Tetrahedron 65 (2009) 5922–5927

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Selective deprotection of the Cbz amine protecting group for the facile synthesis of kanamycin A dimers linked at *N*-3″ position

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ARTICLE INFO

Article history:
Received 5 April 2009
Received in revised form 26 May 2009
Accepted 1 June 2009
Available online 6 June 2009

Keywords:
Regioselective
Aminoglycoside
Kanamycin A
Dimer

ABSTRACT

The optimal conditions for the regioselective deprotection of per-*N*-Cbz-kanamycin A and the reaction mechanism was investigated. We found that the Cbz group at *N*-3″ position was selectively deprotected under milder basic conditions and the cyclic carbamate is an intermediate of the deprotection reaction. The selective deprotection of the amine protecting group enable us to synthesize several kanamycin A dimers linked at *N*-3″ position in a straightforward way.

1. Introduction

Aminoglycosides are clinically important antibiotics to treat infectious diseases caused by Gram-positive and Gram-negative bacteria. These drugs bind to the decoding region of ribosome, inducing codon misreading, inhibiting translocation, and eventually leading to cell death.[1–6] However, the appearance of aminoglycoside-resistant bacteria has significantly reduced their clinical applications.[7–9] In the last several years, aminoglycoside dimers have been synthesized by modifying the hydroxyl groups, such as the 5′-hydroxyl of kanamycin A and 5-hydroxyl of neamine, to enhance the binding affinity to the RNA targets and/or to confer resistance to aminoglycoside-modifying enzymes.[10–12] Recently, the synthesis of neamine dimers linked at the 4′- or the 5-hydroxyl[13a] and at the *N*-1 position[13b] have been reported, which are able to bind to a specific sequence of HIV-1 RNA.

In a previous report, we have described a shorter route to the synthesis of *N*-1 linked neamine dimer based on the selective formation of cyclic carbamate.[14] We report here the synthesis of new kanamycin A dimers linked at the *N*-3″ position via a more effective route. When per-*N*-Cbz-kanamycin A was treated with NaOH under carefully controlled conditions, per-*N*-Cbz-kanamycin A was selectively deprotected at *N*-3″ position in good yield. The deprotected product was used to synthesize several kanamycin A dimmers.

2. Results and discussion

2.1. Selective deprotection of the Cbz groups

There are four free amino groups in kanamycin A, and the regioselective modification of the amines through a simple route is always difficult. In a previous study, we have introduced the cyclic carbamate group to protect the amines, and the carbamate groups could be removed by different basic reagents, which afforded a series of analogs modified at the nitrogen positions of neamine and kanamycin A.[14] Since both the introduction and removal of the cyclic carbamate protecting group could be achieved regioselectively under basic condition, we explored the possibility to remove the Cbz protecting group selectively by controlling the reaction conditions. By careful adjustment of the basic deprotection conditions, we succeeded in deprotecting the *N*-Cbz group at *N*-3″ position of per-*N*-Cbz-kanamycin A specifically. Moreover, the reaction mechanism was elucidated by the separation of the cyclic carbamate intermediate in the deprotection process.

We initially observed that the reaction of per-*N*-Cbz-kanamycin A (**1**) with a 2.5 M NaOH solution in dioxane at 50 °C for 24 h led to the selective deprotection of the Cbz groups at *N*-3″ and *N*-6′ positions and the formation of compound **5**, the 1,3-di-Cbz-kanamycin A, in 85% yield. It suggested that the vicinal hydroxyl groups could aid the deprotection of the Cbz groups and the reaction occurred possibly via the cyclic carbamate intermediate.

Since we have shown that there is selectivity between *N*-3″ and *N*-6′ to form the corresponding cyclic carbamate groups, it is possible to selectively deprotect the Cbz groups at these two positions by

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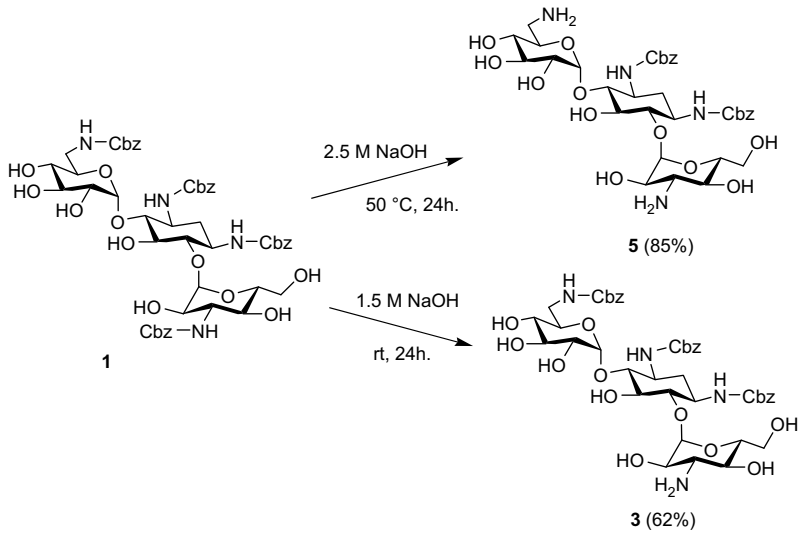
doi:10.1016/j.tet.2009.06.002



Scheme 1. Selective deprotection of per-*N*-Cbz-kanamycin A.

carefully adjusting the reaction conditions. Indeed, when compound **1** was reacted with a 1.5 M NaOH solution in dioxane at room temperature for 24 h, compound **3**, the *N*-3″ selectively deprotected tri-Cbz-kanamycin A, was produced in 62% yield (Scheme 1).

There is only a few related reports on the deprotection of the Cbz group under basic conditions.[15,16] The mechanism via a cyclic carbamate intermediate has never been confirmed, because the cyclic carbamate was unstable under basic conditions and generally could not be separated and identified. In order to confirm the mechanism of the deprotection reaction, the reaction intermediates were isolated and characterized under various conditions.

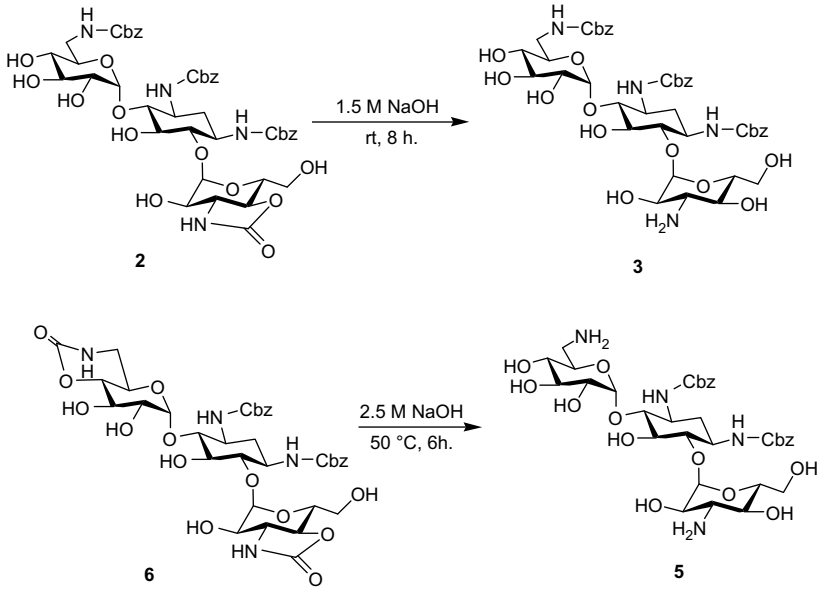
When compound **1** was treated with a 1.5 M NaOH solution at room temperature for 8 h, the intermediate **2** was detected and separated as a by-product in 8% yield. Under this condition, compound **3** was the major product and compound **1** was not consumed completely. When the deprotection reaction was carried out with a 2.5 M NaOH solution at 30 °C for 12 h, multiple products were formed including compound **3**, compound **5** and another intermediate **4** (Scheme 3), which was obtained in 5% yield.

Intermediates **2** and **4** have been synthesized via different routes in our previous work[14] and their structures have been characterized. It's difficult to determine the structures of compounds **3** and **5** directly through spectroscopic analysis. Because the structures of the monocarbamate derivative **2** and the bicarbamate derivative **6** were characterized previously,[14] the formation of compounds **3** and **5** by the basic treatment of compounds **2** and **6**, respectively, confirmed the structures of compounds **3** and **5** indirectly (Scheme 2).

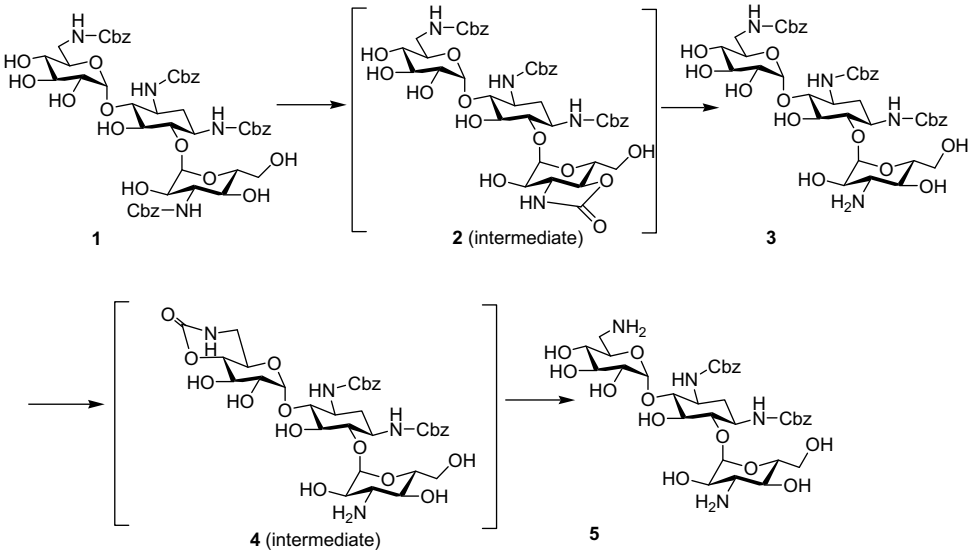
The existence of carbamate intermediate indicated that the rate of cyclic carbamate formation was faster than the rate of corresponding ring opening. Additionally, the rate of the opening of the five-membered cyclic carbamate was faster than the rate of the formation of the six-membered cyclic carbamate; otherwise, compound **3** could not be the main product. Based on the isolation and characterization of the reaction intermediates and the reaction products under various conditions, the deprotection mechanism of Cbz was proposed in Scheme 3.

2.2. Synthesis of kanamycin A dimers

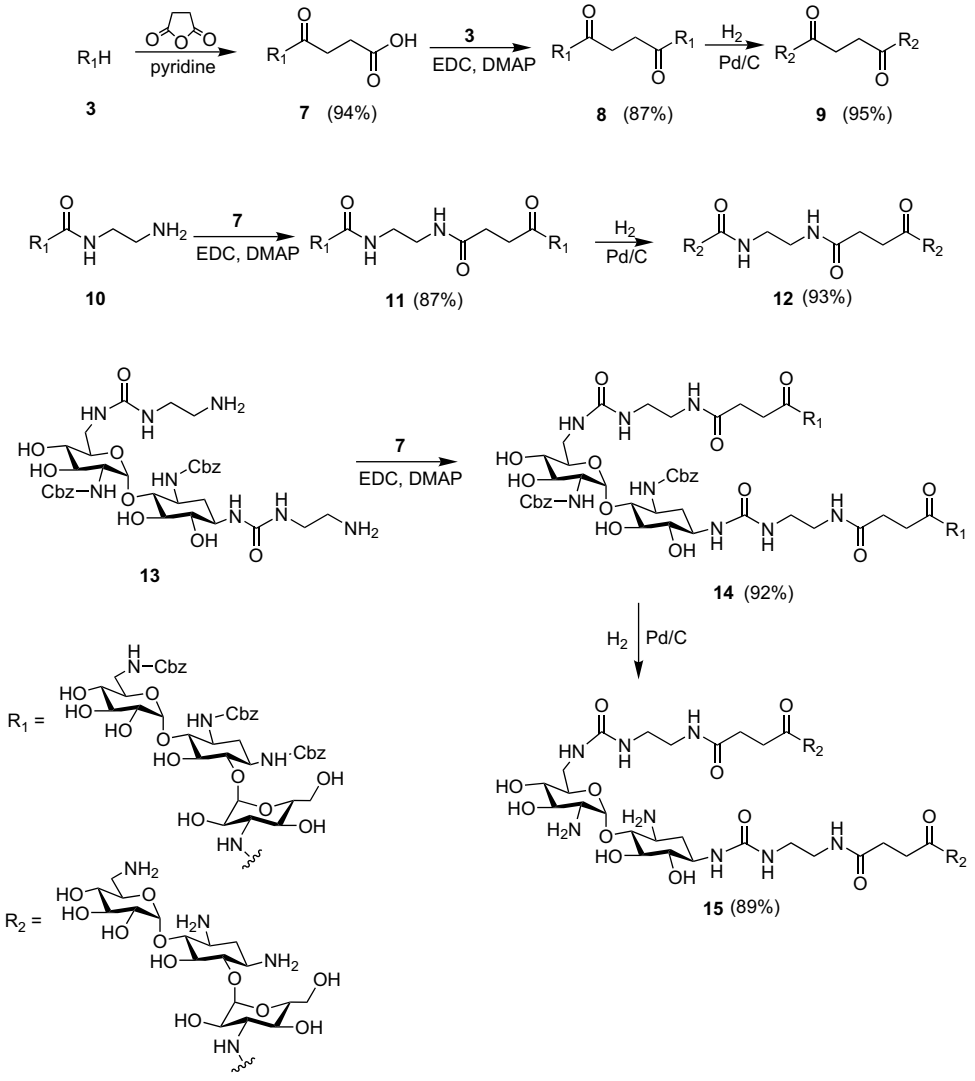
The derivatives obtained through the selective deprotection of the Cbz protecting groups could be used in the selective modification of aminoglycosides. A monomer that has a specific free



Scheme 2. The ring-opening reaction of the cyclic carbamate.



Scheme 3. The mechanism via a carbamate intermediate for the selective deprotection of aminoglycosides.



Scheme 4. Synthesis of dimers of kanamycin A.

amino group, such as compound **3**, is useful for the synthesis of various dimers linked at the nitrogen positions.

Specifically, compound **3** was converted to the corresponding carboxylic acid **7** through the reaction with succinic anhydride in 94% yield (Scheme 4). The coupling of the carboxylic acid **7** with compounds **3**, **10**, and **13**[14] in the presence of EDC/DMAP followed by deprotection with catalytic hydrogenolysis led to the formation of corresponding dimers **9**, **12**, and **15** in 83%, 81%, and 82% yields, respectively.

The new dimer analogs were tested for their ability to suppress the growth of different bacteria, including pathogenic and resistant strains, by measuring the inhibition rate at three concentrations (1 μm, 10 μm, and 100 μm). Aminoglycoside susceptible *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used as the standard reference strains and *E. coli* (440) was used as the resistant strain. And kanamycin A was used as the control.

The results showed that the dimer analogs at 100 μm concentration were active against the two standard strains, but they were not active against the resistant *E. coli* strain (Table 1).

Table 1
The bacteria growth inhibition rates of the aminoglycosides at 100 μm

Entry	Compound	*E. coli* (ATCC 25922)	*S. aureus* (ATCC 25923)	*E. coli* (440)
1	Kanamycin A	88.0	94.0	—
2	**9**	88.7	93.5	—
3	**12**	87.8	28.8	—
4	**15**	87.8	93.4	—

Although the prepared dimmers did not show any activity against the drug resistant *E. coli* strain 440 in our initial study, their activity against other drug resistant strains still needs to be determined. Clearly, there is a need to better understand the structural and functional requirements for the antibacterial activity of kanamycin A dimers. Studies toward this goal are in progress in our laboratories.

3. Conclusion

In conclusion, we developed a novel and effective approach for the synthesis of new kanamycin A dimers and other derivatives at the *N*-3″ position through the selective deprotection of Cbz-protected aminoglycosides. The deprotection mechanism was investigated through the separation and characterization of cyclic carbamate intermediates. Two homodimers of kanamycin A and one heterotrimer of neamine and kanamycin A were prepared with different dicarbonyl linker at the *N*-3″ position.

4. Experimental

4.1. General

Proton magnetic resonance spectra (including ^{1}H–^{1}H COSY, HMQC, and HMBC) were recorded using a Jeol 300 spectrometer. Chemical shifts were reported as parts per million (ppm) downfield from tetramethylsilane in δ unit, and coupling constants were given in cycles per second (Hz). ^{13}C spectra (including DEPT) were obtained using the Jeol 300 spectrometer at 75 Hz. Routine ^{13}C NMR spectra were fully decoupled by broad-band waltz decoupling. All NMR spectra were recorded at ambient temperature. High-resolution electronspray (ESI-HRMS) mass spectra were obtained from Bruker APEX IV-FTMS 7.0T mass spectrometer. Reactions were monitored by thin layer chromatography (TLC) on silica gel 60 F_{254}. Visualization was performed by ultraviolet light and/or by staining with ceric ammonium molybdate or ninhydrin. Preparative column chromatography was performed on silica gel 60. Chemical reagents and starting materials were purchased from Aldrich Chemical Co. or Acros Chemical Co. and were used without purification unless otherwise noted. Pyridine was dried over CaH_2 and kept under nitrogen.

4.1.1. General procedure for hydrogenolysis

To a solution or suspension of glycoside (0.1 mmol) in MeOH/H_2O (10 mL/2 mL), an HCl solution (1 M, 1 mL), and 10% Pd/C (50 mg) was added. The reaction mixture was treated with H_2 for 8 h under pressure (5.06 MPa) at room temperature. The catalyst was removed by filtration and the filtrate was concentrated in vacuum to obtain the desired product.

4.1.2. 1,3,6′-Tri-N-benzyloxycarbonyl-3″,2″-N,O-carbonyl-kanamycin A (*2*) and 1,3,6′-tri-N-benzyloxycarbonyl-kanamycin A (*3*)

To a solution of compound **1**[14] (1 g, 0.98 mmol) in dioxane/H_2O (100 mL/30 mL), a 1.5 M NaOH solution (30 mL) was added. The resulting mixture was stirred for 24 h at room temperature. After completion of the reaction (monitored by TLC, $CHCl_3$/MeOH/$NH_3\cdot H_2O$=4: 3: 1), an HCl solution (1 M) was added to adjust the pH to 2. After removal of the solvent, ice water was added and the solid was filtered. An NaOH solution (1 M) was added to the filtrate to adjust the pH to 9, and the resulting solid was filtered and separated by silica column chromatography ($CHCl_3$/MeOH/$NH_3\cdot H_2O$=12:2:0.2 and $CHCl_3$/MeOH/$NH_3\cdot H_2O$=5:2:0.2) to afford compound **3** as a white solid (0.54 g, 62%).

When the reacting time was shortened to 8 h, compound **2** could be detected and separated by silica column chromatography ($CHCl_3$/MeOH/$NH_3\cdot H_2O$=12:2:0.2) in a low yield (8.2%).

Compound **2**, ^{1}H NMR (DMSO-d_6, 300 MHz) 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 (DMSO-d_6, 75 MHz) 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.

Compound **3**, ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.07 (br s, 2H), 7.34 (s, 16H, Ph and N*H*CO_2), 7.04 (br s, 1H, N*H*CO_2), 5.31 (s, 2H), 5.13–4.89 (m, 11H), 4.35 (br s, 1H), 3.97 (br s, 1H), 3.81–3.73 (m, 2H), 3.30–2.92 (m, 7H), 1.83 (br s, 1H, H-2_{eq}), 1.53 (br s, 1H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 156.7, 155.9, 155.6, 137.3, 137.1, 128.3, 127.7, 99.9, 97.2, 82.5, 80.5, 74.3, 73.0, 72.4, 72.0, 70.0, 68.3, 67.0, 65.3, 65.0, 60.2, 56.4, 50.0, 49.5, 34.4; ESI-HRMS Calcd for $C_{42}H_{55}N_4O_{17}$ ($[M+H]^+$) *m*/*z* 887.3556, found 887.3561.

4.1.3. 1,3-Di-N-benzyloxycarbonyl-6′,4′-N,O-carbonyl-kanamycin A (*4*) and 1,3-di-N-benzyloxycarbonyl-kanamycin A (*5*)

To a solution of compound **1** (1 g, 0.98 mmol) in dioxane/H_2O (100 mL/30 mL), a 2.5 M NaOH solution (30 mL) was added. The resulting mixture was stirred for 24 h at 50 °C. After completion of the reaction (monitored by TLC, $CHCl_3$/MeOH/$NH_3\cdot H_2O$=4:3:1), an HCl solution (1 M) was added to adjust the pH to 10. After removal of the solvent, ice water was added. The resulting solid was filtered and separated by silica column chromatography ($CHCl_3$/MeOH/$NH_3\cdot H_2O$=8:2:0.2 and $CHCl_3$/MeOH/$NH_3\cdot H_2O$=6:3:1) to afford compound **5** as a white solid (0.63 g, 85%).

When the reaction was carried out at 30 °C for 12 h, compound **4** could be detected and separated by silica column chromatography ($CHCl_3$/MeOH/$NH_3\cdot H_2O$=8:2:0.2) as a white solid in a low yield (5%).

Compound **4**, ^{1}H NMR (DMSO-d_6, 300 MHz) 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 (DMSO-d_6, 75 MHz) 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.

Compound **5**, ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.34 (s, 11H, Ph), 7.19 (br s, 1H, N*H*CO_2), 5.52 (br s, 1H), 4.94–4.81 (m, 7H), 4.28–4.24 (m, 2H), 3.72 (br s, 1H), 3.05 (s, 4H), 2.79 (br s, 2H), 1.92–1.89 (m, 1H, H-2_{eq}), 1.40–1.33 (br s, 1H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.8, 155.6, 137.2, 136.9, 128.3, 127.8, 127.5, 127.1, 101.2, 97.5, 84.6, 80.1, 74.8, 72.9, 72.8, 72.7, 72.6, 72.5, 71.2, 69.5, 65.4, 65.2, 60.5, 55.1, 50.2, 49.5, 42.7, 34.9; ESI-HRMS Calcd for $C_{34}H_{49}N_4O_{15}$ ($[M+H]^+$) m/z 753.3189, found 753.3183.

4.1.4. 1,3,6′-Tri-N-benzyloxycarbonyl-kanamycin A-3″-N-succinamide (*7*)

To a solution of compound **3** (400 mg, 0.45 mmol) in pyridine/methanol (100 mL/20 mL), succinic anhydride (120 mg, 1.2 mmol) was added. The resulting mixture was stirred for 8 h at room temperature. After removal of the solvent, ice water (50 mL), and 1 M HCl (5 mL) was added. The resulting solid was filtered, washed with 0.02 M aqueous HCl to afford compound **7** as a white solid (418 mg, 94%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.73 (br s, 1H, N*H*CO), 7.33 (s, 17H, Ph and N*H*CO_2), 7.03 (br s, 1H, N*H*CO_2), 5.50 (br s, 1H), 5.00–4.94 (m, 8H), 3.85 (br s, 1H), 3.55–3.30 (m, 14H), 2.99 (br s, 1H), 2.35 (s, 4H), 1.85 (br s, 1H, H-2_{eq}), 1.48 (br s, 1H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 175.4, 172.8, 156.7, 155.9, 155.6, 137.3, 137.1, 136.9, 128.3, 127.7, 127.6, 101.3, 97.2, 84.5, 80.2, 74.1, 72.9, 72.4, 70.9, 70.2, 67.0, 65.3, 65.0, 60.1, 56.6, 50.0, 49.6, 34.5, 30.5; ESI-HRMS Calcd for $C_{46}H_{59}N_4O_{20}$ ($[M+H]^+$) m/z 987.3717, found 987.3751.

4.1.5. The protected dimer of kanamycin A linked with succinic acid (*8*)

To a solution of compounds **3** (72 mg, 0.081 mmol) and **7** (80 mg, 0.081 mmol) in pyridine (20 mL), DMAP(20 mg, 0.16 mmol), and EDC (100 mg, 0.64 mmol) was added. The resulting mixture was stirred for 8 h at room temperature. After removal of the solvent, ice water was added. The resulting solid was filtered, washed with 0.02 M aq HCl and recrystallized in $CHCl_3$/MeOH to afford compound **8** as a white solid (131 mg, 87%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.71 (br s, 2H, N*H*CO), 7.32 (s, 34H, Ph and N*H*CO_2), 7.06 (br s, 2H, N*H*CO_2), 5.00–4.90 (m, 16H), 4.47 (br s, 13H), 3.84 (br s, 2H), 3.55–3.31 (m, 16H), 3.00 (br s, 2H), 2.38 (s, 4H), 1.86 (br s, 2H, H-2_{eq}), 1.48 (br s, 2H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 172.6, 156.7, 155.9, 155.6, 137.3, 137.1, 136.9, 128.3, 127.7, 127.6, 101.3, 97.2, 84.5, 80.2, 74.2, 72.9, 72.4, 70.9, 70.1, 67.0, 65.7, 65.4, 65.0, 60.1, 56.5, 50.0, 49.6, 42.2, 34.5, 30.4; ESI-HRMS Calcd for $C_{88}H_{111}N_8O_{36}$ ($[M+H]^+$) m/z 1855.7095, found 1855.7159; Calcd for $C_{88}H_{110}N_8O_{36}Na$ ($[M+Na]^+$) m/z 1877.6915, found 1877.6912.

4.1.6. The dimer of kanamycin A linked with succinic acid (*9*)

The title compound was prepared from compound **8** according to the general procedure (12 h) as solid hexahydrochloride (95%).

^{1}H NMR (D_2O, 300 MHz) δ 5.14 (s, 2H), 4.90 (s, 2H), 3.76–3.08 (m, 30H), 2.90 (br s, 2H), 2.37 (s, 4H), 2.06–2.02 (m, 1H, H-2_{eq}), 1.37–1.33 (m, 1H, H-2_{ax}); ^{13}C NMR (D_2O, 75 MHz) δ 175.6, 100.6, 100.1, 85.6, 85.3, 74.3, 72.9, 72.8, 72.0, 71.7, 71.1, 69.5, 66.7, 60.2, 55.4, 50.8, 49.4, 40.3, 32.1, 31.1; ESI-HRMS Calcd for $C_{40}H_{75}N_8O_{24}$ ($[M+H]^+$) m/z 1050.4888, found 1050.4877.

4.1.7. The protected dimer of kanamycin A linked with nine atoms (*11*)

The title compound was prepared from compound **9**[14] according to the procedure for the preparation of compound **7**. Compound **11** was obtained in 89% yield.

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.94 (br s, 1H, N*H*CO), 7.70 (br s, 1H, N*H*CO), 7.32–7.30 (m, 34H, Ph and N*H*CO_2), 7.02 (d, J=8.7 Hz, 1H, N*H*CO_2), 6.87 (br s, 1H, N*H*CO_2), 5.08–4.74 (m, 32H), 3.82 (br s, 2H), 3.55–3.26 (m, 13H), 3.05 (br s, 4H), 2.73 (br s, 1H), 2.30 (br s, 4H), 1.84 (br s, 2H, H-2_{eq}), 1.45 (br s, 2H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 172.7, 171.7, 160.3, 156.7, 156.6, 155.9, 155.8, 155.6, 137.3, 137.2, 137.0, 128.4, 127.8, 127.6, 101.3, 101.1, 97.2, 84.6, 84.3, 80.2, 74.4, 74.1, 72.9, 72.7, 72.3, 70.7, 70.5, 70.2, 68.7, 67.0, 65.3, 65.0, 60.1, 56.6, 55.7, 50.2, 50.0, 49.6, 42.1, 41.7, 34.5, 30.4; ESI-HRMS Calcd for $C_{91}H_{117}N_{10}O_{37}$ ($[M+H]^+$) m/z 1941.7586, found 1941.7558.

4.1.8. The dimer of kanamycin A linked with nine atoms (*12*)

The title compound was prepared from compound **11** according to the general procedure (12 h) as solid hexahydrochloride (93%).

^{1}H NMR (D_2O, 300 MHz) δ 5.39 (s, 1H), 5.29 (s, 1H), 4.92 (s, 2H), 3.76–3.07 (m, 37H), 2.71 (br s, 1H), 2.36 (s, 6H), 1.79–1.75 (m, 2H, H-2_{ax}); ^{13}C NMR (75 MHz, D_2O) δ 175.9, 175.5, 161.3, 101.6, 100.9, 98.5, 95.8, 84.3, 84.0, 79.7, 78.5, 73.7, 73.4, 73.1, 72.9, 72.5, 71.8, 71.6, 71.3, 70.7, 68.6, 68.3, 65.8, 61.1, 60.2, 55.4, 55.2, 50.3, 50.2, 48.8, 48.0, 40.8, 39.9, 39.6, 31.5, 28.0; ESI-HRMS Calcd for $C_{43}H_{82}N_{10}O_{25}$ ($[M+2H]^{2+}$) m/z 569.2715, found 569.2720.

4.1.9. The protected dimer of kanamycin A linked with neamine (*14*)

To a solution of compounds **3** (116 mg, 0.12 mmol) and **13**[14] (45 mg, 0.059 mmol) in pyridine (20 mL), DMAP (30 mg, 0.24 mmol), and EDC (140 mg, 0.89 mmol) was added. The resulting mixture was stirred for 12 h at room temperature. After removal of the solvent, ice water was added. The resulting solid was filtered, washed with 0.02 M aq HCl, dissolved in DMF and crashed with H_2O to afford compound **14** as a white solid (146 mg, 92%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.93 (br s, 2H, N*H*CO), 7.69 (br s, 2H, N*H*CO), 7.32 (s, 44H, Ph and N*H*CO_2), 7.03 (br s, 2H, N*H*CO_2), 6.85 (br s, 2H, N*H*CO_2), 4.99–4.92 (m, 20H), 4.48 (br s, 22H), 3.82 (s, 2H), 3.55–3.29 (m, 18H), 3.02 (s, 8H), 2.71 (br s, 2H), 2.29 (br s, 8H), 1.84 (br s, 2H, H-2_{eq}), 1.45 (br s, 2H, H-2_{ax}); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 172.6, 171.5, 159.1, 158.3, 156.6, 156.2 (s, NHCO_2), 155.9, 155.6, 137.3, 137.1, 137.0, 128.3, 127.8, 127.6, 127.5, 101.3, 98.9, 97.1, 84.6, 81.9, 80.2, 76.8, 75.0, 74.1, 72.9, 72.5, 71.2, 70.8, 70.3, 70.1, 67.0, 65.3, 65.2, 65.0, 60.1, 56.5, 50.2, 50.0, 49.5, 42.1, 35.5, 35.0, 34.5, 30.8, 30.4; ESI-HRMS Calcd for $C_{126}H_{162}N_{16}O_{50}$ ($[M+2H]^{2+}$) m/z 1350.5385, found 1350.5548.

4.1.10. The dimer of kanamycin A linked with neamine (*15*)

The title compound was prepared from compound **14** according to the general procedure (24 h) as solid octahydrochloride (89%).

^{1}H NMR (300 MHz, D_2O) δ 5.50 (s, 1H), 5.29 (s, 2H), 4.93 (s, 2H), 3.72–3.34 (m, 43H), 3.05 (s, 8H), 2.70 (s, 2H), 2.36 (s, 10H), 2.36 (s, 10H), 2.10 (br s, 1H), 1.82–1.74 (m, 2H), 1.53–1.42 (m, 1H); ^{13}C NMR (75 MHz, D_2O) δ 175.9, 175.5, 161.1, 160.4, 101.0, 98.6, 97.3, 84.0, 80.5, 79.7, 76.1, 74.8, 73.5, 73.2, 73.0, 72.8, 72.6, 71.8, 71.6, 70.8, 70.7, 69.3, 68.7, 65.8, 60.2, 55.5, 54.4, 50.2, 49.6, 48.8, 43.3, 40.5, 40.0, 39.7, 31.8, 31.4, 28.2; ESI-HRMS Calcd for $C_{62}H_{116}N_{16}O_{34}$ ($[M+2H]^{2+}$) m/z 814.3909, found 814.3872.

Acknowledgments

We acknowledge the financial support from the National Basic Research Program (973 Program, Grant No. 2004CB518904).

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