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Tandem Exploitation of *Helix pomatia* Glycosyltransferases: Facile Syntheses of H-Antigen-Bearing Oligosaccharides

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Abstract: Snails from the family *Helicidae* produce in their albumen glands a highly branched galactan, which consists almost exclusively of D- and L-galactose. The D-Gal residues are glycosydically β(1→6)- or β(1→3)-linked, whereas the L-Gal moieties are attached α(1→2). Up until the present time, two β(1→6)-D-galactosyl transferases and one α(1→2)-L-galactosyl transferase have been identified in a membrane preparation of these glands. These were used to synthesise various oligosaccharides by successive addition of the NDP-activated (NDP = nucleoside-5′-diphosphate) D-Gal or L-Fuc moieties, up to a heptasaccharide by starting from the disaccharide D-Gal-β-(1→3)-D-Gal-β(1→OMe. Even larger oligosaccharides up to a tridecasaccharide were obtained by starting with the hexasaccharide D-Gal-[β(1→3)-D-Gal]$_4$-β(1→4)-D-Glc as an acceptor substrate. This tandem exploitation process has high potential for the easy introduction of D-Gal and L-Fuc residues into a great variety of oligosaccharides, which can be used in ligand/acceptor studies.

Keywords: enzymes · glycosylation · *Helix pomatia* · snails · synthetic methods

Introduction

The disaccharide sequence L-Fuc-α(1→2)-D-Gal is a commonly recurring motif in bioactive mammalian glycoproteins, glycolipids and oligosaccharides[1,2] and would be required for a variety of biological studies. As glycosyltransferase-catalysed reactions are often characterised by high regio- and stereospecificity and high glycosylation yields,[3–5] this H-antigen sequence may easily be introduced to a D-galactopyranose-containing oligosaccharide by treatment of the corresponding oligosaccharide with an L-fucosyltransferase and GDP-L-Fuc (GDP = guanosine-5′-diphosphate).

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A good enzyme for this type of reaction is the L-galactosyltransferase from albumen glands of *Helix pomatia* and some other snails of the family *Helicidae* which accepts L-Fuc equally as well as L-Gal in their GDP-activated form as a donor, as was elucidated by a time-course study.[6]

In these glands, a highly branched polysaccharide, termed galactan, is synthesised as the sole source of nutrition for embryonic and newly hatched snails.[7–10] This irregularly branched galactan consists almost exclusively of D- (85%) and L-galactose (15%) units.[6,10,11] The L-Gal units are α-(1→2)-linked to a non-reducing terminal D-Gal. The D-Gal moieties are β(1→3)- and β(1→6)-linked,[9–11] and linear stretches are present with predominantly β(1→3)-linkages.[9] The enzymes necessary to form these linkages should thus be expected to appear in the albumen glands of the snails, in which galactan is produced before its secretion into the perivitelline fluid which accumulates around the eggs as they pass through the snail's oviduct.[7,12] Indeed, an α(1→2)-L-galactosyltransferase[13,14] and two β(1→6)-D-galactosyltransferases have been observed in *H. pomatia* albumen glands.[15–17] The *H. pomatia* albumen gland α(1→2)-L-galactosyltransferase transfers either L-Gal or L-Fuc from the corresponding GDP-sugar nucleotide to terminal, non-reducing D-Gal residues of an oligosaccharide and is referred to as L-Gal-T further on.[13,14] One of the two D-galactosyltransferases, (D-Gal-T-I), transfers D-Gal to subterminal, whereas the

other (D-Gal-T-II) transfers D-Gal to terminal D-Gal moieties of an oligosaccharide, both using UDP-D-Gal (UDP = uridine-5′-diphosphate) as a donor (Figure 1).[15,16]

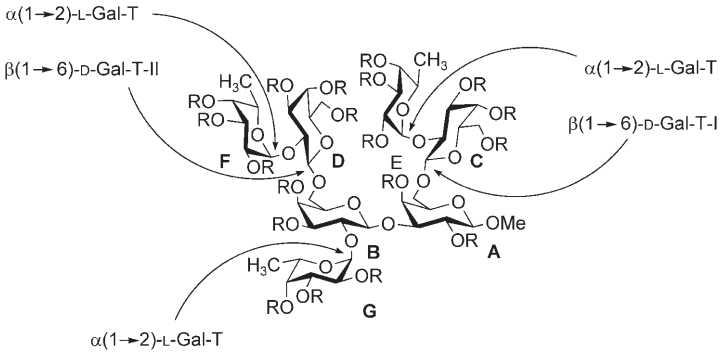


Figure 1. The action of transferases used in this study.

Each of them is specific with respect to the nucleotide donor and the metal ions required for synthesis. The L-Gal-T recognises only GDP-sugar nucleotides, whereas D-Gal-T-I and D-Gal-T-II are specific for UDP-D-Gal. Further, L-Gal-T and D-Gal-T-II require the presence of manganese(II) ions,[15,16] whereas D-Gal-T-I does not.[15]

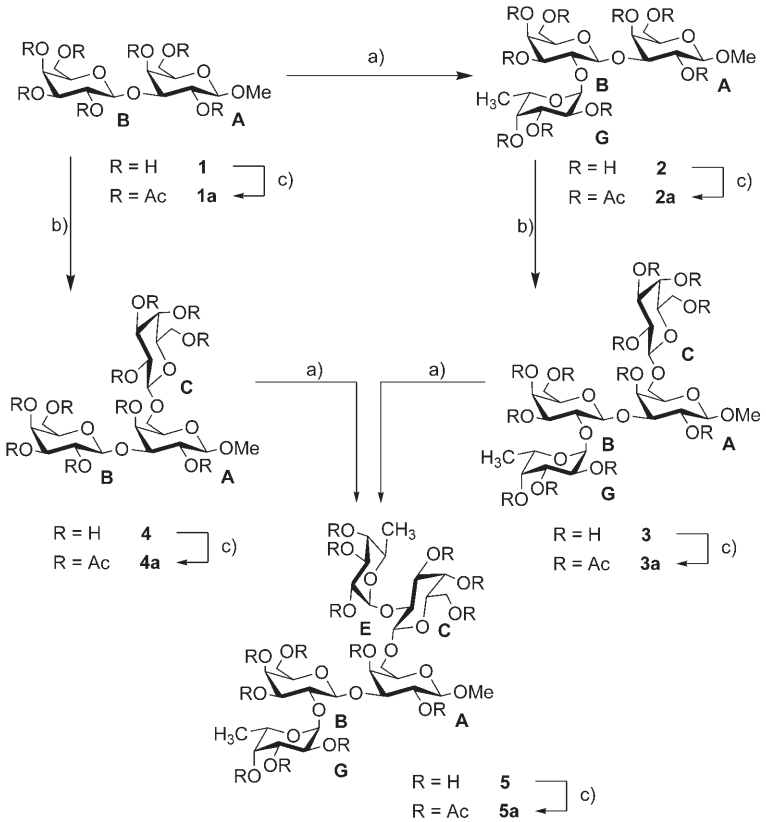
It is presently uncertain how the β(1→3) linkages in the polysaccharide are formed, as a putative D-Gal-transferase has not yet been found.

Though it is often difficult to use membrane-bound enzymes due to unwanted side activities and, in particular, complicated workup procedures, these three enzymes are easy to use. Each of them can be independently activated and they are obtained rapidly by performing simple homogenisation/washing cycles of the albumen glands. Furthermore, as insoluble materials they are rapidly removed from the reaction mixture.

Thus far, mostly di- and trisaccharides have been used as acceptor structures with the aforementioned three enzymes and their corresponding nucleotide donors.[17,18] To evaluate in more detail the potentials of these enzymes, it was tried in this study to combine these different enzymatic reactions and generate more extended and more complex oligosaccharides. Thus it is expected to understand the growth of the native snail polysaccharide in more detail and to explore the use of this enzyme source to obtain a variety of H-antigen bearing oligosaccharides. We refer to this combined, sequential activation of the individual enzymes present in the *H. pomatia* albumen gland sediment as "tandem exploitation".

Results and Discussion

Sequential synthesis of a difucosylated pentasaccharide: Several oligosaccharides have been synthesised by tandem exploitation and their structures were elucidated in their peracetylated forms by one- and two-dimensional NMR experiments. Starting with the disaccharide **1**, tandem exploitation of L-Gal-T and D-Gal-T-I led to the formation of the H-antigen-containing oligosaccharide series **2**–**5** (Scheme 1). Treatment of the β(1→3) galactobiose **1** with GDP-L-Fuc and the *H. pomatia* albumen gland sediment in the presence of Mn^{2+} ions gave the α(1→2)-L-fucosylated trisaccharide **2** (yield 70%).[14] After removing the sediment, the supernatant was deionised and taken to dryness. Synthesis of the tetrasaccharide **3** from this material was initiated with fresh



Scheme 1. Tandem exploitation of two enzymes to synthesise pentasaccharide **5** and its intermediates from disaccharide **2**. a) GDP-L-Fuc, *H. pomatia* albumen gland α(1→2)-L-Gal-T, calf intestine alkaline phosphatase, $MnCl_2$, NaN_3, Tris/HCl buffer (pH 7.6), 28 °C, 24 h; b) UDP-D-Gal, *H. pomatia* albumen gland β(1→6)-D-Gal-T-I, calf intestine alkaline phosphatase, NaN_3, Tris/HCl buffer (pH 7.6), 28 °C, 5 h; c) $(CH_3CO)_2O$, 80 °C, 2 h. Yields: **2a** (70%), **3a** (45%), **5a** (34% from **3**) and **5a** (40% from **4**).

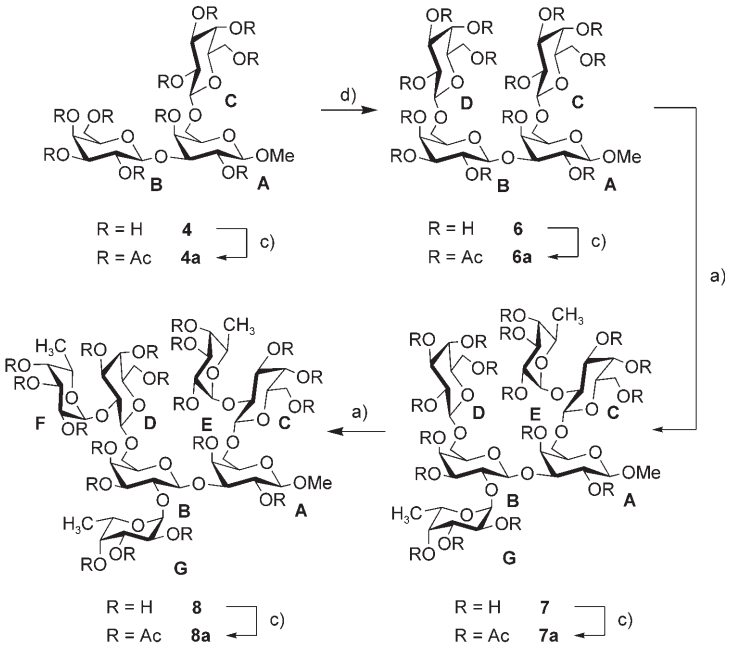
gland extract and UDP-D-Gal utilising D-Gal-T-I which transferred a D-Gal residue to form a β(1→6) linkage to ring A (yield 45%). No further transfer of D-Gal was observed when the reaction product was treated again with fresh gland sediment and UDP-D-Gal as revealed by MALDI-TOF of the peracetylated component **3a** (*m*/*z*: 1191.71 $[M+Na]^+$), indicating that the H-antigen determinant was not an acceptor structure for D-Gal-T-I.

The chemical shifts of **3a** in the ^1H-^1H COSY spectrum indicate three galactopyranosyl ($\delta = 4.21$ (H^A), 4.51 (H^B), 4.46 ppm (H^C)) and one fucopyranosyl ring ($\delta = 5.22$ (H^G)). As expected from the data of the precursor structure **2**, the galactosyl rings A and B are β(1→3)-linked and the fucosyl ring G is α(1→2)-attached to ring B. The newly introduced galactosyl ring C is clearly linked to ring A at C-6, as inferred from the HMBC spectrum and the $J_{1,2}$ coupling constant (7.5 Hz) which is indicative for a β linkage.

Tetrasaccharide **3** was used for further fucosylation after removing undissolved material, concentration under reduced pressure and adding fresh sediments, Mn^{2+} ions and GDP-L-Fuc to form mainly the α(1→2)-L-fucosylated pentasaccharide **5**. A small amount of **3** remained unsubstituted as determined by MALDI-TOF in its peracetylated form (*m*/*z*: 1191.69 $[M+Na]^+$, 1421.42). After repeating the fucosylation once, no acetylated component with a molecular mass of *m*/*z*: 1191.69 was detected anymore, proving clearly that the attachment of the L-Fuc and the D-Gal was carried to completeness (yield 40%).

The pentasaccharide **5** could also be obtained by an alternative pathway. Attaching a D-Gal unit first to the disaccharide **1**, the branched trisaccharide **4** was obtained as reported earlier.[15] This in turn could be subsequently fucosylated at both terminal non-reducing D-Gal residues to give the target **5**. Its structural assignment was determined from the acetylated derivative **5a**. For the five sugar rings there were observed three H-1 doublets ($\delta = 4.21$ (H^A), $\delta = 4.51$ (H^B), $\delta =$ 4.46 ppm (H^C); $J_{1/2} = 7.5$ Hz), indicative for β-linked D-Gal residues and one H-1 doublet ($\delta = 5.22$ ppm (H^E); $J_{1/2} =$ 3.5 Hz) as well as a second H-1 signal under a multiplet ($\delta =$ 5.38 ppm (H^G)), corresponding to the two α-linked L-Fuc rings. A cross peak in the HMBC spectrum between C^B-2 and C^G-1 confirmed the substitution of C^B-2 with L-Fuc as in **3a**. The other L-Fuc residue was linked to ring C as deduced from the shift of C^C-2 to higher field (from $\delta = 5.12$ to 3.88 ppm).

Employment of three transferases allows for formation of larger oligosaccharides: As shown previously,[16–18] another D-Gal unit could be attached to the trisaccharide **4**, if Mn^{2+} ions were added concomitantly with UDP-D-Gal and fresh albumen gland sediment to activate the D-Gal-T-II, resulting in tetrasaccharide **6** as reported previously (yield 40%; Scheme 2).[16,18] However, at that time it was not possible to clarify whether or not ring D was linked either to ring B or C, though its β(1→6) linkage was established by permethylation studies. Thus, tetrasaccharide **6** was synthesised again to establish its structure unambiguously.



Scheme 2. Tandem exploitation of three enzymes for the synthesis of hexa- and heptasaccharides **7** and **8**, respectively. a) GDP-L-Fuc, *H. pomatia* albumen gland α(1→2)-L-Gal-T, calf intestine alkaline phosphatase, $MnCl_2$, NaN_3, Tris/HCl buffer (pH 7.6), 28°C, 24 h; c) $(CH_3CO)_2O$, 80°C, 2 h, d) UDP-D-Gal, *H. pomatia* albumen gland β(1→6)-D-Gal-T-II, calf intestine alkaline phosphatase, $MnCl_2$, NaN_3, Tris/HCl buffer (pH 7.6), 28°C, 24 h. Yields: **6a** (39%), **5a** (12.7%), **7a** (18.2%), **8a** (9.1%).

For unknown reasons, the galactosylation of **4** never went to completion, resulting in a maximal yield of 70% for tetrasaccharide **6** even after repeating the glycosylation procedure, as evaluated by photometric analysis after separation of the acetylated compounds on TLC. The tri- **4** and the tetrasaccharide **6** were separated on a preparative scale in their acetylated forms on TLC by using chloroform/acetone 15:2 as the solvent. The ^1H, and the ^1H-^1H COSY spectra of tetrasaccharide **6a** (*m*/*z*: 1226.37 $[M+Na]^+$) showed that the molecule contained four galactopyranosyl rings ($\delta = 4.22$ (H^A), 4.48 (H^B), 4.50 (H^C), 4.39 ppm (H^D); $J_{1/2} = 7.5$ Hz). Ring A was disubstituted (at the 3- and 6-positions), ring B monosubstituted (6-position), whereas rings C and D were unsubstituted terminal, non-reducing moieties.

All protons could be assigned to their corresponding rings and positions. Examination of the HMBC spectrum showed that the disubstituted ring H-1 coupled to the aglycone methyl group carbon, and that the H-1 of the monosubstituted ring coupled to the C-3 of the disubstituted ring. Further, the H-1 of one of the terminal galactopyranosyl rings coupled to C-6 of the monosubstituted ring B, while H-1 of the other terminal galactopyranosyl ring coupled to C-6 of the disubstituted ring A. This excluded clearly the alternative linkage of ring D to ring C.

By using this oligosaccharide as an acceptor for fucosylation, the influence of neighbouring groups and linkage types on the substitution reactions could be evaluated. Four differ-

ent sites are available for the introduction of an α(1→2)-linkage into **6**, two at the de novo introduced β(1→6)-linked D-Gal units and one at each of the β(1→3)-linked D-Gal entities of the starting material.

In order to use tetrasaccharide **6** for further fucosylations, its synthesis was repeated by starting from disaccharide **1**, but this time the mixture of the tri- **4** and tetrasaccharide **6** was not separated to prevent the loss of valuable material during purification procedures. The oligosaccharide mixture was only separated from the insoluble enzyme preparation, deionised and taken to dryness and replenished with a fresh amount of the *H. pomatia* enzyme fraction, Mn^{2+} ions and GDP-L-Fuc. After 24 h completeness of the reaction was controlled by MALDI-TOF analysis. The presence of unsubstituted tri- **4** and tetrasaccharide **6** concomitant with a penta- **5** and a hexasaccharide **7** indicated incomplete fucosylation. Thus, the fucosylation procedure was repeated with fresh enzyme material and a substantial excess of GDP-L-Fuc. This time MALDI-TOF analysis showed a mixture of three oligosaccharides. The molecular masses of their peracetylated derivatives corresponded to a difucosylated tri- **5** and tetrasaccharide **7**, respectively (*m*/*z*: 1421.42 $[M+Na]^+$, 1709.78), as well as to a trifucosylated tetrasaccharide **8** (*m*/*z*: 1939.74 $[M+Na]^+$). These three acetylated oligosaccharide fractions were separated by TLC using chloroform/acetone 5:1 as the solvent. The relative concentrations of the penta- **5**, hexa- **7** and heptasaccharides **8** were 3:5:2. Five consecutive steps had to be carried out, to go from **1** via **4** to the oligosaccharides **7** and **8**, including the repetitions to bring the reaction to completion. Not each step could be followed quantitatively, but based on initial experiments the yield of the D-Gal-T-I transfer could be conducted with about 78%, whereas the other two transfer reactions resulted in a recovery of less than 50%. Calculations of the yield on this basis amounts to about 7 μmol for the hexasaccharide **7**, whereas only half of it was actually recovered, which is probably due to the necessary repetitions of the glycosylation to achieve a completely fucosylated product.

Compared with the ^{1}H NMR spectrum of **5a** that of **7a** showed four instead of three H-1 doublets ($\delta = 4.43$ (H^A), 4.54 (H^B)), 4.64 (H^C), 4.38 ppm (H^D)) with a coupling constant of $J_{1/2} = 7.5$ Hz, representing four galactopyranoyl rings. Three of the doublets were in an almost identical positions to those in **5a**. An additional doublet (H^D-1) was upfield of H^A-1. Furthermore, two doublets with $J_{1/2} = 3.5$ Hz were identified ($\delta = 5.38$ (H^E), 5.22 ppm (H^G)) in equivalent positions to that of the pentasacharide **5a** resulting from the α-linked fucosyl moieties.

Further signals for the H-2, H-3 and H-4 protons of all four galactosyl rings could be identified in the COSY spectrum. Remarkably, the shifts of H^A-3, H^B-2 and H^C-2 to higher field were virtually similar to those for the pentasaccharide **5a**, indicative of the fucosylation at rings B and C forming α(1→2) linkages. However, neither H^D-2, H^D-3 nor H^D-4 signals were displaced, indicating that the galactosyl ring D remained unsubstituted at C-2, excluding a fucosyl transfer to ring D in this position.

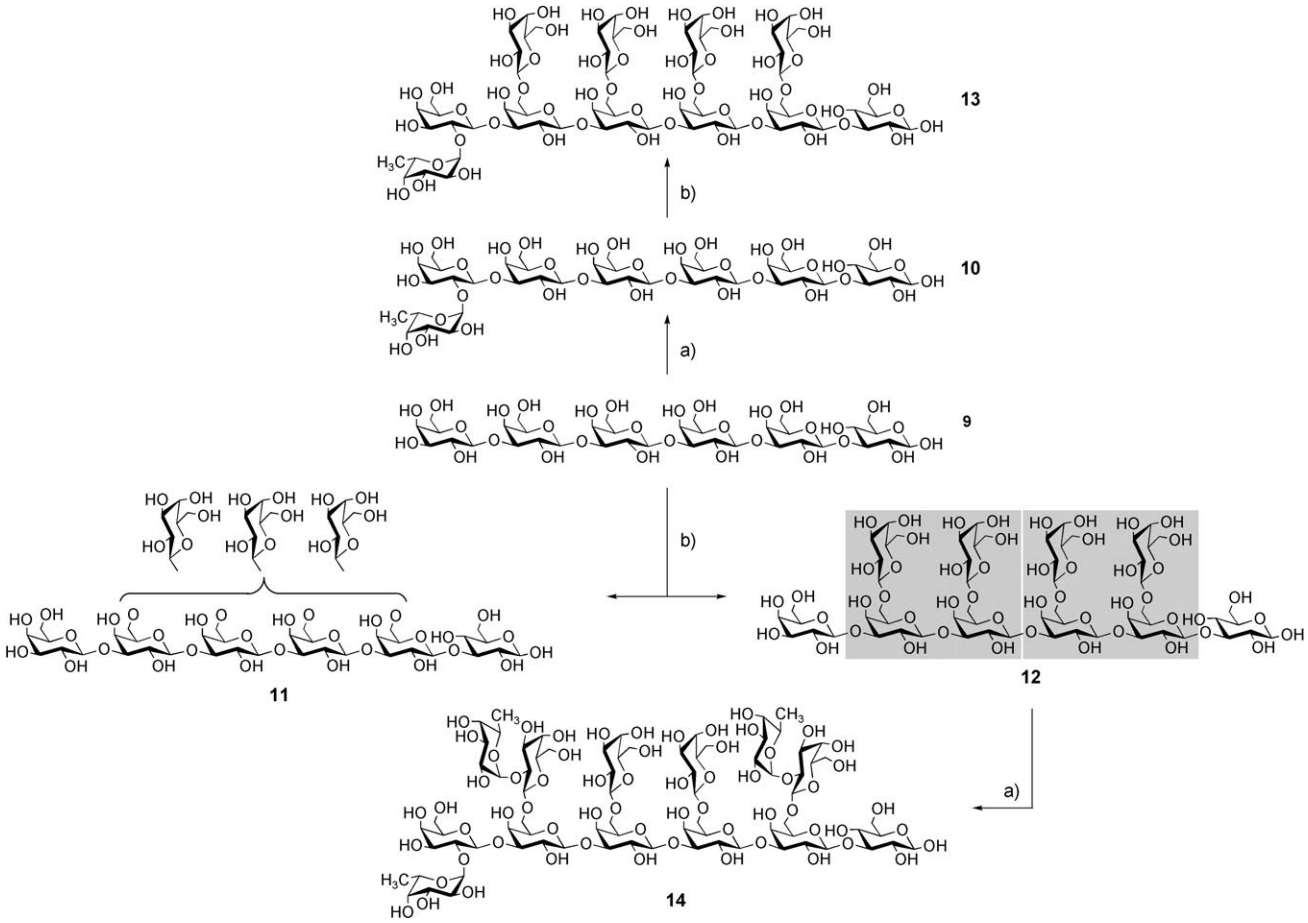
The structure of **6** which was used as the starting material for the synthesis of **7** and **8** (Scheme 2) showed one substitution on each of the two galactosyl rings A and B in position C-6.[18] This was confirmed by analysing the ^{1}H, ^{1}H-^{1}H COSY, HMBC and NOESY spectra of the hexasaccharide **7a**. This conclusion was supported by collision-induced decay studies in electrospray tandem mass spectrometry of **7a**. Among the fragmentation products, a mass of *m*/*z*: 331.09 was found, resulting from the elimination of a 2,3,4,6-tetra-*O*-acetyl galactopyranosyl ion derived from an unsubstituted terminal non-reducing D-Gal moiety. This fragment was only available, if one of the fucosyl rings was attached to ring B and either ring C or D remained unsubstituted. In comparison to **7a**, the heptasaccharide **8a** (*m*/*z*: 1939.74 $[M+Na]^+$) contains a third L-Fuc residue (ring F) as indicated by the de novo appearance of a doublet at $\delta = 5.32$ ppm ($J_{1/2} = 3.5$ Hz). The shift of C^D-2 (from $\delta = 5.05$ to 3.80 ppm) in the COSY spectrum assigned the fucosylation to ring D. In contrast, no other substantial changes were seen for the signals from the galactosyl skeleton nor from the two fucosyl rings detected at rings B and C in the hexasaccharide. When fragmentation studies of the mass *m*/*z*: 1939.74 were carried out by electrospray tandem mass spectrometry, the mass of *m*/*z*: 331.09, derived from an acetylated unsubstituted terminal non-reducing galactopyranosyl moiety (ring D) as seen in **7a** had almost completely disappeared. Summarising, it can be concluded that the newly attached fucosyl ring F was α(1→2) glycosydically linked to ring D.

It could have been assumed that the two terminal galactosyl residues (C and D) in **6** would be preferentially fucosylated and with equal probability followed finally by the substitution of the galactosyl ring B. However, **8** must be derived from the hexasaccharide **7**, as no isomeric forms were observed in the NMR spectrums, therefore ring B was clearly substituted before ring D. Obviously, there was a sequential addition of ring G or E first, followed lastly by F.

Though β(1→3)-linked D-Gal moieties are main elements in the native *Helix pomatia* galactan, forming about 40% of all linkages,[9] no corresponding transferase has yet been identified in its albumen gland. Previously, a galactosyl transferase was reported from the albumen gland of the snail *Lymnaea stagnalis* which linked D-Gal residues β(1→3) glycosidically to lactose.[19] It is doubtful as to whether it is involved in the galactan synthesis, as it prefers β(1→4)- over β(1→3)-linked D-Gal residues and is unable to utilize the *Lymnaea stagnalis* galactan as an acceptor.

Apparently the β(1→6) D-Gal transferases I and II are not sufficient to generate larger oligosaccharides than **6**. Thus, the complex heptasaccharide **8** was the largest component which could be synthesized up to now with snail-derived enzymes, starting from the disaccharide **1**.

Novel acceptors with multiple reaction sites increase the synthetic approaches to higher complex oligosaccharides: To demonstrate that tandem exploitation of the *H. pomatia* glycosyltransferases is not solely limited to smaller oligosaccharides and to further evaluate the influence of neighbour-



Scheme 3. Tandem exploitation with hexasaccharide **9** as an acceptor a) GDP-L-Fuc, *H. pomatia* albumen gland α(1→2)-L-Gal-T, calf intestine alkaline phosphatase, $MnCl_2$, NaN_3, Tris/HCl buffer (pH 7.6), 28 °C, 24 h; b) UDP-D-Gal, *H. pomatia* albumen gland β(1→6)-D-Gal-T-I, calf intestine alkaline phosphatase, NaN_3, Tris/HCl buffer (pH 7.6), 28 °C, 5 h; c) $(CH_3CO)_2O$, 80 °C, 2 h. For further gycosylated intermediate compounds compare Table 1.

ing groups on the growing polysaccharide in the snails, the hexasaccharide **9** [β-D-Gal-(1→3)]$_4$-β-D-Gal-(1→4)-D-Glc[20] was used as an acceptor. This hexasaccharide, isolated from tammar wallaby milk, contains a galactan-like core element with five β(1→3)-linked D-Gal residues and is as such a potential precursor to form dichotomous branching, as found in native snail galactans. As was shown earlier,[21] the L-Gal-T transfers L-Fuc to the terminal non-reducing end, when GDP-L-Fuc was offered in the presence of Mn^{2+} ions as a donor forming **10** (Scheme 3).

Reacting **9** (0.5 mg, 0.504 μmol) with a large excess of UDP-D-Gal in the absence of Mn^{2+} ions formed nonasaccharide **11** (*m*/*z*: 1500.12 $[M+Na]^+$) and decasaccharide **12** (*m*/*z*: 1662.54 $[M+Na]^+$),[16] which were substituted by three and four D-Gal residues, respectively. The decasaccharide was a minor but substantial component.

To investigate what effect, if any, α(1→2)-L-fucosylation at the terminal, non-reducing D-Gal residue would have on subsequent β(1→6)-D-galactosylation, it was decided to react heptasaccharide **10** under exactly the same conditions as those used for the formation of decasaccharide **12**. It was observed that the presence of the terminal α(1→2)-L-fucosyl residue had little or no effect on the D-galactosylation of heptasaccharide **10** to result in **13**, except perhaps to accelerate the initial two D-galactosylation steps. To get some ideas on the influence of densely packed D-Gal units in this reaction and on the L-galactosylation of snail galactans, the mixture of nonasaccharide **11** and decasaccharide **12**, was treated with 7.5 equivalents of GDP-L-Fuc, which corresponded to 1.5 equivalents of donor per terminal D-galactosyl residue. Both oligosaccharides gave rise to a series of mon-, di- and trifucosylated products, in which the trifucosylated ones were the most abundant (Table 1). Repeating the fucosylation procedure did not change the molecular mass of the trifucosylated products.

Decasaccharide **12** can be viewed as two consecutively joined units of the tetrasaccharide **6**, linked at the reducing end to D-Glc and at the non-reducing end to D-Gal (indicated in Scheme 3 by shading). It possessed five unsubstituted terminal, non-reducing D-galactosyl moieties, and four free positions at C-2 in the originally linear D-Gal chain, all together nine potential sites of L-fucosylation.

The oligosaccharide **14** (*m*/*z*: 2099.54 $[M+Na]^+$) was formed with a mass corresponding to a trifucosylated decasaccharide, leaving six D-Gal moieties unreacted. Based on the molecular mass alone, it was not possible to identify the

Table 1. Oligosaccharides identified by MALDI-TOF using hexasaccharide **9** as an acceptor and UDP-D-Gal or GDP-L-Fuc as a donor and the *Helix pomatia* glycosyltransferases after different sequential activations.

Compound	Formula	*m/z*: calcd: $[M+Na]^+$	*m/z*: found: $[M+Na]^+$
hexasaccharide (**9**)	$C_{36}H_{62}O_{31}$	1013.32	1013.60
monofucosylated hexasacharide (**10**)	$C_{42}H_{72}O_{35}$	1159.37	1159.09
nonasaccharide (**11**)	$C_{54}H_{92}O_{46}$	1499.48	1500.12
monofucosylated nonasaccharide	$C_{60}H_{102}O_{50}$	1645.53	1645.46
difucosylated nonasaccharide	$C_{66}H_{112}O_{54}$	1791.59	1791.48
trifucosylated nonasaccharide	$C_{72}H_{122}O_{58}$	1937.65	1937.51
decasaccharide (**12**)	$C_{60}H_{102}O_{51}$	1661.53	1662.04
monofucosylated decasaccharide (**13**)	$C_{66}H_{112}O_{55}$	1807.59	1807.45
difucosylated decasaccharide	$C_{72}H_{122}O_{59}$	1953.64	1953.50
trifucosylated decasaccharide (**14**)	$C_{78}H_{132}O_{63}$	2099.70	2099.55

three L-fucosylation sites. These reaction products could not be separated and analysed by NMR spectroscopic studies due to the small quantities available.

However, compared with the studies of oligosaccharides **3**, **4**, **7** and **8**, the structures of which were unambiguously assigned, some conclusions could be drawn. It was most likely that the four C-2 positions of the β(1→3)-linked D-Gal residues, bearing already a D-Gal moiety at C-6 were not further glycosylated. The remaining five terminal non-reducing D-Gal units are not equivalent. The terminal non-reducing β(1→3)-linked D-Gal moiety in the hexasaccharide **9** was always easily substituted by L-Fuc[21] and should also be glycosylated at the tridecasaccharide **14** as this D-Gal moiety corresponds to ring B in the trisaccharide **2**.

If the terminal non-reducing linked D-Gal moiety was fucosylated, the two other L-Fuc molecules should be attached to sites which would avoid glycosylation of vicinal residues due to steric hindrance, as indicated in structure **14** (Scheme 3). Therefore, fucosylations should most likely take place at the β(1→6)-linked D-Gal residues attached to the backbone closest to the reducing as well as to the non-reducing end. This inference is supported by the substitution of hexasaccharide **7** in which L-Fuc was attached more readily to ring C as to ring D. Tridecasaccharide **14** was not the only fucosylation product, with a difucosylated dodecasaccharide (*m/z*: 1953.50 $[M+Na]^+$) and also a monofucosylated undecasaccharide (*m/z*: 1807.45 $[M+Na]^+$) could be observed. These represent consecutive stages of fucosylation of **11** and in analogy to the fucosylation reactions with **2** and **9**. The first fucosyl transfer leading to the substitution of the terminal, non-reducing D-Gal-linked β(1→3), the second being probably to the D-Gal residue next to the D-Glc at the reducing end.

As the exact galactosylation pattern in the nonasaccharide **11** could not be elucidated exactly, a prediction of the D-Gal and L-Fuc substitution pattern is not reasonable to attempt.

Biological and synthetic aspects of tandem exploitation of *H. pomatia* glycosyltransferases: In the snail galactan, a polysaccharide of four million daltons, corresponding to 25000 D- and L-Gal molecules, large sections are dichotomously branched and thus about 42% of the residues are in terminal non-reducing positions. In the native galactan of *H. pomatia* about 12–15% of all residues are L-Gal. As L-Gal is exclusively found in the periphery, roughly 35% of the peripheral residues are constituted by L-Gal units. Thus, it could be assumed that the introduction of L-Gal into the polysaccharide occurs in a more or less alternating manner, blocking further branching at that residue. The inability of the L-Gal-T to glycosylate all available terminal, non-reducing D-Gal residues may contribute to ensure that growth of the galactan can continue, with reduced steric hindrance. However, this is only one means to restrict extension of the polysaccharide chain to certain branchings, as there are galactans, such as that from the snail *Lymnaea stagnalis*[22,23] or *Biomphalaria glabrata*[24] which are free of L-Gal and still have a comparable irregularly branched structure.

As has been shown here, a variety of H-antigen-related structures can be synthesised by tandem exploitation and the number of possible structures may be even larger, as D-Gal-T-I can also use oligosaccharides as acceptors with D-GalNAc or D-GlcNAc in terminal non-reducing positions as long as D-Gal is subterminal and β-linked. Thus, de novo introduced D-Gal residues can be further substituted by the L-Fuc-T. Furthermore, L-Gal-T can utilise acceptors, in which D-Gal is at the terminal non-reducing end and D-GalNAc or D-GlcNAc are subterminal and linked β(1→3), thus providing the opportunity to synthesise an even larger variety of oligosaccharides. Compounds of this type may have a wide field of applications in specificity studies of lectins,[25,26] monoclonal antibodies directed to bioactive epitops in proteoglycans or cell surface receptors[27] and of a variety of glycosyltransferases, especially when applied in techniques such as surface plasmon resonance spectroscopy, microarrays and immunoassays with fluorescent-labelled carbohydrate conjugates.[28]

Experimental Section

General: The disaccharide D-Galβ(1→3)-D-Galβ(1→OMe was purchased from Sigma-Aldrich and the marsupial oligosaccharide $[D\text{-Gal}\beta(1\rightarrow3)]_4$-D-Gal-β(1→4)D-Glc is that isolated from the tammar wallaby *Macropus eugenii* and was a gift from M. Messer.[20]

Specimens of the snail *H. pomatia* were collected from their natural habitat near Hamburg (Germany) shortly before the main breeding season from May to July, which is when the highest enzyme activity may be expected. The glands were stored at −70°C until processing, whereby no substantial loss of activity could be observed after storage for three years.

The *H. pomatia* albumen glands (2.0 g) were homogenised in Tris-HCl buffer (100 mM, pH 7.6, 10 mL) in a Potter–Elvehjem homogeniser, and centrifuged for 45 min at 4000 rpm at 4°C. The supernatant was removed from the pellet and discarded and this process was repeated five times with 30 min centrifugation at 4000 rpm. The resulting albumen gland sediment was suspended (approximately 1 mL) and homogenised again and either used immediately or stored at −70°C. Albumen gland sedi-

ments, which had been frozen and stored had to be homogenised before use. 300 μL of albumen gland sediment is required to react 2.8 μmol of acceptor. One snail provides one albumen gland, with an average weight of approximately 1.0–2.0 g. Therefore, roughly 9.3 μmol of acceptor can be either α(1→2) fucosylated or β(1→6) galactosylated per gland (this corresponds to 6.7 mg of disaccharide acceptor per snail). No further activity values can be given for the corresponding transferases as they have not been purified to date. However, frequently repeated experiments have shown that these values are reproducible.

Completeness of all reactions was checked by mass spectrometry. MALDI-TOF mass spectra were acquired on a REFLEX IV mass spectrometer (Bruker, Bremen). Each sample (about 1 pM μL^{-1} dissolved in acetonitrile) was mixed 1:1 with 2,5-dihydroxybenzoic acid (saturated aqueous solution containing 10% acetonitrile and 1% trifluoric acid). 1 μL of this mixture was dried on the target. Spectra were taken in positive mode.

ESI MS were acquired in positive mode on a QTOF2 mass spectrometer (Micromass, Manchester). The sample (about 1 pM μL^{-1} in actonitrile) was mixed 1:10 with acetonitrile, containing 1% formic acid. Measurements were performed in nanospray mode by using homemade gold-coated glass capillaries.

^{1}H and ^{13}C NMR spectra were recorded by using either a Bruker DRX-500 (^{1}H: 500.13 MHz, ^{13}C: 125.77 MHz) or for the oligosaccharides **8a** a Brucker DRX-700 (700.13 MHz) instrument. Chemical shifts were calibrated to $(Me)_4Si$ ($\delta = 0.00$ ppm) in $CDCl_3$. TLC of the peracetylated samples was performed on silica gel 60-coated aluminium sheets (E. Merck) by using the given solvent mixtures and developing them three to five times. Spots were visualised by spraying with 10% H_2SO_4 in EtOH and subsequent heating to 150 °C. Preparative TLC was also performed on silica gel 60-coated aluminium sheets. To detect the corresponding fractions, 0.5 cm broad strips were cut off at the edges and in the middle of the sheets and developed as described above. Corresponding fractions were indicated on the untreated sheets and scratched of the aluminium foil. The silica gels were extracted seven times with ethyl acetate. The combined fractions were evaporated to dryness by flushing with N_2 and used for mass spectrometry or NMR spectroscopy. Necessary removal of the ethyl acetate prior to NMR spectroscopic analysis was achieved by repeated up-take in CCl_4 with subsequent evaporation of the solvent. The yield was either determined gravimetrically after the purified material had been dried or by a colorimetric method as described by Kabat and Mayer.[29]

The relative concentrations of components in a mixture were evaluated by the intensity of the corresponding spots after the acetylated products had been separated on TLC and charred by sulphuric acid. Quantification was achieved by using the software ImageMaster-Totallab from Pharmacia.

Enzymatic synthesis of oligosaccharides (GP1): The oligosaccharide acceptor (1 equiv, 100 mg mL^{-1} aqueous solution) was treated with albumen gland sediment (1.5 mL), the corresponding nucleotide donor (1.5 equivalents, 100 mg mL^{-1} aqueous solution), NaN_3 (0.2 M, 20 μL), Tris-HCl buffer (pH 7.6, 0.2 M, 100 μL) and calf intestine alkaline phosphatase (185 mU μL^{-1}, 2 μL). If L-Fuc-T or the D-Gal-T-II were required, they were activated by $MnCl_2 \cdot 6H_2O$ (0.1 M, 30 μL). The reaction mixture was stirred and kept at 28 °C for 18 h, then terminated by dilution with bidistilled water to a total volume of 5 mL and centrifuged at 14000 rpm for 10 min. The pellet was extracted twice with distilled water. The aqueous fractions were combined and deionised with Amberlite MB-3. If incompleteness of the reaction was found by mass spectrometry, the glycosylation procedure was repeated.

Acetylation (GP2): For acetylation, the oligosaccharides were dissolved in acetic anhydride (1 mL) and treated with pyridine (100 μL) and a catalytic amount of 4-dimethylaminopyridine (DMAP) at 80 °C for 1 h. Subsequently, the samples were flushed once with nitrogen and twice with ethyl acetate.

Methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside (2a): According to GP1, disaccharide **1** (9.83 μmol) was treated with GDP-L-Fuc (8.5 mg, 14.4 μmol) as a donor and worked up as described above. Further acetylation following GP2 gave **2a** as a yellow syrup (6.22 mg, 70% yield). $[\alpha]_D^{20} = -33.0$ ($c = 0.10$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.39$ (vd, $^3J(H,H) = 1.0$, 3.8 Hz, 1H; H^A-4), 5.24–5.20 (m, 3H; H^B-4, H^G-4, H^G-1), 5.16 (dd, $^3J(H,H) = 3.6$, 10.4 Hz, 1H; H^G-2), 5.04 (dd, $^3J(H,H) = 3.8$, 9.9 Hz, 1H; H^A-2), 4.90 (dd, $^3J(H,H) = 3.8$, 10.4 Hz, 2H; H^B-3, H^G-3), 4.53 (d, $^3J(H,H) = 7.6$ Hz, 1H; H^B-1), 4.27 (d, $^3J(H,H) = 7.9$ Hz, 1H; H^A-1), 4.23 (m, 1H; H^G-5), 4.15 (dd, 1H, $^3J(H,H) = 6.1$, 11.2 Hz; H^B-6a), 4.10–3.99 (m, 3H; H^A-6a, H^A-6b, H^B-6b), 3.86 (dd, $^3J(H,H) = 3.8$, 9.9 Hz, 1H; H^A-3), 3.78–3.72 (m, 3H; H^B-2, H^B-5, H^A-5), 3.42 (s, 3H; OCH_3); 2.08, 2.07, 2.06, 2.02, 2.01, 2.00, 1.90, 1.90, 1.89 (9×s, each 3H; CH_3COO), 1.08 ppm (d, 3H; H^G-6); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 102.47$ (C^A-1), 101.93 (C^B-1), 96.31 (C^G-1), 76.12 (C^A-3), 74.14 (C^B-3/C^G-3), 72.92, 72.17, 70.99, 70.94 (C^B-2, C^A-5, C^B-5 or C^A-2, in which C^A-2 is either 70.99 or 70.94), 71.69 (C^B-4/C^G-4), 70.56 (C^A-4), 69.06 (C^B-3/C^G-3), 67.65 (C^G-2), 67.27 (C^B-4/C^G-4), 65.38 (C^G-5), 61.98 (C^A-6), 61.25 (C^B-6), 57.28 (OCH_3), 21.29, 21.12, 21.01 (each CH_3COO), 16.01 ppm (C^G-6); MALDI-TOF (DBH): m/z: calcd for $C_{37}H_{52}O_{24}$: 903.2747 $[M+Na]^+$; found: 903.20.

Methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-galactopyranoside (3a): The crude trisaccharide **2** was galactosylated according to GP1 with UDP-D-Gal (12.5 mg, 22.5 μmol). After 5 h, the reaction mixture was centrifuged, the supernatant deionised, acetylated (GP2) and further purified by preparative TLC ($CHCl_3$/acetone 9:1, four times developed) to give **3a** (5.26 mg, 45% yield). $[\alpha]_D^{20} = -8.9$ ($c = 0.37$ in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS) $\delta = 5.31$ (vd, $^3J(H,H) = 3.5$ Hz, 1H; H^G-4), 5.28 (vd, $^3J(H,H) = 3.5$ Hz, 1H; H-4), 5.22 (d, $^3J(H,H) = 4.1$ Hz, 1H; H^G-1), 5.20–5.18 (m, 2H; H^B-4, H^C-4), 5.15 (dd, $^3J(H,H) = 3.5$, 10.4 Hz, 1H; H^C-3), 5.12 (dd, $^3J(H,H) = 3.5$, 10.4 Hz, 1H; H^C-2), 5.02 (dd, $^3J(H,H) = 7.9$, 10.1 Hz, 1H; H^A-2), 4.92–4.87 (m, 3H; H^B-3, H^G-3, H-2), 4.51 (d, $^3J(H,H) = 7.6$ Hz, 1H; H^B-1), 4.46 (d, $^3J(H,H) = 8.2$ Hz, 1H; H^C-1), 4.23–4.19 (m, 2H; H^A-1, H^G-5), 4.11–4.02 (m, 4H; H^B-6a, H^B-6b, H^C-6a, H^C-6b), 3.88–3.81 (m, 3H, H^A-6a, H^A-3, H^B-5 or H^C-5), 3.76–3.68 (m, 3H; H^B-2, H^A-5, H^B-5 or H^C-5), 3.48 (dd, $^3J(H,H) = 8.5$, 10.7 Hz, 1H; H^A-6b), 3.43 (s, 3H; OCH_3), 2.17, 2.07, 2.06, 2.06, 2.06, 1.99, 1.98, 1.96, 1.90, 1.90, 1.89, 1.89 (12×s, each 3H; CH_3COO), 1.07 ppm (d, $^3J(H,H) = 6.3$ Hz, 3H; H^G-6); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 102.81$ (C-1), 102.26 (C^B-1), 101.54 (C^C-1), 96.50 (C^G-1), 75.90 (C^A-3), 74.40, 72.98, 71.25, 70.85 (C^A-5, C^B-5, C^C-5, C^B-2), 74.09, 71.25, 68.96 (C^B-3, C^G-3, C^G-2), 71.64 (C^B-4 or C^C-4), 71.48 (C^A-4), 70.93 (C^A-2), 69.19 (C^C-2), 68.88 (C^A-6), 67.46 (C^C-3), 67.38 (C^G-4), 67.37 (C^B-4 or C^C-4), 65.17 (C^G-5), 61.93, 61.62 (C^B-6, C^C-6), 57.44 (OCH_3), 21.71–20.52 (CH_3COO), 16.06 ppm (C^G-6); MALDI-TOF (DBH): m/z: calcd for $C_{49}H_{68}O_{32}$: 1191.36 $[M+Na]^+$; found: 1191.71.

Methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-galactopyranoside (5a)

*From **3***: Tetrasaccharide **3** was fucosylated with GDP-L-Fuc as a donor (8.5 mg, 15 μmol) and worked up by following GP1. Acetylation (GP2) gave **5a** (4.79 mg, 34% yield).

*From **4***: Trisaccharide **4** (3.5 mg, 9.83 μmol) was fucosylated with GDP-L-Fuc as a donor (17.2 mg, 30 μmol) and worked up following GP1. Acetylation (GP2) gave **5a** (5.6 mg, 40% yield).

*Data for **5a***: $[\alpha]_D^{20} = -23.1$ ($c = 0.39$ in $CHCH_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C, TMS): $\delta = 5.39$ (d, $^3J(H,H) = 3.5$ Hz, 1H; H^G-1/H^E-1), 5.30 (vd, $^3J(H,H) = 3.5$ Hz, 1H; H^A-4), 5.23–5.20 (m, 6H; H^G-1/H^E-1, H^B-4, H^G-4, H^C-4, H^E-4, H^G-2/H^E-2), 5.15 (dd, $^3J(H,H) = 10.7$, 3.1 Hz, 1H; H^G-3/H^E-3), 5.09 (dd, $^3J(H,H) = 7.9$, 10.4 Hz, 1H; H^A-2), 4.92–4.88 (m, 4H; H^B-3, H^C-3, H-3^G/H^E-3, H^G-2/H^E-2), 4.62 (d, $^3J(H,H) = 7.9$ Hz, 1H; H^C-1), 4.55 (d, $^3J(H,H) = 7.6$ Hz, 1H; H^B-1), 4.53 (m, $^3J(H,H) = 6.6$ Hz, 1H; H^G-5/H^E-5), 4.35 (d, $^3J(H,H) = 7.9$ Hz, 1H; H^A-1), 4.22 (vq, $^3J(H,H) = 6.6$ Hz, 1H; H^G-5/H^E-5), 4.13 (dd, $^3J(H,H) = 11.4$ Hz, 6.6 Hz, 1H; H^B-6a/H^C-6a), 4.09–4.01 (m, 3H; H^B-6b/H^C-6b, H^B-6a/H^C-6a, H^B-6b/H^C-6b), 3.93 (dd, $^3J(H,H) = 10.4$, 3.5 Hz, 1H; H^A-3), 3.88 (dd, 1H; H^C-2); 3.85 (dd, $^3J(H,H) = 12.0$, 8.5 Hz, 1H; H^A-6a); 3.81 (vt, 1H; H^B-5/H^C-5); 3.78–3.71 (m, 3H; H^B-2, H^B-5/H^C-5, H^A-5); 3.65 (dd, $^3J(H,H) = 12.0$, 1.9 Hz, 1H; H^A-6b), 3.43 (s, 3H; OCH_3); 2.17, 2.08, 2.07, 2.06, 2.05, 1.97, 1.97, 1.97,

1.93, 1.91, 1.90, 1.90, 1.89, 1.88 (13×s, each 3H; CH_3COO), 1.09 (d, 3H; H^G-6/H^E-6), 1.07 ppm (d, 3H; H^B-6/H^E-6); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=102.34 (C^B-1), 102.22 (C^A-1), 101.89 (C^C-1), 96.36, 95.34 (C^G-1, C^E-1), 75.60 (C^A-3), 75.38, 74.23, 74.02, 73.12, 71.65, 71.54, 71.42, 71.32, 70.87, 70.86, 68.99, 68.83, 68.01, 67.82, 67.71 (C^B-2, C^G-2, C^C-2, C^E-2, C^B-3, C^C-3, C^G- 3/C^E-3, C^A-4, C^B-4, C^G-4, C^C-4, C^E-4; C^A-5, C^B-5, C^C-5), 70.08 (C^A-2), 68.61 (C^A-6), 67.50 (C^G-3/C^E-3), 65.22, 65.00 (C^G-5, C^E-5), 61.61, 60.94 (C^B-6, C^C-6), 55.86 (OCH_3), 19.51, 19.24, 19.16 (each CH_3COO), 16.58, 16.21 ppm (C^G-6, C^E-6); MALDI-TOF (DBH): *m*/*z*: calcd for $C_{59}H_{82}O_{38}$: 1421.43 $[M+Na]^+$; found: 1421.76.

Methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-galactopyranoside (6a): Trisaccharide **4** was treated with UDP-D-Gal (17 mg, 30 μmol) following GP1. Though the reaction was repeated after the removal of the gland sediment, a maximum of 70% of the starting material was converted to tetrasaccharide **6** as was found by spectrophotometric analysis of a small sample run on TLC. Acetylation (GP2) and purification by preparative TLC gave **6a** (5.4 mg, 39% yield). $[\alpha]_D^{20}=-23.1$ (c=0.39 in $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=5.33 (m, ^3J(H,H)=1.0, 3.5 Hz, 1H; H^D-4), 5.31 (m, ^3J(H,H)=1.0, 3.2 Hz, 1H; H^C-4), 5.27 (m, ^3J(H,H)=1.0, 3.2 Hz, 1H; H^A-4), 5.25 (m, ^3J(H,H)=1.0, 3.5 Hz, 1H; H^B-4), 5.12 (dd, 1H; H^C-2), 5.11 (dd, 1H; H^A-2), 5.08 (dd, ^3J(H,H)=7.9, 10.7 Hz, 1H; H^D-2), 4.99 (dd, ^3J(H,H)=7.9, 10.4 Hz, 1H; H^B-2), 4.94 (dd, ^3J(H,H)=3.5, 10.7 Hz, 1H; H^D-3), 4.91 (dd, ^3J(H,H)=3.2, 10.4 Hz, 1H; H^C-3), 4.83 (dd, ^3J(H,H)=3.5, 10.4, 1H; H^B-3), 4.50 (d, ^3J(H,H)=7.9 Hz, 1H; H^C-1); 4.48 (d, ^3J(H,H)=7.9 Hz, 1H; H^B-1); 4.39 (d, ^3J(H,H)=7.9 Hz, 1H; H^D-1), 4.22 (d, ^3J(H,H)=8.2 Hz, 1H; H^A-1), 4.10–4.05 (m, 4H; H^D-6a, H^D-6b, H^C-6a, H^C-6b), 3.88–3.82 (m, 5H; H^D-5, H^C-5, H^A-3, H^A-5, H^A-6a), 3.79–3.70 (m, 2H; H^B-5, H^B-6a), 3.63 (dd, ^3J(H,H)=7.3, 9.5 Hz, 1H; H^B-6), 3.57 (dd, ^3J(H,H)=7.3, 10.1 Hz, 1H; H^A-6b), 3.43 (s, 3H; OCH_3), 2.10, 2.08, 2.07, 2.06, 2.04, 2.00, 1.98, 1.97, 1.95, 1.94, 1.91, 1.91, 1.89 ppm (13×s, each 3H; CH_3COO); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ=102.21 (C^A-1), 101.54, 101.52 (C^B-1, C^C-1), 101.11 (C^D-1), 75.22/74.39 (C^A-3), 72.72 (C^B-5), 71.51 (C^A-2), 74.39/75.22, 71.32, 71.30, 71.26, 71.24, 71.20 (C^D-5, C^C-5, C^A-5, C^B-3, C^D-3, C^C-3), 70.47 (C^A-4), 69.25, 69.23 (C^D-2, C^C-2), 68.89 (C^B-2), 68.87 (C^A-6), 67.67 (C^B-4), 67.42 (C^B-6), 67.36, 67.36 (C^D-4, C^C-4), 61.76, 61.70 (C^D-6, C^C-6), 57.31 ppm (OCH_3); MALDI-TOF (DBH): *m*/*z*: calcd for $C_{59}H_{82}O_{38}$: 1421.44 $[M+Na]^+$; found: 1421.76.

Methyl 3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-3,4-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-galactopyranoside (7a) and methyl 2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-3,4-di-*O*-acetyl-β-D-galactopyranosyl-(1→3)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-galactopyranoside (8a): To obtain a larger amount of tetrasaccharide **6** for further fucosylation, four batches were setup according to GP1, thus quadruplicating the starting material. Fucosylation was repeated once and after acetylation following GP2, the oligosaccharides **5a, 7a** and **8a** were isolated on preparative TLC ($CHCl_3$/acetone 5:1, four times developed). Yield: **5a**: (2.13 mg, 12.7%), **7a**: (5.13 mg, 18.2%) and **8a**: (1.94 mg, 9.1%).

*Hexasaccharide (**7a**)*: ^{1}H NMR (500 MHz, $CDCl_3$, 25°C, TMS): δ=5.37 (d, ^3J(H,H)=3.8 Hz, 1H; H^E-1), 5.30 (d, ^3J(H,H)=2.8 Hz, 1H; H^D-4), 5.26 (d, ^3J(H,H)=3.5 Hz, 1H; H^A-4), 5.25–5.17 (m, 7H; H^G-1, H^B-4, H^C-4, H^E-3, H^G-3, H^E-4, H^G-4), 5.09 (dd, ^3J(H,H)=10.1, 7.9 Hz, 1H; H^A-2), 5.06 (dd, ^3J(H,H)=10.4, 7.9 Hz, 1H; H^D-2), 4.94–4.86 (m, 5H; H^E-2, H^G-2, H^B-3, H^C-3, H^D-3), 4.64 (d, ^3J(H,H)=7.6 Hz, 1H; H^C-1), 4.56–4.51 (m, 1H; H^E-5), 4.54 (d, ^3J(H,H)=7.6 Hz, 1H; H^B-1), 4.41 (d, ^3J(H,H)=7.9 Hz, 1H; H^A-1), 4.36 (d, ^3J(H,H)=7.6 Hz, 1H; H^D-1), 4.24 (br q, ^3J(H,H)=6.6 Hz, 1H; H^G-5), 4.10–4.01 (m, 4H; H^C-6, H^D-6), 3.98 (dd, ^3J(H,H)=10.1, 3.5 Hz, 1H; H^A-3), 3.92–3.85 (m, 3H; H^C-2, H^A-5, H^A-6), 3.85–3.81 (m, 2H; H^C-5, H^D-5), 3.77 (dd, ^3J(H,H)=6.3, 6.3 Hz, 1H; H^B-5), 3.71 (dd, ^3J(H,H)=9.8, 7.6 Hz, 1H; H^B-2), 3.69–3.66 (m, 2H; H^B-6), 3.62 (dd, ^3J(H,H)=11.7, 8.8 Hz, 1H; H^A-6), 3.44 (s, 3H; OCH_3), 2.19–1.87 (17×s, each 3H; $AcCH_3$), 1.10 (d, ^3J(H,H)=6.6 Hz, 3H; H^G-6), 1.04 ppm; (d, ^3J(H,H)=6.3 Hz, 3H; H^E-6); MALDI-TOF (DBH): *m*/*z*: calcd for $C_{89}H_{96}O_{45}$: 1709.52 $[M+Na]^+$; found: 1709.87.

*Heptasaccharide (**8a**)*: ^{1}H NMR (700 MHz, $CDCl_3$, 25°C, TMS): δ=5.36 (d, ^3J(H,H)=3.5 Hz, 1H; H^E-1), 5.31 (d, ^3J(H,H)=3.5 Hz, 1H; H^F-1), 5.30 (d, ^3J(H,H)=2.2 Hz, 1H; H^F-4), 5.27 (d, ^3J(H,H)=3.2 Hz, 1H; H^A-4), 5.25 (d, ^3J(H,H)=4.0 Hz, 1H; H^G-1), 5.24–5.20 (m, 7H; H^C-4, H^B-4, H^E-3, H^F-3, H^G-3, H^E-4, H^G-4), 5.19 (d, ^3J(H,H)=3.5 Hz, 1H; H^D-4), 5.13 (dd, ^3J(H,H)=10.2, 8.0 Hz, 1H, H^A-2), 4.97 (dd, ^3J(H,H)=10.2, 3.5 Hz, 1H; H^D-3), 4.94–4.87 (m, 5H; H^E-2, H^F-2, H^G-2, H^B-3, H^C-3), 4.62 (d, ^3J(H,H)=8.0 Hz, 1H; H^C-1), 4.60 (d, ^3J(H,H)=7.5 Hz, 1H; H^B-1), 4.53 (br q, ^3J(H,H)=6.2 Hz, 1H; H^E-5), 4.41 (d, ^3J(H,H)=8.0 Hz, 1H; H^D-1), 4.37 (d, ^3J(H,H)=8.0 Hz, 1H; H^A-1), 4.30 (br q, ^3J(H,H)=6.2 Hz, 1H; H^F-5), 4.24 (br q, ^3J(H,H)=6.2 Hz, 1H; H^G-5), 4.12 (dd, ^3J(H,H)=11.1, 6.2 Hz, 1H; H^C-6), 4.06–4.03 (m, 2H; H^D-6, H^A-3), 4.02–3.98 (m, 2H; H^C-6, H^D-6), 3.93 (dd, ^3J(H,H)=9.5, 9.5 Hz, 1H; H^B-6), 3.91–3.87 (m, 2H; H^C-2, H^A-6), 3.84 (dd, ^3J(H,H)=7.1, 7.1 Hz, 1H; H^D-5), 3.83–3.78 (m, 3H; H^C-5, H^A-5, H^D-2), 3.74–3.71 (m, 2H; H^B-2, H^B-5), 3.60 (dd, ^3J(H,H)=11.5, 8.9 Hz, 1H; H^A-6), 3.56–3.51 (m, 1H; H^B-6), 3.43 (s, 3H; OCH_3), 2.23–1.87 (19×s, each 3H; $AcCH_3$), 1.12 (d, ^3J(H,H)=6.2 Hz, 3H; H^F-6), 1.09 (d, ^3J(H,H)=6.2 Hz, 3H; H^G-6), 1.08 ppm (d, ^3J(H,H)=6.2 Hz, 3H; H^E-6); MALDI-TOF (DBH): *m*/*z*: calcd for $C_{81}H_{112}O_{52}$: 1939.6018 $[M+Na]^+$; found: 1939.74.

Glycosyltransferase-mediated galactosylation and fucosylation of hexasaccharide 9: Galactosylation of hexasaccharide **9** and heptasaccharide **10** (0.5 mg, 0.5 μmol) to obtain nonasaccharide **11** and decasaccharide **12** as well as undecasaccharide **13** was carried out according to GP1 by using UDP-D-Gal as a donor. Fucosylation of hexasaccharide **9**, nonasaccharide **11** and decasaccharide **12** to yield **10**, **14** and the mono- and difucosylated derivatives of **11** and **12** were carried out by following GP1, supplemented with Mn^{2+} ions and GDP-L-Fuc as the donor. Masses identified by MALDI-TOF of these components are given in Table 1.

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