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Application of 2-Azido-2-deoxythioglycosides for β-Glycoside Formation and Oligosaccharide Synthesis

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Most natural 2-acetamido-2-deoxyglycosides exist in a 1,2-*trans*-β-glycosidic configuration. This study investigated the use of 2-azido-2-deoxythioglycosides for 1,2-*trans*-β-glycosidic bond formation under low-concentration glycosylation conditions. Further application of the 2-azido-2-deoxythioglycosyl substrates for one-pot oligosaccharide synthesis was demonstrated in the synthesis of protected β-(1→6)-*N*-acetylglucosamine trimer, core-6–serine conjugate, and F1–α serine conjugate.

Introduction

2-Acetamidoglycosides including GlcNAc and GalNAc constitute approximately 36.0% of the monosaccharide units in mammalian oligosaccharides, which are often conjugated to proteins and lipids as glycoproteins and glycolipids.[1,2] Such glycoconjugates are prevalent in nature and associated with different biological processes.[3–5] With a few exceptions, most of the 2-acetamidoglycosides are connected to other saccharide units by 1,2-*trans*-β-glycosidic bonds. In general, such bonds are constructed from glycosyl donors bearing a participating function at the C2 position,[6–8] which is applicable for both non-amino- and aminoglycosyl donors. Concerning 2-amino-2-deoxyglycosyl donors, a range of amine protecting groups with a participating function have been developed.[9–11]

In contrast to the participating glycoside donors, 2-azido-2-deoxyglycosides are often recruited for 1,2-*cis*-α-glycosidic bond formation.[12–14] The azido protecting group has a number of advantages over other participating functions such as lower steric bulk, stability under a range of reaction conditions, and a lack of rotamer formation.[11] In addition, the azido function can be reduced to an amine under mild reaction conditions.[15,16] For these reasons, the use of 2-azido-2-deoxyglycosides for β-glycosidic bond formation has always been attractive for synthetic chemists. Previously, 2-azido-2-deoxyglycosyl trichloroacetimidates and phosphates were exploited for β-glycoside formation.[17–21] However, these donors are less, stable and their preparation requires additional synthetic steps. Moreover, trichloroacetimidate derivatives occasionally suffer from glycosyl trichloroacetamide formation. Thus, development of a β-glycosylation method based on 2-azido-2-deoxythioglycoside donors is highly desirable.

In 2009, we presented a low-concentration glycosylation method for the construction of 1,2-*trans*-β-glycosidic bonds that employs nonparticipating thioglycoside donors, particularly in the D-*gluco* and D-*galacto* series.[22] This method employs CH_2Cl_2/alkanenitrile as solvent to generate 1,2-*trans*-β-glycosides under low-substrate-concentration and low-temperature conditions. Subsequent studies revealed a participating role of the C-2 ether function in nitrile solvent (Figure 1a).[23]

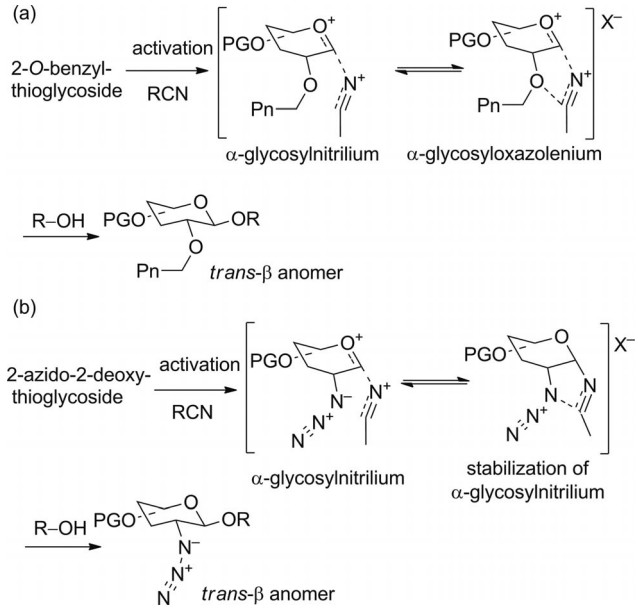

Figure 1. (a) C2 ether participation in low-concentration glycosylations. (b) Possible participation of 2-azido-2-deoxythioglycosides.

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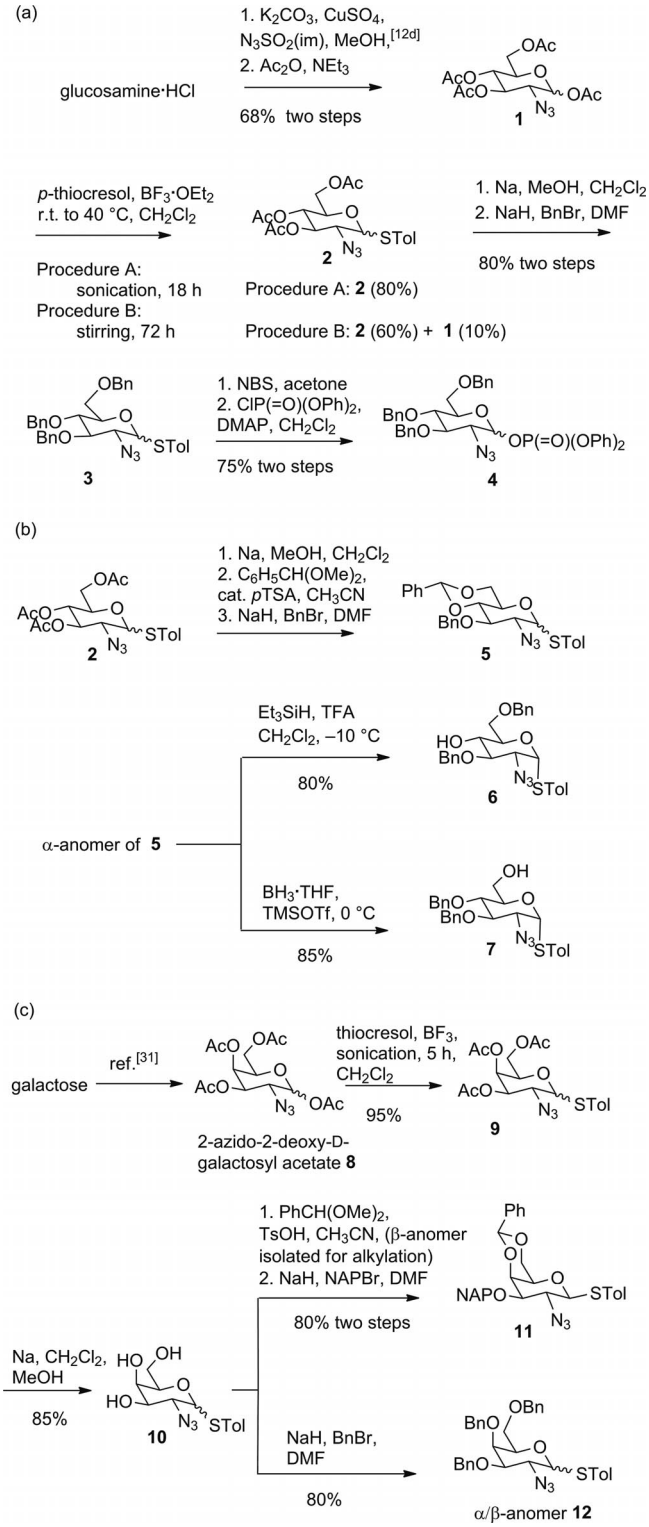
Along this line, we speculated that comparable participation may occur for 2-azido-2-deoxythioglycosyl donors, because the azido function bears lone-electron pairs, which would probably stabilize the electrophilic α-glycosylnitrilium ion (Figure 1b). Putting the idea to practice, we undertook an investigation of the glycosylation behavior of 2-azido-2-deoxythioglycosides under low-concentration glycosylation conditions. The investigation included: (i) development of an efficient thioglycosidation method for peracetyl-2-azido-2-deoxyglycosyl acetates; (ii) exploration of the scope and limitations of 2-azido-2-deoxythioglycoside donors in β-glycosidic bond formation, and (iii) application of 2-azido-2-deoxythioglycoside substrates for one-pot oligosaccharide synthesis.

Results and Discussion

1. Preparation of 2-Azido-2-deoxyglycosyl Substrates

This study commenced with the multigram preparation of 2-azido-2-deoxythioglucosides. Readily available glucosamine–hydrogen chloride was selected as the starting material. Treatment of the glucosamine salt with imidazole-1-sulfonyl azide hydrochloride salt, followed by acetylation, produced per-*O*-acetyl 2-azido-2-deoxyglucosyl acetate **1** (Scheme 1a).[24] However, thioglycosidation of **1** with thiocresol and boron trifluoride–diethyl ether was sluggish when the reaction was performed by stirring at room temp., and the desired thioglycoside **2** was produced in 60% yield accompanied by 5–10% unreacted acetate **1**. Although such an inefficient thioglycosidation was reported in previous studies, no solution has yet been provided.[21,25] To ensure a sufficient supply of thioglycosides, we needed to address this problem. To this end, we explored the use of sonication.[26] When a sonication procedure was applied, the time of thioglycosidation was reduced from 72 to 18 h, and the reaction yield increased from 60 to 80% (Scheme 1a). Upon securing the preparative route, thioglycoside **2** was deacetylated and benzylated to furnish 2-azidoper-*O*-benzyl-2-deoxythioglucoside **3**. To prepare glycosyl donors for an orthogonal glycosylation study, a portion of thioglycoside **3** was converted into 2-azido-2-deoxyglucosyl phosphate **4** by using the procedure developed by Sabesan and Neira.[27]

In addition to 2-azido-2-deoxythioglucoside donor **3**, 2-azido-2-deoxythioglucoside acceptors **6**[28] and **7** were prepared for the orthogonal glycosylation study (Scheme 1b). Thus, deacetylation of 2-azido-2-deoxythioglucoside **2** followed by benzylidenation and benzylation furnished 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxythioglucoside (**5**). The α-anomer of **5** could be separated by precipitation and was further elaborated to thioglycoside acceptors **6** and **7**. It should be noted that use of the pure anomeric form of acceptors **6** and **7** in glycosylations facilitated product characterization. 2-Azido-2-deoxythioglucoside **6** with an unprotected 4-hydroxy function was obtained by treating **5** with triethylsilane (Et_3SiH) and trifluoroacetic acid (TFA).[29] Treatment of **5** with borane–tetrahydrofuran complex (BH_3·THF) and trimethylsilyl triflate (TMSOTf)



Scheme 1. (a) Preparation of 2-azido-2-deoxythioglucosyl donor **3** and glucosyl phosphate donor **4**. (b) Preparation of 2-azido-2-deoxythioglucoside acceptors **6** and **7**. (c) Preparation of 2-azido-2-deoxythiogalactoside donors **11** and **12**.

at 0 °C produced the 2-azido-2-deoxythioglucoside **7** with an unprotected 4-hydroxy function. It should be noted that the previous benzylidene ring-opening reaction employed $CuOTf_2$ as acid catalyst, but, in our hands, the use of $CuOTf_2$ led to the formation of a complex reaction mixture.[30]

In contrast to its D-*gluco* counterpart, preparation of 2-azido-2-deoxythiogalactoside donors followed a different synthetic route that began with inexpensive and readily available galactose (Scheme 1c). First, unprotected galactose was converted into galactal, which was transformed into per-*O*-acetyl-2-azido-2-deoxygalactosyl acetate **8** by standard azidonitration and nitrate → acetate substitution.[31] Similar to acetate **1**, the thioglycosidation of **8** was accelerated by using sonication. Thus, a high yield (95%) of 2-azido-2-deoxythiogalactoside **9** was obtained within 5 h. It should be noted that different reaction times (4 h or 3 d) have been reported for the thioglycosidation of **8**.[14,21] In our hands, the reaction required 24 h to reach completion in the absence of sonication. Subsequent deacetylation of **9** produced unprotected thioglycoside **10**, which was converted into benzylidene-protected 2-azido-2-deoxy-β-thiogalactoside **11** and 2-azidoper-*O*-benzyl-2-deoxy-α-thiogalactoside **12** by standard protecting-group manipulation.

2. Glycosylations with 2-Azido-2-deoxyglycosyl Donors

Once the 2-azido-2-deoxyglycosyl donors were obtained, the stage was set for an investigation into their glycosylation properties. To this end, glycosyl acceptors **7** and **13**–**16**, and aglycon acceptor **17** were employed (Figure 2).[32] Activation of the thioglycoside donors **3**, **11**, and **12** was achieved by using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf),[33] whereas activation of the glycosyl phosphate donor **4** required a stoichiometric amount of TMSOTf.[19,20] The results of the glycosylation reactions are summarized in Table 1.

Glycosylations of galactosyl acceptor **13** and rhamnoside acceptor **14** with 2-azido-2-deoxythioglucoside **3** produced the respective disaccharides **18**[19,22] and **19** in 83 and 60% yields, respectively, with excellent β-selectivities (Table 1, Entries 1 and 3). However, when the same reactions were repeated in CH_2Cl_2, the selectivity was significantly eroded, and the α/β-anomeric ratios of **18** and **19** decreased to 1:2.5 and 1:6, respectively (Table 1, Entries 2 and 4). Other than the 2-azido-2-deoxythioglucoside donor **3**, the low-concentration glycosylation method performed well for 2-azido-2-deoxyglucosyl phosphate donor **4** (Table 1, Entry 5). Besides the glycosylations of primary acceptors, 2-azido-2-deoxythioglucoside **3** was found to be effective for glycosylations of hindered secondary glycosyl acceptors, as witnessed by glycosylations of the glucoside acceptor **15** and mannoside acceptor **16**. Both glycosylations yielded the corresponding disaccharides **20** and **21** in 76 and 55% yields, respectively (Table 1, Entries 6 and 7). It should be noted that GlcNAc-β(1→2)-Man glycoside **21** constitutes the disaccharide unit found in complex *N*-linked glycans.[34]

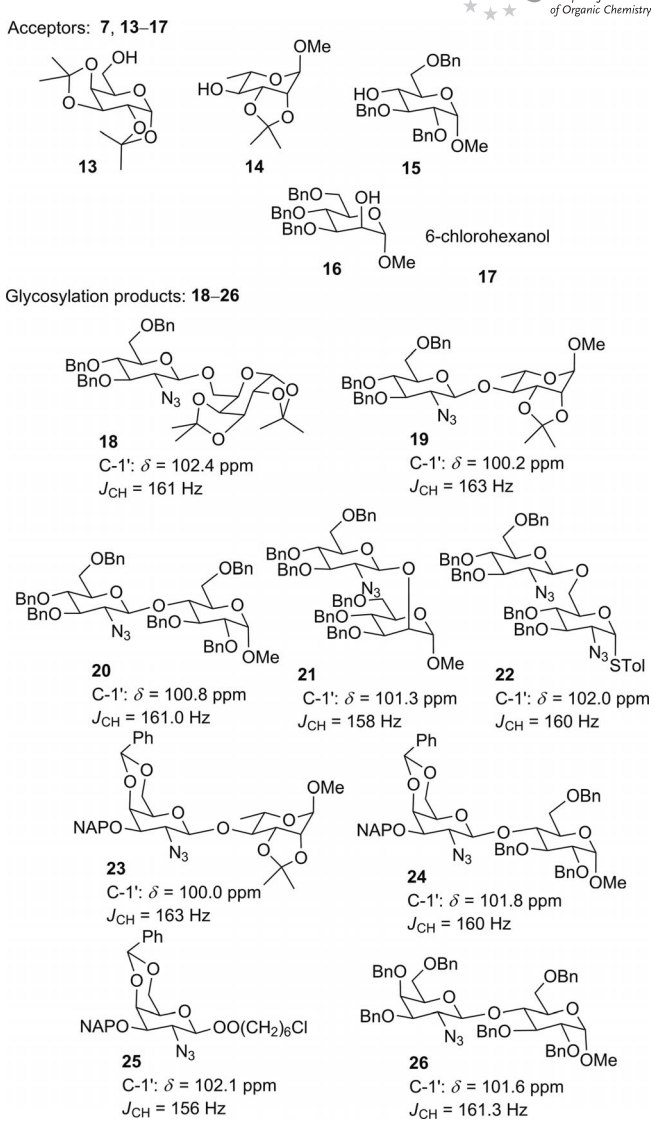


Figure 2. Acceptors **13**–**17** and glycosylation products **18**–**26**.

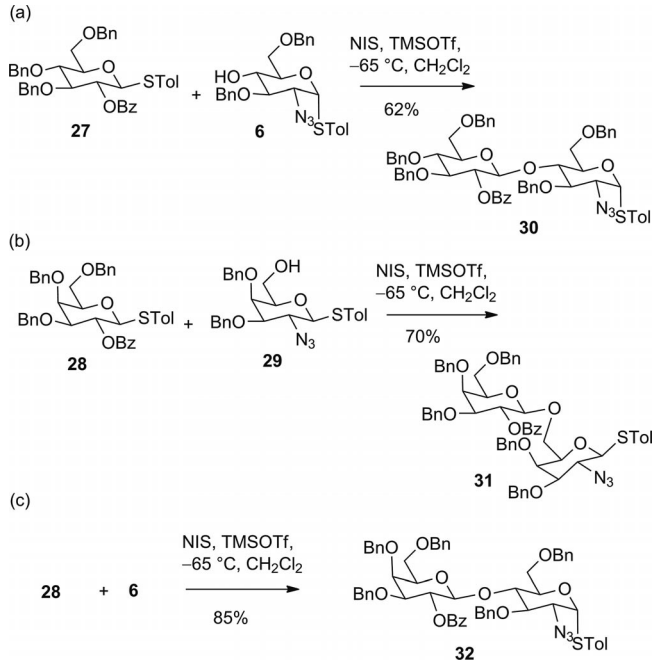
Table 1. Glycosylation of 2-azido-2-deoxythioglycosides **3**, **11**, **12**, and 2-azido-2-deoxyglocosyl phosphate **4**.

Entry	Donor/acceptor	Method[a]	Glycosylation product		
			Product	Yield [%]	Ratio α/β[b]
1	**3/13**	A	**18**	83	1:>49
2	**3/13**	B	**18**	85	1:5
3	**3/14**	A	**19**	60	1:32
4	**3/14**	B	**19**	60	1:6
5	**4/13**	A	**18**	82	1:>49
6	**3/15**	A	**20**	76	1:33
7	**3/16**	A	**21**	55	1:24
8	**4/7**	A	**22**	77	1:19
9	**11/14**	A	**23**	70	1:19
10	**11/15**	A	**24**	80	1:33
11	**11/17**	A	**25**	80	1:19
12	**12/15**	A	**26**	65	1:25

[a] Method A: low-substrate-concentration glycosylation; Method B: conventional glycosylation in CH_2Cl_2. [b] The α/β anomeric ratios were determined by HPLC analysis (Mightysil column: Si-60 250-4.6; hexane/CH_2Cl_2/EtOAc; flow-rate 1.0 mL min^{-1}).

Concerning the orthogonal glycosylation, 2-azido-2-deoxyglucosyl phosphate **4** was coupled with 2-azido-2-deoxythioglucoside acceptor **7**. The glycosylation furnished the desired thiodisaccharide **22** in high yield (77%) with a 1:19 α/β-anomeric ratio (Table 1, Entry 8). Gratifyingly, no self-condensation of acceptor **7** was detected. However, when less reactive 4-hydroxy-unprotected acceptor **6** was employed for glycosylation with **4**, significant levels of thiotransfer reaction occurred, whereby the thioacetal function in **6** was transferred to the anomeric center of **4** (unpublished data).

After studying 2-azido-2-deoxythioglucoside **3**, we shifted the focus to 2-azido-2-deoxythiogalactoside donors **11** and **12**. Although these donors had different protecting-group patterns, this did not affect the glycosylation efficiency as evidenced by the glycosylations of acceptors **14**, **15**, and **17** (Table 1, Entries 9–12). However, such glycosylation properties did not extend to 4,6-*O*-benzylidene-protected 2-azidothioglucoside; in this case, donor activation was ineffective under low-concentration glycosylation conditions (unpublished data). We attributed this behavior to the inherently less reactive nature of D-*gluco* donors. The β-configurations of the newly formed glycosidic bonds in disaccharides **18**–**26** were confirmed by the chemical shifts of the signals of the anomeric carbon atoms at δ = 100.0–102.4 ppm and by the corresponding $^1J_{C-H}$ coupling constant of 156–163 Hz.[35]



Scheme 2. (a) Glycosylation of 2-azido-2-deoxythioglucoside **6** with 2-*O*-benzoyl-protected thioglucoside donor **27**; (b) glycosylation of 2-azido-2-deoxythiogalalctoside **29** with 2-*O*-benzoyl-protected thiogalalctoside donor **28**; (c) glycosylation of 2-azido-2-deoxythioglucoside **6** with 2-*O*-benzoyl-protected thiogalactoside **28**.

3. Glycosylations of 2-Azido-2-deoxythioglycoside Acceptors

After studying the donor properties of the 2-azido-2-deoxythioglycosides, we evaluated their acceptor properties under conventional glycosylation conditions. This information is relevant for the design of saccharide building blocks for oligosaccharide synthesis. It is well recognized that the azido function at the C2 position reduces the reactivity of the anomeric function; however, whether such a disarming effect influences the nucleophilicity of hydroxy functions remains unclear. To investigate this effect, 2-*O*-benzoyl-protected thioglucoside **27**[36] and thiogalactoside **28**[37] were selected as donors for glycosylations of 2-azido-2-deoxythioglucoside **6** and 2-azido-2-deoxythiogalactoside **29** (Scheme 2).[38]

The glycosylations of **6** with **27** and **29** with **28** provided thiodisaccharides **30** and **31**, respectively, in yields of 62 and 70%. No self-glycosylation of thioglycoside acceptors was detected. This result is attributed to the stronger disarming effect of the azido function compared to the 2-benozyl function.[39] Based on the inherently higher reactivity of the D-*galacto* donor and the disarming nature of the 2-azido function, we predicted a highly efficient reactivity-based glycosylation would occur between 2-benzoyl protected thiogalactoside **28** and 2-azidothioglucoside **6**. As expected, the desired LacNAc thioglycoside **32** was produced in excellent yield (85%) despite the hindered 4-hydroxy function of **6** (Scheme 2c).

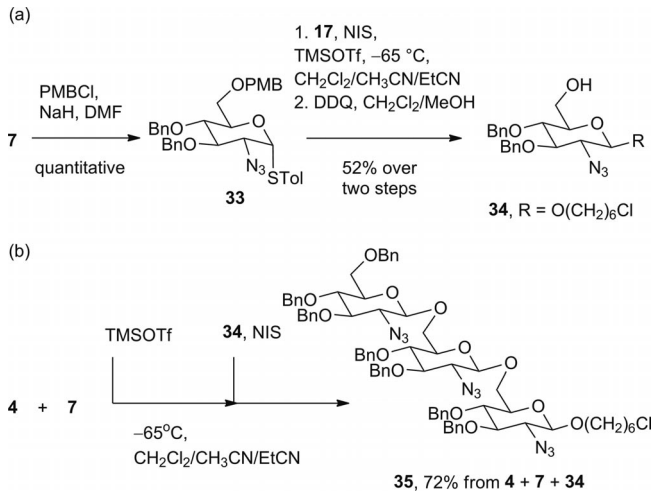
4. Application of 2-Azido-2-deoxythioglycoside Substrates in Oligosaccharide Synthesis

Encouraged by the aforementioned investigations, we attempted the synthesis of oligosaccharides using 2-azido-2-deoxythioglycoside substrates. For model studies, protected β-(1→6)-*N*-acetylglucosamine (PNAG) trimer **35**, core-6–serine conjugate **38**, and F1–α-serine conjugate **39** were selected as the synthetic targets.

The β(1→6)-*N*-acetylglucosamine trimer **35** constitutes the backbone of the β-(1→6)-*N*-acetylglucosamine polymers (PNAG), which is found in various pathogens including *Staphylococcus aureus*,[40] *Escherichia coli*,[41] and *Actinobacter* spp.[42] Previous syntheses of the PNAG oligomers often used the phthalimide function (*N*-Phth) for amine protection.[43,44] However, removal of the *N*-Phth protection is nontrivial and often requires harsh reaction conditions. Thus, an alternative strategy that employs 2-azido-2-deoxythioglycoside building blocks would be desirable.

Assembly of the protected *N*-acetylglucosamine trimer **35** required three 2-azido-2-deoxyglucosyl ($GlcN_3$) building blocks **4**, **7**, and **34**. The reducing-end $GlcN_3$ building block **34** was prepared from the 2-azido-2-deoxythioglucoside **7** by (i) protecting the 6-hydroxy function in **7** with *p*-methoxybenzyl ether (PMB) to obtain thioglycoside **33**; (ii) coupling **33** with 6-chlorohexanol **17** under low-concentration glycosylation conditions to produce fully protected 2-azido-2-deoxy *O*-glucoside, and (iii) removal of the PMB function in the preceding *O*-glycoside leading to the target building block **34** (Scheme 3a). In a one-pot synthesis of **35**, 2-azido-2-deoxythioglucoside **7** was glycosylated with 2-azido-2-de-

oxyglucosyl phosphate **4** under low-concentration glycosylation conditions to obtain thiodisaccharide **22** (Scheme 3b). Without intermediate isolation, a supplementary dose of NIS and the reducing-end $GlcN_3$ building block **34** were added to react with the thiodisaccharide **22**. The glycosylation furnished the desired *N*-acetylglucosamine trimer **35** in 72% yield in one pot.

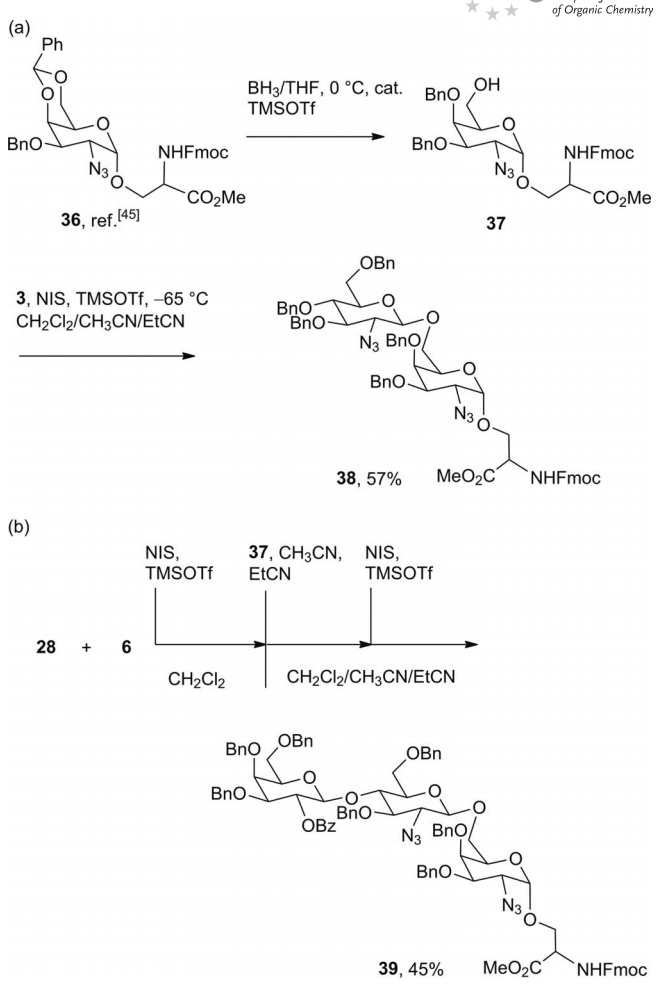


Scheme 3. (a) Preparation of the reducing-end saccharide acceptor **34**; (b) one-pot synthesis of protected β(1→6)-*N*-acetylglucosamine trimer **35**.

Core-6–serine conjugate is composed of a GlcNAc-β(1→6)-GalNAc disaccharide α-linked to the serine of a protein, which belongs to one type of core-unit *O*-linked glycans.[34] Traditional synthesis of the core-6–serine requires different amine protecting functions for the GlcNAc and GalNAc constituent units. The application of 2-azido-2-deoxythioglycoside substrates together with the low-concentration glycosylation conditions can eliminate such a requirement, thereby streamlining the protecting-group manipulation.

Convergent synthesis of the protected core-6–serine conjugate **38** required 2-azido-2-deoxythioglucoside donor **3** and 2-azido-2-deoxy-α-galactosylserine acceptor **37**. The latter was obtained by reductive ring opening of the benzylidene acetal in known 2-azido-4,6-*O*-benzylidene-2-deoxy-α-galactosylserine **36**; TMOSTf was again employed as the acid catalyst for this reaction.[45] With the required building blocks in hand, the protected core-6–serine **38** was readily obtained by glycosylation of 2-azido-2-deoxy-α-galactosylserine **37** with 2-azido-2-deoxythioglucoside donor **3** under low-concentration glycosylation conditions (Scheme 4a). The β-configuration of the newly formed glycosidic bond in **38** was confirmed by the ^{13}C NMR chemical shift of the signal of the anomeric center at δ = 102.0 ppm and the corresponding $^1J_{C-H}$ coupling constant of 160 Hz.[35]

Mucin-related F1–α-antigens are glycoconjugates that contain Gal-β(1→4)-GlcNAc-β(1→6)-GalNAc trisaccharides α-linked to protein serine or threonine. They are an example of aberrant oligosaccharides found on mucins associated with gastric adenocarcinomas.[34,46] Due to their



Scheme 4. (a) Preparation of 2-azido-2-deoxygalactosylserine acceptor **37** and protected core-6–serine **38**; (b) one-pot synthesis of protected F1–α-serine **39**.

high expression in tumorigenic tissue, such *O*-linked mucins are important targets for antitumor immunological studies.[47] As Gal-β(1→4)-GlcNAc-β(1→6)-GalNAc trisaccharide constitutes the carbohydrate component of the F1–α-*O*-linked mucins, an efficient preparation of this target is highly desired. Although the F1–α-antigen was previously synthesized,[48–50] only one sequential one-pot glycosylation method has been reported thus far.[50] By applying 2-azido-2-deoxythioglycoside substrates and low-concentration glycosylation conditions, an alternative, simpler, one-pot glycosylation strategy was realized.

The one-pot synthesis of protected F1–α-antigen **39** employed three saccharide building blocks, namely, 2-*O*-benzoyl-protected thiogalactoside **28**, 2-azido-2-deoxythioglucoside **6**, and 2-azido-2-deoxy-α-galactosylserine **37**. Glucoside **6** was first glycosylated with thiogalactoside **28** under the conventional glycosylation protocol in CH_2Cl_2 to obtain thiodisaccharide **32**, which served as the disaccharide donor in the subsequent glycosylation. Low-concentration conditions were required in the second glycosylation step to effect the β-glycosidic bond formation, which was provided by addition of a CH_3CN/EtCN solution of

37 to the mixture. Subsequently, the coupling reaction of the acceptor **37** and the thiodisaccharide **32** produced the desired F1–α-serine conjugate **39** in 45% yield as a single anomer.

Conclusions

2-Azido-2-deoxythioglucoside and 2-azido-2-deoxythiogalactosides are effective glycosyl donors for 1,2-*trans*-β-glycosidic bond formation under low-concentration glycosylation conditions. The suitability of using such thioglycoside substrates for oligosaccharide synthesis was demonstrated by the synthesis of β-(1→6)-*N*-acetylglucosamine trimers, core-6-unit–serine, and cancer-related F1–α-serine antigens.

Experimental Section

General Experimental: See Supporting Information.

General Low-Substrate-Concentration Glycosylation Procedure for 2-Azido-2-deoxythioglycoside Donors 3, 11, and 12:[22] To a suspension of 2-azido-2-deoxythioglycoside **3**, **11**, or **12** (1.2 equiv.), acceptor **7** or **13**–**17** (1.0 equiv.), flame-dried molecular sieves (4 Å; ca. 5–10 wt.-% of solvent volume) in CH_2Cl_2/CH_3CN/EtCN (1:2:1, v/v) were added NIS (1.2 equiv.) and TMSOTf (0.3–1.5 equiv.) at –70 to –60 °C under N_2. The volume (mL) of solvent mixture used for glycosylation was calculated by referring to the acceptor concentration of 10–15 mM. The reaction was monitored by TLC (hexane/EtOAc or hexane/CH_2Cl_2/EtOAc). After completion of glycosylation, the reaction was quenched with a few drops of NEt_3, satd. aq. $NaHCO_3$ and $Na_2S_2O_3$ (s), followed by stirring at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove MS, and concentrated. The residue was dissolved in CH_2Cl_2 and washed with satd. $NaHCO_3$, H_2O, brine, dried ($MgSO_4$), and concentrated for chromatographic purification.

Typical Sonication Thioglycosidation Procedure for the Synthesis of Peracetyl-2-azido-2-deoxythiogalactoside 9:[14,21,25] Peracetyl-2-azido-2-deoxygalactosyl acetate **8** (31 g, 80 mmol)[31] was dissolved in dried CH_2Cl_2 (150 mL), followed by addition of thiocresol (20 g, 160 mmol) and boron trifluoride–diethyl ether (30 mL, 240 mmol). The reaction mixture was irradiated by ultrasound in a sonication bath (sonication cleaner: Delta DC300) for 40 min then stirred at room temp. for 15 min. The sonication cycle was repeated over 5 h until complete consumption of **8** was observed [reaction analyzed by TLC; R_f of **8** = 0.3 (hexane/CH_2Cl_2/EtOAc, 6:1:1)]. The solution was neutralized and washed with ice-cooled 1 N NaOH (aq.) (4 × 200 mL), then washed with H_2O and brine, dried with $MgSO_4$, and concentrated for chromatographic purification. The thioglycosidation product **9** (35 g, 95%; α/β = 1:1) was obtained as white amorphous solid.

2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (18):[19,22] Figure 2; Table 1, Entry 1. Disaccharide **18** was prepared by glycosylations of acceptor **13** (56 mg, 0.22 mmol) with thioglycoside **3** (151 mg, 0.26 mmol) according to the general low-concentration glycosylation procedure. Compound **18** was obtained as a colorless syrup (154 mg, 83%; α/β = 1:>49) after column chromatography purification (hexane/CH_2Cl_2/EtOAc, 6:1:0 stepwise to 3:1:1, v/v/v). $[\alpha]_D^{30}$ = –25.6 (*c* = 0.14, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.37–7.23 (m, 13 H, ArH), 7.17–7.14 (m, 2 H, ArH), 5.54 (d, *J* = 5.4 Hz, 1 H, C-1), 4.90 (d, *J* = 10.8 Hz, 1 H), 4.81–4.76 (m, 2 H), 4.63–4.50 (m, 4 H), 4.42–4.40 (m, 2 H), 4.32–4.26 (m, 2 H), 4.10–4.03 (m, 2 H), 3.82–3.60 (m, 4 H), 3.46–3.39 (m, 3 H), 1.54 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 1.33 (s, 6 H, 2 × CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.0, 128.41, 128.38, 128.35, 128.0, 127.8, 127.6, 109.3 [*C*(CH_3)$_2$], 108.7 [*C*(CH_3)$_2$], 102.4 (C-1′), 96.3 (C-1), 83.1, 77.7, 75.5, 75.0, 73.5, 71.2, 70.7, 70.5, 68.8, 68.5, 67.6, 66.4, 29.7, 26.0, 25.0, 24.4 ppm.

Methyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→4)-2,3-*O*-isopropylidene-1-α-L-rhamnopyranoside (19): Figure 2; Table 1, Entry 3. Disaccharide **19** was prepared by glycosylations of **14** (68 mg, 0.22 mmol) with **3** (151 mg, 0.26 mmol) according to the general low-concentration glycosylation procedure. Compound **19** was obtained after chromatography purification (hexane/EtOAc, 9:1:0 stepwise to 4:1:1, v/v/v) as a lemon-yellow syrup (145 mg, 60%; α/β = 1:32). $[\alpha]_D^{30}$ = –33.3 (*c* = 0.40, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.26 (m, 13 H, ArH), 7.22–7.21 (d, *J* = 7.0 Hz, 2 H, ArH), 4.91 (d, *J* = 11.0 Hz, 1 H), 4.88 (s, 1 H, 1-H), 4.84 (d, *J* = 8.0 Hz, 1 H, 1′-H), 4.82 (dd, *J* = 8.0, 11.0 Hz, 2 H), 4.63 (d, *J* = 3 Hz, 1 H), 4.59 (dd, *J* = 12, 30 Hz, 2 H),4.29 (t, *J* = 6.0 Hz, 1 H), 4.13 (d, *J* = 5.5 Hz, 1 H), 3.75 (dd, *J* = 4.0, 11.0 Hz, 1 H), 3.70–3.67 (m, 4 H), 3.48 (t, *J* = 9 Hz, 1 H), 3.41 (br. s, 1 H), 3.40 (s, 3 H, OCH_3), 3.37 (d, *J* = 2.0 Hz, 1 H), 1.48 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.35 (d, *J* = 5.0 Hz, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.07, 138.02, 137.99, 128.37, 128.36, 128.33, 128.0, 127.8, 127.7, 127.6, 127.5, 109.3 [*C*(CH_3)$_2$], 100.2 ($J_{C,H}$ = 163.0 Hz, C-1′), 97.9 (C-1), 83.2, 78.4, 78.1, 77.7, 76.0, 75.4, 75.1, 74.9, 73.4, 68.4, 66.4, 63.9, 54.8, 27.8 (CH_3), 26.4 (CH_3), 17.6 (CH_3) ppm. HRMS (ESI): calcd. for $C_{37}H_{45}N_3O_9$ [M + Na]$^+$ 698.3156; found 698.3044.

Methyl 2-Azido-3,4,5-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (20):[19] Table 1, Entry 6. Prepared by glycosylation of **15** (93 mg, 0.2 mmol) with donor **3** (139 mg, 0.24 mmol) according to the general low-concentration glycosylation procedure. Disaccharide **20** was obtained (140 mg, 76%, α/β = 1:33) as a light-yellow syrup after chromatographic purification (hexane/CH_2Cl_2/EtOAc, 6:1:1, v/v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.17 (m, 30 H, ArH), 5.03 (d, *J* = 11.5 Hz, 1 H), 4.83–4.73 (m, 5 H), 4.67 (d, *J* = 12.0 Hz, 1 H), 4.60–4.57 (m, 2 H, including 1-H), 4.54 (d, *J* = 12.0 Hz, 1 H), 4.46 (d, *J* = 12.0 Hz, 1 H), 4.37 (q, *J* = 9.5 Hz, 2 H), 4.25 (d, *J* = 8 Hz, 1 H, 1′-H), 3.99–3.3.89 (m, 3 H), 3.79 (d, *J* = 9.5 Hz, 1 H), 3.70 (d, *J* = 11.0 Hz, 1 H), 3.60 (dd, *J* = 11, 15 Hz, 2 H), 3.49 (m, 2 H), 3.37 (s, 3 H, OCH_3), 3.32 (t, *J* = 9.0 Hz, 1 H), 3.24 (t, *J* = 9 Hz, 1 H), 3.18 (d, *J* = 8.5 Hz, 1 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 139.4, 138.23, 138.19, 137.9, 137.8, 137.7, 128.43, 128.36, 128.29, 128.24, 128.17, 128.0, 127.92, 127.89, 127.77, 127.63, 127.28, 127.47, 127.3, 127.0, 100.8 ($J_{C,H}$ = 161 Hz, C-1′), 98.2 ($J_{C,H}$ = 166 Hz, C-1), 83.2, 80.2, 79.0, 77.8, 76.6, 75.3, 75.2, 75.1, 73.44, 73.39, 73.2, 69.6, 68.5, 68.1, 66.8, 55.2 ppm.

Methyl 2-Azido-3,4,5-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (21): Table 1, Entry 7. A mixture of **3** (140 mg, 0.24 mmol), **16** (93 mg, 0.2 mmol), and activated molecular sieves (4 Å; 1.5 g) in CH_2Cl_2/CH_3CN/EtCN (1:2:1, v/v/v; 16 mL) was stirred under N_2 at –60 °C for 30 min. NIS (55 mg, 0.24 mmol) and TMSOTf (22 μL, 0.12 mml) were added, and the mixture was stirred for 4 h. The reaction mixture was worked up as described in the general low-concentration glycosylation procedure, and the desired disaccharide **21** (100 mg, 55%; α/β = 1:24) was obtained by column chromatographic purification (hexane/CH_2Cl_2/EtOAc, 6.5:1:1, v/v/v). R_f = 0.3 (hexane/

EtOAc, 1:2.5, v/v). $[\alpha]_D^{20}$ = –11.8 (*c* = 0.12, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.38–7.22 (m, 26 H, ArH), 7.17–7.14 (m, 4 H, ArH), 4.92–4.78 (m, 6 H), 4.61–4.41 (m, 7 H), 4.37 (d, *J* = 8.5 Hz, 1 H), 4.27 (s, 1 H), 3.92 (br d, *J* = 8.5 Hz, 1 H), 3.81–3.64 (m, 6 H), 3.61–3.57 (m, 2 H), 3.49–3.46 (m, 1 H), 3.4 (d, *J* = 10 Hz, 1 H), 3.39 (s, 1 H, OCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 138.9, 138.7, 138.5, 138.3, 138.2, 128.9, 128.83, 128.76, 128.7, 128.47, 128.46, 128.4, 128.34, 128.28, 128.1, 128.01, 127.97, 127.8, 101.3 ($J_{C,H}$ = 158 Hz, C-1′), 98.6 (C-1), 83.5, 78.4, 78.0, 75.9, 75.57, 75.54, 75.2, 74.0, 73.8, 73.6, 72.0, 71.2, 70.1, 69.7, 66.6, 55.3 ppm. HRMS (ESI): calcd. for $C_{55}H_{59}N_3O_{10}$ $[M + Na]^+$ 944.4093; found 944.4053.

***p*-Tolyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→6)-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-thio-α-D-glucopyranoside (22):** Table 1, Entry 8. Disaccharide **22** was prepared from glycosylations of 2-azido-2-deoxy-thio-α-D-glucopyranosyl acceptor **7** (107 mg, 0.22 mmol) with 2-azido-2-deoxy-D-glucopyranosyl phosphate **4** (200 mg, 0.26 mmol). To a suspension of donor **4**, acceptor **7**, and flame-dried molecular sieves (4 Å; 2 g) in $CH_2Cl_2/CH_3CN/EtCN$ (1:2:1, v/v/v; 22 mL) was added TMSOTf (36 μL, 0.21 mmol) at –70 to –65 °C under N_2. After completion of the glycosylation, a few drops of NEt_3, satd. aq. $NaHCO_3$, and $Na_2S_2O_3$ (s) were added, and the mixture was stirred at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove molecular sieves, and concentrated as described in the general low-concentration glycosylation procedure. Disaccharide **22** was obtained after column chromatographic purification (hexane/EtOAc/CH_2Cl_2, 16:1:0 stepwise to 8:1:1, v/v/v) as a lemon-yellow syrup (172 mg, 77%; α/β = 1:19). $[\alpha]_D^{30}$ = +74.6 (*c* = 0.39, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.27 (m, 25 H, ArH), 7.18–7.15 (m, 2 H, ArH), 7.07 (d, *J* = 8.1 Hz, 2 H, ArH), 5.53 (d, *J* = 5.4 Hz, 1 H, 1-H), 4.96–4.89 (m, 3 H), 4.58–4.72 (m, 4 H), 4.58–4.47 (m, 3 H), 4.4 (d, *J* = 8.7 Hz, 1 H, 1′-H), 4.14–4.06 (m, 2 H), 3.97–3.92 (m, 1 H), 3.87–3.76 (m, 3 H), 3.70–3.52 (m, 3 H), 3.49–3.34 (m, 3 H), 2.28 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.1, 138.0, 137.7, 137.6, 132.3, 129.8, 129.7, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 102.0 ($J_{C,H}$ = 160 Hz, C-1′), 87.8 (C-1), 83.2, 81.8, 78.0, 77.7, 75.5, 75.3, 74.9, 74.8, 73.4, 71.3, 68.6, 68.0, 66.3, 63.9, 21.0 (CH_3) ppm. HRMS (ESI): calcd. for $C_{54}H_{56}N_6O_8$ $[M + Na]^+$ 965.4558; found 965.4526.

Methyl 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-naphthylmethyl)-D-galactopyranosyl-β(1→4)-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (23): Table 1, Entry 9. Disaccharide **23** was prepared by glycosylation of methyl α-L-rhamnopyranoside **14** (50 mg, 0.23 mmol) with the β-anomer of 2-azido-2-deoxy-D-thiogalactopyranoside **11** (148 mg, 0.28 mmol). To a suspension of donor **11**, acceptor **14**, and flame-dried molecular sieves (4 Å, AW300; 2 g) in $CH_2Cl_2/CH_3CN/EtCN$ (1:2:1, v/v/v; 23 mL) were added NIS (64 mg, 0.29 mmol) and TMSOTf (11 μL, 0.06 mmol) at –70 °C under N_2. Compound **23** was obtained after column chromatographic purification (hexane/EtOAc/CH_2Cl_2, 3:1:1, v/v/v) as a lemon-yellow syrup (102 mg, 70%; α/β = 1:19). $[\alpha]_D^{23}$ = –2.1 (*c* = 1.0, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.83–7.82 (m, 3 H, ArH), 7.79–7.78 (d, *J* = 6 Hz, 1 H, ArH), 7.55–7.52 (m, 3 H, ArH), 7.48–7.35 (m, 5 H, ArH), 5.44 (s, 1 H, benzylidene-*H*), 4.89 (d, *J* = 7.5, 12.5 Hz, 2 H), 4.85 (s, 1 H, 1-H), 4.76 (dd, *J* = 2.0, 8.0 Hz, 1 H, 1′-H), 4.27 (s, 1 H), 4.21 (d, *J* = 12.0 Hz, 1 H), 4.10 (d, *J* = 5.5 Hz, 1 H), 4.03 (d, *J* = 3.0 Hz, 1 H), 3.95 (dd, *J* = 1.5, 12.5 Hz, 1 H), 3.83 (t, *J* = 8.0 Hz, 1 H), 3.69–3.68 (m, 2 H), 3.42 (dd, *J* = 3.5, 10.5 Hz, 1 H), 3.36 (s, 3 H, OCH_3), 3.21 (s, 1 H), 1.47 (s, 3 H, CH_3), 1.33 (d, *J* = 6 Hz, 3 H, CH_3), 1.26 (s, 3 H, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.73, 135.2, 133.1, 133.0, 129.0, 128.2, 127.7, 126.5, 126.3, 126.1, 126.0, 125.7, 109.1, 101.1 (benzylidene-*C*), 100.0 ($J_{C,H}$ = 163 Hz, C-1′), 97.8 ($J_{C,H}$ = 167 Hz, C-1), 78.2, 78.0, 77.5, 76.0, 72.5, 71.7, 69.0, 66.3, 64.0, 62.3, 54.7, 27.8, 26.3, 17.6 ppm. HRMS (ESI): calcd. for $C_{34}H_{39}N_3O_9$ $[M + Na]^+$ 656.2578; found 656.2632.

Methyl 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-naphthylmethyl)-D-galactopyranosyl-β(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (24): Table 1, Entry 10. A mixture of **11** (130 mg, 0.24 mmol), **15** (93 mg, 0.2 mmol), and activated molecular sieves (4 Å; 2 g) in $CH_2Cl_2/CH_3CN/EtCN$ (1:2:1, v/v/v; 16 mL) was stirred at –60 °C under N_2 for 30 min. NIS (55 mg, 0.24 mmol) and TMSOTf (14 μL, 0.08 mmL) were added, and the mixture was stirred for 4 h. The reaction mixture was worked up according to the general low-concentration glycosylation procedure to give the desired disaccharide **24** (141 mg, 80%; α/β = 1:33) as a glassy material after column chromatographic purification (hexane/CH_2Cl_2/EtOAc, 1:1:4.5, v/v/v). R_f = 0.3 (hexane/CH_2Cl_2/EtOAc, 1:1:3, v/v/v). $[\alpha]_D^{20}$ = +34.2 (*c* = 0.11 $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.87–7.80 (m, 4 H, ArH), 7.54 (d, *J* = 8.5 Hz, 1 H, ArH), 7.49–7.47 (m, 6 H, ArH), 7.32–7.14 (m, 16 H, ArH), 5.42 (s, 1 H, benzylidene-H), 5.12 (d, *J* = 10.5 Hz, 1 H), 4.85 (s, 2 H), 4.82–4.77 (m, 2 H), 4.67–4.59 (m, 3 H, including 1-H), 4.37 (d, *J* = 12.5 Hz, 1 H), 4.17–4.14 (m, 2 H, including 1′-H), 4.03 (d, *J* = 11 Hz, 1 H), 3.97–3.93 (m, 3 H), 3.79–3.72 (m, 4 H), 3.52 (br. s, 1 H), 3.38 (s, 3 H, OCH_3), 3.16 (br. d, *J* = 10 Hz, 1 H), 2.79 (s, 1 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 139.6, 138.8, 138.6, 138.2, 135.7, 133.6, 133.5, 129.4, 128.87, 128.80, 128.73, 128.70, 128.58, 128.54, 128.31, 128.21, 128.19, 128.0, 127.7, 126.9, 126.9, 126.7, 126.6, 126.1, 101.8 ($J_{C,H}$ = 160 Hz, C-1′), 101.6 (benzylidene-C), 98.7 (C-1), 80.8, 79.7, 78.7, 77.9, 76.3, 74.0, 73.7, 72.6, 71.8, 70.1, 69.3, 68.9, 66.8, 63.0, 55.8 ppm. HRMS (ESI): calcd. for $C_{52}H_{53}N_3O_{10}$ $[M + Na]^+$ 902.3623; found 902.3692.

6-Chlorohexyl 2-Azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2-naphthylmethyl)-β-D-galactopyranoside (25): Table 1, Entry 11. A suspension of **11** (100 mg, 0.19 mmol), **17** (37 μL, 0.28 mmol), and activated molecular sieves (4 Å; 300 mg) in $CH_2Cl_2/CH_3CN/EtCN$ (1:2:1, v/v/v; 19 mL) was stirred at –70 °C under N_2 for 1 h and then treated with NIS (50 mg, 0.22 mmol) and TMSOTf (9 μL, 0.05 mmol). The mixture was stirred at –70 °C for 2 h. After completion of the glycosylation, a few drops of NEt_3, satd. aq. $NaHCO_3$ and $Na_2S_2O_3$ (s) were added, and the mixture was stirred at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove molecular sieves, and concentrated for chromatographic purification (hexane/CH_2Cl_2/EtOAc, 4:2:1, v/v/v). Glycoside **25** was obtained as a light-yellow oily substance (84 mg, 80%; α/β = 1:19). R_f = 0.3 (hexane/CH_2Cl_2/EtOAc, 4:2:1, v/v/v). $[\alpha]_D^{13}$ = +148.2 (*c* = 0.34). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.82–7.77 (m, 4 H, ArH), 7.54–7.52 (m, 3 H, ArH), 7.48–7.45 (m, 2 H, ArH), 7.37–7.33 (m, 3 H, ArH), 5.44 (s, 1 H, benzylidene-H), 4.88 (s, 2 H), 4.23 (d, *J* = 13 Hz, 1 H), 4.20 (d, *J* = 8.0 Hz, 1 H, 1-H), 4.04 (d, *J* = 3.0 Hz, 1 H), 3.97–3.92 (m, 2 H), 3.87 (dd, *J* = 8, 10 Hz, 1 H), 3.51 (t, *J* = 6.5 Hz, 2 H, CH_2), 3.49–3.47 (m, 1 H), 3.36 (dd, *J* = 5.0, 11.0 Hz, 1 H), 3.20 (s, 1 H), 1.76 (quint, *J* = 7.0 Hz, 2 H, CH_2), 1.66–1.62 (m, 2 H, CH_2), 1.44–1.40 (m, 2 H, CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.6, 135.2, 133.1, 133.0, 128.9, 128.2, 128.1, 127.8, 127.6, 126.5, 126.3, 126.1, 126.0, 125.6, 102.1 ($J_{C,H}$ = 156 Hz, C-1), 101.0 (benzylidene-C), 77.5, 72.4, 71.6, 69.6, 69.0, 66.3, 62.1, 45.0, 32.4, 29.2, 26.5, 25.1 ppm. HRMS (ESI): calcd. for $C_{30}H_{54}ClN_3O_5$ $[M + Na]^+$ 574.2079; found 574.2057.

Methyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-galactopyranosyl-β(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranoside (26):[19] Table 1, Entry 12. A mixture of **12** (150 mg, 0.26 mmol), **15** (100 mg, 0.23 mmol), and activated molecular sieves (4 Å; 2 g) in CH_2Cl_2/

CH_3CN/EtCN (1:2:1, v/v/v; 20 mL) were stirred at –60 °C under N_2 for 30 min. NIS (63 mg, 0.28 mmol) and TMSOTf (28 mL, 0.16 mmol) were added, and the mixture was stirred at –60 °C. Progress of the reaction was monitored by TLC (hexane/CH_2Cl_2/EtOAc, 6:2:1). After 2 h of glycosylation, a few drops of NEt_3, satd. aq. $NaHCO_3$ and $Na_2S_2O_3$ (s) were added, and the mixture was stirred at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove molecular sieves, and concentrated for chromatographic purification (hexane/CH_2Cl_2/EtOAc, 3:1:0.3, v/v/v) Disaccharide **26** was obtained as a glassy solid (150 mg, 65%; α/β = 1:25). R_f = 0.4 (hexane/CH_2Cl_2/EtOAc, 6:2:1, v/v/v). $[\alpha]_D^{20}$ = +12.2 (*c* = 0.12, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.39–7.38 (m, 3 H, ArH), 7.35–7.20 (m, 24 H, ArH), 7.17–7.13 (m, 3 H, ArH), 4.96 (d, *J* = 11.0 Hz, 1 H), 4.88 (d, *J* = 11.0 Hz, 1 H), 4.76 (dd, *J* = 11.0, 29.0 Hz, 2 H), 4.67 (dd, *J* = 5.5, 12.0 Hz, 2 H), 4.61–4.58 (m, 2 H), 4.576 (d, *J* = 3.5 Hz, 1 H, 1-H), 4.50 (d, *J* = 11.5 Hz, 1 H), 4.43 (d, *J* = 11.0 Hz, 1 H), 4.27 (dd, *J* = 12.0, 57.0 Hz, 2 H), 4.14 (d, *J* = 8.0 Hz, 1 H, 1′-H), 3.94 (dd, *J* = 3.0, 10.5 Hz, 1 H), 3.91 (d, *J* = 9.5 Hz, 1 H), 3.85 (t, *J* = 9.0 Hz, 1 H), 3.78–3.72 (m, 2 H), 3.69 (dd, *J* = 1.5, 11.0 Hz, 1 H), 3.50–3.46 (m, 2 H), 3.67 (s, 3 H, OCH_3), 3.30 (dd, *J* = 5.2, 9.1 Hz, 1 H), 3.21 (dd, *J* = 5.4, 8.3 Hz, 1 H), 3.12 (dd, *J* = 2.8, 10.3 Hz, 1 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 139.8, 139.0, 138.8, 138.5, 138.4, 138.1, 128.9, 128.81, 128.80, 128.77, 128.6, 128.5, 128.40, 128.37, 128.33, 128.29, 128.24, 128.22, 128.15, 128.11, 128.07, 127.9, 127.4, 101.6 (C-1′, $J_{C,H}$ = 161.3 Hz), 98.8 (C-1, $J_{C,H}$ = 165 Hz), 81.5, 80.6, 79.5, 77.01, 75.8, 75.15, 74.0, 73.84, 73.75, 73.6, 72.5, 70.1, 68.7, 68.3, 64.2, 55.7 ppm.[19]

6-Chlorohexyl 2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→6)-2-azido-3,4-*O*-di-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→6)-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (35): *N*-Acetylglucosamine trimer in Scheme 3b. A suspension of 2-azido-2-deoxyglucosyl phosphate **4** (226 mg, 0.32 mmol), 2-azido-2-deoxythioglucoside **7** (130 mg, 0.27 mmol), and activated molecula sieves (4 Å; 2 g) in CH_2Cl_2/CH_3CN/EtCN (1:2:1, v/v/v; 26 mL) was stirred at –70 °C under N_2 for ca. 30 min. TMSOTf (48 μL, 0.27 mmol) was then added. Upon completion of the reaction [monitored by TLC; R_f(thiodisaccharide) = 0.6 (hexane/EtOAc, 3:1, v/v)], reducing-end $GlcN_3$ acceptor **34** (150 mg, 0.32 mmol) and NIS (78 mg, 0.35 mmol) were added. Upon completion of the second glycosylation [assessed by TLC; R_f(trisaccharide) = 0.4 (hexane/EtOAc, 3:1, v/v)], a few drops of NEt_3, satd. aq. $NaHCO_3$ and $Na_2S_2O_3$ (s) were added, and the mixture was stirred at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove molecular sieves, and concentrated for chromatographic purification (hexane/CH_2Cl/$EtOAc_2$, 10:1:1 stepwise to 4:1:1, v/v/v) to furnish the expected trisaccharide **35** (260 mg, 72%) as a pale-yellow syrup. $[\alpha]_D^{32}$ = –13.6 (*c* = 0.17 , $CHCl_3$); R_f = 0.4 (hexane/EtOAc, 3:1, v/v). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35–7.25 (m, 33 H, ArH), 7.14–7.13 (m, 2 H, ArH), 4.89–4.85 (m, 4 H), 4.82–4.81 (m, 1 H), 4.79–4.76 (m, 3 H), 4.71 (dd, *J* = 2.5, 10.5 Hz, 1 H), 4.62–4.58 (m, 2 H), 4.560–4.555 (m, 1 H), 4.50 (br. t, *J* = 8.5 Hz, 2 H), 4.385 (d, *J* = 7.5 Hz, 1 H, anomeric-H), 4.379 (d, *J* = 8.0 Hz, 1 H, anomeric-H), 4.30 (br. d, *J* = 7.0 Hz, 1 H, anomeric-H), 4.27 (d, *J* = 3.0, 8.0 Hz, 1 H, anomeric-H), 4.23 (br. d, *J* = 12 Hz, 1 H), 4.17 (d, *J* = 11.0 Hz, 1 H), 4.00–3.98 (m, 1 H), 3.75–3.72 (m, 1 H), 3.67–3.50 (m, 19 H), 1.80–1.74 (m, 2 H, CH_2), 1.68–1.63 (m, 2 H, CH_2), 1.49–1.40 (m, 4 H, 2×CH_2) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.83, 137.78, 137.72, 128.45, 128.42, 128.38, 128.35, 128.0, 127.9, 127.8, 127.7, 127.6, 102.51 ($J_{C,H}$ = 160 Hz, anomeric-C), 102.47 ($J_{C,H}$ = 160 Hz, anomeric-C), 102.0 ($J_{C,H}$ = 159.0 Hz, anomeric-C), 83.0, 78.0, 77.7, 77.5, 75.42, 75.39, 74.92, 74.86, 74.6, 73.4, 69.8, 68.6, 68.4, 66.3, 66.2, 66.1, 45.0 (CH_2), 32.4 (CH_2), 29.3 (CH_2), 26.5 (CH_2), 25.2 (CH_2) ppm. HRMS (MALDI-TOF): calcd. for $C_{73}H_{82}ClN_9O_{13}$ $[M + Na]^+$ 1350.5613; found 1350.5542.

Methyl [2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl-β(1→6)-2-azido-3,4-di-*O*-benzyl-2-deoxy-1-α-D-galactopyranosyl] *N*-[(9-Fluorenylmethoxy)carbonyl]-L-serine Ester 38: Core-6–serine conjugate in Scheme 4b. Prepared from 2-azido-2-deoxythioglucoside **3** (151 mg, 0.26 mmol) and 2-azido-2-deoxygalactosylserine **37** (120 mg, 0.22 mmol) according to the general low-concentration glycosylation procedure. Protected core-6–serine conjugate **37** (180 mg, 57%) was obtained as a lemon-yellow syrup after chromatographiyc purification (hexane/EtOAc/CH_2Cl_2, 6:1:0 stepwise to 3:1:1, v/v/v). R_f = 0.4 (hexane/EtOAc, 4:1, v/v). $[\alpha]_D^{32}$ = +38.3 (*c* = 0.53, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (d, *J* = 7.5 Hz, 2 H, ArH), 7.64–7.60 (m, 2 H, ArH), 7.38–7.23 (m, 27 H, ArH), 7.17–7.12 (m, 2 H, ArH), 5.82 (d, *J* = 8.4 Hz, 1 H, N-H), 4.89–4.65 (m, 7 H), 4.61–4.30 (m, 8 H), 4.23 (t, *J* = 6.9 Hz, 1 H), 4.10–3.91 (m, 5 H), 3.85–3.52 (m, 8 H), 3.45–3.34 (m, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4 (C=O), 155.9 (C=O), 143.8, 141.3, 138.1, 137.8, 137.3, 128.5, 128.4, 128.3, 128.3, 128.2, 127.9, 127.83, 127.77, 127.72, 127.65, 127.0, 125.1, 119.9, 102.0 (C-1′), 99.3 (C-1), 82.9, 75.4, 75.0, 74.8, 73.4, 72.9, 72.0, 70.2, 69.1, 68.4, 67.2, 66.4, 59.5, 54.4, 52.8, 47.1 ppm. HRMS (ESI): calcd. for $C_{55}H_{58}N_6O_9S$ $[M + Na]^+$ 1188.4689; found 1188.4911.

Methyl [2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-D-galactopyranosyl-β(1→4)-2-azido-3,6-*O*-dibenzyl-2-deoxy-D-glucopyranosyl-β(1→6)-2-azido-3,4-dibenzyl-2-deoxy-1-α-D-galactopyranosyl] *N*-[(9-Fluorenylmethoxy)carbonyl]-L-serine Ester (39): F1–α-serine conjugate in Scheme 4c. A suspension of 3,4,6-tri-*O*-benzyl-2-*O*-benzoyl-thiogalactoside donor **27** (323 mg, 0.49 mmol), 2-azido-2-deoxythioglucoside acceptor **6** (200 mg, 0.40 mmol), and activated molecular sieves (4 Å, AW300; 500 mg) in CH_2Cl_2 (8 mL) was stirred at –70 °C for 20 min. NIS (111 mg, 0.5 mmol) and TMSOTf (22 μL, 0.12 mmol) were added to the mixture. When the glycosylation coupling was complete [assessed by TLC; R_f(disaccharide) = 0.2 (hexane/EtOAc, 4:1, v/v)], 2-azido-2-deoxygalactosylserine **37** (288 mg, 0.40 mmol) in CH_3CN (16 mL) and EtCN (8 mL) were added to the reaction mixture, which was stirred at –70 °C for ca. 30 min and then treated with NIS (90 mg, 0.40 mmol) and additional TMSOTf (73 μL, 0.40 mmol). Upon completion of the glycosylation [assessed by TLC; R_f(glycosylation product) = 0.3 (hexane/EtOAc, 2:1, v/v)], a few drops of NEt_3, satd. aq. $NaHCO_3$ and $Na_2S_2O_3$ (s) were added, and the mixture was stirred at room temp. for 20 min. The mixture was diluted with CH_2Cl_2, filtered to remove molecular sieves, and concentrated for chromatographic purification (hexane/EtOAc/CH_2Cl_2, 8:1:0.5 stepwise to 3:1:0.5, v/v/v) to furnish the desired F1–α-serine **39** as a white foam (280 mg, 45%). $[\alpha]_D^{30}$ = +38.5 (*c* = 0.31, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.00 (d, *J* = 7.4 Hz, 1 H), 7.88 (d, *J* = 7.5 Hz, 4 H), 7.73 (t, *J* = 6.1 Hz, 5 H), 7.60 (t, *J* = 7.1 Hz, 7 H), 7.29–7.17 (m, 54 H), 6.14 (t, *J* = 9.4 Hz, 1 H), 5.61 (dd, *J* = 18.8, 10.7 Hz, 1 H), 5.08–4.93 (m, 2 H), 4.81 (d, *J* = 11.0 Hz, 2 H), 4.63–4.55 (m, 8 H), 4.46–4.34 (m, 6 H), 4.25–4.13 (m, 4 H), 3.95 (dd, *J* = 16.3, 6.5 Hz, 7 H), 3.74–3.65 (m, 7 H), 3.46–3.31 (m, 6 H), 3.10 (d, *J* = 9.6 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3 (C=O), 164.9 (C=O), 155.8 (C=O), 143.8, 141.2, 138.8, 138.2, 138.0, 137.8, 137.6, 137.3, 133.0, 129.7, 128.4, 128.4, 128.3, 128.2, 128.18, 128.15, 128.10, 127.9, 127.84, 127.76, 127.71, 127.63, 127.56, 127.4, 127.2, 127.0, 125.1, 119.9, 101.6 (C-1′′), 100.3 (C-1′), 99.4 (C-1), 81.0, 79.6, 75.4, 74.7, 74.4, 73.4, 73.3, 72.3, 71.9, 71.2, 70.1, 69.2, 67.8, 67.1, 65.7, 59.4, 54.3, 52.7, 47.0 ppm. HRMS (ESI): calcd. for $C_{93}H_{93}ClN_7O_{19}$ $[M + Na]^+$ 1634.6526; found 1635.6451.

Supporting Information (see footnote on the first page of this article): Preparation of saccharide building blocks **3**, **4**, **7**, **11**, **12**, **29**,

34, and **37**, and relevant NMR spectra of the glycosylation products **18**–**26**, **30**–**32**, **35**, **38**, and **39**.

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