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De novo asymmetric synthesis of rhamno di- and tri-saccharides related to the anthrax tetrasaccharide

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ABSTRACT

An asymmetric synthesis of the di- and tri-saccharide portion of the naturally occurring anthrax tetrasaccharide from acetylfuran has been developed. The construction of the di- and tri-saccharide subunits is based upon our previously disclosed route to anthrax tetrasaccharide. The approach uses iterative diastereoselective palladium-catalyzed glycosylations, Luche reductions, diastereoselective dihydroxylations, and regioselective protections for the assembly of the rhamno-di- and tri-saccharide. The route was also modified for the preparation of the mixed D-/L-di-saccharide analogue.

1. Introduction

Until late 2001, when the unfortunate event of a powdery sample of *Bacillus anthracis* was sent through the U.S. Postal Service,[1] most cultural references to Anthrax were to the American thrash metal band from New York City. The band was formed in 1981 and named after the disease caused by *B. anthracis*.[2] The spore-forming *B. anthracis* is a Gram-positive bacterium, which when inhaled causes anthrax, a fatal infectious disease in humans and other mammals.[3] The mature endospores exhibit remarkable resistance to extremely harsh conditions, which allow the spores to persist for many years,[4] and make them ideal for use as biological weapon.

While the origin of the weaponized material is still in dispute, the effects of having it sent through the post in 2001 are clear. As a result, 22 people were confirmed to be infected with *B. anthracis* and only 7 survived.[5] In fact, all the 7 survivors are believed to have the more easily treated cutaneous form of anthrax. In response, to the seriousness of the threat, there have been extensive efforts aimed at the discovery of new inexpensive treatments (e.g., vaccines and antibacterials) for the disease,[6] as well as, methods for the detection of the *B. anthracis* spores (e.g., antibodies).[7] As part of the efforts to develop methods for detecting *B. anthracis*, the anthrax tetrasaccharide **1** (Fig. 1) was isolated from BclA, a surface glycoprotein found on the exosporium of *B. anthracis*.[8] The unusual structure of anthrax tetrasaccharide **1** consists of unique tris-L-trisaccharide portion terminated by a never before seen D-aminosugar, called anthrose. These unique structural features in conjunction with the general resistance to evolutionary change of carbohydrates, have made the anthrax tetrasaccharide **1** an excellent target for anthrax detection and in turn total synthesis.[9,10]

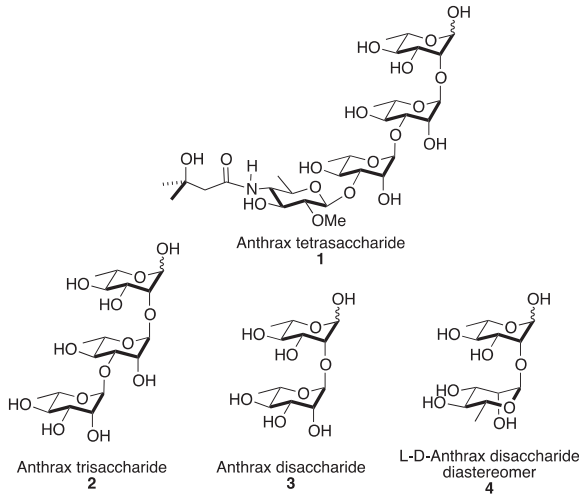

Fig. 1. Anthrax tetrasaccharide and its tri- and di-saccharide fragments.

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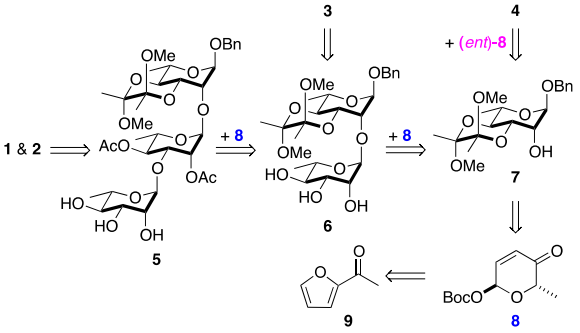
Since its discovery of anthrax tetrasaccharide **1** in 2004, there have been several syntheses of analogues of **1** with various alkyl groups at the reducing end.[11] The addition of functional groups at the anomeric position was made to give the anthrax tetrasaccharide a chemical handle for further conjugation and subsequent manipulations (e.g., antibody/vaccine development). In addition to analogue material with a chemical handle, our de novo approach to the anthrax tetrasaccharide **1** was the only route to prepare synthetic material identical to the isolated natural product.[10] Our synthetic **1** was prepared as part of a broad search aimed at the development of a method for the selective detection of **1** and thus anthrax.[7] To address concern that these methods might focus on the more common tris-rhamnose portion of the molecule, we needed control compounds consisting of the rhamno-di- and tri-saccharide structural motif. Thus, we targeted the all L-tri-saccharide **2** and the all L-di-saccharide **3** as well as the mixed D/L-di-saccharide **4** for synthesis using our previously described approach to anthrax tetrasaccharide **1**.[10] Herein, we describe our successful efforts toward the de novo asymmetric synthesis of anthrax-based rhamno-oligosaccharides **2**, **3**, and **4** (Fig. 1). This de novo asymmetric strategy features iterative use of palladium-catalyzed glycosylation and starts from a commercially available and inexpensive acetylfuran.[12]

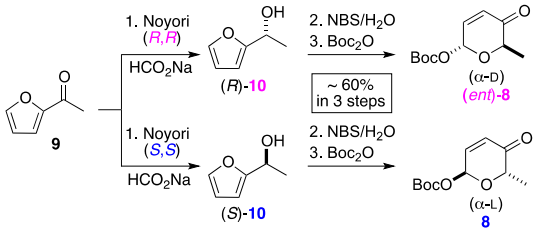
Our de novo approach to anthrax oligosaccharides **2**–**4** is outlined in Scheme 1. Based on our previous route to **1**, we envisioned access the unique rhamno-oligosaccharide scaffold in both anthrax tri-saccharide analogue **2** and di-saccharide analogues **3** and **4** from the protected intermediates **5**–**7**. These precursors, in turn, could be prepared in a highly divergent manner from a glycosylation of Ley-spiroketal di-saccharide **7** with pyranone **8** or (*ent*)-**8**. The rapid assembly of the desired rhamno sugar relies on the iterative use of palladium-catalyzed glycosylation, Luche reduction, and Upjohn dihydroxylation. The required pyranone sugar donor in both D- and L-configuration could be obtained from the achiral starting material acetylfuran **9** through asymmetric Noyori reduction and Achmatowicz oxidative ring expansion.



Scheme 1. Retrosynthetic approach to anthrax saccharides **1**–**4**.

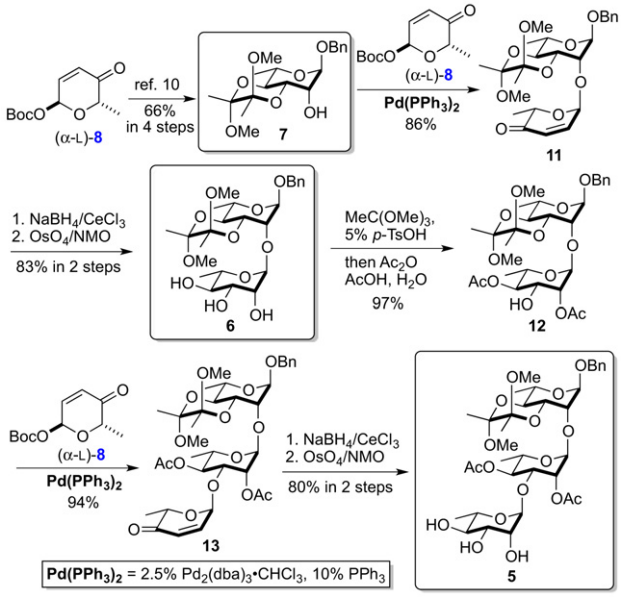
2. Results/discussion

Our approach to the monosaccharide building block began with our efforts to selectively introduce the D- or L-stereochemistry of the rhamnose, which could be derived from the furyl alcohol (*R*)-**10** or (*S*)-**10**. Previously we have shown that either (*R*) or (*S*)-furyl alcohol could be readily prepared by an asymmetric Noyori reduction of commercially available starting material acetylfuran **9** (Scheme 2).[13] Noyori reduction of acetylfuran **9** produced the enantiomerically pure (>96% ee)[14] furfuryl alcohol (*R*)-**10** or (*S*)-**10**, which was exposed in the subsequent Achmatowicz oxidative rearrangement (NBS/H_2O) and *tert*-butoxyl-carbonylation to give the desired Boc-pyranone α-D-(*ent*)-**8** or α-L-**8** with good α-anomeric diastereoselectivity (α:β=4:1).[15] It is worth noting that this route provides Boc-pyranones in 60% overall yield (three steps) with only one chromatographic purification and this route is also scalable for both L- and D-enantiomers.



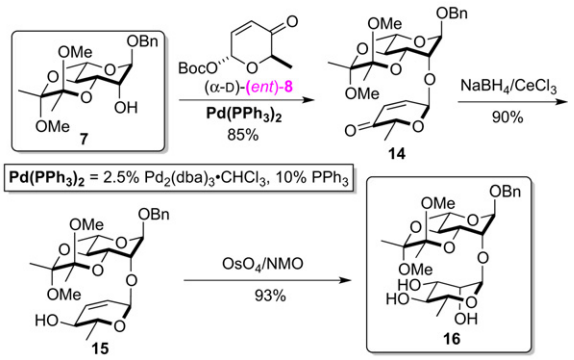
Scheme 2. Preparation of *tert*-butyl-Boc pyranone sugar donors.

Using our previously described route to anthrax tetrasaccharide **1**, we prepared the desired protected known precursors **5**, **6**, and **7** (Scheme 3).[10] In brief, monosaccharide **7** with the free C-2 hydroxyl group can be glycosylated with Boc-pyranone α-L-**8** and in two post-glycosylation transformations ($NaBH_4/CeCl_3$ then OsO_4/NMO) converted into di-saccharide **6**.[16] The conversion of di-saccharide **6** into tri-saccharide **5** began with the selective protection of the C-2/C-4 hydroxyls via a one-pot orthoester/acylation/hydrolysis procedure to give **12**. Once again, a three-step palladium-catalyzed glycosylation/post-glycosylation sequence was used to install the final rhamnose sugar in tri-saccharide **5** with excellent yield and diastereoselectivity.



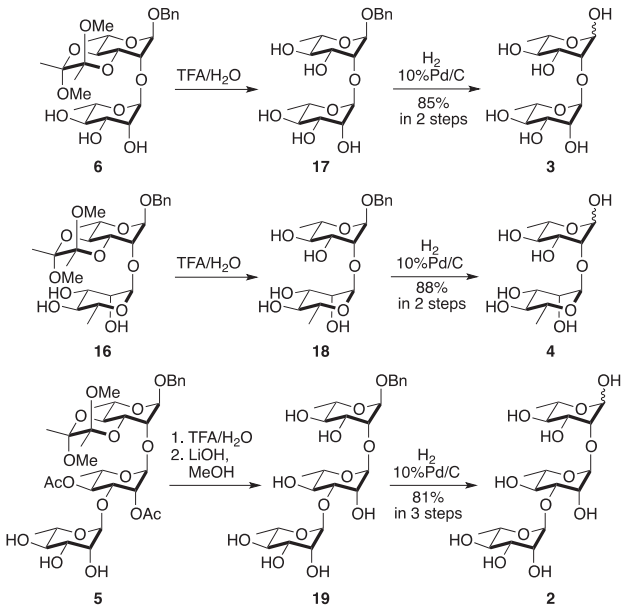
Scheme 3. Previously reported approach to oligosaccharides **5**, **6**, and **7**.

The preparation of the unknown L–D-di-saccharide diastereomer **4** the anthrax di-saccharide analogue **3** began with the common Ley-protected rhamnose intermediate **7**. In a diastereomeric fashion, the C-2 hydroxyl in **7** was glycosylated with Boc-pyranone (*ent*)-**8** to give **14** in a nearly identical yield (85%) for the palladium-catalyzed glycosylation (Scheme 4). Similarly, the resulting enone in L–D-di-saccharide **14** was reduced to give **15** (90%) and dihydroxylated to give **16** (93%), in excellent yield and stereoselectivity. Thus in a diastereomerically divergent fashion, the Ley-protected L-rhamnose **7** can be glycosylated in a three-step sequence to install either a D- or L-rhamnose sugar in nearly identical yields.



Scheme 4. Synthesis of L–D-di-saccharide anthrax precursor.

Finally, all the desired anthrax di- and tri-saccharide analogues **2**, **3**, and **4** were obtained after a two- or three-step deprotection sequence. Simply treating D/L-diastereomers **6** and **16** to aqueous TFA gave di-saccharides **17** and **18**, respectively. The desired fully deprotected diastereomeric di-saccharides **3** and **4** were obtained by hydrogenolysis (1 atm H_2, Pd/C) in excellent yields (85 and 88% over two steps). The deprotection of tri-saccharide **5** was slightly more complicated due to concomitant acetate partial hydrolysis and migration. Thus, the crude product from exposure of **5** to aqueous TFA was immediately followed by the treatment with basic methanol and hydrogenolysis to give tri-saccharide **2** in excellent overall yield (81%) (Scheme 5).



Scheme 5. Preparation of anthrax saccharides **1**–**4**.

3. Conclusion

In conclusion, a highly enantio- and diastereo-controlled approach to the anthrax analogues has been developed in a divergent manner using a Ley-protected rhamno-monosaccharide **7** as the common intermediate for the assembly of *rhamno*-di- and tri-saccharides (i.e., **2** and **3**) and the incorporation of D–L-bis-*rhamno*-di-saccharide diastereomer (i.e., **4**). The feasibility of this was evident in that both the di- and tri-saccharide fragments were readily assembled from achiral starting materials by use of asymmetric catalysis. This de novo asymmetric route illustrates the utilities of Noyori reduction, palladium-catalyzed glycosylation, diastereoselective dihydroxylation, Luche reduction, and selective acylation. Further use of these substrates for the screening of colorimetric assays for anthrax detection is ongoing and will be reported in due course.

4. Experimental section

4.1. (2*R*,6*S*)-6-(((2*R*,3*R*,4*aS*,5*S*,7*R*,8*R*,8*aR*)-7-(Benzyloxy)-2,3-dimethoxy-2,3,5-trimethylhexahydro-2*H*-pyrano[3,4*b*]-[1,4]-dioxin-8-yl)oxy)-2-methyl-2*H*-pyran-3(6*H*)-one (14)

To a solution of Boc-pyranone (*ent*)-**8** (846.6 mg, 3.713 mmol) and Ley-spiroketal rhamnose **7** (171 mg, 0.464 mmol) in dry CH_2Cl_2 (0.9 mL) was added a solution of $Pd_2(dba)_3 \cdot CHCl_3$ (12.01 mg, 0.012 mmol) and PPh_3 (12.2 mg, 0.046 mmol) in 1.0 mL CH_2Cl_2 at 0 °C. The reaction mixture was stirred at 0 °C under argon gas for 4 h. The reaction mixture was quenched with 10 mL of saturated $NaHCO_3$ solution, followed by extraction with Et_2O (10 mL×3). The organic layer was washed with 15 mL saturated brine solution, dried over with Na_2SO_4, and concentrated under reduced pressure. The compound was purified using flash chromatography eluting with 25% EtOAc in hexanes to give the title compound **14** (188 mg, 0.39 mmol, 85%); a yellow oil; R_f 0.50 (30% EtOAc/hexanes); $[\alpha]_D^{25}$ −138 (*c* 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 2995, 2940, 2909, 2836, 1698, 1459, 1374, 1114, 1035, 968; ^1H NMR (600 MHz, $CDCl_3$) δ 7.34–7.26 (m, 5H), 6.79 (dd, *J*=10.2, 3.6 Hz, 1H), 6.03 (d, *J*=10.2 Hz, 1H), 5.12 (d, *J*=3.6 Hz, 1H), 5.02 (q, *J*=6.6 Hz, 1H), 4.78 (d, *J*=0.6 Hz, 1H), 4.68 (d, *J*=12 Hz, 1H), 4.49 (d, *J*=12 Hz, 1H), 4.06 (dd, *J*=3.6, 1.8 Hz, 1H), 4.04 (dd, *J*=10.2, 3.6 Hz, 1H), 3.82 (dq, *J*=10.2, 6.6 Hz, 1H), 3.64 (dd, *J*=10.2, 10.2 Hz, 1H), 3.25 (s, 3H), 3.17 (s, 3H), 1.31 (d, *J*=6.6 Hz, 3H), 1.24 (d, *J*=6.0 Hz, 3H), 1.24 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 198.3, 143.3, 137.3, 128.6 (2C), 128.2 (2C), 128.1, 127.8, 100.0, 99.7, 98.3, 92.8, 75.2, 70.8, 69.3, 68.8, 67.5, 67.4, 48.1, 47.8, 18.0, 17.9, 16.8, 15.3; HRMS (CI): calcd for $[C_{25}H_{34}O_9Na^+]$: 501.20950, found: 501.20966.

4.2. (2*R*,3*S*,6*R*)-6-(((2*R*,3*R*,4*aS*,5*S*,7*R*,8*R*,8*aR*)-7-(Benzyloxy)-2,3-dimethoxy-2,3,5-trimethylhexahydro-2*H*-pyrano[3,4*b*]-[1,4]-dioxin-8-yl)oxy)-2-methyl-3,6-dihydro-2*H*-pyran-3-ol (15)

A solution of di-saccharide pyranone **14** (48 mg, 0.100 mmol) in dry CH_2Cl_2 (0.1 mL) and 0.4 M $CeCl_3$/MeOH (0.1 mL) was cooled to −78 °C. $NaBH_4$ (5 mg, 0.120 mmol) was added and the reaction mixture was stirred for 2 h. The reaction mixture was then diluted with Et_2O (5 mL) and quenched with saturated solution of $NaHCO_3$ (2 mL), then extracted with Et_2O (5 mL×3). The organic layer was dried over with Na_2SO_4, concentrated under reduced pressure, and purified using flash chromatography with 35% EtOAc in hexanes elution to give the title compound **15** (43 mg, 0.09 mmol, 90%); colorless oil; R_f 0.29 (40% EtOAc/hexanes); $[\alpha]_D^{25}$ −92 (*c* 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 3454, 2983, 2940, 2936, 2902, 2836, 1454, 1378, 1138, 1118, 1040, 999, 734; ^1H NMR (600 MHz, $CDCl_3$) δ 7.33–7.25 (m, 5H), 5.92 (d, *J*=10.2 Hz, 1H), 5.74 (ddd, *J*=10.2, 2.4, 1.8 Hz, 1H), 4.87 (d, *J*=2.4 Hz, 1H), 4.75 (s, 1H), 4.67 (d, *J*=12.0 Hz, 1H), 4.57 (d, *J*=12.0 Hz, 1H), 4.04 (dd, *J*=3.6, 1.8 Hz, 1H), 4.02 (dq, *J*=9.0, 6.0 Hz, 1H), 3.99 (dd, *J*=10.2, 3.6 Hz, 1H), 3.80 (dq, *J*=10.2, 6.0 Hz, 1H), 3.63 (dd, *J*=10.2, 9.6 Hz, 1H), 3.25 (s, 3H), 3.21 (s, 3H), 1.26 (d, *J*=6.0 Hz, 3H), 1.26 (s, 3H), 1.25 (s, 3H), 1.24 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.5, 133.9, 128.6 (2C), 128.2 (2C), 128.0, 126.8, 100.0, 99.8, 98.3, 93.1, 73.7, 70.1, 69.3, 68.9, 68.1, 67.2, 67.1, 48.0, 47.9, 18.3, 18.1, 17.9, 16.9; HRMS(CI): calcd for $[C_{25}H_{36}O_9Na^+]$: 503.22515, found: 503.22531.

4.3. (2*R*,3*S*,4*S*,5*S*,6*R*)-2-(((2*R*,3*R*,4*aS*,5*S*,7*R*,8*R*,8*aR*)-7-(Benzyloxy)-2,3-dimethoxy-2,3,5-trimethylhexahydro-2*H*-pyrano[3,4-*b*][1,4]dioxin-8-yl)oxy)-6-methyltetrahydro-2*H*-pyran-3,4,5-triol (16)

To a solution of allylic alcohol **15** (30 mg, 0.063 mmol) in *tert*-butanol/acetone (125 μL, 1:1 (v/v), 0.5 M) at 0 °C was added a solution of *N*-methylmorpholine-*N*-oxide/water (50% w/v, 65 μL, 1 M). Crystalline OsO_4 (0.16 mg, 1 mol %) was added and the reaction mixture was stirred for 12 h at 0 °C. The reaction mixture was then quenched with 500 μL of saturated $Na_2S_2O_3$ solution, extracted with EtOAc (5×2 mL), and dried over with Na_2SO_4 and concentrated under reduced pressure. The compound was purified by flash chromatography eluting with 85% EtOAc in hexanes to give the title compound **16** (29.9 mg, 0.058 mmol, 93%); colorless oil; R_f 0.56 (10% MeOH in EtOAc); $[\alpha]_D^{25}$ −50 (*c* 1.0, CH_2Cl_2); IR (thin film, cm^{-1}) 3304, 2938, 2775, 2100, 1128, 1050; ^1H NMR (600 MHz, $CDCl_3$) δ 7.35–7.25 (m, 5H), 4.75 (d, *J*=0.6 Hz, 1H), 4.74 (d, *J*=1.2 Hz, 1H), 4.67 (d, *J*=12.0 Hz, 1H), 4.47 (d, *J*=12.0 Hz, 1H), 4.26 (dq, *J*=9.6, 6.0 Hz, 1H), 3.99 (dd, *J*=10.2, 3.0 Hz, 1H), 3.96 (m, 1H), 3.95 (dd, *J*=3.6, 1.8 Hz, 1H), 3.81 (dq, *J*=9.6, 6.0 Hz, 1H), 3.64 (dd, *J*=10.2, 10.2 Hz, 1H), 3.40 (dd, *J*=9.6, 9.6 Hz, 1H), 3.23 (s, 3H), 3.22 (s, 3H), 1.25 (s, 3H), 1.24 (d, *J*=6.0 Hz, 3H), 1.24 (s, 3H), 1.24 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 137.4, 128.7 (2C), 128.2 (2C), 128.1, 99.9, 99.7, 98.5, 97.7, 74.00, 73.95, 72.2, 71.3, 69.4, 68.8, 68.0, 67.5, 67.1, 48.2, 47.9, 18.0, 17.9, 17.5, 16.9; HRMS (CI): calcd for $[C_{25}H_{38}O_{11}Na^+]$: 537.23063, found: 537.23074.

4.4. (2*R*,3*S*,4*S*,5*S*,6*R*)-2-(((2*R*,3*R*,4*R*,5*R*,6*S*)-2-(Benzyloxy)-4,5-dihydroxy-6-methyltetrahydro-2*H*-pyran-3-yl)oxy)-6-methyltetrahydro-2*H*-pyran-3,4,5-triol (18)

To a solution of di-saccharide **16** (19.3 mg, 0.0375 mmol) in CH_2Cl_2 (375 μL) was added 75 μL of TFA/H_2O (10:1). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was then quenched with saturated $NaHCO_3$ solution (3.0 mL), dried over with Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography eluting with 8% MeOH in EtOAc to give the title compound **18** (14.7 mg, 0.0367 mmol, 98%); colorless oil; R_f 0.48 (20% MeOH in EtOAc); $[\alpha]_D^{25}$ +5.0 (*c* 1.0, MeOH); IR (thin film, cm^{-1}) 3372, 2071, 1508, 1334, 1120, 973, 786; ^1H NMR (600 MHz, CD_3OD) δ 7.35–7.28 (m, 5H), 4.87 (d, *J*=1.8 Hz, 1H), 4.74 (d, *J*=1.8 Hz, 1H), 4.70 (d, *J*=12.0 Hz, 1H), 4.54 (d, *J*=12.0 Hz, 1H), 3.98 (dq, *J*=9.6, 6.0 Hz, 1H), 3.92 (dd, *J*=3.6, 1.8 Hz, 1H), 3.81 (dd, *J*=3.6, 1.8 Hz, 1H), 3.79 (dd, *J*=9.6, 3.6 Hz, 1H), 3.74 (dd, *J*=9.6, 3.0 Hz, 1H), 3.62 (dq, *J*=9.0, 6.0 Hz, 1H), 3.37 (dd, *J*=9.6, 9.6 Hz, 1H), 3.35 (dd, *J*=9.6, 9.6 Hz, 1H), 1.25 (d, *J*=6.6 Hz, 3H), 1.24 (d, *J*=6.6 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 139.1, 129.6 (2C), 129.2 (2C), 129.0, 100.1, 97.9, 76.9, 74.3, 74.1, 72.6, 72.3, 71.7, 70.5, 70.4, 70.3, 18.2, 18.0; HRMS(CI): calcd for $[C_{19}H_{28}O_9+Na^+]$: 423.16255, found: 423.16269.

4.5. (3*R*,4*R*,5*R*,6*S*)-6-Methyl-3-(((2*R*,3*S*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-tetrahydro-2*H*-pyran-2,4,5-triol (4)

To a solution of benzyl di-saccharide **18** (11.4 mg, 0.030 mmol) in MeOH (0.1 mL) was added 10% Pd/C (60 mg). The reaction suspension was degassed and replaced atmosphere with hydrogen gas three times. The reaction mixture was stirred for 24 h and filtered through a pad of Celite. The solute was concentrated under reduced pressure to give the title compound **4** (8.2 mg, 0.026 mmol, 90%); viscous oil; R_f 0.52 (40% MeOH in EtOAc); $[\alpha]_D^{25}$ +16 (*c* 1.0, MeOH); IR (thin film, cm^{-1}) 3375, 2991, 2942, 1570, 1145, 995; ^1H NMR (600 MHz, CD_3OD) δ 5.10 (d, *J*=1.8 Hz, 1H), 4.79 (d, *J*=1.2 Hz, 1H), 3.98 (dq, *J*=9.6, 6.0 Hz, 1H), 3.86 (dd, *J*=3.0, 1.8 Hz, 1H), 3.83 (dd, *J*=3.6, 1.8 Hz, 1H), 3.81 (dd, *J*=9.6, 3.6 Hz, 1H), 3.80 (dq, *J*=9.6, 6.0 Hz, 1H), 3.75 (dd, *J*=9.6, 3.6 Hz, 1H), 3.38 (dd, *J*=9.6, 9.6 Hz, 1H), 3.32 (dd, *J*=9.0, 9.0 Hz, 1H), 1.25 (d, *J*=6.0 Hz, 3H), 1.24 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 100.1, 92.7, 77.8, 74.6, 74.2, 72.6, 72.3, 71.4, 70.3, 69.8, 18.4, 18.1; HRMS (CI): calcd for $[C_{12}H_{22}O_9Na^+]$: 333.11560, found: 333.11566.

4.6. (3*R*,4*R*,5*R*,6*S*)-6-Methyl-3-(((2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-tetrahydro-2*H*-pyran-2,4,5-triol (3)

To a solution of di-saccharide **6** (26 mg, 0.05 mmol) in CH_2Cl_2 (0.5 mL), was added the 0.1 mL of 10:1 TFA/H_2O. The reaction mixture was stirred at room temperature for 40 min. The reaction mixture was quenched with saturated 5 mL $NaHCO_3$ solution, dried over with Na_2SO_4, and concentrated to remove water and solvent under reduced pressure. The crude compound was purified by passing through a pad of silica gel eluting with 10% MeOH in EtOAc to give the benzyl di-saccharide **17**; To a solution of the benzyl di-saccharide **17** in 0.5 mL MeOH was added 10% Pd/C (100 mg). The reaction suspension was degassed and replaced atmosphere with hydrogen gas three times. The reaction suspension was stirred with hydrogen balloon for 24 h, filtered by passing through a pad of Celite, concentrated under reduced pressure and under vacuo to give the title compound **3** (13 mg, 0.043 mmol, 85%); viscous oil; R_f 0.31 (20% methanol in EtOAc); $[\alpha]_D^{25}$ −20 (*c* 1.0, MeOH); IR (thin film, cm^{-1}) 3351, 2981, 2935, 1645, 1055, 1032; ^1H NMR (600 MHz, CD_3OD) δ 5.20 (d, *J*=1.8 Hz, 1H), 4.98 (d, *J*=1.8 Hz, 1H), 3.89 (dd, *J*=9.6, 3.6 Hz, 1H), 3.85 (dq, *J*=9.6, 6.0 Hz, 1H), 3.84 (dd, *J*=3.6, 1.8 Hz, 2H), 3.78 (dq, *J*=9.6, 6.0 Hz, 1H), 3.75 (dd, *J*=9.6, 3.6 Hz, 1H), 3.44 (dd, *J*=9.6, 9.6 Hz, 1H), 3.39 (dd, *J*=9.6, 9.6 Hz, 1H), 1.31 (d, *J*=6.0 Hz, 3H), 1.29 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 104.2, 94.6, 81.2, 74.7, 74.1, 72.4, 72.2, 71.9, 70.3, 69.5, 18.4, 18.0; HRMS (CI): calcd for $[C_{12}H_{22}O_9Na^+]$: 333.11560, found: 333.11568.

4.7. (3*R*,4*R*,5*R*,6*S*)-3-(((2*S*,3*R*,4*R*,5*S*,6*S*)-3,5-Dihydroxy-6-methyl-4-(((2*S*,3*R*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)oxy)-tetrahydro-2*H*-pyran-2-yl)oxy)-6-methyltetrahydro-2*H*-pyran-2,4,5-triol (2)

To a solution of tri-saccharide **5** (42 mg, 0.05 mmol) in CH_2Cl_2 (0.5 mL) was added 0.1 mL of 10:1 TFA/H_2O. The reaction mixture was stirred at room temperature for 40 min. The reaction mixture was quenched with saturated 5 mL of $NaHCO_3$ solution, dried over with Na_2SO_4, and concentrated to remove water and solvent under reduced pressure. The crude compound was purified by passing through a pad of silica gel eluting with 10% MeOH in EtOAc to give diacetate; to the solution of the subsequent diacetate in MeOH (0.2 mL) was added LiOH (9.6 mg, 0.4 mmol) and stirred for 5 h. The reaction mixture was concentrated under reduced pressure and purified by passing through a pad of silica gel eluting with 15% MeOH in EtOAc to give the benzyl tri-saccharide **19**; the resulting benzyl tri-saccharide **19** was dissolved in 0.5 mL MeOH and added 10% Pd/C (100 mg). The reaction suspension was degassed and replaced atmosphere with hydrogen gas for three times. The reaction suspension was stirred under the hydrogen balloon for 24 h, filtered by passing through a pad of Celite, concentrated under reduced pressure and under vacuo to give the title compound **2** (18.5 mg, 0.041 mmol, 81%); viscous oil; R_f 0.19 (20% MeOH in EtOAc); $[\alpha]_D^{25}$ −82 (*c* 1.2, MeOH); IR (thin film, cm^{-1}) 3356, 2981, 2934, 1572, 1416, 1059, 986; ^1H NMR (600 MHz, CD_3OD) δ 5.18 (d, *J*=1.8 Hz, 1H), 5.10 (d, *J*=1.8 Hz, 1H), 4.98 (d, *J*=1.8 Hz, 1H), 4.12 (dd, *J*=3.6, 1.8 Hz, 1H), 4.03 (dd, *J*=3.6, 1.8 Hz, 1H), 3.86 (m, 7H), 3.57 (dd, *J*=9.6, 9.6 Hz, 1H), 3.45 (dd, *J*=9.6, 9.6 Hz, 1H), 3.43 (dd, *J*=9.6, 9.6 Hz, 1H), 1.35 (d, *J*=6.0 Hz, 3H), 1.31 (d, *J*=6.0 Hz, 3H), 1.29 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 104.0(2C), 94.7, 80.9,

79.4, 74.7, 74.3, 73.5, 72.4, 72.3, 72.0(2C), 70.6, 70.3, 69.5, 18.4, 18.1, 18.0; HRMS (CI): calcd for $[C_{18}H_{32}O_{13}Na^+]$: 479.17351, found: 479.17367.

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Supplementary data

Copies of the spectral data ^{1}H NMR and ^{13}C NMR for all the new compounds can be found in the Supplementary data. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.02.073.

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