A new route for the synthesis of *Streptococcus pneumoniae* 19F and 19A capsular polysaccharide fragments avoiding the β-mannosamine glycosylation step[1]

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Dedicated to Professor Hans Kamerling on the occasion of his 65th birthday.

Abstract – The recently described (*Carbohydr. Res*. **2008**, *43,* 2545-2556) β-D-MaNAc*p*-(1→4)-β-D-Glc*p* thiophenyl glycosyl donor **3** was used in α-glycosylation reactions of OH-2 and OH-3 of the suitably protected *p*-MeO-benzyl α-L-rhamnopyranoside acceptors **7** and **8**. The glycosylation of axial OH-2 of **7** took place in high yield (76%) and with good stereoselectivity (α/β = 3.4) leading to the protected trisaccharide **α-11**, corresponding to the repeating unit of *Streptococcus pneumoniae* 19F. The same reaction on equatorial OH-3 of acceptor **8** gave the trisaccharide **α-15**, constituent of the repeating unit of *S. pneumoniae* 19A, but in lower yield (41%) and without stereoselection (α/β = 1:1.3). Utilizing the introduced orthogonal protection of OH-1 and OH-4'', the trisaccharide **α-11** was transformed into a trisaccharide building block suitable for the synthesis of its phosphorylated oligomers.

Key words: Glycoconjugate vaccines, Thioglycosides, Oligosaccharide synthesis

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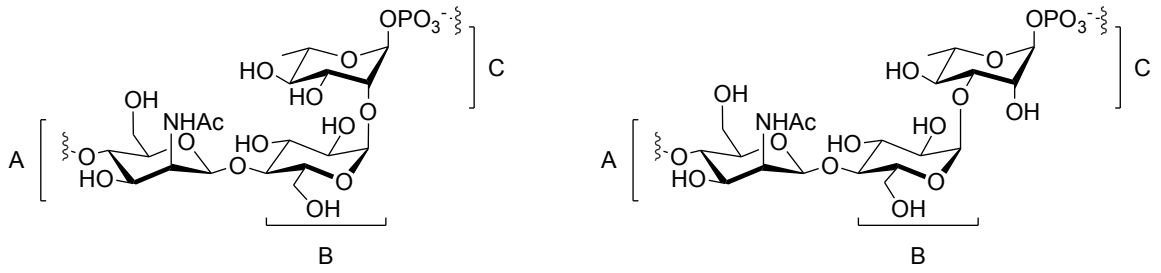
1. Introduction

Encapsulated bacteria present an external carbohydrate coat for protection against the host's immune system and osmotic lysis that is, in the pathogenic strains, responsible for their virulence. The capsular polysaccharide (CPS) structure defines the serotype, and since the discovery that CPS fragments induce a response of the host's adaptive immune system, research has been focused on developing CPS-based vaccines as an alternative to antibiotic therapy.[2] The first attempts to create such vaccines were made by administration of CPS fragments obtained by purification after controlled lysis of the capsule and there are still commercial multivalent vaccines produced with this method. More recently, there has been a development of most efficient glycoconjugate vaccines, were the CPS is conjugated to a carrier protein to allow a T-cell dependent immune response. Also, large efforts have been made to develop synthetic vaccines portrayed by well-defined molecular structures and complete absence of biological contaminants.[3,4] *Streptococcus pneumoniae* serotypes 19F (*SP* 19F) and 19A (*SP* 19A), are responsible of a large number of infections of the upper respiratory system and meningitis, especially in children and immunodeficient subjects, and can be associated to a large number of deaths (1.2 million/year just in developing countries). The repeating units[5] of *SP* 19F and *SP* 19A CPS (Figure 1) both constitute of a trisaccharide containing a *N*-acetyl-D-mannosamine unit (A) linked through a β-1→4 bond to a D-glucose (B) residue which is linked to a L-rhamnose unit (C) through an α-1→2 (*SP* 19F) or an α-1→3 bond (*SP* 19A). The repeating units are linked to each other via an α-1→4 phosphodiester bridge (Figure 1).



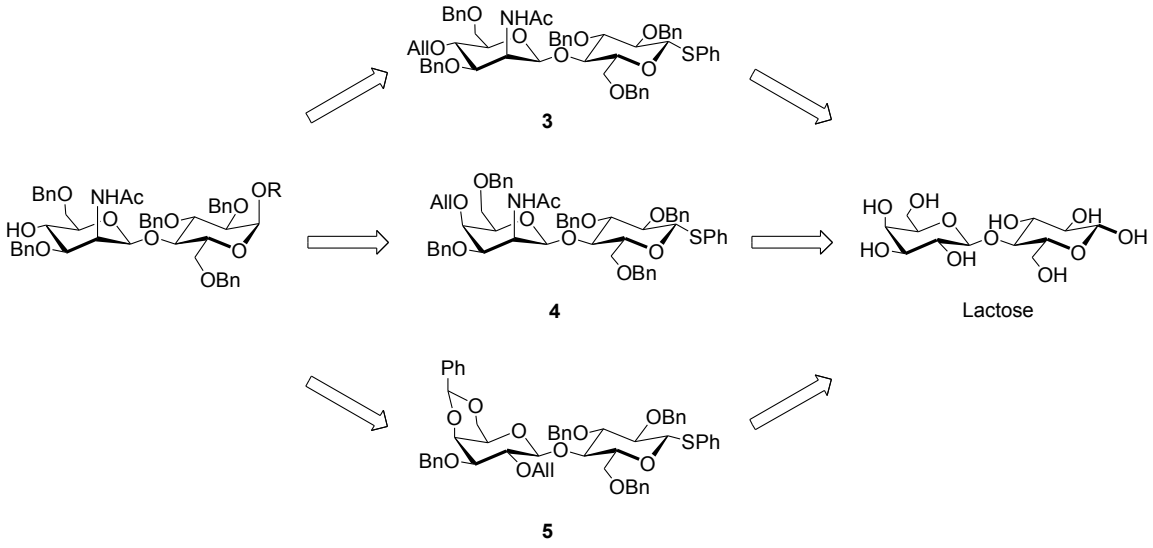
→4)-β-D-ManNAc*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(PO_4)-(1→ →4)-β-D-ManNAc*p*-(1→4)-α-D-Glc*p*-(1→3)-α-L-Rha*p*-(PO_4)-(1→

Figure 1. *SP* 19F and *SP* 19A CPS repeating units.

Since the elucidation of these structures, chemists have been involved with their synthesis, especially with that of *SP* 19F. The reported synthetic approaches involve, in all cases, two different glycosidation reactions, and can be classified into two groups: the first is based on the initial synthesis of the A-B fragment and the successive coupling with the unit C,[6] while the second approach involves the coupling of unit A with the B-C fragment.[7] The most challenging task is the introduction of the *N*-acetyl-β-D-mannosamine linkage, because of the difficulties in stereoselective formation of β-D-mannosamine glycopyranosides by direct glycosidation with D-mannosamine donors.[8] The most employed strategy is based on initial formation of a β-D-glucopyranoside residue, followed by its transformation into a β-D-mannosamine moiety by amination with inversion of configuration at C-2' through an oxidation-oximation followed by reduction of the oximino derivative[6a] or through a S_N2 displacement with sodium azide on a 2-*O*-sulfonate intermediate followed by reduction.[7b,6d] Other methods described are direct glycosidations with 2-azido-2-deoxy-D-mannopyranose donors, either the bromide activated by silver silicate[7a,6b] or with a C-2 oximino glycosyl donor followed by

stereoselective reduction.[6c] Still most of these methods suffer from problems related to low reaction yields and stereoselectivity reducing the efficiency of the syntheses.

A recently reported method[9] for the synthesis of β-D-mannosaminosides and β-D-mannosides is based on the completely stereoselective elaboration in positions 2 (amination with inversion) and 4 (epimerization) of β-D-galactopyranosides. This suggested the possibility for obtaining α-glycosides of the β-D-ManNAc*p*-(1→4)-D-Glc*p* of type **6** from lactose converting its non-reducing end into the *N*-acetyl-D-mannosamine moiety. To this end a systematic investigation[10] (Scheme 1) have been performed on the glycosidation properties of three different disaccharide thiophenyl glycosyl donors obtained from lactose, each carrying at the non-reducing end a D-mannosamine (**3**), a D-talosamine (**4**) or a D-galactopyranose (**5**) unit, with a simple alcoholic acceptor.



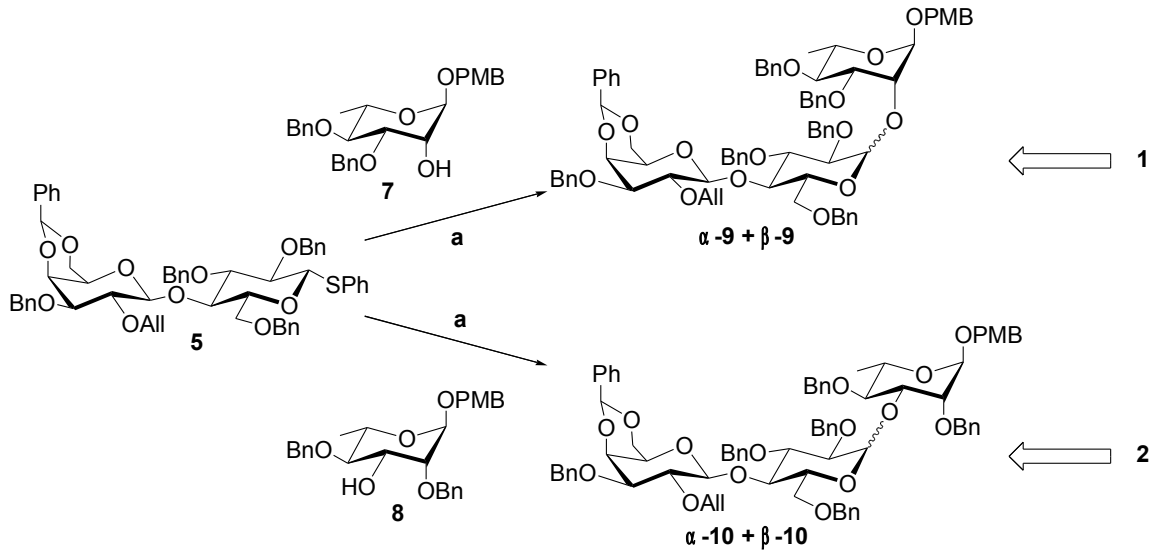
Scheme 1. Complementary approaches to β-D-ManNAcp-(1→4)-α-D-Glcp glycosides from lactose

Using NIS/TfOH or MeOTf as activators, donor **3** gave no glycoside product, whereas donor **4** afforded the desired glycosides but only in a low yield and without any α-stereoselectivity. The best results were obtained with donor **5**. As a continuation of these

results, we herein present an investigation for obtaining the trisaccharides of the repeating units of *SP* 19 F and 19A from lactose avoiding the β-mannosaminylation step.

2. Results and Discussion

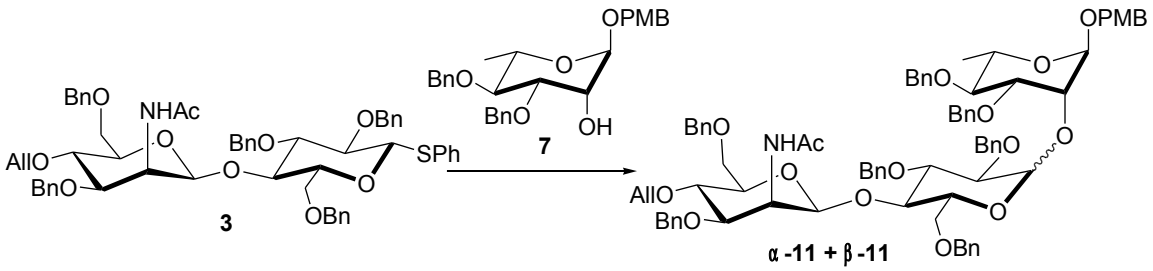
Because of the poor donor properties experienced with compounds **3** and **4**,[10] we initially thought that the best way to obtain the CPS trisaccharide repeating units of *SP* 19F and 19A was to glycosidate acceptors **7**[11] and **8**[12] with donor **5** and subsequently convert the D-galactopyranoside units in the obtained trisaccharides into D-mannosamine residues (Scheme 2).



Scheme 2. Glycosylation of acceptors **7** and **8** with the disaccharide donor **5**. Reagents and conditions: (a) MeOTf, CH_2Cl_2/Et_2O 4:1, 0°C, 30 min.

Thus, acceptors **7** and **8** were coupled with donor **5** using MeOTf[13] (5 eq) as promoter in CH_2Cl_2-Et_2O (4:1, v/v) leading to anomeric mixtures of **9** (75%, α/β 2:1) and **10** (78%, α/β 1.7:1) respectively (Scheme 2). In both cases, the obtained mixtures were

easily separated by chromatographic means, affording pure samples of α-**9** and α-**10**, in 50 and 49% isolated yield, respectively. Successful application of the sequence previously optimised for the analogous isopropyl α-disaccharide[10] to the 2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl unit of **α-9** and **α-10** would afford the protected trisaccharide repeating units of *SP* 19F and *SP* 19A CPS. However, realizing the rather long sequence required for the above procedure (20% overall yield over 12 steps in the reported case[10]), we re-considered the possibility to employ the mannosamine disaccharide **3** as donor to glycosylate the two rhamnoside acceptors **7** and **8**. Taking into account the completely negative results obtained in the preliminary study using NIS-TfOH or MeOTf as promoter,[10] we decided to explore the glycosidation properties of **3** with other activating systems. Hence, glycosidation reactions between donor **3** and rhamnoside acceptor **7** (Scheme 3) were carried out with the most widely used activating systems of thioglycoside donors,[14] but, as in the case of the previously tried NIS-TfOH and MeOTf, also NIS-TMSOTf, PhIO-TMSOTf, MeOTf-collidine or DMTST gave disappointing results. Again no product could be isolated, only retrieved starting materials and decomposition products.



Scheme 3. Glycosidation of acceptor **7** with donor **3**. Reagents and conditions:see Table 1.

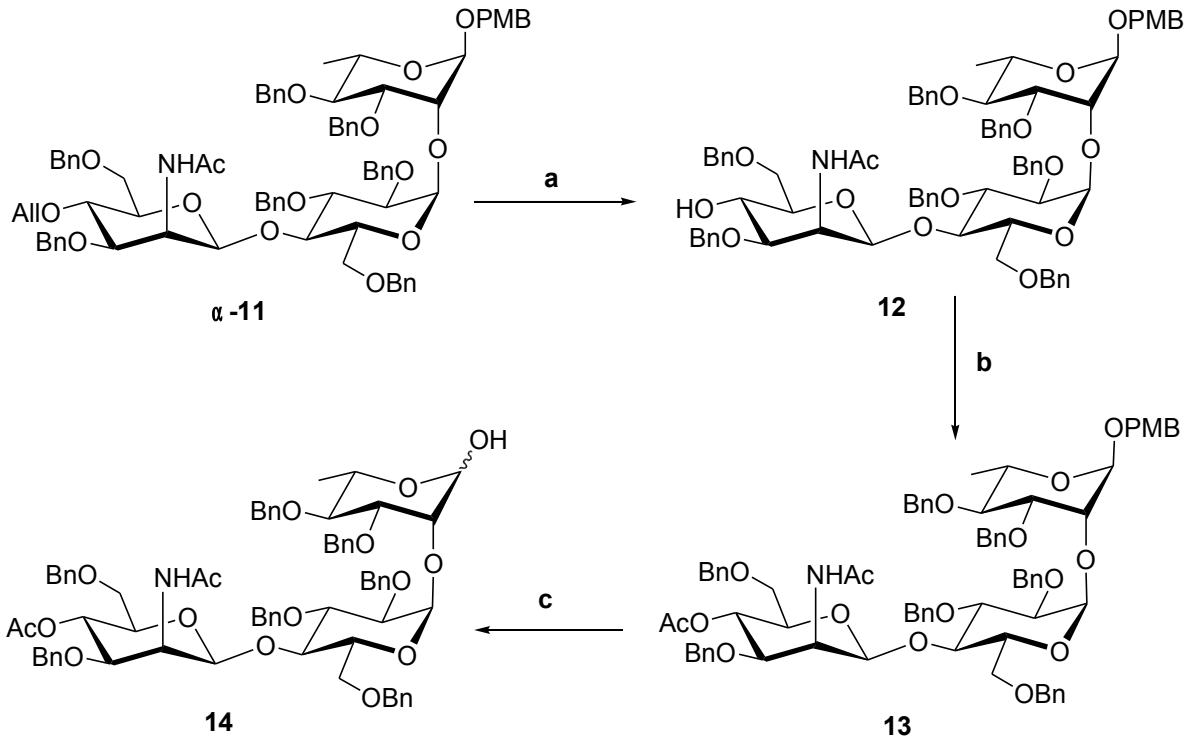
However, an explorative reaction with NIS-AgOTf in CH_2Cl_2 carried out without molecular sieves and temperature control gave a mixture of trisaccharides **α-11** and **β-11** although in minute amounts. Still, encouraged by this result, we started a systematic study on the reaction conditions to optimize the yield of the desired α-anomer. Relevant results of this study are reported in Table 1. Running the reaction at -15 to -8 °C (Entry 1) gave a 34% yield with a 6.5 α/β ratio, even lower temperature and using two equivalents of acceptor resulted in increased yield but lower stereoselectivity (52%, 4.6 α/β ratio, Entry 2). The non-reacted excess of the acceptor is easily retrieved from the reaction mixture by simple flash chromatography. Changing the donor molar concentration (Entry 3) decreased the yield to 31% without any change in stereoselectivity (4.9). The best results were obtained when adding the activating system at –35 °C and keeping the reaction for a longer time (1.3 h) resulting in a total yield of 76% with a stereoselectivity ratio of 3.4 equivalent to afford a 58% yield of the α-anomer (Entry 4). Efforts were made to increase the α/β ratio using a mixture of CH_2Cl_2 and an α-directing[15] ethereal solvent. However, using the suggested[16] 1:1 (v/v) mixture of CH_2Cl_2-Et_2O (Entry 5), the reaction was slower (2 h), needed a triple amount of AgOTf compared to that used for the reaction carried out in just CH_2Cl_2 and, surprisingly, resulted in a complete loss of stereoselectivity. A preparative reaction was performed using the conditions of Entry 4, Table 1, and, most satisfactory, the results were reproducible and the target protected trisaccharide **α-11** was obtained in 59% yield after a simple flash chromatographic purification.

Table 1. Glycosidation of acceptor **7** with disaccharide glycosyl donor **3**[a]

Entry	[3]/[7]	Activating System (eq)	T (°C)	t (h)	Solvent (v/v)	Isolated Yield (%)	α/β
1 [b]	1	NIS (1.2)/ AgOTf (0.5)	-15→-8	0.5	CH_2Cl_2	34	6.5
2 [b]	0.47	NIS (1.4) AgOTf (0.55)	-40→-15	1	CH_2Cl_2	52	4.6
3[c]	0.52	NIS (1.5) AgOTf (0.5)	-35→-12	1	CH_2Cl_2	31	4.9
4[b]	0.52	NIS (1.5) AgOTf (0.5)	-35→-10	1.3	CH_2Cl_2	76	3.4
5 [b,d]	0.44	NIS (1.5) AgOTf (1.67)	-35→-10	2	CH_2Cl_2-Et_2O(1:1)	73	1

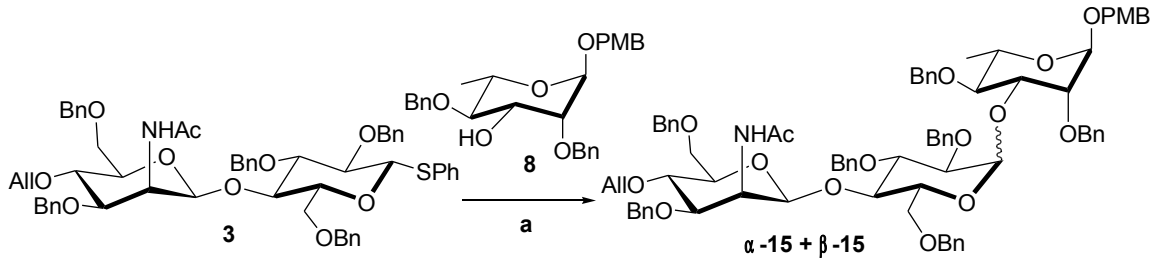
[a]All the reactions were conducted in the presence of 4Å MS (about 150 mg per 0.1 mmol of **3**). [b][Donor]=0.028 M. [c][Donor]=0.056 M. [d]Reaction starts at –15°C

The trisaccharide **α-11**, carrying two orthogonal protecting groups on C-1 and C-4'', is designed to be suitable for the synthesis of oligomers of the *SP* 19F repeating unit. A successful synthesis of hexa- and nonasaccharide phosphorylated fragments of *SP* 19F CPS has already been described by Nilsson and Norberg[6d] using the key intermediate **14**, which **α-11** is easily transformed into (Scheme 4). Exchange of the allyl protecting group at O-4'' to an acetyl group, was accomplished by deprotection with $PdCl_2$ in EtOH-MeOH,[17] obtaining the alcohol **12**, followed by acetylation to give **13** (90% yield over two steps). Removal of the *p*-methoxybenzyl protection group with DDQ in CH_3CN-H_2O then afforded **14** in 85% yield.



Scheme 4: Synthesis of the key intermediate for the preparation of the phosphorylated oligomers of the *SP* 19F CPS repeating unit. Reagents and conditions: (a) $PdCl_2$, MeOH/ EtOH 1:1, room temperature (94%); (b) Ac_2O-Pyridine 1:2, room temperature, 16h (92%); (c) DDQ, CH_3CN-H_2O 9:1, room temperature, 30h (83%).

In the light of these positive results, the synthesis of the trisaccharide repeating unit of *SP* 19A CPS was also attempted by submitting the rhamnosyl acceptor **8** to a glycosylation with disaccharide donor **3** under the same conditions employed for the preparation of **α-11** (Scheme 5). However, in this case the reaction outcome was less satisfactory both in terms of chemical yield (41%) and stereoselectivity (α/β 1:1.3). After chromatography, the pure trisaccharide **α-15** was isolated, although in a modest 18% yield. This result is rather surprising considering the assumed greater reactivity of the equatorial OH-3 of **8** with respect to that of the axial OH-2 of **7**.



Scheme 5. Glycosidation of acceptor **8** with donor **3**. Reagents and conditions: (a) NIS-AgOTf, CH_2Cl_2, 4Å MS, -35 to 10°C (1:1.3 α/β ratio, 43% combined yield).

In conclusion, a new and effective strategy for obtaining a protected derivative of the β-D-MaNAc*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Ham*p* trisaccharide has been developed, using the disaccharide thiophenyl glycosyl donor **3** under appropriate activating conditions. The major novelty of this method, with respect to the previously reported ones,[6,7] is that it avoids the difficult β-mannosamine glycosylation step exploiting the pre-formed β-interglycosidic bond naturally present in lactose. Furthermore, an easy orthogonalization of protecting groups on key positions of the disaccharide donor **3** and the rhamnoside acceptor **7** has been achieved, leading to the previously reported[6d] trisaccharide building block **14** for the synthesis of phosphorylated oligomers of the CPS repeating unit of *SP* 19F.

3. Experimental

3.1 General Methods

Melting points were determined with a Kofler hot-stage apparatus and are

uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at 20±2 °C. NMR were recorded with a Bruker Avance II250 (250.15 MHz for ^{1}H, 63.0 MHz for ^{13}C), respectively) and Varian INOVA 500 (499 MHz for ^{1}H, 125 MHz for ^{13}C) using Me_4Si as internal reference. Assignments were made, when possible, with the aid of DEPT, HETCOR, COSY experiments, and by comparison of values for known compounds. In the case of mixtures, assignments were made by referring to the differences in peak intensities. HRMS were determined with LCT (Liquid Chromatography Time-of-flight) mass spectrometer (Waters Ltd, Micromass MS Technology Centre, Manchester, UK). All reactions were monitored by TLC on Kieselgel 60 F_{254}, with detection by UV light and/or with ethanolic 10% phosphomolybdic or sulphuric acid, and heating. Kieselgel 60 (E. Merck, 70-230 and 230-400 mesh, respectively) was used for column and flash chromatography. Solvents were dried and purified by distillation according to standard procedure,[18] and stored over 4Å molecular sieves activated for at least 24 h at 200 °C. $MgSO_4$ was used as the drying agent for solutions. Donors **3**[10] and **5**[10] and acceptors **7**[11] and **8**[12] were prepared according to literature procedures.

3.2 p-Methoxybenzyl (2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (α-9) and p-methoxybenzyl (2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (β-9)

A mixture of **5** (150 mg, 0.162 mmol), **7** (91 mg, 0.194 mmol) and 4Å molecular sieves (500 mg) in 4:1 (v/v) CH_2Cl_2-Et_2O (5 mL) was stirred for 30 min at room temperature. The suspension was then cooled to 0 °C and MeOTf (89 μL, 0.81 mmol) was added. The reaction mixture was allowed to slowly attain room temperature and stirred overnight, then cooled again to 0 °C and Et_3N (2 mL) was added. After 30 min the mixture was filtered through a short pad of Celite and concentrated. The residue was purified by silica gel flash chromatography (49:1 CH_2Cl_2-Me_2CO) to give **α-9** (103 mg, 50%) and **β-9** (52 mg, 25%).

Data for **α-9**: Colourless syrup; $[\alpha]_D$ + 43.5 (*c* 1.1, $CHCl_3$); ^{1}H-NMR (250 MHz, CD_3CN): δ 7.59-7.16 (m, 37H, Ar-H), 6.92 (m, 2H, Ar-H), 5.78 (ddt, 1H, *J* 5.4, J_{cis} 10.5, J_{trans} 17.3, =C*H*), 5.55 (s, 1H, PhC*H*), 5.16 (dq, 1H *J* 1.7 Hz, J_{trans} 17.3 Hz, =CH_2), 5.16-4.71 (AB system, 2H, $J_{A,B}$ 10.9 Hz PhCH_2), 5.00 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1'), 4.93 (dq, 1H, *J* 1.3, J_{trans} 10.5, =CH_2), 4.88 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1), 4.87-4.70 (AB system, 2H, $J_{A,B}$ 11.1 Hz, PhCH_2), 4.74-4.55 (AB system, 2H, $J_{A,B}$ 12.4 Hz, PhCH_2), 4.71-4.64 (m, 4H, 2 x CH_2), 4.49-4.31 (AB system, 2H, $J_{A,B}$ 11.8 Hz, PhCH_2), 4.40 (d, 1H, $J_{1'',2''}$ 7.6 Hz, H-1''), 4.22 (m, 1H, H-4''), 4.21-4.13 (m, 3H, CH_2O and H-5'), 4.06 (dd, 1H, $J_{2,3}$ 3.0 Hz, H-2), 4.03 (m, 1H, H-6a''), 3.98 (m, 1H, H-6b''), 3.92-3.80 (m, 3H, H-6a', H-6b', H-4'), 3.76 (dd, 1H, $J_{3,4}$ 9.0, H-3), 3.74 (s, 3H, OCH_3), 3.60 (q, 1H, $J_{5,6}$ 6.1 Hz, H-5), 3.50 (dd, 1H, $J_{4,5}$ 9.3 Hz, H-4), 3.48-3.38 (m, 3H, H-3', H-3", H-2"), 3.34 (dd, 1H, $J_{2',3'}$ 9.7 Hz, H-2'), 3.16 (m, 1H, H-5''), 1.22 (d, 3H, H-6). ^{13}C-NMR (63 Mhz, CD_3CN): δ160.2 (MeOAr*C*), 140.7-139.5 (Ar-*C*), 136.6 (=*C*H), 130.6-127.3 (Ar*C*H), 116.3 (=*C*H_2), 114.6 (MeOAr*C*HCH_2), 103.6 (C-1"), 101.6 (Ph*C*H), 97.8 (C-1), 97.7 (C-1'), 80.6, 80.2, 79.6, 78.9 (C-2', C-3', C-2", C-3"), 80.5 (C-4), 79.6 (C-3), 78.0 (C-4'), 75.9 (C-2), 75.6, 75.4, 73.6, 73.1, 72.0, 71.9, 69.3 (Ph*C*H_2, MeOPh*C*H_2), 74.2 (*C*H_2O), 73.9 (C-4"), 71.6 (C-5'),

69.7 (C-6”), 69.2 (C-5), 68.9 (C-6’), 67.2 (C-5”), 55.8 (O*C*H$_3$), 18.4 (C-6). Anal. Calcd for $C_{78}H_{84}O_{16}$: C, 73.33; H, 6.63; Found: C, 73.29; H, 6.66.

Data for **β-9**: Colourless syrup; $[\alpha]_D$ + 11.0 (*c* 1.0, $CHCl_3$); ^{1}H-NMR (250 MHz, CD_3CN): δ 7.58-7.13 (m, 37H, Ar-H), 6.89 (m, 2H, Ar-H), 5.94 (ddt, 1H, *J* 5.4, J_{cis} 10.5, J_{trans} 17.3, =C*H*), 5.55 (s, 1H, PhC*H*), 5.29 (dq, 1H *J* 1.7 Hz, J_{trans} 17.3 Hz, =CH_2), 5.16-5.09 (AB system, 2H, $J_{A,B}$ 11.0 Hz PhCH_2), 5.13 (dq, 1H, *J* 1.4, J_{trans} 10.5, =CH_2), 4.96 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.81-4.51 (m, 4H, PhCH_2), 4.78-4.69 (AB system, 2H, $J_{A,B}$ 10.9 Hz, PhCH_2), 4.67 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1’), 4.49 (d, 1H, $J_{1'',2''}$ 8.2 Hz, H-1’’), 4.43-4.35 (AB system, 2H, $J_{A,B}$ 12.0 Hz, PhCH_2), 4.25 (m, 2H, CH_2O), 4.23 (m, 1H, H-4’’), 4.10 (dd, 1H, $J_{2,3}$ 2.9 Hz, H-2), 4.10-3.98 (2m, each 1H, H-6a”, H-6b”), 3.93 (m, 1H, H-4’), 3.88 (m, 2H, H-6a’, H-6b’), 3.83 (dd, 1H, $J_{3,4}$ 9.3, H-3), 3.64 (q, 1H, $J_{5,6}$ 6.1 Hz, H-5), 3.55 (dd, 1H, $J_{3',4'}$ 8.9 Hz, H-3’), 3.50 (t, 1H, $J_{4,5}$ 9.3, H-4), 3.44 (m, 3H, H-5’, H-4”, H-5”), 3.29 (dd, 1H, $J_{2'3'}$ 9.1 Hz, H-2’), 3.19 (m, 1H, H-5”), 1.23 (d, 3H, H-6). ^{13}C-NMR (63 Mhz, CD_3CN): δ160.2 (MeOAr*C*), 140.4-139.6 (Ar-*C*), 136.6 (=*C*H), 130.7-127.3 (Ar*C*H), 116.4 (=*C*H_2), 114.6 (MeOAr*C*HCH_2), 105.1 (C-1’), 103.6 (C-1”), 101.6 (Ph*C*H), 99.2 (C-1), 83.4 (C-3’), 82.3 (C-2’), 81.1 (C-4), 80.3 (C-3), 80.2 (C-2”), 79.0 (C-3”), 78.0 (C-4’), 77.1 (C-2), 75.6 (C-5’), 75.6, 75.5, 75.0, 73.7, 72.4, 71.9, 69.1 (Ph*C*H_2, MeOPh*C*H_2), 74.0 (*C*H_2O), 73.9 (C-4”), 69.7 (C-6”), 69.2 (C-6’), 68.8 (C-5), 67.2 (C-5”), 55.8 (*C*H_3O), 18.4 (C-6). Anal. Calcd for $C_{78}H_{84}O_{16}$: C, 73.33; H, 6.63; Found: C, 73.30; H, 6.67.

3.4 *p*-Methoxybenzyl (2-*O*-allyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (α-10) and *p*-methoxybenzyl (2-*O*-allyl-3-*O*-

benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (β-10)

A mixture of **5** (498 mg, 0.537 mmol), **8** (300 mg, 0.645 mmol), and 4Å molecular sieves (1.500 g) in 4:1 (v/v) CH_2Cl_2-Et_2O (15 mL) was stirred for 30 minutes at room temperature then cooled to 0 °C and MeOTf (267 μL, 2.43 mmol) was added. The reaction mixture was allowed to slowly attain room temp and stirred overnight and was then cooled again to 0 °C and Et_3N (5 mL) was added followed by stirring for 30 min. After filtration of the mixture through a short pad of Celite and concentration under reduced pressure, the residue was purified by silica gel flash chromatography (13:7 n-hexane-EtOAc) to give two fractions. The first fraction was constituted by **β-10** and unreacted **8** (396 mg total mass) and the second one by pure **α-10** (333 mg, 49%). The first fraction was subjected to acetylation by treatment with a Ac_2O-pyridine mixture (1:2 v/v, 18 mL). After 17 h the solution was repeatedly co-evaporated with toluene and then purified by silica gel flash chromatography (7:3 n-hexane-EtOAc) to give **β-10** (195 mg, 29%).

Data for **α-10**: Colourless syrup; $[\alpha]_D$ + 19.1 (*c* 1.1, $CHCl_3$); R_f 0.24 (13:7 n-hexane-EtOAc); ^{1}H-NMR (250 MHz, CD_3CN): δ 7.59-7.16 (m, 37H, Ar-H), 6.89 (m, 2H, Ar-H), 5.744 (ddt, 1H, *J* 5.3, J_{cis} 10.3, J_{trans} 17.4, =C*H*), 5.54 (s, 1H, PhC*H*), 5.24 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1'), 5.17-4.83 (AB system, 2H, $J_{A,B}$ 11.4 Hz PhCH_2), 5.12 (dq, 1H *J* 1.6 Hz, J_{trans} 17.5 Hz, =CH_2), 4.99 (dq, 1H, *J* 1.4, J_{trans} 10.5, =CH_2), 4.93-4.58 (AB system, 2H, $J_{A,B}$ 11.2 Hz, PhCH_2), 4.89 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.69-4.53 (AB system, 2H, $J_{A,B}$ 11.5 Hz, PhCH_2), 4.68-4.47 (AB system, 2H, $J_{A,B}$ 12.2 Hz, PhCH_2), 4.67-4.59 (AB system, 2H, $J_{A,B}$ 12.0 Hz, PhCH_2), 4.63-4.56 (AB system, 2H, $J_{A,B}$ 11.5 Hz, PhCH_2), 4.49-4.37 (AB

system, 2H, $J_{A,B}$ 12.0 Hz, PhCH_2), 4.44 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1''), 4.21 (m, 1H, H-4'), 4.08 (dd, 1H, $J_{5',6'b}$ 2.9 Hz, $J_{6'a,6'b}$ 9.4 Hz, H-6'b), 4.06-3.82 (m, 9H, CH_2O, H-6"a, H-6"b, H-3', H-4', H-5', H-6'a, H-2), 3.78 (s, 3H, CH_3O), 3.67 (q, 1H, $J_{5,6}$ 6.1 Hz, H-5), 3.55 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-3), 3.53 (t, 1H, $J_{3,4}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz, H-4), 3.52 (dd, 1H, $J_{2',3'}$ 9.6 Hz, H-2'), 3.39 (m, 2H, H-2", H-3"), 3.17 (m, H-5"), 1.23 (d, 3H, H-6). ^{13}C-NMR (63 Mhz, CD_3CN): δ160.2 (MeOAr*C*), 140.5-139.4 (Ar-*C*), 136.4 (=*C*H), 130.6-127.3 (Ar*C*H), 116.1 (=*C*H_2), 114.6 (MeOAr*C*HCH_2), 104.1 (C-1''), 101.5 (Ph*C*H), 97.7 (C-1), 94.1 (C-1'), 80.9 (C-3'), 80.7 (C-4), 80.2-79.1 (C-2', C-3, C-2'', C-3"), 76.4 (C-4'), 75.9, 75.8, 75.6, 73.8, 73.4, 71.9, 69.4 (Ph*C*H_2, MeOPh*C*H_2), 74.2 (*C*H_2O), 73.8 (C-4"), 71.5 (C-5'), 69.6 (C-6"), 69.1, 69.0 (C-6', C-5), 67.2 (C-5"), 58.8 (*C*H_3O), 18.4 (C-6). Anal. Calcd for $C_{78}H_{84}O_{16}$: C, 73.33; H, 6.63; O, 20.04; Found: C, 73.29; H, 6.68.

Data for **β-10**: $[\alpha]_D$ – 0.75 (*c* 1.0, $CHCl_3$); R_f 0.33 (7:3n-hexane-EtOAc); ^{1}H-NMR (250 MHz, CD_3CN): δ 7.45-7.16 (m, 37H, Ar-H), 6.85 (m, 2H, Ar-H), 5.92 (ddt, 1H, *J* 5.3, J_{cis} 10.5 Hz, J_{trans} 17.2 Hz =C*H*), 5.44 (s, 1H, PhC*H*), 5.27 (dq, 1H *J* 1.8 Hz, J_{trans} 17.2 Hz, =CH_2), 5.15-4.73 (AB system, 2H, $J_{A,B}$ 11.1 Hz PhCH_2), 5.13 (dq, 1H, *J* 1.3, J_{trans} 10.5, =CH_2), 4.93-4.79 (AB system, 2H, $J_{A,B}$ 11.4 Hz, PhCH_2), 4.87-4.38 (m, 10H, 4 x PhCH_2), 4.83 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 4.48 (d, 1H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.42 (d, 1H, $J_{1'',2''}$ 8.2 Hz, H-1''), 4.28-4.18 (m. 3H, CH_2O, H-4"), 4.22 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 4.10 (dd, 1H, $J_{3,4}$ 9.6 Hz, H-3), 4.05 (m, 5H, H-6"a, H-6"b, H-6'a, H-6'b, H-4'), 3.68 (q, 1H, $J_{5,6}$ 6.1 Hz, H-5), 3.59 (dd, $J_{3',4'}$ 8.8 Hz, H-3'), 3.51-3.39 (m, 4H, H-4, H-3", H-2", H-5'), 3.38 (dd, 1H, $J_{2',3'}$ 9.1 Hz, H-2'), 3.18 (m, 1H, H-5"), 1.24 (d, 3H, H-6). ^{13}C-NMR (63 Mhz, CD_3CN): δ160.2 (MeOAr*C*), 140.2-139.7 (Ar-*C*), 136.6 (=*C*H), 131.5-127.3 (Ar*C*H), 116.5 (=*C*H_2), 114.6 (MeOAr*C*HCH_2), 104.0 (C-1'), 103.7 (C-1'), 101.5 (Ph*C*H), 98.5 (C-1), 84.2 (C-3'), 82.9 (C-2'), 81.4, 79.0 (C-2", C-3"), 80.2 (C-4), 79.8 (C-4'), 79.3

(C-3), 75.9 (C-5'), 75.4, 75.1, 74.1, 73.9, 73.6, 71.9, 69.3 (Ph*C*H_2, MeOPh*C*H_2), 74.4 (*C*H_2O), 73.7 (C-2, C-4"), 69.4 (C-6"), 69.1 (C-6'), 68.4 (C-5), 67.2 (C-5"), 55.8 (*C*H_3O), 18.4 (C-6). Anal. Calcd for $C_{78}H_{84}O_{16}$: C, 73.33; H, 6.63; Found: C, 73.29; H, 6.68.

3.5 *p*-Methoxybenzyl (2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (α-11) and *p*-methoxybenzyl (2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (β-11).

A solution of **3** (62 mg, 0.0641 mmol) and **7** (56 mg, 0.124 mmol) in anhydrous CH_2Cl_2 (2 mL) containing 4ÅMS (500 mg) was stirred under an argon atmosphere at room temperature for 30 min. The solution was cooled to –35 °C and then NIS (22 mg, 0.0978 mmol, 1.52 eq) followed by AgOTf (8 mg, 0.031 mmol, 0.5 eq) were added. The reaction mixture was allowed to slowly attain –10 °C and was stirred for an additional 1h at that temperature, when TLC (1:1 cyclohexane-EtOAc) showed the complete disappearance of the donor (R_f 0.40) and the formation of two spot at R_f 0.67 and 0.49. The reaction mixture was filtered through a short pad of Celite, diluted with CH_2Cl_2, and washed with 10% aq $Na_2S_2O_3$ (5 mL) and sat aq Na_2HCO_3 (5 mL). The aqueous phases were extracted with CH_2Cl_2 and the collected organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue (135 mg) was purified by silica gel flash chromatography (4:1 to 7:3 cyclohexane-EtOAc) to give first **α-11** (44 mg, 59%) followed by **β-11** (13 mg, 17%).

Data for **α-11**: **C**olourless syrup; $[\alpha]_D$ -22.0 (*c* 0.92, $CHCl_3$); ^{1}H-NMR (500 MHz,

$CDCl_3$): δ 7.37-7.16 (m, 37H, ArH); 6.85-6.84 (m, 2H, ArHOMe); 5.79 (m, 1H, CH_2=C*H*); 5.65 (d, 1H, $J_{2',NH}$ 9.6 Hz, N*H*); 5.16-5.07 (m, 2H, CH_2=CH); 4.83 (m, 2H, H-1, H-1'); 4.50 (m, 1H, H-1''). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.4 (*C*O), 159.6 (Ar*C*OMe), 135.0 (CH_2=*C*H), 139.8-138.1 (Ar*C*), 129.6-126.5 (Ar*C*H), 116.3 (*C*H_2=CH), 99.9 (C-1''), 96.8 (C-1'), 96.3 (C-1), 80.9, 80,67, 80.3, 79.5, 79.1 (C-4, C-4'', C-2', C-3, C-3'), 76.0, 75.4, 75.1, 73.7 (C-2, C-3'', C-4', C-5''), 69.7, 68.4 (C-5, C-5'), 68.5, 68.3, 68.2 (C-6', C-6''), 55.3 (O*Me*), 49.9 (C-2''), 23.3 (*C*H_3CONH), 18.04 (C-6). HRMS: Calcd for $C_{80}H_{90}NO_{16}$ $[M+H]^+$: 1320.6260. Found: 1320.6234.

Data for **β-11**: Colourless syrup; $[\alpha]_D$ -8.0 (*c* 0.84, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.34-7.13 (m, 37H, Ar*H*), 6.84-6.82 (m, 2H, Ar*H*OMe), 5.83-5.75 (m, 1H, CH_2=C*H*), 5.70 (d, 1H, $J_{2',NH}$ 9.7 Hz, N*H*), 5.00 (d, 1H, $J_{1,2}$ 1.43 Hz, H-1), 4.66 (m, 1H, H-1''), 4.62 (d, 1H, $J_{1,2}$ 7.38, H-1'), 3.76 (s, 3H, OCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.5 (*C*O), 159.7 (Ar*C*OMe), 135.1 (CH_2=*C*H), 139.8-138.1 (Ar*C*), 129.6-126.5 (Ar*C*H), 116.4 (*C*H_2=CH), 113.8 (ArCHOMe), 104.7 (C-1'), 99.8 (C-1''), 98.6 (C-1), 55.2 (O*Me*), 49.7 (C-2''), 23.4 (*C*H_3CONH), 18.04 (C-6). HRMS: Calcd for $C_{80}H_{90}NO_{16}$ $[M+H]^+$: 1320.6260. Found: 1320.6234.

3.6 *p*-Methoxybenzyl (2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (12)

To a solution of **α-11** (50.0 mg, 0.0379 mmol) in EtOH-MeOH (1:1 v/v, 7.5 mL) $PdCl_2$ (16 mg, 0.0902 mmol) was added and the reaction mixture was stirred. After 45

minutes another portion of $PdCl_2$ (21 mg, 0.118 mmol) was added. After 1 h and 45 min stirring at room temperature, TLC (2:3 cylcohexane-EtOAc) showed the disappearance of the starting material (R_f 0.9) and the formation of a spot at R_f 0.73. The reaction mixture was filtered through Celite, diluted with CH_2Cl_2 and concentrated to give a crude residue (51 mg) that was purified by silica gel flash chromatography (7:3cylcohexane-EtOAc) to give **12** (45 mg, 94%) as a colourless syrup; $[\alpha]_D$ + 8.9 (*c* 0.88, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.84-7.17 (m, 37H, Ar*H*), 6.85-6.84 (m, 2H, Ar*H*OMe), 5.58 (d, 1H, $J_{2',NH}$ 9.69 Hz, N*H*), 4.84 (m, 2H, H-1, H-1'), 4.48 (m, 1H, H-1"), 3.78 (s, 3H, OC*H*$_3$); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.4 (*C*O), 159.31 (Ar*C*OMe), 139.7-137.7 (Ar*C*), 129.6-126.5 (Ar*C*H), 99.8 (C-1"), 96.9 (C-1'), 96.3 (C-1), 80.6, 80.3, 80.2, 79.5, 79.1, 75.8, 75.1, 75.0 (C-2', C-3, C-3', C-4, C-3", C-2, C-4', C-5"), 69.7, 68.4, 67.3(C-5, C-5', C-4"), 69.2, 68.5, 68.2 (C-6', C-6", CH_2 *p*-MeOBn), 55.2 (O*Me*), 49.4 (C-2'), 23.1 (CH_3CO), 18.0 (C-6). HRMS; Calcd for $C_{77}H_{86}NO_{16}$ $[M+H]^+$: 1280.5947. Found: 1280.6001.

3.7 *p*-Methoxybenzyl (2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α-L-rhamnopyranoside (13)

Compound **12** (45 mg, 0.035 mmol) was dissolved in pyridine (2 mL) and acetic anhydride (1 mL) was added. The reaction mixture was monitored by TLC (7:3 cylcohexane-EtOAc) until, after 16 h the staring material (R_f 0.16) had completely disappeared with concomitant formation of a compound with R_f 0.24. The solution was co-evaporated with toluene and the residue was purified by silica gel flash

chromatography (7:3 cylcohexane-EtOAc) to give **13** (42 mg, 92%) as a colourless syrup; $[\alpha]_D$ + 12.0 (*c* 0.99, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.37-714 (m, 37H, ArC*H*), 6.85-6.84 (m, 2H, ArC*H*OMe), 5.63 (d, 1H, $J_{2',NH}$ 9.6 Hz, N*H*), 4.83 (m, 2H, H-1, H-1'), 4.53 (m, 1H, H-1"); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.5 (CH_3*C*ONH), 169.6 (CH_3*C*O), 159.2 (Ar*C*OMe), 139.6-138.7 (Ar*C*), 129.6-126.6 (Ar*C*H), 113.8 (Ar*C*HOMe), 99.4 (C-1"), 96.9 (C-1'), 96.4 (C-1), 80.8, 80.5, 79.7, 79.3 (C-2', C-3, C-3', C-4), 77.4, 76.0, 75.6, 74.1 (C-3", C-2, C-4', C-5"), 70.0, 68.6, 68.3 (C-5, C-5', C-4"), 69.2, 68.8, 68.4 (C-6', C-6", CH_2 *p*-MeOBn), 55.5 (OMe), 49.7 (C-2"), 23.5 (*C*H_3CONH), 21.1 (*C*H_3CO), 18.3 (C-6). HRMS: Calcd for $C_{79}H_{87}NO_{17}$ $[M+H]^+$: 1322.6052. Found: 1322.6123

3.8 (2-Acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3,4-di-*O*-benzyl-α,β-L-rhamnopyranose (14)

A solution of **13** (40 mg, 0.0302 mmol) in CH_3CN-H_2O (9:1 v/v, 0.5 mL) was portion-wise treated over 29 h with DDQ (88 mg, 0.387 mmol). The reaction was cooled to 0 °C before every addition of DDQ followed by an immediate warming to room temperature. When TLC analysis (1:1 cyclohexane-EtOAc) showed the disappearance of the starting material (30 h) and the formation of two spots at R_f 0.52 and 0.59, the reaction was cooled to 0 °C and Et_3N (1 mL) was added. The mixture was stirred for an additional 10 min, then diluted with CH_2Cl_2 and washed with sat. aq. $NaHCO_3$. The aqueous phase was extracted with CH_2Cl_2 and the collected organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a residue that was subjected to silica gel flash chromatography (7:3 cyclohexane-EtOAc) to give **14** (30 mg,

83% yield) as a colourless syrup. The found data were in full agreement with those already reported in literature.[6d] HRMS: Calcd for $C_{71}H_{79}NO_{16}Na$ $[M+Na]^+$: 1224.5297. Found: 1224.5261.

3.9 *p*-Methoxybenzyl (2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (α-15) and *p*-methoxybenzyl (2-acetamido-4-*O*-allyl-3,6-di-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (β-15)

A solution of **3** (58 mg, 0.06 mmol) and **8** (55 mg, 0.118 mmol) in anhydrous CH_2Cl_2 (2 mL) containing 4Å MS (330 mg) was treated as described above for the synthesis of **α-** and **β-11,** employing NIS (18.12 mg, 0.0806 mmol) and AgOTf (8 mg, 0.031 mmol). After 1h TLC analysis (1:1 cyclohexane-EtOAc) showed the complete disappearance of the donor and the formation of two spots with R_f 0.60 and 0.71. The reaction mixture was filtered through a short pad of Celite, diluted with CH_2Cl_2 and washed with $Na_2S_2O_3$ (10% aq., 7 mL), and $NaHCO_3$ (sat. aq., 6 mL). The aqueous phases were repeatedly extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue (120 mg) was purified by silica gel flash column chromatography (4:1 to 7:3 cyclohexane-EtOAc) to give **α-15** (14 mg, 18%) and **β-15** (18 mg, 23%).

Data for **α-15**: Colourless syrup; $[\alpha]_D$ - 6.0 (*c* 1.5, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.39-7.11 (m, 37H, Ar*H*), 6.85-6.84 (m, 2H, ArC*H*OMe), 5.86-5.78 (m, 1H, CH_2=C*H*),

5.58 (d, 1H, $J_{2',NH}$ 9.59 Hz, N*H*), 4.87 (d, 1H, $J_{1',2'}$ 3.25 Hz, H-1'), 4.77 (d, 1H, $J_{1,2}$ 4.08 Hz, H-1); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 170.3 (*C*O), 159.3 (Ar*C*OMe), 139.5-137.9 (Ar*C*), 135.0 (CH_2=*C*H), 129.5-126.6 (ArC), 116.2 (*C*H_2=CH), 113.8 (Ar*C*HOMe), 99.6 (C-1''), 97.2 (C-1'), 96.3 (C-1), 55.3 (OMe), 49.8 (C-2"), 23.1 (*C*H_3CO), 18.1 (C-6). HRMS: Calcd for $C_{80}H_{90}NO_{16}$ $[M+H]^+$:1320.6260. Found: 1320.6234.

Data for **β-15**: Colourless syrup; $[\alpha]_D$ -23.8 (*c* 1.6, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.38-7.12 (m, 37H, Ar*H*), 6.81-6.79 (m, 2H, Ar*H*OMe), 5.83-5.75 (m, 1H, CH_2=C*H*), 5.66 (d, 1H, $J_{2',NH}$ 9.66 Hz, N*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (*C*O), 159.3 (Ar*C*OMe), 139.2-138.0 (Ar*C*), 134.9 (CH_2=*C*H), 129.3-126.7 (ArC), 116.4 (*C*H_2=CH), 113.7 (Ar*C*HOMe), 103.6 (C-1'), 99.9 (C-1"), 97.7 (C-1), 55.3 (OMe), 49.7 (C-2"), 23.3 (*C*H_3CO), 17.9 (C-6). HRMS: Calcd for $C_{80}H_{90}NO_{16}$ $[M+H]^+$: 1320.6260; Found 1320.6234.

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