# SYNTHESIS OF COMPLEX CARBOHYDRATES



THESIS

SUBMITTED FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

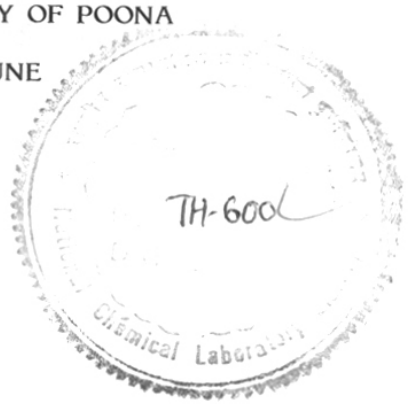
IN CHEMISTRY

TO

UNIVERSITY OF POONA

PUNE



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PUNE - 411 008

1990

DEDICATED WITH LOVE AND AFFECTION

TO

MY GRAND PARENTS AND PARENTS

## DECLARATION

The research work embodied in this thesis has been carried out by me in the Division of Organic Chemistry, National Chemical Laboratory, Pune, under the supervision of **Dr Hari Babu Mereyala,** Scientist, Indian Institute of Chemical Technology, Hyderabad (formerly in NCL, Pune). The work is original and has not been submitted for any research degree of this or other Universities.

**(G VENUGOPAL REDDY)**

**Date:** 29th October 1990

Certified that the work incorporated in the thesis **"Synthesis of Complex Carbohydrates"** submitted by **Mr Gurijala Venugopal Reddy,** was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

M. Hari Babu

**(DR HARI BABU MEREYALA)**
**Supervisor**

**Date:** 29th October 1990

CONTENTS

# ACKNOWLEDGEMENTS

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*I acknowledge the help provided by the staff of the nmr, analytical and library sections of NCL, Pune.*

*I wish to record my appreciation and thanks to* **Mr D Ravi** *and other friends for their help and pleasant company.*

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*Finally I thank the Director, NCL, Pune for providing facilities and CSIR, New Delhi for financial support.*

**(G VENUGOPAL REDDY)**

## GENERAL REMARKS AND EXPERIMENTAL MEMORANDA

1. All dry reactions were performed in oven dried glassware.
2. Dichloromethane was distilled from $P_2O_5$ and stored over 4Å molecular sieves. Diethyl ether and THF were distilled from sodium benzophenone ketyl. Petroleum ether and $CHCl_3$ were distilled over anhydrous $CaCl_2$ and $P_2O_5$. Pyridine was distilled and stored over KOH pellets. Toluene was distilled over sodium and stored over 4Å molecular sieves.
3. Workup procedures include drying organic extracts over anhydrous $Na_2SO_4$ and concentration under diminished pressure.
4. Column chromatography employed silica gel (60-120 mesh and finer than 200 mesh, Acme) unless otherwise stated.
5. Progress of the reactions was monitored by thin layer chromatography on 0.5 mm layers of silica gel-G with 13% $CaSO_4$ binder.
6. Spraying the solution of 2% phosphomolybdic acid, 1% $Ce_2SO_4$ $4H_2O$ in 20% aq. conc. $H_2SO_4$ followed by heating the plates at 130°C for visualization of spots.
7. Melting points were determined in open capillaries and are uncorrected.
8. Nuclear magnetic resonance and carbon magnetic resonance spectra were recorded in $CDCl_3$ solutions containing TMS as an internal standard on Varian J-60, Varian FT-80A, Brucker WH-90 ($^1$H-90 MHz), $^{13}$C-22.3 MHz), Brucker MSL-300 ($^1$H-300 MHz, $^{13}$C-75 MHz) spectrometers. Chemical shifts are expressed in $\delta$ downfield from TMS.

$^{1}$H-nmr ($CDCl_3$, TMS, $\delta$ in ppm, J in Hz)

$^{13}$C-nmr ($CDCl_3$, TMS, $\delta$ in ppm).

9. Optical rotations were recorded on Jasco Dip 181 digital polarimeter using sodium vapour lamp in $CHCl_3$.

10. HPLC was performed on M 6000A pump, M 440 absorbance detector and HP 3330 A integrator of Waters Associates, USA.

11. Ratnam et al[212] have shown the realistic representation of different disaccharides having and glycosidic linkages, however structures drawn in this thesis are according to the normal publication pattern.

# ABSTRACT

The thesis "**Synthesis of complex carbohydrates**" has been divided into five chapters.

**CHAPTER 1 : A REVIEW TO CHEMICAL SYNTHESIS OF OLIGOSACCHARIDES**

Innovations that have comeforth in the chemical synthesis of saccharides have been reviewed (until August 1990) with emphasis on stereoselective formation of O-glycosidic bond.

**CHAPTER 2 : SYNTHESIS OF ALKYL TETRA-O-BENZYL-α-D-GLUCOPYRANOSIDE BY USE OF PER-O-BENZYLATED 2-PYRIDYL THIOGLYCOSIDES AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR. "A NEW GLYCOSIDATION METHODOLOGY".**

A new, mild and efficient glycosidation methodology is reported based on the use of methyl iodide as an electrophilic activator to couple per-O-benzylated 2-pyridyl thioglycosides (**1,2**) with diverse alcohols (ROH: R=Me; i-Pr and t-Bu) to forge the O-glycosidic bond in high axial fidility (1,2-cis-configuration) (Scheme 1).

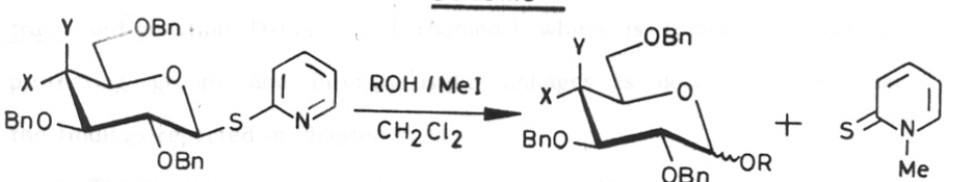
The most significant finding of this reaction is use of even anomeric mixture of 2-pyridyl thioglycosides (**1,2**) as glycosyl donors result in the formation of alkyl α-glycosides (**D**-gluco-, **D**-galacto).

A typical experimental procedure has ensued based on the experiments performed on the (i) effect of solvent (DMF, THF, $CH_2Cl_2$,

$CHCl_3$ and $C_6H_6$) (ii) role of various alkylating agents (MeI, n-BuBr, n-BuI, $Bu_4NBr$, $Bu_4NI$, AgoTf and MeOTf) (iii) reactivity of primary, secondary and tertiary alcohols (iv) reaction time (v) and temperature.

**Typical experimental procedure:** Per-O-benzylated 2-pyridyl-1-thio-β-**D**-glucopyranoside (**1**) (1:10 mmol) was reacted with primary, secondary and tertiary alcohols (MeOH, i-PrOH and t-BuOH) (1.2 mmol) respectively, in dry dichloromethane (10 ml) containing 3% methyl iodide in presence of molecular sieves 4Å at 50°C for 1 to 3 days, to afford (85-90% yield) after workup and chromatographic purification, their corresponding alkyl α-O-glucopyranosides (**1a, 1b** and **1c** respectively) along with a minor amount of β-anomers (**1a** α/β , 65/35; **1b** α/β, 82/18; **1c** α/β, 89/11 from nmr and HPLC data) (Scheme 1).

**Scheme 1**




1) X = OBn , Y = H (Glc)
2) X = H , Y = OBn (Gal)

| | | |
|---|---|---|
| **1a** (Glc) | R = Me | (α/β : 65/35) |
| **1b** (Glc) | R=i-Pr | (α/β : 82/18) |
| **1c** (Glc) | R=t-Bu | (α/β : 89/11) |
| **2a** (Gal) | R=Me | (α/β : 72/28) |
| **2b** (Gal) | R=i-Pr | (α/β : 87/13) |
| **2c** (Gal) | R=t-Bu | (α/β : 91/9) |

Likewise, when per-O-benzylated 2-pyridyl-1-thio-β **-D**-galacto pyranoside (**2**) was reacted with primary, secondary and tertiary alcohols their corresponding alkyl- α-**D**-galactosides (**2a,2b** and **2c**) along with

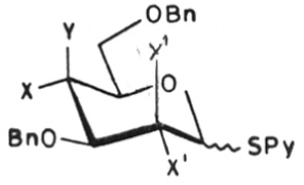
a minor amount of β-anomers (**2a**-α/β, 72/28; **2b**-α/β, 87/13; **2c**-α/β, 91/9 from nmr and HPLC) (Scheme 1).

A comparative account of activation of the 2-pyridylthio moiety of glycosides by methyl iodide and with other well documented modes of activation (heavy metal ion, proton, etc.) is presented to show the efficiency (diastereoselectivity) of the present methodology. The stereochemical outcome of the reaction is rationalized in terms of possible reaction mechanism.

## CHAPTER 3 : A MILD GENERAL METHOD FOR THE SYNTHESIS OF α-LINKED DI- AND TRISACCHARIDES.

A mild, general methodology to obtain various α-linked di- and trisaccharides, (axial) (1,2-cis-configuration, **D**-gluco-**D**-galacto-) (1,2-trans-configuration **D**-manno-, **L**-rhamno-) which is tolerant of various protecting groups and interglycosidic linkages is described based on the findings reported in chapter 2.

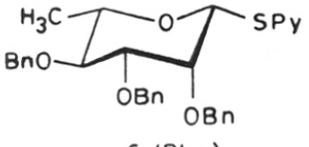
Thus various per-O-benzylated 2-pyridylthioglycosides (**3** to **7**) (with a non participating C-2 neighbouring group) that are easily obtainable from their corresponding sugars viz., **D**-glucose, **D**-galactose, **D**-mannose, **L**-rhamnose and **D**-maltose were successfully coupled under standard reaction conditions (chapter 2) with equimolar amounts of divergent precious sugar alcohols (**8** to **13**) to obtain in good yield (50-85%) α-linked di- and trisaccharides (3+8=**15**, 3+9=**16**, 3+10=**20**, 4+9=**17**, 4+10=**21**, 4+11=**22**, 5+9=**18**, 6+9=**19**, 6+12=**23**, 3+13=**24**, 7+9=**25** and 26+14=**27**).



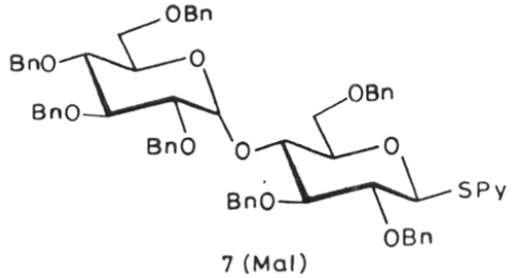

3 X = X' = OBn ; Y = Y' = H (Glc)

4 X = X' = OBn ; Y = Y' = H (Galc)

5 X = X' = OBn ; Y = Y' = H (Man)




6 (Rha)




7 (Mal)

Thus, nine disaccharides and three trisaccharides (eg. panose- a trisaccharide, isolated from amylopectin with protecting groups (24), trisaccharide (25) and the trisaccharide moiety of acarbose (27) were synthesized by this methodology (Scheme 2).

CHAPTER 4 : DIRECTED, ITERATIVE, STEREOSELECTIVE SYNTHESIS OF OLIGOSACCHARIDES BY USE OF 2-PYRIDYL THIOGLYCOSIDES AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR

Directed, iterative and stereoselective methodology to obtain α-linked saccharides (1,2-cis-configuration) is described based on the substantial difference in the rates of glycosidation of 2-O-acetyl (29) and 2-O-benzyl (1) substituted 2-pyridyl thioglycosides on activation

by methyl iodide. Thus coupling of **1** with sugar alcohol (**28**) under standard conditions (Chapter 2) of glycosidation afforded α-linked disaccharides (**30**) (66% yield) (Scheme 3). There was no evidence for the self-condensation of **28**. Acetyl groups of **30** were replaced with benzyl to obtain **31**, the reducing end of which was further coupled with the terminal sugar alcohol "diacetone galactose" (**9**) to obtain the trisaccharide (**32**) (64% yield). The efficiency of this iterative methodology was also demonstrated by use of the disaccharide component such as 2-pyridyl hepta-O-benzyl -1-thio-β-**D**-maltoside(**7**) as glycosyl donor to obtain (7+28= **33**→34+9=**35**) the tetrasaccharide (**35**) (57% yield) (Scheme 3).

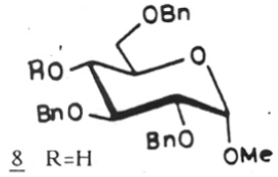
**CHAPTER 5 : STEREOSELECTIVE SYNTHESIS OF β-LINKED (1,2-TRANS-CONFIGURATION) GLYCOSIDES BY USE OF 2-PYRIDYL THIOGLYCOSIDES (WITH A C-2 PARTICIPATING GROUP) AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR.**

Per-O-benzylated 2-pyridyl thioglycoside (**1**) was found to be more reactive than their corresponding per-O-acetylated 2-pyridylthioglycoside (**29**) by methyl iodide activated glycosidation methodology (Chapter 2).

This observation led to the finding of a new glycosidation (1,2-trans) methodology, which is described in this chapter.

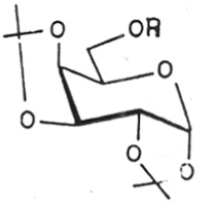
Thus, partnering of 2-acetamido-2-deoxy-2-pyridyl 3,4,6-tri-O-acetyl-1-thio-β-**D**-glucopyranoside (**36**) and 2-pyridyl per-O-benzoylated-1-thio-β-**D**-glucopyranoside (**39**) with simple alcohols [ROH; a) R=i-Pr; b) R=t-Bu] and sugar alcohols (and) under standard conditions (Chapter 2) afforded β-linked (1,2-trans-configuration glycosides (36+a=**36a**, 36+b= **36b**, 36+9=**37**, 36+13=**38**, 39+a=**39a** and 39+9=**40**) in 40-80% yields.

Scheme - 2



8 R=H

15 R =α- tetra-O-benzyl



9 R = H

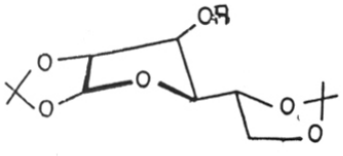
16 R =α - tetra-O-benzyl Glcp

17 R = α- tetra-O-benzyl Galp

18 R = α- tetra-O-benzyl Manp

19 R = α- tri-O-benzyl Rhap

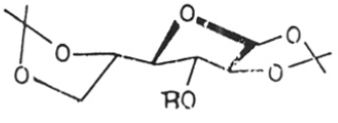
25 R =α - hepta-O-benzyl Malp



10 R = H

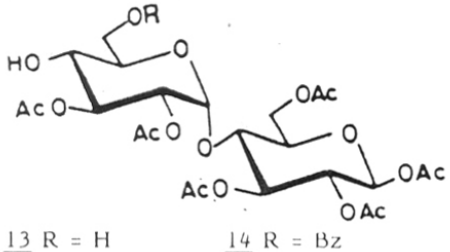
20 R =α- tetra-O-benzyl Glcp

21 R =α- tetra-O-benzyl Galp



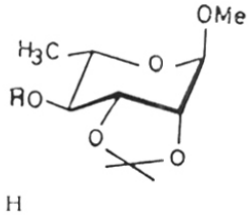
11 R = H

22 R =α- tetra-O-benzyl Galp



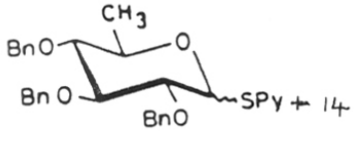
13 R = H 14 R = Bz

24 R = α- tetra-O-benzyl Glcp



12 R = H

23 R = α- tri-O-benzyl Rhap



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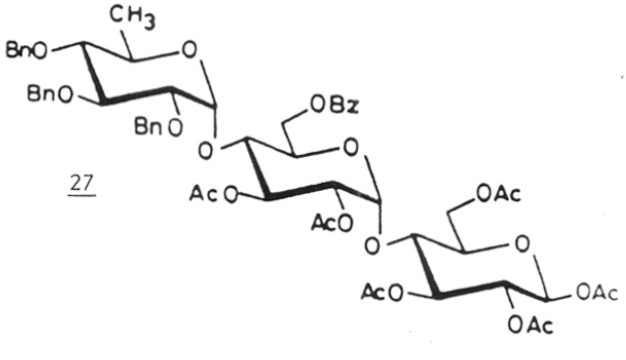
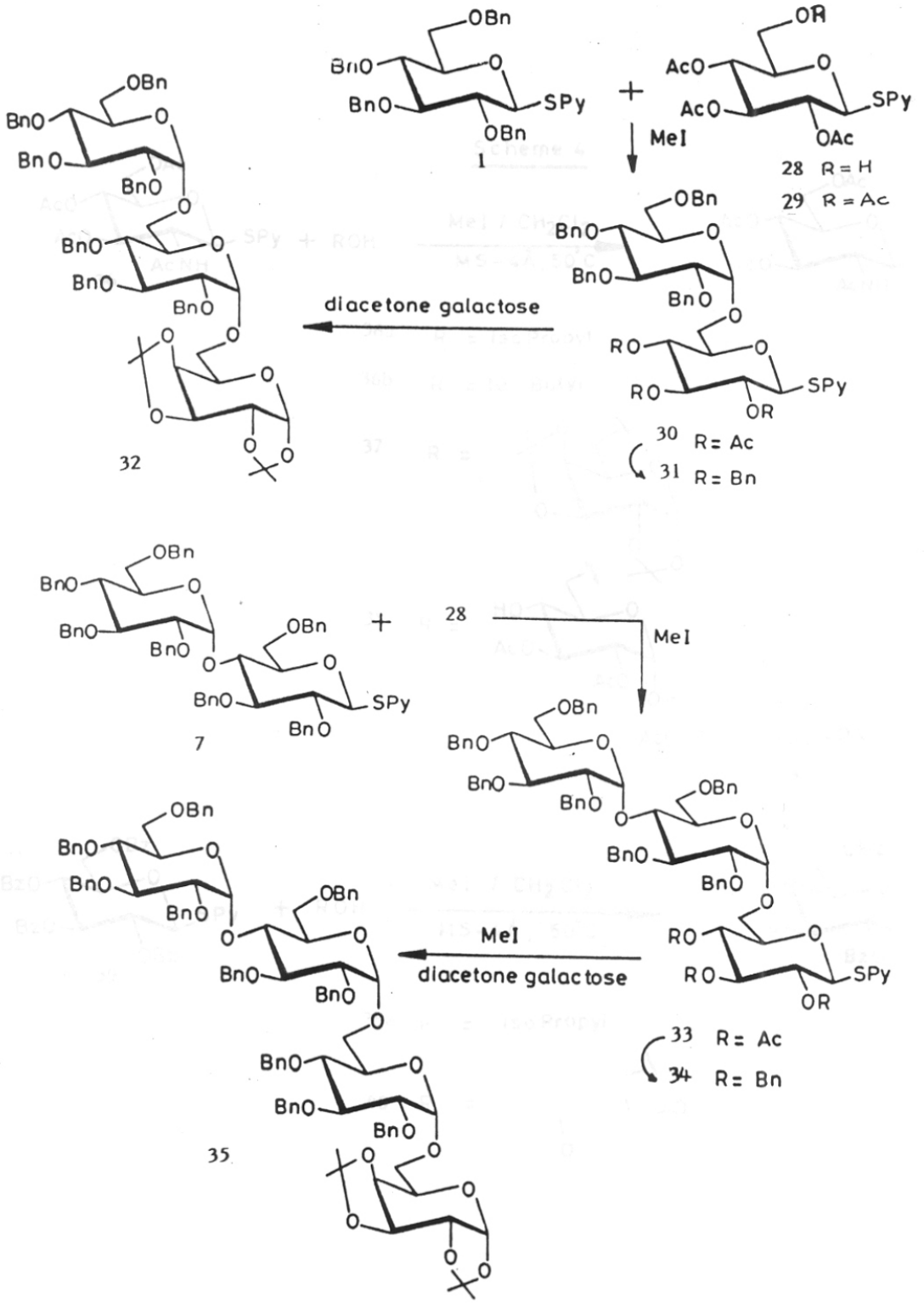
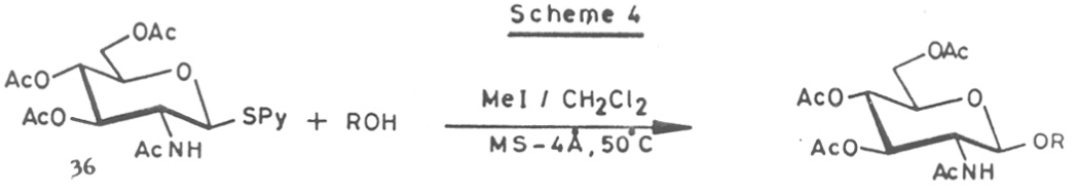


27

**Scheme 3**


OBn
BnO
SPy
OBn
1
+
OR
AcO
SPy
OAc
28 R = H
29 R = Ac
MeI
RO
OR
30 R = Ac
31 R = Bn
diacetone galactose
32
7
28
MeI
33 R = Ac
34 R = Bn
35

Scheme 4

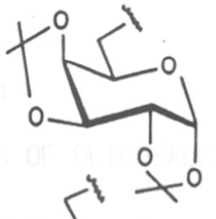



36

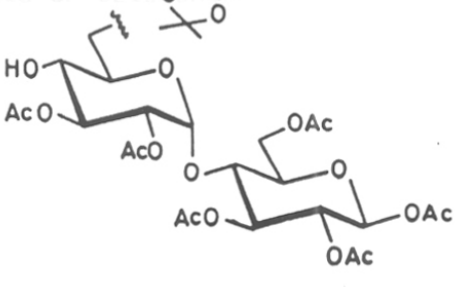
36a R = isoPropyl

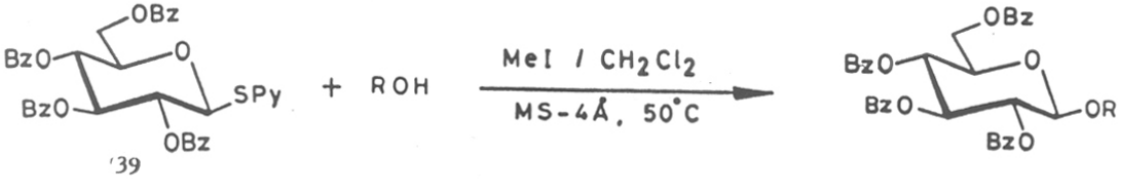
36b R = ter. Butyl

37 R =



38 R =




39

39a R = isoPropyl

40 R =

# CHAPTER 1

# A REVIEW ON CHEMICAL SYNTHESIS OF OLIGOSACCHARIDES

## 1.1 Biological importance and role played by oligosaccharides (Glycoconjugates)

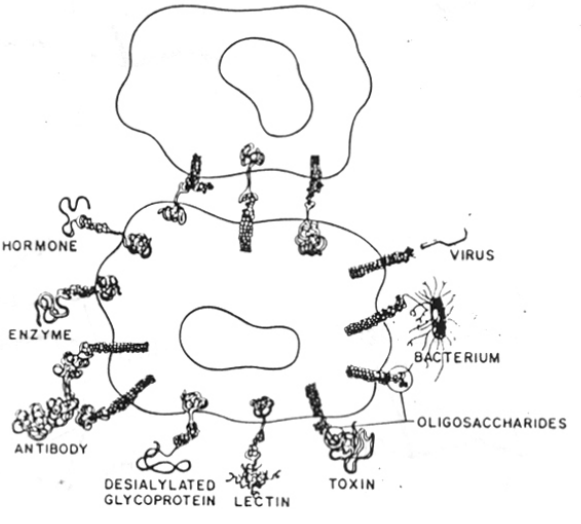
The presence of complex carbohydrate structures as integral constituents of membranes and cell walls has led to manifold activities in recent research[1]. An enormously growing interest, especially in the field of glycoproteins and glycolipids could be observed[2-5]. An increasing number of these glycoconjugates continue to be analysed for their structure.

The oligosaccharide residues are responsible for the inter cellular recognition[6-8], act as receptors for enzymes, hormones, proteins and viruses (Figure 1). They regulate the transportation of proteins between cells, and thus have been regarded as signal substances in cell metabolism[9-11]. The mannan-rich surface glycoprotein , glycosyl phosphatidylinositol residues have been implicated in a second messenger hypothesis for insulin signal transduction[12].

In glycoproteins and glycolipids the oligosaccharide structure often represents the determinant which is responsible for the biological functions. In this case interactions between the carbohydrate residues and other proteins are possible, for example, in the antigen antibody reactions. There are indications that receptor interactions may play an important role for the cell communication[13]. In both cases, molecular structure of the carbohydrate sequence often determines the selectivity of the reaction with the protein.

In glycolipids, the lipid part serves as a means of anchorage in the double layer of the cell membrane, the oligosaccharide component protrudes from the cell membrane and is the determinant and its structure is decisive for their specificity of the immune reaction[14].

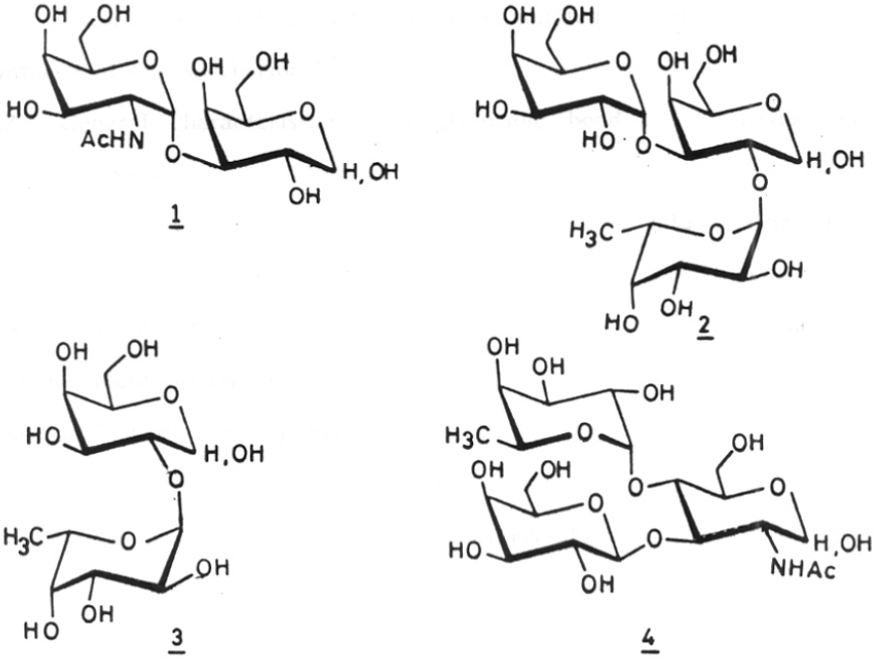
The glycosphingolipids undergo significant changes when malignant cell growth starts. In particular, the structure of the hydrophilic carbohydrate residue becomes simplified[8].




VARIOUS CARBOHYDRATES
PRESENT ON CELL SURFACE

Blood group Lewis a substances that are antigenic determinants are found in the secretions of most individuals who are non secretors of A, B or H substances[15]. The antigenic determinants mainly responsible for Le. a specificity have been shown to be 3-O-(2-acetamido -2-deoxy-α-D-galactopyranosyl)-D-galactopyranose (**1**, the terminal disaccharide unit of the human blood group A determinant), 2-O-(α-L-fucopyranosyl)-3-O-(α-D-galactopyranosyl) D-galactose (**2**, the terminal trisaccharide unit of the human blood group B determinant), 2-O-(α-L-fucopyranosyl)-D-galactose (**3**, the terminal disaccharide unit of the human blood-group

**Scheme 1**



O(H) determinant) and 2-acetamido-2-deoxy-4-O-(α-L-fucopyranosyl)-3-O-(β-D-galactopyranosyl)-D-glucose (**4**, the terminal trisaccharide unit of the human blood-group Le a[16]).

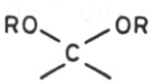
Because of their surface location, oligosaccharides are important agents in bacterial pathogenesis, as they interact directly with the host's immune system[17]. Vaccines developed for the prophylaxis of contagious diseases, have always had certain defects in that they caused adverse reactions in some patients because of the extraneous materials they contained. Thus synthetic vaccines might soon become a reality for making antigens having unique synthetic determinants capable of provoking anti-

bodies that neutralize efficiently a virus or a bacterial toxin thereby "resulting totally in synthetic immunogen"[18].

These significant activities have stimulated interest in chemical synthesis of oligosaccharides[19-31].

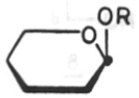
## 1.2 General characteristics of O-glycosidic bond : Formation and Cleavage

The most fundamental structural unit of oligosaccharide chain is the O-glycosidic bond[19]. It is an example of the acetal bond **5** and has the same set of fundamental properties. O-Glycosidic bond has two non-equivalent alkoxy groups attached to the glycosidic center (anomeric center). One of these groups is included in the cyclic system, where as the other is exocyclic and is much more reactive than the former (structure **6** and **7**). So the typical reactions of formation and cleavage



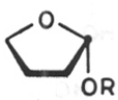

acetal

**5**




cyclic acetal

**6**




• anomeric center

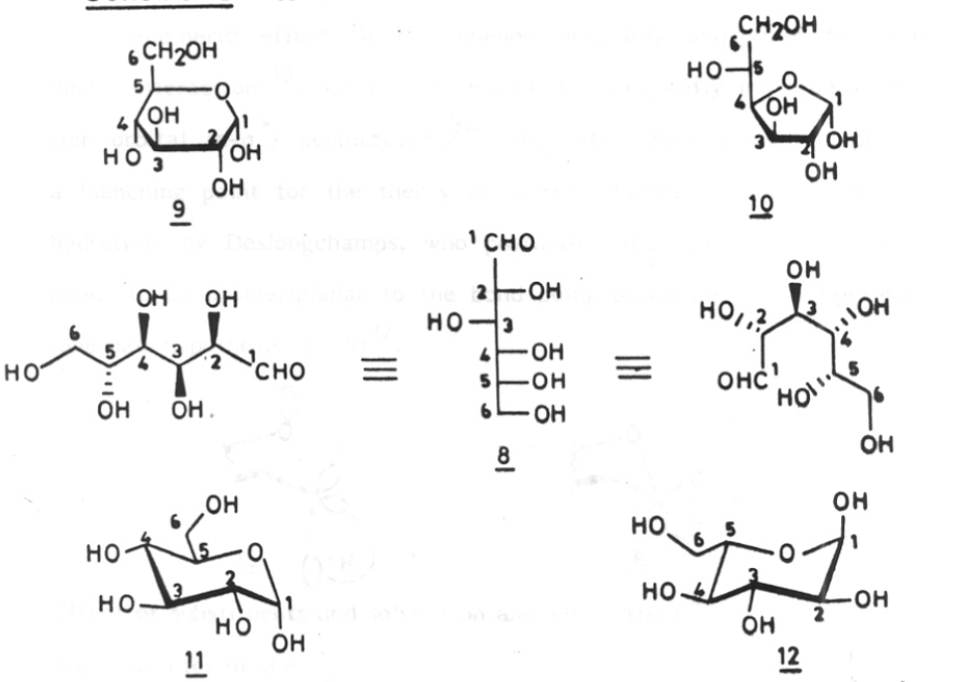
**7**

of the O-glycosidic bond proceed by exchange of exocyclic residues of the acetal system, in which the oxygen heterocycle remains unreacted.

Taking **D**-glucose as an example it has been shown that[32] the acyclic polyhydroxy aldehyde form **8** is in equilibrium with two cyclic forms (Scheme 2). The six membered pyranose ring **9** is favoured over the five **10** and the seven membered forms, as would be expected on conformational grounds. In the $^4C_1$ conformation, α-**D**-glucopyranose **11** has all its substituents equatorial except for the anomeric substituent. This form of **D**-glucose could be conveniently manipulated as a six mem-

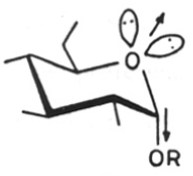
bered cyclic derivative, much in the same way as a cyclohexane derivative, with allowance being made for the stereoelectronic consequences of replacing a ring carbon by an oxygen atom (Scheme 2). The $^{1}C_{4}$ conformation, α-L-glucopyranose **12** also has its anomeric 1-OH in axial and all other substituents in equatorial position.

**Scheme 2** **α-D-Glucose conformations**




### 1.2.1 The anomeric effect

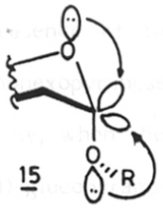
The term anomeric effect introduced first time by Lemieux etal[33,34] in 1958, refers to the tendency of an alkoxy group at C-1 of pyranose ring to assume the axial **13** rather than the equatorial **14** orientation despite unfavourable steric interactions[34].




13


14

Anomeric effect, a phenomenon originally attributed to dipole-dipole interactions[35], has been interpreted subsequently in terms of frontier orbital ($n-\sigma^*$) perturbations[36]. The latter description has provided a launching point for the theory of stereoelectronic control in glycoside hydrolysis by Deslongchamps, who postulated that an electron lone pair needs to be antiperiplanar to the bond being broken i.e. the antiperiplanar lone pair hypothesis, ALPH[37].

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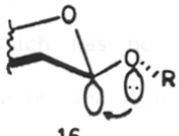



16

**Effect of substituents and solvent on anomeric effect[38].**

**The character of the aglycon:**

The preference for the axial position increases with the electron-withdrawing character of substituent at anomeric center and is most conspicuous for the halogen and alkoxy derivatives. An increase in size of the alkoxy group diminishes the preponderence of the axial form, such that changing from a methoxyl to tertiary butoxyl group[39]. An enhancement of the electronegativity of anomeric substituent by change from the ethoxy to trichloroethoxy derivative increases the population

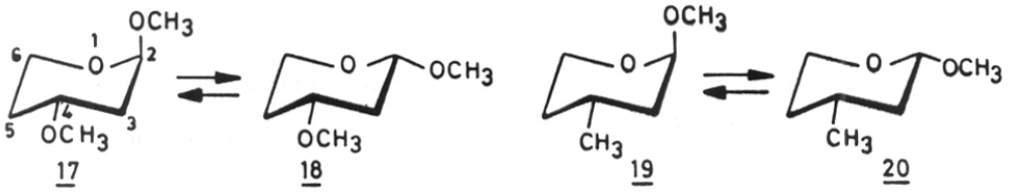
of the axial form from 80 to 95%[40]. Similarly, in halogen derivatives of hexapyranoses, the axial forms are the sole detectable species[41].

The preference for the axial position diminishes with lowered electronegativity of atoms linked to the anomeric center; that is F >O >N >C for the first row of elements in the Periodic Table. As a rough guide it may be stated at the outset that[42,43] the anomeric effect decreases through the series halogen > benzyloxy > acetoxy > acetyl thio > methoxyl > alkyl thio > hydroxyl > amino > methoxy carbonyl > imidizolium pyridinium[43].

**Nature of the other substituents**

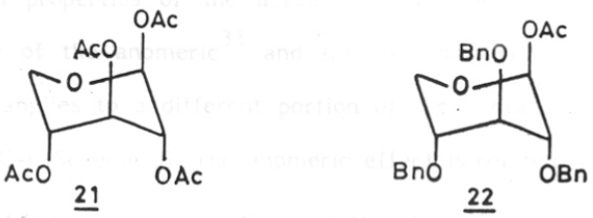
It is well known that the presence and configuration of a hydroxyl on C-2 of the pyranose ring markedly affects the anomeric equilibrium. Thus, in the case of **D**-mannopyranose, the axial hydroxyl group on C-2 increases the presence of the α-anomer (69%)[44] relative to that for 2-deoxy-**D**-arabinohexopyranose (47.5%)[45], which has no hydroxyl group on C-2. Conversely, when the hydroxyl group on C-2 is in the equatorial position, as in **D**-glucopyranose, the position of the α -anomer decreases to 36%, which is due to the Δ 2 effect[46]. In this arrangement, the oxygen atoms around carbon atoms 1 and 2 result in increased conformational instability, due to the placement of three oxygen atoms in close proximity. Whenever this occurs it is indicated by the notation Δ2[46].

The electronegativity of the substituent on C-4 also influences the anomeric equilibrium. Consequently, 2,4-dimethoxy oxane exists in methanol as an equilibrium mixture (**17** and **18**) containing 80% of the isomer having[47] an axial methoxyl group on C-2 (**17**), compared with 67-69% for 2-methoxy-4-methyl oxane (**19**)[48-49].



17 18 19 20

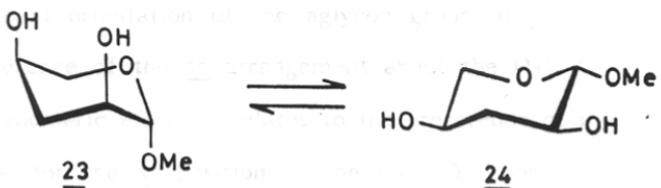
In general, the presence of several bulky substituents on the pyranose ring makes the anomeric equilibrium very intricate, and even the all axial $^{1}C_{4}$ form has been observed[50] both in β -D-xylopyranose tetraacetate (21, 28%) and corresponding tribenzyl β -D-xylopyranosyl acetate (22, 47%).



21 22

**Nature of the solvent**

The influence of dielectric constant of the solvent on the anomeric effect, and hence on conformational equilibria, is often found to be incidental to other, more important solvation effects involving hydrogen bonding. Thus, methyl-3-deoxy β L-erythropentopyranoside (24) exists predominantly as the $^{4}C_{1}$ conformer 23 in solvents such as chloroform which do not form hydrogen bonds with hydrogen atoms of the hydroxyl groups. Indeed, under such circumstances, the $^{4}C_{1}$ conformer is stabilized by an intermolecular hydrogen bond involving the syn axial hydroxyl groups[51].



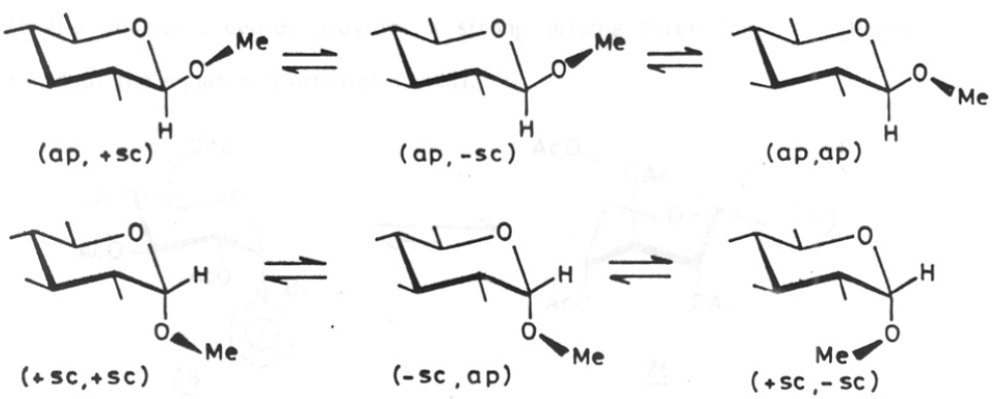
23 24

In summary, experimental data on the isomeric abundances at anomeric equilibrium reveals that the preference for the axial position depends on several interconnected factors like character of aglycon, nature of the solvent and substituents etc, which were clarified in surveys on carbohydrate stereochemistry[42,52].

### 1.2.2 The Exo-anomeric effect

The term exo-anomeric effect was introduced to describe an orientational effect on the aglycon part of a glucopyranoside[53], arising from the special properties of the acetal moiety. There is no difference in the nature of the anomeric[33] and the exo-anomeric effects[53], each of them just applies to a different portion of the acetal segment C-5, O-5, C-1, O-1, C-i (Scheme 3). The anomeric effect is related to the preference

**Scheme 3** **Staggered orientations of the Aglycon in methyl α- and β- D - glucopyranoside. : exoanomeric effect**



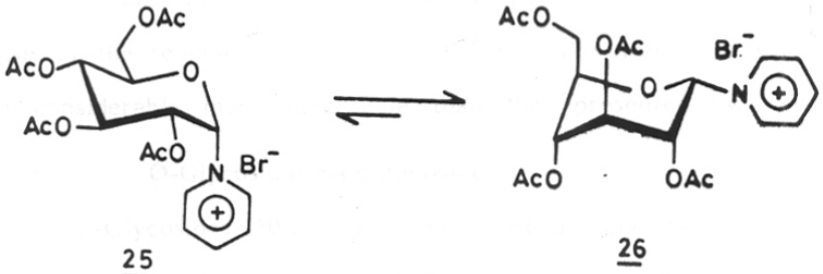

**sc = synclinal or gauche , ap = antiperiplaner or trans**

of the axial orientation of the aglycon group in glycosides, that is, to the preference of the sc arrangement about the O-5, C-1 bond, whereas the exo-anomeric effect[53] relates to the preference of the aglycon carbon atom C-i for the sc position at the C-1, O-1 bond rotational potential

specified by the angle $\theta$. Obviously, the most important outcome of the exo-anomeric effect concerns the relative stability of mutual orientations of the neighbouring saccharide units in oligo and polysaccharides. The exo-anomeric effect is illustrated in Scheme 3, which shows three staggered orientations for rotation about the glycosidic bond in both the $\alpha$ and $\beta$ anomer of methyl D-glucopyranoside[38]. They are referred to as (+sc, +sc), (+sc, ap), and (+sc,-sc) and (ap, +sc), (ap, -sc), and (ap,ap), respectively, using two torsional angles ( $\theta$ and $\phi$ ) for specification of orientations of the C-5, O-5, C-1, O-1-C-i moiety.

### 1.2.3 The Reverse anomeric effect

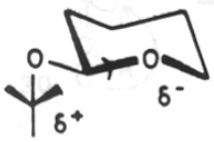
The term "Reverse anomeric effect" has been defined by Lemieux et al as the enhanced trend of the quarternary nitrogen atom to adopt an equatorial orientation[54]. Studies of the protonation of N-glycosyl pyridines[54], imidazoles, and pyrimidines showed[31,38,55] that the preference of the positive charge on the nitrogen atom (**25** and **26**) linked to the anomeric center provides a strong driving force for the aglycon to adopt the equatorial orientation (**26**).



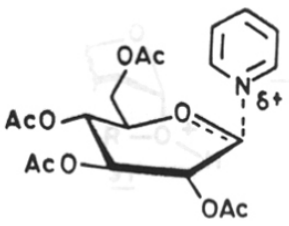

**25** **26**

This preference for the equatorial position in excess above the value that ensued from steric analysis of cyclohexane has been termed as the "Reverse anomeric effect"[54]. The reason explained for the preferential formation of equatorial orientation was, the protonation of an axial

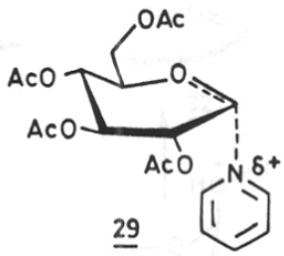
aglycon causes strong destabilization of the conformation of the sugar ring relative to that where in the aglycon is in equatorial orientation. Thus it has been speculated that for α-glycosides, the preferred point of protonation in the course of acid-catalysed hydrolysis would be at the ring-oxygen atom. On the other hand, for β-glycosides, the reverse anomeric effect would tend to lead to protonation at the sterically and electronically most favourable position where in the first carbon of the aglycon is gauche to the ring-oxygen as shown[54] in the structure **27**.




**27**




**28**




**29**

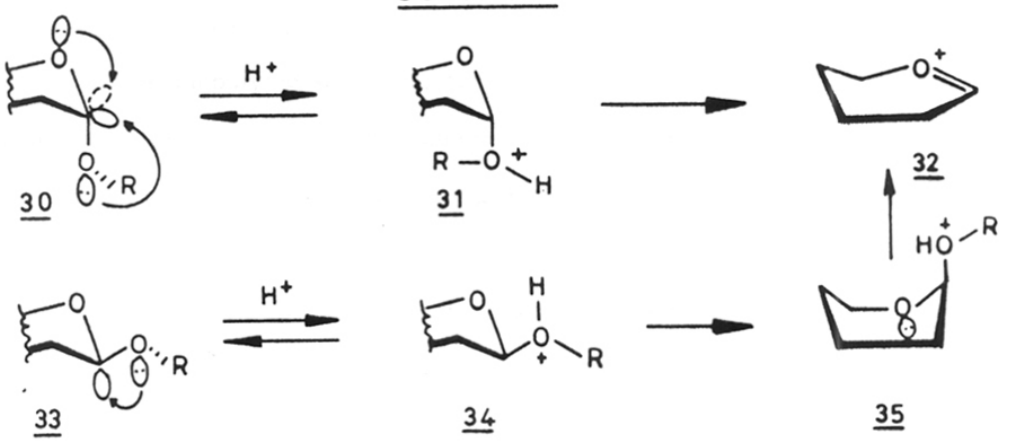
Moreover, an attack by a molecule at the anomeric center which leads to development of a positive charge on the entering group must be expected to be much more favourable when the entering group leads to the equatorial product. For example, it would be expected on the basis of the reverse anomeric effect[54] that the transition state **28** would be considerably more favourable than that presented by structure **29**.

### 1.2.4 O-Glycosidic bond cleavage

α-Glycosides (**30**), on protonation of the glycosidic oxygen directly lead to the reactive intermediate **31** which has the (presumably) optimum geometry for cleavage to the cyclic oxocarbenium ion **32**[56]. However, for the conjugate acid **34** of the β-glycoside **33**, a conformational change, (**34** → **35**) is required in order to meet this antiperiplanar lonepair require-

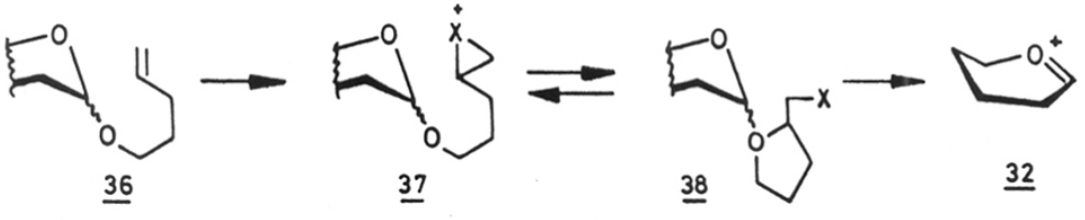
ment[56]. Study of the stereoelectronic requirements for glycoside hydrolysis have relied on kinetic isotopic measurements[56], the presence of "spontaneous" leaving groups at the anomeric center, the study of conformationally biased systems[57], and studies of sulfur analogues. And recently carbohydrate derivatives that are so conformationally restrained that the stereochemical options available are unequivocally defined have also been studied[58].

Scheme 4




The same geometric requirements have been held for the oxidative hydrolysis of n-pentenyl acetals (**36**) and it has been subsequently utilized synthetically for chemospecific liberation of the anomeric center (Scheme 5; **32**, **37** and **38**) by Fraser-Reid et al[59].

Scheme 5



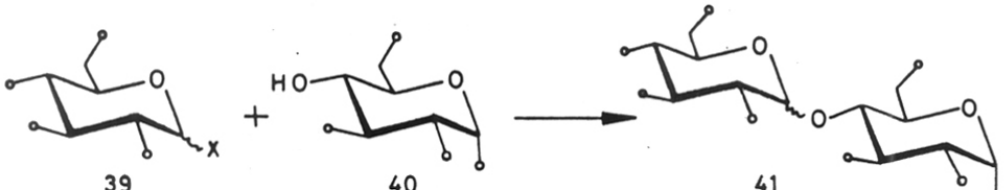
## 1.3 Stereoselective synthesis of oligosaccharides

### General aspects

The stereoselective chemical synthesis of complex oligosaccharides has made remarkable progress over the past fifteen years. Biologically interesting oligosaccharides[60-71] with different building blocks and different types of linkage at the anomeric center have now been systematically synthesized[21]. Improved methods of reaction, selectivity, new catalyst systems, and new separation and analytical techniques have made this possible[22].

Chemical synthesis of oligosaccharides is more complicated than that of other biopolymers. In oligosaccharide synthesis two polyfunctional sugar components are linked (Scheme 6). Regioselectivity is generally achieved by reaction of the glycosylating component **39** (glycosyl donor) possessing selectively protected hydroxyl groups and an activating group at the anomeric carbon atom with a sugar component having one free hydroxyl group (**40**, glycosyl acceptor) and protecting groups at all other hydroxyl functions. Thus, necessitating complicated protecting strategies and suitable procedures for activation at the anomeric carbon atom for saccharide coupling leading to the synthesis of disaccharides (**41**). In addition, the saccharide coupling step should occur diastereo selectively with respect to the formation of an α- or β-linkage (Scheme 6).

**Scheme 6**




39 glycosy donor + 40 glycosy acceptor → 41 disaccharide

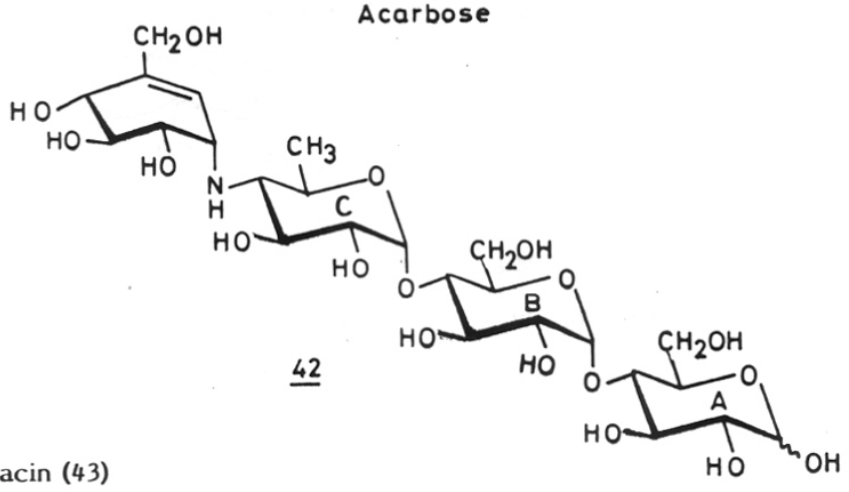
X = activating group ∘ = protecting group

**Some biologically intereesting oligosaccharides**

**Acarbose (42)**

The pseudotetrasaccharide (**42**) containing an unsaturated cyclitol moiety, is a potent inhibitor of intestinal α **D**-glucosidases and saccharases in vitro[60], and may now be used clinically as an effective oral antidiabetic agent has been isolated from strains of Actinoplanes. Considerable interest has therefore been stimulated in the bio-chemistry of this class of inhibitors[61], and extensive synthetic studies have been carried out[62,63].

**Acarbose**

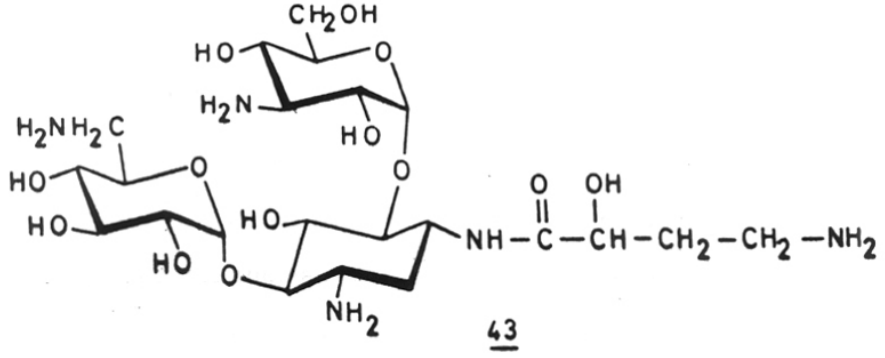



42

**Amikacin (43)**

It is a semi-synthetic aminoglycoside, derived from Kanamycin A[65] and has shown antibacterial and antibiotic[64] activity.

**Amikacin**

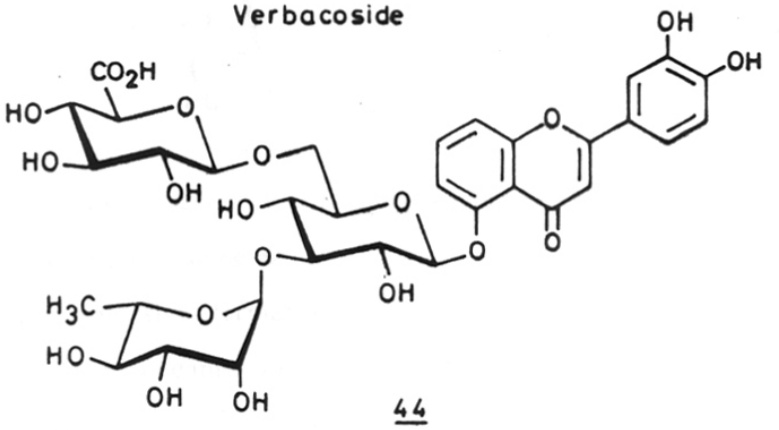



43

**Verbacoside (44)**

A luteolin glycoside from verbascum thapsus is used in the indigenous system of Indian medicine for the treatment of inflammatory disease, asthma and spasmodic coughs[66].

Verbacoside

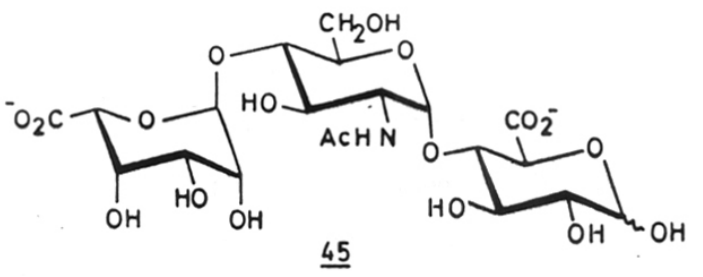


44

**Heparin (45)**

Heparin belongs to the class of glycosaminoglycan (GAG), an alternating copolymer of a hexosamine and an alduronic acid[67] and is used, in therapy as an anticoagulant and antilipemic agent[68].

Heparin

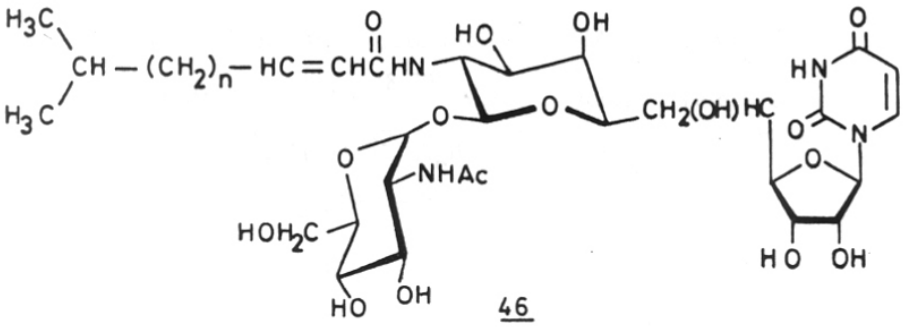


45

**Tunicamycin (46)**

A family of nucleoside antibiotics produced by Streptomyces lysosuperificus[69-71]. It has been shown to interfere with glycoprotein synthesis in yeast and mammalian systems[70]. It is used as a tool in studying glycoproteins in a wide variety of biological systems.

Tunicamycin



46

**1.3.1 β-Glycosidations (1,2-trans-D-gluco-,D-galacto- and 1,2-cis-D-manno-, L-rhamno-).**

For selective formation of β-glycosidic linkages in oligosaccharide synthesis, the fundamental requirement is that the H atom of 2-OH of the glycosyl donor be replaced by a neighbouring group active substituent[22]. 1,2-Trans-glycosidations in **D**-gluco- and **D**-galacto- series and 1,2-cis-glycosidations in **D**-manno- and **L**-rhamno- series have been performed involving neighbouring group participation[21-25].

**1.3.1.1 Glycosyl halides as donors (The Koenigs-Knorr method)**

The classical Koenigs-Knorr method[72] glycoside synthesis dating from 1901 consists of a two-step reaction[21].

1) Introduction of a leaving group at the anomeric center and

2) Catalytic nucleophilic substitution of this leaving group leading to inversion at the anomeric center.

In general, β-coupling proceeds under strict stereocontrol by exploitation of stereoelectronic effects[21] (anomeric[33-37] and reverse anomeric[54] effects), neighbouring group participation and choice of the catalyst.

The following general picture is given in the recent reveiws[21-29].

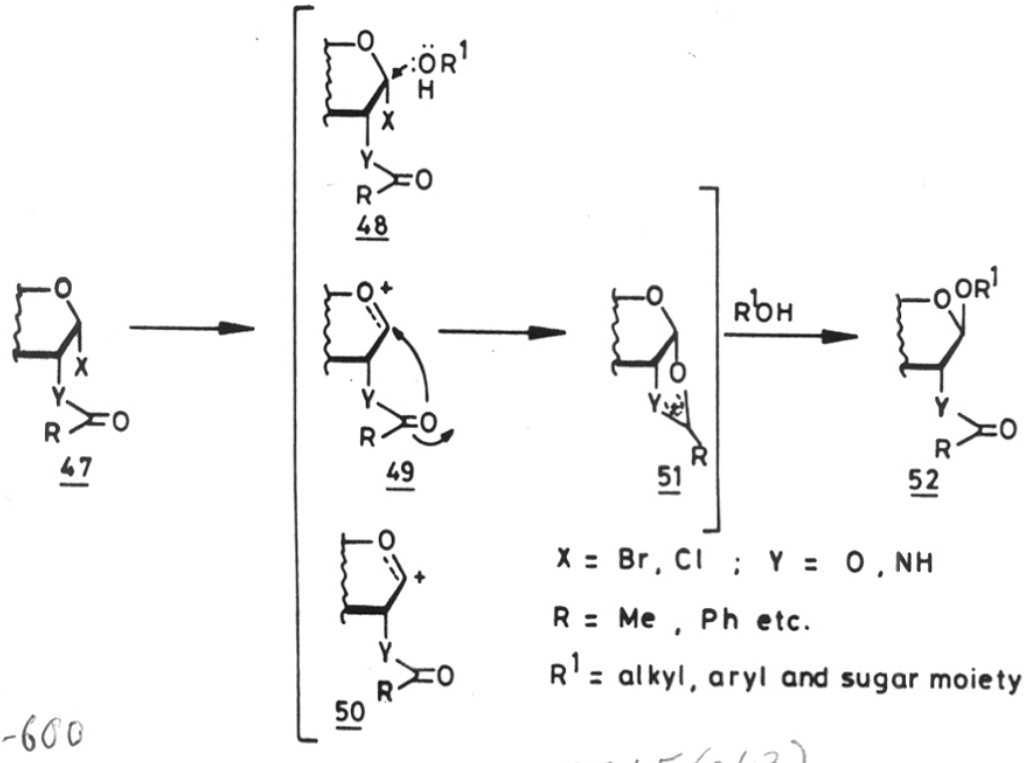
1) The reactivity of the glycosyl donor (i.e. the glycosyl halide) has been varied over relatively wide range by the choice of halogen (Cl, Br), the catalyst[73-87], ($Ag_2CO_3/Ag_2O$[73-76], $Hg(OAc)_2$[77] $HgBr_2/Hg$ $(CN)_2$[78,79] silver hydroxy velerate and salicylate[80], AgOTf[81], AgOTs[82], $AgBF_4$[83], $AgClO_4/Ag_2CO_3$[84,85], Silver zeolites, silver silicate[86], $Ti^+$, $Co^{2+}$, $Cd^{+2}$ salts[87]) and the protecting group pattern.

2) Diastereoselectivity in the coupling is attained by means of participation of the neighbouring group for the formation of β-(1,2-trans)-glycosides of D-gluco-, D-galacto- and α-(1,2-trans)-glycosides of D-manno-[79-88] and L-rhamno-[89] series.

**Mechanism of Koenigs-Knorr reaction**[19]

Condensation of 1,2-cis-acylglycosyl halides **47** with alcohols proceeds usually with total inversion at C-1 leading to 1,2-trans-glycoside **52** (Scheme 7). This result could be the consequence of either an $SN_2$

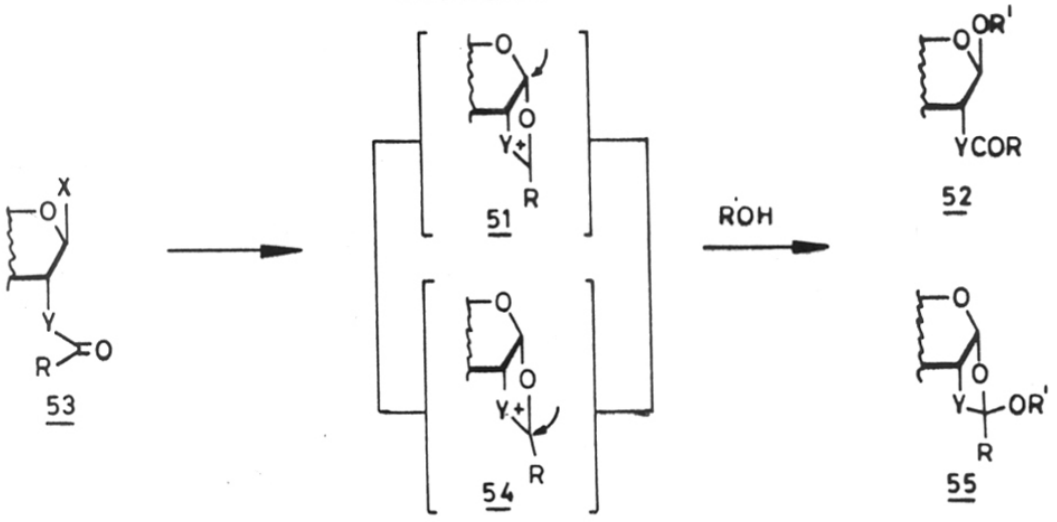
**Scheme 7**. Mechanism of Koenigs - Knorr reaction



reaction (**48**) or heterolysis of C-1 halogen bond leading to glycosyl cation **49**, which stabilizes immediately by intramolecular nucleophilic attack by an ester group at C-2, to give acyloxonium ion **51**, which opens up by a nucleophile from β -face. The initial heterolysis could also occur with the formation of an intimate ion pair **50**, and provides inversion product **52** as a result of condensation with an alcohol.

Reactions of 1,2-trans-acyl-glycosyl halides **53** with alcohols tend to proceed by monomolecular heterolysis of C-1 halogen bond assisted by a neighbouring acyloxy group. Acyloxonium ion **51** thus formed either could react with an alcohol to give 1,2-trans-glycoside (**52**) or by attack of the latter to the center of the charge **54** result in the formation of orthoester **55** (Scheme 8).

**Scheme 8**




Inspite of development of efficient variants, however, severe, partly inherent disadvantages of the Koenigs-Knorr method for the synthe-

sis of oligosaccharides could not be overcome. These disadvantages include the following[21].

1) Relatively harsh conditions are needed for the generation of the glycosyl halide.

2) The glycosyl halides exhibit low thermal stability[21] and have been generated only in situ and at lower temperatures[25], thus necessitating the use of compounds that are sterically nonhomogeneous and sometimes even impure[21].

3) The glycosyl halides are highly sensitive to hydrolysis[19].

4) The heavy-metal salts are expensive[21].

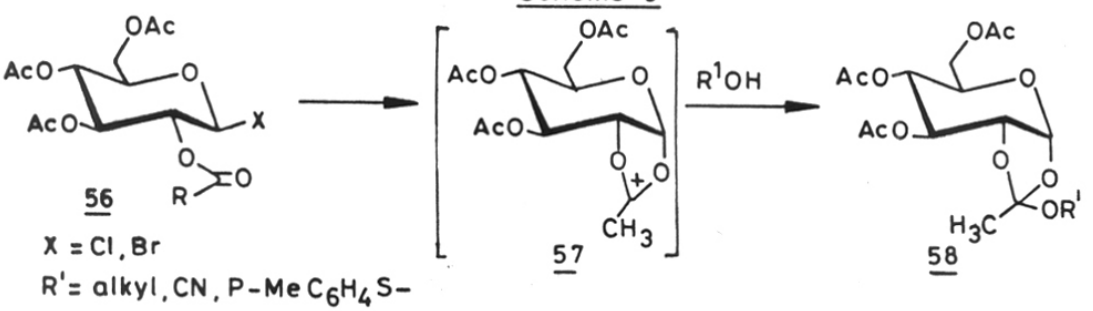
5) The use of heavy-metal salts especially in large scale reactions, is often disadvantageous (toxicity of mercury salts[77,78] explosions[90] with silver perchlorate[79]) though these risks have been reduced sometimes by using catalytic amounts[21].

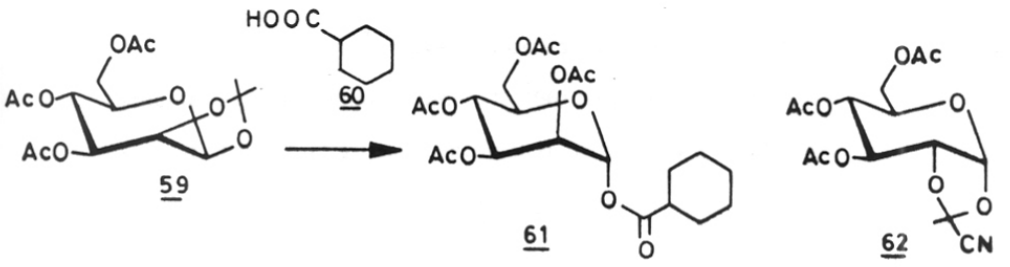
Many attempts have been made[21] to develop competitive methods, which do not require the use of heavy metal salts[91]. However, unitl recently, their general significance has been described as modest[21,22].

### 1.3.1.2 Glycosyl orthoesters as donors

The synthesis of orthoesters (58)[92] (Scheme 9) from 1,2-trans-glycosyl halides **56** and their mercury salt catalysed rearrangement to glycosides and saccharides have been known for a long time[23,24].

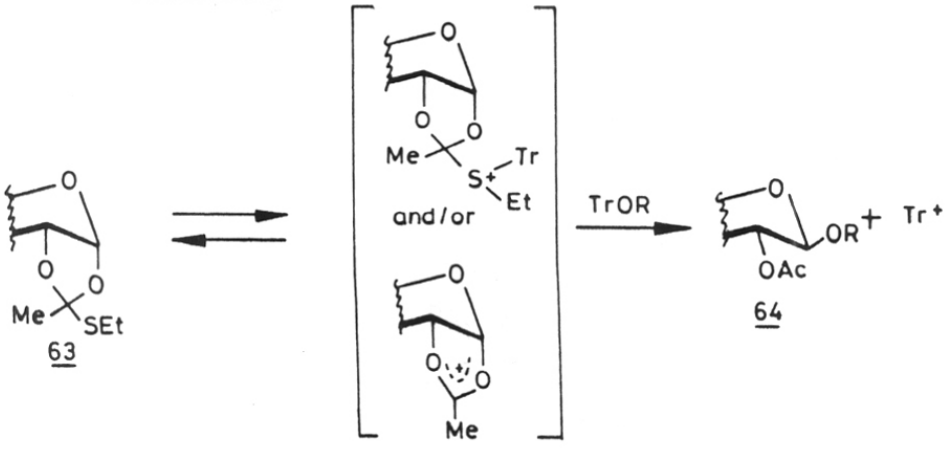
Scheme 9





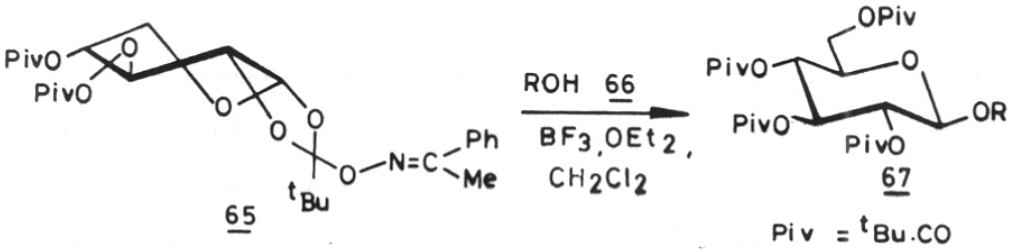

Activation of the $C_1$-O glycosidic bond for glycosyl transfer has resulted from the alkylating character of orthoesters. Thus, as expected, treatment of orthoester **59** with carboxylic acid **60** has resulted in the formation of 1-O-acyl compounds **61**[93]. Kochetkov et al.[94] have developed the orthoester method. The disadvantage of this reaction is the formation of the 2-O-unsubstituted sugar by a competing protonation at the 2-O-atom[95]. This side reaction has been avoided by formation of the acyloxonium intermediate **57** and trapping it with cyanide[59] to give **62**[96] (Scheme 9), or alternatively by the introduction of an alkylthio group[97] (**63**). O-Trityl sugars as acceptors and trityl perchlorate as a co-activator resulted in 1,2-trans-linked disaccharides of glucose, galactose[97,98] eg., **64** in good yield (Scheme 10).

**Scheme 10**



Kunz et al [99] have reported a 1,2-trans-glycosidation reaction (Scheme 11) of complex alcohols (**66**) and phenols with the oxime ortho-ester of O-pivaloyl glucopyranose (**65**) to give β-glycosides **67.**

**Scheme 11**

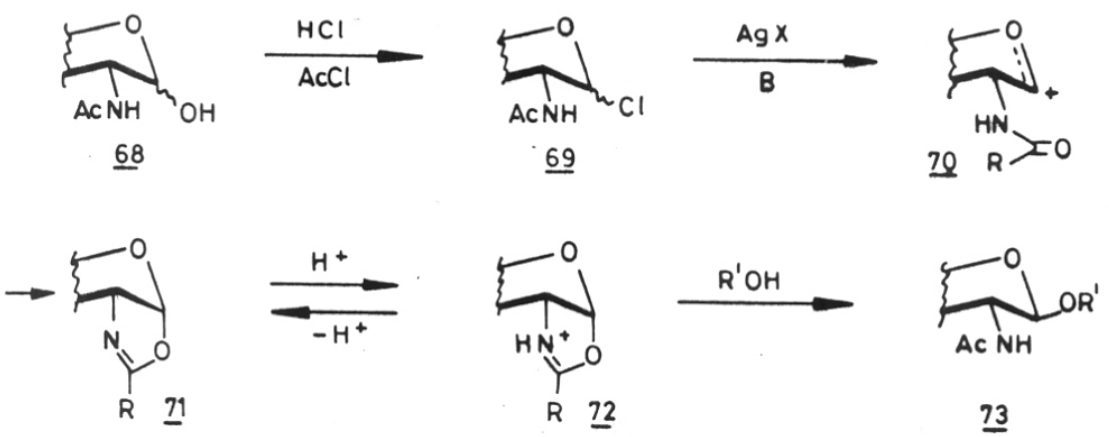



R = Complex steroidal alcohols, steroidol phenols & serine derivative etc.,

**1.3.1.3 Glycosyl oxazolines as donors**

Synthesis of 1,2-trans-glycosides and saccharides of 2-amino-2-deoxy-sugars has been achieved by this method. The oxazoline derivatives of sugars **71** were conveniently prepared[100] by the treatment of N-acylated aminosugar **68** with hydrogen chloride and acetyl chloride to give the N-acyl glycosyl chloride **69.** The reaction of **69** with silver salt and 2,4,6-collidine (Scheme 12) gave oxazoline derivative **71** via **70.**

**Scheme 12**




R = Me , Ph ; X = $NO_3$ , Cl , OTs B = Collidine

R' = alkyl , aryl or diacetone galactose etc.

The oxazoline **71** on protonation[101,102] forms the cation (**72**) which undergoes a nucleophilic attack by an alcohol leading to inversion of configuration at C-1, and formation of 1,2-trans-glycosides **73** (Scheme 12).

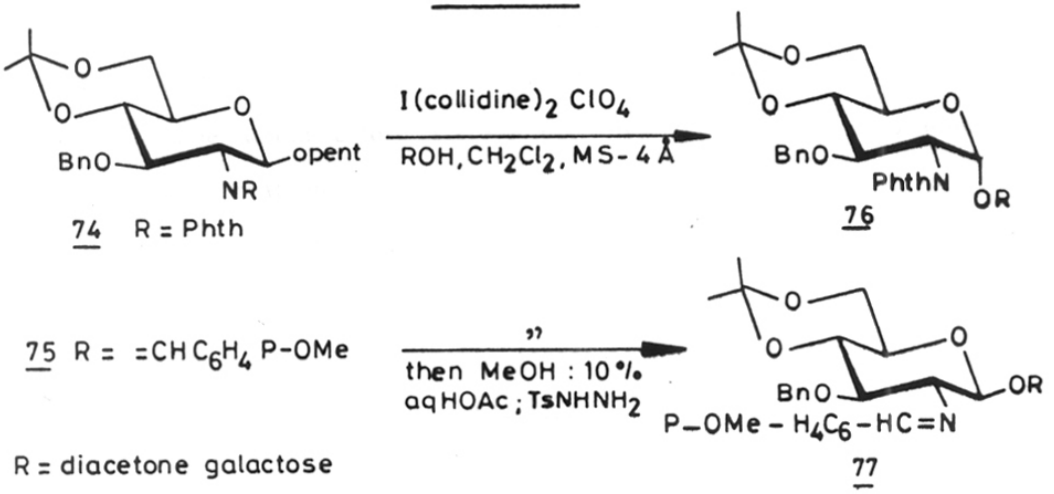
The oxazoline method has been successfully used for the synthesis of 1,2-trans-2-amino-2-deoxy oligosaccharides[103]. 2-Acylamino-2-deoxy-β-D-glucopyranose-1-acetates have been used as a source of glycosyl donors for the synthesis of β-glycosides and disaccharides. Anderson et al[104] have described a direct glycosidation reaction in which the 1-acetate was treated with anhydrous ferric chloride in dichloromethane, oxazolines **71** were formed in the absence of alcoholic reactant[105]. Other related compounds like N-benzoyl, N-chloroacetyl, N-phenoxy acetyl and N-phthaloyl congeners have also been utilized as glycosyl donors, coupling at the 6-position of glycosyl acceptors proceeded smoothly and gave 67-80% yields of disaccharides.

Several improvements in the synthesis of saccharides of N-protected derivatives of 2-amino-2-deoxy-D-glycopyranose have been made making use of various catalysts such as $Hg(CN)_2$[106,107,108], TMS-triflate[109], Sn(II) triflate[110], Tin(IV) chloride[111].

### 1.3.1.4 n-Pent-4-enyl-2-amino-2-deoxy-O-glycosides as donors

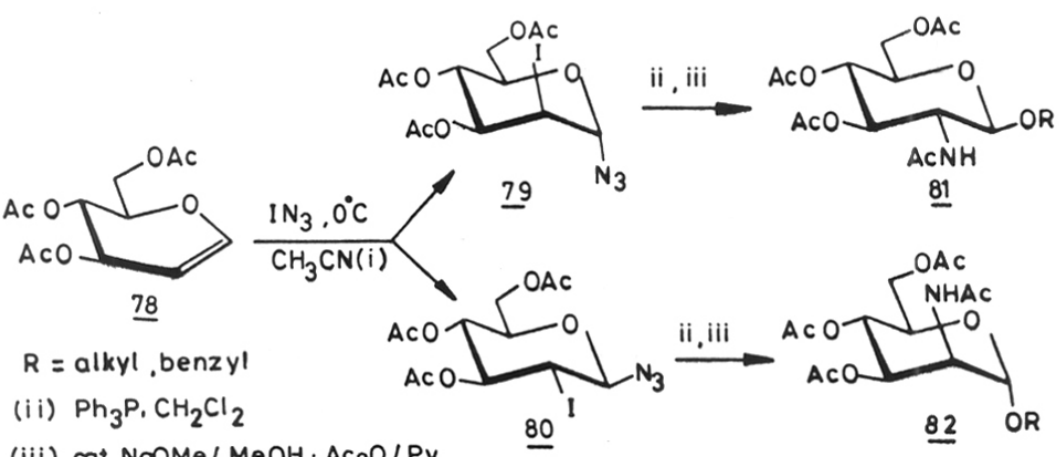
Fraser-Reid et al[112] have developed a new glycosidation reaction making use of n-pent-4-enyl-2-deoxy-2-phthalimido (**74**) and 2-anisylidene amino-2-deoxy-D-glucopyranoside (**75**) as glycosyl donors. Iodonium ion induced saccharide couplings have been performed with a variety of sugar alcohols to obtain selectively either β (eg. **76**) or α (eg. **77**) linked saccharides in moderate to excellent yields (60-90%). The procedure is tolerant of a wide variety of protecting groups (Scheme 13).

Scheme 13




A new synthesis of 1,2-trans-2-acetamido-2-deoxy-glycopyranosides via 1,2-trans-2-deoxy-2-iodo-glycosyl azides has been reported[113]. Trans addition of iodoazide to the double bond in compound **78**, yielded 2-deoxy-2-iodoglycosyl azides **79** and **80**, which are precursors of 1,2-trans-2-amino-2-deoxy glycopyranosides **81** and **82** respectively when treated by an alcohol in the presence of triphenylphosphine (Scheme 14).

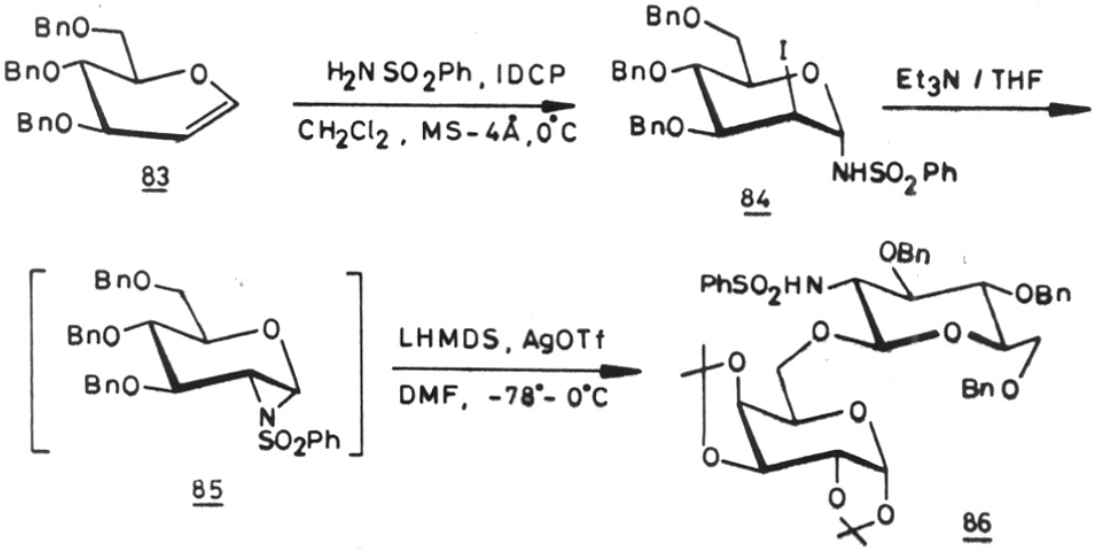
Scheme 14



### 1.3.1.5 $C_1$-$C_2$-Benzenesulfonylaziridine of glycals as donors

Danishefsky et al [114] have shown that the reaction of glycals (**83**) with the combination of iodinium dicollidine perchlorate and benzenesulfonamide would afford stereoselectively, 2-β-iodo-1-α-sulfonamidohexoses (**84**). Treatment of these products with strong base apparently generated $C_1$-$C_2$ sulfonyl aziridine **85**. A 2-β-sulfonamide-1-β-linked disaccharide **86** has been produced when this reaction was carried out with excess base in the presence of a glycosyl acceptor.

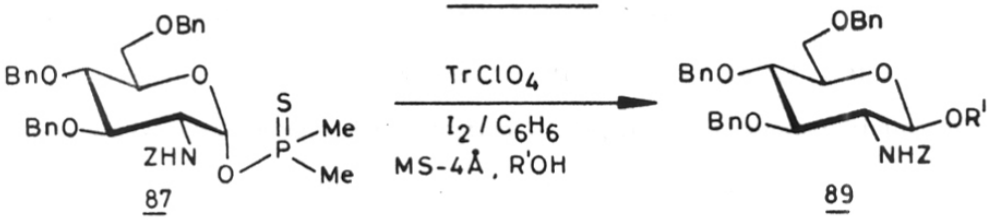
Scheme 15




### 1.3.1.6 2-Amino-2-deoxy dimethylphosphinothiate glycosides as donors

Inazu et al [115] have reported a glycosidation method in which 3,4,6-tri-O-benzyl-2-benzyloxycarbonyl 2-amino-2-deoxy-α-D-glucopyranosyl dimethylphosphinothiate (**87**) with several alcohols (**88a, 88b** etc.) in the presence of iodine and a catalytic amount of tritylperchlorate gave stereoselectively, β-glycosides **89** (Scheme 16).

Scheme 16



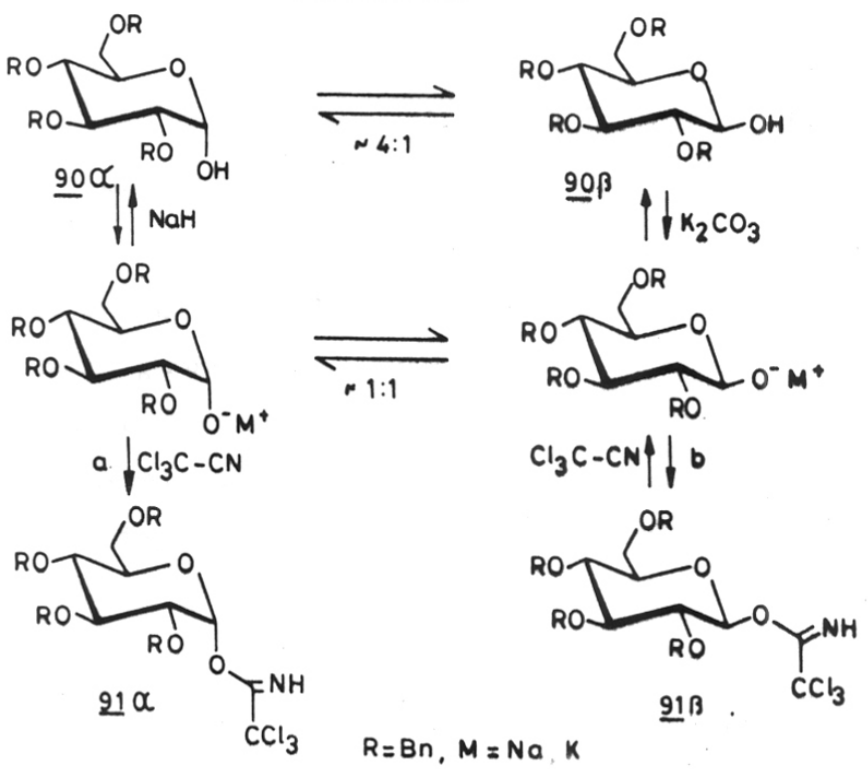

R' = Cholesteryl (88)
R' = Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (88a)
R' = Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (88b)

### 1.3.1.7 O-Glycosyl trichloroacetimidates as donors

The O-glycosyl imidates have been recommended by Schmidt et al. for glycosyl transfer upon activation by acid[21].

The O-α-glucopyranosyl trichloroacetimidate (**91α**) thus has been prepared in almost quantitative yield[116], when tetra-O-benzyl α-D-glucose (**90α**) was reacted with trichloroacetonitrile in the presence of sodium hydride (Scheme 17). Potassium carbonate catalizes the addition relatively

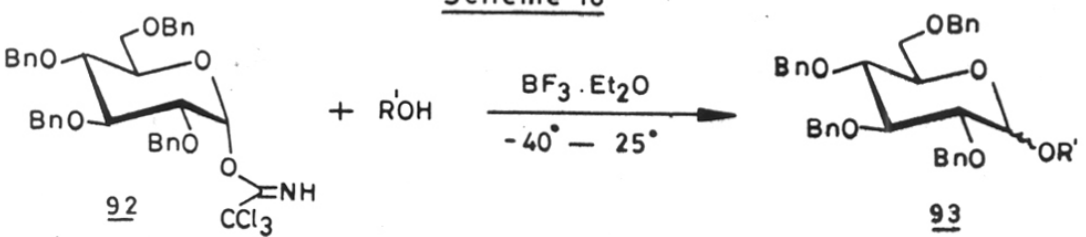
Scheme: 17



rapidly and practically quantitatively. Thus the β-anomer **91β** has been isolated from **90β** as a pure product in 78% yield (Scheme 17).

The stereoselective anomeric activation of carbohydrates and their derivatives through the formation of O-glycosyl trichloroacetimidates has been applied to all important O-protected hexopyranoses (glucose, galactose[117], mannose[118], glucosamine[119] etc ). For reactions of O-glycosyl trichloroacetimidates (**92**) with various alcohol components generally the presence of an acid catalyst is required. Borontrifluoride etherate at -40°C to room temperature has been proved to be suitable[117]. Thus the reactions of α-trichloroacetimidate (**92**) with cholesterol and with other sugar alcohols gave preferentially β-glycosidation product (**93**, α: β ratio 2:3 to exclusive β-) in good yields (32-90%)[21] (Scheme 18).

**Scheme 18**




**92** **93**

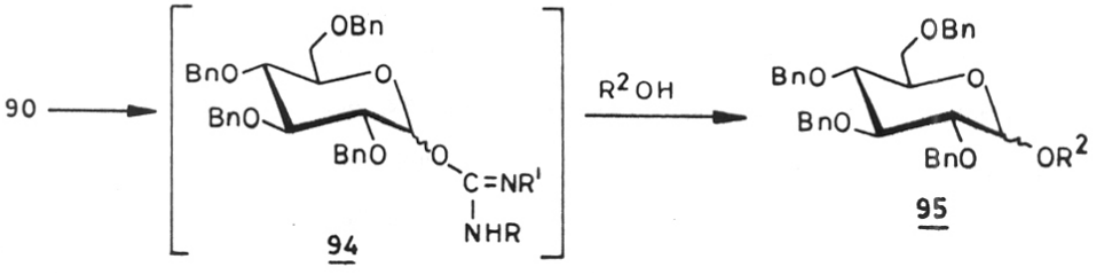
R' = 88 , 88a

R' = 3,4 - Isopropylidene 1,6 - anhydrogalactopyranoside

### 1.3.1.8 Glycosyl ureas as donors

The isourea leaving groups for activating carbohydrates have been introduced by Ishido et al[120] where these were generated in situ from tetra-O-benzyl glucose (**90**) and carbodimide in the presence of copper (I) chloride at about 80°C to give **94**. Phenols, thiophenols, carboxylic acids, peptides and aminoacids[121] have been used as nucleophiles to obtain the corresponding glycosides (**95**; α : β ratio 1:4 to 1:9) in 34-70% yield (Scheme 19).

Scheme 19

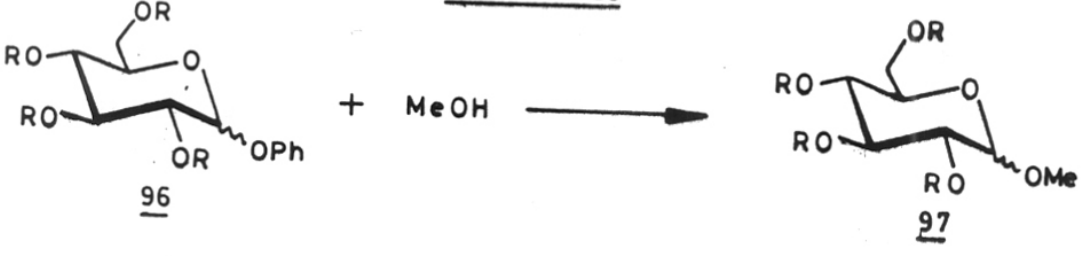



$R^2$ = Z - Tyr - OBz ; Z - Ser - OBz
= Boc - Leu - OH ; Z - Gly - Gly - Phe - OH

### 1.3.1.9 Aryl glycosides as donors

Noyori et al [122] have reported a more unconventional route to the synthesis of glycosides (**97**) using aryl glycosides (**96**) and their substituted derivatives which react with alcohols under mild electrolytic conditions. Acetonitrile has been used as solvent and lithium perchlorate as an electrolyte (Scheme 20). Secondary and tertiary alcohols react with less efficiency. α and β(1 → 6) linked disaccharides were however produced in 65% yield but with poor anomeric selectivity.

Scheme 20

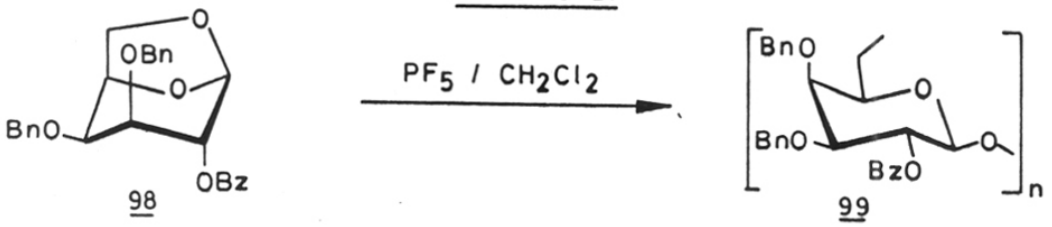



| R | Yield % | α : β |
|---|---|---|
| Ac | 79 | 3 : 97 |
| Bn | 92 | 35 : 65 |

### 1.3.1.10 1,6-, 1,4- and 1,2-Anhydro sugars as donors

(1 → 6)-β-D-Galactopyranan(**99**) has been synthesized[123] by cationic ring opening polymerization of 1,6-anhydro-2-benzoyl-3,4-di-O-benzyl-β-D-galactopyranose (**98**) and subsequent deprotection. The polymerization proceeded at 0° and 20°C, using 10-50 mol% of phosphorus pentafluoride as inhibitor in dichloromethane, to give the polymer of Mn 2.6-3.6 x $10^3$. The formation of the β-(1 → 6) linkage was explained by a neighbouring group participation (Scheme 21).

Scheme 21

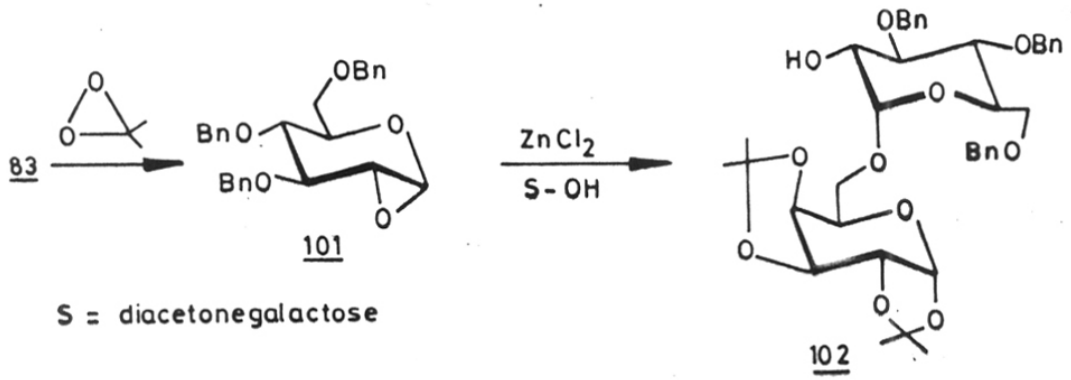



98 99

Michel et al have employed the 1,4-anhydro-α-D-glucopyranose for the synthesis of polymeric sugars[124]. The use of trityl hexafluorophosphate and hexafluoroantimonate resulted exclusively in β (1 → 4) coupling.

Danishefsky et al, have used 1,2-anhydrosugars (**83**) for the synthesis of β-linked saccharides[125]. The anomeric center of **101** has been displaced with clean inversion of configuration by an alcohol component in the presence of anhydrous zinc chloride as catalyst to form, saccharides (**102**) and other conjugates (Scheme 22). 1,2-Anhydrosugars have been

Scheme 22




101 102

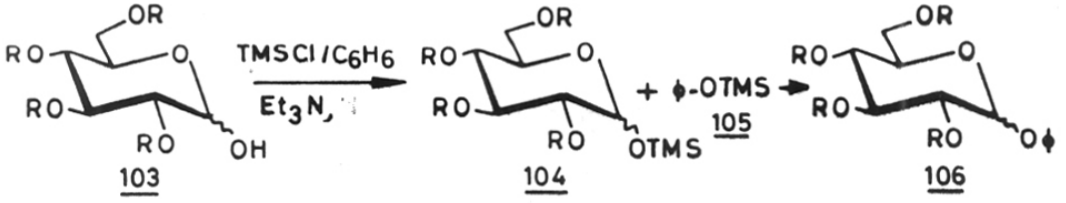
S = diacetonegalactose

prepared from the corresponding glycals (**83**) by reaction with dimethyl dioxirane.

### 1.3.1.11 O-Glycosyl trimethylsilyl ethers as donors

1-O-Trimethylsilyl glycopyranosides (anomeric mixture **104**) with neighbouring acyl protecting groups have been reacted with O-silylated phenols (**105**) in presence of catalytic amounts of trimethylsilyl trifluoromethane sulfonate (TMS-OTf) at 20°C to yield exclusively the aryl β-O-glucosides (**106**) (Scheme 23). With non participating protecting groups, glycosides have been obtained in ratio of 1:10 to 1:16 (α/β)[126]. The 1-O-trimethylsilylglucosides are obtainable in anomerically pure α-form from 1-O-unprotected sugar **103**[127].

**Scheme 23**



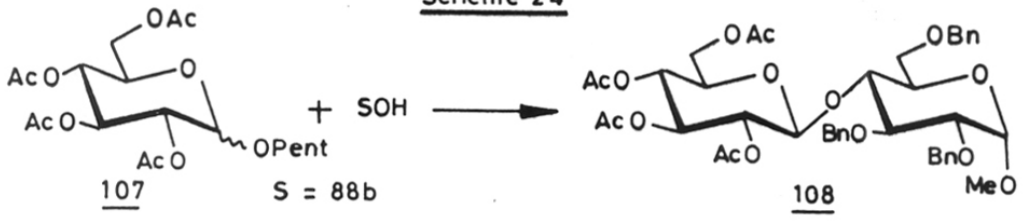

103 104 106

R = Ac, Bn ; ϕ = aryl, p-anisyl, naphthyl, etc.

### 1.3.1.12 n.Pent-4-enyl-O-glycosides as donors[128]

Recently, Fraser-Reid[129] et al have utilized N-iodosuccinimide (NIS) and trifluoromethane sulfonic acid (TfOH) as a powerful source of iodonium ion in the stereoselective β-glycoside (**108**) formation. The 'disarmed' pent-4-enyl glucoside **107** that normally responded sluggishly to iodonium dicollidine perchlorate has reacted rapidly with **88b** in the presence of NIS-TfOH in a glycosyl coupling reaction (Scheme 24).

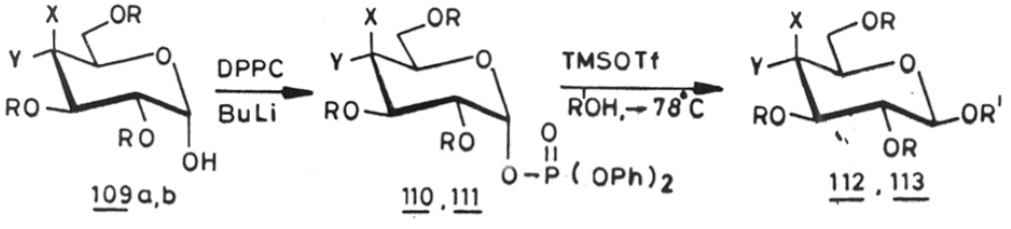
Scheme 24




1.3.1.13 **Glycosyl diphosphates as donors**

A highly stereocontrolled construction of 1,2-trans- β-glycosides with or without neighbouring group (**112,113**) has been achieved by using benzyl or benzoyl protected glycopyranosyl phosphates as glycosyl donors (**110, 111**) in the presence of TMS-OTf (Scheme 25). Benzyl protected glycopyranosyl diphenylphosphates (**110, 111**) have been readily prepared by treatment of the corresponding 1-O-lithium salts with diphenylphosphorochloridate (DPPC)[130].

Scheme 25




**109a**, X = H, Y = OBn; X = OBn, Y = H; **110 → 112** (α:β = 1:9; 1:30)

**109b**, X = OBz, Y = H; Y = OBz, X = H; **111 → 113 (exclusively β)**

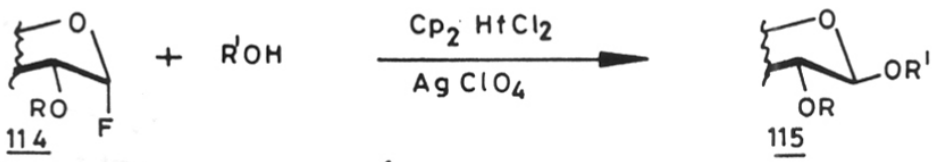
$R^1$ = alkyl, aryl; R = Bn / Bz

The stereochemical outcome was presumably due to the thermodynamically more stable α-ionpair consisting of pyranoxonium ion and phosphate anion TMS-OTf complex, followed by the rear side attack with alcohols on this intermediate[130].

### 1.3.1.14 Glycosyl fluorides as donors

Suzuki et al [133] have reported that the activation of glycosyl fluoride (**114**) with $CP_2H_fCl_2$-$AgClO_4$, in 1:2 ratio provided β-glycoside (**115**) without resorting to neighbouring group assistance. The rapid activation was due to the high fluorophilicity of early transition elements[133] (Scheme 26).

**Scheme 26**



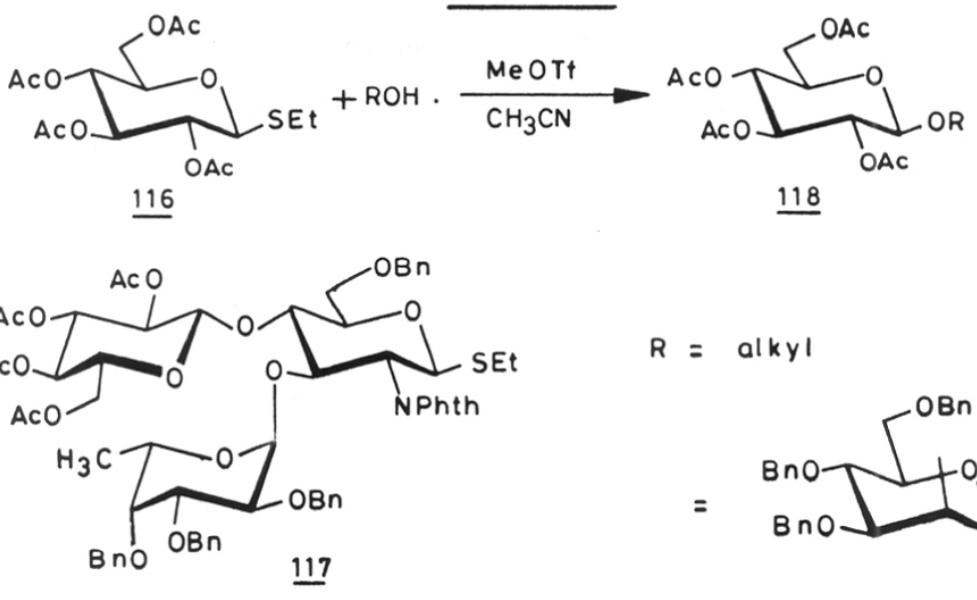

### 1.3.1.15 Alkyl and aryl thioglycosides as glycosyl donors

Thioglycosides are stable and versatile glycosyl donors that allow flexible strategies for the synthesis of complex oligosaccharides[134]. They have been obtained from carbohydrates by a variety of methods (described in detail in page 44) either from 1-O-hydroxy[135,136] or 1-halo hexopyranosides[137,138].

Several methods of activation of thioglycosides for obtaining saccharides have been reported which involve use of 1) bromine[134]; 2) thiophilic metal salts[131,132,145,150,151]; 3) methyltriflate[141,142,143]; 4) nitrosyltetrafluoroborate[146]; 5) benzeneselenyltriflate[148]; 6) boron+ trifluorideetherate[134,152] etc. as promotors to facilitate glycosidations.

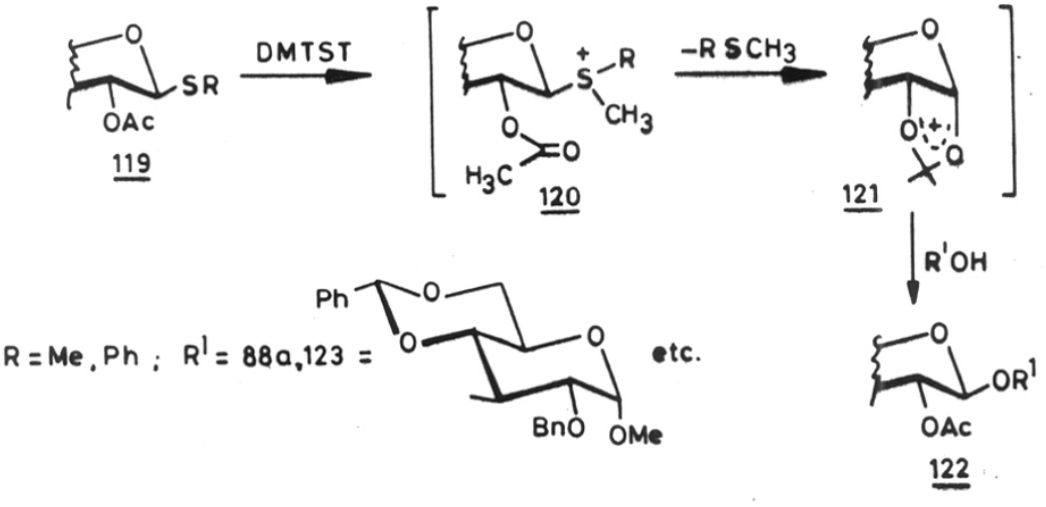
The direct glycosidation of alkyl thioglycosides **116** and **117** with alcohols has been accomplished using thiophilic reagent such as methyltriflate to obtain 1,2-trans-glycosides[141,142] (**118**) (Scheme 27). Stereoselectivity in these reactions is due to the neighbouring group participation from C-2 acyl substituent.

Scheme 27



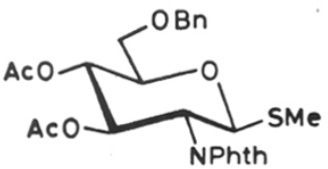

Dimethyl (methylthio) sulfonium triflate (DMTST) has been used as a methylating agent by Garegg et al. for converting alkyl and aryl β-thioglycosides (**119**) with a C-2 participating group, into 1,2-trans oligo saccharides (**122**) in good yields ( 90%)[144].

Scheme 28

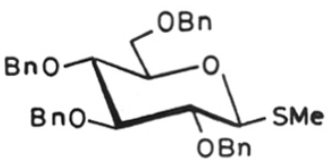


This reaction is presumably proceeding (Scheme 28) through the initial activation of sulfur by methylating agent resulting in a reactive sulfonium ion (**120**). Then the subsequent ejection of methylmercaptan from **120**, followed by intramolecular nucleophilic attack of neighbouring C-2 participating group, leading to the formation of acyloxonium ion (**121**). The required selectivity has been achieved by the attack of alcohol from β-side.

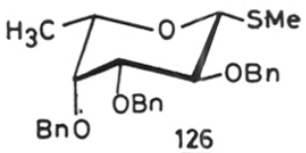
Ogawa et al have described a new methodology, in which the alkyl thioglycosides (**116** and **124**) were activated by the aid of cupric bromide in presence of a catalytic amount of tetrabutylammonium bromide[145] to provide 1,2-trans-saccharides. By use of $CuBr_2$ — $Bu_4NBr$ — AgOTf system, the compounds **125** and **126** afforded a mixture of the 1,2-cis and 1,2-trans products in good yield with **88a** and **123** in which the 1,2-cis products preponderate in the ratio of 6:1 to 10:1.



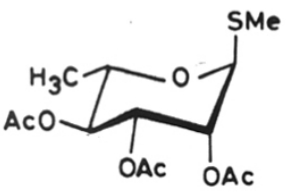

**124**



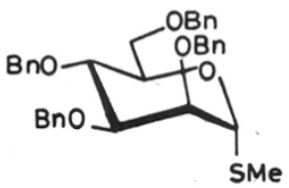

**125**




**126**




**127**




**129**

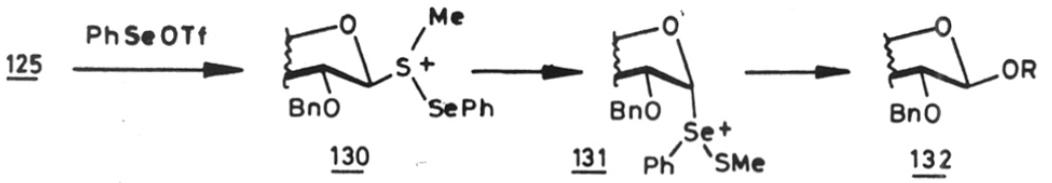
**Methyl 3,4 - di - O - benzyl - α - L - rhamnopyranoside (128)**

Methyl thioglycosides **116, 124, 125** and **127** have been activated[146] under anhydrous conditions with equimolar amount of nitrosyl tetrafluoroborate ($NOBF_4$)[147] in dichloromethane at temperatures between 0-20°

to give in the presence of a glycosyl acceptor (eg. **128**) high yields of O-glycosides. A participating group at C-2 favoured the exclusive formation of a 1,2-trans-O-glycosidic bond. On the other hand, a nonparticipating group at C-2 had no directing effect on the stereoselectivity[146] (2:3 mixture of α:β).

Ogawa et al[148] have developed a new methodology, in which methyl thioglucosides **124, 125** and **129** have been used as glycosyl donors and benzeneselenyl triflate (PhSeOTf)[149] as promotor to obtain β-glycosides on reaction with sugar alcohols (eg. diacetonegalactose). Interestingly perbenzylated thioglycoside (**125**) showed a considerable level of β-selectivity (α : β ratio 1:3 to 1:7). β-Selective glycosidation in the absence of neighbouring group participation has been performed by using insoluble silver catalysts[150,151] or solvents with cation interacting ability such as acetonitrile[142]. The origin of β-selectivity was explained based on the reverse anomeric effect[54].

**Scheme 29**




Thus, a sulfonium salt **130** derived from the thioglycoside **125** and PhSeOTf rearranges to the selenium salt, which in turn reacts mainly in its more reactive α-configuration (**131**) to give β-glycoside **132** predominantly[148] (Scheme 29).

Other activating agents such as methylsulfenyl bromide (MSB) and O-nitrobenzenesulfenyl chloride (NSC) have also been used[153] for glycoside couplings of **116** and **124** with **123** in the presence of silvertriflate (AgOTf) to afford the corresponding β-linked 1,2-trans disaccharides.

Recently, Fraser-Reid et al[154] have also reported a β-glycosidation reaction in which thioglycosides (eg. **119**) have been converted into the corresponding β-disaccharides in the presence of sugar alcohols such as diacetonegalactose by N-iodosuccinimide (NIS) and trifluoromethane sulfonic acid in 83-89% yields.

### 1.3.2 α-Glycosidations (1,2-cis-D-gluco-D-galacto and 1,2-trans-D-manno-, L-fuco-, L-rhamno-).

The prerequisite for the preparation of α-linked saccharide coupling is that the H-atom of C-2-OH of the glycosyl donor be replaced by a non neighbouring group participating substituent. To acheive α-glycoside synthesis, the β-glycosyl donor would be reacted with alcohol component in such a way that it proceeds as completely as possible with inversion in the sense of an $SN_2$ reaction.

Koenigs-Knorr reaction conditions in α-glycoside synthesis involve the use of the 1,2-trans-glucopyranosyl halides and mercury salts as catalyst in low polar solvents[155]. Amongst the most satisfactory approaches by which the required α-diastereoselectivity has previously been achieved, is through the use of, the in situ anomerization procedure, i.e., by the reaction of an α-halogenose donor with an acceptor in the presence of an appropriate catalyst[156]. An alternative method which has claimed comparably good α-stereoselectivity involves the use of 1-O-(N-methyl) acetimidyl per-O-benzylated β-glycopyranoside as glycosyl donors and p-toluenesulfonic acid (PTSA) as a catalyst[157]. The use of glycosyl thiocyanates as glycosylating agents has recently been shown to have good perspectives for stereospecific 1,2-cis-D-gluco and D-galacto-glycosylation[158]. In D-manno-[88], L-fuco-[169] and L-rhamno-[89] series, α-glycosides have been obtained from the neighbouring group participation.

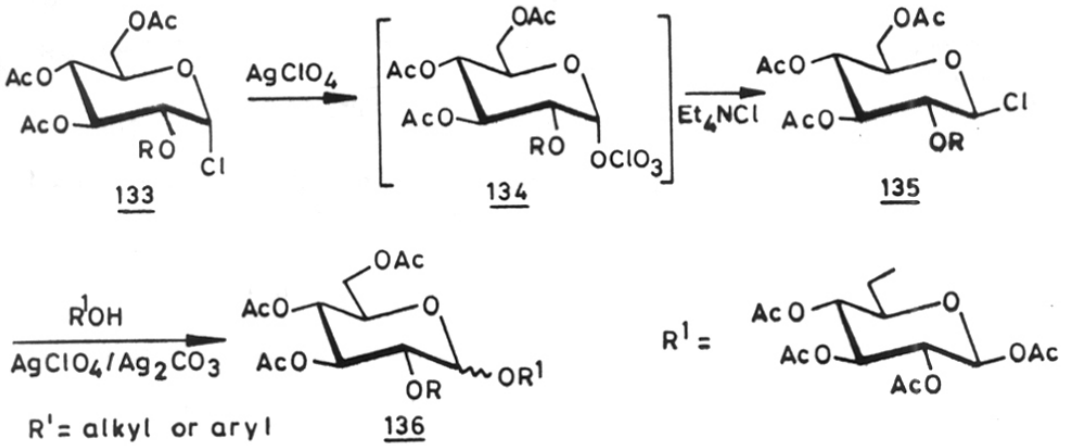
Due to growing importance of 2-deoxy-2-aminosugars, specially N-acetylglucosamine and N-acetylgalactosamine, in complex carbohydrates[22] and glycoconjugates their selective coupling in glycoside and saccharide synthesis is of particular interest[21-24]. For the synthesis of α-glycosides from 2-deoxy-2-amino sugars it is necessary that a non neighbouring group active substituent be present at C-2. Thus, diphenyl phosphono[159] 2,4-dinitroanilino,[160] 2,4-dinitrophenyl P-methoxybenzylidene[161], nitroso[162,163] protecting groups have been used at C-2 for this purpose. The 2-azido-2-deoxy-D-glucopyranosyl halides have been converted selectively into α-glycosides[164,165].

### 1.3.2.1 Glycosyl halides as donors

#### 1.3.2.1.1 The Koenigs-Knorr method[25]

1,2-cis-Glycopyranosides have been synthesized from 1,2-trans-glycopyranosyl halides having a non participating group at C-2. 1,2-trans-Glucopyranosyl chloride (**135**) required for saccharide synthesis has been synthesized from its corresponding 1,2-cis-glucopyranosyl chloride **133** by reaction with silver perchlorate and tetraethylammonium chloride[166] at low temperatures. This β-halide has been used in glycoside coupling[167] in a solvent of low polarity such as dichloromethane or diethyl ether in presence of an active catalyst such as $AgClO_4/Ag_2CO_3$ or $AgClO_4$ or AgOTf with s-colidine[23]. Thus, **135** on reaction with alcohol component gave the α-linked glycosides along with a small (< 10%) amount of β-anomers (**136**, α: β ratio 8:2 to 9:1) (Scheme 30). In polar solvent such as nitromethane a 1:1 mixture of α and β-D-glucopyranosides (**136**) have been obtained[25].

Scheme 30

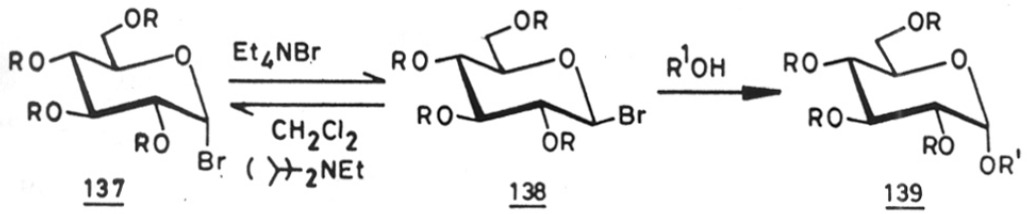



In principle, under $SN_2$ reaction conditions, it should be possible for the β-halide to yield the desired α-glycoside. However, the β-glycosyl halides are rather unstable and incline to anomerization, thus often giving anomeric mixture as reaction products[23].

### 1.3.2.1.2 Halide ion catalysed glycosidations

Lemieux et al,[156] have developed a very efficient α-glycosidation method which has found practical application. α-Glycosyl halides such as **137** with anon participating C-2-substituent (benzyl ether) was in situ anomerized to the more reactive β-halide (**138**) by reacting in presence of excess bromide ions (Scheme 31). This β-halide **138** on reaction with an alcohol acceptor (alkyl and glycosyl) in dichloromethane in the presence of diisopropylethyl amine and molecular seives (4A°) gives rise to α-glycosides[156] (**139**) exclusively in good yields (42-65%).

Scheme 31




R = Bn ; R' = Me, iPr, tBu, diacetone galactose, diacetone glucofuranose, diacetone galactofuranose

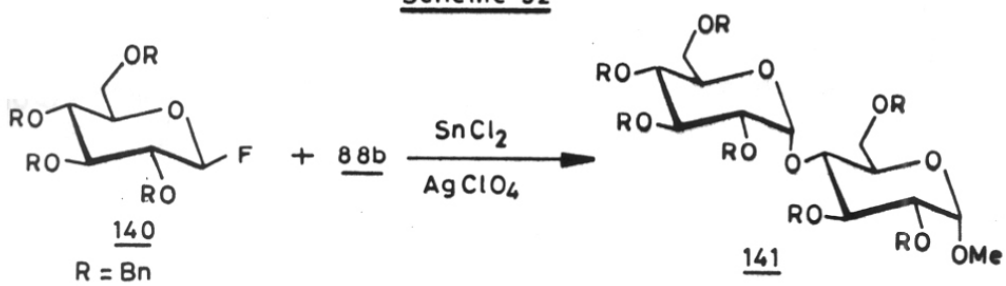
The high α-selectivity and relatively long reaction time indicates that the reaction sequence to be proceeding via the extremely reactive β-halide (**138**). The α-halide **137** exists in equilibrium with a more reactive β-halide (**138**)[168]. The equilibrium in the reaction was catalysed by excess halide ions ($Et_4NBr$) in dichloromethane.

Several disaccharides of **D**-gluco, **D**-galacto[156] and **L**-fuco- pyranosides[169] and the Lewis a blood group antigenic determinants have been synthesized by this method[156].

### 1.3.2.1.3 Glycosyl fluorides as donors

The use of fluoride at the anomeric center as leaving group has arouse interest in the last few years. Being a poor leaving group, fluoride leads to intermediates more stable than glycosyl chlorides and bromides[21]. In the first studies by Mukaiyama et al[170,171] tin (II) chloride/silver-perchlorate have been employed for activation. Synthesis of glycosides and disaccharides starting from the β-glucosyl fluoride (**140**) afforded good α-selectivities with **88b**; maltoside (**141**) has been formed preferentially (91%;α : β ratio 4:1) (Scheme 32).

**Scheme 32**



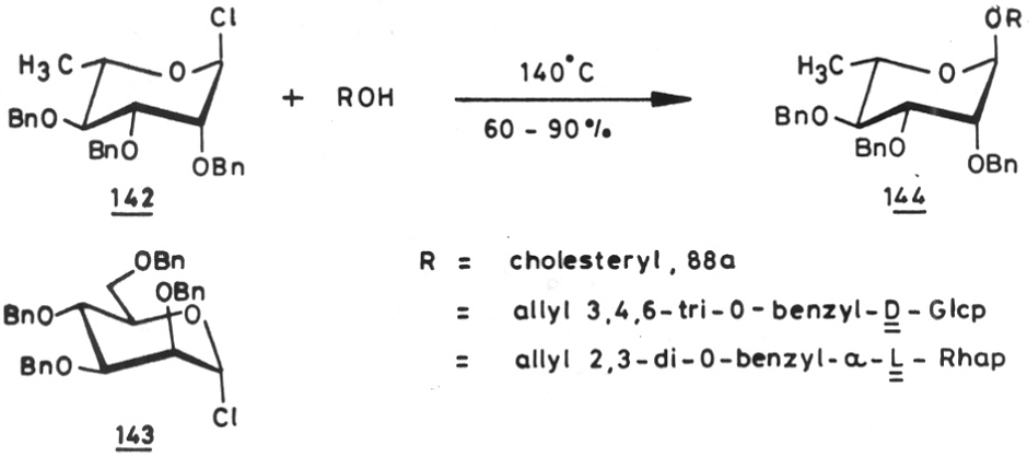

β-Glycosyl fluoride donors required for such synthesis have been obtained either by reaction of 1-O-unprotected sugars by reaction with 2-fluoro-1-methyl-pyridinium toluenesulfonate in the presence of triethylamine[170,171,172] (leading to α/β mixture), or by reaction of phenylthioglycosides with DAST or HF-Py-NBS[173,174].

Activation of glycosyl fluorides by silver perchlorate and tin (II) chloride in stoichiometric ratio with alcohols in the presence of MS-4A° has resulted[174,175] in the formation of glycopyranosides, where α-linked saccharides were predominant (α : β/ 95:5).

### 1.3.2.1.4 Thermal glycosidations : glycosyl chlorides as donors

Recently α -glycosyl chlorides of per-O-benzylated-**L**-rhamno (**142**) and **D**-manno- (**143**) sugars have been utilized[176] as glycosyl donors in the presence of a variety of alcohols under thermal conditions (140°C, >30 min) to obtain α-glycosides (**144**) in good selectivity ( α : β /8:2) and few disaccharides (exclusively α) in 65-75% yield (Scheme 33).

Scheme 33



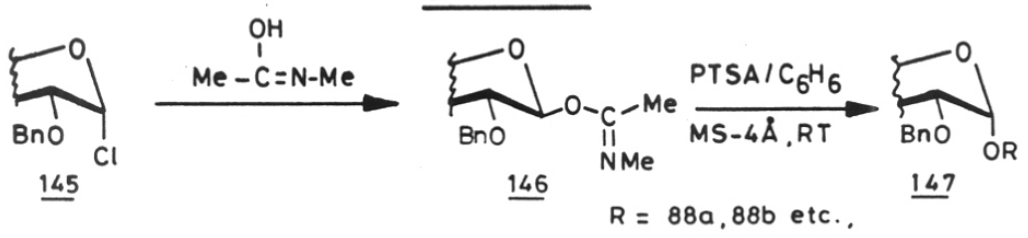

### 1.3.2.2 1-O-Alkyl glycosides as glycosyl donors

#### 1.3.2.2.1 O-Glycosyl N-methyl acetimidates as donors

Sinay et al have shown that N-methyl acetimidoyloxy moiety is a good leaving group for saccharide coupling[157].β -Imidates (**146**) required for coupling have been prepared by reacting glycosyl halide **145** with N-methyl acetamide and freshly prepared silveroxide in the presence of diisopropylamine.

β-Glycosyl imidate **146** has been reacted with hydroxy components in the presence of PTSA and molecular sieves (4Å ) with inversion to give α-glycopyranosides (**147**) (Scheme 34). A number of oligosaccharides have been prepared from D-glucose, D-galactose and L-fucose derivatives by using this method[157,177].

Scheme 34

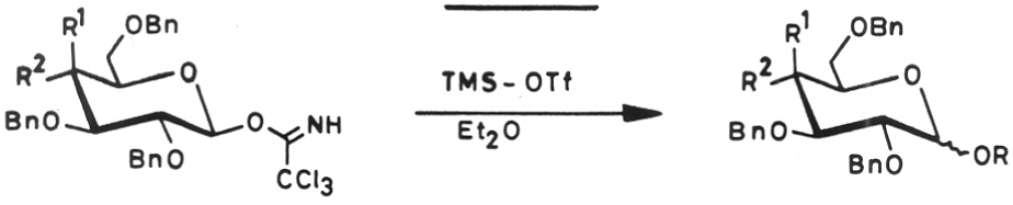


Preparation of β-glycosyl-N-methyl acetimidates from α-glycosyl halides is laborious, furthermore, only the β-imidates are known and these have proved to be relatively unreactive in acid-catalysed glycosidation[21].

### 1.3.2.2.2 O-Glycosyl trichloroacetimidates as donors

Schmidt et al have utilized per-O-benzylated β-trichloroacetimidates as glycosyl donors[116] (**91β** , **148β**) for the synthesis of α-gluco- **149** and α-galactopyranosides (**150**)[1,178] respectively (Scheme 35).

**Scheme 35**




R = Phenyl 2,3,4-tri-O-benzyl-α-D-Glcp. etc.

**91β** : $R^1$ = H; $R^2$ = OBn ⟶ **149**: 83-88% α:β 3:1 to 8:1

**148β** : $R^1$ = OBn; $R^2$ = H ⟶ **150**: 66-86% α:β 5:1 to 36:1

α-Glycosides of glucosamine and galactosamine[179] have also been made by use of this method. The successful application of the trichloroacetimidate method to α-fucosylation[180] has also been described. In all these reactions α-anomers predominated.

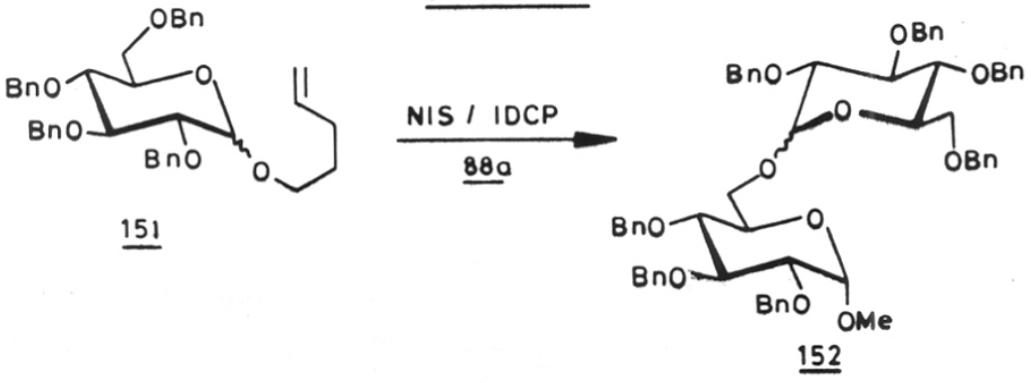
### 1.3.2.2.3 n-Pent-4-enyl-O-glycosides as donors

Fraser-Reid et al have shown that n-pent-4-enyl- glycosides (NPG's) are excellent substrates for a wide variety of reactions occurring at the anomeric center[128,182]. This discovery originated in a serendipitous observation made during the synthesis of ansa chain of streptovaricin[181].

The NPG's (**151**) on treatment with halogenic ions become chemospecifically activated and the resulting oxocarbenium ion is captured

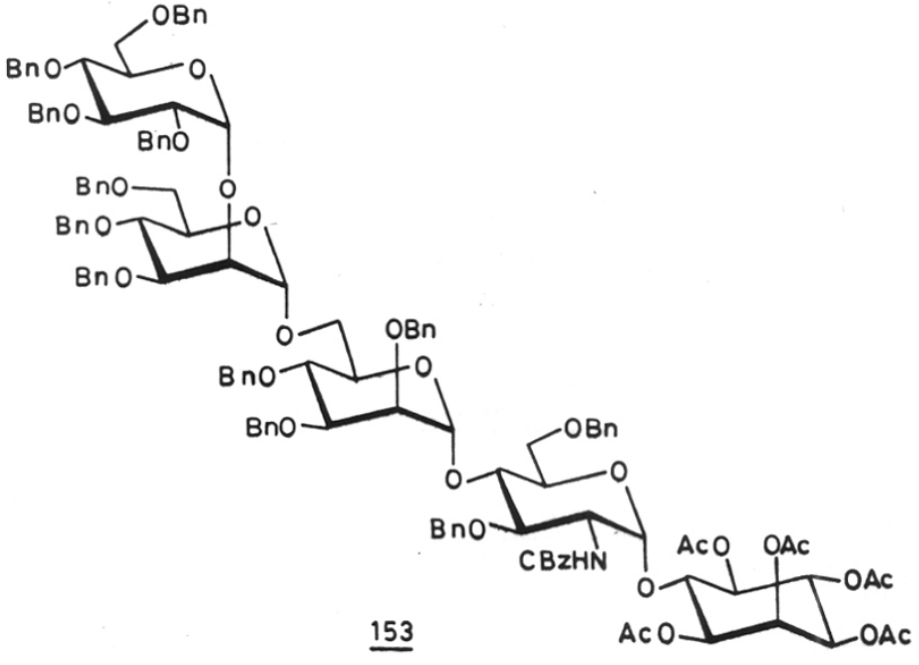
by a sugar alcohol leading to the formation of saccharides **(152)** and the $\alpha/\beta$ selectivity in the coupling process could be controlled by the choice of the solvents[128] (Scheme 36).

Scheme 36




151 152

A variety of disaccharides[128] including the mannan rich pentasaccharide **153**[12] have been synthesized by this method.



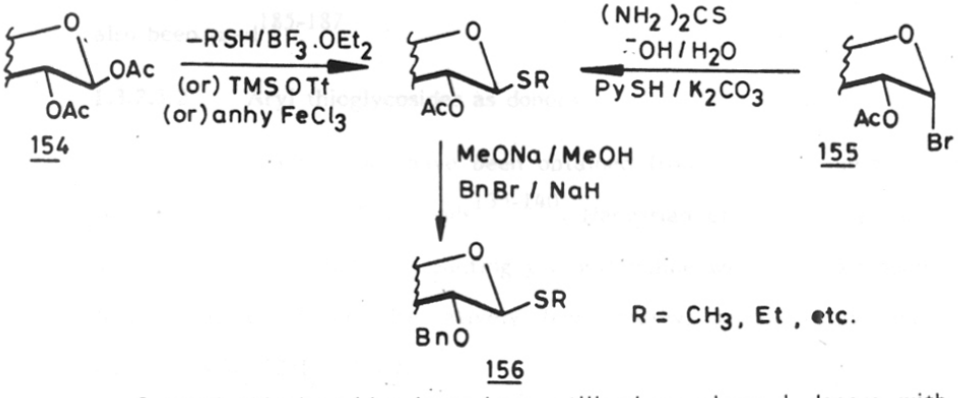

153

### 1.3.2.3 Thioglycosides as donors

#### 1.3.2.3.1 Alkyl thioglycosides as donors[134]

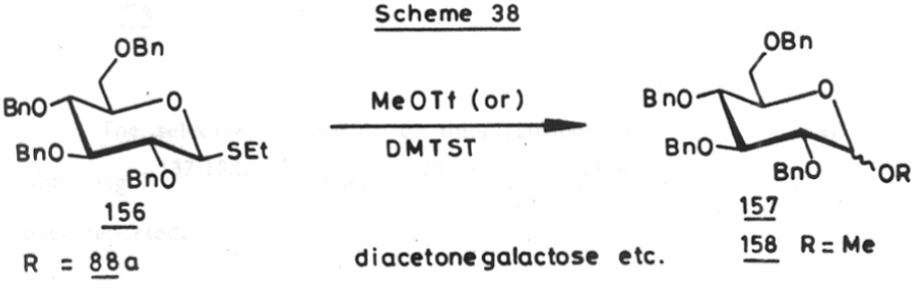
β-Alkyl thioglycosides (156) required for saccharide synthesis have been synthesized either from their corresponding peracetate derivatives (154) on treatment with thioalkanes in the presence of boron trifluoride etherate ($BF_3.OEt_2$)[134] or TMS-OTf[140] and anhydrous ferric chloride[183] from α-acetobromosugars (155) on nucleophilic displacement using alkyl iodide and thiourea[134] (Scheme 37).

Scheme 37




β-Alkyl thioglycosides have been utilized as glycosyl donors with a non participating neighbouring group (156) in presence of strong methylating agents such as methyl triflate[142] and dimethyl (methylthio) sulfonium triflate (DMTST)[143] (Scheme 38).

Scheme 38



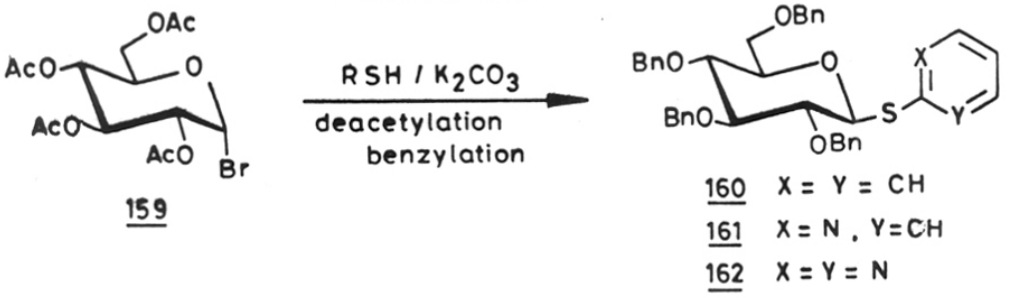
DMTST has proved[184] to be the stronger methylating agent than MeOTf, where the later reagent also gives a certain amount of undesired 1-O-methylated product (158). The yields were high in these reactions (≥90%) but the stereoselectivity (157) (α : β 5:3) was poor.

However, α -selectivity has been achieved[184] by halide ion-catalysed methods of Lemieux[156] by the use of alkyl thioglycosides with the alcohols in the presence of $Et_4N$ Br and DMTST in 3:5 ratio respectively. Other activating groups such as bromine-AgOTf or Hg $(CN)_2$[185] have also been used[185-187]

### 1.3.2.3.2 Aryl thioglycosides as donors

Aryl thioglycosides have been obtained from 1-hydroxy and 1-halo-sugars by a variety of methods[135-140]. Hanessian et al have synthesized aryl thioglycoside (160) by reacting glycosyl halide with the corresponding thiol (Scheme 39) or alternatively from methyl glycoside by reacting with $Me_3SiSPh/ZnI_2/n\text{-}Bu_4NI$[135].

Scheme 39




159

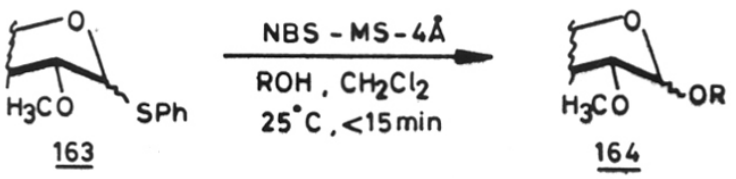
160 X = Y = CH
161 X = N , Y = CH
162 X = Y = N

The selective activation of thioglycosides by thiophilic metal salts like (Hg(II)[137,188,189], Ag(I)[201,202,203], Cu(I)[194]. Pb(I)[135] etc., have been reported.

Ferrier et al[188,189] have shown that β-phenylthioglycosides in the presence of mercury (II) salts readily solvolyse to give alkyl α-D-glucopyranosides. However, combination of mercury (II) salts with in situ generated perchloric acid, the same reaction has been reported to proceed at room temperature in 3 h[190]. Literature evidence on the adaptability of these reactions to oligosaccharide synthesis was not altogether encouraging[188].

Nicolaou et al (Scheme 40) have demonstrated that phenyl thioglycosides (**163**)[191] could be activated with N-bromosuccinimide (NBS) at room temperature and coupled with various alcohols in $CH_2Cl_2$ to yield **164** (α:β /1:1, and in $CH_3CN$ the α/β ratio was 9:1[192].

**Scheme 40**



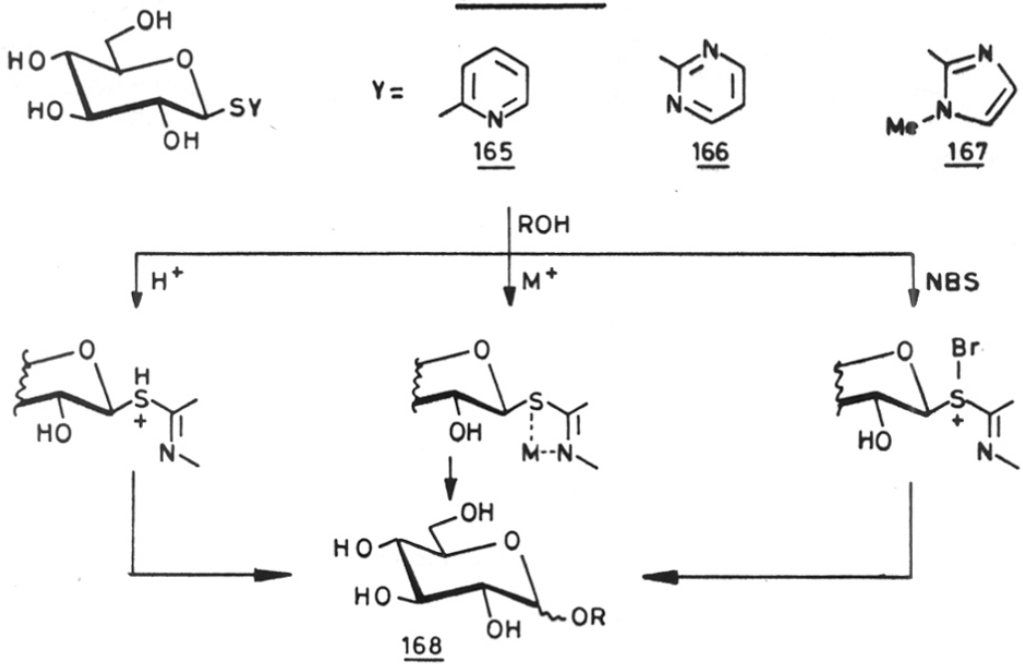

163 164

R = alkyl, aryl and glycosyl

### 1.3.2.3.3 2-Pyridyl thioglycosides as donors

Comparative studies by Hanessian et al[137] on the proton activation of pyridin-2-yl-thioglycopyranosides (**165,166** and **167**) demonstrated the dramatic effect of the heteroatom (remote activation) as a basic anchor for the proton and as a better leaving group, resulting from the negative inductive effect. The reaction of unprotected glucopyranosyl heterocycle with thiophilic acceptors such as mercury (II) nitrate in acetonitrile yielded alkylglycosides in good yields[137] (80-90% α /β anomers 1:1) and the disaccharide, Glu (1→6) Gal in 33% yield with low selectivity (**168**, R = diacetonegalactose) (α/β ratio 3:2).

**Scheme 41**

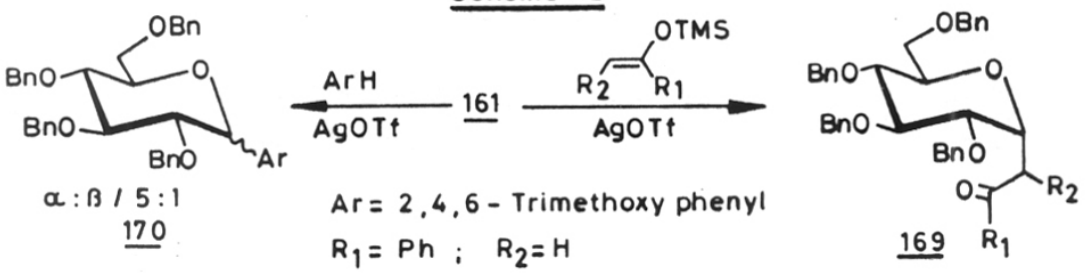



In addition to proton activation, metal ($Hg^{+2}$) salts and bromonium ions (NBS) were also used to activate the thioglycosides[137].

Woodward et al[135] have used 2-pyridyl thioglycoside of L-cladinoside in erythronolide synthesis, its activation with anhydrous $Pb(C10_4)_2$ in the presence of aglycon component to obtain the glycoside with α-selectivity.

Williams et al[193] (Scheme 42) have shown that reaction of **161** with silver (I) triflate and several trimethyl enolethers or electron rich aromatics at room temperature yield the C-glycosides with high α-selectivity (**169** and **170**).

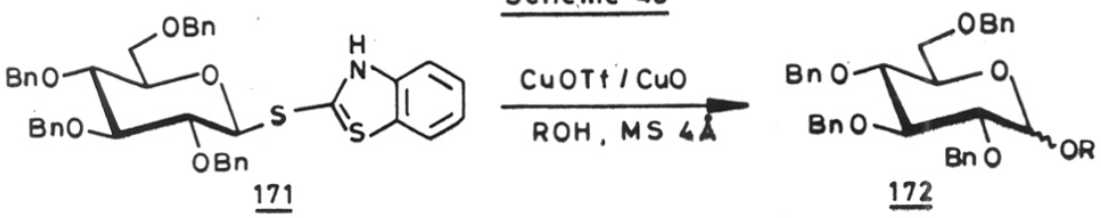
Scheme 42




### 1.3.2.3.4 Benzothiazolyl glycosides as donors

Mukaiyama et al[194] have reported an efficient glycosidation (Scheme 43) method in which 2-benzothiazolyl-2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (**171**) has been reacted with various alcohols including sterically hindered secondary sugar alcohols in the presence of cupric triflate to afford glycosides and saccharides (**172**) (α/β ratio 66:33).

Scheme 43

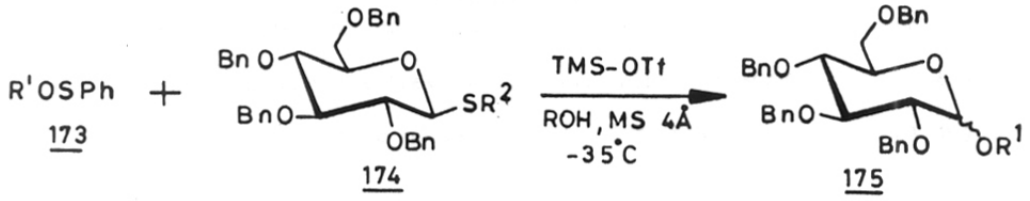



R = cholesteryl, 88a, 88b etc.

### 1.3.2.3.5 Glycosyl sulphenate esters as donors

Ogawa et al[195] have reported that trimethylsilyl triflate (TMSOTf) catalysed reaction of sulphenate esters (**173**) with thioglycosides (**174**) to yield glycosylated products (**175**) (α/β ratio 1:1 to 2:3), under mild conditions (Scheme 44). Sulphenate esters required have been prepared from the corresponding alcohols by the reaction with sulphenylchloride[196].

Scheme 44

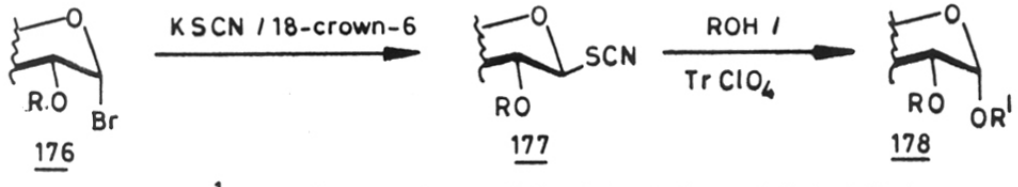



$R^1$ = isoPr, 88a etc., $R^2$ = Me, Ph

1.3.2.3.6 Glycosyl thiocyanates as donors

Kochetkov et al[158] have reported a 1,2-cis-glycosidation method which utilizes β-D-glucopyranosyl thiocyanates bearing a non participating substituent at C-2 as glycosyl donors (Scheme 45). Glycosyl donor has been prepared by reaction of α-halo acetylglucopyranoside (176) with potassium thiocyanate in presence of 18-crown-6. The glycosyl donor 177 has been reacted with per-O-acetylated 2-O-, 3-O-, 4-O-, 6-O-trityl glucopyranosides[197] with triphenyl methylium perchlorate ($TrClO_4$) in dry $CH_2Cl_2$ at room temperature to afford stereospecifically, α-linked disaccharides (178).

Scheme 45



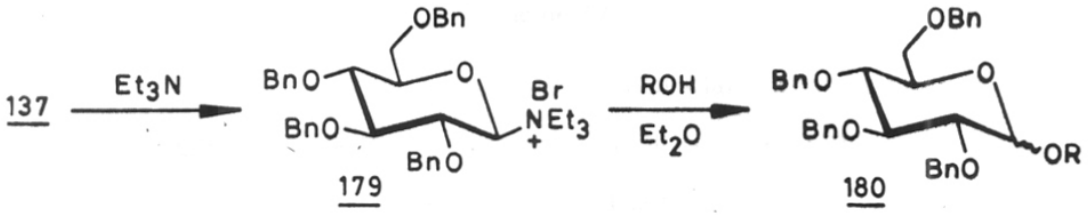

R = Me, Bn ; $R^1$ = Per-O-acetylated 2-O-, 3-O, 4-O-and 6-O-trityl glucopyramosides

1.3.2.3.7 Glycosyl onium salts as donors

Schuerch et al have described the use of quarternary ammonium, phosphonium, and sulfonium salts of glycosyl derivatives in the synthesis of α-glycosides[198-200]. The C-1 salts have been prepared (Scheme 46) from glycosyl bromide derivatives by the reaction of tertiary amines,

trisubstituted phosphines, or disubstituted sulfides. The anomeric configuration of the salts was determined by optical rotation and nmr data, and was predicted from the "reverse anomeric effect" described by Lemieux[54]. The alcoholysis of these onium salts (eg. **179**) gave, by inversion, α -glycosides in good yields and α-linked disaccharides (**180**) in moderate yields (30% α/β ratio 9:1).

**Scheme 46**




R = alkyl, 88a etc.

### 1.3.2.4 Other glycosyl donors

Other methods where nitrobenzene sulfonyl salts-AgOTf[201-203] and N-glycosyl nitrilium-acetonitrile conjugates have also been used for glycosidations. Glycosyl disulfides, sulfonates and sulfones were found not to be practical as glycosyl donors[204].

### 1.3.3 Enzyme catalysed synthesis of oligosaccharides

The area of enzyme catalyzed formation of oligosdaccharides and polysaccharides has recently been reported[205]. The major advantage of enzyme catalysed synthesis is the potential for effecting regiospecific reactions using unprotected carbohydrates under milder conditions in aqueous solutions. There are two strategies available for enzyme catalysed in vitro synthesis of oligosaccharides. The first uses the glycosyl transferase which are used in vivo, by Leloir pathway, to synthesize oligo and polysaccharides. The second strategy uses the glycosidases or glycosyl hydrolases.

**Glycosyl transferases**[205]

In the first step of Leloir pathway sugar (glucose, galactose or mannose) is transformed into 1-phosphate (sugar-1-P). This sugar-1-P reacts with nucleoside triphosphate (NTP) in an enzyme catalysed reaction and forms a chemically active sugar nucleotide diphosphate UDP-Glc, UDP-Gal etc., these enzymes are known as pyrophosphorylases. For example UDP-Glc converts into UDP-Gal (Scheme 47).

**Scheme 47**

Gal → Gal-1-P → UDP-Gal

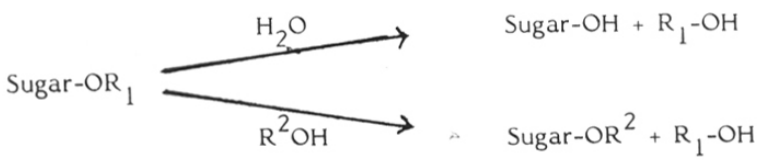
Glc → Glc-6-P → Glc-1-P → UDP-Glc → UDP-xyl

Fru-6-P → UDP-Fru

Then the addition of the activated sugar in a stepwise fashion to a glycoprotein, glycolipid or oligosaccharide by the glycosyl transferases takes place (Scheme 48).

Glycosides have been used synthetically in two ways. The first utilizes a glycosidase, the corresponding monosaccharide, and a nucleophile. This procedure has been referred to as "direct glycosidation". The second route utilizes, a glycosidase which hydrolyses the glycoside. The intermediate is then trapped by a nucleophile (other than water) to yield a new glycoside (Scheme 49).

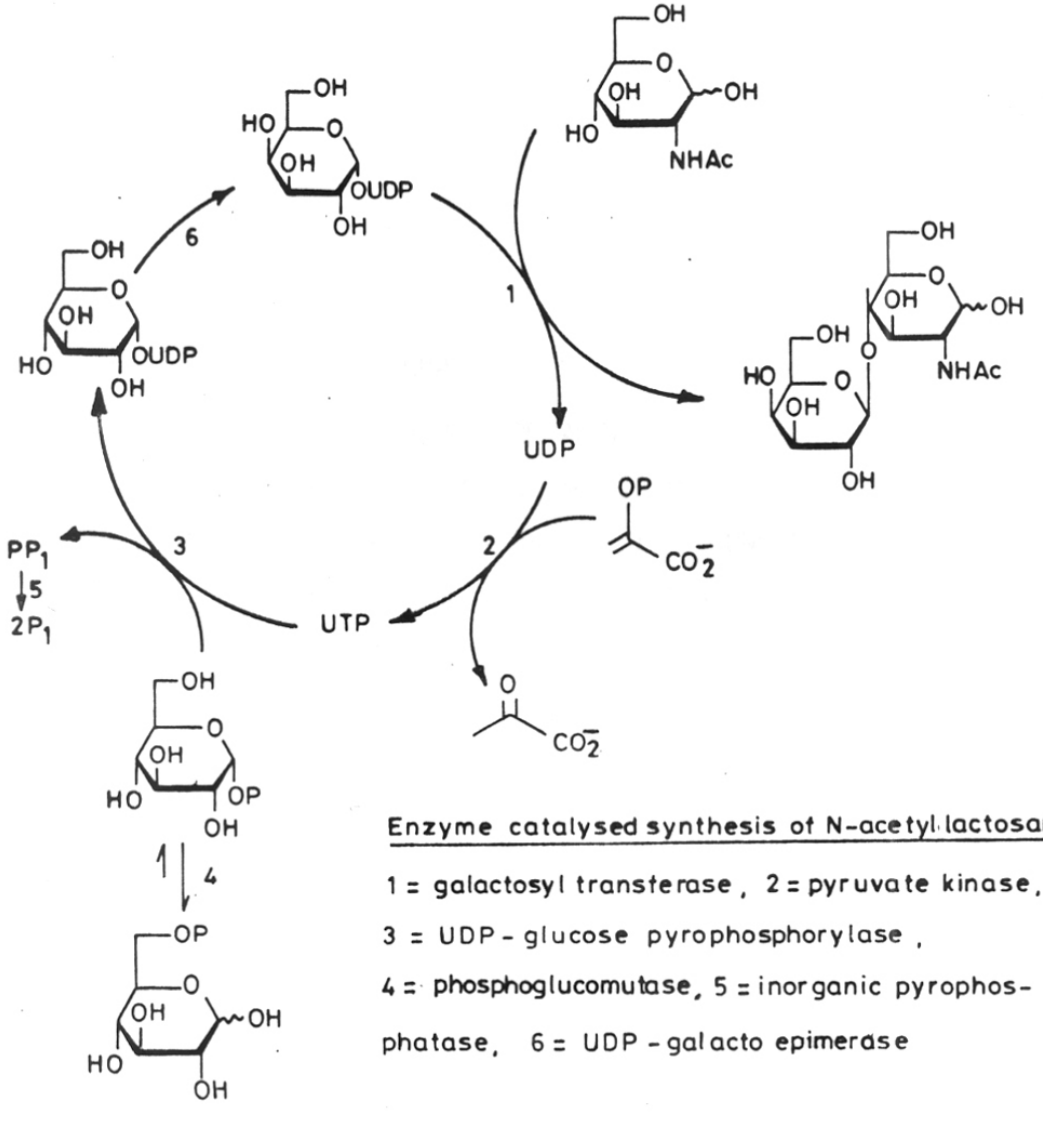
**Scheme 49**




β-Gal-(1 → 4)-Glc

β-Gal(1 → 6)-Glc

Scheme 48




Enzyme catalysed synthesis of N-acetyl lactosamine

1 = galactosyl transferase, 2 = pyruvate kinase,
3 = UDP-glucose pyrophosphorylase,
4 = phosphoglucomutase, 5 = inorganic pyrophosphatase, 6 = UDP-galacto epimerase

# CHAPTER 2

## STEREOSELECTIVE SYNTHESIS OF ALKYL TETRA-O-BENZYL α-D-GLYCOPYRANOSIDES BY USE OF PER-O-BENZYLATED 2-PYRIDYL THIOGLYCOSIDES AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR "A NEW GLYCOSIDATION METHODOLOGY"

## 2.1 Introduction

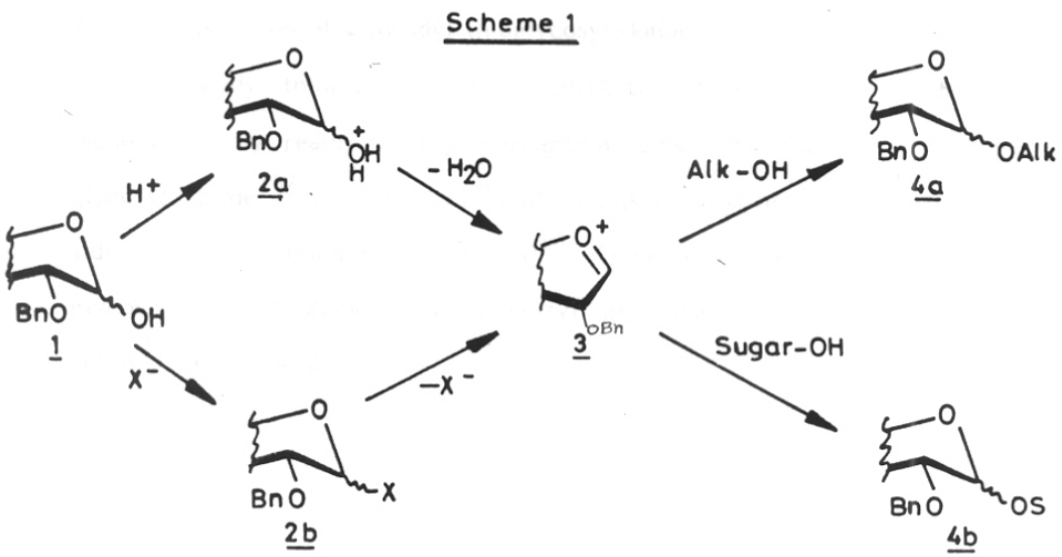
Much effort has been devoted in the last twenty years to the development of efficient and stereocontrolled synthesis of glycosides in 1,2-cis-relationship[156-158].

There are many problems that confront glycoside synthesis[128].

i) Probably the most obvious concerns the development of the multitude of hydroxyl groups that have to be differentiated.

ii) Another problem is, that the delicate anomeric center must be specially activated and/or protected against reaction with the sugar alcohol donor. The use of simple alcohol provides excellent protection for the delicate anomeric center through formation of an alkyl glycoside (**4a**). Where the "alcohol" is a protected monosaccharide, the product is a "disaccharide" (**4b**). In either case, the anomeric center of (**1**) must be converted into an electrophile, which requires activation by development of a good leaving group, and its subsequent ejection to generate the cyclic oxonium ion **3**. In the Fischer glycosidation, where simple alkanols

**Scheme 1**



were used in generous excess with acidic catalyst, bring about a one-pot transformation, **1** → **4a**. In an alternate and more versatile strategy, the anomeric center is activated by prior formation of a stable glycosyl derivative **2b**, from which the ion **3** is generated under controlled conditions (Scheme 1).

This chapter illustrates the results obtained in the development of a new glycosidation methodology by use of 2-pyridyl thioglycosides[206] as glycosyl donors and methyl iodide as an activator. This new methodology has the following unique attributes.

i) Use of stable glycosyl donors.

ii) Their direct preparation from an aldose by modified Fischer glycosidation procedure.

iii) Mild, non-toxic activation of the anomeric center and ultimately high α-stereoselectivity.

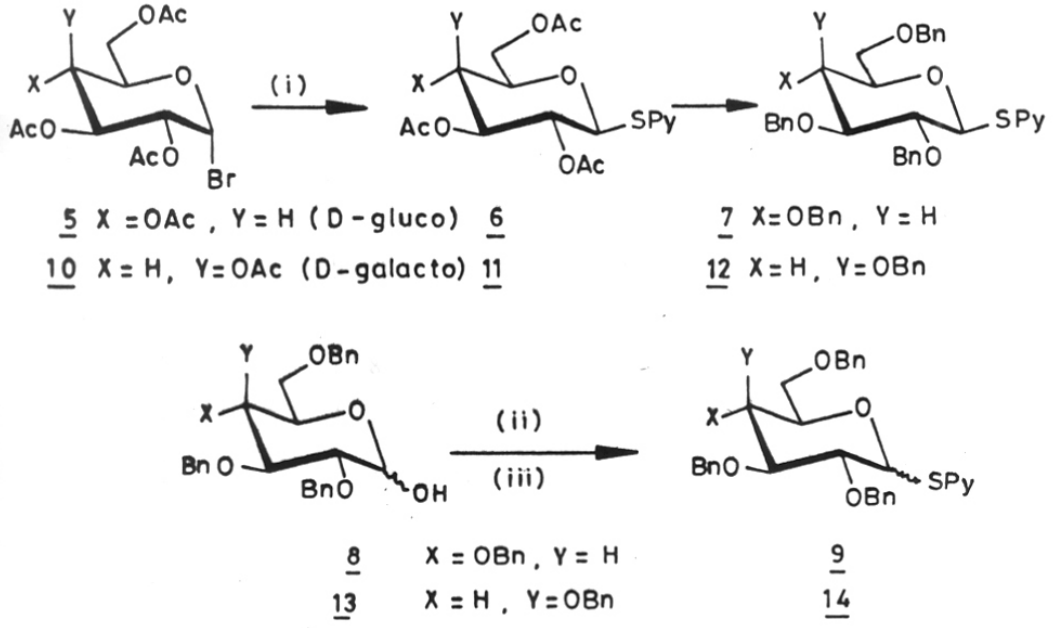
(1,2-cis-D-gluco- and galacto-).

This thesis deals only with pyranoid structures and the term glycoside, for example, implies glycopyranoside.

## 2.2 Preparation of 2-pyridyl thioglycosyl donors

2-Pyridyl thioglycosyl donors **7,9,12** and **14** have been synthesized (Scheme 2) by reaction of 2-mercaptopyridine with the corresponding glycosyl halide[137] (ii) by reaction of 2,3,4,6-tetra-O-benzyl glycopyranoside with 2,2'-dithiodipyridine-$nBu_3P$[135] (Scheme 2) and (iii) by Fischer glycosidation of 2,3,4,6-tetra-O-benzyl glycosides with 2-mercaptopyridine[193] (Scheme 2).

## Scheme 2




5 X = OAc, Y = H (D-gluco) 6 7 X = OBn, Y = H

10 X = H, Y = OAc (D-galacto) 11 12 X = H, Y = OBn

8 X = OBn, Y = H 9

13 X = H, Y = OBn 14

Characterization of the 2-pyridyl thioglycosides **7,9,12** and **14** was done on the basis of $^1$H-nmr spectra (Table 1). Per-O-acetylated β-2-pyridyl thioglycosides (**6,11**) exhibit a doublet at δ 5.75, ($J_{1,2}$ = 9-10 Hz, H-1) and the corresponding per-O-benzylated glycosides (**7,12**) appear at ca. δ 5.31 ($J_{1,2}$ = 9-10 Hz, H-1). For the corresponding α-2-pyridyl thioglycosides (**9,14**) H-1 signals appear at δ ca. 6.46 ($J_{1,2}$ = 5 Hz, H-1).

### 2.3 Results and discussion

Reaction conditions for this new glycosidation procedure was established based on the study of (i) role of activators (MeI, n-BuBr, n-BuI, $Bu_4NBr$, $Bu_4NI$, MeOTf and $AgClO_4$), (ii) effect of solvent (DMF, $CH_2Cl_2$, $CHCl_3$, and $C_6H_6$) (iii) rate of reaction of primary, secondary and tertiary alcohols and (iv) temperature.

**Table 1**

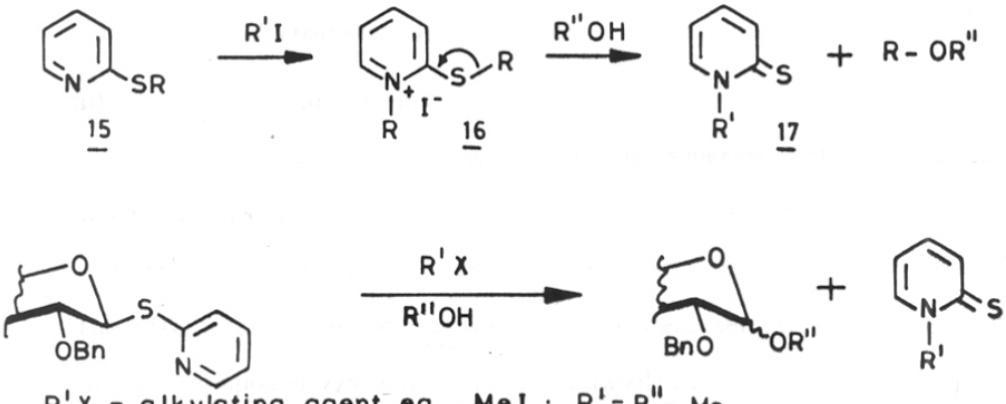
**2-Pyridyl thioglycosides**

| Entry | Compound | Yield % | m.p.°C | Optical rotation in $CHCl_3$ at 25°C | H-nmr δ(J in Hz) H-1 | |
|---|---|---|---|---|---|---|
| | | | | | β-compound | α-compound |
| i. | 6 | 72 | 120-123 | -2.9° (c 1.1) | 5.75 (10) | - |
| ii. | 7 | 87 | 74-76 | +8.8° (c 2.0) | 5.31 (9) | - |
| iii. | 9 | 85 | Syrup | - | 5.31 (9) | 6.62 (5) |
| iv. | 11 | 85 | Syrup | -2.0° (c 1.0) | 5.88 (10) | - |
| v. | 12 | 82 | 82-84 | +2.76° (c 1.0) | 5.26 (10) | - |
| vi. | 14 | 80 | Syrup | - | 5.26 (10) | 6.46 (5) |

**(i) Role of activators**

Several alkyl iodides (RI, R=$CH_3$, $C_2H_5$, n-$C_4H_{11}$) were studied for N-alkylation of the 2-pyridyl thio moiety. Earlier, a wide ranging activators ($Hg^{+2}$, $Ag^{+1}$, $Cu^{+2}$, $Ph_3P$, $Br_2$, NBS protic acids) have been used to activate 2-pyridyl thio moiety[135-137,193]. But activation of 2-pyridyl thio moiety by N-alkylation has not been attempted. The observation by Barlin et al[207], that alkyl 2-thiopyridine **15** on quaternization with alkyl iodide ejects out N-methyl-2-thiopyridone (**17**) has prompted us to investigate into the utility of this finding to develop a new glycosidation methodology.

**Scheme 3**




15 16 17

R'X = alkylating agent eg., MeI ; R' = R" = Me

Thus various alkylating agents such as $CH_3I$, n-BuBr, n-BuI, $Bu_4NBr$, $Bu_4NI$ and methyl triflate (MeOTf) were tried for activation of 2-pyridyl thio moiety on subsequent glycosidation to obtain alkyl-glycosides (Table 2).

In a typical experimental procedure 2-pyridyl thioglycoside **7** was reacted with methanol (equimolar) and the alkylating agent (3 mole equivalents) in dichloromethane at 50°C. The product **18** formed

was characterized by $^1$H-nmr and yield was estimated after purification by column chromatography (Table 2).

The results from Table 2 indicate that methyl iodide (entry i) was superior to all other activators, where as activation with n-BuI, 80% of the starting material was recovered and a very small amount (10%) of product was obtained (entry ii). Experiments by use of n-BuBr, $Bu_4NBr$ and $Bu_4NI$ (entries iii, iv and v) also did not effect glycosidations. Reaction with methyltriflate at room temperature for 24 h also resulted in isolation of 20% of methyl glycoside however no starting material was recovered, which indicated lot of decomposition. Thus the above experiments have confirmed methyl iodide as the suitable activator for glycosidations.

**(ii) The effect of solvent**

Having optimized methyl iodide as superior activating agent, attention was turned to the selection of the solvent. Solvents play profound role in controlling the anomeric ratio of products formed[156]. The effect of solvent on a) yield and b) stereoselectivity in the reaction of compound **7/9** with tertiary butanol was investigated to obtain **20** because it closely represents secondary alcohols of a sugar (Table 3). The yields were higher (over 80%) in benzene and dichloromethane (entres i and ii), somewhat lower (about 65%) in chloroform (entry iii) and only 40-50% yields using N,N-dimethylformamide and tetrahydrofuran (entries iv and v). The reactions in DMF and THF always resulted in the isolation of hydrolysed products in about 15-25% yield due to the presence of traces of water. To ensure strictly anhydrous conditions, molecular sieves 4Å were used while using $C_6H_6$, $CH_2Cl_2$ and $CHCl_3$.

**Table 2**

| Entry | Alkylating Agent | Time (h) | Yield % , **18** |
|---|---|---|---|
| i. | Me I | 22 | 95 |
| ii. | n-BuI | 72 | 10<br>(Starting material recovered) |
| iii. | n-BuBr | 72 | No reaction, (Do) |
| iv. | $Bu_4NBr$ | 72 | No reaction,(Do) |
| v. | $Bu_4NI$ | 72 | No reaction, (Do) |
| vi. | MeOTf | 24 (25°C) | 20<br>(Starting material decomposed) |

**Table 3**

**Effect of solvent on yield and stereoselectivity of formation of 20 in a reaction of compound 7 or 9 with tert-butanol**

| Entry | Solvent | Time (h)/ Temp. (0°C) | Yield % | Stereoselectivity α/β ratio 20 |
|---|---|---|---|---|
| i. | $C_6H_6$ | 48/80 | 80 | 85/15 |
| ii. | $CH_2Cl_2$ | 48/50 | 82 | 89/11 |
| iii. | $CHCl_3$ | 48/70 | 65 | 80/20 |
| iv. | DMF | 34/25 | 15 | 65/35 |
| v. | THF | 36/70 | 25 | 70/30 |

Stereoselectivity of reaction with change of solvent is indicated in Table 3, which is based on $^{1}$H-nmr data.

Because of the ease of purification, high stability, insolubility in water and good solvent properties, dichloromethane was chosen as a suitable solvent for glycosidations.

**(iii) Reactivity of alcohols (1°,2° and 3° alcohols)**

The reactivity of alcohols in the glycosidations was found in the order as follows, MeOH iso PrOH ter. BuOH.

Alcohols were used in equimolar proportions to the 2-pyridyl thioglycosides in dichloromethane at 50°C containing 3% methyl iodide (3 mole equivalents) along with 4 Å molecular sieves. There was no evidence of decomposition of substrates even when excess of methyl iodide was used (Table 4).

**(iv) Effect of temperature**

It was observed that the reaction rate was temperature dependent (Table 5). The reaction at room temperature was slower when compared to the similar reaction performed at the refluxing temperature in dichloromethane.

Thus optimum conditions were established (Table 1 to 4) for performing glycosidations. In a typical experimental procedure, compound 7 was reacted independently with equimolar quantities of methanol, isopropanol and tertiary butanol, in dichloromethane (having 3% methyl iodide) in presence of molecular sieves-4Å at 50°C for 1-3 days (Scheme 4). A similar set of reactions were also performed starting from 2-pyridyl thioglycoside (**12**). In all these glycosidation reactions, products were purified by column chromatography and were characterized by

**Table 4**

| Entry | Glycosyl donor | Alcohol | Product | Time (h) | Yield % | α/β ratio |
|---|---|---|---|---|---|---|
| i. | 7 or 9 | $CH_3OH$ | 18 | 22 | 95 | 65/35 |
| ii. | 7 or 9 | $(CH_3)_2CHOH$ | 19 | 34 | 85 | 82/18 |
| iii. | 7 or 9 | $(CH_3)_3COH$ | 20 | 48 | 82 | 89/11 |
| iv. | 12 or 14 | $CH_3OH$ | 21 | 23 | 96 | 72/28 |
| v. | 12 or 14 | $(CH_3)_2CHOH$ | 22 | 36 | 87 | 87/13 |
| vi. | 12 or 14 | $(CH_3)_3COH$ | 23 | 48 | 80 | 91/9 |

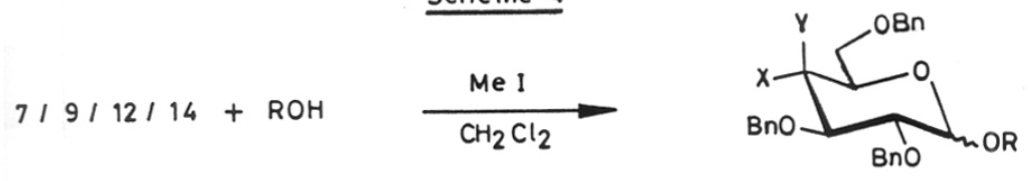
**Table 5**

**Reaction of 2-pyridyl thioglycoside 7**

| Entry | Alcohol | Reaction | | % Yield (glycoside) |
|---|---|---|---|---|
| | | Temp. °C | Time (h) | |
| i. | $CH_3OH$ | 25 | 36 | 92 (18) |
| | | 50 | 22 | 95 (18) |
| ii. | $(CH_3)_2CHOH$ | 25 | 52 | 80 (19) |
| | | 50 | 34 | 85 (19) |
| iii. | $(CH_3)_3COH$ | 25 | 72 | 75 (20) |
| | | 50 | 48 | 82 (20) |

$^1$H-nmr spectra. Anomeric ratio of products were determined by $^1$H-nmr and analytical HPLC (experimental) and yields reported are isolated yields in all cases.

Scheme 4




18) X = OBn; Y = H (Glc) R = Me (α/β, 65/35)

19) X = OBn; Y = H (Glc) R = i-Pr (α/β, 82/18)

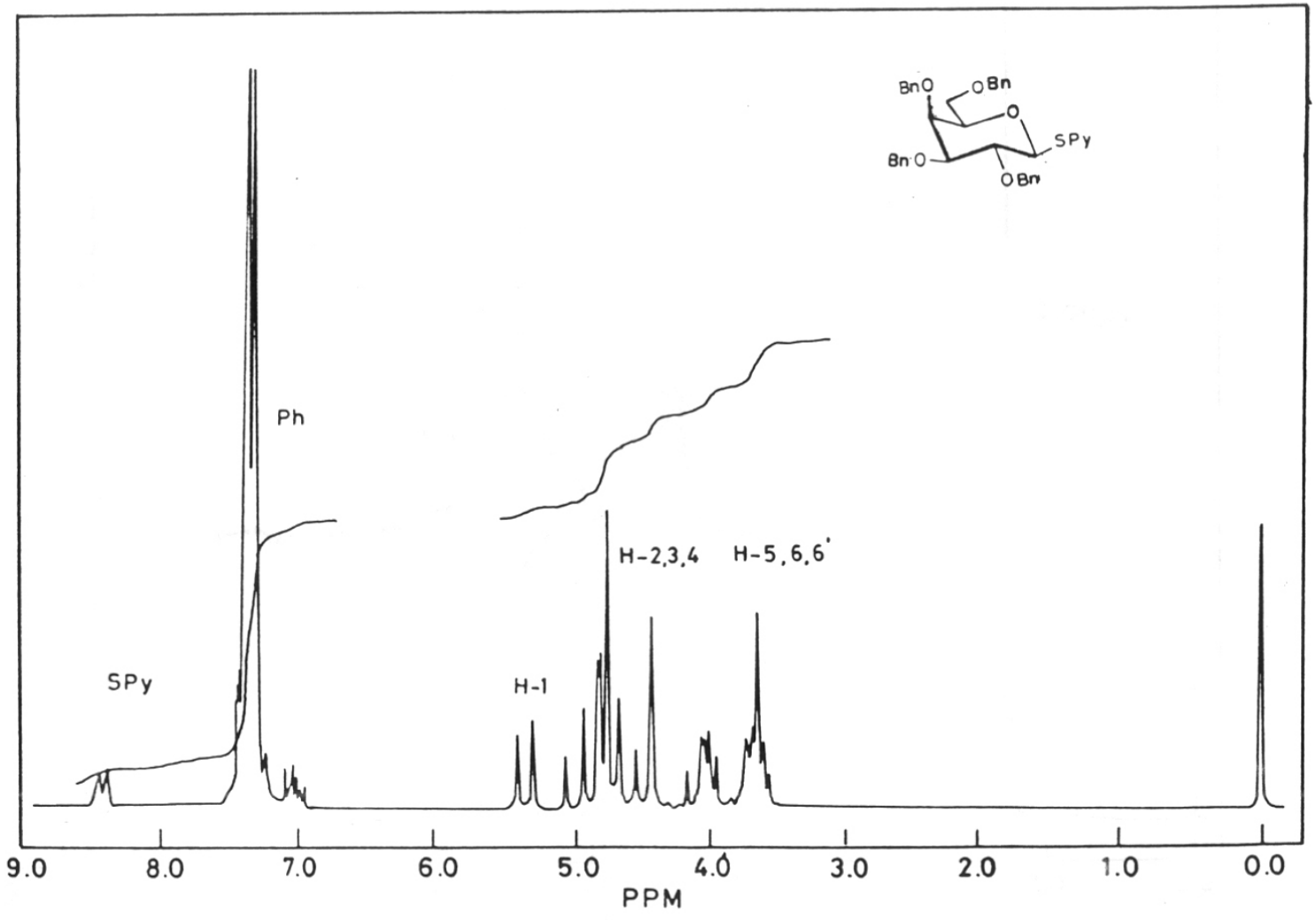
20) X = OBn; Y = H (Glc) R = t-Bu (α/β 89/11)

21) X = H; Y = OBn (Gal) R = Me (α/β, 72/28)

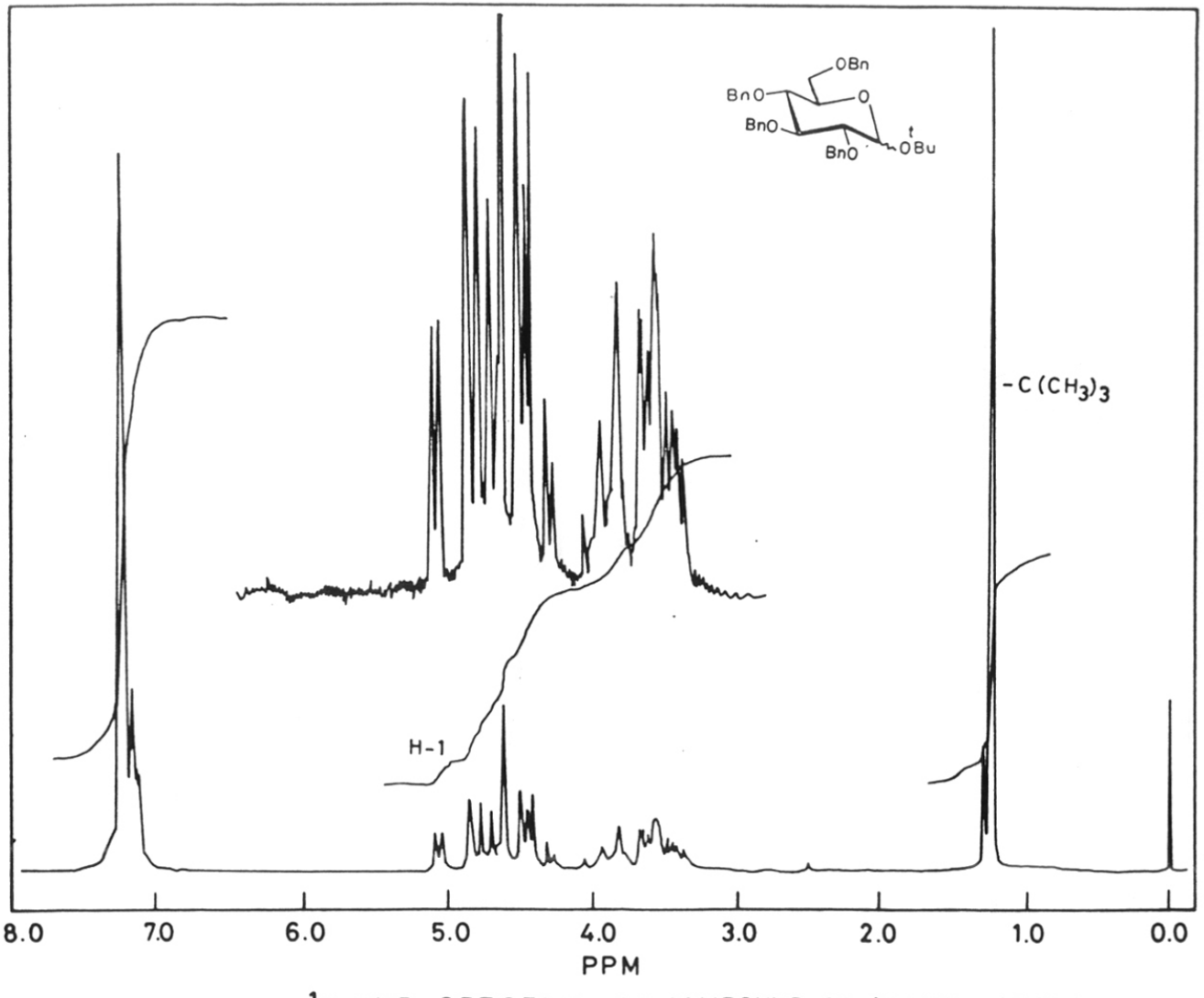
22) X = H; Y = OBn (Gal) R = i-Pr (α/β, 87/13)

23) X = H; Y = OBn (Gal) R = t-Bu (α/β 91/9)

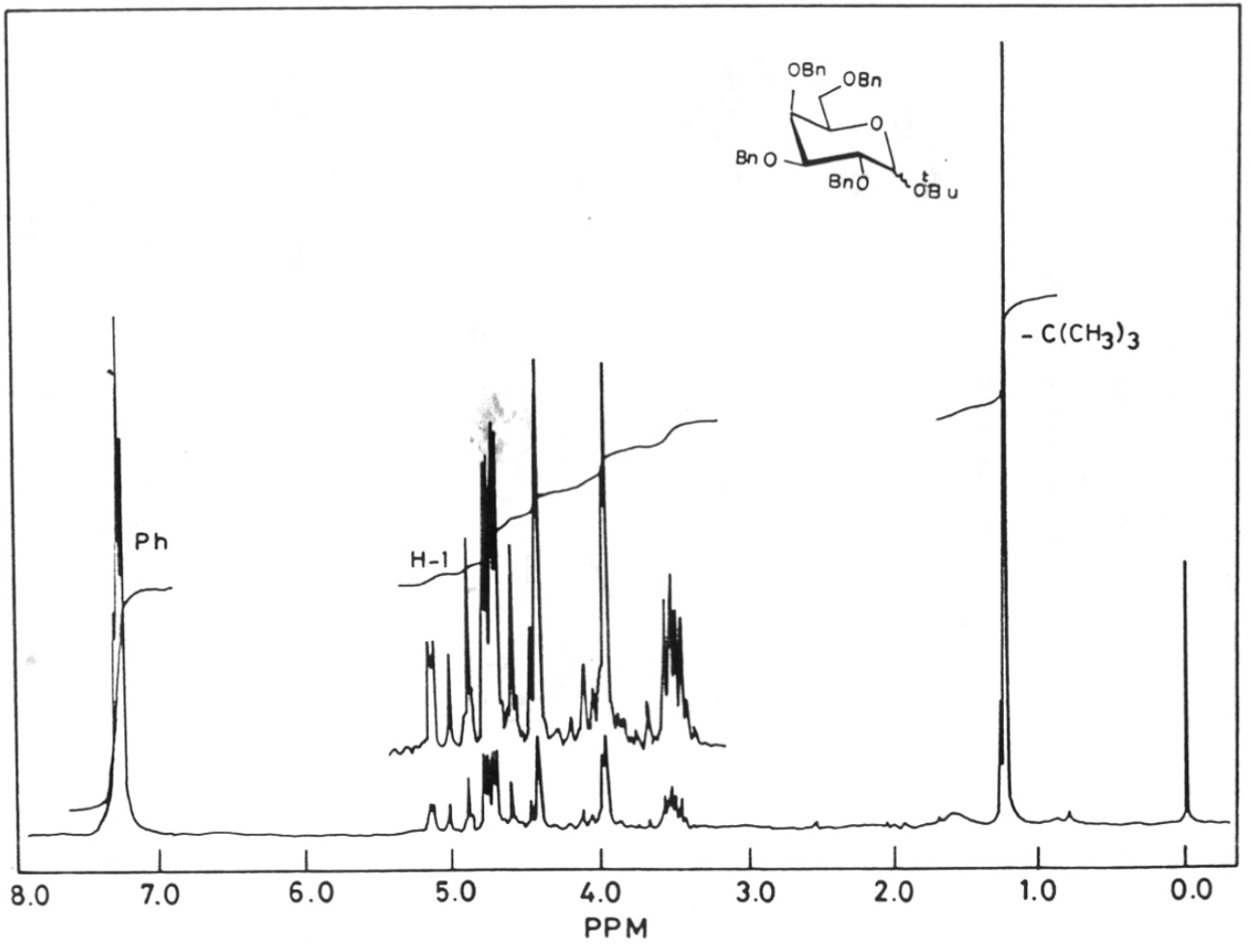
The most significant observation of this glycosidation reaction is anomeric ratio of the alkyl glycosides formed remains unchanged even when anomeric mixture (**9** and **14**) of 2-pyridyl thioglycosides were used as substrates. Thus reaction of **7** (β) or **9** (α/β) with diverse simple alcohols like methanol, isopropanol and tertiary butanol afforded their corresponding glycosides **18, 19** and **20** in α/β ratios 65/35, 82/18 and 89/11 respectively (entires i, ii, iii in Table 4). Similar observations, but in enhanced α-selectivities (**21, 22** and **23**) were obtained from galacto- compounds **12** or **14** (entries iv, v, vi in Table 4). Use of sugar alcohol in place of simple alcohols resulted in the exclusive isolation of α-linked disaccharide (Chapter 3).




$^1$H NMR SPECTRUM OF COMPOUND 12 ( 90 MHz, $CDCl_3$ )




$^1$H NMR SPECTRUM OF COMPOUND 20 (80MHz; $CDCl_3$)



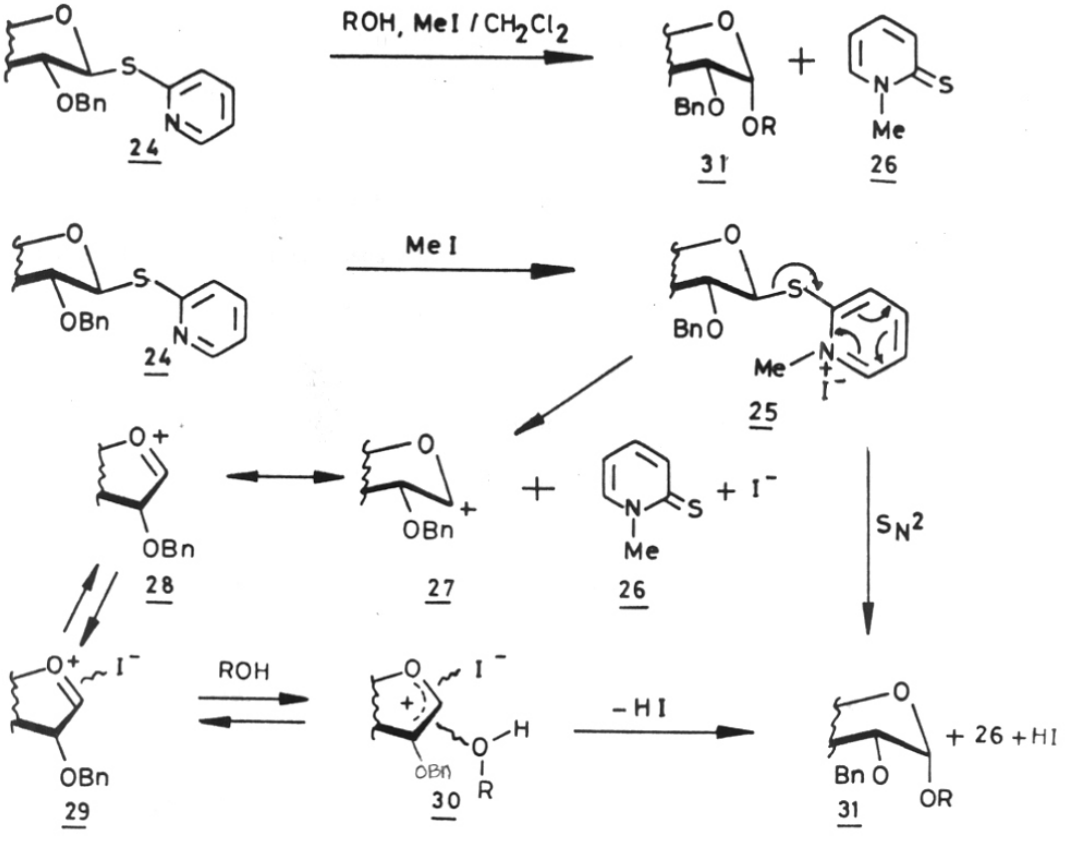

$^1$H NMR SPECTRUM OF COMPOUND 23 (90 MHz, $CDCl_3$)

## 2.4 Mechanism

Reaction of the 2-pyridyl thioglycoside (**24**) (Scheme 5) presumably proceeds through the initial electrophilic attack of methyl iodide on the nitrogen atom rather than sulfur of the pyridyl thio sugar **24** resulting

**Scheme 5**

**Mechanism of glycosidation reaction**




in the formation of N-methyl quaternary salt **25** which immediately ejects out N-methyl-2-thiopyridone (**26**)[208-210] with subsequent $S_N2$ displacement by an alcohol giving the glycoside (**31**) or leads to the formation of a carbonium ion **27** which is in turn captured by the alcohol leading to the formation of alkylglycoside **31.** It is very likely that the

oxocarbeniumion (**28**) forms ion-pair (**29**) with the free iodide which in turn is displaced by an alcohol to give selectively the α-glycoside. α-Glycoside (**31**) would be the thermodynamic product resulting from either $S_N2$ displacement of **25** by an alcohol or through the capture of the glycosyl carbenium ion (**28**).

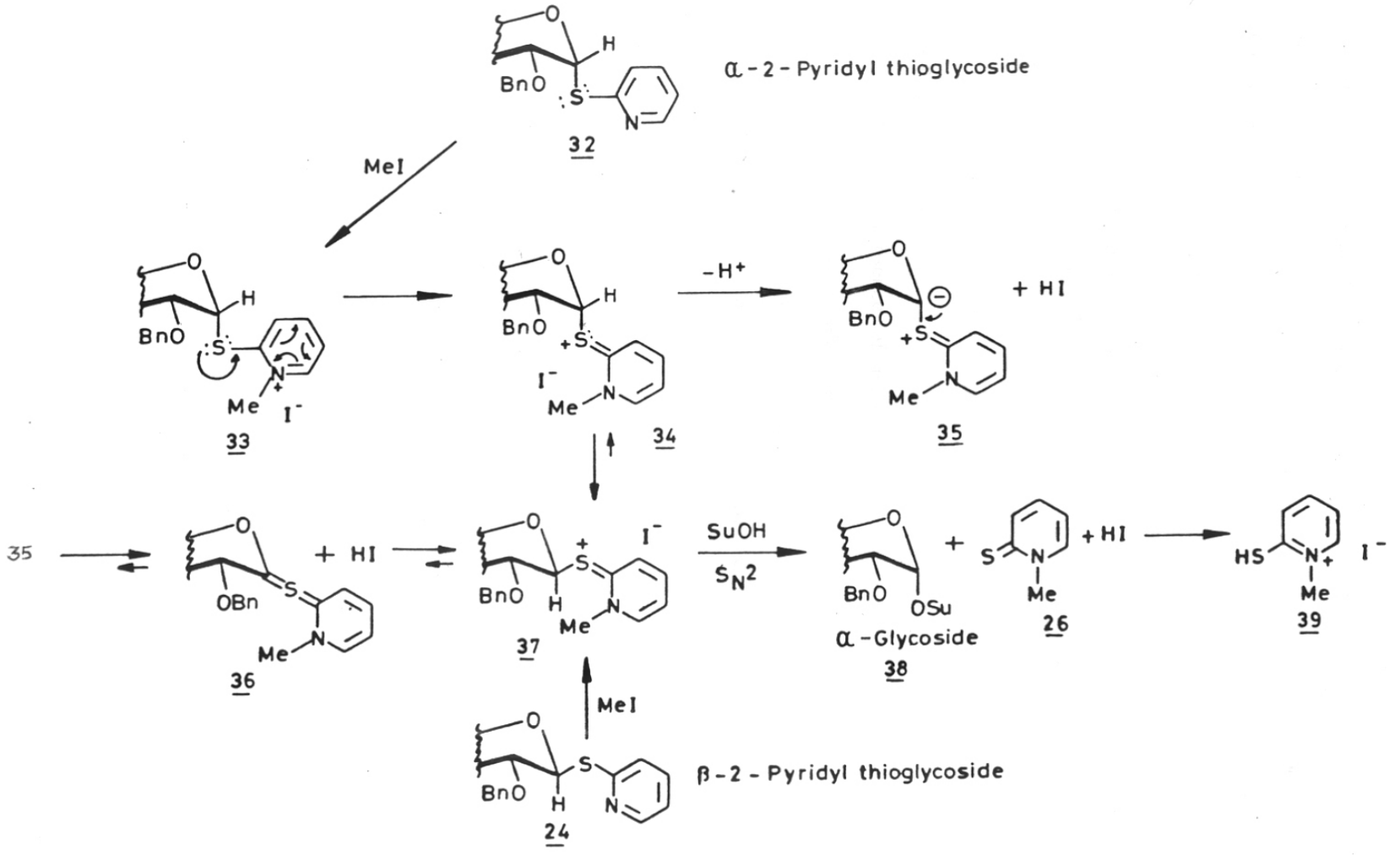
**Mechanism for α-glycosidations**

First step involves N-methylation of the heterocyclic moiety of 2-pyridyl thioglycoside **32** on reaction with methyl iodide leading to the formation of stable N-methyl quaternary thiopyridinium salt **33**. Compound **33** is further stabilized by resonance to form the sulfenium glycoside **34**, which in turn loses the acidic proton at the anomeric center leading to the formation of **35**, which in turn is stabilized by conjugation to form **36**. Protonation of **36** leads to the β-sulfenium ion **37**, which would be stabilized due to reverse anomeric effect[54]. Thus equilibrium is driven from **34** to **37** ultimately. The β-sulfenium glycoside **37** undergoes $S_N2$ displacement on reaction with sugar alcohols (SuOH) leading to the stereoselective formation of α-glycosides (**38**). N-methyl 2-thiopyridone (**26**) which is ejected out, in turn captures the liberated HI to form the salt **39**. Thus α or β -2-pyridyl thioglycosides (**32** and **24**) lead to the formation of α-glycosides (Scheme 6).

The study of anomerization of **32** to **24** was attempted by reaction of **7** with MeI in nmr tube in $CDCl_3$. This reaction did not give any information about anomerization due to rapid hydrolysis of **33** or **25** in the absence of any nucleophile.

In the absence of nucleophile (ROH) in a glycosidation reaction compound **7** shows the formation of the corresponding tetra-O-benzyl

## MECHANISM FOR α- GLYCOSIDATIONS



-α-glucopyranosyl iodide[211], though unstable, is isolated quickly by a short column and was identified from its $^1$H-nmr data. Its $^1$H-nmr spectrum showed a doublet at 6.84 ($J_{1,2}$=4 Hz), consistent with α-configuration. Schuerch et al have synthesized tetra-O-benzyl glycosyl iodides by in situ generation from the reaction of sodium iodide with the corresponding glycosyl chlorides or bromides[211].

Further confirmation of the structure of this unstable α-glycosyl iodide[211] was made by reacting it with isopropanol and AgOTf at 0° for 10 h to obtain isopropyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside as a major compound.

However in presence of nucleophiles formation of the tetra-O-benzyl α-glucopyranosyl iodide was never observed. Thus ruling out α-glycosyl iodides as the possible intermediates in these glycosidations. Normally, it requires a hindered base or a metal salt to effect coupling of glycosyl iodides with alcohol and no such reagent has been used in these reactions[211].

## 2.5 EXPERIMENTAL

### GENERAL PROCEDURES

**2.5.1 Preparation of per-O-acetylated 2-pyridyl-β -thio hexopyranosides**

**A:** To a solution of 2-mercaptopyridine (1.2 molar equivalent) in dry acetone was added potassium carbonate (1.2 molar equivalent) and stirred at 40°C for 30 min. Then crystalline α-acetobromo hexose (1 molar equivalent) dissolved in dry toluene was added gently. Stirring continued at 40°C for another two hours. The reaction mixture was diluted with toluene, stirred for another 10 min and brought to room temperature. Organic phase was washed with water and 1% KOH aqueous solution, dried ($Na_2SO_4$) and solvent was removed on rotavap to yield the per-O-acylated 2-pyridyl- β-thiohexopyranosides (in 78-90%), which were purified either by crystallisation or by column chromatography.

**B:** Methyl per-O-benzyl- α-D-hexopyranosides (5 mmol, D-gluco-D-galacto-) were hydrolysed by heating at 80-85°C for 20-30 min with a mixture of acetic acid (20 ml) and aqueous sulfuric acid (3M, 2.5 ml). After cooling, the mixture was extracted into dichloromethane and washed with cold saturated sodium hydrogen carbonate (2x50 ml) and then with water (3x100 ml). Combined organic extracts were dried ($Na_2SO_4$), evaporated and filtered on a short bed of $SiO_2$ to give the reducing sugar. These were crystallized from solvents like diisopropyl-ether-cyclohexane and diethylether-hexane to give practically pure products (55-65% yields) of hydrolysis. To a solution of 2,3,4,6-tetra-O-benzylated hexopyranose (2 mmol) in dry dichloromethane (10 ml) was added 2,2'-dithiodipyridine (2.2 mmol) followed by the addition of n-tributyl phosphine (2.4 mmol). The reaction mixture was stirred

at room temperature for 1 h. The solvent was removed, resulting residue (yellowish) was chromatographed ($SiO_2$, Petroleum ether:EtOAc, 8:1.5) to obtain the desired 2,3,4,6-tetra-O-benzyl 2-pyridyl thioglycosyl donors (α/β-anomeric mixture) as syrup.

**2.5.2 Deacetylation**

A solution of per-O-acetylated 2-pyridyl thiohexopyranoside (5 mmol) in dry methanol (15 ml) having catalytic amount of sodium methoxide (50 mg, sodium dissolved in 5 ml dry methanol) was kept at room temperature for 2 h. The mixture was briefly warmed to 45°C (about 5 min.), carefully neutralised with IR 120 $H^+$ resin, filtered and solvent removed to obtain a syrupy deacetylated 2-pyridyl thiohexopyranoside in almost quantitative yield.

**2.5.3 Benzylation of 2-pyridyl thiohexopyranosides**

The high vacuum dried deacetylated 2-pyridyl thiohexoses (1 mmol) dissolved in dry DMF (2 ml) were added to sodium hydride (hexane washed, 5 mmol) in dry DMF (2 ml) at 0°C and stirred for 30 min. Then benzyl bromide (4.4 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. When t.l.c. indicated the completion of the reaction, excess sodium hydride was decomposed by the addition of methanol (1 ml), water (100 ml), diluted with petroleum ether:dichloromethane (1:1, 10 ml). The organic phase was washed with water (3x100 ml) dried ($Na_2SO_4$) and evaporated to yield a syrup which was purified by column chromatography ($SiO_2$) to yield the benzylated products (80-89%).

**2.5.4 A typical experimental procedure for glycosidations**

In an oven dried round bottom flask under nitrogen was taken

the perbenzylated 2-pyridyl thioglycoside (1 mmol) and molecular sieves 4 Å (300 mg, activated and powdered). Dichloromethane (15 ml) having 3% methyl iodide was added followed by the addition of glycosyl acceptor (1.2 mmol) and heated at 50°C (oil bath temperature) for 22-36 h. The progress of the reaction was done by thin layer chromatography (t.l.c.). After completion of the reaction, it was filtered on a bed of celite, washed with dichloromethane and evaporated. The residue was purified by column chromatography to obtain the O-glycosides (70-90%). The products were characterised by $^{1}H$, $^{13}C$-nmr and HPLC (analytical).

**2-Pyridyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranoside (6)**[137]

The reaction (2.5.1A) of α-acetobromoglucose (12.3 g, 30 mmol in toluene, 30 ml) with 2-mercaptopyridine (4 g, 36 mmol), anhydrous $K_2CO_3$ (3.48 g, 36 mmol) and acetone (60 ml) at 40°C for 2 h, afforded **6** (10 g, 72% yield) as yellow needles, after workup and recrystallisation (hexane:$CH_2Cl_2$).

m.p.: 120-123°C, $[\alpha]_D^{25}$ -2.9° (c 1.1, $CHCl_3$).

Analysis calcd. for $C_{19}H_{23}NO_9S$: C, 51.70; H, 5.21; N, 3.17; S, 7.25. Found: C, 52.02; H, 5.43; N, 3.25; S, 7.38%.

$^{1}H$-nmr (80 MHz δ ppm, J in Hz): δ 1.94, 2.00, 2.04 (4s, 12H , 4xOAc), 3.6-4.40 (m, 3H, H-5,6,6'), 4.80-5.60 (m, 3H, H-2,3,4), 5.75 (d, 1H, J = 10, H-1), 6.9-7.70 (m, 3H, SPy), 8.36 (m, 1H, SPy).

$^{13}C$-nmr (22.3 MHz, δ ppm): δ 20.2 (4q, 4xO.CO.$\underline{C}H_3$), 71.7 (C-6), 68.1, 69.2, 73.8, 75.6 (C-2,3,4,5), 81.3 (C-1), 120.5, 122.9, 136.2, 149.2, 155.16 (C-2",3",4", SPy), 169.0, 169.6, 170.0 (4s, O.$\underline{C}O.CH_3$).

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (7)**[137]

Compound **6** (7.4 g) was deacetylated (2.5.2) dry methanol 20

ml, cat. NaOMe, RT, 2 h) and neutralised with IR 120 $H^+$ to give the syrupy 2-pyridyl-1-thio-β-**D**-glucopyranoside (4.5 g, 16.7 mmol, DMF, 5 ml) which was benzylated as per the procedure described in 2.5.3. The completion of the reaction was monitored by t.l.c. (Petroleum ether:Ethyl acetate 8:3). Work up and column chromatography yielded the benzylated compound **7** (8.8 g, 87%) as a glassy material.

m.p.: 74-76°C, $[\alpha]_D^{25}$ +8.8° (c 2.0, $CHCl_3$).

Analysis calcd. for $C_{39}H_{39}NO_5S$: C, 73.93; H, 6.16; N, 2.21; S, 5.05. Found: C, 74.12; H, 6.26; N, 2.40; S, 5.03%.

$^1$H-nmr(90 MHz): δ 3.4-3.68 (m, 4H, H-2,5,6,6'), 4.33-4.85 (m, 10H, benzylic and H-3,4), 5.31 (d, 1H, $J_{1,2}$= 9, H-1), 6.78-7.46 (m, 23H, Ph and SPy), 8.28 (m, 1H, SPy).

$^{13}$C-nmr (22.3 MHz): 69.3 (C-6), 73.5, 75.1, 75.5, 75.8 (benzylic), 78.3, 79.6, 81.2, 84.1 (C-2,3,4,5), 87.0 (d, C-1), 120.5, 123.5, 136.7, 149.7, 158.2 (SPy), 126.5, 127.9, 128.5 (Ph), 136.7, 138.2, 138.5, 138.8 (Ph).

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α/β-D-glucopyranosides (9)**

Methyl 2,3,4,6-tetra-O-benzyl-α-**D**-glucopyranoside[213] (2.78 g, 5 mmol) was hydrolysed (general procedure 2.5.1B) to give 2,3,4,6-tetra-O-benzyl-α-**D**-glucopyranose[214] (**8**, 1.5 g, 56%) as a crystalline compound. $[\alpha]_D$ +48° (c 1.0, dioxane)

The reaction of **8** (1.1 g, 2 mmol) in dichloromethane (10 ml) with 2,2'-dithiodipyridine (0.48 g, 2.2 mmol) and n-tributylphosphine (0.59 g, 2.4 mmol) yielded a syrupy compound **9** (1 g, 85%, α/β 2:3 ratio) from $^1$H-nmr (6.62 and 5.31 respectively).

$^1$H-nmr (90 MHz) 3.3-3.95, 4.42-5.0 (m, 14 H, H-2,3,4,5,6,6' and 4x $CH_2$-benzylic), 5.31 (d, 2/3 H, $J_{1,2}$=9, H-1, β-compound) 6.62 (d, 1/3 H, $J_{1,2}$=5, H-1, α-compound) 6.83-8.66 (m, 24H, Ph and SPy).

**2-Pyridyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (11)**

The reaction of α-acetobromogalactose (3.6 g, 8.7 mmol in toluene, 10 ml) with 2-mercaptopyridine (1.08 g, 9.7 mmol) and $K_2CO_3$ (anhydrous) (1.08 g, 11.3 mmol) in dry acetone (23 ml) at 40°C for 2 h afforded compound **11** (2.63 g, 85%).

$[\alpha]_D^{25}$ -2.0° (c 1.0, $CHCl_3$).

Analysis calcd. for $C_{19}H_{23}NO_9S$: C, 51.70; H, 5.21; N, 3.17; S, 7.25. Found: C, 51.86; H, 5.32; N, 3.03; S, 7.17%.

$^1$H-nmr (90 MHz): 1.97 (s, 6H, 2xOAc), 2.04, 2.17 (2s, 6H, 2xOAc), 4.00-4.45 (m, 3H, H-5,6,6'), 5.18 (dd, 1H, $J_{2,3}$=10, $J_{3,4}$=3.5), 5.35 (t, 1H, $J_{1,2}$=10), 5.47 (bs, 1H, H-4), 5.88 (d, 1H, $J_{1,2}$=10, H-1), 7.0-7.8 (m, 3H, SPy), 8.53 (m, 1H, SPy).

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside (12)**

Compound **11** (2 g) was deacetylated (2.5.2) to afford 2-pyridyl-1-thio-β-D-galactopyranoside (1.19 g, 98%) which was benzylated [(2.5.3) (DMF/NaH/BnBr/RT/2 h)] to give compound **12** (0.95 g, 82%).

m.p.: 82-84°C. $[\alpha]_D^{27}$ +2.76° (c 1.0, $CHCl_3$).

Analysis calcd. for $C_{39}H_{39}NO_5S$: C, 73.93; H, 6.16; N, 2.21; S, 5.05. Found: C, 73.98; H, 6.22; N, 2.44; S, 5.16%.

$^1$H-nmr (90 MHz): 3.50-4.25 (m, 3H, H-5,6,6'), 4.3-5.10 (m, 11H, H-2,3,4 and benzylic), 5.26 (d, 1H, $J_{1,2}$=10, H-1), 6.72-7.4 (m, 23H, Ph and SPy), 8.44 (m, 1H, SPy).

$^{13}$C-nmr (22.3 MHz): 69.1, 73.1, 73.8, 74.4, 74.9, 75.8, 77.3, 78.7 (4d, C-2,3,4,5,5t, C-6 and 4xPh-$\underline{C}H_2$-), 84.6 (d,C-1), 120.4, 123.7, 136.5, 149.7, 158.1 (SPy), 126.3, 127.8, 128.1-129.2, 138.4, 138.6, 139.1 (aromatic).

Analysis calcd. for $C_{39}H_{39}NO_5S$: C, 73.93; H, 6.16; N, 2.21; S, 5.05. Found: C, 73.73; H, 6.41; N, 2.01; S, 5.16%.

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α / β -D-galactopyranosides (14)**

Methyl 2,3,4,6-tetra-O-benzyl- α -D-galactopyranose[213] (2.78 g, 5 mmol) was hydrolysed (2.5.1B) to give 2,3,4,6-tetra-O-benzyl- α-D-galactopyranose (**13**, 1.8 g, 66%).

m.p.: 63.66°C, $[\alpha]_D^{20}$ +77° (c 2.3, benzene).

The reaction of compound **13** (1.1 g, 2 mmol) in dichloromethane (10 ml) with 2,2'-dithiodipyridine (0.48 g, 2.2 mmol) and n-tributylphosphine (0.59 g, 2.4 mmol) (general procedure 2.5.1B) afforded compound **14** (0.94 g, 80%) as a syrup in α:β/1:1.

$^1$H-nmr (90 MHz): 3.35-5.11 (m, 14H, H-2,3,4,5,6,6' and 4x$CH_2$-benzylic), 5.26 (d, 1/2 H, $J_{1,2}$=10, H-1, β-compound), 6.46 (d, 1/2 H, $J_{1,2}$=5, H-1, α-compound), 6.85-8.42 (m, 24H, Ph and SPy).

**Methyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosides (18)**

2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio- β -D-glucopyranoside **7** (0.633 g, 1 mmol) was reacted with dry methanol (30.4 μl, 1.2 mmol in 10 ml dichloromethane having 3% methyl iodide, at 50°C for 22 h. (general procedure 2.5.4) to afford compound **18** (0.49 g, 95%) as a syrup (α: β mixture 65:35).

HPLC α:β ratio = 65: 35; Retention time α /β = 7.61/6.33 (min.)

| | | |
|---|---|---|
| Column type | : | RCM C-18 |
| Mobile phase | : | $CH_3CN$:$H_2O$ (70:30) |
| Flow rate | : | 2 ml/min. |
| Detector | : | UV-254 nm |
| Sensitivity | : | 2V |

Analysis calcd. for $C_{35}H_{38}O_6$: C, 75.81; H, 6.86. Found: C, 75.92; H, 6.06%.

$^1$H-nmr (90 MHz): (α/β): 3.30 (s, $OCH_3$), 3.50 (s, $OCH_3$ overlaps with H-5,6,6').

**Methyl 2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosides (21)**

The reaction (2.5.4) of 2-pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-galactopyranoside **12** (0.663 g, 1 mmol) with dry methanol (30.4 μl, 1.2 mmol) yielded compound **21** (0.49, 96%) as a syrup in 23 h. HPLC, α: β ratio = 72:28.

Retention time α/β = 6.52/8.0 (min)

Analysis calcd. for $C_{35}H_{38}O_6$: C, 75.81; H, 6.86. Found: C, 75.92; H, 6.76%.

$^1$H-nmr (80 MHz) (α/β): 3.32 (s, $-OCH_3$), 3.52 (s, $OCH_3$ overlaps with H-5,6,6').

**Isopropyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosides (19)**

Partnering of compound **7** (0.311 g, 0.5 mmol) with isopropanol (56.5 μl, 1.2 mmol) in dichloromethane (5 ml containing 3% methyl iodide) provided compound **19** (0.24 g, 85%) as a syrup in 34 h.

$[\alpha]_D^{29}$ +32.96° (c 1.0, $CHCl_3$).

HPLC, α:β ratio = 82:18; Retention time α/β = 7.87/9.21 (min).

Analysis calcd. for $C_{37}H_{42}O_6$: C, 76.28; H, 7.21. Found: C, 76.31; H, 7.21.

$^1$H-nmr (80 MHz) (α/β): 1.08, 1.14, 1.16, 1.18, 1.22 (2d, 6H, $CH(CH_3)_2$, 3.0-5.10 (m, 14H, H-2,3,4,5,6,6' and Ph-$CH_2$-O-), 6.60-7.90 (m 20H, Ph).

$^{13}$C-nmr (22.3 MHz) (α / β): 21.5, 22.4, 23.4, 23.9 (4q, $\underline{C}H_3)_2$-CH-O), 69.2, 69.7, 70.5, 73.2, 73.7, 75.3, 75.8, 78.4, 80.5, 82.4 (C-2,3,4,5,6,6' and Ph-$\underline{C}H_2$), 95.3 (d, C-1, α), 102.5 (d, C-1,β ), 127.7-139.5 (aromatic).

**Isopropyl 2,3,4,6-tetra-O-benzyl-αβ-D-galactopyranosides (22)**

A reaction (2.5.4) of compound **12** (0.31 g, 0.5 mmol) with dry isopropanol (56.5 l, 24 ul, 1.2 mmol) gave the corresponding isopropyl derivative **22** (0.25 g, 87%) as a syrup in 36 h.

$[\alpha]_D^{27}$ +33.85 (c 1.0, $CHCl_3$).

HPLC ratio α:β = 87:13; Retention time of α/β = 18.15/15.86 (min.)

Analysis calcd. for $C_{37}H_{42}O_6$: C, 76.28; H, 7.21. Found: C, 76.26; H, 7.32%.

$^1$H-nmr (80 MHz)(α/β): 1.15, 1.19, 1.21, 1.25 (2d, 6H, -CH($\underline{C}H_3)_3$), 3.26-5.26 (m, 15H, H-1,2,3,4,5,6,6' and 4xPh-$\underline{C}H_2$-O-), 7.0-7.6 (m, 20H, aromatic).

**t-Butyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranosides (20)**

The reaction of the compound **7** (0.311 g, 0.5 mmol) with t-butanol (69.8 μl, 1.2 mmol) for 48 h yielded compound **20** (0.21 g, 82%).

$[\alpha]_D^{27}$ +37.77° (c 1.0, $CHCl_3$).

HPLC α:β ratio = 89:11; Retention time α /β = 14.97/12.35 (min).

Analysis calcd. for $C_{38}H_{44}O_6$: C, 76.51; H, 7.38. Found: C, 76.63; H, 7.42%.

$^1$H-nmr (80 MHz) (α/β): 1.2, 1.24 (2s, 9H, $(\underline{C}H_3)_3$C- and respectively); 3.0-4.94 (m, 14H, H-2,3,4,5,6,6' and 4xPh-$\underline{C}H_2$O), 5.08 (d, 1H, $J_{1,2}$=3.5, H-1), 6.7-7.7 (m, 20H, aromatic).

**t-Butyl 2,3,4,6-tetra-O-benzyl-α/β-D-galactopyranosides (23)**

Compound **12** (0.311 g, 0.5 mmol) was reacted with dry t-butanol

(69.8 µl, 1.2 mmol) in dichloromethane (5 ml, having 3% methyl iodide) in presence of molecular seives 4Å at 50°C for 48 h to yield after purification, the glycosides **23** (0.219 g, 75%) as a syrup.

$[\alpha]_D^{27}$ +38.77 (c 1.15, $CHCl_3$).

HPLC, ratio of α:β = 91:9, Retention time of α /β = 23.91/22.37 (min). Analysis calcd. for $C_{38}H_{44}O_6$: C, 76.51; H, 7.38. Found: C, 76.62; H, 3.53%.

$^1$H-nmr (90 MHz) (α/β =91/9): 1.22, 1.26 (2s, 9H, $(C\underline{H}_3)_3C$-α and β respectively), 3.22-5.06 (m, 14H, H-2,3,4,5,6,6' and 4xPh-$C\underline{H}_2$-O-), 5.14 (d, $J_{1,2}$=3, H-1), 6.9-7.4 (m, 20H, aromatic).

$^{13}$C-nmr (22.3 MHz) (α/β): 28.9, 29.2 $(C\underline{H}_3)_3C$-), 69.2, 69.5, 73.2, 73.4, 73.7, 75.0, 75.8, 77.1, 79.5 (4d, 5t, C-2,3,4,5,6,6' and 4xPh-$\underline{C}H_2$-O-), 92.6 (d, C-1), 127.6, 128.5, 138.7-139.5 (aromatic).

# CHAPTER 3

# A MILD GENERAL METHOD FOR THE SYNTHESIS OF α-LINKED DI- AND TRISACCHARIDES

## 3.1 Introduction

α-Linked oligosaccharides are of paramount importance as they are constituents of many biologically active natural products[19]. As a consequence, much effort is currently directed to the efficient and stereocontrolled synthesis of such disaccharides and trisaccharides[21-24] Present synthetic methods for construction of such molecules, (described in Chapter 1, section 1.3.2) inspite of some stimulating approaches[128,156-158,174,191,194] however, leave a considerable margin for improvement in terms of, (i) formation of unstable glycosyl halide (classical glycosyl donor), ii) acidic reaction media iii) toxic reagent iv) efficiency v) generality and vi) stereoselectivity.

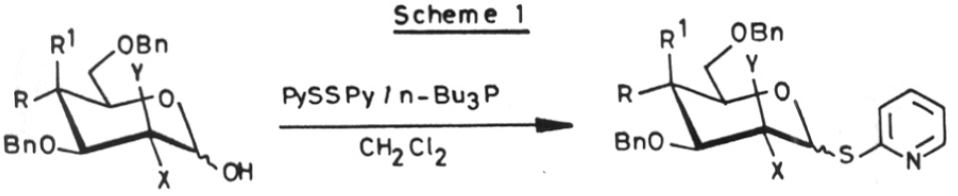
This chapter describes a new mild glycosidation methodology that utilizes stable, readily obtainable anomeric mixture (α and β) of 2-pyridyl thioglycosides as glycosyl donors with a non participating C-2 substituent and methyl iodide as an activator in presence of glycosyl acceptors to obtain α-linked saccharides[206].

## 3.2 Results and discussion:

### 3.2.1 Synthesis of 2-pyridyl thioglycosyl donors

Easily obtainable tetra-O-benzyl α-**D**-glucopyranose[213] (**1**) was transformed into 2-pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α/β-**D**-glucopyranoside (**2**, in α:β ratio 2:3, 85%)[135] (Chapter 2). Similarly per-O-benzylated 2-pyridyl thioglycopyranosides of **D**-galacto- (**4**) and D-manno- (**6**) configurations were prepared from their corresponding per-O-benzylated hexoses[213,214,215] **3** and **5** respectively in good yields (80-85%) (Scheme 1, Table 1).

**Scheme 1**



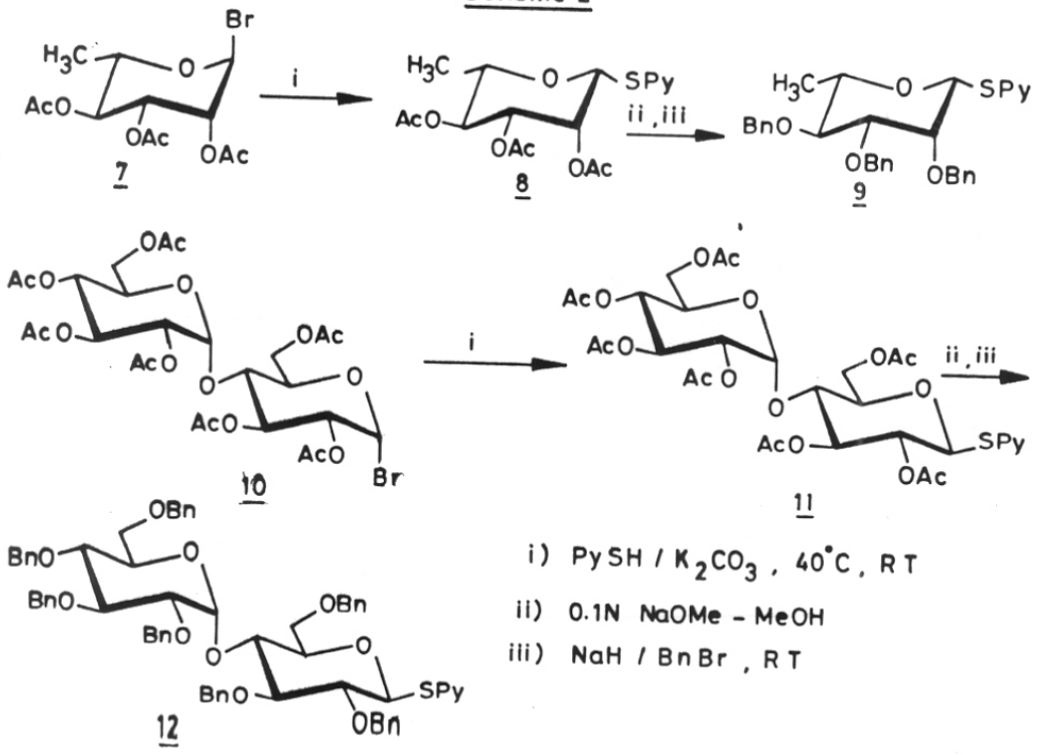

1 R = OBn, $R^1$ = H, X = OBn, Y = H (D-Gluco) 2

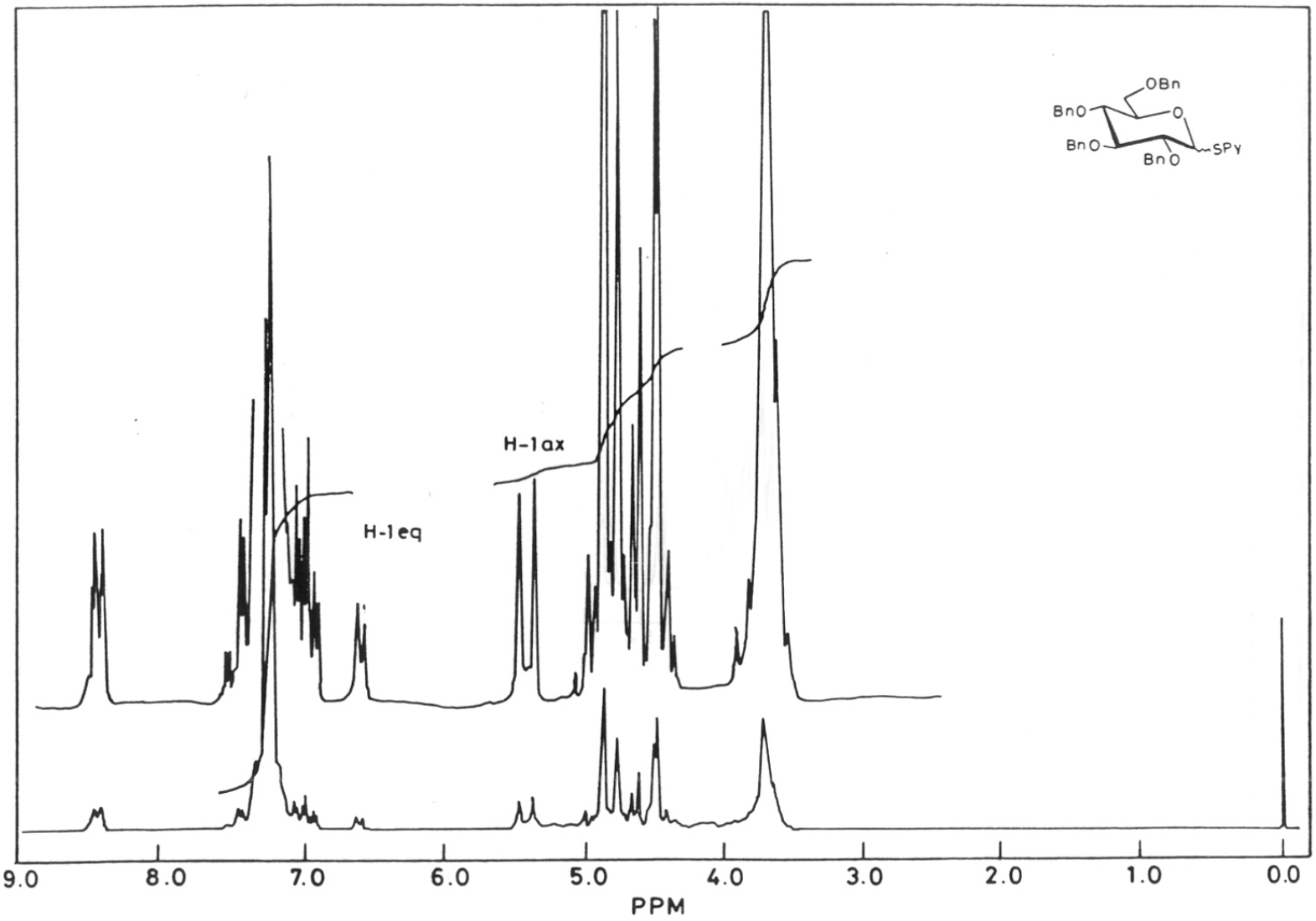
3 R = H, $R^1$ = OBn, X = OBn, Y = H (D-galacto) 4

5 R = OBn, R = H, X = H, Y = OBn (D-Manno) 6

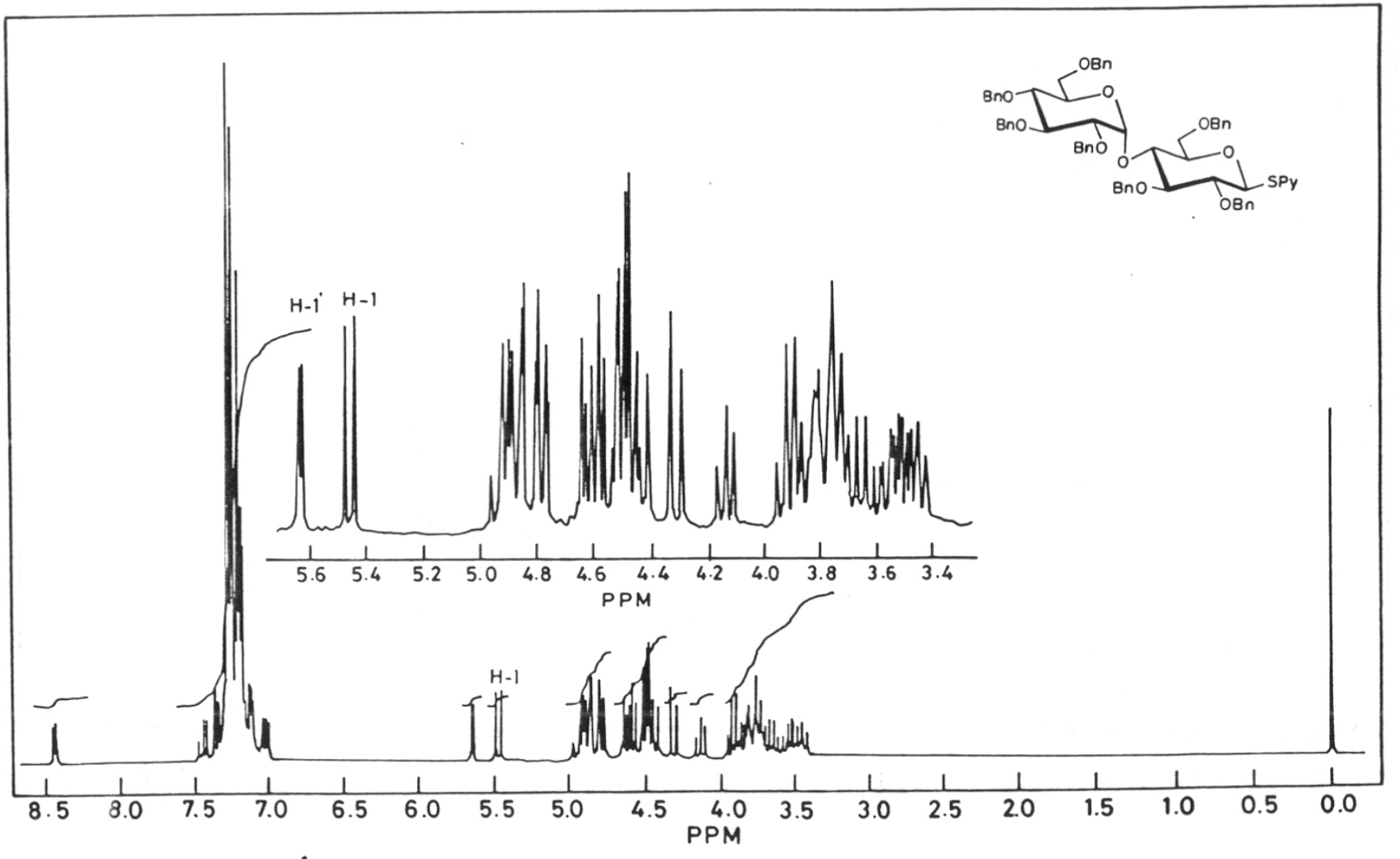
2-Pyridyl 2,3,4,6-tri-O-benzyl-β-L-rhamnopyranoside (**9**) and 2-pyridyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)-1-thio- β-D-glucopyranoside (**12**) were conveniently prepared in four steps (Scheme 2), starting from acetobromosugars (**7** and **10**) by reaction[137] with 2-mercaptopyridene/anhyd. $K_2CO_3$ (Chapter 2) followed by deacetylation and benzylation to obtain **9** and **12** in good yields (74-75% (Table 1).

**Scheme 2**

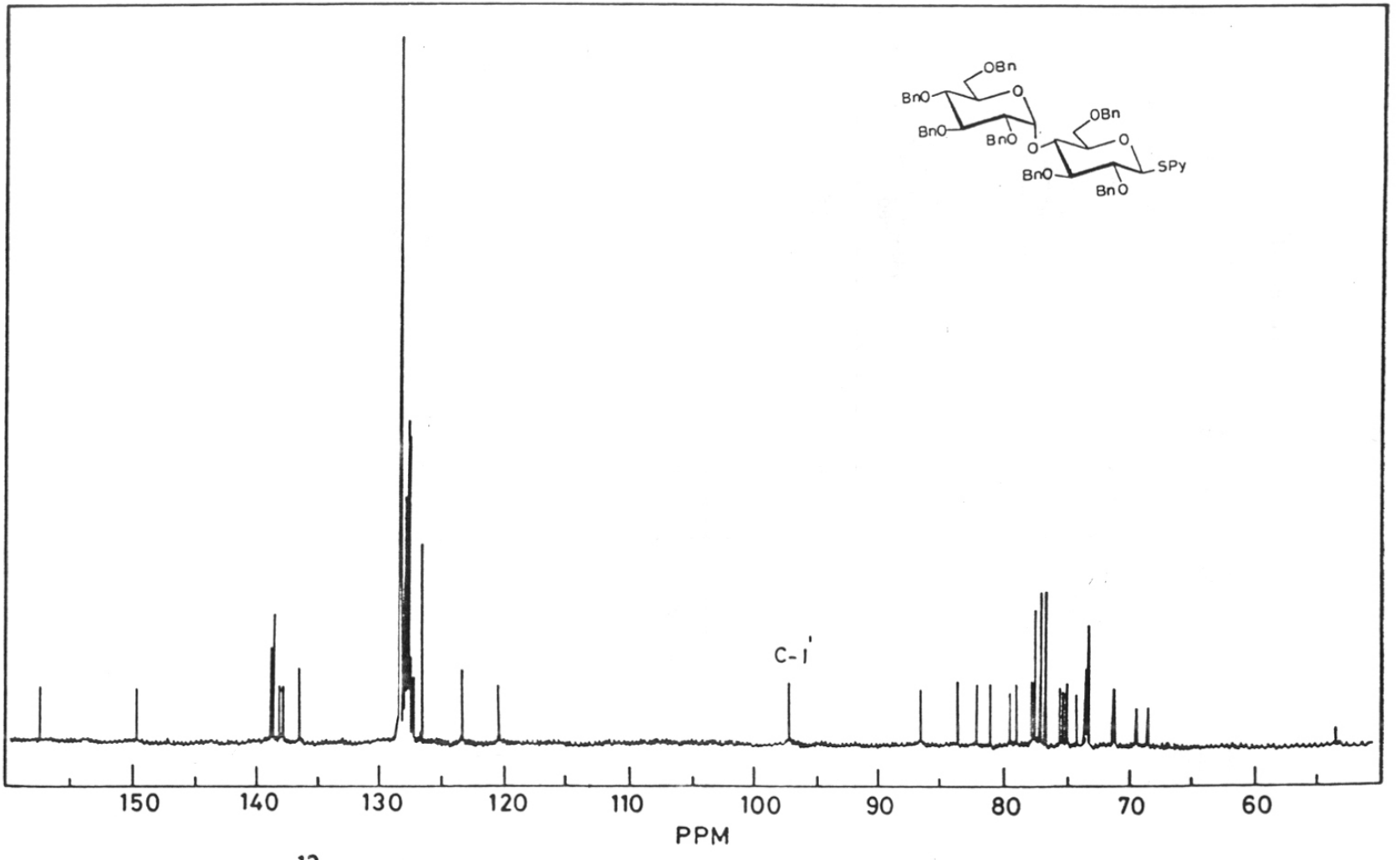





$^1$H NMR SPECTRUM OF COMPOUND 2 (90MHz, $CDCl_3$)



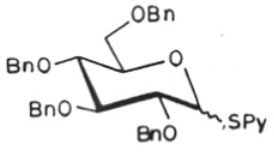
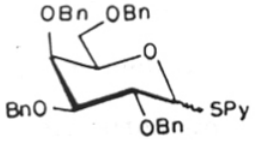
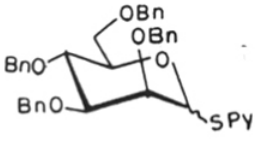
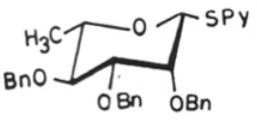
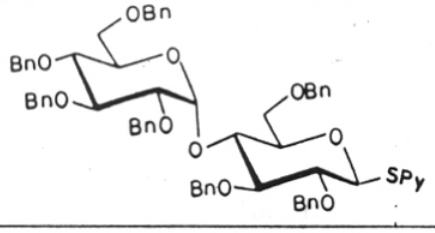

$^1$H NMR SPECTRUM OF COMPOUND 12 ( 300 MHz, $CDCl_3$ )




$^{13}C$ NMR SPECTRUM OF COMPOUND 12 (75MHz, $CDCl_3$)

**Table 1**

**$^1$H-nmr data of 2-pyridyl thioglycosides**

| Structure | Compound No. | Selected $^1$H-nmr data δ ppm, (J in Hz) $CDCl_3$ |
|---|---|---|
|  | 2 | 5.31 (9) H-1ax<br>6.62 (5) H-1eq |
|  | 4 | 5.26 (10) H-1ax<br>6.46 (5) H-1eq |
|  | 6 | 5.60 (0.5) H-1ax<br>6.44 (2) H-1eq |
|  | 9 | 5.66 (2) H-1 |
|  | 12 | 5.45 (10) H-1<br>5.63 (3.6) H-1' |

### 3.2.2 Synthesis of α-linked disaccharides

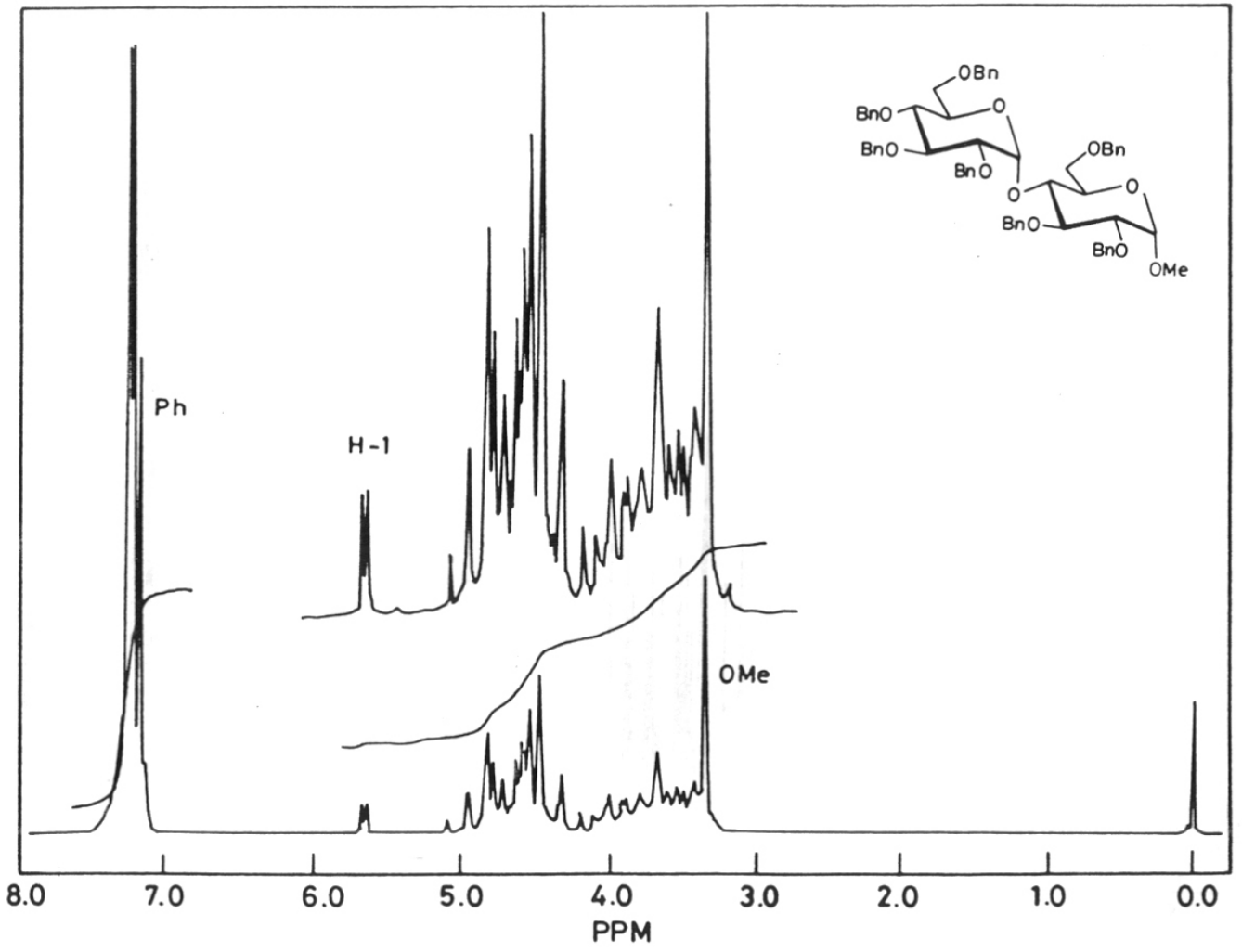
Application of the new glycosidation methodology for the synthesis of oligosaccharides was next taken up[206,241]. In order to study the generality of this glycosidation reaction (Chapter 2), methyl 2,3,6-tri-O-benzyl-α-D-glucopyranose[216] (**13**), which has been reported[156] to resist glycosidation under halide ion catalysed conditions, was considered as the glycosyl acceptor and was reacted with 2-pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α/β-D-glucopyranoside (**2**) at 50°C in dichloromethane (having 3% methyl iodide) in presence of molecular sieves 4Å for 62 h to yield after workup the α-linked disaccharide **14** in 82% yield as a syrup[206].

**Characterization of the α-linked disaccharides was done based on the following criteria.**

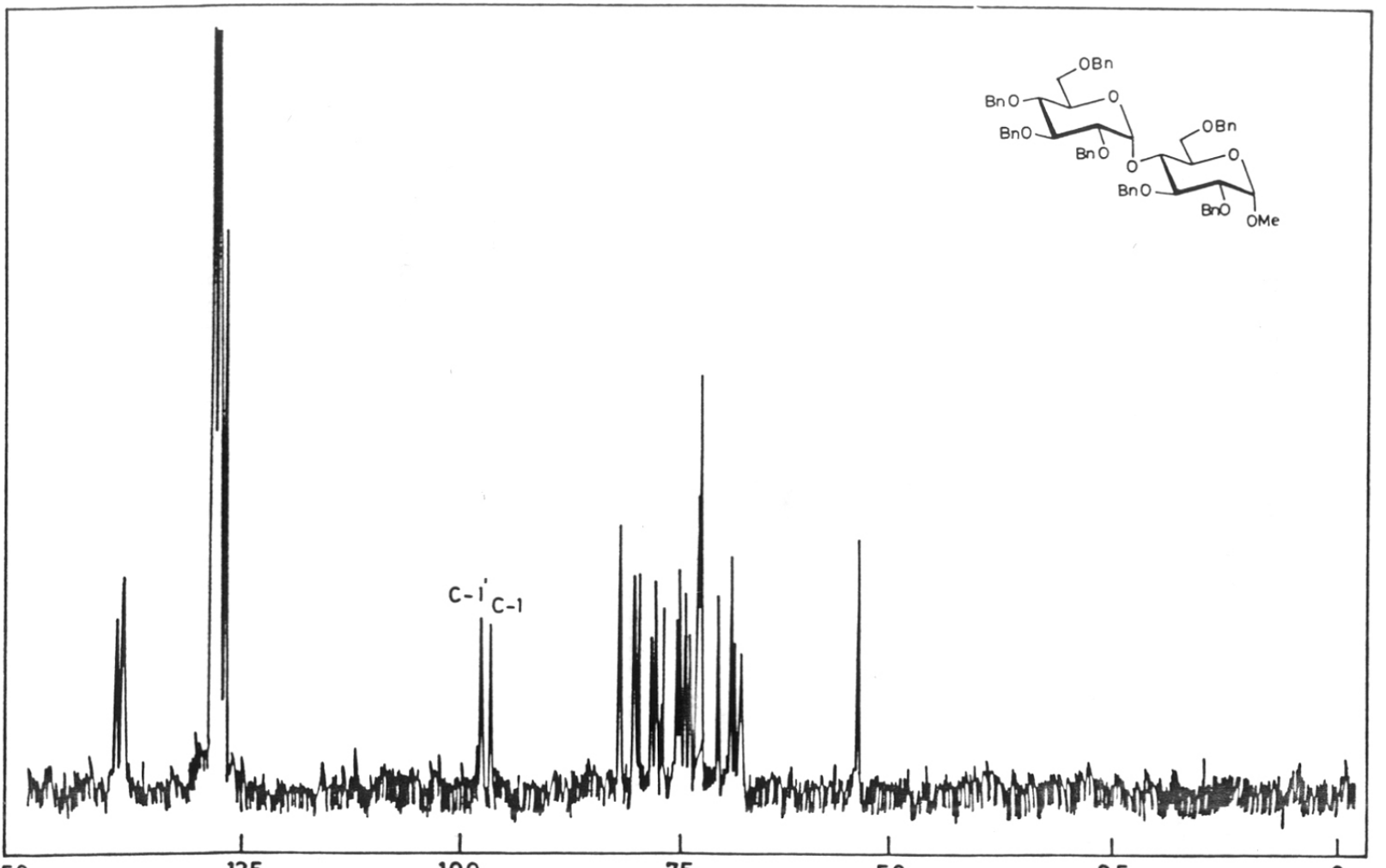
i) $^{1}$H-nmr: The newly formed glycosidic bond showed H-1' signal[217] with a coupling of ($J_{1,2}$) ca.5Hz, consistent with the cis-coupling, corresponding β-linked isomers exhibit a coupling of ($J_{1,2}$) ca. 9-11 Hz (trans)[218]. The relative chemical shift values are also characteristic of α and β-linkages.

ii) $^{13}$C-nmr: α-linked saccharides exhibit[219] in their $^{13}$C-nmr spectra, doublet C-1' at ca. δ 97-100, with characteristic coupling of $JC_1H$ = 168 Hz. The corresponding β-anomeric carbon appears at ca. δ 102-105 and shows[220] a coupling of β-$JC_1H$ = ~ 145 Hz.

iii) Optical rotation : α-linkerd (D-configuration) saccharides exhibit high positive rotations compared to their corresponding β-anomers[156]. The β-anomers exhibit low negative rotations[221].



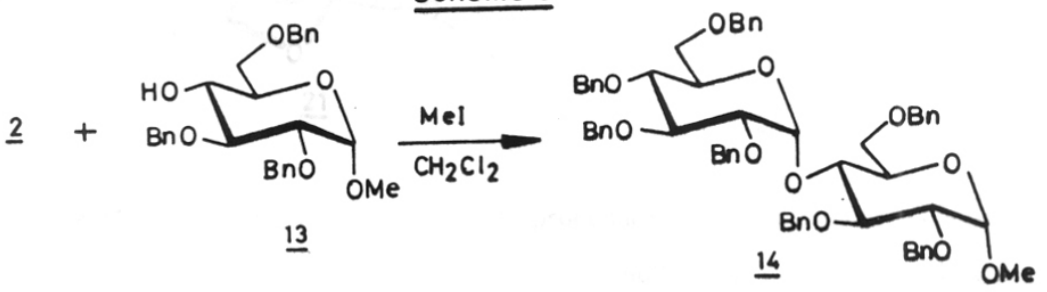

$^1$H NMR SPECTRUM OF COMPOUND 14 (90 MHz, $CDCl_3$)




$^{13}C$ NMR SPECTRUM OF COMPOUND 14 (22.3 MHz, $CDCl_3$)

The α-configuration at the newly formed, interglucosidic linkage in **14** was established by the $^{13}$C-nmr spectrum, which showed a signal for C-1[1] at δ 97.6, with $JC_1H$=168 Hz characteristic of α-linkage[12]. $^1$H-nmr spectrum of **14** showed, a singlet at δ 3.34 for methoxyl protons integrating for 3H, a doublet at δ 5.65 ($J_{1,2}$=4 Hz) for H-1 resonance characteristic of α-linkage. Compound **14** has optical rotation of $[\alpha]_D^{25}$ +48°C (c 1.0, $CHCl_3$) which is comparable with the reported value[157] (+48°).

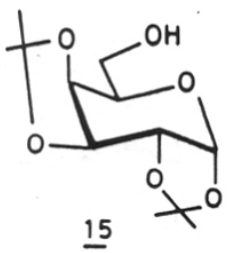
**Scheme 3**



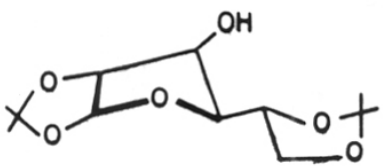

Synthesis of various di- and tri-saccharides was attempted to give this method the status of a general methodology.

Thus, several 2-pyridyl thioglycosides viz., 2-pyridyl 2,3,4,6-tetra-O-benzyl-1-thio – α /β -**D**-glucopyranoside (**2**), 2-pyridyl-2,3,4,6-tetra-O-benzyl-1-thio-α / β-**D**-galactopyranoside (**4**), 2-pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α/β-**D**-mannopyranoside (**6**) and 2-pyridyl 2,3,4-tri-O-benzyl-1-thio- β-**L**-rhamnopyranoside (**9**) were coupled with various glycosyl acceptors viz., 1,2:3,4-Di-O-isopropylidene- α -**D**-galactopyranoside (**15**) 1,2:5,6-Di-O-isopropylidene- α-**D**-glucofuranoside (**17**), 1,2:5,6-Di-O-isopropylidene-α-**D**-glucofuranoside (**21**) and methyl 2,3-isopropylidene-α -**L**-rhamnopyranoside (**26**) to obtain several α -linked disaccharides (2+15 = **16**; 2+17 = **18**; 4+15 = **19**; 4+17 = **20**; 4+21 = **22**; 6+15 = **23**; 9+15 = **24**; 9+26 = **27**).

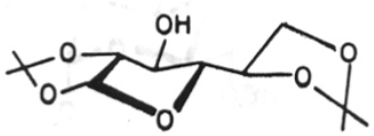
**Glycosyl acceptors**



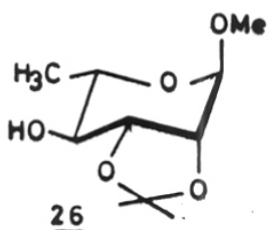
15



17



21



26

**3.2.2A Typical experimental procedure for saccharide coupling**

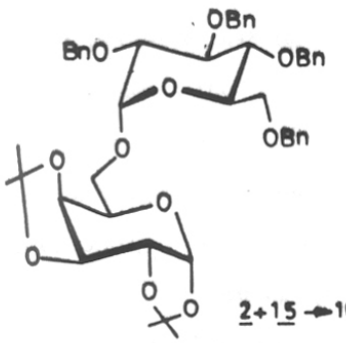
2-Pyridyl thioglycosyl donor (1.0 mmol) (α / β or β anomer) was reacted with the glycosyl acceptor (1.1 mmol) in dry dichloromethane (10 ml, having 3% methyl iodide) in presence of molecular sieves 4 Å (200 mg) at 50°C for 48-72 h. Workup and purification by silica gel column chromatography, gave the α-linked disaccharides in 56-87% yield (Table 2 and 3).

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-α-D-galactopyranoside (16)**

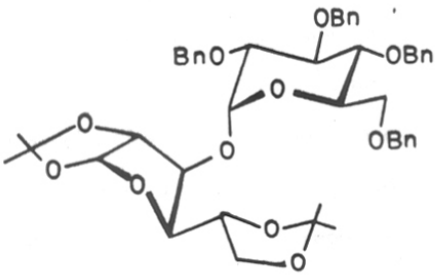
Reaction of 2-pyridyl thioglycosyl donor **2** with diacetone galactose[222] **15** afforded (Scheme 4) the corresponding α-linked disaccharide **16** in 87% yield as a syrup.

Compound **16** was characterized by the appearance of singlets at δ 1.31x2, 1.44 and 1.53 (12H) (Table 3) for the isopropylidene moiety and multiplets between δ 7.0-7.5 (25H) for the aromatic protons; appea-

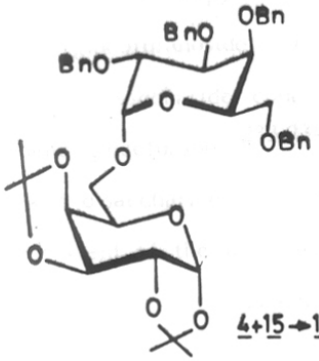
## Scheme 4



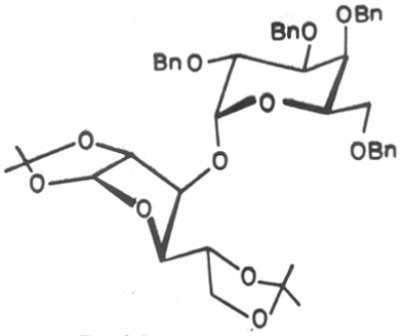
2+15→16



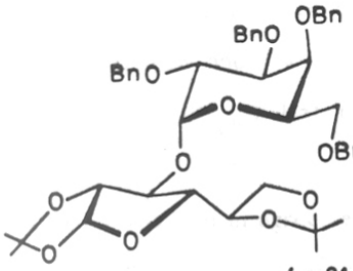
2+17→18



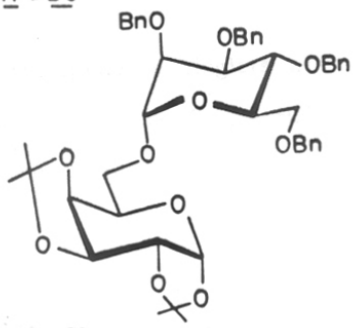
4+15→19



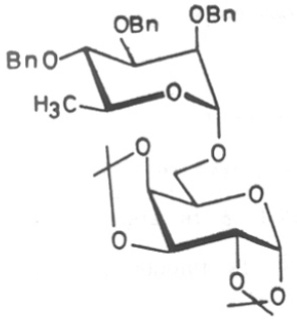
4+17→20



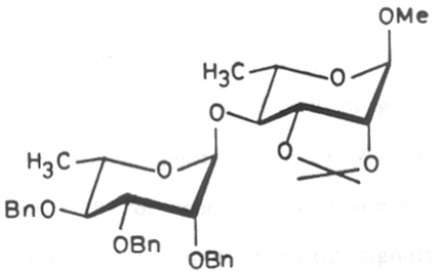
4+21→22



6+15→23



9+15→24



9+26→27

rance of H-1 at δ 5.48 (d, $J_{1,2}$=5 Hz) and finally the disappearance of pyridylthio signals at δ 7.0-8.5 indicated the formation of **16**. The $^{13}$C-nmr of **16** is also characteristic of α-linked sacchardies, by the appearance of C-1 and C-1' at δ 96.5 and 97.2 respectively. The positive optical rotation of $[\alpha]_D^{24}$ +10 (c 1.0, $CHCl_3$) is also in agreement with reported (+10.0) value[156] confirming the structure of **16**.

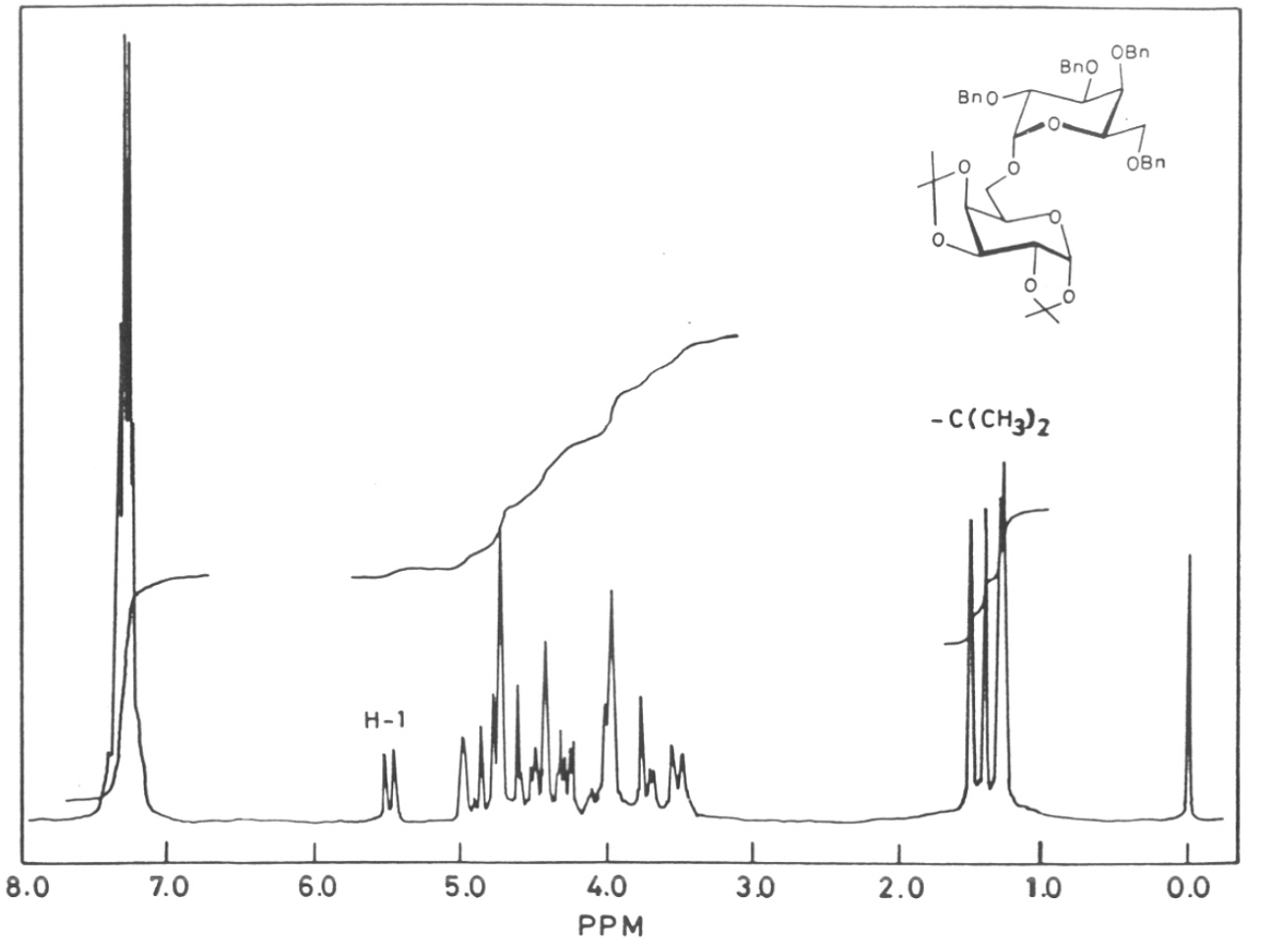
**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-α-D-glucofuranoside (18)**

The saccharide coupling of **2** with acceptor 1,2:5,6-Di-O-isopropylidene glucofuranose[233,234] (**17**) resulted in the isolation of a (1 → 3) α-linked disaccharide **18** in 56% yield as a solid (mp 91°C) (Scheme 4). Lower yield of the disaccharide was due to decomposition of **18** during its purification on column chromatography ($SiO_2$). Compound **18** was characterized as follows.

The $^1$H-nmr showed the presence of isopropylidene singlets at δ 1.17x2, 1.38 and 1.47 (12 H) and two doublets at δ 5.24 ($J_{1,2}$=4 Hz) and δ 5.86 ($J_{1',2'}$=4 Hz) for H-1' and H-1 respectively confirming the formation of α-linked disaccharide. Optical rotation of $[\alpha]_D^{24}$ +46° (c 2.0, $CHCl_3$) was also in agreement with the reported[157] value (+46°).

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-α-D-galactopyranoside (19)**

Coupling of **4** with **15** under methyl iodide activated pyridyl thioglycosidation method gave the (1 → 6)-α-linked disaccharide **19** in good yield (81%) as a syrup (Scheme 4). The $^1$H-nmr spectrum of **19** showed four singlets at δ 1.26, 1.28, 1.4 and 1.48 for isopropylidene methyls (12H), a doublet at δ 5.48 ($J_{1,2}$=5 Hz) for H-1 and aromatic signals




$^1$H NMR SPECTRUM OF COMPOUND 19 (90MHz, $CDCl_3$)

(20H) are indicative of formation of α-linked disaccharide. Optical rotation of $[\alpha]_D^{22}$ +5.1° (c 0.9, $CHCl_3$) was observed for **19**. Where as the reported value[234] for **19** is (+2°). The $^{13}$C-nmr showed characteristic doublet signals at δ 96.2 and 97.5 for C-1' and C-1 respectively, and two singlets at δ 108.4 and 109.1 for isopropylidene carbons, consistent with the α-linked disaccharide.

**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)-α-D-glucofuranoside (20)**

The (1 → 3) α-linked disaccharide **20** was synthesized in 62% yield (syrup) by the saccharide coupling of **4** with **17** (Scheme 4). The $^1$H-nmr spectrum showed, H-1' and H-1 resonances at δ 5.28 ($J_{1',2'}$=4 Hz) and 5.72 ($J_{1,2}$=4 Hz) respectively, which is consistent with 1,2-cis-interglycoside linkage. Compound **20** was further characterized by comparision of its optical rotation of $[\alpha]_D^{20}$ +32.7° (c 1.1, $CHCl_3$) with the reported[157] value (+33°).

**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)-α-D-galactofuranoside (22)**

(1 → 3) α-Linked disaccharide (**22**) was obtained as a crystalline solid (m.p. 120) from the coupling of glycosyl donor **4** (Scheme 4) with the acceptor[225] **21**. The $^1$H-nmr spectrum of **22** showed four singlets at δ 1.24, 1.31, 1.41 and 1.51 (12 H) for isopropylidene methyls, a doublet at δ 5.8 ($J_{1,2}$=4Hz) for H-1 resonance and a multiplet at δ 7.0-7.5 integrated for 20 aromatic protons. The optical rotation of $[\alpha]_D^{25}$ +37° (c 0.84, $CHCl_3$) was also in agreement with the reported[156] value (+36.8). Melting point 120°C is in agreement with the reported[156] value (120-121°C). Thus confirming the structure **22**.

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl- α -D-mannopyranosyl)-α-D-galactopyranoside (23)**

The glycosyl coupling of per-O-benzylated 2-pyridyl thio mannopyranoside **6** with the glycosyl acceptor **15** afforded 55 h) in 60% yield (1 → 6) α-linked disaccharide **23** as a syrup (Scheme 4). The $^{1}$H-nmr of **23** showed, H-1 resonance at δ 5.47 ($J_{1,2}$=4 Hz) and δ 1.30, 1.42, 1.48 (3s, 12H) for isopropylidene methyls and aromatic protons (20H) at δ 7.0-7.5). The optical rotation $[\alpha]_D$ +39° (c 1.0, $CHCl_3$) was also in consistent with the assigned ∝-configuration.

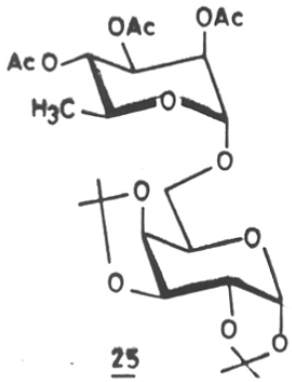
**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-D-galactopyranoside (24)**

As expected per-O-benzylated 2-pyridyl thiorhamnopyranoside also exhibited α-selectivity in this glycosidations by this methodology. Thus α-linked rhamnobioses, that earlier been prepared by neighbouring group assisted orthoester procedure[235] were obtained by this pyridyl-thioglycosidation methodology.

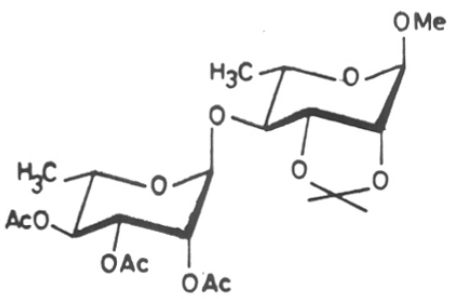
Saccharide coupling of **9** with compound **15** afforded (48 h) **24** in 78% yield as a syrup (Scheme 4). The $^{1}$H-nmr spectrum of **24** showed characteristic isopropylidene methyl singlets at δ 1.28, 1.44 and 1.51 (12H) and C-6' (3H) methyl doublet at δ 1.33 (J=6 Hz). Appearance of H-1 as a doublet with $J_{1,2}$=5 Hz at δ 5.32, multiplet for 15 protons in the region δ 7.0-7.6 for aromatic protons indicated the formation of the disaccharide **24.** $^{13}$C-nmr spectrum showed signals at δ96.5 and 98.3 for C-1 and C-1' respectively confirming the α -linkage at the newly formed interglycosidic bond. Debenzylation (10% Pd-C/$H_2$) and subsequent acetylation afforded compound **25**, which is identical with the reported compound [98], in all respects. $[\alpha]_D$ -89° (c 1.8, $CHCl_3$), m.p.114-115°C.

**Methyl 2,3-O-isopropylidene-4-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (27)**

Similarly, saccharide coupling of compound **9** with methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside[226] **(26)** using pyridylthio methodology, afforded (48 h) the (1 → 4) α-linked disaccharide **27** in 72% yield as a syrup (Scheme 4). The $^{1}$H-nmr of **27** showed H-1 and H-1' resonance at δ 5.28 and δ 5.35 respectively, as two broad singlets. $^{13}$C-nmr of **27** showed, signals at δ 97.6 and δ 98.4 for C-1 and C-1' respectively, consistent with the α -linkage. Optical rotation of $[\alpha]_D^{25}$ -23.9° (c 1.0, $CHCl_3$) is also in agreement with the assigned structure. Further structure confirmation was done by the catalytic hydrogenation of **27** and followed by acetylation to give compound **28**, which was identical in all respects with the physical data optical rotation) reported[98] for **28**. $[\alpha]_D^{20}$ -69° (c 1.5, $CHCl_3$).




**25**




**28**

### 3.2.3 Synthesis of α-linked trisaccharides

**O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-(1 → 6)-(2,3-di-O-acetyl-α-D-glucopyranosyl-(1 → 4)-1,2,3,6-tetra-O-acetyl-β -D-glucopyranoside (panose derivative (30)[227]**

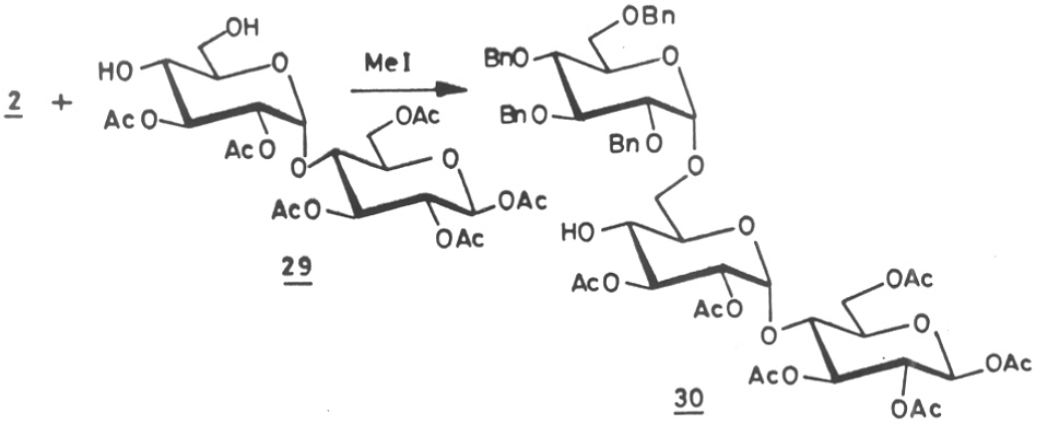
The success of methyl iodide activated 2-pyridylthio- methodology

was demonstrated by synthesis in good yield of α-linked trisaccharide **30**, a panose derivative (Scheme 5). The glycosyl acceptor **29** (1,2,3,6-tetra-O-acetyl-4-O-(2,3-di-O-acetyl- α -D-glucopyranose) was essentially synthesized from the known procedure[227]. The saccharide coupling reaction of **2** (0.62 mmol) with **29** (0.78 mmol) in dry dichloromethane (10 ml, having 3% MeI) in presence of molecular sieves -4Å (300 mg) at 50°C for 48 h afforded after workup and column purification, exclusively the (1 → 6)-α-linked trisaccharide **30**, isolated as a crystalline solid in 62% yield[206], m.p. 150-151°C[227] recrystallized from 30% pet.ether in dry diethyl ether.

The selective formation of (1 → 6) linked saccharide **30** is probably due to the higher reactivity of primary hydroxyl (**6**-OH) over the secondary one (**4**-OH)[227].

Compound **30** in the $^{1}$H-nmr spectrum showed six singlets ca δ 1.98-2,06 (18 H) for 6xOAc, a doublet at ca δ 5.71 ($J_{1,2}$=8 Hz) and a multiplet in aromatic region δ 7.0-7.3 (20H). In the $^{13}$C-nmr spectrum signals at δ 91.6 (C-1), 96.3 (C-1'), 98.2 (C-1'') are in accordance with

**Scheme 5**

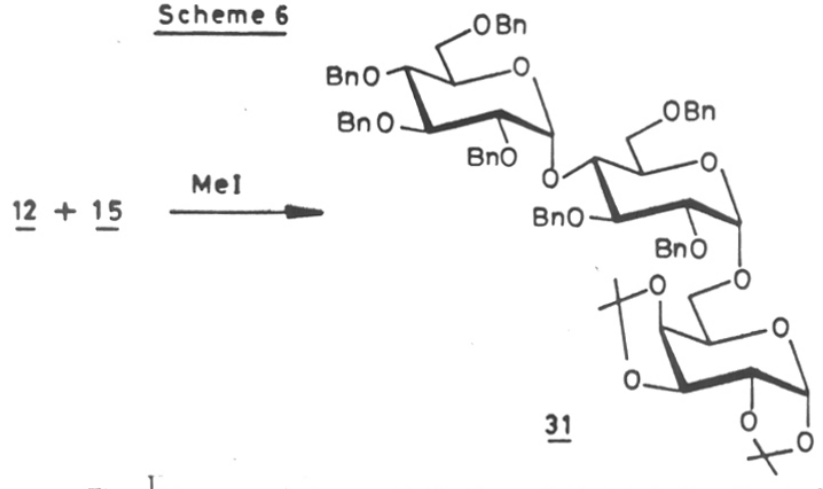


the assigned structure, m.p. (152-153°C) and optical rotation, $[\alpha]_D^{25}$ +51.3° (c 2.0, $CHCl_3$) also are in agreement with the reported data for **30**[227] (+51.3°).

**Synthesis of O-(2,3,4,6-O-benzyl-α -D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl- α-D-glucopyranosyl)-(1 → 6)-1,2:3,4-di-O-isopropylidene-α-D-galacto-pyranoside (31)**

The generality of this new glycosidation method was also illustrated by use of even a disaccharide donor such as 2-pyridyl 2,3,4-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl- α-D-glucopyranosyl)-1-thio-β -D-gluco-pyranoside (**12**) for coupling. Thus the reaction of **12** with diacetone galactose[222] **15** afforded (52 h), the α -linked trisaccharide **31** in 65% yield, as a syrup (Scheme 6).

**Scheme 6**



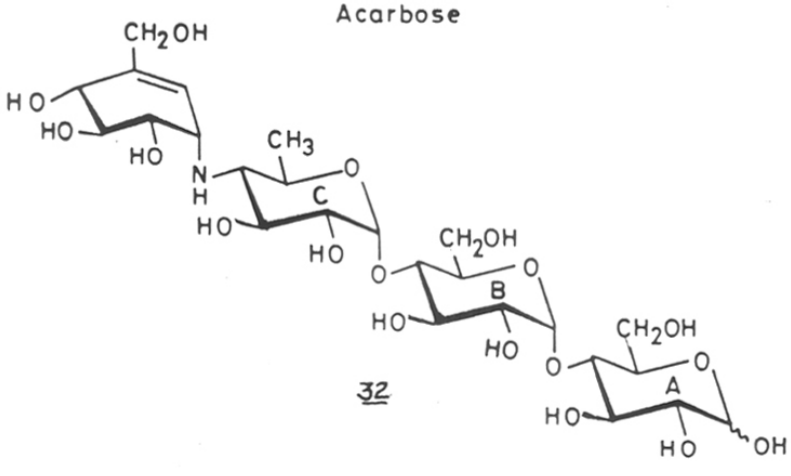

The $^1$H-nmr spectrum of **31** showed doublet signals at δ 5.5 (J=4Hz) and δ 5.67 (J=3.6Hz) for H-1 and H-1" respectively. The H-1' signal was overlaped with the benzylic proton signals in the region of δ 3.3 to δ 5.1. The characteristic isopropylidene signals appeared at δ 1.3, 1.45, 1.57 and 1.6 as four singlets (12H). The $^{13}$C-nmr spectrum of **31** showed signals at 96.1,96.4 & 96.8 for C-1, C-1' and C-1" which is

in agreement with the assigned structure compound **31** showed optical roation of +25.2° (c 1.0, $CHCl_3$).

**Synthesis of trisaccharide moiety (ABC) of Acarbose (37)**

The pseudotetrasaccharide acarbose (**32**) produced by Actinomycetals strains, is a potent inhibitor of intestinal α-D-glucosidase and saccharases[61] *in vitro*[60], and is being used clinically as an effective oral antidiabetic agent. Considerable interest has therefore been shown in the biochemistry of this class of inhibitors[61], which lead to extensive synthetic studies[62,63,228,229,230]. The trisaccharide moiety in acarbose is made of α-Glcp-6-deoxy (1→4)-α-Glcp-(1→4)-β-Glcp.

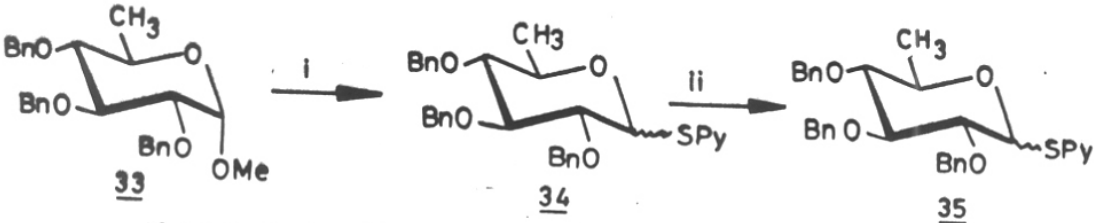
Acarbose



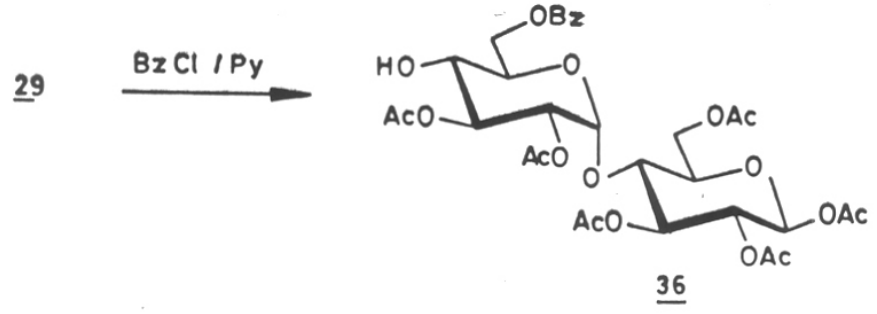

**32**

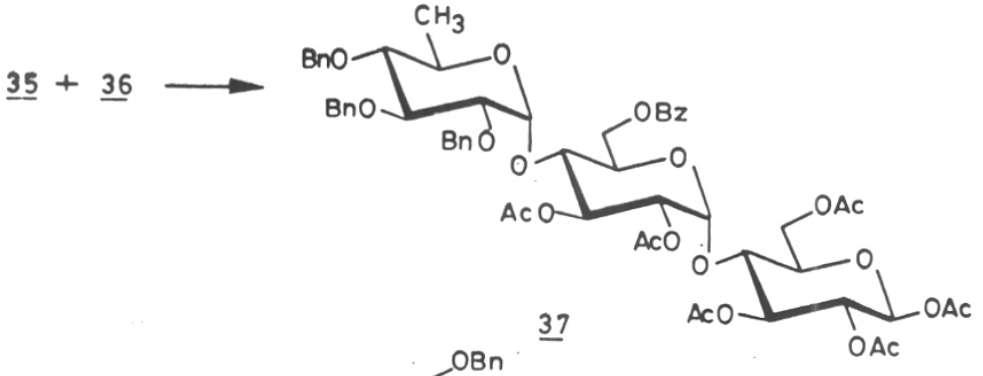
Synthesis of the trisaccharide moiety (ABC) was done by coupling 2-pyridyl 6-deoxy-2,3,4-tri-O-benzyl-1-thio- α/β-glucopyranoside **35** with the glycosyl acceptor **36.** The required α-linked trisaccharide **37** was isolated (96 h) only 12% yield as a solid (m.p. 65-67°C). The low yield is attributed to the steric crowding of the 4-hydroxy group by the bulky protection such as benzoyl ester on C-6 hydroxyl of **36.**

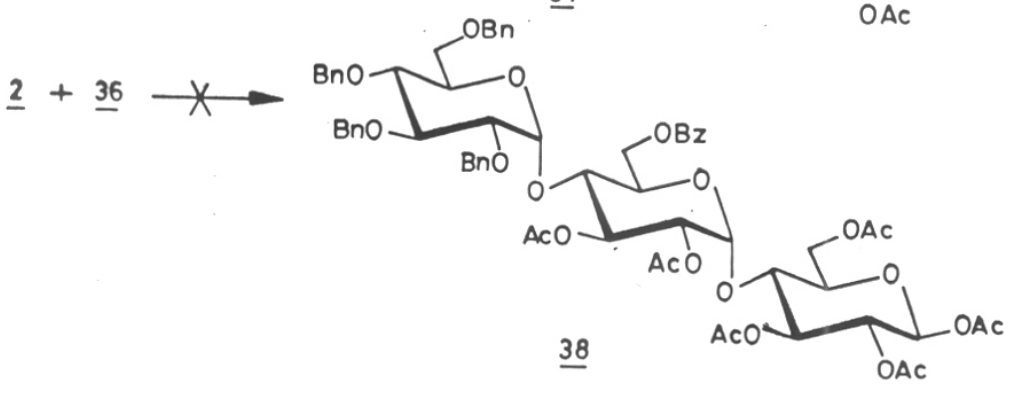
## Scheme 7




i) Hydrolysis ii) PySSPy / n-$Bu_3P$ / $CH_2Cl_2$



A similar observation was made when **2** was reacted with **36**, where 6-hydroxyl was protected as benzoyl ester, to obtain the trisaccharide **38**. This reaction (48 h) did not give any isolable compound **38**, indicating the importance of selecting suitable protecting groups where C-4-hydroxyl reactivity is concerned in glycoside coupling.

The $^{1}$H-nmr of **37** showed signals at $\delta$ 1.16 (d 3H, $J_{5,6}$=6.2 Hz) for three protons of C-6 methyl group and six singlets for acetyl groups in the region of $\delta$ 1.92-2.16. The positive optical rotation of +62.5° (c 1.0, $CHCl_3$) is also in agreement with the assigned structure for **37**.

Compound **35** was synthesized from **33** by known methods[231,232,215] **35** was characterized by $^{1}$H-nmr from the appearence of doublets at $\delta$ 5.44 ($J_{1,2}$=10 Hz, H-1,$\beta$ -anomer) and $\delta$ 6.58 ($J_{1,2}$=5 Hz, H-1,$\alpha$-anomer) as anomeric mixture 3/2.

The glycosyl acceptor **36**[233] was synthesized by selective protection of the diol **29** by reaction with BzCl/Py.

Table 2

Physical data of α-linked saccharides

| Entry | Glycosyl | | Protected disaccharide (m.p.) | Yield % (time) | $[\alpha]_D$ Deg.[b] | |
|---|---|---|---|---|---|---|
| | Donor | Acceptor | | | Observed | Lit. (Ref.) |
| i. | 2 | 13 | 14, m | 82 (62 h) | +48 e,h | +48 (157) |
| ii. | 2 | 15 | 16, m | 87 (72 h) | +10 c,i | +10.1 (156) |
| iii. | 2 | 17 | 18 (91°C)o | 56 (72 h) | +46 c,i | +46 (157) |
| iv. | 4 | 15 | 19, m | 81 (48 h) | +51 f,k | +2 (234) |
| v. | 4 | 17 | 20, m | 62 (72 h) | +32.7 d,l | +33 (157) |
| vi. | 4 | 21 | 22 (120°C),p | 67 (72 h) | +37 g,j | +36.8 (156) |
| vii. | 6 | 15 | 23, m | 60 (55 h) | +39 e,j | a |
| viii. | 9 | 15 | 24, m | 78 (48 h) | -47 e,h | q |
| ix. | 9 | 26 | 27, m | 72 (48 h) | -23.9 e,h | q |
| x. | 2 | 29 | 30 (150-151°C) n | 62 (48 h) | +51.3 e,j | +53 (227) |
| xi. | 12 | 15 | 31, m | 65 (52 h) | +25.2 e,j | a |
| xii. | 35 | 36 | 37 | 12 (96 h) | +62.5 e,j | a |

[a] not known in the literature.

[b] In chloroform, $c_{c2.0}$ [d] $c_{1.1}$ [e] $c_{1.0}$ [f] $c_{0.9}$ [g] $_{0.8}$ [h] $_{27}$ [i] $_{24}$ [j] $_{25}$ [k] $_{22}$ [l] $_{20}$ [m] syrup [n] Lit.m.p.152-153°C

[o] Lit.m.p. 90-91°C [p] Lit.m.p. 120-121°C [q] on debenzylation (Pd-C/$H_2$) acetylation identical with the reported compounds (Ref. 58).

Table 3

Selected $^{1}H$- and $^{13}C$-nmr data of α-linked di- and trisaccharides

| Compound No. | $^{1}H$-nmr, δ (J in Hz) | | | $^{13}C$-nmr, δ ppm | | |
|---|---|---|---|---|---|---|
| | H-1 | H-1' | H-1" | C-1 | C-1' | C-1" |
| **14** | 5.65 (4) | a | - | 96.5 | 97.6 | - |
| **16** | 5.48 (5) | a | - | 96.5 | 97.2 | - |
| **18** | 5.86 (4) | 5.24 (4) | - | 104.9 | 97.7 | - |
| **19** | 5.48 (5) | a | - | 96.2 | 97.5 | - |
| **20** | 5.72 (4) | 5.28 (4) | - | 105.2 | 98.9 | - |
| **22** | 5.8 (4) | a | - | - | - | - |
| **23** | 5.47 (4.5) | a | - | - | - | - |
| **24** | 5.32 (4.5) | a | - | 96.4 | 98.3 | - |
| **27** | 5.35 (brs) | 5.28 (brs) | - | 97.6 | 98.4 | - |
| **30** | 5.71 (8) | a | - | 91.6 | 96.3 | 98.2 |
| **31** | 5.67 (3.6) | a | 5.5 (4) | 96.2 | 96.2 | 96.1 |

## 3.3 EXPERIMENTAL

### 3.3.1 Synthesis of 2-pyridyl thioglycosyl donors

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α/β-D-glucopyranosides (2)**

Compound **2** was synthesized from methyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside on hydrolysis[213] followed by reaction[135] with PySSPy/ $n\text{-}Bu_3P/CH_2Cl_2$ and characterized from its $^1$H-nmr spectrum (see chapter 2, experimental, compound **9**)

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio- α / β -D-galactopyranosides (4)**

Synthesized from methyl 2,3,4,6-tetra-O-benzyl- α-D-galactopyranoside on hydrolysis followed by reaction with PySSPy/ $n\text{-}Bu_3P/CH_2Cl_2$ and characterized from its $^1$H-nmr spectrum (see chapter 2, experimental compound **14**).

**2-Pyridyl 2,3,4,6-tetra-O-benzyl-1-thio-α,β-D-mannopyranosides (6)**

Hydrolysis (2.5.1B) of methyl 2,3,4,6-tetra-O-benzyl- α-D-mannopyranoside (2.78 g, 5 mmol) gave 2,3,4,6-tetra-O-benzyl α / β-D-mannopyranoside (**5**)[213] (1.7 g, 64%). Compound **5** (1 g, 1.8 mmol) was reacted with PySSPy (0.43 g, 1.98 mmol) in dichloromethane (10 ml) and $n\text{-}Bu_3P$ (0.53 g, 2.15 mmol) to give compound **6** (0.98 g, 82%) as a syrup (α: β 1:1 from $^1$H-nmr).

$^1$H-nmr (90 MHz): 3.55-5.0 (m, 14H), 5.6 (bs, 1/2 H, H-1, α -compound) 6.44 (d, 1/2 H, J=2, H-1 β-compound), 7.0-8.5 (m, 24H, Ph and SPy).

Analysis calcd. for $C_{39}H_{39}NO_5S$: C, 73.91; H, 6.20. Found: C, 74.01; H, 6.38%.

**2-Pyridyl 2,3,4-tri-O-acetyl-1-thio-β-L-rhamnopyranoside (8)**

To a solution of 2-mercaptopyridine (1.26 g, 11.4 mmol) in dry acetone (25 ml) was added anhy. $K_2CO_3$ (1.3 g, 13.75 mmol). Then

α-acetobromorhamnose[243] (**7**; 3.1 g, 8.78 mmol) in dry toluene (10 ml) was added slowly and stirred for 2 h at 40°C. After workup and purification (column chromatography, $SiO_2$, pet.ether:ethyl acetate, 2:1) yielded **8** (2.5 g, 75%) as a syrup.

$^1$H-nmr (90 MHz): 1.27 (d, 3H, J=6.2, $CH_3$), 1.97, 2.04, 2.20 (3s, 9H, 3xOAc), 3.70-5.65 (m, 4H, H-2,3,4,5), 6.07 (d, 1H, J=1, H-1), 7.0-8.5 (m, 4H, SPy).

Analysis calcd. for $C_{17}H_{21}NO_7S$: C, 53.25; H, 5.52. Found: C, 53.15; H, 5.48%.

**2-Pyridyl 2,3,4-tri-O-benzyl-1-thio-β-L-rhamnopyranoside (9)**

Compound **8** (2.5 g, 6.78 mmol) was deacetylated [cat.NaOMe-methanol (20 ml)] and neutralized with IR 120 $H^+$ resin, to afford 2-pyridy-1-thio-β-L-rhamnopyranoside (1.74 g, 99%), which was dried and benzylated [1.74 g, 6.77 mmol; NaH (0.57 g, 23.7 mmol), BnBr (2.8 ml, 23.7 mmol), DMF (5 ml)] to afford the syrupy **9** (2.72 g, 75%) after purification by column chromatography (pet. ether:ethyl acetate 8:1.5).

$[\alpha]_D^{27}$ +17.69° (c 1.21, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.33 (d, 3H, J=6.2, $CH_3$), 3.11-5.28 (m, 10H, H-2,3,4,5, 3x-$CH_2$-benzylic), 5.66 (d, 1H, J=2, H-1), 7.0-8.66 (m, 19H, Ph and SPy).

$^{13}$C-nmr (22.3 MHz): 18.3 (d, C-6), 72.6-82.9 (3t, 4d, C-2,3,4,5, benzylic), 84.3 (d, C-1), 120.4, 123.1, 136.5, 149.6, 157.5 (C-2",3",4",5",6"-SPy), 127-128.5, 138.8-139.5 (aromatic).

Analysis calcd. for $C_{32}H_{33}NO_4S$: C, 72.84; H, 6.3. Found: C, 72.79; H, 6.23%.

**2-Pyridyl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl)-1-thio-β -D-glucopyranoside (11)**

To a stirred solution of 2-mercaptopyridine (0.78 g, 7.07 mmol)

and $K_2CO_3$ (0.79 g, 8.1 mmol) in acetone (20 ml) at room temperature was added slowly α-acetobromomaltose 10[243] (3.8 g, 5.4 mmol) in toluene (15 ml). The reaction mixture was further stirred at 40°C for 2 h. The solution was processed as described for the preparation of **8** to give **11** (2.9 g, 74%) as a crystalline yellow solid.

$[\alpha]_D^{27}$ +50.5° (c 1.0, $CHCl_3$); m.p.: 110-113°C.

$^1$H-nmr (300 MHz): 1.99, 2.05, 2.04, 2.06, 2.07, 2.1 (6s, 21H, 7xOAc), 3.8-5.5 (m, 13H), 5.85 (d, 1H, J=10.2, H-1), 7.0-8.5 (m, 4H, SPy).

**Selected $^{13}$C-nmr data (75 MHz):** 80.9 (C-1), 95.3 (C-1').

Analysis Calcd. for $C_{31}H_{39}NO_{17}S$: C, 51.02; H, 5.39. Found: C, 51.17; H, 5.17%.

**2-Pyridyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (12)**

Treatment of **11** (2.75 g, 15 ml MeOH) with cat.NaOMe-MeOH (5 ml) at 40°C for 1 h provided 2-pyridyl-4-O-(α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (1.5 g, 93%) which was dried and benzylated (1.3 g, 3.03 mmol) [NaH (0.73 g, 30.3 mmol) DMF (5 ml) and BnBr (2.9 ml, 24.2 mmol)] at room temperature for 1 h, to give the per-O-benzylated derivative **12** as a colourless syrup (2.0 g, 61%).

$[\alpha]_D^{25}$ +43° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 3.3-5.0 (m, 26H), 5.45 (d, 1H, J=10, H-1), 5.63 (d, 1H, J=3.6, H-1'), 6.6-8.5 (m, 39H, Ph and SPy).

**Selected $^{13}$C-nmr data (75 MHz):** 97.1 (C-1'), 120.4-157.3 (Ph and SPy).

Analysis calcd. for $C_{66}H_{67}NO_{10}S$: C, 74.34; H, 6.33. Found: C, 74.17; H, 6.28%.

### 3.3.2 Synthesis of glycosyl acceptors

The following glycosyl acceptors were made according to the literature methods and were characterized accordingly.

**Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (13)**[216]

$[\alpha]_D^{23}$ +11.9° (c 2.67, $CHCl_3$).

**1,2,3,4-Di-O-isopropylidene-α-D-galactopyranoside (15)**[222]

b.p. 131-135° (0.8 mm) $[\alpha]_D^{25}$ -55 (c 3.6, $CHCl_3$).

**1,2:5,6-Di-O-isopropylidene-α-D-glucofuranoside (17)**[223,224]

m.p.:111-113°C $[\alpha]_D^{20}$ -19.7° ($CHCl_3$).

**1,2:5,6-Di-O-isopropylidene-α-D-galactofuranoside (21)**[225]

m.p.: 97-98°C $[\alpha]_D^{25}$ -37.8° (c 1.0; $CHCl_3$).

**Methyl 2,3-O-isopropylidene-α-L-rhamnopyranoside (27)**[226]

$[\alpha]_D^{27}$ -16.4° (c 3.1, acetone).

**1,2,3,6-Tetra-O-acetyl-4-O-(2,3-di-O-acetyl- α -D-glucopyranosyl)-β -D-glucopyranoside (29)**[227]

m.p.: 205-206° $[\alpha]_D^{20}$ +53.1° (c 2.1, $CHCl_3$).

### 3.3.3 Synthesis of α-linked disaccharides

**Methyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α -D-glucopyranosyl)-α-D-glucopyranoside (14)**[157]

The saccharide coupling (3.2.2A) of **2** (0.41 g, 0.66 mmol, α : β/ 2:3) with **13** (0.36 g, 0.78 mmol) in dry dichloromethane (5 ml, having 3% methyl iodide) in presence of molecular sieves -4Å (200 mg) at 50°C for 62 h, gave compound **14** (0.56 g, 82%) as a syrup, after workup and column chromatographic purification.

$[\ ]_D^{25}$ +48° (c 1.05, $CHCl_3$).

$^1$H-nmr )90 MHz): 3.34 (s, 3H, OMe), 3.38-5.0 (m, 27H), 5.65 (d, 1H, J=4, H-1), 7.0-7.5 (m, 35H, Ph).

$^{13}$C-nmr (22.3 MHz, ppm): 54.9 ($O\underline{C}H_3$), 68.4-81.8 (17C, carbons of hexoses and benzylic) 96.5, 97.6 (C-1 and C-1'), 126.6-139.0 (aromatic). Analysis calcd. for $C_{62}H_{66}O_{11}$: C, 75.43; H, 6.74. Found: C, 75.38; H, 6.69%.

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl)-α-D-galactopyranoside (16)**[156]

The saccharide coupling of **2** (0.4 g, 0.62 mmol) with **15** [(0.2 g, 0.76 mmol) (as described in 3.2.2A)] in 72 h, provided compound **16** (0.42 g, 87%) as a syrupy material after workup and column chromatographic purification ($SiO_2$, pet.ether:ethyl acetate, 8:1.5).

$[\alpha]_D^{24}$ +10° (c 2.0, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.31 (brs, 6H), 1.44, 1.53 (2s, 6H, isopropylidene methyls) 3.33-5.1 (m, 21H), 5.48 (d, 1H, J=5, H-1), 7.0-7.5 (m, 20H, Ph).

$^{13}$C-nmr (22.3 MHz): 24.7, 24.9, 26.2x2 (4q, isopropylidene methyls), 66.0-82.0 (14C, carbons of hexoses and benzylic) 96.5, 97.2 (C-land C-1'), 108.6, 109.3 [2s, 2x$\underline{C}$ $(CH_3)_2$ isopropylidene], 127.5-139.3 (aromatic).

**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α -D-glucopyranosyl)-α-D-glucofuranoside (18)**[157].

The coupling reaction of **2** (0.41 g, 0.66 mmol) with sugar alcohol **17** (0.2 g, 0.76 mmol) in 72 h, yielded **18** (0.24 g, 56%) as a white crystalline compound (50% pet.ether in diethylether) after workup and chromatographic purification ($SiO_2$, pet.ether:diethylether 8:2.5).

$[\alpha]_D^{24}$ +46° (c 2.0, $CHCl_3$); m.p.: 91°C.

$^1$H-nmr (90 MHz): 1.17 (brs, 6H), 1.38, 1.47 (2s, 6H, isopropylidene methyls), 3.33-5.0 (m, 20H), 5.24 (d, 1H, J=4 , H-1'), 5.86 (d, 1H, J=4, H-1), 7.0-7.5 (m, 20H, Ph).

**Selected $^{13}$C-nmr date (22.3 MHz):** 97.7 (d, C-1') 104.9 (d, C-1), 108.7, 111.5 [2x$\underline{C}(CH_3)_2$].

Analysis calcd. for $C_{46}H_{54}O_{11}$: C, 70.57; H, 6.95. Found: C, 70.37; H, 6.84%.

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl-α -D-galactopyranosyl)-α-D-galactopyranoside (19)[234]**

The glycoside coupling reaction of **4** (0.42 g, 0.66 mmol) with the sugar alcohol **15** [(0.2 g, 0.76 mmol) (described in general procedure 3.2.2A)] in 48 h gave **19** (0.4 g, 81%) as a syrup after workup and purification by column chromatography ($SiO_2$, pet.ether:ethyl acetate 8:3).

$[\alpha]_D^{27}$ +5.1° (c 0.9, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.26, 1.28, 1.40, 1.48 (4s, 12H, isopropylidene-$CH_3$), 3.35-5.1 (m, 21H), 5.48 (d, 1H, J=5, H-1), 7.0-7.5 (m, 20H, Ph).

**Selected $^{13}$C-nmr data (22.3 MHz):** 96.2 (d, C-1), 97.5 (d, C-1'), 108.4, 109.1 [2x, $\underline{C}(CH_3)_2$].

Analysis calcd. for $C_{46}H_{54}O_{11}$: C, 70.57; H, 6.95. Found: C, 70.68; H, 6.81%.

**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α -D-galactopyranosyl)-α-D-glucofuranoside (20)[157]**

Saccharide coupling reaction (3.2.2A) of **4** (0.42 g, 0.66 mmol) with the sugar alcohol **17** (0.2 g, 0.76 mmol) in 72 h, afforded **20** (0.26 g, 62%) as a syrup after workup and chromatographic purification ($SiO_2$, pet.ether:diethylether 8:2.5).

$[\alpha]_D^{20}$+32.7° (c 1.1, $CHCl_3$).

$^1$H-nmr (90 MHz) : 1.16, 1.3, 1.38 (3s, 12H, isopropylidene-$CH_3$), 3.1-5.28 (m, 21H), 5.72 (d, 1H, J=4, H-1), 7.0-7.5 (m, 20H, Ph).

**Selected $^{13}$C-nmr data (22.3 MHz):** 98.9 (d, C-1'), 105.2 (d, C-1), 109.1, 111.2 [(2x$\underline{C}$ $(CH_3)_2$].

**1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α -D-galactopyranosyl)-α-D-galactofuranoside (22)**[156]

Compound **4** (0.42 g, 0.66 mmol) was reacted (3.2.2A) with the sugar alcohol **21** (0.2 g, 0.76 mmol), in 72 h, gave a white crystalline compound (50% pet. ether in diethylether) **22** (0.28 g, 67%) after workup and column chromatographic purification ($SiO_2$, pet.ether:ethyl acetate 8:2).

$[\alpha]_D^{25}$ +37° (c 0.8, $CHCl_3$); m.p. 120°C.

$^1$H-nmr (90 MHz): 1.24, 1.31, 1.41, 1.51 (4s, 12H, isopropylidene-$CH_3$), 3.35-5.0 (m, 21H), 5.80 (d, 1H, J=4, H-1), 7.0-7.5 (m, 20H, Ph).

Analysis calcd. for $C_{46}H_{54}O_{11}$: C, 70.57; H, 6.95. Found: C, 70.38; H, 6.78%.

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzyl-α -D-mannopyranosyl)-α-D-galactopyranoside (23)**

Saccharide coupling of **6** (0.42 g, 0.66 mmol) with the sugar alcohol **15** [(0.2 g, 0.78 mmol) (described in 3.2.2A)] in 55 h, to afford **23** (0.3 g, 60%) as a syrup, after workup and chromatographic purification ($SiO_2$, n-hexane:ethyl acetate 4:1).

$[\alpha]_D^{25}$ +39 (c 1.0, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.30, 1.42, 1.48 (3s, 12H, isopropylidene-$CH_3$), 3.5-4.95 (m, 21H, 5.47 (d, 1H, J=4, H-1), 7.0-7.5 (m, 20H, Ph).

Analysis calcd. for $C_{46}H_{54}O_{11}$: C, 70.57; H, 6.95. Found: C, 70.44; H, 6.74%.

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4-tri-O- α -L-rhamnopyranosyl)- α -D-galactopyranoside (24)**

The coupling reaction (3.2.2A) of glycosyl donor **9** (0.34 g, 0.64 mmol) with the sugar alcohol **15** (0.2 g, 0.76 mmol) in 48 h gave **24**

(0.34 g, 78%) as a syrup, after workup and chromatographic purification ($SiO_2$, pet. ether:ethyl acetate 8:3).

$[\alpha]_D^{29}$ -47° (c 1.0, $CHCl_3$).

$^1$H-nmr (80 MHz): 1.28, 1.44, 1.51 (3s, 12H, isopropylidene-$CH_3$), 1.33 (d, 3H, J=6, H-6), 3.5-5.0 (m, 17H), 5.32 (d, 1H, J=5, H-1), 7.0-7.6 (m, 15H, Ph).

$^{13}$C-nmr (75 MHz): 18.1, 24.6, 25.1, 26.1, 26.3 (5 q, 2x $C(\underline{C}H_3)_2$ and C-6), 96.5 (d, C-1), 98.3 (d, C-1'), 108.6, 109.4 [2s, $\underline{C}(CH_3)_2$], 127.6-139.1 (aromatic).

Reductive debenzylation of **24** using 10% Pd-C/$H_2$ followed by acetylation ($Ac_2O$/Py) yielded the disaccharide which has comparable data such as nmr, $[\alpha]_D$ etc., with the reported compound[235].

**Methyl 2,3-O-isopropylidene-4-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (27)**

Compound **9** (0.34 g, 0.64 mmol) was reacted (3.2.2A) with sugar alcohol **26** (0.19 g, 0.78 mmol) in 48 h to afford compound **27** (0.28 g, 72%) as a syrup after workup and chromatographic purification ($SiO_2$, pet.ether:ethyl acetate 8:2).

$[\alpha]_D^{27}$ -23.9° (c 1.0, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.28, 1.33, 1.51 (m, 12H, isopropylidene-$CH_3$, and H-6,6'), 3.2-5.0 (m, 14H), 5.28, 5.35 (2brs, 2H, H-1 and H-1'), 7.0-7.5 (m, 15H, Ph).

$^{13}$C-nmr (22.3 MHz): 18.1, 26.6, 28.1, 29.8 (4q, $C(\underline{C}H_3)_2$ and C-6,6'), 54.9 (q, $O\underline{C}H_3$), 64.3, 69.0, 72.2, 72.7, 75.5, 75.7, 76.4, 78.9, 80.1, 80.7 (8d, 3t carbons of hexose and benzylic), 97.6, 98.4 (C-1 and C-1'), 109.6, [s, $\underline{C}(CH_3)_2$], 127.8-139.1 (aromatic).

Analysis calcd. for $C_{37}H_{46}O_9$: C, 70.01; H, 7.31. Found: C, 70.13; H, 7.25%.

On acatalyic hydrogenation using 10% Pd/C followed by acetylation ($AC_2O$/Py) of **27** yielded the compound **28**, whose physical data was in agreement with the reported compound[235].

### 3.3.4 Synthesis of α-linked trisaccharides

**O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-(1 → 6)-(2,3-di-O-acetyl-α-D-glucopyranosyl- (1 → 4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranoside (panose derivative (30)[227]**

Saccharide coupling of **2** (0.4 g, 0.62 mmol) with sugar alcohol **29** (0.47 g, 0.78 mmol) in dry dichloromethane (10 ml, having 3% methyl iodide) in the presence of molecular sieves 4Å (300 mg), at 50°C for 48 h (as per general procedure 3.2.2A) after workup and chromatographic purification ($SiO_2$, benzene:ethyl acetate 2:1) afforded, the trisaccharide **30** (0.43 g, 62%) as a white crystalline compound (30% pet.ether in diethyl-ether).

$[\alpha]_D^{25}$ +51.3° (c 2.0, $CHCl_3$); m.p.: 150-151°C.

$^1$H-nmr (90 MHz): δ1.98-2.06 (4s, 18H, 6xOAc), 3.35-5.4 (m, 29H), 5.71 (d, 1H, J=8, H-1), 7.0-7.3 (m, 20H, Ph).

**Selected $^{13}$C-nmr data (22.3 MHz):** 20.5, 20.7, 20.9 (q, 6xOAc-$\underline{C}H_3$), 91.6, 96.3, 98.2 (C-1,1',1"), 168.8-170.8 (s, 6x$\underline{C}$-$CH_3$).

Analysis calcd. for $C_{58}H_{68}O_{22}$: C, 62.36; H, 6.14. Found: C, 62.27; H, 6.25%.

**O-(2,3,4,6-Tetra-O-benzyl-α-D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-α-D-glucopyranosyl)-(1 → 6)-1,2:3,4-di-O-isopropylidene-α-D-galacto-pyranoside (31)**

Compound **12** (0.36 g, 0.33 mmol) was reacted with **15** (general

procedure 3.2.2A) in 52 h, to give **31** (0.15 g, 65%) as a syrup after workup and chromatographic purification ($SiO_2$, pet.ether:diethylether:ethyl acetate 2.5:1:0.3).

$[\alpha]_D^{24}$ +25.2° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 1.3, 1.45, 1.57, 1.6 (4s, 12H, isopropylidene-$CH_3$), 3.3-5.1 (m, 19H), 5.5 (d, 1H, J=5, H-1), 5.67 (d, 1H, J=3.6, H-1''), 7.0-7.4 (m, 35H, Ph).

**Selected $^{13}$C-nmr data (75 MHz):** 24.5, 24.7, 25.9, 26.0 [4q, $C(\underline{C}H_3)_2$], 96.1, 96.4, 96.8 (3d, C-1,1',1''), 108.3, 109.0 [2s, $\underline{C}(CH_3)_2$].

Analysis cacld for $C_{73}H_{82}O_{16}$: C, 72.14; H, 6.80. Found: C, 72.01, H, 6.98%.

**2,3,4-Tri-O-benzyl-6-deoxy-α/β-D-glucopyranoside (34)**

Hydrolysis reaction of methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranoside[231,232] (**33**, 1.7 g) using 25 ml of 100:15-AcOH:2M HCl and 5 drops of con.$H_2SO_4$, at 95°C for 2 h[215], yielded compound **34** (1.1 g, 65%).

$[\alpha]_D^{25}$ +10.0 (c 1.0, $CHCl_3$).

$^1$H-nmr (90 MHz): 1.22, 1.25, 1.28 (2d, 3H, J=5, H-6), 5.1 (d, 1/2H, J=3.6, H-1 ).

**Selected $^{13}$C-nmr data:** 17.6 (q, C-6), 90.6 (d, C-1, α -compound), 97.5 (d, C-1, β-compound).

**2-Pyridyl 2,3,4-tri-O-benzyl-6-deoxy-1-thio- α /β -D-glucopyranoside (35)**

Compound **34** (0.5 g, 1.15 mmol) was reacted with PySSPy (0.29 g, 1.3 mmol) in dichloromethane (5 ml) and n-$Bu_3P$ (0.34 ml, 1.4 mmol) as described in 2.5.1B, to give compound **35** (0.51 g, 82%) as a syrup (α:β/3:2 from $^1$H-nmr).

$^1$H-nmr (90 MHz): 1.22, 1.31 (2d, 3H, J=6.2, $CH_3$), 3.0-5.0 (m, 10H), 5.44 (d, 0.5H, J=10, H-1 ax), 6.58 (d, 0.5H, J=5, H-1 eq), 6.9-8.5 (m, 19H, Ph and SPy).

**1,2,3,6-Tetra-O-acetyl-4-O-(2,3-di-O-acetyl-6-O-benzoyl-α-D-glucopyranosyl)-β-D-glucopyranoside (36)**

The compound **29** (1.6 g, 2.4 mmol) was selectively benzoylated[233] using benzoyl chloride (0.4 ml, 2.4 mmol) in pyridine (2 ml) at 0° for 2 h. After workup and chromatographic purification (pet. ether:ethyl acetate 1:1) yielded, **36** (0.8 g, 72%) as colourless crystals.

$[\alpha]_D^{25}$ +49° (c 1.0, $CHCl_3$), m.p.: 95-97°C.

$^1$H-nmr (90 MHz): 1.85, 1.90, 2.0, 2.02, 2.04, 2.06 (6s, 18H, 6xOAc), 3.22 (brs, 1H, -OH), 3.3-5.43 (m, 13H), 5.63 (d, 1H, J=8, H-1), 7.1-8.1 (m, 5H, Ph).

**Selected $^{13}$C-nmr data (22.3 MHz):** 20.2, 20.3 (2q, 6x $OCOCH_3$), 91.0 (d, C-1), 93.7 (d, C-1).

**O-(2,3,4-tri-O-benzyl-6-deoxy-α-D-glucopyranosyl)-(1 → 4)-di-O-(2,3-di-O-acetyl-6-O-benzoyl-α-D-glucopyranosyl)-(1 → 4)-1,2,3,6-tetra-O-acetyl-β-D-glucopyranoside (37)**

The reaction of **35** (0.19 g, 0.35 mmol) with **36** (0.48 g, 0.7 mmol) in dichloromethane (4 ml, having 3% methyl iodide) in presence of molecular sieves 4Å (0.2 g) at 50°C for 96h, gave compound **37** (0.039 g, 12%) upon chromatographic purification (pet.ether:ethyl acetate 2:1) as a solid material (slightly impure from $^1$H-nmr).

$[\alpha]_D^{25}$ +62.5 (c 1.0, $CHCl_3$); m.p.: 65-67°C.

$^1$H-nmr (300 MHz): 1.16 (d, 3H, J=6.2, $CH_3$), 1.92, 2.01, 2.02, 2.06, 2.10, 2.16 (6s, 18H, 6xOAc), 3.0-5.5 (m, 24H), 5.79 (d, 1H, J=8, H-1), 7.21-8.05 (m, 20H, Ph).

# CHAPTER 4

## DIRECTED, ITERATIVE, STEREOSELECTIVE SYNTHESIS OF OLIGOSACCHARIDES BY USE OF 2-PYRIDYL THIOGLYCOSIDES AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR (ARMED / DISARMED SYNTHESIS)

## 4.1 Introduction

Oligosaccharides are implicated in a wide variety of biologically important processes[19-29]. However, the enormous problems encountered in isolations and characterization of oligosaccharide units have frustrated their study as a part of glycoconjugates. Due to the problems encountered in obtaining homogenous saccharide fragements from classical degrative methods researchers have taken to chemical synthesis.

There are many problems that confront oligosaccharide synthesis[128]. The important and crucial ones being

i) Selection of protecting groups to differentiate the multitude of hydroxy groups and minimization of this operation.

ii) Selective activation of the anomeric center for reaction with sugar alcohols without damaging the preformed glycosidic linkages.

iii) Diastereoselectivity : Glycosidic linkages must be formed stereoselectively ($\alpha$ / or $\beta$ ), because separation of anomers is a very tedious and time consuming task.

The general protocol for such syntheses involves either a **stepwise** or a **convergent (block) approach**[134].

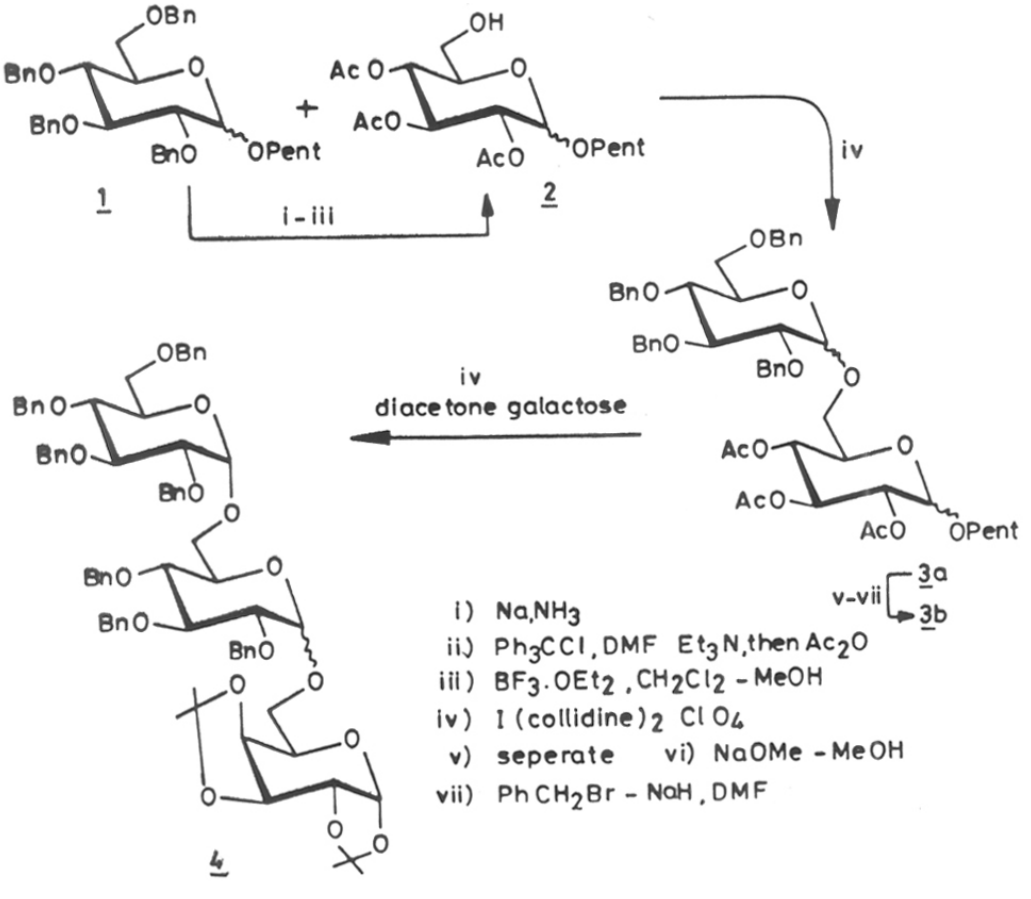
The efficiency of any such method ultimately depends upon the ability to replace the anomeric substituent at the reducing end of the growing oligosaccharide chain without affecting the preformed glycosidic bonds and the protecting groups.

A conceptually new and simple method has been developed by Fraser-Reid et al where n-pent-4-enyl glycosides "arm" or "disarm" the glycosyl donors by means of a protecting group on C-2 oxygen[236]. This result emanated from the hydrolysis rates of n-pent-4-enyl glycosides with various C-2 protecting groups[59]; it was shown that 2-O-acetate

reacted much more slowly than the corresponding 2-O-benzyl ether[236]. Thus the reactivity difference was attributed to the C-2 substituent and it implied that C-2 esters could be considered as being "disarmed" where as C-2 alkyl ethers were "armed" toward reaction with the halonium ion[128]. Thus, the terms "armed"/"disarmed" were coined to indicate the first observed unique reactivity phenomenon.

Thus coupling of 1 and 2, mediated by iodoniumdicollidine perchlorate[237,238] has afforded 62% yield of disaccharide 3a. The anomers of 3a were separated, and the acetyl groups were replaced with benzyl

**Scheme 1**



to obtain **3b**. The reducing end of **3b** was then "armed" by further coupling, with diacetonegalactose[222] to obtain the trisaccharide **4** in 60% yield (α/β anomers) (Scheme 1).

Since, the discovery of "armed"/"disarmed" phenomenon, the effect has also been observed for the couplings of thioglycosides in the laboratories of Van Boom et al[239] (Scheme 3) and of glycals by Danishefsky et al[240] (Scheme 4). For the thioglycosides and glycals respectively the principle is same, 2-O-ethers being more reactive than corresponding 2-O-esters (Scheme 2).

Thus alkyl thioglycoside **5** (armed donors) was coupled with **6** (disarmed acceptor) under iodoniumdicollidineperchlorate promoted glycosidation reaction to afford α-linked disaccharide **7** in 91% yield. Then benzoyl groups were replaced by benzyl groups and reacted with sugar alcohol **6** to obtain the trisaccharide **8**[239] (Scheme 3).

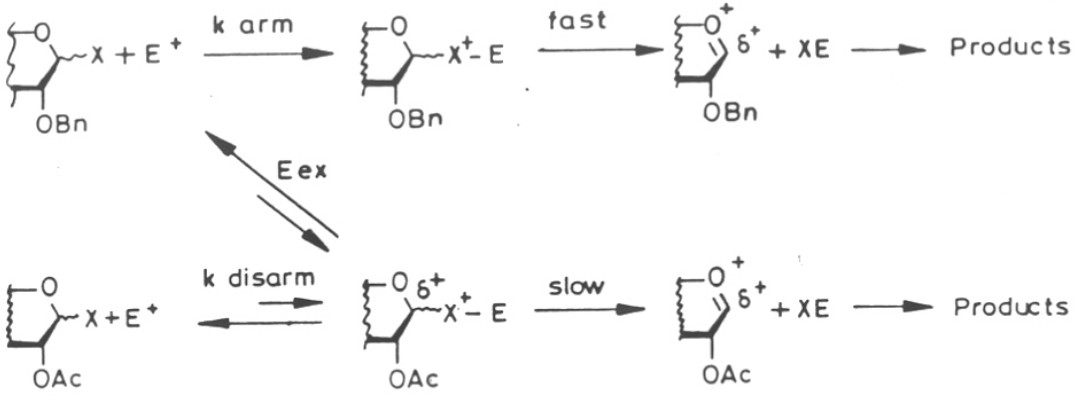
Trisaccharide **12** was synthesized[240] by a reiterative process (Scheme 4) where glycal ether **9** acts as donor and glycal ester **10** as an acceptor.

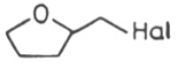
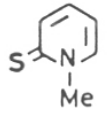
## 4.2 Results and discussion

### 4.2.1 Methyl iodide activated hydrolysis of 2-pyridyl thioglycosides

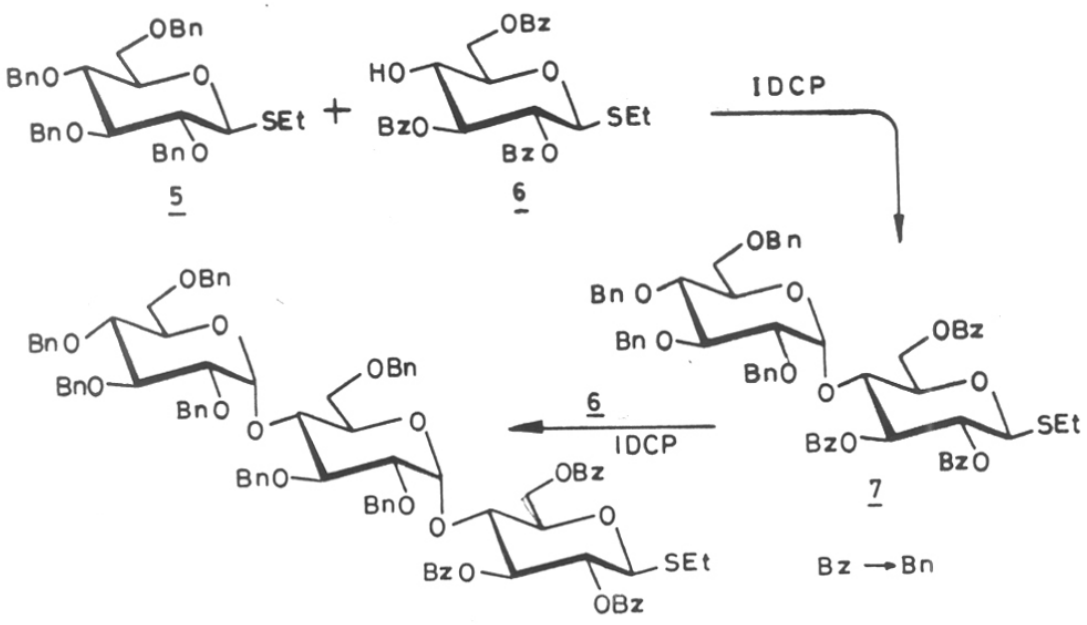
In order to study the effect of 2-O-protecting groups on the activation of 2-pyridyl thioglycosides by methyl iodide, several hydrolysis and glycosidation reactions were performed. Accordingly, per-O-benzyl-**13**, per-O-acetyl-**14**, per-O-benzoyl-**15**, 2-acetamido-2-deoxy-**16** and 2-deoxy-2-bromo-3,4,6-tri-O-acetyl-**17**, 2-pyridyl thioglucosides were subjected to hydrolysis by using 3% methyl iodide in wet DMF at 40°C to obtain their corresponding reducing sugars (Scheme 5).

## Scheme 2

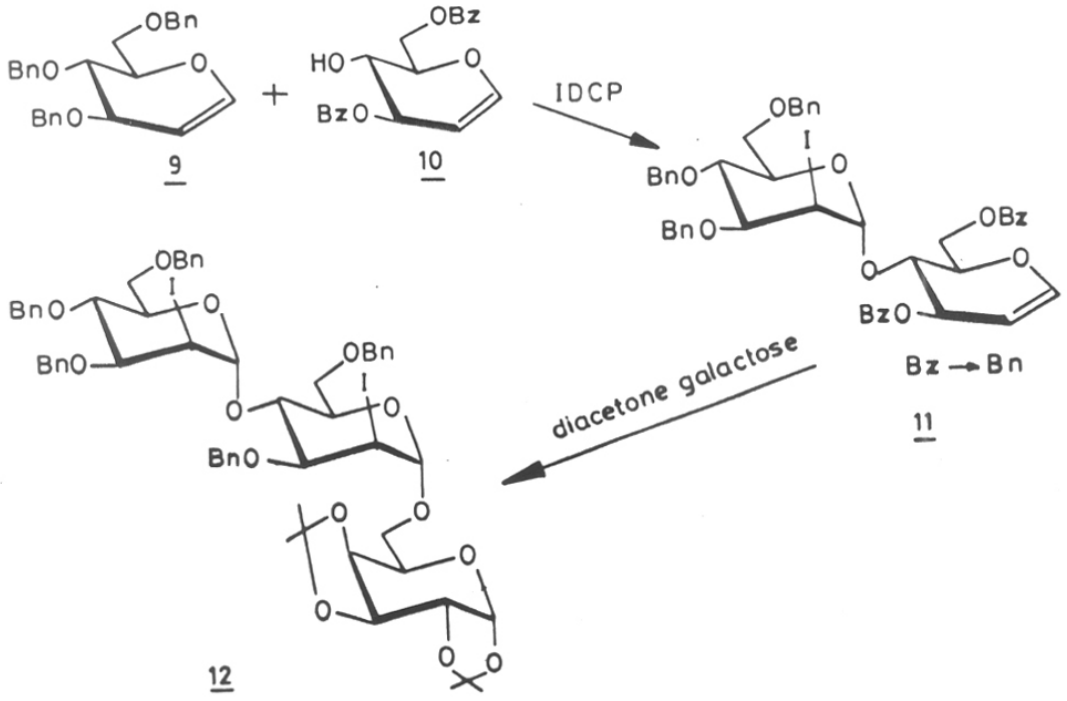



| X | E | XE |
|---|---|---|
| OR | H | ROH |
| SR | $Hal^+$ or $Me^+$ | RSHal or RSMe |
| Br | $Ag^+$ | AgBr |
| OPent | $Hal^+$ |  |
| SPy | $Me^+$ |  |

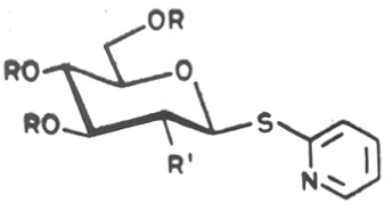
Scheme 3

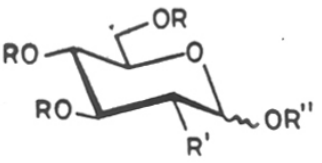



Scheme 4



**Scheme 5**




| | | | | |
|---|---|---|---|---|
| **13** | R = Bn; | $R^1$ = OBn | **18** | **23** |
| **14** | R = Ac; | $R^1$ = OAc | **19** | **24** |
| **15** | R = Bz; | $R^1$ = OBz | **20** | **25** |
| **16** | R = Ac; | $R^1$ = NHAc | **21** | **26** |
| **17** | R = Ac; | $R^1$ = Br | **22** | **27** |

Compound **13** smoothly hydrolysed in 2 h. However under similar experimental conditions **14,15,16** and **17** resisted hydrolysis, starting materials were recovered (24 h; 90-95%); longer reaction time (3-5 days) has resulted in the decomposition of starting material. Thus it was difficult to conclude on the rate of hydrolysis of 2-pyridyl thioglycosides, although, benzyl ether **13** has indicated higher reactivty. Thus due to problems encountered in hydrolysis experiments it was felt worthwhile to study rate of glycosidations with simple alcohol (eg. methanol) (Table 1).

The experimental results indicated higher reactivity of 2-O-ether **13** (entry i) compared to the esters[14-16]. Among esters, 2-deoxy 2-acetamido compound **16** (entry iv) has shown higher reactivity than 2-O-benzoyl ester **15** (entry iii), which in turn was more reactive than 2-O-acetate **14** (entry ii) and 2-bromo compound **17** (entry v). These significant differences in rates of glycosidation indicated their utility in the development of yet another "armed"/"disarmed" glycosidation methodology.

**Table 1**

**Rate of glycosidation of 2-pyridyl thioglycoside derivatives in methanol-MeI**

| Entry | Substrate | Product | Reaction time (h) | Isolated yield (%) |
|---|---|---|---|---|
| i. | 13 | 23 | 20 | 85 |
| ii. | 14 | 24 | 20-72 | a |
| iii. | 15 | 25 | 72 | 68 |
| iv. | 16 | 26 | 48 | 72 |
| v. | 17 | 27 | 24-72 | a |

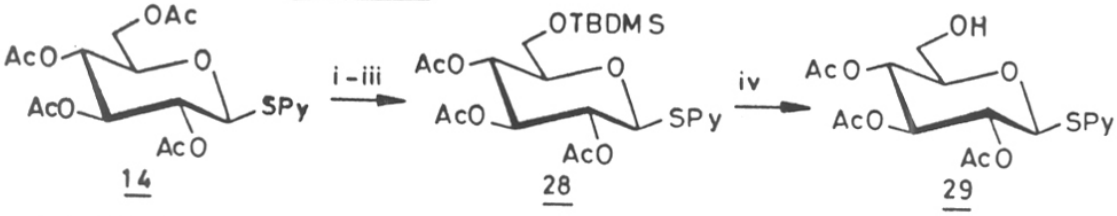
a : Starting material recovered : On longer reaction time (5 days) starting materials decomposed.

### 4.2.2 Synthesis of α-linked saccharides by "Armed/Disarmed" approach

Based on the substantial difference in rates of glycosidation of 2-O-benzyl- and 2-O-acetyl- (OBn >> OAc) 2-pyridyl thioglycosides on activation by methyl iodide (Table 1) a directed, iterative and stereoselective oligosaccharide synthesis has been developed.

2-Pyridyl 2,3,4-tri-O-acetyl- β -D-glucopyranoside (**29**) (disarmed acceptor) was prepared from the per-O-acetyl- 2-pyridyl thioglycoside **14**[137] in a straight forward manner (Scheme 6).

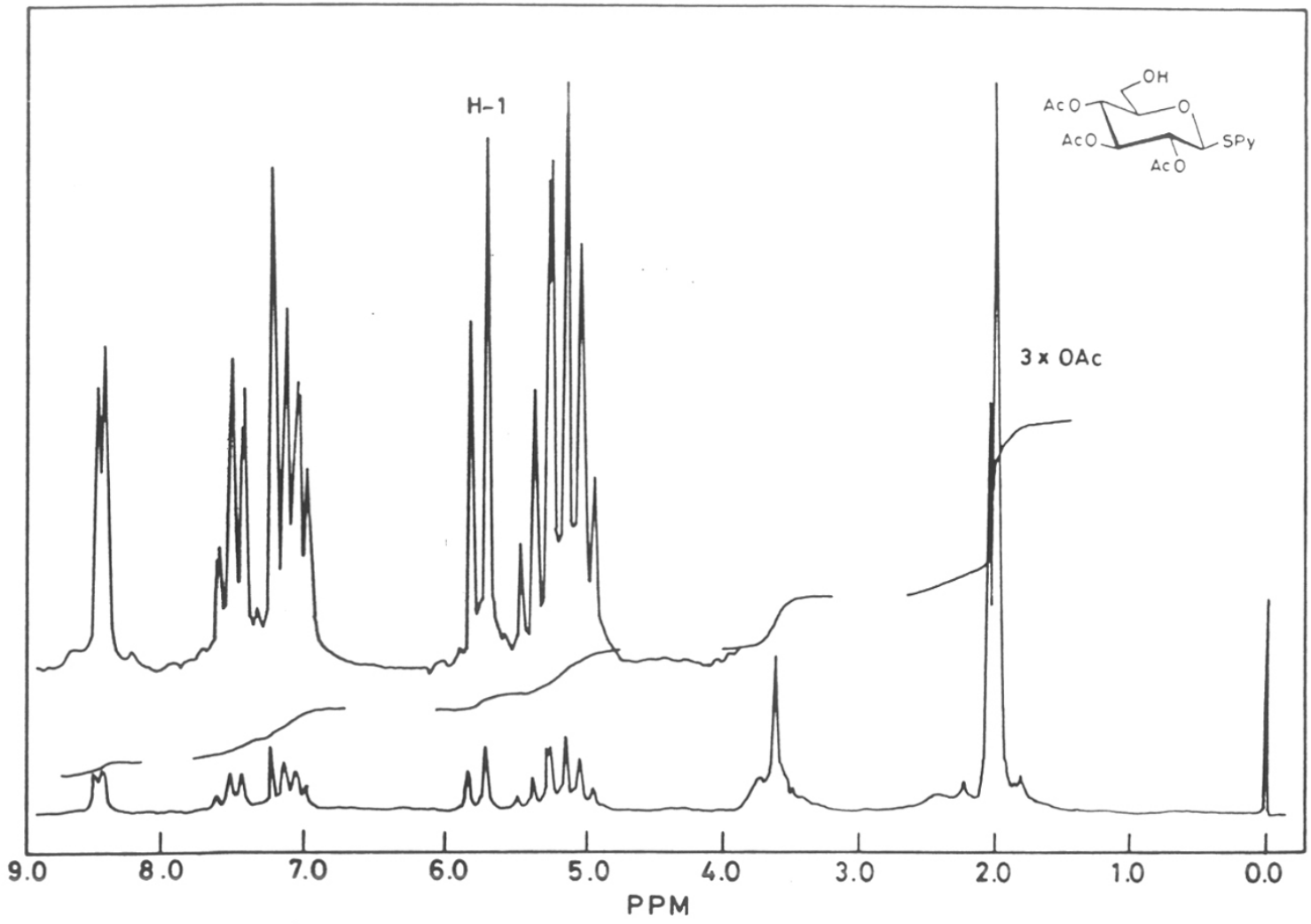
**Scheme 6**



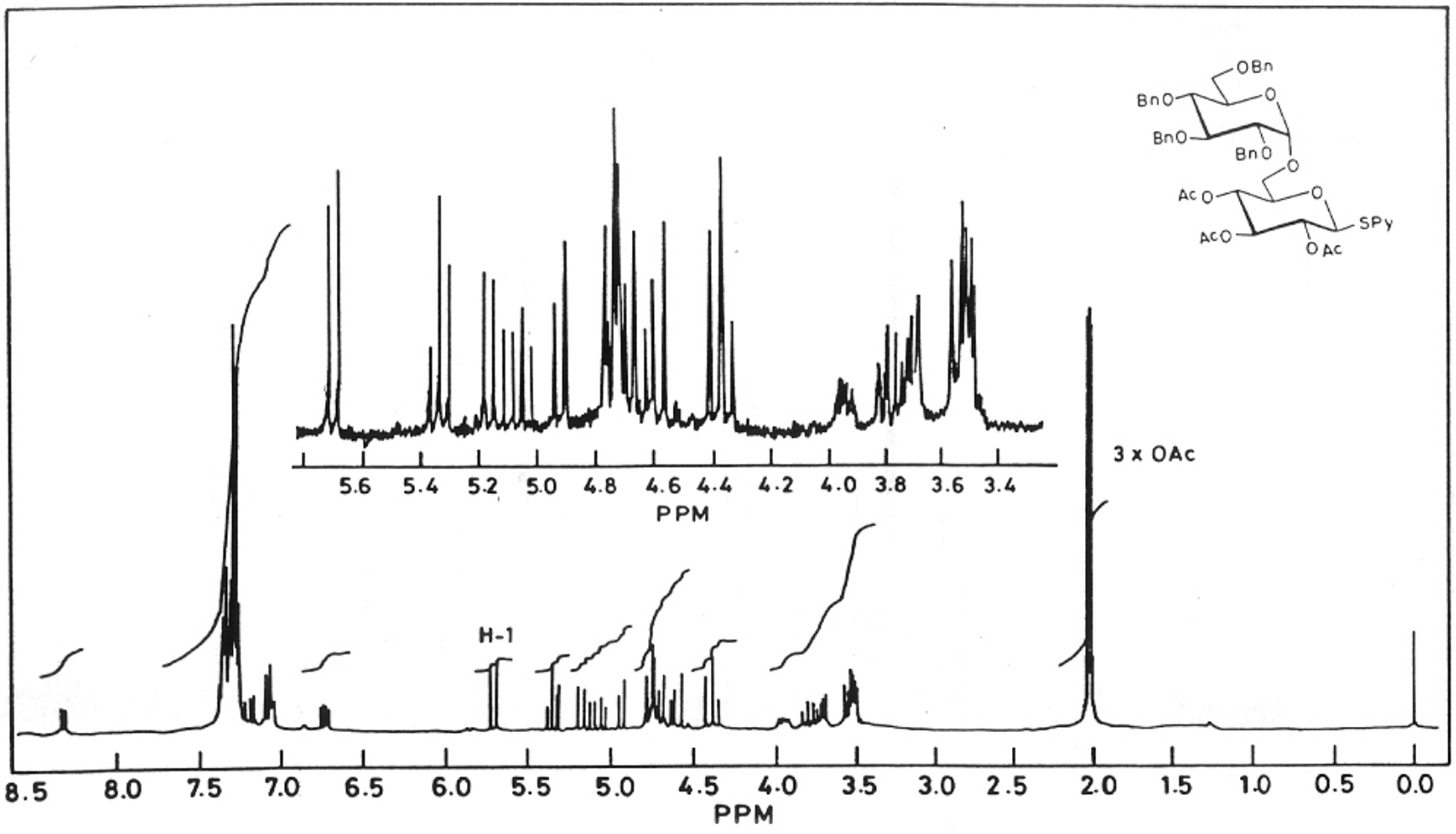

i) 0.1N NaOMe - MeOH, rt, 2h. ii) TBDMSCl - Py, rt, 30 min.
iii) $Ac_2O$ - Py, rt, 19h. iv) 2% PTSA - MeOH, rt, 3h.

Compound **29** was characterized by $^1H$, $^{13}C$-nmr, optical rotation, m.p. and elemental analysis (Experimental). $^1H$-nmr spectrum of **29** showed at δ 2.01, 2.03 (2s, 9H, 3xOAc) a doublet at δ 5.75 ($J_{1,2}$=10 Hz, H-1) and characteristic 2-pyridyl thio protons between δ8-8.5.

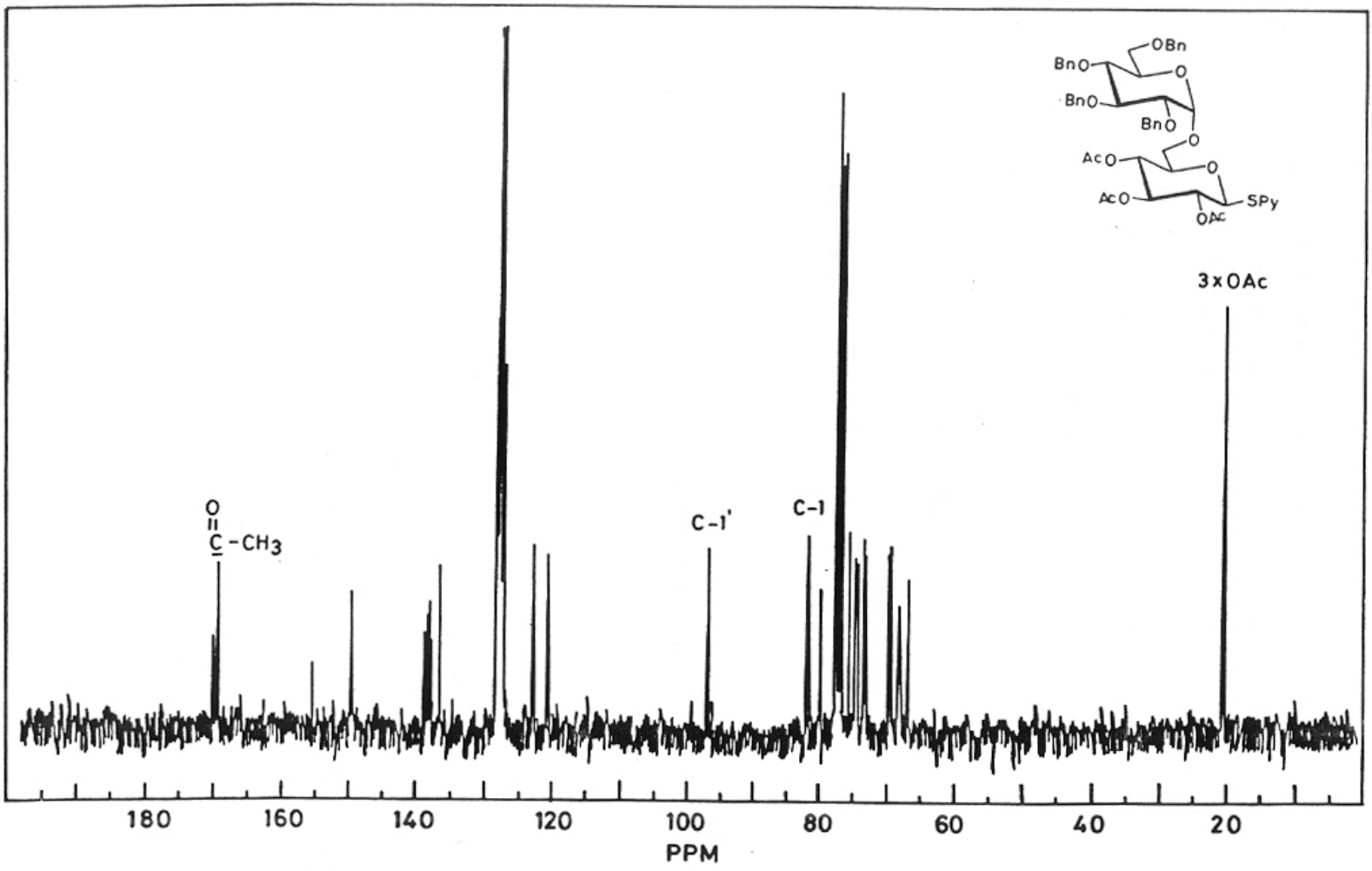
Directed, iterative, stereoselective glycosidations were performed by saccharide coupling of **13 (armed)** (1 mmol) with the sugar alcohol **29 (disarmed)** (1.2 mmol) employing standard conditions of glycosidation (36 h) to afford the α-linked disaccharide **30** (syrup) in 66% yield, $[\alpha]_D^{25}$ +20.2° (Scheme 7). Compound **30** (disarmed) showed in its $^1H$-nmr spectrum (300 MHz; $CDCl_3$), three singlets at δ 2.01, 2.03 and 2.05 for 3xOAc groups, a doublet at δ 5.72, $J_{1,2}$=9 Hz for H-1 and characteristic 2-pyridylthio signals. $^{13}C$-nmr spectrum showed two doublets at δ 81.7 and




$^1$H NMR SPECTRUM OF COMPOUND 29 (90 MHz, $CDCl_3$)



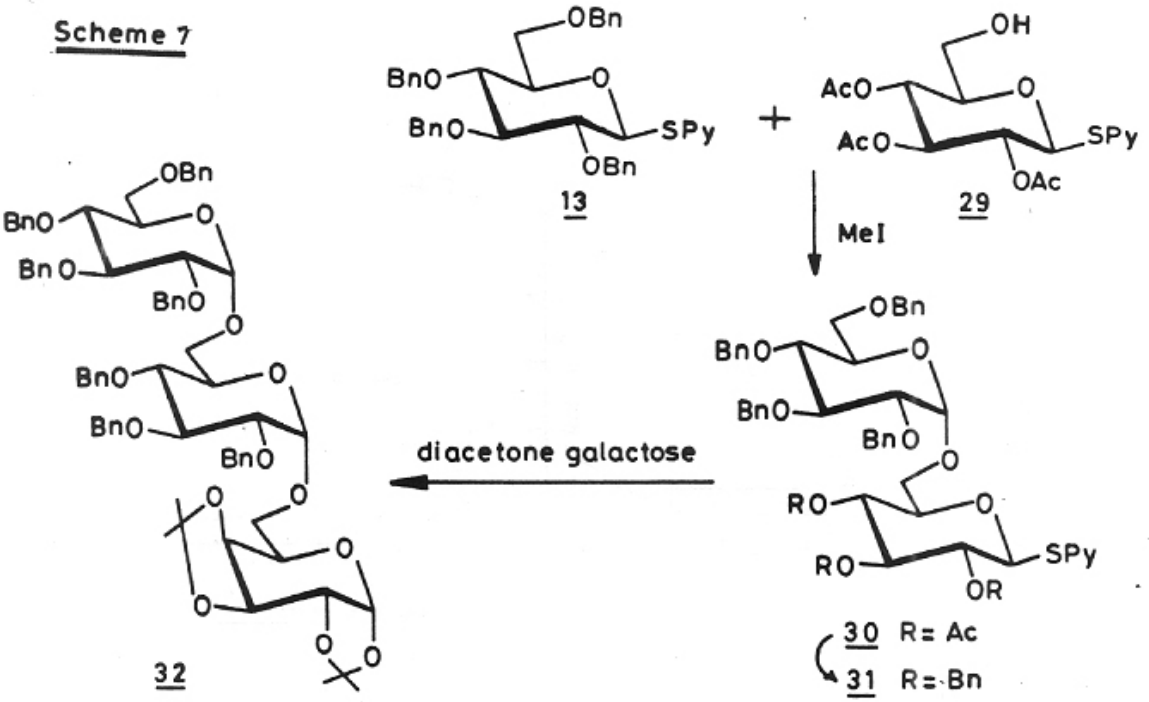

$^1$H NMR SPECTRUM OF COMPOUND 30 (300 MHz, $CDCl_3$)




$^{13}C$ NMR SPECTRUM OF COMPOUND 30 (75 MHz ; $CDCl_3$ )

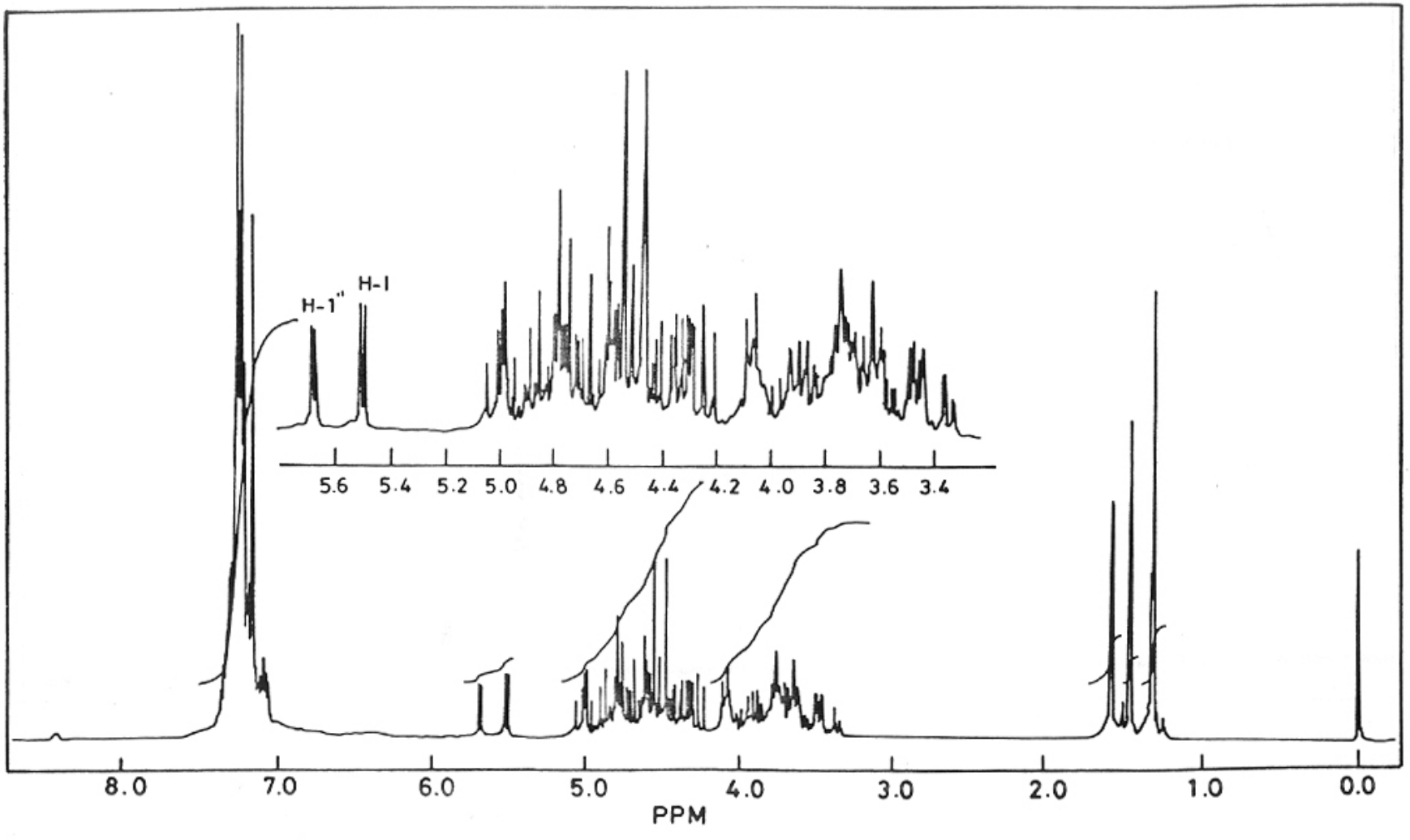
δ 96.9 for C-1 and C-1' respectively confirming α-linkage at the newly formed interglycosidic bond. Other data was also in accordance with the assigned structure for **30**. There was no evidence for self condensation of **29**. Acetyl groups of **30** were replaced by benzyl (0.5 mmol, Cat NaOMe-MeOH, RT, 1h, IR 120 $H^+$, DMF-NaH, BnBr, 2h, RT) to obtain **31** (syrup), $[\alpha]_D^{25}$ +35.8° (85% yield).

**Scheme 7**

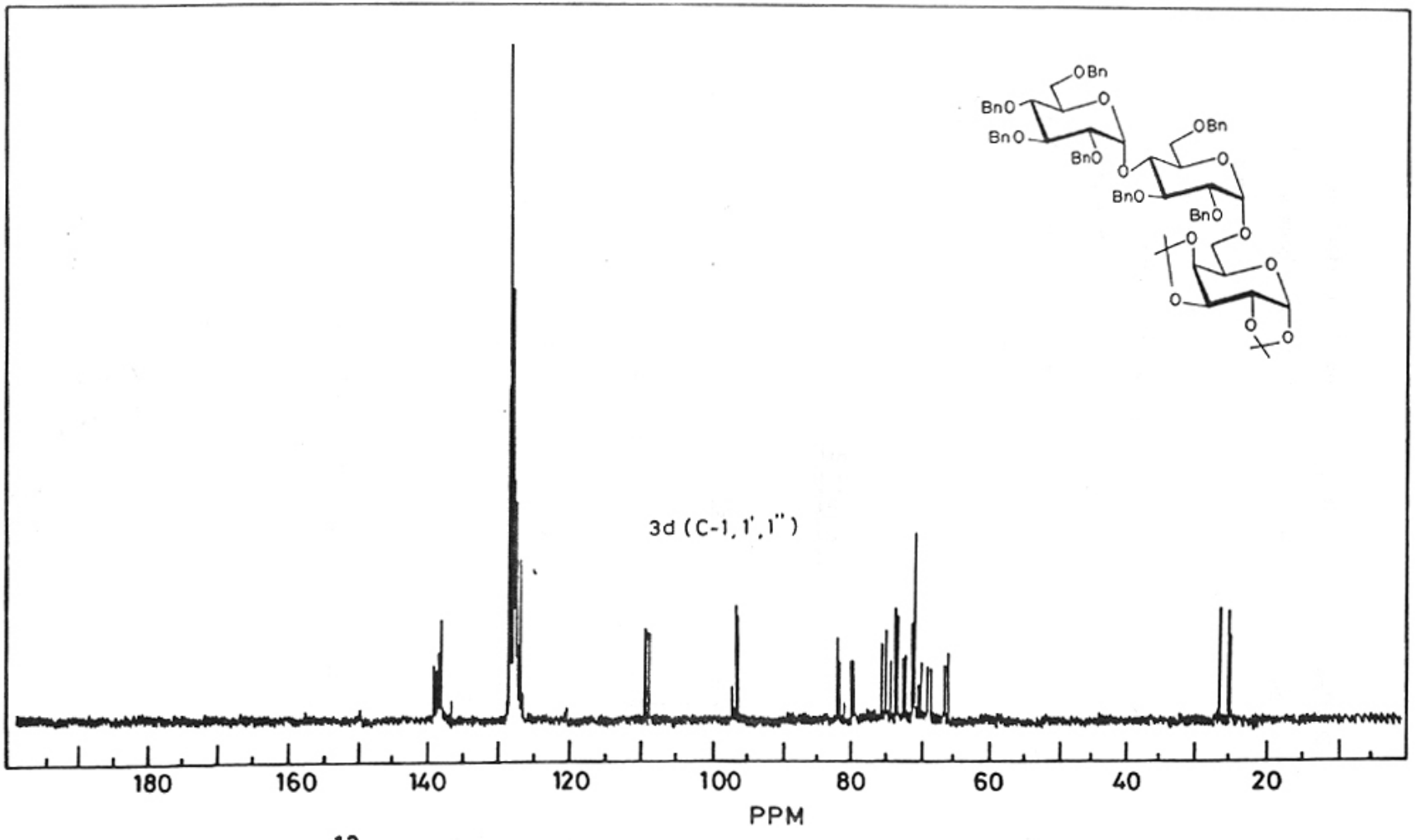



The reducing end of compound **31** (**armed**) (0.1 mmol) was further coupled with diacetonegalactose[222] (0.12 mmol, 26h) to obtain the α-linked trisaccharide **32** in 64% yield (syrup) $[\alpha]_D^{25}$ +46.8°. Compound **32** was characterized from $^1H$, $^{13}C$-nmr optical rotation and elemental analysis.

Compound **32** showed in its $^1H$-nmr spectrum four isopropylidene methyl singlets at δ 1.33, 1.37, 1.5 and 1.57; a doublet at δ 5.55 ($J_{1,2}$= 5 Hz) for H-1 proton, and aromatic protons. $^{13}C$-nmr of **32** showed




$^1$H NMR SPECTRUM OF COMPOUND 31 (300 MHz, $CDCl_3$)

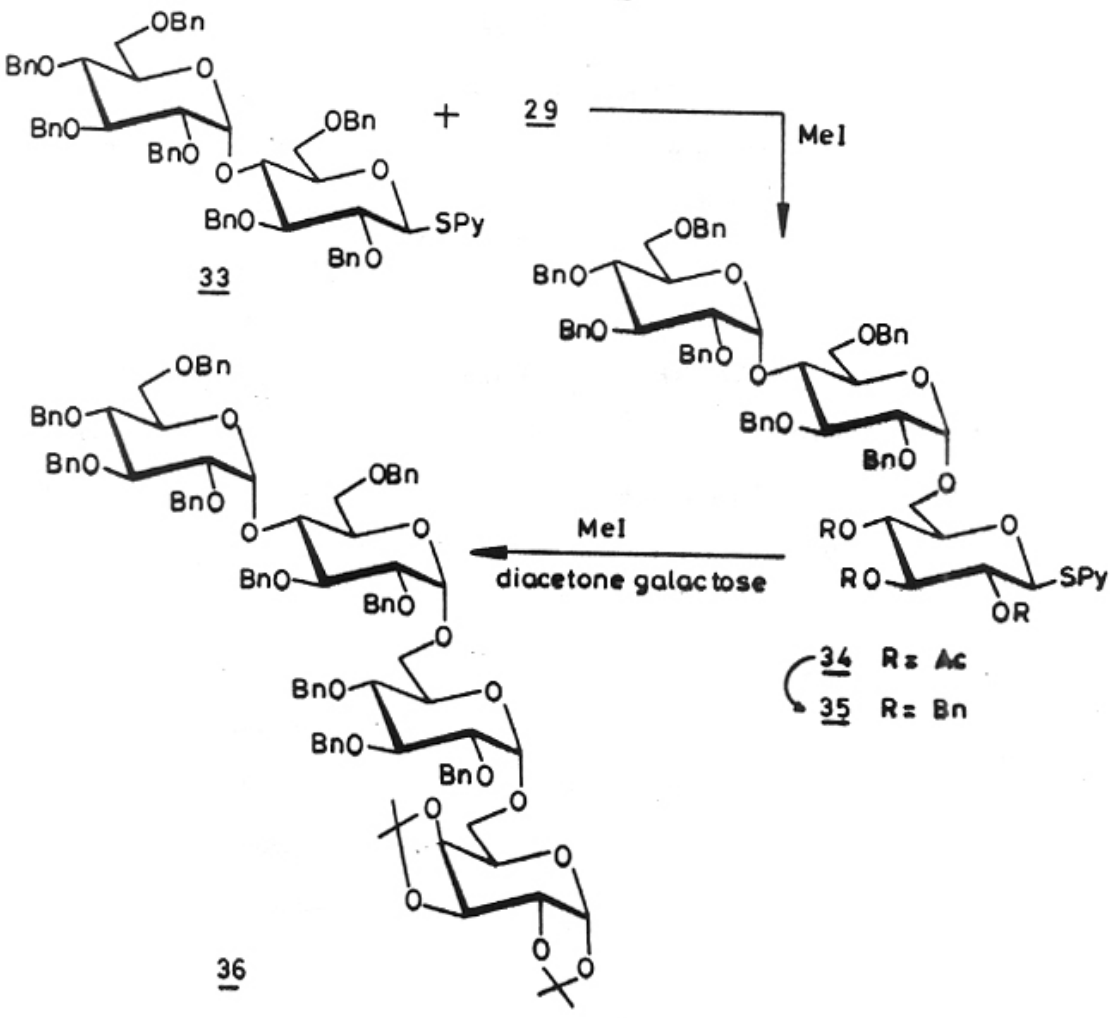



$^{13}$C-NMR SPECTRUM OF COMPOUND 31 (75 MHz, $CDCl_3$)

three doublets at δ 96.2, 96.4, 96.3 for C-1, C-1', C-1" respectively and two singlets at δ 108.1, 108.5 for 2x$\underline{C}(CH_3)_2$ which is in full agreement with the assigned structure.

Efficacy of this iterative methodology was also demonstrated by use of even a disaccharide component such as 2-pyridyl hepta-O-benzyl-thiomaltoside **33 (armed)** as glycosyl donor. Compound **33** was conveniently prepared from α-acetobromomaltose[243]. Saccharide coupling of **33 (armed)** (1 mmol) with **13 (disarmed)** (1.2 mmol) (44 h) gave the trisaccharide **34** in 54% yield $[\alpha]_D^{25}$ +44.7° (Scheme 8).

Scheme 8

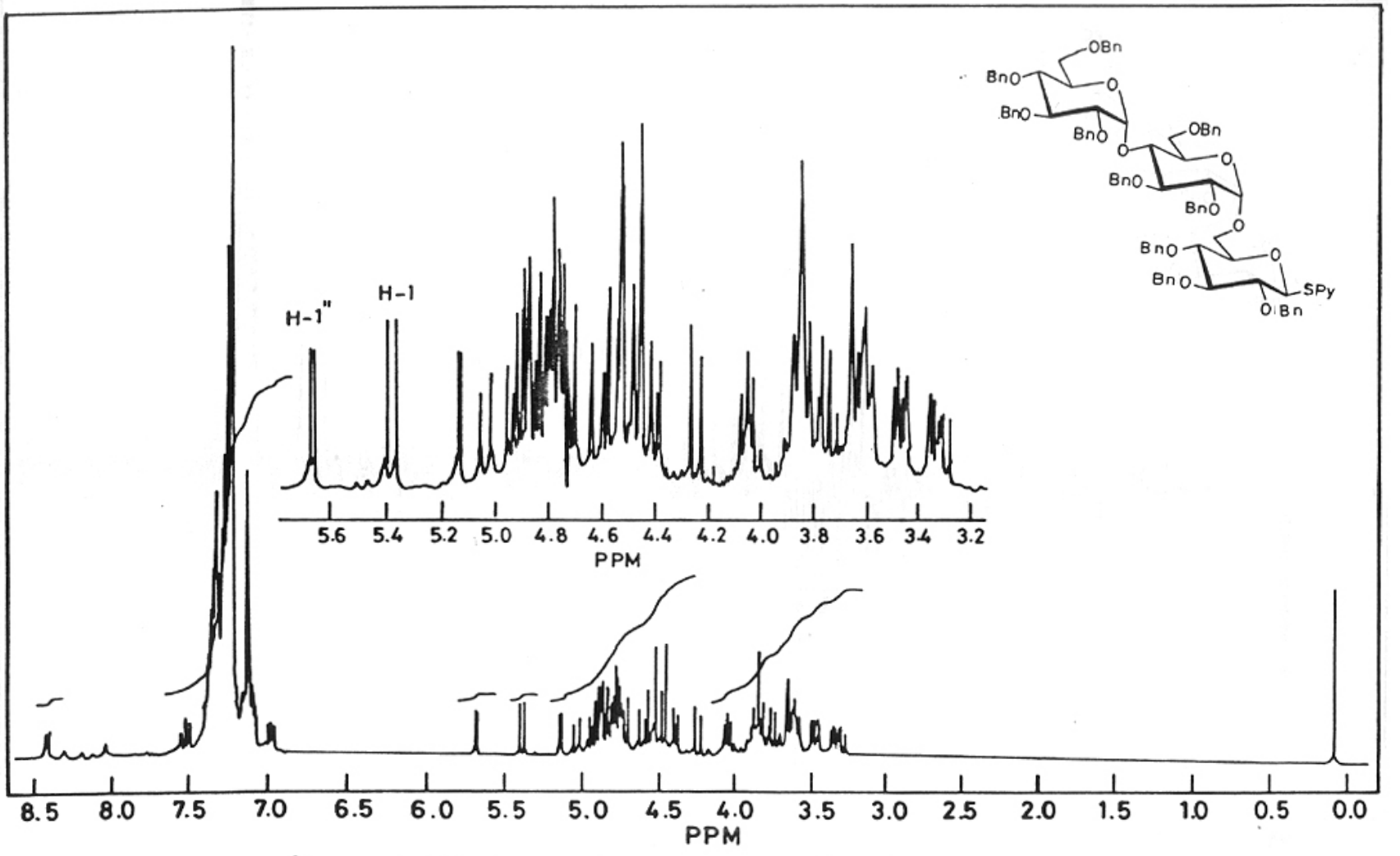


Compound **34** was characterized from its $^{1}$H-nmr spectrum, (300 MHz, $CDCl_3$) which showed three singlets at δ2.0, 2.04 and 2.08 (3xOAc) a doublet at δ 5.61 ($J_{1,2}$=3.6 Hz) for H-1" and a doublet at δ 5.67 ($J_{1,2}$= 10 Hz) for H-1. $^{13}$C-nmr spectrum of **34** showed doublets at δ 96.6 and 96.7 for C-1 and C-1" respectively. **34** was converted into **35** [$[\alpha]_D^{25}$ +43°] in a straight forward manner and was coupled at the reducing end with diacetone galactose[222] (22 h) to obtain the α-linked tetrasaccharide **36** in 57% yield (syrup) $[\alpha]_D^{25}$+26°.

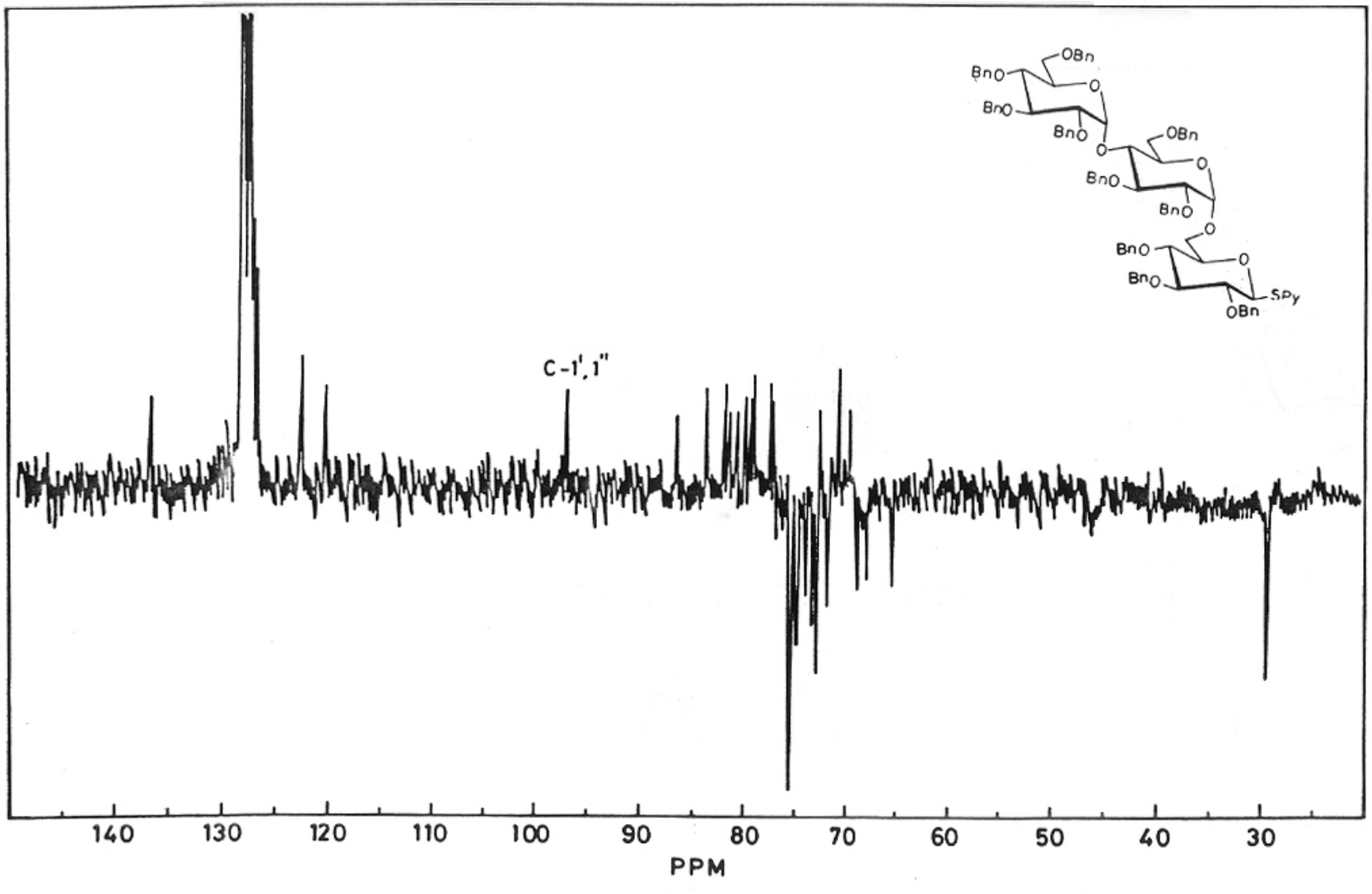
The trisaccharide **35** was characterized on the basis of its $^{1}$H-nmr spectrum (300 MHz, $CDCl_3$), in which a doublet at δ 5.13 ($J_{1',2'}$=3.6 Hz) for H-1', δ 5.37 ($J_{1,2}$=10 Hz) for H-1 and δ 5.67 ($J_{1'',2''}$=3.6 Hz) for H-1" are diagnostic of α-linkages in **35.** $^{13}$C-nmr spectrum of **35** showed signal for C-1' and C-1" at δ 96.7 and 96.9 respectively. Optical rotation and elemental analysis were also in agreement with the assigned structure.

The tetrasaccharide **36** showed in its $^{1}$H-nmr (300 MHz, $CDCl_3$) spectrum characteristic four singlets from the isopropylidene methyls at δ 1.27, 1.33, 1.45 and 1.55 (3H each), four doublets at δ 5.13 ($J_{1'',2''}$= 3.6 Hz), 5.56 ($J_{1,2}$=5.6 Hz), 5.67 ($J_{1''',2'''}$=3.6 Hz), 5.7 ($J_{1',2'}$=3.6 Hz) for H-1", H-1 and H-1''' and H-1' respectively. $^{13}$C-nmr spectrum of compound **36** has characteristic signals at δ24.5, 24.8, 25.9x2 (two singlets signals overlapping) for C-($\underline{C}H_3$)$_2$x2 and δ 96.2, 96.4 96.3 and 96.8 (d, C-1', C-1", C-1''' and C-1 respectively.

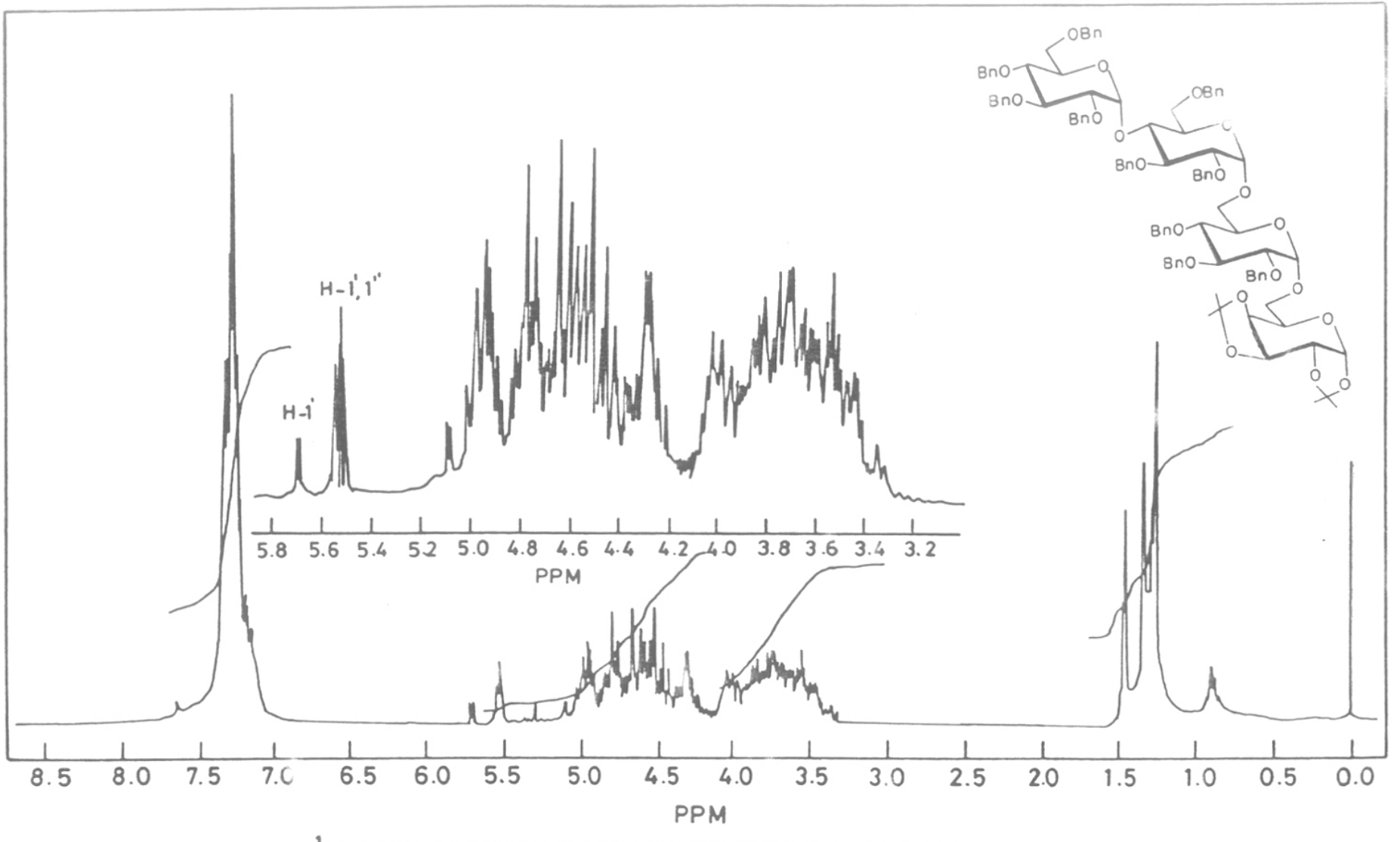
In summary, a useful directed iterative, stereoselective glycosidation methodology based on the substantial difference in the rates of glycosidation of 2-O-acetyl and 2-O-benzyl substituted 2-pyridyl thioglycopyranosides was established for the stereoselective synthesis of oligosaccharides.



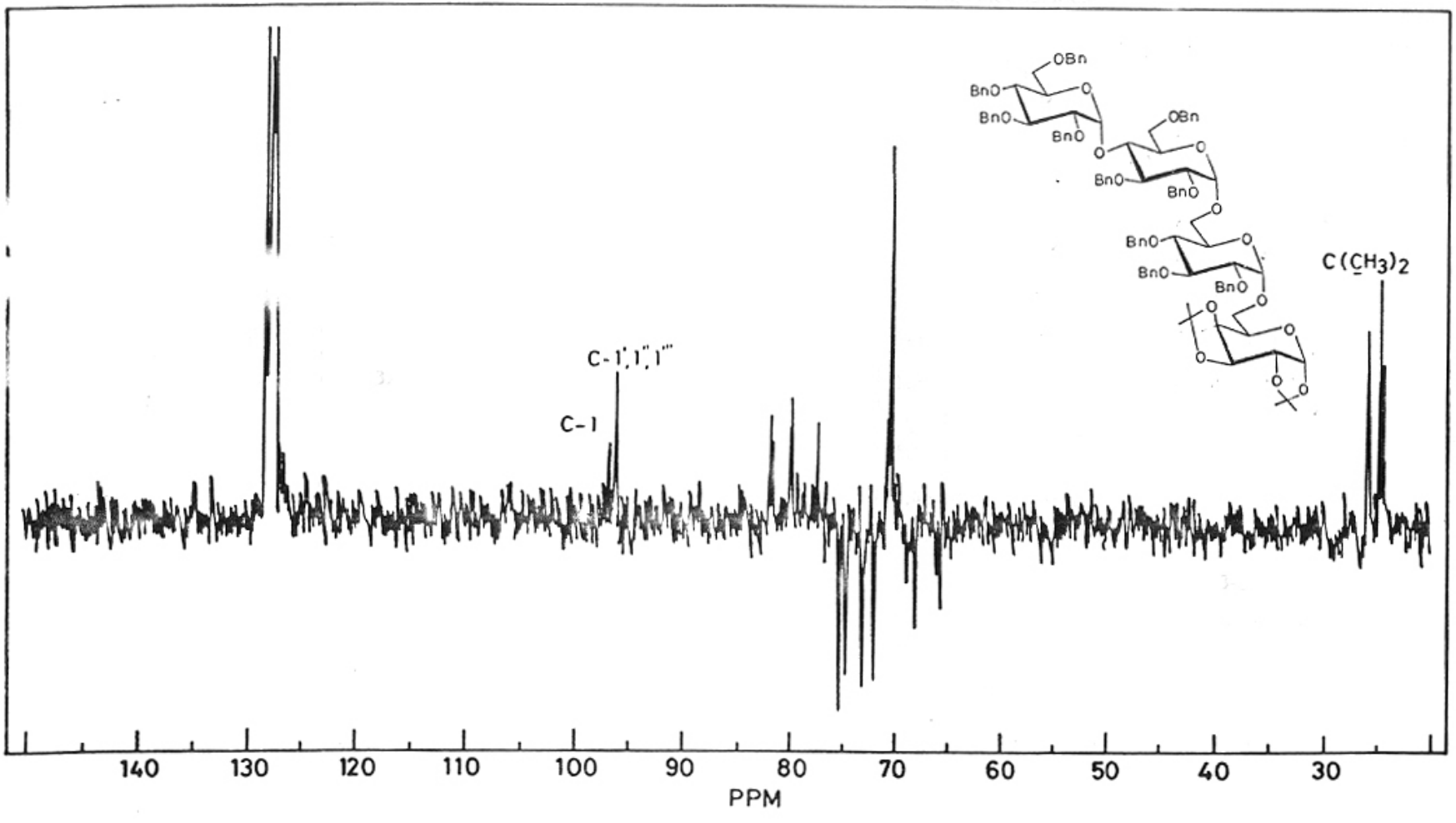

$^1$H NMR SPECTRUM OF COMPOUND 35 (300 MHz, $CDCl_3$)




THE DEPT SPECTRUM OF COMPOUND 35 (75 MHz ; $CDCl_3$ )




$^1$H NMR SPECTRUM OF COMPOUND 36 ( 300 MHz, $CDCl_3$ )




DEPT SPECTRUM OF COMPOUND 36 (75 MHz, $CDCl_3$)

Table 2

Physical characteristics of α-linked oligosaccharides

| Entry | Compound No. | $[\alpha]_D^{25}$ (c1.0, $CHCl_3$) | Yield % | m.p. °C /syrup | Selected $^1$H-nmr data δ (J in Hz) | Selected $^{13}$C-nmr data ( δ ppm) |
|---|---|---|---|---|---|---|
| i. | 29 | +13° | 90 | 139-141 | 5.75 (d, J=10, H-1) | 20.4 (q,3-OCOCH$_3$) |
| ii. | 30 | +20.2 | 66 | Syrup | 2.01, 2.03, 2.05 (3s, 9H, 3-OAc), 5.72 (d, J=9, H-1) | 20.1 (q, 3-OCOCH$_3$) 81.7 (d, C-1), 96.9 (d, C-1) |
| iii. | 31 | +35.8 | 85 | Syrup | 5.4 (d, J=9, H-1) | 97.0 (d, C-1') |
| iv. | 32 | +46.8 | 64 | Syrup | 1.33, 1.37, 1.5, 1.57 (4s, 12H- C(CH$_3$)$_2$ 5.55 (d, J=5, H-1) | 96.2, 96.4, 97.3 (C-1, 1', 1") |
| v. | 33 | +43 | 61 | Syrup | 5.45 (d, J=10, H-1) 5.63 (d, J=3.6, H-1') | 97.1 (C-1') |
| vi. | 34 | +44.7 | 54 | Syrup | 2.0, 2.04, 2.08 (3S, 9H, 3 OAc), 5.61 (d, J=3.6, H-1"), 5.67 (d, J=10, H-1) | 20.5 (q, 3-OCOCH$_3$) 96.6, 96.7 (C-1', 1") |
| vii. | 35 | +43 | 75 | Syrup | 5.13 (d, J=3.6, H-1') 5.37 (d, J=10, H-1), 5.67 (d, J=3.6, H-1") | 96.7, 96.9 (C-1', 1") |
| viii. | 36 | +26 | 57 | Syrup | 1.27, 1.33, 1.45, 1.55 (4s, 12H- C(CH$_3$)$_2$, 5.13, 5.56, 5.67, 5.7 (4d, H-1", 1, 1"', 1') | 96.2, 96.3, 96.4, 96.8 (C-1', C-1"', C-1", C-1) |

## 4.3 Experimental

### Glycosidation reaction

Per-O-benzylated 2-pyridyl thioglycosides (glycosyl donors (0.01-0.05 mmol) were reacted with sugar alcohols (glycosyl acceptors (0.015-0.075 mmol) in dry $CH_2Cl_2$ (having 3% methyl iodide) in the presence of molecular sieves (4Å). The mixture was stirred at 50°C for 26-44 h. Then diluted with $CH_2Cl_2$ (10 ml), filtered, dried and purified by flash chromatography using silica gel (pet. ether:ethyl acetate 8:2-8:3).

**2-Pyridyl 2,3,4-tri-O-acetyl-1-thio-β-D-glucopyranoside (29) - (disarmed sugar)**

2-Pyridyl 2,3,4,6-tetra-O-acetyl-1-thio- β-D-glucopyranoside **14** (2.1 g, 4.75 mmol) was deacetylated (cat. NaOMe-MeOH, 25 ml, 2h, rt) and neutralized (IR 120 $H^+$ resin) to afford the deacetylated product (quantitative). This compound (4.75 mmol) was dissolved in pyridine (5 ml), cooled to 0°C and t-butyl dimethylsilyl chloride (TBDMS-Cl, 0.88 g, 5.86 mmol) was added under stirring. The reaction completed in 30 min by the appearence of a faster moving spot (pet. ether:ethyl acetate 1:2). Then to the reaction mixture was added acetic anhydride (0.77 ml, 7.03 mmol) and stirred at room temperature (19 h). Crushed ice was added to the reaction mixture diluted with dichloromethane (25 ml), the organic layer washed with water (3x50 ml), evaporated and dried to afford a syrup. This syrup was purified by column chromatography ($SiO_2$, pet.ether:ethyl acetate 2:1) and was crystallized in dry ether to obtain yellowish crystals. m.p. 99-100°C. From $^1$H-nmr data it was characterized as 2-pyridyl 2,3,4-tri-O-acetyl-6-O-t-butyl dimethylsilyl 1-thio-β-D-t-butyl glucopyranoside **28** (2.36 g, 95% yield).

$[\alpha]_D^{25}$ +5.6 (c 1.0, $CHCl_3$).

$^1$H-nmr (90 MHz): 0.83 (s, 9H, TBDMS), 2.0, 2.01 (2s, 9H, 3xOAc), 3.70-5.6 (m, 6H), 5.76 (d, 1H, $J_{1,2}$=10, H-1), 7.0-8.5 (m, 4H, SPy).

Compound **28** on treatment with 2% p-toluene sulfonic acid in methanol (10 ml) for 3 h provided **29** (1.8 g, 90%), after neutralization with basic ion-exchange resin (Tulsion A-36 MP). The progress of the desilylation was monitored by t.l.c. in benzene:ethyl acetate 3:1. Filteration of the neutralized solution, drying, followed by crystallization (10% pet.ether in ether) afforded **29** as a crystalline yellowish solid (90% yield).

$[\alpha]_D^{25}$ +13° (c 1.0, $CHCl_3$); m.p. 139-141°C.

$^1$H-nmr (90 MHz): 2.01, 2.03 (2s, 9H, 3xOAc), 3.44-3.88 (m, 3H, H-5,6,6'), 4.88-5.5 (m, 3H, H-2,3,4), 5.75 (d, 1H, $J_{1,2}$=10, H-1), 6.8-8.5 (m, 4H, SPy).

**Selected $^{13}$C-nmr data (22.3 MHz):** 20.4 (q, 3xOCO<u>C</u>$H_3$), 81.5 (d, C-1).

**2-Pyridyl 2,3,4-tri-<u>O</u>-acetyl-6-<u>O</u>-(2,3,4,6-tetra-<u>O</u>-benzyl- α -D-glucopyranosyl)-1-thio-β-D-glucopyranoside (30)**

The saccharide coupling of 2-pyridyl 2,3,4,6-tetra-<u>O</u>-benzyl-1-thio-β-D-glucopyranoside **(13)** (0.64 g, 1 mmol) with the sugar alcohol **29** (0.48 g, 1.2 mmol) for 36 h according to the general glycosidation procedure after work up and chromatographic purification (pet.ether: ethyl acetate 1:1) afforded the α-linked disaccharide **30** (0.6 g, 66%) as a syrup.

$[\alpha]_D^{25}$ +20.2° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 2.01, 2.03, 2.05 (3s, 9H, 3xOAc), 3.45-5.4 (m, 22H), 5.72 (d, 1H, $J_{1,2}$=9, H-1), 6.8-8.5 (m, 24H, Ph and SPy).

$^{13}$C-nmr (75 MHz): 20.1 (q, 3xOCO<u>C</u>H$_3$), 81.7 (d, C-1), 96.9 (d, C-1'), 120.6, 122.8, 136.6, 149.4, 155.3 (SPy), 169.4, 170.0x2 (2s, O<u>C</u>OCH). Analysis calcd. for $C_{51}H_{55}NO_{13}S$: C, 66.43; H, 6.01. Found: C, 66.32; H, 6.13%.

**2-Pyridyl 2,3,4-tri-<u>O</u>-benzyl-6-<u>O</u>-(2,3,4,6-tetra-<u>O</u>-benzyl- α -D-glucopyranosyl)-1-thio-ß-D-glucopyranoside (31)**

The acetyl groups of **30** (0.46 g, 0.5 mmol, cat.NaOMe-MeOH, 20 ml, rt, 1 h, IR 120 $H^+$) were replaced by benzyl groups (0.5 mmol, DMF, BnBr, rt, 1 h) to obtain **31** (0.45 g, 85%) and purified by column chromatography (pet.ether:ethyl acetate 8:1.5) (syrup).

$[\alpha]_D^{25}$ +35.8° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 3.45-5.05 (m, 27H), 5.4 (d, 1H, $J_{1,2}$=9, H-1), 6.8-8.5 (m, 39H, Ph and SPy).

**Selected $^{13}$C-nmr (75 MHz):** 97.0 (d, C-1'), 120.1, 122.4, 136.5, 149.3, 157.2 (SPy), 127.3-138.7 (aromatic).

Analysis cacld. for $C_{66}H_{67}NO_{10}S$: C, 74.34; H, 6.33. Found: C, 74.33; H, 6.31%.

**<u>O</u>-(2,3,4,6-Tetra-<u>O</u>-benzyl-α -D-glucopyranosyl)-(1 → 6)-<u>O</u>-(2,3,4-tri-<u>O</u>-benzyl- α-D-glucopyranosyl)-(1 → 6)-1,2:3,4-di-<u>O</u>-isopropylidene-α -D-galactopyranoside (32)**

The saccharide coupling of **31** (0.22 g, 0.2 mmol) with diacetonegalactose[222] (0.064 g, 0.24 mmol, 5 ml $CH_2Cl_2$) was performed (general glycosidation procedure) in 29 h. The reaction mixture after workup and chromatographic purification (n-hexane:diethylether:ethyl acetate 6:2:1) afforded **32** (0.156 g, 64%) as a syrup.

$[\alpha]_D^{25}$ +46.8° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 1.33, 1.37, 1.5, 1.57 (4s, 12H, isopropylidene methyls), 3.4-5.1 (m, 34H), 5.55 (d, 1H, $J_{1,2}$=5, H-1), 7.0-7.5 (m, 35H, Ph).

$^{13}$C-nmr (75 MHz): 24.6, 24.8, 26.0x2 [(4q, 2x C($\underline{C}H_3)_2$], 96.2, 96.4, 97.3 (3d, C-1, C-1',C-1''), 108.1, 108.5 [2s, -$\underline{C}(CH_3)_2$], 127.3-138.8 (aromatic).

Analysis calcd. for $C_{73}H_{82}O_{16}$: C, 72.14; H, 6.8. Found: C, 72.24; H, 6.71%.

**$\underline{O}$-(2,3,4,6-Tetra-$\underline{O}$-benzyl-α -D-glucopyranosyl)-(1 → 4)-$\underline{O}$-(2,3,6-tri-$\underline{O}$-benzyl-α -D-glucopyranosyl)-(1 → 6)-2-pyridyl 2,3,4-tri-$\underline{O}$-acetyl-1-thio-β -D-glucopyranoside (34)**

The saccharide coupling of 2-pyridyl 2,3,6-tri-$\underline{O}$-benzyl-4-$\underline{O}$-(2,3,4,6-tetra-$\underline{O}$-benzyl-α -D-glucopyranosyl)-1-thio-β -D-glucopyranoside **33** (Chapter 3) (1.07 g, 1 mmol), 10 ml, $CH_2Cl_2$) with the sugar alcohol **29** (0.48 g, 1.2 mmol) employing standard glycosidation (general glycosidation procedure) conditions (44 h) after workup followed by column chromatographic purification (pet.ether:ethyl acetate 2:1) afforded the α-linked trisaccharide **34** (0.73 g, 54%) as a colourless syrup.

$[\alpha]_D^{25}$ +44.7° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 2.0, 2.04, 2.08 (2s, 9H, 3xOAc), 3.38-5.4 (m, 33H), 5.61 (d, 1H, $J_{1'',2''}$=3.6, H-1''), 5.67 (d, 1H, $J_{1,2}$=10, H-1), 6.8-8.5 (m, 39H, Ph and SPy).

$^{13}$C-nmr (75 MHz): 20.5 (q, 3xOCO$\underline{C}H_3$), 96.6, 96.7 (2d, C-1', C-1''), 120.7 -155.6 (Ph and SPy), 169.3, 170.1x2 (3s, O$\underline{C}$OCH$_3$).

Analysis calcd. for $C_{78}H_{83}NO_{18}$: C, 69.16; H, 6.18. Found: C, 69.02; H, 6.27%.

**O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-α -D-glucopyranosyl)-(1 → 6)-2-pyridyl 2,3,4-tri-O-benzyl-1-thio-β-D-glucopyranoside (35)**

The acetyl protecting groups of **34** (0.67 g, 0.5 mmol, cat. NaOMe-MeOH, rt, 1 h, IR 120 $H^+$) were replaced by benzyl groups (DMF-NaH-BnBr, rt, 2 h) to obtain **35** (0.55 g, 75% yield) as a colourless syrup.

$[\alpha]_D^{25}$ +43° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 3.2-5.0 (m, 36H), 5.13 (d, 1H, $J_{1',2'}$=3.6, H-1'), 5.37 (d, 1H, $J_{1,2}$=10, H-1), 5.67 (d, 1H, $J_{1'',2''}$=3.67, H-1''), 6.9-8.5 (m, Ph and SPy).

**Selected $^{13}$C-nmr data:** 96.7, 96.9 (2d, C-1', C-1'').

Analysis calcd. for $C_{93}H_{95}NO_{15}S$: C, 74.53; H, 6.39. Found: C, 74.42; H, 6.25%.

**O-(2,3,4,6-Tetra-O-benzyl- α -D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-benzyl-α -D-glucopyranosyl)-(1 → 6)-O(2,3,4-tri-O-benzyl-α -D-glucopyranosyl)-(1→6)-1,2,3,4-di-O-isopropylidene-α-D-galactopyranoside (36)**

The saccharide coupling of compound **35** (0.3 g, 0.1 mmol in 5 ml $CH_2Cl_2$) with diacetonegalactose[222] (0.12 g, 0.44 mmol) employing general glycosidation procedure provided (22 h) the α-linked tetrasaccharide **36** (0.188 g, 57% yield) as a syrupy material, after the workup and column chromatography( n-hexane:ethyl acetate 8:2).

$[\alpha]_D^{25}$ +26° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): 1.27, 1.33, 1.45, 1.55 (4s, 12H-isopropylidene methyls), 3.38-5.0 (m, 44H), 5.13 (d, 1H, $J_{1'',2''}$=3.6, H-1''), 5.56, 5.67 (2d, 2H, (merged), H-1,H-1'''), 5.7 (d, 1H, $J_{1',2'}$=3.6, H-1'), 7.15-7.45 (m, 50H, aromatic).

**Selected $^{13}$C-nmr data (75 MHz, $CDCl_3$):** 24.5, 24.8, 25.9x2 (4a, 2x$C(\underline{C}H_3)_2$), 96.8 (d, C-1), 96.2, 96.4, 96.3 (3d, C-1',1",1'"), 126.7-128.2 (aromatic). Analysis cacld. for $C_{100}H_{110}O_{21}$: C, 73.29; H, 6.21. Found: C, 72.9; H, 6.11%.

# CHAPTER 5

## STEREOSELECTIVE SYNTHESIS OF β-LINKED (1,2-*trans*-CONFIGURATION) GLYCOSIDES, BY USE OF 2-PYRIDYL THIOGLYCOSIDES WITH ( A C-2 PARTICIPATING GROUP) AS GLYCOSYL DONORS AND METHYL IODIDE AS AN ACTIVATOR

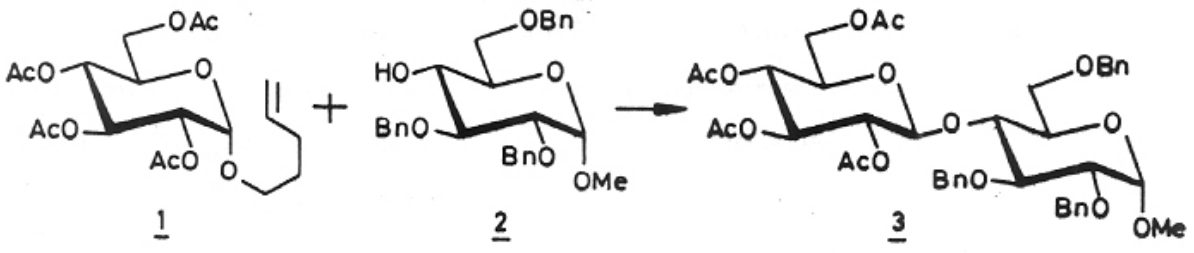
## 5.1 Introduction

A brief review on β-glycosidations is described in Chapter 1. (Section 1.3.1)

During the work on n-pent-4-enyl glycosides Fraser-Reid et al have observed that **"source of iodonium ion"** (promoter) changed the rate of reaction in glycosidations[129]. This observation permitted ready reactivity of even disarmed substrates[129].

Thus the 2-O-acetate (1) which failed to react on activation with IDCP was found to react readily with NIS/TfOH (N-iodosuccinimide/ trifluoromethane sulfonic acid). Thus it was reported that even with disarmed substrates (1), reaction was virtually instantaneous, with 2 yielding 1,2-trans product 3 due to neighbouring group participation[129].

**Scheme 1**




## 5.2 Results and discussion

The successful demonstration of α-glycosidation methodology by use of 2-pyridyl thioglycosyl donors (4) with a non participating group prompted us to look into the use of 2-pyridyl thioglycosides with a C-2 participating group (7) under similar conditions of methyl iodide activation to obtain saccharides with 1,2-trans relationship.

It was however clear that per-O-acetylated 2-pyridyl thioglycoside 12 resisted glycosidation (3 days) resulting in the isolation of the unreacted starting material (Chapter 4; Table 1). Even change of promotors

did not have any effect on the reactivity of **12** (Chapter 2).

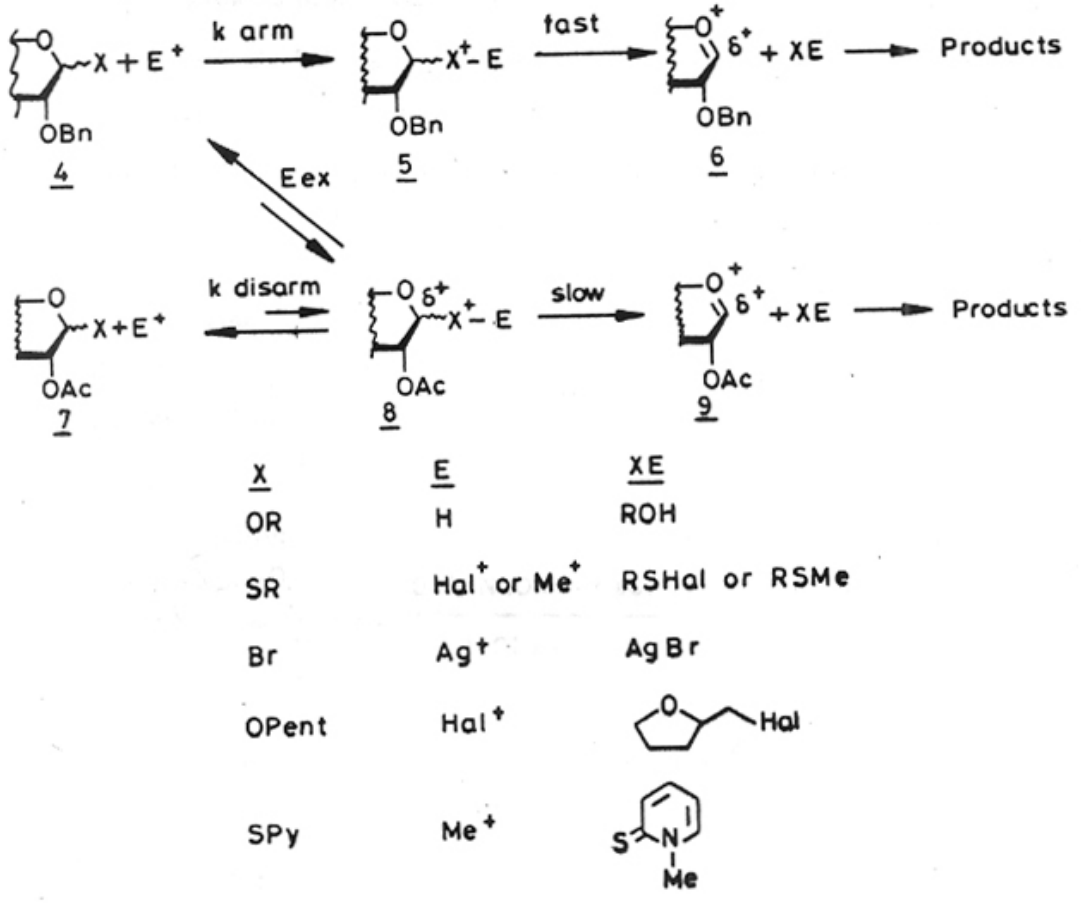
However, a totally useful observation that the benzoyl ester **13** and 2-acetamido-2-deoxy **11** 2-pyridyl thioglycosides were reacted on activation by methyl iodide to yield products with 1,2-trans relationship. These observations are also consistent with the methanolysis experiments (methyl iodide activation) performed with change of substitution on C-2 (Table 1: Chapter 4). It was found that 2-acetamido-2-deoxy-derivative **11** was more reactive than the 2-O-benzoyl ester **13**, which in turn was more reactive than the 2-O-acetyl ester **12**.

### 5.2.1 Rationalization of the Armed/Disarmed methodology, some mechanistic aspects of this phenomenon:

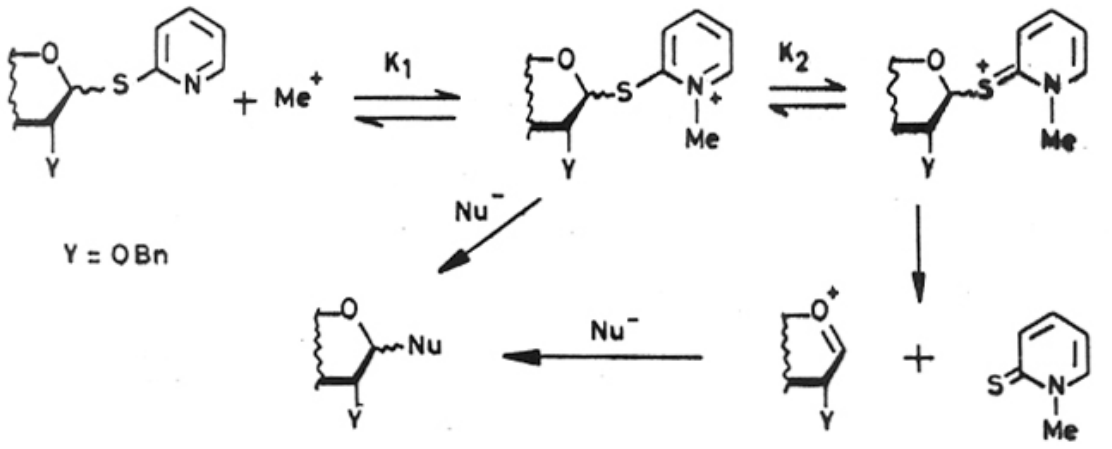
A survey of the carbohydrate literature revealed that the C-2 esters are less reactive than the corresponding ethers[224-226]. Thus (a) alkyl glycosides with a C-2 ester[247] or C-2 halide[248] undergo acid-catalyzed hydrolysis much more slowly than the corresponding C-2 ethers, (b) acetylated glycosyl bromides are more stable than their corresponding benzylated analogs and (c) glycosyl imidate esters have been reported by Schmidt to react more slowly when esterified at C-2 position[249].

The activated intermediates **5** and **6** are formed in equilibrium reactions with the electrophile $E^+$. Thus, the equilibrium constant K arm (or K disarm) is a combination of $K_1$, $K_2$ in Scheme 2a. Intermediate **8** is destabilized by the contiguous ($\delta^+$) and (+) charges, and hence the equilibrium lies on the side of **7**. On the contrary intermediate **5** does not suffer and hence reacts faster to give the reactive intermediate **6**.

## Scheme 2



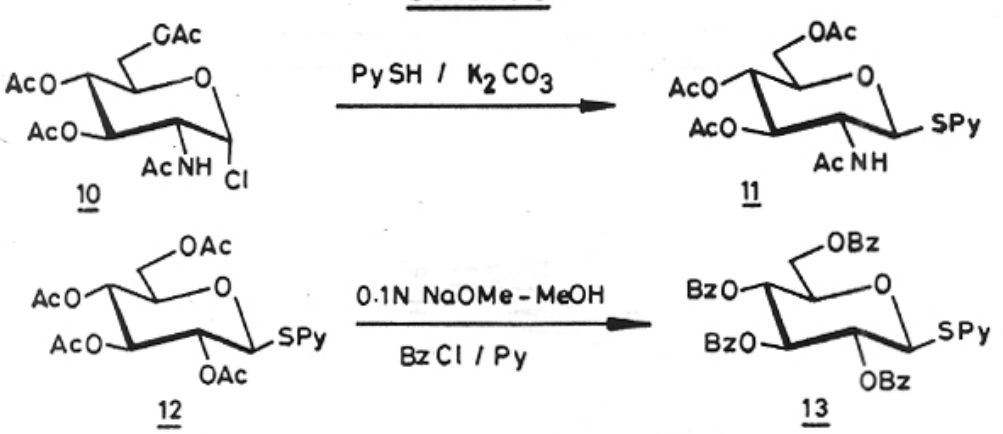

## Scheme 2a



### 5.2.2 Synthesis of β-linked glycosides

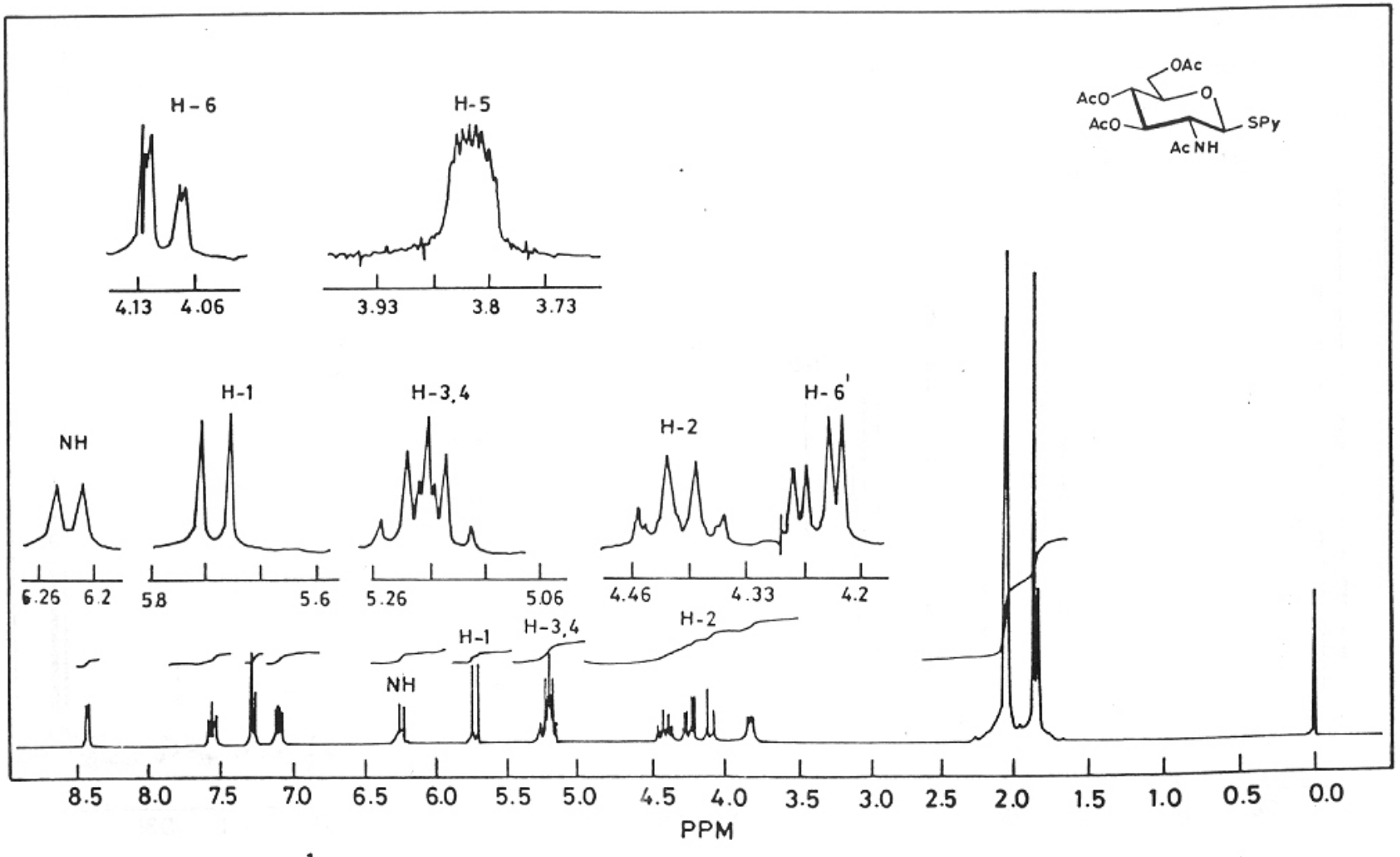
Thus partnering of compound **11** and **13** with simple alcohols and sugar alcohols afforded the β-linked (1,2-trans configuration) glycosides (**14-19**)[251] in good yields (Table). The β-selectivity in these reactions was due to the neighbouring group participation of C-2 substituent.

**Scheme 3**

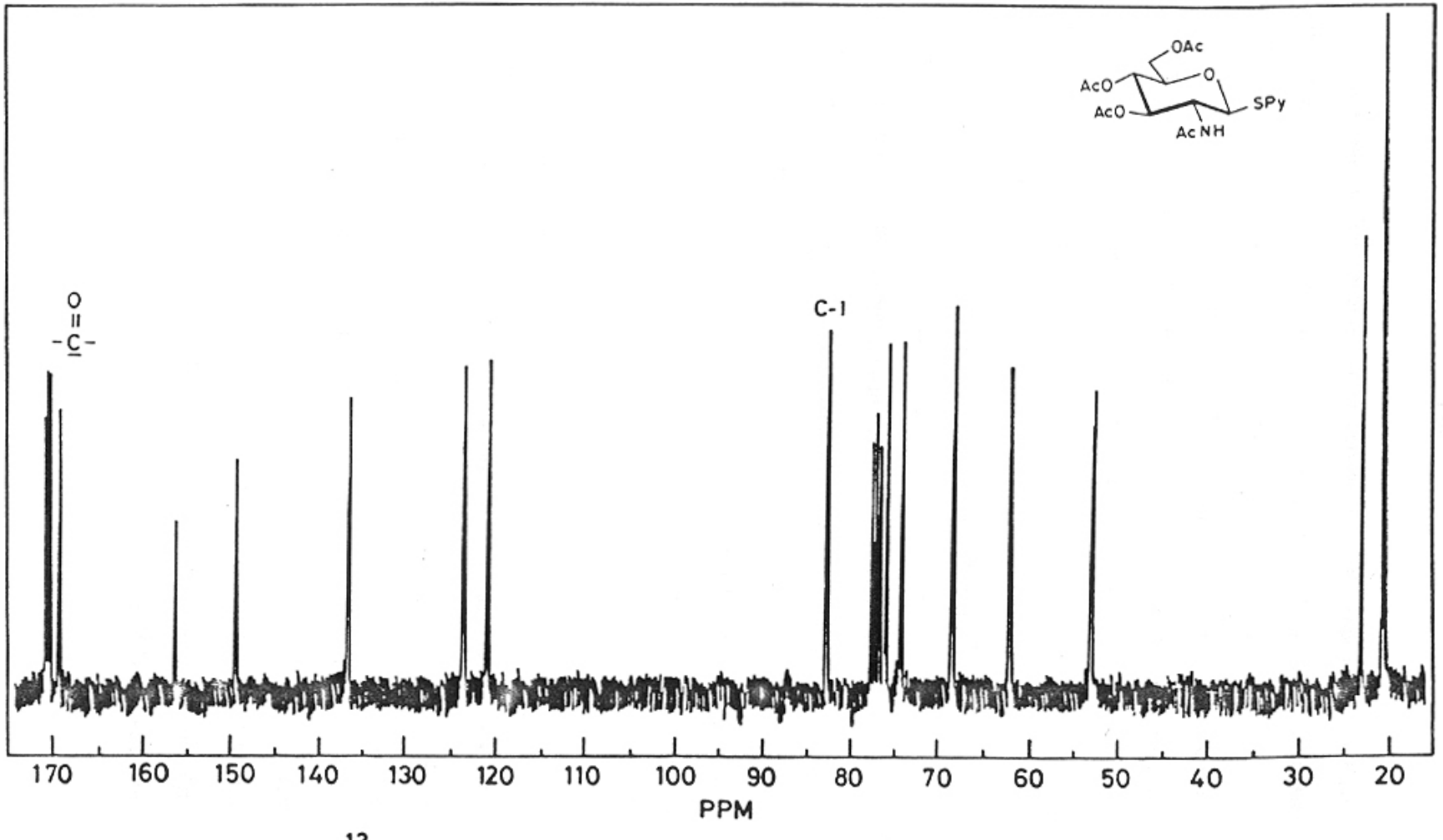



Compound **11** was prepared from known[250] 2-acetamido-2-deoxy 3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride (**10**) by treatment of anhydrous $K_2CO_3$ and 2-mercaptopyridine (Scheme 3). Compound **13** was prepared from **12** on deacetylation (2.5.2) followed by benzoylation (BzCl/Py) in 87% yield.

Compound **11** was characterized from $^1$H-nmr $^{13}$C-nmr, optical rotation, m.p. and elemental analysis. $^1$H-nmr of **11** showed singlets at δ 1.84, 2.0, 2.05, 2.1 for NHAc, 3xOAc, a doublet at 5.74 ($J_{1,2}$= 10.5 Hz) for H-1, consistant with β-configuration at the anomeric center. $^{13}$C-nmr of **11** showed four quartets at δ 20.5, 20.6, 20.7 and 22.9 for 4xCOCH$_3$, a doublet at δ 82.6 for C-1 and characteristic thiopyridyl signals.




$^1$H NMR SPECTRUM OF COMPOUND 11 (300MHz, $CDCl_3$)



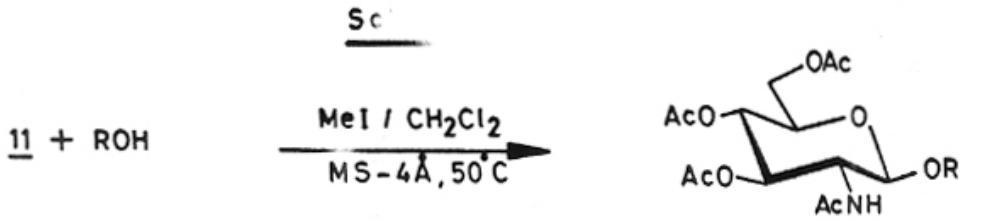

$^{13}$C-NMR SPECTRUM OF COMPOUND 11 (75MHz; $CDCl_3$)

The saccharide coupling of compound **11** with isopropanol, afforded Isopropyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β -**D**-glucopyranoside (**14**) in 80% yield as a syrup (Scheme 4). Compound **14** showed in its $^{1}$H-nmr spectrum a doublet at δ 4.85 ($J_{1,2}$=8.3 Hz) for H-1 and a doublet at δ 5.76 (J=8 Hz) for NH and two doublets at δ 1.13, 1.23 for isopropyl methyl groups. $^{13}$C-nmr spectrum showed at δ 99.1 ppm (C-1) and δ 55.3 ppm for O-CH$(CH_3)_2$ signals that were in accordance with the assigned structure.

Compound **11**, on reaction with t-butanol under standard glycosidation conditions gave β -linked glycoside t-butyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-**D**-glucopyranoside (**15**) in 78% yield as a syrup (Scheme 4). Compound **15** was characterized from its $^{1}$H-nmr spectrum, which showed characteristic H-1 resonance, as a doublet at δ 4.94 ($J_{1,2}$=8.5 Hz), a broad doublet for NH at δ 5.63 and a singlet for nine protons of tertiary butyl group at δ 1.23. $^{13}$C-nmr spectrum of **15** showed a signal at δ 95.1 for C-1 and at δ 20.6 for the tertiary-butyl group (Table 4).

The disaccharide 1,2:3,4-Di-O-isopropylidene-6-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl- β -**D**-glucopyranosyl)-α -**D**-galactopyranoside (**16**) was obtained (36 h) by saccharide coupling reaction of compound **11** with diacetone galactose[222] under standard conditions (Chapter 3.2.2A) in 70% yield as a syrup (Scheme 4).

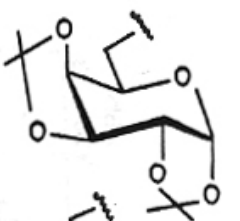
Compound **16** was characterized from its $^{1}$H-nmr spectrum (300 MHz), which showed characteristic doublets at δ 5.53 ($J_{1,2}$=5.5 Hz) for H-1, δ 4.71 ($J_{1',2'}$=8.5 Hz) for H-1' and δ 5.75 (J=8.5 Hz) for NH. $^{13}$C-nmr spectrum showed signals at δ 96.0 (C-1 ), δ 101.4 (C-1') and atδ 108.4, 109.1 (for isopropylidene-C$(CH_3)_3$).



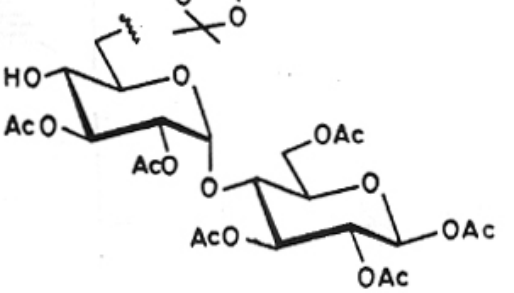

14 R = isoPropyl

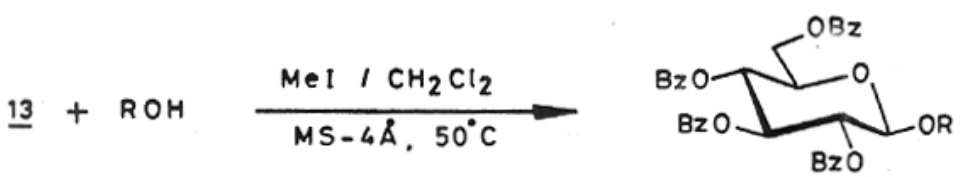
15 R = ter. Butyl

16 R =



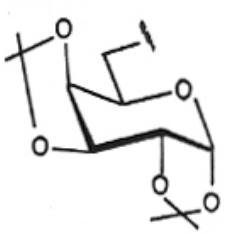
17 R =

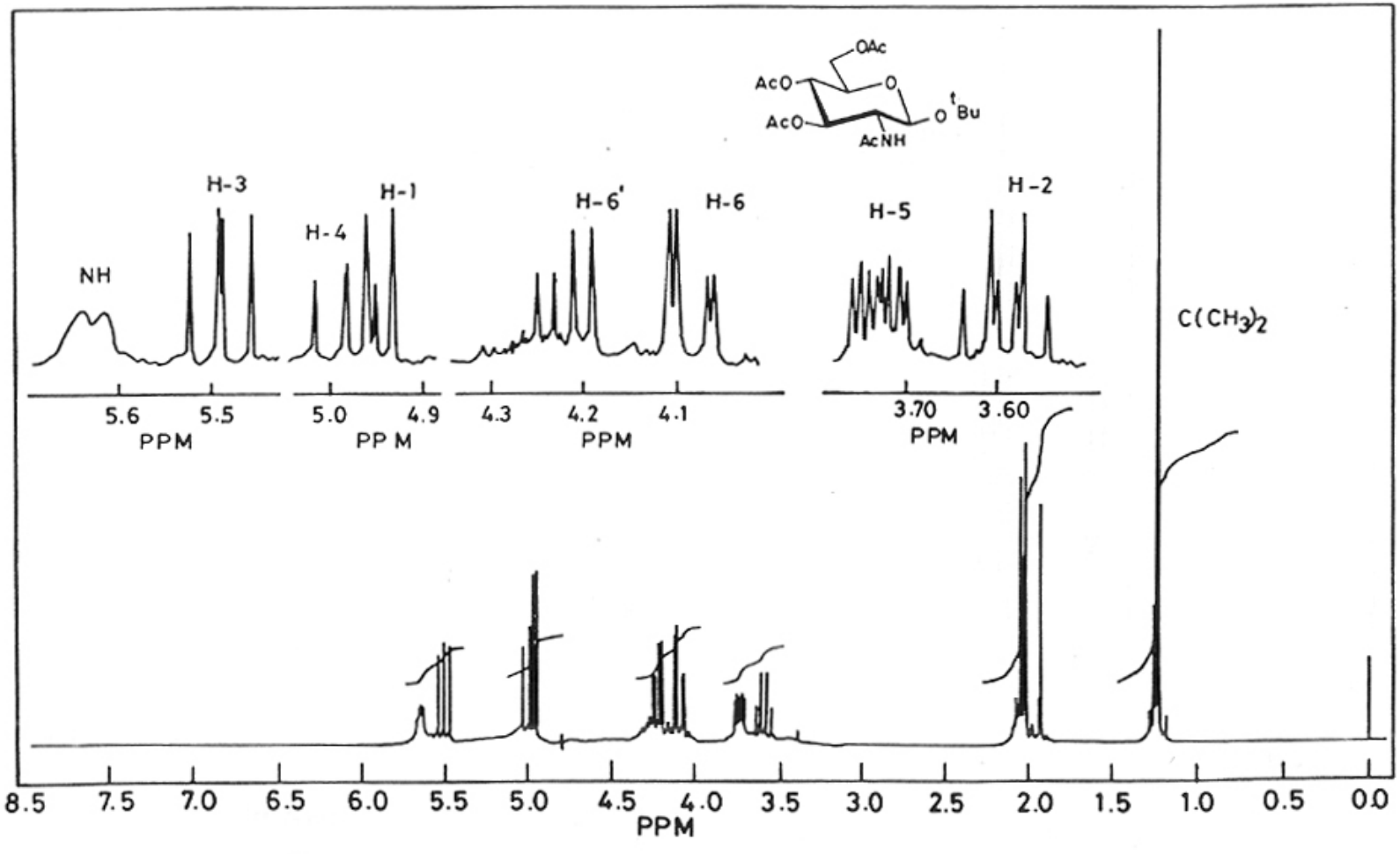


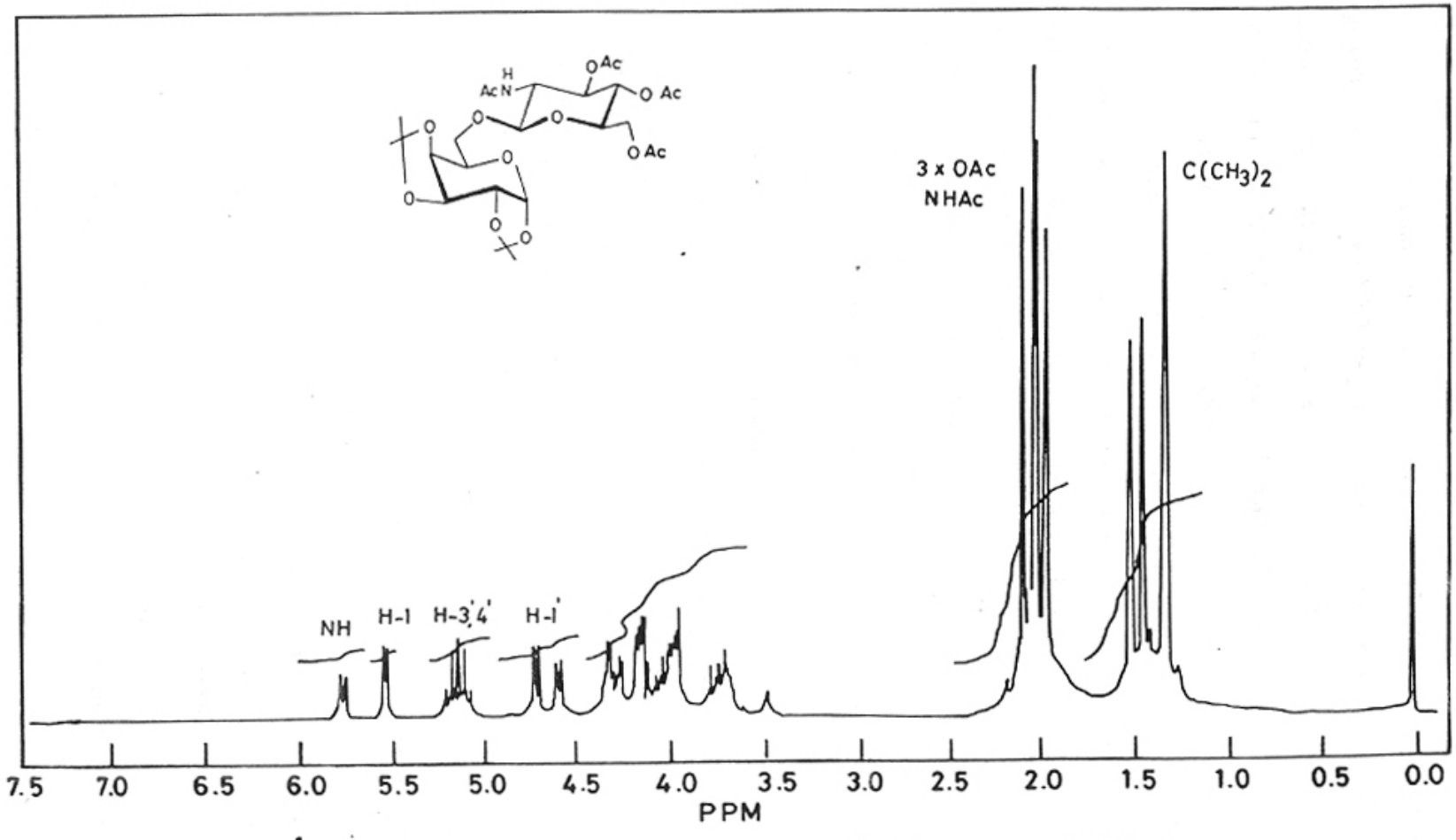

18 R = isoPropyl

19 R =






$^1$H NMR SPECTRUM OF COMPOUND 15 ( 300MHz , $CDCl_3$ )



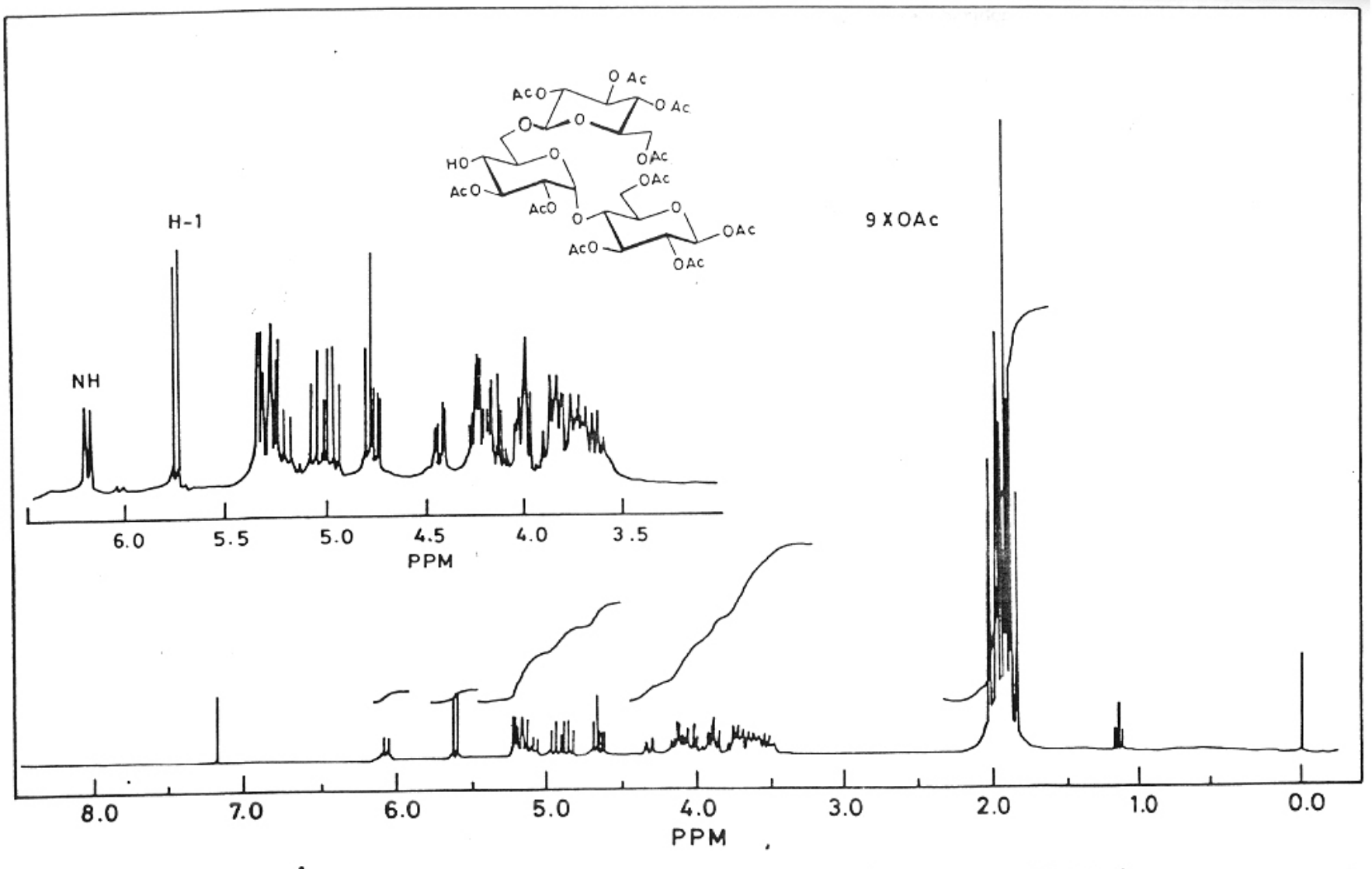

$^1$H NMR SPECTRUM OF COMPOUND 16 (300 MHz, $CDCl_3$)

A trisaccharide O-2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-**D**-glucopyranosyl (1 → 6)-O-2,3-di-O-acetyl-α-**D**-glucopyranosyl-(1 → 4)-1,2,3,4-tetra-O-acetyl-β-**D**-glucopyranoside (**17**) was prepared from the reaction of **11** with 1,2,3,4,2',3'-hexa-O-acetyl maltose[227] (42 h) in 55% yield as a crystalline compound (Scheme 4) recrystallized from ethyl acetate; m.p. 197-198°C). It was characterized from its nmr spectral data. $^{1}$H-nmr (300 MHz) of **17** showed nine singlets between δ 1.94-2.13 (8x-OCOC$\underline{H}_3$, NH-CO-C$\underline{H}_3$) two doublets at δ 5.72 ($J_{1,2}$=8.2 Hz) for H-1 and δ 6.17 (J=8.5 Hz) for NH respectively.

$^{13}$C-nmr spectrum of compound **17** showed signals at δ 91.4, 95.9 and 101.2 for C-1, C-1' and C-1" respectively. Other characteristic signals were in the region δ20.5 to 20.8 for 8xO-CO$\underline{C}H_3$ and NHCO$\underline{C}H_3$.

The saccharide coupling of compound **13** with isopropanol, under standard glycosidation conditions afforded in 72 h the β-linked glycoside Isopropyl 2,3,4,6-tetra-O-benzoyl-β-**D**-glucopyranoside (**18**, 56%) as a syrup. Compound **18** was characterized from its $^{1}$H-nmr spectrum, which showed two doublets at δ 1.07 and 1.28 for O-CH($\underline{C}H_3$)$_2$ and multiplets at δ 7.2-8.24 integrating for 20 protons.

Similarly compound **13** was coupled with the diacetone galactose[222], (Chapter 2) to afford (96 h) the (1 → 6)-β-linked disaccharide 1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzoyl-β-**D**-glucopyranosyl)-α-**D**-galactopyranoside (**19**) in 40% yield as a syrup. Compound **19** was characterized from its $^{1}$H-nmr spectrum, in which isopropylidene methyl singlets (12H) appeared at δ 1.28x2; 1.40, 1.52 and a doublet at δ 6.0 ($J_{1,2}$=8Hz) for H-1. The H-1' proton signal appeared overlaped with the ring protons. $^{13}$C-nmr spectrum of compound **19** showed two doublets at δ 96.2 and δ101.0 for C-1' and C-1 respectively, which was in accordance with the reported value[142].




$^1$H NMR SPECTRUM OF COMPOUND 17 ( 300 MHz, $CDCl_3$ )

Table

Physical characteristics of β-linked glycosides and saccharides

| Compound No. | $[\alpha]_D^{25}$ (c1.0, $CHCl_3$) | m.p. °C | Yield % | Selected $^1$H-nmr δ (J in Hz) | Selected $^{13}$C-nmr data ( δ ppm) |
|---|---|---|---|---|---|
| **11** | +21.4° | 140-141 | 64 | 1.84, 2.0, 2.05, 2.1 (NH Ac, 3xOAc), 5.74 (d, J=10.5, H-1) | 20.5, 20.6, 20.7, 22.9 (4X $COCH_3$), 82.6 (C-1) |
| **13** | +82.1° | 173-175 | 87 | 5.5 - 6.2 (H-1,2,3,4) 6.82 - 8.5 (Ph and SPy) | - |
| **14** | -4.2° | Syrup | 80 | 1.13, 1.23 (-$CH(CH_3)_2$) 4.85 (d, J=8.3, H-1) 5.76 (d, J=8, NH) | 55.3 (O-$CH(CH_3)_2$ 99.1 (C-1) |
| **15** | +10.2° | Syrup | 78 | 1.23 ($C(CH_3)_3$), 4.94 (d, J=8.5, H-1), 5.63 (br d, NH) | 56.1 ($C(CH_3)_3$) 95.1 (C-1) |
| **16** | -10.6° | Syrup | 70 | 5.53 (d, J=5.5, H-1) 4.71 (d-H-1'), J=8.5 5.75 (d, J=8.5, NH) | 96.0 (C-1); 101.4 (C-1'), 108.4, 109.1, $C(CH_3)_2$ |
| **17** | +42.8° | 197-198 | 55 | 1.94-2.13 (NHAc, 9xOAc) 5.72 (d, J=8.2, H-1) 6.17 (d, J=8.5, NH) | 91.4 (C-1'), 95.9 (C-1'') 101.2 (C-1) |
| **18** | -8.1° | Syrup | 56 | 1.07, 1.28 (-$CH(CH_3)_2$) 7.2-8.24 (Ph) | - |
| **19** | -21.5° | Syrup | 40 | 1.28 x 2, 1.4, 1.52 (isopropylidene, $CH_3$) 6.0 (d, J=4.8, H-1) | 96.2 (C-1') 101.0 (C-1) (Ref. 142) |

## 5.3 Experimental

### 5.3.1 Glycosidation procedure

The glycoside coupling of 2-pyridyl-2-acetamido-2-deoxy 3,4,6-tri-O-acetyl-1-thio-β -D-glucopyranoside (**11**) and 2-pyridyl per-O-benzoyl-1-thio-β -D-glucopyranoside (**13**) (0.3-0.5 mmol) with simple alcohols (5 mmol) and sugar alcohols (0.39-0.6 mmol) in dry dichloromethane (10 ml, having 3% methyl iodide) was carried out in the presence of molecular sieves -4A (0.1-0.2 g) at 50°C for 24-96 h workup and column chromatography ($SiO_2$, pet.ether:ethyl acetate 1:1) afforded β-linked glycosides in good yields (40-80%).

**2-Acetamido-2-deoxy 3,4,6-tri-O-acetyl- α -D-glucopyranosyl chloride (10)**[250]

$[\alpha]_D^{25}$ +110°; (c 1.0, $CHCl_3$), m.p.: 127-128°C.

$^1$H-nmr (90 MHz): δ 1.98, 2.01, 2.02, 2.11 (4s, 12H, NHAc, 3xOAc), 4.0-5.5 (m, 6H, H-2,3,4,5,6,6'), 5.84 (d, 1H, $J_{NH,2}$=9, NH), 6.15 (d, 1H, $J_{1,2}$=4.5 Hz, H-1).

**2-Pyridyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-1-thio- β -D-glucopyranoside (11)**

To a stirred solution of 2-mercaptopyridine (1.09 g, 9.8 mmol) and anhydrous $K_2CO_3$ (1.2 g, 10.6 mmol) in dry acetone (20 ml) was added **10** (3 g, 8.2 mmol) dissolved in toluene (10 ml). The reaction mixture was stirred at 50°C for 1 h diluted with toluene (50 ml). Organic layer was washed with water (anhyd. $Na_2SO_4$) evaporated, dried and recrystallized (pet. ether/$CH_2Cl_2$) to give **11** (2.31 g, 64%) as a yellow solid.

$[\alpha]_D$ +21.4° (c 1.0, $CHCl_3$); m.p.: 140-141°C.

$^1$H-nmr (300 MHz): δ 1.84, 2.0, 2.05, 2.10 (4s, 12H, NHAc, 3xOAc), 3.84 (ddd, 1H, $J_{4,5}$=7 Hz, $J_{5,6}$=4.5 Hz, $J_{5,6}$=2.0 Hz, H-5), 4.08 (dd, 1H, $J_{6,6'}$=12 Hz, H-6), 4.20 (dd, 1H, H-6'), 4.64 (ddd, 1H, $J_{1,2}$=10.5, $J_{2,3}$= $J_{2,NH}$=9.5 Hz, H-2), 5.16 (dd, 1H, $J_{3,4}$=7 Hz, H-4), 5.18 (dd, 1H, H-3), 5.74 (d, 1H, $J_{1,2}$=10.5, H-1), 6.24 (d, 1H, NH), 7.12-8.56 (m, 4H, SPy). $^{13}$C-nmr (75 MHz): δ 20.5, 20.6, 20.7, 22.9 (4q, NHCOCH$_3$, 3xOCOCH$_3$), 52.91 (t, C-6), 62.2, 66.5, 74.3, 75.9 (4d, C-2,3,4,5), 82.6 (d, C-1), 120.7, 123.4, 136.6, 149.3 (4d, C-3",4",5",6"), 157.2 (s, C-2"), 169.2, 170.5, 170.2 (4s, NHCOCH$_3$, 3xOCOCH$_3$).

Analysis calcd. for $C_{19}H_{24}N_2O_8S$: C, 51.81; H, 5.49. Found: C, 51.78; H, 5.37%.

**2-Pyridyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (13)**

The saccharide **12** (0.18 g, 0.66 mmol) was deacetylated, dried, dissolved in dry pyridine (3 ml) and cooled to 0° benzoyl chloride (0.34 ml, 2.97 mmol) was added dropwise brought to room temperature and stirred for 1 h. A faster moving spot appeared on t.l.c. (n-hexane:ethyl acetate 1:1) indicating the completion of reaction. The reaction mixture was diluted with cold water (5 ml), extracted into dichloromethane, and the organic phase was washed with cold 2% HCl (2x5 ml), followed by sat.NaHCO$_3$ and water (2x50 ml). Combined organic extracts were concentrated to obtain a syrup, which was recrystallized (dry ether) to give a crystalline **13** (0.4 g, 87%).

$[\alpha]_D^{25}$ +82.1° (c 1.0, CHCl$_3$); m.p.: 173-175°C.

$^1$H-nmr (90 MHz): 4.26-4.56 (m, 3H, H-5,6,6'), 5.5-6.2 (m, 4H, H-1, 2,3,4), 6.82-8.5 (m, 24H, Ph and SPy).

Analysis calcd. for $C_{39}H_{31}NO_9S$: C, 67.91; H, 4.53. Found: C, 67.89; H, 4.42%.

**Isopropyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl- β -D-glucopyranoside (14)**

The reaction of compound **11** (0.44 g, 1 mmol) with isopropanol (235 ml, 5 mmol) in dry $CH_2Cl_2$ (10 ml, having 3% methyl iodide) in the presence of moelcular sieves-4A, at 50°C 24 h, afforded **14** (0.31 g, 80%) after workup and chromatographic purification (pet.ether:ethyl acetate 1:1) as a syrupy material.

$[\alpha]_D^{25}$ -4.2° (c 1.0, $CHCl_3$).

$^1$H-nmr (300 MHz): δ 1.13, 1.23 (2d, 6H, J=6.2, $CH(CH_3)_2$), 1.95, 2.04, 2.05, 2.08 (4s, 12H, NHAc, 3xOAc); 3.6 (2q, 1H, O-CH-$(CH_3)_2$, 3.68 (ddd, 1H, $J_{4,5}$=10.0, $J_{5,6}$=4.5, $J_{5,6'}$=2.0, H-5), 3.95 (ddd, 1H, $J_{1,2}$=8.3, $J_{2,3}$= 9.3, $J_{2,NH}$=8, H-2), 4.13 (dd, 1H, $J_{6,6'}$=12, H-6), 4.24 (dd, 1H, H-6'), 4.85 (d, 1H, $J_{1,2}$=8.3, H-1), 5.03 (dd, 1H, $J_{3,4}$=9.3, H-4), 5.41 (dd, 1H, H-3), 5.76 (d, 1H, J=8, NH).

$^{13}$C-nmr (75 MHz): 20.5x2, 20.6, 21.9x2, 23.2 (6q, $NHCOCH_3$, $3xOCOCH_3$, O-CH-$(CH_3)_2$), 55.3 (d, O-CH$(CH_3)_2$), 62.3 (t, C-6), 69.1, 71.5, 72.3, 72 (4d, C-2,3,4,5), 99.1 (d, C-1), 169.4, 170.3, 170.6x2 (4s, $NHCOCH_3$, $3xOCOCH_3$).

Analysis calcd. for $C_{17}H_{27}NO_9$: C, 52.43; H, 6.99. Found: C, 52.34; H, 6.89%.

**t-Butyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl- β -D-glucopyranoside (15)**

Reaction of compound 11 (0.44 g, 1 mmol) with t-butanol (290 μl, 5 mmol) in $CH_2Cl_2$ (10 ml, having 3% methyl iodide) for 28 h, yielded **15** (0.31 g, 78%) as a syrup after workup and chromatographic purification (pet.ether:ethyl acetate 1:1).

$[\alpha]_D^{25}$ +10.2°.

$^1$H-nmr (300 MHz): δ 1.23 (s, 9H, -O-C(C$\underline{H}_3$)$_3$), 1.93, 2.01, 2.02, 2.05 (4s, 12H, NH$\underline{Ac}$, 3xO$\underline{Ac}$), 3.58 (ddd, 1H, $J_{1,2}$=8.5, $J_{2,3}$=10.5, $J_{2,NH}$=8.5, H-2), 3.73 (ddd, 1H, $J_{5,6}$=5.5, $J_{5,6}$=2.5, $J_{4,5}$=10, H-5), 4.08 (dd, 1H, $J_{6,6'}$/gem=12, H-6), 4.22 (dd, 1H, H-6), 4.94 (d, 1H, $J_{1,2}$=8.5, H-1), 4.98 (dd, 1H, $J_{4,5}$=10, $J_{3,4}$=9.5, H-4), 5.49 (dd, 1H, H-3), 5.63 (brd, 1H, N$\underline{H}$).
$^{13}$C-nmr (75 MHz): 20.7x3, 23.3, 28.5x2 (3q, NHCO$\underline{C}$H$_3$, 3xOCO$\underline{C}$H$_3$, 3x-C($\underline{C}$H$_3$)$_3$), 56.1 (s, -$\underline{C}$(CH$_3$)$_3$), 62.7, 69.4, 71.4, 72.2x2 (C-2,3,4, 5,6), 95.1 (d, C-1), 169.5, 170.1, 170.6x2 (4s, NH$\underline{C}$OCH$_3$, 3xO$\underline{C}$OCH$_3$).
Analysis calcd. for $C_{18}H_{29}NO_9$: C, 53.58; H, 7.25. Found: C, 53.49; H, 7.11%.

**1,2:3,4-Di-O-isopropylidene-6-O-(2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-α-D-galactopyranoside (16)**

The coupling of glycosyl donor **11** (0.44 g, 1 mmol) with diacetone-galactose[222] (0.39, 1.15 mmol) in dichloromethane (10 ml, having 3% methyl iodide) in 36 h yielded a syrupy, compound **16** (0.41 g, 70%) after workup and purification ($SiO_2$, pet.ether : ethyl acetate 1:2).
$[\alpha]_D^{27}$ -10.6° (c 1.0, $CHCl_3$).
$^1$H-nmr (300 MHz): δ 1.32x2, 1.45, 1.51 (4s, 12H,, C(C$\underline{H}_3$), 1.96, 2.02, 2.09, 2.17 (4s, 12H, NH$\underline{Ac}$, 3xO$\underline{Ac}$), m, 10H, H-2,3,4,5,6,6', 2',6'',6'''), 4.71 (d, 1H, $J_{1',2'}$=8.5, H-1'), 5.06-5.2 (m, 2H, H-3',4'), 5.53 (d, 1H, $J_{1,2}$=5.5, H-1), 5.75 (d, 1H, $J_{2,NH}$=8.5, N$\underline{H}$).
$^{13}$C-nmr (75 MHz): δ 20.3, 20.4, 20.5, 22.1, 24.0, 24.7, 25.7, 25.8 (8q, 4x, isopropylidene-$\underline{C}$H$_3$, NHCO$\underline{C}$H$_3$, 3x OCO$\underline{C}$H$_3$), 53.9, 61.9, 68.1, 68.2, 68.3, 68.8x2, 70.0, 70.3, 70.8, 71.5, 72.9 (C-2,3,4,5,6,6',2',3',4',5',6'',6'''), 96.0 (d, C-1'), 101.4 (d, C-1), 108.4, 109.1 (2s, 2x $\underline{C}$(CH$_3$)$_2$), 169.1, 170.4, 170.5, 170.7 (4s, NH$\underline{C}$OCH$_3$, 3xO.$\underline{C}$OCH$_3$).

Analysis calcd. for $C_{26}H_{39}NO_{14}$: C, 52.97; H, 6.67. Found: C, 52.79; H, 6.58 %.

**O-2-Acetamido-2-deoxy-3,4,6-tri-O-acetyl- α -D-glucopyranosyl (1 → 6)-O-2,3-di-O-acetyl- α-D-glucopyranosyl-(1 → 4)-1,2,3,4-tetra-O-acetyl-β -D-glucopyranoside (17)**

Reaction of compound **11** (0.22 g, 0.5 mmol) with 1,2,3,6-tetra-O-acetyl-4-O-(2,3-di-O-acetyl-α -D-glucopyranosyl)-β -D-glucopyranoside[237] (0.23 g, 0.39 mmol) in 42 h, yielded a single compound **17** (0.275 g, 55%), the β -linked trisaccharide, as a crystalline material (ethyl acetate) after workup and chromatographic purification ($SiO_2$, pet.ether:ethyl acetate 1:3).

$[\alpha]_D$ +42.8° (c 1.0, $CHCl_3$); m.p.: 197-198°C.

$^1$H-nmr (300 MHz): δ 1.94, 1.98, 1.99, 2.01, 2.02, 2.03, 2.05, 2.07, 2.08, 2.13 (10s, 30H, NHAc, 9xOAc), 3.5-5.4 (m, 20H), 5.72 (d, 1H, $J_{1,2}$=8.2, H-1), 6.17 (d, 1H, $J_{2,NH}$=8.5, NH).

**Selected $^{13}$C-nmr data (75 MHz):** 20.4x2, 20.5x3, 20.6x2, 20.8x2, 23.1 (10s, $NHCOCH_3$, $9xOCOCH_3$), 54.6, 62.0, 62.9, 68.4, 68.6, 69.2, 70.2, 71.0, 71.5, 71.6, 72.0, 72.2, 72.3, 73.1, 75.3 (15C, C-2,3,4,5,6,2',3',4',5', 6', 2",3",4",5" and 6"), 91.4, 95.9 (C-1,1'), 101.2 (C-1"), 168.7, 169.3, 169.5, 170.01x2, 170.7, 170.8x2, 170.9x2 (10s, $NHCOCH_3$, $9xOCOCH_3$). Analysis calcd. for $C_{38}H_{53}NO_{25}$: C, 49.4; H, 5.78. Found: C, 49.34; H, 5.62%.

**Isopropyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranoside (18)**

The reaction of **13** (0.39 g, 0.54 mmol) with isopropanol (235 nl, 5 mmol) in dry $CH_2Cl_2$ (7 ml, having 3% methyl iodide) in 72 h gave the β-linked glycoside **18** (0.2 g, 56%) upon workup and column purification ($SiO_2$, n-hexane:ethyl acetate 4:1) as a syrup.

$[\alpha]_D^{25}$ -8.1° (c 1.0, $CHCl_3$).

$^1$H-nmr (90 MHz): δ 1.07, 1.28x2 (2d, 6H, -CH($C\underline{H}_3$)$_2$, 3.85-6.26 (m, 8H, H-1,2,3,4,5,6,6' and -O.C$\underline{H}$($CH_3$)$_2$, 7.0-8.24 (m, 2H, aromatic).

**1,2:3,4-Di-O-isopropylidene-6-O-(2,3,4,6-tetra-O-benzoyl-β -D-glucopyranosyl)-αD-galactopyranoside (19)**

Coupling of compound **13** (0.39 g, 0.54 mmol) with diacetonegalactose[222] (0.27 g, 1.08 mmol) in dry $CH_2Cl_2$ (10 ml, having 3% methyl iodide) in 96 h, after workup and chromatographic purification ($SiO_2$, n-hexane:ethyl acetate 4:1) gave compound **19** (0.18 g, 40%), the β-linked disaccharide as a syrup.

$[\alpha]_D^{25}$ -21.5° (c 1.0, $CHCl_3$).

$^1$H-nmr (80 MHz): δ 1.28x2, 1.4, 1.52 (s, 12H, 2x C($C\underline{H}_3$)$_2$ ), 3.32-5.88 (m, 13H, H-2,3,4,5,6,6',1',2',3',4',5',6'',6'''), 6.0 (d, 1H, $J_{1,2}$=4.8, H-1), 7.12-8.12 (m, 20H, aromatic).

**Selected $^{13}$C-nmr data:** δ 96.2 (d, C-1'), 101.0 (d, C-1).

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