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Stereospecific synthesis of methyl 2-amino-2,4-dideoxy-6*S*-deuterio-α-D-*xylo*-hexopyranoside and methyl 2-amino-2,4-dideoxy-6*S*-deuterio-4-propyl-α-D-glucopyranoside: Side chain conformation of the novel aminoglycoside antibiotic propylamycin

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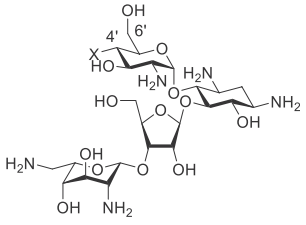
A B S T R A C T

The stereospecific syntheses of methyl 2-amino-2,4-dideoxy-4-C-propyl-α-D-glucopyranoside and of methyl 2-amino-2,4-dideoxy-α-D-*xylo*-hexopyranoside and of their 6*S*-deuterioisotopomers are described as models for ring I of the aminoglycoside antibiotics propylamycin and 4′-deoxyparomomycin, respectively. Analysis of the ^{1}H NMR spectra of these compounds and of methyl 2-amino-2-deoxy-α-D-glucopyranoside, a model for paromomycin itself, reveals that neither deoxygenation at the 4-position, nor substitution of the C–O bond at the 4-postion by a C–C bond significantly changes the distribution of the side chain population between the three possible staggered conformations. From this it is concluded that the beneficial effect on antiribosomal and antibacterial activity of the propyl group in propylamycin does not derive from a change in side chain conformation. Rather, enhanced basicity of the ring oxygen and increased hydrophobicity and/or solvation effects are implicated.

1. Introduction

Propylamycin, 4′-deoxy-4′-C-propylparomomycin, **1** is a novel synthetic 4,5-disubstituted 2-deoxystreptamine class aminoglycoside antibiotic that shows increased antibacterial and antibacterioribosomal activity compared to the parent paromomycin **2**, as well as reduced ototoxicity in the guinea pig model [1]. 4′-Deoxyparomomycin **3** on the other hand is somewhat less active than the parent **2**, with which it shows no significant difference in selectivity for prokaryotic over eukaryotic ribosomes [1,2].



1, X = Pr, propylamycin
2, X = OH, paromomycin
3, X = H, 4′-deoxyparomomycin

In this paper we explore the hypothesis that substitution at the 4′-position in ring I of paromomycin affects the distribution of the ring I side chain between the three staggered conformations, *gauche,gauche* (*gg*), *gauche,trans* (*gt*) and *trans,gauche* (*tg*) (Fig. 1) [3–5] and so influences a critical hydrogen bonding interaction between the aminoglycoside and the drug binding pocket in the decoding A site of helix 44 on the small ribosomal subunit.

It is known that binding of deoxystreptamine type aminoglycosides to the decoding A site on the bacterial ribosome locks the side chain of ring I into the *gt* conformation by virtue of its participation in a pseudo-base pair interaction with A1408 [6,7]. Indeed, we have demonstrated that a model of **2** with the ring I side locked into the *gt* conformation shows activity and selectivity approaching that of propylamycin [8]. On the basis of these observations we were driven to study the influence of the 4′-deoxy and 4′-deoxy-4′-C-propyl modifications on the side chain conformation of ring I in the paromomycin series. In view of the complexity of the NMR spectra of the aminoglycosides, and the need for unambiguous assignment of the diastereotopic side chain protons H6*R* and H6*S*, we elected to study simplified monosaccharide models of ring I. To this end we describe the synthesis of methyl 2-amino-2,4-dideoxy-α-D-*xylo*-hexopyranoside **4α** and methyl 2-amino-2,4-dideoxy-4-C-

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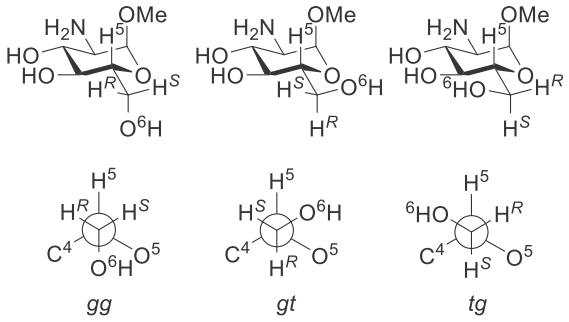
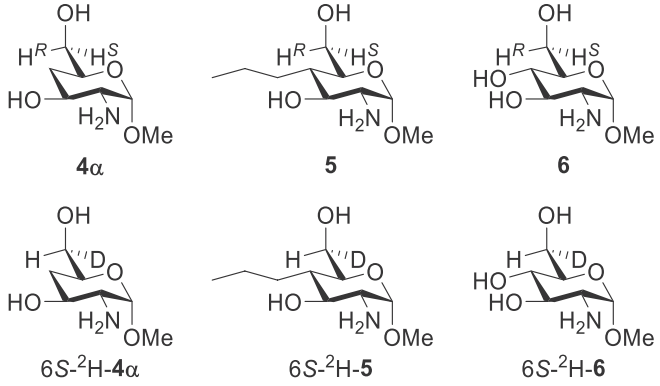


Fig. 1. Staggered conformations of the hexopyranose side chain.

propyl-α-D-glucopyranoside **5**, and for purposes of spectral elucidation their 6*S*-deuterio isotopomers, and the assignment of their side chain conformations by analysis of the 5,6*R* and 5,6*S* ^{1}H,^{1}H ^{3}J coupling constants. Methyl 2-amino-2-deoxy-α-D-glucopyranoside **6** [9] and its 6*S*-deuterio isotopomer is employed as model for ring I of paromomycin itself.



2. Results and discussion

2.1. Synthesis

Methyl 2-amino-2,4-dideoxy-α-D-*xylo*-hexopyranoside **4α** was obtained by hydrogenolysis of the corresponding benzylcarbamate **7**, which was prepared according to Mayer (Scheme 1) [10]. The synthesis of its 6*S*-deuterio isotopomer began with stereospecific introduction of deuterium into the 6-*exo*-position of 1,6-anhydro-D-glucose giving **8** under radical conditions following the literature protocol [11,12], as modified in our laboratory [9] by replacement of tetrachloromethane by the more environmentally friendly α,α,α-trifluorotoluene [13]. Following the established protocol, **8** was converted to the epoxide **10** in two straightforward steps [9,14–16]. Ring opening of **10** with $BF_3{\cdot}OEt_2$ and $NaBH_4$ in dimethoxyethane gave **11** in 99% yield (Scheme 1), which, on treatment with sodium methoxide formed the 2,3-epoxide **12** in 99% yield. Microwave heating of **12 to** 110 °C in DMF with lithium azide and benzoic acid afforded 43% of a 1.3:1 mixture of regioisomeric azidoalcohols **13** and **14** from which 5% of the desired **14** was isolated by HPLC purification. Ring opening of **14** with TFA and acetic anhydride was followed by Fischer glycosylation in methanolic HCl and hydrogenolysis, resulting in an 85% yield of the target 6*S*–^{2}H-**4** in a mixture with the corresponding β-anomer, from which it could not be easily separated. Nevertheless, the NMR spectra of 6*S*–^{2}H-**4α** in the mixture of anomers were well resolved, and permitted unambiguous assignment of the resonances from the diastereotopic 6-H_R and 6-H_S in isotopomers **4α**.

The synthesis of methyl 2-amino-2,4-dideoxy-4-propyl-α-D-glucopyranoside (Scheme 2) began with the 3,4-epoxy tosylate **10**, which was converted to the alcohol **15** by treatment with allylmagnesium chloride in the presence of copper(I) iodide and then to the 2,3-epoxide **16** with sodium methoxide as described in the literature [17,18]. Opening of epoxide **16** with sodium azide under a variety of conditions was unsuccessful, but heating in benzylamine to 155 °C gave the Fürst-Plattner [19] product **17** in 77% yield (Scheme 2). Hydrogenolysis of the benzyl amine and saturation of the double bond gave the amine **18** in 79% yield, which was converted with Stick's reagent [20–23] to the corresponding azido derivative **19** in 91% yield. Ring opening of **19** with TFA and acetic anhydride followed by Fischer glycosylation in methanolic HCl resulted in a 1.5:1 anomeric mixture of **20** in 69% yield, from which the individual anomers **20α** and **20β** were isolated in 22% and 12% yield, respectively. Hydrogenolysis of **20α** gave the acetate salt **5** in 96% yield (Scheme 2). The isotopomer 6*S*–^{2}H-**5** was synthesized analogously and with comparable yield from 6-*exo*-deuterio **10**. Comparison of the ^{1}H NMR spectra of **5** and its 6*S*-deuterio isotopomer enabled assignment of the diastereotopic protons at the 6-position.

The differing regioselectivity in the ring openings of epoxides **12** and **16** with lithium azide and benzylamine, respectively, is of interest. The opening of **16** with benzylamine (Scheme 2) is consistent with the Fürst-Plattner rule and stereoelectronic control, with 1,3-diaxial interactions between the incoming nucleophile and the propyl substituent minimized due to the flattening of the pyranose ring imposed by the 1,6-anhydro bridge [24,25] and consistent with the anti-reflex effect [26,27]. On the other hand, the opening of **12** with azide affords a mixture of regioisomers (Scheme 1) in which the Fürst-Plattner product **14**, formed directly in a chair conformation with *trans*-diaxial substituents, is minor. In contrast the major isomer **13** is necessarily formed in a twist boat conformation before relaxation to the observed chair with its two equatorial substituents. Presumably this diversion from the usual Fürst-Plattner [19,28] is due to the build up of positive charge on carbon during the ring opening of the protonated epoxide by azide, which is better accommodated on C3 adjacent to the methylene group than on C2 adjacent to the electron-withdrawing anomeric center (Scheme 3).

A related example was recently observed in a synthesis of bradyrhizose [29], wherein a cyclohexane-based trisubstituted epoxide underwent ring opening under acidic conditions in an anti-Fürst Plattner manner; an observation that was rationalized by preferential localization of partial positive charge on the tertiary carbon at the transition state (Scheme 4).

2.2. Analysis of side chain populations

The $^{3}J_{H5,H6R}$ and $^{3}J_{H5,H6S}$ coupling constants for **4α** and **5**, taken from ^{1}H NMR spectra recorded at pH 5 in D_2O at room temperature, are reported in Table 1. The corresponding coupling constants for methyl 2-amino-2-deoxy-α-D-glucopyranoside **6** (Table 1) are literature values from our laboratory recorded under the same conditions [9].

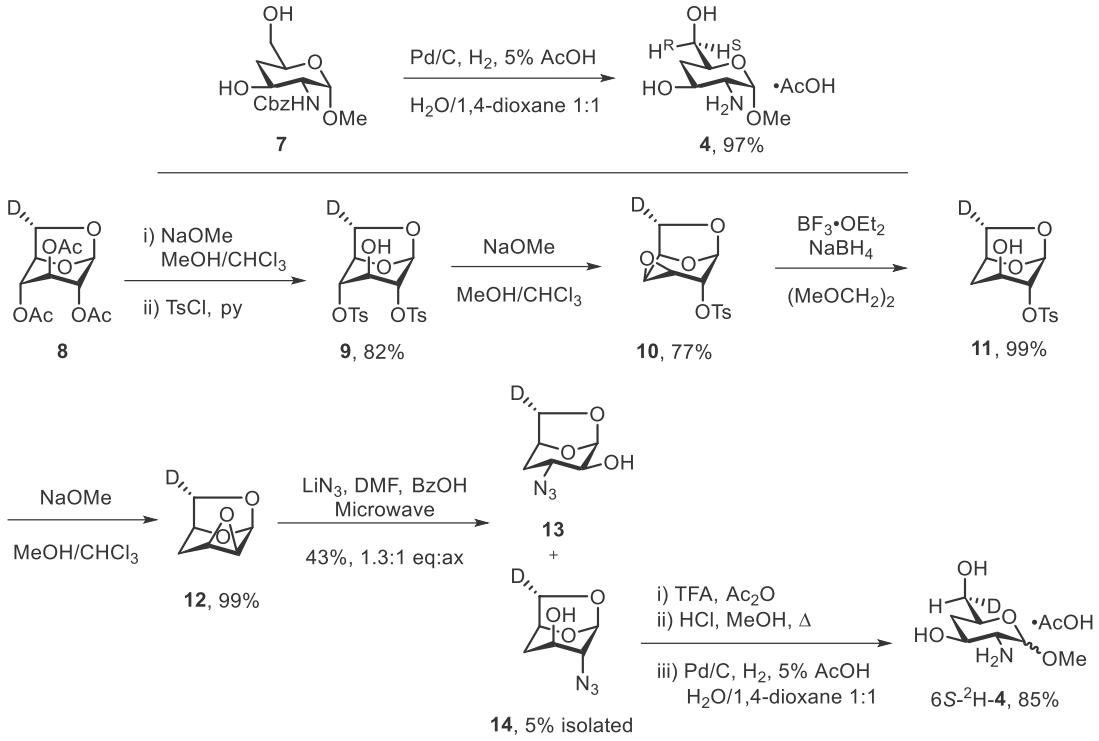
Using limiting values of $^{3}J_{R,gg}$ - $^{3}J_{S,tg}$ for each of the pure *gg*, *gt*, and *tg* conformers (Table 2) taken from a recent study employing a series of conformationally rigid bicyclic models [30], the side chain populations of all compounds ($f_{gg} - f_{tg}$ (Table 3)), were determined in the standard manner [3,31–33] with the aid of eqs (1)–(3). The population of −2% calculated for the *tg* conformers of **6** and **5** is an artifact and arises from the errors in the coupling constants, estimated from the digital resolution of the spectra to be 0.4 Hz, and is considered to be indistinguishable from a 0% population.

$$^{3}J_{H5,H6R} = {}^{3}J_{R,gg}f_{gg} + {}^{3}J_{R,gt}f_{gt} + {}^{3}J_{R,tg}f_{tg} \quad \text{eq 1}$$

$$^{3}J_{H5,H6S} = {}^{3}J_{S,gg}f_{gg} + {}^{3}J_{S,gt}f_{gt} + {}^{3}J_{S,tg}f_{tg} \quad \text{eq 2}$$

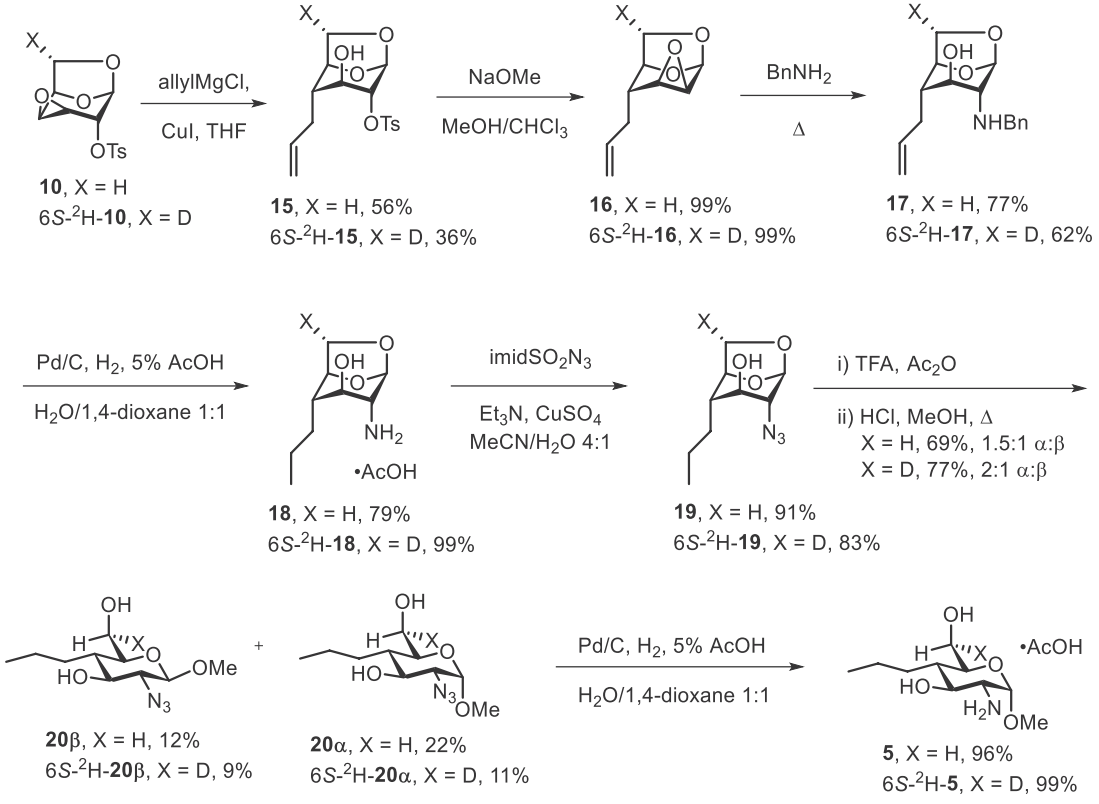
$$1 = f_{gg} + f_{gt} + f_{tg} \quad \text{eq 3}$$

A modest increase in the population of the *gt* conformation, at the expense of a corresponding decrease in the *gg* conformation, is observed on replacement of the equatorial hydroxyl group in methyl 2-amino-2-

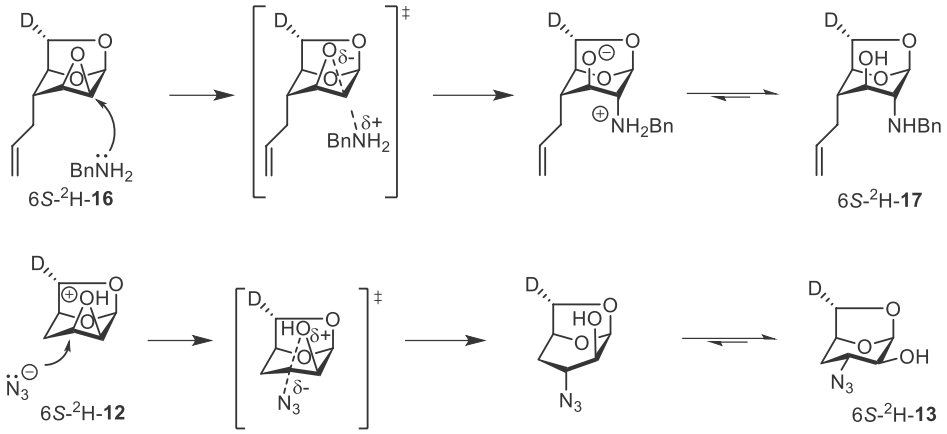


Scheme 1. Synthesis of Methyl 2-amino-2,4-dideoxy–α-D-*xylo*-hexopyranoside **4** and its 6*S*-Deuterio Isotopomer.

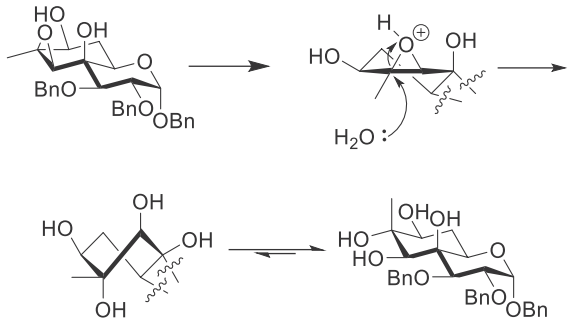
deoxy-α-D-glucopyranoside **6** by a proton in methyl 2-amino-2,4-dideoxy-α-D-glucopyranoside **4α** (Table 3, entries 1 and 2). This pattern of changes mirrors that observed earlier by Bock and Duus [3] on going from methyl α-D-glucopyranoside to methyl 4-deoxy-α-D-glucopyranoside, supporting our earlier conclusions that the presence of protonated amino group at the 2-position has little influence on the side chain conformation [9]. Replacement of the hydroxyl group in **6** by the propyl group in methyl 2-amino-2,4-dideoxy-4-propyl-α-D-glucopyranoside **5** (Table 3, entries 1 and 3) results in a side chain population that is intermediate between that of **6** and **4α**. Clearly, the overall



Scheme 2. Synthesis of Methyl 2-amino-2,4-dideoxy-4-propyl–α-D-glucopyranoside **5** and its 6*S*-Deuterio Isotopomer.



Scheme 3. Differing Mechanisms and Regioselectivities in the Openings of Epoxides **12** and **16**.



Scheme 4. Anti-Fürst-Plattner Regioselectivity in the Opening of an Epoxide en route to Bradyrhizose.

Table 1
Experimental chemical shifts and $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ coupling constants.

Ring I Model	δ H5 (ppm)	δ H6*R* (ppm)	δ H6*S* (ppm)	$^3J_{H5,H6R}$ (Hz)	$^3J_{H5,H6S}$ (Hz)
6	3.53	3.59	3.77	4.9	2.2
4α	3.79	3.43	3.51	5.8	2.5
5	3.62	3.54	3.66	5.3	2.2

Table 2
Limiting coupling constants for the ideal staggered conformers.

	gg	*gt*	*tg*
^3J_R (Hz)	1.0	11.0	4.8
^3J_S (Hz)	2.2	2.5	10.2

Table 3
Side chain populations of the model compounds.

	f_{gg}	f_{gt}	f_{tg}
6	62	40	−2
4	51	47	2
5	58	44	−2

influence of the 4-OH group on the side chain conformation of **6**, whether derived from dipolar interactions, hydrogen bonding, and/or solvation effects, is qualitatively reproduced by the propyl group in **5**.

3. Conclusion

Analysis of the model compounds **6**, **4α**, and **5** indicates that neither deoxygenation nor substitution of the C–O bond at the 4-position by a C–C bond causes a significant change in the relative distribution of the side chain conformation between the three staggered conformations. Consequently, the increased activity of propylamycin in the inhibition of bacterial ribosomes and the inhibition of bacterial growth does not derive from a reduction in the entropic penalty paid on binding to the ribosome with a fixed *gt* side chain conformation. As suggested previously [1] any reduction in binding energy to the ribosome on deoxygenation at the 4′-position of paromomycin, due to the loss of a hydrogen bond to a backbone phosphate, is offset by the increased basicity of the ring oxygen (O5′) and the concomitant increase in the critical hydrogen bond with A1408 in the binding pocket. The difference in activity between the 4′-deoxy derivative **3** and propylamycin **1** with the 4′-deoxy-4′-propyl substitution pattern is therefore presumably related to hydrophobicity and/or solvation.

4. Experimental

4.1. General experimental

All reagents were purchased from commercial sources and used without further purification unless otherwise specified. Chromatographic purifications were carried out in Fisher 230–400 mesh silica gel unless otherwise specified. Microwave reactions were conducted with a Biotage Initiator microwave reactor. High resolution mass spectra were collected on a Waters LCT Premier XE ESI-TOF mass spectrometer. Optical rotations were measured using an automated polarimeter in a 1 dm cell. NMR spectra were collected at 400, 500, or 600 MHz as indicated. ^{1}H NMR spectra were assigned with the aid of 1D and 2D techniques including COSY, HSQC, HMBC, and TOCSY.

Methyl 2-amino-2,4-dideoxy-α-D-*xylo*-hexopyranoside acetate salt (4α). Compound **7** (93.8 mg, 0.30 mmol) was dissolved in a 1:1 mixture of 1,4-dioxane and 10% aqueous AcOH (0.8 mL) followed by addition of Pd/C (20.8 mg). The reaction mixture was stirred under 50 psi H_2 for 5 h followed by filtration over Celite® and lyophilization to obtain **4** as an off white solid (69.1 mg, 97%). $[\alpha]_D^{23}$ = 110.6 (*c* = 0.14, water), ^{1}H NMR (600 MHz, D_2O) δ 4.86 (d, *J* = 3.7 Hz, 1H, H1), 3.91 (td, *J* = 11.0, 5.1 Hz, 1H, H3), 3.79 (ddt, *J* = 11.8, 5.8, 2.5 Hz, 1H, H5), 3.51 (dd, *J* = 12.1, 3.1 Hz, 1H, H6), 3.43 (dd, *J* = 12.1, 6.2 Hz, 1H, H6'), 3.24 (s, 3H, OMe), 3.02 (dd, *J* = 10.5, 3.6 Hz, 1H, H2), 1.86 (ddd, *J* = 12.8, 5.1, 2.1 Hz, 1H, H4eq), 1.76 (s, 3H, AcOH), 1.33 (q, *J* = 12.0 Hz, 1H, H4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.4 (AcOH), 96.6 (C1), 68.8 (C5), 64.3 (C3), 63.3 (C6), 55.2 (C2), 55.0 (OMe), 34.2 (C4), 22.6 (AcOH). ESI-HRMS: *m/z* calcd for $C_7H_{15}NO_4Na$ $[M + Na]^+$ 200.0899, found 200.0891.

1,6-Anhydro-4-deoxy-2-*O*-*p*-toluenesulfonyl-6*S*-deuterio-D-glucopyranose (11). $NaBH_4$ (0.43 g, 11.4 mmol) and $BF_3{\cdot}OEt_2$ (0.85 mL,

6.9 mmol) were added to an ice cold solution of **10** (0.84 g, 2.8 mmol) in 1,2-dimethoxyethane (9.4 mL). After 5 h the reaction mixture was diluted with Et_2O and washed with saturated $NaHCO_3$ solution and brine then dried with Na_2SO_4 and concentrated under vacuum to give **11** as a clear gum (0.85 g, 2.8 mmol, 99%) which was used in the next step without purification. $[\alpha]_D^{23} = -27.0$ (c = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 2H, Ar–H), 7.36–7.33 (m, 2H, Ar–H), 5.27 (d, J = 1.9 Hz, 1H, H-1), 4.52 (d, J = 4.1 Hz, 1H, H-5), 4.20 (q, J = 1.7 Hz, 1H, H-2), 4.09 (s, 1H, H-6), 3.92 (t, J = 6.3 Hz, 1H, H-3), 2.46 (d, J = 6.3 Hz, 1H, –OH), 2.44 (s, 3H, Ar-CH_3), 2.31 (ddd, J = 15.0, 5.7, 4.5 Hz, 1H, H-4ax), 1.70 (ddt, J = 15.0, 1.9, 1.0 Hz, 1H, H-4eq). ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.3, 133.2, 130.1, 127.9 (Ar), 99.3 (C-1), 76.8 (C-2), 71.3 (C-5), 67.5 (t, J = 23.3 Hz, C-6), 66.5 (C-3), 32.5 (C-4), 21.7 (Ar-CH_3). ESI-HRMS: m/z calcd for $C_{13}H_{15}DO_6SNa$ $[M + Na]^+$ 324.0628, found 324.0627.

1,6;2,3-Bisanhydro-4-deoxy-6*S*-deuterio-D-lyxopyranose (12). NaOMe (0.34 g, 6.3 mmol) was added to an ice cold stirred solution of **11** (0.824 g, 2.73 mmol) in 1:1 $CHCl_3$/MeOH (13.6 mL). After 4 h the reaction mixture was diluted with CH_2Cl_2 and washed with water. The aqueous phase was back extracted with CH_2Cl_2 and the combined organic layers were washed with brine then dried with Na_2SO_4 and concentrated under vacuum to give **12** as a colorless oil (0.351 g, 99%). $[\alpha]_D^{23} = -28.6$ (c = 1.0, CH2CL2), ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (dd, J = 3.2, 1.0 Hz, 1H, H-1), 4.38 (br d, J = 6.0 Hz, 1H, H-5), 3.63 (d, J = 1.7 Hz, 1H, H-6), 3.33 (t, J = 3.6 Hz, 1H, H-2), 3.10 (dd, J = 3.3 Hz, 1H, H-3), 2.19 (ddd, J = 15.3, 5.9, 3.2 Hz, 1H, H-4ax), 1.94 (d, J = 15.3 Hz, 1H, H-4eq). ^{13}C NMR (101 MHz, $CDCl_3$) δ 98.0 (C-1), 68.0 (t, J = 23.1 Hz, C-6), 67.2 (C-5), 53.7 (C-2), 46.3 (C-3), 30.0 (C-4). ESI-HRMS: m/z calcd for $C_6H_7DO_3Na$ $[M + Na]^+$ 152.0434, found 152.0438.

1,6-Anhydro-2-azido-2,4-dideoxy-D-*xylo*-hexopyranose (14). Compound **12** (75.0 mg, 0.58 mmol), LiN_3 (145 mg, 2.96 mmol), benzoic acid (109 mg, 0.89 mmol), and DMF (2.0 mL) were added to a microwave vial and heated to 110 °C for 75 min in a microwave reactor. The reaction mixture was then diluted with CH_2Cl_2 and washed with aqueous saturated $NaHCO_3$ solution and brine. The organic layer was dried with Na_2SO_4 and filtered followed by concentration. The residue was subjected to silica gel preparative HPLC eluting with a gradient from 20% to 60% EtOAc in hexanes to give **14** (4.7 mg, 0.027 mmol, 5%). $[\alpha]_D^{23} = 19.6$ (c = 0.2, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.53 (t, J = 2.0 Hz, 1H, H-1), 4.59 (d, J = 4.4 Hz, 1H, H-5), 4.19 (s, 1H, H-6), 4.00–3.96 (m, 1H, H-3), 3.30 (d, J = 2.2 Hz, 1H, H-2), 2.55 (d, J = 7.7 Hz, 1H, –OH), 2.31 (dt, J = 15.2, 4.9 Hz, 1H, H-4ax), 1.84 (ddt, J = 15.3, 2.9, 1.6 Hz, 1H, H-4eq). ^{13}C NMR (151 MHz, $CDCl_3$) δ 100.8 (C-1), 72.0 (C-5), 67.6 (t, J = 23.3 Hz, C-6), 66.9 (C-3), 61.7 (C-2), 33.6 (C-4).

Methyl 2-amino-2,4-dideoxy-6*S*-deuterio-D-*xylo*-hexopyranoside acetate salt (6*S*–^{2}H-4). TFA (0.05 mL) was added to a stirred solution of **14** (4.6 mg, 0.27 mmol) in Ac_2O (0.5 mL) at 0 °C and the reaction mixture was stirred for 3 h with monitoring by TLC. The reaction mixture was then diluted with Et_2O and washed with aqueous saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated to give an inseparable mixture of anomers (8.3 mg, 0.026 mmol, 97%), which were used in the next step without purification. ESI-HRMS: m/z calcd for $C_{12}H_{16}DN_3O_7Na$ $[M + Na]^+$ 339.1014, found 339.1027. The mixture of anomers from the previous step (8.3 mg, 0.026 mmol) were stirred in a 10% HCl methanol solution (0.6 mL) under reflux for 3 h. After the reaction was complete by TLC and LCMS, the reaction mixture was concentrated under vacuum and the resulting inseparable mixture of anomers of methyl glycosides was used in the next step without purification. ESI-HRMS: m/z calcd for $C_7H_{12}DN_3O_4Na$ $[M + Na]^+$ 227.0867, found 227.0864. 10 wt% Pd/C (5.0 mg) was added to a solution of the methyl glycosides (6.1 mg, 0.30 mmol) in 1:1 dioxane/10% aqueous acetic acid (0.4 mL) and the reaction mixture was stirred under 50 psi H_2 for 6 h. The reaction mixture was then filtered through Celite® and concentrated under vacuum to give **6*S*–^{2}H-4** (5.4 mg, 0.023 mmol, 76%) as a mixture of anomers in a ratio of 2:1 α/β as the acetate salts. ESI-HRMS: m/z calcd for $C_7H_{14}DNO_4Na$ $[M + Na]^+$ 201.1962, found 201.1956. **6*S*–^{2}H-4α**: ^{1}H NMR (600 MHz, D_2O) δ 4.87 (d, J = 3.6 Hz, 1H, H-1), 3.92 (td, J = 11.0, 5.0 Hz, 1H, H-3), 3.80 (ddd, J = 12.1, 6.3, 2.2 Hz, 1H, H-5), 3.42 (d, J = 6.0 Hz, 1H, H-6), 3.26 (s, 3H, –OCH_3), 3.03 (dd, J = 10.4, 3.7 Hz, 1H, H-2), 1.87 (ddd, J = 12.1, 5.0, 2.1 Hz, 1H, H-4eq), 1.78 (s, 3H, AcOH), 1.34 (q, J = 12.1 Hz, 1H, H-4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.2 (AcOH), 96.6 (C-1), 68.7 (C-5), 64.3 (C-3), 63.2–62.8 (m, C-6), 55.2 (C-2), 55.0 (-OCH_3), 34.2 (C-4), 22.5 (AcOH). **6*S*–^{2}H-4β**: ^{1}H NMR (600 MHz, D_2O) δ 4.37 (d, J = 8.4 Hz, 1H, H-1), 3.76 (dt, J = 10.9, 5.4 Hz, 1H, H-3), 3.57 (ddd, J = 11.8, 6.7, 2.1 Hz, 1H, H-5), 3.45 (d, J = 6.6 Hz, 1H, H-6), 3.41 (s, 3H, –OCH_3), 2.69 (dd, J = 10.3, 8.4 Hz, 1H, H-2), 1.91 (ddd, J = 12.9, 5.2, 2.0 Hz, 1H, H-4eq), 1.78 (s, 3H, AcOH), 1.31 (dt, J = 12.9, 11.5 Hz, 1H, H-4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.2 (AcOH), 100.1 (C-1), 72.7 (C-5), 66.8 (C-3), 63.1–62.6 (m, C-6), 57.4 (C-2), 57.2 (-OCH_3), 34.4 (C-4), 22.5 (AcOH).

4-*C*-Allyl-1,6-anhydro-2-*N*-benzyl-2,4-dideoxy-D-glucopyranose (17). A solution of **16** (0.319 g, 1.90 mmol) in benzylamine (5.0 mL) was stirred for 3 days at 155 °C. After concentration under reduced pressure the residue was purified by flash column chromatography on silica gel eluting with 40% ethyl acetate and 1% triethylamine in hexanes to afford **17** (0.402 g, 77%). [α]23D = −49.4 (c = 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38–7.23 (m, 5H: aromatic), 5.83 (ddq, J = 16.4, 14.4, 6.2, 5.3 Hz, 1H: CH_2CHCH_2–C4), 5.47 (s, 1H: H1), 5.21–5.10 (m, 2H: CH_2CHCH_2–C4), 4.40 (d, J = 5.0 Hz, 1H: H5), 4.06 (d, J = 7.0 Hz, 1H: H6a), 3.93–3.85 (m, 5H: PhCH_2), 3.80–3.72 (m, 1H, H6b), 3.65 (td, J = 2.9, 1.6 Hz, 1H: H3), 2.68–2.63 (m, 1H: H2), 2.49–2.33 (m, 2H: CH_2CHCH_2–C4), 1.77 (t, J = 7.2 Hz, 1H: H4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.9, 136.1 (CH_2CHCH_2–C4), 128.5, 128.1, 127.2, 117.5 (CH_2CHCH_2–C4), 102.6 (C1), 74.6 (C5), 70.3 (C3), 68.5 (C6), 62.2 (C2), 51.7 (PhCH_2), 44.6 (C4), 36.5 (CH_2CHCH_2–C4). ESI-HRMS: m/z calcd for $C_{16}H_{22}NO_3Na$ $[M+Na]^+$ 276.1600, found 276.1600.

2-Amino-1,6-anhydro-2,4-dideoxy-4-*C*-propyl-D-glucopyranose acetate salt (18). Compound **17** (0.4020 g, 1.46 mmol) and Pd/C (0.1316 g) were stirred in a 1:2 mixture of 10% aqueous acetic acid and 1,4-dioxane (6 mL) under 40 psi H_2 for 7 h. Pd/C (0.23 g) was added after 7 h and the reaction mixture was stirred for an additional 14 h before a final addition of Pd/C (0.39 g). After an additional 28 h the reaction mixture was filtered through Celite® and concentrated to give **18** (0.286 g, 79%). $[\alpha]_D^{23} = -45.9$ (c = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 5.32 (s, 1H, H-1), 4.41 (d, J = 5.2 Hz, 1H, H-5), 3.98 (d, J = 6.8 Hz, 1H, H-6a), 3.68 (dd, J = 6.9, 5.3 Hz, 1H, H-6b), 3.43 (t, J = 4.0 Hz, 1H, H-3), 2.82 (d, J = 4.0 Hz, 1H, H-2), 1.92 (s, 3H, AcOH), 1.64–1.47 (m, 4H, H-4, –CH_2CH_2-), 1.45–1.34 (m, 1H, –CH_2CH_2-), 0.97 (t, J = 7.3 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 104.9 (C-1), 79.1 (C-5), 74.6 (C-6), 72.6 (C-3), 60.7 (C-2), 48.8 (C-4), 37.9 (-**C**$H_2CH_2CH_3$), 25.4 (AcOH), 23.9 (-CH_2**C**H_2CH_3), 16.9 (-CH_2CH_2**C**H_3). ESI-HRMS: m/z calcd for $C_9H_{18}NO_3$ $[M + H]^+$ 188.1287, found 188.1286.

1,6-Anhydro-2-azido-2,4-dideoxy-4-*C*-propyl-D-glucopyranose (19). Stick's reagent (0.466 g, 2.23 mmol) was added to an ice cold stirred solution of $CuSO_4$ (0.024 g, 0.15 mmol), triethylamine (0.62 mL, 4.5 mmol), and **18** (0.278 g, 1.12 mmol) in 4:1 MeCN/water (14.8 mL). The reaction mixture was stirred for 1 h before MeCN was removed under vacuum and the residue was diluted with EtOAc, washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4 and concentrated to give **19** as a colorless gum (0.219 g, 91%). $[\alpha]_D^{23} = -113.2$ (c = 1.0, $CHCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.46 (t, J = 1.4 Hz, 1H, H-1), 4.43 (d, J = 5.1 Hz, 1H, H-5), 4.10 (dd, J = 7.0, 0.8 Hz, 1H, H-6a), 3.78 (dd, J = 7.1, 5.1 Hz, 1H, H-6b), 3.65 (tt, J = 2.4, 1.2 Hz, 1H, H-3), 3.47 (t, J = 2.0 Hz, 1H, H-2), 1.72–1.56 (m, 3H, H-4, -**C**$H_2CH_2CH_3$), 1.55–1.45 (m, 1H, -CH_2**C**H_2CH_3), 1.44–1.34 (m, 1H, -CH_2**C**H_2CH_3), 0.97 (t, J = 7.2 Hz, 3H, –CH_2CH_2**C**H_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 100.6 (C-1), 75.0 (C-

5), 71.2 (C-3), 68.6 (C-6), 63.6 (C-2), 44.3 (C-4), 33.3 ($CH_3CH_2\mathbf{CH_2}$-), 20.5 ($CH_3\mathbf{CH_2}CH_2$-), 14.0 ($\mathbf{CH_3}CH_2CH_2$-).

Methyl 2-azido-2,4-dideoxy-4-*C*-propyl-D-glucopyranoside (20α and 20β). Compound **19** (0.219 g, 1.00 mmol) was dissolved in Ac_2O (8.6 mL) followed by addition of TFA (0.86 mL) and stirred under argon for 45 min. The reaction mixture was then diluted with Et_2O and washed with saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, filtered, and concentrated under vacuum. The residue was then dissolved in 10% HCl MeOH solution (7 mL) and heated to reflux for 4.5 h followed by concentration under vacuum to give a 1.5:1 mixture of **20α** and **20β** (0.174 g, 69%). The resulting residue was subjected to flash column chromatography over silica gel in 45%–50% ethyl acetate in hexanes, which afforded **20α** (55 mg, 22%) and **20β** (31 mg, 12%). **20α**: $[\alpha]_D^{23}$ = 125.4 (*c* = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) *δ* 4.76 (d, *J* = 3.5 Hz, 1H, H1), 3.78 (t, *J* = 10.2 Hz, 1H, H3), 3.74–3.68 (m, 1H, H6), 3.62–3.54 (m, 2H, H5, H6), 3.37 (s, 3H, OMe), 3.10 (dd, *J* = 10.0, 3.5 Hz, 1H, H2), 1.62–1.50 (m, 2H, H4, –CH_2CH_2-), 1.49–1.28 (m, 3H, –CH_2CH_2-), 0.91 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD) *δ* 99.2 (C1), 71.8 (C5), 68.5 (C3), 65.2 (C2), 61.8 (C6), 53.9 (OMe), 43.1 (C4), 28.8 (–CH_2CH_2–), 18.7 (–CH_2CH_2–), 13.7 (-CH_3). ESI-HRMS: *m/z* calcd for $C_{10}H_{19}N_3O_4Na$ [M + Na]$^+$ 268.1273, found 268.1273. **20β**: $[\alpha]_D^{23}$ = −31.8 (*c* = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) *δ* 4.13 (d, *J* = 8.1 Hz, 1H, H1), 3.77 (dd, *J* = 12.1, 2.1 Hz, 1H, H6), 3.59 (dd, *J* = 12.1, 5.5 Hz, 1H, H6), 3.52 (s, 3H, OMe), 3.32–3.24 (m, 2H, H3, H5), 3.03 (dd, *J* = 9.5, 8.1 Hz, 1H, H2), 1.58–1.25 (m, 5H, H4, –CH_2CH_2-), 0.90 (t, *J* = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, CD_3OD) *δ* 102.7 (C1), 76.2 (C5), 72.6 (C3), 68.6 (C2), 61.8 (C6), 55.6 (OMe), 42.6 (C4), 28.7 (–CH_2CH_2–), 18.6 (–CH_2CH_2–), 13.7 (-CH_3). ESI-HRMS: *m/z* calcd for $C_{10}H_{19}N_3O_4Na$ [M + Na]$^+$ 268.1273, found 268.1261.

Methyl 2-amino-2,4-dideoxy-4-*C*-propyl-α-D-glucopyranoside (5). Compound **20α** (13.6 mg, 0.30 mmol) was dissolved in a 1:1 mixture of 1,4-dioxane and 10% aqueous AcOH (0.6 mL) followed by addition of Pd/C (2.9 mg). The reaction mixture was stirred under 50 psi H_2 for 1.5 h followed by filtration over Celite® and lyophilization to obtain **5** as an off white solid (15.4 mg, 99%). $[\alpha]_D^{23}$ = 80.5 (*c* = 0.7, water), ^{1}H NMR (600 MHz, D_2O) *δ* 4.84 (d, *J* = 3.6 Hz, 1H, H1), 3.70 (t, *J* = 10.6 Hz, 1H, H3), 3.66 (dd, *J* = 12.3, 2.2 Hz, 1H, H6), 3.62 (ddd, *J* = 10.9, 5.3, 2.2 Hz, 1H, H5), 3.54 (dd, *J* = 12.3, 5.3 Hz, 1H, H6), 3.24 (s, 3H, OMe), 3.07 (dd, *J* = 10.3, 3.6 Hz, 1H, H2), 1.79 (s, 3H, AcOH), 1.49 (tt, *J* = 10.8, 4.0 Hz, 1H, H4), 1.41–1.32 (m, 1H, –CH_2CH_2-), 1.32–1.24 (m, 1H, –CH_2CH_2-), 1.23–1.06 (m, 2H, –CH_2CH_2-), 0.71 (t, *J* = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, D_2O) *δ* 96.3 (C1), 71.6 (H5), 67.0 (C3), 61.1 (6), 55.3 (C2), 54.9 (OMe), 42.0 (C4), 27.7 (–CH_2CH_2–), 17.8 (–CH_2CH_2–), 13.8 (-CH_3). ESI-HRMS: *m/z* calcd for $C_{10}H_{22}NO_4$ [M + H]$^+$ 220.1549, found 220.1539.

4-*C*-Allyl-1,6-Anhydro-6*S*-deuterio-2,4-dideoxy-2-*O*-*p*-toluenesulfonyl-D-glucopyranose (6*S*–^{2}H-15). Freshly prepared 0.5 M allylMgCl THF solution (16 mL) was added to an ice-cold stirred solution of epoxide **6*S*–^{2}H-10** (0.590 g 1.97 mmol) and CuI (0.38 g, 0.20 mmol) under argon in THF (20 mL). The reaction mixture was stirred for 11 h followed by addition of further allylMgCl solution (8 mL). After another 17 h the reaction mixture was concentrated under vacuum then diluted with Et_2O and washed with aqueous saturated NH_4Cl solution and brine. The organic layer was dried with Na_2SO_4 and concentrated. The residue was then purified using silica gel flash column chromatography in 35–40% EtOAc in hexanes to give **6*S*–^{2}H-15** (0.24 g, 36%). $[\alpha]_D^{23}$ = −51.3 (*c* = 1.0, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 7.83–7.78 (m, 2H, Ar–H), 7.37–7.33 (m, 2H, Ar–H), 5.74 (ddt, *J* = 16.3, 10.5, 7.1 Hz, 1H, $CH_2\mathbf{CH}CH_2$-), 5.27 (d, *J* = 1.5 Hz, 1H, H-1), 5.13 (dq, *J* = 6.1, 1.2 Hz, 1H, $\mathbf{CH_2}CHCH_2$-), 5.10 (t, *J* = 1.3 Hz, 1H, $\mathbf{CH_2}CHCH_2$-), 4.39 (s, 1H, H-5), 4.18 (dt, *J* = 2.5, 1.2 Hz, 1H, H-2), 3.99 (s, 1H, H-6), 3.69 (tt, *J* = 2.7, 1.2 Hz, 1H, H-3), 2.45 (s, 3H, Ar$\mathbf{CH_3}$), 2.37–2.33 (m, 2H, $CH_2CH\mathbf{CH_2}$-), 1.68 (t, *J* = 7.7 Hz, 1H, H-4). ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 135.3 ($CH_2\mathbf{CH}CH_2$-), 130.0 (Ar), 127.9 (Ar), 118.0 ($\mathbf{CH_2}CHCH_2$-), 99.7 (C-1), 78.9 (C-2), 74.2 (C-5), 70.0 (C-3), 68.0 (t, *J* = 23.4 Hz, C-6), 43.0 (C-4), 35.4 ($CH_2CH\mathbf{CH_2}$-), 21.7 (Ar$\mathbf{CH_3}$). ESI-HRMS: *m/z* calcd for $C_{16}H_{19}DO_6Na$ [M + Na]$^+$ 364.0941, found 364.0936.

4-*C*-Allyl-1,6;2,3-bisanhydro-6*S*-deuterio-4-deoxy-β-D-mannopyranose (6*S*–^{2}H-16). NaOMe (0.088 g, 1.63 mmol) was added to an ice-cold stirred solution of compound **6*S*–^{2}H-15** (0.240 g, 0.70 mmol) in 1:1 mixture of methanol and chloroform (3.5 mL) and the solution was allowed to warm to room temperature. After 2 h the reaction mixture was diluted with Et_2O and washed with aqueous saturated NH_4Cl solution and brine. The organic layer was dried with Na_2SO_4 and concentrated to give **6*S*–^{2}H-16** (0.118 g, 99%) as a white waxy solid which was used without further purification. $[\alpha]_D^{23}$ = −15.2 (*c* = 1.0, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 5.82 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, $CH_2\mathbf{CH}CH_2$-), 5.65 (d, *J* = 3.2, 1H, H-1), 5.16–5.10 (m, 2H, $\mathbf{CH_2}CHCH_2$-), 4.23 (s, 1H, H-5), 3.70 (d, *J* = 1.6 Hz, 1H, H-6), 3.34 (ddd, *J* = 3.8, 3.1, 0.7 Hz, 1H, H-2), 2.93 (dd, *J* = 4.0, 1.3 Hz, 1H, H-3), 2.32 (tt, *J* = 7.2, 1.3 Hz, 2H, $CH_2CH\mathbf{CH_2}$-), 2.01 (t, *J* = 7.6 Hz, 1H, H-4). ^{13}C NMR (151 MHz, $CDCl_3$) *δ* 135.2 ($CH_2\mathbf{CH}CH_2$-), 117.8 ($\mathbf{CH_2}CHCH_2$-), 98.0 (C-1), 70.9 (C-5), 68.2 (t, *J* = 23.0 Hz, C-6), 53.8 (C-2), 50.4 (C-3), 39.0 (C-4), 35.1 ($CH_2CH\mathbf{CH_2}$-). ESI-HRMS: *m/z* calcd for $C_9H_{11}DO_3Na$ [M + Na]$^+$ 192.0747, found 192.0746.

4-*C*-Allyl-1,6-anhydro-6*S*-deuterio-2-*N*-benzyl-2,4-dideoxy-D-glucopyranose (6*S*–^{2}H-17). A stirred solution of epoxide **6*S*–^{2}H-17** (0.118 g, 0.427 mmol) in benzylamine (5.0 mL) was heated to 155 °C for 1.5 days before concentration under vacuum. The residue was then purified using silica gel column chromatography in 40% EtOAc and 1% triethylamine in hexanes to give amine **6*S*–^{2}H-17** (0.166 g, 86%). $[\alpha]_D^{23}$ = −32.9 (*c* = 1.0, CH2CL2), ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.36–7.30 (m, 4H, Ar–H), 7.29–7.23 (m, 1H, Ar–H), 5.88–5.76 (m, 1H, $CH_2\mathbf{CH}CH_2$-), 5.46 (s, 1H, H-1), 5.16–5.10 (m, 2H, $\mathbf{CH_2}CHCH_2$-), 4.38 (s, 1H, H-5), 4.03 (s, 1H, H-6), 3.90 (d, *J* = 13.2 Hz, 1H, Ph$\mathbf{CH_2}$N-), 3.86 (d, *J* = 13.2 Hz, 1H, Ph$\mathbf{CH_2}$N-), 3.64 (dq, *J* = 3.0, 1.4 Hz, 1H, H-3), 2.64 (p, *J* = 1.2 Hz, 1H, H-2), 2.50–2.30 (m, 2H, $CH_2CH\mathbf{CH_2}$-), 1.76 (t, *J* = 7.7 Hz, 1H, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) *δ* 139.9 (Ar), 136.1 ($CH_2\mathbf{CH}CH_2$-), 128.5 (Ar), 128.1 (Ar), 127.2 (Ar), 117.5 ($\mathbf{CH_2}CHCH_2$-), 102.6 (C-1), 74.6 (C-5), 70.3 (C-3), 68.2 (t, *J* = 23.4 Hz, C-6), 62.2 (C-2), 51.7 (Ph$\mathbf{CH_2}$N-), 44.6 (C-4), 36.5 ($CH_2CH\mathbf{CH_2}$-). ESI-HRMS: *m/z* calcd for $C_{16}H_{21}DNO_3$ [M + H]$^+$ 277.1657, found 277.1664.

2-Amino-1,6-anhydro-6*S*-deuterio-2,4-dideoxy-4-*C*-propyl-D-glucopyranose acetate salt (6*S*–^{2}H-18). Pd/C (10% w/w, 27 mg) was added to a solution of **6*S*–^{2}H-17** (0.137 g, 0.50 mmol) in a 1:1 mixture of 10% aqueous acetic acid and 1,4-dioxane (0.6 mL) followed by pressurization to 40 psi of H_2. The reaction mixture was stirred vigorously for 9 h before filtration through Celite® and concentration under vacuum to give amine **6*S*–^{2}H-18** (0.122 g, 99%) as the acetate salt, which was used without further purification. $[\alpha]_D^{23}$ = −48.6 (*c* = 4.0, MeOH), ^{1}H NMR (600 MHz, D_2O) *δ* 5.41 (s, 1H, H-1), 4.45 (s, 1H, H-5), 3.87 (s, 1H, H-6), 3.47 (t, *J* = 4.6 Hz, 1H, H-3), 3.01 (dd, *J* = 4.4, 1.0 Hz, 1H, H-2), 1.75 (s, 3H, AcOH), 1.53 (tdd, *J* = 6.8, 4.6, 1.5 Hz, 1H, H-4), 1.43–1.37 (m, 2H, $CH_3CH_2\mathbf{CH_2}$-), 1.32 (dp, *J* = 13.3, 7.2 Hz, 1H, $CH_3\mathbf{CH_2}CH_2$-), 1.21 (tdd, *J* = 15.1, 13.4, 7.1 Hz, 1H, $CH_3\mathbf{CH_2}CH_2$-), 0.76 (t, *J* = 7.3 Hz, 3H, $\mathbf{CH_3}CH_2CH_2$-). ^{13}C NMR (151 MHz, D_2O) *δ* 98.5 (C-1), 75.3 (C-5), 68.7 (t, *J* = 23.5 Hz, C-6), 68.3 (C-3), 55.6 (C-2), 43.6 (C-4), 33.0 ($CH_3CH_2\mathbf{CH_2}$-), 23.0 (AcOH), 19.3 ($CH_3\mathbf{CH_2}CH_2$-), 13.0 ($\mathbf{CH_3}CH_2CH_2$-). ESI-HRMS: *m/z* calcd for $C_9H_{17}DNO_3$ [M + H]$^+$ 189.1349, found 189.1357.

1,6-Anhydro-2-azido-6*S*-deuterio-2,4-dideoxy-4-*C*-propyl-D-glucopyranose (6*S*–^{2}H-19). Stick's reagent (0.209 g, 1.00 mmol) was added to an ice cold stirred solution of $CuSO_4$ (11 mg, 0.07 mmol), triethylamine (0.28 mL, 2.0 mmol), and compound **6*S*–^{2}H-18** (0.123 g, 0.495 mmol) in 4:1 MeCN/water (6.6 mL). The reaction mixture was stirred for 9 h before MeCN was removed under vacuum and the residue was diluted with EtOAc, washed with 1 N HCl, saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4 and concentrated to give **6*S*–^{2}H-19** as a colorless gum (0.101 g, 96%).

$[\alpha]_D^{23} = -40.5$ (c = 1.0, CH_2Cl_2), ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.41 (s, 1H, H-1), 4.38 (s, 1H, H-5), 4.04 (s, 1H, H-6), 3.60 (s, 1H, H-3), 3.42 (s, 1H, H-2), 2.71 (br s, 1H, –OH), 1.66–1.51 (m, 3H, H-4, $CH_3CH_2\mathbf{CH_2}$-), 1.50–1.41 (m, 1H, $CH_3\mathbf{CH_2}CH_2$-), 1.40–1.30 (m, 1H, $CH_3\mathbf{CH_2}CH_2$-), 0.93 (t, J = 7.3 Hz, 3H, $\mathbf{CH_3}CH_2CH_2$-). ^{13}C NMR (151 MHz, $CDCl_3$) δ 100.5 (C-1), 74.9 (C-5), 71.1 (C-3), 68.2 (t, J = 23.2 Hz, C-6), 63.7 (C-2), 44.2 (C-4), 33.3 ($CH_3CH_2\mathbf{CH_2}$-), 20.5 ($CH_3\mathbf{CH_2}CH_2$-), 14.0 ($\mathbf{CH_3}CH_2CH_2$-).

Methyl 2-azido-6*S*-deuterio-2,4-dideoxy-4-*C*-propyl-D-glucopyranoside (6*S*–^{2}H-20α and 6*S*–^{2}H-20β). Compound **6*S*–^{2}H-19** (0.091 g, 0.42 mmol) was dissolved in Ac_2O (4 mL) followed by addition of TFA (0.40 mL) and stirred under argon for 2 h. The reaction mixture was then diluted with Et_2O and washed with saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, filtered, and concentrated. The residue was then dissolved in 10% HCl MeOH solution (3.4 mL) and heated to reflux for 10 h followed by concentration under vacuum to give a 2:1 mixture of **6*S*–^{2}H-20α** and **6*S*–^{2}H-20β** (80.8 mg, 77%). The resulting residue was subjected flash column chromatography over silica gel in 45%–50% ethyl acetate in hexanes which afforded **6*S*–^{2}H-20α** (11.7 mg, 11%), **6*S*–^{2}H-20β** (9.5 mg, 9%), and (11.8 mg, 11%) of a mixture of anomers. ESI-HRMS: m/z calcd for $C_{10}H_{18}DN_3O_4Na$ [M + Na]$^+$ 269.1336, found 269.1326. **6*S*–^{2}H-20α**: $[\alpha]_D^{23}$ = 98.8 (c = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.76 (d, J = 3.5 Hz, 1H, H-1), 3.78 (t, J = 10.2 Hz, 1H, H-3), 3.58 (dd, J = 10.6, 5.4 Hz, 1H, H-5), 3.55 (d, J = 5.5 Hz, 1H, H-6), 3.37 (s, 3H, -OMe), 3.09 (dd, J = 10.1, 3.5 Hz, 1H, H-2), 1.61–1.49 (m, 2H, –CH_2CH_2-, H-4), 1.49–1.27 (m, 3H, –CH_2CH_2-), 0.91 (t, J = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 99.2 (C-1), 71.8 (C-5), 68.5 (C-3), 65.2 (C-2), 61.5 (t, J = 21.3 Hz, C-6), 53.9 (-OMe), 43.1 (C-4), 28.8 (–CH_2CH_2–), 18.7 (–CH_2CH_2–), 13.6 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{18}DN_3O_4Na$ [M + Na]$^+$ 269.1336, found 269.1334. **6*S*–^{2}H-20β**: $[\alpha]_D^{23} = -35.4$ (c = 0.003, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.13 (d, J = 8.1 Hz, 1H, H-1), 3.57 (d, J = 5.5 Hz, 1H, H-6), 3.52 (s, 3H, -OMe), 3.30–3.24 (m, 2H, H-3, H-5), 3.02 (dd, J = 9.5, 8.1 Hz, 1H, H-2), 1.58–1.24 (m, 5H, H4, –CH_2CH_2-), 0.90 (t, J = 7.1 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 102.7 (C-1), 76.1 (C-5), 72.6 (C-3), 68.6 (C-2), 61.5 (t, J = 21.9 Hz, C-6), 55.6 (-OMe), 42.6 (C-4), 28.6 (–CH_2CH_2–), 18.6 (–CH_2CH_2–), 13.6 (-CH_3).

Methyl 2-amino-6-(*S*)-deuterio-2,4-dideoxy-4-*C*-propyl-α-D-glucopyranoside (6*S*–^{2}H-5). Compound **6*S*–^{2}H-20α** (9.0 mg, 0.037 mmol) was dissolved in a 1:1 mixture of 1,4-dioxane and 10% aqueous AcOH (0.6 mL) followed by addition of Pd/C (1.8 mg). The reaction mixture was stirred under 50 psi H_2 for 1 h followed by filtration over Celite® and lyophilization to obtain **6*S*–^{2}H-5** as an off white solid (10.2 mg, 99%). $[\alpha]_D^{23}$ = 56.4 (c = 0.5, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, J = 3.6 Hz, 1H, H1), 3.71 (t, J = 10.5 Hz, 1H, H3), 3.62 (dd, J = 10.9, 5.4 Hz, 1H, H5), 3.53 (d, J = 5.5 Hz, 1H, H6), 3.25 (s, 3H, OMe), 3.08 (dd, J = 10.4, 3.6 Hz, 1H, H2), 1.80 (s, 3H, AcOH), 1.49 (tt, J = 10.8, 4.0 Hz, 1H, H4), 1.40–1.33 (m, 1H, –CH_2CH_2-), 1.32–1.24 (m, 1H, –CH_2CH_2-), 1.24–1.06 (m, 2H, –CH_2CH_2-), 0.72 (t, J = 7.2 Hz, 3H, –CH_3). ^{13}C NMR (151 MHz, D_2O) δ 179.9 (AcOH), 96.3 (C1), 71.6 (C5), 67.0 (C3), 60.99 (t, J = 21.0 Hz, C6), 55.3 (C2), 54.9 (OMe), 42.0 (C4), 27.7 (–CH_2CH_2–), 22.3 (AcOH), 17.8 (–CH_2CH_2–), 13.8 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{21}DNO_4$ [M + H]$^+$ 221.1612, found 221.1605.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: DC and AV are cofounders and have an equity interest in Juvabis AG, a biotech start-up in the area of aminoglycoside antibiotics.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carres.2020.107984.

References

[1] T. Matsushita, G.C. Sati, N. Kondasinghe, M.G. Pirrone, T. Kato, P. Waduge, H.S. Kumar, A. Cortes Sanchon, M. Dobosz-Bartoszek, D. Shcherbakov, M. Juhas, S.N. Hobbie, T. Schrepfer, C.S. Chow, Y.S. Polikanov, J. Schacht, A. Vasella, E.C. Böttger, D. Crich, Design, multigram synthesis, and in vitro and in vivo evaluation of propylamycin: a semisynthetic 4,5-deoxystreptamine class aminoglycoside for the treatment of drug-resistant enterobacteriaceae and other Gram-negative pathogens, J. Am. Chem. Soc. 141 (2019) 5051–5061.

[2] S. Duscha, H. Boukari, D. Shcherbakov, S. Salian, S. Silva, A. Kendall, T. Kato, R. Akbergenov, D. Perez-Fernandez, B. Bernet, S. Vaddi, P. Thommes, J. Schacht, D. Crich, A. Vasella, E.C. Böttger, Identification and evaluation of improved 4'-O-(alkyl) 4,5-disubstituted 2-deoxystreptamines as next generation aminoglycoside antibiotics, mBio 5 (2014), https://doi.org/10.1128/mBio.01827-14.

[3] K. Bock, J.O. Duus, A conformational study of hydroxymethyl groups in carbohydrates investigated by ^{1}H NMR spectroscopy, J. Carbohydr. Chem. 13 (1994) 513–543.

[4] T.B. Grindley, Structure and conformation of carbohydrates, in: B. Fraser-Reid, K. Tatsuta, J. Thiem (Eds.), Glycoscience: Chemistry and Chemical Biology, vol. 1, Springer, Berlin, 2001, pp. 3–51.

[5] V.S.R. Rao, P.K. Qasba, P.V. Balaji, R. Chandrasekaran, Conformation of Carbohydrates, Harwood Academic Publishers, Amsterdam, 1998, p. 359.

[6] A.P. Carter, W.M. Clemons, D.E. Brodersen, R.J. Morgan-Warren, B.T. Wimberly, V. Ramakrishnan, Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics, Nature 407 (2000) 340–348.

[7] Q. Vicens, E. Westhof, Molecular recognition of aminoglycoside antibiotics by ribosomal RNA and resistance enzymes: an analysis of X-ray crystal structures, Biopolymers 70 (2003) 42–57.

[8] A.R. Mandhapati, G. Yang, T. Kato, D. Shcherbakov, S.N. Hobbie, A. Vasella, E.C. Böttger, D. Crich, Structure-based design and synthesis of apramycin-paromomycin analogues. Importance of the configuration at the 6′-position and differences between the 6′-amino and hydroxy series, J. Am. Chem. Soc. 139 (2017) 14611–14619.

[9] T. Kato, A. Vasella, D. Crich, Stereospecific synthesis of methyl 2-amino-2-deoxy-(6*S*)-Deuterio-α,β-D-Glucopyranoside and methyl 2,6-Diamino-2,6-Dideoxy-(6*R*)-Deuterio-α,β-D-Glucopyranoside: side chain conformations of the 2-amino-2-deoxy and 2,6-diamino-2,6-dideoxyglucopyranosides, Carbohydr. Res. 448 (2017) 10–17.

[10] C.E. Lünse, M.S. Schmidt, V. Wittmann, G. Mayer, Carba-sugars activate the GLMS-riboswitch of *Staphylococcus aureus*, ACS Chem. Biol. 6 (2011) 675–678.

[11] H. Ohrui, H. Horiki, H. Kishi, H. Meguro, Synthesis of (6*R*)- and (6*S*)-D-glucose-6-^{2}H through stereospecific photo-bromination of 1,6-anhydro-β-D-glucopyranose derivative, Agric. Biol. Chem. 47 (1983) 1101–1106.

[12] Y. Ichikawa, H. Kuzuhara, Synthesis of 1,6-anhydro-2,3-di-*O*-benzoyl-4-*O*-(methyl 2,3,4-tri-O-benzoyl-α-L-iodpyranosyluronate)-β-D-glucopyranose from cellobiose, Carbohydr. Res. 115 (1983) 117–129.

[13] J.J. Maul, P.J. Ostrowski, G.A. Ublacker, B. Linclau, D.P. Curran, Benzotrifluoride and derivatives: useful solvents for organic synthesis and fluorous synthesis, in: P. Knochel (Ed.), Top. Curr. Chem. vol. 206, Springer, Berlin, 1999, pp. 79–105.

[14] T.B. Grindley, G.J. Reimer, J. Kralovec, R.G. Brown, M. Anderson, Syntheses of 3-deoxy-3-substituted-D-glucose derivatives. Part I. Improvements in preparations of and nucleophilic additions to 1,6:2,3-dianhydro-4-*O*-benzyl-β-D-allopyranose, Can. J. Chem. 65 (1987) 1065–1071.

[15] J.D. White, G.L. Bolton, A.P. Dantanarayana, C.M.J. Fox, R.N. Hiner, R.W. Jackson, K. Sakuma, U.S. Warrier, Total synthesis of the antiparasitic agent avermectin B1a, J. Am. Chem. Soc. 117 (1995) 1908–1939.

[16] T.S. Rasmussen, H.H. Jensen, Synthesis and glycosidase inhibitory activity of noeurostegine - a new and potent inhibitor of β-glucoside hydrolases, Org. Biomol. Chem. 8 (2010) 433–441.

[17] T. Wakamatsu, H. Nakamura, Y. Nishikimi, K. Yoshida, T. Noda, M. Taniguchi, Y. Ban, The oxirane ring openings of the dianhydro sugar with high regioselectivity and its use in preparation of two chiral segments of 6-deoxyerythronolide B, Tetrahedron Lett. 27 (1986) 6071–6074.

[18] H. Nakamura, Alkylated levoglucosan in organic synthesis. A formal total synthesis of elaiophylin, Chem. Pharm. Bull. 38 (1990) 2435–2441.

[19] A. Fürst, P.A. Plattner, Steroids and sex hormones. Clx. 2(α),3(α)- and 2(β),3(β)-oxidocholestane; the configuration of the 2-hydroxycholestanes, Helv. Chim. Acta 32 (1949) 275–283.

[20] E.D. Goddard-Borger, R.V. Stick, An efficient, inexpensive, and shelf-stable diazotransfer Reagent: imidazole-1-sulfonyl azide hydrochloride, Org. Lett. 9 (2007) 3797–3800.

[21] N. Fischer, E.D. Goddard-Borger, R. Greiner, T.M. Klapotke, B.W. Skelton, J. Stierstorfer, Sensitivities of some imidazole-1-sulfonyl azide salts, J. Org. Chem. 77 (2012) 1760–1764.

[22] G.T. Potter, G.C. Jayson, G.J. Miller, J.M. Gardiner, An updated synthesis of the diazo-transfer reagent imidazole-1-sulfonyl azide hydrogen sulfate, J. Org. Chem. 81 (2016) 3443–3446.
[23] H. Ye, R. Liu, D. Li, Y. Liu, H. Yuan, W. Guo, L. Zhou, X. Cao, H. Tian, J. Shen, P.G. Wang, A safe and facile route to imidazole-1-sulfonyl azide as a diazotransfer reagent, Org. Lett. 15 (2013) 18–21.
[24] Z. Miao, M. Xu, B. Hoffmann, B. Bernet, A. Vasella, Functionalised bicyclic exo-glycals by alkynol cycloisomerisation of hydroxy 1,3-diynes and hydroxy haloalkynes, Helv. Chim. Acta 88 (2005) 1885–1912.
[25] P. Uhlmann, A. Vasella, Regioselective glycosylation of diols and triols: intra- and intermolecular hydrogen bonds, Helv. Chim. Acta 75 (1992) 1979–1994.
[26] C.W. Jefford, B. Waegell, The bromination of bicyclo[3.2.1]octanone-3. An example of an α,α'-diaxial dibromocyclohexanone, Tetrahedron Lett. 4 (1963) 1981–1986.
[27] A.J. Baretta, C.W. Jefford, B. Waegell, Stéréochimie des derivés bromés de la diméthyl-6,6 bicyclo[3.1.1]heptanone-3 ou isonopinone. L'Effet anti-réflexe, Bull. Soc. Chim. Fr. (1970) 3899–3908.
[28] G.H. Alt, D.H.R. Barton, Some conformational aspects of neighbouring-group participation, J. Chem. Soc. (1954) 4284–4294.
[29] P. Ngoje, D. Crich, Synthesis of bradyrhizose from D-glucose, Org. Lett. 22 (2020) 523–527.
[30] H. Amarasekara, S. Dharuman, T. Kato, D. Crich, Synthesis of conformationally-locked *cis*- and *trans*-bicyclo[4.4.0] mono-, di- and trioxadecane modifications of galacto- and glucopyranose. Experimental limiting ^{3}JH,H coupling constants for the estimation of carbohydrate side chain populations and beyond, J. Org. Chem. 83 (2018) 881–897.
[31] A. Gaudemer, Determination of configurations by NMR spectroscopy, in: H.B. Kagan (Ed.), Stereochemistry: Fundamentals and Methods, vol. 1, Georg Thieme, Stuttgart, 1977, pp. 44–136.
[32] E.L. Eliel, S.H. Wilen, Stereochemistry of Organic Compounds, Wiley, New York, 1994, p. 1267.
[33] I. Tvaroška, J. Gajdoš, Angular dependence of vicinal carbon-proton coupling constants for conformational studies of the hydroxymethyl group in carbohydrates, Carbohydr. Res. 271 (1995) 151–162.