ACYL TRANSFER IN CHEMICAL SYNTHESIS OF Oligosaccharides

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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University of Toronto

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0-612-34056-2
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Master of Science, 1998,
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ABSTRACT

The most commonly used method of stereoselective synthesis of 1,2-trans glycosidic bonds is protection of the second position of the donor with a participating acyl group. The most commonly used participating group is an acetyl group. However, it has been found that the group at O-2 transfers to acceptors by a transesterification reaction, thus lowering the yields of the desired products. In order to find a way to eliminate this transfer we synthesized five tribenzylated donors each having a different acyl group at O-2, i.e., formyl, acetyl, levulinyl, pivaloyl or benzoyl. The results of the reactions with the acceptor (polyethylene glycol polymer MPEGDOXOH) indicate that presence of the benzyl groups (or absence of acetyl groups) at O-3, 4 and 6 eliminates transfer in the case of benzoyl and pivaloyl groups and reduces it significantly when acetyl and levulinyl groups are present (formyl group transfers significantly). It would be synthetically very useful if only one benzyl group was sufficient to prevent acyl transfer. To investigate this idea I synthesized three triacetylated donors with benzyl groups at O-3, 4 or 6 and one dibenzylated donor with benzyl groups at O-3 and 6 and reacted these donors with MPEGDOXOH. I found that the transfer of the acetyl group was reduced in all cases compared to the tetraacetylated donor but it was significantly higher than from the tribenzylated donor.
ACKNOWLEDGMENTS

I would like to acknowledge the help I received from many people during my studies. First, I would like to express my most sincere thanks and appreciation to my supervisor Dr. Jiri Krepinsky for his valuable guidance and consideration. I am also grateful to all my lab colleagues: Dr. Iwona do Santos Z., Carmin Cautillo, Dr. Nina Lupescu, Dr. Wang Zhi-Guang, Dr. Min Chan, Dr. Enoka Richens, Dr. Steven Douglas, Rudy Furrer, Dr. Jack Chociej, Xu Fang Zhang, Dr. Craig Railton, Catherine Ho and Dr. Anette Nowak for their help, and for creating a warm and jolly atmosphere in the lab. A special thanks goes to Iwona who initiated the project and who helped me tremendously to advance it. I would also like to acknowledge Carmin Cautillo, a former fourth year student in the lab, for carrying out a portion of the work described in this thesis.

I am also grateful to my supervisory committee members: Dr. Rick Collins, Dr. Thomas Tidwell and Dr. Jim Rini, and also to Dr. Marvin Gold, who served on the reading committee, for their help, time, support, and consideration. I would like to extend my thanks to Dr. Dennis Whithfield for providing the reference material related to this thesis.

Very deep thank you goes to my family for their support and also to all the people that I met during my studies for making my life more colorful. The research in this thesis was funded by PENCE, so I would like to thank the network for its support.
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LIST OF ABBREVIATIONS

Ac - acetyl
Bn - benzyl
Bz - benzoyl
DIPEA - diisopropyl ethylamine
Lev - levuliny1
MPEGDOXOH - 4-(hydroxymethyl)benzyl ω-methylpoly(ethyleneglycol)yl ether
Piv - pivaloyl
SPh - thiophenyl
TESOTf - triethylsilyl trifluoromethanesulfonate
Introduction
Biological background

Carbohydrates (saccharides) are essential components of all living organisms and are, in fact, the most abundant class of biological molecules. The basic units of carbohydrates are called monosaccharides. They provide most of the energy used to power biological processes and are principal components of nucleic acids, as well as important elements of complex lipids. Oligosaccharides consist of a small number of covalently linked monosaccharide units (usually less than thirty). They are often covalently linked to proteins (glycoproteins) or lipids (glycolipids) in which they have both structural and regulatory functions. Polysaccharides consist of many covalently linked monosaccharide units and have molecular masses ranging into the millions of daltons. They have essential structural functions in all types of organisms and serve as important nutritional reservoirs in plants (starch) and animals (glycogen).

The ubiquity and enormous variability of structures of carbohydrates explain the great variability of their functions, many of which are just beginning to be discovered. Only in the past decade it has been recognized that carbohydrates, like DNA and proteins, are carriers of specific biological information. That is, as part of glycoproteins and glycolipids they participate in the regulation of many biological processes. Carbohydrate residues can influence the stability, conformation, protease lability, thermal properties, and solubility of proteins. Glycoproteins are found in soluble form in blood and in numerous secretions, and, along with glycolipids, in membranes of endoplasmic reticulum, Golgi apparatus, lysosomes and plasma membrane (Gottschalk, 1972; Horowitz & Pigman, 1977-1982; Singer, 1974; Hirschberg & Snider, 1987). In the cell membranes the carbohydrate portions project

Moreover, fibronectin, an extracellular adhesive glycoprotein helps mediate cell-matrix adhesion and also guides cell migration in both invertebrate and vertebrate embryos (Dufour et al., 1988; Hynes, 1985; Hynes, 1986). Important biological activities have also been determined for glycosaminoglycans, carbohydrate polymers that occur in all animal tissues, mostly as part of a proteoglycan (Ruoslahti, 1989; White & Mecham, 1987; Jackson et al., 1991). A well-known example of this family is the anticoagulant heparin, which is clinically used for the prevention and treatment of thrombosis (Casu, 1985; Lane & Lindahl, 1989; Ofusu et al., 1989).

In addition, many other naturally occurring carbohydrates and analogues thereof have been found to exhibit important pharmacological activities and are used as drugs for treatment of a wide variety of diseases such as bacterial (Umezawa & Hoopers, 1982) and viral infections (de Clercq, 1987), cancer (Sone et al., 1984) and cardiovascular disease (Greef, 1981). Furthermore, it has been demonstrated that carbohydrate derivatives can be used as antigens in vaccines (Peeters et al., 1992; Veeneman et al., 1989).

Thus, although a lot is known about carbohydrates, our knowledge of them lags behind that of proteins and nucleic acids. This can be attributed to the fact that
carbohydrates are present in organisms in complicated mixtures and only in minute quantities. This makes their separation from natural sources impractical. Furthermore, they cannot be studied by the types of genetic analysis that have been invaluable in the study of nucleic acids and proteins because oligosaccharide sequences are built up by sequential action of specific enzymes and are subject to further processing (i.e. cleavage of specific monosaccharide residues) which differs for different glycoconjugates. Also, it has been difficult to design assays for the biological activities of polysaccharides because of their largely structural roles. Therefore, to obtain larger amounts of pure oligosaccharides we must make them synthetically.

Synthesis of oligosaccharides

Currently oligosaccharides can be synthesized using chemical (Khan et al., 1996) or enzymatic (Bednarski & Simon, 1991) methods. At present, chemical synthesis of oligosaccharides is the most utilized approach. This method offers the advantage that structurally modified derivatives (that cannot be made by enzymatic methods) can be prepared which may give additional information concerning the functions of carbohydrates and may lead to the development of new drugs.

In the chemical method, those hydroxyl groups which are not to become glycosylated are protected by chemical groups. Thus, regioselective bond formation is usually achieved by condensing a fully protected glycosyl donor, which is activated at its anomeric centre, with a suitably protected glycosyl acceptor that normally contains only one free hydroxyl group. Some hydroxyl groups need to be selectively protected by short-term protecting groups while other hydroxyl groups need to be
protected by long-term protecting groups so that the short-term protecting groups can be removed in a desired intermediate to make the required hydroxyl groups available for subsequent glycosylation. Once the desired protected oligosaccharide is obtained, then the short-term and the long-term groups can be removed to yield the final product. Often up to five steps are required to selectively protect a monosaccharide for use in a synthetic scheme.

The chemical method of synthesizing oligosaccharides underwent rapid development in the last twenty five years. One important innovation was the identification of better leaving groups than traditionally used anomeric halides. One such improvement was the introduction of the trichloroacetimidate group as a leaving group at the anomeric position (Schmidt, 1986; Schmidt, 1989). The advantage of this group is that it can be used in many different types of glycosylations and often needs only short reaction times. This group was utilized in my studies.

Parallel to the chemical methods of oligosaccharide synthesis, methods of enzymatic oligosaccharide synthesis have been developed in the last decade (David et al., 1991; Bednarski & Simon, 1991; Yuasa et al., 1992; Wang et al., 1994). These methods have the advantage that they require no protecting groups, thereby shortening the synthesis. The glycoside-cleaving enzymes such as glycosidases, which are relatively easy to access, have been investigated first. After having their cleavage site inactivated, these enzymes form glycosidic bonds without cleaving them. These reactions can be done even in organic solvents. However, the yields are limited and also the regio-selectivity is not absolute in some cases, so that purification steps are necessary. On the other hand, glycosylation reactions catalyzed by
glycosyltransferases are much more selective. These enzymes, however, require relatively unstable intermediates, the yields are limited, and unnatural oligosaccharides are unlikely to be formed. It should be noted that the enzymatic and chemical methods of synthesizing oligosaccharides can be combined, especially if complex oligosaccharides are the goal (Ito et al., 1993; Wong et al., 1993).

The chemical synthesis of oligosaccharides can be done in solution, on solid support (Nicolaou et al., 1997; Adinolfi et al., 1996) or in solution on a polymer support (Khan & O'Neil, 1996; Douglas et al., 1995; Verduyn et al., 1993). The last method was utilized in my studies for it combines the anomeric control of solution chemistry with the ease and speed of solid-state supported workup. The polymer used in my studies was polyethylene glycol ω-monomethyl ether (MPEG) attached to an α,α'-dioxyxylyl diether (DOX) linker which allows removal of PEG after the oligosaccharide synthesis is completed (see Figure 1 for the structure of MPEGDOXOH).

A very important goal in the synthesis of oligosaccharides is to synthesize the glycosidic bond stereoselectively, i.e. to form either the α or the β anomer. Protection of the second position with a participating acyl group is the most commonly used method of obtaining 1,2-trans glycosidic linkages. The most commonly used participating group is an acetyl group. However, it has been found to transfer to acceptors during glycosylations by a transesterification reaction, thus lowering the yields of desired products (Lemieux, 1964; Uvarova et al., 1973; Ziegler et al., 1990 a; Ekborg et al., 1983; Kovac, 1985; Zigler et al., 1990 b; Kovac, 1986). This transfer is especially problematic in polymer supported methods since the premature quenching of the growing oligosaccharide gives rise to too many side products and significantly
lowers the yields. To determine from which position of the donor the transfer occurs, Ziegler et al. (1990 a) reacted 2-O-[2,2,2-D₃]acetyl-3,4,6-tri-O-acetyl-α-D-galactopyranosyl bromide with an acceptor and found that only the deuterated acyl group transferred to the acceptor.

Acyl transfer can be overcome in some glycosylation reactions by using donors protected with benzoyl rather than acetyl groups (Garegg & Norberg, 1979). However, in some glycosylations, protection by benzoyl groups shifts the anomeric ratio of the product towards the α anomer (J.J. Krepinsky, private communication). Knowing that, I decided to study acyl transfer in more detail hoping to find a way to prevent it. More specifically, I wanted to investigate the behaviour of different acyl groups at O-2 in the presence of benzyl groups (non-participating, long-term protecting groups) at O-3, 4 and 6 of the donors. Also, I wanted to investigate the transfer of the acetyl group in the presence of only one benzyl group at O-3, 4 or 6.
Methods of Investigations
All the donors were reacted with the acceptor MPEGDOXOH at room temperature in dichloromethane using triethylsilyl trifluoromethanesulfonate (TESOTf) as catalyst (see the Experimental Procedures section). After the disappearance of all the imidate the solution was concentrated and the polymer was precipitated in t-butyl methyl ether or ethanol at 4 °C. The polymer was then isolated by filtration and analyzed by $^1$H-NMR spectroscopy. The spectra indicated whether a mixture of β- and α-glycosylated MPEGDOX, acylated MPEGDOX and unreacted MPEGDOXOH was obtained and the ratios of individual components were deduced from integration values.

In order to obtain the $^1$H-NMR standard spectra of the acylated polymer-linker, MPEGDOXOH was acylated to give formylated, acetylated or benzoylated derivatives (see Appendix, pp. 80-82). The $^1$H-NMR spectrum of the formylated MPEGDOXOH (MPEGDOX-O-formyl) shows that the HCO-O-CH$_2$-C$_6$H$_4$-CH$_2$-(OCH$_2$CH$_2$)$_n$OCH$_3$ signal is shifted to 5.19 ppm from 4.68 ppm in MPEGDOXOH. This same peak appears at 5.09, 5.10 and 5.35 ppm in the acetylated, levulinylated and benzoylated MPEGDOXOH, respectively. [Note: the value for the levulinylated acceptor was obtained from the spectrum of the polymer after its reaction with 3,4,6-tri-O-acetyl-2-O-levulinyl-D-galactopyranosyl trichloroacetimidate (I. do Santos Z., private communication) see Appendix, p. 83.] These values were used as controls during the analysis of the $^1$H-NMR spectra of glycosylated MPEGDOXOH, i.e. the presence and the size of these signals indicated the presence and the extent of acyl transfer during glycosylation reactions. The percent of glycosylation was calculated...
by dividing the integration of a sugar proton (H-2 or H-1) by 1/3 of the integration of the methyl signal of MPEG (which corresponds to a ratio of the number of moles of a sugar attached to MPEG to total MPEG molecules present) and multiplying by 100%.

The percent of acyl transfer was calculated by dividing 1/2 of the acylated methylene DOX signal (i.e. the shifted methylene signal) by 1/3 of the methyl signal of MPEG and multiplying by 100%.

In some reactions, the total percentage of glycosylation and acyl transfer exceeded the theoretical 100%. This was brought about by the overlap of the signal base with neighboring signals. However, this effect was small enough to allow comparisons between different glycosylation reactions.
Results of Investigations
The results of the glycosylation reactions of tetraacetylated donors with MPEGDOXOH are summarized in Table 1. It shows that when tetraacetylated galactopyranosyl trichloroacetimidate was reacted with MPEGDOXOH at room temperature using TESOTf as promoter, the ratio of β-glycosylation to acyl transfer was about 0.5 : 1, i.e. unacceptably high. The \(^1\)H-NMR spectrum of the glycosylated MPEGDOX (see Appendix, pp. 84-85) also indicates presence of two other minor products, although the structure of the sugars and the glycosidic linkage could not be determined from \(^1\)H-NMR. (The \(^1\)H-NMR spectrum clearly shows the presence of only two very weak H-4 signals at 5.34 and 5.36 ppm next to the major H-4 signal at 5.39 ppm. The integration of the signals at 5.34 and 5.36 ppm indicates that the yields of the two minor products were ~2% and 3%, respectively).

It should be noted that similar results were obtained when tetraacetylated glucopyranosyl and mannopyranosyl trichloroacetimidates were reacted with MPEGDOXOH. Even more acyl transfer was obtained when BF_3•Et_2O was used as a promoter in these glycosylation reactions. [The data is not shown (I. do Santos Z., private communication).] On the other hand, the reaction of tetrabenzoylated galactopyranosyl trichloroacetimidate with MPEGDOXOH at room temperature using TESOTf as promoter gave almost 100% β-glycosylation and very little acyl transfer (see Table 1 and Appendix, p. 86).

Therefore, knowing that acyl transfer can be overcome by using tetrabenzoylated rather than tetraacetylated donors (Garegg et al., 1979; Table 1) and that in some reactions protection by benzoyl groups shifts the anomeric ratio of product towards
the α anomer (J.J. Krepinsky, private communication) I decided to investigate the behaviour of a series of acyl groups at O-2 in the presence of benzyl groups (non-participating, long-term protecting groups) at O-3, 4 and 6. Thus, five tribenzylated galactopyranosyl trichloroacetimidates with O-2 formylated (I. do Santos Z., private communication), acetylated, levulinylated, pivaloylated or benzyoylated were synthesized (see Scheme 1). These donors were then reacted with MPEGDOXOH under the same conditions as the tetraacetylated and tetrabenzoylated galactopyranosyl donors and the extent of glycosylation and acyl transfer was measured by $^1$H-NMR (see Appendix, pp. 87-109).

As Table 2 shows, there was significant acyl transfer when a formyl group was present at O-2, very low in the case of the acetyl and levulinyl groups, and it was eliminated completely when C-2 was pivaloylated and benzyoylated. Therefore, benzylation at O-3, 4 and 6 significantly reduces acyl transfer. The glycosides formed were predominantly 1,2-trans (β) although a small amount of the cis (α) isomer was detected in all reactions. Thus, the reaction of 2-O-benzoyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate with MPEGDOXOH was not as stereospecific as was the reaction of 2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate (see Table 1). A similar comparison can be made between the reaction of MPEGDOXOH with 2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate and the tetraacetylated donor (see Tables 1 and 2) since the $^1$H-NMR spectrum of the glycosylation product of the latter indicates that if any α-glycosylation occurred, it was lower than in the reaction of 2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate with MPEGDOXOH (see Appendix,
pp. 84-85). Table 2 also shows that the reactions of the α and β imidates with the acceptor in the cases studied gave approximately the same ratios of glycosylation to acyl transfer.

Benzyl groups at C-3, 4 and 6 also significantly reduce acyl transfer in glucose (see Table 2 and Appendix, p. 110; C. Cautillo, private communication). The synthesis of the 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl trichloroacetimidate was done using a similar synthetic scheme as that used in the synthesis of the galactopyranosyl donors (Scheme 1).

Therefore, knowing that benzyl groups at O-3, 4 and 6 can reduce or inhibit acyl transfer, I wanted to know which of the benzyl groups is responsible for this effect. It would be very useful synthetically if acyl transfer could be prevented by a single benzyl group because it would allow one to vary the protection of other positions. To investigate this possibility I synthesized three triacetylated monobenzylated trichloroacetimidates benzylated at O-3, 4 or 6. I also synthesized one dibenzylated donor with benzyl groups at O-3 and 6 (see Scheme 2), which provided additional information about the requirements for the prevention of acyl transfer. The dibenzylated and the monobenzylated donors were then reacted with MPEGDOXOH and the extent of glycosylation and acyl transfer was measured by $^1$H-NMR (see Appendix, pp. 120-126, 135-138). As Table 3 shows, the extent of acyl transfer was reduced in all cases compared with the tetraacetylated donors although it was significantly greater than the acyl transfer from 2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate. These results indicate that all three positions, i.e. O-3, 4 and 6, have to be benzylated in order to achieve as low acyl transfer as was obtained when 2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate
was reacted with MPEGDOXOH. Interestingly, as shown in Table 3, no α-glycosylation was obtained when the 4-O-benzyl and 6-O-benzyl galactopyranosyl donors were reacted with MPEGDOXOH, but the glycosylation with 3-O-benzyl and 3,6-di-O-benzyl derivatives was less stereoselective and a small amount of α-anomer was obtained.

The following compounds synthesized in this work have not been described in the literature: 3,4,6-tri-O-benzyl-2-O-formyl-D-galactopyranosyl trichloroacetimidate, 3,4,6-tri-O-benzyl-2-O-levulinyld-galactopyranosyl trichloroacetimidate, 3,4,6-tri-O-benzyl-2-O-pivaloyl-D-galactopyranosyl trichloroacetimidate, 2-O-benzoyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate, phenyl 3,6-di-O-benzyl-1-thio-β-D-galactopyranoside, phenyl 2,4-di-O-acetyl-3,6-di-O-benzyl-1-thio-β-D-galactopyranoside, 2,4-di-O-acetyl-3,6-di-O-benzyl-D-galactopyranose, 2,4-di-O-acetyl-3,6-di-O-benzyl-D-galactopyranosyl trichloroacetimidate, 2,3,6-tri-O-acetyl-4-O-benzyl-D-galactopyranosyl trichloroacetimidate and all the MPEGDOXyl galacto- and glucopyranoside derivatives in Tables 1, 2 and 3.

It is noteworthy that the regiospecific benzylation of phenyl 1-thio-β-D-galactopyranoside via stannylation proceeds less smoothly than the same reaction employing methyl β-D-galactopyranoside described by Kovac & Glaudemans (1985 a). That is, the yields of the benzylation of phenyl 1-thio-β-D-galactopyranoside [to give phenyl 3-O-benzyl-1-thio-β-D-galactopyranoside (13%) and 3,6-di-O-benzyl-1-thio-β-D-galactopyranoside (6%)] were much lower than the benzylation of methyl β-D-galactopyranoside described by Kovac & Glaudemans (1985 a) [to give methyl 3-O-
benzyl-β-D-galactopyranoside (65%) using the same reaction conditions. A similar phenomenon was observed (I. do Santos Z., private communication) when phenyl 1-thio-α-D-mannopyranoside was regiospecifically methylated via stannylation to give phenyl 3-O-methyl-1-thio-α-D-mannopyranoside (20%). However, according to Liao & Lu (1996), this reaction gave a 73% yield of the 3-O-methyl derivative when allyl α-D-mannopyranoside was used. Thus, the very low yields of these reactions could be due to the presence of the thiophenyl group at C-1 instead of the methoxy group or the allyl groups.

The acetylation of 53 giving 54 in 87% yield required more acidic conditions than the acetylation of 2-O-acetyl-1,6-anhydro-4-O-benzyl-β-D-galactopyranose (to give the same product, 75% yield) described by Subero et al. (1985). That is, acetylation of 53 required the presence of 0.1 equivalent of sulphuric acid in acetic anhydride/acetic acid (5 : 1) while the acetylation of 2-O-acetyl-1,6-anhydro-4-O-benzyl-β-D-galactopyranose described by Subero et al. (1985) was done only in acetic anhydride/acetic acid (50 : 1).

The removal of allyl and thiophenyl groups (see Schemes 1 and 2) resulted in the formation of unseparable mixtures of the expected products with acyl groups at O-2 and OH at C-1 and also products with acyl groups at O-1 which resulted from the migration of acyl groups from O-2 to O-1 in the acidic conditions of those reactions. The acyl group at O-1 probably migrated back to O-2 (by a base catalyzed migration) during imidate formation. This explanation is based on the observation that very high yield (93%) of 2,4-di-O-acetyl-3,6-di-O-benzyl-D-galactopyranosyl trichloroacetimidate was obtained even though the starting material consisted of 27%
of 1,4-di-O-acetyl-3,6-di-O-benzyl-α-D-galactopyranose. This result agrees with the observation by Lay et al. (1994) that opening the orthoester 3,4,6-tri-O-benzyl-α-D-galactopyranose 1,2-ethyl orthoacetate in the presence of base gave 2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranose, while opening the same orthoester under acidic conditions gave rise to 1-O-acetyl-3,4,6-tri-O-benzyl-α-D-galactopyranose (Hindsgaul et al., 1982).

The above mentioned opening of 3,4,6-tri-O-benzyl-α-D-galactopyranose 1,2-ethyl orthoacetate to give 14 is a faster method of obtaining 22 since it avoids the removal of the allyl group from the anomeric position (see Scheme 1). In addition, 2,4,6-tri-O-acetyl-3-O-benzyl-D-galactopyranosyl trichloracetimidate can be obtained with a higher yield if the thiophenyl group is replaced by a methoxy group (van Steijn et al., 1989; Kovac & Glaudemans, 1985 a). That is, the benzylation of O-3 can be done with 65% yield (Kovac & Glaudemans, 1985 a) which is much higher than the yield in our work (13%).
Discussion of Results
The results of the glycosylation reactions with tribenzylated donors (see Table 2) showed that the 2-O-formyl group is much more prone to transfer to the acceptor than the 2-O-acetyl and 2-O-levulinyl groups when present in the same environment, while the 2-O-pivaloyl and 2-O-benzoyl groups did not transfer at all. Thus, the results indicate that the observed pattern and the magnitude of transfer of these groups is due only to their intrinsic properties, i.e. the more bulky and electron-donating the group, the less it transfers to the acceptor by the transesterification reaction. The reduction/prevention of acyl transfer is probably due to an effect of the more bulky and electron-donating groups on the stability and/or the rate of formation and/or decomposition of intermediates leading to acyl transfer [see Schemes 4 and 5 (Bochkov et al., 1976; Banoub & Bundle, 1979; Hansch & Taft, 1991)]. That is, electron donation by the pivaloyl and benzoyl substituents may stabilize the positive charge of the intermediates in Schemes 4 and 5 and thus change their reactivity. Also, more bulky substituents may reduce the rate of formation and/or the stability of ions 2 and 3 in Scheme 4 due to increased steric interference in the transition states and the intermediates [Note: It is also possible that O-1 may be protonated instead of O-2 of ion 3 in Scheme 4.] (Carey & Sundberg, 1990). This conclusion is supported by the observation that the 2-O-benzoyl and 2-O-pivaloyl groups undergo orthoester formation less readily than 2-O-acetyl groups (Zimmerman, dissertation, 1988; Zimmerman et al., 1988). The observed pattern of acyl transfer to MPEGDOXOH i.e. benzoyl, pivaloyl < levulinyl < acetyl << formyl can be compared with the rate of equilibration of intramolecular migration of benzoyl, acetyl and formyl groups between 2' and 3' positions of uridine, which was
found to increase in the order benzoyl < acetyl < formyl \((k_1 + k_2)\) ratios = 1 : 18 : 670, where \(k_1\) and \(k_2\) are the rate constants for the O-2 \(\rightarrow\) O-3 and O-3 \(\rightarrow\) O-2 migrations, respectively] (Reese & Trentham, 1965).

The reactions of the variously benzylated donors with MPEGDOXOH showed another important result: that replacement of acetyl groups at O-3, 4 and 6 with benzyl groups results in the reduction of acyl transfer. Thus, a question arises - how do benzyl groups (or the absence of acetyl groups) prevent acyl transfer? One explanation can be found in an article by Bochkov et al. (1976). In this publication, the authors report a suppression of acyl transfer after replacement of acetyl groups with methyl groups. That is, 3,4,6-tri-O-acetyl-\(\alpha\)-D-glucopyranose 1,2-(cyclohexyl orthoacetate) transfers acetate to cyclohexanol much more easily than 3,4,6-tri-O-methyl-\(\alpha\)-D-glucopyranose 1,2-(cyclohexyl orthoacetate) under the same glycosylation conditions (ie., 1 equivalent cyclohexanol, 2,4,6-collidinium perchlorate, in chlorobenzene at 90 °C, see Scheme 3). This result, together with the observation that the 2-OH products obtained when the triacetylated orthoester was reacted had the \(\alpha\)-configuration and an anomeric mixture of 2-OH products was obtained when the trimethylated derivative was reacted, suggests that the 3-O-acyl and/or 6-O-acyl group participate at the anomeric centre and facilitate the dissociation of cyclohexyl acetate from ion 4 in Scheme 4. (Note: Participation of 4-O-acyl group is impossible in glucose for steric reasons.)

It is possible that in my case a similar process takes place. That is, when the tetraacetylated donor is reacted with MPEGDOXOH, ion 2 forms as an intermediate which, after protonation at O-2, leads to a homolog of ion 4 in Scheme 4. The acetyl
groups at O-3, 4 and 6 may then participate at C-1 to facilitate the dissociation of ion 4 to ion 5 (see Scheme 4). [Note: A molecular model of 3,4,6-tri-O-acetyl-α-D-galactopyranose 1,2-(alkyl orthoester) indicates that the acyl groups at O-3, 4 and 6 are within a distance from C-1 allowing them to participate at the anomeric centre.] However, when benzyl groups are present at O-3, 4 and 6, the dissociation of ion 4 into the homolog of ion 7 in Scheme 4 may be unfavourable since there are no acyl groups to participate at C-1 and ion 2 may reform preferentially. Ion 2 may then lose MPEGDOXOH and form a β-glycosylated product.

Therefore, now one could ask if there is a preference for a particular position of the acyl group. On the basis of the following factors, Bochkov et al. (1976) suggested that participation of the acetoxy group at O-3 is more likely than participation of the acetoxy at O-6. First, the participation of the acetoxy groups at O-3 and O-6 at the anomeric carbon leads to the formation of six- and seven-membered rings, respectively. In a number of sugars and related compounds six-membered cyclic acyloxonium ions appear to be relatively more stable than their seven-membered isomers (Paulsen, 1971). Second, the X-ray structure of 3,4,6-tri-O-acetyl-α-D-glucopyranose 1,2-(ethyl orthoacetate) shows that the pyranose ring of this compound in the crystalline state has the twist conformation in which the acetoxy group at C-3 occupies the axial position suitable for attack on C-1 (Heitman et al., 1974). Therefore, the above data suggests there is a preference for the participation of the acetyl group at O-3 in substitution at the glycosidic centre above the participation of the acetyl group at O-6.

The molecular model of 3,4,6-tri-O-acetyl-α-D-galactopyranose 1,2-(alkyl orthoester) indicates that the acetyl groups at all three positions (i.e. at O-3, 4 and 6)
can participate at the anomeric centre. However, by applying similar analysis as was done for glucose by Bochkov et al. (1976), we can differentiate between these positions. Thus, the participation of the acetyl group at O-6 would be expected to be least favourable since it would require the group to occupy an axial orientation and would lead to formation of the relatively unstable seven-membered cyclic acyloxonium ion. The choice between the acetyl groups at O-3 and O-4 is harder to make since the participation of the acetyl group at O-3 would require the group to occupy an axial orientation but would lead to formation of the relatively more stable six-membered cyclic acyloxonium ion; however, the participation of the acetyl group at O-4 would not lead to increased steric hindrance between the groups (since O-4 is normally in the axial orientation) but would lead to formation of the relatively less stable seven-membered cyclic acyloxonium ion. It should be noted that my experiments with the monobenzylated donors (see Table 3) suggest that participation of all three acetyl groups at O-3, 4 and 6 is possible since benzylation of all three positions resulted in reduction of acyl transfer. (The data does not clearly indicate a preference for either O-3, 4 or 6.)

Another possible mechanism by which the presence of benzyl groups (or absence of acetyl groups) reduces acyl transfer is by inductive donation of electrons centres (Hansch & Taft, 1991) to the electron-deficient centres. That is, it is possible that benzyl groups donate electrons to the positively charged 2-O-acyl group and thus reduce the attraction of the nucleophile (acceptors hydroxyl) to the 2-O-acyl group. It is also possible that benzyl groups donate electrons to C-1 and thus reduce the need for (or the strength of) the stabilizing participation of the 2-O-acyl group. Thus, if less electron density is donated by the 2-O-acyl group to C-1, then the carbonyl bond
would have more double-bond character and the nucleophile would be less prone to react with it. It should be noted that acetyl groups are electron-withdrawing, and therefore can probably be expected to have the opposite effect from benzyl groups and thus to promote the transfer of the acyl group from O-2.

The above model of inductive effect exerted by substituents at O-3, 4 and 6 is supported by the general observation that O-benzyl- and O-allyl-protected donors appear to be more reactive than O-acetylated derivatives which in turn are more reactive than O-trichloroacetyl-protected donors (Paulsen, 1982). Moreover, studies done by Ishikawa & Fletcher (1969) showed that the replacement of benzyl groups at O-3, 4 and 6 with electronegative para-nitrobenzoyl groups resulted in a greatly reduced rate of anomerization of β-bromide to the more stable α-bromide. That is, the rate of anomerization of 2-O-benzyl-3,4,6-tri-O-p-nitrobenzoyl-β-D-glucopyranosyl bromide and 2,3-di-O-benzyl-4,6-di-O-p-nitrobenzoyl-β-D-glucopyranosyl bromide was slower than the anomerization of 2,4,6-tri-O-benzyl-6-O-p-nitrobenzoyl-β-D-glucopyranosyl bromide, while 2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl bromide seemed to convert to the α-anomer fastest of all derivatives in the series. It is also interesting that, as Ziegler et al. (1990 b) showed, replacement of a benzyl protecting group in 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl chloride by an electronegative bromoacetyl group resulted in a more stereoselective formation of a desired β-product in the glycosylation reaction studied. Thus, as the above examples from the literature show, the protecting groups at O-3, 4 and 6 of the donor can have a profound effect on the reactivity of the anomeric carbon and stereoselectivity of glycosylation reactions.
Another explanation for how benzyl groups reduce acyl transfer is that they interact with the positively charged acyl carbon of the acyloxonium ion, thus sterically preventing the attack of the acceptor on this acyl carbon and therefore preventing formation of ion 2 in Scheme 4 (see Scheme 6). It should be noted that Π-type hydrogen-bonded complexes between the high electron density of benzene and the proton of hydroxyl groups have been described both experimentally (Engdahl & Nelander, 1985; Wanna et al., 1987; Baiocchi et al., 1983; Suzuki et al., 1992; Atwood et al., 1991) and theoretically (Bredas & Street, 1989, Tang et al., 1990; Cheney et al., 1988), and intramolecular Π-type hydrogen bonding in which the aromatic ring is the acceptor have been shown to be one of the stabilizing forces of protein conformations (Burley & Petsko, 1988; Gallo & Gellman, 1992). Thus, it is possible that an analogous interaction exists between the high electron density of the benzene ring of the benzyl group and the positive charge of the cation generated during the glycosylation reaction.

Finally, it is possible that benzyl groups reduce acyl transfer by causing a distortion in the conformation of the pyranose ring which does not allow formation of ion 2 in Scheme 4. However, this effect is probably either unlikely or minimal since high acyl transfer was obtained when a formyl group was present at O-2 (see Table 2) and also, the $^1$H-NMR coupling constants (which are related to the conformation of the ring) are approximately the same in the tetraacetylated, monobenzylated, dibenzylated and tribenzylated trichloroacetimidates (see the Experimental Procedures section).

It is also interesting to note that the results of my experiments with the tribenzylated and monobenzylated donors (Tables 2 and 3) indicate that benzylation
of O-3 results in formation of $\alpha$-glycosylated product. This result agrees with a similar observation by Ziegler et al. (1990 b, described above), who found that replacing a 3-O-benzyl group of the donor with an electronegative bromoacetyl group results in a higher ratio of $\beta/\alpha$ glycosylation. This result can be explained by an increased electron density at C-1 when a benzyl group is present at O-3 (due to inductive donation of electrons) which decreases the need for participation of the acetyl group at O-2. It is also possible that the increase in $\alpha$-glycosylation is caused by an electrostatic attraction between the 3-O-benzyl group and the positively charged C-1 on the $\beta$-face of the sugar which inhibits the $\beta$-attack of the acceptor on the anomeric centre. It should be noted, however, that the molecular model indicates that 4-O-benzyl group can also interact with C-1, although it is possible that for energetic reasons the 3-O-benzyl group is more suitable.

In conclusion, my results showed that the transfer of the 2-O-acetyl group to the acceptor (MPEGDOXOH) can be significantly reduced by tribenzylolation at O-3, 4 and 6. This shows great promise in that the acetyl group may be a suitable participating group in those glycosylation reactions in which benzoyl group is not an effective 1,2-trans directing group (J. Krepinsky, private communication). Moreover, it was shown that the extent of acyl transfer from O-2 of the donors depends on the nature of the acyl group at O-2 as well as on the nature of the substituents at O-3, 4 and 6. More bulky and electron-donating acyl groups at O-2 transfer less to the acceptor, probably due to reduced formation and/or increased decomposition (in the reverse direction) of intermediates leading to acyl transfer (see Scheme 4). Replacement of acetyl groups at O-3, 4 and 6 with benzyl groups probably reduces acyl transfer by eliminating participation of those groups at C-1 and/or by increasing the electron
the positively charged carbonyl carbon of the participating O-2 acyl group. It should be noted that the above explanations for how benzyl groups reduce transfer from O-2 are only speculations at this point. Computer calculations of the energies of the appropriate intermediates would probably give more clues as to the mechanism of this process.
Proposed Future Work
To find support for and possibly to distinguish between the proposed hypothetical mechanisms of prevention of acyl transfer by benzyl groups, one could do computer energy minimization calculations of the intermediate ions.

To determine whether the calculations support the mechanism proposed by Bochkov et al. (1976), one could minimize the energy of ion 1 in Scheme 7. If one of the acyl groups participates at C-1 in the most stable structure, one could also minimize monobenzylated ions and the 3,6-di-O-benzylated ion to find out whether one of the acyl groups participates at C-1.

To determine whether the calculations support the model of inductive donation of electrons by benzyl groups to the 2-O-acyl group (or inductive withdrawal of electrons by acetyl groups), one could minimize the energies of the tetraacetylated ion 3 and the tribenzylated ion 5 in Scheme 7. If the 2-O-acetyl group participates at C-1 in both cases in the most stable structures, then one could compare the charges on C-1 and the 2-O-acetyl carbonyl carbon. If the 2-O-acetyl group is less charged in comparison to C-1 in the tribenzylated donor than in the tetraacetylated donor, then the benzyl groups probably reduce acyl transfer by inductively donating electrons to the 2-O-acetyl group (and/or the acetyl groups increase acyl transfer by withdrawing electrons from the 2-O-acetyl group). Moreover, if the 2-O-acetyl carbonyl bond in the tribenzylated ion 6 is shorter than in the tetraacetylated ion 4, then the 2-O-acetyl group in the former ion participates to a lesser extent than in the latter ion.

The minimization of the 2-O-acetylated tribenzylated ion 3 would also show whether the calculations support the model of interaction of one of the benzyl groups with the positive charge of the ion. The type of calculations that would be used for these minimizations is subject to future investigation.
Experimental Procedures
All starting materials were dried overnight in vacuo at $10^{-3}$ mmHg. All reactions were done under argon. Distilled water was used in reactions requiring water. The progress of the reactions was monitored by Thin Layer Chromatography (TLC) on silica gel ALUGRAM SIL/UV$_{254}$ plates (Macherey-Nagel) in appropriate solvents using starting material as a reference. The TLC results were visualized under UV light (254 nm) and by spraying with 50% sulphuric acid in methanol and heating at 200 °C. The products of the reactions were purified by precipitation, by filtration, by washing with water (and/or appropriate aqueous solution) and/or by silica gel (40-60 μ, Toronto Research Chemicals) column chromatography. Solutions were concentrated at 1mmHg pressure in a rotary evaporator. The structures of the products were determined by $^1$H-NMR and $^{13}$C-NMR spectroscopy at 500 MHz with a Varian Unity Plus spectrometer and by ion spray mass spectrometry with Perkin-Elmer/Sciex API III triple quadrupole mass spectrometer. The NMR spectra were obtained in CDCl$_3$ containing TMS as the internal standard or in CD$_3$OD (compound 33). For ion spray mass spectrometry experiments, the samples were dissolved in dichloromethane or in methanol and methanol was used as the mobile phase. The NMR assignment was confirmed by $^1$H-$^1$H and $^1$H-$^{13}$C correlation spectroscopy. Nuclear Overhauser Effect spectroscopy was used to confirm the assignment of 3 as the exo isomer.

**MPEGDOXyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (1)**

(Hodosi & Krepinsky, 1996)

**Experiment I (using α-imidate):**
Triethylsilyl trifluoromethanesulfonate (TESOTf, 2.0 μL, 8.8 μmol) was added to a solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl trichloroacetimidate (Schmidt & Stumpp, 1983) (27 mg, 55 μmol) and MPEGDOXOH (57 mg, 27 μmol) in 2 mL of dry CH₂Cl₂ at room temperature. After 1 hour stirring TLC [(hexane : ethyl acetate 2 : 1, 2% diisopropyl ethylamine (DIPEA))] indicated complete disappearance of the starting imidate. The reaction was stopped 3 hours after the addition of TESOTf by the addition of NaHCO₃ (and stirring for ~10 min), filtered, and the volume was reduced to ~0.5 mL. Dry t-butyl methyl ether (30 mL) was then added and the polymer was precipitated at 4 °C overnight. The polymer (64 mg) was then isolated by filtration. The ¹H-NMR spectrum indicates that a 40% yield of the β-glycosylated product (1) and a 63% yield of acyl transfer product were obtained.

¹H-NMR (δ; ppm): 5.39 (dd, 1H, J₃,₄=3.4 Hz, J₄,₅=1.0 Hz, H-4), 5.27 (dd, 1H, J₁,₂=8.1 Hz, J₂,₃=10.5 Hz, H-2), 4.98 (dd, 1H, H-3), 4.51 (d, 1H, H-1), 4.21 (dd, 1H, J₅,₆=6.4 Hz, J₆,₆'=11.3 Hz, H-6), 4.16 (dd, 1H, J₅,₆'=6.8 Hz, H-6'), 3.89 (m, 1H, H-5), 2.16 (s, 3H, CH₃CO), 2.07 (s, 3H, CH₃COO), 2.02 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO); 5.09 (s, 2H, CH₃-COO-CH₂-); 3.38 (s, 3H, CH₃(PEG)).

Experiment II (using β-imidate):

TESOTf (3.9 μL, 17 μmol) was added to a solution of 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl trichloroacetimidate (Schmidt & Stumpp, 1983)(43 mg, 87 μmol) and MPEGDOXOH (92 mg, 43 μmol) in 4 mL of dry CH₂Cl₂ at room temperature. After 1 hour stirring TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated complete disappearance of the imidate. Reaction was stopped 3 hours after the addition of TESOTf by adding 3 drops of DIPEA. The solvent was evaporated and the residue was dissolved in 1 mL of CH₂Cl₂. Dry t-butyl methyl ether (42 mL) was then added and the polymer was precipitated at 4 °C overnight to give 93 mg of the polymer. The ¹H-NMR spectrum indicates that a 33% yield of the β-glycosylated product (1) and a 67% yield of acyl transfer product were obtained.
MPEGDOXyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranoside (2)

2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl trichloroacetimidate (Schmidt & Stumpp, 1983) (40 mg, 54 µmol), MPEGDOXOH (57 mg, 27 µmol), TESOTf (2.0 µL, 8.8 µmol), 2 mL of CH₂Cl₂ and 30 mL of t-butyl methyl ether were used. The same procedure was followed as in the synthesis of 1. The polymer (57 mg) was isolated by filtration. The ¹H-NMR spectrum indicates that a 97% yield of the β-glycosylated product (2) and a 7%/0 yield of acyl transfer product were obtained.

¹H-NMR (δ, ppm): 8.10 (d, 2H, J=7.5 Hz, benzoyl aromatic), 8.05 (d, 2H, J=7.5 Hz, benzoyl aromatic), 7.92 (d, 2H, J=7.7 Hz, benzoyl aromatic), 7.78 (d, 2H, J=7.7 Hz, benzoyl aromatic), 5.98 (d, 1H, J₃₄=3.0 Hz, H-4), 5.86 (dd, 1H, J₁₂=8.1 Hz, J₂₃=10.1 Hz, H-2), 5.54 (dd, 1H, H-3), 4.29 (m, 1H, H-5); 5.36 (s, 2H, C₆H₅-COO-CH₂); 3.38 (s,3H, CH₃(PEG)).

3,4,6-tri-O-acetyl-α-D-galactopyranose 1,2-allyl orthoacetate (3)

The solution of 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (1.778 g, 4.555 mmol) in 30 mL of dry CH₂Cl₂ was cooled to 0 °C. Aluminum chloride (0.729 g, 5.47 mmol) was then added and the solution was stirred at 0 °C. After 0.5 hour TLC (hexane : ethyl acetate 2 : 1) indicated complete disappearance of the starting material. One hour after aluminum chloride addition 2,6-lutidine (5.2 mL, 45 mmol) followed by allyl alcohol (3.1 mL, 46 mmol) was then added. Two and a half hours after addition of allyl alcohol TLC (hexane : ethyl acetate 2 : 1) indicated completion of the reaction. The reaction mixture was then diluted with CH₂Cl₂ and washed with 0.2 N aqueous HCl and then with H₂O. After drying (MgSO₄) and concentration the residue was put on a column of silica gel and chromatographed with hexane : ethyl acetate 2 : 1 solvent system, to give 1.481 g (84%) of compound 3.

¹H-NMR spectrum indicates that 6 : 1 mixture of exo and endo isomers was obtained.
\( \delta, \text{ppm} \): Exo isomer: 5.89 (m, 1H, CH\(_2\)=CH-CH\(_2\)-O), 5.82 (d, 1H, J\(_{1,2}\)=4.7 Hz, H-1), 5.44 (m, 1H, H-4), 5.28 (ddd, 1H, J\(_{\text{trans}}\)=17.1 Hz, CH\(_2\)-CH=CH trans), 5.16 (ddd, 1H, J\(_{\text{cis}}\)=10.5 Hz, CH\(_2\)-CH=CH cis), 5.07 (dd, 1H, J\(_{3,2}\)=6.8 Hz, J\(_{3,4}\)=3.4 Hz, H-3), 4.34-4.31 (m, 2H, allyl), 4.17 (dd, 1H, J\(_{6,5}\)=6.8 Hz, J\(_{6,6}\)=11.3 Hz, H-6), 4.12 (dd, 1H, JC\(_5\)=6.4 Hz, H-6'), 2.12 (s, 3H, CH\(_3\)), 2.08 (s, 3H, CH\(_3\)), 1.70 (s, 3H, CH\(_3\)).

\( ^{13}\text{C-NMR} \): 170.47 (CH\(_3\)CO), 170.03 (CHFO), 169.76 (CH\(_3\)CO), 133.99 (WH\(_2\)), 116.71 (=CHp), 97.46 (C-1), 73.82 (C-2 or O-CH\(_2\)), 71.34 (C-3), 69.09 (C-2 or O-CH\(_2\)), 65.92 (C-4), 64.02 (C-5), 61.37 (C-6), 23.78 (CH\(_3\)CO), 20.71 (CH\(_3\)CO), 20.55 (CH\(_3\)CO).

**\( \alpha\)-D-galactopyranose 1,2-allyl orthoacetate (4)**

To a solution of compound 3 (1.602 g, 4.125 mmol) in 20 mL of dry methanol, 2 mL of MeONa (0.5 N) was added. After 20 min TLC (ethyl acetate : methanol : hexane = 7 : 2 : 1) indicated the end of the reaction. The solvent, without neutralization, was evaporated and the residue was purified on a silica column using ethyl acetate : methanol : hexane = 7 : 2 : 1 solvent system with 2% DIPEA, to give 4 (0.992 g, 92%).

\( ^{1}\text{H-NMR} (\delta, \text{ppm}) \): exo isomer: 5.90 (m, 1H, O-CH\(_2\)-CH=), 5.80 (d, 1H, J\(_{1,2}\)=5.1 Hz, H-1), 5.28 (ddd, 1H, J\(_{\text{trans}}\)=17.1 Hz, CH\(_2\)-CH=CH trans), 5.16 (ddd, 1H, J\(_{\text{cis}}\)=10.5 Hz, CH\(_2\)-CH=CH cis), 4.39 (t, 1H, H-2), 1.60 (s, 3H, CH\(_3\)).

**3,4,6-tri-O-benzyl-\( \alpha\)-D-galactopyranose-1,2-allyl orthoacetate (5)**

The solution of compound 4 (0.963 g, 3.67 mmol) in 50 mL of anhydrous N,N-dimethylformamide was cooled to 0 °C. Sodium hydride (60%, 0.844g, 22 mmol) was then added and the reaction mixture was stirred at 0 °C for 2 hours. Benzyl bromide (1.96 mL, 16.5 mmol) was then added dropwise and the reaction was allowed to reach
room temperature. Two hours later TLC (hexane : ethyl acetate = 2 : 1) indicated the disappearance of the starting material. The reaction mixture was neutralized with acetic acid, diluted with CH₂Cl₂, washed with H₂O and dried (MgSO₄). After the concentration the crude product was purified on a column of silica gel (hexane : ethyl acetate = 3 : 1), to give 1.714 g (88%) of compound 5.

1H-NMR (δ, ppm):  7.40-7.26 (m, 15 H, aromatic), 5.90 (m, 1H, OCH₂-CH=), 5.73 (d, 1H, J₁₂=4.6 Hz, H-1), 5.27 (ddd, 1H, Jtrans=17.1 Hz, CH₂-CH=CH trans), 5.14 (ddd, 1H, Jcis=10.5 Hz, CH₂-CH=CH cis), 4.91 (d, 1H, Jgem=11.5 Hz, CH-Ph), 4.80 (d, 1H, Jgem=12.2 Hz, CH-Ph), 4.67 (d, 1H, Jgem=12.2 Hz, CH-Ph), 4.61 (d, 1H, Jgem=11.5 Hz, CH-Ph), 1.61 (s, 3H, CH₃).

Allyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (6) and allyl 3,4,6-tri-O-benzyl-D-galactopyranoside (7)

Compound 5 (0.511 g, 0.96 mmol) was dissolved in 6 mL of dry CH₂Cl₂ and 0.5 g of 4 Å molecular sieves was added, followed by the addition of allyl alcohol (196 µL, 2.88 mmol), and TESOTf (217 µL, 0.960 mmol). After 0.5 hour TLC indicated the the disappearance of the starting material. The reaction was stopped by the addition of 3 drops of DIPEA. The molecular sieves were filtered off and the residue was diluted with CH₂Cl₂, washed with H₂O and dried. After concentration, the crude material was chromatographed on a silica column with heptane : ethyl acetate = 3 : 1 solvent system, to give first compound 6 (0.305 g, 60%) and then compound 7 (90 mg, 19%).

1H-NMR (δ, ppm):  7.36-7.24 (m, aromatic), 5.82 (m, 1H, O-CH₂-CH=CH₂), 5.39 (dd, 1H, J₂₁=8.0 Hz, J₂₃=10.0 Hz, H-2), 5.23 (ddd, 1H, Jtrans=17.3 Hz, CH₂-CH=CH trans), 5.13 (ddd, 1H, Jcis=10.5 Hz, CH₂-CH=CH), 4.93 (d, 1H, Jgem=11.7 Hz, CH-Ph), 4.67 (d, 1H, Jgem=12.2 Hz, CH-Ph), 4.59 (d, 1H, Jgem=11.8 Hz, CH-Ph), 4.52 (d, 1H, Jgem=12.2 Hz, CH-Ph), 4.44 (d, 1H, Jgem=11.8 Hz, CH-Ph), 4.42 (d, 1H, Jgem=11.8 Hz, CH-Ph), 4.39 (d, 1H, H-1), 4.30 (ddt, 1H, Jgem=13.4 Hz, OCH-C=), 4.04 (ddt, 1H,
OCHCH=), 3.95 (m, 1H, H-4), 3.65-3.59 (m, 2H, 2xH-6), 3.55 (m, 1H, H-5), 3.51 (dd, 1H, J₃,₄=2.8 Hz, H-3), 2.03 (s, 3H, CH₃CO); 7 : 7.40-7.24 (m, aromatic), 5.28 (ddd, 1H, Jtrans=17.1 Hz, CH₂-CH=CH trans), 5.92 (m, 1H, OCH₂CH=), 5.00 (d, 1H, J₁₂=4.0 Hz, H-1α), 4.30 (d, 1H, J₁₂=7.6 Hz, H-1β), 3.73 (dd, 1H, J₂,₃=10.0 Hz, J₃,₄=2.8 Hz, H-3α), 3.44 (dd, 1H, J₂,₃=9.8 Hz, J₃,₄=2.8 Hz, H-3β).

**Allyl 3,4,6-tri-O-benzyl-β-D-galactopyranoside (8)**

To a solution of compound 6 (0.295 g, 0.554 mmol) in 20 mL of dry methanol, 6 mL of 0.5 NaOMe was added. After 7 hours TLC (hexane : ethyl acetate 3 : 1) indicated the absence of the starting material. The solution was neutralized with IR-120 (H⁺) Amberlite resin and the solvent was evaporated to give 0.277 g (100%) of compound 8.

¹H-NMR (δ, ppm) : 7.36-7.25 (m, aromatic), 5.93 (m, 1H, OCH₂-CH=), 5.28 (ddd, 1H, Jtrans=17.1 Hz, CH₂-CH=CH trans), 5.18 (ddd, 1H, Jcis=10.5 Hz, CH₂-CH=CH cis), 4.88 (d, Jgem=11.7 Hz, CH-Ph), 4.72 (d, Jgem=12.0 Hz, CH-Ph), 4.66 (d, Jgem=12.0 Hz, CH-Ph), 4.60 (d, Jgem=11.7 Hz, CH-Ph), 4.47 (d, Jgem=11.7 Hz, CH-Ph), 4.43 (d, Jgem=11.7 Hz, CH-Ph), 4.36 (ddt, 1H, OCH-CH=), 4.29 (d, J₁₂=7.6 Hz, H-1), 4.09 (ddt, 1H, OCH-CH=), 3.97 (m, 1H, H-2), 3.92 (m, 1H, H-4), 3.65-3.55 (m, 3H, H-5, 2xH-6), 3.43 (dd, 1H, J₃,₄=2.9 Hz, H-3), 2.35 (d, 1H, J=1.2 Hz, OH); ¹³C-NMR : 133.90 (-OCH₂-CH=CH₂), 117.79 (-OCH₂-CH=CH₂), 102.04 (C-1), 81.98 (C-3), 74.51 (CH₂), 73.74 (C-5), 73.57 (CH₂), 72.82 (C-4), 72.42 (CH₂), 71.32 (C-2), 69.98 (-OCH₂-CH=CH₂), 68.71 (C-6).

**Allyl 3,4,6-tri-O-benzyl-2-O-formyl-β-D-galactopyranoside (9) and 3,4,6-tri-O-benzyl-2-O-formyl-D-galactopyranose (10)**

The solution of compound 8 (0.260 g, 0.530 mmol) in 10 mL of formic acid (96%) was stirred at room temperature for two days. The solvent was then evaporated and the residue was put on a column of silica gel and the compounds were eluted first with
heptane : ethyl acetate = 3 : 1 (300 mL) and then with heptane : ethyl acetate = 2 : 1, to give first 9 (0.10 g, 37%), then 23 mg (9%) of the starting material 13 and finally 91 mg (36%) of compound 10.

1H-NMR (δ, ppm) : 9 : 8.16 (s, 1H, HCOO), 7.37-7.23 (m, 15 H, aromatic), 5.84 (m, 1H, OCH₂-CH=), 5.24 (ddd, 1H, J_{trans}=17.1 Hz, CH₂-CH=CH trans), 5.15 (ddd, 1H, J_{cis}=10.5 Hz, CH₂-CH=CH cis) 5.04 (t, 1H, J_{2,3}=9.0 Hz, H-2), 4.91 (d, 1H, J_{gem}=11.7 Hz, CH-Ph), 4.67 (d, 1H, J_{gem}=12.0 Hz, CH-Ph), 4.59 (d, 1H, J_{gem}=11.7 Hz, CH-Ph), 4.56 (d, 1H, J_{gem}=12.0 Hz, CH-Ph), 4.46 (d, 1H J_{gem}=11.7 Hz, CH-Ph), 4.43 (d, 1H, J_{1,2}=7.8 Hz, H-1), 4.42 (d, 1H, J_{gem}=11.7 Hz, CH-Ph), 4.32 (m, 1H, OCH-CH=), 4.05 (m, 1H, O-CH-CH=), 3.96 (d, 1H, J_{3,4}=2.4 Hz, H-4), 3.65-3.55 (m, 4H, H-3, H-5, 2xH-6); 13C-NMR: 133.47 (-OCH₂-CH=CH₂), 117.45 (-OCH₂-CH=CH₂), 99.65 (C-1), 79.80 (C-3), 74.54 (CH₂), 73.52 (2xCH₂), 72.51 (C-4), 72.33 (CH₂), 69.77 (-OCH₂-CH=CH₂), 68.36 (C-6); 10 : 1H-NMR (δ, ppm) : 8.12 (s, 1H, HCOOβ), 8.10 (s, 1H, HCOOα), 7.36-7.22 (m, aromatic), 5.41 (t, 1H, H-1α), 4.54 (d, 1H, J_{1,2}=8.3 Hz, H-1β).

**Allyl 3,4,6-tri-O-benzyl-2-O-levulinyl-β-D-galactopyranoside (11)**

Levulinic anhydride (0.360 g, 1.68 mmol) was added to a solution of compound 8 (0.158 g, 0.322 mmol) in 3 mL of anhydrous pyridine at room temperature. After overnight stirring TLC (heptane : ethyl acetate 2 : 1) indicated complete disappearance of the starting material. The levulinic anhydride was quenched by addition of several drops of H₂O. It was then diluted with CH₂Cl₂, washed with 0.2 N HCl (aq.), NaHCO₃ (aq., sat.) and finally with H₂O. After concentration the residue was chromatographed on a silica gel column (heptane : ethyl acetate 2 : 1) to give 0.165 g (87%) of compound 11.

1H-NMR (δ, ppm) : 7.37-7.24 (m, aromatic), 5.83 (m, 1H, CH=CH₂), 5.39 (dd, 1H, J_{2,3}=10 Hz, H-2), 5.22 (ddd, 1H, J_{trans}=17.1 Hz, CH₂-CH=CH), 5.14 (ddd, 1H, J_{cis}=10.5 Hz,
CH₂-CH=CH cis), 4.93 (d, 1H, J₆=11.8 Hz, CH-Ph), 4.67 (d, 1H, J₆=12.2 Hz, CH-Ph), 4.59 (d, 1H, J₆=11.8 Hz), 4.55 (d, 1H, J₆=12.2 Hz, CH-Ph), 4.45 (d, 1H, J₆=11.8 Hz, CH-Ph), 4.41 (d, 1H, J₆=11.8 Hz, CH-Ph), 4.38 (d, 1H, J₂=7.9 Hz, H-1), 4.29 (ddt, 1H, O-CH-CH=), 4.04 (ddt, 1H, O-CH-CH=), 3.62-3.55 (m, 3H, H-5, H-6), 3.53 (dd, 1H, J₃₄=2.8 Hz, H-3), 2.73 (m, 2H, levulinyl methylene), 2.56 (m, 2H, levulinyl methylene), 2.16 (s, 3H, CH₃).

**Allyl 3,4,6-tri-O-benzyl-2-O-pivaloyl-β-D-galactopyranoside (12)**

Compound 8 (0.417 g, 0.850 mmol) was dissolved in 13 mL of anhydrous pyridine, pivaloyl chloride (1.26 mL, 10.2 mmol) was added and the reaction was stirred at room temperature. After 2 days TLC (heptane : ethyl acetate 3 : 1) indicated complete disappearance of the starting material. After the workup (see synthesis of 11) the residue was chromatographed on a silica gel column (heptane : ethyl acetate 3 : 1) to give 0.344 g (70 %) of compound 12.

^1H-NMR, δ 7.36-7.23 (m, aromatic), 5.82 (m, 1H, CH=CH₂), 5.44 (dd, 1H, J₂₃=10.0 Hz, H-2), 5.23 (m, 1H, allyl), 5.12 (m, 1H, allyl), 4.93 (d, 1H, J₆=11.8 Hz, CH-Ph), 4.64 (d, 1H, J₆=12.0 Hz, CH-Ph), 4.57 (d, 1H, J₆=11.5 Hz, CH-Ph), 4.56 (d, 1H, J₆=12.0 Hz, CH-Ph), 4.46 (d, 1H, J₆=11.8 Hz, CH-Ph), 4.43 (d, 1H, J₆=10.9 Hz, CH-Ph), 4.41 (d, 1H, J₁₂=7.9 Hz, H-1), 4.31 (m, 1H, allyl), 4.00 (m, 1H, allyl), 3.94 (d, 1H, H-4), 3.64-3.56 (m, 3H, H-5, 2xH-6), 3.56 (dd, 1H, J₃₄=2.8 Hz, H-3), 1.55 (s, 9H, 3xCH₃).

**Allyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (13)**

Benzoyl chloride (200 µL, 1.72 mmol) was added dropwise to a solution of compound 8 (0.272 g, 0.554 mmol) in anhydrous pyridine (3 mL) at room temperature. After 1 hour stirring TLC (hexane : ethyl acetate 3 : 1) indicated the completion of the
reaction. After the workup (see synthesis of 11) the residual pyridine was removed in vacuo to give 0.322 g (98%) of compound 13.

2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranose (14) and 1-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranose (15)
(Ogawa & Nakabayashi, 1981)
Compound 6 (0.200 g, 0.375 mmol) was dissolved in a mixture of acetic acid (10 mL) and H₂O (0.50 mL, 0.28 mmol). Sodium acetate (AcONa•3H₂O, 0.117 g, 0.860 mmol) was added and allowed to dissolve. Palladium chloride (0.311 g, 1.75 mmol) was then added and the reaction was stirred at room temperature overnight [TLC (heptane : ethyl acetate 1 : 1) indicated completion of the reaction]. The reaction was stopped by filtering off palladium and then the mixture was diluted with CH₂Cl₂ and washed with H₂O. The organic layer was concentrated and the residue was chromatographed on a column of silica gel (heptane : ethyl acetate 1 : 1) to give an unseparable mixture of 14 and 15 (0.174 g, 94%). (Note: The ¹H-NMR spectrum indicates that the yield of 14 was 68% and the yield of 15 was 30%)
¹H-NMR (δ, ppm): 14: 5.42 (dd, 1H, J₂,₃=7.9 Hz, J₂,₄=10.0 Hz, H-2 β), 5.35 (dd, 1H, J₂,₁=3.5 Hz, J₂,₃=9.6 Hz, H-2 α), 15: 5.18 (dd, 1H, J₂,₁=8.1 Hz, J₂,₃=10.0 Hz, H-2 β).

3,4,6-tri-O-benzyl-2-O-levulinyl-D-galactopyranose (16) and 3,4,6-tri-O-benzyl-1-O-levulinyl-β-D-galactopyranose (17)
Compound 11 (0.165 g, 0.280 mmol) was dissolved in a mixture of acetic acid (10 mL) and H₂O (0.50 mL, 0.28 mmol). Sodium acetate (AcONa•3H₂O, 95 mg, 0.70 mmol) was added and allowed to dissolve. Palladium chloride (99 mg, 0.56 mmol) was then added and the reaction was stirred at room temperature overnight [TLC (hexane : ethyl acetate 1 : 1) indicated complete disappearance of the starting material]. The reaction was stopped by filtering off palladium and then the mixture was diluted with CH₂Cl₂ and washed with H₂O. After concentration the residue was
chromatographed on a silica gel column (heptane : ethyl acetate 1 : 1) to give an unseparable mixture of 16 and 17 (0.129 g, 84%). (Note: the \(^1\)H-NMR spectrum indicates that the yield of 16 was ~65% and the yield of 17 was ~19%).

\(^1\)H-NMR (δ, ppm): 16: 5.42 (dd, 1H, J\(_{2,1}\) = 7.9 Hz, J\(_{2,3}\) = 10.3 Hz, H-2 β), 5.33-5.30 (m, 1H, H-2 α), 17: 5.19 (dd, 1H, J\(_{2,1}\) = 7.9 Hz, J\(_{2,3}\) = 10.0 Hz, H-2 β).

3,4,6-tri-O-benzyl-2-O-pivaloyl-D-galactopyranose (18) and 3,4,6-tri-O-benzyl-1-O-pivaloyl-D-galactopyranose (19)

Compound 12 (0.235 g, 0.409 mmol) was dissolved in a mixture of acetic acid (10 mL) and water (0.50 mL, 0.28 mmol). Sodium acetate (AcONa•3H\(_2\)O, 0.122 g, 0.896 mmol) was added and allowed to dissolve. Palladium chloride (0.254 g, 1.43 mmol) was then added and the reaction was stirred at room temperature overnight [TLC (heptane : ethyl acetate 3 : 1) indicated complete disappearance of the starting material]. The reaction was then stopped by filtering off palladium, diluted with CH\(_2\)Cl\(_2\), washed with water and concentrated. After silica gel column chromatography (heptane : ethyl acetate 3 : 1) an unseparable mixture of compounds 18 and 19 (0.118 g, 54%) was obtained.

\(^1\)H-NMR (δ, ppm): 18: 5.39 (dd, 1H, J\(_{2,1}\) = 8.0 Hz, J\(_{2,3}\) = 10.0 Hz, H-2 β), 5.28 (dd, 1H, J\(_{2,1}\) = 3.6 Hz, J\(_{2,3}\) = 10.3 Hz, H-2 α), 19: 5.19 (dd, 1H, J\(_{2,1}\) = 7.9 Hz, J\(_{2,3}\) = 10.0 Hz, H-2 β).

2-O-benzoyl-3,4,6-tri-O-benzyl-D-galactopyranose (20)

Compound 13 (0.322 g, 0.541 mmol), PdCl\(_2\) (0.197, 1.11 mmol), AcONa•3H\(_2\)O (0.189 g, 1.39 mmol), H\(_2\)O (0.50 mL, 0.28 mmol) in acetic acid (10 mL) were used. The same procedure was used as in the synthesis of compounds 16 and 17 except that hexane : ethyl acetate 3 : 1 solvent system was used for TLC and for silica gel chromatography. Compound 20 (0.127 g, 42%) was obtained.

\(^1\)H-NMR (δ, ppm): 5.45 (dd, 1H, J\(_{2,1}\) = 7.9 Hz, J\(_{2,3}\) = 10.0 Hz, H-2 β), 3.60 (dd, 1H, J\(_{6,5}\) = 5.9 Hz, J\(_{6,6'}\) = 9.6 Hz, H-6 α), 3.53 (dd, 1H, J\(_{6,5}\) = 6.4 Hz, H-6' α).
3,4,6-tri-O-benzyl-2-O-formyl-α-D-galactopyranosyl trichloroacetimidate (21)
(Urban et al., 1990)
To a solution of compound 10 (0.554 g, 1.16 mmol) in 15 mL of dry CH₂Cl₂, trichloroacetonitrile (0.6 mL, 6 mmol) followed by Cs₂CO₃ (75 mg, 0.232 mmol) was added. After 16 hour stirring at room temperature the catalyst was filtered off and the solvent was removed in vacuo. The crude material was chromatographed on a silica gel column (hexane : ethyl acetate 2 : 1, 1% DIPEA) to give 0.471 g (65%) of compound 21.

^1^H-NMR (δ, ppm): 8.56 (s, 1H, NH), 8.04 (s, 1H, HCOO), 7.38-7.23 (m, 15H, aromat), 6.55 (d, 1H, J₃₋₂= 3.7 Hz, H-1) 5.62 (dd, 1H, J₂₋₃=10.3 Hz), 4.95 (d, 1H, J₋ₓ₋ₓ=11.5 Hz, CH-Ph), 4.73 (d, 1H, Jₓ₋ₓ=12.0 Hz, CH-Ph), 4.65 (d, 1H, J₋ₓ₋ₓ=11.9 Hz, CH-Ph), 4.59 (d, 1H, Jₓ₋ₓ=11.5 Hz, CH-Ph), 4.47 (d, 1H, J₋ₓ₋ₓ=11.5 Hz, CH-Ph), 4.41 (d, 1H, Jₓ₋ₓ=11.7 Hz, CH-Ph), 4.18 (m, 1H, H-5), 4.12 (m, 1H, H-4), 4.07 (dd, J₃₋ₓ₋ₓ=2.7 Hz, H-3), 3.66 (t, J₅₋₆=7.8 Hz, H-6), 3.58 (dd, J₅₋₆=5.4 Hz, J₆₋₆=9.2 Hz, H-6'); ^1^C-NMR: 94.15 (C-1), 75.83 (C-3), 74.94 (CH₂), 73.78 (C-4), 73.50 (CH₂), 73.50 (CH₂), 72.50 (CH₂), 72.23 (C-5), 69.58 (C-2), 67.96 (C-6).

2-O-acetyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate (22a and 22b)
(Amvam-Zollo & Sinay, 1986)
A mixture of compounds 14 and 15 (see above) (0.161 g, 0.327 mmol) was dissolved in 3 mL of dry CH₂Cl₂. Trichloroacetonitrile (0.4 ml, 4 mmol) followed by sodium (0.30 g, 13 mmol) was then added and the reaction was stirred at room temperature. After 1 hour TLC (hexane : ethyl acetate 2 : 1) indicated the disappearance of most of the starting material. The reaction was stopped by filtering off the sodium and concentrating. The crude material was then chromatographed on a silica gel column (hexane : ethyl acetate 2 : 1, 2% DIPEA) to give 79 mg (38%) of the α-anomer 22a and and 21 mg (10%) of the β-anomer 22b.
$^1$H-NMR (δ, ppm) : 22a : 8.50 (s, 1H, NH), 7.34-7.25 (m, aromatic), 6.53 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 5.51 (dd, 1H, $J_{2,3}$=10.5 Hz, H-2), 4.97 (d, 1H, $J_{gem}=11.5$ Hz, CH-Ph), 4.73 (d, 1H, $J_{gem}$=12.2 Hz, CH-Ph), 4.64 (d, 1H, $J_{gem}$=12.2 Hz, CH-Ph), 4.60 (d, 1H, $J_{gem}$=11.3 Hz, CH-Ph), 4.47 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.42 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.16 (m, 1H, H-5), 4.11 (m, 1H, H-5)).

$^1$H-NMR (δ, ppm) : 22b : 8.57 (s, 1H, NH), 7.35-7.24 (m, aromatic), 5.69 (d, 1H, $J_{1,2}$=8.1 Hz, H-1), 5.64 (dd, 1H, $J_{2,3}$=9.8 Hz, H-2), 4.95 (d, 1H, $J_{gem}=11.3$ Hz, CH-Ph), 4.69 (d, 1H, $J_{gem}$=12.4 Hz, CH-Ph), 4.62 (d, 1H, $J_{gem}=11.5$ Hz, CH-Ph), 4.47 (d, 1H, $J_{gem}=11.5$ Hz, CH-Ph), 4.43 (d, 1H, $J_{gem}=11.8$ Hz, CH-Ph), 4.03 (m, 1H, H-4), 3.78 (m, 1H, H-5), 3.69 (dd, 1H, J$_{6,5}$=7.5 Hz, J$_{6,6}$=9.2 Hz, H-6), 3.65-3.60 (m, 2H, H-6 and H-3), 1.98 (s, 3H, CH$_3$COO).

3,4,6-tri-O-benzyl-2-O-levulinyl-$\alpha$-D-galactopyranosyl trichloroacetimidate (23)

A mixture of compounds 16 and 17 (see above) (0.129 g, 0.235 mmol) was dissolved in 5 ml of dry CH$_2$Cl$_2$. Trichloroacetonitrile (0.25 mL, 2.5 mmol) followed by sodium (0.10 g, 43 mmol) was then added. After 3 hour stirring at room temperature TLC (hexane : ethyl acetate 2 : 1) indicated the presence of one major product and of the starting material. The reaction was stopped by filtering off the sodium and concentrating. The residue was then chromatographed on a silica gel column (hexane : ethyl acetate 2 : 1, 2% DIPEA) to give 46 mg (29%) of compound 23.

$^1$H-NMR (δ, ppm) : 8.52 (s, 1H, NH), 7.35-7.26 (m, aromatic), 6.51 (d, 1H, $J_{1,2}$=3.4 Hz, H-1), 5.52 (dd, 1H, $J_{2,1}$=3.5, $J_{2,3}$=10.5 Hz, H-2), 4.97 (d, 1H, $J_{gem}=11.3$ Hz, CH-Ph), 4.72 (d, 1H, $J_{gem}=12.2$ Hz, CH-Ph), 4.65 (d, 1H, $J_{gem}=12.2$ Hz, CH-Ph), 4.60 (d, 1H, $J_{gem}=11.3$ Hz, CH-Ph), 4.47 (d, 1H, $J_{gem}=11.8$ Hz, CH-Ph), 4.41 (d, 1H, $J_{gem}=11.8$ Hz, CH-Ph), 4.16 (m, 1H, H-5), 4.10 (m, 1H, H-4), 4.04 (dd, 1H, J$_{3,4}$=2.8 Hz, H-3), 3.66-3.56
3,4,6-tri-benzyl-2-O-pivaloyl-D-galactopyranosyl trichloroacetimidate (24a and 24b)

A mixture of compounds 18 and 19 (see above) (0.124 g, 0.232 mmol) was dissolved in 2 mL of dry CH$_2$Cl$_2$. Trichloroacetonitrile (0.26 ml, 2.6 mmol) followed by sodium (26.6 mg, 1.16 mmol) was then added and the reaction was stirred at room temperature. After 3 hours TLC (heptane : ethyl acetate 3 : 1) indicated the formation of two products and the presence of the starting material. The reaction was then stopped by filtering off sodium and concentrating. The residue was chromatographed on a silica gel column (heptane : ethyl acetate 2 : 1, 2% triethylamine) to give 17 mg (11%) of the α-anomer 24a and 34 mg (22%) of the β-anomer 24b.

$^1$H-NMR (δ, ppm) : 24a : 8.53 (s, 1H, NH), 7.34-7.24 (m, aromatic), 6.56 (d, 1H, $J_{1,2}$=3.4 Hz, H-1), 5.55 (dd, 1H, $J_{2,3}$=9.8 Hz, H-2), 4.97 (d, 1H, $J_{gem}$=11.3 Hz, CH-Ph), 4.72 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.69 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.58 (d, 1H, $J_{gem}$=11.3 Hz, CH-Ph), 4.46 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.41 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.19 (m, 1H, H-5), 4.10-4.08 (m, 2H, H-3 and H-4), 3.65 (dd, 1H, $J_{6,6'}$=7.8 Hz, J6,6'=9.4 Hz, H-6), 3.57 (dd, 1H, $J_{6,5}$=5.5 Hz, H-6'), 1.16 (s, 9H, (CH$_3$)C); 24b : 8.58 (s, 1H, NH), 7.35-7.22 (m, aromatic), 5.85 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.67 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.92 (d, 1H, $J_{gem}$ = 11.5 Hz, CH-Ph), 4.66 (d, 1H, $J_{gem}$ = 11.8 Hz, CH-Ph), 4.58 (d, 2H, $J_{gem}$ = 11.3 Hz, CH-Ph), 4.47 (d, 1H, $J_{gem}$ = 11.8 Hz, CH-Ph), 4.43 (d, 1H, $J_{gem}$ = 11.8 Hz, CH-Ph), 4.01 (m, 1H, H-4), 3.80 (m, 1H, H-5), 3.70-3.63 (m, 3H, H-3 and 2xH-6), 1.14 (s, 9H, (CH$_3$)$_3$C ).

2-O-benzoyl-3,4,6-tri-O-benzyl-D-galactopyranosyl trichloroacetimidate (25a and 25b)
Compound 20 (0.127 g, 0.229 mmol) was dissolved in 2 ml of dry CH₂Cl₂. Trichloroacetonitrile (0.4 ml, 4 mmol) was then added followed by sodium (30 mg, 1.30 mmol). After 2 hour stirring at room temperature TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated complete disappearance of the starting material and the formation of two products. The reaction was stopped by filtering off sodium and concentrating. The residue was then chromatographed on a silica gel column (hexane : ethyl acetate 2 : 1, 2% DIPEA) to give 94 mg (59%) of the α-anomer 25a and 18 mg (11%) of the β-anomer 25b.

¹H-NMR (δ, ppm) : 25a : 8.40 (s, 1H, NH), 7.96 (m, 2H, benzoyl aromatic), 7.56 (m, 1H, benzoyl aromatic), 7.42-7.23 (m, aromatic), 6.64 (d, 1H, J₃,₂ = 3.4 Hz, H-1), 5.81 (dd, 1H, J₂,₃ = 10.0 Hz, H-2), 5.01 (d, 1H, J₆,₅ = 11.3 Hz, CH-Ph), 4.72 (d, 1H, J₅,₆ = 12.2 Hz, CH-Ph), 4.64 (d, 1H, J₆,₅ = 11.3 Hz, CH-Ph), 4.63 (d, 1H, J₅,₆ = 12.2 Hz, CH-Ph), 4.49 (d, 1H, J₆,₅ = 11.5 Hz, CH-Ph), 4.44 (d, 1H, J₅,₆ = 11.5 Hz, CH-Ph), 4.24-4.18 (m, 3H, H-3, H-4 and H-5), 3.71 (m, 1H, H-6), 3.62 (dd, 1H, J₆,₅ = 5.3 Hz, J₆,₆ = 9.2 Hz, H-6'); 25b : 8.53 (s, 1H, NH), 7.97-7.95 (m, 2H, benzoyl aromatic), 7.56 (m, 1H, benzoyl aromatic), 7.43-7.14 (m, 17H, aromatic), 5.91 (dd, 1H, J₂,₃=9.8 Hz, H-2), 5.84 (d, 1H, J₁,₂=8.1 Hz, H-1), 5.01 (d, 1H, J₆,₅=11.5 Hz, CH-Ph), 4.67 (m, 2H, 2xCH-Ph), 4.52-4.44 (m, 3H, 3xCH-Ph), 4.09 (m, 1H, H-4), 3.84 (m, 1H, H-5), 3.76-3.67 (m, 3H, H-3 and 2xH-6).

MPEGDOXyl 3,4,6-tri-O-benzyl-2-O-formyl-β-D-galactopyranoside (26)

TESOTf (12 µL, 53 µmol) was added to a solution of compound 21 (0.169 g, 0.271 mmol) and MPEGDOXOH (0.275 g, 0.130 mmol) in 3 mL of dry CH₂Cl₂. After 3 hour stirring at room temperature TLC (hexane : ethyl acetate 3 : 1) indicated the absence of the imidate. The reaction was stopped by the addition of 2 drops of DIPEA and the volume was reduced to ~0.5 mL. Dry t-butyl methyl ether (50 mL) was added and the polymer was allowed to precipitate at 4 °C overnight. The polymer (0.278 g) was
then isolated by filtration. The $^1$H-NMR spectrum indicates that a ~25% yield of the β-glycosylated product 26, a ~4% yield of the α-anomer and a 56% yield of acyl transfer product were obtained.

$^1$H-NMR (δ, ppm): 26: 8.14 (s, 1H, HCOO), 5.10 (dd, 1H, J_{1,2}=8.3 Hz, J_{2,3}=9.0 Hz, H-2), 3.96 (d, 1H, J_{3,4}=2.4 Hz, H-4); α-anomer: 8.09 (s, 1H, HCOO); 5.19 (s, 2H, HCOO-CH$_2$-); 3.38 (s, 3H, CH$_3$ (PEG)).

**MPEGDOXyl 2-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (27)**

TESOTf (3 µL, 13 µmol) was added to a solution of compound 22a (27 mg, 42 µmol) and MPEGDOXOH (44 mg, 21 µmol) in 1.5 mL of dry CH$_2$Cl$_2$ at room temperature. After 2.5 hour stirring TLC (hexane : ethyl acetate 3 : 1) indicated complete disappearance of the donor. The reaction was stopped 3 hours after the addition of TESOTf by the addition of NaHCO$_3$ (and stirring ~10 minutes) and then it was filtered. The volume was reduced to ~ 1 mL, t-butyl methyl ether (30 mL) was added, and the polymer was allowed to precipitate overnight at 4°C. The polymer was then isolated by filtration. The $^1$H-NMR spectrum indicates that a 64% yield of the β-glycosylated product 27, a 13% yield of the α-anomer and a 5% yield of acyl transfer product were obtained.

$^1$H-NMR (δ, ppm): 27: 7.36-7.22 (m, aromatic), 5.43 (dd, 1H, J_{1,2}=7.9 Hz, J_{2,3}=10.0 Hz, H-2), 4.37 (d, 1H, H-1), 3.94 (m, 1H, H-4), 1.99 (s, 3H, CH$_3$CO); α-anomer: 5.13 (d, 1H, J_{1,2}=3.9 Hz, H-1); 5.09 (s, 2H, CH$_3$-COO-CH$_2$-); 3.38 (s, 3H, CH$_3$(PEG)).

**MPEGDOXyl 3,4,6-tri-O-benzyl-2-O-levuliny1-β-D-galactopyranoside (28)**

TESOTf (2.2 µL, 9.7 µmol) was added to a solution of compound 23 (23 mg, 33 µmol) and MPEGDOXOH (46 mg, 22 µmol) in 1.5 mL of dry CH$_2$Cl$_2$. The reaction was stopped 2 hours after addition of TESOTf [TLC (hexane : ethyl acetate 2 : 1), indicated complete disappearance of the imidate] by the addition of NaHCO$_3$ (and stirring ~10
minutes). The solution was then filtered and the volume was reduced to ~1 mL. t-Butyl methyl ether (30 mL) was then added and the polymer was allowed to precipitate at 4 °C for 2 hours. The polymer was isolated by filtration. The $^1$H-NMR spectrum indicates that a 49% yield of the β-glycosylated product 28, a 7% yield of the α-anomer and a 2% yield of acyl transfer product were obtained.

$^1$H-NMR (δ, ppm) : 28 : 5.42 (dd, 1H, $J_{2,1}$=7.9 Hz, $J_{2,3}$=10.0 Hz, H-2), 3.93 (m, 1H, H-4), 2.71-2.66 (m, 2H, levulinyl methylene), 2.53-2.49 (m, 2H, levulinyl methylene); α-anomer : 5.12 (d, 1H, $J_{1,2}$=3.6 Hz, H-1); 5.10 (s, 2H, CH$_3$COCH$_2$CH$_2$COO-CH$_2$); 3.38 (s, 3H, CH$_3$(PEG)).

MPEGDOXyl 3,4,6-tri-O-benzyl-2-O-pivaloyl-β-D-galactopyranoside (29)

Experiment I (using α-imidate):

TESOTf [ 1.1 μL (4.9 μmol) diluted in 21 μL of CH$_2$Cl$_2$] was added to a solution of compound 24a (17 mg, 25 μmol) and MPEGDOXOH (27 mg, 13 μmol) in 0.9 mL of dry CH$_2$Cl$_2$ at room temperature. After stirring for 40 minutes TLC (hexane : ethyl acetate 3 : 1) indicated the absence of the donor. The reaction was stopped 3 hours after the addition of TESOTf by the addition of NaHCO$_3$ (and stirring for ~10 minutes) which was then filtered off. The volume was reduced to ~1 mL and then 15 mL of dry t-butyl methyl ether was added. The polymer was precipitated at 4 °C overnight and then was isolated by filtration (26 mg). The $^1$H-NMR spectrum indicates that a 47% yield of the β-glycosylated product 29, a 14% yield of the α-anomer and no acyl transfer product were obtained.

$^1$H-NMR (δ, ppm) : 29 : 5.49 (dd, 1H, $J_{2,1}$=7.9 Hz, $J_{2,3}$=10.0 Hz, H-2), 4.42 (d, 1H, H-1), 3.94 (m, 1H, H-4); α-anomer : 5.28 (dd, 1H, $J_{2,1}$=3.8 Hz, $J_{2,3}$=10.0 Hz, H-2), 5.17 (d, 1H, H-1); 3.38 (s, 3H, CH$_3$(PEG)).

Experiment II (using β-imidate):

The same procedure was followed when the β-imidate was reacted with MPEGDOXOH except for the following amounts of reagents : 24b (34 mg, 50 μmol),
MPEGDOXOH (53 mg, 25 μmol), TESOTf [2.3 μL (10 μmol) diluted in 44 μL of CH₂Cl₂], 2 mL of CH₂Cl₂, 25 mL of t-butyl methyl ether. The same protocol was followed as in the reaction of 24a with MPEGDOXOH. The polymer (55 mg) was isolated by filtration. The ¹H-NMR spectrum indicates that a 42% yield of the β-glycosylated product 29, a 12% yield of the α-anomer and no acyl transfer product were obtained.

MPEGDOXyl 2-O-benzoyl-3,4,6-tri-O-benzyl-β-D-galactopyranoside (30)

Experiment I (using α-imidate):

TESOTf (5.0 μL, 22 μmol) was added to a solution of compound 25a (47 mg, 67 μmol) and MPEGDOXOH (112 mg, 52.9 μmol) in 4 mL of dry CH₂Cl₂ at room temperature. After 1 hour stirring TLC (hexane : ethyl acetate 3 : 1) indicated the absence of the imidate. The reaction was stopped 3 hours after the addition of TESOTf by the addition of NaHCO₃ (and stirring ~10 minutes). The solution was then filtered and the volume was reduced to ~1mL. Dry t-butyl methyl ether (15 mL) was then added and the polymer was allowed to precipitate at 4 °C overnight. The polymer was then isolated by filtration. The ¹H-NMR spectrum indicates that a 93% yield of the β-glycosylated product 30, a 14% yield of the α-anomer and no acyl transfer product were obtained.

¹H-NMR (δ, ppm): 30: 7.98-7.96 (m, 2H, benzoyl aromatic), 7.59 (m, 1H, benzoyl aromatic), 7.47-7.43 (m, 2H, benzoyl aromatic), 5.71 (dd, 1H, J₂,₁=8.0 Hz, J₂,₃=9.8 Hz, H-2), 4.00 (m, 1H, H-4);  α-anomer: 5.57 (dd, 1H, J₂,₁=3.8 Hz, J₂,₃=10.5 Hz, H-2), 5.25 (d,1H, H-1); 3.38 (s, 3H, CH₃(PEG)).

Experiment II (using β-imidate):

TESOTf (2.0 μL, 8.8 μmol) was added to a stirred solution of compound 25b (18 mg, 26 μmol) and MPEGDOXOH (43 mg, 20 μmol) in 1.5 mL of dry CH₂Cl₂, at room temperature. The reaction was stopped 3 hours after the addition of TESOTf [TLC (hexane : ethyl acetate 3 : 1) indicated the absence of the imidate] by the addition of
NaHCO₃ (and stirring ~10 minutes). The solution was filtered and the volume was reduced to ~0.5 mL. Dry ethanol (15 mL) was then added and the polymer was allowed to precipitate overnight at 4 °C. The polymer was then isolated by filtration. The ¹H-NMR spectrum indicates that a 66% yield of the β-glycosylated product 30, a 11% yield of the α-anomer and no acyl transfer product were obtained.

**MPEGDOXyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (31)**

TESOTf (7 µl, 31 µmol) was added to a solution of 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl trichloroacetimidate (60 mg, 94 µmol) and MPEGDOXOH (0.141 g, 66.6 µmol) in 3 mL of dry CH₂Cl₂ at room temperature. The reaction was stopped 3 hours later by the addition of 2 drops of DIPEA. The volume was reduced to ~1mL and then 15 ml of dry t-butyl methyl ether was added. The polymer was allowed to precipitate at 4 °C overnight and was then isolated by filtration. The ¹H-NMR spectrum indicates that a 72% yield of the β-glycosylated product 31 and a 3% yield of acyl transfer product were obtained.

¹H-NMR (δ, ppm) : 31 : 5.07 (dd, 1H, J1,2=8.1, J2,3=9.0, H-2), 4.40 (d, 1H, H-1); 5.09 (s, 2H, CH₃COO-CH₂-).

**Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-galactopyranoside (32)**

(Wang et al., in preparation)

1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose (16.360 g, 41.912 mmol) was dissolved in 266 ml of dry 1,2-dichloroethane at room temperature. Phenylthiotrimethylsilane (26.0 ml, 137 mmol) was then added followed by ZnI₂ (20.100 g, 62.934 mmol). The temperature was increased to 50 °C. After stirring for 1 hour TLC (heptane : ethyl acetate 1 : 1) indicated the completion of the reaction. The reaction was stopped by filtering through a silica gel/celite mixture (17 g/17 g). The silica gel/celite mixture was then washed with 250 ml of CH₂Cl₂. The filtrate and the washings were combined and washed with 200 ml of 0.2 M HCl solution and then with 350 ml of
saturated (aq.) NaCl solution. The organic phase was then concentrated and the residues was purified on a silica gel column (heptane : ethyl acetate 1 : 1) to give 13.671 g (74 %) of compound 32.

\(^1\)H-NMR (δ, ppm) : 7.53-7.51 (m, 2H, aromatic), 7.33-7.30 (m, 3H, aromatic), 5.42 (dd, 1H, \(J_{4,5}=1.0\) Hz, H-4), 5.24 (t, 1H, \(J_{2,3}=9.9\) Hz, H-2), 5.05 (dd, 1H, \(J_{3,4}=3.5\) Hz, H-3), 4.72 (d, 1H, \(J_{1,2}=9.9\) Hz, H-1), 4.20 (dd, 1H, \(J_{5,6}=7.0\) Hz, \(J_{6,6}=11.5\) Hz, H-6), 4.12 (dd, 1H, \(J_{5,6'}=6.2\) Hz, \(J_{6,6'}=11.5\) Hz, H-6'), 3.94 (m, 1H, H-5), 2.12 (s, 3H, CH₃CO), 2.10 (s, 3H, CH₃O), 2.05 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO).

Phenyl 1-thio-β-D-galactopyranoside (33)

A solution of sodium methoxide in methanol (1.6 N, 66 ml) was added to a solution of compound 32 (7.7 g, 17 mmol) in 100 mL of dry methanol at room temperature. After 2 hour stirring TLC (toluene : ethyl acetate 3 : 1) indicated the completion of the reaction. The solution was neutralized with IR-120 (H⁺) Amberlite resin and the solvents were evaporated to give 4.403 g (92%) of compound 33.

\(^1\)H-NMR (δ, ppm) in CD₃OD : 7.55-7.53 (m, 2H, aromatic), 7.30-7.26 (m, 2H, aromatic), 7.22 (m, 1H, aromatic), 4.57 (d, 1H, \(J_{1,2}=9.8\) Hz, H-1), 3.89 (dd, 1H, \(J_{4,5}=0.9\) Hz, H-4), 3.75 (dd, 1H, \(J_{5,6}=6.8\) Hz, \(J_{6,6}=11.3\) Hz, H-6), 3.70 (dd, 1H, \(J_{5,6'}=5.2\) Hz, H-6'), 3.60 (t, 1H, \(J_{2,3}=9.4\) Hz, H-2), 3.55 (m, 1H, H-5), 4.49 (dd, 1H, \(J_{3,4}=3.4\) Hz, H-3).

ESMS : MNa⁺=295 m/z, MNH₄⁺=290 m/z (ie. MW=272 g/mol).

Phenyl 3,6-di-O-benzyl-1-thio-β-D-galactopyranoside (34) and Phenyl 3-O-benzyl-1-thio-β-D-galactopyranoside (35)

(Kovac & Glaudemans, 1985 a)

Compound 33 (1.381 g, 5.071 mmol) was dissolved in 20 ml of benzene under argon and refluxed for 20 hours in Dean-Stark trap. Dibutyltin oxide (1.263 g, 5.074 mmol) was then added and the solution was refluxed overnight. Tetrabutylammonium iodide (1.874 g, 5.134 mmol) followed by benzyl bromide (0.6 ml, 5 mmol) was then
added. After 3 hours TLC (ethyl acetate : methanol 20 : 1) indicated complete disappearance of the starting material. The reaction was stopped by evaporating off the solvent and the residue was chromatographed on a silica gel chromatography column (heptane : ethyl acetate 1 : 1) to give 0.146 g (6%) of compound 34 and 0.300 g (13%) of compound 35.

$$^1\text{H-NMR (δ, ppm)}$$: 34 : 7.57-7.56 (m, 2H, thiophenyl aromatic), 7.38-7.27 (m, 13 H, aromatic), 4.76 (d, 1H, J$_{gem}$=11.8 Hz, CH-Ph), 2.71 (d, 1H, CH-Ph), 4.58 (s, 2H, CH$_2$-Ph), 4.52 (d, 1H, J$_{1,2}$=9.8 Hz, H-1), 4.09 (m, 1H, H-4), 3.83-3.76 (m, 3H, H-2, H-6 and H-6'), 3.65 (t, 1H, H-5), 3.45 (dd, 1H, J$_{2,3}$=9.0 Hz, J$_{3,4}$=3.3 Hz, H-3); 35 : 7.56-7.54 (m, 2H, thiophenyl aromatic), 7.39-7.28 (m, 8H, aromatic), 4.75 (s, 2H, CH$_2$-Ph), 4.53 (d, 1H, J$_{1,2}$=9.8 Hz, H-1), 4.05 (m, 1H, H-4), 3.99 (ddd, 1H, J$_{5,6}$=6.6 Hz, J$_{6,6'}$=11.8 Hz, J$_{6,0H}$=4.0 Hz, H-6), 3.84-3.79 (m, 2H, H-2 and H-6'), 3.56 (t, 1H, H-5), 3.47 (dd, 1H, J$_{2,3}$=9.0 Hz, J$_{3,4}$=3.2 Hz, H-3).

**Phenyl 2,4-di-O-acetyl-3,6-di-O-benzyl-1-thio-β-D-galactopyranoside (36)**

Acetic anhydride (2.5 ml, 26 mmol) was added to a solution of compound 34 (0.142 g, 0.314 mmol) in 2 ml of dry pyridine at room temperature. After overnight stirring TLC (heptane : ethyl acetate 2 : 1) indicated the completion of the reaction. The solution was then diluted with CH$_2$Cl$_2$, washed with distilled H$_2$O and concentrated. The residue was purified on a silica gel column (heptane : ethyl acetate 2 : 1) to give 0.123 g (73%) of compound 36.

$$^1\text{H-NMR (δ, ppm)}$$ : 7.49-7.47 (m, 2H, thiophenyl aromatic), 7.37-7.24 (m, 13H, thiophenyl and benzyl aromatic), 5.62 (d, 1H, H-4), 5.15 (t, 1H, J$_{1,2}$=9.8 Hz, J$_{2,3}$=9.8 Hz, H-2), 4.69 (d, 1H, J$_{gem}$=12.4 Hz, CH-Ph), 4.64 (d, 1H, H-1), 4.55 (d, 1H, J$_{gem}$=11.8 Hz, CH-Ph), 4.46 (d, 1H, J$_{gem}$=11.8 Hz, CH-Ph), 4.41 (d, 1H, J$_{gem}$=12.2 Hz, CH-Ph), 3.78 (m, 1H, H-5), 3.62 (dd, 1H, J$_{5,6}$=6.2 Hz, J$_{6,6'}$=9.6 Hz, H-6), 3.64-3.61 (m, 2H, H-3 and H-6'), 2.07 (s, 3H, CH$_3$CO), 2.04 (s, 3H, CH$_3$CO).
ESMS: $\text{MH}^+ = 537 \text{ m/z, MNH}_4^+ = 554 \text{ m/z (ie., MW} = 536 \text{ g/mol).}$

2,4-di-O-acetyl-3,6-di-O-benzyl-D-galactopyranose (37) and 1,4-di-O-acetyl-3,6-di-O-benzyl-α-D-galactopyranose (38)

(Nicolaou et al., 1983; Z.G. Wang, private communication)

Compound 36 (0.115 g, 0.214 mmol) was dissolved in 5 ml of acetonitrile and 11 µl (0.61 mmol) of H$_2$O was added. The temperature was lowered to 0 °C. N-bromosuccinimide (56 mg, 0.32 mmol) was then added and the solution was stirred at 0 °C. After 40 minutes TLC (heptane : ethyl acetate 1 : 1) indicated complete disappearance of the starting material and the formation of one product. The reaction was stopped by the addition of NaHCO$_3$ (and stirring for ~10 minutes) which was then filtered off. The solvent was evaporated and the residue was purified on a silica gel column (toluene : ethyl acetate 2 : 1) to give 50 mg (52%) of an unseparable mixture of compounds 37 and 38 [the integration of H-1α(1-O-acyl) signal in the $^1$H-NMR spectrum indicates at least a ~14% yield].

$^1$H-NMR (δ, ppm): Three H-4 signals are present (at 5.71, 5.62 and 5.61 ppm) which indicates the presence of at least 3 compounds. The signal at 6.31 ppm (d, 1H, $J_{1,2}$=3.9 Hz, H-1α) indicates the presence of compound 38.

2,4-di-O-acetyl-3,6-di-O-benzyl-D-galactopyranosyl trichloroacetimidate (39a and 39b)

The mixture of compounds 37 and 38 (see above) (50 mg, 0.11 mmol) was dissolved in 1.5 mL of dry CH$_2$Cl$_2$ and the solution was cooled to 0 °C. Trichloroacetonitrile (99 µl, 0.99 mmol) was then added followed by Cs$_2$ CO$_3$ (58 mg, 18 µmol). The solution was stirred at 0 °C. Three hours after the addition of the catalyst TLC (heptane : ethyl acetate 2 : 1) indicated the presence of two products and a small amount of the starting material. The reaction was stopped by filtering off Cs$_2$CO$_3$ and evaporating the solvent. The residue was put on a column of silica gel (hexane : ethyl acetate 2 : 1,
2% DIPEA) to give 28 mg (42%) of the α-imidate 39a and 34 mg (51%) of the β-anomer 39b.

39a : \(^1\)H-NMR (δ, ppm) : 8.55 (s, 1H, NH), 7.36-7.28 (m, 10 H, aromatic), 6.54 (d, 1H, \(J_{1,2}=3.4\) Hz, H-1), 5.75 (d, 1H, H-4), 5.25 (dd, 1H, \(J_{2,3}=10.5\) Hz, H-2), 4.76 (d, 1H, \(J_{\text{gem}}=12.0\) Hz, CH-Ph), 4.56 (d, 1H, \(J_{\text{gem}}=12.0\) Hz, CH-Ph), 4.49 (d, 1H, \(J_{\text{gem}}=12.0\) Hz, CH-Ph), 4.44 (d, 1H, \(J_{\text{gem}}=11.8\) Hz, CH-Ph), 4.30 (m, 1H, H-5), 4.01 (dd, 1H, \(J_{3,4}=3.3\) Hz, H-3), 3.56 (dd, 1H, \(J_{5,6}=5.6\) Hz, \(J_{6,6}=-9.4\) Hz, H-6), 3.49 (dd, 1H, \(J_{5,6}=-7.5\) Hz, H-6'), 2.09 (s, 3H, CH\(_3\)CO), 1.99 (s, 3H, CH\(_3\)CO); \(^{13}\)C-NMR : 170.13 (CH\(_3\)CO), 160.90, 137.44, 128.46 (aromatic), 128.29 (aromatic), 128.05 (aromatic), 127.91 (aromatic), 127.81 (aromatic), 94.12 (C-1), 73.65 (CH-Ph), 72.54 (C-3), 71.40 (CH-Ph), 70.56 (C-5), 68.95 (C-2), 67.51 (C-6), 66.74 (C-4); 39b : \(^1\)H-NMR (δ, ppm) : 8.62 (s, 1H, NH), 7.36-7.28 (m, 10 H, aromatic), 5.71 (d, 1H, \(J_{1,2}=8.3\) Hz, H-1), 5.67 (d, 1H, H-4), 5.38 (dd, 1H, \(J_{2,3}=9.8\) Hz, H-2), 4.73 (d, 1H, \(J_{\text{gem}}=12.4\) Hz, CH-Ph), 4.57 (d, 1H, \(J_{\text{gem}}=12.0\) Hz, CH-Ph), 4.45 (t, 2H, CH\(_2\)-Ph), 3.93 (m, 1H, H-5), 3.63-3.55 (m, 2H, H-3 and H-6), 3.54 (dd, 1H, \(J_{5,6}=-7.5\) Hz, \(J_{6,6}=-9.4\) Hz, H-6'), 2.11 (s, 3H, CH\(_3\)CO), 1.98 (s, 3H, CH\(_3\)CO).

MPEGDOXyl 2,4-di-O-acetyl-3,6-di-O-benzyl-β-D-galactopyranoside (40)

Experiment I (using α-imidate):
TESOTf [2.5 μL (11.1 μmol) diluted 1 : 10 in CH\(_2\)Cl\(_2\)] was added to a solution of compound 39a (26 mg, 44 μmol) and MPEGDOXOH (49 mg, 23 μmol) in 2 mL of dry CH\(_2\)Cl\(_2\) at room temperature. After 1.5 hour stirring TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated complete disappearance of the imidate. The reaction was stopped 3 hours after the addition of the catalyst by the addition of NaHCO\(_3\) (and stirring ~10 minutes) which was then filtered off. The volume was reduced to ~1 ml and 30 ml of dry t-butyl methyl ether was added. The polymer was allowed to precipitate overnight at 4 °C and was then isolated by filtration (49 mg). The \(^1\)H-NMR spectrum indicates that a 61% yield of the β-glycosylated product 40, a ~8% yield of the α-anomer and a 19% yield of acyl transfer product were obtained.
\(^1\)H-NMR (\(\delta\), ppm) : 40: 5.59 (d, 1H, J\(_{3,4}\)=3.0 Hz, H-4), 5.17 (dd, 1H, J\(_{1,2}\)=8.0 Hz, J\(_{2,3}\)=10.0 Hz, H-2); \(\alpha\)-anomer: 5.14 (d, 1H, J\(_{1,2}\)=3.9 Hz, H-1), 5.07 (dd, 1H, J\(_{2,3}\)=10.3 Hz, H-2); 5.09 (s, 2H, CH\(_3\)-COO-CH\(_2\)-); 3.38 (s, 3H, CH\(_3\)(PEG)).

**Experiment II (using β-imidate):**

Compound 39b (27 mg, 46 \(\mu\)mol), MPEGDOXOH (51 mg, 24 \(\mu\)mol), TESOTf [2.2 \(\mu\)l (9.7 \(\mu\)mol) diluted 1 : 10 in CH\(_2\)Cl\(_2\)], 2 mL of CH\(_2\)Cl\(_2\) and 30 mL of t-butyl methyl ether were used. The same protocol was followed as in the reaction of 39a with MPEGDOXOH. The polymer (48 mg) was isolated by filtration. The \(^1\)H-NMR spectrum indicates that a 27% yield of the \(\beta\)-glycosylated product 40, a ~5% yield of the \(\alpha\)-glycosylated product and a 14% yield of acyl transfer product were obtained.

**Phenyl 2,4,6-tri-O-acetyl-3-O-benzyl-1-thio-\(\beta\)-D-galactopyranoside (41)**

Compound 35 (0.153 g, 0.422 mmol), acetic anhydride (2.5 mL) and 2 mL of pyridine were used. The same protocol was followed as in the acetylation of phenyl 3,6-di-O-benzyl-1-thio-\(\beta\)-D-galactopyranoside (34). Compound 41 (72 mg, 36%) was obtained. \(^1\)H-NMR (\(\delta\), ppm) : 7.51-7.49 (m, 2H, thiophenyl aromatic), 7.35-7.24 (m, 8 H, thiophenyl aromatic and benzyl aromatic), 5.53 (dd, 1H, J\(_{3,4}\)=3.2 Hz, J\(_{4,5}\)=0.8 Hz, H-4), 5.16 (t, 1H, J\(_{1,2}\)=9.7 Hz, J\(_{2,3}\)=9.7 Hz, H-2), 4.68 (d, 1H, J\(_{gem}\)=12.2 Hz, CH-Ph), 4.62 (d, 1H, H-1), 4.40 (d, 1H, CH-Ph), 4.17 (d, 2H, J=6.4 Hz, H-6 and H-6'), 3.83 (m, 1H, H-5), 3.57 (dd, 1H, H-3), 2.13 (s, 3H, CH\(_3\)CO), 2.07 (s, 3H, CH\(_3\)CO), 2.06 (s, 3H, CH\(_3\)CO).

ESMS : MH\(^+\)=489 m/z, MNH\(_4\)^+=506 m/z (ie. MW=488 g/mol).

**2,4,6-tri-O-acetyl-3-O-benzyl-D-galactopyranose (42) and 1,4,6-tri-O-acetyl-3-O-benzyl-\(\beta\)-D-galactopyranose (43)**

Compound 41 (74 mg, 0.15 mmol) was dissolved in 3 mL of acetonitrile and 8 \(\mu\)L (0.44 mmol) of H\(_2\)O was added. The solution was cooled down to 0 °C. N-
bromosuccinimide (32 mg, 0.18 mmol) was then added and the solution was stirred at 0 °C. After 1.5 hour TLC (toluene : ethyl acetate 2 : 1) indicated the presence of one major product and a small amount of the starting material. The reaction was stopped by the addition of NaHCO₃ (and stirring ~ 10 minutes) which was then filtered off. The solvent was evaporated. The residue was then chromatographed on a UV active preparative silica plate (Merck) (toluene : ethyl acetate 2 : 1) and eluted with 10% methanol in CH₂Cl₂ to give 22 mg (37%) of an unseparable mixture of compounds 42 and 43. (Note : The 1H-NMR spectrum indicates that the yield of 42 was 26% and the yield of 43 was 11%).

1H-NMR δ: 42: α-anomer: 5.60 (m, 1H, H-4), 5.11 (dd, 1H, J₁₂=3.6 Hz, J₂₃=10.3 Hz, H-2), 3.99 (dd, 1H, J₃₄=3.4 Hz, H-3); β-anomer: 4.96 (dd, 1H, J₁₂=8.3 Hz, J₂₃=10.0 Hz, H-2), 3.61 (dd, 1H, J₃₄=3.4 Hz, H-3); 43: 4.57 (t, 1H, J₁₂=8.8 Hz, J₂₃=8.8 Hz, H-2), 3.54 (d, 1H, H-1₃).

2,4,6-tri-O-acetyl-3-O-benzyl-D-galactopyranosyl trichloroacetimidate (44a and 44b)

The mixture of compounds 42 and 43 (see above) (22 mg, 56 µmol) was dissolved in 1.5 mL of dry CH₂Cl₂ and the temperature was lowered to 0 °C. Trichloroacetonitrile (48 µl, 0.48 mmol) was then added followed by Cs₂CO₃ (4 mg, 13 µmol) and the reaction was stirred at 4 °C overnight [TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated presence of two products and ~50% of unreacted material] and then at room temperature for 3 hours (TLC indicated the presence of two products and a small amount of the starting material). The solution was concentrated and chromatographed on a silica gel column first with hexane : ethyl acetate 3 : 1 (2% DIPEA) to give 10 mg (33%) of the α-imidate 44a and then with hexane : ethyl acetate 1 : 1 (2% DIPEA) to give 8 mg (27%) of the β-anomer 44b.

44a : 1H-NMR (δ, ppm): 8.59 (s, 1H, NH), 7.35-7.28 (m, 5H, aromatic), 6.57 (d, 1H, J₁₂=3.6 Hz, H-1), 5.66 (dd, 1H, H-4), 5.27 (dd, 1H, J₂₃=10.5 Hz, H-2), 4.75 (d, 1H, J₃₄=11.8 Hz, CH-Ph), 4.50 (d, 1H, CH-Ph), 4.35 (m, 1H, H-5), 4.22 (dd, 1H, J₅₆=6.1 Hz, H-5), 3.54 (d, 1H, H-1₃).
HZ, $J_{6,6}' = 11.8$ Hz, H-6'), 4.06 (dd, 1H, $J_{5,6}' = 6.9$ Hz, H-6'), 4.03 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 2.17 (s, 3H, CH$_3$CO), 2.05 (s, 3H, CH$_3$CO), 2.00 (s, 3H, CH$_3$CO); $^{13}$C-NMR : 128.34 (aromatic), 127.98 (aromatic), 109.07 (C-1), 72.46 (C-3), 71.62 (CH$_2$-Ph), 69.55 (C-5), 68.77 (C-2), 66.57 (C-4), 61.93 (C-6), 20.77 (CH$_3$CO); 44b : $^1$H-NMR (δ, ppm): 8.64 (s, 1H, NH), 7.36-7.26 (m, 5H, aromatic), 5.72 (d, 1H, $J_{1,2} = 8.3$ Hz, H-1), 5.57 (m, 1H, H-4), 5.40 (dd, 1H, $J_{2,3} = 10.0$ Hz, H-2), 4.72 (d, 1H, $J_{gem} = 12.2$ Hz, CH-Ph), 4.44 (d, 1H, CH-Ph), 4.24 (dd, 1H, $J_{5,6}' = 6.8$ Hz, $J_{6,6}' = 11.3$ Hz, H-6), 4.18 (dd, 1H, $J_{5,6}' = 6.6$ Hz, H-6'), 4.00 (m, 1H, H-5), 3.64 (dd, 1H, $J_{3,4} = 3.4$ Hz, H-3), 2.19 (s, 3H, CH$_3$CO). 2.08 (s, 3H, CH$_3$CO), 1.99 (s, 3H, CH$_3$CO).

MPEGDOXyl 2,4,6-tri-O-acetyl-3-O-benzyl-β-D-galactopyranoside (45)

Compound 44a (10 mg, 18 μmol), MPEGDOXOH (20 mg, 9.4 μmol), TESOTf [0.9 μL (4 μmol), diluted 1:10 in CH$_2$Cl$_2$], 1 mL of CH$_2$Cl$_2$ and 10 mL of t-butyl methyl ether were used. The same protocol was followed as in the synthesis of compound 45. The polymer (19 mg) was isolated by filtration. The $^1$H-NMR spectrum indicates that a 38% yield of the β-glycosylated product 45, a ~5% yield of the α-anomer and a 19% yield of acyl transfer product were obtained.

$^1$H-NMR (δ, ppm): 45 : 5.50 (m, 1H, H-4), 5.19 (dd, 1H, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 10.0$ Hz, H-2), 4.39 (d, 1H, H-1); α-anomer : 5.15 (d, 1H, $J_{1,2} = 3.9$ Hz, H-1), 5.08 (dd, but one doublet covered by the transfer signal, H-2); 5.09 (s, 2H, CH$_3$-COO-CH$_2$-); 3.38 (s, 3H, CH$_3$(PEG)).

Phenyl 4,6-O-benzylidene-1-thio-β-D-galactopyranoside (46)

(Hall, 1980; can also be synthesized according to Ebara et al., 1996)

With stirring, ZnCl$_2$ (5.255 g, 38.56 mmol) was added to benzaldehyde (36.6 mL, 0.360 mol). After 30 minutes, when the solution was a white gelatinous paste, compound 33 (7.000 g, 25.71 mmol) was added. After 1 hour stirring at room
temperature TLC ($\text{CH}_2\text{Cl}_2$: methanol 13:1) indicated the completion of the reaction. The reaction mixture was poured into a flask containing 70 ml of ice-water and 70 ml of heptane and was shaken vigorously. White precipitate formed immediately. It was isolated by filtration and washed with cold water and cold heptane. After overnight drying in vacuo 8.072 g (87%) of compound 46 was obtained.

$^1\text{H}-\text{NMR}$ ($\delta$, ppm) : 7.71-7.69 (m, 2H, aromatic), 7.41-7.29 (m, 8H, aromatic), 5.53 (s, 1H, benzylidene H), 4.53 (d, 1H, $J_{1,2}$=9.1 Hz, H-1), 4.40 (dd, 1H, $J_{5,6}$=1.7 Hz, $J_{6,6}$=12.4 Hz, H-6), 4.24 (dd, 1H, $J_{2,3}$=3.0 Hz, $J_{3,4}$=1.1 Hz, H-3), 4.05 (dd, 1H, $J_{5,6}$=1.8 Hz, H-6'), 3.72-3.70 (m, 2H, H-2 and H-4), 3.58 (m, 1H, H-5).

Phenyl 6-O-benzyl-1-thio-$\beta$-D-galactopyranoside (47)

(Garegg et al., 1982 and Ehara et al., 1996)

Dry tetrahydrofuran (5mL) was added to a flask containing compound 46 (0.500 g, 1.39 mmol) and 3 Å molecular sieves (0.1 g), and the mixture was stirred at room temperature for 1 hour. NaCNBH$_3$ (1.308 g, 20.81 mmol) was then added. HCl in tetrahydrofuran was added to the reaction mixture until the evolution of gas ceased. After overnight stirring the reaction was neutralized with NaHCO$_3$. The molecular sieves were filtered off and the solution was diluted with CH$_2$Cl$_2$ and washed with distilled H$_2$O. The organic phase was dried with MgSO$_4$, concentrated and chromatographed on a silica gel column (heptane : ethyl acetate 1 : 10) to give 0.396 g (79%) of compound 47.

$^1\text{H}-\text{NMR}$ ($\delta$, ppm) : 7.56-7.52 (m, 2H, aromatic), 7.36-7.18 (m, 8H, aromatic), 4.61 (d, 1H, $J_{1,2}$=9.6 Hz, H-1), 3.87 (d, 1H, $J_{3,4}$=3.3 Hz, H-4), 3.50 (dd, 1H, $J_{2,3}$=9.1 Hz, H-3).

Phenyl 2,3,4-tri-O-acetyl-6-O-benzyl-1-thio-$\beta$-D-galactopyranoside (48)

Acetic anhydride (2.5 ml) was added to a stirred solution of compound 47 (0.357 g, 0.985 mmol) in 5 mL of dry pyridine at room temperature. After 5 hours TLC (toluene : ethyl acetate 5:1) showed only one spot. The reaction was stopped 5 hours
later by diluting with CH$_2$Cl$_2$ and washing with H$_2$O. After washing TLC (toluene : ethyl acetate 5 : 1) showed another slower moving spot. The organic layer was dried with MgSO$_4$, concentrated and the residue was purified on a column of silica gel (toluene : ethyl acetate 5 : 1) to give 0.117 g (24%) of compound 48.

$^1$H-NMR ($\delta$, ppm) : 7.52-7.50 (m, 2H, thiophenyl aromatic), 7.36-7.30 (m, 3H, thiophenyl aromatic), 7.29-7.27 (m, 5H, benzyl aromatic), 5.49 (dd, 1H, $J_{3,4}$=3.6 Hz, $J_{4,5}$=1.1 Hz, H-4), 5.24 (t, 1H, $J_{1,2}$=9.9 Hz, $J_{2,3}$=9.9 Hz, H-2), 5.06 (dd, 1H, H-3), 4.74 (d, 1H, H-1), 4.55 (d, 1H, $J_{gem}$=11.8 Hz, CH-Ph), 4.43 (d, 1H, CH-Ph), 3.90 (m, 1H, H-5), 3.61 (dd, 1H, $J_{5,6}$=6.3 Hz, $J_{6,6'}$=9.9 Hz, H-6'), 3.50 (dd, 1H, $J_{5,6'}$=6.3 Hz, H-6'), 2.09 (s, 3H, CH$_3$CO), 2.04 (s, 3H, CH$_3$CO), 1.97 (s, 3H, CH$_3$CO).

ESMS : M$^+$ = 511 m/z, M$^{Na}$ = 506 m/z (ie. MW = 488 g/mol).

2,3,4-tri-O-acetyl-6-O-benzyl-D-galactopyranose (49) and 1,3,4-tri-O-acetyl-6-O-benzyl-$\alpha$-D-galactopyranose (50)

Compound 48 (0.187 g, 0.383 mmol) was dissolved in 5 mL of acetonitrile and 21 µL (1.2 mmol) of H$_2$O was added. The temperature was lowered to 0 °C and N-bromosuccinimide (85 mg, 0.48 mmol) was added. After stirring for 1.5 hour TLC (toluene : ethyl acetate 5 : 1) indicated almost complete disappearance of the starting material. The reaction was stopped by the addition of NaHCO$_3$ (and stirring for -10 minutes) which was then filtered off. The solvent was evaporated and the residue was chromatographed on a silica gel column (toluene : ethyl acetate 5 : 1) to give 97 mg (64%) of an unseparable mixture of compounds 49 and 50. (Note : The $^1$H-NMR spectrum indicates that the yield of 49 was ~43% and the yield of 50 was ~21%.)

$^1$H-NMR : The integration indicates that 3 compounds are present. The signal at 6.29 ppm (d, 1H, $J_{1,2}$=3.9 Hz, H-1α, 1-O-acetyl) indicates the presence of compound 50. The presence of compound 49 is indicated by the number of acetyl signals (8) and the yield of the imidate 51 (next step, 67%). The following signals are also present in addition to the signal at 6.29 ppm : 5.54 (m, 1H, H-4), 5.41 (dd, 1H, $J_{3,3}$, $J_{3,3'}$=10.7), 4.57
(d, J_{gem}=12.4), 4.54 (d, J_{gem}=11.8), 4.45 (d, J_{gem}=11.5), 4.40 (d, J_{gem}=12.01), 4.22 (m, 1H, H-5), 3.90 (m, 1H, H-5), 2.17 (CH_3CO), 2.10 (CH_3CO), 2.09 (CH_3CO), 2.07 (CH_3CO), 2.06 (CH_3CO), 2.04 (CH_3CO), 1.99 (CH_3CO), 1.99 (CH_3CO).

2,3,4-tri-O-acetyl-6-O-benzyl-\( \alpha \)-D-galactopyranosyl trichloroacetimidate (51)

The mixture of compounds 49 and 50 (see above) (0.197 g, 0.497 mmol) was dissolved in 4 mL of dry CH\(_2\)Cl\(_2\) and the temperature was lowered to 0 °C. Trichloroacetonitrile (0.9 ml, 9 mmol) followed by Cs\(_2\)CO\(_3\) (37 mg, 0.11 mmol) was then added. Solution was stirred at 0 °C for 3 hours and then at room temperature overnight [TLC (hexane : ethyl acetate 1 : 1, 2% DIPEA) indicated the completion of the reaction]. The solution was concentrated and the residue was purified on a silica gel column (hexane : ethyl acetate 1 : 1, 2% DIPEA) to give 0.179 g (67%) of compound 51.

\(^1\)H-NMR (\(\delta\), ppm): 8.62 (s, 1H, NH), 7.34-7.24 (m, 5H, aromatic), 6.58 (d, 1H, J\(_{1,2}\)=3.6 Hz, H-1), 5.64 (dd, 1H, J\(_{3,4}\)=3.0 Hz, J\(_{4,5}\)=1.2 Hz, H-4), 5.43 (dd, 1H, J\(_{2,3}\)=11.0 Hz, H-3), 5.34 (dd, 1H, H-2), 4.54 (d, 1H, J_{gem}=12.1 Hz, CH-Ph), 4.41 (m, 1H, H-5), 4.40 (d, 1H, CH-Ph), 3.55 (dd, 1H, J\(_{5,6}\)=5.8 Hz, J\(_{6,6'}\)=9.6 Hz, H-6), 3.46 (dd, 1H, J\(_{5,6'}\)=7.1 Hz, H-6'), 2.06 (s, 3H, CH\(_3\)CO), 2.02 (s, 3H, CH\(_3\)CO), 2.01 (s, 3H, CH\(_3\)CO).

MPEGDOXyl 2,3,4-tri-O-acetyl-6-O-benzyl-\( \beta \)-D-galactopyranoside (52)

Experiment I (0.4 equivalents of TESOTf):
TESOTf (1.3 \(\mu\)L, 5.7 \(\mu\)mol) was added to a solution of compound 51 (15 mg, 28 \(\mu\)mol) and MPEGDOXOH (30 mg, 14 mmol) in 1 mL of dry CH\(_2\)Cl\(_2\) at room temperature. After 1 hour stirring TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated complete disappearance of the imidate. The reaction was stopped 3 hours after addition of TESOTf by adding few drops of DIPEA. The solvent was evaporated and the residue was dissolved in 0.5 mL of CH\(_2\)Cl\(_2\). The polymer was then precipitated in 15 mL of
dry t-butyl methyl ether at 4 °C overnight. The polymer (32 mg) was then isolated by filtration. The $^1$H-NMR spectrum indicates that a 51% yield of the β-glycosylated product 52 and a 41% yield of acyl transfer product were obtained.

$^1$H-NMR (δ, ppm) : 52 : 5.46 (m, 1H, H-4), 5.25 (dd, 1H, $J_{1,2}$=7.9 Hz, $J_{2,3}$=10.4 Hz, H-2), 4.98 (dd, 1H, $J_{3,4}$=3.4 Hz, H-3), 4.50 (d, 1H, H-1), 3.84 (m, 1H, H-5); 5.09 (s, 2H, CH$_3$-COO-CH$_2$-); 3.38 (s, 3H, CH$_3$(PEG)).

Experiment II (0.8 equivalents of TESOTf):

TESOTf (8.5 µL, 38 µmol) was added to a solution of compound 51 (51 mg, 94 µmol) and MPEGDOXOH (99 mg, 47 µmol) in 4 ml of dry CH$_2$Cl$_2$ at room temperature. After 1 hour stirring TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated complete disappearance of the imidate. The reaction was stopped 3 hours after the addition of TESOTf by adding NaHCO$_3$ (and stirring ~10 minutes) which was then filtered off. The solvent was evaporated to ~0.5 ml of CH$_2$Cl$_2$ and 50 ml of dry t-butyl methyl ether was added. The polymer was allowed to precipitate at 4 °C overnight. The polymer (0.110 g) was then isolated by filtration. The $^1$H-NMR spectrum indicates that a 54% yield of the β-glycosylated product 52 and a 49% yield of acyl transfer product were obtained.

1,6-anhydro-4-O-benzyl-β-D-galactopyranose (53)
(Cruzado & Martin-Lomas, 1988)

A stirred mixture of 1,6-anhydro-β-D-galactopyranose (purchased from Sigma) (1.624 g, 10.02 mmol), 3 Å molecular sieves (8 g), and bis(tributyltin)oxide (7.7 mL, 15 mmol) was heated under argon at 120 °C. After 15 hours benzyl bromide (10.0 ml, 84.1 mmol) followed by N-methylimidazole (1.0 ml, 13 mmol) was added. After 4 hours TLC (CH$_2$Cl$_2$ : methanol 7 : 1) indicated complete disappearance of the starting material. The reaction was stopped by filtering off molecular sieves and washing them with chloroform and then with methanol. The combined filtrate and washings were concentrated and the residue was purified by column chromatography (heptane : ethyl acetate 1 : 4) to give 2.086 g of an unseparable mixture of 1,6-anhydro-4-O-
benzyl-\(\beta\)-D-galactopyranose (53)

(The \(^1\)H-NMR spectrum indicates a 71% yield.) and 1,6-anhydro-3-O-benzyl-\(\beta\)-D-galactopyranose (The \(^1\)H-NMR spectrum indicates a 12% yield).

\(^1\)H-NMR (\(\delta\), ppm): 53: 7.40-7.33 (m, 5H, aromatic), 5.38 (m, 1H, H-1), 4.71 (d, 1H, \(J_{\text{gem}}=4.7\) Hz, CH-Ph), 4.42 (m, 1H, H-5), 4.31 (d, 1H, \(J=7.5\) Hz, H-6\(_{\text{endo}}\)), 4.04 (m, 1H, H-3), 3.84-3.81 (m, 2H, H-2 and H-4), 3.66 (m, 1H, H-6\(_{\text{exo}}\)).

1,2,3,6-tetra-O-acetyl-4-O-benzyl-D-galactopyranose (54)

(Subero et al., 1985)

A mixture of compounds 53 and 1,6-anhydro-3-O-benzyl-\(\beta\)-D-galactopyranose (6: 1, see above) (1.749 g, 6.932 mmol) was dissolved in 9.2 mL of acetic anhydride. Acetic acid (1.8 mL) followed by 37 \(\mu\)L (0.69 mmol) of \(H_2SO_4\) was then added. The reaction was stirred at room temperature. After 2 hours TLC (heptane : ethyl acetate 3:2) indicated complete disappearance of the starting material. The solution was cooled down in ice bath and was neutralized with 5% aqueous NaHCO\(_3\) solution. The solution was then diluted with with \(CH_2Cl_2\), washed with \(H_2O\), dried with MgSO\(_4\) and concentrated. After silica gel column chromatography (heptane : ethyl acetate 3:2) 2.205g (87%) of compound 54 was obtained.

\(^1\)H-NMR (\(\delta\), ppm): \(\alpha\)-anomer (major): 7.38-7.30 (m, 5H, aromatic), 6.36 (d, 1H, \(J_{1,2}=3.8\) Hz, H-1), 5.53 (dd, 1H, \(J_{2,3}=10.9\) Hz, H-2), 5.29 (dd, 1H, \(J_{3,4}=3.0\) Hz, H-3), 4.74 (d, 1H, \(J_{\text{gem}}=11.3\) Hz, CH-Ph), 4.55 (d, 1H, CH-Ph), 4.20 (dd, 1H, \(J_{5,6}=6.1\) Hz, \(J_{6,6}'=10.0\) Hz, H-6), 4.17 (m, 1H, H-5), 4.08 (dd, 1H, \(J_{5,6}'=5.6\) Hz, H-6'), 4.06 (m, 1H, H-4), 2.13 (s, 3H, CH\(_3\)CO), 2.06 (d, 6H, 2 x CH\(_3\)CO), 2.02 (s, 3H, CH\(_3\)CO); \(\beta\)-anomer: 5.67 (d, 1H, \(J_{1,2}=8.1\) Hz, H-1), 5.50 (dd, 1H, \(J_{2,3}=10.5\) Hz, H-2), 5.01 (dd, 1H, \(J_{3,4}=10.3\) Hz, H-3), 3.97 (m, 1H, H-4), 3.85 (m, 1H, H-5).

ESMS: M\(NH_4^+\) = 456 m/z, MNa\(^+\) = 461 m/z \((ie\ . MW = 438\ g/mol)\).

2,3,6-tri-O-acetyl-4-O-benzyl-D-galactopyranose (55) and 1,3,6-tri-O-acetyl-4-O-benzyl-\(\beta\)-D-galactopyranose (56)
Compound 54 (1.700 g, 3.878 mmol) was dissolved in 23 ml of dry N,N-dimethylformamide and the temperature was increased to 50 °C. After 20 minutes TLC (heptane : ethyl acetate 1 : 1) indicated almost complete disappearance of the starting material. The reaction was stopped by diluting with 600 mL of ethyl acetate and washing with 200 mL of cold water. The water fraction was then washed with an equal volume of ethyl acetate. The ethyl acetate fractions were then combined, dried with MgSO₄, concentrated, and chromatographed on a silica gel column (heptane : ethyl acetate 1 : 1) to give 1.253 g (82%) of an unseparable mixture of compounds 55 and 56.

^1H-NMR : the integration indicates the presence of 3 compounds, (δ, ppm) : 55 : 5.23 (dd, 1H, J₁₂=7.9 Hz, J₂₃=10.5 Hz, H-2₃); 56 : 4.63 (dd, 1H, J₁₂=9.8 Hz, J₂₃=7.9 Hz, H-2₃, 1-O-acetyl), 3.41 (d, 1H, H-1).

ESMS : M NH₄⁺ = 414 m/z, M Na⁺ = 419 m/z (ie. MW = 396 g/mol).

2,3,6-tri-O-acetyl-4-O-benzyl-D-galactopyranosyl trichloroacetimidate (57a and 57b)

The mixture of compounds 55 and 56 (see above) (0.948 g, 2.39 mmol) was dissolved in 37 mL of dry CH₂Cl₂. Trichloroacetonitrile (2.4 mL, 24 mmol) followed by sodium (0.15 g, 6.5 mmol) was then added and the solution was stirred at room temperature. After 1.3 hour TLC (hexane : ethyl acetate 2 : 1, 2% DIPEA) indicated presence of two products and the presence of the starting material. The reaction was stopped by removing sodium and concentration. The residue was chromatographed on a silica gel column (hexane : ethyl acetate 2 : 1, 2% DIPEA) to give 0.440 g (34%) of the α-imidate 57a and 0.452 g (35%) of the β-anomer 57b.

57a : ^1H-NMR (δ, ppm): 8.62 (s, 1H, NH), 7.39-7.30 (m, 5H, aromatic), 6.59 (d, 1H, J₁₂=3.7 Hz, H-1), 5.55 (dd, 1H, J₂₃=10.7 Hz, H-2), 5.38 (dd, 1H, J₃₄=2.9 Hz, H-3), 4.74 (d, 1H, J₆₆=11.5 Hz, CH-Ph), 4.56 (d, 1H, CH-Ph), 4.27 (m, 1H, H-5), 4.20 (dd, 1H, J₅₆=6.6 Hz, J₆₆=11.2 Hz, H-6), 4.14-4.09 (m, 2H, H-4 and H-6'), 2.06 (s, 3H, CH₃CO), 2.02 (s, 3H, CH₃CO), 1.99 (s, 3H, CH₃CO); ^13C-NMR : 170.32 (COCH₃), 170.34 (COCH₃), 170.02 (COCH₃), 160.94, 137.18, 128.55 (aromatic), 128.17 (aromatic), 93.83 (C-1), 75.28
(CH₂-Ph), 74.09 (C-4), 70.65 (C-5), 70.47 (C-3), 67.44 (C-2), 62.29 (C-6), 20.90 (CH₃CO), 20.73 (CH₂CO), 20.57 (CH₃CO); **57b**: ¹H-NMR (δ, ppm): 8.65 (s, 1H, NH), 7.38-7.29 (m, 5H, aromatic), 5.80 (d, 1H, J₁₂=7.8 Hz, H-2), 5.06 (dd, 1H, J₃₄=3.1 Hz, H-3), 4.76 (d, 1H, J₃₄=11.6 Hz, CH₂-Ph), 4.57 (d, 1H, CH₂-Ph), 4.30 (dd, 1H, J₅₆=6.4 Hz, J₆₆=11.2 Hz, H-6), 4.15 (dd, 1H, J₅₆=6.7 Hz, H-6'), 4.00 (dd, 1H, J₄₅=1.5 Hz, H-5), 3.92 (m, 1H, H-5), 2.05 (s, 3H, CH₃CO), 2.01 (s, 3H, CH₃CO), 2.00 (s, 3H, CH₃CO); ¹³C-NMR: 170.31 (COCH₂); 169.04 (COCH₂); 161.17; 137.18; 128.52 (aromatic); 128.36 (aromatic); 128.15 (aromatic); 96.11 (C-1), 75.00 (CH₂-Ph); 73.20, 73.15, 73.08 (C-3, C-4 and C-5); 68.39 (C-2), 61.97 (C-6), 20.79 (CH₃CO), 20.77 (CH₃CO), 20.67 (CH₃CO).

**MPEGDOXyl 2,3,6-tri-O-acetyl-4-O-benzyl-β-D-galactopyranoside (58)**

**Experiment I (using α-imidate)**:

Compound **57a** (52 mg, 96 µmol), MPEGDOXOH (0.101 g, 47.8 µmol), 8.6 µl (38 µmol) of TESOTf (8.6 µl, 38 µmol), CH₂Cl₂ (4 mL) and t-butyl methyl ether (50 mL) were used. The same protocol was followed as in the synthesis of **52** (Experiment II). The polymer was isolated by filtration. The ¹H-NMR spectrum indicates that a 58% yield of the β-glycosyalted product **58** and a 39% yield of acyl transfer product were obtained.

¹H-NMR (δ, ppm): 5.44 (dd, 1H, J₁₂=7.8 Hz, J₂₃=10.4 Hz, H-2β), 4.92 (dd, 1H, J₃₄=3.1 Hz, H-3), 4.30 (dd, 1H, J₅₆=6.2 Hz, J₆₆=11.1 Hz, H-6), 4.10 (dd, 1H, J₅₆=6.8 Hz, H-6'); 5.09 (s, 2H, CH₃-COO-CH₂-); 3.38 (s, 3H, CH₃(PEG))

**Experiment II (using β-imidate)**:

The glycosylation of MPEGDOXOH with compound **57b** (β-imidate) was done using the same protocol and the same amounts of reagents as in Experiment II above. The polymer (0.103 g) was obtained. The ¹H-NMR spectrum indicates that a 52% yield of the β-glycosyalted product **63** and a 43% yield of acyl transfer product were obtained.
Figure 1 Structure of MPEGDOXOH

Average MW = 2117 g/mol
Scheme 1 Synthesis of tribenzylated donors
Scheme 1 Continued
Scheme 2 Synthesis of the 3,6-di-O-benzylated donor and monobenzylated donors
Scheme 3 (Bochkov et al., 1976): Results of glycosylation reactions of 3,4,6-tri-O-acetyl-α-D-glucopyranose 1,2-(cyclohexyl orthoacetate) and 3,4,6-tri-O-methyl-α-D-glucopyranosone 1,2-(cyclohexyl orthoacetate) with cyclohexanol under the conditions shown.
Scheme 4 Bochkov et al. (1976): Participation of 3-O-acetyl group of glucose at C-1 facilitates acyl transfer by promoting the dissociation of acylated acceptor from C-1.
Scheme 5  Inductive effect of benzyl and acetyl groups on charge and participation of the 2-O-acyl group. Inductive donation of electrons by benzyl groups (and/or inductive withdrawal of electrons by acetyl groups) decreases acyl transfer by reducing the charge and/or the strength of the participation of the 2-O-acyl group.
Scheme 6 Interaction between a benzyl group and the positively charged 2-O-acyl group. Electrostatic interaction between the electron-rich π orbitals of the benzyl group and the positively charged 2-O-acyl group prevents acyl transfer by sterically preventing the attack of the acceptor on the 2-O-acyl carbonyl carbon (assumption: the interaction can stabilize the positive charge of the oxocarbonium ion).
Scheme 7 Proposed future work: computer energy minimization calculations. a: calculations to determine whether one of the acyl groups in the most stable structure of ion 1 participates at C-1. b: calculations to see whether the benzyl and acetyl groups inductively affect the charge and/or the strength of the participation of the 2-O-acyl group and also to show whether one of the benzyl groups interacts with positively charged centres of the cation.
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<td>0</td>
</tr>
<tr>
<td>ACYL TRANSFER</td>
<td>63</td>
<td>67</td>
<td>7</td>
</tr>
</tbody>
</table>

ND: Not determined; 2 minor compounds are present (~2% and 3% yield) but only their H-4 peaks are clearly visible on the $^1$H-NMR spectrum.

**TABLE 1** Results of reactions of tetraacetylated and tetrabenzoylated donors with MPEGDOXOH
<table>
<thead>
<tr>
<th>R</th>
<th>FORMYL</th>
<th>ACETYL</th>
<th>LEVULINYL</th>
<th>PIVALOYL</th>
<th>BENZOYL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAL</td>
<td>GAL</td>
<td>GLU</td>
<td>GAL</td>
<td>GAL</td>
<td>GAL</td>
</tr>
<tr>
<td>DONOR ANOMER</td>
<td>α</td>
<td>α</td>
<td>α</td>
<td>β</td>
<td>α</td>
</tr>
<tr>
<td>% β GLYCO-SYLATION</td>
<td>25</td>
<td>64</td>
<td>72</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>% α GLYCO-SYLATION</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>ACYL TRANSFER</td>
<td>56</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE 2**  Results of reactions of tribenzylated galactopyranosyl trichloroacetimidates with MPEGDOXOH
**TABLE 3** Results of reactions of monobenzylated donors and the 3,6-di-O-benzylated donor with MPEGDOXOH
REFERENCES


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Appendix

NMR and Mass Spectra
$^1$H-NMR spectrum

CH$_3$O(CH$_2$CH$_2$O)$_n$-CH$_2$·CH$_2$·OH
$^1$H-NMR spectrum
$^{1}H$-NMR spectrum
\(^{1}\text{H-NMR spectrum}\)

\[\text{23}\]

- OBn
- OBn
- BnO
- OL
- C=NH
- Cl\(_3\)
$^1$H-NMR spectrum

![Diagram of molecular structure with chemical shifts and peaks labeled.]
$^1$H-NMR spectrum
$^1$H-NMR spectrum
$^1$H-NMR spectrum

\[ \text{OAc} \quad \text{O-DOX-MPEG} \]

\[ + \quad \text{Ac-O-DOX-MPEG} \]

\[ + \quad \text{HO-DOX-MPEG} \]
$^1$H-NMR spectrum

Experiment 1

HO-DOX-MPEG

$^{29}$
$^1$H-NMR spectrum

Experiment II
$^1$H-NMR spectrum

Experiment 1
$^1$H-NMR spectrum
1H-NMR spectrum

40

Ac-O-DOX-MPEG

HO-DOX-MPEG

Experiment 1

120
$^1$H-NMR spectrum

Ac-O-DOX-MPEG

Experiment II
$^1$H-NMR spectrum
$^{13}$C-NMR spectrum
13C-NMR spectrum