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Finally I would like to express my appreciation to my family for their support.

PREFACE

This thesis consists of two self-contained, independent sections dealing with synthetic chemistry in the areas of higher heterooligosaccharides and anisomycin antibiotics. In each section a separate numbering system for compounds is used, and the same applies to the reaction schemes and literature references.

ABSTRACTS

SECTION I

Improvements were made in the procedures for the preparation of hexa-O-acetyllactal and of the lactosamine donors 3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride, bromide, and trichloroacetimidate. In particular, introduction of halogen by means of dichloromethyl methyl ether and trimethylsilyl bromide was found to be superior to previous methods.

The new disaccharide methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside was synthesized by the Koenigs-Knorr method and was converted, in a one-pot acetolysis reaction, into phenyl-[1,2-dideoxy-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano][2',1':4,5]-2-oxazolinium acetate, a new donor of the lacto-N-biosyl unit in glycosylations. Furthermore, it was discovered that trimethylsilyl triflate-promoted transfer of a 3-O-benzylated D-galactose 1-acetate to OH-3 of a partially blocked methyl N-benzoylglucosaminide led not only to formation of the expected disaccharide linkage but at the same time converted the acceptor glycoside into an oxazoline, thus producing a new lacto-N-biose donor having a temporarily blocked 3'-

position, which should be useful for the design of block syntheses of higher heterosaccharides.

Peracetylated 2-azido-2-deoxylactose and the corresponding 1,3,6,2',6'-pentaacetate (a new lactosamine acceptor equivalent) were synthesized from hexaacetyllactal by the azidonitration method, and a number of other lactosamine and lactose acceptors having unprotected OH-3' and OH-4' groups were prepared by established procedures.

Methyl 4,6-di-O-acetyl-2-benzamido-2-deoxy-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside was synthesized, to be used as a lacto-N-biose acceptor after deprotection of O-3'. Three similarly substituted methyl glycosides of 3-β-D-galactopyranosyl-D-glucose ("isolactose") were prepared by condensations of various D-galactopyranosyl bromides with methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside in order to obtain molecules that may serve as "isolactose" acceptors after deprotection at O-3'. Observations concerning the stability of benzyl ethers in these reactions were recorded.

Treatment of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose with tributyltin benzyloxide in the presence of stannic chloride did not lead to the expected benzyl glycoside but gave in high yield a new disaccharidic

coupling product, *i.e.*, 2,2'-dideoxy-2,2'-diphthalimido-β,β-trehalose hexaacetate, and a minor product which is possibly its α,β-anomer.

SECTION 2

1-Amino-1-deoxy-D-xylitol hydrochloride was sequentially N-carbobenzyloxylated and isopropylidenated. The 1-benzyloxycarbonylamido-1-deoxy-2,3:4,5-di-O-isopropylidene-D-xylitol so obtained was subjected to partial hydrolysis for removal of the 4,5-acetonide, followed by periodate glycol cleavage to give 4-benzyloxycarbonylamido-2,3-O-isopropylidene-L-erythrose. This aldehydo sugar was reacted with p-methoxyphenylnitromethane to furnish 5-benzyloxycarbonylamido-3,4-O-isopropylidene-1-(4-methoxyphenyl)-1-nitropentane-2,3,4-triol. Its 2-acetate was subjected to dehydroacetoxylation, giving the corresponding nitroalkene, 5-benzyloxycarbonylamido-3,4-O-isopropylidene-1-(4-methoxyphenyl)-1-nitro-1-pentene-3,4-diol. Deacetonation then gave the corresponding free diol. Attempts to effect cyclizing Michael addition in these nitroalkenes, to construct the 2-(p-methoxybenzyl)-substituted pyrrolidine-3,4-diol structure of anisomycin have so far been unsuccessful.

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SECTION 2

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LIST OF ABBREVIATIONS

Ac	Acetyl
Ac_2O	Acetic anhydride
All	Allyl
Bn	Benzyl
BnBr	Benzyl bromide
Bz	Benzoyl
BzCl	Benzoyl chloride
Cat	Catalyst
CI	Chemical ionization
COSY	Correlation spectroscopy
DCMME	Dichloromethyl methyl ether
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
E	Electrophile
FAB	Fast atom bombardment spectrometry
Gal	D-Galactose
Glc	D-Glucose

GlcNAc	N-Acetyl-D-glucosamine
HETCOR	Heteronuclear correlation NMR spectroscopy
Hz	Hertz
$M^{\cdot}$	Parent molecular ion
Ms	Mass spectrum
NMR	Nuclear magnetic resonance
Phth	Phthaloyl
TfOH	Trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TMSBr	Trimethylsilyl bromide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
p-TsOH	p-Toluenesulfonic acid

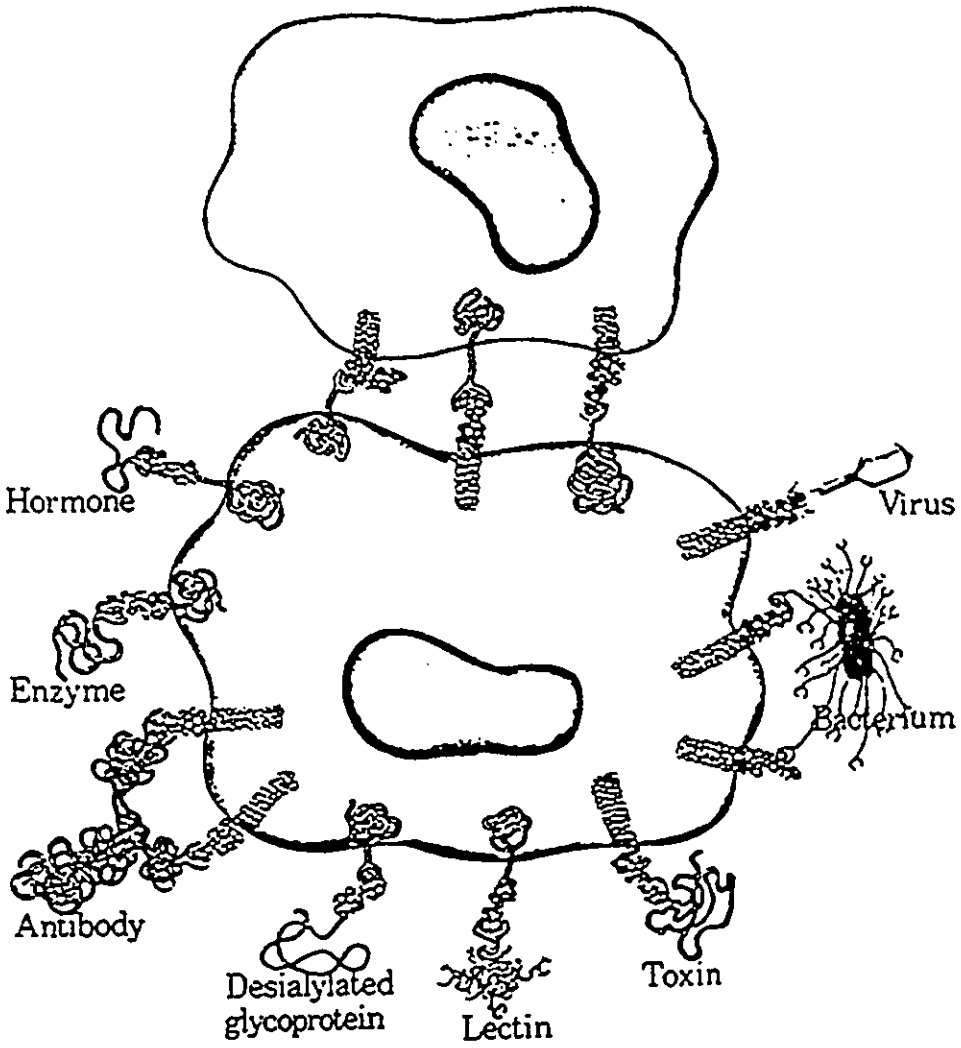
SECTION I

SYNTHESIS OF HIGHER HETEROOLIGOSACCHARIDES

1. INTRODUCTION

Nitrogen-containing oligosaccharides are widespread in nature. They are important components of glycoproteins, glycolipids, and glycophospholipids (glycoconjugates). The oligosaccharide residue is responsible for the intercellular recognition and interaction; it acts as a receptor for proteins, hormones, and viruses and governs immune reactions. These significant activities have stimulated interest in oligosaccharides and glycoconjugates. The membranes of different cells differ from one another in their composition and in the arrangement and mobility of their components. A central role of the immune system of mammalian organisms is the recognition and discrimination of endogenous and exogenous structures. The immune system monitors the constancy of the cellular surfaces in the organism. It responds when the tolerance limits of endogenous surface structure are exceeded. Membrane glycoproteins and glycolipids, specifically their oligosaccharide components, govern these tolerance limits; they are therefore decisive for the specific immune reactions. Moreover, they fulfil important functions in intercellular recognition and interaction, in the control of cell growth and thus tumor formation, and in the interaction with biologically active factors such as enzymes, hormones, bacteriotoxins, and viruses[1-8]. These interactions are schematically represented in the accompanying figure.

Schematic representation of various cell surface carbohydrate interactions (copied from Biocarb chemical catalog 1994).



In 1968, Lloyd *et al.*[9] published a composite structure for the carbohydrate chains of A, B, H, Lewis a, and Lewis b human blood group specific glycoproteins. The structure was presented with the tetrasaccharide, illustrated in Fig. 1, as the structure unit attached to the core protein.

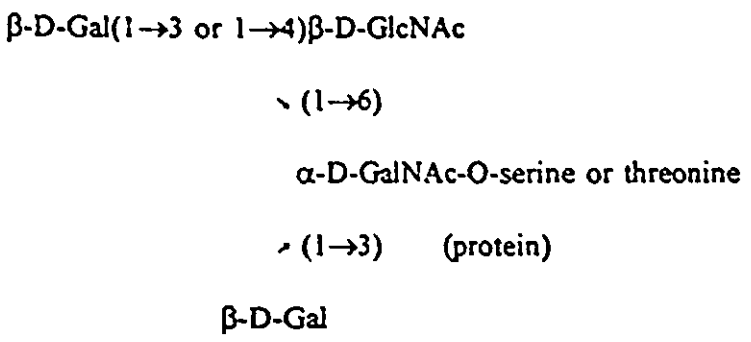


Fig. 1.1. The composite structure for the carbohydrate chains of human blood group substances A, B, and H.

Apart from their occurrence as components of glycoconjugates, a number of aminooligosaccharides have also been found to occur free in some natural materials. The most important instance is human milk, which contains oligosaccharides (tetra-, penta-, and hexasaccharides and even higher homologs), consisting of D-glucose, D-galactose, N-acetyl-D-glucosamine, and L-fucose (lacto-N-polyoses). The study of these important substances, which act as growth factors for the physiological *Lactobacillus bifidus* flora in the intestine of the newborn and which are lacking in cow's milk, was initiated in the 1950's by Richard Kuhn, who was interested in the beneficial effects of human milk

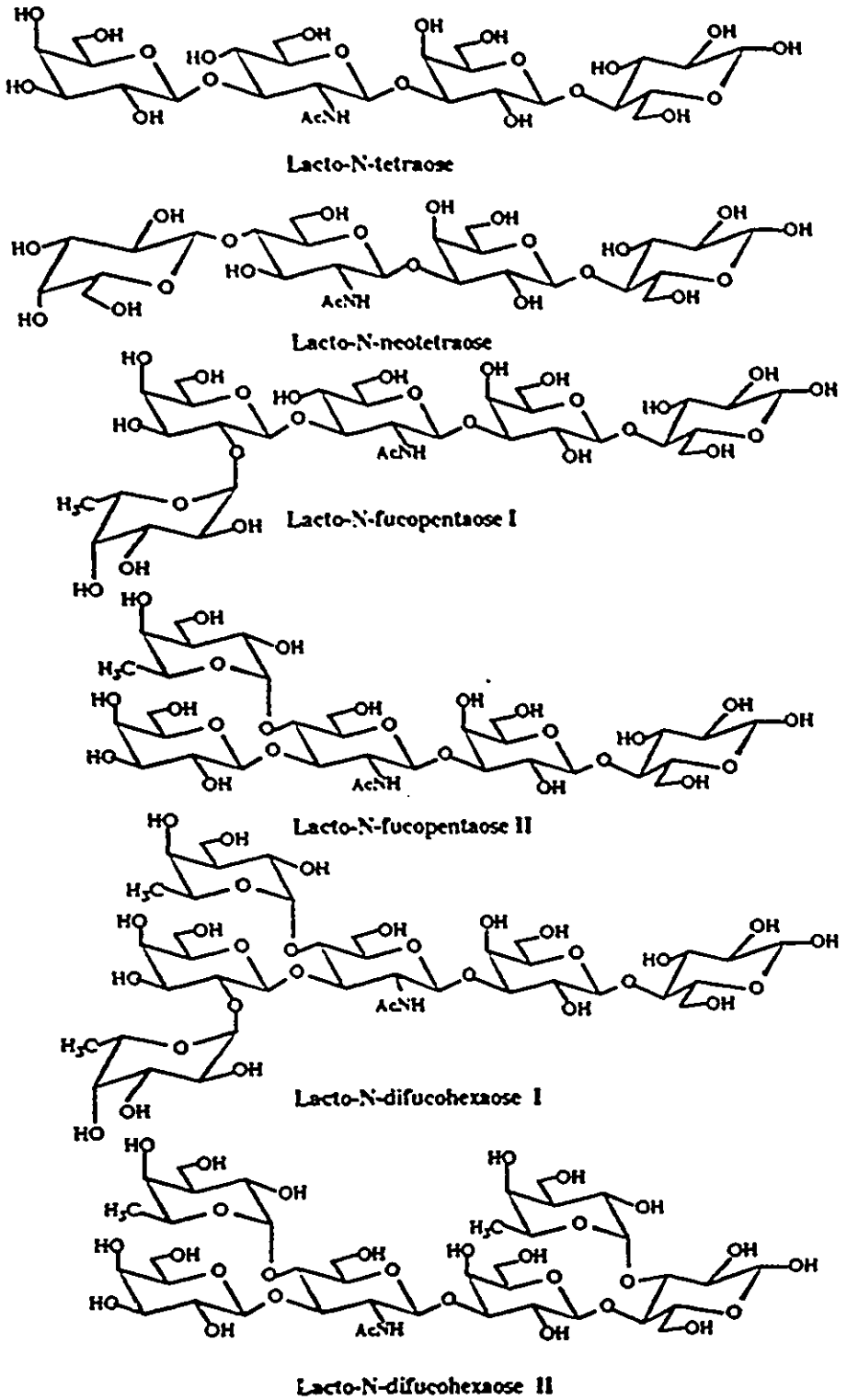


Fig. 1.2. Oligosaccharides isolated from human milk

and in the resistance against bacterial and viral infections which breast-feeding imparts on the newborn. Kuhn, Baer and Gauhe isolated the compounds shown in Fig. 1.2 from human milk and elucidated their structures[10].

This work was of fundamental importance to the development of present-day glycoconjugate chemistry as the types of components and glycosidic linkages present in these crystalline oligosaccharides were subsequently encountered in many different glycoconjugates, which play very important roles in immunochemistry and cell surface recognition. It was considered that the chemical synthesis of closely related but hitherto unknown oligosaccharides would provide useful substances that could serve as tools for future biochemical research in these fields, especially with regard to the investigation of structure-activity relationships. As a project for this thesis research, it was decided to lay some groundwork necessary for synthetic approaches that should ultimately lead to such structures. To start with, analogs of lacto-N-tetraose and lacto-N-neo-tetraose were considered as target molecules, *i.e.*, core oligosaccharides without attached fucose. The compounds shown in Fig.1.3 represent possible structures to be approached; their full formulas are given in Figs.1.3-1 to 1.3-3.

A. Gal(1→3)-GlcNac(1→3)-Gal(1→3)-Glc

B. Gal(1→4)-GlcNac(1→3)-Gal(1→3)-Glc

C. Gal(1→3)-GlcNac(1→3)-Gal(1→4)-GlcNac

D. Gal(1→3)-GlcNac(1→3)-Gal(1→3)-GlcNAc

E. Gal(1→4)-GlcNac(1→3)-Gal(1→4)-GlcNAc

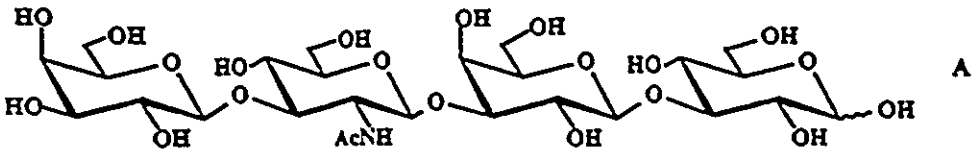
F. Gal(1→4)-GlcNac(1→3)-Gal(1→3)-GlcNAc

G. Gal(1→3)-GlcNac(1→3)-Gal(1→3)-GlcNac(1→3)-Gal(1→4)-Glc

H. Gal(1→4)-GlcNac(1→3)-Gal(1→3)-GlcNac(1→3)-Gal(1→4)-Glc

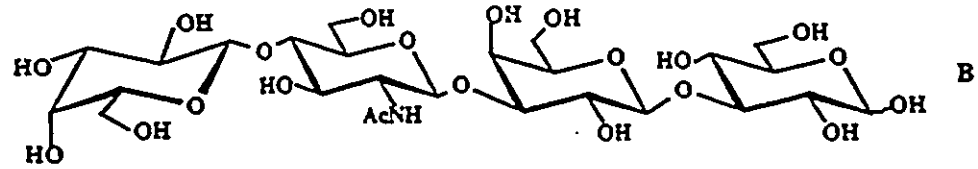
Fig. 1.3. Suggested target oligosaccharides[1]

1. Isomer of lacto-N-tetraose



Lacto-N-biose I Isolactose

2. Isomer of Lacto-N-neotetraose

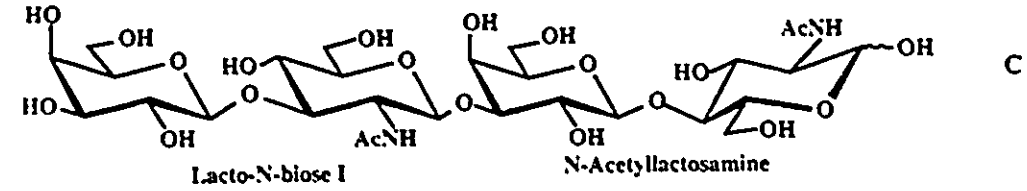


N-Acetyllactosamine Isolactose

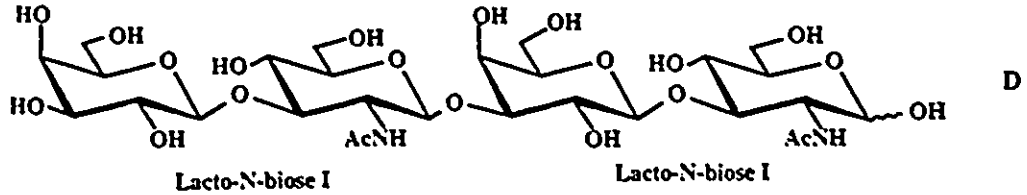
Fig. 1.3-1 Isomers of lacto-N-tetraose and lacto-N-neotetraose

[1] Gal = D-galactopyranosyl; GlcNAc = 2-acetamido-2-deoxy-D-glucopyranosyl (N-acetylglucosaminyl); Glc =D-glucopyranosyl.
For interglycocidic linkages the β-anomeric configuration is implied.

1. Bisacetamido analogs of lacto-N-tetraose

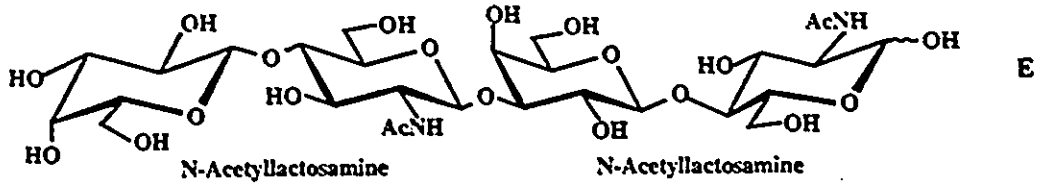


C

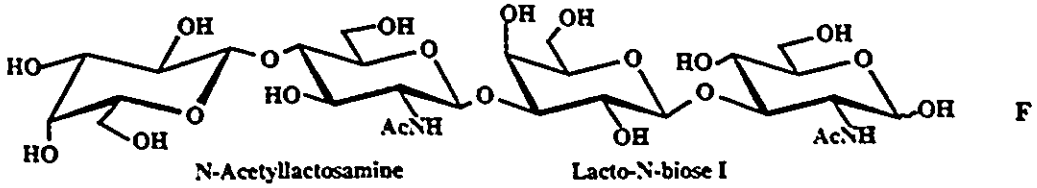


D

2. Bisacetamido analogs of lacto-N-neotetraose



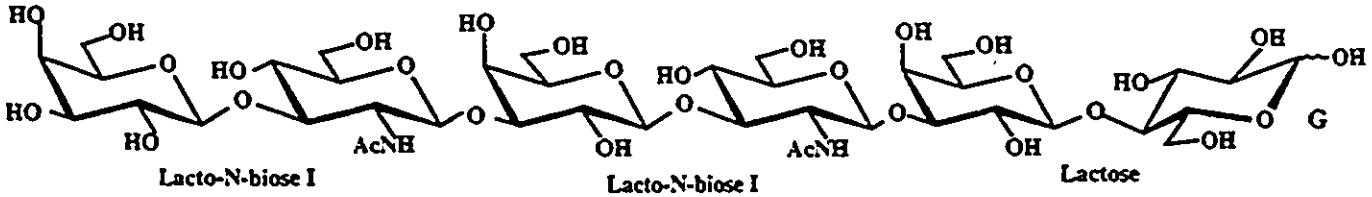
E



F

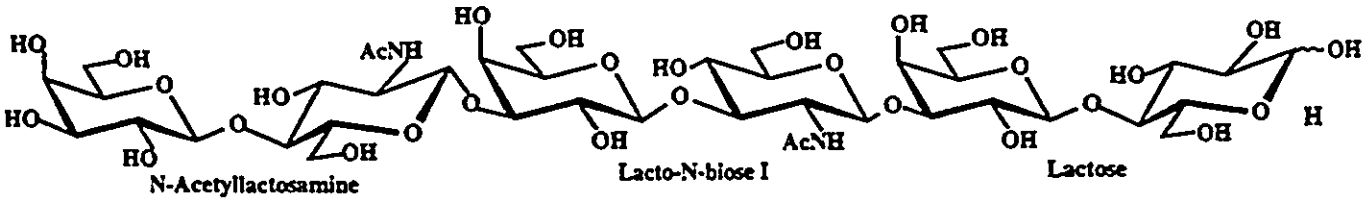
Fig. 1.3-2 Bis acetamido analogs of lacto-N-tetraose and lacto-N-neotetraose

Hexasaccharide homolog of lacto-N-tetraose



G

Hexasaccharide homolog of lacto-N-tetraose



H

Fig. 1.3-3 Hexasaccharide homologs of lacto-N-tetraose

Such oligosaccharides might serve as useful probes in the study of immunochemical problems and cell surface biochemistry; they may serve as substrates for the study of the action of glycosyl transferases; they could be employed for the preparation of affinity adsorbents for isolation of anti-carbohydrate antibodies; they may be conjugated to proteins to furnish such neo-glycoproteins as are increasingly being used as artificial antigens and as substrates for the characterization of lectin specificity[11-13].

Compounds A and B are linkage isomers of lacto-N-tetraose and lacto-N-neotetraose, respectively, having as the reducing half of the molecule a 3-O-β-D-galactopyranosyl-D-glucose unit instead of 4-O-β-D-galactopyranosyl-D-glucose (lactose) unit. Compound C would be an analog of lacto-N-tetraose in which the reducing glucose unit is replaced by N-acetylglucosamine; and D would correspond to a linkage isomer A of lacto-N-tetraose in which the reducing glucose unit is replaced by N-acetylglucosamine; they may be referred to as 'bisacetamido analogs of lacto-N-tetraose'. Compound E would be an analog of lacto-N-neotetraose in which the reducing glucose unit is replaced by N-acetylglucosamine; and F would be the corresponding linkage isomer analog of lacto-N-neotetraose in which the reducing glucose unit is replaced by N-acetylglucosamine; they may be referred to as 'bisacetamido analogs of lacto-N-neotetraose'. Compounds G and H are hexasaccharidic homologs of lacto-N-

tetraose, extended at the non-reducing terminal by 1→3 linked units of lacto-N-biose I and N-acetyllactosamine, respectively.

For the assembly of oligosaccharide structures such as those shown in the preceding Figures, it was necessary first to secure disaccharidic building blocks, namely, suitable glycosyl donors and appropriately blocked glycosyl acceptors that could then be linked together by one of the available glycosylation procedures. Thus, various derivatives of lactose and its β-(1→3) linked isomer ("isolactose"), as well as of lactosamine and its β-(1→3) linked isomer (lacto-N-biose I) were required (Fig. 1.4)[2]:

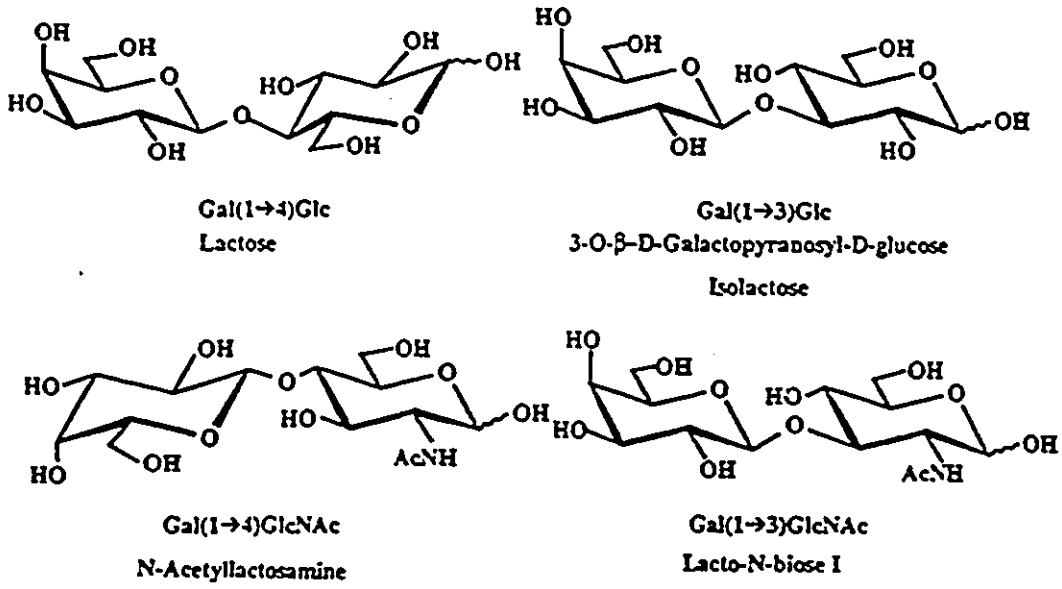
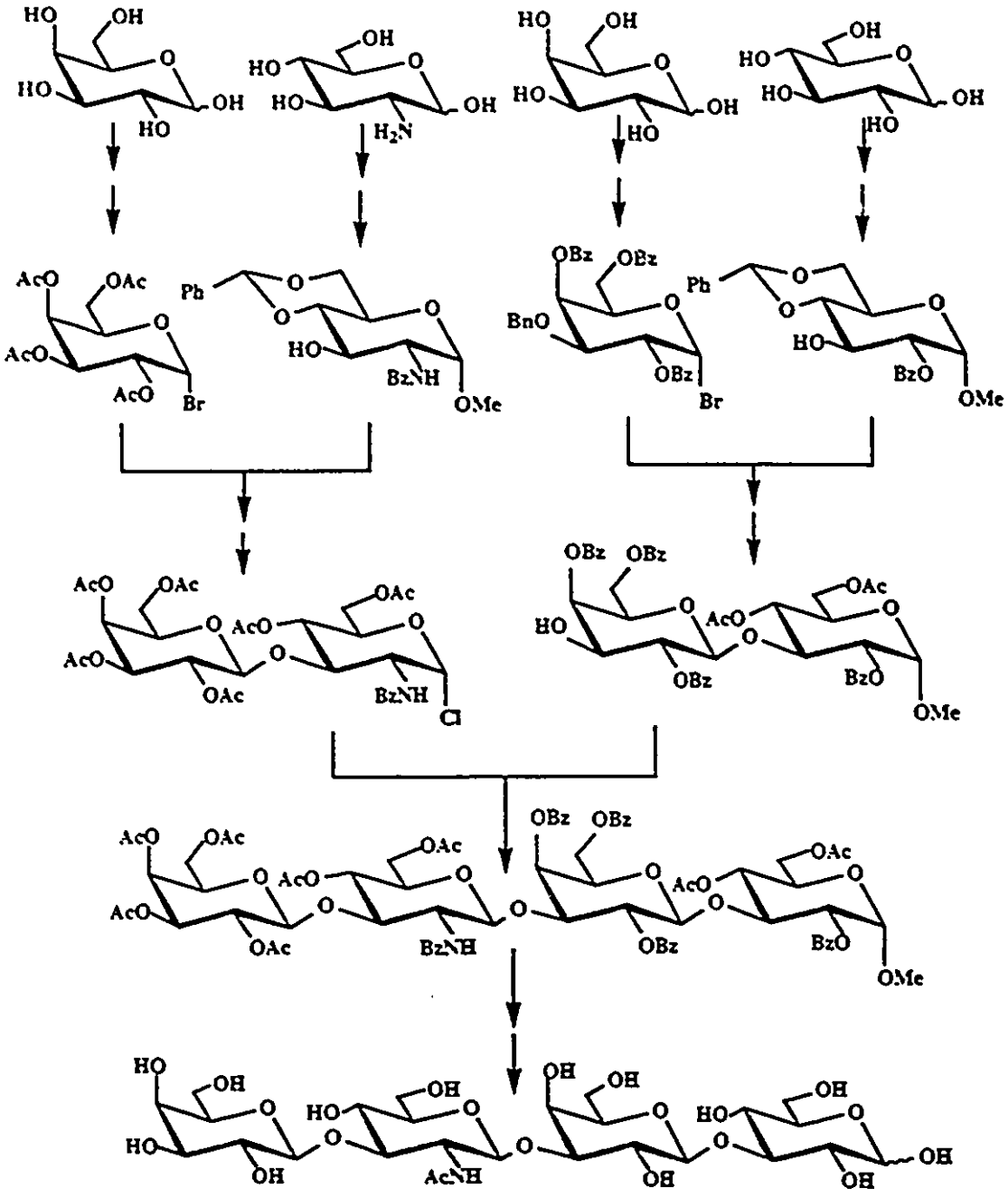


Fig. 1.4 Disaccharide building blocks

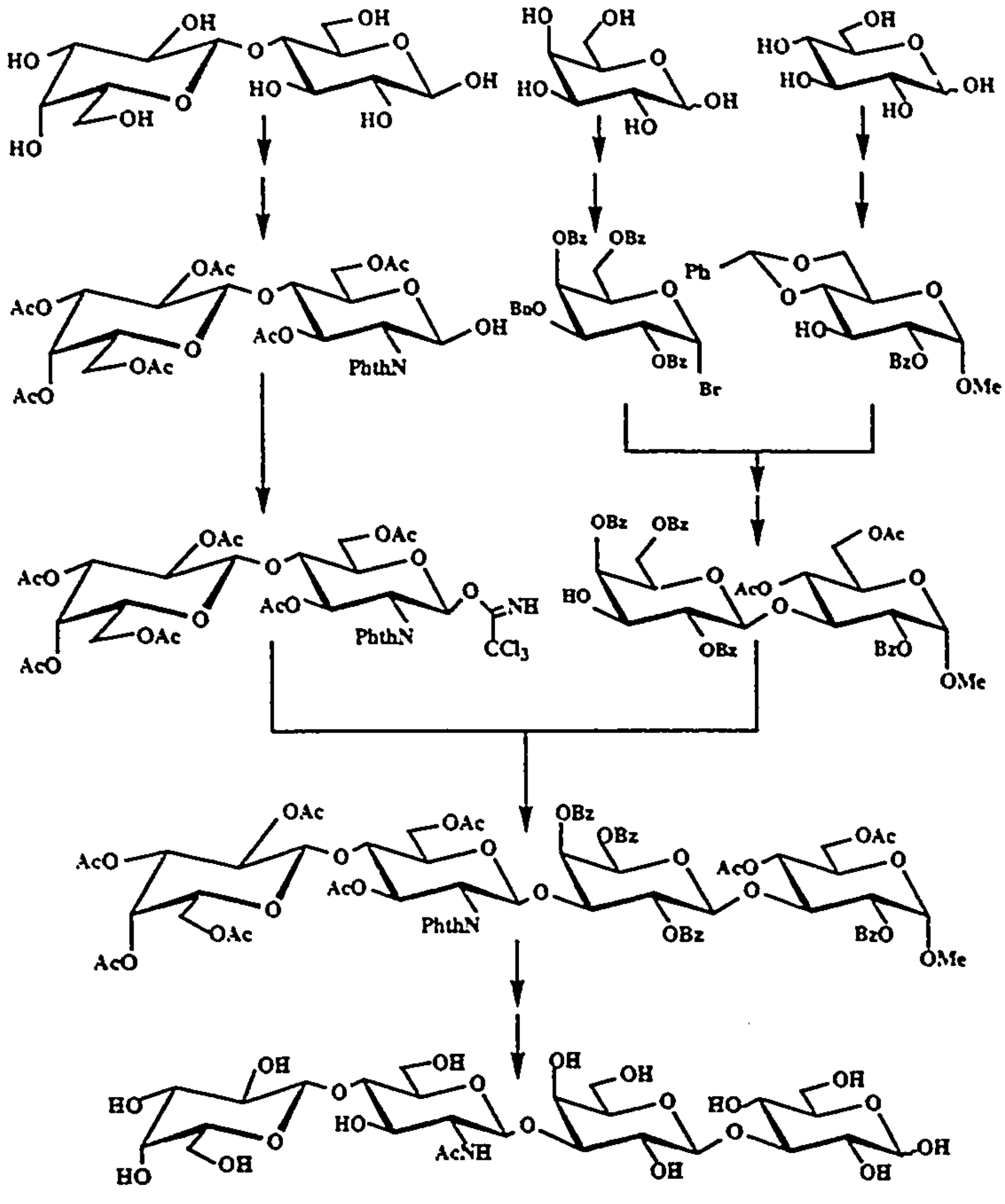
[2] Isolactose is not a generally recognized trivial name for this linkage isomer of lactose; it is used herein for the sake of simplicity. Also, the Roman numeral in lacto-N-biose I will from here on be omitted since its regio-inverted isomer known as lacto-N-biose II [3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-galactose] does not occur in this discussions.

For use as glycosyl donors, the molecules have to be converted into derivatives having an activated reducing terminal (*i.e.*, bearing a good leaving group on the anomeric carbon of the reducing terminal) and being blocked by temporary protecting groups at all other reactive centres. For use as glycosyl acceptors, derivatives must be procured in which the hydroxyl proposed for the linkage is free and all other reactive centres are temporarily blocked.

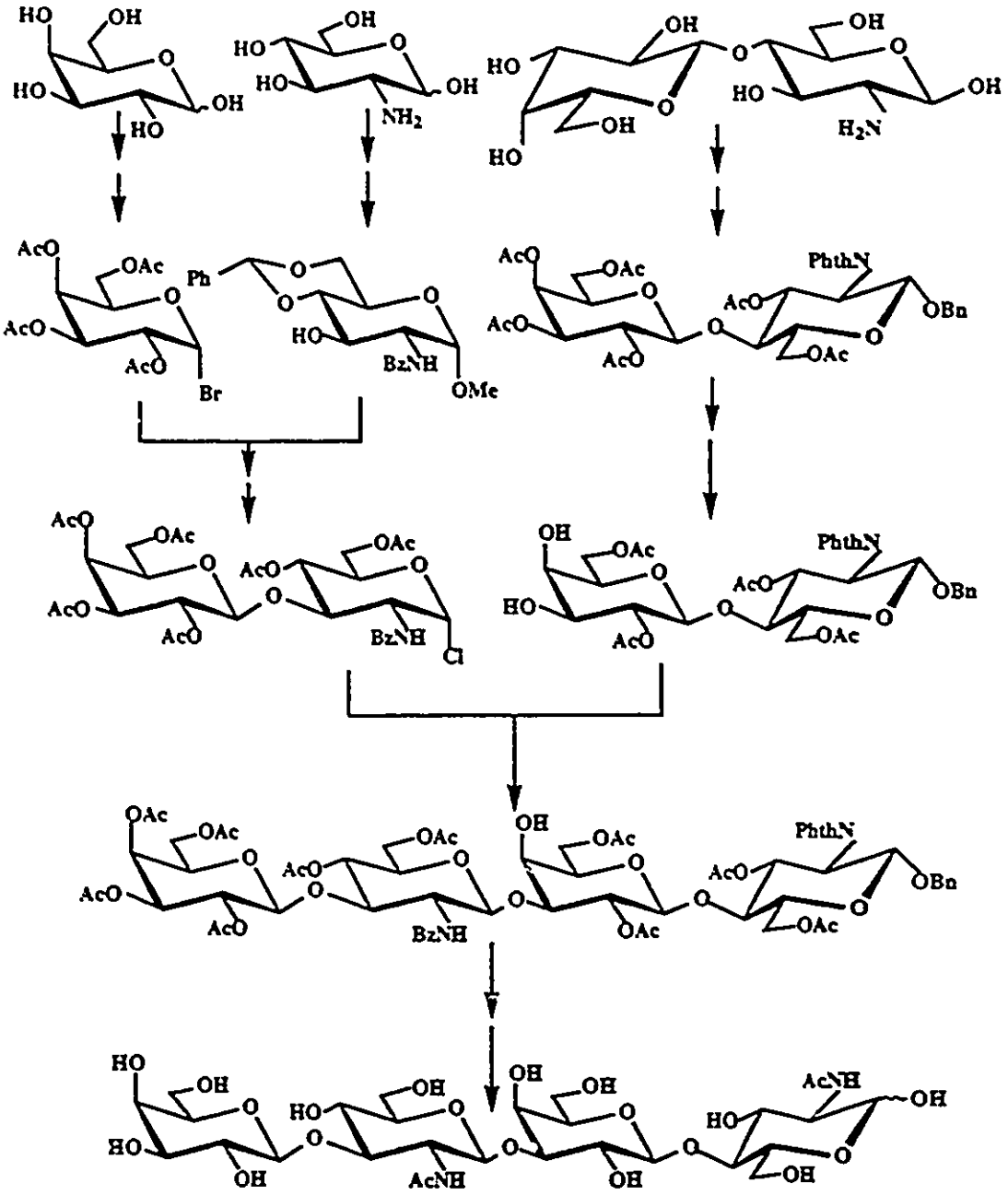
Based on retro-synthetic analyses and once appropriate building blocks are in hand, it should eventually become possible to synthesize some of the target molecules listed in Fig. 1.3 as proposed in Schemes 1.1-1.8. However, it should be recognized that actual production of the target oligosaccharides is a long-term goal; the present investigation, which constituted an initial foray into the field in this laboratory, had to focus on the procurement of useful disaccharidic glycosyl donors and acceptors. To this end, the literature had to be surveyed in search of components already described and possibly suitable for the present purposes; existing procedures for the preparation of requisite donors and acceptors had to be evaluated and, if need be, modified or improved; and new intermediates to be employed in various reaction sequences had to be synthesized and characterized.



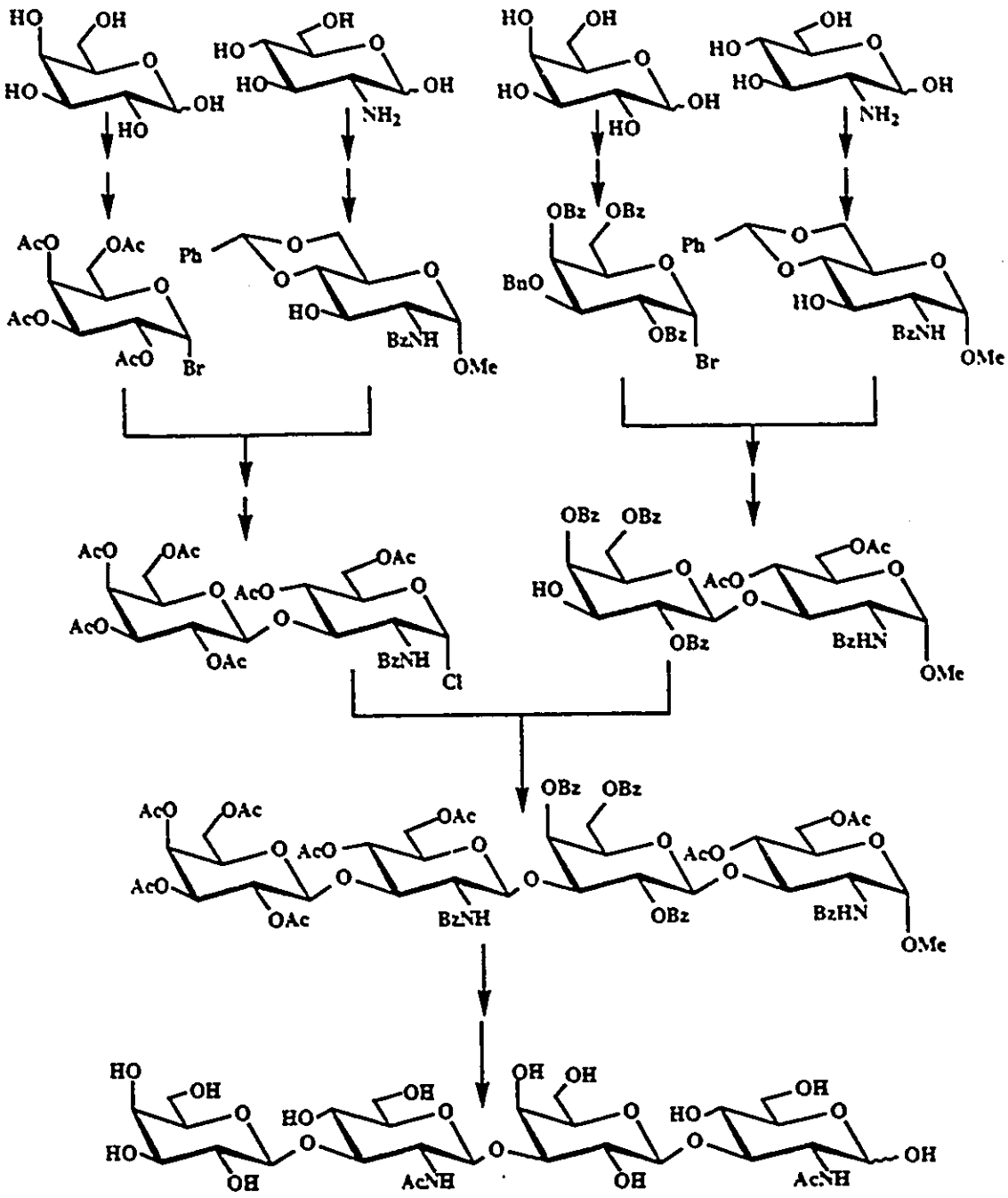
Scheme 1.1 Proposed synthesis of isomer A of lacto-N-tetraose



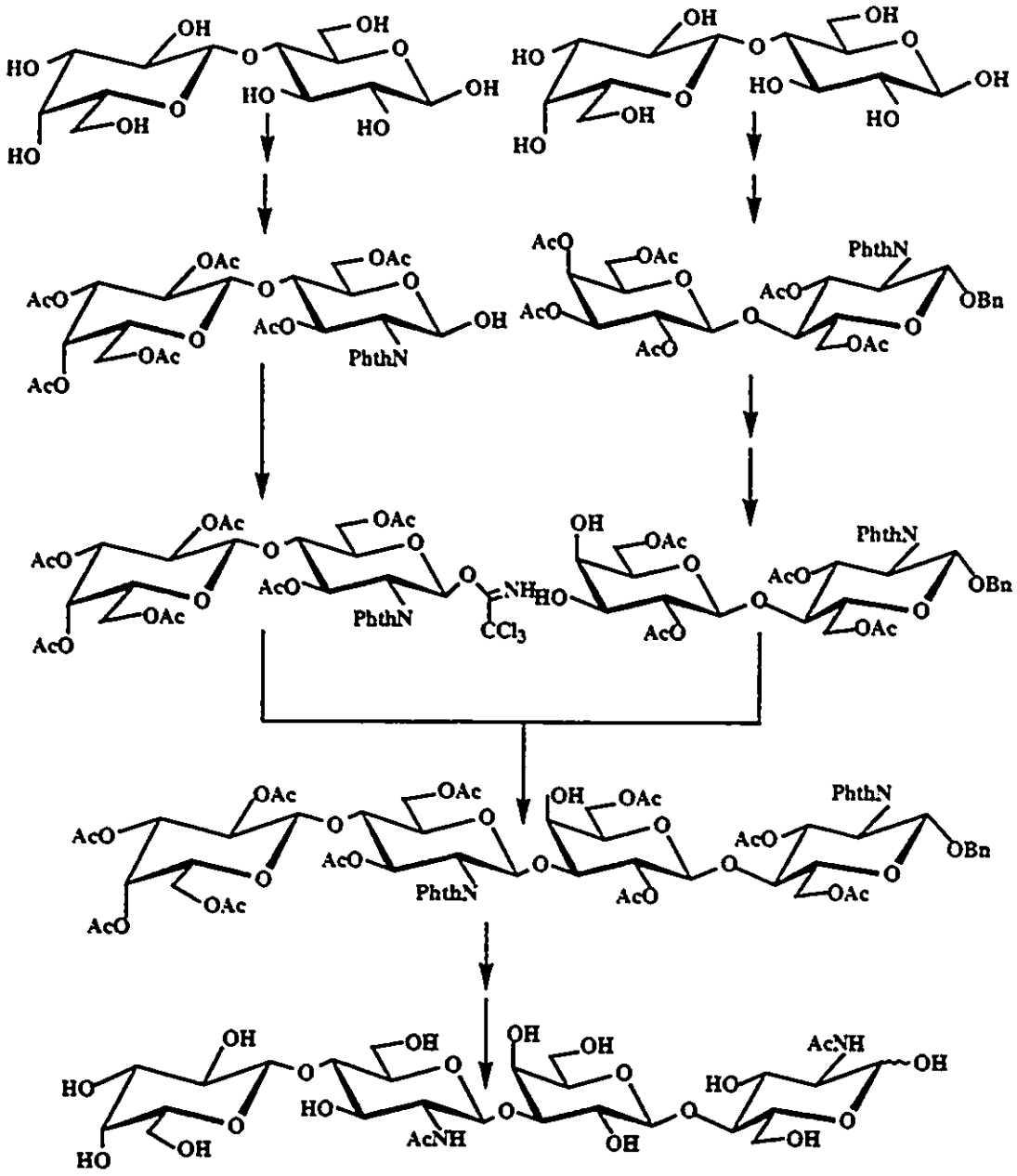
Scheme 1.2 Proposed synthesis of isomer B of lacto-N-neotetraose



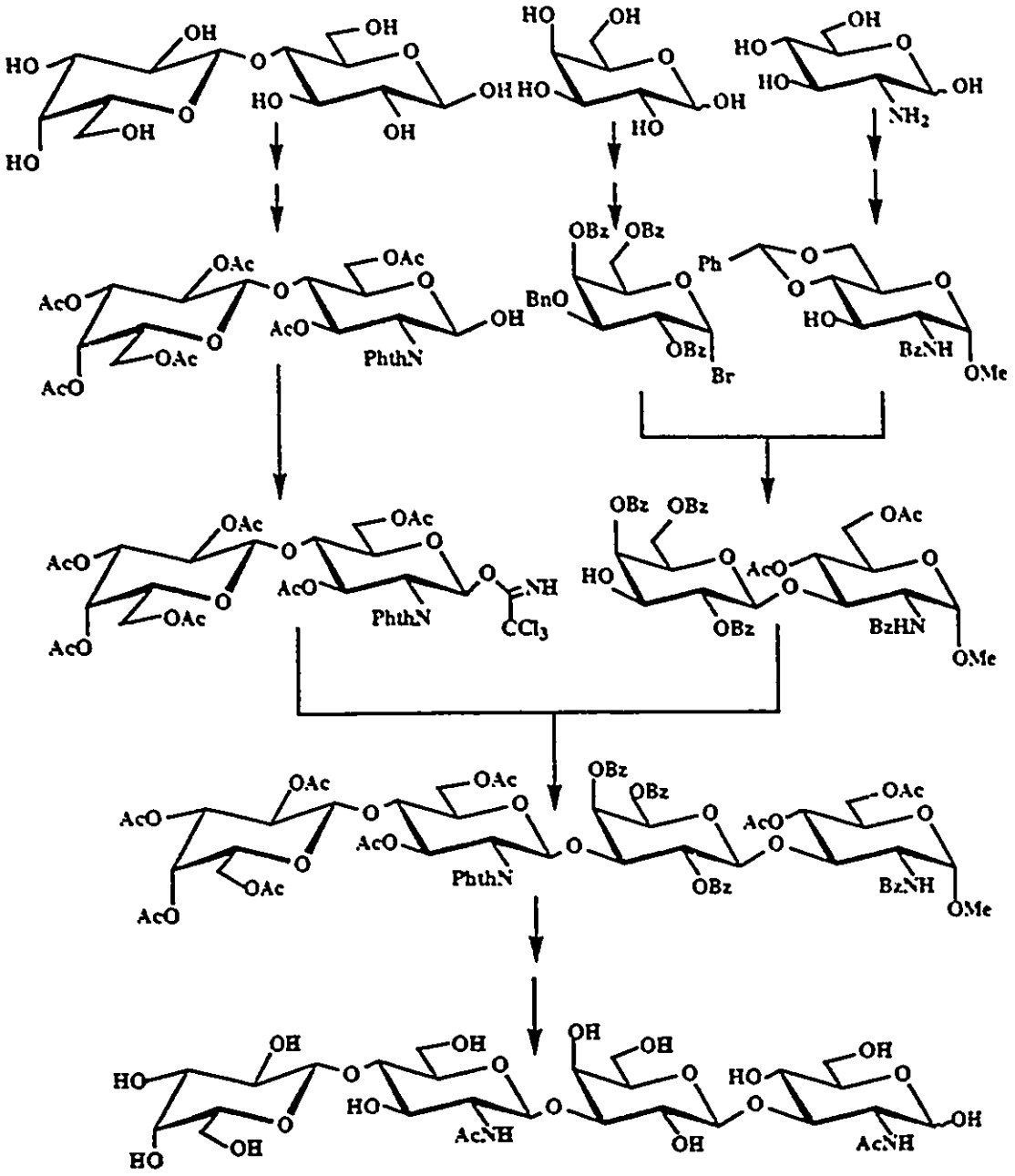
Scheme 1.3 Proposed synthesis of bisacetamido analog C of lacto-N-tetraose



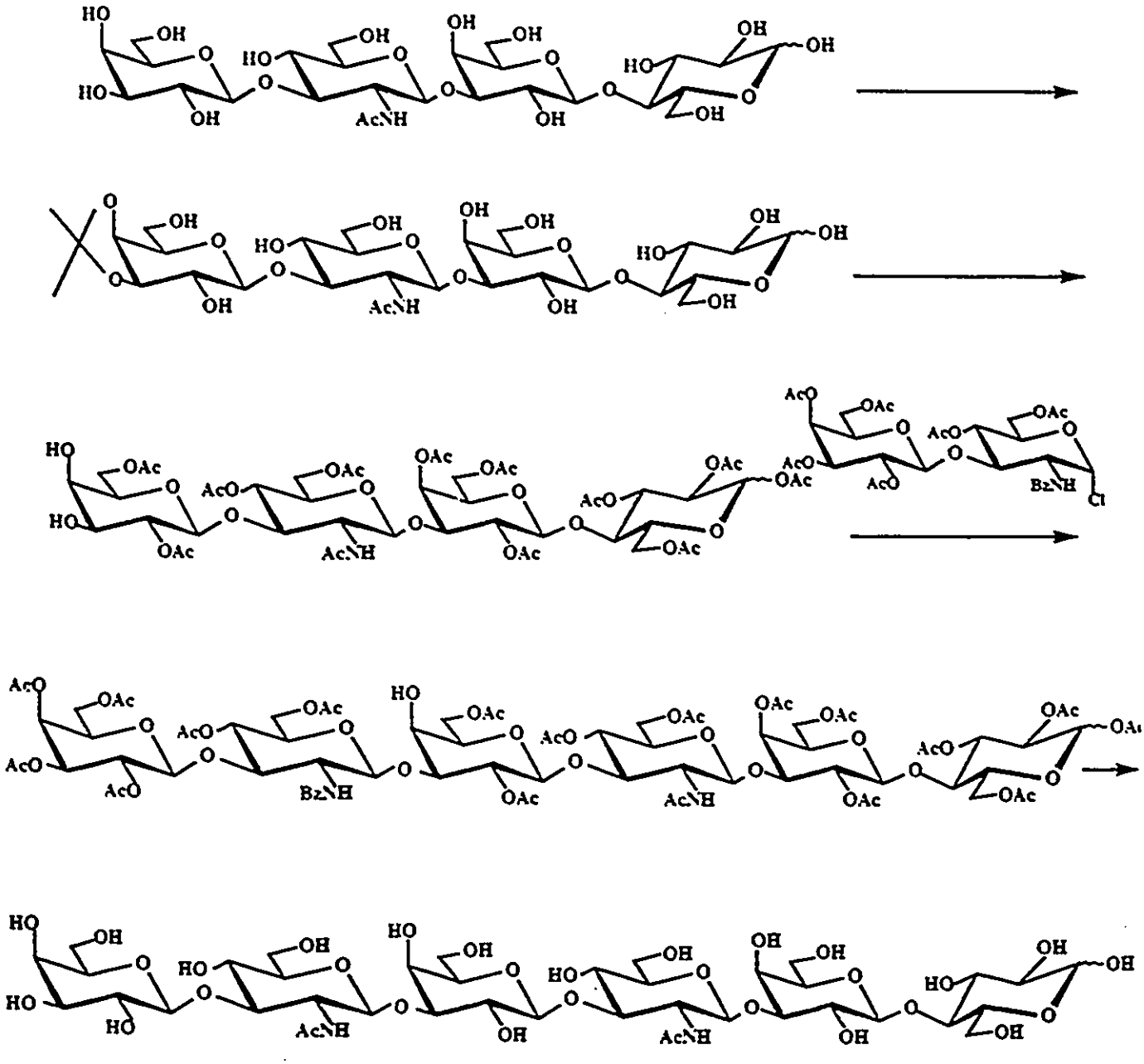
Scheme 1.4 Proposed synthesis of bisacetamido analog D of lacto-N-tetraose



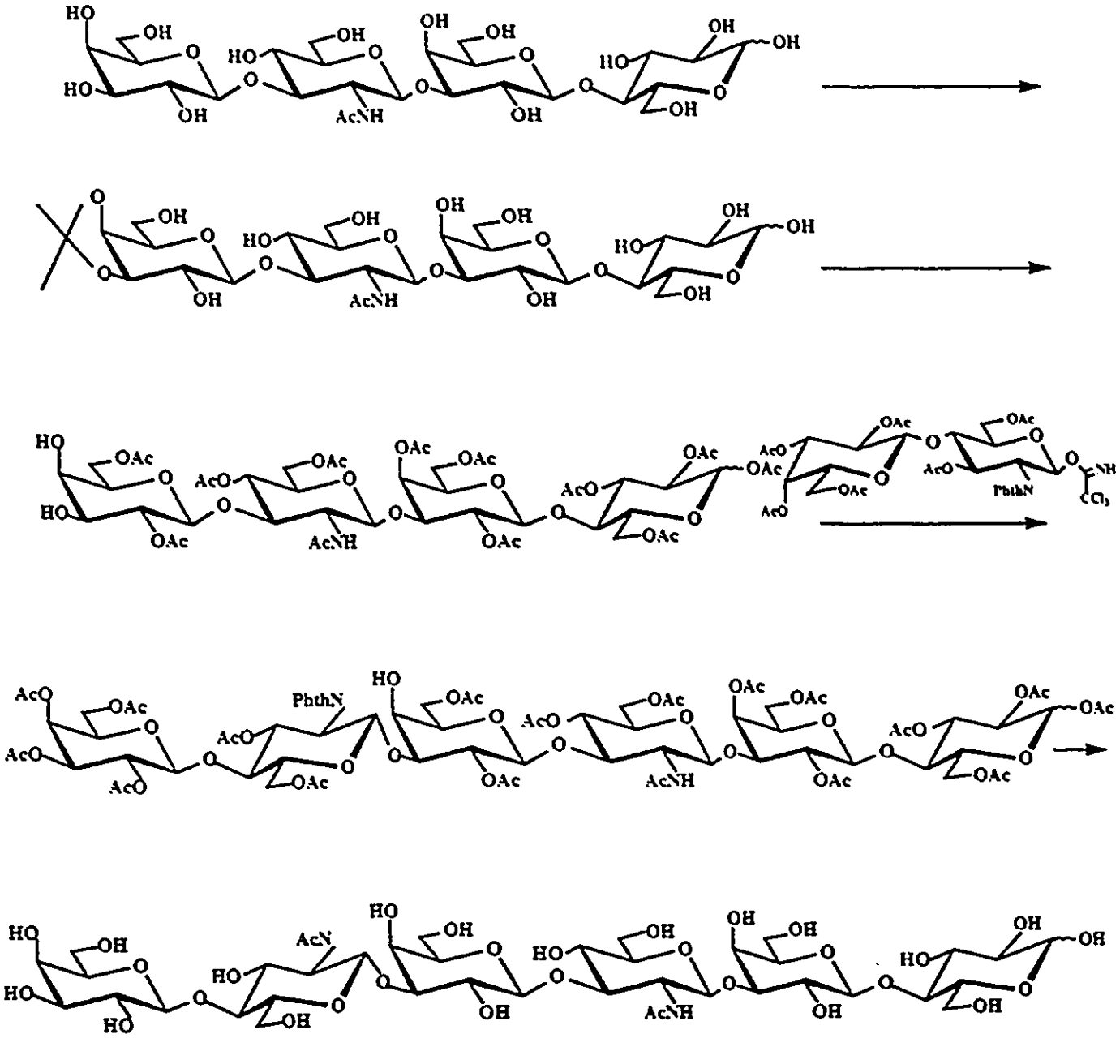
Scheme 1.5 Proposed synthesis of bisacetamido analog E of lacto-N-neotetraose



Scheme 1.6 Proposed synthesis of bisacetamido analog F of lacto-N-neotetraose



Scheme 1.7 Proposed synthesis of hexasaccharidic homolog G of lacto-N-tetraose



Scheme 1.8 Proposed synthesis of hexasaccharidic homolog H of lacto-N-tetraose

2. RESULTS AND DISCUSSIONS

The chemistry discussed in the section that follows concerns the preparation of lactosamine and lacto-N-biose-I donors, of lactose, lactosamine, and 2-azido-2-deoxy-lactose acceptors, and of lacto-N-biose-I as well as 3-β-D-galactopyranosyl-D-glucose (isolactose) acceptors.

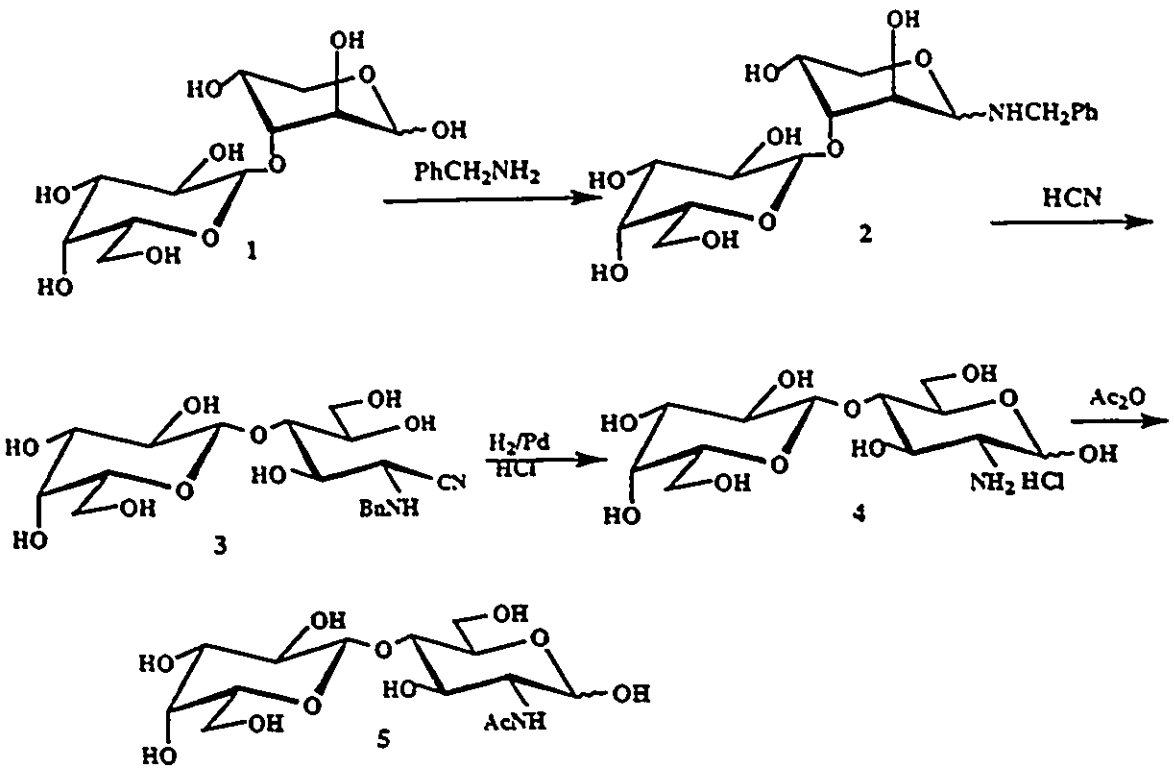
2. 1. Preparation of lactosaminyl donors

2. 1. 1. Introduction

2-Acetamido-2-deoxy-4-O-β-D-galactopyranosyl-D-glucose (N-acetyllactosamine) is a disaccharide of widespread occurrence as a component of the oligosaccharide portions of very many N- and O-linked glycoproteins and the oligosaccharides of human milk[10]. It is also a building unit for the antigenic determinants of a number of certain Ii human blood group activities[14] as well as of the ABH Type 2 determinants[9]. For the synthesis of oligosaccharides containing the N-acetyllactosamine unit, suitable donors of the molecule were required, *i.e.* temporarily protected derivatives featuring an activated anomeric center. Among others, peracetylated N-phthaloyllactosaminyl halides and trichloroacetimidates have been used successfully for the purpose. The simplest approach to such derivatives would start from commercially available lactosamine hydrochloride, but since this product is very expensive it was felt

that a number of known procedures employing more economical starting materials should be evaluated for the convenient preparation of donors on a fairly large scale.

The first practical synthesis of lactosamine was reported by Kuhn and Kirschenlohr.[15] It starts from 3-O-β-D-galactopyranosyl-D-arabinose **1**, a sugar readily prepared by degradation of lactose (and now also available commercially). Key steps are the addition of benzylamine to give the glycosylamine **2**, subsequent addition of hydrogen cyanide (Kiliani-Fischer cyanohydrin reaction) to form the aminonitrile **3**, and catalytic "half-hydrogenation" of **3** by use of a specially developed palladium catalyst in the presence of dilute hydrochloric acid. This hydrogenation converts the nitrile to the imine and simultaneously splits off the N-benzyl group; the imine is trapped by cyclization with OH-5 and the resulting glycopyranosylamine is hydrolyzed to lactosamine hydrochloride **4**, *in situ*. The latter can readily be N-acetylated to **5**, if desired, by standard procedures (Scheme 2.1). Several modifications of the synthesis were reported later[16,17], including generation of HCN *in situ* from potassium cyanide and glacial acetic acid, which avoids the handling of this reagent and allows the preponderant 2-benzylamino-2-deoxy-D-gluconononitrile derivative **3** to crystallize directly from the reaction mixture.



Scheme 2.1 Synthesis of lactosamine by Kuhn and Kirschenlohr

There are several other routes to lactosamine derivatives, two of which start with hexa-O-acetyllactal (9, see Scheme 2.2). This compound belongs to the structural family of "glycals", which are 1,5-anhydro-2-deoxy-hex-1-enitol derivatives. The preparation of reactive lactosamine donors from precursors engendered both by a glycal method and by the foregoing cyanohydrin synthesis will be examined in the sections that follow. Moreover, it is possible to

synthesize lactosamine donors from monosaccharidic components, *i.e.* by galactosylation of properly protected D-glucosamine derivatives[18], and this approach will also be evaluated.

2. 1. 2. Preparation of 3, 6, 2', 3', 4', 6'-hexa-O-acetyllactal

Glycals are versatile intermediates in synthesis, illustrated by many recent applications[19]. 3,4,6-Tri-O-acetyl-D-glucal, 3,4,6-tri-O-acetyl-D-galactal, and 3,6,2',3',4',6'-hexa-O-acetyllactal **9** are important starting materials for the preparation of numerous derivatives of their parent sugars, including those containing a 2,3-double bond[20] and 2-acetamido function[21]. In the traditional method for the preparation of **9** described by Haworth and coworkers[22], 2,3,6,2',3',4',6'-hepta-O-acetyl-α-lactopyranosyl bromide **8** (acetobromolactose, prepared from commercial lactose **6** by reaction of its β-octaacetate **7** with hydrogen bromide) is reacted with zinc dust in aqueous acetic acid, but the yields of peracetylated glycal **9** are usually low (<50 %). Various methods of activation of the zinc have been proposed though it also has been claimed that such activation failed with some zinc samples[23].

A variety of other reductive methods have also been reported to convert glycosyl halides into glycals. Ireland and coworkers[24] used lithium in liquid ammonia as a reagent for converting glycosyl chloride derivatives into the

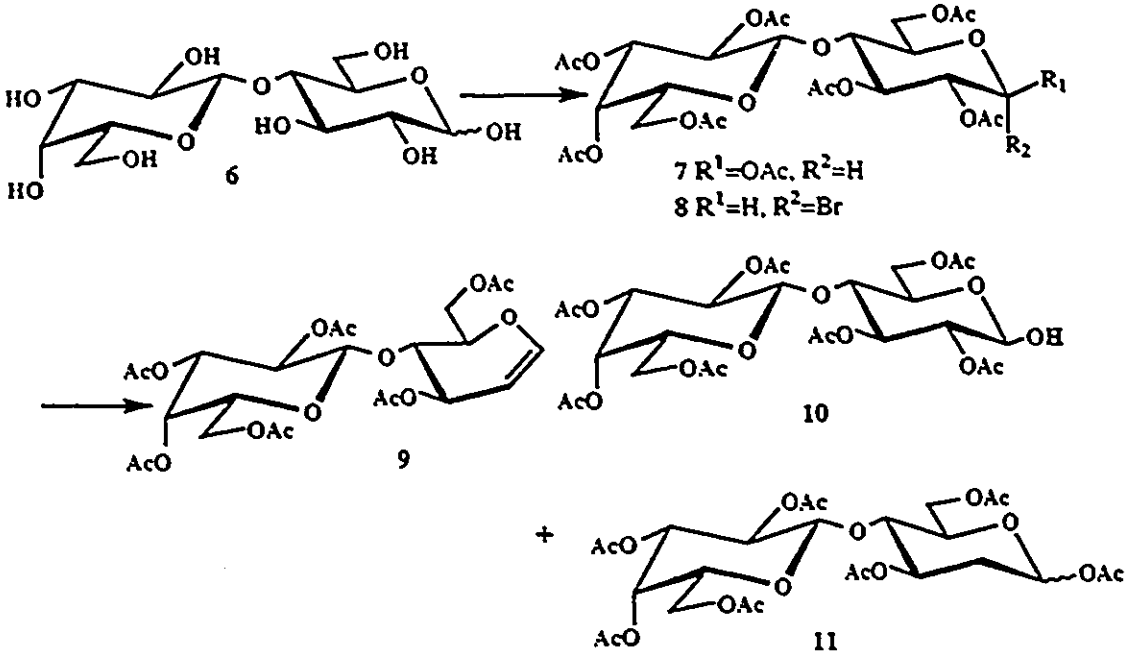
corresponding glycal derivatives. Williams and coworkers[25] reported that peracetylated glycosyl chlorides were readily converted into peracetylated glycals by the reaction with chromium (II) diacetate dimer in dimethylformamide in the presence of 1, 2-ethanediamine. Sinaÿ and coworkers[26] showed the conversion of a variety of thiophenyl glycosides and glycosyl phenyl sulfones into glycal derivatives using lithium naphthalenide, followed by elimination of the substituent at C-2 and also by using samarium diiodide (SmI_2) in tetrahydrofuran-hexamethylphosphoric triamide (THF-HMPA)[27].

Although these new methods provide multiple choices for the preparation of lactal derivatives, some of the techniques involved appeared less simple, and some of the reagent required less common, than those of the classical procedure. Also, a number of additional steps are needed to prepare certain intermediates (*e.g.* thioglycosides and sulfones), which tend to diminish the achievable overall yields based on lactose, the commercial starting material. All things considered, the traditional method[22] referred to above still appeared attractive in view of its simplicity and economy, and it was therefore decided first to try and see if it can perhaps be performed in a more efficient manner.

When the reaction of acetobromolactose **8** with zinc dust was carried out as directed[22], in 50 % acetic acid at 0-5 ℃ for 2 h, the desired lactal **9** (yield, < 50 %) and octaacetyllactose **7** as a by-product (8-10 %) were obtained as

previously described. However, two additional undesired products were found and identified as 2,3,6,2',3',4',6'-hepta-O-acetyl-β-lactopyranose **10** (major) and 1,3,6-tri-O-acetyl-4-O-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-D-glucose **11** (minor). Their formation may be ascribed to the hydrolysis of **8** and addition of an acetyl group to the 1,2 double bond of **9**, respectively.

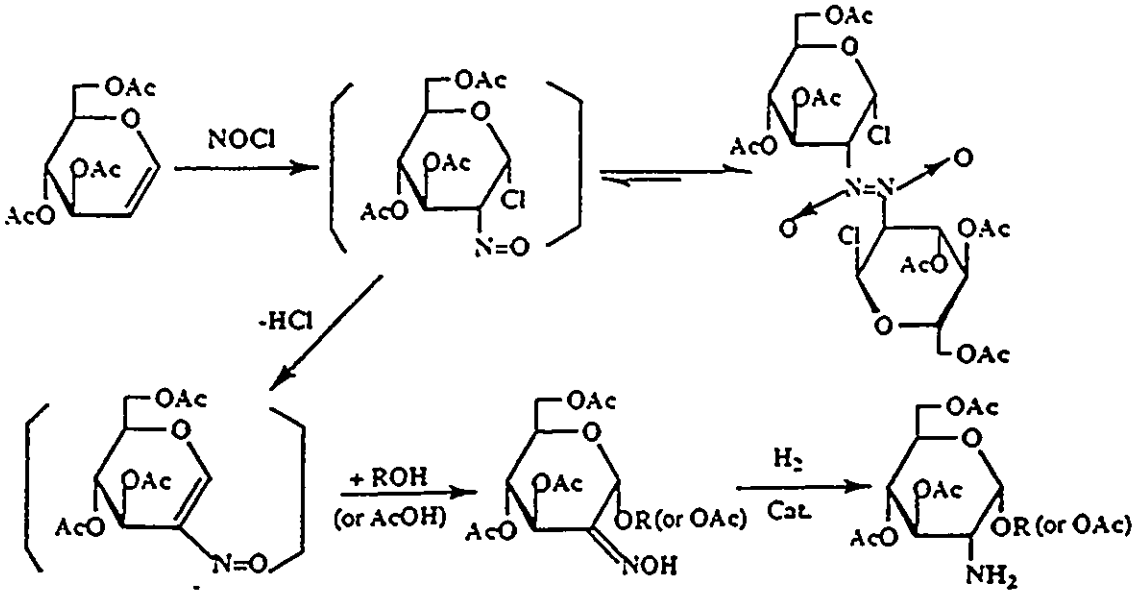
We then performed similar experiments at different temperatures (-15° to +50° C) and with different concentration of acetic acid (30, 50, 70 %) without activating agents for the zinc, and with catalytic amounts of cupric sulphate or chloroplatinic acid as activators. The best result was achieved when the reaction was carried out during 2 h in 50 % acetic acid, at -5 to -10° C, with addition of a catalytic quantity of chloroplatinic acid. Crystalline hexa-O-acetyllactal **9** containing only traces of by-products was obtained in 95 % yield, and the recrystallized material was pure according to its NMR spectra and agreed in melting point and optical rotation with literature[22] data. This modification in procedure represents a welcome advance in the preparation of this important derivative. Its use in the synthesis of lactosaminyl donors will be described in the section that follows.



Scheme 2.2 Synthesis of hexaacetyllactal 9

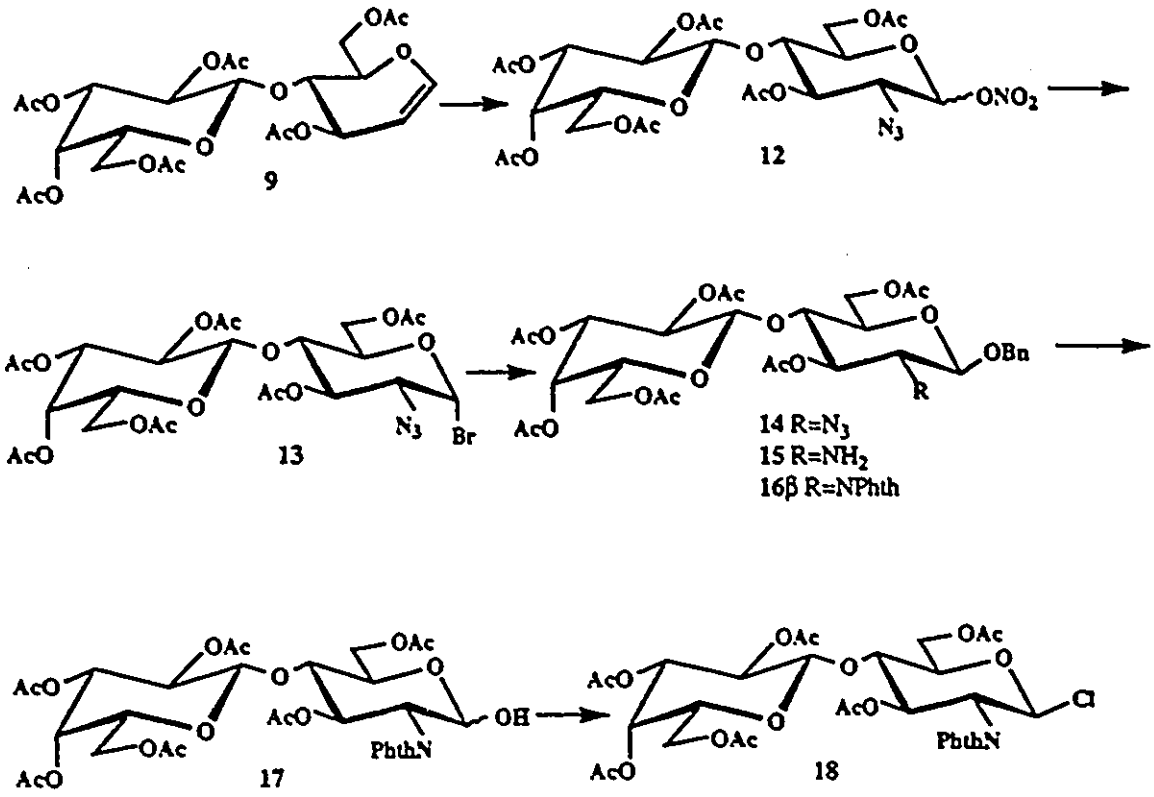
2. 1. 3. Preparation of hexa-O-acetyl-2-deoxy-2-phthalimido-β-lactosyl halides: 3, 6-Di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride 18 and bromide 21.

The first method of converting glycals into amino sugars was developed by Lemieux and coworkers (Scheme 2.3-1). Reaction of glycals with nitrosyl chloride affords a nitroso chloride (isolable as a crystalline dimer), which readily undergoes dehydrochlorination to a (nonisolable) nitrosoalkene. The latter is trapped by adding an alcohol or acetic acid and subsequently tautomerizes to an oxime. Hydrogenation of the oxime provides an amino sugar[28a-c][29].



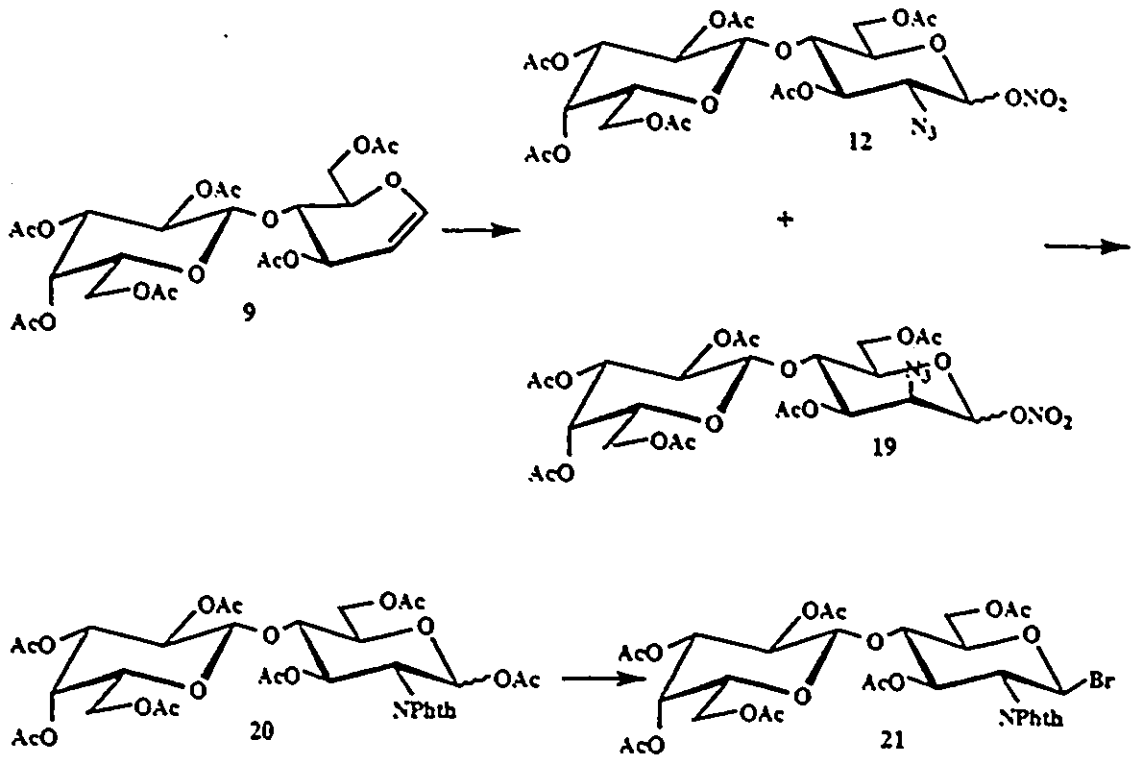
Scheme 2.3-1 Lemieux's nitroso chloride route to amino sugars

However, more recently this process has largely been superseded by the more useful process of azidonitration of glycals, also first introduced by Lemieux[30] (Scheme 2.3-2). It is based on an addition reaction which glycals undergo when treated with cerium (IV) ammonium nitrate and sodium azide. Thus, compound **9** furnished an anomeric mixture **12** of 2-azido-2-deoxy nitrates, from which the lactosaminyl donor **18** has been elaborated as shown in Scheme 2.3-2. It is to be noted that the intermediate azido bromide **13** may also be used as a glycosyl donor in oligosaccharide construction, with the azido group being of particular advantage as it does not engage in neighboring-group participation. It can, of course, be reduced to the amino group at the later stage of a synthesis.



Scheme 2.3-2 Synthesis of β –lactosaminyl chloride (azidonitrate route elaborated by Lemieux)

The azidonitration route has been simplified by Arnarp and Lönngren[31] who prepared the protected lactosaminyl bromide **21** as shown in Scheme 2.4. In their hands, azidonitration of **9** led to an anomeric mixture of nitrates **12**, together with a small proportion of *manno* isomer **19**, with a ratio 12β:12α:**19** = 8:4:1. Direct hydrogenation of the product, followed by N-phthaloylation and O-acetylation, gave hepta-O-acetyl-N-phthaloyl-α,β-lactosamine **20**, which was finally converted into the bromide **21** by the action of anhydrous hydrogen bromide in dichloromethane. Previously, Lemieux and co-workers[28c] had obtained the analogous chloride **18** by treatment of the hexa-O-acetyl-N-phthaloyllactosamine **17** with a Vilsmeier reagent, N,N-dimethylchloroforminium chloride (Scheme 2.3-2).



Scheme 2.4 Synthesis of β –lactosaminyl bromide (simplified azidonitration by Arnarp and Lönngren)

It appeared to us that the method of Arnarp and Lönngren (Scheme 2.4) offered the most convenient access to lactosaminyl halides, and therefore we performed azidonitration of **9** as directed[31]. The crystalline mixture of azidonitrates (**12α**, **12β**, and a small proportion of **19**), obtained in 65 % yield, was immediately hydrogenated over palladium on carbon, and the resulting mixture of amino sugars was *N*-phthaloylated with phthalic anhydride in 90 % ethanol, and *O*-acetylated with acetic anhydride in pyridine. Processing involving column chromatography afforded a 47 % yield of *N*-phthaloyllactosamine heptaacetate **20** as a syrupy mixture of anomers. Although further chromatography gave fractions of the pure, crystalline anomers (**20α** and **20β**), the bulk of the material remained unresolved. However, it was discovered during these studies that treatment of such a mixture with acetic anhydride in the presence of a catalytic amount of perchloric acid at room temperature shifted the anomer ratio in favor of **20β**, and this anomer then crystallized from methanol in 65 % yield, without resort to chromatography.

Alais and Veyrières[32] reported an alternative, convenient synthesis of N-phthaloyllactosamine based on the aforementioned lactosamine synthesis (Scheme 2.1) of Kuhn and Kirschenlohr[15], which we also examined. From commercial 3-O-β-D-galactopyranosyl-D-arabinose **1**, benzylamine, and hydrogen cyanide was produced 2-benzylamino-2-deoxy-4-O-β-D-galactopyranosyl-D-

glucononitrile **3**, catalytic hydrogenation of which in acidic medium afforded an aqueous solution of crude lactosamine hydrochloride **4** and an equivalent amount of ammonium chloride. Reaction of phthalic anhydride with unprotected 2-amino-2-deoxy sugars is usually performed[33] in methanol in the presence of triethylamine to give 2-(2-carboxybenzamido) sugars ("phthalamic acids"), and this reaction can also be done in aqueous acetone with sodium hydrogencarbonate, or in aqueous 1,4-dioxane with triethylamine[34], as was described for amino acids. In the present instance, the solution of **4** and excess sodium hydrogencarbonate was reacted with phthalic anhydride in acetone, and crude N-(2-carboxybenzoyl)-lactosamine so obtained was treated with acetic anhydride and pyridine to achieve full O-acetylation and cyclisation to give the peracetylated phthalimido derivative, obtained as a 2:1 mixture of the α,β-anomers of **20**. Pure crystalline **20β** was isolated in 32 % yield by column chromatography on silica gel. Unresolved material eluted from the column was treated with a catalytic amount of perchloric acid in acetic anhydride to convert part of **20α** into the more stable **20β** which then crystallized, increasing its total isolated yield to 50 %.

For conversion of the lactosaminyl 1-acetate **20** (or of the corresponding, free 1-hydroxy compound **17**) into lactosaminyl halides, there are several different reagents: N,N-dimethylchloroforminium chloride (a Vilsmeier

reagent[35]) was used for the reaction **17** → **18** (Scheme 2.3-2), and hydrogen bromide in dichloromethane for the reaction **20** → **21** (Scheme 2.4) as mentioned before. Aluminum chloride[29] and anhydrous hydrogen chloride[36] served for conversion **20** → **18**.

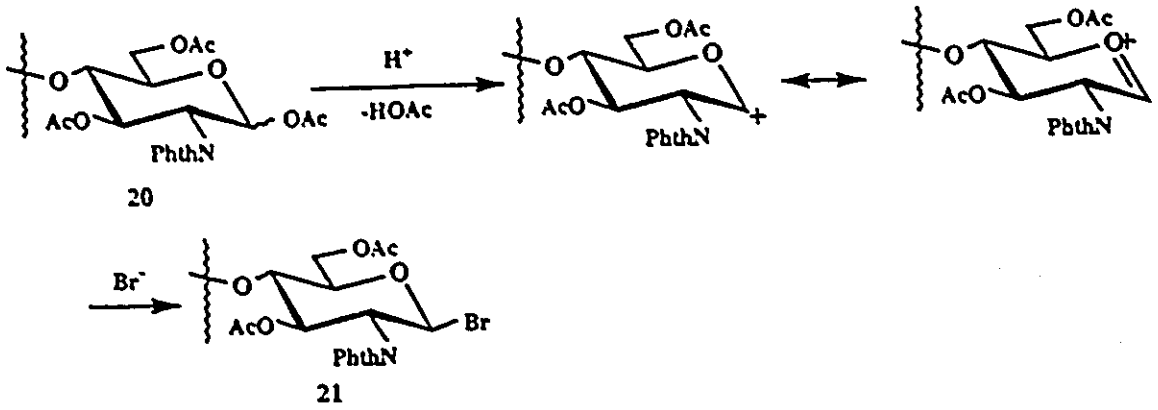
It was decided to explore the utility of other reagents, and indeed, improvements in these halogenations could be accomplished. Thus, whereas repetition of the literature[36a] procedure for chlorination of **20β** using anhydrous hydrogen chloride in dichloromethane gave the glycosyl chloride **18** in 46 % yield (reported: 40 %) after a reaction time of 48 h, treatment of **20β** with 1,1-dichloromethyl methyl ether[37] and zinc chloride in dry chloroform for 30 min furnished **18** in 95 % yield. Similarly, the reaction of **20** (α,β-mixture) with hydrogen bromide in dichloromethane had been reported[31] to give 65 % of glycosyl bromide **21**(α,β-mixture) after a 48 h reaction period. We chose to treat **20** overnight with trimethylsilyl bromide[38] in chloroform solution, which afforded **21** in 90 % yield. The advantages of these new modifications are obvious.

It may be appropriate at this point to present a brief discussion of the mechanism of glycosyl halide formation. To convert a protected sugar or glycoside into a glycosyl halide, a leaving group must first be generated at the anomeric center. The most common and simplest way to do this is protonation

by hydrogen halide. Although all oxygen atoms in the molecule may reversibly become protonated, only at the anomeric oxygen is this productive because departure of the protonated group (as H_2O, ROH, or most commonly AcOH) is promoted by the formation of a resonance-stablized pyranosyl oxocarbonium ion, which then combines with the halide anion (Scheme 2.5-1). Usually in ordinary pyranose systems the halide ion enters axially, to give the α-glycosyl halide owing to the anomeric effect. However, in 2-deoxy-2-phthalimido-pyranoses the β-halides is the preferred product, probably because of the steric requirements of the C-2 substituent.

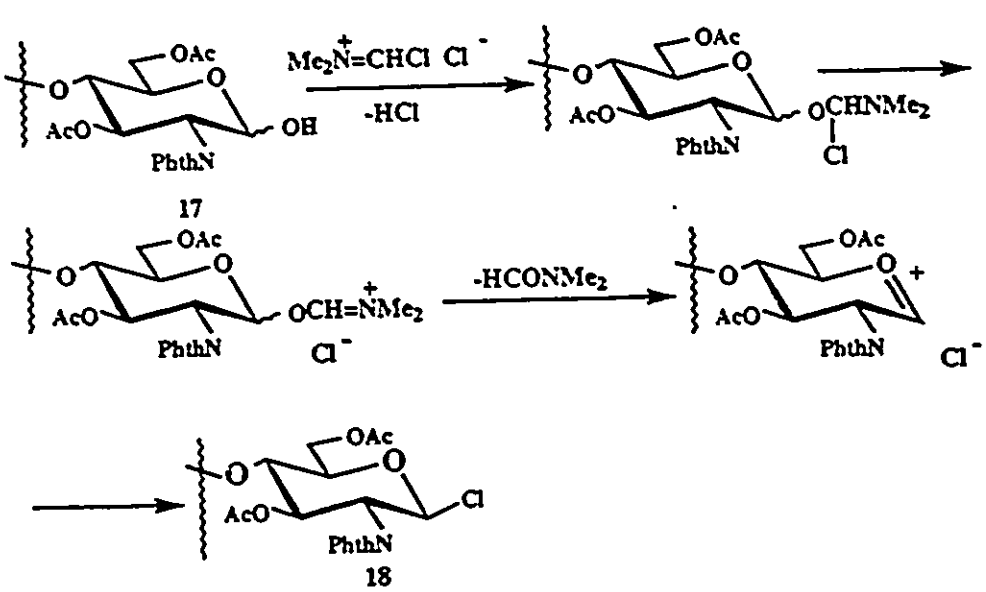
Now, one can also use nonprotonic (Lewis) acids instead of protonic (Bronsted) acids for generating a leaving group. Some of the reagents usable in this sense are titanium tetrahalides, (chloromethylene)dimethyliminium chloride (Vilsmeier reagent), 1,1-dichloromethyl methyl ether, and trimethylsilyl bromide and trifluoromethanesulfonate. For example, the reaction of the 1-hydroxy compound **17** with the Vilsmeier reagent (compare Scheme 2.3-2) is illustrated in Scheme 2.5-2, a. The reaction of 1-acetate **20** with 1,1-dichloromethyl methyl ether can be formulated as shown in Scheme 2.5-2, b. Here the leaving group departs as acetoxychloromethyl methyl ether, a derivative of orthoformic acid which presumebly decomposes immediately to methyl acetate, CO, and HCl (*via* intermediary, unstable formyl chloride). The reaction of **20** with trimethylsilyl

bromide is indicated in Scheme 2.5-2, c.



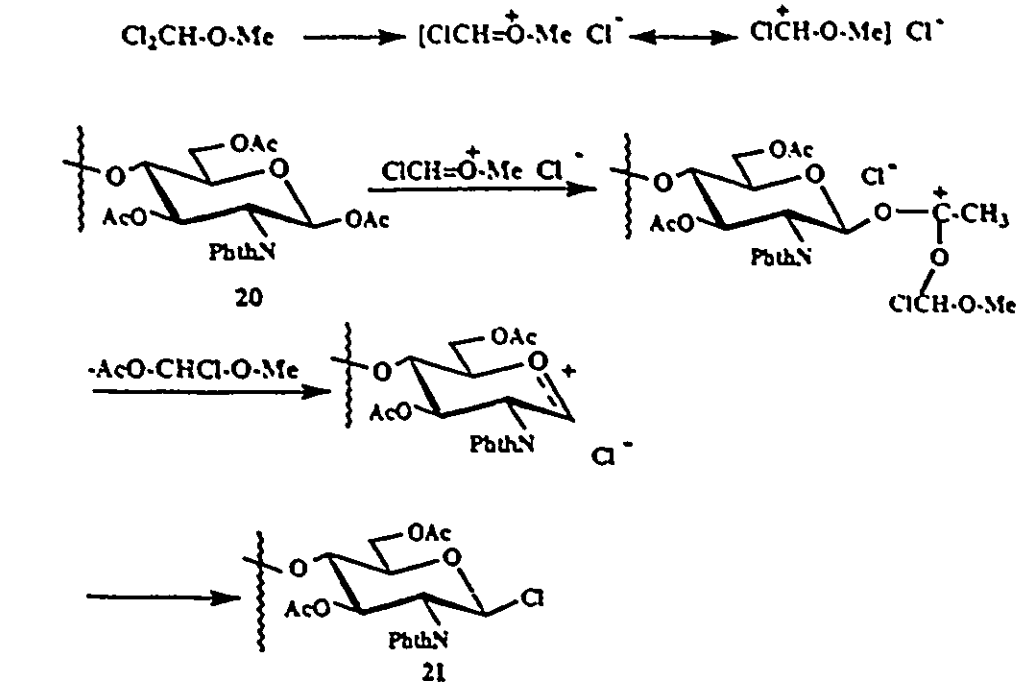
Scheme 2.5-1 Proposed mechanism for glycosyl halide formation using Brønsted acids

a.

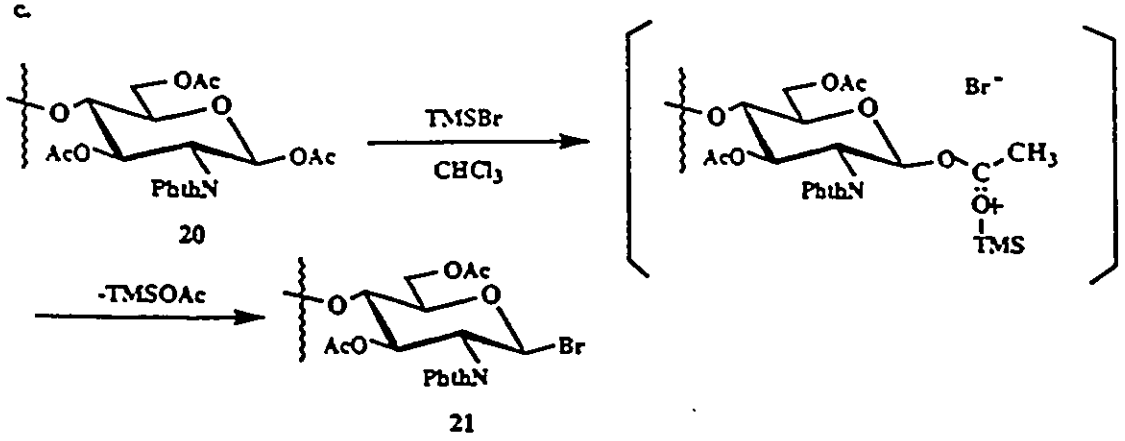


Scheme 2.5-2 Proposed mechanism for formation of glycosyl halides using Lewis acids

b.



c.

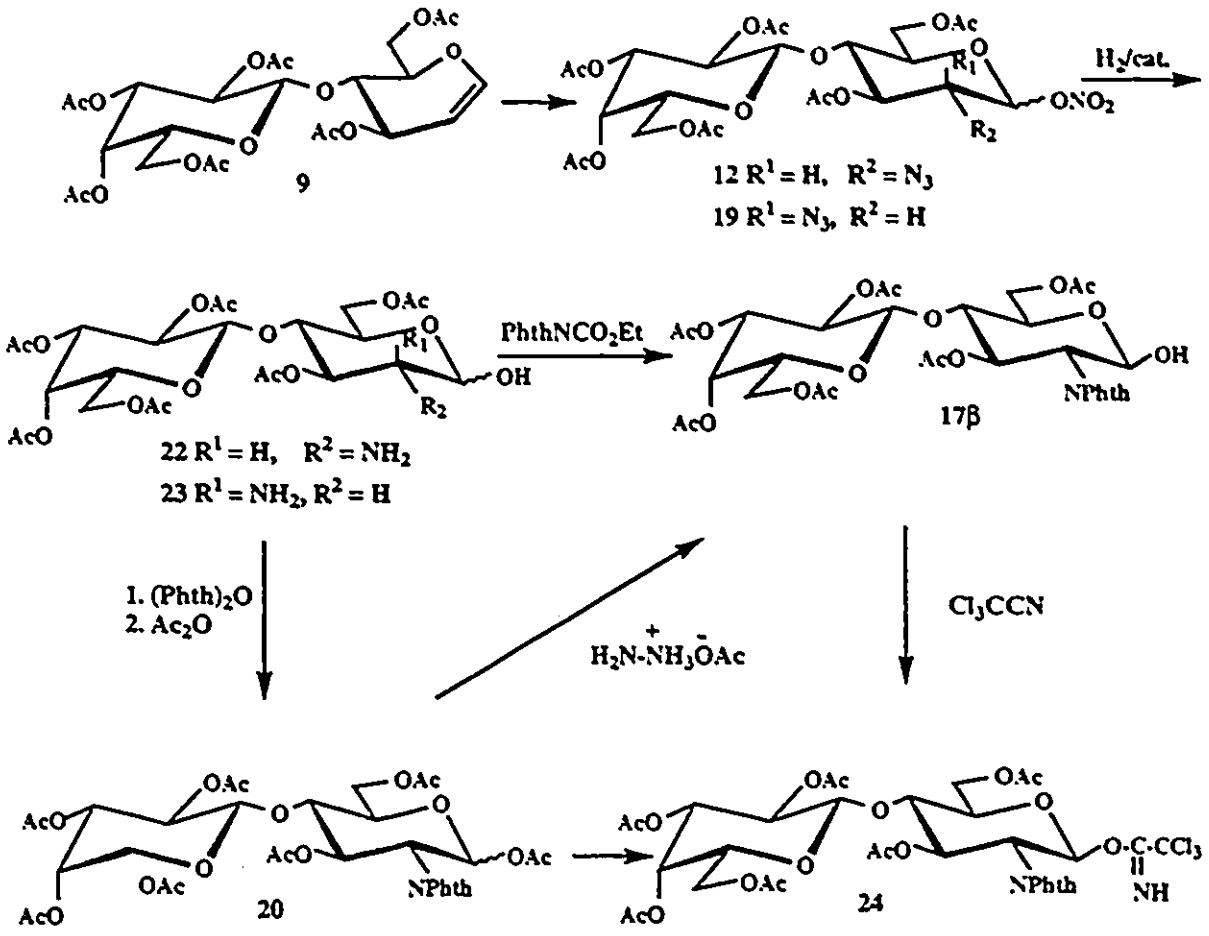


Scheme 2.5-2 Proposed mechanism for formation of glycosyl halides using Lewis acids (continued)

2. 1. 4. Preparation of lactosaminyl trichloroacetimidate donor

Grundler and Schmidt[39a] reported the synthesis of 3,6-di-O-acetyl-2-deoxy-2-phthalimido-4-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-O-trichloroacetimidoyl-β-D-glucose **24**, which may serve as an efficient lactosaminyl donor[39a-c]. They obtained **24** by selective deprotection of the C-1 position in the corresponding *tert*-butyldimethylsilyl β-glycoside to furnish first the reducing disaccharide **17β** (previously prepared along with its anomer **17α** by Lemieux and coworkers[28c] through hydrogenolysis of the benzyl glycoside **16**; compare Scheme 2.3-2), followed by reaction of **17β** with trichloroacetonitrile.

We decided to follow a somewhat shorter and more convenient path to the requisite hemiacetal **17**. Lactal hexaacetate **9** was azido-nitrated according to Arnarp and Lönngren[31], and the product mixture (**12α,β**, and a small proportion of **19**) was immediately hydrogenated as already described in section 2.1.3. However, the resulting crude mixture of amino sugars (mainly **22**, plus some **23**) was then treated, not with phthalic anhydride followed by acetic anhydride as was done before, but with N-carboethoxyphthalimide in refluxing ethanol in the presence of a catalytic amount of triethylamine. Chromatography of the reaction mixture gave crystalline **17β** in 26 % yield (Scheme 2.6). Although this approach to the compound was short, the yield in the phthaloylation was disappointing and we therefore resorted to preparation of the heptaacetate **20** as

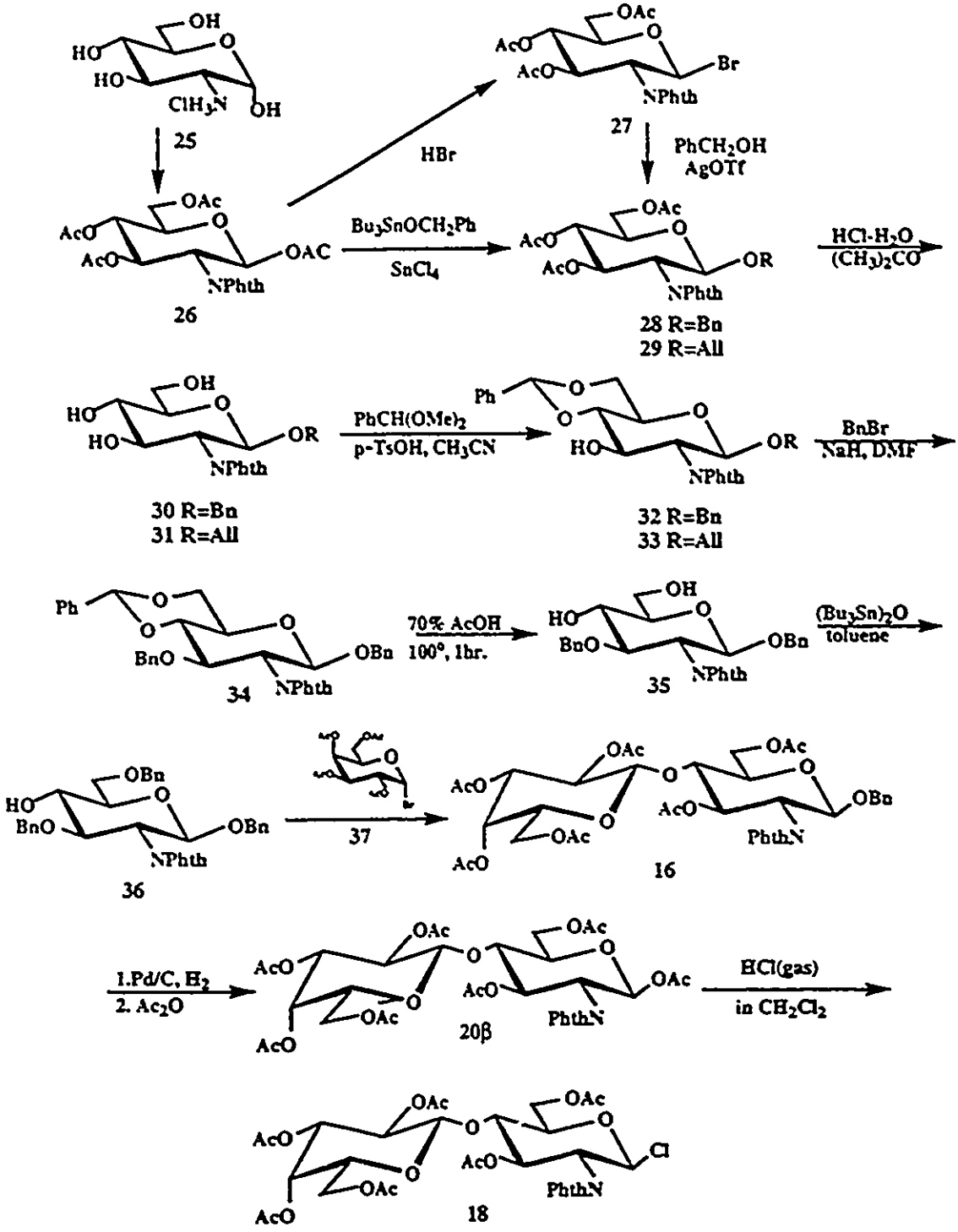


Scheme 2.6 Synthesis of a lactosamine trichloroacetimidate

previously described (compare Scheme 2.4), which is obtainable in good yield and which, as it turned out, could be smoothly deacetylated at O-1 by treatment[32] with hydrazinium acetate in N,N-dimethylformamide at room temperature (yield, 84 %). Reaction of 17β with trichloroacetonitrile[39] then furnished the crystalline target donor **24**; two procedural variants differing in the kind of base present (K_2CO_3 *vs.* NaH) afforded yields of 78 and 85 % (reported[39], 72 %).

2. 1. 5 Synthesis of lactosaminyl donor from monosaccharidic components

In addition to the methods starting from suitable disaccharide derivatives, there is also the possibility of preparing lactosaminyl donors from monosaccharidic building blocks, for example as proposed by T. Ogawa[36a] (Scheme 2.7). Treatment of the known[33] 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **26** with Bu_3SnOBn (tributyltin benzyloxide) in the presence of tin tetrachloride was stated to afford crystalline benzyl β-glycoside **28** in 72 % yield. Deacetylation of **28** was performed under acidic conditions[40], to give the benzyl glycoside **30** in 81 % yield. Benzylidenation of **30** with α,α-dimethoxytoluene and p-toluenesulfonic acid afforded the 4,6-protected derivative **32**, whose free OH group was benzylated to give benzyl ether **34**, and acid-promoted deacetalation of **34** led to the isolation of 3-benzyl ether **35** in 55 % overall yield from triol **30**. Monobenzylation at the primary hydroxyl group of **35** could be conveniently achieved by the stannylidene method, to give the 3, 6-dibenzyl ether **36** in 66 % yield. This key glycosyl acceptor **36** was glycosylated with 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide **37** in the presence of powdered molecular sieves 4A and $HgBr_2$, giving a 71 % yield of disaccharide **16**. The glycosidic benzyl group in **16** was split off by hydrogenolysis in the presence of Pd-C, and subsequent acetylation of the product gave crystalline β-acetate **20-β** in 75 % yield. Transformation of the latter into



Scheme 2.7 Synthesis of a lactosaminyl chloride (Ogawa)

crystalline chloride **18** was achieved in 40 % yield by treatment with hydrogen chloride in 1, 2-dichloroethane. The overall yield for the 10 steps starting from 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **26** was 5 %.

In practical terms, this long route to **18** is of course not competitive with the shorter and higher-yielding approaches departing from disaccharidic precursors as discussed in Section 2. 1. 3, even if the yield in the last step were more than doubled (to 95 % from the reported 40 % or 46 %, as actually achieved during this research) by application of the newly developed Lewis acid chlorination. Nevertheless, numerous reactions along the lines just outlined were performed, not so much in order to reach the target chloride **18** but rather, because some of the intermediates were either required or deemed potentially useful for planned syntheses to be referred to later in this thesis. The products prepared and observations made during these studies will be mentioned in the paragraphs that follow.

Ogawa's starting compound **26** needs to be prepared by N-phthaloylation of D-glucosamine, followed by O-acetylation. According to the original direction[33] a methanolic suspension of D-glucosamine hydrochloride **25** is allowed to react with 1 molar equivalent of sodium methoxide; sodium chloride is supposed to precipitate and to be filtered off, and the solution of free glucosamine is then to be treated with phthalic anhydride and (after processing) with acetic

anhydride. In our hands, adherence to this protocol gave low and erratic yields of **26**. Free glucosamine is only sparingly soluble in methanol and it appeared that a considerable part of it was lost to the phthaloylation through precipitation together with the sodium chloride. A simple remedy was to dispense with filtration and let the phthaloylation proceed in partial suspension. This minor but useful modification afforded **26** as an α,β-mixture in 82 % yield; the pure β-anomer could be obtained by recrystallization in yields of 32-42 %.

The direct conversion of **26** into the corresponding benzyl glycoside **28** has been claimed to be possible with high yield by reaction with tributyltin benzyloxide in the presence of stannic chloride[36a]. However, in our hands that reaction did not yield **28** but led to an unexpected result which will be disclosed in Section 2. 5. Instead, we chose to react **26** with benzyl alcohol and trimethylsilyl triflate and so obtained **28** in 91% yield. The use of TMS-OTf as a coupling reagent for β-glycoside synthesis directly from pyranose β-1-acetates has been first proposed by Ogawa[36b]. Additionally, a detour *via* the glycosyl bromide **27** was undertaken since a need for the latter compound in another context was anticipated. The classical action of hydrogen bromide in glacial acetic acid upon **26**β proved satisfactory, giving a 78 % yield of **27** (probably the α,β-mixture **26** could equally well have been used). The bromide **27** was then allowed to react with benzyl alcohol in the presence of silver triflate and

2,4,6-trimethylpyridine in nitromethane as the solvent, affording **28** in 88 % yield.

Deacetylation of **28** to the triol **30**, previously performed with hydrochloric acid in aqueous acetone[40], was also improved by employing methoxide-catalyzed methanolysis (Zemplén) instead. An 81 % yield of **30** was obtained. Benzylidenation of **30** with α,α-dimethoxytoluene to give the 4,6-acetal **32** (crude yield, 85 %) was performed as reported[36a].

The remainder of Ogawa's sequence (Scheme 2.7) was not pursued as no need for any of the intermediates **34-36** was foreseen. However, it was worthwhile to produce a number of allyl analogs of the benzyl glycosides. The allyl protecting group is selectively cleavable under a variety of conditions and often serves as a useful alternative to the benzyl groups. For example, it can be removed by isomerization to the 1-propenyl group, effected by the action of potassium tert-butoxide[41a] or[41b] by a cationic iridium complex[3], followed by cleavage of the rearranged product with mercuric chloride and mercuric oxide. Deallylation can also be achieved[41c] by means of palladium-on-carbon in aqueous methanol in the presence of p-toluenesulfonic acid.

Thus, the known compounds **29**, **31**, and **33** (Scheme 2.7) were

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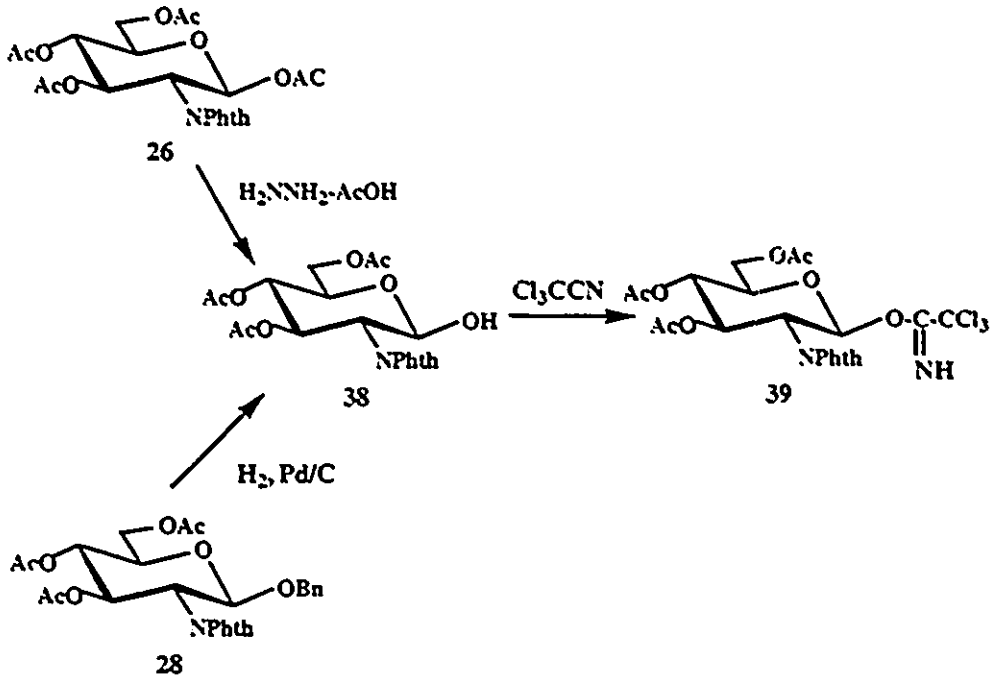
[3] 1,5-Cyclooctadiene-bis(methyldiphenylphosphine)-iridium hexafluoro-phosphate.

synthesized according to the literature procedures[42][43] which involved chemistry analogous to that employed for the benzyl glycosides and afforded excellent yields (89, 91, and 78 %, respectively).

Finally, it was thought that 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose 1-trichloroacetimidate **39** might be useful to have in hand, as a glucosaminyl donor alternative to the bromide **26**. Its preparation required 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **38** as the starting material. The latter was obtained in two ways: *a*, from the tetraacetate **27** by removal of the anomeric acetyl group with hydrazinium acetate in N,N-dimethylformamide at room temperature (yield, 84 %), and *b*, from the benzyl glycoside **28** by palladium-catalyzed hydrogenolysis (yield, 80 %). The reducing sugar **38** was then converted into **39** by the action of trichloroacetonitrile[39a,b] as described earlier for the preparation of the disaccharidic trichloroacetimidate **24**. Again, the two procedural variants (presence of K_2CO_3 *vs* NaH) were applied, giving **39** in yields of 84 and 73 %, respectively (Scheme 2.8).



Scheme 2.8 Synthesis of glucosaminyl donor 39

Summary of Chapter 2. 1

An improvement was made in the procedure for preparing the important starting compound, hexa-O-acetyllactal **9**.

The known lactosamine donors 3,6-di-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride **18**, bromide **21**, and trichloroacetimidate **24** were prepared essentially according to published procedures, but a number of modifications or alternative methods were applied to improve these syntheses. Thus, the yield of one of the key intermediates, 2-deoxy-2-phthalimidolactose-β-heptaacetate **20β**, could be enhanced by partial anomerization of the α,β-mixture with a catalytic amount of perchloric acid, and **20β** could be converted into β-1-hydroxy compound **17β** (another key intermediate) by high-yielding reaction with hydrazinium acetate. The chloride **18** and bromide **21** were prepared from **20** by halogenation with reagents alternative to those used previously, namely, with 1,1-dichloromethyl methyl ether and trimethylsilyl bromide, respectively, which proved superior. Also, the approach to **17β** (and thence **24**) was shortened.

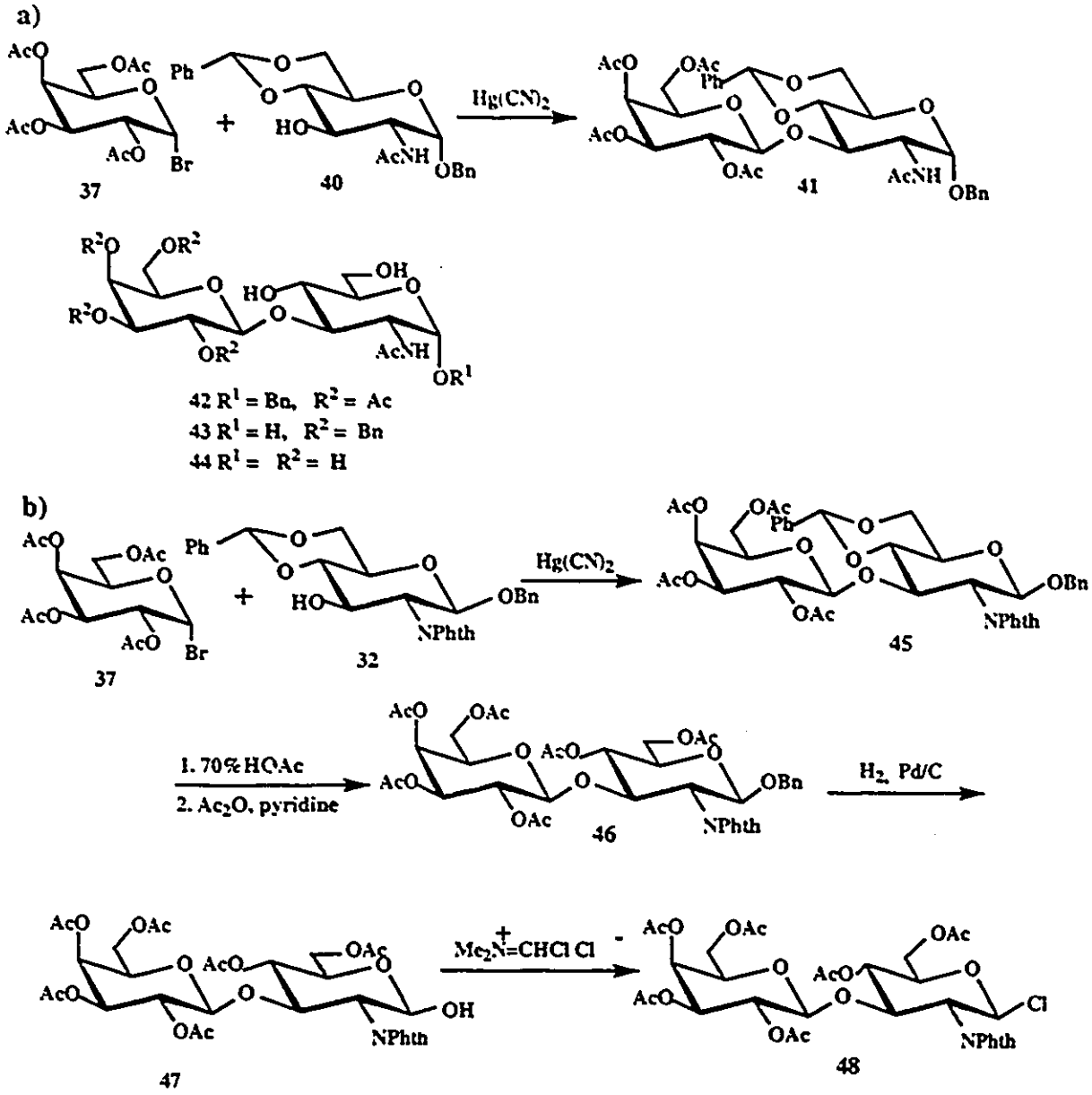
Furthermore, a number of monosaccharidic synthons (**26-37**) were prepared in connection with these studies, for use in subsequent sections of the thesis, whereby procedural variants led to improved yields of some of the products, notably **26**, **28**, and **30**.

Extensive spectroscopic data (Ms, ^{1}H-, and ^{13}C-NMR) were recorded for most of the compounds prepared (see Experimental section).

2. 2. Preparation of lacto-N-biose donors

Lacto-N-biose I [Gal-β(1→3)-GlcNAc, **44**] was first isolated by partial acid hydrolysis of lacto-N-tetraose which has this disaccharide β-linked to the 3-position of lactose. Like N-acetyllactosamine, it is a building unit of several higher oligosaccharides occurring in human milk (Kuhn, Baer, and Gauhe[10]). It was also found among degradation products of a glycoprotein with human blood-group A activity[44][45].

A chemical synthesis of 2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-glucose (lacto-N-biose I) was accomplished by Flowers and Jeanloz[46a] and Paulsen *et al.*[46b] by condensing 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide **37** with benzyl 2-acetamido-4,6-benzylidene-2-deoxy-α-D-glucopyranoside[47] **40** in a mixture of nitromethane-benzene in the presence of mercuric cyanide, to give the derivative **41**. From the latter product, the benzylidene group was removed by mild hydrolysis using acetic acid (→**42**), the O-acetyls were removed by the Zemplén method (→**43**), and the glycosidic benzyl group was finally split off by catalytic hydrogenation, to furnish synthetic **44** (Scheme 2. 9, a).

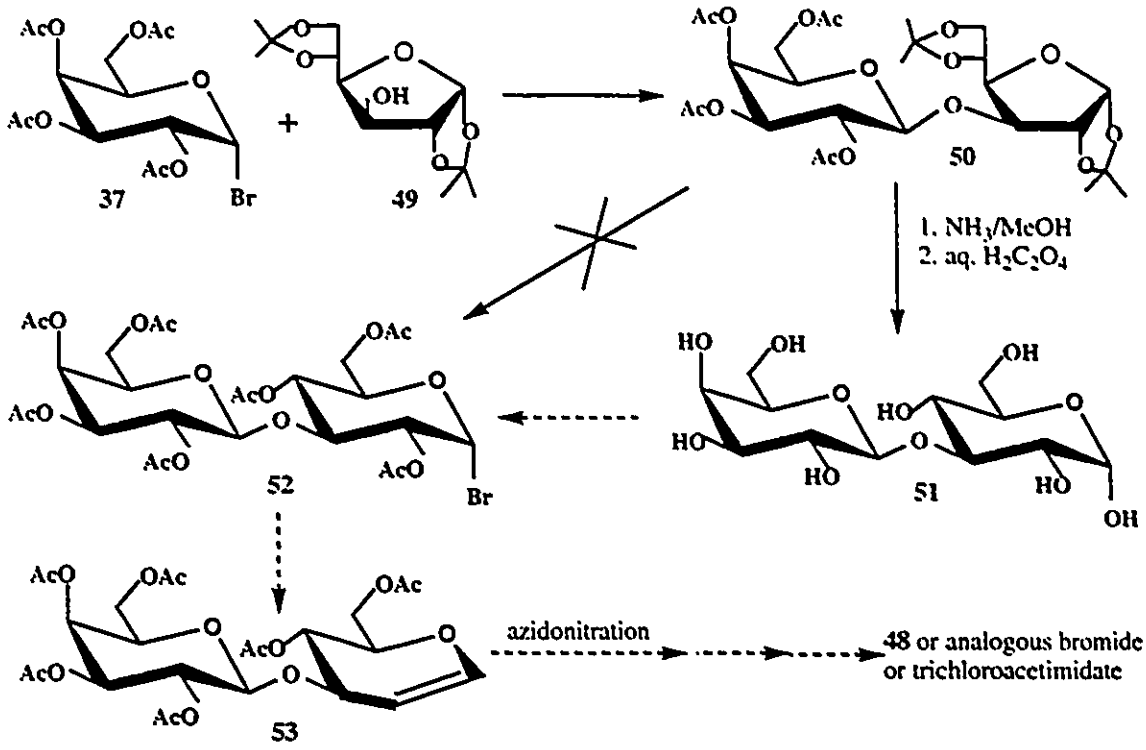


Scheme 2.9 Synthesis of (a) lacto-N-biose (Jeanloz, Paulsen) and (b) a lacto-N-biosyl chloride (Lemieux)

To provide a reactive donor suitable for attaching **44** to other structures, Lemieux and coworkers[40] synthesized the protected lacto-N-biosyl chloride **48** as indicated in Scheme 2. 9. b. The same bromide **37** was similarly condensed with **32** (compare Scheme 2.7) to the disaccharide **45**, which was debenzylidenated and O-acetylated to **46**. Hydrogenolysis of the glycosidic benzyl group (→**47**) followed by replacement of the hydroxyl group with chlorine using the Vilsmeier reagent, N,N-dimethylchloroforminium chloride, afforded the lacto-N-biosyl chloride **48** in 41 % overall yield from **32**. Taking into account the four steps necessary to prepare **32** from commercial D-glucosamine hydrochloride (see Scheme 2.7) and two further steps to make acetobromogalactose **37** from D-galactose, the whole process appeared rather laborious and it was considered worthwhile to investigate whether **48** or a comparable lacto-N-biose donor can perhaps be obtained in a simpler fashion.

The first idea that came to mind was to convert synthetic 3-β-D-galactopyranosyl-D-glucose **51** *via* its acetobromo derivative **52** into the per-acetylated glycal **53**, and to carry on from there with an azidonitration protocol, analogous to the chemistry discussed in the preceding chapter (Scheme 2.10).

The synthesis of the "isolactose" has been reported by Kuhn and Baer[48] who condensed the bromide **37** with readily available 1,2:5,6-di-O-isopropylidene-D-glucofuranose[49][50] **49** and obtained the blocked disaccharide **50** in



Scheme 2.10 Planned synthesis of lacto-N-biosyl donors

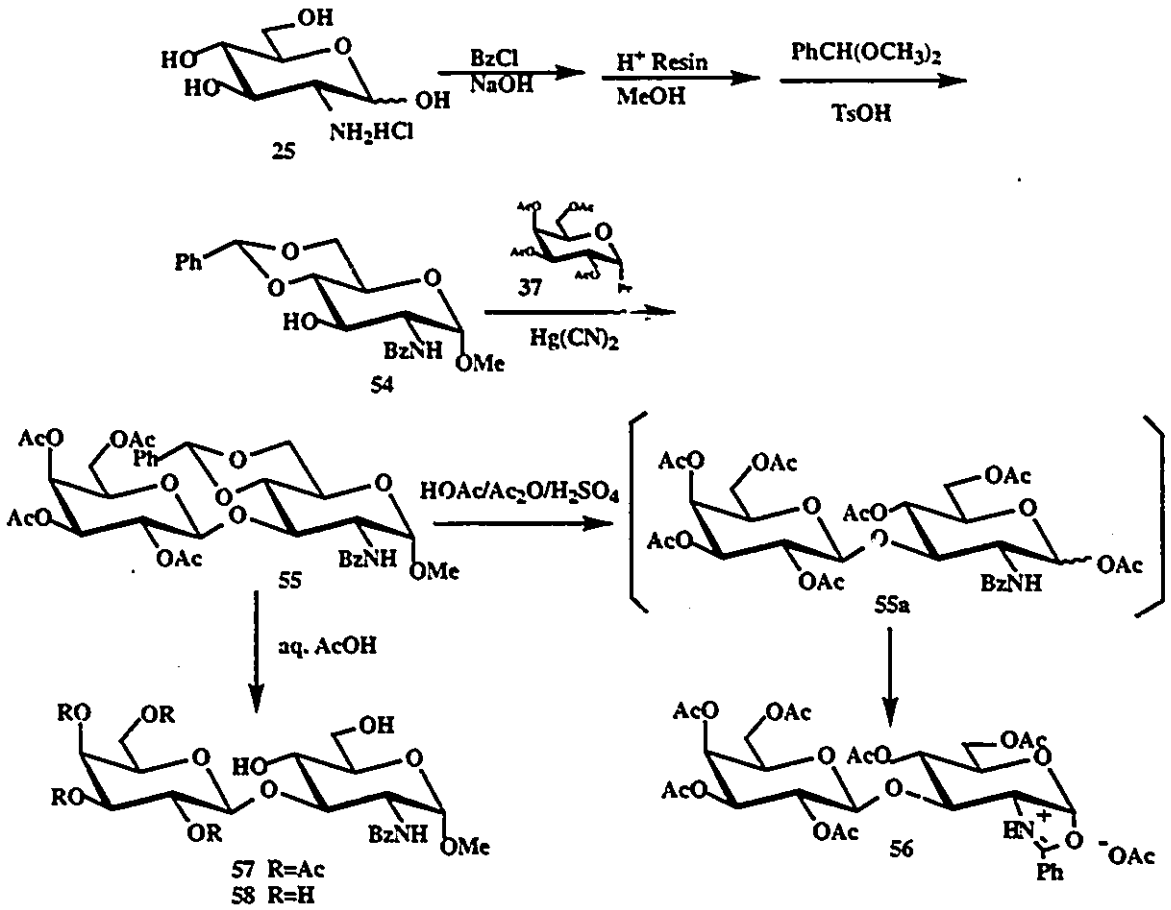
35 % yield. Deacetylation by ammonolysis and deacetonation with oxalic acid then furnished crystalline **51**, but the overall yield from **49** was low (6 %), a problematical operation being hydrolysis of the 1,2-O-isopropylidene group which was invariably accompanied by a significant amount of interglycosidic cleavage (Scheme 2.10). In view of this problem, and since no improved avenue to **51** itself seems to have been recorded in the literature, it was decided to attempt a direct conversion of crude **50** into the bromide **52**. Treatment of **50** with hydrogen bromide in acetic acid was hoped to lead to acetolysis of the isopropylidene groups and concomitant ring expansion of the furanose to the

pyranose system, with subsequent acetylation and bromination. Unfortunately, the experiments did not yield the desired **52**, and the approach was abandoned.

2. 2. 1 A simplified approach to a lacto-N-biose oxazoline

Having failed to procure "isolactal" **53** as a starting point for the azidonitration route to lacto-N-biosyl halides, we resorted to the general methodology employed by Lemieux (Scheme 2.7 and 2.9,b), but attempted to introduce some variation (Scheme 2.11). Instead of the benzyl phthalimido-glucoside **32**, we chose methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** as the point of departure. This key intermediate is easily accessible from D-glucosamine hydrochloride **25** by N-benzoylation (benzoyl chloride in aqueous alkaline solution), glycosidation with methanol catalyzed by cation exchange resin (H^+ form), and 4,6-acetalation with α,α-dimethoxytoluene, processes which all involve only economical, standard operations. Koenigs-Knorr condensation of **54** with acetobromogalactose **37** proceeded very well, giving an 80 % yield of the blocked disaccharidic methyl glycoside **55**. This compound proved amenable to a simple, one-pot transformation by acetolysis (16 h at 0-5 °C) with a mixture of acetic acid, acetic anhydride, and a catalytic amount of conc. sulfuric acid. The process comprised several separate reactions: Removal of the benzylidene group with concomitant acetylation of O-4 and O-6,

and acetolytic cleavage of the methyl glycoside. The original intention was to produce the heptaacetate **55a**, which could subsequently be converted into a glycosyl halide by one or the other of the methods referred to in the preceding chapter. On the other hand, **55a** itself might possibly serve as a glycosyl donor since it has been shown[1][36b] that pyranose 1-acetates (including, *e.g.*, β-octaacetyllactose) can be condensed with alcohols (including secondary hydroxyl groups in partially protected sugars) under catalysis by trimethylsilyl triflate, to form β-glycosidic linkages with high efficiency; compare also the reaction **26** → **28**, page 44. In the event, the acetolysis product obtained from **55** in 92 % yield gave correct microanalytical data for the expected composition $C_{33}H_{41}NO_{18}$ but, instead of **55a**, it proved to be the isomeric oxazolinium salt **56**, 2-phenyl-[1,2-dideoxy-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano][2',1':4,5]-2-oxazolinium acetate. This was concluded from spectral evidence. Thus, the mass spectrum showed at highest m/z (680) a peak attributable to the oxazolinium cation; there was no peak at m/z 739 (for M^+) or 740 (for $M^+ + 1$) assignable to **55a**. The ^{13}C-NMR spectrum lacked the characteristic signal near δ 52-53 required for a benzamido-substituted pyranose C-2 and exhibited, instead, signals at δ 165.2 and 65.5 attributable to C-2 of the oxazoline and C-2 of the glucopyrano ring, respectively. Also, there were 6 (not 7) acetyl carbonyl signals (δ 170.6-169.3).



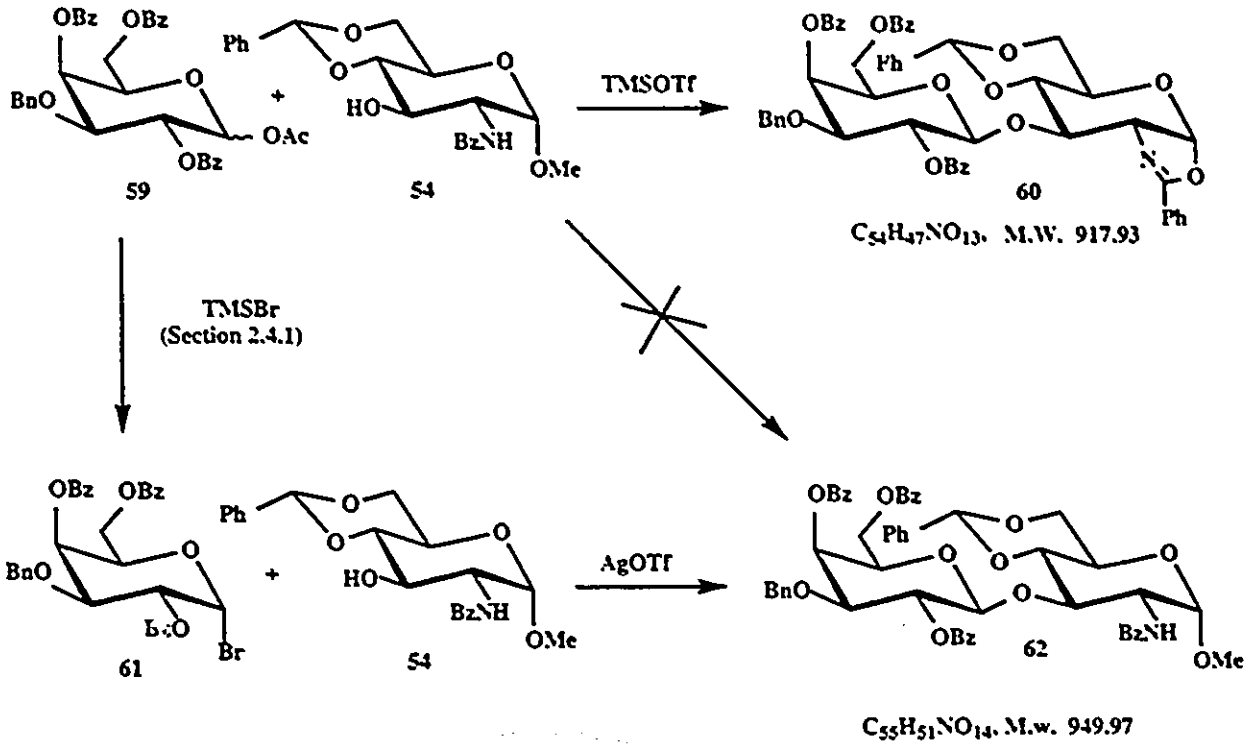
Scheme 2.11 A simplified approach to lacto-N-biose oxazoline

The high-yield formation of **56** in this simple, one-pot operation was a most welcome event as it made unnecessary the preparation of the acetate **55a** and its subsequent conversion into a glycosyl halide or similar donor. Pyranose oxazolines have long been known to be excellent glycosylating agents themselves, useful for introduction of hexosamine units in glycoside and oligosaccharide syntheses. Some of the relevant chemistry will be reviewed in the next section (2.2.2), following the disclosure of another interesting oxazoline formation.

In the course of the studies just described, the condensation product **55** was also debenzylidenated, by hydrolysis with aqueous acetic acid, to furnish the methyl glycoside tetraacetate **57** (yield, 85 %). Acidic deacetylation (HCl/ $CHCl_3$) of the latter gave methyl 2-benzamido-2-deoxy-3-O-(β-D-galacto-pyranosyl)-α-D-glucopyranoside **58**, a compound to be used in another context (see Section 2.4).

2. 2. 2 A novel synthesis of a lacto-N-biose oxazoline

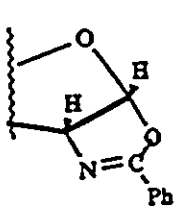
One of the needs for later stages of this research was to make available a β-1→3 linked disaccharide derivative of the lacto-N-biose type whose 3'-position is temporarily blocked and can be selectively liberated so that it may function as an acceptor site. Thus, it was decided to synthesize the disaccharide **62**, which carries a 3'-benzyl ether group removable by hydrogenolysis. To this end 1-O-acetyl-2,4,6-tri-O-benzoyl-3-O-benzyl-α,β-D-galactopyranose **59** (see Section 2.4.1 concerning its preparation) was to be coupled with the glucosaminide **54** by use of the trimethylsilyl triflate method referred to earlier (see Sections 2.2.1 and 2.3.1). When the two components were reacted together with the triflate catalyst in dichloromethane solution in the presence of molecular sieves, a crystalline product was obtained in high yield (Scheme 2. 12). Surprisingly, however, this product lacked the characteristic 3-proton singlet in the δ 3.3 region of its ^{1}H-NMR spectrum, required for a glycosidic methoxyl group, and therefore structure **62** was ruled out. Also, the mass spectrum did not indicate a molecular weight of 950 as expected for **62**, but one of 918 which agrees with the formal loss of CH_3OH. On the basis of additional spectral evidence, to be disclosed in the paragraph that follows, the oxazoline structure **60** was assigned to the new disaccharide. But first it is to be reported that the originally targeted glycoside **62** was indeed obtained, in 88 % yield, when **54**



Scheme 2.12 One-step synthesis of the disaccharide oxazoline **60** and synthesis of the disaccharide methyl glycoside **62**

was glycosylated with the glycosyl bromide **61** (obtainable from **59**, see Section 2.4.1), by use of silver triflate as a condensing agent in dichloromethane solution (Scheme 2.12). Compound **62** did show the expected methoxyl signal (δ 3.27) in its ^{1}H-NMR spectrum, and the $M^+ + 1$ peak at m/z 951 in its mass spectrum, and it differed from **60** also in other significant spectral features (both ^{1}H- and ^{13}C-NMR), and in physical properties ($[\alpha]_D$ +70° *vs.* + 89°; amorphous *vs.* crystalline). Both compounds gave microanalytical data fitting their respective compositions; the C-values in particular underscored their difference (for **60**, calcd. 70.65 %, found 70.76 %; for **62**, calcd. 69.53 %, found 69.34 %).

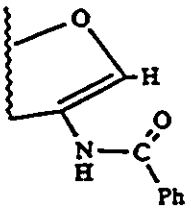
In assigning the structure **60**, based on the above information of an obvious deficit of the elements of CH_3OH as compared to **62**, one had also to consider the structure of an isomeric 2-benzamido-2-deoxyglycal (**60a**) as an alternative:



60

oxazoline

versus



60a

benzamidoglycal

Structure **60a** could readily be ruled out. In the ^{13}C-NMR spectrum it should give a C-1 signal at very low field (compare **9**: δ 145.5), whereas C-1 of the product resonated at δ 95.2. Similarly, C-2 in **60a** should resonate at much lower field than where it was found (δ 66.5). The proton signal for H-1 in **60a** should be expected to be basically a singlet (for lack of vicinal coupling), or at most a broadened singlet or very narrow multiplet due to allylic coupling with H-3 and/or NH-2 (compound **9** shows $J_{1,3}$ 0.7 Hz), whereas the H-1 signal of the product was a clear and rather wide doublet ($J_{1,2}$ 5.1 Hz) at 6.39, coupled with H-2 which gave a triplet with 5.1 Hz spacings at δ 4.79. These data were in harmony with the oxazoline structure **60**. The reason that the 1,2-*cis* proton coupling was somewhat larger, and the 2,3-*trans* coupling smaller, than is normally the case in α-D-glucopyranose derivatives ($J_{1,2}$ ~ 3.5, $J_{2,3}$ ~ 9-10 Hz) can be ascribed to a distortion of the regular $^4C_1$ chair conformation due to the annulation of the heterocycle: C-2 is forced downward and H-2 consequently moved outward, resulting in a diminution of the dihedral angles H-1, 2 from 60° and H-2, 3 from 180°, to smaller values. A further proof of structure was the absence of an NH signal in **60**; such a signal should occur in a spectrum of **60a** as a singlet (or narrow doublet owing to allylic coupling), and did indeed occur in **62** as a large doublet ($J_{2,NH}$ 8.7 Hz) at low field.

Full analyses of the ^{1}H- and ^{13}C- NMR data, obtained from extended 300 MHz and 75.43 MHz spectra, respectively, are recorded in the Experimental. The assignments were aided by COSY and DEPT plots, and by comparison with spectra of compounds having identical or similar molecular moieties. For illustration, some 200 (50.3)-MHz spectra are reproduced in Figures 2.1-2.4.

The facile formation of **60**, admittedly unforeseen, was an event of considerable interest which conceivably may prove very useful as a basis for future studies. As previously mentioned, pyranose oxazolines are useful donors of hexosamine units in glycoside and oligosaccharide synthesis[51], much like glycosyl halides or trichloroacetimidates.

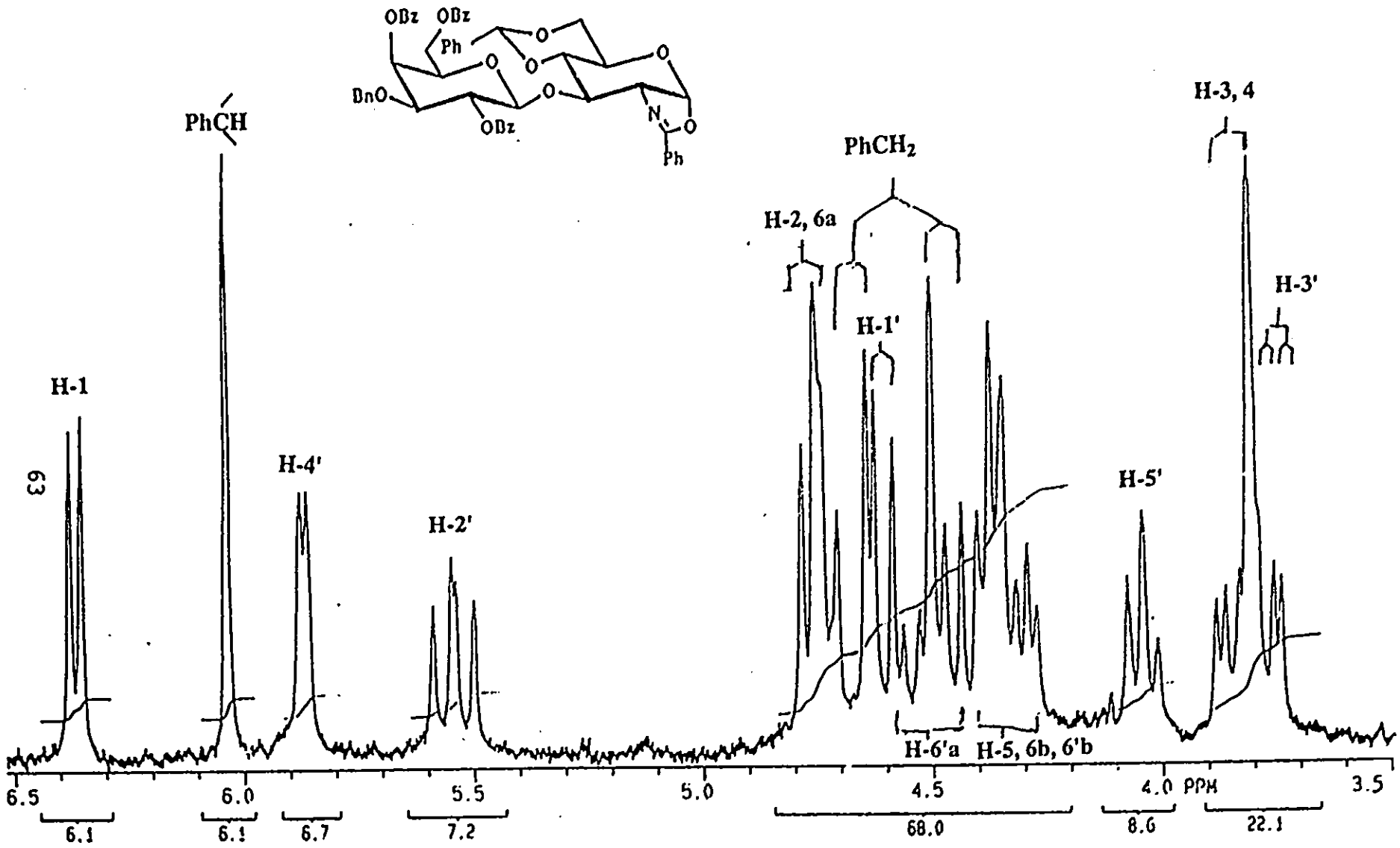


Fig. 2.1. Partial 200 MHz ^{1}H-NMR spectrum of compound 60 (for detailed data obtained from a 300 MHz spectrum, see Experimental.)

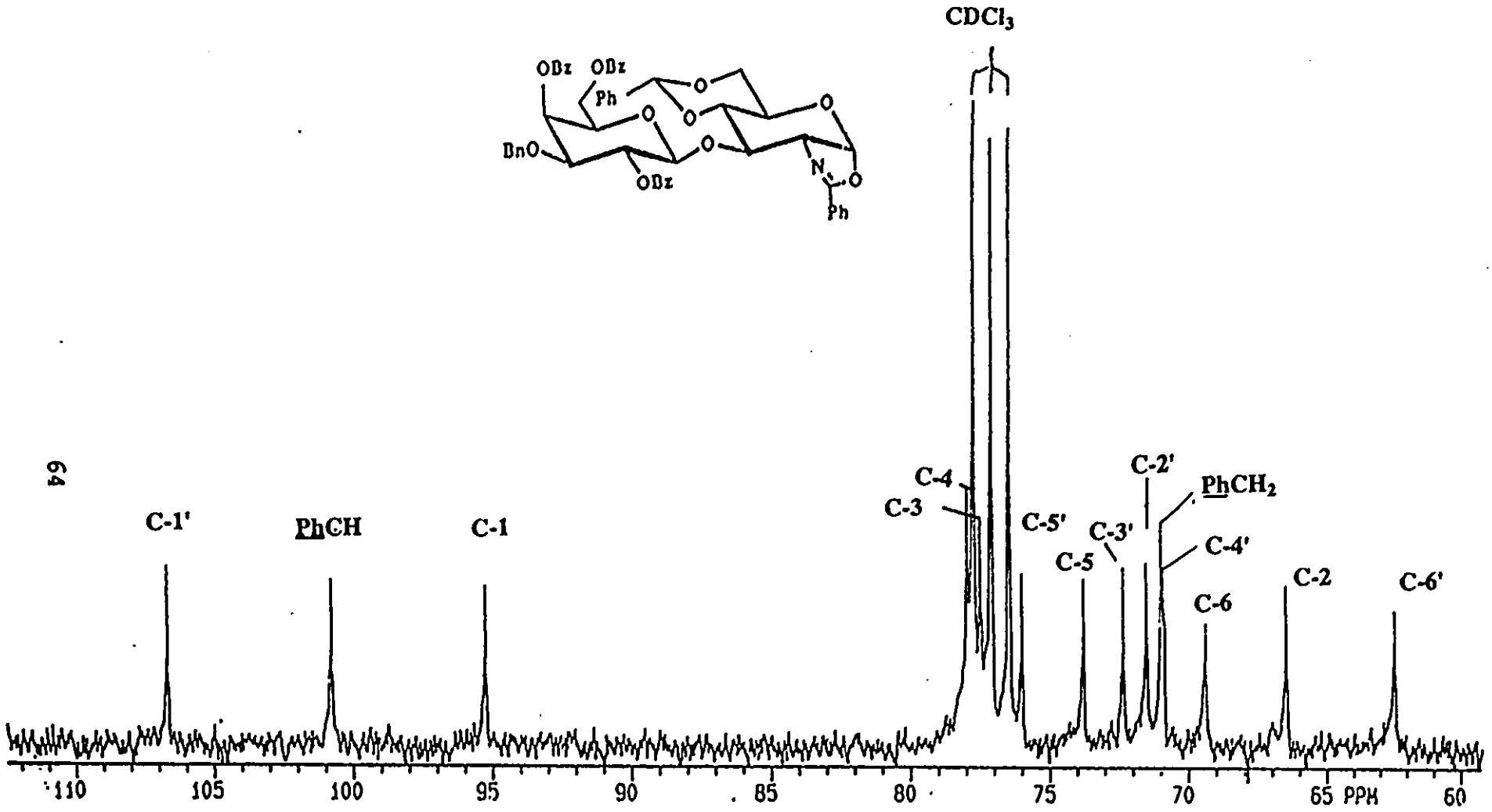
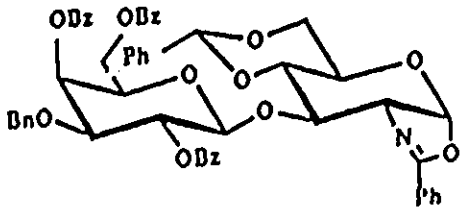


Fig. 2.2. Partial 50 MHz ^{13}C-NMR spectrum of compound 60. The full spectrum shows multiple signals for the aromatic rings in the δ 138-126 region, and 4 signals at δ 166.2, 166.0, 165.1, and 165.0 assignable to the benzoate carbonyls and C-2 of the oxazoline ring. There is no signal below 60 ppm, indicating absence of OCH_3.



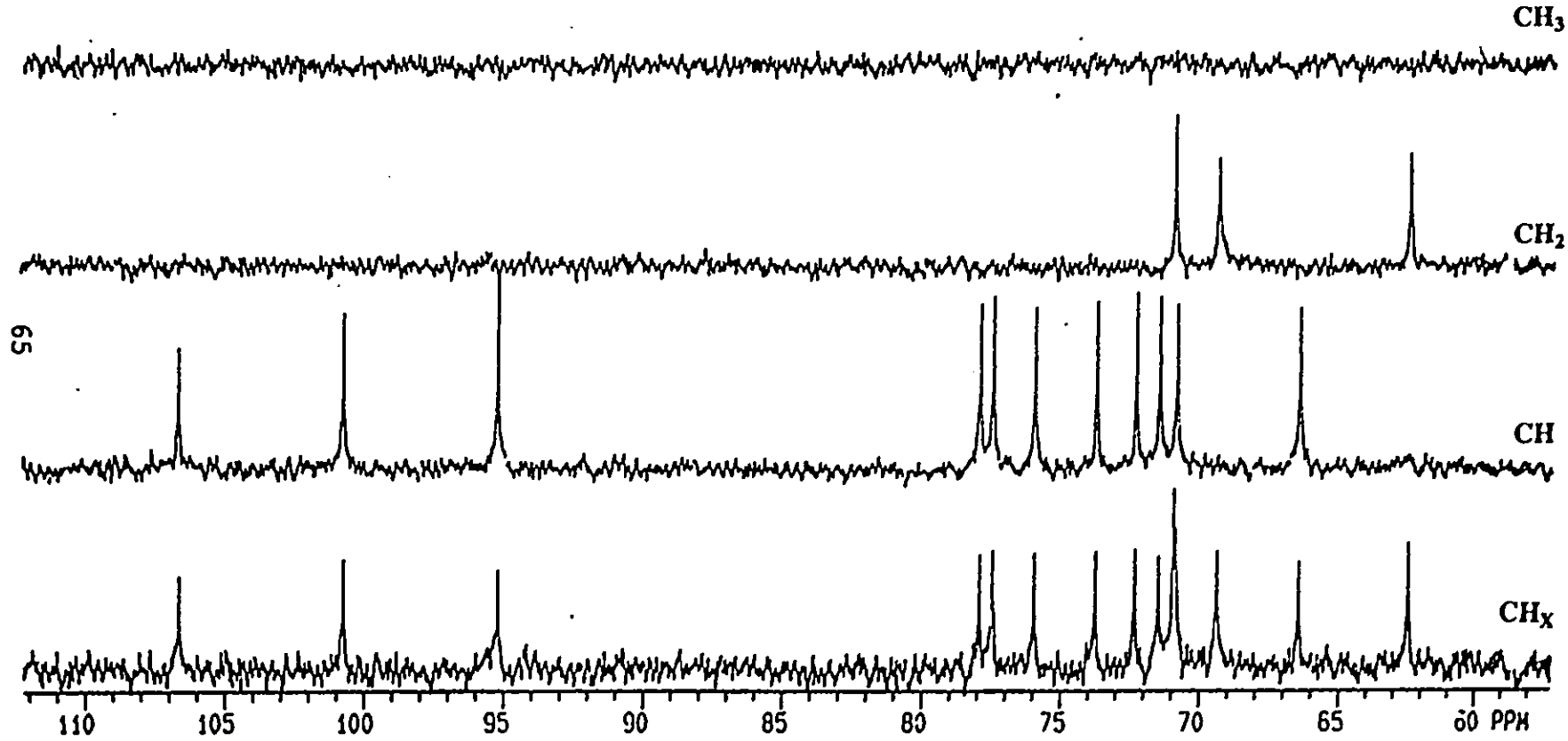


Fig. 2.3. ^{13}C-NMR DEPT spectrum of compound 60.

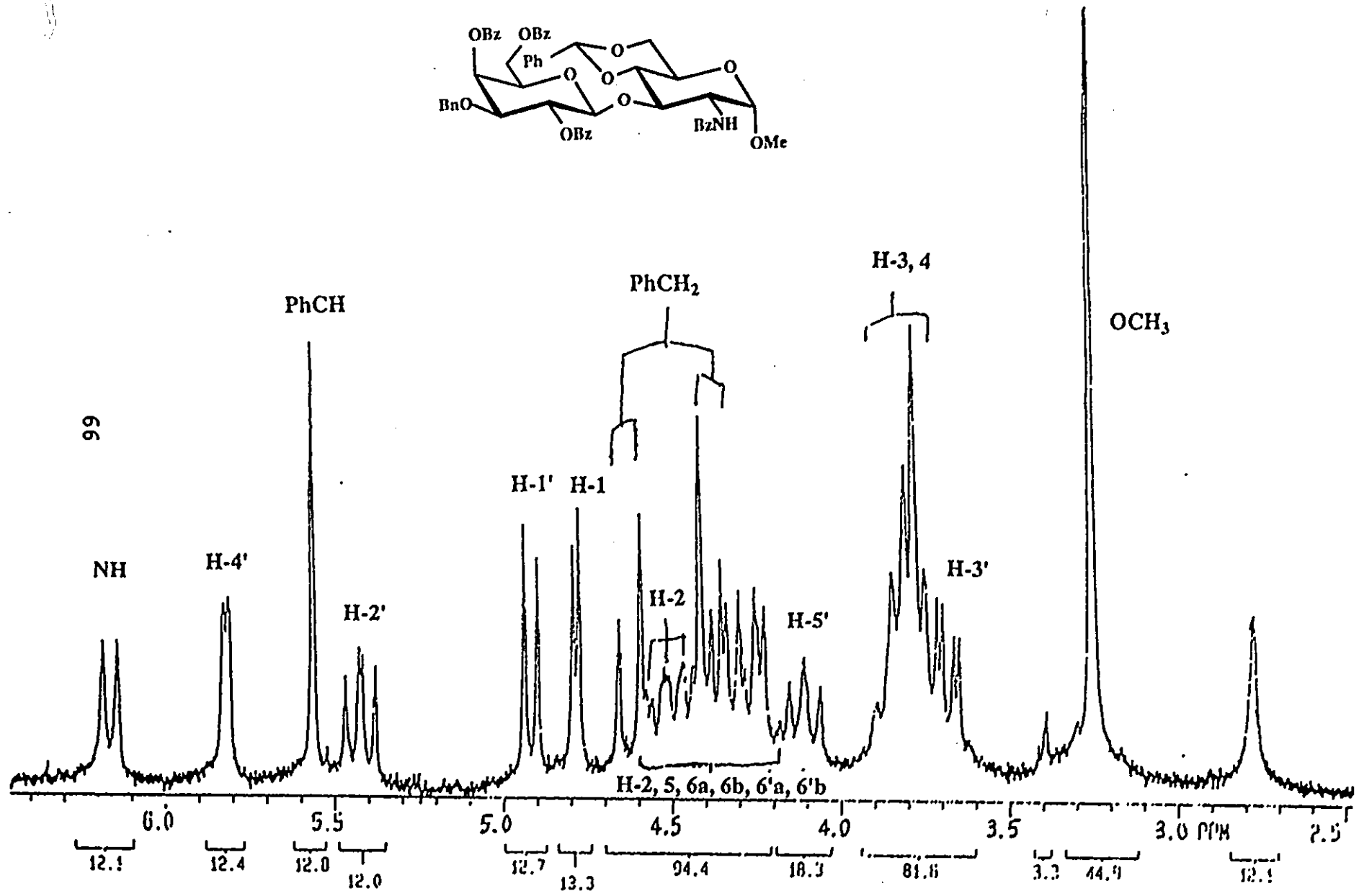
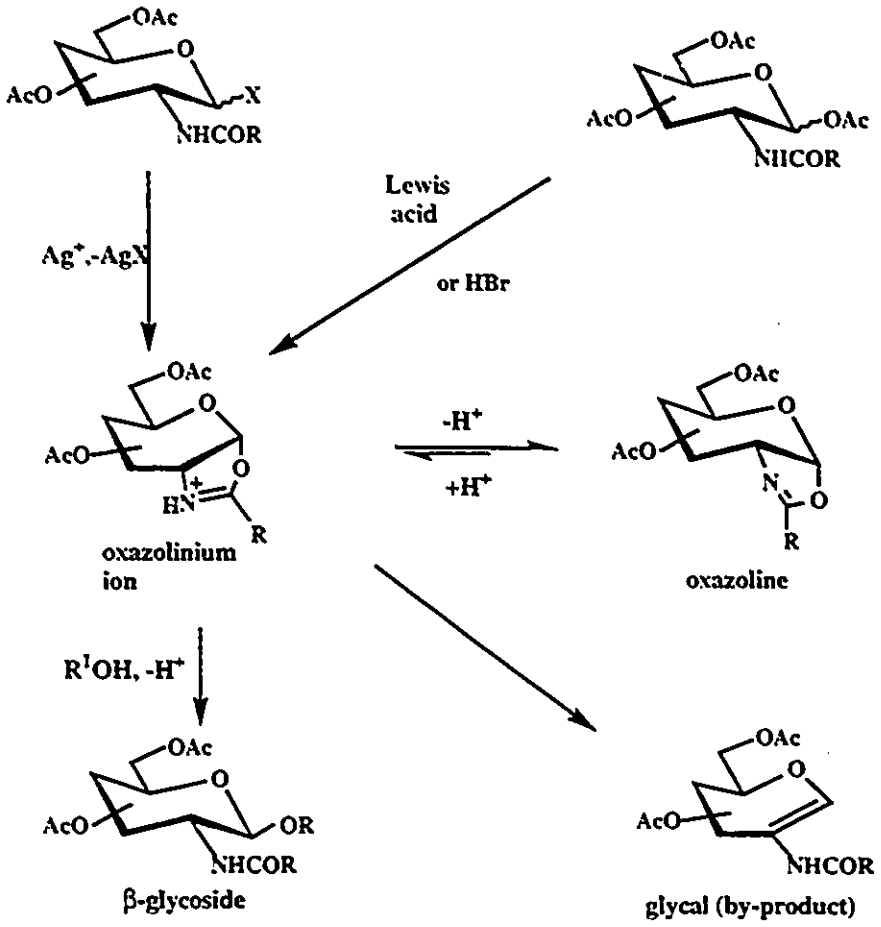


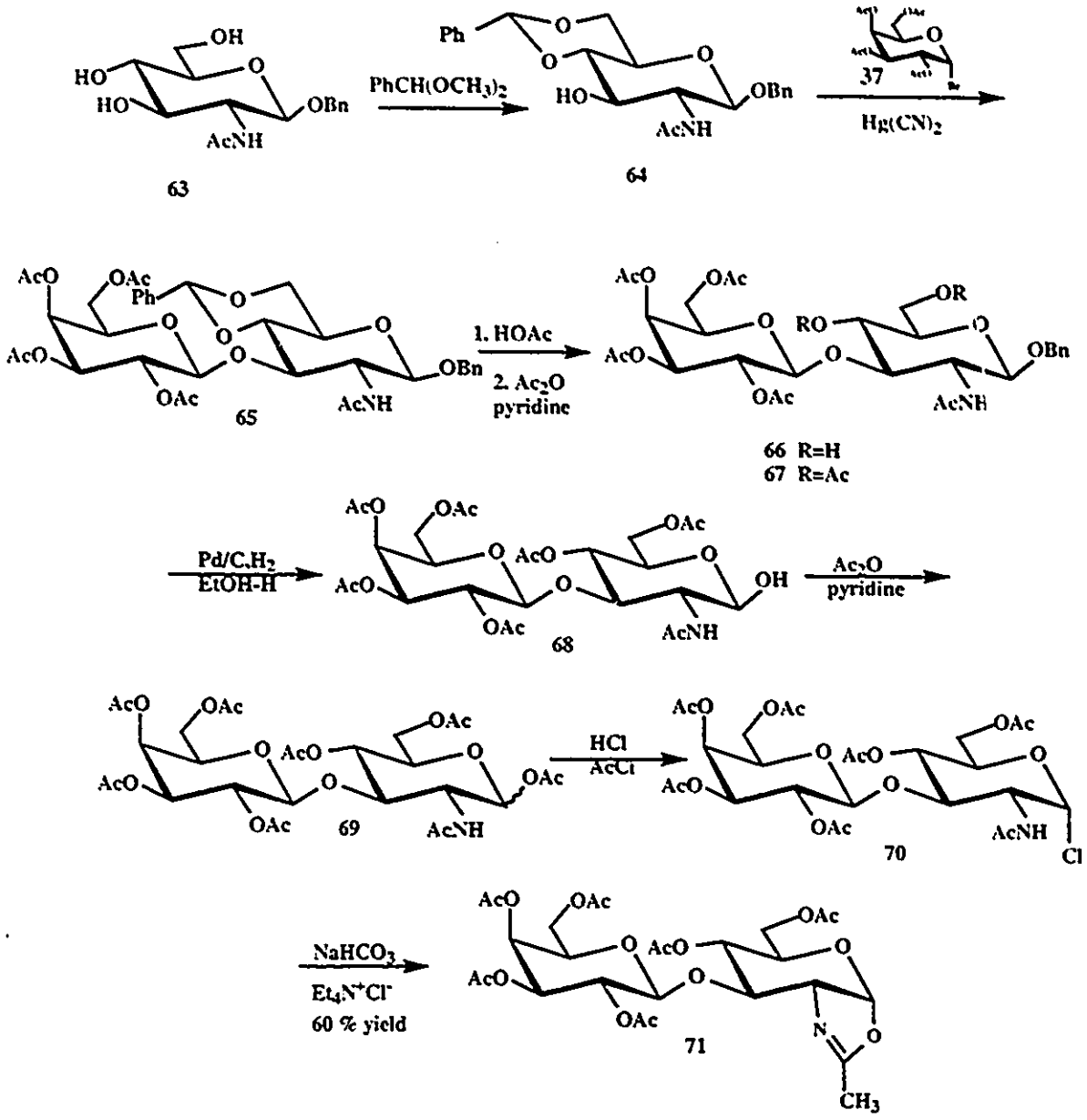
Fig. 2.4. Partial 200 MHz ^{1}H-NMR spectrum of compound 62



Scheme 2.13 Glycosidation using oxazoline donor

Traditionally, oxazolines are obtained from peracylated hexosamines by the action of hydrogen bromide in glacial acid[52a], $FeCl_3$ in dichloromethane[52b], or from peracylated hexosaminyl halides by the action of a silver salt and a base (pyridine or 2,4,6-trimethylpyridine)[51], or through halide-ion catalyzed isomerization (Et_4NCl in the presence of $NaHCO_3$)[53a]. A more recent procedure involves the cleavage of 1-propenyl glycosides (accessible by rearrangement of allyl glycosides) by action of $HgCl_2$[53b]. Unlike glycosyl halides, oxazolines are quite stable and can safely be stored. They are attacked by hydroxy compounds at C-1, usually in nitromethane/benzene solution in the presence of p-toluenesulfonic acid as a catalyst, whereby β-glycosides are stereospecifically formed. A 2-acetamido glycal may be a side product (Scheme 2.13).

Many glycosides and disaccharides have been synthesized by this popular method[51], and disaccharidic oxazolines have been made for use in block syntheses of higher saccharides[54a, b]. As an example may be mentioned the work of Augé and Veyrières[54a] (Scheme 2.14), which led to a lacto-N-biose oxazoline (**71**) similar to **60**, though without a selectively removable protecting group at O-3' (**71** is the 2-methyl oxazoline analog of **56**). Construction of the β-1→3 linked disaccharide benzyl β-glycoside **65** was achieved in full analogy to the synthesis of the corresponding α-glycoside **41** that was shown in Scheme 2.9a, *i.e.*, by the Helferich modification [with $Hg(CN)_2$] of the Koenigs-Knorr reaction



Scheme 2.14 Synthesis of oxazoline disaccharide by Augé and Veyrières

between acetobromogalactose **37** and N-acetylglucosamine derivative **64**. The disaccharide **65** was then transformed into the oxazoline donor **71** in six further steps as indicated in Scheme 2.14. (In subsequent work by the same authors[54b], **71** was successfully coupled with a monosaccharidic acceptor to form a trisaccharide.)

By contrast to the foregoing multistep sequence, the trimethylsilyl triflate-promoted condensation of the two monosaccharide components **54** and **59** furnished the disaccharide oxazoline **60** in a single operation. To our knowledge, methyl glycosides have not previously been cleaved by this reagent and the discovery of this type of oxazoline formation appears unprecedented. Future studies must be awaited to establish the scope of the reaction. As a further illustration of the versatility of the oxazoline procedure it may be mentioned that it was recently used for solid-phase syntheses of glycopeptides[55], whereby N-acetylglucosamine was attached to the terminal serine hydroxyls of the resin-bound polypeptide chains.

Summary of Chapter 2.2

An attempt was made to procure from the known "isolactose" derivative **50** the "isolactal" hexaacetate **53** from which, it was hoped, the azidonitration method could furnish intermediates suitable for the generation of lacto-N-biosyl donors. This approach proved unsuccessful.

Instead, the new disaccharide methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside **55** was synthesized from methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** and acetobromogalactose **37** by Koenigs-Knorr condensation. In a one-pot operation comprising three different reactions the glycoside **55** was converted into a new derivative, the disaccharide oxazolinium salt **56**, which may serve as a donor of the lacto-N-biosyl unit in glycosylations promoted by p-toluenesulfonic acid (oxazoline method).

Further, it was discovered that transfer of the blocked D-galactose 1-acetate **59** to the partially blocked methyl N-benzoylglucosaminide **54**, promoted by trimethylsilyl triflate, led not only to formation of the disaccharide linkage as expected, but at the same time converted the acceptor glycoside unit into an oxazoline, thus producing a new d...or (**60**) having a temporarily blocked 3'-position. For the purpose of comparison the disaccharide methyl glycoside **62**,

which had been expected from the reaction, was synthesized by condensation of **54** with the bromo sugar **61** (that corresponds to **59**) under silver triflate catalysis.

2. 3. Preparation of lactose and lactosamine acceptors

Lactose is the common disaccharide unit at the reducing end of the human milk oligosaccharides referred to in the Introduction (Fig. 1.2) and consequently, that of the two hexasaccharidic homologs of lacto-N-tetraose which were among the synthetic target molecules chosen at the outset of this project (Fig. 1.3-3). In those structures, the site of attachment of the remainder of the molecule is OH-3'. Lactose occurs also at the reducing end of the oligosaccharide chain in glycosphingolipids[4], where it is connected either through OH-3' (*isoglobo*, *lacto*, and *neolacto* series), or through OH-4' (*globo*, *ganglio*, and *muco* series) to various amino-oligosaccharides. In some complex glycosphingolipids both of these positions bear extended chains. Similarly, N-acetyllactosamine constitutes the reducing half of two of the target molecules shown in Fig.1.3-2, again linked at OH-3'. For the pursuit of the originally intended syntheses and for syntheses of analogs linked through OH-4' (which might be added to the list of targets at a late date) it was therefore necessary to prepare derivatives of lactose and lactosamine suitably protected in all positions but OH-3' or OH-4', respectively, which could serve as acceptors for

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[4]Glycosphingolipids are derivatives of the 18-carbon aminodiol sphingosine, $HOCH_2$-$CHNH_2$-CHOH-CH=CH-$[CH_2]_{12}CH_3$, in which the NH_2 group is acylated with a fatty acid (N-acylsphingosine is also called ceramide) and the primary OH group is glycosylated with amino-oligosaccharides. Several series differing in the oligosaccharide chain structure are distinguished.

glycosylations by appropriate donors. For the construction of oligosaccharides having β-1→3' linkages to lactose or N-acetyllactosamine (*i.e.* C, E, G, and H in Figs. 1.3) it was considered that acceptors having free hydroxyls in both the 3'- and the 4'-position should be satisfactory, because the former is known to be much more reactive than the latter so that selective glycosylations should be possible. Reactivity in Koenigs-Knorr and other types of glycosylations varies widely, depending on the particular donor, reaction promoter and solvent employed[1], and choice of a system whose reactivity is not too high should allow galactose derivatives to couple at OH-3 in preference to OH-4, with good regioselectivity. Conversely, if the aim is to glycosylate a galactose unit at OH-4, then OH-3 must be protected, and this can be accomplished by regioselective allylation performed by the stannylation method[56].

The preparation of lactose and lactosamine acceptors is described in the sections that follow.

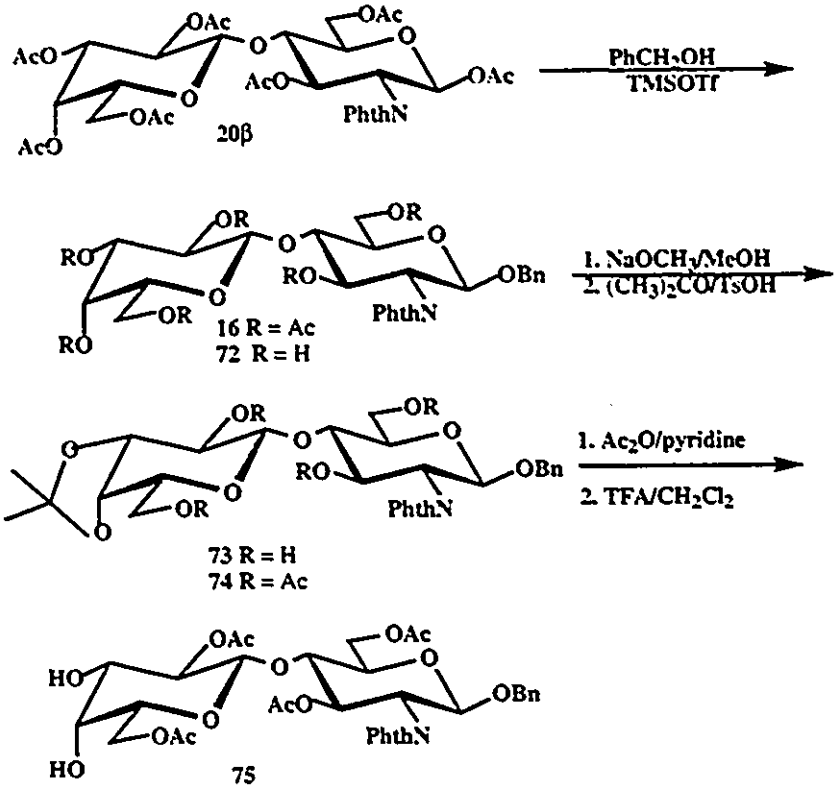
2. 3. 1 Preparation of a lactosamine acceptor

First, it was decided to provide the known[28c][32] 3',4'-diol **75** derived from benzyl β-lactosaminide as an acceptor molecule. This was prepared according to Alais and Veyrières[32], with minor procedural variations (Scheme 2.15). Thus, N-phthaloyl-β-lactosamine heptaacetate **20** was converted into the corresponding benzyl β-glycoside **16** by exchange of the anomeric acetoxy group with benzyl alcohol, catalyzed by trimethylsilyl triflate rather than by stannous chloride as described; the yield was thereby improved from 72 % to 91 % .

Sequential de-O-acetylation (**16** → **72**) by methoxide (Zemplén), conventional acetonation (**72** → **73**) and O-acetylation (**73** → **74**) furnished the 3',4'-O-isopropylidene derivative **74** which was then deblocked to the 3',4'-diol **75** by means of 90 % trifluoroacetic acid in a dichloromethane medium. Prior to this final hydrolysis the position of the acetonide ring was confirmed by examination of ^{1}H-NMR spectrum of **74**: The signals for H-3' and H-4' did not occur in the low-field region of δ 5.4-4.8, whereas compound **16** (in which OH-3' and 4' are acetylated) showed doublets of doublets at δ 4.94 ($J_{3',4'}$ 3.5, $J_{2',3'}$ 10.5 Hz) and 5.31($J_{4',5'}$ 1, $J_{3',4'}$ 3.5 Hz) for these protons due to the strong deshielding effect of acyloxy substituents.

The preparation of a lactosamine acceptor equivalent will be disclosed in

section 2.3.2.

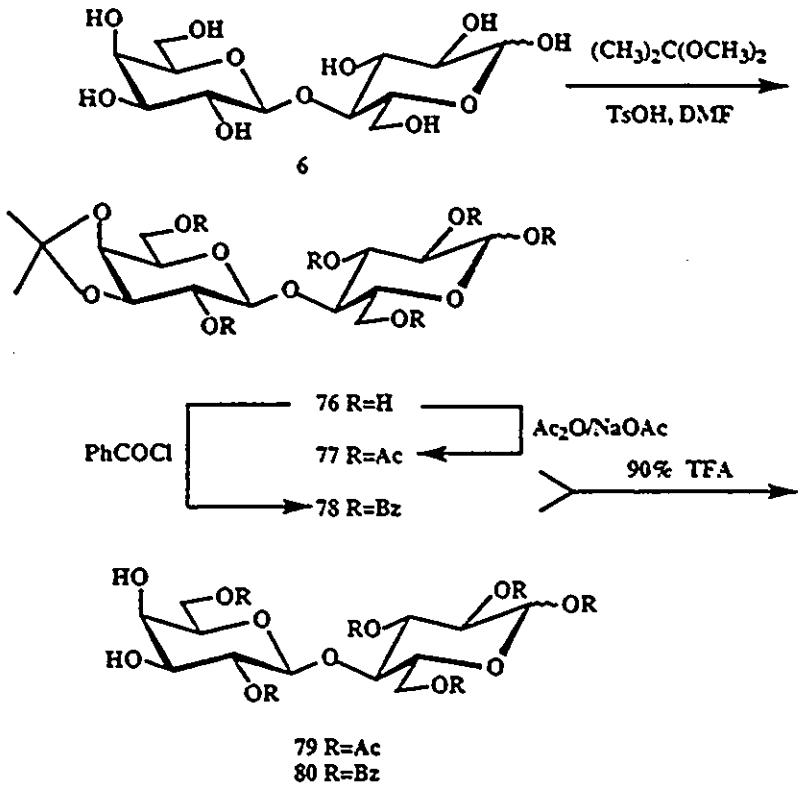


Scheme 2.15 Synthesis of a lactosamine acceptor (Alais and Veyrières[32])

2. 3. 2 Preparation of a lactose acceptor and a 2-azido-2-deoxylactose acceptor

Partially protected disaccharides which contain one or more free hydroxyl groups and are to serve as acceptor molecules for the synthesis of higher oligosaccharides are most often used in the form of glycosides because glycosides usually are stable compounds that tolerate many kinds of chemical manipulations such as are required for protection, glycosylation, and deprotection of hydroxyl groups. The glycosides must of course be selectively cleavable by methods not affecting interglycosidic oligosaccharide linkages if, at the end of a synthetic sequence, the free oligosaccharides are desired. Benzyl glycosides such as 75 described in the preceding section, and also allyl, silyl, and a few other types of glycosides meet this condition, but their preparation at the start and cleavage at the end of a synthetic sequence entails additional efforts. It may therefore be advantageous, occasionally, to use as acceptors O-1 esters instead of glycosides, particularly when other ester protecting groups are present elsewhere in the molecule; preparation and final deprotection may thus be simplified. An example for this concept was provided by Baer and Abbas[57] in their synthesis of 3'-O-α-L-fucopyranosyllactose, in which 1,2,3,6-tetra-O-acetyl-4-O-(2,6-di-O-acetyl-β-D-galactopyrannosyl)-α,β-D-glucopyranose 79 served as a convenient lactose acceptor. For the purposes of the present research it was decided to utilize this compound also and possibly its benzoyl

analog **80**, too. They were prepared as described[57b] (Scheme 2.16).



Scheme 2.16 Synthesis of a lactose acceptor
(Baer and Abbas[57])

Baer and Abbas had found[57a] that kinetically controlled acetonation of lactose **6** with 2,2-dimethoxypropane in N,N-dimethylformamide, catalyzed by p-toluenesulfonic acid and performed under mild conditions (3 h, at room temperature), gives mainly the 4',6'-O-isopropylidene acetal, but when the reaction was performed with the same reagents at 80-85° C for 45 minutes, an acetal mixture in which the thermodynamically more stable 3',4'-O-isopropylidene derivative **76** predominated was obtained[57b]. Acetylation of the latter to **77** followed by acid hydrolysis, furnished **79** in high yield. The corresponding benzoates **78** and **80** were prepared analogously. Interestingly, **79** crystallized as an anomeric mixture in which the β-form predominated, whereas **80** crystallized as the α-form, according to the polarimetric and ^{1}H-NMR spectroscopic evidence.

Frequently, in oligosaccharide syntheses involving 2-amino-2-deoxy sugar building blocks, the corresponding 2-azido sugars are used as equivalents, to be reduced to the amino stage eventually. As was already pointed out (Section 2.1.3), 2-azido sugars have become readily available with the advent of the azidonitration method for glycals. Both glycosyl donors (*e.g.*, compound **13** in

Scheme 2.3-2) and glycosyl acceptors may be azido derivatives[5]. We decided to synthesize hitherto unknown 1,3,6-tri-O-acetyl-2-azido-2-deoxy-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose **85** as a lactosamine acceptor equivalent. To this end, 2-azido-2-deoxylactose **82** was to be made, and then functionalized by the methods just outlined for lactose (Scheme 2.17). The crude mixture of azidonitrates **12** previously referred to was treated a) with hydrazinium acetate for removing the anomeric nitrate esters and then with acetic anhydride/pyridine, or b) with acetic anhydride and pyridine at 98° for one hour, to give 1,3,6-tri-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α,β-D-glucopyranose **81**, which was purified by chromatography. Zemplén deacetylation of **81** gave the unprotected 2-azido-2-deoxylactose **82**, which was isopropylidenated with 2,2-dimethyoxypropane in N,N-dimethylformamide under catalysis with p-toluenesulfonic acid at 80-85°C for 45 min. Acetylation of the crude product **83** with acetic anhydride/sodium acetate afforded the 3',4'-O-isopropylidene derivative **84**, which upon deisopropylidenation provided 1,3,6-tri-O-acetyl-2-azido-2-deoxy-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose **85** (Scheme 2.17).

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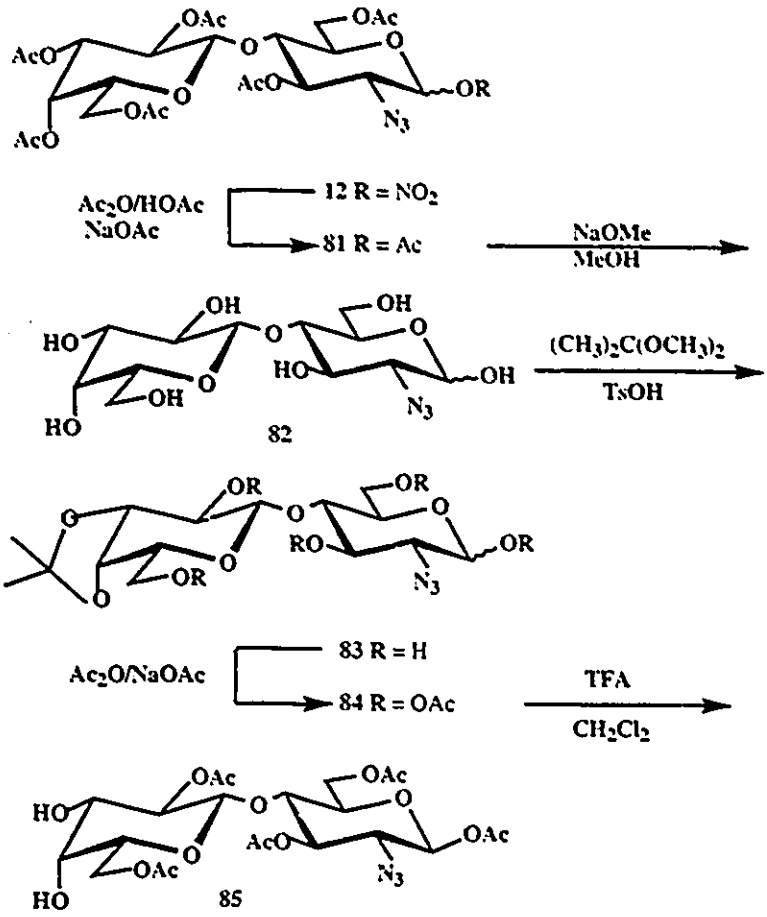
[5]When 2-azido-2-deoxyglycosyl halides such as **13** are used as glycosylating agents it is important to realize that the azido group is a nonparticipating neighboring group, whereas NHAc, NHBz, and NPhth are participating groups. This may have a profound influence upon α,β-selectivity of the glycosylation.



Scheme 2.17 Synthesis of a 2-azido-2-deoxylactose acceptor

Summary of Chapter 2.3

The known lactosamine acceptor having unprotected OH-3' and OH-4' groups, benzyl 2-deoxy-2-phthalimido-β-lactose 3,6,2',6'-tetraacetate **75**, was prepared from 2-deoxy-2-phthalimido-β-lactose heptaacetate **20** in five steps involving established procedures, although the first step (introduction of the benzyl aglycon) was improved by using the trimethylsilyl triflate method.

Starting from lactose, the 1,2,3,6,2',6'-hexaacetate **79** and the corresponding hexabenzoate **80** were prepared as described in the literature. These esters may serve as lactose acceptors in selective glycosylations at the more reactive OH-3' position.

Furthermore, the hitherto unknown 2-azido-2-deoxy-lactose 1,3,6,2',6'-pentaacetate **85** was synthesized from peracetylated 2-azido-2-deoxy-lactose **81** (also new; obtained from hexaacetyllactal by the azidonitration method). Compound **85** may serve as a lactosamine acceptor equivalent.

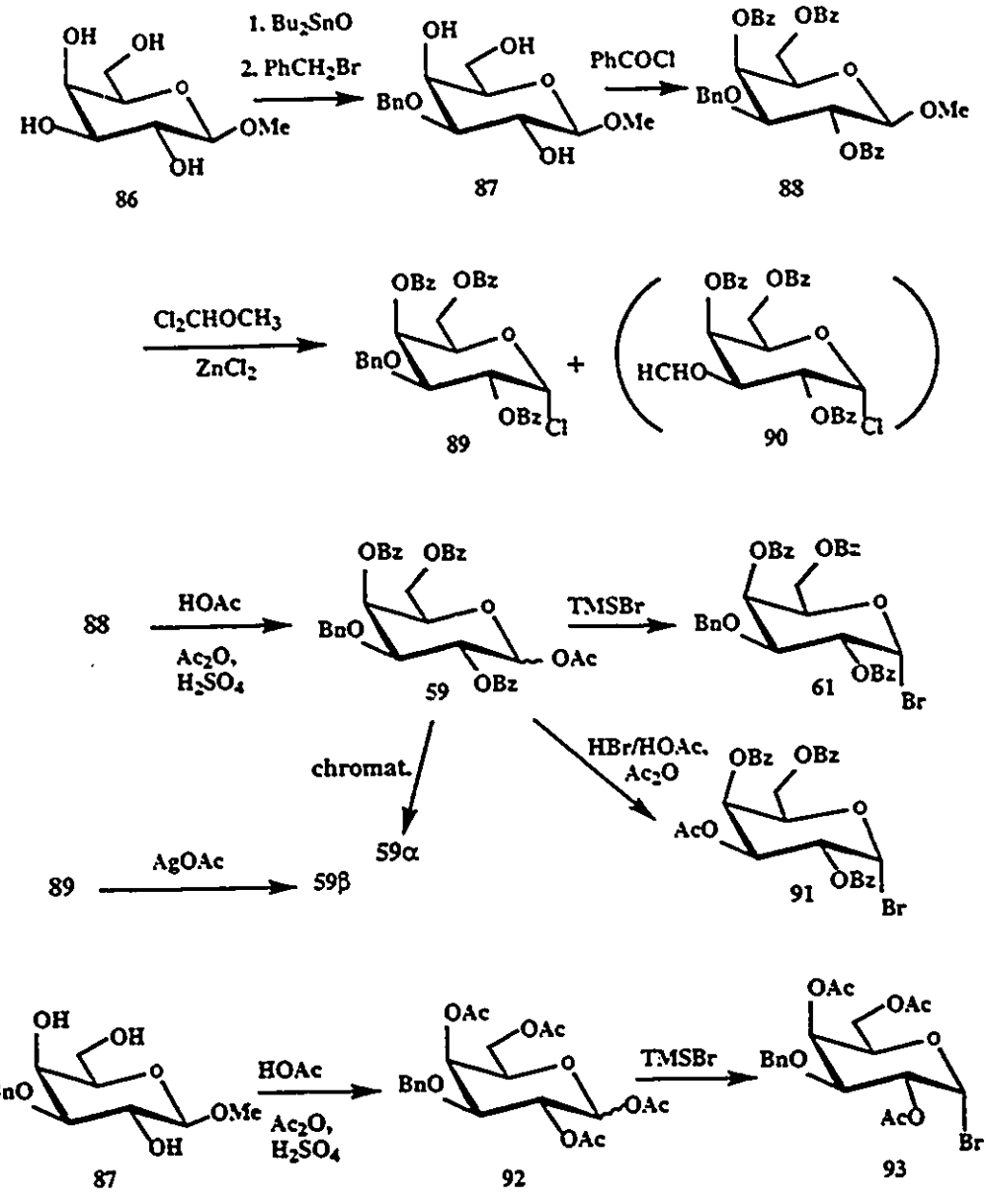
2. 4. Preparation of lacto-N-biose and isolactose acceptors bearing a free OH-3' group

For the synthesis of tetrasaccharides such as A and B (Fig. 1.3-1) or D and F (Fig. 1.3-2), which have isolactose and lacto-N-biose, respectively, as their reducing end units and are linked through O-3' to the other half of the molecule, suitably blocked derivatives with a free OH-3' group of the two disaccharides named were required. Initially we tried to apply, to synthetic isolactose (**51**, Scheme 2.10) and to methyl 2-benzamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-glucopyranoside (**57a**, Scheme 2.11), the method of isopropylidenation which had been successfully applied to lactose (Scheme 2.16) and 2-azido-2-deoxylactose (Scheme 2.17). Unfortunately, these experiments failed; well defined 3',4'-acetonides could not be obtained. As was pointed out in Section 2. 3. 2, isopropylidenation of lactose primarily gives a 4',6'-acetal which under thermodynamic control isomerizes to the desired 3',4'-acetal. Unlike lactose, whose glucose part is fixed in the pyranose form, isolactose may also react in its furanose form and thus become acetonated in the 1,2- and/or 5,6-positions, in addition to the 4',6'- and 3',4'-positions. This was probably the reason that intractable mixtures resulted. Therefore, it was decided to resort to synthesis from monosaccharidic components. For this purpose, one or more D-galactopyranosyl donors possessing a selectively cleavable O-3 protecting group

had to be procured, which could then be condensed with a D-glucose or D-glucosamine acceptor fully blocked except on OH-3. Final deprotection of O-3' in the disaccharides so synthesized would lead to the desired acceptors.

2. 4. 1. Preparation of 3-O-benzylated D-galactopyranosyl donors

2,4,6-Tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl chloride **89** was prepared[37] as shown in Scheme 2.18. The commercially available starting compound, methyl β-D-galactopyranoside **86,** was converted into its 3-benzyl ether **87** by the stannylene method[56,58], which involves first a treatment with dibutyltin (II) oxide to form a 3,4-dibutylstannylidene derivative (not isolated), and a subsequent reaction with benzyl bromide to give the desired 3-benzyl derivative **87** (yield, 65%). Conventional benzoylation of **87** afforded the fully protected, readily crystallizable methyl 2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranoside **88** in 96 % yield. Conversion of **88** into the corresponding glycosyl chloride **89** was accomplished in excellent yield (94 %) by treatment[37] with 1, 1-dichloromethyl methyl ether in chloroform solution in the presence of a catalytic amount of fused zinc chloride, but only if the reaction was performed under careful temperature control (45-50 °C) for 30 min. When the temperature



Scheme 2.18 Synthesis of various α-D-galactopyranosyl halides

was allowed to rise to 55-60 °C and the reaction time was extended to 2 h as described in the original report[37], a mixture was obtained from which only the 3-O-formyl derivative **90** could be isolated by chromatography. Evidently, the conditions for chlorination of **88** predominantly at the anomeric center are critical as the electrophile can competitively attack the benzyl ether structure, displacing a benzyl cation and forming a 1-chloro-methoxymethyl ether which subsequently is decomposed to an O-formyl group.

To generate the bromo analog **61** of **89**, the methyl glycoside **88** was first subjected to acetolysis, to give the 1-acetate **59** as an α,β-mixture from which the pure α-anomer could be isolated by preparative t.l.c. (The β-anomer was obtained pure by reaction of the chloride **89** with silver acetate). Reaction of **59** (α,β-mixture) with trimethylsilyl bromide in chloroform afforded a 90 % yield of 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61**. The same transformation could not be effected by the classical procedure of bromo sugar synthesis from acetates, namely, with hydrogen bromide in a mixture of acetic acid and acetic anhydride. When **59** was treated with that reagent, replacement of the 3-O-benzyl by an acetyl group took place and the bromo sugar **91** was obtained in 78 % yield. This observation, and the formation of **90** noted above, indicated that benzyl ethers may exhibit only limited stability as protective functions in the presence of powerful electrophiles, and therefore, that

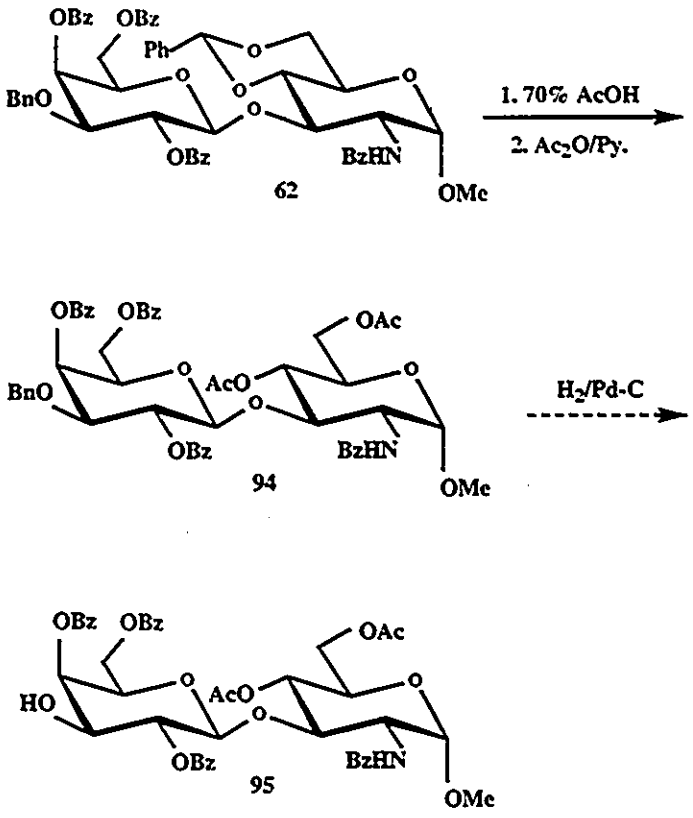
reagents and reaction conditions for the synthesis of benzylated glycosyl halides must be chosen judiciously.

The reactivity of glycosyl halides in glycoside-forming reactions generally depends on the solvent system and on the type of catalyst employed, but also on the substitution pattern of the halide. Bromides are always more reactive than chlorides (but also less stable in storage), and benzyl groups enhance reactivity in comparison with acyl groups. Benzoylated and acetylated halides behave similarly, although the former are sometimes slightly more reactive. High reactivity is of course often desirable, but this is not always so because α,β-selectivity (for halides having no participating 2-substituent) or regioselectivity (when the acceptor has two hydroxyl groups of differential reactivity) will suffer when the halide is too reactive.[1] To optimize results in a given synthetic task it is therefore useful to have a choice of several, structurally equivalent but unequally reactive halides. With this in mind we prepared, in addition to **89** and **61**, the 2,4,6-triacetate analog **93** of the latter bromide. Treatment of the triol **87** with acetic anhydride/acetic acid/conc. sulfuric acid caused simultaneous acetolysis at the anomeric center and acetylation of the hydroxyl groups, to give the tetraacetate **92** as an α,β-mixture in 94 % yield. Reaction with trimethylsilyl bromide as previously performed with **59** then provided 2,4,6-tri-O-acetyl-3-O-benzyl-α-D-galactopyranosyl bromide **93** in 87 % yield.

2. 4. 2 Preparation of lacto-N-biose acceptor and isolactose acceptor

With the donors 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61** and chloride **89** in hand it was easy to approach the desired disaccharidic acceptors. The condensation of **61** with known[59] methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** to give the 3'-O-benzylated disaccharide **62** has already been described in Section 2.2.2 (see Scheme 2.12). This disaccharide was de-O-benzylidenated by mild hydrolysis with hot 70 % acetic acid, and the resulting 4,6-diol was immediately acetylated, without prior isolation, to afford the diacetate **94** in 75 % yield (Scheme 2.19). The crystalline product was well characterized by microanalysis and NMR spectroscopy. A first attempt was then made to split off the 3'-O-benzyl group by palladium-catalyzed hydrogenolysis. Unfortunately, the experiment failed to yield the target compound **95** in satisfactory fashion; an ^{1}H-NMR spectrum of the product suggested incomplete removal of the benzyl group, and one may perhaps suspect that an insufficiently active catalyst sample had been used. Further efforts are needed to complete the task.

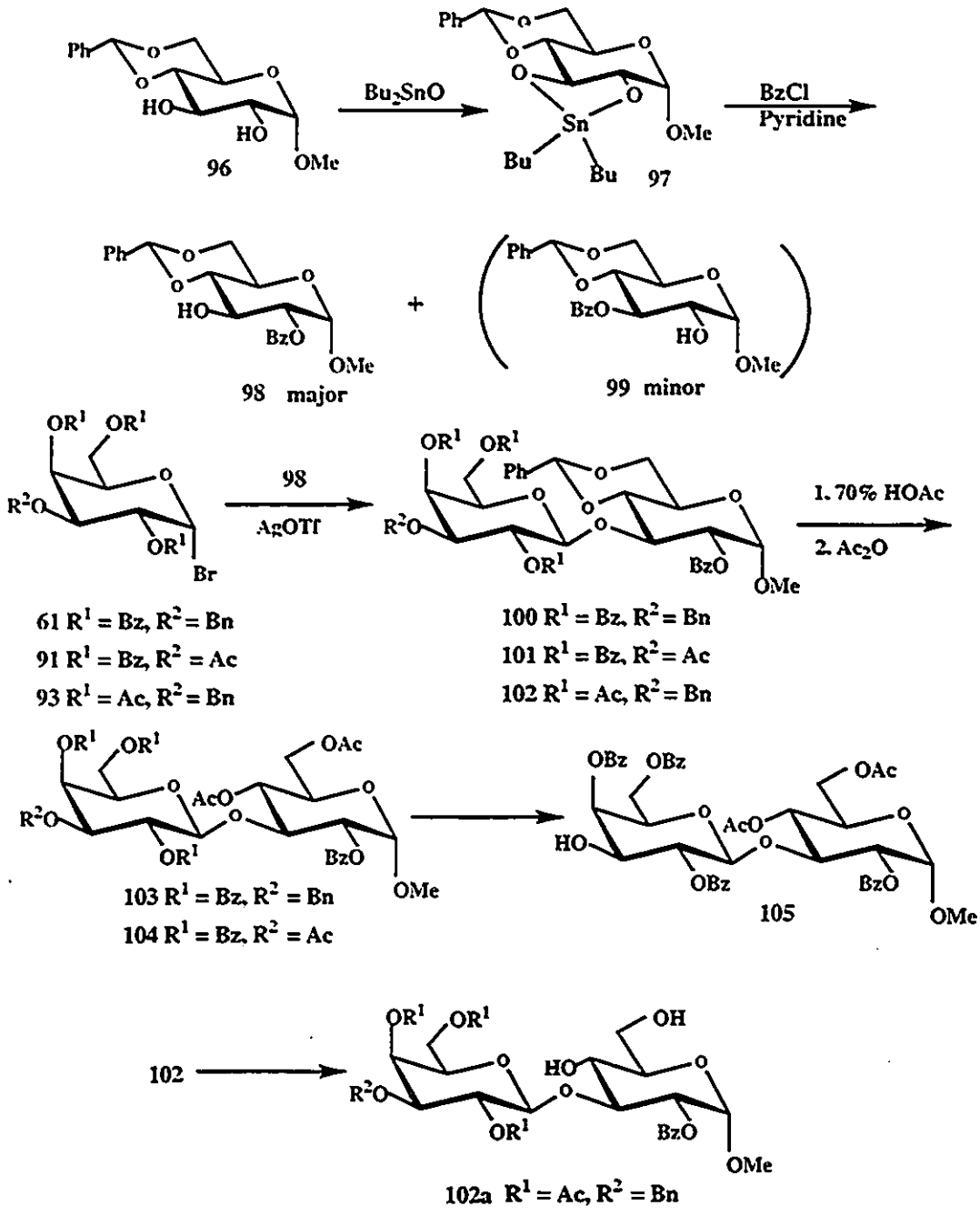
Similarly, 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61** and its 2,4,6-tri-O-acetyl analog **93** were to be condensed with methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside **98** (Scheme 2.20). The requisite 2-benzoate **98** was prepared from methyl 4,6-O-benzylidene-α-D-glucopyra-



Scheme 2.19 Synthesis of a lacto-N-biose acceptor having a free OH-3'

noside **96**[60,61] by selective benzoylation (with benzoyl chloride and pyridine at 0 °C) *via* intermediate dibutylstannylene derivative **97** which is formed quantitatively from **96** by reaction with dibutyltin oxide in benzene-methanol. Compound **98** arises as the major product along with a small proportion of the 3-O-benzoyl regioisomer **99** (see Experimental Section). Coupling of **98** with the bromides **61** and **93** in the presence of silver trifluoromethanesulfonate then furnished the amorphous disaccharides **100** and **102** in yields of 90 % and 91 % respectively. Removal of the benzylidene group from **100** by mild hydrolysis, followed by acetylation with acetic anhydride and pyridine gave the fully blocked disaccharide **103**. Palladium-catalyzed hydrogenolysis finally furnished the desired methyl 4,6-di-O-acetyl-2-O-benzoyl-2-deoxy-3-O-(2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranoside **105**. (Scheme 2.20).

In initial experiments aimed at producing the disaccharide **100**, the glycosyl bromide resulting from treatment of 3-O-benzyl glycoside **59** with hydrogen bromide in acetic acid was condensed with **98**. It had not yet been realized at the time that the new donor was not the expected 3-benzyl ether **61** but in fact the 3-acetate **91**. The condensation, promoted by silver triflate, led to the disaccharide **101** in 97 % yield, and this product was then debenzylidenated and acetylated to furnish methyl 4,6-di-O-acetyl-2-O-benzoyl-3-O-(3-O-acetyl-2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranoside **104**. It



Scheme 2.20 Synthesis of an isolactose acceptor having a free OH-3'

was then determined by spectral and elemental analysis that **104** did not possess a benzyl group. Its loss during the condensation reaction was difficult to visualize, and it soon became clear that cleavage had occurred during the bromination of **59**.

Finally, the fully blocked disaccharide **102** was debenzylidenated with 70 % acetic acid, which afforded the corresponding 4,6-diol **102a**.

Summary of Chapter 2.4

Methyl 2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranoside **88** was prepared according to the literature. Its conversion into the corresponding α-glycosyl chloride **89** by means of dichloromethyl methyl ether was found to be exceedingly sensitive to the reaction conditions. When the recommended literature procedure was followed exactly, the 3-O-benzyl group was converted into a 3-O-formyl group and the chloride **90** resulted; the desired **89** was obtained only after lowering the temperature and shortening the time of the reaction.

Alternatively, the methyl glycoside **88** could be acetolyzed (AcOH, Ac_2O, conc. H_2SO_4) at 0-5 °C without loss the benzyl group, to give the corresponding 1-acetate **59** which on treatment with trimethylsilyl bromide afforded the 3-O-benzylated α-glycosyl bromide **61**. However, conversion of **59** into bromo sugar by the classical method (HBr in $AcOH/Ac_2O$ at 5 °C) again resulted in loss of

the benzyl group and 3-O-acetyl derivative **91** was formed. Acetolysis of methyl 3-O-benzyl-β-D-galactopyranoside **87** (AcOH, Ac_2O, conc. H_2SO_4) at 0 °C smoothly afforded the tetraacetate **92** which in turn gave the 3-O-benzyl-α-glycosyl bromide **93** by treatment with trimethylsilyl bromide. Compounds **59**, **61**, **90**, **91**, **92**, and **93** were not previously described. The facile cleavage of a benzyl ether protecting group under certain conditions was noteworthy.

With the new donor **61** and the partially protected N-benzoylglucosaminide **54** as acceptor the disaccharide **62** was synthesized as already mentioned in Section 2.2.2, and subsequent manipulations led to the new, crystalline derivative methyl 4,6-di-O-acetyl-2-benzamido-2-deoxy-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside **94** which, following removal of the benzyl ether group, should be a suitable lacto-N-biose acceptor.

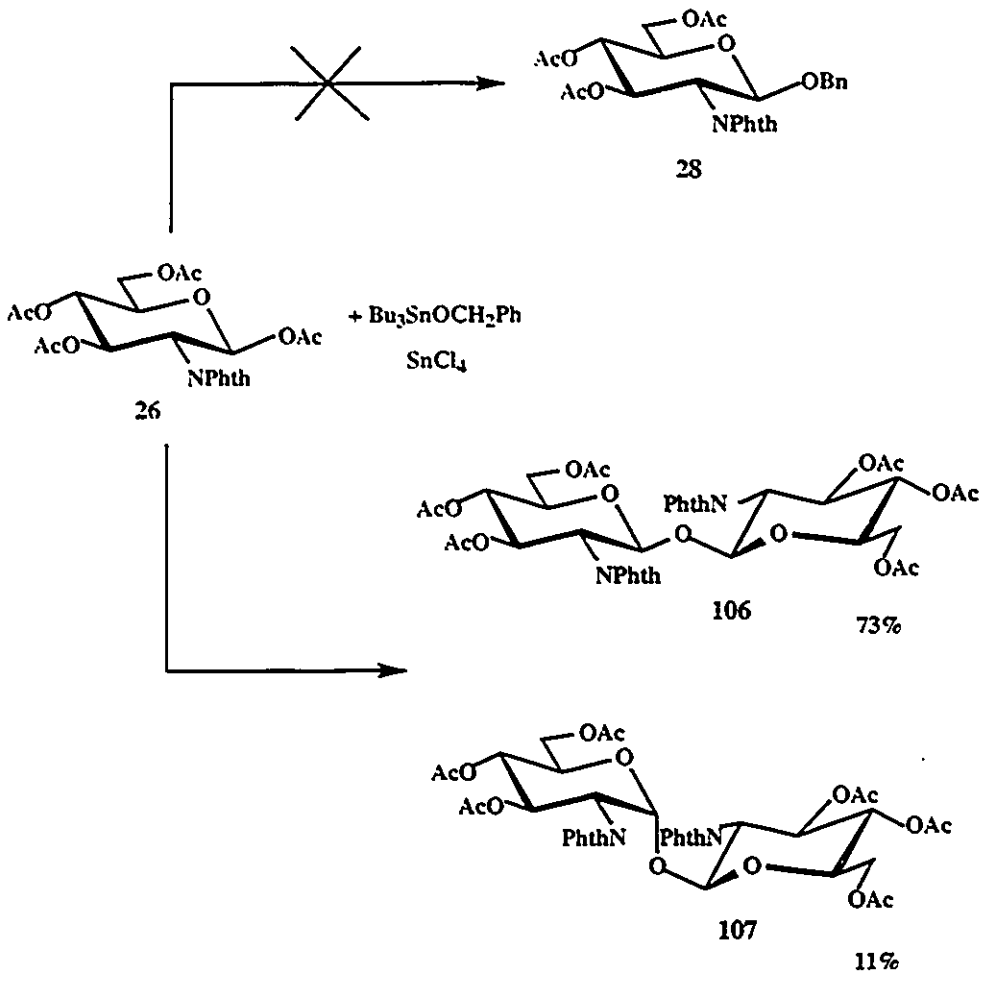
An isolactose acceptor, namely methyl 4,6-di-O-acetyl-2',4',6'-tri-O-benzoyl-α-isolactoside **105** was obtained in analogous fashion by condensation of the bromide **61** with the partially protected α-D-glucopyranoside **98**, followed by appropriate functional group manipulation. As well, the related 3'-O-benzylisolactoside 2',4',6'-triacetate **102a** was synthesized starting with the bromide **93**, and this derivative should be convertible into another isolactose acceptor by cleavage of the benzyl ether after acetylation of the 4,6-positions.

Similar work using the galactosyl bromide **91** produced the 3'-O-acetylated disaccharides **101** and thence **104**. Although these are new compounds, they are of no value as precursors for isolactose acceptors since they do not possess a selectively removable protecting group at O-3'.

2. 5 An unexpected mode of formation of trehalose derivatives

During the studies directed towards the synthesis of a lactosaminyl donor (Scheme 2.7 in Section 2.1.5) an unexpected discovery was made when 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **26** was treated with tributyltin benzyloxide in the presence of stannic chloride, in order to prepare the corresponding benzyl β-glycoside **28** according to the procedure published by Ogawa and co-workers[36a] A mixture of products was obtained, but no **28** could be isolated from it. Column chromatography produced a major product having R_f 0.5 (yield, 73 %), preceded by a minor one of R_f 0.75 (yield, 11 %) (Scheme 2.21).

On the basis of elemental analysis, mass spectrum, and NMR spectra the structure of 2,2'-dideoxy-2,2'-diphthalimido-β,β-trehalose **106** was assigned to the main product. The FAB mass spectrum showed an $(M + 1)^+$ peak at m/z 843.4, and the microanalytical data fitted the composition $C_{40}H_{40}N_2O_{19}$. The ^{13}C-NMR spectrum (Fig. 2.5-2) revealed **106** to be a symmetrical disaccharide, as



Scheme 2.21 Synthesis of 2-deoxy-2-phthalimido-β,β–trehalose and possible structure of byproduct

only a single set of ring and substituent carbon atoms appeared. Thus, there were three acetyl-CO signals (near δ 170) and 3 acetyl-CH_3 signals (near δ 20), as well as one set of 3 signals (δ 134.2, 131.5, and 123.5) for the magnetically nonequivalent pairs of carbon atoms in the phthalimido group. The anomeric carbon atoms C-1 resonated at δ 97.1, which suggested a β,β-interglycosidic linkage; for α,α-trehalose derivatives that signal usually occurs in the δ 90-94 region.[62,63] The ^{1}H-NMR spectrum (Fig. 2.5-1) left no doubt about the structure. The assignments indicated were readily made with the aid of a COSY plot (Fig. 2.5-3) and by comparison with the spectra of several of the phthalimido-glucose derivatives encountered during these studies. Thus, it was immediately evident that **106** was not the expected benzyl glycoside **28** (which exhibits an AB-quartet for CH_2Ph), nor the starting material **26** (which shows 4 O-acetyl singlets), nor the reducing sugar **38** which might have arisen by hydrolysis (it exhibits an exchangeable OH-doublet and a corresponding H-1 triplet); yet all these compounds and structurally comparable ones gave similar patterns for the ring proton signals, albeit with certain chemical-shift differences. Of particular significance in the spectrum of **106** was the large coupling constant $J_{1,2}$ = 8.6 Hz, indicating an H-1,2 *trans* relationship, *i.e.*, the β-configuration. In harmony with the β,β-trehalose structure was also the laevorotation of the new derivative, $[\alpha]_D$ -22.7°. (β,β-Trehalose octaacetate has

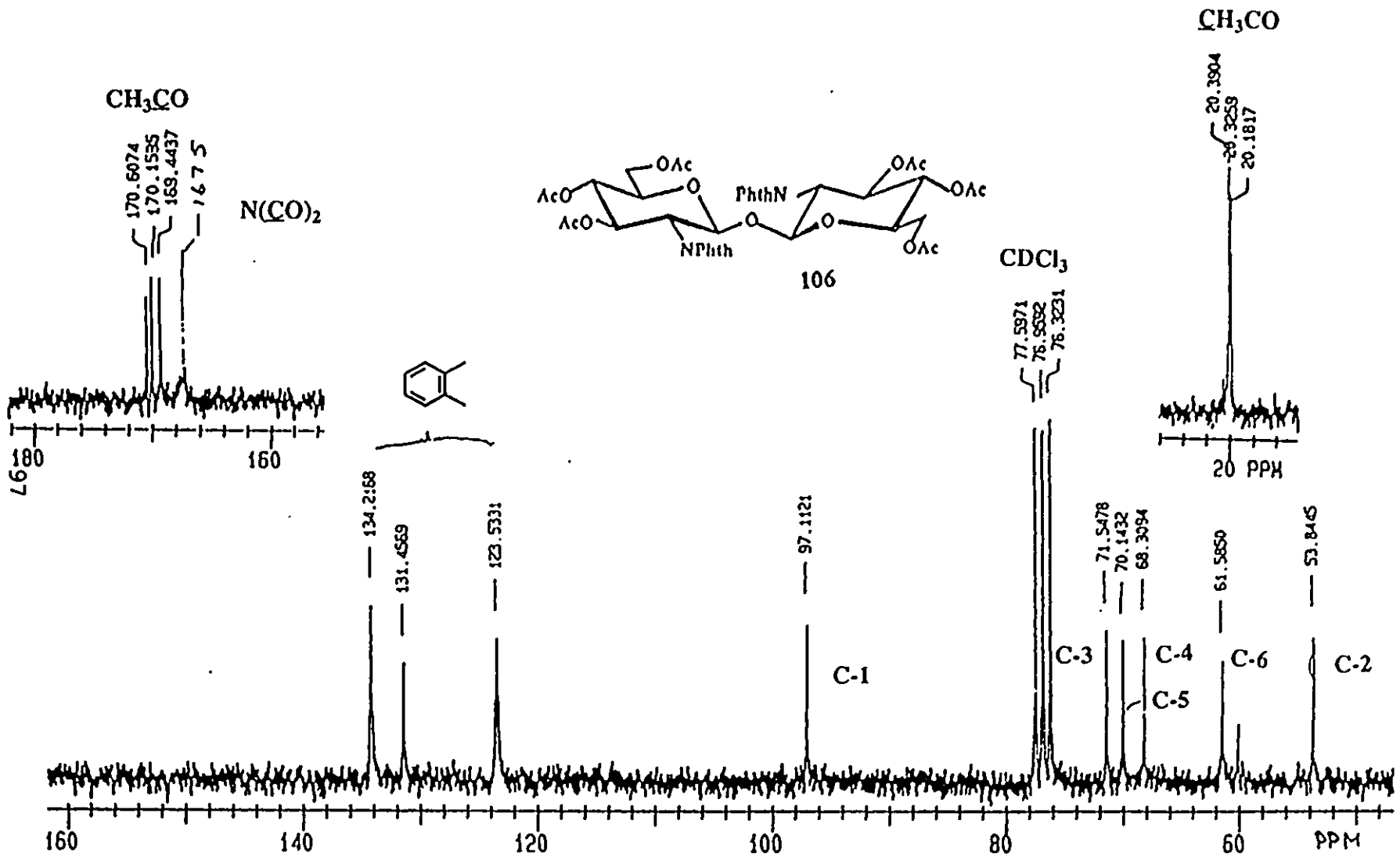


Fig. 2.5-1 ^{13}C-NMR spectrum of compound 106

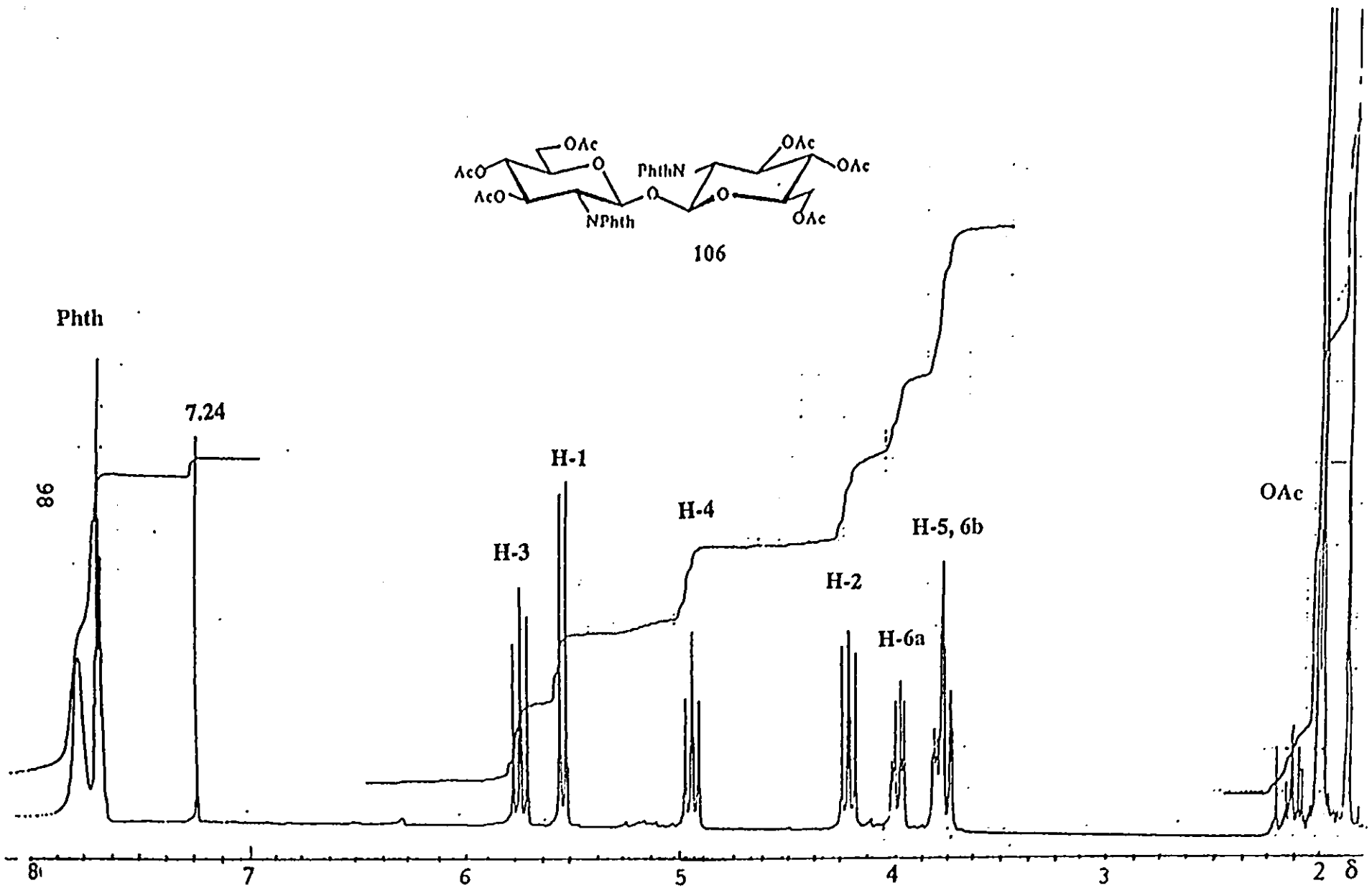
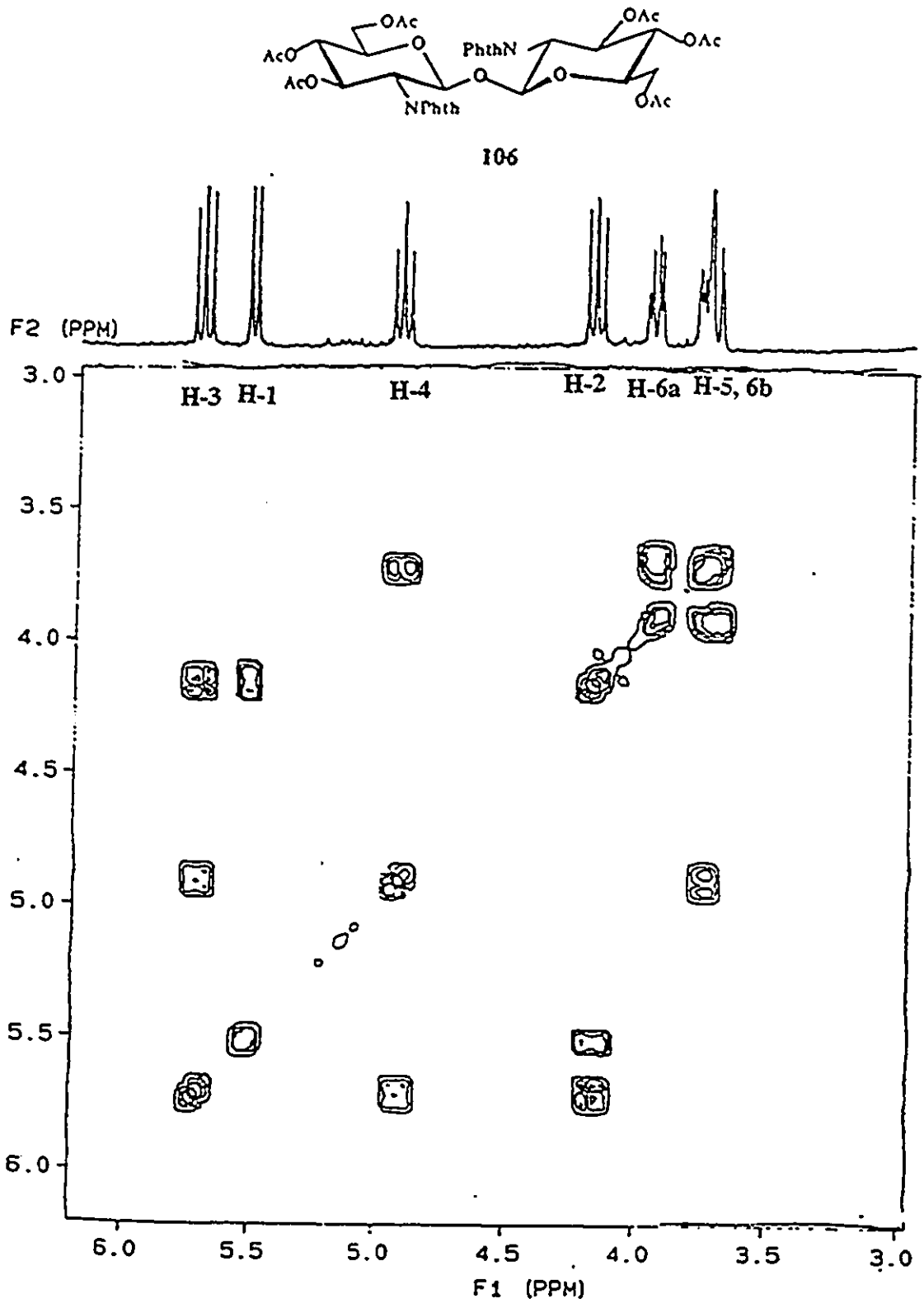


Fig. 2.5-2 300 MHz ^{1}H-NMR spectrum of compound 106



106

Fig. 2.5-3 ^{1}H-NMR COSY plot of compound 106

$[\alpha]_D$ - 19°, whereas the α,α- and α,β-anomers are dextrorotatary, with $[\alpha]_D$ + 162° and $[\alpha]_D$ + 82°, respectively). In summary, all carbon and hydrogen signals in the NMR spectra of **106** are accounted for by a 2-phthalimido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl structure, and in view of the molecular weight, microanalysis, and absence of signals for a benzyl or any other aglyconic group the assigned structure is secure.

The fast-moving, minor component separated on the column from **106** was chromatographically uniform, but not spectroscopically. Its ^{1}H-NMR spectrum revealed that it consisted chiefly of a another new product, which was accompanied by ~20 % of unreacted starting β-tetraacetate **26**; in the ^{13}C-NMR spectrum the signals attributable to **26** were very weak and did not interfere with the analysis of those belonging to the chief product. Presence of benzyl glycoside **28** or the 1-hydroxy triacetate **38** could be excluded by comparison with their spectra.

The structure of the new product could not be elucidated unambiguously, although a close relationship to **106** was apparent. In fact, it gave a mass-spectral peak at m/z 853 like **106**, and the ^{13}C-NMR spectrum was very similar in its general patten to that of **106** but with a number of additional peaks in the ring carbon region (δ 100-50), suggestive of an unsymmetrical trehalose

skeleton. These observations, together with a high dextrorotation[6] of the product, suggested at first glance that it was the α,β-anomer **107** of **106**. This notion was supported by a downfield doublet of doublets (J = 8 and 10 Hz) for one of the H-3 protons at δ 6.47 (superposed on a low-intensity H-1 doublet for the contaminant **26**): none of the fifteen 3-O-acetylated 2-deoxy-2-phthalimido-β-D-glucopyranosyl compounds encountered in this study gave that signal at a field lower than δ 5.9[7], whereas H-3 of the α-anomers **20α** and **27α** evidently experienced greater deshielding due to an axial anomeric substituent and resonated at δ 6.42 and 6.63, respectively.

However, on further examination the spectra revealed some seemingly contradictory features. Firstly, the ^{13}C spectrum showed both of the C-1 signals (clearly attributable because there were no other signals nearby) as having nearly equal chemical shifts (δ 98.7 and 98.1, values even higher than for **106**, see above), instead of showing one of them somewhat more upfield in the δ 94-90 region as expected for an α-pyranosidic C-1 in a trehalose derivative. Secondly, the aromatic proton region of the ^{1}H spectrum displayed a striking

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[6]The optical rotation measured for the material eluted from the column was $[\alpha]_D$ +92.6°. Assuming an approximately 20 % contamination by **26** ($[\alpha]_D$ +74°), a value of approximately +97° is estimated.

[7]These were the following compounds ($\delta_{H\text{-}3}$ in parentheses): **16** (5.73), **17β** (5.76), **18β** (5.74), **20β** (5.83), **21** (5.70), **24** (5.86), **26** (5.85), **27** (5.80), **28** (5.77), **29** (5.80), **38** (5.84), **39** (5.92), **74** (5.72), **75** (5.76), and **106** (5.72).

phenomenon: Whereas *all* the β-compounds (including **106**) listed in footnote 7 *as well as* the aforementioned α-anomers displayed the expected, 4-proton phthalimido group signal (an A_2B_2 doublet of multiplets) centered invariably at δ 7.75, the spectrum of (impure) **107** showed that signal significantly shifted to δ 7.40. (A similar signal having one-fifth the intensity and corresponding to the amount of contaminant **26** present, occurred clearly separated at the usual place; jointly they integrated to 8 protons).

Can such an upfield shift of the phthalimido proton signal be reconciled with the structure **107**? When Dreiding models of **106** and **107** are constructed and the glycosyl residues are rotated about the interglycosidic bonds so that the torsional arrangement of both residues is that which is favoured by the *exo*-anomeric effect (the O—C-1' bond antiparallel to the C-1 — C-2 bond and *vice versa*), the conformations depicted in Fig. 2.5-4 result. It is seen that in these conformations, which are most likely the preferred ones, the phthalimido groups in **107** are located in spatial proximity, and this may perhaps explain a mutual shielding effect. No efforts have so far been made to prove or disprove this hypothesis, mainly because the unforeseen formation of trehalose derivatives from **26** was a diversion from the chief objective of the thesis research, and the formula **107** for the minor by-product in the reaction must remain tentative for the time being. Future work must overcome, first of all, the problem of obtaining

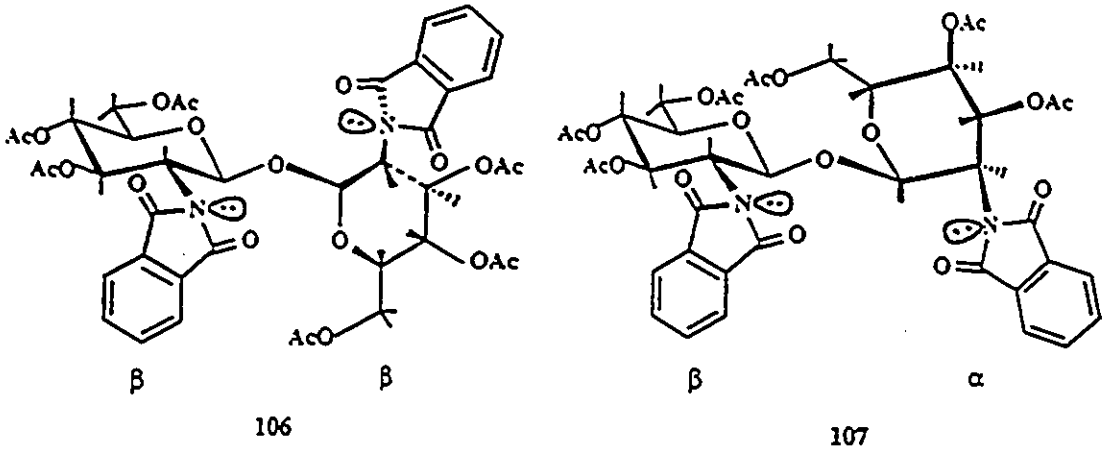

Fig. 2. 5-4 Likely perferred conformations of 106 and 107

the compound free from remnant **26**, so as to enable a more complete analysis of its rather complex ^{1}H-NMR spectrum; heteronuclear correlation spectra and the study of possible nuclear Overhauser effects should then yield data to settle the question.

α,α-Trehalose is one of the most widely distributed disaccharides in nature. It is ubiquitous in insects (where it serves as energy reserve), in fungi, and in mycobacteria where in the form of lipid esters it is part of biologically important cell wall constituents. As well, several amino derivatives of α,α-trehalose have been discovered as fungal metabolites possessing antibiotic activity. Consequently, an enormous amount of research has been devoted to biochemistry and synthetic chemistry of this sugar and its derivatives[64]. By contrast, α,β-trehalose and β,β-trehalose do not play a role as natural products and very little attention has been paid to the synthesis of derivatives. In the realm of amino derivatives, Inouye *et al.*[65] observed the formation of a small amount (2.3 %) of 2,2'-diacetamido-2,2'-deoxy-β,β-trehalose hexaacetate when 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl bromide was treated with silver oxide in the presence of anhydrous calcium sulfate and iodine.

The synthesis of **106** discovered in the present investigations represents a welcome contribution to this rather neglected area of carbohydrate synthesis.

Summary of Chapter 2.5.

Treatment of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyransose **26** with tributyltin benzyloxide in the presence of tin chloride did not give the expected benzyl glycoside **28**. Instead, it led to the discovery of a novel mode of formation of 2,2'-diaminotrehalose derivatives.

3. EXPERIMENTAL

General methods

Melting points were determined with a Buchi apparatus and are uncorrected. Optical rotations were measured at room temperature in a Perkin Elmer 241 polarimeter and refer to chloroform solutions unless otherwise stated. All reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany) with detection by charring with H_2SO_4 or by UV light (if possible). Silica gel 60 (230-400 mesh; E. Merck, Darmstadt, Germany) was used for column chromatography.

Proton and carbon NMR spectra at 300 (or 200) and 75.4 (or 50.3) MHz respectively were recorded with a Varian XL 300 (or XL Gemini 200) spectrometer; the data were obtained from $CDCl_3$ solutions unless otherwise indicated, relative to internal (deutero) chloroform at the δ 7.24 and 77.0 ppm respectively. In ^{13}C spectra, the number of protons attached to each carbon were determined be DEPT or ADEPT spectra. The coupling patterns in ^1H spectra are noted as singlets (s), doublets (d), triplets (t), quartets (q), doublets of doublets (dd), or multiplets (m). In many instances, COSY plots were obtained to aid in signal assignments. Combustion analyses were performed by M-H-W Laboratories (Phoenix, AZ).

Tetrahydrofuran (THF) was distilled over sodium-benzophenone under a nitrogen atmosphere prior to use. Nitromethane, benzene, pyridine and ethyl acetate were distilled from calcium hydride under nitrogen. Dimethylformamide (DMF) was distilled under reduced pressure at 60 °C from calcium sulfate. Dichloromethane was dried by distillation from phosphorus pentoxide under nitrogen. Toluene, reagent grade quality, was dried over sodium. Zinc chloride was freshly fused before use. Organic solutions were evaporated with a Buchi evaporator connected to a water aspirator.

2, 3, 6-Tri-O-acetyl-4-O-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide[22] (8)

Dry β-lactose octaacetate[∞] 7 (20 g, 29.5 mmol) was dissolved in glacial acetic acid (25 mL), and a saturated solution of hydrogen bromide in glacial acetic acid (50 mL) was added. After 2 h at room temperature dichloromethane (50 mL) was added, and the solution was poured into ice-water (600 mL). The dichloromethane layer was washed successively with water, aqueous sodium bicarbonate and water until neutral, and dried over magnesium sulphate for 2 h. Crystallization of heptaacetyllactosyl bromide 8 took place after cautious addition of petroleum ether (b.p. 40-60° C) to the dichloromethane solution (15.5 g, 75 %); m.p.139° C, $[\alpha]_D$ +104° (c 1.0, chloroform); lit.[22] m.p.137° C, $[\alpha]_D$+108° (c 1.0, chloroform).

3, 6-Di-O-acetyl-1,5-anhydro-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-D-*arabino*-hex-1-enitol (9, hexaacetyllactal)

A solution of acetobromolactose 8 (10 g, 14.3 mmol) in 100 mL of 50 % acetic acid was cooled to -5 – -10 °C, zinc dust (10 g) and 2 drops of chloroplatinic acid in 50 % acetic acid were added, and the reaction mixture was stirred vigorously for 2 h. The zinc dust was removed by filtration and the filtrate was poured into 200 mL of ice-cold water; the crystalline lactal

hexaacetate (7.62 g, 94.8 %) which precipitated was separated by filtration and recrystallized from hot, 30 % ethanol, to provide 9 as needles; m.p. 114 °C and $[\alpha]_D$ -18° (c 1 chloroform), as reported[22].

Ms(CI): m/z 501 (M^+ - OAc), 441 (M^+ + 1 - 2 OAc), 381 (M^+ + 1 - 3 OAc), 321 (M^+ + 1 - 4 OAc).

^{1}H-NMR: δ 6.39 (dd, 1 H, $J_{1,2}$ 6.1, $J_{1,3}$ 0.7 Hz, H-1), 5.38 (septet, 1 H, W_H ~ 9.5 Hz, H-3), 5.34 (dd, 1 H, $J_{3',4'}$ 4.2, $J_{4',5'}$ 0.7 Hz, H-4'), 5.17 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.5 Hz, H-2'), 4.97 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.4 Hz, H-3'), 4.82 (dd, 1 H, $J_{1,2}$ 6.1, $J_{2,3}$ 3.3 Hz, H-2), 4.64 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.41 (dd, 1 H, $J_{5,6a}$ 2.1, $J_{6a,6b}$ 11.2 Hz, H-6a), 4.17 (dd, 1 H, $J_{5',6'a}$ 6.2, $J_{6'a,6'b}$ 11.2 Hz, H-6'a), 4.1 (unresolved m, 3 H, H-5, 6b, 6'b), 3.97 (dd, 1 H, $J_{3,4}$ 5.4, $J_{4,5}$ 7.4 Hz, H-4), 3.88 (septet, 1 H, $J_{4',5'}$ 1, $J_{5',6'a} \approx J_{5',6'b}$ 6.2 Hz, H-5'), 2.13, 2.09, 2.06, 2.04, 2.03, and 1.94 (6 s, 18 H, 6 OAc).

^{13}C-NMR: δ 145.5 (C-1), 101.0 (C-1'), 98.9 (C-2), 74.5 (C-4), 74.0 (C-5), 70.8 (C-3'), 70.6 (C-5'), 68.7 (C-2', C-3), 66.6 (C-4'), 61.6 (C-6), 60.8 (C-6').

Benzyl 3, 6-di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (16)

A mixture of 1,3,6-tri-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose 20β (0.77 g, 1 mmol), benzyl alcohol (0.2 mL, 1.9 mmol), and powdered molecular sieves (4 A, 2 g) in dry dichloromethane (20 mL) was stirred under nitrogen for 1 h at room temperature, and then cooled to -20° C. A 0.5 M solution of trimethylsilyl triflate in dichloromethane (2 mL, 1 mmol) was rapidly added, and then the mixture was stirred for 2 h at room temperature. The acid was neutralized with triethylamine, and the solution filtered and washed with water. Evaporation gave a syrup which crystallized from ethyl acetate-hexanes to give **16** (0.74 g, 91 %), m.p. 180-182°C, $[\alpha]_D$ -11° (c 1, chloroform); lit.[28c] m.p. 187-188° C, $[\alpha]_D$ -9.6° (c 2.5, chloroform); lit.[32] m.p. 185-186° C, $[\alpha]_D$ -9° (c 2.1, chloroform).

Ms(FAB): m/z 814.2 (M^+ + 1), 754.2 (M^+ - OAc), 722.2 (M^+ - $PhCH_2$), 706.2 (M^+ - $OPhCH_2$), 646.2 (M^+ - $OPhCH_2$ - HOAc), 586.2(M^+ - $OPhCH_2$ - 2 HOAc),

^{1}H-NMR: δ 7.80-7.68 (m, 4 H, Phth), 7.07 (m, 5 H, Ph), 5.73 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.5 Hz, H-3), 5.35 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 5.31 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 1 Hz, H-4'), 5.09 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd, 1 H, $J_{2',3'}$

10.5 $J_{3',4'}$ 3.5 Hz, H-3') ,4.80 (d, 1 H, J 12 Hz, CH_2Ph), 4.52 (d, 1 H, H-1'), and 1.92, 1.98, 2.05, 2.09, 2.15, 2.19 (6 s, 18 H, 6 OAc).

^{13}C-NMR: δ 101.00 (C-1'), 96.88 (C-1), 76.40 (C-4), 75.17 (C-5), 74.76 (C-3), 74.20 (C-5'), 72.53 (C-3'), 70.43 ($PhCH_2$-), 68.92 (C-2'), 66.40 (C-4'), 62.06 (C-6), 60.55 (C-6'), 54.78 (C-2).

3,6-Di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose (17β)

Method a:

A solution of 1,3,6-tri-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose **20β** (0.77 g, 1 mmol) in N,N-dimethylformamide (10 mL) was treated with hydrazine acetate (0.1 g, 1.1 mmol) for 4 h at room temperature, and then diluted with dichloromethane (40 mL), washed with 10 % aq. NaCl, and concentrated. The residue crystallized from ethanol-hexanes to give **17β** (0.62 g 84 %), m.p. 177-178° C, $[\alpha]_D$ +30° (c 1.0, chloroform); lit.[32] m.p. 139-140° C, $[\alpha]_D$ +34° (c 0.99, chloroform).

Ms (FAB): m/z 724.2 (M^+ + 1), 706.2 (M^+ + 1 - H_2O), 646.2 (M^+ + 1 - H_2O - HOAc).

^{1}H-NMR: δ 7.9-7.70 (m, 4 H, Phth), 5.76 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.0 Hz, H-3),

5.63 (t, 1 H, $J_{1,2} \approx J_{1,OH}$ 7.2 Hz, H-1), 5.31 (dd, 1 H, $J_{3',4'}$ 2.5 Hz, H-4'), 5.08 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.94 (dd ,1 H, $J_{2',3'}$ 10.5, $J_{3',4'}$ 2.5 Hz, H-3'), 4.56 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.54 (dd, 1 H, H-2), 4.21 (t, 1 H, $J_{3,4}$ 8.0, $J_{4,5}$ 8.0 Hz, H-4), 3.45 (d, 1 H, $J_{1,OH}$ 7.2 Hz, OH-1), and 2.20-1.90 (6s, 18 H, 6 OAc);

^{13}C-NMR: δ 170.85-168.37 (six peaks, $CH_3\underline{CO}$), 168.04 (phthaloyl $\underline{CO}$), 100.97 (C-1'), 92.42 (C-1), 76.74 (C-4), 72.58 (C-5), 70.95 (C-3), 70.84 (C-3'), 70.37 (C-5'), 68.93 (C-2'), 66.43 (C-4'), 62.11 (C-6), 60.54 (C-6'), 56.02 (C-2), 20.67 and 20.40 ($\underline{CH_3}CO$).

Method b:

A mixture of free amino sugars **22** (containing some isomer **23**) was prepared by hydrogenation of the crude mixture of azido-nitrates (**12** + some **19**) obtainable from the lactal **9** as detailed in a subsequent section. This amino sugar mixture (5 g, 8 mmol), N-carboethoxyphthalimide (4 g, 16 mmol) and a few drops of triethylamine were dissolved in ethanol (99 %, 50 mL), the solution was heated under reflux and under nitrogen for 3 h, and then was cooled to room temperature, filtered, and concentrated to dryness in vacuo. Purification by chromatography on silica gel using ethyl acetate-hexanes (1:1) as eluent gave the desired compound **17β**, which crystallized from ethanol (1.5 g, 26 %), m.p. 178-179° C, $[\alpha]_D$ +31° (c 1.0, chloroform).

3,6-Di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl chloride (18) from the heptaacetate 20

A. By action of hydrogen chloride:

A solution of **20β** (1.53 g, 2 mmol) in acetic acid (15 mL) and acetic anhydride (5 mL) was saturated with dry HCl at 0 °C, and then kept at room temperature for 48 h. After dilution with dichloromethane (100 mL), the mixture was successively washed with ice-water, cold saturated aq. $NaHCO_3$, and water, dried, and concentrated to a small volume. Addition of dry ether gave crystalline **18** (0.68 g, 46 %), m.p. 171.5° C, $[\alpha]_D$ +35° (c 1.0, chloroform); lit.[28c] m.p. 184-185° C, $[\alpha]_D$ +34.7° (c 1.8, chloroform); lit.[39a] m.p. 189.5-190.5° C, $[\alpha]_D$ +29.5° (c 0.33, chloroform).

^{1}H-NMR: δ 7.90-7.70 (m, 4 H, Phth), 6.11 (d, 1 H, $J_{1,2}$ 9.3 Hz, H-1), 5.67 (dd, 1 H, $J_{2,3}$ 9.5, $J_{3,4}$ 8.0 Hz, H-3), 5.27 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-4'), 5.05 (dd, 1 H, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.4 Hz, H-2'), 4.89 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.3 Hz, H-3'), 4.48 (m, 2 H, consisting of d, $J_{1',2'}$ 7.6 Hz for H-1', superposed on H-6a signal), 4.33 (~ t, 1 H, H-2), 4.13-3.78 (unresolved multiplets, 6 H, H-4, 5, 5', 6_b, $6'_a$, $6'_b$) and 2.12-1.85 (6 s, 18 H, 6 OAc).

^{13}C-NMR: δ 101.07 (C-1'), 85.55 (C-1), 76.30 (C-4), 75.77 (C-5), 71.96 (C-3), 70.72 (C-3'), 70.06 (C-5'), 69.12 (C-2'), 66.65 (C-4'), 62.03 (C-6), 60.81 (C-6'), 57.91 (C-2).

B. By the action of 1,1-dichloromethyl methyl ether:

A mixture of **20β** (1.3 g, 1.7 mmol), 1, 1-dichloromethyl methyl ether (3 mL) and freshly fused zinc chloride (10 mg) in dichloromethane (3 mL) was stirred for 30 min at 40° C with the exclusion of atmospheric moisture. The mixture was concentrated and chromatographed on silica gel with 1:1 ethyl acetate-hexanes as eluent, to give white crystalline **18** (1.2 g, 95 %), m.p. 170 °C, $[\alpha]_D$ +30° (c 1.0, chloroform).

Anal. Calcd. for $C_{32}H_{36}ClNO_{18}$ (742.07): C, 51.79; H, 4.89; N, 1.89; Found: C, 51.74; H, 4.64; N, 1.82.

1,3,6-Tri-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α- and -β-D-glucopyranose (20α and 20β)

A. From lactal hexaacetate by the azidonitration route.

Lactal hexaacetate 9 (20 g, 35.7 mmol) was subjected to azidonitration by the reaction with sodium azide (3.25 g, 50 mmol) and cerium ammonium nitrate (45 g, 82 mmol) according to the procedure of Arnarp and Lönngren.[31] The crystalline mixture of azidonitrate **12α**, **12β**, and **19** obtained in 65 % yield showed ^{1}H-NMR anomeric proton signals at δ 6.28 (d, J 4.2 Hz), 5.58 (d, J 8.5 Hz), and 6.15 (d, J 3.5 Hz), respectively, in a ratio of approximately 4:8:1, and spots at R_f 0.60 , 0.52 and 0.47 as reported[31] (t.l.c. in 1:1 toluene-ethyl acetate).

A mixture of the above azidonitrates (10 g) was then hydrogenated over 10 % Pd/C and the crude product N-phthaloylated and O-acetylated as directed[31], to affored a syrupy mixture (5.4 g, 47 %) of the title compunds 20α and 20β after chromatographic purification on silica gel using 3:1 ethyl acetate-hexane as the eluent.

The authors[31] described a partial separation of the anomers by fractional crystallization but did not record yields of the pure products. In one chromatography of 1.0 g of the mixture, we were able to isolate 300 mg of nearly pure β-acetate 20β from early fractions and 100 mg of nearly pure α-acetate 20α from later fractions, while the bulk of material (0.5 g) emerged unsolved in intermediate fractions.

Compound 20β was crystallized from methanol, m.p. 285-287° C (dec.), $[\alpha]_D$ +35° (c 1.0, chloroform); lit.[31] m.p. 265-266° C, $[\alpha]_D$ +32° (c 1.5, chloroform). Ms (FAB): m/z 766.2 (M^- + 1), 706 (M^- + 1 - HOAc), 646 (M^- + 1 - 2 HOAc). ^{1}H-NMR: δ 7.90-7.70 (m, 4 H, Phth), 6.48 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 5.81 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8.2 Hz, H-3), 5.32 (dd, 1 H, $J_{3',4'}$ 3.3, $J_{4',5'}$ 0.7 Hz, H-4'), 5.11 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.4 Hz, H-2'), 4.95 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.5 Hz, H-3'), 4.50 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.48 (dd, 1 H, downfield part obscured by H-1 signal, H-6a with $J_{5,6a}$ 1.5, $J_{6a,6b}$ ~ 12 Hz, H-5'), 4.34 (dd, 1 H, $J_{1,2}$ 8.9, $J_{2,3}$ 10.4 Hz, H-2), 4.16 (dd, $J_{5,6b}$ 4, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.14-3.84 (2 ill-resolved m, 2

and 3 H, H6'a, 6'b, and H-4, 5, 5'), and 2.13-1.89 (7 s, 21 H, 7 OAc); ^{13}C-NMR: δ 100.85 (C-1'), 89.49 (C-1), 73.17 (C-4), 70.82 (C-5'), 70.63 (C-3), 70.50 (C-5, 3'), 68.82 (C-2'), 66.39 (C-4'), 61.78 (C-6), 60.80 (C-6'), 53.57 (C-2).

Anal. Calcd. for $C_{34}H_{39}NO_{19}$ (765.66): C, 53.33; H, 5.13; N, 1.83; Found: C, 53.04; H, 5.24; N, 1.81.

Compound **20α** was also crystallized from methanol, m.p. 229-230° C, $[\alpha]_D$ +62° (c 1, chloroform); lit[31] m.p.237-238°, $[\alpha]_D$ +67° (c 1.0, chloroform). Ms(FAB): m/z 766.2 (M^+ + 1), 706.2 (M^+ - HOAc).

^{1}H-NMR: δ 7.80-7.70 (m, 4 H, Phth), 6.38 (dd, 1 H, $J_{2,3}$ 11.5, $J_{3,4}$ 9.0 Hz, H-3), 6.20 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.34 (dd, 1 H, $J_{3',4'}$ 3.5 Hz, H-4'), 5.13 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10.5 Hz, H-2'), 4.95 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{2',3'}$ 10.5 Hz, H-3'), 4.65 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 11.5 Hz, H-2), 4.52 (dd, 1 H, $J_{5,6a}$ 1, $J_{6a,6b}$ 9 Hz, H-6a), 4.49 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 4.23-3.8 (poorly resolved m, 6 H, remaining ring protons), and 2.15-1.88 (7 s, 21 H, 7 OAc).

The 300 MHz ^{1}H- and 75.43-MHz ^{13}C-NMR data obtained for **20α** and **20β** accord well with the published[31] 100 (25) MHz NMR data, although either anomer was revealed to contain a trace of the other.

An anomeric mixture of **20α** and **20β** (5.0 g) was dissolved in acetic anhydride (50 mL) containing 70 % aqueous $HClO_4$ (0.35 mL). After 24 h at room temperature, the dark-brown solution was poured into ice-water, stirred for 1 h, and extracted with dichloromethane. The extract was washed with cold saturated $NaHCO_3$ and water, dried, and concentrated. The residue was crystallized from methanol to give pure **20β** (3.25 g, 65 %), m.p. 275-278° C (dec.), $[\alpha]_D$ +33° (c 1.0, chloroform).

B. From the benzylaminonitrile **3**

A solution of 2-benzylamino-2-deoxy-4-O-β-D-galactopyranosyl-D-gluconononitrile[16] **3** (3.2 g, 7.5 mmol) in 0.5 M HCl (35 mL) was hydrogenated at room temperature and atmospheric pressure in the presence of 5 % Pd-$BaSO_4$ (1.1 g). After 19 h, the absorption of H_2 had stopped (300 mL, 85 %), and the catalyst was filtered off and washed with water (5 mL). The filtrate was neutralized with $NaHCO_3$ (1.5 g, 18 mmol), and the resulting solution was added dropwise within 15 min to a stirred solution of phthalic anhydride (2.3 g, 16 mmol) in acetone (80 mL). More $NaHCO_3$ (1.3 g, 16 mmol) was then added portionwise within 30 min under stirring. The mixture was kept overnight at room temperature, and then concentrated. The dry residue was treated with acetic anhydride (20 mL) and pyridine (30 mL) at 100° C. After a few minutes, a vigorous reaction ensued, and the mixture was immediately cooled and kept

for 24 h at room temperature with stirring. Methanol was added to destroy the excess of anhydride. Evaporation and co-evaporation of added toluene gave a residue which was extracted with dichloromethane (50 mL). The extract was washed successively with 1 M HCl, water, saturated $NaHCO_3$, and water, dried, and concentrated. Column chromatography (1:2 ethyl acetate-toluene) of the residue gave three main fractions.

The first-eluted fraction gave pure β-acetate **20β**; (1.8 g, 32 %) which crystallized from methanol, m.p. 272° C (dec), $[\alpha]_D$ +33° (c 1.05, chloroform);

The second fraction contained a mixture of **20α** and **20β** (1.6 g, 28 %), from which 1.0 g (18 %) of crystalline **20β** could be obtained after anomerization as described in section A.

The third fraction gave pure α-acetate **20α** (0.1 g, 2 %) which crystallized from methanol, m.p. 239.5° C, $[\alpha]_D$ +69° (c 1.0, chloroform).

3,6-Di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranosyl bromide (21)

A mixture of heptaacetates **20** (anomeric mixture, 766 mg, 1 mmol) was dissolved in 5 mL of dry acid-free $CHCl_3$, cooled to 0° C, and 750 µl of trimethylsilyl bromide was added. The mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was chromatographed on silica gel with 3:1 hexanes-ethyl acetate as eluent to give the pure β-bromide **21** (700 mg, 90 %), m.p. 104-105° C, $[\alpha]_D$ +21° (c 1, chloroform); lit.[31] m.p. 109-110° C, $[\alpha]_D$ +24.4° (c 1, chloroform).

^{1}H-NMR: δ 7.90-7.70 (4 H, m, Phth); 6.35 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.70 (dd, 1 H, $J_{2,3}$10.3, $J_{3,4}$ 8 Hz, H-3), 5.30 (slightly broadened d, splitting 3.5 Hz, H'-4), 5.08 (dd, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 4.91 (dd, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.4 Hz, H-3'), 4.48 (m, 3 H, H-1', 2, 6a), 4.2-3.8 (ill-resolved m, 6 H, remaining ring protons), 2.14-1.88 (6 s, 18 H, 6 OAc).

^{13}C-NMR: δ 167-170 (C=O), 134.6, 131.2, 123.7 (Phth), 101.0(C-1'), 77.6 (C-1), 77.3 (C-4), 76.19 (C-5), 70.93 (C-3), 70.84 (C-5'), 70.59 (C-3'), 68.94 (C-2'), 66.49 (C-4'), 60.68 (C-6), 60.31 (C-6'), 58.39 (C-2), 21.1-20.4 (6 C, OAc).

3,6-Di-O-acetyl-2-deoxy-2-phthalimido-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-1-O-trichloroacetimidoyl-β-D-glucopyranose (24)

Method a[39a]:

A solution of 17β (1.81 g, 2.5 mmol), anhydrous K_2CO_3 (10 g, 72 mmol), and trichloroacetonitrile (2.5 mL, 25 mmol) in dry dichloromethane (100 mL) was stirred for 4 h at room temperature under N_2, and then filtered over Celite and evaporated. The residue was chromatographed in a short column of silica gel; 1:1 ethyl acetate-hexanes eluted the trichloroacetimidate **24** which crystallized from ether-hexanes (1.6 g, 78 %); m.p. 123° C, $[\alpha]_D$ +39° (c 0.5, chloroform); lit.[39a] m.p. 128° C, $[\alpha]_{578}$ +43° (c 0.5, chloroform).

^{1}H-NMR: δ 8.63 (s, 1 H, NH), 7.84-7.71 (m, 4 H, Phth), 6.58 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 5.83 (dd, 1 H, H-3), 5.30 (narrow d, H-4'), 5.10 (dd, small and large splitting, H-2'), 4.85 (dd, 1 H, small and large splitting, H-3'), 4.45 (m, 3 H, H-1', 2, 6a), 4.2-3.8 (m, 6 H, H-4, 5, 5', 6b, 6'a, 6'b), 2.1-1.9 (s, 18 H, 6 OAc).

^{13}C-NMR: δ 170.3-169.0 (6 Ac<u>C</u>O), 167.40 (phthaloyl <u>C</u>O), 160.32 (imidate C=NH), 134.34, 131.12, and 123.58 (arom. C), 100.82 (C-1'), 93.32 (C-1), 76.21 (C-4), 73.37 (C-5), 70.87, 70.60, 70.56 (C-3, 3', 5'), 68.92 (C-2'), 66.52 (C-4'), 61.75 (C-6), 60.76 (C-6'), 53.76 (C-2), 20.82-20.44 (<u>C</u>H$_3$CO).

Method b[39b]:

A solution of 17β (2.0 g, 2.76 mmol) in dried dichloromethane (15 mL) was

added to a suspension of sodium hydride (70 mg, 1.75 mmol) in dichloromethane (20 mL). Trichloroacetonitrile (5.5 mL, 54.8 mmol) was added and the solution was stirred for 20 min at room temperature. The mixture was placed at the top of a silicic acid column and eluted with ethyl acetate-hexanes (1:1) to give the desired compound 24 (1.92 g, 85 %), m.p. 126° C, $[\alpha]_D$ +40° (c 0.5, chloroform).

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (26)

(Modified from the original Lemieux method[33])

D-Glucosamine hydrochloride 25 (21.6 g, 100 mmol) was added to a sodium methoxide solution prepared from 2.3 g of sodium in 100 mL of methanol. The suspension was shaken for 10 min. in order to liberate the glucosamine base. No clear solution resulted due to the low solubility of free glucosamine and NaCl in methanol. Contrary to the original direction, the mixture was not filtered at this point, but treated directly with finely ground phthalic anhydride (7.4 g, 50 mmol) and shaken for another 10 min. Triethylamine (10.1 g, 100 mmol) was then added and the separated sodium chloride (5.8 g) was removed by filtration and washed with methanol (3 x 20 mL). The combined filtrates were treated with additional phthalic anhydride (8.1 g, 55 mmol). After being kept at 0 °C for 1 h, a solid deposit was collected by

filtration and dried. Evaporation of the filtrate gave a yellow solid which was suspended in diethyl ether (200 mL) and recovered by filtration. The solids were combined and treated with pyridine (200 mL) and acetic anhydride (100 mL) at room temperature for 16 h. The solution was poured into 1 L of ice-water and the aqueous mixture subsequently extracted with dichloromethane (3 x 100 mL). The combined extracts were washed successively with cold water, 3 % hydrochloric acid, saturated sodium bicarbonate solution and water. Solvent removal left a yellow foam which was dissolved in diethyl ether (500 mL) and treated with charcoal. Concentrated to a volume of 150 mL and kept at 0° C overnight, the solution deposited a colourless solid (39.1 g, 82 % yield). The ^{1}H-NMR spectrum of the product showed it to be a mixture of β- and α-anomers. Pure β-anomer **26** (15-20 g) can be obtained by crystallization from ethanol and recrystallization from ethyl acetate; m.p. 90-92°, $[\alpha]_D$ +74° (c 1, chloroform); lit.[33] $[\alpha]_D$ +65.5°, (c 1, chloroform).

Ms(CI): m/z 418 (M^+ - OAc), 358 (M^+ + 1 - 2 HOAc), 298 (M^+ + 1 - 3 HOAc).

^{1}H-NMR: δ 7.86-7.70 (m, 4 H, Phth), 6.49 (d, 1 H, $J_{1,2}$ 9 Hz, H-1), 5.85 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 9.1 Hz, H-3), 5.18 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 10.1 Hz, H-4), 4.44 (dd, 1 H, $J_{1,2}$ 9, $J_{2,3}$ 10.5 Hz, H-2), 4.34 (dd, 1 H, $J_{5,6a}$ 5.3, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.11 (dd, 1 H, $J_{5,6b}$ 6.1, $J_{6a,6b}$ 12.5 Hz, H-6b), 3.99 (ddd, 1 H, $J_{4,5}$ 10.1, $J_{5,6a}$ 5.3, $J_{5,6b}$

6.1 Hz, H-5), 2.09, 2.01, 1.97 and 1.84 (4s, 12 H, 4 OAc).

^{13}C-NMR: δ 89.64 (C-1), 72.46 (C-5), 70.33 (C-4), 68.08 (C-3), 61.34 (C-6), and 53.28 (C-2).

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (27)

A solution of the β-acetate 26 (9.54 g, 20 mmol) and acetic anhydride (5 mL) in a saturated hydrogen bromide solution of glacial acetic acid (30 mL) was kept at room temperature for 24 h. After dilution with chloroform (200 mL), the solution was washed with cold water (3 times), and with saturated sodium bicarbonate solution. Solvent removal after drying left a foamy solid which was crystallized from diethyl ether (7.77 g, 78 %), m.p. 125-126°, $[\alpha]_D$ +65.1° (c 1.0, chloroform); lit.[33] m.p. 122-123°, $[\alpha]_D$ +57.3° (c 1.0, chloroform). ^{1}H-NMR: δ 7.90-7.70 (m, 4 H, Phth), 6.38 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 5.74 (dd, 1 H, $J_{2,3}$ 9, $J_{3,4}$ 10 Hz, H-3); 5.26 (t, 10 Hz, H-4); 4.68 (t, 1 H, $J_{1,2}$ 10, $J_{2,3}$ 9 Hz, H-2), 4.24 (m, H-6 and H-6'); 3.94 (M, H-5), 2.12, 2.04, 1.86 (s, OAc).

Benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (28)

Method a[40]:

A solution of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide **27** (14.95 g, 30 mmol) in dry nitromethane (20 mL) was added to a cooled (-30 °C) solution of benzyl alcohol (3.24 g, 30 mmol), silver trifluoromethanesulfonate (8.0 g, 31 mmol), and 2, 4, 6-trimethylpyridine (3.75 g, 31 mmol) in dry nitromethane (30 mL) under nitrogen. The mixture was stirred at -30° C for 2 h and then diluted with dichloromethane (100 mL). The solid was removed by filtration and the filtrate evaporated in vacuo. The foamy residue was dissolved in dichloromethane (100 mL), and washed sequentially with ice-water, 5 % aqueous hydrogen chloride, and with aqueous sodium bicarbonate solution. Solvent removal after drying left a foam which was crystallized from diethyl ether (13.88 g, 88 %), m.p.106-108° C, raised by recrystallization from ethanol to 108-109 °C; $[\alpha]_D$ -8.3° (c 1, chloroform), lit.[40] $[\alpha]_D$ -11.3° (c 1.6, chloroform).

Ms (CI): m/z 526 (M^+ + 1), 418 (M^+ - $PhCH_2O$), 358 (M^+ - $PhCH_2O$ - AcOH), 298 (M^+ - $PhCH_2O$ - 2 AcOH).

^{1}H-NMR: δ 7.79-7.69 (m, 4H, Phth), 7.06 (s, 5 H, benzyl), 5.77 (dd, $J_{2,3}$ 10, $J_{3,4}$ 9 Hz, H-3), 5.35 (d, $J_{1,2}$ 8.4 Hz, H-1), 5.17 (t, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 4.83 and 4.51 (AB-q, 2 H, J 12 Hz, $PhCH_2$), 4.37 (dd, 1 H, H-2), 4.32 (dd, 1 H, $J_{5,6a}$ 2.5,

H-6a), 4.18 (dd, 1 H, $J_{5,6b}$ 2.5, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.85 (ddd, 1 H, H-5), 2.11, 2.00, and 1.83 (3 s, 9H, 3 OAc).

^{13}C-NMR: δ 171.0, 170.25, 169.6 (3 $CH_3\underline{CO}$), 167.8 (phthaloyl $\underline{CO}$), 136.6-123.6 (3 singals for phthaloyl and 4 for phenyl ring), 97.1 (C-1), 71.7 (CH_2Ph), 71.2 (C-5), 70.5 (C-4), 68.8 (C-3), 61.9 (C-6), 54.4 (C-2), 20.6, 20.4, and 20.2 (3 $\underline{CH_3}CO$).

Method b:

A mixture of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **26** (7.66 g, 10 mmol), benzyl alcohol (2 mL, 19 mmol) and powdered molecular sieves (4 A, 20 g) in dry dichloromethane (100 mL) was stirred under nitrogen for 1 h at room temperature, and then cooled to -20° C. A 0.5 M solution of trimethylsilyl triflate in dichloromethane (20 mL, 10 mmol) was rapidly added, and the mixture was stirred for 2 h at room temperature. The acid was neutralized with triethylamine, and the solution was filtered and washed with water. Evaporation left a syrup which crystallized from ethyl acetate-hexanes, to give **28** (7.40 g, 91 %), m.p. 106-108°C, $[\alpha]_D$ -9° (c 2.1, chloroform).

Allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (29)

A mixture of anhydrous ferric chloride (52 mmol, 8.42 g), dichloromethane (200 mL, dried over 4 A molecular sieves), and allyl alcohol (4.8 mL, 2 mol) was stirred for 5 min, and a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose 26 (16.5 g, 34.56 mmol) in dichloromethane (130 mL) was added. Stirring was continued for 1 h at room temperature, and the mixture was then poured into ice-cold aqueous sodium bicarbonate. After the addition of chloroform and shaking, the organic layer was separated and filtered through Celite, and the filtrate was washed with dilute aqueous sodium bicarbonate and then water, dried, and concentrated. Crystallization of the residue from ethanol-hexanes gave 29 (14.9 g, 89 %), m.p.105-107 °C, $[\alpha]_D$ +33° (c 1.0, chloroform); lit.[41] m.p.109-110 °C, $[\alpha]_D$ +37° (c 0.5, chloroform). Ms(CI): m/z 418 (M^+ - C_3H_5O), 358 (M^+ - C_3H_5O - AcOH), 298 (M^+ - C_3H_5O - 2 AcOH).

^{1}H-NMR: δ 7.9-7.7 (m, 4 H, aromatic protons), 5.77 (dd, 1 H, $J_{3,4}$ 9.1, $J_{2,3}$ 10.7 Hz, H-3), 5.68 (m, 1 H, -CH=), 5.38 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 5.15 (~t, 1 H, $J_{3,4} + J_{4,5}$ = 19.3 Hz, H-4), 5.06 (unsymm. t of narrow d, 2 H, J ~ 1.5, 10, and 17 Hz, =CH_2), 4.32 (dd, 1 H, $J_{1,2}$ 8.6, $J_{2,3}$ 10.7 Hz, H-2), 4.34 - 4.20 (complex m, 2 H, -O$\underline{CH_2}$), 4.15 (dd, 1 H, $J_{5,6a}$ 2.8, $J_{6a,6b}$ 12.7 Hz, H-6a), 4.02 (dd, 1 H, $J_{5,6b}$ 6.3, $J_{6a,6b}$ 12.7 Hz, H-6b), 3.84 (ddd, 1 H, H-5), 2.12, 2.02, and 1.86 (3s, 9 H,

3 OAc).

^{13}C-NMR: δ 170.7, 170.2, 169.5 ($CH_3\underline{CO}$), 168.0 (phthaloyl CO), 134.3, 133.2, 131.3 (arom. ring), 123.5 (CH=), 117.8 (=CH_2), 97.0 (C-1), 71.6 (OCH_2), 70.5 (C-5), 70.0 (C-4), 68.8 (C-3), 61.8 (C-6), 54.3 (C-2), 20.5, 20.3, 20.1 (3 $\underline{C}H_3CO$).

Benzyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (30)

Methanolic 0.3 M sodium methoxide (25 mL) was added to a stirred solution of benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **28** (1.0 g, 1.9 mmol) in dichloromethane-methanol (125 mL, 3:2) at room temperature. After 2 h the solution was neutralized using cation exchange resin, filtered, and concentrated. The residue was crystallized from 2-propanol to give **30** (0.61 g, 81 %), m.p. 169-170° C, $[\alpha]_D$ -50.7° (c 0.5, methanol); lit.[40] m.p. 173-175° C, $[\alpha]_D$ -34.2° (c 0.8, acetone).

Ms (CI): m/z 400 (M^+ + 1), 382 (M^+ + 1 - H_2O), 292 (M^+ - $PhCH_2O$).

^{1}H-NMR(CD_3OD): δ 7.814 (s, 4 H, Phth), 7.04 (s, 5 H, benzyl), 5.15 (d, $J_{1,2}$ 8.5 Hz, H-1), 4.80 and 4.54 (AB-q, 2 H, $PhCH_2$), 4.26 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 8 Hz, H-3), 4.02 (dd, 1 H, $J_{1,2}$ 8.4, $J_{2,3}$ 10.8 Hz, H-2), 3.97 (d with slightly broadened lines, 1 H, $J_{6a,6b}$ 12 Hz H-6a), 3.75 (dd, 1 H, $J_{5,6b}$ ~5, $J_{6a,6b}$ ~12 Hz, H-6b), 3.35 (center of m, 2 H, H-4, 5).

^{13}C-NMR (CD_3OD): δ 98.81 (C-1), 78.37 (C-5), 72.58 (C-3), 72.43 (C-4), 71.92 (CH_2Ph), 62.80 (C-6), and 58.64 (C-2).

Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (31)

Methanolic 0.3 M sodium methoxide (25 mL) was added to a stirred solution of allyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **29** (10.0 g, 19.34 mmol) in dichloromethane-methanol (125 mL, 3:2) at room temperature. After 2 h the solution was neutralized using cation exchange resin, filtered, and concentrated. The residue was crystallized from ethyl acetate-hexanes to give **31** (6.15 g, 91 %), m.p. 186-188 °C, $[\alpha]_D$ -46° (c 0.5, methanol); lit.[42] m.p.190 °C, $[\alpha]_D$ +19° (c 0.8, chloroform). The literature value may be erroneus. In fact, **31** was found to be insufficiently soluble in chloroform for polarimetry or NMR spectroscopy in that solvent.

Ms(CI): m/z 292 (M^+ - C_3H_5O).

^{1}H-NMR [$(CD_3)_2SO$]: δ 5.41 (d, 1 H, $J_{3,OH}$ 4.7Hz, D_2O-exchangeable, HO-3). 5.18 (d,1 H, $J_{4,OH}$ 5.1 Hz, D_2O-exchangeable, HO-4), 4.63 (t, 1 H, J 6.5 Hz, D_2O-exchangeable, HO-6).

^{13}C-NMR [$(CD_3)_2SO$]: δ 126.9 (CH=), 116.5 (=CH_2), 99.8 (C-1), 76.4 (OCH_2), 74.6 (C-5), 69.7 (C-4), 69.0 (C-3), 60.9 (C-6), 56.2 (C-2).

Benzyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (32)

A solution of benzyl 2-deoxy-2-phthalimido-β-D-glucopyranoside **30** (6 g, 15 mmol), α,α-dimethoxytoluene (3.0 g, 20 mmol), and p-TsOH (40 mg) in CH_3CN (70 mL) was stirred for 8 h at 20-25° C, made neutral with Et_3N (0.1 mL), and evaporated in vacuo, to give crude solid **32** (6.2 g, 85 %). A small portion of this crude solid was chromatographed on SiO_2 with 3:1 toluene-EtOAc, to give an analytical sample of **32**, m.p. 187-188° C, $[\alpha]_D$ -81.6° (c 0.25, chloroform); lit.[40] m.p. 183-184° C, $[\alpha]_D$ -75.4° (c 1.2, chloroform).

Ms (CI): m/z 488 (M^+ + 1), 380 (M^+ - OCH_2Ph), 362 (M^+ - OCH_2Ph - H_2O), 274 (M^+ - OCH_2Ph - PhCHO).

^{1}H-NMR: δ 7.8-7.6 (m, 4 H, Phth), 7.55-7.25 (m, 5 H, PhCH-), 7.02 (s, 5 H, benzyl), 5.56 (s, 1 H, CH-Ph), 5.25 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.84 and 4.50 (AB-q, 2 H, J 12 Hz, CH_2Ph), 4.61 (ddd, 1 H, $J_{3,OH}$ 3.5, $J_{3,4}$ 8.7, $J_{2,3}$ 10.5 Hz, H-3), 4.40 (dd, 1 H, $J_{5,6eq}$ 4, $J_{6ax,6eq}$ 10 Hz, H-6eq), 4.27 (dd, 1 H, $J_{1,2}$ 8.5, $J_{2,3}$ 10.5 Hz, H-2), 3.85 (distorted sextet, 1 H, H-5), 3.68-3.54 (m, 2 H, H-4, 6ax), 2.43 (d, 1 H, $J_{3,OH}$ 3.5 Hz, OH-3).

^{13}C-NMR: δ 101.9 (PhCH-), 97.8 (C-1), 82.1 (C-4), 71.1 (CH_2Ph), 68.6 (C-6), 68.4 (C-5), 65.9 (C-3), 56.4 (C-2).

Allyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (33)

Fused zinc chloride (5 g) was added quickly to benzaldehyde (25 mL), and stirred with it for 30 min under exclusion of moisture. Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside **31** (5 g, 14.12 mmol) was then added and stirring was continued for 3 h, after which the syrupy mass was poured into ice-water (50 mL) and hexane (50 mL). After vigorous shaking, the product was collected and crystallized from ethanol-hexanes to give **33** (5.06 g, 82 %), m.p. 176-178°C, $[\alpha]_D$ -29.7° (c 1, chloroform); lit.[42] m.p. 150 °C, $[\alpha]_D$ -39° (c 1, chloroform).

Ms (CI): m/z 438 ($M^+ + 1$), 380 ($M^+ - OCH_2\text{-}CH{=}CH_2$), 362 ($M^+ - OCH_2\text{-}CH{=}CH_2 - H_2O$), 274 ($M^+ - OCH_2\text{-}CH{=}CH_2 - PhCHO$).

^{1}H-NMR : δ 7.87-7.34 (m, 9 H, Phth and Ph), 5.77-5.54 (m, 1 H, CH=), 5.57 (s, 1 H, PhCH-), 5.28 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 5.15-4.98 (m, 2 H, $=CH_2$), 4.63 (ddd, 1 H, $J_{3,OH}$ 2.5, $J_{2,3}$ 9.1, $J_{3,4}$ 10.4 Hz, H-3), 4.36 (dd, 1 H, $J_{5,6eq}$ 4, $J_{6eq,6ax}$ 10.5 Hz, H-6eq), 4.30-4.20 (m, 2 H, consisting of dd for H-2, and dd for allyl H-1a with J_{vic} 5, J_{gem} 13 Hz), 4.00 (dd, 1 H, allyl H-1b with J_{vic} 6, J_{gem} 13 Hz), 3.82 (m, 1 H, H-5), 3.68-3.55 (m, 2H, H-4, 6ax), 2.38 (d, 1 H, D_2O-exchangeable, $J_{3,OH}$ 2.5 Hz,OH-3).

^{13}C-NMR: δ 123.5 (CH=), 117.7 ($=CH_2$), 101.8 (PhCH-), 97.9 (C-1), 82.1 (C-4), 70.0 (OCH_2), 68.5 (C-5), 68.4 (C-6), 66.0 (C-3), 56.5 (C-2).

2,3,4,6-Tetra-O-acetyl-α-D-galactopyranosyl bromide (37)

Compound **37** was prepared by the modified procedure described by C. S. Hudson *et al.*[67] from pentaacetyl-β-D-galactopyranose and hydrogen bromide in glacial acetic acid. After reaction for 10 min at room temperature (instead of 2 h at 0-5°C as originally stated), the reaction mixture was diluted with dichloromethane, washed successively with cold water, saturated $NaHCO_3$ solution and water, and dried over sodium sulfate. Crystalline of **37** was obtained upon concentration of the dichloromethane solution in near quantitative yield; m.p. 82°C, $[\alpha]_D$ +226° (c 1.0, chloroform); lit.[67] m.p. 85 °C, $[\alpha]_D$ +217° (c 1.0, chloroform).

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (38)

Method a:

A solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **26** (477 mg, 1 mmol) in N,N-dimethylformamide (10 mL) was treated with hydrazine acetate (0.1 g, 1.1 mmol) for 4 h at room temperature, and then diluted with dichloromethane (40 mL), washed with 10 % aqueous NaCl, and concentrated. The residue was crystallized from ethanol-hexane to give **38** (365 mg, 84 %), m.p.180-182° C, $[\alpha]_D$+73.5° (c 0.5, chloroform); lit.[42] mp. 178° C, $[\alpha]_D$ +75° (c 0.5, chloroform).

^{1}H-NMR: δ 7.84-7.71 (m, 4 H, Phth), 5.84 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9.2 Hz, H-3), 5.62 (~t, 1 H, $J_{1,2}$ 8.5, $J_{1,OH}$ 7.3 Hz, H-1), 5.16 (t, 1 H, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4), 4.28 (dd, 1 H, H-6a), 4.25 (dd, 1 H, $J_{1,2}$ 8.6, $J_{2,3}$ 10.6 Hz, H-2), 4.18 (dd, 1 H, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.92 (ddd, 1 H, $J_{4,5}$ 10.2, $J_{5,6a}$ 4.7, $J_{5,6b}$ 2.3 Hz, H-5), 3.30 (d, 1 H, $J_{1,OH}$ 7.25 Hz, 1-OH), 2.10, 2.02 and 1.85 (3s, 9 H, 3 OAc).

^{13}C-NMR: δ 92.6 (C-1), 71.9 (C-3), 70.3 (C-5), 68.7 (C-4), 61.9 (C-6), 55.9 (C-2).

Method b:

A mixture of benzyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **28** (526 mg, 1mmol) and 5 % palladium on carbon (0.5 g) in a 1:1 mixture of ethanol and ethyl acetate (15 mL) was hydrogenated at 50 psi at room temperature for 16 h. The solid was removed by filtration and the filtrate evaporated. The resulting foam was applied to a silica gel column and eluted with a mixture ethyl acetate-hexanes (2:1) to give **38** (348 mg, 80 %), m.p.178-180°, $[\alpha]_D$+70.5° (c 0.5, $CHCl_3$).

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose 1-trichloroacetimidate (39)

Method a[39a]:

A solution of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **38** (1.09 g, 2.5 mmol), anhydrous K_2CO_3 (10 g, 72 mmol), and trichloroacetonitrile (2.5 mL, 25 mmol) in dry dichloromethane (100 mL) was stirred for 4 h at room temperature under N_2, and then filtered over Celite and evaporated. The residue was chromatographed in a short column of silica gel; 1:1 ethyl acetate-hexanes eluted **39** which was crystallized from ether-hexanes (1.24 g, 84 %); m.p. 146° C, $[\alpha]_D$ +76° (c 1.0, chloroform), lit.[43a] m.p. 148 °C, $[\alpha]_D$ +70° (c 1.0, chloroform).

^{1}H-NMR: δ 8.67 (s, 1 H, NH); 7.9-7.7 (2m, 4 H, arom.), 6.63 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 5.92 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 5.29 (dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 4.63 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2), 4.40 (dd, 1 H, $J_{5,6a}$ 4.4, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.20 (dd, 1 H, $J_{5,6b}$ 1.8 Hz, H-6b), 4.08 (ddd, 1 H, H-5), 2.13, 2.06, 1.90 (3 s, 9 H, 3 OAc).

Method b[39b]:

To a stirred solution of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose **38** (4.5 g, 10.34 mmol) in dichloromethane (80 mL) containing

molecular sieves 4 A (1.5 g) was added trichloroacetonitrile (6 mL, 59.82 mmol) followed by sodium hydride (200 mg, 8.7 mmol) which was added portionwise. After 10 min the mixture was filtered through Celite and concentrated under diminished pressure. Column chromatography (20:1 dichloromethane-acetone) of the residue and recrystallization from ether gave **39** (4.4 g, 73 %), m.p. 142° C, $[\alpha]_D$ +70° (c 1, chloroform).

1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose (49)

Compound **49** was prepared as described by Schmidt[49] and by Einhorn and Luche[50a] (ultrasonic irradiation). To a suspension of glucose (10 g) in acetone (300 mL) was added conc. sulfuric acid (1 mL), and the mixture was sonicated for 1 h to give **49** (8.7 g, 60 %), as a white solid, m.p. 105-109 °C, $[\alpha]_D$ -13.5° (c 5, chloroform); lit.[49] m.p. 109 -113 °C, $[\alpha]_D$ -18° (c 2, H_2O).

Ms (CI): m/z 261 (M^+ + 1).

^{13}C-NMR: δ 111.8, 109.6 ($2O_2C(CMe_2)$, 105.2 (C-1), 85.0 (C-2), 81.0 (C-4), 75.0 (C-3), 73.3 (C-5), 67.5 (C-6), 26.6, 26.5, 25.9, and 24.9 (4 CH_3), in good agreement with reported[50b] data.

Methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (54)

Compound **54** was prepared by following the procedure of Gibbs *et al.*[59] 2-Benzamido-2-deoxy-D-glucose in dry methanol was heated under reflux with a small amount of Amberlite IR 120 (H^+ form) as a catalyst for 20 h, the resin was removed by filtration, and the crude methyl glucoside which precipitated from the cooled solution was reacted with benzaldehyde and anhydrous zinc chloride to provide compound **54**, m.p. 240-242 °C, $[\alpha]_D$ + 60° (c 0.6, chloroform); lit.[59] m.p. 247-248 °C, $[\alpha]_D$ + 57° (c 2, chloroform).

Ms (CI): m/z 386 (M^++1), 354 (M^+- OCH_3).

^{1}H-NMR: δ 7.82-7.73 and 7.5-7.3 (m, 10 H, 2 phenyl groups), 6.50 (d, 1 H, $J_{2,NH}$ 8.7 Hz, NH), 5.53 (s, 1 H, PhCH=), 4.48 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.43 (octet, 1 H, $J_{1,2}$ 3.7, $J_{NH,2}$ 8.7, $J_{2,3}$ 9.9 Hz, H-2), 4.00 (td, 1 H, $J_{3,OH}$ 3, $J_{3,4}$ 9, $J_{2,3}$ 9.9 Hz, H-3), 4.29 and 3.85-3.50 (complex m, 1 and 3 H, for H-4, 5, 6ax, 6eq; not first order), 3.41 (s, 1 H, OCH_3), 3.15 (d, 1 H, $J_{3,OH}$ 3 Hz).

^{13}C-NMR: δ 101.9 (PhCH), 98.9 (C-1), 82.0 (C-4), 70.8 (C-3), 68.7 (C-6), 62.2 (C-5), 55.3 (OCH_3), 54.3 (C-2).

Methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (55)

A solution of methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** (3.5 g, 9 mmol) in 1:1 (v/v) benzene-nitromethane (500 mL) was boiled until 100 mL of the solvent had distilled. The temperature of the solution was adjusted to 60° C; mercuric cyanide (2.0 g, 7.9 mmol) was added and a solution of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide **37** (3.7 g, 9 mmol) in 1:1 (v/v) benzene-nitromethane (40 mL) was added dropwise with stirring under nitrogen over a period of 4 h. Stirring at 60° was continued for a further 24 h. Additional mercuric cyanide (1.0 g, 4 mmol) and bromide **37** (1.8 g, 4.4 mmol) in 1:1 (v/v) benzene-nitromethane (20 mL) were added, and the solution was stirred for a further 24 h. The reaction mixture was cooled in ice-water and washed with ice-cold saturated aqueous sodium chloride (100 mL), and finally dried (sodium sulfate). Evaporation of the solvents yielded a foam that crystallized on trituration with dry ether. Recrystallization from ethyl acetate-hexanes gave pure **55** as a white solid (5.2 g, 80 %), m.p. 154° C, $[\alpha]_D$ +73.5° (c 1, chloroform).

Ms (FAB): m/z 716.3 ($M^+ + 1$), 685.2 ($M^+ - OCH_3$), 656.2 ($M^+ - OAc$).

^{1}H-NMR: δ 7.8-7.3 (m, 10 H, arom.), 6.26 (d, $J_{2,NH}$ 9.5 Hz, NH), 5.58 (s,

PhCH-), 5.27 (dd, $J_{3',4'}$ 3.4, $J_{4',5'}$ < 1 Hz, H-4'), 5.18 (dd, $J_{1',2'}$ 7.8, $J_{2',3'}$ 10.4 Hz, H-2'), 4.85 (dd, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.4 Hz, H-3'), 4.79 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.66 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 4.56 (td, 1 H, $J_{1,2}$ 3.9, $J_{2,3} \approx J_{2,NH}$ ~9.5-10 Hz, H-2), 4.27 (complex m, 1 H, not first order, H-6eq), 4.05 (t, $J_{2,3} \approx J_{3,4}$ ~ 9.5 Hz, H-3, superposed on m for 6'a), 3.94 (dd, $J_{5',6'b}$ 5.6, $J_{6'a,6'b}$ 11 Hz, H-6'b), 3.85-3.6 (unresolved m, 4 H, H-4, 5, 5', 6ax), 3.35 (s, 1 H, OCH_3), 2.07, 1.95, 1.89, 1.70 (4 s, 3 H each, 4 OAc).

^{13}C-NMR: δ 101.17 (C-1'), 100.84 (PhCH-), 98.97 (C-1), 79.83 (C-4), 76.83 (C-3), 71.09 (C-3'), 70.17 (C-5'), 68.95 (C-2'), 68.71 (C-6), 66.82 (C-4'), 62.83 (C-5), 60.85 (C-6'), 55.25 (OCH_3), 52.95 (C-2).

Anal. Calcd. for $C_{35}H_{41}NO_{15}$ (715.69): C, 58.73; H, 5.77; N, 1.96. Found: C, 58.54; H, 5.96; N, 1.94.

2-Phenyl-[1,2-dideoxy-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyrano][2', 1': 4, 5]-2-oxazolinium acetate (56)

To a cold solution of methyl glycoside **55** (2.15 g, 3 mmol) in acetic anhydride (20 mL) and acetic acid (4 mL) was added 1:10 (v/v) conc.H_2SO_4-acetic acid (1.5 mL). The mixture was kept overnight at 0-5° C, and then diluted with dichloromethane and poured onto ice with stirring. The dichloromethane

layer was washed successively with water, saturated aqueous sodium bicarbonate, and water, and then evaporated to give a residue which after chromatography on silica gel, with 1:1 hexanes-ethyl acetate as eluent, afforded **56** as a white foam (2.04 g, 92 %), $[\alpha]_D$ +34° (c 2.6, chloroform).

Ms (FAB): m/z 680.4 (M^+ - OAc), 620.3 (M^+ - OAc - HOAc).

^{1}H-NMR (COSY): δ 7.98-7.95 and 7.66-7.52 (m, 5 H, arom.), 6.17 (dd, 1 H, $J_{1,2}$ 8.1, $J_{1,3}$ 1.5 Hz, H-1), 5.39 (dd, 1 H, $J_{3',4'}$ 3.5, $J_{4',5'}$ 0.9 Hz, H-4'), 5.24 (dt, 1 H, $J_{2,4} = J_{3,4}$ = 1.5, $J_{4,5}$ 8.4 Hz, H-4), 5.21 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.4 Hz, H-2'), 5.04 (dd, 1 H, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.5 Hz, H-3'), 4.80 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.29 (m, 2 H, H-2, 3), 4.19 (dd, 1 H, $J_{5',6'a}$ 6.4, $J_{6'a,6'b}$ 11 Hz, H-6'a), 4.15-4.05 (m, 3 H, H-6a, 6b, 6'b), 4.00 (td, 1 H, $J_{4',5'}$ ~ 1, $J_{5',6'a} = J_{5',6'b}$= 6.4 Hz, H-5'), 3.62 (septet, 1 H, J 4, 5, and 8.5 Hz, H-5), 2.14, 2.07, 2.05, 2.00, 1.99, and 1.97 (6 s, 3 H each, 6 OAc).

The coupling constants suggest that the glucopyranose ring exists largely in a twist boat conformation in which $\Phi_{1,2}$ is very small (large $J_{1,2}$) and $\Phi_{3,4}$ is < 120° rather than 180° (small $J_{3,4}$), and in which H-1 and H-3 as well as H-2 and H-4 are subject to long-range coupling (close to W arrangements).

^{13}C-NMR: δ 170.65, 170.37, 170.25, 170.05, 169.80, 169.3 (6 CH_3 CO), 165.20 (C-2 of oxazoline ring), 132.2-126.4 region (multiple signals, arom. C),101.93 (C-1'), 99.65 (C-1), 77.84 (C-3), 71.13 (C-3'), 70.87 (C-5'), 68.94 (C-2'), 68.18

(C-4'), 67.54 (C-5), 66.06 (C-4'), 65.51 (C-2), 63.65 (C-6), 61.40 (C-6'), 20.92-20.64 (multiple signals, $\underline{CH_3}CO$).

Anal. Calcd. for $C_{33}H_{41}NO_{18}$ (739.67): C, 53.58; H, 5.59; N, 1.89. Found: C, 53.69; H, 5.61; N, 2.07.

Methyl 2-benzamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-glucopyranoside (57)

A solution of the benzylidene compound **55** (716 mg, 1 mmol) in 60 % acetic acid (10 mL) was stirred at 70° C for 30 min and diluted with water (10 mL). The solution was cooled and evaporated; the residue, after being dried by repeated azeotropic distillation with toluene, left a foam (533 mg, 85 %); $[\alpha]_D$ +38.4° (c 1.2, chloroform); Ms (FAB): m/z 628.2 (M^+ + 1).

^{1}H-NMR: δ 7.75 - 7.45 (m, 5 H, PhCO), 6.29 (d, 1 H, $J_{2,NH}$ 9.7 Hz, 2-NH), 5.31 (d, slightly broadened, 1 H, $J_{3',4'}$ ~3.2, $J_{4',5'}$ < 1 Hz, H-4'), 5.175 (dd, 1 H, $J_{1',2'}$ 7.7, $J_{2',3'}$ 10 Hz, H-2'), 4.88 (dd, 1 H, $J_{2',3'}$ 10.3, $J_{3'4'}$ 3.1 Hz, H-3'), 4.70 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.57 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.46 (td, 1 H, $J_{1,2}$ 3.5, $J_{2,3} \approx J_{2,NH} \approx$ 9.7 Hz, H-2), 4.15 -3.60 (unresolved m, 10 H, H-3, 4, 5, 6a, 6b, 5', 6'a, 6'b, 4-OH, 6-OH), 3.35 (s, 3 H, OCH_3), 2.10, 2.02 and 1.98 (3 s, 12 H, 4 OAc).

^{13}C-NMR: δ 170.5, 170.14, 170.09, 169.4 (4 $CH_3\underline{CO}$), 166.9 (Ph$\underline{CO}$), 134.0, 132.0, 128.8 and 127.1 (Ph ring), 101.8 (C-1'), 98.4 (C-1), 83.8 (C-3), 71.3 (C-

4), 71.0 (C-3'), 70.8 (C-5'), 69.7 (C-5), 68.3 (C-2'), 66.8 (C-4'), 62.83 (C-6), 61.4 (C-6'), 55.2 (OCH_3), 51.8 (C-2), 20.6, 20.6, 20.5 and 20.0 (4 $\underline{CH_3}CO$).

Anal. Calcd. for $C_{28}H_{37}NO_{15}$(627.59): C, 53.58; H, 5.94; N, 2.23. Found: C, 53.41; H, 6.03; N, 2.15.

Methyl 2-benzamido-2-deoxy-3-O-(-β-D-galactopyranosyl)-α-D-glucopyranoside (58)

A solution of the compound **57** (627 mg, 1 mmol) in chloroform (3 mL) was treated with methanolic hydrogen chloride [prepared by addition at 0° C of acetyl chloride (0.2 mL) to dry methanol (5 mL)] at room temperature. The reaction was monitored by t.l.c. (dichloromethane-methanol 9:1). When no starting **57** (R_f 0.9) was left, the mixture was treated with an excess of aqueous potassium hydrogencarbonate, diluted with chloroform (30 mL), washed with water, dried, and concentrated. The product **58** was isolated from the residue by column chromatography on silica gel with 8 % methanol in dichloromethane as eluent, and was obtained as a white amorphous solid (380 mg, 80 %), $[\alpha]_D$ +70° ($\underline{c}$ 0.6, methanol).

Anal. Calcd. for $C_{20}H_{29}NO_{11}\cdot H_2O$ (477.46): C, 50.31; H, 6.54; N, 2.93. Found: C, 50.33; H, 6.68; N, 3.09.

1-O-Acetyl-2,4,6-tri-O-benzoyl-3-O-benzyl-α,β-D-galactopyranose (59)

A. Anomeric mixture and α-anomer

To a cold solution of methyl 2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranoside 88 (596 mg, 1 mmol) in acetic anhydride (10 mL) and acetic acid (1 mL) was added 1:10 (v/v) conc. H_2SO_4-acetic acid (1 mL). The mixture was kept overnight at 0-5 °C, and then diluted with dichloromethane and poured onto ice with stirring. The dichloromethane extract was washed successively with water, saturated $NaHCO_3$, and water, and evaporated to give a residue (650 mg). Chromatography on silica gel with 3:1 hexanes-ethyl acetate gave an anomeric mixture of 1-O-acetyl-2, 4, 6-tri-O-benzoyl-3-O-benzyl-α, β-D-galactopyranose 59 in a 3:1 anomer ratio (574 mg, 92 %). A small portion of the mixture was subjected to preparative t.l.c. affording 59α, $[\alpha]_D$ +123° (c 1.5, chloroform), as a microanalytically pure syrup which, however, still contained a small proportion of 59β (NMR).

Ms (CI): m/z 565 (M^+ - OAc, base peak).

^{1}H-NMR: δ 6.61 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.05 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 5.65 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.3 Hz, H-2), 4.78 and 4.58 (AB-q, 2 H, J 12.5 Hz, CH_2Ph), 4.6-4.3 (m, 3 H, not first order; H-5, 6, 6'), 4.19 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.3 Hz, H-3).

^{13}C-NMR: δ 168.8 (AcCO), 166-165 (3 lines, PhCO), 137-127 (multiple lines,

arom. C), 90.02 (C-1), 72.86 (C-3), 71.31 (CH_2Ph), 69.32 and 68.91 (C-2,5), 67.22 (C-4), 62.43 (C-6), 20.9 ($\underline{CH_3}CO$).

Anal. Calcd. for $C_{36}H_{32}O_{10}$ (624.6): C, 69.22; H, 5.16. Found: C, 69.04, H, 5.12.

B. Pure β-anomer

A mixture of 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl chloride **89** (1 g, 1.7 mmol), silver acetate (0.35 g) and Drierite in acetonitrile (5 mL) was stirred at room temperature until t.l.c. (2:1 hexanes-ethyl acetate) showed that the reaction was complete (24 h). The mixture was diluted with dichloromethane, filtered and concentrated, and the residue was chromatographed on silica gel and eluted with 3:1 hexanes-ethyl acetate to give spectroscopically pure 1-O-acetyl-2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranose **59β** (0.87 g, 77.8 %, amorphous solid), $[\alpha]_D$ +156° (c 1, chloroform); lit.[37] $[\alpha]_D$ +162.5° (c 1.2, chloroform).

^{1}H-NMR: δ 5.99 (broadened d, 1 H, $J_{3,4}$ ~ 3, $J_{4,5}$ <1 Hz, H-4), 5.91 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.71 (t, 1 H, $J_{1,2} + J_{2,3}$ = 18.2 Hz, H-2), 4.74 and 4.53 (AB-q, 2 H, J 12.8 Hz, CH_2Ph), 4.60 (dd, 1 H, $J_{5,6}$ 5.9, $J_{6,6'}$ 11.4 Hz, H-6), 4.45 (dd, 1 H, $J_{5,6'}$ 5.7, $J_{6,6'}$ 11.4 Hz, H-6'), 4.27 (t, 1 H, $J_{5,6} + J_{5,6'}$ = 12.4 Hz, H-5), 3.91 (dd, 1 H, $J_{3,4}$ 3.2, $J_{2,3}$ 9.8 Hz, H-3), 2.05 (s, 3 H, OAc).

^{13}C-NMR.: δ 169.1 ($\underline{CO}CH_3$), 166.0, 165.7, 165.1 (3 $\underline{CO}$Ph), 92.4 (C-1), 76.2

(C-3), 72.5 (C-2), 71.1 (CH_2, benzylic), 70.0 (C-5), 66.4 (C-4), 62.5 (C-6), 21.0 ($\underline{CH_3}CO$).

2-Phenyl-[1,2-dideoxy-4,6-O-benzylidene-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyrano][2', 1': 4, 5]-2-oxazoline (60)

A mixture of 1-O-acetyl-2, 4, 6-tri-O-benzoyl-3-O-benzyl-α, β-D-galactopyranose **59** (687 mg, 1.1 mmol), methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** (385 mg, 1 mmol) and powdered molecular sieves (4 A, 1 g) in dry dichloromethane (20 mL) was stirred under nitrogen for 1 h at room temperature, and then cooled to -20° C. A 0.5 M solution of trimethylsilyl triflate in dichloromethane (2 mL, 1 mmol) was rapidly added, and then the mixture was stirred for 2 h at room temperature. The acid was neutralized with triethylamine, and the solution filtered and washed with water. Evaporation gave a syrup which crystallized from ethanol to give **60** (689 mg, 75 %), m.p. 183.8-184.2 °C, $[\alpha]_D$ +89° ($\underline{c}$ 1, chloroform).

Ms(FAB): $\underline{m/z}$ 918.3 (M^+ + 1).

^{1}H-NMR(300 MHz, COSY): δ 8.1-7.1 (m, 30 H, arom.), 6.39 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1), 6.07 (s, 1 H, PhCH), 5.90 (slightly broadened d, 1 H, $\underline{W_H}$ ~ 5 Hz, $J_{3',4'}$ ~3.5, $J_{4',5'}$ < 1 Hz, H-4'), 5.57 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 10 Hz, H-2'), 4.79 (m, 2 H, consisting of t with $J_{1,2} = J_{2,3}$ = 5.1 Hz for H-2, and the X part of an ABX

system for H-6a), 4.70 and 4.50 (2 d of AB-q, 2 H, J_{gem} 12.7 Hz, $PhCH_2$), 4.63 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1'), 4.54 (dd, 1 H, $J_{5',6'a}$ 6.8, $J_{6'a,6'b}$ 11.3 Hz, H-6'a), 4.42-4.31 (m, 3 H, consisting of dd at δ 4.39 with $J_{5',6'b}$ 5.6 and $J_{6'a,6'b}$ 11.2 Hz for H-6'b, superposed by AB part of ABX system for H-5, 6b), 4.07 (~ t, $J_{5',6'a} + J_{5',6'b}$ = 12.7 Hz, H-5'), 3.89-3.83 (m, 2 H, AB part of ABX system, H-3, 4), 3.80 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.4 Hz, H-3').

^{13}C-NMR: δ 166.2, 166.0, 165.1, 165.0, (3 PhCO and C-2 of oxazoline ring), 138-126 region (multiple signals, arom. C), 106.68 (C-1'), 100.78 (PhCH-), 95.24 (C-1), 77.96 (C-4), 77.50 (C-3), 76.00 (C-3'), 73.78 (C-2'), 72.36 and 71.52 (C-2,5), 70.99 ($PhCH_2$), 70.89 (C-5'), 69.43 (C-6), 66.49 (C-4'), 62.49 (C-6').

Anal. Calcd. for $C_{54}H_{47}NO_{13}$ (917.93): C, 70.65; H, 5.16; N, 1.53. Found: C, 70.76; H, 5.33; N, 1.57.

2,4,6-Tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide (61)

1-O-Acetyl-2,4,6-tri-O-benzoyl-3-O-benzyl-α,β-D-galactopyranose 59 (625 mg, 1 mmol) was dissolved in dry acid-free chloroform (5 mL), cooled to 0° C, and 750 µl of trimethylsilyl bromide was added. The mixture was stirred overnight at room temperature. After evaporation, the residue was chromatographed on silica gel with 3:1 hexanes-ethyl acetate as eluent to give

the pure 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61** (580 mg, 90 %) as a white foam, $[\alpha]_D$ +171.4° (c 1, chloroform).

^{1}H-NMR: δ 6.90 (d, 1 H, $J_{1,2}$ 3.85 Hz, H-1), 6.04 (broadened d, 1 H, $J_{3,4}$ 3 Hz, H-4), 5.41 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.1 Hz, H-2), 4.78 and 4.59 (dd, 2 H, AB-q, J 12.7 Hz, CH_2Ph), 4.71 (broad t, 1 H, $J_{5,6} + J_{5,6'} = 12$ Hz, H-5), 4.46 (dd, 1 H, $J_{5,6}$ 6.5, $J_{6,6'}$ 12 Hz, H-6), 4.45 (dd, 1 H, $J_{5,6'}$ 5.5 Hz, H-6'), 4.34 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.3 Hz, H-3).

^{13}C-NMR: δ 89.5 (C-1), 74.0 (C-3), 72.2 (C-2), 71.8 ($PhCH_2$), 70.0 (C-5), 67.3 (C-4), 62.2 (C-6).

Anal. Calcd. for $C_{34}H_{29}O_8Br \cdot 1.5H_2O$ (672.51): C, 60.72; H, 4.80; Br, 11.88. Found: C, 60.64; H, 4.82; Br, 11.66.

Methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (62)

A solution of 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61** (710 mg, 1.1 mmol) in dry dichloromethane (10 mL) was added dropwise, with rigorous exclusion of moisture and light, to a stirred solution of methyl 2-benzamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside **54** (385 mg, 1.0 mmol), silver triflate (282.7 mg, 1.1 mmol) and 1,1,3,3-tetramethylurea (127.8 mg, 1.1 mmol) in dry dichloromethane (30 mL). The mixture was stirred

at room temperature overnight, and then diluted with dichloromethane and filtered through a Celite pad. Following extraction of the filtrate with aqueous sodium bicarbonate and water, the product obtained after concentration of the organic phase was a syrup. It was purified by elution from a column of silica gel with ethyl acetate-hexanes (1:1) to give **62** (836 mg, 88 %) as a white foam, $[\alpha]_D$ +70° (c 1, chloroform).

Ms (FAB): m/z 951.3 (M^+ + 1).

^{1}H-NMR: δ 8.05-7.01 (m, 30 H, arom. H), 6.17 (d, $J_{2,NH}$ 8.7 Hz, 1 H, NH), 5.82 (dd, $J_{3',4'}$ 2.8, $J_{4',5'}$ 0.5 Hz, 1 H, H-4'), 5.57 (s, 1 H, PhCH), 5.43 (dd, $J_{1',2'}$ 7.8, $J_{2',3'}$ 9.9 Hz, 1 H, H-2'), 4.93 (d, $J_{1',2'}$ 7.8 Hz, 1 H, H-1'), 4.79 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1), 4.63 and 4.39 (2 d, J 12.7 Hz, AB-q, 2 H, $PhCH_2$), 4.53 (o, W ~ 22.3 Hz, 1 H, H-2), 4.47-4.23 (unresolved m, 5 H, H-5, 6a, 6b, 6'a, 6'b), 4.11 (~t, spacings ~ 9Hz, 1 H, H-5'), 3.8 (m, 2 H, H-3, 4), 3.69 (d, $J_{3',4'}$ 3.2, $J_{2',3'}$ 10 Hz, 1 H, H-3'), 3.27 (OCH_3).

^{13}C-NMR: δ 166.96, 165.67, 165.67 and 165.11 (4 PhCO), 137.72-125.96 (arom. C), 101.38 (PhCH), 99.23 (C-1'), 96.89 (C-1), 82.91 (C-4), 73.99 (C-3), 73.05 (C-3'), 72.01 ($PhCH_2$), 69.65 (C-5'), 68.75 (C-6), 68.10 (C-2'), 66.81 (C-4'), 62.64 (C-5), 62.21 (C-6'), 55.39 (OCH_3), 52.01 (C-2).

Anal. Calcd. for $C_{55}H_{51}NO_{14}$ (949.97): C, 69.53; H, 5.41; N, 1.47. Found: C, 69.34; H, 5.51; N, 1.65.

Benzyl 2-deoxy-4-O-β-D-galactopyranosyl-2-phthalimido-β-D-glucopyranoside (72)

To a solution of benzyl glycoside **16** (0.8 g, 1 mmol) in a mixture of methanol and dichloromethane (1:1, 20 mL) was added a solution of M sodium methoxide in methanol (0.5 mL). The mixture was stirred for 3 h at room temperature, then the base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. Filtration and evaporation gave a solid **72** (505 mg, 90 %) which was crystallized from methanol, m.p. 236-237 °C, $[\alpha]_D$-60° (c 1.0, pyridine); lit.[32] m.p. 246-248° C, $[\alpha]_D$-66° (c 1.04, pyridine).

Ms. (FAB): m/z 562.2 (M^+ + 1), 544.2 (M^+ - H_2O + 1).

Anal. Calcd. for $C_{27}H_{31}NO_{12}$ (561.5): C, 57.75; H, 5.56; N, 2.49; Found: C, 57.74; H, 5.24; N, 2.31.

Benzyl 3,6-di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (74)

A mixture of benzyl 2-deoxy-4-O-β-D-galactopyranosyl-2-phthalimido-β-D-glucopyranoside **72** (5 g, 9 mmol) and p-TsOH (0.7 g, 3.7 mmol) in acetone (100 mL) was boiled under reflux for 4 h. T.l.c. (9:1 ether-methanol) showed two new compounds, the 3', 4'-O-isopropylidene derivative **73** at R_f 0.56 and its 4', 6'-O-isopropylidene isomer at R_f 0.12. The mixture was cooled and

the acid was neutralized with solid potassium carbonate. After filtration and evaporation, column chromatography (9:1 ether-methanol) gave the faster migrating product **73** as a syrup (3.21 g, 60 %), which was then O-acetylated overnight with pyridine (30 mL) and acetic anhydride (30 mL). Removal of the solvents gave a residue of the peracetate **74** that crystallized from ethanol, m.p. 140 °C, $[\alpha]_D$ +8.5° (c 1, chloroform); lit.[32] m.p. 141-143° C, $[\alpha]_D$ +9.5° (c 1.05, chloroform).

Ms (FAB): m/z 770.3 (M^+ + 1), 710.3 (M^+ + 1 - AcOH), 678.2 (M^+ + 1 - $PhCH_3$).

^{1}H-NMR: δ 7.78-7.68 (m, 4 H, Phth), 7.08 (m, 5 H, Ph), 5.70 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8 Hz, H-3), 5.34 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.85 (dd, 1 H, H-2'), 4.79 and 4.48 (AB-q, 2 H, J 12 Hz, CH_2Ph), 4.46 (dd, 1 H, H-3'), 4.36 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.3-4.0 (m, unresolved, H-5, 6a, 6b, 6'a, 6'b), 3.89 (t, 1 H, $J_{3',4'} = J_{4',5'}$ ~ 6.5 Hz, H-4'), 3.8 (m, 2 H, unresolved, H-4, 5), 2.16, 2.12, 2.10, 1.90 (4 s, 12 H, 4 OAc), 1.52 and 1.31 (2 s, 6 H, Me_2C).

Benzyl 3,6-di-O-acetyl-2-deoxy-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2-phthalimido-β-D-glucopyranoside (75)

To a solution of the isopropylidene compound **74** (1.14 g, 1.5 mmol) in dichloromethane (20 mL) was added 90 % aqueous trifluoroacetic acid (3 mL),

and the mixture was kept for 1 h at room temperature, and then poured into a cold saturated aqueous solution of sodium hydrogencarbonate. The dichloromethane extract was washed with water, and then evaporated to give a residue of the 3',4'-diol 75 which crystallized from ether-methanol (0.84 g, 78 %), m.p. 193-195° C, $[\alpha]_D$ -10° (c 1.0, chloroform); lit.[32] m.p. 198-200° C, $[\alpha]_D$ -13° (c 1.0, chloroform).

^{1}H-NMR: δ 7.82-7.72 (m, 4 H, Phth), 7.10 (m, 5 H, Ph), 5.76 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 8 Hz, H-3), 5.38 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.90 (dd, 1 H, $J_{1,2}$, 8, $J_{2,3}$ 10 Hz, H-2'), 4.84 (d, 1 H, J 12 Hz, CH_2Ph), 4.52 (d, 1 H, CH_2Ph), 4.39 (d, 1 H, H-1'), 3.20 (d, 1H, J 6 Hz, OH), and 2.16, 2.14, 2.10, 1.88 (4 s, 12 H, 4 OAc).

Compounds 76, 77, 78, 79 and 80 were prepared essentially as described by Baer and Abbas[57].

4-O-(3,4-O-Isopropylidene-β-D-galactopyranosyl)-α, β-D-glucopyranose (76) and 1,2,3,6-Tetra-O-acetyl-4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-β-D-glucopyranose (77)

Compound 76 was made by isopropylidenation of lactose 6 with 2,2-dimethoxypropane in N,N-dimethylformamide, essentially following the published procedure[57]. The 2:1 mixture[57] of 3',4'- and 4',6'-acetals was directly acetylated with boiling acetic anhydride in the presence of sodium acetate. By

column chromatography, the 3',4'-acetal hexaacetate 77 could be separated from its 4',6'-acetal isomer and was obtained in 42 % yield (from lactose), as a white crystalline solid, m.p.170-172 °C, $[\alpha]_D$+24° (c 1, chloroform); lit.[57] m.p.172-174 °C, $[\alpha]_D$ +22.8° (c 0.6, chloroform).

^{1}H-NMR: δ 6.26 (d, $J_{1,2}$ 3.7 Hz, 1 H, H-1α, minor anomer), 5.64 (d, $J_{1,2}$ 8.2 Hz, 1 H, H-1β, major anomer), 5.21 (t, $J_{1',2'} \approx J_{2',3'}$ 7.4 Hz, 1 H, H-2'), 5.02 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 9.5 Hz, 1 H, H-2), 4.86 (m, 1 H, H-5), 4.32 (d, $J_{1',2'}$ 7.4 Hz, 1 H, H-1'), 2.10, 2.08, 2.06, 2.05, 2.03 and 2.00 (6 s, $\underline{CH_3}CO$), 1.50 and 1.28 (2 s, $(CH_3)_2C$).

^{13}C-NMR: β-anomer, δ 170.82, 170.48, 169.90, 169.59, 169.25, and 168.94 (6 $CH_3\underline{CO}$), 110.75 [$Me_2\underline{C}O$], 100.8 (C-1'), 91.44 (C-1), 76.60 (C-4), 75.24 (C-5'), 73.44 (C-4'), 72.85 (C-3'), 72.41 (C-5), 71.98 (C-3), 70.71 (C-2'), 70.30 (C-2), 62.94 (C-6), 61.72 (C-6'), 27.03 and 25.82 (2 isopropylidene CH_3), 20.54, 20.39 and 20.31 (6 $\underline{CH_3}CO$).

1,2,3,6-Tetra-O-benzoyl-4-O-(2,6-di-O-benzoyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-α-D-glucopyranose (78)

Compound **78** was made by benzoylation of the mixture of **76** and its isomeric 4',6' acetal (see the preceding experiment) with benzoyl chloride in pyridine as described[57] to provide compound **78** and its 4',6'-acetal isomer.

Compound 78 was obtained after column chromatography in 38 % yield from lactose, m.p.151-154 °C, $[\alpha]_D$ +53° (c 1, chloroform); lit.[57] m.p.154-157 °C, $[\alpha]_D$ +58.1° (c 1, chloroform).

^{1}H-NMR: δ 8.19-7.81 and 7.64-7.26 (m, 30 H, 6 PhCO), 6.69 (d, $J_{1,2}$ 3.8 Hz, H-1), 5.75 (dd, 1 H, $J_{1',2'}$ 7.9, $J_{2',3'}$ 10.2, H-2'), 5.54 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.2 Hz, H-2), 4.65 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 1.49 and 1.22 (2 s, 6 H, Me_2C).

1,2,3,6-Tetra-O-acetyl-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-α,β-D-glucopyranose (79)

Compound 79 was prepared by deisopropylidenation of 77 with 90 % trifluoroacetic acid at 0 °C, as described[57]; yield 72 %, m.p.188-189 °C, $[\alpha]_D$ +3° (c 1, chloroform); lit.[57] m.p.190-192 °C, $[\alpha]_D$ +4.5° (c 2.4, chloroform).

^{1}H-NMR ($CDCl_3$): δ 6.24 (d, $J_{1,2}$ 3.7 Hz, H-1α, minor anomer), 5.64 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1β, major anomer), 4.31 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1'), 2.06, 2.05, 2.05, 2.03, 2.01 and 2.00 (6 s, 18 H, 6 OAc).

^{13}C-NMR: δ 171.1, 171.1, 170.7, 170.6, 169.6 and 169.0 (6 CH_3CO), 100.8 (C-1'), 91.4 (C-1), 75.6 (C-4), 73.4 (C-5'), 72.7 (C-5), 72.6 (C-3'), 72.1 (C-3), 70.1 (C-4'), 68.5 (C-2), 62.5 (C-2'), 61.7 (C-6'), 60.2 (C-6), 20.8, 20.5, 20.4, 20.3, 20.3 and 20.2 (6 CH_3CO).

1,2,3,6-Tetra-O-benzoyl-4-O-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranose (80)

Compound **80** was prepared by deisopropylidenation of **78** with 90 % trifluoroacetic acid at 0 °C, as just described for **79**; yield 70 %, m.p. 221-223 °C, $[\alpha]_D$ +78° (c 1, chloroform); lit.[57] m.p. 231-233 °C, $[\alpha]_D$ +74° (c 2.7, chloroform).

^{1}H-NMR: δ 8.09-7.83 and 7.60-7.27 (m, 30 H, 6 PhCO), 6.69 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.57 (t, 1 H, $J_{1,2}$ 3.7, $J_{2,3}$ 10.2 Hz, H-2), 5.13 (dd, 1 H, $J_{1',2'}$ 8.0, $J_{2',3'}$ 9.3 Hz, H-2'), 4.66 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1'), 4.85 (dd, 1 H, $J_{5,6a}$ 4.1, $J_{6a,6b}$ 11.7 Hz, H-6a), 4.75 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.45 (m, 2 H, H-6b and H-6'a), 4.26 (dd, 1 H, $J_{5,6b}$ 2.6, $J_{6a,6b}$ 12 Hz, H-6'b).

^{13}C-NMR: δ 166.5, 166.5, 165.3, 165.3, 164.3 and 164.3 (6 PhCO), 133.5-128.2 (m, arom. C), 101.6 (C-1'), 92.3 (C-1), 81.3 (C-4), 73.5 (C-5'), 73.3 (C-5), 73.1 (C-3'), 72.9 (C-3), 72.3 (C-4'), 71.9 (C-2), 68.8 (C-2'), 63.6 (C-6), 62.3 (C-6').

1,3,6-Tri-O-acetyl-2-azido-2-deoxy-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α,β-D-glucopyranose (81)

A mixture of azidonitrates (**12** and **19**) was prepared from hexaacetyl-lactal **9** as previously indicated (see under preparation of **20α** and **20β**, page 114). The crude material was converted into **81** as follows.

A. Reaction of the crude product with hydrazine acetate.

A solution of the product mixture from the aforementioned azidonitration (1.4 g) in N,N-dimethylformamide (10 mL) was treated with hydrazine acetate (0.2 g, 2.2 mmol) for 4 h at room temperature, and then diluted with dichloromethane (20 mL), washed with 10 % aqueous sodium chloride, and concentrated. The residue was then treated with acetic anhydride and pyridine (5 mL each) at room temperature for 8 h, after which the solution was poured into water and extracted with dichloromethane. The organic phase was washed with saturated sodium bicarbonate and water. Concentration followed by chromatographic purification on silica gel with hexane-ethyl acetate (1:1) gave syrupy 2-azido-2-deoxy-α,β-heptaacetyllactose **81** (0.72 g, 53 %).

Anal. Calcd. for $C_{26}H_{35}N_3O_7$ (661.56): C, 47.20; H, 5.33; N, 6.35. Found: C, 47.36; H, 5.46; N, 6.30.

B. Reaction of the crude product with acetic anhydride and pyridine[30]

The mixture from the azidonitration (0.77 g) was treated with acetic

anhydride (5 mL) and pyridine (5 mL) at room temperature for 1 h, and then heated on a steam bath for another hour. After cooling the solution was poured into water and extracted with dichloromethane. The organic phase was washed with saturated sodium bicarbonate and water. Concentration followed by chromatographic purification on silica gel with hexane-ethyl acetate (1:1) gave a syrupy mixture (0.3 g, 45 %) of the 2-azido-2-deoxy-α,β-heptaacetyllactoses **81**. IR: ν^{KBr} 2114 (N_3) and 1752 (OAc) cm^{-1}.

Ms(CI): m/z 662 (0.3 %, $M^+ + 1$), 634 (11 %, $M^+ + 1 - N_2$), 618 (9 %, $M^+ + 1 - CH_3CHO$), 602 (6.5 %, $M^+ + 1$ -AcOH), 574 (29 %, $M^+ + 1 - N_2$ - AcOH), 456 (6.5 %, unassigned), and 331 (100 %, tetra-O-acetylgalactopyranosyl ion).

The NMR spectra were rather complex because the product was a mixture of anomers which additionally contained a small proportion of product originating from isomer **19**. Nevertheless, the following assignments could be made from an expanded 300 MHz ^{1}H-spectrum and a COSY plot.

For **81α** (major anomer): δ 6.23 (d, $J_{1,2}$ 3.7 Hz, H-1), 5.43 (dd, $J_{2,3}$ 10.7, $J_{3,4}$ 9.0 Hz, H-3), 4.45 (d, $J_{1',2'}$ 7.8, H-1'), 3.77 (dd, $J_{3,4}$ 9.1, $J_{4,5}$ 10.2 Hz, H-4), 3.46 (dd, $J_{1,2}$ 3.7, $J_{2,3}$ 10.6 Hz, H-2).

For **81β** (minor anomer): δ 5.50 (d, $J_{1,2}$ 8.5 Hz, H-1), 5.07 (dd, J 8 and 10 Hz, H-3), 4.51 (d, $J_{1',2'}$ 7.8, H-1'), 3.75 (H-4, obscured by signals of impurity [AcOEt] but located by COSY), 3.53 (dd, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2).

For both anomers: δ 5.34 (dd, $J_{3',4'}$ 3.3, $J_{4',5'}$ 1 Hz, H-4'), 5.11 (dd, $J_{1',2'}$ 3.8, $J_{2',3'}$ 10.4 Hz, H-2'), 4.94 (dd, $J_{2',3'}$ 10.4, $J_{3',4'}$ 3.4 Hz, H-3'), 4.38 (dd, $J_{5,6a}$ 2.2, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.20-4.05 (unresolved m, 3 H, H-6b, 6'a, 6b'), 3.95-3.80 (m, 2 H, H-5,5'), 2.18-1.94 (multiple singlets, 21 H, 7 OAc). A low-intensity doublet (J 2 Hz) at δ 6.01 coupled with a double doublet at δ 4.00 were assigned to H-1 and H-2, respectively, of the D-*manno* admixture.

2-Azido-2-deoxy-4-O-(β-D-galactopyranosyl)-α, β-D-glucopyranose (82)

To a solution of 1, 3, 6-tri-O-acetyl-2-azido-2-deoxy-4-O-(2, 3, 4, 6-tetra-O-acetyl-β-D-galactopyranosyl)-α,β-D-glucopyranose **81** (0.8 g, 1 mmol) in a mixture of methanol and dichloromethane (1:1 20 mL) was added a solution of M sodium methoxide in methanol (0.5 mL). The mixture was stirred for 3 h at room temperature, and the base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. Filtration and evaporation gave an amorphous solid which crystallized from methanol, affording 326 mg (85 %) of **82** as a white powder, m.p.246-248° C, $[\alpha]_D$ -66° (c 1.0, pyridine).

Compounds **83**, **84** and **85** were prepared using the procedures of Baer and Abbas[57b], but with 2-azido-2-deoxy-4-O-(β-D-galactopyranosyl)-α,β-D-glucopyranose **82** instead of lactose **6** as the starting material.

2-Azido-2-deoxy-4-O-(3,4-O-isopropylidene-β-D-galactopyranosyl-α,β-D-glucopyranose (83) and 1,3,6-Tri-O-acetyl-2-azido-2-deoxy-4-O-(2,6-di-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosyl)-α, β-D-glucopyranose (84)

Compound **84** was made by isopropylidenation of **82** to give crude **83** (probably containing some 4',6'-acetal isomer), followed by acetylation of the product with Ac_2O/NaOAc and chromatographic purification. The procedures were the same as in the preparation of **77** from lactose. Pure compound **84** was obtained as a white foam in 40 % yield from **82**; $[\alpha]_D$ +18° (c 1, chloroform).

IR: ν^{KBr} 2111 (N_3) and 1746 (OAc) cm^{-1}.

^{13}C-NMR: δ 170.80, 170.59, 169.82, 169.25 ($CH_3\underline{C}O$), 110.86 ($Me_2\underline{C}O_2$), 102.73 (C-1'), 100.25 (C-1), 76.73 (C-4), 75.70 (C-5'), 72.72 (C-4'), 72.62 (C-3'), 71.59 (C-3), 70.77 (C-5), 63.93 (C-2'), 62.98 (C-6), 62.03 (C-6'), 57.24 (C-2), 27.09 and 25.89 ($\underline{Me_2}C$), 20.63 ($\underline{CH_3}CO$).

Anal. Calcd. for $C_{25}H_{35}N_3O_{15}$ (617.55): C, 48.62; H, 5.71; N, 6.80. Found: C, 48.70; H, 5.91; N, 7.04.

1,3,6-Tri-O-acetyl-4-O-(2,6-di-O-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-β-D-glucopyranose (85)

The isopropylidene derivative **84** was deacetonated with 90 % trifluoroacetic acid as previously described[57] for the deacetonation of 77. Compound **85** was obtained as a white foam in 72 % yield; $[\alpha]_D$ +7° (c 1, chloroform).

IR: ν^{KBr} 2112 (N_3) and 1742 (OAc) cm^{-1}.

^{13}C-NMR: 171.10, 170.96, 170.73 ($CH_3\underline{C}O$), 101.03 (C-1'), 98.33 (C-1), 72.45 (C-4), 72.28 (C-5'), 71.94 (C-5), 70.10 (C-3'), 68.28 , 62.59, 61.90 (C-2', 3, 4'), 60.78 (C-6), 60.22 (C-6'), 55.15 (C-2), 20.69, 20.58, 20.49 ($\underline{C}H_3CO$).

Anal. Calcd. for $C_{22}H_{31}N_3O_{15}$ (577.49), calcd. C, 45.75; H, 5.41; N, 7.28. Found, C, 45.59; H, 5.55; N, 7.47.

Methyl 3-O-benzyl-β-D-galactopyranoside (87)

Finely powdered methyl β-D-galactopyranoside **86** (5.82 g, 30 mmol) and dibutyltin (II) oxide (7.5 g, 30 mmol) were stirred under reflux in dry benzene (100 mL) for 48 h with continuous removal of water by passing the condensate through a Soxhlet extractor filled with 3 A molecular sieves (30 mL, bed). The temperature was lowered to 60° C, and benzyl bromide (7.5 mL, 30 mmol) and tetrabutylammonium iodide (11.1 g, 30 mmol) were added. After stirring for 2.5 h at the reflux temperature, t.l.c [5:1 dichloromethane-methanol] showed the presence of a small amount of unchanged **86**, together with several faster moving products, the predominant one having R_f 0.6. The clear yellow solution was concentrated to dryness, the residue was chromatographed on a silica gel column (ethyl acetate) to give the major reaction product **87** (5.5 g, total yield 65 %). A portion was recrystallized from 2-propanol to give material melting at 138-139° C, $[\alpha]_D$ +2.0 ° (c 1.3, methanol), lit.[37] m.p. 135-137° C.

Ms (CI) : m/z 285 (M^+ + 1), 253 (M^+ - OCH_3), 235 (M^+ - H_2O - OCH_3), 217 (M^+ - OCH_3 - $2H_2O$), 199 (M^+ - OCH_3 - $3H_2O$).

^{1}H-NMR (D_2O): δ 7.42 (m, 5 H, arom. H), 4.64 (AB-q, 2 H, J 12.8 Hz, $PhCH_2$), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.69 (m, 6 H, H-2,3,4,5,6,6'), 3.52 (s, 3 H, OCH_3).

^{13}C-NMR (D_2O): δ 140.24, 131.70, 131.64, 131.36 (arom. C), 106.68 (C-1),

82.80 (C-3), 77.95 (C-5), 73.97 (CH_2Ph), 72.67 (C-2) , 68.01 (C-4), 63.87 (C-6), 59.98 (OCH_3). These data were higher by ~2.5 ppm than those reported[37].

Methyl 2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranoside (88)

A solution of methyl 3-O-benzyl-β-D-galactopyranoside **87** (1.42 g, 5 mmol) in pyridine (15 mL) was treated with benzoyl chloride (4.3 mL) for 2 h, after which t.l.c. showed that the reaction was complete. The product, isolated in the usual manner, readily crystallized from ethanol (2.84 g, 95 %). Recrystallization of a portion gave a pure sample of **88**, m.p. 140-141° C, $[\alpha]_D$ +101° (c 1.6, chloroform); lit.[37] m.p. 142-143° C, $[\alpha]_D$ +94° (c 1.6, chloroform). Ms (CI): m/z 597 (M^+ + 1), 565 (M^+ - OCH_3), 475 (M^+ - CO_2Ph).

^{1}H-NMR: δ 5.90 (dd, 1 H, $J_{3,4}$ 3.44 Hz, H-4), 5.52 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 4.69 and 4.48 (AB-q, J 12.7 Hz, CH_2Ph), 4.61 (dd, 1 H, $J_{5,6a}$ 7, $J_{6a,6b}$ 11.3 Hz, H-6a), 4.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.41 (dd, 1 H, $J_{5,6b}$ 6.2, $J_{6a,6b}$ 11.3 Hz, H-6b), 4.07 (td, 1 H, $J_{4,5}$ ~ 1 Hz, $J_{5,6a} \approx J_{5,6b}$ 6.5 Hz, H-5), 3.79 (dd, 1 H, $J_{3,4}$ 3.4, $J_{2,3}$ 10 Hz, H-3), 3.50 (s, 3 H, OCH_3).

^{13}C-NMR: δ 102.3 (C-1), 76.04 (C-3), 71.18 (C-2), 70.97 (C-5), 70.80 (CH_2Ph), 66.43 (C-4), 62.45 (C-6), 56.82 (OCH_3).

2,4,6-Tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl chloride (89) and 2,4,6-Tri-O-benzoyl-3-O-formyl-α-D-galactopyranosyl chloride (90)

A mixture of methyl 2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranoside **88** (1 g, 1.7 mmol), 1, 1-dichloromethyl methyl ether (3 mL) and freshly fused zinc chloride (10 mg) in dry chloroform (3 mL) was stirred for 30 min at 45-50° C under exclusion of moisture. (The reaction temperature was 10° C lower than in the original work[37]). The mixture was concentrated and chromatographed on silica gel with 2:1 hexanes-ethyl acetate to give **89** (R_f 0.6, 1:1 ethyl acetate-hexanes) as an amorphous solid (0.95 g, 94 %); $[\alpha]_D$ +153.7° (c 1.5, chloroform), lit.[37] $[\alpha]_D$ +175° (c 0.86, chloroform).

^{1}H-NMR: δ 6.62 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1), 6.08 (d, 1 H, $J_{3,4}$ 3 Hz, H-4), 5.65 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.2 Hz, H-2), 4.82 and 4.62 (two d of AB-q, 2 H, J 12.5 Hz, CH_2Ph, superposed on multiplets for H-5 and H-6), 4.47 (dd, 1 H, $J_{5,6'}$ 5.8, $J_{6,6'}$ 11.6 Hz, H-6'), 4.33 (dd, 1 H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.2 Hz, H-3).

^{13}C-NMR: δ 166.17, 165.80, 165.80 (COPh), 137.3-127.9 (multiple signals, arom. C), 92.17 (C-1), 72.56 (C-3), 71.65 (CH_2, benzylic), 70.26 (C-2), 70.11 (C-5), 67.24 (C-4), 62.27 (C-6).

When the reaction time was prolonged to 2 h and the temperature allowed to rise to 55-60 °C as originally directed[37], the only product isolated after chromatography was 2,4,6-tri-O-benzoyl-3-O-formyl-α-D-galactopyranosyl

chloride 90 (R_f 0.55, 1:1 ethyl acetate-hexanes); $[\alpha]_D$+154.7° (c 1, chloroform), not the desired compound 89 as reported[37].

^{1}H-NMR: δ 7.95 (s, CHO), 6.64 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 6.03 (dd, 1 H, $J_{3,4}$ 3.3, $J_{4,5}$ 0.9 Hz, H-4), 5.96 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.3 Hz, H-3), 5.71 (dd, 1 H, $J_{1,2}$ 3.9, $J_{2,3}$ 10.4 Hz, H-2), 4.86 (t, $J_{5,6} \approx J_{5,6'}$ ~ 6.4 Hz, H-5), 4.59 (dd, 1 H, $J_{5,6}$ 6.6, $J_{6,6'}$ 11.5 Hz, H-6), 4.40 (dd, 1 H, $J_{5,6'}$ 6.0, $J_{6,6'}$ 11.5 Hz, H-6').

^{13}C-NMR: δ 166.0, 165.6, 165.5 (3 PhCO), 134.0-128.5 (multiple signals, arom. C), 159.7 (CHO), 91.3 (C-1), 69.9 (C-5), 68.4 (C-3), 67.9 (C-2), 66.7 (C-4), 61.6 (C-6).

3-O-Acetyl-2, 4, 6-tri-O-benzoyl-α-D-galactopyranosyl bromide (91)

To a solution of 1-O-acetyl-2, 4, 6-tri-O-benzoyl-3-O-benzyl-α, β-D-galactopyranose 59 (600 mg, 0.96 mmol) in a mixture of glacial acetic acid (5 mL) and acetic anhydride (2 mL) was added 30 % hydrobromic acid in glacial acetic acid (1 mL). The reaction mixture was allowed to stand at +5 °C overnight and then diluted with dichloromethane and poured onto ice with stirring. The dichloromethane extract was washed with water, saturated aqueous $NaHCO_3$, and water, and evaporated to give a residue (600 mg) which was chromatographed on silica gel with 3:1 hexanes-ethyl acetate as eluent. There was obtained pure 2,4,6-tri-O-benzoyl-3-O-acetyl-α-D-galactopyranosyl

bromide **91** (448 mg, 78 %) as white crystals, m.p. 141.5-141.9° C, $[\alpha]_D$ +164.5° (c 1, chloroform).

^{1}H-NMR: δ 6.90 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1), 5.95 (dd, 1 H, $J_{4,5}$ 1, $J_{3,4}$ 3.2 Hz, H-4), 5.76 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.3 Hz, H-3), 5.44 (dd, 1 H, $J_{1,2}$ 4, $J_{2,3}$ 10.5 Hz, H-2), 4.81 (ddd, 1 H, $J_{4,5}$ 1, $J_{5,6} = J_{5,6'}$ 6 Hz, H-5), 4.58 (dd, 1 H, $J_{5,6}$ 6.7, $J_{6,6'}$ 11.5 Hz, H-6), 4.39 (dd, 1 H, $J_{5,6'}$ 6.1, $J_{6,6'}$ 11.5 Hz, H-6').

Anal. Calcd. for $C_{29}H_{25}O_9Br$ (597.4): C, 58.30; H, 4.22; Br, 13.40. Found: C, 58.18; H, 4.33; Br, 13.65.

1,2,4,6-Tetra-O-acetyl-3-O-benzyl-α,β-D-galactopyranose (92)

To a mixture of methyl 3-O-benzyl-β-D-galactopyranoside **87** (0.568 g, 2 mmol) in acetic anhydride (10 mL) and acetic acid (5 mL) at 0° C, 5 drops of conc. sulphuric acid were added. The mixture was stirred at room temperature for 6 h, diluted with dichloromethane and washed successively with water and aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated to give a syrupy **92** as a 3:1 α,β-mixture (824 mg, 94 %). The anomer ratio was deduced from the integrals of the easily distinguishable peaks for H-4α and β.

^{1}H-NMR: δ 7.22 (m, 5 H, arom. H), 6.26 (d, $J_{1,2}$ 3.8 Hz, 1 H, H-1), 6.07 (s, H-4β), 5.15 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10.4 Hz, H-2), 5.11 (d, $J_{3,4}$ 1.2 Hz, 1 H, H-4α), 4.64 and 4.39 (AB-q, J 11.4 Hz, CH_2Ph), 2.06, 2.04, 2.03, 2.01, 1.99, 1.95, 1.94 (m s, α,β OAc).

Anal. Calcd. for $C_{21}H_{26}O_{10}$ (438.4): C, 57.53; H, 5.98. Found: C, 57.30; H, 5.95.

2,4,6-Tri-O-acetyl-3-O-benzyl-α-D-galactopyranosyl bromide (93)

1,2,4,6-Tetra-O-acetyl-3-O-benzyl-α,β-D-galactopyranose **92** (438 mg, 1 mmol) was dissolved in dry acid-free chloroform (5 mL), cooled to 0° C, and trimethylsilyl bromide (750 µl) was added. The mixture was stirred overnight at room temperature, then evaporated, and the residue was chromatographed in a short column of silica gel with 3:1 hexanes-ethyl acetate to give **92** (400 mg, 87.3 %) as a syrup, $[\alpha]_D$ +103° (c 1, chloroform).

^{1}H-NMR: δ 6.71 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 5.62 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 4.96 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 10.1 Hz, H-2), 4.71 and 4.48 (AB-q, J 11.4 Hz, CH_2Ph), 4.40 (t, 1 H, $J_{5,6} + J_{5,6'} \approx 13$ Hz, H-5), 4.22 (dd, 1 H, $J_{5,6}$ 5.7, $J_{6,6'}$ 11.5 Hz, H-6), 4.08 (dd, $J_{5,6'}$ 7.1, $J_{6,6'}$ 11.5 Hz, H-6'), 4.00 (dd, 1 H, $J_{2,3}$ 10.1, $J_{3,4}$ 3.2 Hz, H-3), 2.10, 2.07, 2.05 (3s, 3 OAc).

^{13}C-NMR: δ 89.4 (C-1), 73.7 (C-3), 72.1 (CH_2Ph), 71.6 (C-2), 69.7 (C-5), 66.3 (C-4), 61.4 (C-6).

Methyl 4,6-di-O-acetyl-2-benzamido-2-deoxy-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (94)

A solution of the benzylidene compound **62** (950 mg, 1 mmol) in 60 % acetic acid (10 mL) was stirred at 70° C for 30 min and diluted with water (10 mL). Solvent removal left a foam which was not characterized but acetylated directly with a 2:1 mixture of pyridine-acetic anhydride (9 mL) during 6 h at room temperature. Solvent removal left a foam which was dissolved in dichloromethane (10 mL) and washed with 5 % aqueous hydrochloric acid, sodium bicarbonate solution, and then with ice-water. The residue obtained on solvent evaporation was applied to a silica gel column and eluted with 1:1 ethyl acetate-hexanes, yielding white solid **94** (709 mg, 75 %), m.p. 209-210° C, $[\alpha]_D$ +80.5° (c 1, chloroform).

Ms(FAB): m/z 946.4 (M^+ + 1).

^{1}H-NMR: δ 6.26 (d, $J_{2,NH}$ 9.3 Hz, 1 H, NH), 5.86 (dd, $J_{3',4'}$ 3.3, $J_{4',5'}$ 1 Hz, 1H, H-4'), 5.38 (dd, $J_{1',2'}$ 7.6, $J_{2',3'}$ 10 Hz, 1 H, H-2'), 5.13 (dd, $J_{3,4}$ 9.3, $J_{4,5}$ 9.8 Hz, 1 H, H-4), 4.86 (d, $J_{1',2'}$ 7.6 Hz, 1 H, H-1'), 4.70 (d, $J_{1,2}$ 3.5 Hz, 1 H, H-1), 4.62 and 4.39 (AB-q, J 12.5 Hz, 2 H, $PhCH_2$), 4.60 (dd, $J_{5,6a}$ 7.5, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 4.55 (ddd, $J_{1,2}$ 3.5, $J_{2,NH}$ 9.3, $J_{2,3}$ 11 Hz, H-2), 4.37 (dd, $J_{5,6'b}$ 7.4, $J_{6'a,6'b}$ 11.4 Hz, H-6'b), 4.20 (dd, $J_{5,6a}$ 4.7, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.15-4.10 (m, 3 H, H-3, 5', 6b), 3.82 (ddd, J ~ 2.5, 5, and 10 Hz, H-5), 3.74 (dd, $J_{2',3'}$ 10, $J_{3',4'}$ 3.3 Hz, 1 H,

H-3'), 3.27 (s, 3 H, OCH_3), 2.08 and 1.99 (2 s, 6 H, 2 OAc).

^{13}C-NMR: δ 170.5, 169.0 ($CH_3\underline{C}O$), 166.4, 166.2, 165.8, 165.4 (Ph$\underline{C}O$), 137.0-127.0 (multiple signals, arom. C), 101.03 (C-1'), 98.25 (C-1), 76.14 (C-3), 76.00 (C-3'), 71.96 (C-2'), 70.95 ($PhCH_2$-), 70.87 (C-5'), 68.45 (C-4), 68.00 (C-5), 66.11 (C-4'), 62.30 and 62.01 (C-6,6'), 55.27 (C-2), 52.84 (OCH_3), 21.0 (2 $\underline{C}H_3CO$).

Anal. Calcd. for $C_{52}H_{51}NO_{16}$ (945.94): C, 66.02; H, 5.43; N, 1.48. Found: C, 66.27; H, 5.44; N, 1.45.

Methyl 4,6-O-benzylidene-α-D-glucopyranoside (96)

Compound **96** was prepared by the procedure of Hall[66] and Van Cleve[67]. Zinc chloride-benzaldehyde complex was made by stirring fused zinc chloride with benzaldehyde. Methyl α-D-glucopyranoside was then added and the mixture stirred for 4 h at room temperature, and processed to give **96** (70 %), m.p.160-162 °C, $[\alpha]_D$ +103° (c 1, chloroform); lit.[67] m.p. 166-167 °C, $[\alpha]_D$ +110° (c 1, chloroform).

^{1}H-NMR: δ 7.28-7.5 (m, 5 H, Phenyl), 5.49 (s, 1 H, PhCH-), 4.74 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 2.97 (d, 1 H, $J_{3,OH}$ 2.24 Hz, C(3)-OH), 2.45 (d, 1 H, $J_{2,OH}$ 9.3 Hz, C(2)-OH).

^{13}C-NMR: δ 101.9 (PhCH-), 99.7 (C-1), 80.9 (C-4), 72.8 (C-2), 71.7 (C-3), 68.9

(C-6), 62.3 (C-5), 55.6 (OCH_3).

Methyl 4,6-O-benzylidene-2,3-O-dibutylstannylene-α-D-glucopyranoside (97)

Compound **97** was prepared according to the procedure described by Munavu and Szmant[56]. Methyl 4,6-O-benzylidene-α-D-glucopyranoside **96** (8.50 g, 30 mmol) was refluxed with an equimolar amount of dibutyltin oxide (7.70 g, 30 mmol) in a mixture of benzene (150 mL) and methanol (15 mL) until the solution became clear (45 min). It was then left at 50-55° for 14 h and then the solvents were removed in vacuo, leaving a white solid of **97** (14.5g, 91%), m.p.195-197° C, $[\alpha]_D$ +15° (c 2, chloroform); lit.[56] m.p.194-195 °C, $[\alpha]_D$ not reported.

Ms(CI): m/z 515 ($M^+ + 1$), 483 ($M^+ - OCH_3$).

^{1}H-NMR: δ7.40-7.25 (m, 5 H, phenyl), 5.39 (s, 1 H, PhCH), 4.82 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.22(dd, 1 H, H-6eq), 3.82-3.72 (m, 2 H, H-5, 6ax), 3.62 (t, 1 H, $J_{3,4} = J_{4,5}$ 8.9 Hz, H-4), 3.40 (s, 3 H, OCH_3), 3.40 (overlapped by OCH_3, 1 H, H-3), 3.31 (dd, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 8.9 Hz, H-2).

^{13}C-NMR: δ 136.9, 129.5, 128.3 and 126.4 (multiple signals, arom. C), 102.5 (PhCH), 102.4 (C-1), 82.4 (C-4), 76.7 (C-2), 72.2 (C-3), 69.2 (C-6), 63.6 (C-5), 55.8 (OCH_3), 27.1, 26.9, 26.8, 26.8, 23.4, 21.8, 13.7, and 13.6 (2 n-Bu).

Methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside (98) and Methyl 3-O-benzoyl-4, 6-O-benzylidene-α-D-glucopyranoside (99)

Compound **98** was prepared by the procedure described by Munavu and Szmant[56]. The preceding dibutylstannylene compound **97** was treated with 1.1 equiv. of benzoyl chloride and triethylamine in dioxane at 0 °C for 2 h and then the reaction mixture was stirred at room temperature for 6 h. Compound **98** crystallized as needles from the reaction mixture and was washed with ether; yield 85 %, m.p.163-164° C, $[\alpha]_D$ +108 °(c 1.0, chloroform); lit[56] m.p.168-170° C, $[\alpha]_D$ +111 ° (c 1.0, chloroform).

Ms(CI): m/z 387 (M^+ + 1), 355 (M^+ - OCH_3).

^{1}H-NMR: δ 8.20-8.02 and 7.65-7.30 (m, 10 H, arom. H), 5.56 (s, 1 H, PhCH), 5.06 (dd, 2 H, H-1,2), 4.31 (m, 2 H, H-3,6eq), 3.82 (m, 2 H, H-5,6ax), 3.65 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.3 Hz, H-4), 3.38 (s, 3 H, OCH_3), 2.45 (d, 1 H, $J_{3,OH}$ 2.9 Hz, OH-3).

^{13}C-NMR: δ 166.2 (CO), 137,0 133.4, 130.0, 129.5, 129.3, 128.4, 128.4, 126.3 (multiple signals, arom. C), 102.1 (PhCH), 97.7 (C-1), 81.4 (C-4), 74.1 (C-2), 68.9 (C-3), 68.8 (C-6), 62.0 (C-5), 55.5 (OCH_3).

Compound **99** was separated from the mother liquor by chromatography (silica gel, 1:1 hexanes-ethyl acetate as eluent); yield 11 %, m.p. 219-220° C,

$[\alpha]_D$ +34.5° (c 1.0, chloroform); lit[56] m.p. 209-220° C, $[\alpha]_D$ +34° (c 1.0, chloroform).

Ms(CI): m/z 387 (M^+ + 1), 355 (M^+ - OCH_3).

^{1}H-NMR: δ 8.190-8.00 and 7.56-7.20 (m, 10 H, arom. H), 5.57 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.7 Hz, H-3), 5.51 (s, 1 H, PhCH), 4.84 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.32 (dd, 1 H, $J_{5,6eq}$ 4.2, $J_{6eq,6ax}$ 9.7 Hz, H-6eq), 3.90 (m, 1 H, H-5), 3.82 (m, 3 H, H-2,4,6ax), 3.38 (s, 3 H, OCH_3), 2.35 (d, 1 H, $J_{2,OH}$ 11.3 Hz, OH-2).

^{13}C-NMR: δ 166.6 (CO), 136.9, 133.1, 129.9, 129.0, 128.3, 128.2, 126.1 (multiple signals, arom. C), 101.5 (PhCH), 100.2 (C-1), 78.8 (C-4), 73.0 (C-3), 72.0 (C-2), 69.0 (C-6), 62.8 (C-5), 55.6 (OCH_3).

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (100)

A solution of 2,4,6-tri-O-benzoyl-3-O-benzyl-α-D-galactopyranosyl bromide **61** (710 mg; 1.1 mmol) in dry dichloromethane (10 mL) was added dropwise, with rigorous exclusion of moisture and light, to a stirred solution of methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside **98** (386 mg, 1.0 mmol), silver triflate (283 mg, 1.1 mmol) and 1,1,3,3-tetramethylurea (128 mg, 1.1 mmol) in dry dichloromethane (10 mL). The mixture was stirred at room temperature overnight, and then filtered through a Celite pad. Following extraction of the solution with aqueous sodium bicarbonate and water, the syrup obtained after concentration of the organic phase was purified by elution from a column of silica gel with ethyl acetate-hexanes (1:1). to give **100** as a colorless foam (856 mg, 90 %), $[\alpha]_D$ +73.1° (c 1.01, chloroform).

Ms (FAB): m/z 952.3 (M^+ + 1).

^{1}H-NMR: δ 5.86 (d, $J_{3',4'}$ 3.3 Hz, 1 H, H-4'), 5.61 (s, 1H, PhCH), 5.50 (dd, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.9 Hz, 1 H, H-2'), 4.99 (d, $J_{1,2}$ 3.8 Hz, 1 H, H-1), 4.94 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 9.6 Hz, 1 H, H-2), 4.90 (d, $J_{1',2'}$ 8.1 Hz, 1 H, H-1'), 4.63 and 4.41 (AB-q, J 12.8 Hz, 2 H, $PhCH_2$), 4.50 (dd, $J_{6'a,6'b}$ 11.3 Hz, H-6'a), 4.45-4.25 and 3.88-3.72 (unresolved m, H-3,4,5,6a,6b,6'b), 4.00 (t, J 6.5 Hz, H-5'), 3.67 (dd, $J_{2',3'}$ 9.9, $J_{3',4'}$ 3.3 Hz, 1 H, H-3'), 3.26 (s, 3 H, OCH_3).

^{13}C-NMR.: δ 166.0-165.5 (4 signals, CO), 138-125 (multiple signals, arom. C), 101.38 (C-1'), 100.91 (PhCH), 97.43 (C-1), 79.21 (C-4), 76.39 (C-3), 75.16 (C-3'), 74.06 (C-2'), 71.32 (C-2), 70.93 (C-5'), 70.86 ($PhCH_2$), 68.86 (C-6), 66.31 (C-4'), 62.63 (C-5), 62.04 (C-6'), 55.30 (OCH_3).

Anal. Calcd. for $C_{55}H_{50}O_{15}$ (950.95): C, 69.46; H, 5.30. Found: C, 69.37; H, 5.44.

Methyl 2-O-benzoyl-4, 6-O-benzylidene-3-O-(3-O-acetyl-2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranoside (101)

A solution of 3-O-acetyl-2,4,6-tri-O-benzoyl-α-D-galactopyranosyl bromide **91** (657 mg, 1.1 mmol) in dry dichloromethane (10 mL) was added dropwise, with rigorous exclusion of moisture and light, to a stirred solution of methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside **98** (386 mg, 1 mmol), silver triflate (282.7 mg, 1.1 mmol) and 1,1,3,3-tetramethylurea (128 mg, 1.1 mmol) in dry dichloromethane (10 mL). The mixture was stirred at room temperature overnight, and then filtered through a Celite pad. Following extraction of the solution with aqueous sodium bicarbonate and water, the product obtained after concentration of the organic phase gave a syrup that was purified by elution from a column of silica gel with ethyl acetate-hexanes (1:1). Compound **101** was obtained as a dry white powder (893 mg, 97 % calculated

as monohydrate), $[\alpha]_D$ +74° (c 1.0, $CHCl_3$).

Ms (FAB): m/z 903.87 (M^+ + 1).

^{13}C-NMR ($CDCl_3$): δ 101.42 (C-1'), 100.99 (PhCH-), 97.44 (C-1), 79.26 (C-4), 75.49 (C-3), 73.70 (C-3'), 71.21 (C-2), 70.75 (C-5'), 69.84 (C-2'), 68.86 (C-6), 67.62 (C-4'), 62.58 (C-5), 61.38 (C-6'), 55.31 (OCH_3).

Anal. Calcd. for $C_{50}H_{46}O_{16}\cdot H_2O$ (920.89): C, 65.21; H, 5.25. Found: C, 65.49; H, 5.30.

Methyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,4,6-tri-O-acetyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (102)

A solution of 2,4,6-tri-O-acetyl-3-O-benzyl-α-D-galactopyranosyl bromide **93** (505 mg, 1.1 mmol) in dry dichloromethane (10 mL) was added dropwise, with rigorous exclusion of moisture and light, to a stirred solution of methyl 2-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside **98** (386 mg, 1 mmol), silver triflate (283 mg, 1.1 mmol) and 1,1,3,3-tetramethylurea (128 mg, 1.1 mmol) in dry dichloromethane (20 mL). The mixture was stirred at room temperature overnight, and then filtered through a Celite pad. Following extraction of the solution with aqueous sodium bicarbonate and water, the syrup obtained after concentration of the organic phase was purified by elution from a column of silica gel with ethyl acetate-hexanes (1:1) to give **102** (695 mg, 91

%) as a white foam, $[\alpha]_D$ +80° (c 1, chloroform).

Ms (FAB): m/z 765.2 (M^+ + 1), 733.2 (M^+ - OCH_3), 673.1(M^+ - OCH_3 - HOAc).

^{1}H-NMR: δ 8.05-7.03 (m, arom. H), 5.59 (s, 1 H, PhCH), 5.43 (dd, 1 H, $J_{3',4'}$ 3.4, $J_{4',5'}$ 1.0 Hz, H-4'), 5.11 (dd, 1 H, $J_{1',2'}$ 8.1 $J_{2',3'}$ 10.1 Hz, H-2'), 5.05 (dd, 1 H, $J_{1,2}$ 3.8, $J_{2,3}$ 9.3 Hz, H-2), 5.03 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 4.64 (d, 1 H, $J_{1',2'}$ 8.1 Hz, H-1'), 4.55 and 4.26 (AB-q, 2 H, J 12.5 Hz, $PhCH_2$), 4.32 (t, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), ~4.25 (m, H-6'a), 4.16 (dd, $J_{5,6a}$ 7, $J_{6a,6b}$ 11.1 Hz, H-6a), 4.04 (dd, $J_{5,6b}$ 6.3, $J_{6a,6b}$ 11.2 Hz, H-6b), 3.9-3.7 (unresolved m, 3 H, H-5, 5', 6'b), 3.71 (~t, $J_{3,4} \approx J_{4,5} = 9.4$ Hz, H-4), 3.34 (s, 3 H, OCH_3), 3.33 (dd, 1 H, $J_{2',3'}$ 10.1, $J_{3',4'}$ 3.4 Hz, H-3'), 2.05, 2.03 and 1.31 (3 OAc).

^{13}C-NMR: 170.2, 169.3, 169.3 and 165.5 (3 CH_3CO and PhCO), 137.6-126.0 (m, arom. C), 101.84 (C-1'), 100.83 (PhCH-), 97.53 (C-1), 78.95 (C-4), 76.72 (C-3), 73.86 (C-3'), 71.05 (C-2), 70.69 (C-2',5'), 70.17 ($PhCH_2$-), 68.75 (C-6), 65.65 (C-4'), 62.0 (C-5), 61.84 (C-6'), 55.38 (OCH_3), 20.8, 20.8 and 20.1 (3 CH_3CO).

Anal. Calcd. for $C_{40}H_{44}O_{15}$ (764.75): C, 62.84; H, 5.80. Found: C, 63.01; H, 5.67.

Methyl 2-O-benzoyl-3-O-(2,4,6-tri-O-acetyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (102a)

A solution of the benzylidene compound **102** (765 mg, 1 mmol) in 70 % acetic acid (10 mL) was stirred at 70° C for 30 min and diluted with water (10 mL). The solution was cooled and evaporated; the residue, after being dried by repeated azeotropic distillation with toluene, was triturated with ethanol and chromatographed with $CHCl_3$-MeOH (9:1) to give 4,6-diol **102a** as a white foam (560 mg, 83 %), $[\alpha]_D$ +66° (c 1, chloroform).

Ms (FAB): m/z 677.22 (M^+ + 1), 645.37 (M^+ - OCH_3), 617.20 (M^+ - OAc), 585.17 (M^+ - CH_2Ph).

^{1}H-NMR: δ 8.02-7.10 (m, arom. H), 5.45 (d, $J_{3',4'}$ 3.1 Hz, 1 H, H-4'), 5.07 (dd, $J_{1',2'}$ 8.2, $J_{2',3'}$ 10.0 Hz, 1 H, H-2'), 4.58 and 4.29 (AB-q, 2 H, J 12.5 Hz, $PhCH_2$), 4.52 (d, $J_{1,2}$ 8.2 Hz, H-1'), 3.64 (dd, $J_{2,3}$ 9.6, $J_{3,4}$ 7.9 Hz, H-3), 3.42 (dd, $J_{2',3'}$ 10.0, $J_{3',4'}$ 3.1 Hz, 1 H, H-3'), 3.34 (s, 3 H, OCH_3), 2.12, 2.07 and 1.30 (3 s, 9 H, 3 OAc).

^{13}C-NMR: δ 172.2, 170.3, 169.3 and 165.3 (3 CH_3CO and PhCO), 101.84 (C-1'), 96.79 (C-1), 82.34 (C-3), 77.22 (C-3'), 72.37 (C-2), 71.24 ($PhCH_2$-), 70.86 (C-4), 69.84 (C-2'), 69.56 (C-5'), 65.55 (C-4'), 62.83 (C-5), 62.0 (C-6, 6'), 55.29 (OCH_3), 21.0, 21.0 and 20.1 (3 CH_3CO).

Methyl 4,6-di-O-acetyl-2-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-3-O-benzyl-β-D-galactopyranosyl)-α-D-glucopyranoside (103)

A solution of the benzylidene compound **100** (950 mg, 1 mmol) in 70 % acetic acid (10 mL) was stirred at 70° C for 30 min and diluted with water (10 mL). Solvent removal left a foam which was not characterized but acetylated directly with a 2:1 mixture of pyridine-acetic anhydride (9 mL) at room temperature overnight. Evaporation of the solution gave a foam which was dissolved in dichloromethane (10 mL) and washed with 5 % aqueous hydrochloric acid, sodium bicarbonate solution, and then with ice-water. Solvent removal then left a foam which was applied to a silica gel column and eluted with ethyl acetate-hexanes (1:1), to give **103** as a solid (729 mg, 77 %), $[\alpha]_D$ +94.3° (c 1.8, chloroform).

Ms (FAB): m/z 947.4 (M^+ + 1).

^{1}H-NMR: δ δ 8.12-6.98 (m, 20 H, arom. H), 5.85 (d, $J_{3',4'}$ 3.1 Hz, 1 H, H-4'), 5.43 (dd, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.5 Hz, 1 H, H-2'), 5.09 (t, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, 1 H, H-4), 5.00 (d, $J_{1,2}$ 3.5 Hz, 1 H, H-1), 4.87 (d, $J_{1',2'}$ 8.9 Hz, 1 H, H-1'), 4.85 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.3 Hz, 1 H, H-2), 4.57 and 4.46 (AB-q, J 11.6 Hz, 2 H, $PhCH_2$), 3.89 (m, 1 H, H-5), 3.81 (m, 1 H, H-5), 3.65 (dd, $J_{2',3'}$ 9.5, $J_{3',4'}$ 3.2 Hz, 1 H, H-3'), 3.26 (s, 3 H, OCH_3), 2.10 and 2.04 (2 s, 6 H, 2 OAc).

^{13}C-NMR: δ 170.7, 169.3 (CH_3CO), 166.2-165.0 (4 signals, PhCO), 136.5-127.5

(multiple lines, arom. C), 101.43 (C-1'), 96.59 (C-1), 76.19 (C-3), 75.59 (C-3'), 73.90 (C-2'), 71.05 (C-5'), 70.93 (C-2), 70.74 ($PhCH_2$), 68.06 (C-4), 67.39 (C-5), 66.22 (C-4'), 62.2 (C-6, 6'), 55.35 (OCH_3), 21.0 and 21.0 (2 $\underline{CH_3}CO$).

Methyl 4,6-di-O-acetyl-2-O-benzoyl-3-O-(3-O-acetyl-2,4,6-tri-O-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranoside (104)

A solution of the benzylidene compound **101** (904 mg, 1 mmol) in 70 % acetic acid (10 mL) was stirred at 70° C for 30 min and diluted with water (10 mL). Evaporation of the solvent gave a foam which was not characterized but acetylated directly with a 2:1 mixture of pyridine-acetic anhydride (9 mL), during 16 h at room temperature. The foamy residue obtained upon concentration of the reaction mixture was dissolved in dichloromethane (10 mL) and washed with 5 % aqueous hydrochloric acid, sodium bicarbonate solution, and then with ice-water. Solvent removal then left a foam which, applied to a silica gel column and eluted with ethyl acetate-hexanes (1:1), yielded **104** as a colorless solid (818 mg, 91 %), $[\alpha]_D$ +55.7° ($\underline{c}$ 1, chloroform).

Ms (FAB): $\underline{m/z}$ 899.9 (M^+ + 1).

^{1}H-NMR: δ 8.12-6.98 (m, 20 H, arom. H), 5.78 (d, $J_{3',4'}$ 3.2 Hz, 1 H, H-4'), 5.43 (dd, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.5 Hz, 1 H, H-2'), 5.11 (t, $J_{3,4} \approx J_{4,5}$ 9.6 Hz, 1 H, H-4), 5.00 (d, $J_{1,2}$ 3.5 Hz, 1 H, H-1), 4.92 (d, $J_{1',2'}$ 7.9 Hz, 1 H, H-1'), 4.90 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.3

Hz, 1 H, H-2), 3.89 (m, 1 H, H-5), 3.29 (s, 3 H, OCH_3), 2.15, 2.04 and 1.57 (3 s, 9 H, 3 OAc).

^{13}C-NMR: δ 172.4, 171.2, 169.0, 166.0, 165.2, 163.1 and 164.5 (7 lines, 3 $CH_3\underline{C}O$ and 4 Ph$\underline{C}O$), 101.49 (C-1'), 96.59 (C-1), 75.91 (C-3), 73.67 (C-3'), 71.26 (C-2), 70.79 (C-5'), 69.63 (C-2'), 68.07 (C-4), 67.55, 67.37 (C-4',5), 62.15 (C-6), 61.55 (C-6'), 55.40 (OCH_3), 20.4, 20.4 and 20.1 (3 $CO\underline{C}H_3$).

Methyl 4,6-di-O-acetyl-2-O-benzoyl-3-O-(2,4,6-tri-O-benzoyl-β-D-galacto-pyranosyl)-α-D-glucopyranoside (105)

A solution of the 3'-O-benzyl compound **103** (950 mg, 1 mmol) in 2-methoxyethanol (15 mL) was hydrogenated catalytically with 10 % palladium/ charcoal (200 mg) overnight, at room temperature and atmospheric pressure. The catalyst was removed and the filtrate evaporated to a foamy residue (788 mg, 92 %). Crystalline material (770 mg, 90%) was obtained by chromatography on silica gel with 10 % methanol in dichloromethane; m.p. 165° C, $[\alpha]_D$ +87° (c 1, chloroform); Ms (FAB): m/z 857.2 (M^+ + 1).

^{1}H-NMR: δ 8.21-7.056 (m, 20 H, arom. H), 5.73 (d, $J_{3',4'}$ 3.3 Hz, H-4'), 5.06 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.98 (d, $J_{1',2'}$ 7.9 Hz, H-1'), 3.81 (m, 1 H, H-5), 3.65 (dd, $J_{2',3'}$ 9.5, $J_{3',4'}$ 3.2 Hz, 1 H, H-3'), 3.29 (s, 3 H, OCH_3), 2.10 and 2.05 (2 s, 6 H, 2 OAc).

^{13}C-NMR: δ 172.4, 170.2, 168.5, 166.3, 166.2 and 165.1 (2 $CH_3\underline{CO}$ and 4 Ph$\underline{CO}$), 100.8 (C-1'), 96.6 (C-1), 75.3 (C-3), 74.0 (C-3'), 72.3 (C-2), 71.0 (C-5'), 70.1 (C-2', 4), 67.9, 67.3 (C-4', 5), 62.1 (C-6), 61.9 (C-6'), 55.4 (OCH_3), 20.9, 20.8 (2 $\underline{CH_3}CO$).

3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (2,2'-dideoxy-2,2'-diphthalimido-β,β-trehalose hexaacetate) (106) and a by-product, possibly the α, β-isomer (107)

To a solution of 1,3,4,6-tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **26** (1.9 g, 4 mmol) and Bu_3SnOCH_2Ph (1.75 g, 4.4 mmol) in $Cl(CH_2)_2Cl$ (150 mL) was added $SnCl_4$ (0.46 mL, 4 mmol) dropwise at 5 °C, and then the mixture was stirred for 4 h at 20-25 °C, poured into aqueous $NaHCO_3$, and extracted with ethyl acetate. The extract was washed with water, dried over magnesium sulfate, and evaporated in vacuo. The crude product showed a strong spot at R_F 0.5 and a weak one at R_F 0.75 in t.l.c. Column chromatography on silica gel with 3:1 hexanes-ethyl acetate gave the faster moving component **107** (375 mg, 11 %) as a syrup, $[\alpha]_D$ +92.6° (c 0.95, chloroform); Ms (FAB): m/z 853.4 (M^+ + 1).

^{13}C-NMR: β-glycosidic moiety: δ 98.7 (C-1), 72.6 (C-3), 69.2 (C-5), 68.3 (C-4),

61.8 (C-6), 54.2 (C-2); α-glycosidic moiety: δ 98.1 (C-1), 70.9 (C-3), 68.1 (C-5), 66.9 (C-4), 61.1 (C-6), 53.3 (C-2).

The column fractions containing the component of R_F 0.5 gave **106** (2.49 g, 73 %) as a syrup with $[\alpha]_D$ -22.7° (c 2, chloroform); Ms (FAB): m/z 853.4 ($M^+ + 1$).

^{1}H-NMR: δ 7.80-7.68 (m, 4 H, Phth), 5.72 (dd, 1 H, $J_{2,3}$ 10.7, $J_{3,4}$ 9.2 Hz, H-3), 5.52 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.92 (~ t, 1 H, $J_{3,4}$ 9.2, $J_{4,5}$ 10 Hz, H-4), 4.17 (dd, 1 H, $J_{1,2}$ 8.6, $J_{2,3}$ 10.7 Hz, H-2), 3.94 (dd, 1 H, $J_{5,6a}$ 4.6, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.72 (m, 2 H, $J_{5,6b}$ 2.2 Hz, H-5,6b), 1.94, 1.92, and 1.80 (3 s, 3 H each, 3 OAc).

^{13}C-NMR: δ 170.6, 170.2, 169.4 (3 $CH_3\underline{C}O$), 134.2, 131.5, 123.5 (Phth. C), 97.1 (C-1), 71.5 (C-3), 70.1 (C-5), 68.3 (C-4), 61.6 (C-6), 53.8 (C-2), 20.4, 20.3, 20.2 (3 $\underline{C}H_3CO$).

Anal. Calcd. for $C_{40}H_{40}N_2O_{19}$ (852.74). C, 56.34; H. 4.73; N, 3.29.

Found: C, 56.32; H, 4.82; N, 3.39.

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AN APPROACH TO THE SYNTHESIS OF ANISOMYCIN

1 INTRODUCTION

The antibiotic anisomycin, a fermentation product of various *Streptomyces* species, was originally isolated from culture filtrates of two *Streptomyces* species (*S. griseolus* and *S. roseochromogenes*) by Sobin and Tanner[1] in 1954. Because this antibiotic specifically blocks peptide bond formation on eukaryotic ribosomes, anisomycin has become a valuable tool in molecular biology and exhibits selective action against pathogenic protozoa and several strains of fungi[2]. The drug has been used with some success in clinical trials for the treatment of both amoebic dysentery and vaginitis[3] caused by *Trichomonas vaginalis*. Anisomycin and deacetylanisomycin (Fig. 1) are used as fungicides to eradicate bean mildew and to inhibit other pathogenic fungi in plants[4]. The gross structure of anisomycin was elucidated by Beereboom *et al.*[5] Subsequently the relative stereochemistry was established by NMR[6] and X-ray investigation[7] and the absolute configuration by chemical correlation studies[8]. The systematic name is (2R, 3S, 4S)-2-(4-methoxybenzyl)-3,4-pyrrolidinediol 3-acetate.

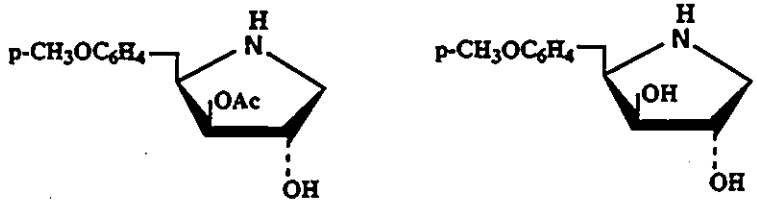
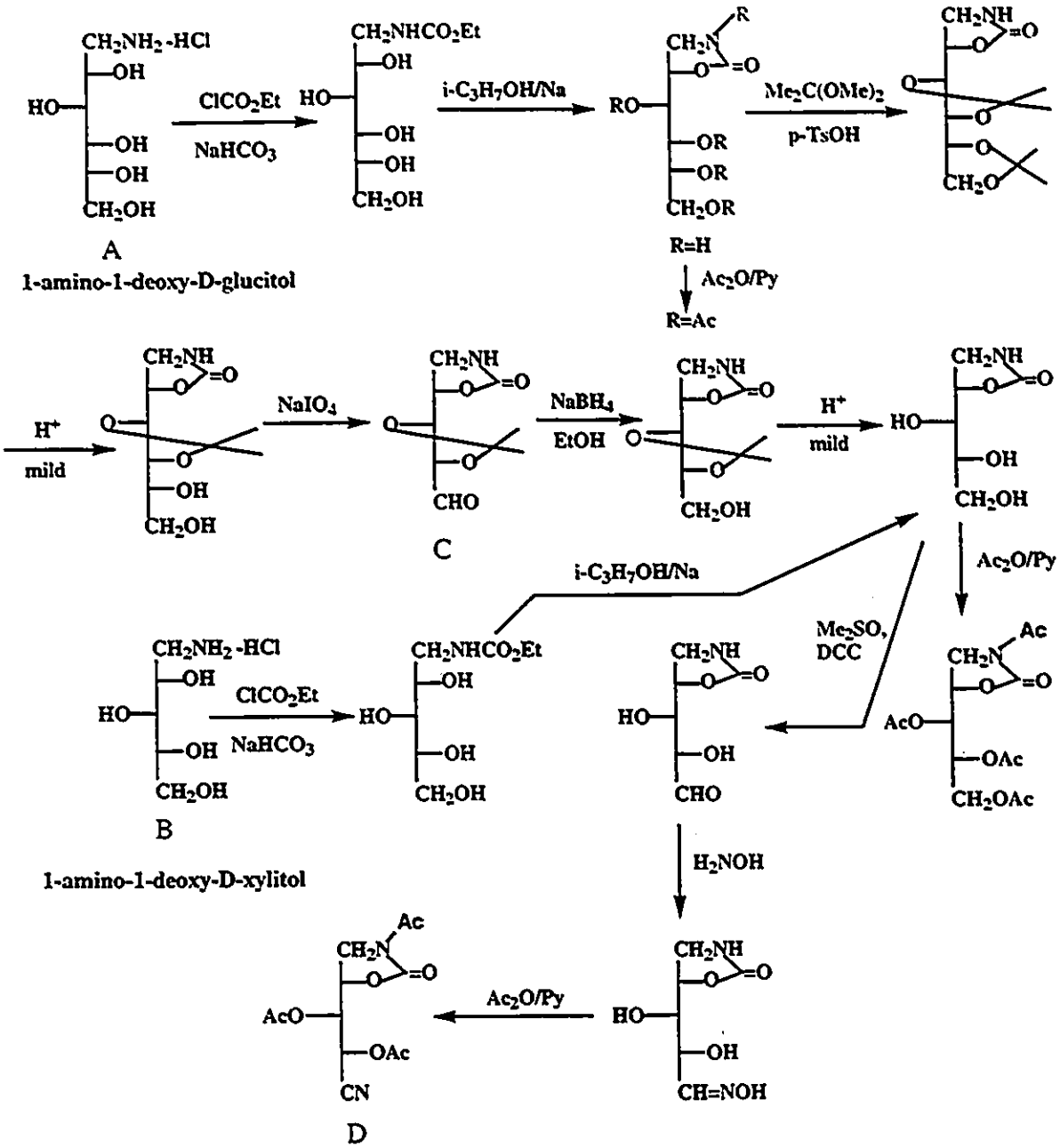


Fig. 1 Anisomycin and deacetylanisomycin

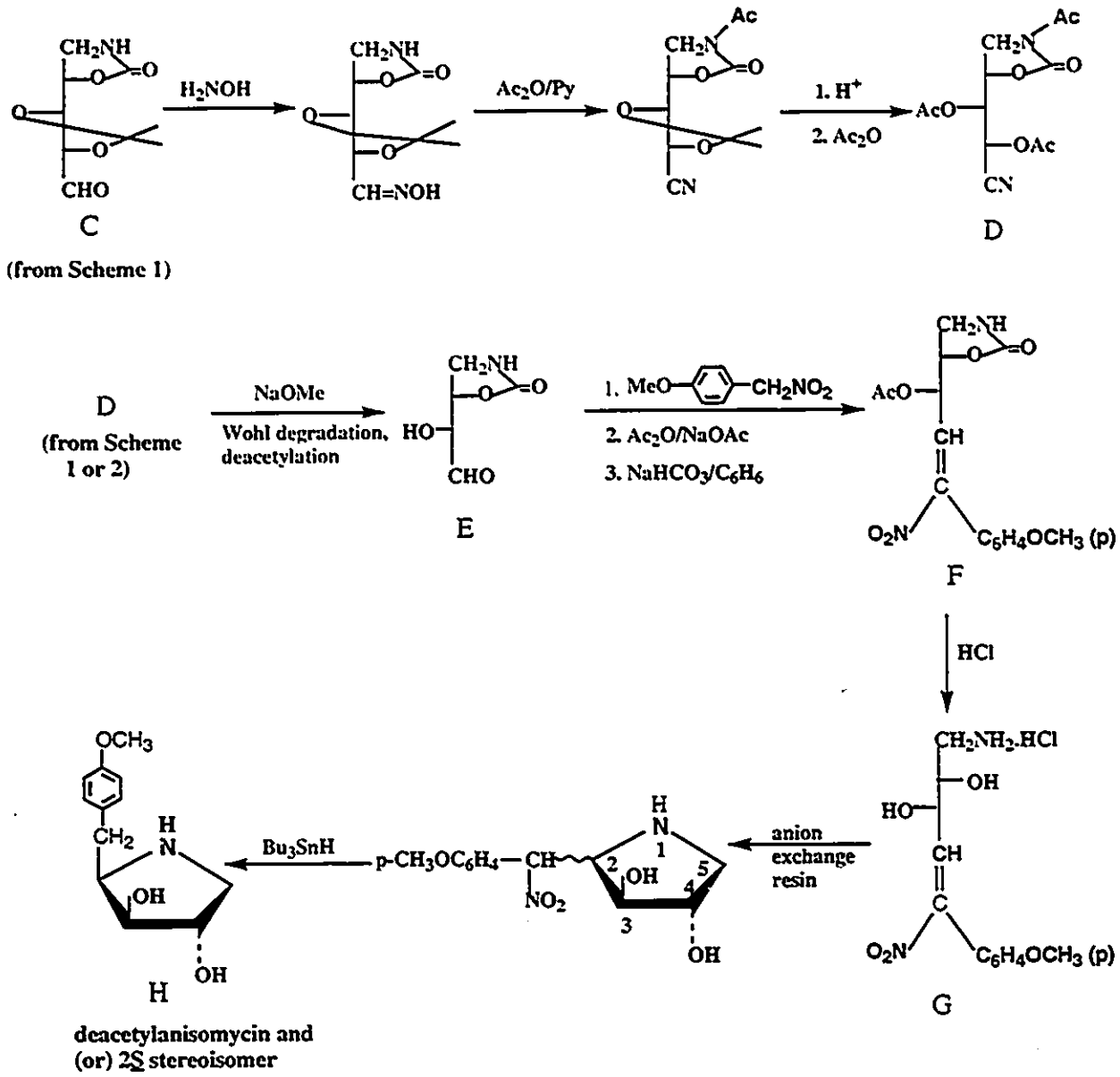
Anisomycin has attracted considerable synthetic interest. Three total syntheses from L-tartaric acid[9], diethyl L-tartrate[10] and 2-(4-methoxybenzoyl)pyrrole[11] were non-stereospecific and gave low overall yields, but a highly selective synthesis from diethyl L-tartrate was accomplished by Iida *et al.*[12] Five chiral syntheses of anisomycin have been reported, three of which were based on the use of carbohydrates as chiral templates. Verheyden *et al.*[13] described an 18-step sequences (8.5 % overall yield) from 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose; Buchanan *et al.*[14] reported a 13-step sequence (6 % overall yield) from D-ribose; and Baer and Zamkanei[15] disclosed an alternative approach, comprising similar strategies but departing ultimately from (more economical) D-galactose in 8 steps with an overall yield of 18 - 20 %.

For the last-mentioned synthesis, the nitrogen atom that was to become part of the pyrrolidine ring was introduced by reaction of hydroxylamine with a derivatized pentose originating from D-galactose. Having completed that synthesis[15], Dr. M. Zamkanei in this laboratory embarked on yet another project of carbohydrate-based anisomycin synthesis. The well-known amino sugar alcohols 1-amino-1-deoxy-D-glucitol and 1-amino-1-deoxy-D-xylitol had recently become available by large-scale, industrial reductive amination of the corresponding sugars at Huls AG, Germany, and generous samples were provided by that company. The idea was to degrade N-protected derivatives of

these starting amino alcohols to an N-protected 4-amino-4-deoxy-L-threose which contains the essential L-*threo* aminodiol structural moiety of anisomycin. The threose would be condensed with anisylnitromethane and, after liberation of the blocked amino function, cyclizing Michael addition to generate the pyrrolidine ring would be attempted. Subsequent denitration by tributylstannane would give deacetylanisomycin (or its 2S-epimer, depending on the stereochemical course of the cyclization). These proposals are represented in Schemes 1 and 2. Scheme 1 shows the transformations of 1-amino-1-deoxy-D-glucitol (A) and 1-amino-1-deoxy-D-xylitol (B) actually completed by Dr. Zamkanei (unpublished results), whereas Scheme 2 illustrates plans for further conversions of some of the products obtained (C and D), including preparation of the N-protected 4-amino-4-deoxy-L-threose (E) and elaboration of the anisomycin structure H as just mentioned. However, the reaction sequences proposed in Scheme 2 were not performed due to the coworker's departure from the laboratory, and thus the project remained unfinished. It became a task for the present thesis to devote some studies toward achievement of the goal.



Scheme 1 Degradation of 1-amino-1-deoxy-D-glucitol (A) and 1-amino-1-deoxy-D-xylitol (B) performed by M. Zamkanei

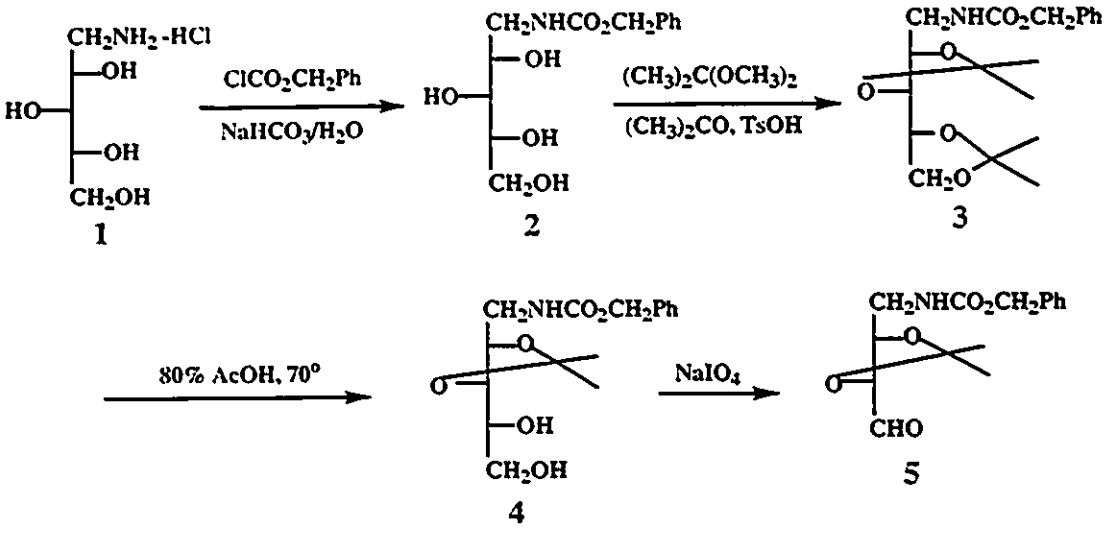


Scheme 2 Projected synthesis of a 4-amino-4-deoxy-L-threose derivative (E) and deacetylanisomycin H (Zamkanei)

2 RESULTS AND DISCUSSION

At the outset, the question was raised whether the strategy of N-protection employed by Zamkanei, namely by generation of cyclic carbamates, might not pose a problem at the late stage of deprotection (step F→G in Scheme 2). An acid hydrolysis is required (base is precluded as it would attack the nitroalkene function). Although carbamates generally are hydrolyzable by mineral acid, rather drastic conditions may be needed and it was feared that these might also affect the nitroalkene part of the molecule. Therefore, an alternative blocking method was to be considered. Furthermore, it was thought that a somewhat simpler approach to a suitable, N-protected 4-amino-4-deoxy-L-threose could be devised. In consequence, the reactions depicted in Scheme 3 were proposed, and these could in fact be realized.

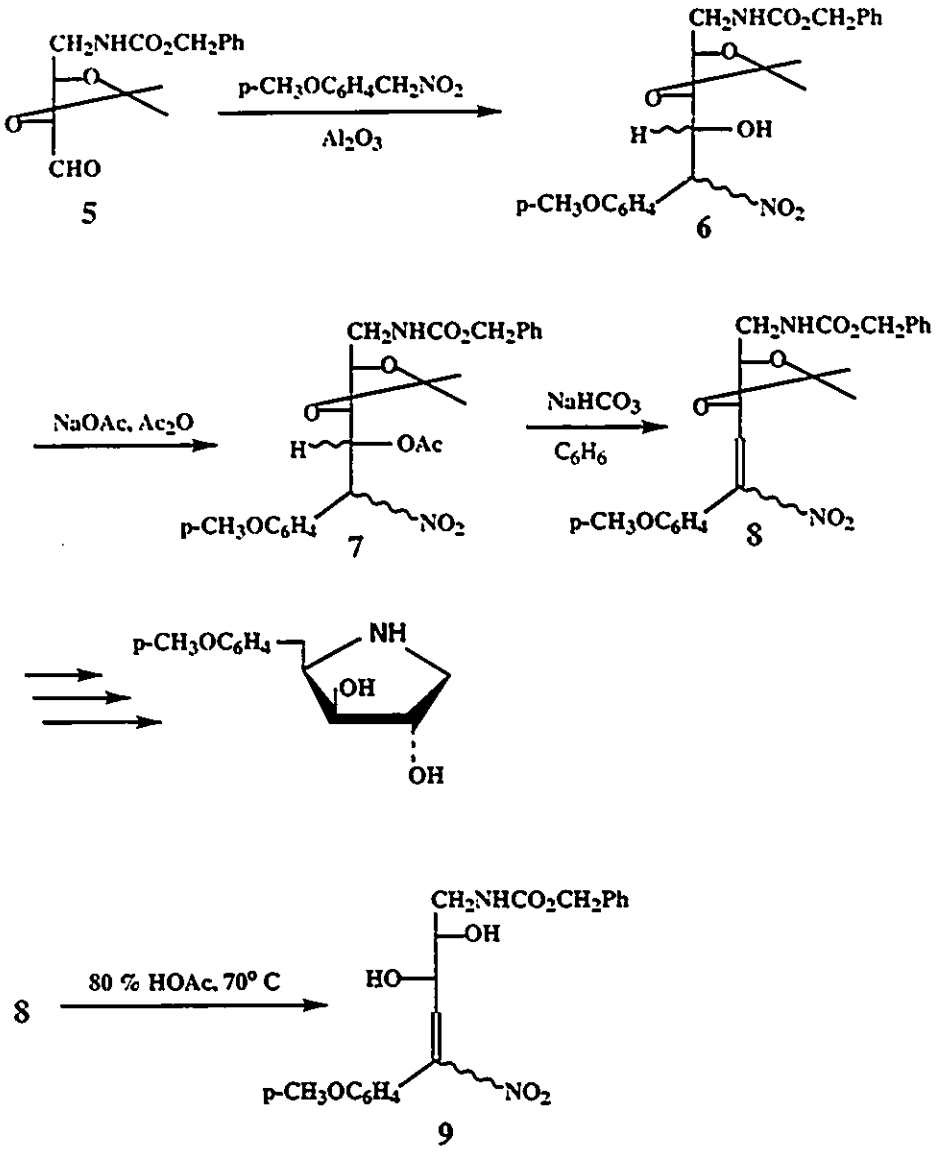
Treatment of 1-amino-1-deoxy-D-xylitol hydrochloride **1** with benzyl chloroformate in the presence of sodium bicarbonate in water at room temperature gave 1-benzyloxycarbonylamido-1-deoxy-D-xylitol **2** as an oil in 80 % yield. The product **2** was acetonated with 2,2-dimethyoxypropane in the presence of a catalytic amount of p-toluenesulphonic acid to furnish 1-benzyloxycarbonylamido-1-deoxy-2,3:4,5-di-O-isopropylidene-D-xylitol **3** as a syrup with 90 % yield. Partial deacetonation of **3** by mild hydrolysis (with 80



Scheme 3 Synthesis of the 4-amino-4-deoxy-L-threose derivative 5 as an intermediate for a new approach to anisomycin

% acetic acid at 70° for 20 min) gave 1-benzyloxycarbonylamido-1-deoxy-2,3-O-isopropylidene-D-xylitol **4**, also in 90 % yield. Oxidation of the diol **4** with sodium periodate quantitatively afforded 1-benzyloxycarbonylamido-1-deoxy-2,3-O-isopropylidene-L-threose **5**. The syrupy sugar was characterized by spectral data and gave correct microanalytical data.

With the key intermediate **5** in hand, chain elongation and cyclization by nitroalkane methodology, essentially in analogy to the proposal of Scheme 2, was to be pursued. The base-catalyzed addition of nitroalkanes to aldehydes (Henry reaction) is a classical procedure which has been utilized many times in carbohydrate Chemistry[16]. The reagent to be used in the present instance, 4-methoxyphenylnitromethane, could not be obtained commercially and was prepared by substitution, with sodium nitrite in N,N-dimethylformamide, of 4-methoxybenzyl bromide which, in turn, was obtained from commercial 4-methoxybenzyl alcohol and tribromophosphine. The nitro reagent was allowed to react with the aldehyde **5** in dichloromethane solution under catalysis by basic aluminum oxide (Scheme 4). The resulting crude mixture of diastereomeric nitro alcohols gave microanalytical data (C, H, N) in excellent accord with the composition $C_{23}H_{28}N_2O_8$ of the expected reaction product, 5-benzyloxycarbonylamido-3,4-O-isopropylidene-1-(4-methoxyphenyl)-1-nitropen-

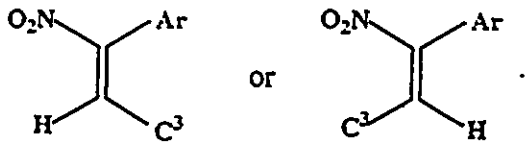


Scheme 4. Synthesis of anisomycin by means of nitroalkene intermediate

tane-2,3,4-triol **6**. It's ^{1}H-NMR spectrum showed substituent resonances for aromatic protons (multiplets at δ 7.50-6.85), methoxyl protons (δ 3.75), and isopropylidene methyl protons (δ 1.4-1.2) in an intensity ratio of approximately 9:3:6, corroborating the expected structure. Likewise, the ^{13}C-NMR spectrum contained all the signals required for **6**, listed in the Experimental section. However, many of signals were doubled or even tripled, indicating that the product was a mixture of closely related stereoisomers. For the same reason the ^{1}H-NMR signals for aliphatic protons in the δ 6.5-2.9 region were poorly resolved and not amenable to detailed analysis although their integration agreed with the structural formula. In the formation of **6** from **5**, two new chiral centers are generated, giving rise to a possible four diastereomers. No efforts were made at separation because the chirality of C-1 and C-2 would disappear again at a later stage of the synthesis. The crude **6** was than acetylated with acetic anhydride in the presence of sodium acetate at room temperature, to provide the corresponding 2-acetates **7** in 80 % yield based on **5**. The CI mass spectrum of **7** (expected molecular weight, 502.5) showed molecular ion peaks at m/z 502 and 503, and in the ^{13}C-NMR spectrum appeared the resonances for O-acetyl (δ 168.5 for C=O and 20.7 for CH_3), indicating successful acetylation. When the acetylation was performed in the presence of pyridine instead of sodium acetate,

an additional acetyl group was introduced according to NMR evidence, namely, at the nitrogen of the benzyloxycarbonylamido function. Similar N-acetylation of carbamates had occurred in three of the reactions of Scheme 1. It appears that such amido nitrogen possesses significant nucleophilicity, and this observation will be recalled at a later stage of the synthesis.

The next step involved dehydroacetoxylation of the β-nitro acetate 7 to generate nitroalkene 8, which is also a procedure widely used in the chemistry of aliphatic nitro compounds and nitro carbohydrates (the Schmidt-Rutz reaction[16][17]). The elimination was performed by action of dry sodium hydrogencarbonate in boiling benzene. It caused the chirality at C-1 and C-2 to be relinquished, but two geometrical isomers (E and Z) could arise. The crude product 8 showed a major and a minor spot in TLC, and column chromatography furnished the major isomer pure, in 70 % yield. It gave correct microanalytical (C,H,N) and mass spectral ($M^+ + 1$ at m/z 443) data for the expected composition $C_{23}H_{26}N_2O_7$, and all the signals of its clear ^{13}C-and ^1H-NMR spectra could be attributed with confidence, aided by a HETCOR plot. However, it was not possible to determine the double bond geometry:

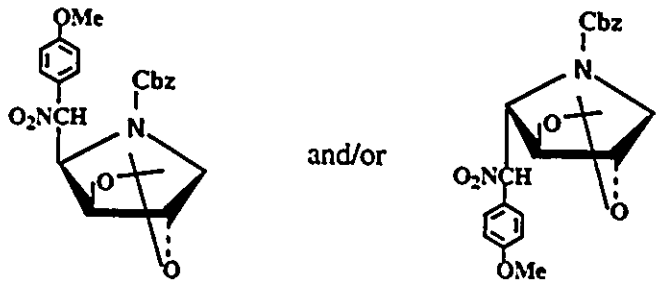


E Z

Compound 8 was deisopropylidenated by hydrolysis with 80 % acetic acid at 70° C to afford the diol 9 (85 %). The product was characterized by microanalysis and NMR spectra (see Experimental). Particularly significant was the disappearance, from the ^{13}C spectrum, of the signals attributable to the isopropylidene group that were present in the spectrum of 8 at δ 110.7, 26.8 and 26.6, and an upfield shift of the C-3 and C-4 signals from 79.5 and 74.3 to 72.8 and 69.0.

The next steps in the project would be, in principle, deprotection of the amino function in 9 and a cyclizing Michael addition of the free amine across the nitroalkene group, to generate the pyrrolidine structure (and finally, reductive removal of the nitro group), analogous to the proposal of Scheme 2 (F → G → H). However, in view of the aforementioned observation that carbamate nitrogen appears to possess significant nucleophilic character it was worthwhile to attempt direct cyclization of the N-carbobenzoxy derivatives 8 or 9; nitroalkenes are excellent Michael acceptors which react even with weak nucleophiles[16][17]. Cyclization in 8 might be impeded by the presence of the *trans*-fused dioxolane ring, but 9 is free from such constraint and should be able to cyclize readily, at least as far as the steric requirements are concerned. In either case, attack of the amido nitrogen could occur on the Re or the Si face

of the nitroalkene double bond, giving rise to the 2R or the 2S pyrrolidine (anisomycin or 2-epi-anisomycin structure), and it would be interesting to study the stereochemistry of this reaction:



Numerous experiments were undertaken to achieve cyclization. These included heating of **8** and **9** in boiling benzene, treating them in benzene at room temperature in the presence of non-nucleophilic bases (t-BuOK or NaH), or under assistance of mercuric acetate. Unfortunately, success evaded all these attempts. While reactions did appear to take place in most of the trials, it was difficult to unravel the complex NMR spectra of the product mixtures, and the desired products of ring closure, if indeed they were formed, could not be isolated.

EXPERIMENTS

1-Benzyloxycarbonylamido-1-deoxy-D-xylitol (2)

To a mixture of 1-amino-1-deoxy-D-xylitol hydrochloride 1 (1.0 g, 5.33 mmol) and sodium bicarbonate (1.5 g, 18 mmol) in 20 mL of water was added benzyl chloroformate (1.82 g, 10.7 mmol) at 0° C, and the reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated, the residue was extracted with 99 % ethanol (3 X 30 mL), and the extracts were concentrated to a clear syrup. A white solid (1.2 g, 80 %) was obtained by triturating the syrup with fresh ethanol; m.p.64-66 °C, $[\alpha]_D$ -9.2° (c 1.7, MeOH), R_f 0.41 (20% MeOH in CH_2Cl_2). Ms(CI): m/z 286 (60 %, $M^+ + 1$).

^{13}C-NMR (D_2O): δ 139.9, 131.8, 131.4, 130.7 (Ph), 75.0, 74.0, 73.1 (C-2,3,4), 69.9 ($PhCH_2$), 65.4 (C-5), 46.0 (C-1).

Anal. Calcd. for $C_{13}H_{19}NO_6$ (285.29): C, 54.73; H, 6.71; N, 4.91.

Found: C, 54.53; H, 6.74; N, 4.86.

1-Benzyloxycarbonylamido-1-deoxy-2,3:4,5-di-O-isopropylidene-D-xylitol (3)

To a mixture of compound **2** (5 mmol, 1.43 g) in 15 mL of 2,2-dimethoxypropane and 30 mL of acetone was added a catalytic amount (50 mg) of p-toluenesulphonic acid. The mixture was stirred overnight and the sugar gradually went into solution. Triethylamine (1 mL) was added and the solution was evaporated. The residue was chromatographed on a column of silica gel by elution with 3:1 hexane-ethyl acetate, which gave crystalline **3** (1.64 g, 90 %); m.p. 44 °C; $[\alpha]_D$ -9.5° (c 1.2, ethyl acetate); Ms (CI): m/z 366 (M^+ + 1), 308 (M^+ + 1 - Me_2CO).

^{13}C-NMR: δ 156.59 (CO), 136.39, 128.55, 128.22, 128.13 (arom. C), 109.73, 109.56 (2 O_2CMe_2), 77.56, 76.11, 74.66 (C-2,3,4), 66.81 (CH_2Ph), 65.44 (C-5), 42.00 (C-1), 26.94, 26.63, 25.89, 25.23 (2 x CMe_2).

Anal. Calcd. for $C_{19}H_{27}NO_6$ (365.42): C, 62.45; H, 7.45; N, 3.83. Found: C, 62.53; H, 7.54; N, 3.83.

1-Benzyloxycarbonylamido-1-deoxy-2,3-O-isopropylidene-D-xylitol (4)

Compound **3** (1.0 g, 2.74 mmol) was treated with 20 mL of 80 % acetic acid at 70° for 20 min. The solution was cooled, neutralized with solid sodium carbonate and evaporated to dryness. The residue was extracted with dichloromethane and the extract concentrated and applied to a small column of silica gel. Elution with 1:1 hexane-ethyl acetate afforded **4** as a syrup (0.80 g, 90 %); $[\alpha]_D$ +9.2° (c 9, ethyl acetate); Ms (CI): m/z 326 (M^+ + 1), 268 (M^+ + 1 - Me_2CO).

^{13}C-NMR: δ 152.3 (CO), 136.5, 128.5, 128.3, 128.0 (arom. C), 109.8, (O_2CMe_2), 77.4, 76.7, 73.5 (C-2,3,4), 66.5 (CH_2Ph), 62.0 (C-5), 46.5 (C-1), 26.9, 26.6 (CMe_2).

Anal. Calcd. for $C_{16}H_{23}NO_6$ (325.35): C 59.06; H, 7.12; N, 4.30.

Found: C, 58.84; H; 7.18; N; 4.27.

4-Benzyloxycarbonylamido-4-deoxy-2,3-O-isopropylidene-L-threose (5)

To compound **4** (1.63 g, 5 mmol) in 20 mL of water was added sodium metaperiodate (1.1 g, 5.1 mmol) at room temperature. After 4 h the solvent was evaporated and the residue was then extracted with dichloromethane (3 x 20 mL). Concentration of the extract gave **5** as a thick oil (1.41 g, 96 %); $[\alpha]_D$ -3.3°

(c 2.5, ethyl acetate); Ms (CI): m/z 294 (M^+ + 1).

^{1}H-NMR: δ 9.74 (d, $J_{1,2}$ 1.2 Hz, aldehydic H-1; this signal was sharp, but of only 0.25 H intensity); there were substituent resonances at δ 7.3 (Ph), 5.1 ($\underline{C}H_2Ph$), and 1.45-1.35 (Me_2C) with intensity ratio 5:2:6.

^{13}C-NMR: δ 171.3 (weak; aldehyde CO), 156.8 (benzyloxycarbonyl CO), 136.4, 128.5, 128.1 (arom. C), 111.3 ($O_2\underline{C}Me_2$), 82.2 (strong; hydrated aldehyde C-1), 75.7 (C-3), 66.7 ($\underline{C}H_2Ph$), 60.0 (C-2), 42.6 (C-4), 26.5 and 25.8 ($C\underline{Me}_2$).

The above NMR data indicate that the spectroscopic sample was partially hydrated. For microanalysis the sample was dehydrated by drying over P_2O_5 in vacuo at 80 °C.

Anal. Calcd. for $C_{15}H_{19}NO_5$ (293.31): C 61.42; H 6.53; N 4.78.

Found: C 61.41; H, 6.60; N, 4.68.

(1RS, 2RS, 3S, 4S)-5-Benzyloxycarbonylamido-3,4-O-isopropylidene-1-(4-methoxyphenyl)-1-nitropentane-2,3,4-triol (6) and its 2-acetate (7)

A solution of the aldehyde 5 (586 mg, 2 mmol) and 4-methoxyphenyl-nitromethane (670 mg, 4 mmol) in dichlorcmethane (20 mL) was stirred with basic aluminum oxide (2 g), and the mixture was boiled under reflux for 4 h and then allowed to stand overnight at room temperature. TLC showed no 5

remaining (1:1 hexane-ethyl acetate). The aluminum oxide was filtered off and washed with dichloromethane (3 x 20 mL). Evaporation of the filtrate at reduced pressure gave the crude mixture of nitro alcohol **6** as a pale yellow syrup (736 mg, 80 %).

Ms (CI): m/z 461 (1.5 %, $M^{\cdot} + 1$), 460 (7 %, $M^{\cdot}$), 459 (23 %, $[M - H^{\cdot}]^{\cdot}$), 414 (34 %, $M^{\cdot} - NO_2$), 413 (98 %, $[M - H^{\cdot}]^{\cdot} - NO_2$), 356 (77 %, $M^{\cdot} - NO_2 - Me_2CO$), 355 (100 %, $[M - H^{\cdot}]^{\cdot} - NO_2 - Me_2CO$).

^{1}H-NMR: δ 7.50-7.25 and 6.85 (m, 9 H, arom.), 5.50, 5.00, 4.50, 4.10, 3.30, and 2.95 (centers of multiplets, 10 H, aliphatic and benzylic H), 3.78 and 3.75 (s, 3 H, OCH_3), 1.39-1.23 (singlets, 6 H, isopropylidene).

^{13}C-NMR: δ 161.03, 160.98, 160.60 (C-4 of methoxyphenyl), 157.32, 156.74 (C=O), 136.28, 136.17 (C-1 of phenyl), 131.61, 130.42, 129.84 (C-2,2' of methoxyphenyl), 128.59, 128.25, 128.04 (C-2,2',3,3',4 of phenyl), 123.87, 123.22, 122.30 (C-1 of methoxyphenyl), 114.48, 114.22, 113.88 (C-3,3' of methoxyphenyl), 109.50 ($\underline{C}Me_2$), 94.28, 92.13, 90.85 (C-1), 76.33, 75.76, 75.44, 73.57, 72.83, 69.79 (C-2,3,4), 67.01, 66.88 ($\underline{C}H_2Ph$), 55.16, 55.09, 55.04 (OCH_3), 42.85, 42.55, 41.05 (C-5), 26.73, 26.68, 26.58, 26.48 ($C\underline{Me}_2$).

Anal. Calc. for $C_{23}H_{28}N_2O_8$ (460.47): C, 59.99; H, 6.13; N, 6.09. Found: C, 60.02; H, 6.13; N, 6.17.

Compound **6** was then acetylated by acetic anhydride (5 mL) and sodium acetate (0.3 g), at room temperature overnight. The reaction mixture was diluted with 20 mL of dichloromethane, washed with saturated sodium bicarbonate and water, and concentrated. Chromatography (silica gel, 1:1 EtOAc-hexane) afforded the diastereomeric acetates 7 as a light yellow syrup (735 mg, 73 % from 5). Ms (CI): m/z 503 (4 %, $M^+ + 1$), 502 (6.3 %, M^+), 501 (2.6 %, $[M - H^-]^+$), 445 (27 %, $M^+ + 1 - Me_2CO$), 444 (100 %, $M^+ - Me_2CO$), 443 (77 %, $M^+ + 1 - AcOH$).

^{1}H-NMR: δ 7.50-7.20 and 6.92-6.80 (m, 9 H, arom.), 5.75, 5.00, 4.42, 4.20, 3.95, 3.75, and 3.35 (centers of multiplets, 10 H, aliphatic and benzylic H), 3.80 and 3.75 (s, 3 H, OCH_3), 2.10 (s, 3 H, CH_3CO), 1.39-1.23 (singlets, 6 H, isopropylidene).

^{13}C-NMR: δ 168.50 (CO of Ac), 156.40 (CO of cbz), 136.28, 128.75, 128.51, 128.11, 127.79, 114.16, 113.76, 113.67 (arom. C of methoxyphenyl and cbz), 110.54 (O_2CMe_2), 87.42, 77.33, 76.61, 76.61, (C-1,2,3,4), 66.92 (CH_2Ph), 55.24 (OCH_3), 42.83 (C-5), 27.16, 26.34 (CMe_2), 20.70 (CH_3CO).

(3S,4S)-5-Benzyloxycarbonylamido-3,4-O-isopropylidene-1-(4-methoxyphenyl)-1-nitro-1-pentene-3,4-diol (8)

A suspension of dry, solid sodium hydrogencarbonate in a solution of the 2-acetate 7 (735 mg, 1.6 mmol) in dry benzene (30 mL) was boiled under reflux for 4 h. The cooled and filtered solution was washed with water, dried (Na_2SO_4), and evaporated. The residue showed 2 spots in TLC (R_F 0.6 and 0.5 in 1:1 hexane-ethyl acetate). It was applied to column chromatography using 1:1 hexane-ethyl acetate as eluent, furnishing the nitroalkene 8 (495 mg, 70 %) as a syrup that appeared uniform according to NMR. $[\alpha]_D$ -6.5° (c 1.1 chloroform). Ms (CI): m/z 443 (M^+ + 1), 411 (M^+ - OCH_3), 385 (M^+ + 1 - Me_2O), 335 (M^+ - OCH_2Ph).

^{1}H-NMR: δ 7.35-6.95 (m, 10 H, 9 aromatic protons and H-2), 5.02 (AB-q, 2 H, $PhCH_2$), 4.86 (t, 1 H, exchangeable, CO-NH), 4.10 (m, 2 H, H-3,4), 3.78 (s, 3 H, OCH_3), 3.38 and 3.10 (ddd and dt, 1 H each, H-5,5'), 1.40 and 1.32 (2s, 3 H each, CMe_2). The assigments were verified by a HETCOR spectrum.

^{13}C-NMR: δ 161.1 (C-4 of p-methoxyphenyl), 156.2 (CO of benzyloxycarbonyl), 154.9 (C-1), 136.2 (C-1 of phenyl), 132.0 (C-2,2' of p-methoxyphenyl), 131.7 (C-2), 128.6, 128.3, 128.1 (C-2,2',3,3'4 of phenyl), 120.2 (C-1 of p-methoxyphenyl), 114.1 (C-3,3' of p-methoxyphenyl), 110.7 (O_2CMe_2), 79.5, 74.3

(C-3,4), 66.9 ($\underline{C}H_2Ph$), 55.2 (OMe), 41.4 (C-5), 26.8 and 26.6 ($O_2C\underline{Me}_2$).

Anal. Calc. for $C_{23}H_{26}N_2O_7$ (442.45): C, 62.43; H, 5.92; N, 6.33.

Found: C, 62.24; H, 5.94; N, 6.15.

(3S,4S)-5-Benzyloxycarbonylamido-1-(4-methoxybenzyl)-1-nitro-1-pentene-3,4-diol (9)

Compound **8** (1.0 g, 2.3 mmol) was treated with 80 % acetic acid (5 mL) at 70° for 20 min, and the reaction mixture was processed as described for the preparation of **4**. Purification of the product by column chromatography (silica gel, 1:1 hexane-ethyl acetate) afforded **9** as syrup (0.8 g, 85 %); $[\alpha]_D$ -1.3° (c 1.0, chloroform).

Ms (CI): m/z 403 ($M^+ + 1$), 402 (M^+), 385 ($M^+ + 1 - H_2O$), 384 ($M^+ - H_2O$), 357 ($M^+ + 1 - NO_2$), 356 ($M^+ - NO_2$), 338 ($M^+ - NO_2 - H_2O$), 312 ($M^+ + 1 - CH_2Ph$), 294 ($M^+ + 1 - CH_2Ph - H_2O$), 266 ($M^+ + 1 - NO_2 - CH_2Ph$).

^{1}H-NMR: δ 7.39-7.19 and 6.90-6.80 (m, 9 H, arom.), 5.65, 5.20, 5.00, 4.05, 3.75, and 3.25 (centers of multiplets, 10 H, aliphatic, benzylic and hydroxyl H), 3.80 and 3.75 (s, 3 H, OCH_3).

^{13}C-NMR: δ 160.9 (C-4 of p-methoxyphenyl), 157.7 (CO of benzyloxycarbonyl), 154.9 (C-1), 136.0 (C-1 of phenyl), 133.7 (C-2), 131.8 (C-2,2' of p-methoxyphenyl), 128.6, 128.3, 128.1 (C-2,2',3,3'4 of phenyl), 120.7 (C-1 of p-

methoxyphenyl), 114.6 (C-4), 114.1 (C-3,3' of p-methoxyphenyl), 72.8, 69.0 (C-3,4), 67.2 ($\underline{C}H_2Ph$), 55.2 (OMe), 43.2 (C-5).

Anal. Calc. for $C_{20}H_{22}N_2O_7 \cdot 0.5\ H_2O$ (402.39+9.01): C, 58.39; H, 5.64; N, 6.81. Found: C, 58.65; H, 5.62; N, 6.52.

p-Methoxybenzyl bromide

To 20 g (0.145 mol) of p-methoxybenzyl alcohol in 40 mL of benzene was added tribromophosphine (6 mL) in 15 mL of benzene dropwise at 0-5 °C, and then the mixture was stirred for 2 h at room temperature. Cold water was added and the organic phase was separated, washed with sodium bicarbonate, and water, and dried over sodium sulphate. After evaporating most of the benzene, the residue was distilled in an oil-pump vacuum to give p-methoxybenzyl bromide as a colourless liquid, b.p. 95-96 °C (18 mm Hg), yield 18.1 g (62 %).

^{13}C-NMR: δ 159.8, 130.7 130.1 and 114.4 (C-4, C-2,2', C-1 and C-3,3' of p-methoxyphenyl), 55.4 ($O\underline{C}H_3$), 34.6 ($\underline{C}H_2Br$).

p-Methoxyphenylnitromethane

4-Methoxybenzyl bromide (18.1 g, 90 mmol) was added to a stirred solution of sodium nitrite (11.2 g, 162 mmol) in dimethylformamide (200 mL). The solution was stirred for 8 h at room temperature, cold water (300 mL) was added, and the mixture was extracted with ether (3 x 150 mL). The combined extracts were dried with sodium sulphate, concentrated, and the residue was distilled in an oil-pump vacuum, to afford the title compound as a pale yellow liquid (9.5 g, 63 %), b.p. 122 °C (22 mm Hg); Ms (CI): m/z 167.9 (M^+ + 1). ^{13}C-NMR: δ 160.7, 131.9 131.5 and 114.3 (arom. C), 79.4 ($\underline{C}H_2NO_2$), 55.2 $O\underline{C}H_3$).

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