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Synthesis of the trisaccharide outer core fragment of *Burkholderia cepacia* pv. *vietnamiensis* lipooligosaccharide

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ABSTRACT

The synthesis of β-Gal-(1→3)-α-GalNAc-(1→3)-β-GalNAc allyl trisaccharide as the outer core fragment of *Burkholderia cepacia* pv. *vietnamiensis* lipooligosaccharide was accomplished through a concise, optimized, multi-step synthesis, having as key steps three glycosylations, that were in-depth studied performing them under several conditions. The target trisaccharide was designed with an allyl aglycone in order to open a future access to the conjugation with an immunogenic protein *en route* to the development of a synthetic neoglycoconjugate vaccine against this *Burkholderia* pathogen.

1. Introduction

Burkholderia cepacia complex (*Bcc*) is a group of closely related Gram-negative bacteria, that comprises at least fifteen species. Originally it was identified as a plant pathogen in the 1950s, when it was recognized as the causative agent of soft onion rot.[1] Its discovery as an opportunistic pathogen in patients with cystic fibrosis (CF) occurred in the 1980s.[2] CF patients colonized with *Bcc* experience a more rapid decline than those colonized with the more commonly acquired pathogen *Pseudomonas aeruginosa*.[3] Actually, the infection by *Bcc* determine, in approximately 20% of CF patients infected with *Bcc*, the 'cepacia syndrome', characterized by fever, pneumonia and bacteraemia.[4] In addition, *Bcc* is inherently resistant to antibiotic treatment,[5] to antimicrobial peptides[6] and increased resistance is observed on formation of *Bcc* biofilms in vitro. Overall, infection with *Bcc* in CF patients correlates with poorer prognosis, longer hospital stays and an increased risk of death. Another typical feature of the *Bcc* strains is their ability to spread among patients. When the use of antibiotics in chronic infections determine the selection of multiple-antibiotic-resistant strains, as usually occurs in CF patients, the development of convenient vaccines represents a desirable resource to prevent infection and for the necessary therapeutic approach. This problem is common to all bacterial infections, but for *Bcc* strains it is more serious due to their inherently acquired resistance to antibiotic treatment.

Among the many types of vaccines, extensive use of polysaccharide carbohydrates has been made against several human pathogens, such as *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae*. An improvement was achieved by covalently coupling the polysaccharide to carrier proteins, thus generating a long-lasting immunity, and making the vaccine effective even on infants and immunocompromised individuals. In the last decade, a higher level of sophistication was brought with the preparation of synthetic neoglycoconjugate vaccines (or vaccine candidates), where a haptenic oligosaccharide epitope is linked by a spacer to an immunogenic protein.[7] The sources of oligosaccharides for vaccines usually arise from bacterial surface carbohydrates as capsular polysaccharides or lipopolysaccharides (LPSs). LPSs are the major component of the outer leaflet of almost all Gram-negative bacteria and are one of virulence factors of pathogenic bacteria. They usually consist of a polysaccharide region, named O-chain, that is linked to an oligosaccharide part—termed core and usually divided into an outer and an inner core—in turn bonded to a glycolipid region, lipid A, which is anchored to the lipid external membrane of bacteria and represents the toxic part of LPSs.[8] The O-chain polysaccharide, also known as O-antigen, is responsible for the serotype classification of the strains. The repeating unit of capsular polysaccharides as well as of the O-chain region of LPSs is usually taken as oligosaccharide hapten for the construction of a vaccine candidate. In the case of *P. aeruginosa* an in-depth investigated glycoconjugate vaccine candidate was

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built as the whole O-chain conjugated to a toxin, showing significant results also on humans.[9] Furthermore, the synthesis of the O-chain repeating unit of the LPS of a *B. cepacia* strain isolated from CF patients *en route* to a potential synthetic neoglycoconjugate vaccine was recently reported.[10]

Some bacteria present no O-chain and the outer core region results as the most external saccharide portion of such lipooligosaccharides (LOSs) on the bacterial surface. It is usually thought that the core can play some roles in place of the O-chain in LOSs and in particular the outer moiety of core can be the source of hapten oligosaccharides. Indeed, the synthesis of some oligosaccharides arising from the outer core of *P. aeruginosa* LOSs has been performed for the future preparation of potential vaccines.[11] Furthermore, the synthesis of some neoglycoconjugates containing *Bcc* inner core epitopes was very recently accomplished.[12] In this work it is reported for the first time a concise, high-yielding synthesis of an outer core fragment of LOS from *Burkholderia cepacia* pv. *vietnamiensis*—the third most prevalent species of the *Bcc* found among CF patients—[13] in order to study its antigenic properties *en route* to the development of a synthetic neoglycoconjugate vaccine candidate.

2. Results and discussion

Full structural characterization of *B. vietnamiensis* LOS was very recently reported (Fig 1).[14] The inner core part showed typical heptose and oct-2-ulosonic acid (Kdo and Ko) constituents, whereas the outer region was constituted of GalNAc and Gal residues.

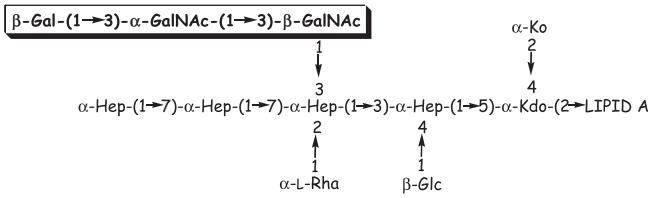
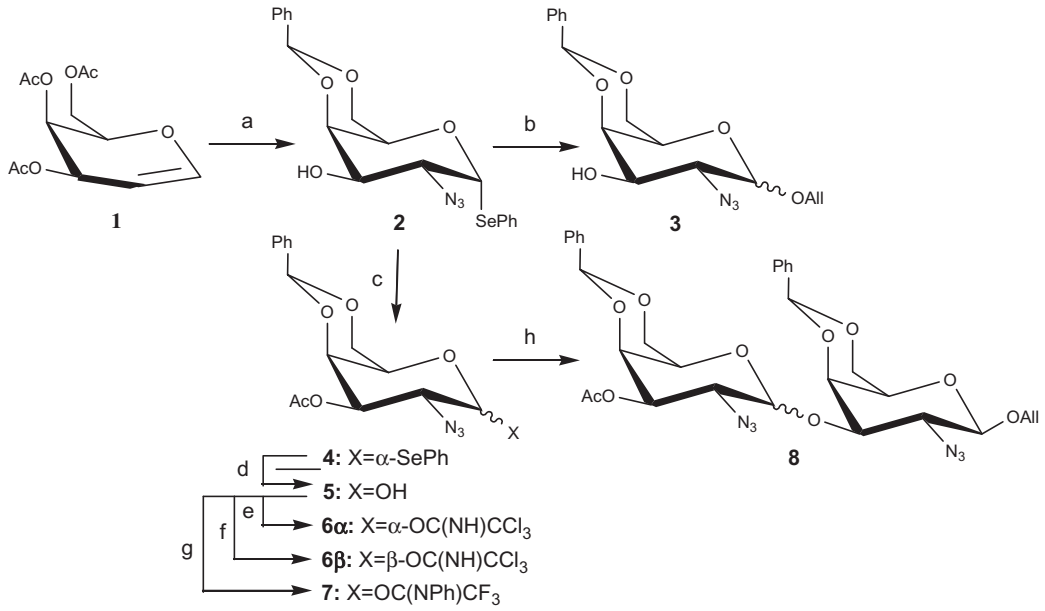


Figure 1

Figure 1. Structure of the core part of LOS from *B. vietnamensis*.

The trisaccharide target of this synthetic work (highlighted in Fig 1) presented two GalNAc units with different stereochemistry at the anomeric position. In order to set up a concise and high-yielding synthetic strategy, in the retrosynthetic analysis we focused on a GalN glycosyl donor, that could be glycosylated with either α- or β-stereoselectivity by a suitable change of reaction conditions. 2-Azido-2-deoxy-GalN[15] and 2-deoxy-2,3-oxazolidinone-GalN donors[16] have been reported in both α- and β-stereoselective glycosylations. Several protocols are known for the straightforward synthesis of 2-azido-2-deoxy-GalN donors from commercially available tri-*O*-acetyl-galactal.[17] We selected **2**[18] as key compound for the synthesis of GalN donor and acceptor. It was obtained from tri-*O*-acetyl-galactal **1** through homogeneous azidophenylselenylation, followed by de-*O*-acetylation and 4,6-benzylidenation (Scheme 1). The synthesis of GalN acceptor required the insertion of a β-linked allyl aglycon for future derivatizations (conjugation with an antigenic protein, coupling with inner core fragments, etc.). We screened several selenoglycoside activation conditions[19] in order to achieve a good yield together with a satisfying β-stereoselectivity (Table 1). The best result was obtained with NBS/$Bi(OTf)_3$ activation system[19a] affording **3β** in 57% yield (Table 1, entry 6). Acetylation of **2** afforded glycosyl donor **4** (99%), that unfortunately gave no coupling product when subjected to glycosylation with **3β** under NBS/$Bi(OTf)_3$ activation (Table 2, entries 1 and 2). Thus, a leaving group exchange at anomeric position was effected in two steps by hydrolysis of selenoglycoside **4** with NBS in aqueous THF and subsequent conversion of the obtained hemiacetal **5** (81%) into trihaloacetimidates **6α** (Cl_3CCN/DBU, 96%) and **7** ($CF_3C(NPh)Cl/Cs_2CO_3$, 79%). Glycosylation of **6α** and **3β** under TMSOTf catalysis in CH_2Cl_2 gave disaccharide **8** in 62% yield but almost no stereoselectivity (Table 2, entry 3) in spite of the α-stereoselectivity reported for Gal trichloroacetimidates with non-participating protecting groups at *O*-2 and *O*-3 and a benzylidene ring system at positions 4 and 6 hindering the attack of the acceptor from the β-face.[20] Attempts to enhance the α-stereoselectivity by conducting the reaction in ethereal solvents lowered the global yield of the glycosylation (entries 4 and 5).



Scheme 1. Synthesis of 2-azido-2-deoxy-GalN donors and acceptor. Reagents and conditions: (a) see Ref. 18; (b) see Table 1; (c) Ac_2O, py, rt, 99%; (d) NBS, 1:1 v/v THF–H_2O, rt, 81% (α/β = 2:1); (e) Cl_3CCN, DBU, CH_2Cl_2, rt, 96%; (f) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 60%; (g) $CF_3C(NPh)Cl$, Cs_2CO_3, acetone, rt, 79% (α/β = 1.4:1); (h) see Table 2.

Table 1
Conversion of selenoglycoside **2** into acceptor **3**

Entry	Activation system	Solvent	*T*	Yield[a] (β/α)[b]
1	AgOTf (3 equiv), K_2CO_3 (5 equiv)	4:1 v/v CH_2Cl_2/AllOH	rt	82% (3:5)
2	NIS (1.1 equiv), TfOH (0.1 equiv)	1:1 v/v $(CH_2Cl)_2$/AllOH	−40 °C	47% (5:1)
3	NBS(1.4 equiv), $Bi(OTf)_3$ (0.08 equiv)	12:4:3 v/v/v CH_2Cl_2/ 1,4-dioxane/AllOH	−30 °C	49% (4:1)
4	NBS(1.4 equiv), $Bi(OTf)_3$ (0.08 equiv)	12:4:3 v/v/v $(CH_2Cl)_2$/ 1,4-dioxane/AllOH	−40 °C	67% (5:2)
5	NBS (1.4 equiv), $Bi(OTf)_3$ (0.08 equiv)	12:4:3 v/v/v $(CH_2C1)_2$/1,4-dioxane/AllOH	−60 °C	51% (4:1)
6	NBS (1.4 equiv), $Bi(OTf)_3$ (0.08 equiv)	2:2:1 v/v/v $(CH_2C1)_2$/ AllOH/1,4-dioxane	−40 °C	76% (3:1)

[a] Isolated yield.
[b] Anomeric ratio measured by separation of the two anomers.

Table 2
Glycosylation reaction to disaccharide **8**

Entry	Donor[a]	Promoter[b]	Solvent	*T*	Yield[c] (α/β)[d]
1	4	NBS (1.3 equiv), $Bi(OTf)_3$ (0.08 equiv)	CH_2Cl_2	−40 °C	No reaction
2	4	NBS (1.3 equiv), $Bi(OTf)_3$ (0.08 equiv)	$(CH_2Cl)_2$	−40 °C	No reaction
3	6α	TMSOTf (0.1 equiv)	CH_2Cl_2	−30 °C	62% (1.3:1)
4	6α	TMSOTf (0.1 equiv)	DME	−40 °C	54% (1:1.7)
5	6α	TMSOTf (0.1 equiv)	1:1 v/v CH_2Cl_2/THF	−30 °C	32% (only α)
6	7	TMSOTf (0.1 equiv)	3:1 v/v CH_2Cl_2/THF	−30 °C	23% (only α)
7	6α	$Bi(OTf)_3$ (0.08 equiv)	3:1 v/v $((CH_2C1)_2$/1,4-dioxane	0 °C	67% (1:1.4)
8	7	$Bi(OTf)_3$; (0.08 equiv)	5:1 v/v $(CH_2C1)_2$/1,4-dioxane	0 °C	55% (1.3:1)
9	6β	$BF_3{\cdot}OEt_2$ (0.26 equiv)	3:2 v/v CH_2Cl_2/*n*-hexane	−78 °C	79% (2.6:1)
10	6α	$BF_3{\cdot}OEt_2$, (0.26 equiv)	3:2 v/v CH_2Cl_2/*n*-hexane	−95 °C	55% (only α)

[a] Donor/acceptor 3β molar ratio = 1.4.
[b] Promoter equivalents calculated with respect to the donor.
[c] Isolated yield.
[d] Anomeric ratio measured by isolation of the two anomers.

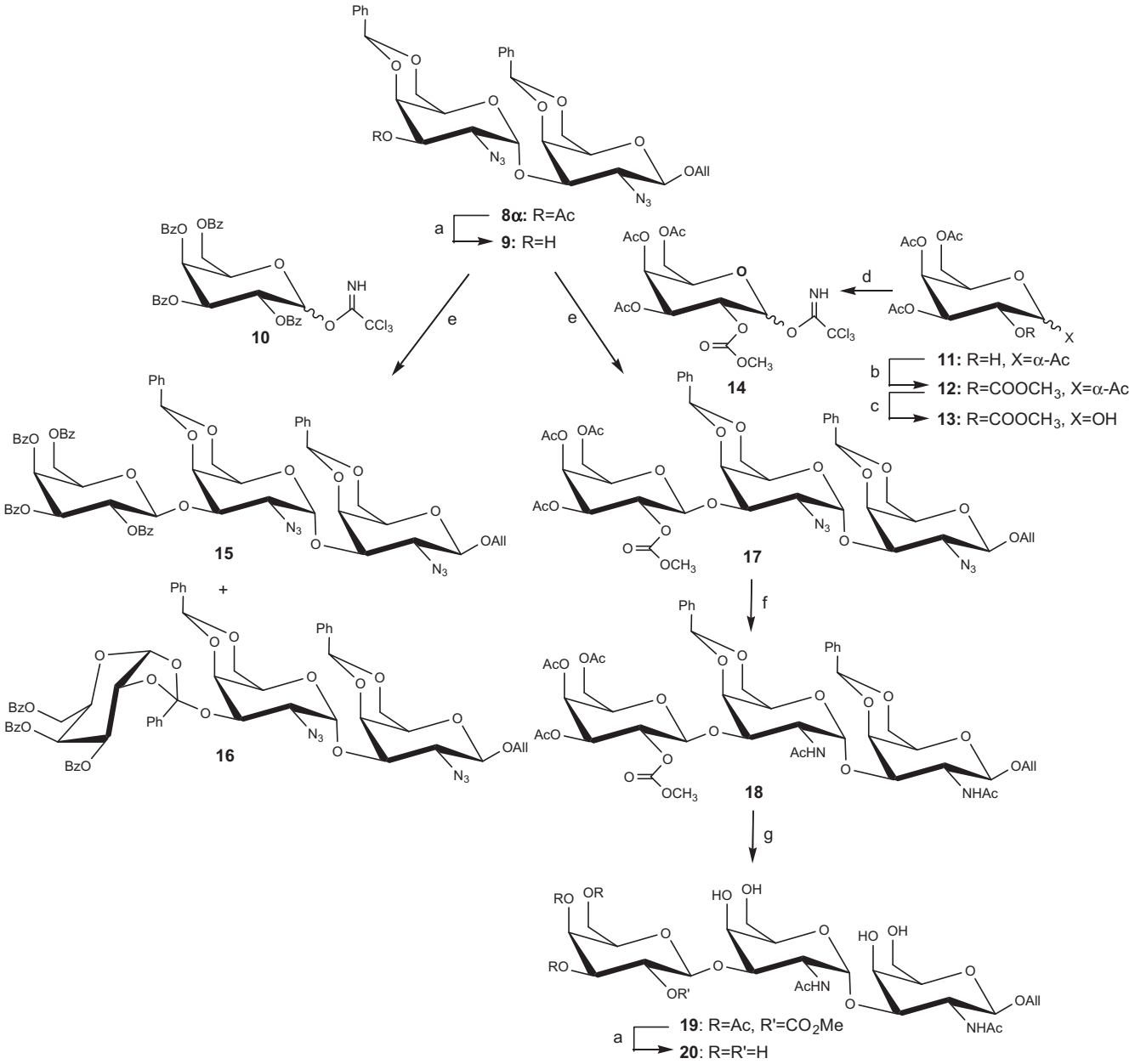
Since *N*-phenyl-trifluoroacetimidate donors[21] sometimes afford higher glycosylation yield than trichloroacetimidate counterparts,[22] donor **7** was also tested, but no better result was obtained (entry 6). The use of $Bi(OTf)_3$—recently reported as a powerful activator of trihaloacetimidate donors in ether-containing solvent systems—[23] afforded satisfying yields but no stereoselectivity again (entries 7 and 8). Since some scattered examples in the literature report that glycosylation of 2-azido-2-deoxyglycosyl trichloroacetimidates shows anomeric inversion under S_N2 reaction conditions,[24] β-trichloroacetimidate **6β** was synthesized from **5** with Cl_3CCN and K_2CO_3 and then coupled with acceptor **3β** under $BF_3{\cdot}OEt_2$ catalysis in CH_2Cl_2–hexane at very low temperature to give disaccharide **8** in satisfying yield and α-stereoselectivity (entries 9 and 10).

Compound **8α** was de-*O*-acetylated under Zemplén conditions to give disaccharide acceptor **9** in quantitative yield (Scheme 2). Glycosylation of **9** with 2,3,4,6-tetra-*O*-benzoyl-galactose trichloroacetimidate **10** under TMSOTf catalysis in CH_2Cl_2 surprisingly afforded trisaccharide **15** in only moderate yield (44%). A by-product was also isolated in 23% yield (Table 3, entry 1), having the same molecular mass of **15** (1186 *m*/*z* as detected by MALDI-MS). NMR signals (δ_H 5.63, δ_C 107.6 ppm) of Gal anomeric position together with a $^1J_{H\text{-}1,H\text{-}2}$ coupling constant of 4.9 Hz[25] revealed that the isolated by-product was the orthoester-linked trisaccharide **16**. This finding was unexpected because it has been reported that benzoyl-protected galactosyl trichloroacetimidate **10** did not lead to orthoester formation in glycosylation with a GalN acceptor very similar to **9**.[26] It is believed that sugar 1,2-orthoesters are acid-labile intermediates usually formed when insufficient acid catalyst is present in the glycosylation reaction mixture.[27] Thus, to achieve in situ conversion of the orthoester to the corresponding glycoside, the addition of more catalyst and/or an increase in reaction time and temperature is usually performed. Nonetheless, no significant enhancement in the yield of **15** was observed in our case (Table 3, entries 2 and 3). Since the use of donors equipped with a methoxycarbonyl protecting group on the *O*-2 has been reported to avoid the generation of orthoester products,[28] we synthesized the novel donor **14** in three steps from commercially available 1,3,4,6-tetra-*O*-acetyl-α-galactose **11**. Methoxycarbonylation of **11** with $ClCO_2Me$/TMEDA[29] gave **12** in quantitative yield. Anomeric acetyl group was then selectively cleaved with $BnNH_2$ in THF to give hemiacetal **13** (74%), that was converted in turn to trichloroacetimidate **14** in 68% yield. Glycosylation of **9** with donor **14** under TMSOTf catalysis gave trisaccharide **17** in good yield (74%) and no orthoester product could be detected (Table 3, entry 4). It is worth noting that trisaccharide **17** has been designed with an allyl aglycone, that could be orthogonally cleaved to activate the anomeric position with a proper leaving group and allow in future further elongation of the fragment with an inner core acceptor.

Global deprotection of **17** was performed in four steps. Azide functions of trisaccharide **17** were transformed into amino groups by reduction with Zn/Cu couple and subsequent N-acetylation afforded **18** (57% over two steps), whose benzylidene groups were then cleaved by aqueous 90% acetic acid treatment (74%). Tetraol **19** was finally de-*O*-acylated by Zemplèn transesterification to give target trisaccharide **20** (94%).

3. Conclusion

The synthesis of β-Gal-(1→3)-α-GalNAc-(1→3)-GalNAc β-allyl glycoside, as the outer fragment of the core region of *Burkholderia cepacia* pv. *vietnamiensis* lipooligosaccharide, was accomplished. An in-depth study of the optimal building blocks and reaction conditions for the three glycosylation steps was performed in order to design a high yielding strategy with the minimal number of synthetic steps. The allyl aglycone of the obtained trisaccharide will allow the



Scheme 2. Synthesis of target trisaccharide **20**. Reagents and conditions: (a) NaOMe, MeOH, rt, quantitative for **9**, 94% for **20**; (b) $ClCO_2Me$, TMEDA, CH_2Cl_2, 0 °C, quantitative; (c) $BnNH_2$, THF, 74% (α/β = 4.8:1); (d) Cl_3CCN, K_2CO_3, CH_2Cl_2, rt, 68% (α/β = 1.1:1); (e) see Table 3; (f) (i) Zn/Cu, 3:2:1 v/v/v THF/Ac_2O/AcOH, rt; (ii) 5:5:1 v/v/v CH_2Cl_2/MeOH/Ac_2O, rt, 57% over two steps; (g) 90% AcOH, 50 °C, 74%.

Table 3
Glycosylation reaction to trisaccharide species

Entry[a]	Donor[b]	Promoter[c]	*T*	*t* (h)	Yield[d]	
					Trisaccharide **15**/**17** (%)	Orthoester **16** (%)
1	10	TMSOTf (0.05 equiv)	−15 °C	1	44	23
2	10	TMSOTf (0.10 equiv)	0 °C	2	45	32
3	10	TMSOTf (0.10 equiv)	rt	o.n.	41	17
4	14	TMSOTf (0.10 equiv)	0 °C	2	74	–

[a] Reaction conducted.
[b] Donor/acceptor 9 M ratio = 2.0.
[c] Promoter equivalents calculated with respect to the donor.
[d] Isolated yield.

conjugation with an immunogenic protein in order to study its antigenic properties *en route* to a synthetic neoglycoconjugate vaccine candidate against *Burkholderia cepacia* pv. *vietnamiensis* pathogen. This work is in progress and will be reported elsewhere.

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on Varian XL-200 (^{1}H NMR: 200 MHz, ^{13}C NMR: 50 MHz), Bruker DRX-400 (^{1}H NMR: 400 MHz, ^{13}C NMR: 100 MHz), Varian INOVA 500 (^{1}H NMR: 500 MHz, ^{13}C NMR: 125 MHz) instruments or on a Bruker-600 DRX (^{1}H NMR: 600 MHz, ^{13}C NMR: 150 MHz) instrument equipped with a cryo probe, in $CDCl_3$ (internal standard, for ^{1}H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0) in CD_3OD (internal standard, for ^{1}H: C*H*D_2OD at δ 3.30; for ^{13}C: CD_3OD at δ 49.9) or in D_2O (internal standard, for ^{1}H: $(CH_3)_2CO$ at δ 2.22; for ^{13}C: $(CH_3)_2CO$ at δ 30.9). Positive MALDI-MS spectra were recorded on an Applied Biosystem Voyager DE-PRO MALDI TOF-MS in the positive mode: compounds were dissolved in CH_3CN at a concentration of 0.1 mg/mL and 1 μL of these solutions were mixed with 1 μL of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH_3CN/H_2O. Optical rotations were measured on a JASCO P-1010 polarimeter. Elemental analysis was performed on a Carlo Erba 1108 instrument. Reagents were purchased from Sigma-Aldrich, except for 1,3,4,6-tetra-*O*-acetyl-α-galactose from Carbosynth. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Merck Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 10% H_2SO_4 ethanolic solution and then heating to 130 °C. Column chromatography was performed on Kieselgel 60 (63–200 mesh).

4.2. Allyl 2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactopyranoside (3)

Compound **2** (82.8 mg, 0.191 mmol) was co-evaporated with toluene (3 × 2 mL), the residue was then mixed under argon atmosphere with NBS (47.6 mg, 0.267 mmol) and freshly activated AW-300 4 Å molecular sieves, cooled to −40 °C and suspended in 1:1 v/v $(CH_2Cl_2)_2$/AllOH (2.5 mL). Upon stirring, a freshly prepared suspension of $Bi(OTf)_3$ (10.0 mg, 15.3 μmol) in 1,4-dioxane (600 μL) was added. After 1 h stirring at −40 °C the reaction was quenched by adding a drop of Et_3N. The mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (4:1–1:1 v/v hexane/ethyl acetate) to give, as first eluted compound, **3α** (11.9 mg, 19%) as a yellowish oil. $[\alpha]_D$ +7.5 (*c* 1.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49–7.38 (m, 5H, H-Ar), 5.90 (m, 1H, $OCH_2CH{=}CH_2$), 5.60 (s, 1H, C*H* benzylidene), 5.34 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.3 Hz, *trans* $OCH_2CH{=}CHH$), 5.24 (dd, 1H, J_{vic} 10.9 Hz, J_{gem} 1.3 Hz, *cis* $OCH_2CH{=}CHH$), 5.07 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 4.31–4.08 (m, 6H, H-3, H-4, H-6a, H-6b, $OCH_2CH{=}CH_2$), 3.78 (br s, 1H, H-5), 3.61 (dd, 1H, $J_{2,3}$ 11.1 Hz, $J_{2,1}$ 3.3 Hz, H-2); ^{13}C NMR (50 MHz, $CDCl_3$) δ 136.8 (C_{ipso}), 133.0 ($OCH_2CH{=}CH_2$), 129.4–126.2 (C-Ar), 118.1 ($OCH_2CH{=}CH_2$), 102.0 (CH benzylidene), 97.5 (C-1), 81.7, 68.8, 68.7, 68.2, 63.0, 62.5 (C-2, C-3, C-4, C-5, C-6, $OCH_2CH{=}CH_2$). MALDI TOF-MS for $C_{16}H_{19}N_3O_5$ (*m/z*): M_r (calcd) 333.13, M_r (found) 356.25 $(M+Na)^+$. Anal. Calcd: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.49; H, 5.99; N, 12.47.

As second eluted compound, **3β** (36.4 mg, 57%) was obtained as a yellowish oil. $[\alpha]_D$ −10 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.52–7.38 (m, 5H, H-Ar), 5.97 (m, 1H, $OCH_2CH{=}CH_2$), 5.57 (s, 1H, C*H* benzylidene), 5.36 (dd, 1H, J_{vic} 16.5 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH{=}CHH$), 5.24 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* $OCH_2CH{=}CHH$), 4.46 (m, 1H, $OCHHCH{=}CH_2$), 4.35 (m, 2H, H-1, H-6a), 4.17 (m, 2H, H-4, $OCHHCH{=}CH_2$), 4.08 (dd, 1H, J_{gem} 10.0 Hz, $J_{6b,5}$ 2.0 Hz, H-6b), 3.65 (dd, 1H, $J_{2,1}$ 10.0 Hz, $J_{2,3}$ 8.0 Hz, H-2), 3.57 (ddd, 1H, $J_{H,OH}$ 9.5 Hz, $J_{3,4}$ 8.0 Hz, $J_{3,2}$ 3.5 Hz, H-3), 3.45 (br s, 1H, H-5), 2.55 (d, 1H, $J_{H,OH}$ 9.5 Hz, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.3 (C_{ipso}), 133.5 ($OCH_2CH{=}CH_2$), 129.3–126.2 (C-Ar), 117.8 ($OCH_2CH{=}CH_2$), 101.4, 100.9 (C-1, CH benzylidene), 74.4, 71.5, 70.2, 69.0, 66.6, 64.1 (C-2, C-3, C-4, C-5, C-6, $OCH_2CH{=}CH_2$). MALDI TOF-MS for $C_{16}H_{19}N_3O_5$ (*m/z*): M_r (calcd) 333.13, M_r (found) 356.39 $(M+Na)^+$. Anal. Calcd: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.39; H, 5.94; N, 12.40.

4.3. Phenyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-1-seleno-α-D-galactopyranoside (4)

A solution of **2** (1.851 g, 4.28 mmol) in 1:1 v/v py/Ac_2O (15 mL) was stirred overnight at rt. Volatiles were then removed in vacuo and the residue was diluted with CH_2Cl_2 (250 mL) and washed with 1 M HCl. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give pure **4** (2.005 g, 99%) as a yellowish oil. $[\alpha]_D$ +311.4 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.60–7.28 (m, 10H, H-Ar), 6.09 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 5.55 (s, 1H, C*H* benzylidene), 5.07 (dd, 1H, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.4 Hz, H-3), 4.53 (m, 2H, H-4, H-6a), 4.11 (m, 3H, H-2, H-5, H-6b), 2.17 (s, 3H, CH_3CO); ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.3 (CO), 137.3 (C_{ipso} benzylidene), 133.9–126.1 (C-Ar), 121.1 (C_{ipso} SePh), 100.8 (CH benzylidene), 84.9 (C-1), 72.9, 68.9, 64.7, 62.4, 58.3 (C-2, C-3, C-4, C-5, C-6), 20.9 ($COCH_3$). MALDI TOF-MS for $C_{21}H_{21}N_3O_5Se$ (*m/z*): M_r (calcd) 475.07, M_r (found) 498.18 $(M+Na)^+$. Anal. Calcd: C, 53.17; H, 4.46; N, 8.86. Found: C, 52.90; H, 4.60; N, 8.60.

4.4. 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactose (5)

A solution of **4** (1.980 g, 4.17 mmol) in 1:1 v/v THF/H_2O (8 mL) was treated with NBS (1.750 g, 9.83 mmol). After 30 min stirring, the solution was diluted with CH_2Cl_2 (250 mL) and washed with 1:1 v/v 10% $Na_2S_2O_3$/1 M $NaHCO_3$. The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give a residue that, after column chromatography (4:1–1:1 v/v hexane/ethyl acetate), afforded **5** (1.135 g, 81%; α/β 2:1) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.37 (m, $5H_\alpha + 5H_\beta$, H-Ar), 5.51 (s, $1H_\alpha$, C*H* benzylidene α), 5.50 (s, $1H_\beta$, C*H* benzylidene β), 5.43 (d, $1H_\alpha$, $J_{1,2}$ 3.5 Hz, H-1_α), 5.34 (dd, $1H_\alpha$, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3_α), 4.71 (dd, $1H_\beta$, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3_β), 4.59 (d, $1H_\beta$, $J_{1,2}$ 8.0 Hz, H-1_β), 4.43 (d, $1H_\alpha$, $J_{4,3}$ 3.5 Hz, H-4_α), 4.29 (d, $1H_\beta$, $J_{4,3}$ 3.5 Hz, H-4_β), 4.20 (d, $1H_\alpha$, J_{gem} 12.5 Hz, H-$6a_\alpha$), 4.13 (app d, $1H_\beta$, J_{gem} 7.0 Hz, H-$6a_\beta$), 4.10 (app d, $1H_\beta$, J_{gem} 7.0 Hz, H-$6b_\beta$), 4.00 (m, $2H_\alpha$, H-2_α, H-$6b_\alpha$), 3.94 (br s, $1H_\alpha$, H-5_α), 3.86 (dd, $1H_\beta$, $J_{2,3}$ 10.5 Hz, $J_{2,1}$ 8.0 Hz, H-2_β), 3.44 (br s, $1H_\beta$, H-5_β), 2.16 (s, $3H_\alpha$, CH_3CO_α), 2.04 (s, $3H_\beta$, CH_3CO_β); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 ($COCH_3$), 137.4 (C_{ipso}), 129.2–126.0 (C-Ar), 100.9 (CH benzylidene α), 99.6 (CH benzylidene β), 96.4 (C-1_β), 92.7 (C-1_α), 73.4, 69.6, 69.2, 62.5, 57.8 (C-2_α, C-3_α, C-4_α, C-5_α, C-6_α), 72.6, 72.3, 69.0, 66.6, 61.9 (C-2_β, C-3_β, C-4_β, C-5_β, C-6_β), 21.0, 20.9 ($COCH_3$). MALDI TOF-MS for $C_{15}H_{17}N_3O_6$ (*m/z*): M_r (calcd) 335.11, M_r (found) 358.35 $(M+Na)^+$. Anal. Calcd: C, 53.73; H, 5.11; N, 12.53. Found: C, 53.51; H, 5.20; N, 12.30.

4.5. 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (6α)

A solution of **5** (154.9 mg, 0.462 mmol) in CH_2Cl_2 (3.5 mL) was treated with Cl_3CCN (700 μL, 6.48 mmol) and then with a 5% v/v DBU solution in CH_2Cl_2 (400 μL). After 1.5 h stirring at rt, the solution was concentrated and the residue was immediately subjected to a column chromatography (7:1–4:1 v/v hexane/AcOEt) to give **6α** (212.8 mg, 96%) as a yellowish oil. $[\alpha]_D$ +131.7 (*c* 3.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (s, 1H, NH), 7.52–7.38 (m, 5H, H-Ar), 6.58 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 5.55 (s, 1H, C*H* benzylidene),

5.33 (dd, 1H, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.3 Hz, H-3), 4.58 (d, 1H, $J_{4,3}$ 3.3 Hz, H-4), 4.32 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{2,1}$ 3.4 Hz, H-2), 4.29 (dd, 1H, J_{gem} 12.8 Hz, $J_{6a,5}$ 1.4 Hz, H-6a), 4.04 (dd, 1H, J_{gem} 12.8 Hz, $J_{6b,5}$ 1.6 Hz, H-6b), 3.98 (br s, 1H, H-5), 2.17 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4 (CH_3CO), 160.5 (C=NH), 137.3 (C_{ipso}), 129.1–126.1 (C-Ar), 100.7 (CH benzylidene), 95.3 (C-1), 72.7, 69.9, 68.7, 64.7 (C-3, C-4, C-5, C-6), 56.7 (C-2), 20.9 (CH_3CO). Anal. Calcd: C, 42.56; H, 3.57; N, 11.68. Found: C, 42.35; H, 3.66; N, 11.51.

4.6. 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranosyl trichloroacetimidate (6β)

A solution of **5** (94.1 mg, 0.281 mmol) in CH_2Cl_2 (3.2 mL) was treated with K_2CO_3 (119.3 mg, 0.863 mmol) and Cl_3CCN (140 μL, 1.39 mmol). The mixture was stirred at rt for 2 h and then filtered over a Celite pad. The filtrate was concentrated and immediately subjected to column chromatography (7:1–2:1 v/v hexane/ethyl acetate) to give **6β** (80.3 mg, 60%) as a colourless oil. $[\alpha]_D$ +43.8 (*c* 1.5, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 8.76 (s, 1H, NH), 7.53–7.38 (m, 5H, H-Ar), 5.70 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 5.54 (s, 1H, C*H* benzylidene), 4.83 (dd, 1H, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.42 (d, 1H, $J_{4,3}$ 3.5 Hz, H-4), 4.36 (dd, 1H, J_{gem} 12.5 Hz, $J_{6a,5}$ 1.5 Hz, H-6a), 4.18 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{2,1}$ 8.5 Hz, H-2), 4.06 (dd, 1H, J_{gem} 12.5 Hz, $J_{6b,5}$ 1.5 Hz, H-6b), 3.67 (br s, 1H, H-5), 2.17 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (CH_3CO), 161.3 (C=NH), 137.5 (C_{ipso}), 129.2–126.2 (C-Ar), 100.9 (CH benzylidene), 96.9 (C-1), 72.4, 72.3, 68.7, 67.2 (C-3, C-4, C-5, C-6), 60.0 (C-2), 20.9 (CH_3CO). Anal. Calcd: C, 42.56; H, 3.57; N, 11.68. Found: C, 42.44; H, 3.60; N, 11.59.

4.7. 3-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactopyranosyl *N*-phenyl-trifluoroacetimidate (7)

A solution of **5** (103.9 mg, 0.310 mmol) in acetone (3.0 mL) was treated with $CF_3C(NPh)Cl$ (50.0 μL, 0.425 mmol) and Cs_2CO_3 (143.6 mg, 0.440 mmol). The mixture was stirred at rt for 2 h and then filtered over a Celite pad. The filtrate was concentrated and subjected to column chromatography (12:1–5:1 v/v hexane/ethyl acetate) to give **7** (123.4 mg, 79%; α/β 1.4:1) as a colourless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 7.53–6.84 (m, $10H_\alpha$ + $10H_\beta$, H-Ar), 6.59 (br s, $1H_\alpha$, H-1_α), 5.60 (br s, $1H_\beta$, H-1_β), 5.56 (s, $1H_\alpha$, C*H* benzylidene), 5.52 (s, $1H_\beta$, C*H* benzylidene), 5.30 (dd, $1H_\alpha$, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.2 Hz, H-3_α), 4.79 (dd, $1H_\beta$, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.0 Hz, H-3_β), 4.57 (d, $1H_\alpha$, $J_{4,3}$ 3.2 Hz, H-4_α), 4.33 (m, $2H_\alpha$ + $2H_\beta$, H-2_α, H-4_β, H-$6a_\alpha$, H-$6a_\beta$), 4.22–4.00 (m, $1H_\alpha$ + $3H_\beta$, H-2_β, H-5_β, H-$6b_\alpha$, H-$6b_\beta$), 3.91 (br s, $1H_\alpha$, H-5_α), 2.19 (s, $3H_\alpha$, CH_3CO_α), 2.18 (s, $3H_\beta$, CH_3CO_β); Anal. Calcd: C, 54.55; H, 4.18; N, 11.06. Found: C, 54.05; H, 4.34; N, 10.87.

4.8. Allyl (3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (8)

A mixture of **3β** (346.5 mg, 1.040 mmol) and **6β** (727.0 mg, 1.521 mmol) was coevaporated three times with toluene (5 mL), the residue was dried and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon atmosphere in 3:2 v/v CH_2Cl_2/*n*-hexane (17.5 mL). The mixture was stirred at −78 °C for 15 min. A 4.5% v/v $BF_3{\cdot}OEt_2$ solution in CH_2Cl_2 (1.1 mL, 0.39 mmol) was then added. The mixture was stirred for 1 h at −78 °C. Few drops of Et_3N were then added. The mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (10:1–4:1 hexane/ethyl acetate) to give, as first eluted compound, **8α** (382.8 mg, 57%) as a colourless oil. $[\alpha]_D$ +127.2 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.57–7.34 (m, 10H, H-Ar), 5.96 (m, 1H, $OCH_2CH{=}CH_2$), 5.60 (s, 1H, C*H* benzylidene), 5.56 (s, 1H, C*H* benzylidene), 5.39 (dd, 1H, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.5 Hz, H-3_B), 5.35 (dd, 1H, J_{vic} 17.0 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH{=}CHH$), 5.30 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1_B), 5.24 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* $OCH_2CH{=}CHH$), 4.56 (d, 1H, $J_{4,3}$ 3.0 Hz, H-4_B), 4.48 (dd, 1H, J_{gem} 12.5 Hz, J_{vic} 5.5 Hz, $OCHHCH{=}CH_2$), 4.39 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1_A), 4.38 (dd, 1H, J_{gem} 12.5 Hz, $J_{6a,5}$ 1.0 Hz, H-$6a_B$), 4.31 (d, 1H, $J_{4,3}$ 3.5 Hz, H-4_A), 4.27 (dd, 1H, J_{gem} 12.5 Hz, $J_{6a,5}$ 1.0 Hz, H-$6a_A$), 4.16 (dd, 1H, J_{gem} 12.5 Hz, J_{vic} 5.5 Hz, $OCHHCH{=}CH_2$), 4.10 (m, 3H, H-5_B, H-$6b_A$, H-$6b_B$), 3.95 (m, 2H, H-2_A, H-2_B), 3.65 (dd, 1H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3_A), 3.41 (br s, 1H, H-5_A), 2.14 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (CH_3CO), 137.5, 137.4 ($2C_{ipso}$), 133.5 ($OCH_2CH{=}CH_2$), 129.1–126.1 (C-Ar), 117.8 ($OCH_2CH{=}CH_2$), 101.0, 100.9, 100.8 (C-1_A, 2CH benzylidene), 94.9 (C-1_B), 74.4, 73.4, 70.7, 70.1, 69.2, 69.1, 69.0, 66.5, 63.1, 61.4, 56.6 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, $OCH_2CH{=}CH_2$), 20.9 (CH_3CO). MALDI TOF-MS for $C_{31}H_{34}N_6O_{10}$ (*m/z*): M_r (calcd) 650.23, M_r (found) 673.40 $(M+Na)^+$. Anal. Calcd: C, 57.23; H, 5.27; N, 12.92. Found: C, 57.00; H, 5.21; N, 12.70.

As second eluted compound **8β** (148.0 mg, 22%) was obtained as a colourless oil. $[\alpha]_D$ +39 (*c* 0.3, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.50–7.32 (m, 10H, H-Ar), 5.94 (m, 1H, $OCH_2CH{=}CH_2$), 5.60 (s, 1H, C*H* benzylidene), 5.52 (s, 1H, C*H* benzylidene), 5.35 (dd, 1H, J_{vic} 17.0 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH{=}CHH$), 5.23 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* $OCH_2CH{=}CHH$), 4.79 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1_B), 4.74 (dd, 1H, $J_{3,2}$ 11.0 Hz, $J_{3,4}$ 3.5 Hz, H-3_B), 4.46 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 5.0 Hz, $OCHHCH{=}CH_2$), 4.38 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1_A), 4.35–4.28 (m, 4H, H-4_A, H-4_B, H-$6a_A$, H-$6a_B$), 4.16 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 5.0 Hz, $OCHHCH{=}CH_2$), 4.12 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{2,1}$ 8.0 Hz, H-2_B), 4.06 (dd, 1H, J_{gem} 12.0 Hz, $J_{6b,5}$ 1.5 Hz, H-$6b_A$), 3.99 (m, 2H, H-2_A, H-$6b_B$), 3.63 (dd, 1H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3_A), 3.48 (br s, 1H, H-5_B), 3.40 (br s, 1H, H-5_A), 2.15 (s, 3H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (CH_3CO), 137.6, 137.5 ($2C_{ipso}$), 133.6 ($OCH_2CH{=}CH_2$), 129.2–126.1 (C-Ar), 117.6 ($OCH_2CH{=}CH_2$), 102.9, 101.3, 100.9, 100.7 (C-1_A, C-1_B, 2CH benzylidene), 76.7, 75.1, 72.5, 72.0, 70.1, 69.0, 68.9, 66.6, 66.3, 62.5, 60.0 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, $OCH_2CH{=}CH_2$), 20.9 (CH_3CO). MALDI TOF-MS for $C_{31}H_{34}N_6O_{10}$ (*m/z*): M_r (calcd) 650.23, M_r (found) 673.17 $(M+Na)^+$. Anal. Calcd: C, 57.23; H, 5.27; N, 12.92. Found: C, 56.92; H, 5.42; N, 12.69.

4.9. Allyl (2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (9)

A solution of **8α** (357.5 mg, 0.549 mmol) in 1:1 v/v CH_2Cl_2/MeOH (24 mL) was treated with a freshly prepared 0.85 M solution of NaOMe in MeOH (1.3 mL) and stirred at rt for 1 h. Amberlyst-15 (H^+ form) was then added until pH was neutral. The mixture was filtered and concentrated to afford pure **9** (333.4 mg, quantitative) as a white foam. $[\alpha]_D$ +114.8 (*c* 1.0, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.58–7.35 (m, 10H, H-Ar), 5.95 (m, 1H, $OCH_2CH{=}CH_2$), 5.61 (s, 2H, 2C*H* benzylidene), 5.34 (dd, 1H, J_{vic} 17.0 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH{=}CHH$), 5.26 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1_B), 5.24 (dd, 1H, J_{vic} 11.0 Hz, J_{gem} 1.5 Hz, *cis* $OCH_2CH{=}CHH$), 4.45 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 4.5 Hz, $OCHHCH{=}CH_2$), 4.39–4.28 (m, 6H, H-1_A, H-4_A, H-4_B, H-$6a_A$, H-$6a_B$, $OCHHCH{=}CH_2$), 4.18–4.10 (m, 3H, H-2_B, H-$6b_A$, H-$6b_B$), 4.05 (br s, 1H, H-5_B), 3.92 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{2,1}$ 8.5 Hz, H-2_A), 3.64 (m, 2H, H-3_A, H-3_B), 3.41 (br s, 1H, H-5_A); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.4, 137.3 ($2C_{ipso}$), 133.5 ($OCH_2CH{=}CH_2$), 129.4–126.1 (C-Ar), 117.8 ($OCH_2CH{=}CH_2$), 101.3, 100.9, 100.8 (C-1_A, 2CH benzylidene), 95.1 (C-1_B), 75.5, 74.5, 70.7, 70.1, 69.2, 69.1, 66.9, 66.5, 63.4, 61.6, 60.1 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, $OCH_2CH{=}CH_2$). MALDI TOF-MS for $C_{29}H_{32}N_6O_9$ (*m/z*): M_r (calcd) 608.22, M_r (found) 631.19

(M+Na)$^+$. Anal. Calcd: C, 57.23; H, 5.30; N, 13.81. Found: C, 56.99; H, 5.45; N, 13.60.

4.10. 1,3,4,6-Tetra-*O*-acetyl-2-*O*-methoxycarbonyl-α-D-galactopyranose (12)

Alcohol **11** (1.131 g, 3.247 mmol) was dissolved in CH_2Cl_2 (18 mL), cooled to 0 °C and then treated with TMEDA (667 μL, 4.42 mmol) and $ClCO_2Me$ (466 μL, 6.03 mmol). After 80 min stirring at 0 °C the mixture was diluted with CH_2Cl_2 (120 mL) and washed with water. The organic phase was collected, dried over anhydrous Na_2SO_4, filtered and concentrated to give pure **12** (1.316 g, quantitative) as a white foam. $[\alpha]_D$ +96.8 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.28 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.36 (d, 1H, $J_{3,4}$ 3.1 Hz, H-4), 5.19 (dd, 1H, $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 3.1 Hz, H-3), 4.98 (dd, 1H, $J_{2,3}$ 9.9 Hz, $J_{2,1}$ 3.5 Hz, H-2), 4.21 (t, 1H, $J_{5,6}$ 6.5 Hz, H-5), 3.94 (m, 2H, H-6a, H-6b), 3.65 (s, 3H, CO_2CH_3), 2.01 (s, 6H, $2COCH_3$), 1.88 (s, 3H, $COCH_3$), 1.85 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 169.6, 169.5, 168.5 ($4COCH_3$), 154.3 (CO_2CH_3), 89.0 (C-1), 69.7, 68.3, 67.0, 66.9, 60.7 (C-2, C-3, C-4, C-5, C-6), 55.0 (CO_2CH_3), 20.4–20.2 ($COCH_3$). MALDI TOF-MS for $C_{16}H_{22}O_{12}$ (*m/z*): M_r (calcd) 406.11, M_r (found) 428.99 (M+Na)$^+$. Anal. Calcd: C, 47.29; H, 5.46. Found: C, 47.05; H, 5.57.

4.11. 3,4,6-Tri-*O*-acetyl-2-*O*-methoxycarbonyl-D-galactose (13)

A solution of **12** (1.338 g, 3.296 mmol) in THF (10 mL) was treated with $BnNH_2$ (558 μL, 5.109 mmol). After overnight stirring at rt, volatiles were removed and the residue was subjected to column chromatography (3:1–2:1 v/v hexane/ethyl acetate) to give **13** (887.9 mg, 74%) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): (α-anomer) δ 5.52 (d, 1H, $J_{4,3}$ 2.5 Hz, H-4), 5.45 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.36 (dd, 1H, $J_{3,2}$ 10.9 Hz, $J_{3,4}$ 2.5 Hz, H-3), 4.96 (dd, 1H, $J_{2,3}$ 10.9 Hz, $J_{2,1}$ 3.8 Hz, H-2), 4.45 (t, 1H, $J_{5,6}$ 6.3 Hz, H-5), 4.12–4.05 (m, 2H, H-6a, H-6b), 3.77 (s, 3H, CO_2CH_3), 2.12 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) (α-anomer) δ 170.7, 170.2, 170.0 ($3COCH_3$), 155.0 (CO_2CH_3), 90.3 (C-1), 71.6, 68.1, 67.2, 66.0, 61.6 (C-2, C-3, C-4, C-5, C-6), 55.1 (CO_2CH_3), 20.6–20.5 ($COCH_3$). MALDI TOF-MS for $C_{14}H_{20}O_{11}$ (*m/z*): M_r (calcd) 364.11, M_r (found) 386.90 (M+Na)$^+$. Anal. Calcd: C, 46.16; H, 5.53. Found: C, 45.89; H, 5.67.

4.12. 3,4,6-Tri-*O*-acetyl-2-*O*-methoxycarbonyl-D-galactopyranosyl trichloroacetimidate (14)

A solution of **13** (99.6 mg, 0.274 mmol) in CH_2Cl_2 (3.0 mL) was cooled to 0 °C and then treated with Cl_3CCN (137 μL, 1.37 mmol) and K_2CO_3 (62.5 mg, 0.452 mmol). The mixture was stirred at rt for 7 h, then filtered over a Celite pad and concentrated. The residue was immediately chromatographed (5:1–2:1 v/v hexane/ethyl acetate) to afford **14** (95.0 mg, 68%; α/β 1.1:1) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, $1H_\beta$, NH_β), 8.69 (s, $1H_\alpha$, NH_α), 6.66 (d, $1H_\alpha$, $J_{1,2}$ 3.5 Hz, H-1_α), 5.83 (d, $1H_\beta$, $J_{1,2}$ 8.2 Hz, H-1_β), 5.57 (d, $1H_\alpha$, $J_{4,3}$ 2.8 Hz, H-4_α), 5.47 (d, $1H_\beta$, $J_{4,3}$ 3.1 Hz, H-4_β), 5.40 (dd, $1H_\alpha$, $J_{3,4}$ 10.8 Hz, $J_{3,2}$ 3.1 Hz, H-3_α), 5.28 (dd, $1H_\beta$, $J_{2,3}$ 10.5 Hz, $J_{2,1}$ 8.2, H-2_β), 5.20 (dd, $1H_\alpha$, $J_{2,3}$ 10.8 Hz, $J_{2,1}$ 3.5 Hz, H-2_α), 5.13 (dd, $1H_\beta$, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.1 Hz, H-3_β), 4.44 (t, $1H_\alpha$, $J_{5,6}$ 6.3 Hz, H-5_α), 4.17 (m, $1H_\alpha$ + $1H_\beta$, H-$6a_\alpha$, H-$6a_\beta$), 4.11 (m, $2H_\beta$, H-5_β, H-$6b_\beta$), 4.07 (dd, $1H_\alpha$, J_{gem} 11.2 Hz, $J_{6b,5}$ 6.7 Hz, H-$6b_\alpha$), 3.79 (s, $3H_\alpha$, $CO_2CH_{3\alpha}$), 3.75 (s, $3H_\beta$, $CO_2CH_{3\beta}$), 2.17 (s, $3H_\beta$, $COCH_{3\beta}$), 2.16 (s, $3H_\alpha$, $COCH_{3\alpha}$), 2.03 (s, $3H_\beta$, $COCH_{3\beta}$), 2.01 (s, $3H_\beta$, $COCH_{3\beta}$), 2.00 (s, $6H_\alpha$, $COCH_{3\alpha}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3–169.9 (3α + 3β *COCH*$_3$), 160.9, 160.8 (C=NH_α + C=NH_β), 154.3, 154.2 ($CO_2CH_{3\alpha}$ + $CO_2CH_{3\beta}$), 96.0 (C-1_β), 93.3 (C-1_α), 71.8, 71.7, 70.5, 70.4, 69.0, 67.5, 67.3, 66.7, 61.2, 60.8 (C-2_α, C-2_β, C-3_α, C-3_β, C-4_α, C-4_β, C-5_α, C-5_β, C-6_α, C-6_β), 55.4, 55.3 ($CO_2CH_{3\alpha}$, $CO_2CH_{3\beta}$), 20.6–20.5 ($COCH_3$). Anal. Calcd: C, 37.78; H, 3.96; N, 2.75. Found: C, 37.49; H, 3.88; N, 2.68.

4.13. Allyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3) -(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (15) and 3,4,6-tri-*O*-benzoyl-α-D-galactopyranose 1,2-[(1→3)-(allyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside)-2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl] orthobenzoate (16)

A mixture of **9** (33.0 mg, 54.2 μmol) and **10** (80.8 mg, 0.109 mmol) was coevaporated thrice with toluene (2 mL), the residue was dried and then mixed with freshly activated AW-300 4 Å molecular sieves, and suspended under argon atmosphere in CH_2Cl_2 (1.6 mL). The mixture was stirred at 0 °C for 15 min. A 3.2% v/v TMSOTf solution in CH_2Cl_2 (62 μL, 10.9 μmol) was then added. The mixture was stirred for 2 h at 0 °C, then a drop of Et_3N was added and the mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (12:1–6:1 v/v toluene/ethyl acetate) to give, as first eluted compound, **16** (20.5 mg, 32%) as a white foam. $[\alpha]_D$ +70 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00–6.98 (m, 30H, H-Ar), 5.99 (m, 2H, H-3_C, $OCH_2CH{=}CH_2$), 5.68 (s, 1H, C*H* benzylidene), 5.63 (d, 1H, $J_{1,2}$ 4.9 Hz, H-1_C), 5.61 (s, 1H, C*H* benzylidene), 5.35 (d, 1H, J_{vic} 17.3 Hz, *trans* $OCH_2CH{=}CHH$), 5.26 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1_B), 5.24 (dd, 1H, J_{vic} 10.5 Hz, *cis* $OCH_2CH{=}CHH$), 4.74 (t, 1H, $J_{2,3} = J_{2,1}$ 4.9 Hz, H-2_C), 4.69 (m, 2H, H-4_C, H-5_C), 4.59 (d, 1H, $J_{4,3}$ 2.8 Hz, H-4_B), 4.46 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 4.8 Hz, OC*H*HCH=CH_2), 4.38 (m, 5H, H-1_A, H-3_B, H-$6a_B$, H-$6a_C$, H-$6b_C$), 4.31 (d, 1H, $J_{4,3}$ 3.2 Hz, H-4_A), 4.23–3.90 (m, 7H, H-2_A, H-2_B, H-5_B, H-$6a_A$, H-$6b_A$, H-$6b_B$, OC*H*HCH=CH_2), 3.65 (dd, 1H, $J_{3,2}$ 10.3 Hz, $J_{3,4}$ 3.2 Hz, H-3_A), 3.41 (br s, 1H, H-5_A); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 165.7, 165.2 (3Ph*C*O), 137.4, 137.3 ($2C_{ipso}$ benzylidene), 133.5–125.7 ($OCH_2CH{=}CH_2$, quaternary C orthoester, C-Ar), 117.8 ($OCH_2CH{=}CH_2$), 107.6 (C-1_C), 100.9, 100.8, 100.5 (C-1_A, 2CH benzylidene), 94.7 (C-1_B), 82.4, 82.0, 77.3, 75.6, 75.4, 74.1, 70.6, 70.2, 70.0, 69.2, 68.3, 66.5, 63.6, 63.3, 61.4, 58.0 (C-2_A, C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, C-6_A, C-6_B, C-6_C, $OCH_2CH{=}CH_2$). MALDI TOF-MS for $C_{63}H_{58}N_6O_{18}$ (*m/z*): M_r (calcd) 1186.38, M_r (found) 1209.44 (M+Na)$^+$. Anal. Calcd: C, 63.74; H, 4.92; N, 7.08. Found: C, 63.41; H, 4.80; N, 6.97.

As second eluted compound, **15** (28.8 mg, 45%) was obtained as a white foam. $[\alpha]_D$ +118 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–7.25 (m, 30H, H-Ar), 5.99 (d, 1H, $J_{4,3}$ 2.9 Hz, H-4_C), 5.96 (m, 1H, $OCH_2CH{=}CH_2$), 5.89 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{2,1}$ 8.1 Hz, H-2_C), 5.57 (dd, 1H, $J_{3,2}$ 10.0 Hz, $J_{3,4}$ 3.1 Hz, H-3_C), 5.53 (s, 1H, C*H* benzylidene), 5.50 (s, 1H, C*H* benzylidene), 5.36 (d, 1H, J_{vic} 17.2 Hz, *trans* $OCH_2CH{=}CHH$), 5.26 (dd, 1H, J_{vic} 10.6 Hz, *cis* $OCH_2CH{=}CHH$), 5.20 (d, 1H, $J_{1,2}$ 3.1 Hz, H-1_B), 5.13 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1_C), 4.76 (m, 1H, H-5_C), 4.53 (d, 1H, $J_{4,3}$ 2.3 Hz, H-4_B), 4.45 (dd, 1H, J_{gem} 12.9 Hz, J_{vic} 4.7 Hz, OC*H*HCH=CH_2), 4.40–4.25 (m, 6H, H-1_A, H-3_B, H-4_A, H-$6a_B$, H-$6a_C$, H-$6b_C$), 4.19–4.06 (m, 3H, H-$6a_A$, H-$6b_B$, OC*H*HCH=CH_2), 3.89 (t, 1H, $J_{2,3} = J_{2,1}$ 10.0 Hz, H-2_A), 3.80 (dd, 1H, $J_{2,3}$ 10.9 Hz, $J_{2,1}$ 3.1 Hz, H-2_B), 3.78 (br s, 1H, H-5_B), 3.66 (m, 2H, H-3_A, H-$6b_A$), 3.38 (br s, 1H, H-5_A); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 165.6, 165.5, 165.2 (4Ph*C*O), 137.6, 137.3 ($2C_{ipso}$ benzylidene), 133.6–126.0 ($OCH_2CH{=}CH_2$, C-Ar), 117.9 ($OCH_2CH{=}CH_2$), 103.1 (C-1_C), 100.9, 100.6, 100.4 (C-1_A, 2CH benzylidene), 94.7 (C-1_B), 76.1, 75.9, 74.2, 72.0, 71.5, 70.4, 70.0, 69.4, 69.1, 68.9, 68.2, 66.4, 63.7, 62.5, 61.4, 57.7 (C-2_A, C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, C-6_A, C-6_B, C-6_C, $OCH_2CH{=}CH_2$). MALDI TOF-MS for $C_{63}H_{58}N_6O_{18}$ (*m/z*): M_r (calcd) 1186.38, M_r (found) 1209.45 (M+Na)$^+$. Anal. Calcd: C, 63.74; H, 4.92; N, 7.08. Found: C, 63.58; H, 4.82; N, 7.00.

4.14. Allyl (3,4,6-tri-*O*-acetyl-2-*O*-methoxycarbonyl-β-D-galactopyranosyl)-(1→3)-(2-azido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (17)

A mixture of **9** (126.4 mg, 0.207 mmol) and **14** (210.5 mg, 0.415 mmol) was coevaporated thrice with toluene (3 mL). The residue was dried and then mixed with freshly activated AW-300 4 Å molecular sieves, suspended under argon atmosphere in CH_2Cl_2 (4.4 mL). The mixture was stirred at 0 °C for 15 min. A 3.2% v/v TMSOTf solution in CH_2Cl_2 (225 μL, 41.5 μmol) was then added. The mixture was stirred for 2 h at 0 °C, then some drops of Et_3N were added and the mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (10:1–3:1 v/v toluene/ethyl acetate) to give **17** (146.4 mg, 74%) as a colourless oil. $[\alpha]_D$ +81.8 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57–7.36 (m, 10H, H-Ar), 5.96 (m, 1H, $OCH_2CH{=}CH_2$), 5.60 (s, 2H, 2C*H* benzylidene), 5.43 (d, 1H, $J_{4,3}$ 3.0 Hz, H-4_C), 5.35 (dd, 1H, J_{vic} 17.0 Hz, J_{gem} 2.0 Hz, *trans* $OCH_2CH{=}CHH$), 5.29 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1_B), 5.24 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 2.0 Hz, *cis* $OCH_2CH{=}CHH$), 5.06 (m, 2H, H-2_C, H-3_C), 4.82 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1_C), 4.47 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 3.5 Hz, $OCHHCH{=}CH_2$), 4.45 (d, 1H, $J_{4,3}$ 3.0 Hz, H-4_B), 4.36 (m, 2H, H-1_A, H-5_C), 4.31 (d, 1H, $J_{4,3}$ 3.5 Hz, H-4_A), 4.28 (dd, 1H, J_{gem} 12.5 Hz, $J_{6a,5}$ 1.0 Hz, H-$6a_B$), 4.24 (dd, 1H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3_B), 4.14 (m, 5H, H-$6a_A$, H-$6a_C$, H-$6b_B$, H-$6b_C$, $OCHHCH{=}CH_2$), 3.95 (m, 4H, H-2_A, H-2_B, H-5_B, H-$6b_A$), 3.72 (s, 3H, CO_2CH_3), 3.69 (dd, 1H, $J_{3,2}$ 10.5 Hz, $J_{3,4}$ 3.5 Hz, H-3_A), 3.40 (br s, 1H, H-5_A), 2.15 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 1.99 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 169.9 ($3CH_3CO$), 154.8 (CO_2CH_3), 137.6, 137.3 ($2C_{ipso}$ benzylidene), 133.4 ($OCH_2CH{=}CH_2$), 129.0–125.3 (C-Ar), 117.9 ($OCH_2CH{=}CH_2$), 102.0 (C-1_C), 100.9, 100.6, 100.5 (C-1_A, 2CH benzylidene), 94.6 (C-1_B), 77.2, 75.6, 75.1, 74.0, 72.6, 70.9, 70.8, 70.4, 70.1, 70.0, 69.1, 67.0, 66.4, 63.7, 61.4, 58.3, 55.1 (C-2_A, C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, C-6_A, C-6_B, C-6_C, $OCH_2CH{=}CH_2$, CO_2CH_3), 21.4, 20.6, 20.5 ($3COCH_3$). MALDI TOF-MS for $C_{43}H_{50}N_6O_{19}$ (*m/z*): M_r (calcd) 954.31, M_r (found) 977.58 $(M+Na)^+$. Anal. Calcd: C, 54.09; H, 5.28; N, 8.80. Found: C, 53.78; H, 5.12; N, 8.60.

4.15. Allyl (3,4,6-tri-*O*-acetyl-2-*O*-methoxycarbonyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (18)

A solution of **17** (141.7 mg, 0.149 mmol) in THF (4.4 mL) was diluted with Ac_2O (2.9 mL) and AcOH (1.5 mL) and then treated with Zn/Cu (405 mg). After 5 h stirring at rt a second aliquot of Zn/Cu (210 mg) was added and the mixture was stirred overnight. It was then filtered over a Celite pad and concentrated. The residue was dissolved in 1:1 v/v CH_2Cl_2/MeOH (8.0 mL) and then treated with Ac_2O (800 μL). The solution was stirred at rt for 6 h, after that volatiles were removed. The residue was subjected to column chromatography (98:2–95:5 v/v CH_2Cl_2/MeOH) to afford **18** (83.5 mg, 57%) as white amorphous crystals. $[\alpha]_D$ +85.5 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.54–7.37 (m, 10H, H-Ar), 5.96 (d, 1H, $J_{H,NH}$ 8.3 Hz, NH_A), 5.92 (d, 1H, $J_{H,NH}$ 9.3 Hz, NH_B), 5.89 (m, 1H, $OCH_2CH{=}CH_2$), 5.54 (s, 1H, C*H* benzylidene), 5.45 (s, 1H, C*H* benzylidene), 5.38 (d, 1H, $J_{4,3}$ 2.0 Hz, H-4_C), 5.28 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH{=}CHH$), 5.20 (dd, 1H, J_{vic} 10.4 Hz, J_{gem} 1.5 Hz, *cis* $OCH_2CH{=}CHH$), 5.09 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_B), 4.95 (m, 2H, H-2_C, H-3_C), 4.71 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1_A), 4.64 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1_C), 4.61 (dt, 1H, $J_{H,NH} = J_{2,3}$ 9.3 Hz, $J_{2,1}$ 3.5 Hz, H-2_B), 4.37 (dd, 1H, J_{gem} 13.0 Hz, $J_{6a,5}$ 5.1 Hz, H-$6a_C$), 4.26 (d, 1H, $J_{4,3}$ 2.8 Hz, H-4_B), 4.21 (d, 1H, J_{gem} 12.5 Hz, H-$6a_B$), 4.14 (m, 4H, H-3_A, H-4_A, H-$6a_A$, $OCHHCH{=}CH_2$), 4.08 (m, 2H, H-$6b_C$, $OCHHCH{=}CH_2$), 3.99 (m, 3H, H-2_A, H-$6b_A$, H-$6b_B$), 3.89 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5_C), 3.84 (dd, 1H, $J_{3,2}$ 11.2 Hz, $J_{3,2}$ 3.1 Hz, H-3_B), 3.69 (s, 3H, CO_2CH_3), 3.65 (br s, 1H, H-5_B), 3.43 (br s, 1H, H-5_A), 2.12 (s, 3H, $OCOCH_3$), 2.01 (s, 3H, $OCOCH_3$), 1.96 (s, 3H, $OCOCH_3$), 1.94 (s, 3H, $NCOCH_3$), 1.45 (s, 3H, $NCOCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3–170.0 ($5CH_3CO$), 154.5 (CO_2CH_3), 137.6, 137.5 ($2C_{ipso}$ benzylidene), 133.9 ($OCH_2CH{=}CH_2$), 129.5–126.1 (C-Ar), 117.7 ($OCH_2CH{=}CH_2$), 102.2 (C-1_C), 101.3, 100.6, 99.0 (C-1_A, 2CH benzylidene), 95.4 (C-1_B), 75.9, 73.9, 72.7, 71.4, 70.6, 69.3, 69.1, 67.0, 66.2, 63.6, 61.1, 55.1, 52.2, 47.4 (C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, C-6_A, C-6_B, C-6_C, $OCH_2CH{=}CH_2$), 55.1, 52.2, 47.4 (C-2_A, C-2_B, CO_2CH_3), 20.7–20.5 ($5COCH_3$). MALDI TOF-MS for $C_{47}H_{58}N_2O_{21}$ (*m/z*): M_r (calcd) 986.35, M_r (found) 1009.15 $(M+Na)^+$. Anal. Calcd: C, 57.20; H, 5.92; N, 2.84. Found: C, 56.89; H, 5.71; N, 2.77.

4.16. Allyl (3,4,6-tri-*O*-acetyl-2-*O*-methoxycarbonyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (19)

Compound **18** (25.6 mg, 26.0 μmol) was dissolved in 90% AcOH (1.25 mL). The solution was stirred at 50 °C for 8 h, after that it was concentrated. The residue was subjected to column chromatography (93:7–85:15 v/v CH_2Cl_2/MeOH) to give **19** (15.6 mg, 74%) as a white foam. $[\alpha]_D$ +62 (*c* 0.9, CH_3OH); ^{1}H NMR (600 MHz, CD_3OD): δ 5.91 (m, 1H, $OCH_2CH{=}CH_2$), 5.40 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_B), 5.27 (dd, 1H, J_{vic} 17.3 Hz, J_{gem} 1.7 Hz, *trans* $OCH_2CH{=}CHH$), 5.15 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.6 Hz, *cis* $OCH_2CH{=}CHH$), 5.11 (dd, 1H, $J_{3,2}$ 10.4 Hz, $J_{3,4}$ 3.5 Hz, H-3_C), 4.92 (m, 2H, H-2_C, H-4_C), 4.77 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1_C), 4.47 (dd, 1H, $J_{2,3}$ 11.1 Hz, $J_{2,1}$ 3.5 Hz, H-2_B), 4.41 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1_A), 4.34 (dd, 1H, J_{gem} 13.3 Hz, J_{vic} 4.9 Hz, $OCHHCH{=}CH_2$), 4.18–4.08 (m, 6H, H-4_A, H-$6a_B$, H-$6a_C$, H-$6b_B$, H-$6b_C$, $OCHHCH{=}CH_2$), 3.97 (d, 1H, $J_{4,3}$ 2.9 Hz, H-4_B), 3.81–3.69 (m, 9H, H-2_A, H-3_B, H-5_B, H-5_C, H-$6a_A$, H-$6b_A$, CO_2CH_3), 3.65 (dd, 1H, $J_{3,2}$ 10.9 Hz, $J_{3,4}$ 3.1 Hz, H-3_A), 3.43 (t, 1H, $J_{5,6}$ 5.9 Hz, H-5_A), 2.12 (s, 3H, $COCH_3$), 2.02 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.95 (s, 3H, $COCH_3$); ^{13}C NMR (50 MHz, CD_3OD) δ 174.8, 174.3, 172.9, 172.7, 172.2 ($5CH_3CO$), 157.5 (CO_2CH_3), 136.4 ($OCH_2CH{=}CH_2$), 118.1 ($OCH_2CH{=}CH_2$), 104.7, 102.9 (C-1_A, C-1_C), 96.8 (C-1_B), 81.0, 78.5, 77.4, 75.1, 73.6, 73.1, 72.8, 71.7, 71.6, 71.0, 69.9, 66.3, 63.5, 63.3 (C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, C-6_A, C-6_B, C-6_C, $OCH_2CH{=}CH_2$), 56.8, 52.8, 50.4 (C-2_A, C-2_B, CO_2CH_3), 24.2, 23.8 (2 $NHCOCH_3$), 21.6, 21.4, 21.3 (3 $OCOCH_3$). MALDI TOF-MS for $C_{33}H_{50}N_2O_{21}$ (*m/z*): M_r (calcd) 810.29, M_r (found) 833.03 $(M+Na)^+$. Anal. Calcd: C, 48.89; H, 6.22; N, 3.46. Found: C, 48.63; H, 6.40; N, 3.31.

4.17. Allyl β-D-galactopyranosyl-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (20)

A solution of **19** (13.9 mg, 17.2 μmol) in MeOH (400 μL) was treated with a 0.55 M methanolic solution of NaOMe (100 μL). After 5 h stirring at room temperature, water (1 mL) was added and the solution was neutralized with Amberlyst-15 (H^+ form). After filtration, volatiles removal and freeze-drying, pure **20** (10.1 mg, 94%) was obtained as a white fluffy solid. $[\alpha]_D$ +13 (*c* 0.6, H_2O); ^{1}H NMR (500 MHz, D_2O): δ 5.92 (m, 1H, $OCH_2CH{=}CH_2$), 5.33 (d, 1H, J_{vic} 17.0 Hz, *trans* $OCH_2CH{=}CHH$), 5.27 (d, 1H, J_{vic} 11.0 Hz, *cis* $OCH_2CH{=}CHH$), 5.07 (br s, 1H, $J_{1,2}$ 3.5 Hz, H-1_B), 4.58 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1_A), 4.44 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1_C), 4.38 (m, 2H, H-2_B, $OCHHCH{=}CH_2$), 4.25 (d, 1H, $J_{4,3}$ 2.0 Hz, H-4_B), 4.20 (dd, 1H, J_{gem} 13.0 Hz, J_{vic} 6.5 Hz, $OCHHCH{=}CH_2$), 4.13 (d, 1H, $J_{4,3}$ 3.0 Hz, H-4_A), 4.08 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{2,1}$ 8.5 Hz, H-2_A), 3.91 (d, 1H, $J_{4,3}$ 3.0 Hz, H-4_C), 3.86–3.74 (m, 9H, H-3_A, H-3_B, H-5_B, H-$6a_A$, H-$6a_B$, H-$6a_C$, H-$6b_A$, H-$6b_B$, H-$6b_C$), 3.65 (m, 3H, H-3_C, H-5_A, H-5_C), 3.51 (dd, 1H, $J_{2,3}$

10.0 Hz, $J_{2,1}$ 8.0 Hz, H-2_C), 1.95 (s, 3H, $COCH_3$), 1.91 (s, 3H, $COCH_3$); ^{13}C NMR (125 MHz, D_2O) δ 175.9, 175.7 ($2COCH_3$), 134.0 ($OCH_2CH{=}CH_2$), 119.2 ($OCH_2CH{=}CH_2$), 106.9 (C-1_C), 100.9 (C-1_A), 94.4 (C-1_B), 78.1 (C-3_B), 75.8 (C-5_A, C-5_C), 75.4 (C-3_A), 73.1 (C-3_C), 71.8 (C-5_B), 70.2 (C-2_C), 70.0 ($OCH_2CH{=}CH_2$), 69.5 (C-4_C), 69.1 (C-4_B), 64.5 (C-4_A), 61.9 (C-6_C), 61.7 (C-6_B), 61.5 (C-6_A), 51.7 (C-2_A), 49.0 (C-2_B), 23.4, 23.2 ($2COCH_3$). MALDI TOF-MS for $C_{25}H_{42}N_2O_{16}$ (m/z): M_r (calcd) 626.25, M_r (found) 649.11 $(M+Na)^+$. Anal. Calcd: C, 47.92; H, 6.76; N, 4.47. Found: C, 47.00; H, 6.98; N, 4.31.

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