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Towards the Synthesis of Aureolic Acid Analogue Conjugates: Synthesis and Glycosidation Reactions of 3-*O*-Acetyl-4-azido-2,4,6-trideoxy-L-glucopyranose Derivatives

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A stereoselective synthesis of 3-*O*-acetyl-4-azido-glucopyranose (6) is described. The derived anomeric acetate (13), and especially the thioglycoside (14), are demonstrated to be good donors for synthesis of α-glycosides of (6). Donor (14) was used in the synthesis of trisaccharide (21), which is targeted for use in the synthesis of the aureolic acid trisaccharide conjugate analogue (5).

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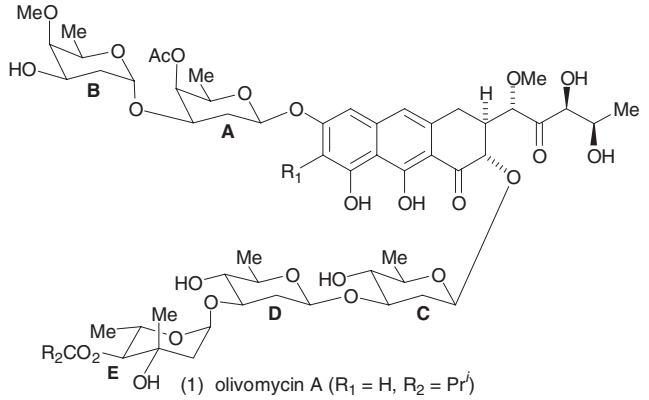
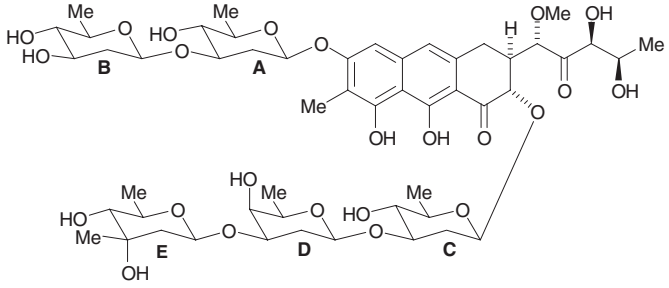
Introduction

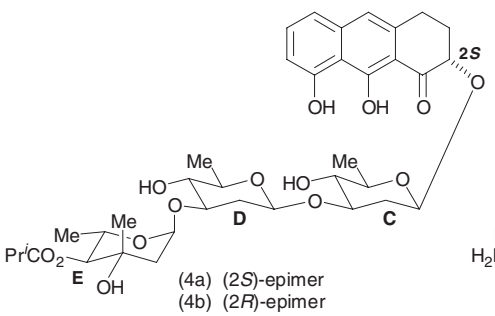
In the preceding paper[1] we described syntheses of trisaccharide conjugates (4a) and (4b), which are simplified analogues of olivomycin A (1) and other members of the aureolic acid antitumour antibiotic family.[2–4] These analogues were synthesized in order to assess the minimum structural features required for biological activity. It is known that the biological effects of the aureolic acids derive from their ability to bind in the minor groove of double-stranded deoxyribonucleic acid (DNA) as 2 : 1 antibiotic/Mg^{2+} complexes, with selectivity for guanine/cytosine (GC)-rich sequences.[5–8] It is also known that mithramycin (3) binds to the GC-rich promoter regions of the c-myc protooncogene and the dihydrofolate-reductase gene, thereby preventing their translation.[9,10] Nuclear magnetic resonance (NMR) studies by Patel[5,6] and Kahne[11,12] have established that the C–D disaccharide of one aureolic acid monomer stacks on the aromatic nucleus of the other aglycone in the 2 : 1 complexes with Mg^{2+}, both when bound to DNA,[5,6] and when free in MeOH solution.[11,12] The intact C–D–E trisaccharide is essential for stable dimer formation (in MeOH)[11,12] and maximal DNA binding,[13,14] as well as for full activity as an antitumour agent.[2–4] However, the contributions of the A–B disaccharide and C3 polyoxygenated side chain to Mg^{2+} dimer formation and DNA binding are not yet known.



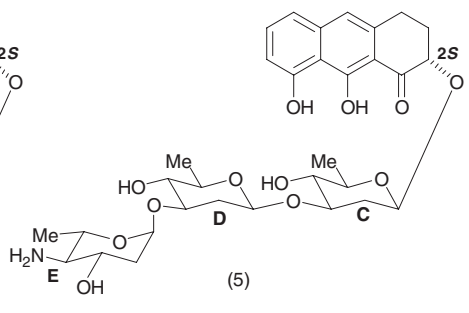
(1) olivomycin A (R_1 = H, R_2 = Pr^i)
(2) chromomycin A_3 (R_1 = R_2 = Me)



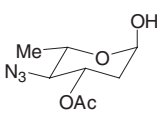
(3) mithramycin



(4a) (2*S*)-epimer
(4b) (2*R*)-epimer



(5)



(6)

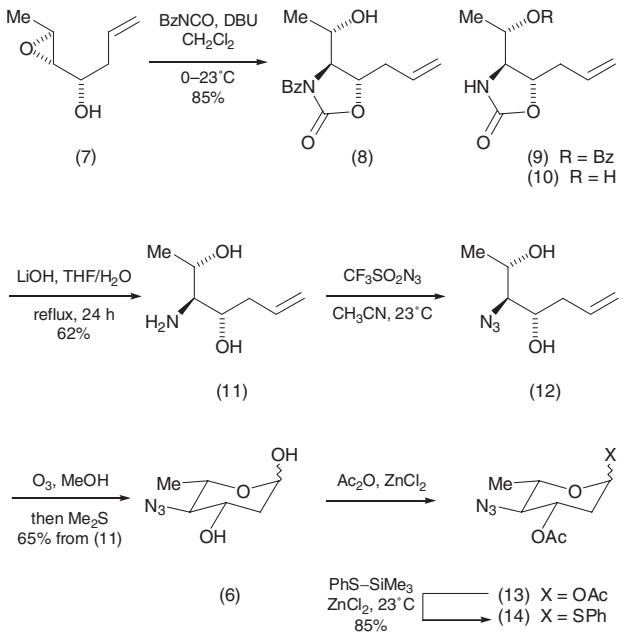
 10.1071/CH01199 0004-9425/02/0100141

One of our long-term goals is to develop aureolic acid analogues with improved properties as DNA binding and chemotherapeutic agents. It is believed that the preferred DNA binding site of the aureolic acids is a tetranucleotide;[7] however, the selectivity for binding among the various GC-rich tetranucleotides is not great in the absolute.[15] One established strategy to improve DNA-binding selectivity is to couple the drug molecule of interest to another DNA-binding element with well established DNA-binding selectivity preferences.[15–19] The NMR structures of DNA-bound aureolic acids reveals that the terminal E sugar makes strong contacts with the floor of the minor groove, and that the C4 acyl substituent is in the middle and projects down the middle of the minor groove. This suggested to us the possibility that an aureolic acid analogue with a C4 amino substituent could be used to couple with other known DNA minor-groove binding agents, thereby leading to the development of more effective and selective DNA binders, and possibly also improved chemotherapeutic agents.

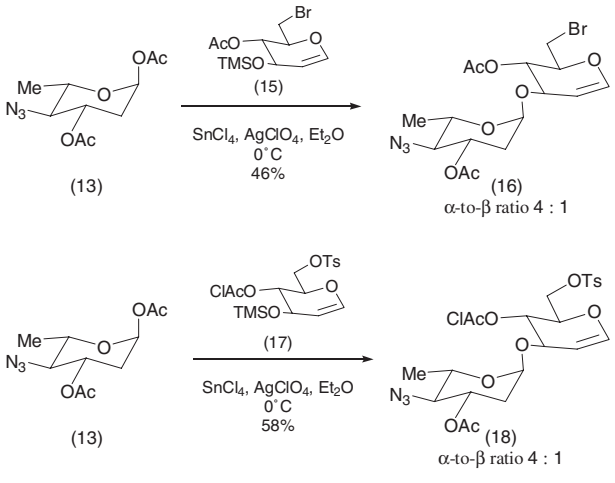
In initial steps towards this goal, we have developed and describe herein a simple, stereoselective synthesis of the 4-azido sugar (6). Glycosidation reactions with suitably activated derivatives of (6) are also described, along with the synthesis of protected forms of the trisaccharide unit [cf. (21)–(23)] of aminotrisaccharide conjugate (5).

Results and Discussion

The synthesis of 4-azido-2,4,6-trideoxy-L-glucopyranose (6) originated from the readily available epoxy alcohol (7) (Scheme 1).[20] Treatment of (7) with benzoyl isocyanate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) provided a mixture of (8) and the benzoyl-transfer isomer (9) in 85% combined yield.[21] Treatment of this mixture with LiOH in aqueous tetrahydrofuran (THF) at reflux for 24 h provided amino diol (11) in 62% yield, along with 33% of carbamate (10) which was hydrolysed by treatment with LiOH to give additional quantities of (11). At this stage we needed to select a protecting group for the amino unit. The main criterion that we applied in our selection process was that the protecting group must be completely compatible with anticipated glycosidation reactions. Carbamate protecting groups were considered undesirable, owing to the propensity for carbamates to participate in neighbouring-group assisted reactions,[22,23] which could compromise the selectivity of the α-glycosidation reactions planned in the synthesis of (6).[24] These considerations prompted us to protect the amino group of (11) as a non-nucleophilic and non-basic azide. Thus, treatment of (11) with trifluoromethanesulfonyl azide and 4-dimethylaminopyridine (DMAP) provided azide (12) in good yield.[25] This intermediate undergoes a facile internal dipolar cycloaddition reaction at ambient temperature, so (12) was used immediately in the following ozonolysis reaction, thereby giving the 4-azido glucopyranose (6) in 65% yield from (11). Finally, treatment of (6) with acetic anhydride and $ZnCl_2$ provided glycosyl acetate (13) almost exclusively as the α-anomer. Treatment of (13) with thiophenyltrimethylsilane and $ZnCl_2$ then provided the thioglycoside (14) in 85% overall yield, as a mixture of anomers.[26]



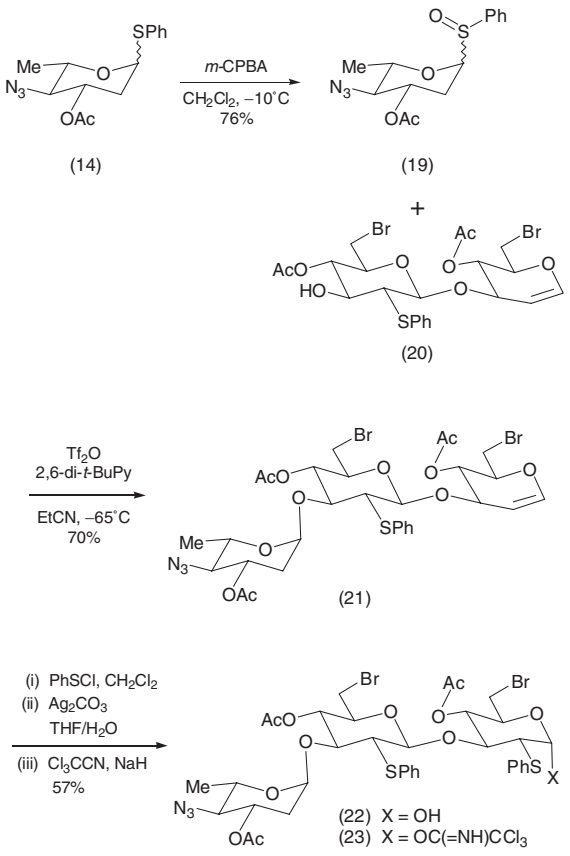
Scheme 1



Scheme 2

Initial glycosidation reactions were performed using glycosyl acetate (13) as the donor (Scheme 2). Best results for the glycosidation reactions of (13) with D-glycal acceptors (15) and (17) were obtained by using $SnCl_4$ and $AgClO_4$ as the activating agents.[27] These reactions provided the indicated disaccharides (16) and (18) in 46 and 58% yields, respectively, both as 4 : 1 α/β anomeric mixtures. Ultimately, much better results were obtained by using thioglycoside (14) as the substrate for the Kahne sulfoxide glycosidation protocol.[28]

Treatment of the mixture of anomeric thioglycosides (14) with *m*-chloroperoxybenzoic acid (*m*-CPBA) at –10°C provided the glycosyl sulfoxides (19) in good yield (Scheme 3). In initial studies directed towards the synthesis of the



Scheme 3

aureolic acid analogue (5), a mixture of (19) and the disaccharide glycal (20) (which was prepared by desilylation of the corresponding *tert*-butyldimethylsilyl (TBS) ether,[29] see Experimental) was treated with triflic anhydride and 2,6-di-*tert*-butylpyridine in propionitrile at –65°C to provide the trisaccharide glycal (21) in 70% yield. The new glycosidic linkage formed in this reaction was exclusively α; none of the β-anomeric product was detected. Treatment of (21) with PhSCl in CH_2Cl_2 and hydrolysis of the intermediate glycosyl chloride with Ag_2CO_3 in wet THF provided trisaccharide pyranose (22).[30] Finally, the trisaccharide trichloroacetimidate derivative (23) was obtained by exposure of (22) to trichloroacetonitrile (as solvent) and NaH at –40°C.[29]

Conclusion

A stereoselective synthesis of 4-azido-glucopyranose (6) has been developed. The anomeric acetate (13), and especially the derived thioglycoside derivative (14), have been shown to be good α-glycosyl donors. Donor (14) was used in the synthesis of trisaccharide (21), which we initially targeted as an intermediate in the synthesis of the aureolic acid trisaccharide conjugate analogue (5). However, in view of the difficulties encountered with the final deprotection of advanced intermediates in our syntheses of (4a,b)[1] and olivomycin A,[31] especially the reductive removal of the thiophenyl substituents, all future work on the synthesis of aureolic acid analogues will utilize our newly introduced method for synthesis of 2-deoxy-β-glycosides that involves the glycosidation reactions of 2-deoxy-2-iodo glycosyl donors.[32–34] Further efforts along these lines, including our efforts to synthesize the targeted aminotrisaccharide conjugate (5),* will be reported in due course.

Experimental Section

General experimental details are provided in the accompanying manuscript.[1]

*(4*R*,5*S*,1′*S*)-4-[1′-Benzoyloxyethyl-5-(prop-2′-enyl)]oxazolidin-2-one (9)*

Benzoyl isocyanate (19.2 mL, 152 mmol) was added dropwise over 10 min to a 0°C solution of the epoxy alcohol (7)[20] (16.3 g, 127 mmol) in anhydrous CH_2Cl_2 (600 mL).[21] The resulting solution was allowed to warm to ambient temperature over 3 h, after which DBU (4.83 g, 31.7 mmol) was added. The resulting mixture was stirred at 23°C for 16 h. Concentration of the reaction mixture, followed by purification of the product by flash chromatography gave a mixture of (8) (unstable) and (9) as a viscous oil (29.5 g, 85% yield). Data for (9): (Found (HRMS): $[M+H]^+$, 276.1234; $[M-C_7H_5O_2]^+$, 153.0836. Calc. for $C_{15}H_{18}NO_4$: $[M+H]^+$, 276.1312; $[M-C_7H_5O_2]^+$, 153.0785). Infrared (IR) ($CHCl_3$) 3460, 3080, 2940, 1760, 1720, 1650, 1640, 1415, 1270, 1245, 1110, 1070 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.47, d, *J* 6.2 Hz, 3H; 2.52–2.46, m, 2H; 3.73, ddd (app. dt), *J* 4.0, 0.8 Hz, 1H; 4.56, qd, *J* 6.2, 4.0 Hz, 1H; 5.24–5.12, m, 3H; 5.80, m, 1H; 6.11, s, 1H, NH; 7.48–7.43, m, 2H; 7.58, m, 1H; 8.03–7.99, m, 2H. ^{13}C NMR ($CDCl_3$) δ 21.3, 34.8, 60.9, 73.5, 75.1, 119.4, 128.6, 129.3, 129.7, 131.8, 133.5, 158.8, 165.8.

*(4*S*,5*S*,6*S*)-5-Aminohept-1-ene-4,6-diol (11)*

A mixture of oxazolidinones (8) and (9) (30.5 g, 111 mmol) and LiOH (18.6 g, 443 mmol) in THF (250 mL) and H_2O (100 mL) was heated at reflux for 36 h. The reaction mixture was cooled to ambient temperature and the solvent removed on a rotary evaporator to give a clear syrup. Subsequent purification of the residue over silica gel (20 : 79 : 1 Et_2O/EtOH/NH_4OH) afforded the desired product (11) as a white *powder* (9.95 g, 62%), and oxazolidinone (10) (5.50 g, 29%) which could be resubjected to the reaction conditions to give additional (11) (Found (HRMS): $[M+H]^+$, 146.1178; $[(M+H)-H_2O]^+$, 128.1075. $C_7H_{16}NO_2$ requires $[M+H]^+$, 146.1249; $[(M+H)-H_2O]^+$, 128.1135). IR (neat) 3700(br) (NH_2, OH), 3100, 3060, 2980, 2920, 1640, 1580, 1430, 1375, 1260, 1060, 980, 920, 790 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.27, d, *J* 6.0 Hz, 3H; 2.41–2.10, m, 6H; 2.57, m, 1H; 3.91–3.87, m, 2H; 5.17–5.09, m, 2H; 5.83, br m, 1H. ^{13}C NMR ($CDCl_3$) δ 19.6, 38.8, 57.6, 69.9, 70.6, 117.8, 134.6.

*(3*S*,5*S*,6*S*)-5-Azidohept-1-ene-4,6-diol (12)*

A solution of aminodiol (11) (247 mg, 1.70 mmol) and DMAP (914 mg, 7.48 mmol) in anhydrous CH_3CN (35 mL) was treated with freshly prepared triflic azide solution[25] (8.81 mL, 0.27 M in CH_2Cl_2), added by syringe over 10 min. (NOTE: Triflic azide is potentially hazardous and must be handled behind a safety shield.[35]) The reaction mixture was stirred at 23°C for 2 h, and then the solvent was removed on a rotary evaporator. The residue was filtered through a small bed of silica gel to give azide (12) as a clear syrup (284 mg, 97%). IR (neat) 3100–3700(br) (OH), 3080, 2980, 2920, 2100, 1640, 1260, 920 cm^{-1}. ^1H NMR ($CDCl_3$) δ 1.34, d, *J* 6.4 Hz, 3H; 2.45–2.33, m, 2H; 2.62, br s, 2H, OH; 3.17, dd, *J* 5.9, 3.0 Hz, 1H; 4.04, dq, *J* 5.9,

* In unpublished preliminary investigations, we have coupled trichloroacetimidate (23) with the same aglycone as in analogues (4a) and (4b). Unfortunately, all attempts to deprotect the trisaccharide conjugate to provide the targeted aminotrisaccharide (5) have also met with considerable difficulty, paralleling the problematic thiophenyl group reduction that plagued our syntheses of (4a), (4b), and olivomycin A itself.

2.7 Hz, 1H; 4.12, dt (app. q), *J* 6.2 Hz, 1H; 5.18, m, 1H; 5.22, m, 1H; 5.81, m, 1H. ^{13}C NMR ($CDCl_3$, 300 MHz) δ 20.7, 39.2, 68.7, 69.4, 70.5, 119.3, 133.9.

Owing to the instability of (12), which undergoes an internal dipolar cycloaddition reaction at ambient temperature, this compound was used directly in the next step without further purification.

Acetyl 3-O-*Acetyl-4-azido-2,4,6-trideoxy*-α-*L-glucopyranoside (13)*

A solution of azido diol (12) (284 mg, 1.66 mmol) in anhydrous MeOH (15 mL) at –40°C was treated with a stream of ozone in O_2 until (12) was consumed as shown by TLC analysis. Excess O_3 was removed with a stream of N_2, and then the ozonide solution was treated with dimethyl sulfide (1.19 mL). The solvent was removed under vacuum and the crude residue was treated with excess acetic anhydride (1.2 mL) in the presence of $ZnC1_2$ (4.4 mg, 0.03 mmol) at 23°C for 23 h. Saturated aqueous Na_2CO_3 solution (10 mL) was carefully added to the reaction mixture and the resulting mixture was stirred at 23°C for 2 h. The mixture was then diluted with EtOAc and the organic layer was collected. The aqueous layer was extracted with EtOAc (3 × 10 mL), washed with saturated aqueous Na_2CO_3, brine, dried (Na_2SO_4), and concentrated under reduced pressure to give the crude *diacetate*. This material consisted primarily of the α-anomer (384 mg, 90%) along with a small amount of the β-anomer. Data for α-anomer (13) (Found (HRMS): $[(M+H)-C_2H_3O_2]^+$, 198.0877; $[(M+H)-C_4H_7N_2O_4]^+$, 110.0610. $C_{10}H_{14}N_3O_5$ requires $[(M+H)-C_2H_3O_2]^+$, 198.0865; $[(M+H)-C_4H_7N_2O_4]^+$, 110.0576). IR (neat) 2989, 2984, 2980, 2939, 2912, 2119, 1760, 1747, 1366, 1249, 1196, 1146, 1033, 993, 898 cm^{-1}. ^{1}H NMR ($CDC1_3$) δ 1.32, d, *J* 6.2 Hz, 3H; 1.83, ddd, *J* 13.4, 11.6, 3.5 Hz, 1H; 2.10, s, 3H; 2.11, s, 3H; 2.27, ddd, *J* 13.4, 5.1, 1.3 Hz, 1H, H2*eq*; 3.21, t, *J* 9.9 Hz, 1H; 3.71, dq, *J* 10.0, 6.2 Hz, 1H; 5.19, ddd, *J* 11.3, 9.7, 5.1 Hz, 1H; 6.17, dd, *J* 3.4,1.3 Hz, 1H. ^{13}C NMR ($CDC1_3$) δ 18.6, 21.1, 34.1, 66.1, 68.8, 69.8, 90.70, 90.72, 169.2, 170.0.

Thiophenyl 3-O-*Acetyl-4-azido-1,2,4,6-tetradeoxy*-α-*L-glucopyranose (14)*

A solution of the glycosyl acetate (13) (1.72 g, 6.69 mmol) in anhydrous CH_2Cl_2 (50 mL) at 23°C was treated with thiophenyltrimethylsilane (3.66 g, 3.7 mmol) followed by tetra-*n*-butylammonium iodide (2.97 g, 20.1 mmol) and zinc(II) iodide (6.41 g, 20.1 mmol).[26] The reaction flask was covered with foil and stirred at 23°C for 1 h. The mixture was then diluted with CH_2Cl_2 (100 mL) and washed with H_2O (2 × 50 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (1 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 10% EtOAc/hexane as eluent. This gave *thioglycoside* (14) (1.92 g, 93%) as a 3 : 1 mixture of α and β anomers. Data for the α-anomer (14α) (Found: C, 54.8; H, 5.6; N, 12.6%; $[M+NH_4]^+$, 325.1324. $C_{14}H_{21}N_4O_3S$ requires C, 54.7; H, 5.6; N, 13.7%; $[M+NH_4]^+$, 325.1334). HPLC (10% EtOAc/hexane, 21 mm column, 12 mL min^{-1}, t_R 9.0 min). ^{1}H NMR ($CDCl_3$) δ 1.35–1.30, d, *J* 6.4 Hz, 3H; 2.16–2.07, m, 4H; 2.52–2.46, dd, *J* 13.2, 5.1 Hz, 1H; 3.23–3.16, t, *J* 9.9 Hz, 1H; 4.21–4.14, m, 1H; 5.27–5.16, m, 1H; 5.60–5.56, d, *J* 5.8 Hz, 1H; 7.33–7.23, m, 3H; 7.46–7.42, d, *J* 1.2 Hz, 2H.

4-O-*Acetyl-3*-O-*(3*-O-*acetyl-4-azido-2,6-dideoxy*-α-*L-glucopyranosyl)-6-bromo-6-deoxy-D-glucal (16α)*

A solution of 4-*O*-acetyl-6-bromo-6-deoxy-D-glucal[29] (61.1 mg, 243 μmol) in anhydrous CH_2Cl_2 (350 μL) was treated with bistrimethylsilyl urea (24.9 mg, 122 μmol) at 23°C for 1 h. Concentration of the reaction mixture under reduced pressure followed by high vacuum for 1 h afforded the trimethylsilyl-D-glucal (15) in quantitative yield.

A solution of $SnC1_4$ (37.4 μL, 37.4 μmol, 1.0 M in CH_2Cl_2) was added to $AgClO_4$ (15.5 mg) suspended in anhydrous Et_2O (1.9 mL) at 23°C. The mixture was shielded from light and stirred for 1 h. This mixture was cooled to 0°C and then an ethereal solution (1.9 mL) of glycal (15) (78.6 mg, 243 μmol) and glycosyl donor (13) (48.1 mg, 187 μmol) was added. The mixture was stirred for 3 h and then saturated aqueous $NaHCO_3$ was added. Standard extractive workup gave the crude disaccharide mixture, which was separated by flash column chromatography (30% EtOAc/hexane). This provided disaccharide (16) as a mixture of anomers (4 : 1 α/β). Further purification by HPLC afforded the desired α-anomer (46%, unoptimized). Partial data for α-anomer: IR (neat) 2978, 2937, 2350 (CO_2), 2107, 1751, 1741, 1649, 1371, 1232, 1041, 997 cm^{-1}. ^{1}H NMR ($CDC1_3$) δ 1.33, d, *J* 6.2 Hz, 3H, H6′; 1.71, ddd, *J* 12.9, 11.5, 3.6 Hz, 1H, H2′*ax*; 2.09, s, 3H; 2.11, s, 3H; 2.21, ddd, *J* 12.9, 5.1, 1.3 Hz, 1H, H2′*eq*; 3.14, t, *J* 10.0 Hz, 1H, H4′; 3.54, dd, *J* 11.0, 8.5 Hz, 1H, H6; 3.62, dd, *J* 11.0, 5.6 Hz, 1H, H6; 3.74, m, 1H, H5′; 4.10, m, 1H, H3; 4.30, dt, *J* 7.6, 4.8 Hz, 1H, H5; 4.92, ddd, *J* 6.4, 4.0, 0.9 Hz, 1H, H2; 5.04, d, *J* 2.7 Hz, 1H, Hl′; 5.16, ddd, *J* 11.6, 9.9, 5.0 Hz, 1H, H3′; 5.21, m, 1H; 6.46, dd, *J* 6.3, 0.8 Hz, 1H. ^{13}C NMR ($CDC1_3$) δ 18.5, 21.0, 39.3, 35.4, 66.3, 66.5, 67.0, 69.2, 70.1, 72.5, 75.3, 93.7, 93.8, 97.6, 144.6, 169.9. Mass spectrum (FAB) *m/z* 470.0 ($[M+Na]^+$, 100%).

3-O-*(3Ef*-O-*Acetyl-4′-azido-2′,6′-dideoxy*-α,β-*L-glucopyranosyl)-4*-O-*chloroacetyl-6-bromo-6-deoxy-D-glucal (18)*

A mixture of donor (13α) (58.8 mg, 0.22 mmol) and acceptor (17) [108 mg, prepared by silylation of the corresponding secondary alcohol by using the procedure outlined for (15)] in anhydrous Et_2O (2.8 mL) was added to a mixture of $SnC1_4$ (22 μL of a 1 M solution, 22 μmol) and $AgClO_4$ (9.0 mg, 44 mmol) suspended in Et_2O (2.8 mL) in the dark at 0°C using the procedure described for preparation of (16). The disaccharide (18) was obtained as a mixture of anomers (4 : 1 α/β) in 58% overall yield after purification on silica gel (30% EtOAc/hexane) and further purification by HPLC (30% EtOAc/hexane, 15 mL min^{-1}). Data for the α-anomer: IR (thin film, $CDC1_3$) 3080, 2960, 2940, 2870, 2110, 1745, 1730, 1650, 1595, 1520, 1410, 1305, 1235, 1180, 1155, 1065, 1030, 820 cm^{-1}. ^{1}H NMR ($CDC1_3$) δ 1.27–1.32, d, *J* 6.4 Hz, 3H, H6′; 1.65–1.75, ddd, *J* 12.9,11.6, 3.8 Hz, 1H, H2′*ax*; 2.10, s, 3H; 2.15–2.22, ddd, *J* 12.9, 4.1, 1.3 Hz, 1H, H2′*eq*; 2.45, s, 3H; 3.10–3.17, t, *J* 9.9 Hz, 1H, H4′; 3.55–3.65, m, 1H, H5′; 4.06, s, 2H; 4.10–4.15, m, 2H, H3 and H6; 4.25–4.31, dd, *J* 10.3, 6.9 Hz, 1H, H6; 4.31–4.35, m, 1H, H5; 4.86–4.90, ddd, *J* 6.8, 3.8, 0.6 Hz, 1H, H2; 5.02–5.04, br. d, *J* 2.7 Hz, 1H, Hl′; 5.04–5.11, m, 2H, H3′and H4; 6.33–6.39, dd, *J* 6.3, 0.9 Hz, 1H, H1; 7.34–7.40, d, *J* 8.6 Hz, 2H; 7.78–7.84, d, *J* 8.6 Hz, 2H. Mass spectrum (fast atom bombardment, FAB) *m/z* 596.1 ($[M+Na]^+$, 100%).

Partial data for β-anomer: ^{1}H NMR ($CDC1_3$) δ 1.34, d, *J* 6.2 Hz, 3H, H6′; 1.57, ddd, *J* 12.3, 11.8, 9.7 Hz, 1H, H2′*ax*; 2.12, s, 3H; 2.25, ddd, *J* 12.5, 5.2, 2.0 Hz, 1H, H2′*eq*; 2.45, s, 3H; 3.12, t, *J* 9.7 Hz, 1H, H4′; 3.24, dd, *J* 9.8, 6.2 Hz, 1H, H5′; 4.06, s, 2H; 4.15, m, 2H, H3 and H6; 4.28, m, 2H, H5 and H6 [dd, *J* 10.2, 6.4 Hz]; 4.65, dd, *J* 9.8, 2.0 Hz, 1H, H1′; 4.85, ddd, *J* 11.8, 9.7, 5.1 Hz, 1H, H3′; 4.92, ddd, *J* 6.2, 3.8, 0.8 Hz, 1H, H2; 5.07, t, *J* 5.1 Hz, 1H, H4; 6.27, dd, *J* 6.3, 1.1 Hz, 1H, H1; 7.34, d, *J* 8.3 Hz, 2H; 7.78, d, *J* 8.3 Hz, 2H.

Phenylsulfoxide 3-O-*Acetyl-4-azido-2,4,6-trideoxy*-α,β-*L-glucopyranose (19)*

A solution of the thioglycoside (14) (1.90 g, 6.2 mmol, 3 : 1 anomer mixture) in anhydrous CH_2Cl_2 (100 mL) was cooled to –78°C and treated dropwise with a solution of *m*-CPBA (1.09 g, 6.2 mmol) in CH_2Cl_2 (15 mL) over 5 min. The reaction mixture was then allowed to warm slowly to –10°C over 1.5 h. It was then quenched with triethylamine (3 mL), diluted with CH_2Cl_2 (100 mL), and immediately poured into a separatory funnel containing saturated aqueous $NaHCO_3$ (100 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography using 35% EtOAc/hexane as eluent to give a mixture of anomeric sulfoxides (19) (1.52 g, 76%) as a clear viscous *syrup*. Data for the α-sulfoxide (19α) (Found: C, 52.2; H, 5.4; N, 12.9%; $[M+Na]^+$ (FAB), 346.3602. $C_{14}H_{17}N_3NaO_4S$ requires C, 52.0; H, 5.3; N, 13.0%; $[M+Na]^+$, 346.3584). R_F 0.27 (40% EtOAc/hexane). IR (thin film, $CHCl_3$) 3050, 2986, 2960, 2925, 2900, 2108, 1445, 1372, 1305, 1297, 1267, 1234, 1157, 1142, 1099, 1079, 1043, 998, 975, 898 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.33, d, *J* 6.0 Hz, 3H, H6; 1.89, ddd, *J* 14.4, 10.9, 6.0 Hz, 1H, H2*ax*; 2.12, s, 3H; 2.84,

ddd, *J* 14.4, 5.3, 1.4, Hz, 1H, H2*eq*; 3.26, t, *J* 9.7 Hz, 1H, H4; 4.01, m, 1H, H5; 4.44, d, *J* 5.3 Hz, 1H, H1; 5.51, m, 1H, H3; 7.53, m, 3H; 7.62, m, 2H. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.6, 20.9, 26.9, 65.6, 72.6, 75.9, 92.1, 124.4, 129.2, 131.5, 140.1, 167.0.

Partial data for the β-sulfoxide (19β): $[\alpha]_D^{25}$ –20° (*c*, 1.04 in EtOH). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.40–1.39, d, *J* 5.8 Hz, 3H; 2.14–1.92, m, 5H; 3.33–3.18, m, 2H; 4.18–4.13, dd, *J* 11.0, 2.9 Hz, 1H; 4.90–4.82, ddd, *J* 11.0, 9.5, 5.5 Hz, 1H; 7.54–7.50, m, 3H; 7.61–7.56, m, 2H. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.6, 20.9, 26.9, 65.6, 72.6, 75.1, 92.1, 124.4, 129.2, 131.5, 140.1, 170.0.

4-O-*Acetyl-3*-O-*[4*-O-*acetyl-3*-O-*(3*-O-*acetyl-4-azido-2,4,6-trideoxy-*α-*L*-gluco-*pyranosyl)-6-bromo-2,6-dideoxy-2-thiophenyl*-β-*D-glucopyranosyl]-6-bromo-2,6-dideoxy-D-arabino-hex-1-enitol (21)*

A solution of 4-*O*-acetyl-3-*O*-(4-*O*-acetyl-6-bromo-3-hydroxy-2,6-dideoxy-2-thiophenyl-β-D-glucopyranosyl)-6-bromo-2,6-dideoxy-D-arabino-hex-1-enitol (20) (487 mg, 0.72 mmol, prepared in 96% yield by deprotection of the corresponding *tert*-butylsilyl ether[29] by using an excess of Et_3N/HF in CH_3CN), sulfoxide (19) (400 mg, 1.23 mmol), and 2,6-di-*tert*-butyl-4-methyl pyridine (386 mg, 1.64 mmol) was dried by evaporation from anhydrous benzene (3 × 15 mL) under reduced pressure. The residue was then dissolved in propionitrile (28 mL, freshly distilled from P_2O_5), cooled to –78°C, and treated with trifluoromethanesulfonic anhydride (245 μL, 1.48 mmol) in one portion. The reaction was allowed to warm to –65°C by removal of dry ice from the cooling bath over 10 min, at which time the appearance of a voluminous precipitate signalled the end of the reaction. The reaction was terminated by adding Et_3N (1.5 mL), followed by addition of saturated aqueous $NaHCO_3$ solution (35 mL) and CH_2Cl_2 (30 mL). The resulting mixture was poured into a separatory funnel and the organic layer was collected. The aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and then concentrated under reduced pressure. The residue was purified by flash chromatography using 40% EtOAc/hexane to give a foam which was further purified by HPLC (30% EtOAc/hexane, 20 mm column, 25 mL min^{-1}). In this way, the desired product (21) (644 mg, 70%) was obtained as a white *foam* (Found: C, 44.4; H, 4.6; N, 5.3%; $[M+Na]^+$ (FAB, 3-nitrobenzyl alcohol (NBA) matrix), 828.0425. $C_{30}H_{37}Br_2N_3O_{11}S$ requires C, 44.6; H, 4.6; N, 5.2%; $[M+Na]^+$, 828.0413). R_F 0.32 (30% EtOAc/hexane, 20 mm column, 10 mL min^{-1}, t_R 23.8 min). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.28–1.24, d, *J* 6.3 Hz, 3H, H6; 1.72–1.62, ddd, *J* 12.6, 9.1, 3.6 Hz, 1H; 2.06, s, 3H; 2.07, s, 3H; 2.12, s, 3H; 2.46–2.39, ddd, *J* 12.6, 5.0, 1.3 Hz, 1H; 3.18–3.06, m, 2H; 3.42–3.28, m, 3H; 3.59–3.48, m, 2H; 3.73–3.62, m, 2H; 4.08–4.04, m, 1H; 4.39–4.33, m, 1H; 4.64–4.60, d, *J* 8.8 Hz, 1H, H1′; 4.84–4.80, m, 1H; 4.89–4.84, dd, *J* 9.7, 8.7 Hz, 1H; 5.21–5.13, ddd, 11.3, 9.8, 4.9 Hz, 1H; 5.32–5.28, m, 2H; 6.46–6.43, dd, *J* 6.3, 0.6 Hz, 1H, H1; 7.39–7.26, m, 3H; 7.5, m, 2H. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.6, 21.0, 21.2, 29.6, 31.3, 35.8, 56.3, 66.7, 67.5, 69.1, 69.7, 69.8, 72.9, 73.8, 75.4, 80.1, 97.6, 99.4, 102.2, 127.6, 129.1, 132.0, 134.1, 144.4, 169.6, 169.7, 169.9.

4-O-*Acetyl-3*-O-*[4*-O-*acetyl-3*-O-*(3*-O-*acetyl-4-azido-2,4,6-trideoxy-*α-*L*-gluco-*pyranosyl)-6-bromo-2,6-dideoxy-2-thiophenyl*-β-*D-glucopyranosyl]-6-bromo-2,6-dideoxy-2-thiophenyl*-α,β-*D-glucopyranose (22)*

PhSCl (50 μL) was added to a stirred solution of the azido trisaccharide glucal (21) (100 mg, 0.124 mmol) in CH_2Cl_2 (2.4 mL) at 0°C. The ice bath was removed and the mixture was stirred for 1 h while warming to ambient temperature. The mixture was concentrated under reduced pressure to give a mixture of crude glycosyl chlorides. This mixture was dissolved in THF (2.4 mL) and H_2O (350 μL), and stirred with Ag_2CO_3 (300 mg, 1.1 mmol) for 24 h. The mixture was diluted with THF and filtered through a pad of Celite. The Celite bed was washed several times with EtOAc and the filtrate was concentrated under reduced pressure to give the crude product, which was purified by flash column chromatography (SiO_2; 20% EtOAc/hexane) to give the *lactol* (22) (88 mg, 76%) as a 12 : 1 α/β mixture of anomers, plus 18 mg of starting glucal. Data for α-anomer (22α) (Found: C, 46.5; H, 4.4%; $[M+Na]^+$ (FAB, NaOAc and NBA matrix), 954.0587. $C_{36}H_{43}Br_2N_3NaO_{12}S_2$ requires C, 46.3; H, 4.6%; $[M+Na]^+$ 954.0553). HPLC (30% EtOAc/hexane, 21 mm column; 10 mL min^{-1}, t_R 23.2 min). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.24, d, *J* 7 Hz, 3H; 1.61, m, 1H; 2.05, s, 3H; 2.11, s, 3H; 2.17, s, 3H; 2.30, m, 1H; 3.19–3.00, m, 4H; 3.27, m, 2H; 3.35, m, 1H; 3.44, m, 2H; 3.70–3.52, m, 3H; 4.25, m, 2H; 4.79, m, 1H; 4.86, m, 1H; 4.94, m, 1H; 5.08, m, 1H; 5.21, m, 1H; 5.28, m, 1H; 7.31–7.18, m, 8H; 7.40, m, 2H. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.6, 21.0, 21.2, 31.8, 35.5, 55.2, 55.5, 66.7, 67.4, 69.6, 69.8, 71.4, 73.2, 73.6, 74.6, 79.6, 92.7, 94.4, 99.1, 102.6, 126.7, 127.5, 128.9, 129.1, 130.4, 131.8, 133.9, 169.7, 169.9, 193.7.

Trichloroacetamido 4-O-*Acetyl-3*-O-*[4*-O-*acetyl-3*-O-*(3*-O-*acetyl-4-azido-2,4,6-trideoxy-*α-*L*-glucopyranosyl)-6-bromo-2,6-dideoxy-2-thiophenyl*-β-*D*-glucopyranosyl]-6-bromo-2,6-dideoxy-*α-*D-glucopyranose (23)*

A solution of the azido trisaccharide lactol (22) (78 mg, 0.084 mmol) in freshly distilled trichloroacetonitrile (2.2 mL) at –40°C was treated with dry NaH powder (16 mg, 0.652 mmol) in portions. The mixture was stirred at –40°C for 1 h and then sealed with Parafilm and placed in the freezer at –20°C for 16–23 h. The resulting yellow solution was filtered through a pad of Celite under N_2 and the Celite pad was washed several times with CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography (20% EtOAc/hexane–1% Et_3N) to give α-imidate (23) (67 mg, 74%). R_F 0.16 (20% EtOAc/hexane). HPLC (20% EtOAc/hexane, 21 mm column, 12 mL min^{-1}, t_R 13.6 min). ^{1}H NMR ($CDCl_3$) δ 1.24, d, *J* 6.1 Hz, 3H, H6′; 1.60, dt, *J* 12.7, 3.8 Hz, 1H, H2′*ax*; 2.05, s, 3H; 2.11, s, 3H; 2.15, s, 3H; 2.29, ddd, *J* 12.7, 5.2, 1.2 Hz, 1H, H2′*eq*; 3.14–3.02, m, 2H; 3.25, dd, *J* 11.0, 10.2 Hz, 1H; 3.49–3.32, m, 4H; 3.61, m, 2H; 3.70, dd, *J* 10.5, 8.3 Hz, 1H; 4.13, m, 1H; 4.37, dd, *J* 11.2, 9.0 Hz, 1H; 4.75, dd, *J* 9.7, 8.4 Hz, 1H; 4.97, dd, *J* 10.3, 10.1 Hz, 1H; 5.05, d, *J* 8.8 Hz, 1H, H1′; 5.07, m, 1H; 5.30, d, *J* 2.9 Hz, 1H, H1′; 6.39, d, *J* 3.2 Hz, 1H, H1; 7.24, m, 4H; 7.29, m, 2H; 7.45, m, 2H; 7.50, m, 2H; 8.72, s, 1H, NH. ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.5, 21.0, 21.1, 21.2, 30.9, 31.2, 35.5, 52.8, 55.0, 55.7, 66.7, 67.3, 69.7, 71.0, 71.6, 73.4, 73.9, 74.1, 79.6, 95.7, 99.1, 102.5, 126.7, 127.9, 128.9, 129.1, 130.4, 132.7, 133.8, 135.2, 160.4, 169.0, 169.4, 169.5. Mass spectrum (FAB) *m/z* 1095.8 ($[M+Na]^+$, 100%).

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