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Regioselective modification of amino groups in aminoglycosides based on cyclic carbamate formation

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ABSTRACT

Conditions for regioselective introduction of cyclic carbamate into per-*N*-Cbz neamine and per-*N*-Cbz kanamycin A have been found. The position and number of cyclic carbamate formed in these two aminoglycosides was controllable. On the base of selective cyclic carbamate formation, regioselective modification on *N*-1, *N*-6′ or both amino groups in neamine, and on *N*-6′, *N*-3″ or both amino groups in kanamycin A was achieved by ring-opening reaction with amines. A new neamine dimer linked at the *N*-1 was also synthesized with this method.

1. Introduction

Aminoglycosides are potent antibacterial agents against serious Gram-negative infections. This kind of antibiotics bind to the ribosomal decoding site and reduce the fidelity of protein synthesis, and eventually kill the bacteria.[1–4] However, relative toxicity and resistance development against aminoglycosides has significantly limited their clinical applications.[5–7] Many aminoglycosides that are effective towards the resistant strain are semi-synthetic products. Many of them were developed by selective modification on specific amino groups in naturally existing aminoglycosides, such as neomycin B **1**, neamine **2** or kanamycin A **3**. The most successful example is amikacin **4** that has an (*S*)-4-amino-2-hydroxybutyryl (AHB) group at the *N*-1 position. This compound holds a wider spectrum than its precursor kanamycin A[8] (Fig. 1). Another neamine derivative Gb-*R*-neamine **5** bearing a nucleobase with an arginine as a linker at *N*-6′ showed some inhibitory activity for HIV TAR–Tat[9] (Fig. 1).

In the search for more molecules that possess antibacterial and antiresistance activities, regioselective modification on the amino groups in aminoglycosides is an important strategy. Methods for protecting unhindered amino groups have been extensively studied, including metal-mediated chelation,[10–14] regioselective Staudinger reduction of azides,[15–17] enzymatic approaches[18] and cyclic carbamate formation.[19–24] The cyclic carbamate method in aminoglycosides was first used by Kumar and Umezawa in late 1970s. They introduced several cyclic carbamates into different aminoglycosides for the purpose of modifying specific hydroxyl groups.[23,24] But they did not find the regioselectivity of cyclic carbamate formation and made no attempt to modify the amino groups in these aminoglycosides.

In order to extend the existing amino group modification methods, we focused on the regioselective cyclic carbamate formation in certain aminoglycosides. It is known that cyclic carbamate could form in *N*-Cbz protected aminoglycosides with the aid of NaH.[23,24] By careful adjustment of ring forming conditions, we succeed in controlling the number and position of cyclic carbamate formed in per-*N*-Cbz neamine and per-*N*-Cbz kanamycin A. The mono and bicyclic carbamates formed this way could easily undergo ring-opening reaction under the attack of amines to form urea derivatives. It is a new method to achieve regioselective modification of certain amino groups in aminoglycosides.

2. Results and discussion

2.1. Selective introduction of cyclic carbamate in aminoglycosides

To achieve the regioselective modification of aminoglycosides, we started to investigate the regioselectivity of cyclic carbamate formation in per-*N*-Cbz neamine and per-*N*-Cbz kanamycin A. In neamine, there are three amino groups, *N*-1, *N*-2′ and *N*-6′, with adjacent hydroxyl group. Kumar and his co-workers had treated compound **6** with NaH to give a bicyclic carbamate neamine derivative **9**, and under their reaction condition, no monocyclic carbamate product had been found.[23,24] By carefully adjusting the

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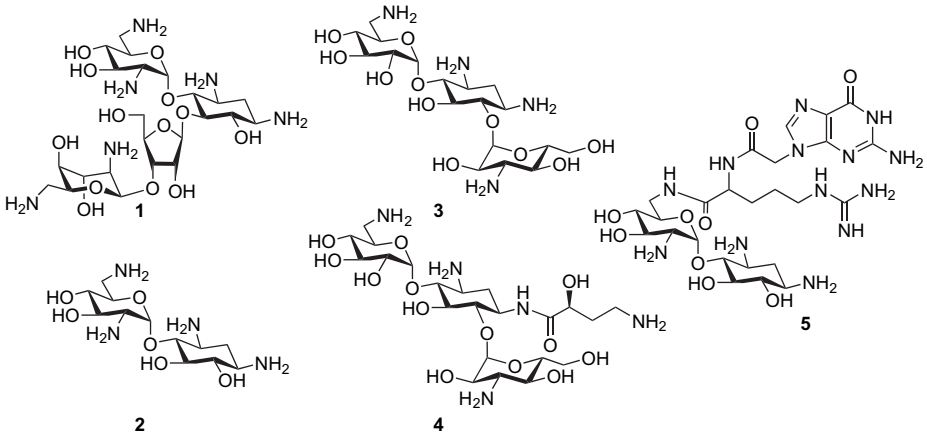


1. neomycin **2**. neamine **3**. kanamycin **4**. amikacin **5**. Gb-R-neamine

Figure 1. Structures of typical aminoglycosides.

combination of NaH amount, reaction temperature and reaction time, monocyclic carbamates **7**, **8** and bicyclic carbamate **9** were obtained, respectively, in considerable yields (Scheme 1). Due to the poor solubility of the resulting cyclic carbamate derivatives in organic solvents and subsequent difficulty of separation, the crude products were acetylated, subjected to column chromatography and then deacetylated to give the pure product. Detailed reaction condition and yields were listed in Table 1.

As indicated by the results, cyclic carbamate formation was not observed for *N*-2′, *O*-3 in neamine, which was consistent with the existing reports.[22–24] This could be the result of ring strain and steric hindrance.

In a similar way, cyclic carbamate could also form in per-*N*-Cbz kanamycin A **10**. Monocyclic carbamates **11** and **12** were obtained at low temperature in 32% and 38%, respectively. At room temperature, bicyclic carbamate **14** was obtained in 63% yield.[25] The monocyclic six-membered-ring carbamate **15** couldn't be synthesized in one step. It was acquired by opening the carbamate on ring I of compound **13** or **14** at a yield of 90% (Scheme 2).

Compounds **7**, **8**, **9**, **11**, **12**, **13** and **14** are the key intermediates for the further structure modification. Their structures were determined by the NMR analysis of corresponding acetyl derivatives. The positions of different protons and carbons were assigned by ^{1}H NMR, ^{13}C NMR and 2D spectrum, and the chemical shift of sugar CH or CH_2 proton adjacent to the acetyl group had a significant downfield shift. By comparing these shifts with their precursors, the position of the acetyl groups could be determined, and subsequently the position of cyclic carbamate could be confirmed.

We found that hydrogenolysis of the Cbz groups in compounds **11**, **12**, **13** and **14** could not be achieved. At the same time, hydrogenolysis of the Cbz groups in compound **15** was completed smoothly under the same condition to give the desired product **16** in 98% yield (Scheme 2). We assumed that the existence of the five-membered-ring carbamate brought about considerable ring strain in ring III of kanamycin A, and it made the glycosidic bond of ring III easy to cleave under the hydrogenolysis condition.

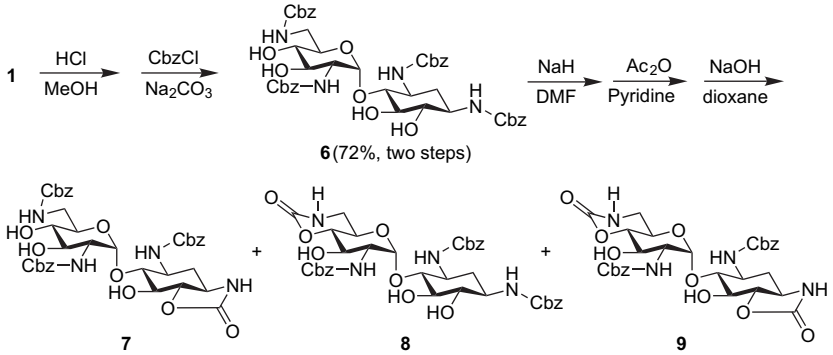
Table 1
The yields[a] of cyclic carbamate derivatives of neamine

Entry	Condition	Compound (%)		
		7	**8**	**9**
1[b]	DMF, 6 equiv NaH, −40 °C to −20 °C, 72 h	53	4	3
2	DMF, 2 equiv NaH, 25 °C, 6 h	10	51	8
3	DMF, 7 equiv NaH, 25 °C, 2 h	4	7	62

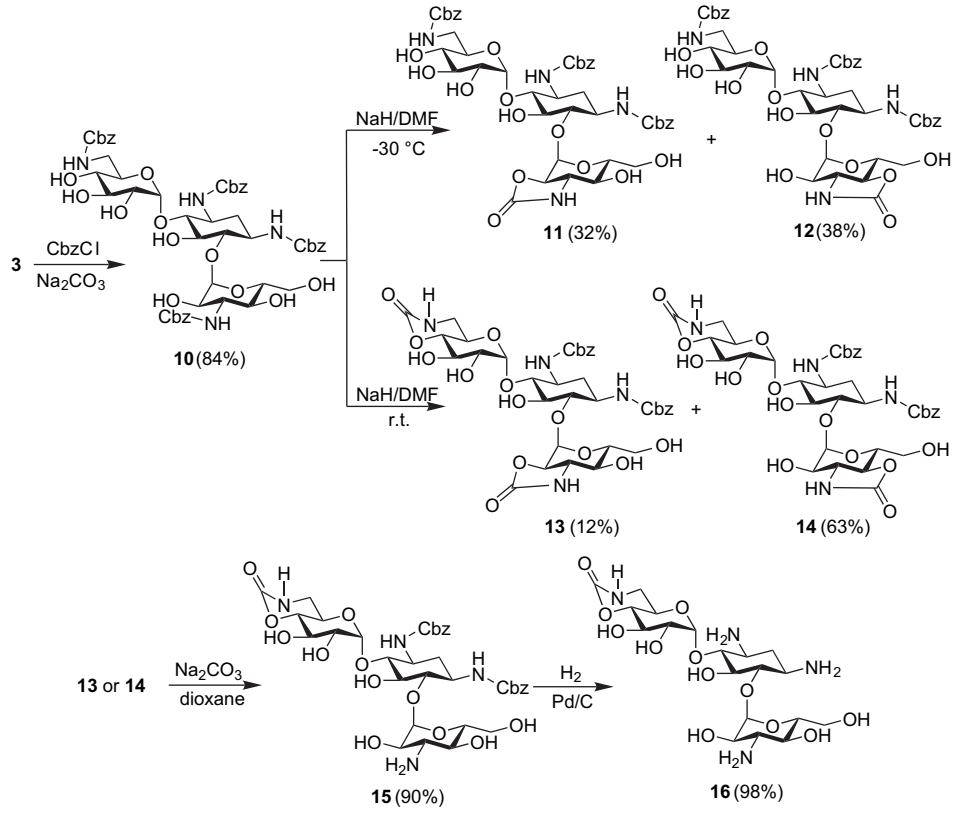
[a] The yields were calculated based on **6** after acetyl derivatizing purification.
[b] Material **6** (32%) was recovered after purification.

2.2. Regioselective modification of aminoglycosides by ring-opening reaction

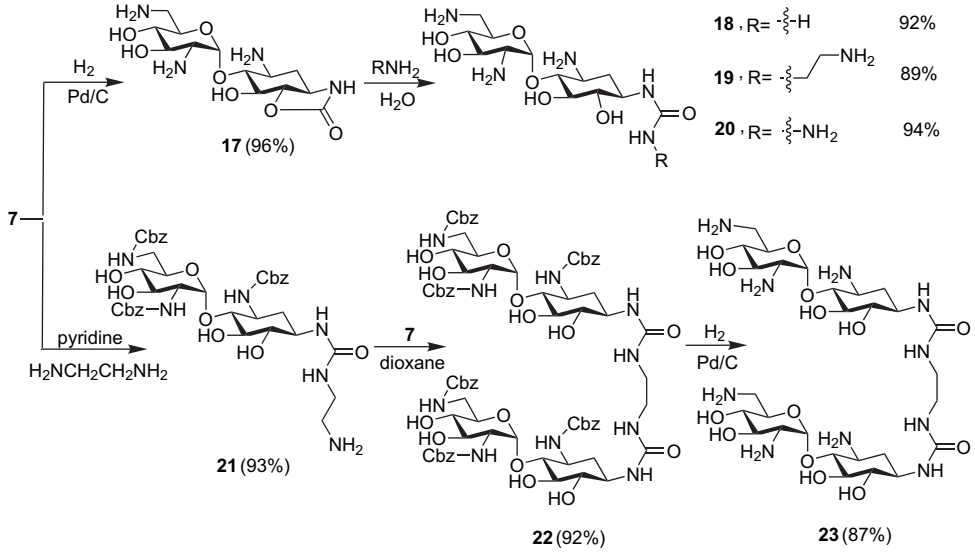
Opening the cyclic carbamate with amine could introduce functional groups into aminoglycosides to produce urea derivatives.[26–28] On the base of regioselective cyclic carbamate formation, we set out to synthesize derivatives with specific structures. Neamine derivative **7** was deprotected by catalytic hydrogenolysis in 96% yield. Derivative **17** underwent smooth ring-opening reaction with different amines in water. The reaction was completed within 2 h to furnish the urea derivatives **18**, **19** and **20** (Scheme 3). This approach provided an effective alternative to



Scheme 1. Regioselective formation of cyclic carbamate in neamine.



Scheme 2. Regioselective formation of cyclic carbamate in kanamycin A.



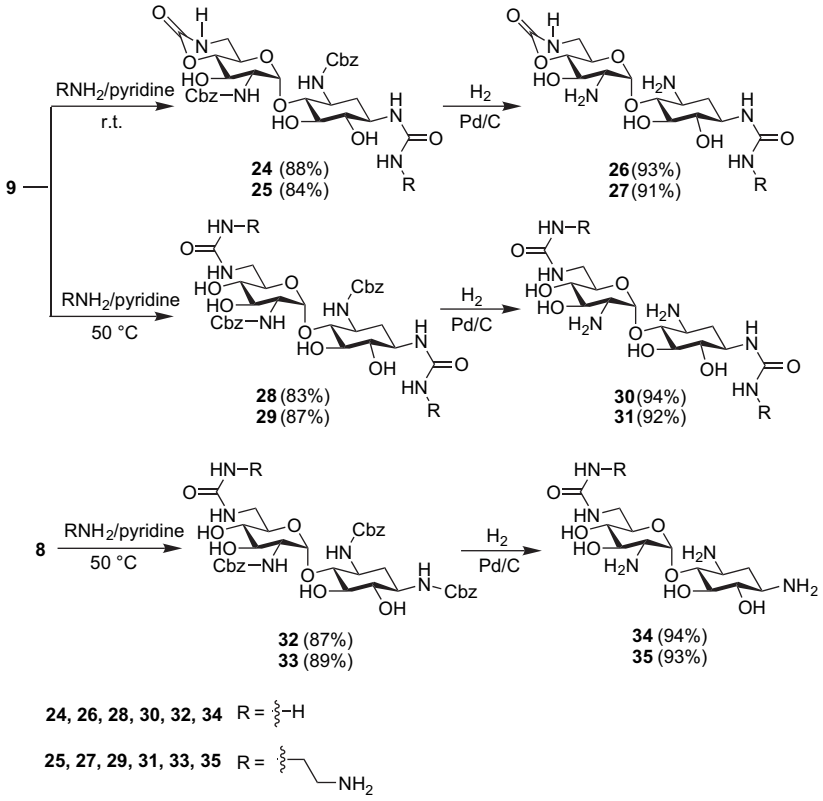
Scheme 3. *N*-1 modification and synthesis of the neamine dimer.

modify the *N*-1 position of aminoglycosides, and previous reports[15,16] showed this to be a formidable task.

Derivative **7** was treated with ethylene diamine in pyridine to produce urea derivative **21** in 93% yield, which was subsequently used in the second ring-opening reaction with compound **7** in dioxane to furnish compound **22** in 92% yield. Hydrogenolysis of the Cbz group in compound **22** gave desired new neamine dimer **23** in 87% yield. To our knowledge, this is the first reported dimer linked at the nitrogen atom of an aminoglycoside (Scheme 3).

When bicyclic carbamate **9** was used as the substrate, the five-membered-ring carbamate was opened selectively under mild condition to produce *N*-1 modified monocyclic carbamates **24** and **25**. The six-membered-ring was kept intact until the reaction temperature rose up to 50 °C. Under this condition, *N*-1 and *N*-6′ modified compounds **28** and **29** were produced. In a same way, compound **8** could be turned into *N*-6′ modified derivatives **32** and **33** (Scheme 4).

After the synthesis of neamine derivatives, we began to introduce amino groups into kanamycin A via the ring-opening



Scheme 4. Regioselective modification of neamine by ring open reaction.

reaction. As a result, all the expected products were produced under different conditions in good yields (Scheme 5).

3. Conclusion

A versatile methodology to achieve the regioselective modification of amino groups in per-*N*-Cbz neamine and per-*N*-Cbz kanamycin A has been developed. The aminoglycoside derivatives with desired cyclic carbamate structure were synthesized. The regioselective modification at specific amino group of these two aminoglycosides by ring-opening reaction was also achieved. These results provided a valuable alternative to the structure modification of certain aminoglycosides.

4. Experimental

4.1. General

Proton magnetic resonance spectra were recorded in $CDCl_3$ or DMSO-d_6 at 300 MHz with Jeol 300 spectrometer. ^{13}C NMR spectra were recorded at 75 Hz. Chemical shifts were reported in parts per million downfield from tetramethylsilane. All NMR spectra were recorded at ambient temperature. High-resolution electronspray spectra were obtained from Bruker APEX IV-FTMS 7.0T Mass spectrometer.

Reactions were monitored by thin layer chromatography on silica gel 60 F_{254}. Column chromatography was performed on silica gel 60. Reagents and starting materials were purchased from Aldrich or Acros and were used without further purification unless otherwise noted. Chloroform was distilled from CaH_2. Pyridine was dried over CaH_2.

4.2. General procedure for hydrogenolysis

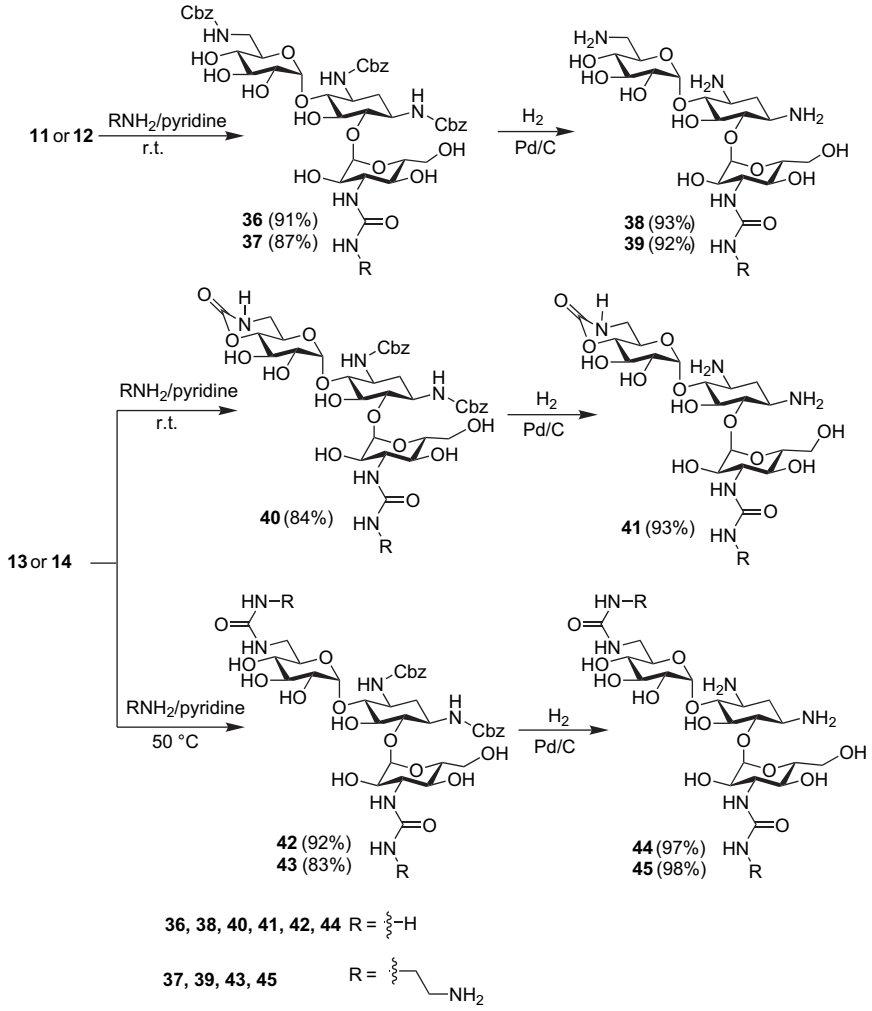
To a solution or suspension of glycoside (0.1 mmol) in MeOH/H_2O (10 ml/2 ml), HCl (1 M, 1 ml) and 10% Pd/C (50 mg) were added. Evacuation was then carried out with hydrogen atmosphere replacements (3×). The mixture was stirred for 8 h at room temperature under an atmosphere of hydrogen at 5 atm. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to furnish the product.

4.3. 1,3,2′,6′-Tetra-*N*-benzyloxycarbonyl-neamine (6)

To a boiling solution of neomycin B trisulfate (20.00 g, 22.02 mmol) in methanol (500 ml), concentrated HCl (18.00 g, 12.1 N) was added dropwise in 30 min. The solution was then refluxed until reactants were consumed. The light yellow reaction solution was cooled to room temperature. This solvent was then evaporated approximately half of its volume and the mixture was cooled to 0 °C. The solid precipitate formed was filtered to give crude neamine hydrochloride (8.01 g).

A solution of CbzCl (16.00 g, 93.84 mmol) in acetone (30 ml) was added dropwise to an aqueous saturated Na_2CO_3 solution (80 ml) of acquired neamine hydrochloride at 0 °C over 30 min. The reaction mixture was vigorously stirred for 2 h in ice bath and 8 h at room temperature to give a white precipitate. The precipitate was then filtered and pulverized in 1 M HCl solution until the Na_2CO_3 was fully neutralized. The white solid was then filtered again and dried in vacuo (13.61 g, 72% for two steps).

^1H NMR (300 MHz, DMSO-d_6) δ 7.38–7.33 (m, 20H, Ph), 7.30–7.14 (m, 2H, N*H*CO_2), 6.96 (d, J=9.9 Hz, 1H, N*H*CO_2), 6.83 (s, 1H, N*H*CO_2), 5.13–4.86 (m, 11H), 3.63 (s, 1H), 3.44–3.10 (m, 8H), 1.75 (br s, 1H, H-2_{eq}), 1.31 (br s, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.5, 156.1, 155.7, 137.2, 136.9, 128.3, 127.7, 127.3, 98.6, 81.5, 76.5,



Scheme 5. Regioselective modification of kanamycin A by ring open reaction.

74.0, 71.4, 71.0, 70.6, 65.2, 65.1, 56.0, 51.1, 50.3, 41.9, 34.8; ESI-HRMS calcd for $C_{44}H_{51}N_4O_{14}$ ($[M+H]^+$) m/z 859.3396, found 859.3400.

4.4. 3,2′,6′-Tri-*N*-benzyloxycarbonyl-1,6-*N*,*O*-carbonyl-neamine (7)

To a solution of compound **6** (0.50 g, 0.58 mmol) in dry DMF (20 ml), NaH (0.14 g, 3.50 mmol, 60% in mineral oil) was added slowly at −40 °C under vigorous stirring. After 12 h, the reaction temperature was raised to −20 °C, and the mixture was stirred for 60 h. HCl (1 M) was added to neutralize the solution. The solvent was removed under reduced pressure, and the residue was pulverized in water to give a white solid (0.48 g). Due to the solubility of the crude product, the purification of the product was achieved through its acetyl derivative as described below.

To the solution of obtained solid in dry pyridine (15 ml), Ac_2O (5 ml) was added. The mixture was stirred for 24 h at room temperature and MeOH (5 ml) was added to quench the reaction. The solvent was removed and the residue was pulverized in water to give a white solid. The solid was purified by chromatography to give triacetyl derivative of compound **7**.

To a solution of compound **7** (0.29 g) in dioxane/H_2O (30 ml/5 ml), NaOH solution (1 M, 5 ml) was added at 0 °C and the resulting mixture was stirred for 45 min in ice bath. After completion of the reaction (monitored by TLC, $CHCl_3$/MeOH 8:1), the reaction was quenched with HCl (1 M, 4.8 ml). After removal of the solvent, ice water was added. The resulting white solid (0.23 g, 92.6%) was filtered and dried without further purification.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (s, 1H, N*H*CO_2), 7.38–7.31 (m, 15H, Ph), 6.98 (s, 1H, N*H*CO_2), 6.73 (d, J=7.5 Hz, 1H, N*H*CO_2), 5.86 (d, J=5.4 Hz, 1H, N*H*CO_2), 5.17–4.88 (m, 8H), 3.91–3.83 (m, 1H), 3.62–3.10 (m, 9H), 1.98–1.94 (m, 1H, H-2_{eq}), 1.47–1.35 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.9, 156.5, 156.1, 155.7, 137.2, 136.8, 128.3, 127.7, 127.6, 127.5, 98.5, 83.3, 82.7, 72.4, 71.3, 71.1, 70.4, 65.4, 65.2, 55.9, 52.8, 51.6, 41.9, 32.5; ESI-HRMS calcd for $C_{37}H_{43}N_4O_{13}$ ($[M+H]^+$) m/z 751.2832, found 751.2804.

4.5. 1,3,2′-Tri-*N*-benzyloxycarbonyl-6′,4′-*N*,*O*-carbonyl-neamine (8)

To a solution of compound **6** (0.50 g, 0.58 mmol) in dry DMF (20 ml), NaH (46 mg, 1.16 mmol, 60% in mineral oil) was added. The mixture was stirred for 6 h at room temperature. HCl (1 M) was added to neutralize the solution. The solvent was removed under reduced pressure, and the residue was pulverized in water to give a white solid (0.46 g). The product was purified through the same process as compound **7** to give a white solid (0.22 g, 50.6%, yield after three steps).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35–7.31 (m, 17H, Ph and N*H*CO_2), 7.15 (br s, 1H, N*H*CO_2), 6.99 (d, J=8.7 Hz, 1H, N*H*CO_2), 5.39 (d, J=5.4 Hz, 1H), 5.13–4.73 (m, 9H), 3.88–3.01 (m, 8H), 1.75 (br s, 1H, H-2_{eq}), 1.34–1.22 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6)

δ 156.2, 155.7, 155.5, 152.3, 137.2, 137.0, 129.0, 128.3, 127.7, 127.4, 99.0, 82.3, 78.6, 76.5, 73.9, 67.8, 65.3, 65.1, 61.4, 55.9, 51.2, 50.0, 42.5, 34.8; ESI-HRMS calcd for $C_{37}H_{42}N_4O_{13}Na$ ($[M+Na]^+$) *m*/*z* 773.2640, found 773.2635.

4.6. 3,2′-Di-*N*-benzyloxycarbonyl-1,6:6′,4′-di-*N*,*O*-carbonyl-neamine (9)

To a solution of compound **7** (0.50 g, 0.58 mmol) in dry DMF (20 ml), NaH (0.16 g, 4.00 mmol, 60% in mineral oil) was added. The mixture was stirred for 2 h at room temperature. HCl (1 M) was added. The solvent was removed under reduced pressure, and the residue was pulverized in water to give a white solid (0.48 g). The product was purified through the same process as compound **7** to give a white solid (0.23 g, 62.0%, yield after three steps).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.70 (s, 1H, N*H*CO_2), 7.49 (d, *J*=8.7 Hz, 1H, N*H*CO_2), 7.36–7.31 (m, 11H, Ph and N*H*CO_2), 6.95 (d, *J*=8.1 Hz, 1H, N*H*CO_2), 5.75 (d, *J*=5.1 Hz, 1H), 5.45 (s, 1H), 5.24 (s, 1H, H-1′), 5.13–4.96 (m, 4H), 4.76 (d, *J*=12 Hz, 1H), 3.88–3.80 (m, 3H), 3.58–3.54 (m, 3H), 3.29 (br s, 1H), 3.03(t, *J*=9.6 Hz, 1H), 1.93 (br s, 1H, H-2_{eq}), 1.50–1.39 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.0, 156.3, 155.6, 152.4, 137.1, 136.9, 128.4, 128.3, 127.8, 127.7, 127.6, 98.9, 83.4, 83.2, 78.5, 72.6, 67.7, 65.4, 65.3, 61.5, 55.8, 53.0, 51.4, 42.6, 32.5.

4.7. 1,3,6′,2″-Tetra-*N*-benzyloxycarbonyl-kanamycin A (10)

Solution of CbzCl (4.25 g, 24.92 mmol) in acetone (15 ml) was added dropwise to an aqueous saturated Na_2CO_3 solution (40 ml) of kanamycin A monosulfate (3.00 g, 5.15 mmol) at 0 °C over 30 min. This reaction mixture was vigorously stirred for 2 h in ice bath and 8 h at room temperature to give a white precipitate. The precipitate was then filtered and pulverized in 1 M HCl until the Na_2CO_3 was fully neutralized. The white solid was then filtered again and dried in vacuo (4.14 g, 84%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37–7.31 (m, 21H, Ph and N*H*CO_2), 7.09 (d, *J*=8.4 Hz, 1H, N*H*CO_2), 7.01 (d, *J*=9 Hz, 1H, N*H*CO_2), 6.84 (br s, 1H, N*H*CO_2), 5.47 (br s, 2H, H-1′ and H-1″), 5.00–4.80 (m, 11H), 4.22 (dd, J_1=4.8 Hz, J_2=5.4 Hz, 1H), 3.86–3.83 (m, 2H), 3.57–3.25 (m, 10H), 3.08–3.06 (m, 1H), 1.83 (br s, 1H, H-2_{eq}), 1.51–1.39 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.6, 156.5, 155.8, 155.6, 137.3, 137.1, 137.0, 128.3, 127.7, 127.5, 101.1, 98.5, 84.3, 80.2, 74.1, 72.8, 72.6, 72.3, 70.6, 70.4, 70.0, 67.0, 65.3, 65.0, 60.1, 56.5, 50.0, 49.6, 41.6, 34.4; ESI-HRMS calcd for $C_{50}H_{64}N_5O_{19}$ ($[M+NH_4]^+$) *m*/*z* 1038.4190, found 1038.4224.

4.8. 1,3,6′-Tri-*N*-benzyloxycarbonyl-3″,2″-*N*,*O*-carbonyl-kanamycin A (11) and 1,3,6′-tri-*N*-benzyloxycarbonyl-3″,2″-*N*,*O*-carbonyl-kanamycin A (12)

To a solution of per-*N*-Cbz kanamycin A (400 mg, 0.39 mmol) in dry DMF (20 ml), NaH (94 mg, 2.35 mmol, 60% in mineral oil) was added slowly at −30 °C under vigorous stirring and the mixture was stirred for 72 h. HCl (1 M) was added to neutralize the solution. The solvent was removed under reduced pressure, and the residue was pulverized in water to give a white solid, most of which was the mixture of compound **11** and **12**. These two compounds could be separated by chromatography on silica gel ($CHCl_3$/MeOH/H_2O 12:2:0.2).

Compound **11**, white solid (113 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.91 (s, 1H, N*H*CO_2), 7.34–7.31 (m, 16H, Ph and N*H*CO_2), 7.21 (d, *J*=8.7 Hz, 1H, N*H*CO_2), 6.87 (br s, 1H, N*H*CO_2), 5.58 (br s, 1H), 5.53 (d, *J*=5.7 Hz, 1H), 5.10–4.86 (m, 10H), 4.73 (dd, J_1=5.7 Hz, J_2=5.1 Hz, 1H), 4.58 (d, *J*=8.7 Hz, 1H), 4.48 (s, 2H), 4.24 (br s, 1H), 3.84–3.72 (m, 2H), 3.69–3.29 (m, 6H), 3.08–3.06 (m, 1H), 1.81–1.77 (m, 1H, H-2_{eq}), 1.50–1.37 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.6, 156.6, 155.8, 155.6, 137.1, 137.0, 128.3, 127.8, 127.3, 101.1, 97.6, 84.3, 79.0, 74.8, 74.3, 72.7, 72.3, 71.3, 70.7, 70.5, 65.3, 59.9, 58.5, 50.4, 49.6, 41.6, 34.7; ESI-HRMS calcd for $C_{43}H_{53}N_4O_{18}$ ($[M+H]^+$) *m*/*z* 913.3360, found 913.3353.

Compound **12**, white solid (135 mg, 38%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.89 (s, 1H, N*H*CO_2), 7.33–7.30 (m, 17H, Ph and N*H*CO_2), 6.85 (br s, 1H, N*H*CO_2), 5.56 (br s, 1H), 5.41 (d, *J*=6.0 Hz, 1H), 5.37 (s, 1H), 5.30 (s, 1H), 5.09–4.85 (m, 9H), 4.35 (s, 1H), 3.81 (s, 2H), 3.56–3.15 (m, 6H), 3.07 (br s, 1H), 1.70 (br s, 1H, H-2_{eq}), 1.49–1.37 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.4, 156.6, 155.7, 155.6, 137.2, 137.0, 128.4, 127.9, 127.8, 127.3, 101.3, 94.3, 84.7, 80.1, 76.8, 74.9, 74.8, 72.7, 72.3, 70.7, 70.5, 68.9, 65.4, 59.4, 56.8, 50.7, 49.8, 41.7, 34.8; ESI-HRMS calcd for $C_{43}H_{53}N_4O_{18}$ ($[M+H]^+$) *m*/*z* 913.3360, found 913.3366.

4.9. 1,3-Di-*N*-benzyloxycarbonyl-6′,4′:3″,2″-di-*N*,*O*-carbonyl-kanamycin A (13) and 1,3-di-*N*-benzyloxycarbonyl-6′,4′:3″,4″-di-*N*,*O*-di-carbonyl-kanamycin A (14)

To a solution of per-*N*-Cbz kanamycin A (600 mg, 0.59 mmol) in dry DMF (20 ml), NaH (235 mg, 5.88 mmol, 60% in mineral oil) was added. The mixture was stirred for 2 h at room temperature. HCl (1 M) was added to neutralize the solution. The solvent was removed under reduced pressure, and the residue was pulverized in water to give a white solid, most of which was the mixture of compound **13** and **14**. These two compounds could be separated by chromatography on silica gel($CHCl_3$/MeOH/H_2O 8:2:0.2).

Compound **13**, white solid (57 mg, 12%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.86 (s, 1H, N*H*CO_2), 7.34–7.29 (m, 13H, Ph and N*H*CO_2), 5.68 (d, *J*=6.0 Hz, 1H), 5.51 (d, *J*=5.1 Hz, 1H), 5.39 (s, 1H), 5.09–4.87 (m, 5H), 4.76–4.68 (m, 2H), 4.58 (d, *J*=8.4 Hz, 1H), 4.18 (br s, 1H), 3.86–3.19 (m, 10H), 2.96 (t, *J*=9.6 Hz, 1H), 1.74–1.70 (m, 1H, H-2_{eq}), 1.49–1.36 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.6, 155.8, 155.5, 152.4, 137.1, 136.9, 128.4, 128.3, 127.8, 127.7, 101.3, 97.6, 84.8, 79.0, 78.0, 74.8, 74.2, 71.9, 71.3, 70.7, 69.9, 65.3, 62.8, 61.5, 59.9, 58.5, 49.4, 42.6, 34.5; ESI-HRMS calcd for $C_{36}H_{44}N_4O_{17}Na$ ($[M+Na]^+$) *m*/*z* 827.2593, found 827.2618.

Compound **14**, white solid (135 mg, 63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.92 (s, 1H, N*H*CO_2), 7.40–7.29 (m, 13H, Ph and N*H*CO_2), 5.79 (d, *J*=6.0 Hz, 1H), 5.56 (d, *J*=5.4 Hz, 1H), 5.44 (d, *J*=6.3 Hz, 1H), 5.30 (s, 1H), 5.24 (s, 1H), 5.14–4.97 (m, 4H), 4.80 (d, *J*=12.3 Hz, 1H), 4.38 (t, *J*=5.4 Hz, 1H), 3.89–3.36 (m, 9H), 3.24 (br s, 1H), 3.01 (t, *J*=9.6 Hz, 1H), 1.72–1.68 (m, 1H, H-2_{eq}), 1.56–1.42 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.4, 155.7, 155.6, 152.4, 137.2, 136.9, 128.5, 128.4, 127.8, 127.7, 101.4, 94.3, 85.3, 80.0, 78.0, 76.7, 74.9, 74.6, 71.9, 70.0, 68.8, 65.4, 65.3, 61.5, 59.4, 56.7, 50.6, 49.5, 42.6, 34.6; ESI-HRMS calcd for $C_{36}H_{45}N_4O_{17}$ ($[M+H]^+$) *m*/*z* 805.2774, found 805.2782.

4.10. 1,3-Di-*N*-benzyloxycarbonyl-6′,4′-*N*,*O*-carbonyl-kanamycin A (15)

To a solution of compound **14** (200 mg, 0.25 mmol) in dioxane/H_2O (20 ml/4 ml), saturated Na_2CO_3 solution (8 ml) was added. The mixture was stirred for 12 h at 40 °C. After the completion of the reaction (monitored by TLC, $CHCl_3$/MeOH/$NH_3\cdot H_2O$ 5:2:0.2), 0.2 M HCl was added to adjust the pH to 8. After removal of the solvent, ice water was added. The resulting white solid, compound **15** (175 mg, 90.3%), was filtered and dried without further purification.

Compound **15** could be prepared from compound **13** in the same way.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (s, 12H, Ph and N*H*CO_2), 5.57–5.51 (m, 1H), 5.14–4.80 (m, 6H), 4.23 (br s, 1H), 3.86 (br s, 1H), 3.70 (br s, 2H), 3.49–3.04 (m, 6H), 2.79–2.72 (m, 1H), 1.88–1.83 (m, 1H, H-2_{eq}), 1.23–1.17 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.9, 155.5, 152.4, 137.1, 136.9, 128.3, 127.8, 101.2, 97.5, 84.9, 80.1,

77.9, 74.6, 72.7, 72.0, 69.9, 66.3, 65.2, 61.4, 60.3, 55.0, 50.0, 49.2, 42.6, 34.4; ESI-HRMS calcd for $C_{35}H_{47}N_4O_{16}$ ($[M+H]^+$) m/z 779.2992, found 779.2990.

4.11. 6′,4′-*N*,*O*-Carbonyl-kanamycin A-trihydrochloride (16)

The title compound was prepared from compound **15** according to Section 4.2 as a white solid (98%).

^{1}H NMR (300 MHz, D_2O) δ 5.34 (s, 1H), 4.92 (s, 1H), 4.10–4.05 (m, 1H), 3.94–3.76 (m, 3H), 3.64–3.37 (m, 10H), 3.12 (q, J=10.2 Hz, 1H), 2.99–2.91 (m, 1H), 2.06–2.02 (m, 1H, H-2_{eq}), 1.41–1.28 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 155.2, 99.6, 98.8, 83.4, 81.1, 77.3, 73.3, 71.8, 70.6, 69.2, 68.9, 66.2, 60.7, 59.3, 54.2, 49.8, 48.1, 41.7, 31.9; ESI-HRMS calcd for $C_{19}H_{35}N_4O_{12}$ ($[M+H]^+$) m/z 511.2246, found 511.2239.

4.12. 1,6-*N*,*O*-Carbonyl-neamine-trihydrochloride (17)

The title compound was prepared from compound **7** according to Section 4.2 (44 mg, 96%).

^{1}H NMR (300 MHz, D_2O) δ 5.86 (d, J=3.3 Hz, 1H, H-1′), 4.13 (t, J=9.0 Hz, 1H), 3.96–3.84 (m, 3H), 3.60–3.32 (m, 5H), 3.18–3.11 (m, 2H), 2.43–2.39 (m, 1H, H-2_{eq}), 1.83–1.70 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 162.8, 96.4, 83.4, 80.0, 72.6, 71.1, 69.8, 68.7, 54.0, 53.6, 50.8, 40.6, 29.4; ESI-HRMS calcd for $C_{13}H_{25}N_4O_7$ ($[M+H]^+$) m/z 349.1717, found 349.1705.

4.13. 1-*N*-[Amino-formyl]-neamine-trihydrochloride (18)

To a stirred solution of compound **17** (60 mg, 0.13 mmol) in H_2O (3 ml), ammonia (0.5 ml) was added at room temperature. After 2 h, the reaction was complete according to TLC (MeOH/$NH_3\cdot H_2O$ 3:1). The mixture was concentrated in vacuo and dissolved in 1 M HCl (0.5 ml). The resulting solution was put on a reversed-phase column (H_2O followed by 10:1 H_2O/MeOH as eluent) to give the urea derivative **18** as a viscous gum (57 mg, 92%).

^{1}H NMR (300 MHz, D_2O) δ 5.69 (s, 1H, H-1′), 3.86–3.66 (m, 3H), 3.52–3.45 (m, 2H), 3.31–3.10 (m, 6H), 2.11 (br s, 1H, H-2_{eq}), 1.47 (br s, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 161.5, 96.7, 79.5, 76.3, 75.0, 71.3, 69.6, 69.3, 54.2, 50.3, 49.3, 40.7, 32.0; ESI-HRMS calcd for $C_{13}H_{28}N_5O_7$ ($[M+H]^+$) m/z 366.1983, found 366.1973.

4.14. 1-*N*-{*N*-[2-Amino-ethyl]-amino-formyl}-neamine-tetrahydrochloride (19)

Compound **19** was synthesized through the similar process as compound **18** with compound **17** and ethylene diamine as a viscous gum (64 mg, 89%).

^{1}H NMR (300 MHz, D_2O) δ 5.72 (d, J=3.6 Hz, 1H, H-1′), 3.84–3.71 (m, 2H), 3.51–3.45 (m, 2H), 3.40–2.91 (m, 10H), 2.13–2.09 (m, 1H, H-2_{eq}), 1.57–1.44 (br s, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 160.5, 96.5, 78.9, 76.3, 74.9, 71.2, 69.6, 68.8, 54.0, 50.1, 49.3, 40.6, 40.4, 37.8, 31.7; ESI-HRMS calcd for $C_{15}H_{33}N_6O_7$ ($[M+H]^+$) m/z 409.2405, found 409.2396.

4.15. 1-*N*-[Hydrazino-formyl]-neamine-trihydrochloride (20)

Compound **20** was synthesized through the similar process as compound **18** with compound **17** and hydrazine hydrate as a viscous gum (61 mg, 94%).

^{1}H NMR (300 MHz, D_2O) δ 5.36 (s, 1H, H-1′), 3.82 (s, 1H), 3.52–3.17 (m, 7H), 2.93–2.81 (m, 3H), 1.86 (br s, 1H, H-2_{eq}), 1.33–1.20 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 162.5, 99.0, 83.6, 76.4, 75.4, 72.6, 71.8, 69.7, 55.1, 50.5, 41.3, 41.1, 34.6; ESI-HRMS calcd for $C_{13}H_{29}N_6O_7$ ($[M+H]^+$) m/z 381.2092, found 381.2084.

4.16. 3,2′,6′-Tri-*N*-benzyloxycarbonyl-1-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-neamine (21)

To a stirred solution of compound **7** (128 mg, 0.17 mmol) in pyridine/MeOH (5 ml/1 ml), ethylene diamine (1 ml) was added at room temperature. After 3 h, the reaction was complete according to TLC ($CHCl_3$/MeOH/$NH_3\cdot H_2O$ 4:3:1). The solvent was removed in vacuo followed by addition of ice water (20 ml), and the resulting white solid (129 mg, 94%) was filtered and dried without further purification.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35–7.29 (m, 16H, Ph and N*H*CO), 5.08–4.85 (m, 7H), 3.49–2.97 (m, 15H), 1.88–1.84 (m, 1H, H-2_{eq}), 1.24–1.12 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.8, 156.9, 156.5, 156.1, 137.6, 137.5, 137.2, 128.7, 128.1, 127.9, 127.7, 98.9, 81.8, 76.9, 75.3, 71.6, 71.2, 70.8, 65.7, 65.6, 65.5, 56.3, 50.5, 50.4, 42.2, 41.8, 35.7; ESI-HRMS calcd for $C_{39}H_{51}N_6O_{13}$ ($[M+H]^+$) m/z 811.3519, found 811.3509.

4.17. The protected dimer of neamine (22)

To a solution of compound **7** (75 mg, 0.1 mmol) in dioxane/H_2O (10 ml/3 ml), compound **21** (81 mg, 0.1 mmol) was added. The resulting mixture was stirred for 6 h at 65 °C. After removal of the solvent, ice water was added. The resulting white solid (143 mg, 92%) was filtered and dried without further purification.

^{1}H NMR (300 MHz, pyridine-d_5) δ 8.03 (br s, 1H, N*H*CO_2), 7.68 (br s, 1H, N*H*CO_2), 7.52 (br s, 1H, N*H*CO_2), 7.21–6.91 (m, 16H, Ph and N*H*CONH), 6.68 (br s, 1H, N*H*CONH), 5.75 (br s, 2H), 5.65 (br s, 1H), 5.13–4.80 (m, 6H), 4.26–4.18 (m, 3H), 3.75–3.67 (m, 7H), 3.39 (br s, 1H), 3.15 (br s, 2H), 2.16 (br s, 1H, H-2_{eq}), 1.45–1.41 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, pyridine-d_5) δ 160.0, 157.5, 156.6, 137.6, 137.5, 128.4, 128.3, 128.0, 127.8, 127.7, 127.5, 100.4, 82.7, 77.7, 77.2, 72.8, 72.4, 72.2, 66.2, 65.9, 65.8, 57.5, 51.3, 51.2, 42.3, 41.0, 35.8; ESI-HRMS calcd for $C_{76}H_{93}N_{10}O_{26}$ ($[M+H]^+$) m/z 1561.6268, found 1561.6210.

4.18. The deprotected dimer of neamine (23)

Compound **23** was obtained from compound **22** according to Section 4.2 as solid trihydrochloride (87%).

^{1}H NMR (300 MHz, D_2O) δ 5.17 (d, J=3.6 Hz, 2H, H-1′), 3.85–3.72 (m, 6H), 3.51–3.46 (m, 4H), 3.30–3.27 (m, 8H), 3.12–3.05 (m, 4H), 3.09 (s, 4H), 2.12–2.08 (m, 1H, H-2_{eq}), 1.57–1.44 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 160.5, 96.5, 78.8, 76.3, 74.9, 71.2, 69.6, 68.8, 54.0, 50.2, 49.4, 40.7, 40.3, 31.8; ESI-HRMS calcd for $C_{28}H_{57}N_{10}O_{14}$ ($[M+H]^+$) m/z 757.4050, found 757.4064.

4.19. 3,2′-Di-*N*-benzyloxycarbonyl-1-*N*-[amino-formyl]-6′,4′-*N*,*O*-carbonyl-neamine (24)

To a solution of compound **9** (109 mg, 0.17 mmol) in pyridine/MeOH (5 ml/1 ml), ammonia (2 ml) was added. The mixture was stirred for 6 h at 25 °C, and the reaction was complete according to TLC ($CHCl_3$/MeOH/$NH_3\cdot H_2O$ 4:3:1). The solvent was removed in vacuo at low temperature (room temperature to 40 °C) followed by addition of ice water (20 ml), and the resulting white solid, compound **24** (98 mg, 88%), was filtered and dried without further purification.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.37–7.26 (m, 11H, Ph and N*H*CO_2), 7.01 (d, J=8.4 Hz, 1H, N*H*CO_2), 5.89 (d, J=6.3 Hz, 1H, N*H*CONH_2), 5.47 (s, 2H, NHCON*H*$_2$), 5.40 (d, J=8.7 Hz, 1H), 5.14–4.97 (m, 6H), 4.75 (d, J=12.6 Hz, 1H), 3.91–3.86 (m, 1H), 3.78 (dd, J_1=8.7 Hz, J_2=9.3 Hz, 1H), 3.62–3.30 (m, 5H), 3.05–2.98 (m, 2H), 1.89–1.85 (m, 1H, H-2_{eq}), 1.25–1.12 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.9, 156.3, 155.5, 152.4, 137.2, 137.0, 128.4, 128.3, 127.7, 127.6, 127.5, 99.0, 82.4, 78.6, 76.8, 75.2, 67.8, 65.3, 65.1,

61.4, 55.9, 50.1, 50.0, 42.6, 35.3; ESI-HRMS calcd for $C_{30}H_{38}N_5O_{12}$ ($[M+H]^+$) m/z 660.2511, found 660.2524.

4.20. 3,2′-Di-*N*-Benzyloxycarbonyl-1-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-6′,4′-*N*,*O*-carbonyl-neamine (25)

Compound **25** was synthesized through the similar process as compound **24** with compound **9** and ethylene diamine as white solid (100 mg, 84%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.29 (br s, 12H, Ph and N*H*CO_2), 5.97 (br s, 2H,), 5.04 (br s, 8H), 3.75–2.49 (m, 13H), 1.83 (br s, 1H, H-2_{eq}), 1.18 (br s, 1H, H-2_{ax}); ^{1}H NMR (300 MHz, pyridine-d_5) δ 8.47 (d, J=9.0 Hz, 1H, N*H*CO_2), 8.33 (s, 1H, N*H*CO_2), 7.42–7.19 (m, 11H, Ph and N*H*CO_2), 6.92 (s, 1H, N*H*C*O*N*H*$_2$), 6.73 (s, 1H, N*H*C*O*N*H*$_2$), 6.18 (s, 1H), 5.56 (d, J=12.3 Hz, 1H), 5.33 (d, J=12.6 Hz, 1H), 5.15–4.61 (m, 9H), 4.37–4.34 (m, 1H), 4.18 (br s, 1H), 4.04 (s, 2H), 3.88 (s, 1H), 3.76–3.74 (m, 1H), 3.53 (s, 1H), 3.42 (s, 2H), 2.83 (t, J=5.4 Hz, 2H), 2.57–2.54 (m, 1H, H-2_{eq}), 1.89–1.77 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.3, 156.2, 155.4, 152.3, 137.2, 137.0, 128.8, 128.7, 128.6, 128.2, 127.5, 127.4, 127.0, 126.9, 98.7, 82.1, 78.5, 76.6, 74.8, 67.6, 65.0, 61.2, 55.8, 50.0, 49.9, 42.5, 41.7, 41.5, 35.2; ESI-HRMS calcd for $C_{32}H_{43}N_6O_{12}$ ($[M+H]^+$) m/z 703.2933, found 703.2933.

4.21. 1-*N*-[Amino-formyl]-6′,4′-*N*,*O*-carbonyl-neamine-dihydrochloride (26)

Compound **26** was obtained from compound **24** according to Section 4.2 as solid dihydrochloride (93%).

^{1}H NMR (300 MHz, D_2O) δ 5.34 (d, J=3.0 Hz, 1H, H-1′), 4.08–4.03 (m, 1H), 3.90 (dd, J_1=9.0 Hz, J_2=9.9 Hz, 1H), 3.77 (t, J=9.3 Hz, 1H), 3.47–3.33 (m, 4H), 3.21–2.92 (m, 4H), 2.00–1.95 (m, 1H, H-2_{eq}), 1.35–1.23 (br s, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 161.6, 156.1, 99.9, 83.3, 78.7, 76.1, 75.1, 69.7, 62.0, 54.8, 50.6, 49.2, 42.7, 33.4; ESI-HRMS calcd for $C_{14}H_{26}N_5O_8$ ($[M+H]^+$) m/z 392.1775, found 392.1775.

4.22. 1-*N*-{*N*-[2-Amino-ethyl]-amino-formyl}-6′,4′-*N*,*O*-carbonyl-neamine-trihydrochloride (27)

Compound **27** was obtained from compound **25** according to Section 4.2 as solid trihydrochloride (91%).

^{1}H NMR (300 MHz, D_2O) δ 5.40 (s, 1H, H-1′), 4.10–4.04 (m, 1H), 3.96–3.80 (m, 2H), 3.47–2.92 (m, 12H), 2.03–1.99 (m, 1H, H-2_{eq}), 1.41–1.29 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 160.5, 156.1, 99.4, 82.4, 78.6, 76.1, 75.1, 69.2, 62.0, 54.6, 50.4, 49.2, 42.7, 40.5, 37.8, 33.0; ESI-HRMS calcd for $C_{16}H_{31}N_6O_8$ ($[M+H]^+$) m/z 435.2197, found 435.2197.

4.23. 3,2′-Di-*N*-benzyloxycarbonyl-1,6′-di-*N*-[amino-formyl]-neamine (28)

To a solution of compound **9** (90 mg, 0.14 mmol) in pyridine/MeOH (5 ml/1 ml), ammonia (2 ml) was added at room temperature. The mixture was stirred for 6 h at 50 °C, and the reaction was complete according to TLC ($CHCl_3$/MeOH/$NH_3\cdot H_2O$ 4:3:1). The solvent was removed in vacuo followed by addition of ice water (20 ml), and the resulting white solid, compound **28** (77 mg, 83%), was filtered and dried without further purification.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (s, 10H, Ph), 7.12 (br s, 1H, N*H*CO_2), 6.85 (br s, 1H, N*H*CO_2), 5.97 (br s, 1H, N*H*C*O*N*H*$_2$), 5.91 (br s, 1H, N*H*C*O*N*H*$_2$), 5.62 (s, 2H, NHCO*N*H_2), 5.48 (s, 2H, NHCO*N*H_2), 5.03–4.84 (m, 7H), 3.55–3.06 (m, 8H), 1.86 (br s, 1H, H-2_{eq}), 1.20–1.16 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.8, 159.0, 156.2, 155.6, 137.3, 137.0, 128.7, 128.3, 127.8, 127.6, 127.4, 99.0, 82.1, 76.8, 75.1, 71.4, 70.8, 70.3, 65.3, 65.1, 56.4, 50.2, 50.1, 43.8, 35.4; ESI-HRMS calcd for $C_{30}H_{41}N_6O_{12}$ ($[M+H]^+$) m/z 677.2777, found 677.2781.

4.24. 3,2′-Di-*N*-benzyloxycarbonyl-1,6′-di-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-neamine (29)

Compound **29** was synthesized through the similar process as compound **28** with compound **9** and ethylene diamine as white solid (93 mg, 87%).

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33–7.31 (m, 10H, Ph), 7.12 (br s, 1H, N*H*CO_2), 6.85 (br s, 1H, N*H*CO_2), 6.15 (br s, 1H, N*H*CONH), 6.06 (br s, 1H, N*H*CONH), 5.95 (br s, 2H, N*H*CONH), 5.06–4.83 (m, 4H), 3.59–3.07 (m, 11H), 2.97 (s, 7H), 2.52 (br s, 2H), 1.86 (br s, 1H, H-2_{eq}), 1.19–1.15 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.2, 158.5, 156.3, 155.7, 137.3, 137.0, 128.3, 127.8, 127.6, 127.5, 98.8, 82.0, 76.8, 75.1, 71.4, 70.9, 70.4, 65.4, 65.2, 56.4, 50.2, 42.4, 42.3, 41.8, 41.5, 35.6; ESI-HRMS calcd for $C_{34}H_{51}N_8O_{12}$ ($[M+H]^+$) m/z 763.3621, found 763.3626.

4.25. 1,6′-Di-*N*-[amino-formyl]-neamine-dihydrochloride (30)

Compound **30** was obtained from compound **28** according to Section 4.2 as solid dihydrochloride (94%).

^{1}H NMR (300 MHz, D_2O) δ 5.37 (s, 1H, H-1′), 3.67–3.11 (m, 10H), 2.07–2.03 (m, 1H, H-2_{eq}), 1.43–1.31 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 162.1, 161.6, 98.3, 82.9, 76.1, 75.0, 72.6, 71.2, 54.8, 50.4, 49.5, 40.8, 32.8; ESI-HRMS calcd for $C_{14}H_{29}N_6O_8$ ($[M+H]^+$) m/z 409.2041, found 409.2030.

4.26. 1,6′-Di-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-neamine-tetrahydrochloride (31)

Compound **31** was obtained from compound **29** according to Section 4.2 as solid tetrahydrochloride (92%).

^{1}H NMR (300 MHz, D_2O) δ 5.12 (s, 1H, H-1′), 3.67–3.61 (m, 1H), 3.53–3.10 (m, 12H), 2.90–2.79 (m, 6H), 1.90–1.85 (m, 1H, H-2_{eq}), 1.24–1.11 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 161.0, 160.6, 100.1, 86.5, 76.2, 75.3, 72.4, 71.6, 55.3, 50.7, 49.6, 41.0, 40.5, 37.9, 37.9, 34.6; ESI-HRMS calcd for $C_{18}H_{39}N_8O_8$ ($[M+H]^+$) m/z 495.2885, found 495.2876.

4.27. 1,3,2′-Tri-*N*-benzyloxycarbonyl-6′-*N*-[amino-formyl]-neamine (32)

Compound **32** was synthesized through the similar process as compound **28** with compound **8** and ammonia in 87% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (s, 15H, Ph), 7.17 (br s, 2H, N*H*CO_2), 6.86 (br s, 1H, N*H*CO_2), 6.00 (br s, 1H, N*H*C*O*N*H*$_2$), 5.63 (br s, 2H, NHCO*N*H_2), 5.04–4.89 (m, 8H), 3.76–3.09 (m, 8H), 1.78 (br s, 1H, H-2_{eq}), 1.39–1.26 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.8, 156.1, 155.7, 155.5, 137.2, 137.1, 136.8, 128.2, 127.7, 127.5, 127.3, 98.9, 81.9, 76.5, 74.0, 71.3, 70.7, 70.2, 65.3, 65.0, 56.3, 51.1, 50.2, 34.9; ESI-HRMS calcd for $C_{74}H_{91}N_{10}O_{26}$ ($[2M+H]^+$) m/z 1535.6100, found 1535.6163.

4.28. 1,3,2′-Tri-*N*-benzyloxycarbonyl-6′-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-neamine (33)

Compound **33** was synthesized through the similar process as compound **28** with compound **8** and ethylene diamine in 89% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35–7.34 (m, 15H, Ph), 7.17 (d, J=6.0 Hz, 1H, N*H*CO), 6.85 (s, 1H, N*H*CO), 6.13 (s, 1H, N*H*CO), 5.95 (br s, 1H, N*H*CO), 5.09–4.86 (m, 7H), 3.57 (br s, 1H), 3.43–3.00 (m, 14H), 2.56 (m, 2H), 1.79 (br s, 1H, H-2_{eq}), 1.39–1.26 (m, 1H, H-2_{ax}); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.2, 156.2, 155.7, 155.6, 137.3, 137.2, 136.9, 128.3, 127.8, 127.6, 127.4, 98.9, 81.8, 76.6, 74.0, 71.4, 70.8, 70.3,

65.3, 65.1, 56.3, 51.2, 50.2, 42.3, 41.8, 34.9; ESI-HRMS calcd for $C_{39}H_{51}N_6O_{13}$ ([M+H]$^+$) *m*/*z* 811.3519, found 811.3519.

4.29. 6′-*N*-[Amino-formyl]-neamine-trihydrochloride (34)

Compound **34** was obtained from compound **32** according to Section 4.2 as solid tetrahydrochloride (94%).

^{1}H NMR (300 MHz, D_2O) δ 5.38 (s, 1H, H-1′), 3.69 (m, 1H), 3.43–3.08 (m, 9H), 2.19–2.15 (m, 1H, H-2$_{eq}$), 1.53–1.43 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 162.0, 97.9, 83.5, 75.6, 73.5, 72.7, 71.2, 70.0, 54.8, 50.7, 49.3, 40.8, 31.0; ESI-HRMS calcd for $C_{13}H_{28}N_5O_7$ ([M+H]$^+$) *m*/*z* 366.1983, found 366.1973.

4.30. 6′-*N*-{*N*-[2-Amino-ethyl]-amino-formyl}-neamine-tetrahydrochloride (35)

Compound **35** was obtained from compound **33** according to Section 4.2 as solid tetrahydrochloride (93%).

^{1}H NMR (300 MHz, D_2O) δ 5.49 (s, 1H, H-1′), 3.73–3.66 (m, 3H), 3.48–3.17 (m, 8H), 2.90 (s, 3H), 2.54 (s, 1H), 2.32–2.28 (m, 1H, H-2$_{eq}$), 1.74–1.62 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 161.1, 97.3, 80.2, 75.4, 73.0, 72.8, 70.8, 69.3, 54.5, 50.3, 40.5, 37.9, 37.1, 29.1; ESI-HRMS calcd for $C_{15}H_{33}N_6O_7$ ([M+H]$^+$) *m*/*z* 409.2405, found 409.2394.

4.31. 1,3,6′-Tri-*N*-benzyloxycarbonyl-3″-*N*-[amino-formyl]-kanamycin A (36)

According to the procedure for compound **24**, compound **36** was obtained from compound **11** or compound **12** in 91% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34–7.31 (m, 16H, Ph and N*H*CO$_2$), 7.19 (d, *J*=8.7 Hz, 1H, N*H*CO$_2$), 6.86 (br s, 1H, N*H*CO$_2$), 6.07 (br s, 1H, N*H*CONH$_2$), 5.82 (s, 2H, NHCON*H*$_2$), 5.49–5.44 (m, 2H), 5.10–4.86 (m, 8H), 4.52 (br s, 1H), 4.25 (br s, 1H), 3.84 (d, *J*=9.3 Hz, 1H), 3.51–3.25 (m, 10H), 3.08 (br s, 1H), 1.84 (br s, 1H, H-2$_{eq}$), 1.46–1.42 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4, 156.5, 155.8, 155.6, 137.1, 137.0, 128.3, 127.7, 127.6, 101.1, 97.1, 84.3, 80.2, 74.4, 72.7, 72.3, 70.8, 70.7, 70.5, 68.9, 65.3, 60.1, 55.5, 50.2, 49.6, 41.6, 34.9; ESI-HRMS calcd for $C_{43}H_{55}N_5O_{18}$ ([M+H]$^+$) *m*/*z* 930.3614, found 930.3627.

4.32. 1,3,6′-Tri-*N*-benzyloxycarbonyl-3″-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-kanamycin A (37)

According to the procedure for compound **25**, compound **37** was obtained from compound **11** or compound **12** in 87% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33–7.31 (m, 16H, Ph and N*H*CO$_2$), 7.20 (d, *J*=7.8 Hz, 1H, N*H*CO$_2$), 6.48 (br s, 1H, N*H*CONH), 6.22 (br s, 1H, N*H*CONH), 5.10–4.86 (m, 8H), 4.16 (br s, 2H, N*H*$_2$), 3.85 (d, *J*=9.0 Hz, 1H), 3.51–3.26 (m, 15H), 3.02 (br s, 3H), 2.60 (br s, 1H), 1.84 (br s, 1H, H-2$_{eq}$), 1.50–1.38 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.7, 156.6, 155.8, 155.6, 137.2, 137.0, 128.7, 128.3, 127.7, 127.6, 101.1, 97.1, 84.2, 80.2, 74.3, 72.7, 72.3, 70.7, 70.5, 68.8, 65.3, 60.1, 55.6, 50.2, 49.6, 41.9, 41.7, 41.1, 34.6; ESI-HRMS calcd for $C_{45}H_{60}N_6O_{18}$ ([M+H]$^+$) *m*/*z* 973.4036, found 973.4036.

4.33. 3″-*N*-[Amino-formyl]-kanamycin A-trihydrochloride (38)

Compound **38** was obtained from compound **36** according to Section 4.2 as solid tetrahydrochloride (93%).

^{1}H NMR (300 MHz, D_2O) δ 5.38 (s, 1H), 4.91 (s, 1H), 3.84–3.48 (m, 12H), 3.29–3.16 (m, 3H), 3.02–2.97 (m, 2H), 2.39–2.34 (m, 1H, H-2$_{eq}$), 1.82–1.70 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 162.5, 101.6, 95.8, 84.4, 78.5, 73.8, 72.7, 72.5, 71.3, 71.2, 71.0, 69.2, 68.2, 61.0, 55.2, 50.3, 48.0, 40.8, 28.0; ESI-HRMS calcd for $C_{19}H_{38}N_5O_{12}$ ([M+H]$^+$) *m*/*z* 528.2511, found 528.2506.

4.34. 3″-*N*-{*N*-[2-Amino-ethyl]-amino-formyl}-kanamycin A-tetrahydrochloride (39)

Compound **39** was obtained from compound **37** according to Section 4.2 as solid tetrahydrochloride (92%).

^{1}H NMR (300 MHz, D_2O) δ 5.38 (s, 1H), 4.92 (s, 1H), 3.60–3.01 (m, 21H), 2.09 (br s, 1H, H-2$_{eq}$), 1.47–1.43 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 161.3, 101.2, 97.3, 85.6, 81.9, 73.9, 73.5, 72.7, 71.5, 71.0, 69.0, 68.4, 60.9, 55.1, 50.8, 48.7, 41.0, 40.6, 38.5, 31.4; ESI-HRMS calcd for $C_{21}H_{43}N_6O_{12}$ ([M+H]$^+$) *m*/*z* 570.2933, found 570.2938.

4.35. 1,3-Di-*N*-benzyloxycarbonyl-3″-*N*-[amino-formyl]-6′,4′-*N*,*O*-carbonyl-kanamycin A (40)

According to the procedure for compound **24**, compound **40** was obtained from compound **13** or compound **14** in 84% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33 (s, 13H, Ph and N*H*CO$_2$), 6.06 (br s, 1H, N*H*CONH$_2$), 5.80 (s, 2H, NHCON*H*$_2$), 5.61 (d, *J*=5.1 Hz, 1H), 5.52 (d, *J*=3.9 Hz, 1H), 5.45 (br s, 1H), 5.13–4.76 (m, 5H), 4.55 (br s, 1H), 4.25 (br s, 1H), 3.83 (br s, 2H), 3.72–3.23 (m, 9H), 3.03 (t, *J*=9.6 Hz, 1H), 1.83 (br s, 1H, H-2$_{eq}$), 1.54–1.42 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4, 155.8, 155.5, 152.4, 137.1, 136.9, 128.4, 128.3, 127.8, 127.6, 101.2, 97.2, 84.8, 80.1, 78.0, 74.3, 72.8, 72.0, 70.8, 70.0, 68.9, 65.3, 61.5, 60.1, 55.5, 50.2, 49.3, 42.6, 34.4; ESI-HRMS calcd for $C_{36}H_{48}N_5O_{17}$([M+H]$^+$) *m*/*z* 822.3039, found 822.3029.

4.36. 3″-*N*-[Amino-formyl]-6′,4′-*N*,*O*-carbonyl-kanamycin A (41)

Compound **39** was obtained from compound **37** according to Section 4.2 as solid tetrahydrochloride (93%).

^{1}H NMR (300 MHz, D_2O) δ 5.46 (s, 1H), 4.89 (s, 1H), 4.03 (br s, 1H), 3.93–3.24 (m, 14H), 3.09 (t, *J*=9.3 Hz, 1H), 2.37–2.34 (m, 1H, H-2$_{eq}$), 1.79–1.67 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 162.5, 156.2, 101.7, 98.3, 84.4, 79.2, 78.3, 73.8, 73.5, 71.2, 71.1, 69.9, 68.1, 62.0, 60.8, 55.2, 50.2, 48.5, 42.8, 28.4; ESI-HRMS calcd for $C_{20}H_{36}N_5O_{13}$ ([M+H]$^+$) *m*/*z* 554.2304, found 554.2319.

4.37. 1,3-Di-*N*-benzyloxycarbonyl-3″,6′-*N*-[amino-formyl]-kanamycin A (42)

According to the procedure for compound **28**, compound **42** was obtained from compound **13** or compound **14** in 92% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (s, 11H, Ph and N*H*CO$_2$), 7.20 (d, *J*=8.4 Hz, 1H, N*H*CO$_2$), 6.10 (br s, 1H, N*H*CONH$_2$), 6.01 (br s, 1H, N*H*CONH$_2$), 5.82 (s, 2H, NHCON*H*$_2$), 5.65 (s, 2H, NHCON*H*$_2$), 5.49–5.44 (m, 2H), 5.07–4.87 (m, 7H), 4.54 (br s, 1H), 4.25 (br s, 1H), 3.83 (d, *J*=9.3 Hz, 1H), 3.51–3.25 (m, 10H), 3.01 (br s, 1H), 1.84 (br s, 1H, H-2$_{eq}$), 1.52–1.40 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.4, 160.0, 155.8, 155.5, 137.2, 136.9, 128.3, 128.1, 127.8, 127.7, 127.6, 101.5, 97.1, 84.9, 80.0, 74.4, 72.7, 72.4, 71.4, 70.8, 69.8, 68.9, 65.4, 65.3, 60.1, 55.5, 50.2, 49.5, 43.4, 34.5; ESI-HRMS calcd for $C_{36}H_{50}N_6O_{17}$ ([M+H]$^+$) *m*/*z* 839.3305, found 839.3284.

4.38. 1,3-Di-*N*-benzyloxycarbonyl-3″,6′-*N*-{*N*-[2-amino-ethyl]-amino-formyl}-kanamycin A (43)

According to the procedure for compound **29**, compound **43** was obtained from compound **13** or compound **14** in 83% yield.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.25 (s, 10H, Ph), 7.13 (br s, 1H, N*H*CO$_2$), 6.48 (br s, 1H, N*H*CO$_2$), 6.26 (br s, 1H, N*H*CONH$_2$), 6.15 (br s, 1H, N*H*CONH$_2$), 5.91 (br s, 1H, N*H*CONH$_2$), 5.77 (br s, 1H, N*H*CONH$_2$), 4.97–4.84 (m, 6H), 4.09 (br s, 7H), 3.73 (br s, 1H), 3.42–2.93 (m, 17H), 2.49 (br s, 2H), 1.76 (br s, 1H, H-2$_{eq}$), 1.33 (br s, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.7, 159.3, 155.7, 155.5, 137.1, 136.9, 128.3, 127.8, 127.5, 101.4, 97.0, 84.6, 79.9, 74.3, 72.7, 72.6, 72.4, 71.3, 70.6, 69.9,

68.9, 65.3, 65.2, 60.1, 55.7, 50.1, 49.5, 42.0, 41.7, 41.5, 34.6; ESI-HRMS calcd for $C_{40}H_{61}N_8O_{17}$ ($[M+H]^+$) m/z 925.4149, found 925.4165.

4.39. 3″,6′-*N*-[Amino-formyl]-kanamycin A-dihydrochloride (44)

Compound **44** was obtained from compound **42** according to Section 4.2 as solid tetrahydrochloride (97%).

^{1}H NMR (300 MHz, D_2O) δ 5.26 (s, 1H), 4.89 (s, 1H), 3.73–3.16 (m, 17H), 2.34–2.30 (m, 1H, H-2$_{eq}$), 1.75–1.63 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 162.5, 162.1, 101.6, 98.9, 84.1, 80.8, 73.7, 72.7, 72.3, 71.7, 71.1, 70.9, 68.1, 60.8, 55.2, 50.4, 49.0, 40.8, 28.7; ESI-HRMS calcd for $C_{20}H_{39}N_6O_{13}$ ($[M+H]^+$) m/z 571.2569, found 571.2547.

4.40. 3″,6′-*N*-{*N*-[2-Amino-ethyl]-amino-formyl}-kanamycin A-tetrahydrochloride (45)

Compound **39** was obtained from compound **37** according to Section 4.2 as solid tetrahydrochloride (98%).

^{1}H NMR (300 MHz, D_2O) δ 5.05 (s, 1H), 4.88 (s, 1H), 3.79–2.78 (m, 23H), 2.69 (t, J=9.0 Hz, 1H), 1.87–1.84 (m, 1H, H-2$_{eq}$), 1.19–1.07 (m, 1H, H-2$_{ax}$); ^{13}C NMR (75 MHz, D_2O) δ 161.4, 160.9, 100.8, 100.7, 88.1, 86.7, 74.6, 73.2, 72.3, 72.2, 71.4, 71.0, 68.5, 60.7, 55.3, 50.9, 49.6, 41.1, 40.6, 40.4, 38.1, 34.3; ESI-HRMS calcd for $C_{24}H_{49}N_8O_{13}$ ($[M+H]^+$) m/z 657.3413, found 657.3415.

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