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Studies on the Formation of β-Mannosidic Linkages by Intramolecular Inversion

BY

Marei Hildegard Erika Griffith

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy.**

Department of Chemistry

Edmonton, Alberta

Fall 1991

ISBN 0-315-70134-X

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: **Marei Hildegard Erika Griffith**

TITLE OF THESIS: **Studies into the Formation of β-Mannosidic Linkages by Intramolecular Inversion**

DEGREE: **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED: **Fall 1991**

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UNIVERSITY OF ALBERTA

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 **Studies into the Formation of a β-Mannosidic Linkage by Intramolecular Inversion** submitted by **Marei Hildegard Erika Griffith** in partial fulfilment for the requirements for the degree of **Doctor of Philosophy**.

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Date June 5/90

Abstract

The production of β-D-mannosidic linkages by intramolecular inversion of the 2 position of glucopyranosides was studied using the *p*-N,N-dimethylaminobenzoyl group and the anisoyl group as intramolecular nucleophiles. Both residues behaved well in initial model studies using methyl 4,6-O-benzylidene-3-O-acyl-2-O-trifluoromethanesulfonate-β-D-glucopyranoside to provide only the 2 and 3-O-acyl-β-D-mannopyranosides. As expected, the *p*-N,N-dimethylaminobenzoyl group was a better nucleophile than the anisoyl group, but it created problems during glycosidation reactions due to protonation of its dimethylamino moiety. While searching for suitable glycosidation conditions, 1,2-orthoacyl fluorides were observed for the first time and characterized by n.m.r. spectroscopy.

When inversion conditions were applied to octyl 4-*O*-benzyl-6-*O*-*t*-butyldiphenylsilyl-3-*O*-acyl-2-*O*-trifluoromethanesulfonate-β-D-glucopyranoside, ring contraction due to 1,2 migration of the ring oxygen was also observed. This side reaction was particularly prevalent for the anisoyl group. The 2-*O*-*p* -N,N-dimethylaminobenzoyl-β-D-mannopyranoside obtained from the inversion was glycosidated to provide the octyl 3-*O*-(2-amino-2-decxy-α-D-mannopyranosyl-)-β-D-mannopyranoside derivative **121**. Due to the limited quantities of β-mannosides obtainable by the inversion method, only a very small amount of this disaccharide could be obtained. Since it was desirable to obtain a larger quantity of this disaccharide to allow enzyme assays, it was also synthesized by an independent method.

The mannosamine residue was introduced into **121** using 3,4,6-tri-*O*-acetyl-2-phthalimido-2-deoxy-α-D-mannopyranosyl bromide. The ring in all 2-phthalimido-2-deoxy-D-mannopyranose derivatives was severely distorted, rendering the assignment of the anomeric configuration by n.m.r. spectrometry

inconclusive. The glycosidation of alcohols by 2-phthalimidomannopyranosyl bromide was therefore investigated using methanol and di-isopropylidene glucose as glycosyl acceptors. In both cases α-mannosidic linkages were formed and furthermore neither ring distortion nor the type of protecting groups employed had much effect on the ^{13}C chemical shifts and coupling constants. These nmr data can therefore be used as indicators for the anomeric configuration of the glycosidic linkage in the protected mannopyranosides.

Acknowledgements :

I would like to acknowledge my supervisor Dr. Ole Hindsgaul for his excellent support and guidance throughout the years of study, as well as my husband Dr. Lee Griffith, without whose continual support during the many years of study I would not have been able to complete my work. I would also like to thank my children, Kirsten and Meike, for providing entertaining distractions from the rigors of research and my parents, Volker and Marion Fluegge, for guiding me towards this choice of career.

I thank the staff of the n.m.r. laboratory and the microanalytical laboratory for their assistance. I would like to also thank the Alberta Heritage Foundation for Medical Research, the Killam Foundation and the Department of Chemistry for financial support.

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4 A = 4 Angstroms

Ac = acetyl

All = allyl

Aloc = allyloxycarbonyl

Asn = asparagine

Bn = benzyl

tBu = *tert*-butyl

Bue = 2-butenyl

Bz = benzoyl

DAST = diethylaminosulfur trifluoride

DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

DMAP = 4-dimethylaminopyridine

DMF = dimethylformamide

Et = ethyl

FAB = fast atom bombardment

gem = geminal

GlcNac = 2-acetamido-2-deoxy-D-glucopyranose

GlcNAcT I = GlcNAc transferase I

GlcNAcT II = GlcNAc transferase II

h.p.l.c. = high performance liquid chromatography

Man = mannose

Me = methyl

Ms = methanesulfonyl

n.m.r. = nuclear magnetic resonance

Ph = phenyl

Phth = phthalimido

p.p.m. = parts per million

r.t. = room temperature

Tf = trifluoromethanesulfonyl

THF = tetrahydrofuran

t.l.c. = thin layer chromatography

triflate = trifluoromethanesulfonate

Ts = p-toluenesulfonyl

UDP = uridine diphosphate

Introduction :

In recent years the field of carbohydrate chemistry and biochemistry has experienced a resurgence of activity as carbohydrates have become recognized as being involved in many important biochemical processes. Although carbohydrates, especially starch and cellulose, have always been important in the industrial sector of food production, paper, textiles and leather (1), the role of carbohydrates as carriers of biological information is relatively new. Due to the complexity of the structures involved, demonstration of this role was greatly hampered by the lack of separation and analysis techniques, but with the advent of high field n.m.r. instruments, hplc and FAB mass spectrometry, increasingly complicated carbohydrate structures were identified and their involvement in biological processes described.

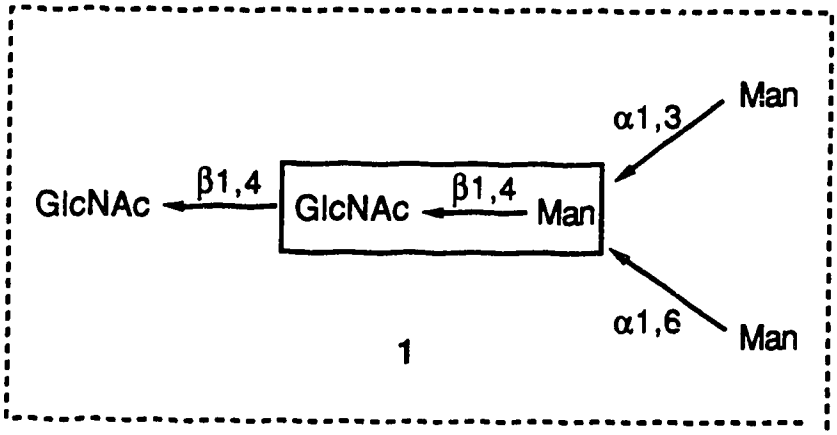
Carbohydrates have now been shown to be involved in diverse biological processes such as the binding and transport of enzymes (2 - 4) and hormones as well as the binding of antibodies (5,6), bacteria (7), toxins (8) and viruses (9,10). They are also involved in the immune response (11) and cell-cell interactions (12 - 15), as for example during embryogenesis and cancer metastasis (16,17).

To allow carbohydrates to act as recognition markers in so many different processes, they must be able to convey a large amount of information and this is indeed possible due to the large number of possible combinations in which they can be assembled. For example, 3 different hexoses can combine to provide 1056 different trisaccharides as compared to only six different tripeptides which can be formed from three different amino acids.

This biologically important versatility in structure makes the investigation of the biological role of carbohydrates quite difficult and an important contribution of the synthetic carbohydrate chemist is therefore to

provide well characterized carbohydrates to more clearly define the function of specific structures or structural components. Eventually the detailed understanding of specific biochemical recognition events will enable us to interpret larger and more complex biological phenomena.

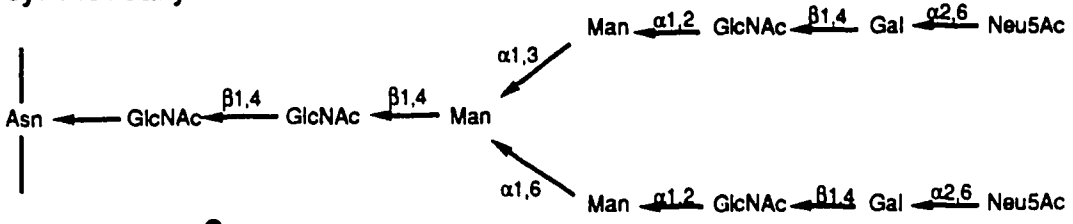
The carbohydrates found on animal cell surfaces are divided into two major groups, those attached to proteins and those linked to lipids. The glycoprotein group is further divided into the O-linked and N- linked oligosaccharides, depending on the type of linkage between the carbohydrate and the protein. In the O-linked glycoproteins, carbohydrates are α-linked via a glycosidic linkage to serine or threonine hydroxyl groups, while in the N-linked glycoproteins they are are attached via a β-N-glycosidic linkage to the amide nitrogen of asparagine residues. These N- linked oligosaccharides are among the most complicated carbohydrate structures found on the cell surface, involving at least 10 to 20 carbohydrate residues. Although there is considerable variation in the structures found among these N-linked oligosaccharides the central core region 1 is identical in all of them (18).



1

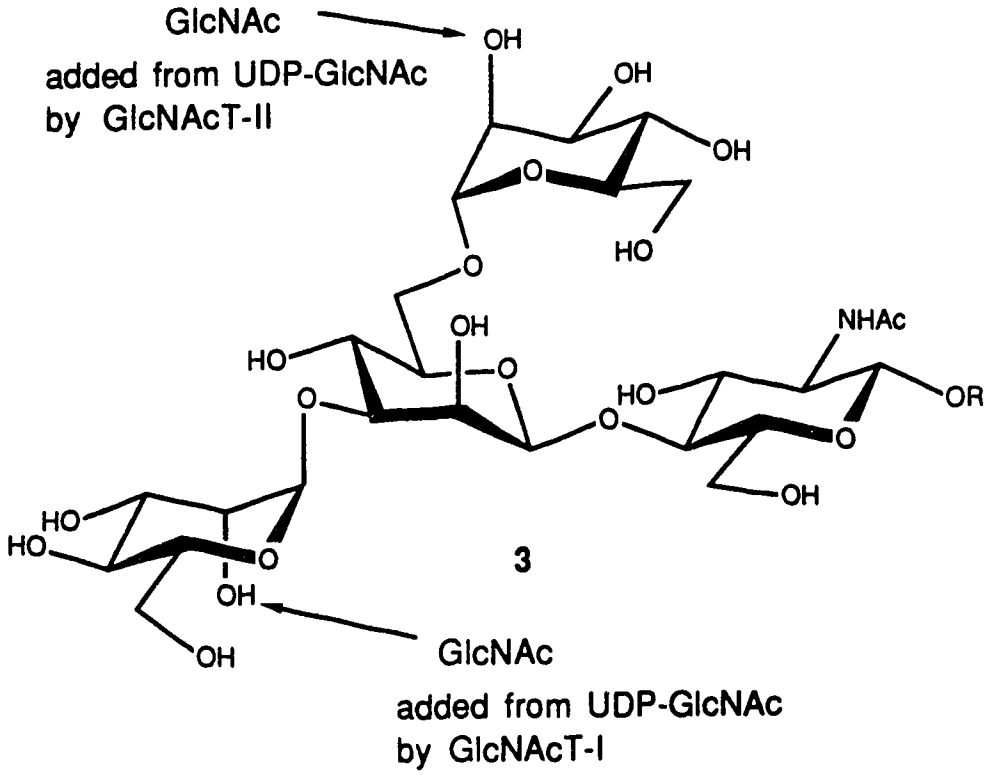
One of the glycosidic linkages in this central core region (see structure 1), the β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-

glucopyranosyl linkage, has remained particularly difficult to form synthetically.



2

The synthesis of analogues or partial structures of complex oligosaccharides such as **2** is synthetically interesting, as di-, tri and tetrasaccharide analogues can serve as substrates and inhibitors for various glycosyltransferases and as ligands for the binding with lectins (19). For example, the tetrasaccharide **3** is a good acceptor for N-acetylglucosaminyl transferases I and II (GlcNAcT I and GlcNAcT II) as indicated. It must be noted that GlcNAcT II acts only after GlcNAcT I has transfered the first GlcNAc residue to the tetrasaccharide **3** (20).



3

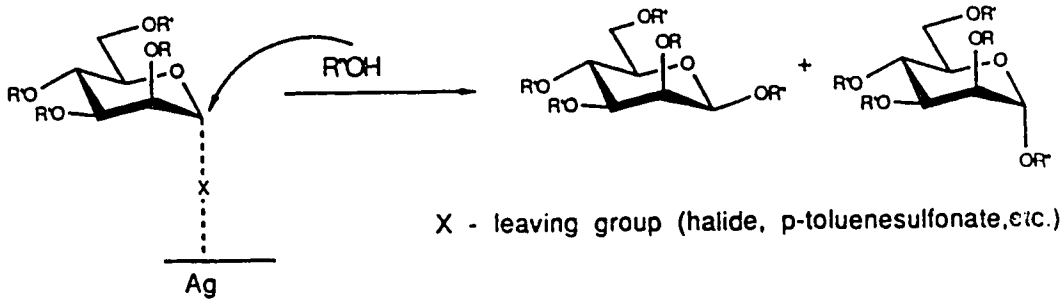
The formation of 1,2-cis glycosidic linkages has been an ongoing problem in carbohydrate chemistry (21) and although satisfactory methods for the formation of cis glycosides with an equatorial substituent at C-2 have been found (22 - 24), the preparation of the corresponding cis glycosides with axial substituents at C-2 is still problematic. To address this problem several methods have been developed over the last 30 years, but most of them fail when applied to large carbohydrate structures.

Scheme 1 depicts an approach to the formation of β-mannosidic linkages, which has been widely applied due to its ease of use. It is based on the traditional Koenigs-Knorr reaction (25) and uses an insoluble silver catalyst to activate the anomeric α-halides by withdrawing the halide anion while simultaneously blocking the α-anomeric side from attack by the alcohol (26). To achieve good results the mannosyl donor has to be protected with a nonparticipating group at the 2-position to prevent neighboring group participation (21,25,27,28). Even then steric factors, as well as the anomeric effect, favor the formation of the α-mannoside. Therefore to achieve the highest possible formation of β-mannoside, both the halide and the alcohol have to be highly reactive to ensure that the glycosidation reaction proceeds under kinetic control (21). The alcohol has to be reactive enough to attack the tight ion-pair,which develops on the way to the oxocarbonium ion, and the halide has to be sufficiently reactive in its α-configuration in order to minimize reaction of the more reactive β-halide, which is always present in small amounts at equilibrium. The formation of β-halides is assisted by the use of soluble catalysts, such as HgX_2 salts or silver perchlorate, and therefore insoluble silver salts were chosen for the preparation of β-mannosidic linkages. Although silver oxide can serve as a suitable catalyst, silver silicate (28) and the easily prepared silver zeolite (29) are more reactive and are thus prefered

catalysts. The effect of the reactivity of the alcohol on the yield of β-mannoside can be seen in scheme 1. While the reaction of the mannosyl bromide with the unreactive 4 hydroxyl group of N-acetylglucosamine provided only a moderate amount of β-mannoside (examples **a** and **b**), the use of the more reactive 1,6-anhydro derivative of an N-acetylglucosamine as glycosyl acceptor produced good yields of the desired β-conformers (example **c**).

Scheme 1 : β-Mannoside Formation by Koenigs-Knorr Type Reactions

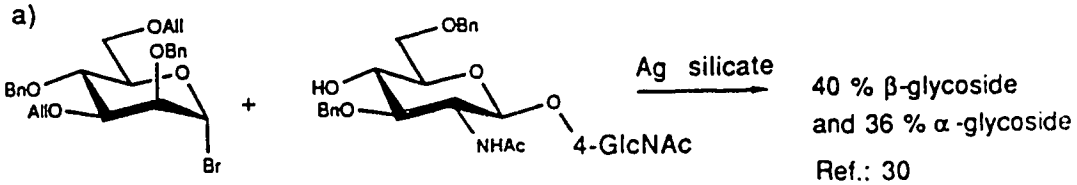
General :



X - leaving group (halide, p-toluenesulfonate,etc.)

Examples :

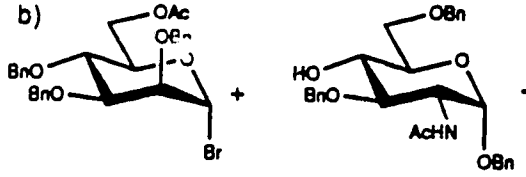
a)



40 % β-glycoside
and 36 % α -glycoside
Ref.: 30

Scheme 1 cont.

b)

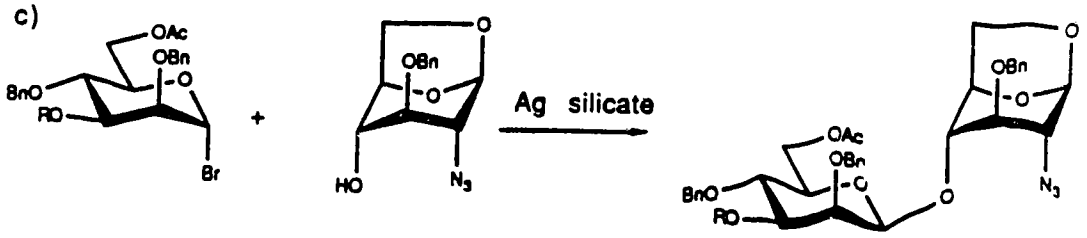


Ag silicate

75 % α-glycoside

Ref.: 31

c)



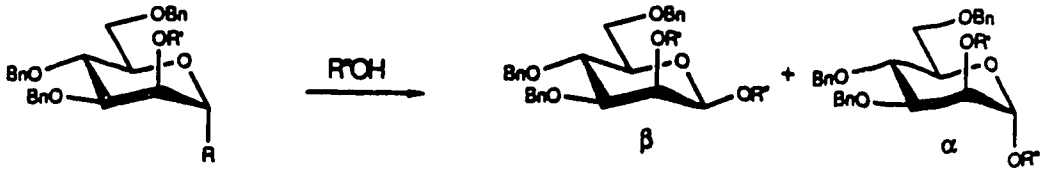
Ag silicate

R= Bn 85 % β

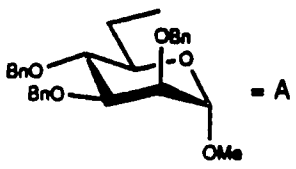
R=Ac 50 % β

R=All 87 % β Ref.: 31,32

d)



R'	R	R"	% β	% α
Ms	OTs	Me	90	-
Ms	OTs	C_6H_{11}	86	14
Ms	$O_3SCH_2CF_3$	C_6H_{11}	93	7
Ms	OTs	A	83	17
Ms	$O_3SCH_2CF_3$	A	>90	-



= A

Ref.:33,34

Example **c** also demonstrates that even small differences in the reactivity of the bromide have a significant effect on the yield of mannoside produced. Thus the less reactive acetylated bromide provided only 50 % of the β-mannoside as compared to 85 - 87 % for the allylated or benzylated halide. It is this sensitivity towards the reactivity of the starting materials that renders this approach unsuitable for block synthesis, as oligosaccharide halides are usually much less reactive than those derived from monosaccharides (21).

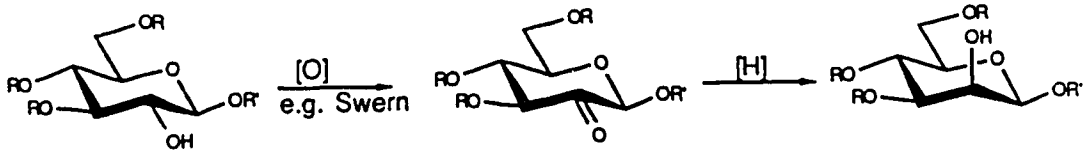
Example **d** in scheme 1 differs from **a-c** in that it does not involve an insoluble catalyst, but the principles underlying this approach are similar to the ones described for examples **a** to **c**. The mannosyl donor is the highly reactive anomeric triflate and the requirement for an active glycosyl donor is therefore fulfilled in this approach as well. Instead of using an insoluble catalyst to ensure that the reaction has a large amount of S_N2 or ion pair character, the 2 hydroxyl group is protected with a strongly electron withdrawing, nonparticipating group such as the mesyl group. When this glycosyl donor was reacted with highly reactive alcohols, good yields of β-mannosides were obtained. Since only the highly reactive primary hydroxyl group of carbohydrates was used in this approach, it is difficult to judge what results would be obtained if the less reactive secondary hydroxyl groups were used as glycosyl acceptors. To be truly useful in synthesis the method has to provide good yields for most carbohydrate hydroxyl acceptors. The regeneration of the 2-hydroxyl group from the mesylate remains problematic as well and therefore more work is needed if this approach should become competitive with the one described in examples **a** - **c** (33,34).

A different approach to the formation of β-mannosidic linkages involves the formation of a β-glucosidic linkage, followed by the oxidation of the OH-2 to the ketone, which is then stereospecifically reduced to provide the β-

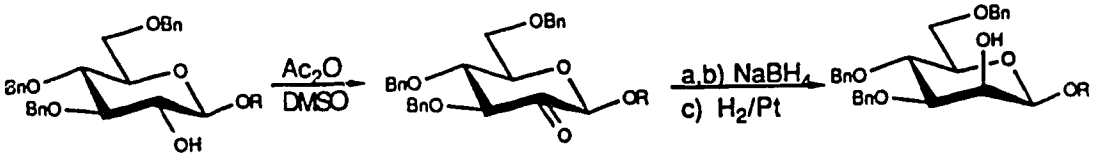
mannoside. This approach is depicted in scheme 2, together with some examples of its synthetic application. The yield of mannosides by this approach seem to be equally good for di and trisaccharides, but the procedure is described as "elaborate" (21) and "laborious" (35) and is therefore not extensively used. The product also contains a free hydroxyl group which must be protected if further synthetic steps are required.

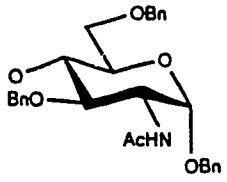
Scheme 2 : β-Mannoside Formation by Oxidation/Reduction of the 2 Hydroxyl Group

General :

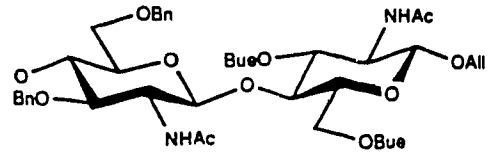


Examples :

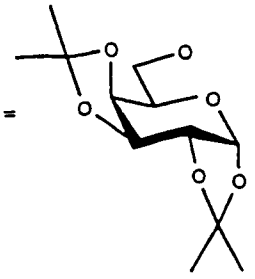


a) RO = 

Ref.: 36 80 - 90 % β-mannoside

b) RO = 

Ref.: 37 72 % β-mannoside

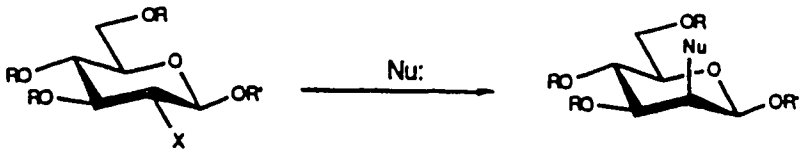
c) RO = 

Ref.: 35,38 90 % β-mannoside

A third method involves the S_N2 displacement of a leaving group at C-2 by an external nucleophile. Positions other than the 2 positions have been commonly epimerized using intermolecular displacements (39 - 43) and the approach described in scheme 3 is an extension of this earlier work (44).

Scheme 3 : β-Mannoside Formation by Intermolecular Inversion of the 2-Position of β-Glucosides

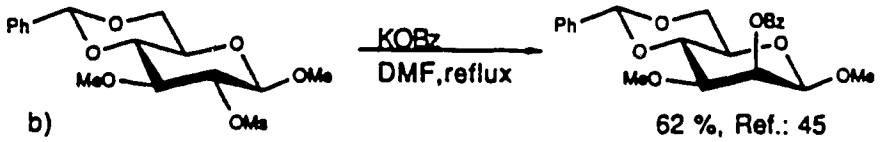
General :



X = leaving group, Nu: = nucleophile

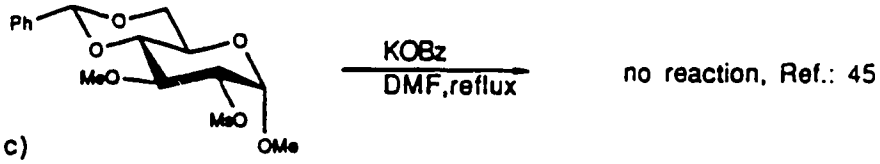
Examples :

a)



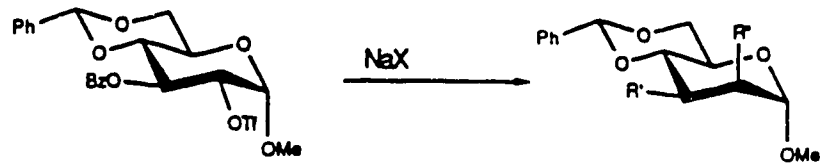
62 %, Ref.: 45

b)



no reaction, Ref.: 45

c)



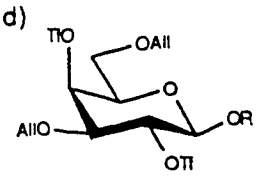
X	Temp./time	R'	R"	yield %
OBz	80 °C/5 hrs	OBz	OBz	82
N_3	80 °C/2 hrs	OBz	N_3	86
MeS	0 °C/1 hr	OBz	MeS	89
PhS	50 °C/6 hrs	OBz	PhS	83
MeO	r.t./overnight	—O—		85

Ref.: 46

The displacement of the 2-O-mesylate of β-glucopyranosides proceeds moderately well to produce 62 % yield of the β-mannopyranoside, but the analogous α-glucopyranoside does not react at all (scheme 3, **a** and **b**). The use of the more reactive triflate as a leaving group can overcome this problem, providing a variety of 2 substituted α-mannosides in greater than 80 % yield (scheme 3, **c**).

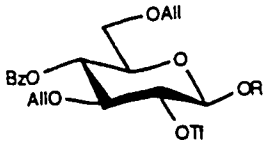
Scheme 3 cont.

d)



$Bu_4N^+BzO^-$

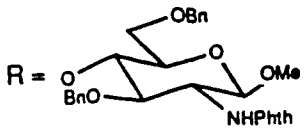
r.t., 30 min.



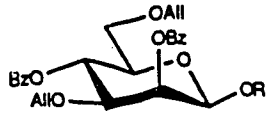
R = Me, Ref. 47

100 °C

R =

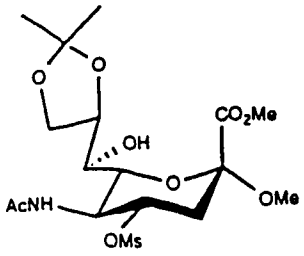


Ref.: 48



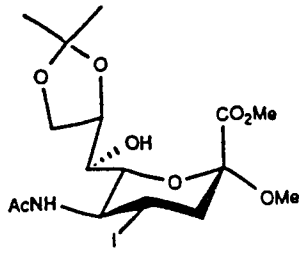
64 %, Ref.: 47
52-55 %, Ref.: 48

e)

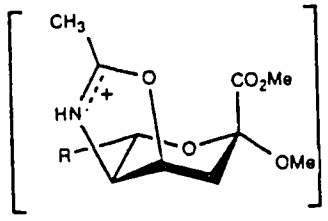


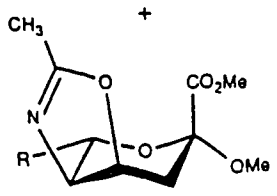
NaI, 100 °C

3 hrs



20 %, with retention





25 %, Ref.: 50

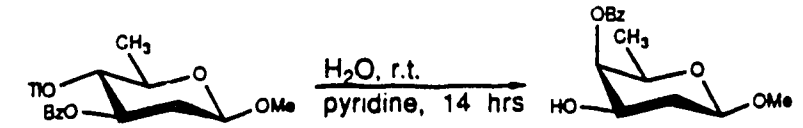
It therefore seems advantageous to use the more reactive triflate in these displacement reactions, especially for the difficult S_N2 reactions at the 2 positions of sugar pyranosides. The low reactivity of the 2 position as compared with other positions is demonstrated in example **d**, where double displacement on a 2,4 ditriflate of galactose proceeded in two distinct steps to provide the β-mannopyranoside in 64 % yield. As example **d** shows, these reactions can be applied to structures larger than monosaccharides, but the yields are only moderate and the displacing nucleophile can cross react with protecting groups if no special precautions are taken (47 - 49).

Neighboring groups can also participate during attempted intermolecular displacement reactions. For example, during the inversion of a 4-O-mesylate of sialic acid with sodium iodide (50), the iodide was introduced with retention of configuration and the oxazoline was formed as a byproduct (example **e**).

Intramolecular inversions are therefore feasible and many examples, including the use of benzoates as internal nucleophiles (scheme 4) are known.

Based on these precedents, we decided to examine whether intramolecular inversion of the 2 position of β-glucosides could provide a general route to β-mannosides. The use of an "internal" nucleophile might have advantages, as attaching the nucleophile to the β-glucoside molecule might focus its action on the inversion and therefore reduce the possible side reactions, that often arise from the use of external nucleophiles. As well, the effects of the molecular structure (e.g. di- or trisaccharides instead of a monosaccharide) on the reaction rate should be diminished during intramolecular inversion, as the approach path of the internal nucleophile is much more limited.

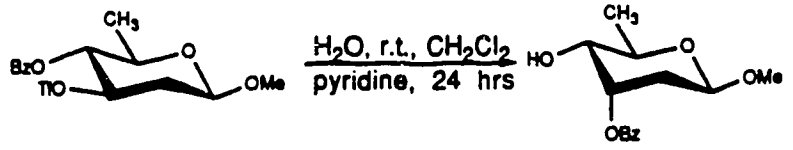
Scheme 4 : Examples of the Formation of Sugar Epimers by Intramolecular Inversion



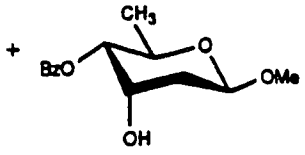
80 %

16 %

proposed intermediate
which was characterized by n.m.r.
spectroscopy



70 %



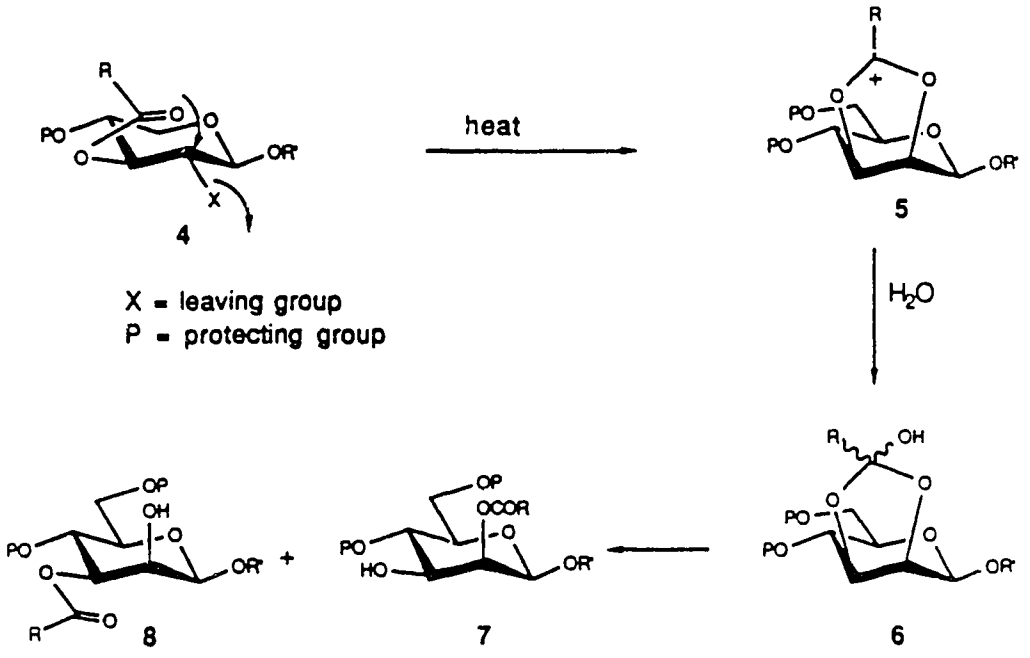
21 %

Ref.: 51 - 53

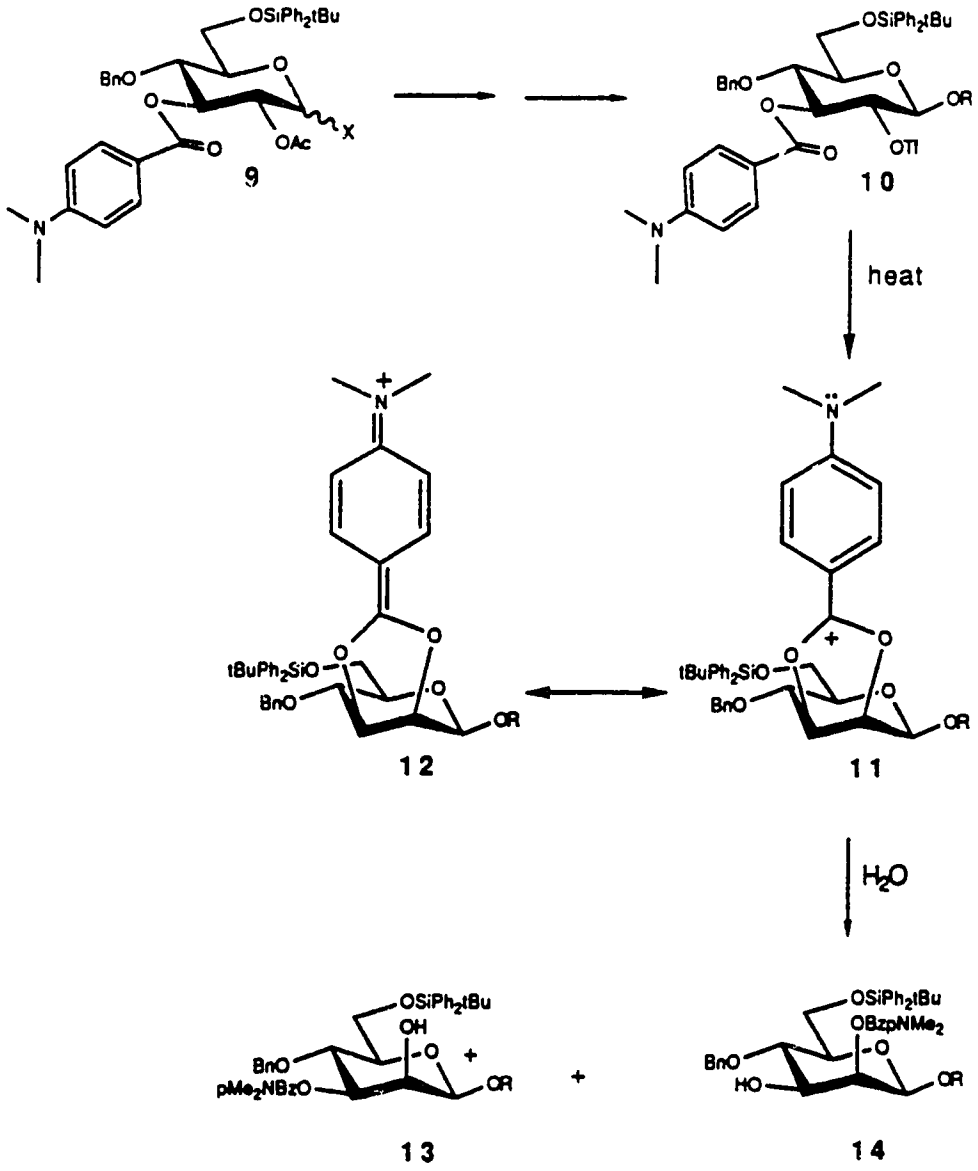
The mechanism of this inversion was expected to proceed as depicted in scheme 5. Upon heating, the activated acyl group would displace the leaving group **X** (structure **4**) to provide the intermediate acyloxonium ion **5**. This cationic species (**5**) should then decompose in the presence of water, via the

orthoacid **6**, to provide the acylated β-mannosides **7** and **8**. The axially acylated structure **7** should predominate substantially as predicted by the results obtained from studies into the acid hydrolysis of orthoesters (33) and also from the results obtained by Binkley et al. (scheme 4, 51,52,53). A preponderant formation of the 2-O-acylated derivative would allow further glycosidation of the unprotected 3-position to provide structural analogues of the complex oligosaccharide **2.** The use of an acyl group as the internal nucleophile would therefore have two desirable effects, the formation of the β-mannosidic linkage and the selective liberation of the 3 hydroxyl group.

Scheme 5 : Possible Mechanism of the Intramolecular Inversion at the 2-Position and Design of the Target Molecule



Scheme 5 cont.



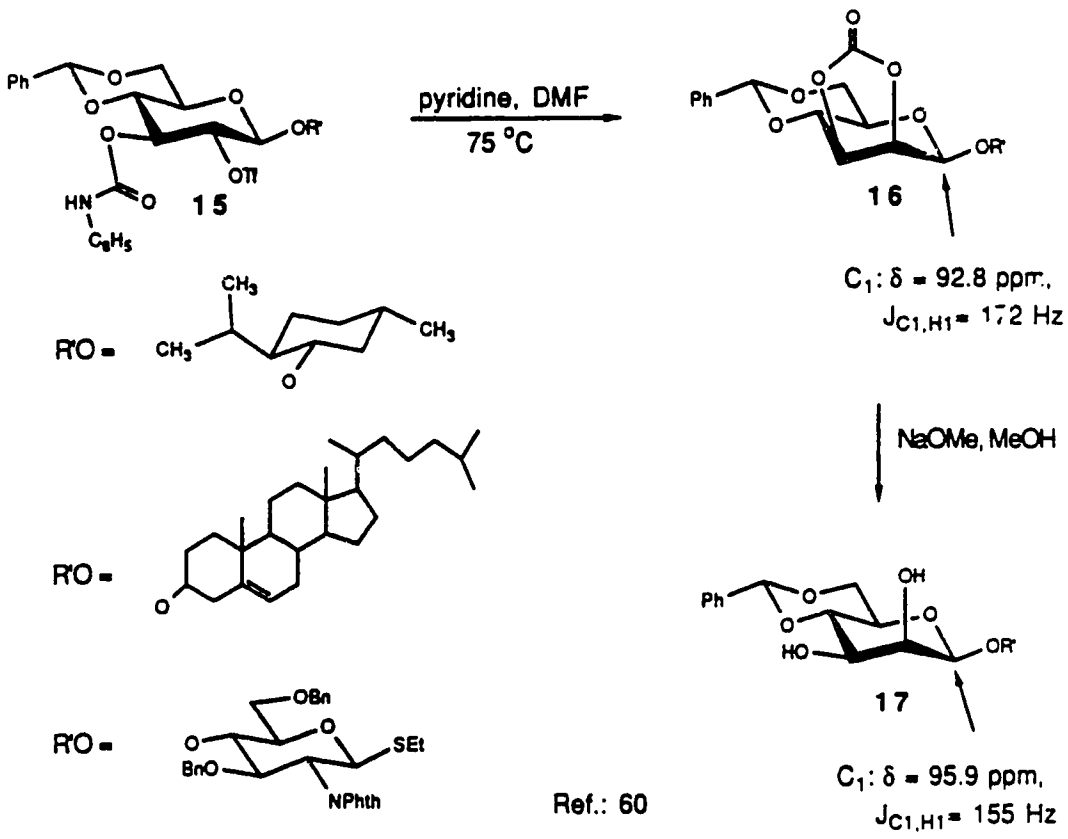
Stabilization of the intermediate acyloxonium ion **5** was expected to facilitate the inversion and therefore the *p*-N,N-dimethylaminobenzoyl group was chosen to serve as the intramolecular nucleophile. It was expected to

stabilize the intermediate acyloxonium cation **11** by delocalization of the positive charge into the aromatic ring as shown in **12**. To provide the desired β-*manno* configuration in the product the *p*-N,N-dimethylaminobenzoyl group had to be attached to the 3 position of a glucopyranoside. A 2-O-triflate ester was further envisioned as the leaving group since it would be highly reactive and the inversion could therefore proceed under the mildest conditions possible (see scheme 3 a,b,c, 45,46). Protection of O-2 in the glycosyl donor **9** by an acetyl group was expected to allow selective access to this position after glycosidation, as there are a number of procedures available for the selective removal of 2-O-acetyl groups (54 - 58). The 2-O-acetate is also a good participating group during glycosidation reactions thus ensuring that the target glycosyl donor **9** would yield predominantly β-glucosides. Deprotection of O-2 in the β-glycoside, triflation and inversion by heating in the presence of water might then provide the β-mannosides **13** and **14**. To avoid the occurrence of further displacement reactions (59) at the 3 position in the intermediate **11**, the 4 position had to be protected with a persistent non-participating protecting group and so the benzyl group was chosen for this purpose. The O-6 was protected with the *t*-butyldiphenylsilyl group, which by deprotection with fluoride ion (60-62) should allow selective access to that position. Compound **9** would therefore combine all the necessary features to allow the selective synthesis of analogs of the complex oligosaccharide **2**.

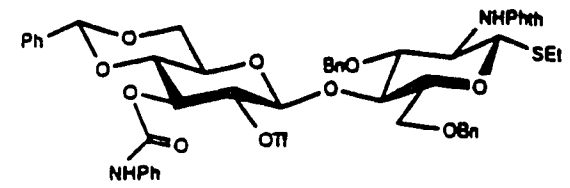
About 1 year into this work, Kunz and coworkers (63) published a very similar approach to the formation of β-mannosidic linkages, but they used a phenylurethane group instead of an acyl group as the intramolecular nucleophile (scheme 6). When the phenylurea derivative **15** was heated to 75 °C in DMF and pyridine, the inversion proceeded smoothly to provide about 90 % of the 2,3-O-carbonyl mannoside **16**. The carbonate group could be

sodium methoxide in methanol to provide the β-mannoside diol **17**. In late 1990 Guenther and Kunz (64) employed this approach in the synthesis of the asparagine linked trisaccharide **18**, where the glucopyranosyl-β-(1→4)-N-acetylglucosamine derivative **19** was inverted to provide the β-mannoside **20**, which was then converted to **17** in an overall yield of 41 %.

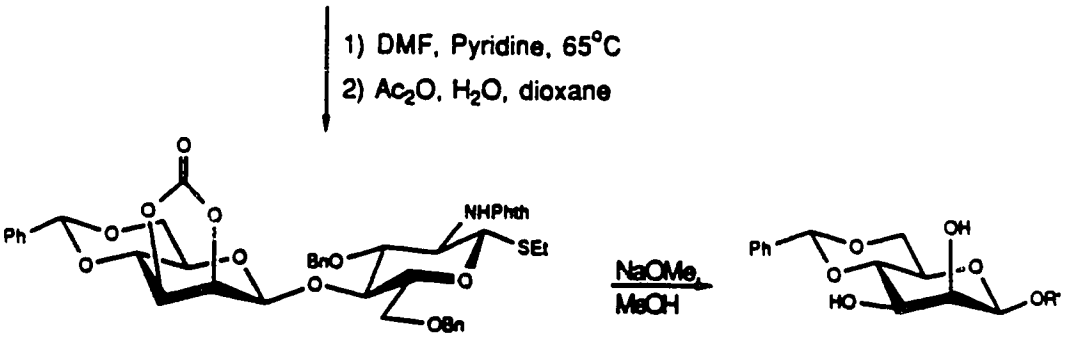
Scheme 6 : Formation of β-Mannosides by Intramolecular Inversion



Scheme 6 cont.

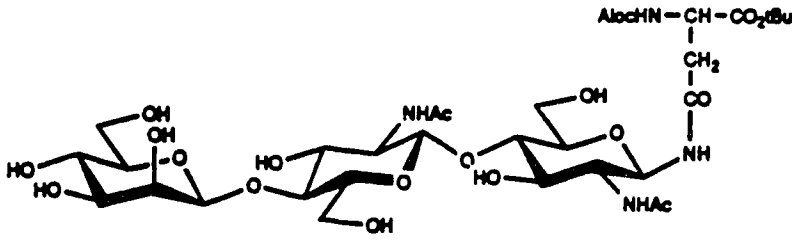


19



20

17



18

Although the yields obtained in this inversion were good, the removal of the benzylidene group by hydrogenation precluded further glycosidations at the terminal mannose residue, as catalytic hydrogenation also causes debenzylation, hydrogenation of allyl groups and sometimes acyl group

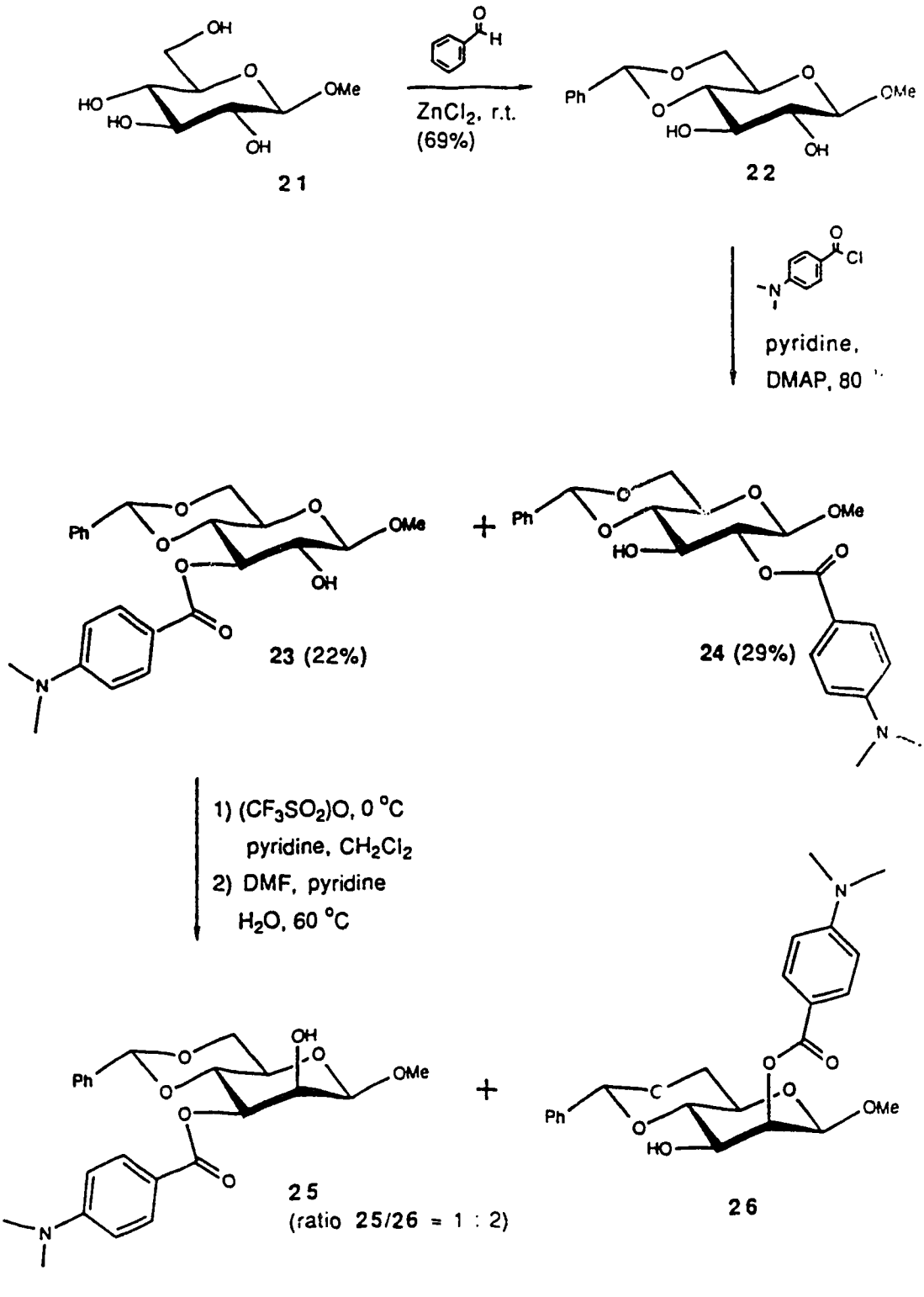
migrations. The removal of the benzylidene by acidic means is a delicate procedure as the labile β-mannosidic bond is prone to hydrolysis or anomerization under those conditions. Furthermore, neither the 2 nor the 3 position of the β-mannoside product was available for selective chain elongation or modification.

We therefore proceeded to investigate the *p*-N,N-dimethylaminobenzoyl approach as it might be more useful for the construction of more diverse oligosaccharide structures.

Results and discussion :

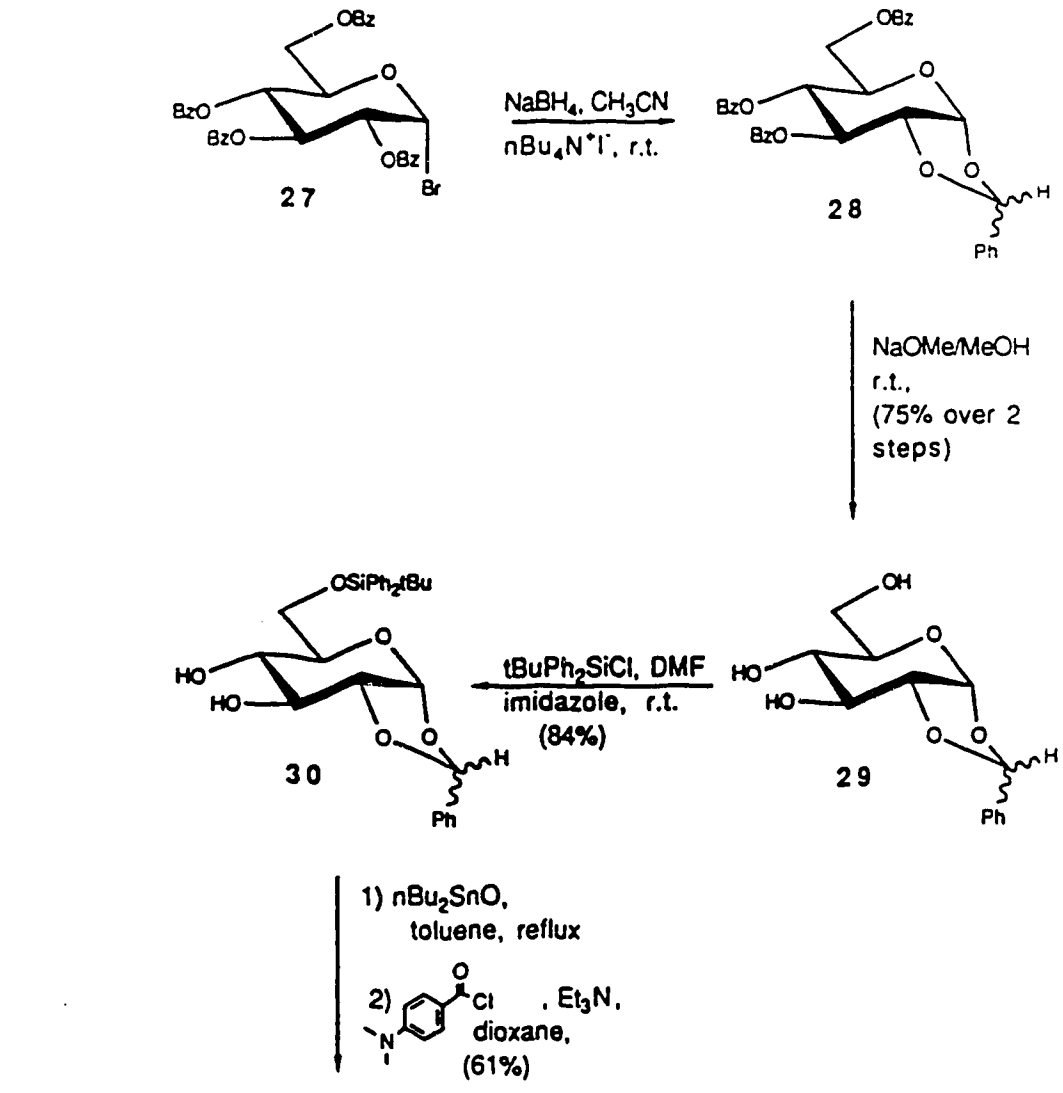
The *p*-N,N-dimethylaminobenzoyl group had not been previously described in inversion reactions and its use for such a purpose was therefore first investigated using the easily prepared model compound **23**. The 4,6 - O - benzylidene derivative **22** was prepared from methyl β-D-glucopyranoside (**21**) using benzaldehyde and zinc chloride in 70 % yield according to standard procedures (65,66). The diol **22** was then esterified with *p*-N,N-dimethylaminobenzoyl chloride in pyridine at 80 °C to provide the desired 3-O-acylated product **23** in 22% yield together with the 2-O-acylated compound **24** (29 %). The derivative **23** was converted cleanly to the trifluoromethanesulfonate by treatment with trifluoromethanesulfonic anhydride in dichloromethane - pyridine at 0 °C. The triflate reacted upon heating to 60 °C in DMF in the presence of pyridine and water to give the mannosides **25** and **26** in a ratio of about 1 : 2. N.m.r. spectroscopy on the crude material revealed only the two products **25** and **26**. Attempts to cause migration of the axial acyl group in **26** to the equatorial position using pyridine in DMF at 60 °C failed, thus indicating that product **25** is not formed from **26** by migration under the reaction conditions but most likely forms directly from the intermediate orthoacid **6**. Although the result of this preliminary study seemed promising, it was essential to proceed towards a more general mono- or disaccharide system that did not contain a trans-decalin type fused ring system. The presence of two trans-fused six membered rings was thought to decrease the ease of inversion by disfavouring the formation of the orthoacid **6**, which requires flattening of the carbohydrate pyranosyl ring.

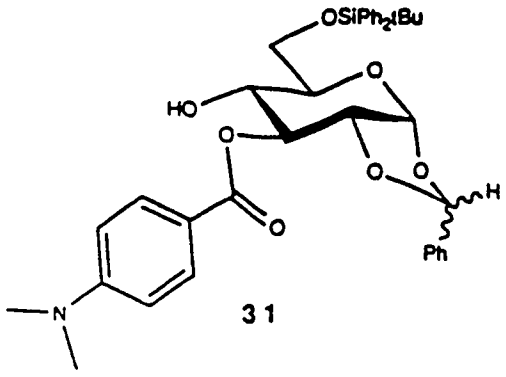
To allow selective protection of the 2 and 3 positions on D-glucose, the bromide **27** was converted to the 1,2-O-benzylidene derivative **28** as


21
ZnCl2, r.t.
(69%)
22
pyridine,
DMAP, 80 °C
23 (22%)
24 (29%)
1) (CF3SO2)O, 0 °C
pyridine, CH2Cl2
2) DMF, pyridine
H2O, 60 °C
25
(ratio 25/26 = 1 : 2)
26

described by Betaneli et al. (67) using sodium borohydride and tetrabutylammonium iodide at room temperature. Compound **28** was debenzoylated using sodium methoxide in methanol without prior purification to provide the triol **29** in 75 % yield over the two steps. Treatment of **29** with *t*-butyldiphenylchlorosilane and imidazole in DMF provided **30** in 84 % yield (60 - 62). Attempts to generate the 3-O-*p*-N,N-dimethylaminobenzoyl derivative **31** in any significant yield by acylation with *p*-N,N-dimethylaminobenzoyl chloride and DMAP in pyridine failed because the reaction proceeded only very slowly at room temperature. Warming to 50 °C did accelerate the reaction, but also caused some decomposition, thus making further temperature increases impossible. Under these conditions the 3-O-acyl product **31** was formed together with the 4-O-acylated and some diacylated product in a ratio of about 8 : 2 : 1. Earlier studies on the selective acylation of the triol **29** using dibutyltin oxide had led to a preponderant formation of 6-O-acylated and 3,6-di-O-acylated products and it was therefore thought that after protection of the 6 position, dibutyltin oxide might favour the formation of **31**. Thus **30** was refluxed with dibutyltin oxide in toluene under azeotropic water removal for 1 - 2 hours.Treatment with *p*-N,N-dimethylaminobenzoyl chloride and triethylamine in dioxane at room temperature then afforded **31** in 61 % yield together with a small amount of diacylated product (68 - 70).

When benzylation of the 4 position of **31** using benzyloxy-2,2,2-trichloroacetimidate and trifluoromethanesulfonic acid (71) was attempted no reaction was observed. The surprising inertness of **31** to the acidic reaction conditions was attributed to the possible protonation of the dimethylaminobenzoyl group by the protic acid, rendering the positively charged compound inert to further approach by a positive benzylating agent.


OBz
BzO
BzO
OBz
Br
27
NaBH4, CH3CN
nBu4N+I-, r.t.
28
Ph
NaOMe/MeOH
r.t.,
(75% over 2
steps)
OSiPh2tBu
HO
HO
30
tBuPh2SiCl, DMF
imidazole, r.t.
(84%)
OH
29
1) nBu2SnO,
toluene, reflux
2)
Cl
, Et3N,
dioxane,
(61%)


OSiPh2tBu
HO
Ph
N

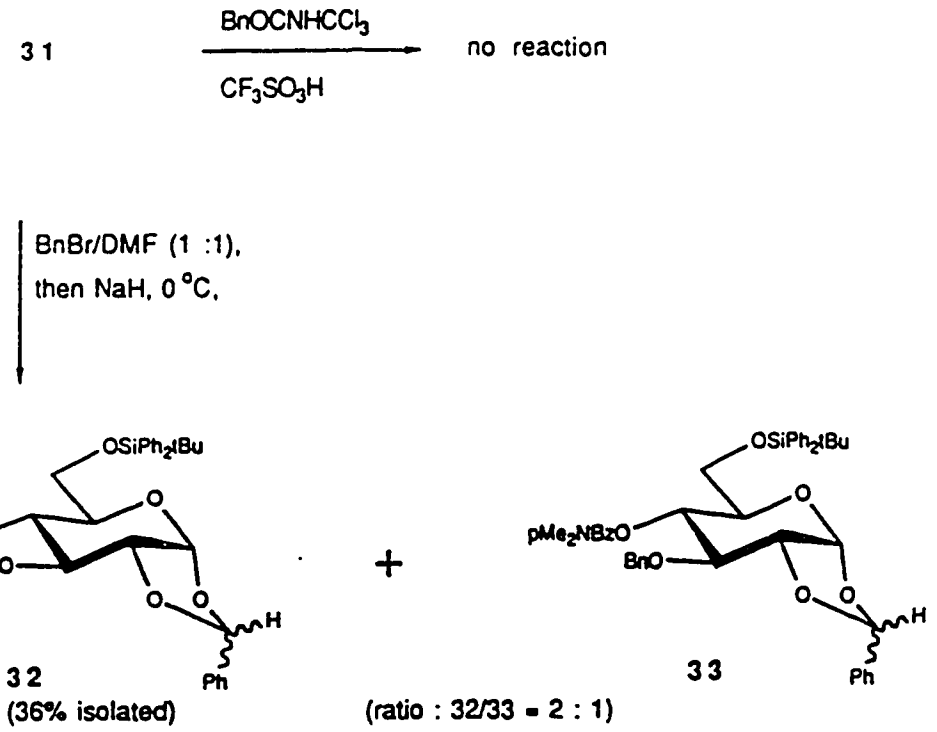
31

Boron trifluoride etherate was therefore substituted for the trifluoromethane sulfonic acid, but under these conditions **31** decomposed.

Migration of acyl groups under basic benzylation conditions is a common occurrence (72). Basic benzylation conditions now had to be considered however, and it was hoped that migration would not be a problem in this case since trans migrations of acetyl groups can often be avoided by using cold temperatures. The much less reactive *p*-N,N-dimethylaminobenzoyl group should migrate even slower than an acetate group. However, upon treatment of **31** with 4 equivalents of benzyl bromide and excess of sodium hydride in DMF, extensive migration took place and both **32** and **33** were formed in a ratio of about 1 : 1.

Flattening of the pyranose ring in **31**, as indicated by the coupling constants ($J_{2,3}$= 3.0 Hz, $J_{3,4}$= 2.8 Hz), brings the 3 and 4 hydroxyl groups into closer proximity and thus eliminates the disfavouring factor for the trans-migration. Later results also indicated that the 3 position of **31** is quite sterically hindered, which might also allow migration to compete with benzylation under these conditions. The ratio of **32** to **33** could only be improved by increasing the amount of benzyl bromide, thus enhancing the rate of the bimolecular reaction over the intramolecular migration. Using benzyl bromide : DMF (1 : 1) as the solvent, **32** and **33** were formed as the only products in a 2 to 1 ratio, but only 36 % of **32** could be obtained in pure form from this mixture due to comigration of the products upon chromatography.

The above benzylation conditions were useful for small scale reactions, but the large amount of benzyl bromide present in the reaction mixture severely complicated the chromatography when the reaction was scaled up even to a one gram scale. After three large columns failed to provide more than

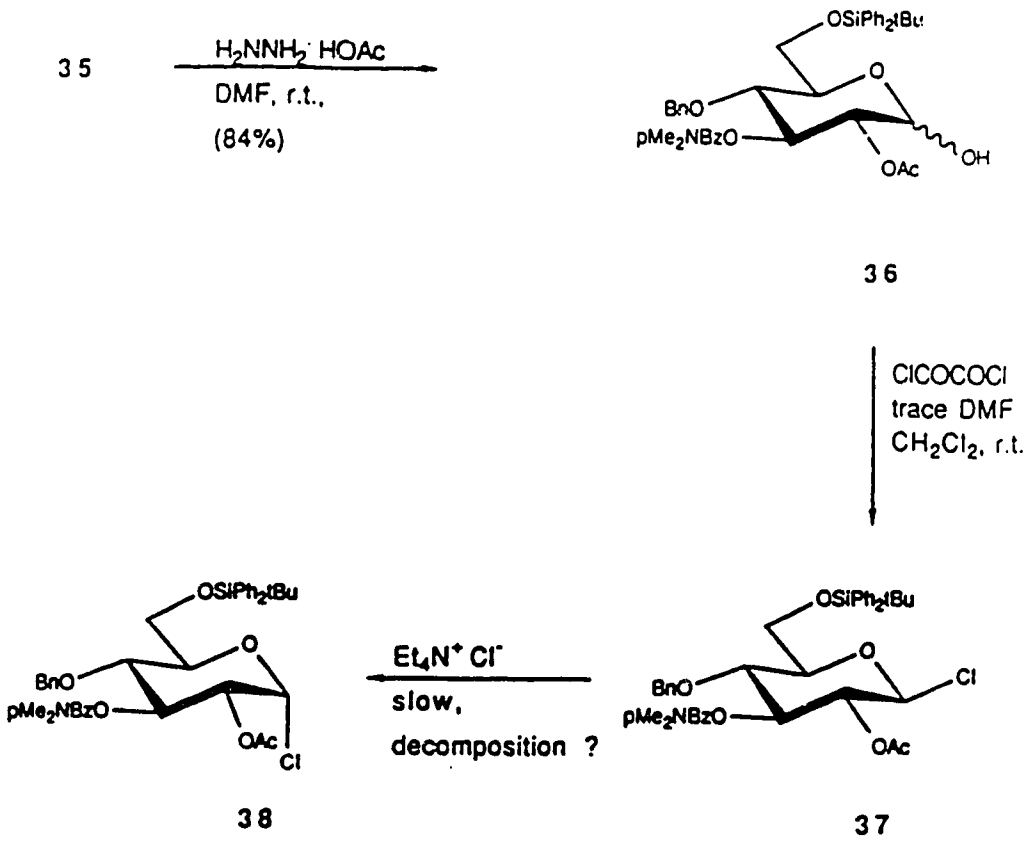

31
BnOCNHCCl3
CF3SO3H
no reaction
BnBr/DMF (1 :1),
then NaH, 0 °C,
OSiPh2tBu
BnO
pMe2NBzO
O
H
Ph
32
(36% isolated)
+
33
(ratio : 32/33 = 2 : 1)
90% CF3CO2H,
CH3CN, 0 °C, (96%)
or
90% CF3CO2H,
CH2Cl2, 0 °C, (90%)
OH
34
Ac2O, DMAP
CH2Cl2, pyridine
r.t.,
(96%)
OAc
35

about 10% of pure product, it was obvious that the benzyl bromide had to be removed somehow prior to chromatography. Conversion of benzyl bromide to benzyl alcohol by hydrolysis with water was slow and removal of the resulting benzyl alcohol by stirring with aluminum oxide was not efficient. Benzyl bromide reacted however exothermically with pyridine or a solution of pyridine in dichloromethane to give the water soluble N-benzylpyridinium bromide salt (73).

Isolation of **32** could be simplified therefore by diluting the reaction mixture with dichloromethane, extracting with water to remove all sodium hydride, then drying and addition of pyridine. Two to twenty four hours later, depending upon the dilution, all the benzyl bromide had reacted and the solution was re-extracted with water to remove the pyridinium salt. The sodium hydride was removed first to avoid the potential formation of an ylid of the pyridinium salt which might cause side reactions. Removal of the benzyl bromide by this procedure did not affect the products and large scale reactions could thus be executed successfully.

The 1,2-O-benzylidene group in **32** was easily removed using 90 % aqueous trifluoroacetic acid in either acetonitrile or dichloromethane to provide **34** in over 90 % yield (67). Compound **32** reacted much faster than 1,2-O-benzylidine-3,4,6-tri-O-benzoyl-β-D-glucopyranoside **28** (10 minutes versus 2 hours), which indicates that **32** is much more electron rich than the tribenzoyl derivative and thus formation of the anomeric carbocation is much easier.

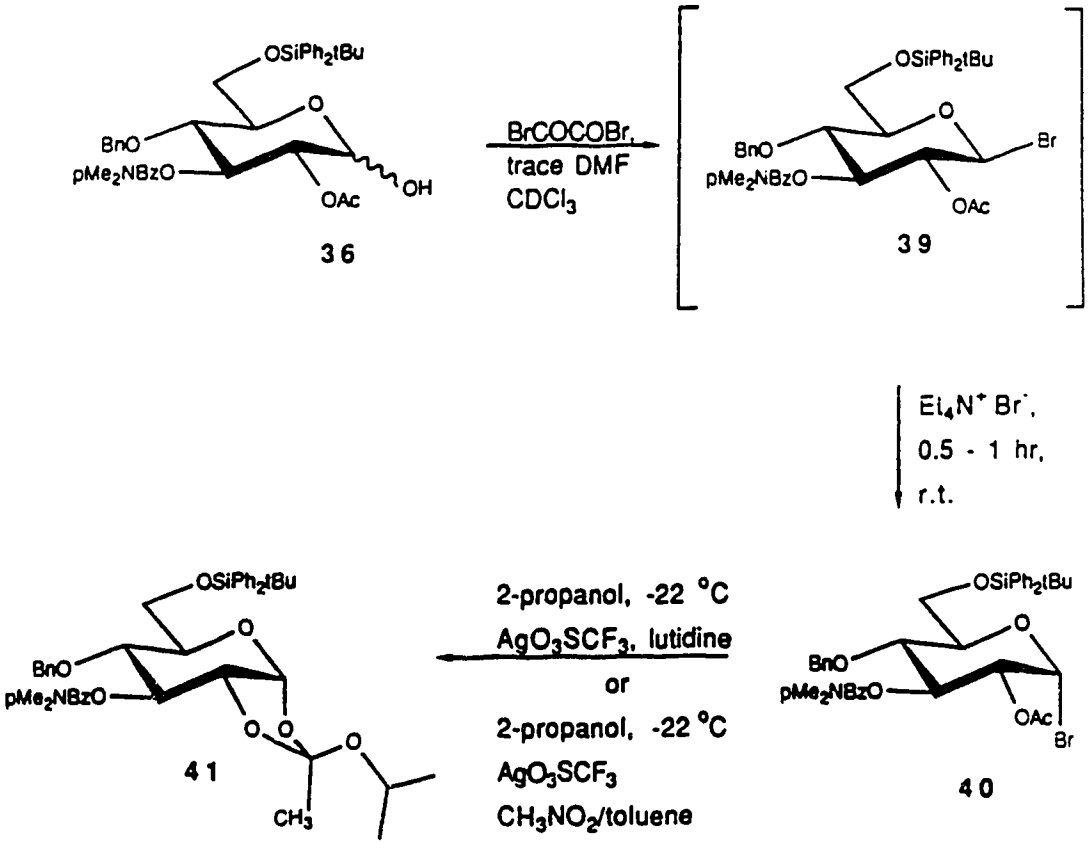
Acetylation of **34** to **35** proceeded smoothly in 96 % yield using acetic anhydride and DMAP in pyridine. Selective removal of the anomeric acetate (74) in **35** was readily achieved using hydrazine acetate in DMF at room temperature providing **36** in 84 % yield.



During the transformation of **36** into the glycosyl chloride **38** using oxalyl chloride and trace DMF (75) in dichloromethane extensive gas evolution was observed, but t.l.c showed only the starting material **36**. The reaction was thus performed in deuterated chloroform and followed by n.m.r. spectroscopy. The spectra revealed the presence of only one compound with a β-anomeric configuration which was thought to be the β-glucopyranosyl chloride **37** (δ = 5.376 p.p.m., d, H_1, $J_{1,2}$=8.5 Hz). Chloride **37** did not react when it was used under standard glycosidation conditions (silver

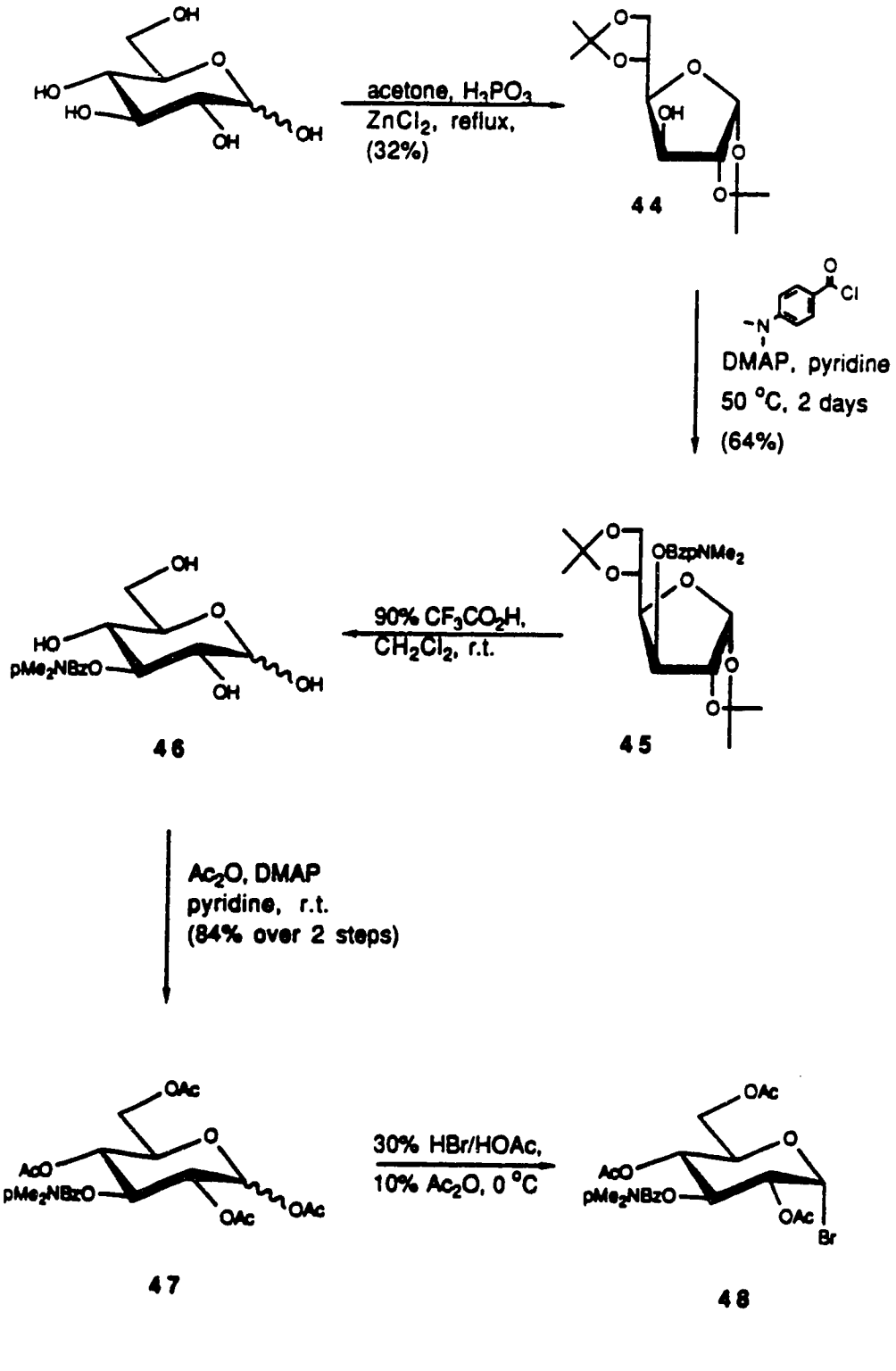
trifluoromethanesulfonate, ROH) (76,77), but decomposed under aqueous work up conditions. The inertness towards glycosylation in contrast to its extreme lability towards water was extremely puzzling and shed doubt on the presence of an anomeric chloride. To investigate whether the compound formed by the reaction of **36** with Vilsmeyer's reagent was indeed the chloride **37**, tetraethylammonium chloride was added to promote what is usually a rapid anomerization of **37** to the α-chloride **38.** Little, if any, rearrangement took place within 2 to 3 days and the reaction was accompanied by what seemed to be extensive decomposition. The failure to rearrange the product to the α-chloride cast even more doubt on whether the β-chloride **37** had indeed formed. It was therefore decided to try to transform **36** into the anomeric bromide using oxalyl bromide/DMF. Initially the n.m.r. spectra showed again the formation of a β-compound which was spectroscopically very similar to the one formed with oxalyl chloride (δ = 5.578 p.p.m., d, H_1, $J_{1,2}$ = 8.5 Hz). This compound seemed to decompose after several hours providing some α-bromide **40** (δ = 6.642 p.p.m., d, H_1, $J_{1,2}$= 4 Hz) and other compounds whose nature could not be identified from the crude spectral data. Thirty minutes to one hour after the addition of tetraethylammonium bromide, however, the mixture had cleanly transformed into the α-bromide **40**. This result suggested that the β-compounds formed initially in both reactions were indeed the β-chloride **37** and β-bromide **39**, even though they behaved rather strangely.

β-Bromides are usually not observed unless special conditions such as low temperatures are applied and an explanation for the abnormal behavior observed in this case had to be found. Careful reexamination of the spectra obtained from the crude products of both reactions showed that there was a significant downfield shift in the methyl proton and ajacent phenyl ring proton signals of the *p*-N,N-dimethylaminobenzoyl group in **37** and **39**.


OSiPh2tBu
BnO
pMe2NBzO
OAc
OH
36
BrCOCOBr,
trace DMF
CDCl3
OSiPh2tBu
BnO
pMe2NBzO
OAc
Br
39
Et4N+ Br-,
0.5 - 1 hr,
r.t.
OSiPh2tBu
BnO
pMe2NBzO
41
CH3
2-propanol, -22 °C
AgO3SCF3, lutidine
or
2-propanol, -22 °C
AgO3SCF3
CH3NO2/toluene
OSiPh2tBu
BnO
pMe2NBzO
OAc
Br
40
R
O
H
N
CH3
6.7 ppm
3.05 ppm
42
H+
lutidine
8.1 ppm
3.3 ppm
43

This shift was attributed to protonation of the dimethylamino group under the acidic reaction conditions (see structures **42** and **43**). Addition of lutidine to the reaction mixture confirmed this interpretation by causing the signals to return to their normal chemical shifts. It was therefore understandable why the β-chloride had showed inertness towards glycosidation, while decomposing instantly with water. The glycosidation reactions are highly acidic and protonation of the dimethylamino group should occur. Having already one positive charge in the molecule, introduction of a second one at the anomeric center should be hampered and thus glycosidation should proceed slowly or not at all. On the other hand addition of water into the reaction mixture should cause rapid transfer of the proton from the dimethylaminobenzoyl group (pKa of the protonated methyl or ethyl p-N,N-dimethylaminobenzoates = 2.30 relative to water, (78)) to the stronger base water and the now unprotonated β-chloride **37** decomposes to the anomeric alcohol **36.**

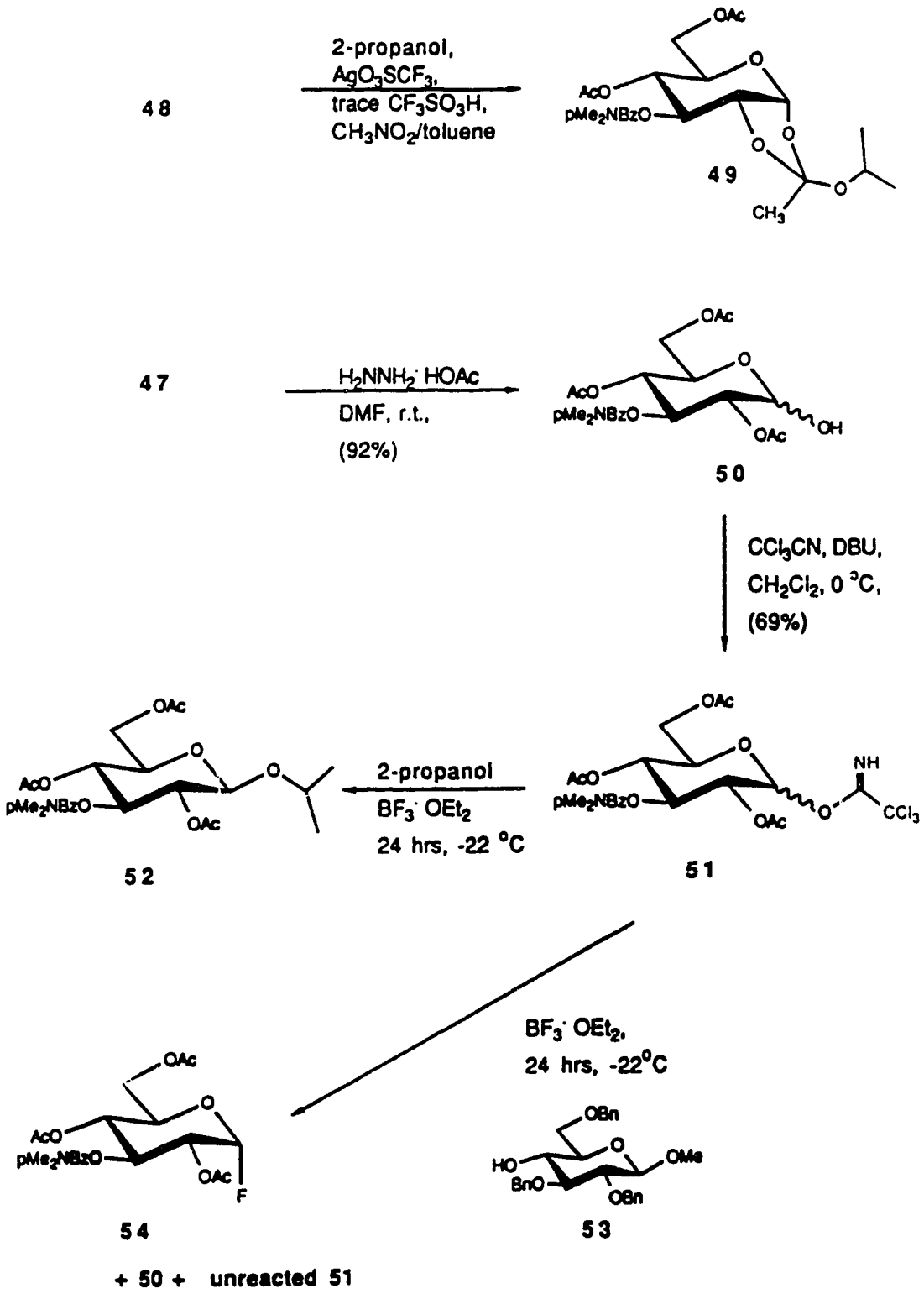
It was possible to purify the α-bromide **40** by aqueous extraction and the bromide was therefore used instead of the β-chloride in the subsequent glycosylation reactions. When **40** was reacted with 2-propanol and silver trifluoromethanesulfonate in the presence of excess lutidine the orthoester **41** was formed as expected under the basic conditions. Even without the addition of lutidine, however, the orthoester **41** was formed as the only product. It was, therefore, obvious that the glycosidation of this glucose derivative would not be facile and although there was indication that the *p*-N,N-dimethylaminobenzoyl group caused most of the trouble in these reactions, steric effects due to the *t*-butyldiphenylsilyl group could not be excluded. A simple analogue (**50**) was therefore prepared in five steps starting from glucose to help clarify the origins of the glycosidation problem. D-Glucose was treated with zinc dichloride and phosphoric acid in acetone (79) to provide


acetone, H_3PO_3
$ZnCl_2$, reflux,
(32%)
44
DMAP, pyridine
50 °C, 2 days
(64%)
90% CF_3CO_2H,
CH_2Cl_2, r.t.
46
45
Ac_2O, DMAP
pyridine, r.t.
(84% over 2 steps)
30% HBr/HOAc,
10% Ac_2O, 0 °C
47
48

the di-O-isopropylidene derivative **44.** The now accessible 3-hydroxyl group was acylated using *p*-N,N-dimethylaminobenzoyl chloride and DMAP in pyridine at 50 °C to provide the compound **45** (64 %). Removal of the isopropylidene protecting group of **45** in 90 % aqueous trifluoroacetic acid at room temperature (79) gave **46**, which was immediately acetylated using acetic anhydride in pyridine/DMAP to yield **47** in an overall yield of 84 % (80). The acetate **47** was then transformed into the anomeric bromide **48** by hydrogen bromide in glacial acetic acid. This procedure was chosen instead of the two step sequence using oxalyl bromide as it provided the α-bromide directly and could therefore be monitored more easily by t.l.c.

The bromide **48** was then reacted again with 2-propanol using silver trifluoromethanesulfonate (75,77) as was done with **40**. Under all conditions employed only the orthoester **49** was produced. Even when a trace of trifluoromethanesulfonic acid was added to ensure an excess of protons over dimethylamino groups (which might have provided an internal base that prevented the rearrangement of the orthoester), only orthoester was observed. The resistance of the orthoester to rearrangement, even in the presence of excess acid, is again most likely due to the protonation of the dimethylaminobenzoyl group which prevents the introduction of another positive charge into the molecule.

To avoid the protonation problem altogether, a glycosidation method was sought that did not involve the generation of protic acids. Anomeric trichloroacetimidates are commonly activated using boron trifluoride etherate (81), which might be less prone to interact with the dimethylamino group and therefore not deactivate the glycosylating agent or the intermediate orthoester. The diacetate **47** was therefore selectively deacylated at the anomeric position using hydrazine acetate in dry DMF (74) to provide **50**

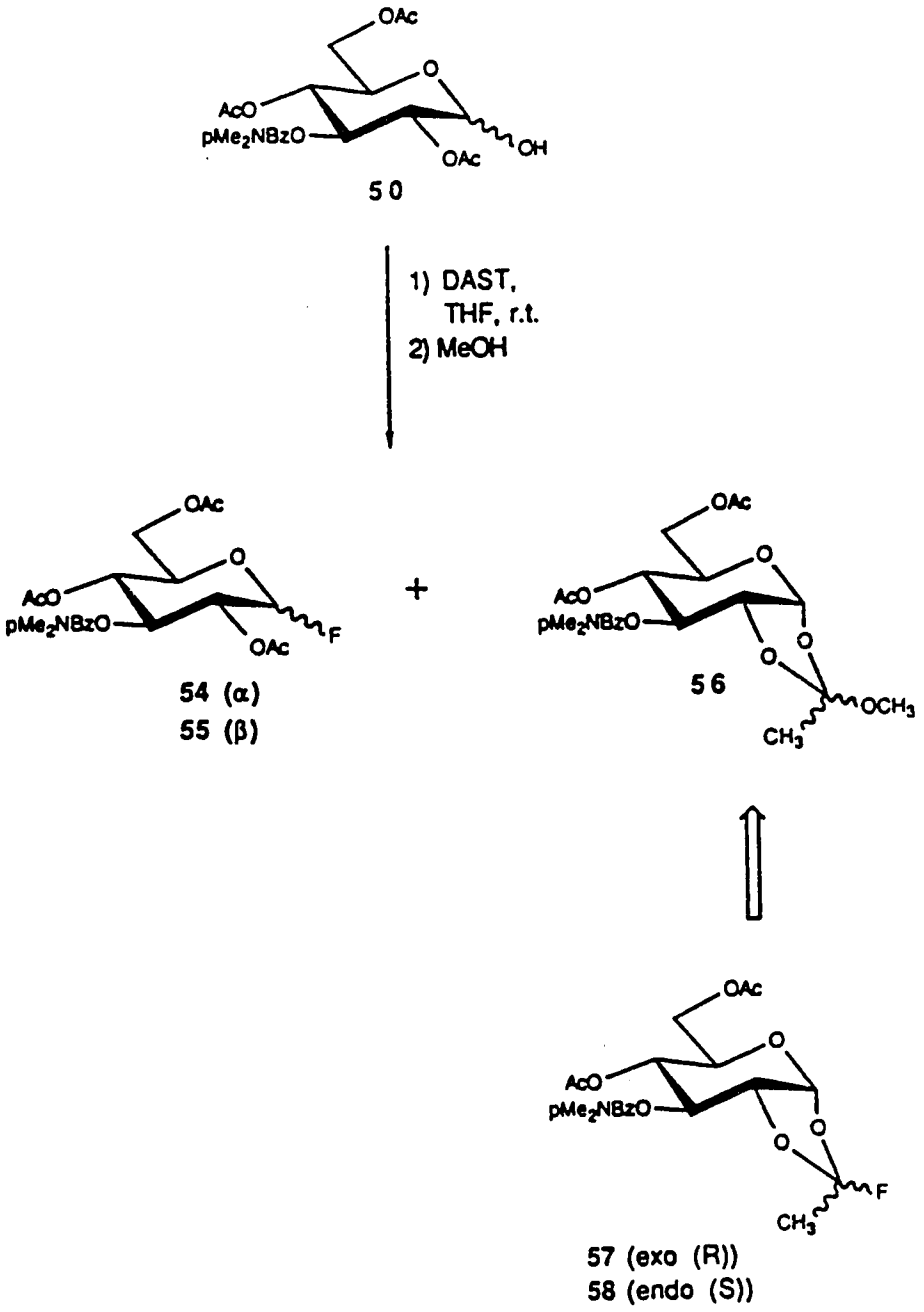

48
2-propanol,
AgO_3SCF_3,
trace CF_3SO_3H,
CH_3NO_2/toluene
49
47
$H_2NNH_2 \cdot HOAc$
DMF, r.t.,
(92%)
50
CCl_3CN, DBU,
CH_2Cl_2, 0 °C,
(69%)
2-propanol
$BF_3 \cdot OEt_2$
24 hrs, -22 °C
52
51
$BF_3 \cdot OEt_2$,
24 hrs, -22°C
54
53
+ 50 + unreacted 51

in 92 % yield, which was then converted to the imidate **51** in 69 % yield using 2,2,2-trichloroacetonitrile and DBU (81).

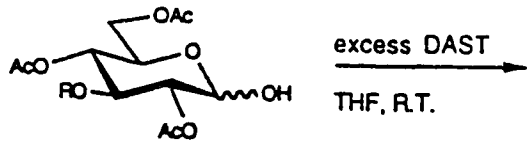
The imidate could be successfully employed in the glycosidation of 2-propanol using boron trifluoride etherate as the catalyst, to provide only the isopropyl β-glycoside **52** as determined by n.m.r. spectroscopy. All attempts to glycosidate the hindered 4-hydroxyl group of the alcohol **53** failed, however, providing only hydrolysis products and a small amount of a compound that seemed to be the α-anomeric fluoride **54**. Alcohol **53** had been previously synthesized from the methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside by reaction with sodium cyanoborohydride (72,82)

To clearly identify the nature of the minor compound isolated in the above reaction as the α-anomeric fluoride, the anomeric fluoride **54** was synthesized separately to allow comparison of the product n.m.r. spectra. There are many methods available for the preparation of anomeric fluorides (83 - 85) and one of the most direct remains the reaction of reducing sugars with diethylaminosulfur trifluoride (DAST). The reducing sugar **50** was thus treated with DAST in tetrahydrofuran at 0 °C according to the procedure of Ogawa et al.(86). After rapid initial progress the reaction stopped at about 50 % fluoride formation and neither further addition of DAST nor increasing the temperature to 22 °C resulted in any further reaction. The reaction was quenched by addition of methanol and, surprisingly, t.l.c. now showed only one spot. It was therefore assumed that the fluorides had formed quantitatively in solution, but hydrolysed on the plate, as is often seen during anomeric chloride and bromide formation. This type of behavior was unexpected for the much more stable anomeric fluorides but no other logical explanation was apparent. Surprisingly the n.m.r. spectrum of the crude product mixture showed not only signals for the the α and β fluorides **54** and **55**, but also those of the methyl

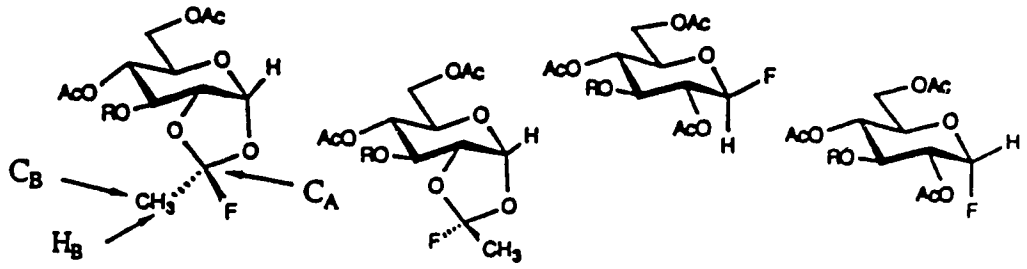
orthoacetates **56**, which must clearly have been formed after the addition of methanol. Under the methanolysis reaction conditions, the orthoester was most likely formed from the intermediate orthoacyl fluorides **57** and **58**.



Orthoacyl halides were thought to be highly unstable and previously had been observed only transiently at low temperatures (87,88) and their existences had not been considered initially as a possible explanation for the strange reaction results.



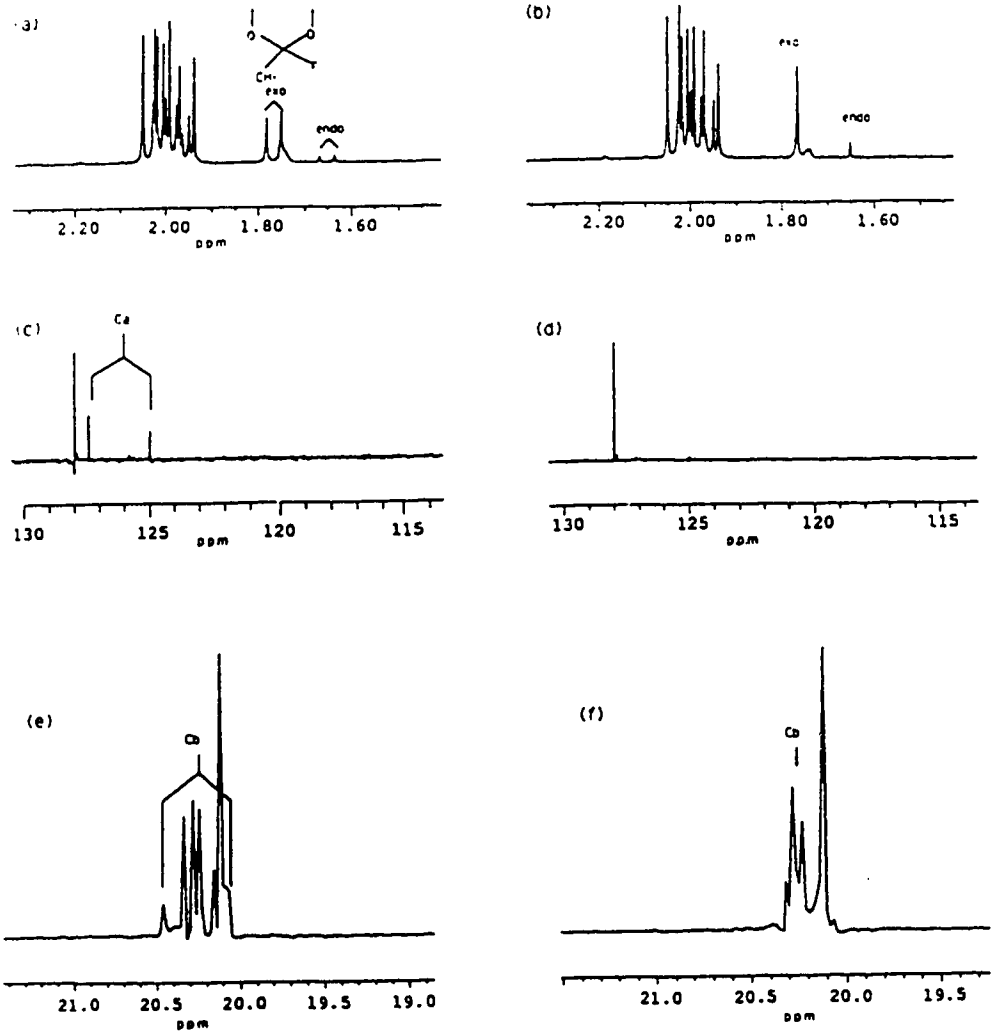
50 : R = p - N,N-Dimethylaminobenzoyl
59 : R = Acetyl



(R = pMe_2NBz)	57 (47%)	58 (5%)	55 (27%)	54 (21%)
(R = Acetyl)	60 (41%)	61 (6%)	62 (38%)	63 (15%)

Orthoacyl halides are postulated intermediates in the formation of anomeric halides involving neighboring group participation (89) and the opportunity to observe and characterize a stable member of this species could not be passed up. The reaction of 50 with DAST was repeated in an n.m.r. tube using deuterated THF as the solvent, and indeed a new species was found that showed the characteristic coupling constants for a distorted pyranose ring

Fig. 1 Partial ^{1}H and ^{13}C-n.m.r. Spectra of the Mixture Produced by the Reaction of **59** with DAST



recorded on a 400 MHz instrument; C_a indicates C-2 of the dioxolane ring C_b indicates the methyl group attached to the C-2 of the dioxolane ring

(a) ^{1}H - n.m.r. spectra : without decoupling; acetate region

(b) with ^{19}F decoupling at the orthoacyl fluoride; acetate region

(c) ^{13}C - n.m.r. spectra : without ^{19}F decoupling; C_a region

(d) with ^{19}F decoupling at the orthoacyl fluoride: C_a region; decoupling power was not sufficient to coalesce the peaks

(e) without ^{19}F decoupling; C_b region

(f) ^{13}C - n.m.r. spectrum with ^{19}F decoupling at the orthoacyl fluoride; C_b region

as seen generally for 1,2-O-orthoesters (J_{12}= 5.5 Hz, J_{23}= 2.8 Hz, J_{34}= 2-3 Hz). Addition of methanol to this mixture led to quantitative formation of the orthoesters from the orthoacyl fluorides, while the anomeric fluorides were not effected in any way (by ratios of n.m.r. product signals).

To investigate whether the unusual properties of the *p*-N,N-dimethylaminobenzoyl group had any influence on the formation of these orthoacyl fluorides, 2,3,4,6-tetra-O-acetyl-D-glucopyranose **59** was also reacted under the same conditions and similar results were obtained. The acetate region (1.5 - 2.1 ppm) of the spectra showed two doublets at 1.767 p.p.m. ($J_{H,F}$ = 12.5 Hz) and 1.652 ppm ($J_{H,F}$ = 13.0 Hz) in a ratio of about 9 : 1. To establish whether these signals corresponded to the fluorine coupled methyl protons (H-B) of the orthoacyl fluorides, fluorine decoupled proton spectra and fluorine decoupled ^{13}C spectra were obtained for the fluorides **60** to **63.** The partial spectra presented in figure 1 clearly show the coupling between fluorine and the methyl protons in spectra **a** and **b** and between fluorine and the methyl carbon (40 Hz) in spectra **e** and **f** as well as the large one bond ^{19}F - ^{13}C coupling (220 Hz) to the C-2 carbon of the dioxalane ring. The covalency of the carbon - fluorine bond as opposed to an ion pair arrangement was therefore established. Table 1 lists the chemical shifts and coupling constants of the ^{1}H and ^{19}F - n.m.r. spectra for compounds **52, 53, 57, 58** and table 2 the ^{1}H, ^{13}C and ^{19}F - n.m.r. data for compounds **60** to **63.** The chemical shifts and coupling constants for the anomeric fluorides **62** and **63** are in general accord with those reported in the literature (90) and the data obtained for the orthoacyl fluorides are similar to those of 1,2 orthoesters or 1,2 acetals (91).

Table 1 : Chemical Shifts [a] and Coupling Constants for Anomeric Fluorides **54,55** and Orthoacyl Fluorides **57,58** [b]

Nucleus	54	55	57	58
H-1 (J_{12}, J_{1F})	5.769 (2.7,54.0)	5.469 (6.8,52.3)	5.867 (5.3)	n.d.
H-2 (J_{23},J_{2F})	n.d. (10)	5.176 (9.0,12.0)	4.595 (3.0, -)	n.d.
H-3 (J_{34})	5.640 (10)	5.452 (9.5)	5.332 (2.5)	n.d.
H-4 (J_{45})	n.d.	5.282 (10.0)	4.16-4.18 (n.d.)	n.d.
H-5 (J_{56})	n.d. (4.5)	4.042 (5.0)	4.16-4.17 nd	n.d.
H-6 ($J_{66'}$)	4.347 (12.0)	4.273 (12.5)	5.052 (m)	n.d.
H-6' ($J_{56'}$)	4.100 (2.0)	4.138 (2.5)	3.914 (4.5)	n.d.
OAc	n.d.	2.005,1.915, 1.858	2.010,1.970	n.d.
H-B[c] ($J_{CH_3,F}$)	----	----	1.785 (12.5)	n.d.
19F (J_{F,H_2})	-150.0 (26.3)	-143.6 (12.0)	-56.8 (-)	-57.0 (-)
(J_{F,H_1})	(54.2)	(52.7)	(-)	(-)
(J_{F,CH_3})	(-)	(-)	(12.5)	(12.8)

The spectral data are reported with more accuracy (signilicant figures) than can be reasonably expected from the experiments to allow distinction of the different chemical shifts of the complex overlapping proton signals.

[a] Chemical shifts of 1H-NMR spectra are relative to TMS (δ = 0); Chemical shifts of 19F-NMR spectra are relative to external CFCl3 (δ = 0); Chemical shifts of 13C-NMR are relative to CDCl3 (δ = 77.05); Splittings are reported as if they were first order coupling constants

[b] 1H and 19F-NMR spectra are recorded in THF-d8; 13C-NMR spectra are recorded in $CDCl_3$

nd not determined

[c] H-B are the protons of the methyl group attached to the C-2 of the dioxalane ring

Table 2: Chemical Shifts [a] and Coupling Constants for the Anomeric Fluorides **62**, **63** and Orthoacyl Fluorides **60** and **61** [b]

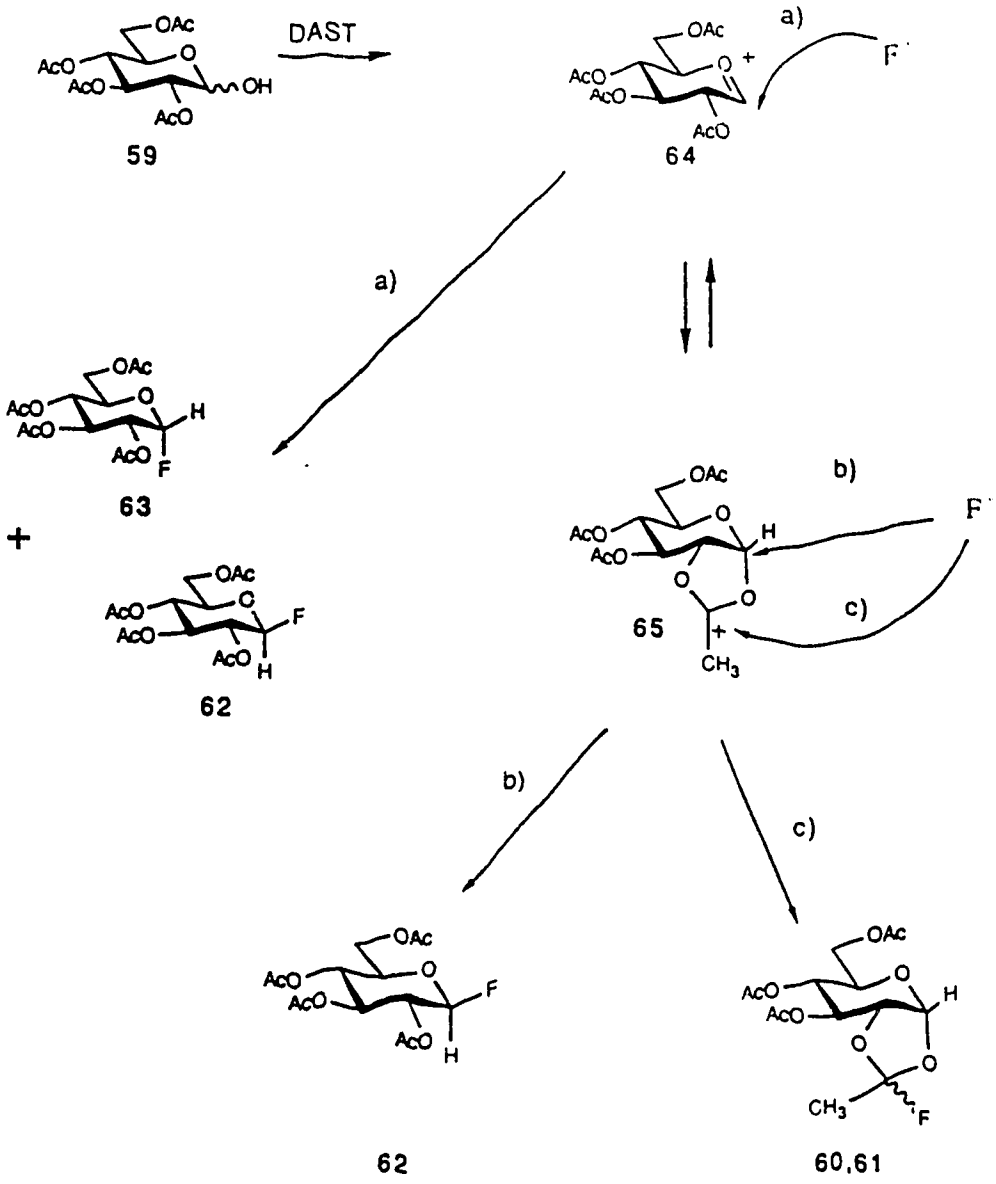
Nucleus	60	61	62	63
H-1 ($J_{1,2}$,$J_{1,F}$)	5.795 (5.5, -)	5 785 (6.0, -)	5.420 (7.0,53.0)	5.742 (2.7,53.0)
H-2 ($J_{2,3}$,$J_{2,F}$)	4.497 (2.8), -)	4.434 (5.0,3.0)	5.030 (9.0,12.0)	4.987 (10,25.0)
H-3 ($J_{3,4}$)	5.130 (2-3)	5.435 (8.0)	5.260 (9.0)	5.415 (10.0)
H-4 ($J_{4,5}$)	4.13-4.14 (n.d.)	n.d.	5.152 (10.0)	≅ 5.13 (10)
H-5 ($J_{5,6}$)	4.13-4.14 (n.d.)	n.d.	3.982 (5.0)	4.176 (4.5)
H-6 ($J_{6,6'}$)	4.884 (9.7)	4.317 (12.3)	4.242 (12.2)	4.360 (12.5)
H-6' ($J_{5,6'}$)	3.850 (m)	4.065 (2.3)	4.128 (2.5)	4.085 (2.1)
OAc	2.052,2.025, 1.992	1.965, n.d., n.d.	2.005,2.017, 1.970,1.937	2.030,2.002, 1.975,1.925
H-B ($J_{CH3,F}$)[c]	1.767 (12.5)	1.652 (13.0)	----	----
C-1 ($J_{C,F}$)	96.80	n.d.	106.05 (220.5)	103.60 (228.0)
C-2 ($J_{C,F}$)	72.55	n.d.	71.00 (28.5)	69.95 (24.5)
C-3 ($J_{C,F}$)	69.20	n.d.	71.53 (8.5)[c]	n.d.
C-4 ($J_{C,F}$)	67.75	n.d.	67.25	67.15
C-5 ($J_{C,F}$)	67.00	n.d.	71.75 (4.0)[c]	69.6 (4.5)
C-6 ($J_{C,F}$)	62.75	n.d.	61.55	61.00
C_a($J_{C,F}$)[c]	126.30 (249.0)	n.d.	----	----
C_b ($J_{C,F}$)[c]	20.20 (41.0)	n.d.	----	----
^{19}F ($J_{F,2}$)	- 56.9 (-)	- 52.6 (3.0)	- 142.0 (11.8)	- 150.4 (24.5)
($J_{F,1}$)	(-)	(5.0)	(53.7)	(53.0)
($J_{F,CH3}$)	(13.0)	(13.0)	----	----

The spectral data are reported with more accuracy (signilicant figures) than can be reasonably expected from the experiments to allow distinction of the different chemical shifts of the complex overlapping proton signals.[a] Chemical shifts of the ^1H- n.m.r. resonances are relative to that of Me_4Si ($\delta = 0$); of ^{19}F-n.m.r. to external $CFCl_3$ ($\delta = 0$) and of ^{13}C-n.m.r. are relative to $CDCl_3$ ($\delta = 77.05$); Splittings are reported as if they were first order coupling constants, [b]^1H and ^{19}F- n.m.r. spectra were recorded for solutions in tetrahydrofuran-d_8, and the ^{13}C-n.m.r. spectra for solutions in $CDCl_3$, **n.d.** not determined. [c] C_a is the C-2 of the dioxolane ring ; C_b is the carbon of the methyl group attached to the C-2 of the dioxolane ring; H-B are the protons of the methyl group attached to the C-2 of the dioxalane ring.

The crude reaction mixture had to be purified somewhat to obtain the ^{13}C- spectra since excess DAST interfered with the observation of the carbon signals. Since aqueous work-up caused instant decomposition of the orthoacyl fluorides, the reaction mixture was simply evaporated *in vacuo* and coevaporated with toluene to remove most of the DAST. The remaining residue could be dissolved in deuterated chloroform and the product mixture remained stable for several days showing no rearrangement of the orthoacyl fluorides. Addition of DAST to this mixture caused slow (about 60% in 2 hours) rearrangement of the orthoacyl fluorides to the anomeric fluorides. Addition of BF_3· etherate to the chloroform solution rearranged the orthoacyl fluorides to the anomeric fluorides within an hour, but failed to do so when added to the orthoacyl fluorides in the THF reaction mixture. Tetra-n-butylammonium fluoride and triflic acid also had no significant effect on the product ratios when added after completion of the reaction to the product mixture in THF. The remarkable stability of the orthoacyl fluoride when water or alcohols were excluded, led us to attempt their crystallization from dry diethyl ether and hexane, but only α and β- fluorides crystallized after a few days and re-examination of the mother liquor showed only minor amounts of orthoacyl fluorides.

The acyloxonium ion **65** is a commonly postulated intermediate in reactions involving neighboring group participation of 2-O-acetyl groups (90,21). It was therefore thought that the orthoacyl fluoride should also be observed in reactions using solvents other than THF. To our surprise, when a larger scale reaction was performed in the less expensive deuterated solvent chloroform, only the anomeric fluorides **62** and **63** were observed in a ratio of 3 to 1 even after only 5 -10 minutes of reaction time. The orthoacyl fluorides once formed were stable in chloroform for several days.

Scheme 7 : Possible pathways to the observed products during the reaction of reducing sugars with DAST



Even after BF_3-etherate or DAST had been added the orthoacyl fluorides rearranged only slowly over a period of an hour or longer to the anomeric fluorides. Hence it seems unlikely that orthoacyl fluorides are formed transiently during the reaction in chloroform and the reaction proceeds probably by different pathway than it does in THF.

Scheme 7 displays the commonly postulated mechanistic pathway for reactions at the anomeric center with neighboring group participation (89) such as glycosidations or anomeric halide formation. From the above observations both the oxocarbonium intermediate **64** and the acyloxonium ion **65** must be involved in the THF reactions, but the same does not seem to hold true for solvents like chloroform, where the intermediate **65** might not be formed. The prevalent formation of β-anomers during reactions with neighboring group participation in solvents like chloroform might therefore have other reasons than the formation of the acyl oxonium ion **65**. In an attempt to synthesize acyl orthoesters Wulff et al. (91) found that these could be formed in good yields by treatment of an anomeric glycosyl bromide with silver carboxylic acid salts in THF. Investigating the effect of THF they found an increase in α- glycoside formation in reactions without neighboring group participation when THF was used instead of other solvents (92). These observations were explained by the formation of a β - adduct between THF and the oxocarbonium ion **64**, thus increasing the amount of α- anomer formed in nonparticipating reactions and facilitating the formation of **65** for reactions involving neighboring participating groups such as 2-O-acetates.

It seems that the reason for the difference in the reaction path for THF as opposed to other solvents is the more facile formation and perhaps enhanced stability of the acyloxonium intermediate **65**. The question thus arises whether the equilibrium between **64** and **65** is established quickly or

whether a significant part of the β- fluoride is formed by attack on **64** and thus the rates of product formation and equilibrium formation are of the same order of magnitude.

Since the oxocarbonium ion **64** is postulated to be formed first in the reaction pathway and then rearranges to the ion **65**, an increase in the fluoride ion concentration prior to the addition of DAST should increase the amount of α and β-fluoride formed (path a) if the rate of attack on the intermediates by fluoride ion is comparable to the rate of transformation of **64** to **65**. If, however, the equilibrium is established quickly compared to the rate of fluoride addition, then the increase in fluoride concentration should have no effect.

Table 3: Ratios of the Orthoacyl Fluorides **60** and **61** to the Anomeric Fluoride **62** and **63** as a Function Varying Fluoride Concentration

Moles sugar ($\times 10^{-5}$)	Moles F^- ($\times 10^{-5}$) [equiv.][b]	[o.a.F(exo) : o.a.F(endo) : β -F : α-F][c] (in %)	o.a.F (**60**,**61**) : α,β-F (**62**,**63**) (in %)
2.920	0 [0.00]	43 : 9 : 35 : 13	52 : 48
2.920	1.62 [0.55]	41 : 12 : 31 : 16	53 : 47
2.920	3.24 [1.11]	42 : 11 : 32 : 15	53 : 47
2.920	6.47 [2.21]	45 : 7 : 32 : 16	52 : 48
2.920	9.71 [3.32]	44 : 7 : 29 : 20	52 : 48

[a] Fluoride ion is added as tetra n-butylammonium fluoride in THF d^8
[b] [equiv.] = equivalent fluoride to sugar concentration
[c] o.a.F = orthoacyl fluoride, α,β-F = α and β - anomeric fluorides
DAST concentration in each experiment was 1.514×10^{-4} moles

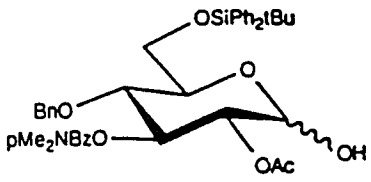
The results of increasing the fluoride concentration from 0 to about 3 equivalents are listed in table 3. As column 4 shows, the overall ratio of orthoacyl fluorides to α,β-fluorides does not change with increasing fluoride concentration, which indicates that **65** is formed quickly from **64** and that the products are mainly produced by attack on the established equilibrium.

Examples of the use of THF as the solvent to prepare anomeric fluorides involving neighboring group participation are rare (93).The reported yields of about 50 % anomeric fluorides with about 40 % recovery of the starting material are consistent with the formation of orthoacyl fluorides in these cases. 1,2-Dimethoxyethane (94 - 96) is more commonly used as the solvent because then quantitative yields of the anomeric fluorides were obtained. It therefore seems that THF is somewhat special in its behavior, but other ethers should also be investigated. As indicated by Wulff et al. (92) diethyl ether, however, does not have the same effect .

The hydrolysis pathway of the orthoacyl fluoride should involve the intermediate orthoacid and some α-1-O-acetate derivative should therefore also be formed during the hydrolysis of the orthoacyl fluorides. This species is indeed observed as a minor component among the products obtained by aqueous decomposition of **60** and **61**.

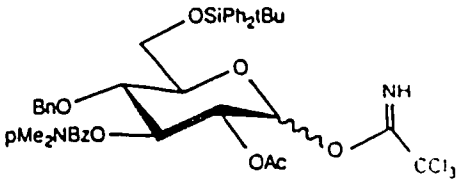
Although much more work could have been performed on the formation, rearrangement and decomposition of this orthoacyl fluoride, we felt that the experiments performed characterized the orthoacyl fluorides sufficiently and returned to the initial synthetic studies into the formation of β-mannosides by intramolecular inversion. A manuscript describing the characterization of the orthoacyl fluorides by n.m.r. spectroscopy was recently accepted for publication (97).

Returning to the glycosidation studies, it had been found, that by the use of anomeric imidates and their activation with BF_3·etherate, β-glycosides of reactive alcohols could be formed even in the presence of the *p*-N,N-dimethylaminobenzoyl group.



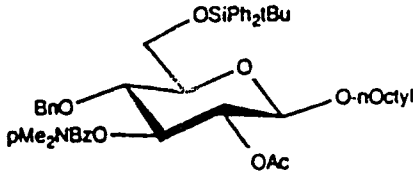
36

CCl_3CN, DBU
-22 °C, CH_2Cl_2
(98%)



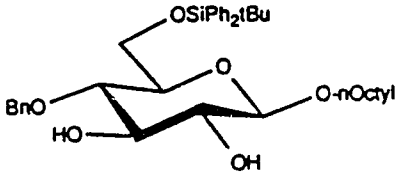
66

n-octanol, BF_3 OEt_2,
CH_2Cl_2, -22 °C,
(36%)

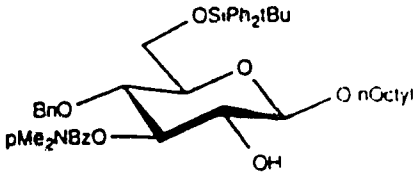


67

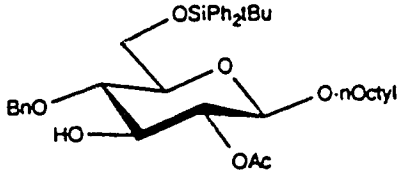
see table 4



69



68



70

It was therefore decided to produce the n-octyl glycoside of compound **36**, since the n-octyl group offers considerable advantages in the isolation and purification of unprotected oligosaccharides using adsorption onto reverse phase matrices.

The reducing sugar **36** was treated with 2,2,2 trichloroacetonitrile and DBU (81) in dichloromethane at -22 °C to provide the imidate **66** in 98 % yield as an α,β mixture. Reaction of **66** with n-octanol in dichloromethane using BF_3·etherate at -22 °C provided the octyl glycoside **67** in only 36 % isolated yield although the reaction appeared almost quantitative by t.l.c.. This yield was not optimized since subsequent selective removal of the 2 -O-acetyl group to provide **68** failed.

There are many methods reported in the literature to remove acetates selectively in the presence of benzoates (56) as well as methods to remove 2-O-acetates in the presence of acetates at other positions of a sugar pyranoside (54,57,58,98). It had therefore been assumed that the removal of a 2-O-acetate in the presence of a 3-O-*p*-N,N-dimethylaminobenzoyl group should proceed with good selectivity, but this was not the case. Table 4 lists all the conditions employed and the ratios of products formed. A reason for the nonselectivity and the slow rates could be that the compound is sterically hindered towards attack at the 2-position and thus reaction at the 3 position also occurs. After one group has been removed, the removal of the next one is then accelerated as the compound becomes less hindered.

Table 4 : Summary of Deacetylation Attempts for Compound **67**

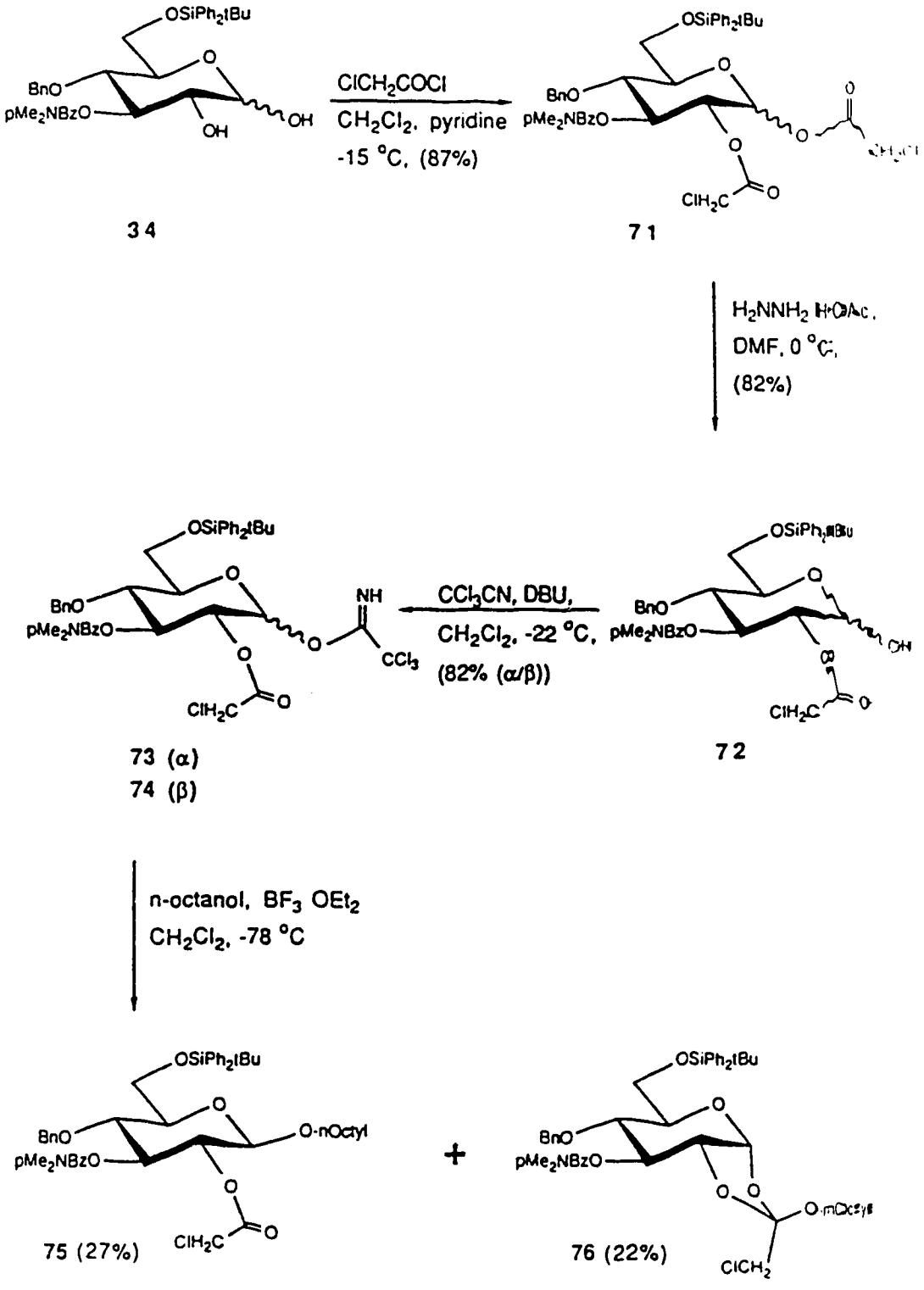
Method of Deacetylation	**approx. Rate** (% **67** converted to Products / Time)	**approx. Ratios of Products** (by TLC) **68 : 69 : 70**	**Ref. #**
NaOMe, MeOH / CH_2Cl_2 (1 : 1), 0 °C	50 % / 2.5 hrs	5 : 5 : 1	54
$H_2NNH_2 \cdot H_2O$, r.t., pyridine	30 % / 24 hrs	7 : 4 : 1	55
sat. H_3N / MeOH, r.t. → 40 °C	no rxn / 24 hrs, add H_2O, then 60 % / 72 hrs	1 : 3 : 3	56
Et_3N, (40 µL H_2O / mL MeOH : CH_2Cl_2 (1 : 1), r.t.	20 % / 24 hrs	2 : 1 : 0	
$H_2NNH_2 \cdot HOAc$, DMF, 40 °C	no rxn.	-	
$H_2NNH_2 \cdot H_2O$, r.t. pyridine / HOAc (4 : 1)	60 % / 24 hrs	1 : 1 : 0	55
KOtBu / tBuOH, r.t.	60 % / 48 hrs	3 : 3 : 1	57
3 % HCl / MeOH, 0 °C	50 % / 24 hrs	decomposition	58,30

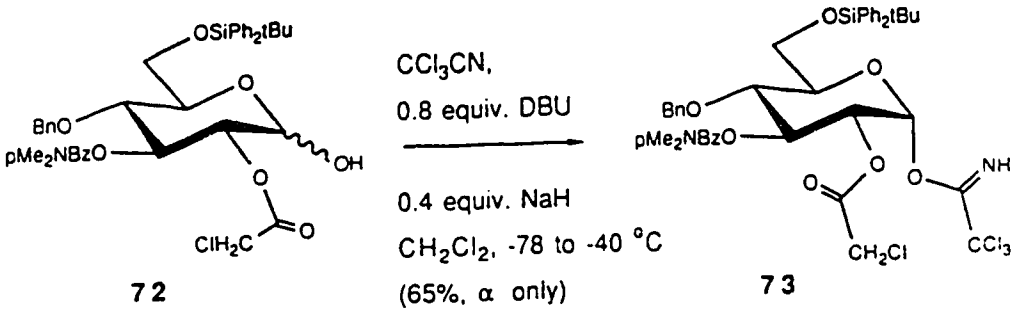
The chloroacetyl group was therefore chosen to replace the acetyl group as a protecting group for the OH-2, since it is a participating group and

can be removed with weak nucleophiles as well as with thiourea (99). If selective removal of the chloroacetate by hydrolysis should fail as seen for the acetate, then the use of thiourea might be successful in removing the acyl group. Thiourea first displaces the chloride at the C-2 of the chloroacetyl group and then removes the acyl group by intramolecular attack. The initial attack of thiourea is therefore specific for the chloroacetyl group and should be little affected by steric hindrance (100).

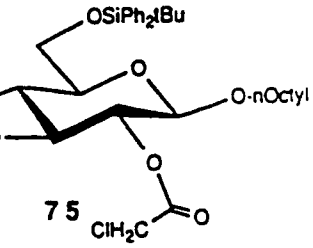
The diol **34** was therefore treated with 2-chloroacetyl chloride in dichloromethane containing pyridine at -15 °C to provide **71** in 87 % yield (101). Chromatography of **71** had to be rapid since the chloroacetyl groups were removed during purification on silica gel columns. The derivative **71** could be selectively deacylated at the anomeric oxygen using hydrazine acetate in DMF (73) at 0 °C to yield 82 % of the reducing sugar **72**. Conversion of **72** to the α,β-imidates **73** and **74** (5 : 2) proceeded in 82 % yield using 2,2,2-trichloroacetonitrile and DBU (81) in dichloromethane at -22 °C. Glycosidation of n-octanol using the imidates and BF_3· etherate in dichloromethane at -78 °C gave the β-glycoside **75** in 27 % yield and the orthoester **76** in 22 % yield. Loss of the chloroacetyl group seemed to occur during the reaction as well as some other decomposition reactions.

Attempts to rearrange the orthoester to the β- glycoside (102) using BF_3· etherate or mercuric acetate at room temperature failed. The sluggishness of the orthoester towards rearrangement had been expected since even octyl orthoacetates rearrange very slowly.The electron withdrawing nature of the chloride in **76** should slow rearrangement even more, but it was unfortunate that no rearrangement could be obtained at all in this case.


OSiPh2tBu
BnO
pMe2NBzO
OH
ClCH2COCl
CH2Cl2, pyridine
-15 °C, (87%)
ClH2C
34
71
H2NNH2 HOAc,
DMF, 0 °C,
(82%)
NH
CCl3
CCl3CN, DBU,
CH2Cl2, -22 °C,
(82% (α/β))
73 (α)
74 (β)
72
n-octanol, BF3 OEt2
CH2Cl2, -78 °C
O-nOctyl
75 (27%)
+
76 (22%)
ClCH2

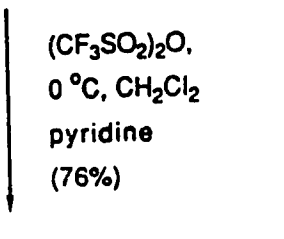

OSiPh2tBu
BnO
pMe2NBzO
OH
ClH2C
CCl3CN,
0.8 equiv. DBU
0.4 equiv. NaH
CH2Cl2, -78 to -40 °C
(65%, α only)
NH
CCl3
CH2Cl

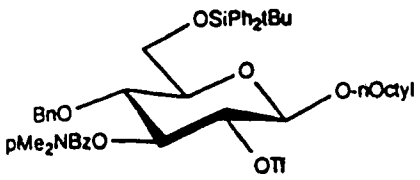
72 73


n-octanol, BF3 OEt2
CH2Cl2 : hexane (1 : 9)
10 volume% CH3CN
-22 °C,
(65%)

OSiPh2tBu
BnO
pMe2NBzO
O-nOctyl
OH
H2NNH2· HOAc
DMF, r.t., (96%)
or
H2NNH2· HOAc
MeOH/CH2Cl2 (1 : 1)
r.t., (69%)
ClH2C

68 75


(CF3SO2)2O,
0 °C, CH2Cl2
pyridine
(76%)


OSiPh2tBu
BnO
pMe2NBzO
O-nOctyl
OTf

77

Changing the solvent to nitromethane resulted in an increase in orthoester formation, while the use of dichloromethane : hexane (1 : 5) or (1 : 9) seemed to give some improvement in the ratio of glycoside to orthoester as judged by t.l.c.. These improvements were however only modest (about 10 % more glycoside).

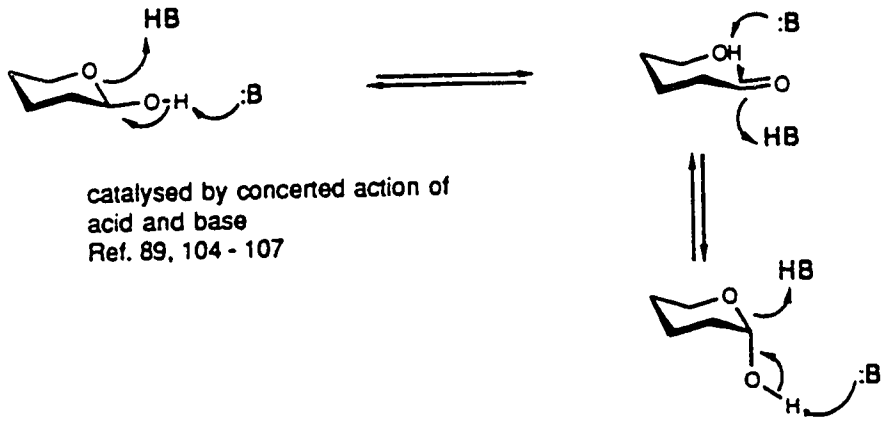
In one reaction, reacting the α,β-imidates **73** and **74** with BF_3· etherate in dichloromethane - hexane (1 : 9) a small reduction in the amount of orthoester as compared with the amount of β-glycoside was observed (about 1 : 3 instead of 1 : 2 by t.l.c.). One possible difference to previous reactions was that a trace contamination with 2,2,2-trichloroacetonitrile could have occurred. Repeating the reaction in the presence of 2,2,2-trichloroacetonitrile indeed improved the amount of β-glycoside from 39 % to 50 % as judged from the integration of crude mixture n.m.r. spectra. The initial assumption, that this improvement was due to rearrangement of the orthoester mediated by 2,2,2-trichloroacetonitrile, proved to be wrong since the initial ratio of orthoester to glycoside did not change after prolonged incubation

It was therefore assumed that 2,2,2-trichloroacetonitrile somehow prevented the orthoester formation during the reaction itself. Recently Sinay et al. (103) used acetonitrile and proprionitrile as a solvent mixture in glycosidation reactions involving no neighboring group participation and obtained mainly β- glycosides, presumably by the formation of an α-adduct between acetonitrile and the oxocarbonium ion. Formation of a similar adduct may also be the reason for the observed reduction in the formation of orthoester.

Chromatography in ethylacetate : dichloromethane : hexane (1 : 2 : 6) allowed the separation of the two anomers of the imidates and thus the reactivity of **73** and **74** were investigated separately. Using only the

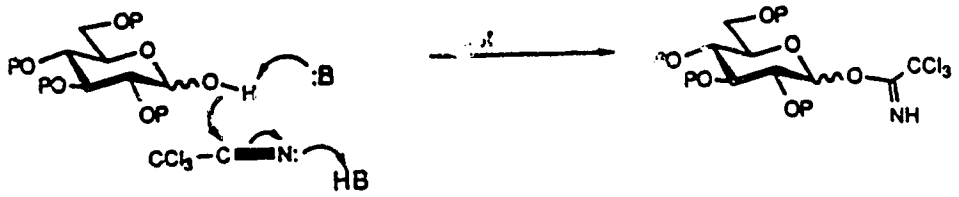
Scheme 8 : Possible Mechanism for the Formation of Anomeric Trichloroacetimidates

a) Mechanism of the mutarotation of anomeric reducing sugars

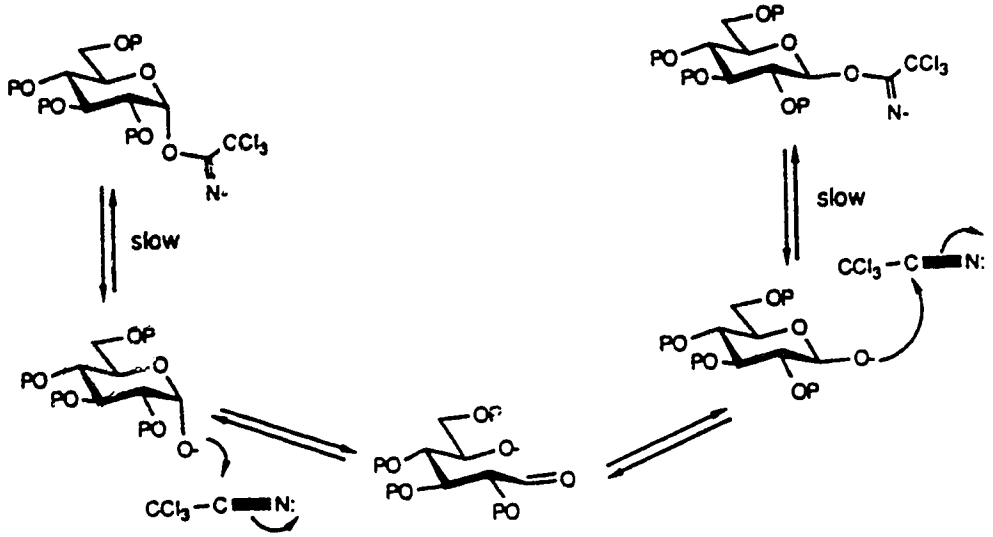


b) Possible mechanism of trichloroacetimidate formation

i) in the presence of a proton source



ii) without proton source

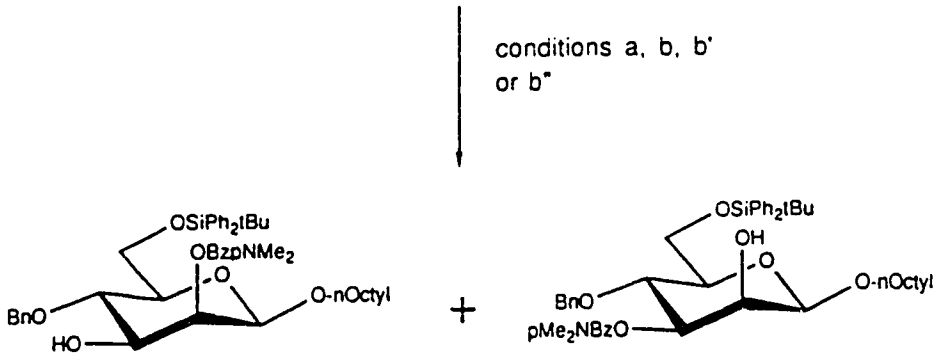


α-imidate **73**, n-octanol was glycosidated using BF_3·etherate in dichloromethane : hexane (1 : 9) containing 10 % acetonitrile to surprisingly provide only β- glycoside (quantitative by n.m.r., isolated yield 65%). Reaction of the β-imidate **74** under identical conditions provided only orthoester. It thus seems that the reaction of the α- imidate does not involve neighboring group participation under these conditions.

In a procedure using sodium hydride and 2,2,2-trichloroacetonitrile in dichloromethane at room temperature , Schmidt et al. (81) obtained 80 % of the α-imidate of 2,3,4,6-tetra-O-benzyl-D-glucopyranose and 2,3,4,6-tetra-O-acetyl-D-glucopyranose, but when the reducing sugar **72** was treated accordingly, the compound decomposed totally and at a lower temperature the reaction did not proceed at all. Considering the possible mechanism of this reaction, it appears that the formation of the imidate should be best catalysed by the presence of both a base and a proton source similar to the mechanism for the mutarotation of reducing sugars (see scheme 8, 89,104,105). The reaction using excess sodium hydride eliminates any proton source from the reaction medium and should therefore be slow compared to reactions using DBU. DBU can act not only as a base but also as a proton donor, once some of its conjugate acid has been formed. Indeed, while the reaction using sodium hydride is slow even at room temperature, the DBU reaction proceeds rapidly even at -78 °C.

Combining the characteristics of both reactions it might therefore be possible to obtain an increased yield of α-imidate at a lower temperature. The imidate has to be deprotonated to allow reversal of the reaction and mutarotation of the sugar, but a proton shuttle has to be present to allow efficient catalysis of imidate formation. The reaction was therefore performed using 2,2,2-trichloroacetonitrile at -40 °C in the presence of 0.8 equivalent

77



78

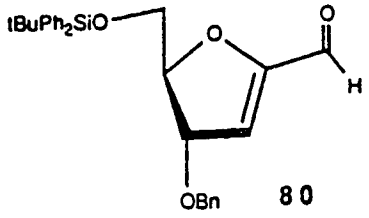
a) 43%
b) 50%
b') 34%
b") 35%

+

79

a) 20%
b) 35%
b') 21%
b") 10%

+



80

a) 27%
b) 15%
b') 3.5%
b") not det.

Conditions : a) DMF, H_2O, pyridine, 60 °C, 5 hrs, (isolated 78 - 80 : 90%)

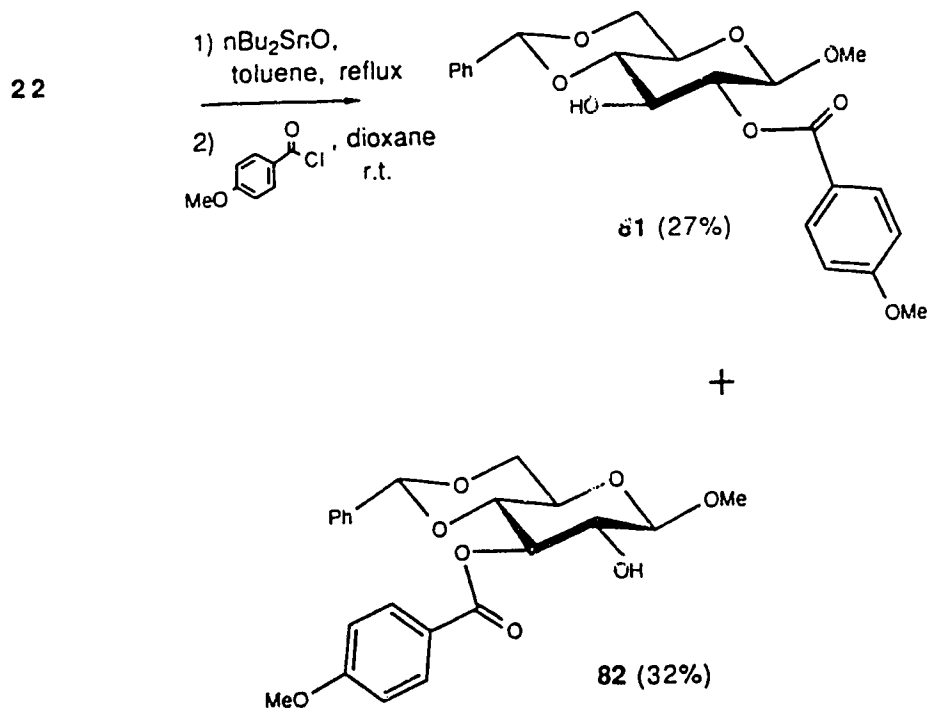
b) CH_3NO_2, H_2O, pyridine, reflux (100 °C), 15 min., (78-80 only by n.m.r. on crude mixture)

b') CH_3NO_2, H_2O, pyridine, 60 °C, 2 hrs, (isolated 78 - 80 : 60%)

b") CH_3NO_2, H_2O, pyridine, 40 °C, 24 hrs, (isolated 78 - 80 : 45%)

DBU, so that its protonated form could serve as a proton shuttle, and 0.4 equivalent of sodium hydride to assure that some of the anomeric hydroxyls or imidates are deprotonated. Although not all imidates would be deprotonated in such a reaction medium, it was hoped that proton transfer from one imidate to another would be fast. The reaction did indeed proceed cleanly to provide only the α and β- imidates **73** and **74** in an improved α to β ratio of about 4 : 1 and in 65 % and 19% isolated yield respectively.

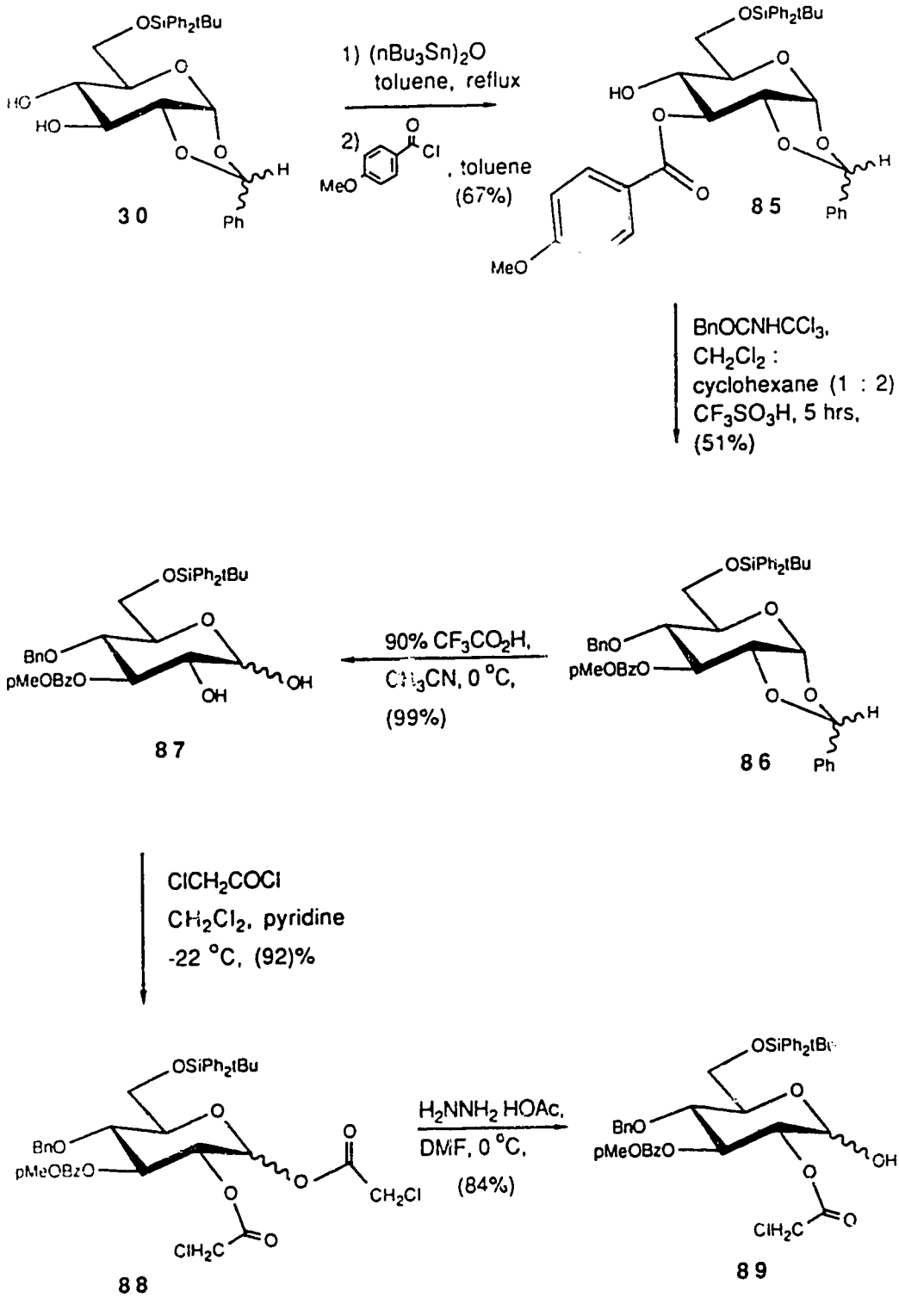
After having obtained the octyl β-glycoside **75**, the chloroacetyl group was removed using hydrazine acetate in either methanol : dichloromethane (1 : 1) or dry DMF to provide **68** in 69 % or 96 % yield respectively. Compound **68** was reacted with trifluoromethanesulfonic anhydride in dichloromethane : pyridine (19 : 1) at 0 °C to provide the triflate **77** in 76 % yield after chromatography. The triflate was then heated to 60 °C in DMF containing pyridine and water to effect the inversion. Apart from the expected mannosides **78** (43 %) and **79** (20 %) a new compound was formed, which proved to be the furfural **80** (27%, H_1 = 9.50 p.p.m.,singlet). The formation of the side product 80 is likely due to the 1,2-migration of the ring oxygen displacing the triflate, which is ideally positioned antiperiplanar to the C1-O bond. This type of migration had been described earlier (108), but it had been hoped that the inversion by the arylacyl group would proceed faster than this side reaction. The lack of ring contracted product in the test reaction (**23** → **25** + **26**, page 20) was most likely due to the presence of the trans decalin system, which would require the formation of an unfavourable trans fused five - six ring system and thus the transition state for the ring contraction would be high in energy. The triflate **77** was heated to 100 °C in wet nitromethane containing pyridine in an attempt to improve the yield of the desired product **78**. The inversion proceeded rapidly to provide **78**, **79** and **80** in 50 %, 35 % and 15%


22
1) nBu2SnO, toluene, reflux
2) dioxane, r.t.
MeO
Ph
HO
OMe
81 (27%)
+
82 (32%)
1) (CF3SO2)O, CH2Cl2, pyridine
2) CH3NO2, H2O, pyridine, reflux
83
(ratio 83/84 = 1 : 2)
84

yield respectively as determined by ^1H - n.m.r. spectroscopy of the crude mixture. In a further attempt to reduce the amount of the sideproduct **80** the temperature of the reaction was decreased, while using otherwise identical conditions in nitromethane. Although the ratios of **78** and **79** to **80** did improve with decreasing temperature, the increase in reaction time required for the transformation caused more decomposition, thus leading to a lowering of overall yields (see **78** conditions b, b' and b").

It became obvious that the *p*-N,N-dimethylaminobenzoyl group was problematic in the glycosidation reactions so another electron rich acyl group, the anisoyl group was investigated. This group lacked a basic amine group and therefore an improvement in the glycosidation reactions was hoped for. To investigate the intramolecular inversion potential of the anisoyl group, the benzylidene derivative **82** was prepared to be used in a preliminary study similar to the one used for the *p*-N,N-dimethylaminobenzoyl group. Methyl 4,6-O-benzylidine-β-D-glucopyranoside **22** was first transformed into the 2,3-O-stannylidine derivative using di-n-butyltin oxide in refluxing toluene with azeotropic water removal. This derivative was then treated with anisoyl chloride in dioxane at room temperature to provide **82** in 32 % yield together with the 2-O-acyl derivative **81** (27 %) (68,69). Compound **82** was triflated with trifluoromethanesulfonic anhydride and then directly inverted using wet DMF and pyridine at 60 °C. The inversion proceeded smoothly, but more slowly than for the *p*-N,N-dimethylaminobenzoyl derivative **23**, and provided the mannosides **83** and **84** in a 1 : 1 ratio. The ratio of **84** to **83** could be improved to 2 : 1 by the use of refluxing wet nitromethane containing pyridine instead of DMF.

The anisoyl analogues of the *p*-N,N-dimethylaminobenzoyl compounds were prepared similarly in the hope that most of the problems in the synthetic

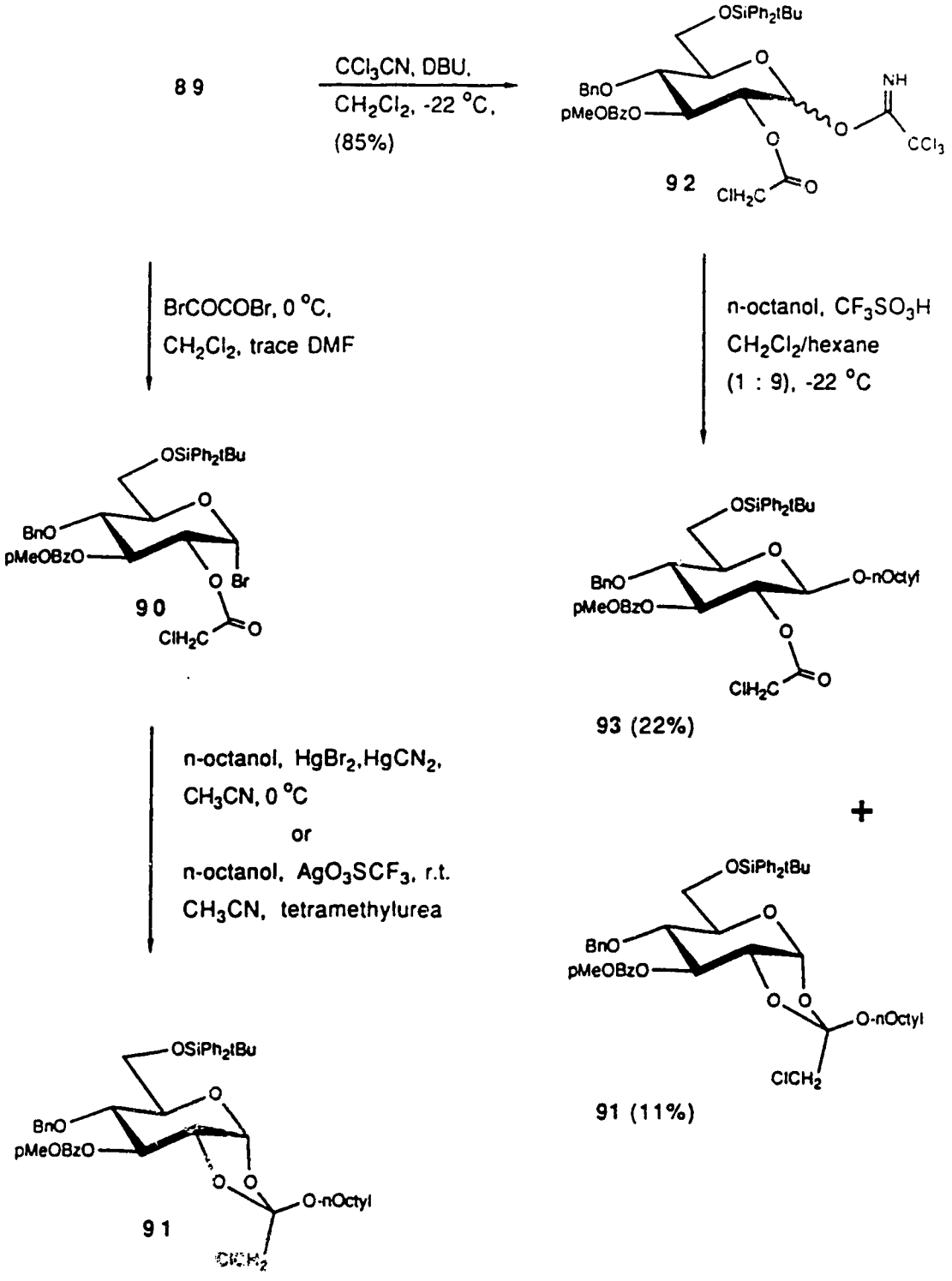

OSiPh2tBu
HO
HO
O
O
O
H
Ph
30
1) (nBu3Sn)2O
toluene, reflux
2)
Cl
O
MeO
, toluene
(67%)
OSiPh2tBu
HO
O
O
O
H
O
MeO
Ph
85
BnOCNHCCl3,
CH2Cl2 :
cyclohexane (1 : 2)
CF3SO3H, 5 hrs,
(51%)
OSiPh2tBu
BnO
pMeOBzO
O
OH
OH
87
90% CF3CO2H,
CH3CN, 0 °C,
(99%)
OSiPh2tBu
BnO
pMeOBzO
O
O
O
H
Ph
86
ClCH2COCl
CH2Cl2, pyridine
-22 °C, (92)%
OSiPh2tBu
BnO
pMeOBzO
O
O
O
CH2Cl
ClH2C
O
88
H2NNH2 HOAc,
DMF, 0 °C,
(84%)
OSiPh2tBu
BnO
pMeOBzO
O
OH
O
ClH2C
O
89

method had already been solved and synthesis would proceed quickly. However some changes had to be made along the way none the less.

Diol **30** and *bis* - tributyltin oxide were refluxed in toluene with azeotropic water removal to provide the tributyltin derivative which was reacted with anisoyl chloride in toluene at room temperature to provide **85** in 67 % yield. The use of di-n-butyltin oxide as employed for the *p*-N,N-dimethylaminobenzoyl compound did not result in the same selectivity as before.

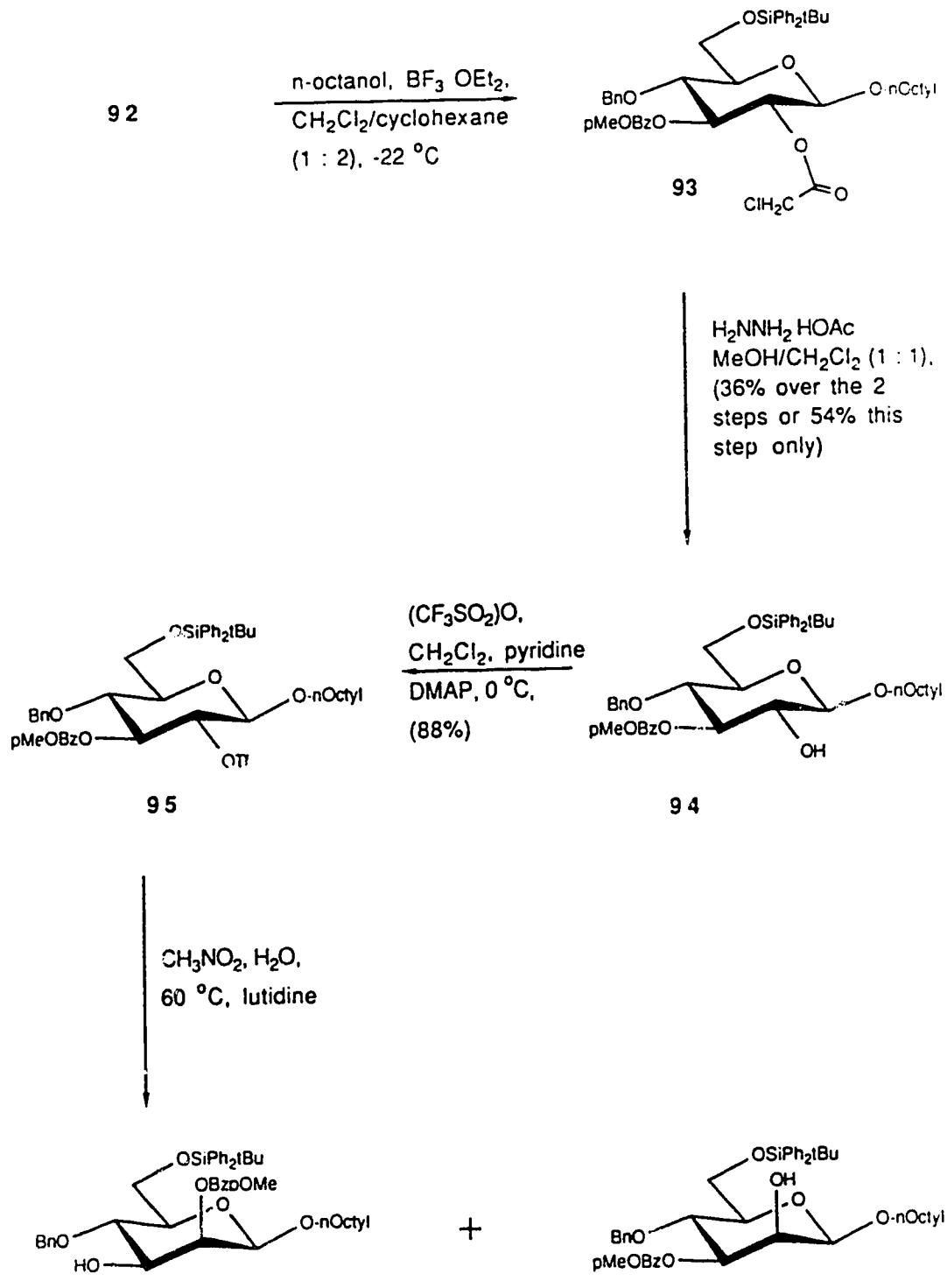
The four position of **85** could not be benzylated under basic conditions due to the increased migratory aptitude of the anisoyl group, but acidic benzylation according to Bundle (71) using benzyloxy-2,2,2-trichloroacetimidate and trifluorosulfonic acid in dichloromethane : cyclohexane (1 : 2) provided **86** in 51 % yield as no interfering *p*-N,N-dimethylaminobenzoyl group was present. The reaction had to be closely watched, as it proceeded to about 70 - 80% completion and then decomposed very rapidly within minutes.The reaction was thus usually terminated at about 60% conversion of starting material and the starting material recovered.

The fully protected derivative **86** could be debenzylidenated using 90 % trifluoroacetic acid in acetonitrile (64) at 0 °C to provide the diol **87** in almost quantitative yield. Chloroacetylation to yield **88** proceeded smoothly in 92 % yield using chloroacetyl chloride in dichloromethane in the presence of pyridine at -22 °C. The loss of the chloroacetyl group during chromatography was even more acute than for the *p*-N,N-dimethylaminobenzoyl derivative **71** and thus chromatography had to be rapid. All chloroacetylated compounds decomposed upon prolonged storage. It was possible to remove the anomeric chloroacetate group selectively using hydrazine acetate in dry DMF as before to give the reducing sugar **89** in 84 % yield.


89
CCl3CN, DBU,
CH2Cl2, -22 °C,
(85%)
OSiPh2tBu
BnO
pMeOBzO
NH
CCl3
92
ClH2C
BrCOCOBr, 0 °C,
CH2Cl2, trace DMF
n-octanol, CF3SO3H
CH2Cl2/hexane
(1 : 9), -22 °C
Br
90
93 (22%)
O-nOctyl
n-octanol, HgBr2,HgCN2,
CH3CN, 0 °C
or
n-octanol, AgO3SCF3, r.t.
CH3CN, tetramethylurea
+
91
91 (11%)
ClCH2

Since alcohol **89** lacked the problematic amino group present in **72**, an attempt was made to use the anomeric bromide as a glycosidating agent. Treatment of **89** with oxalyl bromide and DMF in dichloromethane at 0 °C produced the bromide **90**, which was, without further purification, reacted with n-octanol using either mercuric bromide and mercuric cyanide in acetonitrile or silver trifluoromethanesulfonate and tetramethylurea in dichloromethane. In both cases only the orthoester **91** was obtained. This orthoester was resistant to rearrangement using BF_3·etherate or trifluoromethanesulfonic acid and therefore the imidate method was again applied.

Reaction of **89** with trichloroacetonitrile and DBU at -22 °C provided the imidate **92** as an α,β mixture (ratio about 7 : 4) in 85 % yield. Reaction of **92** and n-octanol in dichloromethane : hexane (1 : 9) using triflic acid as the catalyst provided the glycoside **93** and the orthoester **91** in 22% and 11% yield, respectively. Dechloroacetylation of **93** to give **94** proceeded in 54% yield using hydrazine acetate in methanol : dichloromethane (1 : 1). Since dechloroacetylation was a problem during the glycosylation reaction, the glycoside **93** was also dechloroacetylated directly in the crude product mixture using hydrazine acetate in methanol : dichloromethane (1 :1) to provide **94** in 36 % yield over the two steps. Triflation of **94** with trifluoromethanesulfonic anhydride in dichloromethane : pyridine at 0 °C gave **95** in 89 % yield. Inversion of the triflate in wet nitromethane containing lutidine at 60 °C produced the mannosides **96** and **97** in about 25% and 13% yield respectively. There was extensive formation of some furanoid sideproducts similar to **80**, but they were not identified in detail. The products **96** and **97** could not be obtained in pure form due to interference of the side products during chromatography, but their structures could be assigned by n.m.r. of the partially purified products.

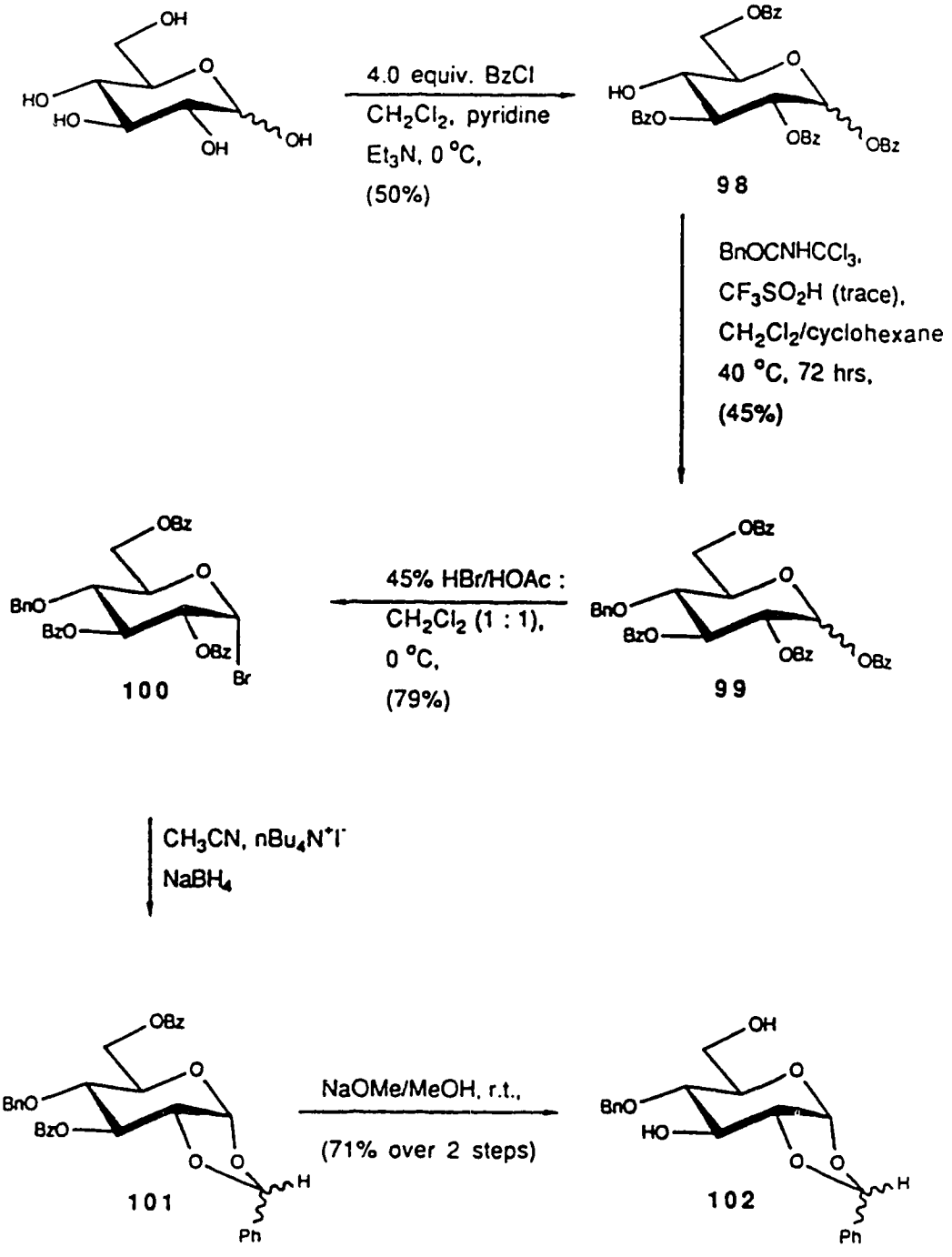

92
n-octanol, BF3 OEt2,
CH2Cl2/cyclohexane
(1 : 2), -22 °C
OSiPh2tBu
BnO
pMeOBzO
O-nOctyl
ClH2C
93
H2NNH2 HOAc
MeOH/CH2Cl2 (1 : 1),
(36% over the 2
steps or 54% this
step only)
(CF3SO2)O,
CH2Cl2, pyridine
DMAP, 0 °C,
(88%)
OTf
95
OH
94
CH3NO2, H2O,
60 °C, lutidine
OBzDOMe
HO
96 (25%)
+
97 (13%)

An attempt was made to trap the transient 2,3 acyloxonium ion **11** (page 14) using methanol and pyridine with the hope of obtaining the methyl orthoester, which might then be hydrolyzed to the predominantly axial acyl derivative **96** by mild acid hydrolysis. Two products were obtained when **95** was heated in methanol and pyridine to 55 °C . These products did not rearrange upon treatment with acetic acid, but could be deacylated using sodium methoxide in methanol (81) indicating that they were not the desired orthoesters but some other rearrangement products. These products were not identified.

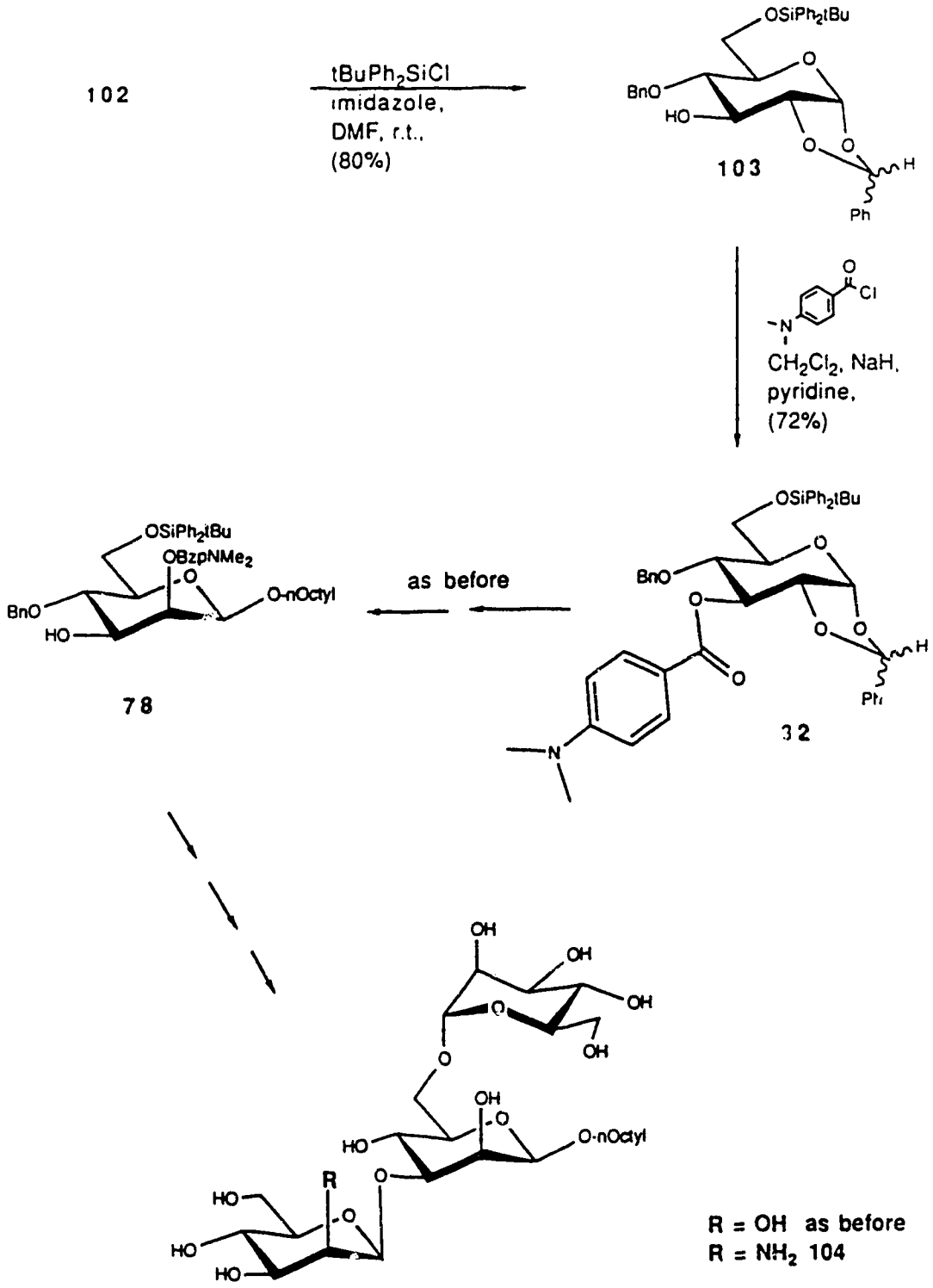
The *p*-anisoyl group was not suitable for the synthesis of the β - mannosides in the inversion reaction due to the extensive side product formation and thus the original *p*-N,N-dimethylaminobenzoyl derivatives remained the better alternative. Since the inverted mannoside with the unprotected 3 hydroxyl group would be a very useful intermediate for the construction of the mannose rich core of asparagine linked oligosaccharides, it was considered important to determine if **78** could be glycosylated. To investigate this glycosidation a larger amount of the mannoside **78** had to be obtained and the previous approach was judged to be too tedious and low yielding for this purpose. A new approach was thus developed.

Starting from glucose, the 1,2,3,6-tetra-O-benzoyl derivative **98** could be prepared in 50% yield by a reaction analogous to one described for the glycoside using four equivalents of benzoyl chloride in the presence of pyridine and triethylamine in dichloromethane at 0 °C (109,110). Acidic benzylation (71) with benzyloxy 2,2,2-trichloroacetimidate and trace trifluoromethanesulfonic acid in dichioromethane : cyclohexane (1 : 2) at 40 °C provided **99** in 45 % yield. It was essential to use very little acid due to the

sensitivity of the anomeric benzoyl group and therefore to accelerate the reaction by increasing the temperature rather than the acid concentration.



The benzoate **99** could be transformed into the bromide **100** in 79 % yield using HBr in glacial acetic acid (111) and dichloromethane at 0 °C. Some hydrolysis was always observed since no acetic anhydride could be added to the HBr solution because the acetoxonium ion formed in the solution would cleave the benzyl group by acetolysis. The bromide (**100**) was converted to the 1,2-O-benzylidene derivative **101**, which was directly debenzoylated under Zemplen conditions to provide **102** in 71 % yield overall. Silylation (65,66) of **102** using *t*-butyldiphenylchlorosilane and imidazole in dry DMF gave 81 % of **103**. Acylation of the 3 hydroxyl group proved to be more difficult than expected. The use of *p*-N,N-dimethylbenzoyl chloride in pyridine containing traces of DMAP (80) at room temperature or 40 °C did not produce any product. Only when the mixture was heated to 80 °C, some (about 20 % after 12 hrs) product was formed but the reaction was accompanied by decomposition. It was therefore decided to add sodium hydride to the reaction mixture to deprotonate the hydroxyl group. The products now started to form even at room temperature and no side reaction was observed. When the reaction was repeated without pyridine, the *p*-N,N-dimethylbenzoyl group was demethylated to provide about 5 to 10 % of 3-O-*p*-methylaminobenzoyl derivative (*p*-CH_3HNBz, d,δ = 2.877 p.p.m., $J_{H,CH3}$=4 Hz, int = 3 H; *p*-CH_2HNBz, d broad, δ = 4.253 p.p.m.) and the reaction was therefore performed using pyridine and sodium hydride to give **32** in 72 % yield. This product was slightly impure due to the presence of a yellow byproduct which could be removed by chromatography only after debenzylidenation of **32** to **34** with 90% trifluoroacetic acid. Aqueous co-evaporation of the crude **34** to remove the benzaldehyde prior to chromatography had to be abandoned as it was accompanied by extensive decomposition of **34**. The synthesis now proceeded as before to finally provide **78** again.


102
tBuPh2SiCl
imidazole,
DMF, r.t.,
(80%)
OSiPh2tBu
BnO
HO
103
Ph
CH2Cl2, NaH,
pyridine,
(72%)
as before
OBzpNMe2
O-nOctyl
78
32
R = OH as before
R = NH2 104

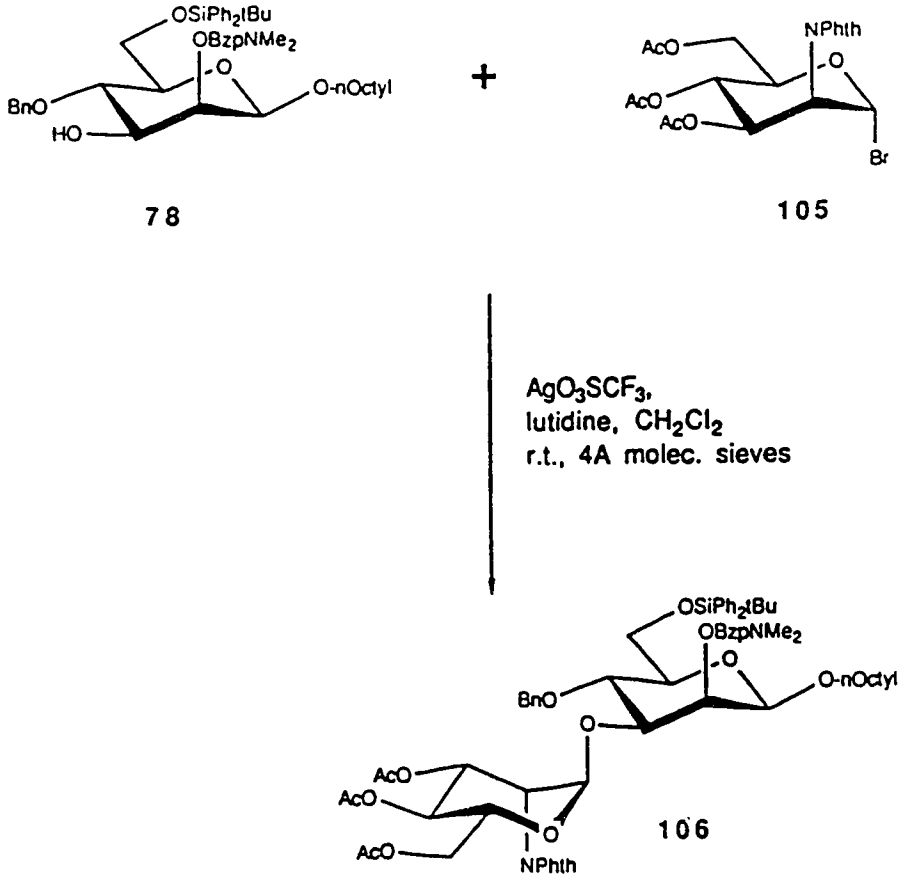
Compound **78** is a potentially very versatile intermediate for the synthesis of oligosaccharides found in the mannose core of asparagine linked carbohydrates, which have the general structure **(3)**, because the 2, 3 and 6 positions are differentially accessible. Ideally, the 4-O-benzyl group should be replaced with an allyl group to allow independent access to the 4 position as well, but in this study the benzyl group had been chosen above other groups to minimize further interference in an already complicated system.

It was thus decided to investigate if **78** could be glycosidated or if again the *p*-N,N-dimethylaminobenzoyl group would interfere. Although the original α-D-mannopyranosyl-(1→3)-β-D-mannopryanoside could have been synthesized, we chose to prepare a 2-amino-2-deoxy-α-D-mannopyranosyl-(1→3)-β-D-mannopyranoside analogue instead because it would be useful in an initial investigation into the acceptance of such substrates by the enzyme GlcNAcT I.

It was decided to use 3,4,6,-tri-O-acetyl-2-deoxy-2-phthalimido-α-D-mannopyranosyl bromide **105** to introduce a 2-amino -2-deoxy-D-mannopyranosyl group α linked to the 3 position of **78**. Although to our knowledge a 2-deoxy-2-phthalimido-mannopyranosyl donor had not been used in such a manner previously, it was expected that it should behave similarly to the 2-deoxy-2-phthalimido glucopyranose analogue and introduce the alcohol trans to the 2-O-phthalimido group, thus yielding predominantly the α -mannoside.

Although there is a standard literature procedure (112) for the formation of 2-phthalimido derivatives of glucosamine, the yields were found to be generally poor and difficult to reproduce in the hands of several coworkers in our research group. A new approach was therefore devised for the mannose derivatives, as mannosamine hydrochloride is much more expensive than

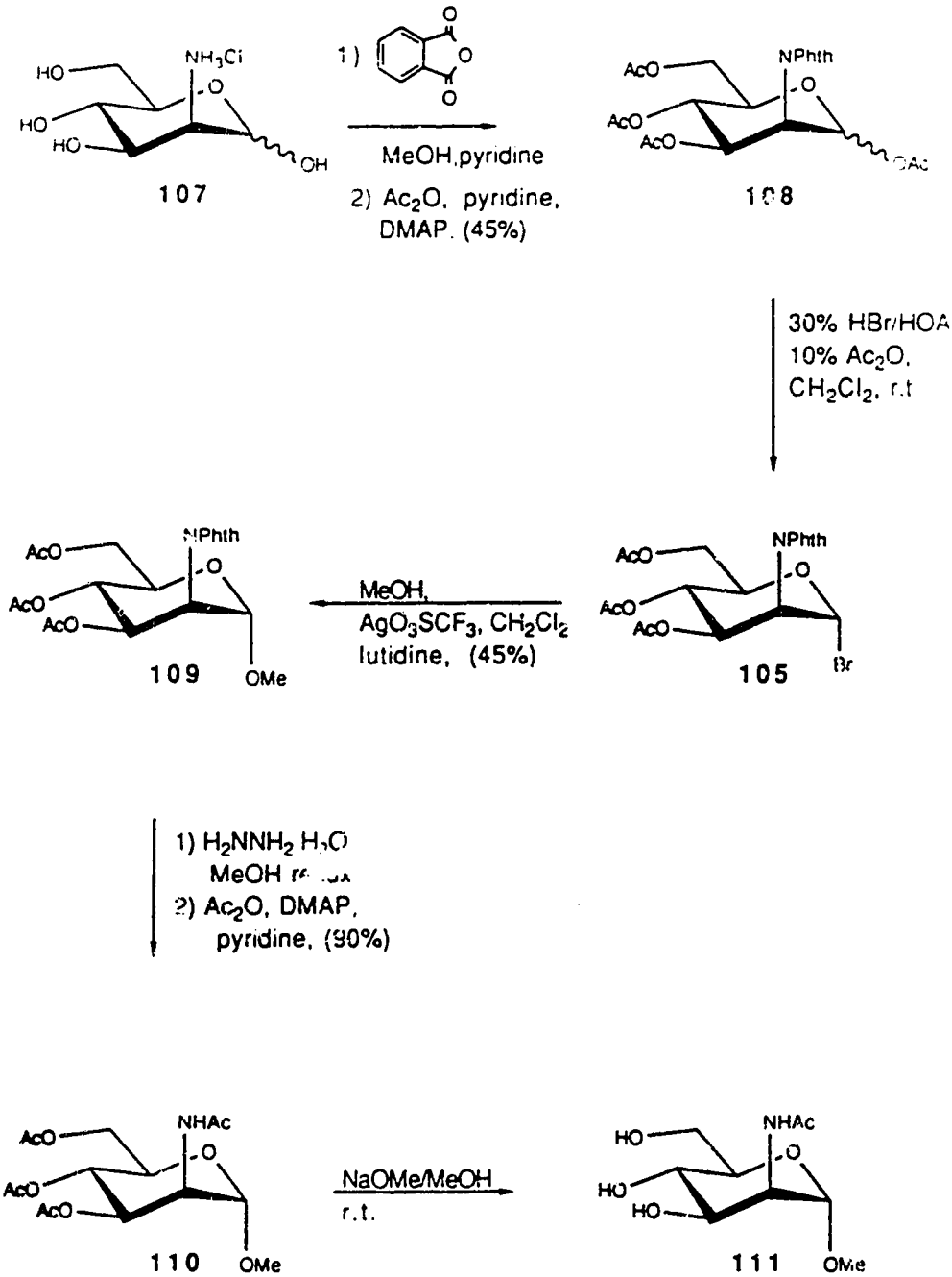
glucosamine hydrochloride and low yields were therefore less acceptable. The new reaction conditions were first tested on 2-amino-2-deoxy-D-glucopyranose and then applied to 2-amino-2-deoxy-D-mannopyranose.



Mannosamine hydrochloride **107** was dissolved in methanol : pyridine (2 : 1) containing 1 equivalent of triethylamine. Three equivalents of phthalic anhydride were added and after about 10 minutes of reaction a ninhydrin positive spot was no longer observable by t.l.c. The crude reaction mixture was diluted with dichloromethane and the product was extracted into the water

layer. After evaporation of water and repeated co-evaporation with toluene, the product was treated with excess acetic anhydride and DMAP in pyridine to provide **108**. On a 100 mg scale 96 % of the product **108** was obtained after chromatography, but on a larger scale (0.8 g) only 45 % could be recovered from the column. There seems to have been extensive crystallization of the almost pure product on the column. Since the objective was to obtain a sufficient amount of the phthalimido-mannose derivative, the reaction was only performed once on the larger scale and was therefore not optimized. Phthalimido-glucose derivatives are now being prepared routinely in 70 % yields for the large scale reactions in the laboratory using this procedure. The acetate **108** was transformed into the bromide **105** by the use of HBr in glacial acetic acid and used without chromatography in the following glycosidation.

Alcohol **78** (10 mg) was reacted with the bromide **105** using silver triflate in dichloromethane and lutidine (74) to provide the disaccharide **106**. Only a small amount of **106** was obtained in pure form after chromatography due to the presence of the 1 : 1 adduct formed by condensation of the bromide with its hydrolysis product which had similar chromatographic mobility. The ^{1}H-n.m.r. spectrum of **106** showed all the signals for the protons of the two rings, but the coupling constants of the sugar ring phthalimido-mannose protons of **106** as well as of **108** and **105** were much larger than normal ($J_{1,2}$= 3 - 5 Hz instead of the usual 0 - 1 Hz, see table 5) and the chemical shifts of some of the ring protons in **108** and **105** were shifted dramatically downfield. The formation of α-mannosidic linkages using **105** should be favoured by factors such as the anomeric effect, steric hindrance to attack from above by the axial hydroxyl group and the possibility of neighboring group participation from the phthalimido group. Since the pyranose ring in **105** was apparently distorted ($J_{1,2}$= 3.5 Hz, $J_{2,3}$= 5.0 Hz), the configuration of the newly formed glycosidic


NH3Cl
HO
HO
HO
OH
107
1)
MeOH,pyridine
2) Ac2O, pyridine,
DMAP, (45%)
NPhth
AcO
AcO
AcO
OAc
108
30% HBr/HOAc
10% Ac2O,
CH2Cl2, r.t
NPhth
AcO
AcO
AcO
109
OMe
MeOH,
AgO3SCF3, CH2Cl2
lutidine, (45%)
NPhth
AcO
AcO
AcO
Br
105
1) H2NNH2 H2O
MeOH r.t.
2) Ac2O, DMAP,
pyridine, (90%)
NHAc
AcO
AcO
AcO
110
OMe
NaOMe/MeOH
r.t.
NHAc
HO
HO
HO
111
OMe

linkage in **106** had to be questioned. The reason for this distortion of the pyranose ring is due to the presence of the phthalimido group and can easily be visualized using an ordinary molecular model. In a perfect chair conformation the carbonyls of the phthalimido group are either interacting with the lone pair of the ring oxygen or with the axial H-4, causing the ring to distort to avoid such interaction. The model also indicates that it is likely that one of the carbonyls has to be aligned parallel to H-1 causing its chemical shift to move downfield in the n.m.r. spectra. A model study using methanol was therefore performed to provide insight into the glycosidation reaction with the bromide **105**.

Bromide **105** was reacted with methanol and silver trifluoromethanesulfonate under the same conditions as were applied during the formation of the disaccharide **106** (74) to provide the methyl glycoside **109** in 45 % isolated yield (based on **105**). The methyl glycoside **109** showed the same unusual shifts and coupling constants as the acetate **108** and bromide **105** (see table 5). Table 6 shows the ^{13}C chemical shifts and the one bond C-1 - H-1 coupling constants for all derivatives formed during the model study.

A coupling constant of 170 Hz or greater is usually taken to indicate an α-mannosidic linkage, but the effect of ring distortion on this coupling constant was not known. Indeed ring distortion was seen to have a dramatic effect on the C_1-H_1 coupling constant in the distorted 4,6-O-benzylidene-2,3,-O-carbonate-β-D-mannopyranoside where it was found to be about 170 Hz for the β-mannoside (61). The methyl mannoside **109** was therefore deprotected and re-acetylated to provide compound **110** in 90% yield using hydrazine hydrate in refluxing methanol followed treatment with acetic anhydride in pyridine. As can be seen from table 5, the proton chemical shifts and coupling

Table 5 : ^{1}H n.m.r. Data for Compounds **108 - 111** in $CDCl_3$ and * in CD_3OD

Nucleus	108 (major)	108 (minor)	105	109	110	111*	α-OMe* (std)	β-OMe* (std)
H_1 (J_{12})	6.589 (3.8)	6.006 (2.8)	6.813 (3.5)	5.270 (3.9)	4.667 (1.5)	4.557 (1.2)	4.565 (1.3)	4.513 (1.5)
H_2 (J_{23})	4.895 (5.2)	5.073 (6.8)	5.220 (5.0)	4.859 (5.0)	4.620 (4.5)	4.273 (4.8)	4.280 (5.0)	4.427 (4.8)
H_3 (J_{34})	5.503 (6.8)	5.478 (9.5)	5.680 (6.8)	5.462 (6.5)	5.323 (10.0)	3.880 (9.5)	3.888 (9.5)	3.625 (9.5)
H_4 (J_{45})	5.562 (8.0)	6.064 (10.0)	5.587 (9.0)	5.517 (7.8)	5.100 (10.0)	3.560 (10.0)	3.567 (10.0)	3.487 (9.5)
H_5 (J_{56})	4.232 (6.0)	3.94 (6.2)	4.49-4.26	4.167 (6.5)	3.977 (5.5)	3.483 (3.2)	3.490 (3.5)	3.220 (3.0)
H_6 ($J_{66'}$)	4.451 (12.0)	4.484 (12.0)	4.49-4.26	4.484 (3.3)	4.287 (2.2)	3.800 (3.2)	3.807 (3.2)	3.840 (3.0)
$H_{6'}$ ($J_{56'}$)	4.315 (3.0)	4.273 (3.0)	4.49-4.26	4.293 (12.0)	4.080 (12.0)	3.800 (0)	3.807 (0)	3.840 (0)
OAc (NAc)	2.155,2.135, 2.105, 1.952	2.166,2.066, 1.91, 11.952	2.17,2.12, 1.96	1.955,2.090, 2.160	2.22, 2.07, 1.99	2.00	2.00	2.00, 1.96
OMe	n.a.	n.a.	n.a.	3.420	3.40	3.350	3.357	3.457
NH (J_{H2})	n.a.	n.a.	n.a.	n.a.	5.860 (9.0)	n.a.	n.a.	n.a.
Phth	7.9 - 7.7	7.95 - 7.7	8.0 - 7.7	7.9 -7.7	n.a.	n.a.	n.a.	n.a.

Table 6 : ^{13}C - n.m.r. Data for Compounds **109 - 111** in $CDCl_3$ and * CD_3OD

Nucleus	109	110	111*	α-OMe* std	β-OMe* std
C1 (J_{CH})	97.60 (171.6)	100.16 (172.0)	101.71 (170.8)	101.72 (171.0)	101.89 (160.0)
C=O (acetyl)	170.73,169.84, 169.71	170.55,170.77, 169.94, 169.87	174.00	174.00	174.65
C=O (Phth)	167.90	n.a.	n.a.	n.a.	n.a.
C6 (J_{CH})	62.73 (147)	62.55 (148)	62.25 (142)	62.26	61.86
CH_3O (J_{CH})	55.38 (143.4)	55.29 (143.0)	55.24 (142.9)	55.25	56.96 (143.1)

Table 7 : ^{1}H and ^{13}C - n.m.r. data for Compounds **106, 112, 113** and **121**

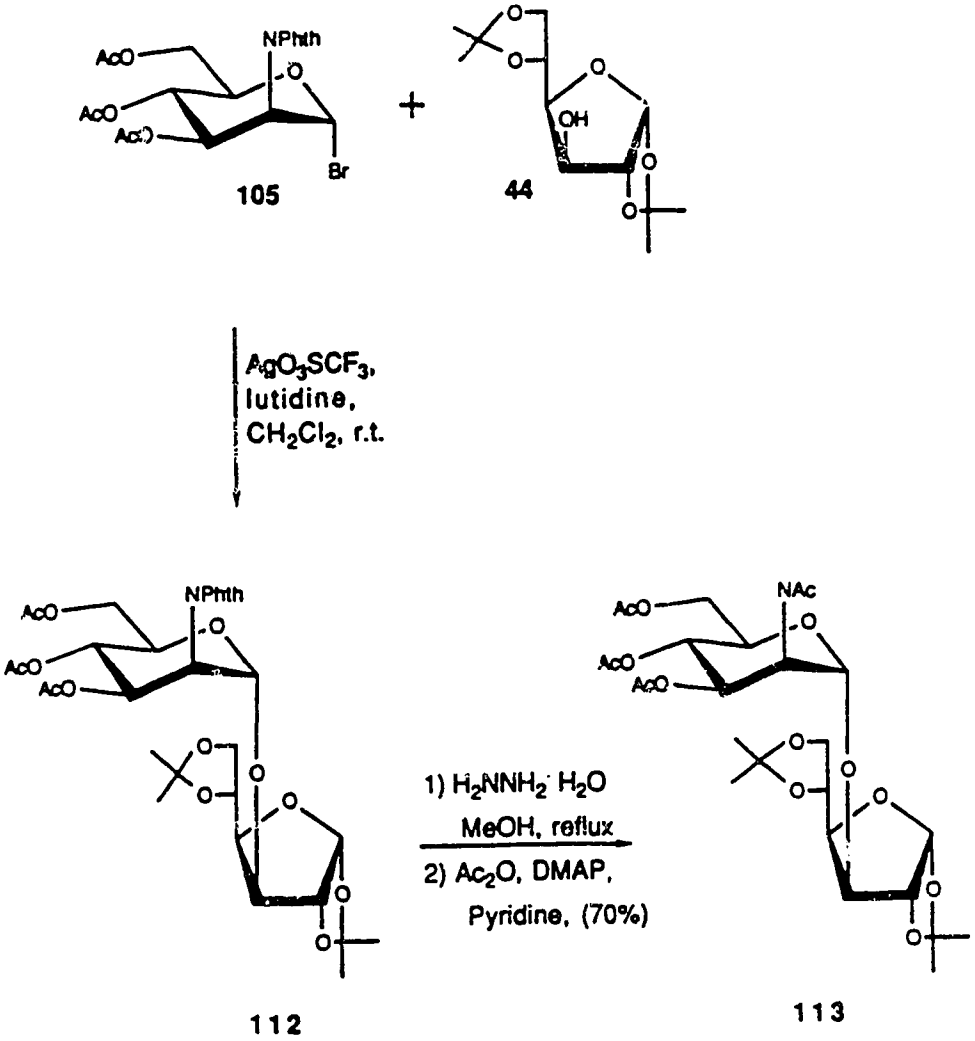
Nucleus	106	112	113	121
H-1 (J_{12})	4.579 (0)	5.832 (3.2)	5.935 (3.8)	n.d.
H-2 (J_{23})	5.204 (0)	4.744 (0)	4.607 (0)	5.513 (3)
H-3 (J_{34})	3.043 (9.5)	n.d.	n.d.	n.d. (9.5)
H-4 (J_{45})	4.162 (9.5)	n.d.	n.d.	4.04 (9)
H-5 (J_{56})	n.d.	n.d.	n.d.	3.257 (-)
H-6 ($J_{66'}$)	n.d.	n.d.	n.d.	n.d.
H-6' ($J_{56'}$)	n.d.	n.d.	n.d.	n.d.
H'-1 ($J_{1'2}$)	4.248 (3)	5.895 (5.2)	5.076 (1.5)	5.720 (3.5)
H'-2 (J_{23})	5.301 (7.5)	4.820 (4.5)	4.659 (4.5)	4.937 (5)
H'-3 (J_{34})	5.664 (9.5)	5.337 (4.5)	5.273 (10)	5.353 (7)
H'-4 (J_{45})	5.859 (10)	5.258 (7.5)	5.067 (10)	5.415 (9)
H'-5 (J_{56})	4.840 (2)	n.d. (7.5)	n.d.	n.d.
H'-6 ($J_{66'}$)	n.d.	4.451 (12)	n.d.	n.d.
H'-6' ($J_{56'}$)	n.d. (2)	4.296 (3)	n.d.	n.d.
OAc	2.20,2.04,1.83	2.16,2.12,2.00	2.12,2.07,2.04,1.99	2.22,2.16,2.08,1.92
NH (J_{2H})	n.a.	n.a.	5.648 (9)	n.a.
C-1 (J_{CH})	n.d.	105.37 (183)	n.d.	98.62 (153.9)
C'-1 (J_{CH})	n.d.	97.12 (175)	n.d.	98.92 (175 [a])
C-6 (J_{CH})	n.d.	63.03 [a]	n.d.	62.87 (148)[a]
C-6' (J_{CH})	n.d.	67.43 [a]	n.d.	62.57 (145)[a]

a could be interchanged; **n.a.** not applicable; **n.d.** not determined due to signal complexity and overlap

constants of **110** are close to the normal values expected for mannopyranosides ($J_{1,2}$= 1.5 Hz, $J_{2,3}$= 4.5 Hz), indicating a decrease in the ring distortion. Again, using a molecular model it was easily seen that the 2-acetamido group could be positioned perpendicular to the ring bringing the NH bond into perfect hydrogen bond distance to the ring oxygen' s lone pair. Interaction between H-4 and the acetamido group are then minimized, allowing the ring to adopt a chair configuration. Interestingly, although there are large changes in the chemical shifts and coupling constants of the proton n.m.r. spectra, the ^{13}C chemical shifts and coupling constants are affected only very slightly (see table 6, compounds **109** and **110**).

The derivative **110** was then deacetylated using sodium methoxide in methanol to provide **111**, which could be compared to standards of methyl 2-acetamido-2-deoxy-α-D-mannopyranoside and methyl 2-acetamido-2-deoxy-β-D-mannopyranoside, which had been graciously provided by Dr. H. Paulsen. As can be seen in table 5, the spectral data of **111** match perfectly with the ones recorded for the α -mannoside standard and it thus appeared that the distortion of the pyranoside ring has no bearing on the preferential formation of the α-glycosidic linkage formation when using the bromide **105** under these conditions. Furthermore as the C-1/H-1 coupling constant in this case is quite unaffected by either changes in protecting groups or even ring distortion, it should be possible to predict the conformation of the glycosidic linkage from the C-1/H-1 coupling constant of the protected sugar in the future. To make quite sure that these observations would also apply to a disaccharide compound **112** was synthesized from di-O-isopropylidene-glucose **44** and the bromide **105** under the usual reaction conditions (74). There are some similarities between the proton spectra of **112** and **109**, but no direct conclusions can be drawn from them as to the nature of the glycosidic

linkage. The proton coupled ^{13}C n.m.r. spectra of **112** however confirmed again the presence of an α-mannosidic linkage by providing a C-1/H-1coupling constant of 175 Hz. Upon removal of the phthalimido group and acetylation to produce compound **113**, the ^{1}H- spectral data compared well with those of **110**.

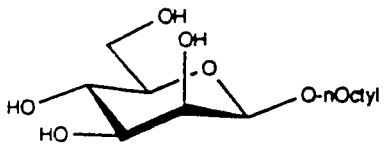


The ^{1}H-n.m.r. spectral data of **109** and **112** were compared with those of **106**, but no conclusions could be drawn from the comparison with regards to

the nature of the glycosidic linkage as the spectrum of **106** showed little similarity with either **112** or **109**.

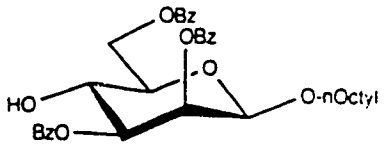
We were very interested in obtaining the octyl 3-O-(2-amino-2-deoxy-α-D-mannopyranosyl)-β-D-mannopyranoside to allow initial tests to be performed with the target enzyme, N-acetylglucosamine transferase I, as it was important to find out if the amino analogue of the native disaccharide would be accepted by the enzyme before setting out to synthesize more complicated structures. As the synthesis of **106** was difficult and time consuming, we chose to synthesize the 2-O-acetyl analogue **121** from octyl-β-D-mannopyranoside **114** instead. It was also hoped that the ^{1}H-n.m.r. spectrum of **106** could be compared to that of **121** thus providing insight into the anomeric linkage of **106**.

n-Octyl β-D-mannopyranoside **114** was prepared from 1-iodoacetate and 2,3,4,6-tetra-O-acetyl-α/β-D-mannopyranose using silver oxide according to the published procedure (113). The product was then tribenzoylated using 3 equivalents of benzoyl chloride in pyridine (109) at -10 °C to provide **115** in 78 % yield. The tribenzoate **115** was next benzylated (71) to provide **116**, which was not isolated, but directly debenzoylated using sodium methoxide in methanol to provide **117** in 71 % yield over the two steps. No attempt was made to isolate **116** because the chromatographic purification is difficult at this stage due to large amounts of benzyl alcohol and similar contaminants which co-elute with the product. Silylation of **117** using *t*-butyldiphenylchlorosilane and imidazole provided **118** in 84 % yield. Using triethylorthoacetate and *p*-toluenesulfonic acid the diol **118** was transformed into the 2,3 orthoester, which upon treatment with acetic acid at 0 °C provided the axial acetate **119** (22) and the 3-O-acetate **120** in a 5 : 2 ratio. Migration of the acetate from the 2 to the 3 position during chromatography could be



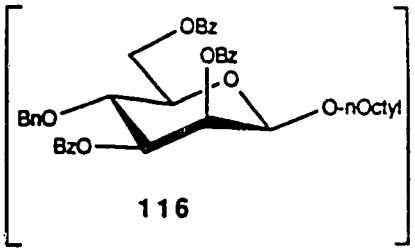
114

3 equiv. BzCl
pyridine,
-10 °C,
(78%)



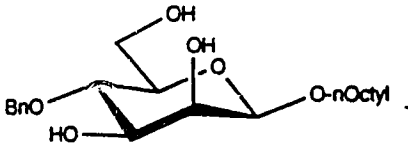
115

$BnOCNHCCl_3$,
CF_3SO_3H, r.t.,
CH_2Cl_2 : cyclohexane
(1 : 2)



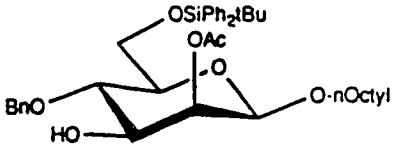
116

NaOMe/MeOH
r.t., (71% over
2 steps)

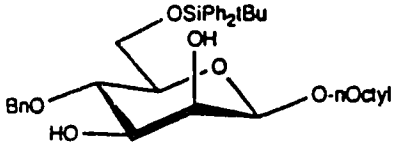


117

$tBuPh_2SiCl$
imidazole
DMF, 0 °C
(84%)



119

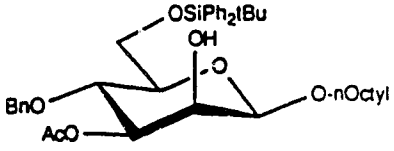


118

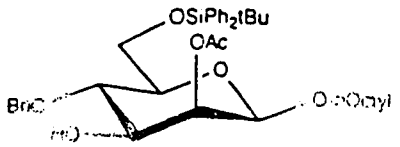
1) $(EtO)_3CCH_3$,
pTsOH

2) 80% AcOH

+

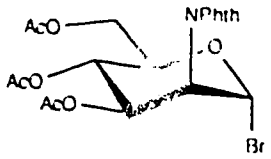


120



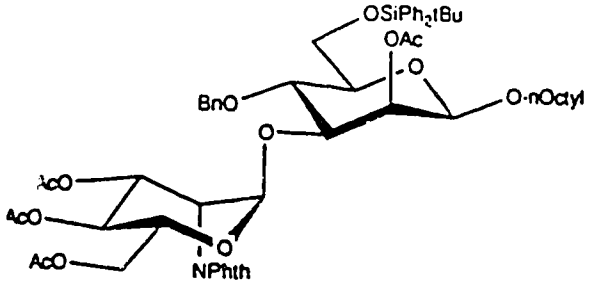
119

+



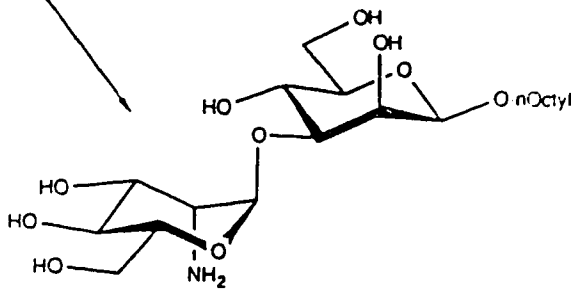
105

AgO_3SCF_3, CH_2Cl_2,
lutidine, 4A sieves,
r.t.



121

a) NaOMe, MeOH
b) $H_2NNH_2 \cdot H_2O$
c) $nBu_4N^+F^- \cdot HF$
d) Pd/C, H_2, MeOH



122

prevented by addition of trace acetic acid to the eluent and the two products **119** and **120** were isolated in 64 % and 24 % respectively. The 3 hydroxy-derivative **119** was next glycosidated using the 2-phthalimido-mannopyranosyl bromide **105** and silver trifluoromethanesulfonate in the presence of lutidine to provide the disaccharide **121** in 50 % isolated yield. The proton coupled ^{13}C-nmr data of **121** showed a C_1-H_1 coupling constant of 175 Hz indicating that the two mannose residues are α-linked. The suitability of the 2-phthalimido-mannopyranosyl donor **105** for the synthesis of amino-analogs of the core region of Asn-linked oligosaccharides is therefore demonstrated.

Studies on the deprotection of **121** are currently in progress. The attempted deprotection of the *t*-butyldiphenylsilyl group with tetrabutylammonium fluoride in dry THF according to the literature procedure (65) caused unexpected side reactions, which most likely involved the acetate and phthalimido groups since re-acetylation with acetic anhydride regenerated largely starting material. The current approach to deprotection therefore involves initial deacetylation using Zemplen conditions (54), followed by removal of the phthalimido group with hydrazine hydrate (112) in refluxing methanol, then desilylation with tetrabutylammonium fluoride in dry THF (65) and finally debenzylation by hydrogenation with palladium on carbon (37,114) to provide the unprotected disaccharide **122**.

Conclusion :

The formation of β-mannosidic linkages by intramolecular inversion was investigated. but the yields obtained were rather modest and the method in its present form will not be able to compete with the already existing methods described in the introduction. The 1,2-migration of the ring oxygen (ring contraction) will remain a problem if temperatures higher than about 40 °C are required for the inversion. Although a more reactive nucleophile could possibly overcome this problem, new difficulties might arise during neighboring group assisted glycosidations since the reactive nucleophile might invert the 2 position during this reaction by attack on the intermediate acyloxonium ion (59). Glycosidation without neighboring group participation would not be useful as the yields of β-glycoside would drastically decline when more hindered alcohols are used (21).

Another problem remaining is the selective deprotection of OH-2 of the β-glycoside especially since activated esters, such as the chloroacetyl group, might not be suitable due to the resistance of their orthoesters towards rearrangement. Introduction of the nucleophile after glycosidation into a 2,3 unprotected glucoside led almost exclusively to 2-O-acylation, when unreactive large acylating agents such as *p*-N,N-dimethylaminobenzoyl chloride were used. Therefore introduction of the nucleophile after the glycosidation step might require several synthetic steps which would render the method inapplicable to the introduction of β-mannosyl residues into larger oligosaccharide structures.

The use of 4,6-O-benzylidenated glucosides in the inversion reactions prevents the 1,2 migration of the ring oxygen, as was seen by Kunz et al., as well as in the model reactions using compounds **23** and **82.** It might therefore

be desirable to introduce the glucopyranosyl moiety as a 4,6-benzylidene derivative or similarly constrained system, then invert the 2 position and finally try to modify the 4,6 ring in such a manner that it can liberate O-4 or O-6 selectively. Methods (115-117) are already available for the selective opening of 4,6,-O-benzylidene rings, but their application to complicated oligosaccharide structures containing sensitive glycosidic linkages may cause new complications.

Attempts to use intramolecular inversion for the formation of β-mannosidic linkages should not be abandoned, as the method is potentially very useful and may be eventually applicable complex structures and block synthesis. The present work shows, however, that it will be much more difficult to find conditions that will avoid all the presently discovered downfalls and not create new ones.

The stable orthoacyl fluoride which was described in this work is potentially useful for the investigation of reaction mechanisms. For example, it might be possible now investigate anomeric orthoacid decomposition, starting with a well described system where the initial amount of orthoacid present in solution is well known from the concentration of the orthoacyl fluoride. The use of chloroacetylated glycosides might also provide more stable orthoacyl fluorides, which might be isolable. Furthermore the solvent dependence of the formation of orthoacylfluorides might provide insight into the factors governing reaction pathways in different solvents and thus allow more informed decisions towards the solvents used in glycosidation reactions. Solvents seem to have profound effects on glycosidations as seen also in the work of Sinay (103) and Wulff et al. (91,92).

Finally, the use of 2-phthalimido-mannosyl donors to attach mannosamine residues to glycosyl acceptors is a useful alternative to the use

of 2-azidomannosyl donors. The phthalimido group does not seem to be compatible with the use of fluoride ion and therefore use of the *t*-butyldiphenylsilyl group should be avoided, as the acidic conditions for its removal are too harsh for the labile β-mannosidic linkage.

Even though the intramolecular inversion was only limited in its success, some of the synthetic procedures developed during the construction of the various mono and disaccharides might prove to be useful in future syntheses.