Oxacarbenium ion intermediates in the stereoselective synthesis of anionic oligosaccharides

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Chapter 7

Summary and Future Prospects

Summary

Synthetic carbohydrate chemistry is an ongoing field of research that studies the preparation of naturally occurring carbohydrates as well as carbohydrate derivatives that are designed to function as tools to elucidate or to influence biological processes. The development of protective group and glycosylation strategies occupy a central position in carbohydrate synthesis. The protective groups of both the donor and acceptor glycosides, the reaction partners in a glycosylation reaction, effect the regioselectivity, the stereoselectivity and the yield of the coupling reaction. Ideally, a suitable glycosyl donor should allow a productive and stereoselective coupling with any hydroxyl function of any acceptor. However, despite major advances in the synthesis of oligosaccharides such as solid phase and one-pot procedures, improvements are still necessary. For instance the stereoselective introduction of some 1,2-*cis* glycosidic bonds still presents a major challenge. Studies on the mechanism of glycosylations may give an entry to the development of stereoselective glycosylations. **Chapter 1** presents a view on the mechanisms involved in glycosylation reactions, which can be roughly classified as S_N2

and S_N1 like pathways. Neighboring group participation, the nature of solvents and anomeric leaving groups are briefly discussed as factors which induce "S_N2" type glycosylations. The stereochemical outcome of "S_N1" type mechanisms and the role of the intermediate oxacarbenium ion allow for more debate. On the one hand a widely supported postulate entails that S_N1 like glycosylation proceed via a late transition state leading to a stereochemical outcome that is governed by the anomeric effect. On the other hand studies on *O*- and in particular *C*-glycosylations support the hypothesis that the stabilities of the half chair oxacarbenium ion conformers are of prime importance for the stereochemical outcome of "S_N1" type glycosylations.

In carbohydrate chemistry thioglycosides are widely used building blocks, as these compounds are stabile during protecting group manipulations and easily activated to induce glycosylations. A general method to hydrolyze thioglycosides is not available. Reported methods are not fail-safe in their application on different thioglycosides. **Chapter 2** reveals a new method for the hydrolysis of thioglycosides. The NIS/TFA reagent combination proved to be an efficient and broadly applicable reagent combination for the hydrolysis of thioglycosides. Both highly reactive (armed) and less reactive (disarmed) thioglycosides were hydrolyzed readily and it was shown that acid labile protective groups tolerated this method. The NIS/TFA mediated hydrolysis was used to attain valuable building blocks en route to hyaluronic acid (HA) oligomers.

In this thesis two routes of synthesis of HA oligomers are described and the first one is discussed in **Chapter 3**. Aith the aid of a new strategy a HA dimer, trimer and pentamer, having a glucuronic acid at the reducing end were prepared. The strategy is based on the finding that donor 1-hydroxysugars can be chemoselectively condensed with acceptor 1-thioglycosides to afford a 1-thiodisaccharide amenable for elongation at both reducing end and non-reducing end. The yields of subsequent glycosylations to higher oligosaccharides proved to be reasonable but required fine-tuning of the amount of base. The acid lability of the benzylidene protective groups on the one hand and the requirement to reduce the amount of base to avoid orthoester formation during the glycosylations on the other hand were conflicting. In the second route of synthesis of HA oligomers, described in **Chapter 4**, the benzylidene group was replaced by the more acid stabile di-*tert*-butylsilylidene (DTBS) protective group. Indeed, the glycosylations using DTBS protected glucosamine building blocks could be executed under acidic conditions and resulted in higher yields. A HA tri, penta and heptasaccharide with a glucosamine at the reducing end were efficiently synthesized using a dimer building block. The required dimer was readily synthesised by the chemoselective condensation of a glucosamine *N*-phenyl trifluoroimidate and a *S*-phenyl glucuronate ester. The glucosamine *N*-phenyl trifluoroimidate donor was prepared in an efficient four step procedure from the HCl salt of glucosamine.

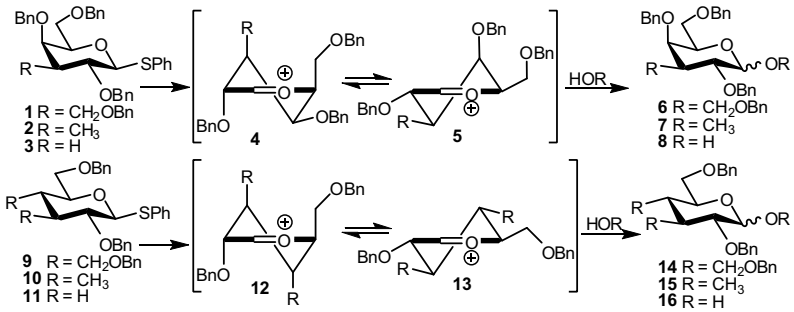
Chapter 5 describes the synthesis of an alginate trisaccharide consisting of 1,4-linked-α-L-guluronic acid residues. It was found that the glycosylations with guluronic acid donors were less *cis*-selective than the corresponding D-mannuronic acid donors. In addition it was shown that L-gulose donors have an intrinsic preference for the formation of 1,2-*cis*-glycosidic bonds. Thus, a gulose trimer was assembled using non-oxidized gulopyranose building blocks and subsequently oxidized to yield the target alginate trisaccharide. The stereoselectivities of gulose and guluronic acid donors were explained by the hypothesis that these glycosylations proceed by an "S_N1" type mechanisms, in which the acceptor attacks the most stable oxacarbenium ion conformer. Theoretical and experimental data indicates that the $^3H_4$ conformation of the oxacarbenium ion of L-gulose is the most favorable. Axial attack from the top side of this conformer leads to the formation of 1,2-*cis* product. The scope of this hypothesis is the subject of **Chapter 6**. With the aid of two model acceptors the glycosylating properties of a series of carbohydrate epimers was investigated. The effect of the nature of the C-5 substituent was investigated using suitably protected D-mannose and D-gulose donors as well as the corresponding C-5 methyl and C-5-uronate ester donors. The results support the finding that the electron withdrawing methyl ester favors a pseudo axial orientation in the oxacarbenium ion intermediate whereas the C-5 methyl group preferentially adopts an equatorial orientation. The hypothesis on the stability of oxacarbenium ions can be used to predict the stereochemical outcome of glycosylations in case all substituents adopt the most favorable position in one of the oxacarbenium ion conformers. In systems having conflicting substituent preferences, steric factors in both the ground state of the ions and product forming transition states become important for the outcome of the reaction, as revealed by experiments using glucose, galactose and allose.

Future Prospects

The research described in this Thesis has contributed to the elucidation of the mechanisms of glycosylation reactions and the development of synthetic strategies towards anionic oligosaccharides. The moderate stereoselective introduction of 1,2-*cis* galactose and the poor stereoselective introduction 1,2-*cis* glucose glycosidic linkages were explained by a less pronounced difference in stability between the $^3H_4$ and $^4H_3$ conformers and destabilizing steric interactions in the respective oxacarbenium ions and the corresponding transition states. An interesting ability to further confirm the proposed glycosylation mechanism is the design, synthesis and evaluation of the glycosylating properties of modified glycosyl donors having the galacto- or gluco configuration. It is envisaged that the unfavorable steric interactions in either the $^3H_4$ or the $^4H_3$ conformer of the oxacarbenium ion can be diminished by the replacement of a particular benzyloxy substituent with either a methyl or a proton. For instance, replacement of the benzyloxy substituent at C-3 in

galactoside **1** with a methyl group (**2**) or an hydrogen atom (**3**) would enhance the stability of the $^4H_3$ conformer (**5**) in comparison with the $^3H_4$ conformer (**4**), resulting in enhanced α-selectivity. Similarly, the benzyloxy substituents at C-3 and/or at C-4 in glucoside **9** can be replaced by a methyl group or a hydrogen atom. The oxacarbenium ion model predicts that both glycosyl donor **10** and **11** are stronger α-directing than the parent tetrabenzyl glucoside **9**.

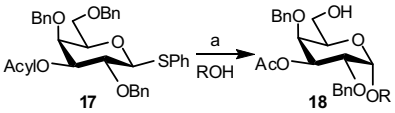
Scheme 1



Glucosyl and galactosyl oxacarbenium ions modified substituents at C3 and C4. Reagents and conditions: Donor, DCM, Ph_2SO, -78 °C, 15 min, acceptor (ROH), warm to 0 °C.

To obtain 1,2-*cis* selectivity in systems having conflicting substituent preferences methods have to be developed to suppress reaction via an oxacarbenium ion pathway. As described in Chapter 1, the 3-*O*-acyl can have an α-directing effect in glycosylations. It is interesting to investigate the influence of an acyl function in **17** with a variety of acceptors.

Scheme 2

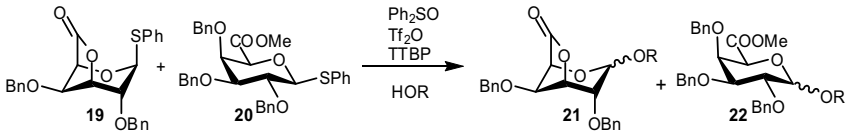


3-*O*-acyl study on galacoside **17**. Reagents and conditions: Donor **17**, DCM, Ph_2SO, -78 °C, 15 min, acceptor (ROH), warm to 0 °C.

From a synthetic point of view, pectin, a linear oligosaccharide composed of all α-1-4 linked galacturonic acids, presents an interesting target. Two general synthetic strategies to pectin oligomers, which use either a post-glycosylation oxidation[1] or a pre-glycosylation oxidation[2] procedure, have been reported, which both strategies have their drawbacks. The post-glycosylation oxidation route entails a precarious oxidation step of multiple primary alcohols at the end of the synthesis while the pre-glycosylation oxidation approach is accompanied by a drop in glycosylation efficiency above the dimer level. The latter is due to the low reactivity of galacturonate esters donors as well as the low nucleophilicity of the

axial 4-OH of α-galactopyranose acceptors.[3] Lactonization of the galacturonic acid C5-carboxylate with the C3 hydroxyl[4] results in a conformational flip, leading to an equatorially oriented C-4-OH which could enhance its nucleophilicity. Moreover, as presented in **Chapters 1**, **5** and **6**, axial hetero atom at the C-3- and C-4 positions in the pyranose core contribute favorably to the reactivity of a glycosyl donor compared to the corresponding equatorial ones. It was revealed by van den Bos *et al.*[4] that galactono-3,6-lactones are reactive, highly α-selective donors making them ideal building blocks for the synthesis of pectin oligosaccharides. On the basis of these considerations, it is proposed to study the donor and acceptor characteristics of galactono-3,6-lactones. Competition experiments with galacto lactone **19** and galacturonate methyl ester **20** will show the difference in reactivity of the lactone donor and the corresponding galacturonate methyl ester galactoside. The ease of activation and the ease of glycosylation are two variables that can be determined (Scheme 3). The difference in ease of activation can be monitored using a limited amount of activator (experiment A) and subsequent reaction with an appropriate acceptor. The difference in glycosylation potential can be monitored using a limited amount of acceptor (experiment B).

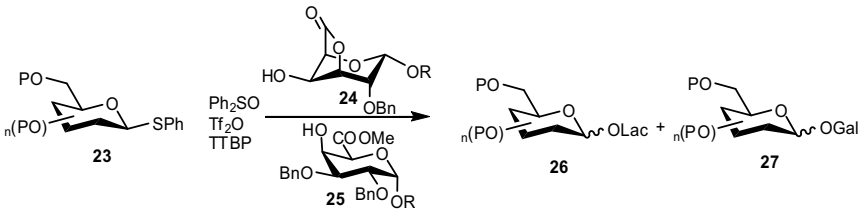
Scheme 3



Competition experiments of a galacturonate methyl ester and galactonolactone donor. Reagents and conditions: Experiment A) Donor **19** : donor **20** : activator : acceptor = 1 :1 : 1 : 2. Experiment B) Donor **19** : donor **20** : activator : acceptor = 1 : 1 : 2 : 1.

The nucleophilic properties of the lactones can be assessed by competition experiments in which equimolar amounts of a lactone acceptor **24** and a galacturonate methyl ester **25** are allowed to react with a half an equivalent of an activated donor **23** (Scheme 4).

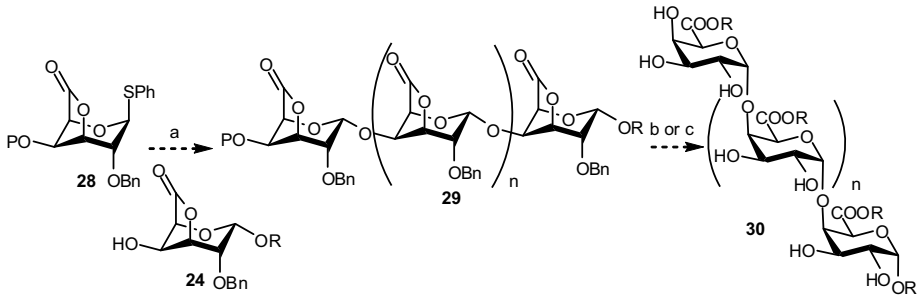
Scheme 4



Competition experiments of galacturonate ester and lactone acceptors. Reagents and conditions: Donor **23** (1 eq), Ph_2SO (1 eq), TTBP (3 eq), -60 °C, Tf_2O (1 eq), 5 min, -78 °C, lactone **24** (1 eq), galacturonate methyl ester **25** (1 eq), warm to 0 °C.

Having the properties of galactono-3,6-lactones as donor and acceptor assessed, the pectin oligosaccharide **30** can be assembled using the Ph_2SO Tf_2O activation system and a stepwise elongation approach (Scheme 5). At the end of the synthesis the lactone rings can be opened using either methanol or water to yield the galacturonate methyl esters or the galacturonic acids, respectively. After hydrogenation the pectin oligomers are obtained.

Scheme 5



Synthesis of pectin oligosaccharides using galactono-3,6-lactones. Reagents and conditions: a) *i*. Ph_2SO, TTBP, DCM, Tf_2O; *ii*. Deprotection 4-OH; b) *i*. MeOH, pTsOH); *ii*. H_2O, MeOH, Pd/C, H_2; c) *i*. THF, LiOH; *ii*. H_2O, MeOH, Pd/C, H_2.

References and notes

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