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Recent developments in the synthesis of glycoconjugates

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Abstract - The biological significance of glycoconjugates has stimulated a great synthetic activity in the synthesis of these structurally demanding compounds. These efforts were in the beginning mainly concentrated on improvements of the well-known KOENIGS-KNORR METHOD providing finally a very valuable methodology for glycoconjugate synthesis. However, due to several, mainly inherent disadvantages other anomeric oxygen exchange reactions have been investigated for the generation of glycosyl donor properties. For instance, GLYCOSYL FLUORIDES and SULFIDES and their activation with fluorophilic and thiophilic catalysts, respectively, was studied in several laboratories, as shortly discussed.

Other activation principles were investigated in our laboratory, namely the base catalyzed activation retaining the anomeric oxygen atom. This led to the direct ANOMERIC O-ALKYLATION procedure with primary triflates as the alkylating agents providing a most convenient method for glycoside bond formation due to its simplicity and the yields and stereoselectivities obtained. However, the direct base catalyzed activation with trichloroacetonitrile afforded O-glycosyltrichloroacetimidates which are in terms of stability, reactivity, and general applicability outstanding glycosyl donors. The scope of this TRICHLOROACETIMIDATE METHOD is summarized in this paper.

INTRODUCTION

The presence of complex carbohydrate structures as integral constituents of membranes and cell walls has led to manifold activities in recent research (ref. 1). An especially important role amongst these glycoconjugates seem to play the glycosphingolipids, the glycophospholipids, and the glycoproteins. Their manifold functions more and more recongnized only very recently (ref. 1-4), are based on a great structural diversity of the oligosaccharide portion, which is inherent to the variability in glycoside bond formation (ref. 1,3). This point was recently impressively illustrated (ref. 1), rendering oligosaccharides ideal as carriers of biological information and specificity.

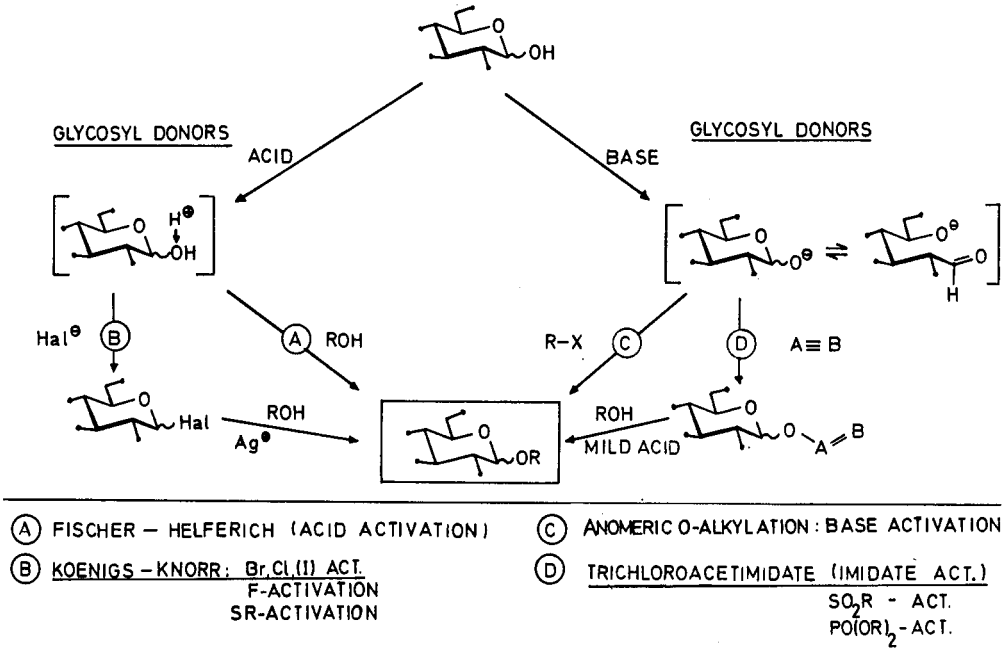
The biological significance of glycoconjugates should be viewed by the synthetic chemist as a major challenge (ref. 1): The synthesis and modification of the oligosaccharidic moieties and their coupling with appropriate lipids, phospholipids, and proteins is essential to extend our knowledge on the molecular mode of action of glycoconjugates and to derive new principles of physiological activity. Due to the great structural variability on the different structural levels, homogeneous compounds are often only with great difficulty accessible from biological material.

GLYCOSYL DONORS VIA ANOMERIC OXYGEN EXCHANGE REACTIONS: THE KOENIGS-KNORR METHOD AND RELATED METHODS

Indeed, the biological significance of glycoconjugates has stimulated a great synthetic activity in the last years (ref. 1,4-6). However, these efforts were in the beginning mainly concentrated on improvements of the well known Koenigs-Knorr method for oligosaccharide and glycoconjugate synthesis (Scheme 1, Path B) which finally led to a very valuable methodology. This methodology has been recently reviewed quite extensively (ref. 4,6). However, several

inherent disadvantages makes the Koenigs-Knorr method often experimentally demanding and certainly not very suitable for large scale preparations (ref. 1). Therefore additional methods are urgently needed.

Scheme 1



SYNTHESIS OF GLYCOSIDES AND SACCHARIDES

For the generation of glycosyl donor properties other anomeric oxygen exchange reactions have been recently investigated quite extensively. Closely related to the Koenigs-Knorr method, where bromine and chlorine are the leaving groups, fluorine has been introduced as the leaving group (Scheme 1) (ref. 1,7). Due to the difference in halophilicity of this element compared with bromine and chlorine, besides silver salts special catalyst systems were found as activators for glycosylation reactions (ref. 7,8). However, due to generally lower glycosyl donor properties of glycosyl fluorides, these intermediates have gained thus far not a general importance for the synthesis of complex glycoconjugates. The main reason for the decrease in reactivity is the strength of the carbon-fluorine bond, which enables even base catalyzed modifications of unprotected hydroxy groups in presence of an anomeric fluorine atom. For instance, successful O-benzylation of completely O-unprotected α-D-glucopyranosyl fluoride was described, providing eventually advantages in terms of the strategy of the glycoconjugate synthesis (ref. 9).

Recently also thioglycosides, where the anomeric oxygen is replaced by an alkyl or arylthio group, have attracted considerable attention as glycosyl donors (Scheme 1) (ref. 10). Similar to the glycosyl fluorides, they appear to offer efficient temporary protection of the anomeric centre, and in addition, several possibilities for regioselective activation into glycosyl donors exist. Earlier attempts for activation include mainly mercury (II), copper (II), and lead (II) salts and N-bromo succinimide. However, besides having disadventageous heavy metal salt activation, the activation was too low to be of general applicability in glycoconjugate synthesis. This problem was partly circumvented by using heterocyclic thioglycosides (ref. 11,12). Not surprisingly, this methodology has been successfuly applied with the more reactive 2-deoxyglycosyl donors, for instance, in the synthesis of erythromycin, avermectin, and digitoxin (ref. 10).

Recent work has demonstrated that, in a two-step process, thioglycosides can be firstly transformed with bromine and chlorine and related halogenating compounds into glycosyl halides, thus enabling subsequently the application of the Koenigs-Knorr method (ref. 10). In addition, two other one-step activations with thiophilic reagents were found. Methyl trifluoromethanesulfonate (methyl triflate) seems to be a good activator of thioglycosides providing

glycosides diastereoselectively, however only with neighboring group participation (ref. 13). An additional disadvantage of this reagent is the suspected health hazard and the formation of O-alkylation products. In contrast, dimethyl (methylthio) sulfonium trifluoromethanesulfonate (DMTST) recently introduced by Fügedi and Garegg (ref. 14) is uniquely thiophilic and gives rise to faster glycosylations than does methyl triflate. Glycosyl donors with neighboring group participation during reaction generate 1,2-trans-linked glycosides in excellent yields and virtually complete stereospecificity. However, with a nonparticipating group in 2-position of the glycosyl donor, the stereoselectivity is thus far poor, limiting the generality of this methodology.

Also some extensions of the Fischer-Helferich method (Scheme 1, Path A) were reported (ref. 1,15). However, due to the reversibility this method has not gained importance for the synthesis of complex oligosaccharides and glycoconjugates.

ACTIVATION THROUGH RETENTION OF THE ANOMERIC OXYGEN: THE ANOMERIC O-ALKYLATION AND THE TRICHLOROACETIMIDATE METHOD

(A) General Aspects. A new, versatile, and generally applicable method for glycoside, oligosaccharide, and glycoconjugate synthesis should meet the following requirements (ref. 1,6):

- For the first step, the activation of the anomeric center generating the glycosyl donor:
  - (1) Convenient formation of a sterically uniform glycosyl donor.
  - (2) Having according to choice either α- or ß-configuration.
  - (3) Thermal stability of the glycosyl donor at least to room temperature, eventually chromatographic purification should be possible.
- For the second step, the glycosyl transfer to the acceptor providing the glycoside, the oligosaccharide, or the glycoconjugate, respectively,
  - (1) Catalysis of the glycosyl transfer by simple means, not by heavy-metal salts.
  - (2) Irreversibility of the reaction.
  - (3) The configuration of other glycosidic bonds must not be affected in the process.
  - (4) High chemical yield.
  - (5) High α- or ß-selectivity via controlled inversion or retention of configuration at the anomeric center.

Only simple means meeting these requirements will lead to a generally acceptable methodology. Therefore, apart from the acid activation (Scheme 1, paths A and B), the simplest form of activation is base activation with formation of an anomeric alkoxide structure of a pyranose or a furanose (Scheme 1, paths C and D). This approach is especially tempting because Nature has a similar approach for generating glycosyl donors.

(B) The Anomeric O-Alkylation Method. Direct anomeric O-Alkylation (Scheme 1, path C), seemed in the beginning very unlikely to fulfill all of the requirements given above for a glycoside synthesis. When all remaining hydroxy groups are blocked by protecting groups, the ring-chain tautomerism between the two anomeric forms and the open-chain form (Scheme 1, path C) give already three possible sites of attack for the alkylating agent. Thus, the yield, the regioselectivity, and the stereoselectivity of the direct anomeric O-alkylation are governed by at least the following factors:

- (1) the stability of the generated, deprotonated species,
- (2) the ring-chain tautomeric equilibrium and its dynamics, and
- (3) the relative reactivities of the three O-deprotonated species.

However, the direct anomeric O-alkylation of carbohydrates by primary triflates has become a convenient method for the synthesis of glycosides and saccharides because of its simplicity and the yields and stereoselectivities obtained (ref. 1,16).

For the furanoses studied, the stereocontrol results primarily from steric and chelate effects, whereas for the pyranoses, the rate of anomerisation and the different basicities and nucleophilicities of the α- and ß-oxide atoms are the governing factors.

In particular, the increased nucleophilicities of the ß-oxide atoms of the gluco-, galacto-, and mannopyranoses studied are noteworthy, because they provide preferentially the thermodynamically less stable anomers. The higher nucleophilicity of the pyranosyl-ß-oxides can be attributed to a steric effect in combination with a stereoelectronic effect, resulting form repulsions of the lone electron pairs or from dipole effects (ref. 17,18). This effect should be more pronounced in pyranosyl ß-oxides than in ß-pyranosides, due to the difference of oxygen lone pair orbitals. In addition, this kinetic anomeric effect should be particularly efficient in the mannopyranosyl-ß-oxide where the thermodynamic anomeric effect, favoring the α-anomer, is also stronger. Thus, ß-mannopyranosides can be obtained selectively in spite of steric hindrance (ref.19), as indicated in Scheme 2. It is assumed, that the mannopyranosyl-ß-oxide serves as a tridentate ligand for the metal ion.

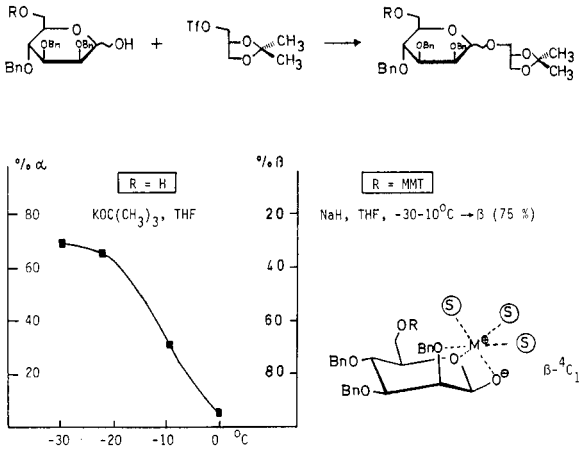
The anomeric O-alkylation method could be recently also successfully applied in the diastereospecific synthesis of KDO-α-glycosides (see below, and ref. 20), which turned out to be a major problem for the Koenigs-Knorr method.

(C) The Trichloroacetimidate Method. The direct anomeric O-alkylation of carbohydrates has demonstrated that pyranoses and furanoses deprotonated at the anomeric O-atom react analogously to alkoxides. Although the higher acidity of the anomeric hydroxy group of the hemiacetal might be expected to result in a lower nucleophilicity of the anomeric oxide, the stereoelectronic effect apparently is compensating for this influence.

These results and the observed stereocontrol suggested that pyranoses and furanoses undergo base-catalyzed addition directly and in a stereocontrolled manner to suitable triple bond systems A ≡ B (or compounds containing cumulative double bonds A=B=C) (Scheme 1, path D), as for instance, to electron deficient nitriles such as trichloroacetonitrile. This procedure has the advantage, that the free imidates can be isolated as stable adducts, which may be activated by mild acid catalysis.

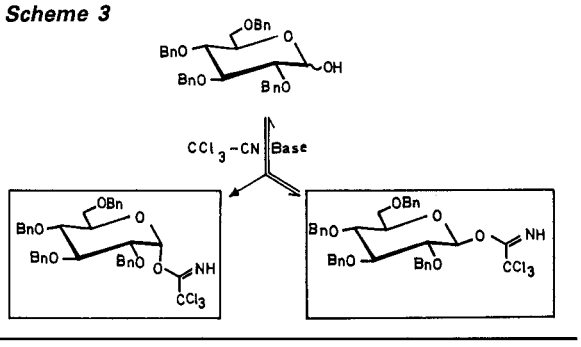
A detailed study of trichloroacetonitrile addition to 2,3,4,6-tetra-O-benzyl-D-glucose and to many other sugar pyranoses exhibited (ref. 1,18) that, following the formation of the anomeric oxide, the equatorial trichloroacetimidate is formed preferentially or even exclusively in a very rapid and reversible addition reaction. However, this product anomerizes in a slow, base catalyzed reaction (via retroreaction, anomeric oxide anomerization, and readdition of trichloroacetonitrile) practically completely to the thermodynamically more stable axial trichloroacetimidate (ref. 17,18) (Scheme 3). The higher reactivity of the equatorial anomeric oxide atom as compared to the axial anomeric oxide can be demonstrated by the use of weak bases, which do not catalyze the retroreaction Thus, for instance, with potassium carbonate the ß-trichloroacetimidate of tetra-O-benzyl-D-glucose can be also isolated as a pure product in very high yield. The ß- and the α-trichloroacetimidate are thermally stable at least up to room temperature; they can be easily stored.

Scheme 2

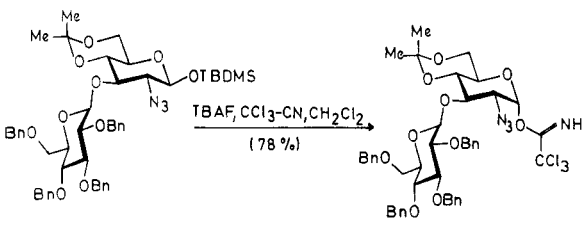


ANOMERIC O-ALKYLATION OF MANNOPYRANOSE

Scheme 3



Scheme 4



DIRECT FORMATION OF O-GLYCOSYL-TRICHLOROACETIMIDATES FROM ANOMERIC O-PROTECTED SUGARS

The convenient stereoselective anomeric O-activation of carbohydrates and their derivatives through the formation of O-glycosyl trichloroacetimidates is applicable to all important O-protected hexopyranoses, hexofuranoses, pentopyranoses, and pentofuranoses and derivatives. This activation principle was recently also successfully applied to a direct transformation of anomeric-O-protected carbohydrates into trichloroacetimidates (Scheme 4) (ref. 21) Thus, the first requirement for a new saccharide synthesis is fulfilled. Ultimately the significance ot the O-glycosyl trichloroacetimidates is derived solely from their glycosylation potential under acidic conditions. This potential has by now been confirmed in many investigations and in various laboratories (ref. 1,4,6,22-29).

The direct glycosylation of Bronsted acids is a particularly valuable property providing, for instance, O-glycosyl carboxylates and phosphates with inversion of configuration at the anomeric center via a most convenient route. A catalyst is not required for this reaction, which is also interesting in terms of the mechanism. Alcohol components for reaction as O-nucleophiles generally require the presence of an acidic catalyst (ref. 1). Boron trifluoride etherate in dichloromethane or dichloromethane/hexane as solvent up to room temperature has proven to be eminently suitable concerning yield and 1,2-trans-diastereoselectivity (with and without neighboring group participation). For 1,2-cis diastereoselectivity good results were obtained with diethyl ether as the solvent and trimethylsilyl triflate as the catalyst (ref. 1,30). Also 2-deoxy-ß-D-glucopyranosides could be obtained recently in excellent yields and diastereoselectivities (ref. 31).

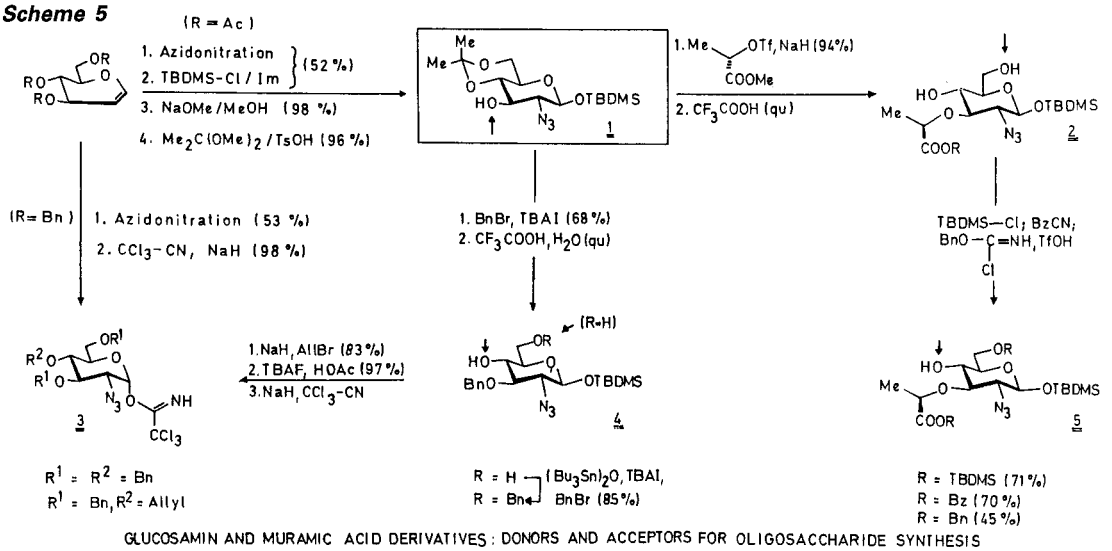
(D) Application of the Anomeric O-Alkylation Method and the Trichloroacetimidate Method to Glycoconjugate Synthesis.

Because of the great importance of glycoconjugates (i) as cell wall constituents of bacteria and (ii) as cell membrane constituents of all vertebrates (essentially glycosphingolipids) some applications of these newly developed methods to their synthesis will be discussed.

(i) Cell Wall Constituents of Bacteria. Amongst cell wall constituents of bacteria the cell wall peptidoglycan (called murein) has become of increasing importance (ref. 32,33). Thus pepticoglycan has a ß(1-4)-linked glycan chain, consisting of alternating N-acetyl-glucosamine and N-acyl-muramic acid units, which are cross-linked by a peptide chain (for instance, by L-Ala-D-iso-Glu-meso-DAP-D-Ala and glycine). The resulting peptidoglycan network and fragments of it exhibit pronounced immunostimulatory and antitumor properties. The minimal structure for the activity of the so-called "Freund's Complete Adjuvant" is a muramyl dipeptide (MDP = N-acetyl-muramyl-L-alanyl-D-isoglutamine). Many investigations were directed towards the synthesis of derivatives of MDP. Thus, it was clearly demonstrated that the carbohydrate portion is responsible for the immunostimulatory activity. Therefore the synthesis of oligosaccharide derivatives of MDP is of special interest because very promising perspectives for combined chemotherapy and immunotherapy based on MPD-analogs were recently reported (ref. 33). This way strong antibacterial, antiviral, antiparasitic, and anticancer effects were found (ref. 33)

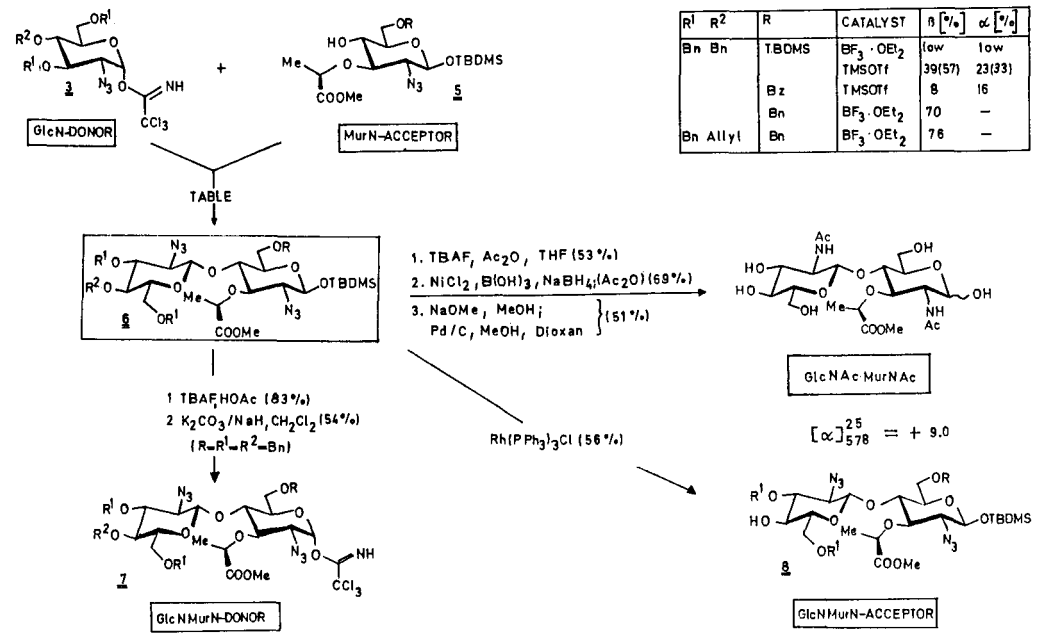
For the synthesis of the required partially protected glucosamine and muramic acid derivatives the very useful 2-azido-2-deoxy-D-glucose building block 1 (Scheme 5) was obtained from tri-O-acetyl-D-glucal. The 3-O-unprotected

Scheme 5



GLUCOSAMIN AND MURAMIC ACID DERIVATIVES: DONORS AND ACCEPTORS FOR OLIGOSACCHARIDE SYNTHESIS

Scheme 6



R^1 R^2	R	CATALYST	ß [%]	α [%]
Bn Bn	TBDMS	$BF_3 \cdot OEt_2$	low	low
		TMSOTf	39(57)	23(33)
	Bz	TMSOTf	8	16
	Bn	$BF_3 \cdot OEt_2$	70	–
Bn Allyl	Bn	$BF_3 \cdot OEt_2$	76	–

DISACCHARIDE UNIT OF THE CELL WALL PEPTIDOGLYCAN OF BACTERIA (MURAMIC ACID AS GLYCOSYL ACCEPTOR)

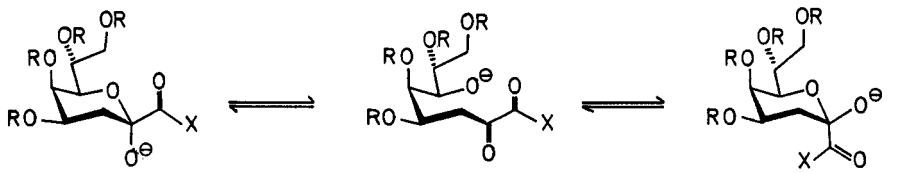
glucosamine derivative 1 was transformed with triflate activated methyl lactate into muramic acid derivative 2 in high yield (ref. 21,34,35). Racemisation was not observed with this method, which is also successful in large scale preparations and which is superior to published procedures (ref.36,37). Compounds 1 and 2, both being interesting glycosyl acceptors, were also regioselectively transformed into the important 4-O-unprotected acceptors 4 and 5, respectively. In addition, from tri-O-benzyl-D-glucal and from compound 4 the required glucosamine donors 3 were obtained.

For the synthesis of the desired GlcNAcß(1-4)MurNAc disaccharide (Scheme 6), based on the trichloroacetimidate method, the donor 3 and the acceptors 5 were treated with an acidic catalyst. However, as indicated in the table, the result was very much dependent on the 6-O-protection of the acceptor 5 (ref.38). Bulky groups (as for instance tert-butyldimethylsilyl = TBDMS) or electron withdrawing groups (as, for instance, benzoyl = Bz) did not improve the known low reactivity of the 4-OH group in glucosamine and derivatives. However, when a benzyl (=Bn) group was introduced for 6-O-protection the trichloroacetimidate method afforded in excellent yields and complete ß-selectivity the desired disaccharides 6. Application of standard deprotection procedures resulted in the GlcNAcß(1-4)MurNAc disaccharide, which was synthesized already independently (ref.35,39). The disaccharide intermediate 6 could be very recently transformed into the donor 7 and the acceptor 8, respectively, (ref.38, non optimized yields) which are useful building blocks for the synthesis of oligomers of the peptidoglycan of bacterial cell walls. All these intermediates are of interest in the synthesis of novel MDP-analogs with potential immunostimulatory activity.

Glucosamine derivative 1 could be also conveniently transformed into muramic acid α- and ß-trichloroacetimidates, which turned out to be very important muramoyl donors for diastereoselctive ß- and α-glycoside syntheses, respectively (ref. 21,35). Acceptors were glucosamine derivative 4 (R=Bn) and several other interesting *glucose, galactose, sphingosine,* and phosphorous acid derivatives, thus leading to a variety of other MDP analogs (ref. 35,40).

Lipopolysaccharides (LPS) are important constituents of the outer membrane of Gram-negative bacteria (ref. 41). The lipophilic part of LPS the Lipid A is the anchor in the membrane; it consists of a ß(1-6)-linked glucosamine disaccharide with phosphate groups in 1- and 4'-position and long chain fatty acids at the N- and partly at the O-atoms. The hydrophilic part of LPS consists of a complex oligosaccharide which is linked via KDO (3-deoxy-D-manno-2-octulosonate) to the glucosamine disaccharide [α(2-6')-connection (ref.42)]. The application of the *Koenigs-Knorr method to generate this α-glycoside bond* with KDO-halogenoses as donors has mainly led to unsatisfactory results due to hydrogen halide elimination and ß-glycoside formation (ref.43). Better results were recently reported for fluoride as the leaving group (ref.44).

Scheme 7



EXPECTED PROBLEMS

(1) DECOMPOSITION DUE TO ELIMINATION IN RING OPEN ISOMER

(2) LOW NUCLEOPHILICITY DUE TO α-COOR GROUP IN PYRANOSE ISOMERS

(3) HIGHER REACTIVITY FOR ß-OXIDE

(4) LOW REACTIVITY OF TRICHLOROACETIMIDATE

SOLUTIONS

(1) VARIATION OF PROTECTIVE GROUPS (!?)

(2) DIANION FORMATION : $X=O^{\ominus}$, $NR^{\ominus}$

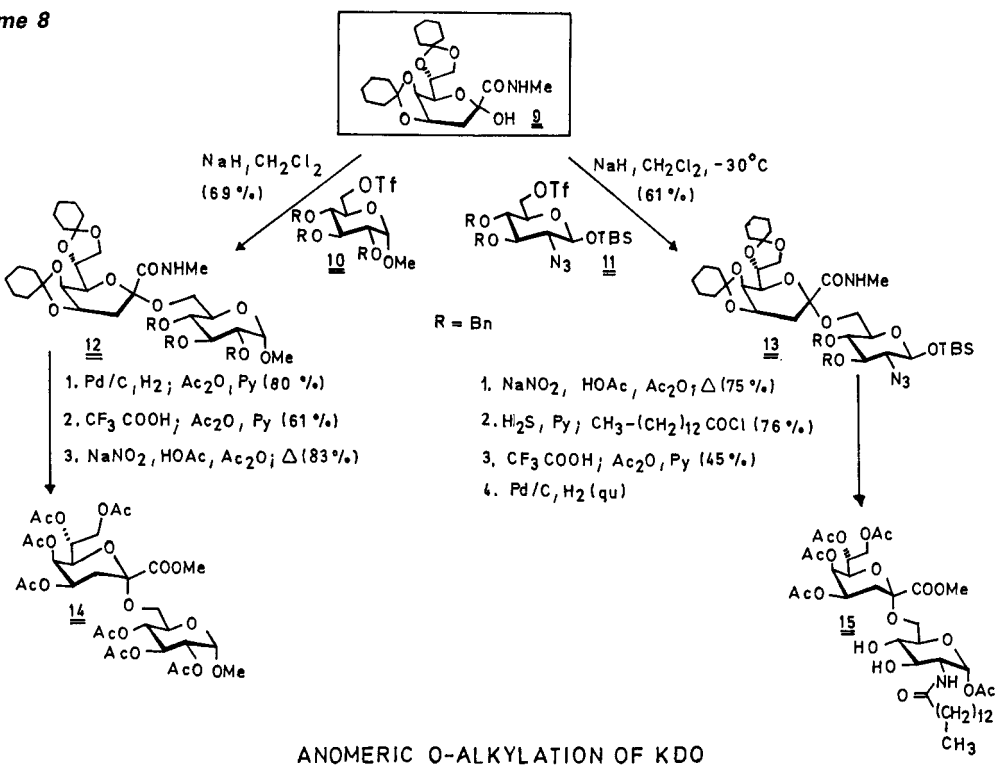
(3) CONFORMATIONAL VARIATIONS: EXPOSING THE ANOMERIC $O^{\ominus}$

(4) CARBOXYL GROUP VARIATION (!?)

BASE-CATALYZED ANOMERIC O-ACTIVATION OF KDO

Base catalyzed anomeric O-activation of KDO seemed to cause serious problems due to ring-chain tautomerism and due to the presence of the carboxylate group (Scheme 7). However, for the application of the anomeric O-alkylation procedure solutions to these problems could be put forward. The desired α-diastereoselectivity appeared to be not attainable with the generally used 4,5,7,8-tetra-O-acyl-protected KDO derivatives because the pyranosyl-ß-oxide should be again more reactive. Therfore we selected the 4,5:7,8-di-O-cyclohexylidene derivative 9 (Scheme 8) which was readily obtained from ß-lithiated α-alkoxyacrylate and 2,3:4,5-di-O-cyclohexylidene-D-arabinose (ref. 45,47). Compound 9 prefers according to the ^{1}HNMR data a boat conformation

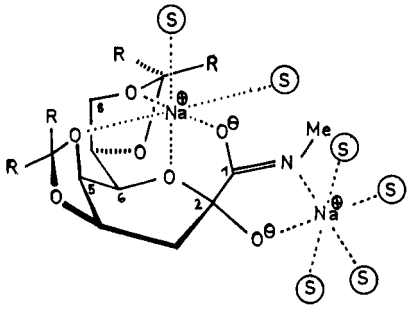
Scheme 8



ANOMERIC O-ALKYLATION OF KDO

providing with the carboxamide group and the oxygens of C-5 and C-8 a tetradentate chelate ligand for the complexation of metal ions. This complexation, generating a dianionic species (Scheme 9), should offer solutions to all the problems especially, however, for the nucleophilic reactivity and the desired α-diastereoselectivity. Indeed, reaction of KDO derivative 9 with two equivalents of base and subsequent addition of glucose triflate 10 or of the triflate of the glucosamine derivative 11, afforded the KDO-α-disaccharides 12 and 13, respectively, in good yields. No ß-connected disaccharides were found in the reaction mixture (ref. 20,45). This reaction could be recently also successfully applied to a glucosamine disaccharide derivative (ref. 45), providing the corresponding α-connected KDO-trisaccharide. For structural proof the KDO disaccharides 12 and 13 were transformed via standard procedures into the O-acetylated compounds 14 and 15, respectively, and compared with analogous compounds (ref. 20).

Scheme 9



2A

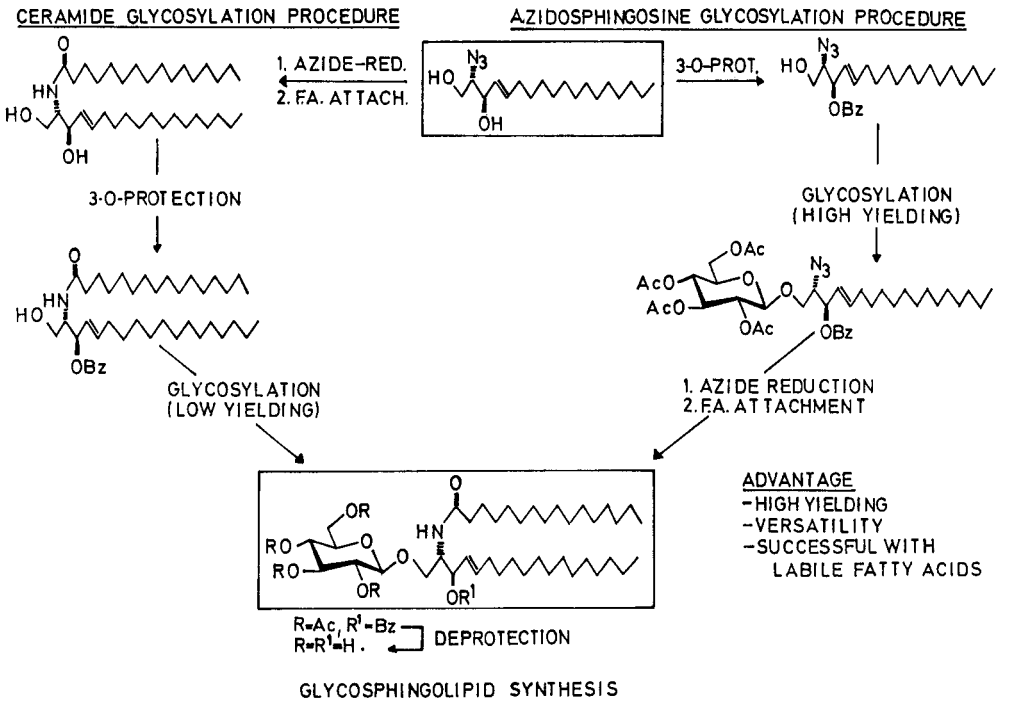
S = Solvens

(ii) Synthesis of Glycosphingolipids. The glycosphingolipids, being membrane constituents, possess a lipophilic ceramide (=N-acylated sphingosine) moiety which is part of the outer plasma membrane bilayer. The hydrophilic oligosaccharide moiety is therefore located on the outer membrane surface; it determines the specificity of the interactions with other cells and various other biofactors leading to the assignment of important biological functions (ref. 1,2).

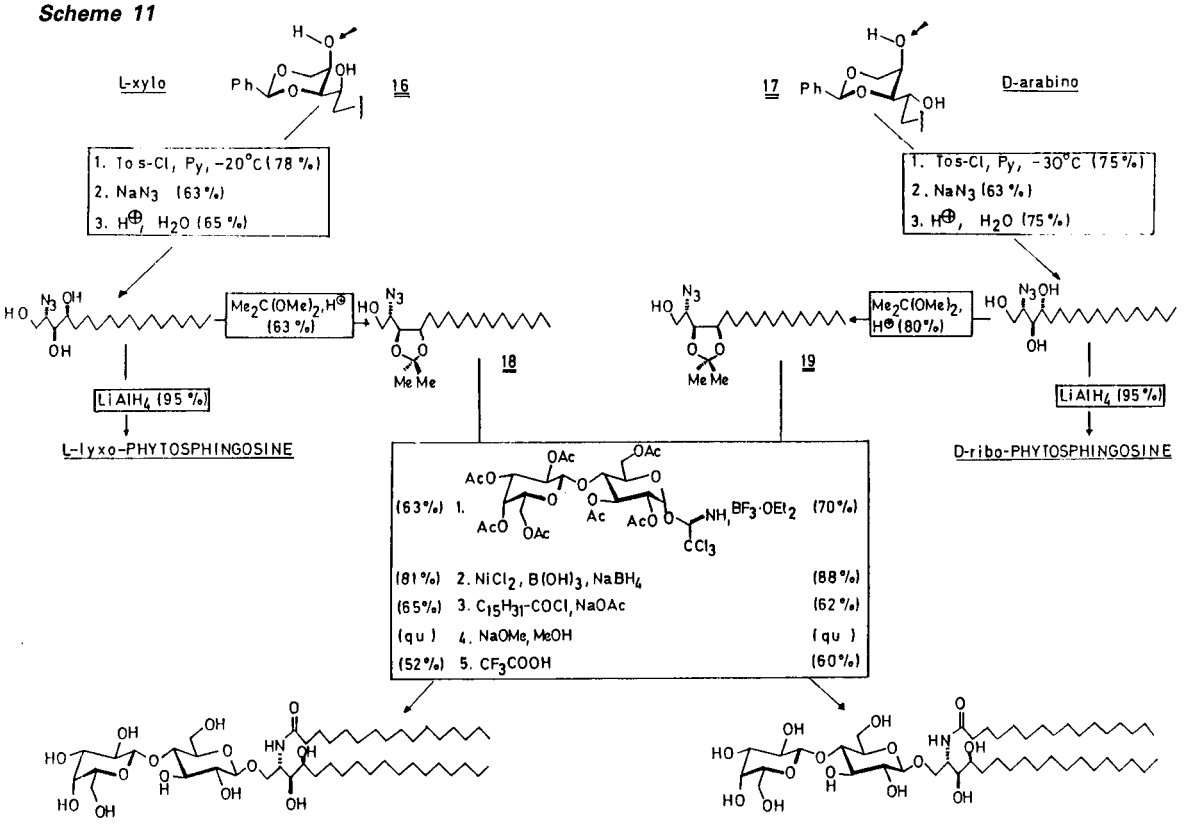
The most important sphingosine base found in the tissue of mammalians is the C_{18}-sphingosine; large amounts of the corresponding C_{20}-homolog were detected in the gangliosides of the brain. The most important N-acyl groups found in the ceramides are derived from the unsubstituted and the α-hydroxy fatty acids of C_{14}- to C_{24}-chain length. However, also lipophilic chains with (additional) double bonds and/or with methyl side chains are encountered (ref.48)

Several rather lengthy syntheses for the required sphingosines have been reported (ref. 1,49). We have developed via an erythro-specific aldol reaction of N,O-persilylated glycine with α, ß-unsaturated aldehydes a two-step synthesis of racemic sphingosine (ref.50). This efficient route provided ceramides very readily. The required racemate resolution and the 3-O-protection could be easily combinend (ref.49). The best method for the glycosylation of these compounds proved to be the trichloroacetimidate method, which afforded better yields in glycosphingolipid syntheses than syntheses applying the Koenigs-Knorr method and variations of it (ref. 51,52). This ceramide glycosylation procedure (Scheme 10) was also successfully applied by Ogawa et al., for instance, in the synthesis of the G_{M3} ganglioside, etc. (ref.27). However, the yields in the glycoside bond forming step are far from satisfactory, due to steric and catalyst binding problems (ref.52). We have therefore developed a new and productive entry to this class of compounds, based on a new and efficient sphingosine synthesis starting from D-galactose or D-xylose (ref.1,49,53,54). In this new methodology glycosylation takes place at the azidosphingosine stage providing the glycosyl bond in high yield (ref.55). For instance, glucosylation with the α-trichloroacetimidate of per-O-acylated D-glucose afforded over 80 % yields (ref.55,56).
This azidosphingosine glycosylation procedure could be also successfully extended to D-ribo-phytosphingosine based glycosphingolipids (ref.57)(Scheme 11) occuring in Nature (ref.58). The required D-ribo-phytoshingosine and its

Scheme 10



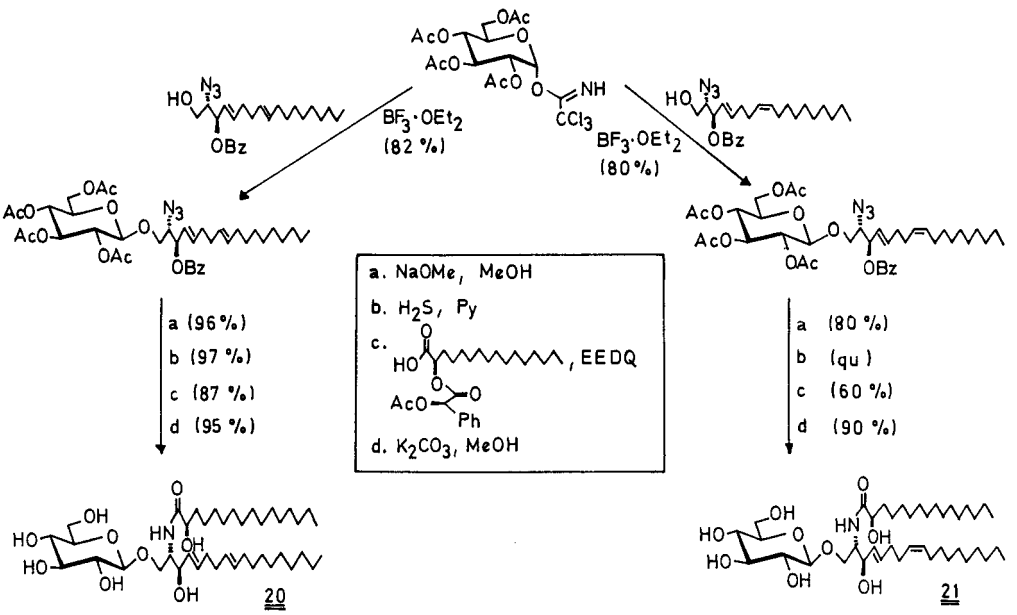
Scheme 11



D-ribo AND L-lyxo-PHYTOSPHINGOSINE SYNTHESIS AND AZIDO-PHYTOPHINGOSINE 1-O-GLYCOSYLATION

L-lyxo isomer were readily obtained from 2,4-O-benzylidene-D-threose by Grignard reaction providing the tetrol derivatives 16 and 17; they were subsequently transformed as indicated in Scheme 11. The generated glycosyl acceptors 18 and 19 were treated with per-O-acetylated O-lactosyl-trichloroacetimidate providing the glycosylated compounds in good yields. Azide reduction, fatty acid attachment, and deprotection completed the reaction sequence.

Scheme 12



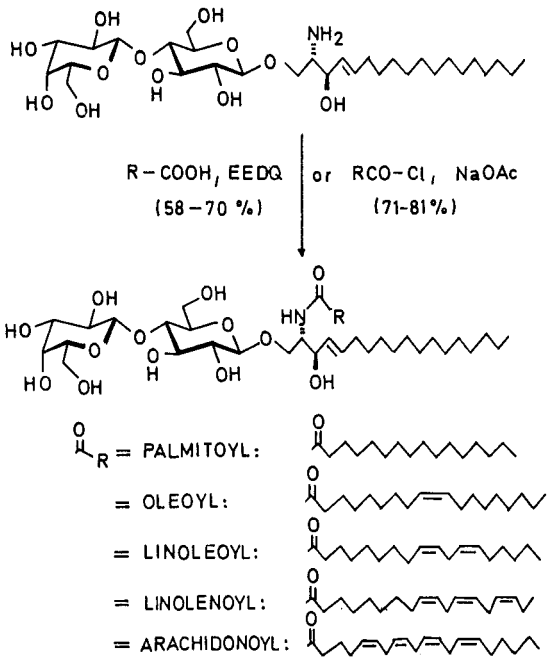
SYNTHESIS OF THE CEREBROSIDES FROM TETRAGONIA TETRAGONOIDES WITH ANTIULCEROGENIC ACTIVITY

During a survey of neurotropic components of oriental crude drugs Okuyama and Yamazaki recognized that the priciples of Tetragonia tetragonoides possessing antiulcerogenic activity are due to glycosphingolipids to which they assigned the ß-D-glucopyranosyl ceramide structures 20 and 21 (Scheme 12)(ref.59). The configuration of the (4E,8Z)- and the (4E,8E)-sphingadienine moieties were suggested to be D-erythro. Both these compounds were synthesized as azido derivatives starting again from 2,4-O-benzylidene-D-threose and then used in the azidosphingosine glycosylation procedure with the fully acetylated O-glucosyl-trichloroacetimidate as the glycosyl donor. Scheme 12 is indicative of the good results obtained (ref.60,61).

The generation of psychosine intermediates in the azidosphingosine glycosylation procedure is an additional advantage because the more sensitive fatty acids (as for instance oleic, linolic, linolenic and arachidonic acid) can be attached at the very last step completing the glycosphingolipid synthesis. This is demonstrated in Scheme 13 for the lactosyl psychosine where the condensation is performed via the acid chloride or with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as the condensing agent (ref.62).

Cell membranes fulfill a variety of different functions. To further the understanding of the complex cell membrane system it is important to investigate the properties of the various membrane constituents independently. The bilayer technique of Müller et al. (ref.63) has been very successfully applied to the important phosphatidylic acid derived membrane constituents. It allows the generation of membrane analogs and the investigation of their physico-chemical properties (ref.63,64). This way the specific electric membrane capacity (C_m) of artificial and also of natural bilayers was measured. This permitted the determination of the thickness of the lipid part of such membranes (Table 1)(ref. 63-65).

Scheme 13



GLYCOSPHINGOLIPIDS VIA PSYCHOSINES

SPECIFIC MEMBRANE CAPACITIES (C_m) AND THICKNESS (d) OF THE LIPID BILAYERS OF D-LACTOSYLCERAMIDES AND COMPARABLE PHOSPHATIDYL CHOLINES [a]

	C-R		C_m [μF/cm²]	d [nm]
PHOSPHATIDYLCHOLINES [b]	DIOLEOYL	(Δ^{9}-C_{18}:1)	0.374 ± 0.013	4.97 ± 0.17
	DILINOLEOYL	($\Delta^{9,12}$-C_{18}:2)	0.416 ± 0.014	4.47 ± 0.14
	DILINOLENOYL	($\Delta^{9,12,15}$-C_{18}:1)	0.582 ± 0.018	3.19 ± 0.11
	DIARACHIDONOYL	($\Delta^{5,8,11,14}$-C_{20}:4)	0.443 ± 0.014	4.20 ± 0.13
D-LACTOSYLCERAMIDES [c]	OLEOYL	(Δ^{9}-C_{18}:1)	0.563 ± 0.072	3.30 ± 0.26
	LINOLEOYL	($\Delta^{9,12}$-C_{18}:2)	0.755 ± 0.073	2.46 ± 0.25
	LINOLENOYL	($\Delta^{9,12,15}$-C_{18}:3)	0.818 ± 0.092	2.27 ± 0.20
	ARACHIDONOYL	($\Delta^{5,8,11,14}$-C_{20}:4)	0.570 ± 0.038	3.26 ± 0.49

[a] MEASUREMENTS AND CALCULATIONS ACCORDING TO P. MUELLER, AL. (1962), P. LÄUGER, AL. (1967).

[b] TAKEN FROM R. BENZ AND K. JANKO (1976); SOLVENT: N-DECANE

[c] TAKEN FROM R.R. SCHMIDT AND T. BÄR (1987); SOLVENT: N-DECANE/N-BUTANOL (100:1)

Bilayer membranes could not be obtained from the glycosphingolipids isolated from natural sources, which are generally not chemically pure on the ceramide level. Therefore we undertook recently investigations towards the generation of bilayer membranes with the glycosphingolipids containing unsaturated fatty acids (Scheme 13 and Table 1), because it is known that unsaturated lipid chains favor membrane formation and membrane fluidity (ref. 62,64). With the help of the above mentioned technique it was indeed possible to generate the desired bilayers and to measure their specific electric membrane capacity

permitting the calculation of their thickness (Table 1)(ref. 62). The values obtained for the thickness of the lipid part of the membrane are lower than those observed for corresponding phosphatidyl derivatives (ref. 65). This may be due to the solvent used for membrane formation, it may be a specific effect of the sugar head group of the membrane constituent, or increased kink formation in the unsaturated lipid part takes place. These and many other questions will have to be persued in the future based on an efficient methodology for glycosphingolipid synthesis.

Scheme 14

ACTIVATION OF THE ANOMERIC CENTER WITH TRICHLOROACETONITRILE

(1) CONVENIENT BASE CATALYZED TRICHLOROACETIMIDATE FORMATION

(2) CONTROLLED ACCESS TO α- OR β-COMPOUNDS BY CHOICE OF THE BASE

(3) THERMAL STABILITY OF α- AND β-TRICHLOROACETIMIDATES UP TO ROOM TEMPERATURE
IF REQUIRED, SILICA GEL CHROMATOGRAPHY CAN BE PERFORMED.

GLYCOSYL TRANSFER

(1) CATALYSIS BY ACIDS (MAINLY LEWIS ACIDS) UNDER VERY MILD CONDITIONS.

(2) IRREVERSIBLE REACTION

(3) OTHER GLYCOSIDIC BONDS ARE NOT AFFECTED

(4) USUALLY HIGH CHEMICAL YIELD
REACTIVITY CORRESPONDS TO THE HALOGENOSE/SILVER TRIFLATE SYSTEM

(5) STEREOCONTROL OF GLYCOSIDE BOND FORMATION IS MAINLY GOOD TO EXCELLENT

(A) PROTECTIVE GROUPS WITH NEIGHBORING GROUP PARTICIPATION ⟶ 1,2 TRANS-GLYCOPYRANOSIDES
β-GLYCOSIDES OF: GLC, GLCN, GAL, GALN, XYL, MUR, 2-DEOXY-GLC
α-GLYCOSIDES OF: MAN, RHA

(B) PROTECTIVE GROUPS WITHOUT NEIGHBORING GROUP PARTICIPATION
CATALYST BF_3,OEt_2: ⟶ INVERSION OF ANOMER CONFIGURATION
β-GLYCOSIDES OF: GLC, GLCN, GAL, GALN, XYL, MUR, GLCUA
CATALYST TMSO-TF: ⟶ THERMODYNAMICALLY MORE STABLE ANOMER
α-GLYCOSIDES OF: GLC, GLCN, GAL, GALN, MAN, FUC, MUR

THE TRICHLOROACETIMIDATE METHOD

(E) Conclusions. The requirements for new glycosylation methods, outlined at the beginning of this paper, are practically completely fulfilled by the trichloroacetimidate method. This is indicated by the examples and references given in the paper and by the summary in Scheme 14. Besides oxygen nucleophiles discussed here, nitrogen (ref. 1,66), carbon (ref. 67), halogen (ref. 1), sulfur (ref. 68), and phosphorous nucleophiles (ref. 69) were meanwhile used successfully as glycosyl acceptors for this in terms of stability, reactivity, and general applicability outstanding glycosyl donors resembling in various aspects the natural nucleoside diphosphate sugar derivatives as glycosyl donors. Thus, the base catalyzed activation of sugars either for direct anomeric O-alkylation or for O-glycosyl-trichloroacetimidate generation has become a very competitive alternative to the acid catalyzed activation of sugars for direct acetalisation (Fischer-Helferich method) or to glycosyl halide (including fluoride) and glycosyl sulfide formation. In addition, both these newly introduced methods can be used in large scale preparations.

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