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COMMUNICATION

Towards a synthetic strategy for the ten canonical carrageenan oligosaccharides – synthesis of a protected γ-carrageenan tetrasaccharide

Christine Kinnaert,[a] and Mads H. Clausen*[a]

Abstract: We herein report a synthetic strategy aiming at synthesizing the ten canonical carrageenan oligosaccharides from one single precursor. The key β-(1→4)-linked disaccharide was synthesized from commercially available galactose pentaacetate. The notoriously difficult formation of β-(1→4)-D-galactan linkages was successfully optimized on the differentially substituted monosaccharides to afford the desired disaccharide in 55% yield. Following a convergent strategy, two disaccharides were then glycosylated to form the fully protected α-(1→4)-linked tetrasaccharide backbone of the carrageenans. The careful selection of protecting groups provides the opportunity to access all ten carrageenan substructures identified in polysaccharides isolated from red algae. Here, we demonstrate how one such target oligosaccharide can be obtained in a protected form.

Introduction

In order to increase understanding of plant cell wall biology and physiology, well-defined plant cell wall oligosaccharides have been used as model compounds to represent domains from larger, more complex polysaccharides. Therefore, synthetic chemists have devoted attention to developing efficient strategies for the chemical synthesis of such oligosaccharide fragments.[1]–[8] However, as outlined by a recent review summarizing all plant and algal cell wall oligosaccharides synthesized to date,[9] there are relatively few examples of synthetic fragments representative of marine polysaccharides. Therefore, we are interested in the synthesis of carrageenans, which are sulfated polysaccharides found in red algae seaweed.[10]–[13] Not only are these targets potentially very valuable for understanding the algal cell wall, but they would also help advance the development of new methodologies in carbohydrate chemistry and more specifically in relation to sulfated oligosaccharides.[14]–[17] Carrageenans are a family of highly sulfated galactans found in the cell walls of red seaweeds of the Rhodophyceae class. These polysaccharides serve as gelling, stabilizing and viscosity-enhancing agents in many sectors including the pharmaceutical and food industry and represent one of the major texturizing ingredients in processed foods.[13]-[18] Their use in pharmaceutical products continues to be investigated, and recent applications in drug delivery systems are summarized in a review by S. Liang Li *et al.*[19] Carrageenan are divided into ten different idealized repeating disaccharides, which differ mainly by their degree of sulfation and the presence or absence of 3,6-anhydrogalactose residues (Figure 1). Naturally occurring carrageenan is a mixture of non-homologous polysaccharides, which makes it challenging to obtain samples of homogenous oligosaccharides by either chemical or enzymatic degradation of the algae cell wall components. We present herein a synthetic strategy aiming at producing the ten canonical carrageenan oligosaccharides from one single protected oligosaccharide precursor.

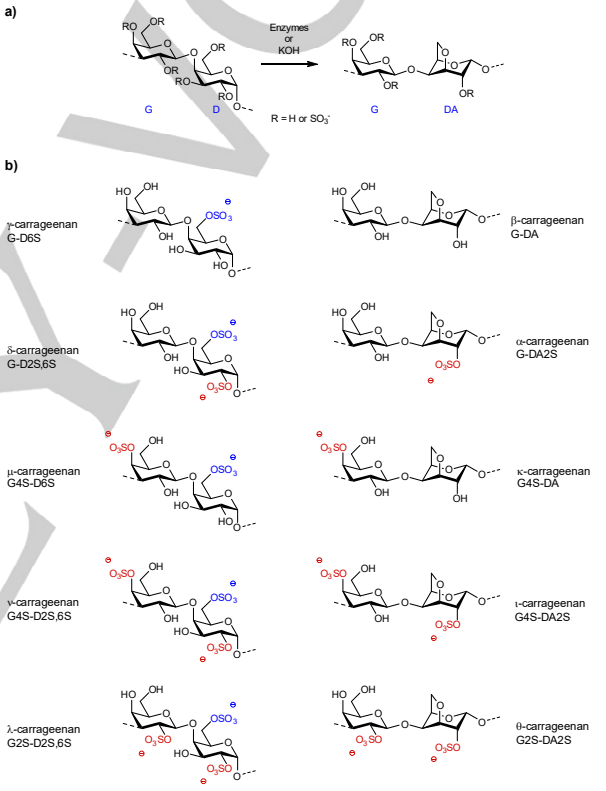

Figure 1. a) Carrabiose unit. **b)** Ten types of idealized repeating units of carrageenans (see nomenclature established by Knutsen, Myslabodski, Larsen and Usov (1994)[20] and integrated into the IUPAC rules).[21]

Carrageenans share a common galactan backbone of alternating 3-linked-β-D-galactopyranose (G) and 4-linked-α-D-galactopyranose (D). The disaccharide repeating units, called carrabioses, are shown in Figure 1 a). It has been shown that hot alkaline treatment of the D6S carrageenans (namely carrageenan having a 4-linked-α-D-galactopyranose residue sulfated at the 6 position) leads to cyclization to form the 3,6-anhydro ring accompanied by a ring flip from $^4C_1$ to $^1C_4$ (see Figure 1 a).[22],[23] In algae this reaction is catalyzed by enzymes such as sulfohydrolases[24],[25] or galactose-6-sulfurylases,[26] which have been isolated from algal cells. The activity of sulfurylases I and II catalyzing the conversion of ν-carrageenan to ι-carrageenan was demonstrated *in vitro* and proved to be specific to this type of carrageenan as it was inactive on μ-carrageenan.[26]
The chemical synthesis of oligocarrageenans requires the formation of a backbone of alternating 3-linked-β-D-

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galactopyranose and 4-linked-α-D-galactopyranose units, followed by selective sulfation of various positions depending on the targeted carrageenan. The strategy we investigated is shown in Figure 2 and uses a blockwise assembly. This approach relies on the synthesis of a key β-(1→4)-linked disaccharide carrying protecting groups suitable for selective modifications later on. This disaccharide will serve as a common building block and will require formation of α-(1→3)-linkages to construct the carrageenan backbone. In order to evaluate the strategy we decided to first target carrageenan tetrasaccharides.

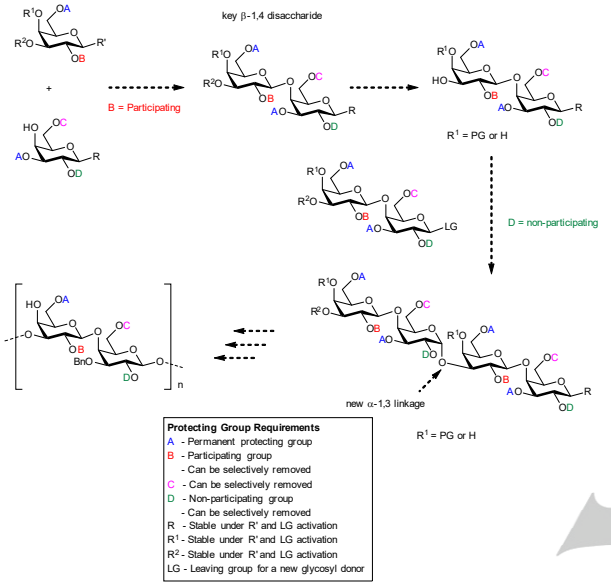


Figure 2. Synthetic strategy based on well-chosen protecting groups.

The protected tetrasaccharide **1a** shown in Figure 3 conforms to all the requirements and was chosen as a potential single precursor for all ten types of carrageenans.

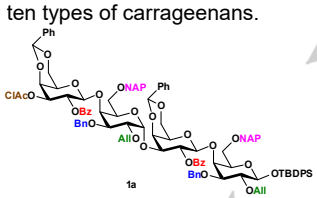
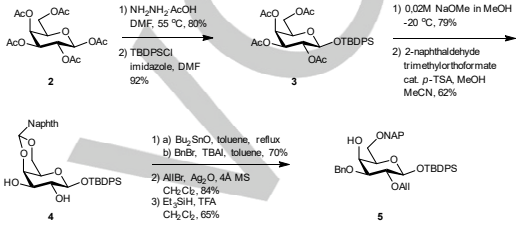


Figure 3. Protected tetrasaccharide precursor of the 10 canonical carrageenans.

Results and Discussion

Based on the criteria set up on the retrosynthetic analysis, we decided to target tetrasaccharide **1a**. The journey to **1a** began with the synthesis of monosaccharide acceptor **5**, achieved in seven steps from commercially available galactose pentaacetate, Scheme 1. First, the anomeric position was deacetylated [27] and protected with TBDPSCl. It was found that the silyl group is prone to migrate under both acidic and basic conditions and as a consequence, all the following steps from TBDPS protected compound **3** had to be carried out under neutral conditions or with very low concentration of acid or base. Zemplén deacetylation of the remaining acetyl groups was performed at low temperature using dilute sodium methoxide in methanol. The C-4 and C-6 positions were protected by reaction with naphthaldehyde dimethyl_acetal, which was formed *in situ* by reacting 2-naphthaldehyde with trimethylorthoformate and a catalytic amount of *p*-TSA in methanol. Unfortunately, a substantial amount of isomerization to the undesired α-anomer was observed under the reaction condition, and therefore the yield was only 62%. The diol **4** was activated with Bu_2SnO *in situ* followed by treatment with benzyl bromide in the presence of TBAI to afford the 3-OBn product. The penultimate step in the sequence was allylation of the 2-OH under neutral conditions using silver oxide and allyl bromide, which afforded the fully protected monosaccharide in 84% yield. The last step to reach the desired acceptor was a regioselective reductive opening of the of the naphthylidene acetal giving a C-6 NAP ether and the free 4-OH. This reaction was performed using triethylsilane and trifluoroacetic acid (TFA) to afford the acceptor **5**. However, hydrolysis of the naphthylidene group was also observed and the corresponding diol was isolated as the major byproduct.

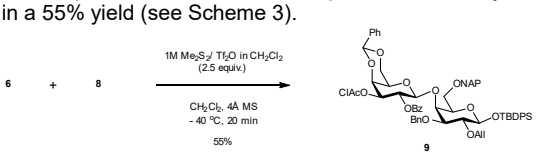


Scheme 1. Synthesis of protected monosaccharide acceptor **5.**

The synthesis of the monosaccharide glycoside donor was more straightforward and achieved in two steps from the known thioglycoside **6**,[28] which in turn is available in three steps from galactose pentaacetate (see Scheme 2). It is worth mentioning that the benzoylation of the remaining unprotected hydroxyl group had to be performed in dichloromethane at low temperature (0 °C) and using Et_3N in order to avoid deprotection of the chloroacetyl group.

Scheme 2. Synthesis of the known[29] protected monosaccharide donor **8.**

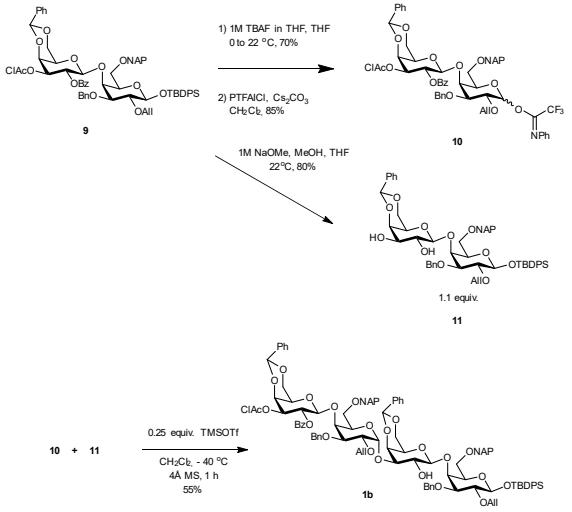
As the synthesis of β-(1→4)-D-galactans is known to be very challenging,[30],[31] the optimization of the glycosylation producing the β-(1→4)-linked disaccharide was cumbersome and even under optimized conditions,[32] the product could only be isolated in a 55% yield (see Scheme 3).



Scheme 3. Optimized conditions to form the key β-(1→4)-linked disaccharide **9**.

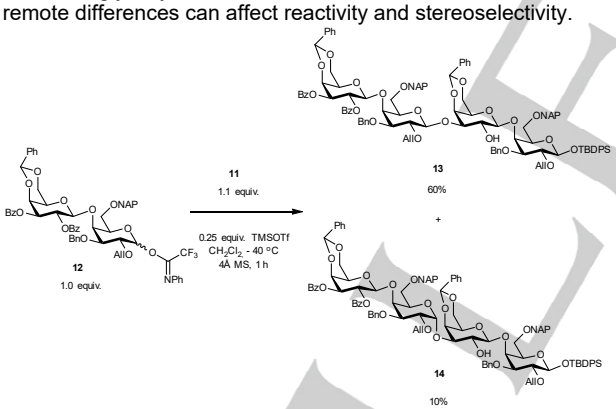
Disaccharide **9** could subsequently be transformed into both the imidate disaccharide donor **10**[33] and a disaccharide acceptor, see Scheme 4. Surprisingly, we found that it was impossible to remove the chloroacetyl group selectively in the presence of the 2'-O benzoyl group without migration of the later (see SI for a detailed account of the conditions that were investigated). Thus, we decided to remove both acyl groups to arrive at **11** and rely on the higher reactivity of the 3-position over the more sterically hindered 2-position for the subsequent glycosylation. Gratifyingly, this proved to be efficient, as the desired α-linked tetrasaccharide could be isolated in 55% yield following the glycosylation reaction. While HMBC correlations confirmed the 1-3 linkage, the configuration of the newly formed anomeric center was

established by a one bond $^{13}C–^{1}H$ coupling constant of 168 Hz along with a ^{13}C chemical shift at 92.2 ppm.[34]



Scheme 4. Synthesis of the α-(1→3)-linked tetrasaccharide **1b**.

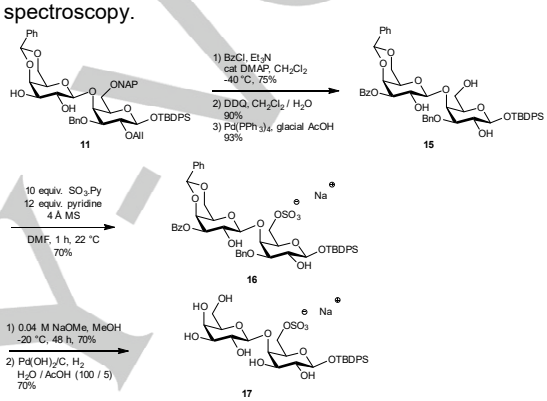
Since the selective deprotection was unfruitful, we wanted to decrease the amount of steps by replacing the chloroacetyl group with another benzoyl group. To our great surprise, glycosylation with donor **12** under the optimized conditions only afforded 10% of the desired α-(1→3)-linked tetrasaccharide **14** (Scheme 5). The major product formed was instead the corresponding β-anomer **13** (60% of β-(1→3)-linked product). The anomeric configuration of **13** and **14** was verified by their one-bond $^{13}C–^{1}H$ coupling constants of 159 and 168 Hz, respectively. That changing the nature of the 3'-ester protecting group can have such a dramatic effect on glycosylation outcome demonstrates how subtle and remote differences can affect reactivity and stereoselectivity.



Scheme 5. Unexpected outcome of [2+2] glycosylation reaction.

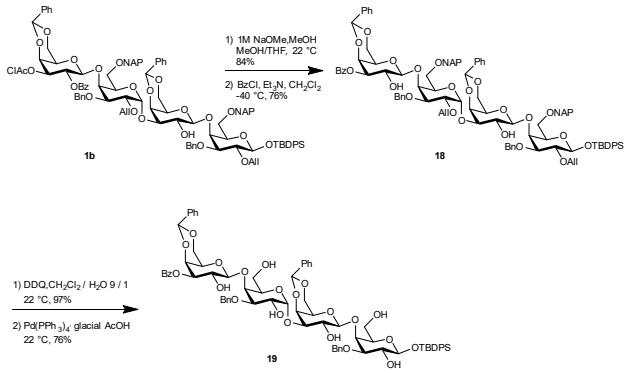
With tetrasaccharide **1b** in hand, the deprotection and sulfation strategy to reach the selectively sulfated carrageenans (λ, γ, δ, μ and ν) was envisioned as shown in Scheme 6. The five corresponding anhydro-carrageenan (θ, β, α, κ and ι) could be reached by performing an alkali treatment of their respective precursor or by incubation with the enzymes mentioned in the introduction.

In order to prove the suitability of the protecting group and test the sulfation step, we first applied the transformations to disaccharide **11**, see Scheme 7. Regioselective benzoylation of diol **11** at the C-3' position was achieved at - 40 °C, using BzCl, Et_3N and a catalytic amount of DMAP in 75% yield with less than 15 % of di-benzoylated adduct isolated. The 6-*O*-NAP protecting group was selectively removed under oxidative conditions using DDQ in a high yield (90%).[35] Selective removal of the allyl group using tetrakis(triphenylphosphine)palladium(0) gave triol **15**, importantly without migration of the TBDPS group or anomerization. Selective sulfation of the C-6 position was performed using 10 equiv. of SO_3-pyridine complex. It was pivotal to add 4Å MS as well as additional pyridine to the reaction mixture to remove any traces of water and acid, respectively, to avoid partial cleavage of the benzylidene protecting group. The monosulfated disaccharide **16** was obtained in 70% yield. Sulfation at C-6 was identified by a downfield shift of the NMR signal of the corresponding carbon atom (~ 8 ppm) and protons (~ 0.5 ppm). According to previous syntheses of heparin oligosaccharides, a downfield shift of ring protons H-2 and H-2' of approximately 1 ppm would indicate the formation of sulfate esters at the C-2 positions.[36],[37] As the chemical shift of H-2 and H-2' did not change, we concluded that sulfation at these two positions did not occur. This result was further confirmed by mass spectroscopy.



Scheme 7. Protection/deprotection and optimized conditions for selective sulfation of disaccharide **11**.

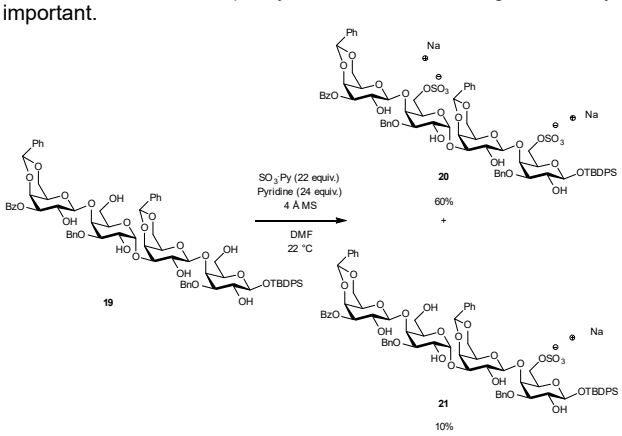
These successful results were then translated to the tetrasaccharide **1b**. Deprotection of the two acyl groups under Zemplén conditions afforded the corresponding tetrasaccharide triol. Regioselective benzoylation of the free C-3''' position under the conditions optimized for the disaccharide analogue gave the monobenzoylated adduct **18** in 76% yield. Oxidative cleavage of the NAP groups using DDQ, followed by deallylation with $Pd(PPh_3)_4$ afforded compound **19** in 74% yield (Scheme 8).



Scheme 8. Deprotection of tetrasaccharide **1b.**

As regioselective sulfation of the two primary alcohols was required, fine-tuning of the amount of SO_3·Py complex used was critical to avoid oversulfation (see conditions used in Scheme 9). We observed large variation in the outcome for this reaction and

careful control of the quality of the sulfation reagent is very important.



Scheme 9. Conditions used for the selective sulfation of the C-6 positions.

Current efforts include further investigation of the sulfation reaction as well as the selective deprotection to reach all canonical carrageenans. The results of these studies will be reported in due course.

Conclusions

In conclusion, a synthesis strategy to afford the alternating 3-linked-β-D-galactopyranose (G) and 4-linked-α-D-galactopyranose (D) backbone of carrageenans was developed and a protected tetrasaccharide precursor for ten different carrageenans was prepared using this approach. Furthermore, the suitability of the protecting group scheme was demonstrated by the synthesis of sulfated D6S di-carrageenan. Selective sulfation of the tetrasaccharide was tested and the results highlighted that fine-tuning of the amount of SO_3·pyridine as well as careful control of the quality of this reagent are key to achieve selective sulfation. We are currently in the process of implementing those results to the synthesis of the other carrageenan targets.

Experimental Section

Experimental details can be found in the Supporting Information.

Acknowledgements

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Keywords: Plant/algal oligosaccharides • sulfated oligosaccharides • carrageenans • α-(1→3)-galactoside • β(1→4)-galactoside

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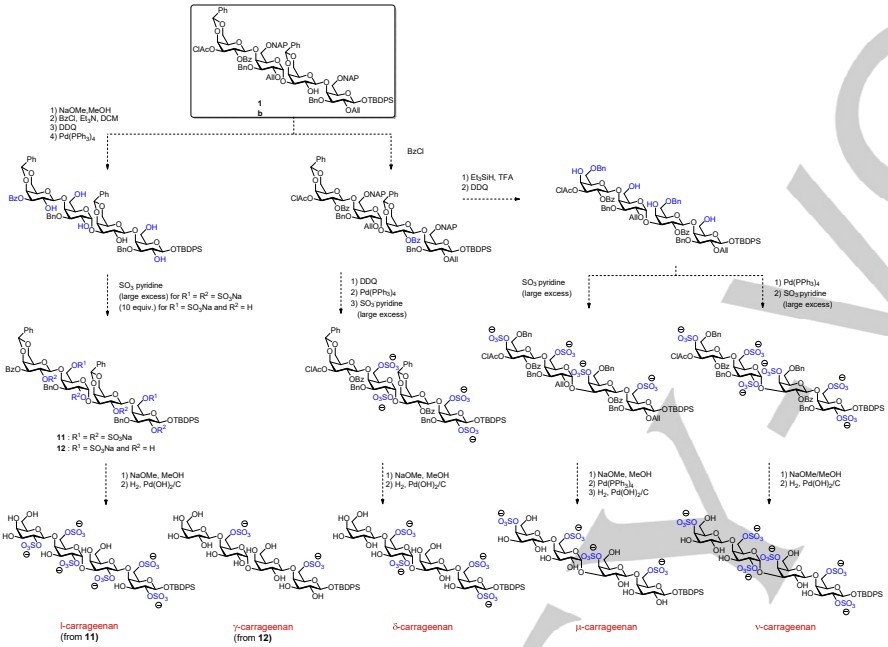

Scheme 6. Deprotection and sulfation strategy for tetrasaccharide **1b**.