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Chantal Grand-Maître

Design and applications of active and latent thioglycosyl donors toward the synthesis of the disaccharide repeating unit of *H. pleuropneumoniæ* serotype 4 CPS.

A thesis submitted in partial fulfilment of the requirements for
the degree of Master of Science in the Department of Chemistry
University of Ottawa
Ottawa, Canada

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UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

To my family
and José

ACKNOWLEDGEMENTS

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PREFACE

This research proposal was intended to prepare the repeating disaccharide unit, joined through monophosphate diester linkage, of *Actinobacillus (Hæmophilus) pleuropneumoniæ* serotype 4 capsular polysaccharide (CPS) and enventually prepare the polysaccharide, by solide-phase synthesis, covalently linked to a spacer suitable for conjugation with carrier proteins, for the construction of synthetic vaccine against *H. pleuropneumoniæ* serotype 4 CPS. It was also intended to design and apply the concept of active and latent thioglycosyl donors toward the synthesis of *H. pleuropneumoniæ* serotype 4 CPS.

ABSTRACT

Chapter 1.

This chapter describes, in a schematic manner, various approaches to the synthesis of the repeating disaccharide unit of *H. pleuropneumoniæ* serotype 4 capsular polysaccharide. These approaches utilize Koenigs-Knorr, Helferich and trimethylsilyl triflate (TMSiOTf) glycosidation conditions. Preparations of the appropriately protected glycosyl acceptors are presented together with their attempted glycosylations with glycosyl halides as glycosyl donors.

Chapter 2.

This chapter describes an overview of the literature procedures that use thioglycosides as glycosyl donors. An indirect use of thioglycosides, by conversion into glycosyl halides, and their direct use with promoters such as dimethyl (methylthio) sulfonium triflate (DMTST) and N-bromosuccinimide (NBS) are discussed. A brief overview of the concept of armed and disarmed glycosyl donors is presented. Fraser-Reid's version utilizes n-pentenyl glycosides while Van Boom's version utilizes thioglycosides. Danishefsky's version involves oxidative coupling of glycals as well as the opening of epoxide linkage at the anomeric center to form oligosaccharides. Condensation of disarmed thioglycosides proposed by Van Boom as well as by Fraser-Reid is also presented.

Chapter 3.

Chapter three describes the development of the concept of active and latent thioglycosyl donors which combines the features of armed and disarmed glycosyl donors is presented. Reactivation of latent donor para-nitrophenyl thioglycoside is also proposed.

Chapter 4.

A brief overview of the synthesis of thioglycosides using Lewis Acid catalyzed reaction, trimethylphenylthio.silane and phase transfer catalysis (PTC) are presented. We describe the synthesis of a complex series of thioglycosides under PTC conditions. During this approach we discovered that the use of the lipophilic PTC catalyst tretrabutylammonium hydrogen sulfate (TBAHS) (1 eq.) and ethyl acetate as solvent greatly improve the reaction rates. The yields were also improved for the less soluble thiols. We also found that the use of PTC occured stereospecifically and afforded higher reaction yields then the previously used Lewis acid catalyzed reactions.

Chapter 5.

This chapter presents our approaches to the stereospecific syntheses of 1,2-trans-aryl-1-thio-ß-D-glycobiosides, active and latent thioglycosyl donors, as well as 1,2-trans-ß-D-glycosyl phosphates by PTC.

Chapter 6.

This chapter describes our approaches to the synthesis of the repeating disaccharide unit of *H. pleuropneumoniæ* serotype 4 (CPS) via thioglycosyl donors using different thiophilic promoters such as NBS, DMTST and N-iodosuccinimide (NIS)/trifluoromethanesulfonic acid (TfOH). In our hands these thiophilic promoters were unsuccessful with N-acetamido sugars as glycosyl acceptors.

Chapter 7.

This chapter presents our approaches to verify the concept of active and latent thioglycosyl donors. During these approaches various disaccharides have been prepared. Synthesis of selectively protected glycosyl donors suitable for further coupling reactions or addition to the phosphate tether as in the synthesis of *H. pleuropneumoniæ* serotype 4 (CPS) is also described.

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LIST OF ABBREVIATIONS

Ac	Acetyl
Ac_2O	Acetic anhydride
AcOH	Acetic Acid
ADEPT	Auto DEPT
Anal	Analytical
arom	Aromatic
Bn	Benzyl
BnBr	Benzyl bromide
Bu	Butyl
t-Bu$(Me)_2$SiOTf	Test-butyldimethylsilyl trifluoromethanesulfonate
Bu_4NF	Tetrabutylammonium fluoride
Bz	Benzoyl
BzCl	Benzoyl chloride
Calc	Calculated
Cat	Catalylic
CBz	Benzyloxycarbonyl
CI	Chemical ionization
cor	Corrected
COSY	Correlation spectroscopy
CPG	Controlled porous glass
CPS	Capsular polysaccharide
DEPT	Distortionless enhanced polarization transfer
DMAP	4-Dimethylaminopyridine
DMF	N, N-Dimethylformamide
DMSO	Dimethylsulfoxide
DMTST	Dimethyl (methylthio) sulfonium triflate
DNA	Deoxyribonucleic acid
E	Electrophile
EDG	Electron donating group
EI	Electron impact
eq	Equivalent
Et	Ethyl
Et_3SiOTf	Triethylsilyl trifluoromethanesulfonate
EWG	Electron withdrawing group
Gal	Galactose
Glc	Glucose
H.	Hæmophilus
HETCOR	Heteronuclear correlation NMR spectroscopy
Hz	Hertz
IDCP	Iodonium dicollidine perchlorate
Im	Imidazolin-2-yl
lit	Literature
M	Molecular ion
# M	# Molar
Me	Methyl
MeO	Methoxy
MeOH	Methanol
MHz	Mega Hertz
min	minutes
mol	Mole
m.p	Melting point
M.S.	Mass spectrum
m/z	Mass to charge ratio

NBS.....N-Bromosuccinimide
NIS.....N-Iodosuccinimide
NMR.....Nuclear magnetic resonance
Pent.....Pentenyl
Ph.....Phenyl
PhCHO.....Benzaldehyde
PhSH.....Thiophenol
Piv.....Pivaloyl
PivCl.....Pivaloyl chloride
ppm.....Parts per million
PTC.....Phase transfer catalysis
py.....Pyridin-2-yl
Rf.....Rate of flow
r.t.....Room temperature
S.....Sugar
Sat.....Saturated
TBAHS.....Tetrabutylammonium hydrogen sulfate
TfOH.....Trifluoromethanesufonic acid
THF.....Tetrahydrofuran
TLC.....Thin layer chromatography
TMSiOTf.....Trimethylsilyl trifluoromethanesulfonate
TMU.....1,1,3,3-Tetramethylurea
p-TsOH.....para-Toluenesulfonic acid
U.V.....Ultraviolet
v.....Volume
w.....Weight

INTRODUCTION

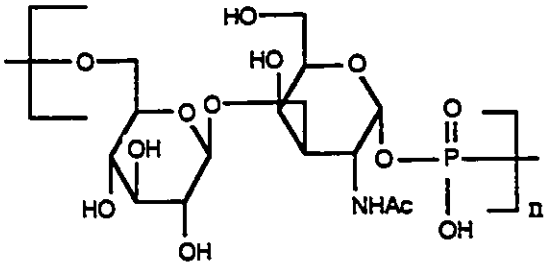
Hæmophilus (Actinobacillus) pleuropneumoniæ is the cause of a highly infectious pneumonia with pleuritis in pigs and is a significant problem in swine-producing areas of the United States and Canada[1-3]. Infections in immunologically naive animals are commonly fatal, and surviving animals are stunted and frequently asymptomatic carriers of *H. pleuropneumoniæ*[4-7]. Presently used methods of controlling this economically important disease have been largely ineffective. Preventing infections by immunization of pigs with *H. pleuropneumonia* bacterins has been only minimally successful. Bacterins provide some serotype-specific resistance to clinical disease but are frequently toxic[8]. Immunization appears to reduce the mortality and the severity of the disease but routinely fails to prevent infection or the occurrence of carrier states[6,9]. In contrast, swines that survive an infection with one serotype of *H. pleuropneumoniæ* develop resistance to reinfection by any of the seven or more other serotypes of *H. pleuropneumoniæ*[5,6].

The organism is encapsulated by a negatively charged, polysaccharides[10] that is serotype specific[11], 10 serotypes, based on capsular polysaccharide antigens, have been recognized. Capsular polysaccharides (CPS) are recognized as important bacterial virulence factors. Capsules enhance bacterial invasion by protecting the bacteria from host defenses[12], in part by inhibiting activation of the complement cascade due to somatic antigens (e.g., lipopolysaccharide)[13]. Therefore, capsules enhance bactericidal serum resistance and prevent phagocytosis in the absence of specific antibody[14-17]. Formation of specific capsular antibody by the host results in opsonization and, for most gram-negative bacteria, *in vitro* bacteriolysis[12]. The presence of antibody specific for capsular polysaccharide is sufficient to protect the host against disease by a variety of bacterial pathogens, including *Hæmophilus influenzæ* type b, *Neisseria meningitidis*, *Streptococcus pneumoniæ*, *Escherichia coli*, and others[12].

The structure of the capsular polysaccharide of *Hæmophilus pleuropneumoniæ* serotype 4 is composed of D-glucose (one part), 2-acetamido-2-deoxy-D-galactose (one part), and phosphate (one part). From hydrolysis, dephosphorylation, methylation, proton-and carbon-NMR studies, the polysaccharide was found to be a high molecular weight polymer of a repeating disaccharide unit, joined through monophosphate diester linkages and having the following structure[18] (scheme 1):

SCHEME 1

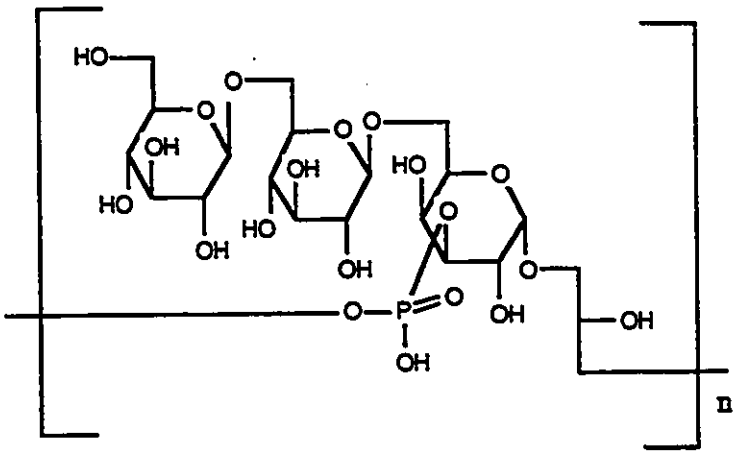
ACTINOBACILLUS (HÆMOPHILUS) PLEUROPNEUMONIÆ SEROTYPE 4



The structure of *Hæmophilus pleuropneumoniæ* serotype 2 (SCHEME 2) consists of repeating tetrasaccharide units which are linked by phosphodiester bonds between the primary HO-3 of glycerol and the secondary HO-3 of D-galactose[19].

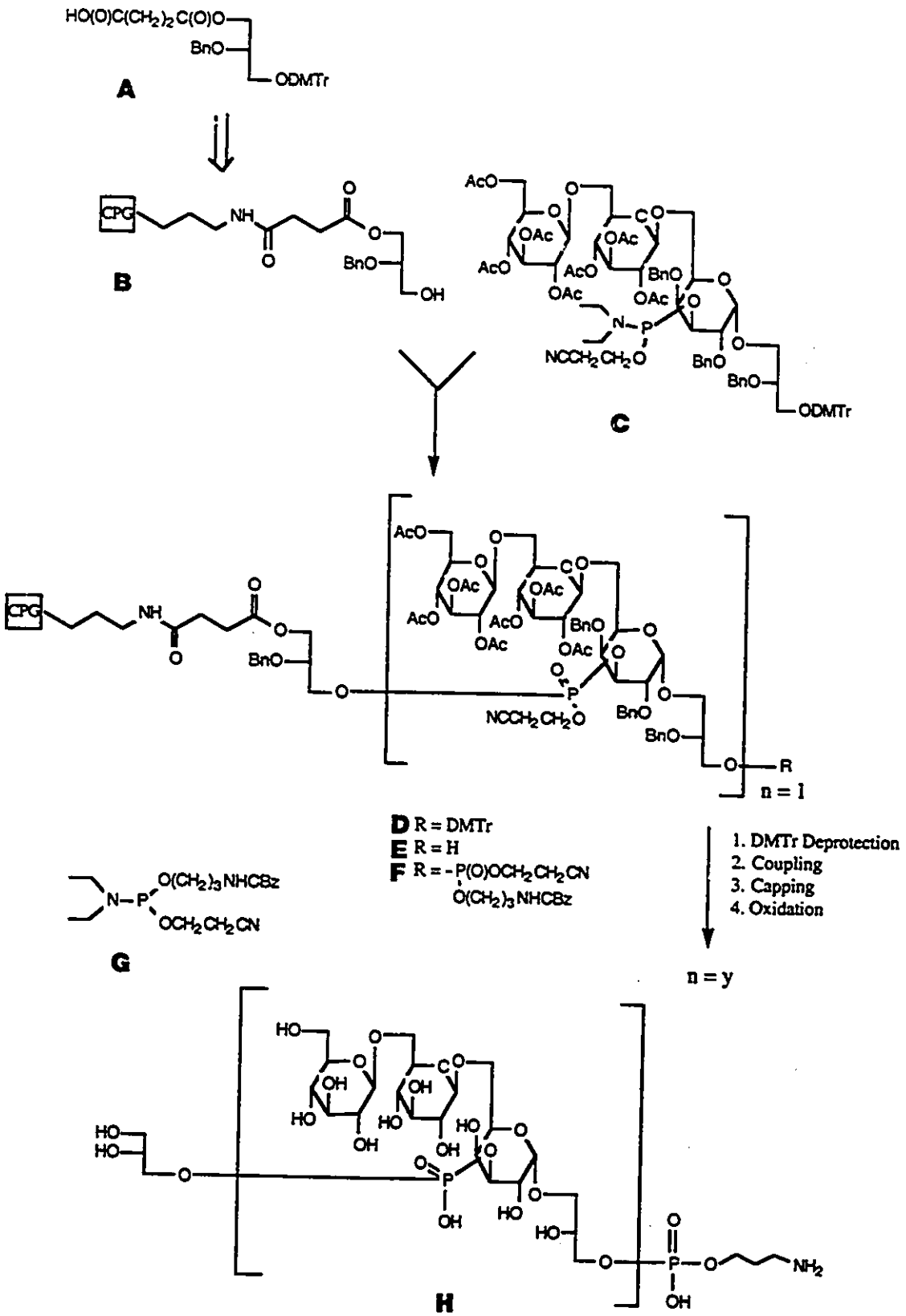
SCHEME 2

ACTINOBACILLUS (HÆMOPHILUS) PLEUROPNEUMONIÆ SEROTYPE 2



Few years ago, Van Boom et al reported[20] the solution synthesis of *H. pleuropneumoniæ* serotype 2 CPS containing two repeating units and a spacer suitable for conjugation with carrier proteins. More recently Van Boom et al reported[21] on a solid-phase synthesis[22] of *H. pleuropneumoniæ* serotype 2 CPS that also contains a covalently linked spacer suitable for conjugation with macromolecular carriers. (Scheme 3). The first step involved preparation of the glycerol unit B attached to a solid support by immobilization of the appropriately protected glycerol unit **A** on aminopropyl-functionalized controlled porous glass (CPG)[23] with diisopropylcarbodiimide and hydroxybenzotriazol to yield immobilized **B**. The second stage involved elongation of **B** with the selectively protected trisaccharide **C** which was achieved by DNA solid-phase synthesis protocol[24], using automated DNA synthesizer. Each following elongation cycle involved 4 chemical steps: detritylation (step 1), coupling (step 2), capping (step 3) and oxidation (step 4). The elongation cycle is then repeated as required. The polysaccharide can then be linked to a spacer by detritylation of **D** and subsequent reaction of **E** with the benzyloxy carbonyl (CBz) protected reagent **G** followed by oxidation to gave immobilized **F**. The final steps involved deprotection procedures to afford the target fragment **H** of *H. pleuropneumoniæ* serotype 2 CPS.

SCHEME 3



The solid-phase approach for the synthesis of the serotype 2 CPS and other[22,25] structurally related teichoic acid-type capsular polysaccharides may also facilitate the synthesis of other naturally occurring teichoic acids, the repeating units of which are linked by phosphodiester bonds. Moreover, the spacer-containing oligomer may open the way to the construction of synthetic vaccine against *H. pleuropneumoniæ* serotype 2 CPS.

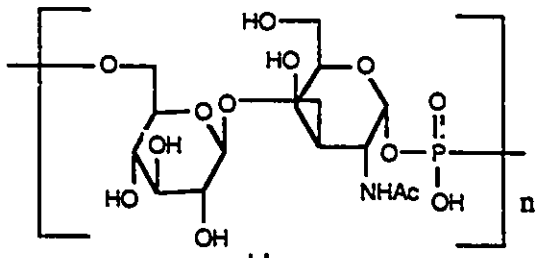
RESULTS AND DISCUSSION

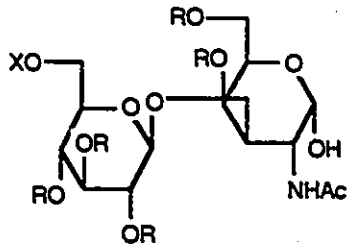
Chapter 1

Approaches to the synthesis of the repeating unit of *H. pleuropneumoniæ* serotype 4 CPS.

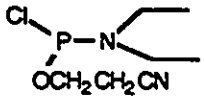
Our approaches, to the synthesis of *H. pleuropneumoniæ* serotype 4 CPS, were based on the retro-synthetic analyses proposed in scheme 4 and 5. As a first disconnection (scheme 4) the phosphate tether can be cleaved to liberate the disaccharide unit **I**. This disconnection suggests that the sixth position, of the non reducing sugar, should be selectively protected for the eventual addition of another disaccharide unit, at the anomeric position, through phosphodiester linkage. Anomerically pure interglycosidic phosphodiester have been reported[26,27] using the monofunctional phosphitylating reagent chloro-ß-cyanoethyl-N,N-diisopropylamino-phosphoramidite **J**. Therefore phosphitylation of the disaccharide **I** would give access to the donor disaccharide **K** ready for the addition of the acceptor disaccharide **L**, selectively deprotected at the sixth position, followed by oxidation to convert the intermediate phosphite to the phosphotriester, the 2-cyanoethyl P-protecting group of which is then removed by a tertiary base. The cycle of deprotection (step 1), coupling (step 2) and oxidation (step 3) can be repeated as required to give the repeating disaccharide **M**.

SCHEME 4

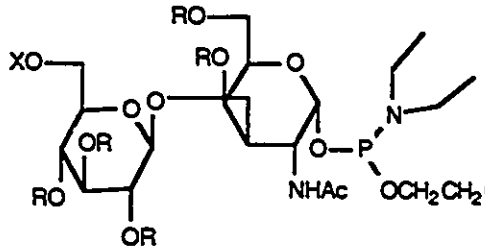




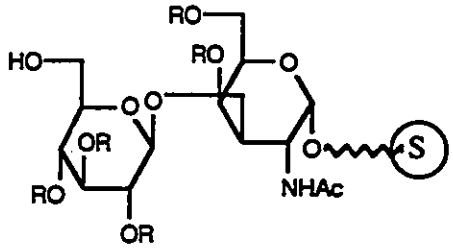
I



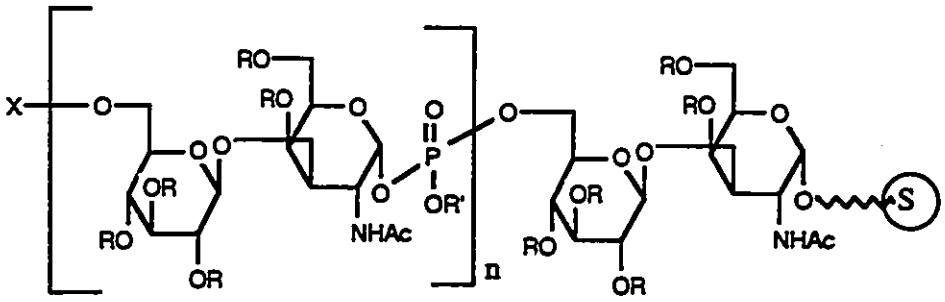
J



K-donor



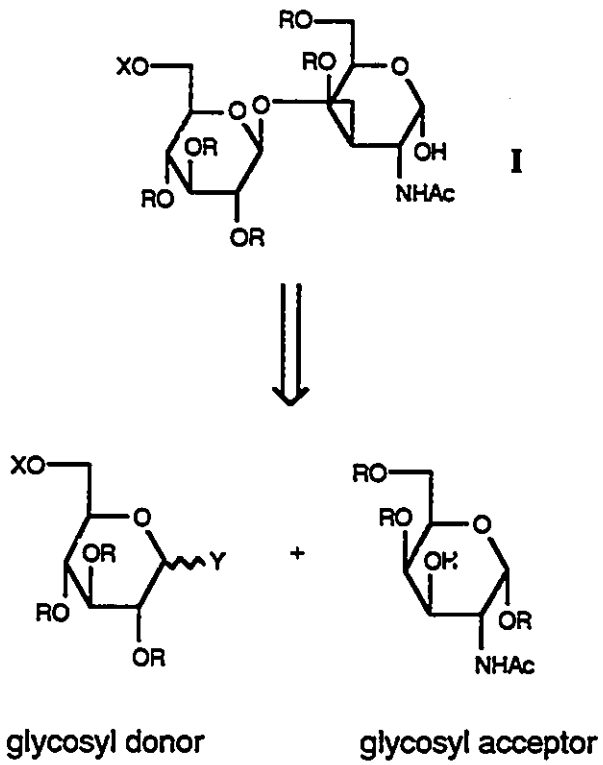
L-accepter



M

The second disconnection that would follow is that of the disaccharide **I**. This disconnection suggests a protected glucose, as glycosyl donor, such that 1,2-trans glycosidic bond can be formed. As glycosyl acceptor a protected N-acetylgalactopyranoside with a free hydroxyl group at position 3 is suggested.

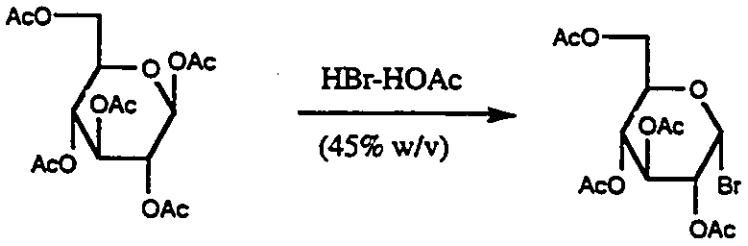
SCHEME 5



Several methods for the preparation of glycosides have been reported[28]. The Fischer methods[28-30], which consists in treatment of a free sugar with an alcohol in the presence of an acid, is particularly suitable for the preparation of lower alkyl glycosides. The classical method for the synthesis of 1,2-trans glycosides is the Koenigs-Knorr method[31]. In this procedure, a glycosyl halide carrying and acyl substituents at O-2 is treated with an alcohol in the presence of a halide ion acceptor ("promoter"), usually a mercury or silver salt. Although silver carbonate or silver oxide is usually used as the acid acceptor in the Koenigs-Knorr reaction, numerous varations and improvements have been reported. Zemplén and coworkers[32-34] used mercury (II) acetate instead of a silver salt. Helferich and coworkers[35-37] used mercury (II) cyanide and mercury (II) bromide. Wulff and Röhle used the silver salts of a number of organic acids.

As glycosyl donor, the glycosyl halide acetobromoglucose **2** was used and prepared in quantitative yield by the standard HBr-HOAc (45% w/v) procedure[38] (scheme 6):

SCHEME 6



1 **2**

The required ß-D-glucopyranose pentaacetate **1** was prepared from anhydrous α-D-glucose in acetic anhydride and anhydrous sodium acetate in 96% yield as a mixture of 88% ß and 12% α anomers. The pure ß-anomer was obtained by recrystallization in 95% ethanol. m.p. 136.2-137.1 °C; lit(39) m.p. 132 °C (cor.). The structure was confirmed by the presence, in the proton NMR sepctrum, of a doublet at 5.69 ppm integrating for one proton with a trans coupling constant for the anomeric hydrogen of 7.8 Hz and by the presence of 5 acetyl groups in the region of 2.09-1.99 ppm.

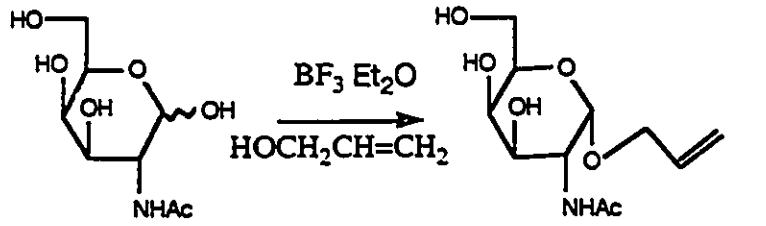
The structure of α-D-acetobromoglucose **2**, m.p. 88.9-89.4 °C; lit (38) m.p. 88-89 °C, was readily assigned on the basis of its spectroscopic data. In particular the ^{1}H-NMR shows a doublet for the anomeric proton at 6.58 ppm with a cis coupling constant of 4.0 Hz. The m/z displayed the expected molecular ions 413 $[M+2]^+$, and 411 $[M]^+$ in the respective ratio of 98%, 100%.

As alcohol acceptor, the allyl α-D- N-acetylgalactopyranoside **3** was prepared[40] (scheme 7). The anomeric position of the aglycon was protected as an allyl group because of its multifunctional transformations and readily cleavage[41,42] to release the reducing oligosaccharide. For example, Hall et al[43] demonstrated that ozonolysis of allyl glycosides generates a terminal aldehydic function that may be linked to protein by reductive amination. In addition, allyl groups also have considerable synthetic utility in that they may be used as TLC tracers[44] as they are selectively detected by the potassium permanganate spray.

The allyl glycosides were prepared according to Fischer glycosidation[40] using allyl alcohol and BF_3Et_2O to give after crystalization 47% of the allyl N-acetylgalactopyranoside **3** m.p.193-194 °C; lit (45) m.p.193-194 °C and 65% for the allyl N-acetylglucopyranoside **4** m.p. 166.2-168 °C; lit (40) m.p.172-174 °C.

SCHEME 7

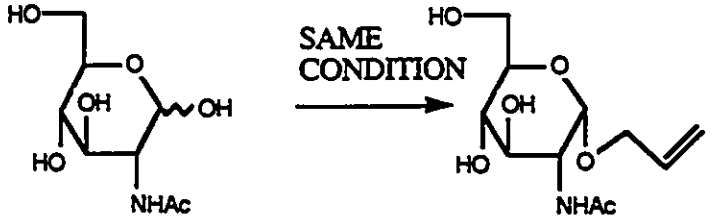
FISCHER GLYCOSIDATIONS



D-GalNAc

47% mp:193-194°C

3



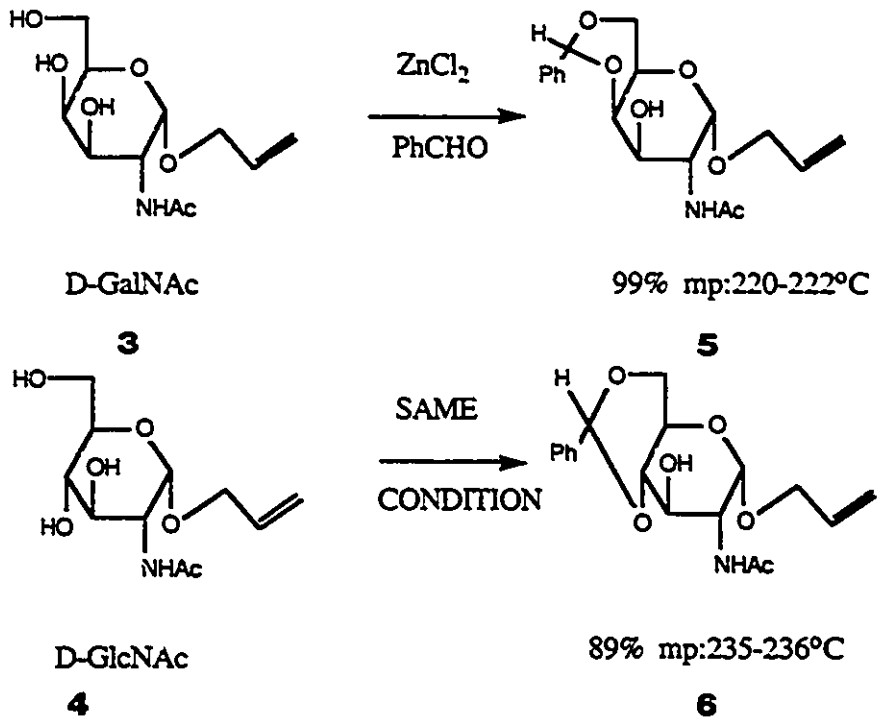
D-GlcNAc

65% mp:166-168°C

4

The structure of the N-acetylgalactopyranoside derivative **3** was confirmed by: the presence of a multiplet at 6.10 to 5.90 ppm integrating for one hydrogen ($\underline{CH}=CH_2$-) and the presence of a multiplet at 5.43-5.25 integrating for 2 protons (-CH=$\underline{CH_2}$). The configuration of the anomeric position was assigned by the cis coupling constant (3.6 Hz) for the doublet at 4.98 ppm corresponding to H-1. The m/z showed a strong molecular ion peak at 262 (100%, [M+H]). The structure of the glucose derivative **4** also contained these expected peaks. The N-acetylglucopyranoside derivative was chosen as a model aglycon because of its low cost compared to the N-acetylgalactopyranoside derivative.

SCHEME 8

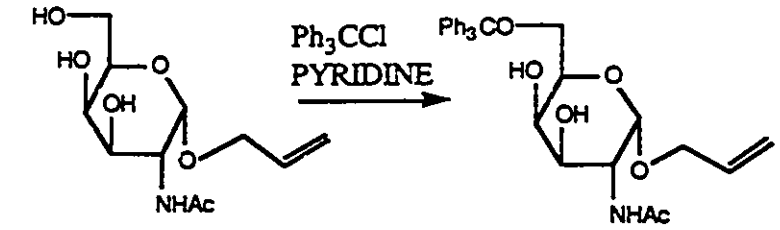


Simultaneous protection of the hydroxyl functions at positions 4 and 6 of galactosamine derivative **3** was accomplished by exposure to benzaldehyde in the presence of zinc chloride (scheme 8). Using these conditions, benzylidene acetal **5** was obtained in near quantitative yield; m.p. 220.7-222 °C; lit (45) m.p. 223-225 °C. The ^{1}H-NMR of N-acetylgalactopyranoside derivative **5** included a multiplet at 7.52-7.31 ppm attributed to the aromatic hydrogens as well as a singlet at 5.55 ppm due to the benzylidene hydrogen. The signal for the anomeric proton was found at 4.98 ppm ($J_{1,2}$ = 3.8 Hz). Using similar conditions to those described above, glucosamine derivative **4** was converted to adduct **6** in 89% yield; m.p. 235-236 °C; lit (44) m.p. 234-237 °C. This material displayed similar spectral characteristics to derivative **5**.

Attempted glycosidations of compounds **5** and **6** with acetobromoglucose in the presence of either silver or mercury salts or TMSiOTf were unsuccessful. The use of thioglycoside **10** (ethyl 2,3,4,6-O-acetyl-1-thio ß-D-glucopyranoside) in the presence of silver triflate and bromine was also unsuccessul.

We then decided to attempt glycosidation using the less hindered glycosyl acceptors **7** and **8** (scheme 9). In these compounds only the primary hydroxyl groups are blocked leaving the hydroxyl groups at positions 3 and 4 free to be glycosidated.

SCHEME 9

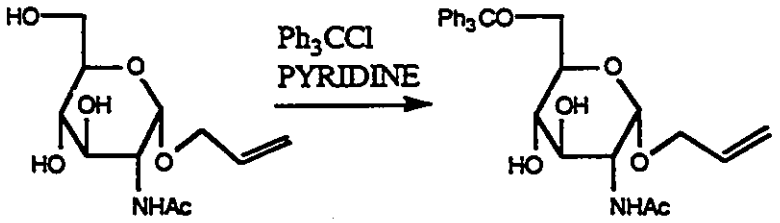


D-GalNAc

3

53% mp:88°C

7



D-GlcNAc

4

42% mp:86-88°C

8

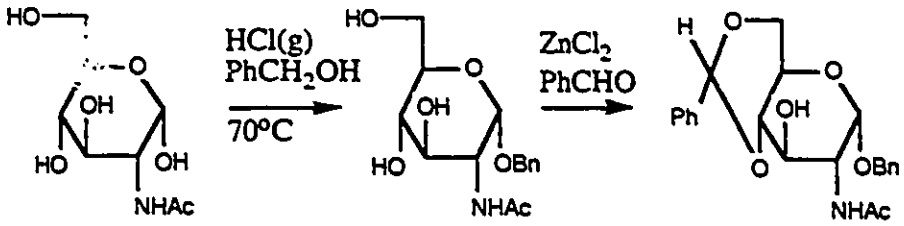
The primary hydroxyl group of compound **3** was protected using chlorotriphenylmethane in pyridine[44]. Unfortunately the trityl group was somewhat labile on chromatography using silica gel and some hydrolysis occured upon separation to give 53% yield of the N-acetylgalactopyranoside derivative **7**. The structure of **7** was confirmed by the presence of 3 phenyl groups (15H) at 7.64-7.15 ppm in the proton NMR spectrum as well as by the presence of a multiplet from 4.39 to 4.20 ppm attributed to the two hydroxyl groups. The anomeric hydrogen was found at 4.87 ppm with a coupling constant of 3.7 Hz.

Under the same conditions, the N-acetylglucopyranoside acetal **8** was obtained in 42 % yield; m.p. 86-88 °C; lit (44) 86-90 °C. These spectroscopic data are in accordance with those found for the corresponding N-acetylgalactopyranoside acetal **7**.

Attempted glycosidation using silver triflate resulted in a poor yield of orthoesters which, on attempt to their rearrangement, gave detritylation and hydrolysis products.

We then decided to replace the allyl glycoside by a benzyl glycoside **9** to increase the solubility of the glycosyl acceptors in the solvents commonly used for the glycosidation. The 4 and 6-O-positions of benzyl 2-acetamino-2-deoxy-α-D-glycophranoside **9.A** were protected using benzaldehyde and zinc chloride[46] to afford a 75% yield of crystalline compound **9** (scheme 10).

SCHEME 10



D-GlcNAc

9.A

75%

9

The structure of compound **9** was determined by the presence, in the ^{1}H-NMR spectrum, of 10 aromatic hydrogens at 7.51-7.29 ppm and by a singlet at 5.54 ppm corresponding to the benzylidene proton as well as by the benzylic protons (doublets) centered at 4.73 and 4.47 ppm with coupling constants of 11.9 Hz. The anomeric proton was found at 4.91 ppm with a characteristic cis coupling constant of 3.7 Hz ($J_{1,2}$).

No glycoside formation occured in the attempted coupling reactions using the Koenigs-Knorr method and unfortunately the solubility was not increased in either benzene/nitromethane or dichloromethane at room temperature. This is in spite of known literature precedent of similar derivatives. We have not identified the source of failure in this particular example.

Chapter 2

Recent glycosydation methods.

I. Indirect and direct use of thioglycosides.

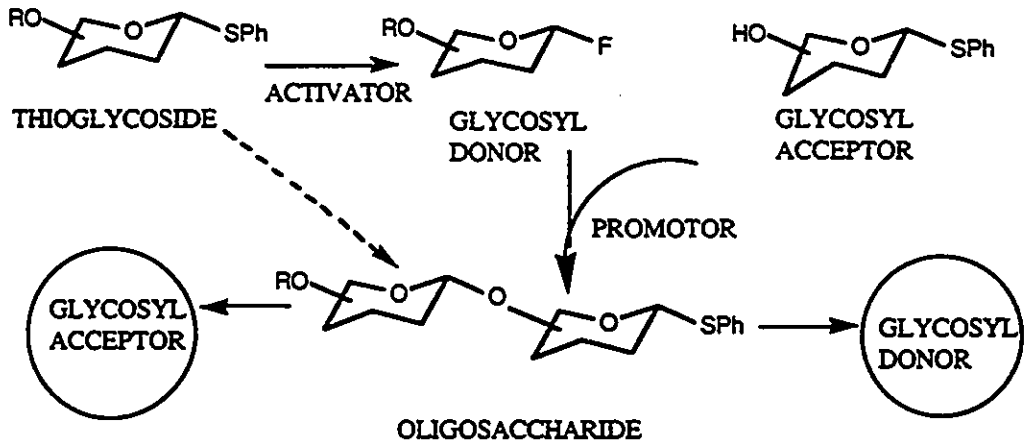
The failure of the Koenigs-Knorr method prompted us to investigate the more recent thioglycosyl donor method.

Thiogycosides are stable and versatile derivatives that allow flexible strategies for the synthesis of complex oligosaccharides[47]. As gycosyl donors, thioglycosides can be activated for glycosilations by conversion into glycosyl halides, usually under mild conditions that are compatible with sensitive protecting groups such as acetals. The glycosyl halides may then be employed in glycoside synthesis using "halophilic" reagents such as silver or mercury salts, or tetraethylammonium bromide[47].

Nicolaou et al advanced a number of years ago a two-stage activation process[48] for oligosaccharide synthesis by combining chemistries of thioglycosides[49] and glycosyl fluorides[50] as outline in scheme 11.

Scheme 11

THE TWO-STAGE ACTIVATION PROCEDURE FOR OLIGOSACCHARIDE SYNTHESIS



Advantages:-Thioglycosides are shelf-stable compounds.
-Glycosyl fluorides can be generated from thioglycosides under mild, neutral conditions in high yield and are stable to chromatography conditions.
-Glycosyl fluorides serve as efficient glycosyl donors under mild conditions.
-Thioglycosides serve as excellent acceptors under the coupling conditions.
-The process is iterative and is adaptable to single unit or block-type synthesis.

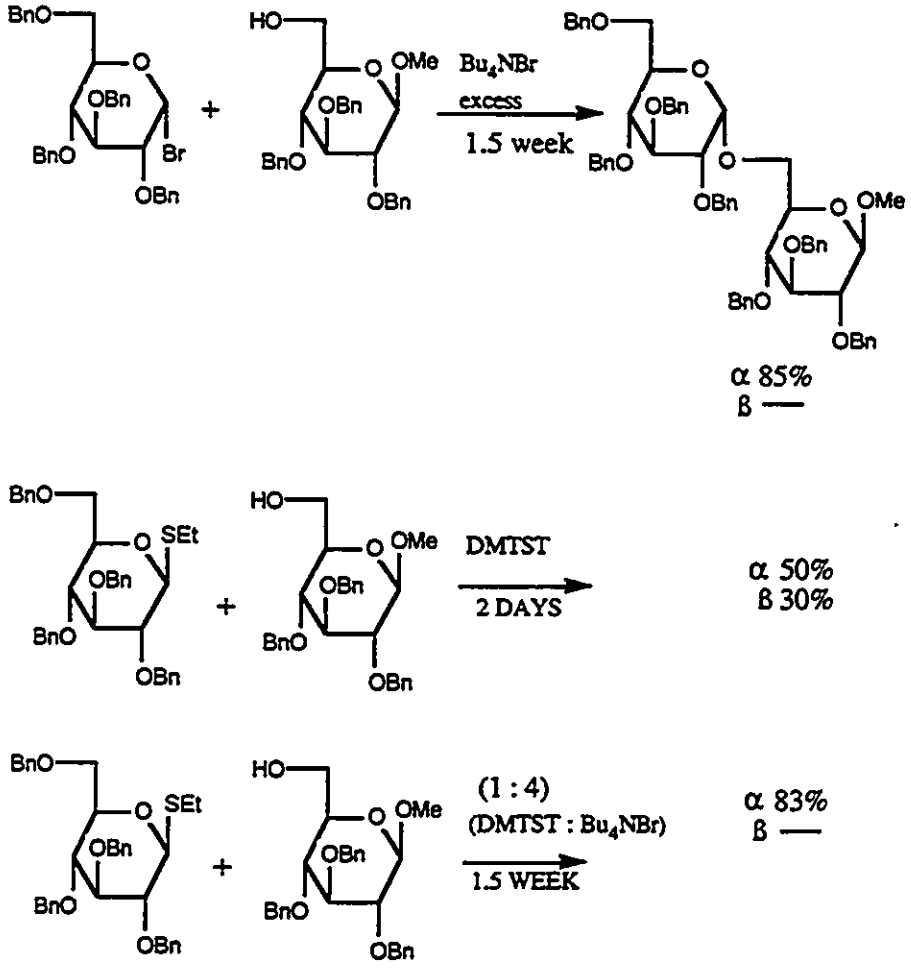
This procedure, involving activation of thioglycosides to glycosyl fluorides under neutral conditions followed by coupling of the resulting glycosyl fluorides to glycosyl acceptors, upon further activation, allows the continuous growth of an oligosaccharide chain without damage to preexisting glycosidic bonds[51].

In complex oligosaccharide synthesis, the block condensation approach has the advantage, over the stepwise addition of one monomer at the time, in that protecting group manipulations are reduced to a minimum[52]. At the same time, however, it necessitates the conversion of oligosaccharides into glycosyl donors, suitable for the construction of glycosidic bonds. By far the most widely used donors are glycosyl halides. The conversion of an oligosaccharide into a glycosyl halide may, however, meet with difficulties, resulting in diminished overall yields[53].

Garegg et al demonstrated that it is possible to obtain stereoselective, halide-assisted, 1,2-cis glycosylations using stable thioglycosides containing a non-participating C-2 substituent as glycosyl donors, in the presence of dimethyl(methylthio)sulfonium triflate and tetrabutylammonium bromide as promoters[54] (scheme 12).

SCHEME 12

HALIDE ASSISTED GLYCOSIDATION



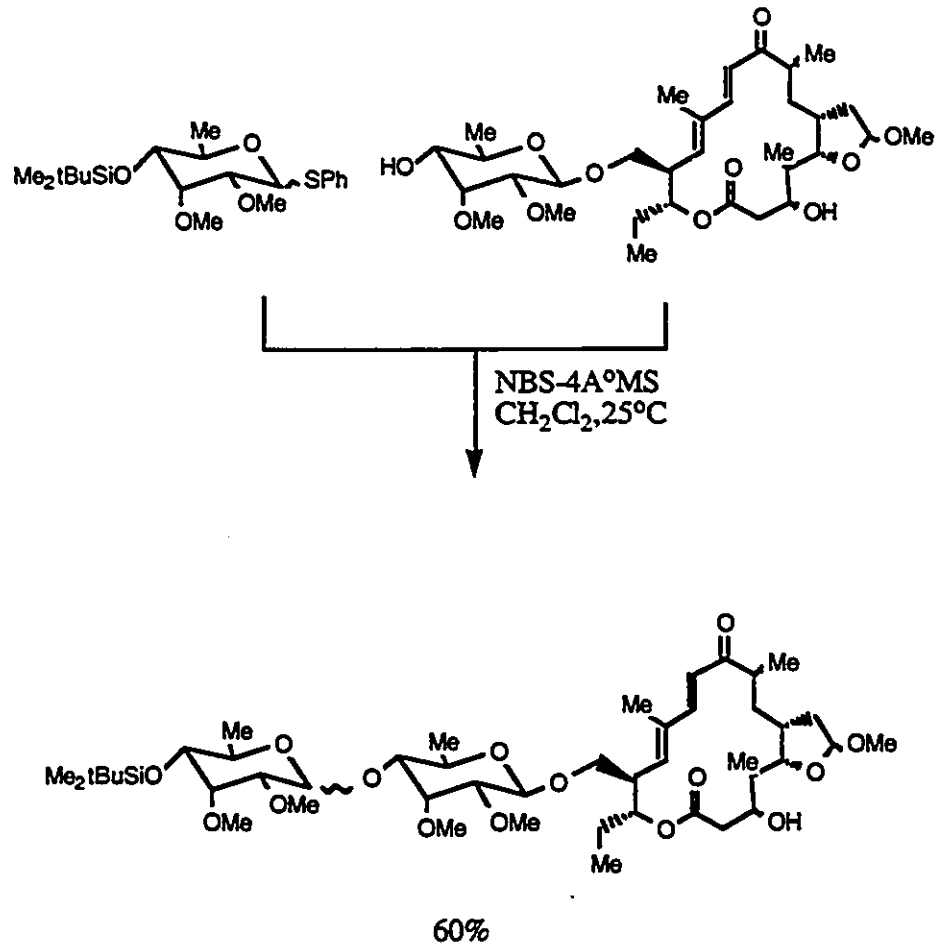
Therefore, it is possible to transfer these reactions into much slower, but highly stereoselective halide-assisted[55] glycosylation.

Thioglycosides may also be used directly as glycosyl donors. Their direct use have been described using a number of thiophilic promoters[47], although complete stereospecificity is only achieved for the 1,2-trans-bonds due to the use of neighbouring group participation from a 2-acyl substituent for controlling the steric outcome of the reaction.

Nicolaou reported a mild and facile procedure that constitutes a convenient and general method for the construction of the O-glycoside bond[56].

SCHEME 13

NBS : A THIOPHILIC PROMOTER



60%

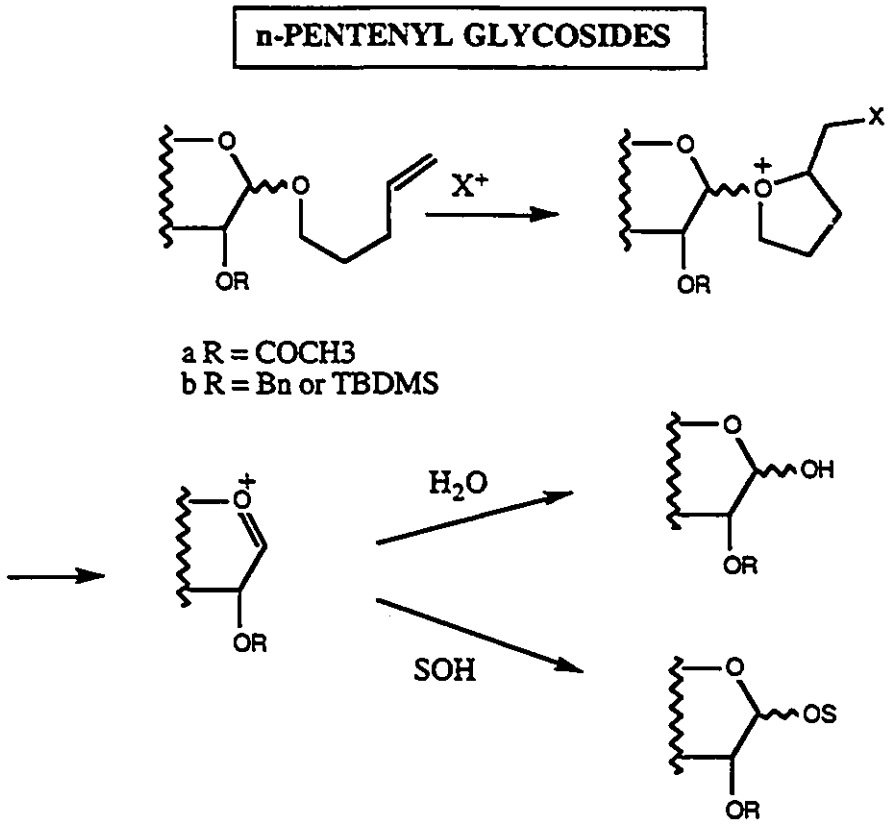
This general method that employs the stable and readily available "armed" (see later) phenyl thioglycoside, which is activated with N-bromosuccinimide, in the presence of the hydroxy component and 4 A° molecular sieves in methylene chloride at 25 °C leads to O-glycosides (α- and ß-anomers) in good to excellent yields, the reaction is complete usually in less than 15 min (scheme 13).

II. Armed and disarmed thioglycosides.

i) Fraser-Reid's version.

Fraser-Reid et al reported that n-pentenyl glycosides undergo chemospecific cleavage with N-bromosuccinimide under conditions that leave a wide variety of other protecting groups unaffected[57].

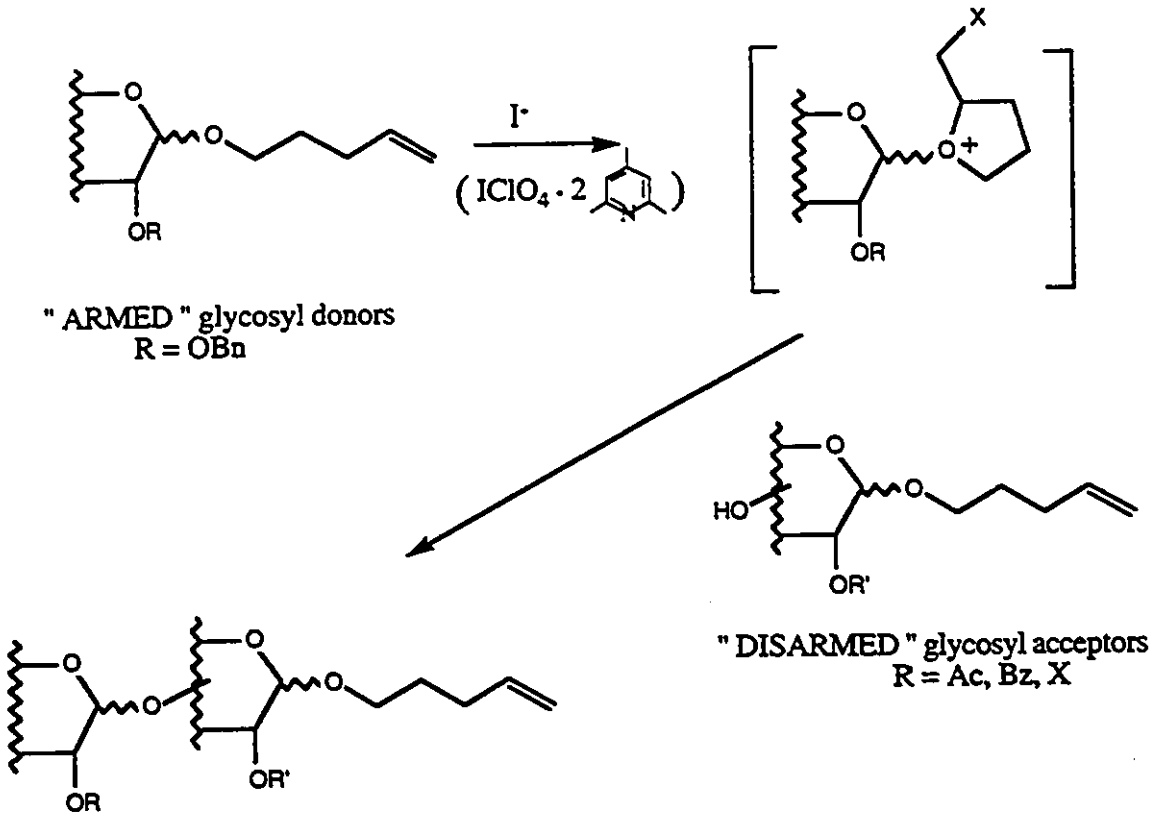
SCHEME 14



According to their proposed mechanism (scheme 14) replacement of water with an alcohol, SOH, should lead to glycoside exchange, a particularly appealing prospect for the synthesis of higher saccharides, where S=sugar[58]. However, the promise of achieving even greater finesse in this exercise emanated from the observation that an ester was hydrolyzed much more slowly than an ether. The latter observations suggested that the pentenyl group could be "armed" (reactive) or "disarmed" (unreactive) by the type of protecting group placed on the C-2 oxygen. The foregoing notion was reduced to pratice, as shown in scheme 15.

SCHEME 15

ARMED and DISARMED Pentenyl Glycosides



Coupling of an "armed" glycosyl donor and a "disarmed" glycosyl acceptor, mediated by iodonium dicollidine perchlorate[59] (IDCP), afforded a good yield of a disaccharide. Therefore, the 2-O-acyl groups had indeed "disarmed" the pentenyl glycoside, thereby ensuring that 2-O-benzyl group served as the only glycosyl donor. Accordingly, there was no evidence for a hexaacyl disaccharide arising from self-condensation of the acyl sugar.

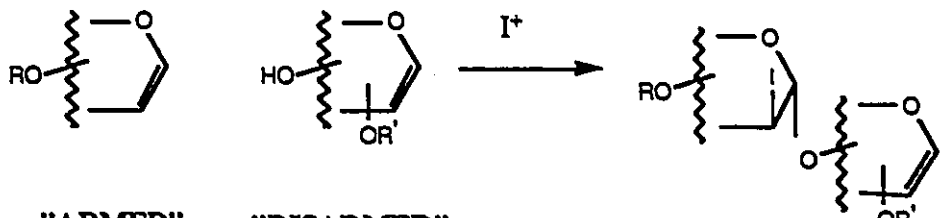
ii) Danishefsky's version.

Danishefsky's version of "armed" and "disarmed" assembly of oligosaccharides involves oxidative coupling of glycals[60] (scheme 16A).

SCHEME 16

DANISHEFSKY'S Version of Armed and Disarmed GLYCALS and EPOXIDES as Glycosyl Donors

A

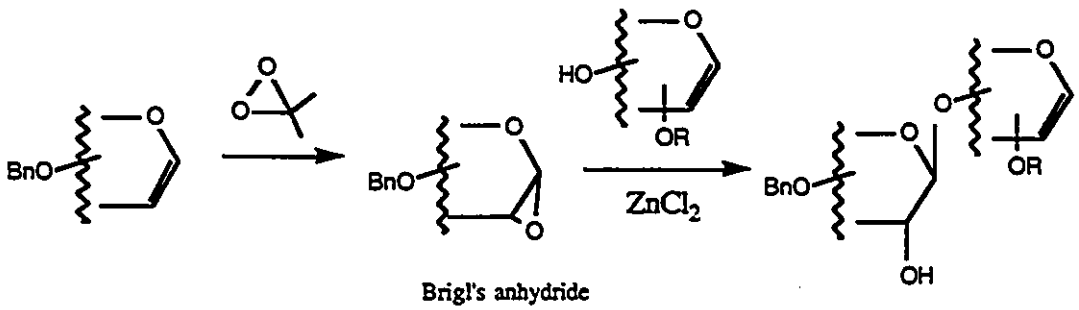


"ARMED" **R = Bn**

"DISARMED" **R' = Ac, Bz**

α-Glycosides (2-deoxy)

B



Brigl's anhydride (1922)

Manipulations at the anomeric centers are unnecessary since coupling is actuated by attack of the oxidant at the donor glycal. Moreover, the next reiteration is straightforward since the disaccharide is itself a glycal, ready for oxidative actuation as before. In principle, however, either glycal can function as a donor. Fortunately, the glycosyl-donating tendencies of the OH-bearing glycal can be suppressed relative to the glycal that lacks a free hydroxyl group. This is accomplished when the intended acceptor containing the free OH group is entered with two acyl protecting groups while the intended donor (no OH groups) is substituted with three ether functions. When a 1:1 mixture of two such glycals is presented to the oxidizing agent, the disaccharide is assembled with strict regiochemical and stereochemical control. To reiterate the scheme, with another glycal, the two ester groups are cleaved and the hydroxyl groups are reprotected as ethers. The coupling reactions, presumably involving a 1,2-iodonium ion intermediate, occur in a clean 1,2-diaxial fashion to afford only the α-glycoside.

Danishefsky also reported on the direct epoxidation of glycals: application of a reiterative strategy for the synthesis of ß-linked oligosaccharides[61]. In a study of the use of glycals as starting materials for glycoside formation, they have considered the direct epoxidation of glycals that would subsequently reacts with a nucleophile (scheme 16B). They have evaluated the use of anhydrous 3,3-dimethyldioxirane as an epoxident[62]. The byproduct, acetone, generated from a successful reaction, would not be expected to react with the 1,2-anhydro sugar. Reaction of tri-O-acetylated glucal with dimethyldioxirane followed by solvolysis in methanol afforded a mixture of products, which was not separated. They reasoned that selectivity might be improved both in epoxidation and in subsequent glycosidation reactions if the protecting groups were of a nonparticipatory nature. They have examined the usefulness of such oxiranes as glycosyl donors in the construction of oligosaccharides. They have used anhydrous zinc chloride as the catalyst in tetrahydrofuran from -78 °C to room temperature for 24 h. In this case, the glycosylation reaction was essentially stereospecific with formation of a ß-glycosidic bond. In addition to their utility in oligosaccharide construction, the 1,2-anhydro sugars synthesized as described above, will be useful glycosylating agents for the formation of lipid conjugates.

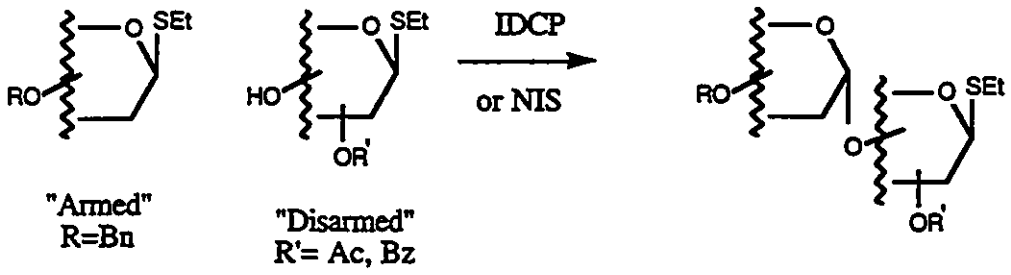
Therefore, Danishefsky and co-workers have described a high-yield, one-step conversion of glycals to 1,2-anhydro sugars. They have also demonstrated that the epoxide linkage at the anomeric center can be displaced with clean inversion of configuration to form oligosaccharides and other conjugates with ß-glycosidic linkages at temperatures as low as -78 °C.

iii) Van Boom's version.

Van Boom's version of "armed" and "disarmed" was anticipated, in analogy with the observed rapid, IDCP promoted, hydrolysis of n-pentenyl glycosides having at C-2, an ether instead of an ester substituent, that a similar difference in rate would also prevail in the case of thioglycosides[63]. This can also be effected with NIS (scheme 17).

SCHEME 17

VAN BOOM'S Version of Armed and Disarmed Thioglycosides



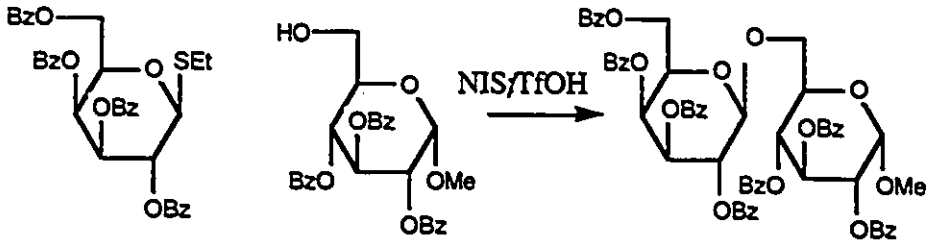
Another interesting and appealing aspect of the in-situ iodonium-ion-promoted thioglycoside approach, is the feasibility to elongate the disaccharide containing a "disarmed" thioglycoside at the reducing end by Zemplén's deacylation and subsequent benzylation to give the "armed" disaccharide ready to be activated. The high chemospecificity of the thiophilic promoter IDCP for "armed" thioglycosides, enables them for the first time to condense a "disarmed" thioglycoside directly with an "armed" thioglycoside resulting in oligosaccharides containing predominantly α-glycosidic bonds.

III. Condensation of disarmed thioglycosides.

It is well-known that the presence of a participating 2-acyl substituent in the donor molecule results in the formation of 1,2-trans linked glycosides. On the basis of this knowledge, together with the availability of effective thiophilic promoters[64,65], it is to be expected that the "disarmed" thioglycosides are also valuable synthons for the introduction of 1,2-trans linkages in a growing oligosaccharide chain. Van Boom et al demonstrated that the use of NIS and catalytic amount of TfOH can promote the condensation of "disarmed" thioglycosides with acceptor molecules and can give access to ß-linked disaccharides[66] (scheme 18).

SCHEME 18

THIOPHILIC ACTIVATION OF "DISARMED" DONORS

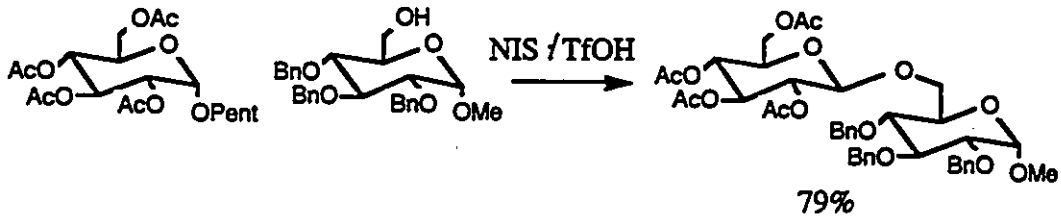


98%
30sec

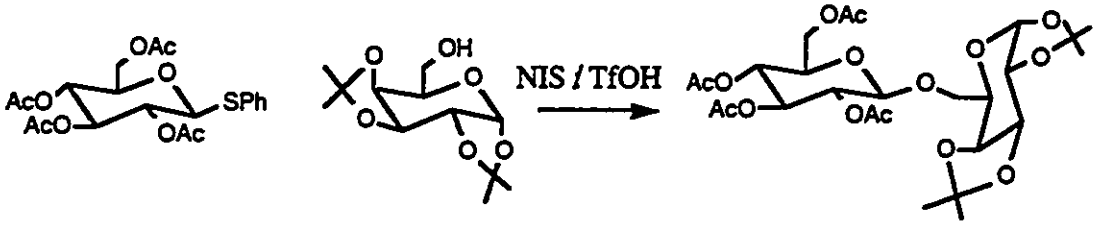
At the same time, Fraser-Reid et al also showed the usefulness of NIS/TfOH to induce the reaction of both "disarmed" n-pentenyl glycosides[67] and thioglycosides[68] (scheme 19).

SCHEME 19

IODONIUM ION INDUCED REACTION OF DISARMED n-PENTENYL GLYCOSIDES AND THIOGLYCOSIDES



79%



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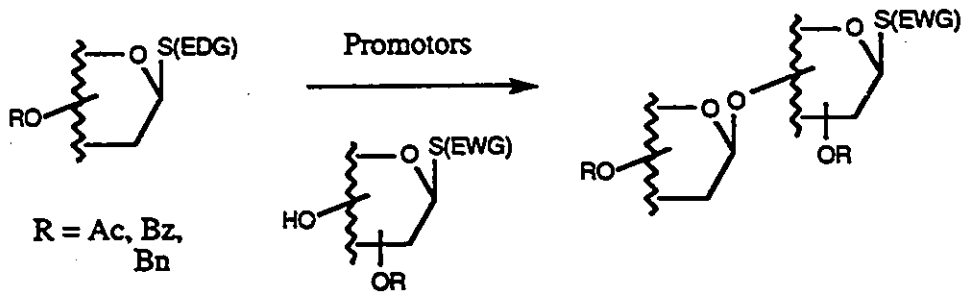
Chapter 3.

I. Active and latent thioglycosides.

We have designed a conceptually new approach which combines the concepts of "armed" and "disarmed" to that of "active" and "latent" thioglycosyl donors (scheme 20).

SCHEME 20

ACTIVE and LATENT THIOglycosyl DONORS



"ACTIVE" DONORS | **"LATENT" or "PASSIVE" Acceptors** | **Oligosaccharides (Re-Activate)**

We anticipated that the use of electron donating groups (EDG) on aryl thioglycosides, readily prepared under PTC conditions (see later), should increase the nucleophilicity of the sulfur atom, and should therefore act as "active" donors by thiophilic promoters while the use of electron withdrawing groups (EWG) should act as "latent" donors toward defined thiophilic promoters.

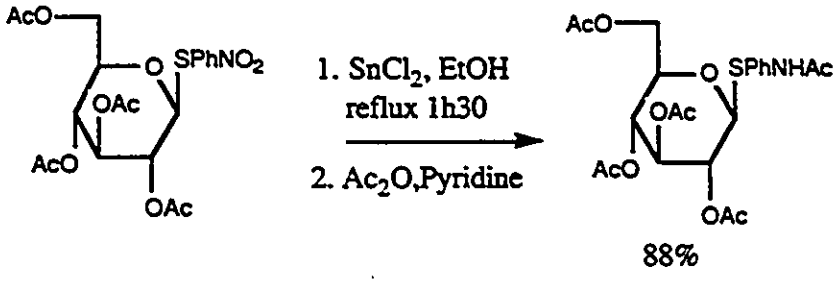
Interestingly, in this approach, the thioglycosyl donors can be designed to produce ß-linked disaccharides using neighbouring groups participation, or predominantly α-linked disaccharides by the use of non-participating 2-ether protecting groups. This methodology should therefore combine the advantages of the "armed" and "disarmed" approaches together with allowing a wide range of reactivities controlled by both the nature of the EDG/EWG groups and by the choice of mild/strong promotors (NBS, NIS, NIS/TfOH).

II. Reactivation of latent thioglycosides.

As for the other approaches of "armed" and "disarmed" glycosyl acceptors and donors, the disaccharide activation can be reiterated by conversion of the "latent" thioglycoside to an "active" thioglycosyl donor. For example, the "latent" para-nitrophenyl thioglycoside **14**, containing the electron withdrawing group (NO_2), had been converted to the "active" glycosyl donor, N-acetylphenyl thioglycoside **18**, containing the electron donating group (NHAc), by reduction with tin (II) chloride dihydrate followed by acetylation in 88 % overall yield (scheme 21).

SCHEME 21

RE-ACTIVATION OF "LATENT" THIOGLYCOSIDES



LATENT **14**

ACTIVE **18**

The structure of the newly reactivated donor has been confirmed by the additional N-acetyl group at 2.12 ppm, in its proton NMR spectrum, corresponding to the NHAc functionality and by the presence of a singlet at 7.80 ppm for the NH group. The anomeric hydrogen is found at 4.57 ppm, a doublet, with a trans-coupling constant of 10.1 Hz. A complete characterization of compound **18** is detailed in the experimental section.

Chapter 4

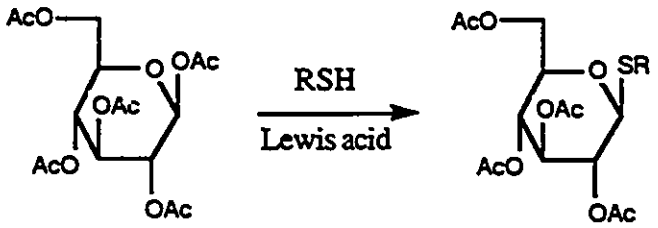
Synthesis of thioglycosides.

i) Lewis acid.

The preparation of a series of thioglycosides was then required in order to verify the concept of "active" and "latent" thioglycosyl donors. A wide variety of methods[69-71] are known for the synthesis of 1,2-trans-thioglycosides, of which the Lewis acid catalyzed reaction[69] between 1,2-trans-glycosyl acetates and thiols appears to be the most common (scheme 22). However, due to undesired side-reactions (formation of mixtures of α and ß anomers[69] as well as formation of dithioacetals[72]) this method sometimes gives yields which are not always satisfactory.

SCHEME 22

LEWIS ACID CATALYZED THIOGLYCOSIDE FORMATIONS



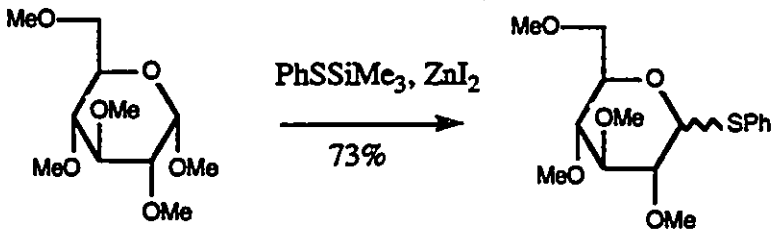
LEWIS ACID : BF_3, $SnCl_4$, $ZnCl_2$, $FeCl_3$

ii) Trimethylphenylthiosilane.

Thioglycosides have also been prepared from their corresponding O-alkyl glycosides[71] (scheme 23). The reaction consists of the treatment of a glycoside or saccharide derivatives with trimethyl [methyl (or phenyl) thio] silane[73] in the presence of zinc iodide which leads to a mixture of anomeric thioglycosides.

SCHEME 23

DIRECT CONVERSION OF GLYCOSIDES INTO THIOGLYCOSIDES



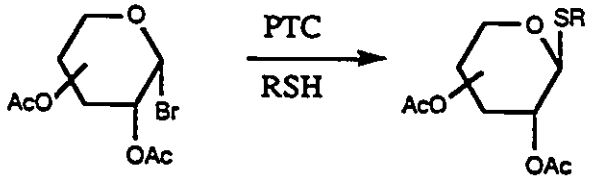
α: ß
2 : 1

iii) Phase transfer catalysis.

The recent use of phase transfer catalysis (PTC), by a Polish group[74], for the synthesis of simple alkyl and phenyl thioglycosides prompted us to attempt synthesis of a complex series of aryl-thioglycosides (scheme 24).

SCHEME 24

PTC Stereospecific Thioglycosides Synthesis

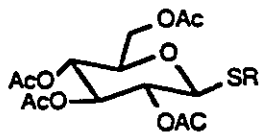


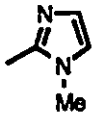
1,2-trans

The Polish method[74] involves the addition of mercaptans to a two-phase system consisting of an aqueous solution of sodium hydroxide and a benzene solution of glycosyl bromide as well as of 25 mol. % tetraalkylammonium salt as catalyst. This method provides high yields of stereospecific 1,2-trans thioglycosides.

Table 1- PTC synthesis of 1-thioglycosides from acetobromoglucose.

THIOGLYCOSYL DONORS



		Benzene		Ethyl acetate		Lewis acid
cpd #	R	reaction time	(yield) %	reaction time	(yield) %	(yield) %
10	SEt	———		———		(70)
11	Ph	20 min	(98)	15 min	(80)	(69)
12	p-MeO-Ph	48 h	(98)	15 min	(92)	
13	p-Me-Ph	12 h	(83)	15 min	(88)	
14	p-NO_2-Ph	4 days	(62)	45 min	(91)	
15	SC(S)OEt	<24h	(44)	3 h	(46)	
16		>24h	(80)	1 h	(92)	
17		48 h	(58)	24 h	(47)	

We first attempted PTC on a series of thioglycosides **11-14, 16** involving the addition of acetobromoglucose **2** (1 eq) to a two-phase system consisting of an aqueous solution of sodium hydroxide (1M) and a dichloromethane solution of the appropriate thiol (1.2 eq) and an equimolar ratio of tetrabutylammonium hydrogen sulfate (TBAHS). This procedure suffered from high contamination of the reaction mixture with products of the substrate deacetylation. The sole exception in this series was the para-methylphenyl thioglycoside **13** which was isolated in 88 % yield after a reaction time of 20 min.

We have therefore decided to prepared the series of thioglycosides **11-17** according to the Polish method (method A) which resulted in good to excellent yields. We have found that the use of one equivalent of tetraalkylammonium salt and the replacement of benzene by ethyl acetate (method B) greatly improve the reaction time. The yield was especially increased for the less soluble para-nitrothiophenol **14** and 2-mercaptopyridine **16** as indicated in table 1.

In the case of ethanethiol **10**, it was reported by the Polish group that only 50% of the expected thioglycoside was isolated and evaluation of the reaction mixture (TLC) showed that it was contaminated with products of the substrate deacetylation. We therefore prepared the ethyl thioglycoside **10** using the Lewis acid catalyzed reaction[66]. We can

observed that in the case of thiophenol **11** the use of PTC conditions afforded much higher yields of the 1,2-trans thioglycosides compared to Lewis acid catalyzed method (table 1). Moreover phase transfer catalysis afforded stereospecific glycosidation products.

The structure of the thioglycosides **10-17** were established by ^{1}H and ^{13}C-NMR spectracopy. The complete assignments of all protons and carbons were confirmed by COSY (figure 1,3) and HETCOR (figure 2). The proton NMR spectrum of the ethylxantate thioglycoside **15** is shown in figure 4. Table 2 includes selected ^{1}H-NMR chemical shifts and coupling constants. Table 3 describes the ^{13}C-NMR data. A complete analysis of these thioglycosides **10-17** can be refered to in the experimental section.

Table 2. Selected ^{1}H-NMR chemical shifts (ppm) and coupling constants (Hz) for the thioglycosides **11-18**.

Compound no.	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$, $J_{5,6'}$)	H-6 ($J_{6,6'}$)	H-6'
10[a]	4.46 (9.9)	5.00 (9.1)	5.20 (9.3)	5.05 (9.8)	3.68 (2.5, 4.8)	4.09 (12.4)	4.22
11[a]	4.68 (10.0)	4.95 (9.1)	5.20 (9.2)	5.02 (9.9)	3.70 (3.0, 4.7)	4.19-4.16 -	4.19-4.16
12[a]	4.52 (10.0)	4.86 (9.2)	5.17 (9.3)	4.97 (10.0)	3.65 (3.7, 3.7)	4.16 -	4.16
13[a]	4.60 (10.0)	4.91 (9.2)	5.18 (9.3)	5.00 (9.8)	3.67 (3.1, 4.3)	4.18-4.16 -	4.18-4.16
14[a]	4.83 (10.0)	5.00 (9.0)	5.24 (9.3)	5.03 (10.0)	3.79 (2.9, 5.0)	4.27-4.09 -	4.27-4.09
15[a]	5.43 (10.4)	5.14 (9.0)	5.29 (9.3)	5.08 (9.9)	3.80 (2.2, 4.8)	4.08 (12.5)	4.22
16[a]	5.80 (10.2)	5.11 (9.2)	5.32 (9.2)	5.17 (9.9)	3.84 (2.2, 4.7)	4.05 (12.4)	4.23
17[b]	4.86 (10.1)	4.95 (9.1)	5.13 (9.2)	4.96 (10.0)	3.57 (2.5, 4.9)	4.01 (12.4)	4.08
18[b]	4.57 (10.1)	4.87 (9.2)	5.16 (9.4)	4.96 (9.9)	3.65 (2.9, 4.4)	4.15-4.13 -	4.15-4.13

[a] At 200 MHz in $CDCl_3$
[b] At 300 MHz in $CDCl_3$

Table 3. Selected ^{13}C-NMR chemical shifts (ppm). (75.4 MHz, $CDCl_3$)

Compound no	C-1	C-2	C-3	C-4	C-5	C-6
10	83.44	69.75	73.83	68.25	75.79	62.09
11	85.65	69.85	73.88	68.13	75.72	62.06
12	85.57	69.78	73.98	68.07	75.64	61.98
13	85.75	69.83	73.94	68.12	75.67	62.05
14	84.27	46.50	52.49	47.54	56.09	40.60
15	85.82	68.45	73.97	68.00	76.69	61.77
16	81.50	69.37	74.06	68.21	75.81	61.88
17	85.95	70.04	73.62	68.00	75.77	61.75
18	85.71	69.83	73.93	68.09	75.74	62.04

Figure 1 COSY spectrum of the phenyl thioglycoside **11** in $CDCl_3$

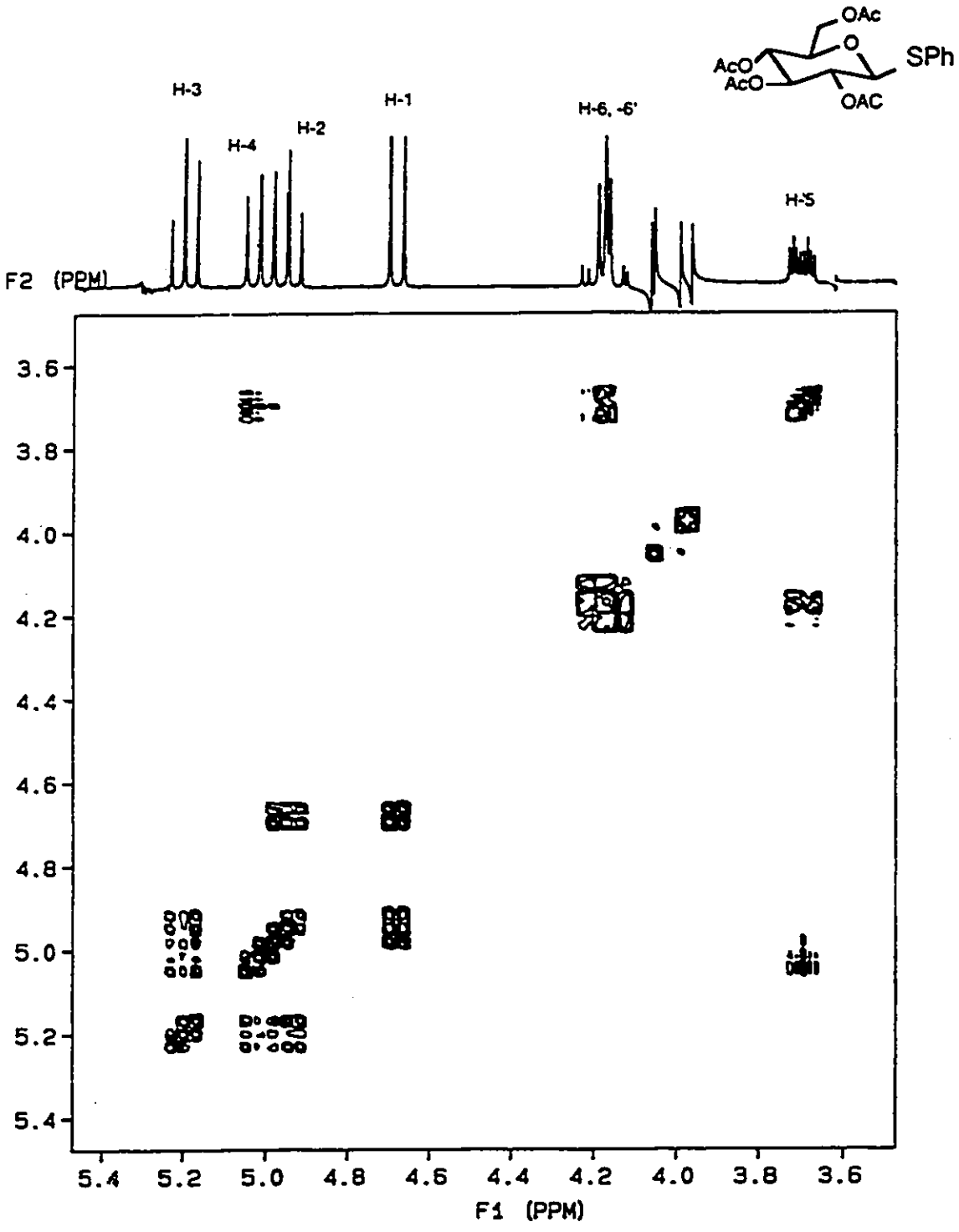


Figure 2 HETCOR spectrum of the phenyl thioglycoside **11** in $CDCl_3$

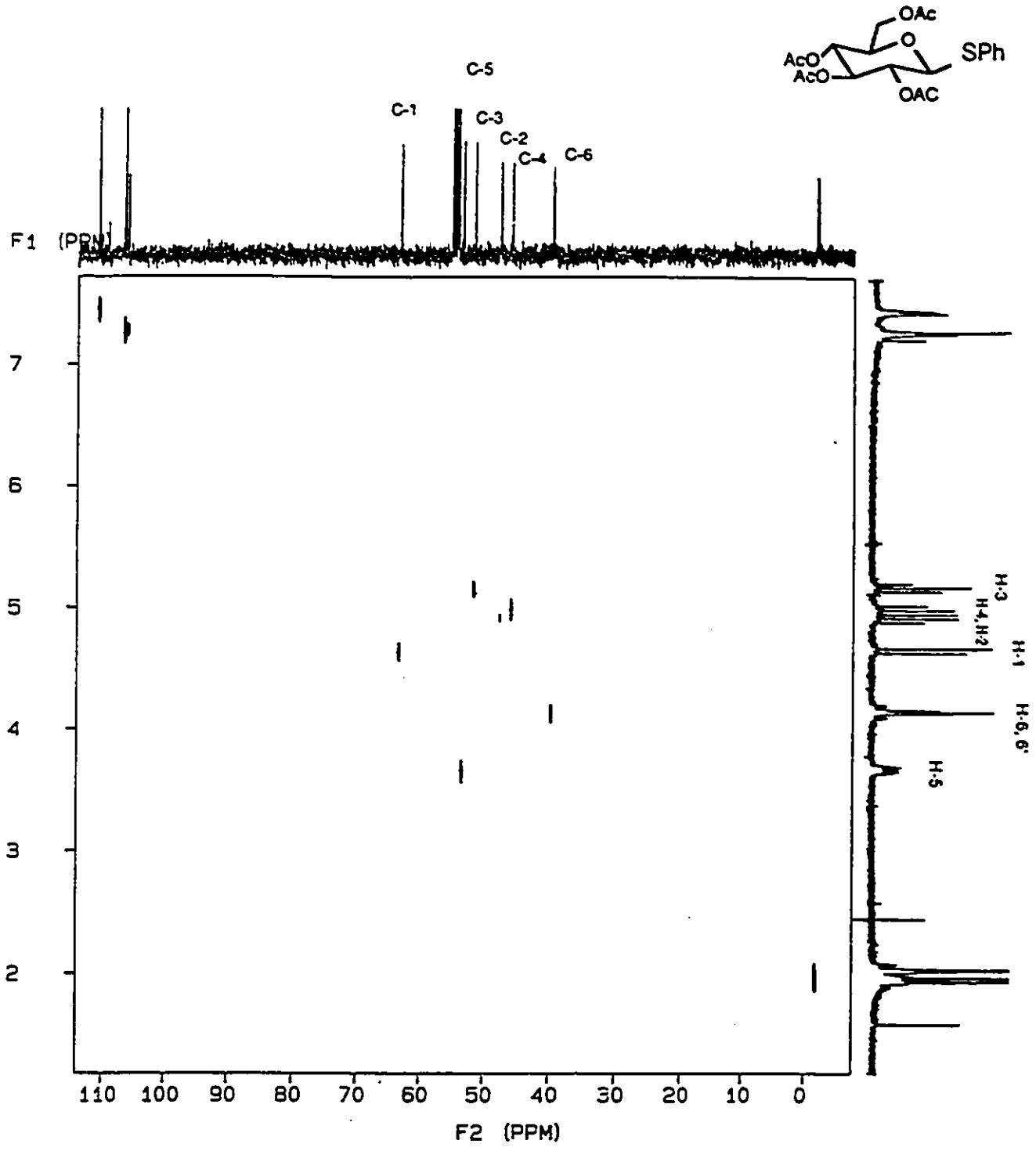


Figure 3 COSY spectrum of the imidazolin-2-yl thioglycoside **17** in $CDCl_3$

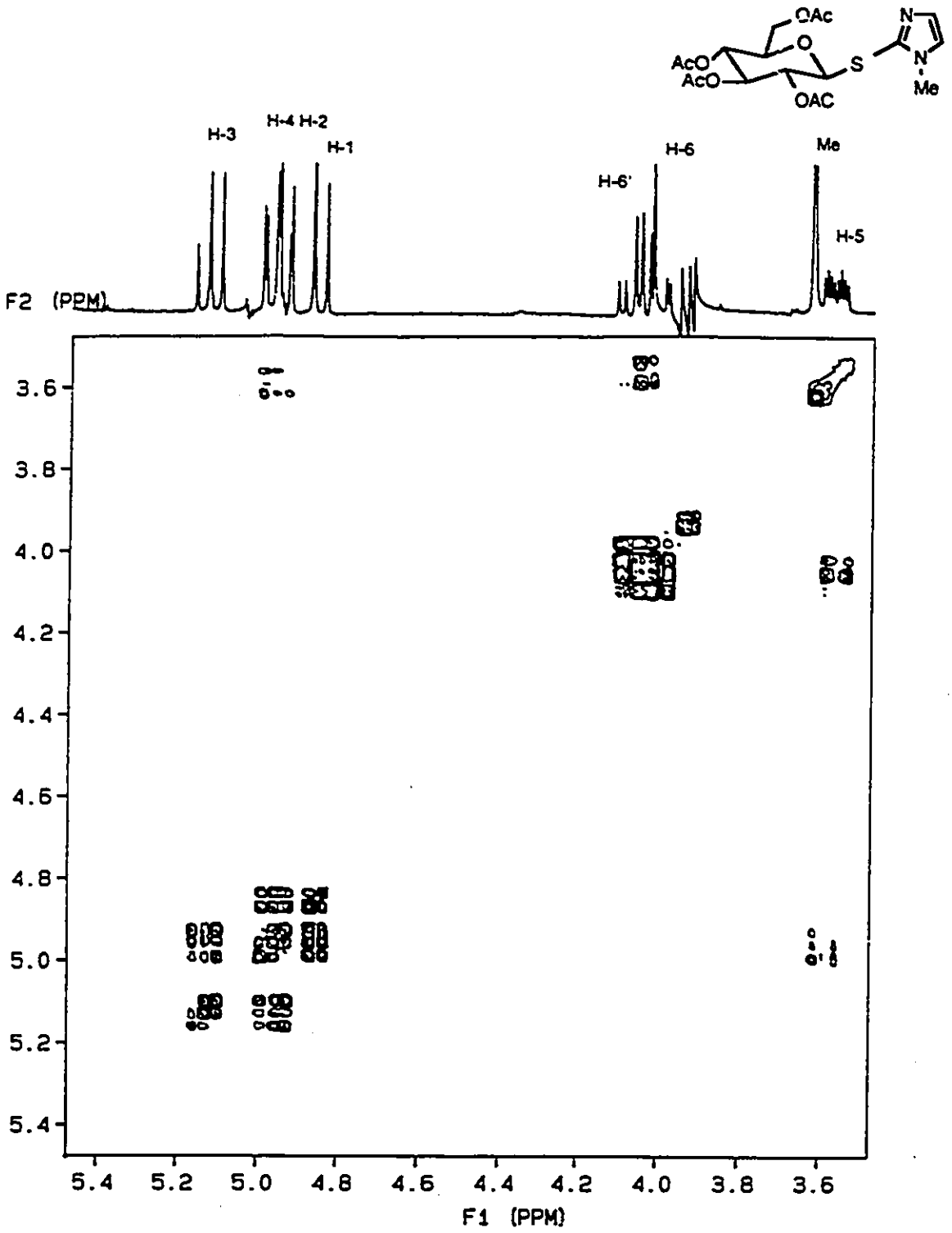
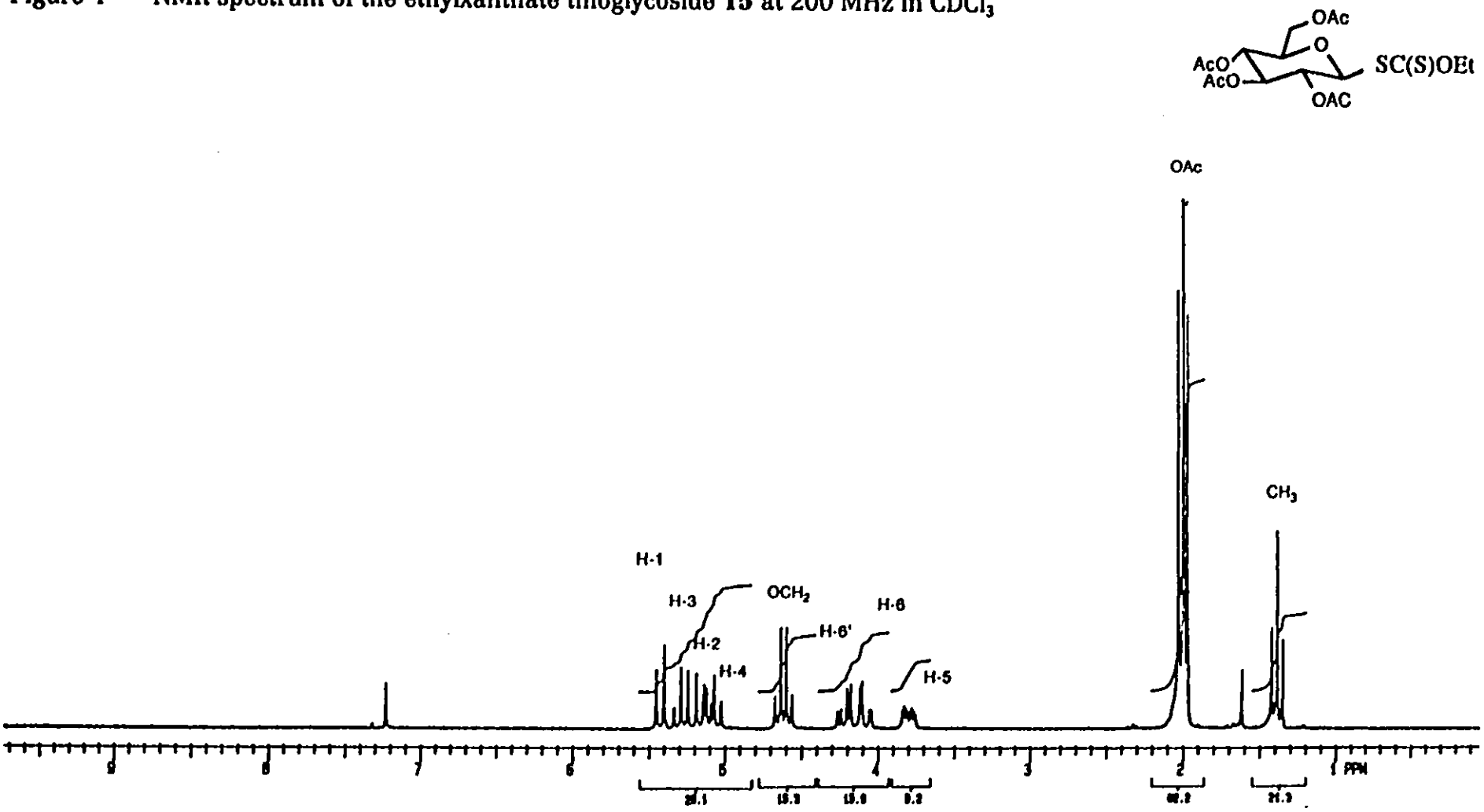


Figure 4 NMR spectrum of the ethylxanthate thioglycoside **15** at 200 MHz in $CDCl_3$



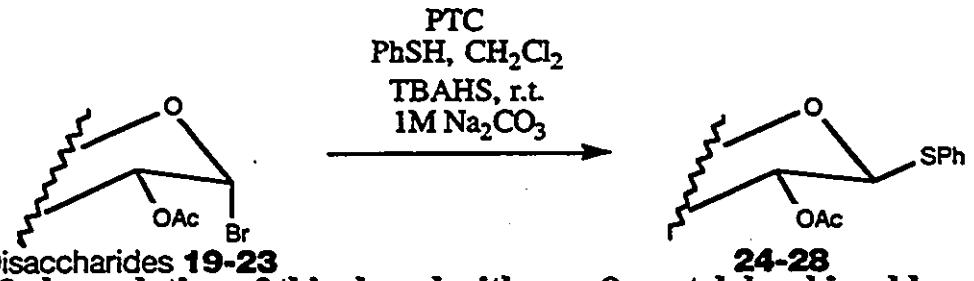
Chapter 5
Phase transfer catalysis.
i) 1,2-trans-Aryl-1-thio-ß-glycobiosides.

The success of the PTC conditions for the synthesis of stereospecific 1,2-trans aryl-1-thio-monosaccharides prompted us to attempt for the first time, the reaction of thiophenols on per-O-acetylated glycobiosyl bromides[75]. Hence, the procedure would give access to phenyl 1,2-trans-1-thio-ß-glycobiosides ready for further oligosaccharide syntheses (Scheme 25).

Disaccharides are of interest since they constitute integral components of a number of polysaccharide repeating units which can be adequately synthesized using the widespread thioglycosyl donor technology[47].

SCHEME 25

Stereospecific Syntheses of 1,2-trans Phenylthio ß-D-Disaccharides Under Phase Transfer Catalysis



Disaccharides **19-23** **24-28**

Table 4. PTC glycosylation of thiophenol with per-O-acetylglycobiosyl bromides

Bromides	Thioglycosides	Yield (%)	mp (°C)
Acetobromocellobiose **19**	**24**	85	224-225
Acetobromolactose **20**	**25**	92	165.1-166.7
Acetobromogentiobiose **21**	**26**	77	167.4-169.0
Acetobromomelibiose **22**	**27**	90	----
Acetobromomaltose **23**	**28**	89	82

Synthesis, Sept. (1991).

Glycosidation of the glycobiosyl bromides **19-21, 23** using 3 equivalents of thiophenols and 1 equivalent of tetrabutylammonium hydrogen sulfate in the two-phase system consisting of an aqueous solution of sodium carbonate (1M) and a dichloromethane solution afforded the corresponding thiodisaccharides **24-26, 28** in more then 77 % yield (table 4). The more slowly reacting bromide **22** gave incomplete conversion under these conditions. The reaction had stopped at ~50 % conversion. Changing the solvents to toluene or ethyl acetate gave, however, the expected thioglycosides **27** in high yields. In this last case, it was suspected that the nucleophilic thiophenoxide was adversely competing for the solvent (dichloromethane). A control experiment was therefore run in which the glycobiosyl bromides were omitted. Using dichloromethane as solvent and under essentially the same reaction conditions as in the general procedure, bis(phenylthio)methane and chloromethylphenyl sulfide were isolated and characterized (EI-MS, ^{1}H-and ^{13}C-NMR). Thus, it was concluded that when the bromides were not converted rapidly enough into the thiodisaccharides, competing nucleophilic displacement with the solvent occured. The situation was obviously avoided in toluene or ethyl acetate without adverse effect on the reaction time.

The bromides **19-23** were derived by prior acetylation of the commercially available disaccharides followed by bromination using the standard HBr-HOAc (45% w/v) procedure[38] in almost quantitative yields.

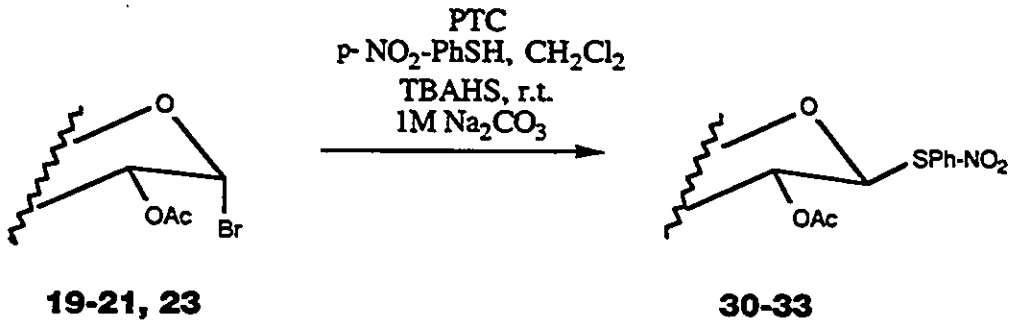
The ^{1}H and ^{13}C-NMR assignments were based on COSY and HETCOR experiments. They can be found in the literature cited[75] for compounds **19-23**. A detailed characterization for the per-O-acetyl gentiobioside **29**, acetobromogentiobiose **21** and 1,2-trans phenylthio-ß-D-gentiobioside **26** is included in the experimental section.

This new and mild procedure gives good to excellent yields of stereospecific 1,2-trans phenylthio ß-D-disaccharide as indicated in table 4 for the corresponding per-O-acetyl glycobiosyl bromides. These "active" phenylthio ß-D-disaccharides can be used as glycosyl donors in block-oligosaccharides synthesis. Gentiobioside **26** constitutes the branch disaccharide repeating unit of *H. pleuropneumoniæ* CPS serotype 2.

Smilarly the "latent" para-nitrophenyl-1-thio-ß-D-glycobiosides **30-33** can also be prepared by treatment of the glycobiosyl bromides **19-21, 23** under catalytic two-phase system using either methylene chloride or ethyl acetate and 1M sodium carbonate and the lipophilic tetrabutylammonium hydrogen sulfate in 70-91 % yields[76] (table 5, Scheme 26).

SCHEME 26

General Synthesis of Para-Nitrophenylthio ß-D-Glycobiosides



19-21, 23 **30-33**

Table5. PTC glycosylation of p-nitrothiophenol with per-O-acetylglycobiosyl bromides

Bromides	Thioglycosides	Yield (%)	mp (°C)
Acetobromocellobiose **19**	**30**	89	233-234°
Acetobromolactose **20**	**31**	91	122.5-124.4°
Acetobromogentiobiose **21**	**32**	70	190.2-191.4°
Acetobromaltose **23**	**33**	71	165.2-166.4°

The reaction, performed at room temperature, occured with complete stereocontrol by nucleophilic displacement with inversion of configuration. No α-thioglycoside could be detected from the crude reaction mixtures by ^{1}H-NMR.

Interestingly, the acetobromo-lactose **20** and -maltose **23** reacted smoothly in methylene chloride within 4-6 h at room temperature, while the acetobromo-cellobiose **19** and -gentiobiose **21** failed to give complete conversion under the same conditions. Thus, using the same three equivalents of p-nitrothiophenol, the reaction stopped at ~50 % conversion with **19** and **21** in methylene chloride. The use of refluxing benzene or toluene as proposed by others[74,77] did not remedy the situation since the p-nitrothiophenoxide ion was not soluble in these solvents. Changing methylene chloride for ethyl acetate was however beneficial since the corresponding thioglycosides **30** and **32** could be obtained in good yields within the same reaction time (4-5.5 h). Obviously, when the reaction is performed in methylene chloride, there is a possible competing reaction between nucleophilic attack at both the anomeric center and on the solvent. Indeed, in one instance, bis(p-nitrothiophenyl)methane ((p-NO_2-PhS)$_2CH_2$) was isolated from the reaction mixture and characterized (^{1}H- and ^{13}C-NMR, MS). Other side reactions such as oxidation to p-nitrophenyl disulfide ((p-NO_2-PhS)$_2$), also isolated and characterized (^{1}H- and ^{13}C-NMR, MS), and mono-substitution on the solvent (p-NO_2PhSCH_2Cl) should be accounted for.

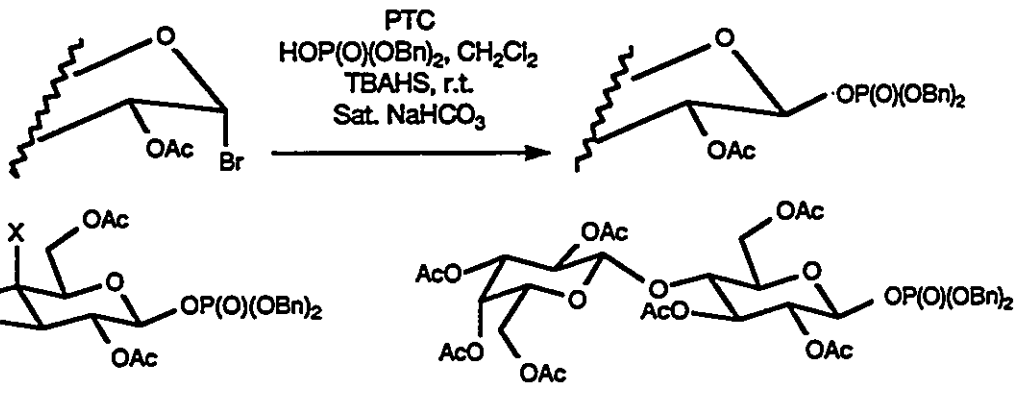
The structures were confirmed by proton and carbon NMR spectroscopy. All assignments were based on COSY and HETCOR experiments. The analysis can be found in reference 76, that for the gentiobiose derivatives is detailed in the experimental section.

ii) 1,2-trans-ß-D-glycosyl phosphates.

We also applied this PTC methodology to the synthesis of 1,2-trans ß-D-anomeric phosphates[78]. It was anticipated that dibenzylphosphate would be a potent nucleophile under a catalytic two-phase system. This procedure would give acces to carbohydrate phosphotriesters from readily available reagents (Scheme 27).

Carbohydrate phosphates are important intermediates in a wide variety of biosynthetic processes[79]. They constitute integral components of the core and lipid A region of lipopolysaccharides, sugar nucleotides, teichoic acids, bacterial polysaccharides, glycophospholipids, and phosphoinositides.

SCHEME 27



35 X = H, Y = OAc, D-Glucose: 83 %
36 X = OAc, Y = H, D-Galactose: 73 %

37 Lactose: 83 %

Treatment of the peracetylated glycosyl bromides of D-glucose **2**, D-galactose **34** and D-lactose in methylene chloride and saturated sodium hydrogen carbonate with dibenzyl phosphate (2 eq) and tetrabutylammonium hydrogen sulfate (1 eq) at room temperature (1.5-3 days) afforded the corresponding ß-D-glycosyl phosphates **35-37** in 73-83 % yields (Scheme 27).

The structures of the above glycosyl phosphates were established by ^{1}H and ^{13}C-NMR spectroscopy (tables 6 and 7). The complete assignments of all the protons and carbons were confirmed by COSY (figure 5, 8) and HETCOR (figure 6,9) experiments. A total characterization for the glucose **35** and galactose **36** derivatives is found in the experimental section that of the lactose **37** can be found in reference 78. The anomeric carbons of **35**, **36**, and **37** appeared at 96.2 ($^2J_{C,P}$ 4.7 Hz), 96.7 ($^2J_{C,P}$ 4.8 Hz), and 96.1 ($^2J_{C,P}$ 4.9 Hz) ppm respectively (table 7). The anomeric proton of the glucosyl phosphate **35** appeared as a doublet of doublets centered at 5.33 ppm with $J_{1,2}$ and $^3J_{1,P}$ couplings of 7.5 Hz (table 6). The same coupling constants were obtained for the anomeric proton (5.30 ppm) of the glucosyl residue of the lactosyl phosphate **37**. The case of the galactosyl phosphate **36** was, however, somewhat ambiguous (in $CDCl_3$) since the H-1 and H-2 protons appeared as an ABX system (300 MHz) (figure 7). Although the C-1 chemical shift and the $^2J_{C,P}$ coupling constant were similar to those observed for the ß-phosphates **35** and **37** and thus indicative of the ß-D-configuration, it was decided to further confirm this assumption. A one-bond ^{13}C-^{1}H coupling constant, which showed a $^1J_{H1,C1}$ of 167 $\pm$<1 Hz for **36**, was obtained by a heteronuclear gated decoupling experiment (300 MHz). This value indicated that the H-1 proton should have been equatorially disposed (α-anomer) since 160 Hz is assumed to be typical of ß-anomers while 170 Hz is typical of the α-anomers[80]. Since the literature cited[80] has not been applied or demonstrated on anomeric glycosyl phosphates, the ambiguity still remained. The anomeric region of the ^{1}H-NMR spectra of **36** was then simulated (figure 10) based on the estimated chemical shifts of H-1, H-2, and H-3 obtained from the ^{1}H-^{1}H homonuclear shift correlated experiment. The data obtained (table 6) showed a $J_{1,2}$ coupling constant of 8.1 Hz ($CDCl_3$), typical of a ß-D-glycosides (axial proton). The apparent ambiguity was finally completely eliminated by running the ^{1}H-NMR spectra of **36** in C_6D_6 (figure 11). The chemical shifts and coupling constants are included in table 6. The H-1 and H-2 system no longer appeared as an ABX pattern and was completely resolved. A complete description is found in the experimental section.

Table 6. Selected ^{1}H-NMR chemical shifts and coupling constants (Hz) of the glycosyl phosphate derivatives.

Compound no.	H-1 ($^3J_{1,P}$, $J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$, $J_{5,6'}$)	H-6 ($J_{6,6'}$)	H-6'
35[a]	5.33 (7.5, 7.5)	5.11 (9.2)	5.20 (9.2)	5.09 (9.9)	3.79 (2.2, 4.8)	4.09 (12.3)	4.22
36[a]	5.30[e] (7.4, 8.1)	5.31[e] (9.8)	5.01[e] (3.5)	5.40 (1.2)	4.00 (6.0, 6.8)	4.08 (11.0)	4.14
	5.51[d] (7.8, 8.0)	5.79 (10.5)	5.14 (3.5)	5.40 (1.1)	3.27 (6.5, 6.5)	4.99-5.09[f] -	4.99-5.09[f]
37[a]GlC	5.30 (7.5, 7.5)	5.01 (9.3)	5.19 (9.2)	3.84 (10.0)	3.71 (2.1, 5.1)	4.48 (12.2)	4.02
Gal	4.47 (7.8)	5.09 (10.4)	4.94 (3.5)	5.33 (<1Hz)	3.85 (6.9, 6.3)	4.11 (11.2)	4.06

[a] At 300 MHz in $CDCl_3$
[b] At 500 MHz in $CDCl_3$
[c] From the simulated spectra, figure 10 other data are observed values.
[d] At 300 MHz in C_6D_6
[e] Unresolved ABX system.

Table 7. ^{13}C-NMR chemical shifts (ppm) and C-P coupling constants (Hz) of the glycosyl phosphate derivatives (75.4 MHz, $CDCl_3$, 25 °C).

Compound no	C-1 ($^2J_{C,P}$)	C-2 ($^2J_{C,P}$)	C-3 ($^3J_{C,P}$)	C-4	C-5	C-6	CH_2Ph ($^2J_{C,P}$)
35	96.2 (4.7)	71.1 (9.2)	72.3 (1.5)	67.8	72.6	61.4	69.62, 69.57 (5.8), (5.5)
36	96.7* (4.8)	68.6 (8.8)	70.4 -	66.6	71.7	61.0	69.60, 69.55 (5.9), (6.1)
37 Gal	96.1 (4.9)	71.5 (9.0)	72.3 -	75.6	73.4	61.5	69.6, 69.5 (6.2), (6.3)
	101.0	69.0	70.9	66.5	70.7	60.8	

* $J_{C\text{-}1,\,H\text{-}1}$ = 167 Hz

In conclusion treatment of halogenoses with dibenzyl phosphate under a catalytic two-phase system afforded 1,2-trans-ß-D-glycosyl phosphotriesters in good yields with complete stereocontrol.

Figure 5 COSY spectrum of the glucosyl phosphate derivative **35** in $CDCl_3$

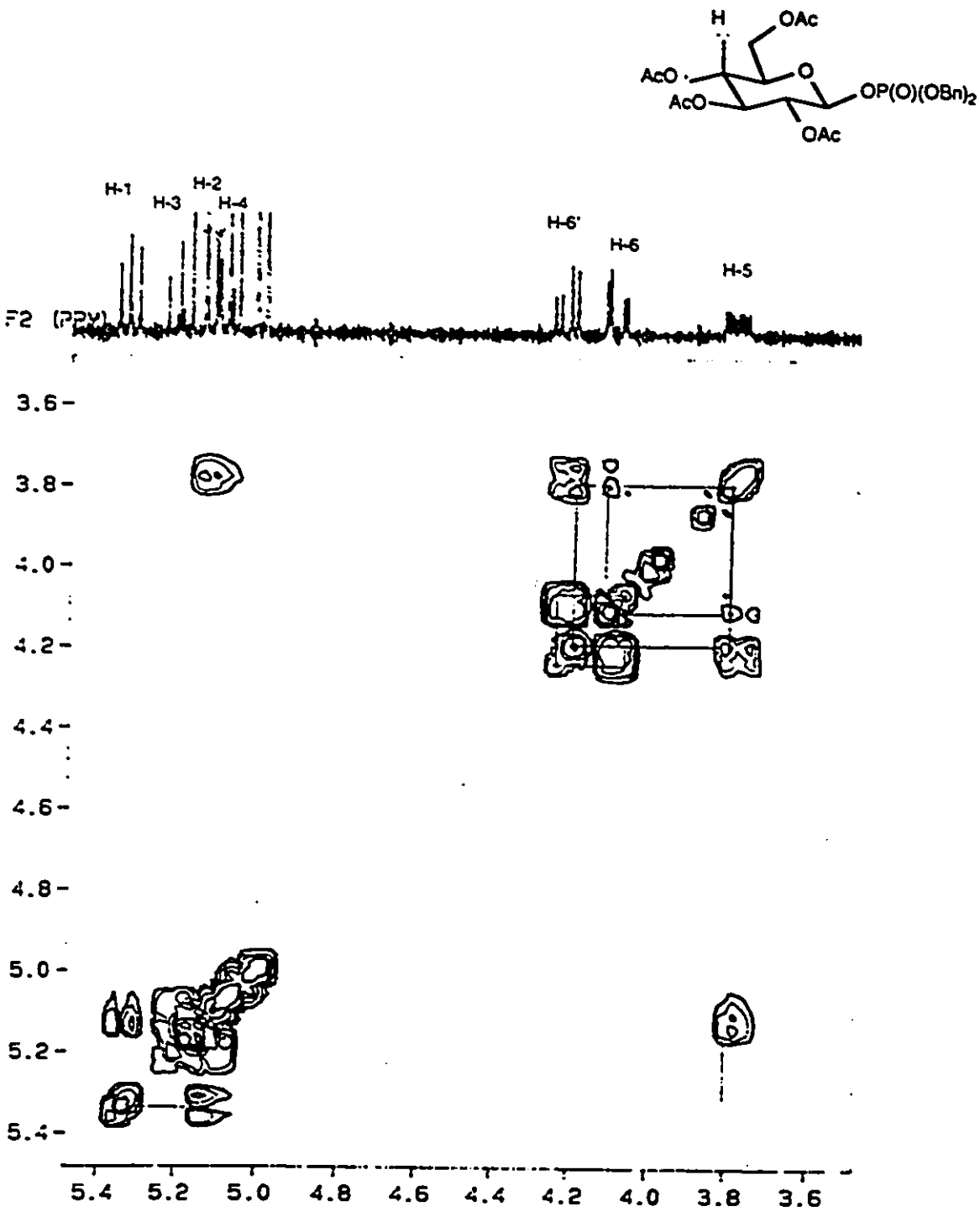


Figure 6 HETCOR spectrum of the glucosyl phosphate derivative **35** in $CDCl_3$

Figure 7 NMR spectrum of the galactosyl phosphate derivative **36** at 300 MHz in $CDCl_3$

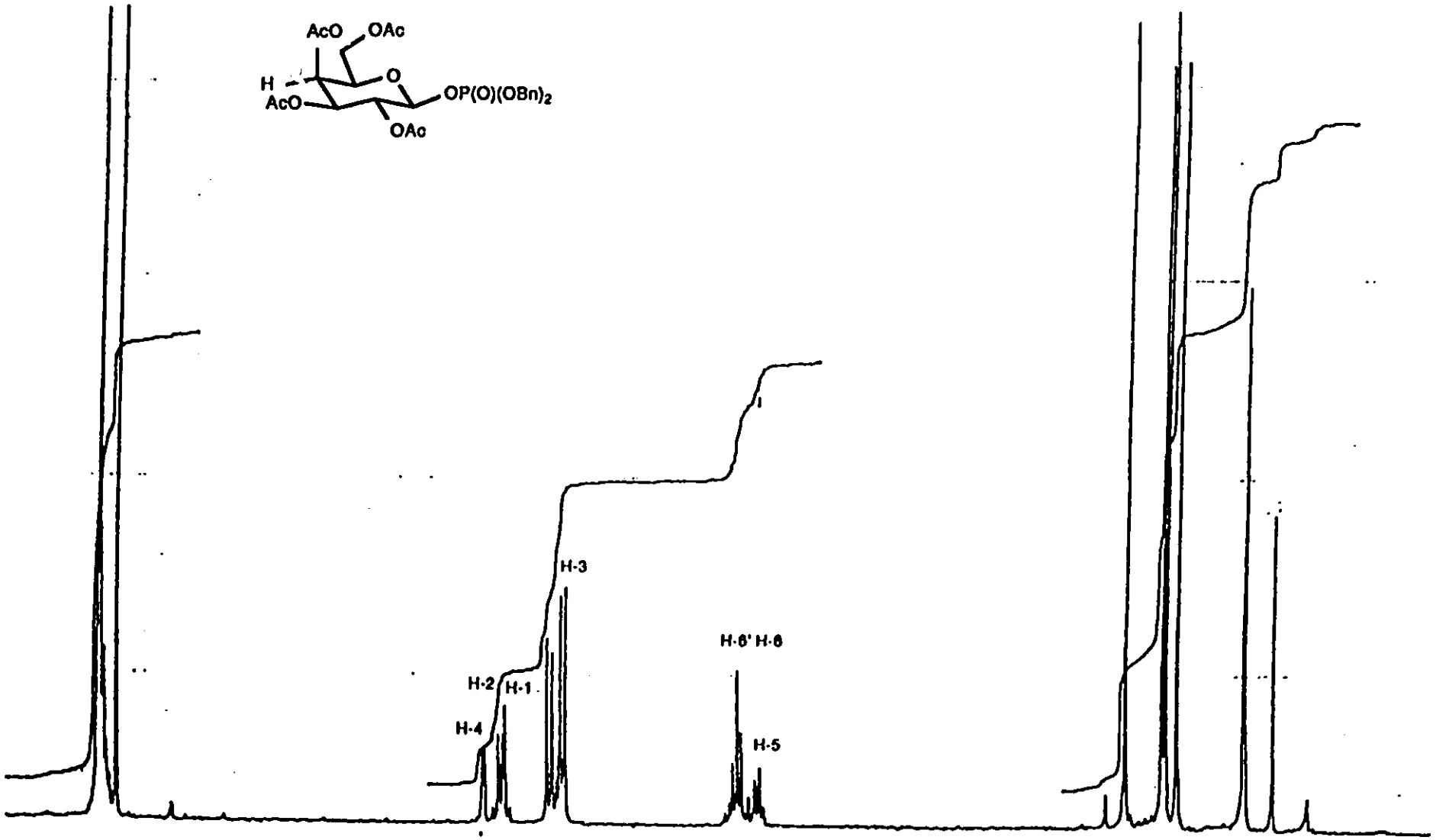


Figure 8 COSY spectrum of the galactosyl phosphate derivative **36** in $CDCl_3$

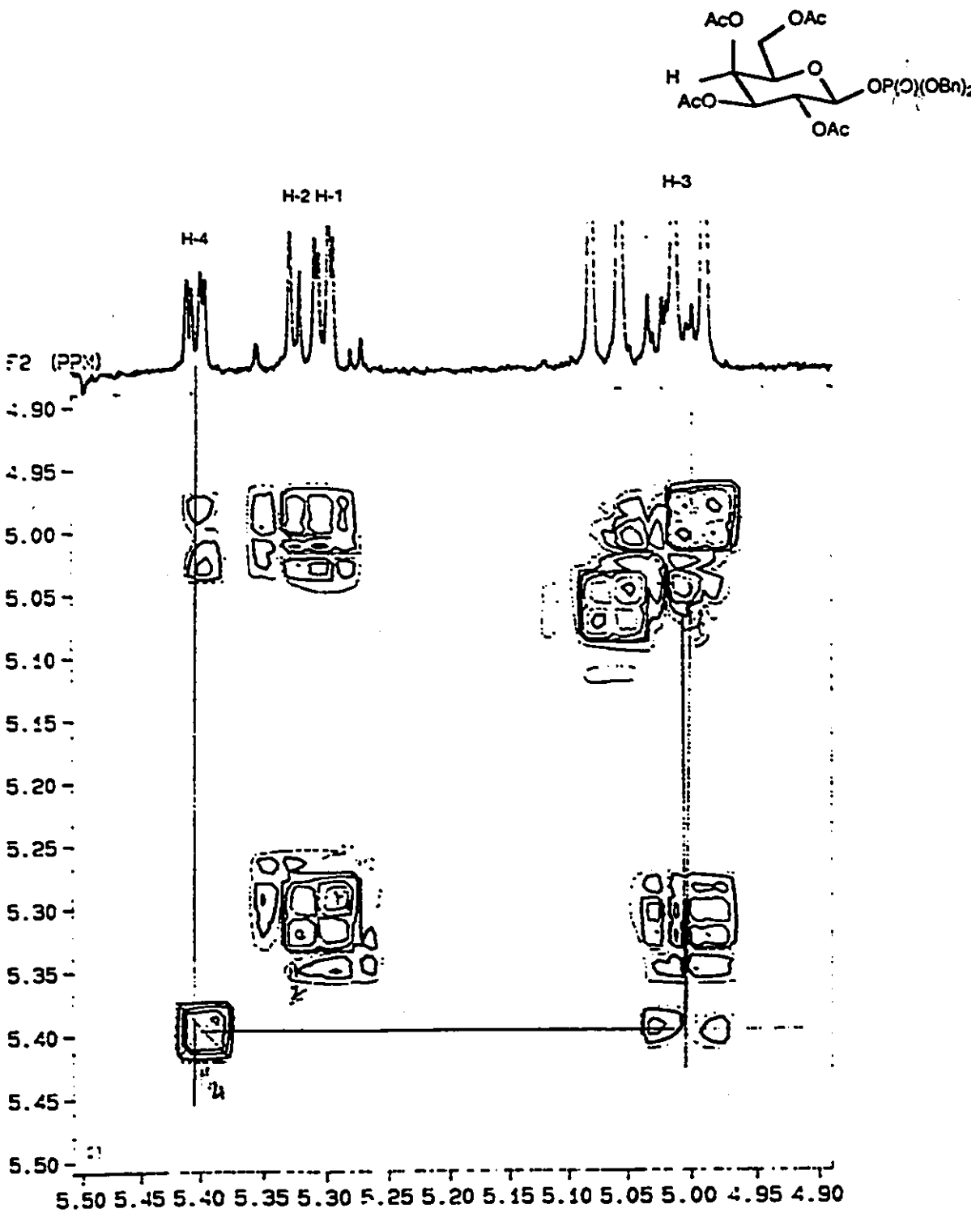


Figure 9 HETCOR spectrum of the galactosyl phosphate derivative **36** in $CDCl_3$

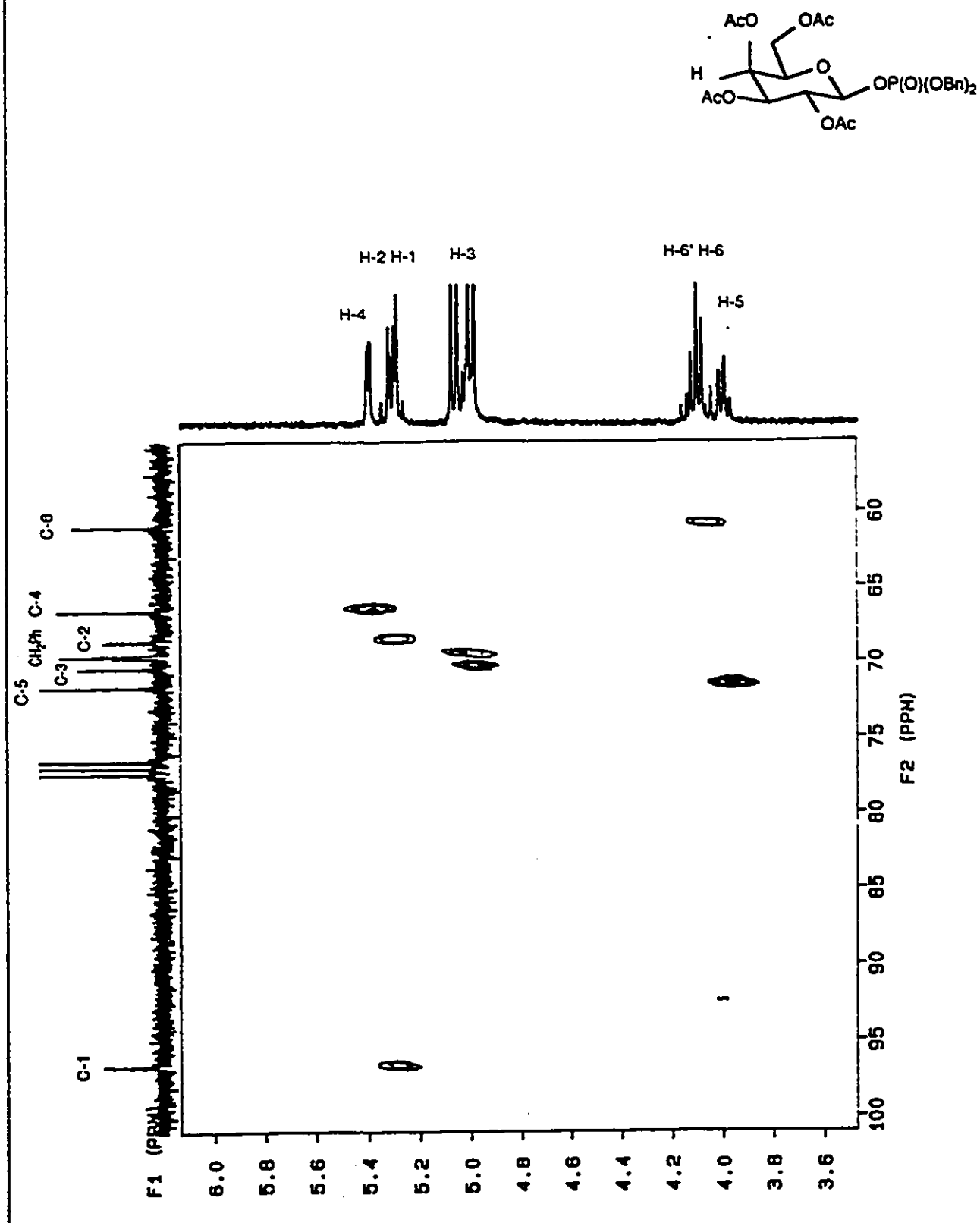


Figure 10 Partial ^{1}H-NMR spectra (300 MHz) of **36** in $CDCl_3$ showing H-1 and H-2 as a second order pattern. Simulated (top), and observed spectrum (bottom)

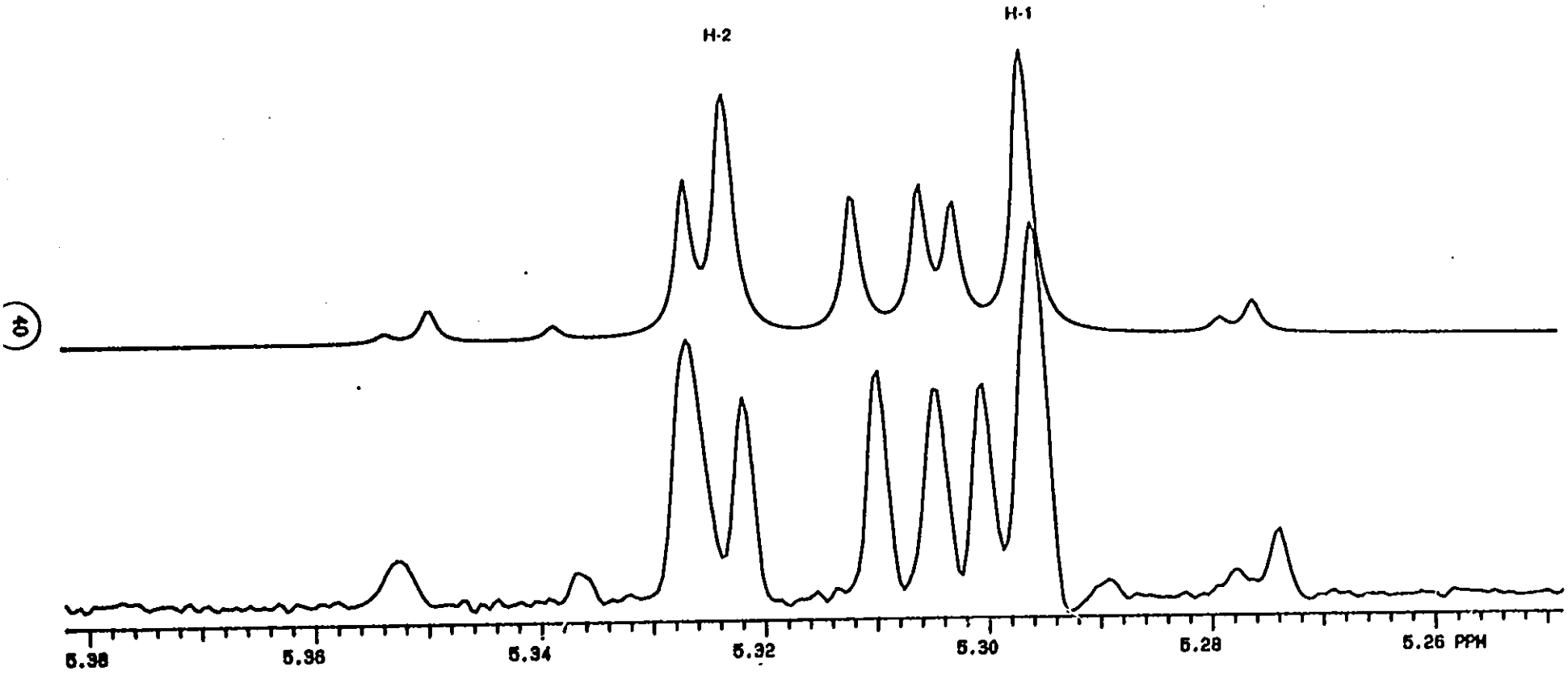
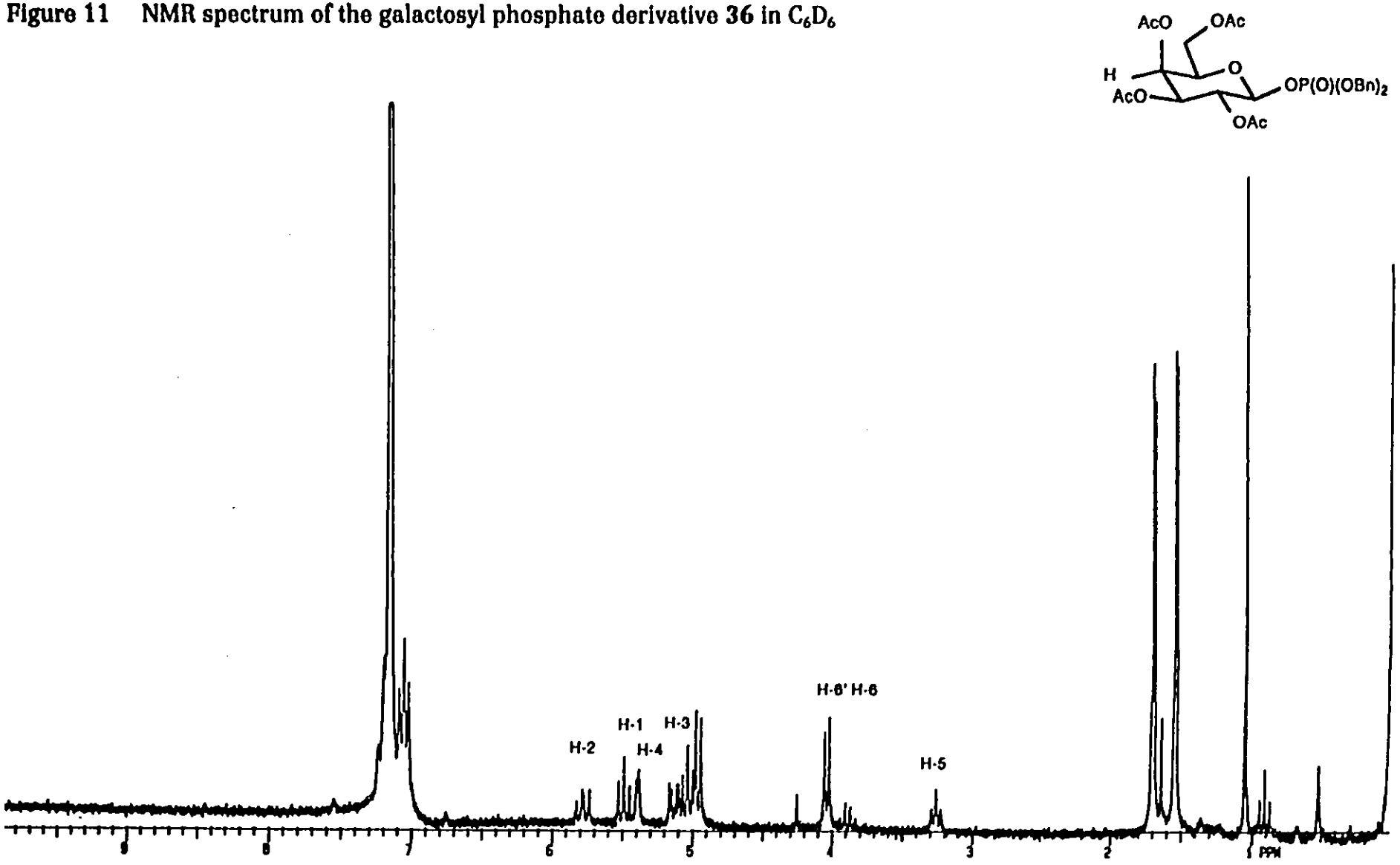


Figure 11 NMR spectrum of the galactosyl phosphate derivative **36** in C_6D_6



Chapter 6.

Attempted to the synthesis of the disaccharide unit of *H. pleuropneumoniæ* serotype 4 CPS using thioglycosides.

A series of glycosidations were attempted using the chemistry of thioglycosides with the previously prepared glycosyl acceptors to provide us the repeating disaccharide unit of *H. pleuropneumoniæ*.

Attempted glycosidation of allyl 2-acetamido-2-deoxy-6-O-trityl-α-D-glucopyranoside **8** and phenyl 6-O-tert-butyldiphenylsilyl-2,3,4-tri-O-acetyl-1-thio-ß-D-glucopyranoside **50** (see later) in the presence of N-bromosuccinimide (1.5 eq) (method H) (see experimental section) showed no reaction after 48 h.

The sugar alcohol, allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **6** and phenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **11** was treated with NIS and a catalytic amount of TfOH (procedure F), after 24 h no reaction occured. The phenyl thioglycoside **11** was replaced by the corresponding ethyl thioglycoside **10**, glycosidation under the same conditions using 1,2-dichloroethane as solvent resulted in no reaction after 48 h. The replacement of the later solvent by acetonitrile did not give the expected disaccharide after one week. Addition of an equimolar ratio of NIS and TfOH in the following combination of solvents dichloromethane, 1,2-dichloroethane and ether was also unsuccessful after 24 h. The thiophilic promotor DMTST was next studied in the presence of the glycosyl donor phenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **11** (method D). Unfortunately no reaction occured after 24 h. While the use of the corresponding ethyl thioglycoside **10** completely hydrolyzed after 2 h. The use of mercuric trifluoroacetate (method G) to a mixture of the phenyl thioglycoside **11** and the aglycon **6** did not gave any products after 48 h.

The benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **9** aglycon was next attempted. The use of DMTST (method D) in the presence of the glycosyl donors phenyl (**11**) or ethyl (**10**) per-O-acetyl-1-thio-ß-D-glucopyranosides in either dichloromethane or acetonitrile resulted in hydrolysis on prolonged reaction time. While the use of NBS (method H) in THF gave no reaction after 3 days, neither did the use of NIS and catalytic amount of TfOH (method F) in THF after 2 days. Although attempted glycosidation in acetonitrile at -50 °C using NIS and catalytic amount of TfOH (method F) resulted in a rapid hydrolysis of the ethyl thioglycoside **10**.

These glycosyl acceptors were not soluble at room temperature in the solvents used for the coupling reactions. This may partly explained the unreactivity of these glycosides in the presence of the thioglycosides and the thiophilic promotors.

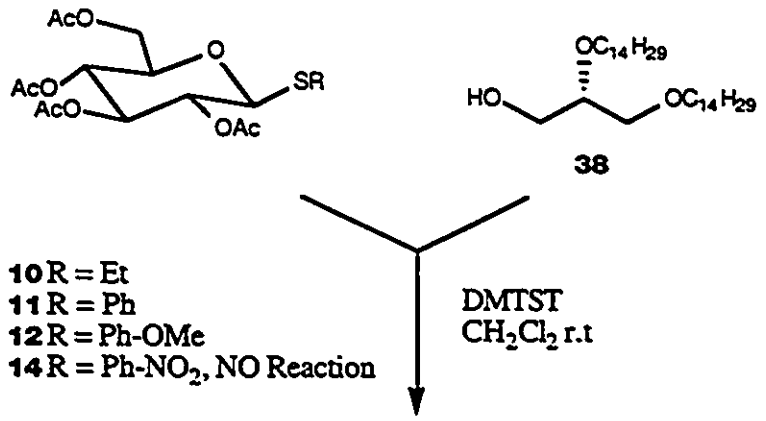
Chapter 7.

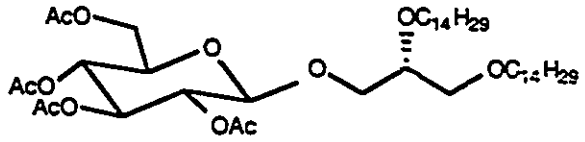
I. Evaluation of the concept of active and latent thioglycosyl donors.

Preliminary evaluation of our concept of "active" and "latent" thioglycosyl donors was quite successful.

SCHEME 28

ACTIVE and LATENT Glycosyl donors : Preliminary Testings





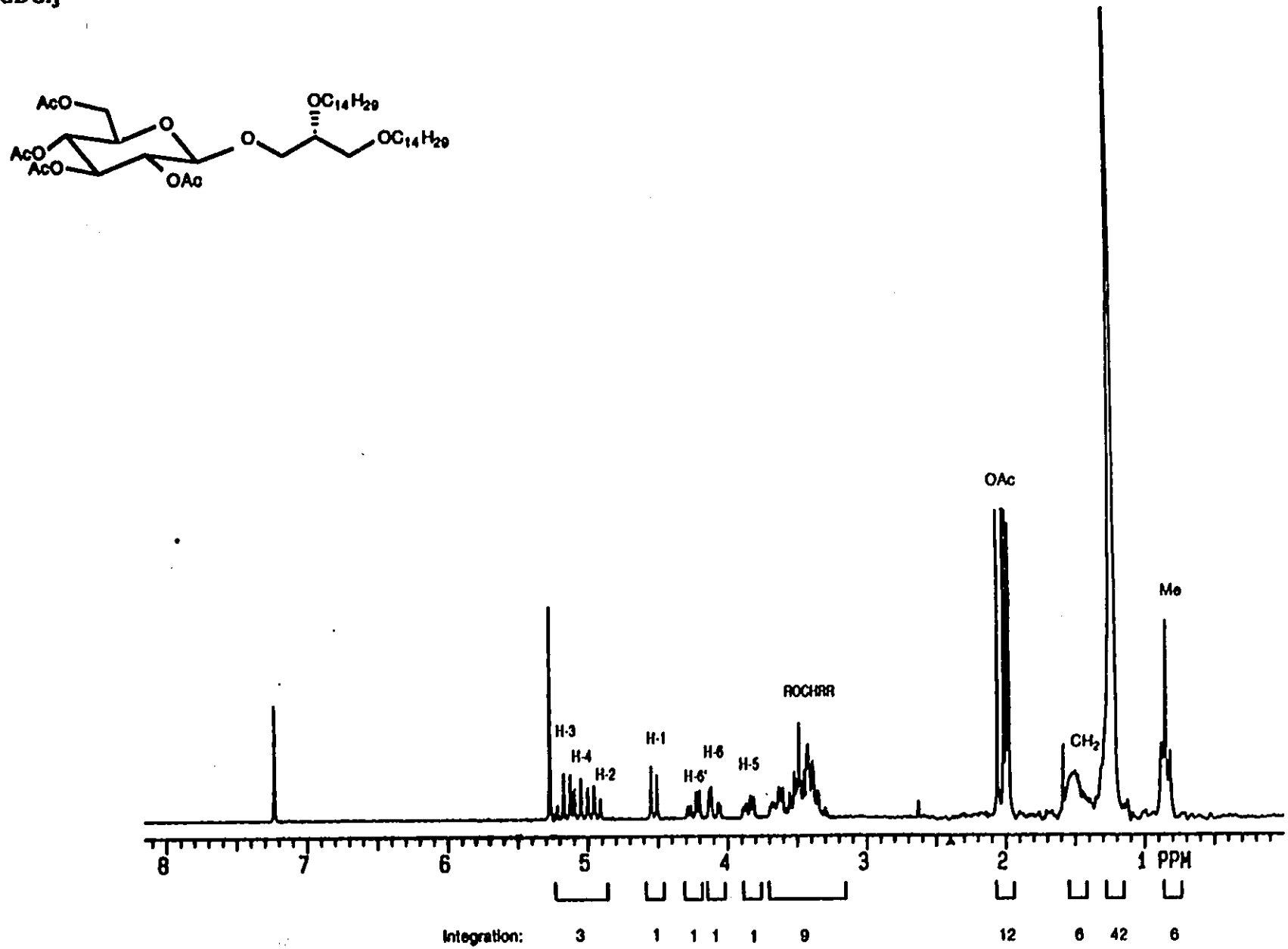
39

reaction rate : SEt > SPh ~ SPh-OMe >>> SPh-NO_2

The reaction of ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **10** with 1,2-O-ditetradecyl Sn-glycerol[81,82] **38** in the presence of DMTST (method D) resulted in the formation of the expected ß-D-glycerol-glycolipid **39** in 22 % yield (Scheme 28). The ethyl thioglycoside **10** was consumed rapidly and the reaction was complete within 1 h. However, an additional equivalent of glycosyl donor **10** was required as there was considerable loss due to the formation of side products (see below) as well as small amounts of the thioglycoside **10** hydrolyzed products.

The structure of the glycerol-glycolipid **39** was confirmed by its proton NMR spectrum (figure 12). The analysis is described in detail in the experimental section. The spectrum shows in particular the anomeric hydrogen at 4.53 ppm with a trans-coupling constant of 7.8 Hz. The presence of the lipid **38** (Scheme 28) is strenghthen by a multiplet from 3.69-3.39 ppm corresponding to 9 (OCHR) hydrogens. The remaining CH_2 groups, 48 protons, are found in the region of 1.52-1.22 ppm and finally two methyl groups appear as a triplet at 0.85 ppm.

Figure 12 NMR spectrum of the glycerol glycolipid **39** at 200 MHz in $CDCl_3$



A series of reactions, done in parallel, using ethyl-**10**, phenyl-**11**, para-methoxyphenyl-**12** and para-nitrophenyl-**14**-thioglycosides with 1,2-O-ditetradecyl-Sn-glycerol **38** were run on TLC scale to study the different donating tendencies of these glycosyl donors in the presence of DMTST (method D).

In the case of the ethyl thioglycoside **10** the same observations were made. The rate of the reaction was considerably reduced by the use of phenyl **11** and para-methoxyphenyl **12** thioglycosides (55 h) relative to the ethyl thioglycoside **10** (1 h). After 1.5 h traces of the ß-glycolipid **39** were found in addition to the above mentioned side products. The para-nitrophenyl thioglycoside **14** under the same coupling conditions was inert over the reaction period of 22 h and no hydrolysis by-products were observed.

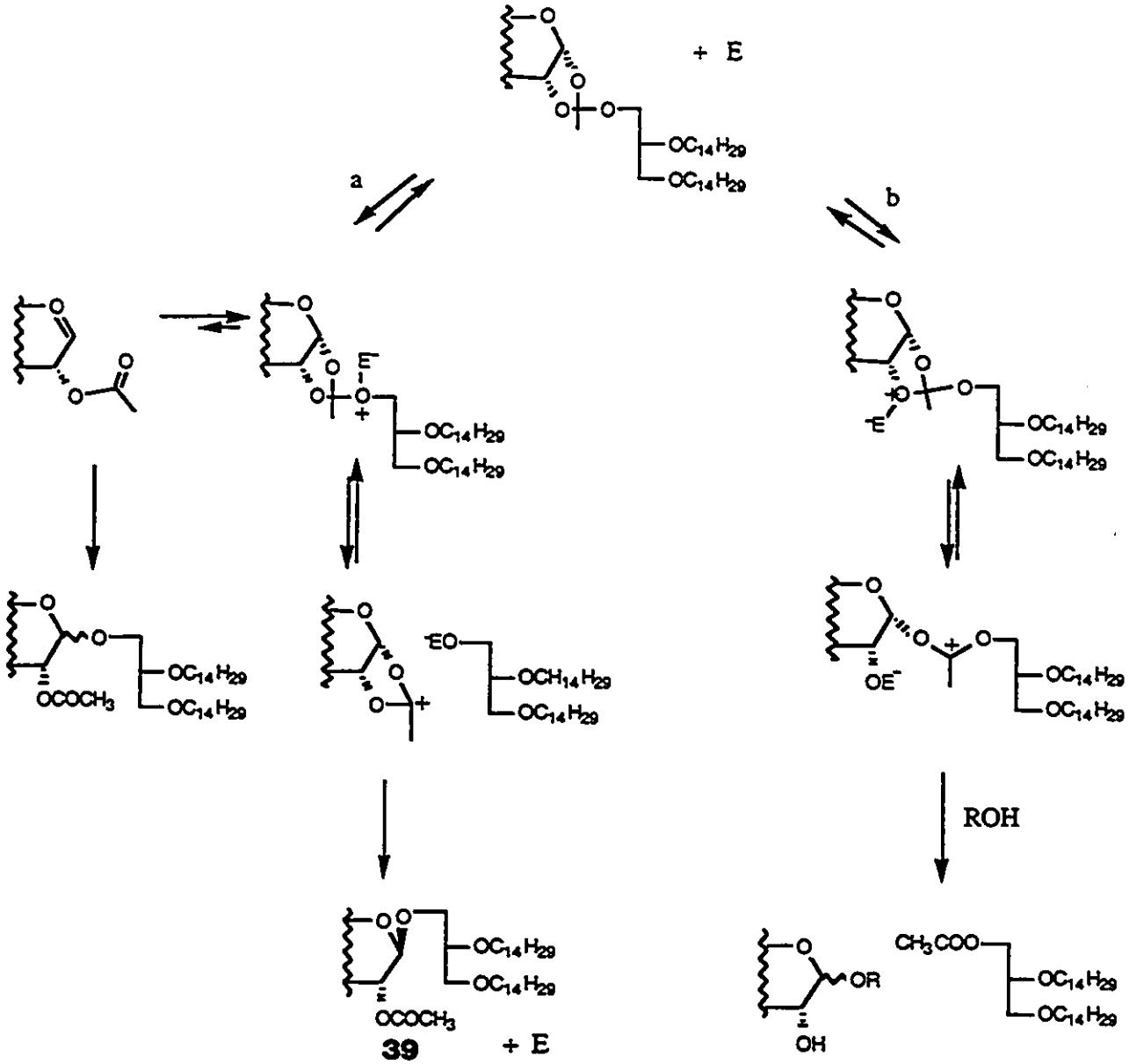
In all of these cases a transient product was formed rapidly, which was slightly more polar then the lipid **38**, and disappeared within 4.5 h for the aromatic thioglycosides. For the more reactive ethyl thioglycoside **10**, the transient product had a shorter life time.

Some qualitative experiments (TLC) were done to determined the nature of the side products. Two of which appeared as an α,ß mixture (vide infra), with lower Rf values than (Rf= 0.30; 0.28 in (7:3) hexane-ethyl acetate) that of the ß-glycolipid **39** (Rf= 0.48) and the remaining side product appeared at a lower polarity (Rf= 0.83) than the model lipid **38** (Rf= 0.65).

It was found that when the thioglycosides were subjected to the same coupling conditions with omission of the aglycon only the hydrolyzis by-products were obtained. Whereas the lipid **38** under the same coupling conditions with the exception of the glycosyl donor resulted in the formation of the transient product which also disappeared after a few hours.

Methylation of the lipid **38**, using sodium hydride and methyl iodide in THF, as well as acetylation of O-methyl α- and ß-glucose confirmed that they did not corresponded to any of these side products. The acetylated lipid which correspond to the same Rf (0.83) as the less polar side product appears to be forming. A proposed mechanism for its formation is shown in scheme 29.

Scheme 29



The proposed mechanism involves initial formation of an orthoester. Opening of the latter by addition of the electrophile onto the oxygen of the aglycon (scheme 29, pathway a) results in the formation of the expected ß-glycolipid **39**. Attack of the electrophile on the C-2 oxygen followed by addition of lipid **38** (scheme 29, pathway b) would give access to the acetylated lipid as well as a mixture of α and ß anomers of 3,4,6-tri-O-acetyl glycoside which may include some of the unidentified side products.

The reaction of the ethyl thioglycoside **10** was attempted at 0 °C. At this temperature the lipid **38** precipitates and no reaction occured until the reaction mixture was warmed up to room temperature.

In summary, it was found that ethyl **10**, phenyl **11**, and para-methoxyphenyl **12** thioglycosides are "active" toward the thiophilic promotor DMTST in the presence of the lipid **38** in moderate yields due to side reactions. The para-nitrophenyl thioglycoside **14** was "latent" under the same conditions. It was also shown that the ethyl thioglycoside **10** reacted at faster rate then the corresponding phenyl **11** and para-methoxyphenyl **12** thioglycosides.

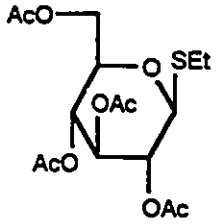
The replacement of the thiophilic promotor DMTST by MeI (method E), as a 3 % solution in dichloromethane, to the same series of thioglycosides **10-12**, **14** and lipid alcohol **38** resulted in no reaction after severals days.

We then attempted the glycosidations using NIS as a reactant and a catalytic amount of TfOH (method F). In the case of ethyl **10**, phenyl **11** and para-methoxyphenyl **12** thioglycosides a small amount of product was formed after a short periode of time (45 min.). The reaction mixtures were stirred for 24 h at room temperature where no changes occured. Therefore additional NIS (1.2 eq) was added without any progress. The reaction mixtures were contaminated by small amounts of a product slightly less polar then the ß-glycolipid **39** as well as by a side product resulting from the reaction of the aglycon **38** with NIS. This later observation was confirmed by a qualitative experiment where the aglycon **38** was allowed to react with NIS in dry dichloromethane. The para-nitrophenyl thioglycoside **14** was unreactive (latent) toward the same coupling conditions.

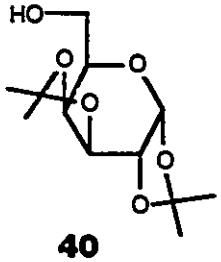
The replacement of phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** by phenyl per-O-benzoyl-1-thio-ß-D-glucopyranoside **55** or by phenyl-6-O-tert-butyldiphenylsilyl-2,3,4-tri-O-benzyl-1-thio-ß-D-glucopyranoside **52** (see later) did not remedy the situation. No glycosidation occured after 5 h in both cases.

Due to the problems encountered with the model lipid **38**, in the testing of the concept of "active" and "latent" thioglycosyl donors, we decided to use the commercially available 1,2:3,4-di-O-isopropylidene-D-galactopyranose 40 as a glycosyl acceptor.

SCHEME 30 **ACTIVE and LATENT Glycosyl donors: Preliminary Testings**

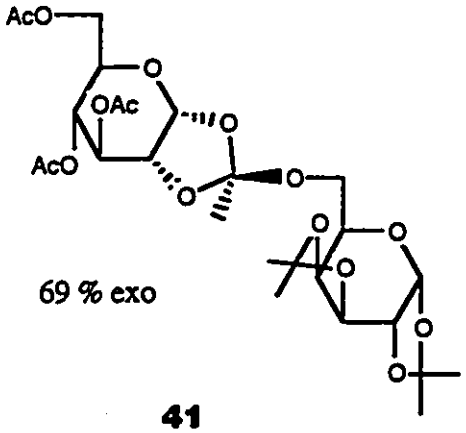


10



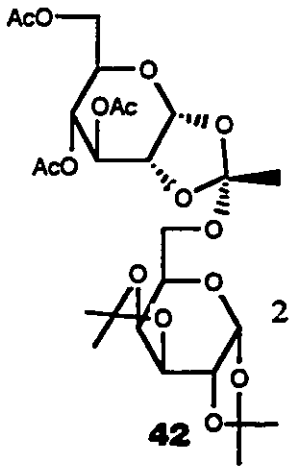
40

NBS



69 % exo

41



21 % endo

42

The coupling reaction between ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **10** and 1,2:3,4-diisopropylidene-D-galactopyranose **40** in the presence of the thiophilic promotor NBS (method H) was evaluated. TLC indicated a complete transformation of the thioglycoside **10** within 1 h to afford 90% yield of a mixture of 69 % and 21 % of the exo **41** and endo **42** orthoesters respectively (Scheme 30).

The presence of a cis coupling constant (5.2 Hz) at 5.69 ppm for the anomeric hydrogen of the D-glucose derivative in addition to the exo **41** and endo **42** CMe groups at 1.69 and 1.58 ppm respectively in the ^{1}H-NMR spectra were indicative of the existence of the orthoesters. The anomeric proton corresponding to the galactose derivative was found at 5.47 ppm, as a doublet, with a cis coupling constant of 5.1 Hz.

Attempted rearrangement of compound **42** with 0.1 eq of TfOH performed in an NMR tube didn't reveal the existence of the expected disaccharide and the spectrum could not be resolved.

The use of 0.12 eq of TfOH in the presence of NBS (method H) or the use of DMTST (4.5 eq) (method D) also gave the orthoesters **41-42**.

Glycosidation of phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** was then attempted using NIS and a catalytic amount of TfOH. The result was not promising, only a small amount of product was obtained after 40 h.

SCHEME 31

ACTIVE and LATENT Glycosyl donors : Preliminary Testings

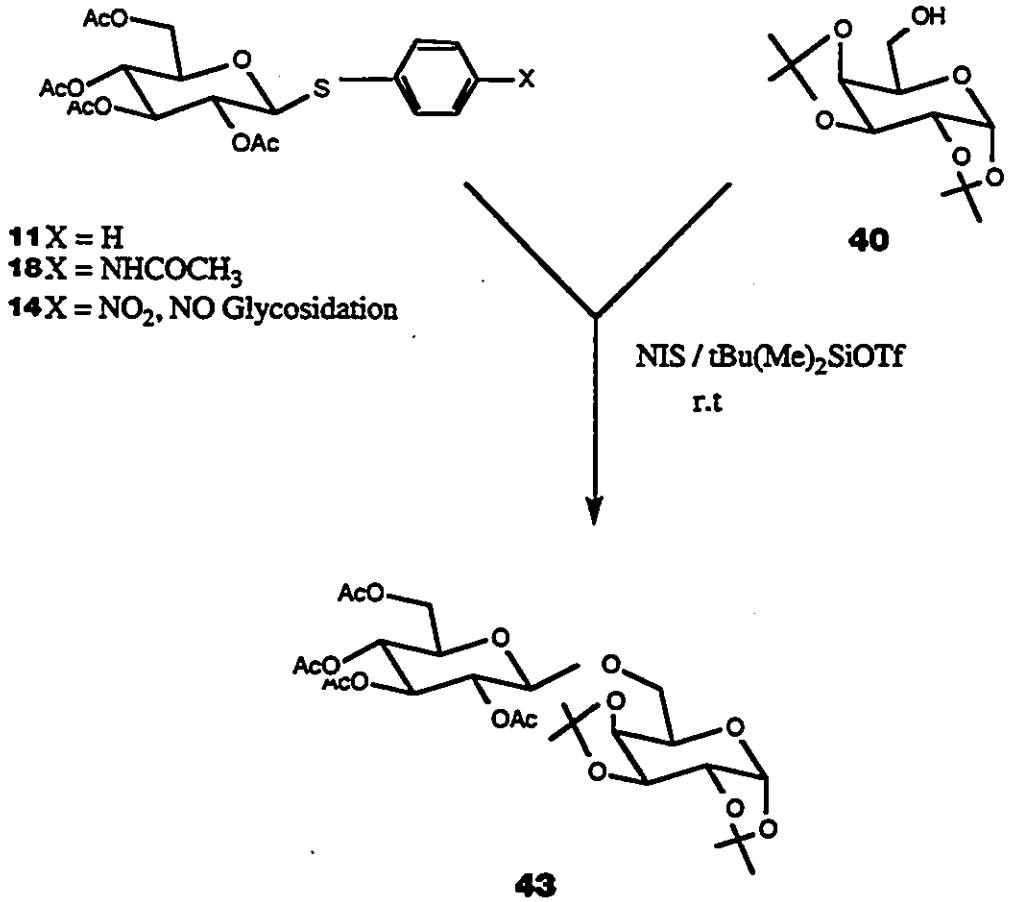


Figure 13 NMR spectrum of the disaccharide **43** at 300 MHz in $CDCl_3$

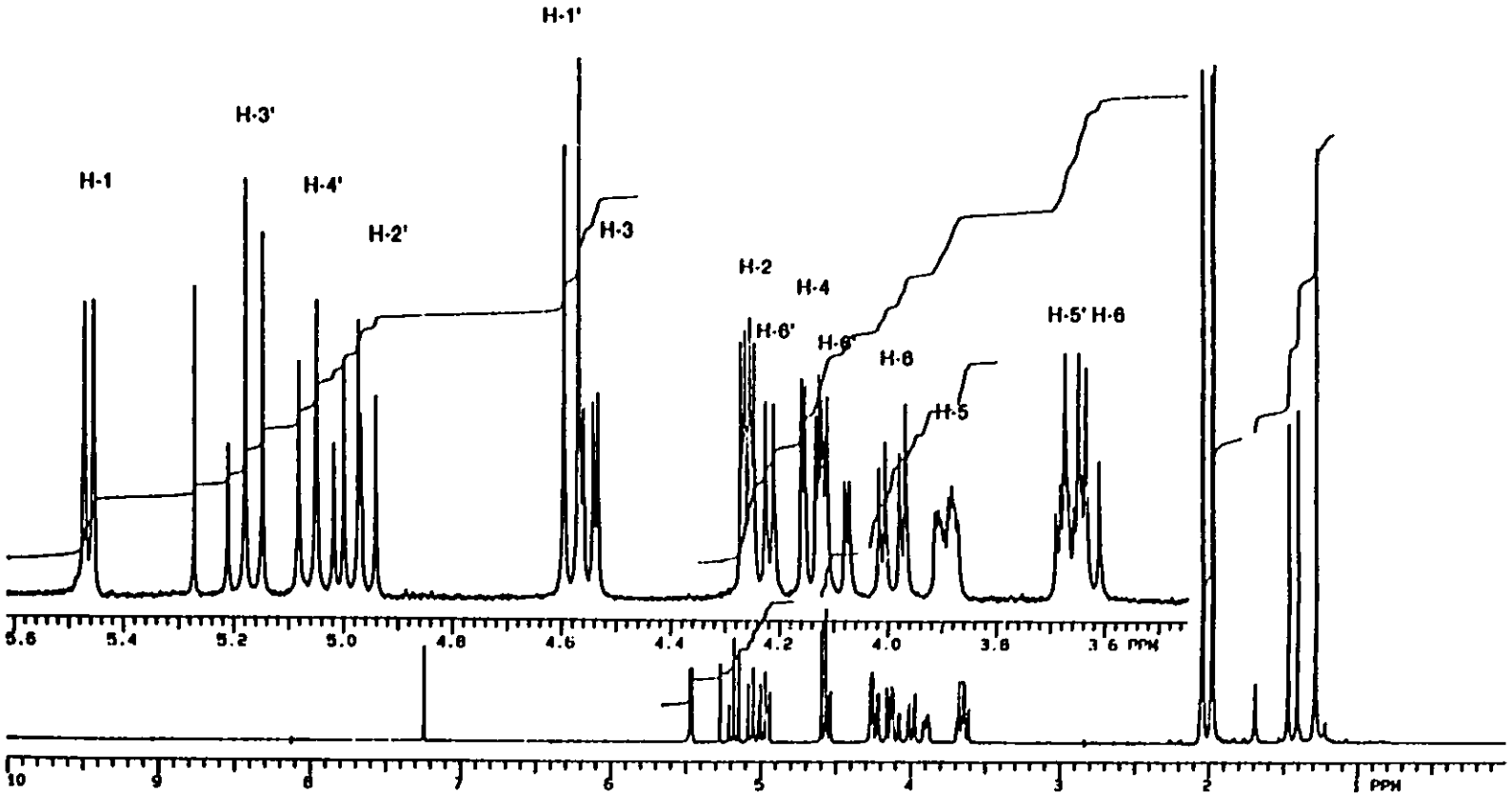
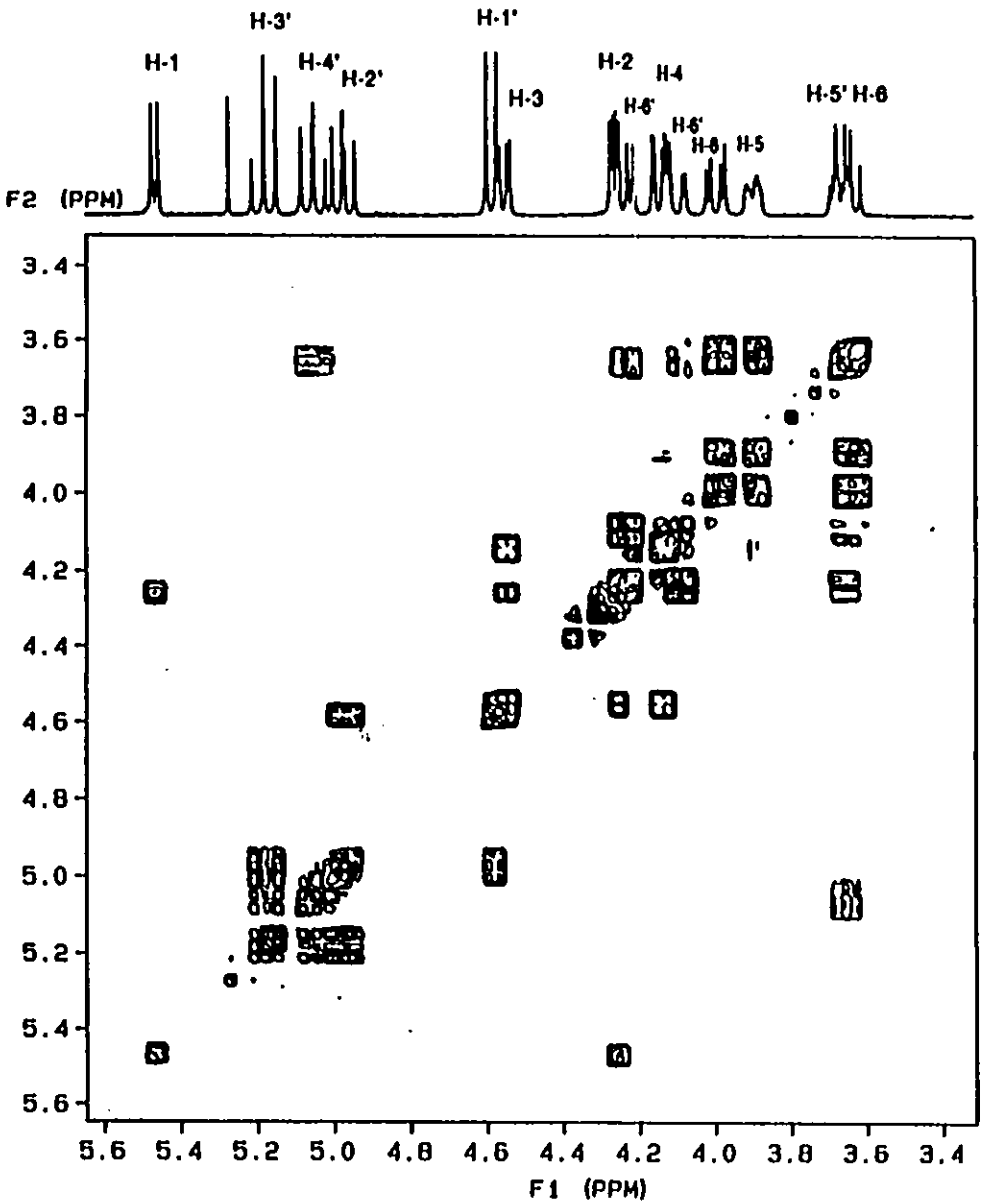
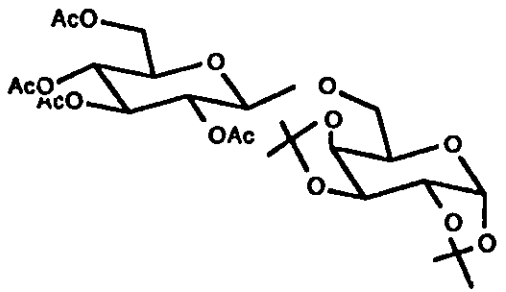


Figure 14 COSY spectrum of the disaccharide **43** in $CDCl_3$





For the first time, glycosidation of phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** with 1,2:3,4-diisopropylidene galactopyranose **40** was attempted in the presence of NIS (1.5 eq) and t-Bu(Me)$_2$SiOTf (1.1 eq) (method I) (Scheme 31). The reaction was completed within 45 min. and afforded 31 % of pure ß-D-disaccharide **43**. The reaction was also completed in the same period of time by replacing t-Bu(Me)$_2$SiOTf with TMSiOTf, although the reaction afforded a lower yield (15 %). In both cases the reaction mixture appeared to be contaminated by products corresponding to the hydrolysis of the isopropylidene groups as well as by small amounts of hydrolyzed products. The reaction mixture also contained two unidentified products which were slightly less polar then the starting thioglycoside.

The structure of the known[83] ß-D-disaccharide **43** was confirmed by proton (figure 13) and carbon NMR. The assignements have been based on the corresponding two dimensional COSY (figure 14) and HETCOR spectra. The ß-configuration of the disaccharide **43** was verified by the doublet at 4.58 ppm integrating for one hydrgen with a coupling constant of 8.0 Hz. The anomeric hydrogen of the galactose residue was found at 5.46 ppm with a cis coupling constant of 4.9 Hz. A full description of the proton and carbon spectra is found in the experimental section.

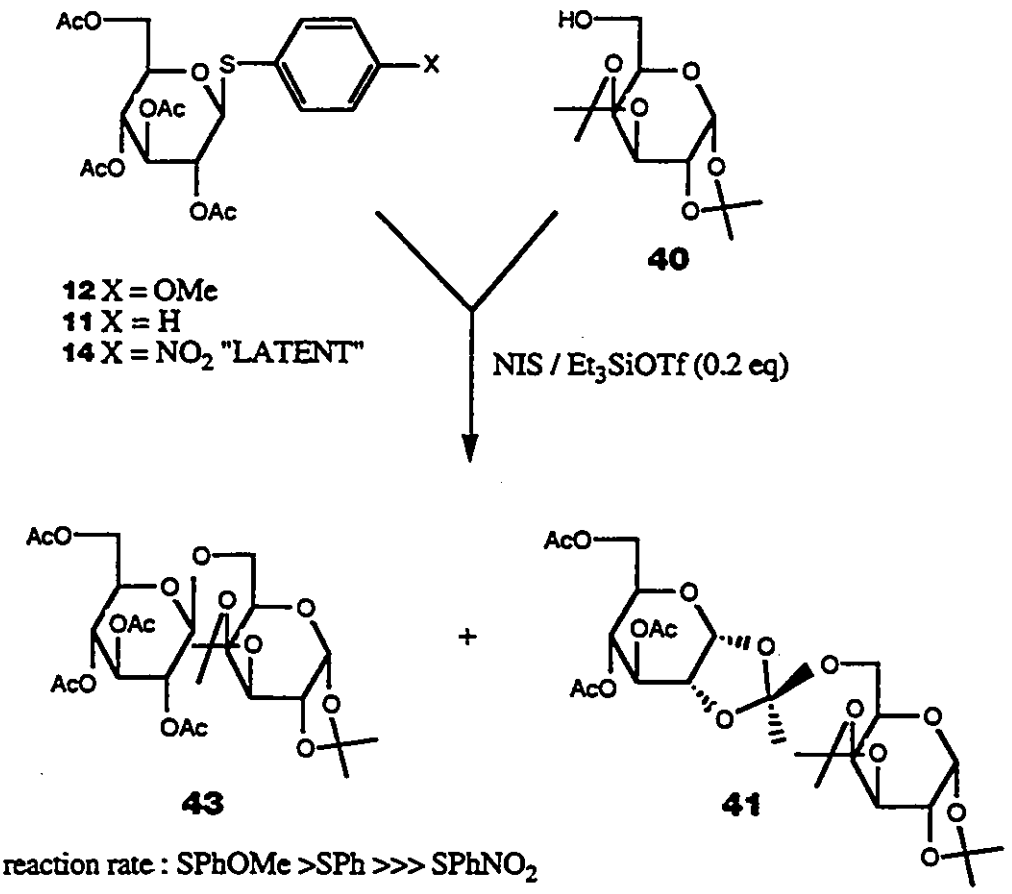
A qualitative test was run (scheme 31) on both the "latent" para-nitrophenyl thioglycoside **14** and is "active" form, the N-acetylphenyl thioglycoside **18**, with NIS (1.5 eq) and t-Bu(Me)$_2$SiOTf (1.1 eq). The results showed that the para-nitrophenyl thioglycoside **14** was intact under these conditions although some decomposition of the aglycon **40** occured. In the case of the N-acetyl form **18** we had indeed "reactivated" the thioglycoside, the expected ß-D-disaccharide **43** was found as well as some side products.

In order to minimize side product formations as well as the hydrolysis of the aglycon portion **40** a catalytic ratio of t-Bu(Me)$_2$SiOTf was used. Glycosidation of phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** in the presence of 1,2:3,4-diisopropylidene galactopyranose **40** (method J) with the addition of t-Bu(Me)$_2$SiOTf (0.2 eq), NIS (0.4 eq) and the aglycon **40** (0.5 eq) after one hour afforded 41 % of a mixture of ß-D-disaccharide **43** (77.5 %) and exo orthoester **41** (22.5 %). Therefore the reduction of the amount of t-Bu(Me)$_2$SiOTf resulted in the formation of the exo orthoester **41**.

The existance of the orthoester was confirmed by the addition, to the proton NMR spectrum of the ß-D-disaccharide **43**, of a doublet at 5.69 ppm with a cis coupling constant (5.1 Hz) corresponding to the orthoester **41** anomeric hydrogen of the glucose derivative and by the singlet at 1.68 ppm for the presence of the CMe group.

SCHEME 32

ACTIVE and LATENT THIOglycosyl donors

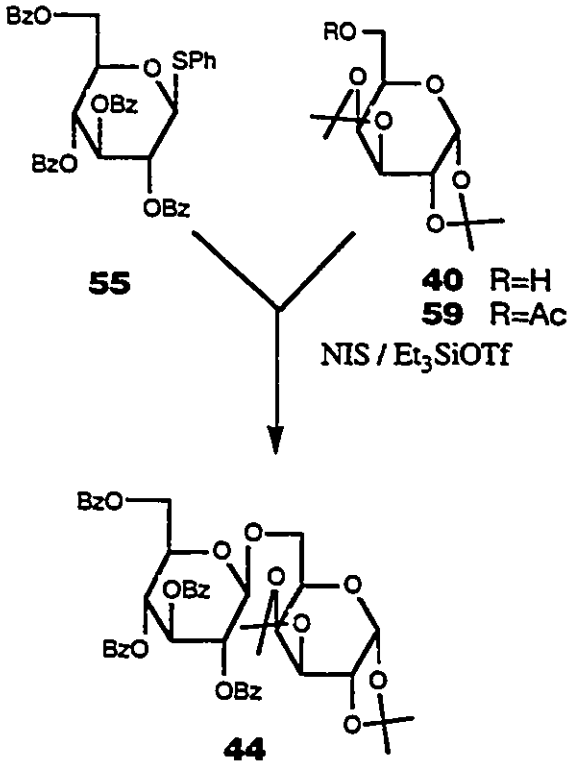


The catalyst t-Bu(Me)$_2$SiOTf was then replaced by Et$_3$SiOTf, according to Fraser-Reid[68], and a series of reactions using NIS (1.15 eq) and Et$_3$SiOTf (0.2 eq) (method J) was studied (Scheme 32). In the case of the more electron donating para-methoxyphenyl thioglycoside **12** the reaction was completed within 1 h and afforded 86 % overall yield of a mixture containing 79 % of the ß-D-disaccharide **43** and 21 % of the exo orthoester **41**. While under the same conditions, the reaction of the corresponding phenyl thioglycoside **11** was not over after 13 h, whereas the use of the electron withdrawing para-nitrophenyl thioglycoside **14** was not active under the same conditions after two days without any side reactions or hydrolysis of the aglycon **40**.

These results suggest that the concept of "active" and "latent" thioglycosyl donors is valid and that under these catalytic conditions (0.2 eq Et$_3$SiOTf) the more electron donating para-methoxyphenyl thioglycoside **12** reacts at a faster rate then the less reactive phenyl thioglycoside **11**.

SCHEME 33

ACTIVE and LATENT THIOglycosyl donors



The replacement of acetyl by benzoyl groups was then investigated to minimize orthoesters formation. Glycosidation of phenyl per-O-benzoyl-1-thio-ß-D-glucopyranoside **55** with 1,2:3,4-diisopropylidene galactopyranose **40** in the presence of NIS and a catalytic amount of Et_3SiOTf (method J) (Scheme 33) required and additional amount of alcohol **40** (0.5 eq), NIS (0.5 eq) and Et_3SiOTf (0.2 eq) after 8 h for completion. The reaction mixture afforded 92% isolated yield of the known[83] ß-D-disaccharide **44** after 32 h.

The structure was determined by ^1H- (figure 15) and ^{13}C-NMR analysis and was supported by COSY (figure 16) and HETCOR (figure 17) experiments. The ^1H-NMR spectrum of compound **44** confirmed the ß-anomeric configuration of the glucose residue at 5.02 ppm a doublet with a 7.8 Hz coupling constant. The anomeric proton of the galactose derivative appears as a doublet at 5.40 ppm with a cis coupling constant of 5.0 Hz.The complete NMR analysis can be refered to in the experimental section.

The following series of reactions were done using NIS and Et_3SiOTf (method J) with the adjusted ratio of NIS (1.5 eq), Et_3SiOTf (0.4 eq) in the presence of the alcohol acceptor 1,2:3,4-diisopropylidene galactopyranose **40** (1.5 eq). The results of the qualitative experiments showed that the reaction of phenyl-**11** and para-methoxyphenyl-**12**-per-O-acetyl-1-thio-ß-D-glucopyranoside was over in 15 min. The reaction of phenyl per-O-benzoyl-1-thio-ß-D-glucopyranoside **55** was not completed after 72 h neither was that of the N-acetylphenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **18**.

These results suggest that the use of benzoyl groups instead of acetyl groups effectively minimized the formation of orthoesters at the expense of a lower reaction rate. Moreover some unexpected complications occured when N-acetylphenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **18** was used as glycosyl donors, at least with NIS/Et_3SiOTf as promoter.

Figure 15 NMR spectrum of the disaccharide **44** at 300 MHz in $CDCl_3$

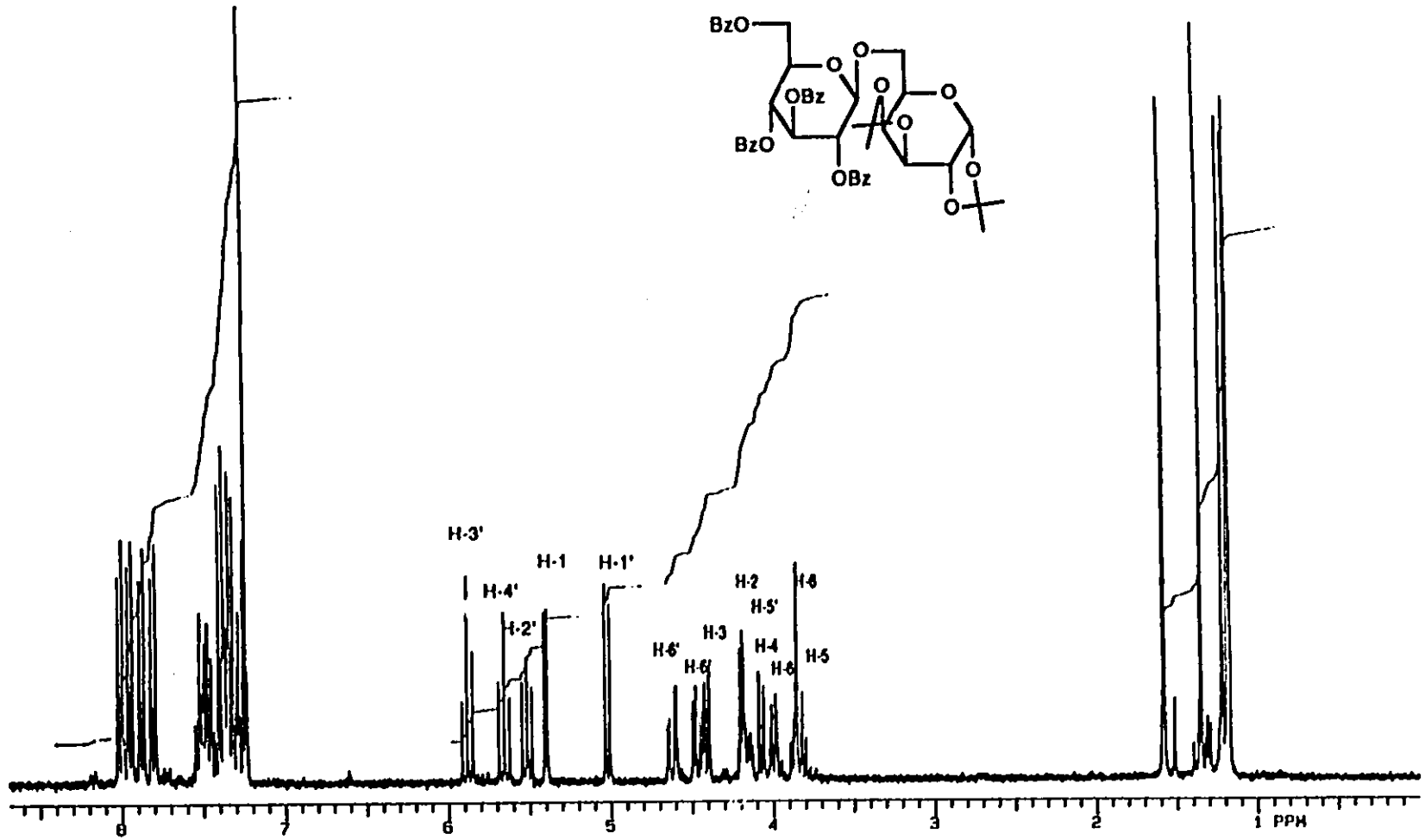


Figure 16 COSY spectrum of the disaccharide **44** in $CDCl_3$

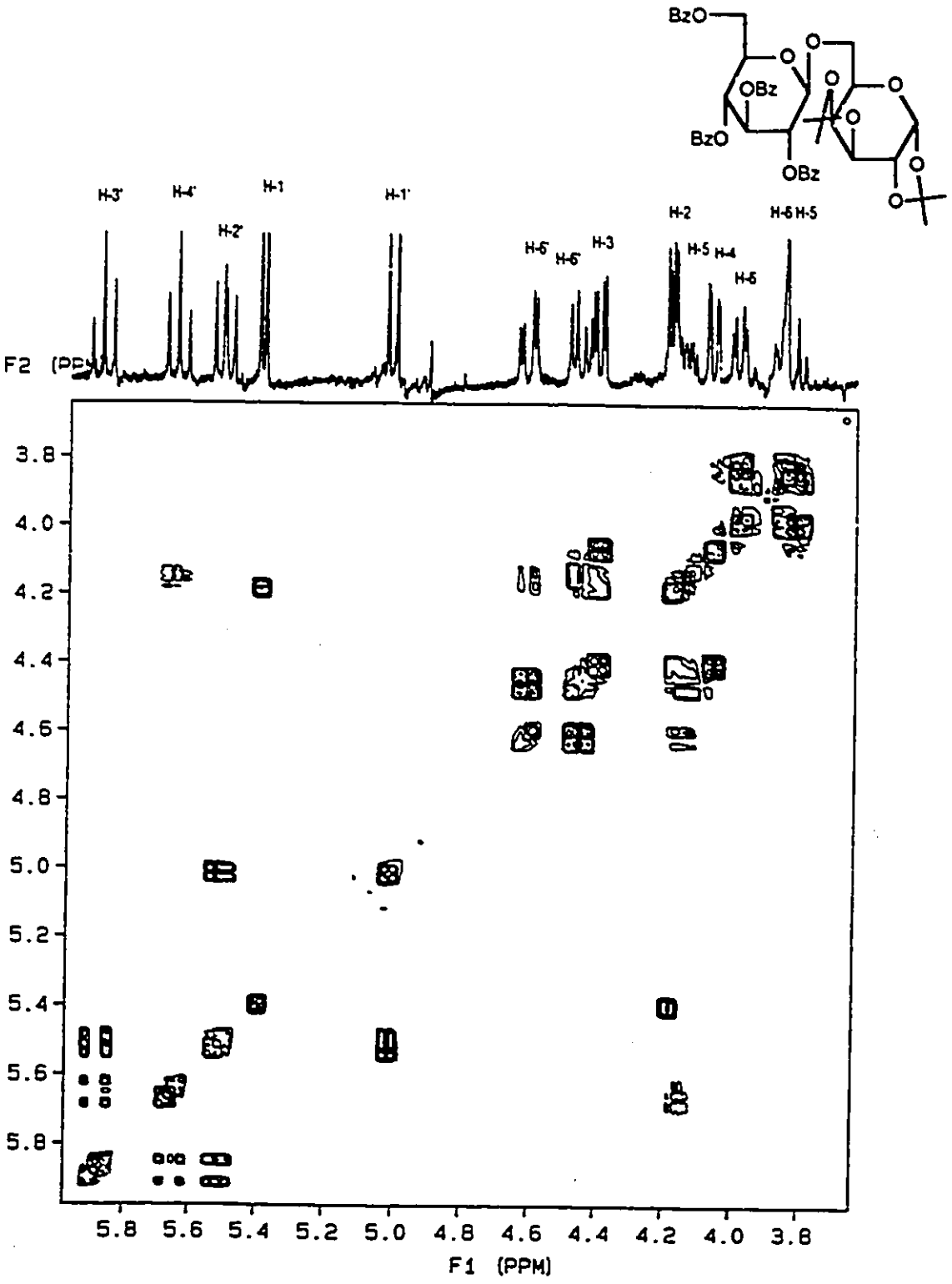
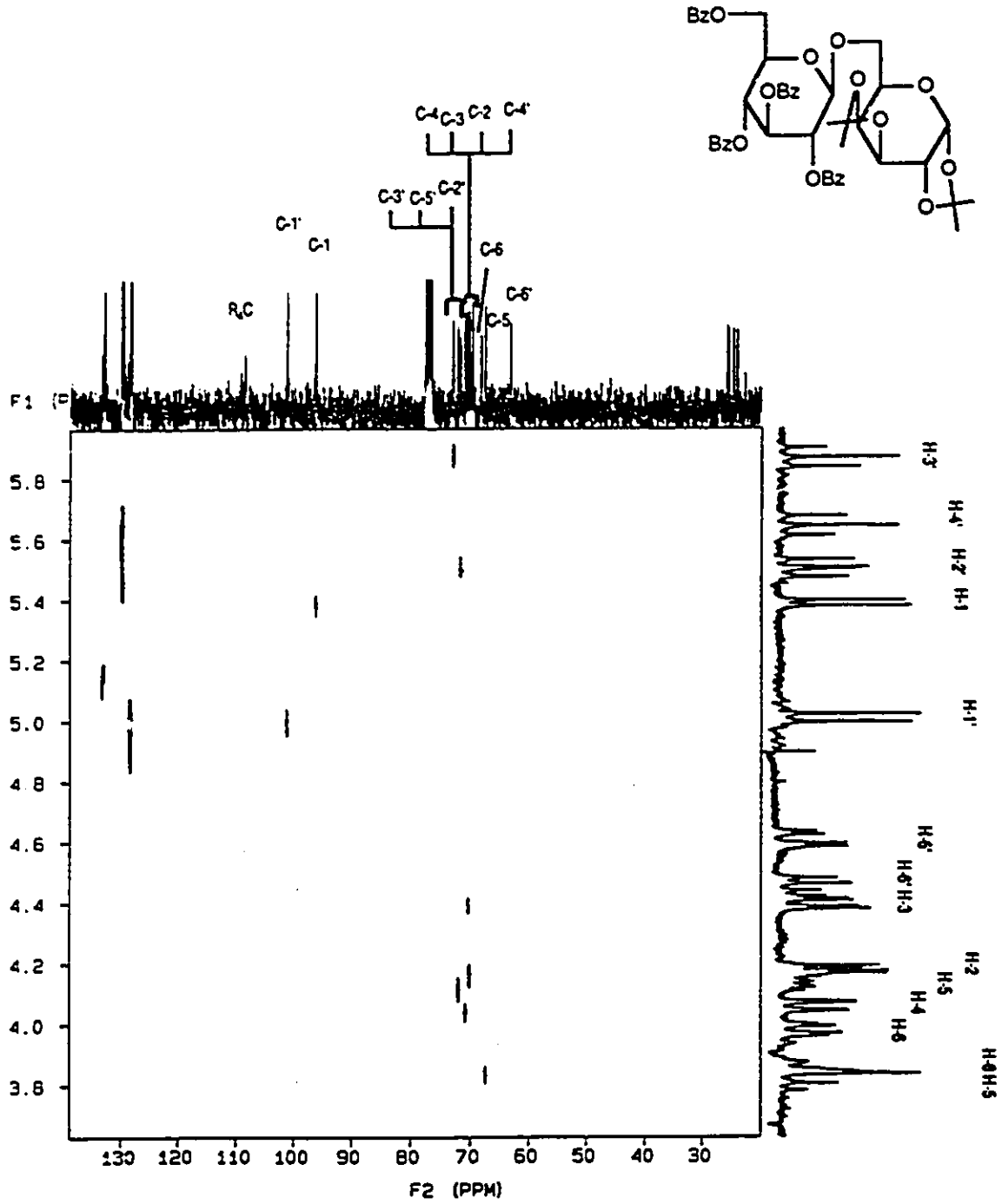


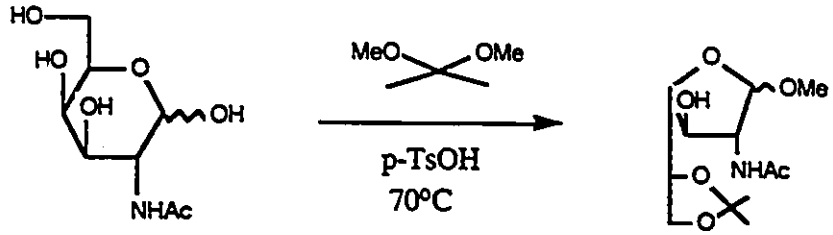
Figure 17 HETCOR spectrum of the disaccharide **44** in $CDCl_3$



The reaction of phenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **11** was repeated under the same conditions and afforded 46 % of the pure ß-D-disaccharide **43**. No orthoesther **41** was found by the initial use of 0.4 eq of Et_3SiOTf. The major side product was isolated and was found to be the acetylated aglycon **59**. The structure of the acetylated aglycon **59** was proved by the presence in the ^{1}H-NMR spectrum of an acetyl group at 2.06 ppm integrating for 3 hydrogens in addition to the 4 CMe groups at 1.49, 1.42, 1.31 and 1.30 ppm. The anomeric proton appeared at 5.51 ppm, a doublet, with a cis coupling constant of 5.0 Hz.

SCHEME 34

PREPARATION OF THE GLYCOSYL ACCEPTOR 47

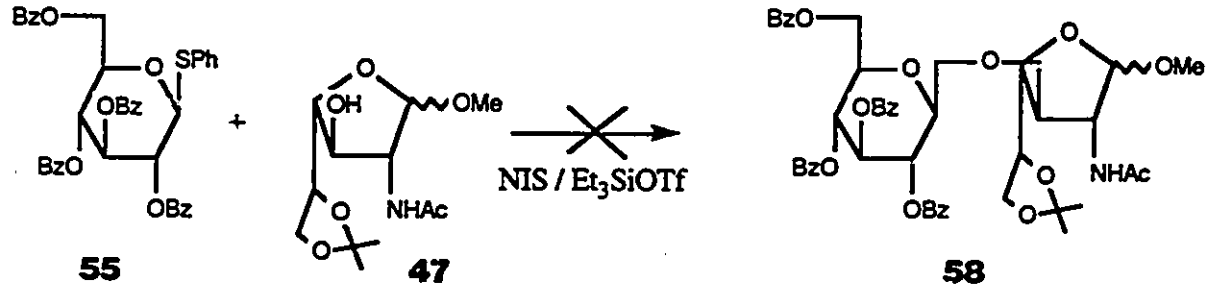


85% **47**

The glycosyl acceptor methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-D-galactofuranoside **47** was prepared for the synthesis of the disaccharide unit of *H. pleuropneumoniæ* serotype 4 CPS. The glycosyl acceptor **47** was synthesized as an α,ß-mixture (1:3) from the commercially available 2-acetamido-2-deoxy-D-galactose in 85 % yield (Scheme 34). The reaction consisted in the treatment of N-acetylgalactopyranoside with 2,2-dimethoxypropane and a catalytic amount of p-toluenesulfonic acid at 65-70 °C in dioxane for 5 h.

The structure of compound **47** was confirmed by the presence in the ^{1}H-NMR spectrum (200 MHz) of two singlets at 3.35 and 3.40 ppm corresponding to the OMe groups of the ß- and α- anomers respectively in addition to the two singlet at 1.41 and 1.37 ppm for the CMe_2 of the ß-anomer. The same functionality for the α-anomer was found at 1.41 and 1.34 ppm. The anomeric hydrogen for the ß-anomer is shown as an apparent singlet at 4.81 ppm, while that of the α-anomer appeared as a doublet at 4.89 ppm with a coupling constant of 5.3 Hz ($J_{1,2}$).

SCHEME 35



55 **47** **58**

The glycosidation of an anomeric mixture of methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-α,ß-D-galactofuranoside **47** with phenyl per-O-benzoyl-1-thio-ß-D-glucopyranoside **55** in the presence of NIS and Et_3SiOTf was attempted (method J) (Scheme 35). After 2 h only traces of two U.V. active products **45-46** were formed. Additional NIS (0.5 eq), Et_3SiOTf (0.2 eq) and thioglycoside **55** (0.5 eq) were added and the reaction mixture was stirred for one week. Since the reaction was not completed

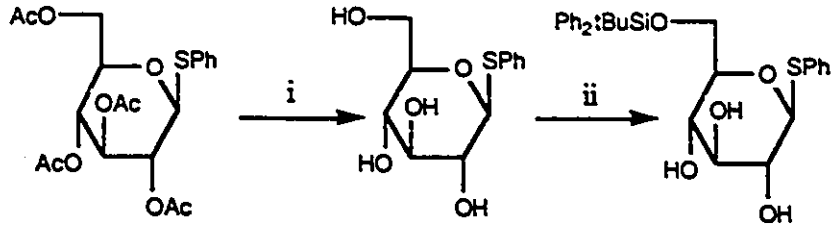
another 0.5 eq of NIS and 0.4 eq of Et_3SiOTf was further added and the reaction mixture was stirred for an additional 24 h. The two U.V. visible products **45-46** isolated were found to be the hydrolyzed products of the thioglycoside **55**.

The structures of the hydrolyzed products **45-46** were determined by proton NMR. The α-anomer **45** was easily assigned by the presence of the anomeric hydrogen at 6.33 ppm with a cis coupling constant of 3.8 Hz in addition to the hydroxyl group at 2.79 ppm a doublet, with a 6.5 Hz coupling constant as well as by the presence of aromatic hydrogenes. The ß-anomer **46** was similarly assigned. The hydroxyl group appeared in the region of 3.66-3.57 ppm as a multiplet while the anomeric hydrogen was found at 5.29 ppm a doublet of doublet coupling with the adjacent H-2 and the OH group with coupling constant of respectively 10.2 and 3.6 Hz. Both spectra are detailed in the experimental section.

II. Preparation of selectively protected glycosyl donors.

Different thioglycosyl donors with selected protecting groups were prepared for further coupling reactions or addition of the phosphate part as in the case of *H. pleuropneumoniæ* serotype 4 CPS.

SCHEME 36



84% cryst.
mp 133.3-134.1°C

65%
mp 61-64°C

11 **48** **49**

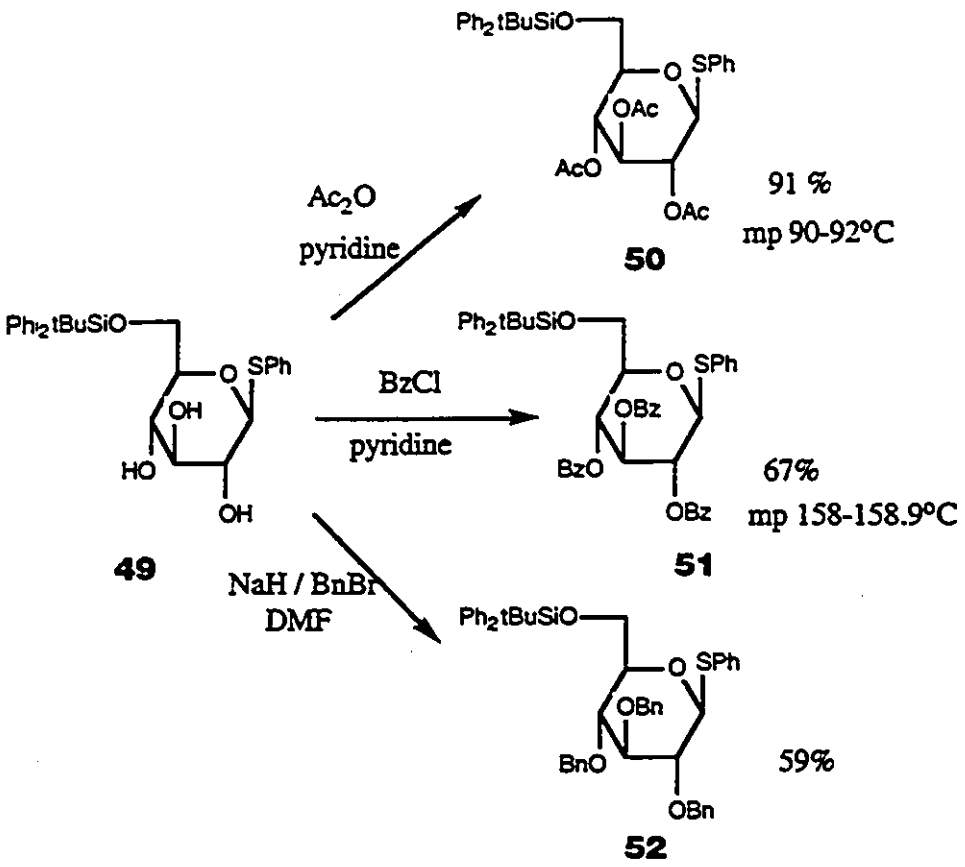
i NaOMe, MeOH

ii $tBu(Ph)_2SiCl$, Imidazole, DMAP, DMF

Phenyl per-O-acetyl-1-thio-ß-D-glucopyranoside **11** was deacetylated under standard Zemplén deacetylation procedure (cat. NaOMe, MeOH) to afford after crystalization compound **48** in 84 % yield (Scheme 36). The structure of the deacetylated thioglycoside **48** was confirmed by the absence in the proton NMR spectrum of the acetyl groups. The anomeric hydrogen was shown at 4.79 ppm with a trans-coupling constant of 10.0 Hz.

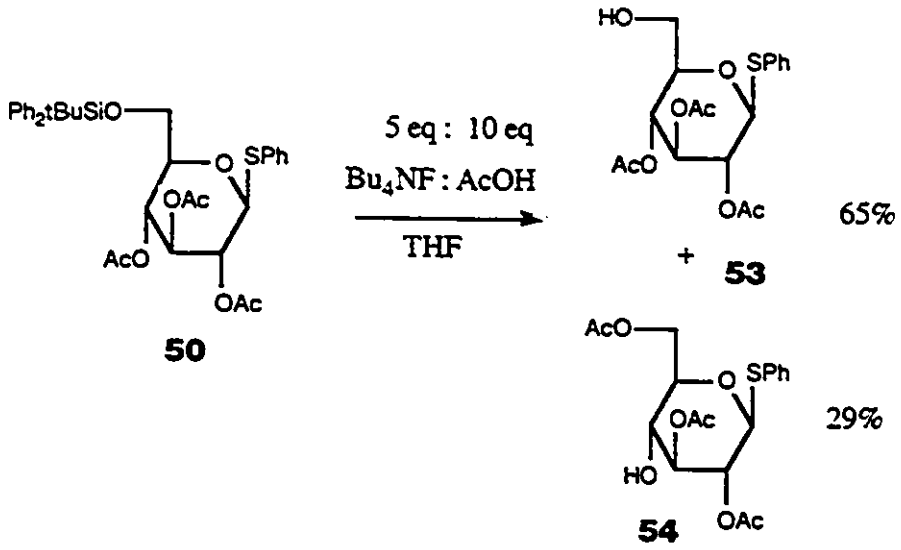
The primary hydroxyl group of 1-thiophenyl-ß-D-glucopyranoside **48** was selectively protected by treatment with tert-butyldiphenylsilyl chloride in the presence of imidazole and DMAP in DMF (Scheme 36). The reaction afforded, after column chromatography in (9:1) CH_2Cl_2: Et_2O, 65 % of pure phenyl-6-O-tert-butyldiphenylsilyl-1-thio-ß-D-glucopyranoside **49**. The structure was confirmed by additional aromatic hydrogens in the ^1H-NMR spectrum as well as by the presence of a singlet at 1.04 ppm for the t-butyl groups.

SCHEME 37



To study the effect of "armed" and "disarmed" thioglycosyl donors toward different thiophilic promoters the remaining hydroxyl groups were acetylated in acetic anhydride / pyridine to give **50** in 91 % yield or either treated with benzoyl chloride in pyridine to afford 67 % of compound **51**, or treated in a suspension of sodium hydride in DMF with benzyl bromide to give product **52** in 59 % yield (Scheme 37).

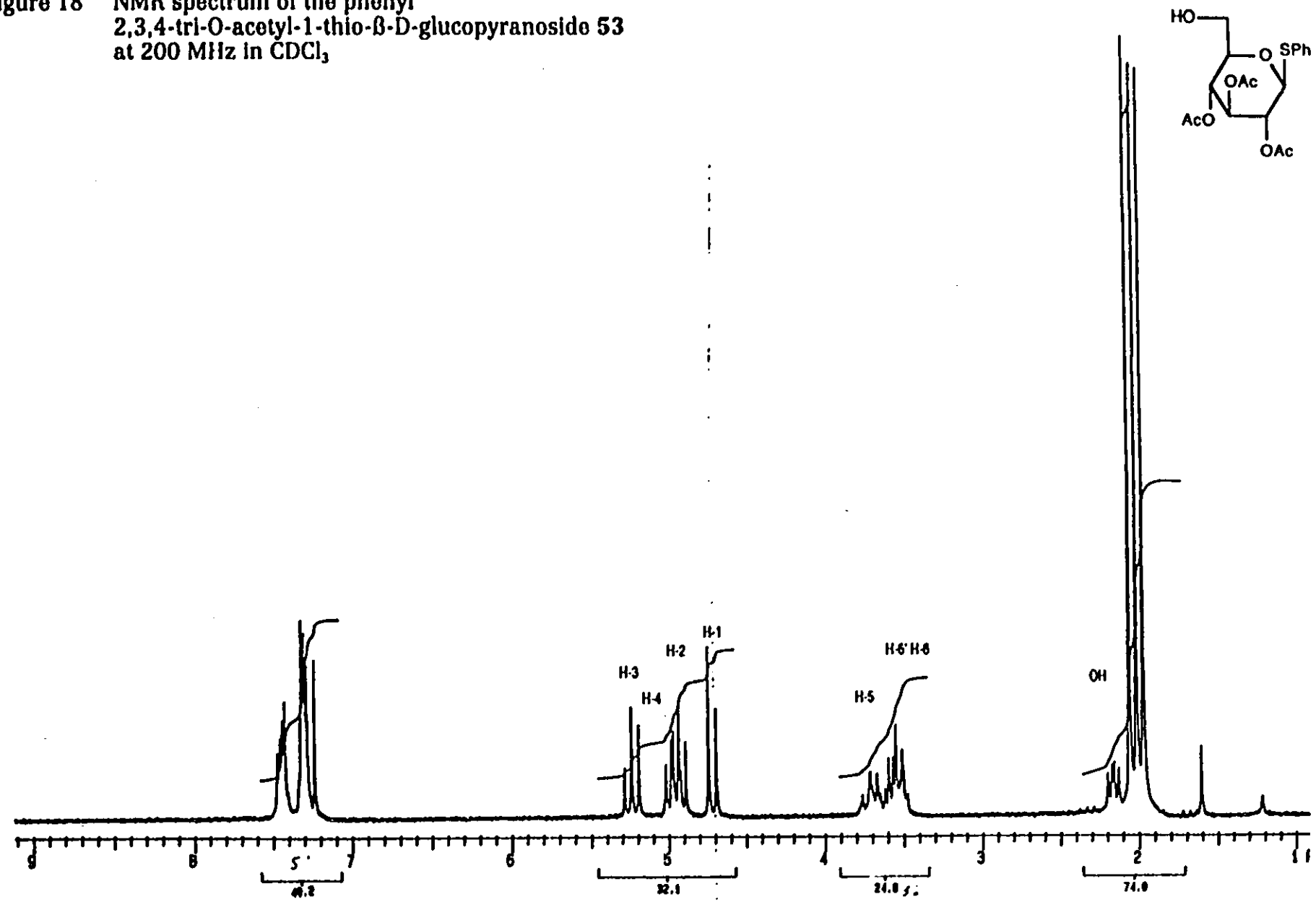
SCHEME 38



Selective deprotection of the silyl ether was optimized using initially 5 equivalents of a 1M solution of tetrabutylammonium fluoride in THF in the presence of 10 equivalents of 1M solution of acetic acid in THF (Scheme 38). Since the reaction was not over after 5 h, additional tetrabutylammonium fluoride (1 eq, 1M solution) and acetic acid (2 eq, 1M solution) were added and the reaction was complete after 13 h.The reaction gave 65 % of the expected phenyl 2,3,4-tri-O-acetyl-1-thio-ß-D-glucopyranoside **53** as well as 29 % of phenyl 2,3,6-tri-O-acetyl-1-thio-ß-D-glucopyranoside **54** the acetyl migration side product. When the reaction was performed in the absence of acetic acid the reaction mixture was contaminated with products resulting from extensive de-O-acetylation.The reaction performed a 0 °C was extremely slow.

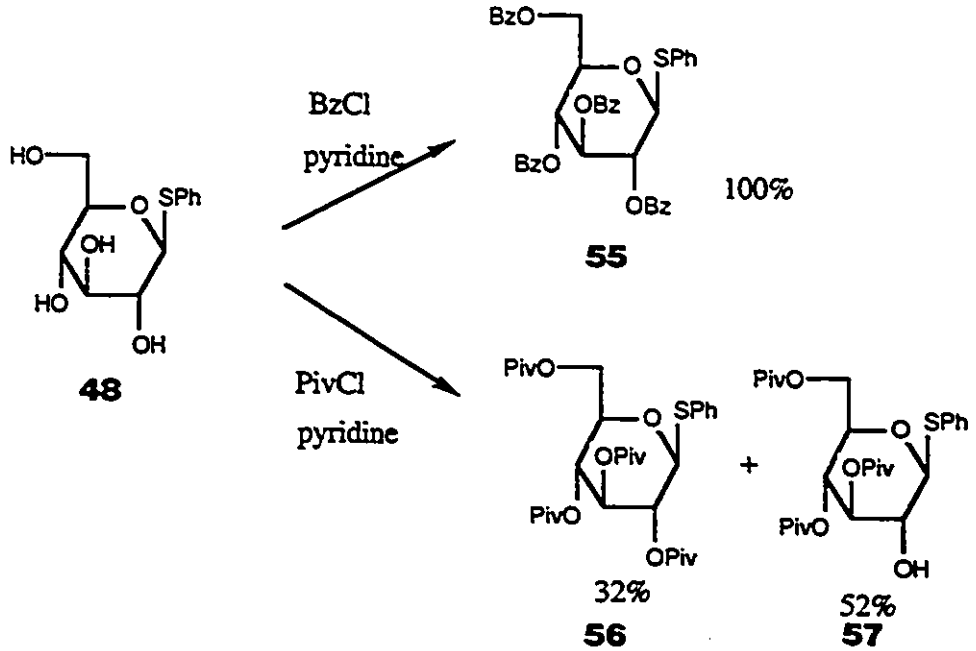
The structure of the alcohol **53** was confirmed by the presence in the proton NMR spectrum (figure 18) of the free hydroxyl group at 2.16 ppm, a doublet of doublet, with coupling constants of 5.4 and 8.2 Hz and by the absence of the methyl groups corresponding to the tert-butyl group. Similarly the alcohol **54** was shown by the presence of the hydroxyl group, in the ^{1}H-NMR spectrum, at 2.93 ppm a doublet (4.4 Hz) coupling with the adjacent H-4 and by the presence of three acetyl groups.

Figure 18 NMR spectrum of the phenyl 2,3,4-tri-O-acetyl-1-thio-ß-D-glucopyranoside **53** at 200 MHz in $CDCl_3$



To prevent orthoesters formation the per-benzoylated and pivaloylated phenyl thioglycosides derivatives 55 and 56 were prepared (Scheme 39).

SCHEME 39



The known phenyl per-O-benzoyl-1-thio-ß-D-glucopyranoside **55** was prepared in quantitative yield by treatment of the tetrol **48** with benzoyl chloride in pyridine. The structure was proven by the additional aromaticity in the proton NMR spectrum. The anomeric hydrogen is shown at 5.04 ppm, a doublet, with a ß-coupling constant of 10.0 Hz. A detailed analysis can be refered to in the experimental section.

Pivaloylation of the tetrol **48** in pyridine using pivaloyl chloride afforded after a long reaction time 32 % of the fully pivaloylated product **56** and 52 % of partly pivaloylated phenyl-3,4,6-tri-O-pivaloyl-1-thio-ß-D-glucopyranoside **57**.

The structure of the phenyl per-pivaloyl-thioglycoside **56** was confirmed by the presence, in the ^{1}H-NMR, of 4 singulets (1.19, 1.18, 1.12 and 1.07 ppm) each integrating for 9 hydrogens corresponding to the 4 CMe_3 groups. The assignments of the proton NMR spectrum (figure 19) of the partly pivaloylated compound **57** was based on COSY (figure 20). The structure was confirmed by the presence of a free hydroxyl group at 2.52 ppm, a doublet, coupling with the adjacent hydrogen, H-2, (3.2 Hz) and by the absence of a t-butyl group (1.19, 1.14 and 1.12 ppm) in its proton NMR spectrum.

Figure 19 NMR spectrum of the partly pivaloylated phenyl thioglycoside **57** at 300 MHz in $CDCl_3$

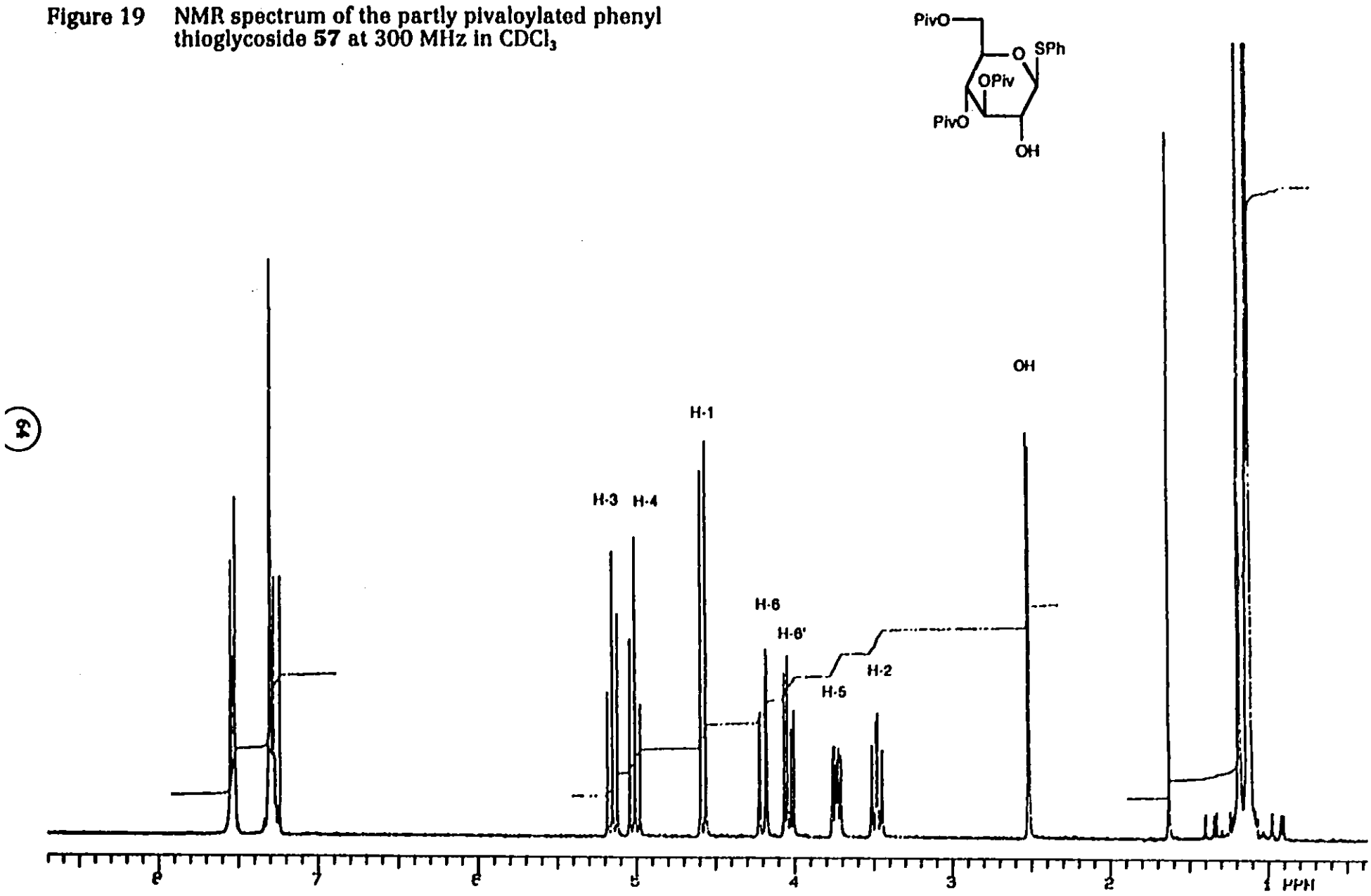
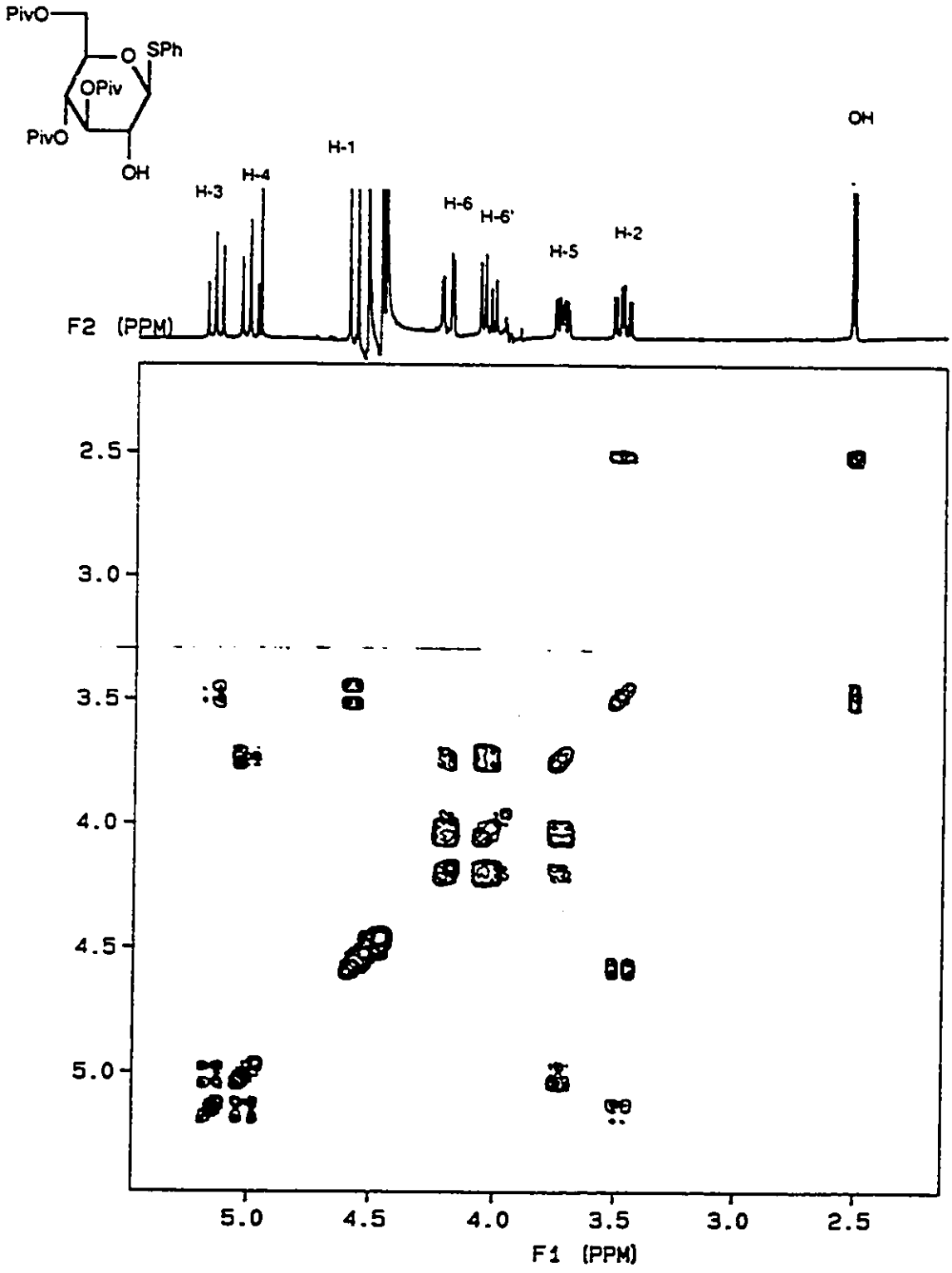


Figure 20 COSY spectrum of the partly pivaloylated phenyl thioglycoside **57** in $CDCl_3$



EXPERIMENTAL

General methods

Melting points were determined by use of a Gallenkamp digital melting point apparatus and are uncorrected. Mass spectra (M.S.) were recorded on an AEIMS 9025 instrument. The peak intensities are given as % of the base peak (100%) intensity. Optical rotations were measured using a Perkin Elmer 241 polarimeter in $CHCl_3$ unless otherwise stated. Combustion analyzes were performed by M-H-W Laboratories (Phoenix, AZ) or Guelph Chemical Laboratories Ltd (Guelph, Ont.).

Proton and carbon NMR spectra at 300 (or 200) and 75.4 MHz respectively were recorded on a Varian XL-300 (or XL Gemini 200) spectrometer. The proton and carbon chemical shifts were taken in deuterochloroform ($CDCl_3$), unless otherwise noted, relative to internal (deutero)chloroform at 7.24 and 77.0 ppm respectively. In ^{13}C spectra, the number of protons attached to each carbon were determined by DEPT or ADEPT spectra. The coupling patterns are noted as singlets (s), doublets (d), triplets (t), quartets (q), doublets of doublets (dd), doublets of doublets of doublets (ddd) or multiplets (m). ^1H-NMR simulations were performed using the standard NMR simulation package of the Varian XL-300 software (version 6.2, revision E). An initial spectrum was obtained using estimates of the chemical shifts (2D-COSY) and typical values for $J_{1,2}$ (7.5 Hz), $J_{2,3}$ (9 Hz), $J_{2,P}$, and $J_{3,P}$ (0 Hz) and a set value of $J_{1,P}$ (6.8 Hz) as measured from the ^{31}P NMR spectrum for compound **36**. This allowed initial assignment of the appropriate transitions. The measured experimental transition frequencies with their appropriate assignments were fixed in the simulation. Iteration on this data set gave the simulation shown in fig.1 and the final data are shown in table 6.

Column chromatography was accomplished using Merck 70-230 mesh silica gel as the adsorbent. Thin layer chromatography (TLC) was performed on Kieselgel 60 F-254 precoated silica gel plates of 0.25 mm thickness and visualized by means of U.V. and by charring with either $CeSO_4$ (1% w/v) / $(NH_4)_6Mo_7O_{24}$ (2.5% w/v) H_2SO_4 (10% aq.) reagent or a solution of 5% H_2SO_4 in MeOH containing isatin. Purifications by radial chromatography were performed on a Harrison Research Chromatotron model 7924 using silica gel coated rotors (1 mm and 2 mm thickness).

Tetrahydrofuran (THF) was distilled over sodium-benzophenone under a nitrogen atmosphere prior to use. Nitromethane, benzene, pyridine and ethyl acetate were distilled from calcium hydride under a nitrogen atmosphere. Dimethylformamide (DMF) was distilled (60 °C), under reduced pressure, from calcium sulfate. Methylene chloride was dried by distillation from phosphorus pentoxide under a nitrogen atmosphere Toluene, reagent grade quality, was dried over sodium. N-bromosuccinimide (NBS) was recrystallized from water. Zinc chloride was fused and poured into carbon tetrachloride. Sodium hydride was obtained as a 50% dispersion and was washed with hexanes prior to use. Organic solutions were evaporated with a Buchi evaporator connected to a water aspirator.

Synthesis of glycosyl donors.

ß-D-glucopyranose pentaacetate 1.

Compound **1** was synthesized according to known literature procedure[39]. The reaction gave 96% of a mixture containing 88% ß and 12% α anomers. Further recrystallization from 95% ethanol produced pure ß-D-glucopyranose pentaacetate **1**; m.p. 136.2-137.1°C; $[\alpha]_D$ +4.7 (C 1, $CHCl_3$); lit (32) m.p. 132 °C (cor.); $[\alpha]_D$ +4 (C 1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 5.69 (d, 1H, $J_{1,2}$= 7.8 Hz, H-1), 5.23 (dd, 1H, $J_{2,3}$= 9.0, $J_{3,4}$= 9.4 Hz, H-3), 5.11 (dd, 1H, $J_{1,2}$= 7.8, $J_{2,3}$= 9.0 Hz, H-2), 5.10 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.6 Hz, H-4), 4.27 (dd, 1H, $J_{5,6'}$= 4.4, $J_{6,6'}$= 12.5 Hz, H-6'), 4.08 (dd, 1H, $J_{5,6}$= 2.2, $J_{6,6'}$= 12.5 Hz, H-6), 3.81 (ddd, 1H, $J_{4,5}$= 9.6, $J_{5,6}$=2.2, $J_{5,6'}$=4.4 Hz, H-5), 2.09, 2.06, 2.01(2 x), 1.99 (4s, 5 x OAc).

Standard HBr-HOAc (45% w/v) procedure:

The per-O-acetylated sugars were dissolved in HBr/HOAc (45% w/v) (~ 3.6 ml/1 g sugar). The reaction mixture was stirred at room temperature until TLC indicated that the reaction was over. The reaction mixture was diluted with chloroform and washed successively with ice water and saturated sodium hydrogen carbonate. The organic extracts were dried with sodium sulfate, filtered, and evaporated in vacuo.

Acetobromoglucose 2.

Compound **2** was prepared by the standard HBr-HOAc (45% w/v) procedure in almost quantitative yield (99%). The product crystallized from anhydrous ether as long white needles; m.p. 88.9-89.4 °C; $[\alpha]_D$ +167.9 (C 1.27, $CHCl_3$); lit (38) m.p. 88-89 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm), 6.58 (d, 1H, $J_{1,2}$= 4.0 Hz, H-1), 5.53 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 9.9 Hz, H-4), 5.13 (dd, 1H, $J_{2,3}$= 10.1, $J_{3,4}$= 9.5 Hz, H-3), 4.81 (dd, 1H, $J_{1,2}$= 4.0, $J_{2,3}$= 10.1 Hz, H-2), 4.33-4.25 (m, 2H, H-6, H-6'), 4.14-4.07 (m, 1H, H-5), 2.08, 2.07, 2.02, 2.01 (4 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): δ (ppm): 170.59, 169.93, 169.87, 169.55 (C=O), 86.47 (C-1), 71.98, 70.42, 69.99, 66.97 (C-2,3,4,5), 60.77 (C-6), 20.41, 20.31 (4 x Ac).

M.S. (C.I. ether) (m/z): 414 (2.4%, $[M+2+H]^+$); 413 (16.1%, $[M+2]^+$); 412 (2.4%, $[M+H]^+$); 411 (16.5%, $[M]^+$); 371 (2.8%, $[M+2+H-Ac]^+$); 369 (3.1%, $[M+H-Ac]^+$); 353 (24.8%, $[M+2-HOAc]^+$); 351 (25.7%, $[M-HOAc]^+$); 331 (85.5%, $[M-HBr]^+$); 289 (65.7%, $[M-Br-Ac]^+$); 271 (19.1%, $[M-HBr-HOAc]^+$); 229 (41.4%, $[M-Br-Ac-HOAc]^+$); 211 (14.3%, $[M-HBr-2(HOAc)]^+$); 169 (100%, $[M-Br-Ac-2(HOAc)]^+$); 127 (25.1%,$[M-HBr-2(OAc)-2(Ac)]^+$); 109 (76.4%, $[M-Br-Ac-3(HOAc)]^+$).

Acetobromogalactose 34.

Compound **34** was prepared by the standard HBr-HOAc (45% w/v) procedure in quantitative yield. The product crystallized from anhydrous ether as long white needles; m.p. 81.5-83.3 °C; $[\alpha]_D$ +217.7 (C 1, $CHCl_3$); lit (84) m.p. 83-84 °C; $[\alpha]_D$ +219 (C 1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 6.67 (d, 1H, $J_{1,2}$= 3.9 Hz, H-1), 5.49 (dd, 1H, $J_{3,4}$= 3.3, $J_{4,5}$=1.2 Hz, H-4), 5.38 (dd, 1H, $J_{2,3}$= 10.6, $J_{3,4}$= 3.3 Hz, H-3), 5.02 (dd, 1H, $J_{1,2}$= 3.9, $J_{2,3}$= 10.6 Hz, H-2), 4.46 (ddd, 1H, $J_{4,5}$= 1.2, $J_{5,6}$= 6.3, $J_{5,6'}$= 7.0 Hz, H-5), 4.16 (dd, 1H, $J_{5,6}$= 6.3, $J_{6,6'}$= 11.4 Hz, H-6), 4.07 (dd, 1H, $J_{5,6'}$= 7.0, $J_{6,6'}$= 11.4 Hz, H-6'), 2.13, 2.09, 2.04, 1.99 (4 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.39, 170.13, 169.98, 169.83(C=O), 88.06 (C-1), 70.91, 67.81, 67.58, 66.80 (C-2,3,4,5), 60.66 (C-6), 20.50, 20.39, 20.33 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 414 (0.3%, $[M+2+H]^+$); 413 (3.1%, $[M+2]^+$); 412 (0.2%, $[M+H]^+$); 411 (3.0%, $[M]^+$); 353 (72.6%, $[M+2-HOAc]^+$); 351 (73.1%, $[M-HOAc]^+$); 331 (100%, $[M-HBr]^+$); 289 (66.6%, $[M-Br-Ac]^+$); 271 (1.9%, $[M-HBr-HOAc]^+$); 229 (8.3%, $[M-Br-Ac-HOAc]^+$); 211 (5.0%, $[M-HBr-2(HOAc)]^+$); 169 (42.3%, $[M-Br-Ac-2(HOAc)]^+$); 127 (12.8%, $[M-HBr-2(OAc)-2(Ac)]^+$); 109 (23.4%, $[M-Br-Ac-3(HOAc)]^+$).

Synthesis of glycosyl acceptors.

Allyl-2-acetamido-2-deoxy-α-D-galactopyranoside 3.

To a solution of N-acetylgalactopyranoside (1 eq) in dry allyl alcohol was added $BF_3.Et_2O$ (0.2 eq). The reaction mixture was refluxed for 3 h and stirred overnight at room temperature. The reaction mixture was then neutralized with triethylamine, evaporated and co-evaporated with ethanol under reduced pressure. Crystallization from absolute ethanol gave 47% of pure **3**; m.p. 193-194 °C; $[\alpha]_D$ +192.6 (C 1, MeOH); lit (45) m.p. 193-194 °C; $[\alpha]_D$ +213 (C 0.35, water).

^{1}H-NMR (200 MHz, D_2O): ∂ (ppm), 6.10-5.90 (m, 1H, $\underline{CH}=CH_2$), 5.43-5.25 (m, 2H, $CH=\underline{CH_2}$), 4.98 (d, 1H, $J_{1,2}$= 3.6 Hz, H-1), 4.29-3.76 (m, 8H, OCH_2-, H-2,3,4,5,6,6'), 2.03 (s, 3H, NHAc).

^{13}C-NMR (75.4 MHz, D_2O): ∂ (ppm), 171.81(C=O), 130.78 (C-1), 115.01 ($\underline{CH_2}$=CHR), 93.35, 68.11, 65.63, 64.83 (C-2,3,4,5), 65.58 (OCH_2), 58.36 (C-6), 47.07 ($\underline{CH}=CH_2$), 19.07 (Ac).

M.S. (C.I. ether) (m/z): 262 (100%, $[M+H]^+$); 230 (2.7%, $[M-OH-Me]^+$); 204 (68.2%, $[M-OCH_2CH=CH_2]^+$); 186 (9.2%, $[M-OCH_2CH=CH_2-H_2O]^+$); 168 (2.8%, $[M-OCH_2CH=CH_2-2(H_2O)]^+$); 150, (5.2%, $[M-OCH_2CH=CH_2-3(H_2O)]^+$).

Allyl-2-acetamido-2-deoxy-α-D-glucopyranoside 4.

Compound **4** was prepared under the same conditions as the corresponding galactose derivative **3**. Crystallization from absolute ethanol gave 65% of pure **4** ; m.p. 166.2-168 °C; $[\alpha]_D$ +145.6 (C 1, MeOH); lit (40) m.p. 172-174 °C; $[\alpha]_D$ +148.8 (C 1.62, water).

^{1}H-NMR (200 MHz, D_2O): ∂ (ppm), 8.20 (d, 1H, J=8 Hz, NH), 6.05-5.86 (m, 1H, -CH=CH_2), 5.39-5.22 (m, 2H, -CH=CH_2), 4.91 (d, 1H, $J_{1,2}$= 3.5 Hz, H-1), 4.22 (dd, 1H, $J_{5,6}$= 5.3, $J_{6,6'}$= 13.0 Hz, H-6), 4.02 (dd, 1H, $J_{5,6'}$= 6.0, $J_{6,6'}$= 13.0 Hz, H-6'), 3.96-3.68 (m, 5H, OCH_2-, H-2,3,4), 3.52-3.43 (m, 1H, H-5), 2.03 (s, 3H, NHAc).

^{13}C-NMR (75.4 MHz, D_2O): ∂ (ppm), 174.10 (C=O), 133.20 (C-1), 117.49 (CH_2=CHR), 95.66, 71.52, 70.61, 69.59 (C-2,3,4,5), 68.03 (OCH_2-), 60.10 (C-6), 53.23 (-CH=CH_2), 21.47 (AC).

M.S. (C.I. ether) (m/z): 262 (97.5%, $[M+H]^+$); 230 (0.7%,$[M-OH-Me]^+$); 204 (55.2%, $[M-OCH_2CH= CH_2]^+$); 186 (6.2%, $[M-OCH_2CH= CH_2-H_2O]^+$); 168 (4.9%, $[M-OCH_2CH= CH_2-2(H_2O)]^+$); 150 (27.8%, $[M-OCH_2CH= CH_2-3(H_2O)]^+$).

Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranoside 5.

A suspension of **3** (0.18 g, 1 eq) in benzaldehyde (3.5 ml) was treated with fused zinc chloride (0.19 g, 2 eq), and stirred under nitrogen for 24 h at room temperature. The mixture was poured into a cold mixture of 10% NH_4Cl solution (15 ml) and hexane (33 ml). After being kept for 1 1/2 h in an ice bath, the solid product that separated was filtered off and washed successively with water, ether, and hexane to give 99% of pure **5**. Recrystallization from methanol gave 0.18 g (75%) of **5** as white needles; m.p. 220.7-222 °C; $[\alpha]_D$ +136.2 (C 1,$CHCl_3$); lit (45) m.p. 223-225 °C; $[\alpha]_D$ +143 (C 0.84, ethanol).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.52-7.31 (m, 5H, Ph), 5.92-5.78 (m, 1H, -$\underline{CH}$=CH_2), 5.71 (d, 1H, J= 9.2 Hz, NH), 5.55 (s, 1H, PhCH), 5.28 (dd, 1H, J= 1.8, J= 15.5 Hz, $\underline{CH_2}$=CH-), 5.21 (dd, 1H, J= 1.8, J= 8.8 Hz, $\underline{CH_2}$=CH-), 4.98 (d, 1H, $J_{1,2}$= 3.8 Hz, H-1), 4.53-3.81 (m, 8H, OCH_2-, H-2,3,4,5,6,6'), 3.68 (d, 1H, J= 1.2 Hz, OH), 2.02 (s, 3H, Ac).

^{13}C-NMR (75.4 MHz, $CDCl_3$, DMSO): ∂ (ppm), 170.57 (C=O), 137.21 (C-ipso), 133.54 (C-1), 128.73 (C-para), 127.72 (2C, C-ortho), 126.21 (2C, C-meta), 117.14 ($\underline{CH_2}$=CH-), 101.47, 96.89, 81.90, 67.99 (C-2,3,4,5), 68.53 (OCH_2-), 68.09 (C-6), 62.60 (PhCH), 54.28 (-$\underline{CH}$=CH_2), 22.79 (Me).

M.S. (C.I. ether) (m/z): 350 (100%, $[M+H]^+$); 292 (31.7%, $[M-OCH_2CH=CH_2]^+$); 186 (4.3%, $[M-OCH_2CH=CH_2-PhCHO]^+$); 168 (2.8%, $[M-OCH_2CH=CH_2-PhCHO-H_2O]^+$); 126 (2.1%, $[M-OCH_2CH=CH_2-PhCHO-OH-Ac]^+$).

Allyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside 6.

Compound **6** was prepared in 89% yield following the method used for the galactose derivative product **5**. Recrystallization in methanol afforded white needles; m.p. 235.2-236 °C; $[\alpha]_D$ 90.4
(C 0.904, DMSO); lit (44) m.p. 234-237 °C; $[\alpha]_D$ +99 (C 1, DMF).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.50-7.31 (m, 5H, Ph), 5.87-5.78 (m, 2H, -CH=CH_2, NH), 5.54 (s, 1H, PhCH), 5.33-5.20 (m, 2H, CH_2=CH-), 4.86 (d, 1H, $J_{1,2}$= 3.8 Hz, H-1), 4.28-3.56 (m, 8H, OCH_2-, H-2,3,4,5,6,6'), 2.93 (d, 1H, J= 3.1 Hz, OH), 2.04 (s, 3H, Ac).

^{13}C-NMR (75.4 MHz, DMSO), 169.78 (C=O), 137.81 (C-ipso), 134.50 (C-1), 129.00 (C-para), 128.15 (2C, C-ortho), 126.50 (2C, C-meta), 116.99 (CH_2=CH-), 101.00, 96.95, 82.11, 67.37 (C-2,3,4,5), 68.09 (OCH_2-), 67.68 (C-6), 62.83 (PhCH), 54.29 (-CH=CH_2), 22.65 (Ac).

M.S. (C.I. ether) (m/z): 350 (100%, $[M+H]^+$); 292 (100%, $[M-OCH_2CH=CH_2]^+$); 274 (10.7%, $[M-OCH_2CH=CH_2-H_2O]^+$); 244 (21.5%, $[M-PhCHO]^+$); 214 (17.7%, $[M-HOCH_2CH=CH_2-C_6H_5]^+$); 186 (26.4%, $[M-OCH_2CH=CH_2-PhCHO]^+$); 168 (14.4%, $[M-OCH_2CH=CH_2-PhCHO-H_2O]^+$); 126 (23.3%, $[M-OCH_2CH=CH_2-PhCHO-OH-Ac]^+$).

Allyl 2-acetamido-2-deoxy-6-O-trityl-α-D-galactopyranoside 7.

A solution of **3** (1 eq) in pyridine was evaporated in vacuo (twice) followed by the addition of chlorotriphenylmethane (1.5 eq) and dry pyridine. The reaction mixture was kept for 4 days at room temperature, when TLC (10:1 chloroform-methanol) showed that **3** had been converted into 6-O-trityl derivative **7** (Rf 0.41). Evaporation and coevaporation with toluene gave a crude product which was purified by column chromatography eluted with 15:1 chloroform-methanol to give 53% of pure **7** as a white foam; m.p. 88 °C sintered; $[\alpha]_D$ +38.7 (C 1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.64-7.15 (m, 15H, Ph), 6.06 (d, 1H, J= 8.8 Hz, NH), 5.99-5.85 (m, 1H, -CH=CH_2), 5.33-5.18 (m, 2H,-CH=CH_2), 4.87 (d,1H, $J_{1,2}$= 3.7 Hz, H-1), 4.39-4.20 (m, 7H, OCH_2-, H-2,4,5,OH,OH), 3.71 (dd, 1H, $J_{2,3}$= 10.6, $J_{3,4}$= 2.7 Hz, H-3), 3.44 (dd, 1H, $J_{5,6'}$= 6.5, $J_{6,6'}$= 9.6 Hz, H-6'), 3.30 (dd, 1H, $J_{5,6}$= 5.1, $J_{6,6'}$= 9.6 Hz, H-6), 1.95 (s, 3H, Ac).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 172.64 (C=O), 143.86, 128.68, 127.87, 127.08 (3 x Ph), 133.55 (C-1), 117.97 (CH_2=CH-), 96.31, 86.82, 71.36, 69.23 (C-2,3,4,5), 68.94 (R_4C), 68.02 (OCH_2-), 63.33 (C-6), 50.62 (-CH=CH_2), 23.11 (Me).

M.S. (C.I. ether) (m/z): 504 (3%, $[M+H]^+$); 262 (28.1%, $[M+H-CH_2CH=CH_2-2(C_6H_5)-HOCH-OH]^+$); 244 (100%, $[M-Ph_3CO]^+$); 243 (100%, $[M-Ph_3COH]^+$); 204 (17.7%, $[M+H-Ph_3CO-CH_2CH=CH_2]^+$); 202 (5.8%, $[M+H-Ph_3CO-Ac]^+$); 186 (4.2%, $[M-Ph_3COH-OCH_2CH=CH_2]^+$).

Allyl 2-acetamido-2-deoxy-6-O-trityl-α-D-glucopyranoside 8.

Compound **8** was prepared according to the procedure described for the galactose derivative **7**, although the reaction time was reduced to one day. Purification under the same conditions afforded 42% of pure **8**; m.p. 86-88 °C; $[\alpha]_D$ +44.7 (C 1, $CHCl_3$); lit (44) m.p. 86-90 °C; $[\alpha]_D$ +44 (C 2.1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.46-7.21 (m, 15H, Ph), 5.89-5.83 (m, 2H, NH, -$\underline{CH}$=CH_2), 5.32-5.19 (m, 2H, -CH=$\underline{CH_2}$), 4.85 (d, 1H, $J_{1,2}$= 3.8 Hz, H-1), 4.20-3.28 (m, 8H, OCH_2-, H-2,3,4,5,6,6'), 3.16-3.15 (m, 1H, OH), 2.52 (d, 1H, J= 2.2 Hz, OH), 2.04 (s, 3H, Ac).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 172.02 (C=O), 146.92, 143.91, 128.72, 127.94, 127.87, 127.26, 127.08 (3 x Ph), 133.64 (C-1), 117.91 ($\underline{CH_2}$=CH-), 96.23, 86.72, 73.72, 72.30 (C-2,3,4,5), 70.47 (R_4C), 67.94 (OCH_2-), 63.61 (C-6), 53.36 (-$\underline{CH}$=CH_2), 23.11 (Me).

M.S. (C.I. ether) (m/z): 262 (4.5%, $[M+H-CH_2CH=CH_2-2(C_6H_5)-HOCH-OH]^+$); 244 (27.7%, $[M-Ph_3CO]^+$); 243 (100%, $[M-Ph_3COH]^+$); 204 (6.5%, $[M+H-Ph_3CO-CH_2CH=CH_2]^+$); 202 (3.9%, $[M+H-Ph_3CO-Ac]^+$); 186 (2.8%, $[M-Ph_3COH-OCH_2CH=CH_2]^+$).

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside 9.

Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside (0.62 g, 1 eq) was dissolved in a mixture of zinc chloride (0.54 g, 2 eq) and benzaldehyde (9 ml). The solution was kept overnight at room temperature. After addition of (30 ml) of diisopropyl ether with vigorous stirring, the solution was kept at -15 °C. After 3 h most of the product had precipitated and was filtered off. The filtrate was shaken with ice water whereupon a second crop precipitated, it was filtered and washed with 2-propanol. Both crops were recrystallized from methanol to give 75% of pure **9**; m.p. 261.7-262.6 °C

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.51-7.29 (m, 10H, 2 x Ph), 5.80 (d, 1H, J= 8.6 Hz, NH), 5.54 (s, 1H, PhCH), 4.91 (d,1H, 3.7 Hz, H-1), 4.73 (d, 1H, J= 11.9 Hz, OCH_2-), 4.47 (d, 1H, J= 11.9 Hz, OCH_2-), 4.25-4.16 (m, 2H, H-6, H-6'), 3.93-3.53 (m, 4H, H-2,3,4,5), 3.03-3.00 (m, 1H, OH), 1.98 (s, 3H, Ac).

^{13}C-NMR (75.4 MHz, DMSO): ∂ (ppm), 169.75 (C=O), 137.80, 137.77, 129.00, 128.37, 128.14, 127.75, 127.70, 126.49 (2 x Ph), 100.98 (C-1), 97.02, 82.13, 67.35, 62.96, (C-2,3,4,5), 68.69 (OCH_2-), 68.09 (C-6), 54.31 (PhCH), 22.62 (Ac).

M.S. (C.I. ether) (m/z): 400 (50.5%, $[M+H]^+$); 292 (26.3%, $[M-OCH_2Ph]^+$); 202 (17.2%, $[M-OCH_2Ph-PhCH]^+$); 186 (2.2%, $[M-OCH_2Ph-PhCHO]^+$).

Methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-α/ß-D-galactofuranoside 47.

2-Acetamido-2-deoxy-D-galactose (1 eq) was treated with 2,2-dimethoxypropane (7 eq) and para-toluenesulfonic acid (0.16 eq) at 65-70 °C in dioxane for 5 h. The reaction mixture was quenched with triethylamine and the solvent was removed under reduced pressure. Column chromatography (ethyl acetate) of the syrupy residue gave the desired product **47** as an α, ß mixture in 85 % yield. The product was composed of 75% ß-anomer and 25% of the α-anomer; m.p. 132.1-136.5 °C; $[\alpha]_D$-26.3 (C 1, $CHCl_3$); lit (85) $[\alpha]_D$ -38.5 (C 1, $CHCl_3$).

Major product ß-anomer:

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 6.74 (d, 1H, J= 7.9 Hz, NH), 4.81 (s, 1H, H-1), 4.36-3.81 (cm, H-2,3,4,5,6,6'), 3.40-3.35 (s, 1H, OH), 3.35 (s, 3H, OMe), 3.83 (s, 3H, NHAc), 1.41, 1.37 (2s, 6H, CMe_2).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 169.49 (C=O), 108.19 (C-1), 84.77, 77.92, 75.61, 60.41 (C-2,3,4,5), 65.64 (C-6), 55.00 (OMe), 26.51 (Ac), 25.92 (2 x Me), 23.06 (R_4C).

Minor product α-anomer:

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 6.19 (d, 1H, J= 5.1 Hz, NH), 4.89 (d, 1H, $J_{1,2}$= 5.3 Hz, H-1), 4.65 (s, 1H, OH), 4.36-3.81 (cm, H-2,3,4,5,6,6'), 3.40 (s, 3H, OMe), 2.01 (s, 3H, NHAc), 1.41, 1.34, (2s, 6H, CMe_2).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 172.59 (C=O), 101.71 (C-1), 84.27, 77.72, 77.21, 61.17 (C-2,3,4,5), 64.98 (C-6), 55.26 (OMe), 25.52 (2 x Me), 25.38 (Ac), 22.82 (R_4C).

M.S. (C.I. ether) (m/z): 276 (100%, $[M+H]^+$); 260 (3.1%, $[M-Me]^+$); 244 (85.7%, $[M-OMe]^+$); 226 (63.9%, $[M-OMe-H_2O]^+$); 168 (23.5%, $[M-OMe-H_2O-NHAc]^+$); 126 (7%, $[M-OMe-H_2O-NHAc-CMe_2]^+$).

General glycosidation procedures:

Mercuric cyanide (Helferich):

The appropriate aglycon (0.075mmol, 1 eq) was dissolved in 1:1 anhydrous nitromethane-benzene (10 ml) containing 4 A° molecular sieve. To ensure dryness, the solution was concentrated by distillation at atmospheric pressure until 6 ml of distillate had been collected in a Dean-Stark trap. Mercuric cyanide (0.070mmol, 0.93 eq) was added and the temperature of the mixture was adjusted to 70 °C. While maintaining this temperature under nitrogen, a solution of acetobromoglucose 2 (0.090mmol, 1.2 eq) in benzene (1 ml) was added dropwise. The mixture was stirred for 4 h at 70 °C, allowed to cool to room temperature, and diluted with benzene. The solution was then successively washed with 10% aqueous potassium iodide, saturated aqueous sodium hydrogen carbonate and water, then dried (magnesium sulfate). The organic layer was evaporated to afford a white precipitate that was chromatographed on a preparative silica gel plate with an appropriate ratio of ethyl acetate-hexane as eluant.

Silver triflate (Koenigs-Knorr):

The appropriate aglycon (1eq) was stirred, in methylene chloride containing 4 A° molecular sieve, silver triflate (1.2 eq) and either collidine (1.2 eq) or TMU (1.2 eq), for a few hours, under a nitrogen atmosphere. To this mixture was added acetobromoglucose 2 (1.2 eq) and the reaction mixture was stirred for 24 hours. More bromide was added (0.5 eq) as well as silver triflate (0.5 eq) and collidine (or TMU) (0.5 eq). The reaction mixture was stirred until TLC indicated that the reaction was over. The reaction mixture was filtered and partioned between dichloromethane and sodium thiosulfate solution (10%). The organic phase was dried (magnesium sulfate) and evaporated to give a white precipitate. The crude product was separated on either preparative silica gel plate or radial chromatography using the appropriate ratio of ethyl acetate-hexane.

Trimethyl silyl triflate:

A solution of ß-D-glucopyranose pentaacetate **1** and the aglycon was stirred under a nitrogen atmosphere in dichloromethane containing 4 A° molecular sieve for 1 h. TMSiOTF was then added dropwise and the reaction mixture was stirred until TLC indicated that the reaction was over. Triethylamine was added and the reaction mixture was evaporated and chromatograph on a preparative plate using a ratio of (7:3) ethyl acetate-hexane.

Silver triflate and bromine:

A solution of the thioglycoside (1.25 eq) and the glycosyl acceptor (1 eq) in dichloromethane was stirred in the presence of molecular sieve 4 A° for one hour. The promoter silver triflate (2 eq) was added followed after 20 min. by bromine. The reaction mixture was stirred at room temperature for 24 h. Since no reaction occured, more silver triflate was added (2 eq) and the reaction mixture was stirred for several days without any progress.

Synthesis of 1-Thioglycoside

IN A CATALYTIC TWO PHASE SYSTEM:

General Procedure

Method A :

A solution of acetobromoglucose (1 eq) in benzene (0.5 ml/100 mg bromide) was added to a stirred solution of thiol (2 eq) in benzene (0.5 ml), sodium hydroxide (1.1 eq) and tetrabutylammonium hydrogen sulfate (0.25 eq) in water (0.2 ml), and the mixture was stirred at room temperature until TLC indicated complete transformation of the glycosyl bromide. The mixture was diluted with benzene and the organic phase was successively washed with saturated sodium hydrogen carbonate, water, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford the crude thioglycosides. The reaction time and yield are reported in table 1.

Method B:

To a solution of the appropriate thiol (3 eq), and tetrabutylammonium hydrogen sulfate (1 eq) in 1M aqueous sodium carbonate (1 ml/100 mg bromide) was added a solution of acetobromoglucose (1 eq) in ethyl acetate (1 ml /100 mg bromide). The two-phase reaction mixture was vigorously stirred at room temperature until TLC indicated complete transformation of the bromide. Ethyl acetate was then added (5 ml). The organic phase was successively washed with saturated sodium hydrogen carbonate, water and saturated sodium chloride. The combined organic extracts were dried with sodium sulfate, filtered and evaporated under reduced pressure to afford the crude thioglycoside **10-17**. The products were purified by crystalization or by chromatography. The reaction time and yield are reported in table 1.

BY LEWIS ACID CATALYSIS

General Procedure

Method C:

To a cool (0 °C) solution of ß-D-glucopyranose pentaacetate (1 eq) in dichloromethane was added the appropriate thiol (1.2 eq) followed by tin tetrachloride (0.15 eq). The reaction mixture was stirred at 0 °C for 3h after which time TLC indicated complete transformation of the ß-D-glucopyranose pentaacetate. The reaction mixture was then diluted with dichloromethane. The organic phase was successively washed with 1M sodium hydroxide and water. The combined organic extracts were dried with magnesium sulfate, filtered and evaporated under reduced pressure. The reaction yield is reported in table1.

Ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 10.

Method C

The residue obtained from the organic extract was directly crystallized from absolute ethanol to provide pure **10** as white long needles; m.p. 82.4 - 83.8 °C; $[\alpha]_D$ - 26.2 (C 1, $CHCl_3$); lit (86) m.p. 84-85 °C; $[\alpha]_D^{22}$ - 27.9 (C 1.9, $CHCl_3$); lit (74) m.p. 78-79 °C; $[\alpha]_D^{20}$ - 38.3 (C 0.78, $CHCl_3$).

Anal. calc. for $C_{16}H_{24}O_9S$: C, 48.97; H, 6.16; S, 8.17
found: C, 49.11; H, 5.97; S, 7.94

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 5.20 (dd, 1H, $J_{2,3}$= 9.1, $J_{3,4}$= 9.3 Hz, H-3), 5.05 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 9.8 Hz, H-4), 5.00 (dd, 1H, $J_{1,2}$= 9.9, $J_{2,3}$= 9.1 Hz, H-2), 4.46 (d, 1H, $J_{1,2}$= 9.9 Hz, H-1), 4.22 (dd, 1H, $J_{5,6'}$= 4.8, $J_{6,6'}$= 12.4 Hz, H-6'), 4.09 (dd, 1H, $J_{5,6}$=2.5, $J_{6,6'}$= 12.4 Hz, H-6), 3.68 (ddd, 1H, $J_{4,5}$= 9.8, $J_{5,6}$= 2.5, $J_{5,6'}$= 4.8 Hz, H-5), 2.68 (q, 1H, J= 7.6 Hz, H-CHMe), 2.66 (q, 1H, J= 7.4 Hz, H-CHMe), 2.04, 2.02, 1.99, 1.97 (4s,OAc), 1.23 (dd, 3H, J= 7.4, J= 7.6 Hz, Me)

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.56, 170.12, 169.33 (2C) (C=O), 83.44 (C-1), 75.79 (C-5), 73.83 (C-3), 69.75 (C-2), 68.25 (C-4), 62.09 (C-6), 24.10 (CH_2), 20.66 (2C), 20.54, 20.52 (4 x Ac), 14.75 (CH_3).

M.S. (C.I. ether) (m/z): 393 (3.9%, $[M+H]^+$); 331 (65.6%, $[M-SEt]^+$); 271 (36.2%, $[M-SEt-HOAc]^+$); 213 (30.0%, $[M-SEt-2(OAc)]^+$).

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 11.

Method A and C.

The crude residue obtained from the organic extract was directly crystallized from absolute ethanol to provide pure **11** as white needles;

Method B

The residue was purified through radial chromatography eluted with 2% ethanol in dichloromethane to give 80% yield. Compound 11 crystallized from absolute ethanol; m.p. 118.2-118.5 °C; $[\alpha]_D$-17.1 (C 1, $CHCl_3$); lit. (70) m.p.116-118°C; $[\alpha]_D$ -14.9 (C 1, $CHCl_3$); lit (86) m.p. 121-122 °C; $[\alpha]_D^{22}$-18.1 (C 2.3, $CHCl_3$).

Anal. calc. for $C_{20}H_{24}O_9S$: C, 54.54; H, 5.49: S, 7.28
found: C, 54.43; H, 5.48; S, 7.06

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.50-7.45 (m, 2H, Ph-meta), 7.31-7.28 (m, 3H, Ph-ortho, para), 5.20 (dd, 1H,$J_{2,3}$= 9.1, $J_{3,4}$= 9.2 Hz, H-3), 5.02 (dd, 1H, $J_{3,4}$= 9.2, $J_{4,5}$= 9.9 Hz, H-4), 4.95 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.1 Hz, H-2), 4.68 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 4.19-4.16 (m, 2H, H-6, H-6'), 3.70 (ddd, 1H, $J_{4,5}$= 9.9, $J_{5,6}$= 3.0, $J_{5,6'}$= 4.7 Hz, H-5), 2.06, 2.05, 1.99, 1.96 (4s, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.48, 170.08, 169.30, 169.16 (C=0), 133.04 (2C, C-meta), 131.55 (C-ipso), 128.86 (2C, C-ortho), 128.34 (C-para), 85.65 (C-1), 75.72 (C-5) , 73.88 (C-3) , 69.85 (C-2) , 68.13 (C-4) , 62.06 (C-6), 20.67 (2C), 20.51 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 441 (0.9 %, $[M+H]^+$); 381 (3.7 %, $[M-OAc]^+$); 331 (100%, $[M-SPh]^+$); 271 (32.4%, $[M-SPh-HOAc]^+$); 213 (33.4%, $[M-SPh-2(OAc)]^+$).

Para-methoxyphenyl 2,3,4,6-tetra-O-acetyl -1-thio-ß-D-glucopyranoside 12.

Method A,B

The crude reaction mixture was purified by radial silica gel chromatography using 1.5% tert-butanol in methylene chloride as eluant to afford the pure thioglycoside where crystallization from benzene-hexane gave fine white needles of analytically pure **12**; m.p. 100.6-101.0 °C; $[\alpha]_D$-26.43 (C 0.98, $CHCl_3$).

Anal. calc. for $C_{21}H_{26}O_{10}S_1$: C, 53.60; H, 5.57; S, 6.80
found: C, 53.76; H, 5.68; S, 6.64

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.42 (d, 2H, J= 8.9 Hz, Ph-meta), 6.82 (d, 2H, J= 8.9 Hz, Ph-ortho), 5.17 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.3 Hz, H-3), 4.97 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 10.0 Hz, H-4), 4.86 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.2 Hz, H-2), 4.52 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 4.16 (d, 2H, $J_{5,6}$= 3.7, $J_{5,6'}$= 3.7 Hz, H-6, H-6'), 3.79 (s, 3H, OMe), 3.65 (dt, 1H, $J_{4,5}$= 10.0, $J_{5,6}$= 3.7, $J_{5,6'}$= 3.7 Hz, H-5), 2.08, 2.05, 1.98, 1.96 (4s, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.49, 170.12, 169.31, 169.17 (C=O), 160.36 (C-para), 136.47 (2C, C-ortho), 120.73 (C-ipso), 114.31 (C-meta), 85.57 (C-1), 75.64 (C-5), 73.98 (C-3), 69.78(C-2), 68.07 (C-4), 61.98 (C-6), 55.25 (OCH_3), 20.74, 20.69, 20.52 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 471 (0.3%, $[M+H]^+$); 411 (4.3%, $[M-OAc]^+$); 331 (100%, $[M-SPhOMe]^+$); 291 (15.2%, $[M-OAc-2(HOAc)]^+$); 271 (30.3%, $[M-SPhOMe-HOAc]^+$); 213 (22.5%, $[M-SPhOMe-2(OAc)]^+$).

Para-methylphenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 13.

Method A,B.

The syrupy residue was purified by silica gel chromatography with 2% tert butanol in methylene chloride as eluant. The thioglycoside **13** crystallyzed from absolute ethanol to give white needles; m.p. 115.5-116.1 °C; $[\alpha]_D$-17.2 (C 1, $CHCl_3$).

Anal. calc. for $C_{21}H_{26}O_9S$: C, 55.49; H, 5.77; S, 7.04
found: C, 55.30; H, 5.82; S, 6.82

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.36 (d, 2H, J= 8.0 Hz, Ph-meta), 7.10 (d, 2H, J= 8.0 Hz, Ph-ortho), 5.18 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.3 Hz, H-3), 5.00 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 9.8 Hz, H-4), 4.91 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.2 Hz, H-2), 4.60 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 4.18-4.16 (m, 2H, H-6, H-6'), 3.67 (ddd, 1H, $J_{4,5}$= 9.8, $J_{5,6}$= 3.1, $J_{5,6'}$= 4.3 Hz, H-5), 2.32 (s, 3H, Me), 2.07, 2.06, 1.99, 1.96 (4s, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.49, 170.11, 169.30, 169.16 (C=O), 138.72, 133.76 (2C), 129.60 (2C), 127.47 (6 x C-aromatic), 85.75 (C-1), 75.67 (C-5), 73.94 (C-3), 69.83 (C-2), 68.12 (C-4), 62.05 (C-6), 21.11 (Me), 20.70, 20.66, 20.51 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 455 (0.4%, $[M+H]^+$); 395 (5.1%, $[M-OAc]^+$); 331 (100%, $[M-SPhMe]^+$); 275 (17.9%, $[M-OAc-2(HOAc)]^+$); 271 (38.7%, $[M-SPhMe-HOAc]^+$); 213 (34.1%, $[M-SPhMe-2(OAc)]^+$).

Para-nitrophenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 14.

Method A,B

The reaction mixture was purified through radial chromatography on silica gel eluted with 2% tert-butanol in methylene chloride. Crystallization from absolute ethanol gave yellowish needles; m.p. 182.5-183 °C; $[\alpha]_D$-21.75 (C 1.03, $CHCl_3$).

Anal. calc. for $C_{20}H_{23}O_{11}N_1S_1$: C, 49.50; H, 4.74; S, 6.61; N, 2.89
found : C, 49.57; H, 4.94; S, 6.72; N, 2.83

^{1}H-NMR (200 MHz, $CDCl_3$); ∂ (ppm),8.11 (d, 2H, J= 9.0 Hz, Ph-meta), 7.55 (d, 2H, J= 9.0 Hz, Ph-ortho), 5.24 (dd, 1H, $J_{2,3}$= 9.0, $J_{3,4}$= 9.3 Hz, H-3), 5.03 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 10.0 Hz, H-4), 5.00 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.0 Hz, H-2), 4.83 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 4.27-4.09 (m, 2H, H-6, H-6'), 3.79 (ddd, 1H, $J_{4,5}$= 10.0, $J_{5,6}$= 2.9, $J_{5,6'}$= 5.0 Hz, H-5), 2.06, 2.03, 1.99, 1.96 (4s, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$); ∂ (ppm), 170.33, 169.97, 169.26, 169.13 (C=O), 146,96, 141.62, 130.94 (2C), 123.81 (2C) (C-arom.), 84.27 (C-1), 56.09 (C-5), 52.49 (C-3), 46.50 (C-2), 47.54 (C-4), 40.60 (C-6), 20.68, 20.59, 20.48 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 486 (13.2%, $[M+H]^+$); 426 (9.2%, $[M-OAc]^+$); 366 (3.3%, $[M-OAc-HOAc]^+$); 331 (100%, $[M-SPhNO_2]^+$); 306 (26.7%, M-OAc-2(HOAc)]$^+$); 271 (100%, $[M-SPhNO_2-HOAc]^+$); 213 (100%, $[M-SPhNO_2-2(OAc)]^+$).

2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl ethylxanthate 15.

Method A,B

The product was purified through radial chromatography, eluant 1% tert-butanol in dichloromethane (method A) or 1% ethanol in dichloromethane (method B) m.p. 82.7-84.8 °C; $[\alpha]_D$ +13.7 (C 1, $CHCl_3$); lit (87) m.p. 75-76 °C; $[\alpha]_D$ +30 (C 1.2, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$) : ∂ (ppm), 5.43 (d, 1H, $J_{1,2}$= 10.4 Hz, H-1), 5.29 (dd, 1H, $J_{2,3}$= 9.0, $J_{3,4}$= 9.3 Hz, H-3), 5.14 (dd, 1H, $J_{1,2}$= 10.4, $J_{2,3}$= 9.0 Hz, H-2), 5.08 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 9.9 Hz, H-4), 4.62 (q, 2H, J= 7.1 Hz, OCH_2), 4.22 (dd, 1H, $J_{5,6'}$= 4.8, $J_{6,6'}$= 12.5 Hz, H-6'), 4.08 (dd, 1H, $J_{5,6}$= 2.2, $J_{6,6'}$= 12.5 Hz, H-6), 3.80 (ddd, 1H, $J_{4,5}$= 9.9, $J_{5,6}$= 2.2, $J_{5,6'}$= 4.8 Hz, H-5), 2.04, 2.00 (2C), 1.98 (4 x OAc), 1.39 (t, 3H, J= 7.1 Hz, CH_3).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.60, 170.07, 169.36, 169.30, 166.95 (4 x C=O), (C=S), 85.82 (C-1), 76.69 (C-5), 73.97 (C-3), 68.45 (C-2), 68.00 (C-4), 70.79 (OCH_2), 61.77 (C-6), 20.74, 20.59 (4 x Ac), 13.70 (Me).

M.S. (C.I. ether) (m/z): 453 (1.5%, $[M+H]^+$); 393 (3.6%, $[M-OAc]^+$); 331 (100%, $[M-SC(S)OEt]^+$); 271 (66.1%, $[M-SC(S)OEt - HOAc]^+$); 245 (2.6%, $[M-C(S)OEt - 2(OAc)]^+$); 213 (38.6%, $[M-SC(S)OEt - 2(OAc)]^+$); 211 (9.4%, $[M-SC(S)OEt - 2(HOAc)]^+$); 169 (63.3%, $[M-SC(S)OEt - HOAc - OAc - Ac]^+$).

Pyridin-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 16.

Method A,B

The product was purified by radial chromatography on silica gel, eluted with 1.5% tert-butanol in methylene chloride. Crystallization from absolute ethanol afforded long yellowish needles; m.p. 128.8-129.2 °C; $[\alpha]_D$-2.5 (C 1, $CHCl_3$).

Anal. calc. for $C_{19}H_{23}O_9N_1S_1$: C, 51.69; H, 5.25; N, 3.17; S, 7.26
found: C, 51.74; H, 5.36; N, 3.13; S, 7.38

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 8.40 (dd, 1H, $J_{4,6}$= 1.8, $J_{5,6}$= 4.9 Hz, H-6 py), 7.50 (ddd, 1H, $J_{2,4}$= 1.8, $J_{3,4}$= 8.2, $J_{4,5}$= 7.2 Hz, H-4 py), 7.17 (dd, 1H, $J_{3,4}$= 8.2, $J_{3,5}$= 1.0 Hz, H-3 py), 7.03 (ddd, 1H, $J_{3,5}$= 1.0, $J_{4,5}$= 7.2, $J_{5,6}$= 4.9 Hz, H-5 py), 5.80 (d, 1H, $J_{1,2}$= 10.2 Hz, H-1), 5.32 (t, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.2 Hz, H-3), 5.17 (dd, 1H, $J_{3,4}$= 9.2, $J_{4,5}$= 9.9 Hz, H-4), 5.11 (dd, 1H, $J_{1,2}$= 10.2, $J_{2,3}$= 9.2 Hz, H-2), 4.23 (dd, 1H, $J_{5,6'}$= 4.7, $J_{6,6'}$= 12.4 Hz, H-6'), 4.05 (dd, 1H, $J_{5,6}$= 2.2, $J_{6,6'}$= 12.4 Hz, H-6), 3.84 (ddd, 1H, $J_{4,5}$= 9.9, $J_{5,6}$= 2.2, $J_{5,6'}$= 4.7 Hz, H-5),1.99, 1.98, 1.97, 1.96 (4s,OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.56, 170.07, 169.44, 169.36 (C=0), 155.21, 149.53, 136.48, 123.21, 120.74 (5 x C-arom.), 81.50 (C-1), 75.81 (C-5), 74.06 (C-3), 69.37 (C-2), 68.21 (C-4), 61.88 (C-6), 20.60 (2C), 20.55 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 442 (99.6%, $[M+H]^+$); 331 (39.7%, $[M-Spy]^+$); 271 (10.7%, $[M-Spy-HOAc]^+$); 213 (69.2%, $[M-Spy-2(OAc)]^+$).

Imidazolin-2-yl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside 17.

Method A,B

The crude reaction mixture was purified by radial chromatography using either a (9:1) ratio of EtOAc : hexane (method A) or (70:30:1) ratio of EtOAc : hexane : ethanol (method B). Crystallization from benzene/Hexane gave a yellowish powder; m.p.114.9-116.5 °C; $[\alpha]_D$ - 11.6 (C 1, $CHCl_3$).

Anal. calc. for $C_{18}H_{24}O_9N_2S$: C, 48.64; H, 5.44; N, 6.30; S, 7.21
found: C, 48.87; H, 5.34; N, 6.23; S, 7.06

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 7.04 (d, 1H, J= 1.2 Hz, H-4 arom.), 6.95 (d, 1H, J= 1.2 Hz, H-5 arom.), 5.13 (dd, 1H, $J_{2,3}$= 9.1, $J_{3,4}$= 9.2 Hz, H-3), 4.96 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 10.0 Hz, H-4), 4.95 (dd, 1H, $J_{1,2}$= 10.1, $J_{2,3}$= 9.1 Hz, H-2), 4.86 (d, 1H, $J_{1,2}$= 10.1 Hz, H-1), 4.08 (dd, 1H, $J_{5,6'}$= 4.9, $J_{6,6'}$= 12.4 Hz, H-6'), 4.01 (dd, 1H, $J_{5,6}$= 2.5, $J_{6,6'}$= 12.4 Hz, H-6), 3.63 (s, 3H, Me), 3.57 (ddd, 1H, $J_{4,5}$= 10.0, $J_{5,6}$= 2.5, $J_{5,6'}$= 4.9 Hz, H-5), 2.03, 1.95, 1.94, 1.92 (4 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.33, 169.99, 169.53, 169.34 (C=O), 136.40, 130.16, 123.83 (C-arom.), 85.95 (C-1), 75.77 (C-5), 73.62 (C-3), 70.04 (C-2), 68.00 (C-4), 61.75 (C-6), 34.01 (Me), 20.68, 20.60, 20.51 (2C) (4 x Ac).

M.S. (C.I. ether) (m/z): 445 (100%, $[M+H]^+$); 385 (4%, $[M-OAc]^+$); 331 (87.5%, $[M-SIm]^+$); 325 (3.2%, $[M-OAc-HOAc]^+$); 271 (23.7%, $[M-SIm-HOAc]^+$); 213 (13.9%, $[M-SIm-2(OAc)]^+$).

4-N-acetamidophenyl-2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside 18.

Compound **14** (1eq) was dissolved in ethanol, in the presence of tin (II) chloride dihydrate (5eq), at 70 °C. The reaction mixture was stirred for 30 min. after which time TLC indicated that the reaction was over. The reaction mixture was diluted with water and the Ph adjusted to 7 with saturated sodium hydrogen carbonate and extracted with ethyl acetate - water. The organic extract was dried with sodium sulfate, filtered and evaporated in vacuo. The crude reaction mixture was then acetylated by the standard acetylation procedure. The product was purified by silica gel radial chromatography to afford 88% of pure **18**; m.p. 164-166.6 °C; $[\alpha]_D$ -30.3 (C 1, $CHCl_3$).

Anal. calc. for $C_{22}H_{27}O_{10}NS$: C, 53.11; H, 5.47; N, 2.82; S, 6.44
found: C, 53.06; H, 5.44; N, 2.65; S, 6.26

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.80 (s, 1H, NH), 7.45 (d, 2H, J= 9.0 Hz, Ph-meta), 7.38 (d, 2H, J= 9.0 Hz, Ph-ortho), 5.16 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.4 Hz, H-3), 4.96 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.9 Hz, H-4), 4.87 (dd, 1H, $J_{1,2}$= 10.1, $J_{2,3}$= 9.2 Hz, H-2), 4.57 (d, 1H, $J_{1,2}$= 10.1 Hz, H-1), 4.15-4.13 (m, 2H, H-6, H-6'), 3.65 (ddd, 1H, $J_{4,5}$= 9.9, $J_{5,6}$= 2.9, $J_{5,6}$= 4.4 Hz, H-5), 2.12 (NHAc), 2.04, 2.03, 1.96, 1.93 (s, 4 x OAc)

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.62, 170.16, 169.38, 169.27, 168.36 (C=O), 138.56 (C=ipso), 134.85 (2C, C-meta), 125.69 (C-para), 119.90 (2C, C-ortho), 85.71 (C-1), 75.74 (C-5), 73.93 (C-3), 69.83 (C-2), 68.09 (C-4), 62.04 (C-6), 24.65, 20.78, 20.59 (5 x Ac).

M.S. (C.I. ether) (m/z): 498 (100%, $[M+H]^+$); 438 (3.6%, $[M-OAc]^+$); 331 (93.7%, $[M-SPhNHAc]^+$); 318 (7.1%, $[M-OAc-2(HOAc)]^+$); 271 (21.8%, $[M-SPhNHAc-HOAc]^+$); 213 (25.1%, $[M-SPhNHAc-2(OAc)]^+$); 211 (4.5%, $[M-SPhNHAc-2(HOAc)]^+$); 169 (47.5%, $[M-SPhNHAc-HOAc-OAc-Ac]^+$); 168 (33.6%, $[M-SPhNHAc-2(HOAc)-Ac]^+$).

Synthesis of 1,2-trans-aryl-1-thio-ß-glycobiosides

Phenyl 2,3,4,2',3',4',6'-hepta-O-acetyl-1-thio-ß-D-gentiobioside 26.

To a solution of thiophenol (3eq) and tetrabutylammonium hydrogen sulfate (1eq) in 1M aqueous sodium carbonate (1 ml / 100 mg bromide) was added acetobromogentiobiose (1eq) in methylene chloride (1 ml / 100 mg bromide). The two-phase reaction mixture was vigorously stirred at room temperature for 15 min after which time TLC indicated complete transformation of the bromide. Methylene chloride was then added and the organic phase was successively washed with 1M NaOH, water and saturated sodium chloride. The combined organic extracts were dried with sodium sulfate, filtered, and evaporated under reduce pressure to afford the crude thiodisaccharide. The syrupy residue was purified by silica gel radial chromatography with 2% ethanol in methylene chloride as eluant to afford **26** in 77% yield. The disaccharide **26** crystallyzed from absolute ethanol as white needles; m.p. 167.4-169.0 °C; $[\alpha]_D$ - 12.8 (C 1, $CHCl_3$).

Anal. calc. for $C_{32}H_{40}O_{17}S$: C, 52.74; H, 5.53; S, 4.40
found: C, 52.56; H, 5.54; S, 4.40

^{1}HNMR (300 MHz, $CDCl_3$): ∂ (ppm), 7.42-7.46 (m, 2H, Ph-meta), 7.32-7.36 (m, 3H, Ph-ortho, para), 5.18 (dd, 1H, $J_{2,3}$= 9.3, $J_{3,4}$= 9.4 Hz, H-3), 5.16 (dd, 1H, $J_{2,3}$= 9.5, $J_{3,4}$= 9.3 Hz, H-3'), 5.06 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 9.7 Hz, H-4'), 4.97 (dd, 1H, $J_{1,2}$= 8.1, $J_{2,3}$= 9.5 Hz, H-2'), 4.92 (dd, 1H, $J_{1,2}$=10.1, $J_{2,3}$= 9.3 Hz, H-2), 4.89 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.7 Hz, H-4), 4.68 (d, 1H, $J_{1,2}$= 10.1 Hz, H-1), 4.54 (d, 1H, $J_{1,2}$= 8.1 Hz, H-1'), 4.23 (dd, 1H, $J_{5,6}$= 4.7, $J_{6,6'}$= 12.3 Hz, H-6'), 4.10 (dd, 1H, $J_{5,6}$= 2.3, $J_{6,6'}$= 12.3 Hz, H-6'), 3.85 (dd, 1H, $J_{5,6}$= 1.9, $J_{6,6'}$= 11.1 Hz, H-6), 3.72 (ddd, 1H, $J_{4,5}$= 9.7, $J_{5,6}$= 1.9, $J_{5,6'}$= 7.4 Hz, H-5), 3.61 (dd, 1H, $J_{5,6}$= 7.4, $J_{6,6'}$= 11.1 Hz, H-6), 3.57-3.65 (m, H-5'), 2.07, 2.05, 2.01, 2.00, 1.99, 1.96, 1.94 (7s,OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$); ∂ (ppm), 170.5, 170.1, 170.0, 169.5, 169.4, 169.3, 169.2 (C=O), 123.3 (C-meta), 131.9 (C-ipso), 129.1 (C-ortho), 128.3 (C-para), 100.6 (C-1'), 85.7 (C-1), 77.3 (C-5), 73.8 (C-3), 72.7 (C-3'), 71.8 (C-5'), 71.0 (C-2'), 69.8 (C-2), 68.7 (C-4), 68.3 (C-6), 68.2 (C-4'), 61-7 (C-6'), 20.7 (3C), 20.5 (4C) (7 x Ac).

M.S. (C.I. ether) (m/z): 729 (2.5%, $[M+H]^+$); 619 (100%, $[M-SPh]^+$); 559 (14.7%, $[M-SPh-HOAc]^+$); 501 (62.5%, $[M-SPh-2(OAc)]^+$); 457 (15.4%, $[M-SPh-HOAc-OAc-Ac]^+$); 381 (14.4%, $[M-SPh-2(OAc)-2(HOAc)]^+$); 330 (100%, $[M-((0H)(OAc)_3GlcSPh)]^+$); 289 (100%, $[M+H-(4(OAc)Glc)-HSPh]^+$); 271 (100%, $[M-((0H)(OAc)_3GlcSPh)-OAc]^+$); 257 (14.5%, $[M-((0)(OAc)_3GlcSPh)-OAc-Me]^+$); 229 (100%, $[M-((0)(OAc)_3GlcSPh)-OAc-Ac]^+$); 211 (67%, $[M-((0H)(OAc)_3GlcSPh)-HOAc-OAc]^+$); 197 (16.8%, $[M-((0H)(OAc)_3GlcSPh)-2(OAc)-Me]^+$).

4-Nitrophenyl 2, 3, 4, 2',3', 4', 6'-hepta-O-acetyl-1-thio-β-D-gentiobioside 32.

Method B

The product was purified through radial chromatography using 2% tert-butanol in methylene chloride. Crystallization from absolute ethanol gave fine white needles of pure **32**; m.p. 190.2-191.4 °C; $[\alpha]_D$-25.0 (C 1, $CHCl_3$).

Anal. calc. for $C_{32}H_{39}NO_{19}S$: C, 49.68; H, 5.08; N, 1.81; S, 4.14
found: C, 49.37; H, 5.08; N, 1.67; S, 4.04

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 8.21 (d, 2H, J= 8.9 Hz, Ph-meta), 7.54 (d, 2H, J= 8.9 Hz, Ph-ortho), 5.23 (dd, 1H, $J_{2,3}$= 9.3, $J_{3,4}$= 9.1 Hz, H-3), 5.17 (dd, 1H, $J_{2,3}$= 9.1, $J_{3,4}$= 9.3 Hz, H-3'), 5.07 (dd, 1H, $J_{4,5}$= 9.8 Hz, H-4'), 5.00 (dd, 1H, $J_{1,2}$= 10.1 Hz, H-2), 4.99 (dd, 1H, $J_{1,2}$= 7.9 Hz, H-2'), 4.92 (dd, 1H, $J_{4,5}$= 9.9 Hz, H-4), 4.81 (d, 1H, $J_{1,2}$= 10.1 Hz, H-1), 4.52 (d, 1H, $J_{1,2}$= 7.9 Hz, H-1'), 4.25 (dd, 1H, $J_{5,6}$= 4.9, $J_{6,6'}$= 12.4 Hz, H-6'), 4.11 (dd, 1H, $J_{5,6}$= 2.3 Hz, H-6'), 3.92 (dd, 1H, $J_{5,6}$= 2.1, $J_{6,6'}$= 10.9 Hz, H-6), 3.81 (ddd, 1H, $J_{5,6'}$= 6.9 Hz, H-5), 3.65 (ddd, 1H, H-5'), 3.59 (dd, 1H, $J_{5,6}$= 6.9 Hz, H-6), 2.07-1.91 (7s, 21H, 7 x OAC).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.5-169.1 (7C=O), 146.9 (C-para), 141.9 (C-ipso), 130.4 (2C, C-meta), 124.2 (2C, C-ortho), 100.7 (C-1'), 84.8 (C-1), 77.5 (C-5), 73.3 (C-3), 72.5 (C-3'), 72.0 (C-5'), 70.9 (C-2'), 69.4 (C-2), 68.4 (C-4), 68.2 (C-6), 68.0 (C-4'), 61.7 (C-6'), 20.5-20.7 (7 x Ac).

M.S. (C.I. ether) (m/z): 743 (7.7%, $[M-2(Me)]^+$); 618 (2.3%, $[M-HSPhNO_2]^+$); 500 (1.9%, $[M-HSPhNO_2-2(OAc)]^+$); 331 (45.2%, $[M-(O)(OAc)_3GlcSPhNO_2]^+$); 271 (4.8%, $[M-(O)(OAc)_3GlcSPhNO_2-HOAc]^+$).

ß-D-gentiobiose octaacetate 29.

Compound **29** was prepared by the standard acetylation procedure in quantitative yield (100%). Crystallization from ethanol / ethyl acetate gave white needles; m.p. 196.4-197.0 °C; $[\alpha]_D$ +1.1 (C 1, $CHCl_3$); lit (88) m.p. 195-196 °C (corr.).

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 5.66 (d, 1H, $J_{1,2}$= 8.2 Hz, H-1), 5.20 (dd, 1H, $J_{2,3}$= 9.5, $J_{3,4}$= 9.4 Hz, H-3), 5.17 (dd, 1H, $J_{2,3}$= 9.4, $J_{3,4}$= 9.4 Hz, H-3'), 5.06 (dd, 1H, $J_{1,2}$= 8.2, $J_{2,3}$= 9.5 Hz, H-2), 5.04 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 10.0 Hz, H-4), 4.97 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 10.1 Hz, H-4'), 4.96 (dd, 1H, $J_{1,2}$= 7.9, $J_{2,3}$= 9.4 Hz, H-2'), 4.52 (d, 1H, $J_{1,2}$= 7.9 Hz, H-1'), 4.24 (dd, 1H, $J_{5,6}$= 4.8, $J_{6,6'}$= 12.5 Hz, H-6'), 4.09 (dd, 1H, $J_{5,6}$= 2.3, $J_{6,6'}$= 12.5 Hz, H-6'), 3.91 (dd, 1H, $J_{5,6}$= 2.4, $J_{6,6'}$= 11.4 Hz, H-6), 3.76 (ddd, 1H, $J_{4,5}$= 10.1, $J_{5,6}$= 2.4, $J_{5,6'}$= 5.8 Hz, H-5), 3.64 (ddd, 1H, $J_{4,5}$= 10.0, $J_{5,6}$= 2.3, $J_{5,6'}$= 4.8 Hz, H-5'), 3.55 (dd, 1H, $J_{5,6}$= 5.8, $J_{6,6'}$= 11.4 Hz, H-6), 2.09, 2.07, 2.05, 2.01, 2.00, 1.99, 1.98 (2C) (8 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.56, 170.16, 170.01, 169.44, 169.31 (2C), 169.14, 168.72 (C=O), 100.55 (C-1'), 91.51 (C-1), 73.82, 72.78, 72.66, 71.83, 70.81, 70.18, 68.34, 68.25 (C-2,3,4,5,2',3',4',5'), 67.42 (C-6), 61.76 (C-6'), 20.71, 20.66, 20.52, 20.49 (8 x Ac).

M.S. (C.I. ether) (m/z): 679 (0.4%, $[M+H]^+$); 619 (100%, $[M-OAc]^+$); 559 (17.8%, $[M-OAc-HOAc]^+$); 499 (9.1%, $[M-OAc-2(HOAc)]^+$); 457 (14.9%, $[M-2(OAc)-HOAc-Ac]^+$); 331 (100%, $[M-(O)(OAc)_4Glc]^+$); 317 (75%, $[M+H-(O)(OAc)_4Glc-Me]^+$); 289 (95.9%, $[M+H-(O)(OAc)_4Glc-Ac]^+$); 271 (100%, $[M-(O)(OAc)_4Glc-HOAc]^+$); 257 (18.7%, $[M-(O)(OAc)_4Glc-OAc-Me]^+$); 229 (85.8%, $[M-(O)(OAc)_4Glc-OAc-Ac]^+$); 211 (43.8%, $[M-(O)(OAc)_4Glc-2(HOAc)]^+$).

Acetobromogentiobiose 21.

Compound **21** was prepared by the standard HBr-HOAc (45% w/v) procedure in quantitative yield (98%) which crystallized from ether; m.p. 122.7 Dec.; $[\alpha]_D$ +105.2 (C 1, $CHCl_3$); lit (89) m.p. 143-144.5 °C; $[\alpha]_D$ +110 (C 1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 6.59 (d, 1H, $J_{1,2}$= 4.0 Hz, H-1), 5.51 (dd, 1H, $J_{2,3}$= 10.0, $J_{3,4}$= 9.5 Hz, H-3), 5.18 (dd, 1H, $J_{2,3}$= 9.3, $J_{3,4}$= 9.2 Hz, H-3'), 5.05 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 8.9 Hz, H-4), 5.04 (dd, 1H, $J_{3,4}$= 9.2, $J_{4,5}$= 9.5 Hz, H-4'), 4.97 (dd, 1H, $J_{1,2}$= 7.9, $J_{2,3}$= 9.3 Hz, H-2'), 4.76 (dd, 1H, $J_{1,2}$= 4.0, $J_{2,3}$= 10.0 Hz, H-2), 4.51 (d, 1H, $J_{1,2}$= 7.9 Hz, H-1'), 4.27-4.16 (m, 1H, H-5), 4.23 (dd, 1H, $J_{5,6}$= 4.8, $J_{6,6'}$= 12.4 Hz, H-6'), 4.09 (dd, 1H, $J_{5,6}$= 2.5, $J_{6,6'}$= 12.4 Hz, H-6'), 3.96 (dd, 1H, $J_{5,6}$= 2.0, $J_{6,6'}$= 11.5 Hz, H-6), 3.65 (ddd, 1H, $J_{4,5}$= 9.5, $J_{5,6}$= 2.5, $J_{5,6'}$= 4.8 Hz, H-5'), 3.58 (dd, 1H, $J_{5,6}$= 5.2, $J_{6,6'}$= 11.5 Hz, H-6), 2.06, 2.02, 1.99, 1.98 (7 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.63, 170.21, 169.85, 169.69, 169.47, 169.34, 169.28 (C=O), 100.69 (C-1'), 86.54 (C-1), 73.09, 72.60, 71.88, 70.78, 70.52, 70.15, 68.20, 67.46 (C-2,3,4,5,2',3',4',5'), 66.61 (C-6), 61.78 (C-6'), 20.69, 20.63 (7 x Ac).

M.S. (C.I. ether) (m/z): 619 (2.7%,[M-Br]$^+$); 559 (1.4%,[M-Br-HOAc]$^+$); 501 (3.7%,[M-Br-2(OAc)]$^+$); 331 (100%,[M-(O)(OAc)$_3$GlcBr]$^+$); 271 (14.9%,[M-(O)(OAc)$_3$GlcBr-HOAc]$^+$); 229 (2.3%,[M-(O)(OAc)$_3$GlcBr-OAc-Ac]$^+$); 213 (2.1%,[M-(O)(OAc)$_3$GlcBr-2(OAc)]$^+$); 211 (2.1%,[M-(O)(OAc)$_3$GlcBr-2(HOAc)]$^+$); 169 (17.4%,[M-(O)(OAc)$_3$GlcBr-HOAc-OAc-Ac]$^+$).

Dibenzyl (2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl) phosphate 35.

To a solution of dibenzyl phosphate (135 mg, 2eq) and tetrabutylammonium hydrogen sulfate (82.5 mg, 1eq) in saturated sodium hydrogen carbonate (2 ml) was added acetobromoglucose (100 mg, 1eq) in methylene chloride (2 ml). The two-phase reaction mixture was stirred at room temperature for 72 h. Care was taken to maintain the Ph of the aqueous phase at Ph 8-9 by addition of a few drops of saturated $NaHCO_3$ solution as required. Ethyl acetate was added (20 ml) and the resulting organic phase was successively washed with saturated $NaHCO_3$, water and saturated NaCl. The organic phase was then dried with magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by radial silica gel chromatography using 2% tert-butanol in methylene chloride as eluant (with a few drops of triethylamine) to afford the pure glycosyl phosphate (123 mg, 83%). Crystallization from di-isopropyl ether gave needles of analytically pure **35**; m.p. 74.6-75.6 °C; $[\alpha]_D$-7.6 (C 1, $CHCl_3$); lit (90) m.p. 76 °C; $[\alpha]_D$-6.5 (C 1, $CHCl_3$); lit (91) m.p. 76.5-78 °C; $[\alpha]_D$-8.4 (C 3, $CHCl_3$).

Anal. calc. for $C_{28}H_{33}O_{13}P$: C, 55.25; H, 5.47; P, 5.09
found: C, 55.07; H, 5.52; P, 4.93

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 7.28 (m, 10H, Ph), 5.33 (dd, 1H, $J_{1,2}$= 7.5, $^3J_{1,P}$= 7.5 Hz, H-1), 5.20 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.2 Hz, H-3), 5.11 (dd, 1H, H-2), 5.09 (dd, 1H, $J_{4,5}$= 9.9 Hz, H-4), 5.07 (d, 2H, $^3J_{H,P}$= 7.4 Hz, CH_2Ph), 5.00 (d, 2H, $^3J_{H,P}$= 7.1 Hz, CH_2Ph), 4.22 (dd, 1H, $J_{5,6'}$= 4.8, $J_{6,6'}$= 12.3 Hz, H-6'), 4.09 (dd, 1H, $J_{5,6}$= 2.2 Hz, H-6), 3.79 (ddd, 1H, H-5), 2.02, 1.99, 1.88 (4s, 12H, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.4, 169.9, 169.3, 169.2 (OAc), 135.4, 135.2, 128.6, 128.5, 127.8, 127.7 (Ph), 96.2 (d, $^2J_{C,P}$= 4.7 Hz, C-1), 72.6 (C-5), 72.3 (d, $^4J_{C,P}$= 1.5 Hz, C-3), 71.1 (d, $^3J_{C,P}$= 9.2 Hz, C-2), 69.62 (d, $^2J_{C,P}$= 5.8 Hz, CH_2Ph), 69.57 (d, $^2J_{C,P}$= 5.5 Hz, CH_2Ph), 67.8 (C-4), 61.4 (C-6), 20.5, 20.45 (2x), 20.36 (OAc).

M.S. (C.I. ether) (m/z): 609 (21%, $[M+H]^+$); 549 (27.1%, $[M-OAc]^+$); 447 (20.8%, $[M-2(OAc)-Ac]^+$); 369 (15.8%, $[M-OAc-3(HOAc)]^+$); 331 (100%, $[M-OP(O)(OBn)_2]^+$); 279 (67.5%, $[M-2(OAc)-2(HOAc)-CH_2C_6H_5]^+$); 271 (100%, $[M-OP(O)(OBn)_2-HOAc]^+$); 211 (26.6%, $[M-OP(O)(OBn)_2-2(HOAc)]^+$); 169 (100%, $[M-OP(O)(OBn)_2-HOAc-OAc-Ac]^+$).

Dibenzyl (2,3,4,6-tetra-O-acetyl-ß-D-galactopyranosyl) phosphate 36.

When the above procedure was applied on acetobromogalactose, the title galactosyl phosphate **36** was obtained as an oil in 73% yield; $[\alpha]_D$ +3.3 (C 1, $CHCl_3$).

Anal. Calc. for $C_{28}H_{33}O_{13}P$: C, 55.25; H, 5.47; P, 5.09
found: C, 55.44; H, 5.57; P, 4.88

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 7.33 (m, 10H, Ph), 5.40 (dd, 1H, $J_{3,4}$= 3.5, $J_{4,5}$= 1.2 Hz, H-4), 5.31 (dd, 1H, $J_{1,2}$= 8.1, $J_{2,3}$= 9.8 Hz, H-2), 5.30 (dd, 1H, $^3J_{1,P}$= 7.4 Hz, H-1), 5.07 (d, 2H, $^3J_{H,P}$= 7.3 Hz, CH_2Ph), 5.03 (dd, 1H, $J_{3,4}$= 3.5 Hz, H-3), 5.00 (dd ,2H, $^3J_{H,P}$= 7.0 Hz, CH_2Ph), 4.14 (dd, 1H, $J_{5,6'}$= 6.8, $J_{6,6'}$= 11.0 Hz, H-6'), 4.08 (dd, 1H, $J_{5,6}$= 6.0, H-6), 4.00 (ddd, 1H, H-5), 2.15, 1.97, 1.95, 1.90 (4s, 12H, OAc).

^{1}H-NMR (300 MHz, C_6D_6): ∂ (ppm), 7.12 (m, 10H, Ph), 5.79 (dd, 1H, $J_{1,2}$= 8.0, $J_{2,3}$= 10.5 Hz, H-2), 5.51 (dd, 1H, $^3J_{1,P}$= 7.8 Hz, H-1), 5.40 (dd, 1H, $J_{3,4}$= 3.5, $J_{4,5}$= 1.1 Hz, H-4), 4.97 (d, 2H, $^3J_{H,P}$= 7.1 Hz, CH_2Ph), 5.14 (dd, 1H, H-3), 4.99-5.09 (2H, ABX, H-6,6'), 3.90 (d, 2H, $^3J_{H,P}$= 7.1 Hz, CH_2Ph), 3.27 (ddd, 1H, $J_{5,6}$= $J_{5,6'}$= 6.5 Hz, H-5), 1.71, 1.70, 1.56, 1.55 (4s, OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.2, 170.0, 169.8, 169.4 (OAc), 135.3, 135.2, 128.6, 128.55, 128.52, 127.8, 127.7 (Ph), 96.7 (d, $^2J_{C,P}$= 4.8 Hz, C-1), 71.7 (C-5), 70.4 (C-3), 69.6 (d, $^2J_{C,P}$= 5.9 Hz, CH_2Ph), 69.55 (d, $^2J_{C,P}$= 6.1 Hz, CH_2Ph), 68.6 (d, $^3J_{C,P}$= 8.8 Hz, C-2), 66.6 (C-4), 61.0 (C-6), 20.6, 20.5 (4 x OAc).

M.S. (C.I. ether) (m/z): 609 (1.1%, $[M+H]^+$); 549 (0.9%, $[M-OAc]^+$); 447 (3%, $[M-2(OAc)-Ac]^+$); 369 (8%, $[M-OAc-3(HOAc)]^+$); 331 (52.9%, $[M-OP(O)(OBn)_2]^+$); 279 (69.7%, $[M-2(OAc)-2(HOAc)-CH_2C_6H_5]^+$); 271 (54%, $[M-OP(O)(OBn)_2-HOAc]^+$); 211 (24%, $[M-OP(O)(OBn)_2-2(HOAc)]^+$); 169 (100%, $[M-OP(O)(OBn)_2-HOAc-OAc-Ac]^+$).

General procedure for glycosidation using thioglycosides:

Method D

Dimethyl (methylthio) sulfonium triflate:

The thioglycoside (1.2 eq) and the aglycon (1 eq) were stirred in dichloromethane containing pulverized 4 A° molecular sieve under a nitrogen atmosphere. The reaction mixture was cooled to 0 °C and stirred for 15 min. before DMTST (4.5 eq) was added. The reaction mixture was warmed to room temperature and stirred until TLC indicated completion of the reaction. When neccessary more thioglycoside (0.5 eq) was added. The reaction mixture was diluted with dichloromethane followed by successive washes with saturated sodium hydrogen carbonate, water and saturated sodium chloride. The organic phase was dried with sodium sulfate, filtered and evaporated under reduced pressure.

Dimethyl (methylthio) sulfonium triflate (DMTST).

Dimethyl disulfide (0.24 g, 1 eq) in dichloromethane (5 ml) was added to an equimolar solution of methyl triflate (0.41 g, 1 eq) in dichloromethane (5 ml) with stirring at room temperature, and the mixture was allowed to stand for 48 h. The product was precipitated out of solution by the addition of ether to give crystals of dimethyl (methylthio) sulfonium triflate; m.p. 50.2-54.5 °C; lit(92) m.p. 28-36 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 3.29 (s, 6H, 2(Me)), 2.68 (s, 3H, Me).

Method E

Methyl iodide:

The appropriate thioglycoside (1.2 eq) and the aglycon (1 eq) were combined in dichloromethane containing pulverized 4 A° molecular sieve followed by the addition of methyl iodide (5 eq) so that a 3 % solution resulted. The reaction mixture was stirred at room temperature for several days.

Method F

N-Iodosuccinimide and catalytic amount of TfOH:

The appropriate glycosyl donor (1.2 eq) and the hydroxy acceptor (1 eq) were dissolved in dichloromethane containing 4 A° molecular sieve. The mixture was allowed to stir for 30 min. followed by the addition of NIS (1.2 eq) and TfOH (0.12 eq). The reaction mixture changed from colorless to dark red. The reaction mixture was stirred at room temperature and more NIS was added if required.

Method G

Mercuric trifluoroacetate:

To a solution of the aglycon (1 eq) in a (1 : 1) ratio of benzene : nitromethane containing 4 A° molecular sieve was added mercuric trifluoroacetate (1.2 eq) followed by the addition of the glycosyl donor phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside. The reaction mixture was stirred under a nitrogen atmosphere at room temperature.

Method H

N-Bromosuccinimide:

The hydroxy component (1.2 eq) and the thioglycoside (1 eq) were combined in dichloromethane containing pulverized 4 A° molecular sieve and a nitrogen atmosphere was secured. The mixture was allowed to stir for 15 min. before recrystallized N-bromosuccinimide (1.8 eq) was added. The reaction completion was followed by TLC. Dilution with dichloromethane followed by successive washes with saturated sodium hydrogen carbonate, 10 % sodium bisulfite, water and drying (sodium sulfate), filtration and evaporation furnished the crude product.

Method I

N-Iodosuccinimide and t-BuMe$_2$SiOTf (or TMSiOTf).

In a typical reaction, a solution of the glycosyl donor (0.22 mmol, 1.5 eq) and sugar alcohol (0.15 mmol, 1 eq), (both starting materials were rotoevaporated with toluene and dried under vaccum) in dry dichloromethane (1.6 ml) containing freshly activated 4 A° molecular sieve was treated with N-iodosuccinimide (0.17 mmol, 1.15 eq) followed by a solution of t-Bu(Me)$_2$SiOTf (or TMSiOTf) (0.16 mmol, 1.1 eq) in 750 µl of dry toluene. The reaction mixture was stirred at room temperature until completion as judged by TLC. The mixture was then diluted with dichloromethane and filtered through celite. The filtrate was washed with 10 % aqueous sodium thiosulfate, saturated sodium bicarbonate and saturated sodium chloride. The solution was dried (Na_2SO_4) and concentrated to an oil which was purified by radial chromatography on silica gel (1 % ethanol in dichloromethane).

Method J

N-Iodosuccinimide and catalytic amount of t-Bu(Me)$_2$SiOTf (or Et$_3$SiOTf).

A solution of the thioglycoside (1 eq) and the sugar alcohol (1.2 eq) were stirred in dry dichloromethane (500 µl / 60 mg thioglycoside) containing 4 A° molecular sieve for 1 h at room temperature. The solution was treated with N-Iodosuccinimide (1.15 eq) followed by the appropriate silyl triflate (0.2 eq) from a stock solution (0.06 mmol / 100 µl) in toluene. The reaction mixture was stirred until TLC indicated that the reaction was over. The mixture was then diluted with dichloromethane and washed successively with 10 % aqueous sodium thiosulfate, saturated sodium bicarbonate, water and saturated sodium chloride. The organic phase was dried (Na_2SO_4), filtered, and concentrated to an oil. Purification by radial chromatography with elution by 1 % ethanol in dichloromethane gave pure products.

Synthesis of disaccharides.

1,2-O-ditetradecyl-3-O-(2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl)-sn-glycerol 39.

Ethyl 2,3,4,6-tetra-O-acetyl-ß-D-glucopyranoside **10** was coupled to 1,2-O-ditetradecyl-Sn-glycerol **38** in the presence of DMTST according to the general procedure of glycosidation method D. The ethyl thioglycoside **10** was consumed rapidly and after 15 min. additional glycosyl donor (1 eq) was added. The reaction was completed within 1 h. The crude residue was purified by radial chromatography eluted with a ratio of (8:2) hexane-ethyl acetate to give 22 % of pure 39 as a white powder; m.p. 53.8-54.4 °C; lit (82) m.p. 54.5-55.5 °C.

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 5.17 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.5 Hz, H-3), 5.05 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 10.1 Hz, H-4), 4.96 (dd, 1H, $J_{1,2}$= 7.8, $J_{2,3}$= 9.2 Hz, H-2), 4.53 (d, 1H, $J_{1,2}$= 7.8 Hz, H-1), 4.24 (dd, 1H, $J_{5,6'}$= 4.7, $J_{6,6'}$= 12.4 Hz, H-6'), 4.09 (dd, 1H, $J_{5,6}$= 2.4, $J_{6,6}$= 12.4 Hz, H-6), 3.85 (ddd, 1H, $J_{4,5}$= 10.1, $J_{5,6}$= 2.4, $J_{5,6'}$= 4.7 Hz, H-5), 3.69-3.30 (m, 9H, 4($ROCH_2R$) (RRCHOR)), 2.06, 2.01, 1.99, 1.97 (4 x OAc), 1.52-1.47 (m, 6H), 1.22 (s, 42H), 0.85 (t, 6H, J= 6.4 Hz, 2(Me)).

2,3,4,6-tetra-O-acetyl-ß-D-glucopyranosyl-(1-6)-α-D-1,2:3,4-di-isopropylidene galactopyranoside 43.

Attempted synthesis of 43

Trial 1

Ethyl 2,3,4,6-terta-O-acetyl-1-thio-ß-D-glucopyranosyl **10** was activated with NBS conformably to the general procedure of glycosidation method H in the presence of 1,2:3,4-diisopropylidene galactopyranose **40**. TLC indicated that the reaction was completed within 1 h. The crude products were purified by column chromatography eluted with 2.5 % ethanol in benzene to afford 69 % and 21 % of the exo **41** and endo **42** orthoesters respectively.

^{1}H-NMR (200 MHz, $CDCl_3$) tentative assignment: ∂ (ppm), 5.69 (d, 1H, $J_{1,2}$= 5.2 Hz, H-1'), 5.47 (d, 1H, $J_{1,2}$= 5.1 Hz, H-1), 5.16 (dd, 1H, $J_{2,3}$= 2.6, $J_{3,4}$= 2.6 Hz, H-3'), 4.86 (m, 1H, H-4'), 4.55 (dd, 1H, $J_{2,3}$= 2.4, $J_{3,4}$= 7.8 Hz, H-3), 4.36 (m, 1H, H-2'), 4.26 (dd, 1H, $J_{1,2}$= 5.1, $J_{2,3}$= 2.4 Hz, H-2), 4.17 (dd, 1H, $J_{3,4}$= 7.8, $J_{4,5}$= 1.7 Hz, H-4), 4.16-4.14 (m, 2H, H-6', H-6'), 3.92-3.82 (m, 2H, H-6, H-5), 3.72-3.58 (m, 2H, H-6, H-5'), 2.06 (s, 9H, 3(OAc)), 1.69 (s, 3H, CMe exo), (1.58 (s, 3H, CMe endo)), 1.48 (s, 3H, Me), 1.41 (s, 3H, Me), 1.29 (s, 6H, 2(Me)).

Trial 2

Coupling of ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **10** with 1,2:3,4-diisopropylidene galactopyranose **40** according to the NBS general procedure of glycosidation (method H) with the adjusted ratio of NBS (1.2 eq) and the addition of TfOH (0.12 eq) was attempted. TLC indicated a rapid consummation of the thioglycoside **10** and formation of orthoesters **41-42** (not isolated).

Trial 3

Glycosidation of ethyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **10** with 1,2:3,4-diisopropylidene galactopyranose **40** was attempted in the presence of DMTST (method D). TLC indicated after 24 h formation of orthoesters **41-42** (not isolated).

Trial 4

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** (1 eq) and the glycosyl acceptor 1,2:3,4-diisopropylidene galactopyranose **40** (1.1 eq) were dissolved in dry dichloromethane under a nitrogen atmosphere, and pulverized activated molecular sieve (4 A°) were added. N-Iodosuccinimide (2.5 eq) was added and a 100 µl saturated solution of TfOH in dichloromethane (ca. 0.15M) was added dropwise. The reaction mixture came redish and discolored in few seconds, more TfOH (0.2 eq) was added in increment of 50 µl until the red color persisted (300 µl). The reaction mixture was stirred for 24 h after which time TLC indicated a small amount of product was formed therefore more TfOH (0.2 eq) was added in increment of 50 µl and the reaction mixture was stirred at room temperature for 40 h without any progress.

Synthesis of 43.

Trial 5

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** was glycosidate with 1,2:3,4-diisopropylidene galactopyranose **40** in the presence of NIS (1.5 eq) and either t-Bu(Me)$_2$SiOTf or TMSiOTf (1.1 eq) according to the general procedure of glycosidation method I. The reaction was completed within 45 min., in both cases, as judge by TLC and afforded 31 % or 15 % respectively of pure **43** as an oil.

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 5.46, (d, 1H, $J_{1,2}$= 4.9 Hz, H-1), 5.18 (dd, 1H, $J_{2,3}$= 9.6, $J_{3,4}$= 9.4 Hz, H-3'), 5.05 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.9 Hz, H-4'), 4.97 (dd, 1H, $J_{1,2}$= 8.0, $J_{2,3}$= 9.6 Hz, H-2'), 4.58 (d, 1H, $J_{1,2}$= 8.0 Hz, H-1'), 4.55 (dd, 1H, $J_{2,3}$= 2.4, $J_{3,4}$= 8.0 Hz, H-3), 4.26 (dd, 1H, $J_{1,2}$= 4.9, $J_{2,3}$= 2.4 Hz, H-2), 4.24 (dd, 1H, $J_{5,6}$= 4.4, $J_{6,6'}$= 12.2 Hz, H-6'), 4.14 (dd, 1H, $J_{3,4}$= 8.0, $J_{4,5}$= 1.9 Hz, H-4), 4.10 (dd, 1H, $J_{5,6}$= 2.4, $J_{6,6'}$= 12.2 Hz, H-6'), 3.99 (dd, 1H, $J_{5,6}$= 3.4, $J_{6,6'}$= 11.3 Hz, H-6), 3.89 (ddd, 1H, $J_{4,5}$= 1.9, $J_{5,6}$= 3.4, $J_{5,6'}$= 7.3 Hz, H-5), 3.66 (ddd, 1H, $J_{4,5}$= 9.9, $J_{5,6}$= 2.4, $J_{5,6'}$= 4.4 Hz, H-5'), 3.64 (dd, 1H, $J_{5,6}$= 7.3, $J_{6,6'}$= 11.3 Hz, H-6), 2.05, 2.04, 1.99, 1.97 (4s, 4(OAc)), 1.47 (s, 3H, Me), 1.41 (s, 3H, Me), 1.29 (s, 6H, 2(Me)).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.58, 170.14, 169.44, 169.33 (C=O), 109.29 (R_4C), 108.57 (R_4C), 101.36 (C-1'), 96.11 (C-1), 72.65 (C-3'), 71.64 (C-5'), 71.15 (C-2'), 70.97 (C-4), 70.55 (C-3), 70.35 (C-2), 69.43 (C-6), 68.41 (C-4'), 67.70 (C-5), 61.83 (C-6'), 25.94, 25.84, 24.95, 24.22 (4 x Me), 20.63, 20.58, 20.52 (4 x Ac).

Trial 6

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** was coupled to 1,2:3,4-di-isopropylidene galactopyranose **40** in the presence of NIS and catalytic amount of t-Bu(Me)$_2$SiOTf conformably to method J. After 1 h TLC indicated that traces of aglycon **40** remained to a fair amount of thioglycoside **11**. Therefore 0.5 eq of the aglycon **40** was added as well as NIS (0.4 eq) and t-Bu(Me)$_2$SiOTf (0.2 eq) and the reaction mixture was stirred for an additional one hour to afford 41% of a mixture of ß-D-disaccharide **43** (77.5 %) and exo orthoester **41** (22.5 %).

Trial 7

Glycosidation of para-methoxyphenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **12** with 1,2:3,4-diisopropylidene galactopyranose **40** conformably to the general procedure of glycosidation method J using Et_3SiOTf as catalyst afforded 86 % overall yield of a mixture containig 79 % ß-D-disaccharide **43** and 21 % of the exo orthoester **41**. The reaction was completed within 1 h.

Trial 8

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-ß-D-glucopyranoside **11** was glycosidated with 1,2:3,4-di-isopropylidene galactopyranose **40** according to the general glycosidation procedure method J with the adjusted ratio of NIS (1.5 eq) and Et_3SiOTf (0.4 eq). The reaction mixture was processed after 15 min. to afford 46 % of the pure ß-D-disaccharide **43** and the side product 6-O-acetyl-1,2:3,4-di-isopropylidene galactopyranose **59**.

6-O-acetyl-1,2:3,4-di-isopropylidene galactopyranose 59:

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 5.51 (d, 1H, $J_{1,2}$= 5.0 Hz, H-1), 4.59 (dd, 1H, $J_{2,3}$= 2.5, $J_{3,4}$= 7.9 Hz, H-3), 4.30 (dd, 1H, $J_{1,2}$= 5.0, $J_{2,3}$= 2.5 Hz, H-2), 4.25-4.14 (m, 3H, H-4, H-6, H-6'), 3.99 (ddd, 1H, $J_{4,5}$= 1.6, $J_{5,6}$= 4.8, $J_{5,6'}$= 7.2 Hz, H-5), 2.06 (s, 3H, OAc), 1.49, 1.42, 1.31, 1.30 (4s, 4(CMe)).

2,3,4,6-tetra-O-benzoyl-ß-D-glucopyranosyl-(1-6)-1,2:3,4-diisopropylidene α-D-galactopyranose 44.

Phenyl 2,3,4,6-tetra-O-benzoyl-1-thio-ß-D-glucopyranoside **55** was coupled to 1,2:3,4-diisopropylidene galactopyranose **40** in the presence of NIS and catalytic amount of Et_3SiOTf based on the general glycosidation procedure method J. TLC indicated that after 8 h the reaction was not over. Therefore additional aglycon (0.5 eq), NIS (0.5 eq) and Et_3SiOTf (0.2 eq) were added. The reaction mixture was stirred for 32 h and afforded 92 % of ß-D-disaccharide **44**: m.p. 89.2-90.7 °C; $[\alpha]_D$-18.2 (C 1, $CHCl_3$); lit (83) $[\alpha]_D$ -18 (C 1, $CHCl_3$).

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 8.01 (dd, 2H, $J_{o,m}$= 8.5, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.95 (dd, 2H, $J_{o,m}$= 8.5, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.88 (dd, 2H, $J_{o,m}$= 8.5, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.81 (dd, 2H, $J_{o,m}$= 8.5, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.55-7.23 (m, 12H, 8 x Ph-meta, 4 x Ph-para), 5.88 (dd, 1H, $J_{2,3}$= 9.5, $J_{3,4}$= 9.6 Hz, H-3'), 5.66 (dd, 1H, $J_{3,4}$= 9.6, $J_{4,5}$= 9.8 Hz, H-4'), 5.52 (dd, 1H, $J_{1,2}$= 7.8, $J_{2,3}$= 9.5 Hz, H-2'), 5.40 (d, 1H, $J_{1,2}$= 5.0 Hz, H-1), 5.02 (d, 1H, $J_{1,2}$= 7.8 Hz, H-1'), 4.62 (dd, 1H, $J_{5,6}$= 3.2, $J_{6,6'}$= 12.1 Hz, H-6'), 4.46 (dd, 1H, $J_{5,6}$= 5.3, $J_{6,6'}$= 12.1 Hz, H-6'), 4.41 (dd, 1H, $J_{2,3}$= 2.4, $J_{3,4}$= 8.0 Hz, H-3), 4.19 (dd, 1H, $J_{1,2}$= 5.0, $J_{2,3}$= 2.4 Hz, H-2), 4.16 (ddd, 1H, $J_{4,5}$= 9.8, $J_{5,6}$= 3.2, $J_{5,6}$= 5.3 Hz, H-5'), 4.07 (dd, 1H, $J_{3,4}$= 8.0, $J_{4,5}$= 1.4 Hz, H-4), 4.00 (dd, 1H, $J_{5,6}$= 2.6, $J_{6,6'}$= 9.5 Hz, H-6), 3.89-3.80 (m, 2H, H-5, H-6), 1.35 (s, 3H, Me), 1.22 (s, 3H, Me), 1.19 (s, 3H, Me), 1.17 (s, 3H, Me).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 165.73, 165.12 (2C), 165.08 (C=O), 133.33-128.13 (24C, 4 x Ph), 109.20 (R_4C), 108.40 (R_4C), 101.16 (C-1'), 96.10 (C-1), 72.95 (C-3'), 72.10 (C-5'), 71.74 (C-2'), 70.92 (C-4), 70.47 (C-3), 70.29 (C-2), 69.75 (C-4'), 68.21 (C-6), 67.47 (C-5), 63.19 (C-6'), 25.82, 25.61, 24.79, 24.19 (4 x Me).

2,3,4,6-tetra-O-benzoyl-ß-D-glucopyranosyl-(1-3)-methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-α-ß-D-galactofuranoside 58.

Attempted synthesis 58:

The glycosidation of methyl 2-acetamido-2-deoxy-5,6-O-isopropylidene-α,ß-D-galactofuranose **47** with phenyl 2,3,4,6-tetra-O-benzoyl-1-thio-ß-D-glucopyranoside **55** in the presence of NIS and Et_3SiOTf was attempted conformably to the general procedure of glycosidation method J. After 2 h TLC indicated the presence of traces of two U.V active products **45-46**. Additional NIS (0.5 eq), Et_3SiOTf (0.2 eq), and thioglycoside **55** (0.5 eq) were added and the reaction mixture was stirred for one week at room temperature. TLC indicated after that time that the reaction was still not over therefore another 0.5 eq of NIS and 0.4 eq of Et_3SiOTf were further added and the reaction mixture was stirred overnight. The reaction mixture was processed according to the general procedure method J to give an α,ß-mixture of the thioglycoside hydrolyzed products **45-46**.

α-anomer **45**:

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 8.18-7.23 (m, 20H, 4 x Ph), 6.33 (d, 1H, $J_{1,2}$= 3.8 Hz, H-1), 5.86 (dd, 1H, $J_{2,3}$= 9.9, $J_{3,4}$= 9.4 Hz, H-3), 5.75 (dd, 1H, $J_{3,4}$= 9.4, $J_{4,5}$= 9.5 Hz, H-4), 4.59-4.37 (m, 3H, H-2, H-6, H-6'), 4.30-4.18 (m, 1H, H-5), 2.79 (d, 1H, J= 6.5 Hz, OH).

ß-anomer **46**:

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 8.05-7.23 (m, 20H, 4 x Ph), 6.24 (dd, 1H, $J_{2,3}$= 10.1, $J_{3,4}$= 9.9 Hz, H-3), 5.77-5.67 (m, 2H, H-2, H-4), 5.29 (dd, 1H, $J_{H\text{-}1,OH}$= 3.6, $J_{1,2}$= 10.2 Hz, H-1), 4.70-4.58 (m, 2H, H-6, H-6'), 4.51-4.36 (m, 1H, H-5), 3.66-3.57 (m, 1H, OH).

Standard Zemplén deacetylation procedure.

The sugars were dissolved in methanol followed by the addition of a 1M solution of sodium methoxide in methanol to a PH~9-10 (PH paper). The reaction mixtures were stirred overnight and neutralized with Amberlite resine IR-120 (H) (PH=7), filtered and evaporated in vacuo.

Standard acetylation procedure.

The sugars were dissolved in 5/1 (v/v) pyridine/acetic anhydride (~40 eq). The reaction mixtures were stirred overnight at room temperature and were then evaporated and coevaporated with toluene in vacuo.

Selective glycosyl donors.

Phenyl-1-thio-β-D-glucopyranoside 48.

Compound **11** was deacetylated using the standard Zemplén deacetylation procedure to afford compound **48** in 84% yield. The resulting residue was recrystallized from ethyl acetate to give **48** as fine needles; m.p. 133.3-134.1 °C; $[\alpha]_D$ - 60.78 (C 1.02, MeOH); lit (93) m.p. 113 °C; $[\alpha]_D$ -72.1 (C 1.8, water).

Anal. calc. for $C_{12}H_{16}O_5S$: C, 52.93; H, 5.92; S, 11.77
found: C, 52.46; H, 5.82; S, 11.59

^{1}H-NMR (200 MHz, D_2O): ∂ (ppm), 7.59-7.55 (m, 2H, Ph-meta), 7.41-7.38 (m, 3H, Ph-ortho, para), 4.79 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 3.88 (dd, 1H, $J_{5,6}$= 2.0, $J_{6,6'}$= 12.3 Hz, H-6), 3.70 (dd, 1H, $J_{5,6'}$= 5.2, $J_{6,6'}$= 12.3 Hz, H-6'), 3.56-3.29 (cm, 4H, H-2,3,4,5).

^{13}C-NMR (75.4 MHz, D_2O): ∂ (ppm), 131.55 (C-ipso), 131.15 (2C, C-meta), 128.88 (2C, C-ortho), 127.65 (C-para), 86.82 (C-1), 79.43 (C-5), 76.77 (C-3), 71.27 (C-2), 68.90 (C-4), 60.35 (C-6).

M.S. (C.I. ether) (m/z): 273 (8.2%, $[M+H]^+$); 255 (30.4%, $[M-OH]^+$); 237 (38.2%, $[M-OH-H_2O]^+$); 219 (3.3%, $[M-OH-2(H_2O)]^+$); 201 (3.3%, $[M-OH-3(H_2O)]^+$); 163 (42.2%, $[M-SPh]^+$); 145 (33.8%, $[M-SPh-H_2O]^+$); 110 (55.7%, $[M-SPh-OH-2(H_2O)]^+$).

Phenyl-6-O-tert-butyldiphenylsilyl-1-thio-ß-D-glucopyranoside 49.

To a solution of phenyl-1-thio-ß-D-glucopyranoside **48** (1 eq), imidazole (1.5 eq) and DMAP (0.5 eq) in DMF was added tert-butyldiphenylsilylchloride (1.5 eq). The reaction mixture was stirred at room temperature until TLC indicated that the reaction was over (3 h). The reaction mixture was then diluted with ether and washed with water. The organic phase was dried (sodium sulfate), filtered and evaporated in vacuo. The crude material was chromatograph on silica gel column and eluted with CH_2Cl_2: Et_2O (9:1) followed by ether and ethylacetate to afford pure **49** in 65% yield as a white foam; m.p. 61-64 °C; $[\alpha]_D$ -30.0 (C 0.96, $CHCl_3$).

Anal. calc. for $C_{28}H_{34}O_5SSi$: C, 65.85; H, 6.71; S, 6.28
found: C, 65.69; H, 6.73; S, 6.10

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.71-7.23 (m, 15H, 3 x Ph), 4.50 (d, 1H, $J_{1,2}$= 9.6 Hz, H-1), 3.92 (d, 2H, $J_{6,6}$= 4.5 Hz, H-6, H-6'), 3.90-3.30 (cm, 4H, H-2,3,4,5), 2.88 (d, 1H, J= 1.9 Hz, OH), 2.77 (d, 1H, J= 1.8 Hz, OH), 2.48 (d, 1H, J= 2.1 Hz, OH), 1.04 (s, 9H, t-Bu).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 135.60 (3C), 135.55 (2C), 132.89, 132.78, 132.36 (2C), 132.02, 129.79 (2C), 128.94 (2C), 127.87, 127.75 (3C) (3 x Ph), 87.77 (C-1), 79.04, 77.72, 71.59, 70.98 (C-2,3,4,5), 64.22 (C-6), 26.76 (3 x Me, tBu), 19.17 (C, tBu).

M.S. (C.I. ether) (m/z): 511 (9.2%, $[M+H]^+$); 493 (14.1%, $[M-OH]^+$); 475 (3.1%, $[M-OH-H_2O]^+$); 401 (12.3%, $[M-SPh]^+$); 383 (76.1%, $[M-SPh-H_2O]^+$); 365 (31.4%, $[M-SPh-2(H_2O)]^+$); 323 (96.3%, $[M-Ph-HSPh]^+$); 305 (100%, $[M+H-SPh-Ph-H_2O]^+$); 221 (96.7%, $[M-OSi(Ph)_2tBu-2(OH)]^+$); 203 (100%, $[M-OSi(Ph)_2tBu-2(OH)-H_2O]^+$).

Phenyl-6-O-tert-butyldiphenylsilyl-2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside 50

The silylated thioglycoside **49** (350 mg, 1 eq) was dissolved in pyridine (6 ml) followed by the addition of acetic anhydride (1.1 ml). The reaction mixture was stirred overnight and was then evaporated and coevaporated with toluene and carbon tetrachloride to yield a white foamy product 50 (91%) which crystallized from absolute ethanol as fine needles; m.p. 90-92 °C; $[\alpha]_D$ +11.04 (C 0.96, $CHCl_3$).

Anal. calc. for $C_{34}H_{40}O_8SSi$: C, 64.13; H, 6.33; S, 5.03
found: C, 63.94; H, 6.31; S, 4.87

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.72-7.21 (m, 15H, 3 x Ph), 5.22 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.3 Hz, H-3), 5.12 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 9.5 Hz, H-4), 4.99 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.2 Hz, H-2), 4.73 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 3.74-3.73 (m, 2H, H-6, H-6'), 3.64-3.58 (m, 1H, H-5), 2.07 (s, 3H, OAc), 1.97, (s, 3H, OAc), 1.86 (s, 3H, OAc), 1.05 (s, 9H, tBu).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.49, 169.41, 169.30 (C=O), 135.74 (2C), 135.67 (2C), 132.99, 132.95, 132.72 (2C), 132.22, 129.76 (2C), 129.01 (2C), 128.14 (2C), 127.76 (3C) (3 x Ph), 85.69 (C-1), 78.75, 74.36, 69.90, 60.08 (C-2,3,4,5), 62.42 (C-6), 26.48 (3 x Me, tBu), 20.58, 20.45, 20.29 (3 x Ac), 18.96 (C, tBu).

M.S. (C.I. ether) (m/z): 637 (3.1%, $[M+H]^+$); 559 (11.5%, $[M-Ph]^+$); 527 (100%, $[M-SPh]^+$); 499 (10.3%, $[M-Ph-HOAc]^+$); 467 (4.2%, $[M-SPh-HOAc]^+$); 439 (4.5%, $[M-Ph-2(HOAc)]^+$); 409 (7.4%, $[M-SPh-2(OAc)]^+$); 407 (3.1%, $[M-SPh-2(HOAc)]^+$); 366 (35.1%, $[M-SPh-2(OAc)-Ac]^+$); 365 (93.4%, $[M-SPh-OAc-HOAc-Ac]^+$); 331 (3.1%, $[M-SPh-OAc-HOAc-PH]^+$).

Phenyl-6-O-tert-butyldiphenyl-2,3-4-tri-O-benzoyl-1-thio-ß-D-glucopyranoside 51.

A solution of the thioglycoside **49** (1 eq) in pyridine was cooled to 0 °C followed by the addition of benzoyl chloride (1.2 eq / OH) in pyridine. The reaction mixture was stirred at room temperature overnight after which time TLC showed that the starting material had disappeared although partly benzoylated products remained therefore another (2 eq) of benzoyl chloride was added. The reaction mixture was stirred for another day and processed as followed: The reaction mixture was quenched with ice water and stirred for 1 hour then extracted with dichloromethane and washed with saturated sodium hydrogen carbonate. The organic extract was dried with sodium sulfate, filtered and evaporated under reduced pressure. The product was purified through radial chromatography eluted with a (1:1) ratio of dichloromethane: hexane to give **51** in 67% yield. Crystallization from absolute ethanol gave fine white needle of pure **51**; m.p. 167.9-168.4 °C; $[\alpha]_D$ + 28.3 (C 1, $CHCl_3$).

Anal. calc. for $C_{49}H_{46}O_8SSi$: C, 71.51; H, 5.63; S, 3.90
found: C, 71.68; H, 5.79; S, 4.01

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 8.03-7.18 (m, 30H, 6 x Ph), 5.94 (dd, 1H, $J_{2,3}$= 9.4, $J_{3,4}$= 9.5 Hz, H-3), 5.72 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 9.2 Hz, H-4), 5.57 (dd, 1H, $J_{1,2}$= 9.9, $J_{2,3}$= 9.4 Hz, H-2), 5.12 (d, 1H, $J_{1,2}$= 9.9 Hz, H-1), 4.02-3.95 (m, 3H, H-5,6,6'), 1.11 (s, 9H, tBu).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 165.84, 165.01, 164.89 (C=O), 135.58 (2C), 135.46 (2C), 133.18 (2C), 133.05, 132.80, 132.74 (2C), 132.31, 129.82 (2C), 129.72 (3C), 129.60, 129.55, 129.28, 129.08, 128.91 (2C), 128.87, 128.31 (3C), 128.29 (3C), 128.18 (2C), 128.05, 127.63 (2C), 127.56 (2C) (6 x Ph), 86.25 (C-1), 79.42, 74.59, 70.61, 68.79 (C-2,3,4,5), 62.74 (C-6), 26.60 (3Me, tBu), 19.11 (C, tBu).

M.S. (C.I. ether) (m/z): 713 (17%, $[M\text{-}SPh]^+$); 471 (9.2%, $[M\text{-}SPh\text{-}2(OBz)]^+$); 349 (2.2%, $[M\text{-}SPh\text{-}2(OBz)\text{-}HOBz]^+$); 273 (2.7%, $[M\text{-}SPh\text{-}3(OBz)\text{-}Ph]^+$); 197 (5.2%, $[M+H\text{-}SPh\text{-}3(OBz)\text{-}2(Ph)]^+$).

Phenyl-6-O-tert-butyldiphenyl-2,3,4-tri-O-benzyl-1-thio-ß-D-glucopyranoside 52.

To a suspension of sodium hydride (1.3 eq / OH) in DMF was added a solution of the alcohol **49** (1 eq) and benzyl bromide (1.3 eq / OH) in DMF dropwise at 0 °C. After 2 days TLC indicated that the reaction was still not over therefore additional benzyl bromide (1 eq / OH) and sodium hydride (1 eq / OH) were added. The reaction mixture was stirred at room temperature over the weekend after which time TLC indicated that the reaction was completed. Methanol was added and the reaction mixture was stirred for 1 hour and was then poured onto ice water and extracted with ether. The organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure. The crude residue was purified by radial chromatography on silica gel plate and eluted with hexane followed by a (1:1) ratio of dichloromethane: hexane and finally by dichloromethane to yield 59% of pure **52** as an oil; $[\alpha]_D$ -16.3° (C 1, $CHCl_3$).

Anal. calc. for $C_{49}H_{52}O_5SSi$: C, 75.35; H, 6.71; S, 4.10
found: C, 75.33; H, 6.61; S, 4.26

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.85-7.18 (cm, 30H, 6 x Ph), 4.98-4.78 (m, 6H, 3 x OCH_2Ph), 4.76 (d, 1H, $J_{1,2}$= 9.7 Hz, H-1), 4.71-3.97 (m, 2H, H-6, H-6'), 3.89 (dd, 1H, $J_{3,4}$= 9.2, $J_{4,5}$= 9.4 Hz, H-4), 3.79 (dd, 1H, $J_{2,3}$= 9.0, $J_{3,4}$= 9.2 Hz, H-3), 3.61 (dd, 1H, $J_{1,2}$= 9.7, $J_{2,3}$= 9.0 Hz, H-2), 3.50-3.43 (m, 1H, H-5), 1.15 (s, 9H, 3 x Me).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 138.28, 138.10, 138.00, 135.82 (2C), 135.58 (2C), 134.10, 133.40, 132.83, 131.60 (2C), 129.61 (2C), 129.57 (2C), 128.86 (2C), 128.45 (2C), 128.38 (4C), 128.11 (2C), 127.93 (2C), 127.82 (2C), 127.70 (3C), 127.61 (2C), 127.20 (6 x Ph), 87.43 (C-1), 86.81, 80.72, 79.80, 77.38 (C-2,3,4,5), 75.96, 75.35, 75.09 (3 x CH_2Ph), 62.61 (C-6), 26.81 (3 x Me, tBu), 19.25 (C, tBu).

M.S. (C.I. ether) (m/z): 563 (3.4%, [M-SPh-$HOCH_2Ph$]$^+$); 455 (2.4%, [M-SPh-2($HOCH_2Ph$)]$^+$); 365 (3.2%, [M-SPh-$HOCH_2Ph$-OCH_2Ph-CH_2Ph]$^+$); 289 (3.4%, [M-SPh-2(OCH_2Ph)-CH_2Ph-Ph]$^+$).

Phenyl 2,3,4-tri-O-acetyl-1-thio-ß-D-glucopyranoside 53.

The thioglycoside **50** (1eq) was dissolved in a 1M solution of acetic acid in THF (10 eq) and cooled to 0 °C followed by the addition of 1M solution of tetrabutylammonium fluoride in THF (5 eq). The reaction mixture was stirred at room temperature for 5 h at which time TLC indicated that the reaction was not over and therefore a 1M solution of acetic acid in THF (2 eq), and 1M solution of tetrabutylammonium fluoride (1 eq) were further added. The reaction mixture was stirred for another 8 h for completion. The reaction mixture was diluted with methylene chloride and successively washed with saturated NH_4Cl and water. The organic phase was then dried with sodium sulfate, filtered and evaporated in vacuo. The residue was purified by radial silica gel chromatography, eluted with 2% tert-butanol in methylene chloride to afford 65% of pure **53** as well as 29% of phenyl 2,3,6-tri-o-acetyl-1-thio-ß-D-glucopyranoside **54** as an acetyl migration side product. Compound **53** crystallized from absolute ethanol; m.p. 94.6-97.5 °C; $[\alpha]_D$ -21.0 (C 1, $CHCl_3$).

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.47-7.43 (m, 2H, Ph-meta), 7.32-7.28 (m, 3H, Ph-ortho, Ph-para), 5.24 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.5 Hz, H-3), 4.97 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 9.6 Hz, H-4), 4.93 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.2 Hz, H-2), 4.72 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 3.79-3.50 (m, 3H, H-5, H-6, H-6'), 2.16 (dd, 1H, $J_{OH,H6}$= 5.4, $J_{OH,H6'}$= 8.2 Hz, OH), 2.06, 2.01, 1.97 (3 x OAc).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 170.13, 169.92, 169.22 (C=O), 132.82 (2C, C-meta), 131.54 (C-ipso), 129.02 (2C, C-ortho), 128.36 (C-para), 85.58 (C-1), 78.18 (C-5), 73.75 (C-3), 70.07 (C-2), 68.37 (C-4), 61.44 (C-6), 20.67, 20.54 (2C) (3 x OAc).

M.S. (C.I. ether) (m/z): 399 (3.2%, $[M+H]^+$); 290 (27.3%, $[M+H-SPh]^+$); 289 (100%, $[M-SPh]^+$); 247 (3%, $[M+H-SPh-Ac]^+$); 229 (47.7%, $[M-SPh-HOAc]^+$); 187 (3.4%, $[M+H-SPh-Ac-HOAc]^+$); 169 (19.0%, $[M-SPh-2(HOAc)]^+$).

Phenyl 2,3,6-tri-O-acetyl-1-thio-ß-D-glucopyranoside 54.

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.49-7.44 (m, 2H, Ph-meta), 7.32-7.26 (m, 3H, Ph-ortho, Ph-para), 5.04 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 8.8 Hz, H-3), 4.90 (dd, 1H, $J_{1,2}$= 9.7, $J_{2,3}$= 9.2 Hz, H-2), 4.67 (d, 1H, $J_{1,2}$= 9.7 Hz, H-1), 4.43 (dd, 1H, $J_{5,6'}$= 3.8, $J_{6,6'}$= 12.2 Hz, H-6'), 4.33 (dd, 1H, $J_{5,6}$= 1.1, $J_{6,6'}$= 12.2 Hz, H-6), 3.53-3.50 (m, 2H, H-4, H-5), 2.93 (d, 1H, $J_{OH,4}$= 4.4 Hz, OH), 2.09, 2.07, 2.05 (3 x OAc).

Phenyl 2,3,4,6-tetra-O-benzoyl-1-thio-ß-D-glucopyranoside 55.

A solution of the thioglycoside **48** (1 eq) in pyridine was cooled to 0 °C followed by the addition of benzoyl chloride (1.5 eq / OH). The reaction mixture was warmed to room temperature and stirred overnight after which time TLC indicated that the reaction was over. The reaction mixture was quenched with ice water and stirred for 1 hour then extracted with dichloromethane and ice water and washed successively with saturated sodium hydrogen carbonate, water and saturated sodium chloride. The organic extract was dried with sodium sulfate, filtered and evaporated and coevaporated with toluene in vacuo to give a quantitative yield (100%) of compound **55**. Crystallization from ethanol afforded pure **55** as white fine needles; m.p. 177.1-178.4 °C; $[\alpha]_D$ + 31.2 (C 1, $CHCl_3$); lit (94) m.p. 167-168 °C; $[\alpha]_D$ +34 (C 1, $CHCl_3$).

Anal. calc. for $C_{40}H_{32}O_9S$: C, 69.76; H, 4.68; S, 4.66
found: C, 69.87; H, 4.60; S, 4.64

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 8.02 (dd, 2H, $J_{o,m}$= 8.4, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.95 (dd, 2H, $J_{o,m}$= 8.4, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.88 (dd, 2H, $J_{o,m}$= 8.4, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.77 (dd, 2H, $J_{o,m}$= 8.4, $J_{o,p}$= 1.4 Hz, Ph-ortho), 7.61-7.07 (m, 17H, 8 x Ph-meta, 4 x Ph-para, SPh), 5.90 (dd, 1H, $J_{2,3}$= 9.4, $J_{3,4}$= 9.6 Hz, H-3), 5.59 (dd, 1H, $J_{3,4}$= 9.6, $J_{4,5}$= 9.7 Hz, H-4), 5.48 (dd, 1H, $J_{1,2}$= 10.0, $J_{2,3}$= 9.4 Hz, H-2), 5.04 (d, 1H, $J_{1,2}$= 10.0 Hz, H-1), 4.67 (dd, 1H, $J_{5,6}$= 2.8, $J_{6,6'}$= 12.3 Hz, H-6), 4.46 (dd, 1H, $J_{5,6}$= 5.9, $J_{6,6'}$= 12.3 Hz, H-6'), 4.19 (ddd, 1H, $J_{4,5}$= 9.7, $J_{5,6}$= 2.8, $J_{5,6'}$= 5.9 Hz, H-5).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 166.00, 165.71, 165.13, 165.00 (C=O), 133.45 (2C), 133.30 (2C), 133.15 (2C), 133.02 (3C), 131.71, 129.79 (3C), 129.68 (3C), 129.54, 129.09, 128.83 (3C), 128.67, 128.60, 128.36 (4C), 128.22 (3C) (5 x Ph), 86.19 (C-1), 76.28, 74.08, 70.39, 69.32 (C-2,3,4,5), 63.14 (C-6)

M.S. (C.I. ether) (m/z): 689 (2%,$[M+H]^+$); 579 (11.1%, $[M-SPh]^+$); 371 (1.4%, $[M+H-3(HBz)]^+$); 337 (2.3%, $[M-SPh-2(OBz)]^+$).

Phenyl 2,3,4,6-tetra-O-pivaloyl-1-thio-ß-D-glucopyranoside 56.

A solution of the thioglycoside **48** (1 eq) in pyridine was cooled to 0 °C followed by the addition of pivaloyl chloride (1.5 eq / OH). The reaction mixture was warmed to room temperature and stirred overnight after which time TLC shown that the reaction was not over, therefore more pivaloyl chloride (0.4 eq / OH) was further added and the reaction mixture was stirred for an additional 3 h. Since the reaction was not completed DMAP (0.1 eq) was added and the reaction mixture was stirred over the weekend. TLC indicated that the reaction was still not over. More pivaloyl chloride (1.5 eq / OH) was added and the reaction was warmed (~60-70 °C) for 4 h. The reaction mixture was then quenched with ice water and stirred for 1 hour then extracted with dichloromethane and washed successively with saturated sodium hydrogen carbonate, water and saturated sodium chloride. The organic phase was dried with sodium sulfate, filtered, evaporated, and coevaporated with toluene under reduced pressure. The crude reaction product was purified through radial chromatography on silica gel eluted with a ratio of (10:1) hexane: ethylacetate to give 32% of the desired product **56** as well as 52% of the partly pivaloylated phenyl 3,4,6-tri-O-pivaloyl-1-thio-ß-D-glucopyranoside **57**. Compound **56** crystallized from absolute ethanol as fine white needles; m.p. 138.7-139.3 °C; $[\alpha]_D$ -6.4° (C 1, $CHCl_3$).

Anal. calc. for $C_{32}H_{48}O_9S$: C, 63.13; H, 7.95; S, 5.26
found: C, 62.98; H, 8.09; S, 5.12

^{1}H-NMR (200 MHz, $CDCl_3$): ∂ (ppm), 7.5-7.44 (m, 2H, Ph-meta), 7.29-7.25 (m, 3H, Ph-ortho, Ph-para), 5.32 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.3 Hz, H-3), 5.06 (dd, 1H, $J_{3,4}$= 9.3, $J_{4,5}$= 10.0 Hz, H-4), 5.00 (dd, 1H, $J_{1,2}$= 10.1, $J_{2,3}$= 9.2 Hz, H-2), 4.70 (d, 1H, $J_{1,2}$= 10.1 Hz, H-1), 4.23 (dd, 1H, $J_{5,6}$= 1.7, $J_{6,6'}$= 12.4 Hz, H-6), 4.02 (dd, 1H, $J_{5,6'}$= 5.9, $J_{6,6'}$= 12.4 Hz, H-6'), 3.74 (ddd, 1H, $J_{4,5}$= 10.0, $J_{5,6}$= 1.7, $J_{5,6'}$= 5.9 Hz, H-5), 1.19 (s, 9H, 3 x Me), 1.18 (s, 9H, 3 x Me), 1.12 (s, 9H, 3 x Me), 1.07 (s, 9H, 3 x Me).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 177.97, 177.06, 176.36, 176.25 (C=O), 132.57 (2C, C-meta), 132.32 (C-ipso), 128.89 (2C, C-ortho), 128.15 (C-para), 86.52 (C-1), 76.33, 73.16, 69.40, 67.60 (C-2,3,4,5), 62.19 (C-6), 38.76, 38.69 (2C), 38.63 (4 x C, tBu), 27.05 (8 x Me), 26.96 (4 x Me) (12 x Me, tBu).

M.S. (C.I. ether) (m/z): 609 (16.7%, $[M+H]^+$); 500 (79.7%, $[M+H-SPh]^+$); 499 (100%, $[M-SPh]^+$); 397 (42.2%, [M-SPh-HOPiv]$^+$); 303 (25.5%, [M-2(HOPiv)-OPiv]$^+$); 297 (22.3%, [M-SPh-2(OPiv)]$^+$); 211 (23%, [M-SPh-HOPiv-OPiv-Piv]$^+$).

Phenyl 3,4,6-tri-O-pivaloyl-1-thio-ß-D-glucopyranoside 57.

Compound **57** crystallized from absolute ethanol as fine white needles; m.p. 136.9-137.8 °C; $[\alpha]_D$ -13.0 (C 1, $CHCl_3$).

Anal. calc. for $C_{27}H_{40}O_8S$: C, 61.81; H, 7.68; S, 6.11
found: C, 61.89; H, 7.65; S, 5.89

^{1}H-NMR (300 MHz, $CDCl_3$): ∂ (ppm), 7.55-7.52 (m, 2H, Ph-meta), 7.32-7.24 (m, 3H, Ph-ortho, Ph-para), 5.16 (dd, 1H, $J_{2,3}$= 9.2, $J_{3,4}$= 9.5 Hz, H-3), 5.01 (dd, 1H, $J_{3,4}$= 9.5, $J_{4,5}$= 10.0 Hz, H-4), 4.58 (d, 1H, $J_{1,2}$= 9.8 Hz, H-1), 4.21 (dd, 1H, $J_{5,6}$= 1.8, $J_{6,6'}$= 12.3 Hz, H-6), 4.04 (dd, 1H, $J_{5,6'}$= 5.7, $J_{6,6'}$= 12.3 Hz, H-6'), 3.74 (ddd, 1H, $J_{4,5}$= 10.0, $J_{5,6}$=1.8, $J_{5,6'}$= 5.7 Hz, H-5), 3.49 (ddd, 1H, $J_{1,2}$= 9.8, $J_{2,3}$= 9.2, $J_{2,OH}$= 3.2 Hz, H-2), 2.52 (d, 1H, $J_{2,OH}$= 3.2 Hz, OH), 1.19 (s, 9H, 3 x Me), 1.14 (s, 9H, 3 x Me), 1.12 (s, 9H, 3 x Me).

^{13}C-NMR (75.4 MHz, $CDCl_3$): ∂ (ppm), 178.10 (2C), 176.50 (C=O), 132.88 (2C, C-meta), 131.34 (C-ipso), 129.01 (2C, C-ortho), 128.32 (C-para), 88.54 (C-1), 76.39, 75.19, 70.89, 67.15 (C-2,3,4,5), 62.20 (C-6), 38.76, 38.70 (3 x C, tBu), 27.02 (9 x Me, tBu).

M.S. (C.I. ether) (m/z): 525 (16.2%, $[M+H]^+$); 507 (16.8%, $[M-OH]^+$); 415 (85.0%, $[M-SPh]^+$); 397 (3.1%, $[M-SPh-H_2O]^+$); 313 (29.5%, [M-SPh-HOPiv]$^+$); 211 (74.0%, [M-SPh-2(HOPiv)]$^+$).

CONCLUSION

Claims to original research:

- Preparation of a series of glycosyl acceptors suitably protected for the synthesis of the repeating disaccharide unit of *(Actinobacillus) Hœmophilus pleuropneumoniœ*.

- Attempted synthesis of the repeating disaccharide unit of *H. pleuropneumoniœ*.

- Stereospecific synthesis of a series of 1,2-trans-thioglycosides by phase transfer catalysis.

- New and mild stereospecific synthesis of 1,2-trans phenyl and para-nitrophenyl-1-thio-ß-D-glycobiosides by phase transfer catalysis.

- Phase transfer catalyzed synthesis of glycosyl phosphates which proceeds by inversion of configuration at the anomeric centers and occurs with complete stereocontrol.

- Design of a conceptually new approach involving "active" and "latent" thioglycosyl donors.

- Reactivation of latent thioglycosyl donors.

- Verified the concept of active and latent thioglycosyl donors:
  i) formation of ß-D-glycolipid
  ii) formation of orthoesters
  iii) formation of ß-D-disaccharides

- Preparation of selectively protected glycosyl donors:
  i) For the synthesis of the repeating unit of *H. pleuropneumoniœ*
  ii) To study the relative reactivities of armed and disarmed thioglycosyl donors
  iii) To prevent orthoesters formation

- Three manuscripts were published.

  René Roy, François D. Tropper, and Chantal Grand-Maître,
  Synthesis of glycosyl phosphates by phase transfer catalysis.
  Can. J. Chem., **69**, 1462 (1991).

  François D. Tropper, Fredrik O. Andersson, Chantal Grand-Maître and René Roy,
  Stereospecific Synthesis of 1,2-trans-1-Phenylthio-ß-D-Disacchrides Under Phase Transfer Catalysis
  Synthesis, 734 (1991).

  François D. Tropper, Fredrik O. Andersson, Chantal Grand-Maître and René Roy,
  Phase Transfer Catalyzed Synthesis of Para-Nitrophenyl 1-thio-ß-D-Glycobiosides
  Carbohydr. Res., in press.

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