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Studies toward a conjugate vaccine for anthrax. Synthesis and characterization of anthrose [4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-D-glucopyranose] and its methyl glycosides

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Abstract—The key step in the first chemical synthesis of anthrose (**16**) and its methyl α- (**6**) and β-glycoside (**22**) was inversion of configuration at C-2 in triflates **10**, **2**, and **18**, respectively, obtained from the common intermediate, methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**1**). To prepare methyl α-anthroside (**6**), methylation at O-2 of the gluco product **3**, obtained from **2**, was followed by hydrogenation/hydrogenolysis of the formed 2-methyl ether **4**, to simultaneously remove the protecting benzyl group and reduce the azido function. Subsequent N-acylation of the formed amine **5** with 3-hydroxy-3-methylbutyric acid gave the target methyl α-glycoside **6**. Synthesis of methyl β-anthroside (**22**) comprised the same sequence of reactions, starting from the known methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-β-D-mannopyranoside (**17**), which was prepared from **1**. In the synthesis of anthrose (**16**), 1-thio-β-glucoside **11**, obtained from **1** through **10**, was methylated at O-2, and the azido function in the resulting benzylated 1-thioglycoside **12** was selectively reduced to give amine **13**. After N-acylation with 3-hydroxy-3-methylbutyric acid, 1-thioglycoside **14** was hydrolyzed to give the corresponding reducing sugar, aldol **15**, which was debenzylated to afford anthrose. Published by Elsevier Ltd.

Keywords: Methyl α-anthroside; Methyl β-anthroside; Thioglycoside hydrolysis; Perosamine

1. Introduction

Anthrax is a serious disease, predominantly of domesticated and wild animals. Humans become infected incidentally when brought into contact with diseased animals or contaminated soil. The most common form of the disease in humans is cutaneous anthrax, which is usually acquired via injured skin or mucous membranes. Untreated contact or cutaneous anthrax has a fatality rate of 5–20%. Inhalational anthrax is much more deadly. Initial symptoms are generally nonspecific: low-grade fever, a dry hacking cough, and weakness. The person may briefly improve after 2–4 days; however, within 24 h after this brief improvement, respiratory distress occurs with shock and death following shortly thereafter. Almost all cases of inhalational anthrax in which treatment was begun after patients have exhibited symptoms result in death, regardless of post-exposure treatment. New concerns regarding anthrax have recently emerged in connection with the use of some form of *Bacillus anthracis*, which is the etiological agent of anthrax, as a biological weapon. As a result, a potent vaccine for anthrax has become a pressing target worldwide.

B. anthracis is a Gram-positive spore-forming bacterium.[1] The spore surface provides the first interaction with the host. It would be beneficial, therefore, to be able to selectively inactivate *B. anthracis* spores. Killing dispersed spores effectively has proven difficult and at present requires the use of agents, which have very broad toxicity, such as chlorine dioxide gas. The exosporium is the outermost integument of the mature spore.

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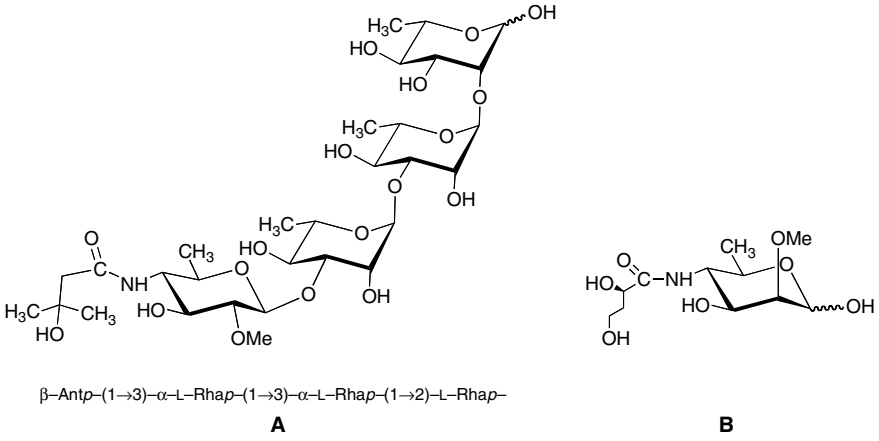


Figure 1. Structure of the terminal tetrasaccharide of the surface glycoprotein of *Bacillus anthracis* (A) and of the terminal monosaccharide of the OPS of *Vibrio cholerae* O:1, serotype Ogawa (B).

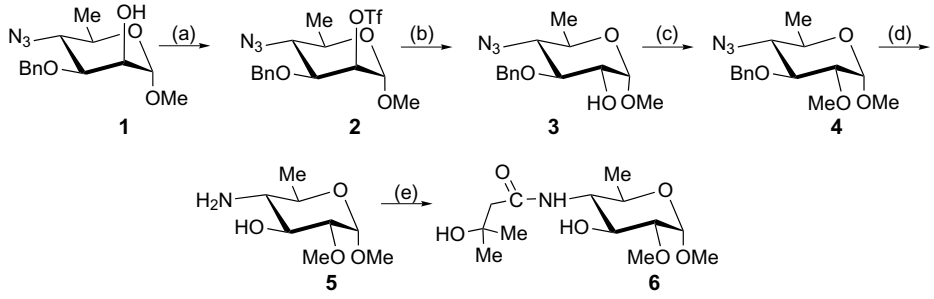
Thus, a vaccine for anthrax could be based on identifying detailed structure of components unique to the spores, and attacking them with specific, neutralizing antibodies.

Daubenspeck et al.[2] have recently established the structure of the tetrasaccharide (Fig. 1A) of the exosporium glycoprotein Bc1A. Since multiple copies of the tetrasaccharide were found to be linked to Bc1A, the tetrasaccharide or its constituents should be suitable targets for specific, homologous antibodies. Accessibility of synthetic derivatives of anthrose is crucial for making its glycosyl donors, to be used for synthesis of anthrose-containing oligosaccharides and conjugate immunogens from them. Within our work toward a carbohydrate-based, conjugate immunogens for anti-anthrax exosporium antibodies, herein, we report the synthesis of anthrose (**16**), the terminal determinant of the *B. anthracis* exosporium glycoprotein tetrasaccharide and methyl α- (**6**) and β-anthroside (**22**). While the syntheses described here, starting from **1**, are not the shortest that could be designed as synthetic routes to anthrose and its derivatives, they are viable alternatives, since the large-scale preparation of starting material **1** is well established.[3–5]

2. Results and discussion

The similarity of the terminal determinant of the *B. anthracis* exosporium glycoprotein tetrasaccharide [anthrose,[2] 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-D-glucose] to the terminal determinant of the O–PS of *Vibrio cholerae* O:1, serotype Ogawa and co-workers[6–8] [Fig. 1B, 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-D-mannose (perosamine)] is remarkable. The two constituent saccharides are epimeric 4,6-dideoxy-4-aminohexoses of the D-series, they are methylated at O-2, and their amino groups are acylated with derivatives of butyric acid. As a result of our involvement in the development of a conjugate vaccine for cholera from synthetic carbohydrate antigens, this laboratory has considerable expertise in the chemistry of perosamine.[9–11] Thus, while other routes could be envisioned, it occurred to us that derivatives of anthrose could be accessible through a suitable precursor of perosamine, and here we report the first synthesis of anthrose and its methyl glycosides from the known methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**1**).

The key step in syntheses of the three title substances from **1** is inversion of configuration at C-2. Literature precedents show that nucleophilic displacements at position 2 in β-manno and β-gluco derivatives usually give better yields of displacement products[12–17] than in their α-counterparts.[12,13] With some nucleophiles, formation of byproducts, resulting from elimination, fragmentation, ring-contraction and neighboring group participation, can be minimized by subjecting 3-hydroxy-2-*O*-triflates to S_N2 displacement reactions.[18] Since our target compounds were to carry a methoxyl group at C-2, the latter approach was not readily applicable. Albert et al.[19] described direct conversion of carbohydrate triflates into *epi*-hydroxy compounds with the nitrite ion as a nucleophile. Accordingly, we have treated the triflyl derivative of **1**, mannopyranoside **2**, with $NaNO_2$ or Bu_4NNO_2 in DMF and obtained crystalline methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-glucopyranoside (**3**) in 50–60% yield after chromatography (Scheme 1). The use of acetonitrile[19] as solvent, with either $NaNO_2$ or Bu_4NNO_2, resulted in much lower yield of **3**. While the yield of **3** was better than those observed for conventional S_N2 displacement in some similar systems,[12,13] the reaction was accompanied by side reactions (TLC). The major byproduct formed in the conversion of **2** was identified (MS, NMR) as the 2,3-olefin, methyl 4-azido-3-*O*-benzyl-2,4,6-trideoxy-α-D-*erythro*-hex-2-enopyranoside. Extensive formation of products of elimination

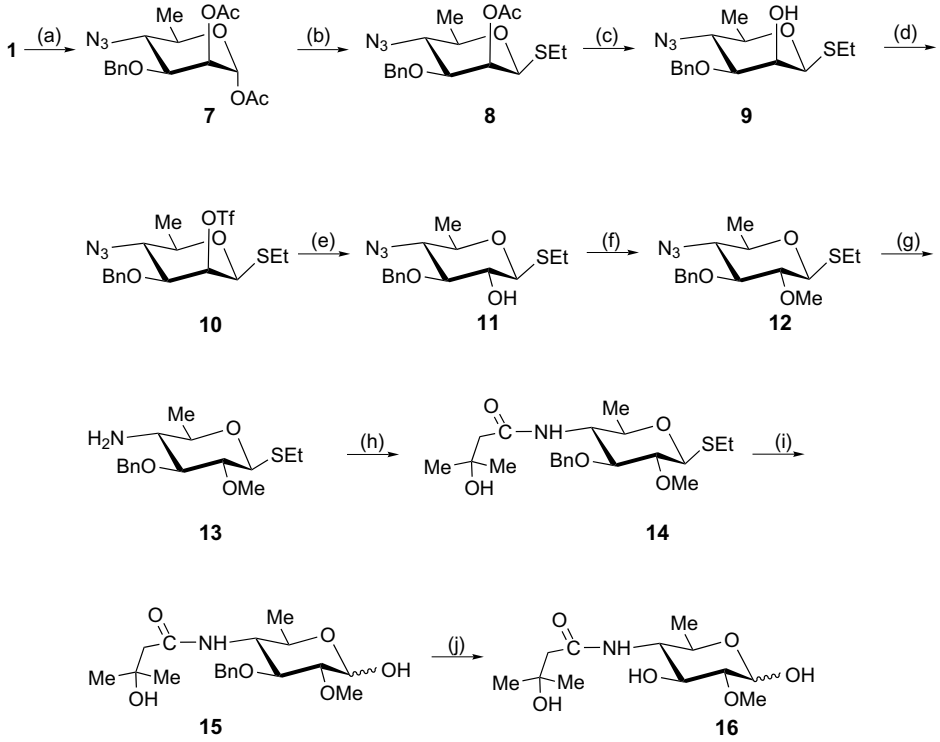


Scheme 1. Reagents and conditions: (a) Tf_2O, Py; (b) $NaNO_2$, DMF, 80 °C; (c) MeI, Ag_2O, Me_2S, DME; (d) H_2, Pd/C, MeOH; (e) 3-hydroxy-3-methylbutyric acid, CH_2Cl_2, HATU, DIPEA.

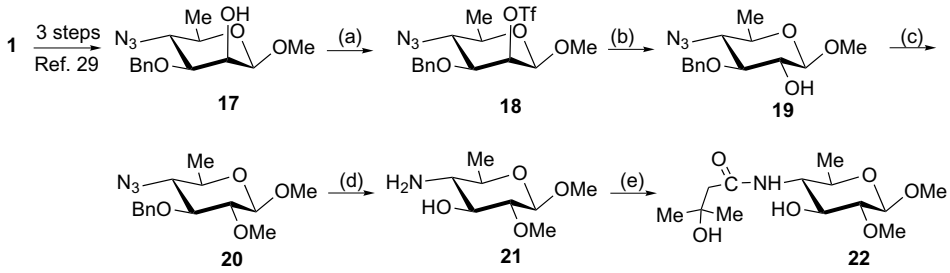
(53–82%) was observed by Haradahira et al.[13] during displacements of 2-triflates in α-gluco- and α-mannopyranosides. Other byproducts formed in our situation, some unstable, were likely analogous to those whose formation was observed in similar systems when inversion of configuration of secondary alcohols was effected by S_N2 displacement reaction using nitrite salts.[20]

Conversion of **3** to methyl α-anthroside **6** by successive methylation[21] at O-2 (→**4**), hydrogenation/hydrogenolysis (→**5**) and N-acylation with 3-hydroxy-3-methylbutyric acid was uneventful, and afforded the target product in crystalline state.

Methyl α-anthroside (**6**) can be made from **1** in only five steps (Scheme 1). Therefore, our original plan was to make anthrose by further conversion of **6**, either by hydrolysis of the glycoside, or by acetolysis followed by deacetylation. Treatment of **6** with dilute mineral or trifluoroacetic acid, under conditions which normally cleaves alkyl glycosides,[22,23] or acetolysis under conditions that yielded the desired product from a related methyl 4-acylamido hexopyranoside[24] did not afford the expected products. Therefore, we chose to prepare anthrose from a thioglycoside, instead from a methyl glycoside. 1-Thioglycosides can be readily converted to reducing sugars (hemiacetals) under mild conditions.[25–27] The use of 1-thio-β-glycoside **9** as the starting material (Scheme 2) for preparation of anthrose, and methyl glycoside **17** as the starting material for preparation of **22** (Scheme 3), offered also an opportunity to compare the effect of anomeric configuration and the nature of



Scheme 2. Reagents: (a) Ac_2O, H_2SO_4, AcOH; (b) EtSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (c) NaOMe, MeOH; (d) Tf_2O, CH_2Cl_2/Py; (e) $NaNO_2$, DMF, 50 °C; (f) MeI, Ag_2O, Me_2S, THF; (g) H_2S, H_2O–Py; (h) 3-hydroxy-3-methylbutyric acid, HATU, DIPEA, CH_2Cl_2; (i) NIS, H_2O, acetone; (j) H_2, Pd/C, MeOH.



Scheme 3. Reagents and conditions: (a) Tf_2O, CH_2Cl_2–Py; (b) $NaNO_2$, DMF, rt; (c) MeI, Ag_2O, Me_2S, DME; (d) H_2, Pd/C, MeOH; (e) 3-hydroxy-3-methylbutyric acid, HATU, DIPEA, CH_2Cl_2.

Table 1. ^1H and ^{13}C chemical shifts[a] (ppm) of the β-anthrosyl residue in **22** and in the tetrasaccharide side chain of the *Bacillus anthracis* exosporium

Residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6 C-6
β-Anthrosyl in **22**	4.108 103.33	2.747 84.00	3.302 72.84	3.397 56.45	3.284 70.33	1.074 17.91
β-Anthrosyl in **A**[b]	4.582 103.4	2.850 84.3	3.301 72.7	3.401 56.4	3.278 70.3	1.082 18.1
	CO	NH	H-2′ C-2′	2C*H*$_3$ 2*C*H$_3$	C*H*$_3$-2 *C*H$_3$-2	C-3′
β-Anthrosyl in **22**	171.38	7.683	2.210 48.61	1.160; 1.148 29.47; 29.36	3.424 59.83	68.61
β-Anthrosyl in **A**[b]	171.4	7.754	2.211 48.6	1.160; 1.148 29.4; 29.5	3.526 59.9	Not reported

[a] Spectra taken in Me_2SO-d_6 at 22 °C, at 600 MHz for ^1H and 150 MHz for ^{13}C.
[b] Data taken from Ref. 2.

the aglycon upon the outcome of the S_N2 displacement reaction at C-2 in similarly protected glycosides. Thus, β-thioglycoside **8**, obtained along with the α-anomer from **1** through **7**,[28] was deacetylated, and the crystalline alcohol **9** was converted to its epimer, the gluco analog **11**, through the same sequence of reactions as in the conversion **1**→**3** (Scheme 1). As with **3**, the formation of **11** from triflate **10** was accompanied by side reactions, and the yields of the desired products in the two S_N2 displacement reactions described above were comparable. After methylation[21] (→**12**) and reduction of the azido group, amidation of the formed amine **13** with 3-hydroxy-3-methylbutyric acid gave ethyl 3-*O*-benzyl-1-thio-β-anthroside (**14**). Hydrolysis of the latter, effected with *N*-iodosuccinimide, gave crystalline hemiacetal **15** as a mixture of anomers. Hydrogenolytic cleavage of the benzyl protecting group then gave anthrose as an anomeric mixture.

Synthesis of methyl β-anthroside (**22**) from the known[29] β-mannopyranoside **17** was readily carried out through the same reaction sequence as for the preparation of α-substance **6** from **1**. The yield of the gluco product **19** of the nucleophilic displacement of the triflate group in **18** with NO_2 anion was higher than those of displacement products from triflates **2** and **10**. Methylation[21] at O-2 (→**20**) and catalytic hydrogenation/hydrogenolysis gave the crystalline amine **21**. Condensation of the latter with 3-hydroxy-3-methylbutyric acid gave methyl β-anthroside **22**, whose NMR data were similar to those for the terminal β-anthrosyl residue in the spectrum of the tetrasaccharide of the exosporium glycoprotein of *B. anthracis* (Table 1). The difference in the chemical shift for H-1 in **22** and the β-anthrosyl residue in **A** (Fig. 1) is attributable to the different substituents at C-1, namely the *O*-methyl group in **22**, versus the rhamnose trisaccharide in **A**.

3. Experimental

3.1. General methods

Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in chloroform, with a Perkin–Elmer automatic polarimeter, Model 341. All reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 coated glass slides (E. Merck). Column chromatography was performed by gradient elution from columns of silica gel with the High-Performance Rapid Flash Chromatography System (RT Scientific) or with the CombiFlash Companion Chromatograph (Isco, Inc.). Solvent mixtures less polar than those used for TLC were used at the onset of separation. Nuclear magnetic resonance

(NMR) spectra were measured with a Varian Gemini or Varian Mercury instrument at 300 MHz (^{1}H) and 75 MHz (^{13}C), or with Bruker Avance 600 spectrometers. Assignments of NMR signals were made by homonuclear and heteronuclear two-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignment of NMR signals, nuclei associated with the *N*-amido side chain are denoted with a prime. Liquid chromatography–electron spray-ionization mass spectrometry (ESIMS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOFMS) was performed with a Shimadzu AXIMA/CFR spectrometer. Attempts have been made to obtain correct analytical data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtain within ±0.4%. Structures of these compounds follow unequivocally from the mode of synthesis, NMR data, and *m*/*z* values found in their mass spectra, and their purity was verified by TLC and NMR spectroscopy. Pd/C catalyst (5%, ESCAT 103) was a product of Engelhard Industries. HATU {*N*-[(Dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]-pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide} was purchased from Applied Biosystems. Solutions in organic solvents were dried with anhydrous Na_2SO_4, and concentrated at 40 °C/2 kPa.

3.2. Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-glucopyranoside (3)

Triflic anhydride (2.7 mL, 16.3 mmol) was added at 0 °C to a stirred solution of methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**1**)[3–5] (4 g, 13.6 mmol) in pyridine (7 mL). The cooling bath was removed, and stirring was continued until TLC (10:1 toluene–acetone) showed that the reaction was complete (∼1 h). After concentration, the residue was chromatographed to give methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-trifluoromethanesulfonyl-α-D-mannopyranoside (**2**, 7 g, ∼100%). ^{1}H NMR ($CDCl_3$): δ 5.05 (br t, 1H, H-2), 4.80, 4.57 (2d, partially overlapped, J 11.5 Hz, 2H, 2CH_2Ph), 4.80 (d, partially overlapped, 1H, $J_{1,2}$ 1.6 Hz, H-1), 3.80 (dd, 1H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.8 Hz, H-3), 3.57–3.45 (m, 1H, H-5), 3.39 (t, partially overlapped, J 9.8 Hz, H-4), 3.37 (s, partially overlapped, OCH_3), 1.35 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.63 (C-1), 80.68 (C-2), 74.84 (C-3), 72.53 (*C*H_2Ph), 67.18 (C-5), 63.57 (C-4), 55.36 (OCH_3), 18.22 (C-6); ESIMS: *m*/*z* 448 ([M+Na]$^+$).

$NaNO_2$ (2 g, 29.6 mmol) was added to a solution of **2** (6.3 g, 14.8 mmol) in DMF (50 mL), and the mixture was stirred at 80 °C for 8 h. TLC (3:1 hexane–EtOAc) then showed that all starting material was consumed, and that one major and several minor byproducts were formed. After concentration, chromatography gave first a mixture of byproducts, which were difficult to resolve. The main component in the mixture, later eluted pure, was the material whose NMR and mass spectra showed that it was methyl 4-azido-3-*O*-benzyl-2,4,6-trideoxy-α-D-*erythro*-hex-2-enopyranoside, $[\alpha]_D$ +249 (*c* 0.5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.98 (br d, distorted by long range coupling, 1H, H-2), 4.91 (dd, 1H, $J_{1,2}$ 3.5, $J_{1,3}$ 1.1 Hz, H-1), 4.85, 4.75 (2d, 1H each, ^2J 11.5 Hz, CH_2Ph), 3.90–3.79 (m, 1H, H-5), 3.72, 3.69 (2t, 1H, $J_{4,5}$ 10.5, $J_{2,4}$ 1 Hz, H-4), 3.37 (s, 3H, OCH_3), 1.34 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.90 (C-3), 96.63 (C-1), 96.37 (C-2), 69.53 (*C*H_2Ph), 65.99 (C-5), 61.62 (C-4), 55.22 (OCH_3), 18.61 (C-6); CIMS: (on addition of LiCl): *m*/*z* 282 ([M+Li]$^+$). Anal. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 61.40; H, 6.28; N, 15.29.

Eluted next was the title glucopyranoside (**3**, 2.3 g, 54.7%), mp 97.5–98 °C (from *i*-Pr_2O), $[\alpha]_D$ +242 (*c* 0.5). ^{1}H NMR (300 MHz, $CDCl_3$ + D_2O): δ 4.93, 4.84 (2d, 1H each, ^2J 11.1 Hz, CH_2Ph), 4.69 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 3.68–3.48 (m, 3H, H-2,3,5), 3.41 (s, 3H, OCH_3), 3.07 (t, 1H, J 9.7 Hz, H-4), 1.30 (d, 3H, $J_{5,6}$ 6.2 Hz, C-6); ^{13}C NMR ($CDCl_3$ + D_2O): δ 99.29 (C-1), 81.10 (C-3), 75.19 (*C*H_2Ph), 72.97 (C-2), 67.53 (C-4), 66.14 (C-5), 55.32 (OCH_3), 18.30 (C-6); ESIMS$^-$: *m*/*z*: 293.15 ([M]$^-$), 279 ([M−N]$^-$), 265 ([M−2N$^-$]). Anal. Calcd for $C_{14}H_{19}N_3O_3$: C, 57.33; H, 6.53; N, 14.33. Found: C, 57.32; H, 6.66; N, 14.29.

A comparable yield of **3** was obtained when the reaction was carried out in DMF as solvent and Bu_4NNO_2 as the source of nucleophile, but diminished yields were observed when CH_3CN was the solvent.

3.3. Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-glucopyranoside (4)

MeI (3 mL), followed by Ag_2O (1.2 g) and a catalytic amount of Me_2S, was added to a solution of alcohol **3** (600 mg, 2 mmol) in 1,2-dimethoxyethane (5 mL), and the mixture was stirred in the dark, overnight at room temperature. TLC (3:1 hexane–EtOAc) showed the reaction was complete, and that one product was formed. The mixture was filtered through a Celite pad, the filtrate was concentrated, and the residue was chromatographed to give **4** (540 mg, 87%), $[\alpha]_D$ +247 (*c* 0.4). ^{1}H NMR ($CDCl_3$): δ 4.89, ∼4.78 (2d, partially overlapped, ^2J 10.7 Hz, CH_2Ph), ∼4.79 (d, partially overlapped, $J_{1,2}$ 3.5 Hz, read from H-2 signal, H-1), 3.75 (t, 1H, J 9.5 Hz, H-3), 3.57–3.51 (m, 4H, H-5, incl. s, 3.52, OCH_3-2), 3.41 (s, 3H, OCH_3-1), 3.32 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.08 (t, 1H, J 9.7 Hz, H-4), 1.29 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.29 (C-1), 82.31 (C-2), 79.73 (C-3), 75.47 (*C*H_2Ph), 67.85 (C-4), 65.82 (C-5), 58.95 (OCH_3-2), 55.21 (OCH_3-1), 18.36 (C-6); ESIMS: *m*/*z* 330

($[M+Na]^+$). Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.57. Found: C, 59.07; H, 7.07; N, 13.50.

3.4. Methyl 4-amino-4,6-dideoxy-2-*O*-methyl-α-D-glucopyranoside (5)

A solution of the foregoing azide **4** (540 mg) in MeOH (15 mL) was treated overnight at room temperature with hydrogen in the presence of 5% Pd/C catalyst (0.5 g). (Caution! Extreme fire hazard.) After filtration, the residue was chromatographed, to give **5** (250 mg, 75%), mp 146–147 °C (from EtOH; the compound sublimes when heated over 70 °C/133 Pa), $[\alpha]_D$ +157 (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 4.84 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 3.58 (t, 1H, *J* 9.5 Hz, H-3), 3.56–3.51 (m, 1H, H-5), 3.49 (s, 3H, OCH_3-2), 3.40 (s, 3H, OCH_3-1), 3.17 (dd, 1H, $J_{2,3}$ 9.4 Hz, H-2), 2.43 (t, 1H, *J* 9.6 Hz, H-4), 1.26 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 96.86 (C-1), 81.68 (C-2), 71.97 (C-3), 67.93 (C-5), 58.77 (C-4), 57.87 (OCH_3-2), 54.72 (OCH_3-1), 17.70 (C-6); ESIMS: *m*/*z* 214 ($[M+Na]^+$). Anal. Calcd for $C_8H_7NO_4$: C, 50.25; H, 8.96; N, 7.32. Found: C, 50.26; H, 8.97; N, 7.24.

3.5. Methyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-α-D-glucopyranoside (6)

To a stirred solution of amine **5** (200 mg, 1.04 mmol) and 3-hydroxy-3-methylbutyric acid (184 mg, 1.56 mmol) in CH_2Cl_2 (2 mL) was slowly added HATU (530 mg, 1.56 mmol) followed by *N*,*N*-diisopropylethylamine (0.2 mL, 1.56 mmol), and the stirring was continued at room temperature. After 0.5 h, TLC (10:1 CH_2Cl_2–MeOH) showed that the reaction was complete. The mixture was concentrated, and the residue was chromatographed to afford the coupling product **6** (236 mg, 79%), mp 128–129 °C (from EtOAc), $[\alpha]_D$ +134 (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 6.05 (d, 1H, $J_{4,NH}$ 8.0 Hz, NH), 4.86 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 3.82–3.76 (m, 2H, H-3,4), 3.71–3.63 (m, 2H, H-5, OH-3′), 3.52 (s, 3H, OCH_3-2), 3.42 (s, 3H, OCH_3-1), 3.25 (dd, 1H, $J_{2,3}$ 9.1 Hz, H-2), 3.00 (br s, 1H, OH-3), 2.41, 2.37 (2d, 2H, ^2J 14.4 Hz, H-2′), 1.31, 1.30 (2s, 3H each, $2CH_{3'}$), 1.25 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 172.93 (CO), 96.89 (C-1), 82.02 (C-2), 71.34 (C-3), 69.80 (C-3′), 66.37 (C-5), 58.50 (OCH_3-2), 56.67 (C-4), 55.28 (OCH_3-1), 48.68 (C-2′), 29.77, 29.16 ($2CH_{3'}$), 17.93 (C-6); ESIMS: *m*/*z* 292 ($[M+H]^+$). Anal. Calcd for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.30; H, 8.56; N, 4.74.

3.6. Ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-β-D-mannopyranoside (8)

This compound was formed in a small amount from 1,2-di-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranose (**7**) following the protocol[28] aimed at the preparation of ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-α-D-mannopyranoside from **1** (6.4 g). Chromatography of the crude product gave first the α-anomer (4.72 g, 74%), whose NMR data agreed with those published.[28]

Continued elution gave the title β-anomer **8** (0.44 g, 6.9%), which solidified on standing, mp 103–104 °C (EtOH), $[\alpha]_D$ −28 (*c* 0.5); lit.[28] $[\alpha]_D$ +107.7 (*c* 0.5) for amorphous material reported as a byproduct of this reaction, whose reported NMR data[28] do not support structure **8**. ^{1}H NMR ($CDCl_3$): δ 5.62 (dd, 1H, $J_{1,2}$ 1.0 Hz, $J_{2,3}$ 3.3 Hz, H-2), 4.76, 4.49 (2d, 1H each, ^2J 11.1 Hz, CH_2Ph), 4.61 (d, 1H, H-1), 3.52 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.41 (t, 1H, H-4), 3.26–3.17 (m, 1H, H-5), 2.77–2.66 (m, 2H, CH_2CH_3), 2.17 (s, 3H, $COCH_3$), 1.39 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6), 1.28 (t, 3H, *J* 7.4 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 81.80 ($J_{C\text{-}1,H\text{-}1}$ 151.0 Hz, C-1), 79.24 (C-3), 75.26 (C-5), 71.44 (CH_2Ph), 68.42 (C-2), 63.55 (C-4), 25.38 (CH_2CH_3), 20.69 ($COCH_3$), 18.67 (C-6), 14.81 (CH_2CH_3). FABMS: *m*/*z* 366 ($[M+H]^+$), 388 ($[M+Na]^+$). Anal. Calcd for $C_{17}H_{23}N_3O_4S$: C, 55.87; H, 6.34; N, 11.50; S, 8.77. Found: C, 55.69; H, 6.22; N, 11.42; S, 8.67.

An intermediate, mixed fraction (0.52 g) was also obtained.

The structure of the compound previously described[28] as the β-anomer **8** is unknown.

3.7. Ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-β-D-mannopyranoside (9)

A solution of thioglycoside **8** (1.6 g, 4.38 mmol) in MeOH (40 mL) was made strongly alkaline by addition of 0.1 M NaOMe, and stirred overnight at room temperature. TLC (3:1 hexane–EtOAc) showed the reaction was complete, and dry ice was added to neutralize the base. The mixture was concentrated, and chromatography gave the deacetylated product **9** (1.35 g, 94%), mp 90–92 °C; $[\alpha]_D$ −235 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.73, 4.68 (2d, 2H, ^2J 11.7 Hz, CH_2Ph), 4.52 (br d, 1H, $J_{1,2} \sim 0.9$ Hz, H-1), 4.09 (br d, 1H, H-2), 3.48–3.44 (m, 2H, H-3,4), 3.20–3.16 (m, 1H, H-5), 2.74–2.67 (m, 2H, CH_2CH$_3$), 2.43 (s, 1H, OH), 1.37 (d, 3H, $J_{5,6}$ 6.3 Hz, H-6), 1.29 (t, 3H, *J* 7.5 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 82.97 (C-1), 80.83 (C-3), 75.07 (C-5), 71.48 (CH_2Ph), 68.40 (C-2), 63.53 (C-4), 25.30 (CH_2CH_3), 18.58 (C-6), 14.88 (CH_2CH_3); CIMS: *m*/*z* 346 ($[M+Na]^{+)}$. Anal. Calcd for $C_{15}H_{21}N_3O_3S$: C, 55.71; H, 6.54; N, 12.99. Found: C, 55.77; H, 6.57; N, 13.11.

3.8. Ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-1-thio-β-D-glucopyranoside (12)

To a stirred solution of the 2-hydroxy compound **9** (1.3 g, 4.02 mmol) in 9:1 CH_2Cl_2–pyridine (40 mL) was

added triflic anhydride (0.87 mL, 5.2 mmol). The mixture was stirred at room temperature for 0.5 h, when TLC (4:1 hexane–acetone) showed that the reaction was complete. The mixture was partitioned between CH_2Cl_2 and aq $NaHCO_3$, the organic layer was concentrated, and chromatography of the residue gave ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-2-*O*-triflyl-β-D-mannopyranoside (**10**, 1.57 g, 86%). ^{1}H NMR ($CDCl_3$): δ 5.22 (br d, 1H, $J_{2,3} \sim 2.8$ Hz, H-2), 4.90, 4.58 (2d, 2H, ^2J 11.7 Hz, CH_2Ph), 4.62 (s, 1H, H-1), 3.51 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 3.40 (t, 1H, J 9.4 Hz, H-4), 3.28–3.18 (m, 1H, H-5), 2.78–2.70 (m, 2H, CH_2CH$_3$), 1.38 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6), 1.29 (t, 3H, J 7.5 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 118.22 (q, CF_3SO_2), 83.39 (C-2), 80.65 (C-1), 78.32 (C-3), 75.87 (C-5), 72.64 (CH_2Ph), 63.36 (C-4), 25.84 (CH_2CH$_3$), 18.56 (C-6), 14.74 (CH$_2$CH_3); CIMS: *m*/*z* 456 ([M+H]$^+$).

$NaNO_2$ (455 mg, 6.6 mmol) was added to a solution of **10** (1 g, 2.2 mmol) in DMF (10 mL), and the mixture was stirred for 1 h at 50 °C. TLC (5:1 toluene–EtOAc) showed that the starting material was consumed and that several products were formed. After concentration, chromatography gave the major product, ethyl 4-azido-3-*O*-benzyl-4,6-dideoxy-1-thio-β-D-glucopyranoside (**11**, 350 mg, 50%) as an oil. ^{1}H NMR ($CDCl_3$): δ 4.96, 4.86 (2d, 2H, ^2J 10.9 Hz, CH_2Ph), 4.26 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 3.54 (ddd, 1H, $J_{2,3}$ 8.7, $J_{2,OH}$ 1.6 Hz, H-2), 3.43 (t, 1H, J 9.5 Hz, H-3), 3.29–3.24 (m, 1H, H-5), 3.16 (t, 1H, H-4), 2.75–2.68 (m, 2H, CH_2CH$_3$), 2.42 (d, 1H, OH), 1.35 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6), 1.31 (t, 3H, J 7.5 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 86.03 (C-1), 83.84 (C-3), 75.10 (C-2), 75.03 (CH_2Ph), 73.46 (C-5), 67.26 (C-4), 24.36 (CH_2CH$_3$), 18.76 (C-6), 15.74 (CH$_2$CH_3); CIMS: *m*/*z* 346 ([M+Na]$^+$).

MeI (2.5 mL, 40 mmol), followed by Ag_2O (1 g, 15 mmol) and a catalytic amount of Me_2S, was added to a solution of alcohol **11** (330 mg, 1.02 mmol) in THF (2.5 mL, 40 mmol), and the mixture was stirred overnight, protected from light, at room temperature. TLC (6:1 hexane–EtOAc) showed that the reaction was complete, and that one product was formed. The mixture was filtered through a Celite pad, the filtrate was concentrated, and the residue was chromatographed, to give pure **12** (265 mg, 87%) as a crystalline solid, mp 36–38 °C, which could not be recrystallized from common organic solvents, $[\alpha]_D$ +14.4 (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 4.90, 4.82 (2d, 2H, ^2J 10.7 Hz, CH_2Ph), 4.31 (d, 1H, $J_{1,2}$ 9.9 Hz, H-1), 3.62 (s, 3H, OCH$_3$), 3.42 (t, 1H, J 8.8 Hz, H-3), 3.25–3.08 (m, 3H, H-5,4,2, in that order), 2.81–2.64 (m, 2H, CH_2CH$_3$), 1.33 (d, 3H, $J_{5,6}$ 5.5 Hz, H-6), 1.30 (t, 3H, J 7.4 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 84.64 (C-1), 84.45 (C-3), 83.65 (C-2), 75.48 (CH_2Ph), 74.55 (C-5), 67.50 (C-4), 60.94 (CH_3), 24.82 (CH_2CH$_3$), 18.57 (C-6), 14.84 (CH$_2$CH_3); FABMS (after addition of LiCl): *m*/*z* 344 [M+Li]$^+$. Anal. Calcd for $C_{16}H_{23}N_3O_3S$: C, 56.95; H, 6.87; N, 12.45. Found: C, 57.25; H, 7.00; N, 12.30.

3.9. Ethyl 3-*O*-benzyl-4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-1-thio-β-D-glucopyranoside (14)

At room temperature, H_2S was passed, for 30 min, through a stirred solution of azido sugar **12** (230 mg, 0.68 mmol) in a 2:1 mixture H_2O–pyridine (12 mL). The solution was stirred at 40 °C for 3 h, when TLC (95:5 CH_2Cl_2–MeOH) showed the reaction to be complete. The mixture was concentrated, and the residue was chromatographed to give ethyl 4-amino-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-1-thio-β-D-glucopyranoside (**13**, 205 mg, 95%). ^{1}H NMR ($CDCl_3$): δ 4.98, 4.69 (2d, 2H, ^2J 11.4 Hz, CH_2Ph), 4.38 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), 3.63 (s, 3H, OCH$_3$), 3.25–3.09 (m, 3H, H-3,5,2, in that order), 2.83–2.67 (m, 2H, CH_2CH$_3$), 2.46 (t, 1H, J 9.3 Hz, H-4), 1.31 (t, 3H, J 7.4 Hz, CH$_2$CH_3) 1.26 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 85.89 (C-2), 84.71 (C-1), 84.22 (C-3), 76.83 (C-5), 75.07 (CH_2Ph), 60.60 (OCH$_3$), 57.95 (C-4), 24.74 (CH_2CH$_3$), 18.11 (C-6), 14.85 (CH$_2$CH_3); CIMS: *m*/*z* 312 ([M+1]$^+$).

To a solution of amine **13** (180 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) was slowly added HATU (102.6 mg, 0.27 mmol) followed by *N*,*N*-diisopropylethylamine (0.054 mL, 0.27 mmol), and the mixture was stirred at room temperature for 1 h. TLC (25:1 CH_2Cl_2–MeOH) showed that the reaction was complete. The mixture was concentrated, and the residue was chromatographed to afford coupling product **14** in virtually theoretical yield, mp 112–114 °C (from hexane containing a few drops of *i*-Pr$_2$O), $[\alpha]_D$ −80.3 (*c* 0.8). ^{1}H NMR ($CDCl_3$): δ 5.53 (d, 1H, $J_{NH,4}$ 8.5 Hz, NH), 4.89, 4.65 (2d, 2H, ^2J 11.7 Hz, CH_2Ph), 4.36 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 3.65–3.58 (m, 4H, H-4, incl. s, 3.64, OCH$_3$), 3.54–3.43 (m, 2H, H-3,5), 3.17 (dd, 1H, $J_{2,3}$ H-2), 2.80–2.68 (m, 2H, CH_2CH$_3$), 2.20, 2.10 (2d, 2H, ^2J 15.1 Hz, H-2′), 1.31 (t, 3H, J 7.2 Hz, CH$_2$CH_3), 1.24, 1.23 (2s, 3H each, 2CH$_{3'}$), 1.21 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 84.52 (C-1), 84.06 (C-2), 81.99 (C-3), 74.99 (C-5), 74.20 (CH_2Ph), 69.52 (C-3′), 60.78 (OCH$_3$), 56.09 (C-4), 47.67 (C-2′), 29.31, 29.22 (2CH$_{3'}$), 24.67 (CH_2CH$_3$), 18.25 (C-6), 14.85 (CH$_2$CH_3); CIMS: *m*/*z* 412 ([M+1]$^+$). Anal. Calcd for $C_{21}H_{33}NO_5S$: C, 61.29; H, 8.08; N, 3.40. Found: C, 61.03; H, 8.00; N, 3.34.

3.10. 3-*O*-Benzyl-4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-α,β-D-glucopyranose (3-*O*-benzylanthrose, 15)

NIS (48 mg, 0.45 mmol), followed by a drop of water, was added at 0 °C, to a stirred solution of thioglycoside **14** (90 mg, 0.21 mmol) in acetone (2 mL). After 1 h, TLC (EtOAc) showed that the reaction was complete

and that one product, showing slower chromatographic mobility than **14**, was formed. After concentration, chromatography of the residue gave **15** (83 mg, 91%), which crystallized as a mixture of anomers (α:β ~ 3:1, NMR), mp 168–170 °C (from *i*-Pr_2O containing a few drops of EtOAc), $[\alpha]_D$ +5.2 (*c* 0.4). ^1H NMR (α-anomer, $CDCl_3$): δ 5.89 (d, $J_{NH,4}$ 9.0 Hz, NH), 5.33 (d, $J_{1,2}$ 3.2 Hz, H-1), 4.83, 4.62 (2d, partially overlapped, ^2J 11.7 Hz, CH_2Ph), 3.96–3.91 (m, H-5), 3.81 (q, *J* 9.4 Hz, H-4), 3.71 (t, partially overlapped, H-3), 3.51 (s, partially overlapped, OCH_3), 3.39 (dd, $J_{2,3}$ 8.6 Hz, H-2), 2.15 (dd, ^2J 14.8 Hz, H-2′a,b), 1.23, 1.20 (2s, $2CH_{3'}$), 1.22 (d, $J_{5,6}$ 6.7 Hz, H-6); ^{13}C NMR (α-anomer, $CDCl_3$): δ 172.40 (CO), 90.23 ($J_{C,H}$ 168 Hz, C-1), 82.46 (C-2), 77.17 (C-3), 73.86 (*C*H_2Ph), 69.55 (C-3′), 67.24 (C-5), 58.62 (OCH_3), 54.83 (C-4), 47.73 (C-2′), 29.30, 29.27 (2*C*$H_{3'}$), 18.06 (C-6); ^1H NMR (β-anomer, $CDCl_3$): δ 5.89 (d, $J_{NH,4}$ 9.0 Hz, NH), 4.85, 4.63 (2d, partially overlapped, ^2J 11.5 Hz, CH_2Ph), 4.61 (d, partially overlapped, H-1), ~3.71 (m, partially overlapped, H-4), 3.62 (s, 3H, OCH_3), 3.56–3.46 (m, partially overlapped, H-3,5), 3.15 (t, *J* 8.2 Hz, H-2), 2.15 (dd, ^2J 14.8 Hz, H-2′a,b), 1.25 (d, $J_{5,6}$ 6.3 Hz, H-6), 1.23, 1.20 (2s, $2CH_{3'}$); ^1H NMR (β-anomer, C_6D_6): δ 5.02 (d, $J_{NH,4}$ 8.9 Hz, NH), 4.79, 4.53 (2d, ^2J 11.7 Hz, CH_2Ph), 4.38 (d, $J_{1,2}$ 8.5 Hz, H-1), ~3.76–3.71 (m, partially overlapped, H-5), 3.56 (q, *J* 9.8 Hz, H-4), 3.49 (s, OCH_3), 3.31 (t, H-3), 2.99 (dd, $J_{2,3}$ 8.3 Hz, H-2), 1.98, 1.87 (2d, ^2J 14.8 Hz, H-2′a,b), 1.13, 1.11 (2s, $2CH_{3'}$), 1.07 (d, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR (β-anomer, $CDCl_3$): δ 172.53 (CO), 97.07 ($J_{C,H}$ 161 Hz, C-1), 85.45 (C-2), 80.14 (C-3), 73.77 (*C*H_2Ph), 70.97 (C-5), 69.53 (C-3′), 60.41 (OCH_3), 55.70 (C-4), 47.83 (C-2′), 29.33, 29.30 ($2CH_{3'}$), 18.17 (C-6); CIMS: *m*/*z* 390 ($[M+Na]^+$). Anal. Calcd for $C_{19}H_{29}NO_6$: C, 62.11; H, 7.96; N, 3.81. Found: C, 61.85; H, 7.87; N, 3.78.

3.11. 4,6-Dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-α,β-D-glucopyranose (anthrose, 16)

A solution of the above anthroside **15** (40 mg, 0.10 mmol) in 1:1 H_2O–MeOH (5 mL) was stirred under hydrogen for 48 h at 50 °C in the presence of 5% Pd/C catalyst (40 mg). TLC (4:1 CH_2Cl_2–MeOH) showed that the reaction was complete and that one product was formed. After filtration and concentration of the filtrate, the residue was chromatographed, mainly to remove residual catalyst. Fractions containing anthrose were concentrated, a solution of the residue in H_2O was filtered through a syringe filter (0.02 μm porosity) and freeze dried, to give anthrose (**16** anomeric mixture, α:β ~ 4.5:5.5, NMR) as a white solid in virtually theoretical yield. ^1H NMR (α-anomer, D_2O): δ 5.44 (d, $J_{1,2}$ 3.6 Hz, H-1), 3.99–3.94 (m, H-5), 3.73 (t, *J* 9.7 Hz, H-3), 3.63 (m, partially overlapped, H-4), 3.48 (s, OCH_3), 3.32 (dd, H-2), 2.48–2.44 (m, H-2′), 1.31, 1.30 (2s, $2CH_{3'}$), 1.17 (d, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (α-anomer, D_2O): δ 176.76 (CO), 92.05 (C-1), 83.86 (C-2), 73.02 (C-3′), 72.41 (C-3), 69.00 (C-5), 60.37 (OCH_3), 59.46 (C-4), 51.60 (C-2′), 31.01, 30.86 ($2CH_{3'}$), 19.80 (C-6); ^1H NMR (β-anomer, D_2O): δ 4.63 (d, $J_{1,2}$ 7.9 Hz, H-1), 3.63 (partially overlapped, H-4), 3.61 (s, CH_3O), 3.58–3.51 (m, H-5, incl. 3.53, t, *J* 9.3 Hz, H-3), 3.04 (dd, 1H, $J_{2,3}$ 9.3 Hz, H-2), 2.48–2.44 (m, H-2′), 1.31, 1.30 (2s, $2CH_{3'}$), 1.20 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR (β-anomer, D_2O): δ 176.76 (CO), 98.29 (C-1), 87.10 (C-2), 75.90 (C-3), 73.64 (C-5), 73.02 (C-3′), 60.37 (OCH_3), 59.29 (C-4), 51.64 (C-2′), 31.04, 30.84 ($2CH_{3'}$), 19.80 (C-6); ESIMS: *m*/*z* 330 ($[M+Na]^+$).

3.12. Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-β-D-glucopyranoside (19)

Triflic anhydride (0.4 mL, 2.5 mmol) was added at 0 °C to a stirred solution of methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-β-D-mannopyranoside (**17**,[29] 500 mg, 1.7 mmol) in CH_2Cl_2 (15 mL) containing pyridine (0.85 mL, 10 mmol). The cooling was removed and, when TLC (4:1 hexane–EtOAc) showed that the reaction was complete (~1 h) the mixture was concentrated. Chromatography gave pure methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside (**18**, 570 mg, ~100%). ^1H NMR ($CDCl_3$): δ 5.12 (br d, 1H, H-2), 4.85, 4.57 (2d, 2H, ^2J 11.3 Hz, CH_2Ph), 4.36 (d, 1H, $J_{1,2}$ ~ 0.6 Hz, H-1), 3.52 (s, 3H, OCH_3), 3.48 (dd, 1H, $J_{2,3}$ 2.8, $J_{3,4}$ 9.9 Hz, H-3), 3.37 (t, 1H, *J* 9.9 Hz, H-4), 3.23–3.13 (m, 1H, H-5), 1.39 (d, 3H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.87 (C-1), 80.76 (C-2), 77.00 (C-3), 72.18 (*C*H_2Ph), 71.30 (C-5), 63.39 (C-4), 56.87 (OCH_3), 18.16 (C-6); ESIMS: *m*/*z* 448 ($[M+Na]^+$).

$NaNO_2$ (267 mg, 3.87 mmol) was added to a solution of the above triflate (550 mg, 1.29 mmol) in DMF (5 mL), and the solution was stirred at room temperature for 24 h. TLC (4:1 hexane–EtOAc) showed that the reaction was complete and that three products, two showing faster and one slower mobility than the starting material, were formed. The mixture containing some precipitate was concentrated, and the residue was chromatographed to give the desired, title compound **19** (276 mg, 73%), mp 68.5–69 °C (from *i*-Pr_2O–hexane), $[\alpha]_D$ −9 (*c* 0.4). ^1H NMR ($CDCl_3$): δ 4.95, 4.85 (2d, 2H, ^2J 11 Hz, CH_2Ph), 4.11 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.54–3.51 (m, 4H, H-2, incl. 3.54, s, OCH_3), 3.44 (t, 1H, *J* 9.1 Hz, H-3), 3.27–3.23 (m, 1H, H-5), 3.15 (t, 1H, *J* 9.5 Hz, H-4), 2.72 (d, 1H, $J_{2,OH}$ 2.6 Hz, OH), 1.34 (d, 3H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 103.38 (C-1), 82.35 (C-3), 74.95 (CH_2Ph), 74.79 (C-2), 70.66 (C-5), 67.25 (C-4), 57.08 (OCH_3), 18.35 (C-6); ESIMS: *m*/*z* 316 ($[M+Na]^+$). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N, 14.33. Found: C, 57.33; H, 6.58; N, 14.33.

3.13. Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranoside (20)

A mixture of alcohol **19** (740 mg), MeI (4 mL), Ag_2O (1.2 g), and a catalytic amount of Me_2S in 1,2-dimethoxyethane (10 mL) was stirred at room temperature for 8 h, protected from light. A fresh portion of MeI (4 mL) and Ag_2O (1.2 g) were added, and stirring was continued overnight. TLC (4:1 hexane–EtOAc) showed that the reaction was complete and that a faster moving product was formed. After filtration and concentration of the filtrate, chromatography gave **20** (690 mg, 89%), mp 67–68 °C (from EtOH), $[\alpha]_D$ +48 (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 4.89, 4.79 (2d, 2H, ^2J 10.8 Hz, CH_2Ph), 4.14 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.58 (s, 3H, OCH_3-2), 3.52 (s, 3H, OCH_3-1), 3.39 (t, 1H, *J* 9.2 Hz, H-3), 3.21–3.16 (m, 1H, H-5), 3.12–3.09 (m, 2H, H-2,4), 1.33 (d, 3H, $J_{5,6}$ 5.9 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 104.21 (C-1), 84.17 (C-2), 82.75 (C-3), 75.38 (*C*H_2Ph), 70.32 (C-5), 67.45 (C-4), 60.52 (OCH_3-2), 56.93 (OCH_3-1), 18.37 (C-6); ESIMS: *m/z* 330 ($[M+Na]^+$). Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67. Found: C, 58.73; H, 6.89; N, 13.72.

3.14. Methyl 4-amino-4,6-dideoxy-2-*O*-methyl-β-D-glucopyranoside (21)

A solution of azide **20** (500 mg) in MeOH (20 mL) was stirred overnight under hydrogen in the presence of 5% Pd/C catalyst (400 mg). (Caution! Extreme fire hazard.) TLC (8:1 CH_2Cl_2–MeOH) showed that the reaction was complete, and that one product was formed. After filtration and concentration of the filtrate, a solution of the residue was eluted from a small column of silica gel, to give amine **20** (270 mg, 87%), mp 145–146 °C (from EtOH–Et_2O; the substance sublimes when heated above 75 °C/133 Pa), $[\alpha]_D$ +13 (*c* 0.5). ^{1}H NMR ($CDCl_3$): δ 4.19 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1), 3.60 (s, 3H, OCH_3-2), 3.53 (s, 1H, OCH_3-1), 3.25 (t, 1H, *J* 9.4 Hz, H-3), 3.22–3.18 (m, 1H, H-5), 2.95 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.49 (t, 1H, *J* 9.6 Hz, H-4), 1.30 (d, 1H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 104.22 (C-1), 83.69 (C-2), 76.03 (C-3), 60.50 (CH_3-2), 58.40 (C-4), 56.70 (OCH_3-1), 17.76 (C-6); ESIMS: *m/z* 192 ($[M+H]^+$). Anal. Calcd for $C_8H_{17}NO_4$: C, 50.25; H, 8.96; N, 7.32. Found: C, 49.99; H, 8.93; N, 7.30.

3.15. Methyl 4,6-dideoxy-4-(3-hydroxy-3-methylbutanamido)-2-*O*-methyl-β-D-glucopyranoside (methyl β-anthroside, 22)

Amine **22** (144 mg, 0.75 mmol) was treated as described for preparation of **6**. After 20 min, TLC (10:1 CH_2Cl_2–MeOH) showed that all starting amine was consumed and that one product was formed. Chromatography gave the title anthroside **22** (185 mg, 85%), mp 149–150 °C (from EtOAc), $[\alpha]_D$ −33.5 (*c*, 0.5). ^{1}H NMR ($CDCl_3$): δ 6.60 (d, 1H, $J_{4,NH}$ 8.8 Hz, NH), 4.19 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.12, 3.78 (2br s, 2H, 2OH), 3.66 (q, 1H, *J* 9.7 Hz, H-4), 3.60 (s, 3H, OCH_3-2), 3.53 (s, partially overlapped, OCH_3-1), 3.52 (t, partially overlapped, *J* 9.7 Hz, H-3), 3.49–3.45 (m, 1H, H-5), 3.00 (dd, 1H, $J_{2,3}$ 8.9 Hz, H-2), 2.41, 2.38 (2d, 2H, ^2J 14.3 Hz, H-2′a,b), 1.31, 1.30 (2s, 6H, 2$CH_{3'}$), 1.28 (d, 3H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 173.02 (CO), 103.97 (C-1), 83.87 (C-2), 74.04 (C-3), 70.67 (C-5), 69.85 (C-3′), 60.58 (OCH_3-2), 57.02 (C-4), 56.77 (OCH_3-1), 48.62 (C-2′), 29.70, 29.21 (2$CH_{3'}$), 17.98 (C-6); ESIMS: *m/z* 314 ($[M+Na]^+$). Anal. Calcd for $C_{13}H_{25}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.34; H, 8.58; N, 4.82.

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