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Synthesis of neoglycoproteins containing O-methylated trisaccharides related to excretory/secretory antigens of *Toxocara* larvae

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Abstract

The disaccharides allyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β- and α-D-galactopyranoside **10a** and **10b** and the trisaccharides allyl 2-*O*-methyl-α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β- and α-D-galactopyranoside **18a** and **18b** have been prepared using stepwise assembly of the sugar units. The glycosidic linkages were formed employing the trichloroacetimidate procedure for the attachment of the galactopyranosyl residue and *N*-iodosuccinimide/triflic acid activation of an ethyl 1-thiofucopyranoside donor for fucosylation. Deprotection furnished the allyl glycosides which were converted into cysteamine-spacered ligands, activated with thiophosgene and subsequently linked to bovine serum albumin. The neoglycoproteins serve as immunoreagents to determine epitope specificities of monoclonal antibodies directed against highly immunogenic *O*-glycans located at the surface of *Toxocara* larvae.

Keywords: Neoglycoprotein; *Toxocara*; Antigen; *O*-Glycan; Fucose

1. Introduction

Toxocara canis and *Toxocara cati* are parasitic roundworms usually occurring in dogs and cats, which may cause severe infections in a human host affecting eyes, liver and the central nervous system.[1] The surface coat of the parasitic nematode larvae may induce a major immunological response of the host immune system.[2] In particular, glycoproteins being present in multiple copies at the outer layer elicit a strong immune reaction, are subsequently released from the surface and divert the immune attack from the mobile nematode. The structure of the excreted and secreted glycoproteins (TES antigens) from *Toxocara canis* investigated by MS analysis of alditol acetates revealed the presence of the trisaccharide backbone α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-D-Gal*p*NAc,[3] with the fucose part being O-methylated at the 2-position. Furthermore, ~50% of the glycans contained a 4-*O*-methyl group at the galactopyranosyl unit, which is also the prominent structure found in *T. cati* glycans. The anomeric configuration of the Gal*p*NAc residue has not yet been determined. Monoclonal anti-carbohydrate antibodies recognizing the TES antigens bind to different sites within the larval parasite.[4] Previously, three disaccharide ligands which are O-methylated either at position 2 of the fucopyranosyl moiety or at position 4 of the galactopyranosyl unit or at both positions have been synthesized.[5] For a more detailed immunochemical characterization of those monoclonal antibodies which should also include the anomeric region of the reducing end as part of the potential epitope, we report on the synthesis of both anomers of the corresponding GalNAc-containing disaccharide allyl glycoside as well as two anomeric trisaccharide ligands containing the terminal 2-*O*-methyl-α-L-fucopyranosyl residue. The allyl glycosides were further converted into spacer-elongated derivatives and coupled to BSA[6] for immunochemical studies.

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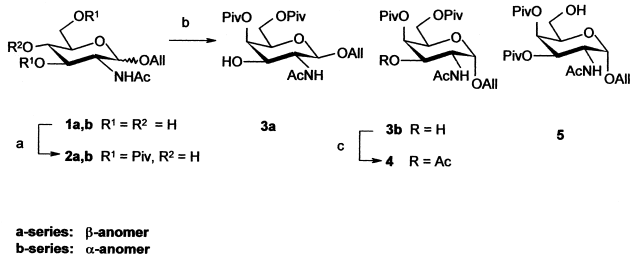
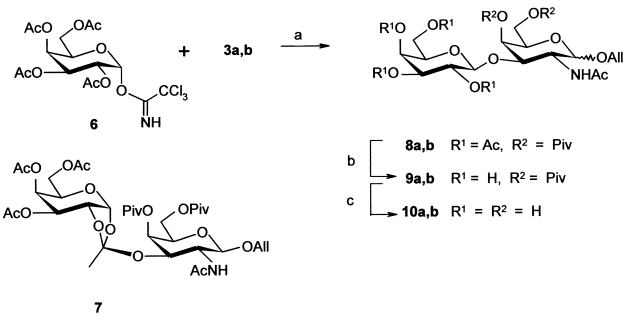
2. Results and discussion

The anomeric allyl 2-acetamido-2-deoxy-galactopyranoside derivatives **3a** and **3b** were prepared separately via epimerization at C-4 of the readily available *N*-acetylglucosamine precursors **1a** and **1b**.[6,7] Thus, reaction of **1a** and **1b** with pivaloyl chloride in pyridine afforded the 3,6-di-*O*-pivaloyl derivatives **2a** and **2b** in 74% and 85% yield, respectively. Following conversion into the corresponding intermediate 4-*O*-triflate derivatives, triflate displacement in the presence of water proceeded smoothly with subsequent acyl migration in the case of the β-allyl glycoside **2a** to furnish the known allyl 2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (**3a**) in 82% yield.[8] By contrast, the intermediate 4-*O*-triflate derivative of α-glycoside **2b** had to be reacted with NaOH to effect epimerization at C-4. Furthermore, the 3,4-di-*O*-pivaloyl derivative **5** was formed as a byproduct, which was separated by column chromatography. For structural confirmation of the substitution pattern, an aliquot of **3b** was O-acetylated (acetic anhydride/pyridine/4-*N*,*N*-dimethylaminopyridine) to give the 3-*O*-acetyl derivative **4**, which displayed a downfield shift of the signal of H-3 to 5.18 ppm in the ^{1}H NMR spectrum (Scheme 1).



Scheme 1. Reagents and conditions: (a) pivaloyl chloride, 1:2 CH_2Cl_2–pyridine, 0 °C, 3 h; (b) Tf_2O, CH_2Cl_2, pyridine, −35 °C→0 °C, 2 h; then H_2O, reflux, 3 h (**3a**) or 1.5 M NaOH, reflux, 15 h (**3b**); (c) $(Ac)_2O$, pyridine, DMAP (cat.), rt, 15 h.

Numerous syntheses for β-Gal*p*-(1→3)-Gal*p*NAc disaccharide units have been reported (for examples see Refs. 9–14) as holds true for fucosylated oligosaccharides of the globo H hexasaccharide.[15–19] For the synthesis of the disaccharide derivatives **8a** and **8b**, the readily accessible 2,3,4,6-tetra-*O*-acetyl-galactopyranosyl trichloroacetimidate **6** (Ref. 20) was first coupled with the glycosyl acceptor derivative **3a** in the presence of boron trifluoride etherate, which produced mainly the exo-orthoester derivative **7** (31%) in addition to a small proportion of the disaccharide **8a** (10%). The presence of the orthoester was deduced from the upfield shifts of the methyl group signal at 1.63 ppm as well as the H-2′ signal (4.31 ppm). Better yields for the β-linked disaccharide derivatives were obtained by adding a solution of donor **6** to a preformed catalyst: acceptor complex.[21] Thus, compounds **8a** and **8b** were isolated in 40% and 45% yield, respectively. Unreacted acceptor derivatives could be recovered in 40–43% yields (Scheme 2).

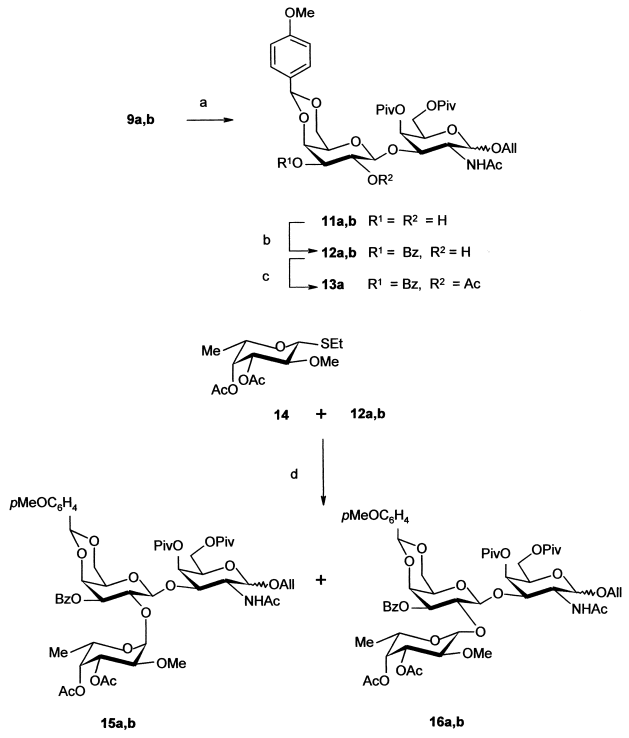


Scheme 2. Reagents and conditions: (a) BF_3-etherate, CH_2Cl_2, −25 °C, 2 h; (b) NaOMe, rt, 3 h; (c) 40% aq Bu_4NOH, 9:1 dioxane–H_2O, rt, 15 h.

The β-anomeric configuration of the galactopyranose residue was confirmed by the value of the coupling constant $J_{1',2'}$ (7.6 Hz for **8a** and 7.9 Hz for **8b**). Selective removal of the acetyl groups was achieved by treatment with sodium methoxide, which produced 4,6-di-*O*-pivaloyl derivatives **9a** and **9b** in 87% and 97% yield, respectively. Finally, the pivaloyl groups were cleaved in the presence of tetrabutylammonium hydroxide in aq dioxane to furnish allyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (**10a**) and allyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (**10b**) in 75% and 89% yield, respectively.

For selective fucosylation of the O-2′ position, compounds **9a** and **9b** were reacted with 4-methoxybenzaldehyde dimethylacetal/toluenesulfonic acid in DMF to give the 4′,6′-*O*-acetals **11a** and **11b** in 72% and 75% yield, respectively. Regioselective protection of the 3′-OH group was accomplished by reaction of **11a** and **11b** with *N*-benzoylimidazole,[22,23] which produced mainly the corresponding 3′-*O*-benzoates **12a** (72%) and **12b** (87% yield) and only minor amounts of disubstituted products. The position of the benzoyl group was confirmed upon subsequent O-acetylation (acetic anhydride/pyridine) which afforded the 2′-*O*-acetyl derivatives **13a** in 95% yield. ^{1}H NMR data showed the expected downfield shift for H-2′ (from 3.55–3.75 ppm in **12a** to 5.40–5.50 ppm in **13a**) (Scheme 3).

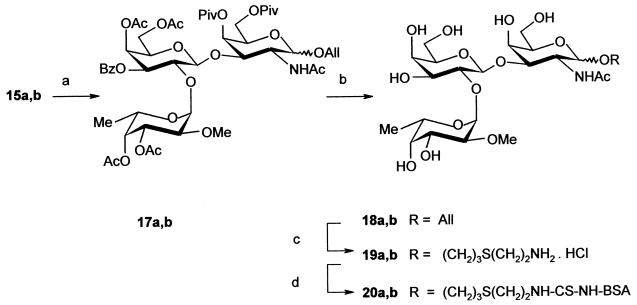
The disaccharide acceptor derivatives **12a** and **12b** were then glycosylated using the previously reported 2-*O*-methyl ethyl-1-thio fucopyranoside donor **14**.[5,24] Whereas iodonium–dicollidinium perchlorate did not effect trisaccharide formation, promotion by *N*-iodosuccinimide/triflic acid furnished a mixture of α- and β-(1→2) linked trisaccharide derivatives which were separated by column chromatography. Thus, the α-fu-



Scheme 3. Reagents and conditions: (a) anisaldehyde dimethylacetal, TsOH, DMF, rt, 15 h; (b) benzoylimidazole, $CHCl_3$, reflux, 48 h; (c) $(Ac)_2O$, pyridine, DMAP (cat.), rt, 15 h; (d) NIS, TfOH, CH_2Cl_2-Et_2O, 5 °C, 15 h.

cosylated derivatives **15a** and **15b** were isolated in 42% and 48% yield, respectively, in addition to the β-(1→2)-linked isomers **16a** (19%) and **16b** (20% yield). The assignment of the anomeric configuration was based on the small value of the coupling constant $J_{1'',2''}$ for the α-fucosyl derivatives, whereas the anomeric protons of the fucopyranosyl units of the β-isomers **16a** and **16b** displayed a spacing of 7.6 and 7.7 Hz, respectively. Deprotection of the trisaccharide compounds **15a** and **15b** was performed *via* removal of the methoxybenzylidene group in 90% aqueous trifluoroacetic acid followed by O-acetylation (acetic anhydride/pyridine/4-dimethylaminopyridine) to afford the tetra-*O*-acetyl derivatives **17a** and **17b** in 94% and 90% yield, respectively.

The acyl groups were finally removed by treatment with tetrabutylammonium hydroxide in aqueous dioxane to give the target allyl trisaccharide derivatives **18a** and **18b** in 82% and 73% yield after final purification of Sephadex LH 20. The structural assignments of the deprotected allyl glycosides were fully confirmed by the NMR data based on COSY, HMQC and HMBC correlations. Thus, glycosylation of O-3 of the galactosamine residue resulted in pronounced downfield shifts of the ^{13}C NMR chemical shift of C-3 (80.78 ppm for **10a** and 77.63 ppm for **10b**), whereas the carbon signals of C-2′ of the β-galactopyranosyl residues displayed a downfield shift upon fucosylation to 76.04 ppm (compound **18a**) and 76.34 ppm (compound **18b**), respectively (Table 1) (Scheme 4).



Scheme 4. Reagents and conditions: (a) 90% TFA, CH_2Cl_2, rt, 1 h, then $(Ac)_2O$, pyridine, DMAP (cat.), rt, 15 h; (b) NaOMe, rt, 15 h, then 40% aq Bu_4NOH, 9:1 dioxane-H_2O, rt, 13 h; (c) cysteamine hydrochloride, H_2O, UV-light (254 nm), rt, 3 h; (d) thiophosgene, 0.1 M aq $NaHCO_3$, rt, 2 h; then BSA, 0.1 M aq $NaHCO_3$, 0.3 M NaCl, rt, 48 h.

For the preparation of neoglycoproteins, the addition of cysteamine to the allylic double bond provided spacer-elongated ligands containing a terminal amino group for further modification.[25,26] Reaction of **18a** and **18b** with cysteamine hydrochloride for 3–6 h afforded the 3-(2-aminoethylthio)propyl glycosides **19a** and **19b** in 70% and 71% yields after purification on a cation-exchange resin followed by desalting on Sephadex LH-20. The structure of the thioether group was based on the NMR spectral characteristics which indicated the absence of the allyl protons and the presence of CH_2N– and CH_2S– at 3.03, 2.73 and 2.54 ppm for **19a** and 3.09, 2.76 and 2.64 ppm for compound **19b**, respectively. Prolonged reaction times of compound **18a** for 2 days, however, led also to formation of N-addition products with subsequent dimerization to a disulfide derivative. The spectrum of the dimer showed two CH_2N– groups in the ^1H NMR spectrum (3.39 and 3.24 ppm) as well as in the ^{13}C NMR assignments (38.96 and 34.47 ppm).

Finally, the trisaccharide ligands were reacted with thiophosgene to give intermediate isothiocyanate derivatives, which were coupled to bovine serum albumin.[27] The neoglycoproteins were purified on Sephadex G-10 and dialyzed against water. According to MALDI MS data, the glycococonjugates **20a** and **20b** contained 6.8 and 3.1 mol ligand/mol protein. Immunochemical results obtained with the neoglycoproteins will be reported elsewhere.

3. Experimental

3.1. General methods

Thin layer chromatography was performed on E. Merck precoated plates (5 × 10 cm, layer thickness 0.25

Table 1
^{13}C NMR data [a] for disaccharides **10a**, **10b** and trisaccharides **18a** and **18b**

Residue	Carbon	δ (ppm)			
		10a	**10b**	**18a**	**18b**
α-2MeFuc*p*-(1→2)	1			97.14	97.38
	2			78.08	78.29
	3			69.39	69.68
	4			72.49	72.74
	5			67.35	67.52
	6			16.04	16.35
β-Gal*p*-(1→3)	1	105.58	105.10	103.01	103.16
	2	71.43	71.01	76.04	76.34
	3	73.32	72.92	74.46	74.65
	4	68.87	68.99	70.08	70.26
	5	75.62	75.37	75.68	75.93
	6	61.75 [b]	61.38 [b]	61.76 [b]	61.99 [b]
α- or β-Gal*p*NAc	1	101.12	96.81	102.52	96.96
	2	52.07	49.01	52.09	50.43
	3	80.78	77.63	77.67	75.09
	4	69.41	69.14	69.39	70.18
	5	75.81	71.01	75.78	71.66
	6	61.83 [b]	61.58 [b]	61.83 [b]	62.22 [b]
OCH_2		71.19	68.83	71.33	69.44
–CH=		134.23	134.09	134.48	134.79
CH_2=		119.05	118.27	118.45	118.64
OMe				58.25	58.50
NHAc		23.05	22.38	23.08	22.94
CO		175.64	174.98	174.61	174.68

[a] Spectra (75.47 MHz) were recorded at 297 K and referenced to 1,4-dioxane (δ 67.40).
[b] Assignments within a column may be reversed.

mm, Silica Gel 60 F_{254}). The spots were detected by UV light and by spraying with anisaldehyde reagent followed by heating to 200 °C. Amine derivatives were additionally detected by ninhydrin spray reagent. Column chromatography was performed on silica gel (0.040–0.063 mm) under normal pressure. Solvents were dried, distilled and kept dry over 4 Å molecular sieves. Evaporation of solvents was performed under reduced pressure at 25–40 °C. Reagents and dry solvents were added *via* oven-dried syringes through septa.^1H NMR spectra were recorded at 300 MHz at 297 K with a Bruker DPX 300F instrument using $CDCl_3$ as solvent and Me_4Si or sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (for solutions in D_2O) as the internal standard.^{13}C NMR spectra were recorded at 75.47 MHz for solutions in D_2O at 24 °C. Chemical shifts (δ) were measured relative to that of 1,4-dioxane, set at δ 67.40 relative to Me_4Si. Homo- and hetero-nuclear 2D NMR spectroscopy was performed with Bruker standard NMR software. MALDI-TOF-MS-ionisation mass spectra were recorded on a Dynamo (Thermo BioAnalysis) instrument in the positive ion mode using CH_3CN or H_2O, both with 2% 2,5-dihydroxybenzoic acid as matrix, by Dr F. Altmann, Institut für Chemie, University of Agricultural Sciences, Vienna. Optical rotations were measured with a Perkin–Elmer 243 B polarimeter. Melting points were determined with a Kofler hot stage and are uncorrected. Elemental analyses were provided by Dr J. Theiner, Mikroanalytisches Laboratorium, Institut für Physikalische Chemie, Universität Wien.

3.2. Allyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (2a)

A mixture of **1a** (2.3 g, 8.8 mmol), pivaloyl chloride (3.25 mL, 26.8 mmol) in CH_2Cl_2 (10 mL) and pyridine (20 mL) was stirred for 3 h at 0 °C under Ar. Methanol (1.0 mL) was added and the mixture was diluted with CH_2Cl_2 (50 mL). The organic phase was washed with 5% aq HCl, water, 5% aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Purification of the residue by column chromatography (1:1 $C_6H_5CH_3$–EtOAc) furnished **2a** as colorless crystals. Yield: 2.8 g (74%); mp 139–140 °C

(*n*-hexane–EtOAc), lit. mp 135–137 °C,[8] $[\alpha]_D^{20}$ −39° (*c* 1.0, $CHCl_3$), lit. $[\alpha]_D^{20}$ −42° (*c* 1.0, $CHCl_3$).[8] ^{1}H NMR ($CDCl_3$): δ 5.87 (d, 1 H, $J_{NH,2}$ 8.8 Hz, NH), 5.86 (m, 1 H, –CH=), 5.25 (dq, 1 H, $=CH_{2trans}$), 5.17 (dq, 1 H, $=CH_{2cis}$), 5.10 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 8.6 Hz, H-3), 4.54 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.45–4.35 (m, 2 H, H-6a, H-6b), 4.31 (ddt,1 H, OCH_2), 4.07 (ddt, 1 H, OCH_2), 3.98 (ddd, 1 H, H-2), 3.60–3.45 (m, 2 H, H-4, H-5), 3.19 (br s,1 H, OH), 1.93 (s, 3 H, Ac), 1.23 and 1.20 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{21}H_{35}NO_8$: C, 58.73; H, 8.21; N, 3.26. Found: C, 58.82; H, 8.21; N, 3.28.

3.3. Allyl 2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (3a)

Compound **2a** (500 mg, 1.16 mmol) was dissolved in CH_2Cl_2 (10 mL) and pyridine (0.5 mL) under Ar at −35 °C. Triflic acid anhydride (0.23 mL, 1.4 mmol) was added dropwise. The mixture was allowed to warm to 0 °C and was stirred for 2 h. Water (1 mL) was added and the mixture was refluxed for 3 h. The solution was diluted with CH_2Cl_2 (50 mL), washed with 5% aq HCl, water, and 5% aq $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated. Purification of the residue on silica gel (40:40:1 EtOAc–*n*-hexane–EtOH) furnished **3a** as a syrup. Yield: (410 mg, 82%); $[\alpha]_D^{20}$ −29° (*c* 0.7, $CHCl_3$), lit. $[\alpha]_D^{20}$ −32.5° (*c* 1.05, $CHCl_3$).[8] ^{1}H NMR ($CDCl_3$): δ 5.92 (m, 1 H, –CH=), 5.72 (d, 1 H, $J_{NH,2}$ 4.9 Hz, NH), 5.32 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 5.30 (dd, 1 H, $J_{3,4}$ 3.8 Hz, H-4), 4.59 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.44–4.30 (m, 2 H, OCH_2, OH), 4.20–4.00 (m, 4 H, H-3, H-6a, H-6b, OCH_2), 3.88 (dd, 1 H, $J_{5,6a} = J_{5,6b}$ 6.8 Hz, H-5), 3.68 (ddd, 1 H, $J_{2,3}$ 10.4 Hz, H-2), 2.05 (s, 3 H, Ac), 1.27 and 1.20 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{21}H_{35}NO_8$: C, 58.73; H, 8.21; N, 3.26. Found: C, 58.23; H, 8.17; N, 3.22.

3.4. Allyl 2-acetamido-2-deoxy-3,6-di-*O*-pivaloyl-α-D-glucopyranoside (2b)

Compound **2b** was prepared from **1b** (1.5 g, 5.74 mmol) in the same fashion as described for **2a** to afford **2b** as crystals. Yield: 2.1 g (85%), mp 75 °C (*n*-hexane–EtOAc); $[\alpha]_D^{20}$ +60° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.86 (m, 1 H, –CH=), 5.74 (d, 1 H, $J_{NH,2}$ 9.7 Hz, NH), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.21 (dq, 1 H, $=CH_{2cis}$), 5.08 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 9.2 Hz, H-3), 4.82 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.41 (dd, 1 H, $J_{6a,5}$ 4.7, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.29 (dd, 1 H, $J_{6b,5}$ 2.3 Hz, H-6b), 4.26 (ddd, 1 H, H-2), 4.18 (ddt,1 H, OCH_2), 3.97 (ddt, 1 H, OCH_2), 3.82 (ddd, 1 H, $J_{4,5}$ 10.5 Hz, H-5), 3.52 (ddd, 1 H, $J_{4,OH}$ 5.0 Hz, H-4), 2.94 (d, 1 H, OH), 1.92 (s, 3 H, Ac), 1.22 and 1.18 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{21}H_{35}NO_8$: C, 58.73; H, 8.21; N, 3.26. Found: C, 58.52; H, 8.28; N, 3.19.

3.5. Allyl 2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (3b) and allyl 2-acetamido-2-deoxy-3,4-di-*O*-pivaloyl-α-D-galactopyranoside (5)

Compound **2b** (500 mg, 1.16 mmol) was dissolved in CH_2Cl_2 (10 mL) and pyridine (0.5 mL) under Ar at −35 °C. Triflic anhydride (0.23 mL, 1.4 mmol) was added dropwise. The mixture was allowed to warm to 0 °C and was stirred for 2 h. Aq NaOH (1.5 M, 1.0 mL) was added and the mixture was refluxed overnight, then diluted with CH_2Cl_2 (50 mL). The organic phase was washed with 5% aq HCl, water, 5% aq $NaHCO_3$, and dried (Na_2SO_4). Purification of the residue on silica gel (40:40:1 EtOAc–*n*-hexane–EtOH) furnished **3b** as crystals. Yield: (225 mg, 45%); mp 120 °C (*n*-hexane–EtOAc); $[\alpha]_D^{20}$ +16° (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.98–5.82 (m, 2 H, NH, –CH=), 5.32 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.30 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 4.95 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.37 (ddd, 1 H, $J_{2,3}$ 11.3, $J_{2,NH}$ 9.7 Hz, H-2), 4.22 (ddt, 1 H, OCH_2), 4.18–3.92 (m, 5 H, H-3, H-5, H-6a, H-6b, OCH_2), 3.21 (br s, 1 H, OH), 2.06 (s, 3 H, Ac), 1.28 and 1.20 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{21}H_{35}NO_8$: C, 58.73; H, 8.21; N, 3.26. Found: C, 58.26; H, 8.12; N, 3.20.

Further elution afforded **5** as crystals. Yield: 150 mg (30%); mp 127 °C (*n*-hexane–EtOAc). $[\alpha]_D^{20}$ +109° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.82 (m, 1 H, –CH=), 5.58 (d, 1 H, $J_{NH,2}$ 9.8 Hz, NH), 5.28–5.10 (m, 4 H, H-3, H-4, $=CH_{2trans}$, $=CH_{2cis}$), 4.85 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.63 (ddd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 4.13 (ddt, 1 H, OCH_2), 4.00 (dd, 1 H, $J_{5,6a} = J_{5,6b}$ 6.8 Hz, H-5), 3.93 (ddt, 1 H, OCH_2), 3.54 (ddd, 1 H, $J_{6a,6b}$ 11.7, $J_{6a,OH}$ 6.9 Hz, H-6a), 3.35 (ddd, 1 H, $J_{6b,OH}$ 6.9 Hz, H-6b), 2.31 (dd, 1 H, OH), 1.87 (s, 3 H, Ac), 1.22 and 1.08 (2 s, 18 H, 2 *t*-Bu). ^{13}C NMR ($CDCl_3$): δ 178.71 and 178.39 (2 (CO), 169.70 (NHAc), 133.23 (–CH=), 118.18 ($=CH_2$), 97.4 (C-1), 69.77 (C-5), 68.64 (OCH_2), 68.39 (C-3), 68.09 (C-4), 60.74 (C-6), 48.10 (C-2), 39.21 and 38.83 [2 Me_3CCO], 27.27 and 26.95 [2 Me_3CCO], 23.27 (CH_3CO). Anal. Calcd for $C_{21}H_{35}NO_8$: C, 58.73; H, 8.21; N, 3.26. Found: C, 58.68; H, 8.06; N, 3.23.

3.6. Allyl 2-acetamido-3-*O*-acetyl-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (4)

A solution of **3b** (10 mg) and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine in dry pyridine (2 mL) was stirred with Ac_2O (200 μL) overnight at room temperature (rt). Methanol (0.5 mL) was added, the solution was coevaporated three times with addition of toluene and concentrated. Purification of the residue on silica gel (2:1 toluene–EtOAc) furnished **4** as syrup. Yield: 10 mg (91%); $[\alpha]_D^{20}$ +72° (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.92 (m, 1 H, –CH=), 5.59 (d, 1 H, $J_{NH,2}$ 9.8 Hz, NH), 5.38 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.29 (dq, 1 H, $=CH_{2trans}$), 5.25 (dq, 1 H, $=CH_{2cis}$), 5.18 (dd,

1 H, $J_{2,3}$ 11.3 Hz, H-3), 4.94 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.61 (ddd, 1 H, H-2), 4.24–4.12 (m, 2 H, H-5, OCH_2), 4.09–3.96 (m, 3 H, H-6a, H-6b, OCH_2), 1.96 (s, 3 H, Ac), 1.27 and 1.19 (2 s, 18 H, 2 *t*-Bu).

3.7. 3,4,6-Tri-*O*-acetyl-1,2-*O*-[1-*exo*-(allyl 2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside)] ethylidene-α-D-galactopyranose (7)

Boron trifluoride etherate (125 μL, 0.98 mmol) was added dropwise for 2 h to a stirred mixture of **3a** (350 mg, 0.82 mmol), **6** (430 mg, 0.87 mmol) and powdered molecular sieves 4 Å (1.5 g) in CH_2Cl_2 (20 mL) under Ar at −25 °C. The suspension was diluted with CH_2Cl_2 (50 mL), neutralized with Et_3N (0.4 mL), filtered over Celite and the filtrate was concentrated. The residue was purified on a column of silica gel (2:3 toluene–EtOAc) to give **7** (exo-isomer, 190 mg, 31%) and the endo-isomer (30 mg, 5%). ^{1}H NMR ($CDCl_3$): δ 5.90 (m, 1 H, –CH=), 5.79 (d, 1 H, $J_{1',2'}$ 4.4 Hz, H-1′), 5.71 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.37 (dd, 1 H, $J_{3',4'}$ $J_{4',5'}$ 3.4 Hz, H-4′), 5.36 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 5.30 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.21 (dq, 1 H, $=CH_{2cis}$), 5.03 (dd, 1 H, $J_{2',3'}$ 6.1 Hz, H-3′), 4.57 (dd, 1 H, $J_{2,3}$ 10.8, $J_{3,4}$ 3.4 Hz, H-3), 4.33 (ddt, 1 H, OCH_2), 4.31 (dd, 1 H, H-2′), 4.27 (ddd, 1 H, $J_{5',6a'}$ 6.7 Hz, H-5′), 4.18 (dd, 1 H, $J_{6a',6b'}$ 11.4 Hz, H-6′a), 4.14–4.02 (m, 4 H, H-6a, H-6b, H-6b′, OCH_2), 3.93 (dd, 1 H, $J_{5,6a}$ 6.6, $J_{5,6b}$ 7.5 Hz, H-5), 3.13 (ddd, H-2), 2.13, 2.06, 2.05 and 1.97 (4 s, 12 H, 4 Ac), 1.63 (s, 3 H, Me), 1.23 and 1.20 (2 s, 18 H, 2 *t*-Bu).

3.8. Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (8a)

A solution of **6** (240 mg, 0.49 mmol) in CH_2Cl_2 (1 mL) was added dropwise for 1 h to a suspension of compound **3a** (200 mg, 0.47 mmol), $BF_3{\cdot}Et_2O$ (64 μL, 0.51 mmol) and powdered molecular sieves 4 Å (1 g) in CH_2Cl_2 (10 mL) at −25° under Ar. The mixture was diluted with CH_2Cl_2 (50 mL), and Et_3N (0.5 mL) was added. The suspension was filtered over Celite, the filtrate was concentrated and the residue was purified on a column of silica gel (40:1 CH_2Cl_2–EtOH) to give **8a** as syrup. Yield: 140 mg (40%); $[\alpha]_D^{20}$ +20° (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.89 (m, 1 H, –CH=), 5.66 (d, 1 H, $J_{NH,2}$ 6.9 Hz, NH), 5.42 (d, 1 H, $J_{3,4}$ 3.6 Hz, H-4), 5.35 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 5.29 (dq, 1 H, $=CH_{2trans}$), 5.22 (dq, 1 H, $=CH_{2cis}$), 5.09 (dd, 1 H, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10.4 Hz, H-2′), 5.07 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.98 (dd, 1 H, H-3′), 4.75 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-3), 4.61 (d, 1 H, H-1′), 4.34 (ddt, 1 H, OCH_2), 4.16 (dd, 1 H, $J_{5',6a'}$ 4.8, $J_{6a',6b'}$ 11.4 Hz, H-6a′), 4.13–4.04 (m, 3 H, H-6a, H-6b, OCH_2), 3.99 (dd, 1 H, $J_{5',6b'}$ 7.9 Hz, H-6b′), 3.92–3.38 (m, 2 H, H-5, H-5′), 3.25 (ddd, 1 H, H-2), 2.14, 2.07, 2.05, 1.99 and 1.98 (5 s, 15 H, 5 Ac), 1.26 and 1.22 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{35}H_{53}NO_{17}$: C, 55.33; H, 7.03; N, 1.84. Found: C, 55.23; H, 7.22; N, 1.87.

Further elution afforded **8a** as syrup. Yield: 60 mg (10%).

3.9. Allyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (8b)

The coupling of donor **6** (320 mg, 0.65 mmol) and acceptor **3b** (250 mg, 0.58 mmol) was performed in the same way as described for **8a** to give **8b** as syrup.Yield: 200 mg (45%); $[\alpha]_D^{20}$ +56° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 5.90 (m, 1 H, –CH=), 5.85 (d, 1 H, $J_{NH,2}$ 9.0 Hz, NH), 5.37 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.34 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4′), 5.29 (dq, 1 H, $=CH_{2trans}$), 5.25 (dq, 1 H, $=CH_{2cis}$), 5.10 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.5 Hz, H-2′), 4.96 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.94 (dd, 1 H, H-3′), 4.59 (d, 1 H, H-1′), 4.56 (ddd, 1 H, $J_{2,3}$ 10.9 Hz, H-2), 4.25–4.05 (m, 5 H, H-5, H-6a, H-6a′, H-6b′, OCH_2), 4.05–3.80 (m, 4 H, H-3, H-6b, H-5′, OCH_2), 2.16, 2.06, 2.05, 1.99 and 1.96 (5 s, 15 H, 5 Ac), 1.25 and 1.21 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{35}H_{53}NO_{17}$: C, 55.33; H, 7.03; N, 1.84. Found: C, 55.16; H, 7.00; N, 1.88.

3.10. Allyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (9a)

A solution of **8a** (140 mg) in dry MeOH (20 mL) was stirred with 0.1 M methanolic NaOMe (0.35 mL) for 3 h at rt. The pH of the solution was adjusted to 7.0 by addition of Dowex 50 (H^+) resin. The resin was filtered off and the filtrate was taken to dryness to give **9a** as a syrup. Yield: 95 mg (87%); $[\alpha]_D^{20}$ +19° (*c* 0.7, MeOH). ^{1}H NMR (D_2O): δ 5.90 (m, 1 H, –CH=), 5.49 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.31 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 4.66 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.36 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.33 (ddt, 1 H, OCH_2), 4.20–4.00 (m, 6 H, H-5, H-6a, H-6b, H-2, H-3, OCH_2), 3.88 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 3.76 (dd, 1 H, $J_{6a',5'}$ 7.5, $J_{6a',6b'}$ 11.5 Hz, H-6a′), 3.71 (dd, 1 H, $J_{6b',5'}$ 4.6 Hz, H-6b′), 3.62 (dd, 1 H, H-5′), 3.57 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3′), 3.38 (dd, 1 H, H-2′), 2.02 (s, 3 H, Ac), 1.26 and 1.19 (2 s, 18 H, *t*-Bu). Anal. Calcd for $C_{27}H_{45}NO_{13}{\cdot}2\,H_2O$: C, 51.95; H, 7.44; N, 2.26. Found: C, 51.67; H, 7.87; N, 2.23.

3.11. Allyl β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (9b)

The de-*O*-acetylation of **8b** (180 mg) was carried out in the same fashion as for **8a** to give **9b** as syrup. Yield: 135 mg (97%); $[\alpha]_D^{20}$ +88° (*c* 0.5, MeOH). ^{1}H NMR (D_2O): δ 5.97 (m, 1 H, –CH=), 5.55 (d, 1 H, $J_{3,4}$ 3.0 Hz,

H-4), 5.35 (dq, 1 H, $=CH_{2trans}$), 5.27 (dq, 1 H, $=CH_{2cis}$), 5.00 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.48 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-2), 4.40 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.24 (dd, 1 H, $J_{3,4}$ 3.0 Hz, H-3), 4.25–4.11 (m, 3 H, H-5, H-6a, OCH_2), 4.06 (ddt, 1 H, OCH_2), 4.00 (dd, 1 H, $J_{6b,5}$ 8.1, $J_{6a,6b}$ 11.3 Hz, H-6b), 3.88 (d, 1 H, $J_{3',4'}$ 3.6 Hz, H-4′), 3.77 (dd, 1 H, $J_{6a',5'}$ 7.5 Hz, H-6a′), 3.71 (dd, 1 H, $J_{6b',5'}$ 4.3 Hz, H-6b′), 3.62 (dd, 1 H, H-5′), 3.58 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3′), 3.38 (dd, 1 H, H-2′), 2.02 (s, 3 H, Ac), 1.26 and 1.19 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{27}H_{45}NO_{13}\cdot H_2O$: C, 53.44; H, 7.55; N, 2.34. Found: C, 53.19; H, 7.77; N, 2.30.

3.12. Allyl β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-β-D-galactopyranoside (10a)

Compound **9a** (15 mg, 0.03 mmol) was dissolved in 90% aq dioxane (3 mL), and then 40% aq tetra-*n*-butylammonium hydroxide (40 μL, 0.22 mmol) was added. After stirring overnight at ambient temperature, the solution was neutralized by addition of Dowex 50 (H^+) resin. The resin was filtered off, the filtrate was concentrated and the residue was purified on a column of silica gel (1:1 EtOH–EtOAc) to give **10a** as an amorphous powder. Yield: 8 mg (75%); $[\alpha]_D^{20}$ −11° (*c* 0.5, MeOH). ^{1}H NMR (D_2O): δ 5.91 (m, 1 H, –CH=), 5.31 (dq, 1 H, $=CH_{2trans}$), 5.26 (dq, 1 H, $=CH_{2cis}$), 4.55 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.43 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.34 (ddt, 1 H, OCH_2), 4.25–4.10 (m, 2 H, H-4, OCH_2), 4.01 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.9 Hz, H-2), 3.9 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.89–3.64 (m, 7 H, H-3, H-5, H-6a, H-6b, H-5′, H-6a′, H-6b′), 3.65 (dd, 1 H, $J_{2',3'}$ 9.9 Hz, H-3′), 3.52 (dd, 1 H, H-2′) and 2.01 (s, 3 H, Ac). Anal. Calcd for $C_{17}H_{29}NO_{11}$: C, 48.22; H, 6.90; N, 3.31. Found: C, 44.26; H, 6.66; N, 2.97.

3.13. Allyl β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-α-D-galactopyranoside (10b)

The deprotection of **9b** (15 mg) was done as described for **9a** to give **10b** as syrup. Yield: 9.5 mg (89%); $[\alpha]_D^{20}$ +132° (*c* 0.7, MeOH). ^{1}H NMR (D_2O): δ 5.97 (m, 1 H, –CH=), 5.35 (dq, 1 H, $=CH_{2trans}$), 5.25 (dq, 1 H, $=CH_{2cis}$), 4.94 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.46 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.34 (dd, 1 H, $J_{2,3}$ 11.1 Hz, H-2), 4.24 (d, 1 H, $J_{3,4}$ 3.2 Hz, H-4), 4.21 (ddt, 1 H, OCH_2), 4.10–3.95 (m, 3 H, H-3, H-5, OCH_2), 3.90 (d, 1 H, $J_{3',4'}$ 3.4, Hz, H-4′), 3.85–3.68 (m, 4 H, H-6a, H-6b, H-6a′, H-6b′), 3.64 (dd, 1 H, $J_{6a',5'}$ 6.9, $J_{6b',5'}$ 4.4 Hz, H-5′), 3.62 (dd, 1 H, $J_{2',3'}$ 10.0 Hz, H-3′), 3.51 (dd, 1 H, H-2′) and 2.01 (s, 3 H, Ac). Anal. Calcd for $C_{17}H_{29}NO_{11}\cdot H_2O$: C, 46.25; H, 7.08; N, 3.17. Found C, 46.34; H, 7.02; N, 3.02.

3.14. Allyl 4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (11a)

A mixture of **9a** (140 mg, 0.24 mmol), anisaldehyde dimethylacetal (170 μL, 0.93 mmol) and *p*-toluenesulfonic acid monohydrate (15 mg) in dry DMF (3 mL) was stirred overnight at rt. Triethylamine (0.1 mL) was added and the solution was concentrated. The residue was purified on silica gel (20:1 CH_2Cl_2–EtOH) to furnish **11a** as colourless syrup. Yield: 120 mg (72%); $[\alpha]_D^{20}$ +63° (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.50–6.80 (m, 4 H, arom. H), 6.05 (d, 1 H, $J_{NH,2}$ 7.4 Hz, NH), 5.88 (m, 1 H, –CH=), 5.47 (s, 1 H, Ph*CHO*), 5.37 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.19 (dq, 1 H, $=CH_{2cis}$), 4.75 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.48 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 3.4 Hz, H-3), 4.40 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1′), 4.35 (ddt, 1 H, OCH_2), 4.24 (dd, 1 H, $J_{5',6a'}$ 1.2, $J_{6a',6b'}$ 12.3 Hz, H-6a′), 4.18 (dd, 1 H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 11.4 Hz, H-6a), 4.15–4.02 (m, 2 H, H-6b, OCH_2), 3.99 (dd, 1 H, $J_{5',6b'}$ 1.6 Hz, H-6b′), 3.86 (dd, 1 H, $J_{5,6b}$ 7.7 Hz, H-5), 3.81 (s, 3 H, OMe), 3.75–3.55 (m, 4 H, H-2, H-2′, H-3′, OH), 3.44 (m, 1 H, H-5′), 2.69 (bs, 1 H, OH), 1.99 (s, 3 H, Ac), 1.24 and 1.20 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{35}H_{51}NO_{14}\cdot 0.5\ H_2O$: C, 58.28; H, 7.16; N, 1.90. Found: C, 58.48; H, 7.29; N, 1.95.

3.15. Allyl 4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (11b)

Compound **11b** was prepared from **9b** (100 mg, 0.17 mmol) and anisalde hydedimethylacetal (125 μL, 0.69 mmol) in the same way as described for the preparation of **11a**. A colorless syrup was obtained after chromatography on silica gel (20:1 CH_2Cl_2–EtOH). Yield: 90 mg (75%); $[\alpha]_D^{20}$ +55° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.45–6.80 (m, 4 H, arom. H), 6.21 (d, 1 H, $J_{NH,2}$ 6.8 Hz, NH), 5.87 (m, 1 H, –CH=), 5.50 (s, 1 H, Ph*CHO*), 5.35–5.51 (m, 4 H, H-1, H-4, $=CH_{2trans}$, $=CH_{2cis}$), 4.46 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.38–4.00 (m, 11 H, H-2, H-3, H-5, OH, H-6a, H-6b, H-4′, H-6a′, H-6b′, 2 OCH_2), 3.85–3.75 (m, 4 H, OMe, H-2′), 3.66 (ddd, 1 H, $J_{2',3'}$ 9.5, $J_{3',4'}$ 3.5, $J_{3',OH}$ 6.4 Hz, H-3′), 3.50 (m, 1 H, H-5′), 2.59 (d, 1 H, OH), 1.99 (s, 3 H, Ac), 1.26 and 1.19 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{35}H_{51}NO_{14}$: C, 59.23; H, 7.24; N, 1.97. Found: C, 58.88; H, 7.29; N, 1.74.

3.16. Allyl 3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1 → 3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (12a)

A mixture of **11a** (115 mg, 0.16 mmol) and benzoyl imidazole (60 mg, 0.35 mmol) in dry $CHCl_3$ (10 mL) was refluxed for 48 h at 80 °C. The mixture was diluted

with CH_2Cl_2 (50 mL) and MeOH (0.2 mL) was added. Concentration of the solution gave a residue, which was purified on silica gel (20:1 CH_2Cl_2–EtOH) to furnish **12a** as syrup. Yield: 95 mg (72%); $[\alpha]_D^{20}$ +63° (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.50–6.80 (m, 9 H, arom. H), 6.10 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 5.87 (m, 1 H, –CH=), 5.45 (s, 1 H, Ph*CHO*), 5.37 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.09 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.4 Hz, H-3′), 4.62 (d, 1 H, $J_{1',2'}$ 8.3 Hz, H-1′), 4.58 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.45–4.38 (m, 2 H, H-3, H-4′), 4.34 (ddt, 1 H, OCH_2), 4.25 (dd, 1 H, $J_{5',6a'}$ 1.1, $J_{6a',6b'}$ 12.2 Hz, H-6a′), 4.18 (dd, 1 H, $J_{5,6a}$ 5.4, $J_{6a,6b}$ 11.4 Hz, H-6a), 4.13–4.04 (m, 3 H, H-6b, H-2′, OCH_2), 4.02 (dd, 1 H, $J_{5',6b'}$ 1.3 Hz, H-6b′), 3.88–3.82 (m, 2 H, H-5, OH), 3.79 (s, 3 H, OMe), 3.76 (ddd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.59 (m, 1 H, H-5′), 1.98 (s, 3 H, Ac), 1.23 and 1.21 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd for $C_{42}H_{55}NO_{15}$: C, 61.98; H, 6.81; N, 1.72. Found: C, 61.76; H, 6.64; N, 1.64.

3.17. Allyl 2-*O*-acetyl-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (13a)

A solution of **12a** (40 mg) and a catalytic amount of 4-*N*,*N*-dimethylaminopyridine in dry pyridine (2 mL) was stirred with Ac_2O (200 μL) overnight at rt. Methanol (1 mL) was added and the solution was coevaporated three times with addition of toluene. The solution was concentrated and purified on silica gel (1:1 *n*-hexane–EtOAc) to furnish **13a** as a syrup. Yield: 40 mg (95%); $[\alpha]_D^{20}$ +90° (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.20–6.80 (m, 9 H, arom. H), 5.96–5.75 (m, 2 H, NH, CH=), 5.50–5.40 (m, 3 H, H-2′, H-4, Ph-*CHO*), 5.26 (dq, 1 H, $=CH_{2trans}$), 5.20 (dq, 1 H, $=CH_{2cis}$), 5.09 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.5 Hz, H-3′), 5.05 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.71 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3), 4.69 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.44 (d, 1 H, H-4′), 4.37–4.26 (m, 2 H, H-6a′, OCH_2), 4.21 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.07 (ddt, 1 H, OCH_2), 4.03–3.94 (m, 2 H, H-6b, H-6b′), 3.88 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{5,6b}$ 7.1 Hz, H-5), 3.79 (s, 3 H, OMe), 3.54 (m, 1 H, H-5′), 3.37 (ddd, 1 H, $J_{2,NH}$ 7.0 Hz, H-2), 1.97, 1.96 (2 s, 6 H, 2 Ac), 1.23 and 1.21 (2 s, 18 H, 2 *t*-Bu).

3.18. Allyl 3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (12b)

Compound **12b** was prepared from **11b** (85 mg, 0.12 mmol) and benzoyl imidazole (45 mg, 0.26 mmol) in the same way as described for the preparation of **12a**. Chromatography on silica gel (20:1 CH_2Cl_2–EtOH) furnished **12b** as a colorless syrup. Yield: 85 mg (87%); $[\alpha]_D^{20}$ +112° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.20–6.80 (m, 9 H, arom. H), 6.28 (d, 1 H, $J_{NH,2}$ 6.7 Hz, NH), 5.87 (m, 1 H, –CH=), 5.49 (s, 1 H, Ph*CHO*), 5.40 (m, 1 H, H-4), 5.28 (dq, 1 H, $=CH_{2trans}$), 5.23–5.15 (m, 2 H, H-1, $=CH_{2cis}$), 5.11 (dd, 1 H, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.4 Hz, H-3′), 4.65 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.47 (d, 1 H, H-4′), 4.38–4.13 (m, 8 H, H-2, H-3, H-5, H-6a, H-2′, H-6a′, OH, OCH_2), 4.13–4.00 (m, 3 H, H-6b, H-6b′, OCH_2), 3.83 (s, 3 H, OMe), 3.66 (m, 1 H, H-5′), 2.03 (s, 3 H, Ac), 1.28 and 1.23 (2 s, 18 H, 2 *t*-Bu). Anal. Calcd. for $C_{42}H_{55}NO_{15}$: C, 61.98; H, 6.81; N, 1.72. Found: C, 61.68; H, 6.43; N, 1.59.

3.19. Allyl 3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→2)-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (15a) and allyl 3,4-di-*O*-acetyl-2-*O*-methyl-β-L-fucopyranosyl-(1→2)-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (16a)

A solution of NIS (16 mg, 0.7 mmol) and TfOH (4 μL) in 1:1 CH_2Cl_2–Et_2O (2 mL) was added to a suspension of **12a** (40 mg, 0.05 mmol), **14** (20 mg, 0.07 mmol) and powdered molecular sieves 4 Å (0.4 g) in 1:1 CH_2Cl_2–Et_2O (2 mL) at −20° under Ar. The suspension was stirred overnight at 5°, diluted with CH_2Cl_2 (50 mL) and filtered over Celite. The filtrate was washed with 5% aq $Na_2S_2O_3$, satd aq $NaHCO_3$ and dried (Na_2SO_4). The solution was concentrated and purified on silica gel (1:1 toluene–EtOAc) to afford **15a** as syrup. Yield: 22 mg (42%); $[\alpha]_D^{20}$ +37° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.10–6.80 (m, 9 H, arom. H), 6.57 (d, 1 H, $J_{NH,2}$ 6.6 Hz, NH), 5.92 (m, 1 H, –CH=), 5.57 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-4), 5.36–5.42 (m, 2 H, H-1″, PhCHO), 5.30 (dq, 1 H, $=CH_{2trans}$), 5.26–5.15 (m, 3 H, H-3′, H-4″, $=CH_{2cis}$), 5.12 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 3.2 Hz, H-3″), 4.96 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.94 (dd, 1 H, $J_{2,3}$ 9.9 Hz, H-3), 4.66 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.45–4.28 (m, 4 H, H-4′, H-6a′, H-5″, OCH_2), 4.25 (dd, 1 H, $J_{5,6a}$ 3.8, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.19 (dd, 1 H, $J_{2',3'}$ 9.8 Hz, H-2′), 4.10 (ddt, 1 H, OCH_2), 4.05–3.90 (m, 3 H, H-5, H-6b, H-6b′), 3.82 (s, 3 H, MeOPh), 3.55 (m, 1 H, H-5′), 3.45 (dd, 1 H, $J_{1'',2''}$ 3.5 Hz, H-2″), 3.34 (ddd, 1 H, $J_{2,3}$ 9.9 Hz, H-2), 3.03 (s, 3 H, OMe), 2.14, 2.00, 1.97 (3 s, 9 H, 3 Ac), 1.26, 1.22 (2 s, 18 H, 2 *t*-Bu) and 1.09 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6″). Anal. Calcd for $C_{53}H_{71}NO_{21}$: C, 60.16; H, 6.76; N, 1.32. Found: C, 60.00; H, 7.00; N, 1.39.

Further elution afforded **16a** as syrup. Yield: 8 mg (19%). ^1H NMR ($CDCl_3$): δ 8.10–6.80 (m, 9 H, arom. H), 6.43 (d, 1 H, $J_{NH,2}$ 7.0 Hz, NH), 5.91 (m, 1 H, –CH=), 5.45 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.41 (s, 1 H, PhCHO), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.18 (dq, 1 H, $=CH_{2cis}$), 5.08 (dd, 1 H, $J_{2',3'}$ 10.8, $J_{3',4'}$ 3.4 Hz, H-3′), 5.06 (d, 1 H, $J_{3'',4''}$ 3.5 Hz, H-4″), 5.01 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.83 (dd, 1 H, $J_{2'',3''}$ 10.3 Hz, H-3″), 4.66 (d, 1

H, $J_{1'',2''}$ 7.6 Hz, H-1″), 4.55 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.50–3.85 (m, 10 H, H-3, H-5, H-6a, H-6b, H-2′, H-4′, H-6a′, H-6b′, 2 OCH_2), 3.79 (s, 3 H, MeOPh), 3.65–3.50 (m, 5 H, H-2, H-5″, OMe), 3.48 (m, 1 H, H-5′), 3.08 (dd, 1 H, H-2″), 2.03, 2.00 and 1.84 (3 s, 9 H, 3 Ac), 1.28, 1.21 (2 s, 18 H, 2 *t*-Bu) and 1.00 (d, 3 H, $J_{5'',6''}$ 6.4 Hz, H-6″).

3.20. Allyl 3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→2)-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (15b) and allyl 3,4-di-*O*-acetyl-2-*O*-methyl-β-L-fucopyranosyl-(1→2)-3-*O*-benzoyl-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (16b)

Compound **12b** (32 mg, 0.04 mmol) was reacted with **14** (15 mg, 0.05 mmol) in the same way as described for the preparation of **15a**. Purification of the residue on silica gel (1:1 toluene–EtOAc) afforded **15b** as syrup. Yield: 20 mg (48%); $[\alpha]_D^{20}$ +103° (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.10–6.80 (m, 10 H, arom. H, NH), 5.81 (m, 1 H, –CH=), 5.43 (s, 1 H, PhCHO), 5.40 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.27 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 4.0 Hz, H-3′), 5.26–5.20 (m, 3 H, H-1″, H-4″, $=CH_{2trans}$), 5.20–5.12 (m, 2 H, H-1, $=CH_{2cis}$), 4.98 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 3.2 Hz, H-3″), 4.50 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.48 (ddd, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.8, $J_{2,NH}$ 8.3 Hz, H-2), 4.40–4.28 (m, 4 H, H-6a, H-4′, H-6a′, H-5″), 4.17–4.04 (m, 3 H, H-3, H-6b, OCH_2), 4.01 (dd, 1 H, $J_{2',3'}$ 10.1 Hz, H-2′), 3.96–3.78 (m, 6 H, H-6b′, H-5, MeOPh, OCH_2), 3.45 (m, 1 H, H-5′), 3.36 (dd, 1 H, $J_{1'',2''}$ 3.5 Hz, H-2″), 3.02 (s, 3 H, OMe), 2.08, 1.98 and 1.85 (3 s, 9 H, 3 Ac), 1.26 and 1.21 (2 s, 18 H, 2 *t*-Bu), 1.07 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6″). Anal. Calcd for $C_{53}H_{71}NO_{21}$: C, 60.16; H, 6.76; N, 1.32. Found: C, 59.64; H, 6.87; N, 1.25.

Further elution afforded **16b** as syrup. Yield: 9 mg (20%). ^1H NMR ($CDCl_3$): δ 8.25–6.75 (m, 10 H, arom. H, NH), 5.84 (m, 1 H, –CH=), 5.42 (s, 1 H, PhCHO), 5.38 (d, 1 H, $J_{3,4}$ 2.8 Hz, H-4), 5.22 (dq, 1 H, $=CH_{2trans}$), 5.20–5.10 (m, 3 H, H-1, H-3′, $=CH_{2cis}$), 5.01 (d, 1 H, $J_{3'',4''}$ 3.4 Hz, H-4″), 4.88 (dd, 1 H, $J_{2'',3''}$ 10.3 Hz, H-3″), 4.62 (ddd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.8, $J_{2,NH}$ 7.7 Hz, H-2), 4.58 (d, 1 H, $J_{1'',2''}$ 7.7 Hz, H-1″), 4.50 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.42–4.30 (m, 3 H, H-6a, H-4′, H-6a′), 4.20–4.05 (m, 4 H, H-3, H-6b, H-2′, OCH_2), 3.95 (ddt, 1 H, OCH_2), 3.92–3.87 (m, 5 H, H-5, H-6b′, MeOPh), 3.61–3.54 (m, 4 H, H-5″, OMe), 3.46 (m, 1 H, H-5′), 3.27 (dd, 1 H, H-2″), 2.00 and 1.88 (2 s, 9 H, 3 Ac), 1.28, 1.21 (2 s, 18 H, 2 *t*-Bu), and 0.85 (d, 3 H, $J_{5'',6''}$ 6.4 Hz, H-6″).

3.21. Allyl 3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-β-D-galactopyranoside (17a)

A solution of **15a** (20 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) was treated with 90% aq CF_3CO_2H (20 μL) at rt. Triethylamine (0.1 mL) was added and the solution was concentrated. The residue was dried, dissolved in pyridine (2 mL) and stirred with a catalytic amount of 4-*N*,*N*-dimethylaminopyridine and Ac_2O (200 μL) overnight at rt. Methanol (0.5 mL) was added and the solution was concentrated. Purification of the residue on silica gel (1:2 toluene–EtOAc) afforded **17a** as syrup. Yield: 18 mg (94%); $[\alpha]_D^{20}$ −11° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 8.00–7.40 (m, 5 H, arom. H), 6.50 (d, 1 H, $J_{2,NH}$ 6.5 Hz, NH), 5.93 (m, 1 H, –CH=), 5.51 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 5.49 (d, 1 H, $J_{3,4}$ 4.0 Hz, H-4), 5.39 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.36–5.27 (m, 2 H, H-3′, $=CH_{2trans}$), 5.26–5.18 (m, 2 H, H-4″, $=CH_{2cis}$), 5.13 (dd, 1 H, $J_{2'',3''}$ 10.6, $J_{3'',4''}$ 3.3 Hz, H-3″), 5.05 (dd, 1 H, $J_{2,3}$ 10.0 Hz, H-3), 5.02 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.69 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.45–4.30 (m, 2 H, H-5″, OCH_2), 4.20–3.88 (m, 7 H, H-6a, H-6b, H-2′, H-5′, H-6a′, H-6b′, H-4″, OCH_2), 3.47 (dd, 1 H, H-2″), 3.21 (ddd, 1 H, H-2), 3.07 (s, 3 H, OMe), 2.15, 2.08 and 2.00 (3 s, 15 H, 5 Ac), 1.29 and 1.23 (2 s, 18 H, 2 *t*-Bu), 1.16 (d, 3 H, $J_{5'',6''}$ 6.5 Hz, H-6″). Anal. Calcd for $C_{49}H_{69}NO_{22}$: C, 57.47; H, 6.79; N, 1.37. Found: C, 57.64; H, 6.77; N, 1.45.

3.22. Allyl 3,4-di-*O*-acetyl-2-*O*-methyl-α-L-fucopyranosyl-(1→2)-4,6-di-*O*-acetyl-3-*O*-benzoyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-4,6-di-*O*-pivaloyl-α-D-galactopyranoside (17b)

A solution of **16b** (22 mg, 0.02 mmol) in CH_2Cl_2 (3 mL) was treated with 90% aq CF_3CO_2H (20 μL) at rt for 1 h. Treatment as described for **17a** gave a residue which was dissolved in pyridine (2 mL) and stirred with a catalytic amount of 4-*N*,*N*-dimethylaminopyridine and Ac_2O (200 μL) overnight at rt. Methanol (0.5 mL) was added and the solution was concentrated. Purification of the residue on silica gel (1:2 toluene–EtOAc) afforded **17b** as syrup. Yield: 19 mg (90%); $[\alpha]_D^{20}$ +45° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 7.97–7.35 (m, 5 H, arom. H), 6.97 (d, 1 H, $J_{NH,2}$ 7.5 Hz, NH), 5.83 (m, 1 H, –CH=), 5.50–5.45 (m, 2 H, H-4, H-4′), 5.38 (dd, 1 H, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.5 Hz, H-3′), 5.30–5.15 (m, 4 H, H-1″, H-4″, $=CH_{2trans}$, $=CH_{2cis}$), 5.14 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.04 (dd, 1 H, $J_{2'',3''}$ 10.5, $J_{3'',4''}$ 3.2 Hz, H-3″), 4.62 (d, 1 H, $J_{1',2'}$ 7.4 Hz, H-1′), 4.50 (ddd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 11.0 Hz, H-2), 4.40 (dd, 1 H, $J_{5'',6''}$ 6.5 Hz, H-5″), 4.38 (dd, 1 H, $J_{5,6a}$ 3.2, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.19–4.00 (m, 5 H, H-3, H-5, H-6a′, H-6b′, OCH_2), 4.00–3.80 (m, 4 H, H-6b, H-2′, H-5′, OCH_2), 3.39 (dd, 1 H, $J_{1'',2''}$ 3.6

Hz, H-2″), 3.00 (s, 3 H, OMe), 2.10, 2.09, 2.07, 2.01 and 1.90 (5 s, 15 H, 5 Ac), 1.29 and 1.22 (2 s, 18 H, 2 *t*-Bu), 1.14 (d, 3 H, H-6″). Anal. Calcd for $C_{49}H_{69}NO_{22}$: C, 57.47; H, 6.79; N, 1.37. Found: C, 57.32; H, 7.04; N, 1.47.

3.23. Allyl 2-*O*-methyl-α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (18a)

A solution of **17a** (16 mg, 0.016 mmol) in dry MeOH (10 mL) was stirred with 0.1 M methanolic NaOMe (0.5 mL) overnight at rt. The pH of the solution was adjusted to 7.0 by addition of Dowex 50 (H^+) resin, the resin was filtered off and the filtrate was taken to dryness. The residue was dissolved in aq 90% 1,4-dioxane (2.5 mL) and aq 40% tetra-*n*-butylammonium hydroxide (50 μL, 0.077 mmol) was added. After stirring overnight at ambient temperature, the solution was neutralized by addition of Dowex 50 (H^+) resin and the resin was filtered off. The filtrate was concentrated and the residue was first purified on column of silica gel (*C*, 3:2 EtOH–EtOAc), then on a column of Sephadex LH-20 (MeOH) to give **18a** as syrup. Yield: 7.5 mg (82%); $[\alpha]_D^{20}$ −55° (*c* 0.6, H_2O). ^1H NMR (D_2O): δ 5.88 (m, 1 H, –CH=), 5.45 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1″), 5.27 (dq, 1 H, $=CH_{2trans}$), 5.23 (dq, 1 H, $=CH_{2cis}$), 4.58 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.35 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.33 (m, 1 H, OCH_2), 4.22 (dd, 1 H, $J_{5'',6''}$ 6.6 Hz, H-5″), 4.15–4.05 (m, 2 H, H-4, OCH_2), 3.98 (dd, 1 H, $J_{2,3}$ 10.9 Hz, H-2), 3.89 (dd, 1 H, $J_{3,4}$ 3.1 Hz, H-3), 3.86 (d, 1 H, $J_{3',4'}$ 3.3 Hz, H-4′), 3.84–3.69 (m, 5 H, H-6a, H-6b, H-3′, H-6a′, H-6b′), 3.69–3.60 (m, 5 H, H-2′, H-5′, H-5, H-3″, H-4″), 3.49 (s, 3 H, OMe), 3.44 (dd, 1 H, $J_{2'',3''}$ 10.3 Hz, H-2″), 2.02 (s, 3 H, Ac), 1.20 (d, 3 H, H-6″). Anal. Calcd for $C_{24}H_{41}NO_{15}$: C, 49.39; H, 7.08; N, 2.40: Found: C, 49.69; H, 6.77; N, 2.41.

3.24. Allyl 2-*O*-methyl-α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside (18b)

The deprotection of **17b** (12 mg) was done as described for **17a**. The residue was purified on a column of silica gel (3:2 EtOH–EtOAc) and then on a column of Sephadex LH-20 to give **18b** as syrup. Yield: 5.0 mg (73%); $[\alpha]_D^{20}$ +14° (*c* 0.6, H_2O). ^1H NMR (D_2O): δ 5.95 (m, 1 H, –CH=), 5.45 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1″), 5.32 (dq, 1 H, $=CH_{2trans}$), 5.24 (dq, 1 H, $=CH_{2cis}$), 4.90 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1), 4.62 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.27–4.10 (m, 5 H, H-2, H-3, H-4, H-5″, OCH_2), 4.07–3.93 (m, 2 H, H-5, OCH_2), 3.87 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 3.85–3.70 (m, 5 H, H-6a, H-6b, H-3′, H-6a′, H-6b′), 3.70–3.60 (m, 4 H, H-2′, H-5′, H-3″, H-4″), 3.52–3.40 (m, 4 H, H-2″, OMe), 2.03 (s, 3 H, Ac), 1.18 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, H-6″). Anal. Calcd for $C_{24}H_{41}NO_{15}$: C, 49.39; H, 7.08; N, 2.40: Found: C, 49.01; H, 7.20; N, 2.42.

3.25. 3-(2-Aminoethylthiopropyl) 2-*O*-methyl-α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside hydrochloride (19a)

A solution of **18a** (3.9 mg, 0.007 mmol), cysteamine hydrochloride (4.2 mg, 0.037 mmol) in water (0.4 mL) was stirred under UV light for 4 h at rt. The solution was then purified on Dowex AG WX8 (NH_4^+ form) using a gradient 0→0.1 M aq NH_3. Carbohydrate-containing fractions were pooled and lyophilized to afford **19a** as an amorphous powder. Yield: 3.1 mg (70%); $[\alpha]_D^{20}$ −26° (*c* 0.3, H_2O). ^1H NMR (D_2O): δ 5.40 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.54 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.25 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 4.16 (dd, 1 H, $J_{5'',6''}$ 6.6 Hz, H-5″), 4.05 (d, 1 H, $J_{3,4}$ 2.1 Hz, H-4), 4.00–3.85 (m, 3 H, H-2, H-3, OCH_2), 3.82 (d, 1 H, $J_{3',4'}$ 3.5 Hz, H-4′), 3.78–3.68 (m, 5 H, H-6a, H-6b, H-3, H-6a′, H-6b′), 3.68–3.53 (m, 6 H, H-5, H-5′, H-2′, H-3″, H-4″, OCH_2), 3.44 (s, 1 H, OMe), 3.40 (dd, 1 H, $J_{2'',3''}$ 10.3 Hz, H-2″), 3.03 (t, 2 H, CH_2N), 2.73 (t, 2 H, SCH_2), 2.54 (t, 2 H, CH_2S), 1.99 (s, 3 H, Ac), 1.77 (m, 2 H, CH_2), 1.20 (d, 3 H, H-6″). MALDI-TOF MS: *m*/*z* 662.03 $[MH]^+$, 684.45 $[M + Na]^+$.

3.26. 3-(2-Aminoethylthiopropyl) 2-*O*-methyl-α-L-fucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranoside hydrochloride (19b)

A solution of **18b** (5.6 mg, 0.01 mmol) and cysteamine hydrochloride (5.9 mg, 0.052 mmol) in water (0.6 mL) was stirred under UV light for 3 h at rt. The solution was then purified on Dowex AG WX8 (NH_4^+ form) using a gradient 0→0.1 M aq NH_3. Carbohydrate-containing fractions were pooled and lyophilized to give **19b** as an amorphous powder. Yield: 4.5 mg (71%); $[\alpha]_D^{20}$ +18° (*c* 0.5, H_2O). ^1H NMR (D_2O): δ 5.41 (d, 1 H, $J_{1'',2''}$ 3.9 Hz, H-1″), 4.82 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 4.56 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.22–4.90 (m, 5 H, H-2, H-3, H-4, H-5, H-5″), 3.82 (d, 1 H, $J_{3',4'}$ 3.4 Hz, H-4′), 3.80–3.65 (m, 6 H, H-6a, H-6b, H-3′, H-6a′, H-6b′, OCH_2), 3.65–3.54 (m, 4 H, H-2′, H-5′, H-3″, H-4″), 3.52–3.37 (m, 5 H, H-2″, OCH_2, OMe), 3.09 (t, 2 H, CH_2N), 2.76 (t, 2 H, SCH_2), 2.64 (t, 2 H, CH_2S), 1.99 (s, 3 H, Ac), 1.84 (m, 2 H, CH_2), 1.14 (d, 3 H, $J_{5'',6''}$ 6.6 Hz, H-6″). MALDI-TOF MS: *m*/*z* 684.45 $[M + Na]^+$.

3.27. Synthesis of the BSA-conjugate 20a

A solution of thiophosgene (2 μL) in $CHCl_3$ (2 mL) was added to **19a** (3.1 mg, 0.0047 mmol) dissolved in 0.1 M

$NaHCO_3$ (1.5 mL). The solution was kept under high speed stirring for 2 h at rt. The organic phase was removed and the aqueous phase was extracted 3 times with $CHCl_3$ (3 mL). Traces of $CHCl_3$ in the aq phase were removed by evaporation under a stream of N_2. The aqueous phase was then added to a solution of BSA (3.1 mg) in a 1:1 mixture of 0.3 M NaCl and 0.1 M $NaHCO_3$ (1.5 mL). The solution was slowly stirred for 48 h at rt and was then separated by gel permeation chromatography on a Sephadex G-25 column (0.01 M $NaHCO_3$). The BSA-glycoconjugate fractions (ninhydrine-positive) were pooled and dialyzed against water for 48 h. Lyophilization gave the BSA-conjugate **20a**. Yield: 2.3 mg. The carbohydrate–BSA ratio was determined via MALDI-TOF-MS: $[M]^+$ 71,227 which corresponds to 6.8 mol ligand/mol BSA.

3.28. Synthesis of the BSA-conjugate 20b

A solution of thiophosgene (2 μL) in $CHCl_3$ (2 mL) was added to **19b** (4.5 mg, 0.0068 mmol) dissolved in 0.1 M $NaHCO_3$ (1.5 mL). The solution was kept under high speed stirring at rt for 2 h. Work-up was done as described for **20a**. The aqueous phase was added to a solution of BSA (3.0 mg) in a 1:1 mixture of 0.3 M NaCl and 0.1 M $NaHCO_3$ (1.5 mL). The reaction mixture was slowly stirred for 48 h at rt. The mixture was separated by gel permeation chromatography on a Sephadex G-25 column. The ninhydrine-positive fractions were combined and dialyzed against distilled water for 48 h. Lyophilization gave the BSA-conjugate **20b**. Yield: 4.2 mg. The carbohydrate-BSA ratio was determined via MALDI-TOF-MS: $[M]^+$ 68,587; (determined for BSA standard: 66,431) which corresponds to 3.1 mol ligand/mol BSA.

4. Supplementary material

The material is available from the authors on request.

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