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 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 rRNA-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 aminoglycosides2 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.3 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,5 but it has also been shown that deoxystreptamine alone shows sequence-specific binding to major groove RNA6 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 antibiotics9 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 (I) 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 chemical12–14 and enzymatic12,15 approaches. However, the chemical routes often proved to be laborious and low-yielding, and enzymatic

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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. Only on few occasions were enantiopure products obtained through 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 (Scheme 2). Although a literature protocol for such a transformation has been published, 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 DIPEA as a base proceeded uneventfully to afford 1 in good yield after purification by chromatography (Scheme 4).

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, 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-acetylated 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-acetylation 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, benzyla tion 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, but the outcome encouraged us to look further into a selective tri-O-acetylation protocol.

Protection with pivaloyl chloride gave mixtures of mono-, di-, tri-, and tetra-O-acetylated 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-acetylation protocol.

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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
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.

**Experimental Section**

**Neamine-4 HCl (2).** To a solution of neomycin sulfate (25 g, 27.5 mmol) in a mixture of H2O/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 Et2O, affording 2 (11.47 g, 24.5 mmol, 89%) as an off-white solid.

\[ ^1H \text{NMR (D}_2\text{O, 400 MHz): } \delta 5.90 (d, J = 3.7 Hz, 1H), 4.03–3.92 (m, 3H), 3.68 (t, J = 9.9 Hz, 1H), 3.59–3.24 (m, 7H), 2.49 (dt, J = 12.6, 4.3 Hz, 1H) 1.89 (q, J = 12.6 Hz, 1H). \]

**13C NMR (D2O, 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. [α]+D = +86.2 (c 1.0, H2O) [lit.22 [α]D = +83 (c 1.0, H2O)].

**1,3,2′,6′-Tetraazidone (3).** To a solution of NaN3 (25.4 g, 388 mmol) in a mixture of H2O/CH2Cl2 (200 mL, 1:1 v/v) at 0 °C was added Tf2O (33 mL, 199 mmol). The reaction mixture was stirred at room temperature for 2 h. After quenching with aqueous NaHCO3, the layers were separated, and the aqueous layer was extracted with EtOAc (200 mL), and a solution of CuSO4 (250 mg) in a mixture of MeOH/H2O/Et3N (50 mL, 3/3/4 v/v/v). The reaction mixture was stirred at room temperature for 2 h. Then solid NaHCO3 (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 (Na2SO4), 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, 16.2 mmol) as a colorless oil.

\[ ^1H \text{NMR (CD}_3\text{OD, 400 MHz): } \delta 5.63 (d, J = 3.7 Hz, 1H), 4.16 (dd, J = 10.4, 5.6, 2.8 Hz, 1H), 3.85 (dd, J = 10.4, 8.8 Hz, 1H), 3.55–3.33 (m, 6H), 3.31–3.21 (m, 2H), 3.32 (dd, J = 10.5, 3.8 Hz, 1H), 2.24 (dt, J = 13.1, 4.2 Hz, 1H). \]

\[ 13C \text{NMR (CD}_3\text{OD, 100 MHz): } \delta 97.8, 79.2, 76.1, 76.0, 71.3, 70.8, 70.3, 62.8, 59.9, 59.0, 50.7, 31.3. \]

**S H E M E 6**

7 steps, 26% overall yield

**Neamine-4 HCl (2).** To a solution of NaN3 (25.4 g, 388 mmol) in a mixture of H2O/CH2Cl2 (200 mL, 1:1 v/v) at 0 °C was added TF2O (33 mL, 199 mmol). The reaction mixture was stirred at room temperature for 2 h. After quenching with aqueous NaHCO3, the layers were separated, and the aqueous layer was extracted with CH2Cl2 (100 mL). The organic layers were combined to afford 200 mL of TfN3 solution. Then, to a solution of NaN3 (25.4 g, 388 mmol) in a mixture of H2O/CH2Cl2 (200 mL, 1:1 v/v) at 0 °C was added TF2O (33 mL, 199 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated to 100 mL, and the formed precipitate was collected by filtration and washed with cold Et2O, affording 2 (11.47 g, 24.5 mmol, 89%) as an off-white solid.

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\[ 13C \text{NMR (D}_2\text{O, 100 MHz): } \delta 98.1, 79.7, 77.2, 74.5, 72.7, 71.2, 70.3, 55.5, 51.7, 50.5, 42.1, 30.3. [α]+D = +86.2 (c 1.0, H2O) [lit.22 [α]D = +83 (c 1.0, H2O)].

**1,3,2′,6′-Tetraazidoneamine (3).** To a solution of NaN3 (25.4 g, 24.5 mmol) in a mixture of H2O/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 Et2O, affording 2 (11.47 g, 24.5 mmol, 89%) as an off-white solid.

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\[ 13C \text{NMR (D}_2\text{O, 100 MHz): } \delta 98.1, 79.7, 77.2, 74.5, 72.7, 71.2, 70.3, 55.5, 51.7, 50.5, 42.1, 30.3. [α]+D = +86.2 (c 1.0, H2O) [lit.22 [α]D = +83 (c 1.0, H2O)].

Finally, oxidation of 8 using NaIO4 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-butyamine, to afford 11 via spontaneous β-elimination of the intermediate diimine 10. The

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ice-cold aqueous NaHCO₃ under stirring. The layers were separated, and the organic layer was washed with H₂O (2 × 50 mL) and then concentrated under reduced pressure. The residue was taken up in EtOAc (100 mL) and washed with aqueous NH₄Cl (2 × 100 mL) and brine (100 mL). The organic layer was dried (Na₂SO₄) 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%). R₆ 0.42 (EtOAc/heptane 2:3). FTIR (ATR) 2357, 2105, 1747, 1371, 1233, 1040 cm⁻¹. ¹H NMR (CDCl₃, 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), 3.66 (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). ¹³C NMR (CDCl₃, 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 C₁₈H₂₄N₁₂O₉ (M+Na)⁺ 575.1682, found 575.1671. [α]D +100.8 (c 0.5, EtOAc).

1,3,2',6'-Tetraazido-5-O-methoxymethyl-6,3',4'-tri-O-acetyl-neamine (7). To a solution of 6 (7.44 g, 13.5 mmol) in a mixture of (CH₂O)₂CH₂ (10 mL) and CH₂Cl₂ (100 mL) was added P₂O₅ (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 NaHCO₃, the layers were separated. The organic layer was washed with H₂O (50 mL) and brine (50 mL), dried (Na₂SO₄), 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. R₆ 0.56 (EtOAc/heptane 1:1). FTIR (ATR) 2104, 1750, 1231, 1037, 614 cm⁻¹. ¹H NMR (CDCl₃, 400 MHz): δ 5.50 (d, J = 3.9 Hz, 1H), 5.04 (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 (dd, J = 10.2, 4.8 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). ¹³C NMR (CDCl₃, 100 MHz): δ 169.6, 169.6, 169.2, 99.4, 97.5, 83.8, 78.3, 74.1, 69.6, 68.9, 68.6, 64.0, 58.0, 57.7, 56.2, 50.3, 31.1, 20.6, 20.2, 20.2. HRMS (ESI) m/z calcd for C₁₉H₂₃N₁₂O₉ (M+Na)⁺ 535.1736, found 535.1736. [α]D +68.1 (c 5.1, EtOAc).

4-O-Acetyl-1,3-diazido-5-O-methoxymethyl-2-deoxy-streptamine (11). To a solution of 8 (4.20 g, 8.20 mmol) in MeOH (300 mL) was added NaIO₄ (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 R₆ 0.54 (2.5% MeOH in CH₂Cl₂)). 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 H₂O (400 mL) and brine (300 mL). The organic layer was dried (Na₂SO₄) 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 quantitatively. The mixture was stirred for 1 h at room temperature and concentrated in vacuo, and the residue was purified by column chromatography (EtOAc/heptane 2:1) to afford 11 (1.77 g, 5.9 mmol, 72%) as a slightly yellow oil. R₆ 0.43 (EtOAc/heptane 1:1). FTIR (ATR) 2100, 1743, 1218, 1033 cm⁻¹. ¹H NMR (CDCl₃, 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), 2.10 (t, J = 13.4, 4.5 Hz, 1H), 2.14 (s, 3H), 1.45 (dt, J = 13.4, 12.5 Hz, 1H). ¹³C NMR (CDCl₃, 100 MHz): δ 169.4, 169.4, 169.2, 99.7, 83.7, 75.0, 73.6, 59.4, 58.0, 31.4, 20.3. HRMS (ESI) m/z calcd for C₁₀H₁₀N₆O₅ (M+Na)⁺ 323.1080, found 323.1109. [α]D +16.7 (c 3.3, EtOAc).

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Supporting Information Available: ¹H NMR and ¹³C 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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