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A straightforward α-selective aromatic glycosylation and its application for stereospecific synthesis of 4-methylumbelliferyl α-T-antigen

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ABSTRACT

A practical and efficient α-selective aromatic glycosylation with simple per-*O*-acetyl glycopyranosyl trichloroacetimidates is reported. The method is particularly effective for L-fucosyl and 2-azido-2-deoxy-D-galatosaminyl imidates, with which exclusive α-selectivity was achieved. The synthetic utility of this method was demonstrated in the stereoselective synthesis of 4-methylumbelliferyl α-T-antigen.

1. Introduction

A wide variety of natural products contain glycosidic linkages to phenols as exemplified by the structures of the potent antibiotic vancomycin and anti-diabetic phlorizin.[1–4] More importantly, the presence of such phenyl aglycons is found to be essential for their biological functions.[5–8] Synthetic *O*-aryl glycosides with chromogenic or fluorogenic aglycons are invaluable substrates for the study of enzyme kinetics;[9–13] therefore, it comes as no surprise that the first synthesis of *O*-aryl glycoside dates back to 1879.[14] A distinct feature of aromatic glycosylation is the poor nucleophilicity of the phenolic oxygen in comparison to an aliphatic oxygen, rendering the glycosylation less efficient in the former cases. To combat this problem, different synthetic measures have been taken that include (1) S_N2 substitution of an anomeric leaving group with the phenolate under basic conditions;[15,16] (2) a Helferich glycosylation procedure with glycosyl acetates;[17–22] (3) Koenig–Knorr glycosylation with glycosyl halides;[23–27] and (4) oxidative condensation of glycosyl hemiacetals and phenol by the Mitsunobu reaction.[28–31] However, most of these methods suffer from some drawbacks that limit their wider application. For example, the harsh reaction conditions in Helferich glycosylation are not suitable for glycosides with fragile aryl glycosidic bonds,[17–22] inadequate selectivity demands tedious chromatography purification, and a large excess of phenol is sometimes required to boost the glycosylation yield.[28–31]

Because of the important role of synthetic aryl glycosides as enzyme substrates or inhibitors, a simple preparatory method is highly desired. Herein, we describe a straightforward synthesis of *O*-aryl α-glycosides from per-*O*-acetyl glycosyl imidates, and their application for the synthesis of fluorogenic 4-methylumbelliferyl α-T-antigen **1** (4-MU α-T-antigen). 4-MU α-T-antigen is widely used as synthetic substrate for *N*-acetyl α-galactosaminidases;[32–34] it comprises the cancer-related β-Gal*p*-(1→3)-Gal*p*NAc disaccharide (known as T-antigen)[35,36] connected with a 4-methylumbelliferyl aglycon (4-MU) via an α-glycosidic linkage (Fig. 1).

2. Results and discussion

2.1. Optimization of α-selective aromatic glycosylation

In a project connected with the kinetic study of α-L-fucosidases, we required chromogenic *p*-nitrophenyl α-L-fucopyranosyl

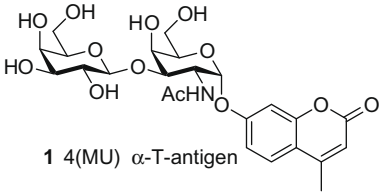

Figure 1. 4-MU α-T-antigen.

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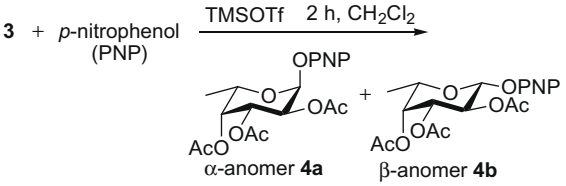
substrates. Based on a Helferich glycosylation procedure,[17,22] coupling of *p*-nitrophenol (PNP) with per-*O*-acetyl L-fucopyranosyl acetate in the presence of $ZnCl_2$ was attempted. Disappointingly, this reaction failed to provide a detectable product under various conditions. We attributed the failure to the harsh conditions, which would be likely damaging to the fragile α-fucosidic bond. A further literature survey revealed that various per-*O*-acetyl glucopyranosyl donors have been employed for the preparation of *O*-aryl α-glucopyranosides,[19,25,26] but the use of per-*O*-acetyl L-fucopyranosyl imidate for such purposes had not been exploited. Given its simplicity of preparation and reactive nature, it was worth exploring the use of 2,3,4-tri-*O*-acetyl-L-fucopyranosyl imidate as glycosyl donor for the synthesis of *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside. Thus, L-fucopyranose was converted to 1,2,3,4-tetra-*O*-acetyl-L-fucopyranose **2**,[37] which was subsequently transformed to imidate donor **3** by standard protocols (Scheme 1).[38] Imidate **3** (1 mol equiv) was then coupled with PNP (1.2 mol equiv). In contemplating a choice of glycosylation promoter, TMSOTf was preferred over $BF_3{\cdot}Et_2O$ because the use of the former was found to favor 1,2-*cis* α-glycosidic bond formation.[26]

To delineate the optimal conditions for reaction, *p*-nitrophenol was first glycosylated with imidate **3** using different amounts of TMSOTf (Table 1, entries 1–3). Glycosylation with either 0.05 or 0.5 mol equiv of TMSOTf at −20 °C resulted in a random anomeric mixture of *p*-nitrophenyl L-fucopyranosides **4a**[39] and **4b**[40] (Table 1, entries 1 and 2). Intriguingly, when 1.0 mol equiv of TMSOTf was used, **4a** was obtained as a single anomer in acceptable 56% yield (Table 1, entry 3). Assignment of 1,2-*cis* α-fucosidic configuration was based on the $^3J_{1,2}$ coupling constant of the anomeric proton (3.6 Hz for **4a** and 9.0 Hz for **4b**).

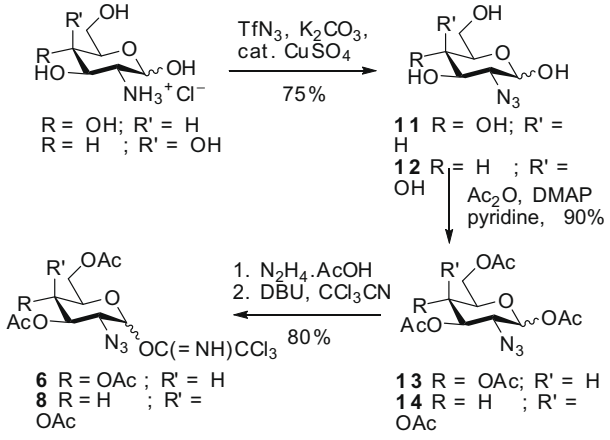
After exploring the optimal concentration of TMSOTf, we next investigated the effect of temperature on the stereochemical outcome of glycosylation (Table 1, entries 3–6). Glycosylation at 0 or 25 °C gave rise to a random anomeric mixture (Table 1, entries 5 and 6), while exclusive α-selectivity was obtained at either −20 or −40 °C (Table 1, entries 3 and 4). Thus, 1 mol equiv of TMSOTf and −20 °C reaction temperature were employed in subsequent glycosylations. It has been argued that the observed α-selectivity could be attributed to β→α anomerization under acidic conditions.[41,42] However, such rationale was ruled out in our case as anomerization to **4a** was not observed when pure β-anomer **4b** was exposed to the same reaction conditions.

Table 1
Glycosylation of PNP with 2,3,4-tri-*O*-acetyl-L-fucopyranosyl imidate **3** under different reaction conditions



Entry	TMSOTf (mol equiv)	*T* (°C)	**4a**[a] (%)	**4b**[a] (%)
1	0.05	−20	40	30
2	0.5	−20	22	30
3	1.0	−20	56	0
4	1.0	−40	54	0
5	1.0	0	26	26
6	1.0	25	22	22

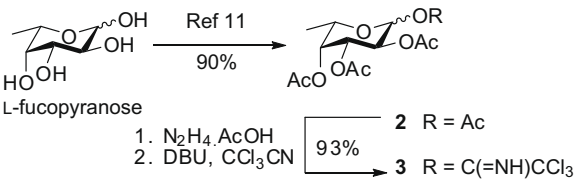
[a] Isolated yield.



Scheme 2. Preparation of 2-azido-2-deoxy-glycopyranosyl imidates **6** and **8**.

2.2. α-Aromatic glycosylation with different per-*O*-acetyl glycosyl imidates

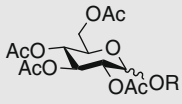
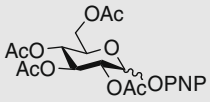
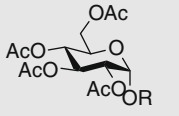
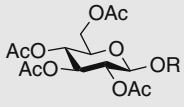
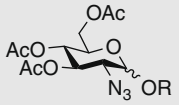
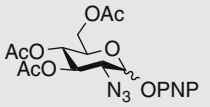
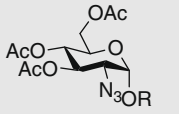
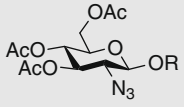
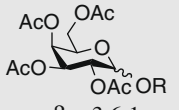
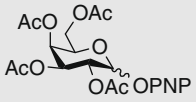
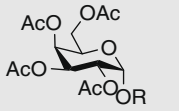
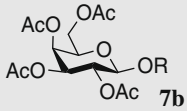
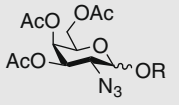
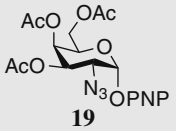
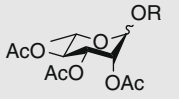
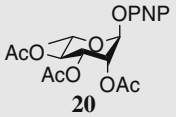
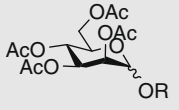
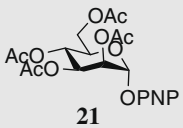
With the appropriate conditions for α-selective aromatic glycosylation in hand, we were prompted to examine the scope of glycosylation with different glycosyl imidates **5**–**10**. Thus, known per-*O*-acetyl glycosyl imidates **5**, **7**, **9**, and **10** were prepared according to literature procedures.[43–46] Syntheses of 2-azido-2-deoxy glycopyranosyl imidates **6** and **8** are outlined in Scheme 2. Hydrogen chloride salts of D-glucosamine and D-galactosamine were converted to 2-azido-2-deoxy glycosyl derivatives **11** and **12**,[47,48] which underwent standard procedures furnishing the desired glycosyl imidates **6**[49] and **8**.[50]



Scheme 1. Preparation of 2,3,4-tri-*O*-acetyl L-fucopyranosyl imidate **3**.

Glycosylation of PNP with the anomeric mixtures of glycosyl imidates **5**–**10** was examined (Table 2). As a comparison, α-glycosyl imidates **5a**, **6a**, **7a**, and β-glycosyl imidates **5b**, **6b**, **7b** were isolated by chromatography and were used for glycosylation studies (Table 2, entries 2, 3, 5, 6, 8, and 9). The α-selectivity of glycosylation with imidates in the D-*gluco* and D-*galacto* series spanned from modest (3.4:1 α- to β-anomer ratio for donor **5**) to α-exclusive (for donor **8**) (Table 2, entries 1–10). No significant difference in α-selectivity was noted using either the α- or β-glycosyl imidate as donor, although the use of β-anomer severely reduced the glycosylation yield (Table 2, entries 2 vs 3, 5 vs 6, and 8 vs 9). The α-selectivity obtained for glycosyl imidates bearing an axial C-4 acetyl function (**3**, **7**, and **8**) was generally higher than that obtained from donors with an equatorial C-4 acetyl function (**5** and **6**) (Table 2, entries 1 vs 7 and 4 vs 10). To aim at higher selectivity, the known α-directing solvent diethyl ether was introduced to form a co-solvent mixture for glycosylation, but no significant improvement was obtained (data not shown).[51] Exclusive α-selectivity observed for L-rhamnopyranosyl and D-mannopyranosyl imidates **9** and **10** was expected owing to the joined forces of

Table 2
Glycosylation of *p*-nitrophenol (PNP) with per-*O*-acetyl glycosyl imidates **5**–**10**

Entry	Glycosyl imidate R = C(=NH)CCl_3	Product	Yield (%)	α:β[a]	Ref.
1	 **5** α:β = 14:1	 **15**	51	3.4:1	54,55
2	 **5a**	**15**	50	3.4:1	–
3	 **5b**	**15**	20	3.8:1	–
4	 **6** α:β = 18:1	 **16**[b]	49	4.5:1	–
5	 **6a**	**16**	50	4.8:1	–
6	 **6b**	**16**	15	4.5:1	–
7	 **7** α:β = 3.6:1	 **18**	60	5.4:1	55
8	 **7a**	**18**	62	5.0:1	–
9	 **7b**	**18**	30	5.6:1	–
10	 **8**	 **19**	60	α nly	–
11	 **9**	 **20**	45	α nly	56
12	 **10**	 **21**	55	α nly	57

[a] Anomeric ratio was determined by HPLC analysis of crude anomeric mixture (elution with 7:3 hexane–EtOAc at 1.2 mL/min over Mightysil Si 60 250–4.6).
[b] Anomeric mixture **16** was deacetylated to give α-anomer **17** for NMR characterization.

anchimeric assistance and anomeric effect (Table 2, entries 11 and 12).[52,53]

2.3. Mechanistic aspect

Based on literature findings and the current experimental data, we propose a mechanism to account for the α-selective aromatic glycosylation (Fig. 2).[25] In the presence of molar equiv of TMSOTf, the glycosyl imidate is completely converted to oxocarbenium ion, which is in association with triflate counterion to form an oxocarbenium-triflate ion pair. For oxocarbenium ions having a C2-acetyl function, neighboring-group participation resulted in an equilibrium mixture of oxocarbenium and acetoxonium ions. It is generally accepted that nucleophilic attack of ordinary acceptor at acetoxonium ion produces the β-glycoside. Nevertheless, for weakly nucleophilic phenols (especially those having electron-withdrawing groups), nucleophilic attack occurred predominantly on the more reactive oxocarbenium–triflate ion pair furnishing a mixture of α- and β-glycosyl oxonium intermediates.[25] Owing to the anomeric effect, the α-glycosyl oxonium ion is more stable than β-glycosyl oxonium ion; thus, formation of the former would be favored based on a product-development control mechanism.[58] In addition, the higher α-selectivity obtained from imidate donor **3**, **7,** or **8** compared to that obtained from imidate donor **5** or **6** should be attributed to the extra stability arising from through-space electrostatic interaction between the axially disposed C-4 acetyl function and ring oxygen atom of the corresponding α-glycosyl oxonium ion.[59] However, validation of the mechanism demands elaborative studies and molecular simulation, which is beyond the scope of present study.

2.4. Synthesis of 4-MU α-T-antigen

To demonstrate the synthetic utility of this α-selective aromatic glycosylation, the synthesis of 4-MU α-T-antigen **1** was attempted. A synthetic challenge for preparation of 4-MU α-T-antigen **1** is the construction of α-aryl glycosidic linkage.[23] Recently, Kiso reported the synthesis of **1** by employing Mitsunobu condensation of an excess of 4-MU (8 equiv) with a 4,6-*O*-di-(*t*-butylsilylidene)-protected disaccharide donor to obtain a less perfect 9:1 α- to β-anomeric mixture of the desired aryl glycoside.[31] With the present glycosylation method in hand, we were confident to tackle this synthetic challenge. Thus, 1.2 mol equiv of 4-MU **22** was coupled with glycosyl imidate **8** to afford 4-MU 2-azido-2-deoxy-α-D-galactopyranoside **23** as a single anomer in 70% yield (Scheme 3). It should be noted that Lemieux reported the synthesis of **23** from the corresponding chloride donor in 33% yield along with 10% β-anomer; thus, our method gave far better result than the previous protocol.[23] Support for the α-glycosidic configuration in **23** was provided by NMR spectroscopy (α-anomer of **23**: ^{1}H, δ = 5.70, $^3J_{1,2}$ = 3.6 Hz; ^{13}C, δ = 97.4). For comparison, the β-anomer of **23**[23] was also prepared by a known phase-transfer catalyzed glycosylation (For β-anomer of **23**: ^{1}H, δ = 5.02, $^3J_{1,2}$ = 7.8 Hz; ^{13}C, δ = 100.5).[60]

Upon deacetylation and benzylidenation, derivative **23** was transformed into galactosaminyl acceptor **24**, which was then glycosylated with galactopyranosyl imidate **7** to give the fully protected 4-MU α-T-antigen **25** in 75% yield. Conversion of **25** to the target 4-MU α-T-antigen **1** was accomplished by standard deprotection. The overall yield of the fully protected 4-MU α-T-antigen **25** from galactosamine·HCl is 21%, which is comparable to that reported by Kiso.[31] However, the present method offers the attractiveness in that only a simple per-*O*-acetyl glycosyl imidate donor is required to react with near stoichiometric amount of a phenol and yet exclusive formation of an aryl α-glycosidic bond is realized.

In summary, we have developed a α-selective aromatic glycosylation for direct access of *O*-aryl α-glycopyranosides. The attractive feature of this method is the use of simple per-*O*-acetyl glycosyl imidates to achieve a respectable level of α-selectivity without resorting to special protecting functions. The synthetic utility of the method was demonstrated by a concise and stereoselective synthesis of a cancer-related fluorogenic 4-MU α-T-antigen **1**. Owing to the prevalent use of aryl glycosides as enzyme substrates in biological studies, the described method should be valuable for their synthesis. Work on the preparation of aryl α-L-fucosides and their subsequent use in the study of human α-L-fucosidase is underway and the results will be reported in due course.

3. Experimental

3.1. General experimental

Chemicals used in experiments were purchased as ACS reagent grade and were used without further purification. Hexane and EtOAc for HPLC elution were purchased as HPLC reagent grade and was degassed by ultrasonication before use. Molecular sieves (MS-4 Å) were activated by microwave irradiation and flame dried intermediately before use. ACS reagent grade CH_2Cl_2 was distillated over calcium hydride under N_2. Amberlite IR-120 H^+ was washed sequentially with deionized H_2O and MeOH, followed by drying in vacuo for 18 h before use. Normal phase and reverse phase flash column chromatography were performed on Silica Gel 60 (70–230 mesh) and 75 μM RP-C18 silica gel, respectively. ^{1}H and ^{13}C NMR spectra of the prepared compounds were recorded either with Bruker 300 MHz and 75 MHz or with Inova 500 MHz and 125 MHz spectroscopies. Chemical shifts (δ ppm) are measured against TMS, generated from the residual $CHCl_3$ lock signal at δ 7.26 ppm, against the residual proton signal of deuterated chloroform, and the ^{13}C resonance signal is calibrated against the ^{13}C signal of deuterated chloroform. HPLC analysis was performed with gradient pump of Hitachi L-2130 and UV-detector L-2400.

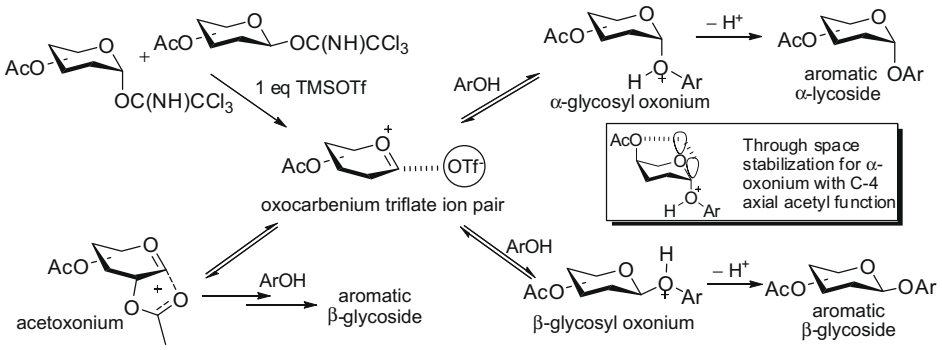
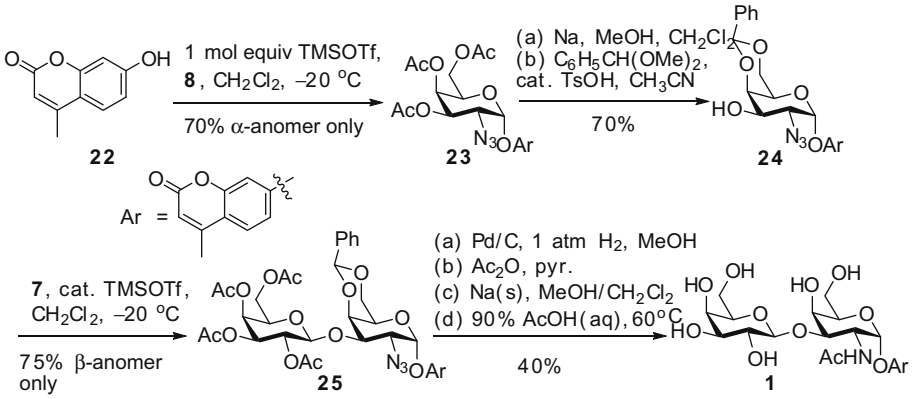


Figure 2. Proposed mechanism for α-selective aromatic glycosylation.



Scheme 3. Stereospecific synthesis of 4-MU α-T-anitgen **1**.

3.2. *p*-Nitrophenyl 2,3,4-tri-*O*-acetyl-L-fucopyranosides 4

To a solution of 2,3,4-tri-*O*-acetyl L-fucopyranosyl trichloroacetimidate **3**[44] (100 mg, 0.23 mmol) in CH_2Cl_2 (2.0 mL), phenol (1.2 mol equiv) and MS-4 Å (300 mg) were added. Upon further addition of TMSOTf (40 μL, 0.23 mmol), the mixture was stirred at −20 °C under N_2. Complete glycosylation was assessed by TLC and the reaction mixture was diluted with CH_2Cl_2 (10 mL). After filtration removal of MS-4 Å, the CH_2Cl_2 solution was washed with sat. $NaHCO_3$ soln (20 mL × 2), water (20 mL × 1), and brine (20 mL × 1), dried over $MgSO_4$, filtered, and concentrated for column chromatography over silica gel to furnish *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-L-fucopyranosides **4**. For *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranoside **4a** (53.0 mg, 56%):[39] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22–8.19 (m, 2H), 7.17–7.13 (m, 2H), 5.86 (d, *J* = 4.0 Hz, 1H), 5.58 (dd, *J* = 6.0, 12.0 Hz, 1H), 5.37 (ddd, *J* = 6.0, 6.0, 12.0 Hz, 2H), 4.29 (q, *J* = 7.0, 14.0 Hz, 1H), 2.19 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 1.21 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.67, 170.65, 170.3, 161.3, 143.0, 126.1, 116.5, 94.9, 70.7, 67.8, 67.6, 66.3, 20.9, 20.9, 20.8, 16.0. HRMS FAB (*m*/*z*) $(M+H)^+$ calcd for $C_{18}H_{21}NO_{10}$ 412.1244, found 412.1245.

3.3. *p*-Nitrophenyl per-*O*-acetyl glycopyranosides 15–21

To a solution of per-*O*-acetyl glycopyranosyl trichloroacetimidates **5**–**10** (100 mg, 1.0 mol equiv) in CH_2Cl_2 (2.0 mL), *p*-nitrophenol (1.2 mol equiv) and MS-4 Å (300 mg) were added at −20 °C. Upon addition of TMSOTf (1.0 mol equiv), the mixture was stirred at −20 °C under N_2 for 0.5–2 h and progress of reaction was monitored by TLC. Upon complete glycosylation, CH_2Cl_2 (10 mL) was added to the mixture, and the organic phase was washed with satd $NaHCO_3$ soln (20 mL × 2), water (20 mL × 1), and brine (20 mL × 1), dried over $MgSO_4$, filtered, and concentrated for column chromatography purification over silica gel. Elution with EtOAc–hexane mixture afforded the respective α- and β-anomers of the titled *p*-nitrophenyl per-*O*-acetyl glycopyranosides **15**, **18**–**21**. To purify **16**, the crude anomeric mixture of glycoside **16** (50 mg, 0.11 mmol) was dissolved in MeOH (2.0 mL) at 0 °C, which was followed by the addition of a piece of freshly cut sodium (ca. 20 mg). The mixture was stirred from 0 °C to rt for 2 h and then neutralized with IR-120 H^+. After filtration removal of resin and removal of solvent by rotary evaporator, the crude deacetylated product was purified by column chromatography over silica gel with 1:20 v/v MeOH–CH_2Cl_2 elution to give the α-anomer **17** as white amorphous solid (29.0 mg, 80%).

3.3.1. *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside 15a

(36 mg, 38%):[54] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.20 (m, 2H), 7.22–7.19 (m, 2H), 5.85 (d, *J* = 3.6 Hz, 1H), 5.72 (t, *J* = 9.9 Hz, 1H), 5.20 (t, *J* = 10.2 Hz, 1H), 5.09 (dd, *J* = 3.6, 10.2 Hz, 1H), 4.26 (dd, *J* = 4.5, 12.3 Hz, 1H), 4.05–4.00 (m, 2H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 170.07, 170.03, 169.4, 143.0, 125.7, 116.4, 94.0, 69.9, 69.5, 68.5, 67.8, 61.2, 20.58, 20.54, 20.50, 20.4. HRMS ES (*m*/*z*) $(M+H)^+$ calcd for $C_{20}H_{23}NO_{12}$ 470.1299, found 470.1308.

3.3.2. *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside 15b

(12 mg, 13%):[55] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.19 (m, 2H), 7.26–7.04 (m, 2H), 5.47–5.07 (m, 3H), 4.31–3.90 (m, 4H), 2.09 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 170.1, 169.3, 169.1, 143.2, 125.7, 116.5, 97.9, 72.4, 72.3, 70.8, 67.9, 61.7, 20.6, 20.59, 20.55, 20.52.

3.3.3. *p*-Nitrophenyl 2-azido-2-deoxy-α-D-glucopyranoside 17

(29.0 mg, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24 (dd, 2H), 7.31 (dd, 2H), 5.76 (d, *J* = 3.6 Hz, 1H), 4.05 (dd, *J* = 8.4, 10.2 Hz, 1H), 3.72–3.68 (m, 2H), 3.64–3.61 (m, 1H), 3.56–3.55 (m, 1H), 3.41–3.35 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.8, 143.1, 125.7, 116.9, 97.3, 74.4, 71.7, 70.4, 63.2, 61.0; HRMS ES (*m*/*z*) $(M+Na)^+$ calcd for $C_{12}H_{14}N_4O_7$ 349.0760, found 349.0760.

3.3.4. *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside 18a

(49.0 mg, 51%):[55] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25–8.19 (m, 2H), 7.20–7.15 (m, 2H), 5.89 (d, *J* = 3.6 Hz, 1H), 5.58–5.51 (m, 2H), 5.33 (dd, *J* = 3.6, 10.2 Hz, 1H), 5.27 (t, *J* = 6.0 Hz, 1H), 4.14–4.02 (m, 2H), 2.17 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 1.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.4, 170.3, 170.2, 170.1, 160.8, 143.0, 125.8, 116.5, 94.6, 67.7, 67.5, 67.3, 67.1, 61.3, 20.68, 20.63, 20.59, 20.54.

3.3.5. *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside 18b

(8.0 mg, 9%):[55] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24–8.21 (m, 2H), 7.15–7.08 (m, 2H), 5.50 (d, *J* = 3.6 Hz, 1H), 5.20 (d, *J* = 8.1 Hz, 1H), 5.16 (dd, 1H), 4.23–4.11 (m, 4H), 2.20 (s, 3H, AcO), 2.08 (s, 6H, AcO), 1.94 (s, 3H, AcO).

3.3.6. *p*-Nitrophenyl 3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranoside 19

(55.0 mg, 60%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25–8.20 (m, 2H), 7.26–7.18 (m, 2H), 5.73 (d, *J* = 3.6 Hz, 1H), 5.58–5.51 (m, 2H), 5.26

(t, J = 6.6 Hz, 1H), 4.12–4.06 (m, 2H), 3.93 (dd, J = 3.6, 10.8 Hz, 1H), 2.19 (s, 3H), 2.15 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 170.2, 170.1, 161.0, 143.7, 126.2, 117.2, 97.2, 68.6, 68.4, 67.4, 61.6, 57.5, 21.0, 20.97, 20.93. HRMS ES (m/z) $(M)^+$ calcd for $C_{18}H_{20}N_4O_{10}$ 452.1179, found 452.1195.

3.3.7. *p*-Nitrophenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside 20

(43.0 mg, 45%):[56] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21–8.16 (m, 2H), 7.18–7.13 (m, 2H), 5.55 (d, J = 3.6 Hz, 1H), 5.54–5.41 (m, 2H), 5.18 (t, J = 9.9 Hz, 1H), 3.90–3.85 (m, 1H), 2.20 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.19 (d, J = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 169.9, 169.7, 160.3, 142.8, 125.7, 116.2, 95.4, 70.4, 69.4, 69.1, 68.4, 20.7, 20.64, 20.60, 17.3; HRMS ES (m/z) $(M)^+$ calcd for $C_{18}H_{21}NO_{10}$ 411.1165, found 411.1168.

3.3.8. *p*-Nitrophenyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside 21

(52.0 mg, 55%):[57] ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25–8.19 (m, 2H), 7.25–7.17 (m, 2H), 5.62 (d, J = 3.6 Hz, 1H), 5.55 (dd, J = 3.3, 9.9 Hz, 1H), 5.46 (dd, J = 3.6, 9.9 Hz, 1H), 5.38 (t, J = 9.9 Hz, 1H), 4.30 (dd, J = 5.4, 12.0 Hz, 1H), 4.12–3.97 (m, 2H), 2.24 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 169.9, 169.6, 160.0, 143.0, 125.8, 116.4, 95.6, 69.7, 68.8, 68.4, 65.5, 61.8, 20.8, 20.7, 20.63, 20.60; HRMS ES (m/z) $(M+H)^+$ calcd for $C_{20}H_{23}NO_{12}$ 470.1299, found 470.1301.

3.4. 4-Methylumbelliferyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranoside 23[23]

To a mixture of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate **8** (600 mg, 1.26 mmol), 4-methylumbelliferone **22** (270 mg, 1.51 mmol), and MS-4 Å (1.20 g) in CH_2Cl_2 (5.0 mL), TMSOTf (220 μL, 1.26 mmol) was added. Upon stirring at −20 °C for 8 h under N_2, the reaction mixture was diluted with CH_2Cl_2 (10 mL). The resulting CH_2Cl_2 solution was washed with satd $NaHCO_3$ soln (20 mL × 2), water (20 mL × 1), and brine (20 mL × 1), dried over $MgSO_4$, filtered, and then concentrated for column chromatography purification over silica gel. Elution with a 1:6 v/v EtOAc–hexane mixture provided **23** as a yellowish oily liquor (430 mg, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, 1H), 7.11 (s, 1H), 7.04 (d, 1H), 6.19 (s, 1H), 5.70 (d, J = 3.6 Hz, 1H), 5.57–5.41 (m, 2H), 4.30 (t, J = 3.6 Hz, 1H), 4.12–4.05 (m, 2H), 3.90 (dd, J = 8.7, 10.8 Hz, 1H), 2.47 (s, 3H), 2.19 (s, 3H), 2.11 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.3, 170.2, 161.2, 159.0, 155.2, 152.7, 126.2, 115.9, 114.3, 113.5, 104.7, 97.4, 68.5, 68.2, 67.5, 61.6, 57.5, 21.0, 20.98, 20.9, 19.0.

3.5. 4-Methylumbelliferyl 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside 24

To a MeOH solution (2.0 mL) of **23** (100 mg, 0.20 mmol), a piece of freshly cut sodium was added and the mixture was stirred initially at 0 °C before being gradually brought up to room temperature. Upon complete deacetylation as assessed by TLC (ca. 2 h), the reaction mixture was neutralized with IR-120 H^+, which was subsequently removed by filtration. After removal of MeOH by rotary evaporator and drying in vacuo for couple of hours, the crude product was re-suspended in CH_3CN (2.0 mL), to which benzaldehyde dimethyl acetal (45 μL, 0.3 mmol) and *p*-toluenesulfonic acid monohydrate (TsOH, 4.0 mg, 0.02 mmol) were added. After stirring at rt for 4 h, the mixture was diluted with CH_2Cl_2 (6.0 mL), and the resulting solution was washed with satd $NaHCO_3$ soln (10 mL × 2), water (10 mL × 1), and brine (10 mL × 1), dried over $MgSO_4$, filtered and concentrated for column chromatography purification over silica gel. Elution with a 1:1 v/v EtOAc–hexane mixture afforded **24** as white amorphous powder (68.0 mg, 70% over two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56–7.51 (m, 3H, ArH), 7.41–7.38 (m, 3H, ArH), 7.11–7.05 (m, 2H, ArH), 6.19 (s, 1H, C=CH), 5.76 (d, J = 3.6 Hz, 1H, H-1), 5.62 (s, 1H), 4.42–4.39 (m, 2H, H-4, H-5), 4.28 (d, J = 12.6 Hz, 1H, H-6), 4.09 (d, J = 12.9 Hz, 1H, H-6′), 3.85–3.81 (m, 2H, H-2, H-3), 2.41 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.3, 159.3, 155.2, 152.7, 137.4, 129.8, 128.7, 126.6, 126.2, 115.7, 113.6, 113.4, 104.7, 101.6, 97.8, 75.5, 69.3, 67.8, 64.3, 60.5, 53.8, 19.1. HRMS ES (m/z) $(M+H)^+$ calcd for $C_{23}H_{21}N_3O_7$ 452.1458, found 452.1452.

3.6. 4-Methylumbelliferyl 2-azido-4,6-di-*O*-benzylidene-2-deoxy-3-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside 25

To a mixture of 2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl trichloroacetimidate **7** (108.0 mg, 0.22 mmol), (4-MU) 2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside **24** (50 mg, 0.11 mmol) and MS-4 Å (300 mg) in CH_2Cl_2 (2.0 mL), TMSOTf (4.0 μL, 0.022 mmol) was added and the mixture was stirred at −20 °C for 2 h. The reaction mixture was then diluted with CH_2Cl_2 (6.0 mL), which was washed with satd $NaHCO_3$ soln (10 mL × 2), water (10 mL × 1), and brine (10 mL × 1), dried over $MgSO_4$, filtered, and concentrated for column chromatography purification over silica gel. Elution with 1:1 v/v EtOAc–hexane elution afforded the titled compound **25** as white amorphous powder (65.0 mg, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57–7.54 (m, 3H), 7.40–7.38 (m, 3H), 7.10–7.07 (m, 2H), 6.19 (s, 1H), 5.80 (d, J = 3.6 Hz, 1H), 5.60 (s, 1H), 5.44–5.30 (m, 2H), 5.10 (dd, J = 3.0, 10.2 Hz, 1H), 4.91 (d, J = 7.8 Hz, 1H), 4.50 (br, 1H), 4.35–3.99 (m, 7H), 3.79 (br, 1H), 2.41 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 170.6, 170.50, 169.8, 161.2, 159.3, 155.3, 152.5, 137.7, 129.4, 128.6, 126.4, 126.3, 115.8, 113.5, 113.3, 105.0, 102.9, 101.0, 98.0, 76.2, 75.8, 71.4, 71.3, 69.3, 69.0, 67.3, 64.6, 61.7, 58.8, 21.15, 21.10, 20.97, 20.90, 19.0. HRMS ES (m/z) $(M)^+$ calcd for $C_{37}H_{39}N_3O_{16}$ 781.2330, found 781.2299.

3.7. 4-Methylumbelliferyl (4-MU) 2-acetamido-2-deoxy-3-(β-D-galactopyranosyl)-α-D-galactopyranoside 1[31]

A mixture of **25** (36.0 mg, 0.046 mmol) and 5% Pd–C (36.0 mg) in MeOH (2.0 mL) was stirred at rt under 1 atm H_2 for 2 h. The reaction mixture was then filtered through Celite and the filtrate was concentrated by rotary evaporator. The crude residue was re-suspended in a mixture of pyridine (1.0 mL) and Ac_2O (1.0 mL), and then stirred at rt for 1 h. Excess solvent and reagent were removed by rotary evaporator, and the residue was dissolved in MeOH (2.0 mL), to which a piece of freshly cut sodium (ca. 20 mg) was added. The resulting mixture was stirred for 2 h from 0 °C to rt and followed by neutralization with IR-120 H^+ resin. After filtration removal of resin, MeOH was reduced by rotary evaporator. The crude residue was then re-suspended in 90% aqueous acetic acid (1.0 mL) and stirred at 60 °C for 1 h. Upon complete deacetylation as assessed by TLC, the solution was concentrated by rotary evaporator for column chromatography purification over reversed phase RP-C18 silica gel with MeOH–H_2O mixture elution (gradient from 25% to 30% MeOH) to afford the 4-MU α-T-antigen **1** (10 mg, 40% over four steps) as white glassy solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.79 (d, 1H), 7.26–7.20 (m, 2H), 6.26 (d, 1H), 5.73 (d, J = 3.6 Hz, 1H), 4.71 (dd, J = 3.6, 11.1 Hz, 1H), 4.61 (d, J = 6.9 Hz, 1H), 4.33 (d, J = 2.4 Hz, 1H), 4.25 (dd, J = 3.0, 11.1 Hz, 1H), 3.99 (t, J = 5.7, 11.1 Hz, 1H), 3.93 (d,

J = 3.0 Hz, 1H) 3.83–3.79 (m, 3H), 3.74–3.70 (m, 2H), 3.60–3.56 (m, 2H), 2.50 (d, 3H), 2.06 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.2, 163.2, 161.4, 156.0, 155.4, 127.3, 116.2, 115.0, 112.9, 106.4, 105.5, 98.3, 78.4, 76.8, 74.7, 73.5, 72.5, 71.4, 70.3, 69.7, 62.6, 62.4, 22.7, 18.6. HRMS ES (m/z) $(M + Na)^+$ calcd for $C_{24}H_{31}NO_{13}$ 564.1693, found 564.1689.

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Supplementary data

^{1}H and/or ^{13}C NMR spectra for the compounds **1**, **4**, **5a**, 5b (unstable), **6a**, 6b (unstable), **7a**, **7b**, **15**, **17**, **18**–**21**, α-anomer **23a**, β-anomer **23b**, 24 (including ^{1}H -^{1}H COSY 2D NMR spectrum) and **25** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.12.013.

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