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An efficient conversion of *N*-acetyl-D-glucosamine to *N*-acetyl-D-galactosamine and derivatives

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ABSTRACT

2-Acetamido-2-deoxy-D-galactose (D-GalNAc) is an important monosaccharide widely distributed in nature. However, unlike its 4-epimer, the 2-acetamido-2-deoxy-D-glucose (D-GlcNAc), D-GalNAc is very expensive to obtain from commercial sources. Herein we report an efficient transformation that allows for the conversion of D-GlcNAc to a D-GalNAc derivative **7** in three steps and in 58.4–75% overall yields. The process was carried out on a greater than 20-g scale without the need of chromatography. The versatility of compound **7** was demonstrated by the synthesis of several useful monosaccharides and thiodisaccharides containing a D-GalNAc residue.

1. Introduction

Glycosides containing 2-acetamido-2-deoxyhexoses are widely distributed in nature.[1,2] Two amino sugars, 2-acetamido-2-deoxy-D-glucose (D-GlcNAc) and the 2-acetamido-2-deoxy-D-galactose (D-GalNAc), are the ones most commonly found. As the constituents of glycoproteins, glycolipids, and polysaccharides, they play many important roles in biology.[1] However, unlike D-GlcNAc which can be readily obtained by the hydrolysis of chitin, D-GalNAc is very expensive to obtain from commercial sources. There has been considerable interest in developing viable strategies to convert D-GlcNAc to D-GalNAc (Fig. 1).

The common strategy involves a selective protection of OH groups at C-1, C-3, and C-6 of D-GlcNAc, leaving the OH-4 unprotected for subsequent epimerization. Since the first attempt reported by Gross et al. in 1967,[3] several approaches have been published in the literature.[4,5] These can be classified into two strategies by comparing the protective group used for the anomeric center. The first involved the use of an alkyl group to protect the anomeric OH group.[3,5] Methyl or benzyl groups were commonly used for this purpose. This strategy usually suffers from long reaction sequences, leading to poor overall yields. Since the anomeric position was protected with a stable group, it was difficult to derivatize the anomeric center further. The second involved the use of an acyl group to protect the anomeric center.[4] This strategy is the most desirable as the three OH groups at C-1, C-3, C-6 can be selectively protected in one step to provide an intermediate with a 4-OH group that can be further activated. One additional advantage is that the acyl group at the anomeric center can be removed and replaced with other groups in a relatively easy manner to allow further transformations. However, this strategy has usually suffered from poor regioselectivity that required tedious separation of other unwanted by-products. Chaplin et al.[4a] reported an elegant enzymatic method to selectively remove a 6-*O*-acetyl group from the peracetylated D-GlcNAc to furnish an intermediate with a free 6-OH group. A subsequent acid treatment resulted in a migration of the acetyl group from O-4 to O-6 to generate the desired derivative with a free 4-OH group. The authors subsequently synthesized the 4-triflate, which was displaced with cesium acetate to obtain the peracetylated D-GalNAc. The entire

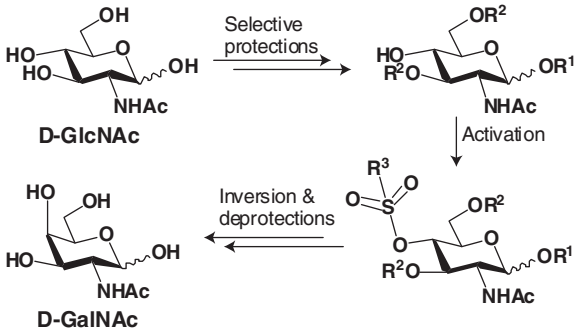

Figure 1. Common strategies to convert D-GlcNAc to D-GalNAc.

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process took six steps from D-GlcNAc to D-GalNAc. Here we report an efficient three-step synthesis of a D-GalNAc intermediate (**7**, see Scheme 1) from D-GlcNAc using pivalate as a protecting group. Our methodology is suitable for both small and large scales and provides a versatile D-GalNAc intermediate that is conveniently protected for further transformations.

2. Results and discussion

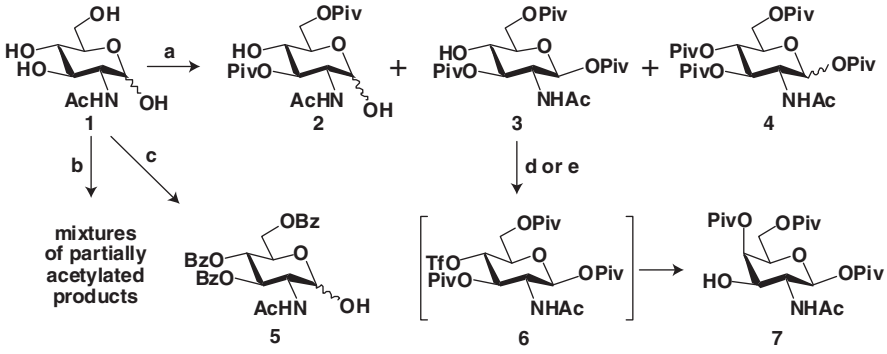
The 3,6-dipivalation of D-GlcNAc glycosides has been reported before.[5a–c,f] In general, the reaction was very selective to afford the corresponding 3,6-dipivalate in high selectivity and high yield. This was usually followed by a triflation of the 4-OH group, and subsequently an inversion of the C-4 center to provide the corresponding D-GalNAc glycoside. Usually, in the formed D-GalNAc glycoside, the 3-*O*-pivaloyl group was observed to undergo a migration to the neighboring O-4 position;[5a,b,f] in one report, Jacquinet's group[5c] also observed the formation of a mixture of benzyl D-GalNAc glycosides containing the non-migrated 3,6-dipivalate as well as the migrated 3,4-dipivalate.

For D-GlcNAc, which has a unprotected anomeric center, its selective pivalation has also been studied. In 1988, Ljevaković et al.[6] reacted D-GlcNAc with 4.8 equiv of pivaloyl chloride in anhydrous pyridine, and obtained a complex mixture containing the 1,3,6-tripivalate (28% yield) and the 1,3,4,6-tetrapivalate (7.5% yield), together with the 3,4,6-tripivalate (6.7%) and the 3,6-dipivalate (21% yield). All compounds were obtained as an α/β-mixture. In another attempt, Santoyo-González's group[4b] prepared a very mild pivalation reagent, the *N*-pivaloylimidazole, and reacted 4 equivalents of the reagent with D-GlcNAc in DMF at 55 °C for 18 h; two partially protected pivalates: the 1,4,6- and 1,3,6-tripivalate were obtained in 28% and 61% yield, respectively, together with the 1,3,4,6-tetrapivalate, which was obtained in 10% yield. The 1,3,6-tripivalated product was subsequently converted on a small scale to a D-GalNAc derivative containing a 4-OH group.

We attempted to repeat the pivalation of D-GlcNAc using pivaloyl chloride as the reagent (Scheme 1). However, under slightly modified conditions, we obtained different results compared to those of Ljevaković et al. The D-GlcNAc was first stirred in a 1:2 mixture of anhydrous pyridine–dichloromethane overnight; after cooling to −10 °C, pivaloyl chloride (3.05 equiv) was added, and the reaction was stirred at room temperature for two days. We observed the formation of a major product with R_f 0.40 (95:5 CH_2Cl_2–MeOH,), together with some less polar products at R_f 0.59 and 0.50. Another more polar product that had an R_f 0.25 was also obtained. The major product was isolated in pure form in 79.5% yield by column chromatography, and it was characterized as the β anomer of the 1,3,6-tripivalate of D-GlcNAc **3**, as shown by NMR studies. The two less polar compounds, isolated in 8.2% yield, were found to be α/β anomers of 1,3,4,6-tetrapivalate **4**. The most polar compound was found to be the 3,6-dipivalate **2**, which was isolated in small amounts (3.1% yield). The reaction could be reproduced in a 20-g scale. We found that compound **2** had a significant solubility in water, as the majority of **2** formed during the reaction were removed by water extraction during the workup.

We attempted to apply the same conditions to the acetylation and benzoylation of D-GlcNAc (Scheme 1), using 3.05 equiv of either acetyl chloride or benzoyl chloride as a reagent. The acetylation gave a complex mixture, while the benzoylation led to the formation of a major product at R_f 0.37 (95:5 CH_2Cl_2–MeOH) that could be isolated in 48.6% yield after a column chromatography on silica gel. NMR spectroscopy confirmed that the major benzoylated product was 3,4,6-tribenzoylated D-GlcNAc **5**. Clearly, the pivalation of D-GlcNAc was the most selective. The differences in the three acylations could be explained by their relative reactivities: the least reactive and most bulky reagent (pivaloyl chloride) provided the highest regioselectivity, while the most reactive but least bulky reagent (acetyl chloride) exhibited the poorest regioselectivity.

The discovery that compound **2** had a significant solubility in water was very important for the development of a practical synthesis for D-GalNAc from D-GlcNAc, as this indicated that after removing compound **2** from the reaction mixture, it should be possible to carry the crude product on to the next step without the need of chromatographic separation. In addition, between the remaining compounds **3** and **4**, since only **3** had an alcohol functionality, the mixture could be used for activation. Indeed, after the pivalation and working up of the reaction mixture as above, the crude product was directly esterified with triflic anhydride at −10 °C in a 1:1 mixture of anhydrous pyridine–dichloromethane to afford the triflate **6**, which was detected by thin-layer chromatography. Water was added to the reaction mixture after 2 h at 0 °C, and the reaction was continued at 60 °C overnight. A major product with R_f 0.28 (95:5 CH_2Cl_2–MeOH) was obtained. At a 5-g scale, we isolated the major compound by recrystallization from a mixture of ethyl acetate–hexane in 75% overall yield from D-GlcNAc. When the reaction was carried out at a 20-g scale, we found that the major product could also be isolated by crystallization, although in slightly lower yield (58.4% for three steps); the highest yield was obtained by chromatography on silica gel (62.7%) using 24% ethyl acetate–hexane as the eluent. The structure of the major product was confirmed to be compound **7** by NMR studies and high-resolution mass spectrometry. The migration of the pivaloyl group from O-3 to O-4 was consistent with the previous finding on the glycosides.[5a,b,f] According to the literature reports,[7,8] the displacement of the 4-triflate in **6** was most probably the result of an intramolecular nucleophilic attack by the carbonyl oxygen of the



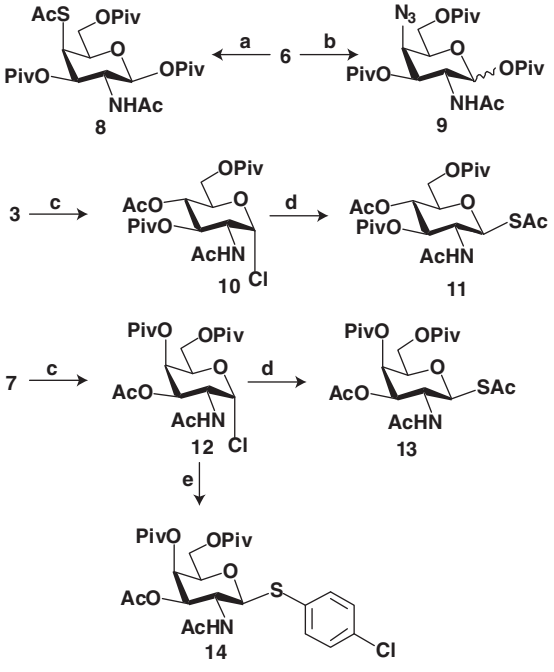
Scheme 1. Selective acylations of D-GlcNAc and the conversion of D-GlcNAc to D-GalNAc. Reagents and conditions: (a) $(CH_3)_3CCOCl$ (3.5 equiv)/CH_2Cl_2-Py (2:1), −10 °C→rt, two days; (b) AcCl (3.05 equiv)/CH_2Cl_2-Py (2:1), −10 °C→rt, two days; (c) PhCOCl (3.05 equiv)/CH_2Cl_2-Py (2:1), −10 °C→rt, two days; (d) Tf_2O/CH_2Cl_2-Py (1:1), −10→0 °C, 2 h; (e) Tf_2O/CH_2Cl_2-Py (1:1), −10→0 °C, 2 h; then H_2O, 60 °C, overnight.

neighboring 3-pivalate to form an orthoacid, which was in the end hydrolyzed to regioselectively place the pivaloyl group at the O-4 position.

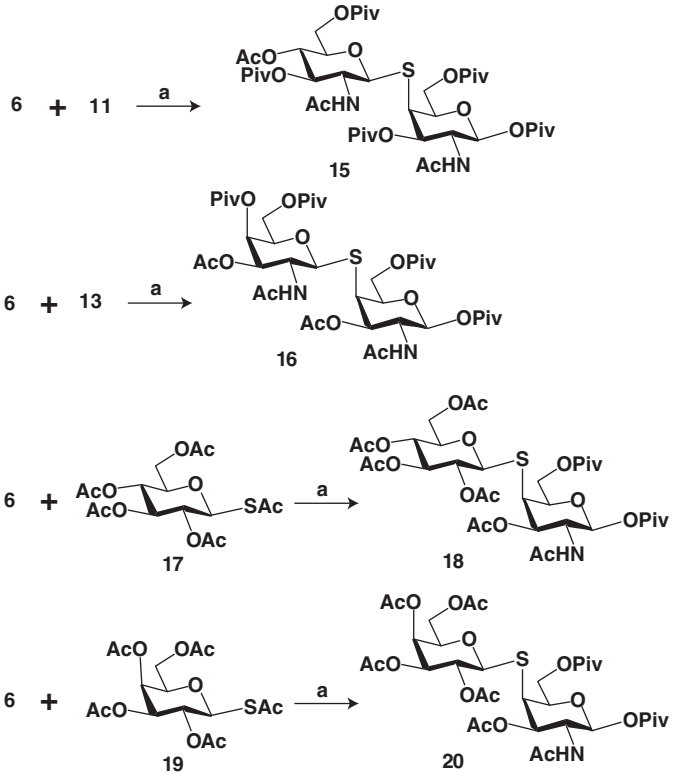
Compound **7** had a free alcohol at C-3 and an ester group at the anomeric center. This suggested that it could be a very useful synthetic intermediate. To demonstrate the versatility of compound **7** as well as other intermediates, we carried out the syntheses of some mono- and disaccharides containing GalNAc.

As shown in Scheme 2, the crude triflate **6** was subjected to S_N2 nucleophilic substitutions with different nucleophiles. When potassium thioacetate was used, compound **8,** which had a galacto configuration, was obtained in 88% from D-GlcNAc, and when sodium azide was used, the 4-azido compound **9** was obtained in 77% yield over two steps. The labile anomeric pivalate in both compounds **3** and **7** was converted to a glycosyl chloride by the treatment with acetyl chloride to furnish the corresponding α-glycopyranosyl chlorides **10** and **12** in almost quantitative yields, according to the proton NMR spectra of the crude materials. In both cases, the free hydroxyl groups that existed in the starting materials were acetylated in situ. The α-chlorides **10** and **12** were converted to the corresponding β-thioacetate **11** and **13** in very good yields. Additionally, we also converted **12** to the *p*-chlorophenyl β-thioglycoside **14** in 62.8% yield. The stable thioglycoside **14** could be derivatized further for glycosylations.[9]

With several functionalized monosaccharides in hand, we proceeded to synthesize four disaccharides containing a GalNAc unit (Scheme 3). The coupling of 4-triflate **6** with **11** in DMF provided disaccharide **15** in 63.4% yield using diethylamine as a base.[10] Compound **15** is a thio analog of the βGlcNAc-(1→4)-GalNAc sequence found in the lipopolysaccharide (LPS) expressed by *Escherichia coli* O48:H21.[11] Similarly, the coupling of 4-triflate **6** with **13** in an analogous fashion provided thiodisaccharide **16** in 70% yield. The disaccharide **16** contains two βGalNAc residues linked together via the β-(1→4)-thio linkage; its natural O-glycoside is present in the LPS structure of *E. coli* 87/D2.[12] Additionally, we also prepared two other disaccharides **18** and **20** using triflate **6** to react with the previously known **17**[13] and **19**,[14] respectively. The O-glycoside counterparts of disaccharides **18** and **20** were, respectively,



Scheme 2. Syntheses of useful intermediates. Reagents and conditions: (a) KSAc/DMF, 65 °C, 2 h; (b) NaN_3/DMF, 65 °C, 2 h; (c) AcCl/MeOH; (d) KSAc/acetone, 0 °C→rt, overnight; (e) *p*-ChPhSH/KOBu-*t*/DMF–THF, overnight.

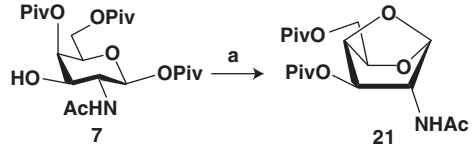


Scheme 3. Syntheses of thio analogs of disaccharides **15**, **16**, **18,** and **20**. Reagents and condition: (a) Et_2NH/DMF, −5 °C, overnight.

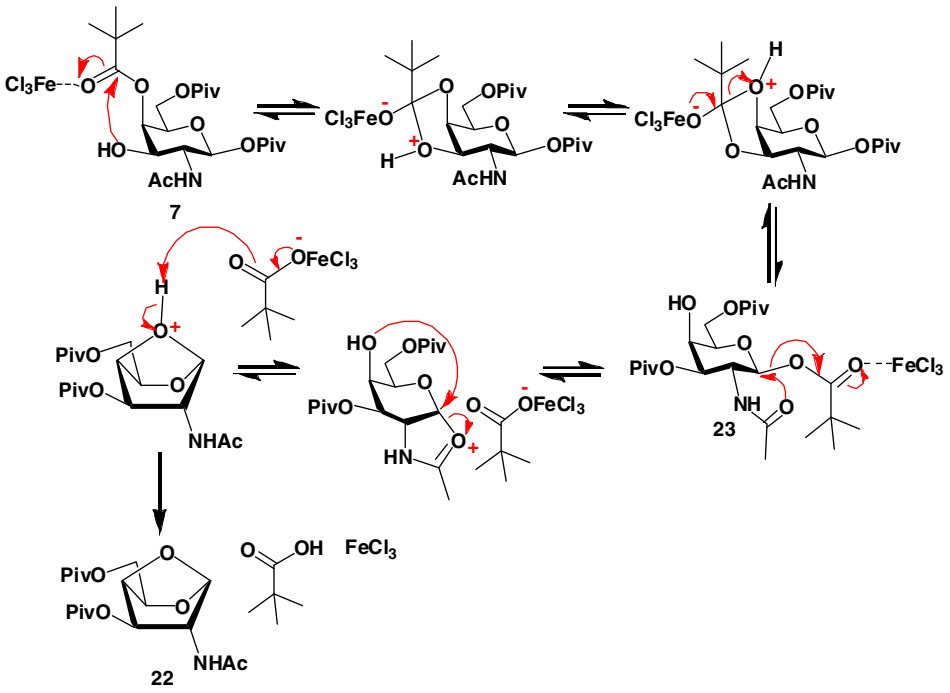
found in the LPS structures of *S. Dakar* serotype O:28[15] and in the capsular polysaccharide of *S. pneumoniae* serotype 10A.[16]

Additionally, since compound **7** had a free hydroxyl group which can act as an acceptor, and its anomeric center was protected with an acyl group, which can act as a donor, we were interested in probing its behavior in carrying out self-condensations when treated with a Lewis acid such as ferric chloride.[17] As shown in Scheme 4, after treating compound **7** with 1.2 equiv of ferric chloride in 1,2-dichloroethane overnight, no self-condensed oligosaccharides were isolated; however, compound **21** was obtained in 27% yield.

Clearly, compound **21** was the result of an intramolecular glycosylation between OH-4 and the anomeric center. Its structure was confirmed by a series of 1D and 2D NMR experiments: for example, in the ^1H NMR spectra, the coupling constants $J_{1,2}$, $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ were consistently small, indicating that the conformation of the original $^4C_1$ chair in **7** has been changed; an 2D ^1H–^{13}C HMBC experiment revealed a strong correlation between H-1 and C-4. Since from a normal HMBC experiment, we can only observe heteronuclear correlations up to three bonds apart; however, in a normal chair conformation of a pyranoside, C-4 is four bonds away from H-1. Thus the observed strong correlation between H-1 and C-4 can only be explained by the formation of a linkage between C-1 and O-4, which brings the distance to three bonds between H-1 and C-4. The structure of compound **21** was also confirmed by high-resolution mass spectrometry.



Scheme 4. The treatment of compound **7** with $FeCl_3$. Reagents and condition: (a) $FeCl_3$/1,2-dichloroethane, 90 °C, overnight.



Scheme 5. Proposed mechanism for the formation of compound **21** from **7**.

The formation of **21** was intriguing because the free hydroxyl group in compound **7** was at C-3. The only way to explain the formation would be a migration of the pivaloyl group from O-4 to O-3 with the assistance of a Lewis acid. As shown in Scheme 5, after coordination of ferric chloride to the carbonyl oxygen of the 4-pivalate, it was possible that an equilibrium would have been established between **7** and intermediate **22** which underwent an intramolecular glycosylation to generate the observed 1,4-anhydro sugar **21**.

3. Conclusions

We have developed a viable method to convert D-GlcNAc to a protected D-GalNAc derivative. The entire process required a three-step transformation which required no chromatography to isolate the desired D-GalNAc derivative in 75% overall yield on a small scale and 58.4% on a larger scale. This is so far the most efficient method reported in the literature. The versatility of the D-GalNAc derivative so obtained and the other intermediates has been illustrated by the synthesis of several mono- and disaccharides.

4. Experimental

4.1. General methods

Optical rotations were determined in a 5-cm cell at 25 ± 2 °C. $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC was performed on Silica Gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in water or with a ceric ammonium molybdate dip. All commercial reagents were used as supplied unless otherwise stated. Column chromatography was performed on Silica Gel 60 (Silicycle, Ontario). Organic solutions from extractions were dried with anhyd Na_2SO_4 prior to concentration under vacuum at <40 °C (bath). ^{1}H NMR spectra were recorded at 300 or 400 MHz on Bruker spectrometers. The first-order proton chemical shifts δ_H and δ_C are reported in δ (ppm) for solutions in $CDCl_3$ and referenced to residual $CHCl_3$ (δ_H 7.24 and δ_C 77.0). ^{1}H and ^{13}C NMR spectra were assigned with the assistance of GCOSY, GHSQC, or GHMBC spectra. High-resolution ESI-QTOF mass spectra were recorded on an Agilent 6520 Accurate Mass Quadrupole Time-of-Flight LC/MS spectrometer. All the data were obtained by the analytical services of the Department of Chemistry, University of Calgary.

4.2. O-Pivalation of 2-acetamido-2-deoxy-D-glucose

2-Acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine, **1**, 20.0 g, 90.4 mmol) was added to a mixture of anhyd CH_2Cl_2 (100 mL) and pyridine (50 mL) and the mixture was stirred overnight. After cooling the reaction mixture to −10 °C, trimethylacetyl chloride (34.0 mL, 276.3 mmol) was added, and the reaction was allowed to warm up to room temperature. After 2 days, the solution was diluted with CH_2Cl_2 (150 mL), and the organic phase was washed with H_2O (2 × 100 mL), brine (1 × 100 mL), and dried over anhyd Na_2SO_4. After evaporation, the crude residue could be used directly for the next step. We also attempted to carry out the purification of the mixture by column chromatography on silica gel using 25% EtOAc–hexane as the eluent to give first a mixture of **3** and **4** (~8.8 g), and subsequently pure compound **3** (29.5 g, 68.9% yield) as a single β anomer. By increasing the polarity of the eluent to 45% EtOAc–hexane, a small amount of compound **2** was obtained as an α/β mixture (1.1 g, 3.1% yield, α/β 25:1). The mixture of **3** and **4** was subjected to another column chromatography using 5% acetone–hexane as the eluent to give compound **4** as an inseparable α/β mixture (4.1 g, 8.2% yield, α/β 1:2) and more pure compound **3** (4.5 g, 10.5%). Compound **3** was obtained in a combined yield of 79.4%.

4.2.1. 2-Acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α,β-D-glucopyranose (2)

R_f: 0.25 (5:95 MeOH–CH_2Cl_2). ^{1}H NMR for **2α** (400 MHz, $CDCl_3$): δ_H 6.55 (d, 1H, J = 9.5 Hz, NH), 5.24 (dd, 1H, J = 10.7, 9.4 Hz, H-3), 5.18 (dd, 1H, J = 3.6, 3.6 Hz, H-1), 5.10 (d, 1H, J = 3.0 Hz, OH-1), 4.35–4.31 (m, 2H, H-6a + H-6b), 4.20 (ddd, 1H, J = 10.0, 10.0,

2.9 Hz, H-2), 4.13–4.05 (m, 1H, H-5), 3.56 (ddd, 1H, J = 7.0, 9.6, 9.6 Hz, H-4), 3.27 (d, J = 6.9 Hz, 1H, OH-4), 1.93 (s, 3H, NHAc), 1.20 (s, 9H, Piv), 1.17 (s, 9H, Piv). ^{13}C NMR for **2α** (100 MHz, $CDCl_3$): δ_C 180.09 (Piv), 179.46 (Piv), 170.64 (NHAc), 91.72 (C-1), 73.07 (C-3), 69.97 (C-5), 69.25 (C-4), 62.96 (C-6), 51.90 (C-2), 38.99 ($C(CH_3)_3 \times 2$), 27.16 (C(CH_3)$_3$), 26.98 (C(CH_3)$_3$), 22.90 (NHAc). HRESIMS: Calcd for $C_{18}H_{32}NO_8$ (M+H$^+$): m/z 390.2122. Found: 390.2115.

4.2.2. 2-*N*-Acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-β-D-glucopyranose (3)

R_f: 0.40 (5:95 MeOH–CH_2Cl_2). $[\alpha]_D$ −3.5 (c 12.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.91 (d, 1H, J = 9.8 Hz, NH), 5.62 (d, 1H, J = 8.8 Hz, H-1), 5.08 (dd, 1H, J = 10.7, 9.1 Hz, H-3), 4.46 (dd, 1H, J = 12.2, 4.6 Hz, H-6a), 4.34 (dd, 1H, J = 12.2, 2.4 Hz, H-6b), 4.31–4.20 (m, 1H, H-2), 3.68 (ddd, 1H, J = 9.8, 4.5, 2.4 Hz, H-5), 3.56 (ddd, 1H, J = 9.4, 5.5 Hz, H-4), 3.24 (d, 1H, J = 5.5 Hz, OH), 1.88 (s, 3H, NHAc), 1.23 (s, 9H, Piv), 1.21 (s, 9H, Piv), 1.19 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 179.78 (Piv), 179.21 (Piv), 177.06 (Piv), 169.86 (NHAc), 92.58 (C-1), 74.85 (C-5), 74.74 (C-3), 69.01 (C-4), 62.75 (C-6), 52.62 (C-2), 38.94 (C(CH_3)$_3$), 38.91 (C(CH_3)$_3$), 38.68 (C(CH_3)$_3$), 27.08 (C(CH_3)$_3$), 26.95 (C(CH_3)$_3$), 26.70 (C(CH_3)$_3$), 22.91 (NHAc). HRESIMS: Calcd for $C_{23}H_{39}NO_9SNa$ (M+Na$^+$): m/z 496.2517. Found: 496.2514. Anal. Calcd for $C_{23}H_{39}NO_9$: C, 58.33; H, 8.30; N, 2.96. Found: C, 58.02; H, 8.20; N, 2.86.

4.2.3. 2-Acetamido-2-deoxy-1,3,4,6-tetra-*O*-pivaloyl-α,β-D-glucopyranose (4)

R_f: 0.59 for **4α**, 0.50 for **4β** (5:95 MeOH–CH_2Cl_2). ^{1}H NMR for **4α** (400 MHz, $CDCl_3$): δ_H 6.17 (d, 1H, J = 3.7 Hz, H-1), 5.41 (d, 1H, J = 9.0 Hz, NH), 5.31–5.22 (m, 2H, H-3 + H-4), 4.56–4.48 (m, 1H, H-2), 4.20–4.08 (m, 2H, H-6a + H-6b), 3.99 (ddd, J = 9.8, 3.4, 3.4 Hz, 1H, H-5), 1.90 (s, 3H, NHAc), 1.30 (s, 9H, Piv), 1.21 (s, 9H, Piv), 1.18 (s, 9H, Piv), 1.15 (s, 9H, Piv). ^{1}H NMR for **4β** (400 MHz, $CDCl_3$): δ_H 5.64 (d, 1H, J = 9.9 Hz, NH), 5.60 (d, 1H, J = 8.8 Hz, H-1), 5.22–5.11 (m, 2H, H-4 + H-3), 4.48–4.37 (m, 1H, H-2), 4.20–4.08 (m, 2H, H-6a + H-6b), 3.83 (ddd, 1H, J = 9.5, 4.2, 2.6 Hz, H-5), 1.87 (s, 3H, NHAc), 1.22 (s, 9H, Piv), 1.19 (s, 9H, Piv), 1.16 (s, 9H, Piv), 1.14 (s, 9H, Piv). ^{13}C NMR for **4α** (100 MHz, $CDCl_3$): δ_C 179.48 (Piv), 178.00 (Piv), 177.06 (Piv), 175.80 (Piv), 169.53 (NHAc), 90.47 (C-1), 70.34 (C-5), 70.24 (C-3), 66.82 (C-4), 61.59 (C-6), 51.72 (C-2), 39.32 (C(CH_3)$_3$), 38.99 (C(CH_3)$_3$), 38.83 (C(CH_3)$_3$), 38.76 (C(CH_3)$_3$), 27.11 (C(CH_3)$_3$), 27.08 (C(CH_3)$_3$), 27.03 (C(CH_3)$_3$), 27.00 (C(CH_3)$_3$), 22.94 (NHAc). ^{13}C NMR for **4β** (100 MHz, $CDCl_3$): δ_C 178.85 (Piv), 178.06 (Piv), 177.06 (Piv), 176.18 (Piv), 169.40 (NHAc), 92.70 (C-1), 73.08 (C-5), 72.25 (C-3), 67.23 (C-4), 61.45 (C-6), 52.80 (C-2), 38.92 (C(CH_3)$_3$), 38.87 (C(CH_3)$_3$), 38.76 (C(CH_3)$_3$), 38.74 (C(CH_3)$_3$), 27.06 (C(CH_3)$_3$), 27.01 (C(CH_3)$_3 \times 2$), 26.74 (C(CH_3)$_3$), 23.02 (NHAc). HRESIMS: Calcd for $C_{28}H_{48}NO_{10}$ (M+H$^+$): m/z 558.3273. Found: 558.3269.

4.3. 2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-glucopyranose (5)

To a mixture containing anhyd CH_2Cl_2 (4 mL) and pyridine (2 mL), was added *N*-acetyl-D-glucosamine (**1**, 500 mg, 2.3 mmol), and the suspension was stirred overnight. After cooling to −10 °C, benzoyl chloride (787.7 μL, 6.8 mmol) was added, and the reaction mixture was allowed to warm up to room temperature. After two days, the solution was diluted with CH_2Cl_2 (15 mL) and washed with H_2O (2 × 10 mL), then brine (1 × 10 mL); the organic phase was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 2.5% MeOH–CH_2Cl_2 as the eluent to give compound **5**[18] as an α/β mixture (586 mg, 48.6% yield, α/β 16:1) as white solid. R_f: 0.37 (5:95 MeOH–CH_2Cl_2). ^{1}H NMR for **5α** (400 MHz, 2:1 $CDCl_3$–CD_3OD): δ_H 8.02–7.97 (m, 2H, Bz), 7.89–7.84 (m, 4H, Bz), 7.56–7.51 (m, 1H Bz), 7.50–7.44 (m, 2H, Bz), 7.42–7.36 (m, 2H, Bz), 7.36–7.29 (m, 4H, Bz), 5.77 (dd, 1H, J = 9.6, 10.7 Hz, H-3), 5.61 (dd, 1H, J = 9.8, 9.8 Hz, H-4), 5.25 (d, 1H, J = 3.5 Hz, H-1), 4.60 (ddd, 1H, J = 3.6, 3.6, 10.0 Hz, H-5), 4.55 (dd, 1H, J = 3.1 Hz, H-6a, overlapped), 4.50 (dd, 1H, J = 3.5, 10.8 Hz, H-2), 4.41 (dd, 1H, J = 4.2, 12.1 Hz, H-6b), 1.84 (s, 3H, NHAc). ^{13}C NMR for **10α** (100 MHz, 2:1 $CDCl_3$–CD_3OD): δ_C 171.76 (NHAc), 166.84 (Bz), 166.68 (Bz), 165.61 (Bz), 133.37 (Ph), 133.30 (Ph), 133.15 (Ph), 129.58 (Ph), 129.57 (Ph), 129.01 (Ph), 128.91 (Ph), 128.31 (Ph), 128.29 (Ph), 91.55 (C-1), 71.76 (C-3), 70.10 (C-4), 67.28 (C-5), 63.25 (C-6), 52.46 (C-2), 22.02 (NHAc). HRESIMS: Calcd for $C_{29}H_{28}NO_9$ (M+H$^+$): m/z 534.1758. Found: 534.1754.

4.4. 2-Acetamido-2-deoxy-1,4,6-tri-*O*-pivaloyl-β-D-galactopyranose (7)

4.4.1. Small-scale procedure

N-Acetyl-D-glucosamine (**1**, 5.0 g, 22.6 mmol) was reacted with trimethylacetyl chloride (8.5 mL, 69.1 mmol) in a mixture of anhyd CH_2Cl_2 (30 mL) and pyridine (15 mL) as above. After workup, the crude mixture was dissolved in a mixture of anhyd CH_2Cl_2 (20 mL) and pyridine (20 mL). Trifluoromethanesulfonic anhydride (5.3 mL, 31.6 mmol) was added at −10 °C, and the reaction mixture was stirred at 0 °C for 2 h. H_2O (5.0 mL) was added, and the reaction mixture was heated to 60 °C overnight. The solution was diluted with CH_2Cl_2 (40 mL) and washed with H_2O (2 × 30 mL), then brine (1 × 30 mL). The organic solution was dried over anhyd Na_2SO_4 and evaporated. The residue was recrystallized from a mixture of EtOAc–hexane to afford the desired compound **7** (8.0 g, 75.0% yield over three steps).

4.4.2. Large-scale procedure

N-Acetyl-D-glucosamine (**1**, 20.0 g, 90.4 mmol) was reacted with trimethylacetyl chloride (34.0 mL, 276.3 mmol) in a mixture of anhyd CH_2Cl_2 (100 mL) and pyridine (50 mL) as above. After workup, the crude mixture was dissolved in a mixture of anhyd CH_2Cl_2 (50 mL) and pyridine (50 mL). Trifluoromethanesulfonic anhydride (21.3 mL, 126.6 mmol) was added at −10 °C, and the reaction mixture was stirred at 0 °C for 2 h. H_2O (20.0 mL) was added, and the mixture was heated to 60 °C overnight. The solution was diluted with CH_2Cl_2 (150 mL) and washed with H_2O (2 × 100 mL), then brine (1 × 100 mL). The organic solution was dried over anhyd Na_2SO_4 and evaporated. The residue could be recrystallized from a mixture of EtOAc–hexane to afford compound **7** (25.0 g, 58.4% yield over three steps). Alternatively, the residue was purified by column chromatography on silica gel using 24% EtOAc–hexane as the eluent to give compound **7** (26.8 g, 62.7% yield over three steps).

4.4.3. 2-Acetamido-2-deoxy-1,4,6-tri-*O*-pivaloyl-β-D-galactopyranose (7)

R_f: 0.28 (5:95 MeOH–CH_2Cl_2). $[\alpha]_D$ +0.56 (c 1.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.83 (d, 1H, J = 7.8 Hz, NH), 5.66 (d, 1H, J = 8.8 Hz, H-1), 5.32 (dd, 1H, J = 3.3, 0.8 Hz, H-4), 4.20–4.04 (m, 3H, H-2 + H-6a + H-6b), 4.01 (ddd, 1H, J = 6.3, 6.3, 1.0 Hz, H-5), 3.89 (ddd, 1H, J = 10.8, 4.5, 3.6 Hz, H-3), 3.54 (d, 1H, J = 4.7 Hz, OH-3), 1.99 (s, 3H, Ac), 1.30 (s, 9H, Piv), 1.23 (s, 9H, Piv), 1.18 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.02 (Piv), 177.93 (Piv), 177.82 (Piv), 172.24 (NHAc), 92.58 (C-1), 72.64 (C-3), 72.17 (C-5), 68.02 (C-4), 61.33 (C-6), 53.51 (C-4), 39.28 (C(CH_3)$_3$), 38.90 (C(CH_3)$_3$), 38.70 (C(CH_3)$_3$), 27.22 (C(CH_3)$_3$), 27.01 (C(CH_3)$_3$), 26.81 (C(CH_3)$_3$), 23.10 (NHAc). HRCIMS: Calcd for $C_{23}H_{40}NO_9$ (M+H$^+$): m/z 474.2703. Found: 474.2686. Anal. Calcd for $C_{23}H_{39}NO_9$: C, 58.33; H, 8.30; N, 2.96. Found: C, 58.04; H, 8.30; N, 2.82.

4.5. 2-Acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-*O*-trifluoromethanesulfonyl-β-D-glucopyranose (6)

Compound **3** (400 mg, 0.845 mmol) was dissolved in a mixture of anhyd CH_2Cl_2 (2 mL) and anhyd pyridine (2 mL). Trifluoromethanesulfonic anhydride (285.4 μL, 1.69 mmol) was slowly added at −10 °C, and the reaction mixture was stirred at 0 °C for 1.5 h. Then the solution was diluted by CH_2Cl_2 (10 mL) and washed with HCl (1 N, 2 × 10 mL), satd aq $NaHCO_3$ (1 × 10 mL) and H_2O (1 × 10 mL), followed by evaporation to dryness. The crude triflate **6** was used for the following reactions without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.95 (d, 1H, *J* = 9.9 Hz, NH), 5.68 (d, 1H, *J* = 8.8 Hz, H-1), 5.48 (dd, 1H, *J* = 10.8, 9.2 Hz, H-3), 5.12 (dd, 1H, *J* = 9.4, 9.4 Hz, H-4), 4.50–4.37 (m, 2H, H-6a + H-2), 4.11–4.00 (m, 2H, H-6a + H-5), 1.88 (s, 3H, Ac), 1.24 (s, 9H, Piv), 1.23 (s, 9H, Piv), 1.18 (s, 9H, Piv).

4.6. 2-Acetamido-4-*S*-acetyl-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-thio-β-D-galactopyranose (8)

The crude triflate **6** (512 mg, 0.845 mmol) was dissolved in DMF (4 mL). KSAc (193 mg, 1.69 mmol) was added, and the reaction mixture was refluxed at 65 °C for 2 h. The solution was diluted with EtOAc (20 mL) and washed with H_2O (3 × 25 mL). The organic solution was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 20% EtOAc–hexane as the eluent to give compound **8** (395 mg, 88.0% yield over two steps) as a yellow solid. R_f: 0.68 (MeOH/CH_2Cl_2 5:95). $[\alpha]_D$ −17.2 (*c* 2.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.60–5.51 (m, 2H, H-1 + NH), 5.23 (dd, 1H, *J* = 4.4, 11.0 Hz, H-3), 4.33–4.22 (m, 3H, H-4 + H-2 + H-6a), 4.18 (ddd, 1H, *J* = 1.5, 6.2, 6.2 Hz, H-5), 4.10 (dd, 1H, *J* = 6.1, 10.8 Hz, H-6b), 2.36 (s, 3H, SAc), 1.86 (s, 3H, NHAc), 1.17 (s, 9H, Piv), 1.16 (s, 9H, Piv), 1.11 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 193.15 (SAc), 178.09 (Piv), 177.93 (Piv), 177.09 (Piv), 169.49 (NHAc), 93.35 (C-1), 72.40 (C-5), 70.55 (C-3), 62.66 (C-6), 51.49 (C-2), 45.84 (C-4), 38.90 ($C(CH_3)_3$), 38.76 ($C(CH_3)_3$), 38.71 ($C(CH_3)_3$), 30.70 (SAc), 27.01 ($C(CH_3)_3$), 26.80 ($C(CH_3)_3$), 26.77 ($C(CH_3)_3$), 23.08 (NHAc). HREIMS: Calcd for $C_{20}H_{32}NO_8S$ ($M-Piv^+$): *m/z* 446.1849. Found: 446.1838. Anal. Calcd for $C_{25}H_{41}NO_9S$: C, 56.48; H, 7.77; N, 2.63. Found: C, 56.29; H, 7.53; N, 2.46.

4.7. 2-Acetamido-4-azido-2,4-dideoxy-1,3,6-tri-*O*-pivaloyl-α,β-D-galactopyranose (9)

The crude triflate **6** (512 mg, 0.845 mmol) was dissolved in anhyd DMF (4 mL), and NaN_3 (109.9 mg, 1.69 mmol) was added. The reaction was stirred at 65 °C for 2 h. The solution was diluted with EtOAc (20 mL) and washed with H_2O (3 × 25 mL). The organic solution was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 20% EtOAc–hexane as the eluent to give compound **9** as an α/β mixture (323 mg, 76.7% yield over two steps, α/β 1:5). R_f: 0.75 (5:95 MeOH–CH_2Cl_2). ^{1}H NMR for **9β** (400 MHz, $CDCl_3$): δ_H 5.58 (d, 1H, *J* = 8.8 Hz, H-1), 5.51 (m, 1H, NH), 5.22 (m, 1H, H-3), 4.52 (ddd, 1H, *J* = 19.1, 9.5, 1.1 Hz, H-2), 4.30 (dd, 1H, *J* = 11.2, 6.3 Hz, H-6a), 4.20 (dd, 1H *J* = 11.2, 6.7 Hz, H6-b), 4.00–3.91 (m, 2H, H-5 + H-4), 1.87 (s, 3H, Ac), 1.24 (s, 9H, Piv), 1.21 (s, 9H, Piv), 1.18 (s, 9H, Piv). ^{13}C NMR for **9β** (100 MHz, $CDCl_3$): δ_C 178.54 (Piv), 177.92 (Piv), 177.14 (Piv), 169.53 (NHAc), 92.84 (C-1), 72.00 (C-3), 71.65 (C-5), 62.17 (C-6), 60.37 (C-4), 49.95 (C-2), 39.28 ($C(CH_3)_3$), 38.79 ($C(CH_3)_3$), 38.78 ($C(CH_3)_3$), 27.11 ($C(CH_3)_3$), 27.03 ($C(CH_3)_3$), 26.77 ($C(CH_3)_3$), 23.10 (NHAc). HREIMS: Calcd for $C_{23}H_{28}N_4O_8$ (M^+): *m/z* 498.2690. Found: 498.2705.

4.8. 2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-α-D-glucopyranosyl chloride (10) and 2-acetamido-4-*O*-acetyl-1-*S*-acetyl-2-deoxy-3,6-di-*O*-pivaloyl-1-thio-β-D-glucopyranose (11)

Compound **3** (500 mg, 1.06 mmol) was dissolved in AcCl (3 mL). Anhyd MeOH (400 μL) was added at 0 °C, and the reaction mixture was kept at room temperature overnight. The solution was evaporated to dryness and the crude chloride **10** was dissolved in acetone (5 mL) containing 4 Å molecular sieves (500 mg). Potassium thioacetate (165 mg, 1.38 mmol) was added to the solution at 0 °C, and the reaction mixture was kept at room temperature overnight. The solution was then evaporated, and the residue was purified by column chromatography on silica gel using 28% EtOAc–hexane as eluent to give compound **11** (325 mg, 62.9% yield) as a yellow solid.

4.8.1. 2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-α-D-glucopyranosyl chloride (10)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.17 (d, 1H, *J* = 3.7 Hz, H-1), 5.81 (d, 1H, *J* = 8.8 Hz, NH), 5.34 (dd, 1H, *J* = 9.7, 9.7 Hz, H-3), 5.26 (dd, 1H, *J* = 9.8, 9.8 Hz, H-4), 4.56 (ddd, 1H, *J* = 10.5, 9.0, 3.7 Hz, H-2), 4.29 (ddd, 1H, *J* = 10.0, 3.0, 3.0 Hz, H-5), 4.25–4.16 (m, 2H, H-6a + H-6b), 2.04 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.24 (s, 9H, Piv), 1.15 (s, 9H, Piv).

4.8.2. 2-Acetamido-3-*O*-acetyl-1-*S*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-1-thio-β-D-glucopyranose (11)

R_f: 0.52 (4:6 EtOAc–toluene). $[\alpha]_D$ +7.2 (*c* 3.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.12 (d, 1H, *J* = 9.8 Hz, NH), 5.28–5.10 (m, 3H, H-3 + H-4 + H-1), 4.39 (ddd, 1H, *J* = 10.5, 9.0, 3.7 Hz, H-2), 4.21–4.09 (m, 2H, H-6a + H-6b), 3.83 (ddd, 1H, *J* = 9.5, 4.0, 3.1 Hz, H-5), 2.37 (s, 3H, SAc), 2.04 (s, 3H, OAc), 1.89 (s, 3H, NHAc), 1.21 (s, 9H, Piv), 1.15 (s, *J* = 9.4, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 193.27 (SAc), 179.31 (Piv), 178.09 (Piv), 169.69 (NHAc), 168.92 (OAc), 81.69 (C-1), 76.64 (C-5), 73.95 (C-3), 67.78 (C-4), 61.86 (C-6), 51.82 (C-2), 38.93 ($C(CH_3)_3$), 38.84 ($C(CH_3)_3$), 30.75 (SAc), 27.05 ($C(CH_3)_3$), 26.85 ($C(CH_3)_3$), 22.99 (NHAc), 20.52 (OAc). HRESIMS: Calcd for $C_{22}H_{35}NO_9SNa$ ($M+Na^+$): *m/z* 512.1925. Found: 512.1924.

4.9. 2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranosyl chloride (12) and 2-acetamido-3-*O*-acetyl-1-*S*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-1-thio-β-D-galactopyranose (13)

Compound **7** (500 mg, 1.06 mmol) was dissolved in AcCl (3 mL). Anhyd MeOH (400 μL) was added at 0 °C and the reaction mixture was kept at room temperature overnight. The solution was evaporated to dryness and the crude chloride **12** was dissolved in acetone (5 mL) containing 4 Å molecular sieves (500 mg). Potassium thioacetate (165 mg, 1.38 mmol) was added to the solution at 0 °C, and the reaction mixture was kept at room temperature overnight. The solution was then evaporated, and the residue was purified by column chromatography on silica gel using 40% EtOAc–hexane as the eluent to give compound **13** (370 mg, 71.6% yield) as a yellow solid.

4.9.1. 2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranosyl chloride (12)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.30 (d, 1H, *J* = 3.6 Hz, H-1), 5.98 (d, 1H, *J* = 8.8 Hz, NH), 5.48 (dd, 1H, *J* = 3.0, 1.1 Hz, H-4), 5.31 (dd, 1H, *J* = 11.3, 3.2 Hz, H-3), 4.78 (ddd, 1H, *J* = 11.5, 8.9, 3.6 Hz, H-2), 4.54 (ddd, 1H, *J* = 7.0, 7.0, <1 Hz, H-5), 4.18–4.06 (m, 2H, H-6a + H-6b), 2.01 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.27 (s, 9H, Piv), 1.19 (s, 9H, Piv).

4.9.2. 2-Acetamido-3-*O*-acetyl-1-*S*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-1-thio-β-D-galactopyranose (13)

R_f: 0.36 (4:6 EtOAc–toluene). $[\alpha]_D$ +8.5 (*c* 6.2, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.96 (br, 1H, NH), 5.38 (dd, 1H, *J* = 3.2, <1 Hz, H-4), 5.24 (d, 1H, *J* = 10.8 Hz, H-1), 5.10 (dd, 1H, *J* = 10.8, 3.3 Hz, H-3), 4.42 (ddd, 1H, *J* = 10.5, 10.5, 10.5 Hz, H-2), 4.13–3.97 (m, 3H, H-5 + H-6a + H-6b), 2.34 (s, 3H, SAc), 1.94 (s, 3H, OAc), 1.89 (s, 3H, NHAc), 1.23 (s, 9H, Piv), 1.13 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 193.49 (SAc), 177.80 (Piv), 177.42 (Piv), 170.45 (NHAc), 170.20 (OAc), 81.86 (C-1), 75.03 (C-5), 71.71 (C-3), 66.16 (C-4), 61.08 (C-6), 48.44 (C-2), 39.16 ($C(CH_3)_3$), 38.64 ($C(CH_3)_3$), 30.75 (SAc), 27.07 ($C(CH_3)_3$), 26.96 ($C(CH_3)_3$), 23.15 (NHAc), 20.53 (OAc). HRESIMS: Calcd for $C_{22}H_{35}NO_9SNa$ ($M+Na^+$): *m/z* 512.1925. Found: 512.1923.

4.10. *p*-Chlorophenyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-1-thio-β-D-galactopyranoside (14)

The glycosyl chloride **12** was prepared from compound **7** (4 g, 7.17 mmol) and AcCl (20 mL) as above. The crude **12** was dissolved in anhyd THF (15 mL), and the solution was added to a DMF solution (20 mL) containing 4-chlorobenzenethiol (2.1 g, 14.4 mmol) and *t*-BuOK (1.45 g, 13.9 mmol). The reaction mixture was stirred at room temperature overnight. The solution was diluted with EtOAc (80 mL) and washed with H_2O (3 × 100 mL). The organic solution was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 20% EtOAc–toluene as the eluent to give the compound **14** (2.96 g, 62.8% yield over two steps). R_f: 0.60 (8:92 MeOH–CH_2Cl_2). $[\alpha]_D$ −25.8 (*c* 0.73, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.54–7.46 (m, 2H, Ph), 7.31–7.24 (m, 2H, Ph), 5.80 (d, 1H, *J* = 9.1 Hz, NH), 5.35 (dd, 1H, *J* = 3.2, <1 Hz, H-4), 5.23 (dd, 1H, *J* = 10.7, 3.3 Hz, H-3), 4.90 (d, 1H, *J* = 10.3 Hz, H-1), 4.14 (m, 1H, H-6a), 4.10–3.98 (m, 3H, H-2 + H-6b + H-5), 1.98 (s, 3H, NHAc), 1.94 (s, 3H, OAc), 1.17 (m, 18H, Piv × 2. ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 177.82 (Piv), 177.29 (Piv), 170.25 (NHAc), 170.09 (OAc), 134.78 (Ph), 134.54 (Ph), 129.97 (Ph), 128.92 (Ph), 85.66 (C-1), 74.52 (C-5), 71.22 (C-3), 66.32 (C-4), 61.52 (C-6), 49.60 (C-2), 39.05 ($C(CH_3)_3$), 38.64 ($C(CH_3)_3$), 26.98 ($C(CH_3)_3$), 26.93 ($C(CH_3)_3$), 23.35 (NHAc), 20.52 (OAc). HRCIMS: Calcd for $C_{26}H_{37}NO_8SCl$ ($M+H^+$): *m/z* 558.1928. Found: 558.1925. Anal. Calcd for $C_{26}H_{36}NO_8SCl$: C, 55.96; H, 6.50; N, 2.51. Found: C, 56.03; H, 6.55; N, 2.39.

4.11. 4-*S*-(2-Acetamido-4-*O*-acetyl-2-deoxy-3,6-di-*O*-pivaloyl-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-thio-β-D-galactopyranose (15)

Thioacetate **11** (265 mg, 0.541 mmol) and the crude triflate **6** (361 mg, 0.595 mmol) were dissolved in anhyd DMF (7 mL). Under argon, Et_2NH (900 μL) was added at −5 °C. The reaction mixture was kept at −5 °C overnight. The solution was then extracted with EtOAc (20 mL) and H_2O (3 × 30 mL). The organic layer was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 15% acetone–hexane as the eluent to give compound **15** (310 mg, 63.4% yield) as a white solid. R_f: 0.45 (1:1 EtOAc–toluene). $[\alpha]_D$ −26.7 (*c* 1.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.58 (d, 1H, *J* = 9.5 Hz, GlcNAc_NH), 5.57 (d, 1H, *J* = 8.5 Hz, GalNAc_H-1), 5.41 (d, 1H, *J* = 9.5 Hz, GalNAc_NH), 5.20 (dd, 1H, *J* = 9.7, 9.7 Hz, GlcNAc_H-4), 5.16 (dd, 1H, *J* = 10.7, 4.6 Hz, GalNAc_H-3), 5.03 (dd, 1H, *J* = 10.3, 9.7 Hz, GlcNAc_H-3), 4.85 (d, 1H, *J* = 10.4 Hz, GlcNAc_H-1), 4.52 (dd, 1H, *J* = 12.2, 7.9 Hz, GalNAc_H-6a), 4.49–4.40 (m, 1H, GalNAc_H-2), 4.36 (dd, 1H, *J* = 12.5, 2.2 Hz, GlcNAc_H-6a), 4.24 (dd, 1H, *J* = 12.2, 3.3 Hz, GalNAc_H-6b), 4.21–4.12 (m, 2H, GlcNAc_H-2 + GlcNAc_H-6b), 4.05 (ddd, 1H, *J* = 7.9, 3.3, 2.1 Hz, GalNAc_H-5), 3.61 (ddd, 1H, *J* = 10.0, 3.8, 2.4 Hz, GlcNAc_H-5), 3.49 (dd, 1H, *J* = 4.5, 1.9 Hz, GalNAc_H-4), 2.01 (s, 3H, NHAc), 1.99 (s, 3H, NHAc), 1.88 (s, 3H, OAc), 1.24 (s, 9H, Piv), 1.22 (s, 9H, Piv), 1.20 (s, 9H, Piv), 1.18 (s, 9H, Piv), 1.15 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.99 (Piv), 178.31 (Piv), 178.28 (Piv), 178.08 (Piv), 176.96 (Piv), 169.97 (Ac), 169.46 (Ac), 168.77 (Ac), 93.24 (GalNAc_C-1), 84.00 (GlcNAc_C-1), 76.21 (GlcNAc_C-5), 74.32 (GalNAc_C-5), 73.47 (GlcNAc_C-3), 72.10 (GalNAc_C-3), 67.68 (GlcNAc_C-4), 64.41 (GalNAc_C-6), 61.52 (GlcNAc_C-6), 53.67 (GlcNAc_C-2), 50.97 (GalNAc_C-2), 45.63 (GalNAc_C-4), 39.12 ($C(CH_3)_3$), 38.95 ($C(CH_3)_3$), 38.88 ($C(CH_3)_3$), 38.78 ($C(CH_3)_3$), 38.73 ($C(CH_3)_3$), 27.26 ($C(CH_3)$), 27.08 ($C(CH_3)_3 \times 2$), 26.91 ($C(CH_3)_3$), 26.84 ($C(CH_3)_3$), 23.17 (NHAc), 23.16 (NHAc), 20.53 (OAc). HRESIMS: Calcd for $C_{43}H_{71}N_2O_{16}S$ ($M+H^+$): *m/z* 903.4519. Found: 903.4522.

4.12. *S*-(2-Acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-thio-β-D-galactopyranose (16)

Thioacetate **13** (170 mg, 0.347 mmol) and the crude triflate **6** (231 mg, 0.382 mmol) were dissolved in anhyd DMF (4 mL). Under argon, Et_2NH (500 μL) was added at −5 °C. The reaction mixture was kept at −5 °C overnight. The solution was then extracted with EtOAc (15 mL) and H_2O (3 × 20 mL). The organic layer was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 20% acetone–hexane as the eluent to give compound **16** (220 mg, 70.2% yield) as a white solid. R_f: 0.43 (1:1 EtOAc–toluene). $[\alpha]_D$ −6.2 (*c* 0.87, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.77–5.65 (m, 2H, NH × 2), 5.55 (d, 1H, *J* = 8.3 Hz, GalNAc_H-1), 5.37 (dd, 1H, *J* = 2.8, <1 Hz, GalNAc_H-4′), 5.22–5.13 (m, 2H, GalNAc_H-3′ + GalNAc_H-3), 4.98 (d, 1H, *J* = 10.3 Hz, GalNAc_H-1′), 4.53 (dd, 1H, *J* = 12.1, 8.0 Hz, GalNAc_H-6a), 4.45 (ddd, 1H, *J* = 9.7, 8.5, 8.5 Hz, GalNAc_H-2), 4.22 (dd, 1H, *J* = 12.1, 3.7 Hz, GalNAc_H-6b), 4.17 (dd, 1H, *J* = 11.0, 6.6 Hz, GalNAc_H-6a′), 4.10–4.00 (m, 3H, GalNAc_H-2′ + GalNAc_H-5 + GalNAc_H-6b′), 3.93–3.84 (m, 1H, GalNAc_H-5′), 3.52 (dd, 1H, *J* = 4.0, 1.8 Hz, GalNAc_H-4), 1.98 (s, 3H, NHAc), 1.95 (s, 3H, OAc), 1.85 (s, 3H, NHAc), 1.24 (s, 9H, Piv), 1.21 (m, 9H, Piv), 1.15 (m, 18H, Piv × 2), 1.14 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.28 (Piv × 2), 177.72 (Piv), 177.42 (Piv), 176.93 (Piv), 170.43 (NHAc), 170.36 (NHAc), 169.60 (OAc), 93.11 (C-1), 83.18 (C-1′), 74.45 (C-5′), 73.91 (C-5), 72.30 (C-3), 70.95 (C-3′), 66.08 (C-4′), 64.25 (C-6), 61.03 (C-6′), 50.88 (C-2), 50.86 (C-2′), 45.25 (C-4), 39.18 ($C(CH_3)_3 \times 2$), 39.04 ($C(CH_3)_3$), 38.70 ($C(CH_3)_3$), 38.66 ($C(CH_3)_3$), 27.22 ($C(CH_3)_3$), 27.09 ($C(CH_3)_3$), 27.04 ($C(CH_3)_3$), 27.00 ($C(CH_3)_3$), 26.77 ($C(CH_3)_3$), 23.33 (NHAc), 23.07 (NHAc), 20.55 (OAc). HRESIMS: Calcd for $C_{43}H_{71}N_2O_{16}S$ ($M+H^+$): *m/z* 903.4519. Found: 903.4512.

4.13. 4-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-thio-β-D-galactopyranose (18)

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-glucopyranose (**17**) was prepared according to the literature procedure.[13] Compound **17** (947 mg, 2.33 mmol) and the crude triflate **6** (1.6 g, 2.69 mmol) were dissolved in anhyd DMF (10 mL). Under argon, Et_2NH (2 mL) was added at −5 °C, and the reaction mixture was kept at −5 °C overnight. The solution was then extracted with EtOAc (40 mL) and H_2O (3 × 60 mL). The organic layer was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 36% EtOAc–hexane as the eluent to give compound **18** (917 mg, 43.7% yield over three steps). R_f: 0.64 (5:95 MeOH–CH_2Cl_2). $[\alpha]_D$ −5.6 (*c* 8.4, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ_H 5.59 (d, 1H, *J* = 10.5 Hz, NH), 5.52 (dd, 1H, *J* = 9.0 Hz, GalNAc_H-1), 5.16 (dd, 1H, *J* = 10.9, 4.2 Hz, GalNAc_H-3), 5.14–5.03 (m, 2H, Glu_H-3 + Glu_H-4), 4.99 (m, 1H,

J = 10.1, 10.1 Hz, Glu_H-2), 4.70 (d, 1H, J = 10.1 Hz, Glu_H-1), 4.43 (ddd, 1H, J = 10.7, 9.3, 9.3 Hz, GalNAc_H-2), 4.34 (dd, 1H, J = 12.2, 7.5 Hz, GalNAc_H-6a), 4.27 (dd, 1H, J = 12.1, 3.5 Hz, GalNAc_H-6b), 4.21 (dd, 1H, J = 12.4, 2.6 Hz, Glu_H-6a), 4.15 (dd, 1H, J = 12.5, 4.6 Hz, Glu_H-6b), 4.01 (ddd, 1H, J = 7.3, 3.4, 1.6 Hz, GalNAc_H-5), 3.58 (ddd, 1H, J = 9.5, 4.2, 2.6 Hz, Glu_H-5), 3.44 (dd, 1H, J = 4.2, 1.6 Hz, GalNAc_H-4), 2.10 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.83 (s, 3H, NAc), 1.22 (s, 9H, Piv), 1.16–1.14 (m, 18H, Piv × 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.47 (Piv), 178.30 (Piv), 176.95 (Piv), 170.62 (NHAc), 170.11 (OAc), 169.50 (OAc), 169.43 (OAc), 169.24 (OAc), 93.12 (GalNAc_C-1), 83.69 (Glu_C-1), 75.73 (Glu_C-5), 73.98 (GalNAc_C-5), 73.70 (Glu_C-3), 72.25 (GalNAc_C-3), 70.23 (Glu_C-2), 68.24 (Glu_C-4), 64.69 (GalNAc_C-6), 61.74 (Glu_C-6), 50.74 (GalNAc_C-2), 47.21 (GalNAc_C-4), 39.10 ($C(CH_3)_3$), 38.71 ($C(CH_3)_3$), 38.66 ($C(CH_3)_3$), 27.14 (C(CH_3)$_3$), 27.00 (C(CH_3)$_3$), 26.76 (C(CH_3)$_3$), 23.07 (NHAc), 20.63 (OAc), 20.61 (OAc), 20.53 (OAc), 20.50 (OAc). HRESIMS: Calcd for $C_{37}H_{57}NO_{17}SNa$ (M+Na$^+$): m/z 842.3239. Found: 842.3238.

4.14. 4-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-1,3,6-tri-*O*-pivaloyl-4-thio-β-D-galactopyranose (20)

2,3,4,6-Tetra-*O*-acetyl-1-*S*-acetyl-1-thio-β-D-galactopyranose (**19**) was prepared according to the literature procedure.[14] Compound **19** (993 mg, 2.44 mmol) and the crude triflate **6** (1.6 g, 2.69 mmol) were dissolved in anhyd DMF (10 mL). Under argon, Et_2NH (2 mL) was added at −5 °C, and the reaction was kept at −5 °C overnight. The solution was then extracted with EtOAc (40 mL) and H_2O (3 × 60 mL). The organic layer was dried over anhyd Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel using 36% EtOAc–hexane as the eluent to give compound **20** as a white solid. (777 mg, 37.0% yield over three steps). R_f: 0.64 (5:95 MeOH–CH_2Cl_2). $[\alpha]_D$ −3.5 (c 6.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.47 (d, 1H, J = 8.7 Hz, GalNAc_H-1), 5.39 (dd, 1H, J = 3.3, 0.7 Hz, Gal_H-4), 5.33 (d, 1H, J = 9.9 Hz, GalNAc_NH), 5.22–5.11 (m, 2H, Gal_H-2 + GalNAc_H-3), 4.90 (dd, 1H, J = 10.0, 3.4 Hz, Gal_H-3), 4.66 (d, 1H, J = 10.1 Hz, Gal_H-1), 4.48 (dd, 1H, J = 19.5, 10.0 Hz, GalNAc_H-2), 4.37 (dd, 1H, J = 12.2, 7.4 Hz, GalNAc_H-6a), 4.26 (dd, 1H, J = 12.2, 3.4 Hz, GalNAc_H-6b), 4.16–4.04 (m, 2H, Gal_H-6a + Gal_H-6b), 3.98 (1H, ddd, J = 7.2, 3.3, 1.5 Hz, GalNAc_H-5), 3.78 (ddd, 1H, J = 6.6, 6.6, 0.8 Hz, Gal_H5), 3.41 (dd, 1H, J = 3.9, 1.5 Hz, GalNAc_H4), 2.15 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.82 (s, 3H, NHAc), 1.21 (s, 9H, Piv), 1.16 (s, 9H, Piv), 1.15 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.78 (Piv), 178.57 (Piv), 177.34 (Piv), 170.63 (NHAc), 170.51 (OAc), 170.30 (OAc), 170.03 (OAc), 169.57 (OAc), 93.46 (GalNAc_C-1), 84.65 (Gal_C-1), 74.61(Gal_C-5), 74.38 (GalNAc_C-5), 72.75 (GalNAc_C-3), 71.96 (Gal_C-3), 67.67 (Gal_C-2), 67.18 (Gal_C-4), 65.00 (GalNAc_C-6), 61.45 (Gal_C-6), 51.00 (GalNAc_C-2), 47.52 (GalNAc_C-4), 39.37 ($C(CH_3)_3$), 38.99 ($C(CH_3)_3$), 38.94 ($C(CH_3)_3$), 29.89, 27.41 (C(CH_3)$_3$), 27.28 (C(CH_3)$_3$), 27.00 (C(CH_3)$_3$), 23.37 (NHAc), 21.01 (OAc), 20.91 (OAc), 20.83 (OAc), 20.78 (OAc). HRESIMS: Calcd for $C_{37}H_{57}NO_{17}SNa$ (M+Na$^+$): m/z 842.3239. Found: 842.3234.

4.15. 2-Acetamido-1,4-anhydro-2-deoxy-3,6-di-*O*-pivaloyl-β-D-galactopyranose (21)

Compound **7** (500 mg, 1.06 mmol) was added to 1,2-dichloroethane (7 mL) containing 4 Å molecular sieves (500 mg), and the mixture was stirred at room temperature for 0.5 h. Anhyd $FeCl_3$ (200 mg, 1.23 mmol) was added to the suspension and the reaction mixture was refluxed at 90 °C overnight. The mixture was filtered through Celite and the filtrate was evaporated. The residue was purified by column chromatography on silica gel using 15% acetone–toluene as the eluent to give compound **21** (105 mg, 26.8% yield) as a yellow solid. R_f: 0.44 (6:94 MeOH–DCM). $[\alpha]_D$ +47.1 (c 2.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 5.97 (d, 1H, J = 7.7 Hz, NH), 5.58 (d, 1H J = 2.6 Hz, H-1), 4.55 (dd, 1H, J = 1.1, <1 Hz, H-4), 4.46 (dd, 1H, J = 1.8, <1 Hz, H-3), 4.24 (dddd, 1H, J = 7.80, 2.80, 1.60, 1.60 Hz, H-2), 4.05 (dd, 1H, J = 11.1, 5.4 Hz, H-6a), 3.92 (dd, 1H, J = 11.1, 6.8 Hz, H-6b), 3.87 (ddd, 1H, J = 6.7, 5.5, <1 Hz, H-5), 1.99 (s, 3H, NHAc), 1.22 (s, 9H, Piv), 1.20 (s, 9H, Piv). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 178.20 (Piv), 178.14 (Piv), 170.15 (NHAc), 99.23 (C-1), 81.86 (C-4), 77.91 (C-3), 73.25 (C-5), 63.25 (C-6), 60.73 (C-2), 38.77 ($C(CH_3)_3$), 38.63 ($C(CH_3)_3$), 27.12 (C(CH_3)$_3$), 26.95 (C(CH_3)$_3$), 23.05 (NHAc). HRCIMS: Calcd for $C_{18}H_{30}NO_7$ (M+H$^+$): m/z 372.2022. Found: 372.2010.

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Supplementary data

Supplementary data (1D and 2D NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.09.008.

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