JOC The Journal of Organic Chemistry

Article

Design, Synthesis and Antibacterial Activities of Conformationally Constrained Kanamycin A Derivatives

Wenxuan Zhang, Ying Chen, Qingzhao Liang, Hui Li, Hongwei Jin, Liangren Zhang, Xiangbao Meng, and Zhongjun Li

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/jo302247x • Publication Date (Web): 10 Dec 2012

Just Accepted

The Journal of Organic Chemistry is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036

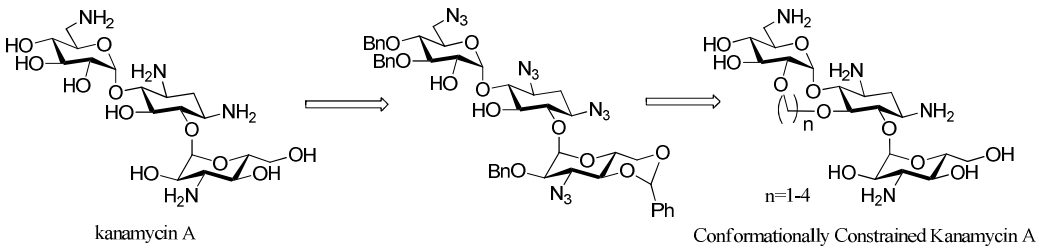
Design, Synthesis and Antibacterial Activities of Conformationally Constrained Kanamycin A Derivatives

Wenxuan Zhang, † Ying Chen, † Qingzhao Liang, † Hui Li, † Hongwei Jin,‡ Liangren Zhang, ‡ Xiangbao Meng,*,† and Zhongjun Li*,†

†The State Key Laboratory of Natural and Biomimetic drugs; Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

‡The State Key Laboratory of Natural and Biomimetic drugs; Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

*Corresponding author. Tel.: 86 10 82801714; e-mail: xbmeng@bjmu.edu.cn; zjli@bjmu.edu.cn



Abstract: A series of conformationally constrained kanamycin A derivatives with 2′-hydroxyl group in ring I and 5-hydroxyl group in ring II tethered by carbon chains were designed and synthesized. Pivotal 5, 2′-hydroxyl groups exposed kanamycin A intermediate was synthesized from 5, 2′, 4″, 6″-di-*O*-benzylidene-protected tetraazido-kanamycin A. Cyclic kanamycin A derivatives with intramolecular eight, nine, ten and eleven-membered ether were then prepared by cesium carbonate mediated Williamson ether synthesis or ring-closing metathesis reaction. The kanamycin A derivatives were assayed against both susceptible and resistant bacterial strains. Although no derivative showed better anti-bacterial activities than kanamycin A, the anti-bacterial activities of these cyclic kanamycin A derivatives indeed varied with the length of the bridge. Moreover, different variations of activities were observed between the susceptible and resistant bacterial strains.

More tightly constrained derivative **2** with one-carbon bridge showed better activity than the others against susceptible strains, but it was much less effective for resistant bacterial strains than derivative **3** with a two-carbon bridge and derivative **6** with an unsaturated four-carbon bridge.

Introduction

Aminoglycosides are frequently prescribed broad spectrum antibiotics, which have been used clinically for 70 years. However, the emergence of resistant bacteria is a threat for their use. One of the major causes of bacterial resistance is the enzymatic modification of the antibiotics.[1] The aminoglycoside-modifying enzymes can be broadly classified as *N*-acetyltransferases (AACs), *O*-adenyl transferases (ANTs), and *O*-phosphotransferases (APHs). Various strategies have been investigated to overcome the resistance.[2] Over the past decade many structural analogues of natural aminoglycosides were designed and synthesized, and some of the structures showed considerable antibacterial activities.[3]

Neamine (**1a**) is a conserved pseudo disaccharide that constitutes the core structures of many aminoglycosides such as neomycin B (**1b**), paromomycin (**1c**), kanamycin A (**1d**) and kanamycin B (**1e**) (Figure. 1). Among the natural aminoglycosides containing neamine motif, neomycin B and paromomycin have a 4, 5-disubstitution on 2-deoxystreptamine (2-DOS, ring II), while kanamycin has a 4, 6-disubstitution on 2-deoxystreptamine. Herein we report the design, synthesis and biological evaluation of conformationally constrained kanamycin A analogues through the linkage of 5- and 2′-hydroxyl groups.

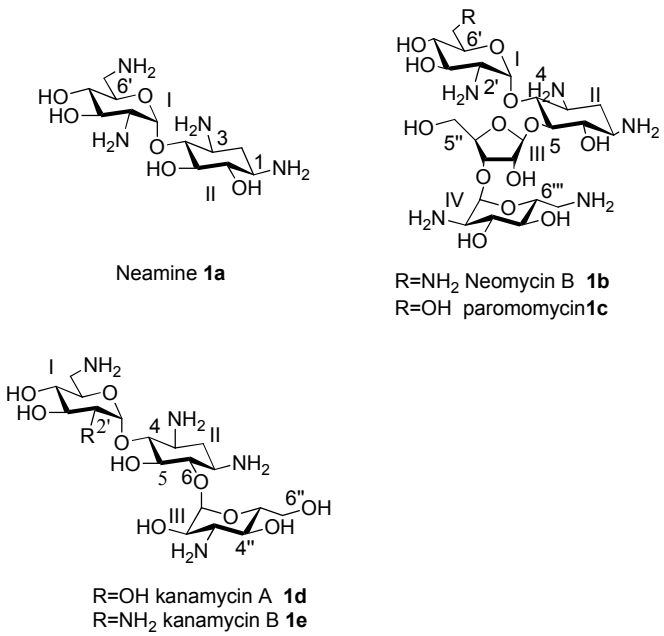


Figure 1. Structures of representative aminoglycosides. The neamine core is composed of ring I and ring II.

Results and Discussion

Design

The 3D structures of kanamycin A in complex with 16S RNA A-site and aminoglycoside-modifying enzymes show that the conformation of kanamycin A recognized by the 16S RNA A-site and the enzymes are different.[4] Indeed, these detailed structural information has already provided insights for the rational design of new aminoglycoside analogues.

Asensio and co-workers have developed a strategy to overcome bacterial resistance with conformationally locked neomycin B (**1b**).[5a] Neomycin-B derivative with a methylene bridge between the 2′-NH_2 of ring I and 5″-OH of ring III represents an improved activity against bacteria expressing *Staphylococcus aureus* ANT(4′). Hanessian's group also synthesized paromomycin (**1c**) analogues by tethering the 6-OH of ring II and the 6‴-NH_2 of ring IV with a five-carbon bridge.[5b] However, the synthesized compounds were inactive at the concentrations lower than 40 μg/mL. Mobashery and van Delft also reported the syntheses of structurally constrained Neamine (**1a**) derivatives, but no biological activity was reported.[5c,d]

The 3D structure of kanamycin A (**1d**) bound to the target ribosomal A-site RNA has been determined.[4l] In addition, several 3D structures of kanamycin A-enzyme complexes have been determined recently.[4] The 3D

structures of the antibiotic in complex with the enzymes significantly differ from that observed in kanamycin A /A-site RNA complex (Figure. 2). For instance, kanamycin A exists in higher energy conformation when bound with ANT(4′)[4a] and AAC(2′)[4g], but it adopts the minimum energy conformation in the RNA-bound state. Therefore, it is possible to design analogues that can retain the ribosomal A-site RNA binding property but resist enzyme modifications.

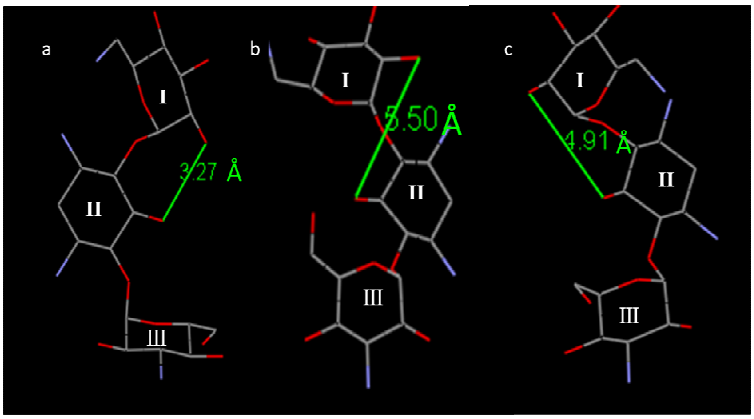


Figure 2. X-ray structures of kanamycin A bound to A-site (a), ANT4′ (b) or AAC2′ (c). Kanamycin A is shown as gray backbone. Atoms are colored by CPK. The 2′O-to-5O distances are indicated.

Previous investigations demonstrated that neamine is the optimal pharmacophore to manifest the antibiotic activity.[6] The 3D structures of RNA–aminoglycoside complexes revealed that aminoglycosides bind to RNA in a similar fashion. The neamine sugar rings of different aminoglycosides adopt the same orientation, bind to relatively the same binding site, and have almost the same conserved contacts .[4i, 4l, 4m] In contrast, the other sugar segments of aminoglycosides looks disorderly. These observations suggest that neamine moiety might be a better target for modifications to gain unique antibacterial properties than other segments. In fact, modifications of ring III of neomycin B or ring IV of paromomycin lead to significant decreases in affinity to 16S RNA in previous reports.[5a,b] We hope a direct restriction of the pseudo-disaccharide of the neamine moiety can reduce the potentially negative impact on the affinity to 16S RNA, while maintaining the conformational restriction. Moreover, electrostatic interaction is considered to be the main mode of aminoglycoside and RNA binding.[7] The ammonium positive ions play a major role in electrostatic interactions. Therefore, the

modifications of the hydroxyl groups may be more likely than the modifications of the amino groups to generate active analogues. The crystal structure of kanamycin A in complex with ribosomal A-site RNA shows that both the 5-hydroxyl group of ring II and the 2′-hydroxyl group of ring I have no critical interaction with the RNA and these two hydroxyl groups are proximate to each other (Figure 3). In addition, the distances between O-5 and O-2′ of kanamycin A are different in the complexes with A-site, ANT(4′) or AAC(2′) (Figure 2). Based on these analyses, kanamycin A derivatives with their structures constrained by linking the 5-hydroxyl group of ring II and the 2′-hydroxyl group of ring I with carbon bridges were designed (compounds **2-5**, Figure 4). Different lengths (1-4 carbons) of the bridges were used to restrict the conformation of the kanamycin A at different degrees. Moreover, a double bond was introduced to enhance the conformational restriction (compound **6**). Because hydrogen bonding interactions also play a key role in the binding of aminoglycosides with RNA, derivative **7** with protected hydroxyl groups was also prepared to evaluate the influence of the modifications of these two hydroxyl groups.

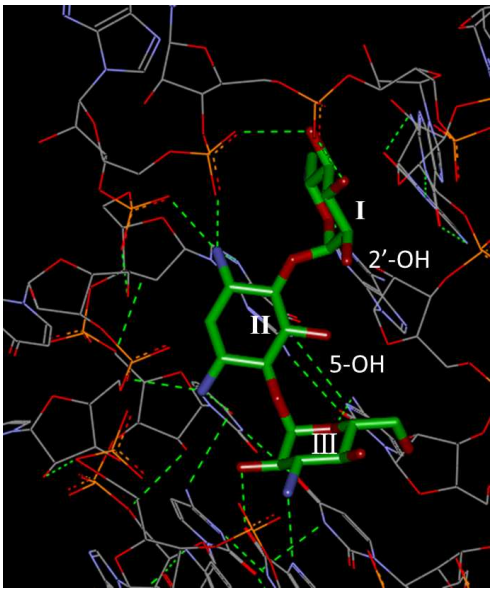


Figure 3. The crystal structure of kanamycin A–A site complex. Kanamycin A is shown as green backbone. The RNA is shown as line. Atoms are colored by CPK. Hydrogen bonds between kanamycin A and RNA are shown with green dash lines.

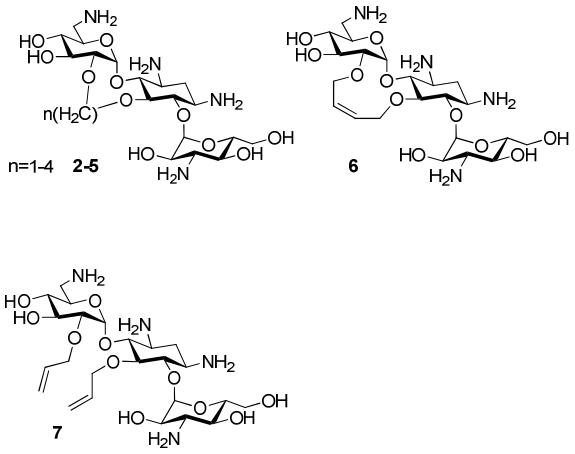


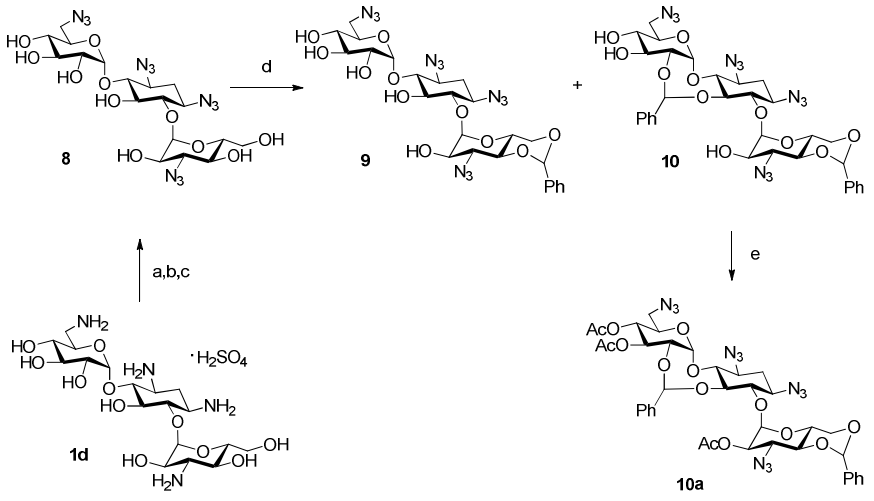
Figure 4. Structures of designed kanamycin A analogues (**2**-**7**)

Synthesis

We prefer to use azido groups as the precursors of amines on kanamycin since the azido derivatives have excellent solubility in organic solvents. Tetraazido-kanamycin A (**8)** was synthesized via a three-step procedure in 80% yield (Scheme 1). The commercially available kanamycin A (**1d**) was firstly converted into perazido species by the modified diazo transfer procedure.[8] In order to facilitate the purification, the reaction residues were treated with acetic anhydride in pyridine to give peracetylated product. After purification by column chromatography, the peracetylated product was then deacetylated with NaOMe–MeOH to provide compound **8**. Our synthesis required the selective protection of the hydroxyl groups of kanamycin A except the 5-hydroxyl and 2′-hydroxyl groups. We previously isolated a by-product 5, 2′, 4″, 6″-di-*O*-benzylidene-protected tetraazido-kanamycin A (**10**) during the synthesis of 4″, 6″-*O*-benzylidene protected tetraazido-kanamycin A (**9**). Its structure was determined by the ^{1}H-^{1}H COSY, HMBC, HMQC, and DEPT of its acetylated derivative **10a** (Scheme 1).[9] The unique 5, 2′-benzylidene group is an eight-membered structure, which should be relatively unstable and could be selectively hydrolyzed. Based on these results, a three-step protection strategy was designed. Compound **8** was firstly treated with 2.4 equiv of benzaldehyde dimethyl acetal and catalytic amount of *p*-toluenesulfonic acid under reduced pressure in DMF at 68ºC on a rotary evaporator for 12h to furnish the desired product **10** in 46% yield. Further increase of the amount of benzaldehyde dimethyl acetal and the

prolongation of reaction time did not improve the yield, but accompanied by the formation of byproducts. When acetonitrile was used as the solvent, compound **10** was afforded in 60% yield with the same proportion of the reactants as above reacted on a rotary evaporator at 40ºC for 5 min (Scheme 2). Compound **10** was then treated with NaH and benzyl bromide in DMF to afford the fully protected product **11**. We subsequently tested HCl (aq), acetic acid, or H_2SO_4 at different concentrations to selectively remove the 5, 2′-*O*-benzylidene group, but they all failed to hydrolyze the 5, 2′-*O*-benzylidene group selectively. It seems that this ring-cross benzylidene group was not as unstable as we previously assumed. Therefore, a two-step reaction was carried out. Complete hydrolysis of the two benzylidene groups with 0.004M HCl (aq) at 50ºC for 3 hours produced compound **12** in 93% yield, which was treated with 1.2 equiv of benzaldehyde dimethyl acetal in acetonitrile at room temperature for 3 hours to afford the 4″, 6″-*O*-benzylidened product **13** in 75% yield.

Scheme 1. Synthesis of compound 10 and 10a

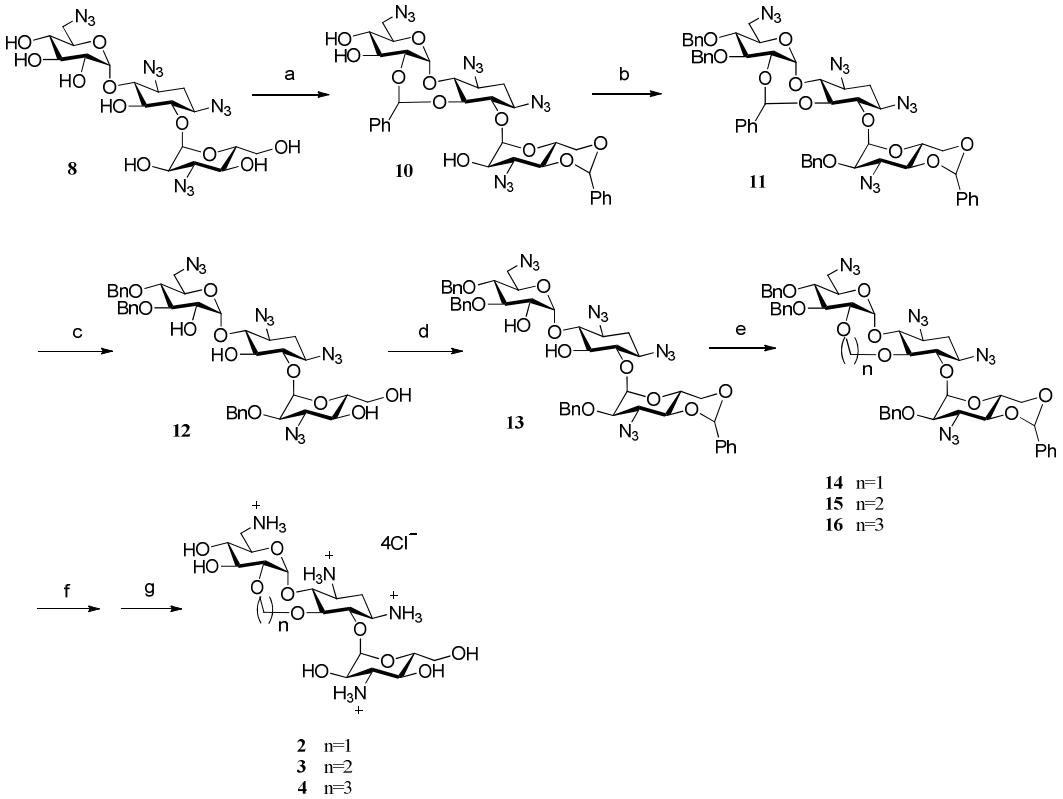


Conditions: (a) (i) Tf_2O, NaN_3, CH_3CN, 0°C ; (ii) $CuSO_4$, H_2O, CH_3CN, NEt_3, r.t; (b) Ac_2O, Pyridine, r.t, yield 82% for two steps; (c) MeOH, MeONa, r.t, 98%;(d) $PhCH(OCH_3)_2$, DMF, p-TsOH, 68°C, 20% for **9**, 46% for **10**; (e) Ac_2O, Pyridine, r.t, 98%.

The synthesis of compounds **2-5** involved the formation of eight, nine, ten and eleven-membered intramolecular ether rings similar to crown ethers. Generally speaking, the preparations of medium sized crown

ethers bearing eight to twelve ring atoms often face considerable difficulties.[10,11] However, a dramatic increase of the yields could be obtained with the use of cesium carbonate as base in the synthesis of crown compounds.[10a] Treatment of compound **13** with dibromomethane in the presence of 8 equiv Cs_2CO_3 in DMF at 90ºC for 24h afforded compound **14** with a methylene bridge in 67% yield (Scheme 2). However, when 1, 2-dibromoethane was used to synthesize compound **15** with Cs_2CO_3 as the base, no product was observed even at higher temperature or longer reaction time. Similarly, 1, 3-dibromo propane could not yield compound **16**. Stronger bases such as NaOH or NaH failed to promote these reactions. When compound **13** was treated with 1 equiv 1, 2-di-methyl sulfonyloxyethane and 8 equiv Cs_2CO_3 in DMF at 80ºC for 2 days, cyclic product **15** was prepared in 10% yield with the formation of 2′ or 5-*O*-sulfonate byproducts. When 3 equiv 1, 2-di-methylsulfonyloxyethane was used, the yield increased to 25% but it was still not satisfactory. However, further increase of the amount of 1, 2-di-methylsulfonyloxyethane did not improve the yield. 1, 2-di-*p*-toluene sulfonyloxyethane (3 equiv) was then used and the yield increased to 45%. As before, further increase of the amount of 1, 2-di-*p*-toluene sulfonyloxyethane, reaction temperature and reaction time did not improve the yield. Compared with previous reports of the similar reactions, the yield here would be satisfactory.[10-13] Similarly, compound **13** treated with 3 equiv 1, 3-di-*p*-toluene sulfonyloxy propane, 8 equiv Cs_2CO_3 in DMF at 80 ºC for 2 days afforded cyclic ether product **16** in 45% yield and 2′ or 5-*O*-allylated byproduct in 30% yield, while 1, 3-di-methyl sulfonyloxy propane under the same condition gave **16** only in 20% yield and 2′ or 5-*O*-allylated byproduct in 40% yield. We attempted to suppress this elimination reaction by using weaker base Na_2CO_3 or K_2CO_3, but no reaction was observed.

Scheme 2. Synthesis of the cyclic kanamycin A derivatives 2-4

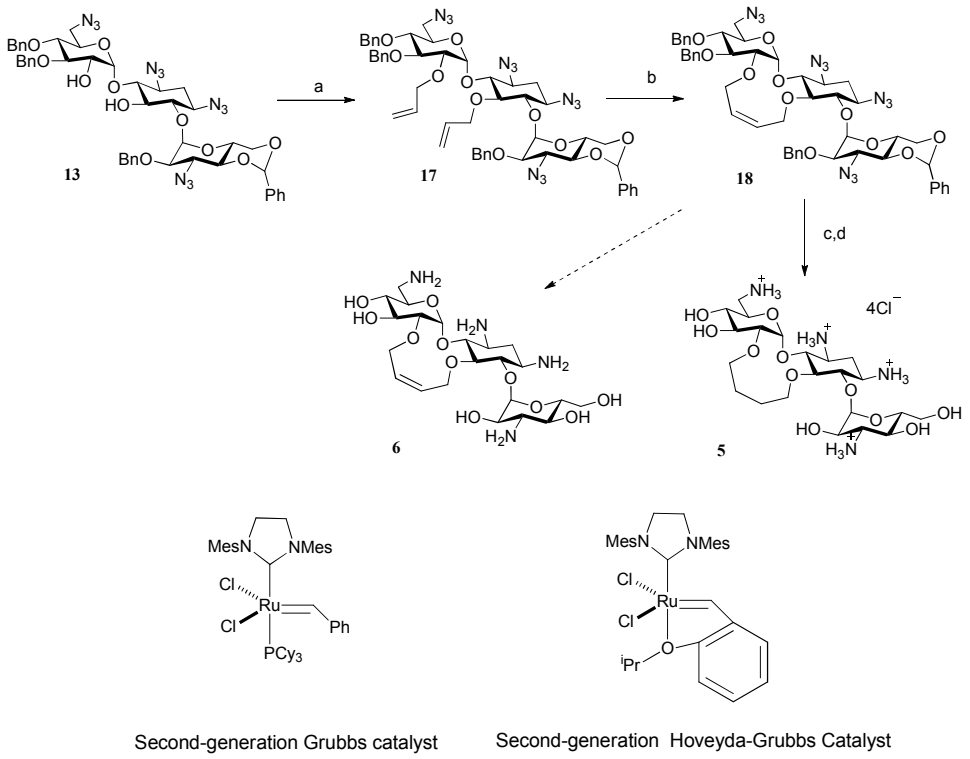


Conditions: (a) 2.4eq $PhCH(OCH_3)_2$, p-TsOH, CH_3CN, 40°C, 60%; (b) BnBr, DMF, NaH, r.t, 95%; (c) THF, MeOH, HCl(aq), 50°C, 93%; (d) 1.2eq $PhCH(OCH_3)_2$, CH_3CN, p-TsOH, r.t, 75%; (e) for **14**, Cs_2CO_3, DMF, CH_2Br_2, 90°C, 24h, 67%; for **15**, $TsOCH_2CH_2OTs$, Cs_2CO_3, DMF, 80°C, 2 days, 45% ; for **16**, $TsOCH_2CH_2CH_2OTs$, Cs_2CO_3, DMF, 80°C, 2 days, 45%; (f) H_2S, Py, Et_3N, H_2O, 6h; (g) HCl(aq), $Pd(OH)_2/C$, H_2, H_2O, 50-60% for two steps.

To construct the four-carbon bridge, ring-closing metathesis reaction was employed. Allylic etherification of 5-hydroxyl and 2′-hydroxyl groups with NaH and ally bromide gave compound **17** in 90% yield (Scheme 3). Phosphine-free second-generation Hoveyda–Grubbs catalyst was applied for ring closure metathesis reaction because of the incompatibility of azido groups with the phosphine ligand of the second-generation Grubbs' catalyst.[5d] Treatment of compound **17** with 0.1 equiv catalyst in toluene for 24h gave only *cis*-cyclic product **18** in 60% yield (J=10.8 Hz). Increase of the amount of catalyst to 0.15 equiv and 0.2 equiv or elevation of temperature did not improve the yield significantly.

The deprotections of **14-16**, **18** were achieved by a two-step process, because direct hydrogenation catalyzed by palladium carbon could not give a clean reaction.[8,9] At first, reduction of azido groups with hydrogen sulfide gas afforded the corresponding amines. After purification with column chromatography, debenzylation was then carried out by palladium carbon catalyzed hydrogenation under acidic conditions (pH=3-4) to give final products **2-5** as hydrogen chloride salt (Scheme 2 and 3). These final products were purified by HPLC for biological activity test. To synthesize compound **6** with an unsaturated four-carbon bridge, boron trichloride[14] was tried to deprotect benzyl groups of compound **18** selectively. However, it did not give a clean reaction. Birch reduction[15] was tried subsequently, but the intramolecular allyl ether bond was decomposed (scheme 3)[16].

Scheme 3. Synthesis of the cyclic kanamycin A derivative 5

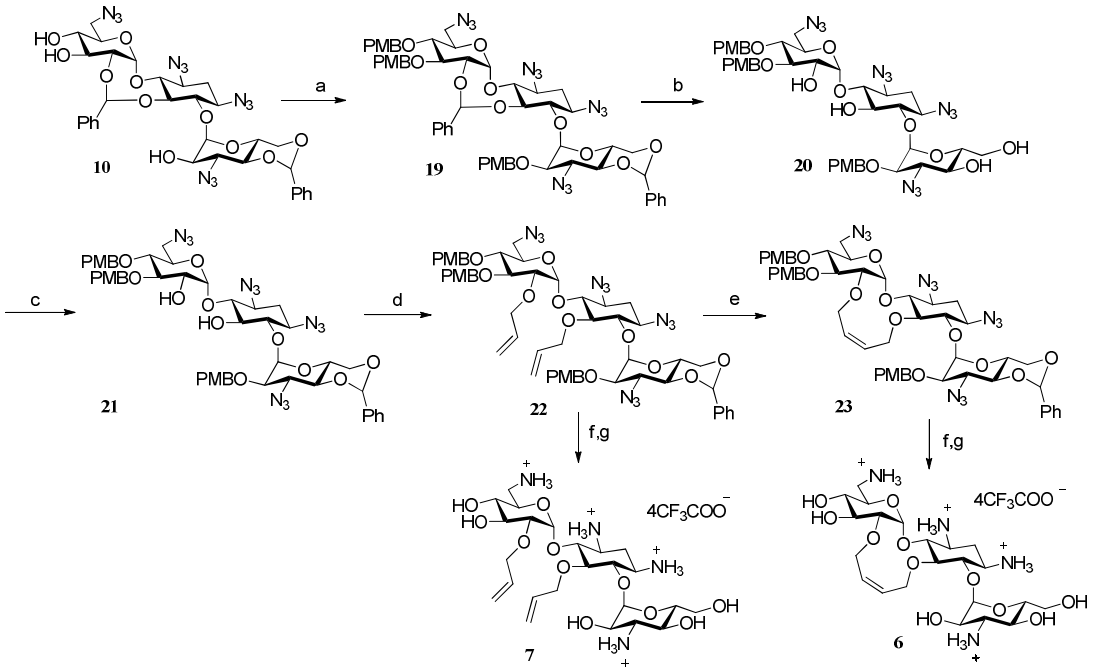


Conditions: (a) NaH, DMF, Allyl bromide, r.t, 3h, 90%; (b) Hoveyda catalyst, toluene, r.t., 24h, 60%; (c) H_2S, Py, Et_3N, H_2O; (d) HCl(aq), $Pd(OH)_2/C$, H_2, H_2O, two-step yields: 60%.

To synthesize compound **6**, different protecting strategy has to be used. The *p*-methoxy benzyl protecting group was used because it could be deprotected by trifluoroacetic acid without affecting the allyl ether.[17]

Treatment of 5, 2′, 4″, 6″-O-benzylidene tetraazido-kanamycin A **(10)** with *p*-methoxy benzyl chloride and NaH in DMF gave compound **19** in 93% yield, which was converted to **23** as shown in Scheme 4 as the synthesis of **18** from **11** in Scheme 3. After reduction of the azides to the corresponding amines with hydrogen sulfide gas, trifluoroacetic acid was then employed to remove PMB protective groups and benzylidene group to give compound **6** as the trifluoroacetate salt. Similarly, 5, 2′-diallyl-kanamycin A (**7**) was also prepared from compound **22.** These two final products were purified by HPLC for biological activity test.

Scheme 4. Synthesis of the cyclic kanamycin A derivative 6 and 5, 2′-diallyl-kanamycin A (7)



Conditions: (a) PMBCl, NaH, DMF, r.t., 6h, 93%; (b) MeOH, THF, HCl, 60°C, 90%; (c) $PhCH(OCH_3)_2$, CH_3CN, p-TsOH, r.t., 70%; (d) NaH, DMF, Allyl bromide, 90%; (e)Hoveyda catalyst, toluene, r.t., 24h, 50%; (f) H_2S, Et_3N, Py, H_2O, 6h; (g) TFA, H_2O, r.t., 2h.

Antibacterial activity

The synthesized aminoglycosides **2-7** were assayed against susceptible bacterial strains, using kanamycin A as the control. Aminoglycoside susceptible *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis (*ATCC 12228) and *Escherichia coli* (ATCC 25922) were used. The minimum inhibitory concentrations (MICs) are summarized in Table 1. All tested compounds showed decreased activity compared with kanamycin A. These results indicated that modifications of 5, 2′ -hydroxyl groups led to the decrease of

activities against susceptible bacterial strains, as previosusly reported.[5a,b,18] However, conformational restrictions lead to higher activity than simple alkylation of hydroxyl groups. In addition, anti-bacterial activities of these cyclic kanamycin A derivatives indeed varied with the length of the bridge. When the number of the carbon atoms in the bridges is less than or equal to three (derivatives **2-4**), the antibacterial activities of kanamycin A derivatives are getting weaker along with the extension of the carbon chain length. When the number of the carbon atoms in the bridges is four, derivative **6** with a double bond in the bridge showed better activity than derivative **5** with a saturated carbon chain, which showed similar activity as derivaive **7**. These results indicate the conformational recognition is important for A-site as a target.[19]

The activities of these synthesized aminoglycosides **2-7** against drug-resistant strains were assayed then. Clinically significant pathogens methicillin-resistant *S. aureus* (MRSA) (Gram positive), *Klebsiella pneumoniae* (Gram negative) and *Escherichia coli* (09-1) were used. Methicillin-resistant *S. aureus* (ATCC 33591) is the leading cause of bacterial infections, which express APH(3′), ANT(4′), and AAC(6′)/APH(2″) and render the bacteria resistant to various aminoglycosides.[20] *K. pneumoniae* (ATCC 700603) is resistant to several aminoglycosides (caused by ANT(2″).[21] *Escherichia coli* (09-1) is another clinical isolate obtained from patients in China in 2009.

The results are summarized in Table 1. As expected, the MIC of kanamycin A for the resistant Methicillin-resistant *S. aureus* (MRSA) is significantly increased compared with the susceptible *S. aureus* (ATCC 25923). Surprisingly, compound **2** showed 8-fold weaker activity against the methicillin-resistant *S. aureus* (MRSA) than the susceptible *S. aureus* (ATCC 25923). In contrast, the cyclic derivative **3** and **6** showed only 2-fold less activity against *S. aureus* (MRSA) than against the nonresistant bacteria. In fact, compound **3** and **6** showed better activity than compound **2** in all the tested resistant bacterial strains. However, these two compounds did not show better activity than kanamycin A. These results indicated that conformational restrictions do not enhance the antibacterial activity of kanamycin A against the resistant bacterial strains. In fact, similar result has been reported by Asensio and co-workers.[22]

Table 1 MIC of the synthesized aminoglycosides against susceptible bacterial strains and resistant bacterial strains [a]

	S. aureus[b]	*S. epidermidis*[c]	*E. coli*[d]	*S. aureus*[e]	*K. pneumoniaeb*[f]	*E. coli*[g]
1d	4	4	4	16	32	16
2	8	8	8	>64	>64	32
3	16	16	16	32	32	16
4	64	64	>64	>64	>64	>64
5	32	32	32	>64	32	>64
6	16	16	32	32	32	32
7	32	32	64	>64	>64	64

[a]Unit: μg/mL. [b]*Staphylococcus aureus* (ATCC 25923). [c] *Staphylococcus epidermidis* (ATCC 12228). [d]*Escherichia coli* (ATCC 25922). [e]*Staphylococcus aureus* (ATCC 33591). [f]*Klebsiella pneumoniae* (ATCC 700603). [g]*Escherichia coli* (09-1).

Conclusion

We have designed and synthesized five conformationally constrained kanamycin A derivatives with modifications at 5, 2′-hydroxyl groups of the neamine core. After 5, 2′-hydroxyl groups exposed kanamycin A derivative was synthesized as the pivotal intermediate, cyclic kanamycin A derivatives with intramolecular 8, 9, 10, 11-membered ether were prepared by cesium carbonate mediated Williamson ether synthesis or ring-closing metathesis reaction. The anti-bacterial activities of these cyclic kanamycin A derivatives indeed varied with the lengths of the bridges. However, they did not showed better anti-bacterial activities against tested resistant bacterial strains.

Experimental Section

1.Chemistry

^{1}H and ^{13}C NMR spectra were recorded on 300M or 400M instruments using TMS as the internal standard. High resolution mass spectra (HRMS) were obtained in positive ion electrospray ionization (ESI) mode using TOF (time-of-flight) analyzer. Optical rotations were measured at 25^{o}C using polarimeter. Column chromatography was performed on Silica Gel H60. Solvents were purified by standard procedures. The purification of the final products is carried out by HPLC (Refractive index detector).

1, 3, 6′, 3″-Tetraazido-kanamycin A (8)

To an ice-cooled suspension of NaN_3 (5.4 g, 83.08 mmol) in anhydrous CH_3CN (50 mL), trifluoromethanesulfonic anhydride (11.56 mL, 69.22 mmol) was added drop-wise over 30 min and the mixture was stirred for 2 h at 0 ºC. The mixture was filtered quickly at 0 ºC and the filtrate was added slowly into a mixture of kanamycin A (5.82 g, 10 mmol), $CuSO_4$ (0.1 g, 0.6 mmol), triethylamine (9.08 mL) and H_2O (25mL). The reaction was stirred for 24 h at room temperature. After completion of the reaction (monitored by TLC, acetone–EtOAc 1:1, v/v), the mixture was concentrated under reduced pressure to a green oil. To this green oil, 100 mL dried pyridine was added, followed by 20 mL acetic anhydride. The mixture was stirred for 4 h at room temperature and MeOH was added to quench the extra Ac_2O in ice bath. The solvent was removed at reduced pressure, and the remained mixture was precipitated by the addition of water to give a yellow solid. This crude product was purified with column chromatography (PE–EtOAc 5:1, v/v) to provide the product as colorless syrup (6.89 g, 8.2 mmol, 82%), Rf=0.43 (PE–EtOAc 3:1, v/v). To a solution of the above product (840 mg, 1 mmol) in 50 mL methanol, sodium methoxide (about 1 M solution in methanol) was added drop-wise to give a pH of 11. After completion of the reaction (in about 4 h, monitored by TLC, CH_2Cl_2–MeOH 3:1, v/v), the reaction was neutralized with cationic resin. Filtered the mixture and concentrated the filtrate under reduced pressure to give **8** (580 mg, 0.98 mmol, 98%) as orange oil: Rf=0. 26 ($CHCl_3$–MeOH 3:1, v/v). $[\alpha]_D^{25}$+80 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.32 (br s, 4H), 5.08 (d, 1H, J=3.6 Hz), 5.06 (d, 1H, J=3.6 Hz), 3.93 –3.82 (m, 2H), 3.69 (m, 1H), 3.56 – 3.06 (m, 14H), 2.28 – 2.24 (m, 1H), 1.59 –1.47 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 100.9, 97.7, 83.3, 79.2, 73.9, 72.7, 72.0, 72.0, 71.8, 70.5, 70.3, 67.5, 67.1, 60.3, 59.7, 58.9, 51.4, 31.9; HR ESI MS: calcd for $C_{18}H_{28}N_{12}NaO_{11}$ ($[M+Na]^+$) 611.1889, found 611.1893.

1, 3, 6′, 3″-Tetraazido-5, 2′, 4″, 6″-di-*O*-benzylidene-kanamycin A (10)

To a solution of compound **8** (5.88 g, 10 mmol) in dry CH_3CN (100 mL) was added *p*-toluenesulfonic acid monohydrate (50 mg), followed by benzaldehyde dimethyl acetal (3.6 mL, 24 mmol). The mixture was rotated under reduced pressure in CH_3CN at 40ºC on the rotary evaporator for 5min. After completion of the reaction (monitored by TLC, CH_2Cl_2–MeOH 12:1, v/v), the reaction was quenched by addition of Et_3N (5 mL) and evaporated to dryness. The resulting crude product was purified with column chromatography (CH_2Cl_2–MeOH 50:1, v/v) to provide colorless foam **10** (4.58 g, 6.0 mmol, 60%). Rf=0.56 (CH_2Cl_2–MeOH 12:1, v/v). $[\alpha]_D^{25}$+92.8 (c 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.26 (m,10H), 5.99 (s, 1H), 5.49 (d, 1H, J= 3.9 Hz), 5.34(s, 1H), 5.17 (d, 1H, J= 3.6 Hz), 4.00 (t, 1H, J=8.4 Hz), 3.87 – 3.23 (m, 15H), 2.90 (s, 1H), 2.29 (m, 1H),1.53 (m, 1H,); ^{13}C NMR (75 MHz, $CDCl_3$): δ 136.5, 134.6, 129.3, 129.1, 128.8, 128.1, 126.4, 126.0, 101.3, 101.0, 100.5, 99.0, 85.4, 81.1, 79.8,79.3, 73.1, 72.0, 71.9, 71.1, 69.2, 68.2, 63.9, 62.9, 59.6, 50.8, 31.5; HR ESI MS: calcd for $C_{32}H_{37}N_{12}O_{11}$ ($[M+H]^+$) 765.2705, found 765.2708.

1, 3, 6′, 3″-Tetraazido-5, 2′, 4″, 6″-di-*O*-benzylidene-3′, 4′, 2″-tri-*O*- benzyl- kanamycin A (11)

To a solution of **10** (764 mg, 1 mmol) in anhydrous DMF (30 mL) was added NaH (3 eq.) at 0 ºC. After stirring for 5 min, BnBr

(4.5 eq.) was added. Then the reaction mixture was warmed slowly to room temperature. After completion of the reaction (in about 6 h, monitored by TLC, PE–EtOAc 5:1, v/v), the reaction was quenched by addition of methanol. The reaction was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (PE–EtOAc 10:1, v/v) to give compound **11** as yellow foam in 95% yield. Rf=0.34 (PE–EtOAc 5:1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43–7.10 (m, 25H), 6.02 (s, 1H), 5.45 (d, 1H, J =4.2 Hz), 5.43 (d, 1H, J= 3.6 Hz), 5.25 (s, 1H), 5.00 (s, 1H), 5.00 (m, 1H), 4.83 (m, 4H), 4.50 (m, 1H), 4.10 (m, 4H), 3.87 (m, 4H), 3.66 – 3.57 (m, 5H), 3.46 – 3.22(m, 6H), 3.19 (m, 1H), 2.25 (m, 1H), 1.50 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.6, 137.9, 137.1, 136.5, 134.8, 129.2, 128.9, 128.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.6, 126.4, 126.2, 101.3, 100.8, 99.1, 96.9, 85.5, 81.8, 79.3, 79.2, 78.2, 76.6, 75.6, 75.1, 73.5, 73.1, 70.5, 68.4, 63.1, 61.2, 60.3, 57.7, 50.8, 32.0; HR ESI MS: calcd for $C_{53}H_{58}N_{13}O_{11}$ ($[M+NH_4]^+$)1052.4367, found 1052.4373.

1, 3, 6′, 3″-Tetraazido-3′, 4′, 2″-tri-*O*- benzyl-kanamycin A (12)

To a solution of **11** (1.25g, 1.21mmol) in THF–MeOH (2:1) 15mL, 60μL 1M HCl (aq) was added. The reaction mixture was kept at 50 ºC. After completion of the reaction (in about 3 h, monitored by TLC, CH_2Cl_2: MeOH =10:1, v/v), the reaction was neutralized with anion resin. Filtered the mixture and concentrated the filtrate under reduced pressure, followed by chromatography (CH_2Cl_2: MeOH =30:1) gave **12** (968mg, 1.13mmol, 93%) as colorless oil. $[\alpha]_D^{25}$+130 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.26 (m, 17H), 5.18-5.16(m, 2H), 5.05(d, J = 3.6 Hz, 1H), 4.92-4.79 (m, 4H), 4.67(d, J = 11.6 Hz, 1H), 4.61(d, J = 10.4 Hz, 1H), 4.17-4.95 (m, 3H), 3.84-3.46 (m, 8H), 3.39-3.21(m, 6H), 2.32-2.29(m, 1H), 155-1.46 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 137.7, 137.1, 128.6, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.7, 101.6, 96.9, 85.1, 82.1, 81.9, 78.1, 77.6, 77.2, 75.3, 75.2, 74.6, 72.9, 72.7, 71.5, 71.4, 69.4, 65.2, 62.2, 60.0, 51.1, 32.1. HR ESI MS: Calcd for $C_{39}H_{46}N_{12}NaO_{11}$ ($[M+Na]^+$) 881.3301, found 881.3289.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-kanamycin A (13)

To a solution of compound **12** (968mg, 1.13mmol) in dry CH_3CN (10 mL) was added *p*-toluenesulfonic acid monohydrate (10mg, 0.05mmol), followed by benzaldehyde dimethyl acetal (0.2ml, 1.35mmol, 1.2eq). The mixture was stirred at room temperature. After completion of the reaction (monitored by TLC, PE–EtOAc 2:1, v/v), the reaction was quenched by addition of Et_3N and evaporated to dryness. The resulting crude product was purified with column chromatography (PE–EtOAc 10:1, v/v) to provide colorless oil **13** (810mg, 0.86mmol, 75%). $[\alpha]_D^{25}$+80 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52-7.25 (m, 20H), 5.53 (s, 1H), 5.17 (d, J = 3.6 Hz, 1H), 5.11 (d, J =3.6 Hz, 1H), 4.94-4.77 (m, 5H), 4.65 (m, 1H), 4.25 (m, 2H), 4.04 (m, 2H), 3.82 (m, 1H), 3.65-3.23 (m, 12H), 2.19 (m, 1H), 1.63 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.1, 137.7, 137.1, 136.8, 129.0, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 126.0, 101.3, 97.6, 85.6, 81.8, 81.3, 79.7, 78.0, 77.4, 75.4, 75.1, 74.1, 73.3, 72.7, 71.4, 68.7, 63.0, 61.8,

60.0, 59.0, 51.1, 32.2. HR ESI MS: calcd for $C_{46}H_{50}N_{12}O_{11}Na$ ([M+Na]$^+$) 969.3614, found 969.3612.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-5, 2′-*O* -methylene-kanamycin A (14)

A mixture of **13** (190mg, 0.2 mmol), dibromomethane(0.3ml) and Cs_2CO_3 (0.5 g, 1.54mmol) in anhydrous DMF (20 mL) was stirred at 90 °C for 24h under argon. After cooling down to r.t., the mixture was filtered, and concentrated to dryness under reduced pressure. The residue was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3×10mL). The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified with column chromatography (PE–EtOAc 10:1, v/v) to provide colorless oil **14** (128mg, 0.13 mmol, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41-7.14 (m, 20H), 5.49 (d, J = 4.2 Hz, 1H), 5.43 (s, 1H), 5.34 (d, J = 3.9 Hz, 1H), 4.90-4.66 (m, 7H), 4.57-4.52 (m, 1H), 4.17-4.12 (m, 1H), 4.04-3.73 (m, 4H), 3.69-3.29 (m, 12H), 2.15 (m, 1H), 1.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 137.9, 136.9, 136.7, 129.1, 128.5, 128.4, 128.3, 128.1, 127.8, 127.7, 127.6, 127.1, 126.9, 126.0, 101.5, 99.2, 96.7, 94.6, 84.8,81.9, 80.4, 79.3, 78.6, 77.7, 77.2, 76.6, 75.6, 75.1, 73.2, 70.8, 68.6, 63.0, 61.6, 60.3, 57.4, 50.7, 32.0. HR ESI MS: calcd for $C_{47}H_{50}N_{12}O_{11}Na$ ([M+Na]$^+$) 981.3614, found 981.3614.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-5, 2′-*O* -1, 2 ethyl- kanamycin A (15)

A mixture of **13** (100mg, 0.11 mmol), 1, 2-di-*p*-toluene sulfonyloxyetane (122.1 mg, 3eq) and Cs_2CO_3 (275mg, 0.845mmol) in anhydrous DMF (20 mL) was stirred at 80 °C for 48h under argon. After cooling down to r.t., the mixture was filtered, and concentrated to dryness under reduced pressure. The residue was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3×10mL). The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified with column chromatography (PE–EtOAc 10:1, v/v) to provide colorless oil **15** (45mg, 0.047 mmol, 45%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45-7.25 (m, 21H), 5.59 (d, J = 3.2 Hz, 1H), 5.51(s, 1H), 5.37(d, J =4.0 Hz, 1H), 4.93(t, 2H), 4.82-4.76(m, 3H), 4.62-4.59(m, 1H), 4.14-4.02(m, 2H), 4.02-3.95(m, 2H), 3.86(t, 1H), 3.71-3.64(m, 2H), 3.56-3.31(m, 12H), 2.38-2.34(m, 1H), 1.60-1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 137.4, 137.0, 136.8, 129.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.5, 126.0, 101.6, 99.3, 96.0, 87.8, 83.0, 79.6, 77.9, 77.5, 77.3, 77.2, 75.3, 75.1, 73.2, 70.6, 68.8, 62.8, 61.5, 60.2, 58.6, 51.3, 32.2. HR ESI MS: Calcd for $C_{48}H_{52}N_{12}NaO_{11}$ ([M+Na]$^+$) 995.3771, found 995.3759.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-5, 2′-*O* -1, 3 propyl- kanamycin A (16)

A mixture of **13** (100mg, 0.11 mmol), 1, 3-di-*p*-toluene sulfonyloxy propane (126.7 mg, 3eq) and Cs_2CO_3 (275mg, 0.845mmol) in anhydrous DMF (20 mL) was stirred at 80 °C for 48h under argon. After cooling down to r.t., the mixture was filtered, and concentrated to dryness under reduced pressure. The residue was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3×10mL). The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified with column chromatography (PE–EtOAc 10:1, v/v) to provide colorless oil **15** (45mg, 0.047 mmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.26 (m, 24H), 5.52-5.50 (s, 2H), 5.20(d, J=3.3 Hz, 1H), 4.96-4.75 (m, 5H), 4.93(t, 2H), 4.62-4.59(m, 1H), 4.23-4.09(m, 4H),

4.00-3.83 (m, 4H), 3.73-3.38(m, 12H), 3.26-3.16(m, 2H), 2.49-2.44(m, 1H), 1.92-1.87(m, 1H), 1.74-1.62(m, 2H), 1.60-1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 136.9, 129.2, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.5, 126.1, 101.9, 99.6, 96.3, 85.4, 82.7, 80.7, 79.6, 77.9, 77.3, 77.2, 75.2, 75.1, 73.2, 70.6, 62.8, 61.8, 61.5, 60.6, 60.4, 58.4, 51.3, 31.8, 28.8. HR ESI MS: Calcd for $C_{49}H_{54}N_{12}NaO_{11}$ ($[M+Na]^+$) 1009.3927, found 1009.3931.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-5, 2′-di-*O* -allyl-kanamycin A (17)

To a solution of **13** (190mg, 0.2 mmol) in anhydrous DMF (10 mL) was added NaH (3 eq) at 0ºC. After stirring for 5 min, allyl bromide (2 eq) was added. Then reaction mixture was warmed slowly to room temperature. After completion of the reaction (monitored by TLC, PE–EtOAc 5:1, v/v), the reaction was quenched by addition of methanol. The mixture was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3×10mL). The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by column chromatography (PE–EtOAc 10:1, v/v) to afford **17** (185mg, 0.18mmol, 90%). $[\alpha]_D^{25}$+60 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48-7.24 (m, 20H), 5.96-5.81 (m, 2H), 5.60 (d, *J*=3.6Hz, 1H), 5.52 (d, *J*=3.6Hz, 1H), 5.47 (s, 1H), 5.34-5.08 (m, 2H), 4.93-4.74 (m, 6H), 4.63-4.50 (m, 2H), 3.70-3.33 (m, 11H), 2.41-2.36 (m, 1H), 1.67-1.55 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 138.0, 137.1, 137.0, 134.2, 133.4, 129.0, 128.5, 128.4, 128.2, 127.8, 127.7, 126.2, 117.8, 116.5, 101.6, 99.1, 96.0, 82.3, 81.8, 79.7, 79.1, 78.3, 77.9, 77.4, 76.6, 75.6, 74.9, 73.9, 73.1, 70.6, 68.7, 62.5, 61.5, 60.2, 59.0, 51.2, 32.1. HR ESI MS: Calcd for $C_{52}H_{62}N_{13}O_{11}$ ($[M+NH_4]^+$) 1044.4685, found 1044.4686.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-benzyl-5, 2′-*O*-(1, 4 butyl-2-ene)-kanamycin A (18)

Compound **17** (51mg, 0.05mmol) was co-evaporated three times with toluene and dissolved in toluene (10mL) again. Argon was bubbled through the solution for 10 min. To the reaction mixture was added Hoveyda–Grubbs catalyst (0.1eq). The mixture was stirred at room temperature. After the reaction was complete (approximately after 24 h, monitored by TLC), the mixture was treated with DMSO (50 equiv, relative to catalyst) overnight. The solution was concentrated in vacuo and purified by column chromatography (PE–EtOAc 8:1, v/v) to provide the RCM product **18** (29.9mg, 0.03mmol, 60%) as colorless oil. $[\alpha]_D^{25}$+84 (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.25 (m, 2H), 5.99-5.92 (m, 1H), 5.72-5.67(m, 1H), 5.58(d, *J* =4.4 Hz, 1H), 5.51(s, 1H), 5.49(d, *J* =3.6 Hz, 1H), 5.11(dd, J_1=9.6Hz, J_2=11.2Hz, 1H), 4.95-4.75(m, 5H), 4.65-4.60(m, 2H), 4.32-4.18(m, 4H), 4.13-4.08 (m, 1H), 3.98 (t, 1H), 3.87(t, 1H), 3.69-3.57(m, 2H), 3.51-3.36(m, 10H), 2.46-2.40(m, 1H), 1.64(dd, J_1=12.4Hz, J_2=24.8Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.0, 137.0, 136.9, 130.6, 130.0, 129.1, 128.6, 128.5, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 126.2, 101.8, 99.2, 96.5, 82.7, 82.3, 82.0, 78.1, 77.7, 77.3, 77.2, 76.0, 75.8, 75.1, 73.2, 70.5, 70.3, 68.7, 62.7, 61.8, 60.8, 60.4, 58.3, 51.2, 32.0. HR ESI MS: Calcd for $C_{50}H_{58}N_{13}O_{11}$ ($[M+NH_4]^+$) 1016.4413, found 1016.4373.

5, 2′-*O*-Methylene-kanamycin A (2)

Compound **14** (96mg, 0.1mmol) was dissolved in Et_3N–pyridine–H_2O (3:2:1), H_2S gas was bubbled into the solution for 1 h. The

mixture was stirred for another 5 h. The solvent was removed under reduced pressure, followed by column chromatography ($CHCl_3$: MeOH: NH_4OH =20: 1: 0.5) to provide benzylated kanamycin A as a colorless oil. The benzylated kanamycin A was added with catalytic amount of $Pd(OH)_2/C$ and 5 mL CH_3OH (pH=3-4). The reaction mixture was stirred at room temperature under hydrogen of 5 atm for 2 day. Then the mixture was filtered through Celite. The residue was washed with water, and the combined solutions were concentrated affording crude product as a HCl salt in 54% yield. The product was purified by HPLC (0.025M CF_3COOH in H_2O and CH_3OH (19:1)) to afford compound **2** as a salt of trifluoroacetic acid. ^{1}H NMR (300 MHz, D_2O) δ 5.56 (s, 1H), 4.95 (s, 1H), 4.84 (s, 2H), 3.85-2.93 (m, 16H), 2.38 (m, 1H), 1.83-1.53 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 101.9, 99.1, 95.5, 82.3, 80.5, 99.6, 78.8, 73.7, 72.5, 70.7, 68.9, 65.7, 60.2, 55.3, 50.5, 48.3, 40.9, 28.4. HR ESI MS: calcd for $C_{19}H_{37}N_4O_{11}$ ($[M+H]^+$) 497.2453, found 497.2440.

5, 2′-*O*-1, 2-Ethyl-kanamycin A (3)

Compound **3** was prepared in the similar way as **2.** Yield: 52% for two steps. ^{1}H NMR (400 MHz, D_2O) δ 5.50 (d, J = 3.6 Hz, 1H), 5.08(d, J =3.2 Hz, 1H), 4.31-4.29(m, 1H), 4.02-3.95(m, 2H), 3.99-3.89(m, 3H), 3.85-3.32(m, 17H), 3.25(t, J =10.0 Hz, 1H), 3.04(dd, J_1=8.4Hz, J_2=13.6Hz, 1H), 2.46-2.43(m, 1H), 1.88 (dd, J_1=12.4Hz, J_2=25.2Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 101.7, 98.5, 88.3, 83.5, 80.5, 80.0, 71.2, 70.5, 68.5, 68.4, 65.0, 59.4, 54.6, 49.7, 47.8, 27.9. HR ESI MS: Calcd for $C_{20}H_{39}N_4O_{11}$ ($[M+H]^+$) 511.2610, found 511.2610.

5, 2′-*O*-1, 3-Propyl-kanamycin A (4)

Compound **4** was prepared in the similar way as **2.** Yield: 59% for two steps. ^{1}H NMR (400 MHz, D_2O) δ 5.50 (d, J = 2.4 Hz, 1H), 5.14(d, J =2.4 Hz, 1H), 4.18-4.16(m, 1H),4.05-4.03(m, 1H), 3.96-3.29(m, 22H), 3.18-3.15(m, 1H), 2.58-2.55(m, 1H), 1.98-1.74 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 101.6, 99.8, 83.4, 83.0, 82.5, 77.2, 73.2, 71.1, 70.5, 70.2, 68.4, 68.3, 59.5, 54.6, 49.9, 48.2, 46.7, 39.8, 28.2, 27.8. HR ESI MS: Calcd for $C_{21}H_{41}N_4O_{11}$ ($[M+H]^+$) 525.2766, found 525.2760.

5, 2′-*O*-1, 4-Butyl-kanamycin A (5)

Compound **5** was prepared in the similar way as **2**. Yield: 55% for two steps. ^{1}H NMR (400 MHz, D_2O) δ 5.50 (d, J = 3.2 Hz, 1H), 5.07(d, J =2.0 Hz, 1H), 3.93-3.63(m, 16H), 3.55-3.33(m, 8H), 3.21-3.17(m, 1H), 2.47(d, J = 12.0 Hz, 1H), 1.92-1.75 (m, 4H), 1.56-1.44(m, 1H); ^{13}C NMR (100 MHz, D_2O) δ101.9, 101.2, 83.2, 82.8, 79.6, 78.2, 75.5, 73.8, 73.3, 70.9, 70.8, 68.6, 68.5, 64.8, 59.1, 54.6, 49.9, 48.2, 40.2, 28.4, 28.1, 24.1. HR ESI MS: Calcd for $C_{22}H_{43}N_4O_{11}$ ($[M+H]^+$) 539.2923, found 539.2922.

1, 3, 6′, 3″-Tetraazido-5, 2′, 4″, 6″-di-*O*-benzylidene-3′, 4′, 2″-tri-*O*-*p*-methoxy- benzyl- kanamycin A (19)

To a solution of **10** (2.5g, 3.27 mmol) in anhydrous DMF (30 mL) was added NaH (3 eq) at 0 ºC. After stirring for 5 min, *p*-methoxy-benzyl chloride (4.5 eq) was added. Then reaction mixture was warmed slowly to room temperature. After completion of the reaction (monitored by TLC, PE–EtOAc 3:1, v/v), the reaction was quenched by addition of methanol. The mixture was poured into H_2O (20 mL), and extracted with CH_2Cl_2 (3×10mL). The combined organic phases were dried (Na_2SO_4), and the solvent was

evaporated. The residue was purified by column chromatography (PE–EtOAc 10:1, v/v) to afford **19** (3.42g, 3.04 mmol, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.25 (m, 15H), 7.19-7.13(m, 4H), 6.93-6.86(m, 7H), 6.06 (s, 1H), 5.47 (d, J = 4.8 Hz, 1H), 5.45 (d, J = 3.2Hz, 1H), 5.27 (s, 1H), 4.94(d, J =10.8 Hz, 1H), 4.78-4.70 (m, 4H), 4.46(d, J =10.8 Hz, 1H), 4.18-4.11 (m, 2H), 3.91-3.78 (m, 14H), 3.74-3.60 (m, 6H), 3.51-3.46 (m, 1H), 3.41-3.31(m, 5H), 3.21(t, J =10.0 Hz, 1H), 2.32-2.29 (m, 1H), 1.61 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 159.3, 136.6, 135.5, 134.9, 130.9, 130.2, 130.0, 129.6, 129.5, 129.2, 129.1, 128.9, 128.7, 127.9, 126.5, 126.3, 113.9, 113.8, 113.7, 101.3, 100.8, 99.1, 96.9, 85.5, 81.8, 79.3, 79.2, 78.2, 77.8, 77.2, 76.5, 75.6, 75.3, 74.8, 73.2, 70.5, 68.4, 63.1, 61.2, 60.3, 57.7, 55.3(2), 50.8, 32.1. HR ESI MS: Calcd for $C_{56}H_{60}N_{12}NaO_{14}$ ($[M+Na]^+$) 1147.4244, found 1147.4227.

1, 3, 6′, 3″-Tetraazido- 4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*- *p*-methoxy-benzyl- kanamycin A (21)

To a solution of **19** (0.625g, 0.56mmol) in THF–MeOH (2:1) 7.5mL, 30μL 1M HCl (aq) was added. The reaction mixture was kept at 50 ºC. After completion of the reaction (in about 3 h, monitored by TLC, CH_2Cl_2: MeOH =10∶1, v/v), the reaction was neutralized with cationic resin. Filtered the mixture and concentrated the filtrate under reduced pressure, followed by chromatography (CH_2Cl_2: MeOH =30∶1) gave **20** (477mg, 90%) as colorless oil. To a solution of compound **20** (477mg, 0.5mmol) in dry CH_3CN (10 mL) was added p-toluenesulfonic acid monohydrate (10mg, 0.05mmol), followed by benzaldehyde dimethyl acetal (0.09ml, 0.6 mmol, 1.2eq). The mixture was stirred at room temperature. After completion of the reaction (monitored by TLC, PE–EtOAc 2:1, v/v), the reaction was quenched by addition of Et_3N and evaporated to dryness. The resulting crude product was purified with column chromatography (PE–EtOAc 10:1, v/v) to provide colorless oil **21** (362mg, 0.35mmol, 70%).Yield for two steps: 63%.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51-7.49 (m, 2H), 7.40-7.34(m, 5H), 7.29-7.22(m, 7H), 6.92-6.86(m, 6H), 5.53(s, 1H), 5.13 (m, 2H), 4.88-4.79(m, 2H), 4.76-4.70 (m, 2H), 4.66(d, J =1.6 Hz, 1H), 4.56(d, J =10.8 Hz, 1H), 4.26-4.23(m, 1H), 4.10-3.99(m, 2H), 3.81-3.79 (m, 7H), 3.77(s, 1H), 3.72-3.63 (m, 2H), 3.59-3.48 (m, 5H), 3.44-3.38(m, 3H), 3.33(t, J =10.4 Hz, 1H), 3.29-3.26(m, 2H), 2.94(d, J =4.4 Hz, 1H), 2.41-2.38 (m, 1H), 1.61 (t, J =5.6Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 159.5, 159.4, 136.7, 135.5, 134.9, 130.4, 130.0, 129.6, 129.5, 129.2, 129.1, 128.3, 126.0, 114.0, 113.9, 113.8, 101.4, 101.3, 97.6, 85.5, 81.8, 81.3, 79.7, 77.8, 77.6, 77.2, 75.1, 74.8, 74.2, 73.0, 72.8, 71.4, 68.8, 63.1, 61.8, 60.0, 59.0, 55.3, 51.1, 32.3. HR ESI MS: Calcd for $C_{49}H_{56}N_{12}NaO_{14}$ ($[M+Na]^+$) 1059.3931, found 1059.3930.

1, 3, 6′, 3″-Tetraazido-4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-*p*-methoxy-benzyl-5, 2′-di-*O*-allyl-kanamycin A (22)

Compound **22** was prepared in the similar way as **17**. Yield: 90%.^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.44 (m, 2H), 7.38-7.33(m, 5H), 7.26(t, J =4.8 Hz, 1H), 7.21(d, J =8.4 Hz, 1H), 6.91(d, J =8.4 Hz, 1H), 6.88-6.84(m, 4H), 5.93-5.85(m, 2H), 5.55(d, J =3.6Hz, 1H), 5.50(d, J =3.6Hz, 1H), 5.47(s, 1H), 5.32-5.22(m, 2H), 5.16(dd, J_1=1.2Hz, J_2=10.4Hz, 1H), 5.09(dd, J_1=1.2Hz, J_2=10.8Hz, 1H), 4.85-4.75(m, 4H), 4.69(d, J =11.2 Hz, 1H), 4.55-4.49 (m, 2H), 4.34-4.30(m, 2H), 4.26-4.05(m, 5H), 3.82-3.79(m, 9H), 3.66-3.31(m, 13H), 2.41-2.37(m, 1H), 1.66-1.58(m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 159.3, 159.2, 137.1, 134.4, 133.5, 130.7, 130.2,

129.9, 129.5, 129.5, 129.2, 129.0, 128.0, 126.2, 117.7, 116.5, 114.0, 113.8, 101.7, 97.2, 96.2, 82.3, 81.7, 79.8, 79.2, 78.0, 77.5, 77.2, 76.9, 76.6, 75.3, 74.6, 73.9, 72.8, 70.7, 68.8, 62.5, 61.5, 60.3, 59.1, 55.3, 51.3, 32.2. HR ESI MS: Calcd for $C_{55}H_{64}N_{12}NaO_{14}$ ($[M+Na]^+$) 1139.4557, found 1139.4561.

1, 3, 6′, 3″-Tetraazido-4″, 6″-*O*-benzylidene-3′, 4′, 2″-tri-*O*-*p*-methoxy-benzyl-5, 2′-*O*-(1, 4 butyl-2-ene)-kanamycin A (23)

Compound **23** was prepared in the similar way as **18**. Yield: 50%, only *cis*-product was detected. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47-7.44 (m, 4H), 7.36-7.34(m, 5H), 7.30-7.26(m, 8H), 7.22(d, J =8.8 Hz, 2H), 6.92-6.86(m, 6H), 5.98-5.93 (m, 1H), 5.72-5.68(m, 1H), 5.56(d, J =4.4 Hz, 1H), 5.51(s, 1H), 5.45(d, J =3.6 Hz, 1H), 5.11(t, J=10.2Hz, 1H), 4.87-4.76(m,4H), 4.69(d, J =11.2 Hz, 1H), 4.54(d, J =10.8 Hz, 1H), 4.33-4.08 (m, 5H), 3.96(t, J =9.6 Hz, 1H), 3.85-3.78(m, 10H), 3.69-3.33(m, 11H), 2.46-2.41(m, 1H), 1.64(dd, J_1=12.4 Hz, J_2=25.6Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.7, 159.4, 159.3, 137.0, 130.8, 130.6, 130.2, 130.1, 130.0, 129.7, 129.6, 129.5, 129.1, 128.9, 128.3, 126.2, 114.0, 113.9, 113.8, 101.8, 99.2, 96.6, 82.7, 82.3, 82.0, 80.1, 79.6, 77.8, 75.5, 74.7, 72.8, 70.5, 70.3, 70.2, 68.7, 62.7, 61.8, 60.6, 58.3, 55.3, 51.2, 32.0. HR ESI MS: Calcd for $C_{53}H_{60}N_{12}NaO_{14}$ ($[M+Na]^+$) 1111.4244, found 1111.4246.

5, 2′-*O*-(1, 4-Butyl-2-ene)-kanamycin A (6)

Compound **23** (100mg, 0.09mmol) was dissolved in NEt_3–pyridine–H_2O (3:2:1), H_2S gas was bubbled into the solution for 1 h. The mixture was stirred for another 5 h. The solvent was removed under reduced pressure, followed by column chromatography ($CHCl_3$: MeOH: NH_4OH =20: 1: 0.5) provided *p*-methoxy benzylated kanamycin A as a colorless oil. To the solution of *p*-methoxy benzylated kanamycin A in H_2O (1mL), 0.5 ml trifluoroacetice acid was added. The mixture was stirred for 2h. The solvent was removed under reduced pressure, followed by column chromatography (ODS, water) to give the crude product in 53% yield. The product was purified by HPLC (0.025M CF_3COOH in H_2O and CH_3OH 19:1) to afford compound **6** as a salt of trifluoroacetic acid. ^{1}H NMR (400 MHz, D_2O) δ 5.87-5.80(m, 2H), 5.64(d, J =4.0 Hz, 1H), 5.04(d, J =2.4 Hz, 1H), 4.47(d, J =15.2Hz, 1H), 4.30(dd, J_1=4.4Hz, J_2=9.2Hz, 1H), 4.02 (dd, J_1=5.2Hz, J_2=15.2Hz, 1H), 3.50-3.25(m, 7H), 2.93(dd, J_1=7.2Hz, J_2=14.4Hz, 1H), 2.41(dd, J_1=4.4Hz, J_2=8.0Hz, 1H), 1.91(dd, J_1=12.4Hz, J_2=25.2Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 131.6, 129.6, 101.7, 99.0, 83.5, 82.8, 79.6, 77.4, 73.2, 71.2, 71.1, 70.6, 68.8, 68.5, 65.0, 59.5, 54.6, 50.0, 48.1, 40.3, 28.2. HR ESI MS: Calcd for $C_{22}H_{41}N_4O_{11}$ ($[M+H]^+$) 537.2766, found 537.2766.

5, 2′-Di-*O*-allyl-kanamycin A (7)

Compound 7 was prepared in the similar way as **6**. Yield: 68%. ^{1}H NMR (400 MHz, D_2O) δ 5.94-5.79(m, 2H), 5.56 (d, J = 2.4 Hz, 1H), 5.28-5.14(m, 4H), 5.07(d, J =2.4 Hz, 1H), 4.30-4.20(m, 4H), 4.11(dd, J_1=6.4Hz, J_2=12.0Hz, 1H), 4.03(t, J=9.6Hz, 1H), 3.90(t, J=9.6Hz, 1H), 3.84-3.63(m, 9H), 3.49-3.26(m, 6H), 3.09(dd, J_1=8.0Hz, J_2=13.6Hz, 1H), 2.41-2.38(m, 1H), 1.93(dd, J_1=12.0Hz, J_2=25.2Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 133.5, 133.5, 119.4, 118.2, 101.1, 95.2, 81.5, 81.4, 77.9, 73.8, 73.6, 73.0, 71.4, 71.3,

71.2, 68.8, 68.5, 64.9, 59.2, 54.6, 49.9, 48.4, 40.4, 27.7. HR ESI MS: Calcd for $C_{24}H_{45}N_4O_{11}$ ($[M+H]^+$)565.3079, found 565.3084.

2. Antibacterial assay

Minimum inhibitory concentrations (MIC) in μg/mL were determined by the microdilution broth method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Inoculum was prepared by diluting actively growing broth cultures to a McFarland value of 0.5 (1–108 cfu/mL). Antibiotic dilutions were made by dispensing 0.1 mL into each of the 96 wells of a standard microtitre tray in 2-fold dilutions. The inoculum was added to give a final concentration of 0.03-64 μg/mL. The trays were covered and incubated at 35ºC for 16–20 h. The MIC was defined as the lowest concentration of drug inhibiting visible growth. The microdilution broth susceptibility testing was performed in triplicate.

Acknowledgements

This work was financially supported by the National Basic Research Program of China (Grant No. 2012CB822100) and the Ministry of Science and Technology of China (2012ZX09502001-001).

Supporting Information

Copies of ^{1}H and ^{13}C NMR spectra of compounds **2-7, 11-19** and **21-23**. This material is available free of charge via the Internet at http:// pubs.acs.org.

References

1. For reviews, see: (a) Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249. (b) Magnet, S.; Blanchard, J. S. *Chem. Rev.* **2005**, *105*, 477.

2. For examples, see: (a) Roestamadji, J.; Grapsas, I.; Mobashery, S. *J. Am.Chem. Soc.* **1995**, *117*, 80. (b) Haddad, J.; Vakulenko, S. B.; Mobashery, S. *J. Am. Chem. Soc.* **1999**, *121*, 11922. (c) Kim, C.; Haddad, J.; Vakulenko, S. B.; Meroueh, S. O.; Wu, Y.; Yan, H.; Mobashery, S.; *Biochemistry* **2004**, *43*, 2373. (d) Gao, F.; Yan, X.; Baettig, O. M.; Berghuis, A. M.; Auclair, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 6859. (e) Magalhaes, M. L. B.; Vetting, M. W.; Gao, F.; Freiburger, L.; Auclair, K.; Blanchard, J. S. *Biochemistry* **2008**, *47*, 579.

3. (a) Li, J.; Chang, C.-W. T. *Anti-Infective Agents in Medicinal Chemistry* **2006**, *5*, 255-271. (b) Hanessian, S.; Szychowski, J.; Adhikari, S. S.; Vasquez, G.; Kandasamy, P.; Swayze, E. E.; Migawa, M. T.; Ranken, R.; François, B.; Wirmer-Bartoschek, J.;

Kondo, J.; Westhof, E. *J. Med. Chem.* **2007**, *50*, 2352. (c) Zhang, J.-J.; Chiang, F.-I.; Wu, L.; Czyryca, P. G.; Li, D.; Chang, C.-W. T. *J. Med. Chem*. **2008**, *51*, 7563. (d) Bera, S.; Zhanel, G. G.; Schweizer, F. *J. Med. Chem.* **2008**, *51*, 6160. (e) Pokrovskaya, V.; Belakhov, V.; Hainrichson, M.; Yaron, S.; Baasov, T. *J. Med. Chem.* **2009**, *52*, 2243. (f) Bera, S.; Zhanel, G. G.; Schweizer, F. *J. Med. Chem.* **2010**, *53*, 3626. (g) Hanessian, S.; Pachamuthu, K.; Szychowski, J.; Giguere, A.; Swayze, E. E.; Migawa, M. T.; Francois, B.; Kondo, J.; Westhof, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7097. (h) Vacas, T.; Corzana, F.; Jimenez-Oses, G.; Gonzalez, C.; Gomez, A. M.; Bastida, A.; Revuelta, J.; Asensio, J. L. *J. Am. Chem. Soc.* **2010** ,*132*, 12074. (i) Yang, L.; Ye, X.-S. *Curr. Med. Chem.***2010**, *10*, 1898. (j)Yan, R.-B.; Yuan, M.; Wu, Y.-F.; You, X.-F.; Ye, X.-S. *Bioorg. Med. Chem.* **2011**, *19*, 30. (k) Shaul, P.; Green, K. D.; Rutenberg, R.; Kramer, M.; Berkov-Zrihen, Y.; Breiner-Goldstein, E.; Garneau- Tsodikova, S.; Fridman, M. *Org. Biomol. Chem.* **2011**, *11*, 4057. (l) Szychowski, J.; Kondo, J.; Zahr, O.; Auclair, K.; Westhof, E.; Hanessian, S.; Keillor, J. W. *chemmedchem.* **2011**, *6*, 1961. (m) Vong, K.; Auclair, K. *medchemcomm* **2012**, *3***,** 397.

4. (a) Pedersen, L. C.; Benning, M. M.; Holden, H. M. *Biochemistry* **1995,** *34*, 13305. (b) Fourmy, D.; Recht, M. I.; Blanchard, A. C.; Puglisi, J. D. *Science* **1996**, *274*, 1367. (c) Yoshizawa, S.; Fourmy, D.; Puglisi, J. D. *EMBO. J.* **1998**, *17*, 22, 6437. (d) Carter, A. P.; Clemons, W. M.; Brodersen, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, 407, 340. (e)Vicens, Q.; Westhof, E. *Structure* **2001**, *9*, 647. (f) Fong, D. H.; Berghuis, A. M. S. *EMBO. J.* **2002**, 21, 2323. (g) Vetting, M. W.; Hegde, S. S.; Javid-Majd, F.; Blanchard, J. S.; Roderick, S. L. *Nat. Struct. Biol*. **2002**, *9*, 653. (h) Vicens, Q.; Westhof, E. *Chem. Biol*. **2002**, *9*, 747. (i)Vicens, Q.; Westhof, E. *Biopolymers* **2003**, 70, 42. (j) Nurizzo, D.; Hewry, S. C.; Erlin, M. H.; Brown, S. A.; Holakia, J. N.; Fuchs, R. L.; Eva, T.; Baker, E. N.; Smith, C. A. *J Mol Biol.* **2003** , *21*, 491. (k) Lynch, S. R.; Gonzalez, R. L., Jr.; Puglisi, J. D. *Structure* **2003**, *11*, 43. (l) Francois, B.; Westhof. E. *Nucleic Acids Research* **2005**, *33*, 5677. (m) Han, Q.; Zhao, Q.; Fish, S.; Simonsen, K. B.; Vourloumis, D.; Froelich, J. M.; Wall, D.; Hermann, T. *Angew. Chem., Int. Ed.* **2005**, 44, 2694. (n) Maurice1, F.; Broutin, I.; Podglajen, I.; Benas, P.; Collatz, E.; Dardel, F. *Embo Rep.* **2008**, *9,* 344-349. (o) Vetting, M. W.; Park, C. H.; Hedge, S. S.; Jacoby, G. A.; Hooper, D. C.; Blanchard, J. S.; *Biochemistry* **2008**, *47*, 9825–9835. (p) Shi, K.; Houston, D. R.; Berghuis, A. M.; *Biochemistry* **2011**, *50*, 6237–6244. (q) Shakya, T.; Stogios, P. J.; Waglechner, N.; Evdokimova, E.; Ejim, L.; Blanchard, J. E.; McArthur, A. G.; Savchenko, A.; Wright, G. D. *Chem.Biol.* **2011**, *18*, 1591-1601.

5. (a) Bastida, A.; Hidalgo, A.; Chiara, J. L.; Torrado, M.; Corzana, F.; Pérez-Canadillas, J. M.; Groves, P.; Garcia-Junceda, E.; Gonzalez, C.; Jimenez-Barbero, J.; Asensio, J. L. *J. Am. Chem. Soc*. **2006**, *128*, 100. (b) Hanessian, S.; Szychowski, J.; Pineda, N. B. C.-R.; Furtos, A.; Keillor, J. W. *Bioorg. Med. Chem. Lett*. **2007**, *17*, 322. (c) Kling, D.; Hesek, D.; Shi, Q.-C.; Mobashery, S. *J. Org. Chem.* **2007**, *72*, 5450. (d) Busscher, G. F.; van den Broek, S. A. M. W.; Rutjes, F. P. J. T. van Delft, F. L. *Tetrahedron* **2007**, *63*, 3183.

6. (a) Greenberg, W. A.; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am.*

Chem. Soc. **1999**, *121*, 6527. (b) Vourloumis, D.; Takahashi, M.; Winters, G. C.; Simonsen, K. B.; Ayida, B. K.; Barluenga, S.; Qamar, S.; Shandrick, S.; Zhao, Q.; Hermann, T. *Bioorg. Med. Chem. Lett.* **2002**, 12, 3367. (c) Venot, A.; Swayze, E. E.; Griffey, R. H.; Boons, G.-J. *ChemBioChem* **2004**, *5,* 1228. (d) Pang, L.-J.; Wang, D.; Zhou, J.; Zhang, L.-H.; Ye, X.-S. *Org. Biomol. Chem.* **2009**, *7*, 4252.

7. Wang, H.; Tor, Y. *J. Am. Chem. Soc.* **1997**, *119*, 8734.

8. Yan, R.-B.; Yang, F.; Wu, Y.-F.; Zhang, L.-H.; Ye, X.-S. *Tetrahedron Lett.* **2005**, *46*, 8993.

9. Chen, Y.; Meng, X.-B.; Chen, G.-H.; Pan, P.; Li, Z.-J. *Journal of Chinese Pharmaceutical Sciences* **2008**, *17*, 138.

10. (a) A. Ostrowicki, E. Koepp, F. Vögtle, *Top. Curr. Chem.* **1992**, *161*, 39. (b)Parker. D. *Macrocycle Synthesis: a practical approach* Oxford University Press, **1996**.77-91.

11. (a) De Voss, J. J.; Sui, Z.-H. *Tetrahedron Lett.* **1994**, *35*(1), 49. (b) Ghosh, A. K.; Gemma, S.; Baldridge, A.; Wang,Y.-F.; Kovalevsky, A. Y.; Koh, Y.; Weber, I. T.; Mitsuya, H. *J. Med. Chem.* **2008,** *51,* 6021.

12. (a) Mohler, D. L; Shen, G. *Org. Biomol. Chem.* **2006**, *4*, 2082. (b) Tahtaoui, C.; Parrot, I.; Klotz, P.; Guillier, F.; Galzi, J. L.; Hibert, M.; Ilien, B. *J. Med. Chem*. **2004**, *47*, 4300.

13. (a) Bogaschenko, T.; Basok, S.; Kulygina, C.; Lyapunov, A.; Lukyanenko, N. *Synthesis* **2002**, *15*, 2266. (b) Mlinaric-Majerski, K.; Kragol, G. *Tetrahedron* **2001**, *57,* 449.

14. Whalen, L. J.; Halcomb, R. L. *Org. Lett.* **2004**, *6*, 3221.

15. Heo, J.-N.; Holson, E. B.; Roush, W. R. *Org. Lett*. **2003**, *5*, 1697.

16. Lindberg, J.; Stralfors, P.; Konradsson, P. *Tetrahedron* **2002**, *58*, 4245.

17. Amer, H.; Hofinger, A.; Puchberger, M.; Kosma, P. *Journal of Carbohydrate Chemistry* **2001**, *20*, 719.

18. Cowan, J. A.; Ohyama, T.; Wang, D. Q.; Natarajan, K. *Nucleic Acids Research* **2000**, *28*, 2935.

19. Blount, K. F.; Zhao, F.; Hermann, T.; Tor, Y. *J. Am. Chem. Soc.* **2005**, *127*, 9818. 20.

20. Ida, T.; Okamoto, R.; Shimauchi, C.; Okubo, T.; Kuga, A.; Inoue, M. *J. Clin.Microbiol.* **2001**, *39*, 3115.

21. Rasheed, J. K.; Anderson, G. J.; Yigit, H.; Queenan, A. M.; Doménech-Sánchez, A.; Swenson, J. M.; Biddle, J. W.; Ferraro, M. J.; Jacoby, G. A.; Tenover, F. C. *Antimicrob. Agents Chemother.* **2000**, *44*, 2382.

22. Revuelta, J.; Vacas, T.; Torrado, M.; Corzana, F.; Gonzalez, C.; Jiménez- Barbero, J.; Menendez, M.; Bastida, A.; Asensio, J. L. *J. Am. Chem. Soc.* **2008**, *130*, 5086