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Synthesis of oligogalacturonates conjugated to BSA

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Dedicated to the memory of Professor Christian Pedersen

Abstract—The synthesis of three oligogalacturonates with an aldehyde spacer attached at the reducing end is described. Trigalacturonates α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→O(CH$_2$)$_7$CHO and α-D-Gal*p*A(Me)-(1→4)-α-D-Gal*p*A(Me)-(1→4)-α-D-Gal*p*A(Me)-(1→O(CH$_2$)$_7$CHO as well as hexagalacturonate α-D-Gal*p*A-(1→4)-[α-D-Gal*p*A-(1→4)]$_4$-α-D-Gal*p*A-(1→O(CH$_2$)$_7$CHO are prepared by stepwise coupling of galactose units followed by oxidation of the 6-positions. The α-linkages are formed by employing *n*-pentenyl galactosides as glycosyl donors and *N*-iodosuccinimide/triethylsilyl triflate as the promoter. Deprotection furnishes the three target oligogalacturonates, which are subsequently linked to bovine serum albumin by reductive amination. These neoglycoproteins will serve as immunogens for generation of new antibodies that can be used for localization and characterization of pectin in plants.

Keywords: Antigen; Galactose; Neoglycoprotein; Pectin; Pentenyl glycoside

1. Introduction

Pectin is an important constituent of the primary cell wall of higher plants. It is composed of 'smooth' homogalacturonan regions and 'hairy' rhamnogalacturonan regions.[1] The function and chemical composition of pectin in plant cell walls, however, is still relatively poorly understood. In order to answer these questions, a number of antibodies binding to various pectic polysaccharides have been prepared. JIM5,[2] JIM7,[2] and LM7[3] recognize partly methylated homogalacturonan, while PAM1[4] is specific for longer stretches of unesterified homogalacturonan. The epitopes have not been characterized in greater detail.[5,6] These antibodies have been raised from complex polysaccharide mixtures, obtained after isolation from plants. This has given the process a somewhat random outcome, since it has been difficult to control the characteristics of the antibodies derived from such immunizations. Recently, however, Knox and co-workers have been able to tailor their immunizations, resulting in antibodies against well-defined haptens. Two antibodies, LM5[7] and LM6,[8] were both raised against synthetic oligosaccharides conjugated to bovine serum albumin (BSA) through the reducing end aldehyde. LM5 and LM6 are specific for the galactan and arabinan sidechains in the hairy regions of pectin. Since homogalacturonan is the most abundant of the pectic polysaccharides, antibodies recognizing parts of this structure are particularly useful. Unfortunately, it has so far not been possible to attach small oligogalacturonates directly to BSA through the reducing end aldehyde. As a result, we envisioned that well-defined synthetic oligogalacturonates could be covalently linked to BSA through a spacer and used for immunization studies. Three different target structures were chosen for these studies: a nonesterified trigalacturonate, a nonesterified hexagalacturonate as well as a fully methyl-esterified trigalacturonate. These three structures were selected based on their difference in size and methyl esterification.

2. Results and discussion

2.1. Synthetic planning

A number of elements required consideration in order to conjugate oligogalacturonates to BSA. The conjugation

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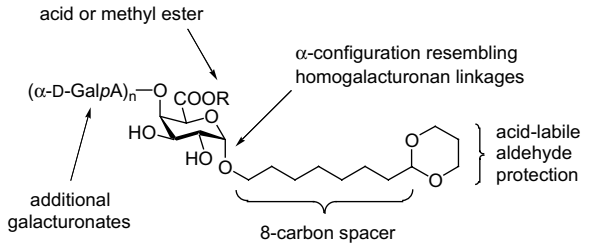
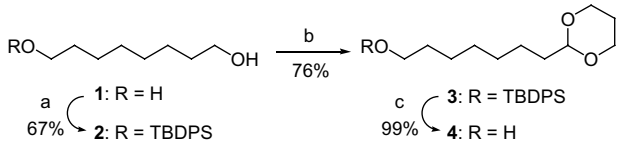


Chart 1. Target oligogalacturonate with spacer.



Scheme 1. Reagents and conditions: (a) TBDPSCl, DIPEA, DMF, rt, 3 h; (b) (1) Me_2SO, $(COCl)_2$, CH_2Cl_2, −70 °C, 30 min, then DIPEA, −70 °C to rt; (2) propane-1,3-diol, TsOH, 4 Å MS, hexane, rt, 10 h; (c) TBAF, THF, rt, 1 h.

method should be compatible with the carboxylic acid and ester groups in the sugar haptens. Furthermore, conjugation of the methyl-esterified trigalacturonate should be performed at neutral pH, since basic conditions will degrade this molecule by β-elimination. Therefore, reductive amination was chosen as the conjugation method.[9] It offers the possibility of conjugating up to 60 sugar moieties to a single protein molecule, because BSA contains 59 lysine residues as well as the *N*-terminal amino group.[10] The use of a hydrocarbon spacer at the reducing end of the carbohydrate hapten was pioneered by Lemieux et al.[11] This prevents the alteration of the reducing end sugar, which occurs when direct conjugation to the reducing end aldehyde is used. Furthermore, the method minimizes the risk of introducing a stronger immunogen close to the sugar, because this type of spacer contains no functional groups in the vicinity of the carbohydrate. Hence, it was planned to synthesize a protected oligogalacturonate with an aglycon spacer containing a masked aldehyde. This will be prepared by repeated coupling of galactose glycosyl donors to a galactose acceptor containing the spacer followed by a single oxidation of all the 6-positions to carboxylic acids. The glycosylations will be performed with *n*-pentenyl galactosides as glycosyl donors in line with our previously developed strategy for assembling oligogalacturonates.[12,13] Conjugation to BSA will then be carried out after removal of the aldehyde protecting group (Chart 1).

In designing the linker, several elements were taken into consideration: an α-linkage was desirable because it would resemble the linkages in native homogalacturonan. The spacer was intended to be eight carbons long, in order to effectively distance the sugar hapten from the protein. Finally, a 1,3-dioxane was chosen as blocking group for the aldehyde, because it is quite stable to acidic conditions[14] thus preventing premature unveiling of the masked aldehyde during the assembly of the oligogalacturonate.

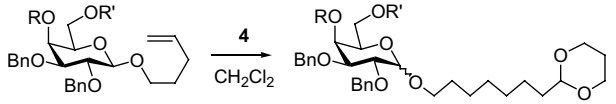
2.2. Attaching the spacer

The protected hydroxyaldehyde **4**, needed as the aglycon, was prepared from octane-1,8-diol (**1**) (Scheme 1). Monoprotection was performed by treatment with *tert*-butyldiphenylsilyl chloride in a mixture of DMF and Hünig's base.[15] Swern oxidation[16] of the remaining primary alcohol in **2** gave the aldehyde, which was trapped by acid-catalyzed acetal formation in the presence of molecular sieves[17] to produce **3**. Lastly, the silyl group was removed quantitatively by treatment with tetrabutylammonium fluoride.

The coupling to galactose was then investigated (Table 1). With the fully protected pentenyl donor **5**,[12] a 1:3.5 ratio was obtained under standard conditions favoring the β-linked product **6β** (entry 1). Cooling the reaction mixture to −78 °C and using stoichiometric amounts of triflic acid as the promoter provided even less of the desired **6α** (entry 2). This is quite surprising since donor **5** has previously given good α-selectivities in couplings to other monosaccharides.[12] Adding ether as co-vent did not significantly alter these ratios. The anomeric configuration of **6α,β** were determined by NMR. The ^{1}H NMR spectrum of **6α** showed H-1 at 4.80 ppm ($J_{H\text{-}1,H\text{-}2}$ 3.7 Hz) while ^{1}H in **6β** was observed at 4.37 ppm ($J_{H\text{-}1,H\text{-}2}$ 7.5 Hz). The ^{13}C NMR spectrum of **6α** showed C-1 at 97.9 ppm ($J_{C\text{-}1,H\text{-}1}$ 172 Hz) while C-1 in **6β** appeared at 102.6 ppm ($J_{C\text{-}1,H\text{-}1}$ 160 Hz).[18]

It seems that **4** is too reactive under these conditions and therefore it was decided to change the coupling method. Lemieux et al. showed that glycosyl bromides could serve as donors when activated with bromide

Table 1. Attaching spacer to galactose

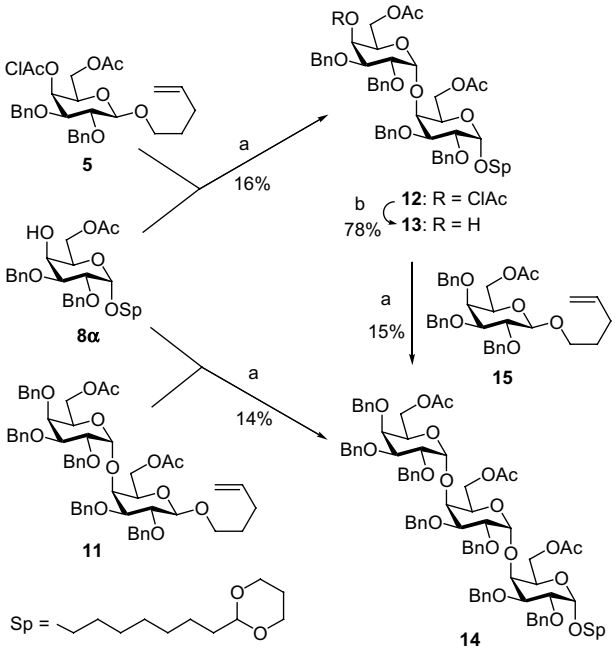


5: R = ClAc, R' = Ac
7: R = H, R' = Ac
9: R = H, R' = PMP

6: R = ClAc, R' = Ac
8: R = H, R' = Ac
10: R = H, R' = PMP

Entry	Donor	Conditions	Yield (%)	α:β
1	**5**	NIS, TESOTf, −20 °C	68	1:3.5
2	**5**	NIS, TfOH, −78 °C	50	1:5.5
3	**7**	Br_2, then Bu_4NI, 20 °C	5	95:5
4	**7**	Br_2, then Et_4NBr, 20 °C	10	>95:5
5	**7**	Br_2, then Et_4NBr, 0–20 °C	25	>95:5
6	**7**	Br_2, then Et_4NBr, 4 Å MS, 0–20 °C	56	>95:5
7	**9**	Br_2, then Et_4NBr, 4 Å MS, 0–20 °C	70	>95:5

ions.[19] Under these conditions, an equilibrium is established between the α- and β-glycosyl bromide, which heavily favors the α-bromide, but the β-bromide is much more reactive. Consequently, only the equatorial bromide reacts with the acceptor, giving rise to the α-glycoside. This method does have limitations, since reaction times are usually rather long, and sterically hindered alcohols cannot be glycosylated in this fashion. However, for the purpose of glycosylating the primary alcohol **4** it was very appropriate, especially since it would not be necessary to protect the less reactive 4-hydroxy group of the donor. The glycosyl bromides were prepared from the corresponding pentenyl glycosides by brominolysis[20] and then subjected directly to the halide mediated glycosylation in the same pot. Initial results with the donor **7**[13] were promising in terms of α-selectivity, albeit the yields were low (entries 3 and 4). Lowering the temperature gave minor improvements (entry 5), but a satisfactory yield was reached only when 4 Å molecular sieves were added (entry 6). The reaction was even more efficient in the case of the PMP-protected donor **9**[13] (PMP = *p*-methoxyphenyl, entry 7), an observation that soon proved important (vide infra).



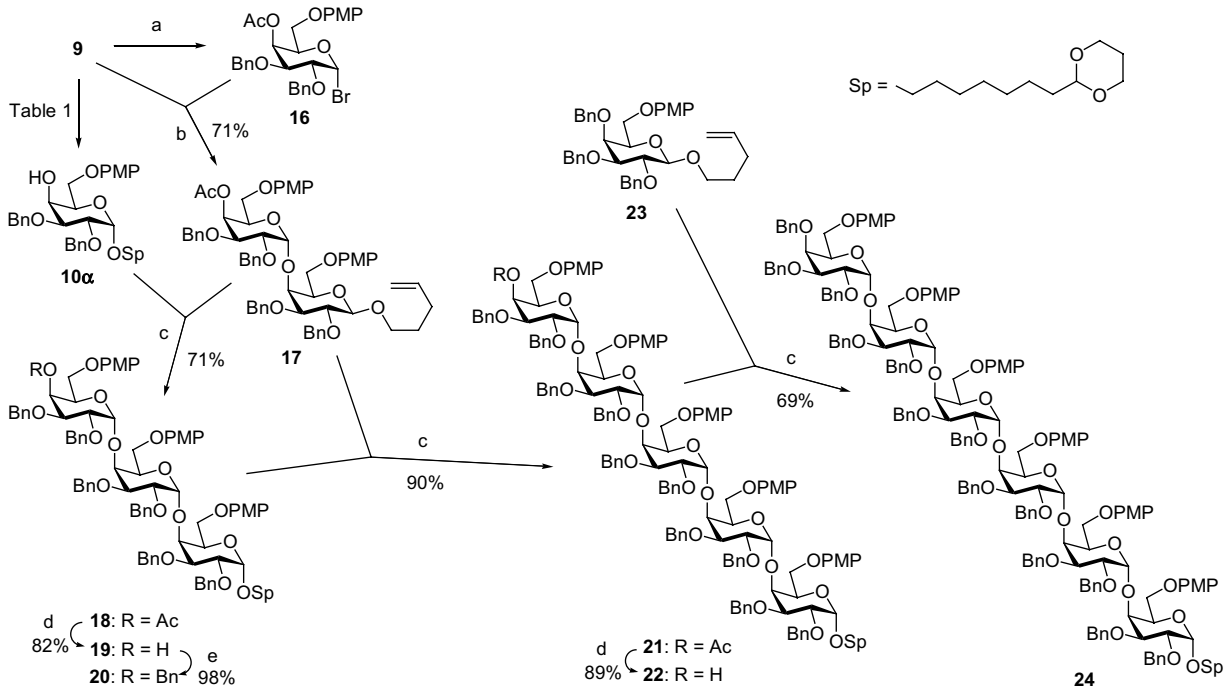
Scheme 2. Reagents and conditions: (a) NIS, TESOTf, CH_2Cl_2, 0 °C, 30 min; (b) thiourea, Bu_4NI, $NaHCO_3$, THF, 55 °C, 24 h.

2.3. Synthesis of neoglycoproteins

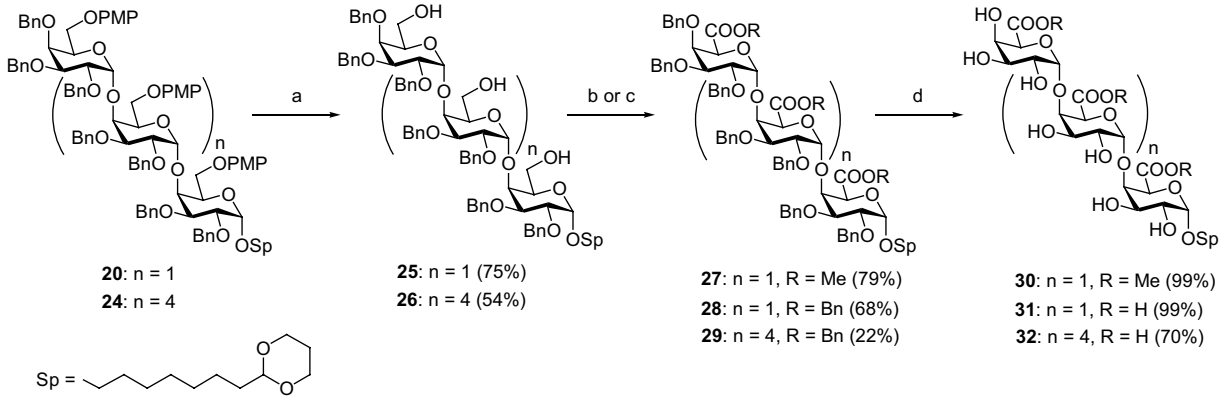
Having prepared the required α-linked spacer-monosaccharide building block **8α**, the stage was now set for targeting the desired tri- and hexasaccharide neoglycoproteins. Unfortunately, glycosylation of the acceptor **8α** with donors **5** and **11**[13] proved surprisingly difficult giving **12** in only 16% yield and **14** in a mere 14% (Scheme 2). None of the corresponding β-linked saccharides were isolated. An attempt to further elaborate **12** into **14** by selective removal of the chloroacetyl group and glycosylation of the resulting disaccharide with **15**[12] resulted in another disappointing result. In all three cases, the donors and acceptors were protected with an acetyl group at the 6-position. Unexpectedly, there seems to be a mismatch between these donors and acceptors, which required the coupling partners to be changed.

Fortunately, this could easily be achieved by using the PMP-protected building blocks (Scheme 3). Initially, disaccharide donor **17** was prepared from **9** by Koenigs–Knorr coupling with bromide **16**, which is also obtained from **9**. The PMP-protected monosaccharide **10α** was then glycosylated with **17** affording the desired α-linked trisaccharide **18** in 71% yield. At this point, bifurcation of the product gave access to two trisaccharides—one that could be taken on to conjugation and one that served as acceptor in the synthesis of the hexasaccharide. Simple deacetylation of **18** yielded alcohol **19**, which upon standard benzylation gave fully protected trisaccharide **20**. Alternatively, alcohol **19** was used as an acceptor and subjected to another coupling with **17**. The product **21**, obtained in an excellent 90% yield, was deprotected to **22** and glycosylated with monosaccharide donor **23**,[12] furnishing hexasaccharide **24** in 69% yield. With **20** and **24** in hand, the stage was set for elaboration to the oligogalacturonates.

CAN-mediated oxidative cleavage of the PMP-groups of **20** and **24** furnished **25** and **26** and was followed by oxidations[12] and esterifications (Scheme 4). For the trigalacturonate both methyl and benzyl esters were desired, giving access to either a fully methylated or a nonmethylated conjugate. Furthermore, it was anticipated that benzyl esterification would facilitate handling and purification, especially for the hexagalacturonate. Thus oxidation to carboxylic acid was performed by a two step procedure using the Dess–Martin periodinane[21] followed by sodium chlorite oxidation. The acids were not purified, but immediately subjected to esterification with cesium carbonate and methyl iodide or benzyl bromide. Hereby, **27** and **28** were obtained in 37 and 42% yield, respectively. Some degradation was observed in both cases, and this proved to be an even more severe problem for the synthesis of hexabenzyl ester **29**, which could be isolated in only 17% yield under these conditions. For the preparation of **27** and **28**, a considerable improvement could be obtained by performing the esterifications under neutral conditions.[13] The use of trimethylsilyl diazomethane for methyl esterification afforded **27** in 79% yield while the tribenzyl ester **28** was obtained in 68% yield by applying phenyl diazomethane. The neutral esterification conditions only led to a slight improvement in the yield of **29** and the



Scheme 3. Reagents and conditions: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2, rt, 16 h, then Br_2, CH_2Cl_2, 0 °C, 15 min; (b) AgOTf, 4 Å MS, CH_2Cl_2, −45 °C, 2 h; (c) NIS, TESOTf, CH_2Cl_2, −20 °C, 30 min; (d) NaOMe, THF, MeOH; (e) BnBr, NaH, Bu_4NI, DMF, rt, 2 h.



Scheme 4. Reagents and conditions: (a) CAN, MeCN, H_2O, 0 °C, 20 min; (b) Dess–Martin periodinane, CH_2Cl_2, rt, 45 min, then $NaClO_2$, NaH_2PO_4, THF, *t*-BuOH, H_2O, rt, 1 h, then $TMSCHN_2$, MeOH, rt, 2 h; (c) Dess–Martin periodinane, CH_2Cl_2, rt, 1 h, then $NaClO_2$, NaH_2PO_4, THF, *t*-BuOH, H_2O, rt, 1 h, then $PhCHN_2$, EtOAc, rt, 2 h; (d) H_2, Pd/C, THF, MeOH, H_2O.

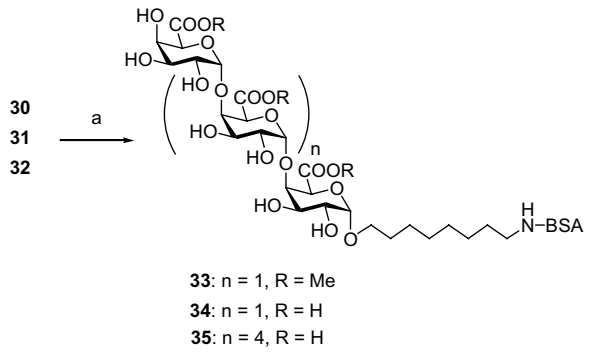
multistep sequence was still plagued by considerable decomposition.

Hydrogenolysis of the fully protected oligogalacturonates proceeded uneventfully. After treatment with aqueous acetic acid to unmask the aldehyde the sugars could be attached to BSA by reductive amination with sodium cyanoborohydride (Scheme 5). According to MALDI-TOFMS data the resulting glycoconjugates **33**, **34**, and **35** contained on average 22.6, 11.5, and 3.8 mol ligand/mol protein. The successful synthesis of the three neoglycoproteins **33**–**35** will now permit the first attempts to raise antibodies toward well-defined homogalacturonan fragments.

3. Experimental

3.1. General procedures

CH_2Cl_2 was distilled from CaH_2. Thin-layer chromatography was performed on aluminum plates precoated with silica gel. Compounds were visualized by heating after dipping in a soln of $Ce(SO_4)_2$ (2.5 g) and $(NH_4)_6Mo_7O_{24}$ (6.25 g) in 10% aq H_2SO_4 (250 mL). Flash chromatography was performed with Silica Gel 60. NMR spectra were recorded on a Varian Unity Inova 500 or a Varian Mercury 300 spectrometer. Optical rotations were measured on a Perkin–Elmer 241 pola-



Scheme 5. Reagents and conditions: (a) AcOH, H_2O, 50 °C, 12 h, then BSA, $NaCNBH_3$, H_2O, rt, 4 d.

rimeter. ESI mass spectra were obtained at the Department of Biochemistry and Molecular Biology, University of Southern Denmark using an Esquire-LC instrument. MALDI-TOF mass spectra were obtained at Danisco Biotechnology in Copenhagen using a Perceptive Biosystems Voyager-De instrument in positive-ion mode with α-cyano-4-hydroxycinnamic acid as the matrix. Microanalyses were conducted at the Department of Chemistry, University of Copenhagen.

3.2. 8-(*tert*-Butyldiphenylsilyloxy)-octan-1-ol (2)

Octane-1,8-diol (**1**) (5.0 g, 34.2 mmol) was disved in DMF (80 mL) and DIPEA (60 mL, 344 mmol). TBDPSCl (5.84 mL, 22.5 mmol) was added dropwise and the reaction mixture was stirred for 3 h at rt. Water (500 mL) was added and the mixture was extracted with Et_2O (3×500 mL). The combined organic phases were washed with 2 M aq HCl (2 ×300 mL) and satd aq $NaHCO_3$ (500 mL), dried, concentrated and purified by flash chromatography (3:1 hexanes–EtOAc), yielding 5.81 g (67%) of **2** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73–7.67 (m, 4H), 7.47–7.36 (m, 6H), 3.69 (t, 2H, *J* 6.4 Hz), 3.64 (t, 2H, *J* 6.6 Hz), 1.81 (br s, 1H), 1.65–1.51 (m, 5H), 1.44–1.26 (m, 7H), 1.09 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.69 (4C), 134.33 (2C), 129.58 (2C), 127.67 (4C), 64.14, 63.21, 32.96, 32.72, 29.53, 29.48, 27.06 (3C), 25.87, 25.52, 19.40. Anal. Calcd for $C_{24}H_{36}O_2Si$: C, 74.95; H, 9.43. Found: C, 74.76; H, 9.45.

3.3. 2-(7-(*tert*-Butyldiphenylsilyloxy)-heptyl)-1,3-dioxane (3)

A soln of anhyd Me_2SO (4.11 mL, 57.9 mmol) in anhyd CH_2Cl_2 (50 mL) under Ar was cooled to −70 °C and oxalyl chloride (2.43 mL, 27.9 mmol) was added dropwise. After 15 min, a soln of **2** (5.50 g, 14.3 mmol) in CH_2Cl_2 (30 mL) was added slowly, keeping the temperature below −65 °C. After 30 min, DIPEA (24.9 mL, 143 mmol) was added, and the reaction mixture was allowed to reach rt. CH_2Cl_2 (150 mL) was added, and the soln was washed with 1 M aq HCl (2×150 mL) and water (150 mL). The combined aq layers were extracted with CH_2Cl_2 (150 mL) and the combined organic phases were dried and concentrated. The residue was suspended in hexane (150 mL) and 4 Å molecular sieves (5 g) was added, followed by propane-1,3-diol (2.53 mL, 35.0 mmol) and TsOH (2.58 g, 13.6 mmol). The mixture was stirred for 10 h, then quenched with id $NaHCO_3$ and filtered through Celite. The pad was rinsed with hexane (3×75 mL). The filtrate was washed with 1 M aq NaOH (200 mL), dried, concentrated and purified by flash chromatography (15:1 hexanes–EtOAc) to give 4.80 g (76%) of **3** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.71–7.67 (m, 4H), 7.46–7.35 (m, 6H), 4.52 (t, 1H, *J* 5.1 Hz), 4.15–4.07 (m, 2H), 3.82–3.75 (m, 2H), 3.67 (t, 2H, *J* 6.6 Hz), 2.09 (m, 1H), 1.65–1.52 (m, 4H), 1.45–1.25 (m, 9H), 1.07 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 135.66 (4C), 134.30 (2C), 129.55 (2C), 127.66 (4C), 102.56, 67.00 (2C), 64.11, 35.38, 32.69, 29.60, 29.39, 27.04 (3C), 26.03, 25.81, 24.06, 19.37. Anal. Calcd for $C_{27}H_{40}O_3Si$: C, 73.59; H, 9.15. Found: C, 73.55; H, 9.20.

3.4. 7-(1,3-Dioxan-2-yl)-heptan-1-ol (4)

A soln of **3** (2.30 g, 5.22 mmol) in anhyd THF (30 mL) was treated with a 1 M soln of TBAF in THF (7.83 mL, 7.83 mmol). Concentrated after 1 h and purified by flash chromatography (2:1 hexanes–EtOAc) yielding 1.05 g (99%) of **4** as a colorless oil. Bp 112–120 °C/0.2 mmHg. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.50 (t, 1H, *J* 5.1 Hz), 4.14–4.06 (m, 2H), 3.81–3.70 (m, 2H), 3.63 (t, 2H, *J* 6.6 Hz), 2.07 (m, 1H), 1.63–1.50 (m, 4H), 1.45–1.25 (m, 9H), 1.21 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 102.33, 66.77 (2C), 62.52, 35.09, 32.64, 29.35, 29.23, 25.78, 25.60, 23.78. ESIMS: *m/z* 203.2 $[M+H]^+$. Anal. Calcd for $C_{11}H_{22}O_3$: C, 65.31; H, 10.96. Found: C, 64.96; H, 11.01.

3.5. General procedure for the pentenyl glycoside couplings with NIS/TESOTf

A mixture of the donor (6.5 mmol) and the acceptor (5.0 mmol) was dried azeotropically with toluene and subjected to high vacuum for 2 h. The mixture was disved in anhyd CH_2Cl_2 (60 mL), cooled to −20 °C, followed by addition of NIS (1.49 g, 6.63 mmol) and TESOTf (0.29 mL, 1.3 mmol). The reaction mixture was stirred at −20 °C until TLC revealed full conversion of the donor (15–45 min). The soln was diluted with CH_2Cl_2 (60 mL) and washed with 10% aq $Na_2S_2O_3$ (100 mL) and satd aq $NaHCO_3$ (100 mL). The combined aq phases were extracted with CH_2Cl_2 (100 mL). The combined organic phases were dried ($MgSO_4$), concentrated, and purified by flash chromatography.

3.6. 7-(1,3-Dioxan-2-yl)-heptyl 6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-chloroacetyl-D-galactopyranoside (6)

Coupling of **5** (2.70 g, 4.94 mmol) and **4** (1.28 g, 6.26 mmol) following the general procedure above afforded 0.50 g (15%) of **6α** as a colorless oil and 1.73 g (53%) of **6β** as a colorless oil.

For **6α**: $[\alpha]_D^{20}$ +54.6 (*c* 1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.21 (m, 10H), 5.59 (d, 1H, *J* 1.7 Hz), 4.80 (d, 1H, *J* 3.7 Hz), 4.79 (d, 1H, *J* 12.1 Hz), 4.73 (d, 1H, *J* 11.0 Hz), 4.61 (d, 1H, *J* 12.1 Hz), 4.58 (d, 1H, *J* 11.0 Hz), 4.50 (t, 1H, *J* 5.1 Hz), 4.18–4.04 (m, 5H), 4.10 (br s, 2H), 4.00 (dd, 1H, *J* 10.1, 3.4 Hz), 3.80–3.69 (m, 3H), 3.60 (dt, 1H, *J* 9.5, 7.0 Hz), 3.44 (dt, 1H, *J* 9.9, 6.6 Hz), 2.03 (m, 1H), 2.02 (s, 3H), 1.69–1.53 (m, 4H), 1.42–1.21 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.58, 167.14, 138.64, 138.11, 128.54 (4C), 128.23 (2C), 128.06 (2C), 127.93, 127.90, 102.58, 97.87 (J_{CH} 172 Hz), 76.06, 75.68, 73.63, 72.79, 70.35, 68.89, 67.10 (2C), 66.53, 62.38, 41.01, 35.47, 29.65, 29.58, 29.49, 26.30, 26.11, 24.12, 20.94. ESIMS: *m/z* 663.4 $[M+H]^+$. Anal. Calcd for $C_{35}H_{47}ClO_{10}$: C, 63.39; H, 7.14; Cl, 5.35. Found: C, 63.63; H, 6.91; Cl, 5.10.

For **6β**: $[\alpha]_D^{20}$ +14.9 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.22 (m, 10H), 5.52 (d, 1H, *J* 1.8 Hz), 4.86 (d, 1H, *J* 11.0 Hz), 4.74 (d, 1H, *J* 11.3 Hz), 4.70 (d, 1H, *J* 11.0 Hz), 4.53 (d, 1H, *J* 11.3 Hz), 4.48 (t, 1H, *J* 5.1 Hz), 4.37 (br d, 1H, *J* 7.5 Hz), 4.22–4.04 (m, 4H), 4.14 (br s, 2H), 3.92 (dt, 1H, *J* 9.5, 6.4 Hz), 3.82–3.68 (m, 3H), 3.58–3.47 (m, 3H), 2.04 (m, 1H), 2.02 (s, 3H), 1.68–1.52 (m, 4H), 1.44–1.22 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.64, 167.28, 138.64, 137.74, 128.58 (2C), 128.49 (2C), 128.33 (2C), 128.25 (2C), 128.03, 127.87, 103.99, 102.61 (J_{CH} 160 Hz), 79.09, 78.87, 75.57, 72.79, 70.73, 70.56, 68.96, 67.12 (2C), 61.88, 41.07, 35.46, 29.91, 29.63, 29.54, 26.23, 26.12, 24.14, 20.96. ESIMS: *m/z* 663.4 $[M+H]^+$. Anal. Calcd for $C_{35}H_{47}ClO_{10}$: C, 63.39; H, 7.14; Cl, 5.35. Found: C, 63.27; H, 6.98; Cl, 5.17.

3.7. 7-(1,3-Dioxan-2-yl)-heptyl 6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranoside (8α)

Prepared from **7** and **4** as described below for **10α**. Colorless oil. $[\alpha]_D^{20}$ +52.3 (*c* 1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.41–7.24 (m, 10H), 4.87–4.81 (m, 5H), 4.50 (t, 1H, *J* 5.1 Hz, 1H), 4.38–4.20 (m, 2H), 4.14–4.05 (m, 2H), 4.02–3.69 (m, 6H), 3.60 (m, 1H), 3.42 (dt, 1H, *J* 9.9, 6.6 Hz), 2.50 (br s, 1H), 2.06 (s, 3H), 2.05 (m, 1H), 1.70–1.53 (m, 4H), 1.44–1.25 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.77, 138.55, 138.24, 128.56 (2C), 128.44, 128.34, 128.15, 128.06, 127.97 (3C), 127.84, 102.48, 97.35, 77.55, 75.94, 73.34, 73.04, 68.43, 67.92, 67.57, 66.99 (2C), 63.80, 35.32, 29.56, 29.44 (2C), 26.24, 25.98, 24.02, 20.98. ESIMS: *m/z* 609.4 $[M+Na]^+$. Anal. Calcd for $C_{33}H_{46}O_9$: C, 67.56; H, 7.90. Found: C, 67.41; H, 7.94.

3.8. 7-(1,3-Dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (10α)

Glycoside **9** (10.4 g, 21.2 mmol) was dried azeotropically with toluene and subjected to high vacuum for 2 h. It was then disved in anhyd CH_2Cl_2 (40 mL), cooled to 0 °C and titrated with a 1 M soln of Br_2 in anhyd CH_2Cl_2 until a faint yellow color persisted. Powdered molecular sieves 4 Å (10 g) was added, followed by a soln of **4** (2.52 g, 12.5 mmol) and Et_4NBr (5.35 g, 25.5 mmol) in anhyd CH_2Cl_2 (40 mL). The mixture was stirred for 40 h, quenched with satd aq $NaHCO_3$ (50 mL) and then stirred for an additional 4 h. The mixture was filtered through Celite and the pad was rinsed with CH_2Cl_2 (50 mL). The organic phase was separated and washed with water. The combined aq phases were extracted with CH_2Cl_2. The combined organic phases were dried, concentrated and purified by flash chromatography (3:1 hexanes–EtOAc) yielding **10α** (5.71 g, 70%) as a id. Mp 74–76 °C (hexanes–EtOAc). $[\alpha]_D^{20}$ +33.1 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.39–7.26 (m, 10H), 6.88–6.77 (m, 4H), 4.86–4.62 (m, 5H), 4.49 (t, 1H, *J* 5.1 Hz), 4.19–4.05 (m, 5H), 3.93 (dd, 1H, *J* 9.9, 3.3 Hz), 3.85 (dd, 1H, *J* 9.9, 3.7 Hz), 3.80–3.60 (m, 4H), 3.76 (s, 3H), 3.43 (dt, 1H, *J* 9.9, 6.6 Hz), 2.49 (br s, 1H), 2.06 (m, 1H), 1.68–1.49 (m, 4H), 1.43–1.24 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.93, 152.72, 138.52, 138.20, 128.40 (2C), 128.29 (2C), 127.84 (3C), 127.77, 127.74, 127.64, 115.54 (2C), 114.58 (2C), 102.33, 97.25, 77.65, 76.01, 73.14, 72.83, 68.27, 67.99, 67.69 (2C), 66.81 (2C), 55.63, 35.22, 29.44, 29.38, 29.33, 26.09, 25.86, 23.94. ESIMS: *m/z* 673.7 $[M+Na]^+$. Anal. Calcd for $C_{38}H_{50}O_9$: C, 70.13; H, 7.74. Found: C, 70.01; H, 7.77.

3.9. Pent-4-enyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (17)

To a soln of **9** (7.28 g, 13.6 mmol) in CH_2Cl_2 (50 mL) were added Ac_2O (1.7 mL, 18 mmol), Et_3N (3.5 mL, 25 mmol), and DMAP (50 mg). The mixture was stirred overnight and then diluted with CH_2Cl_2 (50 mL), washed with water (50 mL), dried, and concentrated. The syrupy residue was purified by flash chromatography (4:1 hexanes–EtOAc) to afford pent-4-enyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (6.39 g) as a id. Mp 63–65 °C (hexanes–EtOAc). $[\alpha]_D^{20}$ +4.6 (*c* 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.22 (m, 10H), 6.85–6.78 (m, 4H), 5.81 (m, 1H), 5.64 (br s, 1H), 5.07–4.95 (m, 2H), 4.89 (d, 1H, *J* 11.1 Hz), 4.78 (d, 1H, *J* 10.9 Hz),

4.75 (d, 1H, *J* 10.9 Hz), 4.57 (d, 1H, *J* 11.1 Hz), 4.41 (m, 1H), 4.08 (dd, 1H, *J* 10.5, 7.2 Hz), 4.02–3.85 (m, 3H), 3.77 (s, 3H), 3.62–3.54 (m, 3H), 2.18 (m, 2H), 2.09 (s, 3H), 1.77 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 154.4, 152.8, 138.2, 138.0, 137.9, 128.5 (4C), 128.3 (2C), 128.2 (2C), 127.9, 127.8, 116.3 (2C), 114.9 (2C), 114.7, 104.0, 79.5, 78.9, 75.6, 72.3, 71.7, 70.0, 67.8, 67.4, 55.9, 30.3, 29.1, 21.4. Anal. Calcd for $C_{34}H_{40}O_8$: C, 70.81; H, 6.99. Found: C, 70.86; H, 6.83.

A soln of this compound (6.50 g, 11.3 mmol) in anhyd CH_2Cl_2 (30 mL) was titrated at 0 °C with a 1 M soln of Br_2 in anhyd CH_2Cl_2 until a faint yellow color persisted. The soln was then cannulated into a mixture of **9** (4.30 g, 8.04 mmol), AgOTf (4.40 g, 17.1 mmol), and flame dried powdered 4 Å molecular sieves (10 g) in anhyd CH_2Cl_2 (30 mL) at −45 °C. The reaction mixture was stirred at −45 °C for 2 h and then quenched by addition of satd aq $NaHCO_3$ (50 mL), allowed to reach room temperature and filtered through Celite. The pad was rinsed with CH_2Cl_2 (50 mL). The organic phase was separated and washed with satd aq $NaHCO_3$ (50 mL) and water (50 mL). The combined aq layers were extracted with CH_2Cl_2 (25 mL). The combined organic phases were dried, concentrated, and purified by flash chromatography (5:1 hexanes–EtOAc) to give **17** (5.83 g, 71%) as a syrup. $[\alpha]_D^{20}$ +25.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39–7.12 (m, 20H), 6.77–6.56 (m, 8H), 5.82 (m, 1H), 5.74 (br s, 1H), 5.08–4.91 (m, 3H), 4.83–4.60 (m, 7H), 4.58–4.44 (m, 3H), 4.39 (d, 1H, *J* 10.3 Hz), 4.17 (d, 1H, *J* 2.1 Hz), 4.13 (dd, 1H, *J* 11.1, 2.0 Hz), 4.01–3.90 (m, 2H), 3.79–3.44 (m, 7H), 3.73 (s, 3H), 3.70 (s, 3H), 2.12 (m, 2H), 1.97 (s, 3H), 1.79 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.91, 153.77 (2C), 152.44, 152.22, 138.49, 138.33, 138.17, 138.07, 137.92, 115.49 (2C), 115.22 (2C), 114.84, 114.48 (2C), 114.25 (2C), 104.01, 100.50, 80.59, 78.46, 76.29, 75.06, 74.85, 73.20, 72.90, 72.74 (2C), 71.62, 69.38, 67.69, 67.27, 66.18, 65.29, 55.55 (2C), 30.11, 28.87, 20.73. ESIMS: *m*/*z* 1047.5 $[M+Na]^+$.

3.10. 7-(1,3-Dioxan-2-yl)-heptyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (18)

Coupling of **17** (1.37 g, 1.34 mmol) and **10α** (621 mg, 0.954 mmol) following the aforementioned general procedure gave 1.07 g (71%) of **18** as a colorless oil. $[\alpha]_D^{20}$ +13.1 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–7.00 (m, 30H), 6.82–6.41 (m, 12H), 5.71 (br s, 1H), 5.10–5.01 (m, 2H), 4.93–4.20 (m, 19H), 4.12–3.89 (m, 9H), 3.83–3.40 (m, 9H), 3.73 (s, 3H), 3.72 (s, 3H), 3.71 (s, 3H), 2.09 (m, 1H), 1.91 (s, 3H), 1.70–1.51 (m, 4H), 1.42–1.23 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.90, 154.03, 153.92, 153.76, 152.53 (2C), 152.33, 138.75, 138.64, 138.49, 138.35, 138.30 (2C), 115.60 (2C), 115.28 (2C), 115.25 (2C), 114.81 (2C), 114.57 (2C), 114.41 (2C), 102.42, 100.42, 100.18, 97.60, 78.77, 78.14, 76.82, 76.26, 75.65, 75.18, 74.73, 74.21, 73.56, 73.12, 73.01, 72.89 (2C), 71.77, 69.46, 69.02, 68.43, 67.63, 67.21, 66.92 (2C), 65.90, 65.43, 64.16, 55.80, 55.73 (2C), 35.31, 29.52, 29.49, 29.39, 26.15, 25.96, 24.00, 20.83. ESIMS: *m*/*z* 1590.0 $[M+H]^+$.

3.11. 7-(1,3-Dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (19)

Trisaccharide **18** (3.01 g, 1.89 mmol) was dissolved in THF (12 mL) and MeOH (24 mL) followed by addition of sodium (100 mg). The soln was stirred overnight and then quenched with Amberlite IRC-50 (H^+) ion exchange resin. The mixture was filtered, concentrated, and the residue purified by flash chromatography (2:1 hexanes–EtOAc) to afford **19** (2.40 g, 82%) as a foam. $[\alpha]_D^{20}$ +18.4 (*c* 1.1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.41–7.06 (m, 30H), 6.81–6.41 (m, 12H), 5.08–5.01 (m, 2H), 4.91–4.41 (m, 19H), 4.33 (s, 1H), 4.28–4.21 (m, 3H), 4.12–3.86 (m, 8H), 3.80–3.58 (m, 5H), 3.75 (s, 3H), 3.74 (s, 3H), 3.69 (s, 3H), 3.56–3.46 (m, 2H), 2.45 (br s, 1H), 2.06 (m, 1H), 1.66–1.54 (m, 4H), 1.41–1.25 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.02, 153.88, 153.77, 152.71, 152.61, 152.37, 138.82, 138.68, 138.56, 138.51, 138.48, 138.26, 115.42 (2C), 115.34 (4C), 114.84 (2C), 114.61 (2C), 114.51 (2C), 102.49, 100.53, 99.98, 97.64, 78.79, 78.31, 78.20, 76.33, 75.57, 75.46, 75.25, 74.43, 73.60, 73.20, 73.04, 72.94 (2C), 72.72, 72.10, 69.53, 69.09, 68.47, 67.98, 66.97 (2C), 66.71, 66.30, 65.53, 55.84 (2C), 55.81, 35.35, 29.56, 29.53, 29.45, 26.19, 26.00, 24.06. ESIMS: *m*/*z* 1569.8 $[M+Na]^+$. Anal. Calcd for $C_{92}H_{106}O_{21}$: C, 71.39; H, 6.90. Found: C, 71.17; H, 6.80.

3.12. 7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (20)

To a soln of **19** (1.22 g, 0.788 mmol) in DMF (10 mL) was added NaH (70 mg of a ~50% oil dispersion, 1.46 mmol) and the mixture was stirred at rt for 15 min. BnBr (0.17 mL, 1.43 mmol) and Bu_4NI (15 mg, 0.04 mmol) were added and the soln was stirred for an additional 2 h. Quenched with MeOH, diluted with CH_2Cl_2 (50 mL), and washed with water (2×40 mL). The organic phase was dried, concentrated and purified by flash chromatography (5:2 hexanes–EtOAc), yielding **20** (1.27 g, 98%) as a foam. $[\alpha]_D^{20}$ +8.4 (*c* 1.1, $CHCl_3$). ^{1}H

NMR (300 MHz, $CDCl_3$): δ 7.42–7.08 (m, 35H), 6.82–6.42 (m, 12H), 5.09–5.02 (m, 2H), 4.96–4.39 (m, 20H), 4.34–4.22 (m, 3H), 4.16–3.88 (m, 9H), 3.86–3.60 (m, 6H), 3.76 (s, 3H), 3.76 (s, 3H), 3.70 (s, 3H), 3.55–3.42 (m, 2H), 2.08 (m, 1H), 1.70–1.53 (m, 4H), 1.46–1.24 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.17, 153.91 (2C), 152.77 (2C), 152.55, 139.04, 138.99, 138.89, 138.85, 138.76, 138.71, 138.67, 115.49 (4C), 115.39 (2C), 114.98 (2C), 114.75 (2C), 114.66 (2C), 102.63, 100.71, 100.31, 97.79, 79.56, 79.05, 78.32, 76.38, 75.94, 75.46, 75.41, 75.16, 74.61, 74.51, 73.65, 73.40, 73.15, 73.11, 72.83, 72.68, 69.74, 69.26, 69.18, 68.60, 67.11 (2C), 65.67, 65.42, 64.54, 56.00 (2C), 55.96, 35.50, 29.68 (2C), 29.60, 26.34, 26.15, 24.21. ESIMS: *m/z* 1660.9 $[M+Na]^+$.

3.13. 7-(1,3-Dioxan-2-yl)-heptyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (21)

Coupling of **17** (2.11 g, 2.06 mmol) and **19** (2.40 g, 1.55 mmol) following the aforementioned general procedure gave 3.47 g (90%) of **21** as a colorless syrup. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41–6.88 (m, 50H), 6.77–6.27 (m, 20H), 5.62 (m, 1H), 5.08–4.19 (m, 38H), 4.16–3.30 (m, 23H), 3.73 (s, 3H), 3.72 (s, 3H), 3.68 (s, 9H), 2.05 (m, 1H), 1.85 (s, 3H), 1.65–1.51 (m, 4H), 1.41–1.22 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.83, 153.98, 153.85, 153.77 (2C), 153.65, 152.49, (2C), 152.38, 152.31, 152.20, 115.53 (2C), 115.24 (2C), 115.20 (2C), 115.14 (4C), 114.79 (2C), 114.63 (4C), 114.50 (2C), 114.36 (2C), 102.42, 100.39, 100.22, 100.17, 100.11, 97.62, 66.91 (2C), 55.81, 55.77 (2C), 55.73 (2C), 35.29, 29.50 (2C), 29.39, 26.14, 25.94, 24.00, 20.79.

3.14. 7-(1,3-Dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (22)

Pentasaccharide **21** (3.45 g) was deacetylated as described above for **19** to afford **22** (3.03 g, 89%) as a foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–6.93 (m, 50H), 6.77–6.26 (m, 20H), 5.04 (d, 1H, *J* 3.0 Hz), 5.02 (d, 1H, *J* 3.4 Hz), 4.99 (d, 1H, *J* 3.4 Hz), 4.97 (d, 1H, *J* 3.4 Hz), 4.94–4.88 (m, 3H), 4.84 (d, 1H, *J* 12.4 Hz), 4.79–4.75 (m, 2H), 4.71–4.21 (m, 25H), 4.18–4.04 (m, 6H), 3.98–3.57 (m, 17H), 3.74 (s, 3H), 3.73 (s, 3H), 3.69 (s, 6H), 3.67 (s, 3H), 3.54 (m, 1H), 3.48 (m, 1H), 3.44–3.37 (m, 2H), 2.40 (br s, 1H), 2.06 (m, 1H), 1.66–1.52 (m, 4H), 1.40–1.23 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.95, 153.77, 153.70, 153.62, 153.52, 152.60, 152.49, 152.37, 152.34, 152.17, 115.28 (4C), 115.24 (2C), 115.17 (2C), 115.12 (2C), 114.77 (2C), 114.62 (2C), 114.47 (2C), 114.40 (4C), 102.40, 100.38, 100.22 (2C), 99.83, 97.54, 66.89 (2C), 55.75 (5C), 35.27, 29.48 (2C), 29.37, 26.12, 25.92, 23.98. Anal. Calcd for $C_{146}H_{162}O_{33}$: C, 71.73; H, 6.68. Found: C, 71.55; H, 6.62.

3.15. 7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (24)

Coupling of **23** (1.55 g, 2.48 mmol) and **22** (3.03 g, 1.24 mmol) following the aforementioned general procedure gave 2.54 g (69%) of **24** as a foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–6.88 (m, 65H), 6.77–6.17 (m, 24H), 5.06–4.18 (m, 46H), 4.11–3.82 (m, 12H), 3.80–3.57 (m, 11H), 3.75 (s, 3H), 3.73 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 3.54–3.24 (m, 6H), 2.06 (m, 1H), 1.67–1.51 (m, 4H), 1.41–1.22 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.95, 153.74, 153.69, 153.65 (2C), 153.59, 152.48 (2C), 152.35, 152.30, 152.27, 152.16, 115.23 (2C), 115.14 (2C), 115.10 (6C), 115.06 (2C), 114.76 (2C), 114.58 (6C), 114.44 (2C), 114.39 (2C), 102.38, 100.36, 100.24, 100.20, 100.17, 99.99, 97.54, 66.86 (2C), 55.78, 55.74 (3C), 55.66, 55.59, 35.27, 29.46 (2C), 29.35, 26.11, 25.90, 23.96. Anal. Calcd for $C_{180}H_{196}O_{39}$: C, 72.46; H, 6.62. Found: C, 72.33; H, 6.58.

3.16. 7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl–D-galactopyranoside (25)

To an ice-cooled soln of trisaccharide **20** (1.64 g, 1.00 mmol) in MeCN (40 mL) was added a soln of CAN (8.23 g, 15.0 mmol) in water (10 mL). The mixture was stirred at 0 °C for 20 min, then diluted with $CHCl_3$ (100 mL) and washed twice with water (80 mL). The combined aq layers were extracted with $CHCl_3$ (80 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated, and the residue purified by flash chromatography (1:1 hexanes–EtOAc) to give **25** (986 mg, 75%) as a foam. $[\alpha]_D^{20}$ +48.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47–7.25 (m, 35H),

5.00–4.89 (m, 4H), 4.86–4.59 (m, 14H), 4.52 (t, 1H, *J* 5.1 Hz), 4.16–3.97 (m, 9H), 3.92 (br s, 1H), 3.90–3.30 (m, 13H), 2.19 (br s, 1H), 2.16 (br s, 1H), 2.09 (m, 1H), 1.81 (br s, 1H), 1.67–1.52 (m, 4H), 1.46–1.26 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.91, 138.75, 138.41, 138.19, 137.91, 137.65, 137.60, 102.47, 100.79, 99.92, 97.54, 79.35, 78.50, 77.77 (2C), 77.49, 76.22, 74.95, 74.76, 74.62, 74.58, 73.22, 73.08, 72.96 (2C), 72.82, 72.20, 71.13, 69.89, 68.35, 66.95 (2C), 62.64, 60.94, 60.66, 60.43, 35.31, 29.49, 29.46, 29.38, 26.14, 25.97, 24.00. ESIMS: *m*/*z* 1342.7 $[M+Na]^+$.

3.17. 7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranoside (26)

Hexasaccharide **24** (2.91 g) was treated with CAN (16.1 g) as described above for **25** to give **26** (1.24 g, 54%) as a foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32–7.20 (m, 65H), 5.04–4.93 (m, 6H), 4.90–4.63 (m, 24H), 4.54 (m, 1H), 4.21–4.00 (m, 13H), 3.98–3.35 (m, 28H), 3.30–3.14 (m, 3H), 2.24 (br s, 2H), 2.21 (br s, 2H), 2.10 (m, 1H), 1.96 (br s, 2H), 1.70–1.57 (m, 4H), 1.50–1.30 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 102.40, 100.79, 100.76, 99.94, 99.89, 99.87, 97.47, 66.88 (2C), 35.24, 29.42 (2C), 29.31, 26.07, 25.90, 23.94. Anal. Calcd for $C_{138}H_{160}O_{33}$: C, 70.63; H, 6.87. Found: C, 70.17; H, 6.83.

3.18. (Methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(methyl (7-(1,3-dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate) (27)

A soln of trisaccharide **25** (986 mg, 0.747 mmol) in anhyd CH_2Cl_2 (15 mL) was added to a suspension of the Dess–Martin periodinane[21] (1.43 g, 3.37 mmol) in CH_2Cl_2 (30 mL). The mixture was stirred at rt for 45 min and then diluted with Et_2O (50 mL), quenched with 10% aq $Na_2S_2O_3$ (60 mL) and stirred for an additional 30 min. The phases were separated and the organic layer washed with satd aq $NaHCO_3$ (50 mL). The combined aq phases were extracted with Et_2O (50 mL). The combined organic phases were dried ($MgSO_4$) and the solvent removed in vacuo to give 1.01 g of the crude trialdehyde. This was taken up in a mixture of THF (9 mL) and *t*-BuOH (24 mL) followed by addition of 2-methyl-but-2-ene (12 mL) and a soln of $NaClO_2$ (2.03 g, 22.4 mmol) and $NaH_2PO_4{\cdot}H_2O$ (2.32 g, 16.8 mmol) in water (11 mL). The mixture was stirred at rt for 1 h and then poured into satd aq NaH_2PO_4 (150 mL) and extracted with EtOAc (3×120 mL). The combined organic phases were dried ($MgSO_4$) and concentrated to yield 1.08 g of the crude triacid. To a soln of this in MeOH (60 mL) was added $TMSCHN_2$ (8.8 mL of a 2.0 M soln in hexanes, corresponding to 5.9 equiv/acid). The mixture was stirred at rt for 2 h and then concentrated, and the residue was purified by flash chromatography (2:1 hexanes–EtOAc) to give 828 mg (79%) of **27** as a foam. $[\alpha]_D^{20}$ +41.6 (*c* 1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38–7.02 (m, 35H), 5.18 (br s, 1H), 5.06 (d, 1H, *J* 2.7 Hz), 4.93 (d, 1H, *J* 3.3 Hz), 4.89 (d, 1H, *J* 2.6 Hz), 4.81–4.34 (m, 19H), 4.25 (br s, 1H), 4.09 (br s, 1H), 4.05–3.96 (m, 2H), 3.91 (dd, 1H, *J* 10.3, 3.1 Hz), 3.83–3.49 (m, 7H), 3.64 (s, 3H), 3.40 (dt, 1H, *J* 9.7, 6.8 Hz), 3.19 (s, 3H), 3.17 (s, 3H), 1.96 (m, 1H), 1.56–1.43 (m, 4H), 1.34–1.12 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.28, 168.85, 168.72, 138.67, 138.55, 138.46, 138.38 (2C), 138.33, 138.27, 102.40, 99.58, 98.77, 97.34, 78.75, 77.67, 76.67, 76.63, 75.95, 74.59, 74.52, 74.21, 73.29, 73.04 (2C), 72.90, 72.74, 72.49, 72.43, 72.37, 71.75, 71.10, 70.04, 68.89, 66.91 (2C), 53.50, 52.30, 51.74, 35.27, 29.44, 29.35, 29.31, 26.00, 25.92, 23.95. ESIMS: *m*/*z* 1424.4 $[M+Na]^+$.

3.19. (Benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl (7-(1,3-dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate) (28)

Prepared from **25** as described above for **27** by using $PhCHN_2$[22] in Et_2O and EtOAc instead of $TMSCHN_2$. Colorless oil. $[\alpha]_D^{20}$ +30.2 (*c* 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.10 (m, 50H), 5.31–5.11 (m, 4H), 5.04 (br s, 1H), 4.97–4.34 (m, 24H), 4.24 (br s, 1H), 4.18–4.08 (m, 3H), 4.00–3.50 (m, 9H), 2.09 (m, 1H), 1.71–1.57 (m, 4H), 1.49–1.25 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.33, 167.95, 167.62, 138.46 (2C), 138.29, 138.23, 138.19, 138.15, 138.05, 135.08, 134.99, 134.83, 102.18, 99.52, 98.81, 97.20, 78.61, 77.66, 76.57, 76.49, 76.20, 76.00, 74.28 (2C), 73.89, 73.26, 72.90, 72.56, 72.50, 72.31 (2C), 71.90, 71.51, 70.92, 70.03, 68.76, 66.71, 66.69 (2C), 66.58, 66.47, 35.09, 29.24, 29.16, 29.08, 25.81, 25.74, 23.73. ESIMS: *m*/*z* 1654.8 $[M+Na]^+$.

3.20. (Benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl (7-(1,3-dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-β-D-galactopyranosid)uronate) (29)

Hexasaccharide **26** (480 mg, 0.205 mmol) was oxidized with the Dess–Martin periodinane[21] (800 mg, 1.89 mmol) and then with $NaClO_2$ (1.1 g) in the presence

of methyl-but-2-ene (6 mL) and $NaH_2PO_4 \cdot H_2O$ (1.3 g) as described above for **27**. The crude hexaacid was dissolved in EtOAc (15 mL) and titrated over 2 h at room temperature with a 1 M soln of $PhCHN_2$[22] in Et_2O until a persisting orange color was obtained. The mixture was concentrated and the residue purified by flash chromatography (3:2 hexanes–EtOAc) to afford 131 mg (22%) of **29** as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–6.92 (m, 95H), 5.19 (d, 1H, *J* 3.4 Hz), 5.15 (d, 1H, *J* 12.4 Hz), 5.12 (d, 1H, *J* 3.4 Hz), 5.09 (d, 1H, *J* 3.4 Hz), 5.08 (d, 1H, *J* 3.4 Hz), 5.05–4.92 (m, 4H), 4.84–4.08 (m, 49H), 4.04 (dd, 1H, *J* 10.2, 3.0 Hz), 3.84 (dd, 1H, *J* 10.7, 3.4 Hz), 3.83–3.51 (m, 14H), 3.46 (dt, 1H, *J* 10.2, 6.8 Hz), 2.08 (m, 1H), 1.64–1.50 (m, 4H), 1.42–1.20 (m, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 168.52, 168.17, 168.12, 167.93, 167.86, 167.55, 102.47, 99.64, 98.73, 98.70, 98.59, 98.51, 97.37, 66.97 (2C), 35.31, 29.47 (2C), 29.33, 26.01, 25.96, 23.97. ESIMS: *m/z* 2994.4 $[M+Na]^+$. Anal. Calcd for $C_{180}H_{184}O_{39}$: C, 72.76; H, 6.24. Found: C, 72.48; H, 6.34.

3.21. (Methyl α-D-galactopyranosyluronate)-(1→4)-(methyl α-D-galactopyranosyluronate)-(1→4)-(methyl (7-(1,3-dioxan-2-yl)-heptyl α-D-galactopyranosid)uronate) (30)

Trisaccharide **27** (118 mg, 0.084 mmol) was dissolved in THF (2 mL) and MeOH (6 mL) and Ar was bubbled through the soln for 5 min. Then 10% Pd/C (72 mg) was added and the reaction mixture was stirred at rt under 1 atm of H_2. Water (2 mL) was added after 15 min and the mixture was stirred under a H_2 atmosphere overnight. Filtered through Celite and concentrated to give **30** (65 mg, quant) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 5.15 (br s, 1H), 5.09 (br s, 1H), 4.94 (d, 2H, *J* 3.8 Hz), 4.87 (d, 1H, *J* 3.8 Hz), 4.53 (t, 1H, *J* 5.1 Hz), 4.51 (br s, 1H), 4.40–4.36 (m, 2H), 4.35 (t, 1H, *J* 6.0 Hz), 4.20 (dd, 1H, *J* 3.0, 1.3 Hz), 4.04 (br dd, 1H, *J* 10.7, 5.1 Hz), 3.91 (dd, 1H, *J* 10.2, 3.0 Hz), 3.89 (dd, 1H, *J* 10.2, 3.0 Hz), 3.81 (s, 3H), 3.80–3.63 (m, 7H), 3.78 (s, 3H), 3.75 (s, 3H), 3.52 (m, 1H), 1.99 (m, 1H), 1.75 (p, 1H, *J* 6.4 Hz), 1.69–1.49 (m, 4H), 1.44–1.26 (m, 8H). ^{13}C NMR (125 MHz, CD_3OD): δ 171.67, 171.14, 170.76, 106.12, 103.63, 102.01, 100.66, 80.39, 80.13, 72.77, 72.03, 71.85, 71.30, 70.75, 70.14, 69.91 (2C), 69.74, 69.72, 67.92 (2C), 60.09, 53.04, 52.86, 52.54, 36.27, 33.71, 30.46, 30.38, 27.08, 27.01, 25.54. ESIMS: *m/z* 795.5 $[M+Na]^+$.

3.22. 7-(1,3-Dioxan-2-yl)-heptyl (α-D-galactopyranosyluronic acid)-(1→4)-(α-D-galactopyranosyluronic acid)-(1→4)-α-D-galactopyranosiduronic acid (31)

Prepared from **28** using the same procedure as described above for **30**. White solid. ^{1}H NMR (500 MHz, D_2O): δ 5.02–4.99 (m, 2H), 4.96 (br s, 1H), 4.95 (d, 1H, *J* 3.8 Hz), 4.93 (d, 1H, *J* 3.4 Hz), 4.67 (m, 1H), 4.47 (m, 1H), 4.39–4.35 (m, 2H), 4.23 (br s, 1H), 4.00–3.89 (m, 3H), 3.82 (dd, 1H, *J* 10.4, 3.2 Hz), 3.72 (m, 1H), 3.67 (dd, 1H, *J* 10.6, 3.4 Hz), 3.64 (dd, 1H, *J* 10.2, 3.8 Hz), 3.61–3.54 (m, 4H), 3.47 (m, 1H), 1.67 (p, 1H, *J* 6.4 Hz), 1.54–1.32 (m, 4H), 1.26–1.12 (m, 9H). ^{13}C NMR (125 MHz, D_2O): δ 173.34, 172.64, 172.31, 103.17, 100.75, 100.64, 99.23, 79.10, 78.83, 71.57, 70.81, 70.62, 70.22, 69.71, 69.40, 68.89, 68.72, 68.52 (2C), 68.41, 67.65, 59.34, 34.87, 34.46, 29.22, 28.96, 28.88, 25.84, 23.84. ESIMS: *m/z* 753.9 $[M+Na]^+$.

3.23. 7-(1,3-Dioxan-2-yl)-heptyl (α-D-galactopyranosyluronic acid)-(1→4)-(α-D-galactopyranosyluronic acid)-(1→4)-(α-D-galactopyranosyluronic acid)-(1→4)-(α-D-galactopyranosyluronic acid)-(1→4)-α-D-galactopyranosiduronic acid (32)

Prepared from **29** using the same procedure as described above for **30**. White solid. ^{13}C NMR (125 MHz, D_2O): δ 173.67, 173.28, 173.21, 173.15 (2C), 173.11, 103.26, 100.65 (2C), 100.59 (3C), 99.18, 67.67 (2C), 34.47, 29.16, 28.91, 25.82, 25.75, 24.61. MALDI-TOFMS: *m/z* 1257.7 $[M-H]^-$.

3.24. BSA-conjugate (33)

A soln of **30** (65 mg, 0.084 mmol) in AcOH (4 mL) and water (1 mL) was heated at 50 °C for 12 h. It was then concentrated and the residue dissolved in water (2 mL). BSA (23 mg, 0.35 μmol) was added and the mixture was stirred for 1 h. Then $NaCNBH_3$ (13 mg, 0.21 mmol) was added, and the stirring was continued at rt for 4 days. Purified by filtration through a Centricon YM-30 filter and lyophilized yielding 18.6 mg (65%) of a white solid. MALDI-TOFMS: *m/z* 82,541 $[M]^+$, which corresponds to 22.6 mol ligand/mol BSA (BSA standard: 66,431).

3.25. BSA-conjugate (34)

A soln of **31** (43 mg, 0.059 mmol) in AcOH (2.8 mL) and water (0.7 mL) was heated at 50 °C for 12 h. It was then concentrated and the residue taken up in water (2 mL) and the pH was adjusted to 9 with $NaHCO_3$. BSA (13 mg, 0.20 μmol) was added and the mixture was stirred for 1 h. Then $NaCNBH_3$ (7.5 mg, 0.12 mmol) was added, and the stirring was continued at rt for 2 days. Purified by filtration through a Centricon YM-30 filter and lyophilized yielding 11.7 mg (81%) of a white solid. MALDI-TOFMS: *m/z* 73,980 $[M]^+$, which corresponds to 11.5 mol ligand/mol BSA.

3.26. BSA-conjugate (35)

A soln of **32** (15 mg, 0.012 mmol) in AcOH (1.25 mL) and water (0.25 mL) was heated at 50 °C for 12 h. The solvent was removed under diminished pressure and the residue taken up in water (1 mL). pH was adjusted to 9 with $NaHCO_3$ and BSA (5 mg, 0.08 μmol) was added. The soln was stirred for 1 h followed by addition of $NaCNBH_3$ (30 mg, 0.48 mmol). The mixture was stirred at rt for 4 days. Purified by filtration through a Centricon YM-30 filter and lyophilized yielding 5.0 mg (94%) of a white solid. MALDI-TOFMS: *m*/*z* 70,996 $[M]^+$, which corresponds to 3.8 mol ligand/mol BSA.

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References

1. *Pectins and their Manipulation*; Seymour, G. B., Knox, J. P., Eds.; Blackwell: Oxford, 2002.
2. Knox, J. P.; Linstead, P. J.; King, J.; Cooper, C.; Roberts, K. *Planta* **1990**, *181*, 512–521.
3. Willats, W. G. T.; Orfila, C.; Limberg, G.; Buchholt, H. C.; van Alebeek, G.-J. W. M.; Voragen, A. G. J.; Marcus, S. E.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Murray, B. S.; Knox, J. P. *J. Biol. Chem.* **2001**, *276*, 19404–19413.
4. Willats, W. G. T.; Gilmartin, P. M.; Mikkelsen, J. D.; Knox, J. P. *Plant J.* **1999**, *18*, 57–66.
5. Willats, W. G. T.; Limberg, G.; Buchholt, H. C.; van Alebeek, G.-J.; Benen, J.; Christensen, T. M. I. E.; Visser, J.; Voragen, A.; Mikkelsen, J. D.; Knox, J. P. *Carbohydr. Res.* **2000**, *327*, 309–320.
6. Clausen, M. H.; Willats, W. G. T.; Knox, J. P. *Carbohydr. Res.* **2003**, *328*, 1797–1800.
7. Jones, L.; Seymour, G. B.; Knox, J. P. *Plant Physiol.* **1997**, *113*, 1405–1412.
8. Willats, W. G. T.; Marcus, S. E.; Knox, J. P. *Carbohydr. Res.* **1998**, *308*, 149–152.
9. Wong, S. S. *Chemistry of Protein Conjugation and Cross-Linking*; CRC: Boca Raton, 1991.
10. Peters, T., Jr. *All About Albumin—Biochemistry, Genetics and Medicinal Applications*; Academic: San Diego, 1996.
11. Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076–4083.
12. Clausen, M. H.; Jørgensen, M. R.; Thorsen, J.; Madsen, R. *J. Chem. Soc., Perkin. Trans. 1*, **2001**, 543–551.
13. Clausen, M. H.; Madsen, R. *Chem. Eur. J.* **2003**, *9*, 3821–3832.
14. Greene, T. W.; Wuts, P. G. M.. In *Protective Groups in Organic Chemistry*, 3rd ed.; John Wiley and Sons: New York, 1997, pp 724–727.
15. Yu, C.; Liu, B.; Hu, L. *Tetrahedron Lett.* **2000**, *41*, 4281–4285.
16. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480–2482.
17. Roelofsen, D. P.; Wils, E. R. J.; van Bekkum, H. *Rec. Trav. Chim. Pays-Bas.* **1971**, *90*, 1141–1152.
18. For assignment of anomeric configuration based on C-1, H-1 coupling constants, see: Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin. Trans. 2*, **1974**, 293–297.
19. Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056–4062.
20. Konradsson, P.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.*, **1989**, 1124–1125.
21. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.
22. Creary, X. *Org. Synth.* **1986**, *64*, 207–216.