JOC Note

Fully Orthogonally Protected 2-Deoxystreptamine from Kanamycin

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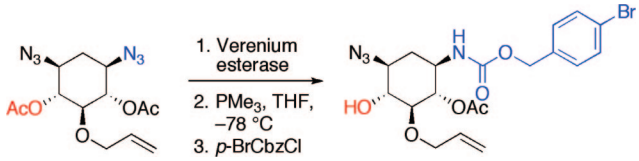
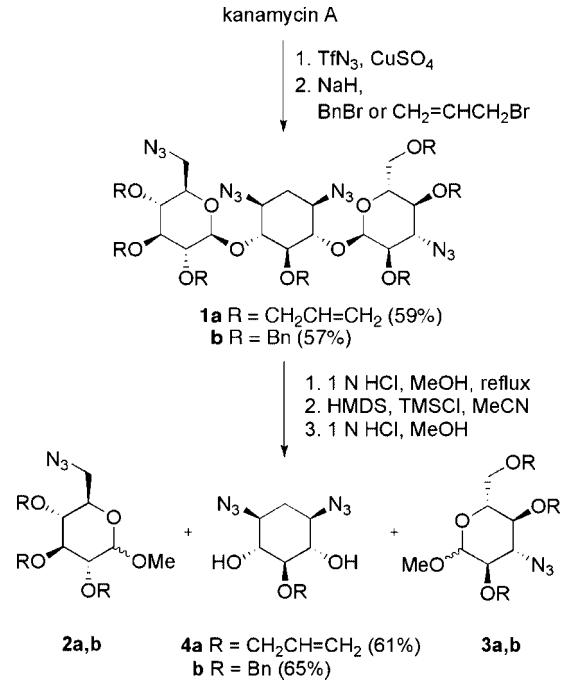


A fully orthogonally protected and enantiopure 2-deoxystreptamine derivative is prepared in a few straightforward steps from commercially available kanamycin. Resolution of a sterically hindered diacetate was effected by a Verenium esterase and was followed by a chemoselective Staudinger reduction−acylation protocol.

Since the discovery of streptomycin in 1944,[1] aminoglycosides find clinical use due to the bactericidal activity and low cost. The mode of action involves binding to the A-site of prokaryotic rRNA, thereby interfering with the fidelity of translation and ultimately causing bacterial cell death.[2] In addition, a number of aminoglycosides have been found to display specific interactions with viral RNA.[3] More recently, emergence of resistance and high human toxicity[4] has stimulated the development of new analogues of aminoglycosides devoid of such limitations.[5] The multiple hydroxy and amino functions, however, thwart a straightforward synthesis of structural analogues due to inevitable protecting group transformations.

SCHEME 1. Synthesis of *meso* 2-DOS Derivatives from Kanamycin



Consequently, a more practical strategy involves the preparation of analogues based solely on the central aminohexitol known as 2-deoxystreptamine (2-DOS). The fundamental role of 2-DOS in binding to RNA[6] makes it a privileged scaffold for development of active structural analogues of the natural aminoglycosides.

Despite appreciable synthetic effort,[7] the most efficient route toward 2-DOS remains acidic degradation of neomycin.[8] However, *meso* 2-DOS thus obtained requires multiple protecting group manipulations before incorporation in novel RNA ligands is ensured. Recently we reported the synthesis of a versatile enantiopure 2-DOS building block by stepwise degradation of neomycin.[9] Herein, we describe a route toward fully orthogonally protected and enantiopure 2-DOS from kanamycin A, a member of the 4,6-substituted aminoglycosides.

Because kanamycin is a 2-DOS 4,6-linked aminoglycoside, it can be conveniently converted into selectively 5-*O*-protected 2-DOS via global protection followed by hydrolysis of the glycosidic bond (Scheme 1). Thus, Cu-catalyzed diazotransfer[10] on kanamycin A was followed by global *O*-allylation and benzylation, to give the fully protected derivatives **1a** and **1b**, respectively, in good yields. Next, acidic hydrolysis of glycosidic bonds of **1a** (1 N HCl, MeOH, reflux) gave an anomeric mixture

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(1) Schatz, A.; Waksman, S. A. *Proc. Soc. Exp. Biol. Med.* **1944**, *55*, 244.
(2) (a) Beaucaire, G. *J. Chemother.* **1995**, *7* (suppl 2), 111. (b) Zhao, F.; Zhao, Q.; Blount, K. F.; Han, Q.; Tor, Y.; Hermann, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 447. (c) Magnet, S.; Blanchard, J. S. *Chem. Rev.* **2005**, *105*, 477.
(3) (a) Zapp, M. L.; Stern, S.; Green, M. R. *Cell* **1993**, *74*, 969. (b) Mei, H. Y.; Cui, M.; Heldsinger, A.; Lemrow, S. M.; Loo, J. A.; Sannes-Lowery, K. A.; Sharmeen, L.; Czarnik, A. W. *Biochemistry* **1998**, *37*, 1420.
(4) (a) Zembower, T. R.; Noskin, G. A.; Postelnick, M. J.; Nguyen, C.; Peterson, L. R. *Int. J. Antimicrob. Agents* **1998**, *10*, 95. (b) Nagai, J.; Takano, M. *Drug Metab. Pharmacokinet.* **2004**, *19*, 159.
(5) (a) Rai, R.; Chen, H. N.; Czyryca, P. G.; Li, J.; Chang, C. W. T. *Org. Lett.* **2006**, *8*, 887. (b) Wang, J.; Li, J.; Chen, H. N.; Chang, H.; Tanifum, C. T.; Liu, H. H.; Czyryca, P. G.; Chang, C. W. T. *J. Med. Chem.* **2005**, *48*, 6271. (c) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Risen, L.; Griffey, R. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 3409.
(6) (a) Yoshizawa, S.; Fourmy, D.; Eason, R. G.; Puglisi, J. D. *Biochemistry* **2002**, *41*, 6263. (b) Liu, X.; Thomas, J. R.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434.
(7) Busscher, G. F.; Rutjes, F. P. J. T.; van Delft, F. L. *Chem. Rev.* **2005**, *10*, 775.
(8) Georgiadis, M. P.; Constantiou-Kokotou, V.; Kokotos, G. *J. Carbohydr. Chem.* **1991**, *10*, 739.
(9) van den Broek, S. A. M. W.; Gruyters, B. W. T.; Rutjes, F. P. J. T.; van Delft, F. L.; Blaauw, R. H. *J. Org. Chem.* **2007**, *72*, 3577.
(10) Nyffeler, P. T.; Liang, C. H.; Koeller, K. M.; Wong, C. H. *J. Am. Chem. Soc.* **2002**, *124*, 10773.

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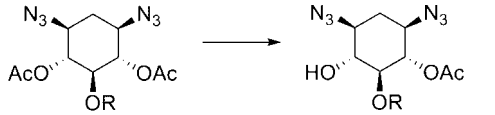
TABLE 1. Enzymatic Desymmetrization of *meso* Diacetates 5a,b

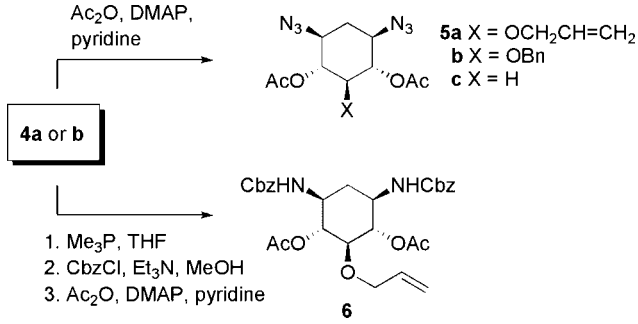


5a R = $CH_2CH{=}CH_2$ **7a** R = $CH_2CH{=}CH_2$
b R = Bn **b** R = Bn

entry	substrate	enzyme[a]	time	conversion	yield (%)	ee (%)[b]
1	**5a**	Verenium 1	6	73		
2	**5a**	Verenium 2	6	36		
3	**5a**	Verenium 3	6	nd[c]		
4	**5a**	Verenium 4	6	19		
5	**5a**	Verenium 5	2	95	92	>99
6	**5a**	Verenium 6	6	100		
7	**5b**	Verenium 5	7	40	22	
8	**6**	Verenium 5	10			

[a] Enzyme, phosphate buffer pH 7.5, MeCN, 37 °C. [b] Determined by chiral HPLC. [c] Mixture of monoacetate, diacetate, and diol.

SCHEME 2. Synthesis of *meso* Compounds 4a and 4b



of methyl glycosides **2a** and **3a** along with the desired 5-*O*-protected 2-DOS **4a**. However, separation of **4a** from the unwanted methyl glycosides **2a** and **3a** was thwarted as a result of near identical retention times on silica. Fortunately, time-consuming chromatographic separation could be avoided by transient trimethylsilylation of the free hydroxyls of 5-*O*-protected **4a** using hexamethyldisilazane (HMDS) and TMSCl. After separation, rapid and quantitative acidic hydrolysis of silyl ethers gave **4a** in pure form. Thus, 5-*O*-allyl-protected 2-DOS **4a** was obtained from kanamycin A in five chemical steps in 36% overall yield. A similar sequence of events gave benzylated compound **4b**. Since compounds **4a** and **4b** are *meso*, enzymatic pathways were explored to convert **4a** and **4b** into building blocks suitable for the assembly of enantiopure aminoglycoside analogues. For reasons of comparison, the 5-deoxy derivative **5c** was also prepared by a known route.[11]

A well-established technology for the desymmetrization of diols involves enzymatic hydrolysis.[12] To this end, **4a** and **4b** were acetylated to **5a** and **5b** and an alternative substrate for enzymatic desymmetrization, compound **6**, was obtained by Staudinger reduction, followed by Cbz-protection and acetylation. Initially, a number of commercially available esterases and lipases were investigated, but none of the enzymes demonstrated hydrolytic activity on **5a** or **5b**. The lack of reactivity is likely due to steric hindrance of the 5-*O*-allyl group, since enzymatic conversion of the analogous 2,5-dideoxy derivative **5c** took place

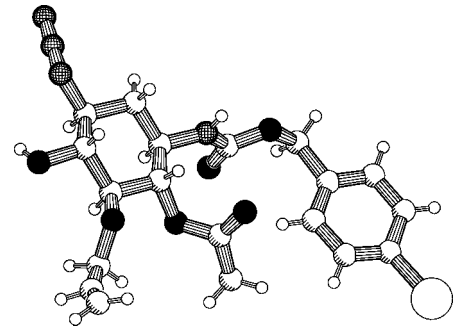


FIGURE 1. Representation of the Crystal Structure of **9**.[17]

rapidly. We next screened a set of esterases containing the GGG(A)X-motif,[13] specifically selected for the capacity to hydrolyze sterically hindered substrates, as provided by Verenium Corporation. Out of 17 enzymes, six were able to convert **5a** and **5b**, although four esterases (entries 1, 2, 4, 6) hydrolyzed both acetate groups and one esterase (entry 3) gave a mixture of monoacetate, diacetate, and diol.

To our delight, in a mixture of acetonitrile and phosphate buffer (pH 7.5, 37 °C) Verenium esterase 5 was able to hydrolyze a single acetate group of substrate **5a** with more than 95% conversion and 92% isolated yield. The resulting monoacetate **7a** was formed in excellent enantiomeric excess as judged by HPLC. Reaction of the same enzyme with more sterically hindered benzylated substrate **5b** (entry 7) gave only 22% isolated yield. In addition, with substrate **6** less than 10% conversion was detected, further supporting the role of steric factors. Further attempts to optimize the reaction conditions, i.e., variation in temperature, pH, or cosolvents, were not successful. Finally, attempts to perform asymmetric acylation of **4a** by subjection to Verenium esterase 5 in neat vinyl acetate were fruitless; no conversion was detected. At this point, the absolute configuration of **7a** was established as depicted in Table 1 by comparison of optical rotation with a sample prepared by a degradative route from D-neamine (see Supporting Information).

To arrive at fully orthogonally protected 2-DOS, differentiation between the two amines is required. A chemoselective azide reduction[10,14] was attempted. Thus, subjection of **7a** to a one-pot Staudinger reduction−Boc protection[15] afforded compound **8** in 40% yield (Scheme 3).

By the same sequence of events, additional proof of the absolute configuration of **8** was obtained by X-ray crystallography. To this end, the aza-ylide intermediate resulting from selective azide reduction was treated with *p*-BrCbzCl, prepared by treating *p*-bromobenzyl alcohol with diphosgene,[16] to give *p*-BrCbz-protected amine **9** in 27% yield (Scheme 3). Crystallization from MeCN followed by X-ray diffraction gave the structure depicted in Figure 1.

In conclusion, a fully orthogonally protected and enantiopure 2-DOS derivative was prepared in eight steps and 12% overall yield from kanamycin. Desymmetrization of the sterically hindered *meso* diol by enzymatic resolution was achieved by a

(11) Seeberger, P. H.; Baumann, M.; Zhang, G. T.; Kanemitsu, T.; Swayze, E. E.; Hofstadler, S. A.; Griffey, R. H. *Synlett* **2003**, *9*, 1323.

(12) (a) Drauz, K.; Waldmann, H. *Enzyme Catalysis in Organic Synthesis: A Comprehensive Handbook*; Wiley-VCH: Weinheim, Germany, 2002. (b) Chenevert, R.; Jacques, F *Tetrahedron: Asymmetry* **2006**, *17*, 1017.

(13) (a) Kourist, R.; Krishna, S. H.; Patel, J. S.; Bartnek, F.; Hitchman, T. S.; Weiner, D. P.; Bornscheuer, U. T. *Org. Biomol. Chem.* **2007**, *5*, 3310.

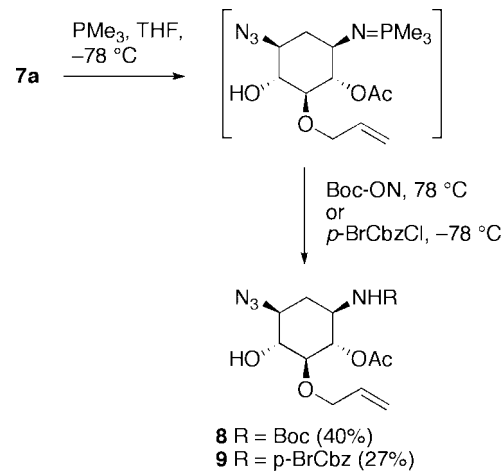
(14) Li, J.; Chen, H. N.; Chang, H.; Wang, J.; Chang, C. W. T. *Org. Lett.* **2005**, *7*, 3061.

(15) Ariza, X.; Urpí, F.; Viladomat, C.; Vilarrasa, J. *Tetrahedron Lett.* **1998**, *39*, 9101.

(16) Waterfield, W. R. *J. Am. Chem. Soc.* **1963**, *85*, 2731.

(17) Spek, A. L. *J. Appl. Crystallogr.* **2003**, *36*, 7.

SCHEME 3. Chemoselective Azide Reduction



Verenium esterase. Finally, chemoselective azide reduction/Boc-protection led to the first fully orthogonally protected 2-DOS based building block, suitable for the synthesis of aminoglycoside type RNA-binding ligands.

Experimental Section

1, 3,6′,3″-Tetraazido-1,3,6′,3″-tetradeaminokanamycin. To a solution of NaN_3 (53.5 g, 0.82 mol) in a mixture of H_2O/CH_2Cl_2 (270 mL, 1:1 v/v) at 0 °C was added Tf_2O (68.3 mL, 0.41 mol, 1.5 equiv/NH_2 based on 50% conversion). The reaction mixture was stirred at room temperature for 2 h. After quenching with aqueous $NaHCO_3$, the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (200 mL). The organic layers were combined to afford 335 mL of TfN_3 solution. Then, to a solution of kanamycin (20 g, 34.3 mmol) and $CuSO_4$ (219 mg) in H_2O (335 mL) were added the TfN_3 solution, MeOH (1.1 L), and Et_3N (43 mL). The reaction mixture was stirred overnight at room temperature. Then solid $NaHCO_3$ (35 g) was added carefully, and the organic solvents were evaporated. The aqueous residue was extracted with EtOAc (5 × 300 mL), and the organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo to give yellow oil. Purification by column chromatography (pure EtOAc to 10% MeOH/EtOAc) afforded the tetraazidoderivative of kanamycin (17 g, 84%) as a colorless oil. R_f 0.44 (MeOH/EtOAc, 1/9). ^{1}H NMR (MeOD, 400 MHz) δ 5.24(d, J = 3.8 Hz, 1H), 5.18 (d, J = 3.8 Hz, 1H), 4.08−3.98 (m, 2H), 3.78−3.28 (m, 15H), 2.33 (dt, J = 4.2, 12.6 Hz, 1H), 1.56 (q, J = 12.4 Hz, 1H). ^{13}C NMR (MeOD, 300 MHz) δ 100.4, 98.0, 83.0, 80.2, 73.8, 72.9, 71.9, 71.5, 70.4, 70.1, 67.9, 66.4, 60.0, 58.8, 50.8, 31.5. HRMS (ESI) *m/z* calcd for $C_{18}H_{28}N_{12}O_{11}$ $(M + Na)^+$ 611.201, found 611.193.

1,3-Diazido-1,3-dideamino-5-*O*-allyl-2-deoxystreptamine (4a). Acetyl chloride (1.5 mL) was added to MeOH (15 mL) at 0 °C. The obtained 1 N HCl/MeOH was added to **1a** (200 mg, 0.23 mmol), and the reaction mixture was refluxed overnight. The reaction was then quenched with $NaHCO_3$, concentrated, and extracted with EtOAc. The organic layer was washed with water and brine, then dried (Na_2SO_4), and concentrated in vacuo. Crude product was treated with 5 equiv of HMDS and catalytic trimethylsilyl chloride in acetonitrile. After purification by column chromatography (EtOAc/heptane, 1:15) the resulting bis-TMS-ether was treated with 1 N HCl in MeOH to afford pure **4a** as a white solid (36 mg, 61%). R_f 0.14 (EtOAc/heptane, 1/3). Mp 85−87 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.01−5.86 (m, 1H), 5.28 (qdd, J = 1.2, 1.4, 10.36, 21.2 Hz, 2H), 4.35 (m, 2H), 4.18 (dt, J = 1.4, 5.6 Hz, 2H), 3.50−3.34 (m, 4H), 3.15 (t, J = 8.9 Hz, 1H), 2.20 (dt, J = 4.6, 12.9 Hz, 1H), 1.38 (dt, J = 12.6, 13.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 134.1, 117.3, 82.5, 75.5, 73.9, 59.8.5, 31.3. HRMS (ESI) *m/z* calcd for $C_9H_{14}N_6O_3$ $(M - H)^-$ 253.1049, found 253.1059.

1,3-Diazido-1,3-dideamino-5-*O*-benzyl-2-deoxystreptamine (4b). Prepared by an analogous procedure with benzyl bromide instead of allyl bromide to afford pure **4b** as a white solid (32 mg, 65%). R_f 0.11 (EtOAc/heptane, 1:3). Mp 135−136 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.49−7.28 (m, 5H), 4.83 (s, 2H), 3.51−3.31 (m, 4H), 3.24 (t, J = 7.8 Hz, 1H), 2.19 (dt, J = 13.2, 3.9 Hz, 1H), 1.27 (dt, J = 13.2, 12.9 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 128.3, 127.8, 127.5, 82.7, 75.0, 59.8, 31.3. HRMS (ESI) *m/z* calcd for $C_{13}H_{16}N_6O_3$ $(M + Na)^+$: 327.1182, found: 327.1177.

1,3-Diazido-1,3-dideamino-5-*O*-allyl-4,6-di-*O*-acetyl-2-deoxystreptamine (5a). To a solution of **4a** (205 mg) in pyridine (10 mL) was added DMAP (catalytic) and acetic anhydride (1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for two hours. Solvents were evaporated under vacuum and residue was purified by column chromatography (EtOAc/heptane, 1:4) to afford **5a** (235 mg, 86%) as a white solid. R_f 0.28 (EtOAc/heptane, 1:3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.75 (ddt, J = 17.3, 10.4, 5.7 Hz, 1H), 5.17 (qdd, J = 1.2, 1.4, 10.3, 13.2, 2H), 5.01 (t, J = 9.9 Hz, 2H), 4.07 (dt, J = 1.4, 5.6 Hz, 2H), 3.45 (m, 3H), 2.28 (dt, J = 13.3, 4.6 Hz, 1H), 2.13 (s, 6H), 1.54 (dt, J = 13.2, 12.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 168.9, 133.4, 116.7, 78.5, 74.2, 73.1, 57.8, 31.4, 20.3. HRMS (ESI) *m/z* calcd for $C_{13}H_{18}N_6O_5$ $(M + Na)^+$ 361.1236, found 361.1250.

1,3-Diazido-1,3-dideamino-5-*O*-benzyl-4,6-di-*O*-acetyl-2-deoxystreptamine (5b). Prepared by an analogous procedure from **4b** to afford **5b** (935 mg, 81%) as a white solid. R_f 0.25 (EtOAc/heptane, 1:3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.38−7.18 (m, 5H), 5.06 (t, J = 9.9 Hz, 2H), 4.60 (s, 2H), 3.52−3.39 (m, 3H) 2.29 (dt, J = 13.5, 4.1 Hz, 1H), 2.02 (s, 6H), 1.57 (dt, J = 13.0, 12.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 168.9, 128.0, 127.4, 127.2, 79.0, 74.3, 57.8, 31.3, 20.2. HRMS (ESI) *m/z* calcd for $C_{17}H_{20}N_6O_5$ $(M + Na)^+$ 411.151, found 411.136.

General Procedure for Enzymatic Resolution. To a solution of a diacetate **5** or **6** (0.088 mmol) in CH_3CN (200 μL) was added phosphate buffer (pH 7.5, 2 mL) and a Verenium enzyme (20 mg). The reaction mixture was shaken for 48 h at 37 °C and then filtered over Celite (flushed with excess of EtOAc). Solvents were evaporated under vacuum, and the residue was purified by column chromatography (EtOAc/heptane, 1:8 to 1:5). HPLC-analysis was performed on a CHIRALCEL OD-H column using racemic compound as reference (column CHIRALCEL OD-H, hexane/*i*-PrOH (9:1), flow rate of 1 mL/min).

(1*R*,3*S*,4*R*,5*S*,6*S*)-1-*N*-Boc-3-azido-3-deamino-5-*O*-allyl-6-*O*-acetyl-2-deoxystreptamine (8). Compound **7a** (20 mg, 0.067 mmol) was dissolved in THF (1 mL) and cooled to −78 °C. To this cooled solution was added 1 M THF solution of PMe_3 (67 μL, 0.067 mmol), and the reaction mixture was stirred at −78 °C for 5 min. The reaction was warmed to room temperature and stirred for 2 h. The reaction was then quenched with Boc-ON (41 mg, 0.167 mmol) and stirred for 4 h. Solvents were then evaporated under vacuum, and the residue was purified with column chromatography (EtOAc/heptane, 1/3) to afford **8** (10 mg, 40%) as a white solid. R_f 0.17 (EtOAc/heptane, 1:3). Mp 138−139 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.85 (m, 1H), 5.22 (qdd, J = 1.25, 1.43, 10.37, 16.44 Hz, 2H), 4.73 (dd, J = 9.5, 10.6 Hz, 1H), 4.20 (ddd, J = 1.55, 2.97, 5.80 Hz, 2H), 3.41 (m, 3H), 2.7 (d, J = 2.36 Hz, 1H), 2.26 (dt, J = 4.20, 12.83 Hz, 1H) 2.09 (s, 3H), 1.41 (s, 9H). 1.28 (m, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 170.6, 164.7, 133.8. 117.0, 80.8, 79.5, 76.7, 74.8, 73.7, 69.6, 48.8, 33.0, 27.7, 20.0. HRMS (ESI) *m/z* calcd for $C_{16}H_{26}N_4O_6$ $(M + Na)^+$ 393.191, found 393.177. $[\alpha]^{20}_D$ +1.6 (EtOAc). HRMS (ESI) *m/z* calcd for $C_{16}H_{26}N_4O_6$ $(M + Na)^+$ 393.1750, found 393.1770.

(1*R*,3*S*,4*R*,5*S*,6*S*)-1-*N*-(4-BrCbz)-3-Azido-3-deamino-5-*O*-allyl-6-*O*-acetyl-2-deoxystreptamine (9). Prepared by an analogous manner from **7a** by quenching with *p*-bromobenzyloxycarbonyl chloride (17 mg, 0.072 mmol) instead of BOC-ON to afford **9a** (9 mg, 27%) as a white crystalline solid. R_f 0.17 (EtOAc/heptane, 1:3). Mp 127−128 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.2 Hz, 2H), 5.86 (tdd, J = 5.6, 10.3, 17.1

Hz, 1H), 5.21 (qdd, $J = 1.2, 1.4, 10.3, 15.1$ Hz, 2H), 5.01 (m, 2H), 4.74 (dd, $J = 9.4, 10.7$ Hz, 1H), 4.20 (ddd, $J = 1.4, 2.7, 5.8$ Hz, 2H), 3.28−3.57 (m, 3H), 2.65 (d, $J = 1.8$ Hz, 1H), 2.30 (dt, $J = 3.9, 12.8$ Hz, 1H), 1.9 (s, 3H), 1.31 (dd, $J = 12.4, 25.1$ Hz, 1H). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 133.7, 131.2, 129.2, 117.0, 80.7, 75.7, 74.5, 73.8, 65.6, 59.4, 49.7, 32.9, 20.3. HRMS (ESI) *m*/*z* calcd for $C_{19}H_{23}N_4O_6Br$ $(M + Na)^+$ 505.0699, found 505.0691.

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Supporting Information Available: General procedures, synthesis, and characterizations of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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