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MODIFIED DONOR AND ACCEPTOR SUBSTRATES FOR FUCOSYLTRANSFERASES

BY

UDAY B. GOKHALE 

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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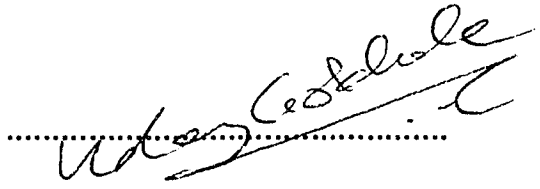

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MODIFIED DONOR AND ACCEPTOR SUBSTRATES FOR FUCOSYLTRANSFERASES.

submitted by UDAY B. GOKHALE

in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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(External Examiner) .....

Date :

Abstract

Fucosyl transferases are enzymes involved in the biosynthesis of the oligosaccharide portion of glycoproteins and glycolipids. The fucosyl transferases are bisubstrate enzymes which catalyze the transfer of L-fucose from guanosine-5'-diphospho fucose (GDP-fucose) onto the hydroxyl groups of oligosaccharide acceptor substrates. βDGal(1-->3)βDGlcNAc (type-1 structure) and βDGal(1-->4)βDGlcNAc (type-2 structure) are known to accept L-fucose at 2-OH of the galactose residue and 3-OH of the N-acetylglucosamine residue giving rise to the mono or di-fucosylated structures. In the present study, modified acceptor and donor substrates were synthesized with the objective of studying the molecular specificity of these enzymes. The acceptor substrate analogues : (2'-deoxy)-βDGal(1-->4)βDGlcNAc and βDGal(1-->4)-(3-deoxy)βDGlcNAc were synthesized as their n-octyl glycosides. The multistep syntheses of these disaccharides involved assembly of the corresponding monosaccharides in the β1-->4 linkage. The deoxygenations were performed at the disaccharide level on selectively functionalized intermediates.

Analogues of GDP-fucose modified on the fucose ring were also prepared. Thus the 3-deoxy, 4-deoxy and 5-nor analogues of

GDP-fucose were synthesized from the corresponding ring-modified fucosyl phosphates. The key reaction in the multistep syntheses of the equatorial fucosyl phosphate analogues was the stereoselective displacement on the corresponding anomeric bromides using tetra-n-butylammonium dibenzyl phosphate. These fucose-1-phosphate analogues were then coupled to activated forms of guanosine-5'-monophosphate to furnish the corresponding sugar-nucleotides. The synthesis of 3-deoxy-GDP-fucose represents the first chemical synthesis of naturally occurring sugar-nucleotide GDP-colitose. The solution conformations of the fucosyl phosphates and the target nucleotides were investigated by ^{1}H, ^{13}C and ^{31}P-nmr spectroscopy.

ACKNOWLEDGEMENTS

I would like to thank my research supervisor Dr. Ole Hindsgaul for his guidance and support throughout this work. The frequent assistance of our collaborator Dr. Monica Palcic and the members of her research group is also highly appreciated. I also thank Prof. R. U. Lemieux for his supervision in the initial stages of the present work.

This work could not have been completed without the informal help and friendship of the support staff of the Dept. of Chemistry. Special thanks are due to Mr. Glen Bigam and Mr. Tom Brisbane of the high field nmr laboratory for their invaluable help.

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I cannot thank enough my wife for her encouraging companionship during the trying period of my graduate studies and her creative assistance in the preparation of this manuscript.

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List of Schemes

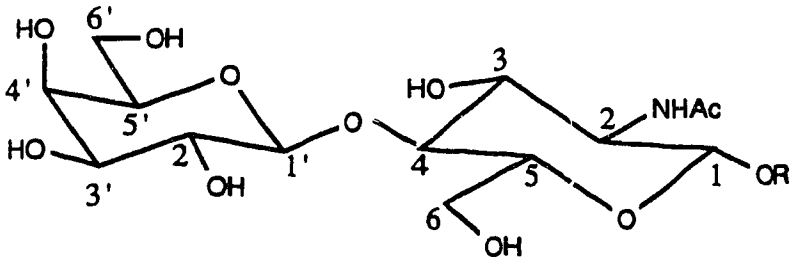
List of Abbreviations

Ac	acetyl
AcOH	acetic acid
AIBN	azo bis isobutyronitrile
Ar	aryl
Bn	benzyl
t-Bu	tertiary-butyl
Bz	benzoyl
CI	chemical ionization
DCC	dicyclohexylcarbodiimide
DMAP	4-dimethylamino pyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
Et	ethyl
FAB	fast atom bombardment
GMP	guanosine-5'-monophosphate
GDP	guanosine-5'-diphosphate
LDA	lithium diisopropylamide
Me	methyl
MS	mass spectrometry
NMR	nuclear magnetic resonance
Ph	phenyl
i-Pr	iso-propyl
PTSA	para-tolunesulfonic acid
Py	pyridine

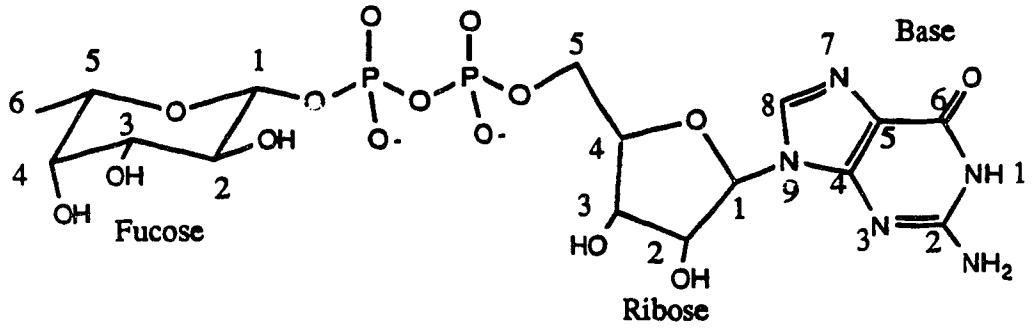
TCAI	trichloroacetyl isocyanate
THF	tetrahydrofuran
tlc	thin layer chromatography
TSP-d4	2,2,3,3-tetradeuterio-3-(trimethylsilyl)-propionic acid, disodium salt.

Numbering convention followed throughout this work

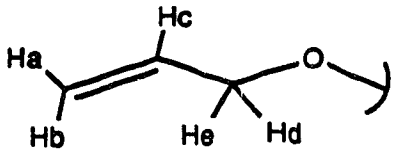
A disaccharide



A sugar-nucleotide



Protons of the allyl group are designated as follows



I. INTRODUCTION

Synthetic carbohydrate chemistry has traditionally been considered a specialized branch of synthetic organic chemistry[1]. A cursory glance at the recent literature of synthetic carbohydrate chemistry reveals that it is undergoing a dramatic transformation. It has contributed *chiral synthons* for the synthesis[1] of complex molecules of biological interest as well as adopted classical synthetic methodologies such as Diels-Alder reactions[2] which provide access to novel carbohydrates. Apart from these mutually enriching interactions with other branches of organic synthesis, synthetic carbohydrate chemistry itself has undergone a remarkable change. Equipped with versatile tools, like stereoselective glycosylation methodologies and selective functional group transformation strategies synthetic carbohydrate chemists have turned their attention to the construction of specific carbohydrate molecules[3] which play crucial roles in various biological processes. These investigations are guided mainly by the ever increasing understanding of the biological functions of the oligosaccharide units of glycoproproteins and/or glycolipids. These biological functions[4,5] include cell-cell recognition, cell adhesion, cell differentiation, the hepatic clearance of glycoproteins from blood, the compartmentalization of lysosomal enzymes, the binding of antibodies or lectins to cell surfaces, and the binding of toxins and microorganisms (viruses, bacteria) to the host cell. These functions are attributed to

glycoproteins[4] and glycolipids[6,7] in both animal and plant kingdoms.

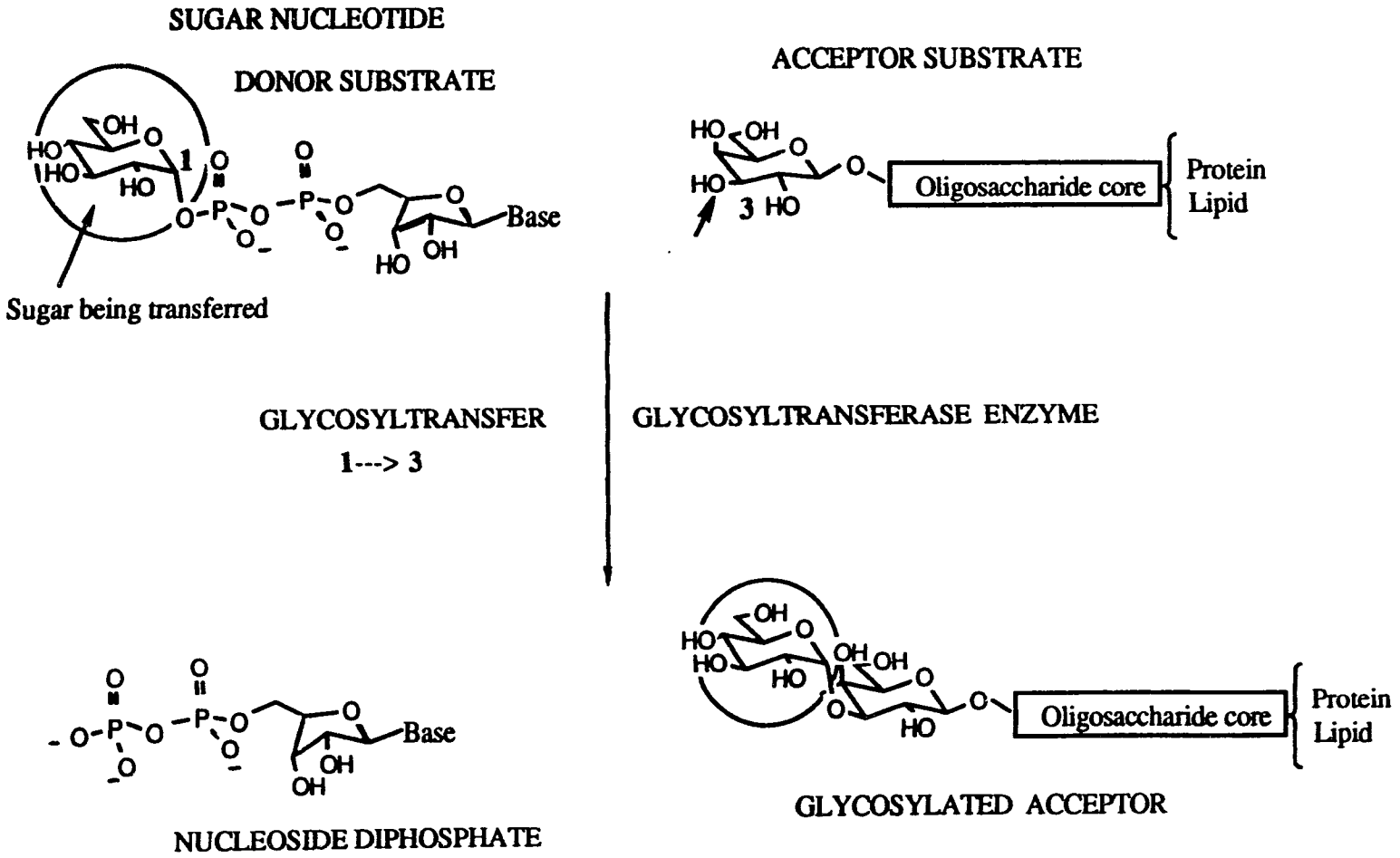
The biological importance of oligosaccharides has made this area one of intense investigation[8-11], especially in areas concerning molecular specificity and the nature of the binding sites of lectins, antibodies, and the enzymes which use oligosaccharide molecules for the expression of their biological activities. The more recent observations regarding the involvement of the cell surface carbohydrates in oncogenic transformation[12,13] have provided a significant impetus for further synthetic[14,15] and immunological[16,17] investigations.

Due to the inherent nature of the issues handled in the above investigations the research efforts need the contribution from such diverse disciplines as synthetic organic chemistry, theoretical chemistry, cell biology, genetics and immunology. This kind of interdisciplinary investigation has been in progress[18,19] in our laboratories with the major goal of understanding more about the molecular specificity of various carbohydrate binding proteins.

The present study addresses itself to the general area of oligosaccharide biosynthesis[20] with a particular emphasis on the biosynthesis of *oligosaccharide chains* of glycoproteins and glycolipids. As depicted in figure 1 the key step in the biosynthesis of oligosaccharides is the enzymatic transfer of the monosaccharide residues from the *donor substrate-sugar nucleotide* to the hydroxyl group of the *acceptor substrate*. The *donor substrate* is generally a nucleoside diphosphate molecule carrying the aldopyranose esterified on the terminal phosphate

FIG.1

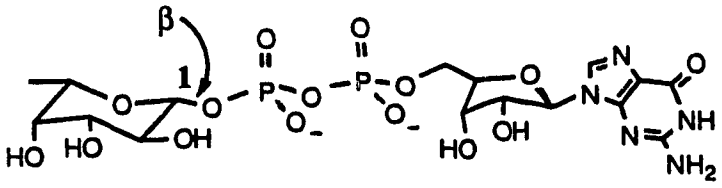
A GLYCOSYL TRANSFER CATALYZED BY A HYPOTHETICAL GLYCOSYLTRANSFERASE ENZYME.



residue. The *acceptor substrate* carries the hydroxyl group onto which the *glycosyl transfer* occurs.

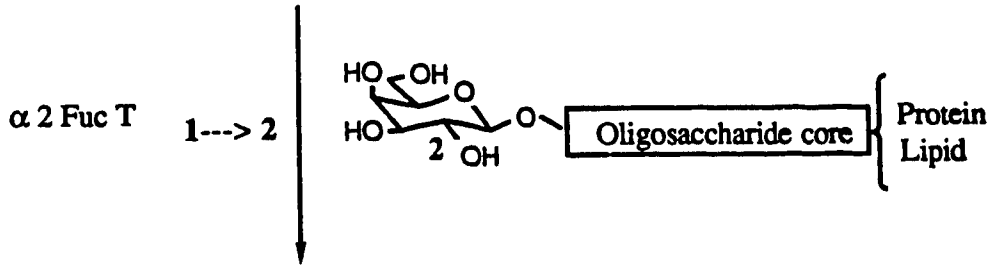
In eucaryotic cells the enzymes-*glycosyltransferases* (EC. 2.4 group) [21] are located in the Golgi apparatus where the multi step biosynthesis of oligosaccharide chains of glycoproteins takes place. One of the most striking features of the glycosyltransferases is the stringency of their specificity requirements that permits complex oligosaccharide structures to be precisely assembled in the absence of a template. As far as the donor substrates are concerned, all glycosyltransferases exhibit specificity toward both the sugar moiety and the nucleotide. In plants and microorganisms the same sugar may be carried by more than one nucleotide[21] although such glycosyltransferases show a marked preference, or absolute specificity, for only one carrier. In mammalian tissues the sugars commonly occurring in the oligosaccharide chains of glycoproteins, glycolipids and proteoglycans have so far been found associated each with only one nucleotide carrier[21]. Thus, although there is a family of mammalian galactosyltransferases capable of catalyzing the addition of D-galactose in either α- or β-anomeric sugars, the donor substrate for this family of enzymes is invariably UDP-galactose. Uridine diphosphate sugars also serve as donor substrates for the formation of glycosides of glucose, N-acetylglucosamine, N-acetylgalactosamine, xylose and glucuronic acid, whereas guanosine diphosphate sugars serve as nucleotide donors for the synthesis of mannosides and fucosides. The donor used by the sialyltransferases differs from the other nucleotide

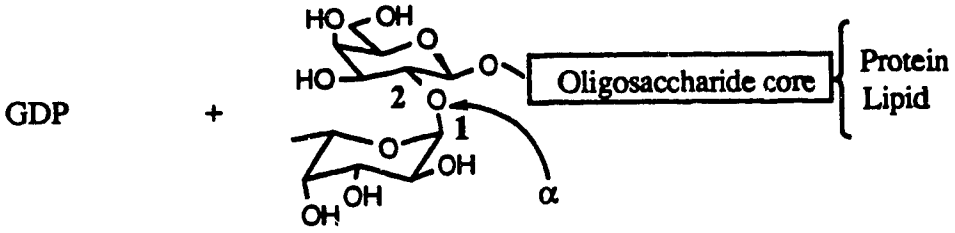
FIG. 2 (1----> 2) FUCOSYLATION CATALYZED BY α 2-FucT



GDP FUCOSE

DONOR SUBSTRATE





FUCOSYLATED PRODUCT
α 1---> 2 Linkage

carriers in being CMP-sialic acid, a monophosphate. The binding specificity of these glycosyltransferases toward the molecular and stereochemical features of both acceptor and donor substrates is being investigated in several laboratories[21,22,23].

The present research project deals with *fucosyl transferases* (FucTs)[21,22]. The reaction catalyzed by a fucosyl transferase[21] is depicted in figure 2 . It results in the transfer of L-fucose from guanosine-5'-diphosphofucose (GDP-fucose) onto an oligosaccharide acceptor :

GDP-Fuc + HO-acceptor --> GDP + Fuc α (1->O)-acceptor

In the acceptor the HO can be either a 2-, 3-, or 6-hydroxyl group of galactose, the 3-, 4- or 6-hydroxyl group of N-acetylglucosamine, or the 3-, or 4- hydroxyl group of glucose.

The acceptor oligosaccharide could be attached to the lipid (glycolipid) or to the protein (asparigine or serine/threonine linked glycoprotein). Fucose occurs exclusively in the α anomeric configuration linked at a nonreducing terminal position to either galactose, N-acetylglucosamine, or to the glucose residues of the acceptor oligosaccharides. These fucosylated structures have attracted a great deal of attention, primarily because they constitute the antigenic determinants of the human ABO and Lewis blood group systems[22]. Also, several fucosylated structures are implicated[13] as *tumor associated antigens* . The fucosyltransferases which catalyze the synthesis of these structures have been extensively studied for their specificity toward the acceptor substrates[22,31].

α2-FucTs, α3-FucTs, and α4-FucTs are the most extensively studied fucosyltransferases.[22] The acceptor substrate for these enzymes is a terminal disaccharide unit Gal β1->3 GlcNAc (the so-called type-1 disaccharide) or Gal β1->4 GlcNAc (type-2 disaccharide) of the oligosaccharide portion of glycoproteins or glycolipids. Figures 3 and 4 depict the enzymatic reaction products of these FucT activities.

α 2-FucTs transfer fucose to the 2-OH of the terminal galactose residue. *α3-FucTs* transfer fucose to the 3-OH of the subterminal N-acetylglucosamine. *α4-FucTs* transfer fucose to the 4OH of the subterminal N-acetylglucosamine residue. The genes which code for these enzymes have been identified[22] and the corresponding enzymes are conveniently named[22] after the gene which codes them. The following table includes four types of FucTs and their acceptor substrate characteristics.

Table-1

FucT	Accept Disac (Gal->GlcNAc)	Gal	GlcNAc	Characteristic Features
H-Enzyme.	Type-2	1->2	-	Expression on Red cells
X-Enzyme.	Type-2	-	1->3	Found in human serum
SeEnzyme	Type-1 Type-2	1->2 1->2	- -	Expressed in secretions
Le-Enzym.	Type-1 Type-2	-	1->4 1->3	Not found in human serum

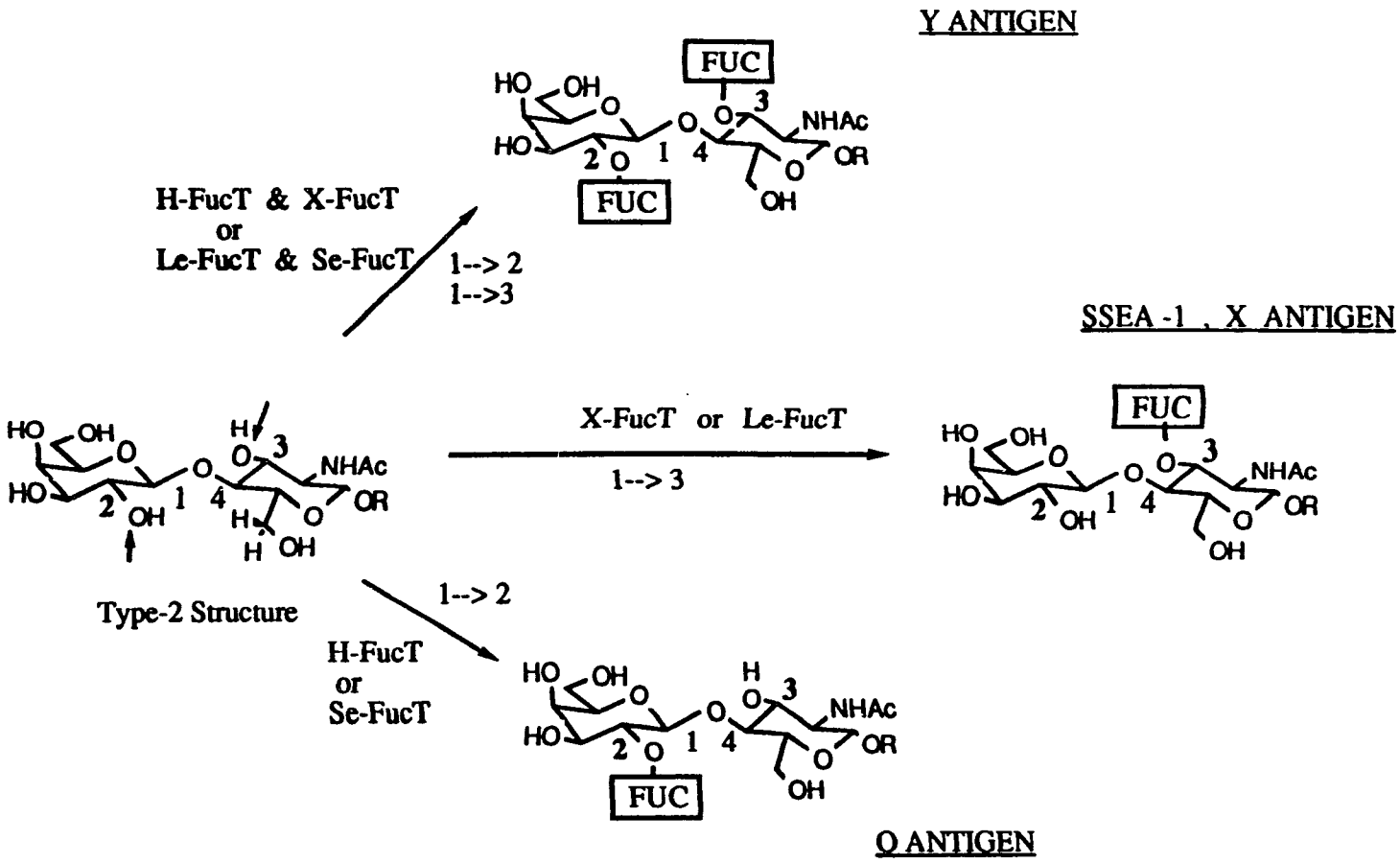


FIG 3 . TYPE-2 STRUCTURE AS ACCEPTOR SUBSTRATE FOR H-FucT, X-FucT, Le-FucT, Se-FucT.

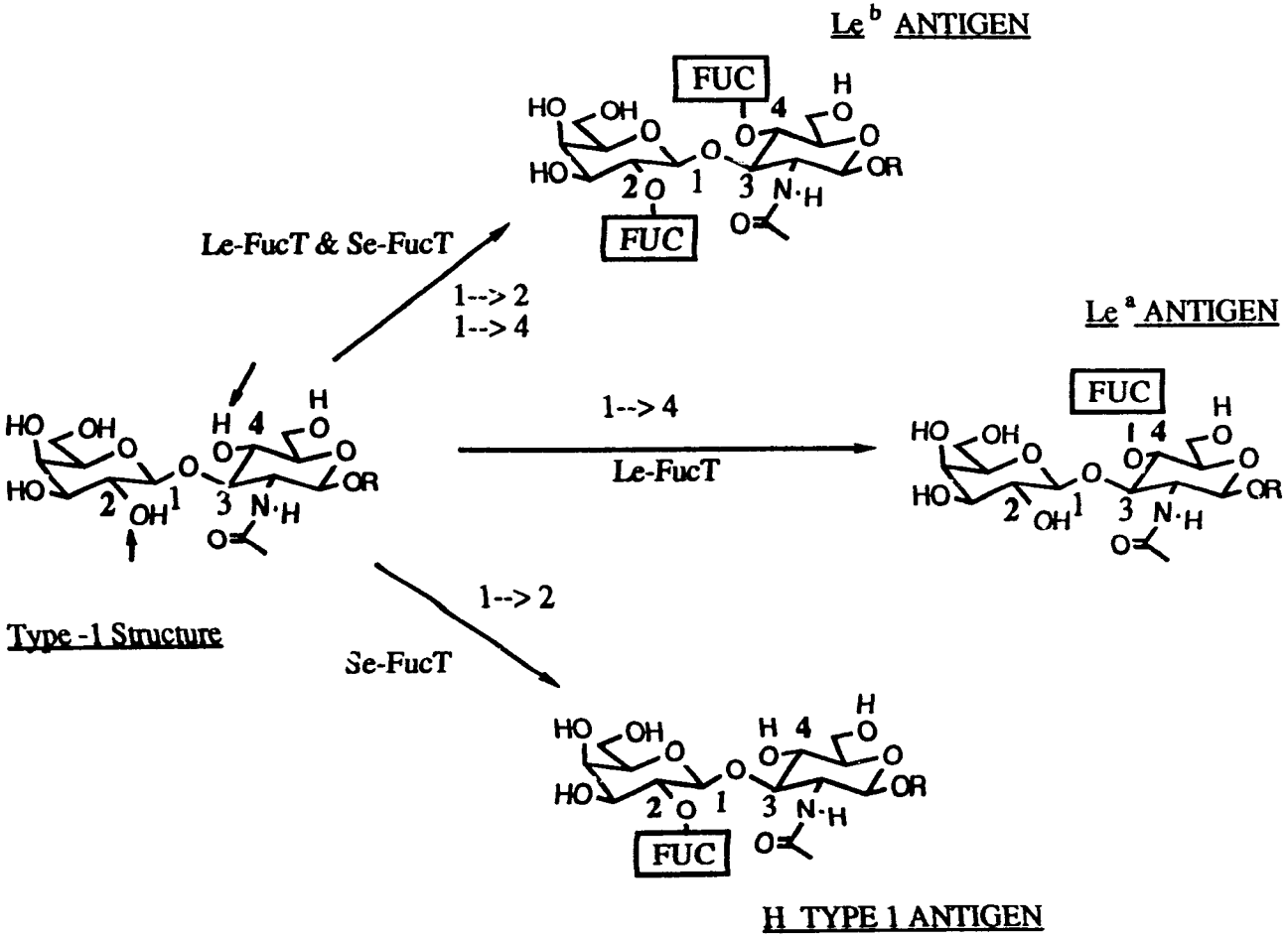


FIG. 4 . TYPE-1 STRUCTURE AS ACCEPTOR SUBSTRATE FOR Se-FucT , Le-FucT .

The H-Enzyme (entry 1 in Table 1) is a fucosyl transferase(**H**-FucT) encoded by the H-gene which transfers **L**-fucose from GDP-fucose onto the 2-OH of the galactose residue of only the *type-2* disaccharide; and the fucosylated products are expressed on the red-blood cell surfaces in humans.

The Se-Enzyme (**Se**-FucT) (entry 3) encoded by Se-gene (secretory gene) also transfers **L**-fucose from GDPfucose onto the 2-OH of the galactose residue. However, it can act on both *type-1* and *type-2* disaccharides to furnish the corresponding fucosylated oligosaccharides. The enzymatic reaction products are expressed on the glycoproteins and glycolipids found in various secretions (e.g., saliva).

The enzymes which transfer **L**-fucose from GDP-fucose onto the N-acetylglucosamine residues of the acceptor disaccharide are encoded by X and Le-genes. The X-Enzymes(**X**-FucT) (entry 2), however, act upon only *type-2* disaccharide, whereas Le-Enzyme(**Le**-FucT) accepts both *type-1* and *type-2* disaccharides as substrates.

Fucosyl-transfer to galactose residue

A careful examination of the type-2 and type-1 disaccharide structures reveals that the 2-OH of the galactose residue has a different environment in these two structures. In the type-2 disaccharide the 2-OH is close to the CH_2OH moiety of the neighbouring GlcNAc residue, whereas in the type-1disaccharide the 2-OH is topographically closer to $NHCOCH_3$

moiety of GlcNAc. In 1978, R.U.Lemieux[3] had predicted that the α2FucTs responsible for the transfer of an L-fucose residue to the 2-OH of type-1 and type-2 substrates should be different because the surfaces presented to them by these substrates in their most favoured conformations are different. Indeed, the two distinct α2-FucTs activities have since been found.[22] One is expressed in red blood cells (**H**-FucT), whereas the other is expressed in salivary glands (**Se**-FucT).

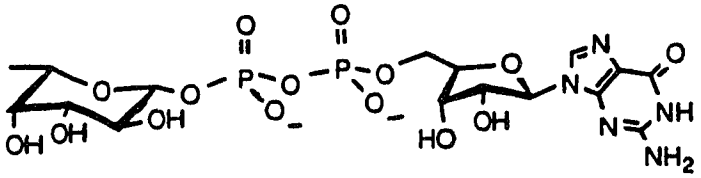
Fucosyl Transfer to N-acetylglucosamine Residues

In the type-2 disaccharide the 3-OH of the GlcNAc residue is fucosylated by X-Enzyme (**X**-FucT) as well as by Le-Enzyme (**Le**-FucT) (figure 3). The fucosylated oligosaccharide is called the X-antigen, Le^x antigen or SSEA-1 antigen. This structure has been identified as a tumor-associated antigen and is found on the glycolipids of human adenocarcinomas.[13] The first total chemical synthesis of SSEA-1 glycoheptosyl ceramide has been recently achieved.[14]

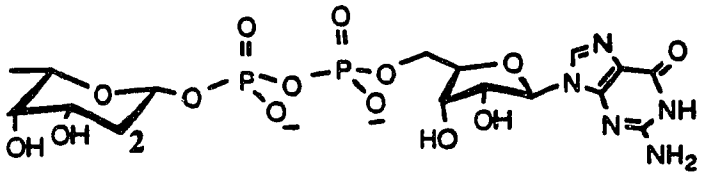
In the type-1 disaccharide the 4-OH of the GlcNAc is fucosylated by the Le-Enzyme (**Le**-FucT) (figure 4) to furnish the Le^a structure. The difucosylated Le^b and Y structures are also observed to accumulate as glycolipid antigens in several human cancers. As shown in figures 3 and 4, the Y structures can be synthesized either by the concerted action of the **H**-FucT and **X**-FucT on the type-2 disaccharide or by the action of **Le**-FucT and **Se**-FucT.

FIG 5 . MODIFIED DONOR SUBSTRATES

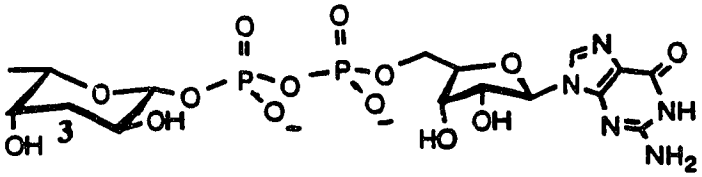
GDP-FUCOSE ANALOGUES



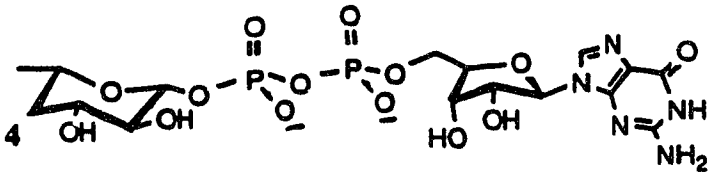
GDP-FUCOSE



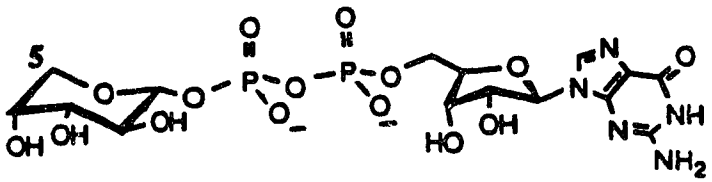
2-DEOXY ANALOGUE



3-DEOXY ANALOGUE



4-DEOXY ANALOGUE



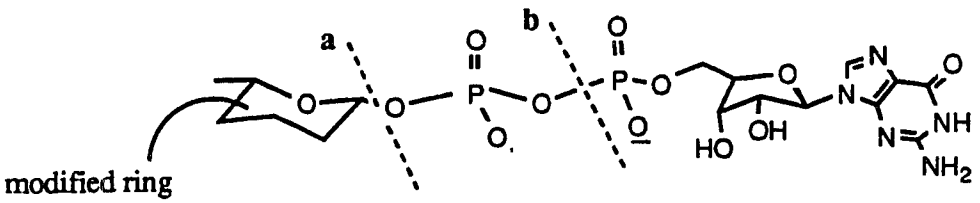
5-NOR ANALOGUE

From the foregoing discussion it is seen that all of the above mentioned fucosyl transferases use the same donor substrate (GDP-fucose) in the *in vivo* fucosylation reaction. Despite the considerable amount of study on the *acceptor-substrate* specificity, a systematic investigation of the *donor-substrate* specificity[23,24] was lacking. We therefore decided to study the donor specificity of fucosyltransferase enzymes by the systematic modification of the natural donor, GDP-fucose. As depicted in figure 5 we decided to attempt the replacement of the 2, 3, and 4 hydroxyl groups of the fucose moiety along with the 5-methyl group with a hydrogen atom.

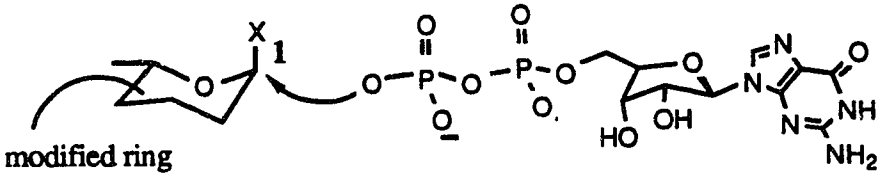
The synthesis of GDP-fucose can in principle be achieved in two ways as shown in figure 6. Bond disconnection **a** would require the displacement of a suitable leaving group on the fucosyl moiety. The bond disconnection **b**, on the other hand, would necessitate the formation of a pyrophosphate bond by displacement at the phosphorus of guanosine-5'-monophosphate. Both approaches were attempted. The latter method, which successfully furnished the target nucleotides, required the preparation of β–fucosyl phosphates (equatorial configuration at C-1) prior to their coupling with the activated guanosine-5'-monophosphate moiety. The results are described in chatpers III and IV.

It was hoped that the target modified GDP-fucose analogues would be recognized and would be transferred by the fucosyl transferases. Examples where C-2 and C-6 halogenated (Cl or F) fucosyl residues appeared to be transferred had been

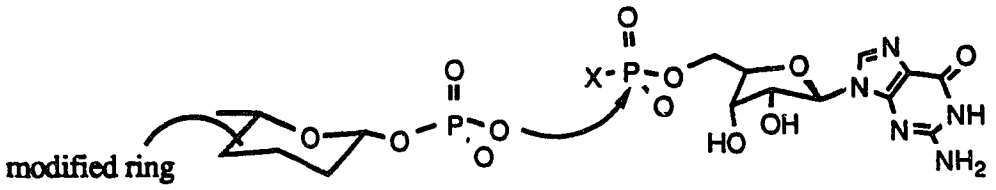
Fig.6 TWO APPROACHES FOR THE SYNTHESIS OF SUGAR NUCLEOTIDES .



DISCONNECTION : **a**



DISCONNECTION : **b**

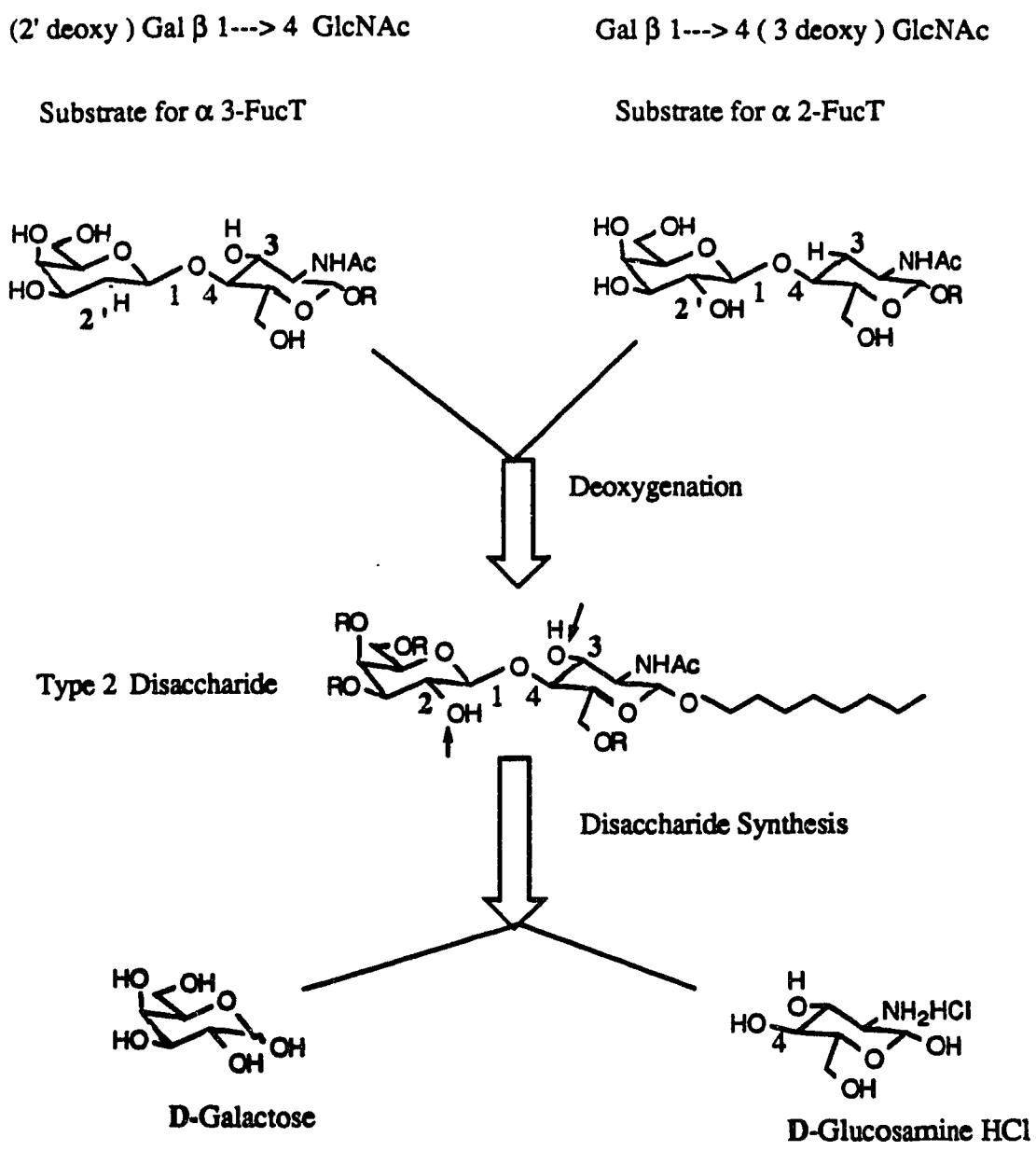


reported[25] in the literature. However, the use of fucose deoxy and demethyl analogues of GDP-fucose in the fucosyl transfer reaction using fucosyl transferases was never attempted. We planned to use these analogues in a preparative *in vitro* fucosylation reaction. In the event that fucosyl transfer from these donor substrates should occur, the product would be a deoxyfucosylated oligosaccharide. In light of the well established[8,18,19] findings that deoxy analogues of the natural antigenic determinants can show an enhanced affinity for antibodies, such chemoenzymatic access to the deoxyfucosylated structures would be synthetically attractive. The synthesis of the analogues was planned to provide us with quantities so as to use them in the production of deoxy-oligosaccharides on the milligram scale.

The α2-FucTs (**H**-FucT)[21] and α3-FucTs[26,27] (**X**-FucT) were chosen as the candidate fucosyl transferases for biological evaluation. These enzymes have been purified and extensively studied with respect to their specificities toward acceptor substrates.[21,27] (The choice of these enzymes was also prompted by their availability in our colaborator's (Dr. Monica Palcic, Dept. of Food Science, U. of A.) laboratories as well as by the reports[28] of their increased levels in tumor cells).

Selective acceptor substrates to be used in the fucosyl transfer reactions involving GDP-fucose analogues were also required. Both, α2-FucT and α3-FucT were known to use the type-2 disaccharide as an acceptor substrate (table 1, *vide supra*).

Fig. 7 SYNTHESIS OF MODIFIED ACCEPTOR SUBSTRATES



As shown in figure 7 the monodeoxy analogues : (2' deoxy) Galβ1->4 GlcNAc and Galβ1->4 (3 deoxy) GlcNAc were synthesized as the selective acceptor substrates for α3-FucT and α2-FucT respectively . The synthesis was achieved by assembling (figure 7) **D**-galactose and **D**-glucosamine in a type-2 (β1->4) linkage. The deoxygenations were carried out on the appropriately protected disaccharide intermediates. In order to facilitate the isolation[33] of the enzymatically fucosylated products, the acceptor disaccharides were synthesized as their n-octyl glucosides.

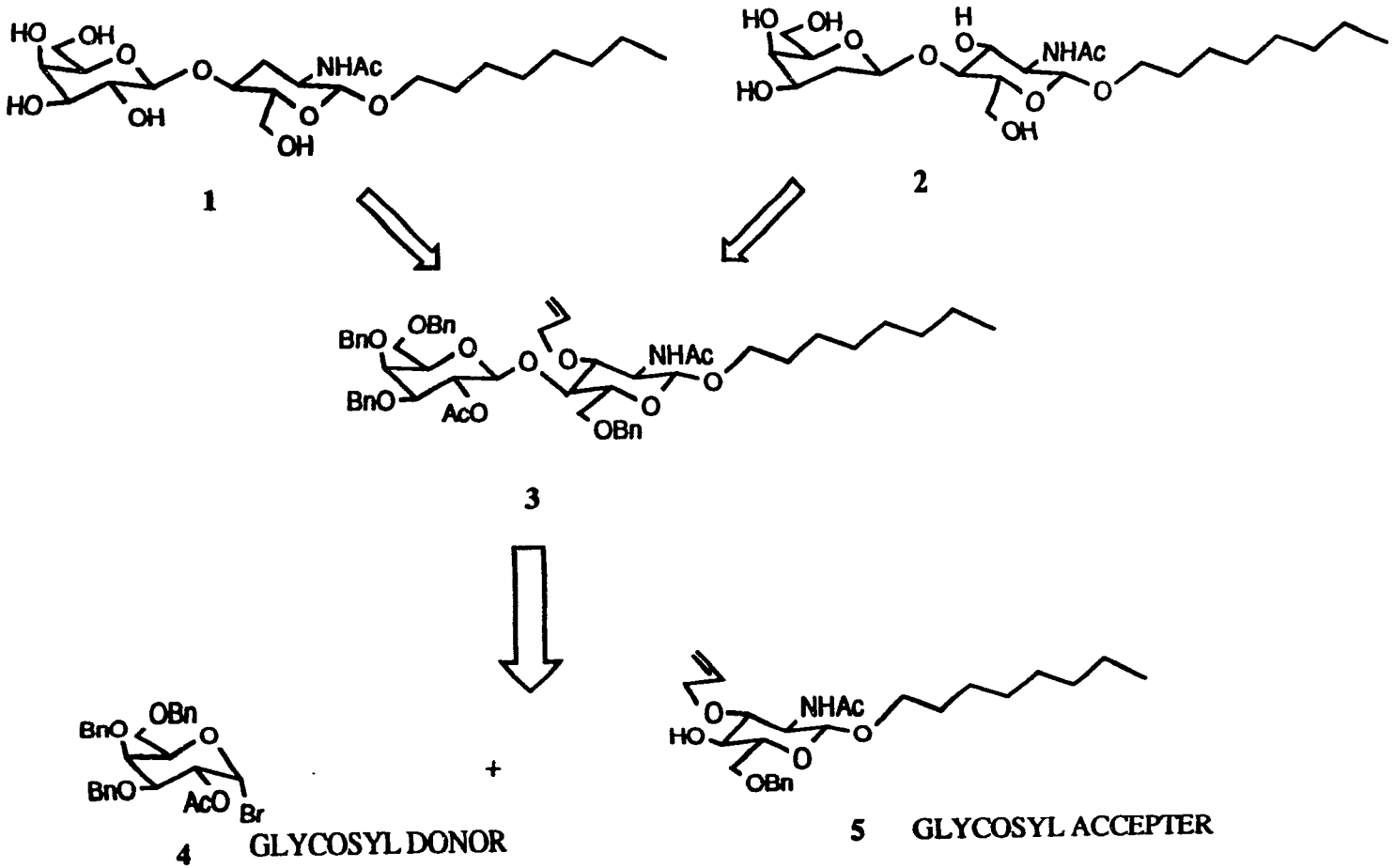
The present thesis reports the results of the synthetic work. First, the synthesis of the deoxy disaccharide acceptors is described (Chapt. II). Description of the synthesis of fucose-1-phosphate analogues and the study[29,30] of their conformational properties is described in Chapt. III. Finally the coupling of modified fucose-1-phosphates to the guanosine-5'-monophosphate to furnish the target sugar nucleotides follows in Chapt. IV.

II. SYNTHESIS OF ACCEPTOR SUBSTRATE ANALOGUES

As summerized in the Introduction (Chapt. I), one of the goals of the project was to synthesize two disaccharides, namely, n-octyl 2-acetamido-2,3-dideoxy-4-O-(β-D-galactopyranosyl)-β-D-ribo-hexopyranoside (1) and n-octyl 2-acetamido-2-deoxy-4-O-(2 deoxy-β-D-lyxo-hexopyranosyl)-β-D-glucopyranoside. (2). The previous syntheses of the two monodeoxy disacharides have been reported,[31] but with a different aglycon, namely 8-methoxycarbonyloctanol.

The use of this aglycon moiety which incorporates an 8 carbon spacer with a carbomethoxy function at the terminus was pioneered by Lemieux et al.[32] The carbomethoxy group offers a functionality with which one can attach the disaccharides to various solid supports, whereas the 8-carbon spacer positions the oligosaccharides at a distance from the support. Indeed, when this aglycone was employed in the earlier[31] syntheses of the disaccharides, the aim was to use the aglyconic moiety as a linking arm for the possible attachment to solid supports. In due course the hydrophobic nature of the aglycone was exploited by Palcic et al.[33] for adsorption onto reverse phase C-18 cartridges, thus giving rise to a novel and efficient assay procedure for the glycosyltransferase enzymes which used hydrophobic glycosides as the acceptor substrates. At the outset of this project, the enzyme assay procedure was optimized in our laboratories with the realization that the simpler octyl aglycone imparted enough hydrophobic

SCHEME 1 . RETROSYNTHESIS OF TARGET DISACCHARIDES INTO MONOSACCHARIDE SYNTHONS .



character to the oligosaccharides thus making the carbomethoxy group unnecesary in the aglycone of the target structures.

A. Retrosynthetic Analysis

As shown in scheme 1, the appropriately protected disaccharide **3** would serve as a central intermediate. The choice of allyl and acetate protecting groups would allow the selective liberation of the respective hydroxyls. The substitution of the specified hydroxyl functions with a hydrogen atom followed by an exhaustive deprotection of the resulting intermediates would yield the desired monodeoxy disaccharides **1** and **2**.

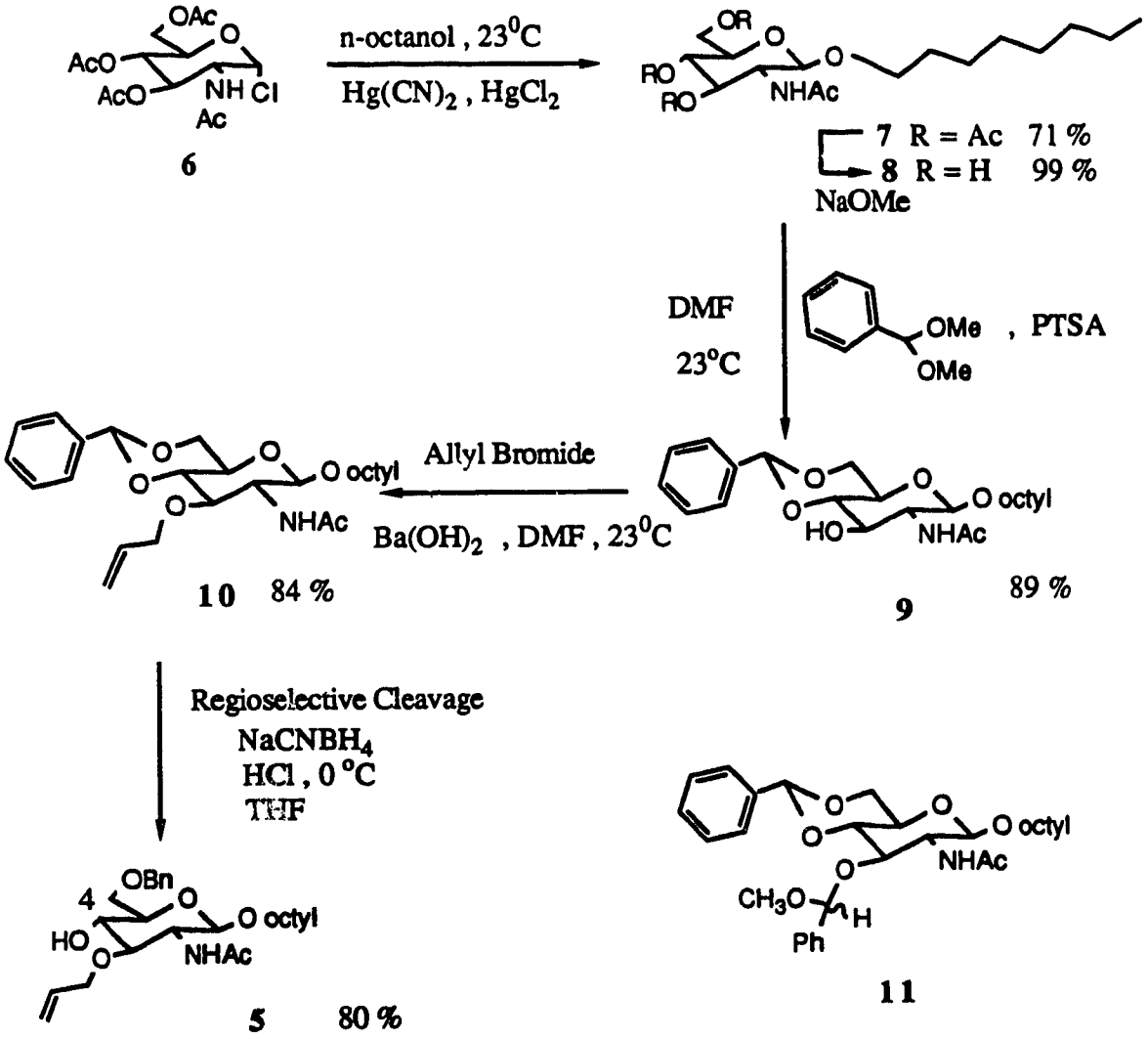
Synthon **3** was retrosynthesized into two appropriately protected monosaccharides (scheme 1). 2-O-acetyl-3,4,6-tri-O benzyl-α-**D**-galactopyranosyl bromide (**4**) would serve as the glycosyl donor for 4-O-glycosylation of n-octyl 2-acetamido-2-deoxy-3-O-allyl-6-O-benzyl-β –**D**-glucopyranoside (**5**).

B. Synthesis of Glycosyl Acceptor and Donor

Glycosyl acceptor **5** was synthesized in five steps from the known[34] 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-**D**-glucopyranosyl chloride (**6**, scheme 2). The glycosylation of **6** with n-octanol under Helferich conditions, using $Hg(CN)_2$ and $HgCl_2$ as promoters, afforded the desired β-glycoside (**7**) in 71% yield. The ^{1}H-nmr spectrum of **7** showed a doublet signal at δ 4.695 for H-1. The coupling constant $J_{1,2}$ = 8.5 Hz was indicative of the β configuration at the anomeric centre. The acetate protecting groups were removed by transesterification in methanol under Zemplen conditions (NaOMe in dry MeOH) to afford triol **8** in near quantitative

SCHEME 2.

SYNTHESIS OF COMPOUND 5

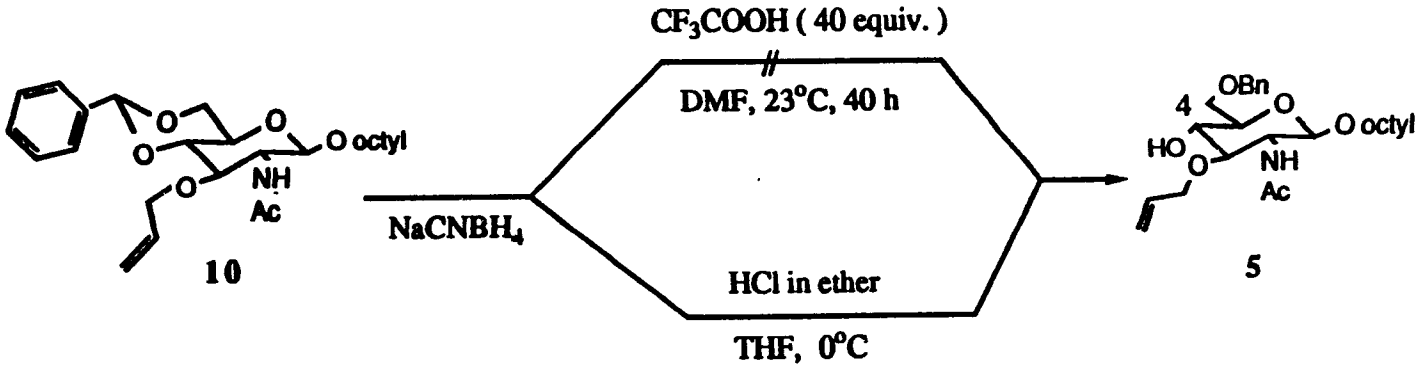


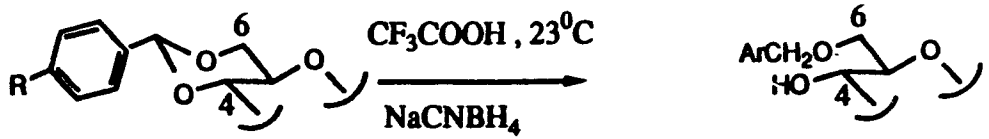
yield. The loss of 3 acetates was confirmed by ^{1}H-nmr spectroscopy. The synthetic strategy for the preparation of **5** required the protection of the 3-hydroxyl group as the allyl ether. Hence hydroxyls at the 4 and 6 positions needed to be protected prior to the 3-O-allylation. Treatment of triol **8** with benzaldehyde di nethyl acetal under acidic conditions afforded the desired benzylidene derivative **9** in 89 % yield. The ^{1}H-nmr spectrum of **9** showed the characteristic singlet at δ 5.560 for benzylic hydrogen and the ^{13}C-nmr spectrum exhibited two resonances in the anomeric region at δ100.59 and δ101.95. The signal for the hydroxylic proton appeared as a broad peak (δ 4.100-4.250) causing H-3 to produce a broad signal at δ4.153 which collapsed to a sharp doublet of doublets ($J_{2,3}$= 9.9 Hz and $J_{3,4}$ = 8.9 Hz) on deuterium exhange. One batch of this benzylidination reaction gave material with an unexpectedly high chromatographic mobility on tlc. This material was different from **9** and resisted crystallization. It was presumably a mixed-benzylidine acetal **11.** When this material was stirred for 30 minutes in dry methanol with Dowex 50-X8 (H^+) resin at ambient temperature, compound **9** was formed as a major product. Compound **11** was not characterized.

3-O-Allylation of **9** was achieved by reaction with allyl bromide and $Ba(OH)_2$ as the acid scavenger. The desired allyl ether **10** was obtained in 84% yield. The ^{1}H-nmr spectrum of **10** displayed the expected resonances for the allyl moiety. The next and final step in the synthesis of **5** was the cleavage of the benzylidene ring in such a way as to liberate the secondary hydroxyl at C-4 for the eventual glycosylation.

Scheme 3.

REGIOSELECTIVE OPENING OF BENZYLIDENE ACETAL 10





R = H , NO REACTION. (THIS STUDY)

R = OCH_3 , REGIOSELECTIVE CLEAVAGE . (REF. 36)

The cleavage of such benzylidene derivatives to yield a free hydroxyl group at a secondary carbon and a benzyl ether functionality at a primary carbon has been achieved[35a] by treatment with $NaCNBH_4$ under acidic conditions. Usually, a crystal of methyl orange is added as an indicator. A rate of dropwise addition of ethereal HCl is maintained so as to impart permanent orange color to the reaction mixture (pH = 3.1). However, in order that the reductive cleavage reaction occurs the addition of ethereal HCl needs to be continued even after the indicator end-point is observed. A close monitoring of the reaction mixture with tlc examination is done routinely in order to assess the progress of the reaction. The experience regarding such benzylidene cleavage reactions in our, as well as other laboratories,[35b] points at the fact that an excessive addition of ethereal HCl poses a potential hazard due to several possible side reactions such as glycosidic bond cleavage. We attempted to avoid this problem by substituting etheral HCl with trifluoroacetic acid. CF_3COOH is known[36] to bring about the desired cleavage on 4-methoxy benzylidene derivatives.

Thus, when a solution of **10** and $NaCNBH_4$ in DMF was treated with several equivalents of CF_3COOH at ambient temperature, less than 5 % of **5** (tlc analysis) was obtained (scheme 3). Up to 40 equivalents of CF_3COOH caused no cleavage reaction on **10.** This differential reactivity of benzylidene and 4-methoxy benzylidene acetals can therefore likely be advantageously used in future selective protection-deprotection reactions.

The transformation of **10** into **5** was however, successfully achieved by the *careful* addition of *dilute* ethereal HCl

until 10% of the starting benzylidene derivative remained unreacted. Under the optimized conditions (see the experimental section) the desired compound **5** was isolated in 80% yield. The regioisomeric product having the primary hydroxyl free was not formed. The absence of the singlet resonance at δ 5.560 in the ^{1}H-nmr spectrum of the product was an indicator of the acetal cleavage. Evidence that the benzyl group was situated on the hydroxyl at C-6 was obtained by ^{13}C-nmr. Usually the hydroxymethyl group carbon (C-6) resonates in the region 60 to 63 ppm downfield from tetramethylsilane.[37] The ^{13}C-nmr of **5** in $CDCl_3$ clearly indicated that the hydroxymethyl group was substituted since the C-6 carbon resonated at 69.89 ppm. Interestingly, when compound **5** was purified by silicagel chromatography using ethyl acetate : hexane as the solvent system, **5** was isolated as a crystalline material which existed as a complex with ethyl acetate (ratio of ethyl acetate to **5** = 2 :1, estimated by ^{1}H-nmr). The solvent molecules could not be removed under high vacuum (0.01 mm, P_2O_5, 24 h). In the ^{1}H-nmr spectrum of this material in $CDCl_3$, the doublet resonance for H-1 was at 4.450 ppm (J=8Hz) and N-H gave a signal at 6.717 ppm (J = 9 Hz). Silica gel purification using CH_2Cl_2 : CH_3OH (9:1) afforded **5** which was devoid of any solvent of crystallization (δ H-1 4.926, J = 8 Hz ; δ N-H 5.659, J = 7 Hz). The upfield shift of H-1 signal in the ethyl acetate-complex was dramatic (Δδ 0.48) and was attributed to the complexation of ethylacetate molecules to the acetamido moiety. The 4-OH and NHAc in **5** are two potential hydrogen-bond donor sites. It is expected that a hydrogen bonding solvent would complex at these groups, thereby affecting the local environment and hence the

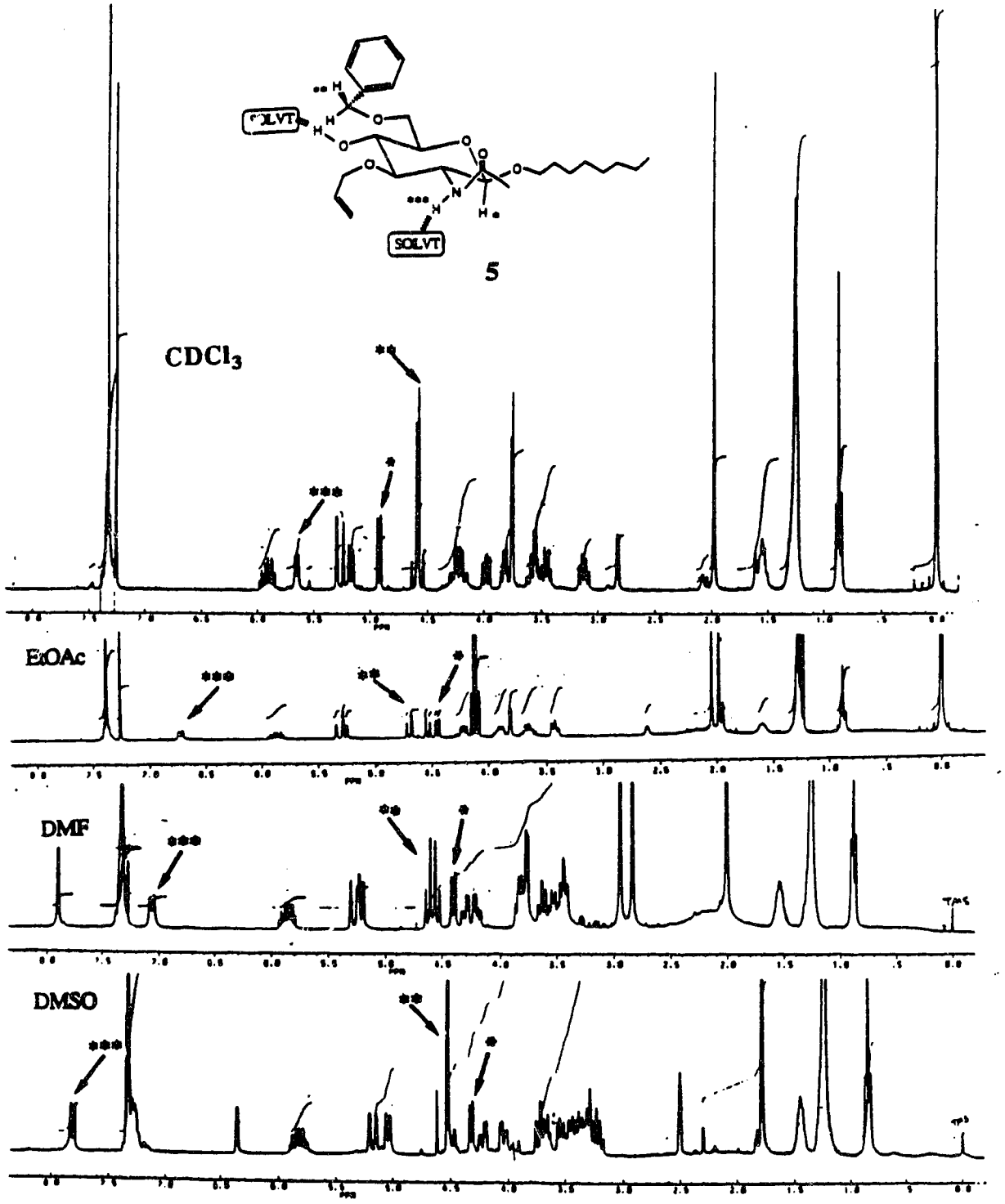


Fig. 8 The 300 MHz ^{1}H nmr spectra of compound **5** in $CDCl_3$ containing EtOAc, DMF, & DMSO.

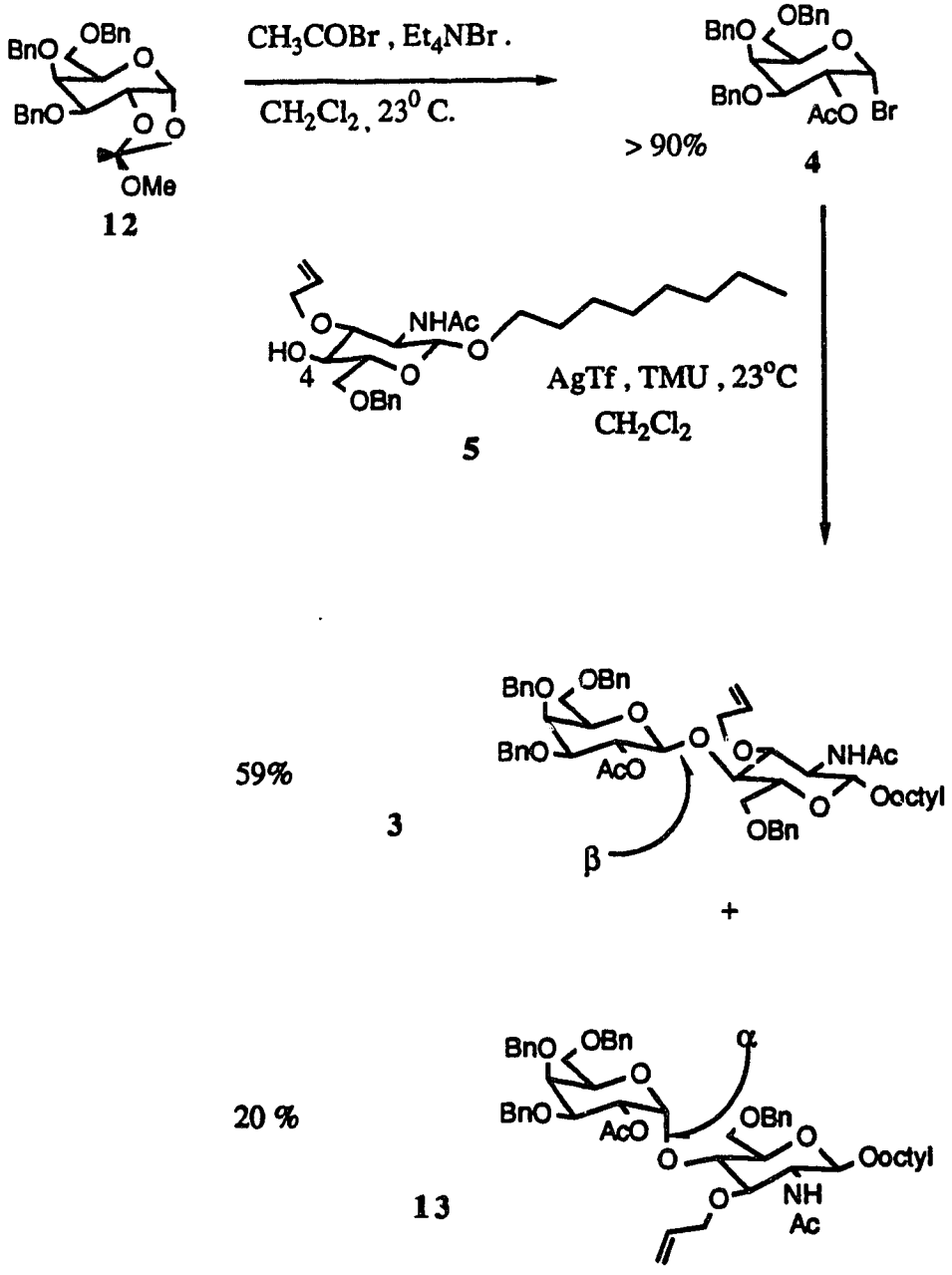
signals of the nuclei not directly involved in the H-bonding may also be affected. Indeed, signals due to the C-6 benzyloxy group and H-1 were dramatically affected due to ethyl acetate complexation. The ^{1}H-nmr spectra are reproduced in figure 8. The addition of DMF and DMSO to the $CDCl_3$ solution of **5** showed the expected deshielding of the N-H signal(designated by ***). The H-1 signal (*) was found around 4.4 ppm in the spectra containing H-bonding solvents. The change in the pattern of the benzyl proton resonance (**) was also apparent in the spectra.

Having prepared the glycosyl acceptor (**5**), 2-O-acetyl-3,4,6-tri-O-benzyl-α-**D**-galactopyranosyl bromide (**4**) was prepared as shown in the scheme 4. The 3,4,6-tri-O-acetyl-α-**D**-galactopyranose-1,2-(methyl orthoacetate)[38] was converted into the corresponding 3,4,6-tri-O-benzyl derivative (**12**) by transesterification under Zemplen conditions followed by treatment of the resulting triol with NaH/benzyl bromide in DMF. The identity and purity of crystalline **12** (exo:endo = 2:1) was assessed by ^{1}H nmr spectroscopy. The 300 MHz ^{1}H-nmr spectrum of **12** is reproduced in figure 9 (The inset shows the 'exo' isomer). Freshly prepared **12** was treated with CH_3COBr in CH_2Cl_2 to generate **4** as a major compound in the reaction mixture. Bromosugar **4** was extracted as a pale brown syrup and immediately used for the glycosylation of **5**.

C. Disaccharide Synthesis

The glycosylation reaction[40] to establish the desired 1–>4 linkage was carried out employing silver triflate as promoter and tetramethylurea as acid acceptor to give 59% yield of the desired β linked product **3**. The separation of β-linked disaccharide

SCHEME 4 . 4-O-GALACTOSYLATION OF 5



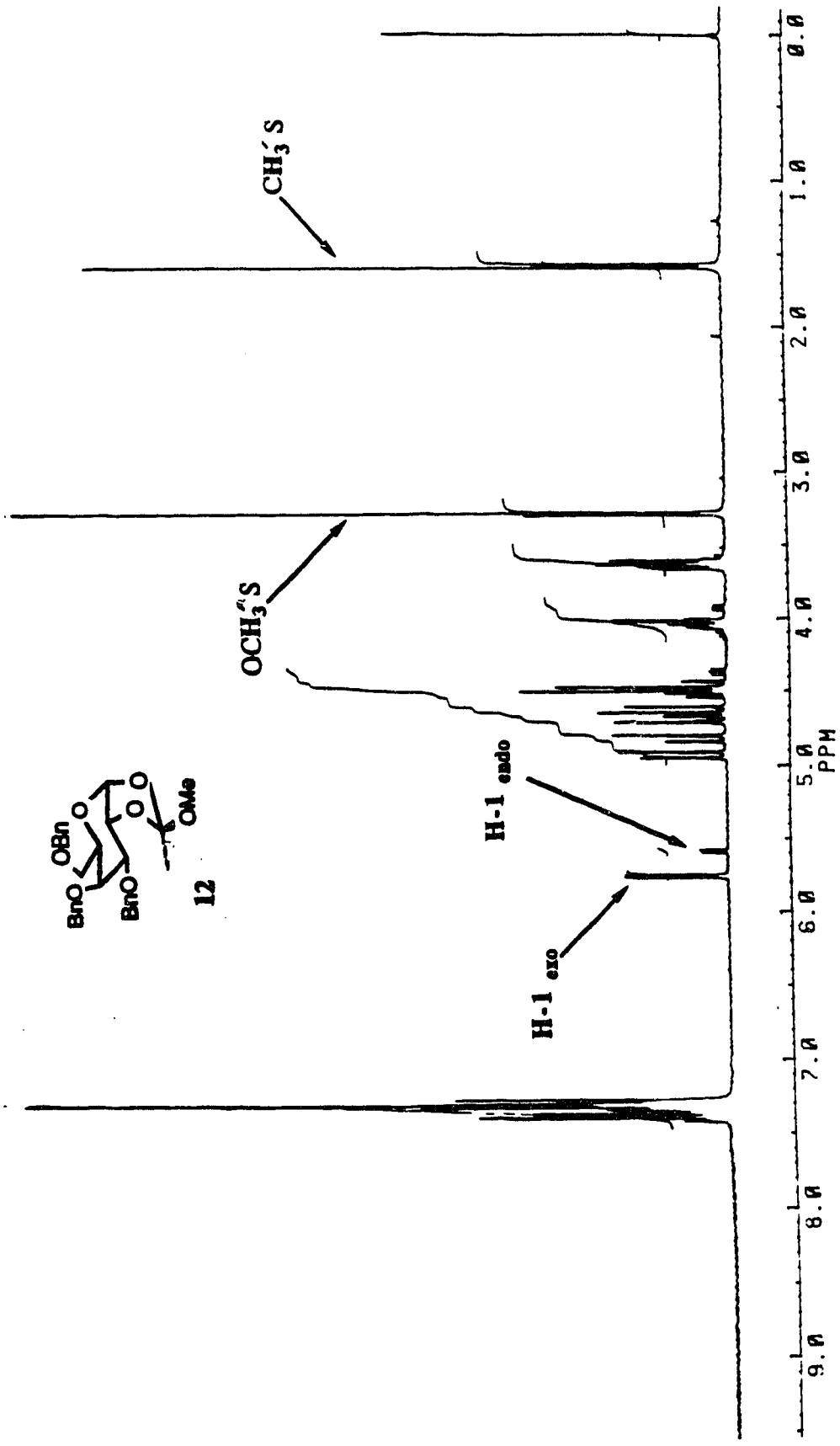


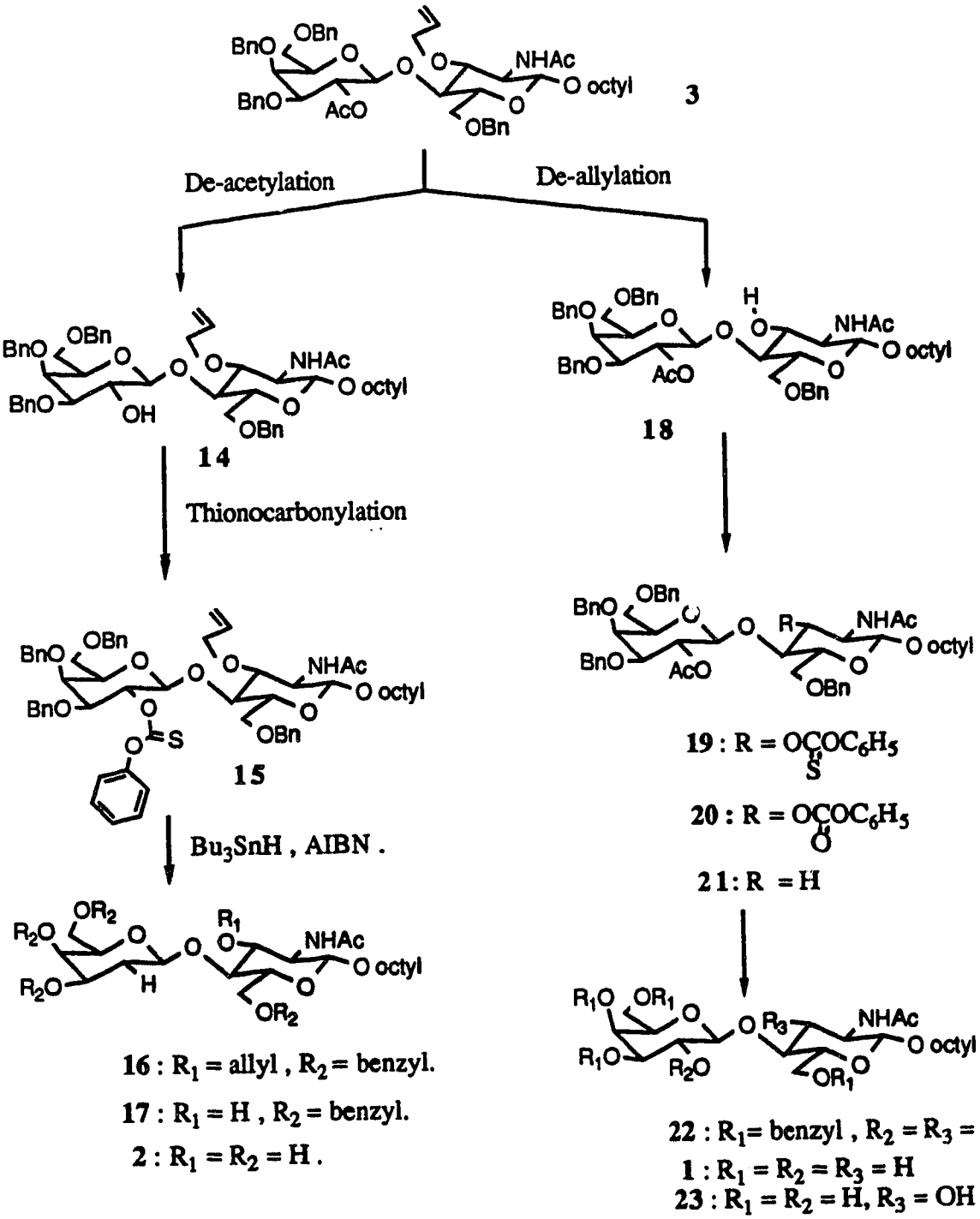
Fig. 9 The 300 MHz ^{1}H nmr spectrum of compound **12** in $CDCl_3$. (exo: endo = 2:1)

(**3**) from the undesired α–linked product (**13**) posed a great deal of difficulty due to their similar mobilities on silica gel in several solvent systems. Silica chromatography using gravity or flash elution methods did not give satisfactory separation. However, preparative HPLC using Prep-Pak-500/silica cartridges and elution with ethyl acetate : hexane (33 : 67) at a rate of 70 ml/min. offered an excellent separation of the α/β mixture into **3** (59%, β glycoside) and **13** (approx. 20%, α glycoside). The β-anomeric configuration of the galactose residue in **3** was established by identifying the H-1' nmr signal at δ4.337. A coupling of 8.0 Hz ($J_{1',2'}$) established the trans diaxial relationship of H-1' and H-2'. Compound **13** on ^{1}H-nmr and combustion analysis proved to be a disaccharide. It is tentatively identified as being an α-glycoside. After preparing the anomerically pure disaccharide (**3**) a sequential deprotection and deoxygenation on the GlcNAc residue at C-3 and on the galactose residue at C-2' was undertaken (scheme 5).

C 1. Synthesis of n-octyl 2-acetamido-2,3-dideoxy-4-O-(β-D-galactopyranosyl)-β- D-ribo-hexopyranoside (1)

As indicated in scheme 5 the free hydroxyl group in **18** was generated by the two-step deallylation procedure.[41] Thus the sequential treatment of **3** with $Rh(PPh_3)_3Cl$ to effect isomerization of the allyl ether moiety to an enol ether followed by hydrolytic cleavage of the enol ether using $HgCl_2$/ HgO in aq. acetone, gave compound **18** in 91% yield. Removal of the allyl protecting group was apparent from the ^{1}H nmr spectrum of **18** where the characteristic allyl signals were absent.

SCHEME 5. DEOXYGENATIONS .



There are various methods available for the substitution of the secondary hydroxyl functions with a hydrogen atom.[42] We chose the widely used method of Robins and Wilson[43] for deoxygenation of **3**. Earlier work[31] in this laboratory had successfully used phenoxythionocarbonylation followed by a tin hydride reduction sequence[43] on a similar disaccharide molecule. However, in our case the yield of **19** from **18** using several equivalents of phenoxychlorothionocarbonate and DMAP never exceeded 30%. Moreover, when pyridine was used along with DMAP, along with the expected thionocarbonylated derivative **19**, a by-product - a carbonate derivative (**20**) was also formed in the reaction in 10-15% yield. Under these conditions 50% of the starting material remained unreacted even after addition of up to 30 equivalents of the reagents. Several attempts at increasing the yield of **19** by changing the solvent, temperature and the duration of the reaction failed. The by-product was identified to be the compound **20**. The combustion analysis of **20** indicated it to be devoid of a sulfur atom. Compound **20** gave the expected molecular ion peak (m/z 1019) in its Fast-atom-bombardment (FAB) mass spectrum .

An alternative derivatization of **18** using thiocarbonyl diimidazole[44] was also unsuccessful. Finally, the deprotonation of **18** using n-BuLi followed by quenching with phenoxychloro thionocarbonate rendered the desired derivative **19** in 94 % isolated yield. Compound **20** was not formed under these conditions. Acetate migration was not observed. Thionocarbonylation at the C-3 hydroxyl was evident from the ^{1}H-nmr spectrum of **19**, wherein the H-3 proton was deshielded to δ 5.667 from δ 3.929 in **18**. Treatment of

thionocarbonate **19** with tri-n-butylstannane in the presence of AIBN gave the desired deoxy disaccharide **21** in 61% yield. It has become customary to use an acetonitrile : hexane extractive work-up[45] for such reductions. The residue obtained after the evaporation of the reaction solvent (usually toluene) is partitioned between acetonitrile and hexane. The material containing tin is soluble in hexane whereas, the desired organic compounds are soluble in acetonitrile. Multiple extractions with hexane ensure removal of the stench associated with the tin residues. Such extractive work up followed by silica chromatography gave the product disaccharide **21** completely free of unwanted tin compounds. The newly created methylene group was apparent in the ^{1}H nmr of **21**: δ H-3$_{ax}$ = 1.800(m), δ H-3eq = 2.175 (m).

Finally, Zemplen deacetylation of **21** gave **22** which on debenzylation using Pd/C (5%) under H_2 at atmospheric pressure furnished the target structure **1** in 66% yield. Anomeric configurations at both anomeric centres were confirmed in the ^{1}H-nmr spectrum of **1** where vicinal coupling constants for H-1 and H-1' signals were 8.0 Hz each. The hydrophobic character of the n-octyl aglycon of disaccharide **1** allowed its purification using C-18 reverse phase cartridges.[33]

C 2. Synthesis of n-Octyl 2-acetamido-2-deoxy-4-O-(2-deoxy-β-D-lyxo-hexopyranosyl) glucopyranoside (2)

As shown in scheme 5 the free hydroxyl group in **14** was generated by deacetylation of **3** under Zemplen conditions. The 2'-deacetylated product **14** was obtained in near quantitative yield. Thionocarbonylation of **14** was first attempted using Robins and

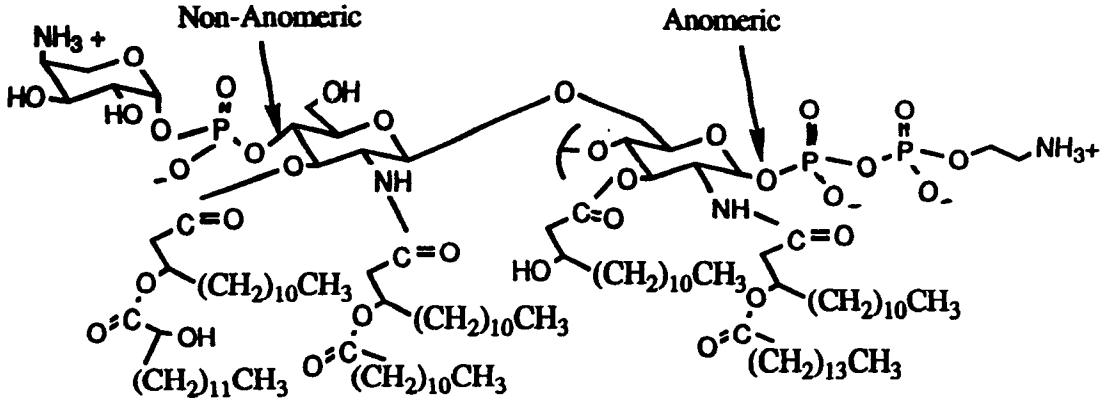
Wilson's procedure. The reaction of **14** with phenylchlorothionocarbonate (10 equiv.) and DMAP (10 equiv.) in refluxing acetonitrile resulted in a 16% yield of the desired thionocarbonate (**15**). Multiple additions of reagents did not result in improvement of the yield. When pyridine was added along with DMAP in the reaction, thionocarbonylation proceeded at a faster rate. Under optimized conditions (see the experimental section) **15** was obtained in 52 % yield. It should be noted that the use of n-BuLi for the deprotonation of the alcohol followed by thionocarbonylation was very effective for the conversion of **18** to **19** in 94% isolated yield (vide supra). The same procedure is likely to give higher yield for **15**. All of the alcohol **14** was however used in the DMAP/pyridine reaction and hence this alternate procedure could not be tested on **14**.The ^{1}H-nmr spectrum of **15** exhibited the expected deshielding of H-2' (δ 5.897). Treatment of **15** with tri-n-butyl stannane gave the expected 2'-deoxygenated disaccharide **16**, whose ^{1}H-nmr spectrum `showed C-2' methylene protons in the high field region (H-2'$_{ax}$ and H-2'$_{eq}$ at δ 1.937-2.000). The two-step deallylation[41] of **16** to give **17** (77% yield) followed by debenzylation gave the desired 2'-deoxy disaccharide **2** in 94% yield. The ^{1}H-nmr spectrum of **2** had two anomeric proton signals: H-1 (δ 4.400, $J_{1,2}$ = 8.0 Hz) and H-1' (δ 4.626, $J_{1,2ax}$ = 10 Hz, $J_{1'2'eq}$ = 2.5 Hz). The magnitudes of the vicinal coupling constants were in accord with those anticipated for the β–anomeric configurations at both sugar residues. The final purification[33] of **2** was achieved by using C-18 reverse phase cartridges.

C 3. Synthesis of n-octyl 2-acetamido-2-deoxy-4-O-(β -D -galactopyranosyl)-β-D-glucopyranoside (23)

The fully protected disaccharide **3** (scheme 5, *vide supra*) was sequentially 3-O-deallylated to give **18** (91% yield) which was 2'-O-deacetylated (98% yield) and finally perdebenzylated (quantitative yield) to furnish **23**. Since **23** was sparingly soluble in water, its final purification on C-18 cartridge[33] was rendered difficult. Repeated precipitations of **23** from its methanolic solution by addition of water furnished analytically and spectroscopically pure material. The ^{1}H-nmr spectrum of **23** exhibited two anomeric proton resonances at δ 4.387 (J = 8.0 Hz) and δ 4.366 (J = 7.0 Hz).

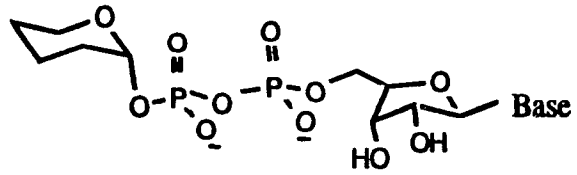
SCHEME 6 . IMPORTANT BIOMOLECULES WHICH CONTAIN A PHOSPHATE MOIETY.

Structure of Salmonella lipid A . (Ref. 46)



24

Nucleoside diphospho glycose . (Ref. 21)



III. SYNTHESIS OF L-FUCOSE PHOSPHATE ANALOGUES

A. Survey of aldopyranose-1-phosphate synthesis

Phosphate esters which contain a carbohydrate moiety play crucial roles in biological chemistry since they are involved at one stage or another in virtually every major metabolic pathway, including the biosynthesis of nucleic acids, proteins, and carbohydrates. Besides being present as metabolites, the phosphate esters of carbohydrates (sugar-phosphates) are the constituents of various biopolymers that are present on the outer surfaces of cells in both animal and bacterial cells. Lipopolysaccharides,[46,47] for example, form a prominent and unique component of the outer membrane of Gram-negative baceteria and have a phosphate ester group situated at the *anomeric* position as well as at the *non-anomeric* (C-4) position (**24** scheme 6). The presence of a phosphoryl group on the mannose residues of the oligosaccharide portion of the lysosomal enzymes is essential for the targeting of these enzymes to the lysosomes[48]. The nucleoside diphosphate sugar molecules (scheme 6), which are the donor substrates[49] in the glycosyl transfer reactions of oligosaccharide biosynthesis, contain the phosphate ester group situated at the anomeric position (C-1) of the hexose or pentose sugar.

Thus the phosphoryl group can be present at anomeric or non anomeric positions. When situated at the anomeric position, the phosphate residue can have either an axial

or equatorial orientation on the aldopyranose ring. As the aim of the project was to synthesize the anomeric phosphates of the aldopyranoses (L-fucose and the fucose ring deoxy analogues) having an equatorial configuration, an appropriate anomeric phosphorylation procedure was required. Both traditional and newer methodologies were examined, with respect to the stereochemical (axial/equatorial) outcome of the phosphorylation step and for the tolerance of various hydroxyl protecting groups toward the reaction conditions.

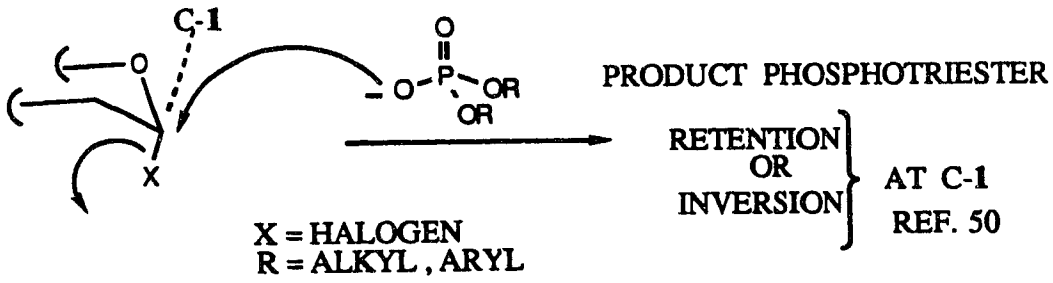
As depicted in scheme 7, there are two conceptually different ways for the introduction of the phosphoryl moiety at the anomeric position of an aldopyranose sugar. The traditional method of synthesis of anomeric phosphates involves the nucleophilic displacement of a leaving group at the hemiacetal carbon (C-1) by a phosphate nucleophile. Anomeric phosphorylation can be alternatively achieved by the displacement of a suitable leaving group at phosphorus by an anomeric hydroxyl group. The latter method is being widely employed in recent attempts at anomeric phosphorylation. The development (up to1977) of phosphorylation methodologies have been reviewed.[50,51,54,55] In what follows I shall briefly outline some preparatively useful procedures which exhibit predictable stereochemistry at the anomeric centre.

A 1. Use of the Phosphate Anion as a Nucleophile

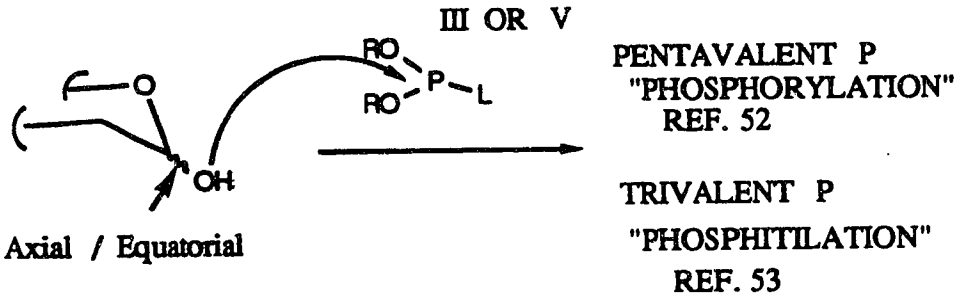
In this approach a good leaving group (e.g. bromine) at C-1, the anomeric carbon, is nucleophilically displaced by the phosphate anion. It is well documented [40, 56]

SCHEME 7 .

A) Displacement reaction at the Anomeric carbon by a phosphate diester anion.

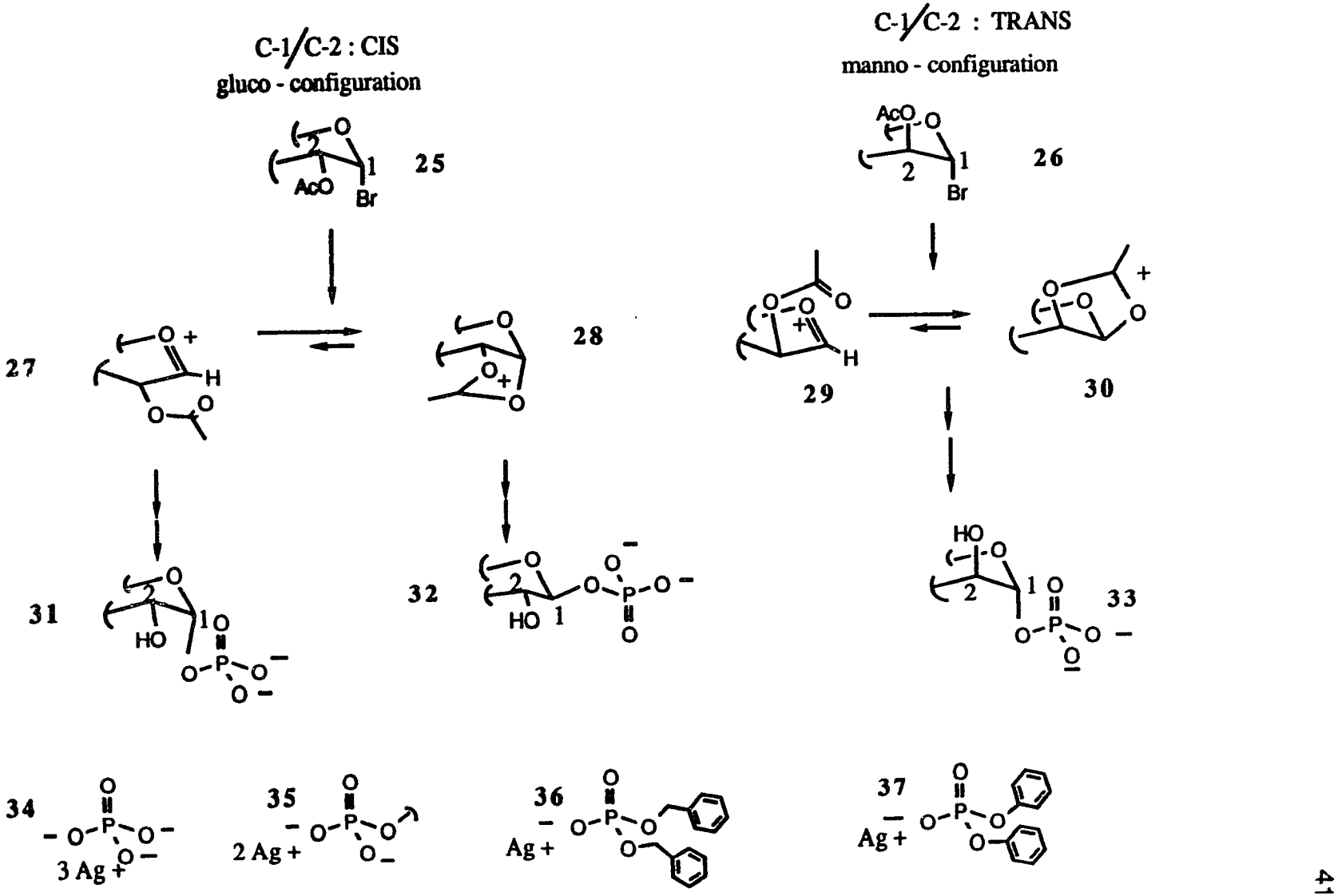


B) Nucleophilic displacement at the Phosphorus centre by an activated or nonactivated Anomeric hydroxyl .



that the configuration at C-2 and the nature of the 0-2 protecting group have a profound stereochemical influence on the nucleophilic displacement reactions at C-1. The *anomeric effect*[57] dictates the thermodynamically most favoured *axial* orientation for polar substituents at C-1. Consequently, the bromine substituent at C-1 is usually in the axial orientation. As indicated in scheme 8, compound **25** has a cis, whereas, **26** has a trans relationship for the C-1 and C-2 substituents. On treatment with the silver salt of a phosphoric acid derivative (**34, 35, 36,** or **37**), these glycosyl bromides undergo an irreversible dissociation of the C1-Br bond to form AgBr and the stabilized oxonium ions **27** and **29** or the corresponding acetoxonium ions **28** and **30**. The reaction of **27** with the phosphate nucleophile on the underface of **27** would give an axial orientation for the phosphoryl moiety, whereas, the nucleophilic attack at C-1 of **28** would give the equatorially disposed phosphoryl group. Indeed, the reaction[50] of tetra-O-acetyl-α-D-glucopyranosyl bromide (e.g. **25**) with silver dibenzylphosphate (**36**) gave, after the removal of benzyl and acetate protecting groups, β-D-glucopyranosyl-1-phosphate (e.g. **32**) in 49% yield. The phosphorylation had occurred with *inversion* of configuration at C-1. Conversely, the similar reaction[50] of tetra-O-acetyl-α-D-glucopyranosyl bromide with silver diphenylphosphate (**37**) afforded α-D-glucopyranosyl-1-phosphate (e.g.**31**) in 29 % isolated yield. The phosphorylation in the latter case had occurred with *retention* of configuration at C-1. The reaction[50] of either **36** or **37** with **26** gave the

SCHEME 8.


C-1/C-2 : CIS
gluco - configuration
C-1/C-2 : TRANS
manno - configuration
25
26
27
28
29
30
31
32
33
34
35
36
37
AcO
Br
HO
H
3 Ag +
2 Ag +
Ag +

product **33** with an *axially* oriented phosphoryl group. Thus the acetohalogenoaldopyranoses (C-1/C-2 : cis) react with **37** to yield an axial glycosyl phosphate, whereas, the reaction with **36** yields an equatorial glycosyl phosphate.

Although the reason for this complementary reactivity of **36** and **37** is not quite clear, the methodology is useful for obtaining anomerically pure phosphates. (Involvement of an orthoester-type intermediate via trapping of **28** or **30** by the phosphodiester anion appears quite plausible.) Multidentate reagents such as **34** and **35** ('disilver phosphate') are now frequently replaced[50] by the phosphodiester salts **36** and **37**. The reactions are carried out in nonpolar aromatic solvents such as benzene or toluene at elevated temperatures. The carbohydrate moiety carries ester blocking groups. After phosphorylation with **36** or **37** is accomplished, the product phosphotriester is subjected to the hydrogenolytic cleavage of benzyl or phenyl groups using palladium on charcoal and platinum dioxide respectively. The ester protecting groups on the carbohydrate can be saponified without affecting the anomeric integrity of the final glycosyl phosphate. *The reactions employing silver phosphate nucleophiles are heterogeneous.* The use of triethylammonium cations instead of silver cations has been pioneered by Khorana et al[58] to more reproducibly achieve homogeneous reaction conditions.

A 2. MacDonald Synthesis

This method which was devised by D. L. MacDonald[51] involves direct fusion at moderate temperatures

(50°C) of fully acetylated sugar derivatives with anhydrous phosphoric acid. Typically, the ratio of the acid to sugar is 8:1 and the duration of the reaction is 3-4 hours. The fusion is carried out with continuous evacuation, thereby removing the volatile by-product of the phosphorylation, namely acetic acid.

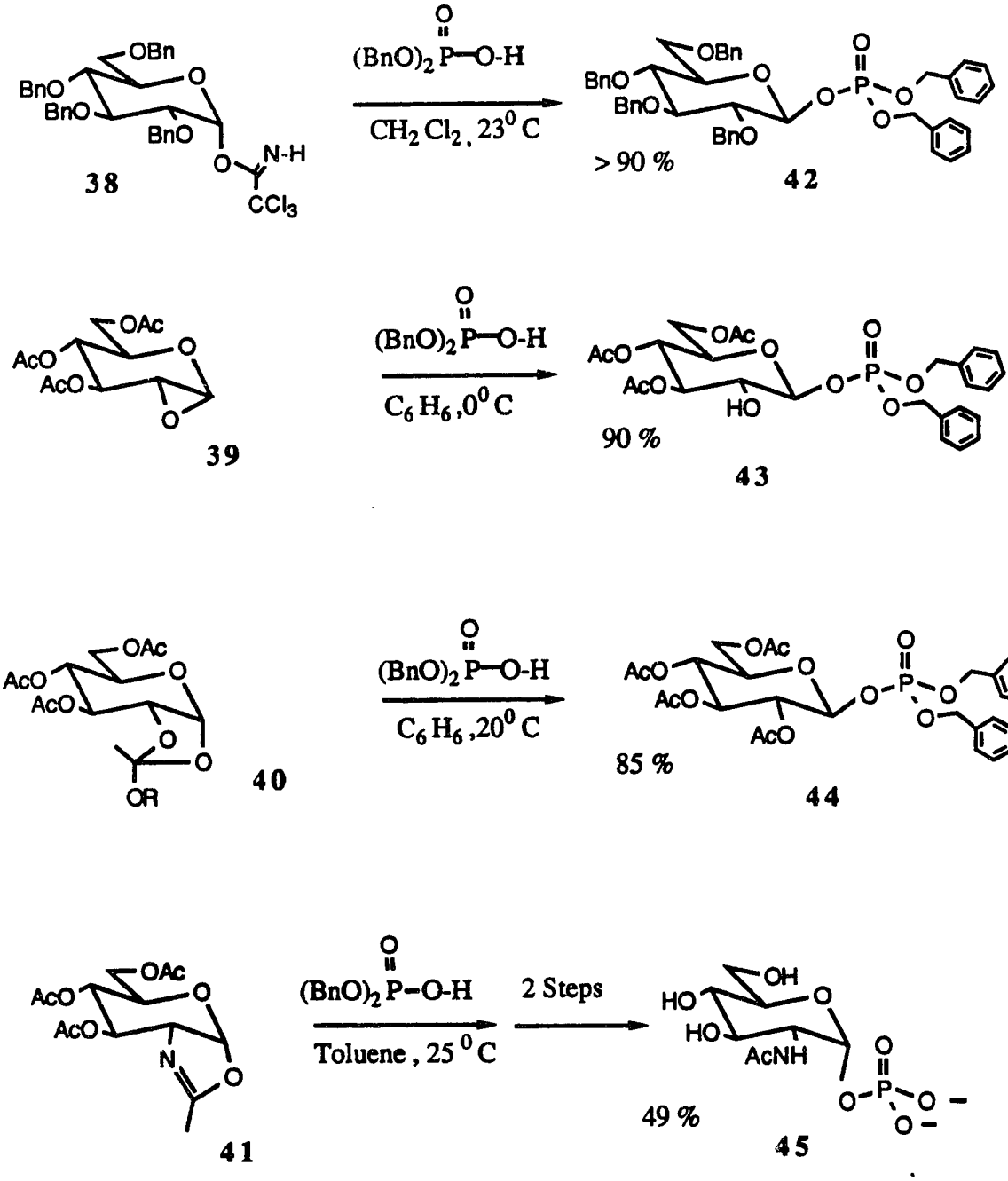
When the reaction is terminated after a few minutes (5-10) the equatorial glycosyl phosphate can sometimes be isolated as the major phosphorylation product (kinetic product), whereas, the continuation of the fusion process over several hours results in the formation of the thermodynamically more stable axial phosphate. The isolated yields of the glycosyl phosphate are generally in the range 40-60% and the method enjoys wide applicability. Using this method the glycosyl phosphates have been prepared from aldoses, ketohexoses, aminosugars[59], methyl pentoses, deoxy sugars and di- as well as, trisaccharides.[60] Most often, this process is used for the synthesis of axial phosphates.

A 3 . Use of Dibenzyl Hydrogen Phosphate as nucleophile.

The first two methods described in this section utilize the halogen and an acetoxy group as the leaving group in the displacement reaction at the anomeric centre. Substrates with 1,2-epoxide,[61] 1,2-oxazoline,[62] 1,2-orthoester[63,64,65] and 1-O-trichloroacetimidate[66] functionalities have been utilized as the electrophiles in anomeric phosphorylation reactions. These functionalities are quite commonplace in the glycosyl donor substrates of glycosylation reactions.[40] Their

SCHEME 9

DIBENZYL PHOSPHATE AS THE PHOSPHORYLATING REAGENT .



application in phosphorylation reactions has supplemented the use of halogens as leaving groups.

The nucleophile used in this method is dibenzyl hydrogen phosphate. The acidity of this reagent provides for the activation of imidate, epoxide, orthoester and oxazoline functionalities. Thus the reaction of **38** (scheme 9) with dibenzyl hydrogen phosphate is initiated by the proton transfer to the imidate nitrogen, thereby creating a good leaving group (protonated imidate moiety) at an anomeric carbon. The subsequent attack of dibenzyl phosphate anion at C-1 from the top face of **38** results in the formation of the equatorial dibenzyl glycosyl phosphotriester **42** in excellent yield.[66] Similarly, the epoxide **39** and the orthoester **40** afford the corresponding equatorial phosphotriesters **43** and **44** in good yields.[61,62] The oxazoline **41** on treatment with dibenzyl hydrogen phosphate however yields an axially oriented phosphotriester intermediate which on exhaustive deprotection yields **45**.[62] As the hydrogenolytic deprotection steps are not expected to induce an anomerization reaction at C-1, the phosphorylation step is considered to furnish the equatorial product which subsequently anomerizes to the thermodynamically more stable axial phosphotriester. All these displacement reactions occur as S_N2-type processes to give inversion of configuration at C-1.

The three above mentioned methods belong to <u>category A</u> in scheme 7, where the axial or equatorial orientation of the anomeric phosphate moiety is achieved by the

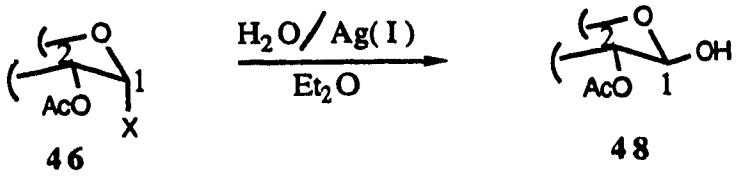
proper choice of the phosphorylating reagent or reaction conditions. Below we shall describe the procedures which involve a displacement reaction at the phosphorus centre.

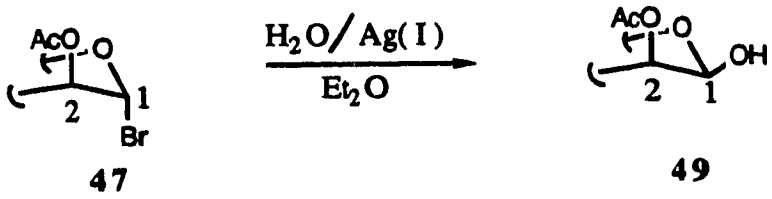
A 4 . Use of O-Phenylene Phosphorochloridate (50)

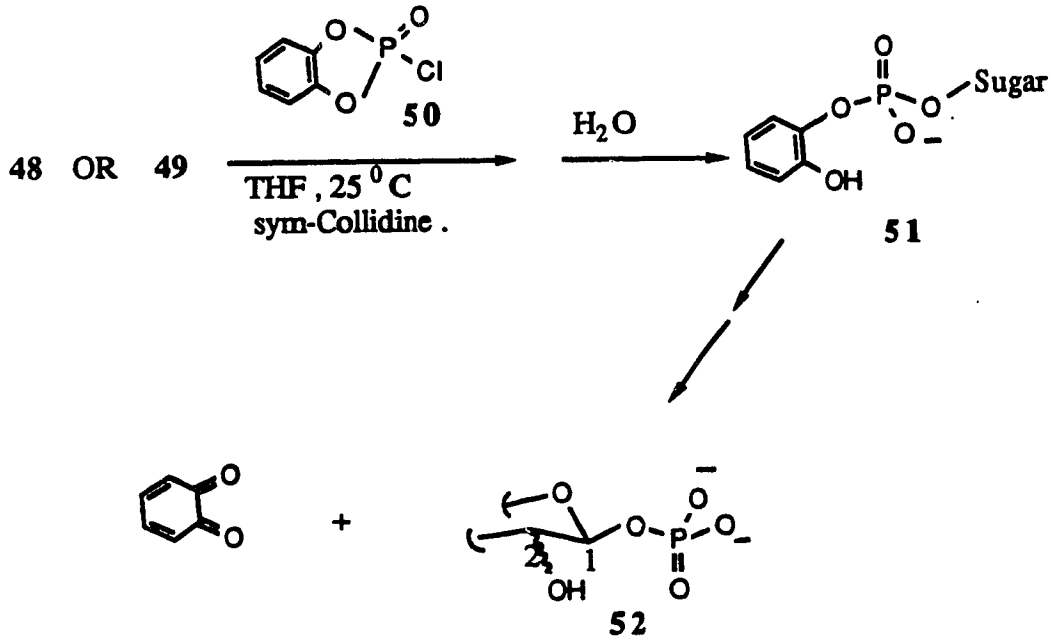
This method is most commonly used for equatorial phosphorylation.[67,68] The desired anomer having the equatorial configuration for the C-1 hydroxyl is synthesized by the stereospecific hydrolysis (Scheme 10) of the C-1 halogeno precursor. Thus the C-1 halogeno substrates having the C-1, C-2 / cis (**46**, scheme 10) or the C-1, C-2 / trans (**47**) relationship yield the equatorial anomers **48** and **49**, respectively, as major products. Usually, fractional crystallization of the reaction product gives the equatorial anomer in over 80% yield. The phosphorylation of these equatorial hydroxyls with the title reagent **50** in tetrahydrofuran containing *sym*-collidine have been reported to yield the *equatorial* phosphate as the principal phosphorylation product.

In solution, reducing sugars tend to attain the anomeric equilibrium favouring the *axial* anomer. The substrates **48** or **49** in the reaction medium (THF containing 10% collidine) at 25°C indeed establish anomeric equilibrium. It was found by Behrman et al.[67] that the half-time for anomerization of 2,3,4-tri-O-acetyl-β–L-fucopyranose under the phosphorylation reaction conditions was 55 hours, whereas, the half-time for 2,3,4-tri-O-acetyl-β-L-rhamnopyranose was 20 hours. The critical issue in this method (and also in all procedures belonging to the category B, scheme 7) is that the rate of

SCHEME 10 .


$H_2O/Ag(I)$
Et_2O
AcO
X
OH
46
48


AcO
Br
$H_2O/Ag(I)$
Et_2O
OH
47
49


Cl
50
48 OR 49
THF , 25 ^{0}C
sym-Collidine .
H_2O
Sugar
OH
51
+
OH
52

phosphorylation be rapid as compared with the rate of anomerization. The phosphorylation of compounds represented by **48** (gluco series) and **49** (manno series) using the reagent **50** is complete within 30 mins. Thus the phosphorylation is at least 100 times faster than the competing anomerization process.

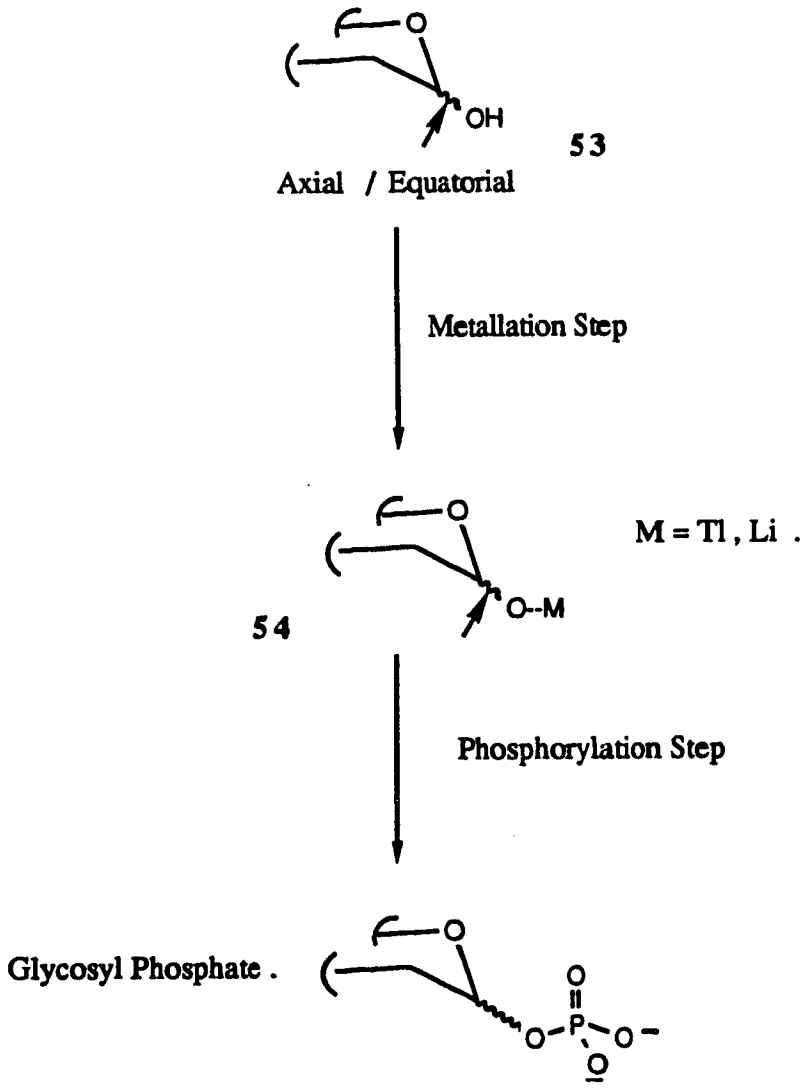
The phosphorylation step (scheme 10) followed by treatment with water yields the phosphodiester intermediate **51**. The oxidative removal of the hydroxyphenyl group in **51** can be achieved[68] by four methods : a) catalytic dehydrogenation, b) bromide water oxidation, c) periodic acid oxidation, or d) by means of lead tetraacetate oxidation. The last reagent is reported[68] to give over 60% yield for phosphorylation based on the starting aldose. The phosphorylation product contained an anomeric equatorial phosphate (**52**) as the major product along with an axial anomeric phosphate and 2-O-aldose phosphate as the minor products The separation of the desired equatorial phosphate is achieved either by fractional crystallization of the barium salt or by means of ion-exchange chromatography. The reported yields for the equatorial glycosyl phosphate vary from 10% to 60% depending on the nature of the substrate.[67,68]

A 5 . Activation of The Anomeric Hydroxyl prior to Phosphorylation.

The anomeric hydroxyl of a suitably protected substrate (**53**, scheme 11) can be activated in the form of a thallium[69] or lithium[52] alkoxide (**54**) which, upon treatment with a phoshorylating reagent, yields 1-O-phosphorylated product. Granata et al.[69] converted 2,3,4,6-tetra-O-benzyl-D-

SCHEME 11

PREACTIVATION OF THE ANOMERIC HYDROXYL GROUP



glucopyranose into the 1-O-thallium salt by using thallous ethoxide as the thallation reagent. The resulting thallium salt was then isolated and treated with diethyl or diphenyl phosphorochloridate. The anomeric composition of the product was found to vary with the solvent used during the phosphorylation step. Acetonitrile was shown to favour the axial phosphotriester when diphenylphosphorochloridate was the reagent, whereas, the use of benzene as the solvent favoured the equatorial product with the same reagent. The effect of the solvent on the stereoselectivity of the phosphorylation was found to be pronounced when the sugar hydroxyls were protected as acetate esters rather than as benzyl ethers. Shiba et al.[52] have employed 1-O-lithium salts of amino-deoxy-sugars for the anomeric phosphorylation.

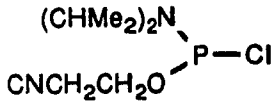
A 6. Phosphitilation Methodology (Use of Trivalent Phosphorus Reagents)

As depicted in scheme 12, Van Boom et al.[53] used the monofunctional phosphitilation reagent **55** for anomeric phosphorylation of 2,3,4-tri-O-benzyl-α-L-fucopyranose (**58**, R = benzyl). The leaving group abilities of the three substituents on the phosphorus (III) centre in **55** are such that the chlorine atom is displaced upon the first nucleophilic attack. Thus the reaction of **58** (100% axial or α anomer, R = benzyl) with **55** gave the phosphite **59** as the isolable intermediate. This phosphitilation step was accompanied by a slight anomerization giving **59** as a mixture of axial (α) and equatorial (β) (ratio = 95 : 5) anomers. The diisopropylamino group on phosphorus was then displaced

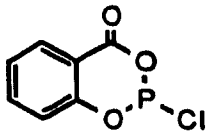
SCHEME 12

PHOSPHITILATION .

Monofuctional reagents .

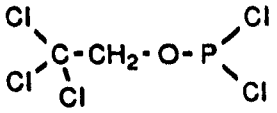


55



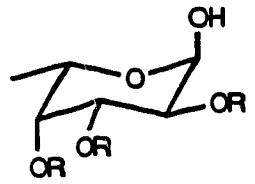
56

Bifunctional reagent .



57

Phosphitilation using **55**



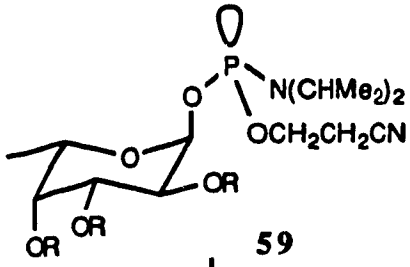
58

R = Benzyl .

55

iPr_2NEt , 20^0C

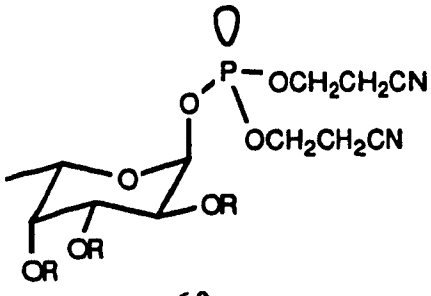
CH_2Cl_2



59

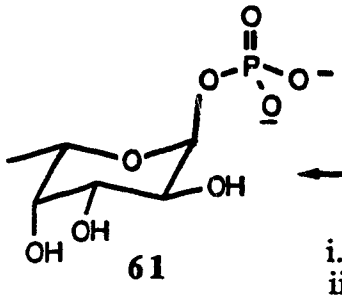
$CNCH_2CH_2OH$

Tetrazole



60

i - iii



61

i. Oxidn. at P

ii. Phosphate deprotection

iii. Carbohydrate deprotection

with cyanoethanol to afford **60**. The oxidation of the phosphorus centre with t-butyl hydroperoxide, followed by the removal of cyanoethyl and benzyl protecting groups afforded α-L-fucopyranosyl phosphate **61** in 89% yield (based on **58**). Compounds **56**[70] and **57**[71] have also been employed for the introduction of a phosphite group at the anomeric position.

So far, the phosphitilation with **55** has been tried only on an axial anomer. The ability to generate the desired axial or equatorial anomer for the phosphitilation and the maintainance of the anomeric integrity throughout the reaction sequence decide the stereochemical purity of the product. The phosphitilation of the equatorial anomer of aldopyranoses has yet to be reported in the chemical literature.

Apart from the usage in the anomeric phosphorylation methodology, the phosphitilation approach (i.e. the use of **55** as a reagent) has also been employed in the construction of phosphodiester linkages between two sugar residues.[53]

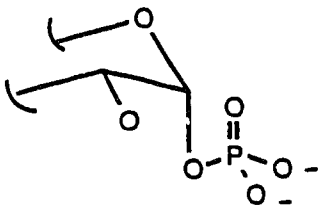
A 7 . Summary

As shown in figure 10, the structures **62-67** summarize the possible stereochemistry (C-1/C-2) for aldopyranosyl phosphates. Methods available for the synthesis of these structures are summarized as follows:

1a) The stereospecific displacement of a halide (Cl or Br) or an imidate at C-1 using dibenzyl phosphate anion was the most

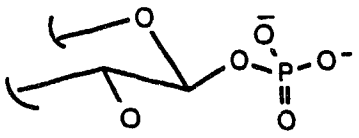
FIG.10

AXIAL PHOSPHATE

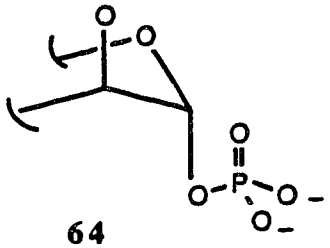


62
C-1/C-2 : CIS

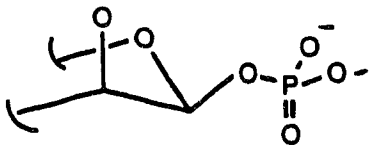
EQUATORIAL PHOSPHATE



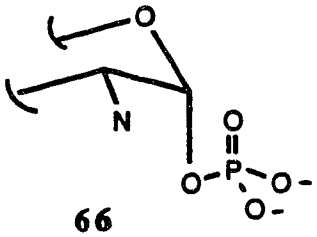
63
C-1/C-2 : TRANS



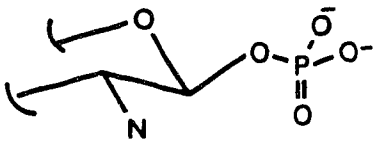
64
C-1/C-2 : TRANS



65
C-1/C-2 : CIS



66
C-1/C-2 : CIS



67
C-1/C-2 : TRANS

STEREOCHEMICAL POSSIBILITIES FOR ANOMERIC PHOSPHATES

promising process for the synthesis of equatorial phosphates of the type **63**[50,66] and **67**[72] (30%- 50% yield).

Minimization of the reaction time (in order to minimize the competing anomerization processes at C-1) and homogeneous reaction conditions (the insolubility of the silver salt of dibenzyl phosphate in the reaction medium) were the most desired modifications needed in this method.

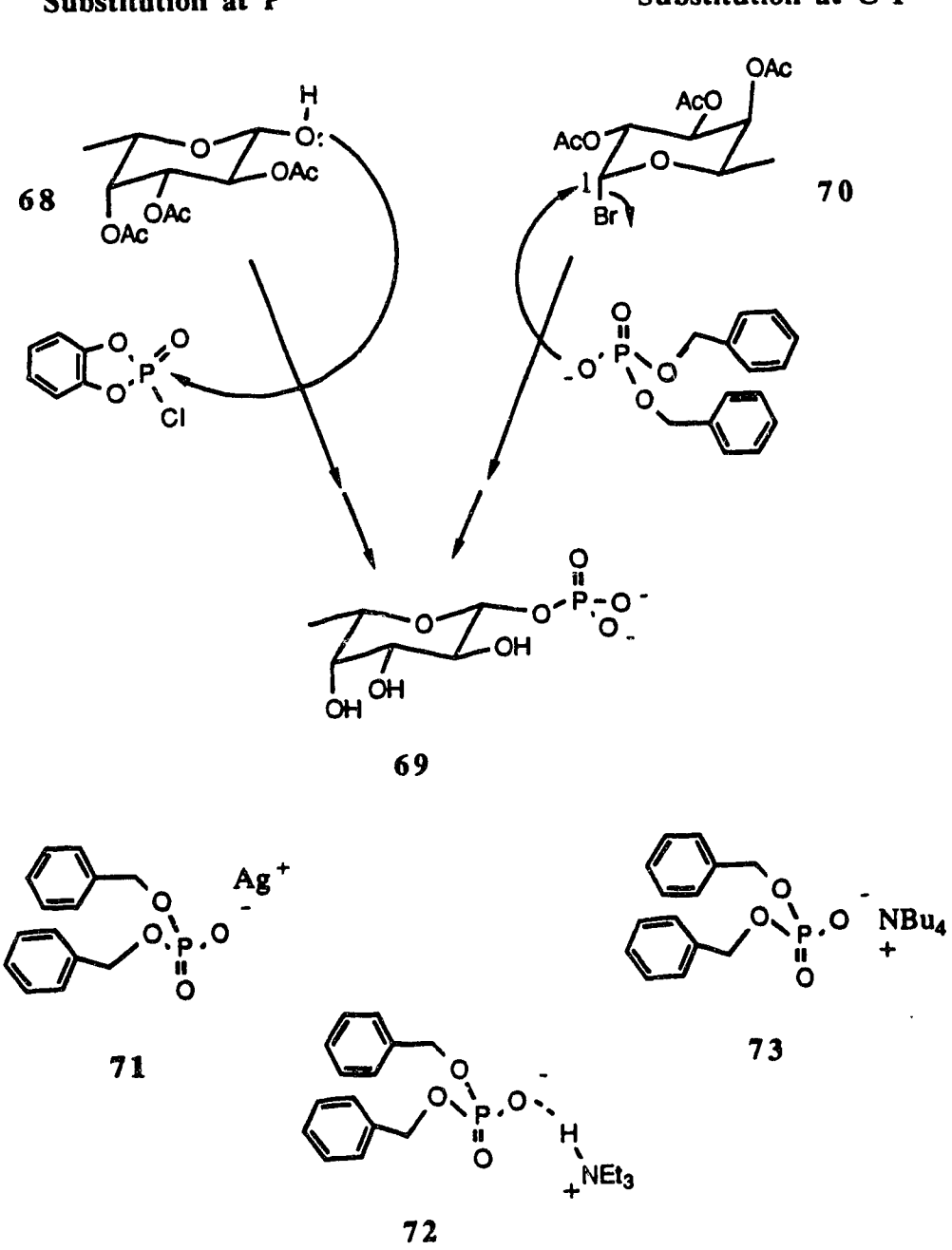
1b) Use of *o*-phenylene phosphorochloridate (**50**) provised an alternative for the synthesis of the compounds of the type **63**[68] and **65**.[73]

2) For obtaining axial phosphates of type **62** and **64**, the McDonald synthesis[51] and the anionic displacement method using silver diphenyl phosphate[50] are the most frequently used procedures. The axial phosphates of aminosugars of the the type **66** are obtained by the oxazoline method[62] and by the 1-O-lithium activation method.[52]

Since our aim was to synthesize equatorial glycosyl phosphates (β-L-fucosyl phosphates), the dibenzyl phosphate displacement method and the use of *o*-phenylene phosphorochloridate as the phosphorylating agent appeared to be the appropriate procedures. Both approaches were investigated and the results will be described in the following section.

B . Anomeric Phosphorylation Studies

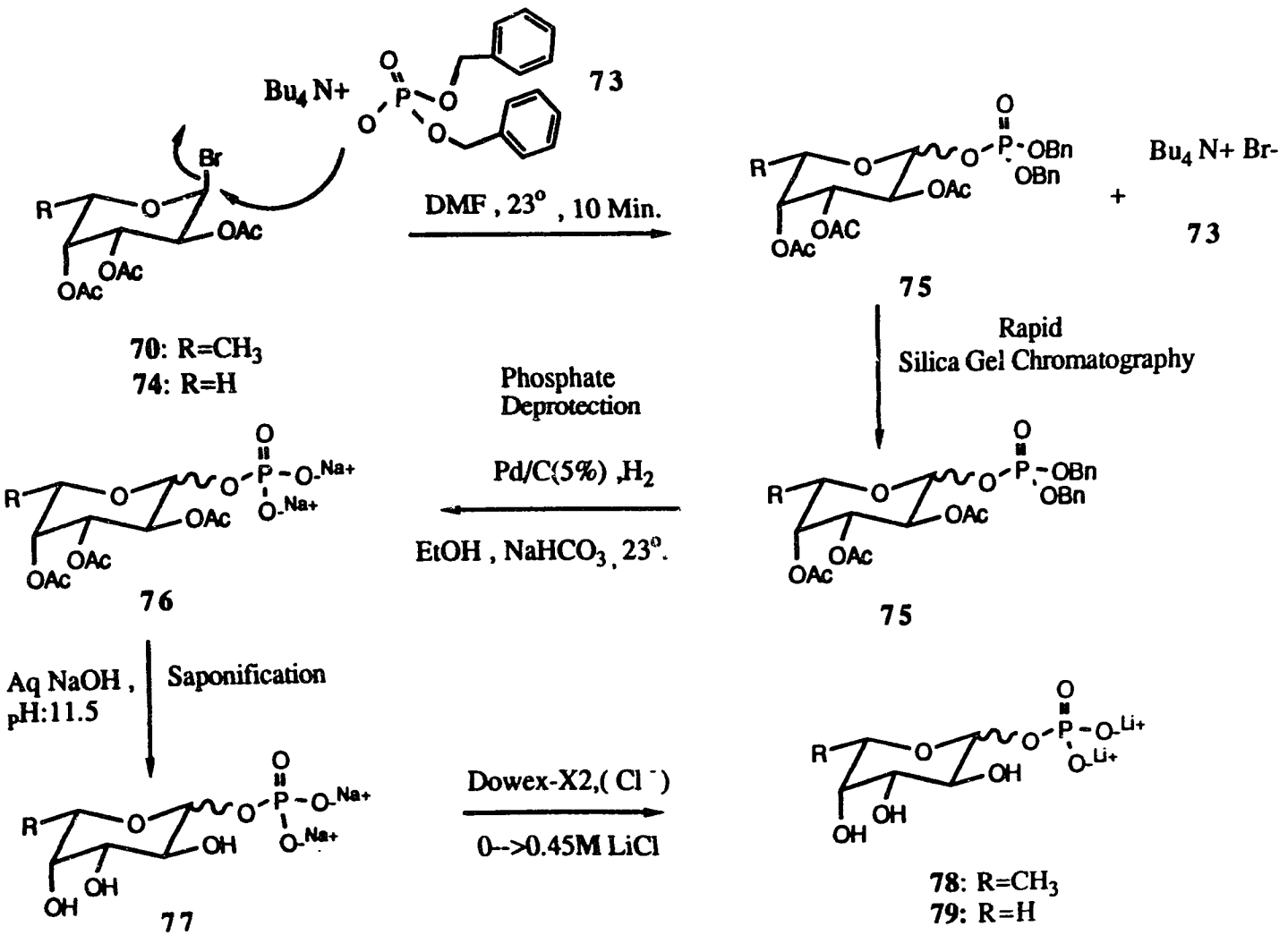
SCHEME 13



As described in the previous section the synthesis of β-**L**-fucopyranosyl phosphate (**69**, scheme 13) had earlier[67,68] been achieved by the reaction of the β-anomer of 2,3,4-tri-O-acetyl-**L**-fucopyranose (**68**) with ortho-phenylene phosphorochroridate (**50**) which afforded the compound in variable yields. Behrman et al.[67] had reported a yield of 11%, whereas Barker et al.[68] obtained the compound in 50% yield. Several attempts to reproduce the Barker procedure however never yielded over 20% of total phosphorylated product and the β-anomer was formed only as a minor product. This approach was therefore abandoned.

The nucleophilic displacement method (**70-->69**, scheme 13) had not been employed in the previous syntheses of **69**. Conventionally, the nucleophiles **71** and **72** are used in such displacement reactions. However, as mentioned earlier, the insolubility of **71** in the reaction medium was frequently problematic and the use of the triethylammonium counterion had been suggested. We envisaged that the use of tetraalkylammonium countercations would offer an additional advantage over trialkylammonium cations. It is likely that the hydrogen bonding between the ion pair as shown for **72** would attenuate the nucleophilicity of the dibenzyl phosphate anion. Such diminution in the reactivity has been found[74] in the displacement reactions using pyrophosphate anion. The anomeric phosphorylation studies using **73** were undertaken with the aim of obtaining high yields of the equatorial isomer.

SCHEME 14


Bu4 N+
73
Br
DMF , 23° , 10 Min.
70: R=CH3
74: R=H
75
Bu4 N+ Br-
73
Rapid
Silica Gel Chromatography
Phosphate
Deprotection
Pd/C(5%) ,H2
EtOH , NaHCO3 , 23°.
76
75
Aq NaOH ,
pH:11.5
Saponification
Dowex-X2,(Cl -)
0-->0.45M LiCl
77
78: R=CH3
79: R=H

B 1. Use of Tetra-n-butylammonium Dibenzyl phosphate (73) as Nucleophile.

The bromosugars **70**[68] and **74**[75] (scheme 14) were synthesized from the corresponding anomeric acetates Commercially available dibenzyl hydrogen phosphate was neutralized with tetra-n-butylammonium hydroxide (30% aq. solution) to afford **73** as a colourless syrup. The displacement reactions were carried out in CH_2Cl_2, toluene DMF as solvents. A solution of **73** was added to **70** or **74** at ambient temperature to obtain homogeneous solutions. The reaction mixtures (0.4-0.5 M in **70** or **74**) were stirred at ambient temperatures and monitored by tlc for the disappearance of the bromosugar and the appearance of the phosphotriesters (**75**). The displacement reaction was found to be exceedingly fast in DMF (3-6 mins) as opposed to 45 mins. in toluene and 1.5 h for CH_2Cl_2. The 6 fold molar excess of **73** was found to be necessary in order to obtain over 90% of equatorial phosphorylation (table 2, *vide infra*). The phosphotriester (**75**) was subjected to debenzylation by using 5%Pd/C /H_2 to obtain **76**. The neutral material contaminating **76** was conveniently extracted into CH_2Cl_2 leaving the desired phosphate in the aqueous layer. ^{1}H-nmr analysis of the compound indicated that the acetate protecting groups had survived the phosphorylation and debenzylation steps. The saponification of O-acetates followed by the purification of **77** by means of ion exchange chromatography afforded the fully deprotected glycosyl phosphates **78** and **79**. The elution of the glycosyl phosphates was carried out by applying a (0-->0.45 M) linear gradient of aq.

solutions of LiCl, NH_4HCO_3 or triethylammonium-bicarbonate[76] salts. The removal of the ammonium bicarbonate salts from the product phosphates was achieved by repeated evaporations of the aqueous solutions under reduced pressure. The lithium chloride could be extracted selectively[77] by a mixture of ethyl ether and ethanol (2:1). The lithium salts of the glycosyl phosphates (**78** or **79**) were insoluble in the same solvent mixture. It was observed that sometimes the buffer salts could not be completely removed from the product. Hence, the quantitation of the product phosphate was done by ^{1}H-nmr using dry TSP-d4 as the internal standard.

Table 2.

Bromosugar	Equiv. of **73**	Solv.	Yield **78/79**	Prod. Distribution Eq.	Ax.
70	2.7	DMF	50%	80%	13%
	6.0	DMF	50%	93%	7%
74	4	TOLUENE	59%	67%	33%
	4	CH_2Cl_2	52%	80%	20%
	6	DMF	47%	97%	3%

As shown in table 2, **78** and **79** were isolated in 50% yield, (4 steps from the peracetylated sugar). The desired equatorial phosphate was formed as the major product. Thus the phosphorylation using **73** as a nucleophile appears to be an

efficient process. The syntheses of ring deoxy analogs of L-fucose phosphate were achieved using this process, details of which will be described in the next section.

B 2 . Synthesis of Equatorial Glycosyl Phosphite (Phosphitilation using 2-chloro-1,3,2-dithiaphosphorinane, 84)

Simultaneously with the phosphorylation studies using tetra-n-butyl ammonium dibenzyl phosphate we undertook the phosphitilation of 2,3,4-tri-O-acetyl-β-L-fucopyranose (**68**). This study was prompted by the fact that no such attempt (i.e. anomeric phosphitilation of an equatorial anomer of an aldopyranose , **80-->81**, scheme 15) has been reported in the literature.

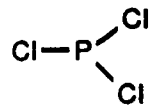
Ogawa et al[71] had reported the first phosphitilation of a hemiacetal moiety. They used phosphorus trichloride (**82**) to phosphitilate the axial anomer of 2,3,4,6-tetra-O-acetyl-D-glucose. Van Boom et al.[53] used the monofunctional reagent **55** for the phosphitilation (scheme 12, *vide supra*) of the axial anomer of 2,3,4-tri-O-benzyl-L-fucopyranose (**58**).

A procedure for the phosphitilation of aliphatic alcohols was reported[78] which used the monofunctional reagent **83**. Thus an alcohol **85** (scheme 15) was converted into the corresponding phoshite **86** which was then oxidized with iodine and water to obtain the phosphate **87** in good yield. The removal of the sulphur substituents and the oxidation of the trivalent phosphorous centre in **86** was achieved in one step.

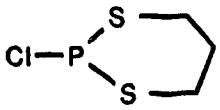
SCHEME 15

PHOSPHITILATION OF THE HEMIACETAL

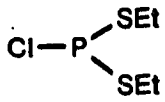
80 **81**



82

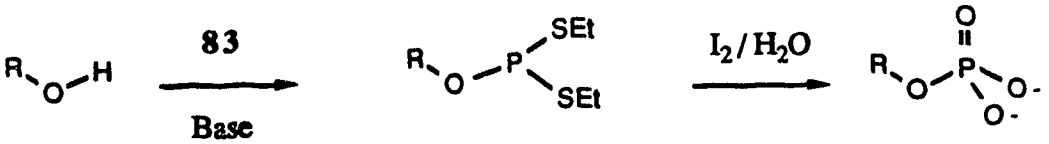


84



83

Phosphitilation of an alcohol



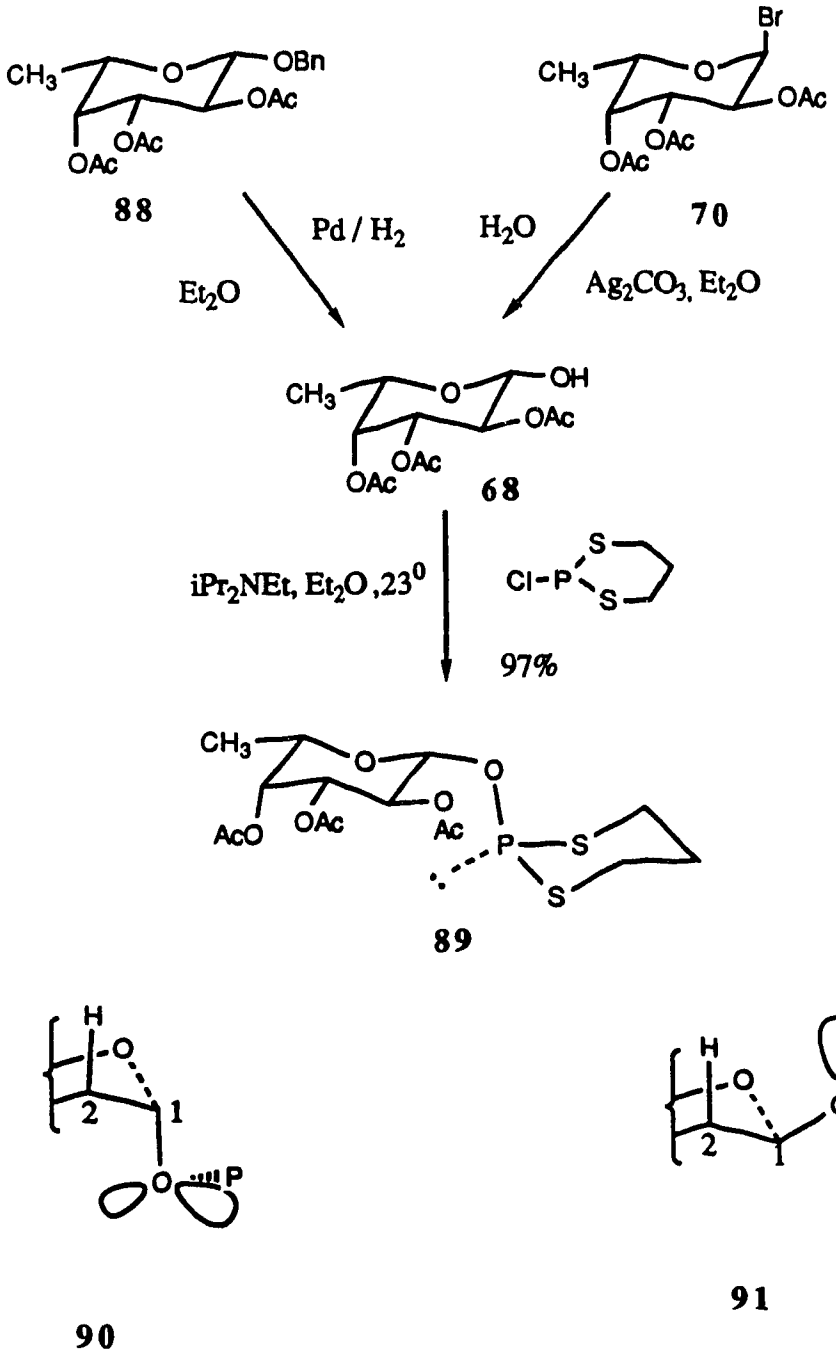
85 **86** **87**

We investigated the use of the title compound **84**[81a,b] (the cyclic counterpart of **83**) for anomeric phosphitilation. (This reagent was obtained as a gift from Prof. M. J. Robins of this department). The substance was obtained as a stable solid ($\delta^{31}P$= 138.9) and was preserved dessicated under argon. As depicted in scheme 16 compound **68** was synthesized from the bromo sugar **70**[68]. The reductive cleavage of β-benzyl fucoside **88**[79] under the conditions described by Ballou et al.[80] (H_2/Pd.C) in absolute ethyl ether as the solvent) also afforded the desired β–anomer **68**. Both approaches gave the mixture of anomers with the desired anomer **68** as the major product. Fractional crystallization in dry ethyl ether afforded the β–anomer in an isolated yield of 40 %.

Treatment of **68** with 2-chloro-1,3,2-dithia phosphorinane **84** in the presence of diisopropyl ethyl amine gave the β-L-fucosylphosphite **89** in near quantitative yield. The phosphite **89** showed remarkable stability to the aqueous work-up conditions and the silica gel chromatography. The β–anomeric configuration of the fucosyl moiety was evident in its ^{1}H-nmr spectrum where H-1 resonated at 4.945 ppm as a doublet of doublets; $J_{1,2}$ = 8 Hz and $J_{1,P}$ = 11.5 Hz. (^{1}H-nmr spectrum is reproduced in figure 11). The trivalent phosphorous in **89** resonated at + 154.1 ppm in its ^{31}P-nmr.spectrum The chemical shift of the phosphorous is in accord with those reported[81c] in the literature for related compounds.

As depicted in scheme 16 the structure **90** displays the preferred conformation for the C1-O-P fragment of

SCHEME 16


CH3
O
OBn
OAc
OAc
OAc
88
Pd / H2
Et2O
H2O
Br
CH3
O
OAc
OAc
OAc
70
Ag2CO3, Et2O
CH3
O
OH
OAc
OAc
OAc
68
iPr2NEt, Et2O ,23°
Cl-P
S
S
97%
CH3
O
O
O
AcO
OAc
Ac
P
S
S
89
H
O
2
1
O
P
90
H
O
2
1
O
P
91

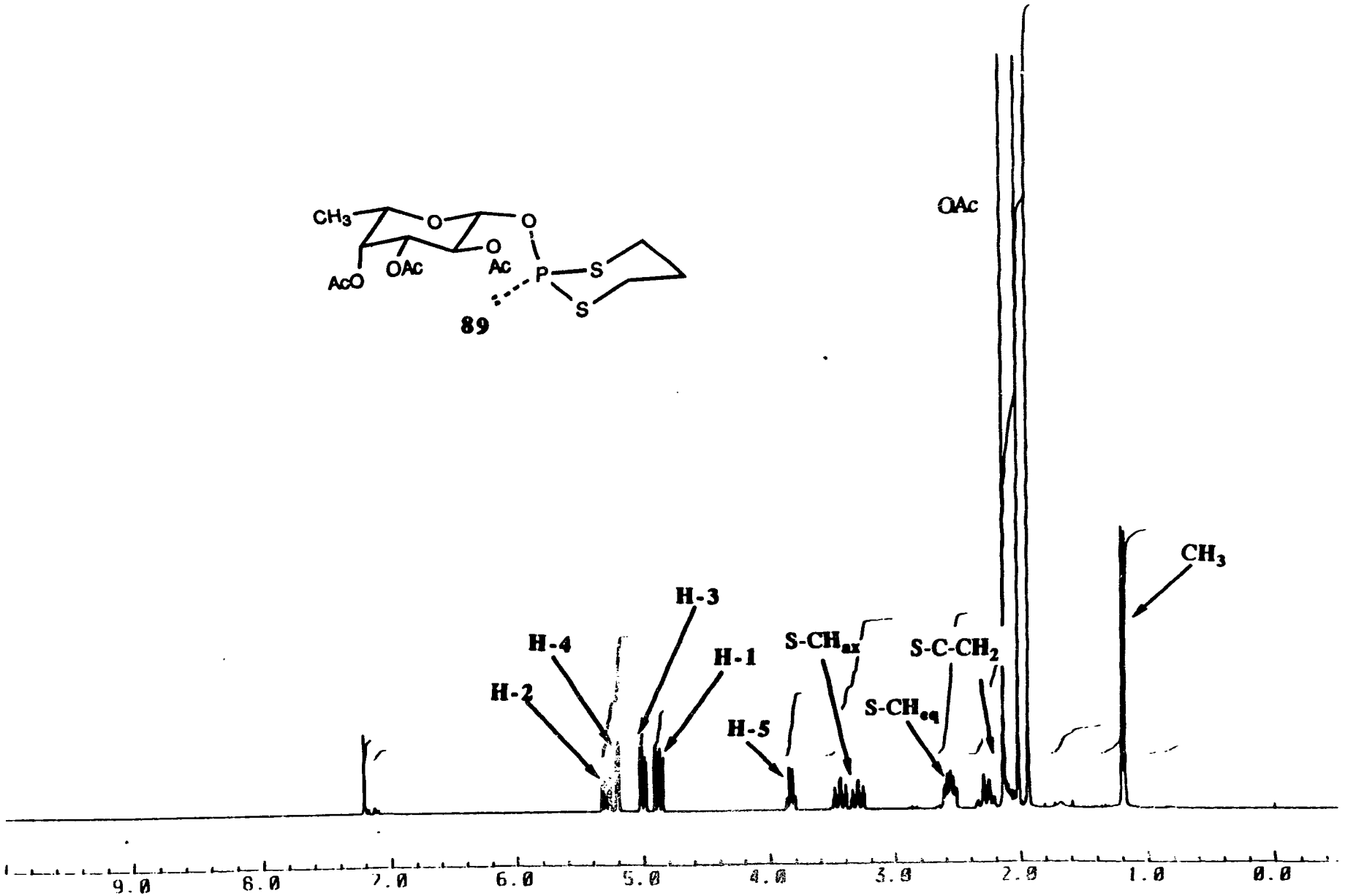


Fig. 11. The 300 MHz ^1H spectrum of compound **89** in $CDCl_3$.

an axial glycosyl phosphate and structure **91** displays a similar conformational preference for the equatorial phosphate. The extended conformation of the C1-O-P fragment in both **90** and **91** is predicted by the exo-anomeric effect.[82] In **90** one obtains a trans-antiperiplanar arrangement of the H-2--C-2--C-1--O-1--P atoms which results in the coupling through four bonds between the phosphorous atom and H-2. In **91**, such coupling does not result owing to the absence of a 'W' type geometric relationship between H-2 and P. In the ^{1}H-nmr spectrum of **90** $^{4}J_{2,P}$ is observed whereas in **91** such coupling is absent. These observations are well-documented.[68,71,83] The phosphite **89**, due to the equatorial configuration at the anomeric centre, did not display any coupling between H-2 and P in its ^{1}H-nmr spectrum(δ H-2 = 5.363, dd, $J_{1,2}$=8Hz, $J_{2,3}$=11Hz). The phosphite **89** represents the first example of an equatorial glycosyl phosphite. Further investigation of the conversion of **89** into the phosphate was judged to be outside the scope of the project.

The synthesis of ring deoxy analogs of L-fucose phosphate were achieved using **73** as a nucleophile, details of which will be described in the following section.

C. Synthesis of the analogues

As described in the Introduction, we proposed to synthesize the GDP-fucose analogues in which the hydroxyl groups at the 2,3, and 4 positions and the methyl group at the 5 position of the fucose moiety were substituted by a hydrogen atom. As depicted in scheme 17, **L**-fucose was chosen as the starting material for the synthesis of 2-deoxy (**92**), 3-deoxy (**93**) and 4-deoxy (**94**) analogues of β-**L**-fucopyranosyl phosphate. The deoxygenations at the 2,3, and 4 positions were planned on the appropriately hydroxyl-protected fucosyl synthons. The synthetic plan will be described in the following section.

For the synthesis of the 5-nor analogue (**79**, scheme 17), the naturally occuring aldopentose **D**-arabinose was the appropriate starting material. This sugar adopts the $^1C_4$ ring conformation in the pyranose ring form and it contains the 2,3 and 4 ring hydroxyls in the appropriate configurations so as to topographically mimic **L**-fucopyranose on its α face.

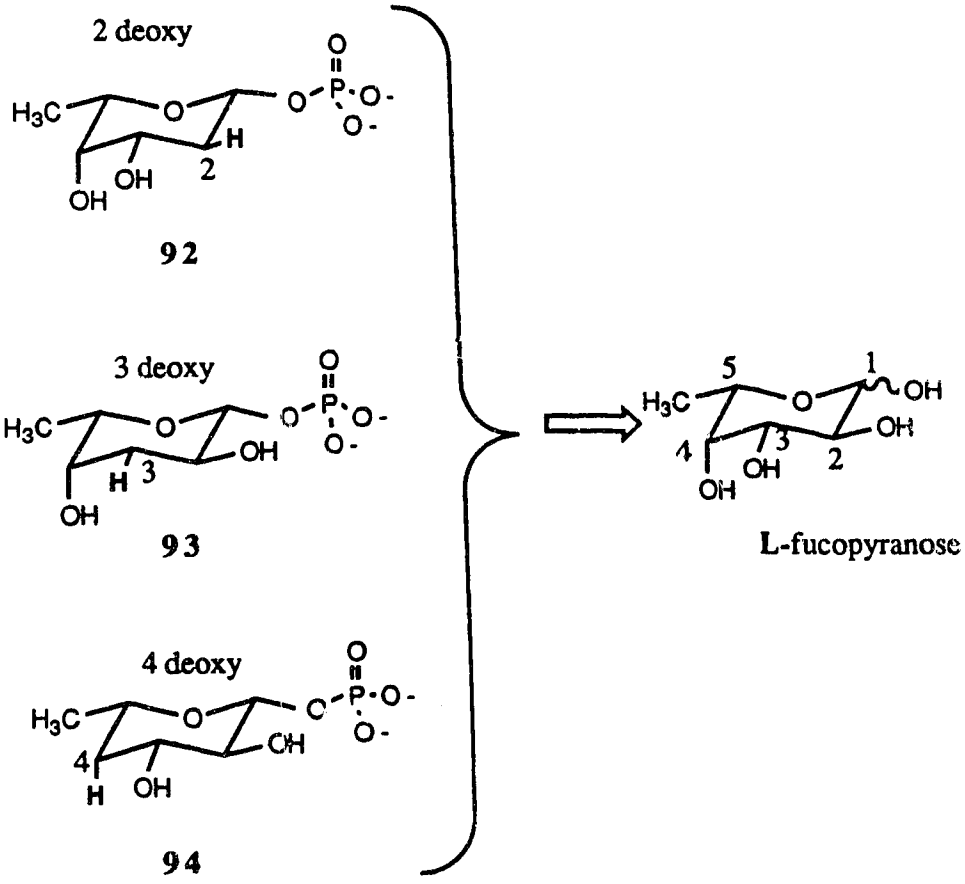
C1 . Synthetic plan

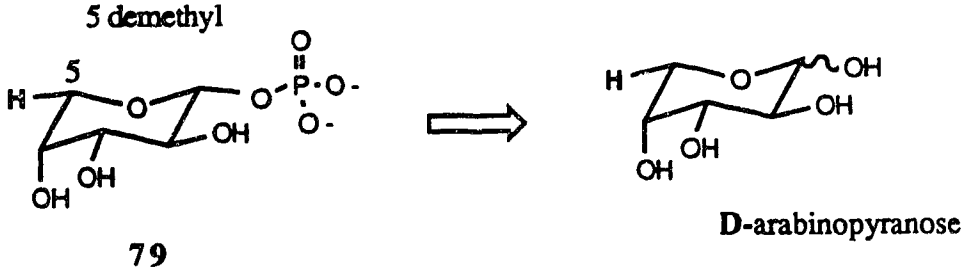
The following aspects were considered while designing the synthetic strategy.

1) Apart from the hydroxyl-protection/ deprotection reactions, the deoxygenation and anomeric phosphorylation were the major bond-forming reactions. The procedure of Robins and Wilson[43] was chosen for the deoxygenation of the ring-hydroxyls. The phosphorylation using tetra-n-butylammonium dibenzyl phosphate was to be performed on the deoxygenated

SCHEME 17

THE ANALOGUES OF L-FUCOSE PHOSPHATE





sugars having a halogen at the anomeric position. Both, the deoxygenation and phosphorylation reaction conditions were compatible with ester type protecting groups for the fucose ring hydroxyls.

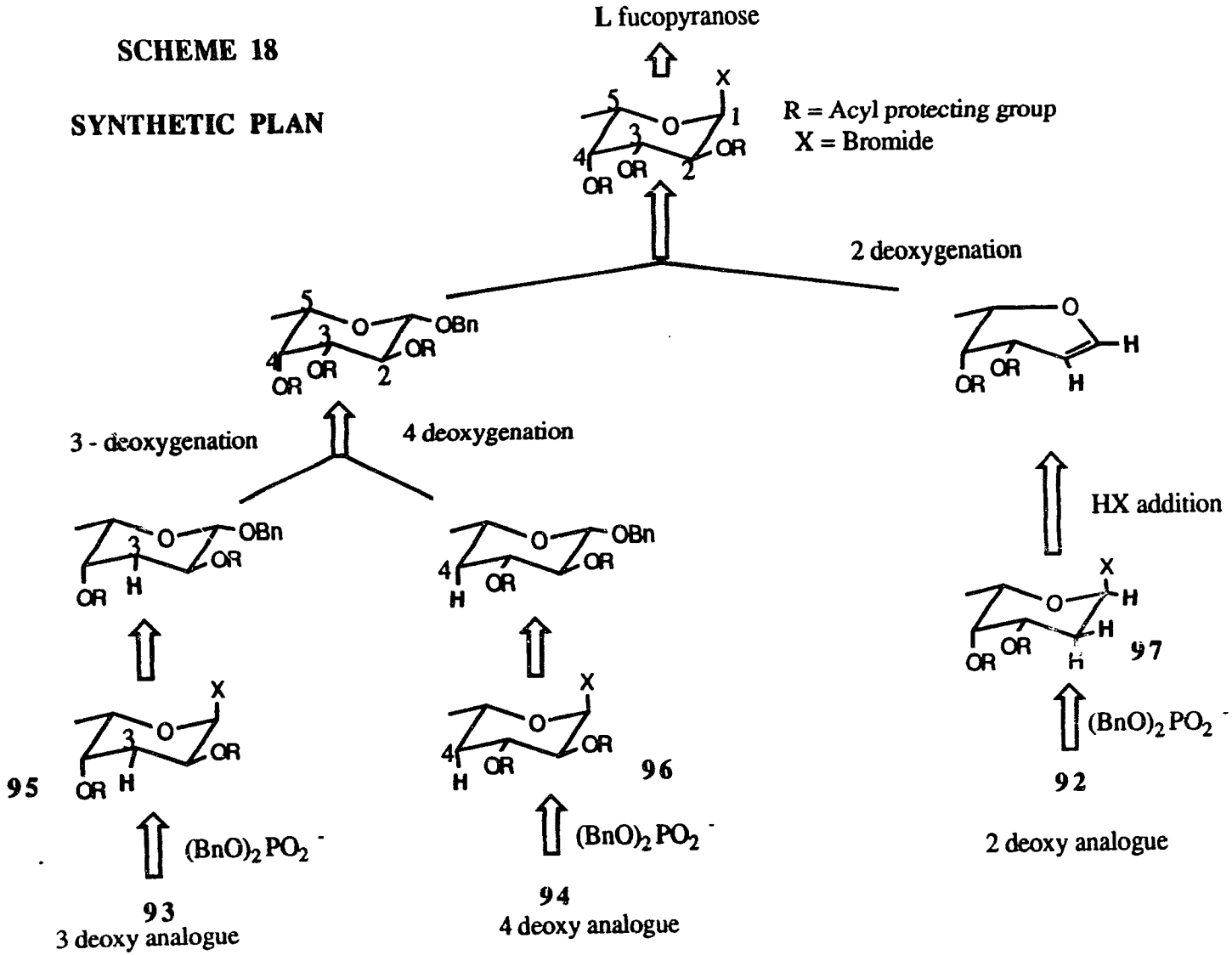
2) The anomeric hydroxyl was not involved in any synthetic transformation until the anomeric phosphorylation, which was the last step (scheme 18). Hence, one needed to have a *persistent* protecting group at O-1 which could survive the intermediate reaction conditions. We decided to protect O-1 as a benzyl ether since these are quite stable toward acidic and basic reaction media and hydrogenolysis under mild conditions achieves their deprotection. Indeed, benzyls have become the persistent protecting group of choice for multi-step oligosaccharide syntheses[40].

3) Among the remaining ring hydroxyls, the 2 and 3 hydroxyls were equatorially disposed and hence were expected to be more reactive than the axial hydroxyl at the 4 position. The differential reactivities of the secondary hydroxyls in pyranosides are well documented[84,85] and selective protection studies were undertaken which will be described in the next section.

4) The deoxy fucosyl halides **95**, **96**, and **97** (scheme 18) were the substrates for anomeric phosphorylations. Their stability toward the competing hydrolytic reactions at C-1 was taken into consideration. The consensus has now been reached[86,87] that as the transition state for the displacement reactions at C-1 is approached, the involvement of the

SCHEME 18

SYNTHETIC PLAN



nonbonding electron pair at O-5 imparts a partial double-bond character to the O5-C1 bond. Thus the structure **98** (scheme 19) can be considered an approximation of the transition state for the departure of the halide leaving group in polar solvents. The initial $^1C_4$ ring conformation is expected to become a distorted chair or "sofa" as shown in **98**. Due to this flattening of the ring, crowding of the ring substituents (eclipsing interactions) is expected. When the deoxygenation replaces the hydroxyl group by a hydrogen atom, one expects a decrease in the crowding for attaining a flattened transition state **98**. Since a hydrogen is less electronegative than an OH group, the charged **98** would form more readily.

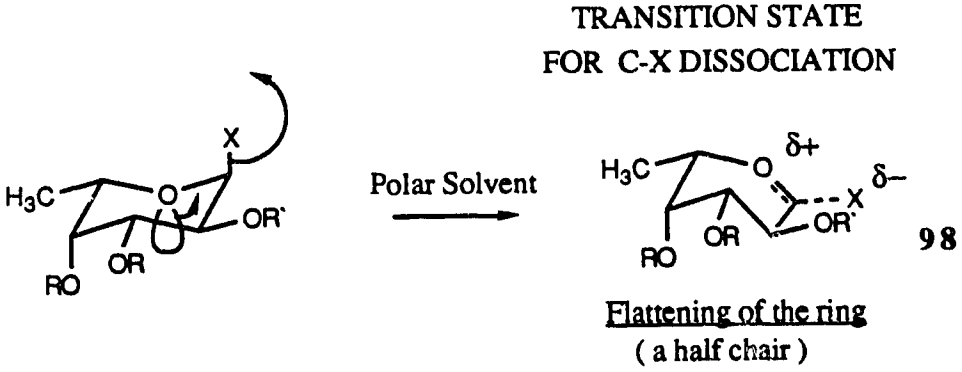
The C-2 hydroxyl in **95** and **96** was protected as a benzoate ester. Potential neighbouring group participation (**98**-->**99**, scheme 19, R' = phenyl) was the possible outcome of 2-O-benzoylation. It was anticipated that the "naked" dibenzyl phosphate anion would compete effectively in trapping the ion pair **98** (**98**-->**100**, Nu = phosphodiester anion) to productively yield the desired phosphorylation.

C 2 . Selective Acylations and Ring Deoxygenations

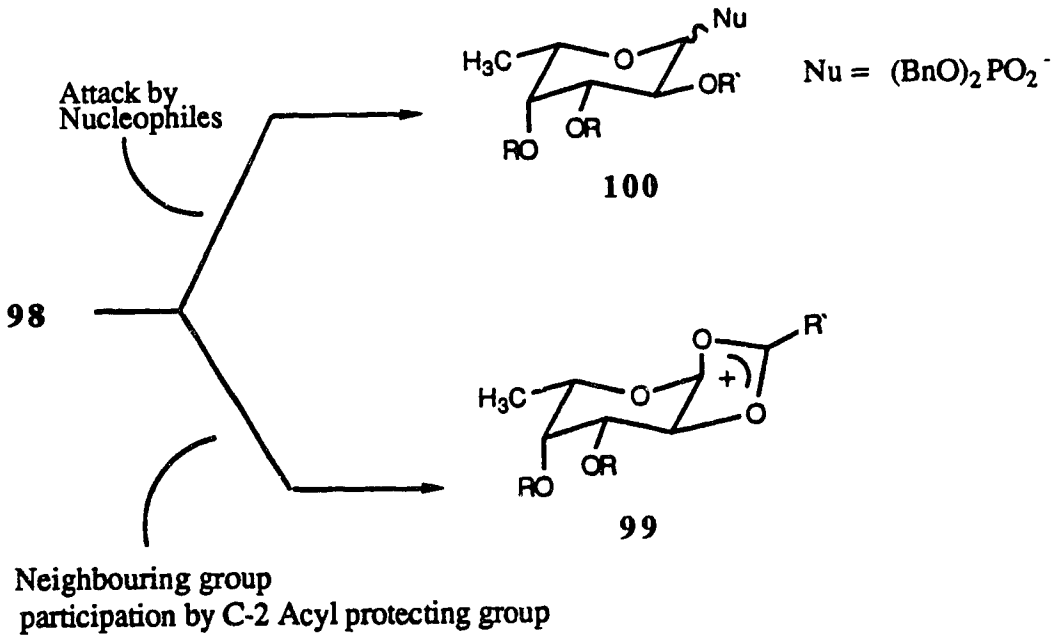
The functionalization of the hydroxyl groups on aldopyranosides and aldofuranosides has been an area of extensive investigation.[85,88,89] The stereochemical disposition (axial/equatorial) of a hydroxyl group on the pyranose ring, has a strong bearing on its reactivity toward sterically discriminating reagents.[85] Equatorial hydroxyls are usually more reactive than the axial ones, the reactivity order being primary > equatorial:

SCHEME 19

DISSOCIATION OF C-1 SUBSTITUENT



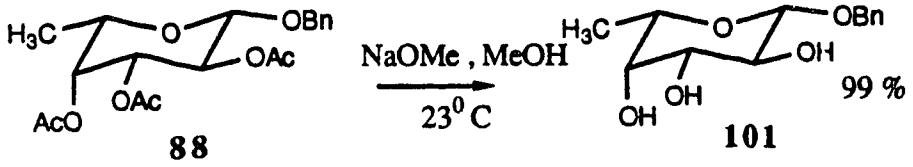
The fate of **98** in the reaction medium



secondary > axial: secondary. These differences are of great practical value for the synthetic transformations on monosaccharides. The regioselective functionalization of the hydroxyls on oligo and polysaccharides remains less predictable.[90]

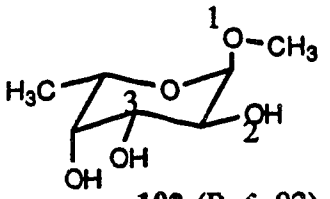
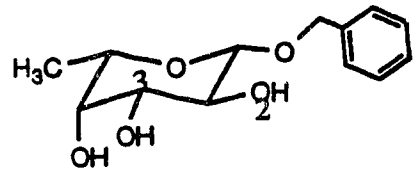
We investigated the reactivity of triol **101**[79] (scheme 20) toward the benzoylation using pyridine and benzoyl chloride in dichloromethane. A literature report[92] described the selective 2,3-di-O-benzoylation of methyl α–L-fucopyranoside **102** in 80% yield. The 3-O-benzoyl derivative was also isolated in 6% yield. Treatment of triol **101** with 2.1 equivalents of benzoyl chloride and 8 equivalents of pyridine in CH_2Cl_2 as the solvent resulted in the formation of the 3-O-benzoate (δ H-3 = 5.082) as the major (60%) product. The 2,3-di-O-benzoate (35%) and tri-O-benzoate (5%) were also formed in the reaction. Several trials of benzoylation consistently resulted in the same product mixture, thereby indicating the reactivity order of 3-OH > 2-OH > 4-OH under the reaction conditions. The well-established phenomenon of nucleophilic catalysis[93] supports the structures **103** and **104** (e.g. use of DMAP in the phenoxythiono carbonylation : Chapt. II : deoxygenations of disaccharides) as the reactive intermediates in such benzoylations. These relatively sterically demanding electrophiles not only discriminate between axial and equatorial hydroxyls, but also discriminate between 3-OH and 2-OH. in **101** The diminished reactivity of the 2-OH in **101** can be contrasted with that in **102**. The free rotation of the C-1 benzyloxy moiety in **101** around the O1-CH_2

SCHEME 20

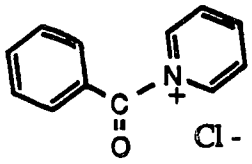


88 101

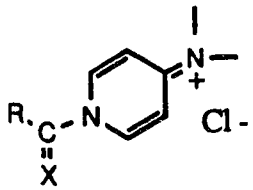
Selective benzoylation using BzCl (2 eq.), Pyridine , CH_2Cl_2

	2, 3 di-O-Benzoate	3-O-Benzoate
 **102** (Ref. 92)	80 %	6 %
 **101** (This study)	35 %	60 %

Nucleophilic Catalysis in benzoylation.



103

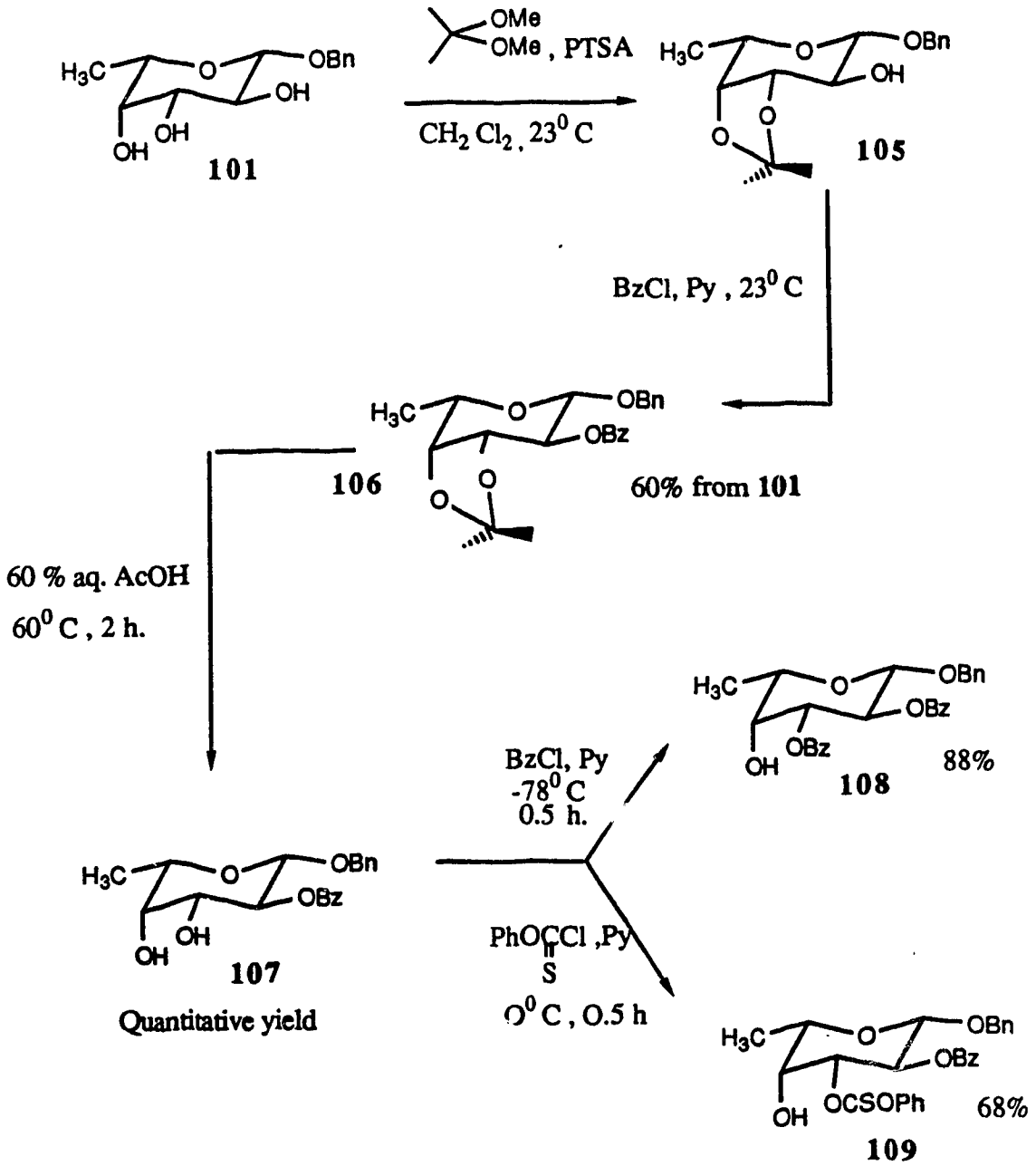


104

bond may be a factor causing steric hindrance for the approach of **103** or **104**.

We decided to proceed with the 2-O-benzoylation of **101** through the three-step sequence described in scheme 21. The protection of the cis-diol moiety in **101** to afford the isopropylidenated compound **105** was achieved by the use of 2,2-dimethoxy propane as an acetone equivalent and para-toluene sulfonic acid as the acid catalyst. The previous synthesis[79] of **105** was achieved by the use of acetone and sulfuric acid wherein the 1,2:3,4-di-O-isopropylidene derivative was also formed as a minor product. The milder reaction conditions in the present case yielded **105** as the only product as judged by tlc and nmr analyses on the crude syrupy product. The signal for the 2-OH in the ^{1}H-nmr spectrum appeared at 2.481 ppm and H-2 gave a signal at 3.593 ppm. On reaction with trichloroacetyl isocyanate (TCAI) the signal for 2-OH disappeared and H-2 resonated at 5.012 ppm as a doublet of doublets $J_{1,2}$ = 8.0 Hz and $J_{2,3}$ = 7.5 Hz which established the free alcohol group to be at C-2. The benzoylation of **105** resulted in the production of desired **106** in 60% yield (from **101**). Introduction of the benzoate functionality resulted in the expected deshielding of H-2 of **106** (δ H-2 = 5.306). The anomeric proton also showed a deshielding (in **105**, δH-1 = 4.226, in **106**, δ H-1 = 4.458). This deshielding is an indication of the electron withdrawing property of acyl protecting groups. The hydrolytic removal of the isopropylidene group in **106** (60% AcOH, 60^{o}C) resulted in the formation of the expected diol **107** in good yield . The position of the benzoate in

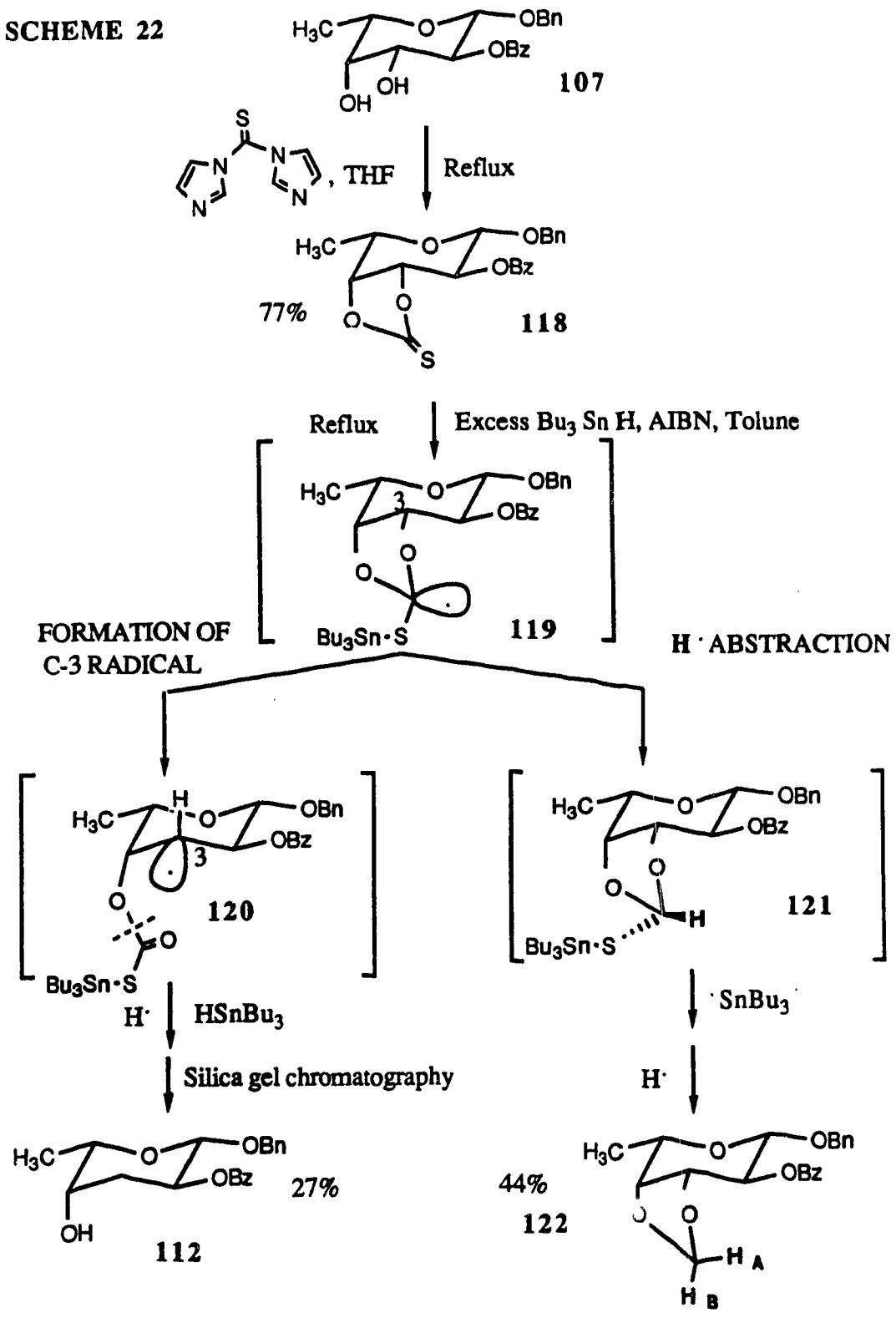
SCHEME 21


OMe
OMe , PTSA
CH2 Cl2 , 23° C
101
105
BzCl, Py , 23° C
106
60% from 101
60 % aq. AcOH
60° C , 2 h.
BzCl, Py
-78° C
0.5 h.
88%
108
107
Quantitative yield
PhOCCl ,Py
S
0° C , 0.5 h
68%
109

107 was established by ^{1}H-nmr spectroscopy δ H-2 = 5.238. Moreover, the reaction of **107** with TCAI resulted in deshielding of H-3 and H-4 thereby excluding the possibility of migration of the benzoyl moiety under the acidic reaction conditions.

Having prepared diol **107**, the next objective was to functionalize the 3-OH and 4-OH selectively to allow their deoxygenation. It was envisaged that the conversion of diol **107** into the cyclic thionocarbonate derivative **118** (scheme 22) followed by the radical deoxygenation[44] should provide an easy access to both the 3-deoxy and 4-deoxy derivatives. Thus the treatment of **107** with N,N-thiocarbonyldiimidazole under neutral conditions (THF, reflux) afforded **118** in 77% yield. Subjecting **118** to the radical deoxygenation conditions (Bu_3SnH : 4 equiv, AIBN) led to the formation of the 3-deoxy derivative **112** in 27% yield. The regioisomeric 4-deoxy derivative was not formed. However, the methylidene derivative **122** was the principal product (44% isolated yield) of the reaction. Since **122** had two types of acetal carbons (C-1 & methylenedioxy carbon) the ^{13}C-nmr spectrum of **122** was recorded by using the method of Rabenstein *et al.*[94] which distinguishes between methyl, methylene, methine, & quaternary carbon atoms . The carbon atom of the methylenedioxy moiety was thus found to resonate at 95.24 ppm while C-1 appeared at 98.64 ppm. The singlet resonances in the ^{1}H-nmr spectrum of **122** at δ 5.050 and δ 5.319 were attributed to H_A and H_B (figure 12). The absence of the geminal coupling for H_A and H_B in such compounds has been well documented.[96] A recent report[97] also mentions the formation of

SCHEME 22


H3C
O
OBn
OBz
OH
OH
107
S
N
N
N
N
, THF
Reflux
H3C
O
OBn
OBz
77%
O
O
O
S
118
Reflux
Excess Bu3 Sn H, AIBN, Toune
H3C
O
OBn
3
OBz
O
O
Bu3Sn·S
119
FORMATION OF
C-3 RADICAL
H · ABSTRACTION
H3C
H
O
OBn
3
OBz
O
O
120
Bu3Sn·S
H·
HSnBu3
Silica gel chromatography
H3C
O
OBn
OBz
OH
27%
112
H3C
O
OBn
OBz
O
O
H
121
Bu3Sn·S
·SnBu3·
H·
H3C
O
OBn
OBz
44%
122
O
O
H A
H B

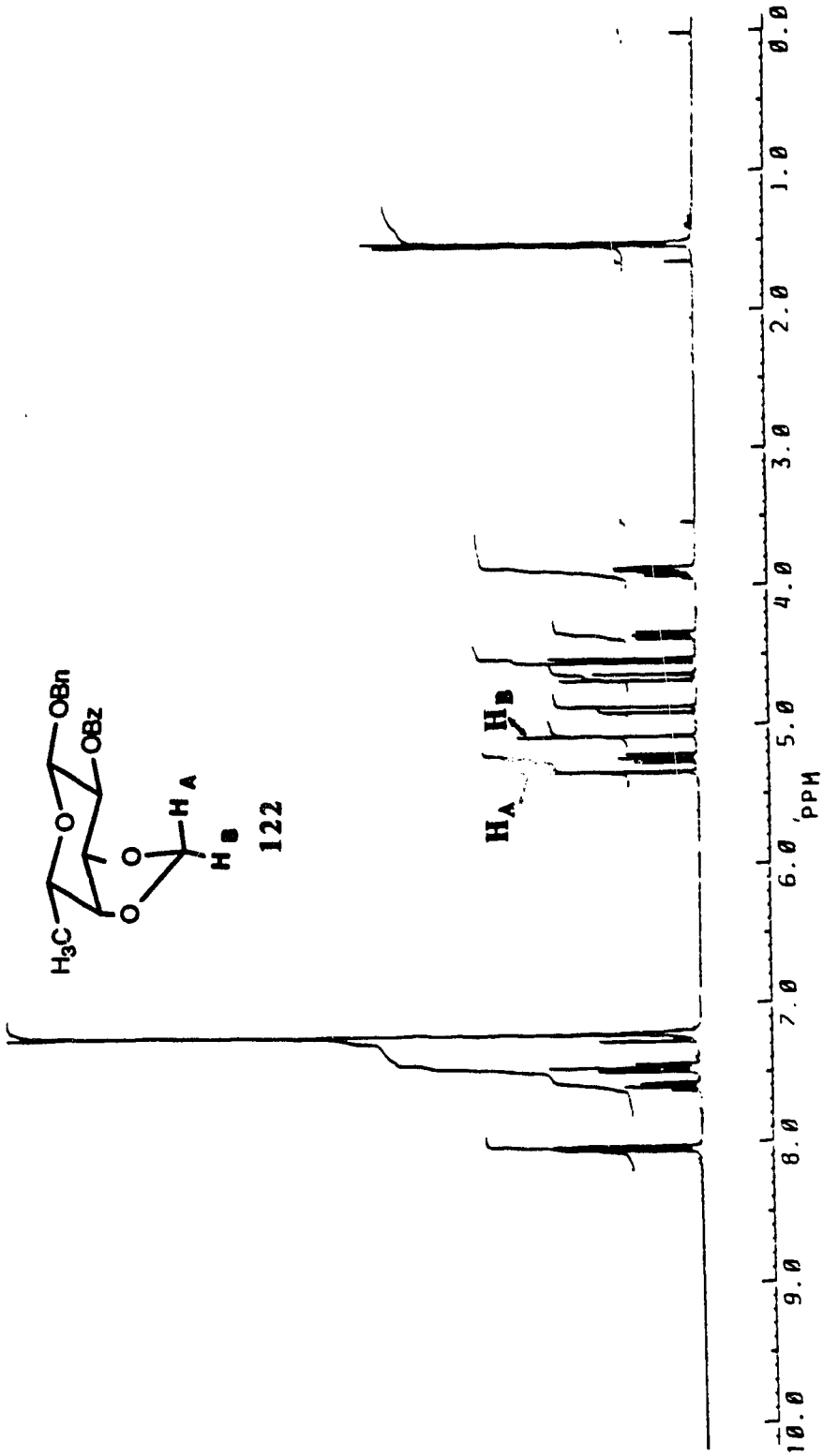


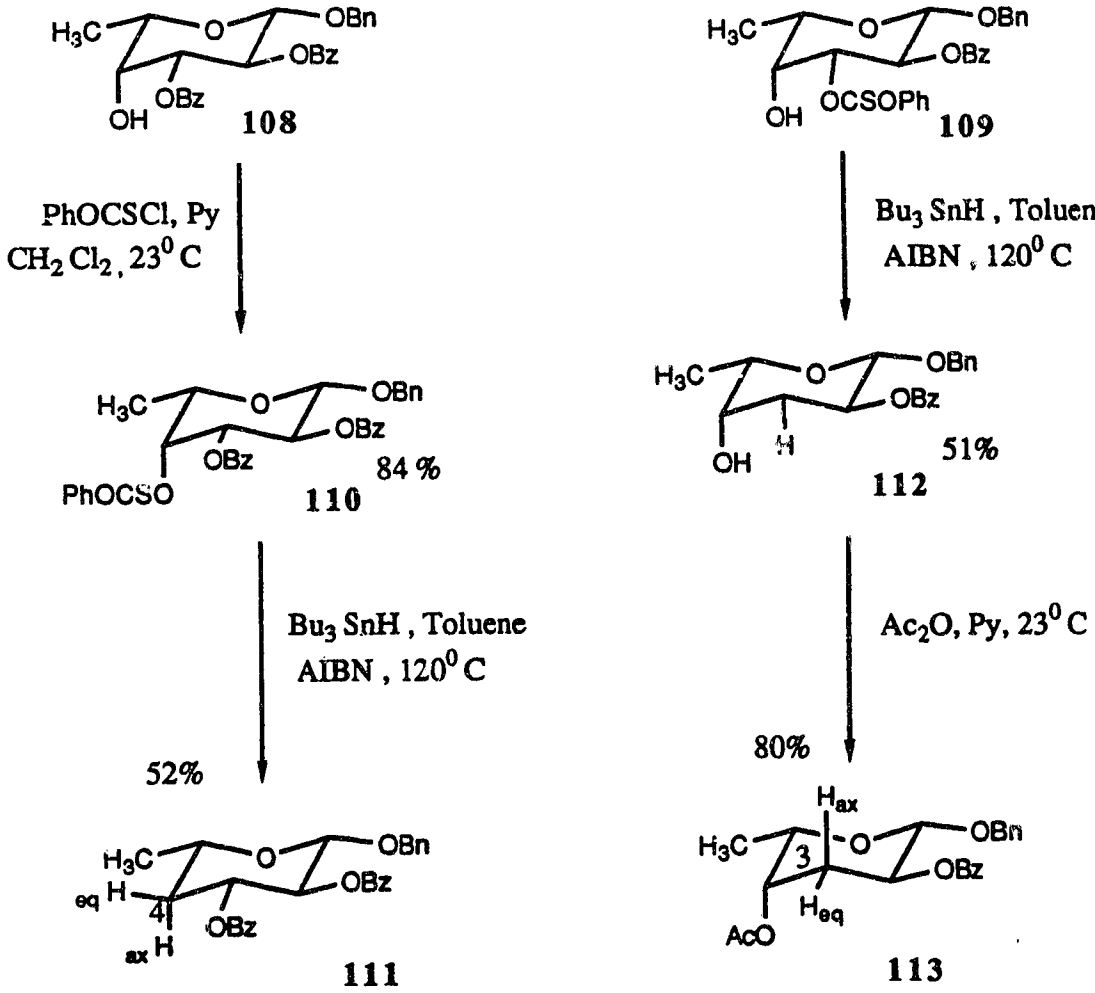
Fig. 12 The 300 MHz ^{1}H nmr spectrum of compound **122** in $CDCl_3$

methylidene derivatives like **122** in the radical deoxygenations of cyclic thionocarbonate derivatives. The anomalous formation of **122** can be rationalized as shown in scheme 22. The initially formed radical intermediate **119** may form **120**, wherein the radical is centred on the ring carbon (C-3). The trapping of the hydrogen radical followed by subsequent silica gel purification would afford **112**. Alternatively, in the presence of a large excess of Bu_3SnH (4 equiv. in the present study) the radical intermedate **119** would abstract the hydrogen radical to give **121** which would desulfurize under the reaction conditions to afford **122**. The synthetic sequence (**118** --> **112+122**) depicted in scheme 22 was repeated several times (with 3 to 4 fold excess of Bu_3SnH) only to end up with **112** (30% yield) and **122** (45% yield). The 4-deoxy derivative was not formed in any of the attempts . Therefore, the following synthetic transformations were undertaken to achieve the desired deoxygenations.

The low temperature (- 78°C) monobenzoylation of **107** to the 3-O-benzoate **108** (δ H-3 = 5.226, δ H-2 = 5.759, δ 4-OH = 2.500) was achieved in 88% yield (scheme 21, *vide supra*). The selective phenoxythioacylation of **107** was attempted to functionalize the equatorial hydroxyl. Thus reaction of **107** with phenoxychlorothionocarbonate (1 equivalent) in the presence of pyridine was investigated. At - 78°C, no acylation was observed by tlc whereas the acylation at 23°C was non-selective and afforded the 3-O-thionocarbonate **109** (δ H-3 = 5.646) along with 3,4-di-O-thionocarbonate (δ H-3 = 5.870 , δ H-4 = 6.240).

SCHEME 23

RADICAL DEOXYGENATIONS



δ H-4_{ax} = 1.700

δ H-4_{eq} = 2.339

δ C-4 = 38.07

δ H-3_{ax} = 1.800

δ H-3_{eq} = 2.445

δ C-3 = 33.17

The reaction of **107** with phenoxychlorothionocarbonate at $0^{o}C$ afforded the desired 3-O-thionocarbonate **109** (scheme 21, *vide supra*) in 68% isolated yield. The fact that 3-O-benzoylation of **107** (scheme 21) occurs at - $78^{o}c$ whereas 3-O-thionocarbonylation requires higher temperatures ($0^{o}C$) is the result of the reduced electrophilicity of the *thionocarbonyl carbon* as compared to that of the *benzoyl carbon.* The dibenzoate derivative **108** was 4-O-thionocarbonylated to **110** (scheme 23) in 84% yield (δ H-4 = 6.107).

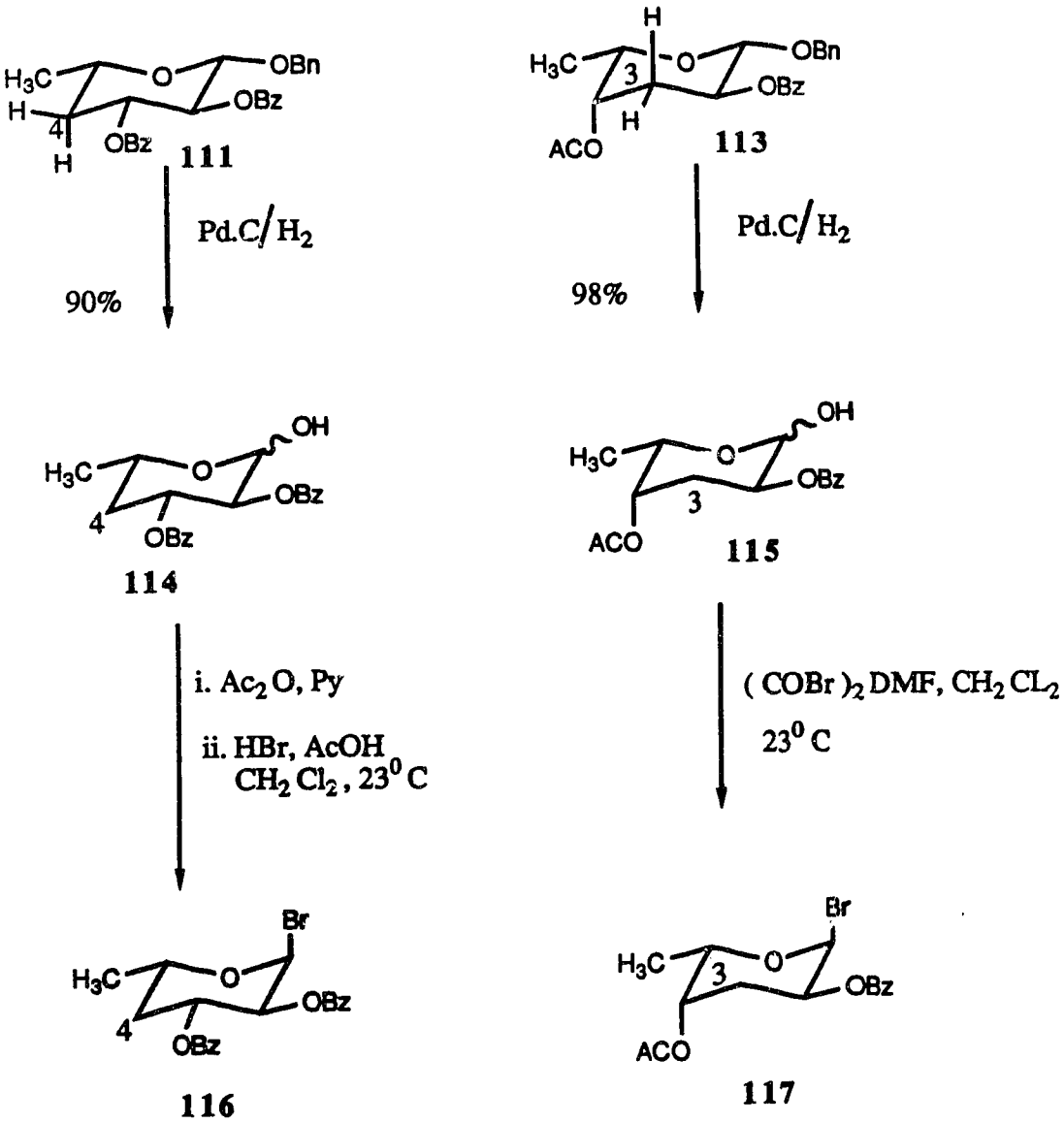
The fully protected thionocarbonate **110** was subjected to radical deoxygenation using tri-n-butyl stannane to afford the 4-deoxy compound **111** (scheme 23). The newly created methylene moiety was confirmed by ^{1}H-nmr spectroscopy on **111** : δ H-4 ax = 1.700, δ H-4 eq = 2.339.

The deoxygenation of **109** likewise yielded the 3-deoxy derivative **112** (51 % yield, δ H-3ax = 1.800, δ H-3eq = 2.445). It should be noted that the 4-OH in **109** need not be protected prior to the deoxygenation of the C-3 position. However, 4-O-acetylation was performed on **112** to furnish **113** (80 % yield). The 3-deoxy (**113**) and 4-deoxy (**111**) benzyl glycosides were then subjected to hydrogenolysis using Pd.C (5 %) under hydrogen atmosphere to yield the corresponding reducing sugars **115** and **114** respectively (scheme 24).

For the conversion of **111** and **113** into the corresponding anomeric bromides, their reactions with oxalyl bromide[95] and DMF in CH_2Cl_2 were investigated. The 3-deoxy derivative **115** reacted smoothly under these conditions to

SCHEME 24

PREPARATION OF ANOMERIC BROMIDES



furnish the corresponding bromo sugar **117** (scheme 24): (Rf 0.78, ethyl acetate: hexane , 1:1). Since **117** failed to survive the aqueous work-up it was not isolated but was subjected to anomeric phosphorylation as described in the following section. Treatment of the 4-deoxy derivative **114** with oxalyl bromide and DMF led to a complex reaction mixture. However, 1-O-acetylation of **114** followed by treatment of the 1-O-acetate derivative with hydrobromic acid in acetic acid furnished the corresponding bromo sugar **116**. The anomeric bromide **116** survived the aqueous work-up and the resulting product (Rf 0.71, ethyl acetate : hexane, 1:3) was used for the synthesis of the anomeric phosphate.

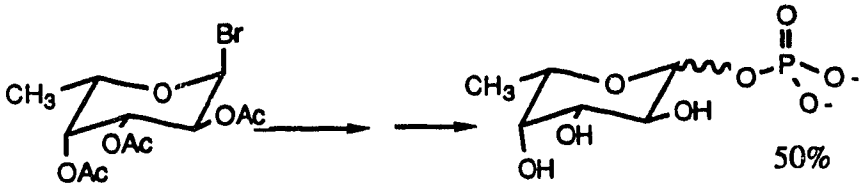
C 3 . Anomeric Displacements

As depicted in scheme 25, the **L**-fucopyranosyl bromide **70**, **D**-arabinopyranosyl bromide **74** and the ring deoxy **L**-fucopyranosyl bromides **116** and **117** were treated with tetra-n-butylammonium dibenzyl phosphate (**73**) to obtain the corresponding phosphotriesters which were purified and deprotected as depicted in scheme 14 (*vide supra*) to obtain the target glycosyl phosphates. The desired equatorial phosphates were obtained as the major products of the reactions. In each case the overall isolated yields of **78** and **79** were 50% and excellent diastereoselectivity was achieved (eq/ax ratio 11:1).

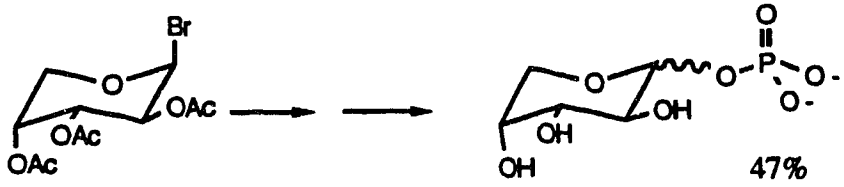
Phosphorylation of the deoxy bromosugars **116** and **117** (scheme 25) required several hours for their conversion into the phosphotriesters. Bromosugar **117** furnished **93** in 14% isolated yield (eq/ax = 5.4 :1). The anomeric displacements on

SCHEME 25

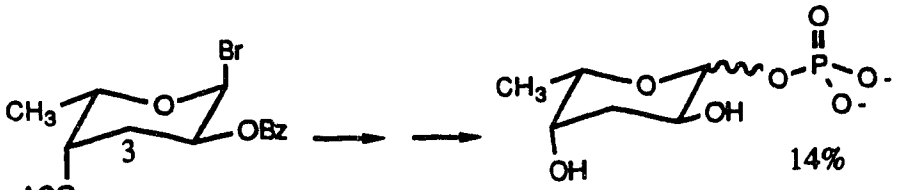
ANOMERIC DISPLACEMENTS USING TETRABUTYL AMMONIUM DIBENZYL PHOSPHATE 73 IN DMF AT 23^0 C



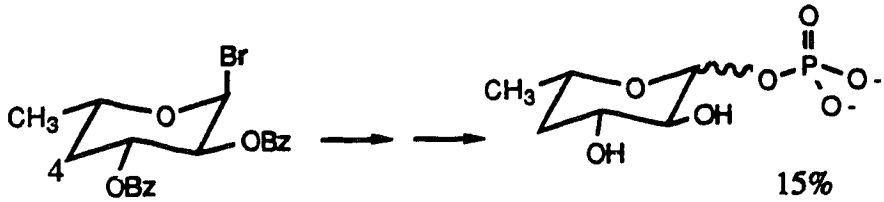
70 → 78 50% eq/ax =12.5 : 1



74 → 79 47% eq/ax =10.7 : 1



117 → 93 14% eq/ax =5.4 : 1



116 → 94 15% eq/ax =1.7 : 1

116 were carried out in relatively dilute solutions (appox. 0.015 molar in bromosugar) to achieve good stereoselectivity and reproducibility of the isolated yield of **94** (15% , 1.7:1). The use of concentrated solution (approx. 0.4 molar in **116**) led to the formation of only the α anomer of **94** . Moderate overall yields for the 3 and 4-deoxy fucose-1-phosphates were attributed to the instability of the anomeric bromides.

The product phosphates were thoroughly characterized by ^{1}H, ^{13}C, ^{31}P-nmr and mass spectroscopic (FAB) analyses. The H-1 of the major equatorial phosphates resonated as a pseudo triplet ($J_{H-1,P}=J_{H-1,H-2}=$ 7.5 - 8 Hz), whereas H-1 of the minor axial phosphates resonated as a doublet of doublets ($J_{H-1,P}$ = 7.0 Hz, $J_{H-1,H-2}$ = 3.6 Hz). In all phosphates C-1 was coupled to the phosphorus atom establishing the anomeric position of the phosphoryl group ($J_{C-1,P}$ = 3.5-4.4 Hz). Compound **79** furnished satisfactory elemental analysis. The compounds **93** and **94** (as their lithium or triethyl ammonium salts) were extremely hygroscopic which precluded their characterization by combustion analysis.

C 4 . Conformational Properties of the Analogues

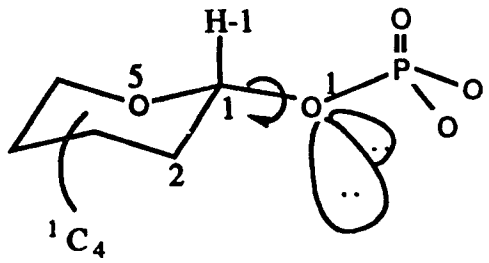
These pyranosyl phosphates embody (scheme 26) two important conformational features :

1) The ring conformation of the pyranose moiety, and
2) The conformation of the C_2-C_1-O_1-P fragment.

The ^{1}H and ^{13}C-nmr parameters of the glycosyl phosphates (in D_2O solutions) have proved to be excellent probes for their conformational preferences in aqueous media. The

SCHEME 26

CONFORMATIONAL FEATURES OF A FUCOSYL PHOSPHATE



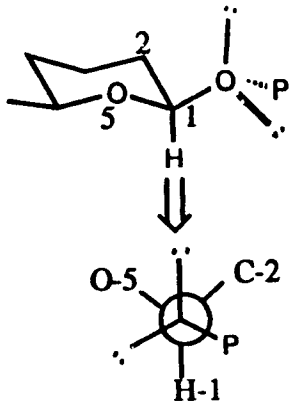
i. PYRANOSE RING CONFORMATION

$^{VIC}J_{H,H}$

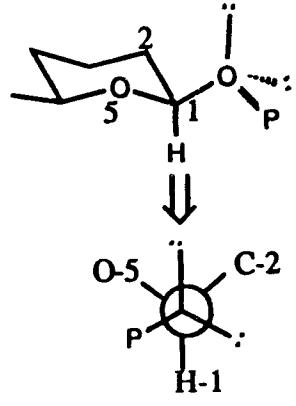
ii. C1----O1 TORSION

$^3J_{H-1,P}$ $^3J_{C-2,P}$

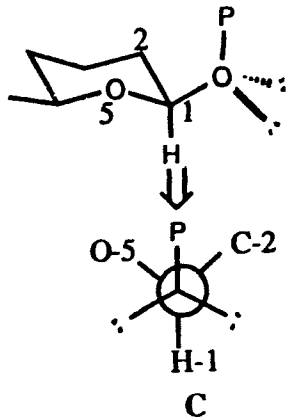
NEWMAN PROJECTIONS FOR THE MOST STABLE ROTAMERS AROUND THE C1---O1 BOND



A



B



C

magnitudes of the vicinal coupling constants for pyranose ring hydrogens can be used to establish the ring conformation ($^1C_4$ in the present case). The elegant studies by Sarma et al.[99] and Barker et al.[83] have demonstrated that the conformational preferences around the C_1-O_1 bond can be assessed based on the magnitudes of the three-bond coupling constants : $^3J_{H-1,\ P}$ and $^3J_{C-2,\ P}$.

The ^1H and ^{13}C-nmr spectra[94] of the phosphate analogues (disodium salts, pH = 7) were recorded. The $^3J_{H,H}$ (for ring hydrogens) and $^3J_{H-1,\ P}$ were measured from their ^1H-nmr spectra, whereas $^3J_{C-2,\ P}$ values were measured from their ^{13}C-nmr spectra. The results are shown below.

Table-3

Formula No.	$^3J_{H1,2}$	$^3J_{H-1,P}$	$^1J_{C-1,P}$	$^3J_{C-2,P}$
78	7.5	7.5	4.4	4.4
79	7.5	7.5	4.2	5.8
93	8.0	8.0	3.5	4.5
94	7.5	7.5	4.4	5.8

Pyranose Ring Conformation

The magnitudes of the $^3J_{H,H}$ coupling constants were found to be consistent with those expected for a $^1C_4$ chair conformation of the pyranose ring moiety (see experimental

section). The parent hexopyranose ; L-fucose, exists in the $^1C_4$ ring conformation, thus equatorial placement of the phosphoryl moiety (as in **78**) at the anomeric centre does not alter the $^1C_4$ conformation. Moreover, the removal of hydroxyls at C-3 (in **93**) and C-4 (in **94**) does not appear to alter the $^1C_4$ conformation for the phosphate analogues **93** and **94**, although slight distortions from the perfect $^1C_4$ geometry are expected. The substitution of the 5-methyl group by a hydrogen atom (in **79**, α-D-arabinose phosphate) also had no detectable effect on the gross $^1C_4$ conformation.

These findings for the phosphate analogues potentially have a significant bearing on the biological behaviour of the corresponding sugar nucleotides, because it has been established[83,99] that the conformational properties of the glycosyl phosphates are not altered in their sugar nucleotides.

Conformers Arising from C1-O1 Torsion

The three gauche rotamers (A, B, and C) arising from rotation around the C1-O1 bond are shown in scheme 26. For each rotamer $^3J_{H\text{-}1,\ P}$ and $^3J_{C\text{-}2,\ P}$ would have a unique value depending upon the dihedral angle between the coupled nuclei[29,30]. Since in aqueous solutions of the glycosyl phosphates at a given pH the rotamers A, B and C could all be populated , the observed value of the coupling constants would have a different contribution from each of these rotamers.

For the equatorial anomers of pyranosyl phosphates the literature[83] values for $^3J_{H\text{-}1,\ P}$ range from 7.5 to 8.8 Hz. In the present study (table 3 *vide supra*) the observed

$^3J_{H\text{-}1\ P}$ values fall in the same range. The $^3J_{C\text{-}2,\ P}$ values[83] for the equatorial pyranosyl phosphates range from 4.4 to 8.1 Hz. In the present study (table 3) $^3J_{C\text{-}2,\ P}$ values for the analogues range from 4.4 to 5.8 Hz.

The mole fractions of these fucose phosphate analogues that exist in the most stable rotamers (A, B, C in scheme 26) can be calculated[83] using values for both $^3J_{C\text{-}2,P}$ and $^3J_{H\text{-}1,P}$. If we denote the mole fraction of A by **a**, and those of B by **b** and C by **c** , we can compute the **a,b** and **c** as follows : In A & C, the phosphorus atom and C-2 are in a gauche relationship hence the contribution of these two rotamers towards the observed $^3J_{C\text{-}2,\ P}$ is : (**a+c**) x J_{gauche}. In B , these two nuclei are in a trans relationship hence (**b**)x J_{trans} is the contribution of B toward the observed $^3J_{C\text{-}2,P}$. The appropriate equation then is

$$^3J_{C\text{-}2,P} = (a+c)\ J_{gauche} + b\ J_{trans} \quad \text{----(eq. 1).}$$

In eq. 1, J_{gauche} = 2.0 Hz and J_{trans} =14 Hz,[83]

Similarly the contribution of A, B, C toward $^3J_{H\text{-}1,P}$ is given by the following equation ,

$$^3J_{H\text{-}1,P} = (a+b)\ J_{gauche} + c\ J_{trans} \quad \text{----(eq. 2).}$$

In eq. 2, J_{gauche}= 2.1Hz and J_{trans}=22.9 Hz[83]

Since **a,b,c** are the mole fraction,

$$a+b+c = 1 \quad \text{----------(eq. 3)}$$

Now one proceeds to compute the mole fraction **c** of C (wherein H and P are trans to each other) from the observed value of $^3J_{H\text{-}1,P}$ and that of B (C and P in trans

relationship) from the observed value of $^{3}J_{C-2,P}$. The mole fraction **a** of A is then obtained by difference .

Hence, to compute **c** , one uses eq. 2 and eq. 3 & to compute **b** eq. 1 and eq. 3 are used . The computed values of **a**,**b**,**c** for the phosphate analogues **79**, **93** and **94** are tabulated in table.4.

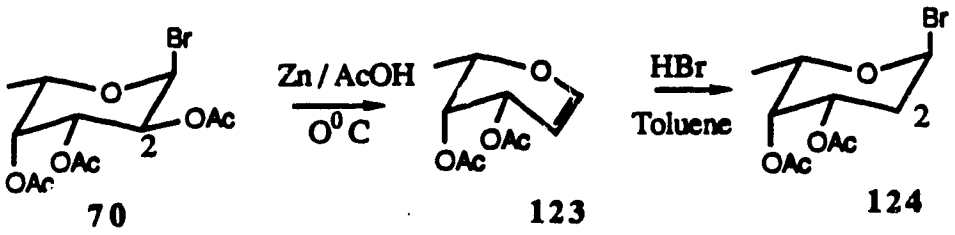
Table 4

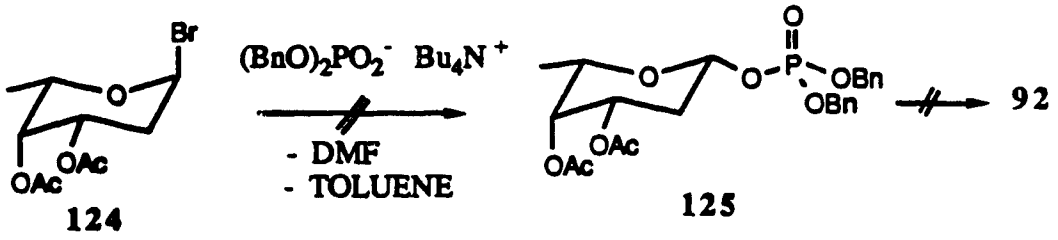
Formula No.		% A	% B	% C
78	Fuc P	56	18	26
79	Arab P	42	32	26
93	3-deoxy Fuc P	51	21	28
94	4-deoxy Fuc P	42	32	26

On stereoelectronic grounds (*exo-anomeric effect*) the conformer B and C with a non-bonding electron pair antiperiplanar to O-5 are expected to contribute to the maximum extent. However, in the present study the observed values of $^{3}J_{C-2,P}$ and $^{3}J_{H-1,P}$ and the mole fractions calculated therefrom clearly indicate a substantial contribution by the conformer A. Since the electron density on O-1 is significantly delocalized into the phosphoryl portion of the O5-C1-O1-PO_3 segment, the exoanomeric contribution toward the rotameric distribution is expected to be decresed.

SCHEME 27

Attempted synthesis of 2-deoxy analogue 92





C 5. Attempted Synthesis of 2-deoxy Analogue (92)

The synthesis of **92** using the 2-deoxy-bromosugar[98] **124** was attempted. The synthesis (scheme 27) of **124** from **70** was achieved by following literature procedures.[98] Thus syrupy 2,3,4-tri-O-acetyl-**L**-fucosyl bromide[68] was treated with Zn/AcOH to obtain 3,4-di-O-acetyl-**L**-fucal[98] (**123**) in 40% yield. A brief treatment of **123** with dry HBr resulted in the formation of **124** (δ H-1 = 6.72) which was isolated as a syrup (>80% purity based on ^{1}H-nmr and tlc analysis) and used in the phosphate displacement reaction. Thus, the reaction of **124** (scheme 27) with excess of tetra-n-butyl ammonium dibenzyl phosphate (**73**) was carried out in DMF as the solvent. The close monitoring of the reaction mixture by tlc revealed that the bromosugar had undergone extensive degradation and no phosphorylation product could be detected. Presumably, the product triester **125** is too unstable to survive the polar reaction conditions. Changing the reaction solvent from DMF to toluene failed to yield the desired product. The instability of the 2-deoxy-equatorial glycosyl phosphates has also been noted by other workers.[59]

Having prepared the analogues of β-**L**-fucosyl phosphates, their coupling with the activated guanosine mono phosphate to establish the *pyrophosphate linkage* was undertaken. The results are desribed in the following chapter.

IV. SYNTHESIS OF GDP-FUCOSE ANALOGUES

Methods for the chemical synthesis of sugar-nucleotides have been under development for the last four decades.[24,100-102] As described in the Introduction, sugar nucleotides serve as donor substrates in enzymatic glycosyl transfer reactions and occur in only small quantities in nature. Their isolation from natural sources is laborious and poses serious practical problems. Efficient methods of chemical[101] and enzymatic[103] synthesis have however made it possible to prepare resonable quantities (from multimilligram up to a gram scale) of both the natural structure and its analogues.

A. Salient Features of Sugar-nucleotide

The key structural features of sugar-nucleotides are displayed in scheme 28.

Pyranoside Moiety **(A)**

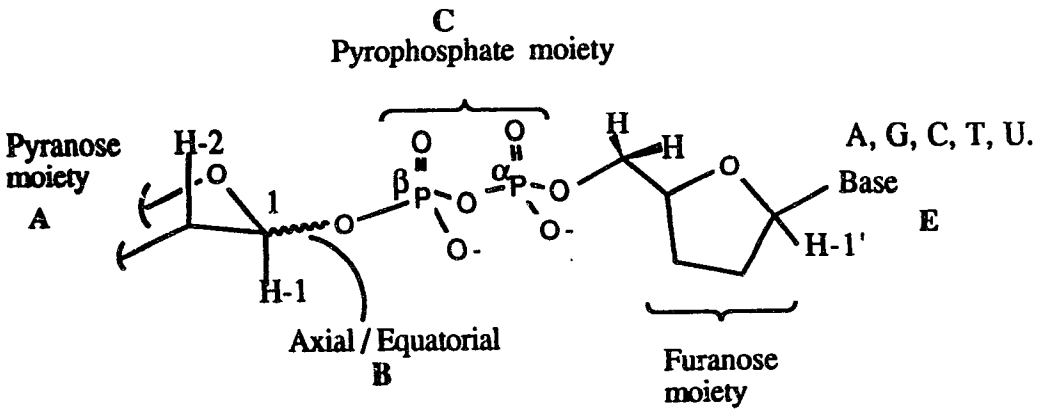
The naturally occurring aldopyranoses of the sugars with a variety of substituents especially related to bacterial polysaccharides are featured in this portion of the molecule. The ring conformation : $^1C_4$ or $^4C_1$ and the anomeric configuration **(B)** are reflected in $^2J_{H\text{-}1,H\text{-}2}$ in the ^1H nmr spectrum.

Pyrophosphate Moiety **(C)**

Conformationally, the pyrophosphate bridge is the most flexible portion of the molecule. The phosphorus attached to the pyranose is described[99] as Pβ and the other phosphorus, being nearest to the furanose portion, is called Pα. Pα and Pβ are linked to each other via an anhydride oxygen and resonate in the ^{31}P-nmr

SCHEME 28

STRUCTURAL FEATURES A-E OF A SUGAR NUCLEOTIDE MOLECULE



SPECTRAL IDENTIFICATION

A . $^{3}J_{H\text{-}1,P\beta}$ and $^{2}J_{C\text{-}1,P\beta}$

B . Axial or Equatorial : $^{vic}J_{H\text{-}1,H\text{-}2}$

C . Pyrophosphate moiety : Mutually coupled doublets in ^{31}P-nmr (- 10 to - 12 ppm)

D . δ H-1' (in D_2O as nmr solvent) at 6 ppm

E . Characteristic Aromatic Resonances in ^{1}H nmr

SEPARATION / PURIFICATION

C . Charged Pyrophosphate moiety at pH =7 : Ion Exchange Separation

E . Aromatic moiety of bases : Adsorption on Carbon

spectrum as mutually coupled doublets. In sugar nucleotides, as compared to the Pα, the Pβ resonates at a higher field. With H_3PO_4 as ^{31}P-nmr reference standard, the sugar nucleotides in their D_2O solutions exhibit mutually coupled doublets in the -5 to -15 ppm range ($^2J_{P,P}$ = 20 Hz, $\Delta\delta$ = 2 ppm). Once GDP-fucose was successfully sythesized (*vide infra*) a heteronuclear (^1H, ^{31}P) decoupling experiment was performed on the synthetic sample : disodium salt (D_2O solution, approx. 10 mg/0.45 ml, 23°C). The ^{31}P-nmr spectrum exhibited a doublet of doublet pattern (δ - 10.8 and - 12.7, $J_{P,P}$=20.8Hz). Decoupling of the high field doublet at - 12.7 ppm resulted in the collapse of the H-1(fucose) signal (dd --> d) in the ^1H-nmr.spectrum Decoupling of the low field doublet at - 10.8 ppm did not affect the H-1(fucose) signal, while peaks due to ribose H-4' and H-5' protons were simplied. At neutral pH, the pyrophosphate bridge carries two negative charges which permit the ion-exchange separation of the sugar nucleotides from neutral as well as other charged compounds. During the ion exchange separations the eluting buffers are chosen in such a way that the pH is close to neutrality. A linear gradient of neutral salts like LiCl or NaCl can efficiently achieve the elution of sugar nucleotides.[104]

Sugar nucleotides are stable compounds when stored as dry lyophilized powders of their alkali metal (Na^+, Li^+) salts. In the solution state at extreme pH conditions extensive hydrolytic processes occur. The protonation of the pyrophosphate moiety (pH = 1-2) makes it labile toward hydrolysis. Thus a pyranose sugar can be quantitatively hydrolyzed at 100°C in 0.01N HCl.[100] Under

alkaline conditions sugar nucleotides are readily hydrolyzed to nucleoside monophosphates and a cyclic 1,2-phosphate of the pyranose sugar.[101] Hydrazinolysis of the pyrophosphate moiety in sugar nucleotides has been studied.[105] The treatment of uridine-5'-(2- acetamido-2-deoxy-α-D-glucopyranosyldiphosphate) with hydrazine at 100 °C results in the cleavage of the anhydride linkage and in deacetylation to furnish 2-amino-2-deoxy-α-D-glucopyranosyl phosphate in 60% isolated yield.

At physiological pH (7.39) the pyrophosphate moiety exists as a doubly charged species. The coordination of divalent metal ions (Mn^{2+}, Mg^{2+}) with pyrophosphate linkages is well known[106] and such interactions are implicated in the case of galactosyl transferases.[107] The fucosyl transferases (α2-FucT and α3-FucT) indeed need divalent metal ions (e.g., Mn^{2+}) for their catalytic activity.[21]

Furanoside Moiety (D)

Most of the natural sugar nucleotides carry a *D-ribose* sugar in the nucleoside part while some have a *2-deoxy ribose*.[104] The characteristic doublet resonance near 6 ppm ($J_{1,2}$ = approx. 6 Hz, D_2O as solvent) of the ribosyl anomeric proton[108] is useful in structure determination of these compounds.

Base (E)

The heterocyclic (pyrimidine or purine) moiety can be adenine, guanine, cytosine, thymine or uracil. The characteristic resonances of the aromatic protons in the ^{1}H-nmr spectrum are excellent probes for the identification of the base. The *aromatic nucleus* of the base portion has traditionally provided a good

handle for hydrophobic adsorption of sugar nucleotides onto activated carbon.[109] Such adsorption of nucleotides onto carbon is a common tool in nucleotide chemistry. A near quantitative adsorption of sugar nucleotides from their aqueous solutions onto activated ('desalting grade') carbon can be easily achieved. The presence of a highly hydrophilic pyrophosphate moiety does not hinder the adsorption of the sugar-nucleotide molecule. This technique is primarily used to isolate the nucleotides away from the buffer salts (desalting). This technique was successfully used during the preliminary pyrophosphate coupling experiments. (vide infra, scheme 32)

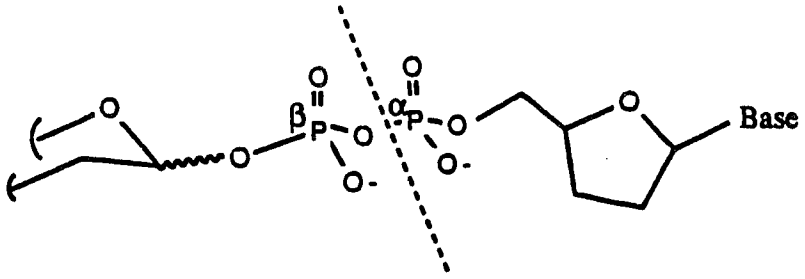
The absorption maxima of the bases fall in the ultra violet region. The ready availability in the literature of the exact values for the extinction coefficient has made the U.V. quantitation of sugar nucleotides a common practice.

Due to the presence of several sensitive structural elements the synthetic design of sugar-nucleotides (especially that of the structurally modified derivatives) requires that the synthetic transformations be done in the following sequence (scheme 29)

1) The modified pyranose phosphate (**126**) portion is synthesized in the requisite salt form. Usually, the phosphate moiety is converted into its trialkylammonium salt form (M^+ in **126** = triethyl or trioctylammonium[110]). Such salts are soluble in organic solvents.[104] The hydroxyl groups on the pyranoside are usually left unprotected.

SCHEME 29

FORMATION OF A PYROPHOSPHATE BOND

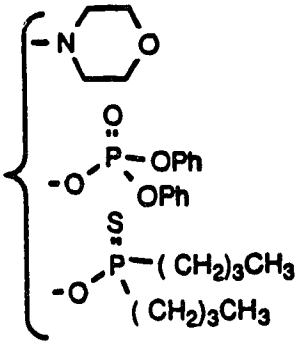


126 **127**

$M^+ = NR_3H$

R = Ethyl, Octyl

X =



2) The nucleoside-5'-monophosphate portion with the desired base is converted into an *activated form* (**127**) in which the phosphorus centre carries a leaving group (X). The anionic displacement of X by **126** yields the pyrophosphate linkage.

3) The pyrophosphate coupling is executed as the last synthetic operation.

The activation of Pα in **127** is usually achieved by the procedures which are reminiscent of the *phosphodiester approach*[111] used during the oligonucleotide synthesis. The following are the most commonly used activation procedures.

Use of phosphoromorpholidates[104]

Most of the pyrophosphate couplings are achieved by this approach (in **127**, X = morpholine). The introduction of morpholine on the phosphorus is achieved via dicyclohexylcarbodiimide[112] coupling or through redox condensation.[113] During the pyrophosphate coupling the ammonium countercations provide a proton for the reversible protonation of the nitrogen atom in the morpholine moiety, thereby making it an excellent leaving group. The couplings are routinely done in dry pyridine as the reaction medium. However, the use of dipolar aprotic solvents like DMF and DMSO has been recommended.[114]

The reported[104,115] coupling yields using this method are quite varied (30%-60%). Also, the insolubility of reagents in the reaction medium is a primary cause of concern during the adoption of this method. In the author's hands the yield of GDP-fucose using this method never exceeded 30%.

Use of diphenoxyphosphoric[116] *anhydride* (in **127**, X = diphenoxyphosphoryl) and more recently[117] the use of di-n-butylphosphinothioic anhydride (in **127**, X = di-n-butylphosphinothioyl) have been tried for pyrophosphate couplings. The latter derivative is particularly attractive because of its solubility in organic solvents, although the reported yields using this derivative also vary considerably (10%-60%).[115,68]

Apart from the above mentioned activation methods several other activated nucleoside-5'-monophosphate derivatives, e.g., in **127** x = imidazole[104], benzimidazole, triazole and benzotriazole etc. have been employed[108] in the synthesis of sugar nucleotides.

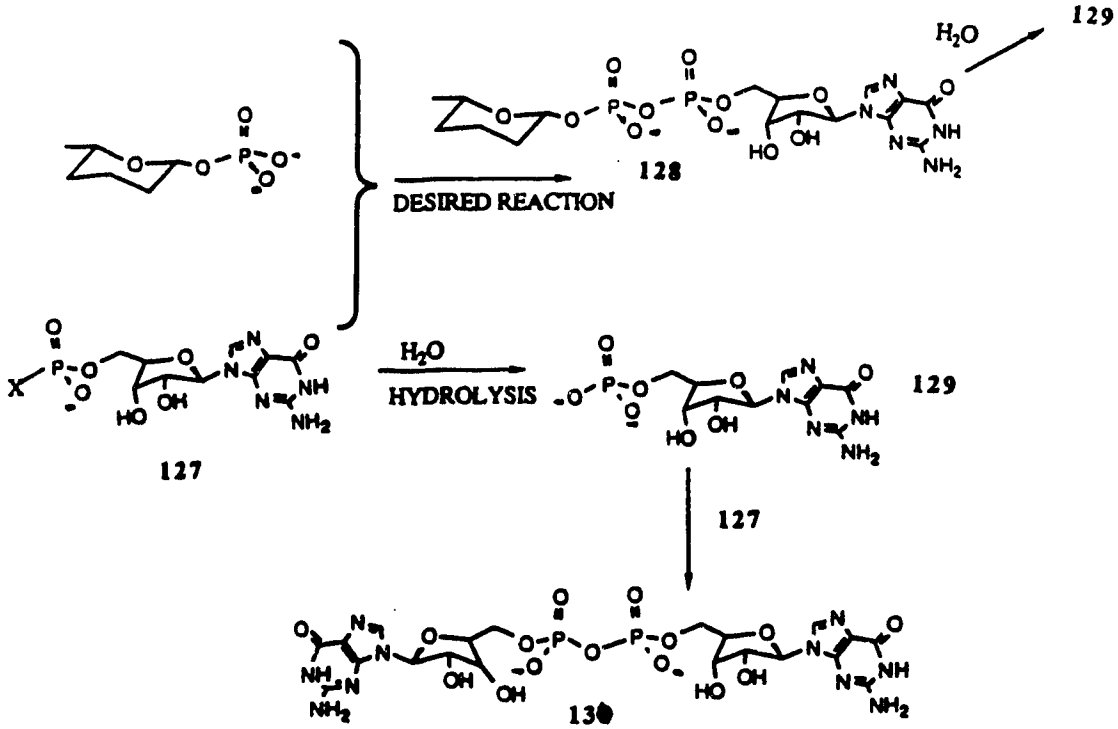
The survey of the reported pyrophosphate coupling procedures and the results of the preliminary coupling studies in this work revealed the following findings (scheme 30).

1) The desired pyrophosphate coupling reaction giving the structure **128** in scheme 30 was accompanied by several side-products[114] such as **129** and **130**. Many of the side-products comigrated with the desired product under paper[114], ion exchange or silica gel (this study) separation conditions.

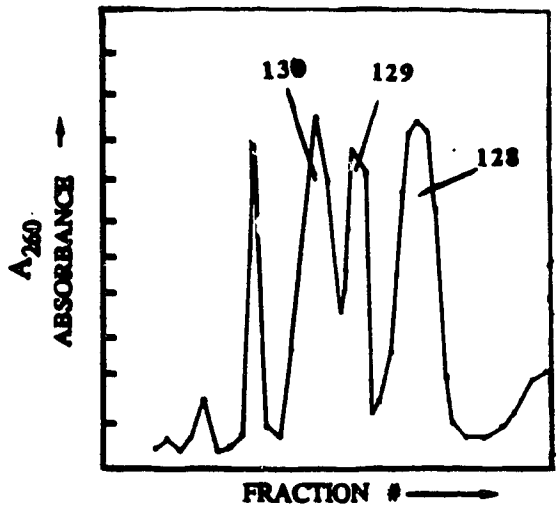
2) The coupling process was sensitive to moisture. The nonproductive hydrolysis of **127** to give **129** was the major side reaction. Moreover, the reaction of **129** with **127** led to the formation of the symmetrical pyrophosphate **130**. The hydrolysis of the desired sugar nucleotide to yield **129** was a possible destructive reaction which was accelerated at higher temperatures.[114]

SCHEME 30

POSSIBLE REACTION PATHWAYS DURING A PYROPHOSPHATE COUPLING PROCESS



A_{260} PROFILE : ION EXCHANGE SEPARATION ON PYROPHOSPHATE COUPLING BETWEEN L- FUCOSE-1-PHOSPHATE AND 131



3) 4-morpholine N,N'-dicyclohexylcarboxamidinium guanosine-5'-phosphomorpholidate[112] remains insoluble in dry pyridine.[104] The use[114] of dry DMF/DMSO or addition of o-chlorophenol to the coupling reaction was suggested in order to obtain homogeneous conditions.[104] In the present study when L-fucose phosphate was engaged in the coupling reaction with GMP-morpholidate with pyridine as the solvent, a completely homogenous reaction could not be achieved even by addition of dry DMF nor by sonication or heating of the reaction mixture. The reaction did not go to completion after stirring for 4-5 days and the yield of GDP-fucose was modest (27%) . The guanosine nucleotides are notoriously insoluble in many organic solvents. The first chemical synthesis of GDP-fucose by Barker et al[68] was achieved by the use of di-n-butylphosphinothioic anhydride of guanosine-5'-monophosphate. We opted for the use of the same reagent for the synthesis of the analogues.

The separation of side-products from the coupling reaction has been traditionally achieved by paper chromatography[119] when the scale of the coupling falls in the micromolar range. Silica gel separations on preparative tlc plates are also possible. The presence of countercations (e.g., NH_4^+) in the elution solvent often helps to neutralize the charged compounds which are then chromatographed as ion-paired species. The scale of separation (up to 10 mg) per tlc plate is often a determining factor for multimilligram scale separations. Ion exchange separation, however, can be carried out on a multigram scale with manageable buffer volumes (10 to 15 l.). Since the sugar-

nucleotides absorb in U. V., the eluent is monitored for U. V. absorption . The profile of the U. V. absorbance (at 260 nm. for purines and at 270 nm for pyrimidine bases) measured over the complete elution volume then indicates the separation (or otherwise) of the compounds. One such profile has been reproduced in scheme 30.

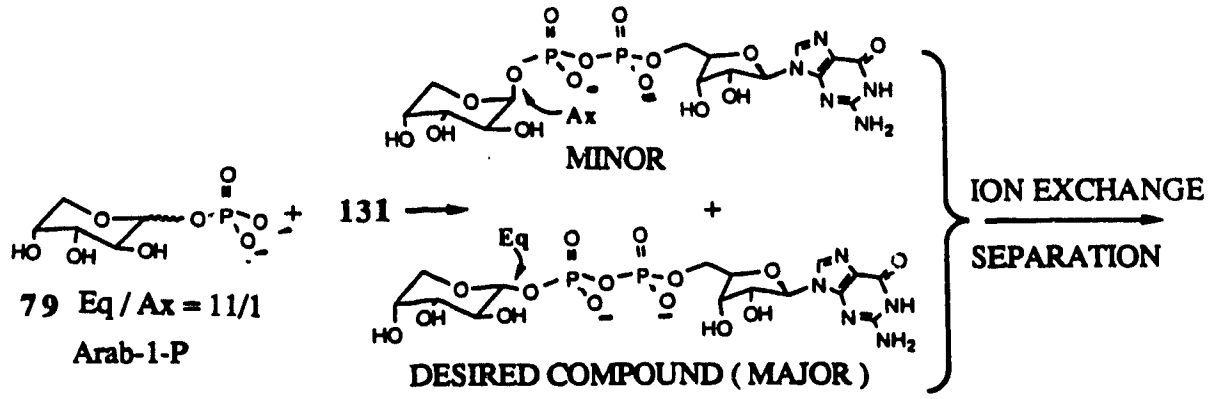
4) This anion exchange separation was performed on a coupling reaction between β-L-fucose-1-phosphate and guanosine-5'-phosphoric-di-n-butylphosphinothioic-anhydride (**131**). The fractions were monitored at a wavelength of 262 nm to detect the guanosine containing compounds. The profile clearly indicates the presence of four major species and two minor compounds. The unreacted fucose-1-phosphate was detected by monitoring the fractions for acid labile phosphates.[69] Similar U.V. profiles were obtained in the separation of GDP-fucose analogues.

The monitoring of the column fractions with ^{31}P-nmr spectroscopy was found to yield some valuable information i.e. the types of phosphorus containing compounds. Since the desired sugar nucleotide molecule invariably gave rise to mutually coupled doublets (- 10 to - 15 ppm) in the ^{31}P-nmr spectrum, it was necessary to focus on the column fractions which were assayed positive for such a pattern.

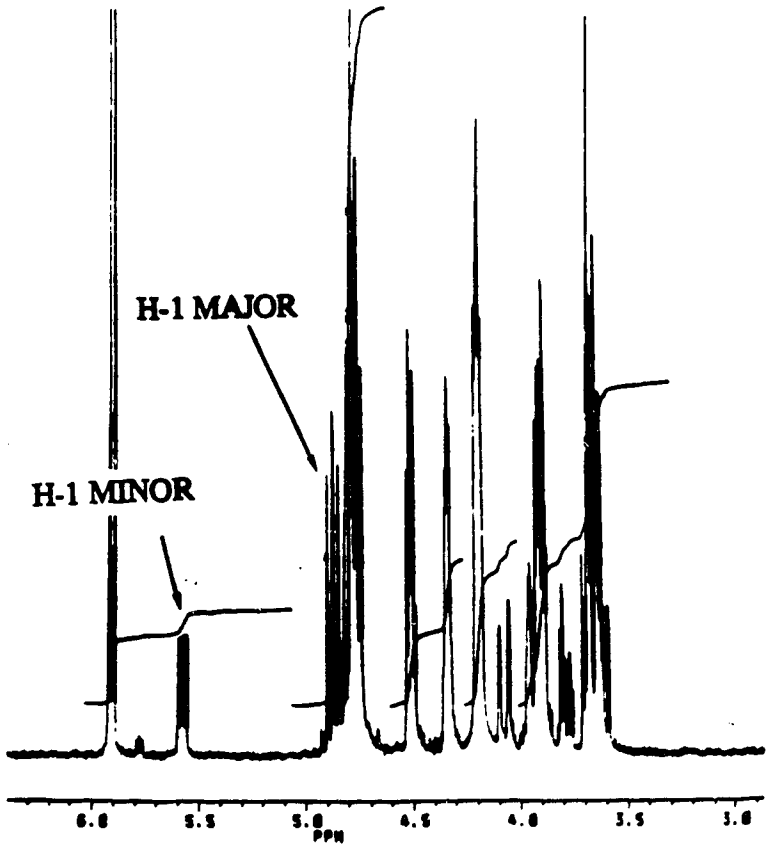
5) The ^{1}H & ^{31}P-nmr spectroscopic monitoring of the eluate was found to be especially useful when ion exchange chromatography on isomeric (ax/eq. mixture at pyranosyl phosphate moiety) sugar nucleotides did not result in baseline separation. An example of this case is illustrated in scheme 31 where the product

SCHEME 31

^{1}H-NMR DETECTION OF MINOR (AXIAL ANOMERIC CONFIG. AT PYRANOSE) SUGAR NUCLEOTIDE



^{1}H NMR ON ONE OF THE EARLY FRACTIONS CONTAINING DESIRED + MINOR PRODUCTS : (FOR ABOVE REACTION)



mixture resulted from the coupling reaction between **D**-arabinopyranosyl-1-phosphate (**79**, eq./ax. ratio = 11:1) and guanosine-5'-phosphoric-di-n-butylphosphinothioic anhydride(**131**) .The minor nucleotide isomer with the axial configuration at the arabinose C-1 was also formed. The ion exchange separation (Dowex 1-X2, Cl^-, linear gradient of LiCl in H_2O) resulted in an excellent separation between unwanted (but invariably generated) by-products and the sugar nucleotides. The isomeric nucleotides however were not separated completely. The minor component eluted before the major one, *albeit* without any baseline separation. Aliquots from the earlier fractions containing the desired nucleotide (U.V. positive & H_2SO_4 charring positive peak) were evaporated to dryness to obtain a solid material which was dissolved in D_2O and analysed by ^{1}H-nmr . The partial ^{1}H-nmr spectrum on one of the fractions is reproduced in scheme 31 . The presence of the minor nucleotide was evident from the low field (δ = 5.600) resonance for H-1 , whereas a triplet resonance for H-1 arising from the major isomer was typically located around δ = 4.8 . Since the undesired isomer was present in minor amounts, the relevant tubes were not pooled with fractions containing the desired nucleotide .

6) Since the objective was to synthesize the GDP-fucose analogues on a multimilligram scale, the choice of ion exchange chromatography as the method of separation was obvious. The linear gradient of LiCl for the elution of the charged compounds followed by U.V., ^{31}P-nmr, and H_2SO_4 spray analyses on

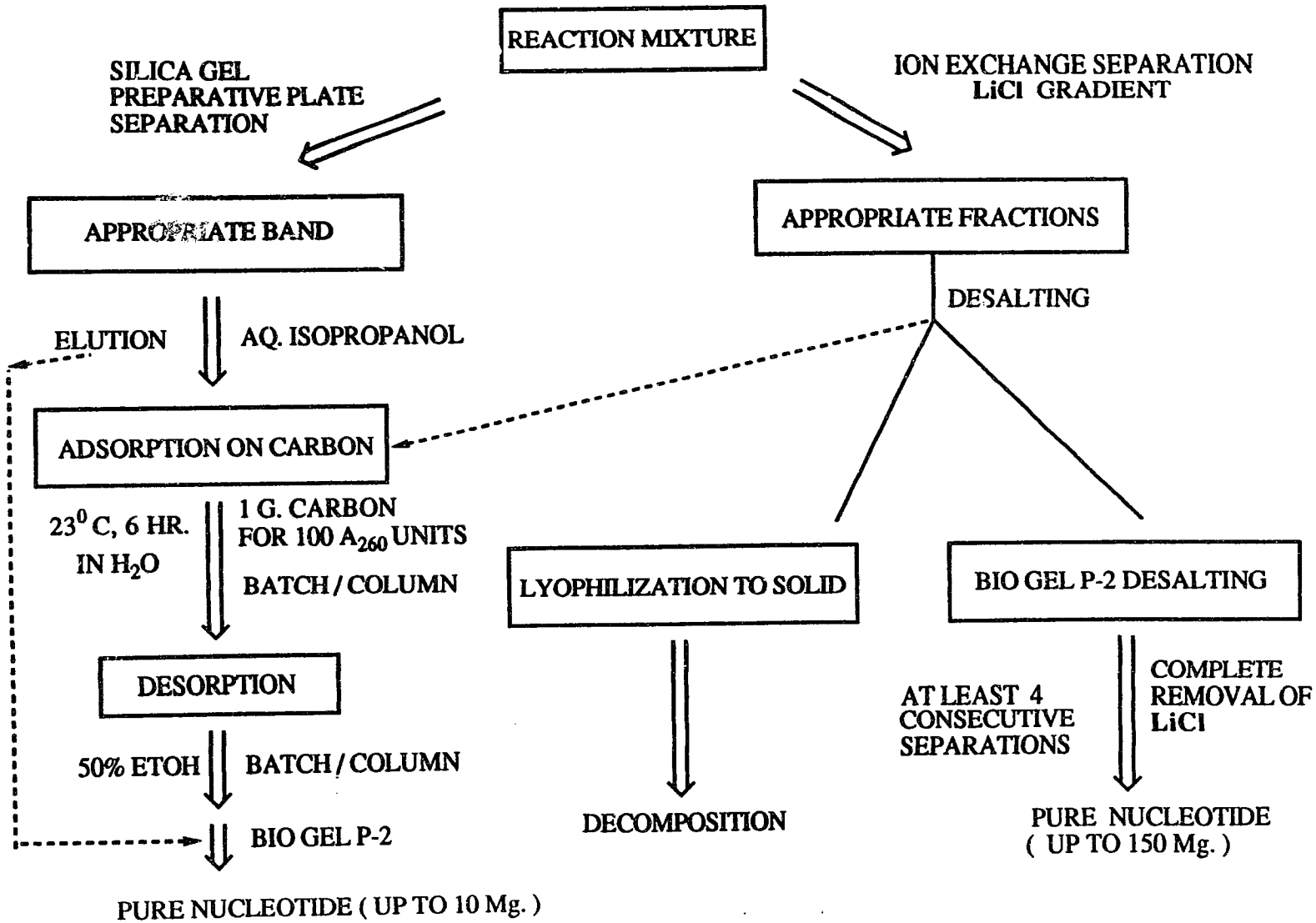
the fractions then indicated the particular set of fractions that contained the desired compound.

It was necessary to isolate the desired nucleotide away from LiCl. As described before the use of desalting grade carbon[109], the selective solvent extraction[77] of LiCl into diethyl ether : ethanol (2 : 1), and size-exclusion chromatography[76] (e.g., Bio gel-P2) were some of the options available. As represented in scheme 32, all three desalting procedures were tried in order to be able to choose the one which would be most effective at a large scale desalting. (100-200 mg of nucleotide in the presence of 3-4 g of LiCl).

When the silica gel preparative plate separations were carried out, the elution of the material was done using aq. i-PrOH. The silicates were also eluted in aq. i-PrOH. Their removal was achieved by the selective adsorbtion of the nucleotides on carbon. Washing of the carbon with water then removed the salts. Desorption of the nucleotides was successfully achieved in aq. ethanol. LiCl can be removed using the same procedure.

For the solvent extraction of LiCl, the appropriate fractions containing the desired nucleotide were lyophilized. For the complete removal of 500 ml of water, a duration of 36 h. was necessary. The dry solid obtained after the lyophilization of the aq. phase was then ready for the extraction of LiCl into ethyl ether : EtOH (2:1) by the solid-liquid extraction procedure. Unfortunately, the solid obtained at the end of lyophilization contained, along with LiCl, the hydrolysis products of the desired sugar nucleotide. It is proposed that on the way to complete lyophilization a stage is

SCHEME 32 SEPARATION AND DESALTING PROTOCOL

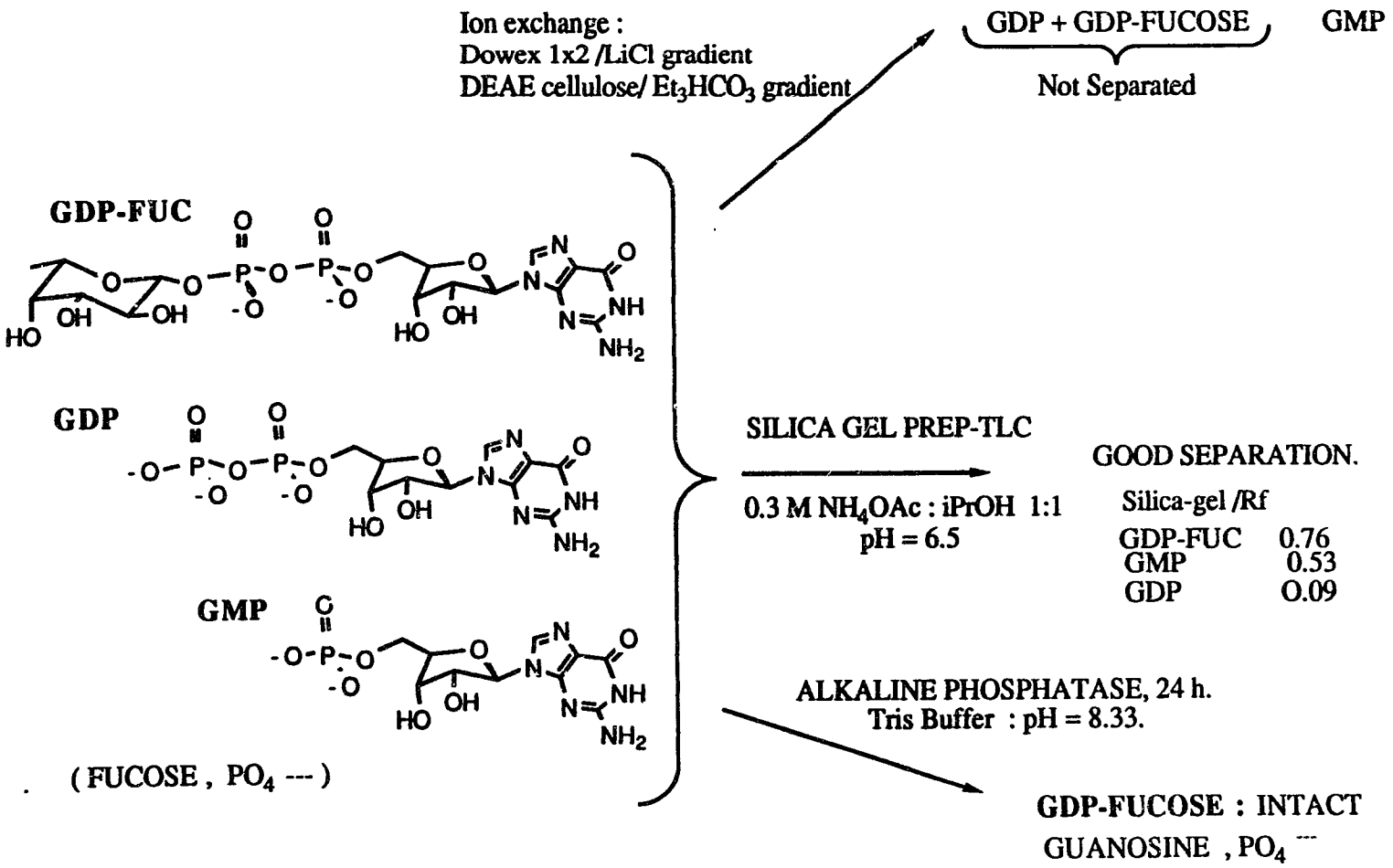


reached when the nucleotide is exposed to an extremely polar environment (concentrated LiCl solution), this results in the hydrolysis of labile pyrophosphate linkage. Such lyophilizations on the preparation of GDP-fucose and GDP-arabinose gave a mixture of GDP, GMP, pyranose sugar and only small quantities of intact sugar-nucleotide. The unexpected hydrolysis of the target nucleotide which was prepared through a multistep synthesis and separation was rather frustrating. Repeated passage of concentrated fractions through a long , wide column of Bio-gel P2 (2 x 60 cm) resulted in complete removal of LiCl (as indicated by detecting Cl^- with aq. $AgNO_3$ solution) from the sugar-nucleotides without any hydrolysis. The nucleotides were eluted in the void volume and the LiCl eluted immediately after the nucleotide band. The fractions were concentrated to about 5 ml. at 27°C/0.05 mm. and reapplied to the Bio-gel column. It was found that a minimum of 4 consecutive bio-gel columns was required before the nucleotide containing fractions could be safely evaporated to dryness. Four passages through Bio-gel finally rendered the nucleotides that were completely devoid of LiCl. Using the above method up to 150 mg of sugar nucleotides could be separated from 3-4 g of LiCl. This method was eventually adopted for the 3 and 4-deoxy analogues as well.

7) As described above an unexpected hydrolysis of GDP-fucose occurred during lyophilization in the presence of large quantities of LiCl. Attempts were made to recover the intact GDP-fucose from its mixture with GDP and GMP. As shown in scheme 33 the ion exchange separations performed on the mixture always

SCHEME 33

RECOVERY OF GDP-FUCOSE FROM DECOMPOSITION PRODUCTS

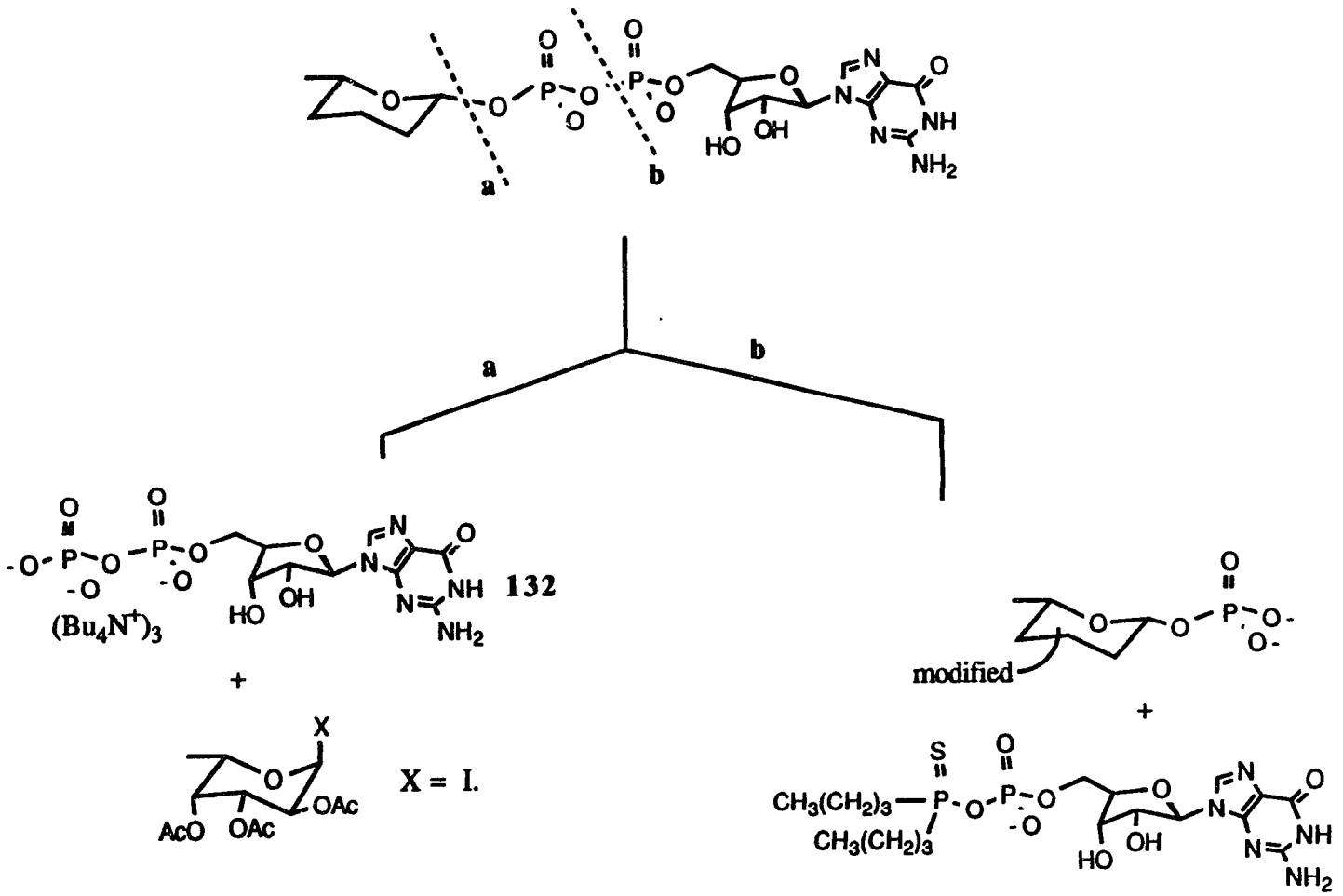


yielded a mixture of GDP and GDP-fucose. The silica gel preparative plate separation could successfully separate all three guanosine containing components. An enzymatic incubation of the mixture with alkaline phosphatase[120] (from E. Coli) resulted in the selective destruction of GMP and GDP to guanosine, leaving the GDP-fucose intact (tlc analysis). A mixture of pure GDP-fucose with GDP (1:1) on incubation with alkaline phosphatase also resulted in the selective hydrolysis of GDP. The ^{31}P-nmr analysis on the product clearly showed the mutually coupled doublets arising from GDP-fucose and a singlet resonance (δ =2) for the inorganic phosphate. In a control experiment pure GDP-fucose remained intact. The preparative use of alkaline phosphatase for the selective destruction of uridine diphosphate in presence of uridine-5'-diphosphoglucose (UDP-glucose) has been recently reported.[121] The substrate specificity and other properties of alkaline phosphatase from E. Coli have been studied.[122] In the present study the incubations were carried out on a small scale (1-2 mg of nucleotides /2 units of Enzyme /0.2 ml of Tris Buffer/23 ^{o}C/12-24 h) and thus the results are qualitative. The use of alkaline phosphatase for the preparative scale purification of GDP-fucose remains to be investigated.

With the experience gained in the preliminary studies of establishing pyrophosphate linkages and the nucleotide separation methods, the synthesis of GDP-fucose analogues was undertaken and the results are described in the following section.

B. Retrosynthetic Analysis

SCHEME 34

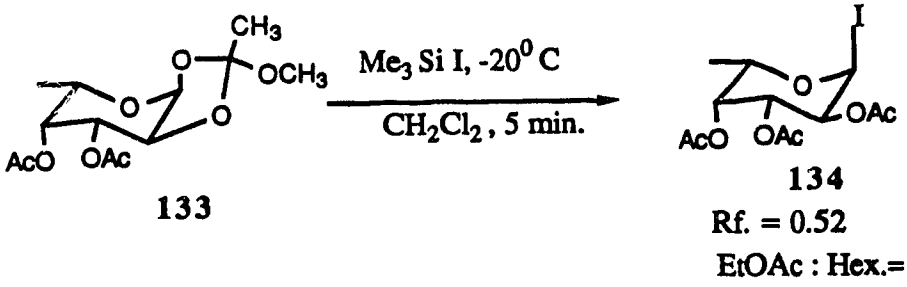


As described in the introduction, the two possible approaches to GDP-fucose synthesis were investigated. Scheme 34 depicts the bond disconnection **a** where guanosine diphosphate was used as a nucleophile to displace a halogen leaving group on per-O-acetylated fucose, thereby establishing the glycosyl diphosphate bond. The alternative approach (bond disconnection **b**, scheme 34) which is traditionally used for the synthesis of the sugar nucleotides was also tried with guanosine-5'-phosphoric di-n-butylphosphinothioic-anhydride (**131**) and guanosine-5'-phosphoromorpholidate (**135**) as the activated forms of GMP.

B 1 Formation of the Glycosyl diphosphate Bond

As shown in scheme 35 2,3,4-tri-O-acetyl-α-L-fucopyranosyl iodide (**134**) was prepared by the treatment of the orthoester **133** with trimethylsilyl iodide in CH_2Cl_2. The iodide being unstable to aq. work-up was generated *in situ* and treated with a DMF solution of tris-(tetra-n-butyl ammonium)-guanosine-di-phosphate (**132**, prepared by the neutralization of the acid form of GDP with tetra-n-butyl ammonium hydroxide). Processing of the reaction mixture (ion exchange separation, desalting and silica prep-tlc purification, see 'experimental' for detailed procedures) afforded a fraction containing GDP-fucose (11% yield : U.V. quantitation). ^{1}H-nmr spectroscopic investigation of this material indicated the presence of 2 methyls (H-6) indicating an α/β anomeric mixture(approx. 1:1) at fucose. Only one example of such an approach has appeared. Behrman et al.[123] have used a similar approach for the synthesis of ADP-glucose(0.5 % yield). The

SCHEME 35

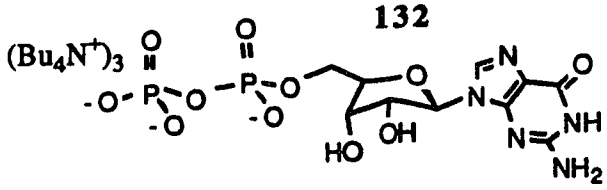


133

134

Rf. = 0.52

EtOAc : Hex.=1:1



132

134

DMF , 23° C , 0.5 h.

i, ii, iii.

11 %

GDP-FUCOSE

(α/β mixture)

i. OH^- : Deacetylation

ii. Ion exchange sep.

iii. Silica gel ptlc.

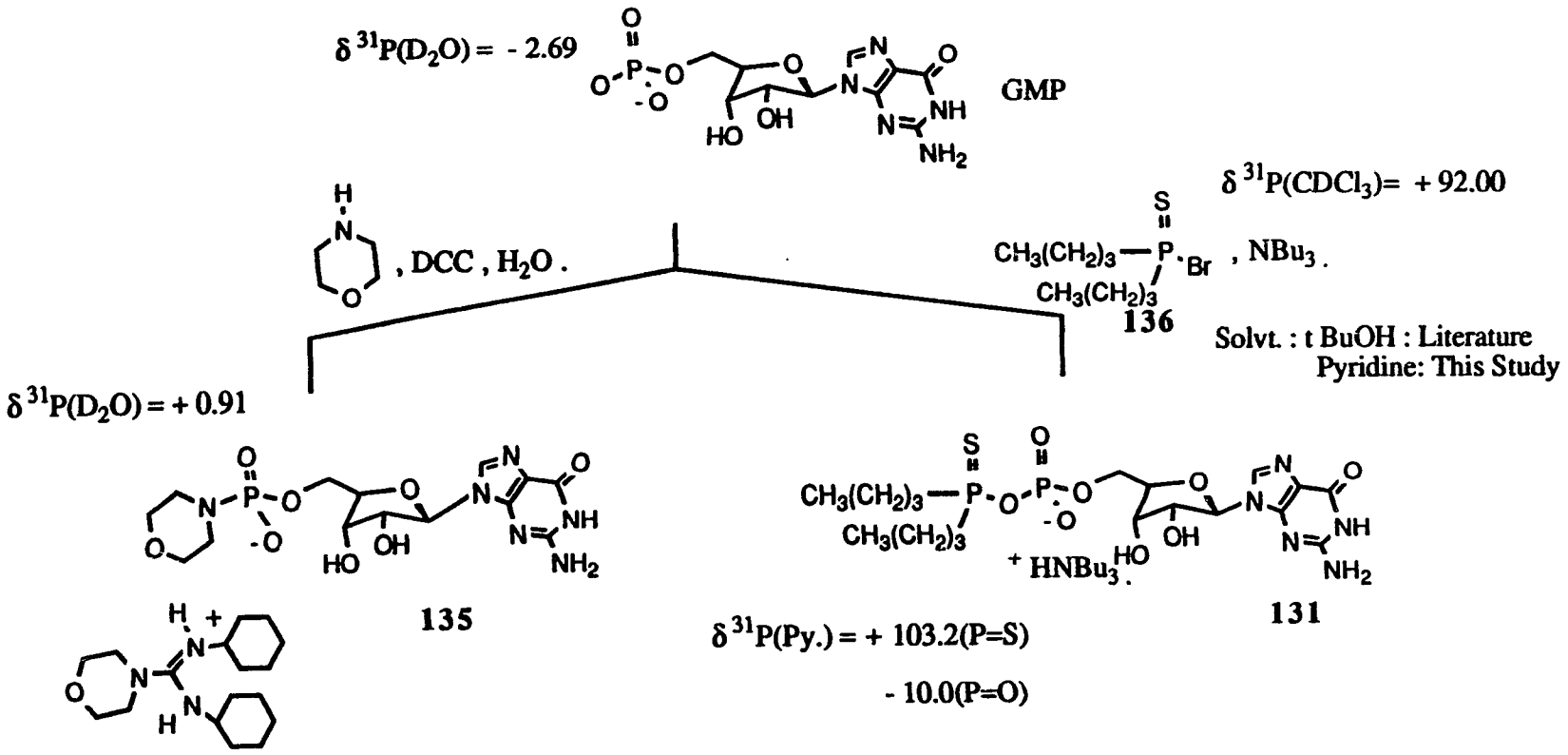
results of the present study and those reported by Behrman[123] point to the fact that the low overall yield and the poor control on pyranose anomeric configuration are the major shortcomings of this approach .

B 2 Formation of the Pyrophosphate Bond

Scheme 36 shows the two activated GMP derivatives **131** and **135** which were employed in the preliminary pyrophosphate coupling reactions. The morpholidate derivative **135** was synthesized using the literature procedure[112] or was commercial. The anhydride derivative **131** has been synthesized before by Furusawa et al.[117] and Barker et al.[68] The conversion of GMP into a salt form (organic solvent soluble) followed by its reaction with di-n-butyl phosphinothioyl bromide[117] (**136**, scheme 36) gives the desired anhydride **131**. Both reported procedures have used t-butanol as the solvent. Barker et al.[68] have used higher temperature (40°C) to achieve a completely homogenous solution. In author's hands, the GMP salts (pyridinium, tributylammonium or trioctylammonium countercations) were marginally soluble in t-butanol even at higher temperatures (40-50 °C). The solubility behaviour was found to vary with different GMP-salt preparations. The use of dry pyridine instead of t-butanol rendered the salts soluble. The addition of tri-n-octylamine to the reaction mixture was found to help the solubilization at ambient temperature. Furusawa et al[117] obtained the anhydride **131** as an oil while Barker et al.[68] obtained it as a crystalline material. Both procedures did not report the yield of the reaction and the product

SCHEME 36

GMP ACTIVATION



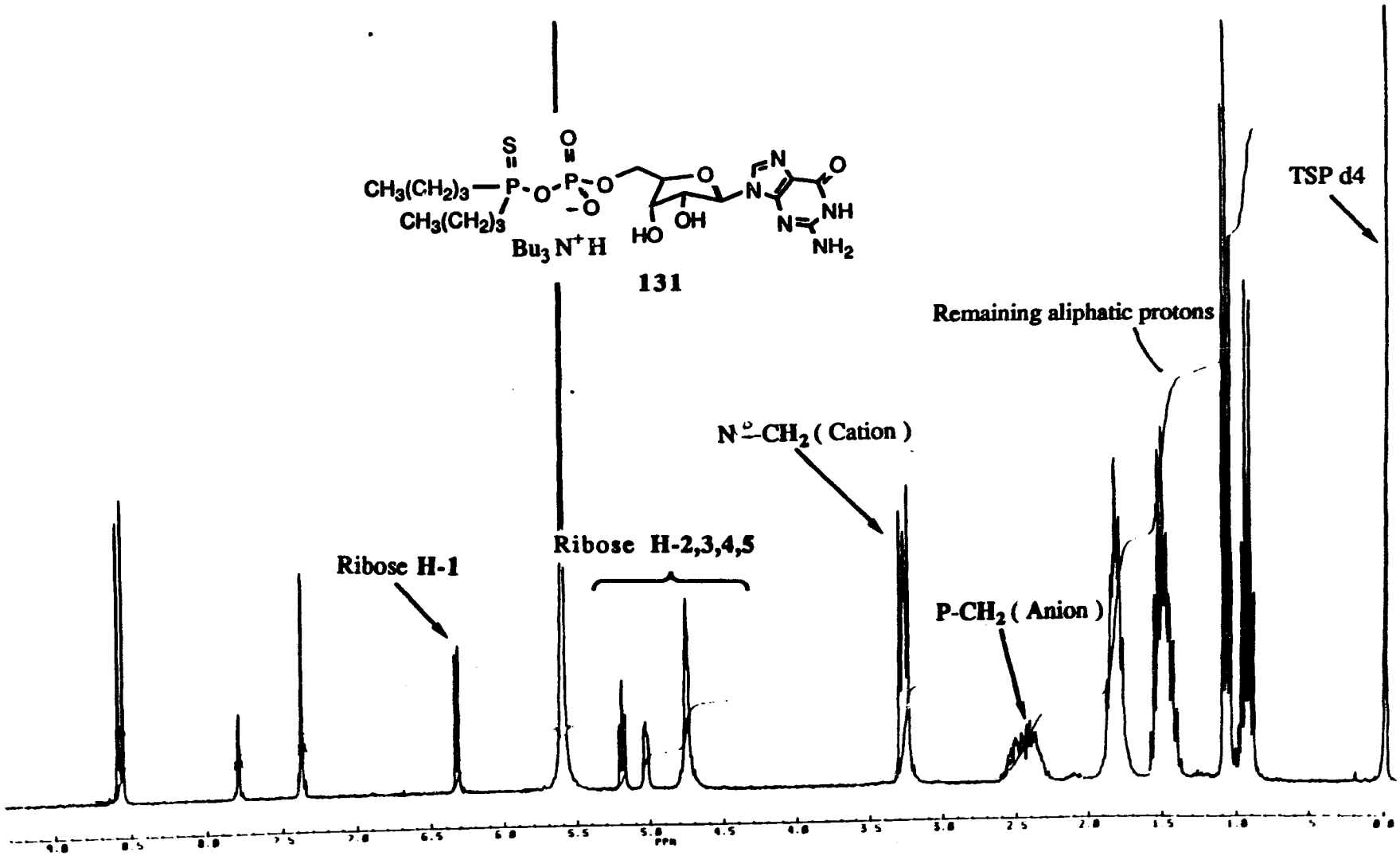


Fig. 13 The 300 MHz ^{1}H nmr spectrum of compound **131** in D_2O : Pyridine .
with TSP d4 ($\delta = 0$) as internal standard.

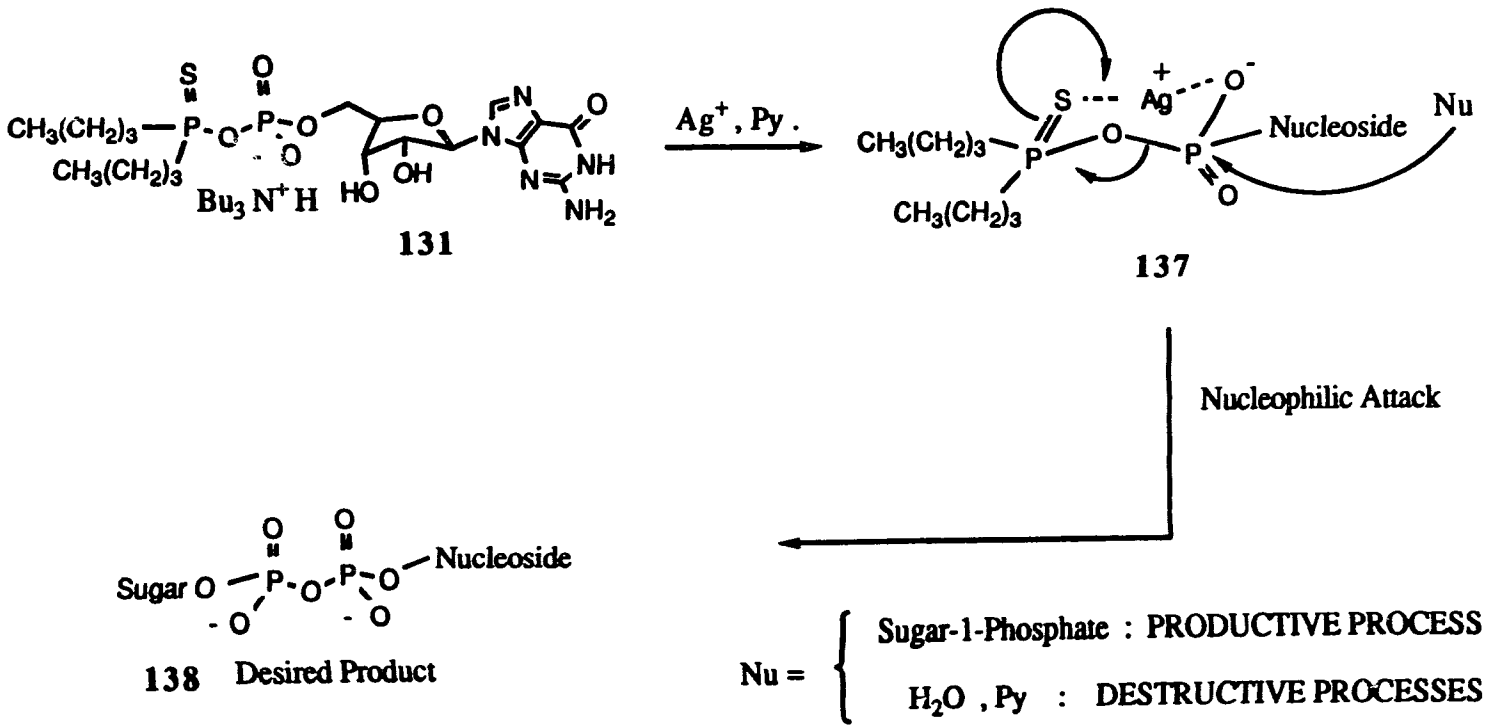
was not characterized. In the present study **131** was synthesized in 28% (crystalline) yield by an improved procedure. The product crystallized as a tributylammonium salt (^{1}H-nmr and combustion analyses). ^{1}H-nmr of **131** is represented in figure13. As pointed out by Furusawa et al[117], the acid form of **131** (Bu_3NH^+ replaced by H^+) has a remarkable stability toward hydrolysis. The acid form of **131** was found to be stable as an aq. solution (23 ^oC, 2 days), whereas in aq. pyridine it underwent hydrolysis.

When **131** is used in the pyrophosphate bond formation reaction (scheme 37) it is treated with a thiophilic inorganic salt such as $AgNO_3$ or AgOAc. The possible complexation of silver with the sulphur atom and the anionic oxygen would result in complex **137** (scheme 37). A variety of nucleophiles present in the reaction medium are then expected to attack at phosphorus. The sugar-1-phosphate present in the reaction mixture is expected to be a better nucleophile than the water (advantitious) and pyridine (solvent).

Relative efficiencies of anhydride **131** or the morpholidate **135** as GMP-donors in the pyrophosphate coupling reaction need to be commented upon. As mentioned above, **131** due to its solubility in organic solvents is considered a better reagent than **135**. In the present study however, the isolated yield of GDP-fucose either by using **131** or **135** was found to be in the same range : 25-30%, (see 'experimental ' for the coupling procedure using **135**). The yield of 70% (by using **131**) for GDP-fucose reported by Barker [68] was not obtained in the present work. The 3-deoxy and 5-nor analogues were obtained in 30-50% yields by

SCHEME 37

COMPOUND 131 IN A PYROPHOSPHATE COUPLING REACTION.



131

137

138 Desired Product

employing **131** as a coupling reagent. It appears that reagent **135** is as good as **131** at least in the synthesis of GDP-fucose analogues. A similar conclusion was reported by Liu et al.[115] in connection with the synthesis of CDP-quinovose.

The quantitation of the product by Barker et al.[68] was based on the purity determination by hplc followed by U.V. absorbance measurements (E_{256}^{M} = 12.2x10^3 for GMP). In the present work all target GDP-fucose analogues were isolated as disodium salts. The homogeneity of each preparation was checked by ^{1}H, ^{13}C , ^{31}P-nmr spectroscopy and silica gel tlc and quantitation was done by weight.

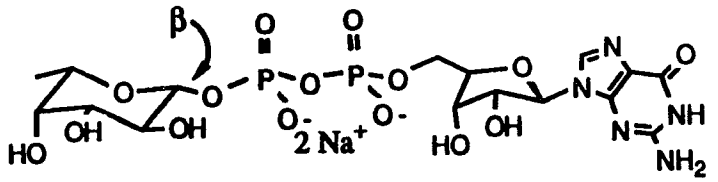
The modified fucose-1-phosphates (as the predominant equatorial anomer) were treated with **131** or **135** in dry pyridine as the solvent. Silver acetate was used as a thiophile when **131** was the reagent. The product nucleotides were purified by ion exchange chromatography. As mentioned earlier, in each case, the minor nucleotide with the axial configuration at the fucose moiety was eluted before the desired sugar-nucleotide. The eluate fractions from the ion exchange chromatography which contained the minor nucleotides (^{1}H-nmr analysis, scheme 31, *vide supra*) were discarded. Bio-gel desalting followed by conversion to the disodium form afforded the target compounds.

C. Synthesis of the 3-deoxy Analogue of GDP-fucose (138)

The engagement of 3-deoxy-L-fucose-1-phosphate **93** (eq/ax : 5.4/1) in the pyrophosphate coupling reaction with **131** (1.5 eq.) and AgOAc in dry pyridine (4 ml) at 23^{o}C for 20 h resulted

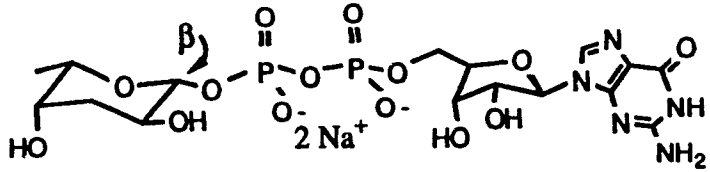
SCHEME 38

TARGET NUCLEOTIDES



GDP-FUCOSE

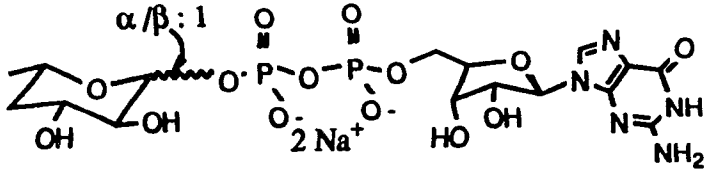
27% YIELD
100 % β



138

GDP 3-DEOXY-FUCOSE
(GDP-COLITOSE)

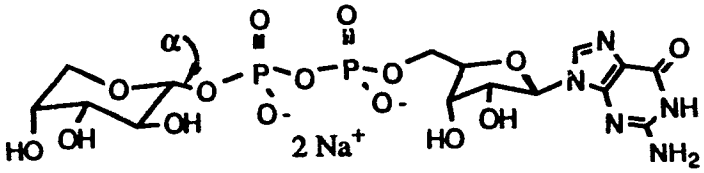
47% YIELD
100 % β



139

GDP 4-DEOXY-FUCOSE

α / β : 1 + impurity



140

GDP 5-Nor-FUCOSE
(GDP-ARABINOSE)

28% YIELD
100 % α

in the formation of the desired compound. The purification of the product by ion exchange chromatography, Bio-gel P-2 desalting, and finally conversion into the disodium salt afforded **138** as a crystalline colourless solid (58 mg, 47% yield, scheme 38) which was extremely hygroscopic. The synthesis of **138** represents the first chemical synthesis of the naturally occurring sugar-nucleotide[101] ' GDP-colitose ' .

D. Synthesis of the 4-deoxy Analogue of GDP-fucose (139)

The 4-deoxy-**L**-fucose-1-phosphate **94** (0.12 mmol, α/β : 1) was reacted with 4-morpholine N,N'-dicyclohexylcarboxamidinium, guanosine-5'-phosphoromorpholidate[112] (1.2 equiv.) to furnish **139** (15 mg, α/β : 1) . The isolated nucleotide was contaminated with at least one guanosine containing impurity (^{1}H, ^{31}P-nmr evidence, ratio of *impurity* : **139** = 1). The presence of the 4-deoxy fucose moiety was established by identifying characteristic resonances arising from the C-4 methylene group of fucose in **139**.

E. Synthesis of the 5-nor Analogue of GDP-fucose (140)

The **D**-arabinose-1-phosphate(**79**, eq./ax :10.7/1) was similarly reacted with **131** to obtain the target nucleotide **140** in 28 % isolated yield(scheme 38).

Thus the pyrophosphate coupling procedure, modified for the detection/separation of the minor isomeric nucleotide provided us with multimilligram quantities of target sugar-nucleotides .The isolated products [**138** (58 mg), **140** (127 mg) and GDP-fucose (150 mg)] were anomerically pure at C-1 of fucose >>95%

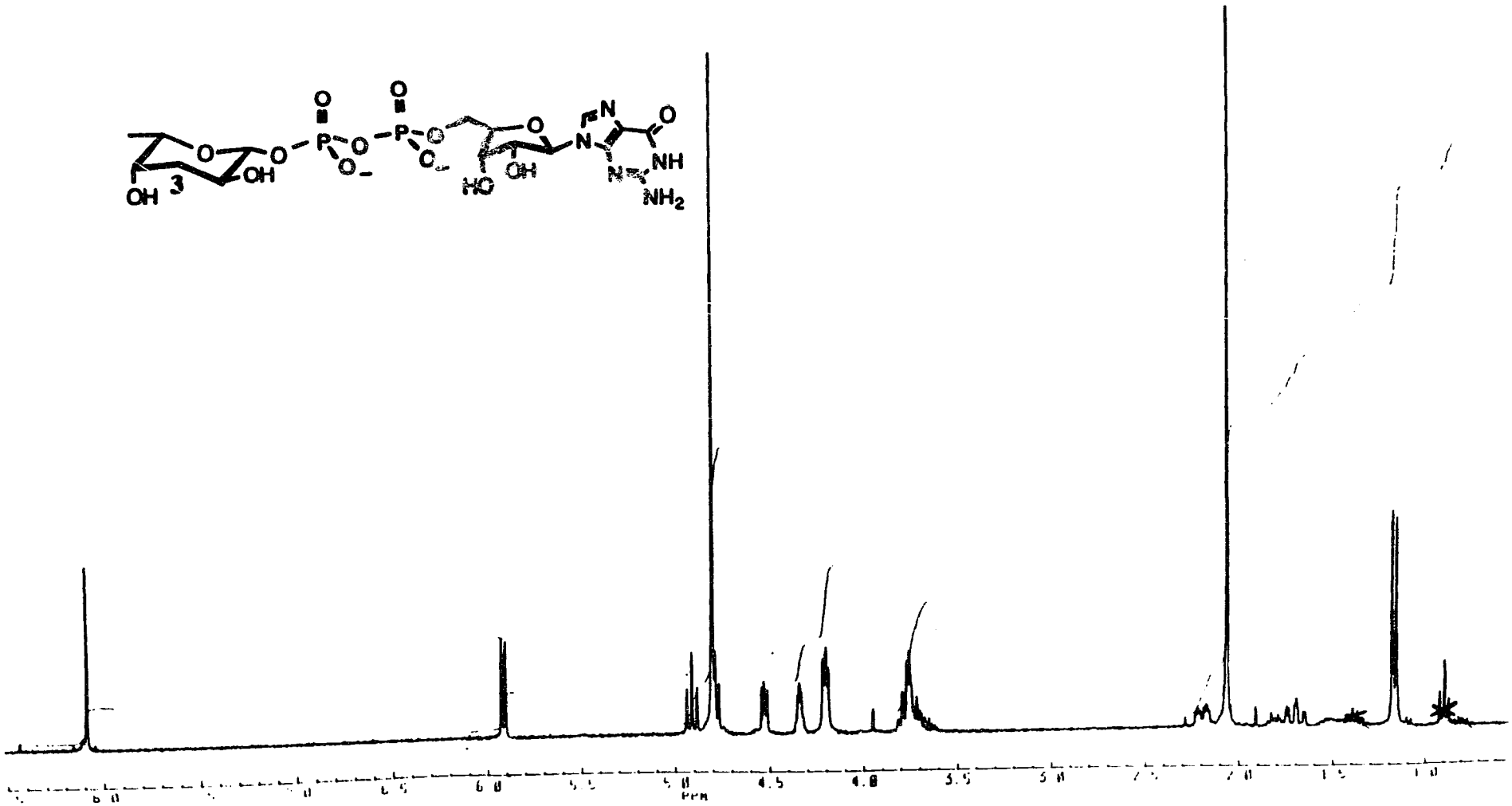


Fig. 14 The 300 MHz ^{1}H-nmr spectrum of compound **138** in D_2O.

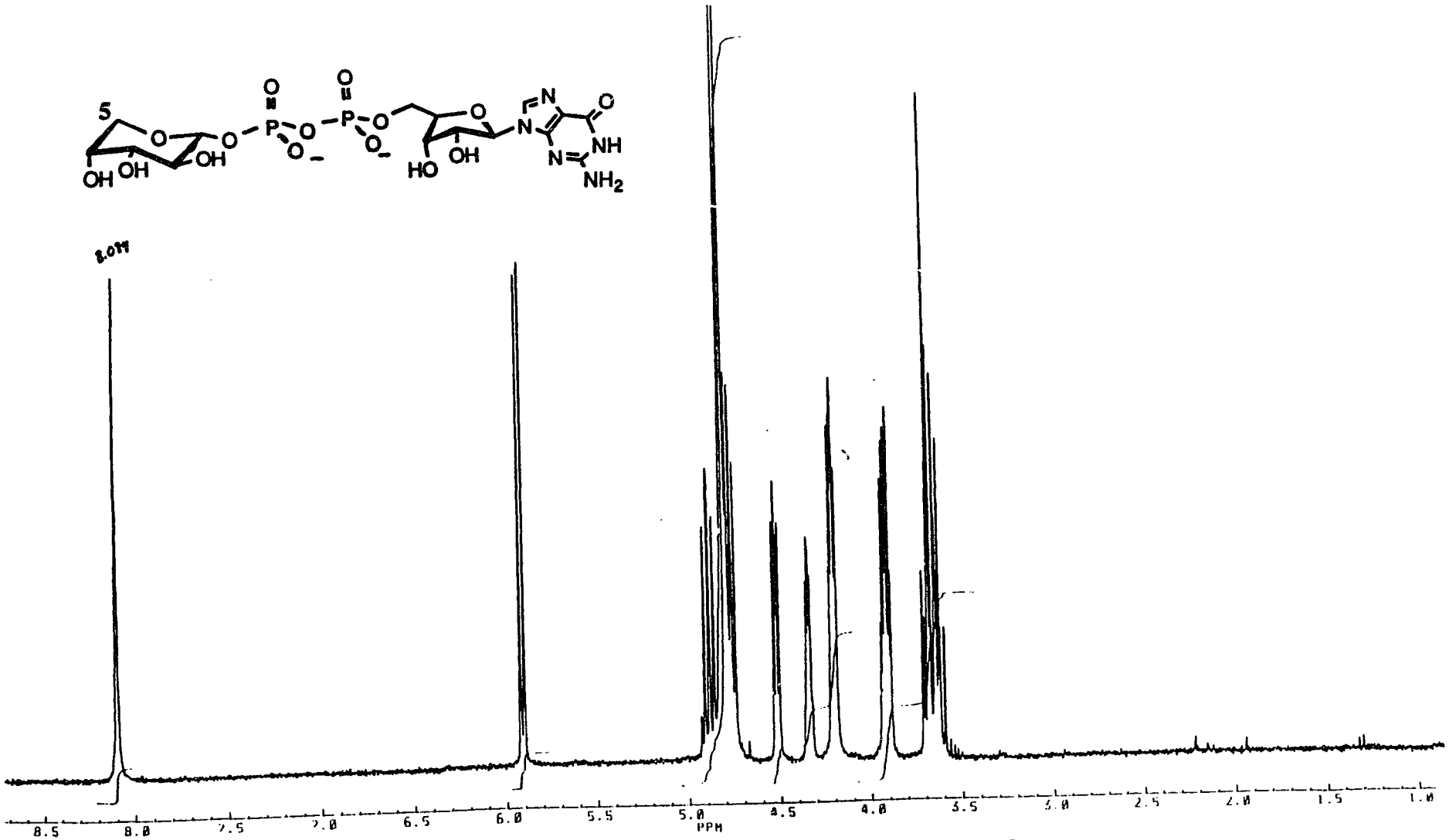


Fig. 15 The 300 MHz ^{1}H-nmr spectrum of compound **140** in D_2O .

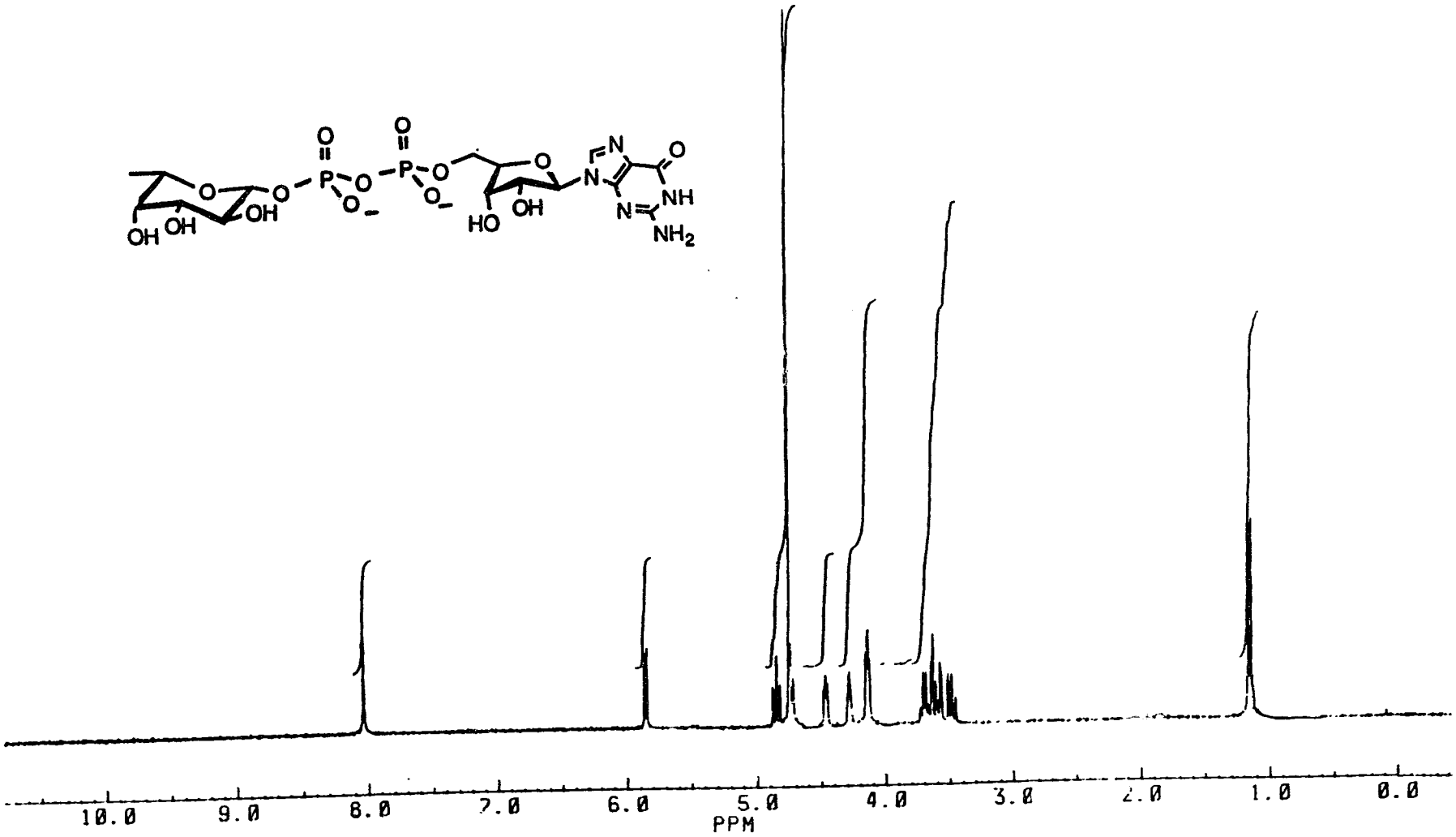


Fig. 16 The 300 MHz ^{1}H-nmr spectrum of GDP-fucose in D_2O

within the detection limits of ^{1}H (300 MHz)-nmr.spectroscopy The identity and homogeneity of the products was assessed by ^{1}H, ^{13}C and ^{31}P-nmr spectroscopic analysis. The ^{1}H-nmr spectra of **138**, **140** and GDP-fucose are reproduced in figures 14, 15, 16.

F. THE CONFORMATIONAL PROPERTIES OF 138 AND 140

Extensive studies on the solution conformations of a variety of nucleoside diphosphate hexoses were reported by Lee and Sarma[99]. Information about the overall conformation was derived by comparing the chemical shift and coupling constants data for the individual components vis-a-vis the integrated sugar-nucleotide molecule . No significant differences were observed in ^{1}H chemical shift data for hexoses and nucleoside-5'-diphosphates as the sugar-nucleotide is formed. The intramolecular interaction between hexose and the nucleoside parts of the molecule was therefore assessed to be insignificant and hence it was reasonably proposed that, in the aqueous solution, the nucleoside diphosphate hexoses exist in a linear (extended) conformation.

The detailed study of the solution conformations of the analogues was out of the scope of the present work. It was however considered important to assess whether or not the structural changes in the fucose part of the molecule had any effect on the overall conformation of the parent GDP-fucose molecule. ^{1}H, ^{13}C, and ^{31}P-nmr spectra of **138** and **140** were compared with those of GDP-fucose. The pyranose ring conformation was $^{1}C_{4}$ in the analogues as evidenced by the expected vicinal coupling constants. The homo and heteronuclear

coupling constants $J_{H,P}$ and $J_{C,P}$ were extracted from ^{1}H and ^{13}C-nmr spectra respectively (Table 5)

Table 5

Formula No.	$^{3}J_{C-2,P}$ fucose	$^{3}J_{C-4',P}$ ribose	$^{3}J_{H-1,P}$ fucose
GDP-Fucose	8.0	9.1	8.0
138	8.3	9.1	8.0
140	8.2	9.2	7.5

The invariance of the coupling constants (and the chemical shifts, see experimental section) in the analogues strongly suggest[99] that the overall conformation of GDP-fucose is not affected by the structural modifications in the fucose part of the molecule. It appears reasonable[99] to anticipate that the overall conformations presented by the modified donor substrates to the interacting fucosyltransferases are similar to those of the native donor substrate:

In conclusion, multistep syntheses of the modified acceptor substrates **1** and **2** (scheme 1) were achieved (chapt. II). The difficulties faced in the derivatization of 2' and 3-OH groups to their corresponding phenoxythionocarbonyl derivatives was presumably due to the steric environments of the particular hydroxyl group in the highly functionalized disaccharide intermediates. Use of a strong base n-BuLi, for the deprotonation of the hydroxyl group however successfully resulted in the desired

functionalization. The biological activity of the final monodeoxy disaccharides **1** and **2** remains to be evaluated.

The syntheses of the modified fucosyl phosphates was achieved by displacements using tetra-n-butylammonium dibenzylphosphate (**73**) on the corresponding anomeric bromides (scheme 25 , chapt. III). Short reaction times were achieved by the use of DMF as solvent. Due to the short reaction times and high diastereoselectivity of the process, this procedure should become a method of choice for the synthesis of thermodynamically less stable equatorial anomeric phosphates of aldopyranoses. Although **L**-fucose-1-phosphate (**78**) and **D**-arabinose-1-phosphate (**79**) were synthesized in high overall yields (50% yield, eq/ax ratios : 12/1), the yields of 3-deoxy (**93**) and 5-nor (**94**) analogues were modest. These lower yields are in all likelyhood attributed to the instability of the corresponding anomeric bromides as well as the product phosphotriesters under the phosphorylation conditions. Substitution of the bromide leaving group by a chloride or an imidiate and their use in the phosphorylation process remains to be tested. The syntheses of the GDP-fucose and its 3-deoxy (**138**) and 5-nor analogues (**140**) are achieved in a manner to furnish anomerically pure molecules. The 4-deoxy analogue **139** was obtained only as a mixture of α and β anomers (ratio : 1:1). Further purification of **139** was not attempted.

The modest overall yields (30-50%) obtained in the pyrophosphate bond formation are due mostly to competing hydrolytic reactions. The anionic pyrophosphate group in the

product can also potentially get involved in a cross reaction with the activated GMP reagent giving rise to destruction of the product.

At this time of writing, evaluation of the newly synthesized deoxy-GDP-fucose analogues as potential donor substrates for fucosyltransferases is in progress. To date, preliminary evidence that both 3-deoxy (**138**) and 5-nor-GDP-fucose (**140**) are substrates for Le-FucT purified from human milk has been obtained using a novel enzyme-linked immunosorbent assay (ELISA) recently developed[124] in this laboratory. Details of these studies will be reported elsewhere.

V. EXPERIMENTAL

A. General Procedures

All solvents and reagents used were reagent grade, and in cases where further purification was required, standard procedures[125] were followed. All solid reactants for glycosylation and for moisture sensitive reactions were dried overnight over phosphorus pentoxide in high vacuum prior to use. Solution transfers where anhydrous conditions were required were done under dry nitrogen or argon using hypodermic syringes. Molecular sieves were purchased from BDH chemicals.

Thin layer chromatograms (tlc) were performed on precoated silica gel 60-F254 plates (E. Merck, Darmstadt) and visualized by quenching of fluorescence and/or by charring after spraying with 5% sulfuric acid in ethanol. For "silica gel chromatography" 40-63 μm. (400-230 mesh) silica gel 60 (E. Merck No. 9385) and distilled solvents were used, and the ratio of silica gel to compound was in the range 50:1-100:1. The Ion exchange resins were washed with methanol or milli-Q-water before use. Milli-Q-water denotes the distilled and deionized water obtained from a millipore water treatment system (model OM-140). During the work-up procedures and ion exchange separations on the water soluble compounds "milli-Q-water" was used.

The evaporation of water was done below 0.1 mm (oil pump vacuum) with a rotary evaporator equipped with a condenser containing acetone and dry ice. Organic solvents were removed on a rotary evaporator under the vacuum of a water aspirator with bath temperaturs of 40 °C or lower. For gel filtration, Bio-Gel P-2 (200-400) mesh (Bio-Rad Laboratories, Richmond, California, USA) was used.

Spectral and elemental analyses were performed respectively by the departmental-nmr Service Laboratory under the supervision of Dr. T. T. Nakashima and the Analytical Service Laboratory under the supervision of Mr. R. Swindlehurst. Proton nuclear magnetic resonance (^{1}H-nmr) spectra were recorded (unless otherwise stated) at 300 MHz (Bruker AM-300) with tetramethylsilane (δ0 in $CDCl_3$) or acetone (δ2.225 in D_2O) or TSP-d4 (δ0 in D_2O) or acetonitrile (δ2.049 in D_2O) as the internal standard at ambient temperature. Carbon-13 nuclear magnetic resonance (^{13}C-nmr) spectra were recorded at 75 MHz (Bruker AM-300) with either external tetramethylsilane (δ0 in $CDCl_3$) or external 1,4-dioxane (δ67.4 in D_2O) as the reference standard. In the description of nmr data the following abbreviations are used : 'bs' : broad singlet,'d' : doublet, 'dd' : doublet of doublet, 'ddd' : doublet of doublet of doublet, 'm' : multiplet, 'pseudo triplet' : overlapping dd, 'q' : quartet, Jgem : geminal coupling constant. ^{1}H chemical shifts and coupling constants are reported as if they were first order. ^{1}H-^{1}H coupling constants are accurate to +/- 0.2 Hz, ^{1}H-^{31}P coupling constants are accurate to +/- 0.5 Hz,

^{31}P-^{13}C coupling constants are accurate to +/- 0.5 Hz. The ^{1}H chemical shifts were reproducible to the three decimal places within one nmr experiment and to the two decimal places between two nmr experiments. Phosphorus-31 nuclear magnetic resonance (^{31}P-nmr) spectra were recorded at 161.97 MHz (Bruker AM-300) with external H_3PO_4 (85%) (δ=0 in D_2O) as the reference standard. The chemical shifts at lower field as relative to external H_3PO_4 are expressd as positive numbers while those at higher field are expressed as negative numbers. For some compounds, consideration of the water of hydration was necessary in order to obtain a match between the combustion analysis results and the expected elemental composition. Mass spectra were measured under the supervision of Dr. Hogg by the mass spectrometry laboratory of this department on an AEI MS-12 instrument for chemical ionization (CI NH_3), or a KRATOS/AEI MS-9 modified for detection of either positive ('pos') or negative ('neg') ions for fast atom bombardment ('FAB') spectra. Optical rotations were determined on a Perkin-Elmer 241 polarimeter in a 1 dm cell at ambient temeprature (23 +/- 1°C). The melting points are uncorrected. High performance liquid chromatography (HPLC) was performed on Waters Delta Prep 3000 preparative chromatography system.

B. Syntheses

n-Octyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside. (7)

To a solution of 2 acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-glucopyranosyl chloride[34] (6, 6.0 g, 16.5 mmol) in dry toluene (125 ml) were added n-octanol (6 ml , 2.3 equiv.), mercuric cyanide (3 g), mercuric bromide (3 g) and powdered calcium sulphate (25 g). The resulting suspension was protected from moisture and stirred for three days. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to a syrup which was then dissolved in dichloromethane (50 ml). Successive washings with water (50 ml), 10 % aq. silver nitrate (2x20 ml), satd. aq $NaHCO_3$ (2x20 ml), water (50 ml) followed by drying ($MgSO_4$) and evaporation of the solvent *in vacuo* left a thick syrup which was dissolved in a minimum quantity of ethyl acetate. Slow diffusion of n-pentane into this solution afforded fine needles of **7** (5.35 g, 71 %); m.p. 126°; $[\alpha]_D$ = - 16.4° (C 0.46, dichloromethane), ^{1}H-nmr ($CDCl_3$) δ = 5.700 (bd, 1H, D_2O exchangeable, NH), 5.319 (dd, 1H, $J_{2,3}$ = 9 H2, $J_{2,3}$ = 10 Hz, H-3), 4.770 (1H, $J_{3,4}$ = $J_{4,5}$ = 10 Hz, H-4), 4. 695 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 3.970 (dd, J_{gem} = 12 Hz, J5,6 = 5 Hz, H-6a), 4.125 (dd, $J_{5,6}$ = 2.5 Hz, H-6b), 3.913-3.764 (m, 2H, H-2, O-CHHCH$_2$), 3.708 (ddd, 1H, H-5), 3.470 (m, 1H, O-CHHCH$_2$), 2.103-1.926 (4xs, 12H, $OCOCH_3$, $NCOCH_3$), 1.613-1.512 (m, 2H, alkyl), 1.317-1.208 (m, 10H, remaining aglycone methylenes), 0.875 (m, 3H, agiycone CH_3). **Anal.** Calcd. for $C_{22}H_{37}NO_9$: C 57.67, H 8.17, N 3.05. Found : C 57.57, H 8.28, N 3.08.

n Octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (8)

To a clear solution of **7** (1.0 g, 2.18 mmol) in dry methanol (40 ml) was added sodium methoxide (100 mg) and the resulting clear solution was stirred for 15 h. The solution was neutralized with IR- 120 (H^+) resin. The resin was removed by filtration. The filtrate was evaporated *in vacuo* to afford a crystalline solid which was dried over P_2O_5 to afford **8** (725 mg, quantitative yield), m.p. 156-60°, $[\alpha]_D$ = - 22° (C 0.46, methanol), Rf 0.47 (toluene : methanol, 2:1). **Anal.** Calcd. for $C_{16}H_3NO_6$: C 57.86, H 9.41, N 4.22. Found : C 57.05, H 9.35, N 4.21.

n Octyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (9)

A solution of dry triol **8** (725 mg, 2.18 mmol), benzaldehyde dimethyl acetal (2.6 ml , 1.2 equiv.) and PTSA (50 mg) in dry DMF (10 ml) was stirred protected from moisture. After 10 hours, the reaction mixture was treated with four drops of triethylamine to neutralize the acid catalyst. The resulting solution was partioned between dichloromethane (40 ml) and water (20 ml). The organic layer was washed with satd. aq. $NaHCO_3$ (20 ml) followed by water (20 ml). and then evaporated in vacuo to afford a pale yellow solid which on trituration with hexane gave a colourless solid. This solid on drying over P_2O_5 furnished **9** (825 mg, 89 % yield). Crystallization from methanol gave the analytical sample. m.p. 250°, $[\alpha]_D$ = - 66° (C O.44, methanol), Rf O.59 (toluene : methanol 2:1), ^{1}H-nmr ($CDCl_3$) δ : 7.520-7.320 (5H, arom), 5.739 (1H, D_2O exchangeable, NH), 5.560 (s, 1H, benzylidene H), 4.697 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.341 (dd,

1H, J_{gem} = 10 Hz, $J_{5,6eq.}$ = 5 Hz, H-6eq), 4.250-4.100 (2H, 3-OH (D_2O exchangeable), H-3 (simplified to sharp dd after D_2O exchange, 4.153 ppm, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 8.9 Hz)), 3.877 (dd, 1H, O-CHH-CH_2), 3.793 (pseudo triplet, 1H, $J_{5,6ax}$ = 10 Hz, H-6ax), 3.603-3.377 (4H, H-2 (3.433 ppm), H-4, H-5, O-CHH-CH_2), 2.046 (bs, 3H, $NCOCH_3$), 1.643-1.519 (m, 2H, O-CH_2-CH_2), 1.374-1.205 (m, 10H, remaining aglycone methylenes), 0.890 (m, 3H, aglycone CH_3). **Anal.** Calcd. for $C_{23}H_{35}NO_6$: C 65.54, H 8.37, N 3.32. Found : C 65.64, H 8.39, N 3.7.

n-Octyl 2-acetamido-3-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (10)

To a solution of **9** (690 mg, 1.64 mmol) and allyl bromide (0.27 ml) in dry DMF (50 ml) were added barium hydroxide octahydrate (282 mg) and barium oxide (960 mg) . The resulting suspension was stirred for six hours at the end of which, according to the tlc analysis of the reaction mixture, over 99 % of **9** had been converted into **10**. The reaction mixture was filtered and the filtrate was partitioned between dichloromethane (40 ml) and water (20 ml). The organic layer was successively washed with 0.1N HCl (20 ml), satd. aq. $NaHCO_3$ (20 ml) and water (20 ml). Drying ($MgSO_4$) and concentration of the organic extract left a microcrystalline mass which on drying over P_2O_5 gave the compound **10** (641 mg, 84.9 %). m.p. 233-35°, $[\alpha]_D$ = - 37° (C 0.31, dichloromethane), Rf 0.78 (ethylacetate : hexane 4:1), ^{1}H-nmr ($CDCl_3$) δ: 7.540-7.300 (5H, arom), 5.963-5.758 (2H, Hc allyl, NH), 5.536 (s, 1NH), 5.239 (1H, Hb allyl), 5.146 (1H, Ha allyl), 5.080 (d,

1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.412-4.228 (3H, H-3, H-6eq., He(d), allyl), 4.133 (1H, Hd(e) allyl), 3.900-3.726 (2H, H-4, -O-CHH-CH_2), 3.626-3.436 (3H, H-5, H-6ax, O-CHH-CH_2), 3.129 (m, 1H, H-2), 2.000 (s, 3H,$NCOCH_3$),1.613-1.500 (m, 3H, CH_2CH_2), 1.359-1.200(m, remaining aglycon methylenes), 0.926-0.839 (m, 3H, CH_2CH_3). **Anal**. Calcd. for $C_{26}H_{39}NO_6$: C 67.67, H 8.52, N 3.04. Found : C 67.53, H 8.53, N 2.93.

n-Octyl 2-acetamido-3-O-allyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (5)

A clear solution of **10** (2.62 g, 5.68 mmol) and sodium borohydride (3.22 g) in dry THF (600 ml) was stirred at 0^o and protected from moisture. A crystal of methyl orange indicator was added to the solution in order to obtain the pale yellow reaction mixture. To this reaction mixture a dilute (30 % by weight) solution of gaseous HCl in dry diethylether was added dropwise at 0^o till the mixture turned permanently pink. The reaction mixture was then closely monitored by tlc (ethyl acetate : hexane 3:1) with simultaneous addition of etheral HCl. The addition of ethereal HCl was terminated when about 20 % of the **10** (Rf 0.69) remained unreacted. The desired compound **5** (Rf 0.31) was found to be the major component of the reaction mixture. A minor compound (Rf 0.06) was also observed. The volume of the reaction mixture was reduced to about 50 ml by evaporation *in vacuo* and the resulting solution was diluted with dichloromethane (300 ml). The insoluble material was removed by filtration. The filtrate was successively washed with satd. aq.

$NaHCO_3$ (2x20 ml) and water (20 ml). Subsequent drying ($MgSO_4$) and evaporation *in vacuo* afforded a syrup which was purified by chromatography on silica gel (dichloromethane : methanol 19:1). The early fractions (Rf 0.69, ethyl acetate: hexane,3:1) contained **10** (582 mg,1.26 mmol). The fractions containing Rf 0.31 compound were pooled and evaporated to afford **5** as a colourless crystalline mass. (1.65g, 80 % based on the recovey of **10**). m.p. 106-7°, $[\alpha]_D$ = - 27° (C. 0.61, dichloromethane), Rf 0.42 (dichloromethane : methanol 19:1),^{1}H-nmr ($CDCl_3$) δ :7.400-7.300 (5H, arom) 5.907 (1H, Hc allyl), 5.659 (d,1H, $J_{N\text{-}H,2}$=7Hz, NH), 5.266 (1H, Hb allyl), 4.926 (d, 1H, $J_{1,2}$=8.0 Hz, H-1) 4.586 (dd, 2H, Jgem=12Hz, $C_6H_5CH_2O$), 4.313-4.126 (m, 2H, Hd,e allyl), 3.984 (dd,1H, $J_{2,3}$=10Hz. $J_{3,4}$=8Hz, H-3), 3.823(m,1H, O-CHH-CH_2), 3.779-3.507 (4H, H4, 5, 6a, 6b), 3.459 (m,1H, OCHHCH2), 3.129 (ddd,1H, H-2), 2.836 (bs,1H, 4-OH), 1.979 (s, 3H, $NCOCH_3$), 1.633-1.500 (m, 2H, OCH_2CH_2), 1.339-1.197 (m, remaining aglycon methylenes), 0.913-0.829 (m, 3H, CH_2CH_3), ^{13}C-nmr ($CDCl_3$) δ : 69.89 (C-6). **Anal.** Calcd. for $C_{26}H_{41}NO_6$: C 67.38, H 8.92, N 3.02. Found : C 67.12, H 8.66, N 3.25 .

n-Octyl 2-acetamido-3-O-allyl-4-0-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-β-D-glucopyranoside. (3)

3,4,6-tri-O-acetyl-α-D-galactopyranose-1,2-(methyl orthoacetate)[38] : Rf 0.61-toluene : methanol : 2:1(syrup, 95 mmol) was dissolved in dry methanol (150 ml) containing $NaOCH_3$ (1.2 g) and stirred at 23°C for 24 h to achieve complete

deacetylation (Rf 0.4). The resulting solution was evaporated to dryness and held under high vacuum(12 h). The resulting syrupy triol was dissolved in dry DMF (700 ml) and stirred at $0^{o}C$. Sodium hydride: 56% oil-dispersion (15.6) was washed with dry hexane to obtain the oil-free material (6.84, 285 mmol) which was added in portions to the above solution at $0^{o}C$. The resulting suspension was stirred at $0^{o}C$ for 2 h and then treated with benzyl bromide (37 ml , 310 mmol). Stirring at $23^{o}C$ afforded 3,4,6-tri-O-benzyl-α-**D**-galactopyranose-1,2-(methyl orthoacetate) (**12**) as the major compound in the reaction mixture (Rf 0.91). Treatment of the resulting solution with methanol (100 ml) followed by evaporation to dryness gave a syrup which was taken up in dichloromethane (300 ml). Washing with cold water (3 x 300 ml), drying of the organic layer ($MgSO_4$), followed by evaporation gave a syrup which was purified by silica chromatography (ethyl acetate : hexane, 1:3) to obtain **12** (15 g, 32%). Glycosyl donor **4** was prepared from freshly prepared **12** as described below.

To a stirred solution of **12** (3.6 g, 7.1 mmol) and tetraethylammonium bromide (dry, 740 mg, 3.5 mmol) in dry dichloromethane (60 ml) was added acetyl bromide (1.2 ml) at ambient temperature. TLC analysis after 1 h indicated complete conversion of **12** (Rf 0.4, ethyl acetate : hexane, 1:3) into **4** (Rf 0.5). Dilution of the reaction mixture with dichloromethane (60 ml) followed by washing with cold water (2x20 ml), drying ($MgSO_4$) of the organic layer and finally evaporation gave **4** as a pale yellow syrup. Bromosugar **4** was added to a stirred solution

of **5** (1.1 g, 2.4 mmol), silver triflate (1.83 g, 3 equiv.) and tetramethylurea (1.13 ml , 4 equiv.) in dry dichloromethane (30 ml). After stirring for 15 h, the reaction mixture was filtered through a celite pad. The filtrate was successively washed with $NaHCO_3$ (2x10 ml), cold water (2x10 ml) and dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the residue was purified by silica chromatography in ethyl acetate: hexane (1:2). The fractions containing the disaccharide material (Rf approx. 0.1, ethyl acetate : hexane 1:2) were pooled and the solvent was evaporated. The residue was purified by preparative HPLC. (PrepPak 500 silica cartridge, ethyl acetate : hexane: 1 :2, Flow rate 70 ml /min) to yield the desired β linked disaccharide **3** (1.3g, 59%). m.p. 100-4 ^{o}C, $[\alpha]_D$ = - 22 ^{o} (C. 0.29, dichloromethane), Rf 0.77 (acetone : hexane, 1:1). ^{1}H-nmr ($CDCl_3$) δ :7.44-7.19 (20 H, arom), 6.273 (d, 1H, D_2O, exchangeable, J = 9 Hz, NH), 5.800 (1H, Hc allyl), 5.279 (dd, 1H, $J_{2,3}$ = 10 Hz, $J_{1,2}$ = 8 Hz, H-2), 5.101 (1H, Hb, allyl), 5.052 (1H, Ha allyl), 4.913 (d, 1H, J_{gem} = 12 Hz, C_6H_5CHHO), 4.700-4.373 (8H, H-1, d 4.534, $J_{1,2}$ = 8 Hz, remaining $C_6H_5CH_2O$), 4.337 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.178-3.467 (14H, H-2 (d 3.95) H-3, H-4, H-5, H-6a, H-6b, H-3, H-4, H-5, H-6'a, H-6'b, $OCHHCH_2$, Hd and He, allyl), 3.374 (m, 1H, $OCHHCH_2$), 2.009-1.984 (2xs, 6H, $NCOCH_3$, $OCOCH_3$), 1.564-1.450 (m, 2H, aliphatic), 1.333-1.187 (m, 10H, remaining aliphatic), o.874 (3H, CH_2CH_3).^{13}C-nmr ($CDCl_3$) δ : 100.14, 90.84 (C-1, C-1). **Anal.** Calcd. for $C_{55}H_{71}NO_{12}$: C 70.41, H 7.63, N 1.49. Found: C 70.16, H 7.77, N 1.53. The α-linked disaccharide **13** was isolated in 20 % yield. m.p. 116-8^{o}, $[\alpha]_D$ = + 54^{o} (C. 0.41, dichloromethane), Rf. o.72

(acetone : hexane,1:1). **Anal**. Calcd. for $C_{55}H_{71}NO_{12}$. H_2O : C 69.09, H 7.69, N 1.46. Found : C 69.02, H 7.54, N 1.45.

n-Octyl 2-acetamido-3-O-allyl-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-2-deoxy-β-D-galactopyranoside (14)

A clear solution of **3** (275 mg, 0.27 mmol) in dry methanol containing sodium methoxide (90 mg) was stirred for three days. Resulting solution was then neutralized with Amberlite IR-120 (H^+) resin. The resin was removed by filtration and washed with methanol (50 ml). The filtrate and the washings were concentrated in vacuo to afford a solid which was then dried over P_2O_5 to afford **14** (243 mg, 99 %). m.p. 101-4 ^oC, $[\alpha]_D$ = - 2.8^o (C. 0.11, dichloromethane), Rf 0.31 (ethyl acetate : hexane, 1:1), ^1H-nmr ($CDCl_3$), δ: 7.44-7.19 (20H, arom), 5.992 (bs, 1H, D_2O, exchangeable, NH), 5.785 (1H, Hc allyl), 5.143 (1H, Hb allyl), 5.017 (1H, Ha allyl), 4.884 (d, 1H, J_{gem} = 12 Hz, C_6H_5CHHO), 4.793 (d, 1H, $J_{1,2}$ = 7 Hz, H-1), 4.733-4.243 (9H, H-1, (δ 4.490, $J_{1,2}$ = 8 Hz), Hd allyl, remaining C_6H_5CHHO), 4.100-3.257 (15 H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2, H-3, H-4, H-5, H-6'a, H-6'b, $OCHHCH_2$, He allyl), 2.510 (bs, 1H, D_2O, exchangeable, OH), 1.930 (S, 3H, $NHCOCH_3$), 1.564-1.450 (m, 2H, aliphatic), 1.333-1.187 (m, 10H, remaining aliphatic), 0.874 (3H, CH_2CH_3). **Anal**. Calcd. for $C_{53}H_{69}NO_{11}$: C 71.04, H 7.76, N 1.56. Found : C 70.89, H 7.55, N 1.43.

n-Octyl 2-acetamido-3-O-allyl-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-2-O-phenoxythiocarbonyl-β-D-galactopyranosyl)-2-deoxy-β-D-glucopyranoside (15)

To a clear solution of **14** (dry, 900 mg, 1.0 mmol), DMAP (610 mg, 5 mmol) and pyridine (0.6 ml , 7 mmol) in dry dichloromethane(40 ml) was added phenoxychlorothionocarbonate (1 ml , 7.2 mmol) and the resulting pale yellow solution was stirred at ambient temperature. After a few minutes, the solution characteristically turned green in colour. Stirring for 1.5 days gave a pale brown reaction mixture. At the end of the stirring period, over 90 % of **14** (Rf 0.31, ethyl acetate : hexane, 1:1) had been converted into the desired derivative **15** (Rf = 0.57). The reaction mixture was partitioned between dichloromethane (60 ml) and cold water (40 ml). The organic layer was washed once more in cold water (20 ml), dried ($MgSO_4$), and concentrated in vacuo to a brown residue which was then purified by silica chromatography (ethyl acetate : hexane, 1:1.5). The appropriate fractions were pooled and evaporated to give a pale brown solid. Drying of this solid over P_2O_5 gave **15** (520 mg, 52 %). m.p. 130-2°C, $[\alpha]_D$ = - 34°(C. 0.1, dichloromethane), Rf 0.57 (ethyl acetate : hexane, 1:1), ^{1}H-nmr ($CDCl_3$) δ: 7.000-7.450 (25H , arom), 5.957 (d 1H, J = 8.5 Hz, NH), 5.897 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10 Hz, H-2), 5.809 (1H, Hc allyl), 5.174 (1H, Hb allyl), 5.043 (1H, Ha allyl), 4.943 (1H, J_{gem} = 12 Hz, C_6H_5CHHO), 4.767-4.677 (2H, H-1 (δ 4.712 ppm), C_6H_5CHHO), 4.636-4.383 (7H, H-1, remaining C_6H_5CHHO), 4.206-4.046 (2H, Hd and He allyl), 4.003 (1H, H-4), 3.954-3.449 (11H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-3, H-5, H-6'a, H-6'b, OCH

HCH_2), 3.400 (m, 1H, $OCHHCH_2$), 1.883 (s, 3H, $NHCOCH_3$), 1.587-1.480 (m, 2H aliphatic), 1.346-1.183 (bs, 10H, remaining aliphatic methylenes), 0.874 (3H, CH_2CH_3). **Anal.** Calcd. for $C_{60}H_{73}NO_{12}S$: C 69.81, H 7.13, N 1.36, S 3.11. Found : C 69.60, H 7.10, N 1.16, S 3.19.

n-Octyl 2-acetamido-3-O-allyl-4-O-(3,4,6-tri-O-benzyl-2-deoxy-β-D-lyxo-hexopyranosyl)-2-deoxy-β-D-glucopyranoside.(16)

A clear solution of **15** (520 mg, 0.5 mmol) in dry toluene (200 ml) was refluxed under dry argon and a continuous stream of dry argon was bubbled through the refluxing solution for 0.5 h. The resulting solution was cooled to ambient temperature and treated with AIBN (50 mg) and tri-n-butyl stannane (6 equiv). The resulting solution was refluxed under argon for 24 hours. At the end of the reflux the thionocarbonate **15** (Rf 0.57, ethyl acetate : hexane, 1:1) had converted into the desired product **16** (Rf 0.48). The tlc analysis of the reaction mixture also indicated that two carbohydrate- containing by-products (Rf's 0.77 and 0.30) were formed, although in small (5%) amounts. The reaction mixture was then evaporated *in vacuo*, the residue was taken up in acetonitrile (100 ml) and washed with hexane (2x20 ml). The hexane extracts (top layers) contained no carbohydrate material. The acetonitrile extract was evaporated *in vacuo* to yield a syrup which was virtually free of the tin residue. Silica gel chromatography (ethyl acetate : hexane, 1:2) of the syrup gave **16** as a colourless oil (160 mg, 36 %), $[\alpha]_D$ = - 15^o (C. 0.21,

dichloromethane), Rf 0.49 (ethyl acetate : hexane, 1:1).^{1}H-nmr ($CDCl_3$) δ : 7.400-7.200 (20H, arom), 5.893-5.746 (m, 2H, Hcallyl, NH), 5.166 (1H, Hb allyl), 5.056 (1H, Ha allyl), 4.926 (d, 1H, Jgem=12Hz, C_6H_5CHH), 4.800 (d,1H, $J_{1,2}$=6.5Hz, H-1), 4.646-4.366 (8H, H-1and remaining $C_6H_5CH_2$), 4.256 (1H, Hd allyl), 4.056 (1H, He allyl), 3.929 (dd, 1H, H-3), 3.873-3.300 (12H, H-2, H-4, H-5, H-6a,b, H-3, H-4, H-5, H-6'a,b , O-CH2), 2.000-1.937 (m, 2H, H-2ax, H-2eq), 1.910 (3H, NHCOCH3), 1.346-1.183 (bs, 10H, remaining aglycon methylenes), 0.874 (3H, CH2CH3). **Anal**. Calcd. for $C_{53}H_{69}NO_{10}$ H_2O : C 70.87, H 7.87. Found : C 71.10, H 7.60.

n-Octyl 2-acetamido-6-O-benzyl-4-O-(3,4,6-tri-O-benzyl-2-deoxy-β-D-lyxo-hexopyranosyl)-2-deoxy-β-D-glucopyranoside (17)

A solution of **16** (150 mg, 0.17 mmol), tris (triphenyl-phosphine) rhodium(I) chloride (20 mg, 0.022 mmol), 1,8-diazabicyclo (2.2.2) octane (6 mg, 0.053 mmol) in ethanol-toluene-water (7:3:1, 15 ml) was refluxed for 18 hours. The reaction mixture had a Rf O.58 compound as a major product (ethyl acetate : hexane, 1:1). The solvent was removed and the residue was dissolved in acetone-water (9:1, 10 ml). To this solution were added mercuric chloride (230 mg, 5 equiv) and mercuric oxide (2 mg). The resulting mixture was stirred at ambient temperature for 8 hours. Following the evaporation of the solvent, the residue was partitioned between dichloromethane (40 ml) and aq. KCl (10 %, 20 ml). The organic phase was dried ($MgSO_4$) and evaporated to a brown solid which was subjected to

silica gel chromatography (ethyl acetate : hexane, 3 : 2). The title compound was obtained as a colourless syrup (110 mg, 77 %), $[\alpha]_D$ = - 18° (C 0.48, dichloromethane), Rf 0.15 (ethyl acetate : hexane, 1:1). ^{1}H-nmr ($CDCl_3$) δ : 7.420-7.200 (20H, arom), 5.576 (d, 1H, $J_{NH,2}$=8Hz, NH), 4.879 (d,1H, Jgem=12Hz, C_6H_5CHH), 4.812 (d,1H, $J_{1,2}$=8Hz, H-1), 4.738-4.279 (8H, H-1and remaining $C_6H_5CH_2$), 4.053-3.253 (13H, H-2, H-3, H-4, H-5, H-6a, b; H-3, H-4, H-5, H-6'a, b, O-CH2), 2.107-2.066 (1H, H-2eq), 1.966 (3H, $NCOCH_3$), 1.826-1.709 (1H, H-2ax), 1.613-1.500 (2H, OCH_2CH_2), 1.333-1.200 (10H, remaining aglycon methylenes), 0.866 (3H, CH_2CH_3). ^{13}C-nmr ($CDCl_3$) δ : 32.85 (C-2). **Anal.** Calcd. for $C_{50}H_{65}NO_{10}$. H_2O : C 67.54, H 7.87. Found : C 67.32, H 7.51.

n-Octyl 2-acetamido-2-deoxy-4-O-(2-deoxy-β-D-lyxo-hexopyranosyl) -β-D-glucopyranoside. (2)

Compound **17** (78 mg, 0.093 mmol) was dissolved in distilled methanol (45 ml) and then 5 % palladium-on-charcoal (400 mg) was added. The mixture was stirred under an atmosphere of hydrogen gas for 15 hours. The catalyst was removed by centrifugation. The catalyst was shaken with methanol (20x3) and the combined methanol solutions were evaporated to afford a colourless solid. Purification[33] on a C-18 cartridge gave **2** as a hygroscopic solid (41 mg, 94 %), $[\alpha]_D$ = + 15.3 (C. 0.3, methanol), Rf 0.74 (dichloromethane : methanol : water, 60:35:6). ^{1}H-nmr (CH_3OD : D_2O, 4 :1) δ : 4.266 (dd,1H, $J_{1,2ax}$=10Hz, $J_{1,2eq}$=2.5Hz, H-1), 4.400 (d,1H, J1,2=8Hz), 3.950-3.425 (11H, H-2, H-3, H-4, H-5, H-6a,b, H-3, H-4, H-5, 6'a,b,

OCH2), 2.00 (4H, H2eq., $NCOCH_3$), 1.775 (m,1H, 2ax),1.525 (2H, $OCHCH_2$),1.300(10H, remaining aglycon methylenes), 0.875 (3H, CH_2CH_3). ^{13}C-nmr (CD_3OD) δ : 102.71 (C-1), 77.93, 76.08, 72.19, 70.60, 62.82, 57.45 (C-2), 32.99, 30.67, 30.47, 27.12, 23.70, 22.99, 14.39 (CH_3 aglycon). Anal. Calcd. for $C_{22}H_{41}NO_{11}$. $4H_2O$: C 47.91, H 8.89, I ..54. Found : C 47.08, H 7.42, N 2.73.

n-Octyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl -β-D-galactopyranosyl)-6-O-benzyl-2-deoxy -β-D-glucopyranoside (18).

Compound **3** (181 ml , 0.19 mmol) was deallylated procedure as described for the preparation of **17**. The purification of the crude product by silica gel chromatography (ethyl acetate : hexane, 7:4) yielded the title compound as a colourless solid (161 ml , 91 %). m.p. 131 °C, $[\alpha]_D$ = + 0.89 °(C. 0.34, dichloromethane), Rf 0.56 (ethyl acetate : hexane, 7:3). ^{1}H-nmr ($CDCl_3$) δ : 7.400-7.120 (arom, 2O H), 5.593 (d, 1H, D_2O exchangeable, $J_{NH,2}$ = 8 Hz, NH), 5.326 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10 Hz, H-2), 4.900 (d, 1H, J_{gem} = 11 Hz, C_6H_5CHHO), 4.741-4.612 (2xd, 2H, H-1 (d 4.702, $J_{1,2}$ = 8.5 Hz) C_6H_5CHHO (J_{gem} = 12 Hz), 4.569-4.328 (8H, H-1 (δ 4.367), remaining C_6H_5CHHO, OH (D_2O exchangeable), 3.988-3.377 (13H, H-2, H-3 (δ 3.929, clean dd pattern on D_2O exch., J = 8 11 Hz) H-4, H-5, H-6a, H-6b, H-3, H-4, H-5, H-6'a, H-6'b, O-CHH-CH_2), 1.967 (m, 6H, $OCOCH_3$, $NCOCH_3$), 1.609-1.492 (m, 2H, OCH_2CH_2), 1.327-1.200 (m, 10H, remaining aglycone methylene protons), 0.867 (3H, $CH2CH_3$). Anal. Calcd. for. $C_{52}H_{67}NO_{11}$. $3H_2O$: C 65.59, H 7.72, N 1.47. Found : C 65.64, H 7.08, N 1.40.

n-Octyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl -β-D-galactopyranosyl)-6-O-benzyl-2-deoxy-3-O-phenoxythiocarbonyl-β-D-glucopyranoside (19)

To a solution of **18** (132 mg, 0.15 mmol) in dry dichloromethane (10 ml) at - 80°C was added n-BuLi (1.6 M in hexane, 0.09 ml , 0.14 mmol) and the resulting clear solution was stirred for 10 minutes at the same temperature. Phenoxychlorothionocarbonate (0.07 ml , 0.51 mmol) was added by syringe at - 80°C. The resulting solution was kept at - 80°C for 0.5 hours, (the tlc at this stage indicated about 10 % thionocarbonylated derivative). After that, the temperature was allowed to reach + 23°C over next 0.5 h. Further stirring at 23°C for 0.5 h resulted in the formation of the desired derivative as the only detectable carbohydrate-containing compound in the reaction mixture. A few drops of cold water were added to the reaction mixture and the resulting solution was partitioned between dichloromethane (40 ml) and water (10 ml). The organic layer was dried ($MgSO_4$) and evaporated to yield a yellow solid which was purified by silica gel chromatography (ethyl acetate : hexane, 1:2). The title compound was obtained as a pale yellow solid. (143 mg 94%) . $[\alpha]_D$ = - 14° (C. 0.55 dichloromethane). Rf 0.61 (ethyl acetate: hexane, 1:1). MS (EtOH/posFAB) m/z 1035 (MH^+). ^{1}H-nmr ($CDCl_3$) δ : 7.370-7.0 (25H, arom), 5.925 (d, 1H, $J_{NH,2}$ = 9 Hz, NH), 5.667 (1H, $J_{2,3} = J_{3,4}$ = 7.5 Hz, H-3), 5. 287 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 10Hz, H-2), 4.897 (d, 1H, J_{gem} = 11.5 Hz, C_6H_5CHHO), 4.7-4.264 (9H, H-1, (δ 4.525,

J1,2 = 8.5 Hz), H-1, (δ 4.379), remaining $C_6H_5CH_2O$), 4.203 (ddd, 1H, H-2), 4.1-3.371 (11H, H-4, H-5, H-6a, H-6b, H-3, H-4, H-5, H-6'a, H-6'b, O-CH_2-CH_2), 1.97 and 1.957 (2xS, 6H, $OCOCH_3$, $NCOCH_3$), 1.600-1.500 (bs, 2H, OCH_2CH_2), 1.30-1.20 (m, 10H, remaining aglycone methylene protons), 0.864 (3H, CH_2CH_3). ^{13}C-nmr ($CDCl_3$) δ : 195.35 (C=S). . **Anal.** Calcd. for $C_{59}H_{71}NO_{13}N$: C 68.51, H 6.92, N 1.35, S 3.10. Found : C 68.29, H 7.00, N 1.82, S 3.50 .

n-Octyl 2-acetamido-4-O-(2-O-acetyl-3,4,6-tri-O-benzyl-β-D-galactopyranopsyl)-6-O-benzyl-2,3-dideoxy-β-D-ribo-hexopyranoside (21)

Compound **19** (131 mg, 0.13 mmol) was subjected to the radical deoxygenation procedure as described for the preparation of **16**. Partitioning between acetonitrile and hexane followed by silica gel chromatography (ethyl acetate : hexane, 1:1) furnished an alcohol **18** (50 mg, 0.056 mmol, Rf 0.24, same solvent system), and the title compound as a syrup (38.4 mg, 61 %, based on the recovery of **18**), $[\alpha]_D$ = - 38.7° (C. 0.38, $CHCl_3$), Rf 0.51 (ethyl acetate : hexane, 1:1).^{1}H-nmr. ($CDCl_3$) δ : Characteristic resonances 5.363 (dd,1H, $J_{1,2}$=10Hz, $J_{2,3}$=8Hz, H-2), 2.175 (m,1H, H-3eq),1.800 (m,1H, H-3ax). ^{13}C-nmr ($CDCl_3$) δ : 33.21 (C-3). **Anal.** Calcd. for $C_{52}H_{67}NO_{11}$: C 70.8, H 7.66. Found : C 71.39, H 7.42.

n-Octyl 2-acetamido-2,3-deoxy-4-O-(β-D-galactopyranosyl) -β-D-ribo-hexopyranoside (1)

Compound **21** (29 mg , 0.033 mmol) was subjected to the Zemplen deacetylation procedure as described for the preparation of **14** to yield the alcohol **22** (Rf 0.39, ethyl acetate : hexane, 2:1) as a tlc-homogeneous syrup. The loss of the 2-O-acetate was confirmed by ^{1}H-nmr analysis on the syrup. Debenzylation of **22** was carried out as described for the preparation of **2**. Purification[33] of the product using C-18 cartridges furnished the title compound as a colourless solid (9.9 mg, 66 % from **22**). m.p. 198-204 ^{o} (dec), $[\alpha]_D$ = - 8.0^o (C. 0.8, methanol.). ^{1}H-nmr. (D_2O) δ . 4.456 and 4.426 (2 x d, 2H, J=11Hz and 10Hz, H-1, H-1), 3.953-3.400 (13H, H-2, H-4, H-5, H-6a, b, OCH2CH2, H-2, H-3, H-4, H-5, H-6'a, b), 2.456 (m, 1H, H-3eq),1.956 (s, 3H, $NCOCH_3$), 1.633 (m, 1H, H-3ax), 1.513 (2H, OCH2CH2), 1.290-1.200 (10H, remaining aglycone methylene), 0.825 (3H, CH_2CH_3). ^{13}C-nmr (D_2O) δ : 22 lines expected , 22 lines observed. 173.59 (carbonyl), 105.08 and 103.85 (C-1,C-1), 79.66, 76.20, 74.84, 74.10, 72.15, 70.85, 69.69, 61.90, 36.56, 32.46, 30.58, 30.31, 30.05, 29.88, 29.29, 26.55, 23.27, 22.92, 14.35. **Anal.** The compound was extremely hygroscopic and failed to furnish expected elemental composition.

n-Octyl 2-acetamido-2-deoxy-4-O-(β-D-galactopyranosyl) β-D-glucopyranoside (23)

A clear solution of **18** (55 mg , 0.061 mmol) and sodium methoxide (3 mg) in dry methanol (4 ml) was left aside for 30 h. The processing of the reaction mixture as described for the preparation of **14** gave the desired deacetylated derivative (54 mg, 98 %, Rf 0.35, ethyl acetate : hexane, 7:3) which was

subjected to hydrogenolytic debenzylation as described for the preparation of **2**. The title compound was obtained as a colourless solid after precipitation (0°C) from methanol / water (32 mg, quantitative yield). m.p : 230-6 °C, $[\alpha]_D$ = - 15.8 °(C. 0.82, methanol), Rf 0.58 ($CHCl_3$: methanol : H_2O, 60 : 35 : 6), ^{1}H-nmr (CD_3OD) δ : 4.387 and 4.366 (2xd, 2H, J = 8.99 Hz, and 6.99 Hz, H-1, and H-1), 3.933-3.351 (14H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2, H-3, H-4, H-5, H-6'a, H-6'b, OCH_2CH_2), 1.953 (3H, $NCOCH_3$), 1.580-1.474 (2H, $O\text{-}CH_2CH_2$), 1.370-1.209 (10H, remaining aglycone methylene protons), 0.886 (3H, CH_2CH_3). ^{13}C-nmr (methanol) : 22 lines expected, 22 lines observed. δ : 173.41(carbonyl) ,105.12 and 102.78 (C-1,C-1), 81.09, 77.17, 76.57, 74.87, 74.22, 72.63, 70.69, 70.34, 62.52, 62.03, 56.78, 33.02, 30.67, 30.49, 27.14, 23.73, 22.97,17.51,14.41. **Anal.** Calcd. for $C_{22}H_{41}NO_{11}.H_2O$: C 51.65, H 8.07. Found : C 51.59, H 8.21.

2-(2,3,4-tri-O-Acetyl-β-L-fucopyranosyl)-1,3,2-dithiaphosphorinane (89)

To a solution of **68**[68](150 mg, 0.52 mmol) in dry ether (4 ml) at 0°C were added N,N-diisopropylethylamine (0.1 ml , 0.6 mmol), followed by **84**[81a] (see Chapt. III B.2,150 mg, 1.7 equiv.) to afford a clear solution. Within a few minutes, a colourless crystalline mass separated out in the reaction mixture. Stirring of the reaction mixture at 0 °C for 2 h resulted in the complete conversion of the starting hemiacetal **68** (Rf 0.11, ethyl acetate : toluene, 1:4) into the desired product (Rf 0.42). Finally, stirring at 23 °C for 0.5 hours, followed by the partitioning of the

reaction mixture in chloroform (20 ml) and cold water (10 ml) gave a pale yellow organic extract. Evaporation of the solvent left a sticky solid (330 mg) which was purified by silica gel chromatography in ethyl acetate : toluene (1:4) to obtain the homogenous oil (216 mg, 97 %) which crystallized with ether and pentane. m.p. 80-3 ^{o}C. $[\alpha]_D$ = + 47.4 ^{o}(C. 0.35, $CHCl_3$). Rf 0.42, (ethyl acetate : Toluene, 1:4) . MS(CI NH_3) m/z 444 (100% M+NH_4). ^{1}H-nmr ($CDCl_3$) δ : 5.363 (dd, 1H, $J_{1,2}$=8Hz, $J_{2,3}$=11Hz, H-2), 5.266 (dd, 1H, $J_{3,4}$=4.5Hz, $J_{4,5}$=1Hz, H-4), 5.069 (dd, 1H, H-3), 4.945 (dd, 1H, $J_{H\text{-}1,P}$=11.5Hz, H-1), 3.893 (dq, 1H, $J_{5,6}$=6.5Hz, H-5), 3.506 (m, 1H, P-S-CH, Hax), 3.366 (m, 1H, P-S-CH, Hax), 2.629 (m, 2H, P-S-CH, Heq), 2.333 (m, 2H, P-S-CH2-CH2), 2.203, 2.076, 2.003 (3xS, 9H, COCH3), 1.259 (d, 3H, H-6). ^{13}C-nmr ($CDCl_3$) δ: 99.54 (d, $J_{C\text{-}1,P}$=20.4Hz, C-1). ^{31}P-nmr ($CDCl_3$) δ : + 154.2. **Anal.** Calcd. for $C_{15}H_{23}O_8PS$: C 42.25, H 5.44, S 15.04. Found: C 42.03, H 5.53, S 15.09.

Benzyl 2-O-benzoyl-3,4-di-O-isopropylidene-β-L-fucopyranoside (106)

To a clear solution of **101**[79](5.32 g, 20.9 mmol obtained by Zemplen deacetylation of **88**) in dichloromethane (80 ml) containing p-toluene sulfonic acid (50 mg) was added 2,2-dimethoxy propane (7.7 ml , 3 equiv.). The resulting solution was protected from moisture and stirred at 23 °C for 6 h. Evaporation of the solvent followed by holding of the resulting syrup under high vacuum (0.1 mm, 23 °C, 0.5 h) gave **105** as a brown oil (single spot tlc : Rf 0.68, ethyl acetate : hexane : EtOH, 10:10:1)

which was dissolved in dichloromethane (15 ml) containing N, N-dimethylaminopyridine (100 mg). To this solution at 23 ^{0}C were then added pyridine (5 ml , 60 mmol) and benzoyl chloride (6.1 ml , 2.5 equiv). After stirring for 10 h at 23 ^{o}C, the solvent was evaporated and the residue was partitioned between dichloromethane (40 ml) and cold water (20 ml). The organic layer was then successively washed with saturated $NaHCO_3$ (20 ml), water (20 ml), dried (MgSO4) and evaporated to a brownish mass, which was purified by silica gel chromatography in ethyl acetate : hexane (1:1). The fractions containing the material with Rf 0.5 were pooled and evaporated to afford the title compound as a crystalline solid (4.96 g, 60 % for two steps). m.p. 100-3 ^{o}C, $[\alpha]_D$ = - 6.2 ^{0}(C. 0.28, $CHCl_3$), Rf 0.64 (toluene : ether, 2:1). ^{1}H-nmr ($CDCl_3$) δ: 7.120-8.060 (arom, 10H), 5.306 (dd, 1H, $J_{1,2}$=8.5Hz, $J_{2,3}$=7.5Hz, H-2), 4.873 and 4.635 (2xd, 2H, J_{gem}=12Hz, $C_6H_5CH_2$), 4.458 (d, 1H, H-1), 4.251 (dd, 1H, $J_{3,4}$=5Hz, H-3), 4.058 (dd, 1H, $J_{4,5}$=2Hz, H-4), 3.894 (dq, 1H, $J_{5,6}$=6.5Hz, H-5), 1.646 and 1.357 (2xS, 6H, isopropylidene methyls), 1.489 (d, 3H, H-6). ^{13}C-nmr ($CDCl_3$) δ: 110.5 (quaternary carbon of isopropylidene), 28.1, 26.6 (isopropylidene methyls). **Anal.** Calcd. for $C_{23}H_{26}O_6$: C 69.33, H 6.58. Found : C 69.27, H 6.77.

Benzyl 2-O-benzoyl-β-L-fucopyranoside (107)

A solution of **106** (4.96, 12.45 mmol) in 60% aq. AcOH (160 ml) was stirred for 2 h in a water bath at 60^{o}C. The solvent was evaporated (15 mm/60^{o}C) and the residue was co-evaporated with toluene (3x50 ml) to afford the title compound as a

colourless solid (4.34 mg, 97%), m.p. 151-2°C, $[\alpha]_D$ = + 64 °(C. 0.2, $CHCl_3$), Rf 0.2 (ethyl acetate : hexane, 1:1). MS(CI NH_3) m/z 376 (30% M+NH_4). ^{1}H-nmr ($CDCl_3$, D_2O exchange) δ: 7.120-8.060 (arom, 10H), 5.238 (dd, 1H, $J_{1,2}$=8Hz, $J_{2,3}$=8.9Hz, H-2), 4.869 and 4.649 (2xd, 2H, J_{gem}=12.5Hz, $C_6H_5CH_2$), 4.522 (d, 1H, H-1), 3.770-3.669 (m, 2H, H-3 and H-4, on treatment with TCAI^{126}: δH-3=5.332, δH-4=5.390), 3.629 (q, 1H, $J_{5,6}$=6.5Hz, H-5), 1.400 (d, 3H, H-6). **Anal.** Calcd. for $C_{20}H_{22}O_6$: C 67.03, H 6.19. Found : C 66.47, H 6.18.

Benzyl 2-O-benzoyl-3,4-O-thionocarbonyl-β-L-fucopyranoside (118)

A bright yellow solution of thionocarbonyldiimidazole (766 mg, 4.3 mmol) and **107** (1.28 g, 3.58 mmol) in dry THF (20 ml) was refluxed under dry argon for 8 h. At the end of the reflux period all of **107** (Rf 0.09, ethyl acetate : hexane, 1:2) had been converted into the desired derivative **118** (Rf 0.27). The yellow residue obtained after the evaporation of solvent was partitioned between dichloromethane (40 ml) and cold water (20 ml). The organic layer was washed with cold water (2x20 ml), dried ($MgSO_4$), and evaporated to afford a yellow solid. The crystallization from dichloromethane : hexane, afforded the title compound as needles (1.099 g, 77%), m.p. 175 °C, $[\alpha]_D$ = + 0.39°(C. 0.23, dichloromethane) , Rf 0.46 (ethyl acetate : hexane, 1:1). MS(CI NH_3) m/z 419(100% M+1). ^{1}H-nmr ($CDCl_3$) δ : 7.975-7.250 (arom, 10H), 5.266 (dd, 1H, $J_{2,3}$=5Hz, $J_{3,4}$=4.8Hz, H-3), 5.103 (dd, 1H, $J_{1,2}$=8Hz, H-2), 4.890-4.787 (7 lines, 3H, H-1, H-4, C_6H_5CHH),

4.636 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.095 (dq, $J_{4,5}$=2Hz, $J_{5,6}$=6.5Hz, H-5), 1.529 (d, 3H, H-6). **Anal.** Calcd. for $C_{21}H_{20}O_6S$: C 62.98, H 5.03, S 8.01. Found : C 63.16, H 4.90, S 7.78.

Benzyl 2-O-benzoyl-3,4-O-methyledene-β-L-fucopyranoside (122)

A degassed solution of **118** (1.033 g, 2.5 mmol) in dry toluene (40 ml) was refluxed with tri-n-butyl stannane (3 ml , 10.3 mmol) and AIBN (50 mg). After 24 h. the reaction was evaporatd to dryness and the residue was purified by silica gel chromatography (ethyl acetate : hexane, 1:3) . The C-3 deoxy compound **112** was isolated (Rf 0.46, ethyl acetate : hexane, 1:1) as a colourless solid (240 mg, 27%). The evaporation of the fractions containing the title compound (Rf 0.7, ethyl acetate : hexane, 1:1) left a crystalline mass which was recrystallized in ethyl acetate : hexane to afford **122** as long colourless needles (420 mg, 44%), m.p. 135 °C, Rf 0.7 (ethyl acetate : hexane, 1:1). MS (CI NH_3) m/z 388 (50% M+NH_4). ^{1}H-nmr ($CDCl_3$) δ : 8.050-7.200 (arom, 10H), 5.319 (S, 1H, methylideneCHH), 5.217 (dd, 1H, $J_{1,2}$=8Hz, $J_{2,3}$=7Hz, H-2), 5.059 (s, 1H, methylideneCHH), 4.873 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.636 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.517(d, 1H, H-1), 4.333 (dd, 1H, $J_{3,4}$=5Hz, H-3), 3,933-3.833 (complex pattern, 2H, H-4, H-5), 1.507 (d, 3H, $J_{5,6}$=6.5Hz, H-6). ^{13}C-nmr ($CDCl_3$) δ : 98.64 (C-1), 95.24 (methylenedioxy carbon). **Anal.** Calcd. for $C_{21}H_{22}O_6$: C 68.11, H 5.95. Found : C 67.79, H 5.95.

Benzyl 2,3-di-O-benzoyl-β-L-fucopyranoside (108)

To a stirred solution of **107** (2.7 g, 7.53 mmol) in dichloromethane (150 ml) at -78°C were added pyridine (13.5 ml) and benzoyl chloride (1.65 ml , 1.2 equiv) in that order. The tlc analysis of the reaction mixture after 20 min. indicated > 95% conversion of **107** (Rf 0.2, ethyl acetate : hexane, 1:1) into **108** (Rf 0.6). Stirring at 23°C for 1 h., followed by partitioning of the reaction mixture in dichloromethane (40 ml) and cold water (20 ml) afforded a colourless organic layer. The organic extract was washed with cold HCl (0.1N, 20 ml) followed by cold saturated $NaHCO_3$ (20 ml). Drying ($MgSO_4$) and evaporation of the organic layer left a solid which was purified by silica gel chromatography (ethyl acetate : hexane, 0.8:1) to afford the title compound as a crystalline solid (3.07 g, 88 % yield); m.p. 109°C, $[\alpha]_D$ = - 53 °(C. 0.61, dichloromethane), Rf 0.6 (ethyl acetate : hexane, 1:1). MS (CI NH_3) m/z 480 (15% $M+NH_4$). ^{1}H-nmr ($CDCl_3$) δ : 8.060-7.120 (arom, 15H), 5.759 (dd, 1H, $J_{1,2}$=8Hz, $J_{2,3}$=10Hz, H-2), 5.226 (dd, 1H, $J_{3,4}$=2.9Hz, H-3), 4.918 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.699 (d, 1H, C_6H_5CHH), 4.668 (d, 1H, H-1), 4.076 (m, 1H, H-4, on treatment with TCAI: δ H-4=5.499), 3.839 (q, 1H, $J_{5,6}$=6.5Hz, H-5), 2.500 (d, 1H, $J_{4,OH}$=6Hz, 4 OH), 1.415 (d, 3H, H-6).

Benzyl 2-O-benzoyl-3-O-phenoxythionocarbonyl-β-L-fucopyranoside (109)

To a stirred solution of **107** (1.09 g, 2.89 mmol) in dichloromethane (50 ml) at - 78°C were added pyridine (1 ml) and phenoxychlorothionocarbonate (0.48 ml , 1.2 equiv). The tlc

analysis after 0.5 h. indicated that no reaction had taken place. The stirring of the reaction mixture at -10°C for 0.5 h. resulted in a slow formation of the title compound (Rf 0.6, ethyl acetate : hexane, 1:1) at the expense of the starting material **107** (Rf 0.2). Finally, stirring at 0 ° to + 2 °C for 0.5 h. resulted in a > 95 % conversion of **107** into the desired compound. The reaction mixture was partitioned between dichloromethane (40 ml) and cold water (20 ml). The organic layer was then dried ($MgSO_4$) and concentrated to a thick brown syrup. For the isolation of the product , a silica column (3x20 cm) was prepared in ethyl acetate : hexane, (1:1) solvent mixture. The syrup was adsorbed on to the silica column and the material was eluted quickly (silica contact time-10 min) with the same solvent mixture to provide the product (Rf 0.6). Evaporation of the appropriate fractions left **109** as a pale brown solid (960 mg, 68 %). m.p. 145-6 °C, $[\alpha]_D$ = + 27.8 ° (C. 0.21, dichloromethane) , Rf 0.6 (ethyl acetate : hexane, 1:1). ^{1}H-nmr ($CDCl_3$) δ : 8.037-6.854 (arom, 15H), 5.741(dd, $J_{1,2}$=7,5Hz, $J_{2,3}$ =10Hz, H-2), 5.646 (dd, $J_{3,4}$=3Hz, H-3), 4.911(d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.676 (d, 1H, C_6H_5CHH), 4.657 (d, 1H, H-1), 4.187 (ddd, $J_{4,5}$=1Hz, $J_{4,OH}$=7Hz, H-4:on treatment with TCAI: δH-4=5.607), 3.807 (dq, 1H, H-5), 2.309 (d, 1H, 4-OH), 1.446 (d, 3H, $J_{5,6}$=6.5Hz, H-6). **Anal**. Calcd. for $C_{27}H_{26}O_7S$. H_2O : C 63.30, H 5.51, S 6.26. Found: C 64.64, H 5.31, S 6.88.

Benzyl 2,3-di-O-benzoyl-4-O-phenoxythionocarbonyl-β-L-fucopyranoside (110)

To a stirred solution of **108** (4.0 g, 8.65 mmol) in dichloromethane (180 ml) at 23°C, were added pyridine (15 ml , 20 equiv) followed by phenoxychlorothionocarbonate (4.5 ml , 4 equiv.) to obtain a green reaction mixture. The addition of the reagents was slightly exothermic. The resulting reaction mixture was stirred at 23°C for 24 h. and then extracted with cold water. Drying ($MgSO_4$) and concentration of the organic layer left a brown syrup which was quickly (silica contact time 0.5 h.) passed through a short (2x20 cm) silica column (ethyl acetate : hexane, 1:3) to isolate the compound with Rf 0.45 (ethyl acetate : hexane, 1:2). Evaporation of the appropriate fractions left **110** as a brown solid (4.34 g, 84 %), m.p. 142-7 °C, Rf 0.45 (ethyl acetate : hexane, 1:2). ^{1}H-nmr ($CDCl_3$) δ : 7.985-6.955 (arom, 20H), 6.107 (dd, 1H, $J_{3,4}$=3.5Hz, $J_{4,5}$=1Hz, H-4), 5.809 (dd, 1H, $J_{1,2}$=10Hz, $J_{2,3}$=7Hz, H-2), 5.497 (dd, 1H, H-3), 4.918 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.755 (d, 1H, H-1), 4.704 (d, 3H, C_6H_5CHH), 4.048 (dq, 1H, $J_{5,6}$=6.5Hz, H-5), 1.440 (d, 3H, H-6). **Anal.** Calcd. for $C_{34}H_{30}O_8S$: C 68.21, H 5.05. Found : C 68.20, H 5.11.

Benzyl 2,3-di-O-benzoyl-4,6-dideoxy-β-L xylohexopyranoside (111)

The compound **110** (180 mg, 0.3 mmol) was subjected to the radical deoxygenation as described for the preparation of **16** . Evaporation of the toluene followed by the silica chromatography of the residue in ethyl acetate : hexane(1:2) gave the title compound as a colourless oil which solidified with hexane. (69.8 mg, 52 %), m.p. 98°C, $[\alpha]_D$ = - 50°, (C. 0.68, dichloromethane) , Rf

O.59, (ethyl acetate : hexane, 1:2). ^{1}H-nmr ($CDCl_3$) δ : 8.000-7.110 (arom, 15H), 5.439 (dd, 1H, $J_{1,2}$=8Hz, $J_{2,3}$=10Hz, H-2), 5.273 (ddd, 1H, $J_{3,4ax}$=11Hz, $J_{3,4eq}$=5Hz, H-3), 4.900 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.679 (d, 1H, C_6H_5CHH), 4.639 (d, 1H, H-1), 3.766 (m, 1H, H-5), 2.339 (ddd, J_{gem}=12Hz, $J_{3,4eq}$=5Hz, $J_{4eq,5}$=2Hz, H-4eq), 1.700 (pseudo q, H-4ax), 1.363 (d, 3H, $J_{5,6}$=6.5Hz, H-6). ^{13}C-nmr ($CDCl_3$) δ : 38.07 (C-4). **Anal**. Calcd. for $C_{27}H_{26}O_6$: C 72.63, H 5.87. Found : C 72.51, H 5.68.

Benzyl 2-O-benzoyl-3,6-dideoxy-β-L-xylohexopyranoside (112)

The compound **109** (960 mg, 1.94 mmol) was subjected to the radical deoxygenation procedure as described for the preparation of **16** . The tlc analysis of the reaction mixture indicated that diol **107** was also formed (20 %) along with the desired compound **112** (Rf 0.45, ethyl acetate : hexane, 1:1). Evaporation of the solvent followed by CH_3CN : hexane work-up afforded a syrup which solidified at 0.1 mm, 23°C, 24 h. The resulting solid was purified by silica gel chromatography (ethyl acetate : hexane, 2 : 3) to provide the title compound as colourless needles (340 mg, 51 %), m.p. 128°C, $[\alpha]_D$ = + 98°, (C. 0.93, dichloromethane). MS (CI NH_3) m/z 360 (96% $M+NH_4$). ^{1}H-nmr ($CDCl_3$) δ : 8.100-7.199 (arom, 10H), 5.292 (ddd, 1H, H-2), 4.900 (d, 1H, J_{gem}=12.5Hz, C_6H_5CHH), 4.694 (d, 1H, C_6H_5CHH), 4.619 (d, 1H, $J_{1,2}$=7.5Hz), 3.746 (m, 2H, H-4, H-5: on treatment with TCAI δH-4 =5.109), 2.523 (ddd, 1H, J_{gem}=13Hz, $J_{2,3eq}$=5Hz, $J_{3eq,4}$=3Hz, H-3eq), 2.269 (d, 1H, $J_{4,OH}$=8Hz, 4 OH), 1.736 (ddd,

$J_{2,3ax}$=12Hz, $J_{3ax,4}$=3Hz, H-3ax), 1.343 (d, 3H, $J_{5,6}$=6.5Hz, H-6). **Anal.** Calcd. for $C_{20}H_{22}O_5$: C 70.16, H 6.48. Found : C 69.74, H 6.33.

Benzyl 4-O-acetyl-2-O-benzoyl-3,6-dideoxy-β-L-xylohexopyranoside (113)

The compound **112** (1.45 g, 4.24 mmol) was dissolved in pyridine (20 ml) and acetic anhydride (10 ml). After 24 h. at 23°C, the reaction mixture was poured onto crushed ice and water (50 ml). The resulting sticky solid was taken up in dichloromethane (40 ml). The organic layer was washed with water (20 ml) and saturated $NaHCO_3$ (2x30 ml). Drying ($MgSO_4$) and concentration of the solvent left a syrup which was purified by silica gel chromatography in ethyl acetate : hexane (1:2) to afford the title compound as a colourless solid (1.3 g, 80 %), m.p. 68 °c, $[\alpha]_D$ = + 89 ° (C. 0.36, dichloromethane). ^{1}H-nmr ($CDCl_3$) δ : 7.825-7.175 (arom, 10H), 5.276 (ddd, 1H, H-2), 5.066 (m, 1H, H-4), 4.924 (d, 1H, J_{gem}=13Hz, C_6H_5CHH), 4.717 (d, 1H, C_6H_5CHH), 4.656 (d, 1H, $J_{1,2}$=8Hz, H-1), 3.822 (dq, 1H, $J_{4,5}$=1Hz, $J_{5,6}$=6.5Hz, H-5), 2.475 (ddd, 1H, J_{gem}=13Hz, $J_{2,3eq}$=5Hz, $J_{3eq,4}$=3Hz, H-3eq), 2.177 (S, 3H, OAc), 1.800 (ddd, $J_{2,3ax}$=12Hz, $J_{3ax,4}$=3Hz, H-3ax), 1.267 (d, 3H, H-6). **Anal.** Calcd. for $C_{22}H_{24}O_6$: C 68.73, H 6.29. Found : C 68.79, H 6.27.

2,3-di-O-Benzoyl-4,6-dideoxy-L-xylohexopyranose (114)

Compound **111** (1.1 g, 2.47 mmol) was dissolved in dry methanol (50 ml) containing Pd/C (5 %, 1.8 g) and the resulting

solution was stirred under H_2 (1 atm.) for 12 h. The hydrogenation mixture was analyzed by tlc to ensure the complete conversion of **111** into the desired reducing sugar **114** (Rf 0.43 and 0.35, α/β mixture, ethyl acetate : hexane, 1:2) The catalyst was filtered and washed with methanol (5x20 ml) *until the washings were completely devoid of any U. V. active material.* The combined filtrate was evaporated and the residue was purified on silica (ethyl acetate : hexane, 1:2) to isolate Rf 0.43 and 0.35 material. The above material was combined and crystallized with ethyl acetate : hexane to afford **113** as a microcrystalline solid. (790 mg, 90 %), m.p. 168°C, $[\alpha]_D$ = - 135° (C. 0.28, methanol, 24 h.). ^{1}H-nmr (200MHz, $CDCl_3$) δ : 5.620 (m, H-1: α anomer), 4.860 (m, H-1: β anomer) : α/β ratio=2.3/1. **Anal**. Calcd. for $C_{20}H_{20}O_6$: C 67.40, H 5.65. Found : C 66.85, H 5.65.

4-O-Acetyl-2-O-benzoyl-3,6-dideoxy-L-xylohexopyranose (115)

Compound **113** (930 mg, 2.42 mmol) was dissolved in ethanol (20 ml) containing Pd/C (5%, 650 mg) and stirred under H_2 (1 atm.) for 24 h. The catalyst was filtered and washed with ethanol (2x40 ml). The combined filtrate was evaporated to dryness to afford the title compound as a syrup (710 mg, 98 %), $[\alpha]_D$ = - 1.6° (C 1.4, methanol, 24 h). ^{1}H-nmr ($CDCl_3$) δ : 5.519 (pseudo triplet, $J_{1,2} = J_{1,OH}$ = 3Hz, H-1:αanomer), 4.793 (pseudo triplet, $J_{1,2}=J_{1,OH}$ = 8Hz, H-1 β anomer) : α/β ratio =1. **Anal**. Calcd. for $C_{15}H_{18}O_6 . H_2O$: C 57.69, H 6.46. Found : C 57.33, H 5.73.

2,3-di-O-Benzoyl-4,6-dideoxy-α-L-xylohexopyranosyl bromide (116)

The compound **114** (125 mg, 0.35 mmol) was acetylated as described for the preparation of **113**. The identity of the 1-O-acetate was confirmed by ^{1}H-nmr in $CDCl_3$: δ 6.401 (d, $J_{1,2}$=4Hz, H-1: α anomer), 5.913 (d, $J_{1,2}$=8Hz, H-1: β anomer): β/α ratio = 3.6 : 1]

The syrupy 1-O-acetate (Rf 0.56, ethyl acetate : hexane, 1:3) was dissolved in dichloromethane (30 ml) and treated with HBr (in acetic acid, 30%, 5.5 ml) containing acetic anhydride (1.0 ml). The resulting pale brown solution was stirred protected from moisture (23^{o}C, 0.5 h). Toward the end of the stirring period tlc analysis of the reaction mixture indicated the presence of the title compound (Rf 0.71, ethyl acetate : hexane, 1:3) as the major product. The reaction mixture was then partitioned between dichloromethane (30 ml) and cold saturated $NaHCO_3$ (20 ml). The organic layer was quickly separated and washed with ice-cold water (2x20 ml). The final aq. extract had neutral pH. The organic layer was dried ($MgSO_4$) and evaporated (15 min, 23^{o}C) to afford the title compound as a syrup. The purity (>80% pure) of **116** was confirmed by the tlc analysis (Rf 0.71, ethyl acetate : hexane, 1:3, a rapid development of the silica tlc-plate was necessary to obtain good results) and it was immediately used for anomeric phosphorylation (i.e. the preparation of **94** *vide infra*) .

4-O-Acetyl-2-O-benzoyl-3,6-dideoxy-α-L-xylopyranosyl bromide (117)

The compound **115** (568 mg, 1.9 mmol) was dissolved in dichloromethane (10 ml) containing DMF (2 ml) and the resulting solution was stirred at -10°C. Into this solution was added oxalyl bromide (1.5 equiv.) at the same temperature. The stirring at 23 °C for 1 h. resulted in near-complete conversion of **115** into the title compound. (Rf 0.78, ethyl acetate : hexane, 1:1). The tlc analysis of the reaction mixture indicated > 80 % of **117** along with hydrolysis products with lower mobility.

Tetra-n-butylammonium dibenzyl phosphate (73)

(Procedure for the preparation of 16.2 mmol of **73**)

A suspension of dibenzyl hydrogen phosphate (4.37 g, 15.7 mmol) in milli-Q-water (50 ml) was stirred at 23 °C. Tetra-n-butyl ammonium hydroxide (approx. 40% aq w/w, 8.42 g) was added dropwise to the suspension to obtain a clear solution. At this stage some lumpy solid was present in the solution. Filtration through Whatman filter paper gave a clear filtrate (pH 8-9). To this was added dibenzyl hydrogen phosphate in small portions untill the pH decreased to 7 (at this stage the solution contained a total of 4.5 g of phosphate, 16.2 mmol). The resulting clear solution was lyophilized to afford **73** as a colourless syrup.

Since commercially available (Sigma) tetra-n-butyl ammonium hydroxide solution tends to solidify on storage, it was essential to warm the container to obtain a homogenous solution. The above commercial preparation should be freshly titrated with aq. potassium phthalate prior to use. (Indicator : *Phenol red*)

L-Fucose-1-phosphate (78)

To a clear solution of fucosyl bromide **70** (freshly prepared[68] from 1.2 g, 3.6 mmol of L-fucose tetraacetate) in dry DMF (3 ml) was added **73** (6 equiv., in dry DMF : 4 ml) to obtain a pale yellow solution. After 10 min. at 23 °C the reaction mixture was partitioned between dichloromethane (30 ml) and cold water (10 ml). The organic layer was washed once more with cold water (10 ml), dried ($MgSO_4$) and evaporated to a syrup. A silica gel column (2.5x20 cm.) packed in dichloromethane : ethyl acetate (1:1) was prepared and the syrup was placed on top of it. Fast elution in the same solvent system afforded a material with Rf 0.8 : the intermediate triester (**75**, scheme 14, R = CH_3). The fractions containing Rf 0.8 material were evaporated (15 mm, 30 °C) to afford a syrup which was not characterized but stirred in ethanol (40 ml) containing aq. $NaHCO_3$ (1 N, 10 ml) and Pd/C (5 %, 300 mg) under an atmosphere of hydrogen. After 2 h at 23 °C (tlc control)the material with Rf 0.8 (dichloromethane : ethyl acetate, 1:1) which absorbed in U. V. had disappeared to give a baseline carbohydrate material . The solids were removed by filtration and the filtrate was concentrated to a syrup which was partitioned between dichloromethane (20 ml) and water (20 ml). The aq. layer was separated and aq. NaOH (1 N, 20 ml) was added. After 0.5 h. at 23 °C the pH was carefully adjusted to 7 with cold AcOH (1 N). The resulting solution was diluted to about 200 ml with water and slowly adsorbed onto a resin bed of Dowex 2 (2.5x10 cm., Cl^-). The column was washed with water (100 ml) and then developed

with a linear gradient[76] of LiCl. The mixing chamber contained 250 ml of water and the reservoir contained 250 ml of 0.45 M LiCl. The column was run at 23 °C at the flow rate of about 120 ml /h and the fractions (4 ml) were collected automatically. The eluate was monitored for the presence of acid-labile-phosphate [H_2SO_4 spraying was used for the detection of the *carbohydrate material* and ammonium molybdate spray was used to detect the *phosphate*[49] . Spotting of the eluate fractions on silica coated plate, then spraying with the above reagents followed by heating on the hot plate resulted in the following positive tests : carbohydrate containing compounds gave a dark yellow brown spot, while the phosphates (organic or inorganic) gave blue spots]. The appropriate fractions containing L-fucose-1-phosphates were pooled and lyophilized to a dry solid. The material was taken in screw-cap centrifuge tubes and shaken (exothermic !) carefully with ethyl ether : ethanol, 2:1. Centrifugation of the resulting suspension afforded clear supernatents which were discarded. The residues were extracted several times with the same solvent system (the supernatents were tested with aq. $AgNO_3$ solution for the presence of Cl^- ions). Residual solids were dried over P_2O_5 and the L-fucose-1-phosphate content was assessed by ^1H-nmr using trimethylsilyl propionate (M.W. 172.28 g/mole, P_2O_5 dried) as the internal standard (**78**,1.8 mmol, 50%, eq./ax.ratio = 12.5/1). The ^1H and ^{13}C-nmr spectral characteristics of **78** were in accord with those in the literature[68]. ^1H-nmr (D_2O, TSP-d4 as int. ref. std.) δ : 4.830 (pseudo triplet. $J_{1,2} = J_{1,P} = 8Hz$. H-1), 3.819 (dq, H-5), 3.759 (dd,

$J_{3,4}$ = 3.5Hz $J_{4,5}$ = 1Hz, H-4), 3.700 (dd, $J_{2,3}$ = 10Hz, H-3), 3.501 (dd, H-2), 1.253 (d, $J_{5,6}$ = 6.5Hz), ^{31}P-nmr (D_2O) d : - 0.86 (s), MS (glycerol/neg FAB) m/z 243 (**78**-dianion minus H). This bis Li salt was dissolved in water and passed through a resin bed of Dowex 50-X8 (NEt_3H^+) to obtain the triethyl ammonium salt. Evaporation of the aq. eluate afforded the monotriethyl ammonium salt which was dried over P_2O_5 prior to its use in the pyrophosphate coupling reaction for the synthesis of GDP-fucose.

D-Arabinose-1-phosphate (79)

Freshly prepared[75] crystalline 2,3,4-tri-O-acetyl-β-**D**-arabinopyranosyl bromide (**74**, 1.0 g, 2.9 mmol, δ ($CDCl_3$) : 6.710 (d, $J_{1,2}$=3.7Hz, H-1) was treated with **73** (6 equiv.) in dry DMF (7 ml). After 10 min. at 23 °C, all bromosugar (**74**, Rf 0.7, ethyl acetate : hexane, 1:1) had been converted into slower moving U. V. absorbing phosphotriester [Rf 0.3, (major),0.32 (minor)]. The reaction mixture was partitioned between dichloromethane (40 ml) and cold water (20 ml). The organic layer was dried ($MgSO_4$) and evaporated to a syrup which was purified by fast elution through a silica gel column (2x20 cm, ethyl acetate : hexane, 3: 2) to the material with Rf 0.3 (ethyl acetate : hexane, 1:1) Evaporation of the appropriate fractions left a syrup which was dissolved in ethanol (40 ml) containing aq. $NaHCO_3$ (1N, 10 ml) and Pd/C (5%, 300 mg) and the resulting solution was stirred under an atmosphere of H_2 for 2 h at 23°C then the solids were removed by filtration. The filtrate was evaporated to a syrup which was partitioned between dichloromethane (20 ml) and

water (30 ml). The aq. layer was separated and aq. NaOH (1N, 20 ml) was added to it. After 0.5 h. at 23 ^{o}C the pH was adjusted to 7 with cold AcOH (1N). The resulting solution was diluted to the total volume of 200 ml with water. Ion exchange chromatography (as described for the preparation of **78**) followed by lyophilization of the fractions containing acid-labile-phosphate afforded the lithium salt of **79** [1.35 mmol, 47%, eq/ax ratio = 10.7/1]. The optical rotation was recorded on a desalted sample of **79** [cyclohexylammonium salt, 99% : α (equatorial) anomer : ^{1}H-nmr analysis] $[\alpha]_D$ - 31.9^{0} (C. 0.14, H_2O) (Lit.[75] - 39.1^{0}). MS (glycerol/negFAB) m/z 229 (**79**-dianion minus H). ^{1}H-nmr (D_2O, TSP-d4 as int. ref. std.) δ : 5.456 (q, $J_{1,2}$=3.6Hz, $J_{1,P}$=7Hz, H-1:axial (β)anomer), 4.800 (pseudo triplet, $J_{1,2} = J_{1,P}$ = 7.5Hz, H-1:equatorial (α) anomer), 3.973-3.636 (m, H-3, H-4, H-5s), 3.586 (dd, H-2, $J_{2,3}$ = 9Hz). ^{13}C-nmr (D_2O) δ : 97.69 (d, $J_{C-1,P}$ = 4.2Hz, C-1), 72.26 (C-3), 72.01(d, $J_{C-2,P}$ = 5.8Hz, C-2), 69.19 (C-4), 66.15 (C-5).

3,6-Dideoxy-L-xylohexopyranose-1-phosphate (3-deoxy-L- Phosphate , 93)

bromosugar **117** (from 1.9 mmol of **115**) was prepared in situ (Rf 0.78, ethyl acetate : hexane, 1:1) and treated with **73** (6 equiv., in dry DMF, 10 ml). Stirring at 23 ^{o}C for 4 h. resulted in a reaction mixture which on tlc analysis showed the presence of a new spot (phosphotriester, Rf 0.47, ethyl acetate : hexane, 1:1). A spot corresponding to the bromosugar **117** persisted. The reaction was terminated at this stage by partitioning the

reaction mixture between dichloromethane (40 ml) and water (20 ml). The organic layer was dried ($MgSO_4$) and evaporated to a syrup which was purified by the fast passage through a short silica gel column (2x20 cm, ethyl acetate : hexane, 13 :10). The fractions containing Rf 0.47 (ethyl acetate : hexane, 1:1) material were pooled and evaporated to a syrup which was then dissolved in ethanol (40 ml) containing aq $NaHCO_3$ (1N, 20 ml) and Pd/C (5%, 1.2 g). The resulting solution was stirred under H_2 atmosphere for 12 h. at 23 ^oC (tlc control). Removal of solids by filtration and evaporation of the filtrate left a syrup which was then dissolved in water (10 ml) and aq. NaOH (1N, 10 ml) was added to obtain pH=11. After 0.5 h. at 23 ^oC, the pH was adjusted to 7 with cold AcOH (1N). Dilution of the resulting solution to 200 ml volume with water followed by the ion exchange chromatography (as described for the preparation of **78**) afforded the title compound **93** as a bislithium salt (0.266 mmol, 14%, eq/ax ratio = 5.4/1). The optical rotation was recorded on a desalted (Bio-Gel-P2) sample of **93** (monotriethyl ammonium salt, β/α ratio: 5.4/1: ^{1}H-nmr analysis), $[\alpha]_D$ - 28.5^o (C. 0.31, H_2O). MS (glycerol/negFAB) m/z 227 (**93**-monoanion). ^{1}H-nmr (D_2O, acetonitrile: int. ref. std. δ = 2.049, 30^{o}C, β anomer) δ : 4.833 (pseudotriplet, $J_{1,2} = J_{1,P} = 7.5$Hz, H-1), 3.863 (dq, $J_{4,5} = 1$Hz, $J_{5,6} = 6.5$Hz, H-5), 3.484 (m, H-4), 3.690 (ddd, H-2), 2.190 (ddd, $J_{gem} = 13$Hz, $J_{2,3eq} = 5$Hz, $J_{3eq,4} = 4$Hz, H-3eq), 1.737 (ddd, $J_{2,3ax} = 12$Hz, $J_{3ax,4} = 3$Hz, H-3ax), 1.223 (d, H-6). ^{13}C-nmr (D_2O) δ : 100.17 (d, $J_{C-1,P} = 3.5$Hz, C-1), 75.23, 69.02 (C-5,C-4), 67.74 (d,

$J_{C-2,P}$=3.5Hz, C-2), 37.37 (C-3), 16.53 (C-6). ^{31}P-nmr (D_2O) δ : + 2.5.

4,6-Dideoxy-L-xylohexopyranose-1-phosphate (4-deoxy-L-fucose phosphate , 94)

The freshly prepared syrupy bromosugar **116** (from 0.35 mmol of **114**) was treated with **73** (6 equiv. in dry DMF: 30 ml). Stirring at 23^{0}C for 2h resulted in a reaction mixture which on tlc analysis showed the presence of a new spot(phosphotriester, Rf 0.16 ethyl acetate: hexane, 1:3). The spot corresponding to bromosugar **116** had disappeared. The reaction mixture was then partitioned between cold water (20 ml) and dichloromethane (40 ml). The organic layer was once washed with cold water (20 ml) dried ($MgSO_4$) and concentrated to a syrup which was passed through a short column (2x20 cm) of silica gel in ethyl acetate: hexane (1:1) to provide material with Rf 0.5 (same solvent system). Evaporation of the appropriate fractions gave a syrup which was taken up in methanol (30 ml) containing aq. $NaHCO_3$ (1 N, 5 ml). Pd/C (5%, 200 mg) was added and the resulting suspension was stirred under an atmosphere of H_2 for 12 h at 23^{o}C. Removal of the solids by filtration and evaporation of the filtrate left a syrup which was dissolved in water (30 ml) and aq. NaOH (1N, approx. 5 ml) was added to obtain pH 11. After 0.5 h at 23^{o}C, pH was adjusted to 7 with cold AcOH (1 N). Dilution of the resulting solution to 120 ml, followed by ion exchange chromatography (as described for the preparation of **78**) afforded the title compound **94** as a bislithium salt (0.053 mmol, 15%, eq/ax ratio : 1.7:1) . The optical rotation was recorded on a

desalted (Bio-Gel) sample of **94** (monotriethyl ammonium salt, β/α ratio : 1.7:1 : ^{1}H-nmr analysis) $[\alpha]_D$ - 26^0 (C. 0.68, methanol). ^{1}H-nmr (D_2O, acetonitrile as int. ref. std. δ = 2.049, 30^{0}C, β anomer) δ : 4.808 (pseudo triplet, $J_{1,2}$=7.5Hz, H-1), 3.800-3.636 (m, H-3, H-5), 3.174 (dd, $J_{2,3}$ = 7Hz, H-2), 1.977 (ddd, J_{gem} = 13Hz, $J_{3,4eq}$ = 5Hz, $J_{4eq,5}$ = 2Hz, H-4eq), 1.363 (ddd, H-4ax), 1.217 (d, $J_{5,6}$ = 6.5Hz, H-6). ^{13}C-nmr (D_2O, α/β mix.) δ : 98.29 (d, $J_{C-1,P}$=4.4Hz, C-1 β anomer), 95.79 (d, $J_{C-1,P}$=5.6Hz, C-1 α anomer), 76.90 (d, $J_{C-2,P}$ = 5.8Hz, C-2 β), 74.41 (d, $J_{C-2,P}$ = 7.5Hz, C-2 α), 70.61, 69.85, 67.40, 66.42 (C-3,C-5 α and β anomers), 40.74 (C-4 α), 40.58 (C-4 β), 24.05 (C-6 α), 20.72 (C-6 β).

Guanosine-5-phosphoric-di-n-butylphosphinothioic anhydride , tributylammonium salt.(131)

Guanosine-5-monophosphate disodium salt,trihydrate (26.3 mmol) was dissolved in water (1.5 l.) and the pH of the solution was adjusted to approximately 2.0 with Dowex-50 (H^+). The resin was removed by filtration and washed with water. The filtrate and the washings were passed through a Dowex 50 (H^+) column (5x30 cm) . The eluate and acidic column washings were pooled and pyridine (150 ml) was added. The resulting solution was evaporated to a small volume to which tri-n-butyl amine (4 equiv) and tri-n-octyl amine (1 equiv) were added. The resulting emulsion was carefully concentrated to a solid which was dissolved in dry pyridine (40 ml) and pyridine was then evaporated in vacuo. Dry pyridine (20 ml x 3)was then added and

evaporated from the sample. Finally, the preparation was dissolved in dry pyridine (400 ml) and concentrated to about 200 ml volume.

Freshly prepared di-n-butyl phosphinothioyl bromide[117] (2 equiv.) was added into the above solution and the resulting solution was stirred at 23 ^{o}C for 10 h. At the end of the stirring , silica gel tlc analysis indicated the presence of a major new spot Rf 0.65 (iPrOH : conc.NH_4OH : H_2O, 7:1:2). The reaction mixture was then filtered to remove the insoluble material. The clear filtrate was evaporated to dryness and resulting material was coevaporated with dry toluene (3x50 ml) and then the residue was partitioned between water (400 ml) and diethyl ether (50 ml). The aq. layer was concentrated to 150 ml and stored at 4 ^{o}C for 12 h to obtain a crystalline material. Filtration, followed by washing the crystals with cold water (5 ml) afforded the title compound as its tri-n-butylammonium salt monohydrate. Drying over P_2O_5 (23^{o}C., 12 h., 0.01 mm) gave a crystalline mass (5.45 g, 28%), m.p 125-130^{o}, $[\alpha]_D$ = -18^{o} (C. 0.18, dry pyridine). Rf 0.65 (iPrOH : conc.NH_4OH : H_2O, 7:1:2). MS (glycerol/negFAB) m/z 539 (anion moiety of **131**) ^{1}H-nmr (pyridine-d5 : D_2O 1.5:1 , TSPd4: int.std.) δ : 7.785 (S, 1H, H-8base), 6.323 (d, 1H, $J_{1,2}$=6Hz, H-1ribose), 5.192 (dd, 1H, H-2ribose), 5.033 (m, 1H, H-3ribose), 4.750 (m, 3H, H-4, H-5a,bribose), 3.250 (m, 6H, +NC**H2**CH2), 2.603-2.283 (m, 4H, PCH_2), 1.806 (m, 10H, methylenes), 1.497 (m, 10H, methylenes), 1.066 (m, 9H, CH_2CH_3 : cation), 0.916 (m, 6H, CH_2CH_3 : anion). ^{13}C-nmr (pyridine-d5) δ : 89.42 (C-1ribose), 84.73 (d, $J_{C-4,P}$=8.3Hz, C-4ribose). ^{31}P-nmr (pyridine-d5), - 9.5

(d, $^2J_{P,P}$ = 32Hz, P=O), + 96.2 (d, $^2J_{P,P}$ = 32Hz, P=S). **Anal.** Calcd. for $C_{30}H_{56}N_6O_8P_2S$. H_2O : C 48.64, H 7.89, N 11.34, S 4.33. Found : C 48.11, H 7.77, N 11.03, S 4.6.

Tetra-n-butyl ammonium, Guanosine-5-diphosphate (132)

Guanosine-5-diphosphate; (disodium salt) was dissolved in water (100 ml water for 1 g of salt) and stirred at 8-10 °C. The solution was adjusted to pH 3 with Dowex 50 (H^+). The resin was quickly filtered and the filtrate was adjusted to pH 7.8 with tetra-n-butyl ammonium hydroxide solution. The resulting clear solution on lyophilization afforded **132** as a colourless, hygroscopic solid. Tlc analysis (silica, NH_4OAc 0.3 M : iPrOH, 1:1, Rf = 0.1) on this solid indicated it to be identical with the authentic sample of GDP. ^{1}H-nmr (D_2O) δ : 8.100 (S, 1H, H-8base), 5.900 (d, 1H, $J_{1,2}$ = 5.8Hz, H-1ribose), 4.738 (dd, 1H, $J_{2,3}$ = 4.7Hz, H-2ribose), 4.607 (dd, 1H, $J_{3,4}$ = 4.3Hz, H-3ribose), 4.300 (bs, 1H, H-4ribose), 4.267-4.091 (m, 2H, H-5a,b ribose), 3.165 (m, 24H, +NC**H**$_2$CH$_2$), 1.623 (m, 24H, +NCH$_2$C**H**$_2$), 1.333 (m, 24H, C**H**$_2$CH$_3$), 0.923 (m, 36H, CH$_2$C**H**$_3$),Ratio of nucleotide: cation =1:3.

3,4-Di-O-acetyl-1,2-O-(methoxyethylidene)-α-L-fucopyranose (133)

2,3,4-tri-O-acetyl-α-L-fucosyl bromide **70** (prepared[68] from 15 mmol of L-fucose tetraacetate) was stirred in dry nitromethane (50 ml) containing molecular sieves (4 A, 4.0 g) and tetraethyl ammonium bromide (2.3 g, 11 mmol). To this suspension at 23°C were added collidine (4.5 ml , 33 mmol)

followed by methanol (2.5 ml , 50 mmol) by syringe and the resulting yellow reaction mixture was stirred at 23 ^{o}C for 24 h. (The product orthoester had a mobility similar to the bromosugar **70** on silica tlc.). The Solids were removed by filtration and the filtrate was partitioned between dichloromethane (300 ml) and cold H_20 (100 ml). Organic layer was washed once with cold HCl (o.1 N, 20 ml), dried ($MgSO_4$) and evaporated to a syrup which was purified by silica gel chromatography (ethyl acetate : hexane, 1:2) to isolate the Rf 0.68 (ethyl acetate : hexane, 1:1) material. Evaporation of the appropriate fractions afforded the title compound as a syrup (2.23 g, 50 %) . $[\alpha]_D$ = -111.7 ^{o} (C 0.58, methanol), Rf 0.68 (ethyl acetate : hexane, 1:1). MS (CI NH_3) m/z 322 ($M+NH_4$). ^{1}H-nmr ($CDCl_3$) δ : 5.807 (d, $J_{1,2}$=5Hz, H-1 major: exo isomer), 5.676 (d, $J_{1,2}$=5Hz, H-1 minor: endo isomer) exo/endo ratio = 3.8 :1. ^{13}C-nmr ($CDCl_3$) δ : 120.98 (orthoester quaternary carbon, minor isomer), 120.47 (orthoester quaternary carbon, major isomer). **Anal**. Calcd. for $C_{13}H_{20}O_8$: C 51.31, H 6.63. Found : C 51.41, H 6.60.

Synthesis of GDP-fucose by displacement using 132

Treatment of the orthoester **133** (1.1 mmol, Rf 0.42) with trimethylsilyl iodide (0.13 ml , 0.9 mmol) in dichloromethane (2 ml) at - 20^{o}C resulted in the formation of 2,3,4-tri-O-acetyl-α-L-fucopyranosyl iodide **134** , Rf 0.52, ethyl acetate : hexane, 1:1. After 5 min the reaction mixture was treated (- 20^{o}C) with a DMF solution (2 ml) of the GDP salt **132** (1.2 mmol) which resulted in an instant disappearance of **134** as evidenced by tlc.

After 0.5 h. at 23^{o}C the reaction mixture was partitioned between dichloromethane (10 ml) and water(5 ml). The pH of the aq. layer was adjusted to 10 with LiOH (1 M) to deacetylate the fucose moiety. After one hour at 23 ^{o}C, the pH of the solution was adjusted to 7 using cold 20% AcOH. This solution was chromatographed on Dowex 1-X2 (Cl^-) resin: 2.5x30 cm, using a linear gradient of 0->0.75 m. LiCl. The U.V. positive fractions eluting at 0.53 M LiCl were pooled (total A_{262} units = 3294). Tlc analysis on silica, NH_4OAc 0.3 M : iPrOH, 1:1 at this stage indicated the presence of the desired GDP-fucose spot (Rf 0.76) along with that of GDP (0.09). Approximately 22 A_{262} units were purified by silica preparative-tlc (NH_4OAc 0.3 M : iPrOH, 1:1). The silica-gel preparative plate was developed over 8 h to obtain a solvent front : approx. 7 cm from the base line . The top most U.V. positive band (Rf 0.76) was scraped off and was extracted with water. The nucleotide content in the water extract was determined by U.V. absorbtion to be 11 A_{262} units corresponding to a total yield of 11.6%, based on GDP. Carbon desalting[109] on the original column eluate was performed using 1.0 g 'desalting grade' (Aldrich) carbon per 100 A_{262} units . The adsorbtion in water was done at 23^{o}C, 6 h, to achieve 85% of adsorbtion as evidenced by U.V. monitoring of the supernatant. The desorbtion (80% recovery) was achieved in 50% ethanol containing ammonium acetate (2 mg per 50 ml). The desalted nucleotide material containing GDP and GDP-fucose, upon silica-gel prep-tlc purification afforded GDP-fucose ^{1}H-nmr on which indicated the

presence of 2 methyls (H-6) indicating an α/β mixture at the fucosyl moiety (α/β ratio = approx. 1:1).

Guanosine-5-(3,6-dideoxy-β-L-xylohexopyranosyl diphosphate) , (3-deoxyGDP-fucose) : 138

The 3-deoxy-**L**-fucose-1-phosphate **93** (mono triehylammonium salt, 0.2 mmol) and **131** (tetra-n-butyl ammonium salt, monohydrate, 211 mg, 0.29 mmol) were dried over P_2O_5 (0.01 mm, 23 °C, 24 h.). The mixture of **93** and **131** was dissolved in dry pyridine (10 ml) and pyridine was evaporated *in vacuo*. The process of evaporation was repeated three times using pyridine (10 ml). After each evaporation, air was slowly admitted to the rotatory evaporator through a long drying tube containing $CaSO_4$. Approximately 5 ml of dry pyridine was then added to the flask by syringe. Dry AgOAc (195 mg, 5.9 equiv.) was quickly added to the flask and the contents were vigorously stirred at 23°C protected from the light and moisture.

After 20 h. at 23°C, H_2O (0.5 ml) was added to hydrolyse any unreacted **131**. After 0.5 h. the contents were transferred to screw-capd centrifige tubes and a slow stream of H_2S was bubbled (5 min) to precipitate the silver sulfide. Centrifugations afforded clear supernatents which were removed. The residues were extracted with pyridine : water, 1:1. The combined supernatents were evaporated *in vacuo* to a syrup which was partitioned between water (30 ml) and diethyl ether (30 ml). The aq. layer was separated, diluted to 100 ml with water and then adsorbed slowly onto a column of Dowex 1-X2 (2.5x15 cm,

Cl^-). The column was washed with water (200 ml) and then eluted with a linear gradient of LiCl. The mixing vessel contained 200 ml water. The reservoir contained 200 ml , 0.8 M LiCl in water. The column was operated at 23 ^oC at a rate of 0.5 ml /min. Fractions (4.5 ml) were collected and monitored by U.V. absorbtion at 262 n.m. Fractions were analyzed for the presence of the acid-labile-phosphate as described for the preparation of **78** . Tubes 43-54 contained **93** (5.5 mg, 0.023 mmol) . A U.V. positive peak eluted in tubes 50 to 69 was a by-product of the reaction, which was not identified. *The fractions which were U.V. positive and showed a positive test with H_2SO_4-charring, contained the desired nucleotide. The* desired nucleotide was eluted toward the end of the gradient (tubes 73 to 89). The First few tubes (73-80) which contained the desired nucleotide were analyzed by ^1H (and ^{31}P)-nmr for the presence of the minor isomeric product (axial configuration at fucose C-1). Fractions 75-89 (which contained **138**) were pooled and concentrated (0.01mm, 23^oC) to 5 ml volume. Desalting was performed by passage of above concentrate through a Bio-Gel P2 column (2x60 cm) in 10% EtOH in water with eluate monitoring at 262 nm. Nucleotide containing fractions were concentrated to 5 ml and four consecutive desalting operations were performed to ensure the complete removal of LiCl. The material obtained after the desaltings was dissolved in water (5 ml) and passed through a bed (1x10 cm) of AG-50(Na^+) resin . Lyophilization of the resulting solution furnished **138** as a white fluffy solid (58.6 mg, 47%, 53% based on the recovery of **93**). $[\alpha]_D$ = - 10.0 (C. 0.12,

H_2O), ^{1}H-nmr (D_2O, CH_3CN as int. ref. std. δ = 2.049) δ : 8.100 (S, 1H, H-8 base), 5.999 (d, 1H, $J_{1,2}$ = 6Hz, H-1 ribose), 4.999 (pseudo triplet, 1H, $J_{1,2} = J_{1,P}$ = 8Hz, H-1 fucose), 4.789 (m, 1H, H-2 ribose), 4.523 (m, 1H, H-3 ribose), 4.336 (m, 1H, H-4 ribose), 4.200 (m, 2H, H-5,5 ribose), 3.819-3.643 (m, 3H, H-2, H-4, H-5, fucose), 2.186 (ddd, 1H, Jgem =13Hz, $J_{2,3eq}$ = 5Hz, $J_{3eq,4}$ = 4Hz, H-3eq fucose), 1.689 (ddd, 1H, $J_{2,3ax}$ = 12Hz, $J_{3ax,4}$ = 3Hz, H-3ax fucose), 1.166 (d, 3H, $J_{5,6}$ = 6.5Hz, H-6 fucose methyl), ^{13}C-nmr (D_2O, dioxane as int. std.) δ : 159.80 (C-6 base), 154.76 (C-2 base), 152.66 (C-4 base), 138.52 (C-8 base), 117.13 (C-5 base), 101.19 (d, $^2J_{C-1,P}$=6.0Hz, C-1 fucose), 87.65 (C-1 ribose), 84.64 (d, $J_{C-4,P}$=9.1Hz, C-4 ribose), 75.58 (C-2 ribose), 74.36 and 68.82 (C-4 and C-5 fucose), 71.29 (C-3 ribose), 66.62 (d, $J_{C-2,P}$=8.3Hz, C-2 fucose), 66.10 (d, $J_{C-5,P}$ <6Hz, C-5 ribose), 37.16 (C-3 fucose), 16.45 (C-6 fucose). ^{31}P-nmr (D_2O) δ : - 10.8 (d, $J_{P,P}$=20.9Hz, P: attached to guanosine), - 12.7 (d, P: attached to fucose)

Guanosine-5-(α-D-arabinopyranosyl diphosphate) (GDP - arabinose) : 140

The **D**-arabinosyl-1-phosphate **79** (triethyl ammonium salt, 0.83 mmol, eq/ax ratio = 10.7 : 1), **131** (900 mg, 1.5 equiv.) and AgOAc (550 mg, 4 equiv.) were engaged in the pyrophosphate coupling reaction as described for the preparation of **138**. The contents were vigorously stirred at 23°C protected from moisture.

After 10 h. at 23°C, H_2O (0.5 ml) was added to hydrolyze any unreacted **131**. After 0.5 h. the contents were transferred to screw-capped centrifige tubes and a slow stream of H_2S was bubbled (5 min) to precipitate the silver sulfide. Centrifugations afforded clear supernatants which were removed. The residues were extracted with pyridine : water, 1:1. The combined supernatants were evaporated *in vacuo* to a syrup which was partitioned between water (30 ml) and diethyl ether (30 ml). The aq. layer was separated, diluted to 100 ml with water and then adsorbed slowly onto a column of Dowex 1-x2 (2.5x30 cm, Cl^-). The column was washed with water (200 ml) and then eluted with a linear gradient of LiCl. The mixing vessel contained 1700 ml water. The reservoir contained 1700 ml , 0.8 M LiCl in water. The column was operated at 23°C at a rate of 0.5 ml /min. Fractions (28ml) were collected and monitored by U.V.absorbtion at 262 n.m. Fractions were analyzed for the presence of the acid-labile-phosphate(as described for the preparation of **78**). Arabinose-1-phosphate was eluted in earlier fractions (0.25 M LiCl). Two U.V. peaks were detected. The first peak eluted at 0.33 M LiCl (a by-product) was discarded. The second peak centered at 0.45 M LiCl contained the desired nucleotide. The first few tubes were analyzed by ^1H-nmr for the presence of the minor nucleotide (scheme 31). The fractions which contained desired nucleotide (**140** -equatorial configuration at arabinose C-1) were pooled and concentrated (0.01 mm, 23°C) to 5 ml volume. Desalting was performed by the passage of above concentrate through a Bio-Gel P2 column(2x60 cm) in 10% EtOH in water. The eluate was

monitored at 262 nm. The nucleotide containing fractions were concentrated to 5 ml and four consecutive desalting operations were performed to ensure the complete removal of LiCl. The material obtained after the desaltings was dissolved in water (5 ml) and passed through a bed (1x10 cm) of AG-Na^+ resin . Lyophilization of the resulting solution furnished **140** as a white solid (145 mg, 28%,). $[\alpha]_D$ - 39^0 (C. 0.6, H_2O), Rf 0.53 (NH_4OH : iPrOH : H_2O, 3 : 5 : 2), ^{1}H-nmr (D_2O, CH_3CN as int. std.) δ : 7.925 (S, 1H, H-8 base), 5.736 (d, 1H, $J_{1,2}$ = 6Hz, H-1 ribose), 4.723 (pseudo triplet, $J_{1,2} = J_{1,P}$ = 7.5Hz, H-1 arabinose), 4.583 (m, 1H, H-2 ribose), 4.349 (m, 1H, H-3 ribose), 4.173 (m, 1H, H-4 ribose), 4.033 (m, 1H, H-5, 5' ribose). 3.790-3.707(m, 2H) and 3.559-3.419(m, 3H), H-2, 3, 4, 5, 5', arabinose ^{13}C-nmr (D_2O, dioxane as int. std.) δ : 159.56 (C-6 base), 154.62 (C-2 base), 152.42 (C-4 base), 138.22 (C-8 base), 116.89 (C-5 base), 99.45 (d, $^2J_{C-1,P}$=5.8Hz, C-1 arabinose), 87.67 (C-1 ribose), 84.46 (d, $^3J_{C-4,P}$=9.2Hz, C-4 ribose), 74.66 (d, C-2, ribose), 72.69 (C-3 arabinose), 71.17 (d, $^3J_{C-2,P}$=8.2Hz, C-2 arabinose), 71.17 (C-3 ribose), 68.79 (C-4 arabinose), 67.25 (C-5 arabinose), 66.10 (d, $J_{C-5,P}$ = 5.6Hz, C-5 ribose). ^{31}P-nmr (D_2O) δ : - 10.4 (d, $J_{P,P}$ = 20.8Hz, P attached to guanosine), - 12.3 (d, P attached to fucose)

Guanosine-5-(4,6-dideoxy-β-L-xylohexopyranosyl diphosphate) (4-deoxy-GDP-fucose) 139

A preparation of 4-deoxy-L-fucose phosphate **94** (triethyl ammonium salt, 0.12 mmol, eq/ax ratio= 1) and guanosine-5'-monophosphate morpholidate **135**[112] (100 mg, 1.2 equiv.) were

dried by coevaporations of their solutions in dry pyridine (4x20 ml). The dried reactants were then dissolved in dry pyridine (3 ml) and resulting solution was stirred at 23°C for 4 days (The reaction mixture remained heterogenous throughout the stirring period). Solvent was evaporated and resulting syrup was dissolved in water (50 ml) which was then slowly passed through Dowex 2-X8 (2x15 cm, Cl^-). The column was washed with water (200 ml) and then eluted with a linear gradient of LiCl. The mixing vessel contained 150 ml water. The reservoir contained 150 ml , 0.8 M LiCl in water. The column was operated at 23°C at a rate of 0.5 ml /min. Fractions (3.5 ml) were collected and monitored by U.V.absorbtion at 262 n.m. Fractions were analyzed for the presence of the acid-labile-phosphate (as described for the preparation of **78**) . Tubes 21-27 (0.2 M LiCl) contained **94** . A U.V. peak in tubes 45-59 (0.5 M LiCl) was a by-product of the reaction. Material eluted in tubes 60-75 (centred at 0.7 M LiCl) was both U.V. and H_2SO_4-charring positive. **All** eluate in tubes 60-75 was pooled and concentrated (0.01mm, 23°C) to 5 ml volume. Desalting was performed by the passage of above concentrate through a Bio-Gel P2 column(2x60 cm) in 10% EtOH in water. The eluate was monitored at 262 nm. Nucleotide containing fractions were concentrated to 5 ml and four consecutive desalting operations were performed to ensure the complete removal of LiCl. The material obtained after the desaltings was dissolved in water (5 ml) and passed through a bed (1 x 5 cm) of AG-50 (Na^+) resin . Lyophilisation of the resulting solution furnished **139** (α/β mixture at C-1 of fucose +

other impurities) as a colourless solid (15 mg). Since the material was contaminated with impurities, optical rotation determination was not performed. ^{1}H-nmr (D_2O, TSP-d4 as ext. std.) d : 8.210,8.132,8.106 (3xS, H-8 base), 5.932 (d, J1,2=6Hz, H-1 ribose), 5.549 (dd, $J_{1,2}$=3.5Hz, $J_{1,P}$=7.0Hz, H-1α, fucose), 4.809 (dd, $J_{1,2} = J_{1,P}$ = 8Hz, H-1_b, fucose), 4.742-4.099 (H-2, H-3, H-4, H-5a,b ribose), 4.013-3.673 (H-3, H-5 fucose), 3.409 (m, H-2α,fucose: complex peak due to a 4 bond phosphorus coupling), 3.409 (pseudo triplet, $J_{1,2}$=$J_{2,3}$ = 8Hz, H-2β fucose), 1.266-1.116 (m, H-6 of fucose ($J_{5,6}$ = 6Hz), + unidentified impuried). ^{31}P-nmr (D_2O) d : - 10.2 --> - 12.7 ppm (two sets of mutually coupled doublets, $J_{P,P}$ =19Hz, due to **139**), - 5.7 (d, J =19Hz, impurity), - 10.3 (d, J = 22Hz, impurity).

Guanosine-5-(6-deoxy-β-L-galactopyranosyl diphosphate) (GDP-fucose)

L-fucose-1-phosphate **78** (triethylammonium salt, 0.89 mmol, eq/ax : 4:1) and guanosine-5'-monophosphate morpholidate **135** [112] (800 mg, 1.2 equiv.) were engaged in the pyrophosphate coupling [104] reaction in dry pyridine (10 ml). The heterogenous reaction was stirred at 23°C for 4 days. Evaporation of the solvent gave a syrup which was diluted to 200 ml volume with water. It was then slowly passed through Dowex 2-X8 (2.5x30 cm, Cl^-). The column was washed with water (300 ml) and then eluted with a linear gradient of LiCl. The mixing vessel contained 1800 ml water. The reservoir contained 1800 ml , 0.8 M LiCl in water. The column was operated at 23°C at a rate of 3 ml /min.

The 15 ml fractions were collected and monitored by U.V. at 262 n.m. Fractions were analyzed for the presence of the acid-labile-phosphate (as described for the preparation of **78**) . The first U.V. peak was eluted at 0.4 M LiCl (a by-product, H_2SO_4-charring : negative), followed by a U.V. peak centred at 0.52 M (strong charring with H_2SO_4). The first peak eluted at 0.4 M LiCl was discarded. The second peak contained the desired nucleotide. First few tubes (belonging to the second U.V. peak) were analyzed by ^{1}H-nmr for the presence of the minor nucleotide.The First two tubes which contained the undesired isomer were disregarded and the eluate in the remaining tubes was pooled and concentrated (0.01 mm, 23^{o}C) to 5 ml volume. Desalting was performed by the passage of above concentrate through a Bio-Gel P2 column (2x60 cm) in 10% EtOH in water. The eluate was monitored at 262 nm. The nucleotide containing fractions were concentrated to 5 ml and four consecutive desalting operations were performed to ensure the complete removal of LiCl. The material obtained after the desaltings was dissolved in water (10 ml) and passed through a resin bed-1x20 cm, AG-50 (Na^+). Lyophilization of the resulting solution furnished GDP-fucose as a white light solid (155 mg, 27%,). $\alpha_{[D]}$ - 34^o (C. 0.18, H_2O), Rf 0.54 (NH_4OH : iPrOH : H_2O, 3 : 5 : 2), 0.76 (iPrOH : NH_4OAc 0.3M , 1:1). MS (glycerol, negFAB) m/z 588 (monoanian). ^{1}H-nmr (D_2O, TSP-d4: as int. std.) δ : 8.125 (s, 1H, H-8 base), 5.950 (d, 1H, $J_{1,2}$=6Hz H-1 ribose), 4.928 (pseudo triplet, 1H, J_{12}=$J_{1,p}$=8Hz, H-1 fucose), 4.800 (HOD peak, H-2 ribose), 4.545 (m, 1H, H-3 ribose), 4.360 (m, 1H, H-4 ribose), 4.220 (m, 2H, H-5a,b ribose),

3.780 (q, 1H, H-5 fucose), 3.720 (dd, 1H, $J_{4,5}$ =1Hz, H-4 fucose), 3.668 (dd, 1H, $J_{3,4}$ = 4Hz, $J_{2,3}$ =10HZ, H-3 fucose), 3.565 (dd, 1H, H-2, fucose), 3.565 (dd, 1H, H-2, fucose), 1.225 (d, $J_{5,6}$ = 6.5Hz, H-6, fucose), ^{13}C-nmr (D_2O) δ : 159.85 (C-6 base), 154.8 (C-2 base), 152.69 (C-4 base), 117.13 (C-5 base), 99.23 (d, $J_{C-1,P}$ = 5.7Hz, C-1 fucose), 57.60 (C-1 ribose), 84.68 (d, $J_{C-4,P}$ = 9.1Hz, C-4 ribose), 73.32 (C-3 fucose), 74.35, 72.25, 72.00, 71.84 (C-2, C-4, C-5, fucose : $J_{C-2,P}$ = approx. 8 Hz : difficult to determine because of the line overlap), 71.32 (C-3 ribose), 66.18 (d, $J_{C-5,P}$ = 5.7Hz, C-5 ribose), 16.24 (C-6 fucose), ^{31}P-nmr (D_2O) δ : - 10.8 (d, $J_{P,P}$ = 20.8Hz, P attached to guanosine), - 12.7 (d, P attached to fucose).

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