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Synthesis of Thiodisaccharides Bearing *N*-Acetylhexosamine Residues: Challenges, Achievements and Perspectives

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Abstract: Carbohydrate-protein interactions are involved in a myriad of biological processes. Thus, glycomimetics have arisen as one of the most promising synthetic targets to that end. Within the broad variety of glycomimetics, thiodisaccharides have proven to be excellent tools to study these processes, and even more, some of them unveiled interesting biological activities. This review brings together research made on the introduction of *N*-acetylhexosamine residues into thiodisaccharides to date, passing through classic substitution (as S_N2, thioglycosylation and ring-opening reactions) and addition (as thiol-ene coupling and Michael-type additions) reactions. Recent and interesting developments regarding addition reactions to vinyl azides, cross-coupling reactions and novel chemoenzymatic methods are also discussed.

Keywords: Biomimetic synthesis, Glycosylation, *N*-Acetylglucosamine, Thiosugars, Thioglycosides.

1. Introduction

Most biological events mediated by carbohydrates are triggered by carbohydrate-protein recognition processes. Among carbohydrate-binding proteins, the activities and functions of glycosyltransferases, glycosidases and lectins are particularly important, as these are main participants in a refined dynamic equilibrium in the cells. Indeed, interactions between these proteins and glycans take part in a myriad of both normal and pathological biological processes, and many excellent reviews appeared on this subject so far.[1–7] Nevertheless, it is worth to make reference to cell-cell communication, adhesion mechanisms and cellular signalling, as processes in which the diversity of carbohydrate structures densely present in the cell surfaces, namely the *glycocalyx*, becomes crucial. These interactions have been studied for several decades as part of the essential processes that mediates information transfer in live beings.[8] As examples, we can mention the participation of galectins as promotors of several pathogen infections by an initial multivalent ligation of both, microorganisms and the host cell glycans,[9] and the interplay between hemagglutinins and neuraminidases in influenza virus infections.[10,11]

Thus, the study of carbohydrate-protein interactions is important to understand these processes, and even more, to develop new therapeutic agents capable of modulating them and, eventually, interfering or blocking pathological events. In the field of synthetic carbohydrate chemistry, one of the most studied approaches is the synthesis of glycomimetics. The term 'glycomimetic' stands for chemically modified carbohydrates or structurally related compounds that can mimic the natural glycans. As it is known, complex carbohydrates are not sufficiently stable in biological media as they are rapidly hydrolysed by enzymes. So, the relevance of glycomimetics relies in their stability and capacity to interfere in these recognition processes, a fact that consequently converts them into powerful tools to study carbohydrate-protein systems, and moreover to develop new drug candidates. This has been largely documented in the last years.[12–15]

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In the search of glycomimetics, a huge number of structural modifications, and concomitantly, readily and useful synthetic methodologies, have been developed, and many excellent reviews appeared on this topic in the last years.[15–17] Among the many approaches to assess glycomimetics, the replacement of either the ring oxygen atom or the glycosidic oxygen atom by carbon, nitrogen or sulphur is one of the most studied. This gives rise to carbasugars, iminosugars, thiosugars and *C*-glycosides, *N*-glycosides, and *S*-glycosides, respectively. In most cases, modification of the interglycosidic oxygen by a different atom is tolerated by biological systems, thus giving more stable products towards acidic or enzymatic hydrolysis.[18,19] Particularly, the potent activity of several iminosugars as inhibitors of glycosidases, encouraged further research on other glycomimetics, strengthening the synthetic carbohydrate field towards the exploration of new therapies based on active small molecules.[20,21]

Within the broad variety of glycomimetics, we are particularly interested in thiodisaccharides, i.e., disaccharides in which the interglycosidic oxygen atom has been replaced by sulphur. Their synthesis have been extensively studied for the last decades,[18,22] and efforts are still being made towards the development of suitable and efficient methodologies to reach them. The particular biological activities of thiodisaccharides rely on the thin balance between the structural similarities with respect to their natural analogues, as mentioned before, and their subtle differences in geometry, conformation, and flexibility. Their distinct physicochemical properties are due to the fact that sulphur is less electronegative and more polarizable than the oxygen atom.[23] It has been demonstrated that thiodisaccharides are more flexible and present more energetically feasible conformers than their oxygenated counterparts. Furthermore, the conformations adopted by thiodisaccharides, this is, the values of the interglycosidic dihedral angles ϕ and

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María Emilia Cano finished her PhD thesis in 2018 at the Universidad de Buenos Aires studying new approaches for the synthesis of carbohydrate multivalent ligands in the search of compounds which can be recognised by β-galactoside binding lectins. Between 2018 and 2020 she held a postdoctoral research position with Dr. José Kovensky (LG2A-UPJV, Amiens, France) to work in the valorisation of industrial fruit waste using analytical techniques to produce and analyse oligosaccharides able to act as plant elicitors. Recently, she returned to Buenos Aires to start a new project in the synthesis of glycomimetics with Dr. María Laura Uhrig.

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ψ, have been studied by spectroscopic and calculation methods to explain its interactions with receptor proteins.[24,25] By X-ray crystallography, it could be verified that the C–S bond (1.78 Å) is longer than the C–O bond (1.41 Å), whereas the C–S–C angle (99°) is smaller than the corresponding C–O–C angle (116°).[24,26] Consequently, as bioisosteres of the natural disaccharides, thiodisaccharides are considered as excellent synthetic targets to be used in carbohydrate-protein recognition studies, and as new potential therapeutics.[24,25] In this respect, it is noteworthy that it has been described the synthesis of a variety of thiodisaccharides having cytotoxic activity against different human cancer cell lines, reason why they have been proposed as anti-cancer drugs.[27–30]

Our research is focused on glycomimetics, particularly thiodisaccharides, bearing *N*-acetylhexosamine (HexNAc) residues (mainly *N*-acetylglucosamine, GlcNAc and *N*-acetylgalactosamine, GalNAc), as these are fundamental monosaccharides, which are constituent of a wide range of glycans, involved in numerous biological processes.[31] These sugars are part of the *N*- and *O*-glycosidic chains of glycoproteins and so, they play important roles in intracellular signalling, cell-cell and cell-pathogen interactions. Also, GlcNAc and GalNAc are the most prevalent sugars in the extracellular matrix polysaccharides, known as glycosaminoglycans (GAGs).[32–34] They also play structural functions as components of the cell wall of bacteria and fungi, and of the exo-skeleton of insects and crustaceans.[31] Remarkably, the incorporation of GlcNAc into several proteins, has tremendous consequences on key cellular processes, and aberrant glycosylation has been associated with the progression of many chronic conditions, such as cardiovascular diseases.[35,36] In this respect, some small-molecule inhibitors of *O*-GlcNAc transferase[37] have been developed with the aim to understand the biochemical mechanisms involved.[38] On the other hand, it has been demonstrated that the introduction of *S*-D-GlcNAc moieties results in enzymatically stable glycoconjugates as glycopeptides and glycoproteins.[19] Even more, its presence can interfere with biosynthetic pathways such as the synthesis of *N*- and *O*-linked glycans, as a result of its resistance towards hexosaminidases.[39]

From a synthetic point of view, the incorporation of HexNAc residues in glycans and/or glycomimetics remains a challenging task. It is well known that the presence of the –NHAc group at the 2-position favours the formation of by-products such as both 1,2- and 2,3-oxazolines, and also aziridines.[40,41] Furthermore, it was demonstrated that this functional group also interacts with neighbouring molecules by stablishing a strong H-bond network, an important reason that have a profound impact in glycosylation reactions.[42,43]

Therefore, considering that synthetic methods to obtain glycomimetics[17] and other thiosugars[44] were recently revised elsewhere, in this review we aim to bring together previous as well as recent work and efforts on the introduction of HexNAc residues into thiodisaccharides. More specifically, we will analyse here the synthetic strategies pursued to overcome the challenges imposed by the *N*-acetyl moiety in C-2, in order to reach the selected stereochemistry. Interesting recent methods involving addition reactions to vinyl azides, Michael addition to acetyl oximes, as well as chemoenzymatic approaches, will also be included.

2. General Strategies for the Access to Thiodisaccharides: the HexNAc Challenge

For the last decades, different synthetic approaches to obtain thiodisaccharides have been explored, i.e., S_N2-like nucleophilic displacement of a good leaving group located at a sugar residue by a thioaldose activated in basic medium, Michael additions of thioaldoses to unsaturated acceptors, epoxide and aziridine ring-opening reactions by thioaldoses, and enzyme-catalysed thiosugar couplings. Even though in 2006 Szilagyi and Varela reviewed the methods reported so far,[45] we will return on those initial works involving HexNAc residues, in order to globally analyse the pursued strategies in the cases of disaccharides containing such sugars.

As most *O*- and *N*-glycans are linked to protein backbones through GlcNAc residues, many thiodisaccharides having GlcNAc/GalNAc at the reducing end have been synthesised, whose general structures are schematised in the centre of Figure 1. Broadly speaking, the available methods to construct the thioglycosidic bonds, can be classified into two groups: a) those involving substitution methods, and b) those involving addition reactions. In both cases, the already mentioned reactivity of the –NHAc group (if present in any of the substrates), should be particularly taken into consideration, not only because of the presence of the H–N group as a strong hydrogen bonding group, but also because of its bidentate nucleophilic nature. Thus, the need for thiodisaccharides bearing HexNAc residues carries a synthetic challenge itself.

2.1. HexNAc-Containing Thiodisaccharides by Substitution Reactions

2.1.1. Thioglycosylation Reactions

Like glycosylation reactions, thioglycosylation reactions involve a glycosyl donor, such as a glycosyl halide or an imidate derivative, and an acceptor (a suitable protected monosaccharide having an SH group), under the presence of a promoter. Thioglycosylation reactions, as *O*-glycosylation reactions, occur through substitution mechanisms within the broad spectrum between pure S_N2 and pure S_N1 mechanisms, even though thiols are stronger nucleophiles than alcohols. Many different conditions have been described to construct a thioglycosidic

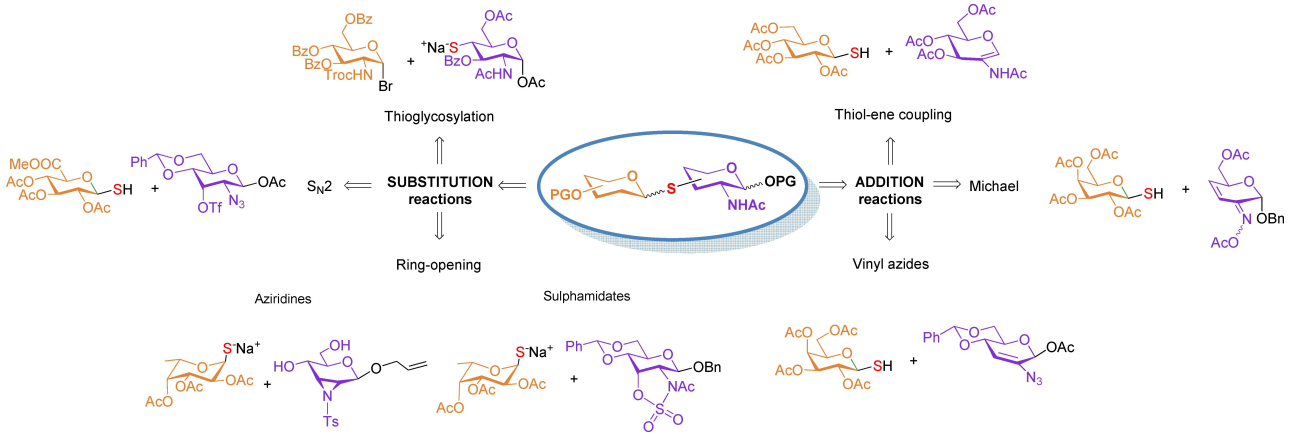


Figure 1. Different strategies pursued for the synthesis of HexNAc-bearing thiodisaccharides.

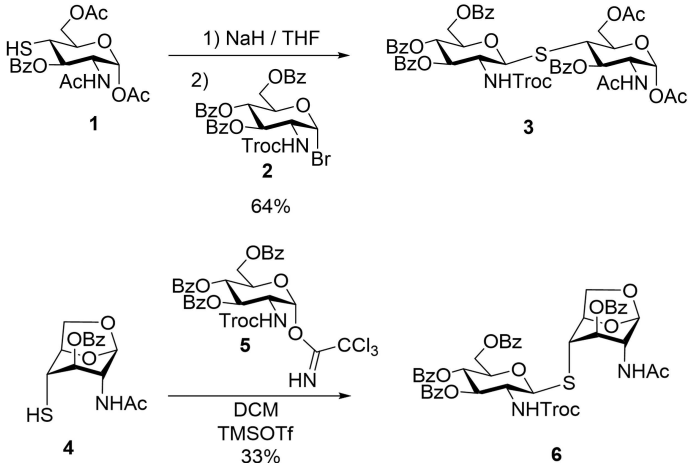
bond, considering that simple thioglycosides are common intermediates widely used in glycosylation reactions.[46–48]

With respect to GlcNAc-containing thiodisaccharides, this methodology was used by Gelas' group, in the search of analogues to the natural chitooligosaccharides produced by soil bacteria that induce symbiotic processes in plant roots, known as nodulation factors (Nod factors). Their work included different synthetic approaches to obtain di- and trisaccharide mimetics bearing the β-*S*-GlcNAc(1→4)GlcNAc fragment, envisaged as inhibitors of chitinases.[49]

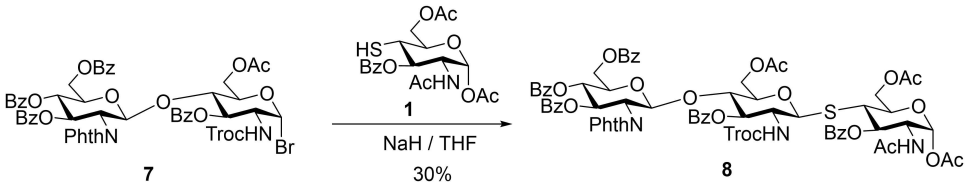
Thus, they reported thioglycosylation reactions using either a 4-thio-α-GlcNAc derivative (**1**) or the 1,6-anhydro thiol **4** as acceptors. Their glycosyl donor counterparts were respectively the 2,2,2-trichloroethoxycarbonyl (Troc) *N*-protected anomeric bromide **2** and the trichloroacetimidate **5**. In the first case, the reaction was carried out in anhydrous THF in the presence of NaH to give thiodisaccharide **3** in 64% yield, while in the second, the reaction was promoted by trimethylsilyl triflate (TMSOTf) in anhydrous DCM giving thiodisaccharide **6** in 33% yield (Scheme 1).

The thiotrisaccharide **8**[50] was also synthesised by base-promoted thioglycosylation reaction of the 4-thiol derivative **1** with the conveniently protected anomeric bromide **7** in 30% yield (Scheme 2).

Later, the synthesis of a trisaccharide having the thio-linkage at the non-reducing end was also reported by Gelas and co-workers.[51] They first used the previously synthesised 1,6-anhydro thiodisaccharide **9** to obtain trichloroacetimidate glycosyl donor **10** in a 3-step sequence. In this case, an azido group was placed at C-2, as precursor of the acetamido substituent, a common strategy employed in the synthesis of HexNAc-containing oligosaccharides. Glycosylation of this donor with the acceptor **11** using $BF_3.Et_2O$ or triethylsilyl triflate (TESTf) as promoters in toluene led to degradation of



Scheme 1. Synthesis of thiodisaccharides **3** and **6** through thioglycosylation reactions.



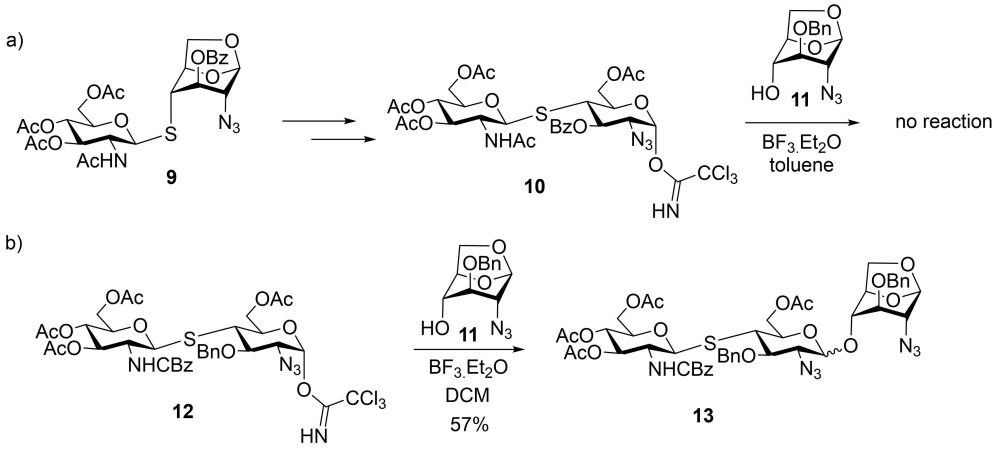
Scheme 2. Synthesis of thiotrisaccharide **8** by thioglycosylation method.

the thiodisaccharide imidate and not to the formation of the desired trisaccharide (Scheme 3a). The authors argued that this issue was due to an incompatible protecting group strategy for the thiodisaccharide donor **10**. The authors then used thiodisaccharide-based glycosyl donor **12** in a glycosylation reaction with the same acceptor **11** (Scheme 3b). Noticeably, the replacement of the benzoate protecting group present at C-3 in **10** by a benzyl group (**12**) was crucial for the success of the reaction involving the axial hydroxyl group present at C-4. Varying the reaction conditions, trisaccharide **13** was achieved in a 57% yield as an anomeric mixture with an α:β 1.0:4.7 stereoselectivity.

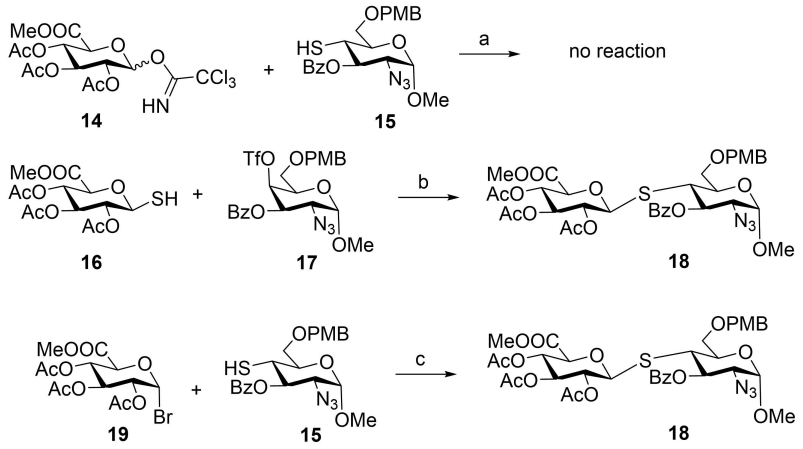
Besides, Cao and Yu developed the synthesis of a thio-linked heparan sulphate trisaccharide to act as a non-hydrolysable substrate and thus inhibitor of heparanase which may give important insight in the study of this enzyme.[52] As the authors state, this is the first report of the construction of oligosaccharides bearing a thioglycosidic linkage between glucuronic acid (GlcA) and glucosamine-derived (GlcN) units. As they employed a Glc-building block as precursor of the GlcA residue, they rationalised that the oxidation of the 6-OH should be carried out before thioglycosylation, as this step might affect the interglycosidic sulphur. Thus, two strategies were envisaged for the construction of the (1→4) thio-linkage: first, a S_N2 displacement of a GalN 4-*O*-triflate derivative with a β-GlcA thiol (βGlcASH **16**), and then, a thioglycosylation reaction between a GlcA donor and a 4-SH GlcN acceptor.

While attempting the synthesis of the desired β-(1→4) thiodisaccharide through thioglycosylation reaction using a GlcA trichloroacetimidate (**14**) no reaction occurred using either TMSOTf or $BF_3.Et_2O$ as promoters. The displacement of a GalN 4-*O*-triflate derivative (**17**) with a GlcA thiolate gave a low 22% yield of the synthetic target **18**. Instead, treatment of GlcA anomeric bromide **19** with a 4-thio-GlcN derivative (**15**) mediated by Cs_2CO_3 in DMF gave a good 56% yield of the desired thiodisaccharide (Scheme 4).

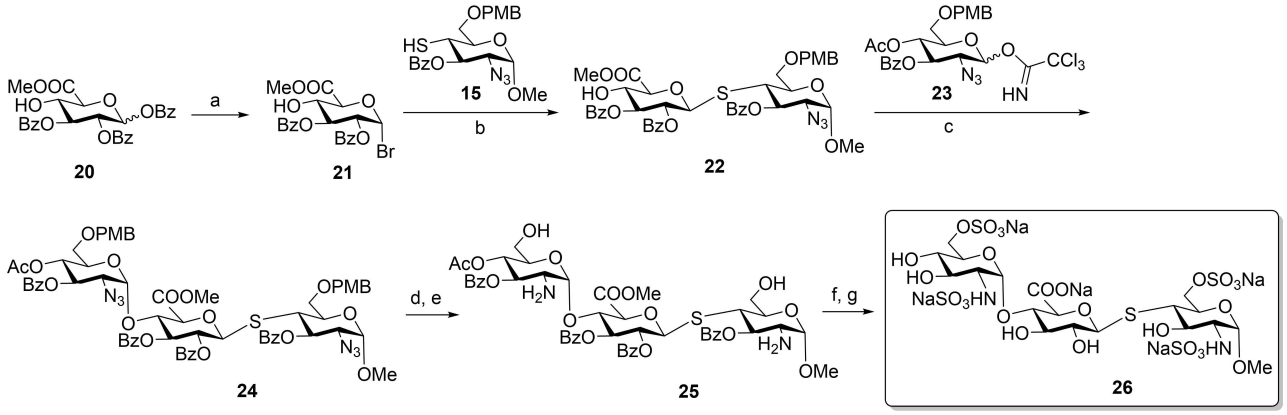
The same procedure using a glycosyl bromide derived from a 4-deprotected GlcA residue (**21**) and the *gluco*-configured 2-azido-4-thio derivative **15**, yielded thiodisaccharide **22**, in a very good 67% yield. Furthermore, thiotrisaccharide **24** was obtained by glycosylation of the free 4'-OH of **22** with the conveniently prepared imidate **23**, promoted by *tert*-butyldimethylsilyl triflate (TBSOTf). Finally, the target thio-trisaccharide **26** was prepared by treating **24** with DDQ to remove both 6- and 6'-*p*-methoxybenzyl groups without altering the thioglycosidic linkage, then reducing both azide groups with 1,3-propanedithiol and Et_3N, simultaneously sulphating 6- and 6'-hydroxyl and 2- and 2'-amino groups with SO_3-pyridine and Et_3N, and last deprotecting both acetyl and benzoyl groups (Scheme 5). Importantly, the authors



Scheme 3. a) No reaction occurred when attempting thioglycosylation reaction of acceptor **11** with glycosyl donor **10**. b) Synthesis of trisaccharide **13** bearing a thio-linkage through glycosylation reaction.



Scheme 4. Reagents and conditions: a) TMSOTf or $BF_3.Et_2O$, DCM, −78 °C to rt; b) NaH, DMF, rt, 22%; c) Cs_2CO_3, DMF, rt, 56%.

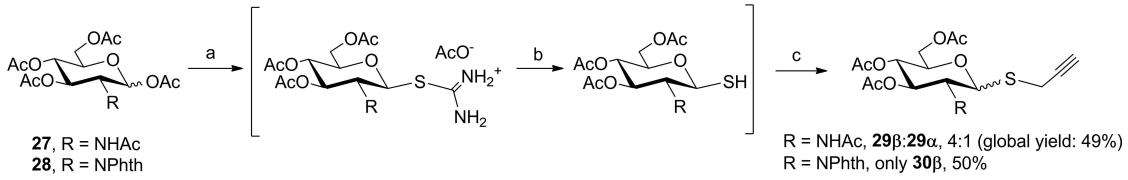


Scheme 5. Reagents and conditions: a) 33% HBr/AcOH, 0 °C, 71%; b) Cs_2CO_3, DMF, rt, 67%; c) TBSOTf, 4 Å MS, toluene, −20 °C, 74%; d) DDQ, DCM : H_2O (10 : 1 v/v), rt, 99%; e) $HS(CH_2)_3SH$, Et_3N, Py/H_2O, rt, 100%; f) SO_3-Py, Et_3N, DCM, rt; g) NaOH (2 N), rt, then neutralisation with IR-120 [H^+], ion exchange with IR-120 [Na^+], desalting with G10 gel, and lyophilization, 23%.

remark that this work represents the first synthesis of a heparan sulphate/heparin oligosaccharide bearing a thioglycosidic linkage.

At this point, it should be mentioned that the synthesis of the GlcNAc and GalNAc 1-thioaldoses and thioglycosides can be in general achieved by following the same methodologies recommended for simple monosaccharides.[47,48,53] Early reports on the synthesis of β-1-thioGlcNAc (βGlcNAcSH) were made by Horton and Wolfrom in 1962.[54] As we are going to discuss later the synthesis of thiodisaccharides bearing two thioglycosidic bonds, we want to make a short reference to the thioglycosylation method involving treatment of a peracetylated derivative with $BF_3.OEt_2$/thiourea and a good electrophile. Initially reported by Ibatullin,[55,56] a one-pot procedure proved to be useful to obtain 1,2-*trans* thioglycosides. Moreover, by optimisation of the experimental conditions, the synthesis of thioalkynyl GlcNAc and GlcNPhth derivatives was achieved, although the yields were lower than in the case of Glc, Gal and other simple monosaccharides.[57] Still, it showed to be convenient as the number of reaction as well as purification steps were sensibly reduced, and also was compatible with both, *N*-acetyl and *N*-phthalimido (NPhth) groups present at C-2 (Scheme 6). Interestingly, a higher stereoselectivity towards the β-anomer was observed in the case of the NPhth derivative, in comparison with the acetamido group, a fact that can be easily explained by the stronger anchimeric participation effect of the C-2 substituent in the case of the former derivative.[58,59]

This strategy proved to be successful when applied to more complex structures, such as thiodisaccharides, as will be discussed later.



Scheme 6. Reagents and conditions: a) Thiourea, $BF_3.OEt_2$, CH_3CN (anh.), 82 °C, 4–12 h; b) Et_3N; c) propargyl bromide, rt, 18 h.

2.1.2. S_N2 Reactions

Taking advantage of the high nucleophilicity of the sulphur atom, the reaction of a thioaldose with a convenient sugar precursor having a good-leaving group, largely proved to be an excellent strategy to obtain thiodisaccharides through S_N2 processes.[44,45,60] The higher stability of the anomeric configuration of 1-thioaldoses with respect to common aldoses is a major advantage.[61] Still, the inherent complexity of sugar chemistry, and the interplay between neighbour group participation and stereochemical aspects should be carefully considered for the success of the reactions, particularly when HexNAc building blocks are involved.

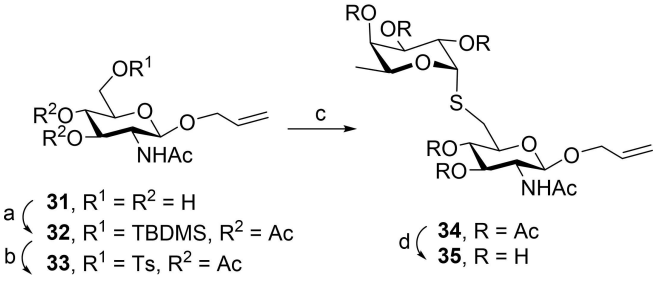
To begin with, the S_N2-approach was used to study the incorporation of α-L-fucopyranosyl residues into HexNAc-bearing thiodisaccharides. The most abundant linkages of L-fucose in glycoproteins and glycolipids are α(1→3), α(1→4) and α(1→6) to D-GlcNAc, so Hashimoto and co-workers explored the synthesis of the corresponding thio-linked disaccharides.[62]

The α(1→6) thiodisaccharide was synthesised by S_N2 displacement of a 6-*O*-tosylate of GlcNAc (**33**), which was obtained from the GlcNAc allyl glycoside **31** through classic methods. Fucosyl thiolate, obtained by treatment of α-1-thiofucose (αFucSH) with sodium hydride, was used as nucleophile (Scheme 7). This synthetic sequence efficiently afforded thiodisaccharide **35**.

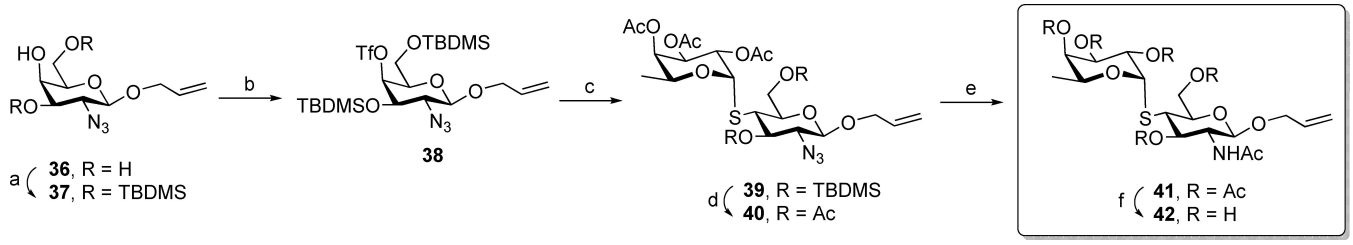
For the synthesis of the α(1→4) thiodisaccharide, the starting material was the allyl 2-azido-2-deoxy-β-D-galactopyranoside **36**. First, its 3,6-di-*O*-TBDMS derivative **37** was obtained and subsequentially subjected to triflation to furnish the 4-*O*-triflate derivative **38**. This compound was not isolated but used next to obtain the desired thiodisaccharide by substitution of the triflate group with αFucSH in the presence of sodium hydride. Thus, compound **39** was obtained in a nice 68% yield from **37**, and further protecting group manipulation led to the desired thiodisaccharide **42** (Scheme 8).

The construction of the α(1→3) thiodisaccharide required a different synthetic approach and will be described later (Section 2.1.3).

In another pioneering work, Wang and Lee presented for the first time the synthesis of thiochitooligosaccharides. The syntheses of di-, tri-, and tetra-thiosaccharides was reported herein.[63] For the construction of the β-GlcNAc-(1→4)-β-GlcNAc thio-linkage the authors used the S_N2 approach. By



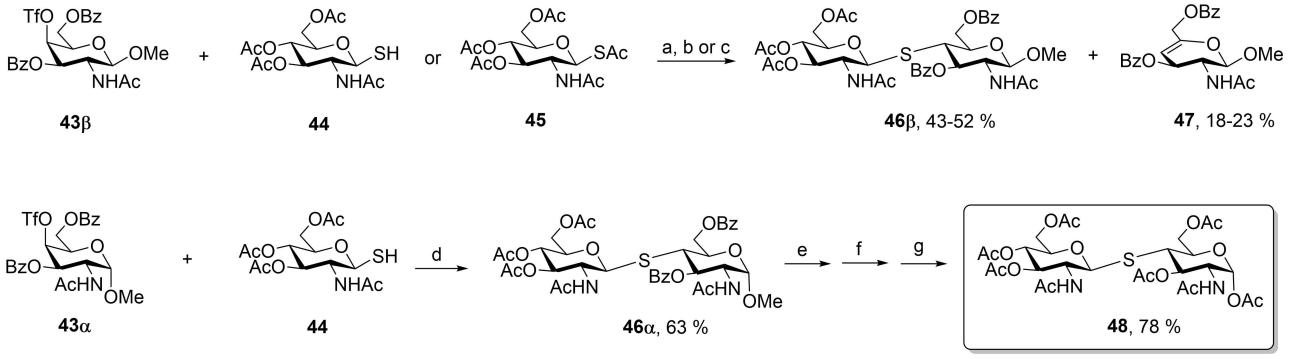
Scheme 7. Reagents and conditions: a) i) TBDMSCl, imidazole, DMF, ii) Ac_2O, DMAP, py, 61% (2 steps); b) i) 60% AcOH, 70 °C, ii) TsCl, py, 60% (2 steps); c) i) αFucSH, NaH, DMF, ii) Ac_2O, DMAP, py, 99%; d) NaOMe, MeOH, 91%.



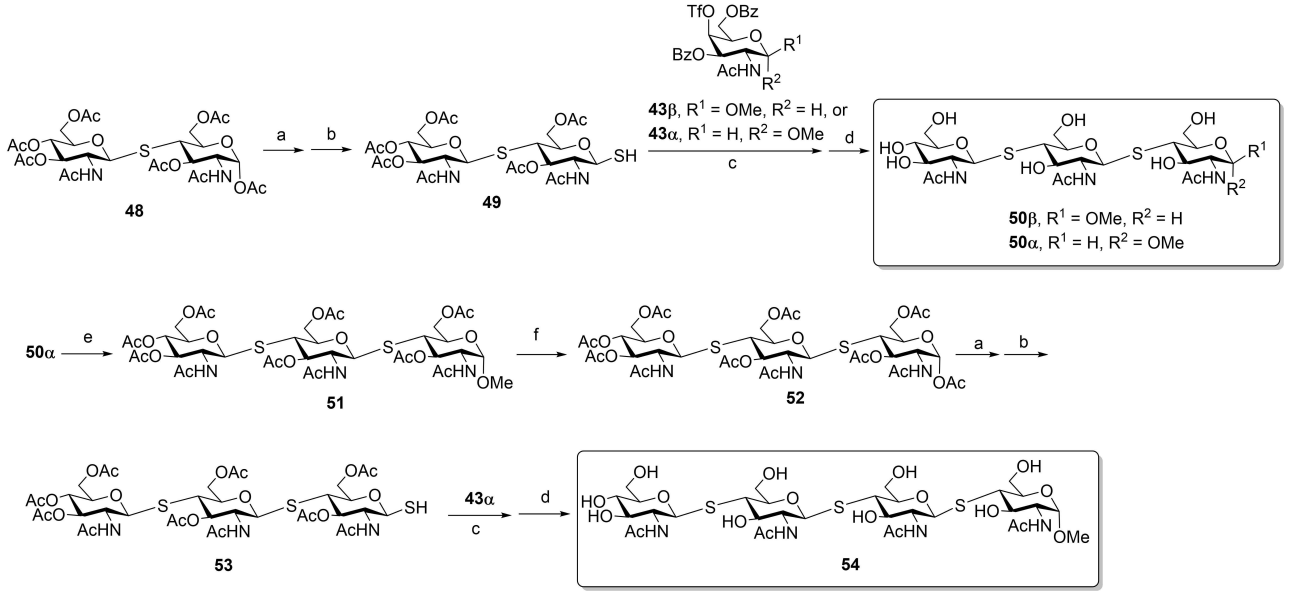
Scheme 8. Reagents and conditions: a) TBDMSCl, imidazole, DMF, 60 °C, 58%; b) Tf_2O, py, DMAP, DCM; c) i) αFucSH, NaH, DMF, ii) Ac_2O, DMAP, py, 68% (from 37); d) i) Bu_4NF, THF, ii) Ac_2O, DMAP, py, 91% (2 steps); e) i) H_2S, py, H_2O, ii) Ac_2O, DMAP, py, 97% (2 steps); f) NaOMe, MeOH, 65%.

displacement of the GalNAc 4-*O*-triflate **43β** with the 1-thiolate formed from βGlcNAcSH (**44**) the authors obtained the thiodisaccharide **46β** in 45% yield, with also 23% yield of the E2 product **47**. The authors intended to improve the yield of thiodisaccharide **46β** and minimise the formation of the eliminated by-product by treating the anomeric GlcNAc thioacetate (**45**) with **43β** in the presence of cysteamine and dithioerythritol (DTE) in DMF. Nonetheless, this method gave 43% yield of the mentioned thiodisaccharide. Direct coupling between triflate **43β** with thiol **44** promoted by cysteamine in DMF gave 52% yield of **46β**. However, the formation of the unsaturated product could not be avoided by none of these approaches. Interestingly, when performing the reaction with α- instead of β-methyl glycoside, 63% yield of the corresponding thiodisaccharide was obtained together with a minimum amount of the eliminated by-product (not quantified). Then, either thiodisaccharide **46β** as well as **46α** were subjected to total deprotection and peracetylation to obtain thiodisaccharide **48** (Scheme 9).

Then the authors applied this method to extend the thiochitobiosides to tri- and tetrasaccharides. To that aim, thiodisaccharide **48** was treated with acetyl chloride saturated with HCl to obtain the anomeric chloride which was subsequently displaced with thiourea in acetone and then reduced with aqueous sodium sulphite to furnish the anomeric thiol **49** (Scheme 10). With this building block in hand, the



Scheme 9. Reagents and conditions: a) **44**, NaH/DMF, 0 to 20 °C, 4 h (**46β**: 45%, **47**: 23%); b) **45**, DTE, cysteamine, DMF, 0 °C to rt, overnight (**46β**: 43%, **47**: 18%); c) **44**, cysteamine, 0 °C to rt, 3 h (**46β**: 52%, **47**: 22%); d) **44**, cysteamine, DTE, DMF, 0 °C to rt, 20 h, 63%; e) NaOMe, MeOH, 20 °C; f) Ac_2O, py, 20 °C; g) Ac_2O:AcOH:H_2SO_4 (8:2:0.1 v/v), 20 °C, 7 h, 78% from **46α**.



Scheme 10. Reagents and conditions: a) acetyl chloride, DCM, HCl (gas), 0–20 °C; b) i) thiourea, acetone, reflux, ii) aq. Sodium sulphite, 20 °C, iii) 5% HCl, (**49**: 83%, **53**: 78%); c) **43β** or **43α**, DTE, cysteamine, DMF, 20 °C; d) i) NaOMe, MeOH, 20 °C, ii) purification by Sephadex G10 column chromatography (**50β**: 30%, **50α**: 35%, **54**: 28%); e) Ac_2O, py, 20 °C, 95%; f) Ac_2O:AcOH:H_2SO_4 (8:2:0.1 v/v), 20 °C, 7 h, 81%.

same S_N2 method was applied by displacement of triflates **43β** or **43α** in the presence of cysteamine and DTE, giving thiotrisaccharides **50β** and **50α**, respectively. The same methodology was applied to obtain the thiotetrasaccharide **54** (Scheme 10).

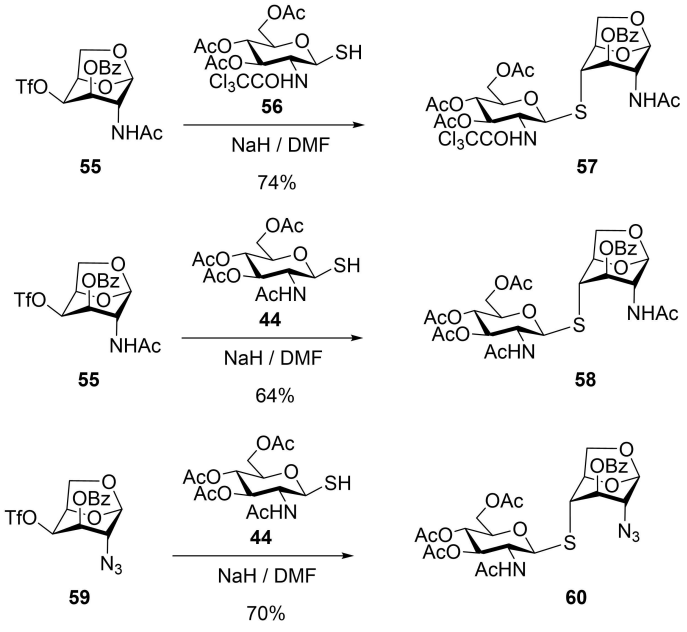
In connection with the previously discussed thioglycosylation methods, Gelas' group also explored S_N2 approaches to reach chitooligosaccharides, in the search of alternative improved conditions.[49] So, the construction of the thioglycosidic bond was achieved by nucleophilic displacement of either the 4-*O*-triflates **55** or **59** with GlcNAc anomeric thiolate derivatives formed from **56** and **44** with NaH in anhydrous DMF. Thiodisaccharides **57**, **58** and **60** were successfully obtained in yields ranging from 64 to 74% (Scheme 11). In these cases, the S_N2 reactions proved to be a better alternative than thioglycosylation, as the yields were considerably higher.

On the other hand, they synthesised thiodisaccharide **63** which bears a carboxybenzyl *N*-protected GlcN moiety at the non-reducing end and a 3-*O*-benzyl GlcN residue at the reducing end. This thiodisaccharide was obtained in 88% yield by S_N2 displacement of the *galacto* 1,6-anhydro-triflate **62** with the GlcNHCBz anomeric thiolate derived from **61**. Prepared thiodisaccharide **63** was then conveniently transformed into imidate **12** (Scheme 12), which was used as glycosyl donor in the synthesis of thiotrisaccharide **13** (Section 2.1.1, Scheme 3b).[51]

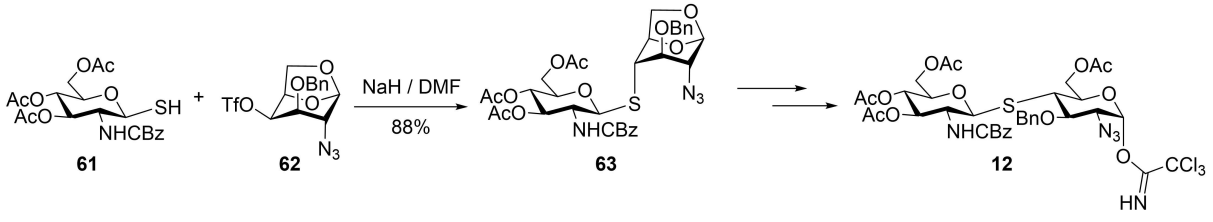
Furthermore, Rye et al. synthesised the *S*-β-GalNAc-(1→4)-GlcA thiodisaccharide which is a mimetic of the repetitive unit of the chondroitin chain. The purpose of this synthesis was to obtain a molecule that could act as inhibitor of chondroitin AC lyases for both therapeutic strategies and structural analysis of the active site of this enzyme. Therefore, the authors used an S_N2 approach to reach the thio-linkage by nucleophilic displacement of the conveniently protected *galacto* 4-*O*-triflate **65** with the corresponding β-1-thiogalactosamine (βGalNAcSH, **64**) anomeric thiolate.[64] The resulting thiodisaccharide (**66**) was obtained in 43% yield and treated afterwards with 10% TFA in DCM to deprotect the 4-methoxybenzyl group giving compound **67** in 86% yield. Successively, the primary alcohol was oxidised under Jones conditions giving compound **68** bearing the GlcA moiety in 73% yield. Remarkably, the oxidising conditions did not alter the thioglycosydic bond. Finally, compound **68** was deprotected with NaOMe/MeOH to obtain the free chondroitin mimetic thiodisaccharide **69** in 48% yield (Scheme 13).

Compound **69** was subjected to inhibition kinetic studies using the chondroitin AC lyase from *Flavobacterium heparinum.* This thiodisaccharide surprisingly binds poorly to the enzyme, acting as competitive inhibitor with a K_i value of 45 mM. The authors conclude that, in this case, differences in bond length and angles between the thioglycosidic bond and its natural *O*-counterpart could be the reason for the low binding.

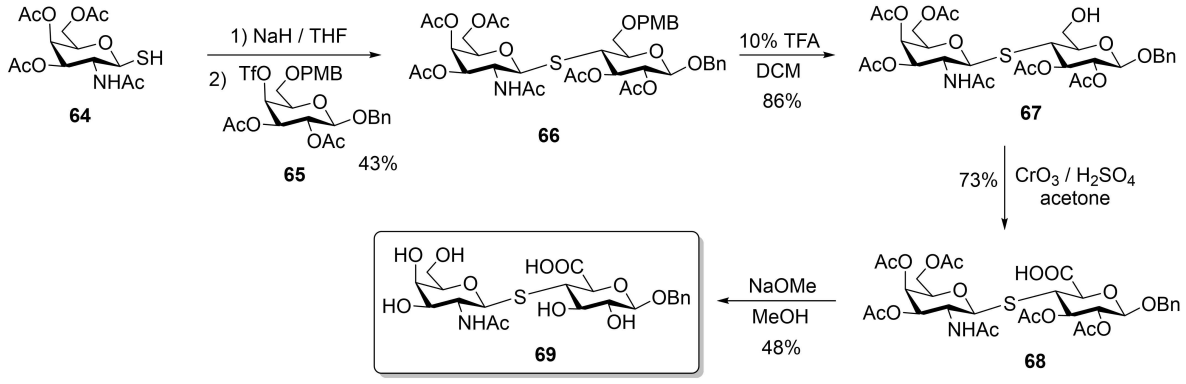
Feng et al. developed a synthetic method to transform D-GlcNAc into D-GalNAc derivatives in a straightforward way, as D-GalNAc is sensitively more expensive to obtain from commercial sources than its 4-epimer.[65] In an attempt to show the versatility of their synthetic method, the authors used one of their intermediates (triflate **70**) to successfully obtain four thiodisaccharides bearing GalNAc residues which are



Scheme 11. Synthesis of thiochitooligosacharides by S_N2 method gave better results than thioglycosylation reactions.



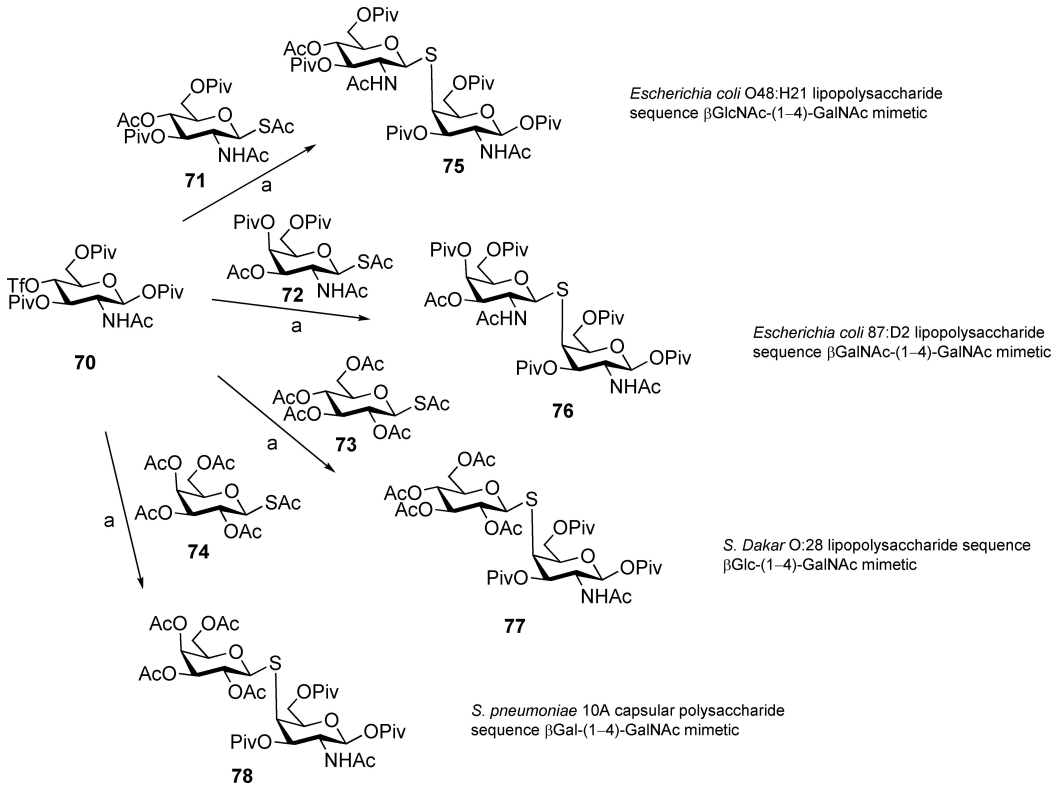
Scheme 12. Synthesis of thiodisaccharide **63** and the imidate derivative **12**.



Scheme 13. Synthesis of the chondroitin thiodisaccharide analogue **69**.

analogues of several sequences of bacteria polysaccharides (**75**–**78**, yields ≈60–70 %). To this aim, S_N2 classic reactions were carried out by reacting a crude triflate with different sugar anomeric thioacetates in the presence of Et_2NH at −5 °C in anhydrous DMF (Scheme 14).

As part of a study on the mechanism and inhibition of chitinases, the synthesis and conformational analysis of four *O*- and *S*-glycosides of *N*,*N*'-diacetylthiochitobiose was reported by Fettke et al.[66] Starting from either GlcNAc anomeric chloride **79** or pentaacetylglucosamine **27β** both β-methyl and β-*p*-methoxyphenyl 3,5-di-*O*-benzoylated glycosides (**80** and **81**) were prepared using standard methods. Next, these glycosides were epimerised using the Lattrell-Dax conditions[67,68] giving the GalNAc derivatives **84** and **85**. After acetylation of the 4-position of compound **85**, this *p*-methoxyphenyl GalNAc glycoside was used as starting material to



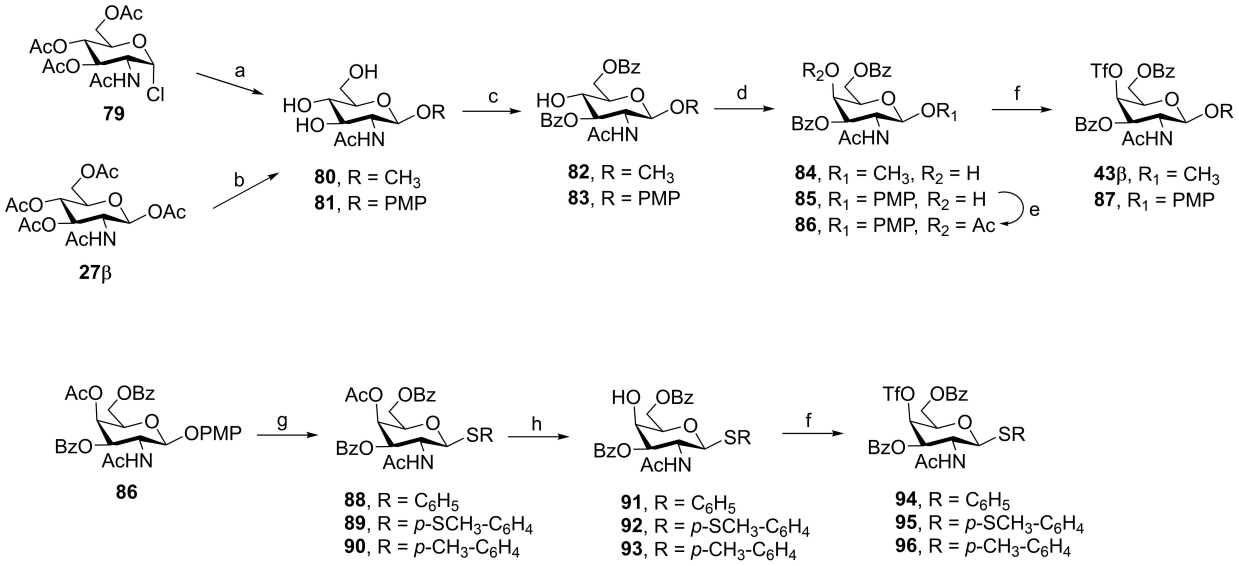
Scheme 14. Reagents and conditions: a) Et_2NH/DMF, −5 °C, overnight.

obtain *S*-aryl derivatives (**88**–**90**) by reaction with aryl thiols and $BF_3.Et_2O$. A final triflation of the free 4-position in **84**, **85**, **91**, **92** and **93** was performed to obtain the precursors **43β**, **87**, **94**, **95** and **96** (Scheme 15). This sequence illustrates the big efforts behind the synthesis of most of the thiodisaccharides referred to here, which require protecting group manipulation and often inversion of the configuration of some stereocentres (as also S_N2 processes occurs with inversion of the sp^3-carbon). In this context, Lattrell-Dax epimerisation reaction proved to be an excellent resource for a clean C-inversion in mild conditions.[67–69]

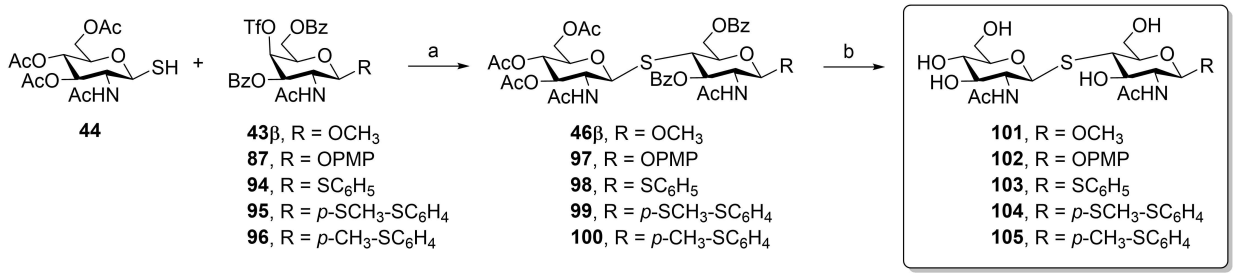
Then, the authors[66] used the GalNAc 4-*O*-triflate derivatives to obtain thiodisaccharides through S_N2 displacement with GlcNAc thiolate in DMF. Therefore, thiodisaccharides bearing *O*- or *S*-glycosidic linkages at the reducing end were prepared (**101**–**105**) (Scheme 16).

The authors[66] also synthesised a thiazoline derivative that mimics the oxazoline produced by several hydrolases (including chitinases and *N*-acetylhexosaminidases) by hydrolysis of chitin. For this purpose, thiodisaccharide **97** was treated with *Lawesson* reagent followed by deprotection to afford the thiazoline **107** (Scheme 17). Besides, the authors performed a complete conformational analysis using NMR techniques and molecular modelling. Finally, they tested compounds (**101**–**105** and **107**) against chitinases and *N*-acetylchitinases.

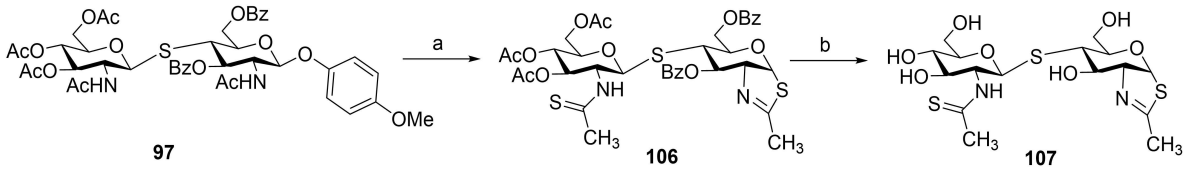
It has been described that Tn (GalNAcα1-Ser/Thr) and STn (Neu5Acα2,6GalNAcα1-Ser/Thr) are carbohydrate antigens associated with tumorigenesis and tumour progression and pointed research attention since they are not normally expressed but highly expressed on tumour cell surfaces. Consequently, they can be considered as targets for cancer immunotherapy. In this context, Huo and Ye presented the



Scheme 15. Reagents and conditions: a) NaOMe, MeOH, DCM, rt, 2 h, 76%; b) i) *p*-methoxyphenol, $BF_3.Et_2O$, DCM, rt, 72 h, 87%, ii) LiOH, MeOH, rt, 4–8 h, 94%; c) BzCl, Py, −60 °C to rt, 16 h (**82**: 87%, **83**: 78%); d) Tf_2O, Py, DCM, −15 °C, 1 h, then $NaNO_2$, DMF, rt, 16 h (**84**: 86%, **85**: 58%); e) Ac_2O, Py. 0 °C to rt, 24 h, 96%; f) Tf_2O, Py, DCM, 0 °C, 2 h (**43β**, **87**, **94**, **95**, **96**: 99%); g) aryl thiol, $BF_3.Et_2O$, 4 Å MS, $CHCl_3$, 60 °C, 24 h (**88**: 79%, **89**: 68%, **90**: 67%); h) AcCl, MeOH, $CHCl_3$, 0 °C to rt, 24–48 h (**91**: 56%, **92**: 54%, **93**: 17%).



Scheme 16. Reagents and conditions: a) NaH, DMF, 0 °C to rt, 5–16 h for **46β**, 57%, NaH, 15-crown-5, THF, rt, 6 h for the rest (**97**: 47%, **98**: 31%, **99**: 28%, **100**: 33%); b) NaOMe, MeOH, rt, 16 h (87–97%).



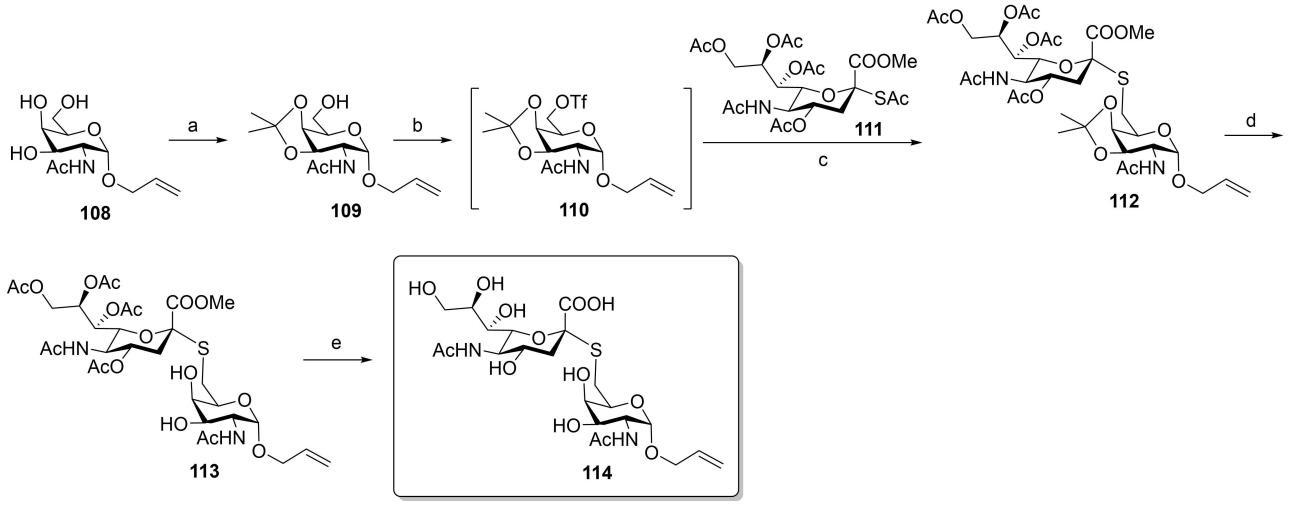
Scheme 17. Reagents and conditions: a) *Lawesson* reagent, toluene, 80 °C, 72 h, 56 %; b) NaOMe, MeOH, rt, 16 h, 98 %.

synthesis of an STn thio-analogue.[70] To synthesise such glycomimetic, the authors started from the GalNAc allyl-glycoside **108** which was treated with 2,2-dimethoxypropane and camphorsulphonic acid (CSA) to furnish compound **109**, where positions 3- and 4- were selectively protected. Compound **109** was then treated with Tf_2O/py in DCM to achieve the GalNAc 6-*O*-triflate **110** which was not isolated from the reaction but subsequently treated with the sialyl thioacetate **111** in the presence of diethylamine in anhydrous DMF. Thus, the desired (2→6) thiodisaccharide **112** was obtained through an S_N2 displacement in 40 % yield (Scheme 18). The moderate yields can be associated to the axial disposition of the substituent at C-4, which partially blocks the access of the nucleophile to C-6. Further deprotection of compound **112** with classic methods afforded the target thiodisaccharide **114**. Still, the obtention of **114** was of great importance due to the previously mentioned relevance of STn antigens. The authors expressed that these results would contribute to the development of carbohydrate-protein conjugates as potential anti-cancer vaccines.[70]

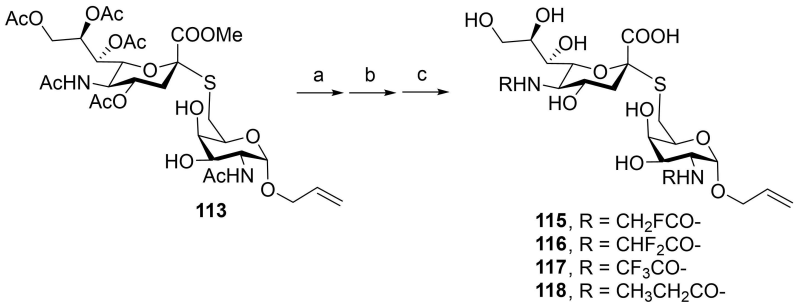
In a more recent work, Huo et al. addressed *N*-acyl modifications of both Neu5Ac and GalNAc residues of thiodisaccharide **114** and used all the synthesised compounds to obtain Keyhole Limpet Hemocyanin (KLH) conjugates.[71] Starting from the previously synthesised thiodisaccharide **113**, both *O*- and *N*-acetyl groups were removed by treatment with NaOMe in MeOH and a subsequent reflux under 2 M NaOH. The resulting completely deprotected thiodisaccharide was treated with either methyl fluroacetate, difluroacetate, trifluoroacetate or propionic anhydride to achieve the *N*-acyl modified derivatives **115–118** (Scheme 19).

To ensemble the *N*-acyl modified thiodisaccharides to the carrier proteins, the authors assayed a photoaddition reaction of 2-aminoethanethiol to the corresponding allyl glycosides **115–118** followed by treatment with the bifunctionalised linker **119**. Thus, compounds **120–123** were obtained in moderate to good yields. Finally, all these carbohydrate derivatives were assembled with the carrier proteins by incubation in PBS buffer, affording the KLH conjugates **124a–127a** and the BSA conjugates **124b–127b** (Scheme 20).

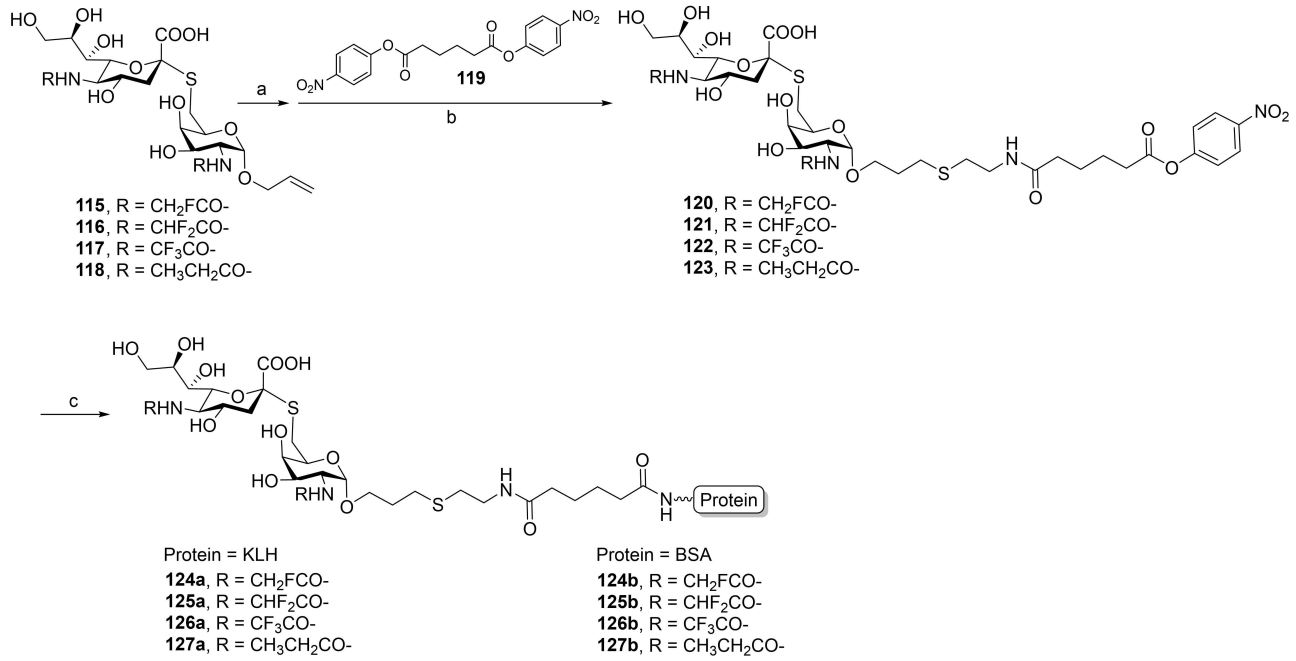
The authors also prepared a series of analogues in which the *N*-acyl modified function was introduced only either at the GalNAc residue or the Neu5Ac residue. In the first case, the *S*-linked derivatives having an *N*-acyl modified GalNAc moiety were prepared by starting the synthesis introducing a trifluor-



Scheme 18. Reagents and conditions: a) 2,2-dimethoxypropane, CSA, rt, 82 %; b) Tf_2O, py, DCM, −78 to 0 °C; c) **111**, Et_2NH, DMF, 0 °C, 40 % from **109**; d) 80 % AcOH, 70 °C, 87 %; e) NaOMe (30 % in MeOH), then 1 N NaOH, rt, 78 %.



Scheme 19. Reagents and conditions: a) NaOMe (30 % in MeOH) rt; b) 2 M NaOH, reflux; c) methyl ester of corresponding carboxylic acid, MeOH, Et_3N, Ar, reflux or propionic anhydride, $NaHCO_3$, MeOH, 0 °C (**115**: 93 %, **116**: 95 %, **117**: 74 %, **118**: 94 %, from **113**).



Scheme 20. Reagents and conditions: a) $HSCH_2CH_2NH_2.HCl$, MeOH, UV (254 nm), rt; b) **119**, Et_3N, DMF, Ar, rt (**120**: 35 %, **121**: 27 % **122**: 70 %, **123**: 60 %, from **115**–**118**), c) PBS buffer, KLH or BSA, rt.

oacetyl protecting group at the 2-position. In the second case, the *S*-linked derivatives bearing an *N*-acyl modified Neu5Ac moiety were synthesised by direct *N*-transacylation with trifluoroacetic anhydride.

The immunogenicity of these glycoconjugates was examined afterwards in a mice model. Mice were vaccinated with KLH conjugates biweekly and the produced antibodies were titrated by ELISA using the corresponding BSA conjugates for the plate coating. All the *N*-acyl modified STn thio-analogues elicited strong antigen-specific immune responses after the fourth vaccination. The authors used a glycoconjugate bearing thiodisaccharide **114** without *N*-acyl modifications as a control. They showed that the conjugate **124a** bearing an *N*-acyl thiodisaccharide modified with two fluoroacetyl groups induced an IgG titer 10-fold greater than that of the control. Better results were obtained in all cases when using fluorinated glycoconjugates.

As previously stated, (1→3) thiodisaccharides bearing HexNAc residues at the reducing end are often difficult to obtain due to side reactions leading to 2,3-oxazoline by-products. Thus, we have recently performed some synthetic studies towards the obtention of such thiodisaccharides. Initial experiments starting from the 3-*O*-triflate derivative of the thioalkynyl GlcNAc precursor **128**, confirmed that the thioaldose S_N2 nucleophilic displacement competed with the intramolecular cyclisation caused by the attack of the acetamido carbonyl oxygen.[72] Thus, a low yield of the desired thiodisaccharide **130** was obtained, even at low temperatures,

such as −45 °C. The oxazoline **131** was also recovered, which was a major drawback as this ring could not be opened under nucleophilic or basic conditions (Scheme 21).[73–75] Nevertheless, oxazoline **131** was attractive as it can serve as precursor for βAllNAc residues. AllNAc is considered a "rare" sugar, and it is naturally found only in *Streptomyces sp.* as part of the pseudotrisaccharide *allosamidin*,[76] which is the most studied insect *endo*-chitinase inhibitor.[77] Hydrolysis of **131** in acid conditions led to a thioalkynyl-βAllNAc clickable building block, which was used further to prepare a resorcinarene-scaffolded octavalent glycocluster. Interestingly, the octavalent βAllNAc glycocluster showed affinity towards the wheat germ agglutinin (WGA), a legume lectin extensively studied due to its ability to recognise GlcNAc residues.[59] This was the first report describing the interaction between AllNAc residues and WGA. and may open the possibility to new chemical and biochemical developments.

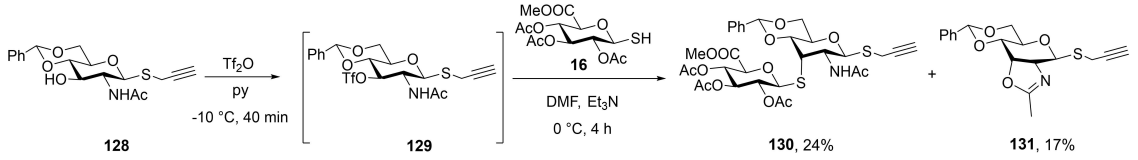
To reach the envisaged products a redesign of the synthetic strategy was necessary. Thus, the installation of an azido group at the C-2 position of a *gluco*-configured precursor replacing the 2-acetamido group was crucial to obtain the desired (1→3) thiodisaccharides. So, starting from **132**, a known 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose precursor,[78] we managed to obtain the *allo*-configured precursor **134** by a standard sequence involving triflation and sodium nitrite treatment (Scheme 22). Gladly, both **133** and **134** showed to be suitable precursors of the (1→3)HexNAc-containing thiodisaccharides, as follows.

On the one hand, treatment of the 3-*O*-triflate intermediate **133** with βGlcASH **16**, led to the $AllN_3$ thiodisaccharide **135** in a good 60% yield, along with an α-*S*-GlcA derived subproduct (**136**) (Scheme 23).[72] Even though there is consensus on the enhanced anomeric stability of thioaldoses, as stated before, the formation of this product can only be explained by a fast anomerisation of the βGlcASH derivative **16**, before the attack to the electrophilic C-3 of the $GlcN_3$ triflate **133**. The presence of the electron withdrawing carboxyl group at C-6 makes the ring-opening and the concomitant anomerisation more feasible.[79] Valuable reports on the mutarotation of thioaldoses during 1-*S*-glycosylation and similar reactions can be found in the literature.[80–83]

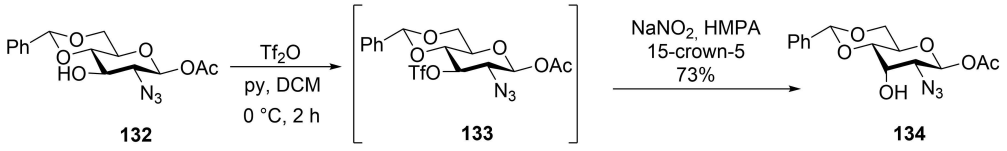
Further protecting group manipulation starting from **135** occurred successfully without affecting the thioglycosydic bond, and gave rise to the (1→3) AllNAc thiodisaccharide **138** (Scheme 24), which was obtained as an α:β 3:2 anomeric mixture in 67% yield (2-steps).

In this way, β- and α-*S*-GlcA(1→3)AllNAc thiodisaccharides were obtained, which were afterwards functionalised with thioalkynyl residues by using the one-pot thioglycosylation procedure described above.[57] Thus, the synthesised derivatives **139β** and **139α**, having two thioglycosydic bonds, are suitably prepared for further click conjugation. Further studies in this respect are on the way (Scheme 25).

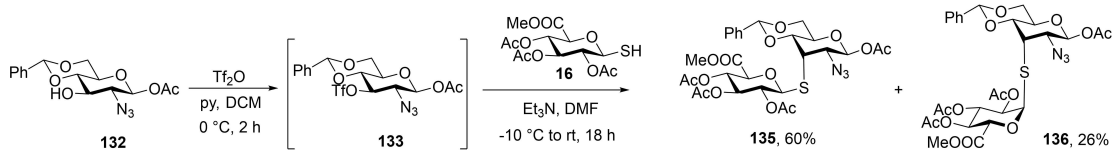
On the other hand, triflation of the 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-allopyranose precursor **134**, led to the *allo*-configured precursor **140** in 82% yield (Scheme 26).



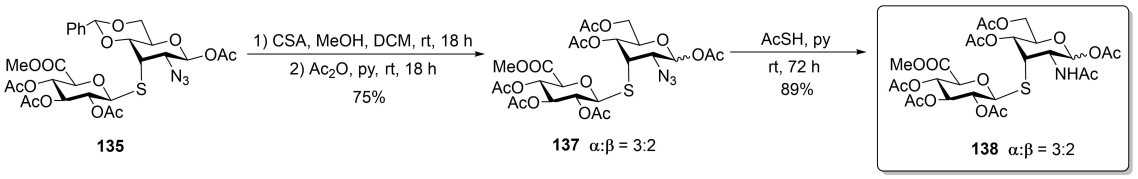
Scheme 21. First approach to the synthesis of β-*S*-GlcA(1→3)β-*S*-AllNAc thiodisaccharide.



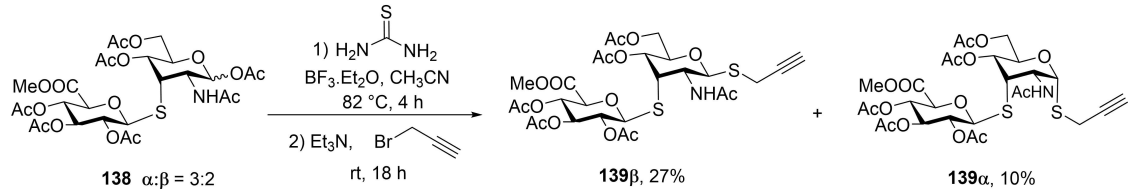
Scheme 22. Synthesis of the *allo* precursor **134**.



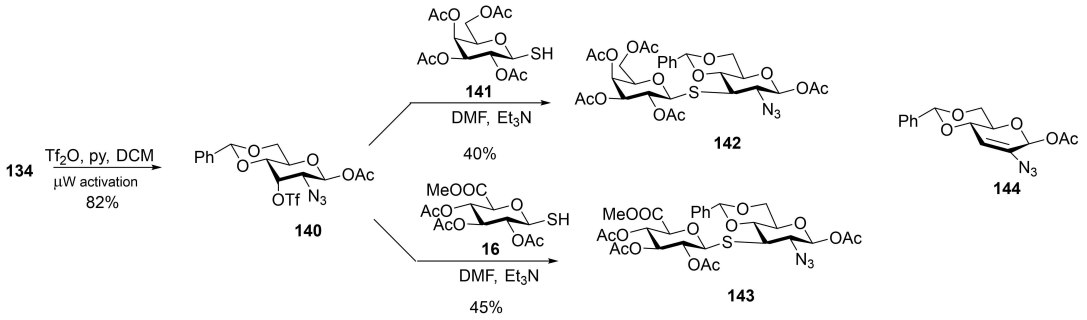
Scheme 23. Synthesis of $AllN_3$ thiodisaccharide **135** and its anomer **136**.



Scheme 24. Transformation of thiodisaccharide **135** into **138α,β**.



Scheme 25. Synthesis of alkynyl thiodisaccharides **139β** and **139α** through one-pot thioglycosylation.



Scheme 26. Synthesis of (1→3) thiodisaccharides **142** and **143**.

This transformation was fundamental and truly challenging as classic triflation conditions were not successful, probably because the axial disposition of HO-3 diminished its reactivity. Thus, the reaction required microwave irradiation to progress. Further S_N2 displacement of the triflate group using suitable protected thioaldoses derived from either glucuronic acid (βGlcASH) and galactose (βGalSH), led to thiodisaccharides **142** and **143**, respectively.[84] This S_N2 step was also challenging, and the reaction conditions should be carefully optimised. Taking into account the complexity of these molecules, the yields were acceptable (40–45 %). Interestingly, together with the desired thiodisaccharides, we could also isolate the vinyl azide **144** and minor amounts of unprecedented (1→2) addition by-products, which will be discussed below.

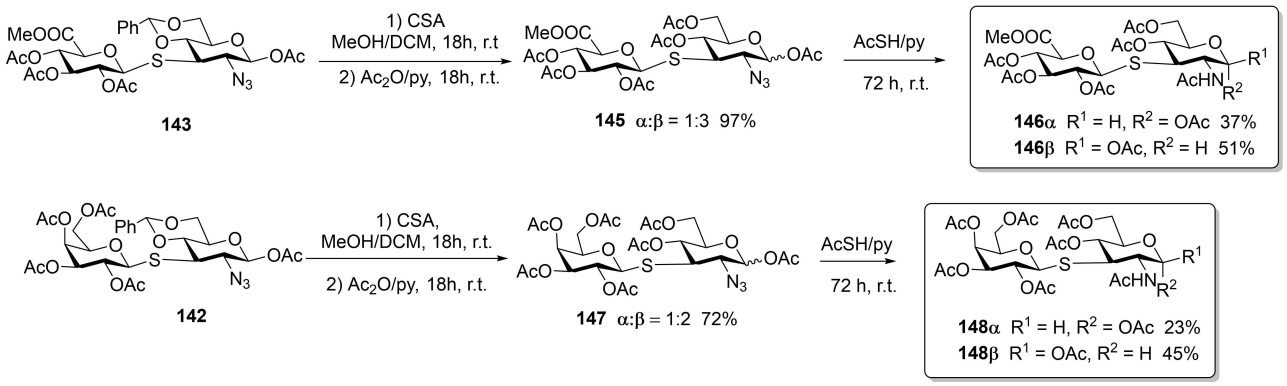
Protecting group manipulation led to the final pursued thiodisaccharides **146α,β** and **148α,β** (Scheme 27) which could be thoroughly separated by column chromatography using silica gel, particle sized < 45 μm.

All these results are related to our project concerning the synthesis of defined GAG-derived glycomimetics for both, the study of biological processes involving these biopolymers and the exploration of multivalent architectures. Thus, β-*S*-GlcA (1→3)GlcNAc, β-*S*-Gal(1→3)GlcNAc and β-*S*-GlcA(1→3) AllNAc thiodisaccharides can be considered mimetics of the repeating units of hyaluronan and keratan GAG structures.[72,84] Indeed, we used the synthesised hyaluronan mimetic thiodisaccharides to construct resorcinarene-based amphiphilic multivalent ligands to assess binding studies towards the C-type lectin Langerin, which is involved in a wide number of critical biological processes (unpublished results).

2.1.3. Ring-Opening Reactions: Aziridines, Sulphamidates and Sulphates as Substrates

In their work on the synthesis of *S*-fucosyl-containing thiodisaccharides, Hashimoto and co-workers also addressed the synthesis of the α-*S*-Fuc-(1→3)GlcNAc thiodisaccharide.[62]

To overcome the difficulties imposed by the –NHAc group in nucleophilic substitutions at C-3, the authors studied the



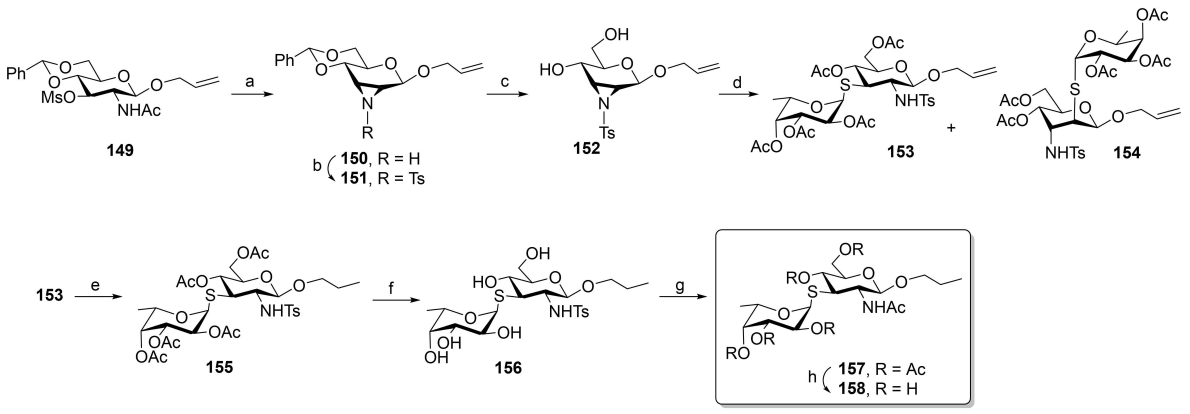
Scheme 27. Transformation of azido-thiodisaccharides **143** and **142** into **146α,β** and **148α,β**.

nucleophilic ring opening of 2,3-aziridines first described by Yamaguchi.[85] The synthesis started from the 3-*O*-mesyl derivative of GlcNAc **149**, and the 2,3-epimino derivative **150** was obtained by treatment with sodium isopropoxide in isopropanol. The aziridine was tosylated to increase the reactivity of the ring, giving compound **151** and the benzylidene acetal group was removed furnishing compound **152**. The ring opening reaction of the aziridine **152** mediated by αFucSH in NaOMe/MeOH proceeded with good stereoselectivity from the upper face of the GlcNAc residue, obtaining the thiodisaccharide **153** and its regioisomer **154** in a 2:1 relationship. Further protecting group manipulation afforded the desired (1→3) thiodisaccharide **158** (Scheme 28).

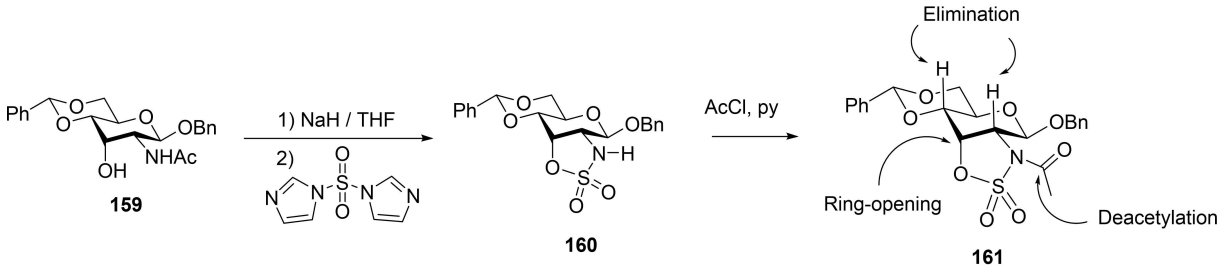
By using *p*-nitrophenyl-α-L-fucopyranoside as substrate, the authors studied the inhibitory activities of the synthesised thiodisaccharide **158**, together with **35** and **42** against α-L-fucosidase from bovine kidney and epididymis. The strongest inhibitor was the α(1→3) thiodisaccharide **158** with *Ki* = 0.65 mM in a competitive mode.

In view to synthesise oligosaccharide inhibitors of neural cell division, Fernández-Mayoralas' group also faced the synthesis of an α-*S*-Fuc(1→3)GlcNAc thiodisaccharide. To avoid dealing with 2,3-oxazoline and 3,4-eliminated by-products,[86] Fernández-Mayoralas' group developed a synthetic methodology which consisted in a nucleophilic opening of a cyclic sulphamidate. This was the key step to reach the α-*S*-Fuc(1→3)GlcNAc thiodisaccharide.[87]

To produce the cyclic sulphamidate, it was necessary to count with the *allo* configured precursor **159** which was obtained through classic synthetic methods. This glycoside was treated with NaH in THF to generate the corresponding 3-alkoxide, which subsequentially reacted with 1,1'-sulphonyldiimidazole to give the 2,3-sulphamidate **160**. In these conditions, the recovered compound was *N*-deacylated, so it required a further *N*-acetylation step by treatment with acetyl



Scheme 28. Reagents and conditions: a) i-PrONa, i-PrOH-dioxane, reflux, 73%; b) TsCl, py, 98%; c) H^+ resin, 80% MeOH, 60 °C, 100%; d) i) αFucSH, NaOMe, MeOH, reflux, ii) Ac_2O, DMAP, py, (**153**: 63%, **154**: 29%, 2 steps); e) H_2, Pd/C, MeOH, 100%; f) NaOMe, MeOH, 90%; g) i) Na, NH_3, THF, −78 °C, ii) Ac_2O, DMAP, py, 85%; h) NaOMe, MeOH, 94%.



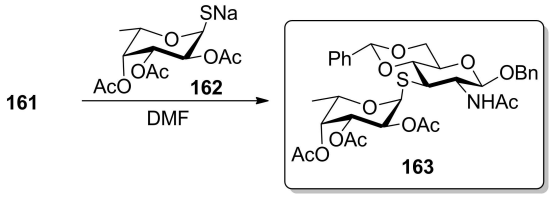
Scheme 29. Synthesis of the cyclic sulphamidate **161**. Different electrophilic centres are shown.

chloride and pyridine, furnishing compound **161** (Scheme 29).

Next, the authors studied nucleophilic opening reactions of the cyclic sulphamidate with nucleophiles of different nature (i.e., S-, N-, O-, and C- nucleophiles). Compound **161** possess several electrophilic centres, namely both H-2 and H-4, C-3 and the *N*-acyl group. In some cases, mostly with C-nucleophiles as for example Grignard or organolithium reagents, 2,3- or 3,4-elimination and/or *N*-deacetylation occurred (Scheme 29).

When treating the cyclic sulphamidate **161** with αFucSNa **162**, the pursued α-*S*-Fuc(1→3)GlcNAc thiodisaccharide derivative **163** was obtained in very good yield (79%) (Scheme 30).

Later, the authors[88] applied this synthetic method to obtain Lewis X trisaccharide glycomimetics. Thus, by acid hydrolysis of the benzylidene acetal in **163** the diol **164** was first obtained. Next, the 6-position was selectively protected, and the authors intended to glycosylate the 4-position with a Gal residue. All the attempts made were unsuccessful (Scheme 31).

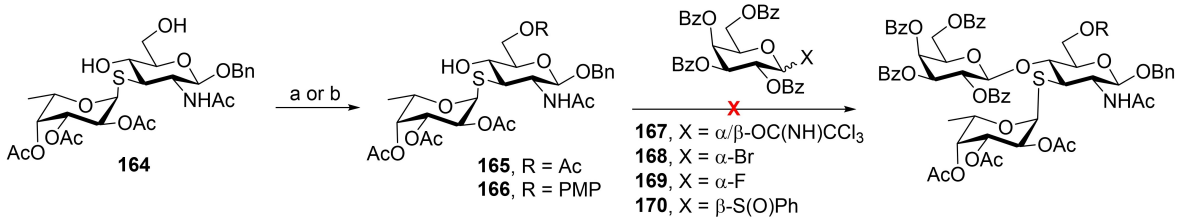


Scheme 30. Synthesis of thiodisaccharide **163** by ring-opening reaction of the cyclic sulphamidate **161**.

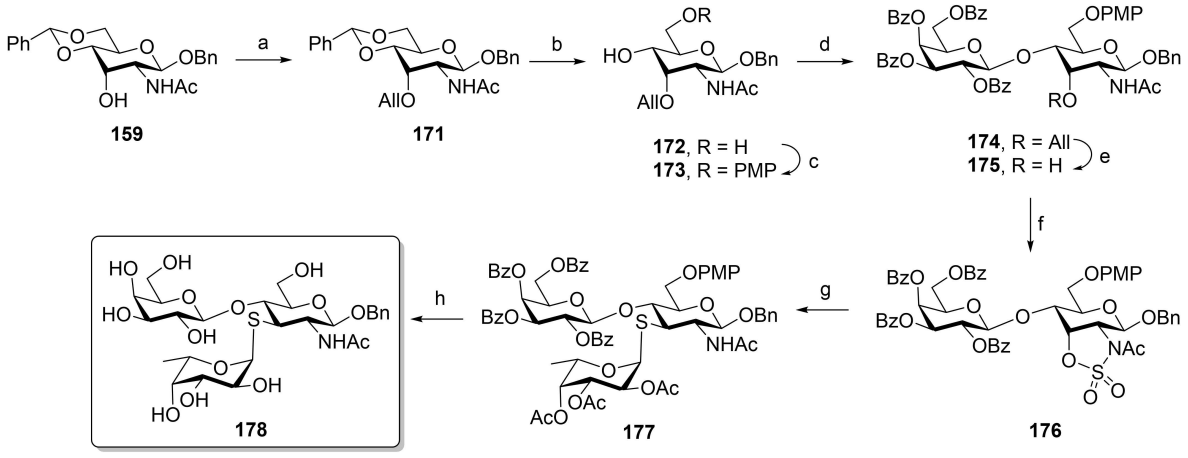
Instead, the authors decided to synthesise in first place the Gal(1→4)AllNAc disaccharide and to incorporate the α-thioFuc residue in later steps. Thus, starting from the AllNAc glycoside **159**, thiodisaccharide **175** was obtained, in which the 3-position was free to react giving the 2,3-sulphamidate **176**. Nucleophilic displacement of the sulphamidate in **176** gave thiotrisaccharide **177** which was further deprotected furnishing thiotrisaccharide **178** as the desired Lewis X mimetic (Scheme 32).

The same sulphamidate-opening reaction was successfully employed later by Chen and Withers to obtain 2-acetamido-2-deoxy-3-thio-β-D-gluco- and β-D-galactopyranoside derivatives,[89] which were then used as substrates for chemoenzymatic thiodisaccharide synthesis (see below, Section 2.3).

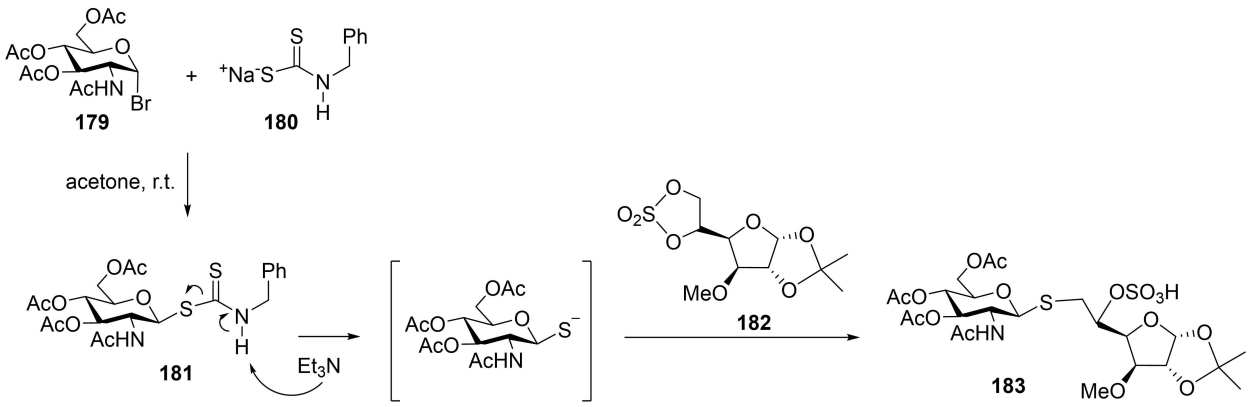
Besides, in 2014, Megia-Fernandez et al.[90] reported the ring-opening reaction of a cyclic sulphate mediated by a thioaldose, as a key reaction for the synthesis of thiodisaccharides. Like cyclic sulphamidates, cyclic sulphates proved to be versatile synthons as epoxide equivalents in organic synthesis.[91] Interestingly, the authors thoroughly studied the synthesis of *S*-pyranosyl-*N*-monoalkyl dithiocarbamates (DTC) as precursors of thioaldoses, which can be gradually released in the presence of Et_3N and then trapped by different electrophilic species. These derivatives were synthesised by reaction of per-*O*-acetylated-α-bromoaldoses with the *N*-benzyl dithiocarbamate sodium salt **180** in mild conditions (Scheme 33).



Scheme 31. Reagents and conditions: a) for **165**, AcCl, collidine, DCM, −78 °C, 82%; b) for **166**, p-methoxyphenol, Ph_3P, DIAD, THF, 70 °C, 95%.



Scheme 32. Reagents and conditions: a) AllBr, NaH, DMF 0 °C to rt, 98 %; b) CSA, MeOH, 60 °C, 91 %; c) *p*-methoxyphenol, Ph_3P, DIAD, THF, 70 °C, 90 %; d) **167**, TMSOTf, DCM, rt, 63 %; e) i) $[Ir(COD)(PMePh_2)_2]PF_6$, H_2, THF, rt, ii) H_2O, I_2, rt, 82 %; f) i) $SOCl_2$, Et_3N, DCM, 10 °C, ii) $NaIO_4$, $RuCl_3.3H_2O$, CH_3CN, H_2O, CCl_4, 0 °C, 61 %; g) i) αFucSH, NaH, DMF, 0 °C to rt, ii) H_2O, H_2SO_4, THF, rt, 77 %; h) i) CAN, CH_3CN-H_2O (4 : 1), 0 °C, ii) NaOMe, MeOH, rt, 66 %.

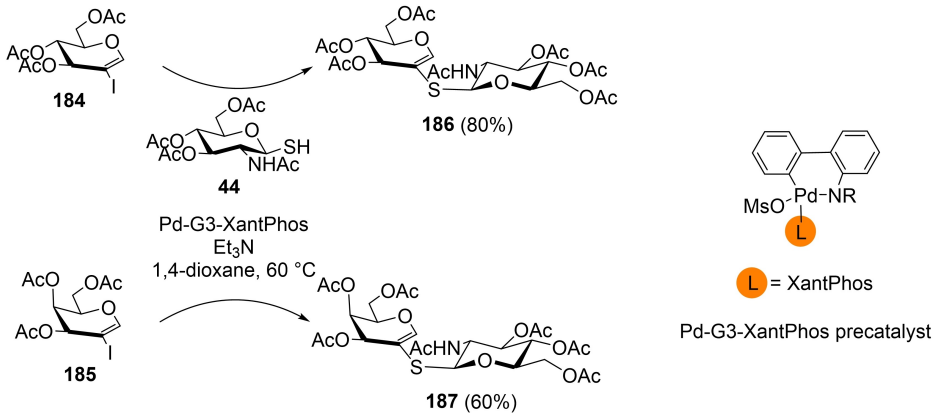


Scheme 33. Synthesis of **183** by cyclic sulphate opening reaction.

Thus, by treating the DTC precursor **181** with the 5,6-cyclic sulphate **182** derived from glucofuranose in the presence of Et_3N, the nucleophilic attack selectively occurred on the primary carbon C-6, to produce the sulphated thiodisaccharide **183**. This was the first report of a thiodisaccharide bearing a GlcNAc residue attached to a glucofuranose derivative.

2.1.4. Cross-Coupling Reactions

2-Iodoglycals have been used before as acceptors in different cross-coupling reactions to introduce an alkyl or aryl substituent at C-2,[92,93] but it was Messaoudi and co-workers[94,95] who used a *S*-nucleophile in this reaction for the first time to synthesise thiodisaccharides. The authors tested first the coupling using alkenyl or aryl halides and glycosyl thiols to form thioglycosides obtaining excellent results.[94] A few years later, they reported the synthesis of different thiodi-, tri- and tetrasaccharides using 2-iodoglucal **184** and 2-iodogalactal **185** as acceptors.[95] In this example, they used βGlcNAcSH and Pd–G3-XantPhos precatalyst to perform the Buchwald-Hartwig-Migita cross-coupling (Scheme 34). In comparison with classical iodoalkenes, 2-iodoglycals **184** and **185** showed to be less reactive. Still, by optimisation of the reaction conditions, the authors found that, when using dioxane as solvent, after 90 minutes at 60 °C, the (1→2) substituted glycals could be obtained in good to very good yields.



Scheme 34. Synthesis of **186** and **187** via a cross-coupling reaction.

2.2. HexNAc-Containing Thiodisaccharides by Addition Reactions to an Unsaturated Sugar Precursor

2.2.1. Thiol-Ene Reactions through Radical Mechanisms

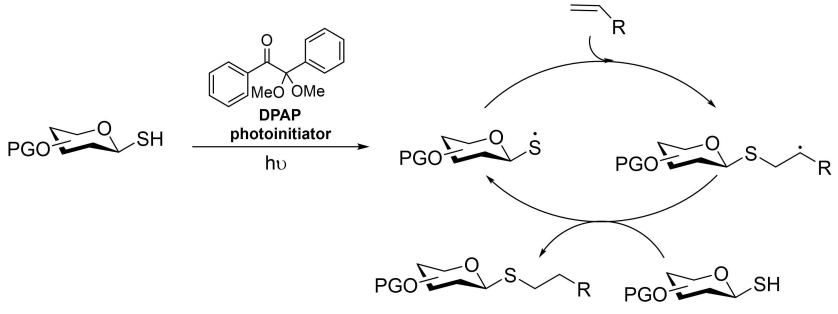
Thiol-ene coupling (TEC), or hydrothiolation reaction, is a well-known reaction which has been largely used to couple a thiol to an olefin to produce a thioether. In the last two decades, this reaction has re-emerged as it can be used in the presence of a wide variety of functional groups with high efficiency under mild reaction conditions. As a result, it has been included in the renowned click reaction group.[96,97] The reaction proceeds by a classic radical mechanism initiated by a thiyl radical, as it is shown in Scheme 35.

In the carbohydrate field, thiyl radicals derived from thioaldoses can be successfully generated in the presence of a radical initiator, but the finding that they could be obtained by a photoinduced process through UV-irradiation in the presence of a photosensitised ketone, rapidly promoted the synthesis of a variety of thiodisaccharides having interesting structures and particular stereochemistry.

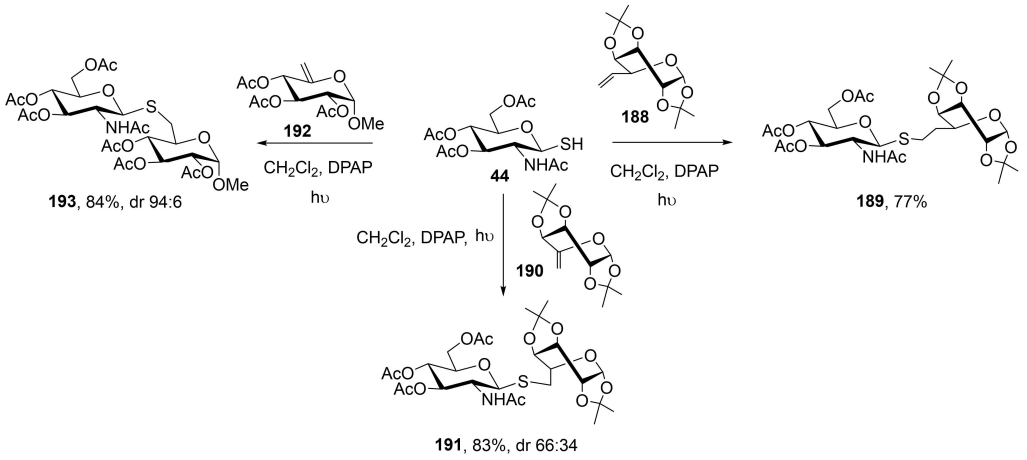
In 2009, Fiore, Marra and Dondoni reported the thiol-ene radical reaction between 1-thiosugars and exocyclic alkenes to obtain 1,6-thiodisaccharides by UV-light irradiation (at 365 nm) using 2,2-dimethoxy-2-phenylacetophenone (DPAP) for the first time.[98] This click reaction performed under mild conditions, proved to be highly efficient and diastereoselective. On the one hand, the authors first optimised the reaction conditions using βGlcSH and the diacetonide **188** and found that in MeOH the disulphide GlcS-SGlc appeared as undesirable by-product. On the other hand, while using CH_2Cl_2 as solvent, disulphide formation was not observed. With the best conditions in hand, the authors tested the reaction with a variety of per-*O*-acetyl 1-thiosugars, including βGlcNAcSH **44**, which successfully reacted with different unsaturated sugars (Scheme 36).

The disaccharides were obtained in very good yields; particularly in the case of **191** and **193**, a mixture of the D-*galacto* (***epi*-191**) and L-*ido* (***epi*-193**) isomers were obtained.

Two years later, the same group published the first reported TEC reaction using glycals as starting materials. Compared with the exocyclic double bond, the glycals needed a higher proportion of **44** (1.2 eq and 6 eq, respectively) to obtain good yields. When **44** was treated with glucal **194** and galactal **195**, the reaction was regioselective. The thiol binds to



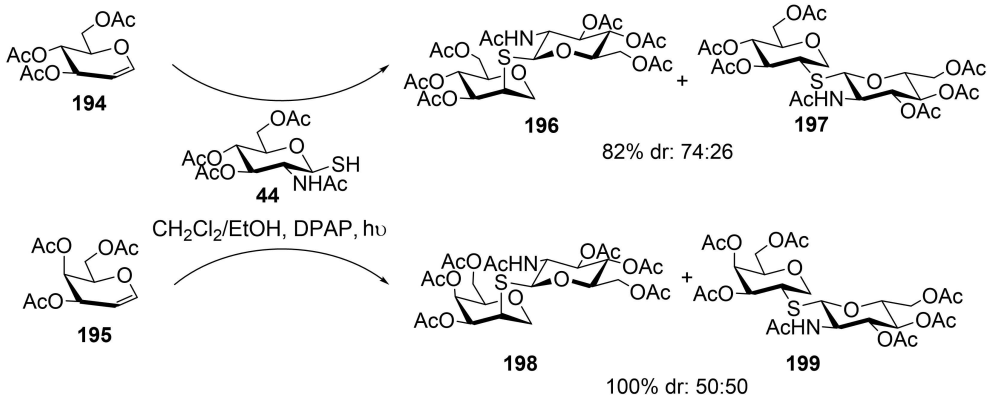
Scheme 35. Radical cycle of thiol-ene coupling using a protected 1-thiosugar and DPAP as initiator.



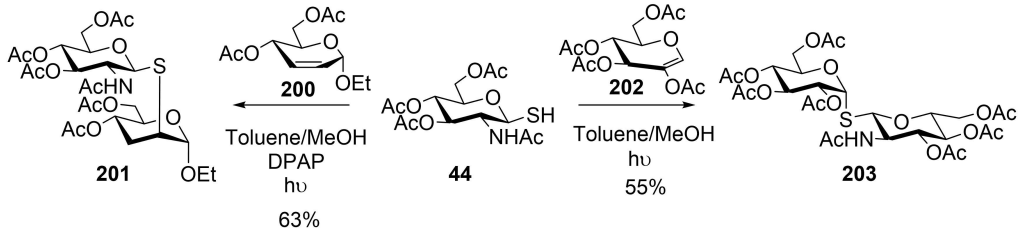
Scheme 36. Free radical thiol-ene reaction with exocyclic double bonds, dr: diastereomeric ratios.

the C-2 of the glycal since the anomeric radical intermediate is stabilised by the adjacent oxygen atom. Additionally, the reaction proceeded with very good yields, although it was not stereoselective (Scheme 37).[99]

Borbás and co-workers[100–102] also made important contributions in this field. They reported the synthesis of α(1→1) and α(1→2) hexosamine-bearing thiodisaccharides, among others, by TEC reaction, using endocyclic enoses and 2-acetoxyglycals as starting materials. They demonstrated that thiol addition to 2-acetoxyglycals and a 2,3-unsaturated glycoside proceeded with total selectivity (Scheme 38).[100] 2-Acetoxyglycals, in turn, led stereoselectively to 1,2-*cis*-α-linked thiodisaccharides, which are difficult to obtain through other methods. Borbás[103] showed that many short cycles of



Scheme 37. Thiodisaccharide synthesis by TEC reaction using glycals as acceptors.



Scheme 38. Synthesis of α(1→1) and α(1→2) thiodisaccharides by TEC reaction.

irradiation with repeated additions of the photosensitiser resulted more beneficial than long-time irradiation periods.

Using the same strategy, this group synthesised a variety of thiodisaccharides having β-1-thioGlcNAc moieties. Interestingly, the GlcNAc-derived glycal **204** resulted a good substrate for the reaction (Scheme 39). Particularly, Eszenyi et al.[101] demonstrated that the conversion increases by diminishing the reaction temperature. They assumed that the stability of the radical intermediate in the thiol-ene reaction increased in these conditions. Another advantage of this method is that the amount of disulphide formed as by-product was substantially diminished.

One year later, the same group optimised the photoinduced hydrothiolation of different glycals, and a trisaccharide (**214**) was successfully synthesised, among a great number of other thiodisaccharides (Scheme 40). The optimal temperature conditions for these reactions showed to be −80 °C. However, for trisaccharide **214**, the reaction gave better results at −40 °C (33 % and 65 % yield, respectively).[102]

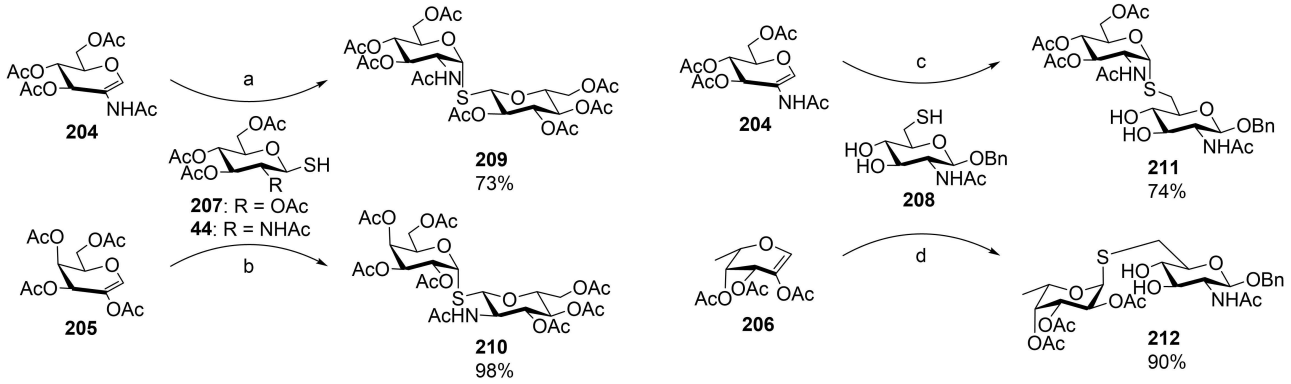
It should be mentioned that Borbás recently published an excellent review which collects all the latest results on the photoinitiated thiol-ene reactions involving unsaturated sugars as precursors, portraying this method as a powerful tool for the construction of thiodisaccharides.[103]

2.2.2. Michael and Michael-Type Addition Reactions

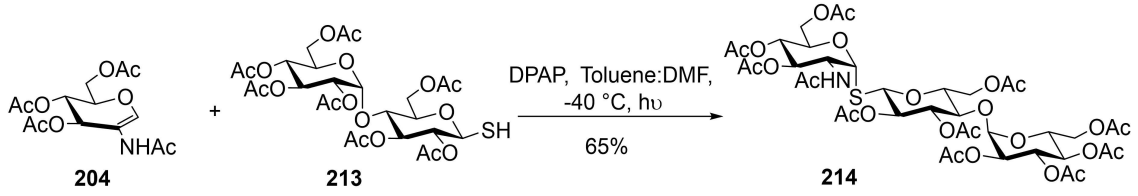
Witczak[27,104–106] and Varela[107–110] groups made relevant contributions to the field of thiooligosaccharide synthesis, using Michael addition reactions. They explored the reactivity of α,β-unsaturated derivatives, mainly the popular levoglucosenone and dihydropyran-2-ones as substrates, and thioaldoses as nucleophiles. Many of the resulting thiodisaccharides proved to be interesting enzyme inhibitors.[104,107,111–114] Moreover, promising anti-cancer activities were also described for some of them.[28–30]

In 1995, Witczak described the synthesis of two α(1→4) thiodisaccharides via Michael addition, one of them bearing a GlcNAc moiety. Particularly, the authors synthesised α-*S*-Fuc (1→4)-3-deoxy-Glc **220** and α-*S*-Fuc(1→4)-3-deoxy-GlcNAc **221** from αFucSH **215** and levoglucosenone **216** (Scheme 41).[104]

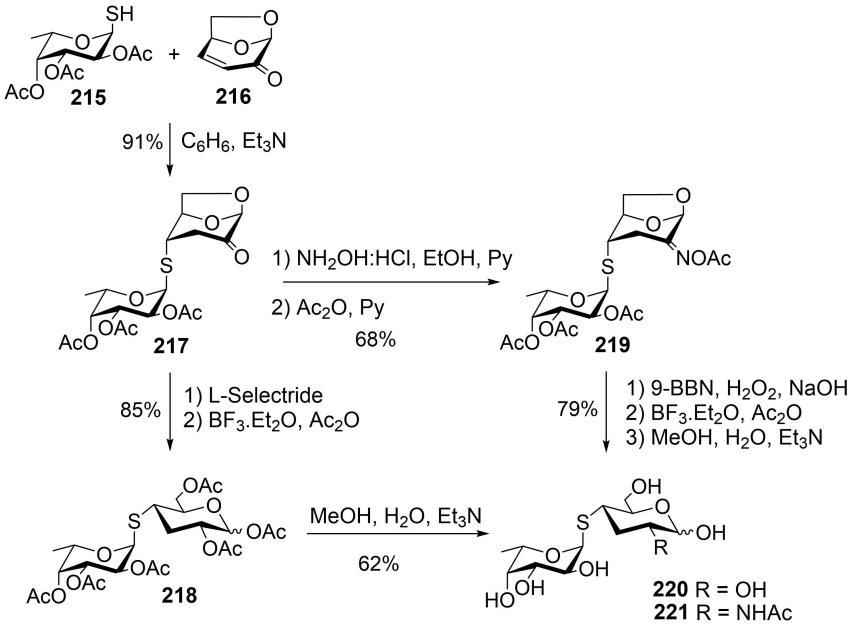
Due to the presence of the anhydro bridge in levoglucosenone, the Michael addition of αFucSH resulted completely stereoselective from the opposite face of the 1,6-anhydro ring. Interestingly, Witczak achieved the conversion of the 2-keto group into a *N*-acetamido through the acetoxime group in intermediate **219**, by treatment of **217** with hydroxylamine, followed by acetylation, and further treatment with 9-BBN (Scheme 41). Final treatment with BF_3 to provoke the opening of the 1,6-anhydro bridge followed by deacetylation, led to the 3-deoxy thiodisaccharide **221**.



Scheme 39. Reagents and conditions: DPAP, −80 °C, hν, a) **207**, Toluene : MeOH 1 : 1; b) **44**, Toluene; c) DMF; d) Toluene : MeOH 1 : 1.



Scheme 40. Synthesis of thiotrisaccharide **214**.

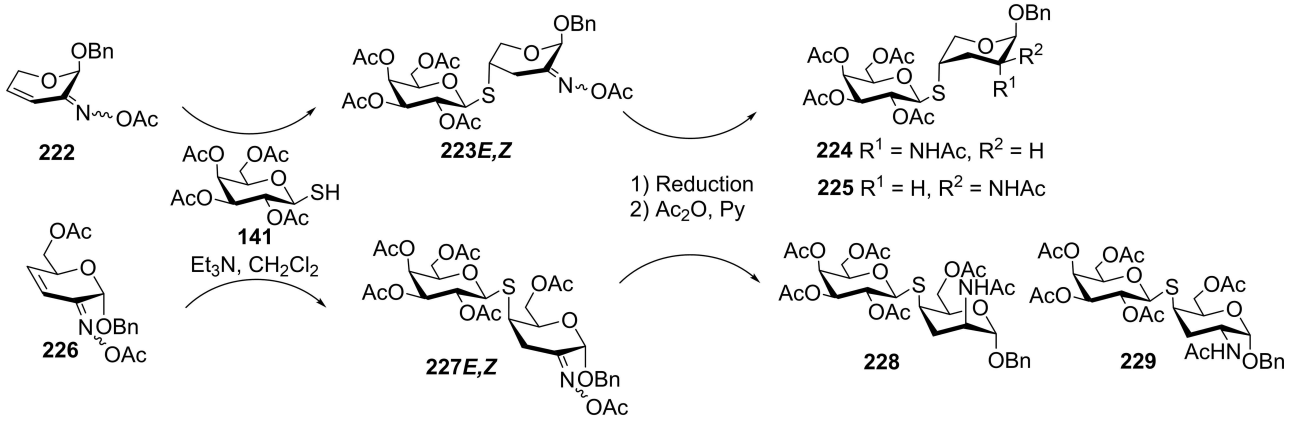


Scheme 41. Synthesis of (1→4) thiodisaccharides via Michael addition.

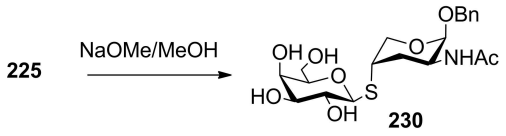
Recently, Varela and co-workers[113] also described the synthesis of 2-acetamido-2,3-dideoxy-(1→4)-thiodisaccharides through a Michael addition reaction of βGalSH to the α,β-unsaturated ketoximes **222** and **226**. These reactions occurred with remarkable stereoselectivity as the thiol attacked from the opposite side of the benzyloxy group (Scheme 42). *Z*-Acetyl oximes were better acceptors and gave higher yields than *E*-oximes, a fact that can be explained by the steric hindrance imparted by the OAc groups in the *E*-isomer. Interestingly, it was observed that the *E*-configured oxime double bond isomerizes upon addition, as **222*E*** led to the **223*Z*** adduct.

The authors finally studied the reduction of the obtained oximes **223*E,Z*** and **227*E,Z*** using a variety of reducing agents. On the one hand, when reducing **223*E,Z***, the use of $NaBH_4$/I_2 gave high yields, but the reaction was not diastereoselective and an unresolved mixture of **224** and **225** was obtained. On the other hand, treatment with $LiAlH_4$ at −18 °C led to the 4-thio-β-D-*threo*-pentopyranoside **225** as a single stereoisomer. Reduction of **227*E,Z*** led to the 2-acetamido-2-deoxy-thiodisaccharides **228** (D-*lyxo*) and **229** (D-*xylo*) in approximately 1 : 1 ratio (Scheme 43).

Thiodisaccharides **225**, **228** and **229** were deacetylated by treatment with NaOMe/MeOH and the products were tested



Scheme 42. Michael reaction followed by oxime reduction using a pentopyrarone as acceptor.



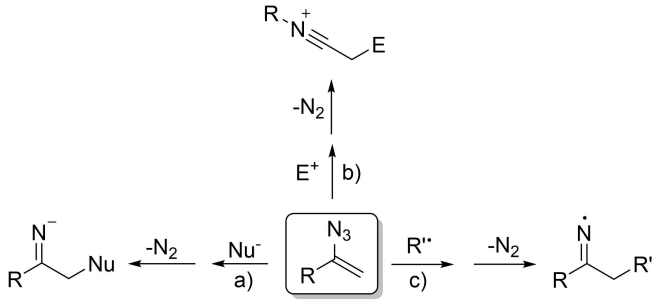
Scheme 43. Deacetylation of compound **225**.

as inhibitors of the β-galactosidase from *E. coli*. Interestingly, **230** showed to be a potent inhibitor of the enzyme with a K_i = 70 μM.[113]

In connection with Michel-type additions, we can mention here a report by Kroutil et al.[115] based on a nucleophilic rearrangement which led to a GlcNAc-bearing thiodisaccharide. The authors described the synthesis of compound **233**, by reaction of per-*O*-acetyl βGlcNAcSH **44** with an unsaturated 1,6-anhydro tosyl hexenose **231**, derived from levoglucosenone (Scheme 44). The reaction proceeded with high stereo and regioselectivity. Final opening of the anhydro bridge led to the final product **233**, which is an interesting precursor of more complex glycomimetics by further functionalization of the double bond.

2.2.3 Addition of Thioaldoses to a Vinyl Azide Sugar Precursor

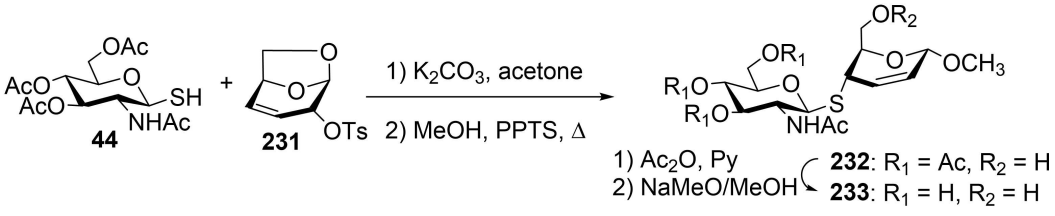
Vinyl azides are valuable precursors in organic synthesis, mainly in the synthesis of heterocycles. Despite some of their applications in heterocycle synthesis are known from decades ago, recently, vinyl azides have been the subject of several works on new and interesting synthetic developments.[116,117] These compounds constitute widely versatile synthons since, aside from the classic reactivity of azides, they can react as radical acceptors, nucleophiles and electrophiles, among other type of reactions (Scheme 45).[117] In 1968, Hanessian described for the first time carbohydrate-based vinyl azides.[118] These were obtained as undesired E2 elimination by-products in a series of S_N2 reactions of triflyl derivatives bearing an azido vicinal group.[119–121] Nonetheless, carbohydrate-derived vinyl azides had not been explored as synthetic precursors of modified sugars until 2020, when we reported the addition reaction of 1-thiosugars to the double bond, as discussed below.



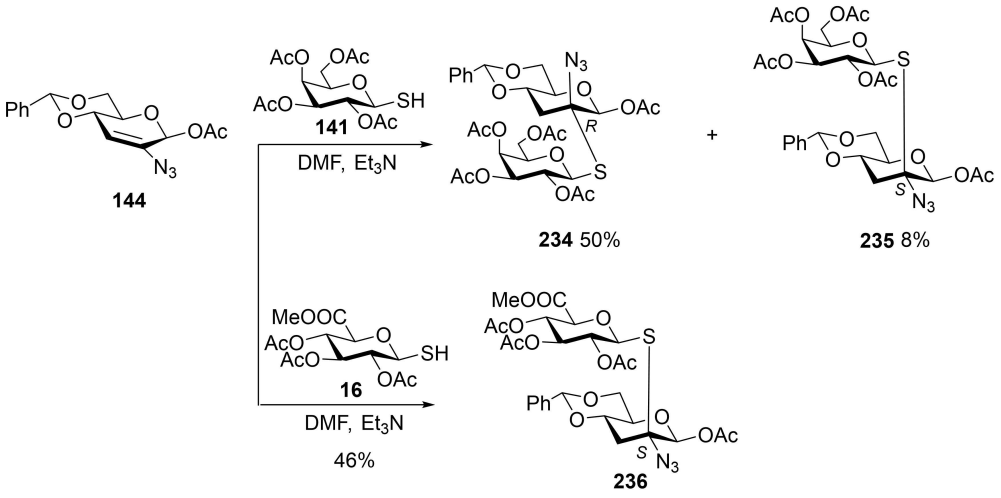
Scheme 45. Reactions of vinyl azides with a) nucleophiles, b) electrophiles, and c) radicals.

We have already mentioned that, on our way to reach (1→3) thiodisaccharides, the vinyl azide derivative **144** was also recovered as a side product from the reaction mixture, in variable yields which depended on the reaction conditions. Also, we detected two other secondary products of intriguing structures, having both the thioaldose and the electrophile fragments, which required further exploration. On the one hand, we could easily demonstrate that the vinyl azide **144** was formed by elimination of the axially disposed triflate substituent placed at the 3-position. On the other hand, we hypothesised that the two other minor products could arise from an addition reaction of the thioaldoses to the double bond present in **144**. Thus, by treatment of **144** with either **141** or **16**, we recovered products **234**–**236**, whose structures were unambiguously determined by a combination of NOESY-NMR experiments together with conformational search molecular modelling (Scheme 46). The selectivity observed may be ascribed to both, electronic and steric effects.

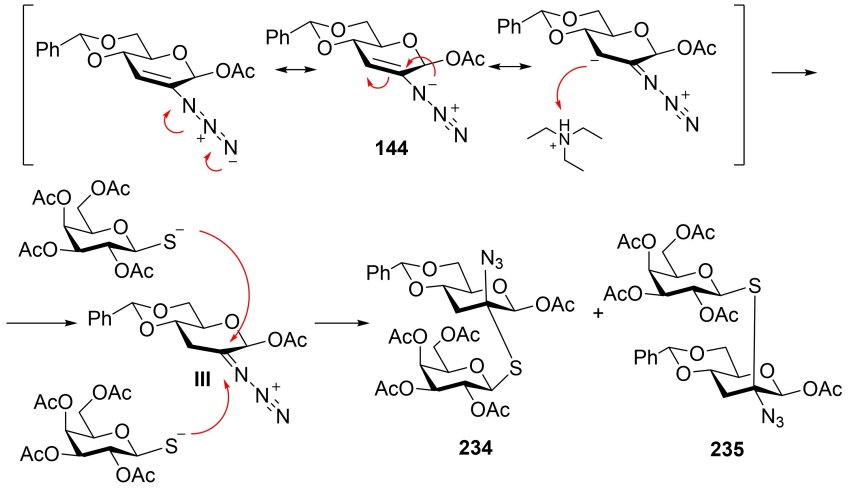
Considering the chemistry and reactivity of vinyl azides, we also were able to propose a mechanism for this addition reaction, involving an iminodiazonium cation intermediate (Scheme 47).[84] Remarkably, the rigidity of the sugar ring, must in some way preclude the classic 1,2-substituent migration observed in iminodiazonium ions with the concomitant loss of N_2, (known as the Schmidt reaction, Scheme 45, path b). Similar results on the addition of nucleophiles to this unsaturated bond were obtained by Wang and co-workers by



Scheme 44. Synthesis of thiodisaccharide **233**.



Scheme 46. Synthesis of (1→2) thiodisaccharide **234**, **235** and **236** from vinyl azide **144**.



Scheme 47. Proposed mechanism for the obtention of (1→2) thiodisaccharides through addition to vinyl azides involving the iminodiazonium cation intermediate **III**.

treating some aliphatic vinyl azides with alcohols in the presence of SelectFluor, as source of electrophilic F^+.[122]

The formation of compounds **234**–**236**, as 2,3-dideoxy-2-azido-(1→2)-thiodisaccharides, revealed the interest in sugar-derived vinyl azides as precursors of new glycomimetics of unprecedented structures.

2.3. Alternative Chemoenzymatic Methods

Withers and co-workers explored the development and use of thioglycoligases, as mutant glycosidases lacking the catalytic acid/base amino acid residue involved in the activity displayed by these retaining-hydrolytic enzymes.[123–125] The successful construction of the thioglycosidic bond relied on the use of suitable donors, such as *p*-nitrophenyl glycosides or glycosyl fluorides, and an appropriate sugar bearing a thiol group as an acceptor, which presents better nucleophilic properties than alcohols. Thus, importantly, the formation of a *S*-glycosidic bond occurs in aqueous medium, avoiding the use of protecting groups. As thiodisaccharides are resistant to enzymatic hydrolysis the reverse reaction does not occur. It should be noted that this approach still requires the preparation of sugar derivatives having the SH functionality in specific positions.

GlcNAc-bearing thiodisaccharides have been synthesised by using this chemoenzymatic method.[126] An enzyme having thioglycoligase activity related to the *Xanthomonas manihotis* β-galactosidase was used, together with 2,4-dinitrophenyl β-D-galactopyranoside as donor and a variety of sugar thiols as

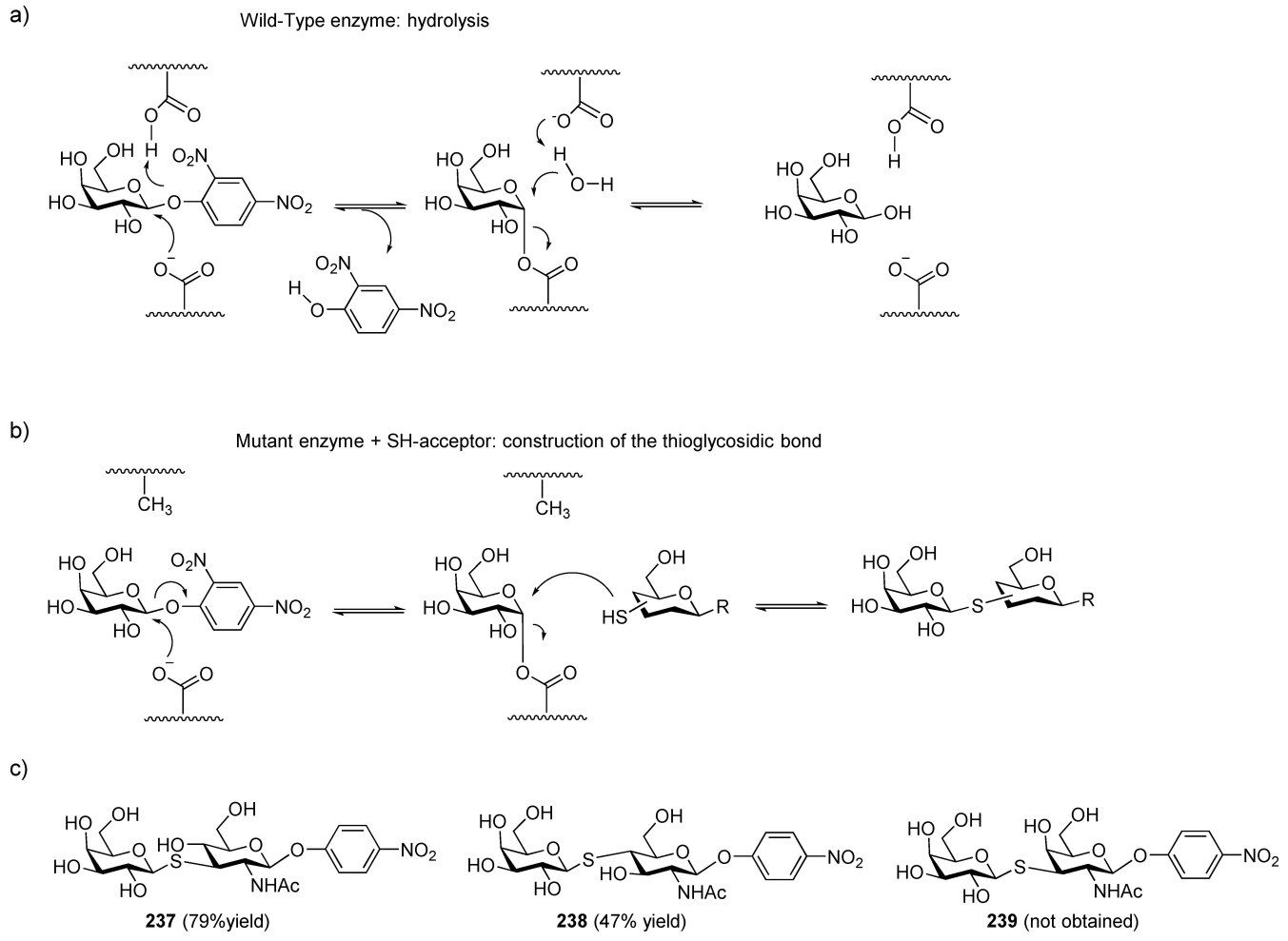
acceptors. Glc or Gal residues having SH-groups in C-3 or 4 led to the corresponding thiodisaccharides in very good yields (79–85%). When their 2-deoxy-2-acetamido counterparts were used as acceptors, an increase in the amounts of both the donor and the enzyme was required and the yields were lower. The best results were obtained when 3-thio-GlcNAc was tested, giving a 79% yield of thiodisaccharide **237**, while the 3-thio-GalNAc derivatives were inactive as acceptors (Scheme 48). These results were ascribed to the fact that the bulky acetamido group probably does not fit properly within the active site of the enzyme.

Variants of the α-*N*-acetyl-glucosaminidase from *Clostridium perfringens* have also been obtained and studied. These modified enzymes proved to catalyse the transfer of an α-GlcNAc residue from a 2-nitrophenyl *N*-acetyl-α-D-glucosaminide to a variety of thiols. When the 4-thio-Glc derivative **241** was used, thiodisaccharide **242** was obtained (Scheme 49).[127]

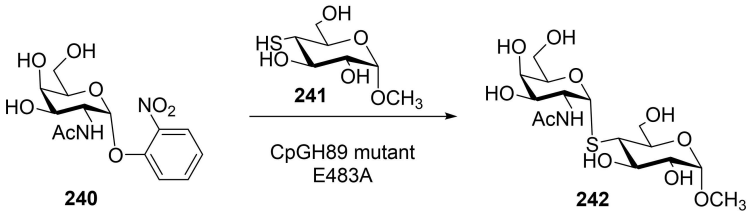
In a recent report, Withers and co-workers[128] used also an hexosaminidase to produce a thioglycoligase. In this case, the authors generated a variant of a glycosidase (GH20) from *Streptomyces plicatus* (SpHex) replacing the Glu314 by an alanine in the active site (SpHex E314A).

As the oxazoline is an intermediate in the catalytic process, either GlcNAc oxazoline **243** or 4-nitrophenyl GlcNAc **244** were used as donors with a wide range of SH-acceptors (including D-GlcNAc, D-Glc, D-GalNAc, D-ManNAc and D-Man configurations) in the thioligation reaction to give thiodisaccharides **245–253** (Scheme 50).

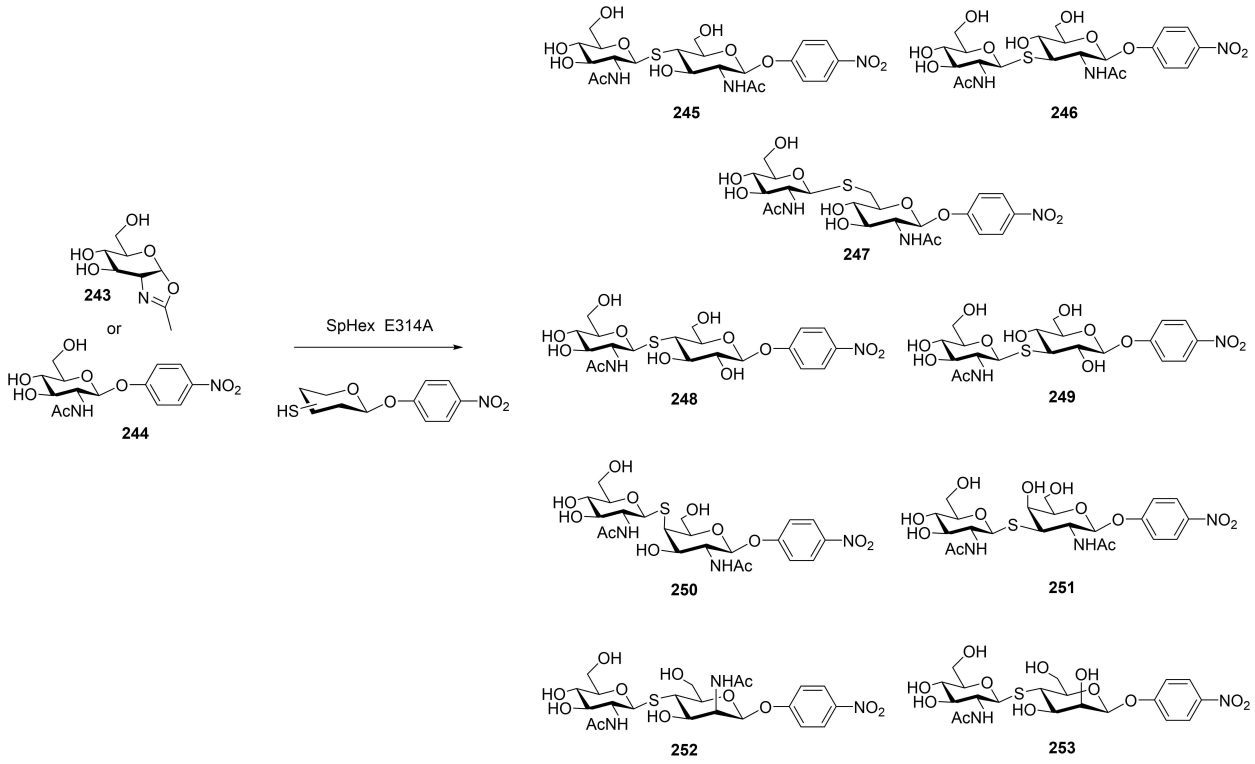
The authors[128] also showed that 4-nitrophenyl-β-GalNAc **254** was an excellent donor obtaining thiodisaccharide **256** in 98% yield (Scheme 51). Importantly, the authors were able to synthesise, in this way, a thiodisaccharide bearing two GalNAc moieties.



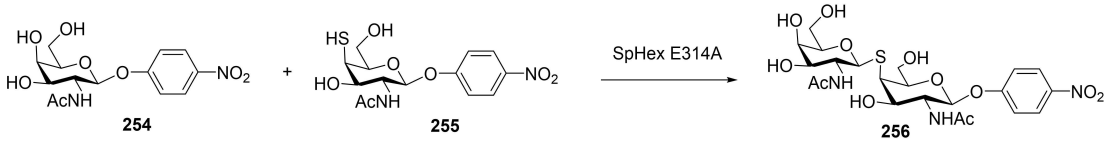
Scheme 48. Chemoenzymatic reaction for the synthesis of thiodisaccharides bearing HexNAc residues. a) Mechanism for WT enzymatic hydrolysis, b) Thioglycoligase-mediated mechanism, c) Target thiodisaccharides



Scheme 49. Synthesis of **242** using a thioglycoligase from an α-*N*-acetyl-glucosaminidase.



Scheme 50. Synthesis of compounds **245**–**253** using a thioglycoligase from *Streptomyces plicatus* GH20 hexosaminidase.



Scheme 51. Synthesis of thiodisaccharide **256** by chemoenzymatic reaction using thioglycoligase SpHex E314A.

3. Summary and Outlook

The increasing knowledge of the importance of carbohydrate-mediated biological processes demands nowadays solid strategies to successfully construct modified sugars which can contribute to this research field. As isosteres of *O*-disaccharides, thiodisaccharides result particularly attractive.

Although reported for the first time many decades ago, classic thioglycosylation and S_N2 approaches are still fruitful, considering the high nucleophilic power of sulphides and that suitable techniques to transform hydroxyls into thiols or good leaving groups and also to obtain glycosyl donors are continuously being developed. Also, the stereoselectivity of the concerted substitutions allows a direct planification of the

reaction sequences keeping in mind the stereochemistry of the target molecule. Thus, (1→6) and (1→4) thiodisaccharides having HexNAc residues at the reducing end have been easily prepared in this way. The more challenging (1→3) thiodisaccharides could also be obtained under controlled conditions and in the presence of additives, while strategically designing the synthetic sequence to avoid side reactions involving the –NHAc group. Alternatively, aziridine and cyclic sulphamidate ring-opening approaches offered excellent possibilities to reach them. Particularly, cyclic sulphate ring-opening reaction provided an interesting example for the access of thiodisaccharides of unusual structures. We consider that further investigation on this topic is still required. Interestingly, cross-coupling reactions also proved to be useful synthetic methods to reach thiodisaccharides, thus broadening the spectrum of accessible sugar mimetics.

Among the addition methodologies, the TEC reaction also proved to be an excellent option for the access to thiodisaccharides with regiochemical control, as mild conditions under UV-light irradiation in the presence of the DPAP ketone are effective for the construction of the thioglycosidic bond between a thioaldose and a sugar-derived alkene. Thus, glycals, 2-acetoxyglycals, 2,3-enuloses and other unsaturated sugars showed to be suitable precursors, although mixtures of stereoisomers are usually obtained. Furthermore, addition reactions of thioaldoses to sugar enone-derived acetoxymes or to vinyl azides, arose recently as alternative methods through ionic intermediates.

Finally, elegant methods based on genetic manipulation of common glycosidases led to enzymes having unique thioglycoligase activities. Despite the chemoenzymatic approach offers the possibility to build the thioglycosidic linkage in a straightforward manner, it is necessary to mention that in all cases a deep synthetic background is needed to reach appropriate sugar acceptors having an SH group in specific positions. Nonetheless, the enzyme specificity and other advantages strongly contribute to strengthen the development of methods for the synthesis of thiodisaccharides which are difficult to obtain otherwise.

Throughout this review we intended to show the importance of thiosugar derivatives in carbohydrate synthetic chemistry as a key part of Glycobiology, mainly focusing on the development of methods to assess HexNAc-containing thiodi- and oligosaccharides.

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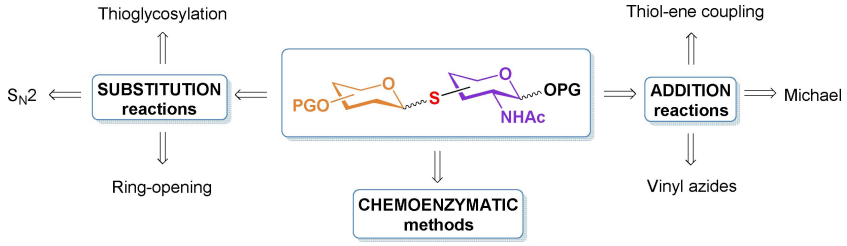

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Synthesis of Thiodisaccharides Bearing *N*-Acetylhexosamine Residues: Challenges, Achievements and Perspectives

In this review, we collected all the excellent reports made to date on the synthesis of HexNAc-containing thiodisaccharides. We organised them into three groups taking into consideration the reactions involved in the construction of the thioglycosidic bond: substitution reactions, addition reactions and chemoenzymatic methods.