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Divergent Synthesis of Three Classes of Antifungal Amphiphilic Kanamycin Derivatives

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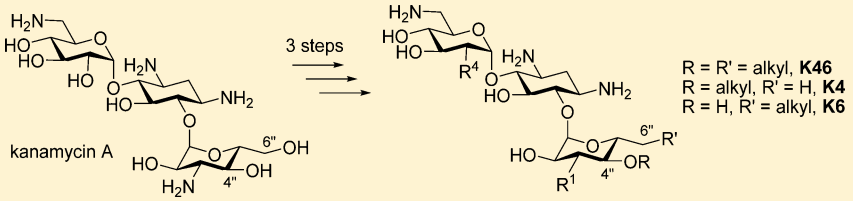
Supporting Information



ABSTRACT: A concise and novel method for site-selective alkylation of 1,3,6′,3″-tetraazidokanamycin has been developed that leads to the divergent synthesis of three classes of kanamycin A derivatives. These new amphiphilic kanamycin derivatives bearing alkyl chains length of 4, 6, 7, 8, 9, 10, 12, 14, and 16 have been tested for their antibacterial and antifungal activities. The antibacterial effect of the synthesized kanamycin derivatives declines or disappears as compared to the original kanamycin A. Several compounds, especially those with octyl chain at *O*-4″ and/or *O*-6″ positions on the ring III of kanamycin A, show very strong activity as antifungal agents. In addition, these compounds display no toxicity toward mammalian cells. Finally, computational calculation has revealed possible factors that are responsible for the observed regioselectivity. The simplicity in chemical synthesis and the fungal specific property make the lead compounds ideal candidates for the development of novel antifungal agents.

INTRODUCTION

Fungal diseases are one of the major threats to human health and food security.[1] Fungal crop diseases like wheat head blight or scab (caused by *Fusarium graminearum*) and stem rust (caused by *Pucciniagraminis*) result in large economic losses and threats to the world's food supplies.[2] Traditional and commonly used antifungals such as amphotericin B and azoles are still in use to treat invasive fungal infections, and fungicidal triazoles, pyrimidines, and strobilurins continue to be used in massive quantities for wheat and other major crops.[3] The effectiveness of the current antifungals keeps decreasing due to fungal resistance, and traditional crop antifungals are causing huge disturbances to natural ecosystems. As a consequence, there is a growing and urgent need to develop novel and effective antifungal agents.

Aminoglycoside antibiotics including kanamycin have been used for treating bacterial infectious disease for almost 70 years (Figure 1).[4] Nevertheless, the prevalence of aminoglycosides-resistant bacterial has significantly reduced the effectiveness of aminoglycosides. For example, kanamycin has been considered clinically obsolete due to bacterial resistance, and thus a surplus of kanamycin sulfate has accumulated and no applicable usage can be implemented.[5]

While the vast amount of effort has been devoted to the development of new aminoglycoside derivatives, studies have shown that most of the modified aminoglycosides are not expected to be effective against aminoglycoside-resistant bacteria due to the evolutionary emergence of aminoglycoside-modifying enzymes (AMEs).[5] Many families of AME manifest substrate promiscuity, rendering extensive structurally modified aminoglycosides, kanamycin, or neomycin classes ineffective.[6] Recent research advances on amphiphilic aminoglycosides, however, may reverse the downward trend in the effectiveness of aminoglycosides for fighting microbial infectious diseases.[7−22]

Traditional aminoglycosides exert their antibacterial activity by binding to the A-site decoding region of the 16S rRNA of bacteria and, thus, interfering with the expression of critical proteins leading to eventual cell death. Amphiphilic aminoglycosides have been shown to have different modes of antibacterial action.[23,24] Rather than targeting cellular nucleic acids, they are capable of disrupting bacterial membranes and display bactericidal effects via this new mode of action. Studies have further shown that the membrane selectivity of these amphiphilic aminoglycosides may be to tuned toward eukaryotic fungi but not plant or mammalian cells.[21,24] These discoveries have revived the interest as well as the usages of aminoglycosides.

In an effort to combat fungal diseases, we have recently developed an amphiphilic kanamycin, **K20**, with antifungal activity but not antibacterial activity.[25,26] Such an invention has

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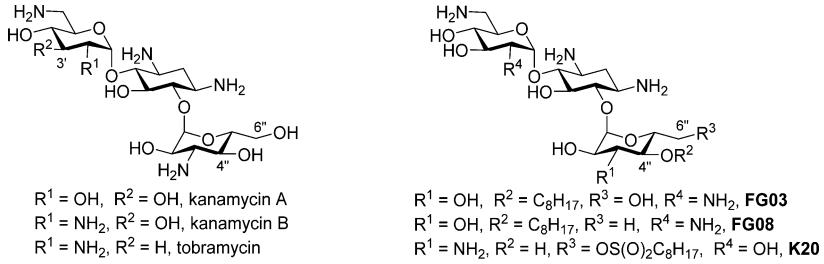
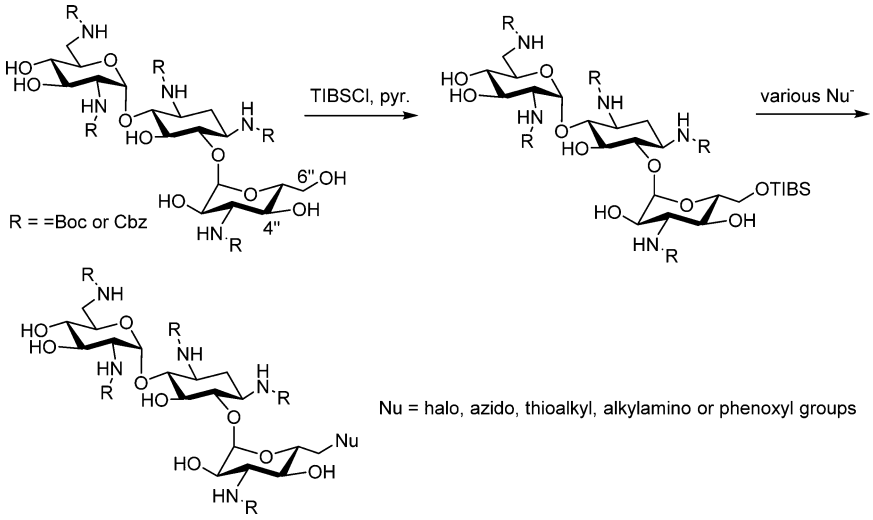


Figure 1. Structure of kanamycin, tobramycin, **K20**, **FG03**, and **FG08**.

Scheme 1



the potential of utilizing the huge stock pile of kanamycin (>90% as kanamycin A) that is otherwise ineffective against bacteria and turning it into novel and high value fungicides. In our continuing studies on the structure activity relationship of amphiphilic antifungal kanamycin, we have revealed that incorporating hydrophobic chain(s) at the *O*-4″ and/or *O*-6″ positions of ring III are crucial for exerting antifungal activity.[16] Various hydrophobic groups, such as phenoxyl, alkylamino, alkylmercapto, and alkylcarbonyl, can be attached to the *O*-6″ position, but it is much more difficult to do so at the *O*-4″ position.[4] Our earlier work on antifungal kanamycins, such as **FG03** and **FG08**, is based upon hydrophobic groups attached at the *O*-4″ and/or *O*-6″ positions of ring III via ether linkage, a much more stable functional group as compared to ester or sulfonate (Figure 1). Nevertheless, tedious synthetic steps prevent the large-scale production of these two compounds. Using a direct modification approach, we reported a three-step synthesis of amphiphilic antifungal aminoglycoside, **K20**.[25,26] While **K20** can be produced in kilogram scale, the octanesunfonyl group attached at the *O*-6″ position is rather unstable, especially in high pH condition. Thus, our group has started to explore the possibility of incorporating hydrophobic groups attached at the *O*-4″ and/or *O*-6″ positions of kanamycin via ether linkages using direct modification approaches.

In general, it is more difficult to introduce functional groups using an ether linkage, which is often synthesized through the Williamson reaction in the presence of strong bases, such as NaH or alkoxides. To selectively incorporate hydrophobic groups attached at the *O*-4″ and/or *O*-6″ positions of kanamycin is even more challenging. Protection of the amino groups on kanamycin is often necessary prior to the modification of hydroxyl groups.

Employing carbamate-protected amino groups (e.g., Boc or Cbz) on kanamycin for the introduction of hydrophobic group via Williamson synthesis is challenging due to the poor solubility of carbamate-protected kanamycin in organic solvents. In addition, the Williamson synthesis often involves the use of strong base, which will lead to the formation of cyclic carbamate adducts.[4c] To circumvent the formation of cyclic carbamate adducts, the use of a weak base, such as $Ba(OH)_2$, is required.[13,27] Following selective sulfonation (using TsCl or TIBSCl) of the only primary hydroxyl group, 6″-OH, on kanamycin, this approach allowed the synthesis of derivatives with halo, azido, thioalkyl, alkylamino, or phenoxyl groups attached at the *O*-6″ position.[13,17,21,22,27] Nevertheless, very few examples for the introduction of alkoxyl groups have been reported (Scheme 1).[27] In addition, the reported methods only offer the possibility of synthesis of kanamycin derivatives with modifications at the *O*-6″ position but not the *O*-4″ position.

In the example using weak base, $Ba(OH)_2$, for *O*-alkylation, only the more reactive Bn and Me groups were reported, and no regioselectivity was noted.[13] When we conducted octylation of Boc-protected kanamycin using $Ba(OH)_2$ as the base, no reaction was noted for over 2 weeks. Alternatively, azido-protected (azido-masked) kanamycin is more suited as the starting material for the incorporation of hydrophobic groups attached at the *O*-4″ and/or *O*-6″ positions of ring III via ether linkage because azido groups are relatively more stable in the presence of strong bases.[28] The challenge is to develop a concise synthetic scheme to make amphiphilic kanamycin

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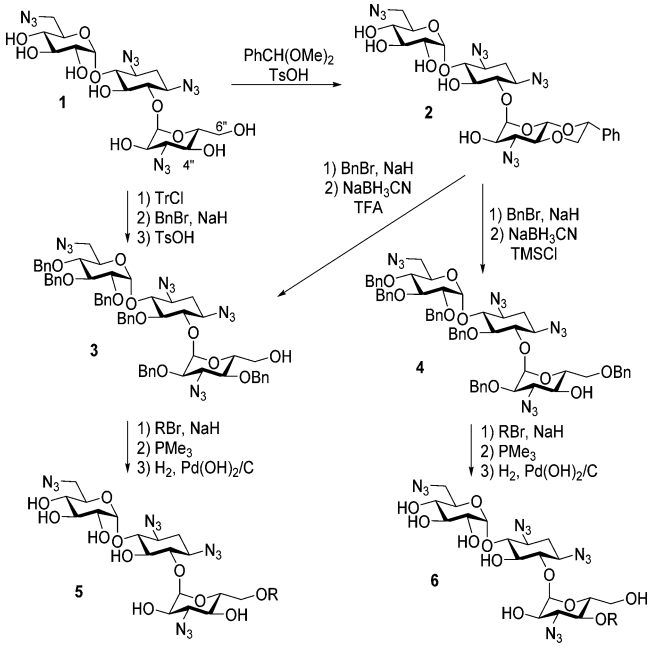
possessing regioselective incorporated ether linkages without excessive protection and deprotection steps.

Chemical Synthesis of Amphiphilic Kanamycin Derivatives. Masking the amino groups on aminoglycosides as azido groups has been utilized for direction modification of aminoglycosides.[4] To achieve the regioselective incorporation of alkyl group(s) at the *O*-4″ and/or *O*-6″ positions, the traditional or conservative approaches usually involve sequential steps including the protection of 4″-OH and/or 6″-OH, the protection of the remaining hydroxyl groups, the removal or selective removal of protecting groups at the 4″-OH and/or 6″-OH, alkylation, and global deprotection (Scheme 2).

Scheme 2



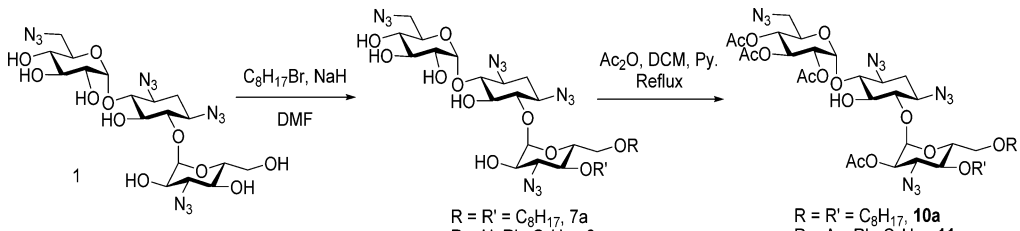
Numerous recent publications have demonstrated the feasibility of similar or identical reactions delineated in Scheme 2.[28] Although these relatively lengthy approaches could ensure the synthesis of desired products, they are not suitable for scale-up or even gram-scale synthesis of lead compounds. Without the capability of producing lead compounds in sufficient quantity, it will significantly hamper further tasks, such as pharmacokinetic study, in vivo efficacy, and clinical trials, which are essential for the development of practical therapeutics.

RESULTS AND DISCUSSION

Because tetraazidokanamycin A can be synthesized in relatively large quantity (10−20 g per batch), the crucial step is to simplify the regioselective alkylation at the *O*-4″ and/or *O*-6″ positions. With the expectation that 6″-OH, the only primary hydroxyl group, should be the most nucleophilic, we attempted the octylation of tetraazidokanamycin A using octyl bromide (1.5 equiv) and sodium hydride (20 equiv). We obtained three main components, which were purified by flash column chromatography. Following the acetylation and spectroscopic analysis using 1D and 2D NMR, we confirmed that these three components were three distinct compounds with octyl group at 4″-OH and 6″-OH (**7a**), 4″-OH only (**8a**), and 6″-OH only (**9a**) (Scheme 3). The yields of octylated compounds, **7a**, **8a**, and **9a**, were then determined to be 25%, 16%, and 14%, respectively. From further increases in the amount of octyl bromide to 2.5 equiv, only **7a** and **8a** were isolated in 46% and 45% yields, respectively. Because compounds **10a**, **11a**, and **12a** are not necessary for the synthesis of final products for biological assay, these compounds were characterized only with ^{1}H NMR and ^{1}H−^{1}H COSY, and the yields were not determined.

In our previous studies, we discovered that a kanamycin derivative bearing an octyl chain at 4″-OH is superior to those with butyl and dodecyl (C12) chains for conferring antifungal activity.[12] Nevertheless, no activity with chain length other than these three was investigated. Therefore, we decided to synthesize amphiphilic kanamycin derivatives with chain length varying from butyl to hexadecyl (C16) for a more complete profile on the structure activity relationship (SAR). All of the reactions were conducted using 20 equiv of NaH and 1.5 equiv of the alkyl bromides using DMF as the solvent. The goal was to generate all three adducts with alkyl group at 4″-OH and 6″-OH (**7b**−**i**), 4″-OH (**8b**−**i**), and 6″-OH (**9b**−**i**) positions rather than optimizing the yields for compounds **7** and **8**. On the basis of R_f values from TLC, the observed polarity of the three products was **8** > **9** ≫ **7**. The greater polarity difference between **7** versus **8** and **9** made the purification of the dialkylated compounds from the monoalkylated compounds readily achievable. However, there is only a very small polarity difference between compounds **8** and **9** as column chromatography purification was successful in separating these two compounds for only one-half of the attempted reactions (Table 1, entries 4−6). Nevertheless, compounds **9b**−**i** were the minor product in all of the reactions. Therefore, in the cases when the isolation of pure 6″-alkylated compounds was unsuccessful

Scheme 3



equiv. of $C_8H_{17}Br$	Yield (%)		
	7a	**8a**	**9a**
1.5	25	16	14
2.5	46	45	Not isolated

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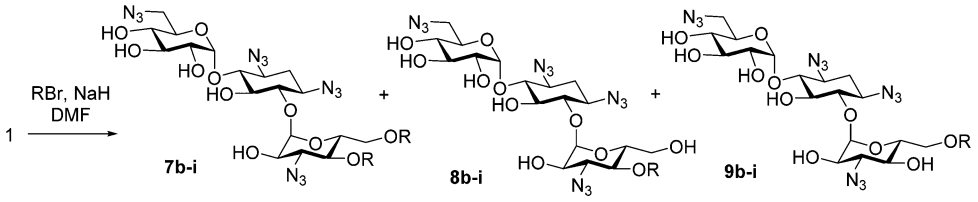
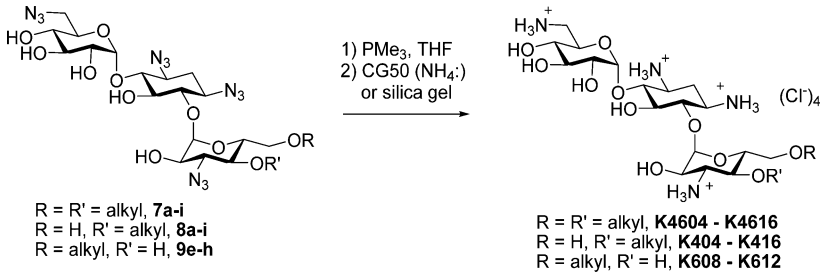
Table 1. Alkylation of Tetraazidokanamycin A



entry	alkyl bromide	7 (yield)	8 (yield)	9 (yield)
1	C_4H_9Br	**7b** (22)	**8b**(22)	**9b** (8[a])
2	$C_6H_{13}Br$	**7c** (28)	**8c**(21)	**9c** (8[a])
3	$C_7H_{15}Br$	**7d** (30)	**8d** (36)	**9d** (9[a])
4	$C_9H_{19}Br$	**7e** (34)	**8e** (25)	**9e** (13)
5	$C_{10}H_{21}Br$	**7f** (30)	**8f** (24)	**9f** (8)
6	$C_{12}H_{25}Br$	**7g** (21)	**8g** (28)	**9g** (13)
7	$C_{14}H_{29}Br$	**7h** (21)	**8h** (16)	**9h** (9[a])
8	$C_{16}H_{33}Br$	**7i** (23)	**8i** (25)	**9i** (7[a])

[a]Estimated from ^{1}H NMR, but the compounds were not isolated due to the low yields.

Table 2. Synthesis of Amphiphilic Kanamycin Derivatives



compounds	alkyl group and site of alkylation	yields (%)
K4604	4″,6″-di-*O*-butyl	71
K404	4″-*O*-butyl	47
K4606	4″,6″-di-*O*-hexyl	48
K406	4″-*O*-hexyl	67
K4607	4″,6″-di-*O*-heptyl	89
K407	4″-*O*-heptyl	46
K4608	4″,6″-di-*O*-octyl	46
K608	6″-*O*-octyl	91
K408	4″-*O*-octyl	47
K4609	4″,6″-di-*O*-noyl	84
K609	6″-*O*-noyl	39
K409	4″-*O*-noyl	42
K4610	4″,6″-di-*O*-decyl	59
K610	6″-*O*-decyl	66
K410	4″-*O*-decyl	41
K4612	4″,6″-di-*O*-dodecyl	62
K612	6″-*O*-dodecyl	52
K412	4″-*O*-dodecyl	38
K4614	4″,6″-di-*O*-tetradecyl	76
K414	4″-*O*-tetradecyl	60
K4616	4″,6″-di-*O*-hexadecyl	65
K416	4″-*O*-hexadecyl	41

(Table 1, entries 1, 2, 3, 7, and 8), the yields for these compounds were estimated on the basis of ^{1}H NMR. Following the acetylation of the isolated compounds (**7**–**9**), which yielded the corresponding compounds **10**–**12**, the sites of alkylation were confirmed using ^{1}H–^{1}H COSY. Again, compounds **10**–**12** were prepared for the purpose of confirming the sites of alkylation. These compounds were characterized only with ^{1}H NMR and ^{1}H–^{1}H COSY, and the yields were not determined. The result is summarized in Table 1.

Overall, the alkylations were very selective despite having seven hydroxyls present in the tetraazidokanamycin A, **1**, in all of the examined reactions. The alkyl chains only attached to the 4″ and 6″ positions of ring III. The major products were either **7** or **8** with **9** as the minor product in all cases. In several alkylations, the yields for compound **9** were too low to enable

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the isolation, and the yields were estimated only with ^{1}H NMR. The compounds that were isolated with column chromatography were subjected to Staudinger reaction followed by purification using ion-exchange resin or silica gel (Table 2). The final amphiphilic aminoglycosides were converted into the corresponding chloride salts and were tested for their biological activities.

We also conducted the analogous syntheses using pentaazidokanamycin B and pentaazidotobramycin as the starting material. Interestingly, no dominant regioselectivity was observed. Several attempts of octylating the pentaazidotobramycin yielded 4′,2″,4″,6″-tetra-*O*-octylpentaazidotobramycin as the major product along with a mixture of mono- and dioctylated adducts. Octylation of pentaazidokanamycin B afforded a complex mixture of alkylated adducts with no dominant products.

Biological Evaluation of Amphiphilic Kanamycin A Derivatives. All of the synthesized kanamycin derivatives were tested against two fungal species (*Aspergillus flavus* and *Fusarium graminearum*), and two bacterial species (*Escherichia coli* and *Staphylococcus aureus*). Both *A. flavus* and *F. graminearum* are significant plant fungal pathogens. *E. coli* and *S. aureus* were selected as model Gram-negative and Gram-positive bacterial species, respectively. The determined minimum inhibitory concentrations (MICs) of the amphiphilic kanamycin A derivatives are summarized in Table 3.

From the MICs, all of the newly synthesized kanamycin derivatives were considered inactive (MIC ≥ 32) against bacteria, except for **K4609** and **K4610**, which showed modest antibacterial activities. The K4 and K46 members appear to have better antifungal activities against *F. graminearum*, especially for those with C8 chain followed by chain length around C8, such as C6, C7, and C9. Further shortening and extending the chain length reduced the antifungal activity significantly. However, no significant activity was observed against *A. flavus*, a plant and animal pathogen known to be difficult to treat. It is notable that Fosso[20] and Shrestha[21] reported antifungal activities of amphiphilic tobramycin analogues against *A. nidulans* as well as *F. graminearum*. Despite being of the same genus, *A. nidulans* and *A. flavus* are phylogenetically quite distinct,[29] and their differences may account for the differing susceptibilities of these *Aspergillus* species to these aminoglycoside analogues. The inhibitory activities of **K408** and **K4608** against *A. nidulans* and of the amphiphilic tobramycin analogues against *A. flavus* remain to be determined. The overall trend of inhibitory activities of **K408** and **K4608** against *F. graminearum* versus alkyl chain length is shown in Figure 2.

Table 3. Antimicrobial Activity of Amphiphilic Kanamycin A Derivatives

		MIC (μg/mL)			
entry	compound	*Aspergillus flavus*	*F. graminearum*	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 25923)
1	**K4604**	>500	125	≥250	≥250
2	**K404**	>500	≥500	≥250	125
3	**K4606**	500	15.6	≥250	64
4	**K406**	>500	31.3	≥250	64
5	**K4607**	**500**	15.6	125	125
6	**K407**	>500	125	≥250	125
7	**K4608**	>500	31.3	≥250	≥250
8	**K608**	>500	125	≥250	≥250
9	**K408**	>500	15.6	≥250	125
10	**K4609**	500	125	16	8
11	**K609**	>500	125	32	≥250
12	**K409**	>500	62.5	≥250	≥250
13	**K4610**	500	≥500	≥250	16
14	**K610**	500	62.5	≥250	64
15	**K410**	>500	62.5	≥250	125
16	**K4612**	>500	≥500	≥250	64
17	**K612**	250	250	32	32
18	**K412**	>500	≥500	125−250	64
19	**K4614**	>500	≥500	≥250	≥250
20	**K414**	>500	≥500	≥250	≥250
21	**K4616**	>500	≥500	≥250	≥250
22	**K416**	>500	≥500	32	125
23	kanamycin			4	1
24	voriconazole	1	32		

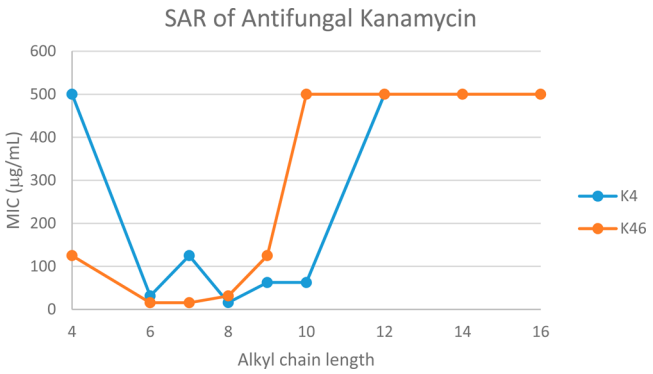

Figure 2. Antifungal activity against *F. graminearum*.

Our group and others have shown that antifungal amphiphilic aminoglycosides often display strong synergism with commonly employed azole-based fungicides. We selected two lead compounds, **K408** and **K4608**, for the synergistic investigation using azole-resistant *Candida albicans* ATCC 64124 as the fungal strain. As expected, both compounds show strong synergistic effect as indicated by the fractional inhibitory concentration index (FICI) with all of the azole-based fungicides (Tables 4 and 5). The synergistic inhibitory effects reported here for **K408** and **K4608** in combination with various azoles are against an azole-resistant strain. The data suggest that such combinations may have potential as antifungal therapeutics against azole-resistant candida mycoses.

The main concern of using amphiphilic aminoglycosides clinically is the toxicity toward mammalian cells. The

Table 4. Synergistic Study of K408[a]

	MIC (μg/mL)			
antifungal compound	alone	azole with **K408**	**K408** with azole	FICI[a] (**K408** with azole)
K408	31.25			
chlotrimazole	3.91	0.24	1.95	0.13
fluconazole	250	15.63	7.81	0.31
itraconazole	250	0.98	7.81	0.25
posaconazole	62.50	0.24	1.95	0.07
voriconazole	7.81	1.95	0.98	0.38

[a]Combination interactions are classified as synergistically inhibitory if the FICI is ≤0.5, indifferent if >0.5−4, and antagonistic if >4.

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Table 5. Synergistic Study of K4608[a]

antifungal compound	MIC (μg/mL) alone	azole with **K4608**	**K4608** with azole	FICI[a] (**K4608** with azole)
K4608	31.25			
chlotrimazole	3.91	3.91	1.95	0.19
fluconazole	>250	31.25	3.91	0.25
itraconazole	>250	3.91	15.63	<0.52
posaconazole	62.50	0.49	3.91	<0.13
voriconazole	7.81	0.98	1.95	0.19

[a]Combination interactions are classified as synergistically inhibitory if the FICI is ≤0.5, indifferent if >0.5−4, and antagonistic if >4.

cytotoxicity of the lead antifungal compounds, **K4608**, **K608**, and **K408**, was evaluated using human cervical cancer cell line HeLa and MTT assay. These compounds were not toxic to HeLa cells (Figure 3). In fact, compounds **K4608** and **K408**

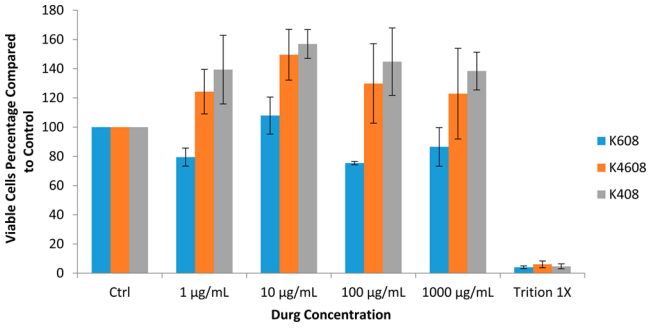

Figure 3. Cytotoxicity of amphiphilic kanamycin with octyl groups.

appeared to slightly promote the growth of cells resulting in a higher number of viable cells in wells treated with the compound. Compound **K608** showed very mild cytotoxicity with ∼80% cell viability at all concentration. IC_{50} values could not be calculated because the compounds were not toxic at the concentrations used for this assay.

Regioselectivity Investigation Using Computational Chemistry. Because the observed regioselective of the alkylation is unprecedented and unexpected, we conducted computational calculation aiming to provide an explanation to two questions: (1) the regioselectivity for the alkylation of tetraazidokanamycin A; and (2) the lack of regioselectivity for the alkylation of pentaazidokanamycin B and pentaazidotobramycin. Because excess NaH was used in the alkylation, our initial model was to illustrate the major structures with ionic bonds between the alkoxyl groups of kanamycin and sodium ions. However, numerous attempts of varying the number of sodium ions in complexing with deprotonated azidokanamycin cannot yield any dominant structure. Therefore, our subsequent effort was directed to employ innate azidokanamycin with hydrogen bonds as the mimics of ionic bonds between the alkoxyl groups of kanamycin and sodium ions and utilized the wealth of known structural information in the public domain.

Using this strategy, the initial guess structure was obtained from the kanamycin structure cocrystallized with kanamycin nucleotidyl transferase (PDB ID: 1kny). All of the amino groups in kanamycin were replaced by azido groups. The resulting structure shows two dihedral angles labeled D1 (O_a−C_b−O_c−O_d) and D2 (O_e−C_f−O_g−C_h), which were used to examine the relative orientation of the sugars through a potential energy scan at B3LYP/6-31G* level (Figure 4). D1 and D2 were fixed at 10° intervals from 0° to 360°, while the

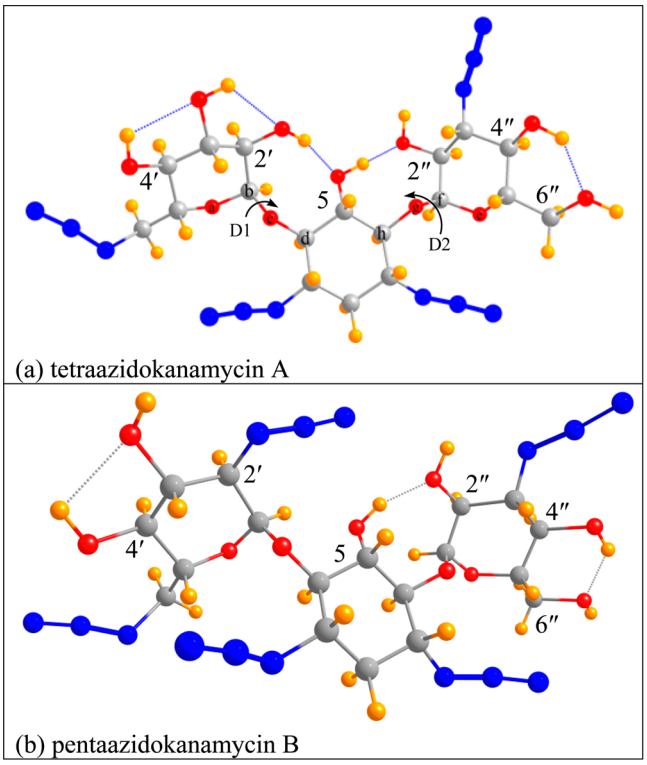

Figure 4. B3LYP/6-31g* optimized structures.

rest of the molecule was allowed to relax. Subsequently, frequency calculation of the global minimum structure was performed at the same level of theory to ensure this structure represents a true minimum. This level of theory has been extensively applied in the literature to study carbohydrates[30−35] using version D01 of Gaussian 09.[36] The most revealing structural feature on tetraazidokanamycin A is the two intramolecular hydrogen-bonding networks (or ionic bonding in the presence of sodium): the one involves 4′, 3′, 2′, 5, and 2″ hydroxyl groups, and the other involves 4″ and 6″ hydroxyl groups (Figure 4a). In contrast, the presence of 2′-azido group on pentaazidokanamycin B and pentaazidotobramycin disrupts the intramolecular hydrogen-bonding networks involving 4′, 3′, 2′, 5, and 2″ hydroxyl groups (Figure 4b).

The transition states were also computed at the same level of theory. The deprotonation free energies and enthalpies for the hydroxyl groups on tetraazidokanamycin A and pentaazidokanamycin B are shown in Table 6. The deprotonation free energy was obtained as the difference between the respective energies of the alkoxide and the alcohol as shown in eq 1. The entropy was also obtained in a similar manner. The positive free energies imply that the deprotonated forms are relatively less stable than the protonated forms. The 4′-OH and 3′-OH turn out to be more positive than other hydroxyl groups, including 4″-OH and 6″-OH. This could imply that the protons associated with *O*-3′ and *O*-4′ are less acidic and hence less prone to deprotonation than other hydroxyl groups. The energetics of 4″-OH and 6″-OH are quite similar; this is due to a rapid proton transfer from *O*-4″ to *O*-6″ leading to a bridge structure were the proton is located at 1.61 Å from *O*-4″ and 1.02 Å from *O*-6″ (Figure 5). From the computational

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Table 6. Thermodynamic Energetics (kcal/mol) of Deprotonation

	Kan A			Kan B		
	ΔG	ΔH	ΔS cal/(mol·K)	ΔG	ΔH	ΔS cal/(mol·K)
2′-OH	322.00	321.30	−2.36			
3′-OH	345.69	345.83	0.50	355.58	354.08	5.02
4′-OH	344.36	344.28	−0.28	365.78	365.29	1.64
2″-OH	322.00	321.3	−2.36	332.01	331.31	2.34
4″-OH	337.48	336.24	−2.07	337.48	336.24	−2.07
6″-OH	337.48	336.24	−2.08	337.48	336.24	−2.08

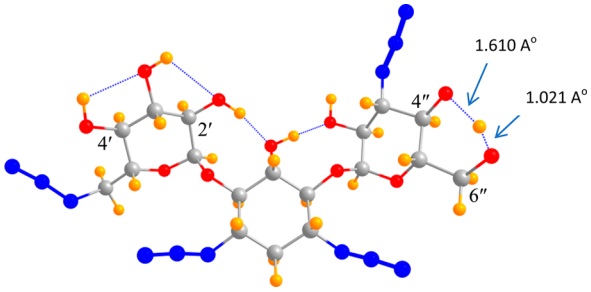

Figure 5. *O*-4″ and *O*-6″ bridge structure gas-phase structures.

calculation, it suggests that 2″-OH and 2′-OH are most prone to deprotonation. Nevertheless, we did not isolate any adducts with alkylation at *O*-2′ or *O*-2″ position from tetraazidokanamycin A. However, these calculations have revealed that 4″-OH and 6″-OH are more prone to deprotonation than 4′-OH and 3′-OH.

$$\mathrm{ROH} + \mathrm{H_2O} \rightarrow \mathrm{RO^-} + \mathrm{H_3O^+}$$

$$\Delta G = \sum (\Delta G_f(\mathrm{RO^-}) + \Delta G_f(\mathrm{H_3O^+})) - \sum (\Delta G_f(\mathrm{ROH}) + \Delta G_f(\mathrm{H_2O})) \quad (1)$$

In an alternative effort to account for the preference in alkylating *O*-4″ and/or *O*-6″, activation energies of alkylation using methyl bromide as the model compound were computed in the gaseous phase. Despite prolonged calculation, the activation energy for the methylation of several hydroxyl groups cannot be acquired. The obtained activation energies are shown in Table 7. In particular, the transition state structures of

Table 7. Activation Energies (kcal/mol) for Methylation

	activation free energy ΔG^* (kcal/mol)	
	Kan A	Kan B
2′-OH	NA[a]	NA
3′-OH	NA	4.29
4′-OH	10.01	3.79
2″-OH	22.06	NA
4″-OH	10.16	10.16
6″-OH	13.05	13.05

[a]No valid number can be acquired.

methylation are shown (Figure 6a and b). From the above results, we notice that *O*-4″ has a small advantage over *O*-6″. On the basis of the above results, the reactivity trend is in the order of *O*-4″ > *O*-6″ for both Kan A and Kan B. Although *O*-4′ on Kan A has activation energy similar to that of *O*-4″, we have not isolated any adduct with *O*-4′ alkylation. It is likely that the regioselectivity is governed by both the deprotonation energy

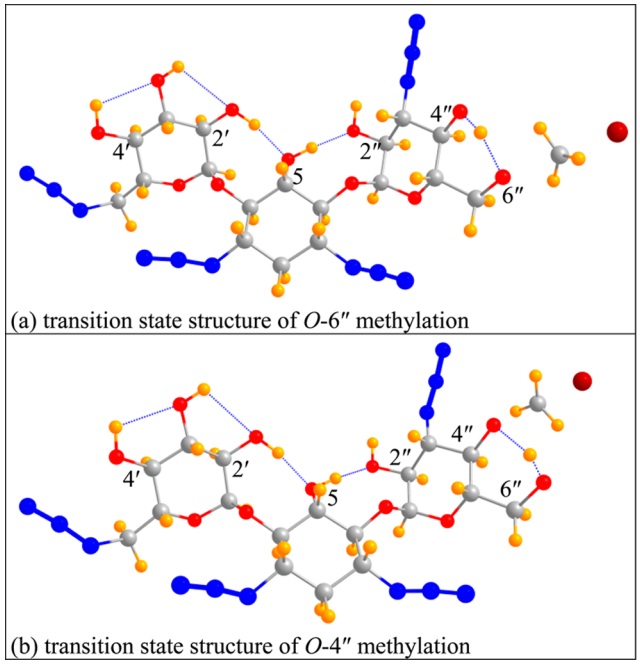

Figure 6. Methylation at *O*-4″ and *O*-6″ of tetraazidokanamycin A.

and the activation energy of alkylation. On Kan B, the activation energies for *O*-3′ and *O*-4′ are much lower than those of *O*-4″ and *O*-6″, which may account for the lack of regioselective alkylation using pentaazidokanamycin B. The difficulty in acquiring valid activation energy for hydroxyl groups, such as 2′-OH and 3′-OH for Kan A, and 2′-OH and 2″-OH for Kan B, may be attributed to the intramolecular hydrogen-bonding networks (or ionic bonding in the presence of sodium) involving 5-OH, which create steric hindrance preventing the calculation of methylation.

Overall, we believe the intramolecular hydrogen-bonding networks (or ionic bonding in the presence of sodium) play a key role in preventing the alkylation at these "interior" hydroxyl groups, 3′-OH (Kan A only), 2′-OH, 5-OH, and 2″-OH. Alkylation at 4″-OH and/or 6″-OH is favored by a combination of both deprotonation energy and the activation energy of alkylation. Finally, the presence of 2′-N_3 on Kan B disrupts the intramolecular hydrogen-bonding networks, and no preferred regioselective alkylation can be achieved.

Additional Synthesis of Kanamycin A Derivatives. We have further examined the feasibility of using the developed synthesis for the regioselective synthesis of mono- and/or di-*O*-alkylated kanamycin derivatives. Commercially available di-, tri-, and tetraethylene glycol's were ditosylated and used for alkylation directly. The results show that the same regioselectivity via an intramolecular dialkylation can be achieved leading to a convenient synthesis of crown ethers (Scheme 4).

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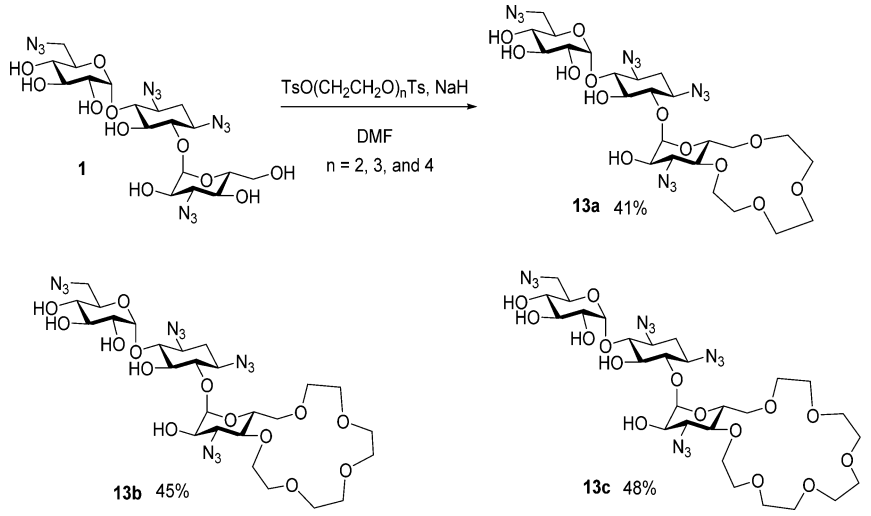
Scheme 4



Further examination of the biological activities and applications of these new derivatives are currently being carried out.

CONCLUSION

We have developed a concise and divergent synthesis of three classes of antifungal aminoglycosides. All three classes of *O*-4″ and/or *O*-6″ alkylated kanamycin A derivatives can be prepared in a divergent three-step process utilizing a novel regioselective Williamson alkylation. The resulting amphiphilic kanamycin derivatives contain alkyl groups linked to the kanamycin core through an ether linkage, which offers more stability and, potentially, can prolong the shelf life for these compounds. The lead antifungal kanamycin derivatives equipped with octyl group show prominent and specific activities against a significant plant pathogen, *F. graminearum*, without showing toxicity to mammalian cells. The lack of activity against *A. flavus* also exemplifies the challenge of developing effective agent against the pathogenic strains of *Aspergilla* sp. From our computational studies, we have revealed that the 4″-OH and 6″-OH are more prone to deprotonation and have a lower activation energy of alkylation, and thus are more reactive toward alkyl bromide.

Further investigation has shown that 4″-OH is more nucleophilic than 6″-OH, which explains the relative yields for all three classes of products. The intramolecular hydrogen-bonding networks (or ionic bonding in the presence of sodium) involving 4′, 3′, 2′, 5, and 2″ hydroxyl groups are likely the cause for the observed selectivity of alkylation at the 4″ and 6″ hydroxyl groups. Lack of selectivity in the cases of pentaazidokanamycin B and pentaazidotobramycin is likely due to the disruption of such an intramolecular hydrogen-bonding network.

In conclusion, we have provided a possible large-scale synthesis route for the synthesis of antifungal aminoglycosides. These new findings may expedite further research for amphiphilic kanamycins to fight against fungal pathogens that are resistant to commonly used azole-based antifungal agents. The synthetic methodology provides quick access for the synthesis of other classes of kanamycin derivatives with modifications at 4″-OH and/or 6″-OH positions for uses in areas such as antibacterial or treatment for genetic diseases.

EXPERIMENTAL PROCEDURES

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 75 or 100 Hz for ^{13}C Nuclei. $CDCl_3$, CD_3OD, and D_2O were used as solvents. Parts per million (ppm) was used to express the chemical shifts on δ scale. The peaks splitting pattern was 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). The synthesis of azidoaminoglycosides and the reduction of azido groups (Staudinger reaction) were conducted as reported in the literature.[16]

General Procedure for O-Alkylation of 1,3,6′,3″-Tetraazidokanamycin A. To a solution of azidokanamycin A (0.50 g, 0.85 mmol) and a catalytic amount of tetrabutylammonium iodide (TBAI) in 20 mL of anhydrous DMF was added NaH (20 equiv). The reaction mixture was stirred for 10 min before the corresponding alkyl bromide (1.5 equiv) was added. The reaction mixture was stirred at room temperature overnight. Completion of the reaction was confirmed by TLC (eluted with EtOAc), and the reaction was quenched by adding MeOH (5 mL). The mixture was concentrated and purified by a gradient column chromatography (eluted from EtOAc/hexane 30:70 to EtOAc/hexane 100:0) to obtain three products. The alkylated 1,3,6′,3″-tetraazidokanamycin A (both monoarmed and diarmed) are colorless or a slight yellowish sticky oil.

General Procedure for Acetylation. The *O*-alkylated tetraazidokanamycin (0.025 g) was dissolved into 15 mL of DCM in a 50 mL round-bottom flask. Eight equivalents of Ac_2O was added followed by addition of 12 equiv of Et_3N and a catalytic amount of DMAP. The reaction mixture was reflexed overnight. The reaction completion was confirm by TLC (EtOAc/hexanes 25:75). The solvent was removed using compressed air. The residue was dissolved in DCM and passed through a short silica gel column. The solvent was removed to afford the acetylated product.

General Procedure for Staudinger Reaction and Preparation of Final Compounds. The *O*-alkylated tetraazidokanamycin (0.2 g) was dissolved into 20 mL of THF in a 50 mL round-bottom flask. Several drops of water and 5 equiv of PMe_3 (1 M solution in toluene) were added to the solution. The reaction mixture was heated at 60 °C for 2 h. The reaction mixture was concentrated and then diluted with water. After been filtered through Celite, the crude product was loaded to a CG50 column (NH_4^+ form) and purified by a mixture of water and ammonium hydroxide (H_2O/NH_4OH 100:0 to 50:50) to afford the

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purified product. Several adducts from Staudinger reaction have poor solubility in water. These adducts were purified by column packed silica gel and eluted with a mixture of methanol and ammonium hydroxide (CH_3OH/NH_4OH 100:0 to 70:30) to afford the purified products. All of the purified products were concentrated, and 1 mL of 5% HOAc in water was added. The solvents were removed, and the salts were redissolved in water. The aqueous solution was loaded to a column packed with Dowex 1 × 8 resin (Cl^- form). The solution containing the desired products was collected and concentrated to afford the final product as chloride salts. All of the final compounds were obtained as amorphous white solid.

*4″,6″-Di-O-butyl-1,3,6′,3″-tetraazidokanamycin (**7b**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 4.11 Hz, 1H), 5.16 (d, J = 3.42 Hz, 1H), 4.1−4.2 (m, 1H), 4.0−4.1 (m, 1H), 3.3−3.7, (m, 18H), 3.18 (t, J = 9.7 Hz, 1H), 2.3−2.4 (m, 1H), 1.5−1.6 (m, 5H), 1.2−1.4 (m, 4H), 0.89 (m, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 83.8, 80.2, 76.6, 74.3, 73.5, 72.6, 72.2 (2C), 71.1 (2C), 70.8, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.3, 32.2, 31.6, 19.2, 19.1, 13.0 (2C). ESI/APCI calcd for $C_{26}H_{44}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 723.3145; measured m/z 723.3140. Yield: 131 mg (0.19 mmol, 22%).

*4″-O-Butyl-1,3,6′,3″-tetraazidokanamycin (**8b**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.42 Hz, 1H), 4.0−4.2 (m, 2H), 3.3−3.8, (m, 16H), 3.17 (t, J = 9.9 Hz, 1H), 2.3−2.4 (m, 1H), 2.0−2.1 (m, 1H), 1.5−1.6 (m, 3H), 1.2−1.4 (m, 2H), 0.89 (t, J = 7.23 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.5, 83.8, 80.3, 76.3, 74.4, 73.5, 72.6, 72.2 (2C), 71.3, 71.1, 70.5, 66.8, 61.0, 60.2, 59.6, 51.5, 32.3, 29.2, 19.1, 13.0. ESI/APCI calcd for $C_{22}H_{36}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 667.2519; measured m/z 667.2516. Yield: 120 mg (0.19 mmol, 22%).

*4″,6″-Di-O-hexyl-1,3,6′,3″-tetraazidokanamycin (**7c**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.78 Hz, 1H), 5.16 (d, J = 3.78 Hz, 1H), 4.1−4.2 (m, 1H), 4.0−4.1 (m, 1H), 3.3−3.7, (m, 18H), 3.19 (t, J = 9.6 Hz, 1H), 2.3−2.4 (m, 1H), 1.5−1.6 (m, 5H), 1.2−1.4 (m, 12H), 0.89 (t, J = 6.4 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 67.0, 61.1, 59.6, 51.5, 32.2, 31.6 (2C), 30.1, 29.5, 25.9 (2C), 22.5 (2C), 13.2 (2C). ESI/APCI calcd for $C_{30}H_{52}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 779.3771; measured m/z 779.3785. Yield: 180 mg (0.24 mmol, 28%).

*4″-O-Hexyl-1,3,6′,3″-tetraazidokanamycin (**8c**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.23 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.1−4.0 (m, 2H), 3.8−3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 6H), 0.89 (t, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.5, 83.8, 80.3, 76.3, 74.4, 73.5, 72.5, 72.2, 71.4, 71.1, 70.8, 66.9, 61.0, 59.6, 51.5, 32.2, 31.6, 30.1, 29.5, 29.2, 25.7, 22.4, 13.1. ESI/APCI calcd for $C_{24}H_{40}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 695.2832; measured m/z 695.2857. Yield: 120 mg (0.18 mmol, 21%).

*4″,6″-Di-O-heptyl-1,3,6′,3″-tetraazidokanamycin (**7d**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 18H), 3.18 (t, J = 10.0 Hz, 1H), 2.34 (ddd, J = 18.9, 9.4, 4.5 Hz, 1H),1.6−1.5 (m, 5H), 1.4−1.2 (m, 16H), 0.89 (t, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.5, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.7, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.1, 59.6, 51.5, 31.8 (2C), 30.2, 29.5 (2C), 29.1 (2C), 26.2, 26.0, 22.6 (2C), 13.2 (2C). ESI/APCI calcd for $C_{32}H_{56}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 807.4084; measured m/z 807.4101. Yield: 200 mg (0.26 mmol, 30%).

*4″-O-Heptyl-1,3,6′,3″-tetraazidokanamycin (**8d**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 16H), 3.18 (t, J = 9.6 Hz, 1H), 2.34 (ddd, J = 12.7, 9.4, 4.5 Hz, 1H)),1.6−1.5 (m, 3H), 1.4−1.2 (m, 8H), 0.86 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.5, 83.8, 80.3, 76.4, 74.5, 73.6, 72.7, 72.5, 72.3, 71.4, 71.1, 70.9, 66.9, 61.1, 59.7, 51.5, 32.2, 31.8, 30.1, 29.5, 29.1, 25.9, 22.4, 13.2. ESI/APCI calcd for $C_{25}H_{42}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 709.2988; measured m/z 709.3000. Yield: 210 mg (0.31 mmol, 36%).

*4″,6″-Di-O-octyl-1,3,6′,3″-tetraazidokanamycin (**7a**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.17 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 18H), 3.18 (t, J = 10.0 Hz, 1H), 2.34 (ddd, J = 18.9, 9.4, 4.5 Hz, 1H)),1.6−1.5 (m, 5H), 1.4−1.2 (m, 20H), 0.88 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.3, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.2, 31.8 (2C), 30.2, 29.5, 29.4 (2C), 29.3 (2C), 26.2, 26.1, 22.6 (2C), 13.3 (2C). ESI/APCI calcd for $C_{34}H_{60}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 835.4397; measured m/z 835.4397. Yield: 173 mg (0.21 mmol, 25%).

*6″-O-Octyl-1,3,6′,3″-tetraazidokanamycin (**9a**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.26 (d, J = 4.1 Hz, 1H), 5.19 (d, J = 3.4 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 17H), 2.34 (ddd, J = 16.8, 8.6, 4.1 Hz, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 10H), 0.86 (t, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 83.7, 80.3, 76.4, 74.5, 73.6, 72.6, 72.2, 71.6, 71.1, 70.9, 69.3, 68.8, 67.3, 60.9, 59.6, 51.5, 32.2, 31.8, 29.5, 29.4, 29.3, 26.0, 22.5, 13.3. ESI/APCI calcd for $C_{26}H_{44}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 723.3145; measured m/z 723.3165. Yield: 83 mg (0.12 mmol, 14%).

*4″-O-Octyl-1,3,6′,3″-tetraazidokanamycin (**8a**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.19 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.34 (ddd, J = 16.8, 8.6, 4.1 Hz, 1H)),1.6−1.5 (m, 3H), 1.4−1.2 (m, 10H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.5, 83.8, 80.4, 76.4, 74.5, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.8, 66.9, 61.0, 59.7, 51.5, 32.2, 31.8, 30.2, 29.5, 29.4, 29.2, 26.1, 22.5, 13.2. ESI/APCI calcd for $C_{26}H_{44}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 723.3145; measured m/z 723.3157. Yield: 95 mg (0.14 mmol, 16%).

*4″,6″-Di-O-nonyl-1,3,6′,3″-tetraazidokanamycin (**7e**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.25 (d, J = 3.8 Hz, 1H), 5.17 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 23H), 3.19 (t, J = 9.8 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 5H), 1.4−1.2 (m, 24H), 0.88 (t, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.3, 31.9 (2C), 30.2, 29.6 (2C), 29.5, 29.4 (2C), 29.3 (2C), 26.2, 26.1, 22.6 (2C), 13.3 (2C). ESI/APCI calcd for $C_{36}H_{64}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 863.4710; measured m/z 863.4710. Yield: 243 mg (0.29 mmol, 34%).

*6″-O-Nonyl-1,3,6′,3″-tetraazidokanamycin (**9e**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.26 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 17H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 12H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 83.7, 80.4, 74.5, 73.6, 72.7, 72.2, 71.6, 71.2, 71.1, 70.9, 69.3, 68.9, 67.3, 60.9, 59.6, 51.5, 32.2, 31.9, 29.5, 29.45, 29.2, 26.0, 25.4, 22.5, 13.2. ESI/APCI calcd for $C_{27}H_{46}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 737.3301; measured m/z 737.3316. Yield: 79 mg (0.11 mmol, 13%).

*4″-O-Nonyl-1,3,6′,3″-tetraazidokanamycin (**8e**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 4.1 Hz, 1H), 5.18 (d, J = 3.4 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 16H), 3.17 (t, J = 9.6 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 12H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.7, 80.4, 74.5, 73.6, 72.7, 72.2, 71.6, 71.2, 71.1, 70.9, 69.3, 68.9, 67.3, 60.9, 59.6, 51.6, 32.2, 31.9, 30.2, 29.5, 29.4, 29.2, 26.0, 22.4, 13.2. ESI/APCI calcd for $C_{27}H_{46}N_{12}O_{11}Na$ ([M + Na]$^+$) m/z 737.3301; measured m/z 737.3312. Yield: 152 mg (0.21 mmol, 25%).

*4″,6″-Di-O-decyl-1,3,6′,3″-tetraazidokanamycin (**7f**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 18H), 3.19 (t, J = 10.0 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 5H), 1.4−1.2 (m, 28H), 0.88 (t, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.3, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.2, 31.9 (2C), 30.1, 29.6 (5C), 29.4 (2C), 29.3 (2C), 26.2, 26.1, 22.6 (2C), 13.3 (2C). ESI/APCI calcd for $C_{38}H_{68}N_{12}O_{11}Na$ ([M + Na]$^+$) 891.5023; measured m/z 891.4995. Yield: 221 mg (0.26 mmol, 30%).

*6″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (**9f**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.26 (d, J = 3.8 Hz, 1H), 5.19 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 17H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 14H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 71.4, 71.1, 70.9, 70.5, 68.9, 67.0, 61.0, 59.6, 51.5, 32.2, 31.9, 29.6 (2C), 29.4, 29.3, 26.2, 26.1, 22.5, 13.3. ESI/APCI calcd for

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$C_{28}H_{48}N_{12}O_{11}Na$ ([M + Na]$^+$) 751.3458; measured m/z 751.3466. Yield: 49 mg (0.07 mmol, 8%).

4″-O-Decyl-1,3,6′,3″-tetraazidokanamycin (**8f**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 4.1 Hz, 1H), 5.18 (d, J = 3.5 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 18H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.5, 83.7, 80.3, 76.3, 74.4, 73.5, 72.6, 72.5, 72.2, 71.4, 71.1, 70.8, 66.9, 61.0, 60.3, 59.6, 51.5, 32.2, 31.8, 30.1, 29.5, 29.4, 29.2, 26.0, 25.4, 22.5, 13.2. ESI/APCI calcd for $C_{28}H_{48}N_{12}O_{11}Na$ ([M + Na]$^+$) 751.3458; measured m/z 751.3477. Yield: 149 mg (0.20 mmol, 24%).

4″,6″-Di-O-dodecyl-1,3,6′,3″-tetraazidokanamycin (**7g**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 18H), 3.19 (t, J = 10.0 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 5H), 1.4−1.2 (m, 36H), 0.88 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.2, 98.4, 83.8, 80.2, 76.7, 74.4, 73.6, 72.7, 72.6, 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 61.0, 59.6, 51.5, 32.2, 31.9 (2C), 30.1, 29.6 (9C), 29.4 (2C), 29.3 (2C), 26.2, 26.1, 22.6 (2C), 13.3 (2C). ESI/APCI calcd for $C_{42}H_{76}N_{12}O_{11}Na$ ([M + Na]$^+$) 947.5649; measured m/z 947.5681. Yield: 165 mg (0.18 mmol, 21%).

6″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (**9g**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.26 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 17H), 2.4−2.3 (m, 1H), 2.1−2.0 (m, 1H), 1.6−1.5 (m, 2H), 1.4−1.2 (m, 18H), 0.88 (t, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 83.7, 80.3, 74.5, 73.6, 72.7, 72.2, 71.6, 71.2, 71.1, 70.9, 69.3, 68.9, 67.3, 60.9, 59.6, 51.5, 32.2, 31.9, 29.6 (6C), 29.3, 26.0, 22.5, 13.2. ESI/APCI calcd for $C_{30}H_{52}N_{12}O_{11}Na$ ([M + Na]$^+$) 779.3771; measured m/z 779.3797. Yield: 83 mg (0.11 mmol, 13%).

4″-O-Dodecyl-1,3,6′,3″-tetraazidokanamycin (**8g**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 4.1 Hz, 1H), 5.18 (d, J = 3.5 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 16H), 3.17 (t, J = 9.8 Hz, 1H), 2.4−2.3 (m, 1H), 2.1−2.0 (m, 1H), 1.6−1.5 (m, 2H), 1.4−1.2 (m, 18H), 0.88 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.5, 83.8, 80.3, 76.4, 74.5, 73.6, 72.7, 72.5, 72.2, 71.4, 71.2, 70.9, 66.9, 61.0, 60.3, 59.7, 51.5, 32.2, 31.9, 30.2, 29.6 (4C), 29.4, 29.2, 26.0, 22.5, 13.2. ESI/APCI calcd for $C_{30}H_{52}N_{12}O_{11}Na$ ([M + Na]$^+$) 779.3771; measured m/z 779.3791. Yield: 180 mg (0.24 mmol, 28%).

4″,6″-Di-O-tetradecyl-1,3,6′,3″-tetraazidokanamycin (**7h**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.25 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.1−4.2 (m, 1H), 4.0−4.1 (m, 1H), 3.3−3.7 (m, 18H), 3.20 (t, J = 9.6 Hz, 1H), 2.3−2.4 (m, 1H), 1.5−1.6 (m, 5H), 1.2−1.4 (m, 44H), 0.89 (t, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 84.0, 80.2, 78.3, 76.7, 74.4, 73.6, 72.6, 72.2, 71.4, 71.1, 70.8, 70.5, 68.9, 67.0, 61.0, 59.6, 51.6, 32.3, 31.3 (2C), 30.2 (2C), 29.7 (10C), 29.6 (2C), 29.4 (2C), 29.4 (2C), 26.3, 26.1, 22.6 (2C), 13.7 (2C). ESI/APCI calcd for $C_{46}H_{84}N_{12}O_{11}Na$ ([M + Na]$^+$) 1003.6275; measured m/z 1003.6308. Yield: 175 mg (0.18 mmol, 21%).

4″-O-Tetradecyl-1,3,6′,3″-tetraazidokanamycin (**8h**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.25 (d, J = 3.78 Hz, 1H), 5.18 (d, J = 3.78 Hz, 1H), 4.0−4.1 (m, 2H), 3.3−3.8 (m, 16H), 3.18 (t, J = 9.9 Hz, 1H), 2.3−2.4 (m, 1H), 1.5−1.6 (m, 3H), 1.2−1.4 (m, 22H), 0.89 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.4, 83.8, 80.1, 76.6, 74.3, 73.5, 72.6, 72.2, 71.3, 71.1, 70.8, 70.4, 68.8, 66.9, 61.0, 59.6, 51.5, 31.8, 30.1, 29.6 (6C), 29.4, 29.2, 26.2, 26.0, 22.5, 13.7. ESI/APCI calcd for $C_{32}H_{56}N_{12}O_{11}Na$ ([M + Na]$^+$) 807.4084; measured m/z 807.4084. Yield: 107 mg (0.14 mmol, 16%).

4″,6″-Di-O-hexadecyl-1,3,6′,3″-tetraazidokanamycin (**7i**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.2−4.1 (m, 1H), 4.1−4.0 (m, 1H), 3.8−3.3 (m, 18H), 3.16 (t, J = 9.6 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 5H), 1.4−1.2 (m, 52H), 0.88 (t, J = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 101.1, 98.3, 83.8, 80.2, 76.7, 74.3, 73.6, 72.6 (2C), 72.2, 71.4, 71.1, 70.9, 70.5, 68.9, 66.9, 60.9, 59.6, 51.5, 32.3, 31.9 (2C), 30.2, 29.7 (15), 29.5 (2C), 29.5 (2C), 29.4 (2C), 26.3, 26.1, 22.6 (2C), 13.4 (2C). ESI/APCI calcd for $C_{50}H_{92}N_{12}O_{11}Na$ ([M + Na]$^+$) 1059.6901; measured m/z 1059.6921. Yield: 196 mg (0.20 mmol, 23%).

4″-O-Hexadecyl-1,3,6′,3″-tetraazidokanamycin (**8i**). ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.1−4.0 (m, 2H), 3.8−3.3 (m, 18H), 3.16 (t, J = 9.6 Hz, 1H), 2.4−2.3 (m, 1H), 1.6−1.5 (m, 3H), 1.4−1.2 (m, 26H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 102.5, 99.9, 85.1, 81.7, 77.7, 75.8, 74.9, 74.1, 74.0, 73.9, 73.6, 72.8, 72.5, 72.2, 68.3, 62.4, 61.6, 61.0, 52.9, 33.6, 33.2 (2C), 31.5, 30.9 (6C), 30.7 (2C), 30.6 (2C), 27.4 (2C), 23.9 (2C), 14.6 (2C). ESI/APCI calcd for $C_{34}H_{60}N_{12}O_{11}Na$ ([M + Na]$^+$) 835.4397; measured m/z 835.4416. Yield: 173 mg (0.21 mmol, 25%).

4″,6″-Di-O-butylkanamycin (**K4604**). ^{1}H NMR (300 MHz, D_2O) δ 5.47 (d, J = 3.78 Hz, 1H), 4.96 (d, J = 3.78 Hz, 1H), 3.3−4.1 (m, 19H), 3.0−3.1 (m, 1H), 2.3−2.4 (m, 1H), 1.7−1.8 (m, 1H), 1.4−1.5 (m, 5H), 1.1−1.3 (m, 4H), 0.7−0.8 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 101.6, 96.8, 84.4, 79.3, 74.0, 73.5, 73.4, 73.1, 72.3, 71.0 (2C), 71.0, 68.8, 68.4, 68.1, 54.5, 50.2, 48.2, 40.5, 31.5, 30.9, 28.8, 18.9, 18.7, 13.4 (2C). ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3701. Yield: 150 mg (0.20 mmol, 71%).

4″-O-Butylkanamycin (**K404**). ^{1}H NMR (300 MHz, D_2O) δ 5.44 (d, J = 3.78 Hz, 1H), 5.00 (d, J = 3.78 Hz, 1H), 3.2−4.0 (m, 17H), 3.0−3.1 (m, 1H), 2.4−2.5 (m, 1H), 1.7−1.8 (m, 1H), 1.4−1.5 (m, 3H), 1.1−1.3 (m, 2H), 0.7−0.8 (t, J = 7.56 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 101.6, 96.0, 84.1, 78.5, 73.5, 73.4, 72.5, 72.4, 72.3, 72.2, 71.9 (2C), 68.8, 68.3, 59.8, 54.0, 49.9, 40.4, 31.5, 27.7, 18.6, 13.3. ESI/APCI calcd for $C_{22}H_{45}N_4O_{11}$ ([M + H]$^+$) 541.3079; measured m/z 541.3082. Yield: 100 mg (0.15 mmol, 47%).

4″,6″-Di-O-hexylkanamycin (**K4606**). ^{1}H NMR (300 MHz, D_2O) δ 5.52 (d, J = 4.11 Hz, 1H), 5.16 (d, J = 3.45 Hz, 1H), 3.3−4.1 (m, 19H), 3.0−3.1 (m, 1H), 2.4−2.5 (m, 1H), 1.8−1.9 (m, 1H), 1.4−1.5 (m, 5H), 1.1−1.3 (m, 12H), 0.7−0.8 (m, 6H); ^{13}C NMR (100 MHz, D_2O) δ 100.8, 97.0, 83.8, 78.2, 73.7, 73.6, 73.2, 72.1, 71.6, 71.1, 71.0, 71.0, 68.8, 68.3, 68.2, 54.4, 50.0, 48.2, 40.5, 31.2 (2C), 29.3, 28.8, 27.8, 25.5, 25.1, 22.3 (2C), 13.7 (2C). ESI/APCI calcd for $C_{30}H_{61}N_4O_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4324. Yield: 101 mg (0.13 mmol, 48%).

4″-O-Hexylkanamycin (**K406**). ^{1}H NMR (300 MHz, D_2O) δ 5.42 (d, J = 3.78 Hz, 1H), 4.97 (d, J = 3.78 Hz, 1H), 2.9−3.8 (m, 17H), 3.0−3.1 (m, 1H), 2.3−2.4 (m, 1H), 1.7−1.8 (m, 1H), 1.4−1.5 (m, 3H), 1.1−1.3 (m, 6H), 0.7−0.8 (t, J = 6.73 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.4, 95.8, 83.8, 78.2, 73.6, 73.5, 72.3, 72.5, 72.1, 72.3, 70.7, 70.7, 68.6, 68.1, 59.7, 54.1, 49.7, 47.6, 40.2, 30.8, 29.1, 27.5, 24.7, 21.8, 13.3. ESI/APCI calcd for $C_{24}H_{49}N_4O_{11}$ ([M + H]$^+$) 569.3392; measured m/z 569.3391. Yield: 141 mg (0.20 mmol, 67%).

4″,6″-Di-O-heptylkanamycin (**K4607**). ^{1}H NMR (300 MHz, D_2O) δ 5.51 (d, J = 3.4 Hz, 1H), 4.98 (d, J = 3.4 Hz, 1H), 4.1−3.3 (m, 18H), 3.1−3.0 (m, 1H), 2.5−2.4 (m, 1H), 1.9−1.8 (m, 1H), 1.6−1.5 (m, 4H), 1.3−1.1 (m, 16H), 0.75 (t, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, D_2O) δ 100.8, 96.7, 83.9, 78.4, 73.8, 73.0, 72.3, 71.8, 71.2, 71.0, 70.9, 68.9, 68.4, 68.3, 68.2, 54.5, 50.1, 48.1, 40.5, 31.4, 31.35, 29.3, 28.8, 28.6 (2C), 37.9, 25.7, 25.3, 22.2 (2C), 13.7 (2C). ESI/APCI calcd for $C_{32}H_{65}N_4O_{11}$ ([M + H]$^+$) 681.4644; measured m/z 681.4643. Yield: 187 mg (0.23 mmol, 89%).

4″-O-Heptylkanamycin (**K407**). ^{1}H NMR (300 MHz, D_2O) δ 5.52 (d, J = 4.1 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0−3.2 (m, 18H), 3.1−3.0 (m, 1H), 2.6−2.5 (m, 1H), 1.9−1.8 (m, 1H), 1.5−1.4 (m, 2H), 1.2−1.1 (m, 8H), 0.75(t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.6, 96.1, 84.1, 78.5, 73.9, 73.7, 72.5, 72.3, 72.25, 70.9 (2C), 68.9, 68.3, 59.9, 54.4, 49.9, 47.8, 40.5, 31.2, 29.4, 28.5, 27.8, 25.2, 22.1, 13.6. ESI/APCI calcd for $C_{25}H_{51}N_4O_{11}$ ([M + H]$^+$) 583.3549; measured m/z 583.3570. Yield: 97 mg (0.13 mmol, 46%).

4″,6″-Di-O-octylkanamycin (**K4608**). ^{1}H NMR (300 MHz, D_2O) δ 5.59 (d, J = 3.8 Hz, 1H), 5.10 (d, J = 3.8 Hz, 1H), 4.1−3.4 (m, 20H), 3.0−2.9 (m, 1H), 2.6−2.5 (m, 1H), 2.1−2.0 (m, 1H), 1.6−1.5 (m, 4H), 1.4−1.2 (m, 20H), 0.88 (t, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, D_2O) δ 100.9, 97.8, 83.8, 78.2, 73.8, 73.7, 72.0, 71.7 (2C), 71.2, 68.9, 68.4, 68.0, 54.5, 50.1, 48.5, 48.2, 43.9, 40.7, 31.9 (2C), 30.3, 29.5 (3C), 29.2, 26.5, 26.2, 25.8, 25.2, 22.8 (2C), 14.0 (2C). ESI/APCI calcd for $C_{34}H_{69}N_4O_{11}$ ([M + H]$^+$) 709.4957; measured m/z 709.4962. Yield: 96 mg (0.11 mmol, 46%).

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*6″-O-Octylkanamycin (**K608**).* ^{1}H NMR (300 MHz, D_2O) δ 5.53 (d, J = 3.8 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0−3.2 (m, 18H), 3.1−3.0 (m, 1H), 2.5−2.4 (m, 1H), 1.9−1.8 (m, 1H), 1.5−1.4 (m, 2H), 1.4−1.2 (m, 10H), 0.74 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.8, 96.4, 84.1, 77.9, 73.1, 72.3, 72.2, 72.0, 71.1, 68.9, 68.6, 68.4, 65.9, 55.3, 50.1, 48.2, 40.7, 31.4, 31.3, 28.8, 28.77, 28.7, 28.6, 25.4, 22.3, 13.8. ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3705. Yield: 192 mg (0.26 mmol, 91%).

*4″-O-Octylkanamycin (**K408**).* ^{1}H NMR (300 MHz, D_2O) δ 5.44 (d, J = 3.8 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0−3.2 (m, 18H), 3.1−3.0 (m, 1H), 2.5−2.4 (m, 1H), 1.9−1.8 (m, 1H), 1.5−1.4 (m, 2H), 1.2−1.1 (m, 10H), 0.74 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.6, 96.1, 84.1, 78.4, 73.8, 73.7, 72.5, 72.3, 72.2, 72.0, 70.9, 68.8, 68.3, 59.9, 54.4, 49.9, 47.8, 40.5, 31.2, 29.3, 28.7, 28.5, 27.7, 25.2, 22.2, 13.6. ESI/APCI calcd for $C_{26}H_{53}N_4O_{11}$ ([M + H]$^+$) 597.3705; measured m/z 597.3697. Yield: 99 mg (0.13 mmol, 47%).

*4″,6″-Di-O-nonylkanamycin (**K4609**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.57 (d, J = 3.8 Hz, 1H), 5.09 (d, J = 3.4 Hz, 1H), 4.1−3.0 (m, 21H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.6−1.5 (m, 4H), 1.3−1.2 (m, 24H), 0.87 (t, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 100.8, 95.2, 84.3, 78.5, 74.0, 73.2, 72.9, 72.3, 71.9, 71.85, 71.8, 71.5, 69.1, 68.9, 68.7, 55.0, 50.4, 41.1, 31.9 (2C), 29.8, 29.6 (4C), 29.5 (2C), 29.3 (2C), 27.7, 26.2, 25.9, 22.5 (2C), 13.2 (2C). ESI/APCI calcd for $C_{36}H_{73}N_4O_{11}$ ([M + H]$^+$) 737.5270; measured m/z 737.5293. Yield: 176 mg (0.20 mmol, 84%).

*6″-O-Nonylkanamycin (**K609**).* ^{1}H NMR (300 MHz, D_2O) δ 5.52 (d, J = 3.4 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 1.9−1.7 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 12H), 0.75 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.7, 96.6, 84.3, 78.5, 73.1, 72.3, 72.2, 71.9, 71.2, 71.0, 68.9, 68.5, 68.3, 65.9, 55.2, 50.1, 48.2, 40.6, 31.4, 28.9, 28.8 (2C), 28.7, 25.3, 25.4, 22.3, 13.6. ESI/APCI calcd for $C_{27}H_{55}N_4O_{11}$ ([M + H]$^+$) 611.3862; measured m/z 611.3862. Yield: 82 mg (0.11 mmol, 39%).

*4″-O-Nonylkanamycin (**K409**).* ^{1}H NMR (300 MHz, D_2O) δ 5.43 (d, J = 3.8 Hz, 1H), 4.99 (d, J = 3.4 Hz, 1H), 4.0−3.0 (m, 23H), 2.3−2.2 (m, 1H), 1.7−1.6 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 12H), 0.75 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.5, 96.9, 84.9, 80.5, 74.3, 73.7, 73.2, 72.4, 72.1, 71.1, 71.0, 68.8, 68.7, 59.9, 54.5, 50.3, 48.2, 40.5, 31.3, 29.8, 29.4, 28.8, 28.7, 28.6, 25.3, 22.2, 13.6. ESI/APCI calcd for $C_{27}H_{55}N_4O_{11}$ ([M + H]$^+$) 611.3862; measured m/z 611.3868. Yield: 89 mg (0.12 mmol, 42%).

*4″,6″-Di-O-decylkanamycin (**K4610**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.57 (d, J = 3.8 Hz, 1H), 5.09 (d, J = 3.4 Hz, 1H), 4.1−3.0 (m, 21H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.6−1.5 (m, 4H), 1.3−1.2 (m, 28H), 0.87 (t, J = 6.9 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 100.8, 95.3, 84.5, 78.9, 74.1, 73.2, 72.9, 72.4, 71.9, 71.8 (3C), 71.5, 69.1, 68.9, 68.7, 55.1, 50.4, 41.1, 31.9 (2C), 29.9, 29.6 (5C), 29.5 (2C), 29.3 (2C), 28.1, 26.2, 25.9, 22.5 (2C), 13.2 (2C). ESI/APCI calcd for $C_{38}H_{77}N_4O_{11}$ ([M + H]$^+$) 765.5583; measured m/z 765.5569. Yield: 123 mg (0.14 mmol, 59%).

*6″-O-Decylkanamycin (**K610**).* ^{1}H NMR (300 MHz, D_2O) δ 5.52 (d, J = 3.4 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 1.9−1.7 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 14H), 0.74 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.7, 96.6, 84.3, 78.5, 73.1, 72.3, 72.2, 71.9, 71.0 (2C), 68.9, 68.5, 68.3, 65.9, 55.2, 50.1, 48.2, 40.6, 31.4, 28.90, 28.87 (2C), 28.8, 28.7, 28.3, 25.4, 22.3, 13.7. ESI/APCI calcd for $C_{28}H_{57}N_4O_{11}$ ([M + H]$^+$) 625.4018; measured m/z 625.4016. Yield: 139 mg (0.18 mmol, 66%).

*4″-O-Decylkanamycin (**K410**).* ^{1}H NMR (300 MHz, D_2O) δ 5.45 (d, J = 4.1 Hz, 1H), 5.00 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 1.9−1.8 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 14H), 0.75 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.6, 96.4, 84.3, 74.0, 73.7, 72.7, 72.4, 72.2, 71.1, 70.9, 68.9, 68.5, 68.4, 68.3, 55.2, 54.5, 50.1, 47.9, 40.5, 31.4, 29.4, 28.9, 28.7 (2C), 28.3, 25.3, 22.2, 13.6. ESI/APCI calcd for $C_{28}H_{57}N_4O_{11}$ ([M + H]$^+$) 625.4018; measured m/z 625.4036. Yield: 86 mg (0.11 mmol, 41%).

*4″,6″-Di-O-dodecylkanamycin (**K4612**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.64 (d, J = 3.8 Hz, 1H), 5.05 (d, J = 3.8 Hz, 1H), 4.0−3.2 (m, 20H), 3.2−3.1 (m, 1H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.6−1.5 (m, 4H), 1.3−1.2 (m, 36H), 0.88 (t, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 100.9, 98.0, 83.8, 77.0, 74.0, 73.9, 73.2, 72.9, 72.1, 71.7 (3C), 71.3, 69.1, 68.4, 63.6, 54.6, 50.1, 40.8, 32.2 (2C), 30.2 (8C), 29.8 (2C), 29.3, 29.1, 29.0, 26.4 (2C), 25.9, 25.3, 22.9 (2C), 14.1 (2C). ESI/APCI calcd for $C_{42}H_{85}N_4O_{11}$ ([M + H]$^+$) 821.6209; measured m/z 821.6208. Yield: 129 mg (0.13 mmol, 62%).

*6″-O-Dodecylkanamycin (**K612**).* ^{1}H NMR (300 MHz, D_2O) δ 5.51 (d, J = 4.1 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 1.9−1.8 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 18H), 0.74 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.8, 96.5, 84.1, 78.1, 72.9, 72.3, 72.1, 71.9 (2C), 70.9, 68.9, 68.4, 68.3, 65.7, 55.2, 49.9, 48.0, 40.6, 31.4, 28.9, 28.7, 27.8, 25.3, 22.2, 13.6. ESI/APCI calcd for $C_{30}H_{61}N_4O_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4342. Yield: 110 mg (0.14 mmol, 52%).

*4″-O-Dodecylkanamycin (**K412**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.53 (d, J = 3.8 Hz, 1H), 5.15 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 18H), 0.86 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 100.2, 94.1, 84.5, 78.7, 74.7, 73.4, 73.0, 71.9 (2C), 71.7, 71.3, 69.1, 68.9, 60.7, 54.8, 50.1, 47.2, 40.9, 31.9, 29.9, 29.5 (5C), 29.3, 25.8, 22.5, 13.2. ESI/APCI calcd for $C_{30}H_{61}N_4O_{11}$ ([M + H]$^+$) 653.4331; measured m/z 653.4337. Yield: 80 mg (0.10 mmol, 38%).

*4″,6″-Di-O-tetradecylkanamycin (**K4614**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.58 (d, J = 3.78 Hz, 1H), 5.10 (d, J = 3.78 Hz, 1H), 3.4−4.2 (m, 19H), 3.0−3.1 (m, 1H), 2.9−3.0, (m, 1H), 2.5−2.6 (m, 1H), 1.5−1.7 (m, 5H), 1.2−1.4 (m, 44H), 0.89 (t, J = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 100.8, 95.0, 84.2, 78.5, 73.9, 73.1, 72.9, 72.1, 71.9, 71.7 (2C), 71.4 (2C), 69.1, 68.9, 68.6, 55.0, 50.3, 41.0, 31.9, 29.8 (13C), 29.6 (2C), 29.5 (2C), 29.3 (2C), 27.6, 26.2, 25.9, 22.5 (2C), 13.1 (2C). ESI/APCI calcd for $C_{46}H_{93}N_4O_{11}$ ([M + H]$^+$) 877.6835; measured m/z 877.6834. Yield: 158 mg (0.16 mmol, 76%).

*4″-O-Tetradecylkanamycin (**K414**).* ^{1}H NMR (300 MHz, D_2O) δ 5.55 (d, J = 3.78 Hz, 1H), 5.00 (d, J = 3.42 Hz, 1H), 3.4−4.2 (m, 17H), 3.29 (t, J = 9.9 Hz, 1H), 2.9−3.0, (m, 1H), 2.4−2.5 (m, 1H), 2.4−2.5 (m, 1H), 1.5−1.7 (m, 3H), 1.2−1.4 (m, 22H), 0.89 (t, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 100.7, 95.6, 84.8, 79.6, 74.1, 73.1, 72.9, 72.5, 71.9, 71.7 (2C), 71.4, 69.0 (2C), 68.6, 55.0, 50.4, 41.0, 31.7, 29.8, 29.8 (6C), 29.4, 29.2, 26.2, 25.8, 22.5, 13.2. ESI/APCI calcd for $C_{32}H_{65}N_4O_{11}$ ([M + H]$^+$) 681.4644; measured m/z 681.4665. Yield: 126 mg (0.15 mmol, 60%).

*4″,6″-Di-O-hexadecylkanamycin (**K4616**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.56 (d, J = 3.8 Hz, 1H), 5.09 (d, J = 3.8 Hz, 1H), 4.0−3.0 (m, 21H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.6−1.5 (m, 4H), 1.3−1.2 (m, 52H), 0.88 (t, J = 6.8 Hz, 6H); ^{13}C NMR (101 MHz, CD_3OD) δ 100.8, 94.8, 84.3, 78.7, 73.9, 73.2, 72.9, 72.1, 71.9, 71.8 (2C), 71.48, 71.44, 71.3, 69.1, 68.9, 68.7, 55.0, 50.4, 31.8 (2C), 29.8, 29.6 (18C), 29.5 (2C), 29.3 (2C), 26.3, 25.9, 22.5 (2C), 13.2 (2C). ESI/APCI calcd for $C_{50}H_{101}N_4O_{11}$ ([M + H]$^+$) 933.7461; measured m/z 933.7452. Yield: 135 mg (0.13 mmol, 65%).

*4″-O-Hexadecylkanamycin (**K416**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.50 (d, J = 3.4 Hz, 1H), 5.13 (d, J = 3.4 Hz, 1H), 4.0−3.0 (m, 19H), 2.5−2.4 (m, 1H), 2.0−1.9 (m, 1H), 1.5−1.4 (m, 2H), 1.3−1.1 (m, 26H), 0.86 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 100.2, 94.6, 85.2, 79.6, 74.8, 73.4, 73.0, 72.9 (2C), 71.9, 71.7 (2C), 69.0, 69.1, 60.5, 54.8, 50.3, 40.9, 31.8, 29.9, 29.6 (10C), 29.3, 25.8, 22.5, 13.2. ESI/APCI calcd for $C_{34}H_{69}N_4O_{11}$ ([M + H]$^+$) 709.4957; measured m/z 709.4958. Yield: 86 mg (0.10 mmol, 41%).

*4″,6″-O-(3,6-Dioxa-1,8-octyl)-1,3,6′,3″-tetraazidokanamycin (**13a**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.22 (d, J = 3.78 Hz, 1H), 5.15 (d, J = 3.78 Hz, 1H), 4.1−4.2 (m, 1H), 3.9- 4.1 (m, 3H), 3.8−3.9 (m, 1H), 3.3−3.7 (m, 24H), 2.3−2.4 (m, 1H), 1.53 (q, J = 12.3 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 100.2, 98.5, 84.0, 80.6, 76.9, 74.3, 73.5,72.6, 72.2, 71.7, 71.2, 70.8, 70.5, 70.4, 70.0, 69.5 (2C), 69.1, 68.7, 67.1, 60.7, 59.6, 51.5, 32.2. ESI/APCI calcd for $C_{24}H_{38}N_{12}O_{13}Na$ ([M + Na]$^+$) 725.2574; measured m/z 725.2571. Yield: 245 mg (0.34 mmol, 41%).

*4″,6″-O-(3,6,9-Trioxa-1,11-undecyl)-1,3,6′,3″-tetraazidokanamycin (**13b**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.25 (d, J = 3.8 Hz, 1H), 5.14 (d, J = 3.8 Hz, 1H), 4.1−4.2 (m, 1H), 3.9−4.0 (m, 1H), 3.2−4.0 (m, 30H), 3.1 (t, J = 9.9 Hz, 1H), 2.3−2.4 (m, 1H), 1.53 (ddd, J =

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12.3 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 100.9, 98.4, 83.3, 81.1, 77.3, 74.3, 73.4, 72.5, 72.0, 71.6, 71.0, 70.6, 70.5, 70.2, 70.1, 70.0 (3C), 69.9, 69.7, 69.0, 66.6, 60.6, 59.5, 51.3, 31.9. ESI/APCI calcd for $C_{26}H_{42}N_{12}O_{14}Na$ ($[M + Na]^+$) 769.2836; measured m/z 769.2829. Yield: 285 mg (0.38 mmol, 45%).

*4″,6″-O-(3,6,9,12-Tetraoxa-1,14-tetradecyl)-1,3,6′,3″-tetraazidokanamycin (**13c**).* ^{1}H NMR (300 MHz, CD_3OD) δ 5.24 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.8 Hz, 1H), 4.1−4.2 (m, 1H), 4.0−4.1 (m, 1H), 3.2−4.0 (m, 35H), 2.3−2.4 (m, 1H), 1.53 (ddd, J = 12.3 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 101.2, 98.5, 83.8, 80.3, 76.9, 74.4, 73.5, 72.6, 72.2, 71.7, 71.2, 70.8 (2C), 70.6, 70.3 (5C), 70.1, 69.4, 66.7, 61.0, 59.5, 51.5, 32.2. ESI/APCI calcd for $C_{28}H_{46}N_{12}O_{15}Na$ ($[M + Na]^+$) 813.3098; measured m/z 813.3099. Yield: 322 mg (0.41 mmol, 48%).

MIC and FICI Determinations. 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 0.08−0.1 at 600 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. FICI determinations with azoles in combination with **K408** and **K4608** were performed using previously described methods.[25] Data presented are based on two separate determinations.

Cytotoxicity Assay. *Cell Culture.* HeLa cells were grown in commercial DMEM 1X (Gibco) with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2. The cells were allowed to adhere for 24 h before drug treatment.

Cell Viability Assay. The cells were seeded in 96-well microtiter plates (10 000/200 μL). After 24 h of incubation in the media, cells were treated with various concentrations of **K608**, **K4608**, and **K408** (0.0, 1.0, 10.0, 100.0, 1000 μg/mL and Trition 1X) for 48 h. Twenty microliters of MTT stock solution (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Upon completion of incubation, the medium was carefully removed and washed twice with 100 mL of pbs buffer. Next, 200 μL of DMSO was added to each well, agitated on orbital shaker for 15 min, and the absorbance at 570 nm with 670 nm filter was determined with a microplate reader (Synergy H4). The results are expressed as viability as compared to that of control. The experiment was carried out in triplicate in three independent experiments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01189.

^{1}H, ^{13}C NMR, and ^{1}H−^{1}H COSY spectra of the synthesized compounds, and tables of atom coordinates (PDF)

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Notes

The authors declare no competing financial interest.

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