1. Introduction and objectives

1.1 *O*-Glycoside bond formation

Glycoside synthesis is a very common reaction in nature providing a great variety of oligosaccharides and glycoconjugates as glycolipids, glycoproteins and glycopeptides. As recognized only recently, the structural diversity of the oligosaccharide portion, which is inherent in the variability in the glycoside bond formation, makes them ideal as carrier of biological information and specificity. For this fact, the field of the synthetic carbohydrate chemistry grew up exponentially in the last twenty years in order to synthesize oligosaccharides for specific purposes which include their use in antibody production, screening of antibodies, lectin and selectin specificity, interaction studies with virus[1,2] and bacterial receptors,[3-5] substrates for glycosidases [6,7] and glycosyltransferases[8] and probes in molecular recognition studies including conformational analysis. To date, it is the challenge for the synthetic chemist to build up glycosidic linkages with high regio- and stereocontrol similar to the naturally occurring ones. Two different approaches are generally used for the *O*-glycoside bond formation:

- Enzymatic *O*-glycoside bond formation
- Chemical *O*-glycoside bond formation

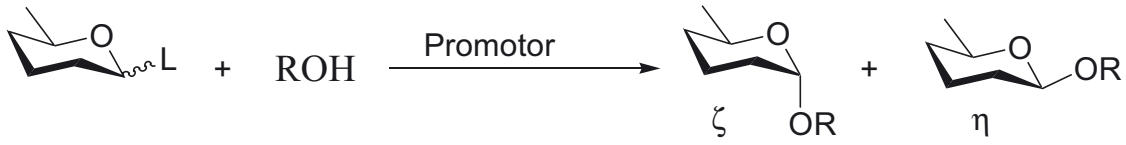
1.1.1 Enzymatic *O*-glycoside bond formation

The enzymatic *O*-glycosylation is generally based on specific glycosyl-transferases which use nucleoside diphosphate or, in some cases, nucleoside monophosphate sugars as glycosyl donors. The nucleoside di- or monophosphate residues are the leaving groups and sugars, or other aglycones are the glycosyl acceptors.[9] The driving force for the irreversible *O*-glycoside bond formation is the cleavage of the nucleoside of the di- or monophosphate residue from the activated sugar, while the glycosyltransferase provides the desired regio- and diastereoselectivity. The limited

availability of the glycosyltransferases, the complex generation of expensive glycosyl donors and the difficulty in carrying out the enzymatic reactions limit the use of this method for the synthesis of complex oligosaccharides. In most of the cases, the fragments of complex oligosaccharides are prepared through a total chemical synthesis and then used as efficient acceptors for specific enzymes (fucosyltransferase, sialyltransferase and galactosyltransferase).

1.1.2 Chemical *O*-glycoside bond formation

The chemical synthesis of oligosaccharides is based on the glycosylation reactions, coupling different building blocks with generating a glycosidic bond. As a general principle of most of the glycosylation methods a glycosyl donor is formed by combining a leaving group with the anomeric centre of one approperiately protected glycosyl building block. In the glycosylation reaction the activated glycosyl donor reacts with one hydroxy group of the completely or partially protected glycosyl acceptor (Scheme 1).



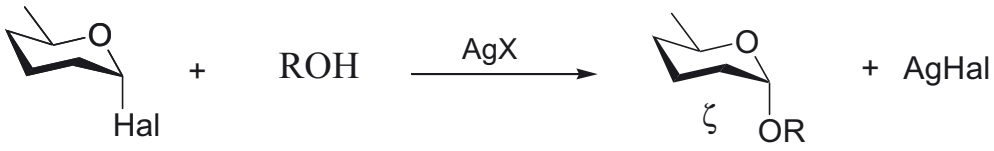
Scheme 1: Glycosylation reactions

When an ζ&η-mixture of the glycoside product is formed, the anomers must be separated by different techniques as chromatography, crystallization, distillation, etc…. Successful glycosylation reactions require high regio- and stereoselectivity preferably leading to only one pure anomer.

1.1.2.1 The Koenigs-Knorr method

The oldest glycosylation method was published by *Koenigs* and *Knorr* in 1901,[10] it was variously modified and it is still in use.[11] The glycosyl donors are usually

chlorides and bromides which are activated with various silver or mercury salts (Scheme 2). Advanced modifications make use of glycosyl fluorides as donor compounds.[12, 13]

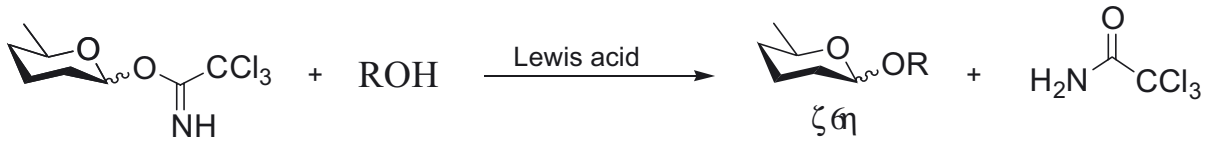


Scheme 2: Koenigs-Knorr glycosylation method

In order to favour a stereocontrolled S_N2-type reaction, solvents of low polarity (dichloromethane, cyclohexane and petroleum ether) and low temperatures are commonly used. The application of this method led to excellent results, for example the synthesis of numerous oligosaccharides including the blood group A-, B-, and Le^a-determinants.[14] However, the main disadvantages of the Koenigs-Knorr method are the need of at least stoichiometric amounts of the promoters and the thermal instability of many glycosyl halides.

1.1.2.2 The trichloroacetimidate method

A universal glycosylation method which avoids the use of heavy metal salts as promoters was developed by *R. R. Schmidt* and *J. Michel*[15] in 1980. *O*-Glycosyl trichloroacetimidates were introduced as a new type of glycosyl donors. It is easily prepared, sufficiently stable and it can be activated for the glycosylation reactions with catalytic amounts of Lewis acids such as TMSOTf, $BF_3.Et_2O$, $Sn(OTf)_2$, AgOTf and $ZnCl_2.Et_2O$[16,17] (Scheme 3).



Scheme 3: The trichloroacetimidate glycosylation method

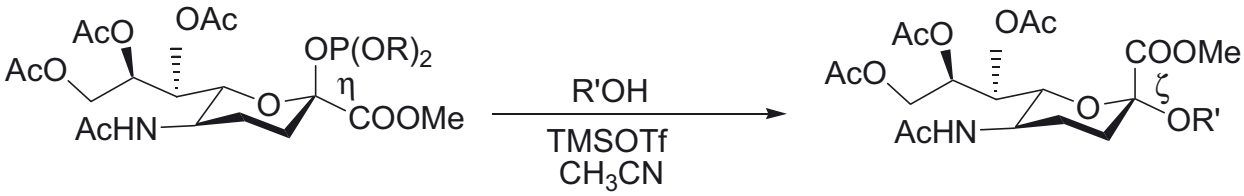
The anomeric configuration /ζ#or#η) of the trichloroacetimidate donors is crucial for the anomeric sterocontrol of the glycosidic bond formation. η-Trichloroacetimidates can be selectively prepared with K_2CO_3 as base[18] (kinetic control), whereas the use of NaH, $CsCO_3$ or KOH[19] with phase transfer catalyst[97] exclusively gives the ζ-trichloroacetimidates (thermodynamic control).

1.1.2.3 Anomeric stereocontrol in *O*-glycoside bond formation

The main advantages of the trichloroacetimidate method include the various possibilities for stereocontrol in the *O*-glycoside bond formation. Excellent stereoconterol can be achieved by using trichloroacetimidates as donors bearing a participating neighbouring group at the 2-position (neighbouring group effect) as well as by performing the reaction in a suitable combination solvent/catalyst (ether and nitrile effect). The trichloroacetimidate glycosylation method will be explained later in detail.

1.1.2.4 The phosphite method

In 1992, R. R. Schmidt and co-workers[21-23] developed the phosphite method as supplementary procedure to the trichloroacetimidate. This method found their best applications in the activation of deoxysugars (KDO and Neu5Ac) and is universally used for the sialylation step in the synthesis of many neuraminic acid glycosides. Glycosyl phosphites are synthesized starting from the unprotected anomeric oxygen of sugars by reaction with phosphorochloridites or phosphoroamidites and Hünig's base. The η-glycosyl phosphites of neuraminic acid can be activated with catalytic amounts of TMSOTf (Scheme 5).

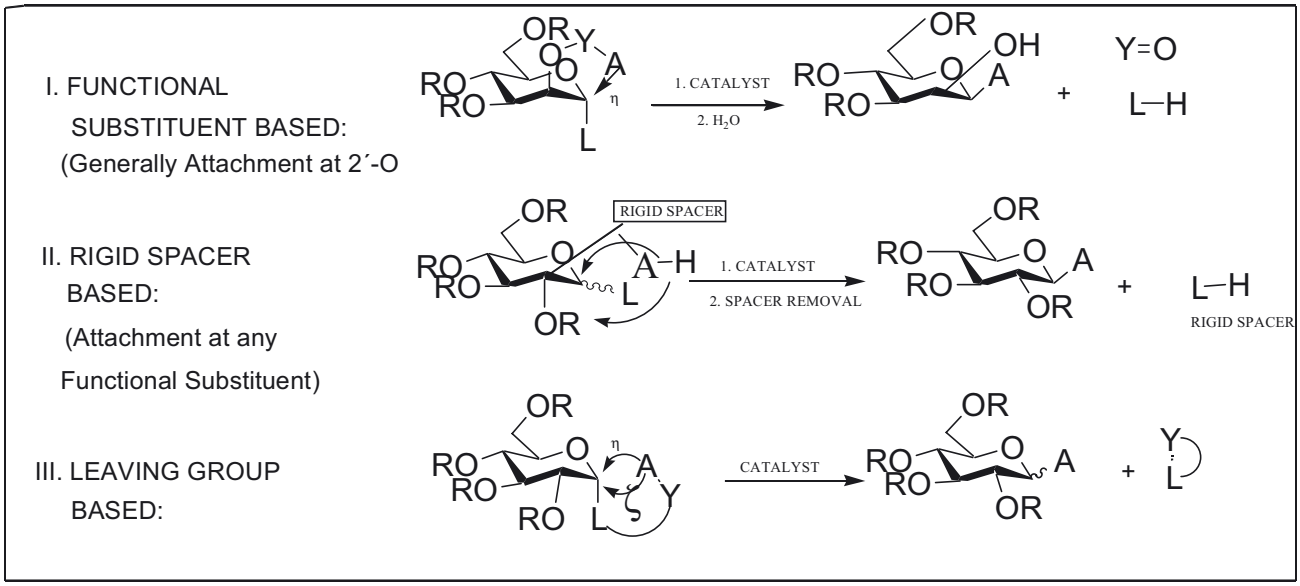


Scheme 5: Phosphite method

1.1.2.5 Intramolecular glycosylation method

An ideal approach which, in principle, could overcome the activation and stereochemical issues involves holding the component sugar donor and acceptor units in appropriate orientations within the same molecule in such a way that they can be forced to couple intramolecularly. Although this method is in its infancy, it appears that it will have high potential for the synthesis of specific sugar-sugar bonds of oligosaccharides. In general, the intramolecular methods are divided into three main classes (Scheme 6)

- Functional substituent based
- Rigid spacer based
- Leaving group based [24]



Scheme 6: Intramolecular glycoside bond formation[25]