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New chemo-enzymatic route toward *N*-acetylneuraminic acid derivatives with alkyl groups at C-7 hydroxyl group

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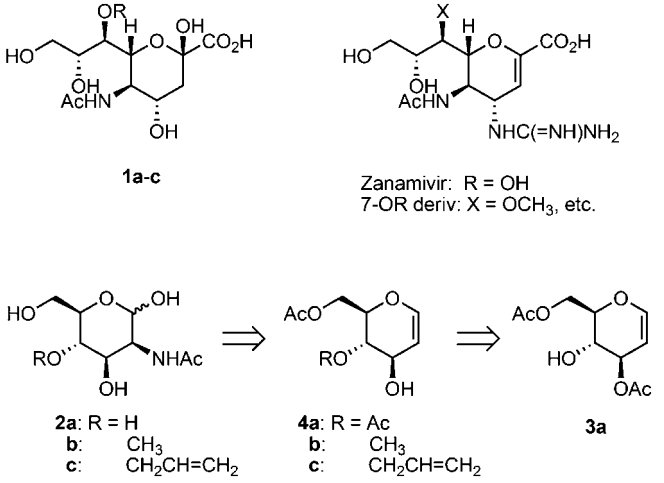
ABSTRACT

Based on chemo-enzymatic regio- and stereoselective reactions, new routes toward C-4 substituted *N*-acetyl-D-mannosamine (ManNAc) and the corresponding sialic acids from D-glucal were established. Lipase-catalyzed regioselective transformation of D-glucal and related substrates furnished precursors on which carbamate and alkyl substituent were properly introduced at C-3 and at C-4, respectively. Cyclic carbamate formation through rhodium-nitrenoid intermediates with iodobenzene pivalate and *tert*-butyl alcohol proceeded in *manno*-configured at C-2 as well as α- at C-1, exclusively. Ring opening and deprotection under mild conditions furnished the target ManNAc derivatives, which were the substrates for aldolase-catalyzed reactions.

1. Introduction

In the course of the extensive synthetic studies on viral neuraminidase inhibitors developed by Honda and co-workers,[1,2] which have been designed from zanamivir, a potent *anti*-flu, derivatives of sialic acid (*N*-acetylneuraminic acid, **1a**) with hydrophobic substituents are recently gaining interests, as their starting materials. Among them, chemo-enzymatic preparation of 7-methoxy derivative **1b** was reported by Wong,[3] via mannosamine derivative **2b**, however, the starting material, *N*-acetyl-D-mannosamine (ManNAc, **2a**) is quite expensive. This situation prompted us to develop expeditious routes toward **2b** and another derivative **2c** with allylic substituent at C-4, together with ManNAc **2a** itself, from D-glucal. New derivatives **1c** and **2c** with allylic pendant are promising, as the elongation with a variety of side chain would be available, by means of Grubbs' cross metathesis reactions. In our plan (Scheme 1), two regioselective transformations are necessary; first, the introduction of two protective groups on C-3 and C-6, to leave only C-4 hydroxy group free (**3a**), where the methyl and allyl groups would be introduced. Second, only the acetate on C-3 should be hydrolyzed for the introduction of nitrogen functional group on C-2 taking advantage of neighboring group participation, while leaving C-6 acetate intact. We turned our attention to the lipase-catalyzed reactions under mild conditions for the above-mentioned two key steps.



Scheme 1. Zanamivir and related aminosugars from D-glucal.

2. Results and discussion

The first step was the *Burkholderia cepacia* lipase (Amano PS-IM)-catalyzed regioselective acetylation of D-glucal **3b** (Scheme 2).[4] We thoroughly examined the reaction conditions especially on co-solvent, and found that *tert*-butyl alcohol greatly enhanced the rate of reaction by the following advantages. The polar triol substrate is highly soluble in polar alcoholic solvent, but the tertiary alcohol itself is inert toward lipase-catalyzed acetylation.[5] The desired product **3a** with C-4 free hydroxyl group was obtained in 81% yield, three times higher than that was obtained by applying the second best co-solvent, 1,4-dioxane.

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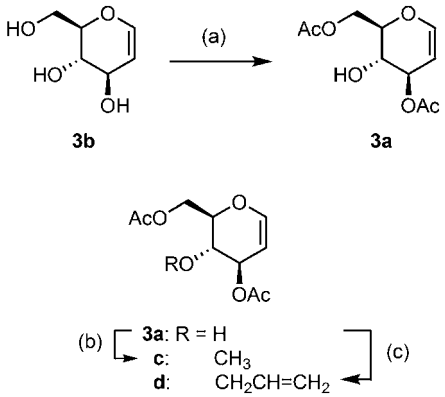
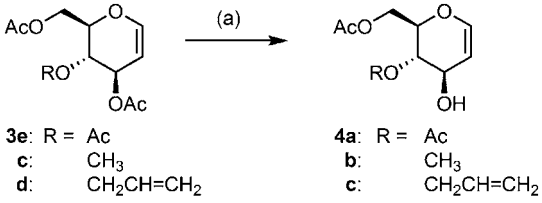


Scheme 2. a) *B. cepacia* lipase (Amano PS-IM), vinyl acetate, *t*-BuOH, 81%; (b) Ag_2O, CH_3I, DMF, 96%; (c) $Pd(PPh_3)_4$, allyl ethyl carbonate, THF, 98%.

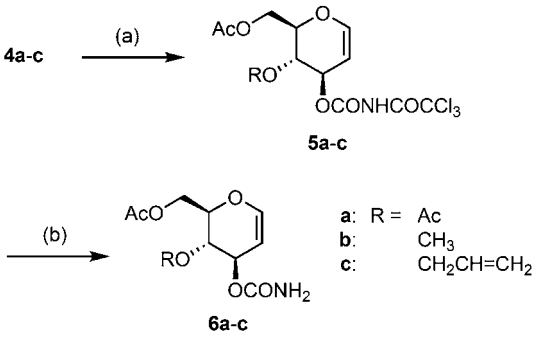
Due to the steric hindrance around the hydroxyl group and the migratory nature of acetyl groups under basic conditions, the alkylation of **3a** was not easy. Among the extensive screening the methylating conditions, only the use of large excess of methyl iodide and silver(I) oxide in DMF was successful to furnish **3c** in 96% yield. In the case of allylation, even a good electrophile, allyl iodide did not work under the above-mentioned conditions. To our delight, the application of π-allyl palladium complex from allyl ethyl carbonate and $Pd(PPh_3)_4$[6] did work, to give **3d** in an excellent yield (98%), by virtue of the highly active intermediate species as well as neutral conditions. The reason for successful allylation was attributable to the superior leaving property of allylic carbonate in the reagent than that of allyl ether in the product.

The second task, to hydrolyze regioselectively the C-3 acetyl group, which is requisite for the next cyclic carbamate formation was realized by *Candida antarctica* lipase B-catalyzed hydrolysis (Scheme 3). Such approach had been reported on triacetyl D-glucal **3e**.[4] In our hand, the introduction of methyl and allyl group at C-4 position did not hamper the reaction, and the regioselective deprotection for **3c** and **3d** also took place in high yields. The regioselectivity was in good accordance with the example that one more carbohydrate moiety was introduced at C-4 position.[7] In our hand, methyl ether **4b** and allyl ether **4c** were also obtained in 86% and 89% yield, respectively.



Scheme 3. (a) *C. antarctica* lipase B (Novozym® 435), quant. for **4a**, 86% for **4b**, 89% for **4c**.

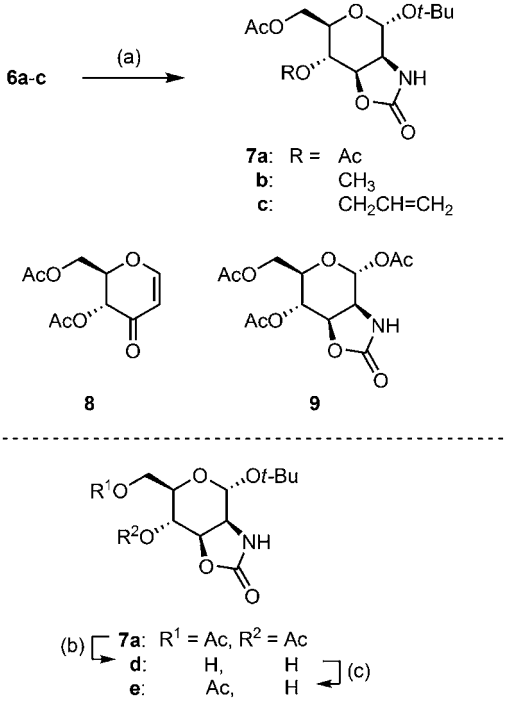
The third task was the introduction of carbamyl group (Scheme 4), toward the precursors for rhodium nitrenoid-mediated cyclization.[8] For carbamylation, the first step was performed by applying trichloroacetyl isocyanate. We found that the reaction should be carefully monitored, and be quenched as soon as possible after the consumption of the starting material. Otherwise, an undesired side product with the migration of double bond between C-2 and C-3 was observed. The subsequent selective deprotection of trichloroacetyl intermediate required as mild conditions as whose acetate located in primary C-6 position survived. Even very weak basic conditions in methanol did not meet with this criterion, in the case of C-4,6 diacetate **5a**, both of primary and secondary acetates easily suffered from hydrolysis in addition to the desired *N*-trichloacetyl group, and the attempt only resulted in a complex mixture.



Scheme 4. (a) CCl_3CONCO, CH_2Cl_2; (b) Al_2O_3, EtOAc, 88%.

It turned out that the treatment with neutral alumina[9] in $CHCl_3$ was effective for furnishing **5a**–**c**. The content of H_2O in alumina was very important for effective and selective deprotection of *N*-trichloroacetyl group. For the reproducibility, the alumina was kept in a closed vessel under air with saturated H_2O vapor by equilibration with small tube containing distilled H_2O at 30 °C, to have the consistent humidity inside.

Next, the reaction conditions for cyclization were examined (Scheme 5). In our case, *tert*-butyl alcohol was introduced for two reasons. The first was the formation of a single anomer, through the introduction of a bulky alkyl substituent from less hindered side, so that the isolation and purification of the product would be easier. The second was the requirement of the protective group at C-1 anomeric position which is stable under basic, but labile under acidic conditions on the occasion of the deprotection at the final stage. We were pleased that the *tert*-butyl group was really introduced in exclusive α-linkage.



Scheme 5. (a) $PhI(OCOt\text{-}Bu)_2$, $Rh_2(OAc)_4$, *t*-BuOH, $ClCH_2CH_2Cl$, 83% for **7a**, 71% for **7b**, 72% for **7c**; (b) LiOH, aq EtOH, 97%; (c) *C. antarctica* lipase B (Novozym® 435), vinyl acetate, 99%.

The contamination of undesired C-1 acetate **9** (mainly α-anomeric form) was another serious side reaction, and we elaborated the reaction conditions. To avoid that, we first attempted the change of oxidant from iodobenzene diacetate to

iodobenzene bistrifluoroacetate. Although trifluoroacetate anion as the leaving group must have lower nucleophilicity, the above-mentioned side reaction caused by the re-attack of acylate anion could not be completely suppressed. Instead, the increase of the bulkiness of acyl leaving group was effective. By applying iodobenzene dipivalate (dimethylpropanoate), the selective formation of desired *tert*-butyl glycoside was much improved.

Second problematic side reaction having so far been reported was the α-elimination of intermediates to give enone functional group (**8**) as shown in Scheme 5. Compared with Rojas' rigid, bicyclic 4,6-benzylidene derivative,[8] our more flexible precursors **6a**–**c** were advantageous to suppress such side reaction. The cyclization proceeded nicely to give **7a**–**c** in 71–83% yield from **6a**–**c**. Even though **6c** with allyl ether substituent at C-4 has another double bond, which would be susceptible for cyclization, the carbamate ring formation was observed exclusively in a desired manner. Kinetically favored five-membered ring formation predominantly occurred, to avoid unstable eight- or nine-membered ring intermediates between external allyl ether.

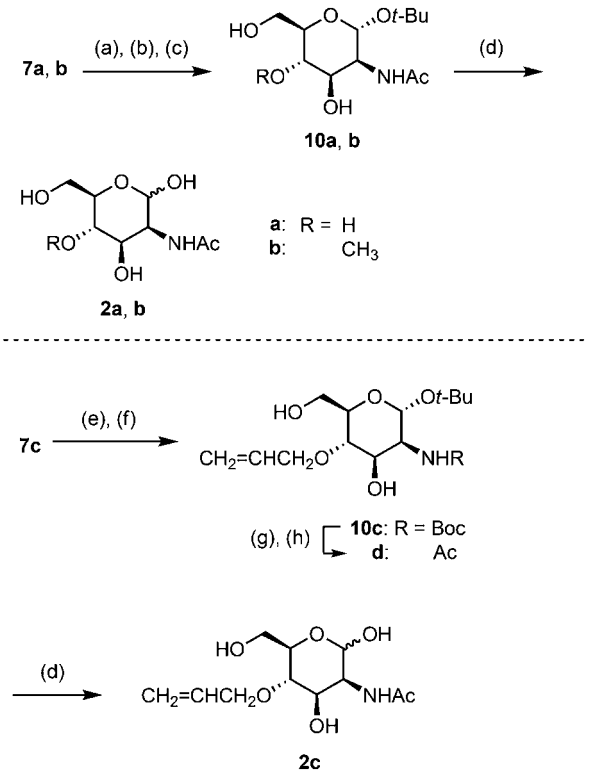
At this stage, another route for **7b** and **7c** through a common intermediate was considered during the preparation of **7a** (Scheme 5). The hydrolysis of two acetyl protective group at C-4 and C-6 in **7a** followed by *C. antarctia* lipase B-catalyzed regioselective acetylation on sterically less hindered hydroxyl group on primary C-6 position provided a requisite precursor (**7e**) with an open C-4 hydroxyl group. The reactivity of resulting hydroxyl group, however, turned out to be extremely low, due to the severe steric hindrance caused by an unexpectedly crooked stereochemistry of bicyclic skeleton in **7**, exemplified by an observation of $J_{1,2}$=0 Hz. Methylation and allylation under various basic conditions only resulted in fruitless results.

The allylation by applying π-allyl palladium complex, which had successfully been applied for C-4 hydroxyl group of **3a**, overreacted on **7e**. *N*-allylation on cyclic carbamate predominantly occurred to give a complex mixture, mainly composed of undesired *N*,*O*-diallylated byproduct. To overcome such situation, a series of tedious transformations involving blocking of less sterically hindered nitrogen atom with TBS protective group, followed by the *O*-allylation and deprotection of TBS was the only way. By comparing the total steps, the route by using of **3d** as the common precursor was obviously advantageous.

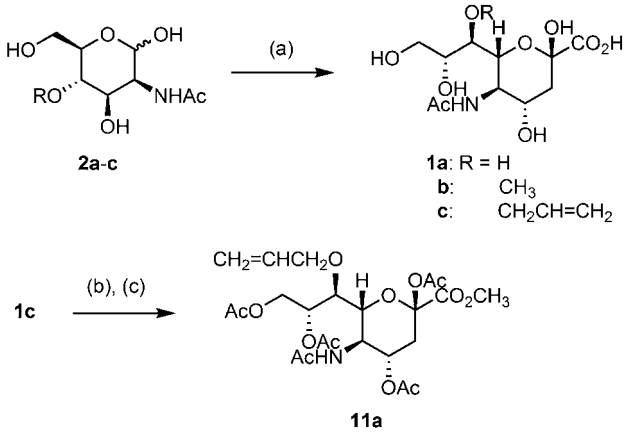
The remaining task was to hydrolyze cyclic carbamate and deprotection at C-1 in a stepwise manner. As seen in Scheme 6, treatment of **7a** with aqueous LiOH at high temperature enabled C–O bond cleavage at C-3 carbamate, followed by hydrolysis of two acetates at C-4 and C-6. In the reaction mixture, the primary product was supposed to be the carbamic acid lithium salt, as when the pH of the mixture was adjusted to be weakly acidic by adding acetic acid, effervescence of carbon dioxide gas as well as the change of TLC appearance were observed. It was noteworthy that if the above hydrolyzate was directly treated with acetic anhydride aiming N-acetylation, a certain proportion of the carbamic acid salt was brought back to the cyclic carbamate, by the activation by way of mixed carbamate anhydride and subsequent five-membered ring formation. After making sure the disappearance of carbamic acid salt under acidic decarboxylation conditions, the mixture was treated with acetic anhydride to give *tert*-butyl *N*-acetylmannosaminide **10a**.

The final step was the deprotection of *tert*-butyl glycoside under acidic conditions. At this stage, the reason and advantage why we chose this protective group is very clear. Mannosamine derivatives, whose C-1 anomeric positions are free, are very prone to epimerize at C-2 to more thermodynamically stable *gluco*-isomers. Any contamination of *gluco*-isomers, the inhibitors for sialic acid aldolase-catalyzed C–C bond formation in the next step, should be avoided. In this case, the treatment of *tert*-butyl glycoside **10a** under acidic conditions with Amberlite® IR-120 (H^+-form) in aqueous ethanol could be applied, to give pure ManNAc **2a** in 85% yield.

The transformation of **10b** with C-4 methoxy substituent to **2b** was effective under similar conditions in total 80% yield. For **10c** with allyl substituent, some unknown more polar spots appeared by TLC analysis, in the course of ring opening of cyclic carbamate under harsh conditions. Then, we temporally introduced *N*-Boc group to give **10c**, for activation of oxazolidinone carbonyl group so that the hydrolysis would be performed under milder conditions (Scheme 6). The successive treatment involving the action of cesium carbonate in methanol at room temperature, *p*-toluenesulfonic acid-mediated Boc deprotection, *N*-acetylation, and hydrolysis of *tert*-butyl group under acidic conditions provided **2c** in total 57% yield.



Scheme 6. (a) LiOH, aq EtOH; (b) AcOH; (c) Ac_2O, quant. for **10a**, 90% for **10b**; (d) Amberlite® IR-120 (H^+-form), aq EtOH, 67% for **2a**, 80% for **2b**, 67% for **2c**; (e) $(Boc)_2O$, DMAP, Et_3N, THF; (f) Cs_2CO_3, MeOH, 72%; (g) *p*-TsOH; (h) Ac_2O, Et_3N, 95%.



Scheme 7. (a) Sialic acid aldolase, CH_3COCO_2Na, 88% for **1b**, 74% for **1c**; (b) Ac_2O, pyridine; (c) CH_2N_2, ether, 20% from **2c**.

We became interested in relative activity of derivatives **2b** and **2c** to the native substrate **2a** in the action of sialic acid aldolase with pyruvate. Judged from the conversion in proper reaction periods, the relative rates of **2b** and **2c** were estimated to be 18 and 9%, respectively. As the product **1c** had previously been unknown,[10] it was fully characterized after derivation to **11a** (Scheme 7).

3. Conclusion

By recent progress on the protein engineering of sialic acid aldolase,[11] the enzymes now accept wide range of substrates with improved specificity and catalytic activity. This situation is promising, by combining the substrates whose new synthetic routes were developed by our present studies in high regio- and stereoselectivity as well as from the naturally abundant, cheap starting material.

4. Experimental section

4.1. Material and methods

Merck silica gel 60 F_{254} thin-layer plates (1.05744, 0.5 mm thickness) and silica gel 60 (spherical and neutral; 100–210 μm, 37560–79) from Kanto Chemical Co., were used for preparative thin-layer chromatography and column chromatography, respectively. Under anhydrous reaction conditions, molecular sieves were pre-dried in vacuo at 100 °C for 1 h. *B. cepacia* lipase (Amano® PS-IM) and *C. antarctica* lipase B (Novozym® 435) show constant specific activity, and are stable immobilized form on solid support. They should be kept in refrigerator, but should never be frozen. Prior to use, to avoid the condensation of the atmospheric moisture on the cold surface of solid supports for lipases, the keeper-bottles were kept for 10–30 min in a dessicator, to equilibrate their temperature with ambient temperature. Invasion of moisture into enzymes, even under immobilized form, lowers their long-term conservativeness.

4.2. Analytical methods

All mps are uncorrected. IR spectra were measured as films for oils or KBr disks for solids on a Jasco FT-IR-410 spectrometer or ATR on a Jeol FT-IR SPX60 spectrometer. ^{1}H and ^{13}C NMR spectra were measured at 400 MHz or 100 MHz on a Varian NMR 400-MR, or at 500 MHz or 126 MHz on a Varian NMR UI-500 spectrometer, respectively. Optical rotation values were recorded on a Jasco P-1010 polarimeter. Mass spectra were recorded on a Jeol JMS-GCmate spectrometer at 70 eV.

*4.2.1. 3,6-Di-O-acetyl-D-glucal (**3a**).* D-Glucal (**3b**, 16.32 g) was prepared by the deacetylation of commercially available tri-*O*-acetyl-D-glucal (**3e**, 30.00 g, 0.110 mol) in a conventional manner and roughly purified by passing through a short column of silica gel. To the solution of **3b** in *t*-BuOH (120 mL), MS 4 Å (2.0 g) and freshly distilled vinyl acetate (50 mL) were added *B. cepacia* lipase (Amano® PS-IM, 2.0 g, see 4.1) and the mixture was stirred at room temperature for 20 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc/MeOH (95:5), R_f for **3b**: 0.23; **3a**: 0.78. The suspension was filtered through a short column of Celite® and the filtrate was concentrated in vacuo. The residue was charged on a silica gel column (300 mL). Elution with hexane/EtOAc (1:1) furnished **3a** (24.39 g, 0.106 mol, 96%) as a colorless oil that crystallized in the freezer. Mp 68–69 °C [lit.[4] mp 65–67 °C]; $[\alpha]_D^{26}$ −5.5 (*c* 0.90, $CHCl_3$), $[\alpha]_D^{25}$ −42.9 (*c* 1.0, MeOH); IR ν_{max}: 3494, 1722, 1705, 1660, 1367, 1242, 1217, 1117, 1074, 1065, 1036, 1024, 949, 918, 843, 823, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.44 (dd, *J*=6.1, 1.6 Hz, 1H), 5.29 (ddd, *J*=6.8, 2.5, 1.6 Hz, 1H), 4.73 (dd, *J*=6.1, 2.5 Hz, 1H), 4.51 (dd, *J*=12.3, 4.5 Hz, 1H), 4.40 (dd, *J*=12.3, 2.5 Hz, 1H), 3.99 (ddd, *J*=9.9, 4.5, 2.5 Hz, 1H), 3.82 (ddd, *J*=9.9, 6.8, 2.6 Hz, 1H), 3.52 (d, *J*=2.6 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 172.5, 171.6, 146.2, 99.5, 76.6, 73.2, 67.4, 62.6, 21.3, 21.0. Its ^{1}H NMR spectrum was identical with that reported previously.[4]

*4.2.2. 3,6-Di-O-acetyl-4-O-methyl-D-glucal (**3c**).* To a solution of **3a** (120 mg, 0.52 mmol) in anhydrous DMF (2.4 mL) and CH_3I (240 μL), Ag_2O (600 mg, 2.5 mmol) was added. The mixture was stirred at room temperature for 24 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **3a**: 0.55; **3c**: 0.77. After consumption of starting material, the mixture was filtered through a short column of Celite® and the filtrate was diluted with EtOAc. The organic layer was washed sequentially with brine, H_2O four times, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was charged on a silica gel column (30 mL). Elution with hexane/EtOAc (3:1) furnished **3c** (122 mg, 0.50 mmol, 96%) as a colorless oil that crystallized in the freezer. Mp 30–31 °C; $[\alpha]_D^{26}$ −52.5 (*c* 0.90, $CHCl_3$); IR ν_{max}: 2931, 2355, 2829, 1738, 1653, 1443, 1375, 1223, 1136, 1107, 1020, 974, 910, 812, 748, 852 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.41 (dd, *J*=6.1, 1.3 Hz, 1H), 5.32 (dddd, *J*=5.7, 3.2, 1.3, 0.7 Hz, 1H), 4.80 (dd, *J*=6.1, 3.2 Hz, 1H), 4.39 (dd, *J*=12.1, 2.9 Hz, 1H), 4.32 (dd, *J*=12.1, 5.8 Hz, 1H), 4.14 (dddd, *J*=7.9, 5.8, 2.9, 0.7 Hz, 1H), 3.55 (dd, *J*=7.9, 5.7 Hz, 1H), 3.49 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.5, 145.5, 99.2, 75.2, 74.9, 69.5, 62.5, 59.2, 21.3, 21.0. Anal. Calcd for $C_{11}H_{16}O_6$: C, 54.09; H, 6.60. Found: C, 53.90; H, 6.64.

*4.2.3. 3,6-Di-O-acetyl-4-O-allyl-D-glucal (**3d**).* To a degassed solution of **3a** (88 mg, 0.40 mmol) in anhydrous THF (3 mL), solid of MS 4 Å (90 mg), tetrakis(triphenylphosphine)palladium (44 mg, 0.04 mmol, 0.1 equiv), and allyl ethyl carbonate (75 μL, 0.57 mmol, 1.4 equiv) were added. The mixture was stirred at 60 °C under argon atmosphere for 30 min. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (2:1), R_f for **3a**: 0.15; **3d**: 0.56. After consumption of starting material, the mixture was concentrated in vacuo. The residue was charged on a silica gel column (30 mL). Elution with hexane/EtOAc (5:1) furnished **3d** (100 mg, 0.39 mmol, 98%) as a pale yellow oil. $[\alpha]_D^{26}$ −12.7 (*c* 1.0, MeOH); IR ν_{max}: 2933, 1736, 1653, 1435, 1367, 1211, 1130, 1099, 1024, 1055, 931, 906, 843, 818, 756, 613 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.41 (dd, *J*=6.1, 1.2 Hz, 1H), 5.86 (dddd, *J*=17.2, 10.4, 5.7, 5.7 Hz, 1H), 5.33 (ddd, *J*=5.8, 3.2, 1.2 Hz, 1H), 5.26 (dddd, *J*=17.2, 1.4, 1.4, 1.4 Hz 1H), 5.19 (dddd, *J*=10.4, 1.4, 1.3, 1.3 Hz 1H), 4.79 (dd, *J*=6.1, 3.2 Hz, 1H), 4.39 (dd, *J*=12.0, 3.1 Hz, 1H), 4.33 (dd, *J*=12.0, 5.7 Hz, 1H), 4.19 (dddd, *J*=12.6, 5.7, 1.3, 1.4 Hz, 1H), 4.15 (ddd, *J*=8.0, 5.7, 3.1 Hz, 1H), 4.11 (dddd, *J*=12.6, 5.7, 1.3, 1.4 Hz, 1H), 3.71 (dd, *J*=8.0, 5.8 Hz, 1H), 2.11 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.5, 145.5, 134.2, 117.8, 99.3, 75.2, 73.1, 72.5, 70.1, 62.5, 21.4, 21.0. Anal. Calcd for $C_{13}H_{18}O_6$: C, 57.77; H, 6.71. Found: C, 57.47; H, 6.69.

*4.2.4. 4,6-Di-O-acetyl-D-glucal (**4a**).* Tri-*O*-acetyl-D-glucal (**3e**, 10.3 g, 37.6 mmol) was dissolved in a mixture of EtOH (100 mL) and phosphate buffer solution (60 mM, 400 mL, pH 7.0). After dissolution, *C. antarctica* lipase B (Novozym® 435, 2.0 g) was added and the mixture was stirred at 30 °C for 12 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **3e**: 0.63; **4a**: 0.27. After the consumption of starting material, the solid of immobilized enzyme was filtered off and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc and washed with brine. The aqueous phase was extracted again with EtOAc three times, the combined organic layer was dried over Na_2SO_4, and concentrated in vacuo. The residue was charged on a silica gel column (200 mL). Elution with hexane/EtOAc (2:1) gave **4a** (8.7 g, 35.7 mmol, 95%) as a colorless oil. $[\alpha]_D^{20}$ +47.7 (*c* 0.86, $CHCl_3$); $[\alpha]_D^{20}$ −11.3 (*c* 0.95, EtOH); IR ν_{max}: 3465, 3072, 2960, 2895,

1745, 1651, 1435, 1371, 1236, 1142, 1097, 1043 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.39 (d, J=5.8 Hz, 1H), 4.96 (dd, J=9.0, 6.3 Hz, 1H), 4.85 (dd, J=5.8, 2.9 Hz, 1H), 4.39 (dd, J=12.2, 5.4 Hz, 1H), 4.30 (ddd, J=6.3, 5.4, 2.9 Hz, 1H), 4.23 (dd, J=12.2, 2.4 Hz, 1H), 4.11 (ddd, J=9.0, 5.4, 2.4 Hz, 1H), 2.43 (d, J=5.4 Hz, 1H), 2.12 (s, 3H), 2.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.8, 170.6, 143.9, 102.8, 73.9, 71.4, 66.9, 61.8, 21.0, 20.8. Its ^1H NMR spectrum was identical with that reported previously.[4]

*4.2.5. 6-O-Acetyl-4-O-methyl-D-glucal (**4b**).* In a similar manner as for **4a**, diacetate **3c** (1.4 g, 5.90 mmol) was treated with *C. antarctica* lipase B (1.0 g) in a mixture of EtOH (10 mL) and phosphate buffer solution (60 mM, 40 mL, pH 7.0). The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **3c**: 0.68; **4b**: 0.40. The workup was performed in a similar manner as in **4a**. The extract was purified with a silica gel column (200 mL). Elution with hexane/EtOAc (2:1) furnished **4b** (1.02 g, 5.04 mmol, 86%) as a colorless oil that crystallized in the freezer. Mp 57–58 °C; $[\alpha]_D^{26}$ +56.0 (*c* 0.95, $CHCl_3$); $[\alpha]_D^{26}$ +42.3 (*c* 0.79, EtOH); IR ν_{max}: 3275, 2918, 2835, 1743, 1643, 1452, 1363, 1329, 1252, 1228, 1101, 1038, 953, 899, 835, 812, 752, 609 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.35 (dd, J=6.0, 1.6 Hz, 1H), 4.75 (dd, J=6.0, 2.6 Hz, 1H), 4.45 (dd, J=12.1, 2.4 Hz, 1H), 4.33 (br s, 1H), 4.30 (dd, J=12.1, 5.7 Hz, 1H), 3.98 (ddd, J=9.1, 5.7, 2.4 Hz, 1H), 3.58 (s, 3H), 3.41 (d, J=13.0 Hz, 1H), 3.34 (dd, J=9.1, 6.5 Hz, 1H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$): δ=170.9, 144.3, 103.2, 79.0, 75.2, 69.1, 63.0, 60.1, 21.0. Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.28; H, 6.98.

*4.2.6. 6-O-Acetyl-4-O-allyl-D-glucal (**4c**).* In a similar manner, diacetate **3d** (2.6 g, 9.62 mmol) was treated with *C. antarctica* lipase B (1.1 g) in EtOH (23 mL) and phosphate buffer solution (57 mL). The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **3d**: 0.47; **4c**: 0.27. The workup was performed in a similar manner as in **4a**, and the extract was purified with a silica gel column (250 mL). Elution with hexane/EtOAc (3:1) furnished **4c** (1.9 g, 8.50 mmol, 89%) as a colorless oil that crystallized in the freezer. Mp 60–61 °C; $[\alpha]_D^{26}$ +49.1 (*c* 1.14, $CHCl_3$); IR ν_{max}: 3267, 2910, 1743, 1651, 1367, 1238, 1215, 1146, 1117, 1093, 1022, 993, 958, 928, 835, 818, 742 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.35 (dd, J=6.0, 1.6 Hz, 1H), 5.93 (dddd, J=17.2, 10.4, 5.8, 5.8 Hz, 1H), 5.30 (dddd, J=17.2, 1.6, 1.2, 1.2 Hz, 1H), 5.21 (dddd, J=10.4, 1.6, 1.6, 1.6 Hz, 1H), 4.75 (dd, J=6.0, 2.5 Hz, 1H), 4.46 (dd, J=12.1, 2.4 Hz, 1H), 4.35 (br d, J=5.5 Hz, 1H), 4.31 (dd, J=12.1, 5.4 Hz, 1H), 4.31 (dddd, J=12.7, 5.8, 1.2, 1.6 Hz, 1H), 4.20 (dddd, J=12.7, 5.8, 1.2, 1.6 Hz, 1H), 4.00 (ddd, J=9.5, 5.4, 2.4 Hz, 1H), 3.50 (dd, J=9.5, 6.7 Hz, 1H), 2.11 (s, 3H), 1.93–1.84 (m, 1H); ^{13}C NMR ($CDCl_3$) δ: 170.9, 144.3, 134.7, 117.8, 103.3, 76.9, 75.4, 73.1, 69.6, 63.0, 21.0. Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07. Found: C, 57.92; H, 7.05.

*4.2.7. 4,6-Di-O-acetyl-3-O-carbamoyl-D-glucal (**6a**).* To a solution of **4a** (1.0 g, 4.35 mmol) in anhydrous CH_2Cl_2 (50 mL), trichloroacetyl isocyanate (825 μL, 6.95 mmol, 1.6 equiv) was added dropwise at 0 °C and under argon atmosphere. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **4a**: 0.19; **5a**: 0.52. The mixture was charged on a column of alumina (100 mL) whose H_2O content was kept as follows: an arbitrary quantity of neutral alumina 90 (Merck, 1.01077) was soaked in distilled H_2O, filtered and kept in a drying oven at 60 °C for 6 h. After cooling, the resulted powder was kept in a closed vessel under the atmosphere with saturated H_2O. It proved convenient to mix the alumina once in a week, to help the equilibration of the system. The column of alumina, which was soaked with reaction mixture, was left at room temperature for 2 h. The product (R_f for **6a**: 0.33 with 1:1 hexane/EtOAc) was then eluted with $CHCl_3$ and concentrated in vacuo. The residue was charged on a silica gel column (200 mL). Elution with hexane/EtOAc (1:1) followed by re-crystallization from hexane/EtOAc gave **6a** (1.0 g, 3.83 mmol, 88%) as colorless needles. Mp 138–139 °C; $[\alpha]_D^{20}$ −34.0 (*c* 0.95, $CHCl_3$); IR ν_{max}: 3442, 3350, 3294, 3207, 1743, 1668, 1622, 1414, 1378, 1336, 1313, 1236, 1036 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.44 (d, J=6.3 Hz, 1H), 5.19–5.29 (m, 2H), 4.88 (dd, J=6.3, 2.9 Hz, 1H), 4.62–4.72 (br, 2H), 4.35 (dd, J=11.5,5.1 Hz, 1H), 4.28–4.48 (m, 1H), 4.22 (dd, J=11.5, 2.9 Hz, 1H), 2.08 (s, 3H), 2.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.5, 169.5, 155.7, 145.3, 99.3, 73.8, 68.1, 67.1, 61.3, 20.9, 20.8; FAB (−)-MS *m*/*z* (%) 296 (M^++Na, 18), 213 (84), 149 (55), 153 (60), 111 (base peak). Anal. Calcd for $C_{11}H_{15}NO_7$: C, 48.35; H, 5.53; N, 5.13. Found: C, 48.31; H, 5.56; N, 5.15.

*4.2.8. 6-O-Acetyl-3-O-carbamoyl-4-O-methyl-D-glucal (**6b**).* In a similar manner as described for **6a**, a solution of **4b** (200 mg, 0.99 mmol) was treated with trichloroacetyl isocyanate (131 μL, 1.09 mmol, 1.1 equiv) in CH_2Cl_2 (20 mL). The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1) R_f for **4b**: 0.18; **5b**: 0.41. After 10 min, the reaction was stopped by adding 13.0 g of alumina as described before and stirred at room temperature for 3 h. The mixture was filtered, and the insoluble materials were washed with $CHCl_3$. The combined filtrate and washings were concentrated in vacuo. The residue was charged on a silica gel column (50 mL). Elution with hexane/EtOAc (1:1) furnished **6b** (226 mg, 0.91 mmol, 93%) as white solid. Mp 121–123 °C; $[\alpha]_D^{25}$ −26.2 (*c* 0.95, $CHCl_3$); $[\alpha]_D^{25}$ −15.4 (*c* 1.37 EtOH); R_f: 0.11 with hexane/EtOAc (1:1); IR ν_{max}: 3408, 3342, 3288, 3205, 2951, 2837, 1736, 1689, 1655, 1624, 1452, 1381, 1327, 1238, 1192, 1261, 1109, 1061, 1009, 984, 958, 906, 746, 611 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.41 (dd, J=6.1, 1.1 Hz, 1H), 5.24 (ddd, J=5.3, 3.5, 1.1 Hz, 1H), 4.85 (dd, J=6.1, 3.5 Hz, 1H), 4.75 (br s, 2H), 4.40 (dd, J=11.9, 3.2 Hz, 1H), 4.30 (dd, J=11.9, 6.5 Hz, 1H), 4.19 (ddd, J=7.2, 6.5, 3.2 Hz, 1H), 3.53 (dd, J=7.2, 5.3 Hz, 1H), 3.50 (s, 3H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.9, 156.0, 145.4, 99.3, 75.3, 74.7, 69.3, 62.4, 59.1, 21.0. Anal. Calcd for $C_{10}H_{15}NO_6$: C, 48.98; H, 6.17; N, 5.71. Found: C, 49.24; H, 6.23; N, 5.53.

*4.2.9. 6-O-Acetyl-4-O-allyl-3-O-carbamoyl-D-glucal (**6c**).* In a similar manner as described for **6a**, allyl ether **4c** (50 mg, 0.22 mmol) was treated with trichloroacetyl isocyanate (32 μL, 0.26 mmol, 1.2 equiv) in CH_2Cl_2 (3 mL). The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1) R_f for **4c**: 0.42; **5c**: 0.63. Similar workup and column chromatography furnished **6c** (52 mg, 0.19 mmol, 88%) as white solid. Mp 115–116 °C; $[\alpha]_D^{28}$ −11.5 (*c* 1.1, $CHCl_3$); $[\alpha]_D^{26}$ −6.6 (*c* 1.1 MeOH); R_f: 0.31 with hexane/EtOAc (1:1); IR ν_{max}: 3398, 3290, 3199, 2937, 1738, 1720, 1686, 1660, 1620, 1450, 1427, 1373, 1319, 1261, 1227, 1153, 1130, 1076, 1045, 1011, 987, 926, 903, 825, 777, 748, 586 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.42 (dd, J=6.1, 1.3 Hz, 1H), 5.89 (dddd, J=17.2,10.4, 5.9, 5.9 Hz, 1H), 5.28 (dddd, J=17.2, 1.7, 1.6, 1.6 Hz, 1H), 5.26 (dddd, J=5.4, 3.4, 1.3, 0.8 Hz, 1H), 5.20 (dddd, J=10.4, 1.7, 1.2, 1.2 Hz, 1H), 4.85 (ddd, J=6.1, 3.4, 0.4 Hz, 1H), 4.64 (br s, 2H), 4.41 (dd, J=12.0, 3.4 Hz, 1H), 4.32 (dd, J=12.0, 6.1 Hz, 1H), 4.22 (dddd, J=12.6, 5.9, 1.6, 1.2 Hz, 1H), 4.19 (dddd, J=6.1, 7.4, 3.4, 0.8 Hz, 1H), 4.11 (dddd, J=12.6, 5.9, 1.6, 1.2 Hz, 1H), 3.70 (ddd, J=7.4, 5.4, 0.4 Hz, 1H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$) δ: 170.9, 156.0, 145.4, 134.3, 117.9, 99.4, 75.0, 73.1, 72.3, 70.0, 62.4, 21.0. Anal. Calcd for $C_{12}H_{17}NO_6$: C, 53.13; H, 6.32; N, 5.16. Found: C, 53.19; H, 6.32; N, 5.15.

*4.2.10. tert-Butyl 4,6-di-O-acetyl-2-amino-2-N,3-O-carbonyl-2-deoxy-α-D-mannopyranoside (**7a**).* A solution of **6a** (511 mg, 1.87 mmol) in a mixture of *t*-BuOH and 1,2-dichloroethane (3:2, total 25 ml) was added on mixture of MS 4 Å (500 mg), rhodium(II) acetate dimer (41 mg, 0.093 mmol, 0.05 equiv), and bis(pivaloyloxy) iodobenzene (1.5 g, 3.74 mmol, 2 equiv). The mixture was stirred under argon atmosphere at 50 °C for 48 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1) R_f for **6a**: 0.33; **7a**: 0.29. After consumption of starting material, the vessel was cooled to room temperature and concentrated in vacuo. The residue was charged on a silica gel column (60 mL). Elution with hexane/EtOAc (1:1) furnished **7a** (535 mg, 83%). This was recrystallized from

hexane/EtOAc to give colorless prisms. Mp 144–145 °C; $[\alpha]_D^{24}$ −10.6 (*c* 1.00, $CHCl_3$); IR (KBr) ν_{max}: 2927, 2857, 1727, 1599, 1461, 1379, 1272, 1123, 1072, 803, 704 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 6.45 (b, 1H), 5.14 (s, 1H), 5.10 (dd, *J*=9.8, 7.3 Hz, 1H), 4.64 (dd, *J*=7.8, 7.3 Hz, 1H), 4.19 (dd, *J*=11.7, 4.9 Hz, 1H), 4.04 (m, 2H), 3.88 (d, *J*=7.8 Hz, 1H), 2.02 (2 s, 6H), 1.20 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 170.6, 169.2, 158.8, 91.2, 76.4, 76.3, 68.3, 65.7, 62.6, 57.4, 28.4, 20.8. Anal. Calcd for $C_{15}H_{23}NO_8$: C, 52.17; H, 6.71; N, 4.06. Found: C, 52.10; H, 6.67; N, 4.01.

*4.2.11. tert-Butyl 6-O-acetyl-4-O-methyl-2-amino-2-N,3-O-carbonyl-2-deoxy-α-D-mannopyranoside (**7b**).* In a similar manner as described for **7a**, carbamate **6b** (225 mg, 0.92 mmol) was treated with $Rh_2(OAc)_4$ (20 mg, 0.046 mmol, 0.05 equiv), bis(pivaloyloxy)iodobenzene (746 mg, 1.84 mmol, 2 equiv), and MS 4 Å (340 mg) in a mixture of *t*-BuOH and 1,2-dichloroethane (3:2, 15 mL) under argon atmosphere at 50 °C for 48 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **6b**: 0.12; **7b**: 0.32. Similar workup and chromatographic purification furnished **7b** (211 mg, 0.66 mmol, 71%) as a yellow oil. $[\alpha]_D^{25}$ +45.5 (*c* 0.67, EtOH); $[\alpha]_D^{25}$ +27.1 (*c* 0.74, $CHCl_3$); IR ν_{max}: 3323, 2974, 2933, 1740, 1645, 1454, 1394, 1365, 1232, 1190, 1122, 1086, 1063, 999, 976, 916, 881, 806, 748, 660 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.17 (d, *J*=1.2 Hz, 1H), 4.67 (dd, *J*=7.9, 6.7 Hz, 1H), 4.34 (dd, *J*=11.8, 2.3 Hz, 1H), 4.21 (dd, *J*=11.8, 5.8 Hz, 1H), 3.96 (ddd, *J*=10.2, 5.8, 2.3 Hz, 1H), 3.92 (dd, *J*=7.9, 1.2 Hz, 1H), 3.50 (s, 3H), 3.35 (dd, *J*=10.2, 6.7 Hz, 1H), 2.05 (s, 3H), 1.27 (s, 9H); ^{13}C NMR (CD_3OD) δ: 172.5, 161.2, 92.6, 80.6, 78.8, 77.1, 67.3, 64.6, 59.5, 59.0, 28.8, 20.7. Anal. Calcd for $C_{14}H_{23}NO_7 \cdot 1/2H_2O$: C, 51.53; H, 7.41; N, 4.29. Found: C, 51.90; H, 7.31; N, 4.09.

*4.2.12. tert-Butyl 6-O-acetyl-4-O-allyl-2-amino-2-N,3-O-carbonyl-2-deoxy-α-D-mannopyranoside (**7c**).* In a similar manner as described for **7a**, carbamate **6c** (926 mg, 3.4 mmol) was treated with $Rh_2(OAc)_4$ (75 mg, 0.17 mmol, 0.05 equiv), bis(pivaloyloxy)iodobenzene (2.8 g, 6.9 mmol, 2 equiv), and MS 4 Å (800 mg) in a mixture of *t*-BuOH and 1,2-dichloroethane (3:2, 50 mL) under argon atmosphere at 50 °C for 48 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:1), R_f for **6c**: 0.28; **7c**: 0.33. Similar workup and purification furnished **7c** (838 mg, 2.44 mmol, 72%) as a yellow oil. $[\alpha]_D^{23}$ +46.0 (*c* 1.2, MeOH); $[\alpha]_D^{24}$ +49.8 (*c* 0.78, $CHCl_3$); IR ν_{max}: 3319, 2981, 1767, 1741, 1392, 1367, 1230, 1186, 1130, 1093, 1061, 993, 918, 806, 762 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.90 (dddd, *J*=17.2, 10.4, 6.2, 5.3 Hz, 1H), 5.29 (dddd, *J*=17.2, 1.7, 1.7, 1.7 Hz, 1H), 5.18 (dddd, *J*=10.4, 1.7, 1.2, 1.2 Hz, 1H), 5.17 (d, *J*=0.9 Hz, 1H), 4.70 (dd, *J*=7.7, 7.0 Hz, 1H), 4.33 (dd, *J*=11.8, 2.3 Hz, 1H), 4.29 (dddd, *J*=12.7, 5.3, 1.7, 1.2 Hz, 1H), 4.22 (dd, *J*=11.8, 5.6 Hz, 1H), 4.09 (dddd, *J*=12.7, 6.2, 1.7, 1.2 Hz, 1H), 4.00 (ddd, *J*=10.2, 5.6, 2.3 Hz, 1H), 3.93 (dd, *J*=7.7, 0.9 Hz, 1H), 3.55 (dd, *J*=10.2, 7.0 Hz, 1H), 2.05 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (CD_3OD) δ: 172.5, 153.4, 135.6, 118.0, 92.6, 80.8, 77.1, 76.1, 73.1, 67.3, 64.6, 59.1, 28.8, 20.7. Anal. Calcd for $C_{16}H_{25}NO_7 \cdot 1/3H_2O$: C, 55.00; H, 7.40; N, 4.01. Found: C, 55.07; H, 7.28; N, 3.91.

*4.2.13. tert-Butyl 2-amino-2-N,3-O-carbonyl-2-deoxy-α-D-mannopyranoside (**7d**).* To a solution of **7a** (487 mg, 1.41 mmol) in EtOH (15 mL), $LiOH \cdot H_2O$ (Kanto Chemical Co. 24129-01, 178 mg, 4.23 mmol, 3 equiv) was added and the mixture was stirred at room temperature for 3 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc, R_f for **7a**: 0.8; **7d**: 0.22. After concentrated in vacuo, the residue was charged on a silica gel column (60 mL). Elution with EtOAc furnished **7d** (356 mg, 1.37 mmol, 97%) as hygroscopic foam. Mp 173–174 °C; $[\alpha]_D^{20}$ +23.3 (*c* 0.7, MeOH); IR ν_{max}: 3297, 2977, 1739, 2927, 1386, 1363, 1234, 1184, 1122, 1049, 987, 925 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.18 (d, *J*=1.2 Hz, 1H), 4.50 (dd, *J*=7.7, 7.4 Hz, 1H), 3.87 (dd, *J*=7.7, 1.2 Hz, H2), 3.80 (dd, *J*=11.2, 2.0 Hz, 1H), 3.75 (ddd, *J*=8.8, 2.6, 2.0 Hz, 1H), 3.74 (dd, *J*=11.2, 2.6 Hz, 1H), 3.70 (dd, *J*=8.8, 7.4 Hz, 1H), 1.26 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (CD_3OD) δ: 161.7, 92.6, 81.3, 76.9, 70.9, 69.0, 62.3, 59.2, 28.9. Anal. Calcd for $C_{11}H_{19}NO_6$: C, 50.57; H, 7.33; N, 5.36. Found: C, 50.28; H, 7.31; N, 5.03.

*4.2.14. tert-Butyl 6-O-acetyl-2-amino-2-N,3-O-carbonyl-2-deoxy-α-D-mannopyranoside (**7e**).* To a solution of **7d** (356 mg, 1.36 mmol) in freshly distilled vinyl acetate (10 mL), *C. antarctica* lipase B (Novozym® 435, 180 mg) was added. The mixture was stirred at room temperature for 3 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc, R_f for **7d**: 0.22; **7e**: 0.52. The suspension was filtered through a short column of Celite® and the filtrate was concentrated in vacuo. The residue was charged on a silica gel column (40 mL). Elution with hexane/EtOAc (1:1) furnished **7e** (412 mg, 1.4 mmol, 99%) as hygroscopic solid. Mp 147–148 °C; $[\alpha]_D^{20}$ +26.6 (*c* 0.6, MeOH); IR ν_{max}: 3425, 3261, 2975, 1765, 1736, 1707, 1373, 1284, 1227, 1092, 1057, 1009, 1005, 974, 933, 864 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.18 (d, *J*=1.2 Hz, 1H), 4.53 (dd, *J*=7.5, 7.5 Hz, 1H), 4.36 (dd, *J*=11.9, 2.3 Hz, 1H), 4.20 (dd, *J*=11.9, 6.0 Hz, 1H), 3.96 (ddd, *J*=10.3, 6.0, 2.3 Hz, 1H), 3.91 (dd, *J*=7.5, 1.2 Hz, 1H), 3.62 (dd, *J*=10.3, 7.5 Hz, 1H), 2.05 (s, 3H, CH_3CO), 1.28 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (CD_3OD) δ: 172.6, 161.5, 92.6, 81.0, 77.1, 69.3, 68.6, 64.7, 59.1, 28.8, 20.7. Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.57; H, 7.04; N, 4.25.

*4.2.15. tert-Butyl 2-acetamido-2-deoxy-α-D-mannopyranoside (**10a**).* To a solution of **7a** (1.20 g, 3.5 mmol) in EtOH (32 mL), $LiOH \cdot H_2O$ (1.02 g, 24.3 mmol, 7 equiv) was added. The mixture was stirred for 24 h at 70 °C. After cooling to room temperature, AcOH (2.2 mL, 47.6 mmol, 13.6 equiv) was added. The solution was further stirred at room temperature for 1 h and Ac_2O (460 μL, 4.6 mmol, 1.3 equiv) was then added. The reaction was checked by silica gel TLC, developed with $CHCl_3$/MeOH (8:1), R_f for **7a**: 0.82; **10a**: 0.18. The mixture was neutralized by the addition of saturated $NaHCO_3$ aq solution and de-salted (Ac-220-10 on Asahi Chemical Micro Acylyzer S1). Removal of H_2O by lyophilization afforded **10a** quantitatively as colorless amorphous solid. This was employed for the next step without further purification. Analytical sample was obtained by re-crystallization from EtOAc containing small amount of EtOH and hexane as fine needles. Mp 167–168 °C; $[\alpha]_D^{25}$ +44.7 (*c* 1.00, EtOH); ^1H NMR ($CDCl_3$) δ: 4.99 (d, *J*=1.5 Hz, 1H); 4.11 (dd, *J*=4.4, 1.5 Hz, 1H), 3.94 (dd, *J*=9.8, 4.4 Hz, 1H), 3.81 (dd, *J*=11.7, 4.4 Hz, 1H), 3.74 (dd, *J*=11.7, 1.9 Hz, 1H), 3.71 (ddd, *J*=9.7, 4.4, 1.9 Hz, 1H), 3.57 (dd, *J*=9.8, 9.7 Hz, 1H), 2.00 (s, 3H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 173.8, 95.1, 76.4, 73.6, 70.7, 68.4, 62.1, 55.9, 28.8, 22.7; IR ν_{max}: 3372, 2977, 1662 cm^{-1}. Anal. Calcd for $C_{12}H_{23}NO_6$: C, 51.97; H, 8.36; N, 5.05. Found: C, 51.72; H, 8.48; N, 4.97.

*4.2.16. tert-Butyl 2-acetamido-4-O-methyl-2-deoxy-α-D-mannopyranoside (**10b**).* In a similar manner as described for **10a**, methyl ether **7b** (188 mg, 0.39 mmol) was treated with $LiOH \cdot H_2O$ (174 mg, 4.1 mmol, 7 equiv) in EtOH (5 mL) at 75 °C for 24 h. After cooling, the mixture was neutralized by adding AcOH (405 μL, 8.9 mmol, 15 equiv). After 1 h, Ac_2O (77 μL, 0.8 mmol, 1.3 equiv) was added for N-acetylation. The progress of the reaction was checked by silica gel TLC, developed with $CHCl_3$/MeOH (8:1), R_f for **7b**: 0.55; **10b**: 0.36. After concentrated in vacuo, the residue was charged on a silica gel column (100 mL). Elution with EtOAc/EtOH (9:1) furnished **10b** (155 mg, 0.53 mmol, 90%) as a solid. Mp 104–106 °C; $[\alpha]_D^{24}$ +42.9 (*c* 0.60, EtOH); IR ν_{max}: 3182, 2970, 2912, 2360, 2333, 1647, 1535, 1462, 1377, 1203, 1124, 1101, 1068, 1047, 1018, 887, 798 cm^{-1}; ^1H NMR (CD_3OD) δ: 4.97 (d, *J*=1.7 Hz, 1H), 4.11 (dd, *J*=4.7, 1.7 Hz, 1H), 4.02 (dd, *J*=9.6, 4.7 Hz, 1H), 3.78 (dd, *J*=11.5, 3.4 Hz, 1H), 3.70 (dd, *J*=11.5, 2.2 Hz, 1H), 3.67 (ddd, *J*=9.6, 3.4, 2.2 Hz, 1H), 3.56 (s, 3H), 3.32 (dd, *J*=9.6, 9.6 Hz, 1H), 2.02 (s, 3H), 1.25 (s, 9H); ^{13}C NMR (CD_3OD) δ: 174.0, 95.1, 78.2, 76.5, 72.7,

70.9, 62.0, 61.0, 56.2, 28.8, 22.7. Anal. Calcd for $C_{13}H_{26}NO_6$: C, 53.59; H, 8.65; N, 4.81. Found: C, 53.66; H, 8.65; N, 4.70.

*4.2.17. tert-Butyl 2-N-tert-butoxycarbonyl-4-O-allyl-α-D-mannopyranoside (**10c**).* To a mixture of **7c** (513 mg, 1.5 mmol) and MS 4 Å (500 mg) in anhydrous THF (35 mL), *tert*-butyl pyrocarbonate (587 mg, 2.69 mmol, 1.98 equiv), triethylamine (228 μL, 1.64 mmol, 1.1 equiv), and DMAP (36 mg, 0.30 mmol, 0.2 equiv) were added. The mixture was stirred at room temperature for 2 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (3:1), R_f for **7c**: 0.10; *N*-Boc derivative: 0.59. The mixture was then concentrated in vacuo, diluted with EtOAc (60 mL). The mixture was washed with aq citric acid solution (50 mM, 3×25 mL), saturated aq $NaHCO_3$ solution (50 mM, 3×25 mL) and brine (2×25 mL). The organic phase was dried over Na_2SO_4 and concentrated in vacuo.

The residue was dissolved in anhydrous CH_3OH (25 mL), and to the resulting mixture Cs_2CO_3 (375 mg, pre-dried in vacuo at 100 °C for 1 h, 0.64 mmol, 0.5 equiv) was added. The mixture was stirred at 0 °C to room temperature for 12 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (3:1), R_f for *N*-Boc: 0.59; **10c**: 0.15. The mixture was concentrated in vacuo and the residue was charged on a silica gel column (100 mL). Elution with hexane/EtOAc (3:1) furnished **10c** (404 mg, 1.08 mmol, 72%, two steps) as a white solid. Mp 63–65 °C; $[\alpha]_D^{25}$ +42.8 (*c* 0.9, EtOH). IR ν_{max}: 3400, 3219, 2972, 2924, 2357, 2158, 1687, 1574, 1387, 1362, 1296, 1254, 1163, 1078, 1043, 1016, 916, 752, 885, 849 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.96 (dddd, *J*=17.2, 10.5, 5.6, 5.6 Hz, 1H), 5.26 (dddd, *J*=17.2, 1.9, 1.8, 1.8 Hz, 1H), 5.11 (dddd, *J*=10.4, 1.9, 1.3, 1.3 Hz, 1H), 4.99 (d, *J*=1.1 Hz, 1H), 4.36 (dddd, *J*=12.5, 5.6, 1.8, 1.8 Hz, 1H), 4.13 (dddd, *J*=12.5, 5.6, 1.3, 1.3 Hz, 1H), 4.01 (dd, *J*=9.5, 4.6 Hz, 1H), 3.77 (dd, *J*=10.5, 4.2 Hz, 1H), 3.77 (dd, *J*=4.6, 1.1 Hz, 1H), 3.68 (dd, *J*=10.5, 2.0 Hz, 1H), 3.68 (ddd, *J*=9.5, 4.2, 2.0 Hz, 1H), 3.42 (dd, *J*=9.5, 9.5 Hz, 1H), 1.46 (s, 9H), 1.25 (s, 9H); ^{13}C NMR (CD_3OD) δ: 136.6, 116.6, 95.6, 80.4, 76.5, 76.4, 74.8, 72.8, 71.0, 61.9, 57.5, 28.8, 28.8. Anal. Calcd for $C_{18}H_{33}NO_7 \cdot 1/2H_2O$: C, 56.23; H, 8.91; N, 3.64. Found: C, 56.34; H, 8.85; N, 3.24.

*4.2.18. tert-Butyl 2-acetamido-4-O-allyl-α-D-mannopyranoside (**10d**).* To a solution of **10c** (340 mg, 0.91 mmol) in a mixture of THF and CH_2Cl_2 (1:2, 15 mL), *p*-toluenesulfonic acid (2.3 g) was added. The mixture was stirred at room temperature for 1 h. The progress of the reaction was checked by silica gel TLC, developed with hexane/EtOAc (1:2), R_f for **10c**: 0.38. Then, triethylamine (2.21 mL, 15.9 mmol) and Ac_2O (170 μL, 1.75 mmol, 1.9 equiv) were added and the mixture was further stirred for 1 h. The progress of acetylation was checked by silica gel TLC, developed with hexane/EtOAc (1:2), R_f for **10d**: 0.11. The mixture was concentrated in vacuo, and the residue was charged on a silica gel column (200 mL). Elution with EtOAc/EtOH (9:1) furnished **10d** (274 mg, 0.86 mmol, 95%) as a white solid. Mp 160–162 °C; $[\alpha]_D^{23}$ +49.5 (*c* 0.6, EtOH); IR ν_{max}: 3275, 3078, 2966, 2933, 2360, 2332, 1651, 1556, 1458, 1371, 1300, 1248, 1196, 1126, 1074, 1043, 1020, 980, 916, 887, 852, 800, 688 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.97 (dddd, *J*=17.2, 10.5, 5.6, 5.6 Hz, 1H), 5.27 (dddd, *J*=17.2, 1.9, 1.8, 1.8 Hz, 1H), 5.12 (dddd, *J*=10.5, 1.9, 1.5, 1.5 Hz, 1H), 4.97 (d, *J*=1.6 Hz, 1H), 4.37 (dddd, *J*=12.5, 5.6, 1.8, 1.5 Hz, 1H), 4.15 (dddd, *J*=12.5, 5.5, 1.7, 1.5 Hz, 1H), 4.12 (dd, *J*=4.6, 1.6 Hz, 1H), 4.05 (dd, *J*=9.5, 4.6 Hz, 1H), 3.79 (dd, *J*=12.0, 4.2 Hz, 1H), 3.71 (dd, *J*=12.0, 2.0 Hz, 1H), 3.71 (ddd, *J*=9.5, 4.2, 2.0 Hz, 1H), 3.47 (dd, *J*=9.5, 9.5 Hz, 1H), 2.02 (s, 3H), 1.25 (s, 9H); ^{13}C NMR (CD_3OD) δ: 174.0, 136.6, 116.7, 95.2, 80.6, 76.5, 74.9, 72.8, 70.9, 61.9, 56.3, 28.8, 22.7. Anal. Calcd for $C_{15}H_{27}NO_6 \cdot 2/3H_2O$: C, 54.70; H, 8.67; N, 4.25. Found: C, 54.93; H, 8.36; N, 4.00.

*4.2.19. 2-Acetoamido-2-deoxy-D-mannopyranose (**2a**).* To a solution of **10a** (51 mg, 0.18 mmol) in H_2O (0.6 mL), Amberlite® IR-120 (H^+ form, 50 mg) was added. The mixture was stirred at 60 °C for 24 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc/MeOH (8:2), R_f for **10a**: 0.30; **2a**: 0.11. Insoluble materials were then filtered and the filtrate was concentrated in vacuo. The residue was charged on a silica gel column (20 mL). Elution with EtOAc/MeOH (8:2) furnished **2a** (43 mg) as a colorless solid. The purity of the product was estimated by ^1H NMR measurement together with methyl β-D-glucoside as the internal standard and the yield was calculated to be 67%. $[\alpha]_D^{18}$ +10.6 (*c* 0.66, H_2O). The optical rotation was in good accordance with commercially available ManNAc monohydrate; $[\alpha]_D^{18}$ +11.1 (*c* 0.71, H_2O). The ^1H NMR data were in agreement with those of commercially available sample.

*4.2.20. 2-Acetamido-4-O-methyl-2-deoxy-D-mannopyranose (**2b**).* In a similar manner as described for **2a**, a solution of **10b** (131 mg, 0.45 mmol) in aq EtOH (10%, 6 mL) was treated with Amberlite® IR-120 (H^+ form, 500 mg) at 60 °C for 24 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc/EtOH (8:2), R_f for **10b**: 0.38; **2b**: 0.27. After similar workup, the residue was charged on a silica gel column (100 mL). Elution with EtOAc/EtOH (8:2) furnished **2b** (84 mg, 0.36 mmol, 80%) as a white hygroscopic solid; $[\alpha]_D^{25}$ +31.7 (*c* 0.75, EtOH); IR ν_{max}: 3292, 2926, 1630, 1541, 1443, 1377, 1294, 1252, 1194, 1088, 1065, 1028, 976, 793, 658 cm^{-1}; ^1H NMR (D_2O) δ: 5.11 (d, *J*=1.2 Hz, H-1α), 5.01 (d, *J*=1.3 Hz, H-1β), 4.45 (dd, *J*=4.4, 1.3 Hz, H-2β), 4.33 (dd, *J*=1.2, 4.5 Hz, H-2α), 4.13 (dd, *J*=9.7, 4.5 Hz, H-3α), 3.93 (dd, *J*=9.5, 4.4 Hz, H-3β), 3.89–3.80 (m), 3.57 (s, OCH_3α), 3.57 (s, OCH_3β), 3.42–3.37 (m), 3.31 (dd, *J*=9.8, 9.8 Hz, H-4β), 2.12 (s, CH_3COβ), 2.08 (s, CH_3COα); ^{13}C NMR (D_2O) δ: 175.6, 174.7, 92.9, 92.8, 76.8, 76.5, 76.2, 71.9, 71.0 (×2), 68.7, 60.2 (×2), 60.1, 54.2, 53.3, 22.0, 21.9. Its NMR spectrum was identical with that reported previously.[3] Judging from its ^1H NMR, this was proved to be a 3:2 mixture of α- and β- anomers.

*4.2.21. 2-Acetamido-4-O-allyl-2-deoxy-D-mannopyranose (**2c**).* In a similar manner as described for **2a**, a solution of **10d** (378 mg, 1.19 mmol) in aq EtOH (10%, 12 mL) was treated with Amberlite® IR-120 (H^+ form, 500 mg) at 60 °C for 24 h. The progress of the reaction was checked by silica gel TLC, developed with EtOAc/MeOH (8:2), R_f for **10d**: 0.50; **2c**: 0.31. After similar workup, the residue was charged on a silica gel column (200 mL). Elution with EtOAc/MeOH (9:1) furnished **2c** (196 mg, 0.75 mmol, 63%) as a white solid. $[\alpha]_D^{23}$ +26.4 (*c* 0.4, EtOH); IR ν_{max}: 3288, 2922, 2353, 1649, 1543, 1448, 1417, 1367, 1298, 1250, 1093, 1061, 1030, 974, 918, 742 cm^{-1}; ^1H NMR (CD_3OD) δ: 5.97 (dddd, *J*=17.2, 10.4, 5.6, 5.6 Hz, 1H), 5.26 (dddd, *J*=17.2, 1.8, 1.8, 1.8 Hz, 1H), 5.12 (dddd, *J*=10.4, 1.8, 1.3, 1.3 Hz, 1H), 4.97 (d, *J*=1.6 Hz, 1H, H-1α), 4.84 (d, *J*=1.5 Hz, 1H, H-1β), 4.37 (dddd, *J*=12.5, 5.6, 1.8, 1.3 Hz, 1H), 4.27 (dd, *J*=4.7, 1.6 Hz, 1H), 4.15 (dddd, *J*=12.6, 5.6, 1.8, 1.3 Hz, 1H), 4.10 (dd, *J*=9.5, 4.6 Hz, 1H), 3.84–3.71 (m, 3H, both α and β H-5, H-6, H-6′), 3.47 (dd, *J*=9.5, 9.5 Hz, 1H, H-4α), 3.39 (dd, *J*=9.6, 9.6 Hz, 1H, H-4β), 2.06 (s, 3H, CH_3COβ), 2.19 (s, 3H, CH_3COα); ^{13}C NMR (CD_3OD) δ: 136.6, 116.6, 94.9, 76.5, 74.8, 72.5, 70.8, 62.0, 55.5, 22.7. Judging from its ^1H NMR, this was proved to be a 3:2 mixture of α- and β- anomers.

*4.2.22. 5-Acetamido-7-O-methyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosulonic acid (**1b**).* A solution of **2b** (75 mg, 0.32 mmol) in phosphate buffer solution (20 mM, 1.6 mL, pH 7.5), sodium pyruvate (176 mg, 1.6 mmol, 5 equiv), and sialic acid aldolase (Toyobo® NAL-301, 190 U, based on the standard assay: the retro-aldol cleavage of *N*-acetylneuraminic acid at pH 7.5) were added and the mixture was stirred at 37 °C for 20 h. The progress of the reaction was checked by ^1H NMR; ^1H NMR [anomeric signals of **2b** at δ 5.11 and 5.01 ppm (3:2, two anomers) in D_2O, and of **1b** at δ 2.22 and 1.89 ppm]. Its relative conversion

rate was 18.2% of that of the native substrate **2a**. Final conversion reached 88%. NMR spectrum of the product **1b** was identical with that reported previously.[3]

*4.2.23. 5-Acetamido-7-O-allyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosulonic acid (**1c**).* In a similar manner as described for **1b**, to a solution of **2c** (166 mg, 0.64 mmol) in phosphate buffer solution (20 mM, 3.2 mL, pH 7.5), sodium pyruvate (350 mg, 3.17 mmol, 5 equiv) and sialic acid aldolase (390 U) were added and the mixture was stirred at 37 °C for 20 h. The progress of the reaction was checked by ^{1}H NMR; ^{1}H NMR [anomeric signals for **2c** at δ 5.13 and 5.03 ppm (3:2, two anomers) in D_2O, and for **1c** at δ 2.20 and 1.86 ppm]. Its relative conversion rate was 9.5% of that of the **2a**. Final conversion reached 74%. The crude product was employed for the next step without further purification.

*4.2.24. Methyl 5-acetamido-2,4,8,9-tetra-O-acetyl-7-O-allyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranosulonate (**11a**).* The above-mentioned crude **1c** was suspended in a mixture of THF (4 mL), pyridine (1 mL), and DMF (1 ml) and cooled with an ice-cold bath. Acetyl chloride (500 μL, 6 mmol, 11 equiv) was then added dropwise. The mixture was stirred for 2 h at room temperature. The mixture was filtered through column of silica gel (20 mL) with EtOAc/MeOH (4:1). After concentration, the residue (104 mg) was suspended in anhydrous CH_2Cl_2 (2 mL) and a solution CH_2N_2 in diethyl ether was added dropwise at room temperature. The progress of the reaction was checked by silica gel TLC, developed with EtOAc/MeOH/H_2O (4:2:1), R_f for **1c**: 0.75; with EtOAc/MeOH (8:1), R_f for **11a**: 0.63. The mixture was concentrated in vacuo and the residue was charged on a silica gel column (50 mL). Elution with EtOAc furnished **11a** (31 mg, 0.058 mmol, 20% from **2c**) as an oil. $[\alpha]_D^{24}$ −25.1 (*c* 1.55, MeOH); IR ν_{max} 3734, 2364, 2335, 2023, 1728, 1645, 1539, 1358, 1234, 1041 cm^{-1}; ^{1}H NMR (CD_3OD) δ: 5.95 (dddd, *J*=17.2, 10.4, 5.8, 5.8 Hz, 1H), 5.29 (dddd, *J*=17.2, 1.9, 1.6, 1.6 Hz, 1H), 5.23 (ddd, *J*=11.5, 9.2, 5.1 Hz, 1H), 5.17 (dddd, *J*=10.4, 1.9, 1.1, 1.1 Hz, 1H), 5.03 (ddd, *J*=6.3, 5.6, 2.4 Hz, 1H), 4.67 (dd, *J*=12.4, 2.4 Hz, 1H), 4.17 (dd, *J*=10.7, 9.2, 1H), 4.17 (dd, *J*=12.4, 6.3 Hz, 1H), 4.12 (dddd, *J*=5.8, 2.9, 1.6, 1.1 Hz, 1H), 4.12 (dddd, *J*=5.8, 2.9, 1.6, 1.1, Hz, H1), 4.02 (dd, *J*=10.7, 1.7 Hz, 1H), 3.75 (s, 3H), 3.74 (dd, *J*=5.6, 1.7 Hz, 1H), 2.48 (dd, *J*=13.3, 5.1 Hz, 1H), 2.13 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H), 1.94 (dd, *J*=13.3, 11.5 Hz, 1H). This was obtained as an anomeric mixture (α/β=95:5); ^{13}C NMR (CD_3OD) δ: 173.4, 172.5, 172.0, 171.6, 170.0, 168.4, 135.8, 117.9, 98.7, 76.5, 75.1, 74.3, 73.9, 70.4, 63.8, 53.5, 50.2, 49.1, 36.8, 22.9, 20.98, 20.79, 20.68, 20.58.

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Supplementary data

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