Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 2026–2036
Carbohydrate RESEARCH

Synthesis of globo- and isoglobotriosides bearing a cinnamoylphenyl tag as novel electrophilic thiol-specific carbohydrate reagents

Mohamed R. E. Aly,[a] Pascal Rochaix,[b] Mohamed Amessou,[b] Ludger Johannes[b] and Jean-Claude Florent[a,*]

[a]*Laboratoire de Chimie UMR 176 CNRS-Institut Curie, 26 rue d'Ulm, F-75248 Paris, France*
[b]*Laboratoire 'Traffic and Signalisation' UMR 144 CNRS-Institut Curie, 26 rue d'Ulm, 75248 Paris, France*

Received 1 December 2005; received in revised form 7 March 2006; accepted 7 March 2006
Available online 13 June 2006

Abstract—The galactosyl donor, 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-D-galactopyranosyl trichloroacetimidate, was efficiently coupled with regioselectively benzylated lactoside acceptors under standard conditions to stereoselectively afford the corresponding globotrioside and isoglobotrioside derivatives in very good yields. These glycosides were smoothly functionalized with a 6-(*p*-cinnamoylphenoxy)-hexyl tether tag as novel electrophilic thiol-specific carbohydrate reagents. Immobilization of the globotrioside conjugate to Thiopropyl Sepharose 6B for purification of B-subunit of Shiga toxin (StxB) and coupling of a model cysteine-containing protein (StxB-Z(n)-Cys) to the isoglobotrioside conjugate were both performed with high efficiency.

Keywords: Globotriose; Isoglobotriose; Chalcone; Thiol-specific reagents; Thiopropyl Sepharose 6B

1. Introduction

The P^k antigen globotriosylceramide (Galα1,4-Galβ1,4-Glcβ1,1-OCer, Gb3), a cell surface glycosphingolipid in various normal tissues,[1] is also well known to be overexpressed in several human tumour cells.[2] The terminal Galα(1→4)Gal or galabiosyl epitope of the trisaccharide core, that is globotriose, or Gb_3 is a ligand for Shiga toxins[3] excreted by *E. coli* strain O-157:H-7. This event is responsible for gastroenteritis and the Haemolytic-Uraemic Syndrome (HUS).[4] A structurally related glycosphingolipid, isoglobotriosyl ceramide (Galα1,3-Galβ1,4-Glcβ1,1-OCer, $isoGb_3$) characterized by the terminal Galα(1→3)Gal moiety, the αGal epitope, of the glycone isoglobotriose ($isoGb_3$) is a known ligand for two exotoxins, toxins A and B, produced by *Clostridium difficile*, which are responsible for human intestinal inflammation.[5] $IsoGb_3$ is also known as animal antigen responsible for immediate hyperacute rejection upon xenotransplantation.[6] Finally, $isoGb_3$ was recently identified as a key endogenous NKT cell antigen present at the surface of antigen presenting cells D1.[7] These trisaccharides, have been often used as probes in biological applications. Thus, several globotriose-based artificial STx ligands were investigated as blockers of the toxin-host cell adhesion.[8–11] Globotriose has been used as a tool to study the retrograde transport (i.e., the directed movement of membrane bound vesicles from recycling endosomes back to the trans-Golgi network where they are recycled for further rounds of transport) and in immunotherapy.[12] For its part, $isoGb_3$, has been used to form artificial isoglobotriosyl polymers as effective antagonists of anti-Gal antibodies to prevent rejection of xenografts.[13] Furthermore the anti-Gal antibody, mediated immunotherapy aimed at redirecting human nature immune defence to kill viruses, bacteria and cancer cells has been investigated by Wang et al.[6] Immobilized isoglobotriose has been used for the separation of toxins A and B.[5]

* Corresponding author. Fax: +33 1 42 34 66 31; e-mail: Jean-Claude.Florent@curie.fr

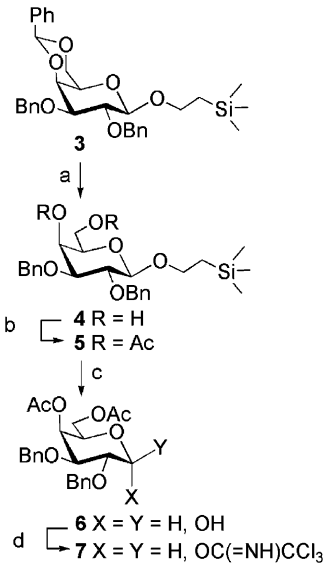
0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.03.044

Although chemical methods routinely used for the synthesis of Gb_3 and $isoGb_3$ epitopes from different galactosyl donors[7,14–17] are well known, these methods frequently lead to β-glycosides as sometimes unseparable byproducts. Our first objective was to investigate the behaviour of 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-D-galactopyranosyl trichloroacetimidate as galactosyl donor. We hypothesized that conjugation of an expected enhanced remote assistance of any of the two acetyl groups, along with the well-established leaving group ability of the trichloroacetimidate[18] for the coupling with reactive benzylated lactosides as galactosyl acceptors might lead to good stereocontrolled α-stereoselectivity. The affinity of the cinnamoylphenyl group (chalcone) to form stable thioether linkages with thiols,[19] prompted us to investigate this group conjugated with these trisaccharides to prepare two novel electrophilic thiol-specific carbohydrate reagents bearing a 6-(*p*-cinnamoylphenoxy)hexyl tether **1** and **2** for neoglycoconjugate synthesis[20] and biosensors preparation.[21] As an example, we realized the immobilization of **1** onto Thiopropyl Sepharose 6B in order to isolate the Shiga toxin StxB from periplasmic extract of *E. coli* strain BH 101, and as an example of neoglycoconjugate synthesis, the coupling of **2** to the protein StxB as thiol-possessing protein (Fig. 1).

2. Results and discussion

2.1. Synthesis of the galactosyl donor 7

The benzylidene group of 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-galactopyranoside **3**[22] was smoothly removed by hydrolysis with a catalytic amount of *p*-TsOH in the presence of an excess of EtSH as scavenger of the released benzaldehyde, resulting in the known diol **4** (97% yield)[22] as depicted in Scheme 1. Acetylation of **4** with Ac_2O–pyridine afforded **5** in 94% yield and subsequent removal of the 2-(trimethylsilyl)ethyl group with 2:1 TFA in CH_2Cl_2 gave the 1-O-unprotected derivative **6** in 82% yield. Further treatment of **6** with trichloroacetonitrile in the presence of 1,8-diazo[5.4.0]bicycloundec-7-ene (DBU) furnished the desired novel galactosyl trichloroacetimidate **7** (α/β mixture 11:1, 93%).



Scheme 1. Reagents and conditions: (a) EtSH, *p*-TsOH, CH_2Cl_2, 97%; (b) Ac_2O, pyridine, 94%; (c) TFA, CH_2Cl_2 2:1, 82%; (d) CCl_3CN, DBU, 93%.

2.2. Synthesis of the globotriosyl chalcone conjugate 1

As outlined in Scheme 2, compound **7** was coupled with the glycosyl acceptor **8**[14b,23] in 1:1 CH_2Cl_2–Et_2O, for the 'ether effect',[24] in the presence of TMSOTf (6 mol %) as promotor at −25 °C and under 'inverse procedure' condition.[25] This stereospecifically led to the α-glycoside **9**, which was obtained in 81% yield. It is noteworthy that this result was obtained with an economical ratio of donor to acceptor of 1.5:1. Assignment of the terminal galactopyranosyl moiety and hence the α-stereoselectivity

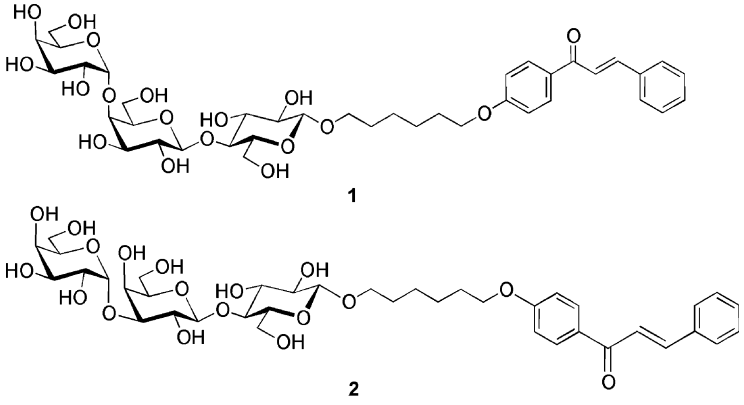
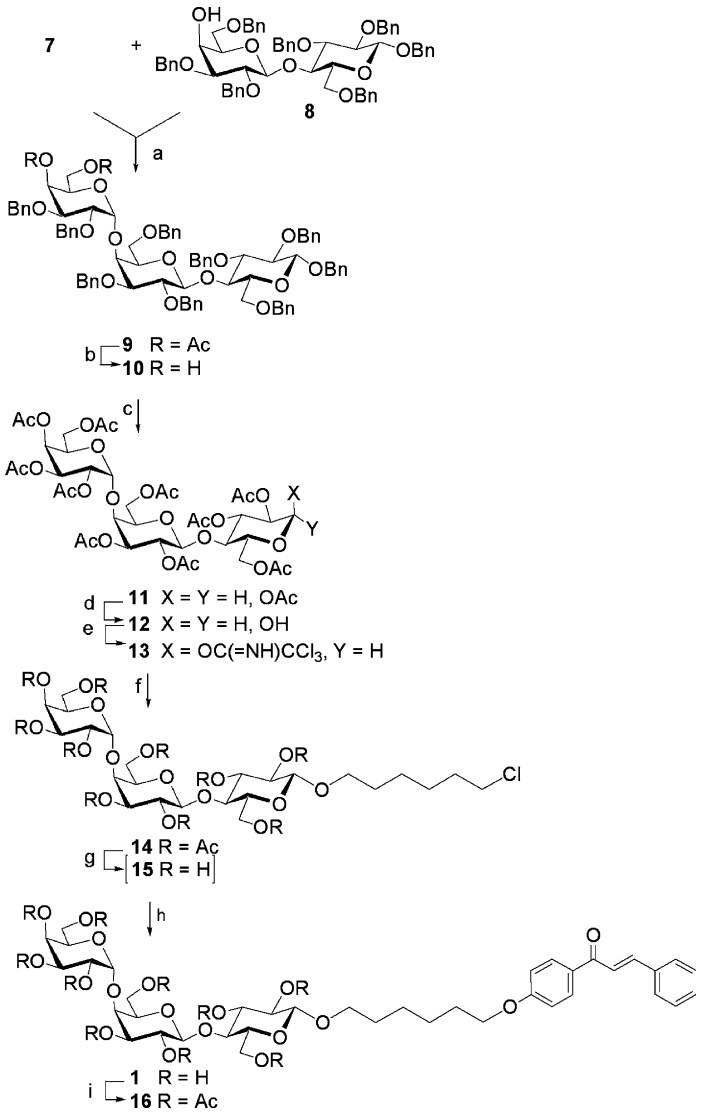


Figure 1.



Scheme 2. Reagents and conditions: (a) TMSOTf (6 mol %), Et_2O–CH_2Cl_2, −25 °C to rt, 81%; (b) NaOMe–MeOH, 98%; (c) 1: H_2, Pd–C, MeOH; 2: Ac_2O, Pyridine, 89%; (d) N_2H_4·HOAc, DMF, 92%; (e) CCl_3CN, DBU, 80%; (f) 6-chloro-1-hexanol, TMSOTf (6 mol %), CH_2Cl_2, −45 °C to rt, 54%; (g) NaOMe–MeOH, 96%; (h) *p*-cinnamoylphenol, K_2CO_3, DMF, 53%; (i) Ac_2O, Pyridine, 88%.

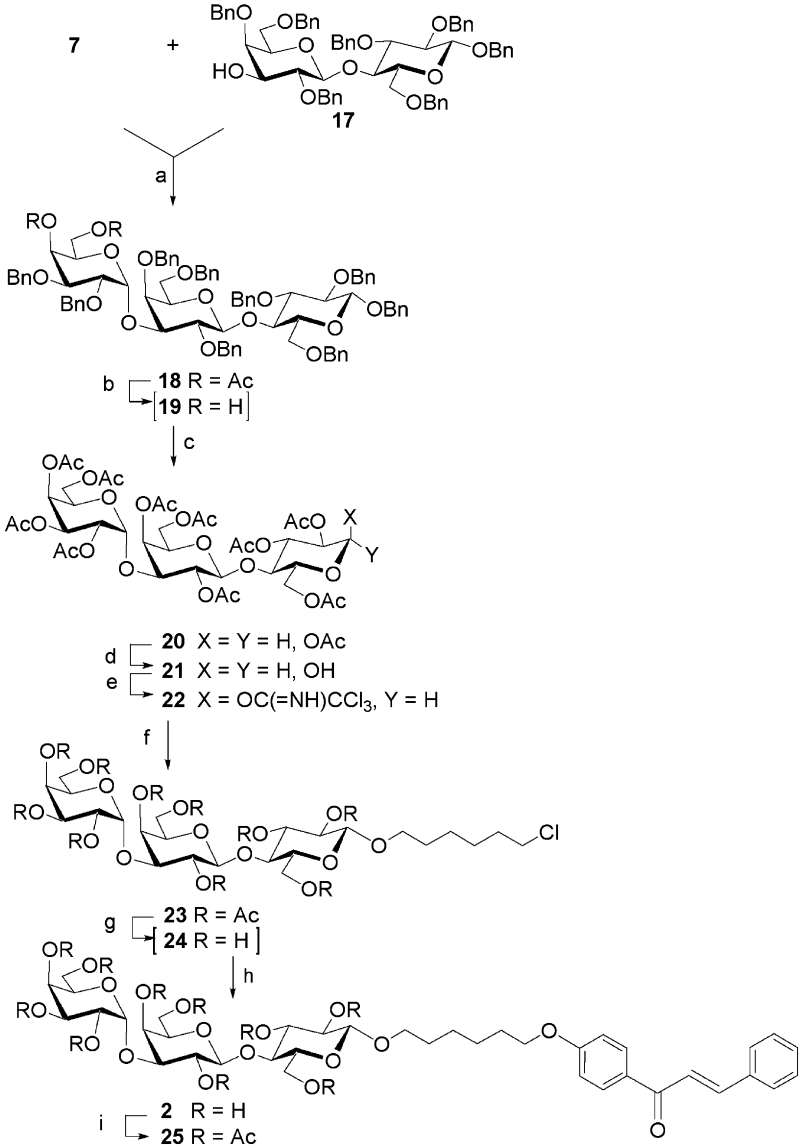
was determined unambiguously by ^1H NMR data of H-1_c ($\delta = 5.14$, $J_{1,2}$ 3.1 Hz) as well as by ^{13}C NMR data C-1_c ($\delta = 100.25$). This high selectivity can be attributed to the participation of any of the remote acetyl groups as well as the reaction conditions used, and thus support our starting hypothesis.

Further important step was the replacement of the benzyl groups by their acetyl counterparts in order to control the β configuration of the spacer. In our first attempt for reductive debenzylation, compound **9** was treated directly with Pd/C (10%, 1:1 w/w) in 3:1 MeOH–AcOH, then acetylated with Ac_2O/pyridine. In these conditions, an array of products was obtained furnishing only a low yield of **11** (data not shown). Fortunately, when **9** was deacetylated first under Zemplén conditions,[26] affording **10** (98%) and the latter product debenzylated in the presence of a catalytic amount of Pd/C (10%) in MeOH at room temperature followed by a treatment with Ac_2O–pyridine, **11**[15d] was obtained in 89% yield. Besides the ease of debenzylation of **10**, deacetylation of **9** removed any traces of unreacted acceptor from the coupling step and was taken into account in estimating the yields of both **9** and **10**. Anomeric deacetylation of **11** was performed by treatment with hydrazinium acetate in DMF to afford **12**[15d] in 92% yield. Subsequent treatment with trichloroacetonitrile in the presence of DBU furnished the known α-trichloroacetimidate **13**[15d] in 80% yield. Reaction of trisaccharide donor **13** with 6-chloro-1-hexanol (under inverse procedure condition)[25] with TMSOTf (6 mol %) as catalyst in MeCN at low temperature, afforded the desired β-linked 6-chlorohexyl glycoside

14 in a moderate (54%) yield. The β-anomeric configuration was determined from the neighbour 2-H proton δ 4.88 ($J_{1,2}$ 8.1 Hz) because of overlapping of signals in the H-1_a region. The modest yield of this coupling reaction could originate from the low donor to acceptor ratio (1:6) and the relatively high acidic conditions used in order to avoid orthoester formation.[15d] Under these conditions, hydrolysis of the donor could be a competitive reaction. It is noteworthy that no glycoside formation was observed using the softer Lewis acid $Et_2O{\cdot}BF_3$. Attempts to obtain **14** directly by reacting **11** with 6-chloro-1-hexanol in the presence of $Et_2O{\cdot}BF_3$ led only to **12** (data not shown). Deacetylation of **14** under Zemplén conditions yielded **15** in 96% yield, then treatment with *p*-cinnamoylphenol and K_2CO_3 in DMF afforded the first target **1** in 53% yield. The reaction proceeded smoothly and could be monitored easily by virtue of both deacetylation and the fluorescent nature of the linker. For detailed confirmation of the structure of **1**, it was transformed into **16** for easier assignment by ^1H NMR spectroscopy.

2.3. Synthesis of the isoglobotriose chalcone conjugate 2

The synthesis of the target compound **2** followed exactly the same sequence as described for the synthesis of **1** (Scheme 3). Thus, treatment of **7** with **17**[27] afforded **18** in higher yield as expected (87%), due to the higher reactivity of the equatorial 3-OH group. The α-stereoselectivity was unambiguously determined by the ^1H NMR data of H-1_c (δ 5.16, $J_{1,2}$ 2.9 Hz) as well from the ^{13}C NMR value for C-1_c (δ 95.50). Subsequent deacetylation



Scheme 3. Reagents and conditions: (a) TMSOTf (6 mol %), Et_2O–CH_2Cl_2, −20 °C to rt, 87%; (b) NaOMe–MeOH, 76%; (c) 1: H_2, Pd–C, MeOH; 2: Ac_2O, Pyridine, 72%; (d) $N_2H_4{\cdot}HOAc$, DMF, 70%; (e) CCl_3CN, DBU, 82%; (f) 6-chloro-1-hexanol, TMSOTf (6 mol %), CH_2Cl_2, −45 °C to rt, 44%; (g) NaOMe–MeOH, quant.; (h) *p*-cinnamoylphenol, K_2CO_3, DMF, 77%; (i) Ac_2O, Pyridine, 91%.

followed by debenzylation and acetylation afforded **20** in good overall yield. Deacetylation of **18** removed easily traces of unreacted acceptor from the coupling step and was taken into account in estimating the yields of both **18** and **19**. Anomeric deacetylation followed by trichloroacetimidate activation gave the trisaccharide donor **22**,[15d] which was coupled with 6-chloro-1-hexanol to afford the β-linked conjugate **23** (44%). The β-anomeric configuration of **23** was unambiguously determined from the signal of the neighbouring H-2 proton at δ 4.86 ($J_{1,2}$ 8.0 Hz) because of overlapping of signals in the H-1_a region as in **14**. Deacetylation and condensation with *p*-cinnamoylphenol afforded the second target compound **2** in 77% yield. Compound **2** was similarly transformed into the acetylated derivative **25,** the structure of which has been completely assigned by both ^{1}H and ^{13}C NMR spectroscopy.

2.4. Purification of StxB using globotriose functionalized Thiopropyl Sepharose 6B

For immobilization of **1** onto Thiopropyl Sepharose 6B, commercially available as the 2-dithiopyridyl gel, thiopyridyl protecting groups were removed before loading into a FPLC column by thorough washing with 2-mercaptoethanol. Then, compound **1** was coupled to Sepharose in two steps. First, Sepharose was treated with **1** (17 mg) dissolved in absolute MeOH in the presence of $InCl_3$ (10 mol %), catalyst accelerator of thiol addition into chalcone derivatives.[19b] Second, to ensure maximum saturation of the column, the recovered compound **1** (10 mg) obtained by evaporation of the flow through, poorly soluble in water, was dissolved in DMF, before being diluted with phosphate buffer (1:1), to avoid precipitation. It was then incubated again with the same gel equilibrated with DMF–phosphate buffer (1:1) at pH 7.5. Unreacted thiol groups of Sepharose were then trapped by *N*-methylmaleimide to prevent disulfide-bond formation with the cysteine residue of the protein.[21a] Periplasmic extracts from bacteria expressing StxB were then passed over the column, and after washing, proteins were eluted with a linear guanidinium chloride gradient. The elution profile showed one large peak at 4.5 M gradient concentration of guanidinium chloride (Fig. 2A). The peak fractions were pooled, and the StxB analyzed by Western Blot with specific antibody (Fig. 2B, left) and by Coomassie blue staining of the gels (Fig. 2B, right). Identical amounts of total protein were loaded in lanes 2–8. The Western Blot allowed identifying the StxB containing fractions. Noteworthy, the column flow through contained only very little amount of StxB (lane 4), indicating that the column retained most of Stx. As shown by Coomassie blue staining, pools 5–7 all contained StxB with purity greater than 99%. For comparison, lane 8 contained the eluted protein from a MonoQ cation exchange column over which the crude periplasmic extract was passed once. The procedure allowed the purification of at least 0.75 mg of StxB from 1 mL of globotriose functionalized column material.

2.5. Coupling of conjugate 2 to the protein StxB/Cys

To demonstrate the ability of compound **2** to fix sulfhydryl containing proteins, we used a variant of StxB to which a Cys was added such as to avoid the formation of inter- and intra-chain disulfide bonds.[28] The coupling reaction was performed with different molar excess of compound **2** over StxB/Cys for 16 h in borate buffer at pH 9 and dialysis against deionized water, then analyzed by MALDI-TOFMS (Fig. 3). It can clearly be seen that, with increasing molar ratios, the coupling product becomes the major species. The ease of synthesis and purification of **2** besides the high coupling efficiency to protein may make this linker an addition to the already available thiol-reactive reagents. Thus, this method

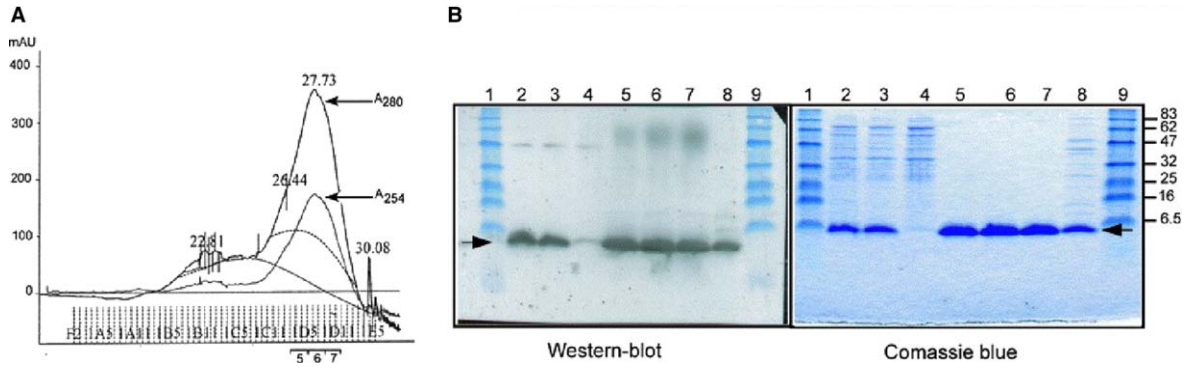


Figure 2. Purification of StxB. (A) Elution profile from globotriose functionalized Sepharose 6B loaded with periplasmic extracts from bacteria expressing StxB. The A_{280} line measures total protein, and the A_{254} line measures tyrosine absorbance (and reflects ribosomal RNA contamination). Fraction 3–5, 6–8 and 9–11 were sampled in pools 5, 6 and 7, respectively. (B) Western blotting and (C) Coomassie blue analysis of the following samples: 1 and 9: markers; 2: crude extract; 3: crude extract after ultracentrifugation at 100,000*g*/30 min; 4: column flow through; 5–7: fraction pools; 8: MonoQ fraction. Arrows indicate StxB.

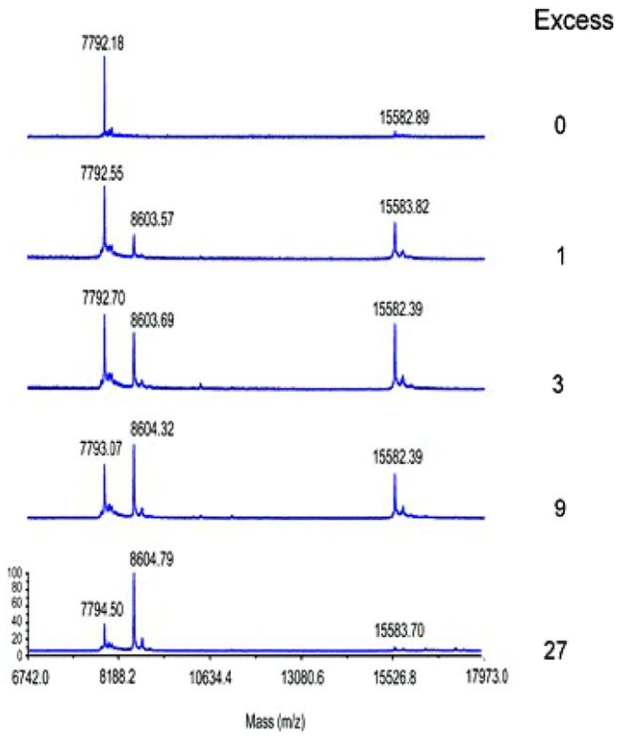


Figure 3. MALDI-TOF analysis of compound **2** coupling to StxB/Cys. The molar excess of compound **2** over StxB B-chain monomer is noted to the right. In the absence of compound **2**, StxB B-chain monomer is the only species at 7793 (theoretical mass: 7791). With increasing excess of compound **2**, the coupling product at 8604 becomes the major product. B-chain dimer was frequently detected at 15,582 as byproduct.

avoids the use of cross-coupling reagents and aminoalkyl glycosides for ligation of glycosides into cysteine-containing proteins[29] or thiol-coated surfaces used for preparation of artificial biosensors.[21a]

2.6. Conclusion

We described here a novel and convenient synthesis of globo- and isoglobotrioses, as well as their grafting to a thiol-reactive chalcone. It is noteworthy that the new glycosyl donor afforded stereospecifically globo- and isoglobotrioses in good yields. Moreover, ligation of the novel conjugates to Thiopropyl Sepharose as well as to a thiol-possessing model-protein was efficiently performed with the new chalcone based spacer. This constitute a valuable addition to methods, which already exist in this context for diverse bio-applications.

3. Experimental

3.1. General methods

Solvents were purified in the usual way. TLC was performed on plastic plates Silica Gel 60 F_{254} (E. Merck, layer thickness 0.2 mm). The detection was achieved by treatment with a soln of 20 g ammonium molybdate and 0.4 g cerium(IV) sulfate in 400 mL 10% H_2SO_4 or with 15% H_2SO_4, and heating at 150 °C. Flash chromatography was carried out on silica gel (Baker, 30–60 μm) or Lichroprep Si 60 (E. Merck; ϕ 15–25 μm), *type-B* silica column. Optical rotation was determined at 20 °C with a Perkin–Elmer 241/MC polarimeter (1 dm cell). NMR spectra were recorded with a Bruker AC 300 DRX instrument with Me_4Si as internal standard; J values are given in Hertz. The assignment of ^1H NMR (300 MHz) spectra was based on chemical shift correlation (DQFCOSY) while, the assignment of ^{13}C NMR (75 MHz) spectra were based on Carbon-Proton Shift-Correlation Heteronuclear Multiple Quantum Coherence (HMQC). Mass spectra were recorded with a MALDI-Kompakt (Kratos) equipment using α-cyano-3,5-dihydroxycinamic acid (CHCA), while FAB spectra were recorded with a Jeol MS700 apparatus at the Ecole Normale Supérieure (Paris). Microanalysis was performed in the Microanalysis Unit of CNRS at Lyon. Thiopropyl Separose 6B was provided from General Electric Amersham Bioscience and the column was equipped with AKTA Purifier 10-General Electric Amersham Bioscience.

3.2. 2-(Trimethylsilyl)ethyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (5)

A mixture of **3** (6.62 g, 12.0 mmol), EtSH (6.5 mL, 87.7 mmol) and *p*-TsOH (0.13 g, 0.70 mmol) in dry CH_2Cl_2 (10 mL) was stirred at rt. After 48 h, the mixture was neutralized with Et_3N, evaporated under diminished pressure and the residue was purified by flash chromatography (3.5:1 toluene–acetone) to yield **4** (5.42 g, 97%) as a colourless powder. R_f 0.22 in 3.5:1 toluene–acetone. The product from the last step was stirred with pyridine (80 mL) and Ac_2O (40 mL) for 24 h at rt, then co-evaporated with toluene under diminished pressure. A purification by flash chromatography (5:1 petroleum ether–EtOAc) furnished **5** (6.13 g, 94%) as a colourless oil; $[\alpha]_D$ +19.6 (*c* 0.3, $CHCl_3$); R_f 0.23 (5:1 petroleum ether–EtOAc); ^1H NMR ($CDCl_3$): δ 7.37–7.26 (m, 10H, 2Ph), 5.49 (d, 1H, $J_{3,4} = J_{4,5}$ 1.8 Hz, H-4), 4.90, 4.73 (2d, 2-H, J_{gem} 10.9 Hz, CH_2Ph), 4.75, 4.53 (2d, 2-H, J_{gem} 11.4 Hz, CH_2Ph), 4.40 (d, 1-H, $J_{1,2}$ 7.3 Hz, H-1), 4.22–4.11 (m, 2-H, H-6′, H-6), 4.06–3.97 (m, 1-H, –OCH*H*), 3.78–3.74 (m, 1-H, H-5), 3.66–3.54 (m, 3-H, –OC*H*H, H-2, H-3), 2.14, 2.07 (2s, 6-H, 2CH_3CO), 1.11–1.01 [m, 2-H, –CH_2Si(CH_3)$_3$], 0.03 [s, 9-H, –Si(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$): δ 170.52, 170.45 (2C=O), 138.64–127.57 (2Ph), 103.26 (C-1), 79.11, 78.89 (C-2, C-3), 76.57, 72.18, (2*C*H_2Ph), 70.56 (C-5), 67.81 (–OCH_2–), 66.50 (C-4), 62.00 (C-6), 20.89, 20.74 (2*C*H_3CO), 18.48 [*C*H_2Si(CH_3)$_3$], −1.48 [Si(CH_3)$_3$]; FABMS *m*/*z* 567.3 $[M+Na]^+$. Anal. Calcd for

$C_{29}H_{40}O_8Si$ (544.78): C, 63:93; H, 7.41. Found: C, 64.10; H, 7.43.

3.3. *O*-(4,6-Di-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosyl) trichloroacetimidate (7)

To a soln of **5** (6.07 g, 11.1 mmol) in anhyd CH_2Cl_2 (30 mL) was added dropwise TFA (70 mL) at rt with stirring. After 5 h, the mixture was co-evaporated with toluene under diminished pressure and dried. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to yield **6** (4.06 g, 82%) as a yellow oil. R_f 0.25 (1.5:1 petroleum ether–EtOAc). Compound **6** (0.89 g, 2.0 mmol) was stirred with CCl_3CN (1.5 mL, 15.0 mmol) and DBU (0.05 mL, 0.3 mmol) in dry CH_2Cl_2 (3.0 mL) at rt. After 5 h, the mixture was co-evaporated with EtOAc under diminished pressure and the residue was purified by flash chromatography (1:1 petroleum ether–EtOAc + 1% Et_3N) to yield **7** (1.11 g, 93%) as a yellow syrup α/β ≈ 11:1. R_f 0.79 (7α), 0.63 (7β) (1:1 petroleum ether–EtOAc + 1% Et_3N); ^1H NMR (300 MHz, $CDCl_3$): δ 8.68 (s, 0.08-H, NH_β), 8.61 (s, 0.92-H, NH_α), 7.30–7.26 (m, 10-H, 2Ph), 6.52 (d, 0.92-H, $J_{1,2}$ <1.0 Hz, H-1_α), 5.78 (d, 0.02-H, $J_{1,2}$ 8.1 Hz, H-1_β), 5.63 (d, 0.92-H, $J_{3,4} = J_{4,5}$ <1.0 Hz, H-4_α), 5.55 (d, 0.08-H, $J_{3,4} = J_{4,5}$ <1.0 Hz, H-4_β), 4.85–4.74 (m, 3-H, 1.5CH_2Ph), 4.58 (d, 1-H, J_{gem} 11.6 Hz, C*H*HPh), 4.33 (m, 1-H, H-5), 4.15 (m, 1-H, H-6′), 4.11–3.96 (m, 3-H, H-6, H-3, H-2), 2.17, 2.07 (2s, 0.48-H, $2CH_3CO$), 2.13, 2.03 (2s, 5.52-H, $2CH_3CO_\alpha$).

3.4. Benzyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9)

Compound **8** (1.4 g, 1.4 mmol) in dry 1:1 CH_2Cl_2–Et_2O (6 mL) was stirred with 0.02 M TMSOTf in CH_2Cl_2 (6.5 mL) under argon at −25 °C while compound **7** (1.5 equiv, 1.25 g, 2.16 mmol) in anhyd CH_2Cl_2 (4.0 mL) was added dropwise. After 1.5 h, the mixture was allowed to warm up to rt over 30 min, then diluted with CH_2Cl_2 (100 mL), washed with satd $NaHCO_3$ (2 × 25 mL), dried over $MgSO_4$ and evaporated under diminished pressure. The residue was purified by flash chromatography (3.5:1 petroleum ether–EtOAc) to yield **9** (1.63 g, 81%) as a yellow syrup. Traces of unreacted acceptor, easily removed after deacetylation of **9** (next step), were taken into account for the calculation of the yield. $[\alpha]_D$ +42.9 (*c* 0.55, $CHCl_3$); R_f 0.22 (3.5:1 petroleum ether–EtOAc); ^1H NMR ($CDCl_3$): δ 7.40–7.18 (m, 45-H, 9Ph), 5.57 (d, 1-H, *J* 1.6 Hz, H-4_c), 5.14 (d, 1-H, $J_{1,2}$ 3.1 Hz, H-1_c), 5.10 (d, 1-H, J_{gem} 11.1 Hz, CH*H*Ph), 5.00 (d, 1-H, J_{gem} 12.1 Hz, CH*H*Ph), 4.95–4.63 (m, 14-H, 7CH_2Ph), 4.60–4.52 (m, 3-H, H-5_c, H-1_a, H-1_b), 4.44 (d, 1-H, J_{gem} 12.1 Hz, CH*H*Ph), 4.32–3.34 (m, 17-H), 2.09, 2.06 (2s, 6-H, $2CH_3CO$); ^{13}C NMR ($CDCl_3$): δ 170.22–170.13 ($2CH_3C{=}O$), 139.03–137.45 (m, 9-ArC), 128.82–127.27 (m, 45-ArCH), 102.80, 102.38 (C-1_a, C-1_b), 100.25 (C-1_c), 82.47–66.60 (m, 30-C), 20.80, 20.65 (2-CH_3CO); FAB+HRMS: Calcd for $C_{85}H_{90}O_{18}$, $[M+Na]^+$ 1421.6006. Found: *m*/*z* 1421.6025.

3.5. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranose (11)

A mixture of **9** (1.52 g, 1.1 mmol) and NaOMe (0.57 g, 10.5 mmol) in dry MeOH (150 mL) was stirred at room temperature for 48 h, then neutralized with Amberlite IR-resin (H^+ form), filtered and evaporated under diminished pressure. The residue was purified by flash chromatography (1.5:1 petroleum ether–EtOAc) to afford **10** (1.41 g, 98%) as a colourless syrup. R_f 0.23 (1.5:1 petroleum ether–EtOAc). This was dissolved in dry MeOH (60 mL) and stirred with 10% Pd–C (0.4 g) under H_2 at rt for 48 h then filtered through Celite, washed with (1:1 MeOH–water), evaporated under diminished pressure and dried well. The residue was stirred with pyridine (50 mL) and Ac_2O (25 mL) at rt for 48 h, then co-evaporated with toluene under diminished pressure. The residue was purified by flash chromatography (3:1 toluene–acetone) to afford **11** (1.03 g, 89%) as colourless foam. R_f 0.42 (2:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 6.21 (d, 0.75-H, $J_{1,2}$ 3.6 Hz, H-$1_{a\alpha}$), 5.65 (d, 0.25-H, $J_{1,2}$ 8.2 Hz, H-$1_{a\beta}$), 5.55 (d, 1-H, *J* <1.0 Hz, H-4_c), 5.44 (dd, 0.75-H, $J_{2,3}$ 10.1, $J_{3,4}$ 9.2 Hz, H-$3_{a\alpha}$), 5.37 (m, 1-H, H-3_c), 5.18–4.98 (m, 4.25-H, H-$3_{a\beta}$, H-2_c, H-2_b, H-$2_{a\alpha}$, H-$2_{a\beta}$, H-1_b), 4.97 (d, 1-H, $J_{1,2}$ 3.4 Hz, H-1_c), 4.71 (m, 1-H, H-3_b), 4.51–4.39 (m, 3-H, H-$6'_{a\beta}$, H-$6'_{a\alpha}$, H-$6'_b$, H-5_c), 4.15–4.06 (m, 4-H, H-$6_{a\beta}$, H-$6_{a\alpha}$, H-$6'_c$, H-6_c, H-6_b), 3.40 (m, 0.75-H, H-$5_{a\alpha}$), 3.99 (d, 1-H, *J* <1.0 Hz, H-4_b), 3.82–3.72 (m, 2.25-H, H-5_b, H-$4_{a\alpha}$, H-$5_{a\beta}$, H-$4_{a\beta}$), 2.14–1.95 (sev. s, 33-H, $11CH_3CO$); ^{13}C NMR ($CDCl_3$): δ 170.57–168.74 (11-CH_3CO), 101.09 (C-$1_{b\alpha}$), 100.85 (C-$1_{b\beta}$), 99.52 (C-1_c), 91.46 (C-1_a), 88.91 (C-$1_{a\alpha}$), 76.82 (C-4_b), 73.37–71.80 (C-3_b, C-4_a, C-5_b, C-$5_{a\beta}$), 70.57–67.00 (C-$5_{a\alpha}$, C-3_c, C-2_c, C-2_b, C-3_a, C-2_a, C-4_c, C-5_c), 61.81–60.17 (C-6_b, C-6_c, C-6_a), 21.34–20.41 (11-CH_3CO); FAB+HRMS: Calcd for $C_{43}H_{50}O_{25}$, $[M+Na]^+$ 989.2750. Found: *m*/*z* 989.2735.

3.6. *O*-[2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tetra-*O*-acetyl-α-D-glucopyranosyl] trichloroacetimidate (13)

Compound **11** (0.93 g, 0.96 mmol) was treated with hydrazinium acetate (0.12 g, 1.3 mmol) in anhyd DMF (3 mL), then co-evaporated with toluene and water under diminished pressure. The residue was purified by

flash chromatography (2.5:1 toluene–acetone) to yield **12** (0.82 g, 92%) as a colourless foam. R_f 0.13 (2.5:1 toluene–acetone). A mixture of **12** (0.29 g, 0.31 mmol) and CCl_3CN (1.0 mL, 10.0 mmol) in dry CH_2Cl_2 (1 mL) was stirred at rt in the presence of DBU (0.05 mL, 0.3 mmol) for 5 h, then co-evaporated with EtOAc under diminished pressure. The residue was purified by flash chromatography (10:1 EtOAc–toluene + 1% Et_3N) to yield **13** (0.27 g, 80%) as a yellow foam. R_f 0.64 (10:1 EtOAc–toluene + 1% Et_3N); ^1H NMR ($CDCl_3$): δ 8.64 (s, 1-H, NH), 6.48 (d, 1-H, $J_{1,2}$ 3.8 Hz, H-1_a), 5.59 (dd, 1-H, $J_{3,4}$ 3.1, $J_{4,5}$ 3.5 Hz, H-4_c), 3.56 (dd, 1-H, $J_{2,3}$ 9.7, $J_{3,4}$ 9.3 Hz, H-3_a), 5.40 (dd, 1-H, $J_{2,3}$ 11.0, $J_{3,4}$ 3.1 Hz, H-3_c), 5.20 (dd, 1-H, $J_{1,2}$ 3.5, $J_{2,3}$ 11.0 Hz, H-2_c), 5.13–5.05 (m, 2-H, H-2_b, H-2_a), 4.99 (d, 1-H, $J_{1,2}$ 7.8 Hz, H-1_b), 4.50–4.42 (m, 3-H, H-$6'_a$, H-5_c, H-$6'_b$), 4.21–4.11 (m, 5-H, H-5_a, H-$6'_c$, H-6_c, H-6_a, H-6_b), 4.02 (dd, 1-H, $J_{3,4} \approx J_{4,5}$ 2.3 Hz, H-4_b), 3.87 (dd, 1-H, $J_{3,4}$ 9.7, $J_{4,5}$ 9.3 Hz, H-4_a), 3.77 (m, 1-H, H-5_b), 2.17–1.98 (sev. s, 33-H, 11CH_3CO).

3.7. 6-Chloro-*n*-hexyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (14)

Compound **13** (0.26 g, 0.24 mmol) in dry CH_3CN (1.5 mL) was added dropwise, under Ar, to 6-chloro-1-hexanol (0.2 g, 1.5 mmol) in CH_3CN (1 mL) and TMSOTf (0.02 M in CH_3CN, 0.76 mL) at −25 °C under stirring, then allowed to reach rt (2.5 h). The mixture was diluted with EtOAc (50 mL), washed with satd $NaHCO_3$ (25 mL), dried over $MgSO_4$ and evaporated under diminished pressure. The residue was purified by flash chromatography (4:1 toluene–acetone) to yield **14** (0.14 g, 54%) as a yellow oil. $[\alpha]_D$ +33.3 (*c* 0.3, $CHCl_3$); R_f 0.15 (4:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 5.58 (d, 1-H, $J_{3,4} = J_{4,5}$ 2.2 Hz, H-4_c), 5.38 (dd, 1-H, $J_{2,3}$ 10.9, $J_{3,4}$ 3.2 Hz, H-3_c), 5.23–5.15 (m, 2-H, H-3_a, H-2_c), 5.10 (dd, 1-H, $J_{1,2}$ 7.7, $J_{2,3}$ 10.9 Hz, H-2_b), 4.98 (d, 1-H, $J_{1,2}$ 3.4 Hz, H-1_c), 4.88 (dd, 1-H, $J_{1,2}$ 8.1, $J_{2,3}$ 9.1 Hz, H-2_a), 4.72 (dd, 1-H, $J_{2,3}$ 10.9, $J_{3,4}$ 1.8 Hz, H-3_b), 4.52–4.40 (m, 5-H, H-1_b, H-5_c, H-1_a, H-$6'_b$, H-$6'_a$), 4.19–4.06 (m, 4-H, H-$6'_c$, H-6_c, H-6_a, H-6_b), 4.00 (d, 1-H, $J_{3,4} = J_{4,5} < 1.0$ Hz, H-4_b), 3.87–3.73 (m, 3-H, –OCH*H*, H-4_a, H-5_b), 3.62 (m, 1-H, H-5_a), 3.56–3.42 (m, 3-H, 1.5 –CH_2–), 2.14–1.69 (m, 30-H, 10CH_3CO), 1.75–1.13 [2m, 8-H, –$(CH_2)_4$–]; ^{13}C NMR ($CDCl_3$): δ 168.87–170.68 (10C=O), 100.53 (C-1_a), 101.11 (C-1_b), 99.63 (C-1_c), 76.91, 76.48, 73.11, 72.79, 72.51, 71.79, 69.86, (C-2_a, C-4_a, C-5_b, C-5_a, C-3_b, C-3_a, C-4_b, –OCH_2–), 69.84, 68.98, 68.83, 67.89, 67.06, (C-5_c, C-3_c, C-4_c, C-2_b, C-2_c), 62.21, 61.32, 60.36 (C-6_c, C-6_a, C-6_b), 44.91 (–CH_2Cl), 32.44, 29.21, 26.47, 25.10 [–$(CH_2)_4$–], 20.90, 20.48 (10*C*H_3CO); FAB+HRMS: Calcd for $C_{44}H_{63}ClO_{26}$, $[M+Na]^+$ 1065.3194 and 1067.3197. Found: *m*/*z* 1065.3132 and 1067.3148.

3.8. 6-(4-Cinnamoylphenoxy)-hexyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (1)

A mixture of **14** (0.13 g, 0.12 mmol) and NaOMe (5.38 M, 50 μL) in dry MeOH (10 mL) was stirred at rt overnight, then neutralized with Amberlite IR 120 resin (H^+-form) and concentrated under diminished pressure to yield **15** (74 mg, 96%) as an amorphous mass. R_f 0.29 (6:3:1 EtOAc–*iso*-PrOH–H_2O). Compound **15** (0.13 g, 0.2 mmol) was stirred with *p*-cinnamoylphenol (1.0 g, 0.42 mmol) and K_2CO_3 (60 mg, 0.43 mmol) in dry DMF (1.0 mL) at 95–100 °C. After 24 h, the mixture was diluted with MeOH, acidified with glacial AcOH (0.2 mL) and co-evaporated with toluene under diminished pressure. The residue was purified by flash chromatography *type-B* silica (6:3:1 EtOAc–*i*-PrOH–water) to yield **1** (90 mg, 53%) as a yellowish white solid. $[\alpha]_D$ +30.9 (*c* 0.11, $CHCl_3$); R_f 0.23 (6:3:1 EtOAc–*i*-PrOH–water); ^1H NMR ($CDCl_3$): δ 8.11–6.76 (m, 11-H, 9Ar, —CH=CH—), 4.94 (d, 1-H, $J_{1,2}$ 3.4 Hz, H-1_c), 4.41 (d, 1-H, $J_{1,2}$ 7.3 Hz, H-1), 4.28 (d, 1-H, $J_{1,2}$ 7.8 Hz, H-1), 4.25–3.20 (m, 22-H), 1.83–1.50 (m, 8-H, 4-CH_2–); (MALDI+MS) Calcd for $C_{39}H_{54}O_{18}$ (810.3310). Found: *m*/*z* 833.7470 $[M+Na]^+$, 849.7293 $[M+K]^+$.

3.9. 6-(4-Cinnamoylphenoxy)-hexyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (16)

A mixture of **1** (9 mg, 11 μmol), pyridine (6 mL) and Ac_2O (3 mL) was stirred at rt for 48 h, then co-evaporated with toluene under diminished pressure. The residue was purified by flash chromatography (3:1 toluene–acetone) to yield **16** (12.0 mg, 88%) as a colourless syrup. R_f 0.32 (3:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 8.04–6.95 (m, 11-H, 9Ar, —CH=CH—), 5.58 (d, 1-H, $J_{3,4} = J_{4,5}$ 3.2 Hz, H-4_c), 5.38 (dd, 1-H, $J_{2,3}$ 11.1, $J_{3,4} = 3.3$ Hz, H-3_c), 5.36–5.15 (m, 2-H, H-3_a, H-2_c), 5.10 (dd, 1-H, $J_{1,2}$ 7.7, $J_{2,3}$ 10.9 Hz, H-2_b), 4.97 (d, 1-H, $J_{1,2}$ 3.4 Hz, H-1_c), 4.88 (dd, 1-H, $J_{1,2}$ 7.8, $J_{2,3}$ 9.4 Hz, H-2_a), 4.72 (dd, 1-H, $J_{2,3}$ 10.9, $J_{3,4}$ 2.4 Hz, H-3_b), 4.52–4.40 (m, 5-H, H-1_b, H-5_c, H-1_a, H-$6'_b$, H-$6'_a$), 4.19–3.97 (m, 7-H, H-4_b, H-$6'_c$, H-6_c, H-6_a, H-6_b, –OCH_2), 3.87–3.73 (m, 3-H, H-4_a, H-5_b, –OCH*H*–), 3.63 (m, 1-H, H-5_a), 3.62 (m, 1-H, H-5_a), 3.47 (m, 1-H, –OC*H*H–), 2.17–1.98 (m, 30-H, 10CH_3CO), 1.61–1.06 [m, 8-H, –$(CH_2)_4$–].

3.10. Benzyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (18)

Compound **17** (1.42 g, 1.4 mol) was treated with **7** (1.2 g, 2.0 mmol) in the presence of TMSOTf (0.02 M in

CH_2Cl_2, 6.6 mL), then worked up exactly as described for **9**. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to yield **18** (1.78 g, 87%) as a light yellow oil. Traces of unreacted acceptor, easily removed after deacetylation of **18** (next step), were taken into account for the calculation of the yield. $[\alpha]_D$ +55.2 (*c* 0.7, $CHCl_3$); R_f 0.15 (3:1 petroleum ether–EtOAc); ^1H NMR ($CDCl_3$): δ 7.32–7.07 (m, 45-H, 9Ph), 5.16 (d, 1-H, $J_{1,2}$ 2.9 Hz, H-1_c), 5.14 (d, 1-H, $J_{3,4} = J_{4,5}$ <1.0 Hz, H-4_c), 5.04 (d, 1-H, J_{gem} 10.6 Hz, 0.5CH_2Ph), 5.01 (d, 1-H, J_{gem} 10.0 Hz, 0.5CH_2Ph), 4.93 (d, 1-H, J_{gem} 12.0 Hz, 0.5CH_2Ph), 4.90 (d, 1-H, J_{gem} 10.7 Hz, 0.5CH_2Ph), 4.83 (d, 1-H, J_{gem} 11.4 Hz, 0.5CH_2Ph), 4.79–4.59 (m, 8-H, 4CH_2Ph), 4.51–4.34 (m, 4-H, H-1_a, H-1_b, CH_2Ph), 4.40–4.23 (m, 4-H, H-5_c, 1.5CH_2Ph), 4.05–3.75, 3.67–3.32 (m, 13-H), 2.09, 1.91 (2s, 6-H, 2CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.38 (C═O), 139.19–127.06 (9Ph), 102.94, 102.48 (C-1_a, C-1_b), 95.49 (C-1_c), 82.94–62.23 (24C), 20.94, 20.92 (2CH_3CO); FAB+HRMS: Calcd for $C_{85}H_{90}O_{18}$, $[M+Na]^+$1421.6025. Found: *m*/*z* 1421.6060.

3.11. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*O*-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-acetyl-D-glucopyranose (20)

Compound **18** (1.77 g, 1.3 mmol) was treated with NaOMe and worked up as described for the synthesis of **10**. Flash chromatography (1:1 petroleum ether–EtOAc) yielded **19** (1.26 g, 76%). Treatment of **19** (1.2 g, 0.9 mmol) with Pd–C, followed by acetylation as described for the synthesis of **11** and purification by flash chromatography (2.5:1 toluene–acetone) gave **20** (0.64 g, 72%) as a colourless foam. R_f 0.29 (2.5:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 6.22 (d, 0.5-H, $J_{1,2}$ 3.6 Hz, H-$1_{a\alpha}$), 5.65 (d, 0.5-H, $J_{1,2}$ 8.2 Hz, H-$1_{a\beta}$), 5.47–3.74 (m, 20-H), 2.17–1.92 (sev. s, 33-H, 11CH_3CO). These ^1H NMR data are in good agreement with the published data.[15d]

3.12. (2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-*O*-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl) trichloroacetimidate (22)

Compound **20** (0.17 g, 0.17 mmol) was treated with hydrazinium acetate, then worked up as described for the synthesis of **12**. The residue was purified by flash chromatography (2.5:1 toluene–acetone) to yield **21** (0.11 g, 70%) as a colourless foam R_f 0.31 (2:1 toluene–acetone), which was next stirred with CCl_3CN (0.8 mL, 8.0 mmol) in dry CH_2Cl_2 (1 mL) at rt for 4 h, then co-evaporated with EtOAc under diminished pressure. The residue was purified by flash chromatography (10:1 EtOAc–toluene + 1% Et_3N) to yield **22** (0.11 g, 82%) as a yellow foam. R_f 0.64 (10:1 + 1% Et_3N EtOAc–toluene); ^1H NMR ($CDCl_3$): δ 8.65 (s, 1-H, NH), 6.19 (d, 1-H, $J_{1,2}$ 3.9 Hz, H-1_a), 5.56 (dd, 1-H, $J_{2,3}$ 10.0, $J_{3,4}$ 9.5 Hz, H-3_a), 5.44 (d, 1-H, $J_{3,4}$ 2.2, $J_{4,5}$ <1.0 Hz, H-4_c), 5.33 (d, 1-H, $J_{3,4} = J_{4,5}$ 2.8 Hz, H-4_b), 5.28–5.24 (m, 2-H, H-2_c, H-1_c), 5.18 (dd, 1-H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.1 Hz, H-2_b), 5.09 (dd, 1-H, $J_{2,3}$ 10.4, $J_{3,4}$ 2.2 Hz, H-3_c), 4.93 (dd, 1-H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.0 Hz, H-2_a), 4.48–4.43 (m, 2-H, H-$6'_a$, H-1_b), 4.26–4.02 (m, 7-H, H-5_c, H-6_c, H-$6'_c$, H-6_a, H-6_b, H-$6'_b$, H-5_b), 3.85–3.79 (m, 3-H, H-3_b, H-4_a, H-5_a), 2.16–1.94 (sev. s, 30-H, 10CH_3CO); ^{13}C NMR ($CDCl_3$): δ 170.41–168.63 (10CH_3CO), 100.75 (C-1_b), 93.43 (C-1_c), 89.92 (C-1_a), 74.98–61.20 (18C), 20.83–20.63 (10CH_3CO).

3.13. 6-Chlorohexyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,3,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (23)

To compound **22** (0.43 g, 0.4 mmol) in dry CH_3CN (3 mL) were added 6-chloro-1-hexanol (0.4 g, 3 mmol) and TMSOTf (0.02 M in CH_3CN, 1.3 mL) at −25 °C. The soln was then stirred and worked up as described for the synthesis of **14**. The residue was purified by flash chromatography on Type-B Silica Gel E. Merck (6:1 then 5:1 toluene–acetone) to yield **23** (0.18 g, 44%) as a colourless foam. $[\alpha]_D$ +40.0 (*c* 0.1, $CHCl_3$); R_f 0.15 (5:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 5.43 (dd, 1-H, $J_{3,4} = J_{4,5}$ 2.5 Hz, H-4_c), 5.30 (dd, 1-H, $J_{3,4} = J_{4,5}$ 2.2 Hz, H-4_b), 5.26–5.11 (m, 4-H, H-3_a, H-2_b, H-2_c, H-1_c), 5.07 (dd, 1-H, $J_{2,3}$ 10.2, $J_{3,4}$ 2.5 Hz, H-3_c), 4.86 (dd, 1-H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.5 Hz, H-2_a), 4.48–4.38 (m, 3-H, H-$6'_a$, H-1_a, H-1_b), 4.20–4.00 (m, 6-H, H-6_b, H-$6'_b$, H-5_c, H-6_a, H-6_c, $6'_c$-H), 3.85–3.73 (m, 4-H, H-5_b, H-4_a, H-3_b, –C*H*–) 3.63–3.41 (m, 4-H, H-5_a, 1.5 –CH_2–), 2.15–1.93 (sev. s, 30-H, 10CH_3CO), 1.76–1.24 (m, 8-H, 4-CH_2–); ^{13}C NMR ($CDCl_3$); δ 170.63–168.71 (10CH_3CO), 101.10 (C-1_b), 100.51 (C-1_a), 93.40 (C-1_c), 67.10–60.97 (16C), 44.92 (CH_2Cl), 32.44, 29.20, 26.46, 25.09 (4-CH_2–), 20.79–20.41 (10CH_3CO); FAB+HRMS: Calcd for $C_{44}H_{63}ClO_{26}$, $[M+Na]^+$ 1065.3194 and 1067.3197. Found: *m*/*z* 1065.3132 and 1067.3179.

3.14. 6-(4-Cinnamoylphenoxy)-hexyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2)

To a soln of compound **23** (0.17 g, 0.16 mmol) in MeOH (10 mL) was added NaOMe (5.38 M, 60 μL). The mixture was stirred at rt overnight. After neutralization with IR 120Amberlite resin (H+ form), **24** (0.1 g, quant) was obtained as an amorphous yellowish residue. (MALDI+MS) Calcd for $C_{24}H_{43}ClO_{16}$ (623.12). Found: *m*/*z* 645.1946, 647.1907 $[M+Na]^+$; 661.2392, 663.1588 $[M+K]^+$. The residue from the last step was treated with 4-cinnamoylphenol then worked up and purified exactly under the same condition used for synthesis of **1** to yield **2** (0.1 g, 77%) as amorphous colourless mass. $[\alpha]_D$ +42.4

(*c* 0.62, $CHCl_3$); R_f 0.31 (6:3:1 EtOAc–^{iso}PrOH–H_2O); ^1H NMR (CD_3OD): δ 8.03–7.03 (m, 11-H, Ph, Ar, —CH=CH—), 5.03 (d, 1-H, $J_{1,2}$ 1.0 Hz, H-1_c), 4.41 (d, 1-H, $J_{1,2}$ 7.3 Hz, H-1), 4.29 (d, 1-H, $J_{1,2}$ 7.8 Hz, H-1), 4.22 (t, 1-H, $J_{5,6}$, 6.1 Hz), 4.09 (t, 2-H, *J* 6.3 Hz, –OCH_2–), 4.03 (dd, 1-H, $J_{3,4} \approx J_{4,5}$ <1.0 Hz, H-4), 3.92–3.49 (m, 16-H), 3.39 (m, 1-H, H-5), 3.24 (dd, 1-H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.4 Hz, H-2), 1.83 (t, 2-H, *J* 6.7 Hz, –CH_2–), 1.67 (t, 2-H, *J* 6.5 Hz, –CH_2–), 1.5 (m, 4-H, 2(–CH_2–)); ^{13}C NMR (CD_3OD): δ 190.75 (C=O), 164.97–115.55 (Ar, —CH=CH—), 10511, 104.24 (C-1a, C-1b), 97.75 (C-1c), 81.00–58.33 (17C), 30.69, 30.18, 26.90, 26.83 (4-CH_2–); MALDI+MS: *m*/*z* 833.2652 $[M+Na]^+$, 849.2392 $[M+K]^+$.

3.15. 6-(4-Cinnamoylphenoxy)-hexyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (2)

Compound **23** (0.17 g, 0.16 mmol) was treated with NaOMe, then worked up as described for the synthesis of **15** to yield **24** (0.1 g) as an amorphous yellowish residue. (MALDI+MS) Calcd for $C_{24}H_{43}ClO_{16}$ (623.12). Found: *m*/*z* 645.1946, 647.1907 $[M+Na]^+$; 661.2392, 663.1588 $[M+K]^+$. The residue from the last step was treated with 4-cinnamoylphenol, then worked up and purified exactly under the same conditions as that used for the synthesis of **1** to yield **2** (0.1 g, 77%) as an amorphous colourless mass. $[\alpha]_D$ +42.4 (*c* 0.62, $CHCl_3$); R_f 0.31 (6:3:1 EtOAc–^{iso}PrOH–H_2O); ^1H NMR (CD_3OD): δ 8.03–7.03 (m, 11-H, Ph, Ar, —CH=CH—), 5.03 (d, 1-H, $J_{1,2}$ 1.0 Hz, H-1_c), 4.41 (d, 1-H, $J_{1,2}$ 7.3 Hz, H-1), 4.29 (d, 1-H, $J_{1,2}$ 7.8 Hz, H-1), 4.22 (t, 1-H, $J_{5,6}$, 6.1 Hz), 4.09 (t, 2-H, *J* 6.3 Hz, –OCH_2–), 4.03 (dd, 1-H, $J_{3,4} \approx J_{4,5}$ <1.0 Hz, H-4), 3.92–3.49 (m, 16-H), 3.39 (m, 1-H, H-5), 3.24 (dd, 1-H, $J_{1,2}$ 7.8, $J_{2,3}$ 8.4 Hz, H-2), 1.83 (t, 2-H, *J* 6.7 Hz, –CH_2–), 1.67 (t, 2-H, *J* 6.5 Hz, –CH_2–), 1.5 (m, 4-H, 2(–CH_2–)); ^{13}C NMR (CD_3OD): δ 190.75 (C=O), 164.97–115.55 (Ar, —CH=CH—), 10511, 104.24 (C-1a, C-1b), 97.75 (C-1c), 81.00–58.33 (17C), 30.69, 30.18, 26.90, 26.83 (4-CH_2–); (MALDI+MS) Calcd for $C_{39}H_{54}O_{18}$ (810.3310). Found: *m*/*z* 833.2652 $[M+Na]^+$, 849.2392 $[M+K]^+$.

3.16. 6-(4-Cinnamoylphenoxy)-hexyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (25)

Compound **2** (24 mg, 2.9 mmol) was acetylated and worked up as described for the synthesis of **16**. The residue was purified by flash chromatography (3:1 toluene–acetone) to yield **25** (33 mg, 91%) as a yellowish foam. R_f 0.5 (3:1 toluene–acetone); ^1H NMR ($CDCl_3$): δ 8.03–6.94 (m, 11-H, Ph, Ar, —CH=CH—), 5.43 (dd, 1-H, $J_{3,4}$ 2.9, $J_{4,5}$ 2.1 Hz, H-4_c), 5.31 (dd, 1-H, $J_{3,4} = J_{4,5}$ 2.1 Hz, H-4_b), 5.12–5.27 (m, 4-H, H-2_b, H-3_a, H-2_c, H-1_c), 5.08 (dd, 1-H, $J_{2,3}$ 10.5, $J_{3,4}$ 2.9 Hz, H-3_c), 4.87 (dd, 1-H, $J_{1,2}$ 8.0, $J_{2,3}$ 9.4 Hz, H-2_a), 4.46–4.39 (m, 3-H, H-$6'_a$, H-1_a, H-1_b), 4.21–4.00 (m, 8-H, H-5_c, H-6_c, H-$6'_c$, H-6_a, H-6_b, H-$6'_b$, –OCH_2–), 3.88–3.73 (m, 4-H, H-4_a, H-3_b, H-5_b, –OC*H*–), 3.60 (m, 1-H, H-5_a), 3.46 (m, 1-H, –OC*H*–), 2.15–1.93 (several s, 30-H, $10CH_3CO$), 1.79–124 (m, 8-H, 4-CH_2–); ^{13}C NMR ($CDCl_3$): δ 188.64 (–*C*O—CH=CH—), 170.35–168.0 ($10CH_3CO$), 162.94–142.87 (–HC=CH–), 135.04–114.23 (Ph, Ar), 101.08 (C-1_b), 100.50 (C-1_a), 93.27 (C-1_c) 76.58–60.93 (20C), 30.89–25.55 (4-CH_2–), 20.78–20.45 ($10CH_3CO$).

3.17. Coupling of 6-(*p*-cinnamoylphenoxy)hexyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl(1→4)-β-D-glucopyranoside 1 to thiopropyl Sepharose 6B

Thiopropyl Sepharose 6B (1 g) was suspended in water (200 mL), filtered over sintered glass, then suspended again in a soln containing 2-mercaptoethanol (0.5 M), $NaHCO_3$ (0.3 M, pH 8.3) and disodium EDTA (1 mM), and mixed gently for 40 min at rt. The gel was filtered on a sintered glass then washed thoroughly with AcOH (0.1 M, 400 mL) containing NaCl (0.5 M) and disodium EDTA (1 mM) to remove 2-mercaptopyridine. The gel (1 mL) was transferred into a C10/10 column, washed with water (20 mL), MeOH (20 mL) then gently mixed for 4 h at rt with a soln of **1** (17 mg) in MeOH (2 mL) containing $InCl_3$ (10 mol %). The gel (1 mL) was eluted and the eluent was evaporated under diminished pressure to recover the non-coupled **1** (10 mg), which was taken up in DMF (1 mL) and diluted with phosphate buffer (pH 7.4, 1 mL). The column was equilibrated by washing with (20 mL) of (1:1 DMF–phosphate buffer), then incubated with the above-mentioned soln of **1** overnight at 45 °C then drained. The unreacted thiol groups on the gel were capped by gentle mixing with *N*-methylmaleimide (1%) in a phosphate buffer at pH 6.8 for 2 h at rt. The column was drained and equilibrated with PBS at pH 7.5 and a periplasmic extract of *E. coli* strain BH 101 (10 mL) was run through it at a rate of 0.1 mL/min then washed with a phosphate buffer containing NaCl (0.5 M, 5 mL). The attached protein was eluted at a rate of 0.1 mL/min with a linear gradient ranging from 0 to 6 M of guanidinium chloride and the protein in the eluted fractions (250 μL/fraction) was monitored by absorbance at 280 and 254 nm. Fractions corresponding to the peaks in the elution profile were dialyzed against PBS and analyzed on a PAGE gel and by WB.

3.18. Coupling of StxB-cys with 2

StxB-cys (23 μg) in borate buffer pH 9 (20 mM, 9 μL) including NaCl (150 mM) was incubated for 16 h at rt at four different concentrations (2.34, 7.10, 21.32 and 63.96 mg/mL) of compound **2** suspended in Me_2SO

(1 μL) corresponding to (1:1, 1:3, 1:9 and 1:27) protein to **2**. The mixture was dialyzed against deionized water (2 L) to remove uncoupled **2** and the residue including the glycoprotein was analyzed by MALDI-TOFMS.

Acknowledgements

Our work was supported by grants from Institut Curie (PIC Vectorization), Fondation de France, and Association pour la Recherche sur le Cancer (no. 3105 and 5177). We are particularly grateful to Lucien Cabanié and Wolfgang Faigle for technical help.

References

1. Makita, A.; Taniguchi, N. In *Glycolipids*; Wiegandt, H., Ed.; New Comprehensive Biochemistry; Elsevier: Amsterdam, 1985; Vol. 10, pp 20–23.
2. Gariepy, J. *Crit. Rev. Oncol. Hematol.* **2001**, *39*, 99–106.
3. O'Brien, A. D.; Holmes, R. K. *Microbiol. Rev.* **1987**, *51*, 206–220.
4. Neild, G. H. Haemolytic uraemic syndrome. In *Oxford Textbook of Medicine*; Weatherall, D. J., Ledingham, J. G. G., Warrell, D. A., Eds., 3rd ed.; Oxford University Press: New York, NY, 1996; pp 3196–3202.
5. (a) Ratcliffe, R. M.; Kamath, V. P.; Yeske, R. E.; Gregson, J. M.; Fang, Y. R.; Palcic, M. M. *Synthesis* **2004**, *14*, 2293–2296, and references cited therein; (b) Ratcliffe, R. M.; Fang, Y. R.; Palcic, M. M. *Carbohydr. Res.* **2004**, *339*, 1141–1146.
6. Chen, Y.; Zhang, W.; Chen, X.; Wang, J.; Wang, P. G. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1716–1722, and references cited therein.
7. Zhou, D.; Mattner, J.; Cantu, C., III; Schrantz, N.; Yin, N.; Gao, Y.; Sagiv, Y.; Hudspeth, K.; Wu, Y.-P.; Yamashita, T.; Teneberg, S.; Wang, D.; Proia, R. L.; Levery, S. B.; Savage, P. B.; Teyton, L.; Bendelac, A. *Science* **2004**, *306*, 1786–1789.
8. Hansen, H. C.; Magnusson, G. *Carbohydr. Res.* **1998**, *307*, 233–242.
9. Kitov, P. I.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 838–853.
10. (a) Gargano, J. M.; Ngo, T.; Kim, J. Y.; Acheson, D. W. K.; Lees, W. J. *J. Am. Chem. Soc.* **2001**, *123*, 12909–12910; (b) Uzawa, H.; Ito, H.; Izumi, M.; Tokuhisa, H.; Taguchi, K.; Minoura, N. *Tetrahedron* **2005**, *61*, 5895–5905.
11. Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.-ichiro; Ito, S.; Mori, H.; Kobayashi, K. *Org. Lett.* **2002**, *4*, 355–357, and references cited therein.
12. (a) Ishitoya, S.; Kurazono, H.; Nishiyama, H.; Nakamura, E.; Kamoto, T.; Habuchi, T.; Terai, A.; Ogawa, O.; Yamamoto, S. *J. Urol.* **2004**, *171*, 1309–1313; (b) Salhia, B.; Rutka, J. T.; Lingwood, C.; Nutikka, A.; Van Furth, W. R. *Neoplasia* **2002**, *4*, 304–311; (c) Mallard, F.; Johannes, L. In *Methods Mol. Med. Shiga Toxin Methods and Protocols*; Philpott, D., Ebel, F., Eds.; 2003; Vol. 73, Chapter 17, pp 209–220; (d) Johannes, L.; Decaudin, D. *Gene Ther.* **2005**, *16*, 1360–1368; (e) LaPointe, P.; Gariepy, J. In *Tumor Targeting in Cancer Therapy*; Page, M., Ed.; Humana Press: Totowa, New Jersey, 2002; pp 307–318.
13. Zang, J.-Qiang; Chen, X.; Zhang, W.; Zacharek, S.; Chen, Y.; Wang, P. G. *J. Am. Chem. Soc.* **1999**, *121*, 8174–8181, and references cited therein.
14. (a) Muller, D.; Vic, G.; Critchley, P. D.; Crout, H. G.; Lea, N.; Roberts, L.; Lord, J. M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2287–2294; (b) Koike, K.; Sugimoto, M.; Sato, S.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 189–208; (c) Miura, T.; Tsujimo, S.; Satoh, A.; Goto, K.; Mizuno, M.; Noguchi, M.; Kajimoto, T.; Node, M.; Murakami, Y.; Imai, N.; Inazu, T. *Tetrahedron* **2005**, *61*, 6518–6526; (d) Nicolaou, K. C.; Caulfield, T.; Kataoka, H.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 7910–7912.
15. (a) Hashimoto, S.-I.; Sakamoto, H.; Honda, T.; Abe, H.; Nakamura, S. I.; Ikegami, S. *Tetrahedron Lett.* **1997**, *38*, 8969–8972; (b) Dohi, H.; Nishida, Y.; Tanaka, H.; Kobayashi, K. *Synlett* **2001**, *9*, 1446–1448; (c) Bhattacharyya, S.; Magnusson, B. G.; Wellmar, U.; Nilsson, U. F. *J. Chem. Soc., Perkin Trans. 1* **2001**, 886–890; (d) Qiu, D.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 217–224.
16. (a) Dejeter-Juszynski, E.; Flowers, H. M. *Carbohydr. Res.* **1971**, *18*, 219–226; (b) Windmueller, R.; Schmidt, R. R. *Tetrahedron Lett.* **1994**, *35*, 7927–7930; (c) Danishefsky, S. J.; Gervay, J.; Paterson, J. M.; McDonald, F. M.; Koseki, K.; Griffith, D. A.; Oriyama, T.; Marsden, S. P. *J. Am. Chem. Soc.* **1995**, *117*, 1940.
17. (a) Van Boeckel, C. A. A.; Beetz, T.; Van Aelst, S. F. *Tetrahedron* **1984**, *40*, 4097–4107; (b) Kucase, T.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 473–482; (c) Nishida, Y.; Takamori Matsuda, Y. K.; Ohrui, H.; Yamada, T.; Kobayashi, K. *J. Carbohydr. Chem.* **1999**, *18*, 985–997; (d) Dohi, H.; Nishida, Y.; Tanaka, H.; Kobayashi, K. *Carbohydr. Res.* **2002**, *337*, 983–989; (e) Shingu, Y.; Nishida, Y.; Dohi, H.; Kobayashi, K. *Org. Biol. Chem.* **2003**, *1*, 2518–2521.
18. (a) Schmidt, R. R.; Michel, J. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 731–732; (b) Schmidt, R. R.; Michel, J.; Roos, M. *Justus Liebigs Ann. Chem.* **1984**, 1343–1357.
19. (a) Brophy, P. M.; Alison, A. M.; van Eldik, A. J.; Teesdale-Spittle, P. H.; Liebau, E.; Wang, M. F. *Bioorg. Med. Chem. Lett* **2000**, *9*, 979–982; (b) Ranu, B. C.; Dey, S. S.; Samanta, S. *ARKIVOC* **2005**, *iii*, 44–50.
20. Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew. Chem., Int. Ed.* **2004**, *43*, 828–833, and references cited therein.
21. (a) Park, S.; Shin, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 3180–3182; (b) Chen, Y.-Ju; Chen, S.-Hua; Chien, Y.-Yih; Chang, Y.-Wan; Liao, H.-Kai; Chang, C.-Yang; Jan, M.-Dan; Wang, K.-Tseng; Lin, C.-Cheng *ChemBioChem* **2005**, *6*, 1169–1173.
22. Bowers, S. G.; Mahmud, T.; Floss, H. G. *Carbohydr. Res.* **2002**, *337*, 297–304.
23. (a) Manzoni, L.; Lay, L.; Schmidt, R. R. *J. Carbohydr. Chem.* **1998**, *17*, 739–758; (b) Greilich, U.; Brescello, R.; Jung, K.-H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1996**, 663–672, and references cited therein.
24. Wulff, G.; Roehle, G. *Angew. Chem. Int., Ed. Engl.* **1974**, *13*, 157–170.
25. Schmidt, R. R.; Toeper, A. *Tetrahedron Lett.* **1991**, *32*, 3353–3355.
26. Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1555–1564.
27. Jung, K.-H.; Hoch, M.; Schmidt, R. R. *Liebigs Ann. Chem.* **1989**, 1099–1106.
28. Haicheur, N.; Benchetrit, F.; Amessou, M.; Leclerc, C.; Falguières, T.; Fayolle, C.; Bismuth, E.; Fridman, W. H.; Johannes, L.; Tartour, E. *Int. Immunol.* **2003**, *15*, 1161–1171.
29. Naicker, K. P.; Li, H.; Heredia, A.; Song, H.; Wang, L.-Xi. *Org. Biomol. Chem.* **2004**, *2*, 660–664.