Synthesis of 3-azidopropyl-functionalized GalNAc-β-(1→4)-Gal natural disaccharide

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Abstract

Oligosaccharides such as the GalNAc-β-(1→4)-Gal disaccharide are involved in host-pathogen interactions and their synthesis is a continuing challenge for organic chemists. Only a few reports have discussed the synthesis of functionalized GalNAc-β-(1→4)-Gal for its further conjugation and applications in glycobiology. The synthetic route described here is taking advantage of (1) a simple and affordable GlcNAc donor which is epimerized to the more expensive GalNAc donor and (2) a 1,6-anhydro-galactose acceptor exalting the reactivity at the 4-position of galactose. The allyloxycarbonyl (Alloc) protecting group used at the 2-position of the GalNAc residue was important (1) for a successful epimerization of the GlcNAc residue into the corresponding GalNAc donor but also (2) for the stereoselective β-glycosylation through anchimeric assistance. The key disaccharide intermediate was further transformed to a trichloroacetimidate donor which could then be glycosylated with any alcohol. The example chosen here is the 3-azidopropyl aglycon for the design of multivalent glycoclusters.

Introduction

Complex oligosaccharides are present at the surface of human cells or microorganisms and viruses. They are responsible for adhesion or communication between hosts and pathogens. The synthesis of such natural epitopes is of prime importance to decipher the functions and implications of such oligosaccharides. Recent efforts have been devoted to the synthesis of large oligosaccharides structures found in pathogenic bacteria and the challenging steps is usually the proper glycosylation reaction used to connect the monosaccharides building blocks to each other.[1,2]

The *N*-acetyl-galactosaminyl-β-(1→4)-galactose (GalNAc-β-(1→4)-Gal) is one of the smallest epitope units. This simple disaccharide by essence has been identified as the natural ligand involved in pulmonary bacterial infections by *Pseudomonas aeruginosa*[3] but also for urinary-tract infections caused by *Escherichia coli*.[4,5] It is also part of larger oligosaccharidic structures of gangliosides in which the GalNAc-β-(1→4)-Gal core is sialylated. A large family (more than 60) of gangliosides has been identified with difference in the sialylation pattern. Synthetic access to the GalNAc-β-(1→4)-Gal inner core of gangliosides is therefore of great interest. The glycosylation of the galactose reducing residue is the key step of such synthesis and the choice of the *N*-acetyl-galactosaminyl donor is at the center of the strategy. Glycosyl bromides have been initially used under Königs-Knorr conditions towards the synthesis of GM_2 and GM_1 oligosaccharides[6] but also Sd^a tetrasaccharide[7] or even for the synthesis of the GalNAc-β-(1→4)-Gal scaffold with a spacer arm.[8] Other studies have used thioglycosides as *N*-acetyl-galactosaminyl donor towards GD_2[9] or GQ_{1b}[10] oligosaccharides. These

synthetic strategies used the *N*-phthaloyl protecting group at the 2-position of the galactosaminyl residue in order to provide the β-stereoselective disaccharide. A similar trichloroacetimidate was also applied to the synthesis of Sd^a determinants.[11] Although the *N*-phthaloyl protecting group is important for the stereoselective glycosylation, its deprotection under basic and nucleophilic conditions (hydrazine or refluxing ethylenediamine or potassium hydroxide) is often not adapted to the targeted multifunctional oligosaccharides. Another approach towards the GalNAc-β-(1→4)-Gal scaffold was also developed from the 2-azido-galactosaminyl precursor activated with a dithiocarbonate for the synthesis of a de-*N*-acetyl GM_2 oligosaccharide.[12] Galactal precursors were used in a 2+2 strategy towards the tetrasaccharidic scaffold of asialo-GM_1 and the galactosaminyl residue was generated through 2-iodo-1-sulfonamidation followed by rearrangement to the corresponding thiogalactosaminyl donor.[13,14]

Results and Discussion

Strategies towards GalNAc-β-(1→4)-Gal disaccharides reported

A selection of four syntheses of the GalNAc-β-(1→4)-Gal natural epitope will be discussed (Figure 1). The key step of these syntheses was the regio- and stereoselective glycosylation and therefore highly depending on the choice of donor and acceptor used.[15] Another important feature was the epimerization of a glucosamine derivative, cheap and readily available, into a rare and much more expensive galactosamine intermediate.

The very first synthesis of the GalNAc-β-(1→4)-Gal disaccharide was reported by Shapiro and Acher[16] starting from a glycosyl bromide donor **A** incorporating a dichloroacetyl protecting group at the 2-amino position which was then converted into the corresponding acetamide. Glycosylation under Königs-Knorr conditions of the 1,6-anhydro-galactosyl acceptor **B** provided a 3:2 mixture of disaccharides in 45% yield which represented an isolated yield of 27% for the desired disaccharide. Further synthetic steps afforded the target GalNAc-β-(1→4)-Gal disaccharide after removal of the protecting groups. Although the glycosylation step was β-stereoselective, the poor yield and regioselectivity observed called for improvements.

The synthesis of the protected GalNAc-β-(1→4)-Gal disaccharide **G** was reported later by Ito and Ogawa[17] in their studies towards polysialogangliosides. The glycosylation was achieved from a conveniently protected galactose acceptor **F** and the galactosaminyl donor **E** in quantitative yield and total β-stereocontrol. Regioselectivity was controlled by the protecting group pattern on the acceptor **F**. An interesting synthetic route was also reported for the conversion of affordable and readily available glucosamine derivatives (e.g., compound **D**) into the uncommon galactosamine through a 3-*O*-carbamate intermediate in good yields (82%). Although the target GalNAc-β-(1→4)-Gal disaccharide scaffold could be obtained in good yields and high regio- and stereoselectivities, the synthetic steps described later in the manuscript did not report the introduction of a spacer arm at the reducing end.

The group of Roland Pieters and co-workers has then reported two subsequent synthetic strategies towards GalNAc-β-(1→4)-Gal disaccharides functionalized with spacer arm at the reducing end with carboxylic acid or azide moieties for further conjugations.[18,19] Both strategies also reported the acetylated disaccharides which are therefore compatible with conjugations to several biomolecules or scaffolds in comparison to the benzylated precursors presented above requiring hydrogenolysis.

The first synthesis[19] was conducted through the galactosaminyl donor **J** (obtained in three steps and 66% overall yield from galactosamine **H**) and acceptor **K** bearing a 2-trimethylsilylethyl aglycon (obtained from galactose **I** in 7 steps and 32% overall yield). The glycosylation proceeded smoothly (72%) at very low temperature (–70°C) and further elaboration provided the carboxymethyl functionalized disaccharide **L**.

A second approach was later reported improving slightly the synthetic steps for the preparation of the acceptor **M** which was obtained in 6 steps and 42% overall yield from galactose **I**.[18] The anomeric benzyl group was used in replacement of the temporary 2-trimethylsilylethyl protecting group and acetates were used for the protection of the secondary positions while another benzyl ether was necessary at the 6-position. The glycosylation proceeded similarly at very low temperature and in higher yield (90%). Another three synthetic steps were required to provide the desired GalNAc-β-(1→4)-Gal derivative **N** functionalized with the 6-azidohexyl aglycon.

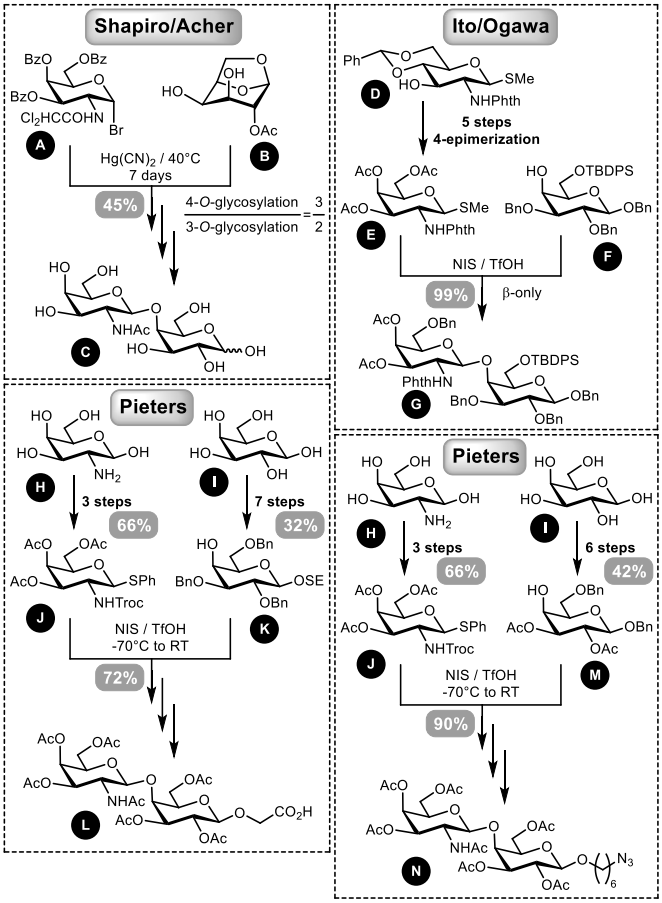


Figure 1. Different strategies towards GalNAc-β-(1→4)-Gal disaccharide

The four different strategies discussed above always required a large number of synthetic steps towards the target disaccharide. Although the stereoselectivity of the glycosylation could be easily controlled, a specific protecting group pattern was required on the galactose acceptor in order to reach high regioselectivity at the 4-position. Access to the GalNAc moieties was smartly accomplished through the 4-epimerization of a more available and affordable GlcNAc moiety. More modern reports used the galactosamine bearing a carbamate protecting group for the tight control of stereoselectivity.

Based on these observations, we have designed a synthetic strategy towards 3-azidopropyl functionalized GalNAc-β-(1→4)-Gal disaccharide (Scheme 1). The total number of steps is still quite high and similar to previous reports. Nevertheless, we have used the 1,6-anhydrogalactose strategy in analogy to the pioneering Shapiro/Acher strategy[16] in order to protect the anomeric position and also enhance the reactivity at the 4-position which is generally low in the galactose series. The 1,6-anhydro system can be easily ring-opened to an anomeric acetate which will be used for a further glycosylation step to introduce the 3-azidopropyl linker arm. Positions 2

and 3 were benzylated in order to improve the regioselectivity in comparison to the 3,4-unprotected Shapiro/Acher[16] strategy. Finally, the galactosamine moiety was prepared from the corresponding glucosamine derivative for a better accessibility.

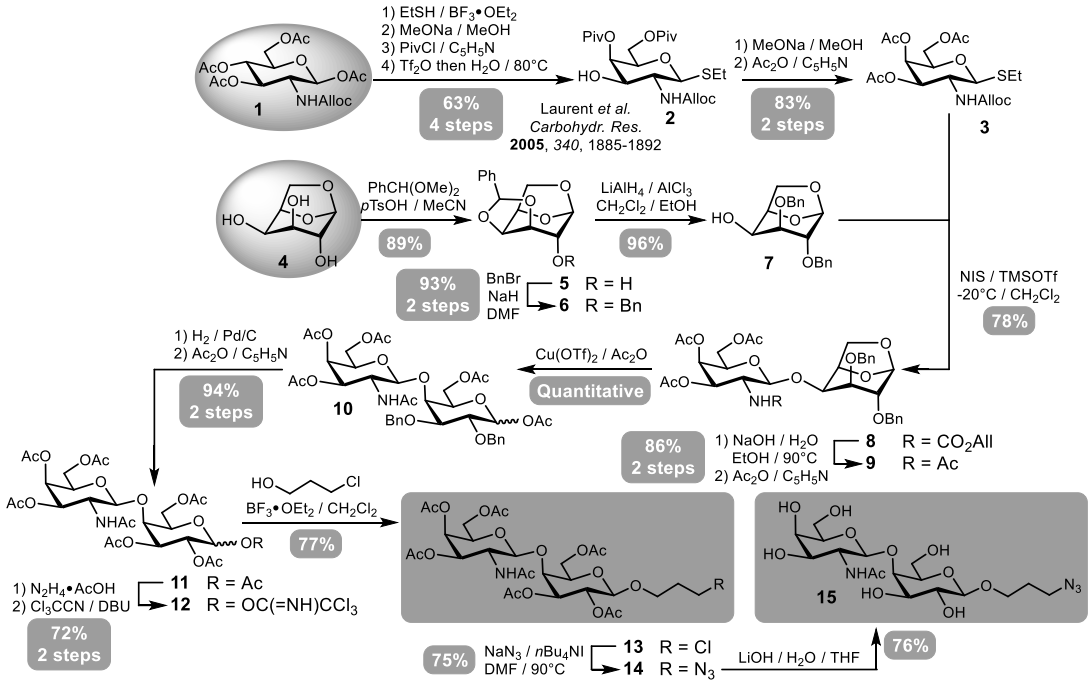
Synthesis of GalNAc-β-(1→4)-Gal disaccharide

The galactosamine-based glycosyl donor **3** can be readily obtained from D-glucosamine through standard protecting group chemistry[20] (**Scheme 1**). The allyloxycarbonyl (Alloc) carbamate protecting group is important for the β-selective glycosylation. The 4-epimerization process has been disclosed by our group[21,22] and can be applied successfully in four steps from the readily available glucosamine derivative **1**. Preparation of an ethyl thioglycoside, de-*O*-acetylation, regioselective *bis*-pivaloylation at *O*-3 and *O*-6, formation of a triflate on the free hydroxyl group at *O*-4 and inversion of configuration at *C*-4 through intramolecular rearrangement yielded the galactosamine derivative **2**[20] in good yield (63%). After de-*O*-pivaloylation and peracetylation, donor **3** was obtained in high yield (83%).

The galactose-based acceptor **7** was synthesized from 1,6-anhydro-β-D-galactopyranose (**4**)[23] which is also commercially available. Compound **4** was protected by a 3,4-*O*-benzylidene group to provide alcohol **5**, followed benzylation at *O*-2 to the fully protected compound **6**. The benzylidene acetal was then reduced regioselectively affording the 1,6-anhydro-2,3-di-*O*-benzyl-β-D-galactopyranose (**7**)[24] in high yield (96%).

Glycosylation reaction of the alcohol acceptor **7** with the thioglycoside **3** afforded the expected β-disaccharide **8** in 78% yield. Hydrogenolysis of the benzyl groups (10% Pd/C, 8 atm H_2 in 1:1 EtOH/CH_2Cl_2) was unsuccessful and only the allyl carbamate was reduced to the propyl carbamate, without cleavage of the benzyl ethers. The disaccharide **8** was therefore treated with sodium hydroxide at 90°C and reacetylated to the *N*-acetyl disaccharide **9**. The 1,6-anhydro ring was then opened using copper triflate in acetic anhydride[25] leading to compound **10** and the benzyl groups were then successfully cleaved by catalytic hydrogenation and the disaccharide **11** was recovered after acetylation.

The remaining steps were performed to introduce the 3-azidopropyl spacer arm. The anomeric position was chemoselectively deprotected using hydrazinium acetate to provide the hemiacetal intermediate which was not isolated and converted to the corresponding trichloroacetimidate glycosyl donor **12**. Glycosylation with 3-chloropropanol afforded the β-disaccharide **13** in good yield (77%) and subsequent azidation afforded the peracetylated GalNAc-β-(1→4)-Gal disaccharide **14** as the target compound for further conjugations. Saponification of the ester protecting groups provided the hydroxylated disaccharide **15** in good isolated yield (76%).



Scheme 1. Synthetic scheme for the preparation of the 3-azidopropyl-functionalized GalNAc-β-(1→4)-Gal

Conclusion

The synthesis of naturally occurring oligosaccharides and the design of synthetic routes towards such molecular targets are a continuing challenge for organic chemists. The GalNAc-β-(1→4)-Gal disaccharidic unit is part of numerous larger oligosaccharidic structures involved in host-pathogen interactions. Even though several approaches have been reported towards the synthesis of the disaccharide's core unit, only a few reports have discussed the synthesis of functionalized carbohydrates for the further conjugation. The synthetic route described here is taking advantage of (1) a simple and affordable GlcNAc donor which is converted to the more expensive GalNAc donor and (2) a 1,6-anhydro-galactose acceptor exalting the reactivity at the 4-position of galactose. The allyloxycarbonyl (Alloc) protecting group used at the 2-position of the GalNAc residue was important (1) for a successful epimerization of the GlcNAc residue into the corresponding GalNAc donor but also (2) for the stereoselective β-glycosylation through anchimeric assistance. The key disaccharide intermediate could be further transformed to a trichloroacetimidate donor which could then be glycosylated with any alcohol. The example chosen here is the 3-azidopropyl aglycon for further conjugation with oligonucleotides or other scaffolds for the design of multivalent glycoclusters.

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Graphical Abstract