The *p*-methoxybenzyl ether as an *in situ*-removable carbohydrate-protecting group: a simple one-pot synthesis of the globotetraose tetrasaccharide

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A one-pot synthesis of the globotetraose tetrasaccharide is reported. The synthetic method relies on the use of a *p*-methoxybenzyl ether as an *in situ*-removable protecting group. *N*-Iodosuccinimide/trifluoromethanesulfonic acid-promoted glycosylation of 2-bromoethyl-2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside **5** at −45 °C with phenyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside **6** gives an intermediate trisaccharide from which the *p*-methoxybenzyl ether is removed by allowing the reaction temperature to rise to 0 °C for 40 min. Again lowering the temperature to −45 °C, followed by addition of methyl 3,4,6-tri-*O*-acetyl-2-trichloroethoxycarbonylamino-2-deoxy-1-thio-β-D-galactopyranoside **8**, affords the globotetraose tetrasaccharide **11** in one-pot in an excellent yield of 76%.

Introduction

A growing appreciation of the importance of glycobiological phenomena[1,2] in life processes has sparked the development of numerous novel powerful methods for glycoside synthesis[3,4] over the last decade. Although a rich plethora of highly efficient glycosylation methods today allows the chemical synthesis of most oligosaccharide structures, many glycosylations are more or less unpredictable with respect to yields and regio/stereoselectivities. Thus, carbohydrate chemists are still often forced to spend many hours in the purification and characterization of glycosylation products (which is in contrast to peptide and nucleotide chemists who often are served by automated equipment for rapid solid-phase synthesis). Various approaches have been pursued in order to simplify glycoside synthesis, such as enzymatic glycoside synthesis,[5,6] solid-phase glycoside synthesis[7,8] and one-pot glycosylations creating two or more glycosidic bonds without the need for intermediate work-up and purification. One-pot glycosylations are attractive, since they minimize the number of both individual reaction steps and, more important, tedious purification steps. (Synthetic oligosaccharides are rarely crystalline and purification is frequently performed using time-consuming silica chromatography.) Hitherto disclosed reports concerning one-pot glycosylations typically involve multiple donors and acceptors possessing different reactivities. Sequential activation of glycosyl donors carrying anomeric leaving groups displaying orthogonal reactivities[9–13] have been used in one-pot oligosaccharide syntheses. Furthermore, the most common approaches towards one-pot multiple glycosylation reactions have involved elegant fine-tuning of glycosyl donor reactivities[14–21] by modifying the leaving group substitution pattern and/or the donor protecting groups, which has allowed the use of a *single* promotor to selectively activate a more reactive glycosyl donor, followed by a less reactive donor. Such fine-tuning of donor reactivities have also been combined with use of glycosyl donors of orthogonal reactivities.[22–24] Reactivity differences between glycosyl acceptors have also allowed one-pot syntheses of di- to hexasaccharides[25] and reaction-rate differences between intra- and intermolecular glycosylations have been exploited in one-pot trisaccharide syntheses.[26] The observation that *n*-pentenyl orthoesters and glycosides show different selectivity towards axial and equatorial inositol hydroxy groups has also been utilized in one-pot trisaccharide synthesis.[27]

Quantification of glycosyl donor reactivities[28–30] has recently been undertaken in order to simplify the planning of one-pot oligosaccharide syntheses. Such quantitative measurements and fine-tuning of glycosyl donor reactivities allowed the construction of a computer program/database as an aid in designing synthetic strategies towards oligosaccharides[29] and an oligosaccharide library.[30]

In the methods described above involving reactivity fine-tuning and the use of only one promotor, the most reactive donor (or acceptor) reacts first, followed by the less reactive donor (or acceptor). Thus, an inherent limitation of these methods is that *reactive* glycosyl donors cannot carry *reactive* hydroxy groups that are to be glycosylated by less reactive glycosyl donors.

In this paper we address this problem by introducing a novel approach in which a *p*-methoxybenzyl ether is used as an *in situ*-removable temporary protecting group for a reactive hydroxy group in a one-pot reaction involving two sequential glycosylations. A *p*-methoxybenzyl ether is stable towards *N*-iodosuccinimide–trifluoromethanesulfonic acid (NIS–TfOH) at −45 °C and can thus block a highly reactive hydroxy group during glycosylation of a less reactive alcohol with a reactive glycosyl donor. When the first glycosylation reaction is complete, the *p*-methoxybenzyl ether is cleaved by simply elevating the temperature to 0 °C to expose a second, more reactive, hydroxy group for glycosylation. A second glycosyl donor can subsequently be added at −45 °C to complete the second glycosylation in one pot.

The usefulness of the present method is demonstrated with the first one-pot synthesis of the globotetraose (Gb4) tetrasaccharide. Apart from being interesting from a biological point of view,[31–33] the Gb4 tetrasaccharide is a rather challenging target for one-pot glycosylations involving the construction of a 1,2-*cis* glycosidic (α-D-*galacto*), as well as a 2-amino-2-deoxy-β-D-galactopyranosidic linkage. Several reports describe step wise[34] or block syntheses[35,36] of the Gb4 tetrasaccharide, as well as of elongated Gb4-containing oligosaccharides.[37–46]

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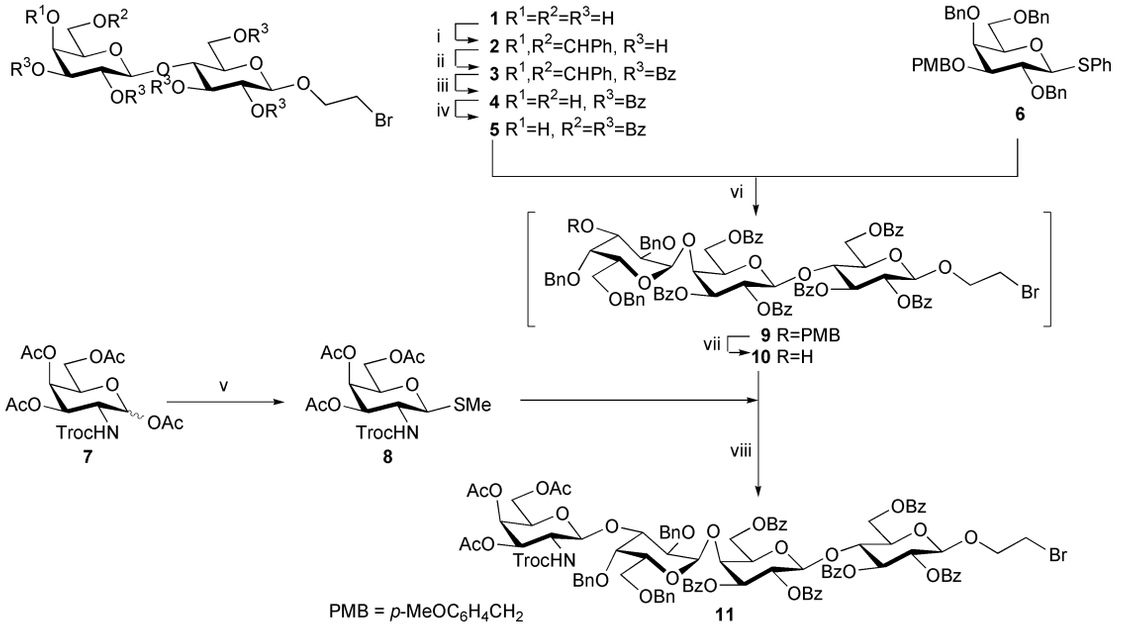


Scheme 1 *Reagents and conditions*: i, $PhCH(OMe)_2$, PTSA, MeCN (89%); ii, BzBr, pyridine, CH_2Cl_2 (71%); iii, AcOH, water, 80 °C (70%); iv, BzCl, pyridine, CH_2Cl_2, −78 °C (82%); v, $MeSSiMe_2$, Me_3SiOTf, MS AW-300, $(CH_2Cl)_2$ (96%); vi, NIS, TfOH, CH_2Cl_2, Et_2O, MS AW-300, −45 °C; vii, TfOH, 0 °C, 40 min; viii, NIS, **8**, −45 °C (76% for vi–viii one pot).

Results and discussion

The design of building blocks for one-pot multiple glycosylation reactions deserves some special attention. Protective-group patterns have to be chosen to ensure complete stereoselectivity in each individual glycosylation reaction in order to avoid the formation of complex product mixtures.

The lactoside acceptor

In the present project a lactoside acceptor had to fulfil two requirements; it should be equipped with an aglycon primed for straightforward conjugation chemistry once the Gb4 tetrasaccharide assembly was completed and it should display low reactivity of the HO-4′ to ensure high stereoselectivity in the α-galactosylation reaction. The first requirement was met by using the 2-bromoethyl aglycon, since it is readily converted into various functionalities *via* bromide substitution reactions.[47] The second requirement was fulfilled by benzoylation of 2-, 3-, 6-, 2′-, 3′- and 6′-OH of the 2-bromoethyl β-lactoside **1** *via* 4′,6′-benzylidenation, benzoylation, acetal hydrolysis and finally regioselective 6′-benzoylation to give the desired lactoside acceptor **5** (Scheme 1). The electron-withdrawing benzoyl groups significantly decrease the nucleophilicity of 4′-OH and such acceptors have proven to yield excellent stereoselectivity in syntheses of the α-D-gal(1→4)-D-gal glycosidic linkage.[48]

The α-D-Gal donor

The known phenyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside[49] **6** was chosen as the α-D-galactosyl donor, because it carries a stable, yet readily activated, anomeric leaving group, a non-participating 2-*O*-benzyl group and a *p*-methoxybenzyl protective group, which can be selectively removed under mildly acidic conditions[50] (Scheme 1).

The β-D-GalNAc donor

The *N*-trichloroethoxycarbonyl (*N*-Troc)-protected methyl thioglycoside donor **8** was chosen for the synthesis of the β-D-GalNAc linkage, because it is activated by the same promotor as the α-D-galactosyl donor **6** discussed above and because the *N*-Troc group has proven to be a reactive and reliable β-directing group removable under mild condtions.[29,51,52] However, earlier reports on the preparation of *N*-Troc-protected thioglycosides of galactosamine have involved laborious multistep syntheses from glucosamine, including inversion of configuration at C-4.[51] During the course of this work, we have found that compound **8** could be obtained in only three high-yielding steps from galactosamine hydrochloride; *N*-Troc protection and acetylation[45] (99% over two steps), followed by treatment with trimethyl(methylthio)silane and trimethylsilyl trifluoromethanesulfonate (96%) (Scheme 1). It is worthwhile to mention that this simple and high-yielding synthesis of the powerful β-D-GalNAc donor **8** is particularly attractive in view of the high cost of galactosamine hydrochloride.

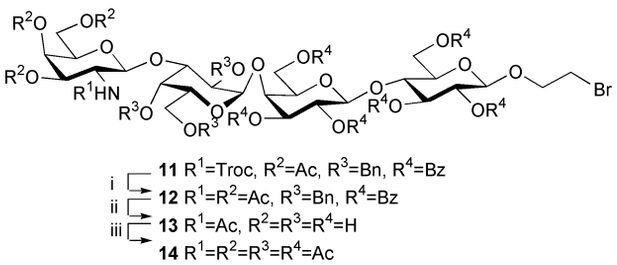
One-pot glycosylations

Initial attempts to glycosylate the lactoside **5** with the galactosyl donor **6** yielded mixtures of the desired product **9** together with the corresponding trisaccharide **10**. The amount of **10** formed was dependent on the reaction temperature, which prompted us to examine the possibilities of performing a one-pot reaction between the lactoside acceptor **5**, the galactosyl donor **6** and the β-D-GalNAc donor **8** towards the protected Gb4-derivative **11**. Indeed, NIS–TfOH (0.2 eq.)-promoted glycosylation[53,54] of **5** with **6** proceeded smoothly in dichloromethane–diethyl ether at −45 °C to consume all of the donor **6** and to give the intermediate trisaccharide **9**. Once all donor **6** was consumed, simply raising the temperature of the reaction mixture to 0 °C, as well as adding more TfOH (0.2 eq.) to speed up *p*-methoxybenzyl ether cleavage, allowed the safe and clean conversion of **9** into **10** in 40 min according to TLC. The temperature was again lowered to −45 °C and the galactosamine donor **8** was added to give the tetrasaccharide **11** in an excellent yield of 76% in one pot (Scheme 1).

Important advantages of our one-pot glycosylation strategy are that only one class of glycosyl donors (simple and stable thioglycosides) and one promotor system (NIS/TfOH) are needed. Yet, our method does not depend on the fine-tuning of donor and/or acceptor reactivities (*i.e.*, fine-tuning leaving group reactivities and/or alcohol nucleophilicities) by introducing special anomeric leaving-groups or hydroxy-protecting groups, since the second hydroxy group to be glycosylated is protected during the first glycosylation reaction. In our example

above, the *most reactive* benzylated galactosyl donor **6** reacts first with the *least reactive* alcohol **5**, followed by cleavage of the *p*-methoxybenzyl ether to expose the *more reactive* alcohol **10** for glycosylation with the *less reactive* Troc-protected galactosamine donor **8**. In comparison, the design of a one-pot protocol for the synthesis of the relatively demanding globotetraose tetrasaccharide, based on donor/acceptor reactivity fine-tuning, is not straightforward.

The *N*-Troc group of **11** was transformed into the corresponding *N*-acetyl derivative **12**, which subsequently was deprotected to give the deblocked 2-bromoethyl spacer-glycoside **13** of globotetraose in high yield (Scheme 2). Finally, compound **13** was per-*O*-acetylated (→ **14**) in order to simplify ^{1}H NMR analyses of the tetrasaccharide structure.



Scheme 2 *Reagents*: i, [a]Zn, AcOH; [b]Ac_2O, MeOH (86%); ii, [a]H_2, Pd/C, AcOH; [b]NaOMe, MeOH (79%); iii, Ac_2O, pyridine, DMAP (88%).

Conclusions

The present report shows that a *p*-methoxybenzyl ether can be used as an *in situ*-removable protecting group during an *N*-iodosuccinimide/trifluoromethanesulfonic acid-promoted glycosylation at −45 °C with a thioglycoside donor. Reaction-temperature manipulation (0 °C) allows convenient removal of the *p*-methoxybenzyl ether, thus setting the stage for glycosylation in one pot with a second thioglycoside donor. The usefulness of this approach was demonstrated with an efficient and high-yielding one-pot synthesis of the globotetraose tetrasaccharide **11**. We believe the strategy of using the *p*-methoxybenzyl ether as a temporary protecting group in one-pot glycosylations is an attractive approach that will become a useful tool for the synthesis of complex oligosaccharides. The strategy is an important complement to and may be used in combination with existing methods based on donor/acceptor reactivity fine-tuning. The scope and limitation of this strategy (*e.g.*, the stability of various carbohydrate *p*-methoxybenzyl ethers at low temperature, the use of multiple *p*-methoxybenzyl ethers for the synthesis of branched oligosaccharides, the use of other acid-labile temporary protecting groups, *etc.*) need to be addressed and are currently under investigation in our laboratory.

Experimental

General methods

^{1}H NMR spectra were recorded with Bruker ARX 500, DRX 400 and ARX 300 MHz instruments (*J*-values are given in Hz). FAB-HRMS spectra were recorded with a JEOL SX102 instrument. Optical rotations were measured with a Perkin-Elmer 241 polarimeter ($[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}). Reactions were monitored by TLC on Silica Gel FG_{254} (E. Merck, Darmstadt, Germany).

2-Bromoethyl 4-*O*-(4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside 2

2-Bromoethyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside[55] **1** (40 g, 89 mmol) was suspended in acetonitrile (2 L) and α,α-dimethoxytoluene (18 mL, 120 mmol) and toluene-*p*-sulfonic acid (PTSA) (0.5 g) were added. Triethylamine (10 mL) was added after 4 h, and the precipitate was filtered off, washed with cold ethanol and left under vacuum overnight to give **2** (42.6 g, 89%); mp 194–197 °C (from EtOH); $[\alpha]_D^{25}$ −21 (*c* 0.3 in MeOH); δ_H (500 MHz; CD_3OD) 7.54 (2H, m, ArH), 7.35 (3H, m, ArH), 5.63 (1H, s, PhC*H*), 4.49 (1H, d, *J* 6.8, H-1′), 4.38 (1H, d, *J* 7.8, H-1), 4.23 (1H, br d, *J* 2.9, H-4′), 4.19 (1H, dd, *J* 1.5 and 8.6, H-6′), 4.10 (1H, ddd, *J* 6.4, 6.9 and 9.9, OCH_2CH$_2$), 3.27 (1H, dd, *J* 8.0 and 8.9, H-2); *m*/*z* 559.0781 (M^+ + Na. $C_{21}H_{29}BrNaO_{11}$ requires *m*/*z*, 559.0791).

2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-β-D-glucopyranoside 3

To a solution of compound **2** (755 mg, 1.40 mmol) in pyridine (30 mL) at 0 °C was added benzoyl bromide (1.16 mL, 9.84 mmol). After 160 min, the reaction mixture was poured onto a mixture of dichloromethane and saturated aq. $NaHCO_3$, and the organic layer was dried (Na_2SO_4), filtered and concentrated. Flash chromatography (SiO_2: heptane–EtOAc 2 : 1) gave **3** (1.05 g, 71%); mp 253–254 °C (from heptane–EtOAc); $[\alpha]_D^{25}$ +114 (*c* 1.1 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.85 (1H, t, *J* 9.2, H-3), 5.80 (1H, dd, *J* 8.0 and 10.5, H-2′), 5.34 (1H, dd, *J* 7.7 and 9.2, H-2), 5.31 (1H, s, PhCH), 5.19 (1H, dd, *J* 3.6 and 10.5, H-3′), 4.85 (1H, d, *J* 7.9, H-1′), 4.78 (1H, d, *J* 7.6, H-1), 4.66 (1H, dd, *J* 2.0 and 11.8, H-6), 4.37 (1H, dd, *J* 4.3 and 11.9, H-6), 4.31 (1H, d, *J* 3.5, H-4′), 4.24 (1H, t, *J* 9.3, H-4), 4.01 (1H, ddd, *J* 5.6, 6.9 and 11.2, OCH_2CH$_2$), 3.89 (1H, ddd, *J* 2.3, 4.3 and 9.7, H-5), 3.79 (1H, br d, *J* 10.9 and H-6′), 3.77 (1H, dt, *J* 7.0 and 11.3, OCH_2CH$_2$), 3.59 (1H, dd, *J* 1.7 and 12.3, H-6′), 3.33 (2H, m, CH_2Br); *m*/*z* 1079.2131 (M^+ + Na. $C_{56}H_{49}BrNaO_{16}$ requires *m*/*z*, 1079.2101).

2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside 4

Compound **3** (1.05 g, 0.99 mmol) was heated to 80 °C in 80% acetic acid (20 mL) for 6 h, then the solution was concentrated. Flash chromatography (SiO_2; CH_2Cl_2–acetone 9 : 1) gave **4** (668 mg, 70%); mp 214–216 °C (from heptane–EtOAc); $[\alpha]_D^{25}$ +78 (*c* 0.8 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.76 (2H, br t, *J* 9.1, H-3 and -2′), 5.43 (1H, dd, *J* 7.6 and 9.4, H-2), 5.11 (1H, dd, *J* 3.2 and 10.4, H-3′), 4.81 (1H, d, *J* 8.0, H-1′), 4.79 (1H, d, *J* 7.7, H-1), 4.64 (1H, dd, *J* 2.0 and 11.9, H-6), 4.42 (1H, dd, *J* 4.9 and 11.9, H-6), 4.21 (1H, t, *J* 9.4, H-4), 4.20 (1H, br d, *J* 4.2, H-4′), 4.04 (1H, ddd, *J* 5.3, 6.8 and 11.3, OCH_2CH$_2$), 3.88 (1H, ddd, *J* 2.0, 4.7 and 9.7, H-5), 3.79 (1H, dt, *J* 6.9 and 11.2, OCH_2CH$_2$); *m*/*z* 991.1792 (M^+ + Na. $C_{49}H_{45}BrNaO_{16}$ requires *m*/*z*, 991.1789).

2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranoside 5

To a solution of compound **4** (247.1 mg, 0.255 mmol) and pyridine (4 mL) in dichloromethane (6 mL) at −78 °C was added benzoyl chloride (32.5 μL, 0.28 mmol). More benzoyl chloride (25 μL, 0.21 mmol) was added after 150 min. The reaction was quenched with methanol (10 mL) after 6 h and the mixture was concentrated. Flash chromatography (SiO_2; heptane–EtOAc 2 : 1) gave **5** (225.1 mg, 82%); mp 197–199 °C (from heptane–EtOAc); $[\alpha]_D^{25}$ +54 (*c* 0.9 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.78 (1H, t, *J* 9.2, H-3), 5.75 (1H, dd, *J* 8.0 and 9.4, H-2′), 5.44 (1H, dd, *J* 7.9 and 9.7, H-2), 5.16 (1H, dd, *J* 3.2 and 10.4, H-3′), 4.80 (1H, d, *J* 7.9, H-1′), 4.77 (1H, d, *J* 7.8, H-1), 4.61 (1H, dd, *J* 2.0 and 12.1, H-6), 4.46 (1H, dd, *J* 4.4 and 12.0, H-6), 4.21 (1H, t, *J* 9.3, H-4), 4.04 (1H, ddd, *J* 5.3, 6.8 and 11.3, OCH_2CH$_2$), 3.87 (1H, ddd, *J* 2.0, 4.7 and 9.7, H-5), 3.79 (1H, dt, *J* 7.1 and 11.3, OCH_2CH$_2$), 3.36 (2 H, m, CH_2Br); *m*/*z* 1095.2070 (M^+ + Na. $C_{56}H_{49}BrNaO_{17}$ requires *m*/*z*, 1095.2051).

Methyl 3,4,6-tri-*O*-acetyl-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy-1-thio-β-D-galactopyranose 8

To a mixture of 1,3,4,6-tetra-*O*-acetyl-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy-α/β-D-galactopyranose[45,56] **7** (α/β 1 : 3; 47.7 g, 91.0 mmol), trimethyl(methylthio)silane (16.7 g, 140 mmol) and MS AW-300 (activated, 10 g) in 1,2-dichloroethane (400 mL) was added trimethylsilyl trifluoromethanesulfonate (20 g, 91.0 mmol). The mixture was filtered after two days, diluted with dichloromethane, washed with saturated aq. sodium hydrogen carbonate, dried (Na_2SO_4), filtered and concentrated. Flash chromatography (SiO_2; heptane–EtOAc 2 : 1 to 1 : 1 gradient) gave **8** (44.6 g, 96%); $[a]_D^{25}$ −18 (*c* 1 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.42 (1H, dd, *J* 0.7 and 3.2, H-4), 5.13 (1H, dd, *J* 3.2 and 10.9, H-3), 5.03 (1H, d, *J* 9.6, NH), 4.82 (1H, d, *J* 12.0, CH_2CCl_3), 4.69 (1H, d, *J* 12.0, CH_2CCl_3), 4.53 (1H, d, *J* 10.3, H-1), 4.18 (1H, dd, *J* 6.8 and 11.3, H-6), 4.14 (1H, dd, *J* 6.6 and 11.3, H-6), 4.04 (1H, br q, *J* 10.2, H-2), 3.96 (1H, dt, *J* 0.9 and 6.7, H-5), 2.25 (3 H, s, SCH_3), 2.18, 2.05, 2.01 (3 H each, 3 s, Ac); *m*/*z* 531.9987 (M^+ + Na. $C_{16}H_{22}Cl_3NNaO_9S$ requires *m*/*z*, 531.9979).

2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-{2,3,6-tri-*O*-benzoyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-(2,2,2-trichloroethoxycarbonylamino)-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside 11

To a solution of **5** (2.27 g, 2.11 mmol) and **6** (2.10 g, 3.17 mmol) in CH_2Cl_2–Et_2O (1 : 2; 66 mL) was added MS AW-300 (activated, 1 g) and the mixture was stirred under N_2 for 4 h. The mixture was then cooled to −45 °C and NIS (922 mg, 4.13 mmol) and TfOH (50 μL, 0.2 eq.) were added. After 40 min, TLC showed the complete disappearance of the donor **6** and formation of the desired product **9**. To the mixture was then added TfOH (50 μL), the temperature was raised to 0 °C and the mixture was stirred for 40 min. The temperature of the reaction mixture was again lowered to −45 °C and the donor **8** (1.20 g, 2.34 mmol) and NIS (600 mg, 2.67 mmol) were added. After 30 min, the reaction mixture was diluted with dichloromethane, filtered through Celite, washed successively with 10% aq. $Na_2S_2O_3$ and 1 M aq. $NaHCO_3$, dried (Na_2SO_4) and concentrated. Column chromatography (SiO_2; toluene–EtOAc, 12 : 1 to 6:1 gradient) gave **11** (3.2 g, 76%); $[a]_D^{25}$ +39 (*c* 1 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.88 (1H, t, *J* 9.3, H-3), 5.73 (2H, dd, *J* 7.7 and 10.5, H-2′ and NH), 5.47 (1H, br d, *J* 2.8, H-4‴), 5.37 (1H, dd, *J* 7.9 and 9.3, H-2), 5.16 (1H, dd, *J* 3.0 and 7.8, H-3‴), 5.13 (1H, dd, *J* 2.7 and 10.4, H-3′), 5.05 (1H, d, *J* 7.7, H-1‴), 4.94 (1H, d, *J* 8.3, H-1′), 4.67 (1H, d, *J* 7.9, H-1), 4.32 (1H, t, *J* 9.6, H-4), 4.25 (3H, m, H-2‴ and OCH_2CH_2), 3.95 (1H, dd, *J* 3.0 and 10.5, H-2″), 3.32 (2H, m, CH_2Br), 2.90 (1H, dd, *J* 5.8 and 8.2, H-6″), 2.15, 2.01, 2.00 (3H each, 3 s, Ac); *m*/*z* 1988.4076 (M^+ + Na. $C_{98}H_{95}BrCl_3NaO_{31}$ requires *m*/*z*, 1988.4035).

2-Bromoethyl 2,3,6-tri-*O*-benzoyl-4-*O*-{2,3,6-tri-*O*-benzoyl-4-*O*-[2,4,6-tri-*O*-benzyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside 12

To a solution of compound **11** (3.0 g, 1.52 mmol) in acetic acid (30 mL) under N_2 was added Zn (1.5 g). The reaction mixture was stirred at room temperature for 24 h, then filtered through a pad of Celite and SiO_2, which was then thoroughly washed with toluene–acetone 1 : 1 containing 1% Et_3N. The filtrate was concentrated and co-concentrated with toluene to give the intermediate amine, which was acetylated with Ac_2O (15 mL) in methanol (30 mL) for 16 h at room temperature. Column chromatography of the crude product (SiO_2; heptane–EtOAc 1 : 1) afforded **12** (2.4 g, 86%) together with unchanged **11** (150 mg, 8% recovery); $[a]_D^{25}$ +34 (*c* 0.8 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 6.25 (1H, d, *J* 9.6, NH), 5.89 (1H, t, *J* 9.3, H-3), 5.75 (1H, dd, *J* 7.6 and 10.6, H-2′), 5.42 (1H, br d, *J* 3.2, H-4‴), 5.37 (1H, dd, *J* 7.9 and 9.2, H-2), 5.17 (1H, t, *J* 3.1, H-3′), 5.14 (1H, t, *J* 3.3, H-3‴), 5.04 (1H, d, *J* 7.7, H-1′), 4.91 (1H, d, *J* 8.4, H-1‴), 4.67 (1H, d, *J* 7.7, H-1), 4.64 (1H, d, *J* 3.4, H-1″), 4.55 (1H, dd, *J* 4.1, 6.3, H-2‴), 4.32 (1H, m, H-2″), 3.70 (2H, m, OCH_2CH_2), 3.33 (3H, m, CH_2Br, H-5′), 2.20, 2.15, 2.00, 1.87 (3H each, 4 s, Ac); *m*/*z* 1856.5060 (M^+ + Na. $C_{97}H_{96}BrNNaNO_{30}$ requires *m*/*z*, 1856.5098).

2-Bromoethyl 4-*O*-{4-*O*-[3-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside 13

A solution of compound **12** (100 mg, 54 μmol) in AcOH (4 mL) was hydrogenolysed over 10% Pd–C (40 mg) for 24 h, filtered and concentrated. The residue was treated with methanolic sodium methoxide (0.09 M; 2.5 mL) at room temperature for 16 h. The solution was neutralized with Duolite C436 (H^+) resin, filtered and concentrated. Column chromatography (SiO_2, CH_2Cl_2–MeOH–H_2O 10 : 5 : 1) gave **13** (35 mg, 79%); $[a]_D^{25}$ +15 (*c* 0.2 in H_2O); δ_H (400 MHz; D_2O) 4.79 (1H, d, *J* 3.9, H-1″), 4.51 (1H, d, *J* 8.4, H-1‴), 4.44 (1H, d, *J* 8.0, H-1), 4.39 (1H, d, *J* 7.7, H-1′), 4.25 (1H, br t, *J* 6.7, H-5″), 4.13 (1H, br d, *J* 2.7, H-4″), 3.46 (1H, dd, *J* 7.8 and 10.3, H-2′), 3.23 (1H, dd, *J* 8.1 and 9.0, H-2), 1.92 (3H, s, Ac); *m*/*z* 836.1831 (M^+ + Na. $C_{28}H_{48}BrNNaO_{21}$ requires *m*/*z*, 836.1800).

2-Bromoethyl 2,3,6-tri-*O*-acetyl-4-*O*-{2,3,6-tri-*O*-acetyl-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranosyl]-β-D-galactopyranosyl}-β-D-glucopyranoside 14

Compound **13** (35 mg, 43 μmol) was acetylated with Ac_2O (0.2 mL), pyridine (0.4 mL) and 4-(dimethylamino)pyridine (DMAP) (10 mg) for 16 h. Concentration and column chromatography of the residue (SiO_2; EtOAc) gave **14** (50 mg, 88%); $[a]_D^{25}$ +44 (*c* 0.5 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 5.65 (1H, d, *J* 8.7, NH), 5.60 (1H, br d, *J* 2.9, H-4″), 5.35 (1H, br d, *J* 3.2, H-4‴), 5.27 (1H, dd, *J* 3.2 and 11.2, H-3‴), 5.23 (1H, t, *J* 9.2, H-3), 5.20 (1H, dd, *J* 3.5 and 10.6, H-2″), 5.13 (1H, dd, *J* 7.6 and 10.8, H-2′), 4.96 (1H, d, *J* 3.5, H-1″), 4.92 (1H, dd, *J* 8.0 and 9.3, H-2), 4.85 (1H, d, *J* 8.2, H-1‴), 4.76 (1H, dd, *J* 2.4 and 10.9, H-3′), 4.56 (1H, d, *J* 7.6, H-1′), 4.55 (1H, d, *J* 7.9, H-1), 4.20 (1H, dd, *J* 3.3 and 10.9, H-3″), 3.91 (1H, dt, *J* 8.7 and 11.0, H-2‴), 3.45 (2H, m, CH_2Br), 2.14, 2.13, 2.12, 2.10, 2.09, 2.07, 2.06, 2.05, 1.99, 1.92 (39 H, 10 s, Ac); *m*/*z* 1340.3063 (M^+ + Na. $C_{52}H_{72}BrNNaO_{33}$ requires *m*/*z*, 1340.3068).

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References

1 A. Varki, *Glycobiology*, 1993, **3**, 97.
2 R. A. Dwek, *Chem. Rev.*, 1996, **96**, 683.
3 G. J. Boons, *Tetrahedron*, 1996, **52**, 1095.
4 B. G. Davis, *J. Chem. Soc.*, *Perkin Trans. 1*, 2000, 2137.
5 C.-H. Wong, R. L. Halcomb, Y. Ichikawa and T. Kajimoto, *Angew. Chem.*, *Int. Ed. Engl.*, 1995, **34**, 412 and 521.
6 M. M. Palcic and O. Hindsgaul, *Trends Glycosci. Glycotechnol.*, 1996, **8(39)**, 37.
7 H. M. I. Osborn and T. H. Khan, *Tetrahedron*, 1999, **55**, 1807.
8 P. Seeberger and W.-C. Haase, *Chem. Rev.*, 2000, **100**, 4349.
9 H. Yamada, T. Harada, H. Miyazaki and T. Takahashi, *Tetrahedron Lett.*, 1994, **35**, 3979.
10 H. Yamada, T. Harada and T. Takahashi, *J. Am. Chem. Soc.*, 1994, **116**, 7919.

11 H. Yamada, T. Kato and T. Takahashi, *Tetrahedron Lett.*, 1999, **40**, 4581.
12 H. K. Chenault and A. Castro, *Tetrahedron Lett.*, 1994, **35**, 9145.
13 H. Xuereb, M. Maletic, J. Gildersleeve, I. Pelczer and D. Kahne, *J. Am. Chem. Soc.*, 2000, **122**, 1883.
14 S. Raghavan and D. Kahne, *J. Am. Chem. Soc.*, 1993, **115**, 1580.
15 S. V. Ley and H. W. M. Priepke, *Angew. Chem.*, *Int. Ed. Engl.*, 1994, **33**, 2292.
16 P. Grice, S. V. Ley, J. Pietruszka, H. W. M. Priepke and E. P. E. Walther, *Synlett*, 1995, 781.
17 P. Grice, S. V. Ley, J. Pietruszka, M. I. Osborn, W. M. Henning, H. W. M. Priepke and S. L. Warriner, *Chem. Eur. J.*, 1997, **3**, 431.
18 R. Geurtsen, D. S. Holmes and G.-J. Boons, *J. Org. Chem.*, 1997, **62**, 8145.
19 S.-F. Lu, Q. O'yang, Z.-W. Guo, B. Yu and Y.-Z. Hui, *J. Org. Chem.*, 1997, **62**, 8400.
20 T. Tsukida, M. Yoshida, K. Kurokawa, Y. Nakai, T. Achiha, T. Kiyoi and H. Kondo, *J. Org. Chem.*, 1997, **62**, 6876.
21 H. I. Duynstee, M. J. van Vliet, G. A. van der Marel and J. H. van Boom, *Eur. J. Org. Chem.*, 1998, 303.
22 M.-K. Cheung, N. Douglas, B. Hinzen, S. V. Ley and X. Pannecoucke, *Synlett*, 1997, 257.
23 L. Green, B. Hinzen, S. J. Ince, P. Langer, S. V. Ley and S. L. Warriner, *Synlett*, 1998, 440.
24 D. K. Baeschlin, L. G. Green, M. G. Hahn, B. Hinzen, S. J. Ince and S. V. Ley, *Tetrahedron: Asymmetry*, 2000, **11**, 173.
25 G.-J. Boons and T. Zhu, *Synlett*, 1997, 809.
26 S. Valverde, M. García, A. M. Gómez and J. C. López, *Chem. Commun.*, 2000, 813.
27 G. Anilkumar, L. G. Nair and B. Fraser-Reid, *Org. Lett.*, 2000, **2**, 2587.
28 N. L. Douglas, S. V. Ley, U. Lucking and S. L. Warriner, *J. Chem. Soc.*, *Perkin Trans. 1*, 1998, 51.
29 Z. Zhang, I. R. Ollman, X.-S. Ye, R. Wischnat, T. Baasov and C.-H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 734.
30 X.-S. Ye and C.-H. Wong, *J. Org. Chem.*, 2000, **65**, 2410.
31 D. M. Marcus, M. A. Naiki and S. K. Kundu, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 3263.
32 H. Leffler and C. Svanborg-Edén, *FEMS Lett.*, 1980, **8**, 127.
33 K. E. Brown, S. M. Anderson and N. S. Young, *Science*, 1993, **262**, 114.
34 U. Nilsson, A. K. Ray and G. Magnusson, *Carbohydr. Res.*, 1994, **252**, 117.
35 H. Paulsen and A. Bünsch, *Carbohydr. Res.*, 1982, **101**, 21.
36 K. Leontein, M. Nilsson and T. Norberg, *Carbohydr. Res.*, 1985, **144**, 231.
37 H. Paulsen and A. Bünsch, *Liebigs Ann. Chem.*, 1981, 2204.
38 H. Paulsen and A. Bünsch, *Carbohydr. Res.*, 1982, **100**, 143.
39 S. Nunomura and T. Ogawa, *Tetrahedron Lett.*, 1988, **29**, 5681.
40 S. Nunomura, M. Mori, Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 1989, **30**, 6713.
41 U. Nilsson, A. K. Ray and G. Magnusson, *Carbohydr. Res.*, 1994, **252**, 137.
42 U. Nilsson and G. Magnusson, *Carbohydr. Res.*, 1995, **272**, 9.
43 J. M. Lassaletta, K. Carlsson, P. J. Garegg and R. R. Schmidt, *J. Org. Chem.*, 1996, **61**, 6873.
44 T. K. Park, I. J. Kim, S. Hu, M. T. Bilodeau, J. T. Randolph, O. Kwon and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1996, **118**, 11488.
45 T. Zhu and G.-J. Boons, *Angew. Chem.*, *Int. Ed.*, 1999, **38**, 3495.
46 W. Zou, J.-R. Brisson, S. Larocque, R. L. Gardner and H. J. Jennings, *Carbohydr. Res.*, 1999, **315**, 251.
47 G. Magnusson, in *Protein-Carbohydrate Interactions in Biological Systems*, ed. D. Lark, Academic Press, London, 1986, p. 215.
48 U. J. Nilsson and G. Magnusson, in *Glycoscience: Chemistry and Chemical Biology*, ed. B. Fraser-Reid, K. Tatsuta and J. Thiem, Springer-Verlag, Berlin/Heidelberg, in press.
49 O. Kanie, Y. Ito and T. Ogawa, *Tetrahedron Lett.*, 1996, **37**, 4551.
50 L. Yan and D. Kahne, *Synlett*, 1995, 523.
51 U. Ellervik and G. Magnusson, *Carbohydr. Res.*, 1996, **280**, 251.
52 M. Nitz and D. R. Bundle, *J. Org. Chem.*, 2000, **65**, 3064.
53 P. Konradsson, U. E. Udodong and B. Fraser-Reid, *Tetrahedron Lett.*, 1990, **31**, 4313.
54 G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 1331.
55 H. Lönn and K. Stenvall, *Tetrahedron Lett.*, 1992, **33**, 115.
56 K. Higashi and H. Susaki, *Chem. Pharm. Bull.*, 1992, **40**, 2019.