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Article

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Structure-Activity Relationships for Antibacterial to Antifungal Conversion of Kanamycin to Amphiphilic Analogs

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ABSTRACT. Novel fungicides are urgently needed. It was recently reported that the attachment of an octyl group at the *O*-4" position of kanamycin B converts this antibacterial aminoglycoside into a novel antifungal agent. To elucidate the structure-activity relationship (SAR) for this phenomenon, a lead compound FG03 with a hydroxyl group replacing the 3"-NH_2 group of kanamycin B was synthesized. FG03's antifungal activity and synthetic scheme inspired the synthesis of a library of kanamycin B analogs alkylated at various hydroxyl groups. SAR studies of the library revealed that for antifungal activity, the *O*-4" position is the optimal site for attaching a linear alkyl chain, and that the 3"-NH_2 and 6"-OH groups of the kanamycin B parent molecule are not essential for antifungal activity. The discovery of lead compound, **FG03,** is an example of reviving clinically obsolete drugs like kanamycin by simple chemical modification and an alternative strategy for discovering novel antimicrobials.

Introduction

Antifungal drug discovery is relatively neglected in medicine when compared to the investment in the development of antibacterial, antiviral and anti-cancer therapeutics. And yet, pathogenic fungi, such as *Aspergillis fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, and *Histoplasma capsulatum* pose serious threats to human health.[1] Additionally, exposure to mycotoxins produced by molds presents great challenges to health and food safety and security. Only a few new fungicides have been introduced since the mid-1980s.[2] Resistance to existing antifungal drugs is a major part of these problems. Many fungicides used in agriculture are chemically synthesized heterocyclic compound-based, such as triazoles and pyrimidines, and they highly resemble the antifungal drugs used for treatment of fungal infections in human. As a consequence, resistant fungi found in agriculture and the environment have counterparts that have evolved among pathogenic fungi found in humans.[3] Thus, there is an urgent need for the development of novel fungicides.

Kanamycin is a class of aminoglycoside antibacterial agent.[4] Nevertheless, it has become clinically obsolete due to the emergence of bacterial pathogens that are resistant to aminoglycoside antibiotics.[5] Extensive efforts have been devoted to the chemical modification of kanamycin with the goal of reviving its activities against resistant bacteria.[6] In the past, these studies have focused primarily on the structure-activity relationship (SAR) of antibacterial activities. More recent discoveries, however, that amphiphilic aminoglycosides can exert unexpected non-bacterial antimicrobial activities have led to new strategies for broadening the applications of kanamycin as well as other aminoglycosides.[7,8]

We have previously reported the synthesis and antifungal investigation of a novel broad-spectrum fungicide (**FG08**) (Figure 1).[8] Plant leaf infection assays and greenhouse studies showed that **FG08** is capable of suppressing wheat fungal infections by *Fusarium graminearum* – the causative agent of Fusarium Head Blight. **FG08** can be viewed as a kanamycin derivative with three distinct structural modifications on ring III: a linear octyl group at *O*-4" position, deoxygenation at *O*-6" position and the replacement of 3"-NH_2 with OH. Among these modifications, the attachment of *O*-4" octyl group is essential for converting the antibacterial kanamycin into an antifungal agent. Further investigation confirms that **FG08** exerts its antifungal activity by specifically increasing the permeability of the fungal plasma membrane - a mechanism of action that differs from the antibacterial action of kanamycin of binding ribosomal RNA and interfering with protein synthesis.[8a,b] Shortening the octyl chain to a butyl group (**FG01**) or extending the chain length to a dodecyl group (**FG02**) diminished the antifungal activity **(**Figure 1**)**. These findings prompt further questions about the structural features that cause the antibacterial to antifungal conversion. Major questions include: 1) Is substitution of 6"-CH_3 for the kanamycin 6"-OH on ring III as in **FG08** needed for antifungal activity? 2) Is the attachment of the octyl chain at the *O*-4" position required for optimal antifungal activity? and 3) Does the presence of the 3"-NH_2 group contribute to the antifungal activity of **FG08**? Herein, we report a comprehensive three-stage SAR study to answers these questions. We designed **FG03** to answer the first question. Since multiple steps are required for the deoxygenation process that produces the 6"-CH_3, keeping the initial

kanamycin 6"-CH_2OH moiety without losing antifungal activity will be crucial for simplifying and scaling up the synthesis of lead antifungal compounds.

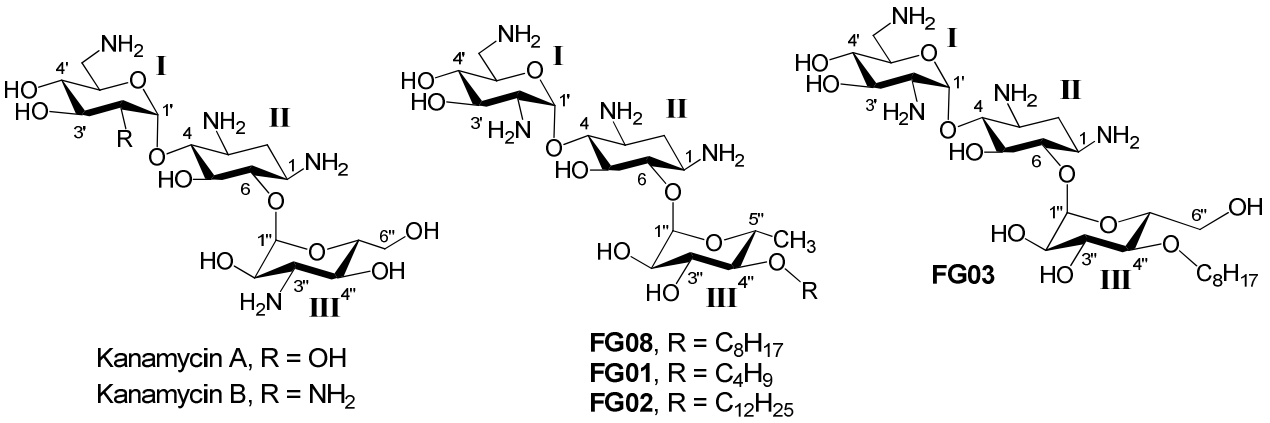


Figure 1. Structures of Kanamycin and Its Amphiphilic Derivatives in the 1st Stage SAR Investigation

Results and Discussion

The synthesis of **FG03** possessing 6"-OH and 4"-octyl groups (Figure 1) started from the 1,3-diol **1**.[9] Tritylation of **1** selectively protected the primary alcohol, leaving a free hydroxyl group at position 4 (Scheme 1). Alkylation of the 4-OH, followed by the acid-catalyzed removal of the trityl group revealed the 6-OH in compound **2**. Benzylation afforded the thiophenylglycoside, **3** as the glycosyl donor. Glycosylation of **4** using the optimal condition[14] developed in our group previously to ensure the formation of α glycosidic bond, and followed by deacetylation, gave **5**. Since reduction of azides and hydrogenolysis of benzyl ether in one-step fashion can be problematic, we decided to adopt a two-step process for the global deprotection.[17] Staudinger reduction of compound **5** that converted the azido groups into amines followed by hydrogenolysis of benzyl ethers, and ion-exchange, provided **FG03** as a chloride salt. The minimum inhibitory concentrations (MICs) of **FG03** were determined (Table 1). Despite possessing a 6"-OH instead of 6"-CH_3 group, **FG03** was found to be as effective as **FG08** in inhibiting the growth of a number of fungi including *F. graminearum* and lack antibacterial activity.

Scheme 1.

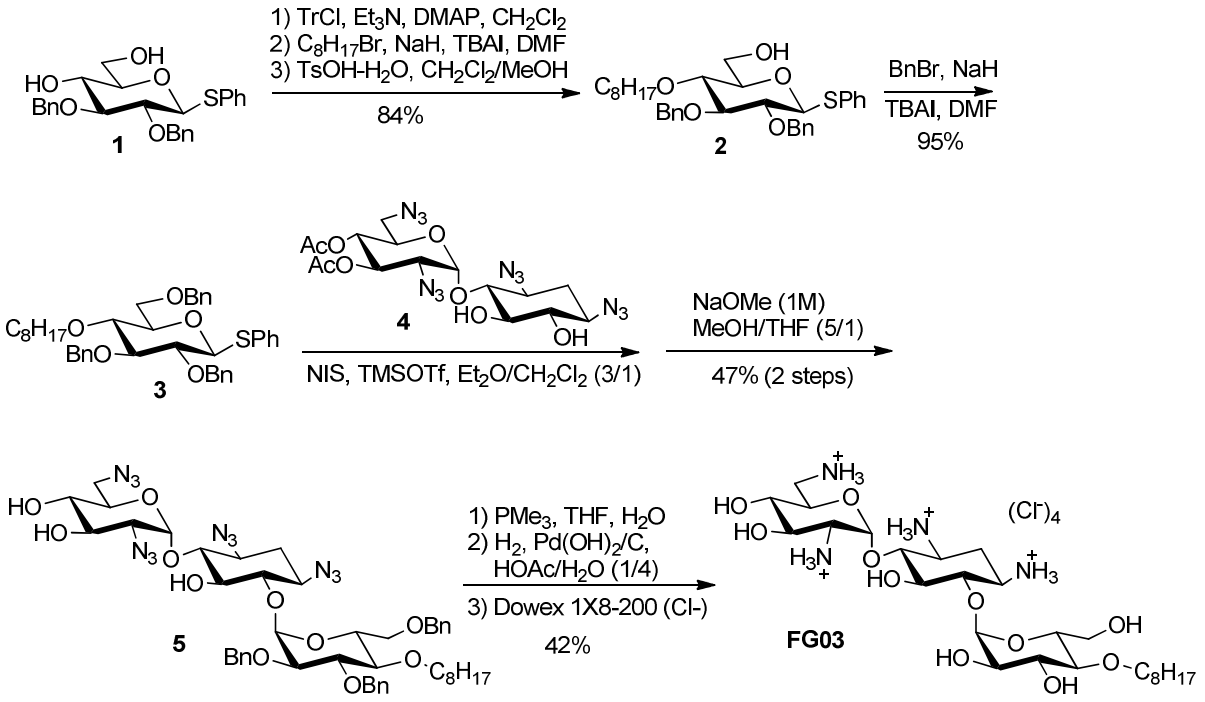


Table 1: MIC (μg/mL) Values of **FG08** and **FG03**[a]

Organism	FG08	FG03
Bacteria		
Escherichia coli TG1[a]	125-250	>500
*Staphylococcus aureus (*ATCC25923)[b]	250	ND[c]
Filamentous fungi		
Fusarium graminearum B-4-5A	7.8	7.8
Pythium ultimum	15.6	62.5
Curvularia brachyspora	31.3	31.3
Bortrytis cinerea	31.3	31.3
Yeasts		
Rhodotorula pilimanae (ATCC26423*)*	7.8	62.5
Candida albicans (ATCC10231)	31.3	62.5

[a] Gram-negative bacteria, [b] Gram-positive bacteria, [c] Not determined

The observed antifungal activities of **FG03** led to the designs of other kanamycin derivatives with 6"-OH and the 2nd stage SAR investigation. In this stage, we planned to determine if octylation at other

hydroxyl groups will enable the antibacterial to antifungal conversion (Figure 2). The design for **FG05** is two-carbon shorter than **FG06** since the position of alkylation is *O*-6" which is considered extended as compared to the octyl group at *O*-4" position (**FG03**). The designs of **FG10** and **FG11** were intended to help understand the role of 3"-NH_2 as well as the effect of the octyl group at the *O*-5 position.

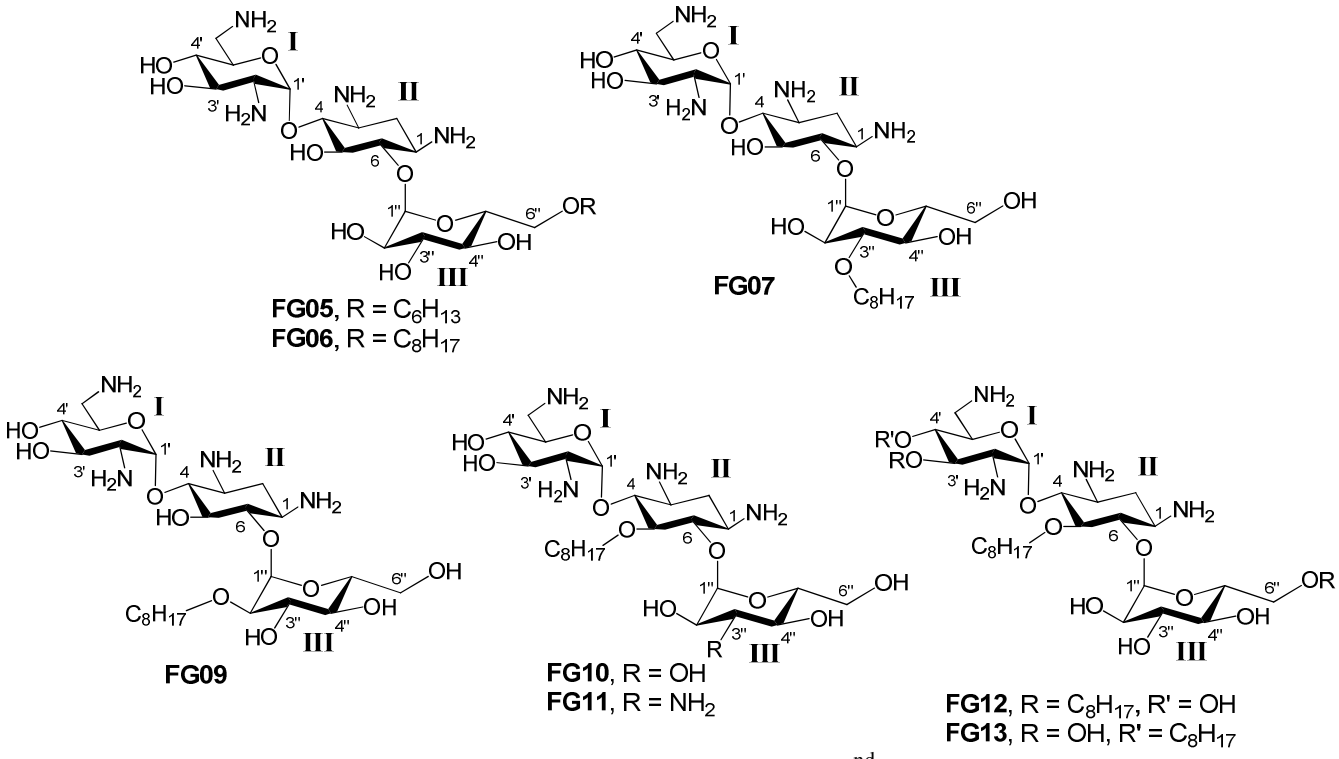
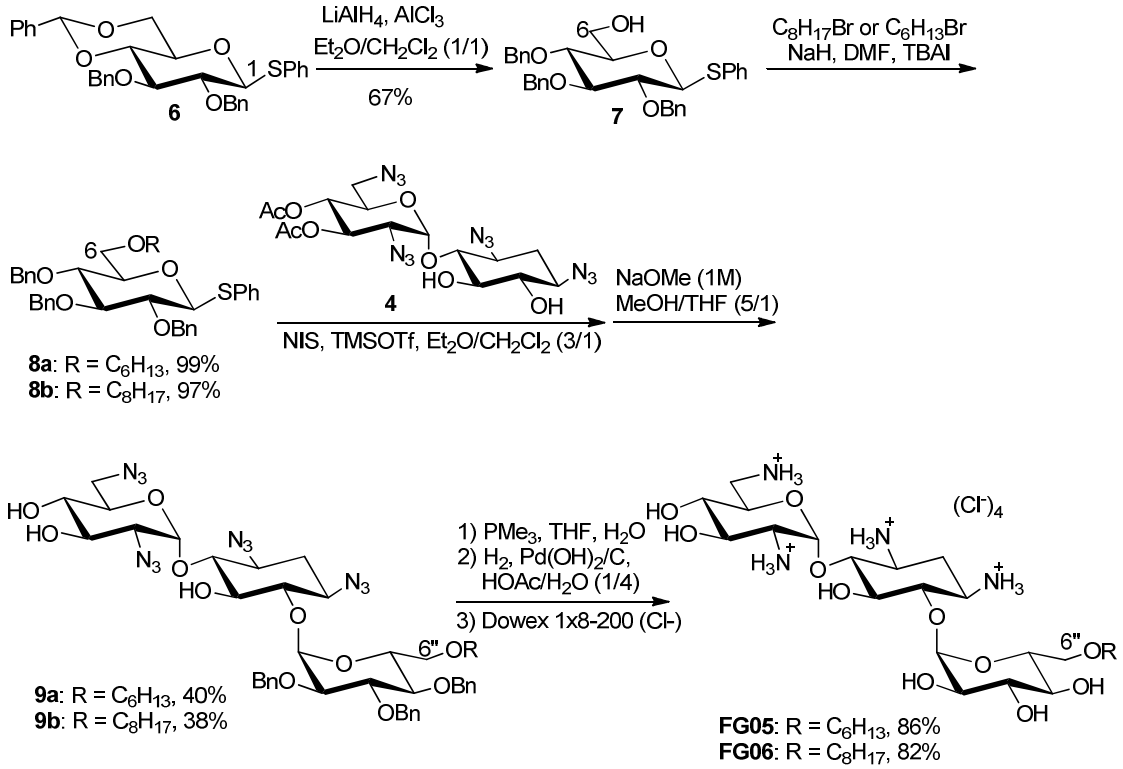


Figure 2. Structures of Amphiphilic Kanamycin Derivatives in the 2nd Stage SAR Investigation

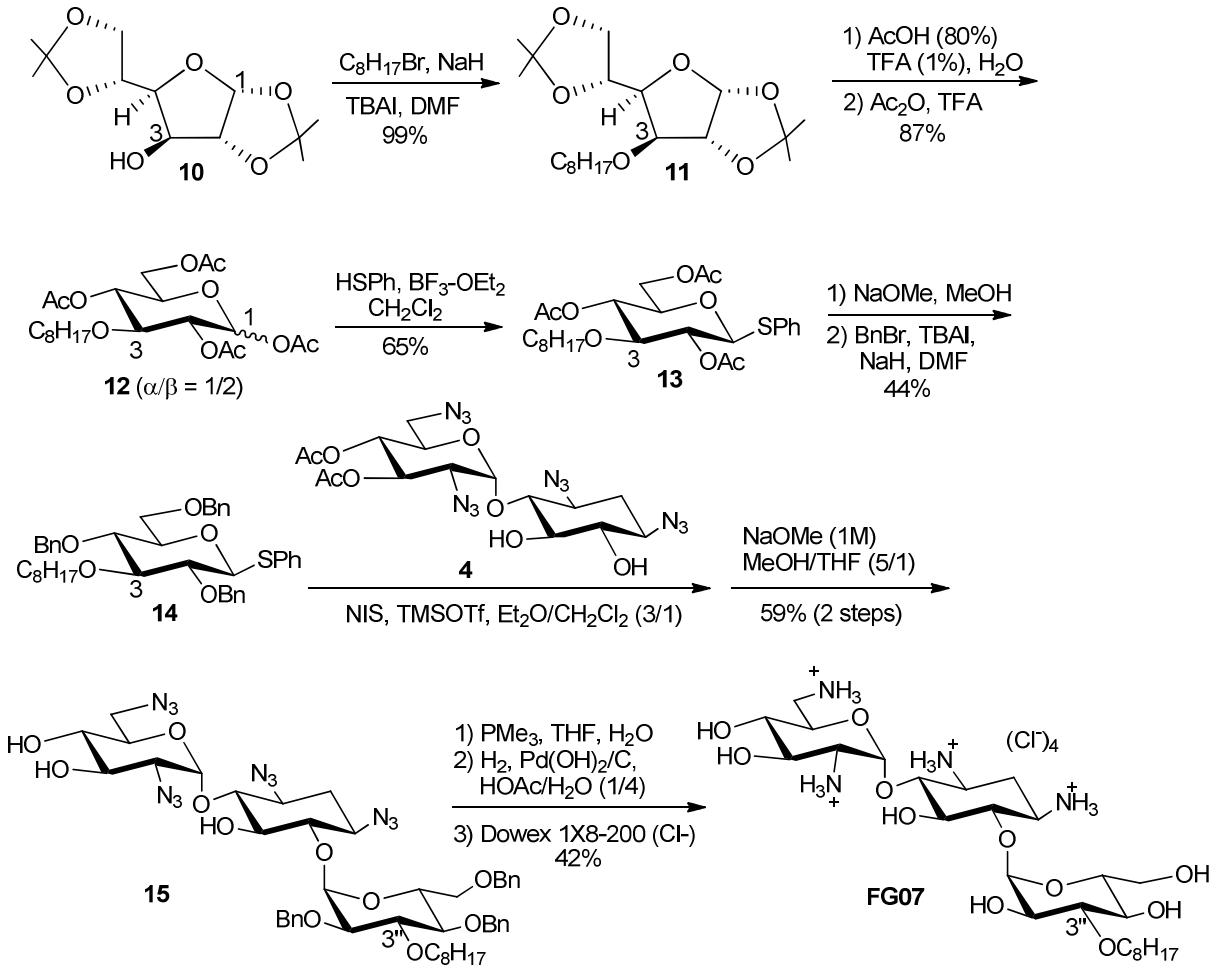
The synthesis of **FG05** and **FG06** commenced with regioselective ring opening of the known compound **6**[10] to obtain **7**[11] with a free 6-OH (Scheme 2). Alkylation using *n*-hexyl bromide and *n*-octyl bromide provided the phenylthioglycosides as the glycosyl donors, **8a** and **8b**, respectively. Glycosylation followed by deacetylation gave **9a** and **9b**. Staudinger reaction, hydrogenation, and ion-exchange afforded **FG05** and **FG06**, with C6 and C8 alkyl chain at the *O*-6" position, respectively.

Scheme 2.



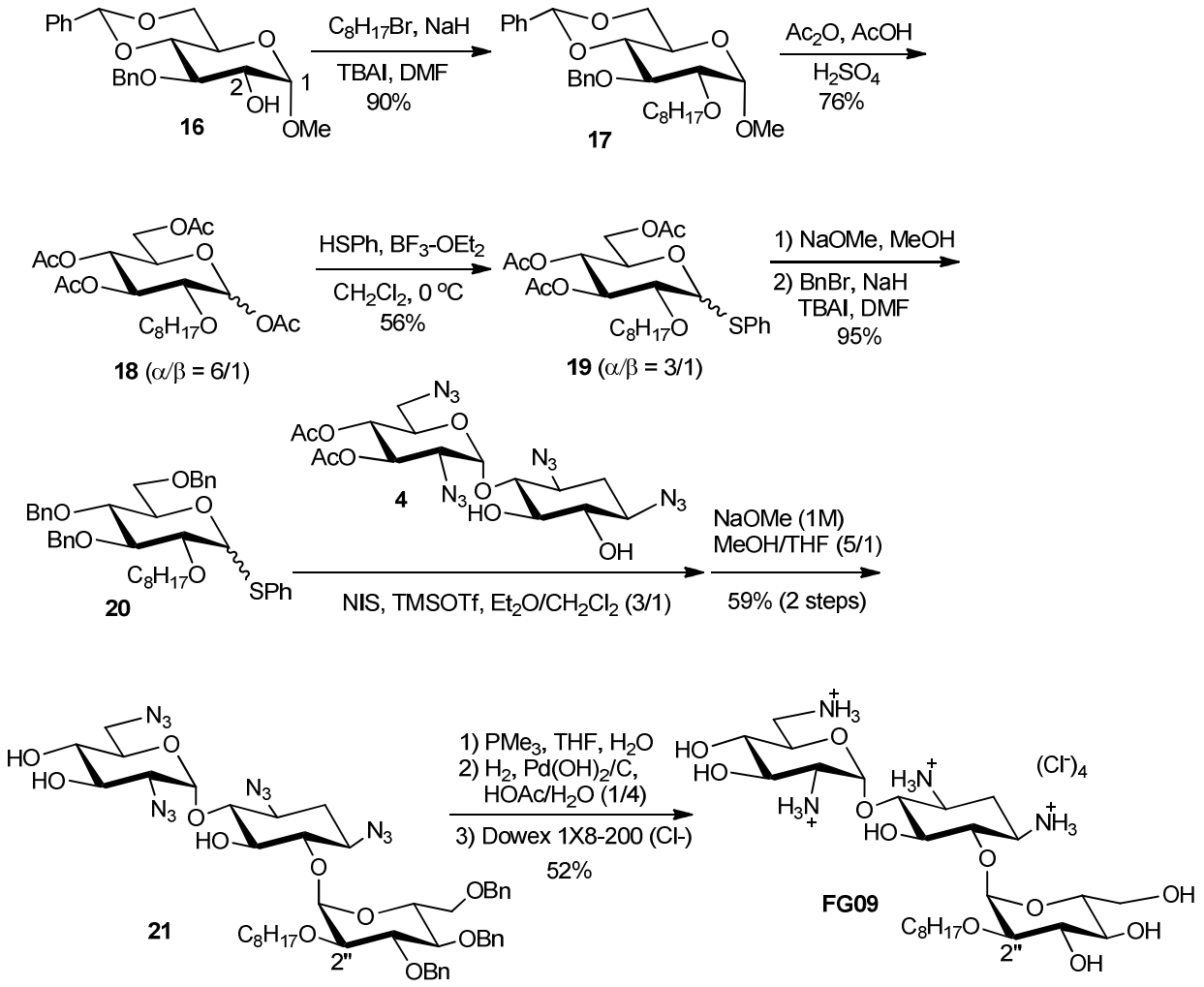
The synthesis of **FG07** started with the alkylation of diacetone-D-glucose **10** using octyl bromide to give the known compound **11**[12] (Scheme 3). Acid-catalyzed hydrolysis of compound **11** followed by acetylation provided **12**. Treatment of **12** with thiophenol in the presence of BF_3-OEt_2 gave **13** as a pure β-anomer. Deprotection of the acetyl groups of **13**, followed by benzylation, afforded the phenylthioglycoside, **14** as the glycosyl donor. Glycosylation of **4** using **14** in the presence of NIS and TMSOTf, followed by deacetylation, gave compound **15**. Staudinger reaction, hydrogenation, and ion exchange afforded **FG07** which has a C8 alkyl chain at the 3" position.

Scheme 3.



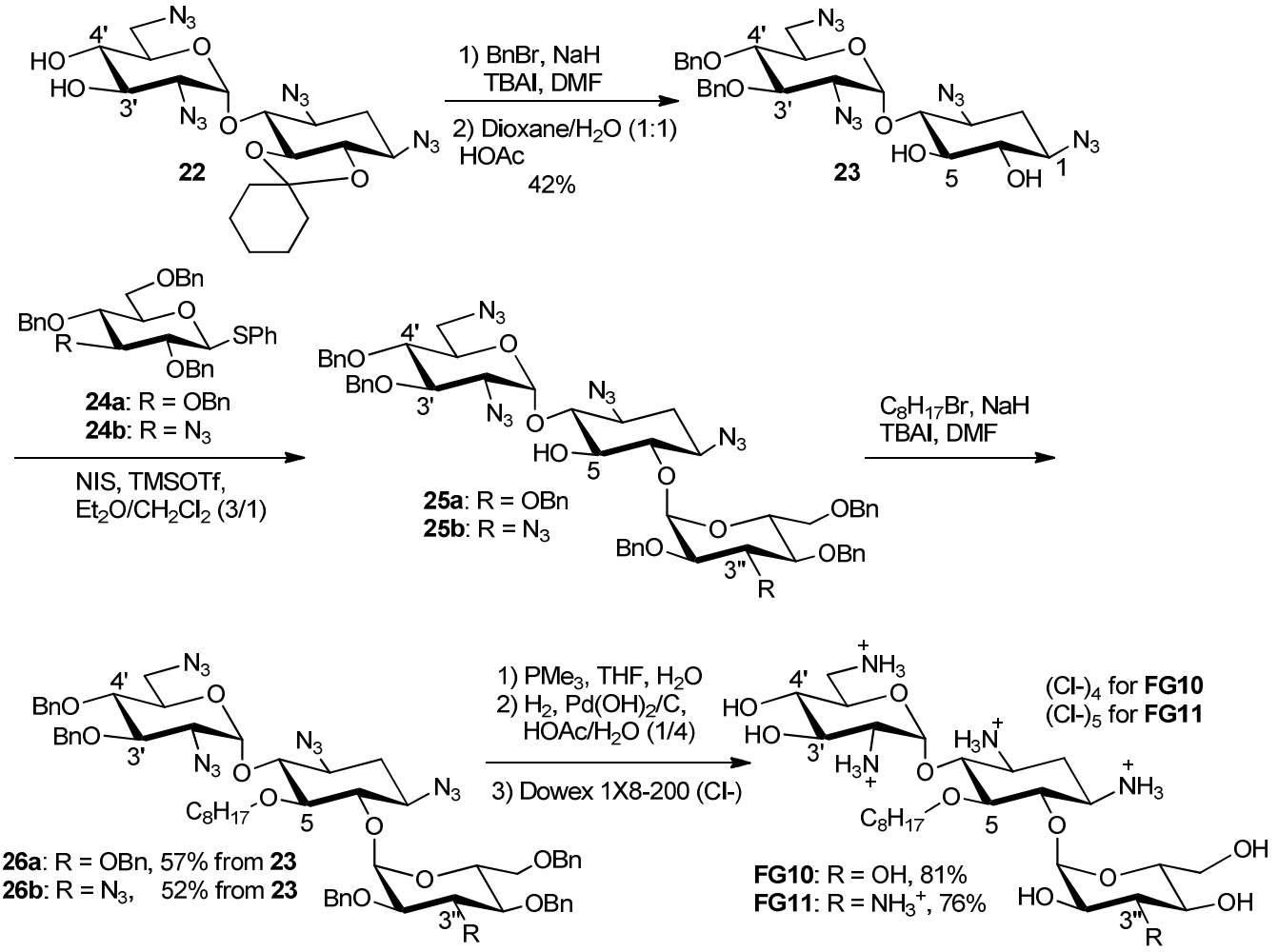
The synthesis of **FG09** started from the known compound **16**[13] (Scheme 4). Alkylation of the 2-OH gave **17**, which upon treatment with $Ac_2O/AcOH/H_2SO_4$ provided **18**. Reaction with thiophenol in the presence of BF_3-OEt_2 gave **19**. Deacetylation, followed by benzylation gave **20**. Following the same glycosylation and global deprotection process as described previously, **FG09** with the C8 alkyl chain at position 2" was prepared as a chloride salt.

Scheme 4.



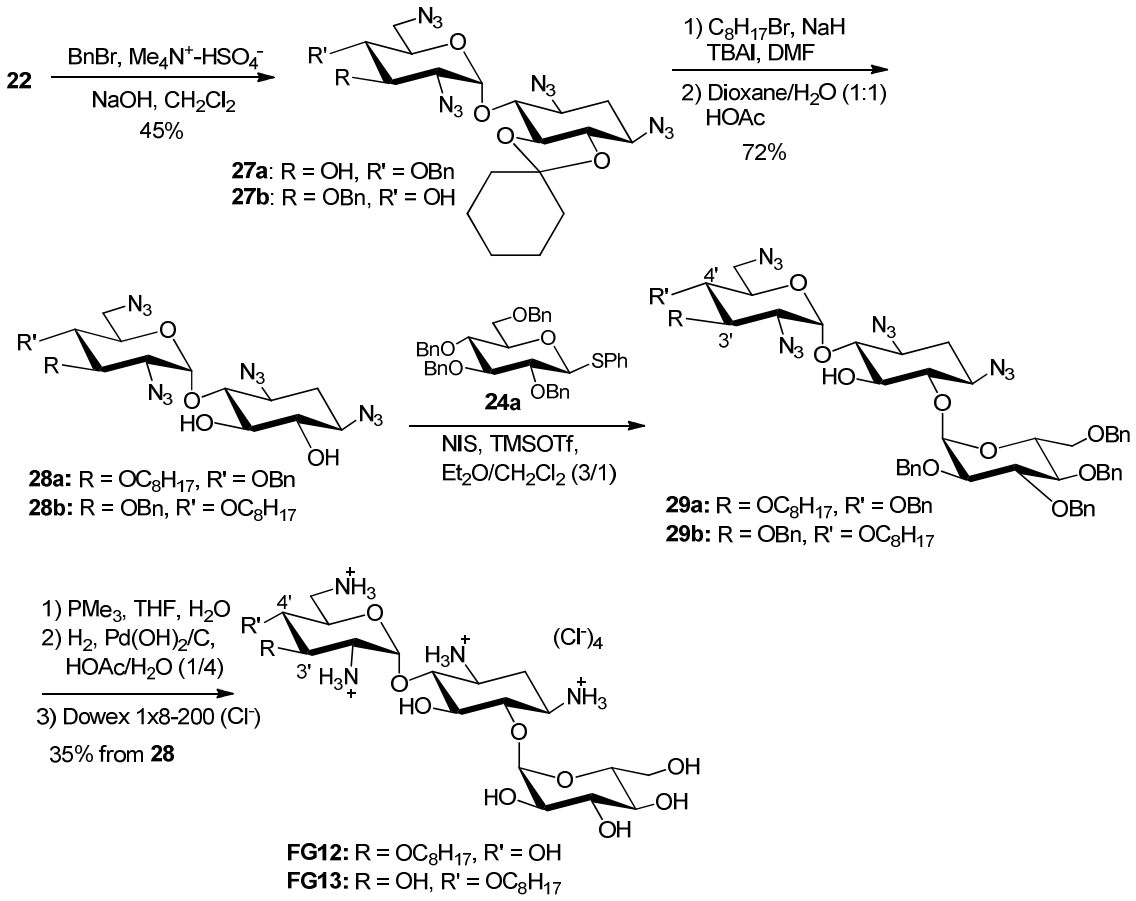
The synthesis of **FG10** and **FG11** began with a benzylation at the 3′ and 4′ positions of the neamine derivative **22**,[14] and was followed by the acid-catalyzed cleavage of the cyclohexylidene protecting group, which gave the glycosyl acceptor **23**[15] (Scheme 5). Glycosylation of **23** with the known thiophenyl donors **24a**[16] and **24b**[17] gave the compounds **25a** and **25b**, respectively. Both compounds have a free hydroxyl group at O-5, which was alkylated directly without purification to provide **26a** and **26b**, respectively. Staudinger reaction, hydrogenation, and ion-exchange afforded **FG10** and **FG11** respectively, with the C8 alkyl chain at *O*-5 position. **FG10** has a free hydroxyl (OH) group at the 3″ position, while **FG11** has an amino (NH_2) group at this position. **FG10** is thus an analog of **FG08,** and **FG11** more closely resembles kanamycin B.

Scheme 5.



The preparation of **FG12** and **FG13** started with selective benzylation of **22**[18] affording a mixture of regioisomers (**27a** and **27b**) (Scheme 6). The regioisomer **27a** has a Bn group at the *O*-4′ position whereas the regioisomer **27b** has the Bn group at the *O*-3′ position. Attempts to separate **27a** and **27b** were unsuccessful. That mixture of **27a** and **27b** was then used as so. Alkylation of the free hydroxyl group in each regioisomer, followed by the acid-cleavage of the cyclohexylidene protecting group gave compounds **28a** and **28b** as an inseparable mixture. Glycosylation of **28a** and **28b** with the donor **24a** afforded **29a** and **29b**, which upon Staudinger reduction, hydrogenolysis, and ion exchange gave a mixture of **FG12** and **FG13**.

Scheme 6.



The synthesized kanamycin B analogs were again tested for growth inhibitory activities against the filamentous fungus *F. graminearum*, G- bacterium *E. coli* (ATCC25922) and G+ bacterium *S. aureus* (ATCC25923, G+) (Table 2). From the MIC's, all of the amphiphilic kanamycin B analogs, regardless of the positions of alkylation, were inactive against bacteria (MIC>32 μg/mL). For antifungal activity, *O*-4" appeared to be the optimal site for antifungal activity while *O*-2" was the next best site. Alkylation at *O*-3" and *O*-6" positions of ring III with octyl groups enabled only moderate antifungal activities. Interestingly, shortening the chain length to a hexyl group (entry 5, Table 2) reduced the antifungal activity dramatically. Finally, alkylation at ring I (*O*-3' or *O*-4') abolishes both antifungal and antibacterial activities.

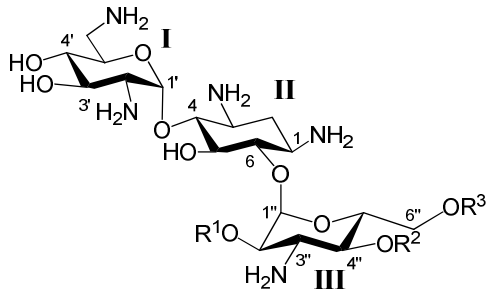
Table 2: MIC Values of **FG** Compounds

Entry	Alkylation site	Compound	MIC (μg/mL)		
			F. graminearum	*E. coli*[a]	*S. aureus*[b]
1	*O*-2"	**FG09**	20	≥250	≥250
2	*O*-3"	**FG07**	62.5	125-250	64-125
3	*O*-4"	**FG03** (6"-OH)	7.8	ND	ND
4	*O*-4"	**FG08** (6"-H)	7.8	64	32-64
5	*O*-6" (C_6H_{13})	**FG05**	125	≥250	≥250
6	*O*-6" (C_8H_{17})	**FG06**	31.3	≥250	≥250
7	*O*-5	**FG10** (3"-OH)	≥500	≥250	≥250
8	*O*-5	**FG11** (3"-NH_2)	31.3	32-64	64-125
9	*O*-3' & *O*-4'	**FG12** & **FG13**	≥500	≥250	≥250
10	-	kanamycin	≥500	4	1

[a]: ATCC25922; [b]: ATCC25923

By comparing the results from **FG10** and **FG11**, the presence of 3"-NH_2 appears to enhance both antifungal and antibacterial activities (entries 7 and 8, Table 2). To further analyze the effect of 3"-NH_2, a 3rd stage SAR investigation was pursued with synthesis of more analogs bearing the 3"-NH_2 group (Figure 3). Additionally, since the presence of an octyl group at the *O*-2" or *O*-6" positions of ring III also promoted modest antifungal activity, we also designed analogs incorporated with two octyl groups at ring III.

Figure 3. Structures of Amphiphilic Kanamycin Derivatives in the 3rd Stage SAR Investigation

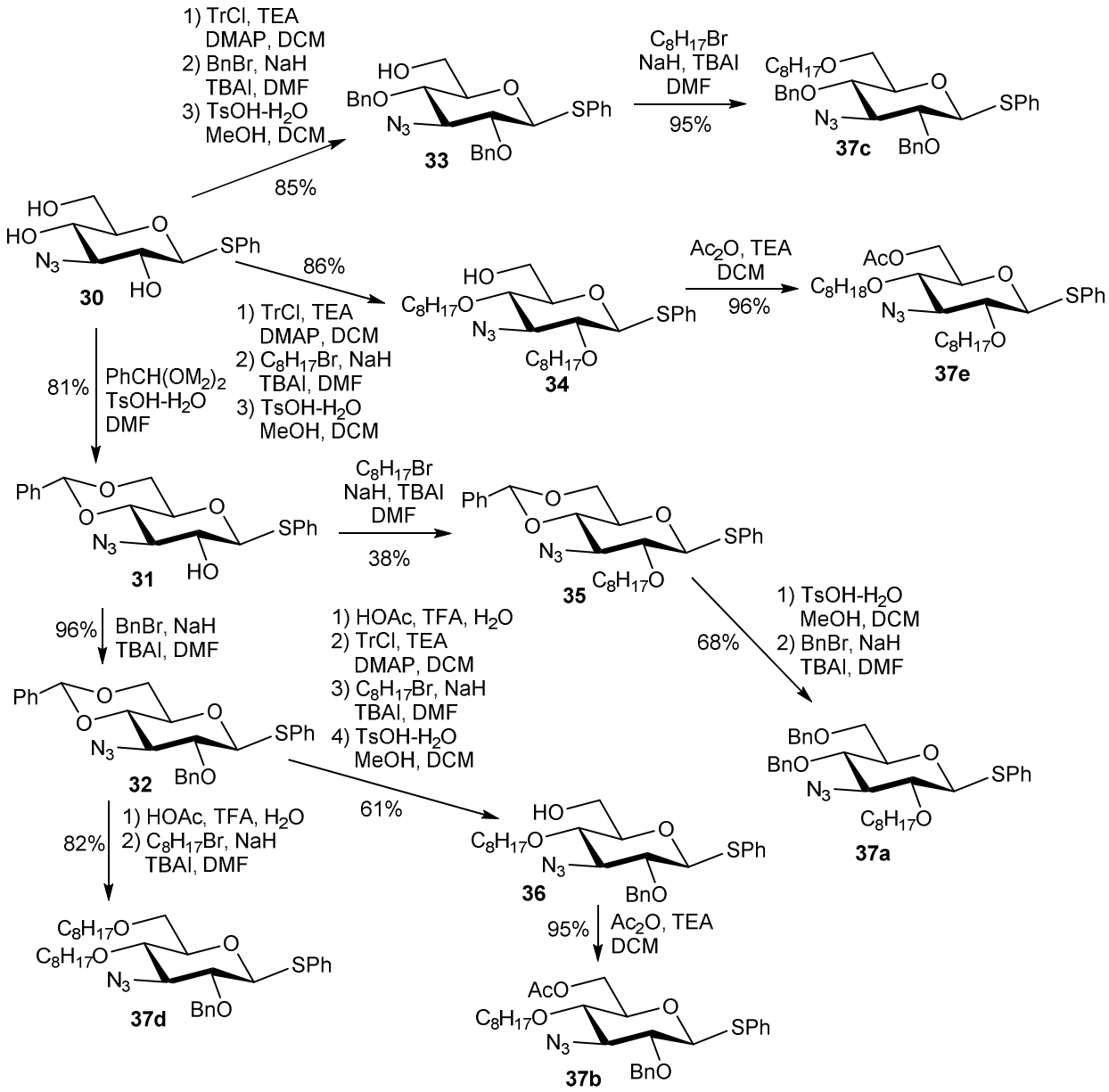


FG14, $R^1 = C_8H_{17}$, $R^2 = H$, $R^3 = H$
FG15, $R^1 = H$, $R^2 = C_8H_{17}$, $R^3 = H$
FG16, $R^1 = H$, $R^2 = H$, $R^3 = C_8H_{17}$
FG17, $R^1 = H$, $R^2 = C_8H_{17}$, $R^3 = C_8H_{17}$
FG18, $R^1 = C_8H_{17}$, $R^2 = C_8H_{17}$, $R^3 = H$

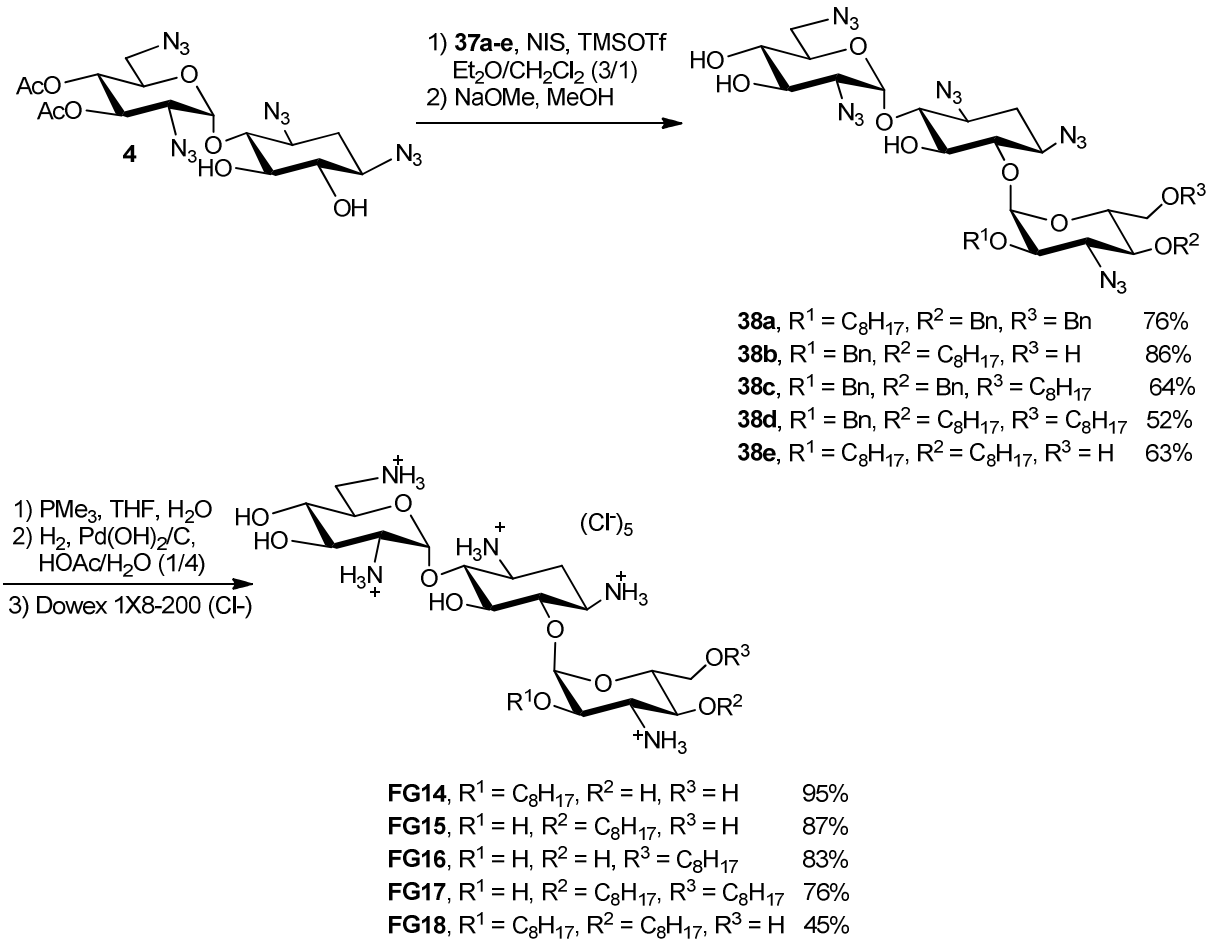
The syntheses of the needed glycosyl donors for these 3"-NH_2 containing kanamycin analogs began with the preparation of phenyl 3-azido-3-deoxy-1-thio-β-D-glucopyranoside, **30**[19] and its derivatives, **31**[19] and **32**[19] (Scheme 6). Using a diversion approach and similar synthetic methodologies, compounds

33 and **34** were prepared with Bn and octyl group incorporated at *O*-4 position, respectively. Alkylation of **33** and acetylation of **34** led to the formation of glycosyl donors, **37c** and **37e**, respectively. Using the common methods reported in the literature,[14,19] glycosyl donor, **37a** was synthesized from **31** whereas glycosyl donors, **37d** were prepared from **32**. Following the glycosylation and deacetylation, compounds were subjected to Staudinger reduction and hydrogenolysis, and the desired kanamycin analogs were purified by column chromatography using either CG50 (NH_4^+) resin or silica gel (Scheme 8). After ion-exchange, these analogs were obtained as chloride salts.

Scheme 7.


1) TrCl, TEA
DMAP, DCM
2) BnBr, NaH
TBAI, DMF
3) TsOH-H2O
MeOH, DCM
85%
33
C8H17Br
NaH, TBAI
DMF
95%
37c
30
86%
1) TrCl, TEA
DMAP, DCM
2) C8H17Br, NaH
TBAI, DMF
3) TsOH-H2O
MeOH, DCM
34
Ac2O, TEA
DCM
96%
37e
81%
PhCH(OM2)2
TsOH-H2O
DMF
31
C8H17Br
NaH, TBAI
DMF
38%
35
96%
BnBr, NaH
TBAI, DMF
32
1) HOAc, TFA, H2O
2) TrCl, TEA
DMAP, DCM
3) C8H17Br, NaH
TBAI, DMF
4) TsOH-H2O
MeOH, DCM
61%
1) TsOH-H2O
MeOH, DCM
2) BnBr, NaH
TBAI, DMF
68%
37a
82%
1) HOAc, TFA, H2O
2) C8H17Br, NaH
TBAI, DMF
37d
36
95%
Ac2O, TEA
DCM
37b

Scheme 8.



The MIC's of the 3rd stage amphiphilic kanamycins are summarized in Table 3. It is clear that the presence of 3"-NH_2 does not increase the antifungal activity (**FG14** vs. **FG09** and **FG15** vs. **FG08**, entries 1 and 2). Again, attachment of the octyl group at the *O*-4" position yielded optimal antifungal activity as compared to other sites at ring III. Compounds bearing with two octyl groups (**FG17** and **FG18**, entries 4 and 5) showed no improvement in antifungal activities, but they had enhanced activities against *S. aureus* (ATCC25923) (Table 3). Perhaps, as the lipophilicity increases, these amphiphilic aminoglycosides are less fungal-specific and behave like other amphiphilic aminoglycosides that contain longer alkyl chain (ex. hexadecyl) and show strong antibacterial activities.[7a,b]

Table 3: MIC Values of **FG** Compounds

Entry	Alkylation site	Compound	MIC (μg/mL)		
			F. graminearum	*E. coli*[a]	*S. aureus*[b]
1	*O*-2"	**FG14**	62.5	125-250	125-250
2	*O*-4"	**FG15**	15.6	125	125

3	*O*-6"	**FG16**	31.3	64-125	125
4	*O*-4" and *O*-6"	**FG17**	31.3	125	32
5	*O*-2" and *O*-4"	**FG18**	31.3	125	32
6	-	kanamycin	>500	4	1

[a]: ATCC25922; [b]: ATCC25923

Conclusion

We have shown that *O*-4" position is the optimal site for attaching a linear alkyl chain that will enable the conversion of antibacterial kanamycin into an antifungal agent. One octyl group at the *O*-4" position of ring III is the best design for inducing antifungal activity. Octylation at the hydroxyl groups on ring I or II causes loss of antibacterial activity and no gain in antifungal activity. We have shown that a 3"-NH_2 group has no or even a negative role in generating antifungal activity. We have also revealed that the site for attaching the linear alkyl chain is essential for the selective antifungal activity. The fact that **FG03** has the same level of antifungal activity as **FG08** indicates that deoxygenation of 6"-OH is not necessary. There is a growing interest in the antibacterial activities of amphiphilic aminoglycosides. Our work here not only offers a new application of amphiphilic aminoglycosides but also detailed SAR that maximizes the antifungal activity. These SAR investigations could lead to the development of new antifungal kanamycin derivative that can be produced in large quantity.[20] Finally, while traditional drug discovery is often laborious and long-term, reviving old drug with simple chemical modifications and new applications may serve as an improved and alternative strategy for new drug development.

Experimental Section

General Procedures.

All chemicals were purchased from the commercially available resources without any further purification. Dry solvents like DMF, DMSO, and THF were dried over molecular sieves. Dichloromethane was dried by distillation over calcium hydride. Mass spectrometry was taken by high resolution mass spectrometry (HRMS) using a TOF mass spectrometer. Two NMR instruments were

used 300 or 400 MHz for the ^{1}H and ^{13}C Nuclei. $CDCl_3$, CD_3OD, and D_2O were used as solvents. The parts per million (ppm) were used to express the chemical shifts on δ scale. The peaks splitting pattern were expressed as (s; for the singlet), (d; doublet), (t; triplet), (q; quadrate), (m; multiplet), and (ddd; doublet of doublets of doublets). Coupling constants J were measured in Hertz (Hz).

General Procedure for *O*-Alkylation of Carbohydrates. To a solution of starting material in anhydrous DMF, octyl bromide or benzyl bromide (2.0 equivalents), NaH (5.0 equivalents), and catalytic amount of TBAI were added. The reaction was stirred overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, and then dried over solid Na_2SO_4. After removal of the solvent and purification with gradient column chromatography (hexane:EtOAc = 100:0 to 60:40), the product was obtained.

General Procedure for the Glycosylation using Phenylthioglycosyl donor and Deacetylation. A solution of glycosyl donor, neamine derivative (1.2 equivalents), and activated powder 4 Å molecular sieve was stirred at room temperature for 2 hours in 12 mL of a mixed anhydrous solution Et_2O:CH_2Cl_2 = 3:1. The mixture was cooled to -70°C and N-iodosuccinimide (1.2 equivalents) was quickly added. After the temperature has warmed up to -40°C, trifluoromethanesulfonic acid (0.15 equivalents) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid $NaHCO_3$, $Na_2S_2O_3$, and Na_2SO_4. After being stirred for 15 minutes, the reaction mixture was filtered through Celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography. The glycosylated compounds were often mixed with inseparable impurities, and were therefore fully characterized after hydrolysis. The glycosylated product was dissolved in THF (1 mL) and MeOH (5 mL), and 1M NaOMe in MeOH (0.5 mL) was added. The mixture was stirred at

room temperature until TLC analysis indicated completion of the reaction (about 30 minutes). The reaction was neutralized with Amberlite IR-120 (H^+), and filtered through Celite. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford the expected product.

General procedure for the synthesis of kanamycin B analogs: To a solution of starting material and THF in a reaction vial equipped with a reflux condenser, 0.1 M $NaOH_{(aq)}$ (0.5 mL) and PMe_3 (1M in THF, 5 - 7 equivalents) were added. The reaction mixture was stirred at 50ºC for 2 hrs. The product has a R_f of 0 when eluted with EtOAc/MeOH (9/1) solution and a R_f of 0.6 when eluted with *i*PrOH/1M NH_4OAc (2/1) solution. After completion of the reaction, the solvents were removed, and the crude benzylated aminoglycoside was added with a catalytic amount of $Pd(OH)_2/C$ (20% Degussa type) and 5 mL of degassed $HOAc/H_2O$ (1/3). After being further degassed, the reaction mixture was stirred at room temperature under atmospheric H_2 pressure. After being stirred for 1 day, the reaction mixture was filtered through Celite. The residue was washed with water, and the combined solutions were concentrated. The crude product was purified with Amberlite CG50(NH_4^+) eluted with a gradient of NH_4OH solution (0% – 20%). The final product was obtained as an HCl salt after elution with water through an ion-exchange column packed with Dowex 1X8-200 (Cl^- form). After collection of the desired fractions and removal of solvent, the final products were characterized by ^1H and ^{13}C NMR before being subjected to bioassay.

Phenyl 2,3-di-*O*-benzyl-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (2). To a solution of **1** (1.80 g, 3.98 mmol) in anhydrous CH_2Cl_2 were added TrCl (1.77 g, 6.36 mmol), Et_3N (1.12 mL, 7.95 mmol) and a catalytic amount of DMAP. The reaction mixture was stirred overnight at room temperature. When complete, the reaction was quenched by addition of MeOH (5 mL). Then the mixture was washed with water, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4 and concentrated. The tritylated crude product was then dissolved in anhydrous DMF, and octyl bromide (1.7 mL, 9.79 mmol), NaH

(0.39 g, 9.79 mmol) and a catalytic amount of TBAI were added. The reaction was stirred overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with water, saturated aqueous $NaHCO_3$ and brine, and then dried over Na_2SO_4. After removal of the solvent, the obtained crude product was dissolved in 50 mL of a mixed solution of CH_2Cl_2:MeOH = 1:1 and *p*-toluenesulfonic acid monohydrate (0.61 g, 3.20 mmol) was added. The resulting mixture was stirred at room temperature overnight. When complete, the reaction mixture was quenched with Et_3N (1.35 mL) and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. After removal of the solvent and purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), **2** was obtained as a white solid, mp 98-99 °C (1.84 g, 3.26 mmol, 84%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.5 (m, 2H), 7.2 – 7.4 (m, 13H), 4.88 (d, *J* = 10.3 Hz, 1H), 4.85 (s, 1H), 4.84 (s, 1H), 4.74 (d, *J* = 10.3 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 1H), 3.9 (m, 1H), 3.5 – 3.8 (m, 4H), 3.43 (t, *J* = 9.6 Hz, 1H), 3.3 (m, 2H), 1.94 (t, *J* = 6.9 Hz, 1H, OH), 1.5 (m, 2H), 1.2 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5, 138.0, 133.5, 131.9 (2 carbons), 129.1 (2 carbons), 128.5 (4 carbons), 128.3 (2 carbons), 128.0, 127.9, 127.82 (2 carbons), 127.75, 87.5, 86.5, 81.0, 79.5, 78.2, 75.9, 75.6, 73.6, 62.3, 31.9, 30.5, 29.6, 29.3, 26.2, 22.7, 14.2; ESI/APCI calcd for $C_{34}H_{44}O_5SNa$ ($[M+Na]^+$) *m/z* 587.2802; measured *m/z* 587.2803.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (3). To a solution of **2** (1.15 g, 2.04 mmol) in DMF (40 mL) were added BnBr (0.49 mL, 4.07 mmol) and a catalytic amount of TBAI. The mixture was then transferred in an ice-water bath and NaH (0.16g, 4.07 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (2 mL) and poured over ice. The mixture was extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, saturated aqueous $NaHCO_3$, water and brine, and then dried over Na_2SO_4. After removal of the solvent and purification with a gradient column chromatography

(hexane:EtOAc = 100:0 to 50:50), **3** was obtained as a yellowish solid, mp 87-88 °C (1.26 g, 1.92 mmol, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.6 (m, 2H), 7.2 – 7.4 (m, 18H), 4.9 (m, 3H), 4.6 – 4.8 (m, 4H), 3.7 – 3.9 (m, 3H), 3.4 – 3.7 (m, 5H), 1.5 (m, 2H), 1.3 (m, 10H), 0.91 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.6, 138.5, 138.2, 134.0, 132.0 (2 carbons), 129.2 (2 carbons), 128.5 (4 carbons), 128.4 (2 carbons), 128.3 (2 carbons), 127.9 (2 carbons), 127.8, 127.7 (3 carbons), 127.6, 127.5, 87.5, 86.8, 80.8, 79.4, 78.2, 75.9, 75.6, 73.5, 73.4, 69.2, 32.0, 30.5, 29.6, 29.4, 26.3, 22.8, 14.3; ESI/APCI calcd for $C_{41}H_{50}O_5SNa$ ($[M+Na]^+$) *m/z* 677.3271; measured *m/z* 677.3280.

6-*O*-(2,3,6-Tri-*O*-benzyl-4-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (5). Please refer to the general procedure for glycosylation and deacetylation. Compound **5** was obtained as a light yellowish oil (0.16 g, 0.17 mmol, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2 – 7.4 (m, 15H), 5.63 (d, *J* = 3.4 Hz, 1H), 5.02 (d, *J* = 3.8 Hz, 1H), 4.92 (d, *J* = 11.0 Hz, 1H), 4.75 (d, *J* = 12.4 Hz, 1H), 4.72 (m, 2H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 12.4 Hz, 1H), 4.1 – 4.2 (m, 1H), 4.0 – 4.1 (m, 1H), 3.96 (d, *J* = 10.3 Hz, 1H), 3.89 (d, *J* = 10.0 Hz, 1H), 3.2 – 3.8 (m, 18H), 2.31 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H), 1.51 (ddd, *J* = 13.0, 12.4, 12.4 Hz, 1H), 1.4 – 1.5 (m, 2H), 1.2 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.8, 138.1, 137.8, 128.55 (2 carbons), 128.49 (2 carbons), 128.4 (2 carbons), 128.13 (2 carbons), 128.06 (2 carbons), 128.0 (3 carbons), 127.9, 127.7, 98.6, 98.2, 86.3, 86.1, 81.4, 79.6, 78.0, 75.9, 75.7, 73.7, 73.5 (2 carbons), 71.6 (2 carbons), 71.4, 71.1, 68.5, 62.9, 59.6, 59.2, 51.3, 32.4, 31.9, 30.4, 29.6, 29.3, 26.2, 22.8, 14.2; ESI/APCI calcd for $C_{47}H_{62}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 993.4553; measured *m/z* 993.4563.

6-*O*-(4-*O*-*n*-Octyl-D-glucopyranosyl)neamine (FG03). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG03** was obtained (0.06 g, 0.11 mmol, 42%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.81 (d, *J* = 3.8 Hz, 1H), 4.93 (d, *J* = 3.8 Hz, 1H), 3.3 - 4.0 (m, 17H), 3.1 – 3.2 (m, 2H), 2.4 (m, 1H), 1.7 - 1.9 (m, 1H), 1.4 – 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.71 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 101.7, 96.1, 83.8, 77.8, 77.6, 74.3,

73.7, 72.9, 72.3, 71.8, 70.9, 69.4, 68.4, 60.4, 53.7, 49.9, 48.5, 40.3, 31.3, 29.4, 28.7, 28.6, 28.2, 25.4, 22.2, 13.7; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}^+$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3708.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*n*-hexyl-1-thio-β-D-glucopyranoside (8a). Please refer to the general procedure for *O*-alkylation of sugars except hexyl bromide has been used. Compound **8a** was obtained as a light yellowish oil (0.69 g, 1.1 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.6 – 7.7 (m, 2H), 7.2 – 7.5 (m, 18H), 4.9 – 5.0 (m, 4H), 4.77 (d, *J* = 10.0 Hz, 1H), 4.70 (d, *J* = 10.0 Hz, 2H), 3.6 – 3.8 (m, 4H), 3.4 – 3.6 (m, 4H), 1.6 (m, 2H), 1.2 – 1.5 (m, 6H), 0.93 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.6, 138.3, 138.2, 134.1, 132.0 (2 carbons), 129.0 (2 carbons), 128.60 (5 carbons), 128.57 (2 carbons), 128.4 (2 carbons), 128.03 (2 carbons), 127.97 (3 carbons), 127.9, 127.5, 87.6, 86.9, 81.0, 79.3, 78.0, 76.0, 75.6, 75.2, 71.9, 69.7, 31.9, 30.0, 26.0, 22.3, 14.3; ESI/APCI calcd for $C_{39}H_{46}O_5SNa$ ($[M+Na]^+$) *m/z* 649.2958, measured *m/z* 649.2971.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (8b). Please refer to the general procedure for *O*-alkylation of sugars. Compound **8b** was obtained as a light yellowish oil (0.70 g, 1.07 mmol, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.6 – 7.7 (m, 2H), 7.2 – 7.5 (m, 18H), 4.9 – 5.0 (m, 4H), 4.79 (d, *J* = 10.3 Hz, 1H), 4.71 (d, *J* = 10.0 Hz, 2H), 3.7 – 3.8 (m, 4H), 3.4 – 3.6 (m, 4H), 1.6 (m, 2H), 1.2 – 1.5 (m, 10H), 0.94 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.6, 138.4, 138.2, 134.2, 132.0 (2 carbons), 129.0 (2 carbons), 128.63 (5 carbons), 128.64 (2 carbons), 128.4 (2 carbons), 128.1 (2 carbons), 128.0 (3 carbons), 127.9, 127.5, 87.7, 86.9, 81.0, 79.4, 78.0, 76.0, 75.6, 75.2, 71.9, 69.8, 32.1, 30.1, 29.7, 29.5, 26.4, 22.9, 14.3; ESI/APCI calcd for $C_{41}H_{50}O_5SNa$ ($[M+Na]^+$) *m/z* 677.3271, measured *m/z* 677.3280.

6-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*n*-hexyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (9a). Please refer to the general procedure for glycosylation and deacetylation. Compound **9a** was obtained as a light yellowish oil (0.37 g, 0.39 mmol, 40%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2 - 7.4 (m, 15H), 5.69

(d, *J* = 3.8 Hz, 1H), 5.05 (d, *J* = 3.8 Hz, 1H), 4.97 (d, *J* = 11.0 Hz, 1H), 4.88 (d, *J* = 10.7 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.75 (s, 1H), 4.74 (s, 1H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.48 (d, *J* = 2.4 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.9 – 4.1 (m, 3H), 3.1 – 3.7 (m, 15H), 2.97 (d, *J* = 3.4 Hz, 1H), 2.92 (d, *J* = 4.1 Hz, 1H), 2.32 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H), 1.6 (m, 2H), 1.50 (ddd, *J* = 13.1, 12.7, 12.7 Hz, 1H), 1.2 – 1.4 (m, 6H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.8, 138.16, 138.07, 128.61 (2 carbons), 128.58 (2 carbons), 128.51 (2 carbons), 128.16 (2 carbons), 128.10 (4 carbons), 128.04 (2 carbons), 127.8, 98.6, 98.2, 85.9, 81.5, 79.7, 79.6, 75.8 (2 carbons), 75.4, 73.5, 71.9 (2 carbons), 71.7, 71.6, 71.4, 71.1, 69.1, 63.0, 59.6, 59.2, 51.3, 32.4, 31.7, 29.4, 25.8, 22.7, 14.2 ; ESI/APCI calcd for $C_{45}H_{58}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 965.4240; measured *m/z* 965.4255.

6-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (9b). Please refer to the general procedure for glycosylation and deacetylation. Compound **9b** was obtained as a light yellowish oil (0.36 g, 0.37 mmol, 38%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2 - 7.4 (m, 15H), 5.69 (d, *J* = 3.8 Hz, 1H), 5.05 (d, *J* = 3.8 Hz, 1H), 4.97 (d, *J* = 11.0 Hz, 1H), 4.88 (d, *J* = 10.7 Hz, 1H), 4.82 (d, *J* = 11.0 Hz, 1H), 4.75 (s, 1H), 4.74 (s, 1H), 4.59 (d, *J* = 10.7 Hz, 1H), 4.48 (d, *J* = 2.4 Hz, 1H), 4.1 – 4.2 (m, 1H), 3.9 – 4.1 (m, 3H), 3.1 – 3.7 (m, 15H), 2.97 (d, *J* = 3.4 Hz, 1H), 2.92 (d, *J* = 4.1 Hz, 1H), 2.32 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H), 1.6 (m, 2H), 1.50 (ddd, *J* = 13.1, 12.7, 12.7 Hz, 1H), 1.2 – 1.4 (m, 6H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.9, 138.3, 138.2, 128.7 (4 carbons), 128.6 (2 carbons), 128.23 (6 carbons), 128.22 (2 carbons), 127.9, 98.7, 98.3, 85.8, 81.6, 79.8 (2 carbons), 77.7, 75.9 (2 carbons), 75.5, 73.6, 72.0, 71.8, 71.7, 71.6, 71.3, 69.2, 63.2, 59.7, 59.3, 51.4, 32.5, 32.1, 29.7, 29.6, 29.5, 26.3, 22.9, 14.3 ; ESI/APCI calcd for $C_{47}H_{62}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 993.4553; measured *m/z* 993.4578.

6-*O*-(6-*O*-*n*-Hexyl-D-glucopyranosyl)neamine (FG05). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG05** was obtained (0.12 g, 0.21 mmol, 86%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.85 (d, *J* = 4.1 Hz, 1H), 4.89 (d, *J* = 3.5 Hz, 1H), 3.2 -

4.0 (m, 18H), 3.1 (m, 1H), 2.4 (m, 1H), 1.8 (m, 1H), 1.3 – 1.5 (m, 2H), 1.0 - 1.2 (m, 6H), 0.69 (t, J = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 101.9, 95.5, 83.8, 77.0, 74.3, 72.9, 72.11, 72.07, 71.7, 70.8, 69.4, 69.3, 68.8, 68.4, 53.6, 49.9, 48.4, 40.4, 31.1, 28.6, 28.1, 25.0, 22.1, 13.6; ESI/APCI calcd for $C_{24}H_{49}N_4O_{11}$ ($[M+H]^+$) *m/z* 569.3392; measured *m/z* 569.3408.

6-*O*-(6-*O*-*n*-Octyl-D-glucopyranosyl)neamine (FG06). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG06** was obtained (0.09 g, 0.16 mmol, 82%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.92 (d, J = 4.1 Hz, 1H), 4.95 (d, J = 3.8 Hz, 1H), 3.3 - 4.0 (m, 18H), 3.2 (m, 1H), 2.4 – 2.5 (m, 1H), 1.9 (m, 1H), 1.4 – 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.74 (t, J = 7.2 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 102.0, 95.6, 83.8, 76.9, 74.3, 72.9, 72.2, 72.1, 71.8, 70.9, 69.5, 69.3, 68.8, 68.4, 53.7, 49.9, 48.6, 40.4, 31.3, 28.8, 28.7, 28.6, 28.0, 25.4, 22.2, 13.7; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3708.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-n-octyl-α-D-glucopyranose (11).[12] Please refer to the general procedure for *O*-alkylation of sugars. Compound **11** was obtained as a light yellowish oil (3.76 g, 10.10 mmol, 99%).

1,2,4,6-Tetra-*O*-acetyl-3-*O*-*n*-octyl-D-glucopyranose (12). A solution of **11** (4.40 g, 11.8 mmol) in 150 mL of a mixed solution of AcOH/TFA/H_2O (80/1/19) was stirred at 55 ^oC overnight. When TLC analysis indicated completion of the reaction, the solvents were removed. After being dried *in vacuo* for a few hours, the crude product was dissolved in Ac_2O (50 mL) and TFA (5 mL), and the mixture was stirred at room temperature overnight. Solid $NaHCO_3$ was then added to neutralize the excess acid. EtOAc was added to dilute the solution and the organic layer was washed with water, saturated aqueous $NaHCO_3$ (3 times), and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 40:60) provided **12** (4.75 g, 10.3 mmol, 87%) as a mixture of α/β anomers in a 1/2 ratio. The obtained

compound was isolated as yellowish oil mixed with inseparable impurities so only ^{1}H NMR characterization was performed. ^{1}H NMR ($CDCl_3$, 300 MHz) (α and β anomers) δ 6.27 (d, *J* = 3. 8 Hz, 1H, H-1α), 5.62 (d, *J* = 8.3 Hz, 1H, H-1β), 4.9 – 5.5.1 (m, 4H), 3.9 – 4.2 (m, 4H), 3.4 – 3.8 (m, 8H), 2.0 – 2.1 (m, 24H), 1.1 – 1.3 (m, 24H), 0.86 (t, *J* = 6.9 Hz, 6H).

Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (13). A solution of **12** (4.04 g, 8.77 mmol) and thiophenol (3.4 mL, 33.3 mmol) in anhydrous CH_2Cl_2 (50 mL) was cooled down to 0 ^oC and BF_3-OEt_2 was slowly added. The reaction was stirred for 2 days at room temperature till completion. Solid $NaHCO_3$, Na_2SO_4 and some few drops of water were then added and the mixture was stirred for 1 hour. The solution was then filtered through a Fritz funnel and the collected solids were washed with EtOAc. After removal of the solvents, purification by gradient column chromatography afforded **13** as a light yellowish oil (2.91 g, 5.70 mmol, 65%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.4 (m, 2H), 7.1 – 7.2 (m, 3H), 4.89 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.88 (dd, *J* = 9.6, 9.6 Hz, 1H), 4.56 (d, *J* = 10.3 Hz, 1H), 3.9 – 4.1 (m, 3H), 3.5 – 3.6 (m, 1H), 3.4 – 3.5 (m, 2H), 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.3 – 1.4 (m, 2H), 1.1 – 1.2 (m, 10H), 0.77 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 169.4, 169.2, 132.9, 132.5 (2 carbons), 129.0 (2 carbons), 128.1, 86.2, 81.9, 76.1, 72.9, 71.5, 69.8, 62.7, 31.9, 30.4, 29.5, 29.4, 26.1, 22.7, 21.1, 20.9, 20.8, 14.2 ; ESI/APCI calcd for $C_{26}H_{38}O_8SNa$ ($[M+Na]^+$) *m/z* 533.2180; measured *m/z* 533.2187.

Phenyl 2,3,6-tri-*O*-benzyl-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (14). To a solution of **13** (2.91 g, 5.70 mmol) in anhydrous MeOH (40 mL), 0.5 mL of a 1M solution of NaOMe in MeOH was added and the mixture was stirred at room temperature for 1 hour. When complete, the reaction was quenched by adding amberlite IR 120 H^+ resin to the mixture, followed by filtration through Celite and concentration of the filtrate. The obtained crude product was dissolved in DMF (40 mL), and BnBr (10.0 mL, 84.0 mmol) and a catalytic amount of TBAI were added. The mixture was then transferred in an ice-water bath and NaH (3.36 g, 84.0 mmol) was slowly added. When TLC analysis performed the

following day indicated completion, the reaction was quenched with MeOH (5 mL) and poured over ice. The mixture was diluted with EtOAc, extracted with 1N aqueous HCl, saturated aqueous $NaHCO_3$, water and brine, and dried over Na_2SO_4. After removal of the solvents, purification by gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) gave **14** as a yellowish solid, mp 79-81 °C (2.00 g, 3.05 mmol, 44%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2 – 7.8 (m, 20H), 5.0 (m, 2H), 4.86 (d, *J* = 10.3 Hz, 1H), 4.6 – 4.8 (m, 4H), 3.8 – 4.0 (m, 4H), 3.5 – 3.7 (m, 4H), 1.7 – 1.8 (m, 2H), 1.3 - 1.5 (m, 10H), 1.01 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.7, 138.65, 138.55, 134.3, 132.3 (2 carbons), 129.2 (2 carbons), 128.75 (4 carbons), 128.67 (2 carbons), 128.5 (2 carbons), 128.3 (2 carbons), 128.1 (2 carbons), 128.0 (2 carbons), 127.9, 127.7, 87.7, 87.1, 81.2, 79.4, 78.1, 75.7, 75.3, 74.4, 73.7, 69.4, 32.2, 31.0, 29.9, 29.6, 26.7, 23.0, 14.5 ; ESI/APCI calcd for $C_{41}H_{50}O_5SNa$ ($[M+Na]^+$) *m/z* 677.3271; measured *m/z* 677.3270.

6-*O*-(2,4,6-Tri-*O*-benzyl-3-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (15). Please refer to the general procedure for glycosylation and deacetylation. Compound **15** was obtained as a light yellowish oil (0.21 g, 0.22 mmol, 59%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.1 – 7.5 (m, 15H), 5.60 (d, *J* = 3.8 Hz, 1H), 5.02 (d, *J* = 3.8 Hz, 1H), 4.83 (d, *J* = 10.7 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 12.4 Hz, 1H), 4.54 (s, 1H), 4.53 (s, 1H), 4.49 (d, *J* = 12.4 Hz, 1H), 4.45 d, *J* = 10.7 Hz, 1H), 4.0 – 4.1 (m, 2H), 3.0 – 4.0 (m, 18H), 2.30 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H), 1.6 – 1.7 (m, 2H), 1.49 (ddd, *J* = 12.7, 12.7, 12.7 Hz, 1H), 1.2 -1.4 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.4, 138.1, 137.9, 128.7 (6 carbons), 128.32 (2 carbons), 128.25 (2 carbons), 128.1 (4 carbons), 128.0, 98.7, 98.4, 85.8, 81.5, 79.9, 79.6, 77.8, 75.9, 75.4, 74.0, 73.6 (2 carbons), 71.7, 71.5 (2 carbons), 71.3, 68.5, 63.0, 59.7, 59.3, 51.4, 32.5, 32.1, 30.9, 29.8, 29.5, 26.5, 22.9, 14.3; ESI/APCI calcd for $C_{47}H_{62}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 993.4553; measured *m/z* 993.4564.

6-*O*-(3-*O*-*n*-Octyl-D-glucopyranosyl)neamine (FG07). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG07** was obtained (0.08 g, 0.14 mmol, 42%) as a chloride

salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.81 (d, *J* = 4.1 Hz, 1H), 4.93 (d, *J* = 4.0 Hz, 1H), 3.3 - 4.0 (m, 18H), 3.16 (dd, *J* = 13.7, 6.9 Hz, 1H), 2.42 (ddd, *J* = 12.4, 4.1, 4.1 Hz, 1H), 1.87 (ddd, *J* = 12.7, 12.4, 12.4 Hz, 1H), 1.4 – 1.5 (m, 2H), 1.0 - 1.3 (m, 10H), 0.71 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 101.8, 96.2, 83.8, 81.3, 77.7, 74.2, 73.4, 73.2, 71.3, 70.8, 69.4, 68.9, 68.4, 60.5, 53.6, 49.9, 48.4, 40.3, 31.3, 29.5, 28.7, 28.6, 28.1, 25.3, 22.2, 13.6; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3720.

Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-*n*-octyl-α-D-glucopyranoside (17). Please refer to the general procedure for *O*-alkylation of sugars. Compound **17** was obtained as a white solid, mp 68-69 °C (2.37 g, 4.89 mmol, 90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.5 (m, 2H), 7.2 – 7.4 (m, 8H), 5.58 (s, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 3.8 Hz, 1H), 4.82 (d, *J* = 11.3 Hz, 1H), 4.30 (dd, *J* = 9.6, 4.1 Hz, 1H), 3.98 (dd, *J* = 9.3, 8.9 Hz, 1H), 3.6 – 3.9 (m, 6H), 3.46 (s, 3H), 1.6 – 1.7 (m, 2H), 1.2 – 1.4 (m, 10H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0, 137.7, 129.1, 128.5 (2 carbons), 128.4 (2 carbons), 128.2 (2 carbons), 127.7, 126.3 (2 carbons), 101.5, 99.2, 82.2, 80.7, 78.6, 75.5, 72.4, 69.3, 62.6, 55.2, 32.1, 30.3, 29.7, 29.5, 26.2, 22.9, 14.4 ; ESI/APCI calcd for $C_{29}H_{40}O_6Na$ ($[M+Na]^+$) *m/z* 507.2717; measured *m/z* 507.2723.

1,3,4,6-Tetra-*O*-acetyl-2-*O*-n-octyl-D-glucopyranose (18). Please refer to the synthesis of **12.** Compound **18** was obtained as a light yellowish oil (1.50 g, 3.26 mmol, 76%) in a mixture of α/β anomers in a 6/1 ratio**.** ^{1}H NMR (α-anomer) ($CDCl_3$, 300 MHz) δ 6.25 (d, *J* = 3.8 Hz, 1H), 5.21 (dd, *J* = 10.0, 9.6 Hz, 1H), 4.94 (dd, *J* = 10.3, 9.6 Hz, 1H), 4.17 (dd, *J* = 13.0, 4.1 Hz, 1H), 3.9 – 4.0 (m, 2H), 3.4 – 3.6 (m, 2H), 3.3 (m, 1H), 2.05 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.3 – 1.4 (m, 2H), 1.1 (m, 10H), 0.75 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 170.1, 169.7, 169.0, 89.3, 76.7, 71.6 (2 carbons), 69.8, 68.1, 61.7, 31.9, 29.8, 29.3 (2 carbons), 25.9, 22.7, 21.0, 20.8, 20.72, 20.66, 14.1; ESI/APCI calcd for $C_{22}H_{36}O_{10}Na$ ($[M+Na]^+$) *m/z* 483.2201; measured *m/z* 483.2192.

Phenyl 3,4,6-tri-*O*-acetyl-2-*O*-*n*-octyl-1-thio-D-glucopyranoside (19). Please refer to the synthesis of **13.** Compound **19** was obtained as a light yellowish oil (0.78 g, 1.53 mmol, 56%) in a mixture of α/β anomers in a 3/1 ratio. The obtained compound was isolated with inseparable impurities so only ^{1}H NMR characterization was performed. ^{1}H NMR (α-anomer) ($CDCl_3$, 300 MHz) δ 7.5 (m, 2H), 7.3 (m, 3H), 5.77 (d, *J* = 5.5 Hz, 1H), 5.30 (dd, *J* = 9.6, 9.6 Hz, 1H), 5.01 (dd, *J* = 10.3, 9.3 Hz, 1H), 4.54 (ddd, *J* = 10.3, 5.2, 2.1 Hz, 1H), 4.29 (dd, *J* = 12.0, 5.2 Hz, 1H), 3.99 (dd, *J* = 12.4, 2.1 Hz, 1H), 3.6 – 3.8 (m, 2H), 3.3 – 3.5 (m, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.5 (m, 2H), 1.2 – 1.4 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 3H).

Phenyl 3,4,6-tri-*O*-benzyl-2-*O*-*n*-octyl-1-thio-α-D-glucopyranoside (20). Please refer to the synthesis of **14.** Compound **20** was obtained as a light yellowish oil (1.21 g, 1.85 mmol, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz) (α anomer) δ 7.5 – 7.7 (m, 2H), 7.2 -7.5 (m, 18H), 5.86 (d, *J* = 4.8 Hz, 1H), 5.10 (d, *J* = 11.0 Hz, 1H), 4.95 (d, *J* = 10.7 Hz, 1H), 4.87 (d, *J* = 10.7 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.59 (d, *J* = 11.0 Hz, 1H), 4.50 (d, *J* = 12.1 Hz, 1H), 3.5 – 4.0 (m, 8H), 1.6 – 1.8 (m, 2H), 1.3 – 1.5 (m, 10H), 0.95 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.2, 138.6, 138.3, 135.1, 132.0, 131.8 (2 carbons), 129.2 (2 carbons), 128.7 (5 carbons), 128.3 (3 carbons), 128.2 (3 carbons), 128.0 (2 carbons), 127.9, 127.3, 87.2, 82.8, 81.0, 77.2, 76.0, 75.4, 73.7, 71.5, 70.7, 68.9, 32.2, 30.4, 29.8, 29.6, 26.5, 23.0, 14.5 ; ESI/APCI calcd for $C_{41}H_{50}O_5NaS$ ($[M+Na]^+$) *m/z* 677.3271; measured *m/z* 677.3277.

6-*O*-(3,4,6-Tri-*O*-benzyl-2-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (21). Please refer to the general procedure for glycosylation and deacetylation. Compound **21** was obtained as a light yellowish oil (0.10 g, 0.11 mmol, 59%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.1 – 7.5 (m, 15H), 5.60 (d, *J* = 3.8 Hz, 1H), 5.02 (d, *J* = 3.8 Hz, 1H), 4.83 (d, *J* = 10.7 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.61 (d, *J* = 12.4 Hz, 1H), 4.54 (s, 1H), 4.53 (s, 1H), 4.49 (d, *J* = 12.4 Hz, 1H), 4.45 d, *J* = 10.7 Hz, 1H), 4.0 – 4.1 (m, 2H), 3.0 – 4.0 (m, 18H), 2.30 (ddd, *J* = 13.1, 4.5, 4.1 Hz, 1H), 1.6 – 1.7 (m, 2H), 1.49 (ddd, *J* = 12.7, 12.7, 12.7 Hz, 1H), 1.2 -1.4 (m, 10H), 0.88 (t, *J* = 6.9 Hz, 3H);

^{13}C NMR ($CDCl_3$, 100 MHz) δ 139.0, 138.1, 137.9, 128.64 (6 carbons), 128.58, 128.26, 128.19, 128.1 (4 carbons), 128.0, 127.8, 98.5, 98.2, 86.5, 81.4, 80.9,, 79.6,, 75.9, 76.4, 76.0, 75.8, 75.4, 73.6, 71.9, 71.7, 71.6, 71.2, 68.5, 63.1, 59.7, 59.3, 51.4, 32.5, 32.0, 30.4, 29.7, 29.5, 26.1, 22.9, 14.3 ; ESI/APCI calcd for $C_{47}H_{62}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 993.4553; measured *m/z* 993.4570.

6-*O*-(2-*O*-*n*-Octyl-D-glucopyranosyl)neamine (FG09). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG09** was obtained (0.04 g, .07 mmol, 52%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.81 (d, *J* = 3.8 Hz, 1H), 5.06 (d, *J* = 3.4 Hz, 1H), 3.0 - 4.0 (m, 19H), 2.4 (m, 1H), 1.4 – 1.5 (m, 3H), 1.1 - 1.2 (m, 10H), 0.72 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 100.3, 96.4, 83.9, 80.1, 78.0, 74.4, 73.3, 73.2, 72.7, 70.8, 69.5, 69.4, 68.4, 60.7, 53.7, 49.8, 48.4, 40.3, 31.3, 29.3, 28.7, 28.5, 28.0, 25.1, 22.2, 13.6; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3701.

3´,4´-Di-*O*-benzyl-1,3,2´,6´-tetraazidoneamine (23).[15] To a solution of **22**[18] (3.60 g, 7.11 mmol) in DMF (40 mL) were added BnBr (3.40 mL, 28.5 mmol) and a catalytic amount of TBAI. The mixture was then transferred in an ice-water bath and NaH (1.14 g, 28.5 mmol) was slowly added. When TLC analysis performed the following day indicated completion, the reaction was quenched with MeOH (2 mL) and poured over ice. The mixture was extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, saturated aqueous $NaHCO_3$, water and brine, and dried over Na_2SO_4. After removal of the solvent, a brownish crude product was obtained, to which 80 mL of mixed solution of dioxane: H_2O = 1:1 was added, followed by 35 mL glacial acetic acid. The resulting mixture was refluxed at 60~65 ^oC overnight. When complete, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. After removal of the solvent followed by purification with a gradient column chromatography (pure hexane to hexane: EtOAc = 40:60), **23** was obtained as a light yellowish oil (2.03 g, 6.62 mmol, 42%).

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-3′,4′-*O*-dibenzyl-1,3,2′,6′-tetraazidoneamine (25a). A solution of **23** (0.20 g, 0.33 mmol), **25a** (0.25 g, 0.40 mmol), and activated powder 4Å molecular sieve was stirred at room temperature for 2 hours in 12 mL of a mixed anhydrous solution Et_2O:CH_2Cl_2 = 3:1. The mixture was cooled to -70 ^oC and *N*-iodosuccinimide (0.09 g, 0.40 mmol) was quickly added. After the temperature has warmed up to -40 ^oC, trifluoromethanesulfonic acid (0.05 mL) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid $NaHCO_3$, $Na_2S_2O_3$ and Na_2SO_4. After being stirred for 15 minutes, the reaction mixture was filtered through Celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford **25a** as a yellowish oil. Because it was mixed with inseparable impurities, it was used as so in the next step.

6-*O*-(3-Azido-3-deoxy-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-3′,4′-*O*-dibenzyl-1,3,2′,6′-tetraazidoneamine (25b). Please refer to the synthesis of **25a.** Compound **25b** was also obtained as a yellowish oil mixed with inseparable impurities and was then used as so in the next step.

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-3′,4′-*O*-dibenzyl-5-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (26a). Please refer to the general procedure for *O*-alkylation of sugars. Compound **26a** was obtained as a light yellowish oil (0.20 g, 0.16 mmol, 57%). ^1H NMR ($CDCl_3$, 300 MHz) δ 7.2 - 7.5 (m, 30H), 5.72 (d, *J* = 3.4 Hz, 1H), 5.62 (d, *J* = 3.8 Hz, 1H), 4.8 – 5.0 (m, 8H), 4.67 (d, *J* = 11.3 Hz, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 11.3 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.32 (d, *J* = 9.6 Hz, 1H), 4.15 (d, *J* = 10.0 Hz, 1H), 4.04 (dd, *J* = 10.3, 8.9 Hz, 1H), 3.9 – 4.0 (m, 1H), 3.3 – 3.8 (m, 15H), 2.4 (m, 1H), 1.5 – 1.7 (m, 3H), 1.0 - 1.4 (m, 10H), 0.86 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.8, 138.7, 138.1 (2 carbons), 137.83, 137.77, 128.64 (3 carbons), 128.57 (3 carbons), 128.49 (4 carbons), 128.25 (3 carbons), 128.20 (4 carbons), 128.1 (3 carbons), 128. 0 (3 carbons), 127.9 (2

carbons), 127.8, 127.7, 127.6 (2 carbons), 127.5, 97.5, 96.0, 83.3, 82.1, 80.2, 79.5, 78.8, 77.7, 77.5, 76.1, 75.8, 75.7, 75.5, 75.2, 75.1, 73.5, 73.4, 71.1, 70.2, 68.5, 63.5, 60.6, 60.5, 59.3, 32.1, 31.9, 30.2, 29.7, 29.6, 26.1, 22.8, 14.2; ESI/APCI calcd for $C_{68}H_{80}N_{12}O_{11}Na$ ($[M+Na]^+$) *m/z* 1263.5962; measured *m/z* 1263.5961.

6-*O*-(3-Azido-3-deoxy-2,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-3′,4′-*O*-dibenzyl-5-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (26b). Please refer to the general procedure for *O*-alkylation of sugars. Compound **26b** was obtained as a light yellowish oil (0.05 g, 0.04 mmol, 52%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.2 -7.5 (m, 25H), 5.70 (d, *J* = 3.5 Hz, 1H), 5.58 (d, *J* = 3.8 Hz, 1H), 4.92 (d, *J* = 11.3 Hz, 1H), 4.91 (s, 2H), 4.82 (d, *J* = 12.0 Hz, 1H), 4.80 (d, *J* = 10.6 Hz, 1H), 4.76 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 11.3 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.3 (m, 1H), 4.11 (d, *J* = 10.0 Hz, 1H), 4.02 (dd, *J* = 10.3, 8.9 Hz, 1H), 3.3 – 3.9 (m, 17H), 2.3 – 2.4 (m, 1H), 1.4 – 1.7 (m, 3H), 1.0 – 1.3 (m, 10H), 0.88 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 138.2, 138.0, 137.9, 137.8, 137.6, 128.8 (5 carbons), 128.7 (2 carbons), 128.43 (2 carbons), 128.40 (2 carbons), 128.3 (5 carbons), 128.2 (2 carbons), 128.04 (4 carbons), 128.00 (2 carbons), 127.9, 97.6, 95.2, 83.3, 80.3, 78.9, 77.5, 76.5 (2 carbons), 76.3, 75.8, 75.5, 75.3, 75.1, 73.8, 73.1, 71.2, 69.9, 68.3, 65.8, 63.6, 60.5, 59.3, 51.2, 32.1, 32.0, 30.3, 29.7, 29.6, 26.0, 22.9, 14.3; ESI/APCI calcd for $C_{61}H_{73}N_{15}O_{10}Na$ ($[M+Na]^+$) *m/z* 1198.5557; measured *m/z* 1198.5527.

6-*O*-(α-D-Glucopyranosyl)-5-*O*-*n*-octylneamine (FG10). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG10** was obtained (0.06 g, 0.10 mmol, 81%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.59 (d, *J* = 3.8 Hz, 1H), 5.01 (d, *J* = 3.4 Hz, 1H), 3.0 - 4.0 (m, 19H), 2.4 (m, 1H), 1.9 (m, 1H), 1.4 – 1.5 (m, 3H), 1.1 - 1.2 (m, 10H), 0.72 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 102.1, 93.1, 81.7, 80.7, 73.7, 73.31, 73.30, 72.8, 72.2, 71.3, 69.9, 68.6, 68.4, 59.9, 53.1, 50.2, 48.7, 40.0, 31.2, 29.4, 29.0, 28.5, 28.2, 25.2, 22.2, 13.6; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3731.

6-*O*-(3-Amino-3-deoxy-α-D-glucopyranosyl)-5-*O*-*n*-octylneamine (FG11). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG11** was obtained (0.06 g, 0.10 mmol, 28%) as a chloride salt. ^{1}H NMR (D_2O, 300 MHz) (chloride salt) δ 5.61 (d, *J* = 3.5 Hz, 1H), 5.08 (d, *J* = 3.5 Hz, 1H), 3.0 - 4.2 (m, 19H), 2.4 (m, 1H), 1.9 (m, 1H), 1.4 – 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.72 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) δ 101.3, 93.0, 82.0, 81.1, 73.7, 73.2, 71.8, 71.1, 69.6, 68.7, 68.2, 64.9, 59.3, 54.9, 53.0, 49.0, 48.7, 39.9, 31.2, 29.4, 29.0, 28.6, 27.9, 25.3, 22.2, 13.6; ESI/APCI calcd for $C_{26}H_{54}N_5O_{10}$ ($[M+H]^+$) *m/z* 596.3865; measured *m/z* 596. 3865.

4´-*O*-benzyl-5,6-*O*-benzylidene-1,3,2´,6´-tetraazidoneamine (27a). To a solution of **12** (3.72 g, 7. 35 mmol) in CH_2Cl_2 (25 mL) was added TBAHS (0.75 g, 2.21 mmol), followed by BnBr (0.97 mL, 8.09 mmol) and NaOH (25 mL, 1N aqueous solution). The mixture was refluxed at 60 ^{o}C overnight. When complete, CH_2Cl_2 was removed from the reaction mixture using a rotavapor and the obtained solution was extracted with EtOAc. The organic layer was then washed with 1 N aqueous HCl, water and brine, and then dried over solid Na_2SO_4. After removal of the solvent and purification with gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), the product **27a** was obtained as a light yellowish oil mixed with its regioisomer **27b** in a 1/1 ratio (1.97 g, 3.31 mmol, 45%). ^{1}H NMR ($CDCl_3$, 300 MHz) (mixture of **27a** and **27b**) δ 7.3 – 7.4 (m, 10H), 5.56 (d, *J* = 3.4 Hz, 1H), 5.52 (d, *J* = 3.8 Hz, 1H), 4.96 (d, *J* = 11.3 Hz, 1H), 4.85 (d, *J* = 11.7 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 2H), 4.0 - 4.1 (m, 4H), 3.7 – 3.9 (m, 2H), 3.3 – 3.7 (m, 13H), 3.23 (dd, *J* = 10.7, 3.8 Hz, 1H), 2.81 (d, *J* = 3.8 Hz, 1H), 2.50 (d, *J* = 3.8 Hz, 1H), 2.2 -2.4 (m, 2H), 1.3 – 1.8 (m, 24H).

3´-*O*-benzyl-5,6-*O*-benzylidene-1,3,2´,6´-tetraazidoneamine (27b). Please refer to the synthesis of compound **27a.**

4′-*O*-benzyl-3′-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (28a). To a solution of a mixture of **27a** and **27b** (1.22 g, 2.04 mmol) in anhydrous DMF (50 mL), *n*-octyl bromide (1.42 mL, 8.18 mmol), NaH (0.33 g, 8.18 mmol), and a catalytic amount of TBAI were added. The reaction was stirred at room temperature overnight. When complete, the reaction was quenched by addition of MeOH (5 mL) and was slowly poured into a mixture of ice and EtOAc. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with 1 N aqueous HCl, water, saturated aqueous $NaHCO_3$ and brine, and then dried over solid Na_2SO_4. After removal of the solvent, a brownish, oily crude product was obtained, to which 70 mL of a mixed solution of dioxane:H_2O = 1:1 was added, followed by 50 mL glacial acetic acid. The resulting mixture was refluxed at 60 ^oC overnight. When complete, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with 1N aqueous HCl, water, saturated aqueous $NaHCO_3$, brine, and dried over solid Na_2SO_4. After removal of the solvent followed by purification with a gradient column chromatography (hexane:EtOAc = 100:0 to 40:60), a mixture of **28a** and **28b** was obtained as a yellowish oil in a 10/7 ratio (0.92 g, 1.46 mmol, 72%). ^{1}H NMR ($CDCl_3$, 300 MHz) (mixture of **28a** and **28b**) δ 7.3 – 7.4 (m, 10H), 5.12 (d, *J* = 3.7 Hz, 1H), 5.11 (d, *J* = 3.4 Hz, 1H), 4.89 (d, *J* = 10.7 Hz, 1H), 4.87 (d, *J* = 10.3 Hz, 1H), 4.83 (d, *J* = 10.3 Hz, 1H), 4.63 (d, *J* = 1.0 Hz, 1H), 4.0 - 4.2 (m, 4H), 3.7 – 3.9 (m, 5H), 3.3 – 3.6 (m, 16H), 3.2 – 3.3 (m, 4H), 2.8 (m, 1H), 2.3 (m, 2H), 1.4 – 1.7 (m, 6H), 1.2 (m, 20H), 0.87 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 137.8 (2 carbons), 128.8 (4 carbons), 128.32, 128.27, 128. 16 (2 carbons), 128.09 (2 carbons), 99.7 (2 carbons), 84.3 (2 carbons), 81.2 (2 carbons), 80.9 (2 carbons), 79.1, 78.7 (2 carbons), 76.1, 75.8, 75.5, 74.2, 73.9, 71.7, 71.5, 64.4 (2 carbons), 59.9 (2 carbons), 59.0 (2 carbons), 51.1 (2 carbons), 32.2 (2 carbons), 32.0 (2 carbons), 30.6 (2 carbons), 29.7 (2 carbons), 29.4 (2 carbons), 26.3 (2 carbons), 22.8 (2 carbons), 14.3 (2 carbons); ESI/APCI calcd for $C_{27}H_{40}N_{12}O_6Na$ ($[M+Na]^+$) *m/z* 651.3086; measured *m/z* 651.3105.

3′-*O*-benzyl-4′-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (28b). Please refer to the synthesis of compound **28a.**

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-4′-*O*-benzyl-3′-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (29a). A solution of the mixture of **28a** and **28b** (0.20 g, 0.32 mmol), **24a** (0.24 g, 0.38 mmol), and activated powder 4Å molecular sieve was stirred at room temperature for 2 hours in 12 mL of a mixed anhydrous solution Et_2O:CH_2Cl_2 = 3:1. The mixture was cooled to -70 ^oC and *N*-iodosuccinimide (0.09 g, 0.38 mmol) was quickly added. After the temperature has warmed up to -40 ^oC, trifluoromethanesulfonic acid (0.05 mL) was added. The solution was stirred at low temperature till the complete consumption of the glycosyl donor. The reaction mixture was quenched by addition of solid $NaHCO_3$, $Na_2S_2O_3$ and Na_2SO_4. After being stirred for 15 minutes, the reaction mixture was filtered through Celite. The residue was washed thoroughly with EtOAc. After removal of the solvents, the crude product was purified with gradient column chromatography (Hexane:EtOAc = 100:0 to 50:50) to afford a mixture of **29a** and **29b** as a yellowish oil mixed with some inseparable impurities that prevented a full characterization.

6-*O*-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl)-3′-*O*-benzyl-4′-*O*-*n*-octyl-1,3,2′,6′-tetraazidoneamine (29b). Please refer to the synthesis of **29a**.

6-*O*-(α-D-Glucopyranosyl)-3′-*O*-*n*-octylneamine (FG12). Please refer to the general procedure for the synthesis of kanamycin B analogs. An inseparable mixture of **FG12** and **FG13** was obtained (0.02 g, 0.003 mmol, 35%) as chloride salts. The spectral information of only one of them (**FG12** or **FG13**) is reported as follows: ^1H NMR (D_2O, 300 MHz) (chloride salt) δ 5.79 (d, *J* = 3.8 Hz, 1H), 4.95 (d, *J* = 3.1 Hz, 1H), 3.3 - 4.0 (m, 19H), 2.4 (m, 1H), 1.7 - 1.9 (m, 1H), 1.4 – 1.5 (m, 2H), 1.1 - 1.2 (m, 10H), 0.71 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (D_2O, 100 MHz) (chloride salt) 101.8, 96.1, 84.0, 79.2, 77.6, 74.3, 73.0, 72.9 (2 carbons), 71.7, 69.8, 69.3 (2 carbons), 60.6, 52.9, 49.9, 48.4, 40.1, 31.3, 29.5, 28.7, 28.5, 25.3, 25.2, 22.2, 13.6; ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ($[M+H]^+$) *m/z* 597.3705; measured *m/z* 597.3716.

6-*O*-(α-D-Glucopyranosyl)-4′-*O*-*n*-octylneamine (FG13). Please refer to the synthesis of **FG12.**

Phenyl 3-azido-2,4-di-*O*-benzyl-3-deoxy-1-thio-β-D-glucopyranoside (33). Compound **33** was prepared using the same procedures for the synthesis of compound **2** and was obtained as a light yellowish oil (0.73 g, 1.53 mmol, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.6 – 7.2 (m, 15H), 4.93 (d, *J* = 10.3 Hz, 1H), 4.86 (d, *J* = 11.0 Hz, 1H), 4.83 (d, *J* = 10.1Hz, 1H), 4.67 (dd, *J* = 12.0 , 10.7 Hz, 1H), 3.9 -3.8 (m, 1H), 3.8 – 3.7 (m, 1H), 3.63 (t, *J* = 8.9 Hz, 1H), 3.43 (t, *J* = 9.6 Hz, 1H), 3.4 – 3.3 (m, 1H), 3.32 (t, *J* = 9.6 Hz, 1H), 1.90 (t, OH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 137.45, 133.4, 132.2 (4 carbons), 129.4 (2 carbons), 128.9 (2 carbons), 128.7 (2 carbons), 128.6 (2 carbons), 128.4 (2 carbons), 128.1, 88.0, 79.9, 79.8, 76.0, 75.7, 75.2, 70.7, 62.0; ESI/APCI calcd for $C_{26}H_{28}N_3O_4S$ ($[M+H]^+$) *m/z* 478.1795; measured *m/z* 478.1793.

Phenyl 3-azido-3-deoxy-2,4-di-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (34). Using benzyl bromide instead of octyl bromide, compound **34** was prepared using the same procedure as for the synthesis of compound **2** and was obtained as a light yellowish oil (0.85 g, 1.64 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5 – 7.4 (m, 2H), 7.3 – 7.2 (m, 3H), 4.59 (d, *J* = 10.0 Hz, 1H), 3.9 – 3.5 (m, 5H), 3.44 (t, *J* = 9.3, 1H), 3.28 (ddd, *J* = 12.0, 9.6, 2.4 Hz, 1H), 3.12 (t, *J* = 9.6, 1H), 3.04 (t, *J* = 9.6, 1H), 1.7 - 1.5 (m, 4H), 1.3 – 1.2 (m, 20H), 0.87 (t, *J* = 5.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.6, 131.9 (2 carbons), 129.2 (2 carbons), 127.9, 87.9, 80.1, 80.0, 73.9, 73.5, 70.7, 62.2, 32.0, 30.3 (2 carbons), 29.9, 29.6, 29.5, 29.4, 26.2 (2 carbons), 22.9, 14.3 (2 carbons); ESI/APCI calcd for $C_{28}H_{47}N_3O_4SNa$ ($[M+Na]^+$) *m/z* 544.3180; measured *m/z* 544.3175.

Phenyl 3-azido-4,6-benzylidene-3-deoxy-2-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (35). Please refer to the general procedure for *O*-alkylation of sugars. Compound **35** was obtained as a light

yellowish oil (1.37 g, 2.76 mmol, 38%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 - 7.5 (m, 10H), 5.55 (s, 1H), 4.70 (d, *J* = 9.6 Hz, 1H), 4.34 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.6 - 3.9 (m, 4H), 3.4 - 3.5 (m, 2H), 3.18 (t, *J* = 9.3 Hz, 1H), 1.6 - 1.7 (m, 2H), 1.2 - 1.6 (m, 10H), 0.90 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.0, 133.4, 132.3, 129.4, 129.3, 129.1, 129.0, 128.6, 128.2, 126.3, 101.7, 89.0, 80.4, 79.1, 74.3, 71.2, 68.8, 67.1, 30.4, 30.1, 29.7, 29.6, 29.4, 26.4, 26.2, 22.9, 14.3; ESI/APCI calcd for $C_{27}H_{36}N_3O_4S$ ([MH^+]) *m/z* 498.2421; measured *m/z* 498.2432.

Phenyl 3-azido-2-*O*-benzyl-3-deoxy-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (36). Please refer to the synthesis of **12.** Compound **36** was obtained as a light yellowish oil (0.7 g, 1.40 mmol, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 – 7.6 (m, 10H), 4.89 (d, *J* = 10.0 Hz, 1H), 4.77 (d, *J* = 10.0 Hz, 1H), 4.67 (d, *J* = 9.6 Hz, H1, 1H), 3.89 (ddd, *J* = 12.0, 5.9, 2.4 Hz, 1H), 3.6 – 3.8 (m, 3H), 3.53 (t, *J* = 9.3 Hz, 1H), 3.3 – 3.4 (m, 1H), 3.25(t, *J* = 8.9 Hz, 1H), 3.19 (t, *J* = 9.6 Hz, 1H), 1.9 – 2.0 (m, 1H, OH), 1.5 - 1.6 (m, 2H), 1.2 – 1.3 (m, 10H), 0.85 (t, *J* = 5.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3, 131.2, 132.0 (3 carbons),129.2 (3 carbons), 128.7 (3 carbons), 128.6 (2 carbons), 128.2 (2 carbons), 127.9 (2 carbons), 87.8, 79.9, 79.4, 75.5, 73.4, 70.5, 62.0, 31.9, 30.3, 29.5, 29.3, 26.1, 22.7, 14.2; ESI/APCI calcd for $C_{27}H_{37}N_3O_4SNa$ ([M+Na]$^+$) *m/z* 522.2397; measured *m/z* 522.2415.

Phenyl 3-azido-4,6-di-*O*-benzyl-3-deoxy-2-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (37a). Please refer to the synthesis of **12** and general procedure for *O*-alkylation of sugars. Compound **37a** was obtained as a light yellowish oil (0.34 g, 0.57 mmol, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5 - 7.6 (m, 2H), 7.2 - 7.4 (m, 13H), 4.81 (d, *J* = 11.0 Hz, 1H), 4.5 - 4.6 (m, 4H), 3.88 (m,1H), 3.7 - 3.8 (m, 3H), 3.4 - 3.6 (m, 3H), 3.14 (t, *J* = 9.3 Hz, 1H), 1.6 - 1.7 (m, 2H), 1.3 - 1.4 (m,10H), 0.90 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.7, 133.9, 132.0, 129.0, 128.6, 128.5, 128.4, 128.2, 127.9, 127.8, 127.6, 88.0, 80.0, 79.5, 76.2, 75.0, 73.8, 73.6, 70.9, 68.9, 32.0, 30.3, 29.6, 29.5, 26.2, 22.8, 14.3; ESI/APCI calcd for $C_{34}H_{47}N_4O_4S$ ([M+NH_4^+]) *m/z* 607.3313; measured *m/z* 607.3323.

Phenyl 6-*O*-acetyl-3-azido-2-*O*-benzyl-3-deoxy-4-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (37b). To a solution of **36** in DCM was added 3 eq. of Ac_2O and 5 eq. of TEA. The mixture was stirred overnight. Solid $NaHCO_3$ was then added to neutralize the excess acid. EtOAc was added to dilute the solution and the organic layer was washed with water, saturated aqueous $NaHCO_3$ (3 times), and brine. The organic layer was then dried over Na_2SO_4, filtered, and concentrated. Purification by gradient column chromatography (hexane:EtOAc = 100:0 to 90:10) provided **37b** as a light yellowish oil (0.72 g, 1.33 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 – 7.6 (m, 10H), 4.90 (d, *J* = 10.0 Hz, 1H), 4.74 (d, *J* = 10.0 Hz, 1H), 4.61 (d, *J* = 9.6 Hz, H1, 1H), 4.36 (dd, *J* = 11.7, 2.1 Hz, 1H), 4.19 (dd, *J* = 12.0, 5.8 Hz, 1H), 3.7 – 3.8 (m, 1H), 3.5 – 3.4 (m, 3H), 3.27 (t, *J* = 9.6 Hz, 1H), 3.11 (t, *J* = 9.6 Hz, 1H), 2.09 (s, 3H), 1.6 - 1.5 (m, 2H), 1.3 – 1.2 (m, 10H), 0.85 (t, *J* = 5.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 137.5, 133.5, 132.3, 129.1, 128.8, 128.7, 128.4, 128.0, 87.9, 79.4, 75.6, 73.6, 70.8, 63.4, 32.1, 32.0, 30.3, 29.9, 29.6, 29.4, 26.2, 22.8, 21.0, 14.3; ESI/APCI calcd for $C_{29}H_{39}N_3O_5S$ ($[M+H]^+$) *m/z* 542.2503; measured *m/z* 542.2510.

Phenyl 3-azido-2,4-di-*O*-benzyl-3-deoxy-6-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (37c). Please refer to the general procedure for *O*-alkylation of sugars. Compound **37c** was obtained as a light yellowish oil (0.85 g, 1.45 mmol, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.2 – 7.7 (m, 15H), 4.95 (d, *J* = 10.3 Hz, 1H), 4.88 (d, *J* = 10.7 Hz, 1H), 4.79 (d, *J* = 10.0 Hz, 1H), 4.6 – 4.7 (m, 2H), 3.7 – 3.8 (m, 2H), 3.65 (t, *J* = 9.3 Hz, 1H), 3.4 – 3.6 (m, 4H), 3.37 (t, *J* = 9.3 Hz, 1H), 1.6 - 1.7 (m, 2H), 1.3 – 1.4 (m, 10H), 0.92 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 137.7, 133.9, 132.2 (2 carbons), 129.2 (2 carbons), 128.9 (2 carbons), 128.8 (2 carbons), 128.7 (2 carbons), 128.6 (2 carbons), 128.5, 128.3, 127.9, 88.30, 79.7, 75.5, 75.1, 72.1, 71.0, 69.6, 32.1, 30.1, 29.8, 29.6, 26.5, 23.0, 14.4; ESI/APCI calcd for $C_{34}H_{43}N_3O_4SNa$ ($[M+Na]^+$) *m/z* 612.2867; measured *m/z* 612.2850.

Phenyl 3-azido-2-*O*-benzyl-3-deoxy-4,6-di-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (37d). Please refer to the synthesis of **12** and general procedure for *O*-alkylation of sugars. Compound **37d** was

obtained as a light yellowish oil (0.18 g, 0.29 mmol, 82%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 - 7.5 (m, 10H), 4.89 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 10.3 Hz, 1H), 4.62 (d, *J* = 9.6 Hz, 1H), 3.2 - 3.8 (m, 10H), 1.5 - 1.7 (m, 4H), 1.2 - 1.4 (m, 20H), 0.9 - 1.0 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137. 9, 134.1, 132.2, 129.3, 129.0, 128.8, 128.4, 127.9, 88.1, 80.0, 79.5, 77.0, 75.6, 73.4, 72.1, 71.1, 69.8, 32.3, 32.2, 32.1, 30.6, 30.2, 29.9, 29.8, 29.7, 28.3, 26.6, 26.5, 23.0, 14.5 (2 carbons); ESI/APCI calcd for $C_{35}H_{57}N_4O_4S$ ([M+NH_4^+]) *m/z* 629.4095; measured *m/z* 629.4112.

Phenyl 6-*O*-acetyl-3-azido-3-deoxy-2,4-di-*O*-*n*-octyl-1-thio-β-D-glucopyranoside (37e). Please refer to the synthesis of **37b.** Compound **37e** was obtained as a light yellowish oil (0.88 g, 1.56 mmol, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5 – 7.4 (m, 2H), 7.3 – 7.2 (m, 3H), 4.54 (d, *J* = 10.0 Hz, H1, 1H), 4.34 (dd, *J* = 12.0, 2.1 Hz, 1H), 4.15 (dd, *J* = 11.7, 5.8 Hz, 1H), 3.9 – 3.6 (m, 3H), 3.5 – 3.4 (m, 3H), 3.06 (ddd, *J* = 12.0, 9.6, 2.4 Hz, 1H) 2.06 (s, 3H), 1.7 - 1.5 (m, 4H), 1.3 – 1.2 (m, 20H), 0.87 (t, *J* = 5.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 133.8, 132.1, 129.1, 128.1, 128.0, 127.8, 88.0, 80.0, 77.0, 73.9, 73.5, 70.8, 63.5, 32.1, 32.0, 30.3 (2 carbons), 29.6, 29.59, 29.5, 29.4, 26.2 (2 carbons), 22.9, 22.8, 21.0, 14.3 (2 carbons); ESI/APCI calcd for $C_{30}H_{49}N_3O_5SNa$ ([M+Na]$^+$) *m/z* 586.3285; measured *m/z* 586.3276.

6-*O*-(3-azido-4,6-di-*O*-benzyl-3-deoxy-2-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (38a). Please refer to the general procedure for the synthesis of kanamycin B analogs. Compound **38a** was obtained as a light yellowish oil (0.07 g, 0.08 mmol, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.4 - 7.9 (m, 10H), 5.5 (d, *J* = 3.8Hz, 1H), 5.25 (d, *J* = 3.4, 1H), 4.80 (d, *J* = 10.7, 1H), 4.4 - 4.7 (m, 4H), 4.1 - 4.2 (m, 2H), 3.2 - 4.0 (m, 19H), 3.31 (m, 1H), 1.6 – 1.7 (m, 3H), 1.2 - 1.4 (m, 10H), 0.90 (t, *J* = 6.7Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.5, 128.7 (2 carbons), 128.5, 128.3, 128.1, 127.9, 98.5, 97.2, 84.5, 80.5, 79.2, 76.9, 76.2, 75.6, 75.2, 73.7, 71.9, 71.4, 71.3, 71.1, 68.2, 64.9, 63.2, 59.7, 59.1, 51.3, 32.4, 32.0, 30.0, 29.5, 29.4, 26.0, 22.8, 14.3; ESI/APCI calcd for $C_{40}H_{59}N_{16}O_{10}$ ([M+NH_4^+]) *m/z* 923.4595; measured *m/z* 923.4610.

6-*O*-(3-azido-2-*O*-benzyl-3-deoxy-4-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (38b). Please refer to the general procedure for the synthesis of kanamycin B analogs. Compound **38b** was obtained as a light yellowish oil (0.11 g, 1.35 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5 – 7.3 (m, 5H), 5.45 (d, *J* = 3.45 Hz, 1H), 5.08 (d, *J* = 3.1 Hz, 1H), 4.74 (s, 2H), 4.1 – 3.0 (m, 30H), 2.3 – 2.2 (m, 1H), 1.5 – 1.3 (m, 3H), 1.2 – 1.1 (m, 10H), 0.87 (t, *J* = 6.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 129.0, 128.8, 128.5, 128.4, 128.3, 99.1, 97.4, 84.1, 81.5, 77.8, 75.5, 73.8, 73.4, 72.1, 71.9, 71.6, 71.3, 65.0, 63.4, 61.8, 59.7, 58.9, 51.3, 32.3, 32.0, 30.4, 29.6, 29.4, 26.2, 22.8, 14.3; ESI/APCI calcd for $C_{33}H_{49}N_{15}O_{10}Na$ ($[M+Na]^+$) *m/z* 838.3679; measured *m/z* 838.3709.

6-*O*-(3-azido-2,4-di-*O*-benzyl-3-deoxy-6-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (38c). Please refer to the general procedure for the synthesis of kanamycin B analogs. Compound **38c** was obtained as a light yellowish oil (0.09 g, 0.10 mmol, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 – 7.5 (m, 10H), 5.55 (d, *J* = 3.5 Hz, 1H), 5.09 (d, *J* = 3.5 Hz, 1H). 4.9 – 4.8 (m, 1H), 4.75 (s, 2H), 4.5 - 4.6 (m, 1H), 3.9 – 4.1 (m, 4H). 3.1 – 3.7 (m, 20H), 2.32 (ddd, *J* = 12.7, 4.1, 4.1 Hz, 1H), 1.4 – 1.7 (m, 3H), 1.1 – 1.2 (m, 10H), 0.72 (t, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.5, 129.0 (2 carbons), 128.8, 128.5 (2 carbons), 128.4 (3 carbons), 98.6, 97.4, 84.3, 80.6, 76.3, 75.6, 75.3, 73.4, 72.1, 71.5, 71.3, 71.1, 68.9, 65.1, 63.3, 59.7, 59.1, 51.4, 32.4, 32.0, 29.6, 29.5, 29.4, 26.3, 22.9, 14.3; ESI/APCI calcd for $C_{40}H_{55}N_{15}O_{10}Na$ ($[M+Na]^+$) *m/z* 928.4149; measured *m/z* 928.4151.

6-*O*-(3-azido-2-*O*-benzyl-3-deoxy-4,6-di-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (38d). Please refer to the general procedure for the synthesis of kanamycin B analogs. Compound **38d** was obtained as a light yellowish oil (0.08 g, 0.08 mmol, 52%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 - 7.4 (m, 5H), 5.57 (d, *J* = 3.7 Hz, 1H), 5.06 (d, *J* = 3.4 Hz, 1H), 4.77 (s, 2H), 4.35 (s, 1H), 3.1 – 4.1 (m, 24H), 2.30 (m, 1H), 1.6 - 1.7 (m, 5H), 1.2 - 1.4 (m, 20H), 0.90 (t, *J* = 6.7 Hz, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 128.9, 128.7, 128.4, 128.3, 98.5, 97.4, 84.4, 80.3, 77.0, 76.9, 76.6, 75.6, 73.5, 73.3, 72.2, 72.0, 71.9, 71.4, 71.3, 68.9, 65.1, 63.2, 59.6, 59.1, 51.3, 32.3, 32.0, 30.3, 29.8, 29.6, 29.5, 29.4, 26.2, 26.2, 22.8; ESI/APCI calcd for $C_{41}H_{65}N_{15}O_{10}Na$ ($[M+Na]^+$) *m/z* 950.4931; measured *m/z* 950.4958.

6-*O*-(3-azido-3-deoxy-2,4-di-*O*-*n*-octyl-α-D-glucopyranosyl)-1,3,2′,6′-tetraazidoneamine (38e). Please refer to the general procedure for the synthesis of kanamycin B analogs. Compound **38e** was obtained as a light yellowish oil (0.13 g, 0.15 mmol, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.48 (d, *J* = 3.4 Hz, 1H), 5.18 (d, *J* = 3.5 Hz, 1H), 4.4 – 4.5 (m, 1H), 4.1-3.3 (m, 25H), 3.21 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.04 (t, *J* = 9.6 Hz, 1H), 2.3 – 2.4 (m, 1H), 1.5 – 1.7 (m, 5H), 1.2 – 1.4 (m, 20H), 0.82 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 98.9, 97.3, 84.3, 81.4, 79.2, 75.3, 73.6, 72.3, 72.1, 71.5, 71.4, 64.9, 63.5, 61.9, 59.8, 59.0, 51.4, 32.4, 32.0 (2 carbons), 30.4, 30.2, 30.1, 29.9, 29.6 (2 carbons), 29.4 (2 carbons), 26.2, 26.0, 22.9 (2 carbons), 14.3 (2 carbons); ESI/APCI calcd for $C_{34}H_{59}N_{15}O_{10}Na$ ($[M+Na]^+$) *m/z* 860.4462; measured *m/z* 860.4490.

6-*O*-(3-Amino-3-deoxy-2-*O*-*n*-octyl-α-D-glucopyranosyl)neamine (FG14). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG14** was obtained as a chloride salt (0.06 g, 0.08 mmol, 95%). ^{1}H NMR (300 MHz, D_2O) δ 5.83 (d, *J* = 3.8 Hz, 1H), 5.23 (d, *J* = 3.1 Hz, 1H). 3.3 – 4.0 (m, 23H), 3.21 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.46 (ddd, *J* = 13.3, 4.1, 4.1 Hz, 1H), 1.94 (ddd, *J* = 13.3, 12.7, 12.4 Hz, 1H), 1.4 – 1.5 (m, 2H), 1.1 – 1.2 (m, 10H), 0.72 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 98.0, 96.4, 93.7, 77.9, 76.5, 74.6, 73.4, 72.7, 70.9, 69.5, 68.4, 65.8, 60.2, 54.0, 53.7, 49.6, 48.5, 40.4, 31.3, 29.2, 28.8, 28.6, 27.9, 25.1, 22.2, 20.7, 13.7; ESI/APCI calcd for $C_{26}H_{54}N_5O_{10}$ ($[M+H]^+$) *m/z* 596.3865; measured *m/z* 596.3879.

6-*O*-(3-Amino-3-deoxy-4-*O*-*n*-octyl-α-D-glucopyranosyl)neamine (FG15). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG15** was obtained as a chloride salt (0.91

g, 1.17 mmol, 87%). ^{1}H NMR (300 MHz, D_2O) δ 5.88 (d, *J* = 3.8 Hz, 1H), 5.23 (d, *J* = 3.7 Hz, 1H). 3.3 – 4.0 (m, 23H), 3.21 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.46 (ddd, *J* = 12.7, 4.1, 4.1 Hz, 1H), 1.94 (ddd, *J* = 12.7, 12.7, 12.4 Hz, 1H), 1.4 – 1.5 (m, 2H), 1.1 – 1.2 (m, 10H), 0.72 (t, *J* = 5.7 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.7, 96.1, 83.9, 77.4, 74.4, 73.7, 73.6, 72.4, 70.8, 69.4, 68.4, 68.2, 59.9, 54.3, 53.7, 49.8, 48.5, 40.3, 31.2, 29.3, 28.7, 28.6, 28.0, 25.3, 22.2, 13.7; ESI/APCI calcd for ESI/APCI calcd for $C_{26}H_{54}N_5O_{10}$ ($[M+H]^+$) *m/z* 596.3865; measured *m/z* 596.3863.

6-*O*-(3-Amino-3-deoxy-6-*O*-*n*-octyl-α-D-glucopyranosyl)neamine (FG16). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG16** was obtained as a chloride salt (0.64 g, 0.82 mmol, 83%). ^{1}H NMR (300 MHz, D_2O) δ 5.91 (d, *J* = 4.1 Hz, 1H), 5.23 (d, *J* = 3.4 Hz, 1H). 3.3 – 4.0 (m, 23H), 3.21 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.46 (ddd, *J* = 12.7, 4.1, 4.1 Hz, 1H), 1.89 (ddd, *J* = 12.7, 12.7, 12.4 Hz, 1H), 1.4 – 1.5 (m, 2H), 1.1 – 1.2 (m, 10H), 0.74 (t, *J* = 5.7 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 101.0, 95.7, 83.9, 77.0, 74.4, 72.2, 72.1, 70.8, 69.48, 68.4, 68.2, 65.6, 55.1, 53.6, 49.8, 48.6, 40.4, 31.3, 28.8, 28.7, 28.6, 28.0, 25.3, 22.2, 20.6, 13.6; ESI/APCI calcd ESI/APCI calcd for $C_{26}H_{54}N_5O_{10}$ ($[M+H]^+$) *m/z* 596.3865; measured *m/z* 596.3857.

6-*O*-(3-Amino-3-deoxy-4,6-di-*O*-*n*-octyl-α-D-glucopyranosyl)neamine (FG17). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG17** was obtained as a chloride salt (0.06 g, 0.07 mmol, 76%). ^{1}H NMR (300 MHz, D_2O) δ 5.93 (d, *J* = 3.8 Hz, 1H), 5.23 (d, *J* = 3.1 Hz, 1H). 3.2 – 4.0 (m, 25H), , 2.43 (ddd, *J* = 13.3, 4.1, 4.1 Hz, 1H), 1.94 (ddd, *J* = 13.3, 12.7, 12.4 Hz, 1H), 1.4 – 1.6 (m, 4H), 1.1 – 1.2 (m, 20H), 0.72 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.9, 94.7, 84.3, 73.8, 72.2, 71.9, 71.5, 69.6, 68.9, 68.7, 54.4, 50.4, 49.1, 41.1, 31.8 (2 carbons), 29.8, 29.5, 29.4 (2 carbons), 29.3 (3 carbons), 26.2, 25.9, 22.5 (2 carbons), 13.3 (2 carbons); ESI/APCI calcd for $C_{34}H_{70}N_5O_{10}$ ($[M+H]^+$) *m/z* 708.5117; measured *m/z* 708.5119.

6-*O*-(3-Amino-3-deoxy-2,4-di-*O*-*n*-octyl-α-D-glucopyranosyl)neamine (FG18). Please refer to the general procedure for the synthesis of kanamycin B analogs. **FG18** was obtained as a chloride salt (0.06 g, 0.07 mmol, 45%). ^{1}H NMR (300 MHz, D_2O) δ 5.81 (d, *J* = 3.8 Hz, 1H), 5.23 (d, *J* = 3.1 Hz, 1H). 3.2 – 4.0 (m, 25H), , 2.43 (ddd, *J* = 13.3, 4.1, 4.1 Hz, 1H), 1.94 (ddd, *J* = 13.3, 12.7, 12.4 Hz, 1H), 1.4 – 1.6 (m, 4H), 1.1 – 1.2 (m, 20H), 0.72 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 98.0, 96.5, 83.8, 78.1, 76.4, 74.5, 73.8, 72.7, 70.7, 69.4, 68.4, 59.9, 53.6, 53.1, 49.5, 48.4, 40.2, 31.2, 29.3, 29.1, 28.7, 28.5, 28.0, 25.2, 25.0, 22.2, 13.6; ESI/APCI calcd for ESI/APCI calcd for $C_{34}H_{70}N_5O_{10}$ ($[M+H]^+$) *m/z* 708.5117; measured *m/z* 708.5126.

Antibacterial MIC Determination. A solution of selected bacteria was inoculated in the Trypticase Soy broth at 35°C for 1-2 h. The bacteria concentration was found and diluted with broth, if necessary, to an absorption value of 0.08 to 0.1 at 625 nm. The adjusted inoculated medium (100 μL) was diluted with 10 mL of broth and then applied to a 96-well microtiter plate (50 μL). A series of solutions (50 μL each in 2-fold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35°C for 12-18 h. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

Antifungal MIC Determination. *In vitro* growth inhibition of yeast strains by FG compounds were determined using MIC microbroth dilution assays in 96-well uncoated polystyrene microtiter plates (Corning Costar, Corning, NY, USA) as described in the M27-A3 reference methods of the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Standards Laboratory Standards) (NCCLS).[16] Serial dilutions of compounds were made in uncoated polystyrene 96-well plates in the range of 0.48 to 500 μg/mL. For MIC determinations with filamentous fungi, previously described methods were used.[17]

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Supporting Information Available ^{1}H and ^{13}C NMR spectra of the synthesized compounds. This material is available free of charge via the internet at http://pubs.acs.org.

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TOC Graphical Abstract

antifungal kanamycin B derivative