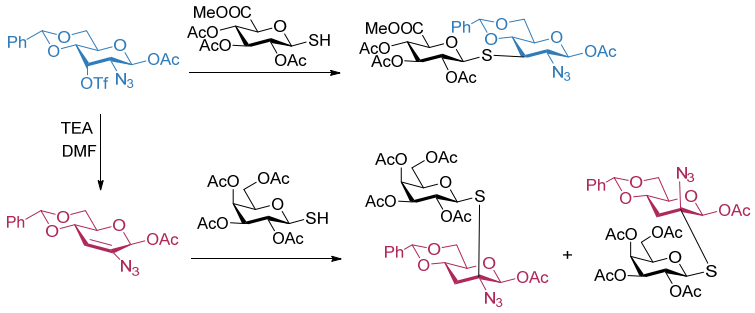
Synthesis of (1→3) thiodisaccharides of GlcNAc and the serendipitous formation of 2,3-dideoxy-(1→2)-thiodisaccharides through a vinylazide intermediate

Alejandro Ezequiel Cristófalo[1,2], Pedro M. Nieto ~~Mesa~~[3] and Maria Laura Uhrig[1,2]*



[1]Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Química Orgánica. Buenos Aires, Argentina; [2]CONICET- Universidad de Buenos Aires. Centro de Investigaciones en Hidratos de Carbono (CIHIDECAR). Buenos Aires, Argentina; [3]Glycosystems Laboratory, Instituto de Investigaciones Químicas (IIQ), cicCartuja, CSIC and Universidad de Sevilla, España.

*Corresponding author, e-mail address: mluhrig@qo.fcen.uba.ar

ORCID: https://orcid.org/0000-0002-6980-4141

Keywords: N-Acetylglucosamine, thiodisaccharides, vinylazides, glycosaminoglycans, β-D-thiogalactose

ABSTRACT

The syntheses of β-*S*-GlcA(1→3)GlcNAc and β-*S*-Gal(1→3)GlcNAc thiodisaccharides were achieved by S_N2 displacement of a triflate group allocated at 3-position of a convenient 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-*allo*pyranose precursor, by the corresponding nucleophilic suitable protected thioaldoses derived from GlcA and Gal. The study of the reaction led to the finding that the vinylazide formed by the competitive E2 reaction of the mentioned triflate, was an interesting precursor of a new kind of 2,3-dideoxy-2-azido-(1→2) thiodisaccharides, through an addition reaction. The determination of the stereochemistry of the new stereocenter at C-2 was achieved by NOESY experiments. Final protecting group manipulation of the (1→3) thiodisaccharides led to a family of derivatives that could be used as building blocks for the synthesis of more complex glycomimetics.

INTRODUCTION

The synthesis of thiodisaccharides, that is, disaccharides in which the interglycosidic oxygen has been replaced by a sulfur atom, has been largely addressed over the past 30 years or more. Still, thiodisaccharides represent a synthetic challenge for carbohydrate synthetic chemists, taking into consideration their incoming importance in the Glycobiology field, as they are considered carbohydrate mimetics with a great potential as enzyme inhibitors or new ligands for lectins with increased resistance in biological media.

Different synthetic approaches have been explored to access these compounds. Nucleophilic replacement of a good leaving group present in one sugar residue by an activated thioaldose in basic medium (through a classic S_N2 mechanism) accounted for the synthesis of a variety of structures.[1–4]

The incorporation of *S*-D-GlcNAc and *S*-D-GalNAc residues, components of a variety of relevant bioactive glycans (as those found in glycosaminoglycans), presents extra challenges. First, the presence of the -NHAc group in 2-position favors the formation of 1,2-oxazoline derivatives when an oxocarbenium ion is involved, thus, hampering glycosylation reactions.[5] On the other hand, it is well known that the NHAc participates through H-bonding, a fact that can led to unexpected and inconvenient secondary products.[6] Also, when a good leaving group, such as a mesylate or a triflate, is attached to the OH-3, both 2,3-oxazolines and 2,3-aziridines can be obtained.[7] The ring-opening reaction of the latter with α-FucSH in strong basic conditions, led to a (1→3) thiodisaccharide, together with its (1→2) isomer, having the nitrogenated function in 3-position.[8] The formation of stable 2,3-oxazoline rings is a major disadvantage as this

group needs strong acid medium to be hydrolyzed, which is incompatible with the required further manipulation of protecting groups.[9,10]

Furthermore, a thiodisaccharide analog to the repetitive unit of chondroitin sulfate, β-GalNAc(1→4)GlcA was also synthesized through an S_N2 reaction, and its binding to the chondroitin AC lyase was studied.[11]

On the other hand, the +bimolecular substitution of the mentioned good leaving group (i. e. a triflate group), usually competes with the elimination E2-type reaction, in a fashion that is dependent on the stereochemistry of the substrate. For example, the treatment of a 4-*O*-triflate derivative of a 2-acetamido-D-galactopyranosyl substrate with GlcNAcSH as nucleophile, gave rise to the expected thiodisaccharide βGlcNAc(1→4)GlcNAc in only modest yields, as the 4,5-elimination product was also obtained.[12] The mentioned disaccharide was alternatively synthesized from a 1,6-anhydro-4-*O*-triflyl GlcNAc derivative and GlcNAcSNa in DMF.[13]

Besides the S_N2 mechanism to construct the thioglycosidic bond, alternative approaches were explored. The nucleophilic opening of a cyclic 2,3-sulfamidate was the key step to obtain the α-*S*-Fuc(1→3)GlcNAc thiodisaccharide. This 2,3-sulfamidate intermediate was generated from an *allo*-configured precursor and 1,1'-sulfonyl diimidazole, followed by treatment with acetyl chloride.[14–16] This ring-opening reaction was later used to obtain the nitrophenyl glycosides of 3-thio-β-GlcNAc and 3-thio-β-GalNAc, designed as precursor for the synthesis of glycomimetics.[17]

In another approach, the Michael addition of thioaldoses to the α,β-unsaturated systems of sugar enones, successfully produced 3- and 4-deoxythiodisaccharides and other structurally related compounds.[18–24] Some of the disaccharides obtained proved to be good inhibitors of glycosidases such as the *E. coli* β-galactosidase[21,24] and others.[25]

The ring-opening reaction of epoxides also yielded (1→3) and (1→4) thiodisaccharides under stereoselective processes.[8,26] Similarly, when starting with episulfides as precursors, branched thiotrisaccharides through an S_N2-like mechanism were also obtained, in some cases with variable amounts of sugar disulfides.[27,28]

In a very recent approach, a complete study on the Michael addition of β-D-GalSH to *E* and *Z* acetyl oximes derived from sugar enones was reported. This strategy broadened the possibilities/perspectives for the access to disaccharides having a GlcNAc or GalNAc residue in the reducing end.[29]

On the other hand, with respect to vinyl azides, it is worth to make reference that they have been proved to be valuable precursors in the organic synthesis field, mainly for the synthesis of heterocyclic compounds.[30,31] For example, a number of methodologies involving vinyl azides and 1,3-dicarbonyl compounds under catalysis of metals proved to be unique to obtain a wide variety of heterocyclic structures. Thermal- or photo-induced reactions of these precursors also conducted to heterocycles through vinyl nitrenes and 2H-azirines as intermediates.[32]

Vinyl azides can also behave as enamine-like nucleophiles to give iminodiazonium ions. The most explored fate of the latter is the 1,2-substituent-migration (Schmidt reaction) which finally conducts to amides by elimination of nitrogen.[33] Moreover, iminodiazonium ions can also react with nucleophiles, to give addition products. In this respect the fluoro- and bromo-alkoxylation of vinyl azides was developed, providing α-alkoxy-β-haloalkyl azides in good yields.[34]

Vinyl azides derived from carbohydrates were first described by Hanessian in 1968.[35] They have been obtained as undesired collateral E2-products in a number of S_N2 reactions of triflyl derivatives having a vicinal azide group, as mentioned above.[36–38] Yet,

as far as we know, carbohydrate-derived vinyl azides have not been explored as synthetic precursors of modified carbohydrates, even though, this functional group has demonstrated a great potentiality in an extensive variety of applications.

Thus, we present herein the results obtained in our way to the thiodisaccharide β-S-GlcA(1→3)GlcNAc, mimetic of the repetitive unit of hyaluronane, through a 2-azido-3-*O*-triflate having *allo*- configuration as key intermediate. The reaction was studied also by using GalSH as nucleophile, and thus, β-*S*-Gal(1→3)GlcNAc thiodisaccharide was also synthesized. Unexpectedly, the reaction led also to a particular class of derivatives, namely 2,3-dideoxy-2-azido-(1→2)-thiodisaccharides. We demonstrated that the latter were obtained by addition of the thioaldose to a vinylazide intermediate formed by E2 reaction of the mentioned *allo*-configured triflate. The determination of the configuration of these compounds was achieved by a combination of NMR experiments (2D-NOESY) and molecular modeling.

RESULTS AND DISCUSSION

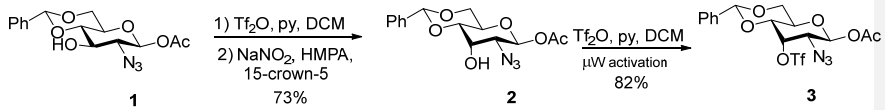
Inspired on the structure of hyaluronane, constituted by the repetitive disaccharide [→4)βGlcA(1→3)βGlcNAc(1→], we envisioned the synthesis of (1→3) thiodisaccharides of GlcNAc through a C-3 double inversion strategy from a *gluco*-configured precursor, conveniently protected in 4,6-positions. The presence of an *N*-acetamido group in the 2-position should be avoided, because of its participation in S_N2 displacements to give a 2,3-oxazoline.[15] Thus, we synthesized **1**, as a suitable precursor, in a 3-step sequence from D-$GlcNH_2$ hydrocloride (see SI).[39] It should be mentioned that the installation of the

2-azide group was performed using imidazole-1-sulfonyl azide hydrogen sulfate, as azide transfer reagent, in a more convenient procedure than that involving TfN_3.[40]

Thus, **1** was treated with Tf_2O and pyridine in anhydrous DCM and subsequently displaced with sodium nitrite in HMPA in the presence of 15-crown-5,[39] to obtain **2**. The signal corresponding to H-3 in the ^{1}H-NMR spectrum appeared at 4.45 ppm, showing a $J_{2,3} = J_{3,4} = 2.7$ Hz, confirming the *allo* configuration. These values are in accordance with those reported by Hung and coworkers for their 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-*allo*pyranosyl benzoate.[39]

Then, we faced the synthesis of **3**, the 3-*O*-triflate of **2**. Treatment of **2** with Tf_2O and pyridine in anhydrous DCM in standard conditions was not effective. The reaction required low power microwave irradiation, probably because the axial disposition of HO-3 lessens its reactivity as described for *gulo*-configured derivatives.[41] On the other hand, surprisingly, compound **2** and its triflate **3** had the same chromatographic mobility in a number of solvents, a fact that complicated the analysis. The ^{1}H-NMR spectrum of **3** showed that H-3 appeared strongly deshielded at 5.39 ppm. This signal was again very characteristic of *allo* configuration, appearing as a triplet with $J_{2,3} = J_{3,4} = 2.7$ Hz.

Scheme 1. Synthesis of 1-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-3-*O*-trifluoro methanesulfonyl-β-D-*allo*pyranoside 3.



1 **2** **3**

Compound **3** was stable enough to be isolated and purified by column chromatography for characterization. Despite this, in the following reactions, it was used without purification, after verification by ^{1}H-NMR that the complete transformation of **2** into **3** has occurred, by observing the signals of the H-3 described above. Thus, nucleophilic displacement of the triflate group with the 1-thioaldoses **4** and **5** in the presence of Et_3N led to the desired thiodisaccharides β-Gal(1→3)$GlcN_3$ (**6**) and β-GlcA(1→3)$GlcN_3$ (**10**), respectively (Scheme 2). As in our initial experiments these products were obtained in low yields together with a number of byproducts the reaction was studied under different conditions. The effects of temperature, concentration of the reactants and base, and the solvent were explored to optimize the yield of the thiodisaccharides (Table 1), which were isolated from the other products by column chromatography.

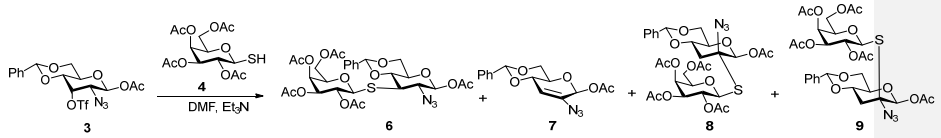
First, it was determined that in the absence of base, the reaction did not proceed (Table 1, entry 1). On the other hand, in the presence of an excess of base, the vinyl azide **7** was the main product obtained (Table 1, entry 2). Using GalSH (**4**) as nucleophile, **6** was obtained in 40% yield (Table 1, entry 3) when the reaction was carried out in the presence of 1.2 equivalents of Et_3N at -10 ºC. The ^{1}H-NMR spectrum of **6** showed a triplet at 2.97 ppm, with $J_{2,3} \cong J_{3,4}$ = 10.8 Hz, diagnostic for the H-3, which correlated with a signal at 50.0 ppm in the ^{1}H-^{13}C-HSQC NMR spectrum. On the other hand, when GlcASH (**5**) was used, the reaction required 2.4 equivalents of base and HMPA as co-solvent, giving 45% yield of **10** (Table 1, entry 5). Similarly, H-3 appeared as a triplet at 3.02 ppm ($J_{2,3} \cong J_{3,4}$ = 10.7 Hz) in the ^{1}H-NMR spectrum, while C-3 was observed at 49.5 ppm in the ^{13}C-NMR spectrum. Noteworthy, despite all the attempts made, compound **7** was obtained in all cases, as a result of the E2 elimination of the 3-*O*-triflate group and the H-2 which are in antiperiplanar disposition.

Compound **7** was characterized on the basis of its ^{1}H and ^{13}C NMR spectra, together with the corresponding 2D-HSQC, COSY and HMBC experiments. The H-1 appeared at 6.34 ppm as a double-doublet with a $J_{1,3}$ = 0.7 Hz and $J_{1,4}$ = 2.1 Hz. The signal for H-3 at 5.87 ppm appeared as a broad singlet. In the ^{13}C-NMR spectrum, a signal at 115.7 ppm was assigned to the C-3, as it correlated with the H-3 signal in the ^{1}H-^{13}C HSQC spectrum. The 2D-HMBC experiment was important for the assignment as the system H-1/H-3/H-4 was coupled**.** The coupling between the carbonylic carbon of the anomeric acetate and the proton at 6.34 ppm confirms the assignation of the latter to H-1.

On the other hand, unexpected additional compounds (indicated as compounds **8**, **9** and **11**), were obtained, as showed in Table 1 (entries 3 and 7, and Scheme 2). NMR spectra of these products presented the signals of both the thioaldose and the benzylidene-protected precursor. Interestingly, in all cases the H-1 appeared as deshielded singlets, consistent with the presence of the 1-*O*-acetyl group, and two protected signals appeared in the range 2.70-2.00 ppm suggesting the presence of a methylene, which meant, an unfunctionalized carbon. Shielding of C-3 signal to ~40 ppm was also diagnostic for the presence of a methylene.

Further studies to confirm the structures of **8**, **9** and **11** were carried out as described below.

Scheme 2. Synthesis of (1→3) thiodisaccharides 6 and 10 and formation of byproducts 7, 8, 9 and 11.


Ph
O
O
O
OAc
OTf
N3
3
AcO
OAc
AcO
O
SH
OAc
4
DMF, Et3N
6
7
8
9
S

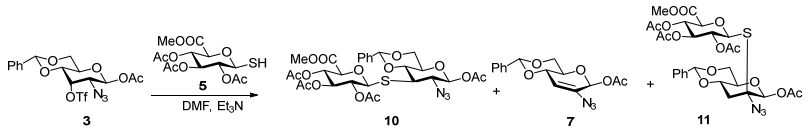
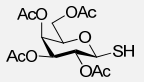
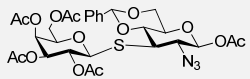
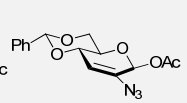
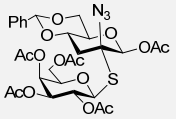
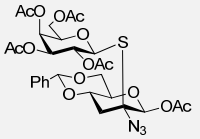
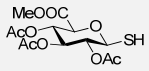
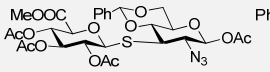
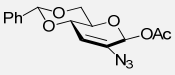
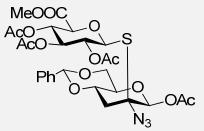

Ph
O
O
O
OAc
OTf
N3
3
MeOOC
AcO
AcO
O
SH
OAc
5
DMF, Et3N
10
7
11
S

Table 1. Reaction conditions for (1→3) thiodisaccharides 6 and 10

Entry	**3**:**4**	Equiv base	Temperature	Solvent	Thioaldose	Yield **6** (%)	Yield **7** (%)	Yield **8** + **9** (%)
								 
					4	**6**	**7**	**8** **9**
1	1:1.1	0.0	-10	DMF		-	-	-
2	1:1.5	4.5	-30	DMF/HMPA 1:1		19%	79%	<2%
3	1:1.1	1.2	-10	DMF		40%	<1%	55%
4	1:1.2	2.4	-10	DMF/HMPA 9:1		27%	14%	35%

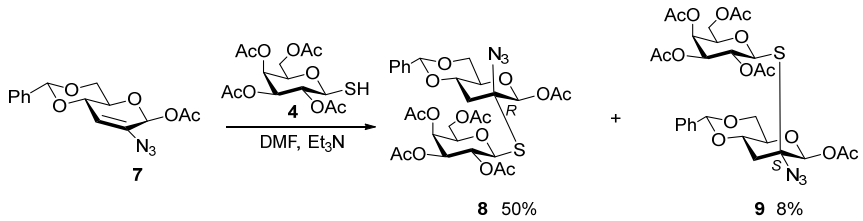
Entry	**3**:**5**	Equiv base	Temperature	Solvent	Thioaldose	Yield **10** (%)	Yield **7** (%)	Yield **11** (%)
								
					5	**10**	**7**	**11**
5	1:1.2	2.4	-10	DMF/HMPA 9:1		45%	51%	n.d.
6	1:1.3	1.6	-30	DMF/HMPA 9:1		36%	42%	<1%
7	1:1.7	5.1	-30	DMF		30%	39%	8%

Synthesis and structural elucidation of (1→2) thiodisaccharides 8, 9 and 11

As mentioned in the Introduction section, vinyl azides have been reported as versatile intermediates for the synthesis of organic compounds. It was recently reported that by reaction with an electrophilic halonium, an iminodiazonium cation is formed, which suffers from the attack of an alcohol as nucleophile, to give α-alkoxy-β-haloalkyl azides as addition products.[34] This report was encouraging to consider that **8**, **9** and **11** were the addition products of thioaldoses **4** or **5** to the olefin present in **7**, formed through a similar mechanism. If that were the case, vinyl azide **7** could be an unexpected useful precursor for the synthesis of (1→2) thiodisaccharides of unreported structures. To confirm this hypothesis, it was necessary to carry out the reaction starting from vinyl azide **7**.

Thus, **7** was prepared in 83% yield by treatment of **3** with Et_3N in DMF, for 18 h at room temperature. Then, compound **7** was treated with **4** and Et_3N in DMF at -10 °C for 2 h and we were glad to verify that the same mixture of products **8** and **9** were obtained in 50% and 8% yield, respectively (Scheme 3). Compounds **8** and **9** could be successfully isolated by careful column chromatography using silica gel < 45 μm (for thin layer chromatography) as stationary phase and mixtures of EtOAc/hexane.

Scheme 3. Synthesis of (1→2) thiodisaccharides 8 and 9 from vinylazide 7.



Spectroscopic analysis by NMR confirmed the proposed structures of **8** and **9**, as stereoisomers differing in the stereochemistry of C-2. As stated above, the signals in the ^{1}H-NMR corresponding to the anomeric protons appeared as singlets, and the protons corresponding to H-3ax and H-3eq, as multiplets at 2.70-2.00 ppm. Chemical shifts of H-3ax and H-4 were the main difference between the ^{1}H-NMR spectra of **8** and **9**. Also, the low-intensity carbon signal appearing at 72-74 ppm in the ^{13}C spectrum was assigned to C-2, a quaternary carbon, as it did not correlate with any proton in the 2D ^{1}H-^{13}C-HSQC spectrum. The challenged step was the determination of the stereochemistry at C-2. This was unambiguously achieved by 2D ^{1}H-NOESY NMR experiments, as follows.

In the case of **8**, the 2D ^{1}H-NOESY spectrum revealed the following inter-residue NOE contacts: H-3ax/H-1', H-3eq/H-1' and H-1/H-1'. For **9**, in turn, only H-3eq/H-1' inter-residue NOE contact was observed. This confirmed the equatorial disposition of the thiogalactosyl residue in **8**, which allows the proximity of H-1 and H-1', in contrast to **9**, where the thiogalactosyl residue is axially disposed (Figure 1). Thus, the absolute configuration of C-2 was *R* in the case of **8** and *S* in the case of **9**.

Molecular modeling of both structures confirmed this fact. Conformational analysis of compounds **8** and **9** was performed with MacroModel (Schrödinger Suites) applying a procedure that includes a final step of mixed Monte Carlo Multiple Minimum/Low-mode sampling (MCMM/LMOD). Conformers **8(1)**, **8(3)** and **9(1)** are shown in Figure 1 (see also Figure S1 and Table S1). H-1/H-1' interprotonic distance measured in conformer **8(1)** was 2.3 Å while H-3ax/H-1' and H-3eq/H-1' distances determined in conformer **8(3)** were 3.1 Å and 2.2 Å respectively. Then, the experimental results are compatible with a fast equilibrium, in the chemical shift scale, between **8(1)** and **8(3)** conformers, resulting

Con formato: Fuente: Negrita

Con formato: Fuente: Negrita

in averaged distances. Conversely, the only NOE contact observed for **9** (H-3eq/H-1') was consistent with the distance determined in conformer **9(1)** of 2.6 Å.

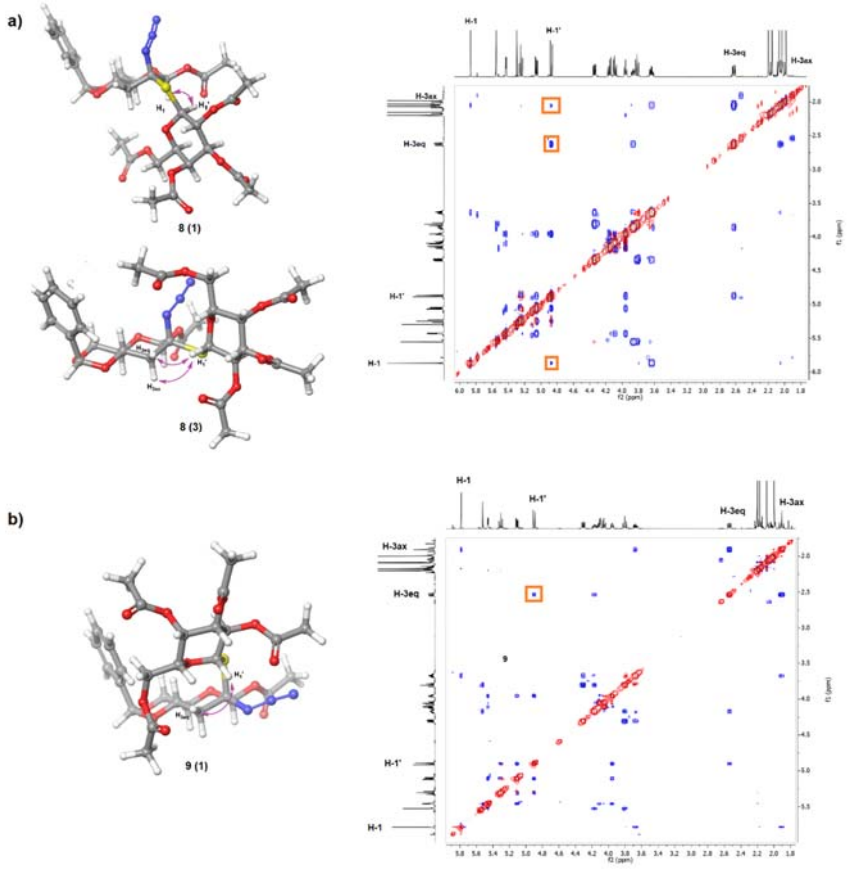
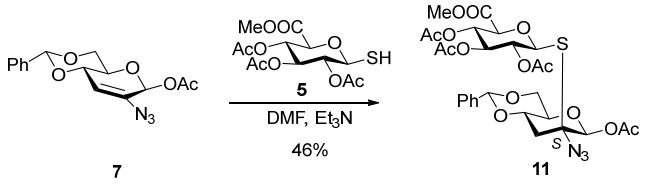


Figure 1. 3-Dimensional models of **8** (a) and **9** (b) representing low energy conformers obtained by MCMM/LMOD (MacroModel) and NOESY experiments (mixing time: 0.9 s, T = 298.2 K). Diagnostic NOE contacts are labeled.

The addition product of **5** to the vinylazide **7** under the same conditions described for **8** and **9**, was thiodisaccharide **11** (Scheme 4), which was obtained in 48% yield. The axial disposition of the *S*-GlcA residue, and thus, the *S* configuration for C-2, were determined by 2D ^{1}H-NOESY as described for the case of **9**, as the only inter-residue NOE contact

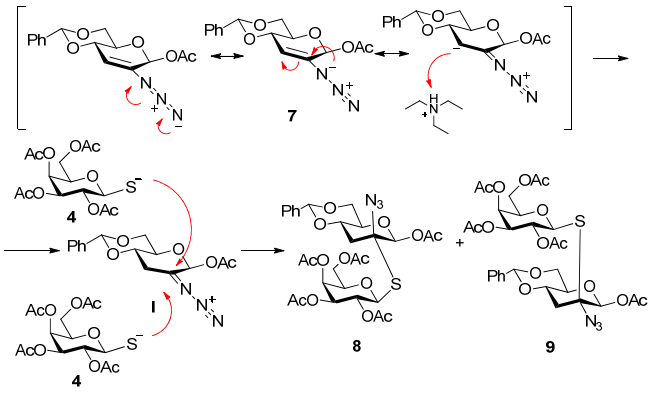
observed was that corresponding to H-3eq/H-1' (Figure S1, Table S1). Conformational analysis of compound **11** confirmed this, as for the most stable conformer **11(1)**, the H-3eq/H-1' distance was 2.7 Å.

Scheme 4. Synthesis of (1→2) thiodisaccharide 11 from vinylazide 7.



To explain the formation of **8**, **9** and **11**, we propose a mechanism involving an initial reaction of the vinylazide **7** with the electrophile H^+ to give the iminodiazonium cation **I**, which suffers nucleophilic attack of the thioaldose.[34] The selectivity observed in each case probably results as a consequence of electronic and steric factors and was not yet further investigated.

Scheme 5. Proposed mechanism for the synthesis of (1→2) thiodisaccharides 8 and 9.



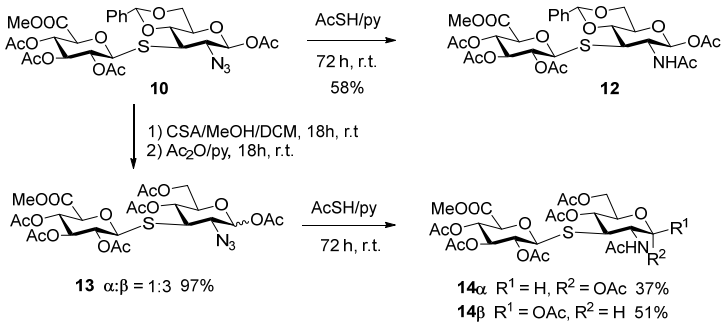
Modification of the (1→3) thiodisaccharides 6 and 10

At this moment it became interesting to have alternative derivatives that could be used as building blocks for the synthesis of more complex glycomimetics, as thiodisaccharides **6** and **10** might be considered starting materials in glycosylation reactions. Thus, our particular interest in glycosaminoglycan mimetics led us to modify first compound **10**, by two different approaches. On one hand, the installation of the -NHAc group in 2-position was evaluated by treatment of **10** with AcSH in pyridine[42,43] at room temperature during 72 h, giving compound **12** in a moderate 58 % yield (Scheme 6).

On the other hand, by treatment of **10** with CSA in DCM/MeOH at room temperature during 18 h, followed by acetylation under standard conditions, compound **13** was obtained in almost quantitatively yield (97%) although as a mixture of anomers, probably as a consequence of the long treatment in acidic conditions (Scheme 6). Finally, successful reductive acylation of the azide group was achieved by treatment of **13α,β** with AcSH/py during 72 h at room temperature, and thus, **14** was obtained as an α:β

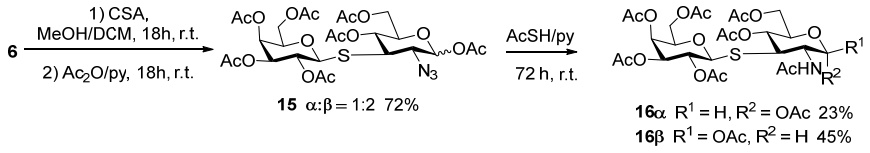
(0.7:1.0) mixture of anomers in 88% global yield. The anomers were successfully resolved by column chromatography as described in the Experimental section.

Scheme 6. Transformation of 10 in the thiodisaccharides 12, 13α,β and 14α,β



Similarly, compound **15α,β** was obtained in 72% yield, by treatment of **6** with CSA in DCM/MeOH at room temperature during 18 h, followed by acetylation (Scheme 7). Further reduction of the azide group with AcSH/py led to **16α,β** in a 68% combined yield, as an α:β (1:2) mixture of anomers. Again, resolution of the anomers was successfully achieved by column chromatography.

Scheme 7. Transformation of 6 in the thiodisaccharides 15α,β and 16α,β



CONCLUSIONS

It is well known that N-acetyl D-glucosamine is a relevant sugar because of its presence in numerous glycoconjugates, such as *N*- and *O*- linked glycans in glycoproteins, and also as main component of glycosaminoglycans. Thus, its incorporation in glycomimetic oligosaccharides may lead to useful derivatives for the study of biological processes associated to such glycoconjugates.

As a common procedure in carbohydrate synthetic chemistry, the challenges imposed by the presence of the –NHAc group can be overcome by the incorporation of an azide group at C-2 of the selected sugar precursor, as this azide substituent can be efficiently reduced at the final stages of a glycan synthesis. Consequently, we adopted this approach in an attempt to avoid the participation of the –NHAc in the 3-position functionalization.

Thus, in this report, we successfully obtained (1→3)-thiodisaccharides having a GlcNAc residue. The key precursor was a 3-*O*-triflate prepared from a 2-azido derivative having *allo*-configuration. The azido-thiodisaccharides obtained in this way (**6** and **10**) can be considered useful armed glycosyl donors for the synthesis of more complex glycomimetics by classic glycosylation methods, as they still possess the azide group at C-2. Hydrolysis of the 4,6-*O*-benzylidene protecting group present in **6** and **10**, caused the anomerization of the derivatives. *In situ* further peracetylation, led efficiently to **15α,β** and **13α,β** respectively. These anomeric mixtures constitute alternative building blocks for further conjugation, taking into consideration that the absence of the benzylidene group make them also compatible with thioglycosylation methods involving strong Lewis acids, such as $BF_3.OEt_2$.[44]

On the other hand, reduction of the azide present in the thiodisaccharides obtained could be effectively achieved by the classical reported procedure using AcSH/py.[42,43]

A relevant finding associated to the S_N2 reaction explored in this work, was that vinyl azide **7** was formed by elimination reaction of triflate **3**. The addition reaction of thioaldoses **4** and **5** to **7**, serendipitously gave rise to a new class of 2-azido-(1→2) thiodisaccharides, having a thioaminal type linkage at C-2. Compounds **8**, **9** and **11**, were fully characterized and the stereochemistry of the newly tetrasubstitued stereogenic centers at C-2 were unambiguously determined by a combination of 2D-NOESY methods and molecular modeling calculations.

To the best of our knowledge, the addition reaction of nucleophiles to sugar-derived vinyl azides has not been reported previously. Taking into account that vinyl azides have been extensively used as precursors for the synthesis of a variety of heterocycles, and in most cases the elimination of nitrogen is concomitant with cyclization, the reactivity observed here is remarkable as the azide group remains at C-2. This probably is due to the partial rigidity established by the sugar cycle and the restoring of a $^4C_1$ conformer. **Ver esto**. Besides the potentiality of compounds **8**, **9** and **11** as building blocks of more complex glycomimetics, as stated above for **6** and **10**, the 2-azide group make them interesting substrates for a variety of reactions as, for example, the CuAAC reaction. Due to the particularities of these unexplored structures, further studies on its reactivity as well as binding experiments with proteins would be relevant.

Additional studies on both, the use of **6**, **10**, **13 α,β**, **14 α,β**, **15 α,β** and **16 α,β** as glycosyl donnors in glycosylation and/or thioglycosylation reactions, and the use of **8**, **9** and **11** as recognition elements in the construction of multivalent ligands by click-chemistry are on the way.

EXPERIMENTAL

General Methods

Solvents were distilled before use. Thin layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck). The compounds were detected with 5% (v/v) sulfuric acid in EtOH, containing 0.5% *p*-anisaldehyde. Column chromatography was performed on silica gel 60, particle sizes of 40-63 μm or < 45 μm (for thin layer chromatography) from Merck, by elution with the solvents indicated in each case. Thioaldoses **4** and **5** were prepared by reported methods.[26,28,45,46] Reactions under microwave irradiation were carried out in an Anton-Paar Monowave 300 instrument with a System Internal IR probe type, under low power activation (T = 35 °C, t = 1.5 h). ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded at 25 °C at 500 and 125.7 MHz, respectively, in a Bruker Avance Neo 500 spectrometer. For ^{1}H, ^{13}C chemical shifts are reported in parts per million relative to tetramethylsilane or a residual solvent peak ($CHCl_3$: ^{1}H: δ 7.26 ppm, ^{13}C: δ 77.2 ppm). Assignments of ^{1}H, ^{13}C and stereochemistry were determined by analysis of coupling constants and assisted by 2D ^{1}H COSY, 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{13}C HMBC and 2D ^{1}H NOESY (mixing time 0.9 s) experiments. High resolution mass spectra (HRMS) were obtained by Electrospray Ionization (ESI) and Q-TOF in a Bruker micrOTOF-Q II spectrometer. Optical rotations were determined in a Perkin-Elmer 343 polarimeter, at 20 °C in a 1 dm cell. Melting points were measured in a Fisher-Jones apparatus.

1-*O*-Acetyl-2-Azido-4,6-*O*-benzylidene-2-deoxy-β-D-*allo*pyranose (2)

To a stirred solution of **1** (400 mg, 1.2 mmol) in anhydrous DCM (4.0 mL) and pyridine (1.6 mL) at 0 °C, Tf_2O (240 μL, 1.4 mmol) was added dropwise. After 2 h, MeOH was added and the mixture concentrated under vacuum. The residue was dissolved in EtOAc (40 mL) and extracted with HCl (1 × 10 mL), $NaHCO_3$ s.s. (1 × 15 mL) and brine (1 × 15 mL), then dried over $MgSO_4$ and concentrated under vacuum.

The crude triflate (*gluco*-configured) was re-dissolved in HMPA (6.4 mL) and sodium nitrite (890 mg, 12.9 mmol) and 15-crown-5 (265 µL, 1.3 mmol) were added. The reaction mixture was stirred for 3 h. The mixture was diluted with EtOAc (40 mL) and washed with water (4 x 20 mL), dried ($MgSO_4$) and concentrated under vacuum. Column chromatography (9:1→4:1 hexane-EtOAc) of the residue gave compound **2** (294 mg, 73%), as white solid mp 94-95 °C (from H_2O): R_f = 0.48 (7:3 hexane-EtOAc); $[\alpha]_D^{20}$ -18.3 (*c* 1, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.49-7.47 (m, 2 H, H-aromatic), 7.39-7.37 (m, 3 H, H-aromatic), 6.08 (d, 1 H, $J_{1,2}$ = 8.6 Hz, H-1), 5.58 (s, 1 H, PhC*H*O), 4.45 (dd, 1H, $J_{2,3}$ = 2.8, $J_{3,4}$ = 2.4 Hz, H-3), 4.41 (dd, 1 H, $J_{5,6eq}$ = 5.1, $J_{6eq,6ax}$ = 10.5 Hz, H-6eq), 4.19 (ddd, 1 H, $J_{5,6eq}$ = 5.1, $J_{4,5}$ = 9.5, $J_{5,6ax}$ = 10.5, H-5), 3.74 (t, 1 H, $J_{6eq,6ax} \cong J_{5,6ax}$ = 10.5 Hz, H-6ax), 3.62 (dd, 1 H, $J_{3,4}$ = 2.4, $J_{4,5}$ = 9.5 Hz, H-4), 3.53 (dd, 1 H, $J_{2,3}$ = 2.8, $J_{1,2}$ = 8.6 Hz, H-2), 2.19, (s, 3 H, C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 168.9 (*C*O), 136.8, 129.6, 128.5, 126.3 (C-aromatic), 102.2 (Ph*C*HO), 91.7 (C-1), 78.2 (C-4), 68.8, 68.6 (C-6, C-3), 64.1 (C-5), 61.9 (C-2), 21.1 (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{15}H_{17}N_3NaO_6$: 358.1010, found: 358.1002

1-*O*-Acetyl-2-Azido-4,6-benzylidene-2-deoxy-3-*O*-trifluoromethansulfonyl-β-D-*allo*pyranose (3)

To a solution of compound **2** (200 mg, 0.6 mmol) in anhydrous dichloromethane (6 mL) and pyridine (170 µL, 2.2 mmol) in an Anton-Paar microwave capped vessel was added dropwise trifluoromethansulfonic anhydride (160 µL, 1.0 mmol) at 0 °C. The ice bath was removed and the reaction flask was gradually warmed up to room temperature. Low-power activation was performed under microwave irradiation for 1.5 h, at 35 °C. Column chromatography (9:1→4:1 hexane-EtOAc) of the residue gave compound **3** (228 mg,

82%), as white solid mp 112 °C (d) (from EtOH/H_2O): R_f = 0.50 (7:3 hexane-EtOAc); $[\alpha]_D^{20}$ -18.5 (*c* 1, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.46-7.36 (m, 5 H, H-aromatic), 5.92 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1), 5.57 (s, 1 H, PhC*H*O), 5.40 (t, 1 H, $J_{2,3} \cong J_{3,4} \cong$ 2.6 Hz, H-3), 4.42 (dd, 1 H, $J_{5,6eq}$ = 5.1, $J_{6ax,6eq}$ = 10.6 Hz, H-6eq), 4.07 (ddd, 1 H, $J_{5,6eq}$ = 5.1, $J_{4,5}$ = 9.5, $J_{5,6ax}$ = 10.3 Hz, H-5), 3.85 (dd, $J_{2,3}$ = 2.6, $J_{1,2}$ = 8.4 Hz, H-2), 3.77 (dd, 1 H, $J_{3,4}$ = 2.6, $J_{4,5}$ = 9.5 Hz, H-4), 3.75 (dd, 1 H, $J_{5,6ax}$ = 10.3, $J_{6ax,6eq}$ = 10.6 Hz, H-6ax), 2.20 (s, 3H, C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 168.4 (*C*O), 136.1, 129.7, 128.6, 126.4 (C-aromatic), 102.7 (Ph*C*HO), 91.8 (C-1), 81.9 (C-3), 75.5 (C-4), 68.6 (C-6), 64.7 (C-5), 60.1 (C-2), 21.0 (*C*H_3CO). ESI-HRMS: $[M+H]^+$ calcd for $C_{16}H_{17}F_3N_3O_8S$: 468.0683, found: 468.0698

General conditions for the synthesis of the (1→3) thiodisaccharides

The synthesis of triflate **3** was performed as described above, starting from **2**. As compounds **2** and **3** showed the same mobility by TLC (R_f = 0.50, 7:3 hexane-EtOAc), the consumption of the starting material **2** was checked by NMR, by observing the total disappearance of the signal corresponding to H-3 (4.45 ppm) and the presence of the one corresponding to **3** (5.39 ppm). The solution was then transferred to a round-bottom flask and solvents were evaporated under vacuum. This led to the *allo*-configured crude triflate **3**, which was used subsequently without any purification. To a solution of the latter in dry DMF and HMPA (in the proportion indicated in each case, see below and Table 1) was added sequentially the thioaldose (**4** or **5**) and Et_3N, and the reaction was stirred for 2 h. The mixture was diluted with EtOAc (10 mL), washed with LiCl 5% (3 x 2 mL), dried ($MgSO_4$) and concentrated. The residues were purified by column chromatography as described in each case. Variable amounts of compound **7**

were recovered from the first fractions of the column. Characterization of **7** is described below, when obtained by elimination reaction of **3**.

1-*O*-Acetyl-2-azido-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2,3-dideoxy-3-thio-β-D-glucopyranose (6)

Compound **6** was prepared from crude triflate **3**, obtained from **2** (100 mg, 0.30 mmol), and **4** (120 mg, 0.33 mmol) in anh. DMF (1.5 mL) at -10 ºC, using Et_3N (0.36 mmol, 50 μL), as described above. Column chromatography (9:1→7:3 hexane-EtOAc) gave first the mixture of compounds **8** and **9** (112 mg, 55%), which were characterized afterwards, when obtained by reaction between **7** and **4** (see below). Further elution of the column gave compound **6** (82 mg, 40%) as white solid, mp 198-199 °C (from hexane/EtOAc): R_f = 0.37 (3:2 hexane-EtOAc); $[\alpha]_D^{20}$ -17.9 (*c* 1, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.48-7.46 (m, 2 H, H-aromatic), 7.36-7.35 (m, 3 H, H-aromatic), 5.56 (d, 1 H, $J_{1,2}$ = 8.3 Hz, H-1), 5.55 (s, 1 H, PhC*H*O), 5.39 (d, 1 H, $J_{3',4'}$ = 2.9 Hz, H-4'), 5.20 (t, 1 H, $J_{1',2'} = J_{2',3'}$ = 10.0 Hz, H-2'), 5.05 (dd, 1 H, $J_{2',3'}$ = 10.0, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.83 (d, 1 H, $J_{1',2'}$ = 10.0 Hz, H-1'), 4.36 (dd, 1 H, $J_{6a,6b}$ = 10.5, $J_{5,6b}$ = 4.6 Hz, H-6b), 4.09 (dd, 1 H, $J_{6a',6b'}$ = 11.3, $J_{5',6a'}$ = 6.2 Hz, H-6a'), 3.94 (dd, 1 H, $J_{6a',6b'}$ = 11.3, $J_{5',6b'}$ = 6.9 Hz, H-6b'), 3.82 (dd, 1 H, $J_{5',6a'}$ = 6.2, $J_{5',6b'}$ = 6.9 Hz, H-5'), 3.74 (t, 1 H, $J_{6a,6b} = J_{5,6a}$ = 10.5 Hz, H-6a), 3.62–3.55 (m, 3 H, H-2, H-4, H-5), 2.97 (t, 1 H, $J_{2,3} \cong J_{3,4}$ = 10.8 Hz, H-3), 2.18, 2.14, 2.04, 1.97, 1.93 (5 s, 15 H, C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.4 (× 2), 170.2, 169.7, 168.7 (*C*O), 136.7, 129.5, 128.5, 126.2 (C-aromatic), 102.2 (Ph*C*HO), 94.7 (C-1), 83.4 (C-1'), 77.5 (C-4), 74.6 (C-5'), 71.8 (C-3'), 70.3 (C-5), 68.4 (C-6), 67.9 (C-2'), 67.2 (C-4'), 65.2 (C-2), 61.8 (C-6'), 50.0 (C-3), 21.0,

20.8 (× 2), 20.7 (× 2) (*C*H$_3$CO). ESI-HRMS: [M+Na]$^+$ calcd for $C_{29}H_{35}N_3NaO_{14}S$: 704.1732, found: 704.1723, [M+K]$^+$ calcd for $C_{29}H_{35}KN_3O_{14}S$: 720.1471, found: 720.1485.

1-*O*-Acetyl-2-azido-4,6-*O*-benzylidene-2,3-dideoxy-3-*S*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-3-thio-β-D-glucopyranose (10)

Compound **10** was obtained from crude triflate **3**, obtained from **2** (250 mg, 0.75 mmol) and **5** (315 mg, 0.90 mmol) in a mixture of anh. DMF (4 mL) and HMPA (0.6 mL), using Et_3N (1.8 mmol, 250 μL), as described above. Column chromatography (9:1→ 3:2 hexane-EtOAc) gave first compound **7** (122 mg), and then thiodisaccharide **10** (226 mg, 45%) as white solid, mp 175-176 °C (from hexane/EtOAc): R_f = 0.45 (1:1 hexane-EtOAc); $[\alpha]_D^{20}$ -31.1 (*c* 1, $CHCl_3$). δ 7.47-7.45 (m, 2 H, H-aromatic), 7.37-7.35 (m, 3 H, H-aromatic), 5.75 (d, 1 H, $J_{1,2}$ = 8.2 Hz, H-1), 5.54 (s, 1 H, PhC*H*O), 5.24 (t, 1 H, $J_{3',4'} \cong J_{4',5'} \cong$ 9.5 Hz, H-4'), 5.17 (dd, 1 H, $J_{2',3'}$ = 8.9, $J_{3',4'}$ = 9.5 Hz, H-3'), 5.00 (dd, 1 H, $J_{2',3'}$ = 8.9, $J_{1',2'}$ = 10.2, Hz, H-2'), 4.91 (d, 1 H, $J_{1',2'}$ = 10.2 Hz, H-1'), 4.35 (dd, 1 H, $J_{5,6eq}$ = 4.5, $J_{6ax,6eq}$ = 10.6 Hz, H-6eq), 3.88 (d, 1 H, $J_{4',5'}$ = 9.5 Hz, H-5'), 3.74 (dd, $J_{5,6ax}$ = 9.0, $J_{6ax,6eq}$ = 10.5 Hz, H-6ax), 3.70 (s, 3H, C*H*$_3$O), 3.63–3.53 (m, 3 H, H-2, H-4, H-5), 3.02 (t, $J_{2,3} \cong J_{3,4}$ = 10.7 Hz, H-3), 2.19, 2.01, 2.00, 1.95 (4 s, 12 H, 4 × C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.2, 169.5, 169.4, 168.7, 166.7 (*C*O), 136.7, 129.4, 128.5, 126.2 (C-aromatic), 102.1 (Ph*C*HO), 94.6 (C-1), 83.1 (C-1'), 78.2 (C-4), 76.1 (C-5'), 73.0 (C-4'), 70.8 (C-2'), 70.3 (C-5), 69.1 (C-3'), 68.4 (C-6), 65.0 (C-2), 53.0 (O*C*H$_3$), 49.5 (C-3), 21.0, 20.7 (× 2), 20.6 (*C*H$_3$CO). ESI-HRMS: [M+Na]$^+$ calcd for $C_{28}H_{33}N_3NaO_{14}S$: 690.1575, found: 690.1582, [M+H]$^+$ calcd for $C_{28}H_{34}N_3O_{14}S$: 668.1756, found: 668.1757

1-*O*-Acetyl-2-Azido-4,6-*O*-benzylidene-2,3-dideoxy-β-D-*erythro*-hex-2-enopyranose (7)

To a solution of compound **3** (80 mg, 0.17 mmol) in anhydrous DMF (2 mL) at room temperature, Et_3N was added (0.36 mmol, 50 µL) and the reaction mixture was stirred for 18 h. After this, solvents were evaporated under vacuum. Column chromatography of the residue (hexane→4:1 hexane-EtOAc) gave compound **7** (45 mg, 83%) as an amorphous solid, R_f = 0.40 (4:1 hexane-EtOAc); $[\alpha]_D^{20}$ -51.8 (*c* 0.6, $CHCl_3$); ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.50-7.37 (m, 5 H, H-aromatic), 6.34 (dd, 1 H, $J_{1,3}$ = 0.7, $J_{1,4}$ = 2.1 Hz, H-1), 5.87 (brs, 1 H, H-3), 5.62 (s, 1 H, PhC*H*O), 4.45 (dt, 1 H, $J_{1,4} \cong J_{3,4}$ = 2.1, $J_{4,5}$ = 8.3 Hz, H-4), 4.34 (dd, 1 H, $J_{5,6eq}$ = 4.2, $J_{6eq,6ax}$ = 10.2 Hz, H-6eq), 3.89 (t, $J_{5,6ax} \cong J_{ax,eq}$ = 10.2 Hz, H-6ax), 3.80 (ddd, 1 H, $J_{5,6eq}$ = 4.2, $J_{4,5}$ = 8.3, $J_{5,6ax}$ = 10.2 Hz, H-5), 2.17 (s, 3 H, C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 169.3 (*C*O), 136.9 (C-aromatic), 135.6 (C-2), 129.5, 128.5, 126.3 (C-aromatic), 115.7 (C-3), 102.1 (Ph*C*HO), 89.4 (C-1), 74.1 (C-4), 71.2 (C-5), 68.7 (C-6), 21.0 (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{15}H_{15}N_3NaO_5$: 340.0904, found: 340.0907

General procedure for the synthesis of the 2-*C*-azido-(1→2) thiodisaccharides

Compound **7** was dissolved in DMF, the solution was cooled to -10 °C and the thioaldose (**4** or **5**) and Et_3N were added. The reaction mixture was stirred for 2 h, then diluted with EtOAc and washed twice with LiCl 5%. Column chromatography of the residue in silica gel < 45 µm (for thin layer chromatography), gave the desired products as described below.

1-*O*-Acetyl-2-*C*-azido-2-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2,3-dideoxy-2-thio-β-D-*gluco*pyranose (8) and 1-Acetyl-2-*C*-azido-2-*S*-

(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-4,6-*O*-benzylidene-2,3-dideoxy-2-thio-β-D-*manno*pyranose (9)

Compounds **8** and **9** were obtained by reaction of **7** (62 mg, 0.19 mmol) and **4** (90 mg, 0.24 mmol) in the presence of Et_3N (100 µL) in DMF (1.30 mL). Column chromatography of the residue using silica gel < 45 µm (for thin layer chromatography) and hexane-EtOAc mixtures (9:1→3:1), gave first **9** (11 mg, 8%) and then, **8** (67 mg, 50%) as white solids.

8: mp 105-106 °C (from hexane/EtOAc), R_f = 0.44 (3:2 hexane:EtOAc); $[\alpha]_D^{20}$ +33.7 (*c* 1, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.46-7.44 (m, 2 H, H-aromatic), 7.38-7.36 (m, 3 H, H-aromatic), 5.87 (s, 1 H, H-1), 5.55 (s, 1 H, PhC*H*O), 5.43 (dd, 1 H, $J_{4',5'}$ = 1.2, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.25 (t, 1 H, $J_{1',2'} \cong J_{2',3'}$ = 10.1 Hz, H-2'), 5.06 (dd, 1 H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.1 Hz, H-3'), 4.87 (d, 1 H, $J_{1',2'}$ = 10.1 Hz, H-1'), 4.35 (dd, 1 H, $J_{5,6eq}$ = 4.9, $J_{6ax,6eq}$ = 10.5 Hz, H-6eq), 4.16 (dd, 1 H, $J_{5',6a'}$ = 7.0, $J_{6a',6b'}$ = 11.5 Hz, H-6a'), 4.09 (dd, 1 H, $J_{5',6b'}$ = 6.0, $J_{6a',6b'}$ = 11.5 Hz, H-6b'), 3.96 (ddd, 1 H, $J_{4',5'}$ = 1.2, $J_{5',6b'}$ = 6.0, $J_{5',6a'}$ = 7.0 Hz, H-5'), 3.86 (ddd, 1 H, $J_{3eq,4}$ = 4.2, $J_{4,5}$ = 9.2, $J_{3ax,4}$ = 11.3 Hz, H-4), 3.82 (t, 1 H, $J_{5,6ax} \cong J_{6ax,6eq}$ = 10.5 Hz, H-6ax), 3.64 (ddd, 1 H, $J_{5,6eq}$ = 4.9, $J_{4,5}$ = 9.2, $J_{4,6ax}$ = 10.5 Hz, H-5), 2.62 (dd, 1 H, $J_{3eq,4}$ = 4.2, $J_{3ax,3eq}$ = 13.0 Hz, H-3eq), 2.20, 2.15, (2 s, 6 H, 2 × C*H*$_3$CO), 2.09-1.98 (m, 10 H, H-3ax, 3 × C*H*$_3$CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.6, 170.3, 170.1, 169.5, 168.2 (*C*O), 136.9, 129.5, 128.5, 126.2 (C-aromatic), 102.2 (Ph*C*HO), 95.9 (C-1), 81.2 (C-1'), 74.8 (C-5'), 73.6 (C-4), 72.1 (C-2), 72.0 (C-3'), 71.9 (C-5), 68.5 (C-6), 67.2 (C-4'), 67.0 (C-3'), 61.7 (C-6'), 40.0 (C-3), 21.0, 20.8 (× 2), 20.7 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{29}H_{35}N_3NaO_{14}S$: 704,1732, found: 704.17154, $[M+NH_4]^+$ calcd for $C_{29}H_{36}N_4O_{14}S$: 699,2178, found: 699.21505.

9: mp xxx-xxx °C (from xxx), R_f = 0.49 (3:2 hexane:EtOAc); $[\alpha]_D^{20}$ -39.4 (*c* 0.9, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.47-7.40 (m, 2 H, H-aromatic), 7.38-7.32 (m, 3 H, H-aromatic), 5.78 (s, 1 H, H-1), 5.52 (s, 1 H, PhC*H*O), 5.46 (dd, 1 H, $J_{4',5'}$ = 1.1, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.31

(t, 1 H, $J_{1',2'} \cong J_{2',3'}$ = 10.1 Hz, H-2'), 5.11 (dd, 1 H, $J_{3',4'}$ = 3.4, $J_{2',3'}$ = 10.1 Hz, H-3'), 4.90 (d, 1 H, $J_{1',2'}$ = 10.1 Hz, H-1'), 4.31 (dd, 1 H, $J_{5,6eq}$ = 4.8, $J_{6ax,6eq}$ = 10.3 Hz, H-6eq), 4.16 (ddd, 1 H, $J_{3eq,4}$ = 4.1, $J_{4,5}$ = 9.3, $J_{3ax,4}$ = 11.4 Hz, H-4), 4.11 (dd, 1 H, $J_{5',6b'}$ = 5.1, $J_{6a',6b'}$ = 11.5 Hz, H-6b'), 4.05 (dd, 1 H, $J_{5',6b'}$ = 7.3, $J_{6a',6b'}$ = 11.5 Hz, H-6a'), 3.95 (ddd, 1 H, $J_{4',5'}$ = 1.1, $J_{5',6b'}$ = 5.1, $J_{5',6a'}$ = 7.3 Hz, H-5'), 3.81 (t, 1 H, $J_{6ax,6eq} \cong J_{5,6ax}$ = 10.3 Hz, H-6ax), 3.68 (ddd, 1 H, $J_{5,6eq}$ = 4.8, $J_{5,6ax}$ = 9.8, $J_{4,5}$ = 9.3, Hz, H-5), 2.54 (dd, 1 H, $J_{3eq,4}$ = 4.1, $J_{3ax,3eq}$ = 13.2 Hz, H-3eq), 2.20, 2.17, 2.09, 2.00 (4s, 12 H, CH_3CO), 1.91 (dd, 1 H, $J_{3ax,4}$ = 11.4, $J_{3ax,3eq}$ = 13.2 Hz, H-3ax); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.3, 170.1, 169.8, 168.4 (*C*O), 137.1, 129.5, 128.5, 126.3 (C-aromatic), 102.5 (PhCHO), 96.6 (C-1), 82.1 (C-1'), 75.2 (C-5'), 74.1 (C-4), 74.0 (C-2), 72.7 (C-5), 72.0 (C-3'), 68.5 (C-6), 67.5 (C-4'), 66.8 (C-3'), 62.2 (C-6'), 39.8 (C-3), 21.1, 20.9 (× 2), 20.7, 20.4 (*C*H_3CO). ESI-HRMS: $[M+H]^+$ calcd for $C_{29}H_{36}N_3O_{14}S$: 682.1913, found: 682.1905

1-*O*-Acetyl-2-*C*-azido-2-*S*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-4,6-*O*-benzylidene-2,3-dideoxy-2-thio-β-D-*manno*pyranose (11)

Compound **11** was obtained by reaction of **7** (100 mg, 0.32 mmol) and **5** (130 mg, 0.37 mmol) in the presence of Et_3N (130 µL) in DMF (2 mL). Column chromatography of the residue using silica gel of < 45 µm (for thin layer chromatography) and hexane-EtOAc mixtures (9:1→7:3), gave **11** (96 mg, 46%) as white solid, mp 197-198 °C (from hexane/EtOAc), R_f = 0.55 (1:1 hexane:EtOAc); $[\alpha]_D^{20}$ -9.8 (*c* 1, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7xx-xxx (m, 2 H, H-aromatic), xx-xx (m, 3 H, H-aromatic), 5.85 (s, 1 H, H-1), 5.55 (s, 1 H, PhC*H*O), 5.29 (dd, 1 H, $J_{2',3'}$ = 8.6, $J_{3',4'}$ = 9.5 Hz, H-3'), 5.22 (t, 1 H, $J_{3',4'} \cong J_{4',5'}$ = 9.5 Hz, H-4'), 5.04 (dd, 1 H, $J_{2',3'}$ = 8.6, $J_{1',2'}$ = 10.3 Hz, H-2'), 4.96 (d, 1 H, $J_{1',2'}$ = 10.3 Hz, H-1'), 4.33 (dd, 1 H, $J_{5,6eq}$ = 4.9, $J_{6ax,6eq}$ = 10.5 Hz, H-6eq), 4.02 (d, $J_{4',5'}$ = 9.5 Hz, H-5'), 3.86 (ddd,

1 H, $J_{3eq,4}$ = 4.2, $J_{4,5}$ = 9.2, $J_{3ax,4}$ = 11.4 Hz, H-4), 3.80 (t, 1 H, $J_{5,6ax} \approx J_{6ax,6eq}$ = 10.5 Hz, H-6ax), 3.75 (s, 3 H, OCH_3), 3.62 (ddd, 1 H, $J_{5,6aq}$ = 4.9, $J_{4,5}$ = 9.2, $J_{5,6ax}$ = 10.5 Hz, H-5), 2.59 (dd, 1 H, $J_{3eq,4}$ = 4.2, $J_{3ax,3eq}$ = 13.1 Hz, H-3eq), 2.16 (s, 3H, CH_3CO), 2.07- 2.02 (m, 13H, H-3ax, 3 × CH_3CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.1, 169.4, 169.3, 169.8, 168.1, 166.5 (*C*O), 136.9, 129.5, 128.5, 126.2 (C-aromatic), 102.2 (PhCHO), 96.1 (C-1), 80.9 (C-1'), 76.3 (C-5'), 73.5 (C-4), 73.1 (C-3'), 72.0 (C-2), 71.9 (C-5), 69.6 (C-2'), 69.1 (C-4'), 68.5 (C-6), 53.2 (OCH_3), 39.6 (C-3), 21.9, 20.8, 20.7, 20.6 (CH_3CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{28}H_{33}N_3NaO_{14}S$: 690.1575, found: 690.1567chequear espectro

1-*O*-Acetyl-2-acetamido-4,6-*O*-benzylidene-2,3-dideoxy-3-*S*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-3-thio-β-D-glucopyranose (12)

To a stirred solution of compound **10** (100 mg, 0.15 mmol) in pyridine (3.2 mL), thioacetic acid was added (215 µL, 3 mmol) under argon atmosphere. The mixture was stirred at room temperature for 72 h. After this, the mixture was concentrated under vacuum, re-disolved in EtOAc (20 mL), extracted with HCl 1 M (1 x 5 mL), sat. $NaHCO_3$ (1 x 10 mL) and brine (1 x 10 mL), dried ($MgSO_4$) and concentrated. Column chromatography (hexane:EtOAc 3:1 → 1:3) of the residue gave compound **12** as white solid (60 mg, 58%), mp 188-189 °C (from xxx): R_f = 0.46 (EtOAc); $[\alpha]_D^{20}$ -50.5 (*c* 0.5, $CHCl_3$). ^{1}H RMN (500 MHz, $CDCl_3$) δ 7.47-7.45 (m, 2 H, H-aromatic), 7.35-7.34 (m, 3 H, H-aromatic), 6.28 (d, 1 H, $J_{NH,2}$ = 7.4 Hz, N*H*), 6.05 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 5.52 (s, 1 H, PhC*H*O), 5.30 (t, 1 H, $J_{2',3'} \cong J_{3',4'}$ = 9.3 Hz, H-3'), 5.12 (dd, 1 H, $J_{3',4'}$ = 9.3, $J_{4',5'}$ = 10.0 Hz, H-

4'), 4.90 (d, 1 H, $J_{1',2'}$ = 10.1 Hz, H-1'), 4.77 (dd, 1 H, $J_{2',3'}$ = 9.3, $J_{1',2'}$ = 10.1 Hz, H-2'), 4.36 (dd, 1 H, $J_{5,6eq}$ = 3.5, $J_{6eq,6ax}$ = 9.1, Hz, H-6eq), 3.97 (d, 1 H, $J_{4',5'}$ = 10.0 Hz, H-5'), 3.76–3.59 (m, 7 H, H-2, H-3, H-5, H-6ax, OCH_3), 3.49 (dd, $J_{3,4} \cong J_{4,5}$ = 9.3 Hz, H-4), 2.10, 2.02, 1.97, 1.96, 1.74 (5 s, 15 H, CH_3CO); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 171.0, 170.1, 169.6, 169.4, 169.2, 167.5 (*C*O), 136.9, 129.3, 128.4, 126.4 (C-aromatic), 102.3 (Ph*C*HO), 93.6 (C-1), 80.2 (C-1'), 78.6 (C-4), 75.2 (C-5'), 72.7 (C-3'), 71.2 (C-2'), 70.4 (C-5), 68.9 (C-4'), 68.7 (C-6), 55.0 (C-3), 53.2 (O*C*H$_3$), 46.1 (C-2), 23.3, 21.0, 20.7, 20.6 ($\times$ 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{30}H_{37}NNaO_{15}S$: 706.1776, found: 706.1786, $[M+H]^+$ calcd for $C_{30}H_{38}NO_{15}S$: 684.1957, found: 684.1971

1,4,6-tri-*O*-acetyl-2-azido-3-*S*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,3-dideoxy-3-thio-α,β-D-glucopyranose (13α,β)

Compound **10** (100 mg, 0.15 mmol) was treated with CSA (63 mg, 0.27 mmol) in a solution of MeOH:DCM 1:1 (12.8 mL) and the reaction mixture was stirred for 18 h at room temperature, then quenched with Et_3N and concentrated under vacuum. The residue was treated with acetic anhydride (3 mL, 32 mmol) in pyridine (3 mL) during 18h at room temperature. After this, MeOH was added and solvents were evaporated under vacuum. Column chromatography (8:2→1:1 hexane-EtOAc) of the residue gave **13α,β** (96 mg, 97%) in a relation α:β 1:3 as white solid, R_f = 0.39 (1:1 hexane:EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.28 (d, 0.34 H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1β), 5.29 (2 t superimposed, 1.35 H, $J_{2',3'} \cong J_{3',4'} \cong$ 9.2 Hz, H-3'α + H-3'β), 5.20 (2 t superimposed, 1.35 H, $J_{3',4'} \cong J_{4',5'}$ = 9.7 Hz, H-4'α + H-4'β), 5.00-4.90 (m, 2.7 H, H-2'α + H-2'β + H-4α + H-4β), 4.88 (2 d superimposed, 1.35 H, $J_{1',2'}$ = 10.1 Hz, H-1'α + H-1'β), 4.26-4.23 (2 dd superimposed, 1.35 H, H-6aα + H-6aβ), 4.10-4.00 (m, 3.00 H, H-5'α, H-5'β, H-

5α, H-6bα, H-6bβ), 3.90 (dd, 0.35 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.7 Hz, H-2α), 3.77-3.72 (m, 5.00 H, H-5β + OC*H*$_3$α + OC*H*$_3$β), 3.10 (t, 0.35 H, $J_{2,3} \approx J_{3,4} \approx$ 11.7 Hz, H-3α), 2.75 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 11.2 Hz, H-3β), 2.2-2.0 (m, 24.3 H, 6 C*H*$_3$CO-α + 6 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.1, 169.6, 169.5, 169.3, 168.8, 168.7, 166.8, 166.7 (*C*O), 94.3 (C-1β), 89.7 (C-1α), 83.9 (C-1'β), 83.8 (C-1'α), 76.0 (C-5'α), 75.9 (C-5'β), 75.4 (C-5β), 73.1 (C-3'α + C-3'β), 70.8 (C-5α), 70.0 (C-2'α + C-2'β), 69.3 (C-4'α), 69.2 (C-4'β), 65.5 (C-4α), 65.4 (C-4β), 65.3 (C-2β), 63.1 (C-2α), 62.1 (C-6α), 62.0 (C-6β), 53.0 (O*C*H$_3$-α), 62.0 (C- O*C*H$_3$-β), 51.4 (C-3β), 49.2 (C-3α), 21.1, 20.9 (× 2), 20.7 (× 3), 20.6 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{25}H_{33}N_3NaO_{16}S$: 686.1474, found: 686.1487

Revisar este espectro

1,4,6-tri-*O*-acetyl-2-*N*-acetamido-3-*S*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-2,3-dideoxy-3-thio-α- and β-D-glucopyranose (14α and 14β)

The anomeric mixture **13α,β** (87 mg, 0.13 mmol) was dissolved in pyridine (2.8 mL). Under argon atmosphere, AcSH (190 µL, 2.6 mmol) was added. The reaction mixture was stirred at room temperature for 72 h. Then solvents were evaporated under vacuum and column chromatography of the residue using silica gel of < 45 µm (for thin layer chromatography) and hexane:EtOAc mixtures (3:7→1:9 hexane-EtOAc) gave first **14β** (30 mg, 51%) and then **14α** (22 mg, 37%) as white solids.

14α: R_f = 0.37 (EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.28 (d, 0.34 H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1β), 5.29 (2 t superimposed, 1.35 H, $J_{2',3'} \cong J_{3',4'} \cong$ 9.2 Hz, H-3'α + H-3'β), 5.20 (2 t superimposed, 1.35 H, $J_{3',4'} \cong J_{4',5'}$ = 9.7 Hz, H-4'α + H-4'β), 5.00-

4.90 (m, 2.7 H, H-2'α + H-2'β + H-4α + H-4β), 4.88 (2 d superimposed, 1.35 H, $J_{1',2'}$ = 10.1 Hz, H-1'α + H-1'β), 4.26-4.23 (2 dd superimposed, 1.35 H, H-6aα + H-6aβ), 4.10-4.00 (m, 3.00 H, H-5'α, H-5'β, H-5α, H-6bα, H-6bβ), 3.90 (dd, 0.35 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.7 Hz, H-2α), 3.77-3.72 (m, 5.00 H, H-5β + OC*H*$_3$α + OC*H*$_3$β), 3.10 (t, 0.35 H, $J_{2,3} \approx J_{3,4} \approx$ 11.7 Hz, H-3α), 2.75 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 11.2 Hz, H-3β), 2.2-2.0 (m, 24.3 H, 6 C*H*$_3$CO-α + 6 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.1, 169.6, 169.5, 169.3, 168.8, 168.7, 166.8, 166.7 (*C*O), 94.3 (C-1β), 89.7 (C-1α), 83.9 (C-1'β), 83.8 (C-1'α), 76.0 (C-5'α), 75.9 (C-5'β), 75.4 (C-5β), 73.1 (C-3'α + C-3'β), 70.8 (C-5α), 70.0 (C-2'α + C-2'β), 69.3 (C-4'α), 69.2 (C-4'β), 65.5 (C-4α), 65.4 (C-4β), 65.3 (C-2β), 63.1 (C-2α), 62.1 (C-6α), 62.0 (C-6β), 53.0 (O*C*H$_3$-α), 62.0 (C- O*C*H$_3$-β), 51.4 (C-3β), 49.2 (C-3α), 21.1, 20.9 (× 2), 20.7 (× 3), 20.6 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{27}H_{37}NNaO_{17}S$: 702.1674, found: 702.1669

14β: R_f = 0.42 (EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.28 (d, 0.34 H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1β), 5.29 (2 t superimposed, 1.35 H, $J_{2',3'} \cong J_{3',4'} \cong$ 9.2 Hz, H-3'α + H-3'β), 5.20 (2 t superimposed, 1.35 H, $J_{3',4'} \cong J_{4',5'}$ = 9.7 Hz, H-4'α + H-4'β), 5.00-4.90 (m, 2.7 H, H-2'α + H-2'β + H-4α + H-4β), 4.88 (2 d superimposed, 1.35 H, $J_{1',2'}$ = 10.1 Hz, H-1'α + H-1'β), 4.26-4.23 (2 dd superimposed, 1.35 H, H-6aα + H-6aβ), 4.10-4.00 (m, 3.00 H, H-5'α, H-5'β, H-5α, H-6bα, H-6bβ), 3.90 (dd, 0.35 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.7 Hz, H-2α), 3.77-3.72 (m, 5.00 H, H-5β + OC*H*$_3$α + OC*H*$_3$β), 3.10 (t, 0.35 H, $J_{2,3} \approx J_{3,4} \approx$ 11.7 Hz, H-3α), 2.75 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 11.2 Hz, H-3β), 2.2-2.0 (m, 24.3 H, 6 C*H*$_3$CO-α + 6 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.1, 169.6, 169.5, 169.3, 168.8, 168.7, 166.8, 166.7 (*C*O), 94.3 (C-1β), 89.7 (C-1α), 83.9 (C-1'β), 83.8 (C-1'α), 76.0 (C-5'α), 75.9 (C-5'β), 75.4 (C-5β), 73.1 (C-3'α + C-3'β), 70.8 (C-5α), 70.0 (C-2'α + C-2'β), 69.3 (C-4'α), 69.2 (C-4'β), 65.5 (C-4α), 65.4 (C-4β), 65.3 (C-2β), 63.1 (C-2α), 62.1 (C-6α), 62.0 (C-6β), 53.0

(O*C*H$_3$-α), 62.0 (C- O*C*H$_3$-β), 51.4 (C-3β), 49.2 (C-3α), 21.1, 20.9 (× 2), 20.7 (× 3), 20.6 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{27}H_{37}NNaO_{17}S$: 702.1674, found: 702.1671

1,4,6-tri-*O*-acetyl-2-azido-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3-dideoxy-3-thio-α,β-D-glucopyranose (15α,β)

Compound **6** (70 mg, 0.10 mmol) was treated with CSA (43 mg, 0.19 mmol) in a solution of MeOH:DCM 1:1 (8.4 mL) and the reaction mixture was stirred for 18 h at room temperature, then quenched with Et_3N and concentrated under vacuum. The residue was treated with acetic anhydride (1.4 mL, 14.8 mmol) in pyridine (1.4 mL) during 18h at room temperature. After this, MeOH was added and solvents were evaporated under vacuum. Column chromatography (8:2→1:1 hexane-EtOAc) of the residue gave **15** (50 mg, 72%) as white solid, R_f = 0.35 (1:1 hexane:EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.25 (d, 0.45 H, $J_{1,2}$ = 3.7 Hz, H-1α), 5.45-5.43 (m, 2.45 H, H-1β + H-4'α + H-4'β), 5.22-5.16 (2 t superimposed, 1.45 H, $J_{1',2'} \cong J_{2',3'} \cong$ 9.9 Hz, H-2'α + H-2'β), 5.10-5.05 (m, 1.45 H, H-3'α + H-3'β), 5.04-4.95 (m, 1.45 H, H-4α + H-4β), 4.91-4.88 (2 d superimposed, 1.45 H, $J_{1',2'}$ = 10.0 Hz, H-1'α + H-1'β), 4.30-4.24 (2 dd superimposed, 1.45 H, H-6aα + H-6aβ), 4.17-3.97 (m, 6.25 H, H-5'α, H-5'β, H-5α, H-6bα, H-6bβ, H-6'aα H-6'aβ, H-6'bα, H-6'bβ), 3.90 (dd, 0.45 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.6 Hz, H-2α), 3.79 (dd, 1 H, $J_{1,2}$ = 8.4, $J_{2,3}$ = 9.5 Hz, H-2β), 3.75 (m, 1 H, $J_{4,5}$ = 2.2, $J_{5,6a}$ = 4.9, $J_{5,6b}$ = 7.1 Hz, H-5β), 3.12 (t, 0.45 H, $J_{2,3} \approx J_{3,4} \approx$ 11.3 Hz, H-3α), 2.74 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx$ 11.2 Hz, H-3β), 2.20-1.87 (m, 34.80 H, 8 C*H*$_3$CO-α + 8 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.5, 170.4, 170.3, 170.1, 170.0, 169.6, 169.4, 168.7 (*C*O), 94.3 (C-1β), 89.8 (C-1α), 83.0 (C-1'β), 82.9 (C-1'α), 75.5 (C-5β), 74.6 (C-5'α +

C-5'β), 71.8 (C-3'α + C-3'β), 70.8 (C-5α), 67.4, 67.3, 67.2 (C-2'β, C-4'α, C-4'β), 65.2 (C-4α, C-4β), 64.6 (C-2β), 62.5, 62.3, 62.2, 62.1 (C-6α, C-6β, C-6'α, C-6'β), 51.4 (C-3β), 48.7 (C-3α), 21.1 (× 2), 20.9 (× 2), 20.8 (× 4), 20.7 (CH_3COα). ESI-HRMS: $[M+Na]^+$ calcd for $C_{26}H_{35}N_3NaO_{16}S$: 700,1630, found: 700.1644

Revisar este espectro

1,4,6-tri-*O*-acetyl-2-*N*-acetamido-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3-dideoxy-3-thio-α- and β-D-glucopyranose (16α and 16β)

The anomeric mixture **15α,β** (50 mg, 0.07 mmol) was dissolved in pyridine (1.6 mL). Under argon atmosphere, AcSH (110 µL, 1.5 mmol) was added. The reaction mixture was stirred at room temperature for 72 h. Then solvents were evaporated under vacuum and column chromatography of the residue using silica gel of < 45 µm (for thin layer chromatography) and hexane:EtOAc mixtures (3:7→1:9 hexane-EtOAc) gave first **16β** (23 mg, 45%) and then **16α** (11 mg, 23%) as white solids.

16α: R_f = 0.28 (EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.28 (d, 0.34 H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1β), 5.29 (2 t superimposed, 1.35 H, $J_{2',3'} \cong J_{3',4'} \cong$ 9.2 Hz, H-3'α + H-3'β), 5.20 (2 t superimposed, 1.35 H, $J_{3',4'} \cong J_{4',5'}$ = 9.7 Hz, H-4'α + H-4'β), 5.00-4.90 (m, 2.7 H, H-2'α + H-2'β + H-4α + H-4β), 4.88 (2 d superimposed, 1.35 H, $J_{1',2'}$ = 10.1 Hz, H-1'α + H-1'β), 4.26-4.23 (2 dd superimposed, 1.35 H, H-6aα + H-6aβ), 4.10-4.00 (m, 3.00 H, H-5'α, H-5'β, H-5α, H-6bα, H-6bβ), 3.90 (dd, 0.35 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.7 Hz, H-2α), 3.77-3.72 (m, 5.00 H, H-5β + OCH_3α + OCH_3β), 3.10 (t, 0.35 H, $J_{2,3} \approx J_{3,4} \approx$ 11.7 Hz, H-

3α), 2.75 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx 11.2$ Hz, H-3β), 2.2-2.0 (m, 24.3 H, 6 C*H*$_3$CO-α + 6 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.1, 169.6, 169.5, 169.3, 168.8, 168.7, 166.8, 166.7 (*C*O), 94.3 (C-1β), 89.7 (C-1α), 83.9 (C-1'β), 83.8 (C-1'α), 76.0 (C-5'α), 75.9 (C-5'β), 75.4 (C-5β), 73.1 (C-3'α + C-3'β), 70.8 (C-5α), 70.0 (C-2'α + C-2'β), 69.3 (C-4'α), 69.2 (C-4'β), 65.5 (C-4α), 65.4 (C-4β), 65.3 (C-2β), 63.1 (C-2α), 62.1 (C-6α), 62.0 (C-6β), 53.0 (O*C*H$_3$-α), 62.0 (C- O*C*H$_3$-β), 51.4 (C-3β), 49.2 (C-3α), 21.1, 20.9 (× 2), 20.7 (× 3), 20.6 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{28}H_{39}NNaO_{17}S$: 716.1831, found: 716.1832

14β: R_f = 0.38 (EtOAc). ^{1}H RMN (500 MHz, $CDCl_3$) δ 6.28 (d, 0.34 H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.45 (d, 1 H, $J_{1,2}$ = 8.4 Hz, H-1β), 5.29 (2 t superimposed, 1.35 H, $J_{2',3'} \cong J_{3',4'} \cong 9.2$ Hz, H-3'α + H-3'β), 5.20 (2 t superimposed, 1.35 H, $J_{3',4'} \cong J_{4',5'} = 9.7$ Hz, H-4'α + H-4'β), 5.00-4.90 (m, 2.7 H, H-2'α + H-2'β + H-4α + H-4β), 4.88 (2 d superimposed, 1.35 H, $J_{1',2'} = 10.1$ Hz, H-1'α + H-1'β), 4.26-4.23 (2 dd superimposed, 1.35 H, H-6aα + H-6aβ), 4.10-4.00 (m, 3.00 H, H-5'α, H-5'β, H-5α, H-6bα, H-6bβ), 3.90 (dd, 0.35 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 11.7 Hz, H-2α), 3.77-3.72 (m, 5.00 H, H-5β + OC*H*$_3$α + OC*H*$_3$β), 3.10 (t, 0.35 H, $J_{2,3} \approx J_{3,4} \approx 11.7$ Hz, H-3α), 2.75 (t, 1 H, $J_{2,3} \approx J_{3,4} \approx 11.2$ Hz, H-3β), 2.2-2.0 (m, 24.3 H, 6 C*H*$_3$CO-α + 6 C*H*$_3$CO-β); ^{13}C RMN (125.7 MHz, $CDCl_3$) δ 170.7, 170.1, 169.6, 169.5, 169.3, 168.8, 168.7, 166.8, 166.7 (*C*O), 94.3 (C-1β), 89.7 (C-1α), 83.9 (C-1'β), 83.8 (C-1'α), 76.0 (C-5'α), 75.9 (C-5'β), 75.4 (C-5β), 73.1 (C-3'α + C-3'β), 70.8 (C-5α), 70.0 (C-2'α + C-2'β), 69.3 (C-4'α), 69.2 (C-4'β), 65.5 (C-4α), 65.4 (C-4β), 65.3 (C-2β), 63.1 (C-2α), 62.1 (C-6α), 62.0 (C-6β), 53.0 (O*C*H$_3$-α), 62.0 (C- O*C*H$_3$-β), 51.4 (C-3β), 49.2 (C-3α), 21.1, 20.9 (× 2), 20.7 (× 3), 20.6 (× 2) (*C*H$_3$CO). ESI-HRMS: $[M+Na]^+$ calcd for $C_{28}H_{39}NNaO_{17}S$: 716.1831, found: 716.1833

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

The SI file includes the synthesis of **1**, the analysis of the conformers of **8**, **9** and **11** obtained by molecular modeling and copies of ^{1}H and ^{13}C-NMR spectra for all compounds synthesized. 2D ^{1}H-COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-NOESY experiments of **6**-**10** are also included.

AUTHOR INFORMATION

*Corresponding Author, E-mail: mluhrig@qo.fcen.uba.ar

ORCID: https://orcid.org/0000-0002-6980-4141

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Support for this work from the Universidad de Buenos Aires and the Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET is gratefully acknowledged. We thank Prof. Oscar Varela and Gerardo Burton for inspiring discussions. We also thank the AUIP for the mobility fellowship granted to Alejandro E. Cristófalo. Alejandro E. Cristófalo is a PhD fellow from CONICET. María Laura Uhrig is a research member of CONICET.

References

(1) Szilagyi, L.; Varela, O. Non-Conventional Glycosidic Linkages: Syntheses and Structures of Thiooligosaccharides and Carbohydrates with Three-Bond Glycosidic Connections. *Curr. Org. Chem.* **2006**, *10* (14), 1745–1770. https://doi.org/10.2174/138527206778249874.

(2) Reed, L. A.; Goodman, L. Synthesis of Thiolactose (4-S-β-d-Galactopyranosyl-4-Thio-d-Glucopyranose). *Carbohydr. Res.* **1981**, *94* (1), 91–100. https://doi.org/10.1016/S0008-6215(00)85599-8.

(3) Contour-Galcera, M. O.; Guillot, J. M.; Ortiz-Mellet, C.; Pflieger-Carrara, F.; Defaye, J.; Gelas, J. Synthesis of Sulfur-Linked Analogues of Nigerose, Laminarabiose, Laminaratriose, Gentiobiose, Gentiotriose, and Laminaran Trisaccharide Y. *Carbohydr. Res.* **1996**, *281* (1), 99–118. https://doi.org/10.1016/0008-6215(95)00339-8.

(4) Moreau, V.; Norrild, J. C.; Driguez, H. Synthesis of Methyl 4-Thio-β-Cellobioside. A Reinvestigation. *Carbohydr. Res.* **1997**, *300* (3), 271–277. https://doi.org/10.1016/S0008-6215(97)00046-3.

(5) Nigudkar, S. S.; Demchenko, A. V. Stereocontrolled 1,2-Cis Glycosylation as the Driving Force of Progress in Synthetic Carbohydrate Chemistry. *Chem. Sci.* **2015**, *6* (5), 2687–2704. https://doi.org/10.1039/c5sc00280j.

(6) Fowler, P.; Bernet, B.; Vasella, A. A1H-NMR Spectroscopic Investigation of the Conformation of the Acetamido Group in Some Derivatives OfN-Acetyl-D-Allosamine and -D-Glucosamine. *Helvetica Chimica Acta*. 1996, pp 269–287. https://doi.org/10.1002/hlca.19960790127.

(7) Karban, J.; Kroutil, J. *Chemistry of Carbohydrate Aziridines*; 2006; Vol. 60. https://doi.org/10.1016/S0065-2318(06)60003-6.

(8) Hashimoto, H.; Shimada, K.; Horito, S. Synthesis of α-L-Fucopyranosyl Disaccharides with Thioglycosidic Linkages and Characterization of α-L-Fucosidases from Bovine Kidney and Epididymis by Their Inhibitory Activities. *Tetrahedron: Asymmetry* **1994**, *5* (12), 2351–2366. https://doi.org/10.1016/S0957-4166(00)80385-7.

(9) Cai, Y.; Ling, C.-C.; Bundle, D. R. Concise and Efficient Synthesis of 2-Acetamido-2-Deoxy-Beta-D-Hexopyranosides of Diverse Aminosugars from 2-Acetamido-2-Deoxy-Beta-D-Glucose. *J. Org. Chem.* **2009**, *74* (2), 580–589. https://doi.org/10.1021/jo801927k.

(10) Okazaki, H.; Hanaya, K.; Shoji, M.; Hada, N.; Sugai, T. A New Route toward 2-Acetamido-4-O-Methyl-2-Deoxy-d-Mannopyranose from a Ferrier Derivative of Tri-O-Acetyl-d-Glucal, Which Contributes to Aldolase-Catalyzed Synthesis of Laninamivir (CS-8958). *Tetrahedron* **2013**, *69* (37), 7931–7935. https://doi.org/10.1016/j.tet.2013.07.018.

(11) Rye, C. S.; Withers, S. G. The Synthesis of a Novel Thio-Linked Disaccharide of Chondroitin as a Potential Inhibitor of Polysaccharide Lyases. *Carbohydr. Res.* **2004**, *339* (3), 699–703. https://doi.org/10.1016/j.carres.2003.12.011.

(12) Wang, L.; Lee, Y. C. Stereoselective Synthesis of N-Acetyl Thiochitooligosaccharides. Different Behaviours of Methyl N-Acetyl-a- and -b-Thiochitobiosides during Acetolysis. *J. Chem. Soc., Perkin Trans. 1* **1996**, No. 6, 581–591.

(13) Auzanneau, F.-I.; Bennis, K.; Fanton, E.; Promé, D.; Defaye, J.; Gelas, J. Synthesis of S-Linked Thiooligosaccharide Analogues of Nod Factors. Part 1: Selectively N-Protected 4-Thiochitobiose Precursors. *J. Chem. Soc. Perkin Trans. 1* **1998**, No.

21, 3629–3636. https://doi.org/10.1039/a802932f.

(14) Aguilera, B. and Fernández-Mayoralas, A. Nucleophilic Displacements on a Cyclic Sulfamidate Derived from Allosamine: Application to the Synthesis of Thiooligosaccharides. Chem. Commun. 1996, pp 127–128.

(15) Aguilera, B.; Fernández-Mayoralas, A.; Jaramillo, C. Use of Cyclic Sulfamidates Derived from D-Allosamine in Nucleophilic Displacements: Scope and Limitations. *Tetrahedron* **1997**, *53* (16), 5863–5876. https://doi.org/10.1016/S0040-4020(97)00246-9.

(16) Aguilera, B.; Fernandez-Mayoralas, A. Synthesis of a Thio-Analogue of Lewis X by Regioselective Opening of Cyclic Sulfamidates. *J. Org. Chem.* **1998**, *63* (8), 2719–2723. https://doi.org/10.1021/jo971784a.

(17) Chen, H. M.; Withers, S. G. Syntheses of the 3- and 4-Thio Analogues of 4-Nitrophenyl 2-Acetamido-2-Deoxy-??-D-Gluco- and Galactopyranoside. *Carbohydr. Res.* **2007**, *342* (15), 2212–2222. https://doi.org/10.1016/j.carres.2007.05.023.

(18) Witczak, Z. J.; Chen, H.; Kaplon, P. Thio-Sugars. Part 5: From d-Glucal to 3-Deoxy-(1→2)-2-S-Thiodisaccharides through Isolevoglucosenone—a Simple Approach. *Tetrahedron: Asymmetry* **2000**, *11* (2), 519–532.

(19) Witczak, Z. J.; Kaplon, P.; Kolodziej, M. Thiosugars VI: A Simple Stereoselective Approach to (1→3)-3-S-Thiodisaccharides from Levoglucosenone. *Monatshefte fur Chemie* **2002**, *133* (4), 521–530. https://doi.org/10.1007/s007060200025.

(20) Witczak, Z. J.; Kaplon, P.; Dey, P. M. Thio-Sugars VII. Effect of 3-Deoxy-4-S-(β-D-Gluco- and β-D-Galactopyranosyl)-4-Thiodisaccharides and Their Sulfoxides and Sulfones on the Viability and Growth of Selected Murine and Human Tumor Cell

Lines. *Carbohydr. Res.* **2003**, *338* (1), 11–18. https://doi.org/10.1016/S0008-6215(02)00394-4.

(21) Uhrig, M. L.; Manzano, V. E.; Varela, O. Stereoselective Synthesis of 3-Deoxy-4-S-(1→4)-Thiodisaccharides and Their Inhibitory Activities towards β-Glycoside Hydrolases. *European J. Org. Chem.* **2006**, *4* (1), 162–168. https://doi.org/10.1002/ejoc.200500457.

(22) Witczak, Z. J.; Lorchak, D.; Nguyen, N. A Click Chemistry Approach to Glycomimetics: Michael Addition of 2,3,4,6-Tetra-O-Acetyl-1-Thio-β-d-Glucopyranose to 4-Deoxy-1,2-O-Isopropylidene-l-Glycero-Pent-4-Enopyranos-3-Ulose - a Convenient Route to Novel 4-Deoxy-(1→5)-5-C-Thiodisaccharides. *Carbohydr. Res.* **2007**, *342* (12–13), 1929–1933. https://doi.org/10.1016/j.carres.2007.06.005.

(23) Repetto, E.; Marino, C.; Uhrig, M. L.; Varela, O. Two Straightforward Strategies for the Synthesis of Thiodisaccharides with a Furanose Unit as the Nonreducing End. *European J. Org. Chem.* **2008**, No. 3, 540–547. https://doi.org/10.1002/ejoc.200700874.

(24) Cagnoni, A. J.; Uhrig, M. L.; Varela, O. Synthesis of Pentopyranosyl-Containing Thiodisaccharides. Inhibitory Activity against β-Glycosidases. *Bioorganic Med. Chem.* **2009**, *17* (17), 6203–6212. https://doi.org/10.1016/j.bmc.2009.07.055.

(25) Witczak, Z. J.; Sun, J.; Mielguj, R. Synthesis of L-Fucopyranosyl-4-Thiodisaccharides from Levoglucosenone and Their Inhibitory Activity on α-L-Fucosidase. *Bioorganic Med. Chem. Lett.* **1995**, *5* (18), 2169–2174. https://doi.org/10.1016/0960-894X(95)00377-6.

(26) Manzano, V. E.; Uhrig, M. L.; Varela, O. Straightforward Synthesis of

Thiodisaccharides by Ring-Opening of Sugar Epoxides. *J. Org. Chem.* **2008**, *73* (18), 7224–7235. https://doi.org/10.1021/jo8012397.

(27) Repetto, E.; Manzano, V. E.; Uhrig, M. L.; Varela, O. Synthesis of Branched Dithiotrisaccharides via Ring-Opening Reaction of Sugar Thiiranes. *J. Org. Chem.* **2012**, *77* (1), 253–265. https://doi.org/10.1021/jo2018685.

(28) Manzano, V. E.; Uhrig, M. L.; Varela, O. Convenient Synthesis of 4-Thiolactose, 3,4-Dithiolactose and Related Thiooligosaccharides and Disulfides. Inhibitory Activity of the Glycomimetics against a β-Galactosidase. *Org. Biomol. Chem.* **2012**, *10* (44), 8884–8894. https://doi.org/10.1039/c2ob26388b.

(29) Dada, L.; Manzano, V. E.; Varela, O. Design and Synthesis of 2-Acetamido-2,3-Dideoxythiodisaccharides via Diastereoselective Conjugate Addition to Sugar Enone O-Acetyl Oximes. Galactosidase Inhibition Studies. *Org. Lett.* **2018**, *20* (19), 6225–6228. https://doi.org/10.1021/acs.orglett.8b02692.

(30) Hayashi, H.; Kaga, A.; Chiba, S. Application of Vinyl Azides in Chemical Synthesis: A Recent Update. *J. Org. Chem.* **2017**, *82* (23), 11981–11989. https://doi.org/10.1021/acs.joc.7b02455.

(31) Anderson, E. A.; Bi, X.; Zanoni, G.; Anderson, E. A. A-Substituted Vinyl Azides: An Emerging Functionalized Alkene. *Chem. Soc. Rev.* **2017**, *46* (23), 7208–7228. https://doi.org/10.1039/C7CS00017K.

(32) Hu, B.; Dimagno, S. G. Reactivities of Vinyl Azides and Their Recent Applications in Nitrogen Heterocycle Synthesis. *Org. Biomol. Chem.* **2015**, *13* (13), 3844–3855. https://doi.org/10.1039/c5ob00099h.

(33) Wrobleski, A., Coombs, T. C., Huh, C. W., Li, S., Aubé, J. THE SCHMIDT REACTION AARON. *Org. React.* **2012**, *78*.

(34) Wang, Y. F.; Hu, M.; Hayashi, H.; Xing, B.; Chiba, S. Linking of Alcohols with Vinyl Azides. *Org. Lett.* **2016**, *18* (5), 992–995. https://doi.org/10.1021/acs.orglett.6b00116.

(35) Hanessian, S., Plessas, N. R. Synthesis of Unsaturated Sugars Containing Vinylic Substituents. *Chem. Commun.* **1968**, No. 0, 706b – 708.

(36) Franconetti, A.; Álvarez, E.; Cabrera-Escribano, F. Efficient Two-Step Multifunctionalization of Substituted 2-Hydro-Xyglycopyranosides. *Synlett* **2017**, *28* (2), 201–206. https://doi.org/10.1055/s-0036-1588624.

(37) Emmadi, M.; Kulkarni, S. S. Synthesis of Rare Deoxy Amino Sugar Building Blocks Enabled the Total Synthesis of a Polysaccharide Repeating Unit Analogue from the LPS of Psychrobacter Cryohalolentis K5T. *J. Org. Chem.* **2018**, *83* (23), 14323–14337. https://doi.org/10.1021/acs.joc.8b02037.

(38) Rakotomanomana, N., Lacombe, J., Pavia, A. Rbduction-Acetylation Sélective Des Azido-Sucres Par Le Mélange Acide Thioacétique-Thioacétate de Potassium. *Carbohydr. Res.* **1990**, *197*, 318–323.

(39) Luo, S.; Kulkarni, S. S.; Chou, C.-H.; Liao, W.; Hung, S. A Concise Synthesis of Tetrahydroxy-LCB, r-Galactosyl Ceramide, and 1,4-Dideoxy-1,4-Imino-L-Ribitol via D-Allosamines as Key Building Blocks. *J. Org. Chem.* **2006**, *71* (3), 1226–1229.

(40) Potter, G. T.; Jayson, G. C.; Miller, G. J.; Gardiner, J. M. An Updated Synthesis of the Diazo-Transfer Reagent Imidazole-1-Sulfonyl Azide Hydrogen Sulfate. *J. Org. Chem.* **2016**, *81* (8), 3443–3446. https://doi.org/10.1021/acs.joc.6b00177.

(41) Jakab, Z.; Fekete, A.; Borbás, A.; Lipták, A.; Antus, S. Synthesis of New Sulfonic Acid-Containing Oligosaccharide Mimetics of Sialyl Lewis A. *Tetrahedron* **2010**, *66* (13), 2404–2414. https://doi.org/10.1016/j.tet.2010.01.100.

(42) Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Petillo, P. A. Synthesis of Two Hyaluronan Trisaccharides. *Org. Lett.* **2000**, *2* (9), 1279–1282. https://doi.org/10.1021/ol0057075.

(43) Kolakowski, R. V.; Shangguan, N.; Sauers, R. R.; Williams, L. J. Mechanism of Thio Acid/Azide Amidation. *J. Am. Chem. Soc.* **2006**, *128* (17), 5695–5702. https://doi.org/10.1021/ja057533y.

(44) Cristófalo, A. E.; Montenegro, H. O.; Cano, E.; Colomer, J. P.; Uhrig, M. L.. Synthesis of 2-Propynyl 2-Acetamido-3,4,6-tri-O-Acetyl-2-Deoxy-1-Thio-β-D-Glucopyranoside, 2-Propynyl 3,4,6-tri-O-Acetyl-2-Deoxy-2-Phthalimido-1-Thio-β-D-Glucopyranoside and their 2-(2-Propynyloxyethoxy)ethyl Analogs in: Tanja Wrodnigg guest editor, Pavol Kovac Series editor (Eds.), Carbohydr. Chem. Proven Synth. Methods, vol. 5, **2019**, in press

(45) Floyd, N.; Vijayakrishnan, B.; Koeppe, J. R.; Davis, B. G. Thiyl Glycosylation of Olefinic Proteins: S-Linked Glycoconjugate Synthesis. *Angew. Chem. Int. Ed. Engl.* **2009**, *48* (42), 7798–7802. https://doi.org/10.1002/anie.200903135.

(46) Yerien, D. E.; Barata-Vallejo, S.; Camps, B.; Cristófalo, A. E.; Cano, M. E.; Uhrig, M. L.; Postigo, A. Electron-Catalyzed Radical Perfluoroalkylation of Organic Sulfides: The Serendipitous Use of the TMEDA/I_2 Complex as a Radical Initiator. *Catal. Sci. Technol.* **2017**, *7* (11), 2274–2282. https://doi.org/10.1039/C7CY00236J.