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Carbohydrate mimic of 2-deoxystreptamine for the preparation of conformationally constrained aminoglycosides

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Abstract—The synthesis of a carbohydrate mimic of 2-deoxystreptamine (2-DOS) is described starting from D-ribose. Crucial steps of the synthesis involve a stereoselective nitroaldol condensation and deoxygenation via elimination–in situ reduction. Moreover, glycosylation of the carbohydrate 2-DOS derivative with a phenyl thioglycoside donor in the presence of TTBP and AgOTf followed by ring-closing metathesis yielded a conformationally restricted aminoglycoside analogue.

1. Introduction

Aminoglycosides form a large class of clinically important antibiotics with a broad antibacterial spectrum and proven efficacy, particularly against Gram-negative bacteria.[1] The key structural feature of nearly all aminoglycosides is a 1,3-diaminocyclohexanetriol termed 2-deoxystreptamine (2-DOS). The central position of 2-deoxystreptamine suggests that 2-DOS is a crucial scaffold for correct positioning of the aminoglycosides during binding to bacterial RNA for interference with protein translation. Not surprisingly, therefore, the vast majority of novel RNA-binding ligands reported in literature are structures derived from 2-DOS. It is obvious that such an approach requires that the availability of substantial amounts of 2-DOS is secured. However, although many synthetic routes toward 2-DOS have been developed,[2] the majority of these routes necessitate a large number of synthetic steps. We therefore reasoned that a carbohydrate-derived entity like compound **A** (Fig. 1) could be an interesting alternative for 2-DOS, taken into consideration that the amino group at C-1 is modified in several clinically used aminoglycosides and the C-6 hydroxyl is not essential for antibacterial activity (2-DOS numbering). Furthermore, starting from a (cheap) carbohydrate precursor leads to enantiomerically pure product. To date, two syntheses of such carbohydrate analogues are known. A carbohydrate analogue was synthesized starting from benzyl-α-D-glucopyranoside by Meyer zu Reckendorf and co-workers (not depicted), in a 10-step synthesis leading to a compound with structural resemblance to 2-DOS, but as a mixture of anomers.[3] Secondly, the assumption that carbohydrates may be worthy alternatives for 2-DOS has also been pursued by Boons and co-workers (vide infra).[4,5]

Retrosynthetic analysis of β-configured carbohydrate analogue **A** suggests a synthesis from nitroglucopyranoside **B**, by means of deoxygenation at C-4, nitro reduction, and azide introduction at C-6 (carbohydrate numbering). The nitroglucopyranoside in turn was projected to result from D-ribose, a cheap and enantiopure carbohydrate, via oxidative glycol cleavage followed by tandem Henry reaction with nitromethane. Although it is clear that the final carbohydrate analogue lacks the C-6 hydroxyl, the fact that the 6-OH of 4,5-linked aminoglycoside antibiotics is only involved in indirect binding events with the RNA backbone (Fig. 2)[6,7] led us to assume that its omission would not necessarily result in a large penalty in binding enthalpy. Furthermore, such a synthetic strategy would result in a compound with a homologated amino group, as in several semi-synthetic aminoglycoside analogues.

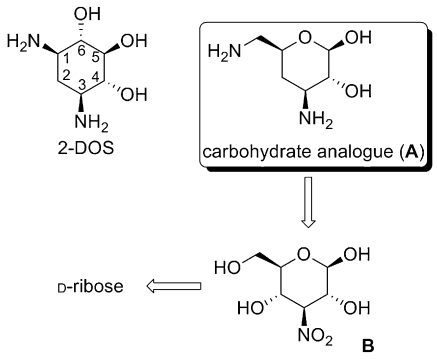

Figure 1. Retrosynthesis of carbohydrate derivative (**A**) as a mimic for 2-deoxystreptamine.

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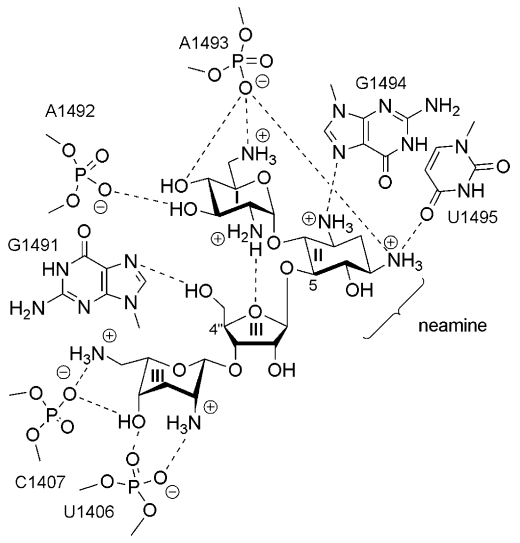
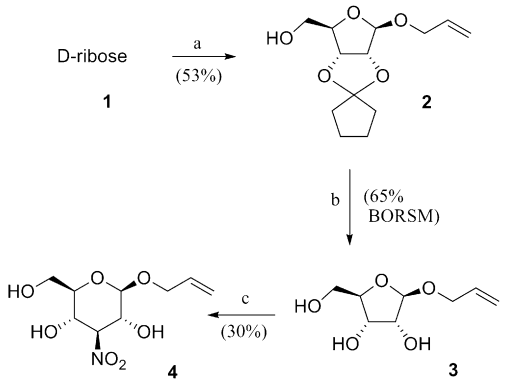


Figure 2. Neomycin complexed to nucleobases and the phosphate backbone of 16S rRNA.

2. Preparation of 2-DOS carbohydrate mimic

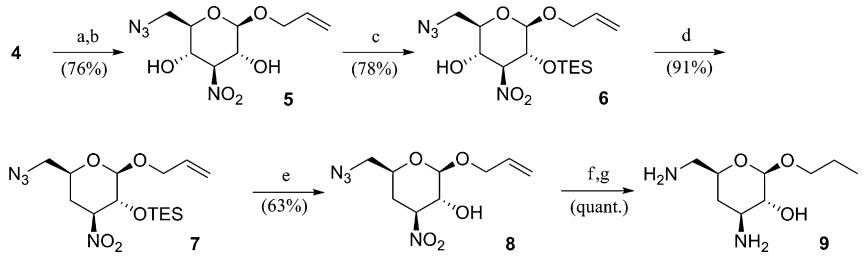
The first synthetic step in the preparation of carbohydrate analogue **A** is triple protection of D-ribose. In a one-pot reaction, both C-2 and C-3 hydroxyls were protected with a cyclopentylidene group and the C-1 hydroxyl with an allyl group yielding the protected allyl β-D-ribofuranoside **2**. A cyclopentylidene function is employed instead of the more common isopropylidene because various acidic conditions led to unacceptable amounts of fully hydrolyzed product.[8–11] However, the more labile cyclopentylidene group[12,13] was chemoselectively removed with acetic acid and water (80/20), leading to allyl β-D-ribofuranoside (**3**) in reasonable yield, although in this case it was mandatory to quench the reaction after 48 h to avoid hydrolysis of the anomeric protective group.[14] Next, a challenging synthetic transformation was undertaken, involving oxidative cleavage of the diol, followed by nitroaldol condensation with nitromethane.[15] The fact that the desired stereoisomer **4** has the thermodynamically most favorable all-equatorial configuration was an important stimulus to undertake this endeavor and we were delighted to find that the diastereomerically pure product 3-nitro-β-D-glucopyranoside **4** was isolated after acidification and crystallization from EtOAc (Scheme 1).[16] Two other isomers, formed in smaller proportions, presumably the *galacto* and *manno* configured sugars,[16,17] were not further characterized. Having the requisite nitrosugar **4** in hand, the desired regioselective deoxygenation at C-4 could be investigated. To this end, the 6-OH was first converted into an azide by treatment of **4** with tosyl chloride followed by displacement with sodium azide to give the 6-azidopyranoside **5** in good yield (Scheme 2). Much to our satisfaction, subsequent protection of the 2-hydroxyl with a triethylsilyl group proceeded with good regioselectivity to yield monosilylated glucopyranoside **6**.[18] Subsequent acetylation of O-4 with acetyl chloride[19] and addition of sodium borohydride to the crude reaction product led to a one-step elimination–reduction affording compound **7**.[20] Finally, removal of the TES group in 2 M HCl in EtOAc afforded the desired structure **8** in a total of eight synthetic steps. As projected, compound **8** possesses the correct stereochemistry to function as a carbohydrate mimic of 2-deoxystreptamine, with the 2-OH (5-OH in 2-DOS numbering) selectively free for glycosylation. Before such a glycosylation of **8** was undertaken, reduction of the nitro or azido group was investigated. Indeed, in the presence of Raney-Ni, hydrogenation of nitroglucopyranoside **8** led to full reduction of the azido, nitro, and allyl groups. Subsequent removal of the TES group under aforementioned conditions yielded the carbohydrate derivative **9** quantitatively.



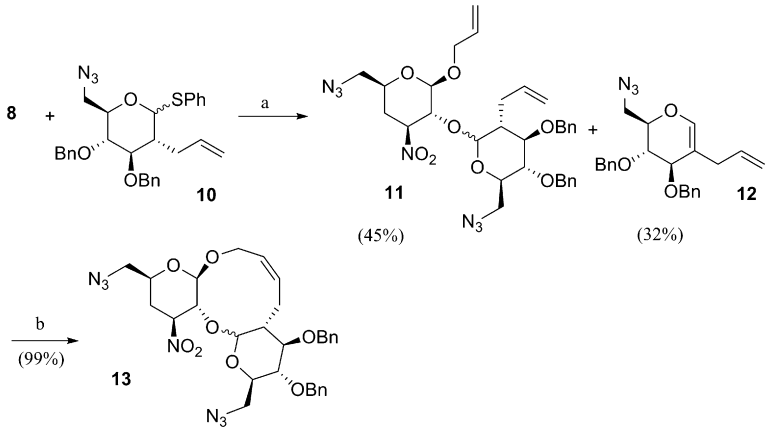
Scheme 1. Reagents and conditions: (a) $CuSO_4$, cyclopentanone, allyl alcohol, H_2SO_4, 40 °C, 48 h; (b) CH_3COOH 80%, 40 °C, 48 h; (c) $NaIO_4$, H_2O, 0 °C to rt, 30 min, then NaOMe, NO_2Me, MeOH, rt, 45 min.

3. Conformationally constrained aminoglycoside ligand

NMR spectroscopy and X-ray crystallography studies have indicated that neamine is the minimal motif for selective binding to the A-site of 16S rRNA.[6,7] It has also been shown that 2,6-diamino-2,6-dideoxyglucopyranoside is responsible



Scheme 2. Reagents and conditions: (a) TsCl, CH_2Cl_2/pyridine (1/1), 0 °C to rt, o.n.; (b) NaN_3, DMF, 55 °C, o.n.; (c) TESOTf, Et_3N, CH_2Cl_2, 0 °C, 30 min; (d) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 30 min, then $NaBH_4$, EtOH, rt, 2 h; (e) 2 M HCl, EtOAc, rt, 10 min; (f) Ra-Ni, MeOH, H_2, 3 bar, rt, 24 h; (g) 2 M HCl, EtOAc, rt, 10 min.



Scheme 3. Reagents and conditions: (a) AgOTf, 4 Å MS, TTBP, CH_2Cl_2, 0 °C to rt, o.n. ($\alpha/\beta \approx 3/1$); (b) Hoveyda–Grubbs catalyst, toluene, rt, 4 h.

for most of the key interactions with RNA. Studies by Wong and co-workers have demonstrated that substitution patterns other than 2,6-diamines lead to loss of activity of these antibiotics.[21] Studies by Boons and co-workers[4,5] on a library of 24 disaccharide mimics of neamine including α(1–3) or β(1–3) and α(1–4) or β(1–4) linked dimers containing 2–4 amino groups indicated that the compound with the best binding affinity was the one with four amino groups (C-2, C-2′, C-6, C-6′), displaying an affinity similar to that of neamine. On the downside of aminoglycosides, extensive clinical use is limited due to the associated nephro- and ototoxicities,[22] as well as the global development of microbial resistance as the result of structural modification by bacterial enzymes.[23,24] In this respect, it is known that most aminoglycosides bind rather promiscuously to a variety of RNA targets. The NMR structure derived from paromomycin complexed to 16S rRNA revealed that several intermolecular contacts between the aminoglycosides and RNA recognition sites are important for binding.[6a] Our attention was drawn to a hydrogen bond between N-2′ and O-4″ (Fig. 2) and it was reasoned that a neamine analogue covalently linked between C-2′ and O-5 (O-2 in 2-DOS analogue **8**) would be locked in the appropriate conformation for binding to rRNA and may therefore show enhanced binding specificity (Scheme 3). A similar reasoning was followed by Jiménez-Barbero and co-workers, who recently prepared a conformationally restricted neomycin B analogue and found it to be less susceptible to resistance.[25] Another report on conformationally restricted aminoglycoside analogues was recently published by Blount et al.[26]

These observations stimulated us to investigate incorporation of nitroanalogue **8** in a conformationally restricted analogue of neamine via coupling to a thioglycoside followed by macrocycle formation. Thus, glycosylation of the carbohydrate acceptor **8** with thioglycoside donor **10**[27] was carried out in the presence of 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and AgOTf (Scheme 3).[28] The product was formed in a yield of 45% and the α/β ratio was 3:1.[29] Apart from that, formation of a substantial amount (32%) of an undesirable side-product was observed, NMR analysis of which revealed that elimination product **12** was formed. Next, after separation of the two products (**11** and **12**) ring-closing metathesis was attempted. It was found that exposure of disaccharide **11** to Grubbs' catalyst failed to give the desired product, due to incompatibility of azide functionality with the phosphine ligand of the catalyst.[30] Consequently, phosphine-free Hoveyda–Grubbs catalyst was applied instead and much to our satisfaction, the cyclic product **13** was formed in excellent yield (99%). We are currently applying the methodology for the preparation of a library of neamine analogues for application in novel antimicrobial therapy.

4. Conclusion

A carbohydrate analogue of 2-DOS was synthesized successfully in eight steps and an overall yield of 6.6%. The analogue is enantiopure and orthogonally protected. Model reduction and deprotection of the analogue proceeded in only two steps in a high yield. The carbohydrate precursor is conveniently protected for incorporation in new aminoglycoside entities, suitable for the synthesis of 4,5-linked aminoglycoside antibiotics. Moreover, the presence of an allyl moiety at O-1 makes the carbohydrate mimic highly suitable for the synthesis of conformationally restricted RNA binders as was illustrated in the successful coupling of the carbohydrate derivative to thioglycoside **10**, to form neamine mimic **11**. A conformationally restricted analogue was synthesized by ring-closing metathesis with the Hoveyda–Grubbs catalyst. Further application of the thus developed route, to prepare a library of conformationally restricted neamine analogues is currently under investigation.

5. Experimental

5.1. General

All solvents were distilled from appropriate drying agents prior to use. Chemicals were purchased from Sigma–Aldrich and used as such. Reactions were carried out under argon atmosphere. Standard syringe techniques were applied to the transfer of dry solvents and air- or moisture sensitive reagents. R_f values were obtained using thin layer chromatography (TLC) on silica gel-coated plates (Merck 60 F_{254}) with the indicated solvent mixture. Melting points were analyzed with a Büchi melting point apparatus B-545. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer; absorption in cm^{-1}. GC was performed on a

Hewlett Packard 5890, containing an HP1 column (25 m×0.32 mm×0.17 μm), with FID detection and HP3393A integrator. NMR spectra were recorded on a Bruker DMX 300 (300 MHz), and a Varian 400 (400 MHz) spectrometer in $CDCl_3$ solutions (unless otherwise reported) using TMS as internal standard; chemical shifts are given in parts per million. Coupling constants are reported as *J*-values in hertz. Peak assignment in ^{13}C spectra is based on 2D-GHSQC and GHMBC spectra. Enantiomeric purities were determined on a Shimadzu HPLC, with indicated column and solvent mixture. Column or flash chromatography was carried out using ACROS silica gel (0.035–0.070 mm and ca. 6 nm pore diameter). Optical rotations were determined with a Perkin Elmer 241 polarimeter. Mass spectra were obtained with a Fisons (VG) Micromass 7070E apparatus and/or a Finnigan MAT900S. MALDI-TOF-MS spectra were obtained on a Bruker Biflex III machine, with dihydroxybenzoic acid (DHB) as matrix. Elemental analyses were carried out using a Carlo Erba Instruments CHNS-O EA 1108 element analyzer.

5.1.1. Allyl 2,3-*O*-cyclopentanone-β-D-ribofuranoside (2). Powdered D-ribose (**1**, 5.85 g, 38.9 mmol) and anhydrous cuprous sulfate (12.4 g) were suspended in a mixture of cyclopentanone (110 mL) and allyl alcohol (32 mL) containing a catalytic amount of H_2SO_4 (0.2 mL). The resulting mixture was stirred at 40 °C for 48 h and then neutralized with $NaHCO_3$, filtered, and the solvents were evaporated. The crude product was extracted with EtOAc and washed with brine, dried (Na_2SO_4), and the solvent was evaporated to give the crude product, which was purified by flash chromatography (EtOAc/*n*-heptane, 1/10), to obtain **2** (3.32 g, 53%) as a colorless oil.[13] $[\alpha]_D^{20}$ −70.8 (*c* 0.35, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3475, 2964, 1338, 1102, 1043, 1001, 914, 742. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 5.90 (m, 1H, allyl), 5.30 (m, 2H, allyl), 5.13 (s, 1H, CH), 4.78 (d, 1H, *J*=6.0 Hz, CH), 4.55 (d, 1H, *J*=6.0 Hz, CH), 4.41 (br s, 1H, OH), 4.21 (m, 1H, CH), 4.08 (m, 1H, CH), 3.69 (m, 2H, CH), 3.22 (m, 1H, CH), 1.68 (m, 8H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 132.9, 121.6, 118.2, 107.6, 88.1, 85.6, 81.3, 68.8, 63.9, 35.6, 35.5, 23.5, 23.0. HRMS (EI) *m/z* calcd for $C_{13}H_{20}O_5$ $(M)^+$: 256.1311, found: 256.1312.

5.1.2. Allyl β-D-ribofuranoside (3). Compound **2** (2.05 g, 8.00 mmol) was dissolved in 80% acetic acid and heated to 40 °C for 48 h. The reaction mixture was neutralized with $NaHCO_3$ and evaporated. Elution by flash column chromatography (EtOAc/*n*-heptane, 1/5) gave first recovered starting material (0.41 g, 25%) followed by **3** as a colorless oil (604 mg, 40%). R_f 0.38 (EtOAc). $[\alpha]_D^{20}$ −57.1 (*c* 0.77, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3347, 2926, 1640, 1084, 1033, 993, 931, 633, 559. ^1H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.04–5.97 (m, 1H, allyl), 5.28 (dd, *J*=1.6, 1.8 Hz, 1H, allyl), 5.20 (dd, *J*=1.8, 1.6 Hz, 1H, allyl), 4.97 (s, 1H, CH), 4.20–3.91 (m, 7H, CH), 3.72–3.59 (m, 2H, OH), 3.67 (br s, 1H, OH). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 135.4, 116.8, 107.7, 84.8, 76.2, 72.7, 69.1, 65.0. HRMS (CI) *m/z* calcd for $C_8H_{15}O_5$ $(M+H)^+$: 191.0919, found: 191.0920.

5.1.3. Allyl 3-nitro-3-deoxy-β-D-glucopyranoside (4). $NaIO_4$ (7.68 g, 35.9 mmol) was dissolved in H_2O (90 mL). After cooling the reaction mixture to 0 °C, compound **3** (6.83 g, 35.9 mmol) was added. The reaction mixture was slowly warmed to room temperature. After stirring for 30 min, TLC analysis (EtOAc) indicated complete disappearance of starting material. EtOH was added to the reaction mixture before cooling to 0 °C and filtration. The filtrate was evaporated, the residue dissolved in EtOH, and filtered again. The resulting dialdehyde was co-evaporated twice with MeOH, dissolved in MeOH, and cooled to 0 °C. Nitromethane (2.0 mL, 37.34 mmol) was added, followed by freshly prepared NaOMe (2.48 g, 35.9 mmol) and the reaction was stirred at room temperature for 45 min. To the reaction mixture was added a cold suspension of Amberlite IR-120 (H^+) in MeOH. The yellow reaction mixture became colorless after 10 min. The reaction mixture was passed through a column of Amberlite IR-120 and thoroughly rinsed with MeOH. After evaporation of the solvent EtOAc was added and evaporated, EtOAc was added again, and the solution was stored in the fridge for one night leading to precipitation of **4**. EtOAc was filtered off to yield compound **4** as a white powder (2.54 g, 30%). R_f 0.62 (EtOAc). Mp: 158.6 °C. $[\alpha]_D^{20}$ −24.9 (*c* 1.0, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3285, 1556, 1124, 1079, 1042, 1012. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 7.09–5.96 (m, 1H, CH), 5.37 (ddt, *J*=1.6, 1.8 Hz, 1H, CH), 5.31 (ddt, *J*=1.3, 1.5 Hz, 1H, CH), 4.46 (t, *J*=10.1 Hz, 1H, H-3), 4.37 (d, *J*=7.7 Hz, 1H, H-1), 4.41–4.33 (m, 1H, CH_2), 4.13 (ddt, *J*=13.0, 6.0, 1.4 Hz, 1H, CH_2), 3.96 (t, *J*=10.0 Hz, 1H, H-4), 3.86 (dd, *J*=12.0, 2.3 Hz, 1H, H-6a), 3.78 (dd, *J*=10.2, 7.8 Hz, 1H, H-2), 3.71 (dd, *J*=8.0, 5.2 Hz, 1H, H-6b), 3.27 (ddd, *J*=9.7, 5.1, 2.3 Hz, 1H, H-5). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 135.1 (CH), 117.3 (CH_2), 102.7 (CH), 95.8 (CH), 78.1 (CH), 72.3 (CH), 71.1 (CH_2), 69.2 (CH), 62.0 (CH_2). HRMS (ESI) *m/z* calcd for $C_9H_{15}O_7N$ $(M+Na)^+$: 272.0746, found: 272.0745. Elemental analysis: calculated for $C_9H_{15}O_7$: C 43.37, H 6.07, N 5.62, found C 43.00, H 6.10, N 5.51.

5.1.4. Allyl 3-nitro-3-deoxy-6-*O*-tosyl-β-D-glucopyranoside. Compound **4** (1.07 g, 5.13 mmol) was dissolved in a mixture of CH_2Cl_2 and pyridine (1/1, 0.2 mL). After cooling the mixture to 0 °C tosyl chloride (1.17 g, 6.16 mmol) was added. The reaction was stirred overnight. The reaction mixture was quenched with water, extracted with EtOAc, and dried with Na_2SO_4 to yield after flash column chromatography (EtOAc/*n*-heptane, 1/10) the compound as a colorless oil (1.74 g, 91%). R_f 0.78 (EtOAc). IR ν_{max} film: (cm^{-1}) 3482, 1559, 1173, 1039, 981, 814, 730, 533. ^1H NMR (CD_3OD, 400 MHz, ppm): δ 7.79, (d, *J*=8.2 Hz, 2H, arom), 7.35 (d, *J*=8.1 Hz, 2H, arom), 5.94–5.82 (m, 1H), 5.33–5.19 (m, 2H), 4.54 (t, *J*=10.1 Hz, 1H), 4.41–4.33 (m, 2H), 4.34–4.25 (m, 2H), 4.18–4.03 (m, 3H), 3.94 (dq, *J*=9.7, 4.1, 2.1 Hz, 1H), 3.57–3.51 (m, 1H), 1.54 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 145.3, 132.9, 130.0, 128.0, 118.6, 101.2, 92.2, 74.1, 70.9, 67.7, 22.1.

5.1.5. Allyl 6-azido-3-nitro-3,6-dideoxy-β-D-glucopyranoside (5). Tosyl glucopyranoside (33 mg, 0.082 mmol) was dissolved in DMF (1 mL). The reaction mixture was heated to 55 °C and NaN_3 (10 mg, 0.16 mmol) was added. After stirring overnight the reaction mixture was quenched with water and extracted with EtOAc, to yield after flash column chromatography (EtOAc/*n*-heptane, 2/3) compound **5** as a white solid (17 mg, 84%). R_f 0.28 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ −16 (*c* 0.45, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3426, 2103, 1559, 1372, 1281, 1065. ^1H NMR ($CDCl_3$,

400 MHz, ppm): δ 6.04–5.81 (m, 1H), 5.52–5.20 (m, 2H), 4.54 (t, *J*=10.0 Hz, 1H), 4.42 (d, *J*=7.8 Hz, 1H), 4.36 (d, *J*=7.5 Hz, 1H), 4.21–4.30 (m, 2H), 4.18 (t, *J*=9.5 Hz, 1H), 3.63 (br s, 1H, OH), 3.59–3.38 (m, 3H), 3.24 (br s, 1H, OH). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 132.9, 118.9, 100.9, 92.8, 75.5, 70.9, 70.8, 69.0, 51.2. HRMS (CI) *m/z* calcd for $C_9H_{15}O_6N_4$ $(M+H)^+$: 275.0992, found: 275.0981.

5.1.6. Allyl 6-azido-3-nitro-3,6-dideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside (6). The glucopyranoside **5** (13 mg, 0.047 mmol) was dissolved in CH_2Cl_2 (1 mL). After cooling the reaction mixture to 0 °C, triethylsilyl trifluoromethanesulfonate (16 μL, 0.071 mmol) and Et_3N (17 μL, 1.2 mmol) were added and the reaction mixture was stirred for 30 min before quenching with satd aq NH_4Cl, extraction with CH_2Cl_2, and drying with Na_2SO_4. Flash column chromatography (EtOAc/*n*-heptane, 2/3) gave compound **6** (18 mg, 78%) as a colorless oil. R_f 0.52 (EtOAc/*n*-heptane, 1/10). $[\alpha]_D^{20}$ −14 (*c* 0.46, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3425, 2876, 2099, 1557, 1067, 815, 745. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): 6.05–5.90 (m, 1H), 5.42–5.18 (m, 2H), 4.49 (t, *J*=6.4 Hz, 1H), 4.45–4.31 (m, 1H, CH), 4.37 (d, *J*=7.6 Hz, 1H), 4.19–3.92 (m, 3H), 3.52–3.49 (m, 3H), 1.14–0.79 (m, 9H, TES), 072–0.45 (m, 6H, TES). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 133.1, 118.5, 101.6, 95.0, 75.3, 72.3, 70.9, 69.4, 51.4, 6.9, 5.1. HRMS (FAB) *m/z* calcd for $C_{15}H_{29}O_6N_4Si$ $(M+H)^+$: 389.1856, found: 389.1863.

5.1.7. Allyl 6-azido-3-nitro-3,4,6-trideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside (7). Compound **5** (100 mg, 0.26 mmol) was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. Et_3N (64 μL, 0.47 mmol), Ac_2O (49 μL, 0.51 mmol), and DMAP (cat.) were added and the reaction was stirred for 30 min. The reaction mixture was quenched with a 0.1 M HCl solution and extracted with CH_2Cl_2 and dried with Na_2SO_4. The compound was dissolved in EtOH (2 mL) and $NaBH_4$ (20 mg, 0.51 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 2 h and quenched with acetone and evaporated. Flash column chromatography (EtOAc/*n*-heptane, 1/5) gave the compound **7** (87 mg, 91%) as colorless oil. R_f 0.60 (EtOAc/*n*-heptane, 2/3). $[\alpha]_D^{20}$ −29 (*c* 0.32, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 2954, 2877, 2099, 1558, 1130, 1006, 743. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.10–5.82 (m, 1H, allyl), 5.49–5.18 (m, 2H, allyl), 4.64–4.54 (m, 1H, H-3), 4.46–4.38 (m, 1H, H-7a), 4.32 (d, 1H, *J*=7.6 Hz, H-1), 4.15–4.00 (m, 2H, H-7b and H-2), 3.70–3.65 (m, 1H, H-5), 3.62–3.39 (m, 1H, H-6a), 2.20 (dd, 1H, *J*=3.5 Hz, H-6b), 2.20 (ddd, 1H, *J*=1.9 Hz, H-4a), 2.04 (q, 1H, *J*=5.9 Hz, H-4b), 1.01–0.81 (m, 9H, TES), 0.71–0.51 (m, 6H, TES). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 133.2, 118.4, 101.8, 88.1, 72.5, 71.7, 70.7, 54.0, 33.6, 6.9, 5.2. HRMS (FAB) *m/z* calcd for $C_{15}H_{29}O_5N_4Si$ $(M+H)^+$: 373.1907, found: 373.1904.

5.1.8. Allyl 3-nitro-3,4,6-trideoxy-6-azido-β-D-glucopyranoside (8). Compound **7** (28 mg, 0.075 mmol) was dissolved in a 2 M HCl solution and stirred for 10 min at room temperature. The reaction mixture was evaporated and flash column chromatography (EtOAc/*n*-heptane, 1/5) gave **8** (12 mg, 63%) as colorless oil. R_f 0.55 (EtOAc/*n*-heptane, 1/1). $[\alpha]_D^{20}$ −26 (*c* 1.4, CH_2Cl_2). IR ν_{max} film: (cm^{-1}) 3431, 2924, 2099, 1556, 1065, 1036. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 6.07–5.84 (m, 1H), 5.32 (dd, *J*=35.2, 17.1 Hz, 2H), 4.59 (ddd, *J*=5.0, 10.0, 12.5 Hz, 1H), 4.38 (d, *J*=6.6 Hz, 1H), 4.51–4.31 (m, 1H), 4.14 (ddd, *J*=1.1, 6.5, 12.6 Hz, 1H), 4.01 (dd, *J*=8.0, 9.7 Hz, 1H), 3.75–3.64 (m, 1H), 3.47 (dd, *J*=7.2, 13.0 Hz, 1H), 3.21 (dd, *J*=3.4, 13.0 Hz, 1H), 2.71 (br s, 1H), 2.41–2.19 (m, 1H), 2.02 (q, *J*=12.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz, ppm): δ 133.3, 118.8, 101.3, 85.7, 71.8, 71.2, 70.5, 53.8, 33.1. HRMS (CI) *m/z* calcd for $C_9H_{14}O_5N_4$ $(M+H)^+$: 259.1042, found: 259.1049.

5.1.9. Propyl 3,6-diamino-3,4,6-trideoxy-2-*O*-triethylsilyl-β-D-glucopyranoside. Compound **8** (30 mg, 0.081 mmol) was dissolved in MeOH, Raney-Ni in MeOH (7 mL) was added and the hydrogenation was performed in a Parr apparatus overnight at 3 bar, followed by filtration over Hyflo-supercel, and evaporation of the solvent to yield the compound as a white powder. IR ν_{max} film: (cm^{-1}) 3369, 2955, 1582, 1113, 1078, 823, 741. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 4.13 (br d, *J*=7.4 Hz, 1H), 3.77 (dd, *J*=7.3, 16.4 Hz, 1H), 3.57–3.34 (m, 2H), 3.27 (br s, 1H), 3.12–2.94 (m, 1H), 2.79–2.58 (m, 2H), 1.75 (dd, *J*=3.0, 12.8 Hz, 1H), 1.19 (dq, *J*=7.2, 14.3 Hz, 2H), 0.92 (dd, *J*=11.9, 24.2 Hz, 1H), 1.04–0.83 (m, 12H), 0.77–0.58 (m, 6H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 104.6, 79.0, 75.0, 72.0, 54.7, 46.7, 36.5, 24.3, 11.1, 7.4, 6.3. HRMS (ESI) *m/z* calcd for $C_{15}H_{35}O_3N_2Si$ $(M+H)^+$: 319.2417, found: 319.2404.

5.1.10. Propyl 3,6-diamino-3,4,6-trideoxy-β-D-glucopyranoside (9). The diamino glucopyranoside (26 mg, 0.081 mmol) was dissolved in a 2 M HCl solution in EtOAc (5 mL). The reaction mixture was stirred for 1.5 h and the solvent was evaporated and the residue was dissolved in *t*-BuOH and evaporated. This was repeated two times, to yield compound **9** as a white powder (22 mg, quant, two steps). $[\alpha]_D^{20}$ −3.5 (*c* 1.1, H_2O). ^{1}H NMR (D_2O, 300 MHz, ppm): δ 4.53 (d, 1H, *J*=7.2 Hz), 4.10–3.87 (m, 2H), 3.71–3.60 (m, 1H), 3.51–3.42 (m, 2H), 3.40–2.30 (m, 1H), 3.22–3.05 (m, 1H), 2.32–2.15 (m, 1H), 1.81–1.58 (m, 3H), 0.95 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (D_2O, 75 MHz, ppm): δ 103.1, 73.3, 71.5, 69.5, 52.3, 43.2, 32.2, 23.2, 10.7. HRMS (CI) *m/z* calcd for $C_9H_{20}O_3N_2$ $(M+H)^+$: 205.1539, found: 205.1552.

5.1.11. Allyl (2′-*C*-allyl-3′,4′-di-*O*-benzyl-2′,6′-dideoxy-α/β-D-glucopyranosyl)-(1′,2)-6-azido-3,4,6-trideoxy-3-nitro-β-D-glucopyranoside (11). AgOTf (142 mg, 0.552 mmol) was co-evaporated three times with toluene and activated molecular sieves (50 mg) were added. In a separate flask, compounds **8** (45 mg, 0.18 mmol) and **10** (93 mg, 0.19 mmol) were co-evaporated three times with toluene after which DCE (3 mL) and TTBP (137 mg, 0.552 mmol) were added and the mixture was stirred for 30 min, before transferring the solution under argon to the flask containing AgOTf (at 0 °C). The reaction mixture was stirred overnight at room temperature. The reaction mixture was quenched with 50 equiv pyridine and filtered through a layer of Hyflo-supercel. The solvents were evaporated and flash column chromatography (EtOAc/*n*-heptane, 1/20 to 1/2) gave product **11** (54 mg, 45%) as colorless oil and **12** (23 mg, 32%) as well as remaining starting material (4 mg, 9%). R_f 0.45 (EtOAc/*n*-heptane, 1/1). IR ν_{max} film: (cm^{-1}) 2915, 2098, 1557, 1453, 1371, 1283, 1066, 919,

740, 699. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.42–7.21 (m, 10H), 6.09–5.69 (m, 2H), 5.51–5.35 (m, 1H), 5.27–5.21 (m, 1H), 5.15–4.95 (m, 3H), 4.92–4.81 (m, 3H), 4.72–4.65 (m, 4H), 4.49–4.31 (m, 2H), 4.32–4.04 (m, 2H), 3.82–3.62 (m, 1H), 3.51–3.35 (m, 6H), 2.52–2.32 (m, 1H), 2.28–2.10 (m, 2H), 1.91–1.82 (m, 1H). HRMS (FAB) *m/z* calcd for $C_{32}H_{40}O_8N_7$ $(M+H)^+$: 650.2938, found: 650.2918.

5.1.12. 1,5-Anhydro-2-allyl-2,6-dideoxy-D-arabino-hex-l-enitol (12). Yield 23 mg, 32%: R_f 0.75 (EtOAc/*n*-heptane, 1/2). IR ν_{max} film: (cm^{-1}) 3336, 2070, 1120, 1090, 973, 823, 697. ^{1}H NMR (CD_3OD, 400 MHz, ppm): δ 7.39–7.23 (m, 10H), 6.23 (s, 1H), 5.81–5.52 (m, 1H), 5.11–4.91 (m, 2H), 4.66 (s, 2H), 4.52 (dd, J=11.5, 45.9 Hz, 2H), 4.22–4.07 (m, 1H), 3.94 (d, J=4.3 Hz, 1H), 3.84 (dd, J=4.3, 5.5 Hz, 1H), 3.65 (dd, J=7.2, 13.2 Hz, 1H), 3.45–3.22 (m, 1H), 2.72 (dq, J=6.3, 15.4 Hz, 2H). ^{13}C NMR (CD_3OD, 75 MHz, ppm): δ 139.4, 135.8, 114.6, 110.5, 75.0, 73.3, 73.3, 71.8, 70.9, 49.4, 32.4. HRMS (EI) *m/z* calcd for $C_{23}H_{26}O_3N_3$ $(M+H)^+$: 392.1974, found: 392.1979.

5.1.13. (2*R*,3*S*,4*R*,4*R*,9*R*,11*S*,13*S*,13*R*)-2,11-Bis(azidomethyl)-3,4-bis(benzyloxy)-13-nitro-2,3,4,4,5,8,9,11,12,13,13,14-dodecahydrodipyrano[2,3-2′,3′][1,4]dioxecine (13). Compound **11** (6 mg, $9\,10^{-3}$ mmol) was co-evaporated three times with toluene and dissolved in toluene (0.5 mL) again. Argon was bubbled through the solution for 10 min. To the reaction mixture was added Hoveyda–Grubbs catalyst[31] (9.8 mg, $15\,10^{-3}$ mmol). The mixture was stirred for 4 h at room temperature and evaporated. Column chromatography (EtOAc/*n*-heptane, 1/10 to 1/1) gave **13** (6 mg, 99%) as a white solid. R_f 0.34 (EtOAc/*n*-heptane, 1/1). IR ν_{max} film: (cm^{-1}) 2924, 2100, 1557, 1067, 1028, 916. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 7.39–7.21 (m, 10H), 5.41–4.32 (m, 12H), 4.30–4.04 (m, 2H), 3.81–3.62 (m, 1H), 3.51–3.32 (m, 6H), 2.52–2.32 (m, 1H), 2.28–2.10 (m, 2H), 1.91–1.82 (m, 1H). HRMS (ESI) *m/z* calcd for $C_{30}H_{35}O_8N_7$ $(M+Na)^+$: 644.2445, found: 644.2450.

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