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A Short and Scalable Route to Orthogonally *O*-Protected 2-Deoxystreptamine

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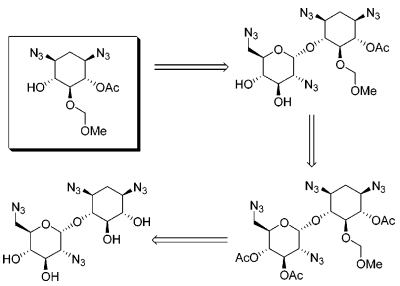

A seven-step synthesis of orthogonally *O*-protected 2-deoxystreptamine has been developed from readily available neomycin, with an overall yield of 28%. Key chemical transformations include a chemoselective glycosidic bond hydrolysis and two regioselective protective group manipulations involving acetylation and deacetylation. The synthetic route is amenable to scale-up for the production of multigram quantities of enantiopure and orthogonally *O*-protected 2-deoxystreptamine, a versatile scaffold for the generation of libraries of RNA-targeting ligands.

Aminoglycosides form a diverse class of naturally occurring substances with strong bactericidal activity. The antibacterial effect is the result of a specific binding of aminoglycosides to the 16S tRNA-acceptor site in prokaryotic ribosomal RNA, resulting in the production of non-sense proteins due to extensive misreading of mRNA.[1] As a consequence, several members of the aminoglycoside family, most notably gentamicin, have been applied as clinically important antibiotics for more than 60 years now. However, the toxicity, growing bacterial resistance, and poor oral bioavailability of most aminoglycosides[2] creates the need for new structures with antibiotic activity. Apart from that, the generation of new aminoglycoside-type structures is interesting from a broader perspective because aminoglycosides show rather promiscuous binding to A-form RNA in general.[3] For example, neomycin was found to compete with the Rev protein for HIV RRE, resulting in attenuated HIV replication in tissue culture cells.[4] Moreover, aminoglycosides also act as inhibitors of several ribozymes, including hammerhead, group I introns, and RNase P.[5] The molecular structure of aminoglycosides is characterized by a highly conserved diaminocyclohexitol termed 2-deoxystreptamine and suggests an essential role in binding to RNA. Such an assumption is corroborated by NMR studies that reveal substantial hydrogen bonding between 2-deoxystreptamine and RNA,[6] but it has also been shown that deoxystreptamine alone shows sequence-specific binding to major groove RNA[7] or in dimeric form to RNA hairpin loops.[8] Thus, it may be postulated that 2-deoxystreptamine can serve as a key building block in the development of new high-affinity RNA-targeting compounds for the development of antibiotics[9] or novel drugs against pathogenic viruses such as HIV or hepatitis.

Throughout the years, a large number of preparations of (protected) 2-deoxystreptamine and direct analogues thereof have been published.[10] Nevertheless, to date the simplest way to obtain 2-deoxystreptamine (**1**) is by acidic degradation of neomycin (Scheme 1). Treatment of neomycin, readily available by *Streptomyces* fermentation, with 50% aqueous HBr at elevated temperatures affords 2-deoxystreptamine in a single transformation.[11]

It must be noted, however, that the fully unprotected 2-deoxystreptamine **1** may be easily obtained, but it is a meso compound and contains only equatorial functional groups that are difficult to differentiate. The latter features limit the potential of raw 2-deoxystreptamine as starting material for the synthesis of novel RNA-binding constructs, despite the fact that several synthetic procedures toward enantiopure 2-deoxystreptamine derivatives have been developed, including chemical[12−14] and enzymatic[12,15] approaches. However, the chemical routes often proved to be laborious and low-yielding, and enzymatic

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(1) Beaucaire, G. *J. Chemother*. **1995**, *7* (suppl. 2), 111.

(2) Zembower, T. R.; Noskin, G. A.; Postelnick, M. J.; Nguyen, C.; Peterson, L. R. *Int. J. Antimicrob. Agents* **1998**, *10*, 95.

(3) Arya, D. P.; Xue, L.; Willis, B. *J. Am. Chem. Soc.* **2003**, *125*, 10148.

(4) Van Ahsen, G.; Umemura, M.; Yokota, M. *Nature* **1991**, *353*, 368.

(5) (a) Schroeder, R.; Waldsich, C.; Wank, H. *EMBO J.* **2000**, *19*, 1. (b) Vourekas, A.; Kalavrizioti, D.; Stathopoulos, C.; Drainas, D. *Mini-Rev. Med. Chem.* **2006**, *6*, 971.

(6) (a) Yoshizawa, S.; Fourmy, D.; Puglisi, J. D. *EMBO J.* **1998**, *17*, 6437. (b) Hobbie, S. N.; Pfister, P.; Brüll, C.; Westhof, E.; Böttger, E. C. *Antimicrob. Agents Chemother.* **2005**, *49*, 5112.

(7) Yoshikawa, S.; Fourmy, D.; Eason, R. G.; Puglisi, J. D. *Biochemistry* **2002**, *41*, 6263.

(8) Liu, X.; Thomas, J. R.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2004**, *126*, 9196.

(9) (a) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Griffey, R. H. *Org. Lett.* **2001**, *3*, 1621. (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) Vourloumis, D.; Winters, G. C.; Simonsen, K. B.; Ayida, B. K.; Shandrick, S.; Zhao, Q.; Hermann, T. *ChemBioChem* **2003**, *4*, 879. (d) Ding, Y.; Hofstadler, S. A.; Swayze, E. E.; Griffey, R. H. *Chem. Lett.* **2003**, *32*, 908.

(10) For a recent review, see: Busscher, G. F.; Rutjes, F. P. J. T.; van Delft, F. L. *Chem. Rev.* **2005**, *105*, 775.

(11) Georgiadis, M. P.; Constantiou-Kokotou, V.; Kokotos, G. *J. Carbohydr. Chem.* **1991**, *10*, 739.

(12) Canas-Rodriguez, A.; Ruizpoveda, S. G. *Carbohydr. Res.* **1977**, *59*, 240.

(13) Greenberg, W. S.; 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.

(14) Tona, R.; Bertolini, R.; Hunziker, J. *Org. Lett.* **2000**, *12*, 1693.

(15) Orsat, B.; Alper, P. B.; Moree, W.; Mak, C-P.; Wong, C-H. *J. Am. Chem. Soc.* **1996**, *118*, 712.

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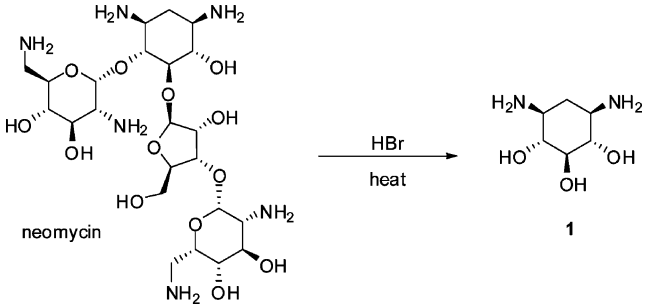
SCHEME 1



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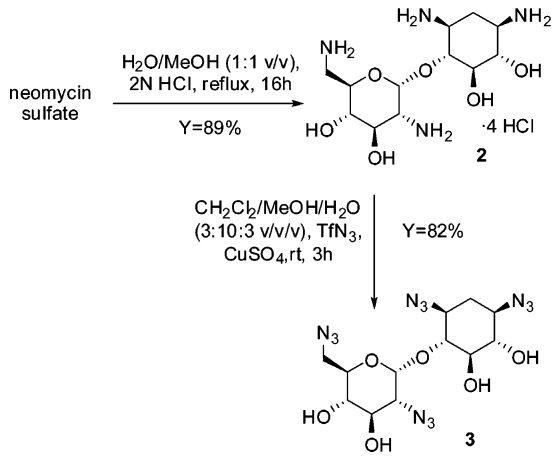


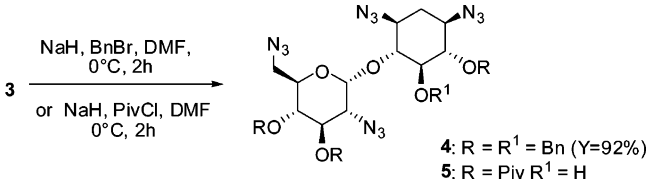
processes imposed restrictions on the protecting groups. Furthermore, the 2-deoxystreptamine derivatives obtained via the aforementioned methods were not fully orthogonally protected.

To overcome these drawbacks, a number of de novo syntheses of 2-deoxystreptamine have appeared in literature over the years.[10,16,17] Only on few occasions were enantiopure products obtained though and several times with impractical protective groups on the nitrogen atoms.

For these reasons, we set out to develop a practical and scalable route toward a versatile 2-deoxystreptamine scaffold with a suitable protective group pattern. We now wish to report a short and straightforward synthetic sequence for the multigram preparation of enantiomerically pure orthogonally *O*-protected 2-deoxystreptamine.

The first step in our route was the selective hydrolysis of commercially available neomycin sulfate to neamine (**2**, Scheme 2). Although a literature protocol for such a transformation has been published,[18] modification of the protocol proved to be necessary, since the yield of the original procedure significantly dropped upon scale-up. In particular we found the addition of H_2O to the MeOH suspension highly beneficial to give, after concentration and precipitation from MeOH, neamine in 89% yield on a multigram scale. Subsequent transformation of the amino groups into azides via diazotransfer[19] with freshly prepared triflyl azide afforded **3** in good yield.

SCHEME 3



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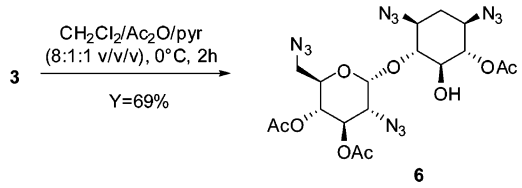


In order to efficiently arrive at the desired *O*-orthogonally protected 2-deoxystreptamine, we then investigated whether a selective protection of the hydroxyl functionalities on either the 4- or 5-position of the 2-deoxystreptamine moiety could be achieved. It is known that reactivity of these alcohols differs significantly,[19] and therefore tetraol **3** was subjected to a range of protection reactions. Application of standard benzylation conditions (NaH, benzyl bromide, DMF) led to the fully protected neamine derivative **4** in excellent yield (Scheme 3). Protection with pivaloyl chloride gave mixtures of mono-, di-, tri-, and tetra-*O*-acylated products, with the tri-*O*-pivaloylated compound **5** predominating. Difficulties during purification made this reaction impossible to implement in our intended synthetic route, but the outcome encouraged us to look further into a selective tri-*O*-acylation protocol.

Dissolving **3** in a 1:1 mixture of acetic anhydride and pyridine and addition of a catalytic amount of DMAP led to the formation of a tetra-*O*-acetylated neamine derivative. However, when the reaction was performed in dichloromethane in the absence of DMAP, a mixture of tri- and tetraacetates was obtained. Optimization of the reaction conditions eventually led to a ratio of 5:1 in favor of the desired tri-*O*-acetylated product **6**, isolated in good yield after purification by chromatography (Scheme 4).

With compound **6** in hand, protection of the remaining 5-hydroxyl and hydrolysis of the α-glucopyranosyl bond were the only remaining transformations required to arrive at *O*-orthogonally protected 2-deoxystreptamine. To this end, benzylation of the alcohol was attempted, but upon deprotonation with NaH or LiHMDS an unexpected acetyl shift occurred, leading to a 1:1 mixture of the 5- and 6-*O*-acetylated product. To circumvent the base-catalyzed acetyl migration, introduction of the methoxymethyl (MOM) protective group was undertaken, since much milder conditions are required for its installation. Thus, protection of the alcohol using MOMCl in the presence of DIPEA as a base proceeded uneventfully to afford **7** in 72% yield (Scheme 5). At this point, it was recognized that MOM-protection can also be effected under acidic conditions. Much to our satisfaction, we found that the yield of **7** could be further improved to 91% by applying a procedure involving dimethoxymethane and P_2O_5.[20]

Next, in order to arrive at the desired 2-deoxystreptamine derivative, a variety of hydrolytic conditions was investigated for the selective cleavage of the glycosidic bond with respect to the MOM protective group. However, treatment of **7** with a

(16) Busscher, G. F.; Groothuys, S.; De Gelder, R.; Rutjes, F. P. J. T.; van Delft, F. L. *J. Org. Chem.* **2004**, *69*, 4477.

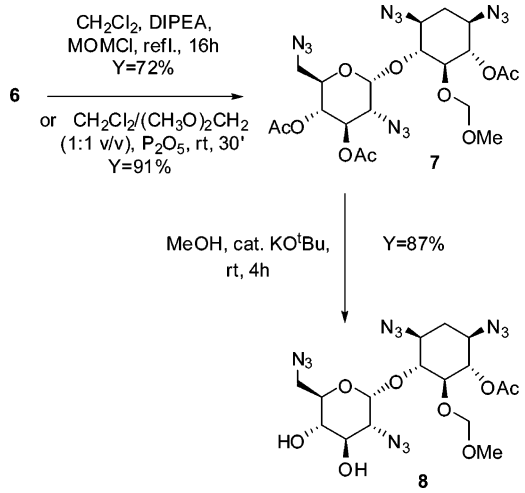
(17) Busscher, G. F.; Rutjes, F. P. J. T.; van Delft, F. L. *Tetrahedron Lett.* **2004**, *45*, 3629.

(18) Park, W. K. C.; Auer, M.; Jaksche, H.; Wong, C-H. *J. Am. Chem. Soc.* **1996**, *118*, 10150.

(19) Alper, P. J.; Hung, S-C.; Wong, C-H. *Tetrahedron Lett.* **1996**, *37*, 6029.

(20) Vedejs, E.; Larsen, S. D. *J. Am. Chem. Soc.* **1984**, *106*, 3030.

SCHEME 5



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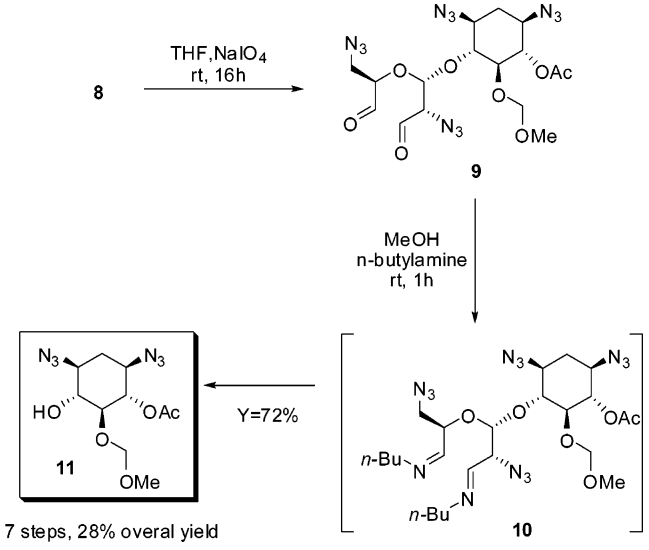

variety of acids under numerous conditions either gave no conversion, a phenomenon we ascribe to the presence of the electron-withdrawing 2′-azido functionality, or resulted in the formation of a complex mixture of products. For this reason, an alternative route was considered involving glycol cleavage with $NaIO_4$ followed by based-induced elimination, a strategy applied to neamine derivatives before.[12] Obviously, application in our case required the liberation of the vicinal 3′,4′-diol. Importantly, in order to retain the asymmetric protective group pattern on 2-deoxystreptamine, a selective removal of acetyl functions from the glucose was required, while leaving the 5-acetyl unaffected. Therefore, a variety of hydrolytic conditions were investigated. Gratifyingly, treatment of **7** with a catalytic amount of potassium *tert*-butoxide in MeOH afforded the desired monoacetate **8** in good yield.

Finally, oxidation of **8** using $NaIO_4$ smoothly afforded dialdehyde **9** (Scheme 6). Not unexpectedly, **9** proved to be only moderately stable and therefore was immediately subjected to elimination. The most satisfactory conditions involved treatment of dialdehyde **9** with *n*-butylamine, to afford **11** via spontaneous β-elimination of the intermediate diimine **10**. The desired orthogonally *O*-protected and enantiomerically pure 2-deoxystreptamine (**11**) was thus obtained in 72% yield over two steps.

In conclusion, we have developed a seven-step synthesis to orthogonally *O*-protected and enantiopure 2-deoxystreptamine from readily available neomycin, with an overall yield of 28%. The synthesis is characterized by a chemoselective glycosidic bond hydrolysis, two regioselective protective group manipulations involving acetylation and deacetylation, and an oxidation−elimination sequence. The synthetic route allows the production of multigram quantities of suitably protected 2-deoxystreptamine, a scaffold that may find particular application in the development of RNA-targeting ligands as novel drugs.[21]

Experimental Section

Neamine·4 HCl (2). To a solution of neomycin sulfate (25 g, 27.5 mmol) in a mixture of H_2O/MeOH (400 mL, 1:1 v/v) was added concentrated HCl (80 mL), and the reaction was stirred at 75 °C for 16 h. The reaction mixture was concentrated, and the crude solid was poured into MeOH (400 mL). Concentrated HCl (80 mL) was added, and the resulting mixture was stirred for another 12 h at 75 °C. The mixture was concentrated to 100 mL, and the formed precipitate was collected by filtration and washed with cold Et_2O, affording **2** (11.47 g, 24.5 mmol, 89%) as an off-white solid. ^{1}H NMR (D_2O, 400 MHz): δ 5.90 (d, J = 3.7 Hz, 1H), 4.03−3.92 (m, 3H), 3.68 (t, J = 9.9 Hz, 1H), 3.59−3.24 (m, 7H), 2.49 (dt, J = 12.6, 4.3 Hz, 1H) 1.89 (q, J = 12.6 Hz, 1H). ^{13}C NMR (D_2O, 100 MHz): δ 98.1, 79.7, 77.2, 74.5, 72.7, 71.2, 70.3, 55.5, 51.7, 50.5, 42.1, 30.3. $[\alpha]^{21}_D$ +86.2 (*c* 1.0, H_2O) {lit.[22] $[\alpha]^{25}_D$ +83 (*c* 1, H_2O)}.

1,3,2′,6′-Tetraazidoneamine (3). To a solution of NaN_3 (25.4 g, 388 mmol) in a mixture of H_2O/CH_2Cl_2 (200 mL, 1:1 v/v) at 0 °C was added Tf_2O (33 mL, 199 mmol). 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 (100 mL). The organic layers were combined to afford 200 mL of TfN_3 solution. Then, to a solution of **2** (7.58 g, 16.2 mmol) in H_2O (200 mL) were added the TfN_3 solution, MeOH (600 mL), and a solution of $CuSO_4$ (250 mg) in a mixture of MeOH/H_2O/Et_3N (50 mL, 3/3/4 v/v/v). The reaction mixture was stirred at room temperature for 2 h. Then solid $NaHCO_3$ (20 g) was added carefully, and the organic solvents were evaporated. The aqueous residue was extracted with EtOAc (2 × 200 mL), and the organic layers were combined, dried (Na_2SO_4), and concentrated in vacuo to give a yellow oil. Purification by column chromatography (EtOAc/heptane 1:2 to 1:1) afforded **3** (5.68 g, 13.3 mmol, 82%) as a colorless oil. R_f 0.56 (EtOAc/heptane 2:1). FTIR (ATR) 2357, 2105, 1747, 1233, 1040 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz): δ 5.63 (d, J = 3.7 Hz, 1H), 4.16 (ddd, J = 10.4, 5.6, 2.8 Hz, 1H), 3.85 (dd, J = 10.4, 8.8 Hz, 1H), 3.55−3.33 (m, 6H), 3.31−3.21 (m, 2H), 3.12 (dd, J = 10.5, 3.8 Hz, 1H), 2.24 (dt, J = 13.1, 4.2 Hz, 1H), 1.89 (q, J = 12.5 Hz, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 97.8, 79.2, 76.1, 76.0, 71.3, 70.8, 70.3, 62.8, 59.9, 59.0, 50.7, 31.3. HRMS (ESI) *m*/*z* calcd for $C_{12}H_{18}N_{12}O_6$ $(M + Na)^+$ 449.1370, found 449.1401. $[\alpha]^{21}_D$ +121.5 (*c* 1.4, EtOAc).

1,3,2′,6′-Tetraazido-6,3′,4′-tri-*O*-acetylneamine (6). To a solution of **3** (6.68 g, 15.6 mmol) in a mixture of CH_2Cl_2/pyridine (100 mL, 1:1 v/v) at 0 °C was added Ac_2O (100 mL). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and slowly poured on

(21) Zaman, G. J. R.; Michiels, P. J. A.; van Boeckel, C. A. A. *Drug Discovery Today* **2003**, *8*, 297.

(22) Peck, R. L.; Hoffhine, C. E., Jr.; Gale, P. H.; Folkers, K. *J. Am. Chem. Soc.* **1953**, *75*, 1018.

ice-cold aqueous $NaHCO_3$ under stirring. The layers were separated, and the organic layer was washed with H_2O (2 × 50 mL) and then concentrated under reduced pressure. The residue was taken up in EtOAc (100 mL) and washed with aqueous NH_4Cl (2 × 100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo, and the residue was purified by column chromatography (EtOAc/heptane 1:2 to 1:1) to afford **6** a colorless oil (7.44 g, 12.1 mmol, 69%). R_f 0.42 (EtOAc/heptane 2:3). FTIR (ATR) 2357, 2105, 1747, 1371, 1233, 1040 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.48 (dd, J = 10.6, 9.2 Hz, 1H), 5.34 (d, J = 3.7 Hz, 1H), 5.04 (dd, J = 10.2, 9.3 Hz, 1H), 4.92 (t, J = 9.9 Hz, 1H), 4.33 (ddd, J = 10.2, 5.0, 2.8 Hz, 1H), 3.68 (dd, J = 10.5, 3.6 Hz, 1H), 3.58 (d, J = 3.4 Hz, 1H), 3.54 (ddd, J = 12.5, 10.0, 4.6 Hz, 1H), 3.46−3.35 (m, 4H), 2.40 (dt, J = 13.1, 4.3 Hz, 1H), 2.18 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 1.61 (q, J = 13.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.9, 169.7, 169.2, 98.3, 83.4, 74.4, 73.9, 70.8, 69.0, 68.8, 61.3, 57.8, 57.4, 50.4, 31.5, 20.3, 20.2, 20.1. HRMS (ESI) m/z calcd for $C_{18}H_{24}N_{12}O_9$ $(M + Na)^+$ 575.1687, found 575.1671. $[\alpha]^{21}_D$ +100.8 (c 0.5, EtOAc).

1,3,2′,6′-Tetraazido-5-*O*-methoxymethyl-6,3′,4′-tri-*O*-acetylneamine (7). To a solution of **6** (7.44 g, 13.5 mmol) in a mixture of $(CH_3O)_2CH_2$ (10 mL) and CH_2Cl_2 (100 mL) was added P_2O_5 (7.66 g, 4 equiv), and the reaction mixture was stirred for 30 min at room temperature. After carefully pouring the mixture on ice-cold aqueous $NaHCO_3$, the layers were separated. The organic layer was washed with H_2O (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/heptane 1:1) to give **7** (6.68 g, 11.2 mmol, 91%) as a colorless oil. R_f 0.56 (EtOAc/heptane 1:1). FTIR (ATR) 2104, 1750, 1231, 1037, 614 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.53 (d, J = 3.8 Hz, 1H), 5.48 (dd, J = 10.9, 9.2 Hz, 1H), 5.05 (dd, J = 10.2, 9.3 Hz, 1H), 4.97 (t, J = 9.8 Hz, 1H), 4.82 (d, J = 6.8 Hz, 1H), 4.74 (d, J = 6.8 Hz, 1H), 4.52 (ddd, J = 10.2, 4.8, 2.8 Hz, 1H), 3.63−3.56 (m, 2H), 3.50−3.27 (m, 8H), 2.39 (dt, J = 13.2, 4.5 Hz, 1H), 2.16 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 1.63 (q, J = 12.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.6, 169.6, 169.2, 99.4, 97.5, 83.8, 78.3, 74.1, 69.6, 68.9, 68.8, 60.4, 58.0, 57.7, 56.2, 50.3, 31.1, 20.6, 20.2, 20.2. HRMS (ESI) m/z calcd for $C_{20}H_{28}N_{12}O_{10}$ $(M + Na)^+$ 619.1949, found 619.1938. $[\alpha]^{21}_D$ +192.4 (c 0.8, EtOAc).

6-*O*-Acetyl-1,3,2′,6′-tetraazido-5-*O*-methoxymethylneamine (8). To a solution of **7** (6.68 g, 11.2 mmol) in MeOH (100 mL) was added KO^{t}Bu (1.37 g, 11.2 mmol, 1 equiv), and the resulting mixture was stirred at room temperature for 2 h. The solution was neutralized with Amberlite IR 120 plus, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc/heptane 1:1) to afford **8** (5.24 g, 10.2 mmol, 87%) as a colorless oil. R_f 0.31 (EtOAc/heptane 2:1). FTIR (ATR) 2102, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 5.50 (d, J = 3.8 Hz, 1H), 4.96 (t, J = 9.5 Hz, 1H), 4.83 (d, J = 6.8 Hz, 1H), 4.76 (d, J = 6.8 Hz, 1H), 4.22 (ddd, J = 9.9, 4.5, 3.0 Hz, 1H), 3.99 (t, J = 9.6 Hz, 1H), 3.65−3.35 (m, 7H), 3.34 (s, 3H), 3.23 (dd, J = 10.4, 3.8 Hz, 1H), 2.79 (broad s, 1H), 2.66 (broad s, 1H), 2.35 (dt, J = 13.3, 4.5 Hz, 1H), 2.16 (s, 3H), 1.57 (q, J = 12.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.7, 99.4, 97.6, 83.7, 77.7, 74.1, 71.1, 70.9, 70.7, 62.4, 58.6, 57.8, 56.2, 50.6, 31.3, 20.6. HRMS (ESI) m/z calcd for $C_{16}H_{24}N_{12}O_8$ $(M + Na)^+$ 535.1738, found 535.1736. $[\alpha]^{21}_D$ +68.1 (c 5.1, EtOAc).

4-*O*-Acetyl-1,3-diazido-5-*O*-methoxymethyl-2-deoxystreptamine (11). To a solution of **8** (4.20 g, 8.20 mmol) in MeOH (300 mL) was added $NaIO_4$ (13.15 g, 61.5 mmol, 7.5 equiv) at 0 °C. The resulting mixture was allowed to warm to room temperature and stirred for 16 h (TLC R_f 0.54 (2.5% MeOH in CH_2Cl_2)). The formed white precipitate was filtered off, and the filtrate was concentrated in vacuo. The residue was taken up in EtOAc (400 mL) and washed with H_2O (400 mL) and brine (300 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was then dissolved in MeOH (400 mL), and butylamine (2.43 mL, 24.6 mmol, 3 equiv) was added dropwise. The mixture was stirred for 1 h at room temperature and concentrated in vacuo, and the residue was purified by column chromatography (EtOAc/heptane 1:2) to afford **11** (1.77 g, 5.9 mmol, 72%) as a slightly yellow oil. R_f 0.43 (EtOAc/heptane 1:1). FTIR (ATR) 2100, 1743, 1218, 1033 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 4.96 (t, J = 10.0 Hz, 1H), 4.70 (d, J = 6.8 Hz, 1H), 4.60 (d, J = 6.8 Hz, 1H), 4.29 (d, J = 1.6 Hz, 1H), 3.54−3.31 (m, 7H), 3.45 (s, 3H), 2.21 (dt, J = 13.4, 4.5 Hz, 1H), 2.14 (s, 3H), 1.45 (dt, J = 13.4, 12.5 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.4, 97.9, 83.7, 75.0, 73.6, 59.4, 58.0, 55.8, 31.4, 20.3. HRMS (ESI) m/z calcd for $C_{10}H_{16}N_6O_5$ $(M + Na)^+$ 323.1080, found 323.1109. $[\alpha]^{21}_D$ +6.7 (c 3.3, EtOAc).

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Supporting Information Available: ^1H NMR and ^{13}C NMR spectra for compounds **2**, **3**, **6**, **7**, **8**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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