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General methods for the synthesis of glycopyranosyluronic acid azides

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Abstract

Per-*O*-acetylated D-glycopyranoses derived from both mono- and disaccharides were first converted to glycosyl iodides and subsequently reacted with an azide source to achieve the stereoselective synthesis of β-D-glycosyl azides after deacetylation. Low-temperature (4 °C) TEMPO oxidation of the monosaccharides provided the corresponding uronic acids, which were purified as the free acids. Oxidation of the lactosyl- and cellobiosyl azides resulted in diacid formation. However, 4′,6′-*O*-benzylidene protection enabled selective oxidation of the C-6 hydroxyl. 2-Acetamido-2-deoxy-D-glycopyranosyl azides were also prepared and converted to uronic acids completing the library synthesis.

Keywords: Glycosyl azides; TEMPO oxidation; Uronic acid azides, unprotected

1. Introduction

The design and synthesis of novel molecular scaffolds with unique structural and biological properties is an increasingly active area of chemical research.[1] In many of these studies, carbohydrates containing both carboxylic acid and amine functionalities have been used as building blocks for the construction of amide-linked oligomers.[2] We have found solid-phase synthesis to be an efficient method for coupling the monomer units, and in most cases the hydroxyl groups do not require protection. As we expand our studies to include diverse combinations of carbohydrate units, it has become important to develop general methods for the synthesis of appropriately functionalized precursors. Since compounds containing carboxylic acid and azide functionalities can be directly incorporated into solid-phase strategies,[3] we have targeted the synthesis of these important carbohydrate analogs.

There are two general strategies for the synthesis of glycopyranosyluronic acid azides, which differ primarily by the sequence of events. Most often per-*O*-acetylated glycopyranoses are converted to glycosyl azides and then deprotected to the free sugars, which are subsequently subjected to selective oxidation of the primary hydroxyl groups. Alternatively, esters of per-*O*-acetylated uronic acids can be converted to glycosyl azides. Györgydeák and Thiem demonstrated the feasibility of these methods on a number of monomeric sugars.[4] However, due to the formation of salts in the oxidation step, the unprotected hydroxy acids were not isolated. Instead, after oxidation of the glycopyranosyl azide, the acid was converted to a methyl ester, and the resulting compound was acetylated for purification.

Since we required unprotected azido acids for the incorporation into solid-phase oligomer synthesis, we decided to re-examine the Györgydeák approach with the goal of establishing methods for the purification of the free glycopyranosyl azide uronic acids. We also sought to extend the Györgydeák protocol to include the preparation of disaccharides containing two glycuronic acids as well as singly oxidized analogs. Finally, to increase diversity in the sugar acid azide library, preparation of 2-acetamido-2-deoxy-D-gluco-, D-galacto- and D-mannopyranosyluronic azides was targeted.

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2. Results and discussion

Initially, our work focused on developing a convenient and stereoselective method for the preparation of β-D-glycosyl azides. Classically, per-*O*-acetylated glycosyl chlorides and bromides have been reacted with metal azides (lithium, sodium, or silver) to afford good to moderate yields of glycosyl azides, but typically high

Table 1
Conversion of per-*O*-acetylated sugars to β-D-glycopyranosyl azides

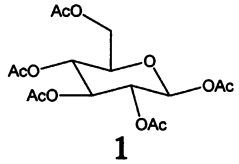
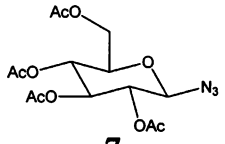
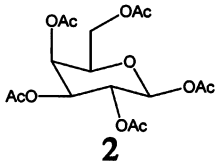
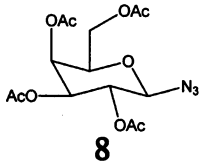
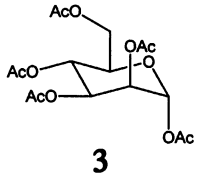
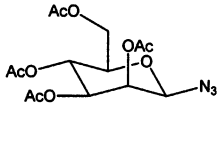
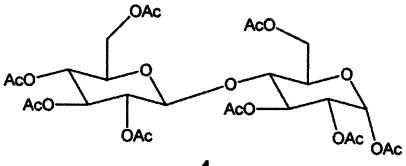
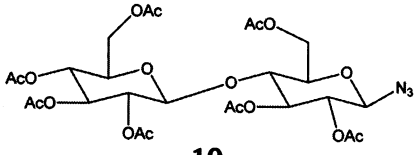
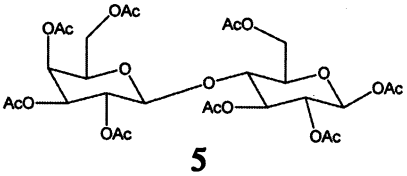
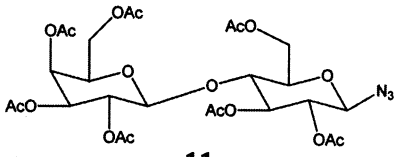
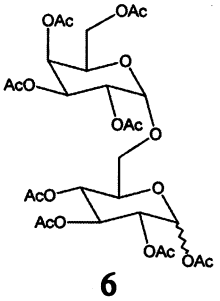
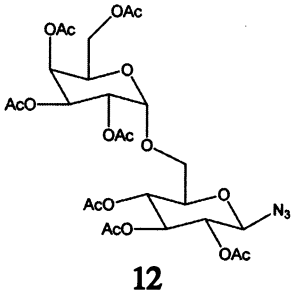
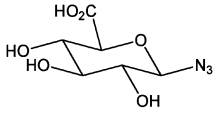
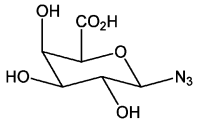
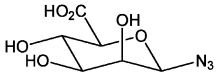
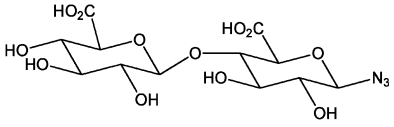
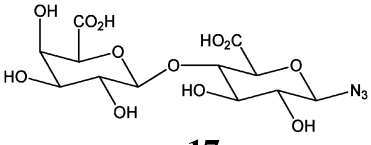
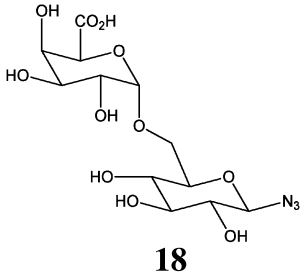
Starting Material	TMSI; $TBAN_3$ 2-step yield	TMSI; TMGA 2-step yield	Product
 **1**	88%	90%	 **7**
 **2**	91%	76%	 **8**
 **3**	88%	71%	 **9**
 **4**	82%	73%	 **10**
 **5**	87%	84%	 **11**
 **6**	70%	64%	 **12**

Table 2
Oxidation of D-glycopyranosyl azides to the corresponding uronic acids

Starting Material	Method (Yield)	Product
7	A (76%)	 **13**
8	A (71%)	 **14**
9	B (20%)	 **15**
10	A (76%)	 **16**
11	A (71%)	 **17**
12	A (64%)	 **18**

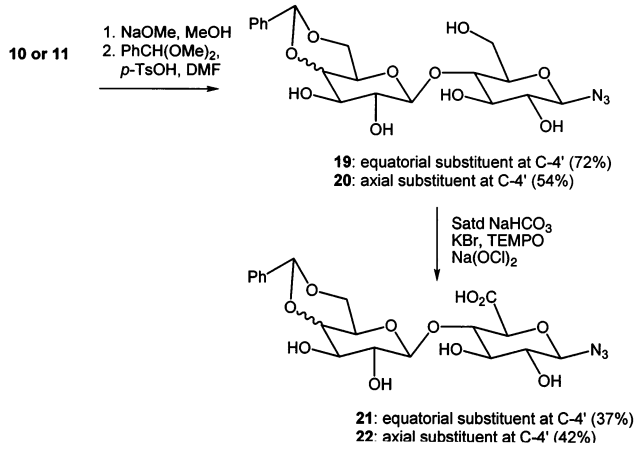
Method A: NaOMe, MeOH; dilute product in aq $NaHCO_3$, add TEMPO and KBr, then add NaOCl in portions. Stir at 4 °C for 48 h. Method B: Same as method A only $Ca(OCl)_2$ was used as oxidant.

boiling polar solvents such as HMPA or DMF and heating are required.[5] Alternatively, per-*O*-acetylated sugars can be activated with a Lewis acid in the presence of azidotrimethylsilane to give the 1,2-trans product.[6] However, in the case of mannosyl azides, only the α anomer has been prepared efficiently. In view of our recent studies on the use of glycosyl iodides in organic synthesis,[7] we decided to probe the potential of these donors in the preparation of glycosyl azides.

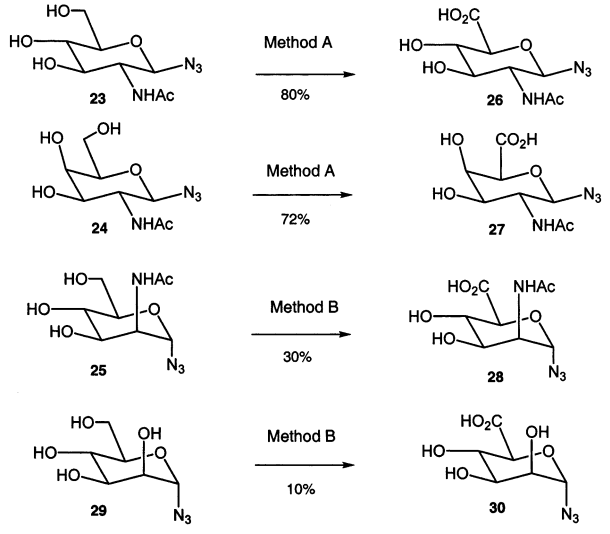
The glycosyl iodides were readily obtained from the anomeric acetate upon treatment with iodotrimethylsilane in refluxing CH_2Cl_2 for 30 min. The trimethylsilyl acetate generated in the reaction was easily removed by evaporation, yielding pure per-*O*-acetylated glycosyl iodides, which were quite stable and could even be chromatographed. More typically, the crude iodide donors were directly reacted with tetrabutylammonium azide ($TBAN_3$) or tetramethylguanidinum azide (TMGA)[8] in CH_2Cl_2 to afford the per-*O*-acetyl glycosyl azides. The results from the above experiments appear in Table 1. The yields represent a one-pot, two-step process involving glycosyl iodide generation and azide displacement to give the β-D-glycosyl azides (**7**–**12**).[9]

We next turned our attention to the oxidation step. The glycopyranosyl azides were first deprotected using catalytic amounts of sodium methoxide in methanol.[4] When TLC indicated that the reaction was complete, the methanolic solution was acidified with Dowex 50 (H^+) resin and evaporated to dryness. The crude glycopyranosyl azides were dissolved in saturated sodium bicarbonate solution, and KBr (5 mol%) and TEMPO (6 mol%) were added. The oxidant (NaOCl 1.3 M in H_2O, 3 equiv) was added slowly in portions, making certain that the reaction mixture remained cold. In preliminary studies, we observed that over-oxidation occurred when the substrates were reacted at room temperature, but this problem could be overcome by performing the oxidation at 4 °C. Due to salt formation, it was difficult to follow the reaction by TLC, but the progress of the reaction could be monitored by working up a small amount of the reaction mixture. In general, the reactions were carried out for 48 h at 4 °C to insure that the reaction was complete.

When the reaction was complete, any solids that had formed were removed by filtration, and the aqueous solution was washed with CH_2Cl_2 to remove the TEMPO byproducts. The aqueous layer was carefully acidified to pH 2.0, making sure that the mixture remained cold. The resulting yellow solution was concentrated on the rotary evaporator until it became clear, after which time it was lyophilized to afford a white powder. The white powder was dissolved in methanol, the salts were filtered, and the methanol was removed by evaporation. This process was repeated three times, and the resulting solid was purified using reversed-phase (RP) HPLC. Salts eluted first from the column with the desired salt free uronic acids eluting several minutes later. Typically, the salt-containing fractions were analyzed for the presence of carbohydrate, which was evidenced by the blue color produced upon charring a TLC spot of the fraction with molybdate. If carbohydrate was present, the fraction was acidified with HCl and resubjected to RP-HPLC. As shown in Table 2, high yields of the glucosyl (**13**), galactosyl (**14**), and melibiosyl (**18**) uronic acid azides were obtained for the two-step process. Similarly, the cellobiosyl and lactosyl azides were efficiently oxidized to the diacids **16** and **17**. The yield of the mannosyl-



Scheme 1.



Scheme 2.

uronic acid (**15**) was only 8% using NaOCl as the oxidant.[†] The reaction was slightly improved using $Ca(OCl)_2$ but even then only about 20% yield was realized. However, the starting material could be retrieved from the reaction and re-subjected to the reaction conditions. In this manner multigram quantities of **15** were produced. The disaccharide diacids (**16** and **17**) are attractive compounds for generating branched amide-linked oligomers; however, we also recognized the need for single oxidation products. In order to achieve that goal, **10** and **11** were deacetylated and subsequently treated with α,α-dimethoxytoluene in the presence of a catalytic amount *p*-toluenesulfonic acid to provide the corresponding 4′,6′-*O*-benzylidene compounds **19** (72%) and **20** (54%), respectively. Subsequent oxidation proceeded smoothly yielding the glucuronic acids **21** and **22** after the aforementioned purification procedure (Scheme 1).

In order to add further diversification to our library of glycopyranosyluronic acid azides, glycosyl azides[10] of 2-acetamido-2-deoxy-D-glucose (**23**), D-galactose (**24**), and D-mannose (**25**) were subjected to the oxidation conditions. As can be seen in Scheme 2, **23** and **24** underwent efficient conversion to the uronic acids **26** and **27**, respectively. In contrast, **25** gave poor yields of **28**, just as was seen for β-D-mannopyranosyl azide. We also attempted the oxidation of α-D-mannopyranosyl azide **29**, and here again, the uronic acid (**30**) was formed in only 10% yield, indicating that oxidation is generally sluggish for substrates of the D-manno configuration.

3. Conclusions

Methods have been developed for the mild conversion of per-*O*-acetylated sugars to β-glycopyranosyl azides via glycosyl iodides and their subsequent oxidation to the corresponding uronic acids. A protocol for desalting the unprotected hydroxy acids and purifying them on RP-HPLC has been established providing carboxylic acid azides ready for solid-phase oligomer synthesis. Compounds derived from D-gluco and D-galacto sugar azides were smoothly converted, whereas oxidation of substrates with the D-manno configuration did not proceed to completion. Nevertheless, the mannuronic acids could be separated from the starting material; and by recycling the starting mannopyranosyl azides, multigram quantities of both α- and β-D-mannopyranosyluronic acid azides and α-2-acetamido-2-deoxy-mannopyranosyluronic acid azide could be prepared.

4. Experimental

4.1. General methods

All chemicals were used as supplied without further purification. Solvents (MeOH 99.8%, CH_2Cl_2 99.8%, PhMe 99.8%) were purchased in anhydrous Sure/Seal™ bottles from Aldrich, used without further purification, and stored under argon. TMSI was purchased from Fluka ($\geq 98\%$) and used without purification unless the color had changed to a dark brown shade, in which case, it was discarded or distilled under an argon atmosphere from quinoline. TMSI was stored at $-15\,^{\circ}C$ under a desiccated atmosphere. Dowex 50WX8 (200 mesh) acidic resin was purchased from Aldrich, washed copiously with MeOH, and used without further purification. NaOMe–MeOH (0.5 M) was purchased from Aldrich. Glass-backed EM Science TLC plates (Silica Gel 60 with a 254 nm fluorescent indicator) were pur-

[†] Low yields in the oxidation of mannopyranosyl azides with NaOCl was also observed by Györgydeák and Thiem. See Ref. 4.

chased from VWR International, cut into 2×5-cm strips, used without further manipulation, and stored over dessicant. Developed TLC plates were visualized under a short-wave UV lamp, stained with a cerium–molybdate solution and charred. Column chromatography was conducted using flash silica gel (32–63 μm) available from Scientific Adsorbents, and solvents were purchased from EM Science. NMR experiments (1D and 2D) were conducted on Inova 400 MHz and/or Bruker DRX500 MHz spectrometers at 298 K. Optical data were taken on a JASCO DIP-370 Digital Polarimeter. RP-HPLC preparative separations were carried out on Dynamax-60A C18 column. Solvents: (A) $H_2O + 0.1\%$ TFA; and (B) H_2O–CH_3CN gradient 100% to 50% H_2O, with UV detection at 190 and 220 nm.

4.2. General procedure for the synthesis of per-*O*-acetyl glycosyl azides

To a solution of per-*O*-acetyl pyranoses (1.0 mmol) in dry CH_2Cl_2 (0.5 M solution) was added TMSI (1.1 mmol) under argon. The solution was refluxed under argon for 30 min and concentrated in vacuo. The resulting residue was azeotroped with dry toluene twice to afford the crude glycosyl iodide, which was redissolved in dry CH_2Cl_2, before adding $TBAN_3$ or TMGA (1.5 mmol). The solution was stirred under argon for 10 min, CH_2Cl_2 (20 mL) and water (20 mL) were added to the reaction mixture successively, and the organic layer washed with 10% $Na_2S_2O_3$, 10% $NaHCO_3$, water, brine, and dried (anhyd Na_2SO_4) and evaporated. The residue was purified by column chromatography to give the corresponding per-*O*-acetyl glycosyl azide.

4.3. General procedure (A) for TEMPO oxidation

To a solution of the unprotected glycosyl azides (1.0 mmol) in satd aq $NaHCO_3$ (5 mL) were added KBr (12 mg) and TEMPO (10 mg), after which a solution of NaOCl (2.4 mL, 1.3 M) was added dropwise at 0 °C. The mixture was stirred for 2 days at 4 °C, then the mixture was washed with CH_2Cl_2 to remove TEMPO byproducts. The aq layer was acidified to pH 1–2 using 4 N HCl. The yellow solution was concentrated until it became clear, after which time it was lyophilized to afford a white powder. The white powder was extracted with MeOH, the salts were filtered, and the MeOH was removed by evaporation. This process was repeated three times and the resulting solid was purified by RP-HPLC.

4.4. General procedure (B) for TEMPO oxidation

To a solution of the unprotected glycosyl azides (1.0 mmol) in satd aq $NaHCO_3$ (5 mL) were added KBr (12 mg) and TEMPO (10 mg), after which solid $Ca(OCl)_2$ (200 mg) was added in portions at 0 °C. The mixture was stirred for 2 days at 4 °C, then the mixture was filtered and washed with CH_2Cl_2 to remove TEMPO byproduct. The aqueous layer was acidified to pH 1–2 by 4 N HCl. The yellow solution was concentrated until it became clear, after which time it was lyophilized to afford a white powder. The white powder was extracted with MeOH, the salts were filtered, and the MeOH was removed by evaporation. This process was repeated three times, and the resulting solid was purified by RP-HPLC.

4.5. β-D-Glucopyranosyluronic acid azide (13)

Prepared in 76% yield using TEMPO oxidation method A. Purified by RP-HPLC using solvent system A. $[\alpha]_D^{26}$ $-22.5°$ (*c* 1.1, H_2O); ^1H NMR (D_2O): δ 4.84 (d, 1 H, *J* 8.5 Hz, H-1), 4.09 (d, 1 H, *J* 9.0 Hz, H-5), 3.54–3.61 (m, 2 H, H-3, H-4), 3.32 (applt, 1 H, *J* 8.5 Hz, H-2); ^{13}C NMR (D_2O): δ 177.0 (C-6), 92.2 (C-1), 79.6 (C-5), 77.7, 74.8 (C-2), 73.6; ESIMS: m/z 241.9 $[M + Na]^+$.

4.6. β-D-Galactopyranosyluronic acid azide (14)

Prepared in 71% yield using TEMPO oxidation method A. Purified by RP-HPLC using solvent system A. $[\alpha]_D^{26}$ $-31.9°$ (*c* 0.8, H_2O); ^1H NMR (D_2O): δ 4.83 (d, 1 H, *J* 9.0 Hz, H-1), 4.37 (d, 1 H, *J* 3.0 Hz, H-4), 4.35 (s, 1 H, H-5), 3.88 (dd, 1 H, *J* 10.0, 3.5 Hz, H-3), 3.63 (applt, 1 H, *J* 9.5 Hz, H-2); ^{13}C NMR (D_2O): δ 174.2 (C-6), 90.6 (C-1), 77.2 (C-5), 73.0 (C-3), 70.3 (C-2, C-4); ESIMS: m/z 242.0 $[M + Na]^+$.

4.7. β-D-Mannopyranosyluronic acid azide (15)

Prepared in 20% yield using TEMPO oxidation procedure B. Purified by RP-HPLC using solvent system A. $[\alpha]_D^{26}$ $-70.8°$ (*c* 1.0, H_2O); ^1H NMR (D_2O): δ 4.94 (s, 1 H, H-1), 4.01 (d, 1 H, *J* 3.0 Hz, H-2), 3.96 (d, 1 H, *J* 9.5 Hz, H-5), 3.80 (applt, 1 H, *J* 9.5 Hz, H-4), 3.67 (dd, 1 H, *J* 3.0, 9.5 Hz, H-3); ^{13}C NMR (D_2O): δ 176.3 (C-6), 87.9 (C-1), 78.6, 73.3 (C-3), 71.6 (C-2), 69.0; ESIMS: m/z 242.0 $[M + Na]^+$.

4.8. β-Cellobiosyldiuronic acid azide (16)

Prepared in 76% yield using TEMPO oxidation procedure B. Purified by RP-HPLC using solvent system A. ^1H NMR (D_2O): δ 4.91 (d, 1 H, *J* 9.0 Hz, H-1), 4.61 (d, 1 H, *J* 7.5 Hz, H-1′), 4.23 (d, 1 H, *J* 9.5 Hz, H-5), 4.06 (d, 1 H, *J* 9.5 Hz, H-5′), 3.88 (applt, 1 H, *J* 9.0 Hz, H-4), 3.75 (applt, 1 H, *J* 9.0 Hz, H-3), 3.64 (applt, 1 H, *J* 9.0 Hz, H-4′), 3.58 (applt, 1 H, *J* 9.5 Hz, H-3′), 3.42–3.37 (m, 2 H, H-2, H-2′); ^{13}C NMR (D_2O): δ 172.7, 171.8, 102.4 (C-1′), 90.38 (C-1), 79.7 (C-4), 75.5

(C-5), 75.4 (C-3′), 74.6 (C-5′), 74.2 (C-3), 73.0, 72.6, 71.5 (C-4′); ESIMS: m/z 418.0 $[M + Na]^+$.

4.9. β-Lactosyldiuronic acid azide (17)

Prepared in 71% yield using TEMPO oxidation procedure B. Purified by RP-HPLC using solvent system A. $[\alpha]_D^{26}$ −58.6° (*c* 0.5, H_2O); ^1H NMR (D_2O): δ 4.89 (d, 1 H, *J* 8.5 Hz, H-1), 4.50 (d, 1 H, *J* 8.0 Hz, H-1′), 4.45 (d, 1 H, *J* 0.5 Hz, H-5′), 4.29 (d, 1 H, *J* 2.0 Hz, H-4′), 4.24 (d, 1 H, *J* 10.0 Hz, H-5), 3.88 (applt, 1 H, *J* 9.0 Hz, H-4), 3.76 (applt, 1 H, *J* 8.5 Hz, H-3), 3.74 (dd, 1 H, *J* 8.0 Hz, 3.5 Hz, H-3′), 3.56 (applt, 1 H, *J* 8.0 Hz, H-2′), 3.37 (applt, 1 H, *J* 8.5 Hz, H-2); ^{13}C NMR (D_2O): δ 171.7, 171.6, 102.7 (C-1′), 90.3 (C-1), 80.1 (C-4), 75.2 (C-5), 74.3 (C-5′), 74.2 (C-3), 72.3 (C-3′), 72.1 (C-2), 70.3 (C-2′), 69.6 (C-4′); ESIMS: m/z 418.0 $[M + Na]^+$.

4.10. β-Melibiopyranosyluronic acid azide (18)

Prepared in 64% yield using TEMPO oxidation procedure A. Purified by RP-HPLC using solvent system A. $[\alpha]_D^{26}$ +49.5° (*c* 3.2, H_2O); ^1H NMR (D_2O): δ 5.02 (d, 1 H, *J* 3.5 Hz, H-1′), 4.73 (d, 1 H, *J* 9.0 Hz, H-1), 4.64 (s, 1 H, H-5′), 4.32 (d, 1 H, *J* 2.0 Hz, H-4′), 3.94–3.81 (m, 4 H, H-3′, H-6′, H-6, H-2′), 3.68 (m, 1 H, H-5), 3.50–3.41 (m, 2 H, H-3, H-4), 3.24 (applt, 1 H, *J* 9.0 Hz, H-2); ^{13}C NMR (D_2O): δ 174.0 (C-6′), 98.7 (C-1′), 90.8 (C-1), 76.8 (C-5), 76.4, 73.3 (C-2), 71.4 (C-5′), 69.7 (C-3′), 69.6, 68.4 (C-2′), 66.7 (C-6); FABMS: m/z 404.0911 $[M + Na]^+$.

4.11. 4′,6′-*O*-Benzylidene-β-cellobiopyranosyl azide (19)

To a solution of cellobiosyl azide 10 (1.0 mmol) in dry DMF (5 mL) were added α,α-dimethoxytoluene (1.5 mmol) and *p*-toluenesulfonic acid (10 mg). The mixture was stirred at 40 °C in a vacuum. After 2 h, more DMF (4 mL), dimethoxytoluene (1.5 mmol) and *p*-toluenesulfonic acid (10 mg) were added, and stirring was continued for an additional 2 h. The solution was concentrated, and the residue was purified by column chromatography to give **19** in 72% yield. ^1H NMR (CD_3OD): δ 7.42 (m, 2 H, Ar), 7.27 (m, 3 H, Ar), 5.50 (s, 1 H, C*H*Ar), 4.49 (d, 1 H, *J* 8.5 Hz, H-1), 4.47 (d, 1 H, *J* 8.5 Hz, H-1′), 4.23 (m, 1 H, H-6‴), 3.87–3.80 (m, 2 H, H-6, H-6′), 3.71 (m, 1 H, H-6″), 3.59 (applt, 1 H, *J* 9.0 Hz, H-3′), 3.55 (applt, 1 H, *J* 9.0 Hz, H-4′), 3.48–3.42 (m, 5 H, H-3, H-4, H-5, H-5′), 3.28 (applt, 1 H, *J* 8.5 Hz, H-2′), 3.15 (applt, 1 H, *J* 8.5 Hz, H-2); ^{13}C NMR (CD_3OD): δ 139.0, 129.9, 129.0, 127.5, 105.0 (C-1′), 102.9 (*C*HPh), 91.8 (C-1), 81.9 (C-5′), 80.0 (C-4′), 78.5 (C-5), 76.1 (C-3), 75.8 (C-2), 74.5 (C-2), 74.5 (C-3′), 69.4 (C-6′), 67.7 (C-4), 61.5 (C-6). FABMS: m/z 456.1630 $[M + H]^+$.

4.12. 4′,6′-*O*-Benzylidene-β-lactopyranosyl azide (20)

To a solution of lactosyl azide (**11**) (1.0 mmol) in dry DMF (5 mL) were added α,α-dimethoxytoluene (1.5 mmol) and *p*-toluenesulfonic acid (10 mg). The mixture was stirred at 40 °C in a vacuum. After 2 h, more DMF (4 mL), dimethoxytoluene (1.5 mmol) and *p*-toluenesulfonic acid (10 mg) were added, and stirring was continued for an additional 2 h. The solution was concentrated, and the residue was purified by column chromatography to give **20** in 54% yield. ^1H NMR (CD_3OD): δ 7.45 (m, 2 H, Ar), 7.27 (m, 3 H, Ar), 5.54 (s, 1 H, C*H*Ar), 4.47 (d, 1 H, *J* 9.0 Hz, H-1), 4.40 (d, 1 H, *J* 7.0 Hz, H-1′), 4.13–4.08 (m, 3 H, H-4′, H-6″, H-6‴), 3.84 (m, 2 H, H-6, H-6′), 3.60–3.54 (m, 4 H, H-2′, H-3′, H-4, H-5′), 3.49 (applt, 1 H, *J* 9.0 Hz, H-3), 3.43 (m, 1 H, H-5), 3.14 (applt, 1 H, *J* 9.0 Hz, H-2); ^{13}C NMR (CD_3OD): δ 139.4, 129.9, 129.0, 127.4, 104.6 (C-1′), 102.18 (CHPh), 91.7 (C-1), 79.3 (C-5′), 78.5 (C-5), 77.2 (C-4′), 76.2 (C-3), 74.5 (C-2), 73.3 (C-3′), 71.7 (C-2′), 70.1 (C-6′), 68.3 (C-4), 61.4 (C-6). FABMS: m/z 478.1420 $[M + Na]^+$.

4.13. 4′,6′-*O*-Benzylidene-β-cellobiopyranosyluronic acid azide (21)

Prepared in 37% yield using TEMPO oxidation procedure A. Purified by RP-HPLC using solvent system B. $[\alpha]_D^{26}$ −37.7° (*c* 0.8, H_2O); ^1H NMR (D_2O): δ 7.53 (m, 2 H, Ar), 7.46 (m, 3 H, Ar), 5.72 (s, 1 H, C*H*Ar), 4.77 (d, 1 H, *J* 9.0 Hz, H-1), 4.60 (d, 1 H, *J* 8.3 Hz, H-1′), 4.36 (dd, 1 H, *J* 5.0, 10.5 Hz, H-6′), 3.93–3.85 (m, 2 H, H-5, H-6), 3.78–3.60 (m, 5 H, H-3′, H-4′, H-4, H-5′, H-3), 3.41 (applt, 1 H, *J* 8.3 Hz, H-2′), 3.32 (applt, 1 H, *J* 9.0 Hz, H-2); ^{13}C NMR (D_2O): δ 176.8 (C-6), 138.3, 132.1, 130.9, 128.5, 105.2 (C-1′), 103.9, 92.0 (C-1), 82.1 (C-4′), 82.0 (C-4), 79.4 (C-5), 76.4, 76.3, 74.6, 74.5, 70.0, 68.1; FABMS: m/z 470.1403 $[M + H]^+$.

4.14. 4′,6′-*O*-Benzylidene-β-lactopyranosyluronic acid azide (22)

Prepared in 42% yield using TEMPO oxidation procedure A. Purified by RP-HPLC using solvent system B. $[\alpha]_D^{26}$ −20.2° (*c* 0.6, H_2O); ^1H NMR (D_2O): δ 7.53 (m, 2 H, Ar), 7.46 (m, 3 H, Ar), 5.74 (s, 1 H, C*H*Ar), 4.85 (d, 1 H, *J* 9.0 Hz, H-1), 4.53 (d, 1 H, *J* 7.5 Hz, H-1′), 4.34 (d, 1 H, *J* 3.5 Hz, H-4′), 4.25 (m, 2 H, H-6, H-6′), 4.14 (d, 1 H, *J* 10.0 Hz, H-5), 3.84–3.73 (m, 5 H, H-2′, H-3′, H-5′, H-3, H-4), 3.34 (applt, 1 H, *J* 9.0 Hz, H-2); ^{13}C NMR (D_2O): δ 172.5 (C-6), 137.0, 129.9, 128.8, 126.4, 102.9 (C-1′), 101.30 (*C*HPh), 90.3 (C-1), 79.6 (C-5′), 75.9 (C-5), 75.9 (C-4′), 74.2, 72.3 (C-2), 71.4, 70.5, 69. (C-6′), 66.9; ESIMS: m/z 492.0 $[M + Na]^+$.

4.15. 2-Acetamido-2-deoxy-α-D-mannopyranosyl azide (25)

Prepared in 85% yield using the published method.[6] ^1H NMR (D_2O): δ 5.39 (s, 1 H, H-1), 4.23 (s, 1 H, H-2),

3.92–3.79 (m, 4 H, H-3, H-5, H-6, H-6′), 3.59 (applt, 1 H, *J* 9.5 Hz, H-4), 2.01 (s, 3 H, NHAc); ^{13}C NMR (CD_3OD): δ 174.0, 90.6, 76.5, 69.9, 67.8, 62.1, 53.9, 22.6. ESIMS: m/z 246.9 $[M+H]^+$.

4.16. 2-Acetamido-2-deoxy-β-D-glucopyranosyluronic acid azide (26)

Prepared in 80% yield using TEMPO oxidation procedure A. $[\alpha]_D^{26}$ −37.8° (*c* 1.5, H_2O); ^{1}H NMR (D_2O): δ 4.81 (d, 1 H, *J* 9.5 Hz, H-1), 3.86 (d, 1 H, *J* 9.0 Hz, H-5), 3.77 (applt, 1 H, *J* 9.5 Hz, H-2), 3.65–3.57 (m, 2 H, H-3, H-4), 2.08 (s, 3 H, NHAc); ^{13}C NMR (D_2O): δ 177.0, 176.0, 90.8 (C-1), 79.1 (C-5), 75.5, 73.7, 56.9 (C-2), 24.2 (NHAc); FABMS: m/z 283.0649 $[M+Na]^+$.

4.17. 2-Acetamido-2-deoxy-β-D-galactopyranosyluronic acid azide (27)

Prepared in 72% yield using TEMPO oxidation procedure A. $[\alpha]_D^{26}$ −10.8° (*c* 0.9, H_2O); ^{1}H NMR (D_2O): δ 4.73 (d, 1 H, *J* 9.0 Hz, H-1), 4.31 (d, 1 H, *J* 2.0 Hz, H-4), 4.18 (s, 1 H, H-5), 3.97 (applt, 1 H, *J* 9.5 Hz, H-2), 3.87 (dd, 2 H, *J* 3.5, 10.5 Hz, H-3), 2.10 (s, 3 H, NHAc); ^{13}C NMR (D_2O): δ 175.6, 172.8, 89.3 (C-1), 76.5 (C-5), 70.8 (C-3), 69.2 (C-4), 51.7 (C-2), 22.8 (NHAc); FABMS: m/z 283.0647 $[M+Na]^+$.

4.18. 2-Acetamido-2-deoxy-α-D-mannopyranosyluronic acid azide (28)

Prepared in 30% yield using TEMPO oxidation procedure B. $[\alpha]_D^{26}$ +78.8° (*c* 1.1, H_2O); ^{1}H NMR (D_2O): δ 5.40 (d, 1 H, *J* 3.2 Hz, H-1), 4.23 (applt, 1 H, *J* 3.8 Hz, H-2), 4.11 (d, 1 H, *J* 8.8 Hz, H-5), 3.92 (dd, 1 H, *J* 4.4, 8.8 Hz, H-3), 3.78 (t, 2 H, *J* 8.8 Hz, H-4), 2.04 (s, 3 H, NHAc); ^{13}C NMR (D_2O): δ 176.0, 174.7, 88.0 (C-1), 75.5, 69.1, 68.5, 51.4, 22.0 (NHAc); ESIMS: m/z 283.0 $[M+Na]^+$.

4.19. α-D-Mannopyranosyluronic acid azide (30)

Prepared in 10% yield using TEMPO oxidation procedure B. $[\alpha]_D^{26}$ +128.4° (*c* 1.7, H_2O); ^{1}H NMR (D_2O): δ 5.44 (d, 1 H, *J* 3.5 Hz, H-1), 4.32 (d, 1 H, *J* 8.5 Hz, H-5), 3.94 (applt, 1 H, *J* 8.5 Hz, H-4), 3.84 (applt, 1 H, *J* 3.5 Hz, H-2), 3.79 (dd, 1 H, *J* 3.5, 8.5 Hz, H-3); ^{13}C NMR (D_2O): δ 176.4 (C-6), 89.6 (C-1), 75.0, 70.0, 69.7, 68.9; ESIMS: m/z 242.0 $[M+Na]^+$.

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