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Synthesis of cancer-associated glycoantigens: stage-specific embryonic antigen 3 (SSEA-3)

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Abstract—The synthesis of the tumor-associated carbohydrate antigens SSEA-3 and Gb3 in a semi-convergent fashion using building blocks bearing a *S*-thiazolinyl (STaz) moiety is reported. Complete stereoselective control of a difficult α-(1→4)-galactosylation and high overall yields were achieved.

Keywords: Convergent strategy; Glycosylation; Stereoselective synthesis; Selective activation

1. Introduction

Glycosphingolipids of the globo series (globosides) are commonly associated with different types of human carcinomas including lung, breast, kidney, and ovarian.[1] Over the last few years, anti-cancer immunotherapy has emerged as a new promising direction for controlling tumor development and growth. It has been demonstrated that carbohydrate antigens in the form of their protein conjugates can be used as suitable candidates for highly immunogenic anti-cancer vaccines.[2] The high demand for the globosides for quantitative studies and the difficulty in their isolation in pure form have underscored the need for their chemical synthesis.

The stage-specific embryonic antigen-3 (SSEA-3) represents the core pentasaccharide sequence β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Glc*p*, which is common to a number of structurally related glycosphingolipids (Fig. 1). The trisaccharide composing the reducing end of the molecule is commonly referred to as globotriaosyl ceramide (Gb3, Fig. 1). Other related structures include glycosphingolipids of the globo series: Globo-H bearing an additional L-fucose moiety at C-2 of the terminal galactose unit; SSEA-4, which is commonly referred to as monosialosyl galactosyl globoside (MSGG), bearing a (2→3)-linked terminal sialic acid unit; and disialosyl galactosyl globoside (DSGG), bearing an additional sialic acid unit at C-6 of the subterminal galactosamine residue.

In spite of the considerable progress that has been made in the area of convergent,[3,4] polymer-supported,[5] one-pot,[6] and automated[7] oligosaccharide synthesis, the availability of synthetic glycostructures remains low. Indeed, in the current state of knowledge, successful total syntheses of complex carbohydrate structures demand significant resources. As a consequence, synthetic glycoconjugates of the globo series had been mainly accessible to an elite circle of chemists: SSEA-3—Ogawa,[8] Magnusson,[9] Danishefsky,[10] and Seeberger;[11] Globo-H—Danishefsky,[12] Schmidt,[13] Boons,[14] Wong,[15] and Seeberger,[11] or SSEA-4—Hasegawa[16] and Schmidt/Garegg.[17]

As a part of a program to develop convergent strategies for rapid oligosaccharide synthesis, we describe herein the synthesis of the spacer-containing carbohydrate antigens Gb3 and SSEA-3 using *S*-thiazolinyl (STaz) glycosides, an approach that has been recently developed in our laboratory.[18] The methodological importance of synthesizing the SSEA-3 pentasaccharide derives from the fact that its backbone structure is structurally related to other complex glycosphingolipids of

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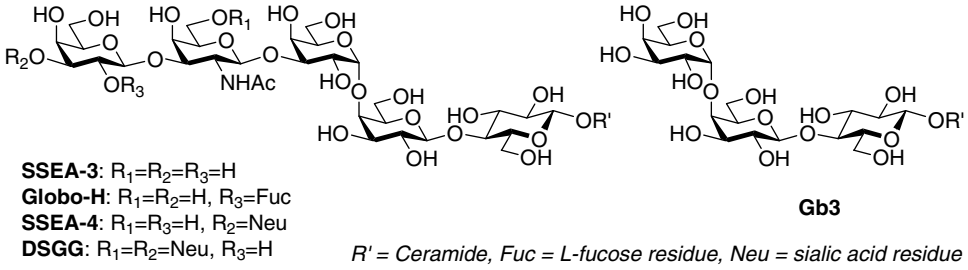


Figure 1. Major tumor-associated antigens of the globo series and Gb3.

the globo series (see Fig. 1). Therefore, it is anticipated that the discoveries made during this synthesis could be translated to future syntheses of other compounds shown in Figure 1. The spacer unit, introduced in place of the ceramide arm, should enable conjugation to an immunogenic carrier protein to alter the poor immunogenicity of the bare oligosaccharide or glycosphingolipid molecules.

2. Results and discussion

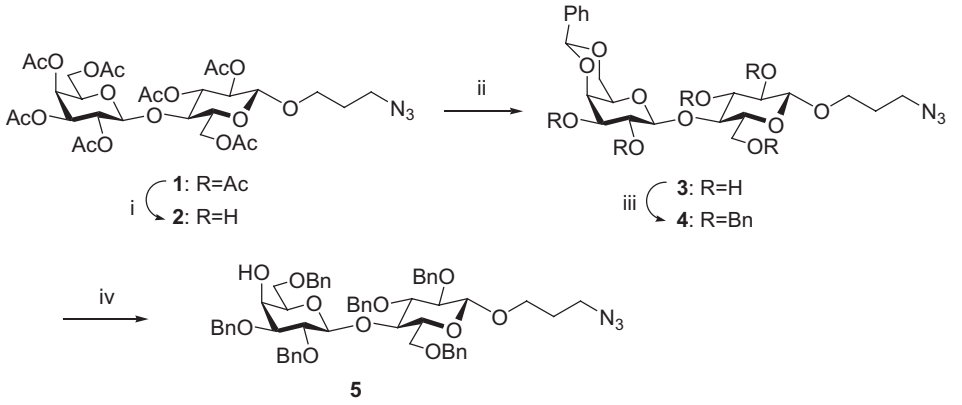
The carbohydrate part of SSEA-3 is a core pentasaccharide consisting of three galactose units, one galactosamine, and one glucose unit, all of the D-pyranose series. It was our intention to execute the synthesis in a 2+3 fashion from the terminal disaccharide β-D-Gal*p*-(1→3)-D-Gal*p*NAc and the reducing end trisaccharide α-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-D-Glc*p* building blocks. The latter oligosaccharide represents the carbohydrate sequence of Gb3 (Fig. 1).

2.1. Synthesis of the Gb3 trisaccharide

The construction of the Gb3 target molecule began with the preparation of a suitably protected lactose building block. For this purpose, we selected known lactose derivative **1** bearing a 3-azidopropyl spacer at the anomeric position.[19] In this context, the azido group can serve as a suitable masking functionality for the amino functionality, which can be removed by simple reduction.[20] Compound **1** was deacetylated ($NaOCH_3$ in CH_3OH) and positions 4′ and 6′ were protected as benzylidene acetal with dimethoxytoluene (DMT) in the presence of camphorsulfonic acid (CSA) in CH_3CN to afford selectively protected compound **3** (Scheme 1).[21] The remaining hydroxyl groups of **3** were protected under standard benzylation conditions (BnBr, NaH, DMF), and the benzylidene acetal was reductively opened with $NaCNBH_3$ in an acidic medium[22] to afford **5** with complete regioselectivity in a high overall yield of 60% over four synthetic steps starting from **1**.

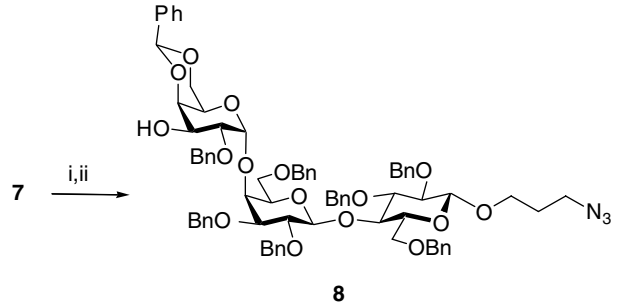
The introduction of the α-(1→4)-linked galactose residue at the terminal end of the GB3 trisaccharide is arguably a major challenge in the synthesis of the tumor antigens of the globo series.[23] A variety of glycosyl donors have been previously tested in this synthetic step: fluoride,[12] trichloroacetamidate,[13] and glycosyl phosphate.[11] In many cases, the stereoselectivity could be improved by elaborate optimizing of reaction conditions, in turn influencing the stereoselectivity of glycosylation.[11,12,17] Typically, only average yields, rarely exceeding 60%, could be achieved at this stage of the synthesis.

As recently demonstrated in our laboratory, glycosyl thioimidates often provide excellent yields and elevated 1,2-cis stereoselectivity in a variety of applications.[24] Therefore, we decided to investigate whether the use of



Scheme 1. Synthesis of the lactose acceptor **5**. Reagents and conditions: (i) $NaOCH_3$, CH_3OH, 89%; (ii) $PhCH(OCH_3)_2$, CSA, CH_3CN, 86%; (iii) BnBr, NaH, DMF, 93%; (iv) $NaCNBH_3$, HCl/Et_2O, THF, 3 Å molecular sieves, 84%.

the STaz glycosyl donor **6**[18] bearing a non-participating benzyl group at C-2 would prove advantageous for this key step of the synthesis. Initially, the standard reaction conditions for activation of the STaz moiety with the use of AgOTf or CH_3OTf as promoters in 1,2-dichloroethane at room temperature were investigated.[23] In both cases, the glycosylation proceeded smoothly leading to the formation of target disaccharide **7** in yields of 87% or 80%, respectively (Scheme 2). Very importantly, complete α-stereoselectivity was obtained in each case, which was sufficient to halt cumbersome experimenting with the reaction conditions.



Scheme 3. Synthesis of the trisaccharide acceptor **8**. Reagents and conditions: (i) $NaOCH_3$, CH_3OH, 99%; (ii) $PhCH(OCH_3)_2$, CSA, CH_3CN, 73%.

2.2. Preparation of the SSEA-3 pentasaccharide

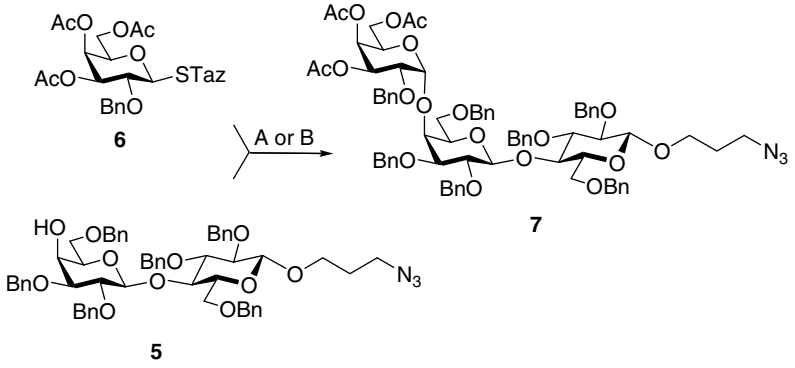
With the desired Gb3 trisaccharide **7** in hand, we turned our attention to the assembly of the remaining sequence of the title pentasaccharide. Trisaccharide **7** needed to be modified to be able to act as the glycosyl acceptor. This was achieved by simple protecting group chemistry involving the two-step deacetylation-benzylidene acetal installation sequence. These manipulations were performed under similar reaction conditions to those described for the synthesis of the building block **5**. As a result, the 3″-OH acceptor **8** was obtained in 73% yield over two synthetic steps (Scheme 3).

In addition, the synthesis of two new building blocks of the D-galacto and D-galactosamino series for the introduction of the terminal and subterminal units, respectively, was required. Our intention to assemble the final sequence in a highly convergent manner required the use of two leaving groups, one of which could have been activated over another under a suitable set of reaction conditions (selective activation).[4] We have already demonstrated that the STaz moiety can be selectively activated over the *O*-pentenyl moiety using AgOTf as a promoter.[25] Therefore, the *O*-pentenyl protection was chosen for the synthesis of the D-galactosamino building block.

Throughout the course of our studies, we investigated a variety of temporary protecting groups for the 2-amino functionality, including *N*-phthalimido, *N*-acetyl, *N*-trichloroethoxycarbonyl (*N*-Troc), and azido. Amongst these, the 2-*N*-Troc substituent,[26,27] in combination with the *O*-pentenyl moiety,[28] seemed to be the most attractive. Considerations such as the ease of the synthesis, regioselective modification, and glycosylation have contributed to this selection. The synthesis of the building block was started from known acetate **9**,[27] which was converted into *O*-pentenyl glycoside **11** via the two-step procedure, involving anomeric bromination (AcBr, AcOH) and O-glycosylation of pent-4-enol under Helferich conditions.[29] Compound **11** was then deacetylated in the presence of guanidine and $NaOCH_3$,[30] and the resulting triol was protected as 4,6-benzylidene acetal **13**. This synthesis was accomplished in 58% yield over four steps starting from **9** (Scheme 4).

The last building block required for the introduction of the terminal galactose residue of SSEA-3, **14**, was obtained from known ethyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside[31] by the previously reported two-step procedure involving anomeric bromination followed by the introduction of the STaz moiety.[25,32] This two-stage procedure afforded the requisite compound **14** in the total yield of 78%.

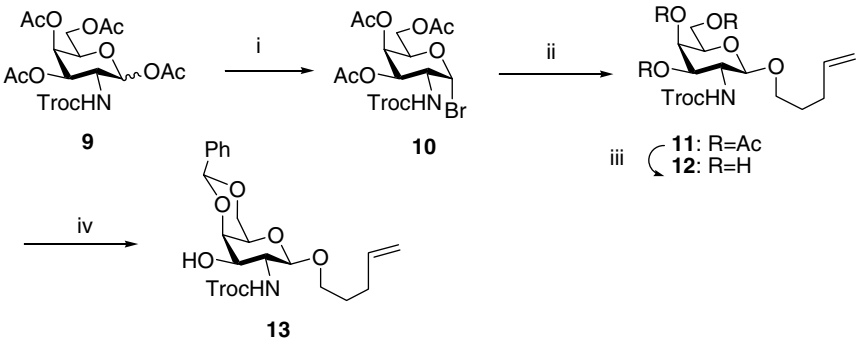
Having obtained building blocks **8**, **13**, and **14**, we refocused our attention on the final assembly of the pentasaccharide of SSEA-3. To achieve this goal, we



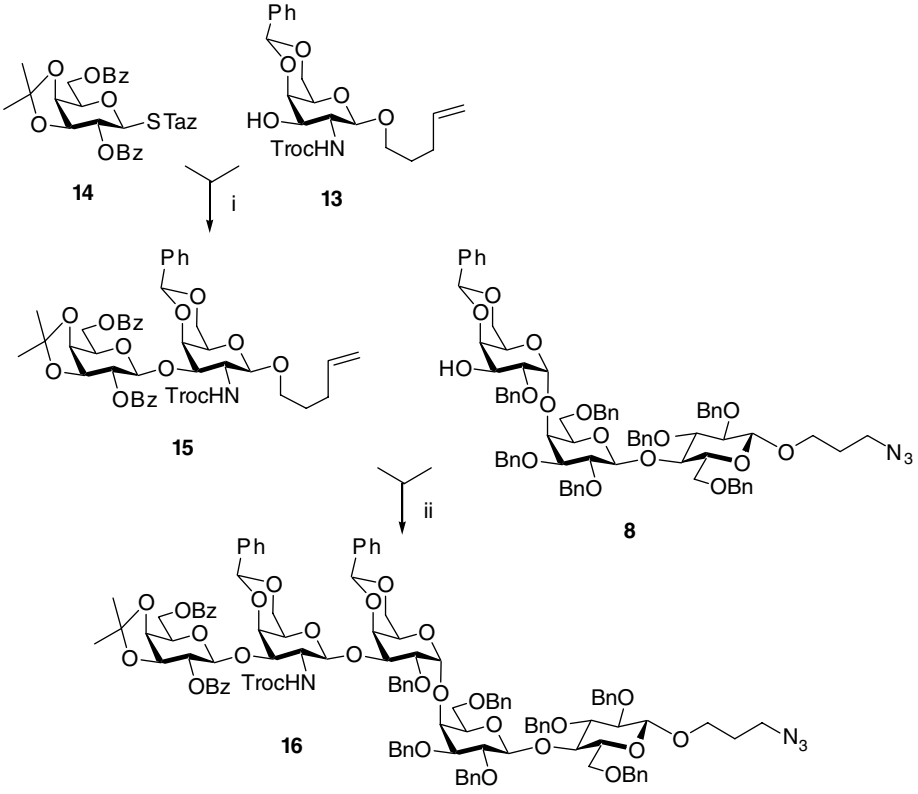
Scheme 2. Synthesis of Gb3 trisaccharide **7**. Reagents and conditions: (A) AgOTf, 1,2-dichloroethane, 25 h, 87% (α only); (B) CH_3OTf, 1,2-dichloroethane, 16 h, 80% (α only).

performed two sequential glycosylations in a highly convergent manner.[4] First, the STaz moiety of the glycosyl donor **14** was activated over the *O*-pentenyl moiety of the glycosyl acceptor **13**. This selective glycosylation was achieved in the presence of AgOTf, conditions under which the *O*-pentenyl moiety remained entirely inert. As a result, the disaccharide derivative **15** was obtained in 79% yield with complete β-stereoselectivity (Scheme 5). In this context, the reported experimental evidence implies the difficulty of glycosylations at the C-3 position of glycosamines. These examples include a relatively low reactivity of the 3-OH that has been observed with *N*-acetamido derivatives[33] or steric factors caused by bulky *N*-phthalimido group.[34]

It has to be also noted that the use of *S*-ethyl glycoside as glycosyl donor did not seem to be feasible. Although we have already demonstrated that the *S*-ethyl moiety can be selectively activated in the presence of *O*-pentenyl glycosides,[35] the necessity to use CH_3OTf as a promoter for such activation could also cause undesirable (and



Scheme 4. Synthesis of the glycosyl acceptor **13**. Reagents and conditions: (i) 30% HBr/AcOH, CH_2Cl_2, 95%; (ii) 4-pentenol, HgO/$HgBr_2$, CH_2Cl_2, 4 Å molecular sieves, 74%; (iii) guanidine·HCl, CH_3OH/CH_2Cl_2, Na, 99%; (iv) $PhCH(OCH_3)_2$, CSA, CH_3CN, 83%.



Scheme 5. The final assembly of the SSEA-3 pentasaccharide **16**. Reagents and conditions: (i) AgOTf, 1,2-dichloroethane, 3 Å molecular sieves, 79%; (ii) NIS/TMSOTf, 1,2-dichloroethane, 4 Å molecular sieves, 73%.

irreversible) 2-N-methylation[36] of the glycosyl acceptor **13** and/or the disaccharide **15**.

The final step of the oligosaccharide assembly involved coupling of the disaccharide **15** with the trisaccharide acceptor **8**. This reaction was performed in the presence of NIS and catalytic TMSOTf, conventional reaction conditions for the *O*-pentenyl moiety activation.[28] As a result, the target pentasaccharide **16** was isolated in 73% yield.

3. Conclusions

In summary, we have synthesized SSEA-3, the tumor-associated antigen of the globo series using STaz glycosides for the installation of two critical glycosidic bonds of the sequence. In parallel, the synthesis of Gb3 was also accomplished. This strategy can be applied for the synthesis of the other tumor-associated antigens, the syntheses of which are currently under pursuit in our laboratory.

4. Experimental

4.1. General methods

Column chromatography was performed on Silica Gel 60 (EM Science, 70–230 mesh). Reactions were monitored by TLC on Kieselgel 60 F_{254} (EM Science). The compounds were detected by examination under UV light and by charring with 10% sulfuric acid in CH_3OH. Solvents were removed under reduced pressure at <40 °C. CH_2Cl_2, $ClCH_2CH_2Cl$, and CH_3CN were distilled from CaH_2 directly prior to use. CH_3OH was dried by heating at reflux over magnesium methoxide, distilled, and stored under argon. Anhydrous DMF (EM Science) and THF were used as received. Molecular sieves (3 or 4 Å) used for reactions, were crushed and activated in vacuo at 390 °C for 8 h in the first instance and then for 2–3 h at 390 °C directly prior to use. AgOTf (Acros) was co-evaporated with toluene (3 × 10 mL) and dried in vacuo for 2–3 h directly prior to use. Optical rotations were measured on a Jasco P-1020 polarimeter. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz (Bruker Advance), ^{13}C NMR spectra were recorded in $CDCl_3$ at 75 MHz (Bruker Advance) or at 125 MHz (Bruker ARX-500). HRMS determinations were made with the use of JEOL MStation (JMS-700) Mass Spectrometer.

4.2. 3-Azidopropyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-4-*O*-β-D-glucopyranoside (4)

A 1.0 M solution of $NaOCH_3$ in CH_3OH (1 mL) was added to a stirred suspension of **1**[19] (310 mg, 0.43 mmol) in dry CH_3OH (10 mL) and the reaction mixture was stirred for 1 h at rt. The reaction was then neutralized with Dowex (H^+), filtered, and concentrated in vacuo to yield crude **2** as white foam (163 mg, 89% yield). The product **2** was dissolved in dry CH_3CN (7 mL) containing $PhCH(OCH_3)_2$ (0.11 mL, 0.74 mmol) and CSA (5 mg, 0.02 mmol). The reaction mixture was kept for 2 h at rt, then neutralized with Et_3N and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH gradient elution) to allow **3** as a colorless syrup (168 mg, 86% yield). The crude **3** was dissolved in dry DMF (5 mL) and benzyl bromide (0.27 mL, 2.29 mmol) followed by portionwise addition of NaH (60% in mineral oil, 137 mg, 3.44 mmol) at rt over 15 min. The stirred reaction mixture was kept for additional 1 h, then quenched by adding crushed ice and stirred until cessation of H_2 evolution. The mixture was then extracted with EtOAc/Et_2O (3 × 20 mL, 1:1, v/v) and the combined organic layer was washed with water (3 × 10 mL). The organic extract was separated, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford **4** as white solid (293 mg, 93% yield). ^{1}H NMR: δ 1.89 (m, 2H, $CH_2CH_2CH_2$), 2.95 (s, 1H, H-5′), 3.37–3.45 (m, 5H, H-2′, H-3, H-5, OCH_2), 3.60–3.70 (m, 3H, H-3′, CH_2N_3), 3.70–3.80 (m, 2H, $J_{2,3} = 7.9$ Hz, H-2, H-6b), 3.80–3.92 (m, 2H, H-4, H-6b′), 3.92–4.05 (m, 3H, $J_{4',5'} = 3.6$ Hz, H-4′, H-6a, CH_2N_3), 4.20–4.25 (dd, 1H, H-6a′), 4.33 (d, 1H, CH_2Ph), 4.38 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.46 (d, 1H, $J_{1,2} = 6.7$ Hz, H-1), 4.56 (d, 1H, CH_2Ph), 4.73–4.88 (m, 7H, CH_2Ph), 5.20 (d, 1H, CH_2Ph), 5.48 (s, 1H, CHPh), 7.17–7.55 (m, 30H, aromatic); FABMS calcd for $C_{57}H_{61}N_3NaO_{11}$ $[M+H]^+$: 986.4204. Found: 986.4204.

4.3. 3-Azidopropyl 2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-4-*O*-β-D-glucopyranoside (5)

A mixture of the acetal **4** (900 mg, 0.93 mmol) and molecular sieves (3 Å, 4 g) in THF (36 mL) was stirred under argon for 1 h. $NaCNBH_3$ (0.78 g, 12.4 mmol) was then added, followed by the dropwise addition of 2 M HCl/Et_2O (6.2 mL, 12.4 mmol) until cessation of H_2 evolution. The reaction mixture was kept for an additional 30 min at rt and the solid was filtered-off and washed with CH_2Cl_2. The combined filtrate (200 mL) was washed with water (50 mL), 20% aq $NaHCO_3$ (50 mL), and water (2 × 50 mL). The organic extract was separated, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford acceptor **5** as colorless syrup (757 mg, 84% yield): $R_f = 0.55$ (2:3, EtOAc/hexanes); $[\alpha]_D^{28}$ +16.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR: 1.81 (m, 2H, $CH_2CH_2CH_2$),

3.20–3.34 (m, 6H, H-2′, H-3, H-5, H-5′, OCH_2), 3.34–3.42 (dd, 1H, $J_{5,6a} = 5.2$ Hz, $J_{6a,6b} = 9.7$ Hz, H-6a), 3.45–3.63 (m, 5H, H-2, H-3′, H-6a′, H-6b, CH_2N_3), 3.72 (dd, 1H, $J_{5,6a} = 4.2$ Hz, $J_{6a',6b'} = 10.9$ Hz, H-6b′), 3.85–3.95 (m, 3H, H-4, H-4′, CH_2N_3), 4.27–4.36 (m, 5H, $J_{1,2} = 7.4$ Hz, $J_{1',2'} = 7.8$ Hz, H-1, H-1′, CH_2Ph), 4.47 (d, 1H, CH_2Ph), 4.55–4.70 (m, 6H, CH_2Ph), 4.75 (d, 1H, CH_2Ph), 4.91 (d, 1H, CH_2Ph), 7.10–7.30 (m, 30H, aromatic); ^{13}C NMR: δ 29.5, 48.5, 66.3, 66.7, 68.6, 72.2, 73.0, 73.4, 73.7, 75.2, 75.3, 75.5, 75.57, 76.8, 77.4, 79.6, 81.3, 82.0, 83.1, 102.7, 103.8, 127.5, 127.7 (×2), 127.8 (×2), 127.9 (×4), 128.0 (×4), 128.1 (×4), 128.3 (×4), 128.5 (×6), 128.6 (×2), 128.7 (×2), 138.1, 130.4, 138.5, 138.8 (×2), 139.3; FABMS calcd for $C_{57}H_{63}N_3NaO_{11}$ $[M+Na]^+$: 988.4360. Found 988.4360.

4.4. 2-Thiazolinyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-1-thio-β-D-galactopyranoside (6)

NaSTaz (1.16 g, 8.22 mmol) was added to a stirred solution of 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl bromide[37] (2.5 g, 5.48 mmol) in dry CH_3CN (15 mL) under argon. The reaction mixture was stirred for 30 min at rt. Upon completion, the mixture was diluted with toluene (200 mL) and washed with 1% aq NaOH (50 mL) and water (3 × 50 mL), the organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford the STaz glycoside **6** (2 g, 73%) as white solid: $R_f = 0.48$ (1:4, EtOAc/CH_2Cl_2); $[\alpha]_D^{23}$ +28.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 1.95, 2.05, 2.15 (3s, 9H, 3 × $COCH_3$), 3.40 (dd, 2H, CH_2N), 3.79 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 4.02 (dd, 1H, $J_{5,6} = 6.8$ Hz, H-5), 4.09–4.19 (m, 3H, H-6a, H-6b, CH_2S), 4.22–4.35 (m, 1H, $J_{CH_2S,CH_2N} = 7.5$ Hz, CH_2S), 4.62 (d, 1H, CH_2Ph), 4.80 (d, 1H, CH_2Ph), 5.07 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 5.39–5.44 (dd, 2H, H-1, H-4), 7.27–7.34 (m, 5H, aromatic); ^{13}C NMR: δ 20.9 (×3), 61.5, 64.3, 67.8, 74.3, 74.7, 75.5, 75.8, 77.4, 85.1, 128.2 (×2), 128.6 (×2), 137.6, 170.0, 170.3, 170.6; FABMS calcd for $C_{22}H_{28}NO_8S_2$ $[M+H]^+$: 498.1256. Found: 498.1254.

4.5. 3-Azidopropyl 3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7)

Method A. A mixture of glycosyl donor **6** (20 mg, 0.04 mmol), glycosyl acceptor **5** (25.8 mg, 0.027 mmol), and freshly activated molecular sieves (3 Å, 60 mg) in $ClCH_2CH_2Cl$ (0.5 mL) was stirred under argon for 1.5 h. Freshly conditioned AgOTf (20.6 mg, 0.08 mmol) was added and the reaction mixture was stirred for 25 h at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq $NaHCO_3$ (10 mL) and water (3 × 10 mL), the organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford trisaccharide **7** as white solid (47 mg, 87% yield) with complete α-stereoselectivity. *Method B*. A mixture of glycosyl donor **6** (20 mg, 0.04 mmol), glycosyl acceptor **5** (25.8 mg, 0.027 mmol), and freshly activated molecular sieves (3 Å, 60 mg) in $ClCH_2CH_2Cl$ (0.5 mL) was stirred under argon for 1.5 h. CH_3OTf (15 μL, 0.12 mmol) was added and the reaction mixture was stirred for 16 h at rt, then diluted with CH_2Cl_2, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq $NaHCO_3$ (10 mL) and water (3 × 10 mL), the organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford trisaccharide **7** (43.2 mg, 80% yield) with complete α-stereoselectivity: $R_f = 0.45$ (2:3, EtOAc/hexanes); $[\alpha]_D^{28}$ +38.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 1.84, 1.85, 2.02 (3s, 9H, 3 × $COCH_3$), 1.87 (m, 2H, $CH_2CH_2CH_2$), 3.25–3.46 (m, 7H, H-2′, H-3, H-5, H-5′, H-6b, CH_2N_3), 3.49–3.70 (m, 5H, H-2, H-3′, H-6a, H-6a′, CH_2^aO), 3.79–4.07 (m, 6H, H-2″, H-4, H-4′, H-5″, H-6b′, CH_2^bO), 4.28 (s, 2H, CH_2Ph), 4.33 (d, 1H, CH_2Ph), 4.34 (d, 1H, $J_{1',2'} = 9.4$ Hz, H-1′), 4.43 (d, 1H, $J_{1,2} = 7.7$ Hz, H-1), 4.47–4.93 (m, 12H, H-6a″, H-6b″, CH_2Ph), 5.04 (d, 1H, CH_2Ph), 5.14 (d, 1H, $J_{1'',2''} = 3.4$ Hz, H-1″), 5.32–5.37 (dd, 1H, $J_{3'',4''} = 3.2$ Hz, H-3″), 5.40 (dd, 1H, $J_{4'',5''} = 1.1$ Hz, H-4″), 7.20–7.40 (m, 35H, aromatic); ^{13}C NMR: δ 20.8, 20.9, 29.4, 48.5, 61.0, 66.4, 66.7, 67.4, 68.4, 68.7, 70.3, 72.9, 73.3, 73.6, 74.0, 75.1, 75.2, 75.4, 77.4, 79.6, 80.9, 81.9, 82.9, 99.9, 103.2, 103.7, 127.2, 127.7, 127.7, 127.8, 127.9, 128.0, 128.1 (×2), 128.2, 128.3, 128.4, 128.5, 128.5, 128.5, 128.6, 128.6, 138.2, 138.3, 138.5, 138.6, 138.8, 139.6; HR-FAB MS calcd for $C_{76}H_{85}N_3NaO_{19}$ $[M+Na]^+$: 1366.5675. Found: 1366.5682.

4.6. 3-Azidopropyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8)

The title compound was obtained from **7** by the deacetylation-benzylidene acetal formation sequence as described in the synthesis of **4** in 72% yield as colorless syrup: $R_f = 0.52$ (2:3, EtOAc/hexanes); $[\alpha]_D^{28}$ +51.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 1.90 (m, 2H, $CH_2CH_2CH_2$), 3.29–3.50 (m, 7H, H-2′, H-3, H-5, H-5′, H-6b, OCH_2), 3.55–3.68 (m, 4H, H-2, H-3′, H-6a, CH_2N_3), 3.75 (dd, 1H, H-6b′), 3.80–3.90 (m, 2H, $J_{2'',3''} = 3.0$ Hz, H-2″, H-6a′), 3.95–4.03 (m, 2H, H-4′, CH_2N_3), 4.07–4.14 (m, 4H, H-4″, H-5″, H-6a″, H-6b″), 4.18 (dd, 1H, $J_{3'',4''} = 10.2$ Hz), 4.23–4.43 (m, 4H, $J_{1',2'} = 8.1$ Hz, H-1′,

CH_2Ph), 4.50 (d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 4.55–4.90 (m, 10H, CH_2Ph), 5.08 (d, 1H, CH_2Ph), 5.20 (d, 1H, $J_{1'',2''} = 3.1$ Hz, H-1″), 5.40 (s, 1H, CHPh), 7.20–7.40 (m, 40H, aromatic); ^{13}C NMR: δ 29.5, 48.6, 63.0, 66.7, 67.4, 68.6, 68.9, 69.4, 72.4, 73.1, 73.3, 73.4, 73.7, 74.3, 75.1, 75.2, 75.3, 75.3, 76.6, 77.0, 77.5, 78.9, 81.5, 81.9, 82.9, 100.5, 101.1, 103.1, 103.7, 126.5, 127.3, 127.4, 127.7, 127.8, 127.8, 127.9, 128.2, 128.2, 128.3, 128.4, 128.5, 128.5, 128.5, 128.6, 128.6, 128.7, 129.2, 137.9, 138.4, 138.6, 138.6, 138.8, 139.5; FABMS calcd for $C_{77}H_{83}N_3NaO_{16}$ $[M+Na]^+$: 1328.5671. Found 1328.5681.

4.7. 4-Pentenyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranoside (13)

To a stirred solution of **9**[27] (3.2 g, 6.13 mmol) in CH_2Cl_2 (25 mL), 30% HBr in acetic acid (20 mL) was added. The solution was stirred at rt for 1 h, then diluted with CH_2Cl_2 (250 mL) and washed with cold water (60 mL), 20% aq $NaHCO_3$ (2×60 mL), and cold water (3×60 mL). The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo to obtain compound **10** as white foam. To a stirred solution of the crude bromide **10** in CH_2Cl_2 (20 mL), 4-pentenol (0.91 mL, 8.84 mmol), freshly activated molecular sieves (4 Å, 9.6 g), HgO (1.27 g, 5.89 mmol), and $HgBr_2$ (106 mg, 0.29 mmol) were added and the reaction mixture was stirred under argon for 2 h. Upon completion, the solid was filtered-off and the combined filtrate was separated and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford *O*-pentenyl glycoside **11** as white foam (2.4 g, 74% yield). Compound **11** was then dissolved in the solution containing guanidine hydrochloride (2.5 g, 26.2 mmol), Na metal (280 mg, 12.2 mmol) in CH_3OH/CH_2Cl_2 (260 mL, 9:1, v/v). The reaction was stirred for 20 min at rt to allow **12** as white solid (1.82 g, 99% yield). Crude compound **12** (1 g, 2.37 mmol) was dissolved in dry CH_3CN (15 mL) containing dimethoxytoluene (0.71 mL, 4.75 mmol) and CSA (28 mg, 0.12 mmol). The reaction mixture was kept for 3 h at rt, then neutralized with Et_3N. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with H_2O (20 mL), 20% aq $NaHCO_3$ (20 mL), and H_2O (3×20 mL). The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (acetone/toluene gradient elution) to afford **13** as white solid (1 g, 83% yield): $R_f = 0.16$ (1:1, EtOAc/hexanes); $[\alpha]_D^{28}$ −3.14 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.71 (m, 2H, $CH_2CH_2CH_2$), 2.11 (m, 2H, CH_2CH=), 3.45–3.49 (m, 2H, H-5, OCH_2), 3.56 (m, 1H, H-2), 3.90–3.97 (m, 2H, H-3, OCH_2), 4.04–4.09 (dd, 1H, $J_{5,6a} = 1.6$ Hz, H-6a), 4.18 (d, 1H, $J_{4,5} = 3.2$ Hz, H-4), 4.30–4.35 (dd, 1H, $J_{5,6b} = 1.1$ Hz, H-6b), 4.55 (d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 4.72 (s, 2H, CH_2CCl_3), 4.95–5.05 (m, 2H, CH_2=), 5.30 (d, 1H, $J_{NH,2} = 6.8$ Hz, NH), 5.56 (s, 1H, CHPh), 5.79 (m, 1H, CH=), 7.35–7.55 (m, 5H, aromatic); ^{13}C NMR: δ 29.0, 30.5, 56.1, 66.9, 69.3, 69.6, 75.0, 75.5, 77.7, 95.9, 101.0, 101.7, 115.4, 126.8 (×2), 128.7 (×2), 129.7, 137.9, 138.5, 155.2; FABMS calcd for $C_{21}H_{27}Cl_3NO_7$ $[M+H]^+$: 510.0853. Found: 510.0854.

4.8. 2-Thiazolinyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (14)

The solution of ethyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside[31] (1.6 g, 3.39 mmol) and activated molecular sieves (3 Å, 1.7 g) in CH_2Cl_2 (51 mL) was stirred under argon for 1 h. A freshly prepared solution of Br_2 in CH_2Cl_2 (32 mL, 1/165, v/v) was then added and the reaction mixture was kept for 5 min at rt, then concentrated under reduced pressure at rt. The crude residue was then treated with NaSTaz (2.39 g, 16.9 mmol, obtained by mixing stoichiometric amounts of $NaOCH_3$ and 2-mercaptothiazoline in distilled CH_3OH at rt)[18] in dry CH_3CN (20 mL) under argon for 20 min at rt. Upon completion, the mixture was diluted with toluene, then filtered. The solid was washed with toluene. The combined filtrate (200 mL) was washed with 1% aq NaOH (50 mL) and H_2O (3×50 mL), the organic layer was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford STaz glycoside **14** as white foam (1.4 g, 78% yield): $R_f = 0.43$ (1:1, EtOAc/hexanes); $[\alpha]_D^{28}$ +39.2 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.36 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 3.14–3.20 (m, 2H, CH_2N), 4.01 (dd, 2H, CH_2S), 4.35–4.41 (m, 2H, H-4,5), 4.47 (dd, 1H, $J_{3,4} = 6.2$ Hz, H-3), 4.55–4.61 (m, 1H, H-6a), 4.65 (dd, 1H, H-6b), 5.41 (dd, 1H, $J_{2,3} = 6.5$ Hz, H-2), 5.57 (d, 1H, $J_{1,2} = 9.3$ Hz, H-1), 7.38–7.55 (m, 6H, aromatic), 8.01–8.08 (m, 4H, aromatic); ^{13}C NMR: δ 26.2, 27.5, 35.1, 63.8, 64.1, 71.3, 73.6, 74.4, 76.5, 77.4, 82.2, 111.1, 128.4 (×4), 129.3, 129.8 (×2), 130.0 (×2), 133.2, 133.5, 163.3, 165.4, 166.3; FABMS calcd for $C_{26}H_{27}S_2NaNO_7$ $[M+Na]^+$: 552.1127. Found: 552.1129.

4.9. 4-Pentenyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranoside (15)

The title compound was obtained by Method A as described for the synthesis of **7** (AgOTf) as white solid, 79% yield: $R_f = 0.32$ (1:9, acetone/toluene); $[\alpha]_D^{29}$ +25.6 (*c* 1.0, $CHCl_3$); ^1H NMR: δ 1.36 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 1.60 (m, 2H, $CH_2CH_2CH_2$), 2.03 (m, 2H, CH_2CH=), 3.17 (s, 1H, H-5′), 3.32–3.42 (m, 2H, H-2′,

OCH_2), 3.71 (d, 1H, H-6b′), 3.81–3.93 (m, 1H, OCH_2), 4.10–4.25 (m, 3H, H-4, H-6a′, H-6b), 4.28–4.50 (m, 4H, H-1′, H-3, H-3′, H-5), 4.58 (dd, 1H, $J_{4',5'} = 3.8$ Hz, H-4′), 4.69 (d, 1H, H-6a), 4.76–4.89 (m, 3H, $J_{1,2} = 8.1$ Hz, H-1, CH_2CCl_3), 4.90–5.05 (m, 2H, CH_2═), 5.20 (m, 1H, NH), 5.25 (dd, 1H, $J_{2,3} = 7.1$ Hz, H-2), 5.46 (s, 1H, CHPh), 5.74 (m, 1H, CH═), 7.32–8.11 (m, 15H, aromatic); ^{13}C NMR: δ 26.5, 27.8, 28.8, 30.2, 54.2, 64.0, 65.5, 66.7, 69.2, 69.2, 71.4, 73.6, 73.7, 76.2, 77.4, 95.9, 100.9, 101.7, 111.3, 115.0, 126.5 (×3), 128.2 (×3), 128.7 (×2), 128.8 (×2), 129.0, 129.9 (×2), 130.0 (×2), 133.5, 133.6, 138.0, 138.3, 154.0, 165.3, 166.4; FABMS calcd for $C_{44}H_{48}Cl_3NaNO_{14}$ $[M+Na]^+$: 942.2038. Found: 942.2023.

4.10. 3-Azidopropyl 2,6-di-*O*-benzoyl-3,4-*O*-isopropylidene-β-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (16)

A mixture of the glycosyl donor **15** (27.7 mg, 0.03 mmol), glycosyl acceptor **8** (19.7 mg, 0.015 mmol), and freshly activated molecular sieves (4 Å, 90 mg) in $ClCH_2CH_2Cl$ (0.5 mL) was stirred for 1 h under argon. NIS (13.6 mg, 0.06 mmol) and TMSOTf (1.1 μL, 0.006 mmol) were added and the reaction mixture was stirred for 5 min at rt. Upon completion, the solid was filtered-off and the residue was washed with CH_2Cl_2. The combined filtrate (30 mL) was washed with 20% aq. $Na_2S_2O_3$ (10 mL) and water (3 × 10 mL). The organic phase was separated, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane gradient elution) to afford the title pentasaccharide **16** in 73% yield (23.6 mg) as color syrup. Selected data: $R_f = 0.45$ (2:3, EtOAc/hexane); $[\alpha]_D^{28}$ +42.2 (*c* 0.2, $CHCl_3$); ^{1}H NMR: δ 1.34 (s, 3H), 1.57 (s, 3H), 1.88 (m, 2H), 3.25–3.30 (m, 2H), 3.30–3.45 (m, 5H), 3.50–3.65 (m, 8H), 3.65–3.75 (dd, 1H), 3.75–3.85 (dd, 1H), 3.85–4.04 (m, 6H), 4.05–4.23 (m, 6H), 4.40–4.68 (m, 7H), 4.70–4.90 (m, 10H), 5.01 (d, 1H), 5.12 (d, 1H), 5.20 (dd, 1H), 5.36 (s, 1H), 5.39 (s, 1H), 7.20–8.20 (m, 55H, aromatic); ^{13}C NMR: δ 100.7, 100.78, 101.5, 103.3, 103.7; FABMS calcd for $C_{116}H_{121}Cl_3N_4NaO_{29}$ $[M+Na]^+$: 2163.7113. Found: 2163.6946.

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