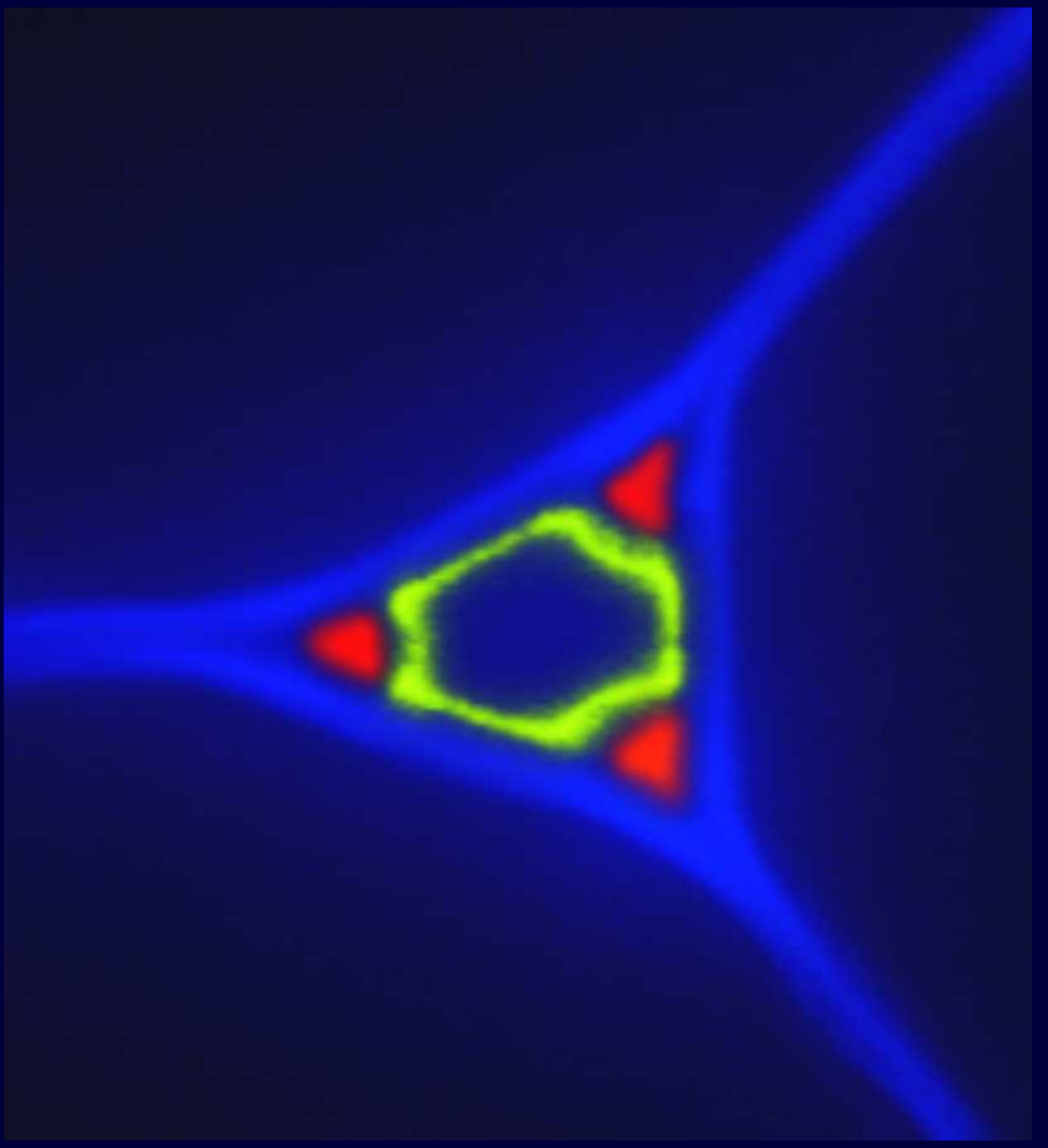
Synthesis and Application of Pectic Oligosaccharides



Mads H. Clausen

Department of Chemistry
Technical University of Denmark

Synthesis and Application of Pectic Oligosaccharides

Ph.D. Thesis

Mads H. Clausen

Technical University of Denmark

2002

Synthesis and Application of Pectic Oligosaccharides

Mads H. Clausen
Department of Chemistry
Technical University of Denmark
2002

Cover illustration: Immunofluorescent labeling of pea tissue with the anti-homogalacturonan monoclonal antibodies LM7 and PAM1. For further information see Chapters 1 and 6 of this volume.

Printed by DTU-tryk
Building 101A
Anker Engelundsvej
2800 Kgs. Lyngby

Printed in Denmark

PREFACE

This thesis is the result of 3 years research, as part of the Danish program to obtain a Ph.D. degree.

The work has been carried out at the Department of Chemistry, Technical University of Denmark, under the supervision of Associate Professor Robert Madsen and Professor Inge Lundt.

Chapter one is a brief introduction to pectic polysaccharides. Chapter two deals with the synthetic planning, initial methodology development and synthesis of trisaccharides. Chapter three contains various methods to produce disaccharide glycosyl donors. Chapter four describes the synthesis of hexasaccharides. In chapter five work involving neoglycoconjugates is outlined. Chapter six tells of different aspects of antibody technology applied. The work described in this chapter was carried out at the Centre for Plant Sciences, University of Leeds, UK. Chapter seven is a conclusion and Chapter eight contains experimental protocols and compound data.

Appendix A contains a list of abbreviations used throughout the thesis.
Appendix B is an abstract in Danish.

An article describing the work in Chapter 2 has been published in 2001:
M. H. Clausen, M. R. Jørgensen, J. Thorsen, R. Madsen, "A strategy for chemical synthesis of selectively methyl-esterified oligomers of galacturonic acid" *J. Chem. Soc., Perkin Trans 1* **2001**, 543-551.
An article describing the work in Chapter 3 and 4 has been submitted:
M. H. Clausen, R. Madsen, "Synthesis of Hexasaccharide Fragments of Pectin", *J. Org. Chem.*, submitted.
An article describing the work in Chapter 5 is under preparation.
An article describing part of the work in Chapter 6 has been submitted:
M. H. Clausen, W. G. T. Willats, J. P. Knox, "Synthetic methyl hexagalacturonate hapten inhibitors of anti-homogalacturonan monoclonal antibodies LM7, JIM5 and JIM7", *Carbohydr. Res.*, submitted.
Another article concerning the work described in Chapter 6 is under preparation.

I hope you will enjoy this thesis.

Mads H. Clausen

ACKNOWLEDGMENTS

I would like to thank the following:

Dr. Robert Madsen for 3 years of dedicated supervision, never failing to inspire and guide me. Professor Inge Lundt for always taking time for me. Jytte Grove-Rasmussen and Janne Borg Rasmusssen for their skillful technical assistance. The students of the Madsen group, especially Philip R. Skaanderup and Morten Jørgensen, for valuable discussions.

The students involved in the project through 3 years: Malene R. Jørgensen, Jesper Thorsen, Birgitte V. Gundersen, Karen-Margrethe Juul, Eva Bundgaard, Charlotte B. Pipper, Mette Mikkelsen, Henriette Holm, Dorthe M. Skytte, Anders Piper, and Niels M. E. Jensen. Not all contributions have made their way to this thesis, but they have all been valuable.

Dr. Kudzai Mutenda, University of Southern Denmark, for mass spectroscopy. Clive P. Walter, Danisco, for mass spectroscopy. Professor Jørn D. Mikkelsen and Dr. Tove M. I. E. Christensen, Danisco for valuable discussions and allowing me access to the laboratory facilities at Danisco.

Dr. J. Paul Knox for allowing me to work in his group for 2 months, and Dr. William G. T. Williams and Dr. Lesley McCartney for their patience in showing me how to.

The Danish Research Agency Center Contract Program for financial support.

Henriette for love, support, and valuable critique.

Contents

Abstract

Development of strategies for chemical synthesis of pectic oligosaccharides is described. The protocols utilize the *n*-pentenyl glycosylation methodology and preparation of suitably protected mono- and disaccharide glycoside donors is reported. A novel method for the direct conversion of an *n*-pentenyl glycoside into a glycosyl fluoride has been developed, along with a new application of the *armed-disarmed* method for saccharide coupling. Tri- and hexagalacturonates with a selective pattern of methyl esterification have been prepared and the latter have been successfully applied as substrates for pectic enzymes and haptens for anti-homogalacturonan monoclonal antibodies. Furthermore, the synthesis of neoglycoconjugates in the form of tri- and hexasaccharides linked to bovine serum albumin has been achieved. The characterization of a new antibody originating from *in vivo* immunization with one of these glycoprotein antigens is outlined.

1. Pectin – An Overview

Origin, Structure, Biological and Industrial Importance

Introduction

Pectin is a common name given to a class of polysaccharides of plant origin. They are found in the primary cell walls of all higher plants where they constitute approximately 22-35% of the dry mass.[1] Pectic polysaccharides contribute to a number of important functions of cell walls, and thus of the plant.[2] The pectic matrix resists compressive forces resting upon cell walls, determines porosity, contributes to ionic status, and has important roles in the plant's defense mechanisms.[3]

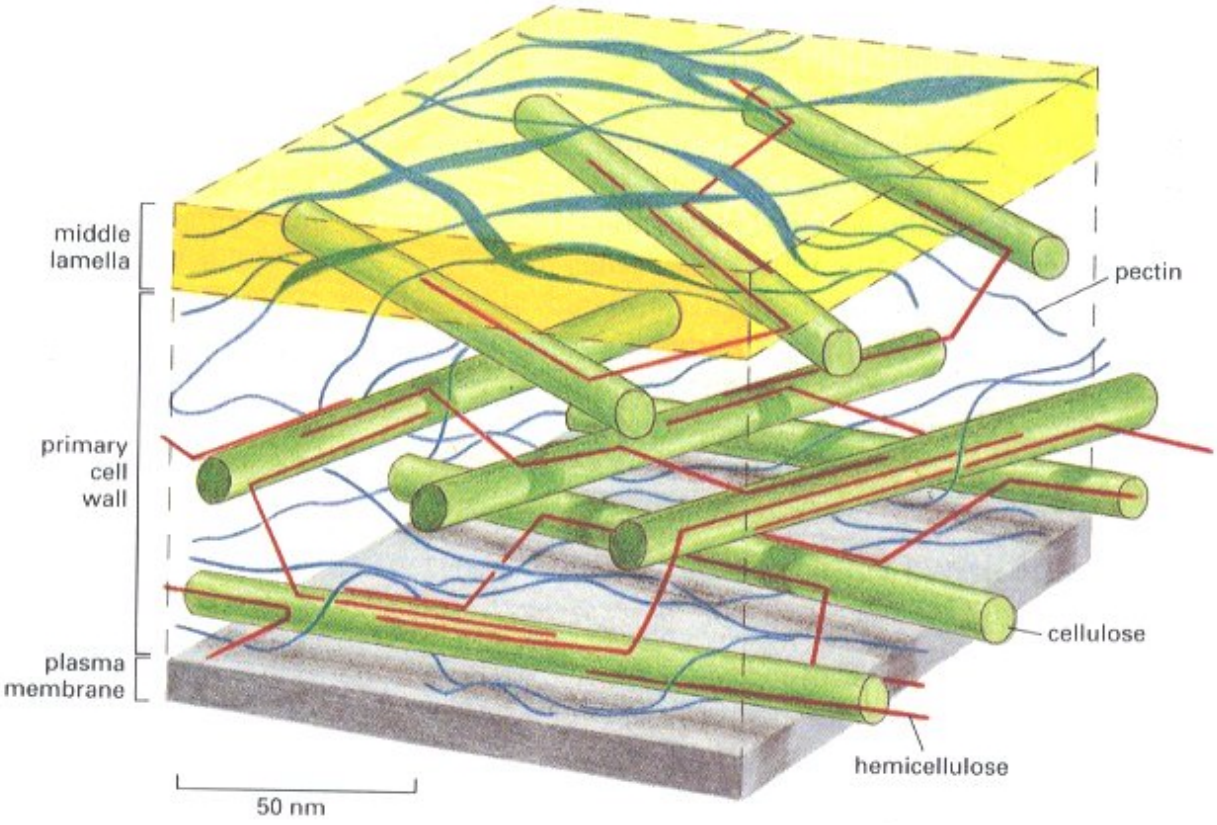


Figure 1

[1] Carpita, N. C.; Gibeaut, D. M. *Plant J.* **1993**, *3*, 1.
[2] McCann, M. C.; Bush, M.; Milioni, D.; Sado, P.; Stacey, N. J.; Catchpole, G.; Derfernez, M.; Carpita, N. C.; Hofte, H.; Ulvskov, P.; Wilson, R. H.; Roberts, K. *Phytochemistry* **2001**, *57*, 811.
[3] Basic, A.; Harris, P.; Stone, B. In *The Biochemistry of Plants*, *vol. 14, Carbohydrates*, Academic Press, London, **1988**, pp. 297-369.

Primary plant cell walls are a highly complex cooperative assembly of many different glycans and proteins. The composition of cell walls is highly variable, but some of the major features are shown in Figure 1.[4]
The figure illustrates the primary cell wall between the plasma membrane and the middle lamella. The main glycans found here are cellulose, the most abundant carbohydrate in plants, hemicellulose, and the pectic polysaccharide fibers. These are shown in blue and are intertwined in the cellulose network in the tissue.
Pectic polysaccharides are immensely complex. Their common feature is D-galacturonic acid, the backbone of the majority of pectic polysaccharides. Apart from the abundant uronic acid, neutral sugars such as L-rhamnose, D-galactose, D-xylose, and L-arabinose are also major components of pectic polysaccharides (Figure 2).

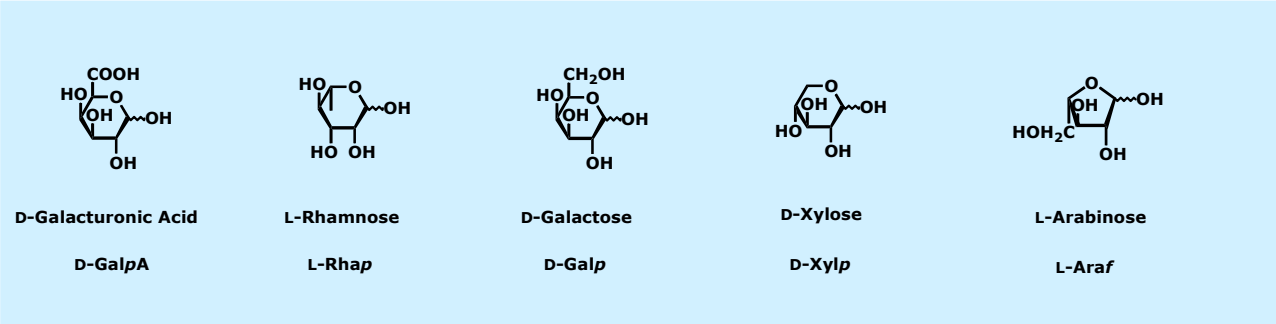


Figure 2

In general, three types of pectic polysaccharides have been identified in plants, homogalacturonan (HG) and rhamnogalacturonan (RG) I and II. A schematic illustration of the structures of HG and RG-I is given in Figure 3.[5] Whether these polymers are joined by covalent linkages, and whether they are further linked to other cell wall components is not known with certainty, but several experimental observations indicate it to be so.[6]
Because HG is the most abundant of the pectic polysaccharides, it has been subject to most studies. It consists of α-(1→4)-linked D-galacturonic acids,

[4] From: Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K. *Molecular Biology of THE CELL*, *3rd ed.*, Garland Publishing, New York, **1994**, p. 1002.
[5] Adapted from: a) Schols, H. A.; Voragen, A. G. J. In *Pectins and their Manipulation*, Seymore, G. B.; Knox, J. P. (Eds.), Blackwell Publishing, Oxford, **2002**, pp. 1-29 and b) Ridley, B. L.; O'Neill, M. A.; Mohnen, D. *Phytochemistry* **2001**, *57*, 929.
[6] a) Ishii, T.; Matsunaga, T. *Phytochemistry* **2001**, *57*, 969; b) Albertsheim, P.; Darvill, A. G.; O'Neill, M. A.; Schols, H. A.; Voragen, A. G. J. In *Pectins and Pectinases*, Visser, J.; Voragen, A. G. J. (Eds.), Elsevier, Oxford, **1996**, pp. 47-56; c) Mort A. J. In *Pectins and their Manipulation*, Seymore, G. B.; Knox, J. P. (Eds.), Blackwell Publishing, Oxford, **2002**, pp. 30-51.

which are methyl esterified to a degree dependent on the plant in question and to the localization of the polysaccharide within the plant tissue. Furthermore, the polymer may be acetylated at *O*-2, *O*-3 or both, but acetylated HG is believed to be present only within certain plant species.[7] It is generally accepted, that the degree and pattern of methyl esterification, although very complex, is not random, but rather an intrinsic part of the plants regulation of cell wall growth and structure.[8]

RG-I consists of a backbone made up of a repeating unit of galacturonic acid and rhamnose: [-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)]. The backbone is functionalized with sidechains of neutral sugars, e.g. arabinan and galactan, and the galacturonic acid residues may be acetylated or methyl esterified.

RG-II, the third major component of pectic polysaccharides, is a complex, but highly conserved structure within plants. It has a backbone of (1→4)-α-D-Gal*p*A as HG, but numerous sidechains containing rare sugars are appended, the precise location and connectivity of which is not fully known.

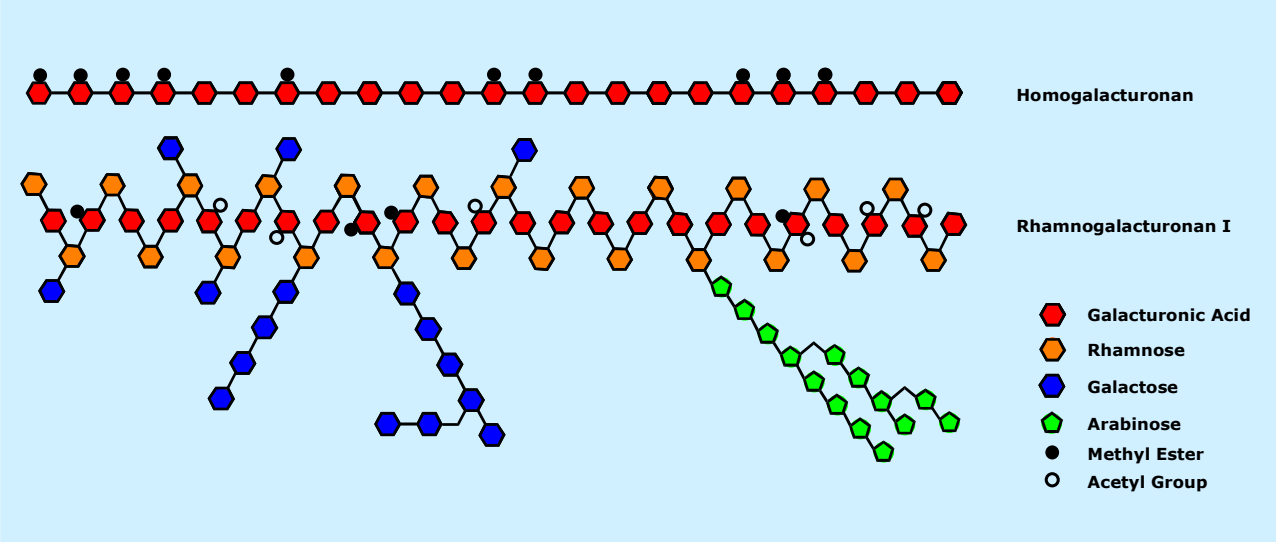


Figure 3

Plants obviously play a vital role in the ecosystem. They produce dioxygen and are a source of nutrition for microorganisms, fungi, and animals. The cell walls of plants are cornerstones of plant development and life, and an understanding of the mechanisms governing their structure and function is important for comprehending plant biology. An important part of obtaining such understanding is the study of pectic polysaccharides *in planta.* To appreciate the magnitude of this task, one only has to consider for instance the fact that not only does HG isolated from different plant sources differ in composition,

[7] Ishii, T. *Plant Physiol.* **1997**, *113*, 1265.
[8] Mort, A. J.; Qiu, F.; Maness, N. O. *Carbohydr. Res.* **1993**, *247*, 21.

but even within a single cell wall, HG with differing esterification pattern has been localized (*vide infra*).[9]
Extracts of pectic polysaccharides from plants, especially citrus peel and apple pomace, have important applications in the food industry and are termed pectin.[10] They are used primarily as gelling agents and stabilizers.[11] The ability of pectin to form gels is being ascribed to the HG and its ability to form a rigid network. The presence of Ca^{2+} ions mediates the gelling by forming dimers between stretches of unesterified HG.[12] This crosslinking is also present in plants, where it contributes to the strength of the matrix formed by the pectic polysaccharides.

Pectic Enzymes

The enzymes involved in biosynthesis, modification, and degradation of pectic oligosaccharides are many and varied. Glycosyltransferases, acetyl transferases, and methyl transferases are responsible for synthesizing the polymers *in planta* and esterases, lyases, and various glycosylases degrade and modify the polysaccharides. If the assumption is made, that one discrete glycosyltransferase is responsible for each individual glycosidic linkage found in the pectic polysaccharides, at least 53 enzymes are involved in their synthesis.[13] Of these, only a few have been isolated, and none have so far been cloned.
Enzymes degrading pectic polysaccharides, and especially HG, have been subject to most studies, mainly due to their importance as molecular tools for investigating the carbohydrates extracted from various plants. *endo*-Polygalacturonase, pectin and pectate lyase, and pectin methyl esterase from both plants and fungi are the most important of the HG degrading enzymes used in biochemical studies and are of paramount importance for the understanding of HG structure-activity relationships, both in plants and in food applications. The action sites and products formed by these enzymes are depicted in Figure 4. It has been shown that polygalacturonase from *Aspergillus niger* hydrolyses the HG polymer between two unesterified

[9] 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.
[10] Pilnik, W. *Pectin – a many splendored thing*, In *The Chemistry and Technology of Pectin*, Walter, R. H. (Ed.), Academic Press Inc, San Diego, **1991**, pp. 209-221.
[11] Pilgrim, G. W.; Walter, R. H.; Oakenfull, D. G. *Jams, Jellies, and Preserves*, In *The Chemistry and Technology of Pectin*, Walter, R. H. (Ed.), Acedemic Press Inc, San Diego, **1991**, pp. 24-50.
[12] Jarvis, M. C. *Plant Cell Environ.* **1984**, *7*, 153.
[13] For a review of pectin biosynthesis, see: Mohnen, D. In *Comprehensive Natural Product Chemistry, Vol. 3, Carbohydrates and their Derivatives Including Tannins, Cellulose, and Related Lignins*, Pinto, B. M. (Ed.), Elsevier, Oxford, **1999**, pp. 497-527.

galacturonic acid residues and is incapable of complete degradation of methyl esterified HG.[14] The requirements for cleavage by pectin and pectate lyases are less well established. They operate in methylated and unmethylated regions of HG respectively, and degrade the polymer by β-elimination, generating a 4-5 unsaturation at the non-reducing end of the fragment released.[15] Pectin methyl esterases from fungi forms HG with a distributed, random pattern of esterification,[14] whereas similar enzymes of plant origin are also known to generate blocks of deesterified HG.

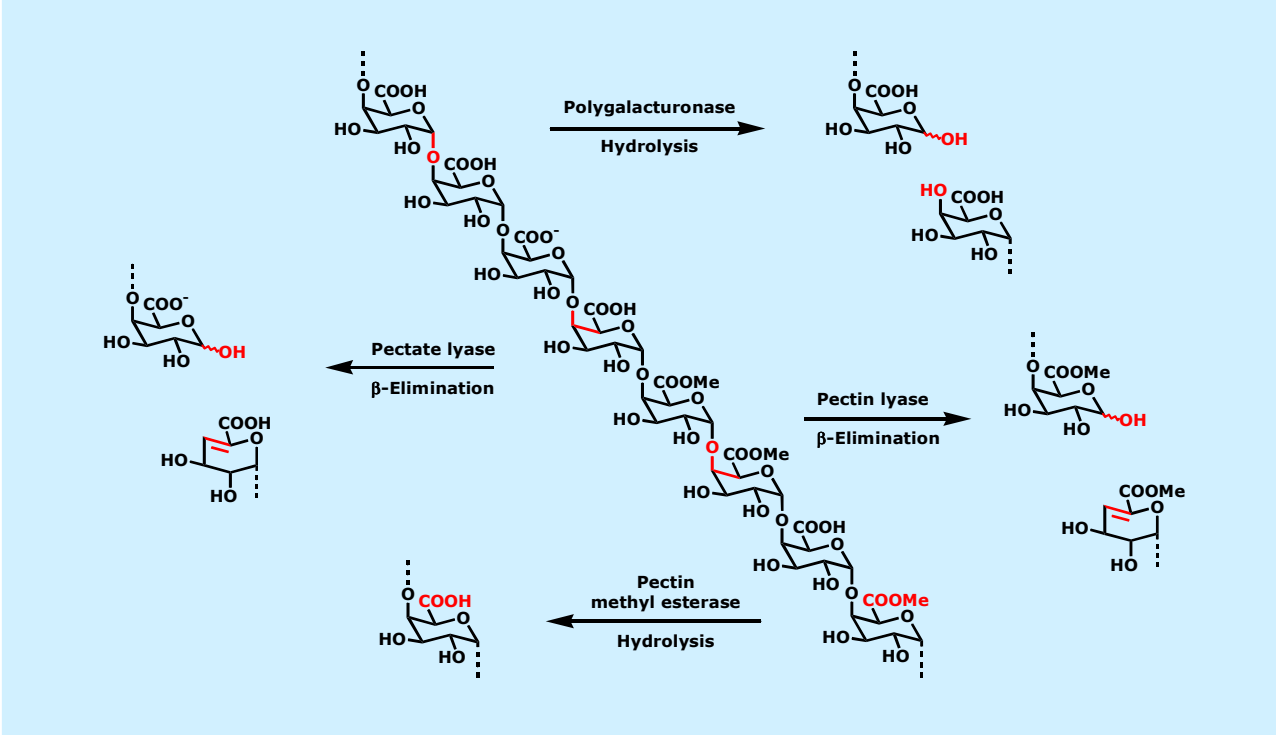


Figure 4

Studying Pectic Polysaccharides *in planta* Using Antibody Probes

The difficulties involved in correlating the structure and property of isolated polysaccharides obtained from various plant sources with their function *in planta* have instigated a desire to study them *in situ*, within the context of intact cell wall architecture. This not only means that the carbohydrates are unaffected by the isolation processes, but furthermore provides valuable

[14] Limberg, G.; Körner, R.; Buchholt, H. C.; Christensen, T. M. I. E.; Roepstorff, P.; Mikkelsen, J. D. *Carbohydr. Res.* **2000**, *327*, 293.

[15] a) Pilnik, W.; Voragen, A. G. J. In *Food Enzymology*, Fox, P. F. (Ed.), Elsevier Applied Science, London, **1991**, pp. 303-336; For recent contributions to the study of pectin lyases, see: b) Mutenda, K. E.; Körner, R.; Christensen, T. M. I. E.; Mikkelsen, J.; Roepstorff, P. *Carbohydr. Res.* **2002**, *337*, 1217; c) van Alebeek, G.-J. W. M.; Christensen, T. M. I. E.; Schols, H. A.; Mikkelsen, J. D.; Voragen, A. G. J. *J. Biol. Chem.* **2002**, *277*, 25929.

information about their distribution and developmental importance. To this end, a number of anti-pectin antibodies have been produced, each recognizing different epitopes, i.e. a unique chemical structure or substructure.
HG with different degrees and patterns of methyl esterification is recognized by various antibodies,[16] polygalacturonates dimerized by Ca^{2+} is the target of an antibody,[17] and several antibodies have been raised to distinct RG-I domains.[18] An example of the application of antibody technology is given in Figure 5, showing pea tissue labeled with two different antibodies. The blue fluorescence in (a), (b), and (c) stems from Calcofluor, a fluorophore binding to cellulose. This highlights the cell walls of three adjacent cells, with the intracellular space in the middle. In (a), PAM1,[19] which recognizes approximately 20-30 contiguous unesterified galacturonic acid residues in HG, shows up as green fluorescence. In (b), the green fluorescence is the antibody LM7,[9] which binds to a distinct, but unknown, epitope of partially methyl esterified HG (see also Chapter 6). Both antibodies have been applied in (c), PAM1 fluorescing green and LM7 red. It is evident, that the epitope of LM7 is only present at the corners of the intercellular space formed by three cells, whereas the PAM1 epitope of unesterified HG is ubiquitous along the intercellular space cell wall tissue.

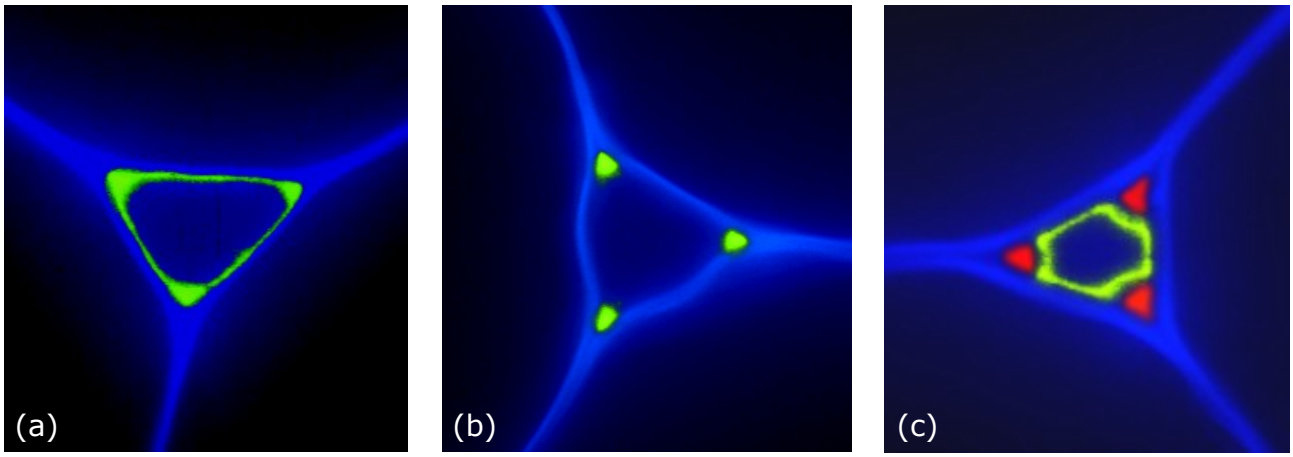


Figure 5

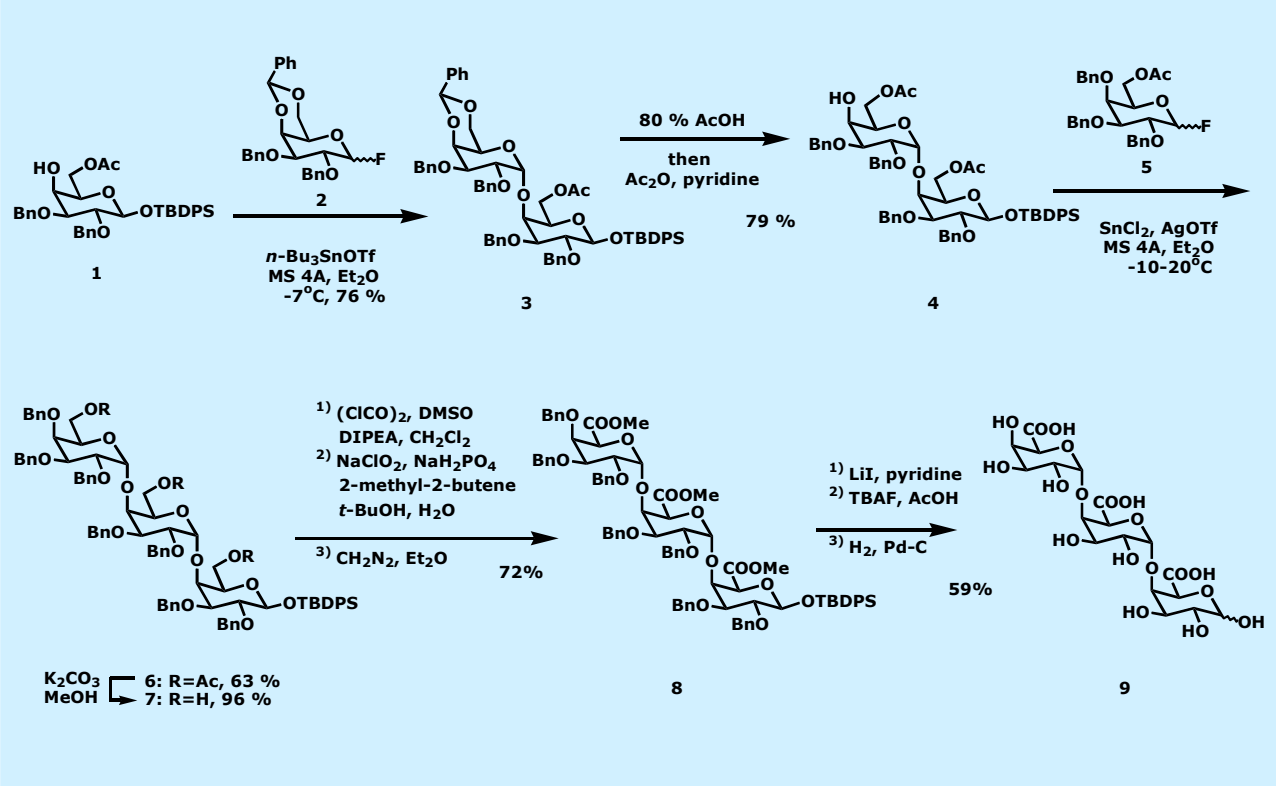
[16] Willats, W. G. T.; Limberg, G.; Buchholt, H. C.; van Alebeek, G.-J.; Benen, J.; Christensen, T. M. I. E.; Visser, J.; Voragen, A.; Mikkelsen, J.; Knox, J. P. *Carbohydr. Res.* **2000**, *327*, 309 and references herein; Ref. 9.
[17] Liners, F.; Leteson, J.-J.; Didembourg, C.; van Cutsem, P. *Plant Physiol.* **1989**, *91*, 1419.
[18] For a discussion of these, see: Knox, J. P. In *Pectins and their Manipulation*, Seymore, G. B.; Knox, J. P. (Eds.), Blackwell Publishing, Oxford, **2002**, pp. 131-149.
[19] Willats, W. G. T.; Gilmartin, P. M.; Mikkelsen, J. D.; Knox, J. P. *Planta* **1999**, *18*, 57.

Synthetic Studies of Homogalacturonan Oligosaccharides

The difficulties associated with obtaining pure samples of oligomers of galacturonic acid for various investigations have prompted several efforts to make such oligomers available by chemical synthesis.[20] Nakahara and Ogawa published the synthesis of a trigalacturonate in 1990,[21] and later that year the synthesis of a dodecamer.[22] Their strategy for the synthesis of the former compound (**9**), outlined in Scheme 1, relied on coupling of galactosyl fluoride glycosyl donors (**2** and **5**) and galactose acceptors (**1** and **4**) to arrive at the fully protected trigalactan **6**. They then removed the 6-*O*-acetyl protecting groups and oxidized the liberated alcohols of **7** to the corresponding uronic acids, using Swern oxidation[23] followed by treatment with sodium chlorite.[24] In order to purify the triacid, it was subjected to ethereal diazomethane[25] to produce the triester **8**. Removal of the methyl esters using lithium iodide,[26] desilylation with tetrabutylammonium fluoride[27] and hydrogenolysis provided the desired compound **9**. A similar strategy was applied for the synthesis of the dodecagalacturonic acid, only here, they took advantage of the possibility of removing the *tert*-butyldiphenylsilyl protection group from the anomeric position. Treatment of the resulting hemiacetal with diethylaminosulfur trifluoride[28] yielded a glycosyl fluoride, which could then serve as a glycosyl donor. This allowed a convergent synthesis applying di- and trisaccharide glycosyl donors.

---

[20] For reviews of various aspects of carbohydrate chemistry, see: a) David, B. G. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2137; b) Various authors, *Glycobiology*, in *Chem. Rev.* **2002**, *102*, 283-603, Dwek, R. A.; Butters, T. D. (Eds.).
[21] Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *200*, 363.
[22] Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *205*, 147.
[23] Omura, K.; Swern, D. *Tetrahedron* **1978**, 1651.
[24] Kraus, G.; Roth, B. *J. Org. Chem.* **1980**, *45*, 4825.
[25] Resitz, M.; Maas, G. In *Diazo Compounds, Properties and Synthesis*, Academic Press, Orlando, **1986**.
[26] McMurry, J. *Org. React.* **1976**, *24*, 187.
[27] Kinzy, W.; Smith, R. R. *Justus Liebigs Ann. Chem.* **1985**, 1537.
[28] a) Middleton, W. J. *J. Org. Chem.* **1975**, *40*, 574; b) Posner, G. H.; Haines, S. R. *Tetrahedron Lett.* **1985**, *26*, 5.

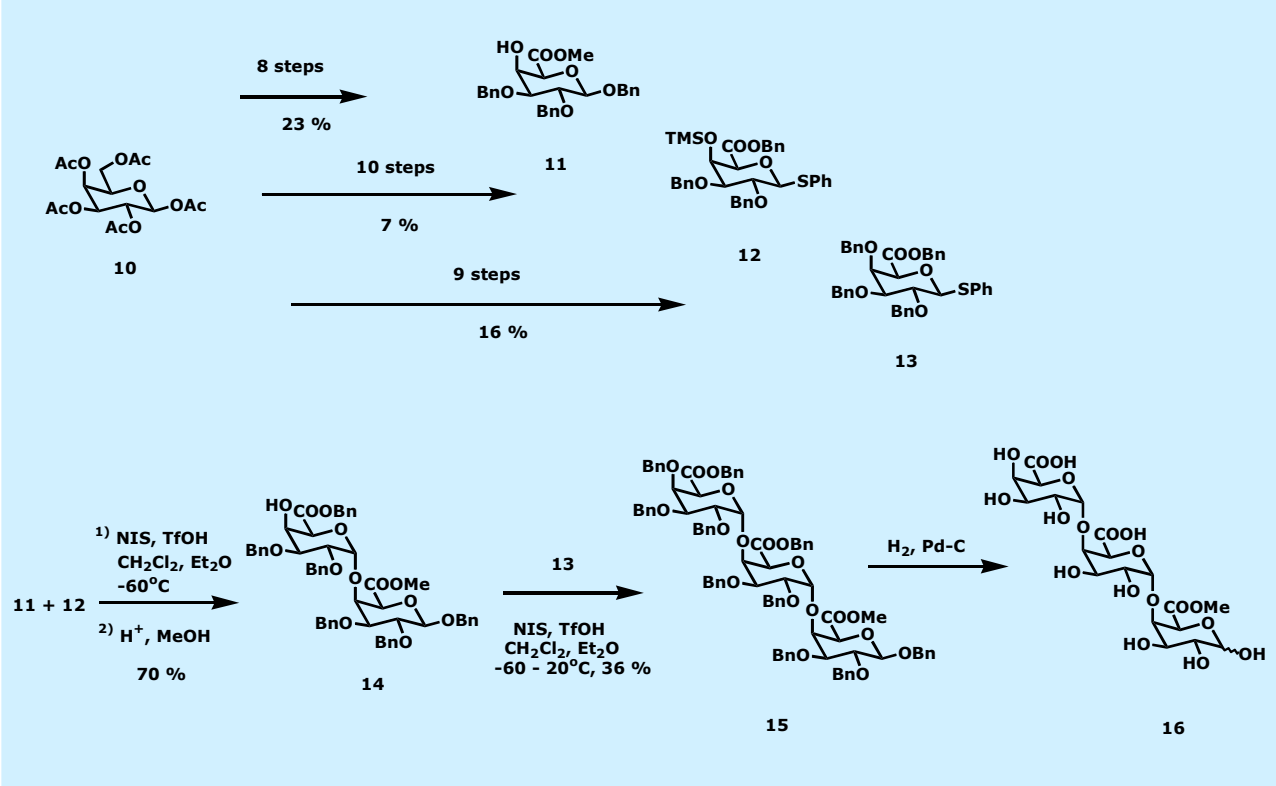


Scheme 1

Where Ogawa applied suitably protected galactose building blocks and then oxidized the galactans to uronic acids at a late stage in the synthesis, others have since chosen to work with protected galacturonic acid glycosyl donors and acceptors. Anker, Doutheau and co-workers have for some time been engaged with the preparation of HG oligomers using this strategy.[29] Their efforts culminated in 1999 with the preparation of three monomethylated trigalacturonates, the first example of selectively methylated oligomers of galacturonic acid.[30] The route to one of these is shown in Scheme 2. The three required building blocks **11**, **12**, and **13** were all prepared from galactose pentaacetate **10**. The coupling of **11** and **12** proceeded smoothly to give disaccharide acceptor **14** after *in situ* removal of the trimethylsilyl protecting group. Glycosylation of **14** with donor **13** yielded trisaccharide **15**, however in a modest 36% yield. Finally, hydrogenolysis afforded the desired monomethylated trisaccharide **16**.

[29] a) Magaud, D.; Grandjean, C.; Doutheau, A.; Anker, D.; Schevchik, V.; Cotte-Pattat, N.; Robert-Baudouy, J. *Tetrahedron Lett.* **1997**, *38*, 241; b) Magaud, D.; Grandjean, C.; Doutheau, A.; Anker, D.; Schevchik, V.; Cotte-Pattat, N.; Robert-Baudouy, J. *Carbohydr. Res.* **1998**, *314*, 189.

[30] Kester, H. C. M.; Magaud, D.; Roy, C.; Anker, D.; Doutheau, A.; Schevchik, V.; Hugouvieux-Cotte-Pattat, N.; Benen, J. A. E.; Visser, J. *J. Biol. Chem.* **1999**, *274*, 37053.



Scheme 2

In a similar fashion, the other two possible monomethylated trisaccharides were synthesized, and the compounds were used as enzyme substrates for a number of pectic enzymes.[30]

A very similar approach to the synthesis of pectic oligomers was used by Vogel and co-workers. Applying ethyl thioglycosides as glycosyl donors,[31] a protected, trimethylated trigalacturonate was reported in 2000.[32] The main difference between the work described by them and the approach taken by Anker, Doutheau and co-workers is the preparation of the building blocks. Vogel and co-workers synthesized their glycosyl acceptors directly from D-galacturonic acid, thereby bypassing the oxidation of a protected galactoside. They were not able to prepare a thioglycoside donor in a similar fashion,[32] but they have later disclosed the use of trichloroacetimidate glycosyl donors[33] for the synthesis of rhamnogalacturonan fragments,[34] thus bypassing this hindrance.[35] However, they have never published the synthesis of any fully

[31] For a review describing the use of thioglycosides as glycosyl donors, see: Fügedi, P.; Garegg, P. J.; Lönn, H.; Norberg, T. *Glycoconjugate J.* **1987**, *4*, 97.
[32] Kramer, S.; Nolting, B.; Ott, A.-J.; Vogel, C. *J. Carbohydr. Chem.* **2000**, *19*, 891.
[33] See: a) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212; b) Schmidt, R. R. *Pure Appl. Chem.* **1989**, *61*, 1257.
[34] See also: Nolting B.; Boye, H.; Vogel, C. *J. Carbohydr. Chem.* **2000**, *19*, 923.
[35] Nolting B.; Boye, H.; Vogel, C. *J. Carbohydr. Chem.* **2001**, *20*, 585.

deprotected HG oligomers, although they declare to have prepared such compounds.[36]
In the summer of 1999, the only known synthetic work addressing the preparation of HG oligomers was the papers of Ogawa and co-workers. No partially methyl esterified oligomers had been prepared and there was a need for these compounds in order to advance the knowledge of pectic enzymes and anti-HG antibodies. Therefore, a program aimed at developing reliable methods for synthesizing such oligosaccharides was initiated, the results of which is described in the following chapters.

[36] Vogel, C. *personal communication*, Royal Danish Veterinary and Agricultural University, February 2002.

2. Method Development

Synthesis of Monosaccharides and Monomethylated Trigalacturonates

Introduction

The investigation of pectic enzymes and epitopes of pectin-recognizing antibodies is retarded by lack of access to well-defined, oligomeric galacturonates. With the current separation technologies, milligram amounts of demethylated oligogalacturonates with DP=3-20 can be obtained from mixtures of oligomers originating from enzymatic degradation of polygalacturonates.[37] However, the challenging task of separating oligomers with the same degree of polymerization (DP) and methylation (DM), differing only in methylation pattern, is yet to be achieved. Although it would be desirable to have access to well-defined fragments of pectic polysaccharides from simple degradation of the natural polymer, this is not possible yet. This has prompted synthetic studies towards selectively methylated oligogalacturonates (cf. Chapter 1). When this project was initiated, only few examples existed in the literature of application of uronic acid glycosyl donors. At the time, glycosylation yields were moderate, and the only example of a galacturonic acid donor, a phenyl thioglycoside, had afforded a trisaccharide in 45% yield.[29a] Knowing that galactosyl donors are more reactive, a strategy similar to the one used by Ogawa and co-workers in their syntheses of non-esterified oligogalacturonates (see Chapter 1) was planned. Thus, the oligomers were to be assembled using galactose glycosyl donors and acceptors and then oxidized to uronic acids.[38]

In order to explore synthetic methodology towards selectively methylated oligogalacturonates, trisaccharides **16**, **17**, and **18** (Figure 6) were chosen as the first targets.

[37] Hotchkiss, Jr.; A. T.; Lecrinier, S. L.; Hicks, K. B. *Carbohydr. Res.* **2001**, *334*, 135.

[38] For a similar approach to glucuronic acid containing heparin oligomers, see: Haller, M.; Boons, G.-J. *J. Chem. Soc., Perkin Trans. 1* **2001**, 814.

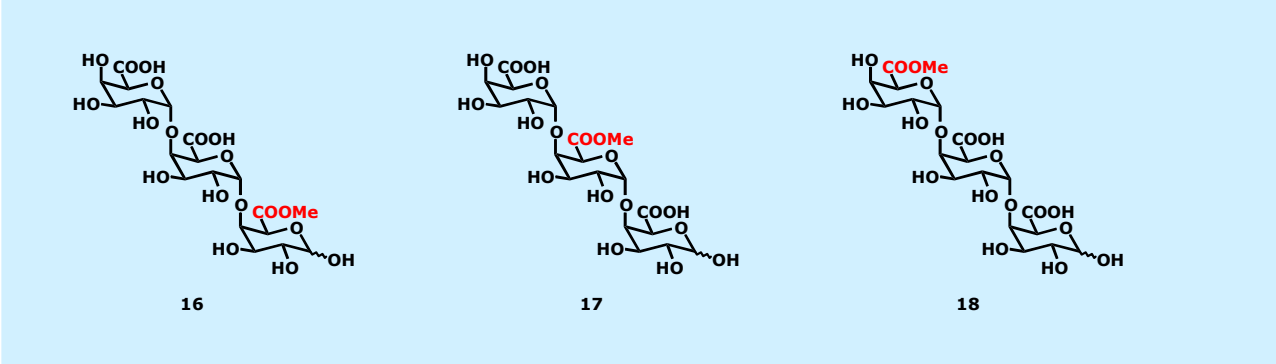
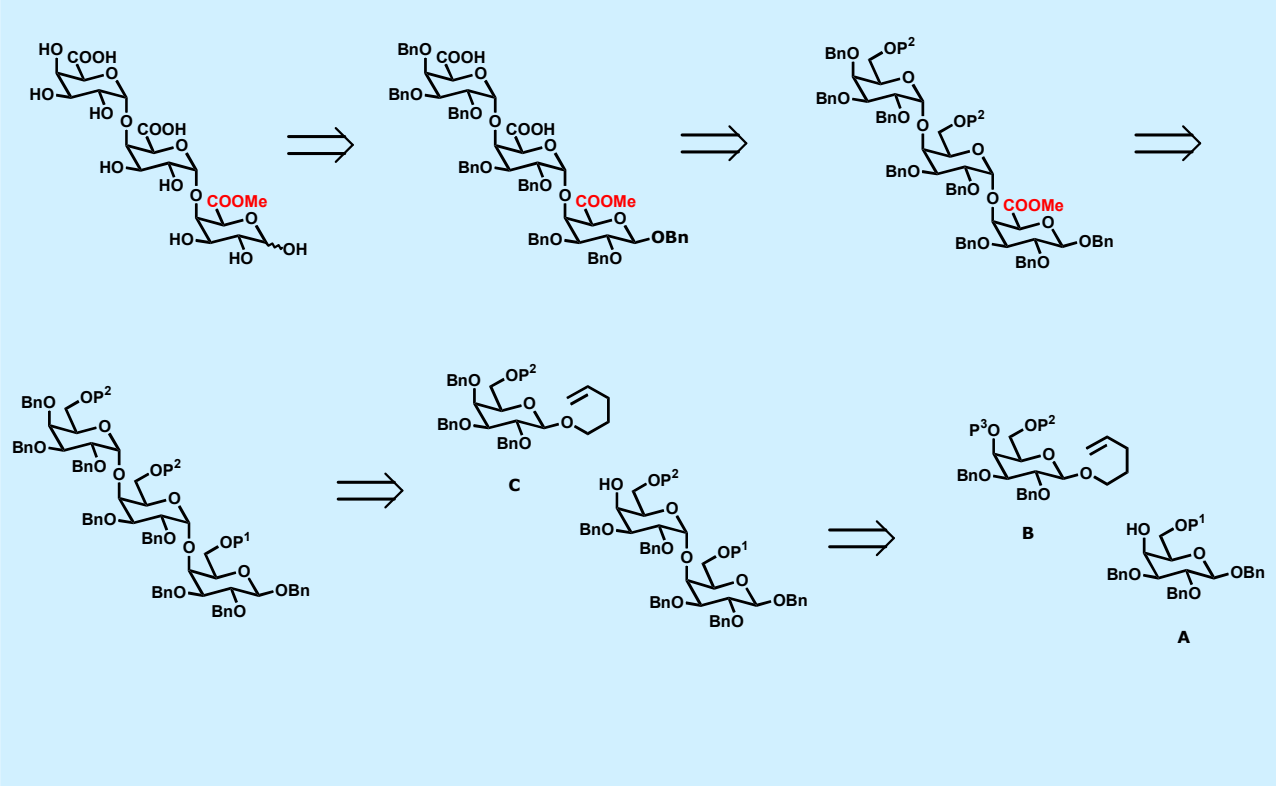


Figure 6

The successful synthesis of these compounds would establish that methyl esters could be introduced to any uronic acid residue in pectic oligosaccharides. Furthermore, the simplicity of these targets made them ideal for method development.[39]

Retrosynthesis



Scheme 3

[39] Incidentally, we were not the only group with these considerations, see ref. 30.

The retrosynthetic analysis of a monomethylated trigalacturonate is shown in Scheme 3. The target molecule can be obtained by hydrogenolysis of the benzyl groups,[40] used for permanent blocking of the hydroxy functionalities that are not manipulated during the synthesis. The choice of benzyl ethers for protection of the 2- and the 3-positions should furthermore aid in obtaining a high α-selectivity in the glycosylation reactions because they are non-participating groups.[41] The acid groups are installed after the single methyl ester, by removal of P^2 followed by oxidation of the primary alcohols. This requires that P^2 can be cleaved in the presence of both benzyl groups and the methyl ester and that P^2 is stable to the reaction conditions used earlier in the synthesis. Benzyl ethers are stable towards most mild deprotection conditions, but the methyl esters are vulnerable to base, with both transesterification and β-elimination as potential deleterious side-reactions. The *para*-methoxyphenyl (PMP) group was chosen as P^2, since it can be cleaved under mildly acidic conditions using a one-electron oxidant.[42]

The methyl ester is introduced by oxidation and esterification after selective removal of P^1. An acetyl group was chosen as blocking group for this primary alcohol, since it is perfectly stable to the glycosylation conditions and can be removed under mildly basic conditions.

The trigalactoside ensues from glycosylation of a disaccharide acceptor with the glycosyl donor **C**. The disaccharide is obtained after coupling of glycosyl acceptor **A** and glycosyl donor **B** and removal of the P^3 protecting group. This deprotection should leave the P^1 and P^2 blocking groups intact and the chloroacetyl group was selected as P^3 since numerous examples exist of selective cleavage in the presence of acetyl groups.[43]

n-Pentenyl glycosides were chosen as glycosyl donors.[44] This was done because of the stability of the pentenyl group towards a number of transformations needed for the installment of protecting groups, the high reactivity when activated with appropriate electrophiles, and its easy conversion into other glycosyl donor types (see Chapter 3).

---

[40] King, A. O.; Larsen, R. D. *Palladium-Catalyzed Hydrogenolysis*, In *Organopalladium Chemistry for Organic Synthesis, Vol. 1*, Negishi, E. (Ed.), John Wiley & Sons, New York, **2002**, pp. 995-1050.

[41] The use of an acyl protecting group at the 2-position of the glycosyl donor is a widely used method of obtaining 1,2-*trans* glycosides. For the first paper describing the phenomenon see: Frush, H. L.; Isbell, H. S. *J. Res. Natl. Bur. Stds.* **1941**, *27*, 413.

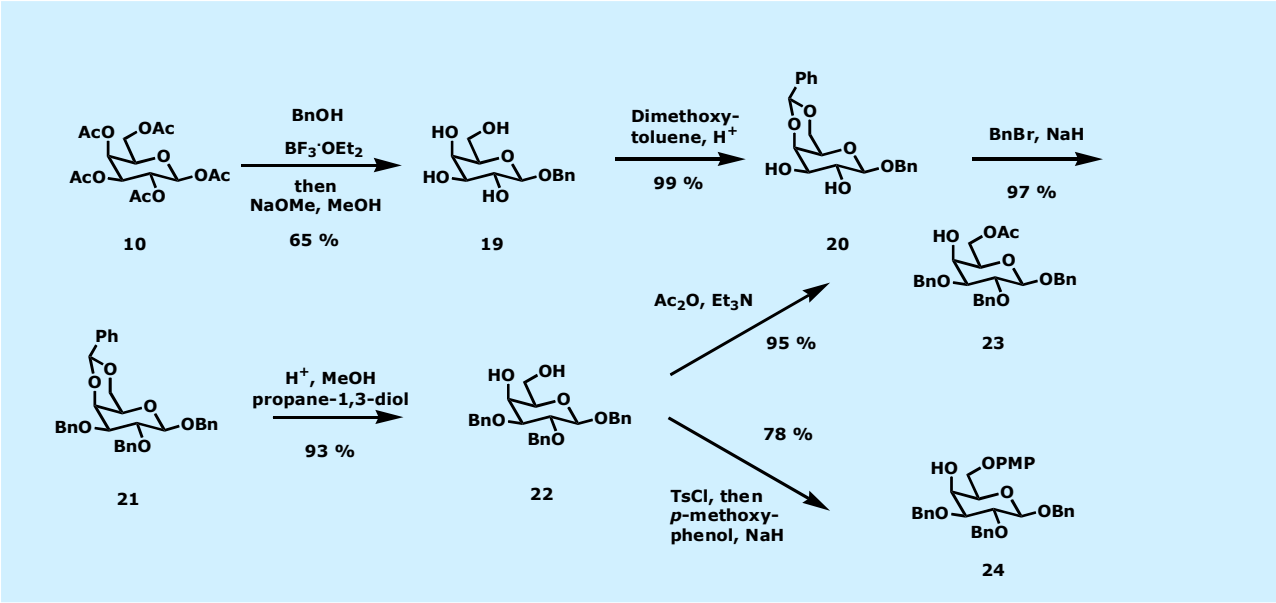
[42] Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *26*, 6291.

[43] a) Naruto, M.; Ohno, K.; Naruse, N.; Takeuchi, H. *Tetrahedron Lett.* **1979**, 251; b) van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *24*, 3775; c) Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1987**, 1026; d) Lefeber, D. J.; Hamerling, J. P.; Vliegenthart, J. F. G. *Org. Lett.* **2000**, *2*, 701.

[44] Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927.

Synthesis of Protected Monosaccharides

As outlined in the retrosynthetic scheme, a number of protected monosaccharides were required for the syntheses of the oligogalacturonates. In general, three types of protected galactosides were needed. Benzyl galactopyranosides for the reducing end residues of the oligosaccharides (**A**, Scheme 3). Pentenyl galactosides with a temporarily blocked 4-OH (**B**, Scheme 3), and pentenyl glycosides with a permanent benzyl protection group at the axial 4-position for the non-reducing end of the oligosaccharides (**C**, Scheme 3). All three types of building blocks should be available with both a PMP and an acetyl group at the 6-position in order to prepare **16**-**18**.

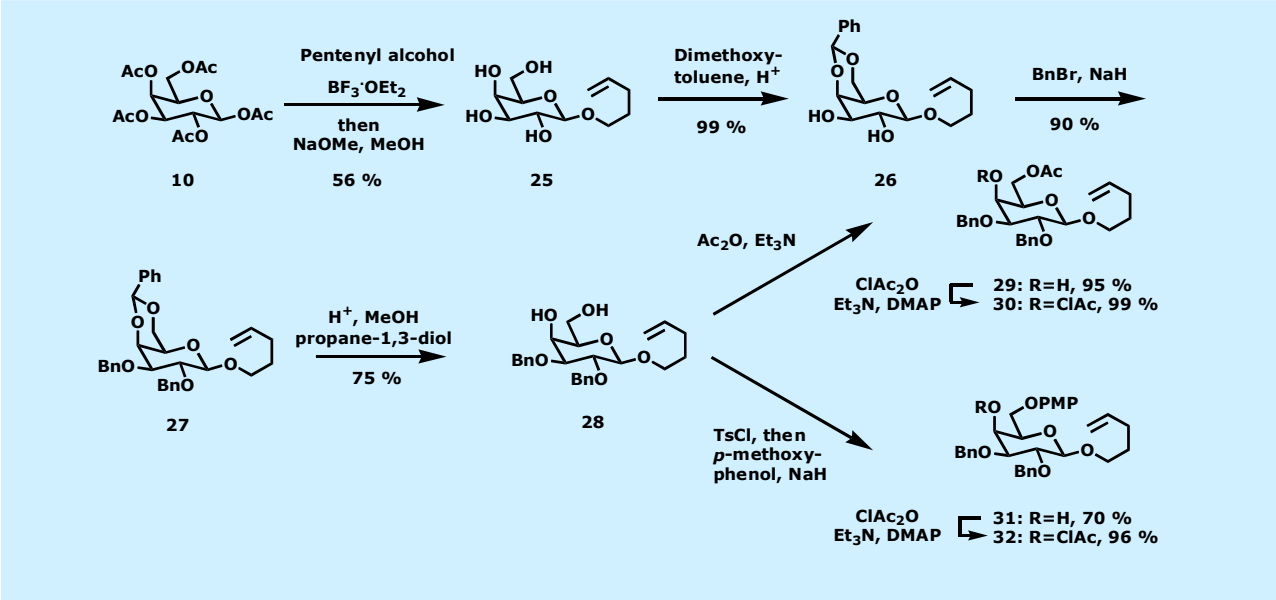


Scheme 4

The synthesis of the two desired A-type galactosides is outlined in Scheme 4. Benzyl alcohol was glycosylated with galactose pentaacetate (**10**) and Zemplén deacetylation[45] afforded the crystalline tetrol **19**. The 4- and the 6-position were blocked as a benzylidene acetal giving **20** and benzyl ethers introduced at the 2- and the 3-position providing **21**. Hydrolysis of the acetal proved troublesome. The reaction gave unsatisfactory yields when standard methanolysis or hydrolysis (*p*-toluenesulfonic acid in methanol and dichloromethane or 80% aq. acetic acid) was attempted. Addition of propane-1,3-diol to trap the liberated benzaldehyde as the phenyldioxane provided a solution, giving access to crystalline diol **22** in 93% yield, which upon

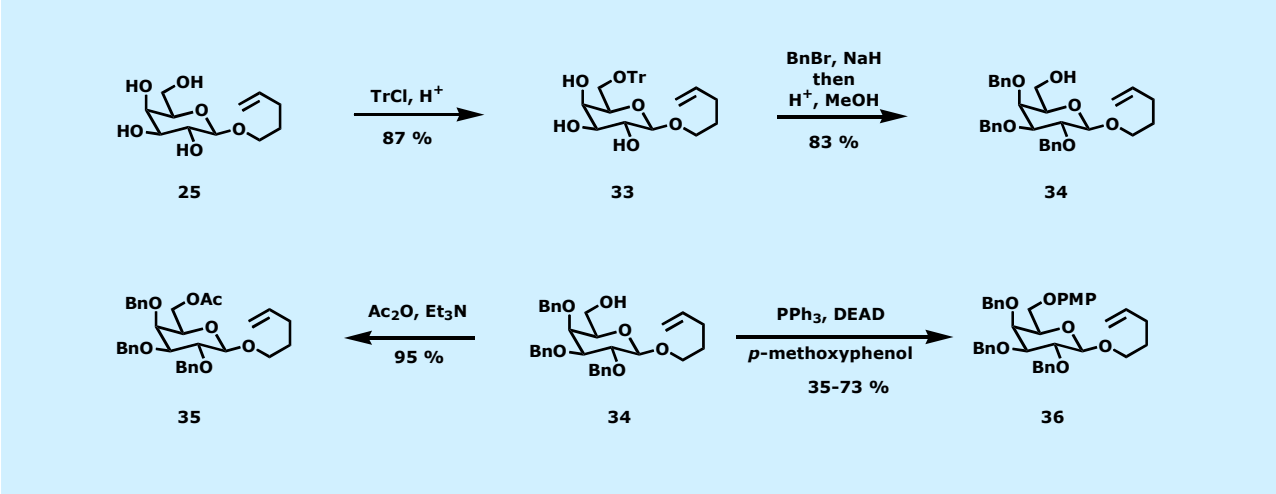
[45] Zempén, G.; Kunz, A. *Ber.* **1923**, *56*, 1705.

acetylation yielded **23**. Alternatively, a tosylgroup was introduced selectively at the primary alcohol of **22** and the leaving group was displaced with the sodium salt of *para*-methoxyphenol affording **24**.



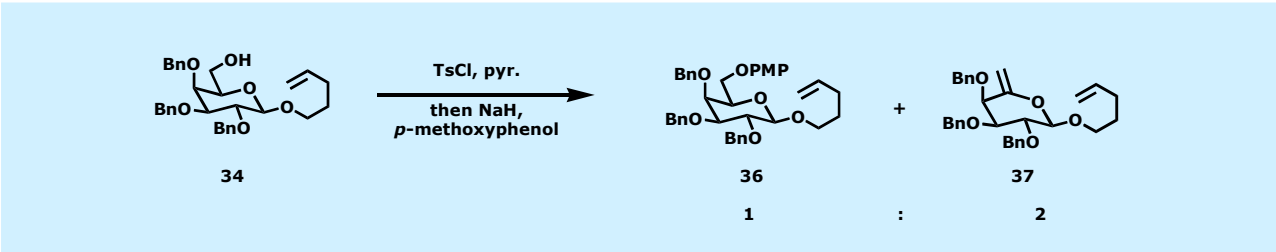
Scheme 5

Similar reactions were applied for the synthesis of the B-type galactosides (Scheme 5). Pentenyl galactopyranoside **25** was prepared, protected as benzylidene adduct **26**, benzylated to give **27**, and the benzylidene group removed by hydrolysis providing easy access to crystalline diol **28**. After introduction of an acetyl group at *O*-6 (**29**), chloroacetylation furnished fully protected galactoside **30**. Tosylation and treatment of the intermediate with *para*-methoxyphenolate yielded **31**, which in turn was easily converted to the chloroacetylated compound **32**.



Scheme 6

Tetrol **25** also served as the starting point for the synthesis of C-type glycosides (Scheme 6). The 6-position was protected with a trityl group to give **33**. Benzylation and acidic hydrolysis of the trityl ether furnished alcohol **34**. This could be acetylated providing **35** or alternatively, a Mitsunobu reaction[46] with *para*-methoxyphenol provided glycosyl donor **36**. This transformation was sensitive to scale-up however, resulting in decreased yields. Attempts to use the tosylation-displacement method analogous to the synthesis of **24** and **31** were disappointing. The formation of the desired adduct **36** was accompanied by the preferential formation of the hexenopyranoside **37** (Scheme 7). The unreliability of the Mitsunobu reaction prompted a design of the glycosylation strategy favoring application of **35** over **36** when possible (see Chapter 4).

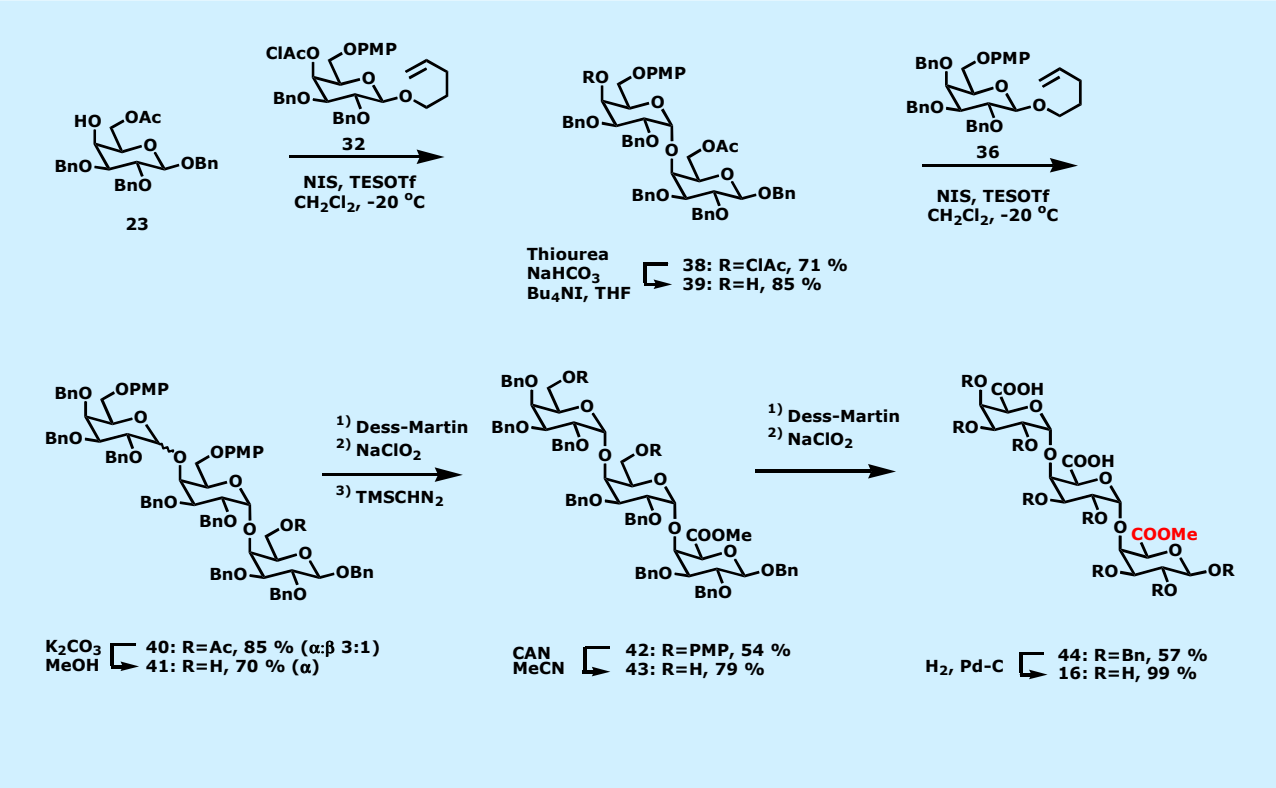


Scheme 7

[46] Mitsunobu, O. *Synthesis*, **1981**, 1.

Monomethylated Trisaccharides

The synthesis of **16**, commencing with the assembly of **23**, **32**, and **36**, is shown in Scheme 8. Glycosylation of acceptor **23** with donor **32** furnished disaccharide **38**. Generally, 1.3-1.5 equivalent of donor relative to acceptor was applied in glycosylations with pentenyl donors. A smaller surplus of donor decreased the coupling yields, but increasing the donor/acceptor ratio did not increase yields significantly. The reactions were run at –20 °C and were usually complete within 5-15 minutes affording the desired α-linked glycoside as the major product. Lowering the temperature resulted in slower conversion and raising the temperature diminished the α/β ratio.



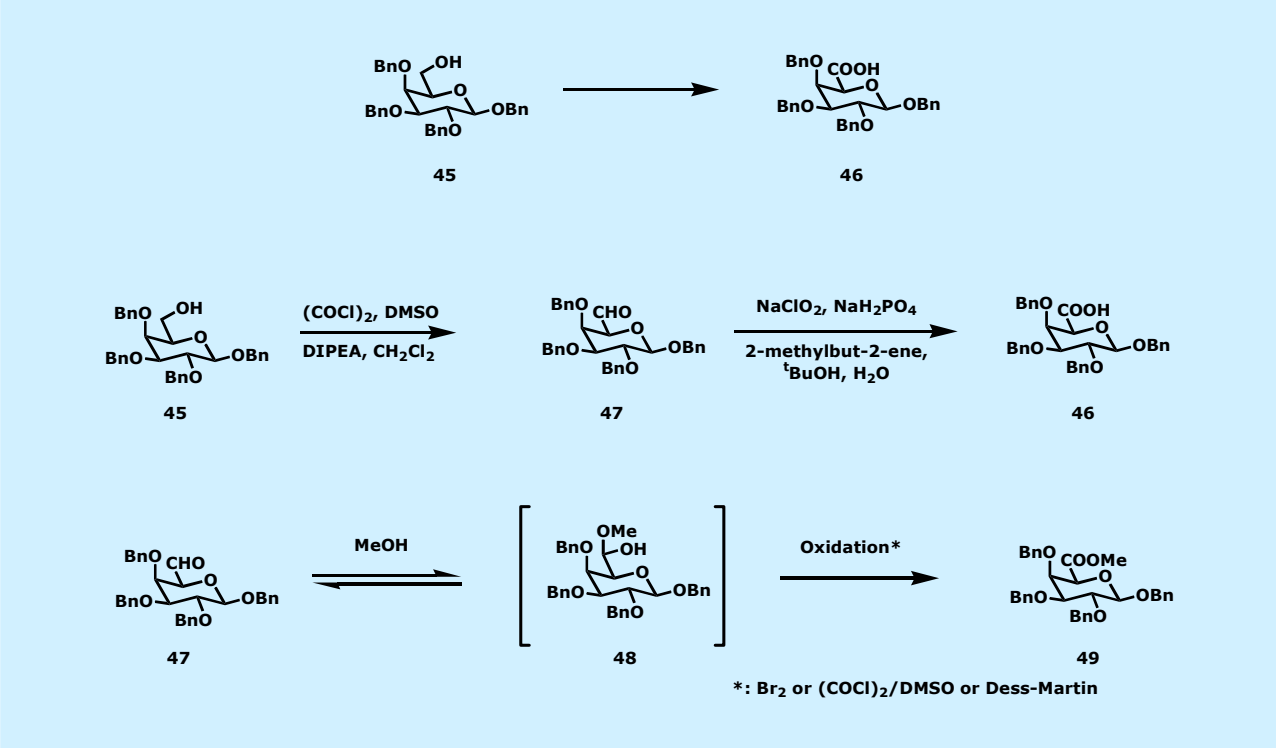
Scheme 8

The 4-hydroxy group was reconstituted by removal of the chloroacetyl group. This was achieved by treatment of **38** with thiourea. Traditionally, this reaction is performed in refluxing methanol.[47] When this was attempted, concomitant removal of the primary acetyl group was observed. Apparently, the added bicarbonate is sufficiently basic to produce a catalytic amount of sodium methoxide, which probably caused this sidereaction. This could be initiated by the thermal decomposition of bicarbonate into carbon dioxide and carbonate.

[47] See ref. 43a.

However, changing the solvent to the aprotic THF alleviated this problem and ensured a completely chemoselective reaction.[48]
Glycosylation of **39** with C-type glycosyl donor **36** yielded trisaccharide **40** as a 3:1 α:β-mixture. Saponification removed the single acetyl group and the desired α-anomer **41** could be isolated by chromatography.
Now the stage was set for transforming the primary alcohol to the corresponding carboxylic acid ester. This was achieved using a two-stage protocol. The aldehyde was obtained using the Dess-Martin periodinane,[49] and this was immediately taken on by oxidation with sodium chlorite. The resulting carboxylic acid was esterified using trimethylsilyl diazomethane in methanol,[50] affording **42**. Removal of the PMP protection groups[51] was best performed with cerium(IV) ammonium nitrate in wet acetonitrile,[52] furnishing **43**. The second round of oxidations was performed analogous to the first, and **44** was obtained. Finally, hydrogenolysis furnished the target monomethylated trigalacturonate **16**.
In all cases, the presence of the desired α-linkage in the di- and trisaccharides were confirmed by NMR spectroscopy. In general, the ^{13}C signals of α-linked anomeric carbon atoms can be found between 98 and 101 ppm, whereas the corresponding β-linked carbon atoms resonate at 102-105 ppm.[53] In some cases, the $^{1}J_{C1\text{-}H1}$ were determined, either from coupled ^{13}C NMR spectra or from coupled gHSQC 2D spectra. Generally, these values differed by approximately 10 Hz, the equatorial protons of α-linked saccharides having the highest C-H coupling constants (~165-175 Hz).[54]

[48] A similar chemoselectivity could be obtained using hydrazinedithiocarbonate (Ref. 43b). This reaction was significantly less practical to use, however.
[49] Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.
[50] Hashimoto, N.; Aoyama, T.; Shioiri, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475.
[51] Petitou, M.; Duchaussoy, P.; Choay, J. *Tetrahedron Lett.* **1988**, *29*, 1389.
[52] CAN and DDQ were compared in a TLC experiment. Treatment of the 6-*O*-*para*-methoxyphenylated monosaccharide **24** with DDQ resulted in several spots beside the desired diol **22**. CAN gave a clean conversion to **22**.
[53] This observation has been done for many galactose containing saccharides, see e.g. Bock, K.; Pedersen, C.; Pedersen, H. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 193.
[54] Again, this observation is general, see: a) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett.* **1973**, 1037; b) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.

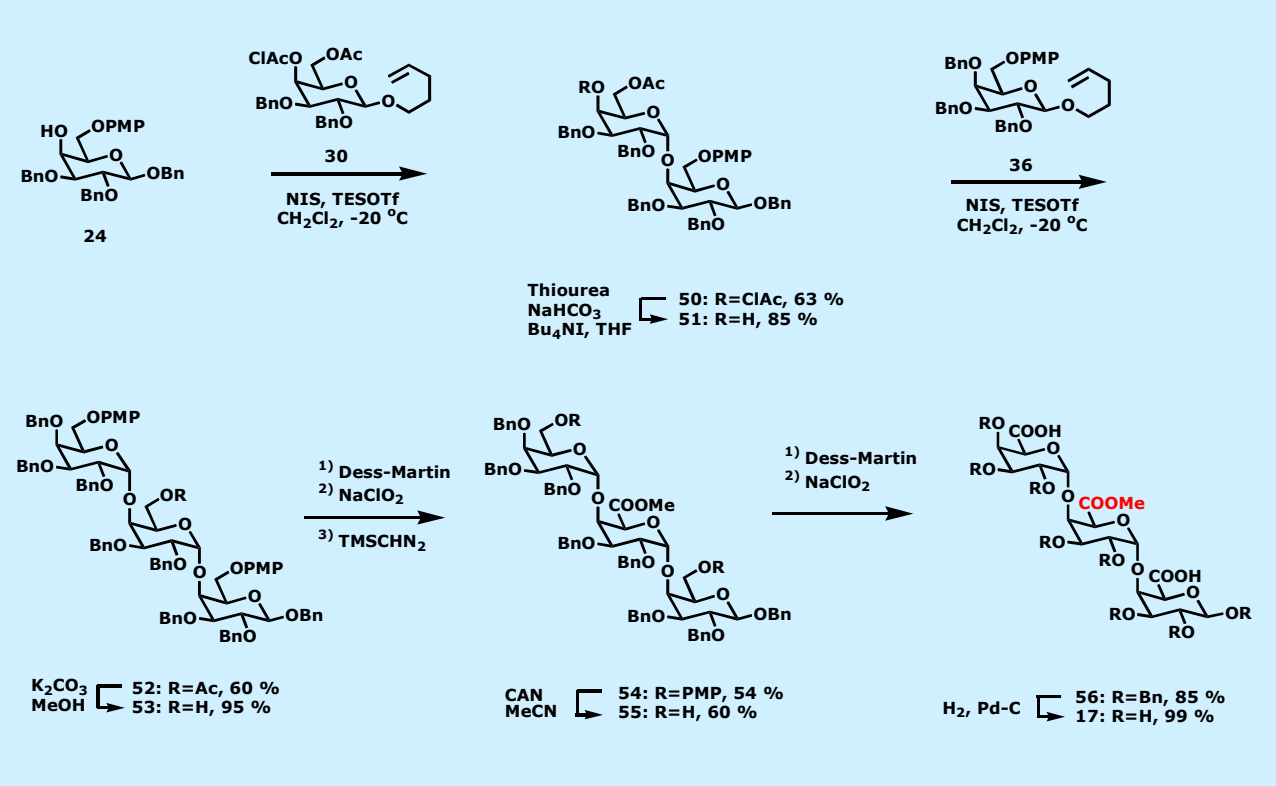


Scheme 9

The choice of oxidizing agents was subject to careful scrutiny (Scheme 9).[55] The lability of the substrates, and the fact that the oxidations were performed at a late stage in the synthesis, made effective and mild conditions mandatory. Initial model studies were performed on a known model substrate **45**, prepared in 60% yield from **21** by $AlCl_3$-mediated reductive opening of the benzylidene acetal by $LiAlH_4$.[56] These experiments revealed that a direct transformation of the monosaccharide **45** to the uronic acid **46** using chromium(VI)-based reagents[57] was not favorable, primarily due to difficulties associated with removal of residual chromium salts.[58] TEMPO-catalyzed oxidation[59] was attempted, but the yields were unsatisfactory.[58] Focus was thus put on a two-stage oxidation, starting with Swern oxidation to yield the aldehyde **47**, followed by further oxidation with sodium chlorite to **46** (Scheme 9).

[55] For a review of oxidation methods in carbohydrate chemistry, see: Madsen, R. *Glycoscience* **2001**, *1*, 195.
[56] Lipták, A.; Jodál, I.; Nánási, P. *Carbohydr. Res.* **1975**, *44*, 1.
[57] Lee, D. G. *The Oxidation of Organic Compounds by Permanganate Ion and Hexavalent Chromium*, Open Court Publishing, La Salle, **1980**, pp. 65-70.
[58] Thorsen, J.; Madsen, R., *unpublished results*.
[59] a) Miyazawa, T.; Endo, T.; Siihashi, S.; Okawara, M. *J. Org. Chem.* **1985**, *50*, 1332; b) Anelli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.* **1987**, *52*, 2559.

Attempts to convert the intermediate aldehyde **47** directly to the methyl ester **49** *via* the transient hemiacetal **48**[60] using either bromine in methanol,[61] or Swern conditions were troubled by low yields (less than 50% isolated yield). The Dess-Martin periodinane failed altogether in this transformation resulting only in recovery of the starting aldehyde.
However, although Swern conditions were very reliable for the first round of oxidation of the trisaccharide **41** (affording **42** in 57% yield), when applied for the oxidation of the diol **43**, severe decomposition occurred, and no product was obtained. This presumably ensued from β-elimination due to the acidity of the C-5 proton α to the methyl ester in **43**. This was completely depressed by switching to the Dess-Martin oxidation protocol, which is performed under neutral conditions.

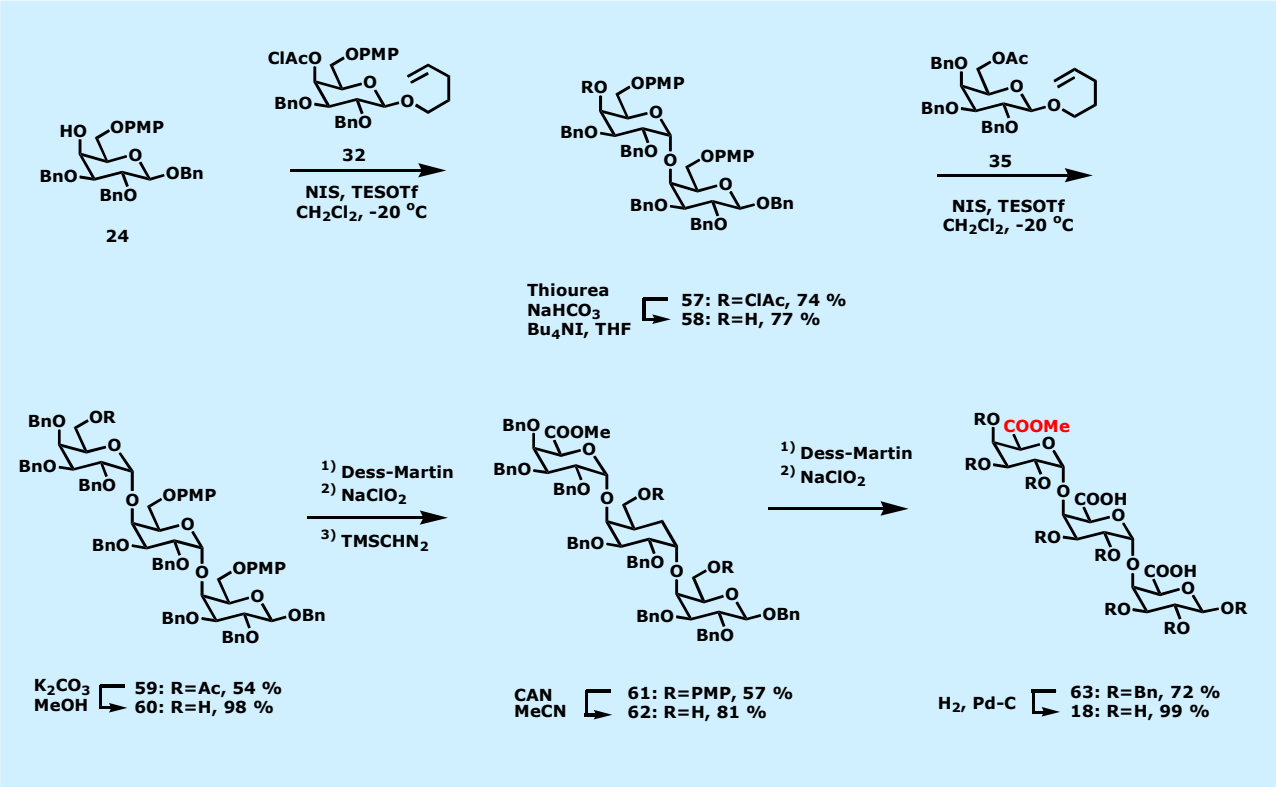


Scheme 10

Scheme 10 depicts the synthesis of the second monomethylated trisaccharide **17**. As can be seen, all reagents and conditions are identical to the ones applied for the synthesis of **16**. In this case, it was possible to isolate both desired α-linked di- and trisaccharides immediately upon glycosylation.

[60] **48** was formed in >95% yield upon addition of 2 equiv. of methanol to a solution of **47** in $CDCl_3$, according to ^{13}C NMR.
[61] Lichtenthaler, F. W.; Jarglis, P.; Lorenz, K. *Synthesis* **1988**, 790.

The third trigalacturonate **18**, having a methyl ester at the non-reducing end residue was prepared accordingly (Scheme 11). It is noteworthy that having established convenient conditions for each step in the synthesis no modification of the methods was called for – all transformations were carried out under analogous conditions. This fact, together with the observation that the yields in the individual steps were comparable, strongly indicated that the strategy applied was reliable and efficient.
Analysis of the three products **16, 17**, and **18** was assessed by ^{1}H- and ^{13}C-NMR together with ESI MS. The NMR data was identical to literature values.[29] Due to the repetitive nature of the individual saccharide residues in the oligomers, and the presence of both the α- and β-anomer in solution, the spectral data were appreciably complex. The α:β ratio in water was approximately 2:3.



Scheme 11

Due to this complexity, emphasis was put on mass spectral analysis. Roepstorff and co-workers have developed methods for sequencing methyl esterified oligogalacturonates by tandem mass spectrometry (MS-MS).[62] The analyses rely on the fact that homogalacturonan preferentially fragments along

[62] Körner, R.; Limberg, G.; Christensen, T. M. I. E.; Mikkelsen, J.D.; Roepstorff, P. *Anal. Chem.* **1999**, *71*, 1421.

the glycosidic bonds. This produces two types of fragment ions from the non-reducing end (B and C, Figure 7) and two other types from the reducing end (Y and Z, Figure 7). B, Z and C, Y have identical masses respectively, but can be differentiated by selective ^{18}O-labeling of the reducing end hemiacetal (Figure 7). Furthermore, experience has shown that in the negative ion mode predominantly C and Z ions are observed.

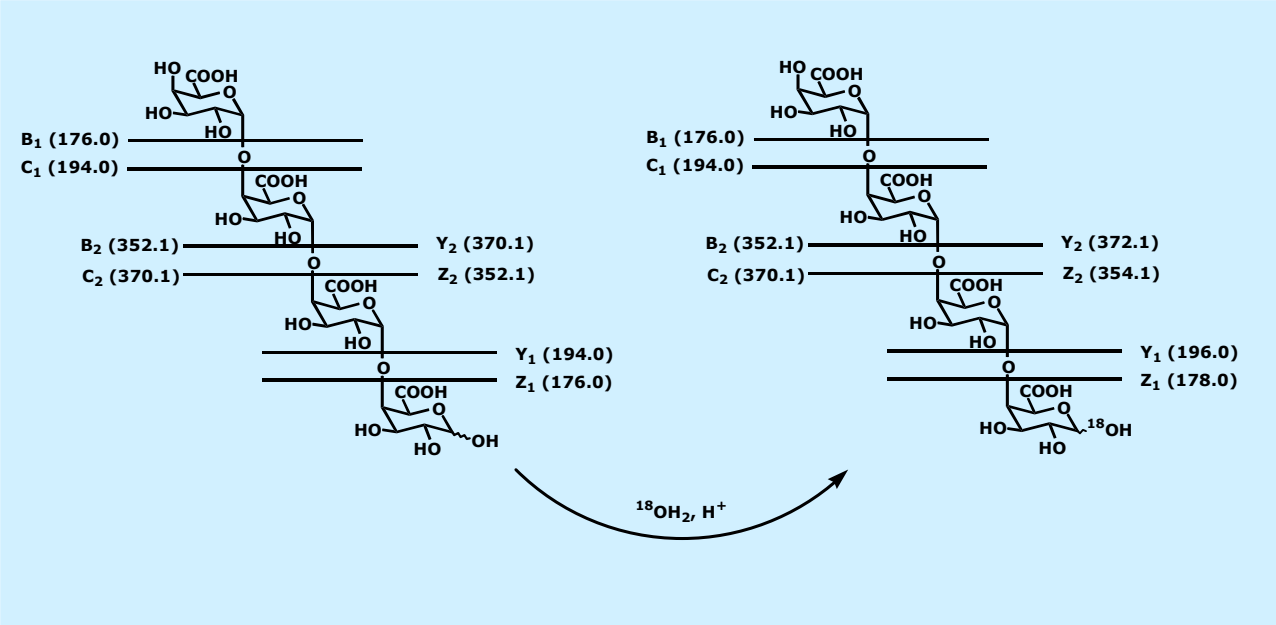


Figure 7

For the monomethylated trigalacturonates the methyl ester pattern was verified by MS-MS. Using **18** as an example, the parent compound had m/z 559 $[M-H]^-$ and 561 with ^{18}O-labeling of the reducing end, see Figure 8. From the peak at 559, daughter ions were observed at 383 (M-H-176), C_2, and 351 (M-H-208), Z_2. These assignments were confirmed by O_{18}-labeling, with C_2' at 383 (M-H-178) and Z_2' at 353 (M-H-208). Analogous observations were done with the other two trigalacturonates **16** and **17** (see Chapter 8).

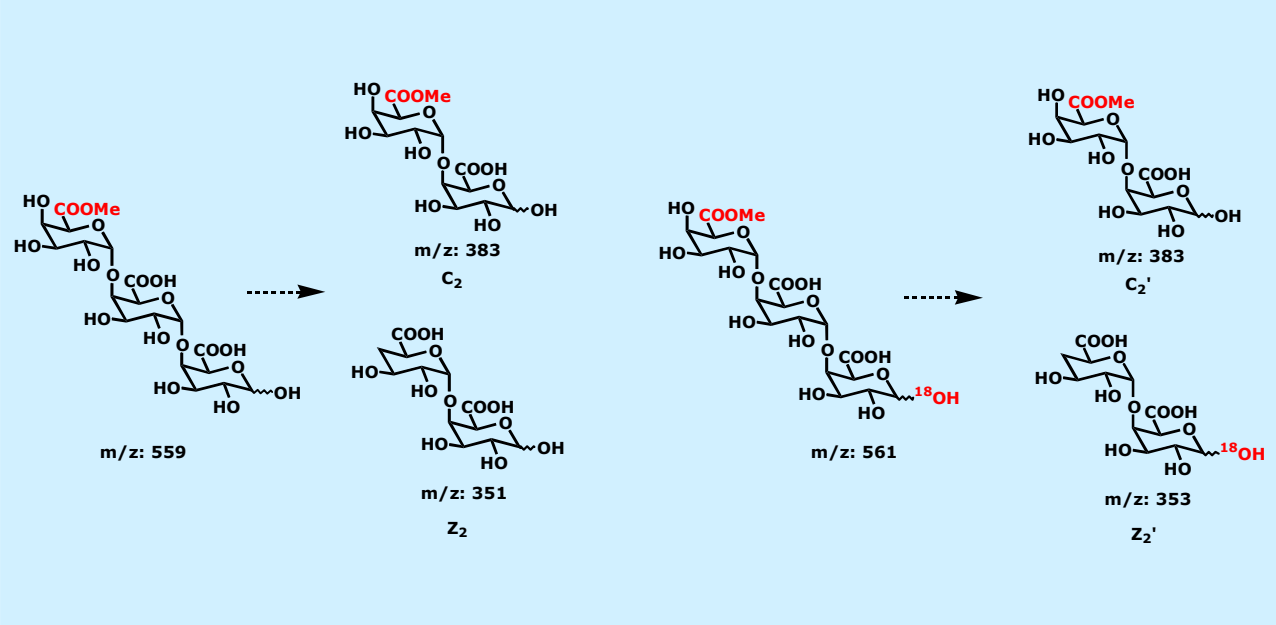


Figure 8

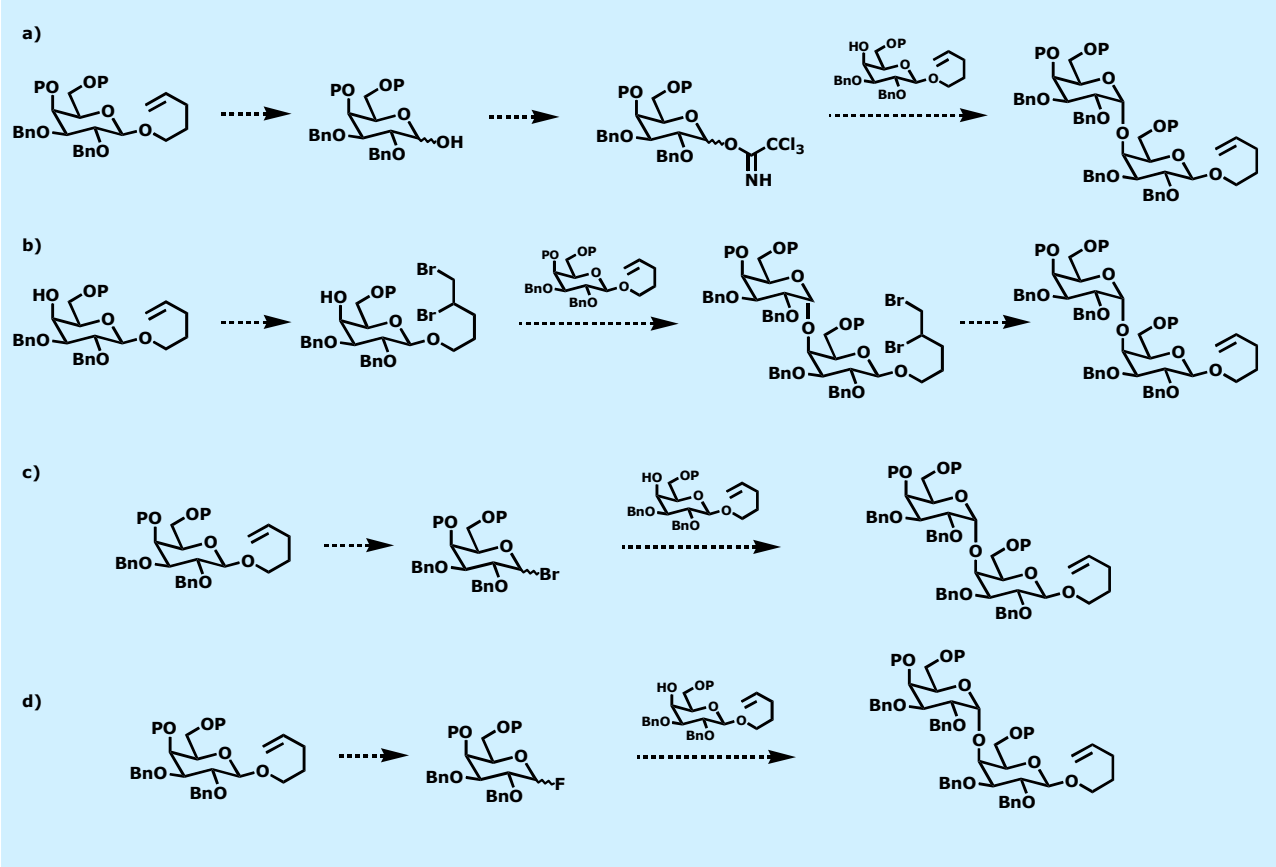
Although seemingly trivial, this technique became indispensable for confirming the methyl esterification pattern of larger oligomers (see Chapter 4).

3. Pentenyl Disaccharides

Synthesis of Disaccharide Donors for Convergent Assembly of Oligosaccharides

Introduction

For the synthesis of larger oligogalacturonates, a convergent strategy was desirable. It was envisioned that this could be achieved by way of pentenyl disaccharides, applied as glycosyl donors. Several entries to these molecules can be taken, as outlined in Scheme 12.



Scheme 12

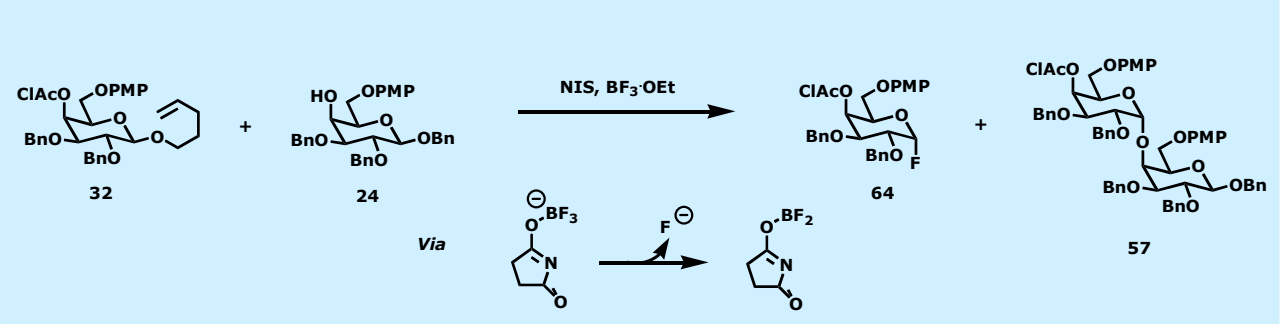
Pentenyl glycosides are easily hydrolyzed to reducing sugars by e.g. NBS/H_2O.[63] Conversion of these to the very reactive trichloroacetimidate donors developed by Schmidt is straightforward.[33] These donors can be selectively activated in the presence of a pentenyl group using a strong Lewis acid (Scheme 12a).

[63] Lopez, J. C.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1991**, 159.

A second method to circumvent problems with dual activation of donor and acceptor has been developed by Fraser-Reid.[64] The acceptor is converted to a vicinal dibromide under the action of bromine and excess bromide. Upon glycosylation, the pentenyl group is regenerated using a reductive debromination (Scheme 12b).
In the absence of bromide ions, bromine transforms a pentenyl glycoside to a glycosyl bromide. This can in turn be activated by e.g. silver salts and serve as the glycosyl donor in a Koenigs-Knorr coupling,[65] another method developed by Fraser-Reid (Scheme 12c).[66]
The reagents used for activation of glycosyl fluorides are typically also compatible with the pentenyl moiety, making these yet another alternative as glycosyl donors (Scheme 12d).[67]
The first two methods outlined in Scheme 12 were judged as too roundabout for practical, large-scale synthesis and thus focus was put on the use of glycosyl fluorides and -bromides.

Glycosyl Fluorides

The glycosyl fluorides were the first to be examined, due to a serendipitous discovery of a possible easy route to their synthesis.[68] In an attempt to substitute TESOTf with $BF_3{\cdot}OEt_2$ in the preparation of **57**, the glycosyl fluoride **64** was obtained as a major byproduct (Scheme 13). Presumably, the succinimidyl-trifluoroborate complex liberated a fluoride ion leading to **64**.



Scheme 13

[64] a) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068. For an application of this technique, see: b) Merritt, J. R.; Naisang, E.; Fraser-Reid, B. *J. Org. Chem.* **1994**, *59*, 4443.
[65] Koenigs, W.; Knorr, E. *Ber.* **1901**, *34*, 957.
[66] Konradsson, P.; Fraser-Reid, B. *J. Chem. Soc., Chem. Commun.* **1989**, 1124.
[67] For a review describing preparation of glycosyl fluorides, see: Yokoyama, M. *Carbohydr. Res.* **2000**, *327*, 5.
[68] Thorsen, J.; Madsen, R., *unpublished results*.

This encouraged an investigation of a one-pot synthesis of glycosyl fluorides from pentenyl glycosides. The results of these efforts are summarized in Table 1. Initial attempts to increase the yield of glycosyl fluoride by treatment with stoichiometric amounts of boron trifluoride etherate and NIS proved futile (entry 1). The main product isolated was the *N*-succinimide glycoside (not shown). The addition of an external fluoride source proved successful (entries 2 and 3), but the yields were still unsatisfactory. Knowing that thioglycosides can be transformed to glycosyl fluorides in one step by treatment with NIS and diethylaminosulfur trifluoride (DAST), these conditions were investigated (entry 4). Interestingly, the yield improved but the α/β ratio deteriorated. Further investigation of compounds **65**-**68** (Figure 9),[69] in order to explore the generality of this transformation, proved that NIS can be substituted with the cheaper NBS.[70] Furthermore, the protocol worked excellently for galacto- and glucopyranosides **65**-**67** (entries 5-7). In the case of the mannoside **68**, only the α-linked glycosyl fluoride was observed, but the reaction was not very efficient (entry 8).

Table 1

Entry	Glycoside[a]	Conditions[b]	Yield [%]	α:β[c]
1	**30**	A	17	>10:1
2	**35**	B	50	>10:1
3	**35**	C	55	>10:1
4	**35**	D	80[d, e]	5:2
5	**65**	E	73	5:3
6	**66**	E	74	5:1
7	**67**	E	76	3:2
8	**68**	E	41	α only

[a] Cf. Figure 9. [b] **A**: NIS (1.2 equiv.), $BF_3{\cdot}OEt_2$ (1.2 equiv.), CH_2Cl_2, -20 °C; **B**: NIS (1.05 equiv.), $BF_3{\cdot}OEt_2$ (0.2 equiv.), CsF (1.2 equiv.), CH_2Cl_2, -20 °C; **C**: NIS (1.05 equiv.), $BF_3{\cdot}OEt_2$ (0.2 equiv.), Bu_4NBF_4 (1.2 equiv.), CH_2Cl_2, -20 °C; **D**: NIS (1.2 equiv.), DAST (1.2 equiv.), CH_2Cl_2, 0 °C; **E**: NBS (1.3 equiv.), DAST (1.3 equiv.), CH_2Cl_2, 0 °C. [c] Determined by ^{1}H NMR. [d] Including traces of (5-fluoro-4-iodo-pentanyl) glycoside. [e] Average of two runs.

[69] a) **65** and **67**: Ref 66; b) **66**: Udodong, U. E.; Rao, C. S.; Fraser-Reid, B. *Tetrahedron* **1992**, *48*, 4731; c) **68**: Roberts, G.; Madsen, R.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 1546.
[70] Juul, K. M.; Piper, A.; Jensen, N. M. E.; Clausen, M. H.; Madsen, R., *unpublished results*.

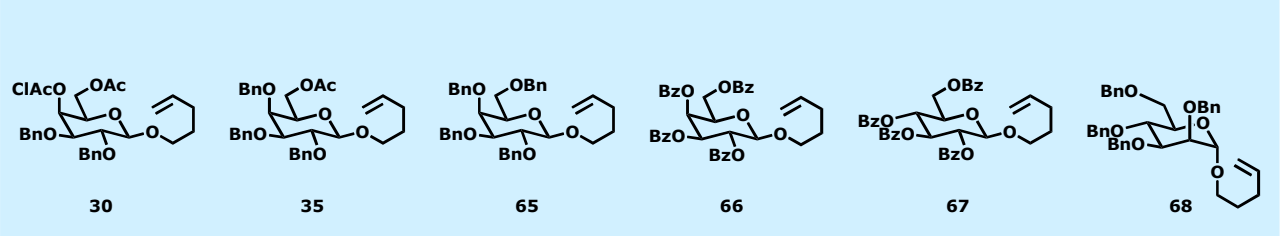
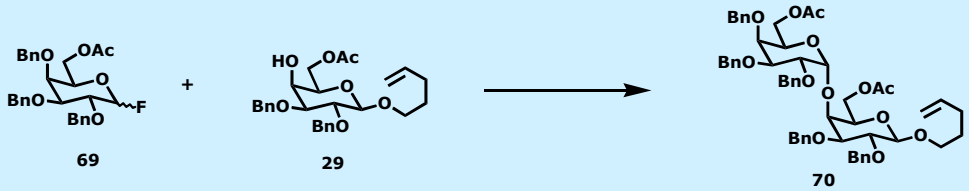


Figure 9

A number of activation methods are available for glycosyl fluorides.[71] The most widely applied method involves a combination of silver and tin salts and was developed by Mukaiyama[72] and Ogawa.[73] Table 2 sums up the results of the glycosylation of pentenyl acceptor **29** with fluoride **69**.[74] Using AgOTf alone gave a very low yield of the desired disaccharide **70** (entry 1). The reaction mixture was very acidic, and presumably the reactants and the products were not stable under these conditions. Changing to $AgClO_4$ and adding molecular sieves as scavengers of perchloric acid was beneficial (entry 2). Surprisingly, pentenyl alcohol was liberated during the reaction, and served as a glycosyl acceptor – this was discovered after isolation of an α-linked pentenyl galactoside. This indicated that the glycosidic linkage of the acceptor (and presumably the product) was not stable under the reaction conditions. The addition of collidine to buffer the solution mainly led to isolation of unreacted starting materials (entry 3). However, lowering the temperature had an advantageous effect on the reaction (entry 4). Using these conditions, disaccharide **70** could be isolated in 73% yield as a 3:1 α:β mixture.

[71] Review: Toshima, K. *Carbohydr. Res.* **2000**, *327*, 15.
[72] Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431.
[73] a) Ogawa, T.; Takahashi, Y.; *Carbohydr. Res.* **1985**, *138*, C5; b) Takahashi, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *164*, 277.
[74] **69**α and **69**β have been synthesized from the corresponding reducing sugar using DAST: Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1987**, *28*, 2731.

Table 2



Entry[a]	Ag source	Additive	Temp. [°C]	Yield [%]	α:β[b]
1	AgOTf	-	0	5	n.d.
2	$AgClO_4$	MS 4A	0	20[c]	α only
3	$AgClO_4$	Collidine	0	10	n.d.
4	$AgClO_4$	MS 4A	-30	73[d]	3:1

[a] $SnCl_2$ (1.5 equiv.), silver salt (1.5 equiv.), CH_2Cl_2. [b] Determined by ^1H NMR. [c] Pentenyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-(α- and β)-D-galactopyranosides were also isolated. [d] Partly determined by NMR since the β-linked product was inseparable from the acceptor.

Although the two step protocol involving conversion of the pentenyl glycoside to a glycosyl fluoride and subsequent coupling under Mukaiyama conditions had now been developed, this method was not completely satisfactory. The overall yield of the desired α-linked product was only 44% and the purification of the products was rather laborious. It was therefore decided not to pursue this any further, but instead to explore the formation of glycosyl bromides from pentenyl glycosides as an alternative route to pentenyl disaccharides.

Glycosyl Bromides

In order to convert the pentenyl galactoside **35** to a glycosyl bromide, it was titrated with bromine. This cleanly afforded the known[75] α-glycosyl bromide **71**, as judged by NMR (Figure 10). It was anticipated that the glycosyl bromides would have limited stability and be incompatible with chromatographic purification. Therefore, the bromides were formed *in situ* and used immediately thereafter. Silver triflate was chosen as promoter for the glycosylations and in all cases, molecular sieves were added to attenuate the acidity and thus avoid decomposition. The reactions were performed at –50 °C and quenched with sat. aq. $NaHCO_3$ upon completion. Using these reaction conditions, pentenyl disaccharides **70**, **72**, and **73** (Figure 11) were synthesized in 40, 61, and 75% yield, respectively.

[75] Paulsen, H.; Lockhoff, O. *Chem. Ber.* **1981**, *114*, 3079.

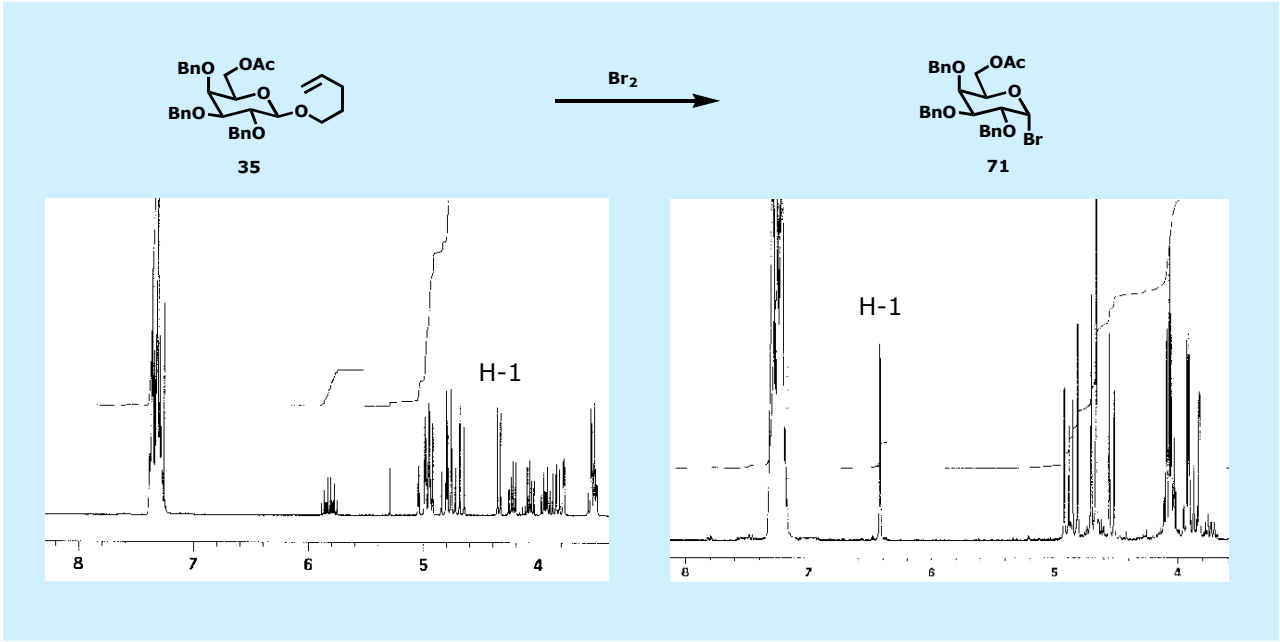


Figure 10

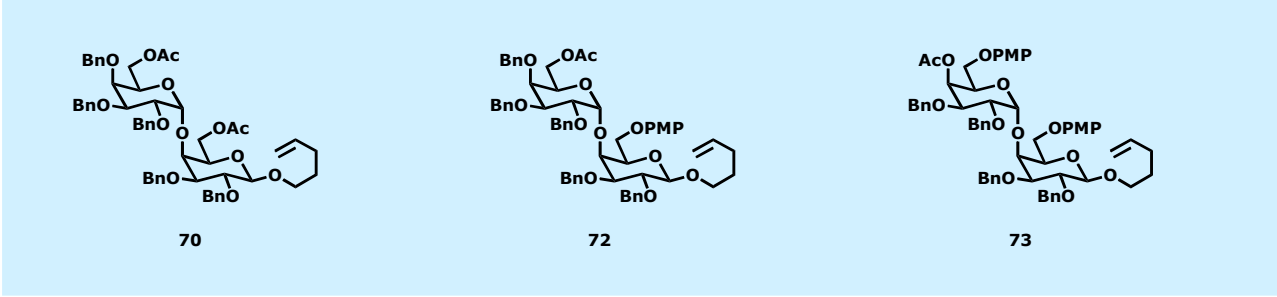
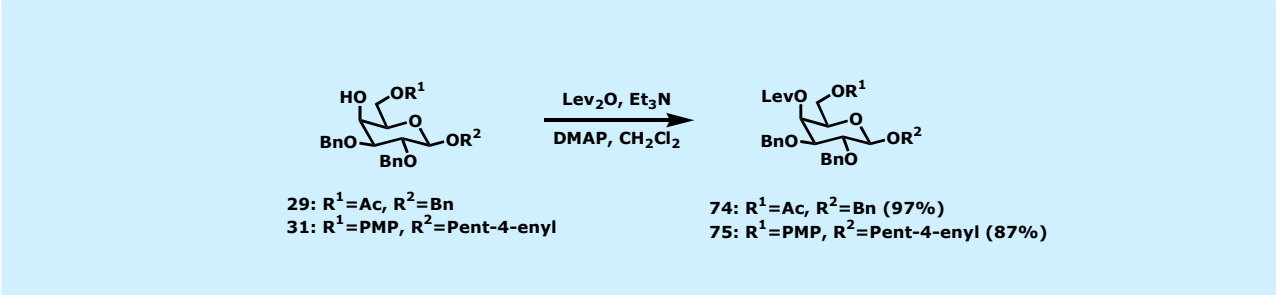


Figure 11

Regrettably, the chloroacetyl protecting group that had served well in the synthesis of trigalactosides was incompatible with the reaction conditions. The reaction failed to provide the desired product and in all cases, the chloroacetyl group had been lost from the reactants. The Lewis acidity of AgOTf and the high halophilicity of silver is a plausible explanation for this observation. In order to access pentenyl digalactosides with a temporary protection group at the 4' position, another orthogonal blocking group had to be found. To test this, the levulinyl-protected galactosides **74** and **75** were prepared (Scheme 14). The levulinyl (4-oxypentanoyl) protecting group[76] can be selectively removed in the presence of an acetyl group by reaction with hydrazine.[77, 78]

[76] Hassner, A.; Strand, G.; Rubenstein, M.; Patchornik, A. *J. Am. Chem. Soc.* **1975**, *97*, 1614.
[77] van Boom, J. H.; Burgers, P. M. J. *Tetrahedron Lett.* **1976**, 4875.
[78] For compound **74** it was found that treatment with hydrazine acetate in refluxing THF/water led to complete removal of the levulinate, leaving the acetyl group untouched.

Unfortunately, conversion of **75** to the corresponding bromide and coupling under Koenigs-Knorr conditions did not provide the desired pentenyl disaccharide. A number of unidentified byproducts and recovered acceptor were observed as the sole products of the reaction.
The allyl group is another orthogonal protection group that could be investigated for the synthesis of pentenyl disaccharides. However, the endpoint of titration of the pentenyl olefin with bromine would be hard to identify, since the bromine was expected to react, albeit slower, with the allyl group as well. The idea of using the allyl group as temporary protection for the 4-position inspired another possible route to pentenyl disaccharides though.



Scheme 14

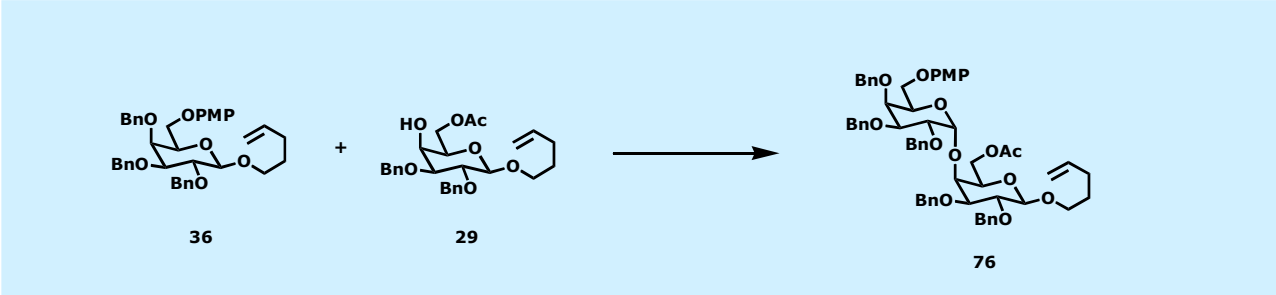
Armed-Disarmed Strategy for the Synthesis of Pentenyl Disaccharides

Fraser-Reid and co-workers were the first to realize the tremendous impact of the nature of blocking groups on the reactivity of glycosyl donors in their investigation of pentenyl glycosides.[79] The effect was termed the "armed-disarmed effect" and exploited the distinct reactivity of differentially protected glycosyl donors for selective couplings. Conversely, armed donors typically had electron-donating protection groups (e.g. benzyl ethers) and disarmed donors were bearing electron-withdrawing blocking groups like esters.
Later, these effects were shown by van Boom and co-workers to be equally useful for thioglycoside donors[80] and selenoglycosides,[81] and have since gained wide acceptance as a mean to control reactivity in glycosidic couplings.[82]

---

[79] Mootoo, D. R.; Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 2662.; Ref. 64a.
[80] a) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 27; b) Veeneman, G. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275; c) Veeneman, G. H.; Brugghe, H. F.; van den Elst, H.; van Boom, J. H. *Carbohydr. Res.* **1990**, *195*, C1.
[81] Zuurmond, H. M.; van der Meer, P. H.; van der Klein, P. A. M.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1993**, *12*, 1091.
[82] See e.g.: Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc., Perkin Trans. 1* **1998**, 51.

Wong and co-workers have developed a strategy for preparing tri- to pentasaccharides using a plethora of protected thioglycosides, and relying on differences in their reactivity for one-pot multi-sequence couplings.[83] The donors used for their investigations were protected with a number of protecting groups, and the exact position and nature of these groups determined the reactivity of the donor in question. This method was not practical for our purpose since the 2- and 3-positions had to be permanently blocked with benzyl ethers. Furthermore, the 6-positions have to be protected with two different orthogonal protecting groups in order to control the positions of the methyl esters in the final products. One could envision, however, that the electronic difference between a pentenyl glycoside with a PMP group and one with an acyl group at the 6-position could be exploited for selective couplings.

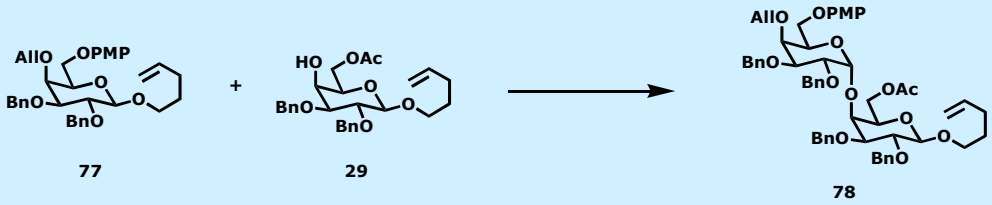


Scheme 15

The initial experiment with donor **36** and acceptor **29** gave the disaccharide product **76** in 22% yield, together with a variety of homocoupling products of **29** (Scheme 15). Although this was a low yield, it was promising and suggested that the method could be applicable, since it was conceptually simple and practical. The above mentioned allyl group was perfectly suitable as protection for the 4-position of the donor. It is electron-donating and can be removed selectively in the presence of the other protecting groups employed. Hence, the allyl protected pentenyl galactoside **77** was synthesized by allylation of **31** using sodium hydride and allyl bromide in THF (91% yield), and the coupling was investigated with this donor (Table 3).

[83] Zhang, Z.; Ollmann, I. A.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734.

Table 3

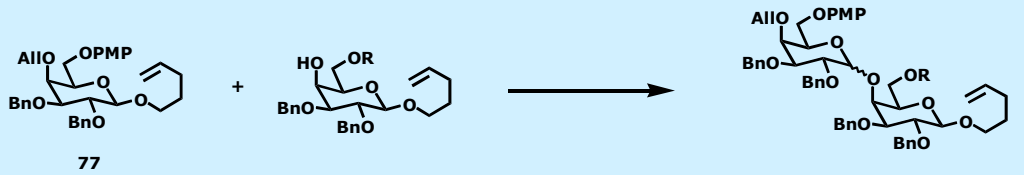


Entry[a]	77 [mmol]	29 [mmol]	NIS [mmol]	Solvent	Yield [%]	α:β[b]
1	1.3	1	1.5	CH_2Cl_2	20	6:1
2	1	1	1.1	CH_2Cl_2	22	6:1
3	1	1.3	1.2	CH_2Cl_2	27	6:1
4	1	1.3	1.2	Toluene	23	n.d.

[a] Approximately 0.1 M in NIS, 0.2 equiv. TESOTf relative to NIS, -20 °C. [b] Determined by ^{1}H NMR.

The stoichiometry of the two coupling partners **77** and **29** was investigated (entries 1-3). These experiments showed that employing a surplus of acceptor **29** in the reaction was slightly superior, with 27% as the best yield. An attempt to improve the yield by changing the solvent to toluene was in vain (entry 4). Clearly, acceptable yields could not be achieved using this donor and acceptor. Evidently, the reactivity difference was not sufficient to facilitate an efficient coupling. In order to further disarm the acceptor relative to the donor, a number of acyl *O*-6 protection groups were tested (Table 4). All acceptors were synthesized by selective acylation of the diol **28** by treatment with the acyl chloride or anhydride in dichloromethane in the presence of triethylamine. It would be convenient to be able to correlate the reactivity of the glycosyl donor with its physical properties. Wong and co-workers have described a correlation between the reactivity and the chemical shift of the anomeric proton for some thiogalactosides.[83] No such relationship could be detected for the pentenyl glycosides examined here, but the electron-withdrawing effect of the different acyl groups could be tentatively probed by determining the ^{13}C NMR chemical shift of the carbonyl carbon (see Table 4).

Table 4



28: R=H
29: R=$COCH_3$
79: R=$COCH_2Cl$
80: R=$COCCl_3$
81: R=CO(4-$NO_2C_6H_4$)
82: R=CO(3,5-di-$NO_2C_6H_3$)
83: R=COC_6F_5

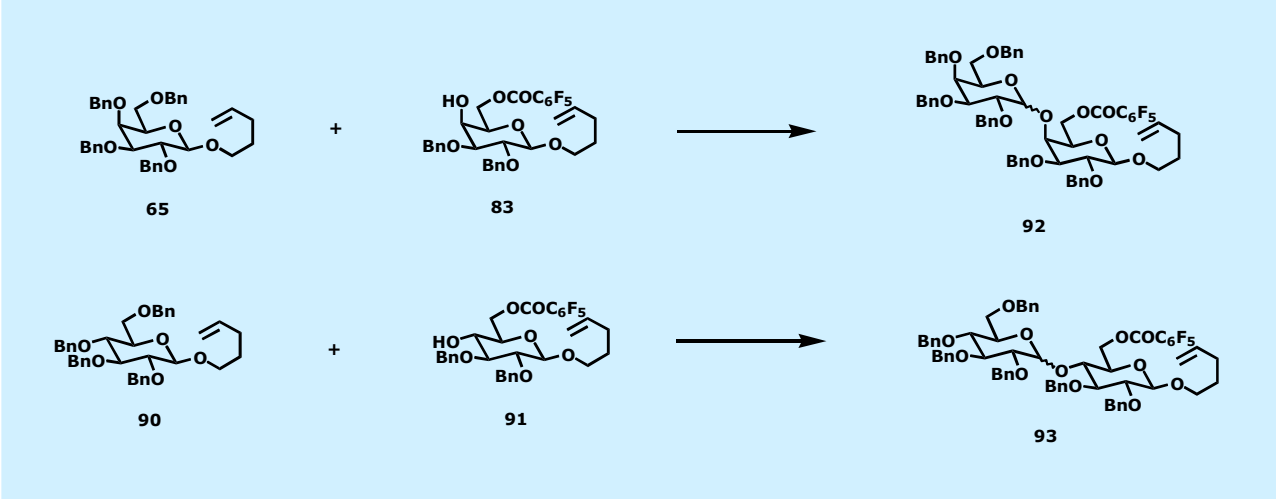
84: R=H
78: R=$COCH_3$
85: R=$COCH_2Cl$
86: R=$COCCl_3$
87: R=CO(4-$NO_2C_6H_4$)
88: R=CO(3,5-di-$NO_2C_6H_3$)
89: R=COC_6F_5

Entry[a]	Acceptor	Acylation yield [%]	δ C=O	Product	Yield [%]	α:β[b]
1	**29**	95	170.9	**78**	27	6:1
2	**79**	83	167.2	**85**	33	6:1
3	**80**	87	161.8	**86**	35	6:1
4	**81**	72	164.6	**87**	42	6:1
5	**82**	83	162.4	**88**	46	6:1
6	**83**	91	158.7	**89**	52	6:1

[a] Ratio donor:acceptor:NIS 1:1.3:1.3, approximately 0.1 M in NIS, 0.2 equiv. TESOTf relative to NIS, -20 °C, CH_2Cl_2. [b] Determined after deacylation – except for **87**, the α and β mixtures of protected disaccharides were inseparable by chromatography on silica.

It is evident from the results in Table 4 that more electron-withdrawing acyl groups improve the yield of the glycosylation. The chloroacetyl and trichloroacetyl protected acceptors **79** and **80** gave disaccharides **85** and **86** in 33 and 35% yield, respectively (entries 2 and 3). Attempts to use the trifluoroacetyl protection group (not shown) was impeded by the lack of stability of this group to purification by silica gel chromatography. Although the acylation reaction was virtually quantitative according to TLC, only 42% of the desired protected galactoside could be isolated due to hydrolysis during work-up regenerating **28**. This was also the case for the disaccharide product of the couplings with this acceptor and hence the yield of the reaction could not be determined. Instead, aromatic esters were investigated. Both mono- and dinitrobenzoyl protected acceptors **81** and **82** performed well in the reaction (entries 4 and 5), but the best result was obtained with the pentafluorobenzoyl protected galactoside **83**, affording disaccharide **89** in 52% yield (entry 6). In general, the products were formed as a 6:1 α:β mixture. These mixtures were inseparable by standard column chromatography, but the α:β-ratio could be determined after deacylation, and the disaccharides **84α** and **84β** were fully characterized.

The present method is a good supplement to the use of glycosyl bromides and fluorides with a benzyl group in the 4-position of the donor described earlier. The consequence of having the allyl group at the 4'-position of the pentenyl disaccharide is that this building block can be incorporated in a growing oligomer chain. Moreover, the reaction was simple to perform, the pentafluorobenzoyl group was installed in a high yield, giving crystalline alcohol **83**, and it was stable to the glycosylation conditions and to purification by silica gel chromatography.[84]



Scheme 16

It was of interest to investigate, whether this reaction was sufficiently general to be applicable as an easy route to disaccharide glycosyl donors. Consequently, the coupling of pentafluorobenzoyl protected pentenyl galacto- and glucopyranosides **83** and **91** with perbenzylated pentenyl donors **65** and **90** were examined (Scheme 16).[85] Surprisingly, the coupling of **65** and **83** resulted in a mere 32% yield of the α-linked product **92α** (**92β** was not isolated). Attempts to use the weaker promoter iodonium dicollidine perchlorate (IDCP)[86] only resulted in incomplete conversion of the starting materials. Cooling the reaction mixture to −78 °C did not improve the selectivity, and neither did omitting TESOTf in an attempt to promote the reaction with NIS only – this slowed the reaction tremendously (approximately

[84] Although the application of fluorinated benzoyl protection groups in carbohydrate chemistry has recently been reported (Sjölin, P.; Kihlberg, J. *J. Org. Chem.* **2001**, *66*, 2957), the present work constitutes the first application of the pentafluorobenzoyl group.
[85] **90**: Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1805.
[86] a) Lemieux, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2190. For an example of application, see: b) Fraser-Reid, B.; Iley, D. E. *Can. J. Chem.* **1979**, *57*, 645.

20% conversion in 5 days).[87] It appears that the reaction is very sensitive to the nature of the protecting groups (in this case, the substitution of the PMP and allyl groups of the donor with benzyl groups significantly reduced the yield). In the case of the *gluco*-configured coupling partners **90** and **91**, the product **93** was isolated in 39% yield as a 3:1 α:β mixture. Further investigations are needed in order to ascertain whether this strategy can be used as a general route to disaccharide glycosyl donors.

[87] Very long reaction times for this method have been reported, see: Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. E. *J. Chem. Soc., Chem. Commun.* **1988**, 823.

4. Methyl Hexagalacturonates

Substrates for Enzymatic Studies and Epitope Identification

Introduction

The primary purpose of synthesizing higher oligomers of galacturonic acid was to provide substrates for enzymatic studies. Trisaccharides had been used as such before, but the activities of the studied enzymes towards these substrates were very low.[88] Obviously, enzymes with polymers as their natural substrates will have the highest activities with polymeric substrates. However, the chemical synthesis of e.g. a dodecamer or a higher oligomer is very time-consuming and therefore a compromise had to be reached between enzyme activity and practicality of the syntheses.

Table 5

Entry	Substrate	PME[a, b, c]	PG I[a, d, e]	PG II[a, e, f]	PL[a, g, h]
1	$(GalA)_3$	1	1	1	0
2	$(GalA)_4$	15	115	94	1
3	$(GalA)_5$	54	118	169	16
4	$(GalA)_6$	1070	260	225	147
5	$(GalA)_7$	n.d.	287	272	904

[a] Data has been normalized so that the lowest enzymatic activity is 1. [b] Pectin methyl esterase – substrate: methyl esterified oligomers. [c] Ref. 88a. [d] *endo*-Polygalacturonase I – substrate: non-esterified oligomers. [e] Ref. 88b. [f] *endo*-Polygalacturonase II – substrate: non-esterified oligomers. [g] Pectic Lyase A – substrate: methyl esterified oligomers. [h] Ref. 88c.

Investigations of HG oligomers with either full or no methyl esterification had shown that pectic enzymes have reasonable activity towards hexagalacturonates (Table 5).
Having access to a range of oligomers with different degrees and patterns of methyl esterification would be valuable, since comparison of the results obtained with the individual oligomers could reveal cleavage- and subsite requirements of the enzymes. It was believed that hexagalacturonates were the smallest oligomers that could give reliable results when applied as substrates for pectic enzymes, and thus it was decided to pursue such

[88] a) Kester, H. C. M.; Benen, J. A. E.; Visser, J.; Warren, M. E.; Orlando, R.; Bergmann, C.; Magaud, D.; Anker, D.; Doutheau, A. *Biochem. J.* **2000**, *346*, 469; b) Benen, J. A. E.; Kester, H. C. M.; Visser, J. *Eur. J. Biochem.* **1999**, *259*, 577; c) Ref. 15c; d) Ref. 30.

oligomers. In collaboration with researchers working with pectic enzymes,[89] it was decided to target the five hexagalacturonates **94**-**98** (Figure 12).

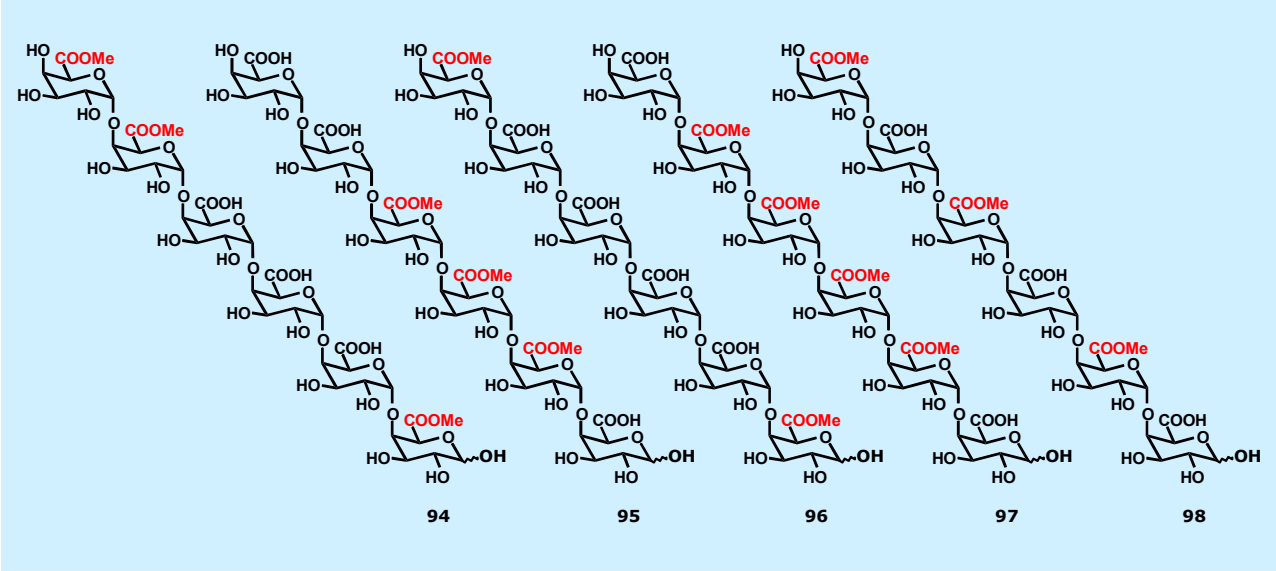


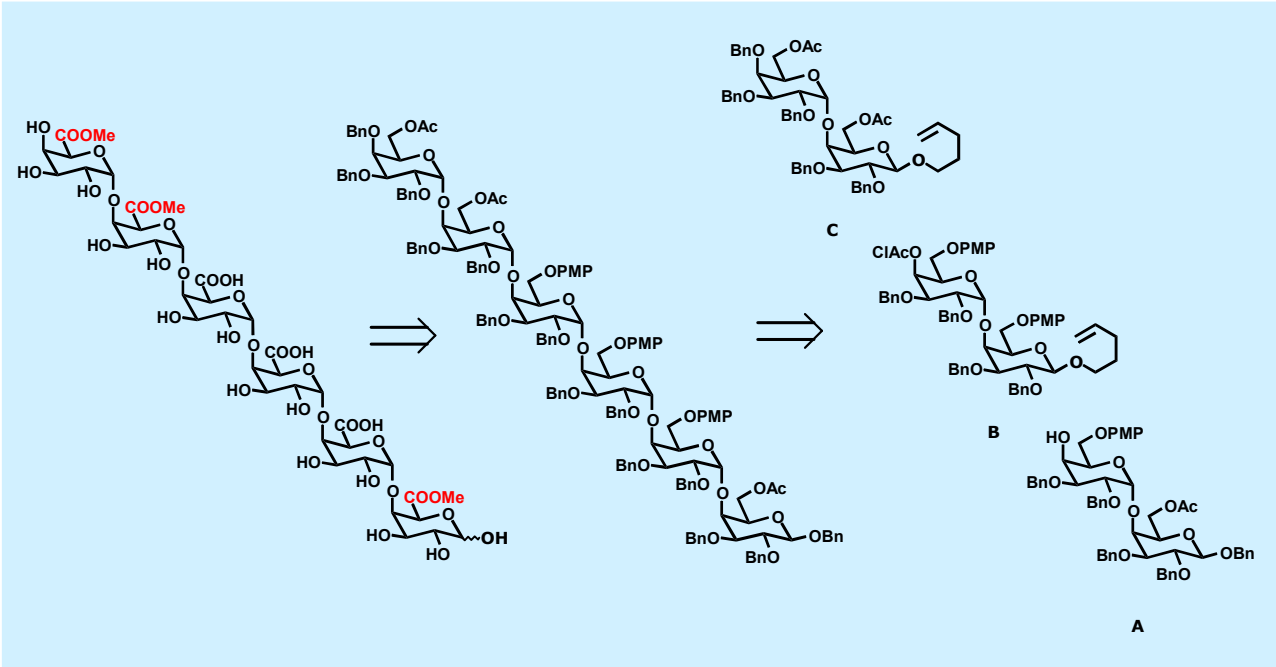
Figure 12

94 and **96** both contained contiguous unesterified residues (3 and 4 respectively), thus making them possible substrates for polygalacturonases. **95** and **97**, on the other hand, contained uninterrupted stretches of methyl esterified residues, enabling the investigation of pectin lyases, known to have a preference for degrading HG with a high DM. Finally, **98** was included as a negative control, since both types of enzymes should have a very low activity towards an oligomer with an alternating pattern of methyl esterification. It was expected, that in all cases the enzyme activities would be fairly low, meaning that a negative control would be very valuable in establishing the relative turn-over rates of the enzymatic degradations.

Retrosynthesis

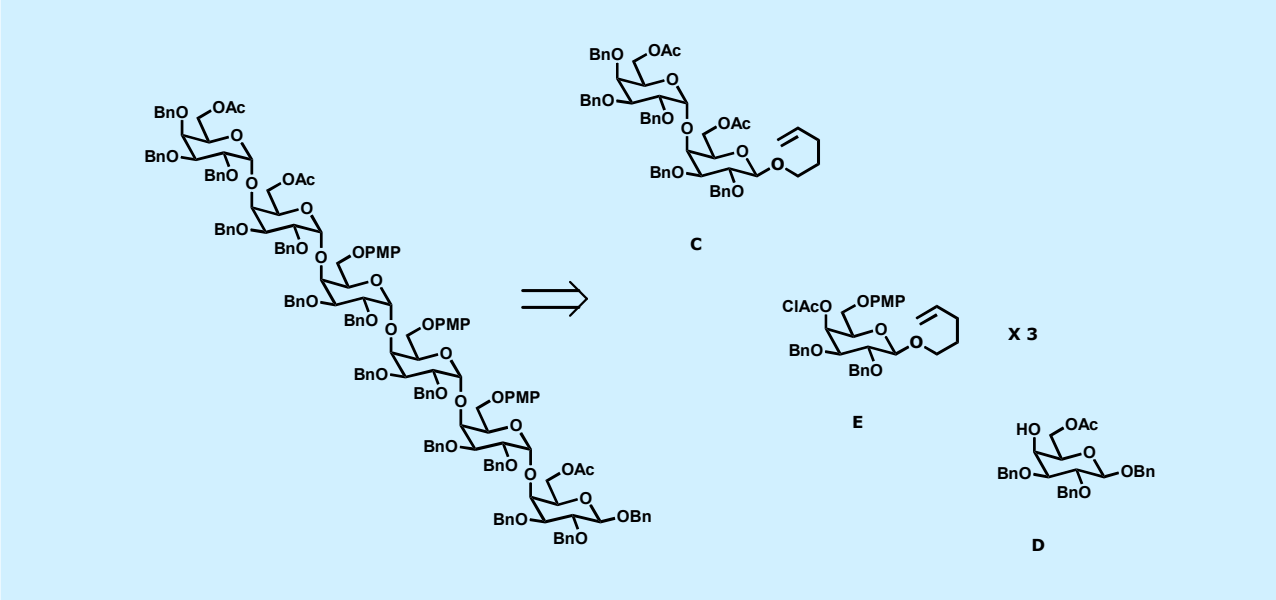
Based on the synthetic strategy used for the synthesis of the monomethylated trigalacturonates, a straightforward retrosynthetic analysis could be envisioned, as demonstrated for the trimethylated hexagalacturonate **94** (Scheme 17).

[89] Thanks to Dr. Tove M. I. E. Christensen and Professor Jørn D. Mikkelsen, Danisco, Copenhagen, for valuable discussions regarding the most appropriate methyl esterification patterns of **94**-**98**.



Scheme 17

However, even though fragments as **A** in Scheme 17 had already been synthesized during preparation of the trisaccharides (cf. e.g. **39**, Chapter 2) and fragments of type **C** were available using bromination-Koenigs-Knorr coupling (cf. **70** and **72**, Chapter 3), fragment **B** caused a problem. No methods were available for the direct synthesis of **B**-type disaccharides, and although they were accessible in the case shown in Scheme 17 by replacing the acetyl group of **73** with a chloroacetyl group, this was neither general nor efficient. Instead, it was decided to use stepwise coupling of monosaccharide glycosyl donors until a tetrasaccharide had been synthesized, and then use the **C**-type fragments for the final coupling. Scheme 18 shows a retrosynthesis based on such a strategy. This approach does on first inspection appear less elegant than the one presented in Scheme 17, but it involves the same number of glycosylation reactions and furthermore allows the use of fragment **E** three times, reducing the number of required building blocks.

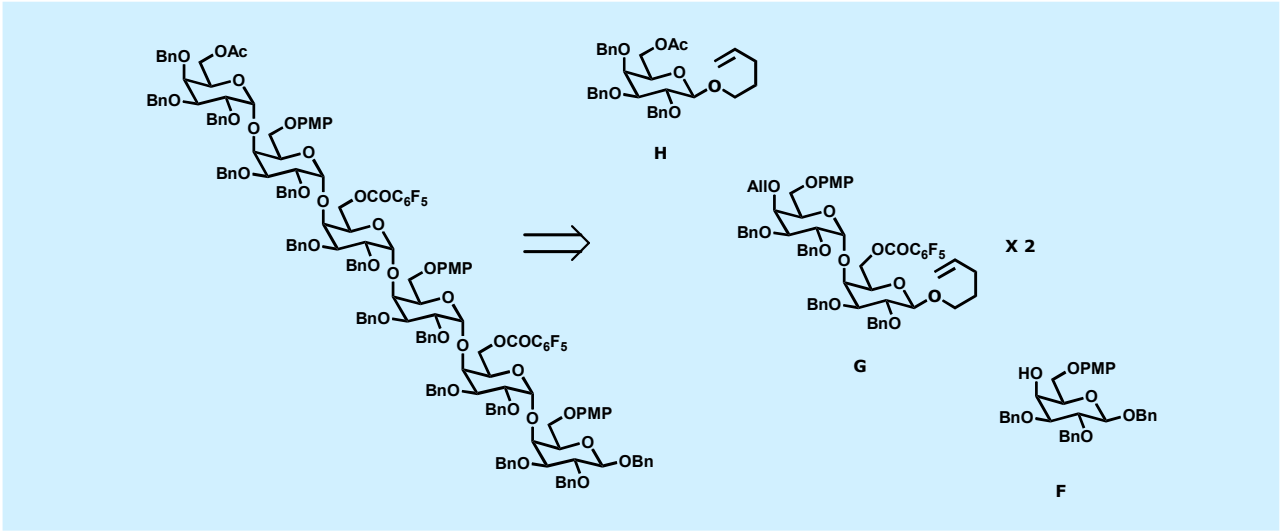


Scheme 18

Another important aspect of this route to protected hexagalactosides appeared during the planning. Given that benzyl esters could be installed equally facile as the methyl esters synthesized earlier, one hexagalactoside could serve as precursor for two hexagalacturonates. This would only require, that in one case, the sites of the acetyl protecting groups should be oxidized and methyl esterified, while in the second case, this series of transformations was performed on the PMP-protected alcohols. Such an approach reduces the number of steps involved for the production of a pair of hexagalacturonates with the reverse methylation pattern dramatically. **94**-**95** and **96**-**97** are pairs with this reversed methyl ester motif.

Inspection of the first four targets in Figure 12 also disclose a common intermediate in the tetrasaccharide obtained upon three rounds of glycosylation and deprotection (cf. **100**, Scheme 20 and Scheme 23). This feature adds to the convergency of these syntheses, in that only the **C**-type glycosyl donor is different for the synthesis of four of the five selected targets.

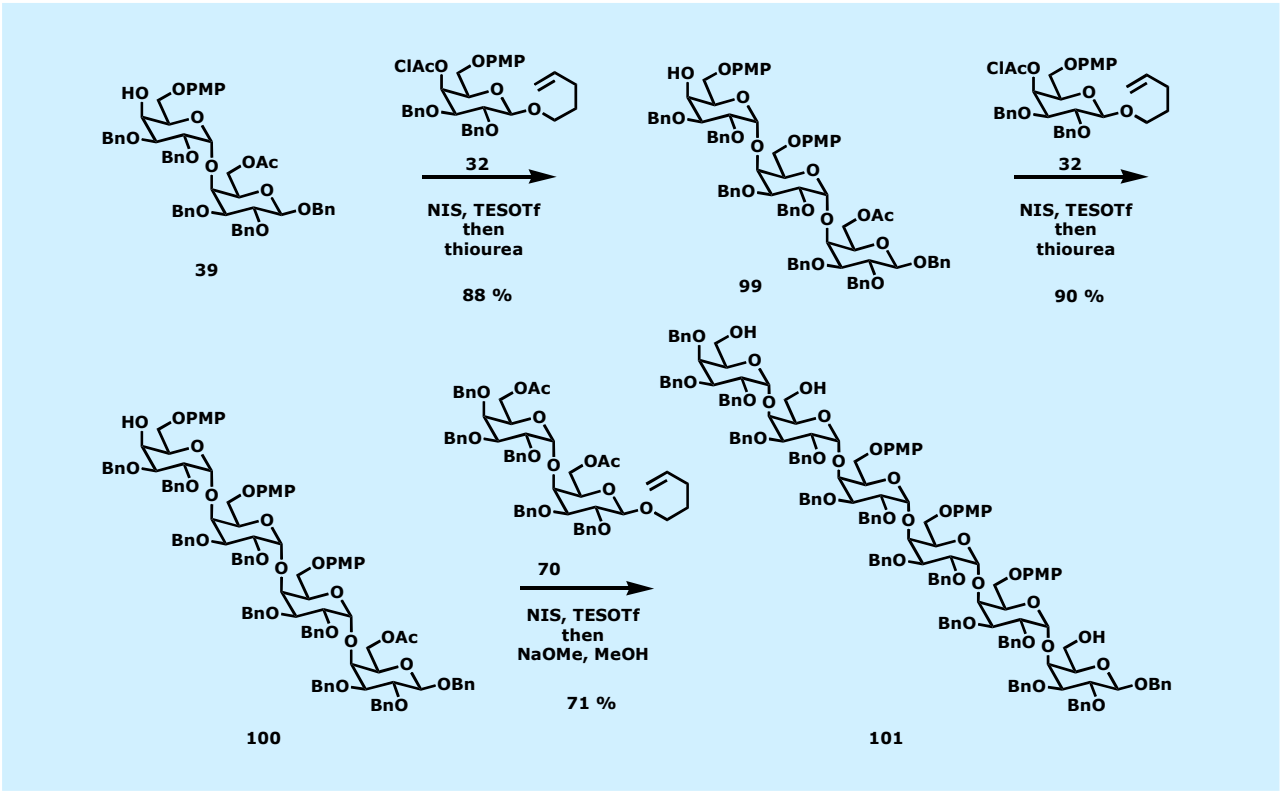
The fifth hexagalacturonate **98** on the other hand prompted a different strategy, as this served as an excellent opportunity to apply the disaccharide donor obtained using the armed-disarmed methodology described in Chapter 3. The retrosynthesis is illustrated in Scheme 19. The **F** and **H** fragments are described earlier, since they were used for the preparation of the monomethylated trigalacturonates (see Chapter 2). Fragment **G** (disaccharide **89**) is accessible *via* the armed-disarmed method, albeit as a 6:1 α:β mixture.



Scheme 19

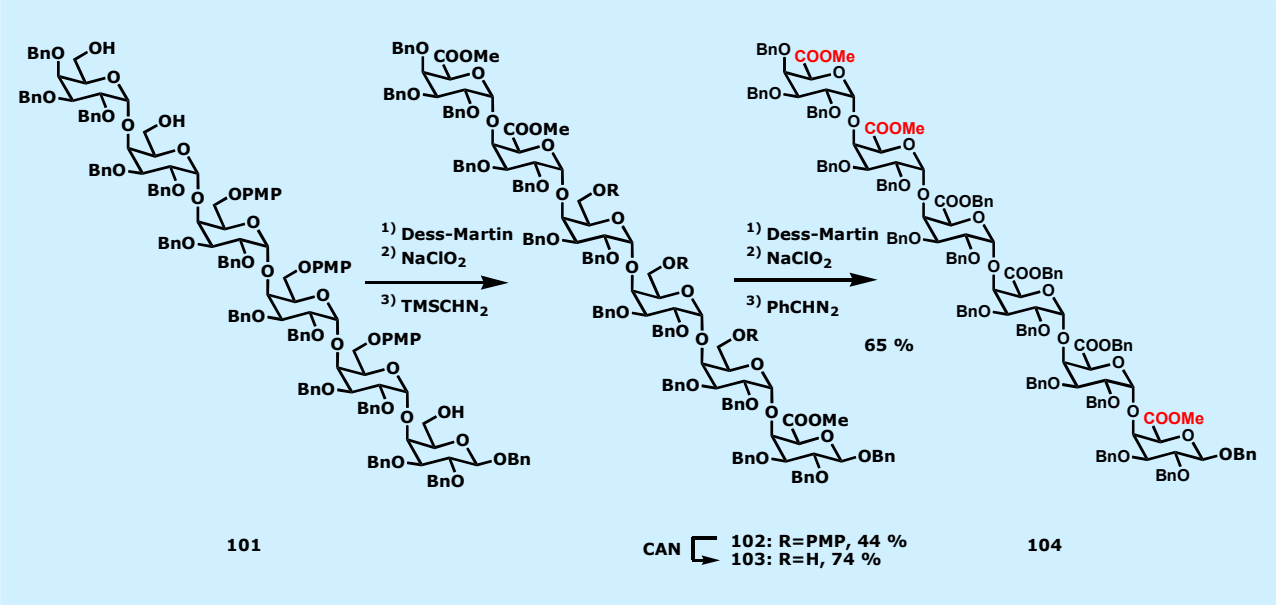
Synthesis of Selectively Methylated Hexagalacturonates

The assembly of the hexagalactoside serving as precursor for target molecules **94** and **95** is shown in Scheme 20. Starting from known disaccharide **39**, glycosylation with donor **32** and selective removal of the chloroacetyl group affords trisaccharide **99**. Another round of coupling and deprotection yields tetrasaccharide acceptor **100**. Both reactions provide the desired α-linked



Scheme 20

products in remarkably high yields. Glycosylation with pentenyl disaccharide **70** and deacetylation gives the triol **101**, again in an excellent yield. Compared to the glycosylation reactions performed during the synthesis of the trigalacturonates, the use of larger building blocks does not serve a problem. Contrarily, the yields and the selectivities improve – this can be a consequence of decreased reactivity of the coupling partners, leading in turn to a higher yield of the desired α-configured glycoside, which is favored by the anomeric effect.[90]



Scheme 21

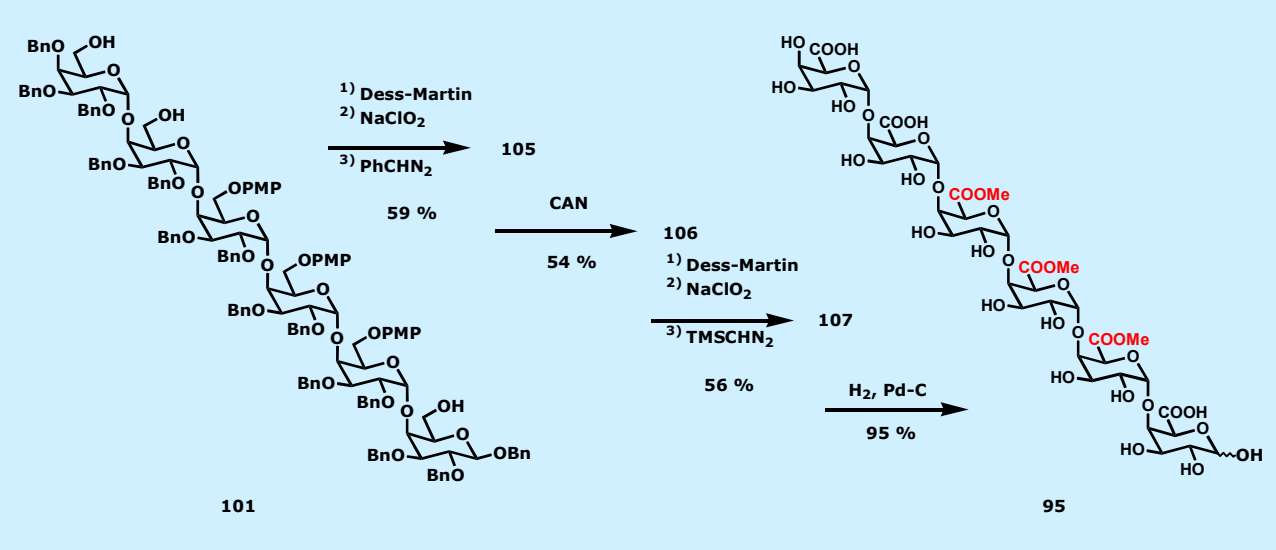
The oxidation and methyl esterification of **101** occurred smoothly implying the Dess-Martin-$NaClO_2$-$TMSCHN_2$ three step protocol applied earlier, giving intermediate **102** (Scheme 21). Not surprising, considering that nine transformations were performed in this step, the yield was slightly lower compared to what had been obtained in the synthesis of trigalacturonates. The three remaining primary alcohols were unmasked by removing the PMP groups affording **103** (74%) and then oxidized in two steps and benzyl esterified with phenyl diazomethane[91] (65%). The introduction of benzyl esters prior to global deprotection served two purposes. The product **104** could be isolated and characterized efficiently, and at the same time, it was established that benzyl

[90] For treatises on the anomeric effect see: a) Kirby, A. J. *Anomeric Effect and Related Stereoelectronic Effects at Oxygen*, Springer Verlag, Berlin, **1982**; b) *The Anomeric Effects and Associated Stereoelectronic Effects, ACS Symposium Series, Vol. 539*, Thatcher, G. R. J. (Ed.), American Chemical Society, Washington, **1994**; c) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*, Elsevier Science, Amsterdam, **1983**.

[91] Prepared according to: Creary, X. *Org. Synth.* **1986**, *64*, 207. WARNING: phenyl diazomethane ignites at temperatures above 30 °C and should be handled with care.

esters could be introduced with a mild protocol.[92] This set the stage for exploring the synthesis of the target molecule with the reverse pattern of methyl esterification (**95**). Finally, the benzyl groups were removed from **104** affording the desired trimethylated hexagalacturonate **94** in 95% yield (not shown).

The preparation of the second target molecule **95** is summarized in Scheme 22. Starting from **101**, oxidation and benzyl esterification with $PhCHN_2$ produced **105** and PMP removal gave **106**. Another round of oxidations and methyl esterification yielded **107** and lastly, hydrogenolysis afforded the desired hexagalacturonate **95** in 17% overall yield.



Scheme 22

The ^{1}H and ^{13}C NMR spectra of **94** and **95** were very complex due to the similarity of the individual residues in the oligomer, and the presence of **94** and **95** as α:β mixtures in solution. Based on the synthetic routes and the NMR data of the precursors **104** and **107**, the structures of **94** and **95** were not in doubt, but it was nevertheless comforting to be able to verify the degrees and patterns of methyl esterification by tandem mass spectroscopy (see Chapter 2). Figure 13 shows the ESI MS-MS of **95** in the negative ion mode.[93] As seen, the fragmentation occurred almost exclusively from the reducing end, cleaving one monosaccharide residue at a time (giving C_5-C_2 fragments, see Chapter 2). In all cases, it was possible to verify the structure of the synthetic oligogalacturonates using this powerful analytical technique (see Chapter 8).

[92] Esterification under basic conditions had been tested in a different context and led to degradation of the oligomers, see Chapter 5.

[93] Thanks to Dr. Kudzai E. Mutenda, University of Southern Denmark, Odense, for performing the MS-MS analyses.

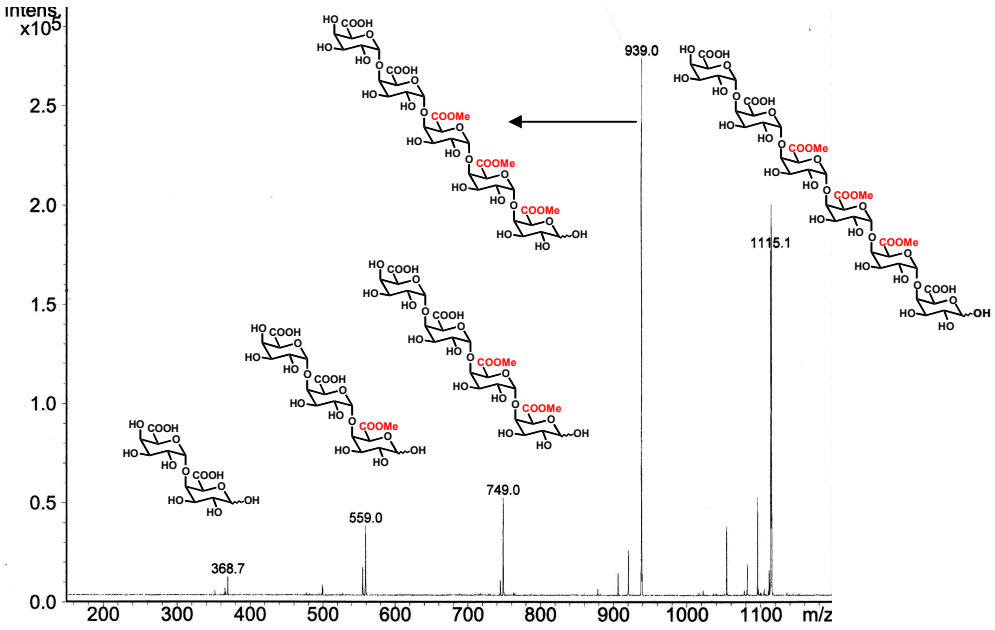
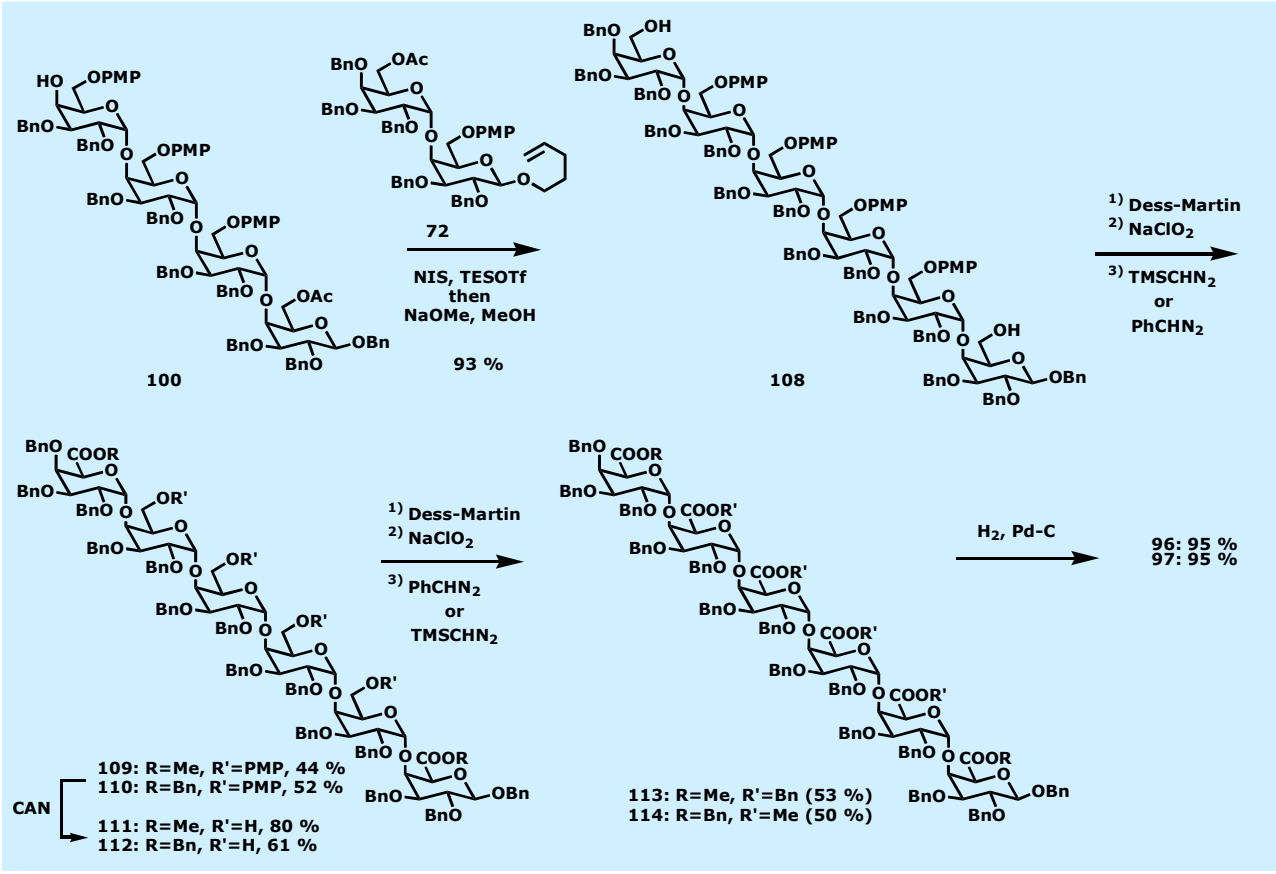


Figure 13

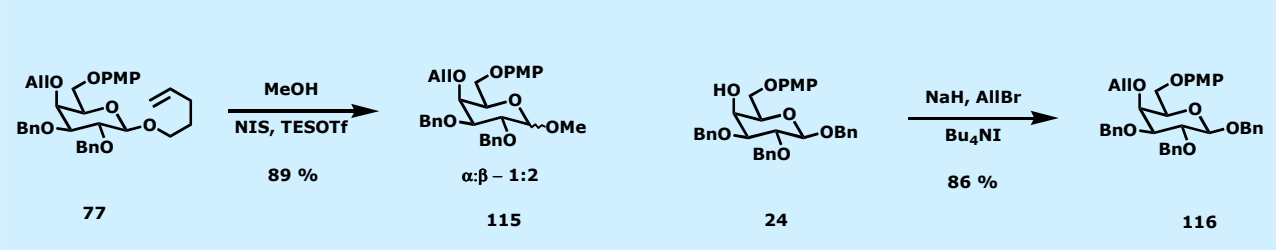
The two hexagalacturonates **96** and **97**, with two and four methyl esters respectively, were conveniently prepared starting from the tetrasaccharide acceptor **100** (Scheme 23). Glycosylation with pentenyl disaccharide donor **72** and subsequent Zemplén deacetylation provided hexasaccharide diol **108** in an excellent yield. Oxidation in two stages was followed by introduction of either methyl (compound **109**, 44%) or benzyl esters (**110**, 52% yield). The PMP groups were removed from both intermediates affording **111** (80%) and **112** (61%). Further oxidations and esterifications gave the fully protected hexagalacturonates **113** and **114**, which led to the desired compounds **96** and **97** upon hydrogenolysis. It was comforting that applying the very same conditions used previously for the synthesis of **94** and **95** in the present syntheses was straightforward. The overall yields were comparable and all reactions were performed according to identical protocols. In fact, only minor alterations to the methods used for the preparation of trisaccharides **16**-**18** were necessary.



Scheme 23

As shown in Scheme 19, the synthesis of the fifth hexagalacturonate **98** involved two couplings with pentenyl disaccharide **89**. This called for a convenient and mild method for removing the allyl group in the presence of both the pentafluorobenzoyl and the PMP group. A survey of the literature revealed a plethora of methods for cleaving an allyl group.[94] In order to test those that seemed most appropriate, two allyl protected monosaccharides were synthesized (Scheme 24). Methanol was glycosylated with donor **77** affording **115** as a 1:2 α:β mixture, and model substrate **116** was prepared by allylating **24**.

[94] a) Green, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, 3rd ed.*, John Wiley & Sons, New York, **1999**, pp. 67-74; b) Guibé, F. *Tetrahedron* **1997**, *53*, 13509, and references herein.

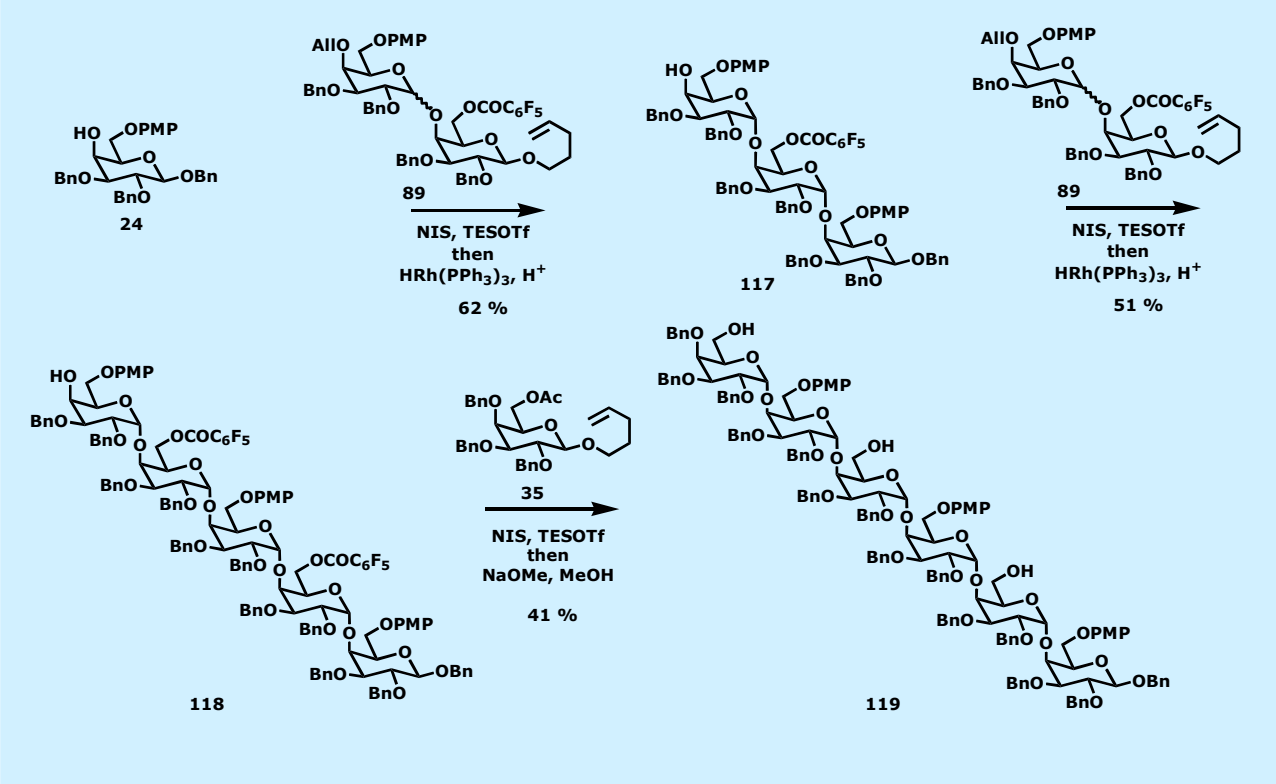


Scheme 24

The first deallylation method tested involved heating **115** in methanol with 10% Pd·C and TsOH.[95] 10, 20 and 25 mol% Pd and acid was tested but in all cases, no more than approximately 50% conversion could be obtained, as judged from TLC. Changing the acid to $HClO_4$ gave full conversion within 4 hours, but the reaction was very acidic, and it was doubtful, whether these conditions would be applicable for larger, more sensitive substrates. The next method tested involved treating **115** with 2 equivalents of $Pd(OAc)_2$ in 95% aq. acetic acid at 40 °C.[94b] These conditions led to full conversion within 4 hours, without any formation of byproducts, as judged by TLC. Unfortunately, when these conditions were applied to the trisaccharide formed after coupling of **24** and **89** (see Scheme 25), a substantial amount of the propan-2-onyl-substituted trisaccharide was isolated together with the desired product **117**. This presumably originated from competing Wacker oxidation[96] of the olefin. Other means of removing the allyl group had to be sought. It has been reported, that Wilkinson's catalyst $ClRh(PPh_3)_3$ is capable of isomerizing allyl groups to vinyl ethers that, in turn, can be hydrolyzed to liberate the hydroxy functionality.[97] However, in experiments with this catalyst isomerization of the allyl group of **116** was accompanied by hydrogenation, leading to a propyl ether. Instead, focus was put on the application of a modified catalyst, first reported by Boons and co-workers.[98] The procedure involved treatment of Wilkinson's catalyst with *n*-BuLi, presumably forming $HRh(PPh_3)_3$,[99] a compound known to isomerize double bonds.[100] When **116** was mixed with a solution of this rhodium catalyst and refluxed in anhydrous THF, NMR showed complete isomerization to a 2:1 *Z*:*E* mixture after 15 minutes. When a solution of NBS in wet THF was added and reflux continued, complete hydrolysis was obtained within 30 min. This, however, turned out to serve a problem when

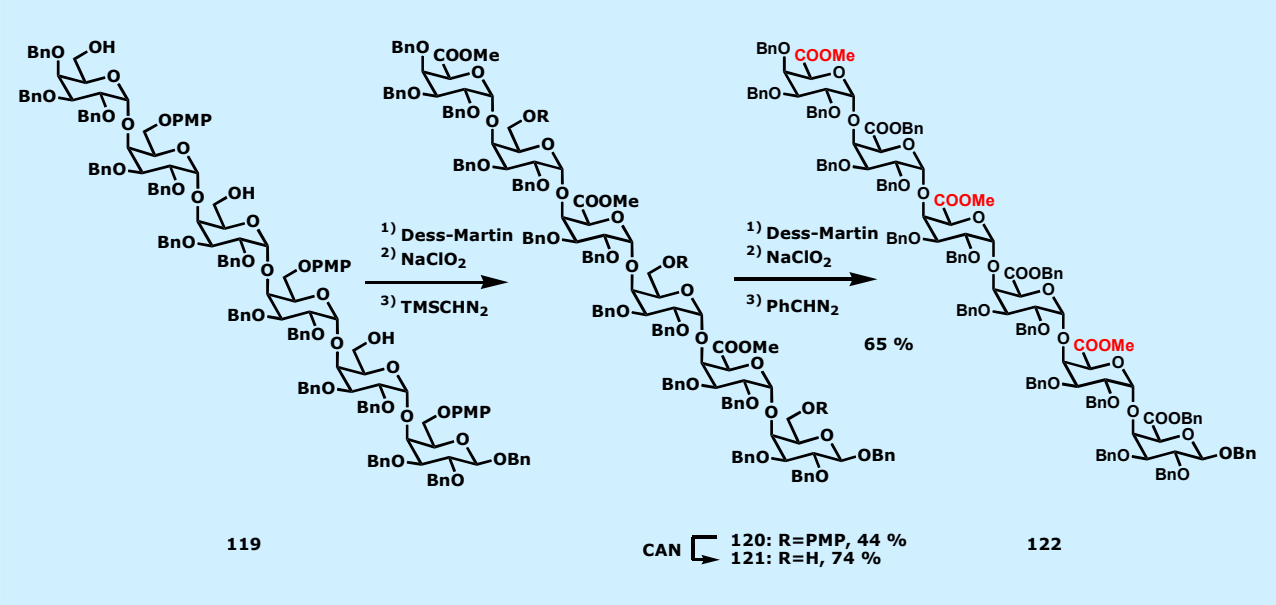
[95] a) Boss, R.; Scheffold, R. *Angew. Chem.* **1976**, *88*, 578; b) Carless, H. A. J.; Haywood, D. J. *J. Chem. Soc., Chem. Commun.* **1980**, 980.
[96] First report: a) Smidt, J.; Hafner, W.; Jira, R.; Sedlmeier, J.; Sieber, R.; Rüttinger, R.; Kojer, H. *Angew. Chem.* **1959**, *71*, 176; For a review, see: b) Tsuji, J. *Synthesis* **1984**, 369.
[97] Corey, E. J.; Suggs, J. W. *J. Org. Chem.* **1973**, *38*, 3224.
[98] Boons, G.-J.; Burton, A.; Isles, S. *Chem. Commun.* **1996**, 141.
[99] Dewhirst, K. C.; Keim, W.; Reilly, C. A. *Inorg. Chem.* **1968**, *7*, 546.
[100] Attridge, C. J.; Maddock, S. J. *J. Chem. Soc. C* **1971**, 2999.

used for the preparation of the larger tri- and pentasaccharides **117** and **118** (see Scheme 25), since low yields were obtained and the products were contaminated with succinimide from the NBS. Therefore, the THF solution of *E*- and *Z*-vinyl ethers, produced upon treating **116** with $HRh[PPh_3]_3$, was diluted with methanol and stirred with acidic ion-exchange resin. Despite the fact that the methanolysis was slow (24 hours for full conversion), the conditions are mild and no byproducts were formed. This method also proved useful for the larger tri- and pentasaccharides, although the reactions were generally more sluggish – 20% of the modified catalyst was employed, and the isomerizations took up to 6 hours to go to completion. The acidic methanolysis occurred smoothly, but to form the pentasaccharide **118**, a reaction time of 36 hours was needed in order to obtain good conversion.



Scheme 25

The synthesis of hexagalactoside **119** is outlined in Scheme 25. Glycosylation of monosaccharide acceptor **24** with pentenyl disaccharide donor **89** (as a 6:1 α:β mixture) occurred smoothly, and after removal of the allyl group, the desired, α-linked trisaccharide **117** could be isolated in 62% overall yield. Another glycosylation with **89** afforded **118** after deprotection (51% overall). Finally, glycosylation of **118** with donor **35** and concomitant removal of the three acyl protecting groups under Zemplén conditions gave triol hexagalactoside **119**, in a moderate 41% yield.



Scheme 26

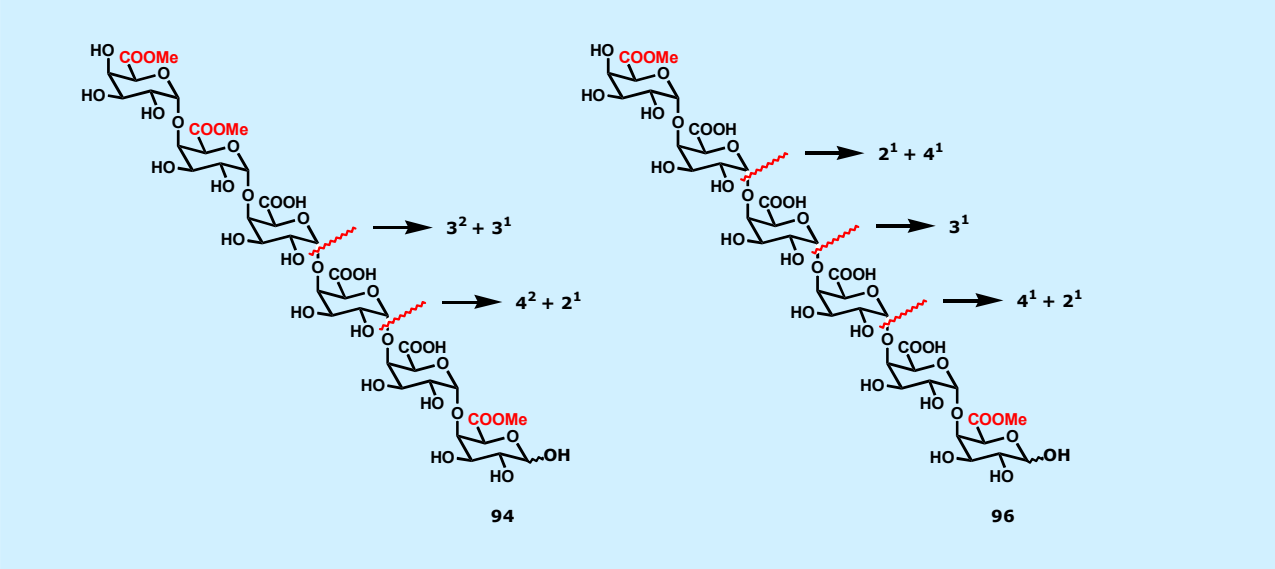
With **119** in hand, the stage was set for completing the synthesis of the fifth hexagalacturonate **98** (Scheme 26). Oxidation and treatment of the resulting tricarboxylic acid with $TMSCHN_2$ afforded **120**. Removal of the PMP protecting groups gave **121**, and oxidation and benzyl esterification provided **122**. **98** was obtained after hydrogenolysis (not shown).
With all five partially methyl esterified hexagalacturonates **94**-**98** in hand, their ability to serve as substrates for pectic enzymes could now be investigated. Approximately 100 mg of each hexasaccharide was made, providing sufficient material for thorough biological studies.

Initial Studies of PG I and PG II with Synthetic Hexagalacturonates

In order to ascertain, whether the synthetic hexasaccharides **94**-**98** had a sufficiently high DP to be used as substrates for pectic enzymes, their degradation by *endo*-polygalacturonase I (PG I) and II (PG II) were examined by researchers at Danisco.[101]
PG I is known to function by attaching to a HG polymer, and then degrade it one residue at a time, while moving towards the non-reducing end of the chain. It has been shown by total digest of partially esterified HG and analysis of the products by mass spectrometry that the enzyme will not cleave between two methylated galacturonic acid residues.[88b]

[101] Thanks to B.Sc. Jesper Harholt and Dr. Tove M. I. E. Cristensen, Danisco, Copenhagen for performing the enzymatic studies of PG I and PG II.

PG II has the same preference for cleavage between unmethylated GalA residues, but it releases the two products of the degradation after cleavage, and reattaches to the polymer in a different position.[102]
In these studies, solutions of the hexamers were made in sodium acetate buffer (pH=4.5) and incubated with the enzyme under study for periods of 5 minutes, 1 hour, and 24 hours. After the incubation, the solutions were boiled to stop the reactions and analyzed using ESI MS. For both enzymes, no activity was found towards substrates **95**, **97**, and **98**. This was comforting, considering that these hexamers did not contain any contiguous non esterified GalA residues (except for the two residues at the non-reducing end of **95** – but cleavage at this position would not be expected of *endo*-polygalacturonases).
94 on the other hand was a substrate, both for PG I and PG II. The activities of the enzymes were low, incubation for 24 hours were needed to obtain more than 90% conversion of the starting hexagalacturonate to smaller oligomers. In both cases, the main product was a tetrasaccharide with two methyl esters (4^2, Scheme 27).[103] A smaller amount of trisaccharides with one and two methyl ester groups (3^1 and 3^2, Scheme 27) was also observed.



Scheme 27

Not surprisingly, **96** was an even better substrate for these enzymes. After incubation for 5 minutes, a level of degradation similar to what was observed for **94** after 24 hours was detected. PG I produced a monomethylated tetrasaccharide (4^1) and the corresponding monomethylated digalacturonate

[102] Benen, J. A. E.; Kester, H. C. M.; Parenicová, L.; Visser, J. In *Pectin and Pectinases, Progress in Biotechnology, Vol 14*, Visser, J.; Voragen, A. G. J. (Eds.), Elsevier, Amsterdam, **1996**, p. 221.
[103] The nomenclature X^Y refers to a fragment with DP=X and Y methyl esters.

(2^1) as the primary products. After further incubation, 4^1 was degraded forming a 3^1, after which no further activity could be detected within the time scale of the experiment. PG II formed the same initial products as PG I, but then no further degradation was detected.

The observations made so far are very promising in that it has been established that the synthetic hexagalacturonates **94**-**98** are potential substrates for pectic enzymes. This is the first application of well-defined, partially esterified HG oligomers with a DP of more than three in enzymatic studies.[104] The preliminary results have confirmed previous finding based on total digestion of HG polymers, and this method can potentially reveal more details about cleavage preferences and subsite requirements of pectic enzymes. Further investigations of these and other enzymes will reveal the full potential of synthetic HG oligomers in pectin research. Such explorations are in progress, and the results will be reported in due course.[105]

[104] For the applications of synthetic trigalacturonates in studies of pectic enzymes see Refs. 30 and 88a.

[105] Tove M. I. E. Christensen, *personal communication*.

5. NEOGLYCOCONJUGATES

Synthesis of BSA-Conjugated Oligogalacturonates for Immunization Studies

Introduction

Antibodies are very useful tools in biological sciences. When using *in vivo* techniques (see Chapter 6) for the production of an antibody, a key issue to consider is the immunogenicity of the antigen applied, i.e. the ability of the antigen to raise an immune response in the animal used for producing the antibody. In general, antigens need to be of a certain size for the immune system to react to them. With carbohydrates, this has traditionally been obtained by conjugation to a larger, immunogenic body, typically a protein.[106] This method has proven successful for preparation of e.g. antibodies to various blood group determinants,[107] tumor-associated antigens,[108] and bacterial receptor glycans.[109]

Antibodies towards pectic polysaccharides have traditionally been raised to complex polysaccharides mixtures, obtained after isolation from plants. This has given the process a somewhat random outcome, since it has been difficult to control the nature of the epitopes 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. For the two antibodies in question, LM5[110] and LM6,[111] a single, defined oligosaccharide was conjugated to bovine serum albumin (BSA) through the reducing end aldehyde, and rats were immunized with these conjugates. Since homogalacturonan is the most abundant of the pectic polysaccharides, antibodies recognizing parts of this structure are very useful. A number of such antibodies exist (see Chapter 6), but it would be desirable to be able to raise novel antibodies with well-defined oligosaccharide antigens. In order to explore this, it was envisioned that synthetic oligogalacturonates could be covalently linked to BSA and used for immunization studies. Three target structures were chosen for these studies, a fully methylated trigalacturonate and non-esterified

[106] For recent reviews, see: a) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579; b) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215.
[107] Lemieux, R. U. *Chem. Soc. Rev.* **1978**, *7*, 423.
[108] For examples see: a) Ragupathi, G.; Park, T. K.; Zhang, S.; Kim, I. J.; Graber, L.; Adluri, S.; Lloyd, K. O.; Danishefsky, S. J.; Livington, P. O. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 125; b) Allen, J. R.; Ragupathi, G.; Livingston, P. O; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 10875.
[109] See e.g.: a) Ray, A. K.; Magnusson, G. *Acta Chem. Scand.* **1992**, *46*, 487; b) Sekljic, H.; Wimmer, N.; Hofinger, A.; Brade, H.; Kosma, P. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1973.
[110] Jones, L.; Seymore, G. B.; Knox, J. P. *Plant Physiol.* **1997**, *113*, 1405.
[111] Willats, W. G. T.; Marcus, S. E.; Knox, J. P. *Carbohydr. Res.* **1998**, *308*, 149.

tri- and hexagalacturonates. The ability to prepare such conjugates would enable the future option of preparing neoglycoconjugates with a well-defined, customized pattern of methyl esterification.

Synthetic Planning and Method Development

In planning the conjugation of oligogalacturonates to bovine serum albumin, a number of elements required consideration. Reductive amination was chosen as the conjugation method, since this is a well-proven and reliable protocol for functionalization of proteins.[112] 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.[113, 114] The use of a hydrocarbon spacer at the reducing end of the carbohydrate hapten was pioneered by Lemieux and co-workers.[115] 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 monosaccharide with an aglycon spacer containing a protected aldehyde to serve as glycosyl acceptor in the preparation of oligosaccharides (cf. Figure 14).

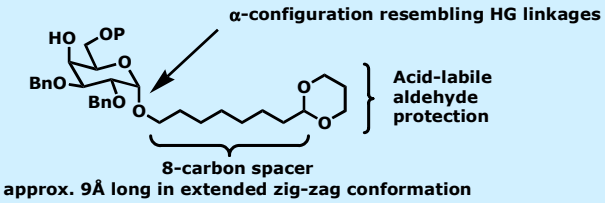
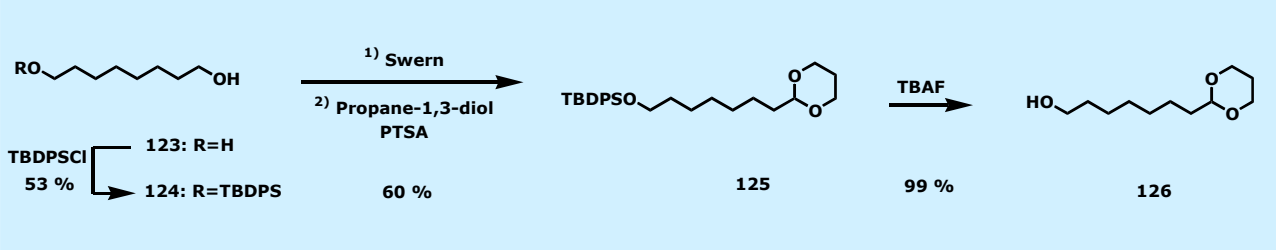


Figure 14

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 8 carbons long, which corresponds to roughly 9Å in an extended zig-zag conformation, 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 removed by

[112] Wong, S. S. *Chemistry of Protein Conjugation and Cross-Linking*, CRC Press, Boston, **1991.**
[113] Peters, T. *All About Albumin: Biochemistry, Genetics and Medicinal Applications*, Academic Press, London, **1995**.
[114] Detailed studies have shown that reductive amination of BSA preferably functionalizes the lysine residues, see: Schwartz, B. A.; Gray, G. R. *Arch. Biochem. Biophys.* **1977**, *181*, 542.
[115] Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076.

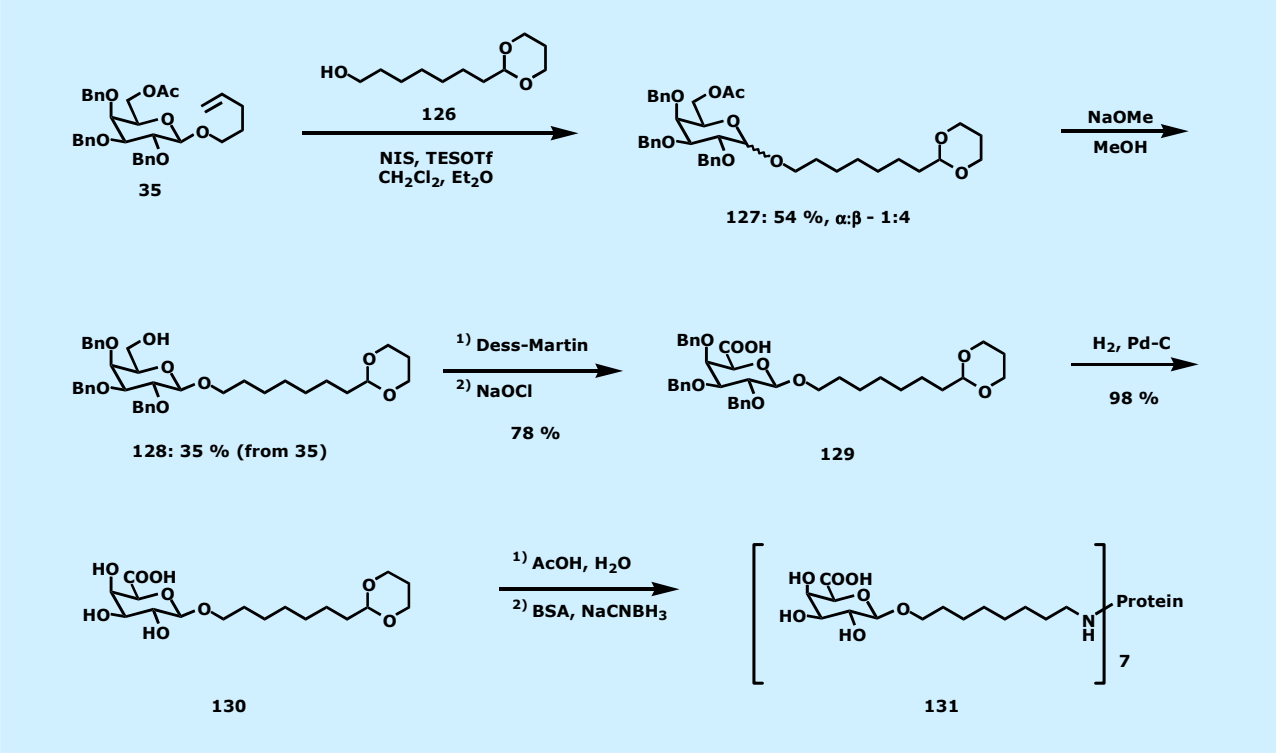
treatment with acid, avoiding base-mediated degradation of the sugar. Moreover, this blocking group is stable down to pH 2,[116] preventing premature unveiling of the masked aldehyde throughout the assembly of the oligosaccharide.



Scheme 28

The protected ω-hydroxyaldehyde **126**, needed as the aglycon of the target saccharides, was prepared from octane-1,8-diol (**123**), as outlined in Scheme 28. Monoprotection was obtained by treatment with *tert*-butyldiphenylsilyl chloride in a mixture of DMF and Hünig's base.[117] Swern oxidation of the remaining primary alcohol of **124** gave the aldehyde, which was trapped by acid-catalyzed acetal formation,[118] producing **125**. Lastly, the silyl group was removed quantitatively by treatment with tetrabutylammonium fluoride.
To test the conjugation conditions, monosaccharide **130** was synthesized (Scheme 29). Expecting poor α-selectivity for the glycosylation of the reactive primary alcohol **126** with **35**, the reaction was performed in dichloromethane/ether 2:1, since the addition of ether has been reported to favor formation of the α-linked product.[119] Nonetheless, the β-glycoside **127β** was formed predominantly, which was also the case when ether was omitted as co-solvent. This is in agreement with earlier observations comparing tri- and tetrasaccharides with monosaccharides as acceptors in glycosylations (see Chapter 2 and 4). In these cases, a higher yield and preference for the α-linked product was obtained for the larger substrates.

[116] Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis, 3rd ed.*, John Wiley & Sons, New York, **1999**, pp. 308-309, p. 725.
[117] Yu, C.; Liu, B.; Hu, L. *Tetrahedron Lett.* **2000**, *41*, 4281.
[118] Roelofsen, D. P.; Wils, E. R. J; van Bekkum, H. *Rec. Trav. Chim. Pays-Bas* **1971**, *90*, 1141.
[119] a) Wulff, G.; Röhle, G. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 157; b) Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1987**, *28*, 4701; c) Ref. 87.



Scheme 29

Since the anomeric configuration was believed to be irrelevant for testing the conjugation conditions, it was decided to carry on despite the disappointing outcome of the glycosylation. Deacetylation of **127** enabled the isolation of β-linked glycoside **128**. Oxidation with Dess-Martin periodinane and sodium chlorite provided **129**, and removal of the benzyl ethers afforded **130**. Treatment with 80% aq. acetic acid efficiently hydrolyzed the dioxane, and subsequent sodium cyanoborohydride-mediated reductive alkylation of BSA with the crude aldehyde installed an average of seven sugar residues on the protein in conjugate **131**, as determined by MALDI-TOF MS. The unmasking of the aldehyde could not be monitored by TLC due to the polar nature of the starting material and the product. However, an NMR experiment proved unequivocally that virtually complete hydrolysis was obtained within 2 hours at 50 °C (Figure 15). **130** was dissolved in CD_3COOD:D_2O 4:1, heated to 50 °C and a spectrum was recorded every 15 minutes. Generally, these deprotections were performed for 12 hours to ensure complete hydrolysis.

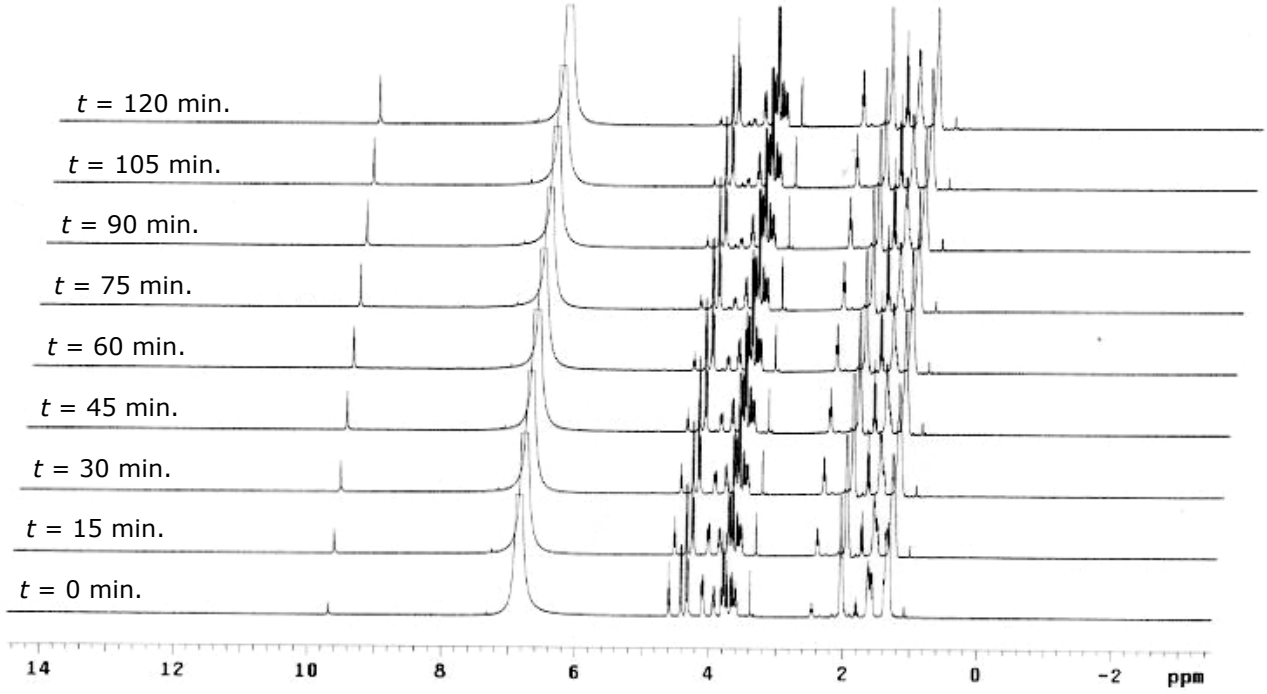
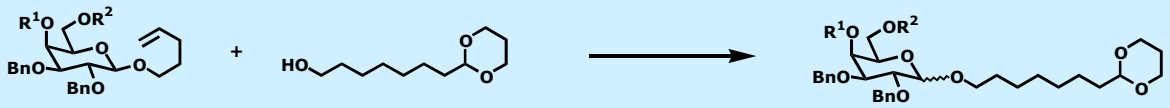


Figure 15

Still, the problem of obtaining an α-linked glycoside had to be solved. Lemieux and co-workers reported a method for very stereoselective glycosylations.[120] They showed that glycosyl bromides could serve as donors in the absence of silver and mercury salts when activated with bromide ions. Supposedly, an equilibrium is established between the α- and β-glycosyl bromide, the so-called *in situ* anomerization. The equilibrium heavily favors the α-bromide, but the β-bromide is much more reactive than the axial anomer. Consequently, only the equatorial bromide acts as a nucleofuge, giving rise to the α-glycoside through an S_N2-reaction with the acceptor. This method does have limitations, since reaction times are usually rather long, and very sterically hindered alcohols cannot be glycosylated in this fashion. However, for the purpose of glycosylating the primary alcohol **126** it was very appropriate, especially since it would not be necessary to protect the 4-OH of the donor. This was a direct consequence of the large difference in reactivity between the hydroxy groups, which minimized homocoupling of the donor.

[120] Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056.

Table 6



30: R^1=ClAc, R^2=Ac
29: R^1=H, R^2=Ac
31: R^1=H, R^2=PMP

132: R^1=ClAc, R^2=Ac
133: R^1=H, R^2=Ac
134: R^1=H, R^2=PMP

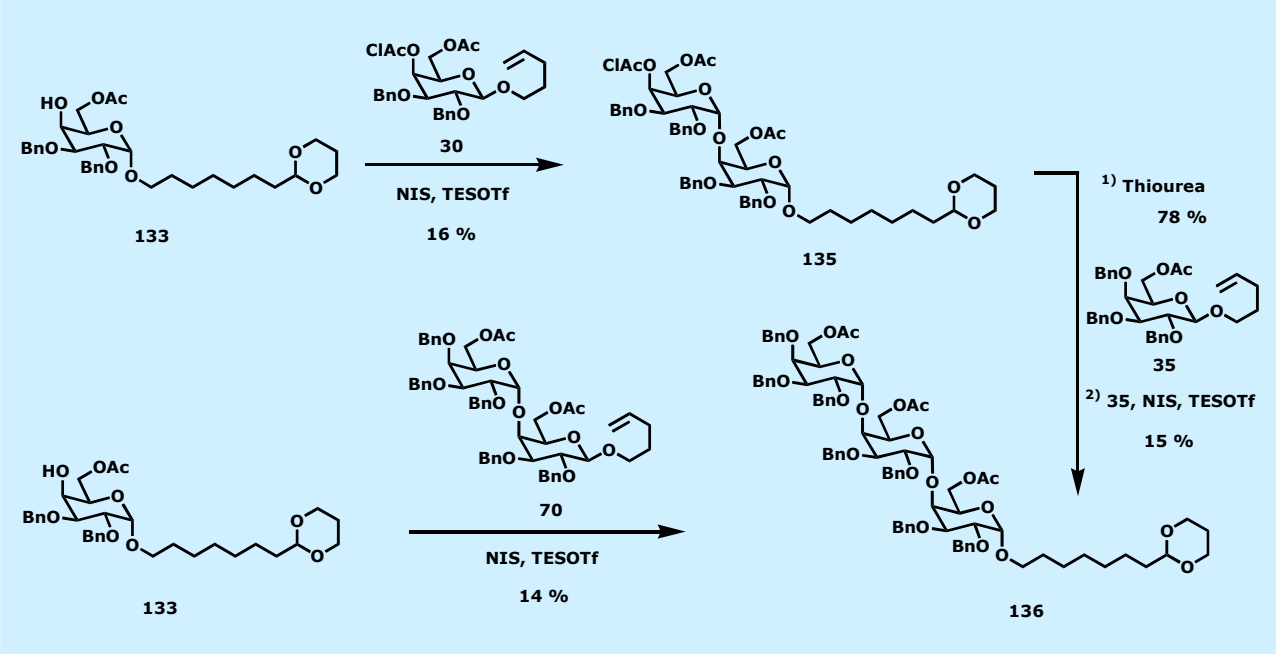
Entry	Donor	Conditions[a]	Temp. [°C]	Yield [%]	α:β[b]
1	**30**	NIS, TESOTf	-20	58	1:3.5
2	**30**	NIS, TfOH	-78	50	1:5.5
3	**29**	Br_2 then Bu_4NI	20	5	95:5
4	**29**	Br_2 then Et_4NBr	20	10	>95:5[c]
5	**29**	Br_2, Et_4NBr	0-20	25	>95:5 [c]
6	**29**	Br_2, Et_4NBr, MS 4A	0-20	56	>95:5 [c]
7	**31**	Br_2, Et_4NBr, MS 4A	0-20	70	>95:5 [c]

[a] CH_2Cl_2 as solvent. [b] Determined by ^1H NMR. [c] At most, traces of the β-anomer were observed.

Table 6 summarizes the efforts to obtain an α-glycoside. Entries 1 and 2 show experiments with the fully protected pentenyl donor **30**. Under standard conditions, a 1:3.5 ratio was obtained favoring the β-linked product **132β**. Cooling the reaction to −78 °C and using stoichiometric amounts of triflic acid as promoter provided even less of the desired **132α**. Initial results with the donor **29** using brominolysis and *in situ* anomerization were promising in terms of α-selectivity, albeit the yields were low (entries 3 and 4). Lowering the temperature improved the reaction (entry 5), but a satisfactory yield was reached only when 4A molecular sieves were added to quench the hydrobromic acid formed (entry 6). Entry 7 illustrates that the reaction is even more efficient in the case of the PMP-protected donor **31**, an observation that soon proved important (*vide infra*).

Synthesis of Neoglycoconjugates

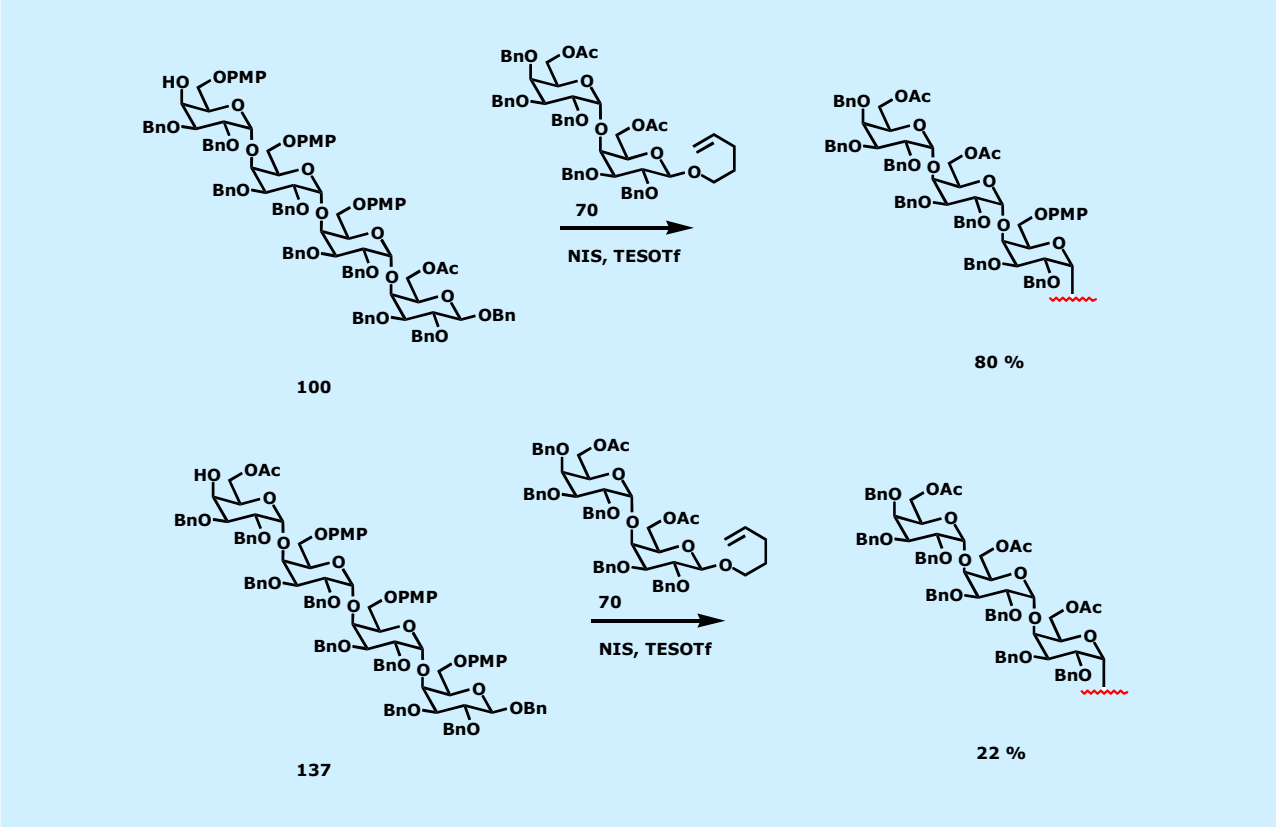
Having established reliable conditions for the conjugation of galacturonates to BSA and with the desired α-linked spacer-monosaccharide building block **133** in hand, the stage was set for targeting the desired tri- and hexasaccharide neoglycoconjugates. Glycosylation of the acceptor **133** with donors **30** and **70** proved surprisingly difficult (Scheme 30). **135** was obtained in only 16% yield and **136** in mere 14%. An attempt to further elaborate **135** to **136** by selective removal of the chloroacetyl group and glycosylation of the resulting disaccharide with **35** resulted in another disappointing result (Scheme 30).



Scheme 30

In all three cases, the donors and acceptors were protected with an acetyl group at the 6-position. There seems to be a mismatch between these donors and acceptors. Another example is shown in Scheme 31, where the couplings of two different tetrasaccharide acceptors with the same disaccharide donor displayed very different efficiencies.[121] The only apparent difference of the substrates **100** and **137** is the *O*-6 protective group at the non-reducing end. It appears that the combination of a relatively disarmed donor with an acceptor bearing an electron-withdrawing substituent proximate to the site of glycosylation is detrimental to the coupling. One could speculate that a 6-membered cyclic intermediate is formed on the acceptor *en route* to migration of the acetyl group from *O*-6 to *O*-4,[122] but no answer presents itself as to why no 1-6 linked saccharides are observed and why this is not a problem, when the donor has a PMP group at the 6-position. Nonetheless, the observation is ruinous to the current strategy. Fortunately, this could easily be refurbished by using PMP-protected building blocks (Scheme 32).

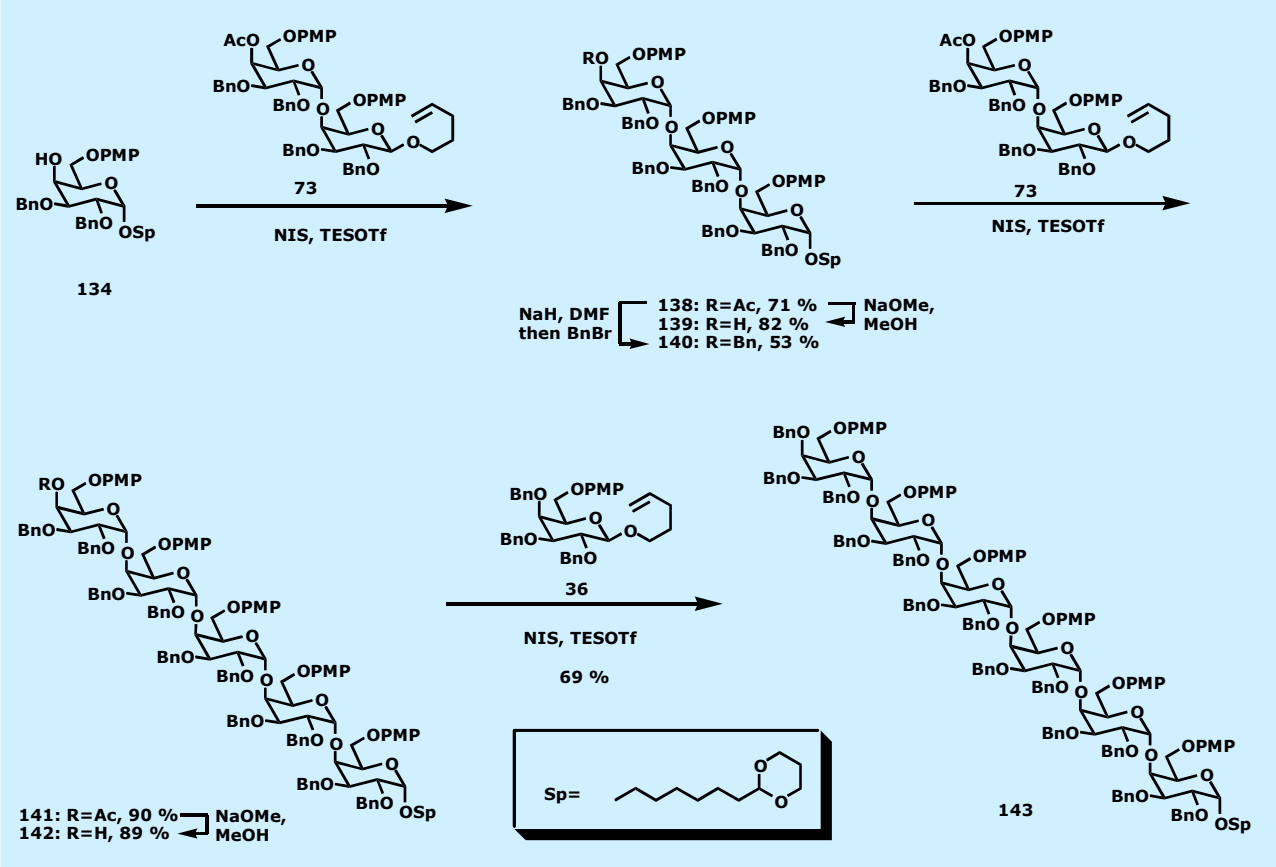
[121] Tetrasaccharide **137** was synthesized in a similar fashion as **100** (for experimental details see Chapter 8).
[122] Acetyl groups are known to be prone to migration, see e.g.: Reinhard, B.; Faillard, H. *Liebigs Ann. Chem.* **1994**, 193.



Scheme 31

The PMP-protected monosaccharide **134** (cf. Table 6) was glycosylated with donor **73**, made by Koenigs-Knorr coupling (cf. Chapter 3), affording trisaccharide **138** 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. Treatment of **138** with sodium hydride in DMF until complete deacetylation had ensued and then adding benzyl bromide provided **140** in a one-pot procedure.[123] Alternatively, simple deacetylation yielded **139**, which could undergo another coupling with **73**. The product **141**, obtained in impressive 90% yield, was deprotected to **142** and glycosylated with monosaccharide donor **36**, furnishing hexasaccharide **143** in 69% yield. With **140** and **143** in hand, the stage was set for elaboration to oligogalacturonates.

[123] In a test experiment, **73** had undergone a similar one-pot transformation in 90% yield.

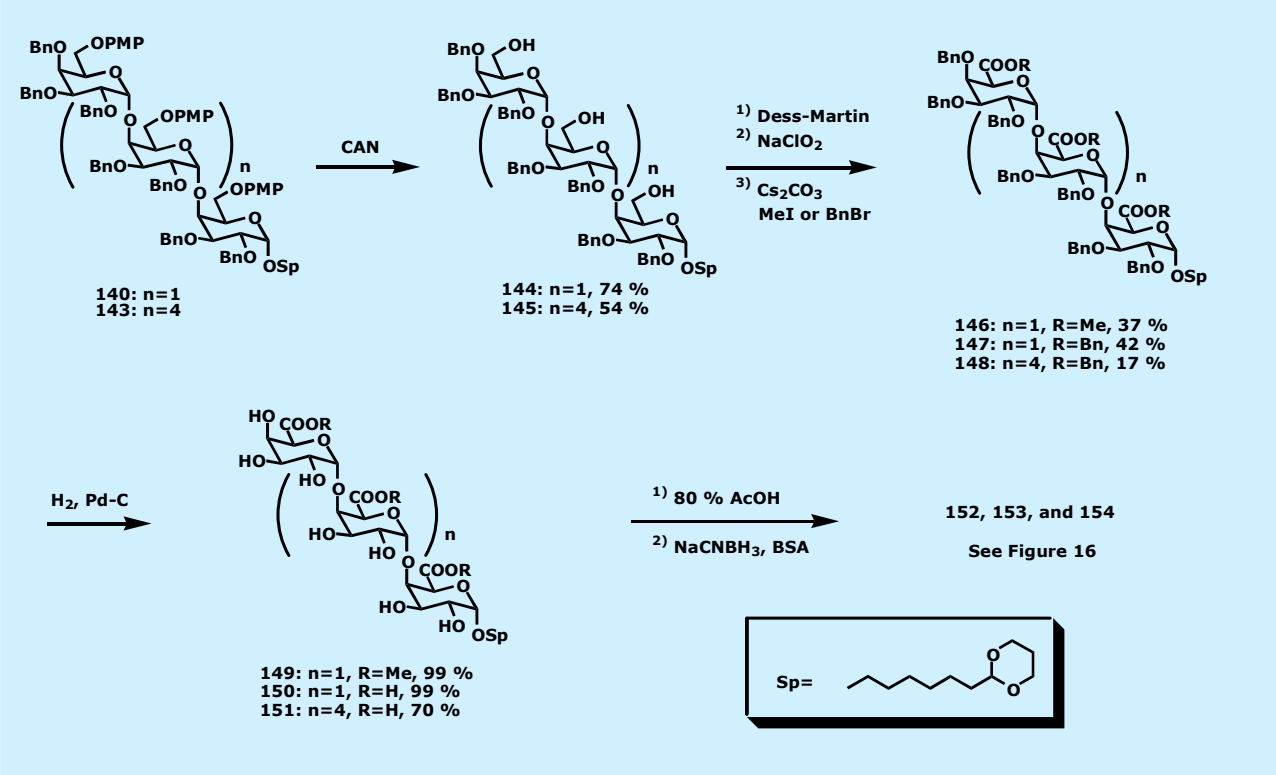


Scheme 32

The efforts towards completing the glycoconjugate syntheses are depicted in Scheme 33. CAN-mediated oxidative cleavage of the PMP-groups of **140** and **143** furnished **144** and **145** and was followed by oxidations and esterifications. For the trigalacturonate both methyl and benzyl esters were desired, giving access to either a fully methylated or non-esterified conjugate. Furthermore, it was anticipated that benzyl esterification would facilitate handling, especially for the hexagalacturonate. In a model study, galacturonate **46** had been esterified by treatment with cesium carbonate and methyl iodide or benzyl bromide. The yields of these transformations were higher than 90% in both cases, and no β-elimination of benzyl alcohol was observed. Therefore, these conditions were applied for esterifying the trisaccharide **144**, affording **146** and **147** in acceptable 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 **148**, which could be isolated in only 17% yield.[124] Hydrogenolysis of the fully protected oligogalacturonates proceeded uneventfully and after treatment with aqueous acetic acid to unmask the

[124] It was these results that encouraged the use of phenyldiazomethane for benzyl esterifications (cf. Chapter 4).

aldehyde the sugars could be attached to BSA by reductive amination with sodium cyanoborohydride.[125] The structures of the conjugates **152-154** are shown in Figure 16. The average number of conjugated sugar residues per protein were determined by MALDI-TOF MS. Evidently, the conjugation of the neutral sugar **149** was more facile than for the corresponding acidic sugars **150** and **151**.



Scheme 33

The successful synthesis of the three neoglycoconjugates **152**-**154** permitted the first attempts to raise antibodies towards well-defined HG fragments. Initial experiments[126] with *in vivo* immunization of rats with **152** and **153** were not lucrative in terms of raising anti-HG antibodies, but surprisingly, a different antibody could be isolated following these efforts (see Chapter 6). However, **154** has not yet been tested as immunogen and no experiments have so far been done with phage display technology employing any of the neoglycoconjugates. This work is currently being pursued and the results will be published in due course.[127]

[125] Roy, R.; Katzenellenbogen, E.; Jenning, H. J. *Can. J. Biochem. Cell Biol.* **1984**, *62*, 270.
[126] Marcus, S. E.; Knox, J. P., *unpublished results*.
[127] Dr. J. Paul Knox, University of Leeds, UK, *personal communication*.

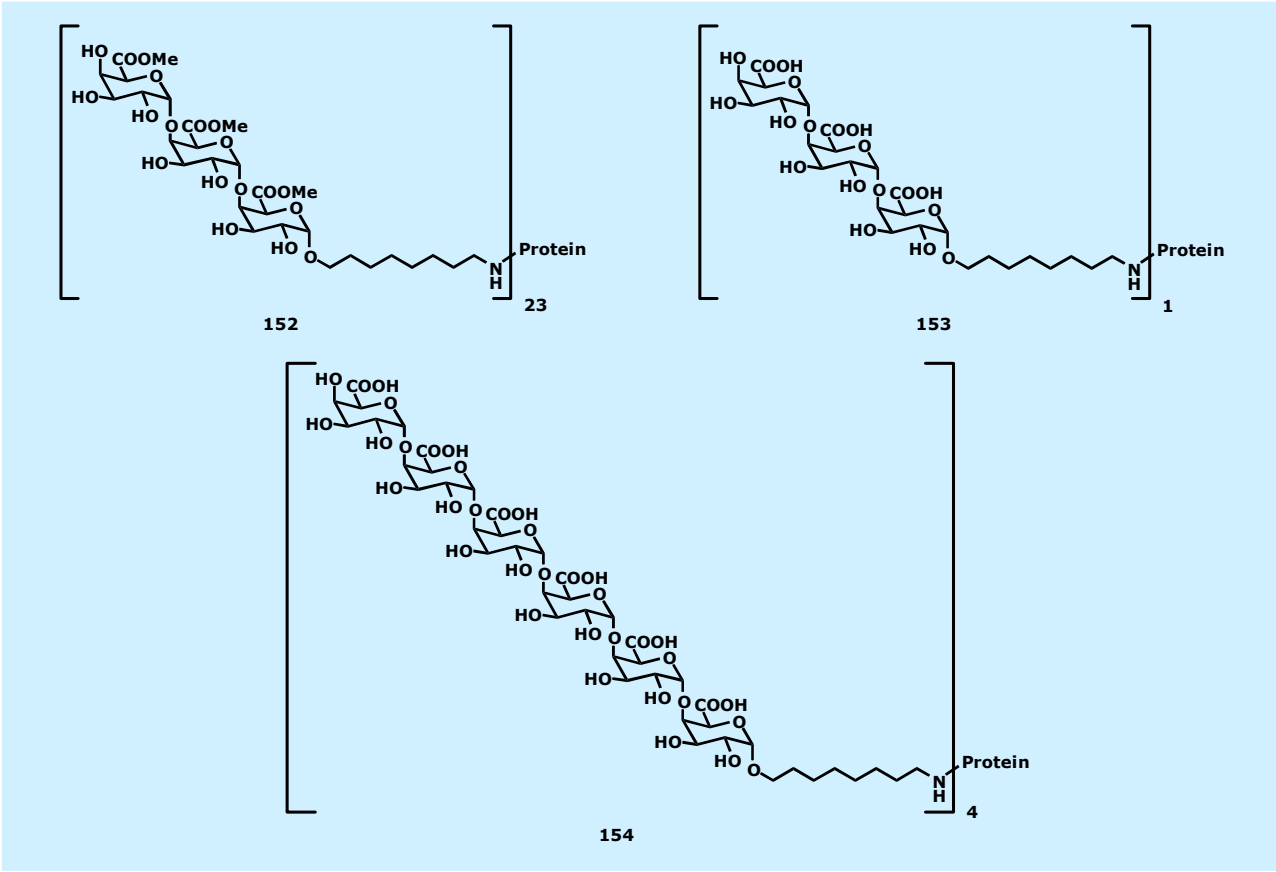

HO
COOMe
O
HO
HO
O
COOMe
O
HO
HO
O
COOMe
O
HO
HO
O
N
H
Protein
23
152
HO
COOH
O
HO
HO
O
COOH
O
HO
HO
O
COOH
O
HO
HO
O
N
H
Protein
1
153
HO
COOH
O
HO
HO
O
COOH
O
HO
HO
O
COOH
O
HO
HO
O
COOH
O
HO
HO
O
COOH
O
HO
HO
O
COOH
O
HO
HO
O
N
H
Protein
4
154

Figure 16

6. Pectic Antibodies

Epitope Mapping of Known Antibodies and Identification of a Novel Pectic Antibody

Introduction

Antibodies recognizing specific chemical entities are very powerful tools in the study of plant physiology, growth, and development. Knowledge of the plant cell wall is of great importance to the field of plant science, since the cell wall is vital to plant life. The function of pectic polysaccharides herein and the importance of their chemical composition is still relatively poorly understood. In order to answer these questions a number of antibodies binding to various pectic polysaccharide structures have been prepared and characterized, some of which are shown in Table 7.[18]

Table 7

Antibody	Antigen/epitope	References
JIM5	HG – low degree of methylation	16, 128
JIM7	HG – high degree of methylation	16, 128
PAM1	HG – 20-30 unesterified residues	19
LM5	RG-1 – (1→4)-β-D-galactan	110. 129
LM6	RG-1 – (1→5)-α-L-arabinan	111, 129
LM7	HG – specific pattern of methylation	9

JIM5 and JIM7 both recognize methylated HG – the latter preferring a higher degree of methylation. PAM1 is specific to unesterified HG, whereas LM7 binds to partially methylated HG and has an epitope with a very distinct localization in plant tissue (see chapter 1). LM5 and LM6 were both raised against synthetic oligosaccharides conjugated to BSA (see also Chapter 5). They are specific for oligosaccharides often associated with the sidechains of RG-1 – LM5 for galactan and LM6 for arabinan.

Using these and other antibodies, the role of pectic polysaccharides in a number of plants has been studied. Nonetheless, a more detailed knowledge of the epitopes of the known pectic antibodies, as well as preparation of novel antibodies that recognize different epitopes, are important subjects in plant cell wall research.

[128] Knox, J. P.; Linstead, P. J.; King, J.; Cooper, C.; Roberts, K. *Planta* **1990**, *181*, 512.
[129] Willats, W. G. T.; Steele-King, C. G.; Marcus, S. E.; Knox, J. P. *Plant J.* **1999**, *20*, 619.

Antibody research can be said to occur in three distinct, but often overlapping, areas: preparation of an antibody; characterization of the epitope; and application of the antibody probe to answer relevant questions in biology.[130]
The preparation of antibodies is done using one of two techniques: *in vivo* immunization, illustrated in Figure 17[131] or phage display expression of proteins, a method which is outlined in Figure 18.[132]

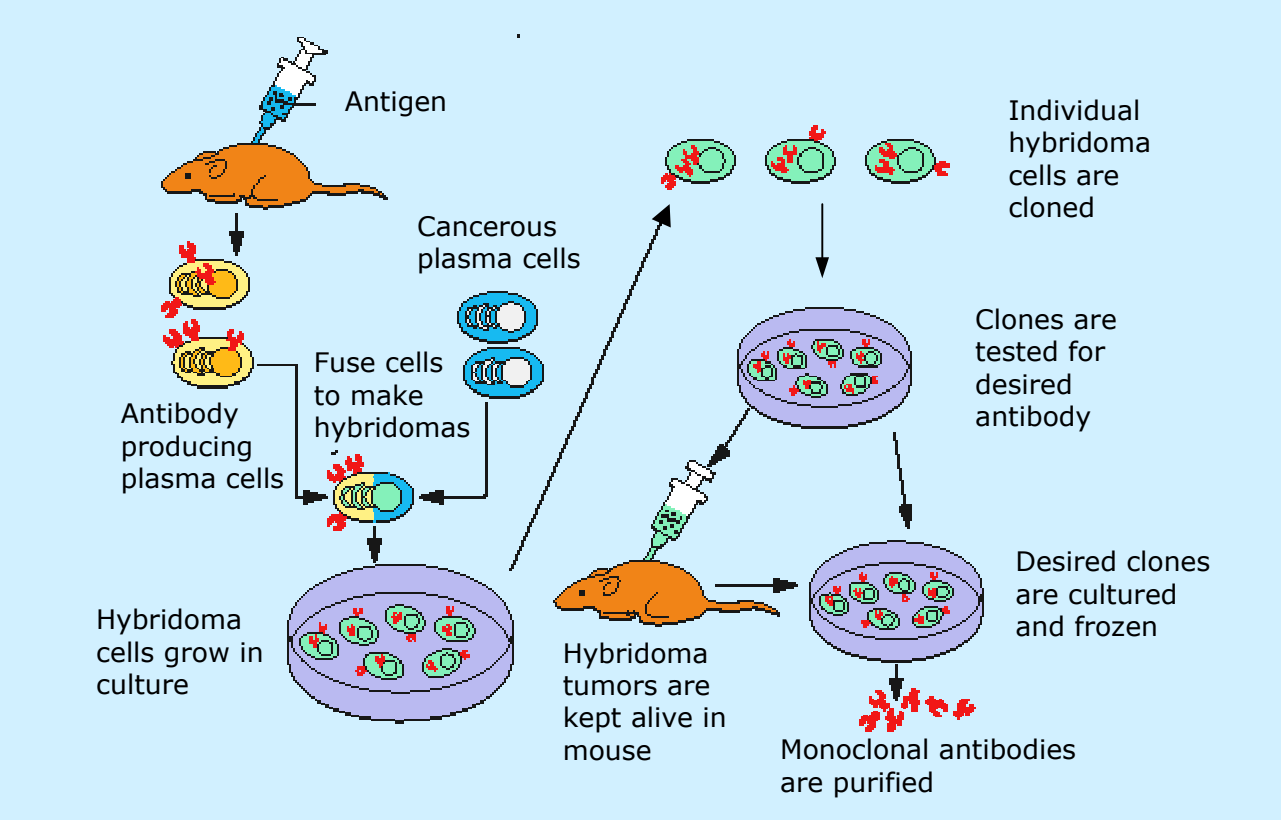


Figure 17

In vivo immunization relies on the ability of a suitable living organism (e.g. mouse, rat, or rabbit) to produce antibodies after being exposed to a relevant immunogen, i.e. a substance that both contains the desired epitope structure and is able to trigger the immune system of the animal. When the existence of relevant antibodies in blood from the animal has been established, fusion of the antibody producing spleen cells of the animal with myeloma cells[133]

[130] The following discussion focuses on methods used in the laboratories of Dr. J. Paul Knox, Centre for Plant Sciences, Leeds University, UK, and is not meant to be comprehensive.
[131] Adapated from National Cancer Research Center, Internet Immune System Tutorial Pages, see: http://newscenter.cancer.gov./sciencebehind/immune.immune00.htm
[132] From Willats, W. G. T. *Plant Mol. Biol.* **2003**, *in press.*
[133] Myeloma cells are cancerous cells that ideally do not produce antibodies of their own, but are immortal and thus can be cultured and stored easily.

produces hybridoma cells. These cells contain abilities from both sources, i.e. they produce the relevant antibody, they secrete it into solution, and they are immortal and easily grown in suspension tissue cultures, thus constituting a reliable source of antibody containing solutions.[134]

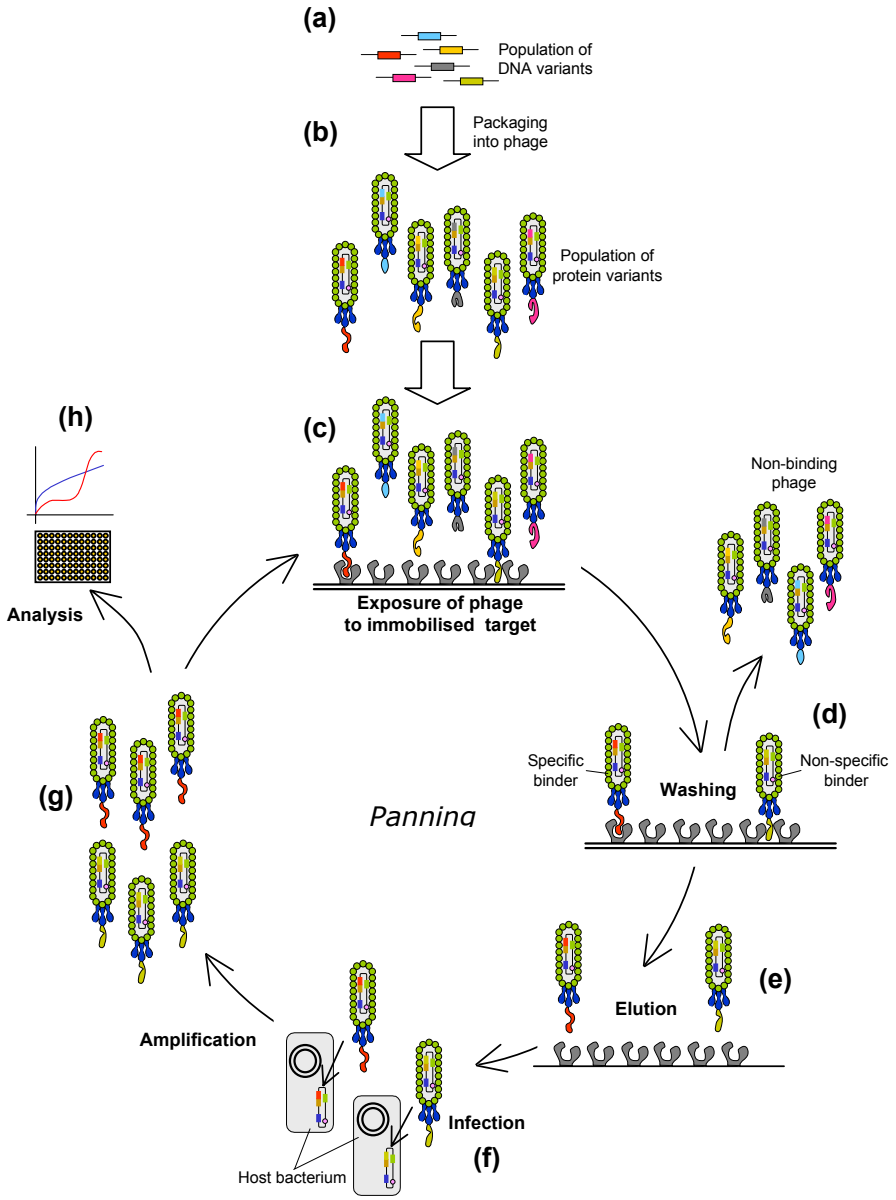


Figure 18

Phage display technology relies on the fusion of DNA that encodes phage[135] coat proteins to the genetic sequence encoding a desired protein - including

[134] For an introduction to antibody technology, see: Liddell, E.; Weeks, I. *Antibody Technology*, BIOS Scientific Publishers, Oxford, **1995.**

[135] Phage are bacterial vira, i.e. organisms containing genetic material, but relying on the infection of a bacterial host for proliferation.

antibody binding domains. This achieves the expression of the protein as an extension of the coat protein, exposing it to the surrounding environment. In a library of phage expressing a range of proteins, those containing the desired binding properties toward a given epitope can be selected and amplified in a process known as *panning* (Figure 18c-h). This eventually provides a monoclonal phage population, i.e. a solution of phage expressing a single protein. This phage solution can then be used directly as antibody, or the binding protein can be harvested and applied instead. Furthermore, the protein sequence can be easily obtained by sequencing of the encoding DNA.[136]

Characterization of the antibody involves establishing the epitope it recognizes. Ideally, this includes knowledge of the precise chemical structure of the epitope as well as the three-dimensional arrangement of the epitope. Often antibodies are applied with great success, although their epitopes are not this rigidly characterized. In order to pin down the exact nature of the epitope, a range of suitable potential haptens must be available and screened for antibody binding. In case of large antigens, this can be done using immuno-dot assays (IDAs)[137] or enzyme-linked immuno-sorbent assays (ELISAs).[138] When dealing with small hapten molecules, competitive inhibition ELISAs (ciELISAs)[139] are more convenient, because the antigens do not need immobilization.

Naturally, these same techniques used for the characterization of the antibody are also useful for the application of the antibody in probing the presence of the epitope in unknown samples. In addition to these methods, the *in planta* study of the occurrence of the epitope is made possible using immunomicroscopy of plant tissue. This tissue can be whole cells, protoplasts, or tissue sections. The latter can either be made by hand sectioning or by embedment in a wax or resin matrix, followed by cutting in ultrathin sections using an ultramicrotome. Immunofluorescence microscopy employs visualization of antibody binding by a conjugated fluorophore, visible in a light microscope (*vide infra*). Immunogold microscopy is visualized in a transmission electron microscope *via* conjugated gold particles.[140]

---

[136] For a review of phage-display antibodies, see: Winther, G.; Griffiths, A. D.; Hawkins, R. E.; Hoogenboom, H. R. *Ann. Rev. Immunol.* **1994**, *12*, 433.

[137] In an IDA experiment, the antigens are immobilized on a nitrocellulose sheet, followed by treatment with the antibody to be studied.

[138] In an ELISA experiment, the antigens are immobilized in the wells of a microtiter plate, followed by treatment with the antibody to be studied. Eventually, the results can be conveniently read using a suitable colorimetric response.

[139] ciELISA is grounded on the ability of a soluble hapten to compete with an immobilized antigen for antibody binding.

[140] For a discussion of the above mentioned techniques, as well as detailed protocols, see: Willats, W. G. T.; Steele-King, C. S.; Marcus, S. E.; Knox, J. P. *Antibody Techniques, in Molecular Plant Biology Volume Two: A Practical Approach*, Gilmartin, P.; Bowler, C. (Eds.), Oxford University Press, Oxford, **2002**, pp. 199-219.

In all the above-mentioned techniques, it is necessary to detect the binding, i.e. establish the presence of an antibody-antigen complex. Unless an antibody directly linked to a desired marker is available, this is done with so-called *indirect detection*. Here, the relevant material (whether a nitrocellulose sheet, an ELISA plate, or a plant section) is exposed to the primary antibody (1° AB), i.e. the antibody recognizing the epitope under investigation, for a given period and then washed to remove unbound antibody. It is then probed with a secondary antibody (2° AB), conjugated to a relevant detectable component. The 2° AB is often a commercially available antibody, binding strongly to hybridoma cells of e.g. rat origin (in case of a 1° AB made in rats). In the case of IDAs and ELISAs, the 2° AB is conjugated to for example the enzyme horseradish peroxidase. This enzyme catalyzes the conversion of an appropriate colorless substrate to a colored product, thus enabling detection of the antigen-1° AB-2° AB complex formed during incubation. When using immunofluorescence microscopy, the 2° AB is conjugated to a fluorophore, e.g. fluorescein isothiocyanate (FITC), emitting green light (515 nm) upon excitation at 495 nm.
In the following, two applications of these techniques will be described: epitope mapping of known anti-HG antibodies and the characterization of a novel antibody raised in rats.

Epitope Mapping of Antibodies LM7, JIM5, and JIM7

The epitopes of the three anti-HG antibodies LM7, JIM5, and JIM7 have previously been studied using a combination of engineered pectic polymers and the effect of pectin degrading enzymes on the antibody binding.[9, 16] In all cases, the results showed that the three antibodies bound to partially methyl esterified HG. The model pectins applied in these investigations contained HG with varying degrees of methyl esterification. They were produced by treating a lime pectin with DM of 81% with either a plant pectin methyl esterase (P series), a fungal pectin methyl esterase (F series), or subjecting it to a basic solution for a given period (B series). All model pectins mentioned in this chapter are summarized in Table 8.[141] In the previous epitope mapping studies of LM7, JIM5, and JIM7 optimal binding of these monoclonal antibodies was obtained with different model pectin samples, indicating that they had different epitopes. It was therefore of great interest to investigate their binding to the five synthetic hexagalacturonates with varying patterns of methyl esterification (**94**-**98**, Chapter 4).

[141] For details on the model pectins, see: a) Ref. 14; b) Limberg, G.; Körner, R.; Buchholt, H. C.; Christensen, T. M. I. E.; Roepstorff, P.; Mikkelsen, J. D. *Carbohydr. Res.* **2000**, *327*, 321.

Table 8

Model Pectin	Esterification[a]	Acetylation[a]	Amidation[a]	Origin	Esterification pattern[b]
E81	81	-	-	Lime	Native
P53	53	-	-	Lime	Blockwise
F43	43	-	-	Lime	Non-blockwise
F31	31	-	-	Lime	Non-blockwise
B34	34	-	-	Lime	Non-blockwise
A4322	43	-	22	Lime	Non-blockwise
SBP6230	62	30	-	Sugar Beet	Native
P3429	34	29	-	Sugar Beet	Blockwise
F3331	33	31	-	Sugar Beet	Non-blockwise
B0100	1	0	-	Sugar Beet	Non-blockwise

[a] In % [b] Given as either *native*, i.e. as isolated from the plant, *blockwise*, i.e. after treatment with a plant pectin methyl esterase, or *non-blockwise*, i.e. after treatment with either a fungal pectin methyl esterase or by chemical deesterification.

To this end, a model pectin (F31, see Table 8) containing an epitope of all three antibodies was selected as immobilized antigen for competitive inhibition ELISAs. The titer of the individual antibody solutions towards F31 binding were determined using ELISA. The concentration of antibody resulting in 90% binding to the immobilized antigen F31 was identified for LM7, JIM5, and JIM7, as well as LM5 used as a negative control (results not shown). At 90% binding concentration, the sensitivity of the ciELISAs is optimized, because a small change of binding efficiency will give rise to a large change in the signal. These antibody concentrations were then used in a series of ciELISAs with **94**-**98** and appropriate controls as the soluble hapten competitors. The results are shown in Figure 19 and Table 9. The IC_{50} values of all samples were determined up to a concentration of 1000 $\mu g \cdot mL^{-1}$ and are shown in the table together with a graphic representation of the methylation patterns of **94**-**98**. As expected, the results shown in Figure 19a confirmed that none of the synthetic hexagalacturonates contained an epitope for LM5. For the F31 control, a decrease in signal was observed with increase of competitor concentration. This indicated that the soluble hapten (F31 itself in this case) was effectively competing for antibody binding, leaving less antibody bound to the immobilized antigen. In the end, this was seen as a lowering of the absorption, because the antigen-1° AB-2° AB complex was absent from the plates during visualization. That the antibody bound to the F31 sample may seem surprising, but in all of the model pectins, sidechains of e.g. galactan from RG-1 were present.

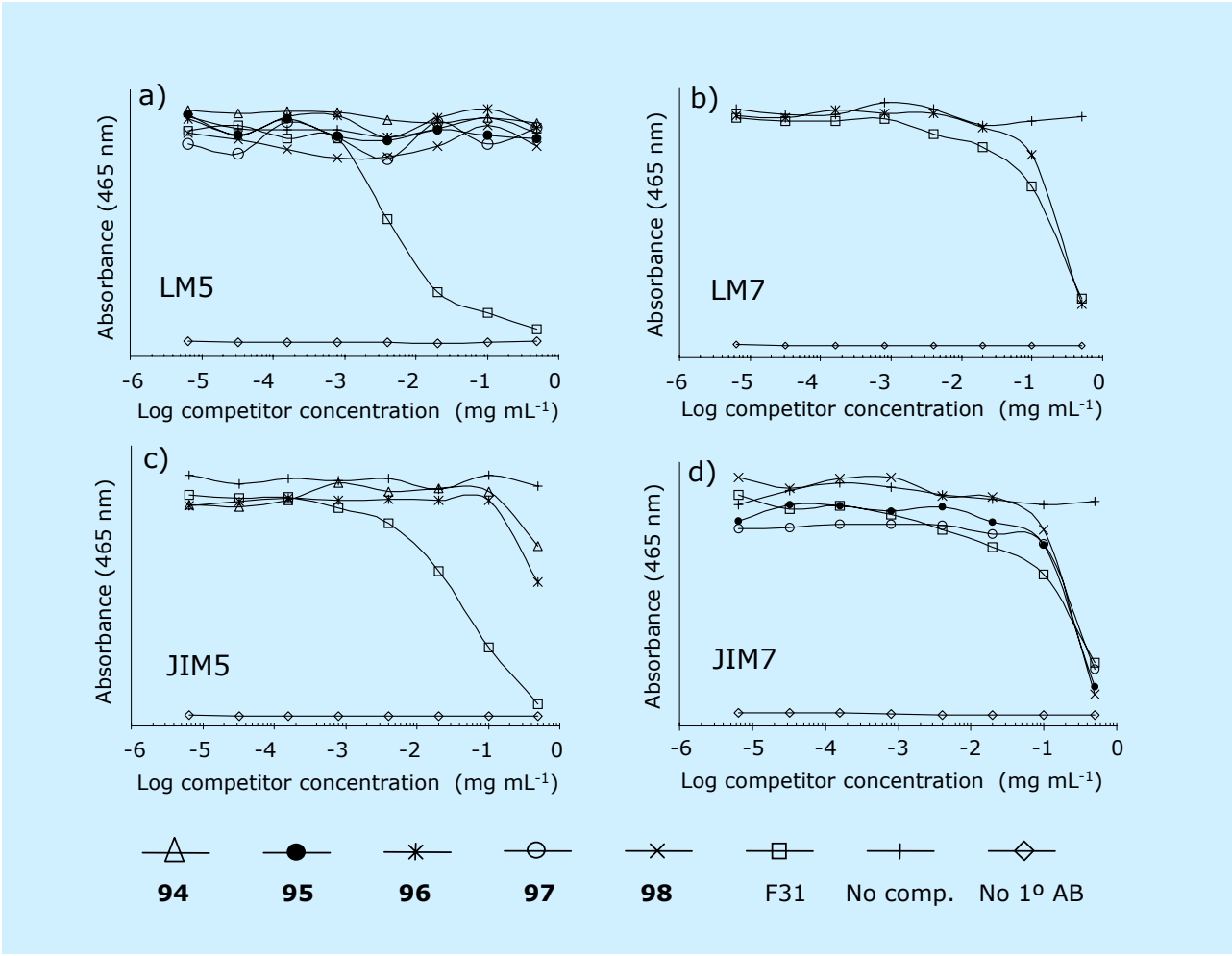


Figure 19

LM7 only bound to one of the hexasaccharides, namely **96** (Figure 19b). This is in good agreement with what has been observed earlier that the LM7 epitope is less abundant in plants than JIM5 and JIM7. The fact that LM7 bound to **96**, but not **94** indicated that the optimal epitope contains at least 4 contiguous galacturonic acid residues, flanked by one or more methyl esterified residues.
JIM5 bound to both **94** and **96**, although relatively weakly (Figure 19c). Previously, similar binding by JIM5 has been observed with unesterified oligomers with DP 7-9.[16] This implies that the epitopes presented by **94** and **96** are at best sub-optimal. The fact that binding was also obtained with unesterified oligomers suggests that the optimal epitope may contain a long stretch of unesterified residues, perhaps as much as 6 to 8, flanked or interrupted by a single or two methyl esterified residues. This would explain the binding to **94** and **96**, in that they represented the esterified part of the epitope, while the galacturonic acid oligomers represented the unesterified part, but none of them offered optimal binding conditions.

Table 9

Entry	Compound	LM7[a]	JIM5[a]	JIM7[a]	Structure[b]
1	**94**	>1000	900	>1000	
2	**95**	>1000	>1000	200	
3	**96**	220	600	>1000	
4	**97**	>1000	>1000	230	
5	**98**	>1000	>1000	260	

[a] The values shown are IC_{50}s, i.e. the concentration of inhibitor where 50% of the binding to the immobilized antigen F31 was lost. [b] An open circle represents a galacturonic acid residue, a closed circle represents a methyl group (see also Figure 12, Chapter 4).

JIM7 binding quite remarkably was inhibited by three of the synthetic hexagalacturonates, **95**, **97**, and **98** (Figure 19d), at comparable levels (Table 9). The three compounds does not have a unique methyl ester pattern in common – however, if one considers the possibility that one of the residues is not participating in binding, two possible epitopes (or sub-epitopes) can be identified, namely Me-?-Me-H and H-Me-?-Me. This could be a consequence of the folding of the oligomer, exposing some residues to binding, while the location of others preclude them from recognition by the protein, thus making the presence of an acid or ester irrelevant to binding. Again, this is in good agreement with previous findings that a certain level of methyl esterification is a prerequisite for JIM7 binding to HG samples.[16]

The fact that none of the synthetic oligomers were better inhibitors than the control sample F31 implies that none of the compounds contained a complete epitope for any of the antibodies. The number of monomeric galacturonic acid residues present in solution was approximately the same for the hexasaccharides and the polymer. Hence, if the full epitopes were contained within the six residues of **94**-**98**, comparable inhibition by the polymer would only be obtained if it were composed entirely of repeating epitopes, which is obviously not the case. However, another plausible explanation for the relatively lower inhibition levels observed for **94**-**98** could be found in the three-dimensional presentation of the individual binding elements. Given that the folding of the HG chain is important to the recognition by the antibodies, it is reasonable to assume that the folding of the polymeric sample F31 will induce a higher resemblance to the epitopes present in the polymeric antigens used to raise the antibodies.

In conclusion, this study has added significantly to the knowledge of the epitope structures of the three widely applied HG antibodies LM7, JIM5, and

JIM7. Furthermore, this is the first example of synthetic HG oligomers used as haptens for the study and characterization of antibodies.

Characterization of the Novel Antibody 5C4

In an attempt to produce an antibody recognizing three contiguous methyl esterified residues of HG, rats were immunized with a synthetic trisaccharide conjugated to BSA (**152**, Chapter 5). In the initial screening for antibodies in the polyclonal hybridoma population produced from the spleen cells of the immunized rats, binding to the model pectin P41 was observed. However, upon further cloning towards a monoclonal antibody, this binding was lost.[142]

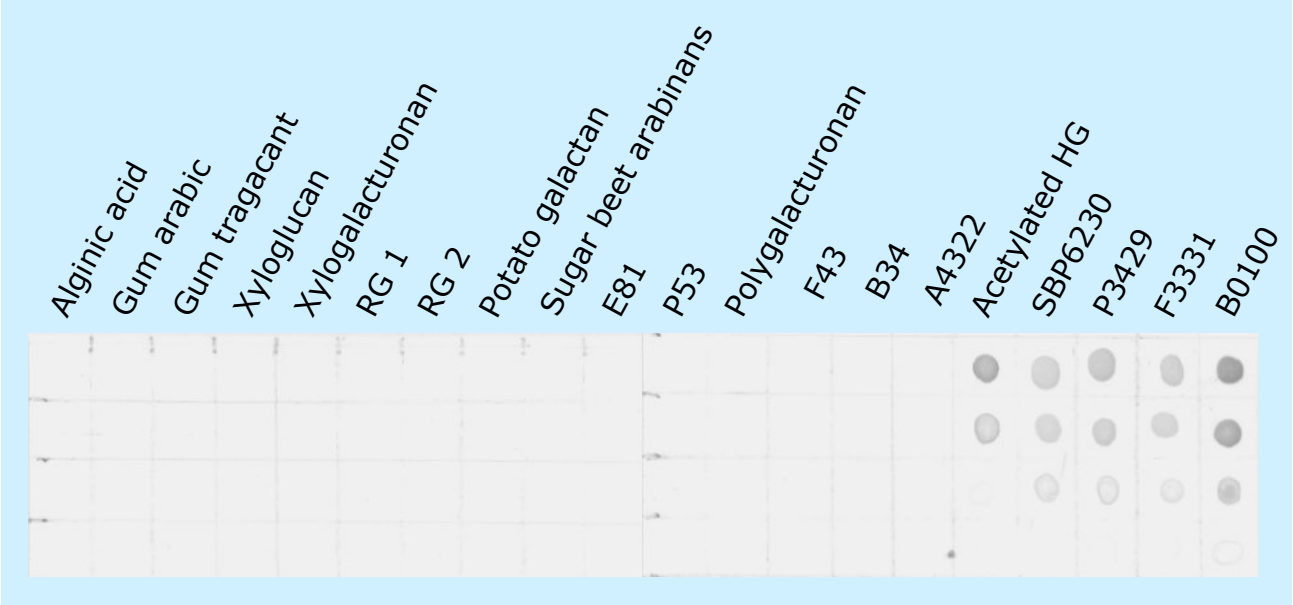


Figure 20

With the purpose of characterizing the antibody 5C4, IDAs were performed with a range of plant polymer samples (Figure 20). As can be seen from the figure, no binding was observed for a number of plant polymers, including model lime pectins with a degree of methylation ranging from 81 to 0%. The only samples, which the antibody bound to in this initial screening, were sugar beet pectins. Very weak binding was also obtained with a sample consisting primarily of arabinans from sugar beet, although not visible in Figure 20. This binding was confirmed by ELISA. Sugar beet HG is known to contain acetyl groups at *O*-2 and *O*-3 of the galacturonic acid residues.[143] Initially, the epitope of 5C4 was thought to be acetylated HG. However, even though the antibody bound to both native sugar beet samples (Acetylated HG and

[142] Susan E. Marcus, J. Paul Knox, *unpublished results*.
[143] Rombouts, F. M.; Thibault, J.-F. *in Chemistry and Function of Pectins, ACS Symposium Series 310*, Fishman, M. L.; Jen, J. J. (Eds.), American Chemical Society, Washinton DC, **1986**, pp. 49-60.

SBP6230, Figure 20) and samples with a lowered DM (P3429 and F3331, Figure 20), it also bound to a sample containing no acetyl groups (B0100, Figure 20). In order to confirm that binding was not related to acetylation, ciELISAs were performed with two additional samples, a sugar beet pectin treated vigorously with base to remove acetyl and methyl ester groups and a lime pectin treated with pectin acetyl transferase to introduce acetyl groups.[144] In both cases, no binding by 5C4 was detected (results not shown), confirming that acetylated HG was not part of the epitope. A ciELISA was also performed with the original antigen used for immunizing the rats, **152**, together with the other two neoglycoconjugates **153** and **154** (cf. Chapter 5). All three samples failed to inhibit binding of 5C4 (data not shown). This validated that either the antigen had been altered *in vivo* to resemble a new epitope, or the antibody was already present in the immune system of the rats prior to immunization. The latter possibly because the epitope could have been present in their food.

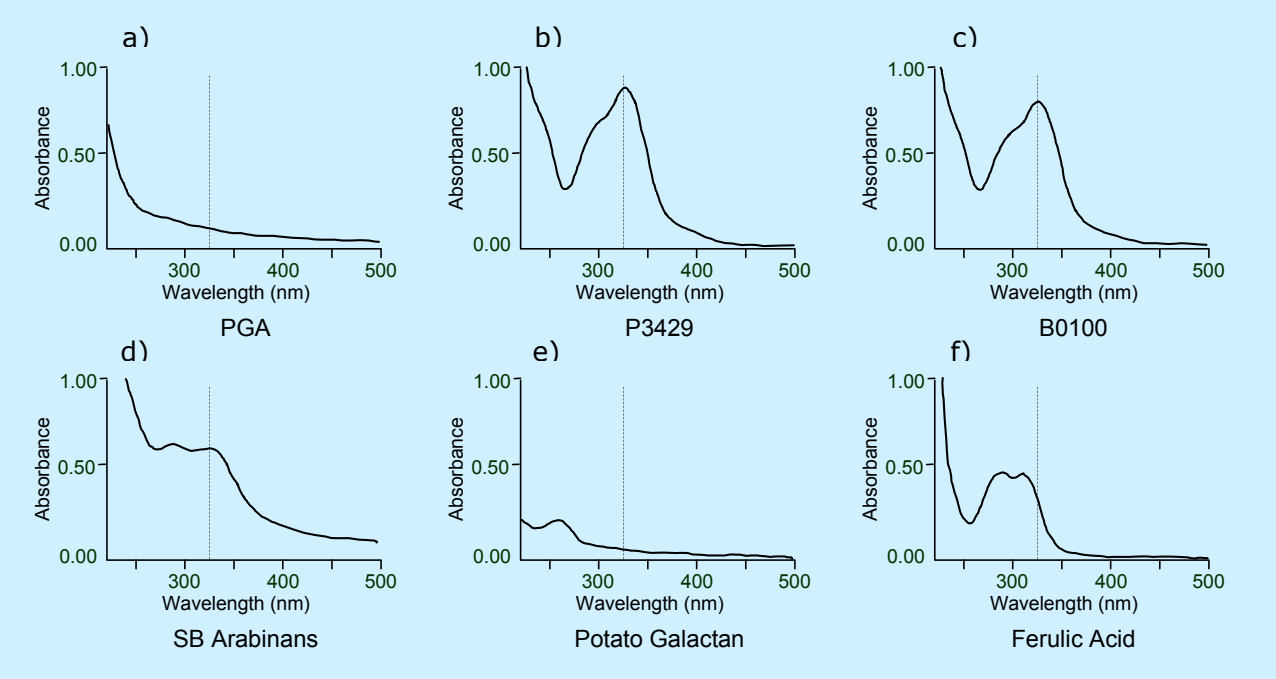


Figure 21

Instead, focus was put on identifying an entity present in sugar beet pectin but absent from lime pectin. It is known that sugar beet and similar plants contain ferulic esters (4-hydroxy-3-methoxy-*E*-cinnamates) attached to the galactan and arabinan side-chains of RG-1.[145] Ferulates have a distinct UV absorption at

[144] Thanks to Dr. Hans Christian Buchholt, Danisco, Copenhagen, for providing these two samples.
[145] Ralet, M.-C.; Thibault, J.-F.; Faulds, C. B.; Williamson, G. *Carbohydr. Res.* **1994**, *263*, 227.

324 nm, making them easy to detect by spectrofotometry.[143] Figure 21 shows the UV spectra of a number of model pectins as well as ferulic acid itself. Encouragingly, all the samples, which were positive for 5C4 binding in the IDAs, absorbed at 324 nm (Figure 21b-d), whereas the samples missing an epitope for the antibody showed no absorption in this region (Figure 21a, e). Ferulic acid (Figure 21f) absorbed at a slightly lower wavelength than the ferulates (310 nm).

This data strongly hinted at a ferulated epitope. Fortunately, a number of ferulated mono-, di-, tri-, and tetrasaccharides isolated from plants were available for screening.[146] The structure of ferulic acid (**155**), ethyl ferulate (**156**), and the ferulated glycans **157**-**161** are given in Figure 22, together with the structure of the digalactan **162** used as a control (*vide infra*). The saccharides **157** and **159** were both isolated from sugar beet, **158** was of maize origin, **160** and **161** stemmed from wheat, and **162** was a commercial sample.

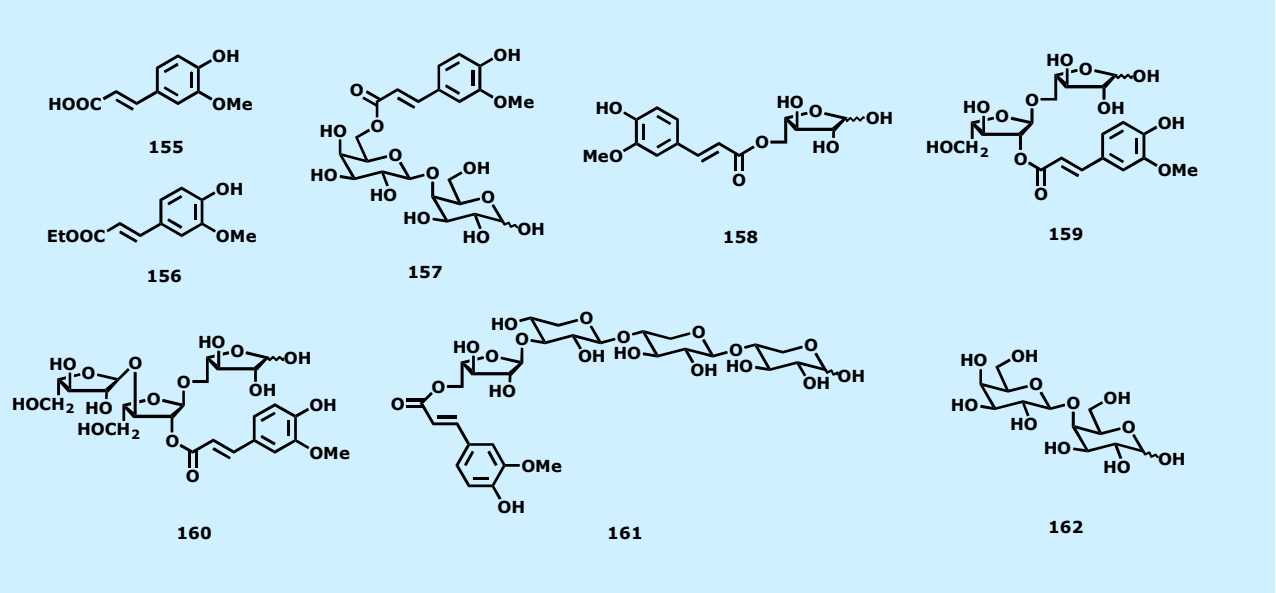


Figure 22

[146] Dr. Marie-Christine Ralet, Institut National de la Recherche Agronomique, Nantes, France is gratefully acknowledged for providing samples **157**-**161**. See also: Colquhoun, I. J.; Ralet, M.-C.; Thibault, J.-F.; Faulds, C. B.; Williamson, G. *Carbohydr. Res.* **1994**, *263*, 243.

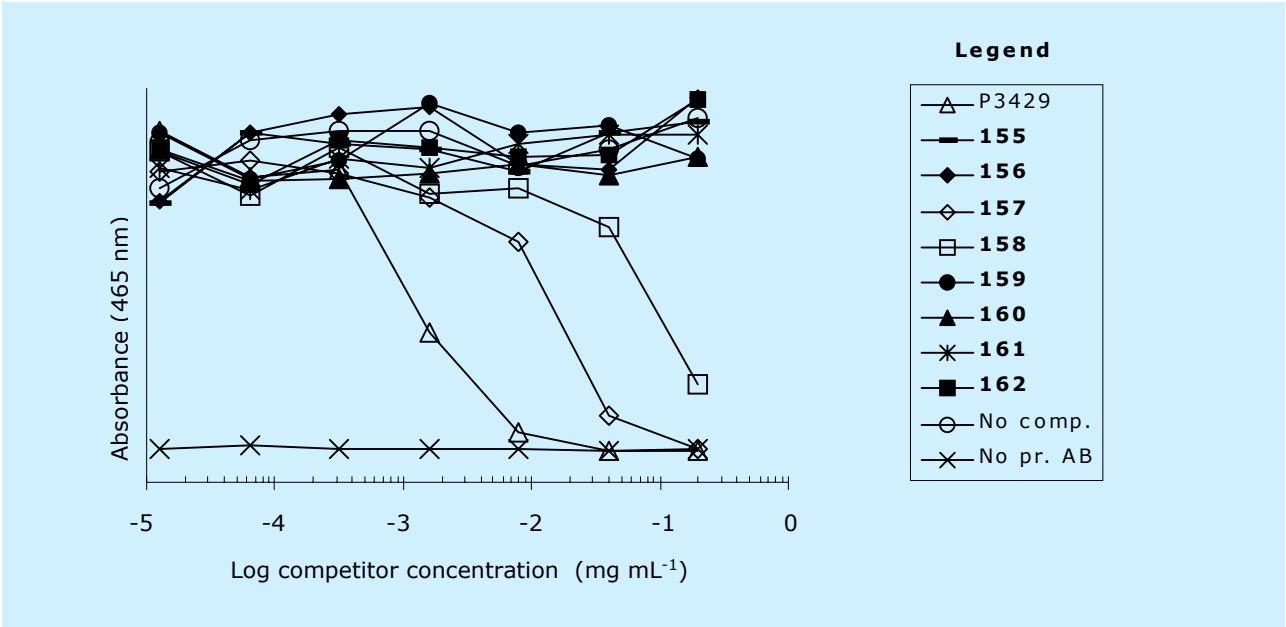
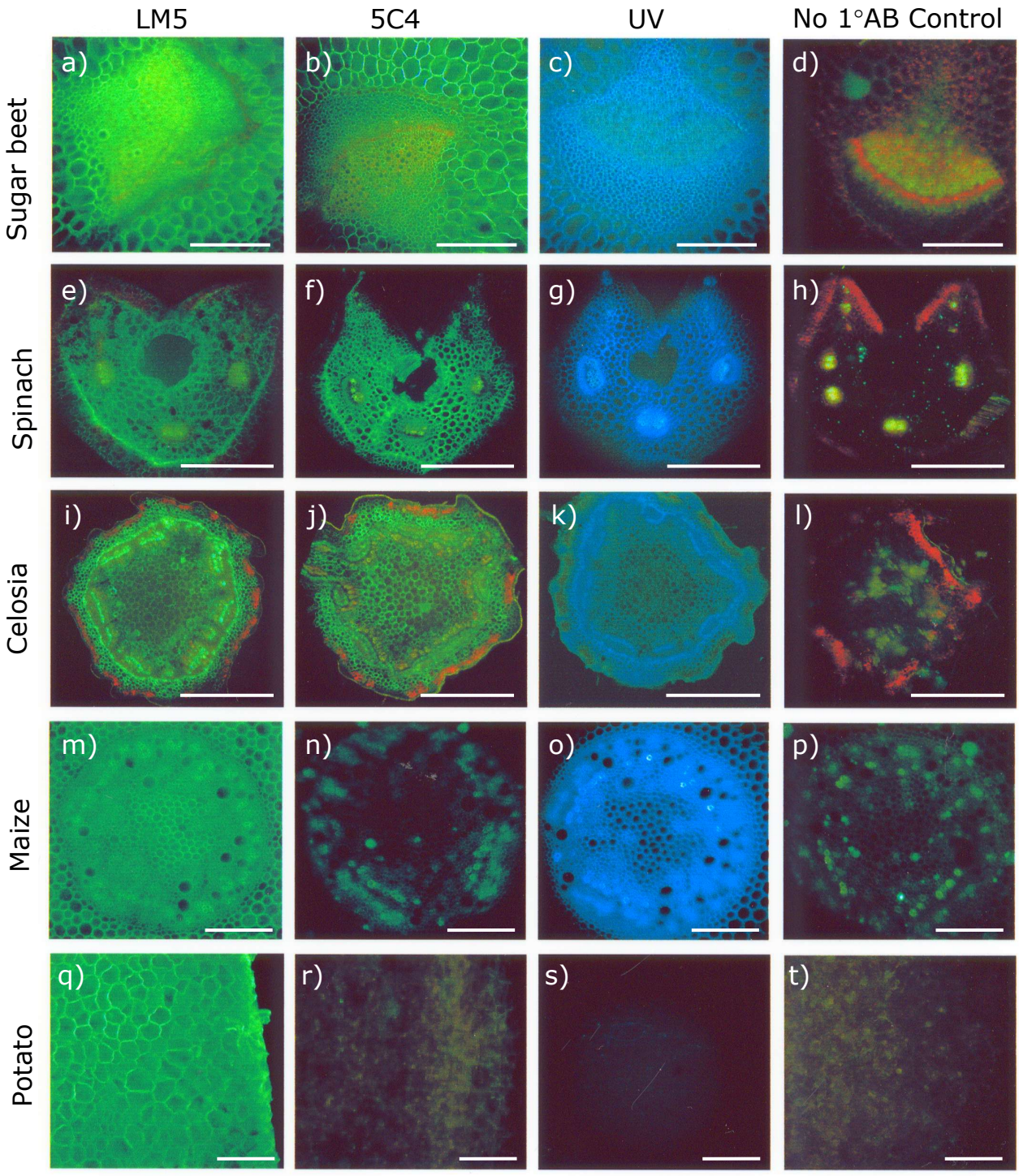


Figure 23

The binding of 5C4 to the samples in Figure 22 were explored in a series of ciELISAs employing the model sugar beet pectin P3429 as the immobilized antigen, see Figure 23. It was evident from the ciELISA data that **157** was an inhibitor of 5C4 binding to the immobilized antigen P3429. A significant, but weaker, inhibition was displayed by sample **158**, ferulated arabinose. None of the other ferulated glycans were able to compete with P3429 for antibody binding, and neither were the simple ferulates **155** and **156**. That no binding was observed to the non-ferulated digalactan **162** emphasized that the epitope of 5C4 was a ferulated galactan. Arabinans isolated from sugar beet are ferulated in the 2-position, and no binding was detected for the two representative samples **159** and **160**. The fact that some inhibition was seen for the 5-*O*-ferulated arabinose from maize (**158**) did not weaken the evidence for ferulated galactan as the epitope. Rather, this sample constituted a sub-optimal epitope due to its resemblance to **157** - both compounds were esterified at their primary alcohol. Evidence to support this observation came from the inability of 5C4 to bind to maize tissue (*vide infra*).

At this point, it was crucial to determine whether the antibody would bind to actual plant tissue. All observations made so far indicated that 5C4 would bind to plant tissue containing ferulated galactans, but nonetheless absence of binding to plant tissue would severely limit the applicability of the antibody. Figure 24 shows immunofluorescence microscopy of hand sectioned plant material employing LM5 (binding to β-(1→4)-linked galactans) and 5C4, as well as the UV signal of similar sections and sections subjected only to the 2° AB (a negative control). UV fluorescence established the presence or absence of

ferulates in the plant tissue and has been widely used to determine ferulation in plants. First, there was no doubt that 5C4 actually bound to plant tissue.



Scale bars. a-d: 0.5 mm, e-l: 1 mm, m-t: 0.25 mm

Figure 24

Comparing e.g. sugar beet tissue treated with 5C4 (b) with the no 1° AB control (d) (signal amplified for clarity), the green fluorescence of FITC was easily distinguishable from the background autofluorescence of the control. Binding of 5C4 was detected in sugar beet (b), spinach (f), and celosia (j), three plants that are closely related taxonomically, and known to contain ferulates – this was confirmed by the UV fluorescence seen in (c), (g), and (k), respectively. Esters of ferulic acid are also abundant in maize (o), but 5C4 did not bind to maize sections (compare (n) and the control (p)). This was not due to the absence of galactan from maize tissue, confirmed by the strong binding of LM5 seen in (m). Rather, it was an indication that ferulates are present on other glycans, e.g. arabinoxylans. Galactan is known to be abundant in potatoes and accordingly, the LM5-signal was intense in potato sections (q). Ferulates, however, have never been reported, and this was verified by the absence of a signal from both 5C4 (r) and UV fluorescence (s).

Having established that 5C4 binds to plant tissue with a high affinity and selectivity, it only remains to identify the immunoglobulin subtype and assure that the antibody is monoclonal. This work is currently in progress, and all results concerning 5C4 will be reported in due course.

In conclusion, the characterization and application of the novel anti-pectin antibody 5C4 has added to the arsenal of molecular probes used to study plant cell wall composition. One question remains, however: can neoglycoconjugates be trusted to provide antibodies recognizing a desired pectic epitope? The present work indicates that this is not the case – but the presence of a strong immunogen in the form of ferulated galactans may have led to the loss of an antibody having the initially desired binding properties. Further investigations may enable the isolation of such an antibody.

7. Conclusion

The aim of the project was to develop reliable and efficient methods for synthesizing defined fragments of pectic polymers. A method for preparation of selectively methylated oligogalacturonates is the major outcome of the project. The strategy utilizes *n*-pentenyl galactosides as glycosyl donors and relies on assembly of the oligomers as suitably protected galactosides and subsequent oxidation of the 6-position to yield galacturonates. The synthesis of three monomethylated trigalacturonates (**16**-**18**) exemplified the feasibility of the protocols (Chapter 2).

The study of pectic enzymes was one of the main applications of the synthetic pectic oligosaccharides prepared in this project. To make sure that the sugars would be substrates for the enzymes, i.e. that good enzymatic activity could be obtained, it was decided to synthesize selectively methylated hexagalacturonates. In order to facilitate the assembly of these larger sugars, a convergent approach was sought. The use of disaccharide glycosyl donors in the coupling reactions was believed to be a good way to achieve this goal. Various routes to *n*-pentenyl digalactosides were investigated (Chapter 3). A new protocol for direct conversion of *n*-pentenyl donors to glycosyl fluorides was developed, and one of the resulting fluorides was used in a Mukaiyama-type coupling to a glycosyl acceptor bearing a pentenyl group at the anomeric position. This resulted in one of the desired disaccharide donors. The conversion to glycosyl bromides and subsequent glycosylation under Koenigs-Knorr conditions proved a good alternative, resulting in three key building blocks for further work (**70**, **72**, and **73**). In order to make a fourth building block, a novel application of the *armed-disarmed* glycosylation method was used. The method relies on disarming the glycosyl acceptor with a strongly electron withdrawing protecting group, the pentafluorobenzoyl group, in the 6-position. This is the first application of this protection group in carbohydrate chemistry and the strategy resulted in the *n*-pentenyl disaccharide donor **89**.

With these protected digalactoside donors in hand, the synthesis of selectively methylated hexagalacturonates were explored. Five such sugars (**94**-**98**) were selected as targets - with methylation patterns that would facilitate enzymatic studies. Their preparation is described in Chapter 4 and in all cases the assembly of the hexagalactosides went smoothly, with good to excellent yields in the coupling reactions and high selectivity for the desired α-linked products. These were then elaborated to the target molecules *via* oxidations and esterifications. The yields of the individual steps were moderate in all cases, but considering the number of transformations involved in each step, and the nature of the substrates, the results were satisfactory. Proof of concept for the

significance of the synthetic pectin fragments was obtained by researchers at Danisco in Copenhagen. They used **94**-**98** as substrates for two pectic enzymes and were able to detect enzymatic activity with two of these, in compliance with previous findings about the substrate specificity of the enzymes. Further studies are currently being undertaken.
In collaboration with researchers at Leeds University in Great Britain, it was decided to investigate the possibility of obtaining monoclonal antibodies against specific homogalacturonan structures by using synthetic carbohydrate antigens. Three such sugars were made and coupled to bovine serum albumin, giving neoglycoconjugates **152**-**154** (Chapter 5). The preparation of the precursors for these compounds were made according to the methods used earlier, but limiting the structures to either fully methylated or unmethylated oligomers. Furthermore, in these syntheses the base sensitivity of the substrates was demonstrated when β-elimination occurred in a key step. This highlighted the importance of employing neutral or weakly acidic conditions when working with oligogalacturonates.
The result of the immunization of rats with **152** was somewhat surprising, but interesting nonetheless (Chapter 6). A novel antibody resulted from these studies - but not with the anticipated homogalacturonan epitope. Instead, the epitope was characterized as β-(1→4)-linked galactan functionalized with an ester of ferulic acid. Nevertheless, this turned out to be a very useful antibody and taxonomical investigations were initiated. Additionally, another application of the synthetic hexasaccharides **94**-**98** was found. Three known anti-homogalacturonan antibodies were tested for binding to these compounds, and all of them displayed binding. The fact that they bound to different hexagalacturonate samples gave new information about their epitopes (Chapter 6).

In conclusion, the project has been very rewarding. Methods for synthesizing selectively methylated oligogalacturonates have been developed and exemplified by the preparation of tri- and hexasaccharides. Additionally, two successful applications of these molecules have been demonstrated. They have proved useful as substrates for pectic enzymes, giving hope that they can aid in obtaining more detailed information about the substrate specificity of such enzymes. Secondly, they have added to the current knowledge of anti-pectin antibodies by narrowing the range of possible epitopes of three such antibodies.
The project has shown the value of synthetically prepared pectic oligomers in various aspects of pectin research – hopefully the future will bring more examples of such gratifying interactions between chemistry and biology to this field.

8 EXPERIMENTAL

General and Specific Procedures and Compound Data

Notes on format

General procedures are given in most cases. References to these are given under the relevant compounds. Compounds are listed in order of appearance in the preceding chapters. Methods used for the work described in Chapter 6 are given in Materials and Methods.

General

Optical rotations were determined with a Perkin-Elmer 241 polarimeter.
NMR spectra were recorded using a Varian Mercury 300 spectrometer or a Varian Unity Inova 500 spectrometer. Chemical shifts were measured in ppm and coupling constants in Hz, and the scanning frequency is indicated in each case.
MALDI-TOF mass spectra were obtained at Danisco, Copenhagen, Denmark using a Perseptive Biosystems Voyager-De instrument in positive-ion mode with α-cyano-4-hydroxycinnamic acid as the matrix.
ESI mass spectra were obtained at University of Southern Denmark, Odense, Denmark using an Esquire-LC instrument, operating in either positive or negative mode.
ESI HRMS spectra were obtained at University of Copenhagen, Denmark.
TLC was performed on aluminum sheets precoated with silica gel (Merck 1.05554). Compounds were visualized by charring after dipping in a solution of cerium(IV) sulfate (2.5 g) and ammonium molybdate (6.25 g) in 10% aq. H_2SO_4 (250 mL). Flash column chromatography was performed using silica gel 60 (Amicon 85040). Microanalyses were obtained from the Department of Chemistry, University of Copenhagen, Denmark or the Department of Physical Chemistry, University of Vienna, Austria.

Materials and Methods

Antibodies
Monoclonal antibodies LM5, LM7, JIM5, and JIM7 were described earlier (see Chapter 6), and the hybridoma supernatants were used as described. 5C4 was generated using hybridoma technology subsequent to the immunization of a group of rats with neoglycoconjugate **152**: Three male Wistar rats were immunized subcutaneously with 100 μL of an emulsion of **152** at 2 mg/mL in phosphate buffered saline (PBS)[147] and an equal volume of Freund's complete adjuvant on day 0. On days 51 and 79 the injections were repeated using Freund's incomplete adjuvant. Tail bleeds were taken on day 61 and 90, serum was prepared and the immune response assessed. The rat with the best response was selected and given a pre-fusion intraperitoneal boost of 100 μg **152** in PBS on day 328 and the spleen was removed on day 331. Lymphocytes were isolated and fused with the IR983F rat myeloma cell line[148] using standard hybridoma preparation and limiting dilution cloning procedures.[149] Hybridoma supernatants were screened for the presence of anti-pectin antibodies in ELISAs using model pectin P41 as antigen. A number of positive antibody-secreting cell lines were identified. After further cloning however, this binding was lost. Further screening using IDAs revealed one cell line (5C4) with binding to sugar beet pectins, and this was selected for characterization and use in further studies.

---

[147] PBS: 8 g NaCl, 2.86 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KCl, 0.2 g KH_2PO_4 in 1L H_2O, pH 7.2.
[148] Bazin, H. *Prot. Biol. Fluids* **1982**, *29*, 615.
[149] Liddel, J. E.; Cryer, A. *A Practical Guide to Monoclonal Antibodies*, John Wiley & Sons, New York, **1991**.

Immuno-Dot Assays
The samples were applied to a nitrocellulose sheet in 5-fold dilution series starting with 1 μg (1 μL of a 1 mg/mL solution) per dot. PBS containing 5% milk powder (MP/PBS) were used to block the nitrocellulose sheet for 1 h. The primary antibody was applied as a 5-fold dilution of hybridoma supernatant in MP/PBS for 1.5 h. The sheet was then washed with water and the secondary antibody (rabbit anti-rat IgG coupled to horseradish peroxidase (anti-rat-IgG-HRP), from Sigma), diluted 1000-fold in MP/PBS, was added for 1.5 h. The sheets were then washed thoroughly and antibody binding was detected by addition of a chloronapthol based substrate and H_2O_2.

ELISAs
ELISAs were performed in 96-well microtiter plates (Maxisorb, NUNC) coated with 100 μL per well of 50 μg/mL of antigen in PBS either 2 h at 20 °C or 12-96 h at 4 °C. Unbound antigen was washed out with water and 200 μL of a blocking solution of 3% bovine serum albumin in PBS (BSA/PBS) was applied for 2 h. After washing 100 μL per well of hybridoma supernatant was added as a 5-fold dilution series in BSA/PBS. After 1.5 h, washing was repeated and 100 μL per well of anti-rat-IgG-HRP was added. After 1.5 h, the plates were washed thoroughly and antibody binding was detected by addition of 150 μL per well of HRP substrate (0.1 M sodium acetate buffer pH 6, 1% tetramethyl benzidine, 0.006% H_2O_2). The reaction was stopped by the addition of 35 mL per well of 2 M H_2SO_4 and the absorbance was read at 465 nm on a microtiter plate reader. All data reported were done at least in triplicates.

ciELISAs
Competitive inhibition ELISAs were performed by serially diluting haptens 5-fold in BSA/PBS in a microtiter plate coated with an appropriate antigen (50 μL per well) and adding primary antibody (50 μL per well, diluted in BSA/PBS to 90% of maximum binding concentration as determined by ELISAs). All other steps were carried out as described above. Concentrations of haptens resulting in 50% inhibition of antibody binding (IC50s) were obtained by plotting absorbance against inhibitor concentration. Values from controls with no added inhibitor were taken as 0% inhibition and values from controls with no added primary antibody was taken as 100% inhibition.

Immunolabeling of Plant Material
Sugar beets, spinach, celosia, and potato were obtained from commercial vendors. Maize seeds were imbibed overnight in tap water and allowed to grow roots for 3 days in the dark. Sections of stem, petiole, midrib, tuber, or root were made by hand to a thickness of approximately 100-300 μm. Sections were placed immediately in a fixative containing 4% paraformaldehyde. Following 1 h of fixation, sections were washed with PBS (x3) and then incubated for 1 h in primary antibody diluted 2-fold in MP/PBS. Sections were washed with PBS (x3) and then incubated with secondary antibody (rabbit anti-rat IgG coupled to fluorescein isothiocyanate, from Sigma) diluted 100-fold in MP/PBS in the dark for 1 h. Sections were then washed with PBS (x3) in the dark, mounted onto microscopy slides in an anti-fade agent (Citifluor, Agar Scientific) and examined on a microscope equipped with epifluorescence illumination (Olympus BH-2).

General Procedures

General Procedure for Acylation Reactions - A
The diol **28** (4.28 g, 10.0 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL). The solution was cooled to 0 °C, Et_3N (2.23 mL, 16.0 mmol) and the acyl anhydride or chloride (11 mmol) was added. The reaction mixture was stirred until TLC revealed full conversion (0.5-2 h). Silica gel (10 g) was added and the reaction mixture was concentrated and purified by flash chromatography.

General Procedure for Glycosylation Reactions using Pentenyl Glycosides - B
A mixture of the donor (6.5 mmol) and the acceptor (5.0 mmol) was dried azeotropically with toluene (x2) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous 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 solution was diluted with CH_2Cl_2 (60 mL) and washed with 10% aq. $Na_2S_2O_3$ (100 mL) and sat. aq. $NaHCO_3$ (100 mL). The combined aqueous phases were extracted with CH_2Cl_2 (100 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography.

General Procedure for Glycosylation Reactions using Glycosyl Bromides - C
The donor (9.0 mmol) was dissolved in anhydrous CH_2Cl_2 (30 mL), cooled to 0 °C, and titrated with a 1M sol. of bromine in anhydrous CH_2Cl_2 until a faint yellow color persisted. The solution was cannulated to a solution of the acceptor (5.0 mmol), MS 4A (10 g) and AgOTf (3.47 g, 13.5 mmol) in anhydrous CH_2Cl_2 (15 mL) at –50 °C. The reaction mixture was stirred at –50 °C while monitored by TLC (2 – 4 h), then quenched by addition of sat. aq. $NaHCO_3$ (50 mL), allowed to reach room temperature and filtered through celite. The pad was washed with CH_2Cl_2 (100 mL), and the aqueous phase extracted with CH_2Cl_2 (50 ml), the combined organic phases were dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography.

General Procedure for Armed-Disarmed Glycosylation Reactions - D
A mixture of the donor (1.0 mmol) and the acceptor (1.3 mmol) was dried azeotropically with toluene (x2) and subjected to high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (13 mL), cooled to –20 °C, followed by addition of NIS (293 mg, 1.3 mmol) and TESOTf (0.06 mL, 0.26 mmol). The reaction mixture was stirred at –20 °C for 30 min. The solution was diluted with CH_2Cl_2 (13 mL) and washed with 10% aq. $Na_2S_2O_3$ (25 mL) and sat. aq. $NaHCO_3$ (25 mL). The combined aqueous phases were extracted with CH_2Cl_2 (25 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography.

General Procedure for Removal of the Chloroacetyl Protecting Group - E
The protected saccharide (5 mmol) was dissolved in THF (50 mL). Thiourea (1.14 g, 15 mmol), $NaHCO_3$ (1.39 g, 16.5 mmol), and tetrabutylammonium iodide (369 mg, 1 mmol) was added and the suspension heated to 55 °C. Monitored by TLC until full conversion was observed (12-24 h). The mixture was cooled, filtered, concentrated and purified by flash chromatography.

General Procedure for Removal of the Allyl Protecting Group - F
Wilkinson's catalyst ($ClRh(PPh_3)_3$), 463 mg, 1.0 mmol) was dissolved in anhydrous THF (27 mL) and the solution was degassed. n-BuLi (0.75 mL, 1.6 M in hexanes, 1.2 mmol) was added, and the mixture was stirred for 10 min. A degassed solution of the protected saccharide (5 mmol) in anhydrous THF (40 mL) was heated to reflux, and the solution of the catalyst was added. The reaction mixture was refluxed until ^1H NMR revealed full conversion to the vinyl ether (1-6 h), then cooled to 50 °C, MeOH (140 mL) and Amberlite IR-120 (H^+) (15 mL) was added, and the resulting mixture was stirred until TLC revealed full conversion (24-36 h). The resin was filtered off, washed with CH_2Cl_2 (100 mL), and the filtrate was concentrated and purified by flash chromatography.

General Procedure for Removal of Acyl Protecting Groups - G
The protected saccharide (1 mmol) was dissolved in THF (25 mL) and MeOH (50 mL). Sodium (12 mg, 0.5 mmol) was added. Stirred at 20 °C until full conversion was observed by TLC (1-24 h). The reaction was quenched with Amberlite IR-120 (H^+) (5 mL) and stirred for an additional 30 min. The resin was filtered off, washed with CH_2Cl_2 (50 mL), and the filtrate was concentrated and purified by flash chromatography.

General Procedure for Removal of the 4-Methoxyphenyl Protecting Group - H
The protected saccharide (0.5 mmol) was dissolved in MeCN (20 mL) and cooled to 0 °C. A solution of CAN (5 equiv./PMP group) in 5 ml water was added. Stirred 15 min., diluted with chloroform (50 mL), washed with water (2x40 mL). The combined aqueous phases were extracted with $CHCl_3$ (40 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated and purified by flash chromatography.

General Procedure for Oxidation to Uronic Acid - I
To a suspension of the Dess-Martin periodinane (1.5 equiv./alcohol) in anhydrous CH_2Cl_2 (36 mL) was added a solution of the saccharide (0.5 mmol) in CH_2Cl_2 (24 mL). The reaction was stirred 45-60 min., diluted with Et_2O (120 mL), quenched with 10% aq. $Na_2S_2O_3$ (120 mL) and stirred 30 min. The organic phase was separated and washed with sat. aq. $NaHCO_3$ (100 mL), the combined aqueous phases were extracted with Et_2O (100 mL), the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The crude aldehyde was taken up in THF (11 mL). ^tBuOH (26 mL) and 2-methyl-but-2-ene (50 equiv./aldehyde) was added, followed by a solution of $NaClO_2$ (10 equiv./aldehyde) and $NaH_2PO_4 \cdot H_2O$ (7.5 equiv./aldehyde) in 11 mL H_2O. Stirred at 20 °C until full conversion was observed by TLC (1-2 h). The reaction mixture was partly concentrated, and acidified with 1M HCl. The aqueous phase was extracted with EtOAc (x3), the combined organic phases were dried ($MgSO_4$), filtered and concentrated to afford the crude acid.

General Procedure for Methyl Esterification - J
The crude product of the oxidations was taken up in THF (2.8 mL) and MeOH (25 mL) and $TMSCHN_2$ (2M sol. in hexanes, 6 equiv./acid) was added. Stirred 12-24 h while monitored by TLC - more $TMSCHN_2$ added if necessary (up to an additional 6 equiv./acid). When full conversion was observed, the reaction mixture was concentrated and purified by flash chromatography.

General Procedure for Benzyl Esterification - K
The crude product of the oxidations was taken up in EtOAc (30 mL) and titrated with $PhCHN_2$ (approximately 0.5M sol. in Et_2O) until full conversion was observed by TLC (1.5-2 equiv./acid, 1-2 h). The reaction mixture was concentrated and purified by flash chromatography.

General Procedure for Hydrogenolysis of Galacturonic Acids - L
The partly protected galacturonate (0.1 mmol) was dissolved in MeOH (20 mL) and H_2O (5 mL). 125 mg 10% Pd/C was added, and an atmosphere of H_2 (1 atm.) was installed. Stirred until TLC indicates full conversion (12-30 h), filtered through celite, and concentrated yielding a white solid.

General Procedure for Hydrogenolysis of Fully Protected Galacturonates - M
The protected galacturonate (0.1 mmol) was dissolved in THF (5 mL) and MeOH (15 mL). 125 mg 10% Pd/C was added, and an atmosphere of H_2 (1 atm.) was installed. Stirred 2-3 h, then 5 ml H_2O was added. Stirred until TLC indicates full conversion (12-30 h), filtered through celite, and concentrated yielding a white solid.

Compounds

O-α-D-Galactopyranosyl uronic acid-(1→4)-*O*-α-D-galactopyranosyluronic acid-(1→4)-(methyl D-galacturonate) (16)

Prepared from **44** according to General Procedure L.
Foam, R_f 0.28 (MeOH-H_2O 3:1 + 3% AcOH).
^{1}H NMR (D_2O, 300 MHz) δ 5.30 (d, *J* = 3.8 Hz, 0.4H, $H_{1\alpha}$), 5.01-4.97 (m, 3H, 2xH5, $H_{1'}$), 4.92 (d, *J* = 3.5 Hz, 1H, $H_{1''}$), 4.79-4.53 (signals hidden under HDO-peak), 4.45-4.34 (m, 2.6H), 4.26 (d, *J* = 2.2 Hz, 1H), 3.98 (m, 0.4H), 3.95 (m, 1H), 3.86 (dd, *J* = 10.5, 3.4 Hz, 1H), 3.77-3.64 (m, 3.4H), 3.75 (s, 1.6H), 3.75 (s, 1.4H), 3.42 (dd, *J* = 10.0, 7.7 Hz, 0.6H).
^{13}C NMR (D_2O, 75 MHz) δ 172.9, 172.2, 170.9, 170.0, 100.6, 100.1, 96.6, 92.6, 79.2, 78.4, 73.4, 71.5, 71.3, 71.1, 70.4, 70.1 (2C), 70.0, 68.9, 68.2, 68.0 (2C), 67.8, 53.2, 53.1.
ESI MS-MS: m/z 558.8, 368.7 (C_2), 364.8 (Z_2).

O-α-D-Galactopyranosyl uronic acid-(1→4)-*O*-(methyl α-D-galactopyranosyluronate)-(1→4)-D-galacturonic acid (17)

Prepared from **56** according to General Procedure L.
Foam, R_f 0.51 (MeOH-H_2O 2:1 + 3% AcOH).
^{1}H NMR (D_2O, 300 MHz) δ 5.29 (d, *J* = 4.1 Hz, 0.4H, $H_{1\alpha}$), 5.09 (s, 1H), 5.06 (d, *J* = 3.9 Hz, 1H, $H_{1'}$), 4.94 (bs, 1H), 4.88 (d, *J* = 3.9 Hz, 1H, $H_{1''}$), 4.70-4.63 (signals hidden under HDO-peak), 4.59 (d, *J* = 7.7 Hz, 0.6 H, $H_{1\beta}$), 4.42-4.39 (m, 1.6H), 4.35 (d, *J* = 2.8 Hz, 0.6H), 4.28-4.26 (m, 1.6H), 4.00 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.85 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.76 (s, 3H), 3.74 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.70 (m, 1H), 3.67 (dd, *J* = 10.7, 4.0 Hz, 1H), 3.43 (dd, *J* = 10.1, 7.7 Hz, 0.6H).
^{13}C NMR (D_2O, 75 MHz) δ 175.9, 175.6, 174.8, 173.5, 103.1, 102.7, 99.0, 95.1, 81.5 (2C), 80.7, 76.0, 74.5, 74.0, 73.4, 72.9, 72.5, 71.6, 70.7 (2C), 70.6, 55.7.
ESI MS-MS: m/z 558.8, 382.7 (C_2), 364.7 (Z_2).

O-(Methyl α-D-galactopyranosyluronate)-(1→4)-*O*-α-D-galactopyranosyluronic acid-(1→4)-D-galacturonic acid (18)

Prepared from **63** according to General Procedure L.
Foam, R_f 0.43 (MeOH-H_2O 2:1 + 3% AcOH).
^{1}H NMR (D_2O, 300 MHz) δ 5.26 (d, *J* = 3.7 Hz, 0.4 H, $H_{1\alpha}$), 5.07 (m, 2H), 5.08 (d, *J* = 3.8 Hz, 1H, $H_{1'}$), 4.76 (d, *J* = 0.8 Hz, 0.4H), 4.71 (s, 1H), 4.68-4.63 (signals hidden under HDO-peak), 4.55 (d, *J* = 8.0 Hz, 0.6H, $H_{1\beta}$), 4.40-4.32 (m, 2.6H), 4.27 (m, 1H), 4.01-3.92 (m, 2H), 3.87 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.77 (m, 0.6H), 3.74 (s, 3H), 3.72-3.68 (m, 1.6H), 3.67 (dd, *J* = 10.0, 3.8 Hz, 1H), 3.44 (dd, *J* = 9.5, 8.0 Hz, 0.6H).
^{13}C NMR (D_2O, 75 MHz) δ 175.4, 174.6, 171.7, 99.6, 99.3, 99.1, 96.4, 92.4, 78.8, 78.2, 77.3, 74.4, 72.5, 71.7, 71.6, 71.4, 70.8, 70.3, 69.0 (2C), 68.4, 68.2, 53.0.
ESI MS-MS: m/z 558.9, 382.7 (C_2), 350.7 (Z_2).

Benzyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (22)

A solution of galactose pentaacetate (**10**) (25 g, 64 mmol), BnOH (12 mL, 116 mmol) and BF_3·OEt_2 (10 mL, 79 mmol) in CH_2Cl_2 (200 mL) was stirred at room temperature for 12 h, and then worked up and deacetylated according to General Procedure G. Purified by flash chromatography (acetone-EtOAc 1:1) to give 15.8 g of a solid which on recrystallization from MeCN gave 12.4 g of benzyl β-D-galactopyranoside (**19**), mp 106-108 °C (lit.[150] 99-100 °C). A mixture of this material (10 g, 37 mmol), $PhCH(OMe)_2$ (7.5 mL, 50 mmol) and camphorsulphonic acid (200 mg) in $CHCl_3$ (175 mL) was heated at reflux for 1 h in a flask equipped with a distillation head. About 50 mL of $CHCl_3$-MeOH mixture distilled off. Benzyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**20**) crystallized directly, 12.4 g, mp 195-198 °C (lit.[150]

[150] Turvey, J. R.; Williams, T. P. *J. Chem. Soc.* **1962**, 2119.

209-210 °C). This was benzylated as described below for **26** to give after direct crystallization from EtOAc 16.8 g of benzyl 4,6-*O*-benzylidene-2,3-di-*O*-benzyl-β-D-galactopyranoside (**21**), mp 162-164 °C (lit.[150] 169.5-170.5 °C). This was deprotected as described below for **27** to give after direct crystallization from EtOAc-hexane 13.6 g of **22**, mp 113-115 °C (lit.[150] 116-117 °C).

Benzyl 6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (23)

A mixture of **22** (3.0 g, 6.7 mmol), Ac_2O (0.7 mL, 7.4 mmol) and Et_3N (1.4 mL, 10 mmol) in dry CH_2Cl_2 (20 mL) was stirred overnight and then quenched with MeOH. The solution was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (30 mL). The organic phase was dried and concentrated to give a solid which was recrystallized from EtOAc-hexane to afford **23** (3.1 g, 95%), R_f 0.31 (hexane-EtOAc 2:1).
mp 99-103 °C (EtOAc-hexane).
$[\alpha]_D^{20}$ −17.7 (c 1.5, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.25 (m, 15H), 4.96 (d, *J* = 11.0 Hz, 1H), 4.94 (d, *J* = 11.0 Hz, 1H), 4.77-4.65 (m, 4H), 4.45 (d, *J* = 7.8 Hz, 1H), 4.39-4.35 (m, 2H), 3.93 (dd, *J* = 3.6, 0.9 Hz, 1H), 3.72 (dd, *J* = 9.4, 7.8 Hz, 1H), 3.59 (dt, *J* = 6.3, 0.9 Hz, 1H), 3.50 (dd, *J* = 9.4, 3.5 Hz, 1H), 2.10 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.0, 138.7, 138.0, 137.5, 128.7-127.8 (15C), 102.5, 80.7, 79.0, 75.4, 72.9, 72.1, 71.1, 67.0, 63.3, 21.1.
Anal. Calcd for $C_{29}H_{32}O_7$: C, 70.72; H, 6.55. Found: C, 70.62; H, 6.62.

Benzyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (24)

To an ice-cooled solution of **22** (3.5 g, 7.77 mmol) in dry pyridine (10 mL) was added TsCl (2.08 g, 10.9 mmol). The mixture was stirred at room temperature for 6 h and then diluted with CH_2Cl_2 (60 mL) and washed with 1 M aq. HCl (3x50 mL). The organic phase was dried and concentrated. To the residue in DMF (15 mL) was added a solution of sodium *p*-methoxyphenolate in DMF (prepared from 1.4 g of *p*-methoxyphenol and 0.54 g of ~50% NaH oil dispersion in 5 mL of DMF). The mixture was stirred for 16 h and then diluted with Et_2O (70 mL) and washed with H_2O (2x50 mL). The organic layer was dried, concentrated and purified by flash chromatography (hexane-EtOAc 3:1, R_f 0.31) to give **24** (3.37 g, 78%) as an oil which crystallized from EtOH to give 3.12 g.
mp 87-87.5 °C (EtOH).
$[\alpha]_D^{20}$ −34.4 (c 1.0, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.28 (m, 15H), 6.88 (m, 4H), 4.97 (d, *J* = 10.5 Hz, 1H), 4.95 (d, *J* = 11.8 Hz, 1H), 4.80-4.71 (m, 3H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.51 (d, *J* = 7.7 Hz, 1H), 4.31-4.18 (m, 2H), 4.11 (d, *J* = 3.3 Hz, 1H), 3.79 (s, 3H), 3.76-3.72 (m, 2H), 3.56 (dd, *J* = 9.7, 3.5 Hz, 1H), 2.09 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.4, 153.0, 138.7, 138.0, 137.6, 128.7-127.8 (15C), 116.1 (2C), 114.9 (2C), 102.7, 80.9, 79.2, 75.5, 73.0, 72.9, 71.1, 67.7, 66.9, 56.0.
Anal. Calcd for $C_{34}H_{36}O_7$: C, 73.36; H, 6.52. Found: C, 73.47; H, 6.55.

Pent-4-enyl β-D-galactopyranoside (25)

To a solution of galactose pentaacetate (**10**) (35 g, 89.7 mmol) and pent-4-en-1-ol (20 mL, 194 mmol) in CH_2Cl_2 (250 mL) was added $BF_3{\cdot}OEt_2$ (14 mL, 110 mmol). The mixture was stirred at room temperature under an atmosphere of nitrogen for 10 h, and then diluted with CH_2Cl_2 (100 mL) and washed with sat. aq. $NaHCO_3$ (350 mL). The organic layer was dried and concentrated. The syrupy residue was dissolved in 0.04 M NaOMe in MeOH (300 mL) and stirred for 2 h. The mixture was quenched with Amberlite IR-120 (H^+) (15 mL) and stirred for an additional 30 min. The resin was filtered off and the filtrate concentrated and purified by flash chromatography (CH_2Cl_2-MeOH 6:1, R_f 0.30) to give 17.5 g of a greasy solid. Recrystallization from EtOAc afforded **25** (12.5 g, 56%) as white crystals.
mp 88-90 °C (EtOAc).
$[\alpha]_D^{20}$ −10.0 (c 1.6, H_2O) (lit.[69b] −9.02 (c 1.23, H_2O)).

^{1}H NMR (D_2O, 300 MHz) δ 5.82 (m, 1H), 5.01 (d, *J* = 17.4 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.30 (d, *J* = 8.0 Hz, 1H), 3.87-3.82 (m, 2H), 3.72-3.51 (m, 5H), 3.42 (t, *J* = 8.9 Hz, 1H), 2.07 (m, 2H), 1.65 (m, 2H).
^{13}C NMR (D_2O, 75 MHz) δ 139.1, 115.0, 103.0, 75.3, 73.1, 71.0, 70.1, 68.9, 61.1, 29.6, 28.3
Anal. Calcd for $C_{11}H_{20}O_6$: C, 53.22; H, 8.12. Found: C, 53.42; H, 7.98.

Pent-4-enyl 4,6-*O*-benzylidene-β-D-galactopyranoside (26)

To a solution of $PhCH(OMe)_2$ (33 mL, 220 mmol) and camphorsulphonic acid (450 mg) in $CHCl_3$ (980 mL) was added **25** (38.5 g, 155 mmol). The flask was equipped with a distillation head and the mixture heated at reflux for 1.5 h during which time about 250 mL of a $CHCl_3$-MeOH mixture distilled off. The reaction mixture was quenched with Et_3N (0.7 mL) and concentrated to a solid which was recrystallized from EtOAc to give **26** (51.7 g, 99%), R_f 0.53 (EtOAc).
mp 158-160 °C (EtOAc).
$[\alpha]_D^{20}$ −36.5 (c 1.5, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (m, 2H), 7.35 (m, 3H), 5.81 (m, 1H), 5.54 (s, 1H), 5.04 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.97 (dq, *J* = 10.4, 1.7 Hz, 1H), 4.32 (dd, *J* = 12.5, 1.5 Hz, 1H), 4.26 (d, *J* = 7.3 Hz, 1H), 4.20 (dd, *J* = 3.6, 1.5 Hz, 1H), 4.07 (dd, *J* = 12.5, 1.9 Hz, 1H), 3.98 (dt, *J* = 9.7, 6.6 Hz, 1H), 3.74 (dd, *J* = 9.6, 7.3 Hz, 1H), 3.69 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.52 (dt, *J* = 9.4, 7.0 Hz, 1H), 3.46 (m, 1H), 2.24-2.06 (m, 4H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.4, 137.8, 129.4, 128.4 (2C), 126.7 (2C), 115.1, 103.1, 101.6, 75.7, 73.0, 72.0, 69.6, 69.4, 66.9, 30.4, 28.9.
Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.51; H, 7.23.

Pent-4-enyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (27)

A mixture of **26** (51.6 g, 153 mmol) and NaH (28 g of ~50% oil dispersion, 583 mmol) in DMF (500 mL) was stirred at room temperature for 30 min. and then cooled to 0 °C. BnBr (55 mL, 462 mmol) and Bu_4NI (4 g, 11 mmol) were added and the solution stirred at room temperature overnight. Quenched with MeOH, diluted with CH_2Cl_2 (750 mL) and washed with H_2O (750 mL). The organic phase was dried, concentrated and the residue crystallized from EtOAc-hexane to afford **27** (71.2 g, 90%), R_f 0.33 (hexane-EtOAc 3:1).
mp 114-117 °C (EtOAc-hexane).
$[\alpha]_D^{20}$ −27.8 (c 1.5, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (m, 2H), 7.41-7.26 (m, 13H), 5.82 (m, 1H), 5.50 (s, 1H), 5.07-4.90 (m, 3H), 4.81-4.72 (m, 3H), 4.39 (d, *J* = 8.0 Hz, 1H), 4.31 (d, *J* = 12.0 Hz, 1H), 4.12-3.96 (m, 3H), 3.84 (t, *J* = 8.7 Hz, 1H), 3.58-3.50 (m, 2H), 3.32 (s, 1H), 2.19 (m, 2H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 139.1, 138.7, 138.4, 138.1, 129.1, 128.6 (2C), 128.5 (2C), 128.3 (2C), 128.2 (2C), 128.0 (2C), 127.9, 127.8, 126.8 (2C), 115.1, 103.9, 101.6, 79.4, 78.7, 75.6, 74.2, 72.3, 69.5 (2C), 66.6, 30.5, 29.2.
Anal. Calcd for $C_{32}H_{36}O_6$: C, 74.40; H, 7.02. Found: C, 74.17; H, 6.73.

Pent-4-enyl 2,3-di-*O*-benzyl-β-D-galactopyranoside (28)

A solution of **27** (13.0 g, 25.2 mmol), propan-1,3-diol (9.1 mL, 126 mmol) and *p*-toluenesulphonic acid (100 mg, 0.6 mmol) in CH_2Cl_2 (70 mL) and MeOH (70 mL) was heated at reflux for 96 h. The mixture was cooled, concentrated and purified by flash chromatography (EtOAc-hexane 1:1, R_f 0.15) to afford **28** (8.11 g, 75%) as a solid.
mp 59-63 °C (EtOAc-hexane).
$[\alpha]_D^{20}$ −2.5 (c 1.6, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.27 (m, 10H), 5.81 (m, 1H), 5.07-4.90 (m, 3H), 4.77-4.71 (m, 3H), 4.37 (d, *J* = 7.7 Hz, 1H), 4.01-3.93 (m, 3H), 3.82 (dd, *J* = 11.5, 4.8 Hz, 1H), 3.69-3.42 (m, 4H), 2.37 (bs, 2H), 2.16 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.8, 138.2, 138.0, 128.7-127.8 (10C), 115.1, 104.0, 80.7, 79.1, 75.4, 74.2, 72.8, 69.6, 67.7, 62.8, 30.4, 29.2.
Anal. Calcd for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 69.88; H, 7.56.

Pent-4-enyl 6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (29)

Prepared according to General Procedure A.
$[\alpha]_D^{20}$ +2.0 (*c* 2.8, $CHCl_3$).
IR (KBr) 1723 cm^{-1}.
^1H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.26 (m, 10H), 6.83 (m, 1H), 5.03 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.98 (bd, *J* = 10.2 Hz, 1H), 4.93 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.39-4.31 (m, 3H), 3.96 (dt, *J* = 9.4, 6.4 Hz, 1H), 3.93 (bs, 1H), 3.65 (dd, *J* = 9.4, 7.7 Hz, 1H), 3.61-3.54 (m, 2H), 3.51 (dd, *J* = 9.4, 3.4 Hz, 1H), 2.49 (bs, 1H), 2.18 (m, 2H), 2.08 (s, 3H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.88, 138.66, 138.18, 137.92, 128.62 (2C), 128.44 (2C), 128.22, 128.08 (2C), 127.96 (2C), 127.77, 115.01, 103.80, 80.56, 78.96, 75.33, 72.80, 71.88, 69.46, 66.85, 63.17, 30.36, 29.13, 21.02.
Anal. Calcd for $C_{27}H_{34}O_7$: C, 68.92; H, 7.27. Found: C, 68.84; H, 7.30.

Pent-4-enyl 6-*O*-acetyl-4-*O*-chloroacetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (30)

To a solution of **29** (2.2g, 4.12 mmol) in anhydrous CH_2Cl_2 (25 mL) was added $(ClAc)_2O$ (1.2 g, 7.0 mmol), Et_3N (1 mL) and DMAP (10 mg). The mixture was stirred for 3 h, and then concentrated and purified by flash chromatography (hexane-EtOAc 3:1, R_f 0.44) to give **30** (2.5 g, 99%) as a syrup.
$[\alpha]_D^{20}$ +17.5 (c 1.1, $CHCl_3$).
^1H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.27 (m, 10H), 5.81 (m, 1H), 5.53 (m, 1H), 5.06-4.95 (m, 2H), 4.87 (d, *J* = 11.0 Hz, 1H), 4.74 (d, *J* = 11.0 Hz, 1H), 4.72 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 11.0 Hz, 1H), 4.38 (m, 1H), 4.21-4.14 (m, 4H), 3.94 (dt, *J* = 9.5, 6.4 Hz, 1H), 3.80 (dt, *J* = 6.8, 0.9 Hz, 1H), 3.62-3.52 (m, 3H), 2.16 (m, 2H), 2.07 (s, 3H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6, 167.2, 138.6, 138.1, 137.7, 128.6-127.9 (10C), 115.2, 104.0, 79.1, 78.9, 75.6, 72.8, 70.6, 70.0, 68.9, 61.9, 41.1, 30.4, 29.1, 21.0.
Anal. Calcd for $C_{29}H_{35}ClO_8$: C, 63.67; H, 6.45. Found: C, 63.25; H, 6.47.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (31)

To an ice-cooled solution of **28** (5.0 g, 11.7 mmol) in pyridine (35 mL) was added TsCl (3.3 g, 17.3 mmol). The mixture was stirred at room temperature overnight and then diluted with CH_2Cl_2 (100 mL) and washed with 1 M aq. HCl (2x75 mL). The organic phase was dried and concentrated. To the residue in DMF (10 mL) was added a solution of sodium *p*-methoxyphenolate in DMF (prepared from 2.5 g of *p*-methoxyphenol and 1.2 g of ~50% NaH oil dispersion in 15 mL of DMF). The mixture was stirred for 12 h and then diluted with Et_2O (100 mL) and washed with H_2O (2x60 mL). The organic layer was dried, concentrated and purified by flash chromatography (hexane-EtOAc 4:1) to give **31** as a syrup (4.43 g, 70%).
$[\alpha]_D^{20}$ +9.7 (*c* 2.7, $CHCl_3$).
^1H NMR ($CDCl_3$, 500 MHz) δ 7.42-7.26 (m, 10H), 6.92-6.83 (m, 4H), 5.85 (m, 1H), 5.05 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.99 (bd, *J* = 10.2 Hz, 1H), 4.95 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 11.1 Hz, 1H), 4.76 (s, 2H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.27 (dd, *J* = 9.8, 6.4 Hz, 1H), 4.18 (dd, *J* = 9.8, 6.0 Hz, 1H), 4.12 (d, *J* = 3.1 Hz, 1H), 3.98 (dt, *J* = 9.4, 6.4 Hz, 1H), 3.78 (s, 3H), 3.74 (t, *J* = 6.0 Hz, 1H), 3.70 (dd, *J* = 9.4, 7.7 Hz, 1H), 3.61-3.56 (m, 2H), 2.63 (bs, 1H), 2.19 (m, 2H), 1.78 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.18, 152.85, 138.70, 138.15, 137.94, 128.54 (3C), 128.37, 128.14 (3C), 127.97, 127.89, 127.67, 115.90 (2C), 114.92, 114.73 (2C), 103.81, 80.70, 79.05, 75.27, 72.74, 72.63, 69.37, 67.57, 66.69, 55.78, 30.33, 29.09.
Anal. Calcd for $C_{32}H_{38}O_7$: C, 71.89; H, 7.16. Found: C, 71.62; H, 6.96.

Pent-4-enyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (32)

31 (4.43 g, 8.29 mmol) was treated overnight with $(ClAc)_2O$ (2.2 g), Et_3N (1.8 mL), and DMAP (20 mg) in CH_2Cl_2 (50 mL) followed by evaporation of the solvent and flash chromatography (hexane-EtOAc 5:1, R_f 0.35) to afford **32** (4.86 g, 96%) as an oil.

$[\alpha]_D^{20}$ +2.5 (c 0.9, $CHCl_3$).
^1H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.27 (m, 10H), 6.87-6.80 (m, 4H), 5.82 (m, 1H), 5.72 (d, *J* = 2.4 Hz, 1H), 5.00 (m, 2H), 4.88 (d, *J* = 11.0 Hz, 1H), 4.78 (d, *J* = 11.1 Hz, 1H), 4.74 (d, *J* = 11.1 Hz, 1H), 4.57 (d, *J* = 11.5 Hz, 1H), 4.43 (d, *J* = 7.3 Hz, 1H), 4.11 (m, 1H), 4.10 (s, 2H), 4.01-3.87 (m, 3H), 3.77 (s, 3H), 3.60 (m, 3H), 2.17 (m, 2H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.1, 154.6, 152.6, 138.6, 138.1, 137.8, 128.6-127.9 (10C), 116.2, 115.2, 114.9, 104.0, 79.3, 79.0, 75.6, 72.7, 71.6, 70.0, 69.2, 67.1, 55.9, 41.0, 30.4, 29.2.
Anal. Calcd for $C_{34}H_{39}ClO_8$: C, 66.82; H, 6.43. Found: C, 66.75; H, 6.53.

Pent-4-enyl 6-*O*-trityl-β-D-galactopyranoside (33)

A mixture of **25** (10.2 g, 41.1 mmol) and TrCl (12.6 g, 45.2 mmol) in dry pyridine (130 mL) was heated at 90 °C for 3 h, and then concentrated and co-concentrated with toluene. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with H_2O (150 mL). The organic layer was dried and concentrated. The residue was crystallized from Et_2O and recrystallized from EtOAc-hexane to give **33** (17.6 g, 87%), R_f 0.48 (EtOAc).
mp 89.0-91.5 °C (EtOAc-hexane).
$[\alpha]_D^{20}$ −27.6 (c 0.7, $CHCl_3$).
^1H NMR ($CDCl_3$, 300 MHz) δ 7.48-7.42 (m, 6H), 7.34-7.21 (m, 9H), 5.83 (m, 1H), 5.02 (dq, *J* = 17.2, 1.7 Hz, 1H), 4.96 (dq, *J* = 10.2, 1.7 Hz, 1H), 4.20 (d, *J* = 7.2 Hz, 1H), 4.02 (t, *J* = 3.3 Hz, 1H), 3.93 (dt, *J* = 9.7, 6.2 Hz, 1H), 3.65-3.35 (m, 6H), 2.55 (bd, *J* = 5.9 Hz, 1H), 2.37 (bs, 1H), 2.28 (d, *J* = 4.3 Hz, 1H), 2.13 (m, 2H), 1.75 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.3 (3C), 135.5, 126.1 (6C), 125.3 (6C), 124.5 (3C), 112.4, 100.6, 84.3, 71.2, 71.1, 69.1, 66.8, 66.7, 60.2, 27.5, 26.2.
ESI MS: *m/z* calcd for $C_{30}H_{34}NaO_6$ $[M+Na]^+$ 513.23, found 513.30.

Pent-4-enyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (34)

To a solution of **33** (16.5 g, 33.7 mmol) in DMF (130 mL) was added NaH (8 g of ~50% oil dispersion, 167 mmol). The mixture was stirred at room temperature for 30 min. and then cooled to 0 °C followed by addition of BnBr (18 mL, 152 mmol) and Bu_4NI (1 g, 2.7 mmol). The mixture was stirred at room temperature overnight, and then quenched with MeOH, diluted with Et_2O (700 mL) and washed with H_2O (700 mL). The organic layer was dried, concentrated and the residue dissolved in 1% H_2SO_4 in MeOH (290 mL). After stirring at room temperature for 1 h solid Na_2CO_3 (15 g) was added and the stirring continued until neutral pH. The mixture was filtered and the filtrate concentrated to a syrup. This was dissolved in CH_2Cl_2 (300 mL), washed with H_2O (200 mL), dried, concentrated and purified by flash chromatography (hexane-EtOAc 2:1, R_f 0.31) to give **34** (14.5 g, 83%) as a syrup.
$[\alpha]_D^{20}$ −23.8 (c 0.8, $CHCl_3$).
^1H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.25 (m, 15H), 5.89-5.74 (m, 1H), 5.05-4.92 (m, 4H), 4.85-4.64 (m, 4H), 4.36 (d, *J* = 7.6 Hz, 1H), 3.95 (dt, *J* = 9.5, 6.4 Hz, 1H), 3.84 (dd, *J* = 9.7, 7.7 Hz, 1H), 3.81-3.73 (m, 2H), 3.57-3.46 (m, 3H), 3.37 (t, *J* = 6.2 Hz, 1H), 2.16 (m, 2H), 1.75 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.5, 138.3, 138.1, 138.0, 128.6-127.5 (15C), 114.7, 103.9, 82.1, 79.5, 75.1, 74.3, 74.0, 73.3, 72.6, 69.2, 61.9, 30.1, 28.8.
Anal. Calcd for $C_{32}H_{38}O_6$: C, 74.11; H, 7.39. Found: C, 74.14; H, 7.39.

Pent-4-enyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (35)

A solution of **34** (4.0 g, 7.71 mmol), Ac_2O (1.0 mL, 10.6 mmol), Et_3N (1.6 mL, 11.4 mmol) and DMAP (16 mg) was stirred at room temperature for 2 h and then concentrated and purified by flash chromatography (hexane-EtOAc 3:1, R_f 0.35) to give **35** (4.1 g, 95%) as a syrup.
$[\alpha]_D^{20}$ −22.0 (c 1.3, $CHCl_3$).
^1H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.20 (m, 15H), 5.79 (m, 1H), 5.11-4.88 (m, 4H), 4.80-4.62 (m, 4H), 4.33 (d, *J* = 8.0 Hz, 1H), 4.20 (dd, *J* = 11.0, 8.0 Hz, 1H), 4.05 (dd, *J* = 11.0, 8.0 Hz, 1H), 4.08-3.70 (m, 3H), 3.58-3.40 (m, 3H), 2.15 (m, 2H), 1.95 (s, 3H), 1.75 (m, 2H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 138.6, 138.3, 138.1 (2C), 128.5-127.5 (15C), 114.7, 103.9, 82.2, 79.4, 75.1, 74.2, 73.3, 72.8, 71.9, 69.3, 62.9, 30.1, 28.8, 20.7.
Anal. Calcd for $C_{34}H_{40}O_7$: C, 72.83; H, 7.19. Found: C, 72.25; H, 7.24.

Pent-4-enyl 2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (36)

To a solution of **34** (5.9 g, 11.4 mmol), PPh_3 (6.0 g, 23 mmol), and *p*-methoxyphenol (5.8 g, 47 mmol) in dry THF (45 mL) was added DEAD (3.6 mL, 23 mmol). The mixture was stirred at room temperature for 2 days during which time an additional amount of PPh_3 (3 g) and DEAD (1.8 mL) was added. Concentrated and purified by flash chromatography (hexane-EtOAc 4:1, R_f 0.54) to afford **36** (5.2 g, 73%) as a solid.
mp 77.5-79 °C (EtOH).
$[\alpha]_D^{20}$ −15.3 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.18 (m, 15H), 6.78 (m, 4H), 5.81 (m, 1H), 5.05-4.91 (m, 4H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.79 (d, *J* = 11.1 Hz, 1H), 4.75 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.40 (d, *J* = 7.7 Hz, 1H), 4.04-3.96 (m, 3H), 3.95 (m, 1H), 3.86 (t, *J* = 8.5 Hz, 1H), 3.78 (s, 3H), 3.69 (t, *J* = 6.2 Hz, 1H), 3.58 (m, 1H), 3.54 (m, 1H), 2.16 (m, 2H), 1.75 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.3, 152.8, 139.0, 138.7, 138.6, 138.4, 128.7-127.7 (15C), 115.7 (2C), 115.0, 114.8 (2C), 104.3, 82.5, 79.8, 75.4, 74.8, 73.5, 73.4, 73.1, 69.6, 67.1, 56.0, 30.5, 29.2.
Anal. Calcd for $C_{39}H_{44}O_7$: C, 74.98; H, 7.10. Found: C, 74.73; H, 7.02.

Pent-4-enyl 2,3,4-tri-*O*-benzyl-6-deoxy-α-L-*arabino*-hex-5-enopyranoside (37)

Syrup, R_f 0.43 (hexane-EtOAc 7:1).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.21 (m, 15H), 5.83 (m, 1H), 5.08-4.95 (m, 2H), 4.91-4.77 (m, 4H), 4.62 (s, 2H), 4.53-4.41 (m, 3H), 4.06-3.97 (m, 3H), 3.63-3.54 (m, 2H), 2.19 (m, 2H), 1.78 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.64, 138.32, 138.21, 138.04 (2C), 128.53 (3C), 128.51 (3C), 128.33 (3C), 128.20, 127.92 (2C), 127.82, 127.79, 127.76, 115.12, 104.65, 100.18, 78.99, 78.82, 75.30, 73.63, 72.32, 69.73, 69.65, 30.44, 29.18.

Benzyl *O*-(2,3-di-*O*-benzyl-4-*O*-chloroacetyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-acetyl-β-D-galactopyranoside (38)

Prepared from **23** and **32** according to General Procedure B.
Foam, R_f 0.10 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +21.9 (c 0.6, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.09 (m, 25H), 6.65-6.45 (m, 4H), 5.72 (bd, *J* = 2.0 Hz, 1H), 5.19 (s, 1H), 4.93-4.47 (m, 11H), 4.38 (m, 3H), 4.05 (m, 1H), 3.91 (s, 2H), 3.84 (d, *J* = 2.9 Hz, 1H), 3.73 (dd, *J* = 10.3, 3.5 Hz, 1H), 3.65 (m, 1H), 3.61 (s, 3H), 3.59-3.42 (m, 3H), 3.33 (dd, *J* = 9.9, 2.7 Hz, 1H), 1.98 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7, 166.8, 154.2, 152.5, 138.5-137.6 (5C), 128.7-127.9 (25C), 115.9 (2C), 114.7 (2C), 103.0, 101.0, 80.8, 78.7, 76.6, 76.5, 75.3 (2C), 74.2, 73.5, 72.5, 72.3, 71.4, 70.2, 67.5, 66.3, 62.4, 55.9, 41.1, 21.2.
ESI MS: *m/z* calcd for $C_{58}H_{60}ClNa_2O_{14}$ $[M-H+2Na]^+$ 1061.35, found 1060.98.

Benzyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-acetyl-β-D-galactopyranoside (39)

Prepared from **38** according to General Procedure E.
Crystalline, R_f 0.08 (hexane-EtOAc 3:1).
mp 103-104 °C (EtOH).
$[\alpha]_D^{20}$ +22.3 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43-7.18 (m, 25H), 6.74-6.61 (m, 4H), 5.02-4.61 (m, 11H), 4.52-4.43 (m, 4H), 4.25 (m, 1H), 4.08-3.91 (m, 4H), 3.77-3.72 (m, 2H), 3.70 (s, 3H), 3.54 (t, *J* = 6.5 Hz, 1H), 3.42 (dd, *J* = 9.9, 2.9 Hz, 1H), 2.05 (s, 3H), 1.70 (bs, 1H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.4, 153.7, 152.6, 138.3-137.3 (5C), 128.3-127.5 (25C), 115.3 (2C), 114.3 (2C), 102.6, 100.6, 80.5, 78.5, 76.3, 75.9, 75.6, 74.9, 73.7, 72.8, 72.2, 72.1, 71.0, 68.1, 66.8, 66.6, 52.3, 55.5, 20.8.
Anal. Calcd for $C_{56}H_{60}O_{13}$: C, 71.47; H, 6.43. Found: C, 71.35; H, 6.37.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (40)

Prepared from **39** and **36** according to General Procedure B.
Foam, R_f 0.13 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +15.9 (c 1.1, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.08 (m, 40H), 6.75-6.58 (m, 4H), 6.55-6.48 (m, 4H), 5.30 (m, 1H), 5.08-3.36 (m, 36H), 3.74 (s, 3H), 3.64 (s, 3H), 2.10 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7, 153.9 (2C), 152.8, 152.6, 139.0-137.7 (8C), 128.5-127.4 (40C), 115.5 (2C), 115.4 (2C), 114.7 (2C), 114.7 (2C), 102.9, 101.1, 100.3, 80.9, 79.7, 78.9, 78.3, 76.1, 76.0, 75.7, 75.2 (2C), 75.0, 74.5, 73.7, 73.5, 73.0, 72.9, 72.7, 72.6, 71.2, 69.8, 69.2, 65.6, 64.9, 62.7, 56.0, 55.9, 22.9.
Anal. Calcd for $C_{90}H_{94}O_{19}$: C, 73.05; H, 6.40. Found: C, 72.51; H, 6.46.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (41)

Prepared from **40** according to General Procedure G.
Foam, R_f 0.10 (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +22.5 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.15 (m, 40H), 6.76-6.55 (m, 8H), 5.14 (d, *J* = 2.9 Hz, 1H), 5.07 (d, *J* = 3.0 Hz, 1H), 4.96-4.50 (m, 18H), 4.45 (d, *J* = 7.5 Hz, 1H), 4.37 (m, 2H), 4.31 (d, *J* = 2.0 Hz, 1H), 4.21 (s, 1H), 4.11-3.97 (m, 5H), 3.91 (t, *J* = 8.8 Hz, 1H), 3.83-3.62 (m, 5H), 3.75 (s, 3H), 3.66 (s, 3H), 3.53-3.42 (m, 3H), 3.33 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.0 (2C), 152.8 (2C), 139.0-137.8 (8C), 128.8-127.5 (40C), 115.6 (2C), 115.5 (2C), 114.8 (2C), 114.7 (2C), 103.2, 100.5, 100.5, 81.2, 79.5, 79.3, 78.1, 76.4 (2C), 76.0, 75.8, 75.2 (3C), 74.6 (3C), 74.4, 73.8, 73.1, 72.8, 72.5, 71.4, 70.2, 69.5, 65.9, 65.5, 60.5, 56.0, 55.9.
Anal. Calcd for $C_{88}H_{92}O_{18}$: C, 73.52; H, 6.45. Found: C, 73.22; H, 6.26.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (42)

Prepared from **41** according to General Procedures I and J.
Foam, R_f 0.11 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +15.0 (c 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48-7.07 (m, 40H), 6.68 (m, 4H), 6.53 (m, 4H), 5.19 (d, *J* = 3.1 Hz, 1H), 5.07-5.02 (m, 2H), 4.92-4.37 (m, 19H), 4.29 (m, 2H), 4.15 (s, 1H), 4.06-3.92 (m, 4H), 3.91-3.73 (m, 4H), 3.77 (s, 3H), 3.67 (s, 3H), 3.65 (s, 3H), 3.52-3.48 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.7, 153.9 (2C), 152.8 (2C), 139.1-137.7 (8C), 128.6-127.4 (40C), 115.6 (2C), 115.4 (2C), 114.7 (2C), 114.7 (2C), 103.0, 100.2, 99.7, 80.7, 79.6, 78.5, 78.0, 77.5, 76.2, 75.9, 75.7, 75.3, 75.1, 74.9, 74.5, 74.0, 73.7, 72.9, 72.8, 72.7, 71.4, 70.1, 69.2, 65.5, 65.4, 56.0, 55.9, 52.6.
Anal. Calcd for $C_{89}H_{92}O_{19}$: C, 72.93; H, 6.33. Found: C, 72.57; H, 6.42.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (43)

Prepared from **42** according to General Procedure H.

Foam, R_f 0.09 (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +34.3 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45-7.23 (m, 40H), 5.14-5.05 (m, 2H), 4.96-4.87 (m, 4H), 4.83-4.60 (m, 12H), 4.48 (d, *J* = 7.6 Hz, 1H), 4.41 (d, *J* = 2.2 Hz, 1H), 4.15 (t, *J* = 6.0 Hz, 1H), 4.08 (dd, *J* = 10.2, 3.5 Hz, 1H), 4.02-3.91 (m, 5H), 3.87 (m, 1H), 3.84-3.73 (m, 2H), 3.66-3.50 (m, 3H), 3.54 (s, 3H), 3.47 (dd, *J* = 9.7, 2.6 Hz, 1H), 3.30 (dd, *J* = 11.2, 4.1 Hz, 1H), 2.3 (bs, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.5, 139.2-137.7 (8C), 128.8-127.6 (40C), 103.0, 100.8, 99.1, 80.2, 79.7, 79.4, 78.7, 77.3, 77.1, 76.4, 75.7, 75.4, 75.2, 74.8, 74.7, 73.9, 73.2 (2C), 73.1, 72.9, 72.3, 71.5, 71.0, 62.9, 61.4, 52.5.
Anal. Calcd for $C_{75}H_{80}O_{17}$: C, 71.87; H, 6.43. Found: C, 71.53; H, 6.32.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronic acid)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyluronic acid)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (44)

Prepared from **43** according to General Procedure I, purified by flash chromatography (EtOAc-hexane 2:1 + 3% AcOH, R_f 0.10) and obtained as a foam.
$[\alpha]_D^{20}$ +80.6 (c 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50-7.18 (m, 40H), 5.31-5.07 (m, 3H), 4.95-4.41 (m, 22H), 4.05-3.60 (m, 7H), 3.69 (s, 3H), 3.47 (m, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.2, 170.3, 168.5, 138.7-137.5 (8C), 128.8-127.5 (40C), 102.9, 99.5, 99.0, 79.1, 78.4, 78.1, 76.4, 76.3, 76.2, 75.6, 75.3 (2C), 75.1, 73.7 (3C), 73.1 (3C), 72.8, 71.5 (2C), 71.1, 52.7.
ESI MS: *m/z* calcd for $C_{75}H_{76}NaO_{19}$ $[M+Na]^+$ 1303.49, found 1304.60.

Benzyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranosyluronate (46)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.22 (m, 20H), 4.99-4.86 (m, 3H), 4.82-4.63 (m, 5H), 4.58 (d, *J* = 6.7 Hz, 1H), 4.27 (bs, 1H), 4.04 (bs, 1H), 3.92 (dd, *J* = 10.3, 6.7 Hz, 1H), 3.60 (dd, *J* = 10.3, 2.1 Hz, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.00, 138.58, 138.23, 138.18, 137.32, 128.73 (2C), 128.70 (3C), 128.52 (3C), 128.49, 128.33 (3C), 128.23 (2C), 128.14 (2C), 128.04 (2C), 128.00, 127.86, 102.93, 81.25, 78.77, 75.57, 75.39, 75.34, 74.14, 73.33, 72.04.

Benzyl (methyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranosyluronate) (49)

Prepared from **46** using different methods of esterification ($TMSCHN_2$/MeOH or Cs_2CO_3/MeI/MeCN) or from **45** *via* **47** (Br_2/MeOH).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43-7.21 (m, 20H), 5.11 (d, *J* = 10.9 Hz, 1H), 5.00 (d, *J* = 11.2 Hz, 1H), 4.96 (d, *J* = 10.9 Hz, 1H), 4.83-4.67 (m, 5H), 4.52 (d, *J* = 7.2 Hz, 1H), 4.26 (bs, 1H), 4.05 (d, *J* = 1.1 Hz, 1H), 4.01 (dd, *J* = 11.1, 7.2 Hz, 1H), 3.71 (s, 3H), 3.61 (dd, *J* = 11.1, 3.1 Hz, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.4, 138.9, 138.6 (2C), 137.7, 128.7, 128.6 (4C), 128.5 (2C), 128.4 (2C), 128.2 (2C), 128.0 (2C), 127.9 (4C), 127.8 (3C), 102.4, 81.9, 79.0, 75.6, 75.5, 74.8, 74.3, 73.5, 71.3, 52.3.

Benzyl *O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (50)

Prepared from **24** and **30** according to General Procedure B.
Syrup, R_f 0.37 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +43.9 (c 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45-7.16 (m, 25H), 6.72 (m, 4H), 5.61 (m, 1H), 5.02-4.97 (m, 3H), 4.88-4.43 (m, 11H), 4.20-4.09 (m, 4H), 4.05-3.97 (m, 4H), 3.81-3.63 (m, 2H), 3.75 (s, 3H), 3.50 (dd, *J* = 10.9, 2.9 Hz, 1H), 1.95 (s, 3H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.3, 167.1, 154.3, 152.5, 138.8, 138.6, 138.4, 138.0, 137.7, 128.7-127.6 (25C), 115.7 (2C), 114.9 (2C), 103.2, 100.3, 80.4, 79.0, 76.2, 75.2 (3C), 73.7, 73.3, 73.2, 72.3, 71.4, 70.0, 66.7, 65.7, 61.4, 56.0, 41.0, 20.9.
Anal. Calcd for $C_{58}H_{61}ClO_{14}$: C, 68.46; H, 6.04. Found: C, 68.08; H, 5.94.

Benzyl *O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (51)

Prepared from **50** according to General Procedure E.
Foam, R_f 0.25 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +32.7 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46-7.25 (m, 25H), 6.78-6.68 (m, 4H), 5.05 (d, *J* = 3.5 Hz, 1H), 4.99 (d, *J* = 11.4 Hz, 1H), 4.98 (d, *J* = 11.4 Hz, 1H), 4.97-4.84 (m, 3H), 4.80-4.67 (m, 5H), 4.59 (d, *J* = 12 Hz, 1H), 4.57 (d, *J* = 7.5 Hz, 1H), 4.47-4.43 (m, 2H), 4.48-4.02 (m, 6H), 3.89-3.67 (m, 3H), 3.76 (s, 3H), 3.51 (dd, *J* = 9.8, 2.9 Hz, 1H), 1.94 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.7, 154.2, 152.6, 138.9, 138.9, 138.6, 138.3, 137.7, 128.7-127.6 (25C), 115.8 (2C), 114.9 (2C), 103.1, 100.2, 80.4, 79.2, 77.8, 76.1, 75.2 (2C), 73.7, 73.3, 72.7, 72.5, 71.3, 68.0, 67.4, 66.3, 63.0, 56.0, 21.1.
Anal. Calcd for $C_{56}H_{60}O_{13}$: C, 71.47; H, 6.43. Found: C, 71.02; H, 6.47.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (52)

Prepared from **51** and **36** according to General Procedure B.
Syrup, R_f 0.15 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +14.7 (c 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46-7.12 (m, 40H), 6.82-6.54 (m, 8H), 5.11 (d, *J* = 3.3 Hz, 1H), 5.05-3.54 (m, 35H), 3.78 (s, 3H), 3.77 (s, 3H), 3.49 (dd, *J* = 9.8, 2.9 Hz, 1H), 1.93 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.1, 154.0 (2C), 152.8, 152.7, 139.1-137.9 (8C), 128.9-127.5 (40C), 115.7 (2C), 115.5 (2C), 114.9 (2C), 114.7 (2C), 103.1, 100.6, 100.3, 79.9, 79.7, 79.3, 77.9, 76.5, 75.9, 75.2, 75.0, 74.8, 74.5, 74.2, 73.6, 73.3, 73.2, 72.9, 72.8, 72.5, 71.3, 69.5, 69.4, 66.1, 65.7, 62.1, 56.0, 55.9, 21.2.
Anal. Calcd for $C_{90}H_{94}O_{19}$: C, 73.05; H, 6.40. Found: C, 72.99; H, 6.56.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (53)

Prepared from **52** according to General Procedure G.
Foam, R_f 0.50 (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +20.0 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48-7.17 (m, 40H), 6.87-6.55 (m, 4H), 6.78 (s, 4H), 5.09-4.52 (m, 19H), 4.38 (m, 1H), 4.29-3.45 (m, 23H), 3.24 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.2, 154.1, 152.8, 152.7, 139.0-137.9 (8C), 128.9-127.2 (40C), 115.7 (2C), 115.5 (2C), 114.9 (2C), 114.8 (2C), 103.2, 101.0, 100.7, 80.4, 79.6, 79.1 (2C), 78.1, 76.7, 75.6, 75.3 (2C), 75.0, 74.7 (2C), 73.5, 73.3, 72.9, 72.8, 72.7, 71.3, 71.0, 69.8, 65.9 (2C), 61.3, 56.0 (2C).
Anal. Calcd for $C_{88}H_{92}O_{18}$: C, 73.52; H, 6.45. Found: C, 72.99; H, 6.38.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (54)

Prepared from **53** according to General Procedures I and J.
Syrup, R_f 0.42 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +21.9 (c 1.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36-7.03 (m, 40H), 6.75-6.40 (m, 4H), 6.69 (s, 4H), 5.11 (d, *J* = 3.1 Hz, 1H), 4.95-4.36 (m, 20H), 4.29 (dd, *J* = 8.3, 5.2 Hz, 1H), 4.21 (d, *J* = 3.3 Hz, 1H), 4.08-3.34 (m, 12H), 3.67 (s, 3H), 3.66 (s, 3H), 3.17 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 154.3, 154.0, 152.9, 152.5, 139.2-137.8 (8C), 128.7-127.3 (40C), 115.6 (2C), 115.6 (2C), 115.0 (2C), 114.7 (2C), 103.3, 100.3, 100.1, 80.4, 79.5, 78.6, 77.9, 77.6, 76.0, 75.2 (2C), 75.0, 74.9, 74.3, 74.0, 73.5 (2C), 73.1, 72.9, 72.2, 71.6, 71.4, 69.8, 66.2, 65.0, 56.0, 55.9, 52.1.
Anal. Calcd for $C_{89}H_{92}O_{19}$: C, 72.93; H, 6.33. Found: C, 72.67; H, 6.50.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (55)

Prepared from **54** according to General Procedure H.
Foam, R_f 0.12 (EtOAc-hexane 3:2).
$[\alpha]_D^{20}$ +39.0 (c 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.23 (m, 40H), 5.13 (s, 1H), 5.02 (d, *J* = 3.0 Hz, 1H), 4.95 (d, *J* = 10.9 Hz, 1H), 4.93-4.86 (m, 3H), 4.84-4.53 (m, 13H), 4.47 (s, 1H), 4.43 (d, *J* = 7.5 Hz, 1H), 4.06-3.89 (m, 6H), 3.85 (s, 1H), 3.81-3.76 (m, 2H), 3.69-3.54 (m, 2H), 3.44-3.31 (m, 3H), 3.37 (s, 3H), 2.12 (bs, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.1, 138.7-138.1 (8C), 128.7-127.6 (40C), 103.2, 100.1, 99.7, 80.2, 79.3, 79.0, 77.8, 77.4, 76.8, 76.0, 75.8, 75.7, 75.2, 74.7, 74.5, 74.4, 73.6 (2C), 73.1, 72.4, 71.9, 71.5 (2C), 62.8, 60.9, 52.2.
Anal. Calcd for $C_{75}H_{80}O_{17}$: C, 71.87; H, 6.43. Found: C, 71.45; H, 6.41.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronic acid)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranosyluronic acid (56)

Prepared from **55** according to General Procedure I, purified by flash chromatography (EtOAc-hexane 2:1 + 3% AcOH, R_f 0.11) and obtained as a foam.
$[\alpha]_D^{20}$ +65.2 (c 0.6, $CHCl_3$).
^{1}H NMR (CD_3OD, 300 MHz) δ 7.51-7.20 (m, 40H), 5.30 (d, *J* = 2.8 Hz, 1H), 5.10 (d, *J* = 11.5 Hz, 1H), 4.98 (d, *J* = 3.3 Hz, 1H), 4.90-4.78 (6H, hidden under HDO-signal), 4.75-4.50 (m, 13H), 4.34-4.21 (m, 3H), 3.95-3.81 (m, 3H), 3.69-3.54 (m, 3H), 3.19 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.0, 169.5, 169.0, 138.7-137.4 (8C), 128.9-127.4 (40C), 103.1, 99.8, 99.1, 79.3, 78.6, 77.7, 77.6, 76.3, 75.4, 75.2 (2C), 74.6 (2C), 73.7 (2C), 73.3, 73.0, 72.9, 72.6, 72.4, 72.2, 71.6, 71.4, 52.1.
ESI MS: *m/z* calcd for $C_{75}H_{76}NaO_{19}$ $[M+Na]^+$ 1303.49, found 1304.54.

Benzyl *O*-(2,3-di-*O*-benzyl-4-*O*-chloroacetyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (57)

Prepared from **24** and **32** according to General Procedure B.
Syrup, R_f 0.54 (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +18.5 (c 3.3, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47-7.09 (m, 25H), 6.83-6.49 (m, 8H), 5.81 (d, *J* = 2.0 Hz, 1H), 5.05-4.44 (m, 13H), 4.19 (m, 1H), 4.12 (d, *J* = 7.0 Hz, 1H), 4.08-3.96 (m, 2H), 3.95 (s, 2H), 3.83-3.45 (m, 12H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 166.5, 153.9 (2C), 152.2 (2C), 138.3-137.3 (5C), 128.3-127.2 (25C), 115.5 (2C), 115.2 (2C), 114.5 (2C), 114.3 (2C), 102.9, 100.5, 80.6, 78.5, 76.2, 75.3, 74.9, 74.8, 73.3, 73.0, 72.8, 71.9, 71.0, 69.9, 67.0, 65.9, 65.2, 55.5 (2C), 40.7.
Anal. Calcd for $C_{63}H_{65}ClO_{14}$: C, 69.96; H, 6.06. Found: C, 69.57; H, 6.14.

Benzyl *O*-(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 (58)

Prepared from **57** according to General Procedure E.
Foam, R_f 0.32 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +21.6 (c 2.6, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.13 (m, 25H), 6.76-6.57 (m, 8H), 5.07-4.40 (m, 14H), 4.26 (m, 1H), 4.18 (d, *J* = 3.0 Hz, 1H), 4.12-3.42 (m, 14H), 2.05 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.8 (2C), 152.6, 152.6, 138.4-137.4 (5C), 128.3-127.2 (25C), 115.3 (4C), 114.5 (2C), 114.3 (2C), 102.8, 100.3, 80.7, 78.7, 77.7, 75.6, 75.0 (2C), 73.3, 72.9, 72.6, 72.0, 71.0, 68.0, 66.8, 66.6, 65.6, 55.6 (2C).
Anal. Calcd for $C_{61}H_{64}O_{13}$: C, 72.89; H, 6.42. Found: C, 72.43; H, 6.28.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-acetyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-(4-methoxyphenyl)-2,3-di-*O*-benzyl-β-D-galactopyranoside (59)

Prepared from **58** and **35** according to General Procedure B.
Syrup, R_f 0.49 (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +36.1(c 1.1, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.11 (m, 40H), 6.78 (s, 4H), 6.65-6.44 (m, 4H), 5.11-5.03 (m, 2H), 5.00-4.81 (m, 5H), 4.81-4.36 (m, 15H), 4.29-4.17 (m, 3H), 4.14-3.79 (m, 8H), 3.78-3.63 (m, 3H), 3.76 (s, 3H), 3.67 (s, 3H), 3.49 (dd, *J* = 9.9, 2.8 Hz, 1H), 1.78 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.0, 154.2, 154.0, 152.7, 152.5, 139.0-137.8 (8C), 128.6-127.3 (40C), 115.6 (2C), 115.6 (2C), 114.9 (2C), 114.7 (2C), 103.2, 100.6, 99.6, 81.2, 79.5, 79.0, 77.5, 77.3, 76.1, 75.3 (2C), 75.0 (2C), 74.7, 74.3, 73.7, 73.2, 73.0 (2C), 72.6, 71.2, 69.7, 68.5, 65.8, 64.9, 62.5, 56.0, 55.9, 20.9.
ESI MS: *m/z* calcd for $C_{90}H_{94}NaO_{19}$ $[M+Na]^+$ 1501.63, found 1502.91.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-(4-methoxyphenyl)-2,3-di-*O*-benzyl-β-D-galactopyranoside (60)

Prepared from **59** according to General Procedure G.
Foam, R_f 0.45, (hexane-EtOAc 2:1).
$[\alpha]_D^{20}$ +31.9 (c 2.3, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.12 (m, 40H), 6.76 (s, 4H), 6.65-6.46 (m, 4H), 5.12 (d, *J* = 3.3 Hz, 1H), 5.03 (d, *J* = 3.2 Hz, 1H), 4.98 (d, *J* = 11.9 Hz, 1H), 4.92-4.83 (m, 4H), 4.79-4.44 (m, 14H), 4.30-4.21 (m, 3H), 4.11-4.02 (m, 3H), 3.98-3.90 (m, 3H), 3.87-3.62 (m, 4H), 3.76 (s, 3H), 3.67 (s, 3H), 3.50 (m, 1H), 3.33 (m, 2H), 2.9 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.2, 153.9, 152.7, 152.6, 138.9-137.8 (8C), 128.6-127.4 (40C), 115.6 (2C), 115.5 (2C), 114.9 (2C), 114.7 (2C), 103.2, 100.5, 99.9, 81.1, 79.4, 79.0, 77.5, 76.2, 75.9 (2C), 75.5, 75.3, 75.1, 74.7, 73.7, 73.2, 73.1, 73.0 (2C), 72.9, 71.2, 70.8, 69.8, 65.9, 65.0, 62.5, 56.0, 55.9.
ESI MS: *m/z* calcd for $C_{88}H_{92}NaO_{18}$ $[M+Na]^+$ 1459.62, found 1460.49.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-(4-methoxyphenyl)-2,3-di-*O*-benzyl-β-D-galactopyranoside (61)

Prepared from **60** according to General Procedures I and J.
Foam, R_f 0.14 (hexane-EtOAc 3:1).
$[\alpha]_D^{20}$ +3.5 (c 2.4, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.04 (m, 40H), 6.78 (s, 4H), 6.65-6.47 (m, 4H), 5.18 (d, *J* = 3.8 Hz, 1H), 5.07 (d, *J* = 3.4 Hz, 1H), 4.99-4.81 (m, 6H), 4.76-4.41 (m, 13H), 4.35 (d, *J* = 1.7 Hz, 1H), 4.24 (t, *J* = 1.9 Hz, 1H), 4.22-4.17 (m, 2H), 4.09-4.04 (m, 2H), 3.97 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.88 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.80 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.76 (s, 3H), 3.75-3.62 (m, 4H), 3.67 (s, 3H), 3.48 (dd, *J* = 10.0, 2.8 Hz, 1H), 3.22 (s, 3H).

^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.5, 154.0, 153.8, 152.5, 152.2, 138.6-137.6 (8C), 128.3-126.9 (40C), 115.3 (4C), 114.7 (2C), 114.5 (2C), 102.9, 100.5, 99.4, 81.0, 78.6 (2C), 77.9, 76.7, 74.9 (3C), 74.5, 74.4, 74.3, 73.6, 73.0, 72.8, 72.7 (2C), 71.8, 71.5, 71.0, 69.1, 65.4, 64.2, 55.7, 55.6, 51.7.
ESI MS: *m/z* calcd for $C_{89}H_{92}NaO_{19}$ $[M+Na]^+$ 1487.61, found 1487.29.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (62)

Prepared from **61** according to General Procedure H.
Foam, R_f 0.12 (hexane-EtOAc 3:2).
$[\alpha]_D^{20}$ +48.3 (c 0.8, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42-7.22 (m, 40H), 5.01 (d, *J* = 3.4 Hz, 1H), 4.98 (d, *J* = 3.4 Hz, 1H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.91-4.55 (m, 16H), 4.41 (d, *J* = 7.7 Hz, 1H), 4.34 (t, *J* = 2.1 Hz, 1H), 4.14 (dd, *J* = 10.2, 3.4 Hz, 1H), 4.08 (d, *J* = 2.9 Hz, 1H), 4.04 (t, *J* = 6.2 Hz, 1H), 4.00-3.95 (m, 3H), 3.88 (dd, *J* = 10.7, 3.2 Hz, 1H), 3.72-3.62 (m, 4H), 3.59 (dd, *J* = 11.1, 6.0 Hz, 1H), 3.48-3.37 (m, 2H), 3.43 (s, 3H), 1.73 (bs, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 169.7, 138.9-137.8 (8C), 128.9-127.4 (40C), 103.3, 100.5, 100.3, 80.8, 79.4, 78.9, 77.6, 77.1, 76.8, 76.4, 76.2, 75.7, 75.4, 75.0, 74.7, 74.6, 74.3, 73.1 (2C), 72.0 (3C), 71.5, 61.6, 60.5, 52.3.
ESI MS: *m/z* calcd for $C_{75}H_{80}NaO_{17}$ $[M+Na]^+$ 1275.53, found 1276.27.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-α-D-galactopyranosyluronic acid)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranosyluronic acid (63)

Prepared from **62** according to General Procedure I, purified by flash chromatography (EtOAc-hexane 2:1 + 3% AcOH, R_f 0.15) and obtained as a foam.
$[\alpha]_D^{20}$ +72.6 (c 0.6, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52-7.08 (m, 40H), 5.38 (bs, 1H), 5.23 (d, *J* = 3.3 Hz, 1H), 5.05 (d, *J* = 11.7 Hz, 1H), 4.94-4.39 (m, 20H), 4.18-4.11 (m, 3H), 3.90 (s, 3H), 3.86 (m, 1H), 3.69-3.52 (m, 4H).
^{13}C NMR (CD_3OD, 75 MHz) δ 170.8, 170.4, 170.0, 138.9-137.9 (8C), 128.6-127.2 (40C), 102.8, 98.3, 97.7, 79.7, 77.8, 77.6, 76.6, 76.1, 76.0, 75.1, 74.6 (2C), 74.5, 73.2, 73.1, 73.0, 72.4 (2C), 72.2, 71.5, 71.3, 71.2, 70.8, 51.2.
ESI MS: *m/z* calcd for $C_{75}H_{76}NaO_{19}$ $[M+Na]^+$ 1303.49, found 1304.46.

Pent-4-enyl *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (70)

Prepared from **29** and **35** according to General Procedure C or from **29** and **69** (see Table 2, Chapter 3).
$[\alpha]_D^{20}$ +38.3 (*c* 0.5, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46-7.18 (m, 25H), 5.82 (m, 1H), 5.03 (dq, *J* = 16.8, 1.5 Hz, 1H), 4.99-4.66 (m, 11H), 4.59 (d, *J* = 11.2 Hz, 1H), 4.44-4.29 (m, 4H), 4.13-4.02 (m, 3H), 3.98-3.86 (m, 4H), 3.64 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.55 (dt, *J* = 9.7, 7.1 Hz, 1H), 3.49 (t, *J* = 6.6 Hz, 1H), 3.38 (dd, *J* = 10.2, 3.1 Hz, 1H), 2.17 (m, 2H), 2.01 (s, 3H), 1.81 (s, 3H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 170.59, 170.22, 138.83, 138.79, 138.60, 138.54, 138.43, 138.17, 115.03, 104.07, 100.46, 80.23, 79.14, 79.05, 76.54, 75.57, 75.15, 74.71, 74.60, 73.99, 73.10, 72.78, 72.41, 69.69, 69.01, 62.70, 62.65, 30.37, 29.14, 21.02, 20.89.
ESI MS: *m/z* calcd for $C_{56}H_{64}NaO_{13}$ $[M+Na]^+$ 967.4, found 967.5.

Pent-4-enyl *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (72)

Prepared from **31** and **35** according to General Procedure C.
$[\alpha]_D^{20}$ +33.0 (*c* 1.2, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.21 (m, 25H), 6.73-6.64 (m, 4H), 5.84 (m, 1H), 5.04 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.03 (d, *J* = 3.4 Hz, 1H), 4.98 (bd, *J* = 10.2 Hz, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.93 (d, *J* = 11.1 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.81 (d, *J* = 12.4 Hz, 1H), 4.80 (d, *J* = 11.1 Hz, 1H), 4.79 (d, *J* = 11.9 Hz, 1H), 4.73 (d, *J* = 12.6 Hz, 1H), 4.71 (d, *J* = 12.6 Hz, 1H), 4.58 (d, *J* = 12.4 Hz, 1H), 4.57 (d, *J* = 11.1 Hz, 1H), 4.43-4.35 (m, 3H), 4.13 (d, *J* = 3.0 Hz, 1H), 4.09 (dd, *J* = 10.2, 7.7 Hz, 1H), 4.08-3.89 (m, 6H), 3.74 (s, 3H), 3.69 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.63 (dd, *J* = 7.7, 6.0 Hz, 1H), 3.58 (dt, *J* = 9.4, 6.8 Hz, 1H), 3.46 (dd, *J* = 9.8, 3.0 Hz, 1H), 2.19 (m, 2H), 1.80 (s, 3H), 1.79 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 170.19, 153.87, 152.29, 138.71, 138.52, 138.43, 138.36, 138.32, 137.94, 115.36 (2C), 114.87, 114.54 (2C), 103.89, 99.92, 80.24, 78.89, 78.74, 76.29, 74.84, 74.47, 74.38, 74.33, 73.30, 72.87, 72.73, 72.39, 69.21, 68.61, 65.87, 62.47, 55.52, 30.18, 28.99, 20.63.
ESI MS: *m/z* calcd for $C_{61}H_{68}NaO_{13}$ $[M+Na]^+$ 1031.5, found 1031.6.

Pent-4-enyl O-(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 (73)

The donor (Pent-4-enyl 4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside) was prepared by acetylation of **31**, data:
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.22 (m, 10H), 7.85-7.78 (m, 4H), 5.81 (m, 1H), 5.64 (bs, 1H), 5.07-4.95 (m, 2H), 4.89 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 10.9 Hz, 1H), 4.75 (d, *J* = 10.9 Hz, 1H), 4.57 (d, *J* = 11.1 Hz, 1H), 4.41 (m, 1H), 4.08 (dd, *J* = 10.5, 7.2 Hz, 1H), 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 ($CDCl_3$, 75 MHz) 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.
This compound and **31** were treated according to General Procedure C, affording **73**.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39-7.12 (m, 20H), 7.77-7.56 (m, 8H), 5.82 (m, 1H), 5.74 (bs, 1H), 5.08-4.91 (m, 3H), 4.83-4.60 (m, 7H), 4.58-4.44 (m, 3H), 4.39 (d, *J* = 10.3 Hz, 1H), 4.17 (d, *J* = 2.1 Hz, 1H), 4.13 (dd, *J* = 11.1, 2.0 Hz, 1H), 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 ($CDCl_3$, 75 MHz, partial) 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
ESI MS: *m/z* calcd for $C_{61}H_{68}NaO_{14}$ $[M+Na]^+$ 1047.5, found 1047.5.

Benzyl 6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-(4-oxopentanoyl)-β-D-galactopyranoside (74)

Prepared by acylation of **23** with freshly prepared levulinic anhydride (levulinic acid, DCC, Et_2O).[76]
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41-7.23 (m, 15H), 5.50 (d, *J* = 2.8 Hz, 1H), 4.95 (d, *J* = 12.1 Hz, 1H), 4.90 (d, *J* = 11.0 Hz, 1H), 4.75 (d, *J* = 10.6 Hz, 1H), 4.72 (d, *J* = 11.2 Hz, 1H), 4.68 (d, *J* = 11.9 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.49 (d, *J* = 7.5 Hz, 1H), 4.27-4.10 (m, 2H), 3.76 (bt, *J* = 6.8 Hz, 1H), 3.65 (dd, *J* = 9.3, 7.5 Hz, 1H), 3.56 (dd, *J* = 9.7, 3.4 Hz, 1H), 2.77-2.63 (m, 4H), 2.16 (s, 3H), 2.08 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.89, 172.02, 170.39, 138.34, 137.66, 137.02, 128.29 (2C), 128.16 (3C), 128.10 (3C), 127.96 (2C), 127.87 (2C), 127.73, 127.56, 127.46, 102.30, 78.95, 78.65, 75.23, 72.04, 71.04, 70.74, 66.75, 62.02, 37.96, 29.65, 28.00, 20.68.

Pent-4-enyl 2,3-di-*O*-benzyl-6-O-(4-methoxyphenyl)-4-*O*-(4-oxopentanoyl)-β-D-galactopyranoside (75)

Prepared by acylation of **31** with freshly prepared levulinic anhydride.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39-7.24 (m, 10H), 6.89-6.78 (m, 4H), 5.84 (m, 1H), 5.63 (d, *J* = 2.5 Hz, 1H), 5.02 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.97 (bd, *J* = 10.2 Hz, 1H), 4.89 (d, *J* = 11.4

Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 1H), 4.74 (d, *J* = 11.5 Hz, 1H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.42 (m, 1H), 4.10 (dd, *J* = 11.2, 6.4 Hz, 1H), 4.01-3.91 (m, 2H), 3.86 (bt, *J* = 6.4 Hz, 1H), 3.76 (s, 3H), 3.61-3.52 (m, 3H), 2.79-2.57 (m, 4H), 2.18 (m, 2H), 2.11 (s, 3H), 1.78 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.31, 172.32, 154.48, 152.84, 138.83, 138.23, 138.14, 128.51 (2C), 128.49 (3C), 128.28 (2C), 128.52, 127.88, 127.81, 116.34 (2C), 115.16, 114.87 (2C), 104.00, 79.42, 79.13, 75.55, 72.34, 72.07, 69.87, 67.66, 67.51, 55.95, 38.34, 30.44, 29.99, 29.21, 28.37.

Pent-4-enyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-6-*O*-(4-methoxyphenyl))-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (76)

Prepared from **29** and **36** according to General Procedure D.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44-7.12 (m, 25H), 6.80-6.58 (m, 4H), 5.82 (m, 1H), 5.06-7.71 (m, 10H), 4.63 (d, *J* = 11.3 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.56-4.40 (m, 4H), 4.38 (d, *J* = 7.2 Hz, 1H), 4.18-4.12 (m, 2H), 4.01-3.68 (m, 8H), 3.78 (s, 3H), 3.42 (dd, *J* = 10.3, 2.3 Hz, 1H), 2.22 (m, 2H), 2.04 (s, 3H), 1.79 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.5, 154.1, 153.0, 138.9, 138.7, 138.6 (2C), 138.4, 138.2, 128.6 (3C), 128.5 (8C), 128.3 (5C), 128.3 (3C), 127.9 (2C), 127.8 (2C), 127.7 (2C), 115.5 (2C), 115.2, 114.8 (2C), 104.1, 101.2, 80.8, 79.2, 78.8, 76.7, 76.1, 75.3 (2C), 74.7, 74.1, 73.0, 72.9, 72.7, 70.0, 69.6, 66.0, 62.4, 56.0, 30.5, 29.3, 21.2.

Pent-4-enyl 4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (77)

Alcohol **31** (5.35 g, 10 mmol) was dissolved in anhydrous THF (30 mL). NaH (720 mg, 30 mmol) was added and the resulting suspension stirred for 2 h before addition of allyl bromide (1.3 mL, 15 mmol). The mixture was stirred 24 h, then diluted with Et_2O and washed twice with water. The combined aqueous phases were extracted with Et_2O, and the combined organic phases were dried, concentrated, and purified by flash chromatography to afford a white solid (6.32 g, 91%).
mp 52.5-53 °C.
$[\alpha]_D^{20}$ –36.3 (*c* 0.9, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.26 (m, 10H), 6.91-6.81 (m, 4H), 5.90-5.78 (m, 2H), 5.17 (bd, *J* = 17.5 Hz, 1H), 5.07 (bd, *J* = 10.2 Hz, 1H), 5.02 (dq, *J* = 17.1, 2.1 Hz, 1H), 4.99 (bd, *J* = 10.2 Hz, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.79 (d, *J* = 12.2 Hz, 1H), 4.78 (d, *J* = 11.1 Hz, 1H), 4.77 (d, *J* = 12.2 Hz, 1H), 4.39 (d, *J* = 7.7 Hz, 1H), 4.37 (dd, *J* = 12.4, 5.6 Hz, 1H), 4.16 (t, *J* = 8.3 Hz, 1H), 4.14 (dd, *J* = 12.8, 6.8 Hz, 1H), 4.07 (dd, *J* = 9.0, 5.1 Hz, 1H), 3.99-3.92 (m, 2H), 3.80 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.78 (s, 3H), 3.70 (dd, *J* = 7.3, 5.6 Hz, 1H), 3.58-3.51 (m, 2H), 2.17 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.14, 152.63, 138.86, 138.52, 138.18, 135.43, 128.39, 128.29 (3C), 128.10 (3C), 127.60 (2C), 127.55, 117.22, 115.61 (2C), 114.85, 114.74 (2C), 104.05, 81.96, 79.68, 75.29, 73.97, 73.08, 73.00, 72.76, 69.37, 66.67, 55.74, 30.32, 29.06.
Anal. Calcd for $C_{35}H_{42}O_7$: C, 73.15; H, 7.37. Found: C, 73.12; H, 7.37.

Pent-4-enyl *O*-(4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl -β-D-galactopyranoside (78α)

Major product from subjecting **77** and **29** to General Procedure D.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43-7.18 (m, 20H), 6.80-6.61 (m, 4H), 5.97-5.78 (m, 2H), 5.20-4.60 (m, 14H), 4.51-4.32 (m, 6H), 4.14-4.05 (m, 2H), 4.00-3.86 (m, 2H), 3.74 (s, 3H), 3.72-3.48 (m, 5H), 3.51 (dd, *J* = 9.2, 3.3 Hz, 1H), 2.21 (m, 2H), 2.04 (s, 3H), 1.79 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.8, 154.1, 152.8, 138.9, 138.8 (2C), 138.3, 137.9, 135.7, 128.7 (3C), 128.5 (6C), 128.4 (3C), 128.3 (2C), 128.0 (2C), 127.8, 127.7 (3C), 117.5, 115.5 (2C), 115.2, 114.8 (2C), 104.2, 101.0, 81.2, 79.1, 78.9, 76.0, 75.0, 74.0 (2C), 73.9, 73.1, 72.8 (2C), 72.6, 69.7, 69.4, 65.8, 62.3, 55.9, 30.5, 28.9, 21.2.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-chloroacetyl-β-D-galactopyranoside (79)

Prepared according to the General Procedure A
mp 62-63.5 °C.
$[\alpha]_D^{20}$ +0.7 (*c* 0.9, $CHCl_3$).
IR (KBr) 1745 cm^{-1}.
^1H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.26 (m, 10H), 5.83 (m, 1H), 5.03 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.98 (bd, *J* = 10.2 Hz, 1H), 4.92 (d, *J* = 11.1 Hz, 1H), 4.76 (d, *J* = 11.7 Hz, 1H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.49 (dd, *J* = 11.1, 6.8 Hz, 1H), 4.42 (dd, *J* = 11.5, 5.1 Hz, 1H), 4.34 (d, *J* = 8.1 Hz, 1H), 4.08 (s, 2H), 3.94 (dt, *J* = 9.8, 6.6 Hz, 1H), 3.92 (m, 1H), 3.66-3.61 (m, 2H), 3.56 (dt, *J* = 9.4, 6.8 Hz, 1H), 3.51 (dd, *J* = 9.4, 3.4 Hz, 1H), 2.46 (bs, 1H), 2.17 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.12, 138.56, 138.08, 137.78, 128.56, 128.37 (2C), 128.12 (3C), 128.06, 127.90 (2C), 127.71, 114.98, 103.72, 80.34, 78.80, 75.24, 72.82, 71.61, 69.44, 66.81, 64.82, 40.81, 30.29, 29.05.
Anal. Calcd for $C_{27}H_{33}ClO_7$: C, 64.22; H, 6.59. Found: C, 64.14; H, 6.53.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-trichloroacetyl-β-D-galactopyranoside (80)

Prepared according to General Procedure A.
mp 52.5-54.5 °C.
$[\alpha]_D^{20}$ +0.2 (*c* 1.0, $CHCl_3$).
IR (KBr) 1757 cm^{-1}.
^1H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.27 (m, 10H), 5.81 (m, 1H), 5.01 (dd, *J* = 17.1, 1.7 Hz, 1H), 4.97 (bd, *J* = 10.2 Hz, 1H), 4.93 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 11.5 Hz, 1H), 4.74 (d, *J* = 11.1 Hz, 1H), 4.70 (d, *J* = 11.5 Hz, 1H), 4.68 (dd, *J* = 11.5, 7.7 Hz, 1H), 4.54 (dd, *J* = 11.5, 5.1 Hz, 1H), 4.36 (d, *J* = 8.1 Hz, 1H), 3.93 (dt, *J* = 9.8, 6.4 Hz, 1H), 3.91 (m, 1H), 3.71 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.64 (dd, *J* = 9.4, 8.1 Hz, 1H), 3.54-3.50 (m, 2H), 2.48 (bs, 1H), 2.15 (m, 2H), 1.74 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.77, 138.57, 138.05, 137.76, 128.65, 128.43 (3C), 128.17 (2C), 127.97 (3C), 127.78, 115.07, 103.73, 80.24, 78.77, 75.27, 73.09, 71.40, 69.44, 67.63, 66.91, 53.55, 30.33, 29.01.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-(4-nitrobenzoyl)-β-D-galactopyranoside (81)

Prepared according to General Procedure A.
mp 97-98.5 °C.
$[\alpha]_D^{20}$ –5.4 (*c* 0.9, $CHCl_3$).
IR (KBr) 1718, 1527, 1296 cm^{-1}.
^1H NMR ($CDCl_3$, 500 MHz) δ 8.30 (m, 2H), 8.21 (m, 2H), 7.39-7.26 (m, 10H), 5.80 (m, 1H), 5.01 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.95 (bd, *J* = 10.2 Hz, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.75 (d, *J* = 11.1 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.66-4.63 (m, 2H), 4.38 (d, *J* = 8.1 Hz, 1H), 3.97 (m, 1H), 3.93 (dt, *J* = 9.4, 6.4 Hz, 1H), 3.75 (t, *J* = 6.2 Hz, 1H), 3.67 (dd, *J* = 9.4, 8.1 Hz, 1H), 3.58-3.52 (m, 2H), 2.52 (bs, 1H), 2.16 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.55, 150.79, 138.57, 138.05, 137.85, 135.43, 130.92 (2C), 128.65 (2C), 128.47, 128.21 (3C), 128.15, 127.97 (2C), 127.82, 123.68 (2C), 115.06, 103.88, 80.48, 78.88, 75.35, 73.04, 71.87, 69.51, 67.10, 64.62, 30.34, 29.11.
Anal. Calcd for $C_{32}H_{35}NO_9$: C, 66.54; H, 6.11; N, 2.42. Found: C, 66.31; H, 6.05; N, 2.45.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-(3,5-dinitrobenzoyl)-β-D-galactopyranoside (82)

Prepared according to General Procedure A.
mp 119-121 °C.
$[\alpha]_D^{20}$ +2.5 (*c* 1.0, $CHCl_3$).
IR (KBr) 1736, 1545, 1345 cm^{-1}.
^1H NMR ($CDCl_3$, 500 MHz) δ 9.24 (t, *J* = 2.1 Hz, 1H), 9.16 (d, *J* = 2.1 Hz, 2H), 7.39-7.28 (m, 10H), 5.80 (m, 1H), 5.00 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.93 (bd, *J* =

10.2 Hz, 1H), 4.81-4.68 (m, 5H), 4.41 (d, *J* = 7.7 Hz, 1H), 4.00-3.94 (m, 2H), 3.79 (t, *J* = 6.2 Hz, 1H), 3.68 (dd, *J* = 9.4, 7.7 Hz, 1H), 3.61-3.55 (m, 2H), 2.56 (bs, 1H), 2.17 (m, 2H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.48, 148.92, 138.62, 138.14, 137.83, 133.89, 129.67 (2C), 128.76 (2C), 128.55 (2C), 128.29 (2C), 128.22, 128.15 (2C), 128.06, 127.92, 122.69, 115.10, 103.95, 80.38, 78.91, 75.45, 73.16, 71.87, 69.69, 67.25, 65.81, 30.39, 29.19.
Anal. Calcd for $C_{32}H_{34}N_2O_{11}$: C, 61.73; H, 5.50; N, 4.50. Found: C, 61.58; H, 5.48; N, 4.50.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-galactopyranoside (83)

Prepared according to the General Procedure A.
mp 94-95 °C.
$[\alpha]_D^{20}$ –2.3 (*c* 0.9, $CHCl_3$).
IR (KBr) 1729 cm^{-1}.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39-7.27 (m, 10H), 5.81 (m, 1H), 5.01 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.96 (m, 1H), 4.94 (d, *J* = 11.1 Hz, 1H), 4.77 (d, *J* = 11.7 Hz, 1H), 4.74 (d, *J* = 11.1 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.69 (dd, *J* = 11.5, 7.3 Hz, 1H), 4.59 (dd, *J* = 11.5, 5.1 Hz, 1H), 4.36 (d, *J* = 7.7 Hz, 1H), 3.98-3.92 (m, 2H), 3.71 (t, *J* = 6.2 Hz, 1H), 3.65 (dd, *J* = 9.2, 7.9 Hz, 1H), 3.55 (dt, *J* = 9.4, 6.4 Hz, 1H), 3.53 (dd, *J* = 9.4, 3.4 Hz, 1H), 2.48 (bs, 1H), 2.17 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.73, 145.68 (bd, $J_{C\text{-}F}$ = 258 Hz, 2C), 143.27 (bd, $J_{C\text{-}F}$ = 259 Hz, 1C), 138.62, 138.07, 138.02 (bd, $J_{C\text{-}F}$ = 251 Hz, 2C), 137.81, 128.58 (2C), 128.39 (2C), 128.14 (2C), 128.07, 127.91 (2C), 127.73, 114.91, 108.06 (dt, $J_{C\text{-}F}$ = 11, 4 Hz, 1C), 103.76, 80.34, 78.84, 75.26, 72.89, 71.72, 69.34, 66.91, 65.40, 30.30, 29.02.
Anal. Calcd for $C_{32}H_{31}F_5O_7$: C, 61.73; H, 5.02. Found: C, 61.71; H, 4.91.

Pent-4-enyl *O*-(4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (84α)

Major product from subjecting **78**, **85**, **86**, **87**, **88**, or **89** to General Procedure G.
$[\alpha]_D^{20}$ +32.7 (*c* 0.7, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44-7.13 (m, 20H), 6.78-6.69 (m, 4H), 5.89-5.76 (m, 2H), 5.16 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.09-5.04 (m, 2H), 5.01 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.96 (bd, *J* = 10.2 Hz, 1H), 4.91 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 11.1 Hz, 1H), 4.81 (d, *J* = 11.5 Hz, 1H), 4.76 (d, *J* = 11.5 Hz, 1H), 4.69 (d, *J* = 11.9 Hz, 1H), 4.68 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.1 Hz, 1H), 4.64 (d, *J* = 11.9 Hz, 1H), 4.38 (dd, *J* = 8.5, 5.1 Hz, 1H), 4.34 (ddq, *J* = 12.8, 6.8, 1.3 Hz, 1H), 4.31 (d, *J* = 7.3 Hz, 1H), 4.10 (bdd, *J* = 12.4, 6.0 Hz, 1H), 4.08-4.02 (m, 4H), 3.96 (t, *J* = 8.7 Hz, 1H), 3.91 (dt, *J* = 9.8, 6.4 Hz, 1H), 3.79-3.72 (m, 2H), 3.72 (s, 3H), 3.64 (m, 1H), 3.62 (dd, *J* = 9.8, 7.3 Hz, 1H), 3.51 (dt, *J* = 9.8, 6.8 Hz, 1H), 3.47 (bt, *J* = 7.0 Hz, 1H), 3.42 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.27 (bs, 1H), 2.15 (m, 2H), 1.74 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 153.88, 152.72, 138.60, 138.46, 138.41, 138.02, 137.58, 135.30, 117.14, 115.45 (2C), 114.90, 114.58 (2C), 104.05 ($J_{C\text{-}H}$ = 156.7 Hz), 100.39 ($J_{C\text{-}H}$ = 168.1 Hz), 81.23, 79.08, 78.69, 77.54, 75.83, 75.02, 74.84, 74.13, 74.07, 73.97, 72.96, 72.31, 69.59, 69.57, 66.18, 60.18, 55.66, 30.21, 28.98.

Pent-4-enyl *O*-(4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (84β)

Minor product from subjecting **78**, **85**, **86**, **87**, **88**, or **89** to General Procedure G.
$[\alpha]_D^{20}$ +10.1 (*c* 1.1, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.46-7.13 (m, 20H), 6.81-6.67 (m, 4H), 5.91-5.76 (m, 2H), 5.23 (d, *J* = 11.1 Hz, 1H), 5.22 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.14 (bd, *J* = 10.2 Hz, 1H), 5.00 (dq, *J* = 17.1, 1.7 Hz, 1H), 4.95 (bd, *J* = 10.2 Hz, 1H), 4.83-4.68 (m, 7H), 4.44 (d, *J* = 11.1 Hz, 1H), 4.43 (ddt, *J* = 12.8, 5.1, 1.3 Hz, 1H), 4.35 (d, *J* = 7.3 Hz, 1H), 4.22 (d, *J* = 2.6 Hz, 1H), 4.13 (dd, *J* = 9.4, 7.7 Hz, 1H), 4.07 (bdd, *J* = 12.8, 6.4 Hz, 1H), 4.01 (dd, *J* = 9.8, 5.1 Hz, 1H), 3.97 (m, 1H), 3.94 (dt, *J* = 9.4, 6.6 Hz, 1H), 3.92 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.77 (s, 3H), 3.74

(d, *J* = 3.0, 1H), 3.73-3.67 (m, 2H), 3.62 (m, 1H), 3.56 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.52 (dt, *J* = 9.4, 6.8 Hz, 1H), 3.50-3.44 (m, 2H), 3.41 (bs, 1H), 2.15 (m, 2H), 1.76 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 154.53, 152.30, 139.29, 138.86, 138.73, 138.63, 138.23, 135.21, 117.17, 115.97 (2C), 114.94, 114.91 (2C), 104.90 (J_{C-H} = 161.9 Hz), 104.05 (J_{C-H} = 161.3 Hz), 81.66, 81.29, 80.34, 79.53, 75.42, 75.12, 73.99, 73.85, 73.81, 73.59, 73.31, 73.14, 72.83, 69.55, 67.96, 59.79, 55.88, 30.36, 29.11.
Anal. Calcd for $C_{55}H_{64}O_{12}$: C, 72.03; H, 7.03. Found: C, 71.81; H, 7.20.

Pent-4-enyl *O*-(4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-galactopyranoside (89α)

Major product from subjecting **83** and **77** to General Procedure D.
$[\alpha]_D^{20}$ +32.5 (*c* 1.7, $CHCl_3$).
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44-7.17 (m, 20H), 6.76-6.63 (m, 4H), 5.87-5.77 (m, 2H), 5.13 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.06-4.99 (m, 3H), 4.96 (bd, *J* = 10.2 Hz, 1H), 4.88 (d, *J* = 11.5 Hz, 1H), 4.86 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.82 (d, *J* = 11.9 Hz, 1H), 4.78-4.62 (m, 6H), 4.43 (dd, *J* = 9.0, 4.7 Hz, 1H), 4.36-4.31 (m, 2H), 4.12-4.02 (m, 4H), 3.97-3.89 (m, 3H), 3.72 (s, 3H), 3.69 (dd, *J* = 8.1, 4.7 Hz, 1H), 3.66 (dd, *J* = 10.8, 8.3 Hz, 1H), 3.60 (t, *J* = 6.4 Hz, 1H), 3.54 (dt, *J* = 9.4, 6.8 Hz, 1H), 3.41 (dd, *J* = 9.8, 2.6 Hz, 1H), 2.17 (m, 2H), 1.77 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 158.36, 153.86, 152.66, 145.40 (bd, J_{C-F} = 263 Hz, 2C), 143.20 (bd, J_{C-F} = 253 Hz, 1C), 138.70, 138.57, 138.50, 138.26, 137.98, 137.59 (bd, J_{C-F} = 242 Hz, 2C), 135.43, 116.97, 115.31 (2C), 114.87, 114.53 (2C), 107.91 (m, 1C), 103.99, 101.17, 80.67, 79.07, 78.76, 76.98, 76.51, 75.18, 74.39, 74.21, 73.99, 73.16, 72.58, 72.31, 69.67, 69.46, 65.79, 64.79, 55.57, 30.23, 28.95.
ESI MS: *m/z* calcd for $C_{62}H_{63}F_5NaO_{13}$ $[M+Na]^+$ 1133.4, found 1133.2.

Pent-4-enyl 2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-glucopyranoside (91)

Prepared according to General Procedure A, except that pent-4-enyl 2,3-di-*O*-benzyl-β-D-glucopyranoside[151] was the starting material.
mp: 48-49.5 (EtOAC/Hexane).
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40-7.25 (m, 10H), 5.80 (m, 1H), 5.03-4.92 (m, 4H), 4.72 (d, *J* = 10.9 Hz, 1H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.57 (bd, *J* = 10.5 Hz, 1H), 4.42 (bs, 1H), 3.92 (dt, *J* = 9.3, 6.5 Hz, 1H), 3.59-3.41 (m, 5H), 2.23 (bs, 1H), 2.18 (m, 2H), 1.77 (m, 2H).

Pent-4-enyl *O*-(2,3,4,6-tetra-*O*-benzyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-galactopyranoside (92α)

Prepared from **83** and **65** according to General Procedure D.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39-7.23 (m, 30H), 5.97 (m, 1H), 5.22-4.67 (m, 12H), 4.58-4.01 (m, 11H), 3.91-3.40 (m, 7H), 2.30 (m, 2H), 1.92 (m, 2H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.3, 146.0 (bd, J_{C-F} = 258 Hz, 2C), 143.4 (bd, J_{C-F} = 259 Hz, 1C), 139.1, 138.9 (2C), 138.8 (2C), 138.6 (2C), 137.9 (bd, J_{C-F} = 250 Hz, 2C), 128.7 (2C), 128.6 (6C), 128.5 (6C), 128.4 (3C), 128.1 (3C), 128.0 (3C), 127.9 (3C), 127.8 (4C), 115.3, 108.1, 104.1, 101.2, 80.8, 79.6, 79.0, 76.2, 75.7, 75.5, 75.4, 74.3, 73.6, 73.1, 72.8, 72.5, 70.1, 70.0, 69.7, 68.1, 64.4, 30.6, 29.3.

Pent-4-enyl *O*-(2,3,4,6-tetra-*O*-benzyl)-α-D-glucopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-β-D-glucopyranoside (93α)

Major product from subjecting **91** and **90** to General Procedure D.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50-7.20 (m, 30H), 5.91 (m, 1H), 5.63 (d, *J* = 2.9 Hz, 1H), 5.28-4.50 (m, 18H), 4.19-3.48 (m, 11H), 2.25 (m, 2H), 1.83 (m, 2H).

[151] Juul, K. M.; Madsen, R., *unpublished results*.

^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.4, 145.8 (bd, $J_{C\text{-}F}$ = 259 Hz, 2C), 143.2 (bd, $J_{C\text{-}F}$ = 258 Hz, 1C), 139.2, 139.0, 138.7, 138.6, 138.5, 138.2 (2C), 137.8 (bd, $J_{C\text{-}F}$ = 250 Hz, 2C), 128.7 (4C), 128.6 (4C), 128.5 (4C), 128.4 (3C), 128.3 (4C), 128.2 (2C), 128.1 (4C), 128.0 (2C), 127.9 (2C), 127.0, 115.5, 108.1, 103.9, 98.2, 84.3, 82.2, 82.0, 79.7, 78.1, 75.9, 75.8, 75.2, 74.4, 74.2, 73.8, 73.6, 72.9, 72.0, 69.3, 68.3, 66.0, 30.8, 29.2.

_O_-(Methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-(methyl D-galacturonate) (94)

Prepared from **104** according to the General Procedure M.
^{13}C NMR (D_2O, 75 MHz) δ 171.62, 170.94, 170.08, 100.67, 99.95, 99.85, 96.08, 92.76, 79.12, 78.87, 78.30, 78.19, 73.47, 71.69, 71.50, 71.32, 70.77, 70.22, 68.84, 68.64, 68.27, 68.17, 68.05, 67.99, 53.22, 53.14, 53.04.
ESI MS/MS: *m/z* 1114.6, 925.1, 749.0, 572.8.
ESI HRMS calcd for $C_{39}H_{56}NaO_{37}$ $[M + Na]^+$ *m/e* 1139.2398, found *m/e* 1139.2382.

_O_-α-D-Galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-D-galacturonic acid (95)

Prepared from **107** according to the General Procedure M.
^{13}C NMR (D_2O, 75 MHz) δ 171.48, 171.38, 101.08, 100.30, 96.90, 82.49, 79.67, 79.52, 79.21, 78.75, 78.70, 72.79, 72.72, 72.02, 71.28, 71.23, 69.97, 69.31, 69.07, 68.87, 68.73, 68.67, 68.51, 53.60.
ESI MS/MS: *m/z* 1115.1, 939.0, 749.0, 559.0, 368.7.
ESI HRMS calcd for $C_{39}H_{56}NaO_{37}$ $[M + Na]^+$ *m/e* 1139.2398, found *m/e* 1139.2406.

_O_-(Methyl α-D-galactopyranosyluronate)-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-(methyl D-galacturonate) (96)

Prepared from **113** according to the General Procedure M.
^{13}C NMR (D_2O, 75 MHz) δ 171.67, 170.93, 170.05, 99.93, 96.59, 92.63, 78.83, 78.41, 78.12, 73.45, 71.66, 71.44, 71.30, 70.25, 70.06, 68.90, 68.74, 68.16, 68.03, 53.19, 53.14, 53.03.
ESI MS/MS: *m/z* 1100.9, 910.9, 734.9, 558.9, 382.8.
ESI HRMS calcd for $C_{38}H_{54}NaO_{37}$ $[M + Na]^+$ *m/e* 1125.2242, found *m/e* 1125.2218.

_O_-α-D-Galactopyranosyluronic acid-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-D-galacturonic acid (97)

Prepared from **114** according to the General Procedure M.
^{13}C NMR (D_2O, 75 MHz) δ 170.96, 170.86, 100.60, 99.90, 96.40, 79.22, 78.90, 78.24, 72.18, 71.54, 70.81, 70.61, 69.31, 68.04, 53.14.
ESI MS/MS: *m/z* 1128.9, 952.9, 762.9, 572.8, 382.8.
ESI HRMS calcd for $C_{40}H_{58}NaO_{37}$ $[M + Na]^+$ *m/e* 1153.2555, found *m/e* 1153.2551.

_O_-(Methyl α-D-galactopyranosyluronate)-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-_O_-α-D-galactopyranosyluronic acid-(1→4)-_O_-(methyl α-D-galactopyranosyluronate)-(1→4)-D-galacturonic acid (98)

Prepared from **122** according to the General Procedure M.
^{13}C NMR (D_2O, 75 MHz) δ 171.67, 171.02, 99.90, 94.45, 92.61, 82.00, 78.77, 78.68, 78.16, 72.14, 71.45, 70.77, 70.25, 68.92, 68.59, 68.53, 68.22, 53.13, 53.05.
ESI MS/MS: *m/z* 1115.1, 939.1, 749.0, 572.9, 382.9.
ESI HRMS calcd for $C_{39}H_{56}NaO_{37}$ $[M + Na]^+$ *m/e* 1139.2398, found *m/e* 1139.2435.

Benzyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (99)

Prepared from **39** and **32** according to General Procedures B and E.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50-7.16 (m, 35H), 6.79-6.54 (m, 8H), 5.15 (d, *J* = 3.0 Hz, 1H), 5.08 (d, *J* = 3.4 Hz, 1H), 5.02 (d, *J* = 11.9 Hz, 1H), 4.98 (d, *J* = 11.9 Hz, 1H), 4.94-4.84 (m, 4H), 4.80-4.55 (m, 11H), 4.52 (d, *J* = 7.3 Hz, 1H), 4.47 (dd, *J* = 9.4, 5.1 Hz, 1H), 4.38-4.32 (m, 3H), 4.13 (dd, *J* = 10.7, 2.6 Hz, 1H), 4.08 (dd, *J* = 10.7, 3.4 Hz, 1H), 4.04 (t, *J* = 8.5 Hz, 1H), 4.01 (d, *J* = 3.0 Hz, 1H), 3.91 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.90 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.77 (s, 3H), 3.76-3.67 (m, 3H), 3.69 (s, 3H), 3.62 (t, *J* = 6.4 Hz, 1H), 3.47 (dd, *J* = 10.2, 3.0 Hz, 1H), 2.60 (bs, 1H), 2.15 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 170.50, 153.87, 153.78, 152.71, 152.41, 138.62, 138.52, 138.45 (2C), 138.31, 138.26, 137.53, 115.42 (2C), 115.39 (2C), 114.52 (2C), 114.47 (2C), 102.75, 100.88, 99.91, 80.81, 78.66, 78.39, 78.02, 75.91, 75.80, 75.60, 75.02, 74.74, 73.62, 73.26, 72.87, 72.74, 72.41, 72.02, 71.05, 69.59, 68.06, 66.70, 66.37, 64.69, 62.55, 55.64 (2C), 21.00.
Anal. Calcd for $C_{83}H_{88}O_{19}$: C, 71.74; H, 6.38. Found: C, 71.59; H, 6.36.

Benzyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (100)

Prepared from **99** and **32** according to General Procedures B and E.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.76-7.08 (m, 45H), 6.75-6.42 (m, 12H), 5.12 (d, *J* = 3.0 Hz, 1H), 5.09 (d, *J* = 3.4 Hz, 1H), 5.04 (d, *J* = 3.8 Hz, 1H), 5.00 (d, *J* = 11.5 Hz, 1H), 4.97 (d, *J* = 11.5 Hz, 1H), 4.93 (d, *J* = 11.9 Hz, 1H), 4.90 (d, *J* = 11.9 Hz, 1H), 4.88-4.23 (m, 25H), 4.10 (dd, *J* = 10.7, 2.1 Hz, 1H), 4.01 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.98 (d, *J* = 2.6 Hz, 1H), 3.95-3.87 (m, 3H), 3.84-3.68 (m, 4H), 3.76 (s, 3H), 3.71 (s, 3H), 3.69 (s, 3H), 3.65 (dd, *J* = 8.1, 5.1 Hz, 1H), 3.58 (t, *J* = 6.8 Hz, 1H), 3.50 (dd, *J* = 8.5, 4.3 Hz, 1H), 3.44 (dd, *J* = 9.8, 2.6 Hz, 1H), 2.49 (bs, 1H), 2.12 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 170.55, 153.81, 153.76 (2C), 152.67, 152.53, 152.33, 115.34 (4C), 115.29 (2C), 114.68 (2C), 114.58 (2C), 114.46 (2C), 102.81, 100.91, 100.28, 100.00, 80.75, 79.12, 78.70, 78.35, 78.28, 75.79 (2C), 75.42, 75.38, 75.07, 74.66, 73.97, 73.58, 73.30, 73.05, 72.90, 72.76, 72.67, 72.38, 72.08, 71.09, 69.67, 69.44, 67.94, 66.62, 66.12, 64.43, 64.25, 62.44, 55.80, 55.75 (2C), 21.05.
ESI MS: *m/z* calcd for $C_{110}H_{116}NaO_{25}$ $[M+Na]^+$ 1860.8, found 1860.8.
Anal. Calcd for $C_{110}H_{116}O_{25}$: C, 71.88; H, 6.36. Found: C, 71.64; H, 6.41.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (101)

Prepared from **100** and **70** according to General procedures B and G.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40-7.05 (m, 70H), 6.70-6.32 (m, 12H), 5.11 (d, *J* = 3.4 Hz, 1H), 5.07 (d, *J* = 3.0 Hz, 1H), 5.06 (d, *J* = 3.0 Hz, 1H), 5.01 (d, *J* = 3.0 Hz, 1H), 4.95-4.28 (m, 38H), 4.21 (m, 1H), 4.14 (dd, *J* = 9.8, 8.5 Hz, 1H), 4.09-4.00 (m, 4H), 3.97-3.88 (m, 3H), 3.85-3.61 (m, 13H), 3.68 (s, 3H), 3.67 (s, 3H), 3.66 (s, 3H), 3.50-3.39 (m, 5H), 3.29-3.11 (m, 4H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 153.85, 153.81, 153.63, 152.59, 152.39, 152.19, 115.29 (2C), 115.25 (2C), 115.23 (2C), 114.69 (2C), 114.62 (2C), 114.47 (2C), 103.02, 100.80, 100.31 (2C), 100.02, 99.37, 55.74 (3C).
ESI MS: *m/z* calcd for $C_{155}H_{164}NaO_{34}$ $[M+Na]^+$ 2594.1, found 2593.9.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (102)

Prepared from **101** according to General Procedures I and J.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.88 (m, 70H), 6.79-6.41 (m, 12H), 5.16 (d, *J* = 3.5 Hz, 1H), 5.09 (d, *J* = 3.4 Hz, 1H), 5.07-5.01 (m, 2H), 5.01 (d, *J* = 3.4 Hz, 1H), 4.94-4.15 (m, 42H), 4.10-4.05 (m, 2H), 4.01 (dd, *J* = 10.2, 2.6 Hz, 1H), 3.99 (bs, 1H), 3.91 (dd, *J* = 7.3, 3.4 Hz, 1H), 3.89 (dd, *J* = 7.3, 3.4 Hz, 1H), 3.79-3.62 (m, 6H), 3.72 (s, 3H), 3.70 (s, 3H), 3.66 (s, 3H), 3.63 (s, 3H), 3.61-3.49 (m, 4H), 3.47 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.17 (s, 3H), 3.04 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 169.07, 168.54, 168.39, 153.69 (3C), 152.45, 152.24, 151.97, 115.14 (4C), 115.02 (2C), 114.57 (4C), 114.50 (2C), 102.71, 99.92, 99.72, 99.58, 99.47, 98.93, 55.69 (2C), 55.62, 52.26, 51.57, 51.49.
ESI MS: *m/z* calcd for $C_{158}H_{164}NaO_{37}$ $[M+Na]^+$ 2678.1, found 2678.0.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (104)

Prepared from **102** *via* **103** according to the General Procedures H, I, and K.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.98 (m, 85H), 5.22 (d, *J* = 3.4 Hz, 1H), 5.11 (d, *J* = 3.1 Hz, 2H), 5.07-4.98 (m, 4H), 4.90 (d, *J* = 11.9 Hz, 1H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.84-4.21 (m, 41H), 4.16 (m, 1H), 3.98 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.89-3.82 (m, 4H), 3.81-3.76 (m, 2H), 3.68 (dd, *J* = 10.2, 2.6 Hz, 1H), 3.65-3.51 (m, 4H), 3.64 (s, 3H), 3.30 (dd, *J* = 9.8, 2.6 Hz, 1H), 3.24 (s, 3H), 3.11 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 169.12, 168.28, 168.19, 168.06, 167.88, 167.70, 102.66, 99.32, 98.68, 98.55 (2C), 98.54, 52.21, 51.66, 51.51.

Benzyl *O*-(benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (105)

Prepared from **101** according to General Procedures I and K.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.81 (m, 85H), 6.70-6.43 (m, 12H), 5.18 (d, *J* = 3.4 Hz, 1H), 5.10-4.98 (m, 4H), 5.09 (d, *J* = 3.4 Hz, 1H), 4.94-4.84 (m, 4H), 4.81-4.13 (m, 42H), 4.07-3.99 (m, 4H), 3.97 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.89 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.82-3.62 (m, 7H), 3.72 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.57-3.46 (m, 5H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 168.43, 167.77, 167.62, 153.82 (2C), 153.77, 152.56, 152.34, 152.09, 115.25 (4C), 115.10 (2C), 114.66 (2C), 114.59 (2C), 114.58 (2C), 102.73, 99.94, 99.78 (2C), 99.11, 98.93, 55.81 (2C), 55.75.
ESI MS: *m/z* calcd for $C_{176}H_{176}NaO_{37}$ $[M+Na]^+$ 2906.2, found 2905.9.

Benzyl *O*-(benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (107)

Prepared from **105** *via* **106** according to General Procedures H, I and J.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.95 (m, 85H), 5.20 (d, *J* = 3.4 Hz, 1H), 5.12-4.98 (m, 7H), 4.88, (d, *J* = 11.3 Hz, 1H), 4.86 (d, *J* = 11.3 Hz, 1H), 4.82-4.28 (m, 38H), 4.23 (d, *J* = 12.4 Hz, 1H), 4.20 (d, *J* = 13.2 Hz, 1H), 4.06 (m, 1H), 4.00 (dd, *J* = 10.2, 3.5 Hz, 1H), 3.93 (bs, 1H), 3.91 (dd, *J* = 10.7, 2.6 Hz, 1H), 3.84 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.79-3.72 (m, 3H), 3.66-3.47 (m, 5H), 3.39 (s, 3H), 3.38 (m, 1H), 3.36 (s, 3H), 3.29 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 168.81, 168.76, 168.62, 168.43, 167.51, 167.41, 102.67, 99.65, 98.67, 98.62, 98.40, 98.34, 51.78 (2C), 51.70.
ESI MS: *m/z* calcd for $C_{158}H_{158}NaO_{37}$ $[M+Na]^+$ 2672.0, found 2671.9.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-β-D-galactopyranoside (108)

Prepared from **100** and **72** according to General Procedures B and G.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40-6.97 (m, 70H), 6.69-6.29 (m, 16H), 5.10 (d, *J* = 3.2 Hz, 1H), 5.08 (d, *J* = 3.7 Hz, 1H), 5.04 (d, *J* = 3.7 Hz, 1H), 5.03 (d, *J* = 3.7 Hz, 1H), 4.96-4.79 (m, 8H), 4.77-4.27 (m, 31H), 4.21 (d, *J* = 9.0 Hz, 1H), 4.19 (d, *J* = 9.0 Hz, 1H), 4.10-4.03 (m, 3H), 3.97-3.61 (m, 15H), 3.69 (s, 3H), 3.68 (s, 3H), 3.67 (s, 3H), 3.66 (s, 3H), 3.55-3.40 (m, 4H), 3.26-3.10 (m, 3H), 2.66 (bs, 1H), 2.49 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 154.05 (2C), 153.91, 153.87, 152.79, 152.60, 152.53, 152.35, 115.50 (2C), 115.41 (4C), 115.38 (2C), 114.90 (4C), 114.79 (2C), 114.69 (2C), 103.22, 100.53 (3C), 100.06, 99.63, 56.02, 55.99 (2C), 55.95.
ESI MS: *m/z* calcd for $C_{162}H_{170}NaO_{35}$ $[M+Na]^+$ 2700.2, found 2700.2.
Anal. Calcd for $C_{162}H_{170}O_{35}$: C, 72.68; H, 6.40. Found: C, 72.47; H, 6.46.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (109)

Prepared from **108** according to General Procedures I and J.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.88 (m, 70H), 6.69-6.26 (m, 16H), 5.16 (d, *J* = 3.4 Hz, 1H), 5.08 (d, *J* = 3.4 Hz, 1H), 5.06 (d, *J* = 12.0 Hz, 1H), 5.03 (d, *J* = 2.6 Hz, 1H), 4.99 (d, *J* = 3.4 Hz, 1H), 4.96 (d, *J* = 3.4 Hz, 1H), 4.94-4.12 (m, 42H), 4.03-3.96 (m, 3H), 3.90 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.86 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.84-3.62 (m, 7H), 3.80 (s, 3H), 3.79 (s, 3H), 3.78 (s, 3H), 3.66 (s, 3H), 3.63 (s, 3H), 3.61-3.54 (m, 3H), 3.50-3.42 (m, 2H), 3.40 (dd, *J* = 7.7, 5.1 Hz, 1H), 3.09 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 169.38, 168.52, 153.81, 153.76, 153.73, 153.70, 152.60, 152.46, 152.34, 152.00, 115.27 (2C), 115.23 (2C), 115.11 (4C), 114.67 (4C), 114.60 (2C), 114.50 (2C), 102.82, 100.12, 100.08, 100.00, 99.55, 99.32, 55.83, 56.82, 55.77, 55.74, 52.39, 51.67.
ESI MS: *m/z* calcd for $C_{164}H_{170}NaO_{37}$ $[M+Na]^+$ 2756.1, found 2756.1.
Anal. Calcd for $C_{164}H_{170}O_{37}$: C, 72.07; H, 6.27. Found: C, 71.85; H, 6.27.

Benzyl *O*-(benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (110)

Prepared from **108** according to General Procedures I and K.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.89 (m, 80H), 6.69-6.27 (m, 16H), 5.18 (d, *J* = 3.0 Hz, 1H), 5.09 (d, *J* = 3.4 Hz, 1H), 5.08-5.02 (m, 2H), 5.00 (d, *J* = 3.4 Hz, 1H), 4.93 (d, *J* = 3.4 Hz, 1H), 4.90 (d, *J* = 3.4 Hz, 1H), 4.88-4.16 (m, 43H), 4.08 (t, *J* = 2.1 Hz, 1H), 4.03 (dd, *J* = 10.2, 2.1 Hz, 1H), 4.01-3.96 (m, 2H), 3.90 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.86 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.83-3.64 (m, 8H), 3.70 (s, 3H), 3.69 (s, 3H), 3.68 (s, 3H), 3.66 (s, 3H), 3.61-3.56 (m, 2H), 3.54 (dd, *J* = 10.7, 3.0 Hz, 1H), 3.48 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.46 (m, 1H), 3.41 (dd, *J* = 8.1, 5.1 Hz, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 168.63, 167.75, 153.78, 153.73, 153.69, 153.66, 152.55, 152.33, 152.30, 151.97, 115.23 (4C), 115.07 (4C), 114.62 (4C), 114.55 (2C), 114.46 (2C), 102.69, 100.14, 100.03 (2C), 99.94, 99.14, 55.78, 55.77, 55.73, 55.70.
ESI MS: *m/z* calcd for $C_{176}H_{178}NaO_{37}$ $[M+Na]^+$ 2908.2, found 2908.3.
Anal. Calcd for $C_{176}H_{178}O_{37}$: C, 73.26; H, 6.22. Found: C, 72.98; H, 6.14.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(methyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (113)

Prepared from **109** *via* **111** according to General Procedures H, I and K.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-6.92 (m, 90H), 5.21 (d, *J* = 3.7 Hz, 1H), 5.12 (d, *J* = 3.1 Hz, 1H), 5.09 (d, *J* = 3.7 Hz, 1H), 5.07 (d, *J* = 3.1 Hz, 1H), 5.04 (d, *J* = 12.1 Hz, 1H), 5.02 (d, *J* = 3.2 Hz, 1H), 4.99 (d, *J* = 12.1 Hz, 1H), 4.92 (d, *J* = 12.1 Hz, 1H), 4.86-4.28 (m, 42H), 4.23-4.18 (m, 2H), 4.14 (m, 1H), 4.02 (dd, *J* = 10.0, 3.2 Hz, 1H), 3.87-3.76 (m, 6H), 3.68 (dd, *J* = 10.0, 2.6 Hz, 1H), 3.64 (s, 3H), 3.60-3.61 (m, 4H), 3.30 (dd, *J* = 10.0, 3.1 Hz, 1H), 3.21 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 168.99, 168.13, 167.99, 167.85, 167.67, 167.37, 102.60, 99.45, 98.63, 98.46 (2C), 98.40, 52.15, 51.57.
ESI MS: *m/z* calcd for $C_{164}H_{162}NaO_{37}$ $[M+Na]^+$ 2748.1, found 2748.1
Anal. Calcd for $C_{164}H_{162}O_{37}$: C, 72.28; H, 5.99. Found: C, 71.97; H, 5.91.

Benzyl *O*-(benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (114)

Prepared from **110** *via* **112** according to General Procedures H, I and J.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-7.01 (m, 80H), 5.29 (d, *J* = 3.2 Hz, 1H), 5.13-4.96 (m, 7H), 4.89-4.27 (m, 40H), 4.07 (bs, 1H), 3.97-3.90 (m, 3H), 3.85 (dd, *J* = 10.5, 3.2 Hz, 1H), 3.81-3.74 (m, 3H), 3.67-3.61 (m, 2H), 3.59-3.52 (m, 3H), 3.41 (s, 3H), 3.38 (m, 1H), 3.37 (s, 3H), 3.29 (s, 3H), 3.12 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 168.74, 168.69, 168.54, 168.39, 168.36, 167.32, 102.58, 99.39, 98.60, 98.52, 98.48, 98.25, 51.71, 51.69, 51.62, 51.51.
ESI MS: *m/z* calcd for $C_{152}H_{154}NaO_{37}$ $[M+Na]^+$ 2596.0, found 2595.9.
Anal. Calcd for $C_{152}H_{154}O_{37}$: C, 70.96; H, 6.03. Found: C, 70.67; H, 6.13.

Methyl 4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (115β)

Major product from glycosylation of methanol with **77** according to General Procedure B.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43-7.21 (m, 10H), 6.90-6.79 (m, 4H), 5.83 (m, 1H), 5.19 (d, *J* = 11.4 Hz, 1H), 5.09 (m, 1H), 4.92-4.66 (m, 4H), 4.88 (d, *J* = 11.1 Hz, 1H), 4.42-4.33 (m, 2H), 4.20-3.92 (m, 5H), 3.81-3.68 (m, 1H), 3.79 (s, 3H), 3.58 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.3, 153.1, 138.9, 138.5, 137.7, 128.6 (2C), 128.5 (2C), 128.3 (2C), 127.8 (2C), 127.7 (2C), 117.8, 115.8 (2C), 115.0 (2C), 105.3, 82.1, 79.9, 75.4, 74.2, 73.3, 73.1, 73.0, 66.4, 57.3, 56.0.

Benzyl 4-*O*-allyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (116)

Prepared by allylating **24**, as for the preparation of **77**.
^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.39-7.22 (m, 15H), 7.83 (bs, 4H), 5.81 (m, 1H), 5.17 (dq, *J* = 17.1, 1.7 Hz, 1H), 5.04 (bd, *J* = 10.2 Hz, 1H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.91 (d, *J* = 11.5 Hz, 1H), 4.78-4.75 (m, 3H), 4.63 (d, *J* = 11.3 Hz, 1H), 4.49 (d, *J* = 6.7 Hz, 1H), 4.38 (dd, *J* = 10.9, 3.2 Hz, 1H), 4.20-4.04 (m, 3H), 3.95 (d, *J* = 3.2 Hz, 1H), 3.87 (dd, *J* = 10.9, 6.7 Hz, 1H), 3.78 (s, 3H), 3.72 (m, 1H), 3.54 (dd, *J* = 10.9, 3.2 Hz, 1H).

Benzyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (117)

Prepared from **24** and **89** according to General Procedures B and F.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-7.14 (m, 35H), 6.81-6.58 (m, 8H), 5.06 (d, *J* = 3.4 Hz, 1H), 4.99 (d, *J* = 3.4 Hz, 1H), 4.97 (d, *J* = 12.0 Hz, 1H), 4.90-4.63 (m, 15H), 4.54-4.42 (m, 5H), 4.28 (d, *J* = 1.8 Hz, 1H), 4.16 (d, *J* = 2.6 Hz, 1H), 4.10-4.07 (m, 2H), 4.01 (t, *J* = 8.5 Hz, 1H), 3.92 (dd, *J* = 9.9, 3.4 Hz, 1H), 3.90 (dd, *J* = 9.4, 3.1 Hz, 1H), 3.81 (dd, *J* = 9.9, 3.1 Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.73-3.65 (m, 3H), 3.44 (dd, *J* = 9.9, 2.9 Hz, 1H), 2.50 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 157.90, 154.06, 153.91, 152.73, 152.47, 145.39 (bd, $J_{C\text{-}F}$ = 257 Hz, 2C), 143.15 (bd, $J_{C\text{-}F}$ = 260 Hz, 1C), 138.66, 138.57, 138.51, 138.29, 138.19, 138.15, 137.80, 137.61 (bd, $J_{C\text{-}F}$ = 257 Hz, 2C), 115.45 (2C), 115.43 (2C), 114.75 (2C), 114.53 (2C), 107.78 (dt, $J_{C\text{-}F}$ = 15, 3.5 Hz, 1C), 102.91, 100.42, 100.20, 79.84, 79.25, 78.46, 77.35, 76.86, 75.90, 75.08, 74.98, 74.59, 74.31, 73.02, 72.96, 72.77, 72.43, 72.12, 71.09, 69.00, 68.38, 66.66, 66.43, 65.65, 63.77, 55.72, 55.69.
ESI MS: *m/z* calcd for $C_{88}H_{85}F_5NaO_{19}$ $[M+Na]^+$ 1463.6, found 1563.7.

Benzyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-pentafluorobenzoyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (118)

Prepared from **117** and **89** according to General Procedures B and F.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.41-7.02 (m, 55H), 6.77-6.45 (12H), 5.06 (d, *J* = 1.7 Hz, 1H), 5.02 (d, *J* = 3.4 Hz, 1H), 4.96 (d, *J* = 3.7 Hz, 1H), 4.95 (d, *J* = 11.4 Hz, 1H), 4.93 (d, *J* = 3.4 Hz, 1H), 4.88-4.01 (m, 40H), 3.93-3.88 (m, 2H), 3.86 (dd, *J* = 9.9, 3.4 Hz, 1H), 3.83-3.76 (m, 4H), 3.74 (s, 6H), 3.71-3.62 (m, 3H), 3.69 (s, 3H), 3.51 (dd, *J* = 8.2, 4.5 Hz, 1H), 3.42 (dd, *J* = 9.9, 2.8 Hz, 1H), 2.45 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 157.99, 157.66, 154.09, 153.91, 153.89, 152.68, 152.48, 152.35, 145.45 (bd, $J_{C\text{-}F}$ = 257 Hz, 4C), 143.22 (bd, $J_{C\text{-}F}$ = 260 Hz, 2C), 137.69 (bd, $J_{C\text{-}F}$ = 257 Hz, 4C), 115.47 (2C), 115.38 (2C), 115.27 (2C), 114.79 (2C), 114.71 (2C), 114.54 (2C), 107.56 (m, 2C), 102.94, 100.59, 100.43, 100.15, 99.79, 55.80 (3C).
Anal. Calcd for $C_{142}H_{134}F_{10}O_{31}$: C, 67.50; H, 5.35. Found: C, 67.17; H, 5.15.

Benzyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (119)

Prepared from **118** and **35** according to General Procedures B and G.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-6.97 (m, 70H), 6.73-6.34 (m, 12H), 5.01 (d, *J* = 4.0 Hz, 1H), 5.00 (d, *J* = 3.6 Hz, 1H), 4.99-4.94 (m, 4H), 4.91 (d, *J* = 11.0 Hz, 1H), 4.89 (d, *J* = 11.0 Hz, 1H), 4.83-4.43 (m, 28H), 4.27-3.99 (m, 14H), 3.95-3.74 (m, 10H), 3.72 (s, 3H), 3.71-3.52 (m, 6H), 3.69 (s, 3H), 3.67 (s, 3H), 3.43 (dd, *J* = 10.0, 2.9 Hz, 1H), 3.40 (m, 1H), 3.32-3.24 (m, 3H), 3.08 (bs, 1H), 2.81 (m, 1H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 153.97, 153.74, 153.72, 152.46, 152.33, 152.29, 115.43 (2C), 115.20 (4C), 114.70 (2C), 114.59 (2C), 114.52 (2C), 102.99, 100.34, 100.26, 100.19, 99.84, 99.77, 55.74 (2C), 53.52.
Anal. Calcd for $C_{155}H_{164}O_{34}$: C, 72.41; H, 6.43. Found: C, 72.16; H, 6.31.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(methyl-2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-β-D-galactopyranoside (120)

Prepared from **119** according to General Procedures I and J.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-6.86 (m, 70H), 6.80-6.28 (m, 12H), 5.15 (d, *J* = 3.1 Hz, 1H), 5.10 (d, *J* = 3.3 Hz, 1H), 5.06 (d, *J* = 3.1 Hz, 1H), 5.02 (d, *J* = 3.1 Hz, 1H), 5.00-4.03 (m, 46H), 3.99 (t, *J* = 9.0 Hz, 1H), 3.88 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.85 (dd, *J* = 10.5, 3.3 Hz, 1H), 3.81-3.58 (m, 9H), 3.75 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H), 3.45-3.35 (m, 2H), 3.33 (s, 3H), 3.14 (s, 3H), 3.12 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 169.62, 169.60, 169.39, 154.38, 154.09, 153.99, 152.46, 152.40, 152.29, 115.63 (2C), 115.50 (2C), 115.46 (2C), 115.05 (2C), 114.91 (2C), 114.75 (2C), 103.27, 100.25, 100.11, 100.09, 99.57 (2C), 56.02 (2C), 53.80, 52.17, 52.05, 51.92.
Anal. Calcd for $C_{158}H_{164}O_{37}$: C, 71.48; H, 6.23. Found: C, 71.26; H, 6.47.

Benzyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-β-D-galactopyranosyluronate) (122)

Prepared from **120** *via* **121** according to General Procedures H, I and K.
^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.42-6.95 (m, 85H), 5.25 (d, *J* = 3.2 Hz, 1H), 5.14 (d, *J* = 12.4 Hz, 1H), 5.09-4.94 (m, 7H), 4.87-4.28 (m, 42H), 4.22 (d, *J* = 12.7 Hz, 1H), 4.12 (m, 1H), 4.00 (dd, *J* = 10.2, 3.2 Hz, 1H), 3.92 (bs, 1H), 3.87 (dd, *J* = 10.2, 2.2 Hz, 1H), 3.82-3.75 (m, 3H), 3.63 (dd, *J* = 10.2, 2.6 Hz, 1H), 3.61-3.52 (m, 3H), 3.38 (s, 3H), 3.36 (dd, *J* = 10.2, 2.9 Hz, 1H), 3.27 (s, 3H), 3.21 (s, 3H).
^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 169.14, 168.80, 168.53, 168.02, 167.52, 167.41, 102.64, 99.66, 98.65, 98.52 (2C), 98.40, 51.74, 51.68, 51.65.
ESI MS: *m/z* calcd for $C_{158}H_{158}NaO_{37}$ $[M+Na]^+$ 2672.0, found 2671.7.
Anal. Calcd for $C_{158}H_{158}O_{37}$: C, 71.64; H, 6.01. Found: C, 71.27; H, 5.88.

8-(*tert*-Butyl-diphenyl-silanyloxy)-octan-1-ol (124)

Octane-1,8-diol (**123**) (2.5 g, 17.10 mmol) was dissolved in DMF (51 mL) and DIPEA (30 mL, 171.0 mmol) was added. TBDPSCl (4.42 mL, 17.27 mmol) was added dropwise and the reaction mixture was stirred 3 h at room temperature. 300 mL water was added and the

mixture was extracted with Et_2O (3x300 mL). The combined organic phases were washed (2M HCl, 2x300 mL, sat. $NaHCO_3$, 250 mL), dried, concentrated and purified by flash chromatography (hexane-EtOAc 3:1), yielding 3.48 g (53%) of **124** as a colorless oil.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.73-7.67 (m, 4H), 7.47-7.36 (m, 6H), 3.69 (t, *J* = 6.4 Hz, 2H), 3.64 (t, *J* = 6.6 Hz, 2H), 1.81 (bs, 1H), 1.65-1.51 (m, 5H), 1.44-1.26 (m, 7H), 1.09 (s, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz) 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.

2-(7-(*tert*-Butyl-diphenyl-silanyloxy)-heptyl)-1,3-dioxane (125)

A solution of anhydrous DMSO (2.60 mL, 36.19 mmol) in anhydrous CH_2Cl_2 under argon was cooled to −70 °C and oxalyl chloride (1.54 mL, 18.10 mmol) was added dropwise, keeping T ≤ -70 °C. After 15 min., a solution of **124** (3.48 g, 9.05 mmol) in CH_2Cl_2 (20 mL) was added slowly, keeping T ≤ -65 °C. After 30 min., DIPEA (15.73, 90.48 mmol) was added, and the reaction mixture was allowed to reach room temperature. CH_2Cl_2 (100 mL) was added, and the reaction mixture was washed (1M HCl, 2x100 mL, water, 100 mL), the combined aqueous phases were extracted (CH_2Cl_2, 100 mL) and the combined organic phases were dried, concentrated and suspended in hexane (100 mL). MS4A (5 g) was added, followed by propane-1,3-diol (1.6 mL, 22.63 mmol) and TsOH (1.63 g, 18.10 mmol). Stirred 10 h, quenched with solid $NaHCO_3$, filtered through celite, pad washed with hexane (3x50 mL), filtrate washed (1M NaOH, 150 mL), dried, concentrated and purified by flash chromatography (hexane-EtOAc 19:1, R_f 0.13) yielding 2.39 g (60%) of **125** as a colorless oil.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.71-7.67 (m, 4H), 7.46-7.35 (m, 6H), 4.52 (t, *J* = 5.1 Hz, 1H), 4.15-4.07 (m, 2H), 3.82-3.75 (m, 2H), 3.67 (t, *J* = 6.6 Hz, 2H), 2.09 (m, 1H), 1.65-1.52 (m, 4H), 1.45-1.25 (m, 9H), 1.07 (s, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz) 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.

7-(1,3-Dioxan-2-yl)-heptan-1-ol (126)

A solution of **125** (2.30 g, 5.22 mmol) in anhydrous THF (30 mL) was treated with TBAF (1M sol. in THF, 7.83 mL, 7.83 mmol). Concentrated after 1 h and purified by flash chromatography (hexane-EtOAc 2:1, R_f 0.13) yielding 1.05 g (99%) of **126** a colorless oil.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 4.50 (t, *J* = 5.1 Hz, 1H), 4.14-4.06 (m, 2H), 3.81-3.70 (m, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 2.07 (m, 1H), 1.63-1.50 (m, 4H), 1.45-1.25 (m, 9H), 1.21 (bs, 1H).
^{13}C NMR ($CDCl_3$, 75 Mhz) δ 102.33, 66.77 (2C), 62.52, 35.09, 32.64, 29.35, 29.23, 25.78, 25.60, 23.78.
ESI MS: *m/z* calcd for $C_{11}H_{23}O_3$ $[M+H]^+$ 203.2, found 203.2.

7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (128)

Prepared from **126** and **35** *via* **127** according to General Procedures B and G.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.41-7.22 (m, 15H), 4.99-4.90 (m, 2H), 4.85-4.62 (m, 4H), 4.48 (t, *J* = 5.1 Hz, 1H), 4.35 (t, *J* = 7.7 Hz, 1H), 4.13-4.04 (m, 2H), 3.97-3.68 (m, 6H), 3.54-3.45 (m, 4H), 3.37 (t, *J* = 6.0 Hz, 1H), 2.06 (m, 1H), 1.83-1.51 (m, 5H), 1.43-1.20 (m, 8H).
^{13}C NMR ($CDCl_3$, 75 Mhz) δ 138.84, 138.55, 138.38, 128.70, 128.48 (2C), 128.34 (3C), 128.18 (3C), 127.97 (2C), 127.73, 127.68, 127.62 (2C), 104.17, 102.51, 82.40, 79.78, 75.28, 74.66, 74.26, 73.49, 73.19, 70.23, 66.99 (2C), 62.15, 35.31, 29.85, 29.49, 29.44, 26.15, 25.98, 24.03.

7-(1,3-Dioxan-2-yl)-heptyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranosyluronic acid (129)

Prepared from **128** according to General Procedure I, purified by flash chromatography (hexane-EtOAc 1:1 + 3% AcOH), and obtained as a foam.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.38-7.23 (m, 15H), 4.94-4.86 (m, 2H), 4.80-4.71 (m, 3H), 4.63 (d, *J* = 11.0 Hz, 1H), 4.49 (t, *J* = 5.1 Hz, 1H), 4.42 (d, *J* = 7.7 Hz, 1H), 4.24 (m, 1H), 4.13-4.04 (m, 4H), 3.87 (dt, *J* = 9.5, 6.6 Hz, 1H), 3.83 (dd, *J* = 9.7, 7.7 Hz, 1H), 3.74 (dt, *J* = 12.3, 2.2 Hz, 1H), 3.60-3.50 (m, 2H), 2.04 (m, 1H), 1.70-1.50 (m, 5H), 1.44-1.23 (m, 8H).

^{13}C NMR ($CDCl_3$, 75 Mhz) δ 170.13, 138.37, 138.00, 137.87, 128.30 (2C), 128.17 (2C), 128.06 (4C), 128.03 (2C), 127.99, 127.62, 127.52, 127.51, 127.48, 103.50, 102.34, 80.90, 78.48, 75.10 (2C), 74.87, 73.60, 72.94, 70.44, 66.75 (2C), 35.05, 29.57, 29.30, 29.23, 25.93, 25.74, 23.81.

7-(1,3-Dioxan-2-yl)-heptyl β-D-galactopyranosyluronic acid (130)

Prepared from **129** according to General Procedure L.
^{1}H NMR (CD_3OD, 500 Mhz) δ 4.52 (t, *J* = 5.0 Hz, 1H), 4.26-4.11 (m, 3H), 4.06-4.00 (m, 2H), 3.93 (m, 1H), 3.80-3.73 (m, 2H), 3.56-3.49 (m, 3H), 1.98 (m, 1H), 1.66-1.59 (m, 2H), 1.55-1.47 (m, 2H), 1.42-1.27 (m, 9H).
^{13}C NMR (CD_3OD, 125 Mhz) δ 165.74, 104.67, 103.71, 75.37, 74.61, 72.03, 71.46, 71.12, 67.94 (2C), 36.21, 30.75, 30.53, 30.49, 27.05, 26.98, 24.94.

8-[N-BSA]-octyl β-D-galactopyranosyluronic acid (131)

A solution of **130** (60 mg, 0.16 mmol) in AcOH-H_2O 4:1 was heated at 50 °C for 12 h, cooled, concentrated and taken up in water (4 mL). BSA (50 mg, 0.75 μmol, "0.05 mmol NH_2") was added, the mixture was stirred for 1 h, then $NaCNBH_3$ (30 mg, 0.48 mmol) was added, and the mixture was stirred at room temperature for 4 days.
MALDI-TOF MS: 66434 (BSA), 68460 (BSA+2026 = 6.7 sugar residues).

7-(1,3-Dioxan-2-yl)-heptyl 6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-galactopyranoside (132α)

Minor product from **126** and **30** using General Procedure B.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.37-7.21 (m, 10H), 5.59 (d, *J* = 1.7 Hz, 1H), 4.80 (d, *J* = 3.7 Hz, 1H), 4.79 (d, *J* = 12.1 Hz, 1H), 4.73 (d, *J* = 11.0 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 58 (d, *J* = 11.0 Hz, 1H), 4.50 (t, *J* = 5.1 Hz, 1H), 4.18-4.04 (m, 5H), 4.10 (bs, 2H), 4.0 (dd, *J* = 10.1, 3.4 Hz, 1H), 3.80-3.69 (m, 3H), 3.60 (dt, *J* = 9.5, 7.0 Hz, 1H), 3.44 (dt, *J* = 9.9, 6.6 Hz, 1H), 2.03 (m, 1H), 2.02 (s, 3H), 1.69-1.53 (m, 4H), 1.42-1.21 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz) 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_{C-H} = 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.

7-(1,3-Dioxan-2-yl)-heptyl 6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-D-galactopyranoside (132β)

Major product from **126** and **30** using General Procedure B.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.37-7.22 (m, 10H), 5.52 (d, *J* = 1.8 Hz, 1H), 4.86 (d, *J* = 11.0 Hz, 1H), 4.74 (d, *J* = 11.3 Hz, 1H), 4.70 (d, *J* = 11.0 Hz, 1H), 4.53 (d, *J* = 11.3 Hz, 1H), 4.48 (t, *J* = 5.1 Hz, 1H), 4.37 (bd, *J* = 7.5 Hz, 1H), 4.22-4.04 (m, 4H), 4.14 (bs, 2H), 3.92 (dt, *J* = 9.5, 6.4 Hz, 1H), 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 ($CDCl_3$, 75 Mhz) 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_{C-H} = 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.

7-(1,3-Dioxan-2-yl)-heptyl 6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranoside (133)

Prepared from **126** and **29** as for **134**, below.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.41-7.24 (m, 10H), 4.87-4.81 (m, 5H), 4.50 (t, *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, *J* = 9.9, 6.6 Hz, 1H), 2.50 (bs, 1H), 2.06 (s, 3H), 2.05 (m, 1H), 1.70-1.53 (m, 4H), 1.44-1.25 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz) 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.

7-(1,3-Dioxan-2-yl)-heptyl 2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranoside (134)

31 (10.45 g, 21.24 mmol) was dried azeotropically with toluene (x2), subjected to high vacuum for 2 h, dissolved in anhydrous CH_2Cl_2 (40 mL), cooled to 0 °C and titrated with a 1M sol. of bromine in anhydrous CH_2Cl_2 until a faint yellow color persisted. MS 4A (10 g) was added, followed by a solution of **126** (2.52 g, 12.49 mmol) and Et_4NBr (5.35 g, 25.49 mmol) in anhydrous CH_2Cl_2 (40 mL). Stirred 40 h, quenched with sat. $NaHCO_3$ (50 mL), stirred 4 h, filtered through celite, pad washed with CH_2Cl_2 (50 mL), organic phase separated and washed with H_2O, combined aqueous phases extracted (CH_2Cl_2), combined organic phases dried, concentrated and purified by flash chromatography (hexane-EtOAc 3:1, R_f: 0.08) yielding **134** (5.71 g, 70%).
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.39-7.26 (m, 10H), 6.88-6.77 (m, 4H), 4.86-4.62 (m, 5H), 4.49 (t, *J* = 5.1 Hz, 1H), 4.19-4.05 (m, 5H), 3.93 (dd, *J* = 9.9, 3.3 Hz, 1H), 3.85 (dd, *J* = 9.9, 3.7 Hz, 1H), 3.80-3.60 (m, 4H), 3.76 (s, 3H), 3.43 (dt, *J* = 9.9, 6.6 Hz, 1H), 2.49 (bs, 1H), 2.06 (m, 1H), 1.68-1.49 (m, 4H), 1.43-1.24 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz) 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.
ESI MS: *m/z* calcd for $C_{38}H_{50}NaO_9$ $[M+Na]^+$ 673.3, found 673.7.
Anal. Calcd for $C_{38}H_{50}O_9$: C, 70.13; H, 7.74. Found: C, 70.01; H, 7.77.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-4-*O*-chloroacetyl-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranoside (135)

Prepared from **133** and **30** according to General Procedure B.
^{1}H NMR ($CDCl_3$, 500 Mhz) δ 7.39-7.23 (m, 20H), 5.58 (d, *J* = 3.4 Hz, 1H), 4.90 (bs, 1H), 4.87 (d, *J* = 3.4 Hz, 1H), 4.83-4.72 (m, 6H), 4.65 (d, *J* = 11.9 Hz, 1H), 4.57 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.50 (dd, *J* = 10.7, 5.1 Hz, 1H), 4.49 (d, *J* = 10.7 Hz, 1H), 4.38 (dd, *J* = 11.1, 4.3 Hz, 1H), 4.30 (dd, *J* = 11.5, 6.4 Hz, 1H), 4.15-4.04 (m, 4H), 4.02-3.95 (m, 2H), 3.92 (dd, *J* = 10.2, 3.4 Hz, 1H), 3.90-3.85 (m, 3H), 3.77-3.71 (m, 3H), 3.64-3.55 (m, 2H), 3.46 (dt, *J* = 9.9, 6.6 Hz, 1H), 2.09 (m, 1H), 2.04 (s, 3H), 1.94 (s, 3H), 1.67-1.52 (m, 4H), 1.42-1.23 (m, 9H).
^{13}C NMR ($CDCl_3$, 125 Mhz) δ 171.75, 170.99, 170.70, 167.58, 139.16, 139.15, 138.71, 138.54, 129.23 (2C), 129.03 (3C), 128.97 (2C), 128.77 (2C), 128.66 (2C), 128.46 (2C), 128.41 (2C), 128.36 (2C), 128.28, 128.23 (2C), 103.09, 100.77, 97.77, 77.62, 76.91, 76.05, 75.44, 74.85, 74.07, 73.33, 72.82, 70.22, 69.20, 69.05, 67.57 (2C), 67.09, 63.12, 61.64, 41.45, 35.94, 30.13, 30.04, 30.00, 26.78, 26.58, 24.60, 21.53, 21.33.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranoside (136)

Prepared from **133** and **70** according to General Procedure B, or from **135** and **35** according to General Procedures E and B.
^{13}C NMR ($CDCl_3$, 125 Mhz) δ 171.14, 170.57, 170.54, 139.58, 139.45, 139.39 (2C), 139.19, 139.17, 138.94, 129.01-127.98 (35C), 103.12, 100.41, 100.28, 97.96, 80.14, 78.17, 77.32, 76.95, 76.56, 76.47, 76.13, 75.61, 75.35, 75.27, 74.79, 74.72, 74.17, 74.10, 73.63, 73.44, 73.40, 69.35, 69.26, 69.01, 67.60 (2C), 63.56, 62.93, 62.28, 35.96, 30.15, 30.07, 30.03, 26.79, 26.60, 24.63, 21.60, 21.52, 21.40.

Benzyl *O*-(6-*O*-acetyl-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (137)

Prepared from **58** and **32** according to General Procedures B and G (87%) followed by reaction of the resulting trisaccharide acceptor with **30** according to General Procedures B and E (68%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.44-7.07 (m, 45H), 6.79 (s, 4H), 6.60 (m, 4H), 6.55 (bd, 4H), 5.09 (d, *J* = 3.4 Hz, 1H), 5.07 (d, *J* = 3.4 Hz, 1H), 5.03 (d, *J* = 3.0 Hz, 1H), 4.98 (d, *J* = 11.9 Hz, 1H), 4.93 (d, *J* = 10.7 Hz, 1H), 4.91 (d, *J* = 10.7 Hz, 1H), 4.89 (d, *J* = 4.89 Hz, 1H), 4.84 (d, *J* = 11.9 Hz, 1H), 4.78 (d, *J* = 11.1 Hz, 1H), 4.74-4.44 (m, 16H), 4.42-4.32 (m, 3H), 4.28 (bs, 1H), 4.24 (d, *J* = 2.6 Hz, 1H), 4.19-4.06 (m, 4H), 3.97 (bs, 1H), 3.90-3.84 (m, 2H), 3.82-3.65 (m, 7H), 3.76 (s, 3H), 3.70 (s, 3H), 3.69 (s, 3H), 3.59 (dd, *J* = 8.1, 4.7 Hz, 1H), 3.50 (dd, *J* = 9.8, 3.0 Hz, 1H), 2.34 (bs, 1H), 1.90 (s, 3H).

^{13}C NMR ($CDCl_3$, 75 MHz, partial) δ 170.35, 154.24, 154.00, 153.94, 152.71 (2C), 152.41, 115.58 (2C), 115.57 (2C), 115.50 (2C), 114.98 (2C), 114.82 (2C), 114.73 (2C), 103.20 ($J_{C\text{-}H}$ = 159 Hz), 100.83 ($J_{C\text{-}H}$ = 172 Hz), 100.19 ($J_{C\text{-}H}$ = 170 Hz), 99.50 ($J_{C\text{-}H}$ = 174 Hz), 81.15, 79.00, 78.47, 78.23, 78.03, 75.81, 75.40, 75.34, 75.25, 75.04, 74.95, 74.48, 73.72, 73.24, 73.17, 72.98, 72.89, 72.84, 72.71, 72.40, 71.26, 69.73, 69.43, 67.37, 66.91, 65.65, 64.44, 64.39, 62.46, 56.00 (2C), 55.95, 21.02.

Anal. Calcd for $C_{110}H_{116}O_{25}$: C, 71.88; H, 6.36. Found: C, 71.48; H, 6.30.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (138)

Prepared from **134** and **73** according to General Procedure B.

^{1}H NMR ($CDCl_3$, 500 Mhz) δ 7.42-7.00 (m, 30H), 6.82-6.41 (m, 12H), 5.71 (bs, 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 ($CDCl_3$, 75 Mhz, partial) 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (139)

Prepared from **138** according to General Procedure G.

^{1}H NMR ($CDCl_3$, 500 Mhz) 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 (bs, 1H), 2.06 (m, 1H), 1.66-1.54 (m, 4H), 1.41-1.25 (m, 9H).

^{13}C NMR ($CDCl_3$, 75 Mhz, partial) 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.

Anal. Calcd for $C_{92}H_{106}O_{21}$: C, 71.39; H, 6.90. Found: C, 71.17; H, 6.80.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (140)

To a solution of **138** (3.33 g, 2.09 mmol) in DMF (25 mL) was added NaH (600 mg of ~50% oil dispersion, 12.54 mmol) and the mixture was stirred until TLC showed full conversion (2 days). BnBr (0.75 mL, 6.28 mmol) was added, stirred 1 h, quenched with MeOH, diluted with CH_2Cl_2 (75 mL) and washed with H_2O (75 mL). The organic phase was dried, concentrated and purified by flash chromatography (hexane-EtOAc 5:2), yielding **140** (1.82 g, 53%) as a foam.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.42-7.08 (m, 35H), 7.82-7.42 (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 ($CDCl_3$, 75 Mhz, partial) 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.
ESI MS: *m/z* calcd for $C_{99}H_{112}NaO_{21}$ $[M+Na]^+$ 1660.8, found 1660.9.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(4-*O*-acetyl-2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (141)

Prepared from **139** and **73** according to General Procedure B.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.41-5.88 (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 ($CDCl_3$, 75 Mhz, partial) 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (142)

Prepared from **141** according to General Procedure G.
^{1}H NMR ($CDCl_3$, 500 Mhz) δ 7.40-6.93 (m, 50H), 6.77-6.26 (m, 20H), 5.04 (d, *J* = 3.0 Hz, 1H), 5.02 (d, *J* = 3.4 Hz, 1H), 4.99 (d, *J* = 3.4 Hz, 1H), 4.97 (d, *J* = 3.4 Hz, 1H), 4.94-4.88 (m, 3H), 4.84 (d, *J* = 12.4 Hz, 1H), 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), 3.79 (s, 6H), 3.77 (s, 3H), 3.54 (m, 1H), 3.48 (m, 1H), 3.44-3.37 (m, 2H), 2.40 (bs, 1H), 2.06 (m, 1H), 1.66-1.52 (m, 4H), 1.40-1.23 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz, partial) 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, 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-6-*O*-(4-methoxyphenyl)-α-D-galactopyranosyl)-(1→4)-*O*-(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 (143)

Prepared from **142** and **36** according to General Procedure B.
^{1}H NMR ($CDCl_3$, 500 Mhz) 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 ($CDCl_3$, 75 Mhz, partial) 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, 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranoside (144)

Prepared from **140** according to General Procedure H.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.47-7.25 (m, 35H), 5.00-4.89 (m, 4H), 4.86-4.59 (m, 14H), 4.52 (t, *J* = 5.1 Hz, 1H), 4.16-3.97 (m, 9H), 3.92 (bs, 1H), 3.90-3.30 (m, 13H), 2.19 (bs, 1H), 2.16 (bs, 1H), 2.09 (m, 1H), 1.81 (bs, 1H), 1.67-1.52 (m, 4H), 1.46-1.26 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz, partial) 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.
ESI MS: *m/z* calcd for $C_{78}H_{94}NaO_{18}$ $[M+Na]^+$ 1342.6, found 1342.7.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-(1→4)-2,3-di-*O*-benzyl-α-D-galactopyranoside (145)

Prepared from **143** according to General Procedure H.
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.50-6.28 (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 (bs, 2H), 2.21 (bs, 2H), 2.10 (m, 1H), 1.96 (bs, 2H), 1.70-1.57 (m, 4H), 1.50-1.30 (m, 9H).
^{13}C NMR ($CDCl_3$, 75 Mhz, partial) δ 102.40, 100.79, 100.76, 99.94, 99.89, 99.87, 97.47, 66.88 (2C), 35.24, 29.42, 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate) (146)

Prepared from **144** according to General Procedure I and treatment of the product with Cs_2CO_3 (3.5 equiv.) and MeI (6 equiv.) in MeCN for 12 h. Taken up in Et_2O, washed (H_2O), aqueous phase extracted (Et_2O), combined organic phases dried, filtered, concentrated and purified by flash chromatography (hexane-EtOAc 2:1, R_f: 0.13).

^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.38-7.02 (m, 35H), 5.18 (bs, 1H), 5.06 (d, *J* = 2.7 Hz, 1H), 4.93 (d, *J* = 3.3 Hz, 1H), 4.89 (d, *J* = 2.6 Hz, 1H), 4.81-4.34 (m, 19H), 4.25 (bs, 1H), 4.09 (bs, 1H), 4.05-3.96 (m, 2H), 3.91 (dd, *J* = 10.3, 3.1 Hz, 1H), 3.83-3.49 (m, 7H), 3.64 (s, 3H), 3.40 (dt, *J* = 9.7, 6.8 Hz, 1H), 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 ($CDCl_3$, 75 Mhz, partial) 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.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-*O*-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate) (147)

Prepared from **144** according to General Procedure I and treatment of the product with Cs_2CO_3 (3.5 equiv.) and BnBr (6 equiv.) in MeCN for 12 h. Taken up in Et_2O, washed (H_2O), aqueous phase extracted (Et_2O), combined organic phases dried, filtered, concentrated and purified by flash chromatography (hexane-EtOAc 5:2, R_f: 0.11).
^{1}H NMR ($CDCl_3$, 300 Mhz) δ 7.50-7.10 (m, 50H), 5.31-5.11 (m, 4H), 5.04 (bs, 1H), 4.97-4.34 (m, 24H), 4.24 (bs, 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 ($CDCl_3$, 75 Mhz, partial) 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, 66.69, 66.58, 66.47, 35.09, 29.24, 29.16, 29.08, 25.81, 25.74, 23.73.
ESI MS: *m/z* calcd for $C_{99}H_{106}NaO_{21}$ $[M+Na]^+$ 1654.7, found 1654.8.

7-(1,3-Dioxan-2-yl)-heptyl O-(benzyl 2,3,4-tri-O-benzyl-α-D-galactopyranosyluronate)-(1→4)-O-(benzyl 2,3-di-O-benzyl-α-D-galactopyranosyluronate)-(1→4)-O-(benzyl 2,3-di-O-benzyl-α-D-galactopyranosyluronate)-(1→4)-O-(benzyl 2,3-di-O-benzyl-α-D-galactopyranosyluronate)-(1→4)-(benzyl 2,3-di-O-benzyl-β-D-galactopyranosyluronate) (148)

Prepared from **145** according to General Procedure I and treatment of the product with Cs_2CO_3 (7 equiv.) and BnBr (12 equiv.) in MeCN for 12 h. Taken up in Et_2O, washed (H_2O), aqueous phase extracted (Et_2O), combined organic phases dried, filtered, concentrated and purified by flash chromatography (hexane-EtOAc 3:2, R_f: 0.14).
^{1}H NMR ($CDCl_3$, 500 Mhz) δ 7.43-6.92 (m, 95H), 5.19 (d, *J* = 3.4 Hz, 1H), 5.15 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 3.4 Hz, 1H), 5.09 (d, *J* = 3.4 Hz, 1H), 5.08 (d, *J* = 3.4 Hz, 1H), 5.05-4.92 (m, 4H), 4.84-4.08 (m, 49H), 4.04 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.84 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.83-3.51 (m, 14H), 3.46 (dt, *J* = 10.2, 6.8 Hz, 1H), 2.08 (m, 1H), 1.64-1.50 (m, 4H), 1.42-1.20 (m, 9H).
^{13}C NMR ($CDCl_3$, 125 Mhz, partial) 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, 29.33, 26.01, 25.96, 23.97.
ESI MS: *m/z* calcd for $C_{180}H_{184}NaO_{39}$ $[M+Na]^+$ 2994.2, found 2994.4.

7-(1,3-Dioxan-2-yl)-heptyl *O*-(methyl α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl α-D-galactopyranosyluronate)-(1→4)-(methyl α-D-galactopyranosyluronate) (149)

Prepared from **146** according to General Procedure M.
^{1}H NMR (CD_3OD, 500 Mhz) δ 5.15 (bs, 1H), 5.09 (bs, 1H), 4.94 (d, *J* = 3.8 Hz, 2H), 4.87 (d, *J* = 3.8 Hz, 1H), 4.53 (t, *J* = 5.1 Hz, 1H), 4.51 (bs, 1H), 4.40-4.36 (m, 2H), 4.35 (t, *J* = 6.0 Hz,

1H), 4.20 (dd, *J* = 3.0, 1.3 Hz, 1H), 4.04 (bdd, *J* = 10.7, 5.1 Hz, 1H), 3.91 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.89 (dd, *J* = 10.2, 3.0 Hz, 1H), 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, *J* = 6.4 Hz, 1H), 1.69-1.49 (m, 4H), 1.44-1.26 (m, 8H).
^{13}C NMR (CD_3OD, 125 Mhz) 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.
ESI MS: *m/z* calcd for $C_{32}H_{52}NaO_{21}$ $[M+Na]^+$ 795.3, found 795.5.

7-(1,3-Dioxan-2-yl)-heptyl *O*-α-D-galactopyranosyluronate-(1→4)-*O*-α-D-galactopyranosyluronate-(1→4)-α-D-galactopyranosyluronate (150)

Prepared from **147** according to General Procedure M.
^{1}H NMR (D_2O, 500 Mhz) δ 5.02-4.99 (m, 2H), 4.96 (bs, 1H), 4.95 (d, *J* = 3.8 Hz, 1H), 4.93 (d, *J* = 3.4 Hz, 1H), 4.67 (1H, signal hidden under HDO peak), 4.47 (m, 1H), 4.39-4.35 (m, 2H), 4.23 (bs, 1H), 4.00-3.89 (m, 3H), 3.82 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.75-3.70 (m, 1H), 3.67 (dd, *J* = 10.6, 3.4 Hz, 1H), 3.64 (dd, *J* = 10.2, 3.8 Hz, 1H), 3.61-3.54 (m, 4H), 3.47 (m, 1H), 1.67 (p, *J* = 6.4 Hz, 1H), 1.54-1.32 (m, 4H), 1.26-1.12 (m, 9H).
^{13}C NMR (D_2O, 125 Mhz) 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.
ESI MS: *m/z* calcd for $C_{29}H_{46}NaO_{21}$ $[M+Na]^+$ 753.2, found 754.9.

7-(1,3-Dioxan-2-yl)-heptyl O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-α-D-galactopyranosyluronate (151)

Prepared from **148** according to General Procedure M.
^{13}C NMR ($CDCl_3$, 125 Mhz, partial) 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-TOF MS: *m/z* calcd for $C_{47}H_{69}O_{39}$ $[M-H]^-$ 1257.34, found 1257.70.

8-[*N*-BSA]-octyl *O*-(methyl α-D-galactopyranosyluronate)-(1→4)-*O*-(methyl α-D-galactopyranosyluronate)-(1→4)-(methyl α-D-galactopyranosyluronate) (152)

A solution of **149** (65 mg, 0.084 mmol) in AcOH-H_2O 4:1 was heated at 50 °C for 12 h, cooled, concentrated and taken up in water (2 mL). BSA (23 mg, 0.35 μmol, "0.02 mmol NH_2") was added, the mixture was stirred for 1 h, then $NaCNBH_3$ (13 mg, 0.21 mmol) was added, and the mixture was stirred at room temperature for 4 days. Purified by filtration through a Centricon YM-30 filter and lyophilized.
MALDI-TOF MS: 66431 (BSA), 82541 (BSA+16110 = 22.6 sugar residues).

8-[*N*-BSA]-octyl *O*-α-D-galactopyranosyluronate-(1→4)-*O*-α-D-galactopyranosyluronate-(1→4)-α-D-galactopyranosyluronate (153)

A solution of **150** (152 mg, 0.208 mmol) in AcOH-H_2O 4:1 was heated at 50 °C for 12 h, cooled, concentrated and taken up in water (7 mL). BSA (55 mg, 0.84 μmol, "0.05 mmol NH_2") was added, the mixture was stirred for 1 h, then $NaCNBH_3$ (62 mg, 0.99 mmol) was added, and the mixture was stirred at room temperature for 2 days. PH adjusted to 9 with $NaHCO_3$, and BSA (25 mg) and $NaCNBH_3$ (15 mg, 0.24 mmol) was added, stirred for 3 days. Purified by filtration through a Centricon YM-30 filter and lyophilized.
MALDI-TOF MS: 66431 (BSA), 67097 (BSA+666 = 1.0 sugar residue).

8-[*N*-BSA]-octyl O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-O-α-D-galactopyranosyluronate-(1→4)-α-D-galactopyranosyluronate (154)

A solution of **151** (15 mg, 0.012 mmol) in AcOH-H_2O 4:1 was heated at 50 °C for 12 h, cooled, concentrated and taken up in water (1 mL). pH adjusted to 9 with $NaHCO_3$, BSA (5 mg, 0.08 μmol, "0.005 mmol NH_2") was added, the mixture was stirred for 1 h, then $NaCNBH_3$ (30 mg, 0.48 mmol) was added, and the mixture was stirred at room temperature for 4 days. Purified by filtration through a Centricon YM-30 filter and lyophilized.
MALDI-TOF MS: 66431 (BSA), 70996 (BSA+4565 = 3.8 sugar residues).

Appendix A

Abbreviations

Ac ..... *Acetyl*
All ..... *Allyl, prop-2-ene-1-yl*
b..... *Broad signal, in NMR*
Bn..... *Benzyl*
Bz ..... *Benzoyl*
BSA..... *Bovine serum albumin*
CAN ..... *Cerium(IV)ammonium nitrate*
ClAc ..... *Chloroacetyl*
d..... *Doublet, in NMR*
DAST..... *Diethylaminosulfur trifluoride*
DCC ..... *Dicyclohexylcarbodiimide*
DEAD ..... *Diethylazodicarboxylate*
DIPEA..... N,N-*Diisopropyl ethylamine, Hünig's base*
DM..... *Degree of methylation*
DMAP ..... 4-(N,N-*Dimethylamino)-pyridine*
DMF ..... N,N-*Dimethylformamide*
DMSO..... *Dimethylsulfoxide*
DP..... *Degree of polymerization*
Equiv..... *Equivalents*
ESI..... *Electrospray ionization*
Et ..... *Ethyl*
FITC..... *Fluorescein isothiocyanate*
gHSQC..... *Pulse field gradient Heteronuclear single quantum coherence*
h..... *Hour(s)*
HG ..... *Homogalacturonan*
IDCP ..... *Iodonium dicollidine perchlorate*
J ..... *Coupling constant, in NMR*
Lev..... *Levulinyl, 4-oxo-pentanoyl*
m..... *Multiplet, in NMR*
M ..... *Molecular ion, in MS*
MALDI-TOF..... *Matrix assisted laser desorption ionization time of flight*
Me ..... *Methyl*
min ..... *Minutes*
MP ..... *Milk powder, fat free milk protein*
MS ..... *Mass spectrometry*
NBS ..... N-*Bromosuccinimide*
NIS ..... N-*Iodosuccinimide*
NMR ..... *Nuclear magnetic resonance*
p..... *Pentet, in NMR.*
P, P^1, P^2, P^3 ..... *Protection groups*
PBS ..... *Phosphate buffered saline*
PG..... *Polygalacturonase*
Ph ..... *Phenyl*
PL ..... *Pectin lyase*
PME..... *Pectin methyl esterase*
PMP..... para-*Methoxyphenyl*
ppm ..... *Parts per million*

PTSA ..... para-*Toluenesulfonic acid*
q..... *Quartet, in NMR*
RG ..... *Rhamnogalacturonan*
s..... *Singlet, in NMR*
t ..... *Triplet, in NMR*
TBAF ..... *Tetrabutylammonium fluoride*
TBDPS ..... tert-*Butyldiphenylsilyl*
t-Bu ..... tert-*Butyl*
TEMPO..... *2,2,6,6-Tetramethylpiperidine-1-oxyl*
TES ..... *Triethylsilyl*
Tf..... *Triflic, Trifluoromethanesulfonyl*
THF ..... *Tetrahydrofuran*
TLC ..... *Thin layer chromatography*
TMS ..... *Trimethylsilyl*
Tr ..... *Trityl, triphenylmethyl*
Ts ..... *Tosyl,* p-*toluenesulfonyl*
Å ..... *Angstrom,* 10^{-10} *m*

APPENDIX B

Dansk resumé

En strategi for kemisk syntese af oligosakkarider med relation til plante-polymeren pektin bliver beskrevet. De udviklede protokoller anvender *n*-pentenyl glykosyl donorer og sådanne mono- og disakkarider med passende beskyttelsesgrupper er fremstillet. En ny metode til fremstilling af glykosyl fluorider fra *n*-pentenyl glykosider er udviklet og forskelle på reaktivitet mellem glykosyl donorer bliver udnyttet i selektive koblingsreaktioner. Tri- og hexagalakturonsyrer med et selektivt methylesterificeringsmønster er blevet fremstillet. Sidstnævnte er anvendt som substrater for pektinnedbrydende enzymer og til karakterisering af monoklonale antistoffer, som genkender pektin. Derudover er glykoproteiner blevet produceret ved kobling af tri- og hexasaccharider til bovin serum albumin. Desuden skildres karakterisering af et nyt antistof, som opstod efter immunisering af rotter med et af ovennævnte glykoproteiner.