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ORIGINAL ARTICLE

Chemical synthesis of 4-azido-β-galactosamine derivatives for inhibitors of *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase

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Abstract

Chondroitin sulfate E (CS-E) plays a crucial role in diverse processes ranging from viral infection to neuroregeneration. Its regiospecific sulfation pattern, generated by *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST), is the main structural determinant of its biological activity. Inhibitors of GalNAc4S-6ST can serve as powerful tools for understanding physiological functions of CS-E and its potential therapeutic leads for human diseases. A family of new 4-acylamino-β-GalNAc derivatives and 4-azido-β-GalNAc derivatives were synthesized for their potential application as inhibitors of GalNAc4S-6ST. The target compounds were evaluated for their inhibitory activities against GalNAc4S-6ST. The results revealed that 4-pivaloylamino- and 4-azido-β-GalNAc derivatives displayed evident activities against GalNAc4S-6ST with IC_{50} value ranging from 0.800 to 0.828 mM. They showed higher activities than benzyl D-GalNAc4S that was used as control.

Keywords Sulfotransferase · *N*-Acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase · Inhibitor · Chemical synthesis

Abbreviations

CS-A	chondroitin sulfate A
CS-E	chondroitin sulfate E
GalNAc4S	2-acetamido-2-deoxy-4-*O*-sulfonato-D-galactopyranose
GalNAc4S-6ST	*N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase
GalNAc4S6S	2-acetamido-2-deoxy-4,6-di-*O*-sulfonato-D-galactopyranose
GlcA	D-glucuronic acid
PAP	3′-phosphoadenosine 5′-phosphate
PAPS	3′-phosphoadenosine 5′-phosphosulfate

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Introduction

Chondroitin sulfate E (CS-E), chondroitin sulfate isomer containing GlcAβ1-3GalNAc4S6S repeating unit, is found in various mammalian cells. CS-E is implicated in several physiological functions in various mammalian systems such as mast cell maturation [1], regulation of procoagulant activity of monocytes, neuronal polarization [2], promotion of osteoblast differentiation by binding to both *N*-cadherin and cadherin-11 [3], basic fibroblast growth factor [4], inhibition of P-selectin binding to a human breast cancer cell line *in vitro* [5], processes of neural development in brain [6], migration of neuronal precursors during cortical development [7], pulmonary metastasis [8], and enhancement of plasminogen activation [9]. In addition, CS-E has been reported to be strongly expressed in human ovarian adenocarcinomas. CS-E is not expressed in normal ovary [10] to function in metastasis of a Lewis lung carcinoma cell line [11] and to prevent excitatory amino acid-induced neuronal cell death [12]. Moreover, CS-E

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expressed in murine osteosarcoma LM8G7 cells is involved in focal formation of liver tumors [13].

N-Acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST: EC 2.8.2.33) is the sulfotransferase responsible for biosynthesis of highly sulfated CS-E that is expressed in the anterior visceral endoderm and accordingly is possible to play an active role during early mouse development [14]. Sulfated carbohydrate chains in glycoproteins and glycolipids play important roles in infection by microorganisms and diseases. Inhibitors of sulfotransferases, which are responsible for biosynthesis of these carbohydrate chains, are medical agents against such infections and diseases [15]. Previously, 4-[6-(morpholin-4-yl)-5-nitropyridin-2-yl] morpholine and 5-(4-fluorophenyl)-3-(4-methylphenyl)-1-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole, have been reported, which serve as inhibitors for GalNAc4S-6ST [16, 17]. Recently, (2*E*)-3-(3-bromo-4-hydroxy-5-methoxyphenyl)-2-cyano-*N*-(2,4-dichlorophenyl)prop-2-enamide has been found to be the first cell-permeable, small molecule to selectively inhibit the activity of GAG sulfotransferases as well as to reduce CS-E and overall sulfation levels on cell-surface [18]. We previously cloned GalNAc4S-6ST, that transfers sulfate from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the C-6 hydroxy group of the GalNAc4S residue of chondroitin sulfate A (CS-A). Thus CS-E containing GlcA-GalNAc4S6S repeating units is formed (Fig. 1).

The development of specific inhibitors of GalNAc4S-6ST is important to investigate the function of CS-E. Because GalNAc4S-6ST requires a group attached to the C-4 hydroxy group of the GalNAc residue as the acceptor, the sulfated GalNAc residue is expected to interact with GalNAc4S-6ST and affect its activity. We furthermore synthesized phenyl α- or β-2-acetamido-2-deoxy-D-galactopyranosides containing a sulfate group at the C-3, C-4, or C-6 hydroxy group and examined their inhibitory activities against recombinant GalNAc4S-6ST. We found that phenyl β-GalNAc4S inhibited GalNAc4S-6ST competitively and also served as an acceptor. The sulfated product derived from phenyl β-GalNAc4S was identical to phenyl β-GalNAc4S6S. These observations indicate that β-GalNAc4S derivatives are possible specific inhibitors of GalNAc4S-6ST [19]. After demonstrating that phenyl GalNAc4S could serve as an acceptor for GalNAc4S-6ST and thereby inhibit GalNAc4S-6ST competitively, we compared the inhibitory effects of various glycosides in which various hydrophobic aglycons were attached to D-GalNAc4S via β-anomeric configuration. *p*-Nitrophenyl and *p*-chlorophenyl D-GalNAc4S were stronger inhibitors than phenyl D-GalNAc4S. Among the examined inhibitors (Fig. 2) here, 3-estradiol β-D-GalNac4S was the strongest inhibitor. The K_i of 3-estradiol β- D-GalNac4S for the competitive inhibition was 0.008 mM, which was much lower than that of phenyl D-GalNAc4S, 0.98 mM [20]. Inspired by these results, we investigated and synthesized cost-effective and simple compounds that can serve as effective inhibitors for GalNAc4S-6ST.

In this report, we pursued multiple structural modification strategies (Fig. 3) to discover a novel class of small molecular inhibitor candidates for GalNAc4S-6ST. The sulfate group of phenyl β-GalNAc4S (**A**, R^1 = phenyl) was exchanged for substituted amides at 4-position, resulting in 4-acylamino-β-galactosamine derivatives **B**. In addition, 4-azido-β-galactosamine derivatives **C** were generated via the modification of GalNAc4S derivatives **A** by installing an azido group and different aglycons (R^1 = cyclohexyl, benzyl) into 4-position and 1-position respectively. 4-Acylamino-β-galactosamine derivatives **B** are sulfate analogue having deceased negative charge and high cell membrane permeability. One of our purposes was to synthesize selective cell membrane permeable inhibitors for GalNAc4S-6ST.

Results and discussion

Synthesis of amide derivatives B

As described in scheme 1, 2,2,2-trichloroethoxycarbonyl (Troc) protecting group having temporary amino-masking functionality was used as because it performed neighboring

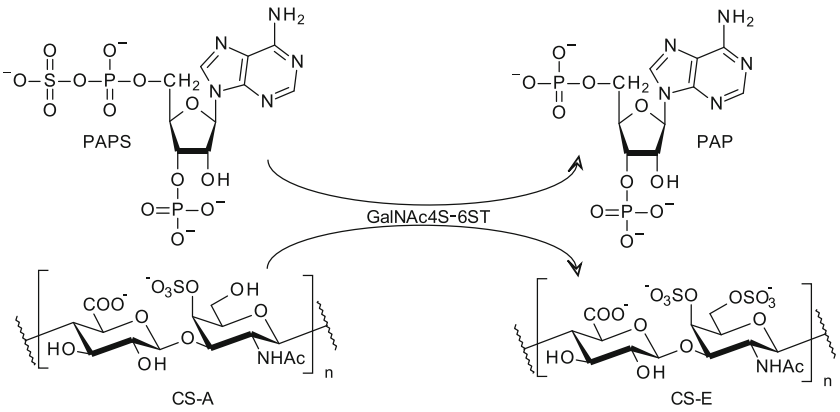


Fig. 1 Function of GalNAc4S-6ST

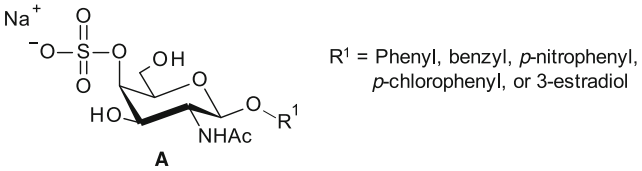


Fig. 2 Previous inhibitors **A**

group participation and accordingly allowed the introduction of β-glycosides. Phenyl glycoside **2** that has been reported previously [21] can be prepared by β-selective glycosylation of phenol with imidate donor **1** at −20 °C in CH_2Cl_2 in the presence of BF_3•OEt_2. We also conducted a TfOH-promoted glycosylation of phenol with thioglycoside donor at −20 °C to 0 °C in the presence of NIS in CH_2Cl_2. A complex mixture, however, was provided. Conversion the phenyl glycoside **2** to acetylated glucosamine **3** that has been documented [22] was accomplished by a two-step procedure. First, the Troc protecting group was deprotected with Zn and AcOH in MeOH and CH_2Cl_2. The acetylation of the amino group with Ac_2O was then performed in the presence of pyridine. Then, the transesterification of the acetyl esters of **3** afforded known compound **4** using sodium methoxide in MeOH [22–28]. Compound **4** has acetamido group at 2-position and three hydroxy groups. Subsequently, attention was focused on the regioselective installation of azido into the compound at 4-position. Numerous of conditions were examined to improve the selectivity of 3,6-di-*O*-pivaloylation of compound **4**. We found that the treatment of compound **4** using pivaloyl chloride in pyridine at 0 °C formed 3,4,6-tri-*O*-pivaloylated compound. A high selectivity was obtained when compound **4** was reacted with pivaloyl chloride in pyridine at −20 °C to give compound **5**. Compound **6** was afforded via the reaction between compound **5** and Tf_2O at −10 °C in the presence of pyridine and CH_2Cl_2 followed by S_N2 reaction with sodium azide. Compound **7** was easily prepared in 91% yield by depivaloylation of **6** using sodium methoxide in MeOH. The azido moiety of **7** was reduced with hydrogen over Pd-C in the mixture of 95% EtOH-H_2O to give amine. Subsequently, the amino group was selectively *N*-acylated with acetic anhydride, benzoyl chloride, and pivaloyl chloride to afford compound **8**, **9**, and **10**, respectively.

Synthesis of 4-azido-β-galactosamine derivatives C

As shown in scheme 2, NIS-promoted glycosylation of selected alcohols (in this study: cyclohexanol, benzyl alcohol) with glycosyl donor **11** in CH_2Cl_2 at −10 °C gave compound **12a** [29–34] in 90% yield, and **12b** [35–37]. Next, the Ac protecting groups of **12a** were removed by sodium methoxide in MeOH to give GalNAc derivative **13a** in a near-quantitative yield. Selective 3,6-di-*O*-pivaloylation of **13a** with pivaloyl chloride and pyridine in CH_2Cl_2 at 0 °C afforded glucosamine **14a** in good yield. In this approach, to avoid pivaloylation of hydroxy group at 4-position due to the steric hindrance which might be caused by Troc group, the selective 3,6-di-*O*-pivaloylation had been conducted before removing the Troc group. Then, removal of Troc group of **14a** using Zn and AcOH in MeOH and CH_2Cl_2 gave a free amine derivative that was converted into acetamido derivative **15a** by reaction with Ac_2O in the presence of pyridine in CH_2Cl_2. Compound **16a** was obtained by the treatment of **15a** with Tf_2O at −10 °C in the presence of pyridine and CH_2Cl_2 followed by azidation with NaN_3 at rt. in DMF. Thence, compound **17a** was obtained by depivaloylation of **16a**.The same reaction sequence in high yield was applied to precursor **12b** to afford **13b** [37], **14b**, **15b** [38, 39], **16b**, and **17b**.

Biological activity

The compounds (**7**, **8**, **9**, **10**, **17a**, and **17b**) were tested as inhibitors of GalNAc4S-6ST. The result of the GalNAc4S-6ST test was expressed as the inhibition that indicates the inhibitor concentration at which 50% of inhibitory activity occurs (IC_{50}) (Figs. 4 and 5). The inhibition is then compared

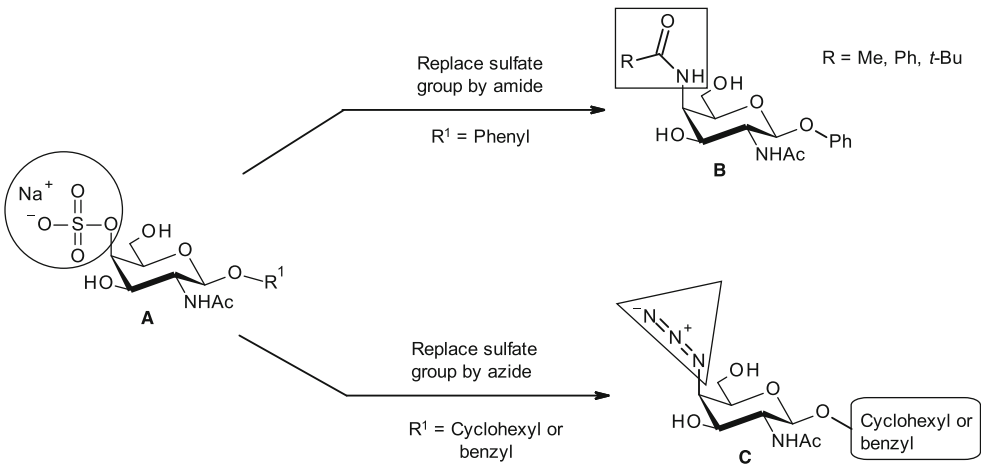
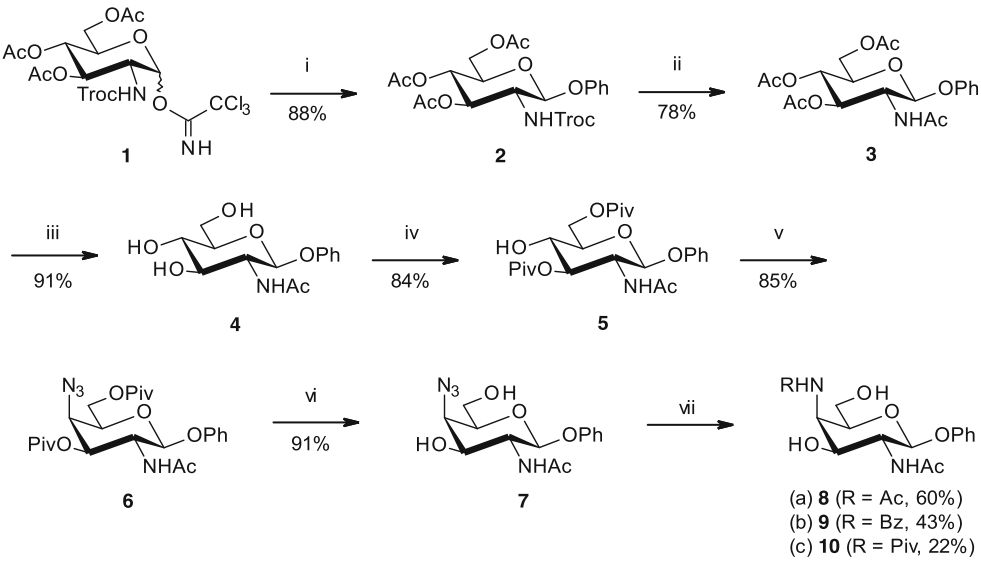


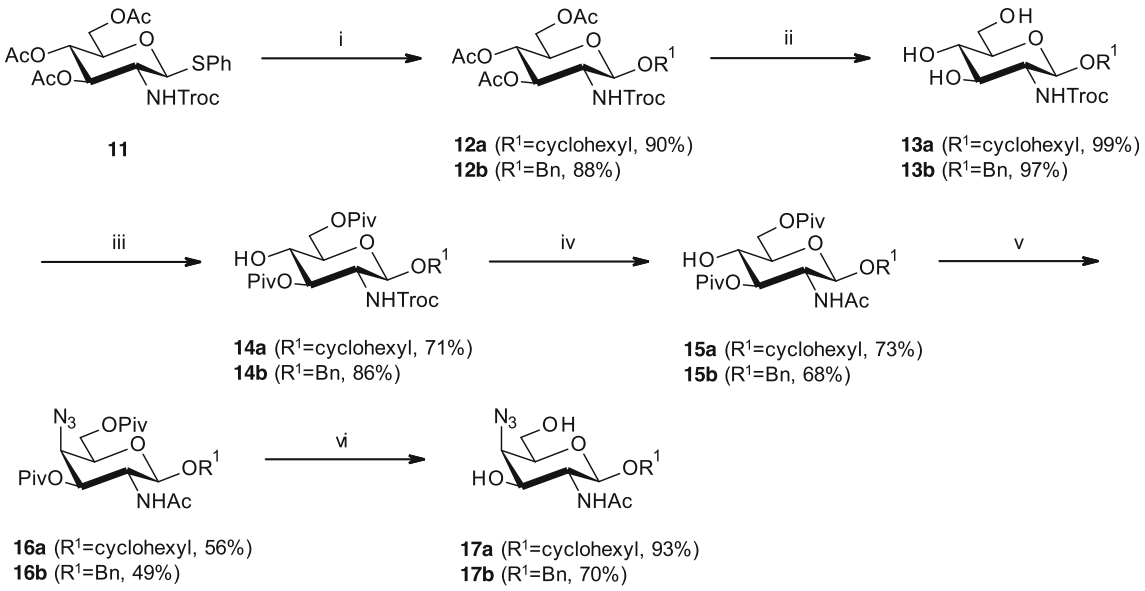
Fig. 3 Structural modification



Scheme 1 Synthesis of amide derivatives **8**, **9**, and **10**. i) Phenol, BF_3·OEt_2, MS4A, CH_2Cl_2, −20 °C, 2.5 h; ii) 1) Zn, AcOH, MeOH, CH_2Cl_2, rt., 2.5 h, 2) Ac_2O, pyridine, rt., 2.5 h; iii) NaOMe, MeOH, rt., 1.5 h; iv) PivCl, pyridine, −20 °C, 14 h; v) 1) Tf_2O, pyridine, CH_2Cl_2, −10 °C, 1 h, 2) NaN_3, DMF, rt., 28 h; vi) NaOMe, MeOH, rt., 27.5 h; vii) 1) H_2, Pd-C, 95%EtOH-H_2O, rt., 14.5 h, 2) (a) Ac_2O, 0 °C, 2 h, (b) BzCl, 0 °C, 2 h, (c) PivCl, 0 °C, 40 min

with that of a reference inhibitor such as benzyl D-GalNAc4S, which is a small molecular inhibitor against GalNAc4S-6ST, to give relative inhibition. The results of the tested compounds are summarized in Table 1 below.

Compounds **7**, **10**, **17a**, and **17b** showed approximately 1.36-fold improvement in activity relative to **benzyl D-GalNAc4S**, which were more effective than compounds **8**, **9**. This result implied that 4-azido-β-GalNAc derivatives are more preferable for the activity because of the linear structure of azido group to enhance its proximity to the GalNAc4S-6ST binding site and the assistance from the binding site-orientated 6-hydroxy group's lone pairs. The result also suggested that functional groups at 4-position were more influential in inhibitory activity than aglycons. Moreover, modification of the linear azido group to 1,1,1-trimethylacetamido group (**10**) that has bulky and restrictive conformation led to no change in potency. The modification to acetamido (**8**) and benzamido (**9**) groups decreased the potency by 42 and 26%, respectively. These results suggest that the bulky group at 4-position restricted its conformation and the rotation of 6-hydroxy group resulted in strong hydrogen bonds. Thus, specific structure and electronic properties of the amide would be important for the interaction with GalNAc4S-6ST.



Scheme 2 Synthesis of 4-azido-β-galactosamine derivatives **17a** and **17b**. i) R^1OH, NIS, TfOH, MS4A, CH_2Cl_2, −10 °C, 1 to 23 h; ii) NaOMe, MeOH, −5 to 0 °C, 1.5 to 2.5 h; iii) PivCl, pyridine, CH_2Cl_2, −10 to 0 °C, 3 to 20 h; iv) 1) Zn, AcOH, MeOH, CH_2Cl_2, rt., 2 to 4 h, 2) Ac_2O, pyridine, CH_2Cl_2, rt., 16 to 18 h; v) 1) Tf_2O, pyridine, CH_2Cl_2, −10 °C, 1.5 to 2.5 h, 2) NaN_3, DMF, rt., 17 to 17.5 h; vi) NaOMe, MeOH, 0 °C, 17.5 to 22.5 h

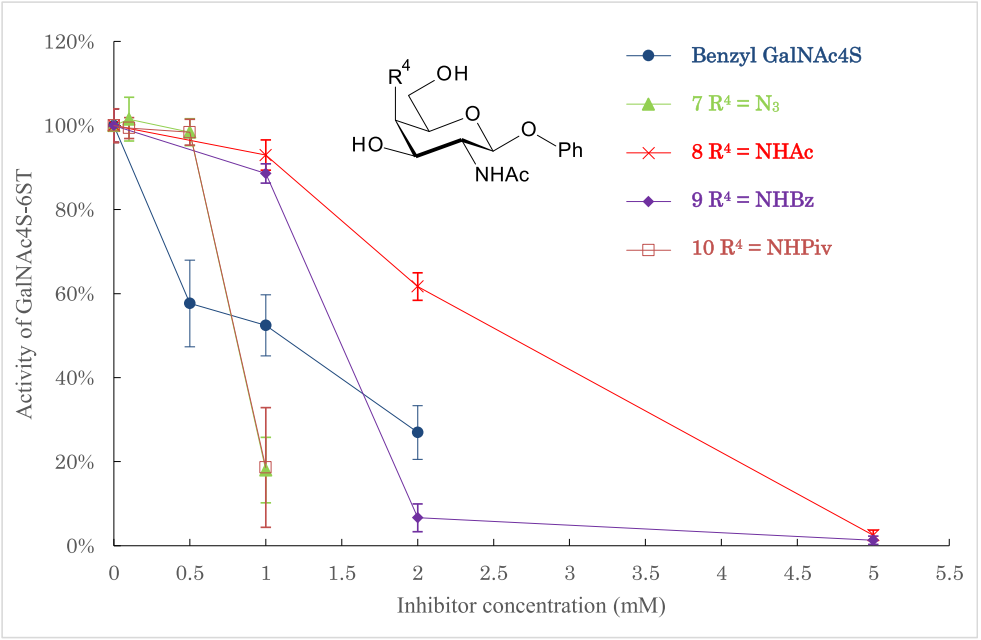


Fig. 4 Inhibitory effects on GalNAc4S-6ST by azido and amido derivatives **7**, **8**, **9**, and **10**. Bars show standard deviation of triplicate measurements in each experiment

Conclusion

In summary, we described a new approach for the chemical synthesis of 4-azido-β-GalNAc derivatives. In addition, we discovered that 4-azido-β-GalNAc derivatives greatly inhibited the activity of GalNAc4S-6ST. A specific functional group rather than aglycon was important for the inhibitory activity. Moreover, specific structure and electronic properties of the amide would be important for the interaction with GalNAc4S-6ST. The applications of click chemistry and Staudinger ligation reaction on these molecules to afford libraries of potential inhibitors for GalNAc4S-6ST and monosaccharide-linked biomolecules are currently underway.

Experimental

General methods

All solvents were of reagent grade quality and purchased commercially. Structures of synthetic compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and two-dimensional NMR (COSY, HSQC, HMBC, and NOESY) spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVANCE III instrument operating at 400.13 and 100.62 MHz, respectively. Chemical shifts were referenced to TMS in $CDCl_3$ and δ values (ppm) of water in D_2O (^{1}H: δ = 4.70) as internal standard. Electrospray ionization

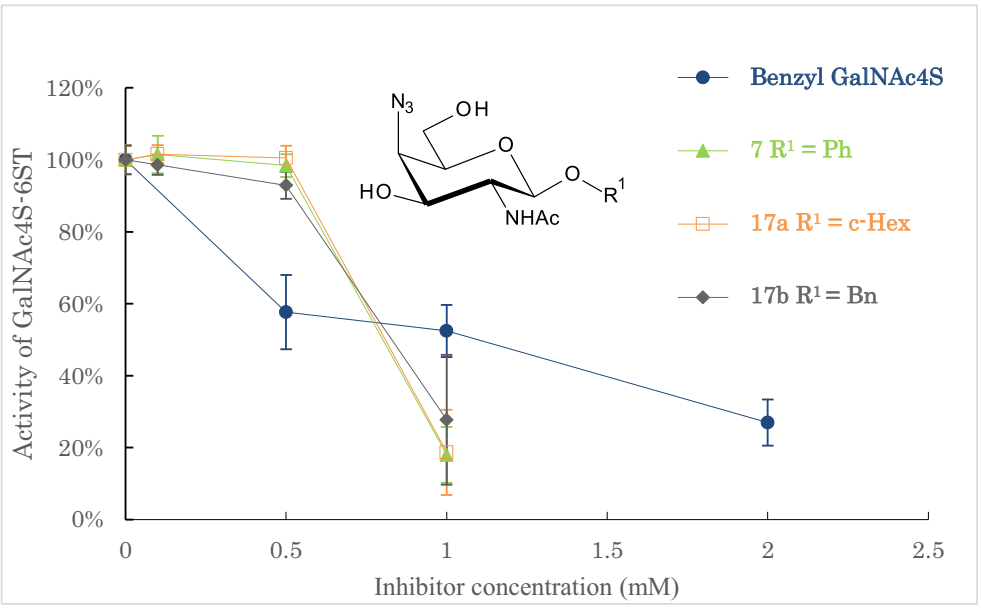


Fig. 5 Inhibitory effects on GalNAc4S-6ST by **benzyl GalNAc4S** and azido compounds **7**, **17a**, and **17b**. Values are averages of three determinations. Bars show standard deviation of triplicate measurements in each experiment

Table 1 Inhibition of GalNAc4S-6ST

Test compound (R^1, R^4)	IC_{50} (mM)	Relative inhibition
Benzyl D-GalNAc4S (Bn, OSO_3^-)	1.09	1
7 (Ph, N_3)	0.800	1.36
8 (Ph, NHAc)	2.59	0.421
9 (Ph, NHBz)	1.47	0.741
10 (Ph, NHPiv)	0.802	1.36
17a (c-Hex, N_3)	0.804	1.36
17b (Bn, N_3)	0.828	1.32

(ESI) and atmospheric pressure chemical ionization (APCI) mass spectra were obtained on a Bruker Daltonics micrOTOF-QII. Thin layer chromatography (TLC) was performed on pre-coated Silica gel 60 F_{254} plates. Column chromatography was performed on silica gel 60 N (spherical neutral) that was purchased from Kanto Chemical Company, Japan.

General procedure a (Troc removal and *N*-acetylation)

Activated zinc and AcOH were add to a solution of respective monosaccharide in CH_2Cl_2 and MeOH. The mixture was stirred at temperature and reaction time as described individually. After quenching with saturated aq $NaHCO_3$, the mixture was passed through a column of the Celite washing with chloroform. The filtrate was washed using saturated aq $NaHCO_3$, saturated aq NaCl, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2, and the solution was cooled to 0 °C. After adding pyridine and Ac_2O, the solution was stirred at temperature and reaction time as described individually. The reaction mixture was quenched using MeOH before concentrated under reduced pressure. The residue was dissolved in $CHCl_3$. The resulting solution was washed with 5% aq HCl, saturated aq $NaHCO_3$, and saturated aq NaCl, before it was dried over Na_2SO_4, and concentrated under reduced pressure to provide the respective crude product that was purified as described individually.

General procedure B (pivaloylation)

Pivaloyl chloride was added to a cooled solution of respective monosaccharide in dry pyridine. The mixture was stirred at temperature and reaction time as mentioned individually. After the addition of MeOH, the mixture was concentrated under diminished pressure. The residue was diluted with $CHCl_3$ and washed with 2.5% aq HCl, saturated aq $NaHCO_3$, and saturated aq NaCl. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give the respective crude product that was purified as described individually.

General procedure C (azidation)

Trifluoromethanesulfonic anhydride was added to a cooled solution of respective monosaccharide in pyridine and CH_2Cl_2. The mixture was stirred at temperature and reaction time as recorded individually. After quenching with 5% aq HCl, the mixture was extracted with $CHCl_3$. The organic layer was washed with 5% aq HCl, saturated aq $NaHCO_3$, and saturated aq NaCl, before it was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in DMF, and sodium azide was added to the resulting solution. The mixture was stirred at temperature and reaction time as described individually. Then, the reaction mixture was extracted using ethyl acetate. The organic layer was washed with saturated aq $NaHCO_3$, saturated aq NaCl, dried over Na_2SO_4, and concentrated under reduced pressure to afford the respective crude product that was purified as reported individually.

General procedure D (deacylation)

A solution of the respective starting material in dry MeOH was treated with 1.0 M sodium methoxide in MeOH. The mixture was then stirred at temperature and reaction time as reported individually. Next, the mixture was neutralized with Amberlite IRC-50 H^+ resin and concentrated under diminished pressure to give the respective crude product that was purified as described individually.

General procedure E (reduction and *N*-acylation)

Pd-C (5%) was added to a solution of the respective monosaccharide in 95% *v*/v EtOH-H_2O. The mixture was stirred under hydrogen at temperature and reaction time as described individually. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was dissolved in EtOH, and the solution was cooled to 0 °C. Ac_2O was added to the solution. The mixture was stirred at temperature and reaction time as documented individually. The mixture was concentrated under reduced pressure to give the respective crude product that was purified as described individually.

General procedure F (glycosylation)

A mixture of thioglycoside, respective alcohol, *N*-iodosuccinimide, and MS4A in dry CH_2Cl_2 was stirred at temperature and reaction time as described individually. A solution of 1.0 M trifluoromethanesulfonic acid in CH_2Cl_2 was added to the solution and stirred. After quenching with saturated aq $NaHCO_3$, the reaction mixture was passed through a column of the Celite washing $CHCl_3$. The filtrate was washed with 5% aq $Na_2S_2O_3$, saturated aq $NaHCO_3$,

saturated aq NaCl, before it was dried over Na_2SO_4 and thence concentrated under reduced pressure. The respective crude product was afforded and purified as described individually.

General procedure G (zinc activation)

Zinc powder was added to a 5% aq HCl and then the mixture was sonicated for 30 min. Next, the mixture was filtered with suction. The residue (activated zinc) was first washed using H_2O, MeOH, and finally Et_2O.

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamino-β-D-glucopyranoside (2).

Boron trifluoride diethyl etherate (0.325 mL, 367 mg, 2.59 mmol, 2.0 equiv) was added to a cooled (−20 °C) mixture of **1** (824 mg, 1.32 mmol, 1.0 equiv), phenol (0.500 mL, 536 mg, 5.69 mmol, 4.3 equiv), and MS4A in dichloromethane (10 mL). The mixture was stirred for 2.5 h. After quenching with saturated aq $NaHCO_3$ (50 mL), the reaction mixture was passed through a column of the Celite washing with chloroform (50 mL). The organic layer was washed using saturated aq $NaHCO_3$, saturated aq NaCl, before it was dried over Na_2SO_4 and thence concentrated under reduced pressure to give, after separated by silica gel column chromatography eluting with 50% *v*/v ethyl acetate-hexane, product **2** (643 mg, 1.15 mmol, 88%) as colorless crystals. R_f = 0.48 (50% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 2.06 (s, 3 H, 4-OCOCH_3), 2.07 (s, 3 H, 3-OCOCH_3), 2.09 (s, 3 H, 6-OCOCH_3), 3.87 (ddd, 1 H, $J_{4,5}$ = 10.1 Hz, $J_{5,6b}$ = 5.3 Hz, $J_{5,6a}$ = 2.2 Hz, H-5), 3.90 (dt, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{1,2}$ = 8.2 Hz, $J_{2,NH}$ = 8.0 Hz, H-2), 4.17 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.2 Hz, H-6a), 4.32 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 5.3 Hz, H-6b), 4.71 (d, 1 H, J_{gem} = 12.0 Hz, Ha of Troc), 4.76 (d, 1 H, J_{gem} = 12.0 Hz, Hb of Troc), 5.15 (t, 1 H, $J_{4,5}$ = 10.1 Hz, $J_{3,4}$ = 9.7 Hz, H-4), 5.23 (d, 1 H, $J_{2,NH}$ = 8.0 Hz, 2-N*H*COOCH$_2$CCl$_3$), 5.25 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1), 5.43 (t, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.7 Hz, H-3), 6.98–7.01 (m, 2 H, *o*-arom. H), 7.04–7.09 (m, 1 H, *p*-arom. H), 7.26–7.31 (m, 2 H, *m*-arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz):δ 20.64 (q, 4-OCO*C*H$_3$), 20.66 (q, 3-OCO*C*H$_3$), 20.71 (q, 6-OCO*C*H$_3$), 56.33 (d, C-2), 62.06 (t, C-6), 68.54 (d, C-4), 71.50 (d, C-3), 72.01 (d, C-5), 74.45 (t, 2-NHCOO*C*H$_2$CCl$_3$), 95.35 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 99.00 (d, C-1), 116.96 (d, *o*-arom. CH), 123.36 (d, *p*-arom. CH), 129.56 (d, *m*-arom. CH), 153.99 (s, 2-NH*C*OOCH$_2$CCl$_3$), 156.88 (s, arom. C), 169.47 (s, 4-O*C*OCH$_3$), 170.58 (s, 3-O*C*OCH$_3$), 170.61 (s, 6-O*C*OCH$_3$); HRMS (APCI) m/z [M + H]$^+$ calcd for $C_{21}H_{25}Cl_3NO_{10}^+$ 556.0539, found 556.0538.

Phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (3)

Following general procedure A, a solution of compound **2** (5.606 g, 10.07 mmol, 1.0 equiv) in dichloromethane (10 mL) and methanol (20 mL) was treated with activated zinc (11.252 g, 0.172 mol, 17 equiv) and acetic acid (10 mL) at room temperature for 2.5 h followed by acetylation with acetic anhydride (2.80 mL, 3.02 g, 29.6 mmol, 2.9 equiv) in the presence of pyridine (0.780 mL, 0.764 g, 9.66 mmol, 0.96 equiv) at room temperature for 2.5 h to give, after separated by silica gel column chromatography eluting with a gradient from 33% *v*/v ethyl acetate-hexane to 100% ethyl acetate, compound **3** (3.312 g, 7.82 mmol, 78%) as colorless crystals. R_f = 0.40 (5% v/v methanol-chloroform); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.96 (s, 3 H, 2-NHCOCH_3), 2.05 (s, 3 H, 4-OCOCH_3), 2.07 (s, 3 H, 3-OCOCH_3), 2.08 (s, 3 H, 6-OCOCH_3), 3.87 (ddd, 1 H, $J_{4,5}$ = 9.9 Hz, $J_{5,6b}$ = 5.4 Hz, $J_{5,6a}$ = 2.5 Hz, H-5), 4.13 (dt, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{2,NH}$ = 8.7 Hz, $J_{1,2}$ = 8.2 Hz, H-2), 4.16 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.5 Hz, H-6a), 4.30 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 5.4 Hz, H-6b), 5.15 (t, 1 H, $J_{4,5}$ = 9.9 Hz, $J_{3,4}$ = 9.3 Hz, H-4), 5.27 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1), 5.41 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.60 (d, 1 H, $J_{2,NH}$ = 8.7 Hz, 2-N*H*COCH$_3$), 6.99 (d, 2 H, $J_{o,m}$ = 8.5 Hz, *o*-arom. H), 7.06 (t, 1 H, $J_{m,p}$ = 7.4 Hz, *p*-arom. H), 7.28 (dd, 2 H, $J_{o,m}$ = 8.5 Hz, $J_{m,p}$ = 7.4 Hz, *m*-arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 20.65 (q, 6-OCO*C*H$_3$), 20.71 (q, 3,4-OCO*C*H$_3$), 23.37 (q, 2-NHCO*C*H$_3$), 54.86 (d, C-2), 62.15 (t, C-6), 68.51 (d, C-4), 71.98 (d, C-3), 72.03 (d, C-5), 98.94 (d, C-1), 116.89 (d, *o*-arom. CH), 123.18 (d, *p*-arom. CH), 129.55 (d, *m*-arom. CH), 157.00 (s, arom. C), 169.42 (s, 4-O*C*OCH$_3$), 170.32 (s, 2-NH*C*OCH$_3$), 170.64 (s, 6-O*C*OCH$_3$), 170.87 (s, 3-O*C*OCH$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{20}H_{26}NO_6^+$ 424.1602, found 424.1613.

Phenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (4)

Following general procedure D, compound **3** (3.312 g, 7.822 mmol, 1.0 equiv) was treated with 1.0 M NaOMe-MeOH (1.50 mL, 1.50 mmol, 0.19 equiv) at room temperature for 1.5 h to provide **4** (2.126 g, 7.151 mmol, 91%) as a colorless solid. R_f = 0.40 (20% *v*/v methanol-chloroform); ^{1}H NMR (D_2O, 400.13 MHz): δ 1.91 (s, 3 H, 2-NHCOCH_3), 3.43 (t, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, H-4), 3.51 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 5.3 Hz, $J_{5,6b}$ = 2.1 Hz, H-5), 3.53 (dd, 1 H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 3.67 (dd, 1 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 5.3 Hz, H-6a), 3.83 (dd, 1 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 2.1 Hz, H-6b), 3.86 (dd, 1 H, $J_{2,3}$ = 10.1 Hz, $J_{1,2}$ = 8.4 Hz, H-2), 5.04 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 6.96 (d, 2 H, $J_{o,m}$ = 7.6 Hz, *o*-arom. H), 7.03 (t, 1 H, $J_{p,m}$ = 7.5 Hz, *p*-arom. H), 7.24–7.29 (m, 2 H, *m*-arom. H); ^{13}C NMR (D_2O, 100.62 MHz): δ 22.04 (q, 2-NHCO*C*H$_3$), 55.48 (d, C-2), 60.49 (t, C-6), 69.65 (d, C-4), 73.51 (d, C-3), 76.06 (d, C-5), 99.57 (d, C-1), 116.62 (d, *o*-arom. CH), 123.41 (d, *p*-arom. CH), 129.92 (d, *m*-arom. CH), 156.66 (s, arom. C), 174.87 (s, 2-NH*C*OCH$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{14}H_{20}NO_6^+$ 298.1285, found 298.1296.

Phenyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (5)

Following general procedure B, compound **4** (2.126 g, 7.151 mmol, 1.0 equiv) was reacted with pivaloyl chloride (3.60 mL, 3.528 g, 29.26 mmol, 4.1 equiv) in dry pyridine (30 mL, 0.24 M) at −20 °C for 14 h to give, after silica gel chromatography eluting with a gradient from 30% *v*/v ethyl acetate-hexane to 100% ethyl acetate, compound **5** (2.798 g, 6.010 mmol, 84%) as colorless crystals. R_f = 0.33 (50% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.205 (s, 9 H, 6-OCOC (CH_3)$_3$), 1.208, (s, 9 H, 3-OCOC (CH_3)$_3$), 1.89 (s, 3 H, 2-NHCOCH_3), 3.26 (d, 1 H, $J_{4,OH}$ = 6.2 Hz, 4-O*H*), 3.58 (td, 1 H, $J_{4,5}$ = 9.6 Hz, $J_{3,4}$ = 9.2 Hz, $J_{4,OH}$ = 6.2 Hz, H-4), 3.79 (ddd, 1 H, $J_{4,5}$ = 9.6 Hz, $J_{5,6a}$ = 6.6 Hz, $J_{5,6b}$ = 2.0 Hz, H-5), 4.20 (dt, 1 H, $J_{2,3}$ = 10.4 Hz, $J_{2,NH}$ = 9.0 Hz, $J_{1,2}$ = 8.3 Hz, H-2), 4.31 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 6.6 Hz, H-6a), 4.47 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 2.0 Hz, H-6b), 5.14 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 5.28 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 6.05 (d, 1 H, $J_{2,NH}$ = 9.0 Hz, 2-N*H*COCH$_3$), 7.00 (d, 2 H, $J_{o,m}$ = 7.7 Hz, *o*-arom. H), 7.02 (t, 1 H, $J_{p,m}$ = 7.4 Hz, *p*-arom. H), 7.24 (t, 2 H, $J_{o,m}$ = 7.7 Hz, $J_{p,m}$ = 7.4 Hz, *m*-arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.17 (q, 2-NHCO*C*H$_3$), 27.01 (q, 3-OCOC (*C*H$_3$)$_3$), 27.14 (q, 6-OCOC (*C*H$_3$)$_3$), 38.85 (s, 6-OCO*C* (CH$_3$)$_3$), 39.00 (s, 3-OCO*C* (CH$_3$)$_3$), 54.07 (d, C-2), 63.56 (t, C-6), 69.54 (d, C-4), 74.36 (d, C-5), 74.73 (d, C-3), 99.25 (d, C-1), 116.80 (d, *o*-arom. CH), 122.86 (d, *p*-arom. CH), 129.40 (d, *m*-arom. CH), 157.29 (s, arom. C), 170.13 (s, 2-NH*C*OCH$_3$), 179.01 (s, 6-O*C*OC (CH$_3$)$_3$), 179.86 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (FAB) *m/z* [M + H]$^+$ calcd for $C_{24}H_{36}NO_8^+$ 466.2435, found 466.2455.

Phenyl 2-acetamido-4-azido-2,4-dideoxy-3,6-di-*O*-pivaloyl-β-D-galactopyranoside (6)

Following general procedure C, compound **5** (0.532 g, 1.14 mmol, 1.0 equiv) in pyridine (2 mL) and dichloromethane (2 mL) was reacted with trifluorometha nesulfonic anhydride (0.288 mL, 0.495 g, 1.76 mmol, 1.5 equiv) at −10 °C for 1 h followed by treatment with sodium azide (167.6 mg, 2.578 mmol, 2.3 equiv) in DMF (5 mL) at room temperature for 28 h to afford, after silica gel column chromatography eluting with 35% *v*/v ethyl acetate-hexane, compound **6** (0.476 g, 0.970 mmol, 85%) as colorless crystals. R_f = 0.48 (50% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.22 (s, 9 H, 6-OCOC (CH_3)$_3$), 1.26 (s, 9 H, 3-OCOC (CH_3)$_3$), 1.93 (s, 3 H, 2-NHCOCH_3), 4.01 (dd, 1 H, $J_{5,6b}$ = 6.9 Hz, $J_{5,6a}$ = 5.5 Hz, H-5), 4.02 (d, 1 H, $J_{3,4}$ = 3.7 Hz, H-4), 4.24 (dt, 1 H, $J_{2,3}$ = 11.1 Hz, $J_{2,NH}$ = 8.5 Hz, $J_{1,2}$ = 8.3 Hz, H-2), 4.25 (dd, 1H, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 5.5 Hz, H-6a), 4.32 (dd,1 H, $J_{6a,6b}$ = 11.2, Hz, $J_{5,6b}$ = 6.9 Hz, H-6b), 5.26 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 5.55 (dd, 1 H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 3.7 Hz, H-3), 5.59 (d, 1 H, $J_{2,NH}$ = 8.5 Hz, 2-N*H*COCH$_3$), 6.99 (d, 2 H, $J_{o,m}$ = 7.7 Hz, *o*-arom. H), 7.03 (t, 1 H, $J_{m,p}$ = 7.3 Hz, *p*-arom. H), 7.24 (t, 2 H, $J_{o,m}$ = 7.7 Hz, $J_{m,p}$ = 7.3 Hz, *m*-arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.32 (q, 2-NHCO*C*H$_3$), 27.04 (q, 3-OCOC (*C*H$_3$)$_3$), 27.12 (q, 6-OCOC (*C*H$_3$)$_3$), 38.75 (s, 6-O-CO*C* (CH$_3$)$_3$), 39.24 (s, 3-O-CO*C* (CH$_3$)$_3$), 52.08 (d, C-2), 60.49 (d, C-4), 63.00 (t, C-6), 71.04 (d, C-5), 71.21 (d, C-3), 98.89 (d, C-1), 116.90 (d, *o*-arom. CH), 123.02 (d, *p*-arom. CH), 129.42 (d, *m*-arom. CH), 157.03 (s, arom. C), 170.17 (s, 2-NH*C*OCH$_3$), 178.00 (s, 6-O-*C*OC (CH$_3$)$_3$), 178.09 (s, 3-O-*C*OC (CH$_3$)$_3$); HRMS (FAB) *m/z* [M + H]$^+$ calcd for $C_{24}H_{35}N_4O_7^+$ 491.2500, found 491.2486.

Phenyl 2-acetamido-4-azido-2,4-dideoxy-β-D-galactopyranoside (7)

Following general procedure D, compound **6** (646 mg, 1.32 mmol, 1.0 equiv) in dry methanol (13 mL, 0.10 M) was treated with 1.0 M NaOMe-MeOH (5.27 mL, 5.27 mmol, 4.0 equiv) at room temperature for 27.5 h to provide, after silica gel chromatography eluting with a gradient from 10% *v*/v to 50% v/v methanol-chloroform, compound **7** (388 mg, 1.20 mmol, 91%) as a colorless solid. R_f = 0.50 (20% *v*/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 2.00 (s, 3 H, 2-NHCOCH_3), 3.72–3.79 (m, 3 H, H-5, H-6a, H-6b), 4.00 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4), 4.07 (dd, 1 H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.5 Hz, H-3), 4.13 (dd, 1 H, $J_{2,3}$ = 10.6 Hz, $J_{1,2}$ = 8.0 Hz, H-2), 5.02 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1), 7.00–7.03 (m, 3 H, *o*-arom. H, *p*-arom. H), 7.28 (t, 2 H, $J_{m,p}$ = 7.8 Hz, $J_{o,m}$ = 8.2 Hz *m*-arom. H); ^{13}C NMR (CD_3OD, 100.62 MHz): δ 21.57 (q, 2-NHCO*C*H$_3$), 53.23 (d, C-2), 60.74 (t, C-6), 62.39 (d, C-4), 71.51 (d, C-3), 73.76 (d, C-5), 99.75 (d, C-1), 116.34 (d, *o*-arom. CH), 122.21 (d, *p*-arom. CH), 129.07 (d, *m*-arom. CH), 157.68 (s, arom. C), 172.81 (s, 2-NH*C*OCH$_3$); HRMS (FAB) *m/z* [M + Na]$^+$ $C_{14}H_{18}N_4O_5Na^+$ 345.1169, found 345.1185.

Phenyl 2,4-diacetamido-2,4-dideoxy-β-D-galactopyranoside (8)

The general procedure E with compound **7** (32 mg, 99.3 μmol, 1.0 equiv), 5% Pd-C (34 mg) and acetic anhydride (0.10 mL, 108 mg, 1.06 mmol, 10.7 equiv), followed by silica gel column chromatography eluting with 5% *v*/v methanol-chloroform gave **8** (20 mg, 59.1 μmol, 60%) as colorless crystals. R_f = 0.41 (20% v/

v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 2.01 (s, 3 H, 2-NHCOCH_3), 2.09 (s, 3 H, 4-NHCOCH_3), 3.58 (dd, 1 H, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6a}$ = 5.6 Hz, H-6a), 3.64 (dd, 1 H, $J_{6a,6b}$ = 11.7 Hz, $J_{5,6b}$ = 6.9 Hz, H-6b), 3.80 (ddd, 1 H, $J_{5,6b}$ = 6.9 Hz, $J_{5,6a}$ = 5.6 Hz, $J_{4,5}$ = 1.4 Hz, H-5), 3.92 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 4.4 Hz, H-3), 4.16 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{1,2}$ = 8.5 Hz, H-2), 4.47 (dd, 1 H, $J_{3,4}$ = 4.4 Hz, $J_{4,5}$ = 1.4 Hz, H-4,), 5.00 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 7.02 (t, 1 H, $J_{m,p}$ = 7.3 Hz, *p*-arom. H), 7.04 (d, 2 H, $J_{o,m}$ = 8.7 Hz, *o*-arom. H), 7.29 (dd, $J_{o,m}$ = 8.7 Hz, $J_{m,p}$ = 7.3 Hz, *m*-arom. H); ^{13}C NMR (CD_3OD, 100.62 MHz), δ 21.08 (q, 4-NHCO*C*H$_3$), 21.55 (q, 2-NHCO*C*H$_3$), 50.13 (d, C-4), 53.29 (d, C-2), 60.83 (t, C-6), 70.21 (d, C-3), 74.94 (d, C-5), 100.24 (d, C-1), 116.29 (d, *o*-arom. CH), 122.18 (d, *p*-arom. CH), 129.10 (d, *m*-arom. CH), 157.78 (s, arom. C), 172.78 (s, 2-NH*C*OCH$_3$), 173.70 (s, 4-NH*C*OCH$_3$); HRMS (FAB) *m/z* $[M + H]^+$ calcd for $C_{16}H_{23}N_2O_6^+$ 339.1551, found 339.1562.

Phenyl 2-acetamido-4-benzamido-2,4-dideoxy-β-D-galacto pyranoside (9)

The general procedure E with compound **7** (32 mg, 99.3 μmol, 1.0 equiv), 5% Pd-C (30 mg) and benzoyl chloride (30 μL, 36.4 mg, 0.260 mmol, 2.6 equiv), followed by silica gel column chromatography eluting with 5% *v*/v methanol-chloroform gave **9** (17 mg, 42.5 μmol, 43%) as colorless crystals. R_f = 0.60 (20% v/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 2.02 (s, 3 H, 2-NHCOCH_3), 3.70 (d, 2 H, $J_{5,6}$ = 6.2 Hz, H-6), 3.92 (td, 1 H, $J_{5,6}$ = 6.2 Hz, $J_{4,5}$ = 1.2 Hz, H-5), 4.04 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 4.6 Hz, H-3), 4.28 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, $J_{1,2}$ = 8.4 Hz, H-2), 4.72 (dd, 1 H, $J_{3,4}$ = 4.6 Hz, $J_{4,5}$ = 1.2 Hz, H-4), 5.08 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 7.03 (t, 1 H, $J_{m,p}$ = 7.4 Hz, *p*-arom. H of 1-OPh), 7.07 (d, 2 H, $J_{o,m}$ = 8.5 Hz, *o*-arom. H of 1-OPh), 7.30 (dd, 2 H, $J_{o,m}$ = 8.5 Hz, $J_{m,p}$ = 7.4 Hz, *m*-arom. H of 1-OPh), 7.50 (t, 2 H, $J_{m,p}$ = 7.5, Hz, $J_{o,m}$ = 7.1 Hz, *m*-arom. H of 4-NHBz), 7.57 (t, 1 H, $J_{m,p}$ = 7.5, Hz, *p*-arom. H of 4-NHBz), 7.92 (d, 2 H, $J_{o,m}$ = 7.1 Hz, *o*-arom. H of 4-NHBz); ^{13}C NMR (CD_3OD, 100.62 MHz): δ 21.55 (q, 2-NHCO*C*H$_3$), 50.92 (d, C-4), 53.30 (d, C-2), 60.94 (t, C-6), 70.28 (d, C-3), 75.13 (d, C-5), 100.28 (d, C-1), 116.34 (d, *o*-arom. CH of 1-OPh), 122.30 (d, *p*-arom. CH of 1-OPh), 127.39 (d, *o*-arom. CH of 4-NHBz), 128.05 (d, *m*-arom. CH of 4-NHBz), 129.15 (d, *m*-arom. CH of 1-OPh), 131.40 (d, *p*-arom. CH of 4-NHBz), 134.22 (s, arom. C of 4-NHBz), 157.70 (s, arom. C of 1-OPh), 170.89 (s, *C*O of 4-NHBz), 172.95 (s, 2-NH*C*OCH$_3$); HRMS (APCI) *m/z* $[M + H]^+$ calcd for $C_{21}H_{25}N_2O_6^+$ 401.1707, found 401.1710.

Phenyl 2-acetamido-2,4-dideoxy-4-pivaloylamino-β-D-galac topyranoside (10)

The general procedure E with compound **7** (31 mg, 96.2 μmol, 1.0 equiv), 5% Pd-C (34 mg) and pivaloyl chloride (0.24 mL, 235 mg, 1.95 mmol, 20 equiv), followed by silica gel column chromatography eluting with a gradient from 5% *v*/v to 10% v/v methanol-chloroform gave **10** (8 mg, 21.0 μmol, 22%) as colorless crystals. R_f = 0.60 (20% v/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 1.17 (s, 9 H, 4-NHCOC (CH_3)$_3$), 1.89 (s, 3 H, 2-NHCOCH_3), 3.46 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 5.8 Hz, H-6a), 3.50 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 6.5 Hz, H-6b), 3.72 (td, 1 H, $J_{5,6b}$ = 6.5 Hz, $J_{5,6a}$ = 5.8 Hz, $J_{4,5}$ = 1.3 Hz, H-5), 3.84 (dd, 1 H, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 4.5 Hz, H-3), 3.98 (dd, 1 H, $J_{2,3}$ = 10.9 Hz, $J_{1,2}$ = 8.3 Hz, H-2), 4.35 (dd, 1 H, $J_{3,4}$ = 4.5 Hz, $J_{4,5}$ = 1.3 Hz, H-4), 4.91 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 6.91 (t, 1 H, $J_{m,p}$ = 7.3 Hz, *p*-arom. H), 6.93 (d, 2 H, $J_{o,m}$ = 8.7 Hz, *o*-arom. H), 7.18 (dd, 2 H, $J_{o,m}$ = 8.7 Hz, $J_{m,p}$ = 7.3 Hz, *m*-arom. H); ^{13}C NMR (CD_3OD, 100.62 MHz): δ 21.53 (q, 2-NHCO*C*H$_3$), 26.42 (q, 4-NHCOC (*C*H$_3$)$_3$), 38.83 (s, 4-NHCO*C* (CH$_3$)$_3$), 50.31 (d, C-4), 53.30 (d, C-2), 60.84 (t, C-6), 70.04 (d, C-3), 74.95 (d, C-5), 100.21 (d, C-1), 116.29 (d, *o*-arom. CH), 122.31 (d, *p*-arom. CH), 129.14 (d, *m*-arom. CH), 157.64 (s, arom. C), 172.88 (s, 2-NH*C*OCH$_3$), 181.40 (s, 4-NH*C*OC (CH$_3$)$_3$; HRMS (FAB) *m/z* $[M + Na]^+$ calcd for $C_{19}H_{28}N_2NaO_6^+$ 403.1840, found 403.1849.

Cyclohexyl 3,4,6-tri-*O*-aceyl-2-deoxy-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (12a)

The general procedure F with compound **11** (2.000 g, 3.49 mmol, 1.0 equiv), cyclohexanol (0.55 mL, 0.521 g, 5.21 mmol, 1.5 equiv), *N*-iodosuccinimide (1.476 g, 6.56 mmol, 1.9 equiv), and MS4A (2.012 g) in dry dichloromethane (35 mL) and 1.0 M trifluoromethanesulfonic acid (1.0 M in dichloromethane, 0.70 mL) at −10 °C for 1 h, followed by silica gel column chromatography eluting with a gradient from 15% *v*/v to 30% v/v ethyl acetate-hexane gave **12a** (1.767 g, 3.14 mmol, 90%) as colorless crystals. R_f = 0.53 (50% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.18–1.23 (m, 1 H, H-4ax of cyclohexyl), 1.25–1.38 (m, 3 H, H-3ax, H-5ax, H-2ax of cyclohexyl), 1.41–1.44 (m, 1 H, H-6ax of cyclohexyl), 1.46–1.51 (m, 1 H, H-4 eq of cyclohexyl), 1.66–1.75 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.78–1.81 (m, 1 H, H-2 eq of cyclohexyl), 1.86–1.92 (m, 1 H, H-6 eq of cyclohexyl), 2.026 (s, 3 H, 4-OCOCH_3), 2.032 (s, 3 H, 3-OCOCH_3), 2.08 (s, 3H, 6-OCOCH_3), 3.53 (ddd, 1 H, $J_{2,3}$ = 9.9 Hz, $J_{1,2}$ = 8.6 Hz, $J_{2,NH}$ = 8.1 Hz, H-2), 3.63 (tt, 1 H, $J_{H\text{-}1ax,H\text{-}2ax}$ = 9.1 Hz, $J_{H\text{-}1ax,H\text{-}2eq}$ = 3.8 Hz, H-1ax of cyclohexyl), 3.70 (ddd, 1 H, $J_{4,5}$ =

9.8 Hz, $J_{5,6b}$ = 4.8 Hz, $J_{5,6a}$ = 2.4 Hz, H-5), 4.11 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.4 Hz, H-6a), 4.28 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 4.8 Hz, H-6b), 4.68 (d, 1 H, J_{gem} = 11.9 Hz, Ha of Troc), 4.76 (d, 1 H, J_{gem} = 11.9 Hz, Hb of Troc), 4.79 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 5.05 (t, 1 H, $J_{4,5}$ = 9.8 Hz, $J_{3,4}$ = 9.6 Hz, H-4), 5.18 (d, 1 H, $J_{2,NH}$ = 8.1 Hz, 2-N*H*COOCH$_2$CCl$_3$), 5.37 (t, 1 H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 9.6 Hz, H-3); ^{13}C NMR (CDCl$_3$, 100.62 MHz): δ 20.59 (q, 4-OCO*C*H$_3$), 20.63 (q, 3-OCO*C*H$_3$), 20.70 (q, 6-OCO*C*H$_3$), 23.65 (t, C-3 of cyclohexyl), 23.77 (t, C-5 of cyclohexyl), 25.43 (t, C-4 of cyclohexyl), 31.58 (t, C-2 of cyclohexyl), 33.23 (t, C-6 of cyclohexyl), 56.62 (d, C-2), 62.25 (t, C-6), 68.95 (d, C-4), 71.58 (d, C-5), 71.76 (d, C-3), 74.43 (t, 2-NHCOO*C*H$_2$CCl$_3$), 77.96 (d, C-1 of cyclohexyl), 95.38 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 98.94 (d, C-1), 153.87 (s, 2-NH*C*OOCH$_2$CCl$_3$), 169.46 (s, 4-O*C*OCH$_3$), 170.55 (s, 3-O*C*OCH$_3$), 170.66 (s, 6-O*C*OCH$_3$); HRMS (FAB) *m/z* [M + Na]$^+$ calcd for $C_{21}H_{30}Cl_3NNaO_{10}^+$ 584.0828, found 584.0842.

Cyclohexyl 2-deoxy-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (13a)

Following general procedure D, compound **12a** (0.767 g, 1.36 mmol, 1.0 equiv) was treated with 1.0 M NaOMe-MeOH (0.27 mL, 0.27 mmol, 0.20 equiv) at 0 °C for 1.5 h to give **13a** (0.591 g, 1.35 mmol, 99%) as a colorless solid. R_f = 0.28 (20% *v*/v methanol-chloroform); ^{1}H NMR (D$_2$O, 400.13 MHz): δ 1.12–1.25 (m, 5 H, H-4ax, H-3ax, H-5ax, H-2ax, H-6ax of cyclohexyl), 1.34–1.36 (m, 1 H, H-4 eq of cyclohexyl), 1.53–1.54 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.69–1.75 (m, 2 H, H-6 eq, H-2 eq of cyclohexyl), 3.24–3.31 (m, 3 H, H-2, H-5, H-4), 3.41–3.42 (m, 1 H, H-3), 3.62 (d, 1 H, $J_{6a,6b}$ = 11.3 Hz, H-6a), 3.61–3.66 (m, 1 H, H-1ax of cyclohexyl), 3.79 (d, 1 H, $J_{6a,6b}$ = 11.3 Hz, H-6b), 4.53 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.61 (d, 1 H, J_{gem} = 12.7 Hz, Ha of Troc), 4.80 (d, 1 H, J_{gem} = 12.7 Hz, Hb of Troc); ^{13}C NMR (D$_2$O, 100.62 MHz): δ 23.02 (t, C-3 of cyclohexyl), 23.24 (t, C-5 of cyclohexyl), 24.95 (t, C-4 of cyclohexyl), 30.95 (t, C-2 of cyclohexyl), 32.54 (t, C-6 of cyclohexyl), 57.58 (d, C-2), 60.71 (t, C-6), 69.89 (d, C-4), 73.83 (d, C-3), 74.18 (t, 2-NHCOO*C*H$_2$CCl$_3$), 75.78 (d, C-5), 78.28 (d, C-1 of cyclohexyl), 94.93 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 99.12 (d, C-1), 156.78 (s, 2-NH*C*OOCH$_2$CCl$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{15}H_{25}Cl_3NO_7^+$ 436.0691, found 436.0688.

Cyclohexyl 2-deoxy-3,6-di-*O*-pivaloyl-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (14a)

Following general procedure B, compound **13a** (68 mg, 0.156 mmol, 1.0 equiv) was reacted with pivaloyl chloride (0.10 mL, 98 mg, 0.813 mmol, 5.2 equiv) and pyridine (0.18 mL, 2.23 mmol, 5.2 equiv) in dichloromethane (2 mL, 78 mM) at 0 °C for 3 h to afford, after silica gel chromatography eluting with 20% *v*/v ethyl acetate-hexane, compound **14a** (67 mg, 0.111 mmol, 71%) as colorless crystals. R_f = 0.40 (30% v/v ethyl acetate-hexane); ^{1}H NMR (CDCl$_3$, 400.13 MHz): δ 1.20 (s, 9 H, 3-OCOC (C*H*$_3$)$_3$), 1.22, (s, 9 H, 6-OCOC (C*H*$_3$)$_3$), 1.20–1.28 (m, 3 H, H-3ax, H-4ax, H-5ax of cyclohexyl), 1.30–1.37 (m, 1 H, H-2ax of cyclohexyl), 1.41–1.43 (m, 1 H, H-6ax of cyclohexyl), 1.46–1.49 (m, 1 H, H-4 eq of cyclohexyl), 1.66–1.73 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.77–1.80 (m, 1 H, H-2 eq of cyclohexyl), 1.86–1.89 (m, 1 H, H-6 eq of cyclohexyl), 3.31 (br s, 1 H, 4-OH), 3.50 (td, 1 H, $J_{4,5}$ = 9.3 Hz, $J_{3,4}$ = 9.2 Hz, $J_{4,OH}$ = 4.8, H-4), 3.58–3.67 (m, 3 H, H-1ax of cyclohexyl, H-5, H-2), 4.29 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 6.3 Hz, H-6a), 4.43 (dd, 1 H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 1.7 Hz, H-6b), 4.64 (d, 1 H, J_{gem} = 11.9 Hz, Ha of Troc), 4.66 (d, 1H, $J_{1,2}$ = 6.5 Hz, H-1), 4.74 (d, 1 H, J_{gem} = 11.9 Hz, Hb of Troc), 5.20 (t, 1 H, $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 5.63 (d, 1 H, $J_{2,NH}$ = 9.2 Hz, 2-N*H*Troc); ^{13}C NMR (CDCl$_3$, 100.62 MHz): δ 23.64 (t, C-3 of cyclohexyl), 23.73 (t, C-5 of cyclohexyl), 25.47 (t, C-4 of cyclohexyl), 26.97 (q, 3-OCOC (*C*H$_3$)$_3$), 27.09 (q, 6-OCOC (*C*H$_3$)$_3$), 31.58 (t, C-2 of cyclohexyl), 33.27 (t, C-6 of cyclohexyl), 38.84 (s, 6-OCO*C* (CH$_3$)$_3$), 38.94 (s, 3-OCO*C* (CH$_3$)$_3$), 56.18 (d, C-2), 63.65 (t, C-6), 70.04 (d, C-4), 73.77 (d, C-5), 74.52 (t, 2-NHCOO*C*H$_2$CCl$_3$), 74.84 (d, C-3), 77.73 (d, C-1 of cyclohexyl), 95.42 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 99.47 (d, C-1), 154.19 (s, 2-NH*C*OOCH$_2$CCl$_3$), 179.00 (s, 6-O*C*OC (CH$_3$)$_3$), 179.72 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{25}H_{41}Cl_3NO_9^+$ 604.1841, found 604.1838.

Cyclohexyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (15a)

Following general procedure A, a solution of compound **14a** (0.252 g, 0.417 mmol, 1.0 equiv) in dichloromethane (6.0 mL) and methanol (6.0 mL) was treated with activated zinc (3.543 g, 54.17 mmol, 20.8 equiv) and acetic acid (1.19 mL) at room temperature for 2 h, followed by acetylation with acetic anhydride (0.82 mL, 0.886 g, 8.67 mmol, 20.8 equiv) at room temperature for 18 h to give, after separated by silica gel column chromatography eluting with 15% *v*/v ethyl acetate-hexane, compound **15a** (0.144 g, 0.305 mmol, 73%) as colorless crystals. R_f = 0.30 (30% v/v ethyl acetate-hexane); ^{1}H NMR (CDCl$_3$, 400.13 MHz): δ 1.20 (s, 9 H, 3-OCOC (C*H*$_3$)$_3$), 1.22 (s, 9 H, 6-OCOC (C*H*$_3$)$_3$), 1.19–1.27 (m, 3 H, H-3ax, H-4ax, H-5ax of cyclohexyl), 1.29–1.31 (m, 1 H, H-2ax of cyclohexyl), 1.38–1.42 (m, 1 H, H-6ax of cyclohexyl), 1.45–1.49 (m, 1 H, H-4 eq of cyclohexyl), 1.68–1.73 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.75–1.79 (m, 1 H, H-2 eq of

cyclohexyl), 1.85–1.89 (m, 1 H, H-6 eq of cyclohexyl), 1.92 (s, 3 H, 2-NHCOCH_3), 3.10 (d, 1 H, $J_{4,OH}$ = 5.4 Hz, 4-OH), 3.49 (td, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{3,4}$ = 9.1 Hz, $J_{4,OH}$ = 5.4 Hz, H-4), 3.58 (ddd, 1 H, $J_{4,5}$ = 9.5 Hz, $J_{5,6a}$ = 5.8 Hz, $J_{5,6b}$ = 2.5 Hz, H-5), 3.58–3.61 (m, 1 H, H-1ax of cyclohexyl), 3.80 (dt, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{2,NH}$ = 8.8 Hz, $J_{1,2}$ = 8.5 Hz, H-2), 4.33 (dd, 1 H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6a}$ = 5.8 Hz, H-6a), 4.40 (dd, 1 H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 2.5 Hz, H-6b), 4.67 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 5.16 (dd, 1 H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 5.78 (d, 1 H, $J_{2,NH}$ = 8.8 Hz, 2-NH); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.25 (q, 2-NHCO*C*H_3), 23.77 (t, C-3 of cyclohexyl), 23.87 (t, C-5 of cyclohexyl), 25.52 (t, C-4 of cyclohexyl), 27.05 (q, 3-OCOC (*C*H_3)$_3$), 27.17 (q, 6-OCOC (*C*H_3)$_3$), 31.75 (t, C-2 of cyclohexyl), 33.33 (t, C-6 of cyclohexyl), 38.90 (s, 6-OCO*C* (CH$_3$)$_3$), 39.01 (s, 3-OCO*C* (CH$_3$)$_3$), 54.65 (d, C-2), 63.54 (t, C-6), 69.96 (d, C-4), 74.02 (d, C-5), 75.13 (d, C-3), 77.53 (d, C-1 of cyclohexyl), 99.38 (d, C-1), 169.85 (s, 2-NH*C*OCH$_3$), 178.96 (s, 6-O*C*OC (CH$_3$)$_3$), 179.89 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (FAB) *m/z* [M + H]$^+$ calcd for $C_{24}H_{42}NO_8^+$ 472.2905, found 472.2877.

Cyclohexyl 2-acetamido-4-azido-2,4-dideoxy-3,6-di-*O*-pivaloyl-β-D-galactopyranoside (16a)

Following general procedure C, compound **15a** (0.603 g, 1.28 mmol, 1.0 equiv) in pyridine (1.03 mL) and dichloromethane (12 mL) was reacted with trifluoromethanesulfonic anhydride (0.43 mL, 0.740 g, 2.62 mmol, 2.1 equiv) at −10 °C for 1.5 h, followed by treatment with sodium azide (0.210 g, 3.23 mmol, 2.5 equiv) in DMF (12 mL) at room temperature for 17 h to give, after silica gel column chromatography eluting with 20% *v*/v ethyl acetate-hexane, compound **16a** (0.358 g, 0.720 mmol, 56%) as colorless crystals. R_f = 0.20 (30% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.21 (s, 9 H, 6-OCOC (*C*H_3)$_3$), 1.24 (s, 9 H, 3-OCOC (C*H*$_3$)$_3$), 1.19–1.28 (m, 4 H, H-3ax, H-4ax, H-5ax, H-2ax of cyclohexyl), 1.35–1.45 (m, 1 H, H-6ax of cyclohexyl), 1.47–1.50 (m, 1 H, H-4 eq of cyclohexyl), 1.67–1.72 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.76–1.78 (m, 1 H, H-2 eq of cyclohexyl), 1.86–1.90 (m, 1 H, H-6 eq of cyclohexyl), 1.92 (s, 3 H, 2-NHCOCH_3), 3.58 (tt, 1 H, $J_{H\text{-}1ax,H\text{-}6ax}$ = 9.1 Hz, $J_{H\text{-}1ax,H\text{-}2eq}$ = 3.9 Hz, H-1ax of cyclohexyl), 3.76 (dt, 1 H, $J_{2,3}$ = 11.0 Hz, $J_{1,2}$ = 8.3 Hz, $J_{2,NH}$ = 7.9 Hz, H-2), 3.86 (td, 1 H, $J_{5,6b}$ = 6.7 Hz, $J_{5,6a}$ = 6.5 Hz, $J_{4,5}$ = 0.9 Hz, H-5), 3.96 (dd, 1 H, $J_{3,4}$ = 3.7 Hz, $J_{4,5}$ = 0.9 Hz, H-4), 4.17 (dd, 1 H, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6a}$ = 6.5 Hz, H-6a), 4.30 (dd, 1 H, $J_{6a,6b}$ = 11.2 Hz, $J_{5,6b}$ = 6.7 Hz, H-6b), 4.88 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 5.54 (d, 1 H, $J_{2,NH}$ = 7.9 Hz, 2-N*H*COCH$_3$), 5.55 (dd, 1 H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.7 Hz, H-3); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.31 (q, 2-NHCO*C*H$_3$), 23.77 (t, C-3 of cyclohexyl), 23.87 (t, C-5 of cyclohexyl), 25.48 (t, C-4 of cyclohexyl), 27.04 (q, 3-OCOC (*C*H$_3$)$_3$), 27.11 (q, 6-OCOC (*C*H$_3$)$_3$), 31.72 (t, C-2 of cyclohexyl), 33.32 (t, C-6 of cyclohexyl), 38.74 (s, 6-OCO*C* (CH$_3$)$_3$), 39.19 (s, 3-OCO*C* (CH$_3$)$_3$), 52.99 (d, C-2), 60.73 (d, C-4), 62.72 (t, C-6), 70.45 (d, C-5), 71.27 (d, C-3), 77.72 (d, C-1 of cyclohexyl), 98.80 (d, C-1), 170.03 (s, 2-NH*C*OCH$_3$), 177.91 (s, 6-O*C*OC (CH$_3$)$_3$), 177.93 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{24}H_{41}N_4O_7^+$ 497.2970, found 497.2962.

Cyclohexyl 2-acetamido-4-azido-2,4-dideoxy-β-D-galacto pyranoside (17a)

Following general procedure D, compound **16a** (338 mg, 0.681 mmol, 1.0 equiv) in dry methanol (7 mL, 0.097 M) was treated with 1.0 M NaOMe-MeOH (2.72 mL, 2.72 mmol, 4.0 equiv) at 0 °C for 17.5 h to provide, after silica gel chromatography eluting with a gradient from 10% *v*/v to 20% v/v methanol-chloroform, compound **17a** (208 mg, 0.633 mmol, 93%) as a colorless solid. R_f = 0.55 (20% *v*/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 1.21–1.33 (m, 4 H, H-2ax, H-3ax, H-4ax, H-5ax of cyclohexyl), 1.36–1.41 (m, 1 H, H-6ax of cyclohexyl), 1.44–1.49 (m, 1 H, H-4 eq of cyclohexyl), 1.69–1.72 (m, 2 H, H-3 eq, H-5 eq of cyclohexyl), 1.77–1.81 (m, 2 H, H-2 eq, H-6 eq of cyclohexyl), 1.96 (s, 3 H, 2-NHCOCH_3), 3.55 (td, 1 H, $J_{5,6}$ = 6.5 Hz, $J_{4,5}$ = 0.9 Hz, H-5), 3.60–3.66 (m, 1 H, H-1ax of cyclohexyl), 3.67 (d, 2 H, $J_{5,6}$ = 6.5 Hz, H-6), 3.75 (dd, 1 H, $J_{2,3}$ = 10.6 Hz, $J_{1,2}$ = 8.4 Hz, H-2), 3.90 (dd, 1 H, $J_{3,4}$ = 3.7 Hz, $J_{4,5}$ = 0.9 Hz, H-4), 3.97 (dd, 1 H, $J_{2,3}$ = 10.6 Hz, $J_{3,4}$ = 3.7 Hz, H-3), 4.50 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1); ^{13}C NMR (CD_3OD, 100.62 MHz): δ 23.08 (q, 2-NHCO*C*H$_3$), 24.56 (t, C-3 of cyclohexyl), 24.73 (t, C-5 of cyclohexyl), 26.81 (t, C-4 of cyclohexyl), 32.61(t, C-2 of cyclohexyl), 34.38 (t, C-6 of cyclohexyl), 55.18 (d, C-2), 62.22 (t, C-6), 64.04 (d, C-4), 72.99 (d, C-3), 74.80 (d, C-5), 78.06 (d, C-1 of cyclohexyl), 101.22 (d, C-1), 173.94 (s, 2-NH*C*OCH$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{14}H_{25}N_4O_5^+$ 329.1820, found 329.1811.

Benzyl 3,4,6-tri-*O*-aceyl-2-deoxy-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (12b)

The general procedure F with compound **11** (2.001 g, 3.49 mmol, 1.0 equiv), benzyl alcohol (0.54 mL, 0.56 g, 5.22 mmol, 1.5 equiv), *N*-iodosuccinimide (1.4775 g, 6.57 mmol, 1.9 equiv), trifluoromethanesulfonic acid (65 μL, 111 mg, 0.741 mmol, 0.21 equiv), and MS4A (2.012 g) in dry dichloromethane (35 mL) at −10 °C for 23 h, followed by silica gel column chromatography eluting with a gradient from 13% *v*/v to 27% v/v ethyl acetate-

hexane, gave **12b** (1.758 g, 3.08 mmol, 88%) as colorless crystals. R_f = 0.44 (50% *v*/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 2.02 (s, 3 H, 4-OCOCH_3), 2.03 (s, 3 H, 3-OCOCH_3), 2.12 (s, 3 H, 6-OCOCH_3), 3.67 (ddd, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{5,6b}$ = 4.5 Hz, $J_{5,6a}$ = 2.2 Hz, H-5), 3.73 (dt, 1 H, $J_{2,3}$ = 10.3 Hz, $J_{2,NH}$ = 9.1 Hz, $J_{1,2}$ = 8.8 Hz, H-2), 4.17 (dd, 1 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6a}$ = 2.2 Hz, H-6a), 4.30 (dd, 1 H, $J_{6a,6b}$ = 12.3 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.617 (d, 1 H, $J_{1,2}$ = 8.8 Hz, H-1), 4.621 (d, 1 H, J_{gem} = 12.0 Hz, Ha of 1-OC$H_2C_6H_5$), 4.72 (s, 2 H, CH_2 of Troc), 4.92 (d, 1 H, J_{gem} = 12.0 Hz, Hb of 1-OC$H_2C_6H_5$), 4.99 (d, 1 H, $J_{2,NH}$ = 9.1 Hz, 2-N*H*COOCH$_2$CCl$_3$), 5.10 (t, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{3,4}$ = 9.6 Hz, H-4), 5.23 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 9.6 Hz, H-3), 7.26–7.37 (m, 5 H, arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 20.64 (q, 4- or 3-OCO*C*H$_3$), 20.66 (q, 3- or 4-OCO*C*H$_3$), 20.80 (q, 6-OCO*C*H$_3$), 56.22 (d, C-2), 62.04 (t, C-6), 68.61 (d, C-4), 70.85 (t, 1-O*C*H$_2$C$_6$H$_5$), 71.90 (d, C-3), 71.90 (d, C-5), 74.51 (t, 2-NHCOO*C*H$_2$CCl$_3$), 95.36 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 99.24 (d, C-1), 128.06 (d, *o*-arom. CH), 128.19 (d, *p*-arom. CH), 128.57 (d, *m*-arom. CH), 136.50 (s, arom. C), 154.00 (s, 2-NH*C*OOCH$_2$CCl$_3$), 169.46 (s, 4-O*C*OCH$_3$), 170.67 (s, 3-O*C*OCH$_3$), 170.73 (s, 6-O*C*OCH$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{22}H_{27}Cl_3NO_{10}^+$ 570.0695, found 570.0679.

Benzyl 2-deoxy-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (13b)

Following general procedure D, compound **12b** (0.947 g, 1.66 mmol, 1 equiv) in dry methanol (23.7 mL, 0.070 M) was treated with 1.0 M NaOMe-MeOH (0.30 mL, 0.30 mmol, 0.18 equiv) at −5 °C for 2.5 h to provide **13b** (0.719 g, 1.62 mmol, 97%) as a colorless solid. R_f = 0.18 (10% *v*/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 3.27 (ddd, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{5,6a}$ = 5.9 Hz, $J_{5,6b}$ = 2.2 Hz, H-5), 3.34 (t, 1 H, $J_{3,4}$ = 10.0 Hz, $J_{4,5}$ = 9.7 Hz, H-4), 3.41–3.49 (m, 2 H, H-3, H-4), 3.71 (dd, 1 H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6a}$ = 5.9 Hz, H-6a), 3.91 (dd, 1 H, $J_{6a,6b}$ = 11.9 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b), 4.48 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 4.61 (d, 1 H, J_{gem} = 11.9 Hz, Ha of 1-OC$H_2C_6H_5$), 4.65 (dd, 1 H, J_{gem} = 12.0 Hz, Ha of Troc), 4.87 (dd, 1 H, J_{gem} = 12.0 Hz, Hb of Troc), 4.91 (d, 1 H, J_{gem} = 12.0 Hz, Hb of 1-OC$H_2C_6H_5$), 7.23–7.33 (m, 5 H, arom. H); ^{13}C NMR (CD_3OD, 100.62 MHz): δ59.37 (d, C-2), 62.97 (t, C-6), 71.82 (t, 1-O*C*H$_2$C$_6$H$_5$), 72.34 (d, C-4), 75.69 (t, 2-NHCOO*C*H$_2$CCl$_3$), 75.90 (d, C-3), 78.14 (d, C-5), 97.32 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 102.17 (d, C-1), 128.75 (d, *p*-arom. CH), 128.90 (d, *o*-arom. CH), 129.44 (d, *m*-arom. CH), 139.24 (s, arom. C), 157.23 (s, 2-NH*C*OOCH$_2$CCl$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{16}H_{21}Cl_3NO_7^+$ 444.0378, found 444.0370.

Benzyl 2-deoxy-3,6-di-*O*-pivaloyl-2-(2,2,2-trichloroethoxy) carbonylamino-β-D-glucopyranoside (14b)

Following general procedure B, compound **13b** (0.709 g, 1.59 mmol, 1.0 equiv) was reacted with pivaloyl chloride (0.585 mL, 0.573 g, 4.75 mmol, 3.0 equiv) in pyridine (0.78 mL) and dichloromethane (5.9 mL) at −10 °C for 20 h to afford, after silica gel chromatography eluting with a gradient from 10% v/v to 30% v/v ethyl acetate-hexane, compound **14b** (0.841 g, 1.37 mmol, 86%) as colorless crystals. R_f = 0.48 (30% *v*/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.19 (s, 9 H, 3-OCOC (CH_3)$_3$), 1.26 (s, 9 H, 6-OCOC (CH_3)$_3$), 2.91 (s, 1 H, 4-OH), 3.48–3.55 (m, 2 H, H-4, H-5), 3.75 (dt, 1 H, $J_{2,3}$ = 10.2 Hz, $J_{2,NH}$ = 9.1 Hz, $J_{1,2}$ = 8.4 Hz, H-2), 4.40 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6a}$ = 1.9 Hz, H-6a), 4.44 (dd, 1 H, $J_{6a,6b}$ = 12.0 Hz, $J_{5,6b}$ = 3.5 Hz, H-6b), 4.47 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 4.61 (d, 1 H, J_{gem} = 12.3 Hz, 1-OC*H* (H)C$_6$H$_5$), 4.62 (d, 1 H, J_{gem} = 12.0 Hz, Ha of Troc), 4.76 (d, 1 H, J_{gem} = 12.0 Hz, Hb of Troc), 4.90 (d, 1 H, J_{gem} = 12.3 Hz, 1-OCH (*H*)C$_6$H$_5$), 4.96 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 8.5 Hz, H-3), 5.00 (d, 1 H, $J_{2,NH}$ = 9.1 Hz, 2-N*H*COCH$_3$), 7.29–7.36 (m, 5 H, arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 27.00 (q, 3-OCOC (CH_3)$_3$), 27.24 (q, 6-OCOC (CH_3)$_3$), 39.00 (s, 6-OCO*C* (CH_3)$_3$), 39.01 (s, 3-OCO*C* (CH_3)$_3$), 55.84 (d, C-2), 63.05 (t, C-6), 69.64 (d, C-4), 70.37 (t, 1-O*C*H$_2$C$_6$H$_5$), 74.36 (d, C-5), 74.50 (d, C-3), 74.65 (t, 2-NHCOO*C*H$_2$CCl$_3$), 95.27 (s, 2-NHCOOCH$_2$*C*Cl$_3$), 99.49 (d, C-1), 127.90 (d, *o*-arom. CH), 128.04 (d, *p*-arom. CH), 128.51 (d, *m*-arom. CH), 136.80 (s, arom. C), 154.12 (s, 2-NH*C*OOCH$_2$CCl$_3$), 179.16 (s, 6-O*C*OC (CH_3)$_3$), 179.65 (s, 3-O*C*OC (CH_3)$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{26}H_{37}Cl_3NO_9^+$ 612.1528, found 612.1524.

Benzyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-β-D-gluco pyranoside (15b)

Following general procedure A, a solution of compound **14b** (0.803 g, 1.31 mmol, 1.0 equiv) in dichloromethane (9.4 mL) and methanol (9.4 mL) was treated with activated zinc (10.379 g, 158.7 mmol, 121 equiv) and acetic acid (3.56 mL) at rt. for 4 h, followed by acetylation with acetic anhydride (0.89 mL, 0.96 g, 9.4 mmol, 8.3 equiv) at rt. for 16 h to give, after separated by silica gel column chromatography eluting with 15% *v*/v ethyl acetate-hexane, compound **15b** (0.427 g, 0.305 mmol, 68%) as colorless crystals. R_f = 0.20 (40% *v*/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.19 (s, 9 H, 3-OCOC (CH_3)$_3$), 1.26 (s, 9 H, 6-OCOC (CH_3)$_3$), 1.90 (s, 3 H, 2-NHCOCH_3), 2.86 (d, 1 H, $J_{4,OH}$ = 4.2 Hz, 4-OH), 3.49 (ddd, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{5,6b}$ = 4.5 Hz, $J_{5,6a}$ = 2.3 Hz, H-5), 3.53 (ddd, 1 H, $J_{4,5}$ = 9.7 Hz, $J_{3,4}$ = 8.4 Hz, $J_{4,OH}$ = 4.2 Hz, H-4), 4.06 (ddd, 1 H,

$J_{2,3}$ = 10.8 Hz, $J_{2,NH}$ = 9.4 Hz, $J_{1,2}$ = 8.5 Hz, H-2), 4.38 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6a}$ = 2.3 Hz, H-6a), 4.43 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1), 4.46 (dd, 1 H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.60 (dd, 1 H, J_{gem} = 12.3 Hz, 1-OC*H* (H)C_6H_5), 4.88 (dd, 1 H, J_{gem} = 12.3 Hz, 1-OCH (*H*)C_6H_5), 4.91 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 8.4 Hz, H-3), 5.32 (d, 1 H, $J_{2,NH}$ = 9.4 Hz, 2-N*H*COCH$_3$), 7.29 (dd, 2 H, $J_{o,m}$ = 7.6 Hz, *o*-arom. H), 7.28–7.33 (m, 1 H, *p*-arom. H), 7.35 (ddd, 2 H, $J_{m,p}$ = 8.4 Hz, $J_{o,m}$ = 7.6 Hz, *m*-arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.31 (q, 2-NHCO*C*H$_3$), 27.03 (q, 3-OCOC (*C*H$_3$)$_3$), 27.24 (q, 6-OCOC (*C*H$_3$)$_3$), 39.02 (s, 3,6-OCO*C* (CH$_3$)$_3$), 53.64 (d, C-2), 63.05 (t, C-6), 69.27 (d, C-4), 70.07 (t, 1-O*C*H$_2$C$_6$H$_5$), 74.41 (d, C-5), 74.79 (d, C-3), 99.55 (d, C-1), 128.01 (d, *p*-arom. CH), 128.04 (d, *o*-arom. CH), 128.47 (d, *m*-arom. CH), 137.07 (s, arom. C), 169.80 (s, 2-NH*C*OCH$_3$), 179.29 (s, 6-O*C*OC (CH$_3$)$_3$), 179.77 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{25}H_{38}NO_8^+$ 480.2592, found 480.2599.

Benzyl 2-acetamido-4-azido-2,4-dideoxy-3,6-di-*O*-pivaloyl-β-D-galactopyranoside (16b)

Following general procedure C, compound **15b** (0.520 g, 1.08 mmol, 1.0 equiv) in pyridine (1.6 mL) and dichloromethane (4.7 mL) was reacted with trifluoromethanesulfonic anhydride (0.45 mL, 0.774 g, 2.74 mmol, 2.5 equiv) at −10 °C for 2.5 h, followed by treatment with sodium azide (0.231 g, 3.55 mmol, 3.3 equiv) in DMF (3.7 mL) at rt. for 17.5 h to give, after silica gel column chromatography eluting with 15% *v*/v ethyl acetate-hexane, compound **16b** (0.2704 g, 0.536 mmol, 49%) as colorless crystals. R_f = 0.50 (30% v/v ethyl acetate-hexane); ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ 1.23 (s, 9 H, 3-OCOC (C*H*$_3$)$_3$), 1.24 (s, 9 H, 6-OCOC (C*H*$_3$)$_3$), 1.90 (s, 3 H, 2-NHCOC*H*$_3$), 3.81 (dt, 1 H, $J_{5,6a}$ = 6.6 Hz, $J_{5,6b}$ = 6.5 Hz, $J_{4,5}$ = 1.0 Hz, H-5), 3.92 (dd, 1 H, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ = 1.0 Hz, H-4), 4.12 (dt, 1 H, $J_{2,3}$ = 11.1 Hz, $J_{2,NH}$ = 8.8 Hz, $J_{1,2}$ = 8.4 Hz, H-2), 4.22 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6a}$ = 6.6 Hz, H-6a), 4.35 (dd, 1 H, $J_{6a,6b}$ = 11.3 Hz, $J_{5,6b}$ = 6.5 Hz, H-6b), 4.57 (d, 1 H, J_{gem} = 12.1 Hz, 1-OC*H* (H)C_6H_5), 4.59 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 4.87 (d, 1 H, J_{gem} = 12.1 Hz, 1-OCH (*H*)C_6H_5), 5.22 (d, 1 H, $J_{2,NH}$ = 8.8 Hz, 2-N*H*COCH$_3$), 5.30 (dd, 1 H, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 3.6 Hz, H-3), 7.26–7.36 (m, 5 H, arom. H); ^{13}C NMR ($CDCl_3$, 100.62 MHz): δ 23.35 (q, 2-NHCOC*H*$_3$), 27.04 (q, 3-OCOC (*C*H$_3$)$_3$), 27.18 (q, 6-OCOC (*C*H$_3$)$_3$), 38.81 (s, 6-OCO*C* (CH$_3$)$_3$), 39.24 (s, 3-OCO*C* (CH$_3$)$_3$) 51.71 (d, C-2), 60.63 (d, C-4), 62.60 (t, C-6), 70.33 (t, 1-O*C*H$_2$C$_6$H$_5$), 70.72 (d, C-5), 71.59 (d, C-3), 99.36 (d, C-1), 128.07 (d, *p*-arom. CH), 128.13 (d, *o*-arom. CH), 128.48 (d, *m*-arom. CH), 136.95 (s, arom. C), 169.88 (s, 2-NH*C*OCH$_3$), 177.97 (s, 6-O*C*OC (CH$_3$)$_3$), 178.23 (s, 3-O*C*OC (CH$_3$)$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{25}H_{37}N_4O_7^+$ 505.2657, found 505.2658.

Benzyl 2-acetamido-4-azido-2,4-dideoxy-β-D-galacto pyranoside (17b)

Following general procedure D, compound **16b** (109.1 mg, 0.216 mmol, 1.0 equiv) in dry methanol (1.08 mL) was treated with 1.0 M NaOMe-MeOH (0.86 mL, 0.86 mmol, 4.0 equiv) at 0 °C for 22.5 h to give, after separated by reversed phase chromatography eluting with a gradient from 30% v/v to 25% v/v water-methanol, compound **17b** (50.7 mg, 0.151 mmol, 70%) as a colorless solid. R_f = 0.44 (10% v/v methanol-chloroform); ^{1}H NMR (CD_3OD, 400.13 MHz): δ 1.95 (s, 3 H, 2-NHCOC*H*$_3$), 3.58 (dt, 1 H, $J_{5,6b}$ = 6.7 Hz, $J_{5,6a}$ = 6.2 Hz, $J_{4,5}$ = 0.9 Hz, H-5), 3.70 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6a}$ = 6.2 Hz, H-6a), 3.75 (dd, 1 H, $J_{6a,6b}$ = 11.0 Hz, $J_{5,6b}$ = 6.7 Hz, H-6b), 3.90–3.93 (m, 3 H, H-2, H-3, H-4), 4.42–4.44 (m, 1 H, H-1), 4.57 (d, 1 H, J_{gem} = 12.5 Hz, Ha of 1-OC*H*$_2$C$_6$H$_5$), 4.84 (d, 1 H, J_{gem} = 12.5 Hz, Hb of 1-OC*H*$_2$C$_6$H$_5$), 7.24–7.34 (m, 5 H, arom. H); ^{13}C NMR (CD_3OD, 100.62 MHz): δ 23.15 (q, 2-NHCO*C*H$_3$), 54.74 (d, C-2), 62.41 (t, C-6), 64.06 (d, C-4), 71.70 (t, 1-O*C*H$_2$C$_6$H$_5$), 73.20 (d, C-3), 75.10 (d, C-5), 102.22 (d, C-1), 128.84 (d, *p*-arom. CH), 128.99 (d, *o*-arom. CH), 129.48 (d, *m*-arom. CH), 139.24 (s, arom. C), 174.16 (s, 2-NH*C*OCH$_3$); HRMS (APCI) *m/z* [M + H]$^+$ calcd for $C_{15}H_{21}N_4O_5^+$ 337.1507, found 337.1500.

Sulfotransferase activity assay

GalNAc4S-6ST activity was assayed by the radioisotope labeling method using [^{35}S] PAPS (Perkin Elmer, Boston, MA) as described previously [20, 40]. The reaction mixture in a final volume of 50 μL at pH 6.8 consisted of 50 mM sodium potassium phosphate, 2 mM dithiothreitol (DTT), [^{35}S] PAPS (1 nmol, 0.1 μCi), 10 μg of CS-A from whale cartilage (Seikagaku, Tokyo, Japan), and the recombinant human GalNAc4S-6ST [41] in the absence or presence of the synthesized GalNAc derivatives (0.1–5.0 mM). The reaction mixture was incubated at 37 °C for 30 min and stopped by heating at 100 °C for 1 min. The radiolabeled products were isolated by gel filtration using a Superdex Peptide HR 10/300 column and quantified by liquid scintillation counting.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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