study seriously curtailed the activity of paromomycin **2**, indicating them to act at either the 3’, or the 5”-positions, or both. In contrast, the strains carrying APH(3’)-IIa, APH(3’)-IIb, and APH(3’)-VI were all susceptible to 3’-deoxyparomomycin (lividomycin B) **3**, while only the APH(3’)-Ia carrying strain was not. APH(3’) isoforms IIa, IIb and VI therefore act only at the 3’-position, while isoform Ia acts at both the 3’ and 5”-positions. Thus, it is clear that 4’-deoxy-4’-propyl modification that characterizes propylamycin is effective in blocking the action of APHs at the adjacent 3’-position, but does not hinder the action of isoforms acting at the 5”-position. Modification of propylamycin at the 5”-position to remove the hydroxy group is effective at overcoming the action of APH(3’)-Ia and affords a series of compounds that retain excellent activity in the presence of each of the four APH(3’) isoforms investigated. Consistent with the activity levels against the ESKAPE pathogens (Table 1) the optimal compound in this series was the 5”-deoxy-5”-formamide **11**. The profile of 3’-deoxypropylamycin **14** in the face of the various APH(3’) isoforms was essentially unchanged with respect to propylamycin itself indicating the high level of protection afforded by the 4’-deoxy-4’-propyl modification against those isoforms acting solely at the 3’-position. Interestingly, the 6’,5”-(bishydroxyethylamino) derivative **15** was susceptible to inactivation by APH(3’)-Ia suggesting that this isoform is sufficiently accommodating to accept the hydroxy group of the 5”-(hydroxyethylamino)-substituent as substrate. Most compounds retained their full activity in the presence of the AAC(3)-IV, ArmA and RmtB resistance determinants suggesting that the various modifications introduced at the 5”-position little affect resilience toward the AAC(3)-IV itself or to target modification by ArmA or RmtB.

To further assess the susceptibility of the compounds prepared to AMEs we screened against a series of clinical isolates with acquired resistance determinants (Table 3). With respect to the isolates carrying the APH(3’) and AAC(3) determinants, the results are consistent with those reported in Table 2 for the engineered strains. Notably, strain AG163 carrying the APH(3’)-I resistance determinant inactivated those compounds with a hydroxy group at the 5”-position and additionally the 5”-(hydroxyethylamino) derivative **15** fully consistent

with the observations from the genetically engineered strains. No compounds were found to display significant susceptibility to the presence of AAC(6').

Activity and Selectivity at the Drug Target

Turning to the investigation of activity at the target level, the ribosomal decoding A site is the well-established target for aminoglycosides in general[2, 11] and for propylamycin **1** in particular.[1] We employed cell-free translation assays to test the ability of the various compounds to inhibit protein synthesis by wild-type bacterial ribosomes as previously described (Table 4).[38] Consistent with expectation, and the mode of action of the DOS type AGAs by binding to the ribosomal decoding A site,[11, 39–43] the pattern of IC_{50} values for inhibition of protein synthesis by the bacterial ribosome largely followed that for antibacterial activity against the wild-type ESKAPE pathogens (Table 1), with the most active compounds being propylamycin **1**, and 5"-deoxy-5"-formamidopropylamycin **11**.

As AGA binding to the cognate decoding A sites of the human mitochondrial and especially the A1555G mutant mitochondrial ribosomes in the cochlea is considered to be one of the main causes of AGA-induced ototoxicity,[11, 44–50] we also screened for inhibition of protein synthesis by a set of humanized bacterial ribosomes in which the complete bacterial decoding A site has been replaced by that of the human mitochondrial (Mit13) or A1555G mutant mitochondrial ribosome (A1555G) (Figure 3).[51] Finally, to assess the possibility of broader systemic toxicity, we screened for inhibition of protein synthesis by similarly engineered bacterial ribosomes carrying the human cytosolic decoding A site (Cyt14) (Figure 3). Consistent with our earlier observations and as borne out by in-vivo studies using the guinea pig model for ototoxicity,[1] propylamycin **1** exhibits increased selectivity over the parent paromomycin **2** for the A site of the bacterial over the human mitochondrial and especially the mutant mitochondrial ribosome; it also exhibits consistently high selectivity for the A site of the bacterial over the human cytosolic ribosome (Table 1). The selectivity profile of 3'-deoxyparomomycin **3** on the other hand is close to that of paromomycin itself. In contrast, the 5"-deoxy-5"-amino derivatives of propylamycin **8**, **9**; and **10** show much reduced selectivity for each of the mitochondrial, mutant mitochondrial and cytosolic ribosomes suggestive of a reduced therapeutic index for these compounds. Compound **13**, which also carries a basic amino group at the 5"-position albeit with an acetyl spacer, also suffers from this phenomenon. These observations are intriguing as we have previously demonstrated that apralogs carrying basic amino groups at the 5-position of the appended ribofuranosyl ring, as exemplified by **6**, show reduced affinity for each of the three humanized ribosomes that is reflected in increased selectivity and reduced outer hair cell loss in the ex-vivo mouse cochlear explant system.[18, 19] In contrast, Baasov and coworkers have demonstrated that 5"-amino-5"-deoxyribostamycin derivatives show increased affinity for the cytosolic ribosome which, coupled with reduced antibacterial activity, renders them attractive for the treatment of genetic diseases due to the mutation of an amino acid codon to a premature stop codon, and is reflected in the ongoing clinical trials of **ELX-02** (Figure 4).[52–54]

In contrast to the 5"-amines, the 5"-formamido derivative **11** showed excellent selectivity for the bacterial ribosome over the three humanized ribosomes surpassing that of propylamycin

itself, while the ureido derivative **12** showed across the board selectivity comparable to propylamycin. 3-Deoxypropylamycin **14** was similar to propylamycin itself and showed good across the board selectivity. Finally, the activity of the formamido derivative **11** at the target level is comparable to that of the clinical AGAs gentamicin and plazomicin, while its selectivity is vastly superior.

Conclusions

Replacement of the 5”-hydroxy group in propylamycin by either an amino group or a derivatized amino group overcomes the susceptibility of this AGAs to inactivation by APH(3’) isozymes capable of acting at the 5”-position. Unfortunately, and in contrast to the apralog series, this type of substitution conveys a small reduction in inhibition of the bacterial ribosome and an increase in inhibition of the eukaryotic ribosomes resulting overall in significantly reduced selectivity. In contrast, the 5”-formamido derivative of propylamycin combines propylamycin-like levels of antibacterial activity with increased selectivity for the bacterial over the eukaryotic ribosomes, all while overcoming the APH(3’)-resistance determinants that act at the 5”-position. The 3’-deoxypropylamycin derivative shows no advantage over propylamycin itself, with the presence of the propyl group at the 4’-position being sufficient to retard the action of the APH(3’) AMEs on the 3’-hydroxy group.

Methods.

Cell-free luciferase translation assays.

Cell-free in-vitro translation inhibition assays were performed using luciferase mRNA and bacterial S30 extracts containing either wild-type bacterial or human hybrid ribosomes.[55] In brief, firefly luciferase mRNA was transcribed in vitro using T7 RNA polymerase (Thermo) using a plasmid as template in which the mammalian promoter in pGL4.14 (Promega) has been replaced by theT7 bacteriophage promoter. Test articles in aqueous solution containing 0.3% Tween20 were dispensed into white 96-well plates (Eppendorf) using the TECAN D300e digital dispenser. The test article dispension volume was balanced to a total of 1.5 μL by 0.3% Tween20 in water. The reaction volume was brought to 15 μL by addition of 13.5 μL Translation Master Mix comprised of bacterial S30 extract, 0.2 mM amino acid mix, 6 μg tRNA (Sigma), 0.4 μg hFluc mRNA, 0.3 μL protease inhibitor (cOmplete, EDTA-free, Roche), 12 U RNAse inhibitor (Ribolock, Thermo Scientific), and 6 μL S30 premix without amino acids (Promega). Dispension and mixing of reagents was performed on ice prior to incubating the sealed plates at 37 °C. After 30 minutes of incubation, the reaction was stopped on ice and 75 μL of luciferase assay reagent (Promega) was added to each well. Luminescence was recorded with a plate reader (BIO-TEK FLx800, Witec AG, Littau, Switzerland).

Antibacterial inhibition assays.

The minimal inhibitory concentrations (MIC) of synthesized compounds were determined by broth microdilution assays according to CLSI reference methodology M07[56] as described previously.[57] A summary of bacterial strains used in this study is provided in Table S1. Clinical bacterial isolates were obtained from the diagnostic laboratories of the

Institute of Medical Microbiology, University of Zurich. Whole genome sequencing of the bacterial isolates and bioinformatic annotation of resistance genes was done as described previously.[57]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments.

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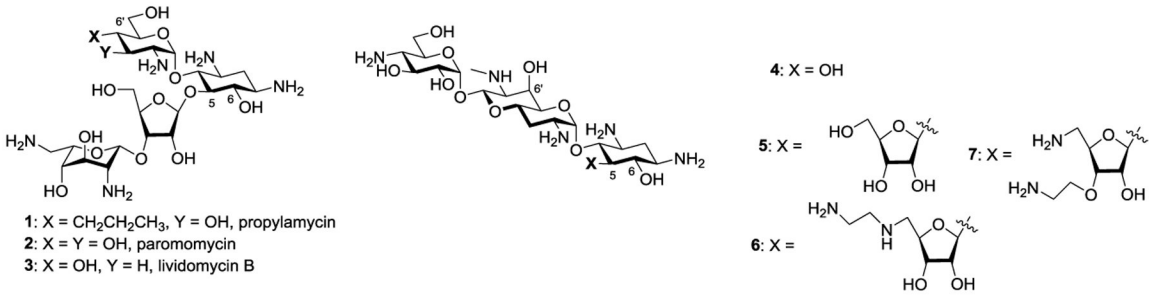


Figure 1.
Propylamycin, Paromomycin, Lividomycin B and the Apralogs.

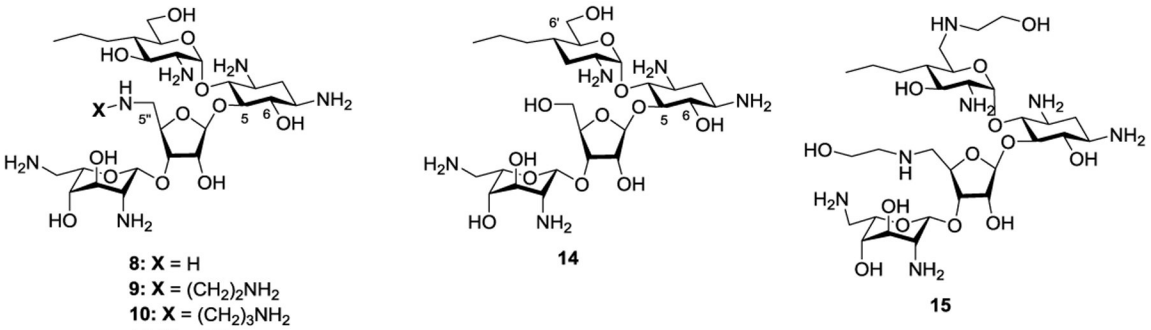


Figure 2.
Target Propylamycin Derivatives.

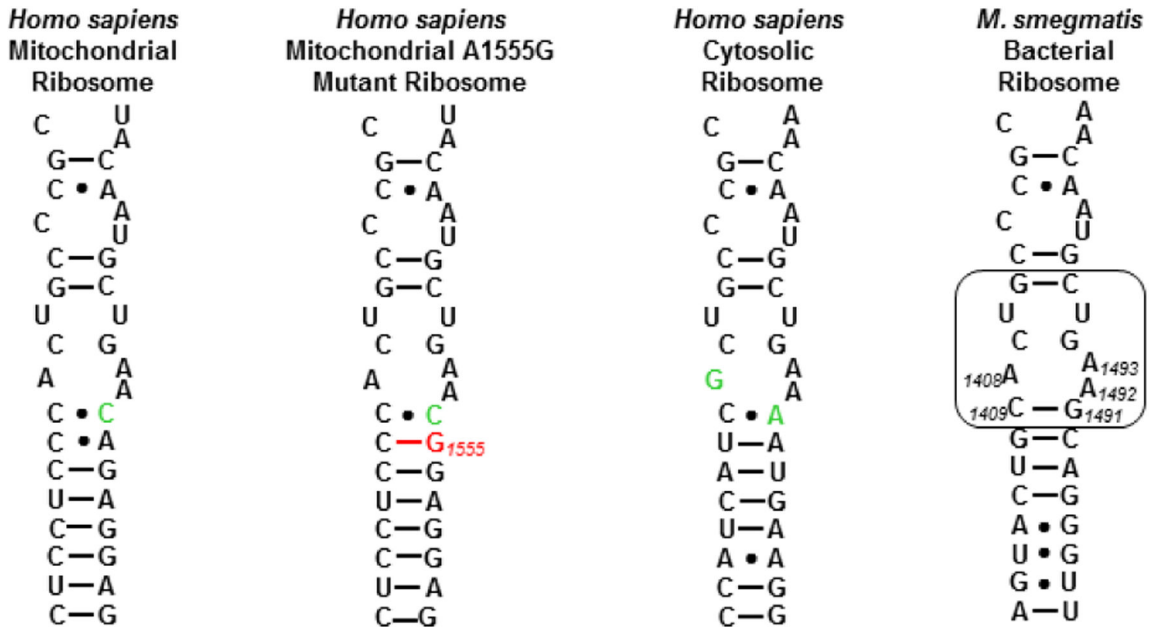


Figure 3.

Decoding A sites of prokaryotic and eukaryotic ribosomes. The bacterial AGA binding pocket is boxed. The bacterial numbering scheme is illustrated for the AGA binding pocket. Changes from the bacterial ribosome binding pocket are coloured green. The A1555G mutant conferring hypersusceptibility to AGA ototoxicity is coloured red.

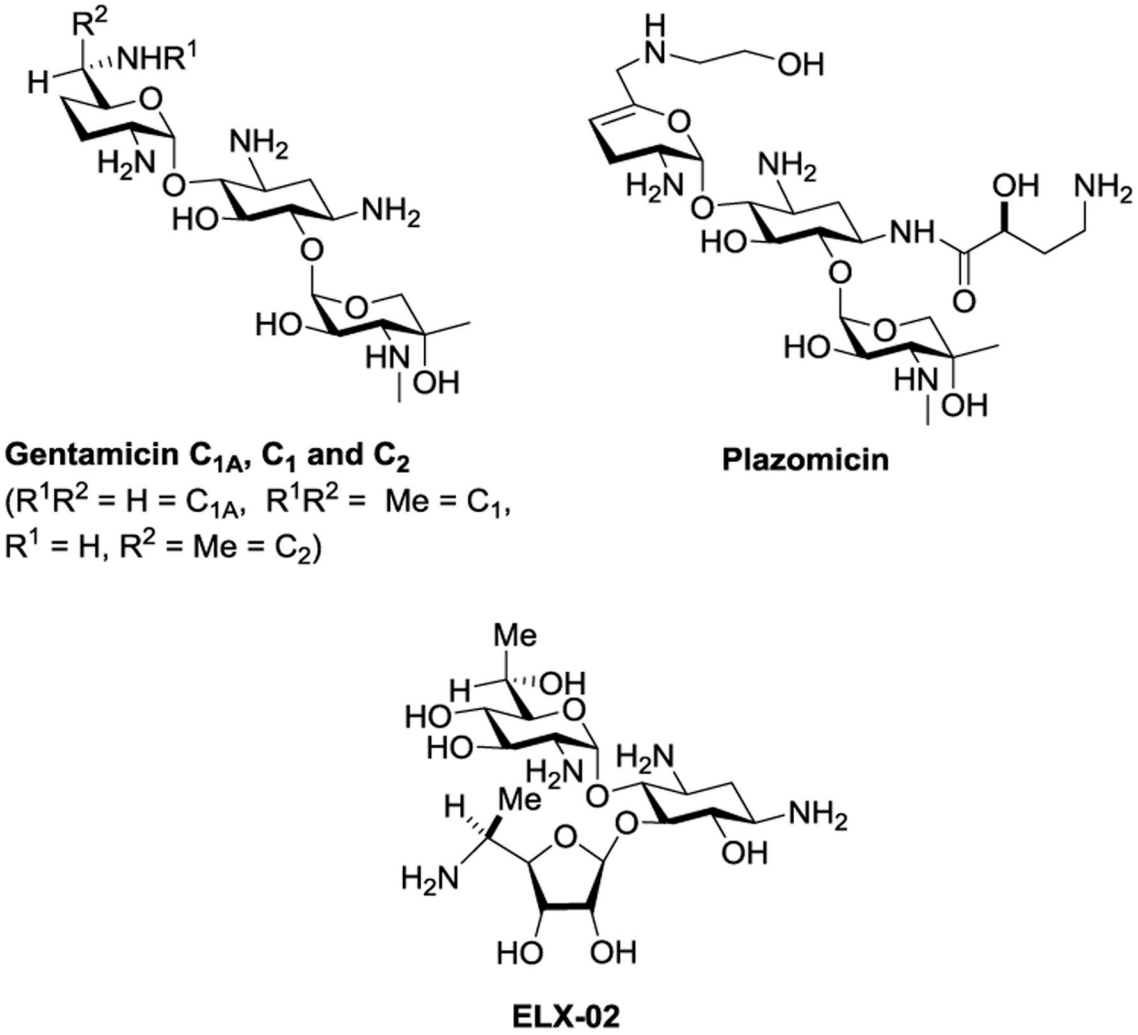
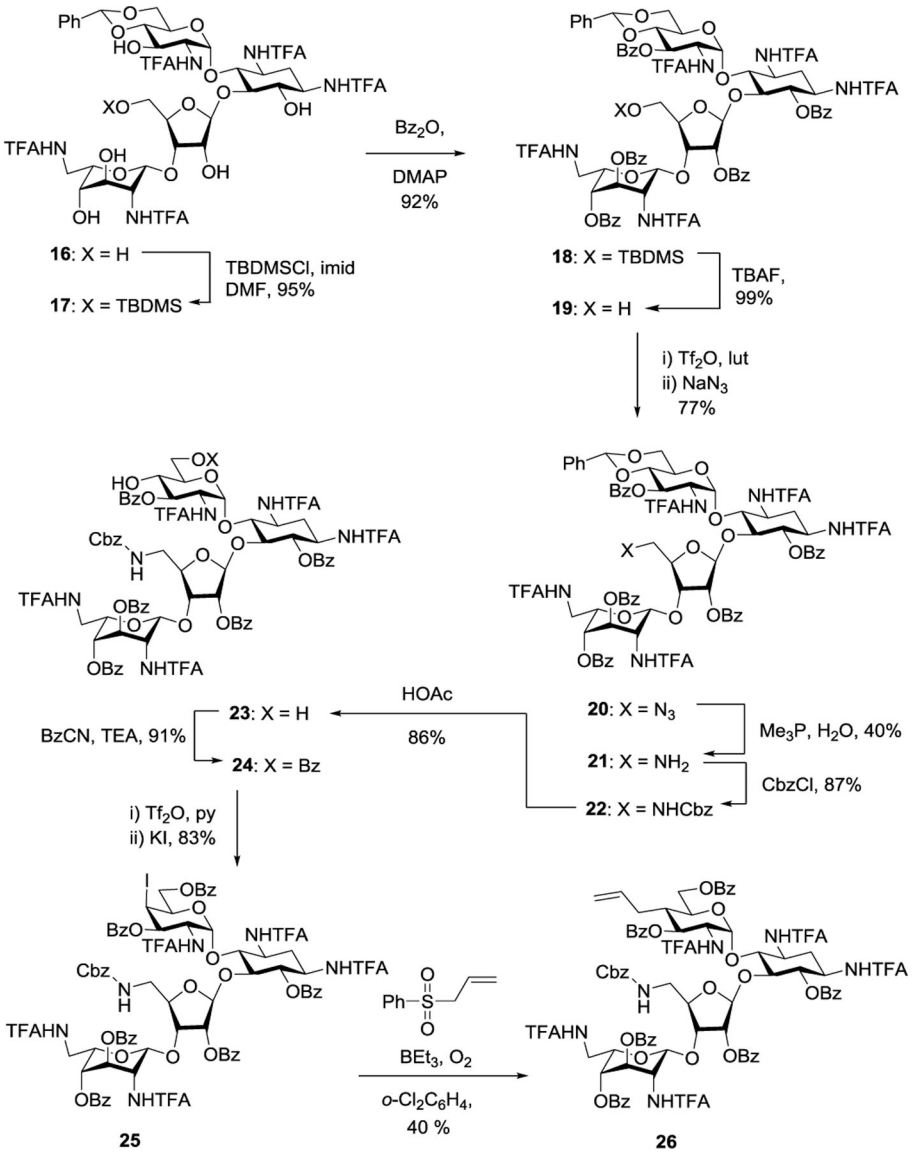
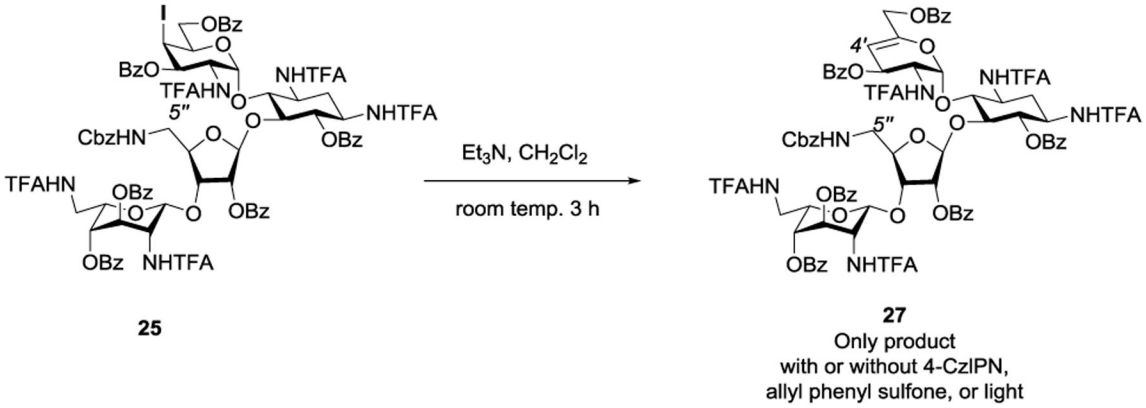


Figure 4.
Structures of the Clinical Drugs Gentamicin and Plazomicin and of the Experimental Drug ELX-02



Scheme 1.

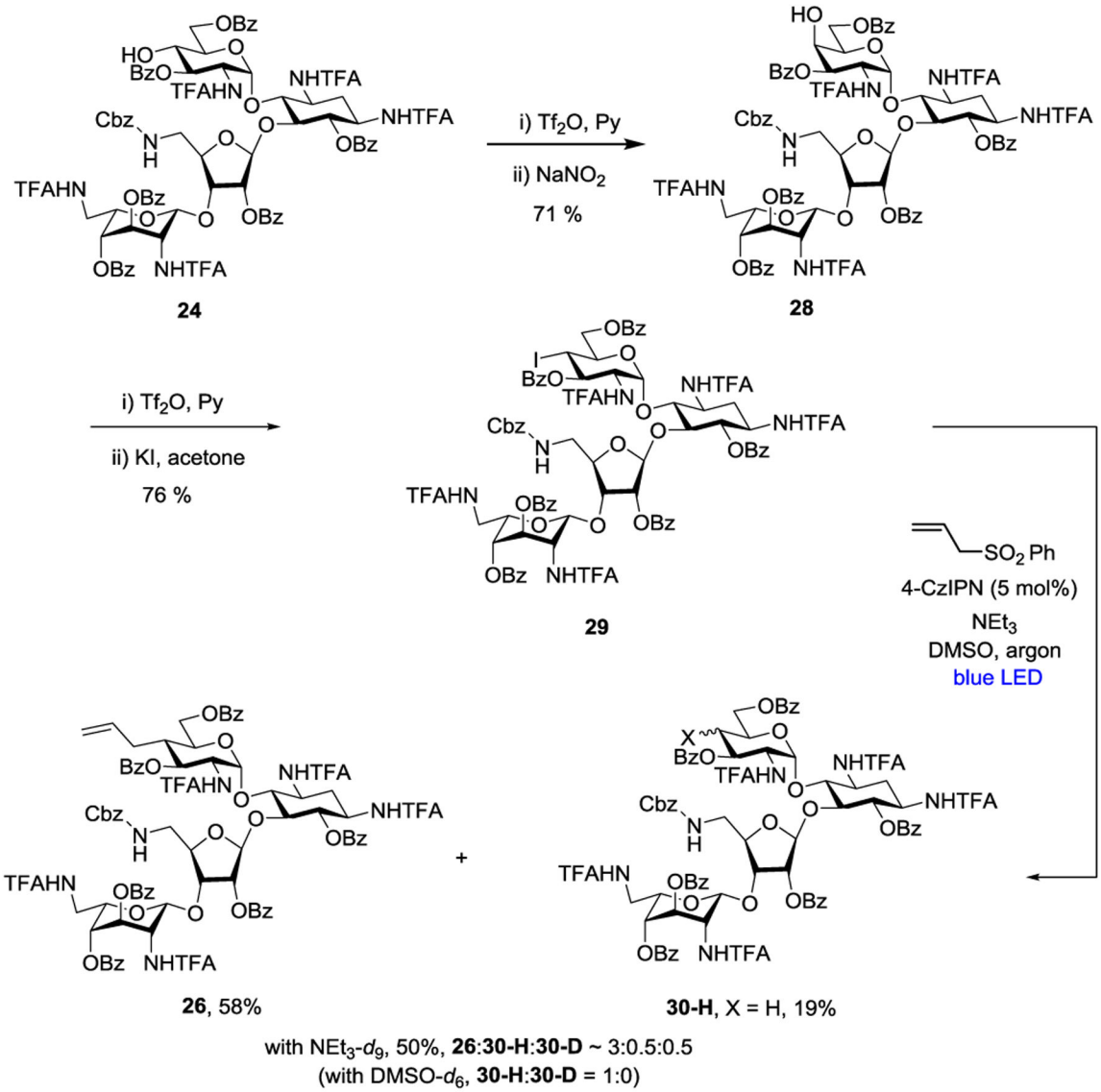
Synthesis of Key Intermediate **26** en route to propylamycin derivatives **8–13**



25

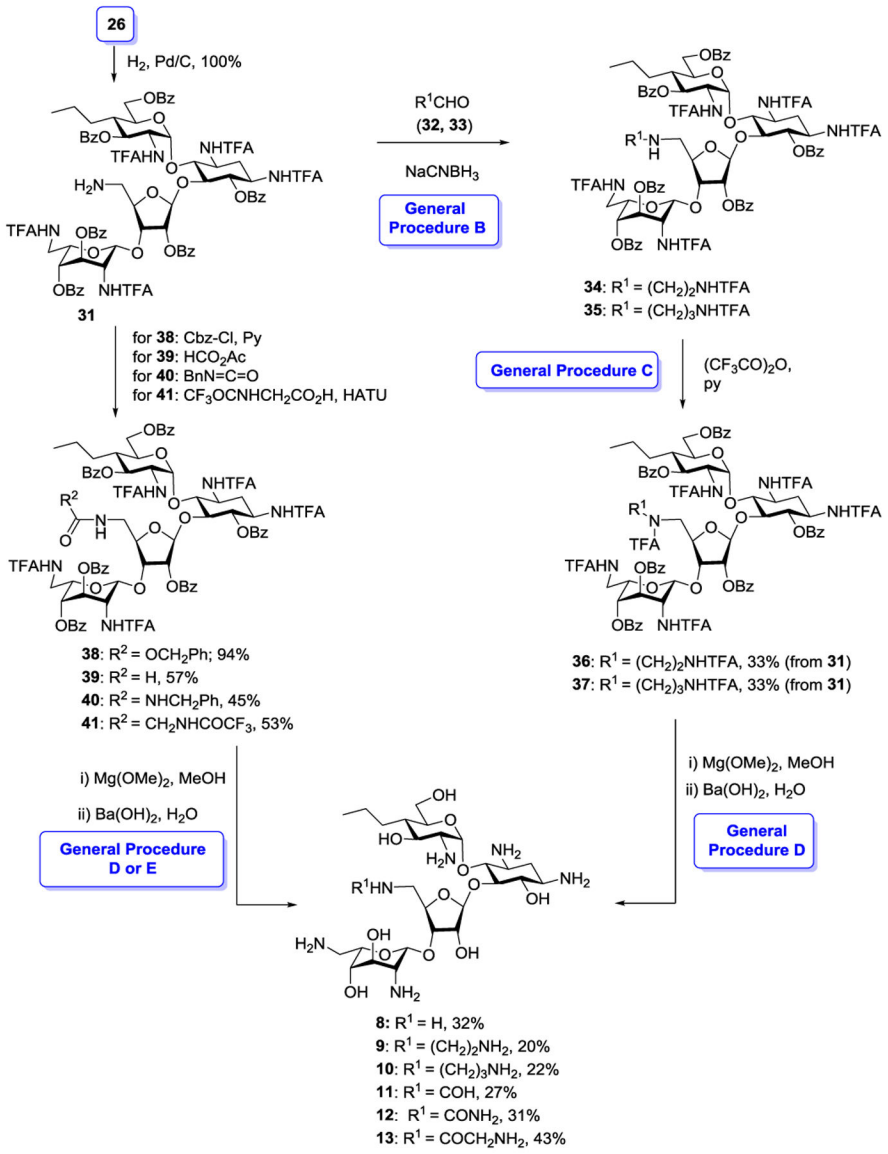
27
Only product
with or without 4-CzIPN,
allyl phenyl sulfone, or light

Scheme 2.
Competing Elimination of Attempted Photocatalytic Allylation of **25**



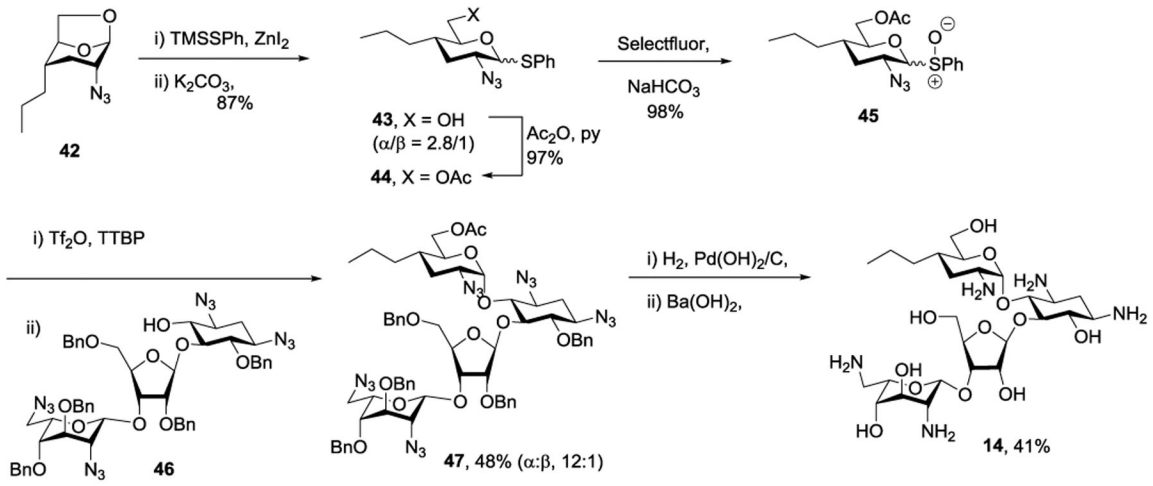
Scheme 3.

Competing Photocatalytic Allylation and Reduction of Equatorial Iodide **29**

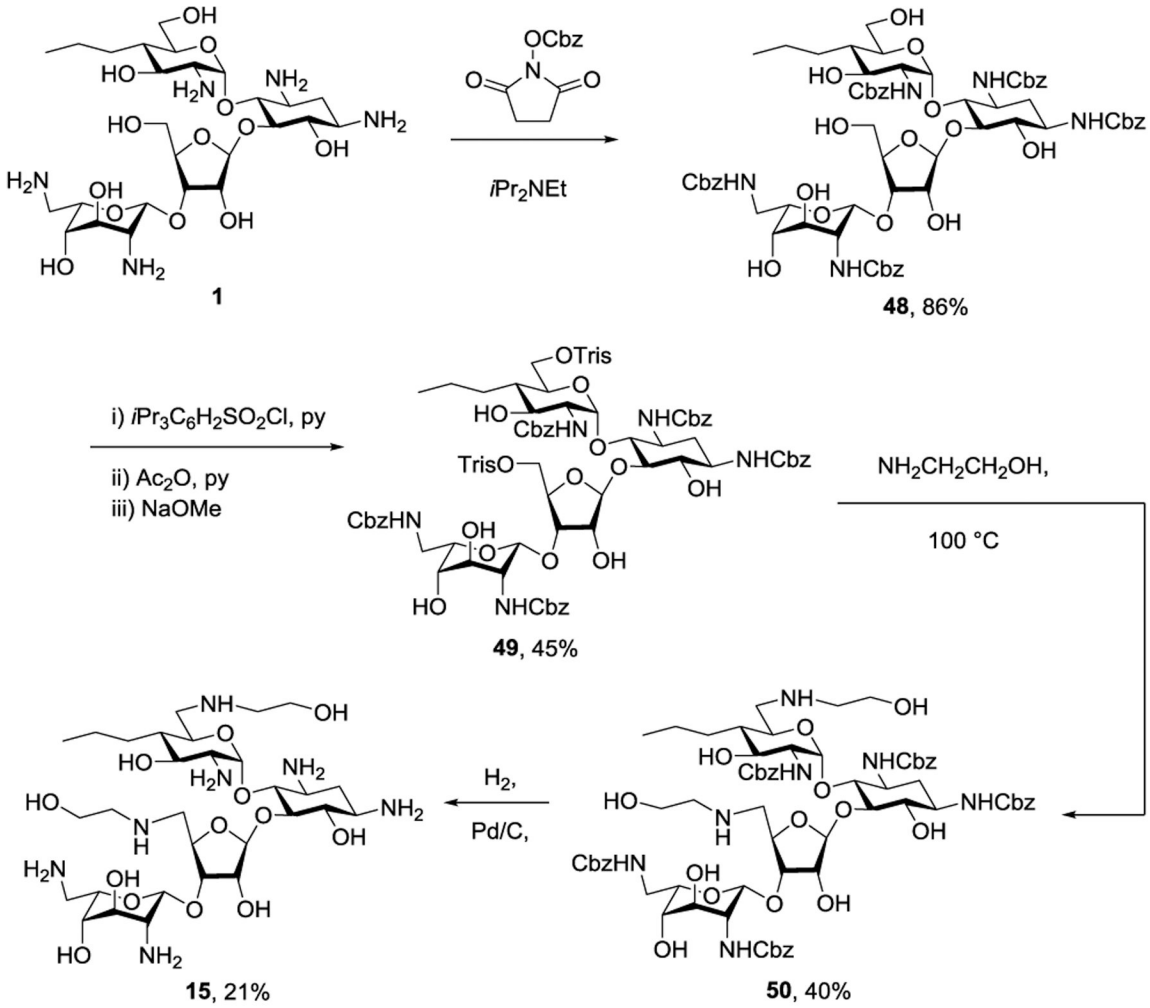


Scheme 4.

Completion of 5”-*N*-Functionalized Propylamycin Derivatives **8–13**



Scheme 5.
Synthesis of 3'-Deoxypropylamycin **14**



Scheme 6.

Synthesis of 6',5"-Dideoxy-6',5"-bis(ethanolamino)propylamycin **15**

Table 1.

Antibacterial Activities Against Wild-Type *E. coli* and ESKAPE Pathogens (MIC, μg/mL)[a]

Species	MRSA	*E. coli*	*K. pneu.*	*Enterob.*	*A. baum.*	*P. aerug.* [b]
Strain	**AG038**	**AG001**	**AG215**	**AG290**	**AG225**	**AG220**
Propylamycin 1	1–2	1	0.25–0.5	0.5	1–2	8–16
Paromomycin 2	2	2–4	1	2	2–4	>128
Lividomycin B 3	4	4–8	2	2	4	4
8	2	2	1	1	2	4
9	1–2	2–4	1	4	4	4
10	2	2–4	2	8	8	16
11	1	1	1	1	2	8
12	2	4	2–4	2–4	2–4	128
13	2	2–4	2	2	2	4
14	2	1–2	1	1	2	4
15	4	4	2	2	32	>64
Gentamicin	1–2	0.5–1	0.25	0.25	0.5–1	1
Plazomicin	2	1	0.5	0.5	2	2–4

[a] All values were determined in duplicate using twofold dilution series.

[b] *P. aeruginosa* carries a chromosomal APH(3') gene, which principally affects the 3'-hydroxy group.

Table 2.

Activities Against *E. coli* in the Presence of Specific Resistance Determinants (MIC, μg/mL)[a]

Resistance det	**WT-parental**	**APH(3')-Ia**	**APH(3')-IIa**	**APH(3')-IIb**	**APH(3')-VI**	**AAC(3)-IV**	**ArmA**	**RmtB**
Strain	**EC026**	**EC189**	**EC191**	**EC125**	**EC141**	**EC118**	**EC102**	**EC103**
Propylamycin 1	0.25–0.5	64–128	0.5–1	0.5–1	0.5	1–2	0.5	0.5
Paromomycin 2	0.5–1	128	64	64–128	64	1–2	0.5–1	0.5–1
Lividomycin B 3	1–2	>128	1–2	0.5	1	2	1	1–2
8	0.5–1	2–4	1–2	0.5–1	0.5–1	2–4	2–4	2–4
9	0.5–1	4	1–2	0.5–1	0.5	2	1–2	2
10	1–2	8	4	4	1–2	8	4	4–8
11	0.5–1	2	0.5–1	0.5	0.25	2	2	4
12	1	8–16	2–4	2–4	0.5–1	16	8	4
13	0.5–1	2–4	1–2	0.5–1	0.5	4	4	2–4
14	1	>128	1–2	0.5–1	1	2–4	1	2
15	2	>32	4	4	1–2	16	4	4–8

[a)] All values were determined in duplicate using twofold dilution series.

Table 3.

Activities Against *E. coli* in the Presence of Acquired Resistance Determinants (MIC, μg/mL)[a]

Resistance det	**WT-parental**	**AAC(3)-II**	**AAC(3')-IV**	**APH(3')-I**	**APH(3')-II**	**AAC(6')**
Strain	**AG001**	**AG003**	**AG173**	**AG163**	**AG166**	**AG175**
Propylamycin 1	1	2	2	>128	1–2	2–4
Paromomycin 2	2–4	4	8–16	>128	>128	4
Lividomycin B 3	4–8	8	8–16	>128	8	8
8	2	4–8	4	16	2–4	4–8
9	2–4	2–4	4	4–8	4	4
10	2–4	4–8	8–16	16–32	4	4–8
11	1	2	4–8	4–8	2	2–4
12	4	4–8	32	32	16–32	4–8
13	2–4	4–8	8	8–16	4	4–8
14	1–2	4	4–8	>128	4	4
15	4	8	4	>32	16	8–16

[a] All values were determined in duplicate using twofold dilution series.

Table 4.

Antiribosomal Activities and Selectivities (IC_{50}, μM).

	Antiribosomal Activity				Selectivity		
	wt	**Mit13**	**A1555G**	**Cyt14**	**Mit13**	**A1555G**	**Cyt14**
Propylamycin 1	0.022±0.005	150±51	56±17	61±14	6818	2545	2773
Paromomycin 2	0.033±0.006	128±67	12±3	38±6	3879	364	1152
Lividomycin B, 3	0.084±0.030	182±35	24±1	98±12	2167	286	1140
8	0.14±0.04	62±24	38±9	9.0±1.6	443	271	64
9	0.091±0.008	55±10	36±8	31±2	604	396	341
10	0.46±0.09	41±12	25±4	24±2	89	54	52
11	0.034±0.014	505±208	118±12	175±14	14991	3471	5147
12	0.078±0.007	318±69	189±66	194±15	4077	2423	2487
13	0.090±0.018	40±16	10±4	6.1±0.4	444	111	68
14	0.064±0.005	204±25	93±31	305±58	3188	1453	4766
15	0.40±0.04	89±6	54±6	68±3	222	135	170
Plazomicin	0.065±0.020	107±33	5.9±2.1	299±82	1633	90	4569
Gentamicin	0.029±0.009	15±4	0.80±1.5	74±41	517	28	2552